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Ram Naresh Bharagava *Editor*

Environmental Contaminants: Ecological Implications and Management

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Editor

Environmental Contaminants: Ecological Implications and Management

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This book is truly dedicated to my parents for their unfailing patience, contagious love, forgiveness, selflessness, and endless support, my wife for trusting and believing me, and my kids for always a hope to move forward in life.

Ram Naresh Bharagava

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Babasaheb Bhimrao Ambedkar
University (A Central University),
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January 31, 2019

Ram Naresh Bharagava

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Naveen Kumar Arora, Ph.D., microbiology, Professor in the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh, India, is a renowned researcher in the field of environmental microbiology and biotechnology. His specific area of research is rhizosphere biology and PGPRs. He has more than 50 research papers published in premium international journals and several articles published in magazines and dailies. He is editor of 10 books, published by Springer. He is a member of several national and international societies, Secretary General of Society for Environmental Sustainability, in editorial board of four journals, and reviewer of several international journals. He is also the Editor in Chief of the journal *Environmental Sustainability* published by Springer Nature. He has delivered lectures in conferences and seminars around the globe. He has a long-standing interest in teaching at the PG level and is involved in taking courses in bacteriology, microbial physiology, environmental microbiology, agriculture microbiology, and industrial microbiology. He has been an advisor to 118 postgraduate and 8 doctoral students. Recently, he was awarded for excellence in research by the Honorable Governor of Uttar Pradesh. Although an academician and researcher by profession, he has a huge obsession for the wildlife and its conservation and has authored a book, *Splendid Wilds*. He is the President of Society for Conservation of Wildlife and has a dedicated website www.naveenarora.co.in for the cause of wildlife and environment conservation.

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Ram Naresh Bharagava was born on 1977. He received his B.Sc. degree (1998) in Zoology, Botany, and Chemistry from the University of Lucknow, India; his M.Sc. degree (2004) in Molecular Biology and Biotechnology from Govind Ballabh Pant University of Agriculture and Technology (GBPUAT), India; his Ph. D. degree (2010) in the area of Microbiology jointly from Environmental Microbiology Division, Council of Scientific and Industrial Research (CSIR)-Indian Institute of Toxicology Research (IITR), Lucknow, and Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India. He was a Junior Research Fellow (JRF) during his Ph.D., and passed the CSIR-National Eligibility Test (NET) twice and the Graduate Aptitude Test in Engineering (GATE) once. He has written one book and edited six books. He has also authored and coauthored numerous research/review papers and two book reviews in national and international journals. He has also written several chapters for national and international edited books as well as a number of scientific articles, and has presented papers at national and international conferences. Further, he is a reviewer for various national and international journals. After a postdoctoral appointment at CSIR-IITR, Lucknow, he joined Babasaheb

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Chapter 1
Agro-industrial Wastes: Environmental Toxicology, Risks, and Biological Treatment Approaches

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Abstract The great challenge for agro-industries, the "green chemistry" market, and related areas in recent years is to find viable alternatives to reduce dependence on the use of fossil fuels and traditional chemical solvents in energy production. These can have negative effects on human and environment health, combined with insecurity due to their high potential for flammability, volatility, and toxicity. As a solution, natural origin sources have become a scientific and industrial trend in biofuel generation. Lignocellulosic biomass, which includes solid organic waste consisting mainly of cellulose, hemicellulose, and lignin (LiP) and liquid waste composed of organic matter and suspended solids, is an economically viable alternative, since it comes from natural and renewable sources. Besides that, when used as raw material, it promotes a significant reduction in the production process final cost; and it conforms to sustainable development. However, cellulosic content pretreatment is necessary, mainly to break the lignin chemical structure and to remove other recalcitrant components. Biological treatment, specifically with

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white rot fungi (WRF), emerges as an excellent technology in organic product degradation, mainly to those which resist conventional treatment. They have a nonspecific and oxidative ligninolytic enzyme system, composed of the enzymes laccase (Lac) and manganese peroxidase (MnP), which are able to convert bioavailable polysaccharides in the vegetal wall structure (cellulose and hemicellulose) into sugars of easy assimilation and promote degradation of various agroindustrial wastes and effluents. In addition, toxicity is importantly addressed by new scientific, industrial, and political strategies which assist in the evaluation of these wastes' toxic interactions and their consequences that affect the ecosystem water bodies. In this context, this chapter focuses mainly on several aspects of biological treatment with white rot fungi and their ligninolytic enzyme production as a sustainable technology to degrade agro-industrial residues and components of lignocellulosic biomass into products with high added value. As well, it aims to study the eco-toxicity to estimate maximum permissible levels of toxic compounds. In general, this new approach is an alternative method for obtaining technological products and processes that enable the improvement of industrial sustainability through the use of ecologically renewable inputs, as well as reducing costs and increasing process efficiency.

Keywords Agro-industrial wastes · Environmental toxicology · Risks · Management · Enzyme production

1.1 Introduction

The steady increase in the consumption of environmentally friendly products, such as renewable resources, has occurred as a result of strict government regulations around the world and increased awareness among the population to minimize environmental pollution. For this, fossil fuel substitution for biofuels has been extremely important to stimulate the growth of economies based on sustainability through the use of these resources (Moraes et al. 2015; Martini et al. 2018). Brazil is one of the biggest agricultural commodity producers, since it is considered the world's biggest producer of sugar, coffee, orange juice, and soybeans and the biggest exporter of beef and poultry (CNA 2018). According to the Confederation of Agriculture and Livestock of Brazil (CNA 2018), in 2017, the agribusiness reached approximately R$536.5 billion of gross production value (GPV), R$342.6 billion in agricultural production and R$193.9 in the farming segment. Within this scenario, agro-industrial production accounts for approximately 5.9% of the gross domestic product (GDP), generating an average of 108 million tons of waste per year; and this sector is therefore recognized as a crucial vector of Brazilian economic growth (IBGE 2018).

The agro-industry is responsible for the generation of various residues and by-products inherent to agricultural activities (Bilal et al. 2017). Lignocellulosic biomass appears as a potential source for second-generation bioethanol production once it presents a high level of cellulose and hemicellulose in its composition. In addition, it presents surplus availability and a relatively low cost (Arevalo-Gallegos et al. 2017). Soybean straw, sugarcane leaves, bark and corn husk, and sugarcane bagasse are the most suitable biomass types to be used as cellulose raw material (Araújo et al. 2018). However, lignocellulosic biomass conversion to degradable inputs is directly attributed to its chemical and morphological characteristics, since the structural factors reflect its recalcitrance property (Bilal et al. 2017). Several pretreatment methods have been proposed and developed (Bharathiraja et al. 2017; Huzir et al. 2018; Kumari and Singh 2018). The wastewater produced by this sector is usually characterized by high chemical (COD) and biochemical oxygen demand (BOD), high staining and aromatics presence. From this problem, there is a growing interest in the treatment of agro-industrial residues that contain high amounts of organic matter and toxic components by researchers and industries (Ghangrekar 2018).

Therefore, agro-industries are looking for alternative treatments for their disposal and/or reuse in agriculture, in order to reduce or eliminate the use and generation of toxic substances that affect human and environment health (Ghangrekar 2018). An excellent alternative is the use of processes that involve mycoremediation, which replace traditional processes by the use of microorganisms (Voběrková et al. 2018). In this case, white rot fungi that are part of the class *Basidiomycetes* stand out because they have high catalytic potential and specific characteristics, especially the possibility of resistance mechanisms in adverse environmental conditions (Zhuo et al. 2017; Naraian et al. 2018). Besides that, these fungi produce a large amount of ligninolytic extracellular enzymes, such as laccase (Lac) and manganese peroxidase (MnP), which are capable of degrading and/or mineralizing various recalcitrant aromatic dyes (Naraian et al. 2018; Finimundy et al. 2018).

Although the waste treatment by these processes is advantageous in relation to conventional treatments, it is necessary to study the overall efficiency, just as it is important that the final product is within the release maximum limits of liquid effluents in water bodies (Ghangrekar 2018; Mo et al. 2018). From this, the use of bioassays with organisms is essential to evaluate the toxic effects of chemical substances, industrial effluents, and environmental samples such as water and sediment (Vilar et al. 2018).

In this scenario, in order to attend to the principles of technological development processes and sustainable products that enable the improvement of industrial sustainability through the acquisition and use of ecologically renewable inputs, the bioremediation importance has been studied by several authors, based on the use of ligninolytic enzymes, since they present a direct transformation potential of the recalcitrant compounds during the fermentation process, in some cases with significant mineralization rates. In this sense, the proposal of this work is to have an overview of the lignocellulosic biomass and agro-industrial residue processing main methods, in order to turn them into products with high added value, as well as to analyze the agro-industrial residues' (solid or liquid) suitability as raw material in biological treatment, from the use of white rot fungi and their ligninolytic enzymes.

In addition, a review on toxicity is proposed to address environmental protection issues considering the deleterious effects of the chemical composition of agro-industrial wastes.

1.2 Agro-industrial Residues

Agro-industrial residues are products generated during the processing of a certain agricultural crop or animal material, coming from the industrial sector (Vallejos et al. 2017). Depending on the residue nature, it can be found in solid or liquid form (Panesar et al. 2015). Moreover, in the applications associated with the agronomy sector, this category is composed of several organic materials, such as by-products from the sugarcane industry (sugarcane bagasse, filter cake, molasses, oilseed cake, and sugar whitening sludge), agricultural production (manure), charcoal production (tar and coal tar), fruit processing (coconut, banana, mango, apple, orange, and cashew), and vegetable (tomato, bean, beet, potato, and soybean), cereal (wheat, rice, corn, barley, and sorghum), grain (coffee and cocoa), vegetable oil (palm), leather, and wood production (Ying et al. 2016). These natural origin inputs are processed during agricultural harvest and include all seeds, straws, stems, barks, lint, bran, stones, stubble, and pulps (Kumari and Singh 2018). In general, these residues are generally easily adaptable in arid and semiarid conditions in tropical developing countries, and their production is characterized by being seasonal (Pérez-Pimienta et al. 2018; Ying et al. 2016) (Fig. 1.1).

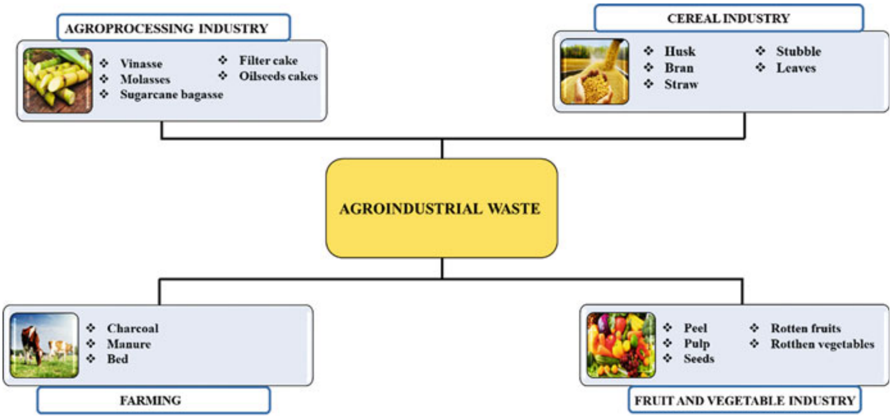

Fig. 1.1 Types of agro-industrial waste generated from different industries

1.3 Chemical Composition of Agricultural Wastes

Agro-industrial wastes are an important part of new scientific, industrial, and political strategies that help reduce dependence on fossil fuels, once they are derived from renewable resources and present high nutritional quality and valuable properties for the "green" fuel sector (Huzir et al. 2018). The composition of these includes primarily carbohydrates, proteins, minerals, vitamins, and bioactive compounds, depending on the source specificity. From this, they can be destined for human consumption, promoting biomass reduction and nutrient loss in the productive chain. Waste use provides important environmental benefits with respect to resource renewability, in order to develop more efficient and sustainable by-products with high added value (Arevalo-Gallegos et al. 2017).

Among the available sources of renewable raw material that integrate a large part of solid agro-industrial residues, lignocellulosic materials have achieved an increasing emphasis in the global scenario, with a relatively low cost advantage, stimulated by the advances in biotechnological processes (Bilal et al. 2017). In particular, lignocellulosic biomass derived from the plant cell wall is a rich organic source of biopolymers, chemicals, and sugars which accumulate from agricultural, forestry, municipal, and other activities (Zheng et al. 2014). Moreover, it is characterized by being multifunctional since it promotes the generation of several products, such as textile fibers, composites, dispersants, flocculants, and activated carbon due to its carbon-rich chemical composition. Another important characteristic is its surplus availability which does not allow it to compete with food production or animal feed (Kumari and Singh 2018).

Lignocellulosic biomass consists mainly of cellulose, hemicellulose, and lignin (Fig. 1.2), which are natural organic polymers joined by non-covalent forces and by covalent crosslinks, as well as smaller amounts of proteins (nitrogen compounds),

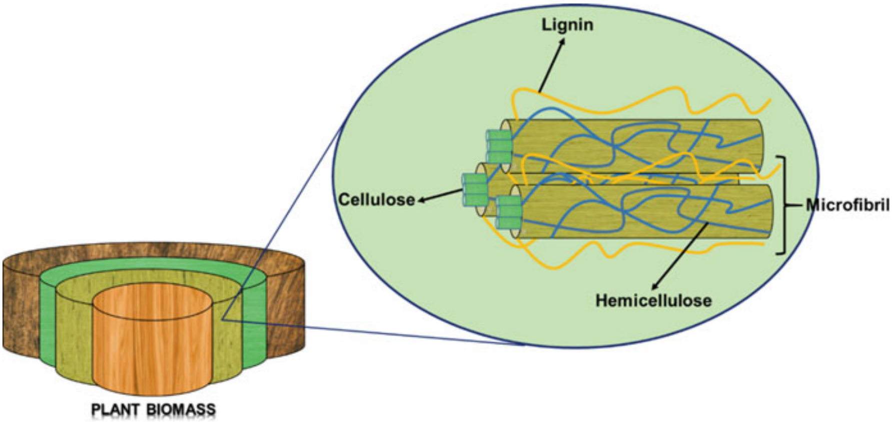

Fig. 1.2 Structure of the cellulose, hemicellulose, and lignin fractions in the lignocellulosic biomass

resins, pectin, extractives, fatty acids, phenols, and salts (WEI 2016). Component contents vary according to the biomass type, genetic variability, geographic location, and growing conditions. Usually, cellulose ($C_6H_{10}O_5$) contains 40–50% of the wood dry weight (Sohni et al. 2018). This is considered the main biopolymer present in plant cell walls, since it consists of D-glucopyranose units strongly connected via β-1,4-glycosidic covalent bonds formed from the linear repeat of cellobiose units, which can be disrupted in glucose monomers (Taha et al. 2016). When molecules crystallize into long and parallel chains of glucose (high crystallinity), microfiber packets are formed, which results in the recalcitrance property of the lignocellulosic biomass (Gupta et al. 2016). However, formation of small amounts of amorphous cellulose (low crystallinity) allows it to be more susceptible to biodegradation. Thus, the crystallinity index is an essential parameter in the orientation of the most degradable region.

Hemicellulose of general formula $(C_5H_4O_8)_b$ comprises 25–35% of the lignocellulosic biomass, characterized as a polysaccharide of linear and branched structure. The hemicellulosic chain is formed by successive units of the same sugar (homopolymer) or by mixtures of different sugars (heteropolymer) (Kumari and Singh 2018). Furthermore, these sugars can be classified as glucose, xylose, galactose, arabinose, mannose, fructose, and uronic acid. The fact that it has an amorphous structure with low polymerization degree causes it to be removed under softer reaction conditions, once it hydrolyzes easily when compared to cellulose, which confers less resistance to chemical products and microorganisms (Mo et al. 2018).

On the other hand, the amorphous heteropolymer lignin $(C_9H_{10}O_3(OCH_3)_{0.9-1.7})_c$ is a phenyl propanol composed of three different strongly branched hydrophobic monomers (coniferyl alcohol, syringyl alcohol, and coumaril) with high molecular weight (WEI 2016). Characteristics of impermeability, resistance to chemical and biological degradation, and insolubility in water make this component the most recalcitrant of the vegetal cell walls. Besides that, this component hinders the enzymatic hydrolysis process, since during lignocellulosic biomass conversion to fermentable sugars, it acts as a physical barrier by which the enzymes are prevented from entering the polysaccharides. In general, this compound has 10–25% of lignocellulosic material and forms a complex structure in the cell wall, depending on the bonds formed between cellulose and hemicellulose during the polymerization process (Kumari and Singh 2018). Table 1.1 shows the average values of the main components that constitute some agro-industrial residues.

1.4 Agro-industrial Waste Management

Agro-industrial waste management contributes to economic and social growth potential, just as it promotes the rise of the agro-processing industry. Moreover, large volumes of these by-products are generated throughout the year, which is why they have attracted researchers and industries' interest, once they are abundantly available, cheap, and renewable. Nevertheless, despite providing prospects and

Table 1.1 Percent composition of lignocellulose components (% dry mass) in various lignocellulosic materials

Agro-industrial residues	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Barley hull	34	36	19	Kim et al. (2008)
Nut shells	25–30	25–30	30–40	Abbasi and Abbasi (2010)
Forage sorghum	36	20	18	Li et al. (2010)
Sugarcane bagasse	41.6	25.1	20.3	Kim and Day (2011)
Sorgo doce	44.6	27.1	20.7	Kim and Day (2011)
Rice straw	47.2	31.8	18.7	Chen et al. (2011)
Rice	32.6	27.3	18.4	Zheng et al. (2012)
Corn stalks	49.22	25.57	17.18	Egüés et al. (2012)
Olive wood	31.48	11.33	23.5	Garcia-Maraver et al. (2013)
Wheat straw	30	26	16	Abdolali et al. (2014)
Bamboo	26	15	21	Abdolali et al. (2014)
Rape straw	37.6	31.4	21.3	Hamidreza et al. (2015)
Barley straw	34.8	27.9	14.6	Hamidreza et al. (2015)
Flax fibers	75.9	20.7	3.4	García et al. (2016)
Oat straw	37.6	23.34	12.85	Ying et al. (2016)
Grass	30	23	7	Rodriguez et al. (2016)
Peel fruit	53.6	22.5	22.6	Selvaraju et al. (2017)
Corncob	25.1	26.1	12.3	Menezes et al. (2017)
Palm waste	22–40	19–33	12–25	Bensidhom et al. (2017)
Banana waste	38	28	33.8	Sango et al. (2018)
Sawdust	42.96	19.99	26.8	Saffe and Fernandez (2018)
Stalk	16.02	5.78	30.79	Saffe and Fernandez (2018)

presenting good characteristics for general agricultural use, there is a new interest in these organic wastes' treatment, since if they are discarded without proper treatment in the water bodies, they promote pollution of the entire ecosystem and consequently cause aquatic organisms' death, as well as they impair the photosynthetic activity which results in dissolved oxygen decrease in water (Ghangrekar 2018). Additionally, since they are considered to be chemically stable and difficult to break down, they can also generate acute toxic effects to humans.

Generally, these residues in the liquid form are responsible for most of the wastewater contamination, since they are constituted by high organic load, color, high turbidity, and high content of oil and grease. For these reasons, it is necessary to develop treatment processes that can promote greater treatment efficiency and require less energy demand, once conventional methods are not considered satisfactory. Among these alternative processes are the biological ones, among which the anaerobic treatment is the most used in the fermentation industry, due to its reduced mechanization and low energy consumption, where an upflow anaerobic sludge blanket (UASB) is commonly used because it is capable of receiving higher organic load amount (Arevalo-Gallegos et al. 2017). Therefore, the appropriate technology development for effluent treatment has attracted a great interest, since the main objective is the removal and degradation of residues harmful to the environment. Furthermore, these agro-industrial residues present several chemical characteristics that can hinder the water treatment process, especially the biological one, once its application is sensitive to toxicity of certain chemical products (Nascimento et al. 2018), whose legal limits must comply with the control standards established by the National Environmental Council (NEC).

1.4.1 Pretreatment Method of Solid Residue

Within the scope of bioenergy and biofuels sustainable production, the lignocellulosic biomass used in conversion processes is still not well understood; and its technological domain may allow the production of high added value products, low energy consumption, and non-toxicity. The conversion can occur by thermochemical and biochemical processes, where this thermochemical conversion is based on the lignocellulosic material heating (600–900 °C) in the presence of gasification reactions, pyrolysis, and combustion in order to produce biogas (H_2, CO_2, CH_4, C_2H_2), solids (coal), and liquids (bio-oil) (Chen et al. 2015; Hejazi et al. 2017). Biochemical conversion involves biomass processing by biological and/or chemical routes in order to produce biofuels (Domínguez et al. 2017). These processes refer to the biomass pretreatments that promote component separation and solubilization which in turn enable the biomass to be more degradable to additional biological or chemical treatments.

The several existing methods for lignocellulosic biomass pretreatment can be classified as physical, chemical, physical-chemical, biological, and combined (Bilal et al. 2017). The pretreatment must be suitable for the raw material, facilitating access and separation of the main components (cellulose, hemicellulose, and lignin), as well as preserving hemicellulose, raising the material porosity, and decreasing the cellulose crystallinity (Shirkavand et al. 2016). Table 1.2 summarizes the main effects of pretreatment techniques on the chemical composition and lignocellulosic biomass physical structure.

Physical pretreatment consists of methods that do not use microorganisms and/or chemicals during the treatment process. This method offers advantages with regard to particle size reduction and surface area increase of lignocellulosic materials (Huzir et al. 2018). Some physical pretreatment methods include combinations (milling and grinding), steam explosion (autohydrolysis), extrusion and irradiation (ultrasound and microwave), liquid hot water (hydrothermolysis), and freezing. Chemical pretreatment refers to the use of chemical substances, which include acids, bases, organic solvent, ionic liquid, catalyzed vapor explosion, wet peroxide oxidation and

Table 1.2 Pretreatment effect on the chemical and structural lignocellulosic biomass composition

Pretreatment	Increase of accessible surface area	Decrystallization of cellulose	Solubilization of hemicellulose	Solubilization of lignin	Alteration of lignin structure	Formation of furfural/ hydroxymethylfurfural (HMF)
Combination	•	•				
Irradiation	•	❖	❖			❖
Extrusion	•	❖	❖	❖		
Steam explosion	•		•	❖	•	•
Liquid hot water	•	ND	•	❖	❖	❖
Catalyzed steam explosion	•		•	•	•	•
Acid	•		•	❖	•	•
Alkaline	•		❖	•	•	❖
Oxidative	•	ND		•	•	❖
Ionic liquids	•	•	❖			
Ozonolysis	•	❖	•	•	•	
Biological	•	ND	•	•	•	
Combined	•	ND	•	•	•	ND

• = major effect
❖= minor effect
ND = not determined
Blank = no effect
Adapted from Zheng et al. (2014)

ozonolysis. This method is used mainly in hemicellulose and lignin decomposition (Bharathiraja et al. 2017).

The cellulose rupture occurs through physical-chemical pretreatment, where the ligning and hemicellulose are removed due to the operational conditions of pressure and temperature, in the presence or absence of a chemical agent. Methods include ammonia fibre explosion (AFExp), ultrasonic (US), autohydrolysis (AutHy), and carbon dioxide explosion (CO_2 Exp) (Kumari and Singh 2018). Biological pretreatment uses microorganisms in the processes, mainly fungal consortia (white and brown rot), in which the enzymes produced are able to degrade lignocellulose. In addition, it is considered the safest and most ecological method, since it does not require substantial energy input and chemical addition, as in the physical and chemical pretreatments, respectively. For combined pretreatment, two or more consecutive methods are used to improve overall process productivity (Haghighi et al. 2013).

In general, pretreatment aims to reduce energy demands and biofuel production cost, facilitate substrate enzymatic hydrolysis, avoid the loss or degradation of formed sugars, and avoid inhibitory agent production (Huzir et al. 2018). These are formed during carbohydrate degradation and may be furan aldehydes; aliphatic acids composed of acetic acid, formic acid, and levulinic acid; and phenolic compounds which are contributed by lignin (Huzir et al. 2018).

1.4.2 *Pretreatment Method of Liquid Residue*

Pretreatment methods for surface water and groundwater are used daily in numerous conventional processes, especially when these contain agro-industrial wastes (Mo et al. 2018). These methods include combination of physical, chemical, and biological treatments that can occur simultaneously and synergistically, increasing pollutant removal process efficiency (Ghangrekar 2018). When more unit processes are included, the desired effluent quality is achievable, but the treatment costs are increased. Biological treatment systems stand out for offering the possibility of using aerobic and/or anaerobic microorganisms capable of degrading organic compounds. Additionally, they have the advantage of treating large volumes of effluents with high rates of organic matter removal, due to the reduction of the BOD, COD, and suspended solids, enabling the reuse of these after the treatment (Ghangrekar 2018).

Another alternative to minimize the possible negative effects of the organic effluents' disposal in nature to the environment is the treatment with physical processes (decantation, flotation, filtration, and adsorption) which are characterized by residue separation; but, depending on the compound used, there is a need for sequential treatment (Prajapati and Chaudhari 2015). Chemical treatments are based on oxidation of biorefractory contaminants or biodegradable products through reactions of strongly oxidant species produced, such as H_2O_2, Cl_2, ClO_2, and MnO_4^-. However, intermediate products may remain in the solution and, consequently,

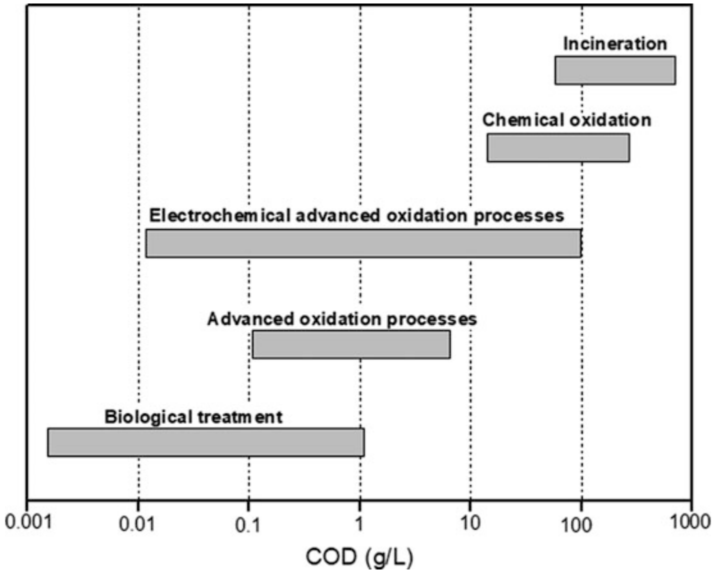

Fig. 1.3 Treatment technologies applicability based on the organic load amount. (Adapted from SIRÉS et al., 2014)

offer a similar or higher toxicity than the initial compounds, besides not promoting the residue total mineralization (Faust and Aly 2018).

Another possibility is the traditional incineration method, but it presents emission problems if the treatment is not controlled, besides having a high cost and being applied only in controlled solutions (Xian et al. 2018). For these reasons, residues can still be treated by advanced oxidative processes (AOPs), specifically using electrochemical technologies (AEOPs), which are based on electron transfer, since the electron is considered a clean reagent in the generation of hydroxyl radicals (·OH) (Flores et al. 2017; Vilar et al. 2018). According to Sirés et al. (2014), these different treatment system types are based on chemical oxygen demand (COD) concentration (Fig. 1.3) of the industrial waste present in water which generally contains a mixture of organic and inorganic compounds.

1.5 White Rot Fungi (WRF)

White rot fungi (WRF) belonging to the class *Basidiomycetes* are used in numerous mycoremediation processes, mainly in the biotechnology area, since they present low-cost technology use, high potential for recalcitrant compound degradation, and resistance mechanisms in adverse environmental conditions (Zhuo et al. 2017). This *Basidiomycetes* group is known as lignin-degrading agents, and they are distinguished from other microorganisms when applied to bioremediation due to the presence of septate filaments called hyphae that easily cross the substrate and reach pollutants. Moreover, they are the most favorable fungi for biological pretreatment since they have a nonspecific multienzyme system capable of catalyzing complex organic macromolecules in simple compounds of easy degradation via co-metabolism or catabolism (Finimundy et al. 2018).

In general, these fungal consortia destabilize chemically stable molecules due to their aerobic degradability and secretion of extracellular enzymes in the substrate that colonize them, including hydrolases and oxidoreductases, the latter playing an important role in lignin removal once they promote conversion of the bioavailable polysaccharides in the vegetal wall structure (cellulose and hemicellulose) into sugars of easy assimilation (Saha et al. 2016; Fasanella et al. 2018). Laccase (Lac), manganese peroxidase (MnP), and lignin (LiP) are the most important oxidoreductase enzymes; and the expression pattern of these enzymes depends on the organisms that secrete them. For these reasons, not all white fungi secrete these enzymes. The delignification process varies according to the fungal strain. In this sense, the genera of white rot fungi most used in inoculation are *Phanerochaete*, *Pleurotus*, *Trametes*, *Ganoderma*, *Lentinula*, *Irpex*, *Ceriporiopsis*, *Pycnoporus*, and *Polyporus* (Strap and Silva 2013; Sindhu et al. 2015; Xu et al. 2017). These genera can also use various agro-industrial by-products as growth substrates, and they can be cultivated from these to act directly in ligninolytic enzyme production, depending on the culture conditions (Rouches et al. 2016a, b) as Table 1.3 shows.

Fungal growth can be achieved from two fermentation types, the solid-state (SSF) and submerged fermentation (SmF), when organic wastes are used. Solid-state fermentation consists of microbial growth into insoluble solid particles in the absence of or low water activity, in which such solid material functions as carrier substrates. Furthermore, this fermentation has achieved an increasing emphasis in biocatalysis, since it provides less susceptibility to inhibition and greater enzyme stability when subjected to changes in temperature and pH (Rouches et al. 2016a, b). In submerged fermentation, the microorganisms are cultivated in liquid medium containing essential nutrients. Nonetheless, when compared to the SSF, it presents lower operating cost, once it requires less aeration, heating, mixing, and water (Dey et al. 2016). Thereafter, during the fermentation processes, several extracellular enzymes are produced and simultaneously used for the extraction and/or removal of recalcitrant compounds present in the substrate matrices (Dey et al. 2016).

1.6 Enzyme Production

Agro-industrial wastes are rich in lignocellulosic materials that inevitably are produced in large quantities by agricultural and industrial activities. Most of these materials are partially or totally transformed into environmental pollutants. So, the interest in enzymatic technological advances comes up in favor of transforming this huge quantity of raw materials into new products. According to Abdel-Hamid et al. (2013), the function of each ligninolytic enzyme, involved in the set of reactions that

Table 1.3 Different agricultural substrates application in the ligninolytic and cellulolytic enzyme production from cultivation of white rot fungi

Substrate	Strains	Enzyme	Application	Pretreatment conditions	Lignin removal (%)	Reference
Cornstalk	*Irpex lacteus*	ND	Delignification and xylan removal	28 °C, 15 days	16.97	Yu et al. (2010)
Cotton stalk	*Pleurotus eryngii*	ND	Delignification	60 °C, 140 days	69.68	Yildirim (2011)
Corn stover	*Trametes versicolor*	Lac	Lignocellulose degradation	30 °C, 21 days	34.8	Zhu et al. (2011)
Banana peels	*Phoma* sp. and *Pleurotus ostreatus*	Lac	Chemical product removal with endocrine disruption	14–28 °C, 18 days	ND	Junior et al. (2012)
Grape stalks	*Trametes trogii*, *Stereum hirsutum*, and *Coriolus antarcticus*	Lac and MnP	Dye decolorization	28 °C, 5 days	ND	Levin et al. (2012)
Rubber wood	*Ceriporiopsis subvermispora* and *Trametes versicolor*	ND	Enzymatic hydrolysis	28 °C, 30 days	18.80 and 13.34	Nazarpour et al. (2013)
Sugarcane bagasse	*Phlebia* sp.	ND	Ethanol production	28 °C, 5 days	23.4	Khuong et al. (2014)
Coconut shell	*Trametes villosa*	MnP	Agricultural waste delignification	28 °C, 7 days	39.6	Lordêlo et al. (2014)
Rice straw	*Phanerochaete chrysosporium*	Lac, MnP, LiP	Ligninolytic enzyme production	30 °C, 10 days	ND	Munir et al. (2015)
Wheat straw	*Schizophyllum commune*	Lac, MnP, LiP	Ligninolytic enzyme production and delignification	30 °C, 7 days	67.2	Asgher et al. (2016)
Oak sawdust	*Ganoderma applanatum*	Lac, MnP	Ligninolytic material degradation	25 °C, 14 days	40.9	Cilerd et al. (2016)
Corncobs	*Pleurotus eryngii*	Lac, MnP, LiP	Ligninolytic enzyme production	30 °C, 7 days	48.05	Letters (2016)

(continued)

Table 1.3 (continued)

Substrate	Strains	Enzyme	Application	Pretreatment conditions	Lignin removal (%)	Reference
Banana peels	*Pleurotus eryngii*	Versatile peroxidase (VP)	Peroxidase enzyme production	28 °C, 10 days	ND	Palma et al. (2016)
Barley	*Lentinus crinitus*	Lac, MnP, LiP	Ligninolytic enzyme production	28 °C, 28 days	ND	Conceição et al. (2017)
Shredded oil palm	*Ganoderma boninense*	ND	Delignification	28 °C, 7 days	61.97	Jumali and Ismail (2017)
Pineapple waste	*Pleurotus pulmonarius*	Lac, MnP	Dye synthetic decolorization	28 °C, 14 days	ND	Silva et al. (2017)
Wheat bran	*Pleurotus ostreatus* and *Pleurotus eryngii*	Lac, MnP	Ligninolytic compound degradation	23 °C, 21 days	ND	Wanzenböck et al. (2017)
Vinasse	*Pleurotus sajor-caju*	Lac, MnP	Vinasse degradation	28 °C, 15 days	ND	Vilar et al. (2018)
Asparagus	*P. ostreatus* and *P. nebrodensis*	Lac, MnP, xylanase	Lignin degradation	25 °C	68.4 and 51.2	Zhai and Han (2018)
Wheat bran	*Inonotus obliquus*	CMCase, FPase, and β-glucosidase	Cellulolytic enzyme production	28 °C, 7 days	ND	Xu et al. (2018)

leads to the lignin degradation, are grouped between phenoloxities (oxidoreductases and peroxidases) and accessory enzymes. Oxidoreductases such as laccases (EC 1.10.3.2) catalyze electron transfer reactions, in other words oxidation reactions that promote molecule reduction while they oxidize the interest molecule and are independent of H_2O_2 to act. Peroxidases such as lignin peroxidases (EC 1.11.1.14), manganese peroxidases (EC 1.11.1.13), and versatile peroxidases (VPs) (EC 1.11.1.16) catalyze a set of oxidative reactions and hydroxylations using hydrogen peroxide H_2O_2 as an electron acceptor. H_2O_2 producers comprise accessory enzymes such as glucose oxidases (EC 1.1.3.4), pyranose-2-oxidases (EC 1.1.3.10), methanol oxidases (EC 1.1.3.13), aryl alcohol oxidases (AAO, EC 1.1.3.7), and glyoxal oxidases (EC 1.1.3.9). The enzymes aryl alcohol dehydrogenase (AAD) and quinone reductase (QR) reduce the derived compounds from lignin degradation (Demarche et al. 2012).

Among the ligninolytic enzymes, Lac and MnP have been increasingly prominent in the mycoremediation scenario, since they have a highly versatile nature. Lac belongs to a group of blue multicoated oxidases with molecular mass ranging from 50 to 130 KDa (Agrawal et al. 2018). Generally, they are involved in the oxidation of phenolic and non-phenolic compounds; and their potential is widely used in several industrial areas, such as in food/waste, tannery, agro-industrial, textile, cosmetics, pharmaceutical, and pesticide industries (Olajuyigbe et al. 2018; Qiang et al. 2018; Vallecillos et al. 2017; Yesilada et al. 2018; Zeng et al. 2017). In addition, due to their low specificity, they may directly oxidize a variety of substrates or indirectly through a chemical mediator whose purpose is to act on target substrates with high redox potential (Jamil et al. 2018; Theerachat and Guieyesse 2016).

Figure 1.4 shows the Lac three-dimensional structure, where it is composed of four copper molecules distributed in three different types of nuclear bonds (T1, T2, and T3). Monocular copper (T1) is responsible for removing electrons from the substrate and transferring them to T2 and T3 through the tripeptide sequence (His-Cys-His), besides promoting molecular oxygen (O_2) reduction in water, due to its strong interaction with T2. For these reasons, its catalytic mechanism has attracted the attention of several research groups, mainly because it is considered a "green process," since it requires O_2 as the only co-substrate for the catalytic reactions, which excludes H_2O_2 participation and consequently offers the advantage of degrading lignin with nontoxic derivatives (Agrawal et al. 2018).

In contrast, MnP is a heme glycoside with a lower molecular weight (ranges from 40 to 50 KDa) when compared to Lac. Its catalytic size (Fig. 1.4) promotes oxidation of Mn^{2+} to Mn^{3+} through the H_2O_2 substrate (Zhang et al. 2018), where Mn^{3+} is responsible for the generation of the Mn^{3+} (organic) complex, since it undergoes stabilization of the organic acid and consequently exerts the redox mediator function, by which it allows breaking of aromatics rings in recalcitrant organopollutant compounds (Yee et al. 2018).

As regards lignocellulolytic enzymes, these are classified as hydrolases and oxidases. Aside from being employed in synergistic lignocellulosic degradation and being contained in a versatile enzymatic complex, they are also characterized by the heterogeneity of their composition. Synergistic actions of these enzymes occur by endoglucanases (e.g., endo-1,4-β-D-glucanases or EC 3.2.1.4) which

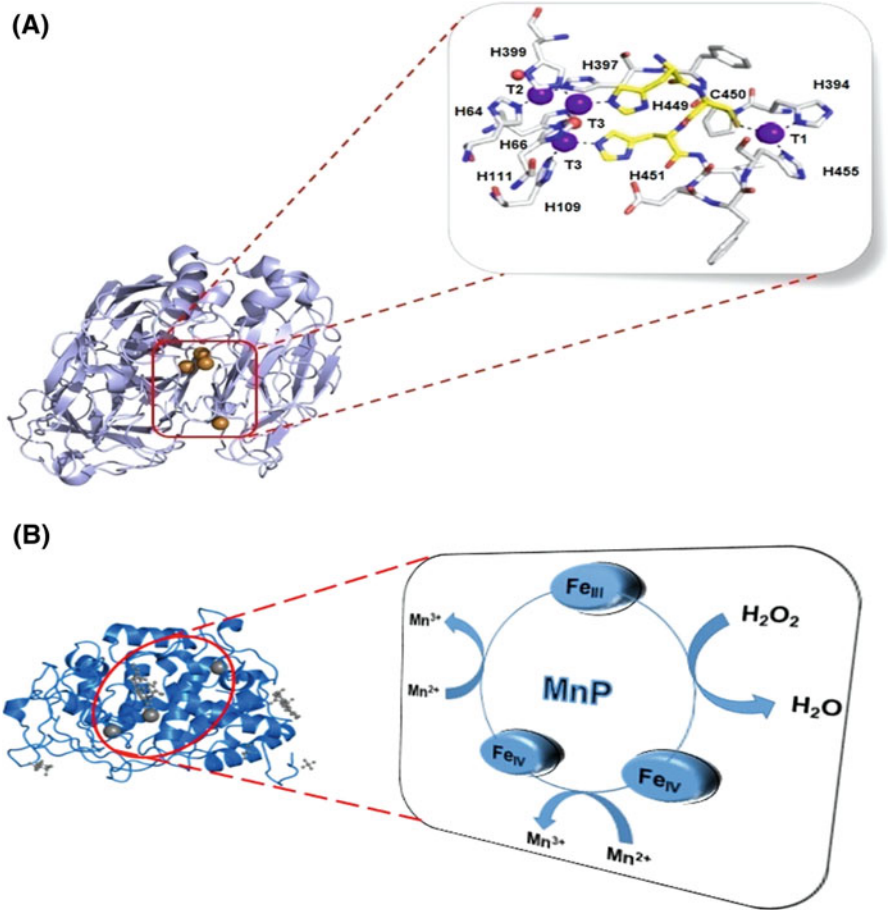

Fig. 1.4 Three-dimensional structure of the enzymes' active site: (a) laccase and (b) manganese peroxidase belonging to the Basidiomycetes class. (Adapted from Furukawa et al. 2014; Zheng et al. 2014)

hydrolyze the cellulose bonds via random cleavage at internal cellulose chain sites (Rouches et al. 2016a, b). Exo-glucanases or cellobiohydrolases (CBH, 1,4-β-D-glucan-cellobiohydrolases, or EC 3.2.1.9) hydrolyze the bonds in cellobiose via sequential cleavage both in a reducing and non-reducing manner at the ends of the cellulose, and β-glucosidases (BGL, cellobiases, or EC 3.2.1.21) specifically convert cellobiose and cello-oligosaccharides to glucose (WEI 2016). In general, these enzymes' attributes become possible in the performance of waste degradation processes and pollutant treatment, because they are useful in relation to conventional treatments, besides providing a lower cost.

1.7 Environmental Toxicology of Agro-industrial Waste

Environmental toxicology is the field that studies the chemical harmful effects on living organisms (including humans) and assesses the consequences of toxic interactions that affect the normal physiology of each individual present in the ecosystem (Kahru and Dubourguier 2010; Rubach et al. 2011). Additionally, this segment that covers several sub-areas of (eco)-toxicology (Fig. 1.5) is able to determine the levels of contaminants present in the environment (biology) and in the organisms' cells (physiology and genetics), to estimate the maximum permitted levels of toxic compounds (chemistry) and indicate the danger degree of their metabolites (biochemistry) (Walker 2012; Hassan et al. 2016). The ecotoxicology studies are based on the organisms (plants and animals) biotic integrity protection and on the measures control to prevent or remedy their intoxication, once they are submitted to chemical stressors (Tarazona and Ramos-Peralonso 2014; Menéndez-Helman et al. 2015).

Evaluations of the deleterious effects of particular chemical substances associated with toxic mixtures (including agro-industrial wastes, dyes, pesticides, pharmaceuticals, biocides, etc.) present in the wastewater are carried out by means of eco-toxicological tests. These are considered to be effective biological response methods, since they show the synergistic, additive, antagonistic, and potentiating effects resulting from interactions between substances which do not appear in traditional physical-chemical analyses or in isolated contaminant evaluations (Lopez-Roldan et al. 2012). For these reasons, the analyzed effluents can still remain toxic which consequently leaves the biota unprotected. However, bioassays have the advantage of determining the total pollutant's impact, once living organisms respond to the integral pollutant effect (Verma 2011; Figuerêdo et al. 2016).

Toxicity tests may be classified as acute and chronic, depending on the organism's test exposure time (Seeland et al. 2012). Generally, most of this exposure

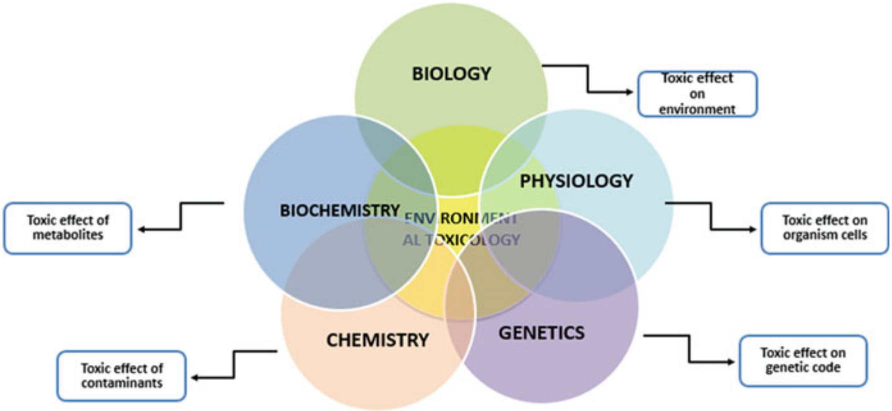

Fig. 1.5 Main study areas of environmental technology

occurs acutely, as it has a short and severe response to lethal effects over the organism's life cycle (24–96 h), and the observed effect is based on lethality or total immobility. The result is expressed as the mean lethal concentration (LC_{50}) or the mean effective concentration (EC_{50}), which is the toxic agent concentration value that promotes mortality or immobility, respectively, to 50% of the test-exposed organisms (Clément et al. 2013; Medeiros et al. 2013). Thus, in the literature, it is possible to observe an increase, in the last years, of different studies aiming at toxicity test application using different test organisms during the treatment processes of agro-industrial effluents present in the water bodies and soils, as well as of the crude residues.

Young et al. (2012) evaluated the phytotoxicity of industrial effluents and organic solid residues (cereals and olive oil, respectively), using the seeds of `Lactuca sativa`. The study results indicated an EC_{50} of 57.61% for the solid residues and a germination index (GI) of 63.8% for the effluents. In a later study, Melo et al. (2018) studied the toxicity of two agro-industrial by-products (cheese whey and corn steep liquor), using seeds of `Allium cepa`. And the results revealed a GI of 80.5% for cheese whey by-product and 62% for corn steep liquor. The study realized by Vilar et al. (2018) used *Raphidocelis subcapitata* algae to indicate the vinasse residue toxicity, and the result found was an IC_{50} of 3.13%. In this context, toxicology covers not only the identification of the chemicals responsible for the toxic effects but also these chemicals' sources in order to find solutions by applying control measures. This principle has a direct connection with the remediation objective, since these effects can be from behavioral changes, growth, or reproduction until the organisms' death.

1.8 Conclusion

The fossil fuel depletion and growing concern about the negative impact of fossil fuels on the environment have led to the critical need to explore alternative sources of renewable energy. From this, the agro-industrial resources with the highest number of natural origin inputs are used as alternatives. This increases their participation as raw material for biofuel production. However, future research should be focused on the development of economic treatment strategies, since adequate pretreatment is necessary for different lignocellulosic biomass types. Furthermore, liquid waste must reach the discarding standards with less environmental impact with the general treatment cost reduction. More importantly, it is necessary to reduce suspended solids and organic matter, as well as to provide biological fermentation technology for industrial implementation. The biological pretreatment presented several advantages over chemical pretreatment, especially in the mycoremediation context, where the white rot fungi are promising, once they have potential of xenobiotic compounds direct transformation during the fermentation process. However, it is necessary to determine these wastes' toxic effects, as toxicity is not well understood and its technological domain aims to meet industrial and social sustainability through integrated environmental quality assessment.

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Chapter 2
Remediation and Management of Petrochemical-Polluted Sites Under Climate Change Conditions

Pankaj Kumar Gupta and Brijesh Kumar Yadav

Abstract Groundwater serves as a major drinking water resource throughout the world. The water quality in the soil–water system has been impacted in emerging industrialized countries mainly by improper disposal of waste and wastewater from population growth and industrial manufacturing of products. Remediation of polluted sites requires a better understanding of climate change conditions. The traditional treatment technology involving physicochemical and biological methods is not efficient or effective to treat the contaminants to acceptable levels. Thus, the focus has now shifted in favor of using green and sustainable approaches that employ native microorganisms to destroy pollutants. Therefore, the aim of this chapter is to present a detailed framework for application of engineering principles and economic and regulatory constraints to determine a remedial action strategy and to select technologies to implement the strategy for a given data set at a site. This chapter discusses the physical, chemical, and biological methods used to remediate petrochemically polluted soils and groundwater. The emphasis of the chapter is on present practical applications and technologies that employ sustainable methods. The knowledge pool of this chapter will help in applying decontamination techniques to petrochemically polluted soil–water systems.

Keywords Petrochemical pollutants · Bioremediation · Groundwater resources · Economic and regulatory constraints

2.1 Introduction

One of the fastest emerging needs nationally and internationally in the twenty-first century is the need for clean water. The available freshwater reserves are being fast depleted globally because of rapid urbanization and population increase. On the

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other hand, extensive industrialization has caused novel contaminants to be introduced into environmental systems. Environmental pollution has become a global concern consequent to the rapid increases in industrialization, urbanization, and modern development under climate change conditions (Gupta and Sharma 2018). Technological innovations in industries have given rise to new products and new pollutants at abundant levels which are greater than the self-cleaning capacity of the environment (Joshi and Gupta 2018; Kumari et al. 2019; Gupta and Yadav 2019).

A large number of industrial chemicals such as total petroleum hydrocarbons (TPH), polychlorinated biphenyl (PCBs), polycyclic aromatic hydrocarbons (PAHs), heavy metals, and pesticides are released in the environment without proper treatment (Kumar et al. 2013; Gupta et al. 2018; 2019). Therefore, it is reported that the most common organic contaminants in groundwater include aromatic hydrocarbons, pesticides, and chlorinated compounds (Alvarez and Illman 2006). These chemicals pose potential threats to human as well as ecological health (Farhadian et al. 2008). These pollutants are released by various anthropogenic activities such as accidental spillage, industries, leaks from underground storage tanks, agricultural practices, and poor waste disposal in landfills. These releases may cause ultimate sinks for such toxic pollutants into major components of the soil–water system. Therefore, the biogeochemical properties of these natural resources have been transformed, resulting in the continuous loss of soil–water functions in sustaining living organisms. Large-scale production, transport, use, and disposal of petroleum have made it as one of the leading subsurface contaminants.

The petrochemicals are generally referred to as nonaqueous-phase liquids (NAPL), mainly classified as light and dense nonaqueous-phase liquids (LNAPLs and DNAPLs, respectively), based on their density compared to water. When released in sufficient amounts at the (sub)surface, the NAPL move downward through the unsaturated zone and are generally retained by the water table because these are of lighter density than soil water, whereas DNAPLs penetrate the water table and move downward until they are retained by an impermeable layer (USEPA 1995). The NAPLs in partially saturated zones are fractionized and cause multiphase partitioning: that is, air-phase, aqueous-phase, solid-phase, and pure-phase NAPL itself. The LNAPL pool retained at the water table starts dissolving with groundwater and subsequently moves to surrounding locations by advection, diffusion, and dispersion mechanisms of mass transport (Dobson et al. 2007; Powers et al. 1991). Water table fluctuations cause the (up)-downward movement of the plume, causing the entrapment of NAPLs in pore space, which increases the wide coverage of the NAPL masses. Thus, NAPLs entrapped in the form of isolated blobs or ganglia increase water interfacial area, which enhanced NAPL dissolution (Dobson et al. 2007; Soga et al. 2004). NAPLs trapped in the porous media become residual and long-lasting pollution sources which are very slowly attenuated (Yadav and Hassanizadeh 2011).

The biogeochemical characteristics of the subsurface environment are under the direct influence of the surface or atmospheric conditions; thus, the variations in ambient temperature, precipitation, stream flow, and other dominating variables affect subsurface water quality. This effect may cause alternation in soil moisture regimes and groundwater table fluctuations under climate change conditions (Dobson et al. 2007). Therefore, the variations in groundwater flow and the groundwater table cause more advective transport with enhanced dissolution of these pollutants in the subsurface. Thus, the subsurface pollutants are a particular concern under climate change conditions because of their higher water solubility and wide coverage in the subsurface. To meet the growing global demand for clean water, there is a great need to develop efficient decontamination techniques for polluted soil–water resources and to develop innovative strategies for management of water resources.

Petrochemicals can be removed from soil–water systems by chemical methods such as dispersants, chemical oxidation, and photocatalysis (Mascolo et al. 2008). Such physical techniques as physical containment, booming and skimming, mechanical removal, water flushing and sediment relocation, electro-remediation, air sparging, carbon adsorption, filtration, and adsorption by zeolites are also used (Zhu et al. 2004). The physical and chemical treatments of these hydrocarbons are usually expensive and harmful to the indigenous biota. The other promising and environmentally benign technique is bioremediation, which causes no harm to the contaminated ecosystem. Bioremediation is a grouping of biological processes using (native)-microorganisms under favorable conditions that act on pollutants to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil or groundwater resources. This cost-effective technique has been applied to clean up spills of petroleum products under a broad range of environmental conditions, soil and groundwater systems, marine shorelines, and surface water.

Various studies have evaluated bioremediation techniques and have shown a significant removal rate of petrochemical pollutants from the soil–water system. Because the natural attenuation of these compounds is quite slow, the microbial populations and their environmental conditions are technically modified to hasten the process of biodegradation. Further, the accelerated (co)-metabolism of the potential microbes by providing favorable subsurface conditions causes degradation or transformation of pollutants to nontoxic end products, which method is environmentally friendly and self-sustainable.

2.2 Petrochemical Pollutants in the Subsurface

Total petroleum hydrocarbons (TPH) originated from petroleum industries are the main source of pollutants in the subsurface environment (Al-Baldawi et al. 2014). The term TPH refers to a broad family of chemical compounds in water, soil, or air that indicate petroleum content (Pawlak et al. 2008). Their main constituents are diesel (C_6–C_{40}), petrol, benzene, toluene, xylene (BTEX), and kerozene, generally referred to as NAPL (Balachandran et al. 2012). Diesel is a complex fuel mixture composed of hundreds of organic compounds (Al-Baldawi et al. 2014). Diesel is composed of 65–85% saturates, 5–30% aromatics, and 0–5% olefins, although the percentage may vary with manufacturer, mining locations, refining processes, and sulfur content (Liang et al. 2005), as well as the sources of crude petroleum (Agarwal et al. 2013).

Crude petroleum can be divided into three general classes: saturated hydrocarbons, aromatic hydrocarbons, and polar organic compounds. Saturated hydrocarbons can be separated further into straight-chain and branched alkanes, as well as cyclic alkanes with varying numbers of saturated rings and side chains. Aromatic hydrocarbons contain one or more aromatic rings ranging from simple monoaromatic compounds, such as benzene and toluene, to polyaromatic compounds, such as pyrene. The polar fraction is made of compounds containing "polar" heteroatoms, such as nitrogen, sulfur, and oxygen. Similarly, other petrochemicals such as kerosene, gasoline, and jet fuels (JP-4, JP-5) may contain thousands of hydrocarbons including *n*-paraffins, cycloparaffins, and aromatics. Similarly, PAHs, also called polynuclear aromatics, constitute a class of hazardous organic chemicals, made up of two or more fused benzene rings in linear, angular, or cluster arrangements, containing carbon and hydrogen (Cerniglia 1992).

2.3 Fate and Transport of Petrochemical Pollutants in Porous Media

Accidental release of petroleum hydrocarbons and chlorinated hydrocarbons by leakages, spills, or improper disposal has deteriorated large volumes of soil and groundwater in all industrial and urban regions of the world (Clement et al. 2000). If a petroleum hydrocarbon like NAPL is spilled at the (sub)-surface, and it infiltrates into the subsurface, it will move into the partially saturated zone. The multifluid porous media systems are characterized by a solid phase within which interconnected pore space allows fluids to flow along with nonaqueous liquids, such as petrochemical products. Because water and air already form a two-fluid system in the patrially saturated soil–water system, introduction of a second liquid results in a three-fluid, porous-medium system. In two fluid systems, two fluids and one solid, and among these three substances form three possible interface combinations: interfaces between the two fluids, interfaces between one fluid and the solid, and interfaces between a second fluid and the solid. When three fluids are present, there are six possible interface types (interface pairs), so that the system is becoming quite complex. An additional complication to the overall multifluid problem is the observation that an immiscible organic fluid is often composed of a number of components. For example, gasoline contains a number of components, some of which are lighter fractions such as benzene, toluene, ethylbenzene, and xylene (BTEX).

Partially saturated zones cause partitioning into air-phase, aqueous-phase, and solid-phase NAPLs, and pure-phase NAPL itself. The remaining mass of NAPLs moves downward through the partially saturated zone, in which LNAPLs are generally retained by the water table because their density is lighter compared to soil water; DNAPLs penetrate the water table and move downward until being retained by an impermeable layer (USEPA 1995). The LNAPL pool retained at the water table starts dissolving with groundwater and subsequently moves to surrounding downgradient locations by advection, diffusion, and dispersion mechanisms of mass transport (Dobson et al. 2007; Powers et al. 1991). Similarly, the DNAPLs also form a dissolved plume, but most of the pure-phase mass penetrates the water table and moves downward by gravity.

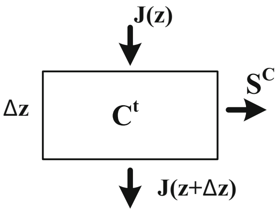
Advection, dispersion, and liquid diffusion contribute to contaminant spreading in the water-saturated area, with the last being the slowest process. Advective transport driven by groundwater flow depends on groundwater velocity. Mechanical dispersion causes migration in a parallel as well as in a perpendicular direction with respect to the main groundwater flow direction. Dispersion dominates over diffusion at groundwater velocities higher than 0.1 m day^{-1} (McCarthy and Johnson 1993). Diffusion, driven by solute concentration gradients over space, may dominate at low groundwater velocities.

Let us consider the petrochemical contaminant mass balance in an elementary root zone layer of Δz thickness, as shown next. The petrochemical contaminant influx into the layer is denoted by $J(z)$ (mg per unit area per unit time) [ML^{-2} S^{-1}], and outflux from the layer is $J(z+\Delta z)$. S_C is the sink term denoting amount of solute taken up by plant roots and degradation of the petrochemical contaminant by microbial biomass [ML^{-3} S^{-1}]. Then, the change in total petrochemical contaminant concentration C^t over time t can be written as

$$\frac{\partial C^T}{\partial t} = \frac{J(z) - J(z + \Delta z)}{\Delta z} - S_C \tag{2.1}$$

Expressing Eq. 2.1 as partial derivatives, we can write Eq. 2.2:

$$\frac{\partial C^T}{\partial t} = -\frac{\partial J}{\partial z} - S_C \tag{2.2}$$



where influx (J_z) and outflux ($J_{z+\Delta z}$) with sink term (S^C).

Here, the total petrochemical contaminant concentration in soil volume (C^t) is the sum of contaminants in the soil solution and the contaminants adsorbed on porous media and expressed as

$$C^T = \theta.C + \rho_S S_D \tag{2.3}$$

Here, C is the contaminant concentration in the soil solution (mg per unit volume of soil solution) [ML^{-3}], θ is the volumetric moisture content [L^3 L^{-3}], ρ_S is the bulk density of the porous media [ML^{-3}], and S_D is the contaminant adsorbed to the porous media [MM^{-1}]. The total flux J in Eq. 2.1 includes the change in the contaminant concentration from advection, dispersion, and diffusion, as is given below:

$$J = J_{adv} + J_{dis} + J_{diff} \tag{2.4}$$

where J_{adv}, J_{dis}, and J_{diff} are the advective, dispersive, and diffusive fluxes, respectively.

Advective Flux Advection causes the contaminant to move because of the velocity of the flow: hence, the advective flux is given as

$$J_{adv} = \nu\theta C = qC \tag{2.5}$$

where v is the pore water velocity [LT^{-1}], q is the soil water flux [LT^{-1}], and θ is the volumetric moisture content.

Diffusive Flux Diffusion is mathematically described by Fick's law, which states that the net rate of contaminant transport is proportional to the negative gradient of its concentration and can be modified for an unsaturated porous medium as

$$J_{diff} = -\tau D_O \theta \frac{\partial c}{\partial z} = -D_m \theta \frac{\partial c}{\partial z} \tag{2.6}$$

where τ is the tortuosity factor (dimensionless) that accounts for the increased distance of transport resulting from the tortuous path of the solute particle in a porous media. D_o and D_m are the free water diffusivity and molecular diffusion coefficients, respectively [L^2 T^{-1}].

Dispersive Flux Dispersion at a microscopic scale occurs from the variation of velocity within the pores and the tortuous movement of the fluid around the soil grains. Macroscopic dispersion refers to the dispersion resulting from the interfingering of materials of different permeability. Mechanical dispersion is mathematically described in the same way as molecular diffusion by using Fick's law:

$$J_{dis} = -\alpha_L \nu \frac{\partial C}{\partial z} = -\alpha_L \frac{q}{\theta} \frac{\partial C}{\partial z} \tag{2.7}$$

where α_L is the longitudinal dispersivity of the porous media in the direction of flow [L] and v is the pore velocity. Now, by adding all the aforementioned fluxes, we get the resultant flux as in Eq. 2.8:

$$J = J_{adv} + J_{dis} + J_{diff} = qC - D\theta \frac{\partial C}{\partial z} \tag{2.8}$$

where, $D = \tau D_O + \alpha_L \frac{q}{\theta} = \tau D_O + \alpha_L \nu$

Here, D is the diffusion-dispersion or hydrodynamic dispersion coefficient, which is the pore water velocity-dependent function [$L^2\ T^{-1}$]. The modified form of advection-dispersion (Eq. 2.9) is obtained by substituting Eq. 2.2 and Eq. 2.3 into Eq. 2.8:

$$\frac{\partial(\theta c + \rho_b s)}{\partial t} = -\frac{\partial}{\partial z}\left(qC - \theta D \frac{\partial C}{\partial z}\right) - S_C \tag{2.9}$$

A general expression of hydrocarbon depletion in soil, in which only microbial densities and the contaminant concentration determine the degradation kinetics, can be written as (Lyman et al. 1992):

$$-\frac{\partial C}{\partial t} = \mu_{\max} C \frac{(C_O + X_O - C)}{(K_S + C)} \tag{2.10}$$

where μ_{max} is the maximum growth rate, C is the contaminant concentration at time t, C_0 is the initial contaminant concentration, X_0 corresponds to the contaminant required to produce initial microbial density, and K_s is the half-saturation constant, also known as the growth-limiting concentration.

The foregoing equation reflects a linear relationship of changes in microbial density as well as a nonlinear relationship of changes in contaminant concentration on the rate of contaminant degradation. Furthermore, different simplified degradation kinetic models can be approximated considering extreme ratios of initial contaminant concentration (C_0) to K_s or initial microbial densities (X_0) to C_0 in Eq. 2.10 and are listed in Table 2.1 (Yadav and Hassanizadeh 2011).

Then, retardation factor (R) of the used porous media was calculated using the following equation:

$$R = 1 + \frac{\rho_b}{n} K_d \tag{2.11}$$

where R = retardation factor, ρ_b = soil bulk density (g/cm^3), n = porosity, and K_d = soil distribution coefficient. Similarly, the solid-phase mass partition, that is, adsorption, is governed by

$$S = K_d C \tag{2.12}$$

Table 2.1 The governing mechanisms of petrochemical fate and subsurface transport

<table>
<tr><th colspan="2">Mechanisms</th><th colspan="2">Governing equations</th><th>Descriptions</th></tr>
<tr><td colspan="2">1. Contamination partition</td><td colspan="2" rowspan="3">$C_{\text{gas}} = H\frac{C_{\text{liq}}}{\text{RT}}$</td><td rowspan="3">Where H is expressed in Pa L mol^{-1}, R is the universal gas constant (8.31 J mol^{-1} K^{-1}) and T is the absolute temperature (K)</td></tr>
<tr><td colspan="2">(i) Liquid–gas: Henry's law</td></tr>
<tr><td colspan="2">(Volatilization)</td></tr>
<tr><td colspan="2" rowspan="2">(ii) Solids–liquid partition</td><td colspan="2">$C_{\text{soil}} = K_d C_{\text{Liq}}$</td><td rowspan="2">$K_{\text{d}}$ is the partitioning coefficient in L kg^{-1}, K_{oc} (L kg^{-1}) is the organic carbon water partitioning coefficient, and f$_{oc}$ is the mass fraction of organic carbon</td></tr>
<tr><td colspan="2">$K_d = K_{\text{oc}} f_{\text{oc}}$</td></tr>
<tr><td colspan="2" rowspan="2">1.2. Advective transport</td><td colspan="2">$F_z = V_z C$</td><td rowspan="2">Where $V_x = \frac{K}{n}\frac{\partial h}{\partial l}$; V_x = linear velocities (L/T), K is hydraulic conductivity (L/T), n = effective porosity, $\frac{\partial h}{\partial l}$ = hydraulic gradient (L/L), C = concentration of LNAPLs $_{\text{(water, gas)}}$, t = time (T)</td></tr>
<tr><td colspan="2">$\frac{\partial C}{\partial t} = -V_z \frac{\partial C}{\partial z}$</td></tr>
<tr><td colspan="2" rowspan="5">3. Dispersive transport</td><td colspan="2">$\frac{\partial C}{\partial t} = \text{nD}_z \frac{\partial C}{\partial z} \partial A$</td><td>Where dA is the cross-sectional area of REV</td></tr>
<tr><td colspan="2">$D_L = \alpha_L v_z + D^*$</td><td>Dz = hydrodynamic dispersion coefficient</td></tr>
<tr><td colspan="2" rowspan="3">$D_T = \alpha_T v_z + D^*$</td><td>α_L = longitudinal dynamic dispersity</td></tr>
<tr><td>α_T = transverse dynamic dispersity</td></tr>
<tr><td>D* = diffusion coefficients</td></tr>
<tr><td colspan="2">Mechanisms</td><td colspan="2">Governing equations</td><td>Descriptions</td></tr>
<tr><td colspan="2">4. Adsorption</td><td colspan="2">(i) $S_{\text{eq}} = K_d C_{\text{eq}}$</td><td rowspan="5">$S_{\text{eq}}$ = the adoption concentration, K_{d} = linear adsorption coefficient, K = Freundlich constant, m = fitting parameters. $S_{\max}$= the maximum adsorbed concentration mass fraction, K_L = Langmuir adsorption coefficient</td></tr>
<tr><td colspan="2">(LNAPLs to solids mass transfer)</td><td colspan="2">(i)(ii) $S_{eq} = \text{KC}_{\text{eq}}^{m}$</td></tr>
<tr><td colspan="2">(i)(i) Linear isotherm</td><td colspan="2" rowspan="3">(i)(i)(iii) $S_{eq} = \frac{K_L S_{\max} C_{\text{eq}}}{1+K_L C_{\text{eq}}}$</td></tr>
<tr><td colspan="2">(ii) Freundlich isotherm</td></tr>
<tr><td colspan="2">(iii) Langmuir isotherm</td></tr>
<tr><td colspan="2">5. Dissolution</td><td colspan="2">$\frac{\partial C}{\partial t} = K_{\text{dissolution}}(C_{\max} - C_w)$</td><td rowspan="2">$K_{\text{dissolution}}$ = dissolution rate constant [T^{-1}], $C_{\max}$ = equilibrium concentration, C_w = NAPL concentration in water, S = solubility limits, X = mole fractions</td></tr>
<tr><td colspan="2">(LNAPLs to water mass transfer)</td><td colspan="2">$C_{\max}$ = SX</td></tr>
<tr><td colspan="4">6. Natural attenuation/biodegradation</td><td rowspan="6">Where $\mu_{\max}$ is the maximum specific growth rate, C is the contaminant concentration at time t, C_o is the initial concentration, and X_o corresponding to contaminate required to produce initial microbial density, K_s is the substrate affinities constant. The two kinetics parameters are $\mu_{\max}$ and K_s and the other two stoichiometric parameters are $Y_{s/x}$ and S$_{\min}$</td></tr>
<tr><td colspan="4">$-\frac{\partial C}{\partial t} = \mu_{\max} C \left(\frac{\text{Co}+\text{Xo}-C}{\text{Ks}+C}\right)$</td></tr>
<tr><td>Kinetics model</td><td>Condition</td><td>Equation</td><td>Rate constant</td></tr>
<tr><td>Constant or zero order</td><td>$X_o >> C_o$;
$C_o >> K_s$</td><td>$-\frac{\partial C}{\partial t} = k_{\text{o}}$</td><td>$k_{\text{o}} = \mu_{\max} X_o$</td></tr>
<tr><td>Linear of first order</td><td>$X_o >> C_o$;
$K_s >> C_o$</td><td>$-\frac{\partial C}{\partial t} = k_1$ C</td><td>$k_1 = \mu_{\max} X_o / K_s$</td></tr>
<tr><td>Monod</td><td>$X_o >> C_{\text{o}}$</td><td>$-\frac{\partial C}{\partial t} = k_m$ C/($K_s + C$)</td><td>$k_m = \mu_{\max} X_o$</td></tr>
</table>

(continued)

Table 2.1 (continued)

Mechanisms		Governing equations		Descriptions
Logistic	$K_s >> C_o$	$-\frac{\partial C}{\partial t} = k_1$ $C/(C_o + X_o - C)$	$k_1 = X_o/K_s$	
Logarithmic	$K_s >> C_o$	$-\frac{\partial C}{\partial t} = k/(C_o + X_o - C)$	$k = \mu_{max}$	

where S is mass of adsorbed petrochemicals, C is mass of aqueous-phase petrochemicals, and K_d is the distribution coefficient, the product of organic carbon partitioning coefficient (K_{oc}) and organic carbon content of the soil f_{oc} as

$$K_d = K_{oc} f_{oc} \tag{2.13}$$

The mass transfer from pure-phase petrochemicals to the dissolved-phase plume is mainly governed by dissolution, that is, an interface mass-transfer process that is frequently slow and rate limited (Powers et al. 1991; Sulaymon and Gzar 2011).

$$\frac{\partial C}{\partial t} = K_{\text{dissolution}}(C_{\max} - C_w) \tag{2.14}$$

Furthermore, the mass transfer coefficient (k^*) is determined by Eq. 2.15 as reported by Power and Heermann (1999).

$$k^* = n\sqrt{\frac{4D_t V_x}{\pi l_{c(e)}}} \tag{2.15}$$

where $l_{c(e)}$ is the length of the pool, D_t is the vertical dispersion coefficient (D_T), corresponding to respective pore water velocities (V_x).

2.4 Role of Climate Variability and Changes

The alteration in climatic variability, and subsequently climatic boundary conditions, affects the soil–water–atmosphere interaction, resulting in quantitative and qualitative changes in subsurface water resources. Climatic variability affects the subsurface physical interaction by changes in the land use/land cover patterns and soil regime characteristics (Bates et al. 2008), which alter the functional interaction caused by the changing evapotranspiration (ET) rates resulting from increasing temperature (Woldeamlak et al. 2007). This condition may cause reduced infiltration rates from precipitation and consequently reduced subsurface recharge. Furthermore, the changes in the seasonality and intensity of rainfall, although rarely considered, significantly influence the timing and magnitude of subsurface recharge and consequently the changes in subsurface water storage and contamination flow conditions (Van Dijck et al. 2006).

Higher variability in precipitation and in temperature may thus negatively impact subsurface recharge in general. The climate change accelerates the ET rate, and high temperatures in many areas cause evaporation losses from streams/rivers, creating an increased hydraulic gradient between river and groundwater system (Burnett et al. 2006). The result is an enhanced discharge rate of groundwater along with pollutants toward the streams/rivers system (Wang et al. 2009). These variations not only change the subsurface yield or discharge, but also modify the groundwater flow network; for example, gaining streams may suddenly become losing streams, and groundwater divides may move position.

The reduced subsurface recharges and/or increased discharge cause alterations in the moisture flow pattern, which may affect the biogeochemical characteristic of the subsurface regimes (Green et al. 2011). Therefore, the fate and transport of contaminants including petrochemicals are directly affected by climate change conditions. The literature suggests the following effects of climate change on the fate and transport of petrochemicals in the subsurface environment:

- Environmental variability and changes considerably affect groundwater resources and are responsible for frequent fluctuations in the groundwater table and its flow velocities, particularly in shallow unconfined aquifers. Rapid groundwater table fluctuations along with high pore water velocities are expected in shallow aquifers, causing the enhanced mobilization of pollutants (Dobson et al. 2007).
- Water table fluctuations cause the (up)-downward movement of the plume with entrapment in pore space, which increases the wide coverage of the pollutant masses. Thus, pollutants entrapped in the form of isolated blobs or ganglia increased the water interfacial area, which enhanced dissolution (Soga et al. 2004). Pollutants trapped in the porous media become residual and long-lasting pollution sources that are very slowly attenuated (Yadav and Hassanizadeh 2011).
- Large area coverage of pollutants transported with advective flow under fast groundwater flow is caused by climatic variations. The fast groundwater velocity also enhanced the dissolution of pollutants, which may increase the pollution load in downgradient locations (Gupta and Yadav 2017).
- Distribution of water and air has direct effects on pollutant fate and transport as well as on the soil microbial processes responsible for degradation of subsurface pollutants, especially organic pollutants (Yadav and Hassanizadeh 2011).
- Low soil moisture content results in greater air-filled porosity, which should improve oxygen mass transfer to the pollutant-degrading microbial assemblage. However, there is likely to be a trade-off between improved oxygen availability and the detrimental influences of scarce soil moisture content (Alvarez and Illman 2006).

- Similarly, the mass transfer of pollutants through soils is dependent on soil water content (English and Loehr 1991). At high soil moisture content, pollutant mass transfer is impeded by the reduced air-filled porosity and partitioning of pollutants into soil water (Papendick and Campbell 1981).
- However, pollutant molecules may be retarded by adsorption onto organic and/or mineral components of soil solids when air-filled porosity increases at low water content (Petersen et al. 1994).
- Similarly, bioavailability and solubility of pollutants are temperature dependent. Low-temperature conditions usually result in increased viscosity, reduced volatilization, and decreased water solubility of pollutants and thus delayed onset of the biodegradation process (Margesin and Schinner 2001).
- Temperature has a significant role in controlling the nature and extent of microbial metabolisms that are responsible for degradation of several pollutants, especially hydrocarbons (Yadav and Hassanizadeh 2011).
- To summarize, the climatic variation response to dynamic soil moisture flow, nature of underlying groundwater flow, ambient temperature profile, and pollutant regimes affects not only the microbial degradation rate but also the transport of soil air and substrate throughout the variably saturated zone.

2.5 Engineered Bioremediation Techniques: Comparative Accounting

Bioremediation is an emerging technology that holds great promise for the cost-effective removal of a wide variety of environmental pollutants. Successful applications of bioremediation have been well documented for many sites contaminated with hazardous petroleum hydrocarbons. Bioremediation offers several advantages and limitations compared to traditional site remediation approaches such as pump-and-treat or soil excavation followed by other physicochemical remediation techniques.

Bioremediation practices can be categorized as in situ and ex situ. The in situ methods remediate soils and groundwater in place, whereas ex situ practices include the elimination of the contaminated soil–water resources away from the challenging site. In situ bioremediation of an aquifer contaminated by petrochemicals has been in existence for more than four decades and is mostly reliant on native microbes to reduce the pollutants. It does not require any mining; therefore, it is supplemented by slight or no disruption to soil morphology. Preferably, these methods should to be less expensive compared to ex situ bioremediation, as there is no additional cost prerequisite for excavation practices; nevertheless, the cost of the plan and in-site setting up of some sophisticated tools to increase microbial growth during bioremediation is of major concern. Some in situ bioremediation might be enhanced as biostimulation and phytoremediation, whereas with other methods the effect persists without any form of enrichment, that is, intrinsic bioremediation or natural attenuation. The native microbial population is familiarized to degrade the pollutant being exposed for a long time, but it takes a significantly long time for cleanup.

Engineered bioremediation emerges to accelerate bioremediation using modification to the environmental conditions and native microflora. This advanced bioremediation influences microbial activities and their neighboring environmental condition for accelerating the practice of biodegradation, categorized as biostimulation and bioaugmentation. Biostimulation is enhanced by adding of nutrients, electron acceptors, oxygen, and other relevant compounds to the polluted sites, which enhances the (co)-metabolic actions of the microflora. Bioaugmentation is a microorganism-seeding practice for cultivating the volume of a petrochemical degrader by adding potential microbial cultures that are grown independently in well-defined conditions.

Furthermore, plants also accelerate petrochemical removal by promoting microbial reestablishment in polluted soils and water by the constantly delivery of oxygen by root-zone aeration and nutrients for microbial development by fixation and exudation. Similarly, the constructed wetlands techniques are a concurrent treatment for polluted soil–water resources. The nature, depth, and degree of pollution, the type of situation, and location are among the selection measures that are reflected when selecting any bioremediation strategies (Azubuike et al. 2016). Furthermore, performance measures such as the amount of nutrient that control the success of bioremediation are also given major attention before a bioremediation project. In this chapter, the art pertaining to bioremediation of petrochemically polluted soil–water resources is presented with special emphasis on engineered bioremediation strategies.

2.5.1 Aerobic Versus Anaerobic Bioremediation

The oxidation state of target contaminants is the single most important factor determining whether the bioremediation process should be aerobic or anaerobic. Aerobic bioremediation is thermodynamically most favorable for the cleanup of reduced pollutants such as hydrocarbons. Such pollutants degrade faster under aerobic conditions than under anaerobic conditions, and oxygen availability is a common rate-limiting factor. In these cases, the pollutants serve as electron donors and a carbon source to support microbial growth. On the other hand, highly chlorinated compounds such as perchloroethylene (PCE, $CH_2 = CCl_2$) and hexachlorobenzene (HCB, C_6Cl_6) are already oxidized (by chlorine), and their aerobic degradation is not as feasible thermodynamically. Such compounds degrade faster anaerobically by reductive dechlorination. In such cases, the pollutants do not serve as carbon sources for growth but as electron acceptors in co-metabolic or respiratory processes (i.e., dehalorespiration), and the availability of suitable electron donors can be rate limiting.

2.5.2 Biostimulation Versus Bioaugmentation

Based on the source of microorganisms used, there are two main bioremediation approaches. Biostimulation involves the addition of nutrients, electron acceptors (or electron donors), and sometimes auxiliary substrates to stimulate the growth and activity of specific indigenous microbial populations. Enhanced biostimulation is a special case of engineered bioremediation, wherein the existing conditions of polluted sites may be modified by providing a favorable environment or nutrients for the growth of potential microbial populations. The introduction of pollutants to the subsurface causes reduction in oxygen level and nutrients, etc., which directly affects the microbial activities. Thus, native microbes need more (micro)-nutrients, electron acceptors, and favorable environmental conditions to achieve the complete degradation of pollutants. Therefore, the modification in polluted sites by providing such essential components stimulates the microbial growth and ultimately the removal of pollutants. Generally, the petrochemical-polluted sites are modified by providing (1) oxygen sources, (2) nutrients, (3) electron acceptors, (4) commercial products, and (5) by maintaining favorable environmental conditions.

Bioaugmentation refers to the addition of exogenous, specialized microorganisms with enhanced capabilities to degrade the target pollutant. Biostimulation is commonly selected for the cleanup of hydrocarbon-contaminated sites, often through the addition of oxygen and macronutrients such as nitrogen and phosphorus. Bioaugmentation is often unnecessary in such cases because the indigenous bacteria that degrade hydrocarbons under aerobic conditions are ubiquitous in nature. The continuous release of petrochemicals causes adverse effects on microbial activities and a decline in microbial population in the (sub)-surface.

To maintain the optimal population of such potential microbes, seeding of the native potential microbes is needed. This microbial seeding technique to the polluted sites to achieve the maximum removal of petrochemical mass is known as an bioaugmentation technique (Atlas 1991; Sarkar et al. 2005). This technique mostly prefers the polluted area having continuous release with high substrate concentrations, lower microbial counts, and dynamic environmental variables. Da Silva and Alvarez (2004) investigated enhanced biodegradation of NAPLs in microbially seeded aquifer columns and showed removal of the NAPL mass increased up to 88%. Joo et al. (2008) used the microbial strain of *Candida catenulata* (CM1) to treat contaminated soil and showed that 84% NAPL mass removal had taken place in the next 13 days. Similarly, toluene degradation by seeding of *Corynebacterium variabilis* (SVB74) and *Acinetobacter radioresistens* (SVB65) shows high rates in the vadose zone.

2.5.3 Microbial Versus Plant-Based Bioremediation

There are two principal biological approaches to treat the contamination in situ: traditional/engineered bioremediation systems that rely on microbial metabolism for site cleanup, and phytoremediation, which relies on vegetation. Phytoremediation is a biological treatment process that utilizes natural processes harbored in (or stimulated by) plants to enhance degradation and removal of petrochemicals in contaminated soil or groundwater. Broadly, phytoremediation can be cost effective for (1) large sites with shallow residual levels of contamination by petroleum pollutants, where contamination does not pose an imminent danger and only "polishing treatment" is required; and (2) where vegetation is used as a final cap and closure of the site (Basu et al. 2015).

Plant-assisted bioremediation refers to the use of selective plant species for the targeted pollutant to mitigate the toxic effects and remove the pollutant mass from the subsurface. This technique used the plant–geochemical interaction to modify the polluted site and also supply (micro)-nutrients, oxygen, etc. to the subsurface for better performance of petrochemical degrader on targeted pollutants (Susarla et al. 2002). Petrochemicals are mostly removed by degradation, rhizoremediation, stabilization, and volatilization, with mineralization being possible when some plants such as *Canna generalis* are used (Basu et al. 2015). The plant–geochemical interaction enhances (1) the physical and chemical properties of sites, (2) nutrient supply by releasing root exudates (Shimp et al. 1993), (3) aeration by transfer of oxygen (Gupta and Yadav 2017), (4) intercepting and retarding the movements of chemicals, (5) plant enzymatic transformation, and (6) resistance to the vertical and lateral migration of pollutants (Narayanan et al. 1998). Similarly, the plant–microbe interaction increased (1) mineralization in the rhizosphere and (2) numbers of degraders, shortening the lag phase until disappearance of the compound.

Some key factors to consider when choosing a plant include its root system, which may be fibrous or tap subject to the depth of contaminant, toxicity of pollutant to plant, plant survival and its adaptability to prevailing environmental conditions, plant growth rate, and resistance to diseases and pests. The deep root systems of plants improve the subsurface aeration, which maintains oxygen level in the deep vadose zone. The root exudates, dead root hair, and fine roots serve as an important source of the carbon for microbial growth (Shimp et al. 1993). The root exudates also accelerate the enzyme synthesis of microbial metabolisms (Dzantor 2007). Overall, the plant has a crucial role in removal of petrochemical mass, but many issues related to plant application to petrochemically polluted sites are required to investigate before implementation of such techniques. Further, study of the impacts of static and dynamic environmental variables on the pollutant removal, the combination of other bioremediation techniques with plant-assisted bioremediation, and multi-scale investigation are needed.

2.5.4 *Phytoremediation Versus Constructed Wetland*

Phytoremediation is generally referred to the application of selective plant species for the targeted pollutant to mitigate the toxic effects and removal of pollutant mass from the subsurface. This technique uses plant–geochemical interactions to modify the polluted site and also to supply micronutrients and oxygen, etc., into the subsurface for better performance of the petrochemical degrader on targeted pollutants (Susarla et al. 2002). A constructed wetland system is a specifically engineered structure having selected plant species for water quality improvement. Wetlands, either constructed or natural, offer a cheaper, low-cost alternative technology for remediation of industrially polluted sites. Constructed wetland treatment systems (CWTS) are treatment systems that use natural processes involving wetland vegetation, soils, and their associated microbial assemblages to improve water quality.

The remediation potential of both techniques results from rhizospheric and endophytic microorganisms associated with the plants. These microbes use the contaminant as a carbon source or energy source for growth and secondary substrate in co-metabolic pathways. CWTS have been previously assessed at different mesocosm or pilot levels for their ability to remove petroleum hydrocarbons (Cottin and Merlin 2008), among other substances. In aquatic plant-based systems, complex physical, chemical, and biological processes may occur simultaneously, including volatilization, sorption and sedimentation, phytodegradation, and plant uptake and accumulation, as well as microbial degradation (Matamoros et al. 2005).

2.6 Recent Advances in Remediation Techniques

2.6.1 *Biobarriers*

Biobarriers is an emerging technology as an alternative to traditional impermeable barriers (e.g., slurry walls, grout curtains, sheet pile cutoff walls), which are containment tools designed to prevent or control groundwater flow into, through, or from a certain location. Such impermeable barriers keep fresh groundwater from contacting a contaminated aquifer zone or contaminated groundwater from moving into pristine areas. In contrast, biobarriers are permeable and rely on the creation of a biologically active zone perpendicular to the path of the plume to intercept and degrade contaminants that flow through it (Yerushalmi et al. 1999). The general goal of biobarriers is to establish and maintain a biological treatment system with a high density of competent microorganisms under a controlled process that protects specific degraders and prevents their loss under environmental stress conditions.

2.6.2 Permeable Reactive Barriers

A permeable reactive barrier (PRB) is an engineered zone of reactive material placed in an aquifer that removes contamination from groundwater flowing through it (Essaid et al. 2015). The ability of reactive materials in PRBs to remove both organic and inorganic contaminants from groundwater is well established. The technology relies on natural gradients to move groundwater through the treatment media and, therefore, has cost benefits compared with active pumping methods. But, for treatment of petrochemicals, PRBs are not the most effective remediation tools, because the nature of the petrochemical attenuation in the subsurface depends on the microbial activities rather than adsorption or other physicochemical mechanisms. Therefore, the biobarriers are the most recommended remediation tool for petrochemical treatment in subsurface (Fig. 2.1).

2.6.3 Treatment Wetland

Treatment wetlands (TWs) are traditionally seen as black boxes where the contaminated water enters and treated water leaves the system. However, in recent years emphasis is given to understanding the different physical, biological, and chemical processes occurring in the wetlands. These TWs are constituted of water, solutes, plants, and various microbes that create optimal conditions for removing the pollutants. The plants promote microbial degradation by providing suitable conditions and root exudates such as short-chain organic acids, amino acids, and sugars that support a variety of aerobic and anaerobic microorganisms (Williams 2002). The basic classification of wetlands is based on the type of macrophytic (plant) growth and further bifurcated based on the water flow regime.

The type of wetlands with plants include free-floating, floating-leaved, emergent, and submerged plants such as the common reed (*Phragmites australis*), cattail (*Typha* spp.), or soft rush (*Juncus* spp.) growing on gravel, sediment, or in hydroponic solution. Treatment wetlands are mainly divided into surface-flow (SF) and subsurface-flow (SSF) wetlands. The surface-flow wetlands have water usually less than 0.4 m deep and are densely vegetated. Subsurface-flow TWs are subdivided into horizontal flow (HF) and vertical flow (VF) types based on the direction of the water flow through the porous media (Langergraber 2008; Mustapha et al. 2018). The polluted water flows horizontally (i.e., the inlet and outlet are placed horizontally opposite) through the artificial filter bed of porous media, which usually consists of a matrix of sand or gravel and the aquatic macrophytes, roots, and rhizomes. In the vertical-flow type, water is added intermittently to prevent flooding. The water infiltrates into the substrate, and then gradually drains down vertically before collecting in a drainage network at the base.

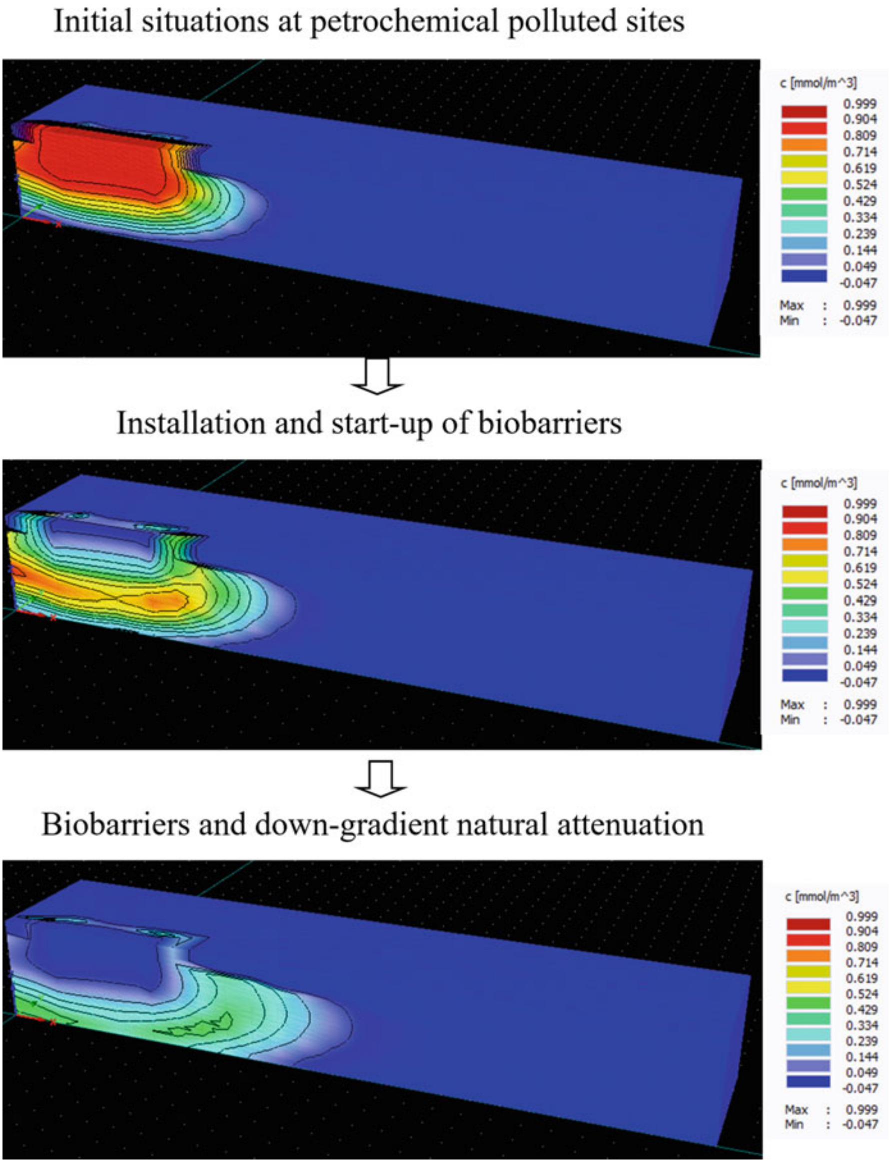


Fig. 2.1 Installation of biobarriers for remediation of petrochemically polluted soil–water system. *Top*: initial condition or pre-installation condition; *lower*: post-installation condition

2.7 Modeling of Petrochemical Behavior in Subsurface: A Case Study

A two-dimensional subsurface domain was simulated to investigate the fate and transport of petroleum hydrocarbons under basic groundwater flow conditions. The dissolved LNAPL, selected representative of petroleum hydrocarbons, is transported in a two-dimensional homogeneous porous medium under steady-state uniform flow conditions; for non-decaying dissolved organics and if sorption occurs under local equilibrium conditions, it is governed by

$$D_x \frac{\partial^2 C(t,x,y)}{\partial x^2} + D_y \frac{\partial^2 C(t,x,y)}{\partial y^2} - V_x \frac{\partial C(t,x,y)}{\partial x} = R_f \frac{\partial C(t,x,y)}{\partial t} \tag{2.16}$$

where V_x is the average unidirectional interstitial groundwater velocity; R_f is the dimensionless retardation factor; and D_x, D_y, and D_z are the longitudinal, transverse, and vertical hydrodynamic dispersion coefficients, respectively. The two-dimensional (2D) simulation domain, having element size 150 × 60 × 10 cm, was generated in a Hydrus simulator. One nodal recharge point at the left side and one nodal drain point in the right side of tank were incorporated in the study domain to maintain the constant water table. The van-Genuchten-Mualem single porosity model was selected for the domain simulation, and the material properties of the water flow and solute transport for sand media. The space discretization followed the Galerkin finite-element approaches, and time discretization followed the Crank–Nicholson scheme for solute transport. Finally, the mass balances were calculated for the study domain. The initial condition was pressure head for water flow and zero concentration gradient for solute transport.

The boundary conditions were atmospheric boundary condition at the top of the domain and no flux boundary condition at the other five sides, that is, left side, right side, back side, front side, and bottom boundary for water flow. Similarly, for solute transport, no flux boundary conditions were applied at all side boundaries. To mimic the surface spill of petrochemicals, a few top boundary nodes were selected as release nodes. The relative concentration of toluene was plotted as a function of time at different depths, known as breakthrough curves (BTCs), for a 2D study domain. The BTCs show that the degradation of LNAPL started after 25 days and that 98% removal was achieved in the domain. Figure 2.2 shows the soil moisture profile in the 2D domain. Similarly, the pattern of biodegradation at different observation points is plotted in Fig. 2.3. The concentration isolines are presented in the figure, showing the transport of LNAPL in 2D domain with time. The results show that the advective transport is the dominating mechanism in LNAPL transport.

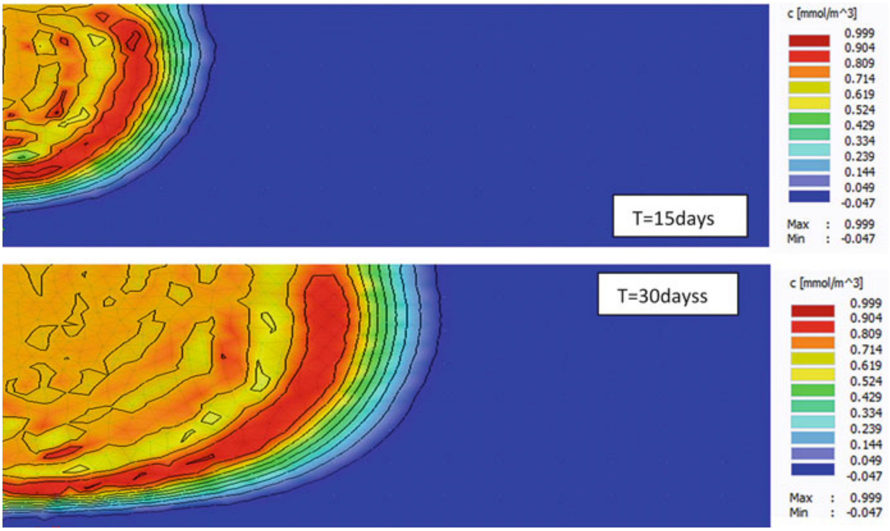
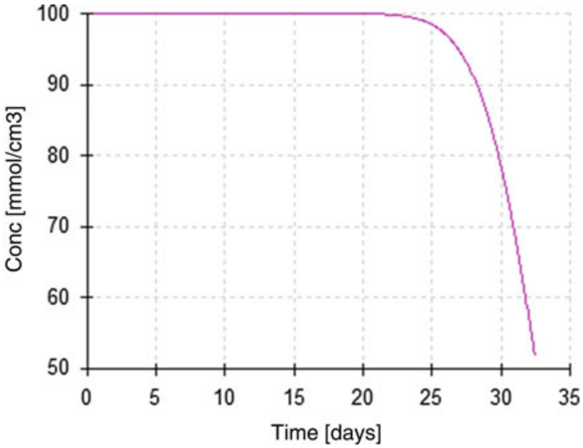


Fig. 2.2 Simulated 2D transport of petrochemical pollutants in soil–water system at time (*top*) 15 days; (*lower*) 30 days

Fig. 2.3 Simulated breakthrough curves (BTCs) for petrochemical pollutants in 2D numerical domain showing the degradation pattern of petrochemicals in subsurface



2.8 Conclusion and Future Prospects

Bioremediation is a promising cost-effective technique causing no harm to the contaminated ecosystem as compared to the traditional physicochemical methods. Natural microbes degrade contaminants from polluted soil–water resources in bioremediation; however, this process of natural bioremediation is quite slow under the prevailing environmental conditions of a typical polluted site. So, to enhance the degradation rate, engineered bioremediation is practiced by the addition of seeded cultures and/or nutrients, popularly known as bioaugmentation and biostimulation.

Another key role in the success of bioremediation application is filled by various site-specific environmental conditions such as temperature, moisture content, and oxygen availability.

Research has also proven that plants have an important role in accelerating the degradation rate cost-effectively in an enhanced bioremediation technique. Furthermore, the recent advanced techniques such as biobarriers, PRBs, and treatment wetlands are more effective for in situ remediation of petrochemically polluted natural resources. Therefore, this chapter presents a state-of-the-art review on the fate and transport of petrochemicals in the subsurface under climate change conditions and the most effective feasible method of bioremediating contaminated soil–water. On the basis of the literature review, the following future recommendations are suggested for better implementation of management of polluted natural resources.

(a) A complete assessment of biogeochemical and lithological characteristics with pollutant type, nature, and extent is required for effective performance of bioremediation techniques in the field.
(b) Based on the initial biogeochemical and lithological investigation, the selection of appropriate bioremediation techniques is more significant in management of polluted natural resources.
(c) Consideration of heterogeneity of porous media, associated microbial community, and subsurface conditions will enhance the understanding of the fate and transport of petrochemical pollution in the subsurface.
(d) A better understanding of plant–microbe interactions and its modeling is required for the engineered bioremediation of these pollutants.
(e) Multi-scale and multi-dimensional investigations are required to frame the methodology of selected bioremediation techniques for field application.
(f) To understand the treatment performance of bioremediation techniques, various modeling approaches ranging from simple empirical to more complex physically based models have been developed.

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Chapter 3
Phycoremediation: Role of Algae in Waste Management

Polur Hanumantha Rao, Ramanathan Ranjith Kumar, and Natarajan Mohan

Abstract Phycoremediation is a green technology because of its vast environmental benefits, and in the past decade, there has been a steady rise in the number of phycoremediation plants throughout the world. The success in the widespread use of this technology will rely on joint efforts by entrepreneurs, academicians and policy-makers. Considering the replacement of existing secondary treatment systems with more environment-friendly and profitable phycoremediation technology or at least integrating into the conventional systems, wherever feasible, is of paramount importance.

Keywords Phycoremediation · Algae · Bioremediation · Metal removal · Wastewater Treatment · Biofuel

3.1 Introduction

Anthropogenic activities from time immemorial and rapidly paced development of technology have resulted in the deterioration of the pristine nature of the environment, thereby leading to near-irreversible ecological degradation. In particular, only during the last two centuries has it become very difficult to maintain sustainability with respect to environment protection. Population explosion, rapid urbanization and industrialization, modernization of agriculture and ever-increasing vehicle emissions have led to environmental damage. In other words, the harm occurs due to the release of pollutants at undesirable levels into the environment. By the twenty-first century, pollution had extended from a primarily localized problem to one of global

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consequences in which pollutants not only persisted in the environment but also altered atmospheric and climatic conditions. Hence, environment management has become a more serious global issue, with a great deal of attention being given to waste generation and waste disposal practices, in particular hazardous waste disposal. Over the years, various methodologies using physical, chemical and biological tools have been/are being developed for minimizing the impact of waste generation. Of these, biological methods are often preferred because of their sustainability and harmless biotransformation mechanisms. Therefore, much research at the laboratory and pilot-scale is needed to develop advanced and highly efficient biotechnological tools for effective waste management.

3.2 Waste Generation and Its Global Impact

Huge amounts of waste are generated every year globally, of which a relatively lesser portion is recycled and a majority of it goes dumped or untreated, leading to cascades of problems for people and the environment. Wastes belong to any one of the following categories: solid, liquid or gas. Solid wastes are residues commonly referred to as trash, garbage, rubbish, refuse, discards and throwaways that are no longer of any relevance to the disposer, for example, broken glass, cans, plastics, paper, battery casings and nylon (Adedibu 1982). Liquid wastes, generally referred to as effluents, comprise of domestic wastewater, agricultural runoff water and wastewater discharged from industrial processes (NISP 2003). Examples of gaseous wastes are greenhouse gas emissions and waste gases from stack, cement factories, stone crushing excavation activities, lime dust, asbestos dust, acid fumes and cigarette fumes (NISP 2003).

3.2.1 Solid Wastes

Solid wastes can be classified in a number of ways, on the basis of source, environmental risks, utility and physical property. On the basis of source, which is a common classification, solid wastes are classified as municipal solid wastes, industrial solid wastes, agricultural solid wastes, mining and mineral wastes, construction and demolition wastes, healthcare wastes, radioactive (nuclear) wastes and human and animal wastes (Omofonmwan and Eseigbe 2009). The generation of solid waste from household, industries, markets, institutions, abattoir and shops results in improvement in the standard of living of the inhabitants. However, it leads to huge levels of pollution, including enormous methane gas as well as CO_2 emissions. Although source reduction, waste minimization, recycling, incineration, landfilling, etc. are very vital implementation measures, waste processing/transformation by biological and chemical means should remain the ultimate area of interest for sustainable technology development and successful waste management.

3.2.2 Liquid Wastes

Liquid wastes comprise of black water, grey water or sullage and wastewaters from commercial establishments: black water is domestic wastewater, which contains human excreta including urine and faeces; sullage or grey water is discharged from domestic dwellings, which will not contain human excreta; and wastewater from industrial establishments includes primarily industrial wastewaters. Globally, around 80% of wastewater is released into the environment without treatment. The untreated wastewater is either infiltrated into aquifers or diluted in or transported downstream into aquatic environments (WWAP 2017). Therefore, development of sustainable technologies is needed for effective disposal of liquid wastes. “Improved wastewater management generates social, environmental and economic benefits, and is essential to achieving the 2030 Agenda for Sustainable Development” (WWAP 2017).

3.2.3 Gaseous Wastes

Gaseous wastes comprise a wide category of atmospheric pollutants generated predominantly due to anthropogenic activities. They include primarily greenhouse gases such as CO_2, methane and chlorofluorocarbons, in addition to oxides of nitrogen (NOx), oxides of sulphur and carbon monoxide. All gaseous waste components lead to severe atmospheric pollution levels and ultimately reach terrestrial and aquatic environments during precipitation. Management of gaseous wastes is an area of serious concern; even though treatment processes like filtration are available, they are not considered a viable option, and hence control measures are given due consideration. Much research is needed for developing effective treatment technologies.

3.2.4 Toxic Wastes

Toxic wastes can be categorized under any of the above wastes, and their treatment remains a great challenge in environment protection. The presence of toxic contaminants such as heavy metals, pesticides, plastics, etc. poses a major problem in developing a suitable remediation technology. Although various physical, chemical and biological treatment methods are available, all these processes lead to accumulation of toxic elements in the environment resulting in bioaccumulation and biomagnification. Development of successful remediation technology will highly depend on use of biotransformation processes, where toxic constituents are converted into safe, disposable non-toxic forms. Therefore, to sustain environmental, economic and social development principles, efficient waste and recycling management plans have to be developed for treating all the above types of wastes (Demirbas 2011).

3.3 Phycoremediation: An Overview

Phycoremediation can be defined as the use of microalgae or macroalgae for sequestration of carbon dioxide (CO_2), or for the removal of toxic and non-toxic substances from solid, liquid or gaseous wastes, where the organisms do so by metabolic uptake, accumulation or biotransformation processes. It is considered as a method of bioremediation systems, but of late, it is being referred to as phycoremediation. The term phycoremediation was first coined by John (2000) to refer to the remediation carried out by algae. However, the use of algae in the remediation of wastewater has been in vogue for nearly 50 years, with one of the first descriptions of this application being reported by Oswald, considered one of the pioneers in biological treatment of wastewaters (1957). Since then, various studies have shown phycoremediation potentials of numerous algal species for different types of wastewater possessing varying nutrient, pathogen and organic loads (Gupta et al. 2016, Shriwastav et al. 2014).

3.4 Conventional Bioremediation and Phycoremediation: A Comparison

Bioremediation, in general, refers to treatment of wastes using living systems, including microorganisms, plants and animals. Although categorized under this, phycoremediation is being recently identified as a unique technology. Of all bioremediation systems, use of bacteria is more common, which is now widely regarded as conventional bioremediation technology. On the other hand, phycoremediation, which has recently gained prominence, offers lots of advantages over conventional bioremediation systems (Table 3.1) (Rao and Sivasubramanian 2016). Wastewater offers ideal conditions for bacterial growth and decomposition of organic matter by oxygenation; however, bacteria are less efficient in the removal of inorganic nutrients such as phosphorus, which is usually the main cause of eutrophication of freshwater ecosystems. Activated sludge process (ASP) and biofilm systems are the most commonly used biological treatment (tertiary) in wastewater treatment plants. These processes consume more energy (ASP, 1.3–2.5 MWh per million gallons (MG) of wastewater, and biofilm systems, 0.8–1.8 MWh per MG) when compared to that of algal ponds (0.4–1.4 MWh MG-1 d-1) (Lundquist et al. 2010). Roughly 1 kg of biochemical oxygen demand (BOD) removed in an ASP requires 1 kWh of electricity for aeration, which produces 1 kg of fossil CO_2 from power generation. By contrast, 1 kg of BOD removed by photosynthetic oxygenation requires no energy inputs and produces enough algal biomass to generate methane that can produce 1 kWh of electric power (Oswald 2003).

Table 3.1 Phycoremediation and bioremediation: a comparison

Phycoremediation	Other bioremediation systems, in particular bacterial treatment methods
Some microalgal strains are capable of growing in multiple modes of nutrition such as autotrophic, heterotrophic and mixotrophic, thereby making them versatile in removing wide range of pollutants	Even though high-efficiency organisms are used, most of them remove organic load
The technology can be used case-specifically particularly for the removal of toxic materials such as heavy metals, individually or in tandem or concomitantly	Processes like Bardenpho target predominantly towards removal of only nitrogen and phosphorus
Energy consumption is comparatively less	Energy consumption can be high, e.g. in surface aeration or in diffused aeration systems
Construction and maintenance costs are typically less	Construction and maintenance costs can be very high as in activated sludge process or sequence batch reactors
Algal biomass is highly suitable for liquid biofuel production such as ethanol and biocrude/biodiesel, which are truly carbon-neutral	Bacterial biomass usually undergoes anaerobic digestion
The technology is robust, and the algae can withstand high range of pH	The systems are very sensitive to pH ranges
Certain phycoremediating strains can be used as biofertilizers	Usually strains/consortia used here do not serve as biofertilizers
Odour generation during the process is very less	Comparatively, the odour generation is more, particularly in open treatment systems
Can be effective in removing BOD when heterotrophic/mixotrophic cultures are used. Preferably, mixotrophic cultures increase in dissolved oxygen due to photosynthetic activity	Aerobic heterotrophic bacteria are highly efficient in removing BOD, but the requirement of oxygen at high rates is inevitable
Compatible with many physical and chemical treatment processes, e.g. pH neutralization and flocculation using chemicals do not affect the treatment	Process specific and hence narrowly compatible with certain conventional methods
Highly environment-friendly as the organisms are capable of mitigating CO_2	In fact, CO_2 is released into the atmosphere during growth of the bacterial systems

3.5 Phycoremediation and Its Applications

3.5.1 Nitrogen and Phosphorus Removal

Over the last few decades, efforts have been made to apply potential microalgal cultures in biological secondary/tertiary treatments of domestic wastewaters (Oswald and Gotaas 1957; De la Noüe et al. 1992). Microalgae use CO_2 as a carbon source and hence can grow photoautotrophically without the addition of an additional organic carbon source. Unicellular green algae such as *Chlorella* sp. and *Scenedesmus* sp. have been widely used in wastewater treatment as they are commonly found organisms in sewage and have fast growth rates and high nutrient removal capabilities. Successful deployment of microalgae for nutrient removal, particularly nitrogen and phosphorus, from different wastes has been described. Few studies have specifically described the removal of nitrogen and phosphorus from wastewater by microalgae (De la Noüe and Pruix 1988; Oswald 1988; Thomas et al. 2016; Ghosh 2018). Microalgae have the potential to remove various pollutants including NOx (Nagase et al. 2001). Microalgae assimilate a significant amount of nitrogen and phosphorus nutrients for the synthesis of proteins (45–60% of microalgae dry weight), nucleic acids and phospholipids. Nitrogen and phosphorus removal can be further enhanced by stripping of ammonia and by precipitation of phosphorus due to the rise in the pH associated with photosynthesis (Oswald 2003).

3.5.2 Removal of Organic Load

Conventional biological treatment systems, involving bacterial consortia, such as activated sludge are known to reduce the organic load of industrial and domestic wastewaters. However, phycoremediation is gaining prominence in the removal of organic content because of the versatility of the algal system in remediating the wastewaters. In this context, some strains of microalgae are capable of utilizing the organic matter present in the effluents, thereby leading to a reduction in chemical oxygen demand (COD), BOD and total organic carbon (TOC). Although different species of microalgae exhibit varying modes of growth, they, in particular, grow either by autotrophic or mixotrophic modes of nutrition. Few species have the ability to concurrently assimilate both organic and inorganic carbon, which may result in their mixotrophic growth. Cultivation of microalgae by mixotrophic mode of nutrition overcomes the limitation of light requirement presented during the photoautotrophic mode of nutrition, and thus they can utilize high levels of organic carbon from wastewater for rapid growth under mixotrophic or photoheterotrophic conditions in the presence of light (Li et al. 2011). Thus the efficiency of nutrient scavenging by microalgae from municipal, agricultural or industrial wastewaters is higher under aerobic, dark heterotrophic conditions as well as mixotrophic conditions than under photoautotrophic conditions (Zhou et al. 2013). According to Usha et al. (2016), microalgal cultivation in pulp and paper mill effluent resulted in 82%, 75% and 75% reduction in BOD, COD and TOC, respectively. In a nutshell, the efficiency of the effluent treatment by microalgae is related to their ability to reduce the COD, BOD and TOC levels in the effluent.

3.5.3 Removal of Anions/Cations

The metabolism of algae includes mainly four basic elements: carbon, nitrogen, phosphorous and sulphur. In addition, other cationic components like potassium, sodium, iron, calcium and magnesium can also be helpful in their effective growth system (Umamaheswari and Shanthakumar 2016). The presence and characteristics of specific binding sites determine cation accumulation necessitating further study to determine the ion removal capabilities of the particular microalgal species. Bender et al. (1994) found that metal-tolerant cyanobacterial mats could remove 96% zinc and 85% manganese when exposed at 14 mg L^{-1}. Likewise, removal of anionic components apart from phosphates, nitrates and nitrites has been a challenge in the secondary treatment of wastewater. In particular, removal of sulphate, one of the major anionic components present in many industrial wastewaters, remains the biggest hurdle in bioremediation technology. However, microalgae offer promise in removing the sulphate ions, and as reported by Ballen-Segura et al. (2016), there was a 92% reduction during phycoremediation of tannery wastewater. Similarly, reduction of sulphates, silicates and chlorides was also reported by Vignesh et al. (2006).

3.5.4 pH Correction

The pH of wastewaters to be treated determines the solubility and biological availability of chemical constituents such as phosphorus, nitrogen, carbon and heavy metals (lead, copper, cadmium, etc.). With regard to heavy metals, the degree of solubility determines their toxicity. Metals tend to be more toxic at lower pH because of their increased solubility. The ability of certain algal species to increase the alkalinity of the surrounding medium as a by-product of their inorganic carbon accumulating mechanism is well known (Shiraiwa et al. 1993). During photosynthesis, algae remove CO_2 from the water, which increases the hydroxide (OH^-) levels, resulting in an increase in pH. Thus microalgae can very well be employed for the treatment of acidic wastewaters. Casiot et al. (2004) have reported that microalgae simultaneously control acidity and reduce metals in acid mine drainage (AMD) leading to natural attenuation of the environmental impact. Sivasubramanian et al. (2009) have successfully implemented the world's first full-scale phycoremediation plant for treating the effluent from an alginate industry. *Chroococcus turgidus*, a blue-green microalga, was used to neutralize the highly acidic effluent by continuous mode of operation, and the plant has been in operation since 2006.

3.5.5 Scavenging of Heavy Metals

Many studies have shown that algal biomass possesses high metal-binding capacities because of the presence of polysaccharides, proteins or lipids on the cell wall surface. These biomolecules in turn provide amino, hydroxyl, carboxyl and sulphate functional groups, which can act as binding sites for metals (Ramelow et al. 1992; Holan and Volesky. 1994). The cell wall matrix of green algae, which constitutes about 10–70% of the cell, contains complex hetero-polysaccharides and proteins (Andrade and Rollemberg. 2005). The biosorption of heavy metals by microalgae involves (a) entrapment by cellular components; (b) active transport across the cell membrane; (c) cation exchange or complexation; and (d) adsorption (Harris and Ramelow 1990). The first two mechanisms are associated with living cells, whereas the latter two can only occur with the dead cells. The entrapment can happen by covalent bonding, surface precipitation, redox reactions or crystallization on the cell surface. Active uptake that often involves the transport of the metals into the cell interior is often a defensive tool, and it serves to accumulate essential trace elements. Microalgae can also release extracellular metabolites, which are capable of chelating metal ions. Moreover, the increase in pH associated with microalgal growth can enhance heavy metal precipitation (Chojnacka et al. 2005).

3.5.6 Pesticide Removal

Microorganisms can use a range of organic pollutants, including pesticides, as an energy source for their growth and at the same time detoxify and mineralize compounds (Massoud et al. 2008). The main mechanism of biodegradation of pesticides is reductive dehalogenation/dechlorination, which is accelerated by photochemical transformation in autotrophic microorganisms. In such reductive dehalogenation processes, microorganisms facilitate electron transfer from reduced organic compounds for oxidation–reduction, which results in the removal of halogen atoms from complex halogenated compounds. Another mode of degradation is when microorganisms produce enzymes that break the bonds in the pesticide molecules without the microorganisms deriving any benefit from it (Cerniglia et al. 1980; Kobayashi and Rittmann 1982). Many studies show that microalgae are capable not only of bioaccumulating pesticides but also of bio-transforming some of these environmental pollutants. Therefore, there is a lot of scope for field application of microalgae in the treatment of agricultural wastewaters containing high amounts of pesticides. However, the major disadvantages with the metabolization of pesticides are that some are exceedingly difficult to break down and some degradation products can be highly hazardous (Gadd 2009).

3.5.7 Sludge Reduction

Biological treatment systems generate huge amounts of biosludge, which has to be properly used or disposed off. This type of sludge becomes all too problematic when there are hazardous/toxic components involved in the treatment processes. Different types of microorganisms are used for secondary treatment of domestic/industrial wastewaters. Among these, bacterial systems, aerobic bacterial treatment processes in particular, generate lots of sludge due to the faster growth rate of organisms.

However, use of microalgae for the treatment offers promise with respect to the quantities of sludge generated. They have slower growth rates and higher nutrient removal capabilities compared to those of bacterial cells. In addition, microalgal biomass has huge potential for commercial applications. Sivasubramanian et al. (2009) demonstrated almost nil/negligible sludge generation during continuous full-scale phycoremediation treatment of the acidic effluent from an alginate industry in South India.

3.6 Phycoremediation as a Technology

Algae have historically been utilized for domestic wastewater treatment and have led to the development of specific processes (e.g. raceway ponds and photo-bioreactors [PBRs]). Various reactor designs have been constructed and utilized for wastewater treatment using microalgae and have been standardized for the best treatment efficiency, or tailor-made according to the raw wastewaters. The treatment systems may comprise of suspended algal systems, attached systems or closed systems. Integration of this technology with other sustainable technologies can be highly beneficial (Fig. 3.1). Some of the processes that have been developed so far are discussed below.

3.6.1 Photo-Bioreactors

Photo-bioreactors are usually closed cultivation systems that are characterized by the regulation and control of nearly all the major parameters to attain the maximum benefits. The benefits of using a PBR for microalgal cultivation or for phycoremediation include reduced contamination risk, no CO_2 losses, reproducible cultivation conditions, controllable hydrodynamics and temperature and flexible technical design (Pulz 2001). To be specific, they offer cultivation under a wide variety of conditions or prevent, to some extent, outcompeting of the production strain by other microalgae or contamination with undesirable microorganism. The other major benefits of closed bioreactor systems are higher areal productivities and the prevention of water loss by evaporation (Posten 2009). Different types of PBRs such as tubular, vertical column and flat panel have been developed. To choose the optimal type of PBRs, it is mandatory to understand the major phenomena limiting the performance of microalgal cells such as light availability, nutrient supply including CO_2, environmental conditions including temperature and solar radiation and mixing (Acién et al. 2018). Although these types of systems have been suggested for use in wastewater treatment, many drawbacks including investment and operational costs make them non-conducive for wastewater treatment.

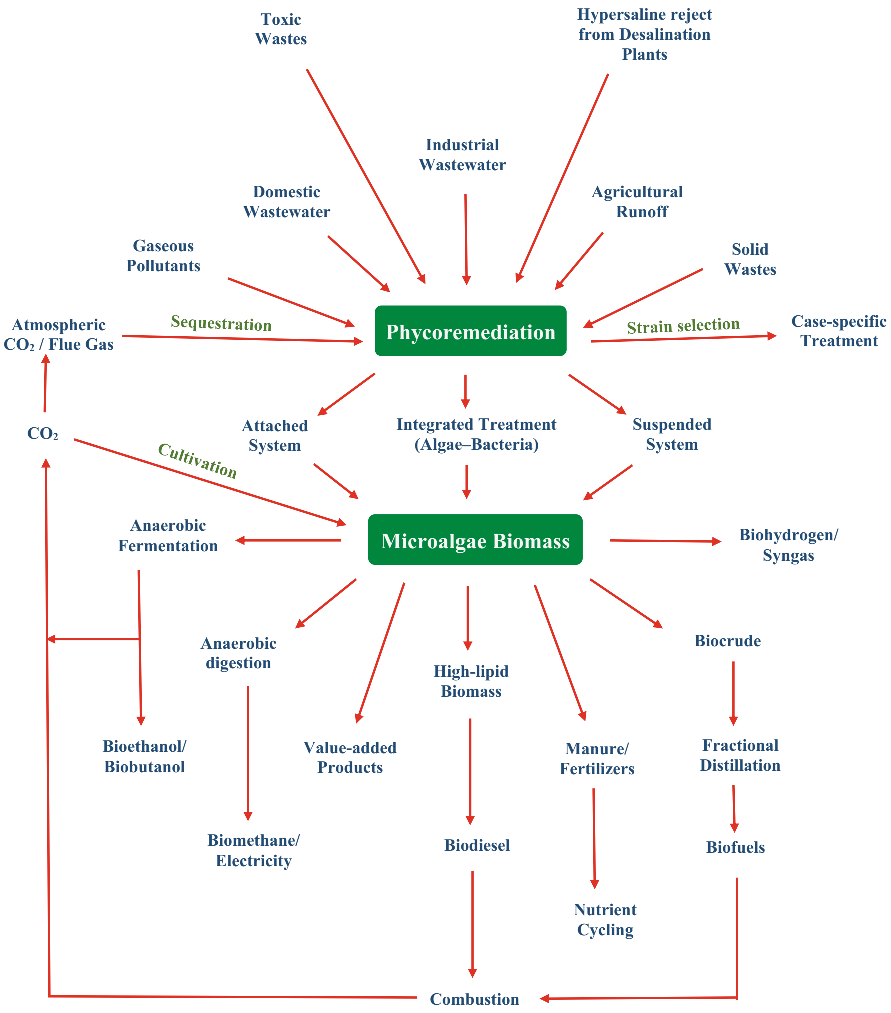

Fig. 3.1 Microalgae and environmental sustainability

3.6.2 *Open Pond Treatment Systems*

Microalgal mass culture has always been preferred in closed culture systems when they are grown for high value products; however, for phycoremediation and for production of biofuels, large commercial open systems are used with varying degrees of efficiency because closed culture systems are very expensive and are difficult to scale up. Furthermore, most closed systems are operated indoors with artificial lighting, and this contributes to high energy costs. A classic example of an open suspended system is a high rate algal pond (HRAP). The concept of the HRAP was developed by Oswald and co-workers in the mid-1950s and since then has been used in various countries (Oswald et al. 1957). The system typically consists of a primary settlement lagoon with a shallow (0.2–0.6 m depth) meandering open channel in which the effluent is propelled by a motorized paddle wheel to prevent settling (Oswald, 1988). Most ponds are operated at average velocities ranging from 10 to 30 cm/s to avoid deposition of algal cells (Dodd 1986). Such an optimized pattern will maximize the exposure of algal cells to favourable light conditions, thereby maximizing the quantity of algal oxygen production and reducing the area or volume requirement of the HRAP. HRAPs are very appropriate because of their simplicity of operation in comparison with conventional technologies such as activated sludge treatment methods.

Wastewater treatment in HRAPs allows a significant reduction of not only the organic matter but also nitrogen and phosphorus. HRAPs provide a more efficient wastewater treatment method by also reducing other parameters such as bacteria load, BOD and even toxic nutrients (García et al. 2000). In a recent study for treating slaughterhouse wastewater, high removal efficiencies of 86% and 91% were achieved in HRAP for chemical oxygen demand and soluble phosphorous, respectively (Hernández et al. 2016).Although there are difficulties in separation of biomass after treatment, this remains one of the widely used processes because of its high treatment efficiency (Mohan et al. 2010).

3.6.3 Attached Systems/Sloping Pond Technology

Immobilization of microalgae has been known for a long time, and this concept was introduced for wastewater treatment nearly three decades ago by de la Noue and his collaborators (Chevalier and de la Noue 1985; de la Noue et al. 1990). Immobilization of algae can be effected by either growing microalgae by trapping inside a matrix or on attached systems. Algae growing on the surface can be harvested by simple mechanical means such as suction or scraping. In addition, when the algae are harvested, the remaining algal colonies on the surface can be used as inoculum for the next batch of growth, and thus this harvesting method aids in a semi-continuous culturing of microalgae (Johnson and Wen 2010; Liu et al. 2013). In an earlier study, rotating algal biofilm system was developed, in which microalgal cells were grown on the surface of a material rotating between nutrient-rich liquid and CO_2-rich gaseous phase (Gross et al. 2013). Gao et al. (2015) developed a novel algal biofilm membrane photo-equipped with solid carriers and a submerged membrane module for attached growth of *Chlorella vulgaris* and for subsequent secondary effluent treatment with high efficiency. Thus attached systems are preferred for ease of operation and harvesting the biomass.

A more robust form is the use of a cost-effective sloping pond, which is a combination of attached and suspended systems for effluent treatment. The principle of the sloping design is to create a turbulent flow while the algal suspension flows through sloping channels or surfaces. The suspension is kept circulating during those hours of the day when sufficient incidence of radiation is present. During the remaining hours, the suspension is stored in tanks where aeration and mixing is provided (Setlik et al. 1970). The advantage is that the efficiency of the system will not be affected seriously by deviations occurring from the hydrodynamic balance (Becker 1994). Another successful method of attached system was described by Adey (1982). The technology involves creating an algal turf by growing benthic macroalgae/microalgae for use as a scrubber of CO_2, nutrients and pollutants as well as biomass production.

3.6.4 Integration of Algal Treatment System into Other Conventional Biotreatment Systems

Microalgal treatment is an upcoming technology, and there is a need to design the novel process train, if not integrate into conventional treatment processes. Phycoremediation is highly cost-effective and can be easily integrated as part of the existing secondary/tertiary treatment systems (Rao and Sivasubramanian 2016). The integration of algal technology into conventional biological treatment systems depends on the quality of the wastewater being treated. Being very compatible, the efficiency of nutrient removal can be very high when bacterial and algal systems are integrated (Nithiya et al. 2016). In particular, when the wastewater contains high organic load as well as highly toxic heavy metals, this approach could be meaningful (Fig. 3.2).

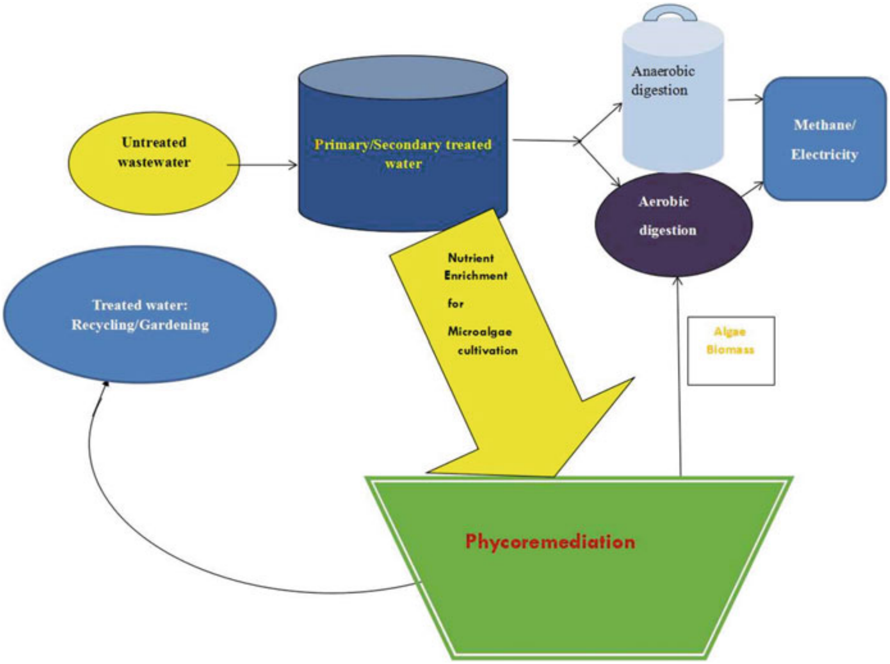

Fig. 3.2 Flowchart of phycoremediation process

3.7 Phycoremediation of Various Wastes

3.7.1 Domestic Wastewater Treatment

Domestic wastewater is a combination of water and other wastes originating from homes, commercial and industrial facilities and institutions. Untreated wastewater generally contains alarming levels of organic matter, numerous pathogenic microorganisms as well as nutrients and toxic compounds. The untreated domestic wastewater represents an ideal medium for microalgal growth as it contains high concentrations of all necessary nutrients such as organic carbon, ammoniacal nitrogen, nitrate nitrogen, phosphorus, etc. Moreover, microalgae offer a low-cost and effective approach to remove excess nutrients and other contaminants during secondary/tertiary treatment of domestic wastewaters, in which they are cultivated according to specific bioremediation needs. In particular, microalgae can be cultivated in facultative or aerobic high-rate ponds for use in municipal wastewater in small- and middle-scale treatment facilities (Oswald, 1988). Many microalgae have been reported to be suitable for domestic wastewater treatments such as *C. vulgaris*, *Micratinium* sp., *Desmodesmus* sp., *Scenedesmus obliquus*, *Scenedesmus acutus*, *Botryococcus* sp. and *Botryococcus braunii* (Gani et al. 2016); *Scenedesmus* sp., *Chlorella* sp. and *Spirulina* sp. (Abdel-Raouf et al. 2012); and *Scenedesmus dimorphus*, *Nostoc muscorum*, *Anabaena variabilis*, *Plectonema* sp., *Oscillatoria* sp., *Phormidium* sp., *Spirulina* sp., *Chlorella pyrenoidosa*, *Euglena* sp. and *Chlorella* sp. (Dewangan 2016). Thus, phycoremediation of domestic wastewaters offers immense scope for better cycling of waters as well as for effective generation of biomass. The microalgal biomass thus generated has huge commercial applications where value-added and commodity products can be produced.

3.7.2 Industrial Wastewater Treatment

3.7.2.1 Textile/Dyeing Industry

Textile and dyeing industry effluents have been increasing proportionally making it one of the main sources of severe pollution problems across the globe (IPPC 2003). The worldwide dye production is over 7×10^5 tons per annum (Daneshvar et al. 2005), which in turn results in the release of large volume of wastewater discharged on land with partial or without treatment. Kumar et al. (2006) reviewed that removal of colour from textile industry wastewater is a multifaceted problem due to the difficulty faced in treating such dye wastewaters by conventional treatment methods. The wastewater is generated from the various processing units of textile industries such as desizing, scouring, bleaching, mercerizing, dyeing, printing and packing units. The major parameters that need to be removed/reduced in the textile industry effluent are pH, electrical conductivity, chloride, sulphate, phenols, total dissolved solids, biochemical oxygen demand and chemical oxygen demand and other toxic substances. All physical/chemical treatment methods lead to accumulation of huge amounts of toxic sludge, which is very difficult to dispose of. Daneshvar et al. (2005) reported that algae have very high potential to degrade dye effluents, thus postulating that the reduction appears to be related to the molecular structure of dyes and the species of algae used. It was also reported that more than 30 azo compounds were biodegraded and decolorized by *C. pyrenoidosa*, *C. vulgaris* and *Oscillatoria tenuis*, in which azo dyes were decomposed into simpler aromatic amines (Yan and Pan 2004). Phycoremediation of dye effluents has been gaining attention since the last two decades, and Sivasubramanian et al. (2010a) reported colour removal of textile dyeing wastewater using *Chroococcus* sp., *Chlorococcum* sp., *Desmococcus* sp. and *Cosmarium* sp. (Table 3.2).

3.7.2.2 Mining/Metallurgy Industry

Leaching of metals from mining and metallurgy industries into the soil/groundwater leads to serious environmental concerns typically characterized by contamination of various ecosystems (Kalin et al. 2006). Some of these industries include chrome plating industry, other electroplating plants, goldsmith workshops, steel industries, etc. Conventional technologies such as ion exchange or lime precipitation are often ineffective and/or very expensive when used for treating such wastes. Use of algae for treating mining or metallurgy industry effluents is a novel approach, in particular for AMD. Therefore, hyperaccumulating/hypersequestering capabilities of microalgae have been extensively studied in the last few decades (Lee et al. 1992; Gupta et al. 2000). Nutrient removal has been shown to be more efficient using algal strains with special attributes (Table 3.2). Such special attributes include tolerance to extreme temperature, chemical composition with predominance of high value-added products, quick sedimentation behaviour or a capacity to remove nutrients more efficiently (Tang et al. 1997).

3.7.2.3 Food Industry

The food-processing industry is one of the rapidly growing industries that have occupied worldwide global market in terms of increasing economy. During food production/processing, tons of portable water is used, which in turn results in huge amounts of wastewater, apart from the water being used for washing and sterilizing vessels. The effluent is rich in total organic carbon, nitrogen and phosphate with a high range of COD, which may result in major environmental issues. Many food industries use conventional biological treatment systems using microorganisms to reduce parameters such as COD. Although bacterial systems can clean up food industry wastewater efficiently, microalgae are considered as a more potential alternative. High rates of nutrient removal by algae have been reported with monocultures of cyanobacteria such as *Spirulina* (Lincoln et al. 1996; Olguin 2003) and *Phormidium* (Blier et al. 1996) grown on effluents from dairy industries. Gani et al. (2016) have used phycoremediation technology for the treatment of wastewater of a food-processing industry. In their study, they obtained a total reduction in COD and

Table 3.2 Reduction of nutrients/pollutants from various industrial effluents treated with microalgae

Effluents/ wastewater	Parameters	Algae	Family	References
Chrome plating sludge	TDS, conductivity	*Oscillatoria animalis*, *Stigonema dendroideum*	Cyanophyceae	Manoharan and Subramanian (1992)
	TDS, Na, K, free ammonia, nitrite, nitrate, chloride, fluoride, sulphate, phosphate, COD	*Chlamydomonas pertusa*, *Dactylococcopsis raphidiodes*, *Desmococcus olivaceus*, *Chlorococcum humicolo*, *Chroococcus turgidus*, *Chlorella vulgaris*, *Scenedesmus dimorphus* and *Scenedesmus incrassatulus*	Cyanophyceae Chlorophyceae Cyanophyceae Chlorophyceae Chlorophyceae Cyanophyceae Chlorophyceae Chlorophyceae Chlorophyceae Chlorophyceae	Sivasubramanian et al. (2010a, b)
Electroplating plant	pH, nitrite, nitrate, ammonia and phosphorus	*Scenedesmus* sp.	Chlorophyceae	Ahluwalia et al. (1989)
	TDS, sodium, potassium, chloride and phosphate	*Desmococcus olivaceus*	Cyanophyceae	Sivasubramanian et al. (2010a, b)
Gold smith workshop	TDS, conductivity	*Oscillatoria animalis*	Cyanophyceae	Kamaleswari et al. (2007)
Steel industry	Turbidity, total solids, TDS, calcium, sodium, iron, chloride, TSS, potassium, oil and grease	*Oscillatoria animalis*, *Chlorella vulgaris*, *Scenedesmus obliquus* and *Pithophora polymorpha*	Chlorophyceae	Jinqi and Houtian (1992)
			Chlorophyceae Pithophoraceae	Elizabeth and Hema Priyadarshini (2004)
Tannery	Turbidity, total solids, conductivity, iron, free ammonia, nitrite, BOD, COD, calcium, copper, chromium	*Oscillatoria animalis*	Cyanophyceae	Vignesh et al. (2006)
	COD, nitrogen, phosphorus, chloride, sulphate	*Scenedesmus dimorphus and Chlorella vulgaris*	Chlorophyceae	Sakthivel and Elumalai (2016)

(continued)

Table 3.2 (continued)

Effluents/ wastewater	Parameters	Algae	Family	References
	Sulphide, calcium, sodium and chromium		Chlorophyceae	Rao et al. (2011a)
	Calcium, sodium			
	Copper, manganese,			
	BOD and COD			
Dairy	Turbidity, TSS, conductivity	*Spirulina platensis* and *Pithophora polymorpha*	Chlorophyceae	Kotteswari et al. (2007)
	Magnesium, free ammonia	*Chlorella vulgaris*, *Botryococcus braunii*	Cyanophyceae	Sreekanth et al. (2014)
	Sulphate, silica, fluoride, BOD and COD	*Chlorella pyrenoidosa*	Chlorophyceae	Kothari et al. (2012)
	COD, TDS, ammonia, nitrogen, nitrate	*Chlorella vulgaris*	Chlorophyceae	Choi (2016)
	Ammonium, phosphorus and *COD*		Chlorophyceae	
	BOD, *COD*, *suspended solids*, *total nitrogen and total phosphorus*			
Paper mill	TDS, conductivity, chloride, nitrate, nitrite, ammonia	*Pithophora polymorpha*	Cladophoraceae	Tarlan et al. (2002)
	Colour, calcium, magnesium, nitrite, nitrate, phosphate, sulphate, COD and BOD	*Phormedium tenue*	Oscillatoriaceae (Cyanophyceae)	Nagasathya and Thajuddin (2008)
Pulp industry	Colour, AOX and COD	*Chlorella* sp.	Chlorophyceae	Tarlan et al. (2002)
Pharmaceutical	Oil and grease, COD, nitrate, BOD, pH, BOD, COD, TSS, BOD and COD	*Oscillatoria animalis*, *Nostoc muscorum*, *Spirulina platensis*,	Cyanophyceae	Kannan et al. (2004)

(continued)

Table 3.2 (continued)

Effluents/ wastewater	Parameters	Algae	Family	References
		Chroococcus turgidus,		
		Chlorella vulgaris and *Pithophora polymorpha*	Chlorophyceae	Vignesh et al. (2006)
		Scenedesmus quadricauda	Cladophoraceae	Vanerkar et al. (2015)
		Spirulina	Cyanophyceae	Kshirsagar (2010)
			Chlorophyceae	

BOD of 70.68% and 61.11%, respectively. Similarly, there was a significant reduction in TOC at 76.66%. Therefore, phycoremediation offers promise for treating high TOC-containing food industry wastewaters (Table 3.2).

3.7.2.4 Tanneries/Chemical Industries

Tanneries and chemical industries produce huge amounts of effluents during production processes, which contain a wide variety of inorganic and organic pollutants, including toxic substances such as chromium, particularly due to dehairing during leather processing. Sakthivel and Elumalai (2016) reported that in India alone about 2000–3000 tons of chromium escapes into the environment annually from tannery industries, with chromium concentrations ranging between 2000 and 5000 mg/l in the aqueous effluent compared to the recommended permissible discharge limits of 2 mg/l. Although the less toxic trivalent chromium is commonly present in tannery effluents, the presence of certain naturally occurring minerals, especially MnO_2 oxides, can enhance the oxidation of Cr (III) to more toxic Cr (VI) in the soil environment. Microalgae-based treatment methods are preferred particularly due to high binding affinity for heavy metals (Table 3.2) (Rao et al. 2011b). Microalgae have high potential to absorb, entrap and volatilize heavy metals from wastewaters, and the separation of metal-saturated algae from the medium is also an economical method resulting in high-quality reusable effluent water and valuable biomass which could be used for different purposes (production of biogas, fertilizer, fodder, etc.).

3.7.2.5 Dairy Industry

A steady rise in the demand of milk and milk products in many countries has led to the enormous growth of dairy industries throughout the world. Being a major agricultural economy, India tops the world in milk production and shares about 13.1% of the total milk produced in the world (Kothari et al. 2012). Kushwaha et al.

(2011) opined that dairy wastes are biodegradable; however, they produce undesirable colour and odour, along with high levels of BOD, COD and TOC. Dairy wastes also contain metals like Na, Cl, K, Ca, Mg, Fe, CO, Ni, Mn, etc. along with significant amount of nutrients, viz. total nitrogen and total phosphorus. According to Kothari et al. (2012), most of the studies have used fungi and bacteria for reducing the organic load of dairy wastewater. However, in recent years, the use of microalgae in treatment and recycling of dairy wastewater has attracted great interest due to their treatment efficiency and their role in CO_2 fixation. According to Kotteswari et al. (2012), the treatment of dairy effluents by *Nostoc* sp. is very efficient, and it also proved to be a cost-effective and eco-friendly treatment. In the above study, *Nostoc* sp. played a vital role in the reduction of COD, BOD, total suspended solids (TSS), total dissolved solids (TDS) and other metals. However, few studies exist regarding the potential use of microalgae for dairy wastewater treatment. Therefore, a thorough understanding of these systems and implementation on a larger scale are needed to develop new approaches to remediation of dairy effluents (Table 3.2).

3.7.2.6 Paper/Pulp Industry

The utilization of plant fibre for paper production leads to the generation of effluents with huge amounts of lignocellulosic derivatives (Sharma et al. 2014). Therefore, pulp and paper mill industry sectors release chlorinated lignosulphonic acids, chlorinated resin acids, chlorinated phenols and chlorinated hydrocarbons in the effluent. The highly toxic and recalcitrant compounds, dibenzo-p-dioxin and dibenzofuran, are also found in some paper and pulp industry effluents. Sharma et al. (2014) reported that physical and chemical processes are quite expensive to remove high-molecular-weight chlorinated lignins, colour, toxicants, suspended solids and COD. In addition, BOD and low-molecular-weight compounds are not removed efficiently. Hence, the biological process is particularly attractive. One of the specific problems that have not yet been solved is the strong black-brown colour of the effluent, which is primarily due to lignin and its derivatives released into the effluents, mainly from pulping, bleaching and chemical recovery stages. In several studies, investigators have suggested that microalgae can be used to remove colour and absorbable organic halides (AOX) (Lee et al. 1978; Tesmer and Joyce 1980). *Chlorella*, *Ankistrodesmus* or *Scenedesmus* species have been used to treat wastewater containing organic pollutants from pulp and paper mills and olive oil mills (Table 3.2) (Munoz and Guieysee 2006). Therefore, phycoremediation may be highly beneficial for treating effluents from paper and pulp industries.

3.7.2.7 Pharmaceutical Industry

Pharmaceutical industries, during the drug-manufacturing process, use lot of water, natural raw materials such as plant extracts and complex constituents of alkaloids, heavy metals and solvents. It is extremely important to use eco-friendly treatment technologies because of the nature of the pharmaceutical effluents. Vanerkar et al. (2015) evaluated the toxicity of herbal pharmaceutical wastewater after its treatment with the green microalga *Scenedesmus quadricauda*. Physico-chemical characteristics such as pH, COD, BOD, total solids, sodium, potassium and heavy metals were significantly reduced after the treatment. Kshirsagar (2010) also reported based on the experimental result of a pharmaceutical industry that the wastewater treatment using *Spirulina* was very effective and there was a massive reduction in BOD and COD by 73% and 70%, respectively. However, more information is needed on morphological changes and secondary metabolites and pigment production in microalgae (Table 3.2).

3.7.3 Management of Oil Spills

The transport of petroleum across the world presents a great challenge leading to frequent oil spills in the marine environment (Harayama et al. 1999). Actually, it is widely recognized that petroleum hydrocarbon contamination has impacted and damaged the world oceans, seas and coastal zones and represents a constant threat to sustaining the health of the Earth (Mc Genity et al. 2012). Many methods have been trialled for cleaning up the same, but practical difficulties including cost pose a great hurdle. Among the many methods, use of microorganisms is considered one of the potential methods. The microbes of most interest have been bacteria and fungi, and only during the last two decades has the question of how algae figure in the catabolism of these compounds attracted a degree of interest (Semple et al. 1999). Some species of algae are capable of heterotrophic growth on organic carbon sources (Neilson and Lewin 1974). Walker et al. (1975) had performed experiments with a green alga *Prototheca zopfii*, which was found to degrade petroleum hydrocarbons and motor oils. In crude oil, 38–60% of saturated aliphatic hydrocarbons and 12–41% of aromatic compounds were degraded, whereas in motor oil, 10–23% of saturated aliphatic hydrocarbons and 10–26% of aromatic compounds were degraded. Many marine microalgae can withstand high concentrations of crude oil, and some cyanobacteria appear to accumulate hydrocarbons without degrading them in inter-thylakoid spaces (Al-Hasan et al. 2001). Moreover, the organic compounds produced by microalgae may influence hydrocarbon degradation in different ways; for example, extracellular polysaccharides could serve to emulsify hydrocarbons (Cohen 2002). Further research in this area will strengthen our understanding about management of oil spills and in developing technologies using microalgae and other microbial consortia.

3.7.4 Carbon Sequestration

Industrialization and increasing transportation needs have resulted in enormous release of greenhouse gases, especially CO_2, which in turn has led to the global rise in temperature. In this context, sequestration of CO_2 is of utmost importance and currently one of the most researched areas around the globe. Although many physical and chemical methods have been suggested, biological sequestration remains a promising one. Terrestrial plants mitigate vast amounts of CO_2 from the atmosphere; however, because of the relatively small percentage of CO_2 (0.036%) in the atmosphere, the use of terrestrial plants is not an economically feasible option. Furthermore, discharge gases from heavy industries commonly contain CO_2 levels significantly higher than that found in the atmosphere (10–20%). Therefore, it would be wise to develop strategies based on the emissions mentioned above. Thus, biofixation of CO_2 using microalgae has emerged as a potential option. Biochemical analyses have shown that microalgal biomass consists of 40–50% carbon, which suggests that about 1.5–2.0 kg of CO_2 is required to produce 1 kg of biomass (Sobczuk et al. 2000).

Microalgae have the advantages of efficient photosynthesis superior to that of C4 plants, faster proliferation rates and wider tolerance to extreme environments and potential for intensive culturing techniques. Several microalgal species have been tested with CO_2 concentrations of above 15%. One of the high CO_2-tolerant species is *Euglena gracilis*, and the growth of this species was found to be higher with 5–45% concentrations of CO_2 (Nakano et al. 1996). *Chlorella* sp. can be cultivated under 40% CO_2 conditions (Hanagata et al. 1992). Maeda et al. (1995) reported a strain of *Chlorella* sp. which could grow in 100% CO_2, although the maximum growth rate occurred at 10% concentration, while *Scenedesmus* sp. could grow in 80% CO_2 conditions, but the maximum cell mass was observed in 10–20% CO_2 concentrations (Hanagata et al. 1992). *Cyanidium caldarium* (Seckbach et al. 1971) and some other species of *Cyanidium* can also be cultivated in pure CO_2 (Graham and Wilcox 2000). The CO_2 supply not only serves as the carbon source for microalgal growth but also controls the pH of the culture (Brown 1996).

A significant amount of research has been carried out on the growth of microalgae using flue gases for CO_2 mitigation. The average efficiency of flue gas CO_2 capture in algal biomass was found to be 70% (Benemann et al. 1982; Weissman and Goebel 1987; Benemann and Oswald 1996). In another report, a carbon fixation rate of 14.6 g C m^{-2} (basal area) day^{1} at a growth rate of 30.2 g dry wt m^{-2} day^{-1} was reported (Watanabe and Hall, 1995). Likewise, Kurano et al. (1996) reported a range of 0.65–4.0 g CO_2 L^{-1} day^{-1} at growth rates of 0.4–2.5 g dry wt L^{-1} day^{-1}. Marine microalgal open farming is another area that has significant potential in global biological carbon sequestration.

3.8 Carbon-Neutral Biofuels from Algae for Mitigation of Global Temperature Rise

3.8.1 A Replacement for Fossil Fuels

Global climate change is currently the most pressing environmental problem, and it is widely accepted that use of fossil fuels has been the major cause. Therefore, fossil fuels as a source of energy should be substituted with renewable, clean energy sources to reduce CO_2 emissions (Amin 2009). Moreover, the continued use of fossil fuels as a primary source of energy is now widely recognized to be unsustainable because of depleting resources (Khan et al. 2009). Among many renewable fuels, biofuels have attracted immense interest because of increased sustainability, possibility of converting into a cheaper technology and environment friendliness. First-generation biofuels derived from oil crops/other food crops are a potential renewable and carbon-neutral alternative to petroleum fuels; unfortunately, these cannot realistically satisfy even a small fraction of the existing demand for transport fuels and may need extensive land areas and huge amounts of freshwater (Chinnasamy et al. 2012). Moreover, use of the above resources may lead to food-versus-fuel conflict. Likewise, large-scale production of second-generation biofuels from lignocellulosic and other agricultural wastes is not without its bottlenecks. Commercialization of both the above biofuels faces huge challenge because of the seasonal availability of raw materials. Production of third-generation biofuels using microorganisms remains the only viable option, and microalgae are considered far superior because of the production of carbon-neutral fuels.

In addition, oil productivity of many microalgae greatly exceeds the oil productivity of the high-oil-yielding crops. Microalgae can provide several different types of renewable biofuels such as methane, biodiesel, biohydrogen, bioethanol, biobutanol, etc.; these include methane produced by anaerobic digestion of the algal biomass (Spolaore et al. 2006), biodiesel derived from microalgal oil (Roessler et al. 1994; Dunahay et al. 1996; Sheehan et al. 1998; Banerjee et al. 2002; Gavrilescu and Chisti 2005) and photobiologically produced biohydrogen (Ghirardi et al. 2000; Akkerman et al. 2002; Melis 2002; Fedorov et al. 2005; Kapdan and Kargi 2006).

3.8.2 Biodiesel

Biodiesel is produced by a mono-alcoholic transesterification process, in which triglycerides react with a mono-alcohol (most commonly methanol or ethanol) with the catalysis of alkali, acids or enzymes (Meher et al. 2006). Microalgae find their use in biodiesel production, because certain species contain high amounts of oil. Microalgal biodiesel production is typically performed by oil extraction followed by the transesterification of algal oil (Miao and Wu 2006). The Aquatic Species Program was initiated by the US National Renewable Energy Laboratory in 1978 for the first large-scale collection and screening of oleaginous microalgae for the production of biodiesel. More than 3000 isolates were collected, and around 300 species were identified as oil-rich algae (Sheehan et al. 1998). Therefore, production of microalgal biodiesel is highly sustainable and carbon-neutral, but future research is warranted for successful commercial deployment.

3.8.3 Bioethanol

In the past few decades, there has been an interest in the potential use of microalgae to produce biofuels through other biomass energy conversion technologies such as anaerobic fermentation (Harun and Danquah 2011). Anaerobic alcoholic fermentation using yeast is the conversion of biomass materials which contain sugars, starch or cellulose into ethanol (McKendry 2002). With the exception of some microalgal species, a majority of microalgae have relatively low carbohydrate content. However, microalgal biomass composition can be manipulated by creating various stress conditions during cultivation. Under stressed conditions, such as nutrient starvation or high light intensity, many microalgal species accumulate carbohydrates in their biomass, which can amount to a significantly high level of up to 65% (Markou et al. 2012). *Chlorella vulgaris* has been found to be a good source for ethanol due to its high carbohydrate content (ca. 37% dry wt.), and 65% ethanol conversion efficiency has been recorded (Hirano, et al. 1997). Ueno et al. (1998) produced ethanol from microalgae and achieved a maximum ethanol productivity of 450 mmol g^{-1} dry wt. at 30 °C. From the outlined concepts, it is arguable that ethanol production from microalgae is technically viable.

3.8.4 Biomethane

Various feedstocks have always been searched for cost-efficient production of biogas/biomethane. Biomethane is produced by anaerobic digestion of biomass/biowaste and is used both as a cooking fuel and for generating electricity. Compared to plants, for example, maize, where the whole plant is used for biomethane production, algae might be more productive under optimal conditions. Specific biogas yields of microalgae vary between 287 and 611 L/kg volatile solids (VS) and specific methane yields between 100 and 450 L/kg VS. According to Heerenklage et al. (2010) and Golueke et al. (1957), thermophilic digestion of microalgae results in higher biogas yields than during mesophilic digestion. Likewise, Zamalloa et al. (2012) noted that thermophilic digestion of *S. obliquus* resulted in a biogas production 1.3 times that of mesophilic digestion. The added advantage of microalgae feedstock is that lipid-extracted biomass can be used for anaerobic digestion, thereby producing both biodiesel and biomethane.

3.8.5 Biocrude/Syngas

Hydrothermal liquefaction of biomass is the thermochemical conversion of biomass into liquid fuels by processing in a hot, pressurized water environment for sufficient time to break down the solid biopolymer structure to mainly liquid components. At a temperature range of 520 and 647 K, the process results in the production of a liquid fuel known as biocrude. Biocrude is similar to petroleum crude and can be upgraded to the whole distillate range of petroleum-derived fuel products. At temperatures above 647 K, gasification reactions occur, and the process is defined as hydrothermal gasification, resulting in the production of a synthetic fuel gas. Various authors have dealt with studying the characteristics of algal biomass as a feedstock. Dote et al. (1994)

carried out thermochemical liquefaction at 300 °C on *B. braunii* to achieve a maximum yield of 64% dry wt. basis of oil with HHV of 45.9 MJ kg^{-1}. In a similar study, an oil yield of 42% dry wt. was obtained from *Dunaliella tertiolecta* with an HHV of 34.9 MJ kg^{-1} (Rosenberg et al. 2008). Reports also indicate that bio-oils from microalgae are of a higher quality than those extracted from lignocellulosic materials (Demirbas 2006). These results indicate that hydrothermal/thermochemical liquefaction is a viable option for the conversion of algal biomass to carbon-neutral liquid biocrude.

3.8.6 Biohydrogen

Hydrogen is a clean and renewable energy source that does not produce CO_2 as a by-product when used in fuel cells for electricity generation. Although hydrogen biogas can be efficiently produced at the laboratory level, there is no known commercially operating hydrogen from biomass production facility in the world (Zhu and Beland 2006). Research into hydrogen production from biological sources is being carried out since many decades. It was observed that, under anaerobic conditions, the green alga *S. obliquus* can either use H_2 as electron donor in the CO_2 fixation process in the dark (Gaffron 1944) or evolve H_2 in the light (Gaffron and Rubin 1942). Many microorganisms produce hydrogen as their by-product during anaerobic fermentation processes. In biological hydrogen production, hydrogen formation and consumption are coupled, so that hydrogen is available as the final product (Reith et al. 2003). Biological production of hydrogen using microorganisms is an exciting area of technology development that offers the potential production of usable hydrogen from a variety of renewable resources. Biological systems provide quite a range of approaches to generate hydrogen, which include direct biophotolysis, indirect biophotolysis, photofermentation and dark fermentation (Das and Veziroglu 2001).

3.9 Phycoremediation and Biofuel Production: A Concomitant Approach

Due to decreasing fossil fuel resources and increasing greenhouse gas emissions, we look at the prospects of producing alternative eco-friendly, carbon-neutral, renewable and sustainable biofuels such as biodiesel, biomethane, biobutanol, biohydrogen and bioethanol. Microalgae are viewed as a potential source for the production of these fuels. However, the life-cycle analysis at the demonstration scale shows that the biofuels produced are not economically viable. Thus, cultivation of microalgae in marine water or in domestic/industrial/agricultural wastewaters will be a viable option (Rawat et al. 2011). Moreover, studies have shown the potential for moderate lipid production (less than 10–30% DW) and high lipid productivity (up to 505 mg L^{-1} day^{-1}) in microalgae grown on wastewater. This suggests the potential of lowering the cost of algal biofuel production, which is currently not economically feasible. The concomitant approach will offer lots of advantages such as use of coastal wastelands, saving valuable freshwater resources, effective waste management, generation of algal biomass for biofuel production, reduction in the cost of biofuels and an effective approach to global warming.

3.10 Value-Added Products from Algal Biomass: Wealth from Waste Approach

In the past decades, microalgae have been studied for use as supplementary food, fodder, fertilizers and pharmaceuticals, biofuels, etc. They become all too important when useful products are derived while treating industrial effluents (Venkataraman 2005). In the early 1950s, the increase in the world's population and prediction of an insufficient protein supply led to the search for new alternatives and unconventional protein sources, and algal biomass appeared, at that time, as a good candidate (Becker 2004). Research into application aspects continued with the search for antimicrobials, particularly antibiotics. Later, it was even suggested that algae can be used as photosynthetic gas exchangers for space travel (Borowitzka 1995). As microalgal cells are capable of synthesizing all amino acids, they can provide all the essential amino acids to humans and animals. Carbohydrates in microalgae can be found in the form of starch, glucose, sugars and other polysaccharides. Their overall digestibility is high, which is why there is no limitation in using dried whole microalgae in foods or feeds (Becker 2004). The average lipid content of algal cells ranges between 1% and 70% but can reach up to 90% of dry weight under stressed conditions (Metting 1996). Algal lipids are composed of glycerol, sugars or bases esterified to saturated or unsaturated fatty acids (Guil-Guerrero et al. 2004). Among all the fatty acids in microalgae, some fatty acids of the ω3 and ω6 families are of particular interest. Microalgae also represent a valuable source of nearly all essential vitamins (e.g. A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid and pantothenic acid) (Becker 2004). Thus generation of such valuable products during phycoremediation offers immense potential and hence will lead to the production of wealth from waste.

3.11 Genetically Engineered Algae for Phycoremediation/Biofuels

Genetic engineering has the potential to improve not only algal growth but also lipid productivity and the types of lipids produced. High-quality lipids with fatty acid mixture of 16:1, 18:1 and 14:0 in the ratio of 5:4:1 can be obtained by engineering microalgae (Schenk et al. 2008). Another successful approach is to engineer algae to increase biomass yields, which is by downregulating the expression of light-harvesting antenna complexes (Beckmann et al. 2009; Mussgnug et al. 2007).

Although the application of genetic engineering to improve biofuel production in algae is still in its infancy, significant inroads have been made in the development of genetic manipulation tools in the past years. However, if these genetically engineered algae are not constrained during their cultivation, they could pose a threat to the environment (Beer et al. 2009). It is possible, and perhaps inevitable, that genetically modified algae grown in open ponds may escape and replicate in the environment leading to genetic cross-contamination. To enhance the wastewater treatment potentials, genetically engineered algal strains can be developed and employed for better wastewater management. For the development of suitable genetically engineered algal strains that would be effective in increased uptake of nitrogen and phosphorus, selection of suitable genes with increased nitrogen and phosphorus uptake capacity is needed. Such genes, when overexpressed under the influence of a suitable promoter system, will lead to increased uptake of nitrogen and phosphorus in the modified algal strain. Therefore, development of genetically transformed algal strains with enhanced absorption and metabolism characteristics can help in improving the phycoremediation potential. However, the problems associated with outdoor treatments using GM algae have to be addressed.

3.12 Safety and Environmental Impact of Phycoremediation

The safety and environmental impact of any emerging technology has to be thoroughly studied before full-scale implementation of the same. Phycoremediation is a safe technology where only photosynthetic oxygenic organisms that are non-pathogenic are used. In fact, the many organisms exert antagonistic effects and can get rid of bacterial loads (García et al. 2000). The toxicity of the algal sludge generated depends on the quality of wastewater treated, and in cases where the toxicity levels are high, algal sludge is subjected to treatment before being disposed of. Surprisingly, some species exhibit phycovolatilization, where toxic substances are converted to non-toxic volatile substances (Rao et al. 2011b). With respect to the environmental impact, phycoremediation has far more positive aspects such as biological carbon sequestration, effective nutrient removal and oxygenation by photosynthesis. The phycoremediated algal sludge has been used as aquatic feed and as a plant growth promoter without any adverse effects (Rao et al. 2010).

3.13 Current Global/National Scenario

There has been increasing awareness around the globe on the potential benefits of phycoremediation technology. Lots of pilot-scale and commercial-scale trials are being conducted worldwide; phycoremediation is very effective for the treatment of various types of wastewaters, i.e. domestic sewage, agricultural waste substrates, agro-industrial wastewater, livestock wastewater, piggery wastewater, food-processing effluents as well as other industrial wastes (Abdel-Raouf et al. 2012; Cai et al. 2013; Gupta et al. 2016; Ansari et al. 2019). Several studies have demonstrated excellent wastewater treatment potentials by various microalgal species (Ogbonna et al. 2000; Kim et al. 2013; Gupta et al. 2016; Rawat et al. 2016; Ghosh 2018). In India, the success story of commercialization of phycoremediation technology began with the implementation of the world's full-scale phycoremediation plant at SNAP Alginate Pvt. Ltd., India, by Sivasubramanian et al. (2009). Subsequently, the India-based Phycospectrum Environment Research Centre has installed full-scale plants in numerous industries across India as well as in many other countries. During the last few years, the algal technology company has installed/is in the process of installing large-scale plants in reputed industries such as Brintons Carpets, UK; Pacific Rubiales oil-drilling site, Colombia; KH Exports, India; Ranitec CETP, India; etc. (Rao and Sivasubramanian 2016).

3.14 Drawbacks/Constraints in Full-Scale Implementation

Although microalgae-based wastewater treatment has lots of advantages, certain constraints plague the smooth transition to the field scale that include construction of ponds, hassles in operations, harvesting/separation of biomass, strain selection, biological contamination, culture inoculum size, grazing protozoans, land requirements, light requirements particularly during winters in temperate zones, temperature variations and rainfall/snowfall. However, these challenges can be overcome in the future decades by carrying out research in the above specific problems or by developing tailor-made technologies.

3.15 Future Prospects

Environmental sustainability, benefits of carbon credits and creation of wealth from waste are some of the advantages that make phycoremediation technology a promising one for the future. On the other hand, with the ever-increasing prices and finite nature of fossil fuels, there is an urgent need for sustainable generation of biomass at a very cheaper cost. Therefore, the integrated approach of waste remediation and generation of biomass is being considered by many biofuel industries. Such innovations will help overcome biomass constraints, thereby providing greater commercial gains. Likewise, suitable policy amendments by the government pertaining to environment management and industrial waste management will certainly facilitate implementation of such eco-friendly technologies without any difficulty. For example, providing technical as well as financial support, subsidies, tax benefits, etc. will certainly encourage both the start-up and well-established industries to implement phycoremediation technology and derive multiple benefits.

3.16 Conclusion

To conclude, considering replacement of existing secondary treatment systems with more environment-friendly and profitable phycoremediation technology or at least integrating into the conventional systems, wherever feasible, is of paramount importance. Phycoremediation is a real green technology because of its vast environmental benefits, and in the past decade, there has been a steady rise in the number of phycoremediation plants throughout the world. The success in the widespread use of this technology will rely on joint efforts by entrepreneurs, academicians and policymakers.

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Chapter 4
Aspects of CO_2 Injection in Geological Formations and Its Risk Assessment

Shachi, Pankaj Kumar Gupta, and Brijesh Kumar Yadav

Abstract Anthropogenic emission of greenhouse gases (GHG) in atmosphere is equivocal and the major cause of global warming, significantly impacting the global climate change. Scientists, researchers, expert organizations, and policymakers suggest that in order to combat the instabilities caused by global warming, further investigation in the direction of safe CO_2 storage should be at utmost priority. To reduce GHG concentration in atmosphere, carbon capture and storage (CCS) has emerged as a promising bridge technology, by capturing CO_2 from the major sources such as cement factories, fossil fuel-based production plants, etc. and subsequently storing it in subsurface. CCS technology greatly reduces CO_2 concentration and restricts and protects subsequent migration by safely storing within geologic formations for millions of years. The CO_2 is injected into suitable geological formations at depths below 800 m or more, and various trapping mechanisms would prevent further migration of the stored CO_2 to the surface. Potential candidates for safe storage of CO_2 are geological storage (in geological formations, such as un-mineable coal seams, depleted oil and gas fields, and deep saline formations), ocean storage (direct release into ocean), and industrial fixation into inorganic carbonates. This chapter provides a detailed knowledge on the sources of GHG emissions into the atmosphere and the status of CCS process worldwide emphasizing on the various trapping mechanisms of CO_2 in geological formations. The storage of CO_2 in geological formations is greatly affected by environmental factors, so impact of these variables is described. In order to study the impact of these variables on CO_2 sequestration, a state-of-the-art modeling techniques along with the numerical methods are reviewed comprehensively.

Keywords Greenhouse gases (GHGs) · Carbon capture and storage (CCS) · Geological formations · Numerical investigations

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4.1 Introduction

Global warming due to the anthropogenic emissions of greenhouse gases in atmosphere becomes a major threat to the environment. The concentration of greenhouse gases already crossed the mark of 415 ppm this year (CO2.earth, 2019) contributing toward global warming, which is much above (approximately 45% (Celia et al. 2015)) of the pre-industrial time concentration of 280 ppm (Orr Jr. 2009a). The anthropogenic emissions have already impacted global average temperatures, decrease in ocean pH, sea level rise, leaching of soil nutrients, high-latitude ice cover, changes in precipitation pattern, changes in snow cover, etc. Now, it is the time to think about the potential impact of future emissions, which cannot be ignored (in terms of present energy demand), currently projected to rise substantially in near future lacking significant changes in energy systems. The energy production to cater the demands of increasing population by keeping the level of greenhouse gases in limit has become one of the challenging tasks for the researchers, policymakers, and scientific communities. In order to reduce global warming, Energy Technology Perspectives 2012 (IEA) emphasizes to limit the global average temperature rise by 2 °C by 2050; CCS can contribute one-sixth of CO_2 reduction until 2050. Intergovernmental Panel on Climate Change concluded that in order to stabilize the CO_2 concentrations, reduction in emissions at significantly higher-level present is required for keeping a check on 2 °C global average temperature rise. Thus, the CCS technologies gain more attention as effective mitigation option to reduce the GHGs concentration. In this direction, the most widely perceived method of disposing unwanted CO_2 permanently is sequestering it into the deep geological formations (Orr 2009b) on a temporal scale ranging from tens to hundreds to thousands of years. Deep geological formations are economically feasible under site-specific conditions for storage of CO_2 in saline formations, oil and gas fields, and storage in coal seams at shallower depths.

According to (IPCC 2005) carbon dioxide (CO_2) capture and storage (CCS) is a process in which CO_2 is separated from energy production and industrial sources, further transported to a storage location by injecting into the deep geological formations. This process enables isolation from the atmosphere for longer time period and stabilizes the atmospheric greenhouse gas concentrations. For deep underground storage sites, the options for CO_2 storage include un-mineable coal seams, deep saline aquifers, depleted oil and gas reservoirs, and abandoned coal mines (Piessens and Dusar 2004; Jalili et al. 2011). The CO_2 storage trapping mechanisms are classified as physical and chemical trapping mechanisms. Structural/stratigraphic trapping and residual trapping are referred as physical trapping whereas solubility trapping, mineral trapping, and adsorption trapping (in coal) as chemical trapping mechanisms (CSLF 2007). Thus, the focus of this chapter is to provide a detailed account of the sources of GHG emissions into the atmosphere and the status of CCS process worldwide emphasizing on the various trapping mechanisms of CO_2 in geological formations. The storage of CO_2 in geological formations and environmental factors affecting the CO_2 storage process are described in depth.

4.2 Current Status of Carbon Capture and Storage (CCS)

The sequestration of anthropogenic CO_2 in the subsurface porous rocks appears to be the most viable method for CO_2 mitigation and, however, has many challenges to overcome (Orr 2004). To address the challenges of geological sequestration of CO_2, a proliferation of projects worldwide for CO_2 injection into underground formations is expected (Benson and Myer 2002). Recently in Paris COP 21, 195 countries agreed to reduce their carbon output in near future to keep the global warming below to 2 °C target (United Nations 2015). Almost every country needs to urgently establish the efforts for the CO_2 capture and storage. In this direction, International Energy Agency, IPCC, USGS, UNFCCC, etc. have evaluated the potential geological formation for CCS throughout the globe (IEA 2008). The IEA 2009 CCS roadmap highlighted the need to develop 100 CCS projects between 2010 and 2020, storing around 300 Mt of CO_2 per year (International Energy Agency 2013). The carbon sequestration leadership forum completed many projects on various sites throughout the globe. The important CCS projects include CarbonNet Project at southeastern Australia in the Latrobe Valley and offshore Gippsland Basin; CO_2 store in a saline formation at the North Sea; Fort Nelson Carbon Capture and Storage Project in the northeast British Columbia; Frio Project in the eastern Texas region of the USA; Quest CCS Project, in Fort Saskatchewan, Alberta, Canada; and SECARB III in the USA.

The deep saline aquifers are usually considered as potential sites for CO_2 storage due to its high saline proportion and its depth. The Sleipner field in the North Sea pioneered the concept of injecting CO_2 into an underlying saline formation for permanent storage. The Utsira sandstone formation at Sleipner in the North Sea is one of the examples where a large amount of CO_2 have been injected almost 1Mton of CO_2 per year (Bentham and Kirby 2005). CO_2 sequestration in abandoned coal mines have been inspired by the storage of natural gases in coal mines since 1961. The storage of natural gas has demonstrated the technical feasibility to use abandoned coal mines as CO_2 reservoir (Piessens and Dusar 1961). The capacity of CO_2 storage at Beringen colliery (Belgium) has been estimated to be 13 Mton at an injection rate of 300,000–500,000 Mton/year for 25 years (Jalili et al. 2011). Though modeling and experimental studies have been performed to understand the postinjection processes in geological formations, complete geochemical interactions of the injected CO_2 in deep geological formations are still not very well understood. Therefore, most of the international agencies strongly recommended the investigation on CO_2 sequestration and its reactive transport in subsurface (International Energy Agency 2013). Laboratory and simulation experiments were performed by Soong et al. (2004) to investigate the effect of various conditions on the reaction of CO_2 with the brine samples collected from the Oriskany Formation in Indiana Country, PA, in the autoclave reactor. Few laboratory studies were also performed to investigate the CO_2 flow in small-scale setup to provide a description of two-phase flow in sequestration and to develop a better understanding of the displacement of brine by CO_2 (Ji et al. 2001; Ogunsola et al. 2000).

Mathematical model developed by Juanes et al. (2010) for CO_2 migration in deep saline aquifers accounted gravity override, capillary trapping, natural groundwater flow, and the shape of the plume during and after injection period. To estimate the transport and dissolution of supercritical CO_2, simulation experiments were performed for the carbonated aquifer of the Dogger (Paris Basin) and the sandstone aquifer of the Bunter (North Sea) Lagneau et al. (2005). Doughty and Pruess (2004) investigated the physical processes that occur during the sequestration of CO_2 in liquid-saturated, brine-bearing geologic formations using the numerical simulator TOUGH2. (Karsten Pruess and Keni Zhang 2008) developed the numerical model of the dissolution-diffusion-convection process for CO_2 storage in saline aquifers. Further, (Hajime Yamamoto 2010) investigated the impact of CO_2 pool on regional groundwater flow. The interactions of CO_2 with coal were investigated under a set of conditions to determine the effects of the nature of the coal and the sequestration environment including parameters such as rank and moisture content of the coal, temperature, pressure, and pH. Some of the researchers investigated the trapped CO_2 leakage and seepage from geological sites and their migration through the variably saturated zones (Oldenburg and Unger 2003; Pruess 2006).

4.3 Potential Sources of Greenhouse Gas (GHG) Emissions

Carbon dioxide concentration is continuously increasing in atmosphere since the pre-industrial times as a result of anthropogenic activities. Methane concentration in atmosphere has been increased by 150% since 1750 and is mostly from anthropogenic sources, but its emission rates are diffucult to quantify (Houghton et al. 2001). Nitrous oxide main contributor is combustion of coal, and its concentration is increased by 16% since pre-industrial era and is contributing significantly toward global warming. Hydrochlorofluorocarbons (HCFCs) and chlorofluorocarbons (CFCs) are used as coolants in air conditioners and refrigerators and, because of their longer lifetimes in atmosphere, considered significant greenhouse gases but contribute little toward global warming. Ozone sources from vechicle emissions increased from 20% to 50% during the twentieth century; its warming capacity is not known but predicted to be around 15% (Hardy 2003). Among all the greenhouse gas, carbon dioxide concentration has drastically increased in recent decade mainly contributed by burning of fossil fuels, cement industries, thermal power plants, etc. The use of fossils cannot be avoided as it meets the 75% (Bachu and Adams 2003) and 84% (between 2005 and 2030) of the world's energy demand (IEA 2007). The fossils will remain on the top for the next century to meet the world's energy supply because of its widespread availability throughout the globe, ease of transportation and storage, low cost, and large resources. The industry and power sector together accounts for 60% of world's CO_2 emissions (IPCC 2005), which in near future is likely to decline to 50% by 2050. Coal (fossil fuel available greatly) is the worst CO_2 emitter per unit of energy produced. So, the greatest challenge for the engineers of twenty-first century is to develop systems for the capture of CO_2 produced by burning fossil fuel and sequestering it away from the atmosphere.

4.4 Mechanisms of CO_2 Trapping in Subsurface

The technique with which carbon dioxide is injected and flows through the storage reservoir, together with the time the CO_2 remains in geological formation, defines the trapping mechanisms over time. Once the CO_2 is injected into a reservoir, it can be stored using different mechanisms, temporally ranging on a scale from century to millennium. There are four foremost trapping mechanisms as shown in Figs. 4.1–4.4, which determine the geological storage of CO_2. The CO_2 is stored: (i) physically trapped as a free, buoyant supercritical fluid confined by a structure leading to structural/stratigraphic trapping, (ii) retained in the pore space by capillary forces as immobile bubbles at irreducible gas saturation leading to residual trapping, (iii) dissolved into formation water as solubility trapping, and (iv) in the form of soluble minerals as mineral trapping. In the deep geological formations, different trapping mechanisms interact with each other even though they come into picture at different time periods of entire storage process.

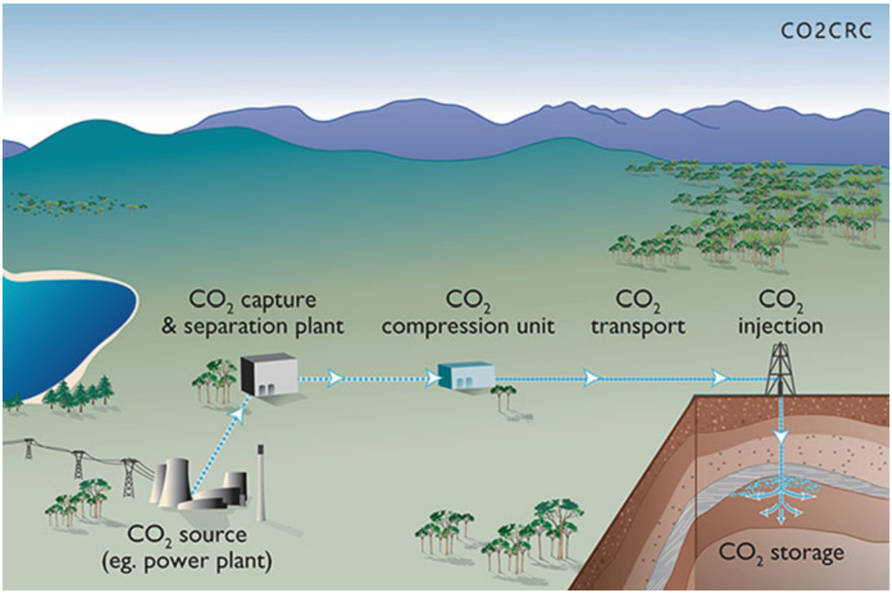

Fig. 4.1 Picture shows the process of CO_2 capture and storage process in geological formations

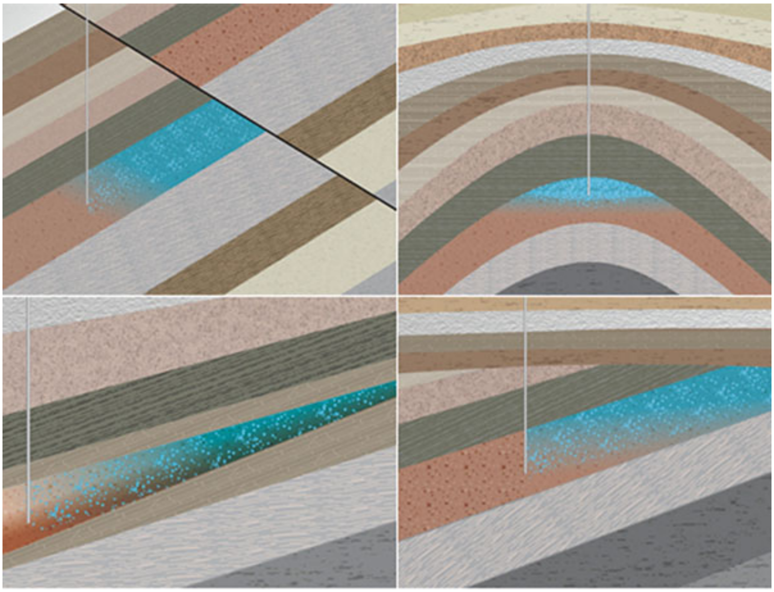

Fig. 4.2 Structural/stratigraphic trapping involved during CO_2 storage in deep geological formations

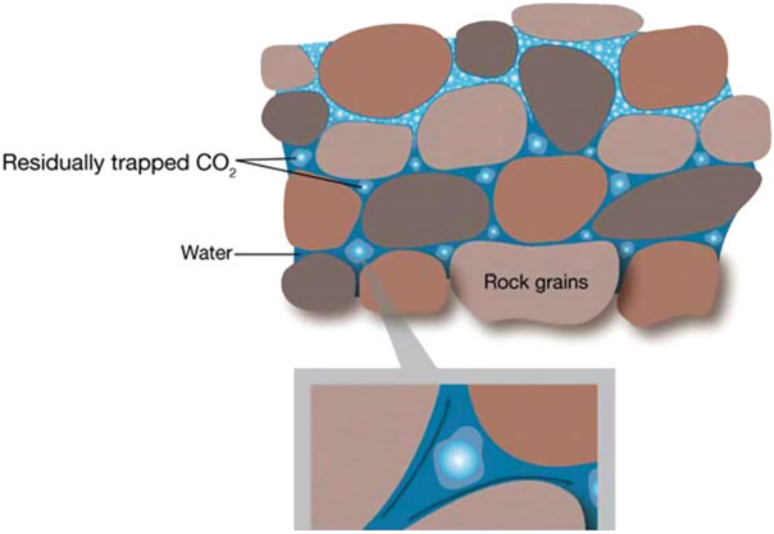

Fig. 4.3 Residual trapping of CO_2 in pore space in geological formations

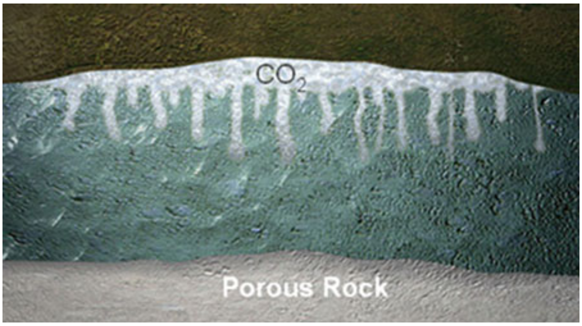

Fig. 4.4 Dissolution trapping of CO_2 in deep geological formations

4.4.1 *Structural/Stratigraphic Trapping*

It is the dominant among all other mechanisms and involves a physical trap of CO_2 in reservoir, where its further migration is ceased due to the presence of impermeable barriers (Macminn et al. 2010). Here the CO_2 is trapped as buoyant free phase beneath a low-permeability caprock having different geometrical arrangements leading to fault trap, anticline trap, facies change trap, and unconformity trap. The maximum storage capacity with structural trapping can be obtained at the injection end and during the injection period only. Recent studies concluded reduction in structural trapping, if the CO_2 is injected at a favorable distance from the reservoir boundaries (Vishal and Singh 2016).

4.4.2 *Residual Trapping*

The CO_2 is retained in pore spaces of reservoir rocks under the influence of capillary forces, also known as capillary trapping, phase trapping, or mobility trapping (Christine Doughty 2010). When the injected CO_2 displaces the formation brine, being less buoyant migrates as separate phase until it is residually trapped (IPCC 2005) as disconnected droplets in pore spaces, which are immobile. It occurs in the postinjection phase, and displaced brine invades back by hysteresis effect (Khudaida and Das 2014). It is the quickest and safest mechanism of trapping CO_2 in the form of bubbles (Celia et al. 2015).

4.4.3 *Solubility Trapping*

Solubility trapping refers to the CO_2 dissolution in formation brine, which further increases the density and acidity causing convective mixing. The CO_2-enriched brine being denser than the native brine, which help CO_2 to sink deeper into the formation, thus enables more dissolution to occur as more CO_2 come in contact of formation brine (Ennis-King and Paterson 2005; Silin et al. 2009). The effectiveness of solubility trapping lies in the fact that CO_2 no longer exists as a separate phase, and hence its upward migration is constrained.

4.4.4 Mineral Trapping

It is a long-term storage mechanism and most secured form of storage and involves the precipitation of CO_2 in the form of solid carbonate minerals. The quantification of CO_2-brine-mineral interactions is highly uncertain at reservoir conditions. Supercritical CO_2 being buoyant displaces the formation water and dissolves it to react with minerals well, cements, and pipes. The CO_2 sequestration is a transient process depends on reaction kinetics. It begins with CO_2 dissolution in formation brine to produce weak carbonic acid and bicarbonate ions which further interact with the divalent cations to produce carbonate minerals. The production of carbonate minerals increases the soluble carbonates and bicarbonates in solution further enhancing the solubility trapping process. The shell of organisms is formed by reaction of calcium and magnesium carbonates with carbonic acid, which later on are deposited as limestone sediment.

4.5 Environmental Aspects of CO_2 Storage in Geological Formations

Sequestration of carbon dioxide in various geological formations is greatly affected by the environmental parameters such as temperature, pressure, salinity, pH, effective stress, and moisture content. In the case of coal formation, adsorption is the main phenomenon responsible for the storage of carbon dioxide. The temperature increase lowers the adsorption capacity of coal (Azmi et al. 2006), while the pressure increase enhances the adsorption capacity (Day et al. 2008). The sorption capacity is also dependent on moisture content and decreases to a critical value (40% – 80%) with increase in moisture content, and beyond critical value there is no change in sorption capacity (Weishauptová et al. 2015). The less acidic the water, i.e., low pH value, higher is the adsorption capacity (Schroeder et al. 2002) of coal, while increase in effective stress reduces the adsorption capacity (Hol et al. 2011). In saline aquifers solubility or dissolution plays a major role in securing carbon dioxide safely. As we move more deeper in saline aquifers, the salinity increases with depth, and hence solubility of CO_2 decreases (Spycher and Pruess 2005). With the increase in pressure, the CO_2 solubility increases, while with temperature increase the solubility of CO_2 decreases (Iglauer 2011). Role of environmental parameters on CO_2 sequestration in geological media is summarized in Table 4.1.

Table 4.1 Summary of environmental factors affecting the adsorption capacity of coal

Studied parameter	Relation with sorption capacity	Reference
Temperature	Higher the temperature, lower is the adsorption capacity of coal	Azmi et al. (2006)
Pressure	Higher the pressure, higher is the adsorption capacity	Day et al. (2008), Pone et al. (2009)
Moisture content	Sorption capacity decreases as moisture content increases to a critical value (40%–80%). With further increase in moisture content beyond critical value, there is no change in sorption capacity	Weishauptová et al. (2015) Siemons and Busch (2007)
pH value	The less the pH value, higher is the adsorption capacity	Azmi et al. (2006), Schroeder et al. (2002)
Effective stress	Adsorption capacity reduces with increasing effective stress	Hol et al. (2011)

4.6 Numerical Modeling of CO_2 Storage in Subsurface

Numerical modeling is prerequisite to understand the flow dynamics of system for improving decisions related to field applications of the phenomenon in practical situations. Dynamic simulation is required to answer the questions like storage capacity estimation of a site, pressure buildup due to CO_2 injection, and physical and chemical processes occurring due to CO_2 injection. The numerical solution is trade-off between the computational cost, complexity, and accuracy as the process takes place on a large spatial and temporal scale with no data available and highly complex equations representing the processes are solved (Mykkeltvedt 2014). Mathematically, the CO_2 injection into a subsurface formation solves complex fluid pressure and fluid transport phenomenon using coupled system of nonlinear partial differential equations. Thus, the investigation related to CO_2 storage and leakage is very complex by its thermal, mechanical, chemical, and hydrodynamic processes, involving large spatial and temporal scale. Hence, numerical modeling can enhance the knowledge about a particular site for its suitability for storage and operational parameters and plays a vital role in decisionmaking process.

CO_2 sequestration in subsurface depends on properties of both CO_2 and formation fluid along with the characteristic geological formation under specific environmental conditions. Thus, modeling CO_2 geological sequestration is highly complex and requires formulation of governing equations incorporating all the processes in subsurface. The CO_2 density depends upon the temperature, pressure, and geothermal gradients. At optimal depth (600–1000 m), CO_2 density ranges from 250 to 800 kg/m3 are suitable for sequestration (Stefan Bachu 2003). The CO_2 exists in supercritical state having smaller density than brine of 1200 g/m3 (Celia et al. 2015) at such depths. The solubility of CO_2 in formation water depends on temperature, pressure, and salinity, which decreases with increasing temperature and water salinity and increases with increasing pressure as shown in Table 4.2 (Iglauer 2005). In the case of coal formation, CO_2 is adsorbed on the coal surface (Celia

Table 4.2 Summary of environmental factors affecting solubility of carbon dioxide

Parameter	Relation with solubility	Reference
Salinity	The salinity increases with increasing depth, and the solubility of CO2 decreases with increasing salinity	Spycher and Pruess (2005)
Temperature	With increasing temperature solubility decreases	Iglauer (2011)
Pressure	With pressure increase solubility increases	Iglauer (2011)

et al. 2015), while in saline aquifers, it dissolves in water forming weak carbonic acid ultimately precipitating as minerals (Rochelle et al. 2004).

The multiphase system having CO_2 as a non-wetting phase and brine as wetting phase is incorporated in modeling. A multiphase system consisting of CO_2-rich phase and brine and multicomponent system having CO_2, water, and salts dissolved in brine are considered for injection. The dissolution of CO_2 in brine lowers the pH of the formation thus initiating the geochemical reactions; and phase partitioning into CO_2-rich phase generates corrosive wet CO_2. The governing equation for phase partitioning, chemical reactions, non-isothermal effects (temperature contrast of injected CO_2 and ambient fluid), and geo-mechanical effects (formation pressurization) is to be considered in the modeling on the basis of site prevailing conditions (Celia et al. 2015).

4.6.1 Mass Balance Equation

The modeling involves the injection of supercritical CO_2 (31.1 °C and 7.38 MPa) in saline aquifers which represents the movement of two immiscible fluids, water (brine) as a wetting phase and CO_2 as a non-wetting phase in a porous medium. All the models involving injection of CO_2 in geological formations are governed by mass conservation equation:

$$\sum_{\gamma}\left[\frac{\partial}{\partial t}\left(S_{\gamma}\phi\rho_{\gamma}m_{\gamma}^{i}\right)+\nabla.\left(\rho_{\gamma}v_{\gamma}m_{\gamma}^{i}+j_{\gamma}^{i}\right)\right]=\sum_{\gamma}\psi_{\gamma}^{i} \tag{4.1}$$

where S_{γ} is phase γ (water or CO_2) saturation, φ is porosity, ρ_{γ} is density, t is elapsed time, v_{γ} is average pore velocity, m_{γ}^{i} is mass fraction of component i(CO_2, H_2O, and salt) in phase γ, j_{γ}^{i} is diffusion or mechanical dispersion flux vector (non-advective), ψ_{α}^{i} is external source or sink term, and interphase mass transfer is eliminated by taking summation.

4.6.2 Constitutive Equations

From the generalized Darcy's law (equation of momentum), velocity vector v_γ (for multiphase) can be calculated as:

$$v_\gamma = -\frac{k_{r\gamma}}{\mu_\gamma} K \cdot \left(\nabla p_\gamma - \rho_\gamma g\right) = -\lambda_\gamma K \cdot \left(\nabla p_\gamma - \rho_\gamma g\right) \tag{4.2}$$

$k_{r\gamma}$ is the relative permeability of phase γ, μ refers to dynamic viscosity, p_γ is the pressure, K is the tensor of absolute permeability, g is the vector of gravity, and λ_γ is phase mobility. The phase permeability k_γ (effective permeability) is related to the relative permeability ($k_{r\gamma}$) as

$k_{r\gamma} = \frac{k_\gamma}{K}$ where K is the domain permeability for a single-phase flow.

Equation 4.2 relates the capillary pressure (P_c), saturation (S), and relative permeability (k_r). Capillary pressure is given by the difference of non-wetting phase pressure (P_n) and wetting phase pressure (P_w).

$$p_n - p_w = p_c \tag{4.3}$$

Constraints on mass and volume fractions are given by:

$$\sum_i m_\gamma^i = 1 \tag{4.4a}$$

$$\sum_\gamma S_\gamma = 1 \tag{4.4b}$$

The kinetic expressions for component transfer are given by:

$$\frac{\partial}{\partial t}\left(S_\gamma \phi \rho_\gamma m_\gamma^i\right) + \nabla.\left(\rho_\gamma v_\gamma m_\gamma^i + j_\gamma^i\right) = \psi_\gamma^i + \sum_{\beta \neq \gamma} \psi_{\gamma,\beta}^i \tag{4.5}$$

$\psi_{\gamma,\beta}^i$ is the mass exchange of component I between γ and β phase.

In the systems where individual component behavior is not prominent, the bulk phase equation is used by taking summation over the components within phase.

$$\frac{\partial}{\partial t}\left(S_\gamma \phi \rho_\gamma\right) + \nabla.\left(\rho_\gamma v_\gamma + \sum_i j_\gamma^i\right) = \psi_\gamma^i + \sum_{\beta \neq \gamma} \psi_{\gamma,\beta}^i \tag{4.6}$$

In the case of immiscible fluid phases, second term of RHS tends to zero ($\psi_{\gamma,\beta}^i = 0$) with no internal component gradients $j_\gamma^i = 0$; the above equation reduces to:

$$\frac{\partial}{\partial t}\left(S_\gamma \phi \rho_\gamma\right) + \nabla .\left(\rho_\gamma v_\gamma\right) = \psi_\gamma \tag{4.7}$$

Equation (4.7) is the simplest form for modeling the CO_2 brine system.

4.6.3 Energy Balance Equation

The systems in which thermal effects are also considered are required to couple mass balance equation with the energy balance equation which is given by:

$$\frac{\partial e_t}{\partial t} = \nabla \cdot f_t = Q_t \tag{4.8}$$

$$e_t = \varphi \sum_\gamma S_\gamma \rho_\gamma s_\gamma + (1-\varphi)\rho_s C_s T \tag{4.9}$$

$$f_t = \sum_\gamma \rho_\gamma s_\gamma h_\gamma + j_t^\gamma \tag{4.10}$$

where $s_\gamma$is specific internal energy in phaseγ, $s_\gamma$is specific heat, h_γ is specific enthalpy, T is temperature, and j_t^γ is conductive heat flux equation obtained from Fourier law. Equations 4.8, 4.9, and 4.10 are sufficient to incorporate temperature effects in modeling CO_2 migration, for obtaining temperature of injected CO_2 (more complex process) as density varies with temperature.

4.6.4 Geomechanics Equations

When the fluid is injected in the geological formation, mechanical response is expected which makes the necessity of introducing variables mechanical displacement vector w and mechanical stress tensor σ given by Hooke's law.

$$\sigma = C \nabla w \tag{4.11}$$

C is material stiffness tensor.

In order to couple the fluid flow with mechanical deformation, mechanical stress must balance effective fluid pressure and gravity, or effective fluid volume depends on volumetric change of displacement.

$$\nabla \cdot (\sigma - \alpha_\sigma p_\sigma I) = \left((1-\varphi)\rho_s + \varphi \sum_\gamma \rho_\gamma\right) g \tag{4.12}$$

$$\varphi = \varphi_0 + \alpha_\sigma \nabla . w \tag{4.13}$$

$\alpha_\sigma$is Biot coupling coefficient and is the measure of fluid pressure strength in momentum balance equation; material-dependent parameter satisfies $\varphi_0 \leq \alpha_\sigma \leq 1$ (Lewis and Schrefler 1998). The above formulation can be applied in region where geomechanical deformation is caused by storing CO_2, while for large fracturing and deformation, more complex models should be used. The coupling of fluid flow with geomechanics can be performed by solving simultaneous equations of fluid pressure and geomechanics, rock deformation with flow properties porosity and permeability.

The injection of CO_2 is associated with a wide range of processes occurring simultaneously in the subsurface such as geomechanical effect, geochemical reactions, and energy transport giving highly nonlinear PDE. The equations obtained after coupling are extremely complex and computationally demanding, so a fully coupled simulation for carbon storage has not yet been achieved. Thus, it is necessary to ignore one or more processes and apply further assumptions for simplifying the equations for mathematical formulation.

4.7 Risk Analysis of CO_2 Migration from the Storage Zone

The risk can be defined as the effect of uncertainty on objective (Purdy 2010) or uncertainty together with damage (Kaplan and Garrick 1981). Geological carbon sequestration is associated with both natural and technological risks. The leakage of either the CO_2 from the storage formation or the brine by the injected CO_2 is a challenge associated with geological storage of CO_2 for mitigating global warming. The geological sites selected for storing CO_2 are bounded by impermeable seal layers in addition to the Underground Sources of Drinking Water (USDWs). For secure storage the overlying caprock must be impervious. However, geological discontinuities such as faults and wells act as potential sources for leakage of CO_2 (Chang et al. 2008). The leakage allows the movement of CO_2 in shallower geological units and further to the atmosphere thus enhancing the pollution of underground water, soil, and air (Fig. 4.5). The risk assessment consists of processes: (i) identifying risks, (ii) analyzing risks, and (iii) evaluating risks.

The certification framework, CO_2 FEP (feature, event, and process), and RISQUE (risk identification and strategy using quantitative evaluation) are some qualitative and quantitative methods. There are various modeling studies (Oldenburg and Unger 2003; Oldenburg et al. 2009; Carpenter and Aarnes 2013) emphasize to select the geological storage sites and analyze risks within the certification framework. The injected CO_2 increases the formation pressure that will change effective stresses, enhances permeability, and propagates movement along faulted zone (Pruess 2006) with seismicity resulting in leakage from the storage reservoir (Rutqvist and Tsang 2002).

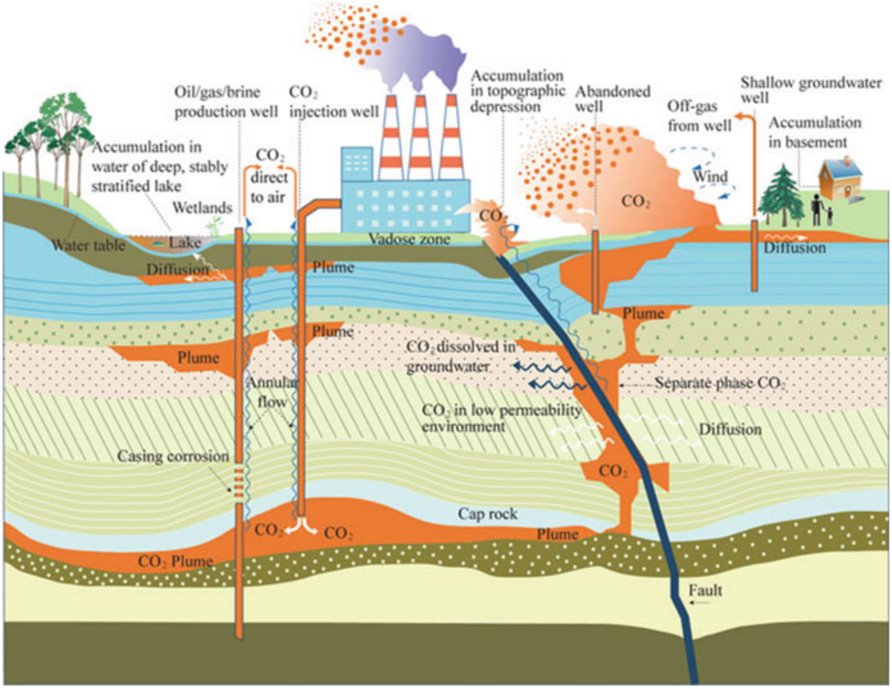

Fig. 4.5 Possible leakage pathways of sequestered carbon dioxide

4.8 Conclusion and Future Prospects

A various mitigation technologies addressing the stabilization of the concentration of atmospheric CO_2 have been premeditated and investigated in the past. Among those intensively studied, carbon capture and storage (CCS) technology emerges to be effective potential and viable option to reduce point source emissions when combustion of fossil fuel for energy production remains the core source (IPCC 2005). CO_2 capture and storage process involves capturing CO_2 from large sources like power plants to produce a concentrated stream that can be easily transported to a CO_2 storage sites. Generally, deep saline aquifers, active and depleted oil and gas reservoirs, un-mineable deep coal seams, and mined salt domes are used for underground storage media for CO_2 geo-sequestration. Of the various proposed underground geological structures suitable for carbon storage, deep saline aquifers are considered to be the best option. Injection of carbon dioxide in such structures may not have the added value of injecting it in hydrocarbon reservoirs for enhanced oil or gas recovery, but in return, deep saline aquifers provide the largest capacity of underground geological storage for carbon sequestration. Saline aquifers are also abundant in sedimentary basins all around the world. The geological sequestration involves structural, residual, mineral, and dissolution trapping mechanisms in the geological formations. In structural trapping, CO_2 is trapped as buoyant free phase beneath a low permeability caprock having different geometrical arrangements leading to fault trap, anticline trap, facies change trap, and unconformity trap. Likewise, CO_2 is retained in pore spaces of reservoir rocks under the influence of capillary forces in residual trapping and dissolved with brine in solubility trapping. Further, the CO_2 react with the formation and mineralized in mineral trapping. These trapping mechanisms are significantly affected by environmental factors especially temperature and pressure. Based on the literatures, following recommendations are listed below for future research:

(a) Investigation on different trapping mechanisms is required for estimation of CO_2 storage in geological formations.
(b) Role of geohydrological/environmental variables on the CO_2 storage in geological formation is needed for long-term CO_2 sequestration in geological media.
(c) Literature shows few investigations based on the laboratory experiments; most of the studies reported are based on numerical experiment. Therefore, the practical experiments are needed to investigate the CO_2 storage potential in different geological media.
(d) When investigating the effect of change in the reservoir stress field on the multiphase flow characteristics during subsurface CO_2 disposal, in order to eliminate the effects of other variable factors, apart from the change in net effective pressure, and draw a more solid conclusion, a new sample needs to be used with every value of the net effective pressure. However, the samples need to be as close as possible to each other in terms of physical properties and homogeneity. Furthermore, in order to investigate the effects of change in properties of the fluids as well, the change in reservoir stress field needs to be applied by increasing the pore pressure rather than reducing the overburden pressure.
(e) Furthermore, CO_2 migration in deep subsurface zones is required to investigate the risk associated with its upward movements using laboratory and numerical experiments. Impact of CO_2 leakage on groundwater system is needed to frame sustainable groundwater management and remediation, especially for organic and metals contaminants (Gupta and Yadav 2017; Mustapha et al. 2018; Joshi and Gupta 2018; Gupta and Yadav 2018; Kumari et al. 2019; Gupta and Yadav 2019)
(f) A complete methodological framework is required for effective implementation of CCS technology in field under climate change conditions.

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Chapter 5
Microbial Degradation of Polyaromatic Hydrocarbons

Tayssir Kadri, Agnieszka Cuprys, Tarek Rouissi, and Satinder Kaur Brar

Abstract Polycyclic aromatic hydrocarbons (PAHs) belong to the large group of compounds that are composed of multiple aromatic rings. These are found in natural and anthropogenic sources like coal and petrol and can be detected in various environmental compartments, which is a strong concern due to their toxic, cancerogenic, and genotoxic properties. It is crucial to develop methods that would decrease their concentration in water and soils. The most potent degradation of PAHs is usage of microorganisms to biotransform the compounds in situ. In this chapter, the characterization of PAHs is presented. Biodegradation may be performed by bacteria and fungi and also in anaerobic conditions. The factors influencing the biodegradation of PAH are also discussed.

Keywords Polycyclic aromatic hydrocarbons · Aromatic rings · Coal and petrol · Cancerogenic and genotoxic · Microorganisms · Biodegradation

5.1 Introduction

PAHs are extensively present in the environment. Since, these can be generated through the incomplete combustion of many organic substances such as petroleum gas, oil, coal, and wood. These pollutants exhibit a major concern since these represent the most structural diversity in the environment. Additionally, the concentrations of PAHs in water and soil are growing with continued oil production and transportation (Arun et al. 2008; Chowdhary et al. 2018). Their fused rings are scarcely degraded in nature and, thus, tend to bioaccumulate, and their persistence and resistance to biodegradation increase with increase in the number of rings.

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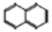
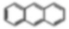
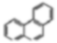
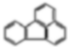
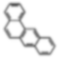
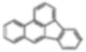
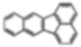
Furthermore, the PAHs are reported as potentially mutagenic and carcinogenic agents (Albanese et al. 2015; Wang et al. 2014; Zhao et al. 2014; Saxena et al. 2017).

Different promising treatment methods have been performed and developed through the years to prevent further environmental and economic consequences among which bioremediation using microorganisms is a promising key method, which is eco-friendly and offers long-lasting and low-cost solutions for PAH degradation (Mulla et al. 2017). Microorganisms that were widely investigated by researchers and demonstrated effective in PAH biodegradation comprise bacteria (Sun et al. 2014; Cybulski et al. 2003; Arulazhagan and Vasudevan 2011; Hamamura et al. 2013; Singh et al. 2015) and fungi (Hadibarata et al. 2009, Hadibarata and Kristanti 2014; Li et al. 2005; Wunch et al. 1999; Bonugli et al. 2015; Jove et al. 2016; Young et al. 2015). Biodegradation implies the breakdown of organic compounds through biotransformation into less complex metabolites and through mineralization into inorganic minerals, H_2O, CO_2 (aerobic), or CH_4 (anaerobic) (Haritash et Kaushik 2009; Bharagava et al. 2017). Biodegradation efficiency is dependent on many parameters such as the type of microorganisms, environmental conditions, chemical structure, and nature of pollutants. This book chapter is an overview of different aspects of microbial degradation of polycyclic aromatic hydrocarbons using bacterial and fungal species (Saxena and Bharagava 2017; Bharagava and Chandra 2010). Further, anaerobic degradation has also been discussed including various factors influencing the biodegradation process.

5.2 Characteristics of PAHs

PAHs are organic compounds composed of carbon and hydrogen atoms joined together into at least two benzene rings in linear, angular, or clustered arrangements (Kim et al. 2013). PAHs with less than four aromatic rings are considered as low molecular weight compounds whereas those with four or more rings are considered as high molecular weight compounds and called as large PAHs. In pure chemical form, PAHs are usually colorless, white, or pale yellow-green solids having a faint, pleasant odor (Ghosal et al. 2016). These aromatic hydrocarbons are hydrophobic, and their persistence in the environment is mainly due to their low water solubility (Cerniglia 1997). In addition, these have higher melting and boiling points and lower vapor pressure. The vapor pressure of PAHs as well as their solubility decreases with increased number of fused benzene rings; however, the melting and boiling points increase (Kadri et al. 2017). Other PAH properties comprise light sensitivity, heat resistance, conductivity, imitability, corrosion resistance, and physiological action (Masih et al. 2012). Most PAHs are also fluorescent, emitting characteristic wavelengths of light when they are excited (Masih et al. 2010). The physicochemical properties of some selected PAHs are given in Table 5.1.

Table 5.1 Physical-chemical characteristics of different polycyclic aromatic hydrocarbons

Compound	Formula	Mol. wt. (g mol^{-1})	CAS registry no.	V.P. (Pa at. 25 °C)	B.P. (°C)	M.P. (°C)	Aqueous solubility (mg/l)	IP (eV)[a]	Structure
Naphthalene	C10H8	128	91-20-3	11.9	218	80.2	30	–	
Anthracene	C14H10	178	120-12-7	3.4×10^{-3}	340	216.4	0.015	7.43	
Phenanthrene	C14H10	178	85-01-8	9.07×10^{-2}	339–340	100.5	1–2	8.03	
Fluoranthene	C16H10	202	206-44-0	1.08×10^{-3}	375–393	108.8	0.25	7.90	
Pyrene	C16H10	202	129-00-0	5.67×10^{-4}	360–404	393	0.12–0.18	7.53	
Benz[*a*]anthracene	C18H12	228	56-55-3	14.7×10^{-3}	438	162	0.0057	<7.35	
Benz[*a*]pyrene		252	50-32-8	0.37×10^{-6}	495	179	0.0038	<=7.45	
Benzo[b] fluoranthene	C20H12	252	205-99-2	1.07×10^{-5}	168	168.3	–	7.70	
Benzo[k] fluoranthene	C20H12	252	207-08-9	1.28×10^{-8}	217	215.7	–	7.48	
Benzo(ghi) perylene	C22H12	276	191-24-2	1.33×10^{-8}	525	277	–	7.31	

[a] (Pysh and Yang 1963; Simonsick and Hites 1986)

5.3 Production of PAHs

PAHs are mainly produced from the combustion of organic materials such as coal, tar, oil and gas, automobile exhaust, tobacco, or smoked food (Blumer 1976; Cerniglia and Yang 1984). Naturally, PAHs are formed during forest fires and volcanic eruptions or by plant and bacterial reactions (Blumer 1976). Nevertheless, the anthropogenic sources of PAHs such as fuel combustion, pyrolysis of organic substances, petroleum products, accidental discharge, waste incinerators, and domestic heaters (Cerniglia 1984; Chandra et al. 2008; Freeman and Cattell 1990) far exceed the natural sources.

5.4 Microbial Degradation of PAHs

5.4.1 Bacterial Degradation

Bacteria have been actively involved in the degradation of organic pollutants from contaminated sites through years, and this is due to their capability to develop pathways that enable them to obtain energy from almost every compound. Naphthalene and phenanthrene have been reported to be degraded by many bacterial species (Cerniglia 1984; Peng et al. 2008; Mallick et al. 2011). There are two major strategies to degrade PAHs. The first is aerobic degradation where the oxygen is not only the final electron acceptor but also a co-substrate for the hydroxylation and oxygenolytic ring cleavage of the aromatic ring, whereas second is in the absence of oxygen, primarily based on reduction reactions.

However, bacteria tend toward aerobic degradation of PAHs through oxygenase-mediated pathway. Figure 5.1 shows the main steps in this pathway, which are the oxidation of benzene ring by dioxygenase enzymes to form *cis*-dihydrodiols. Then dihydrodiols are dehydrogenated to dihydroxylated intermediates by the action of a dehydrogenase, which are metabolized to carbon dioxide and water via catechols (Cerniglia and Yang 1984; Mallick et al. 2011). There is a large range of bacteria that are able to oxidize PAHs using dioxygenase enzymes such as bacteria belonging to the genus *Pseudomonas* and *Rhodococcus* (Bamforth and Singleton 2005).

A small number of bacteria such as *Mycobacterium* sp. are able to oxidize PAHs to trans-dihydrodiols using the cytochrome P450 monooxygenase enzymes (Moody et al. 2004) (Fig. 5.1). Rockne and colleagues showed the ability of marine methanotrophs in degrading PAHs via the action of the methane monoxygenase gene (Rockne et al. 1998). Many studies have investigated the naphthalene-degrading bacteria due to their widespread presence in the environment (Cerniglia 1984; Peng et al. 2008; Mallick et al. 2011; Ghosal et al. 2016). One of the bacterial species is *Pseudomonas* that has been reported as the principal degrader of naphthalene. Along with *Pseudomonas*, the sphingomonad genera, principally

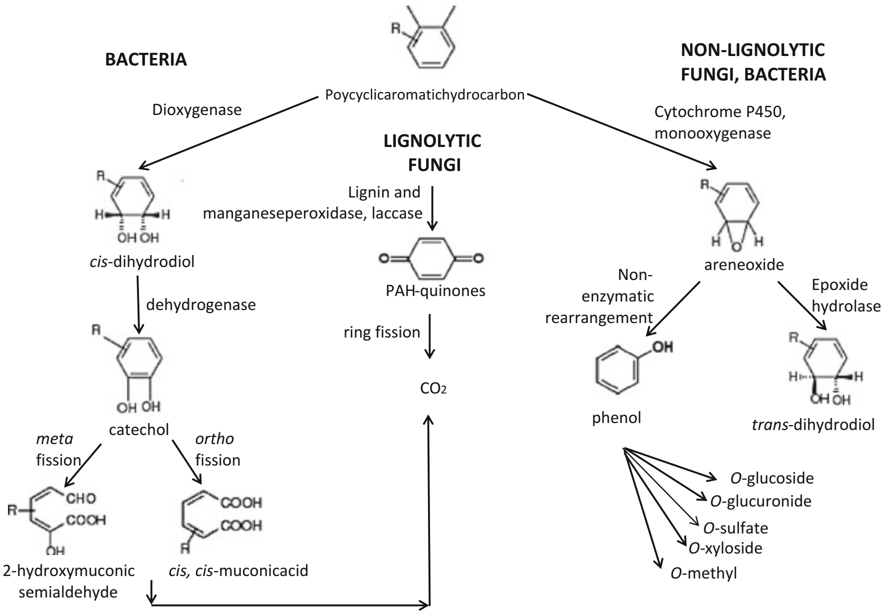


Fig. 5.1 Summary of polyaromatic hydrocarbon main degradation pathways by bacteria and fungi (Bamforth et al. 2005)

Sphingomonas, *Sphingobium*, and *Novosphingobium*, have been presented as important aromatic degraders including high molecular weight PAHs (Ghosal et al. 2016).

Along with naphthalene, numerous reports have been also investigating phenanthrene degradation by different bacterial strains (Peng et al. 2008; Mallick et al. 2011). Mallick et al. (2007) revealed the degradation of phenanthrene by S*taphylococcus* sp. (Mallick et al. 2007). Later on, Ghosal et al. (2010) studied the degradation of phenanthrene, which was used as a sole source of carbon and energy by *Ochrobactrum* sp. strain isolated from municipal waste-contaminated soil (Ghosal et al. 2010). Romero et al. (1998) have claimed that *Rhodotorula glutinis* and *Pseudomonas aeruginosa* are the chief microorganisms using phenanthrene and that degradation of this pollutant correlated directly to their density and biomass growth. The same author found that high concentrations of phenanthrene were totally removed in 30 days by the strain *Pseudomonas aeruginosa* isolated from a stream deeply contaminated by petroleum.

Other low molecular weight PAHs such as anthracene, fluorene, acenaphthene, and acenaphthylene have been also reported to dominate in polluted sites and used by different bacterial strains as sole carbon and energy sources (Moody et al. 2001; Peng et al. 2008; Mallick et al. 2011). Ghosal et al. (2013) reported the assimilation of acenaphthene and acenaphthylene by *Acinetobacter* sp. AGAT-W isolated from municipal waste-contaminated soil sample using acenaphthene as a sole source of carbon and energy (Ghosal et al. 2013). High molecular weight PAHs, such as pyrene, benzo[a] pyrene, and benzo[a]anthracene, are of significant environmental concern due to their high toxicity, persistence, and mutagenic and carcinogenic effects (Cerniglia 1984; Kanaly and Harayama 2000; Peng et al. 2008). Biodegradation of these classes of PAHs have been studied and discussed adequately in the previous reports as well as pathways (Kanaly and Harayama 2000; Peng et al. 2008). Nevertheless, there are still some areas to be exploited such as the regulatory mechanism, biodegradation of a mixture of high molecular weight PAH with other hydrocarbons, and the interactions of complex microbial community during higher degradation. The outcome of these investigations will facilitate the environmental fate of these recalcitrant compounds and help in the development of practical as well as cost-effective bioremediation process.

5.4.2 Fungal Degradation

Numerous fungi have been extensively studied for their degradation capability of PAHs. Most fungi are not able to use PAHs as sole source of carbon and energy. Nevertheless, they may co-metabolize PAHs to different oxidized products and sometimes to CO_2. The enzymes that are used by fungi for the degradation of PAHs are principally monooxygenases (Cerniglia and Sutherland 2010; Kadri et al. 2017). Yet, the pathways used by fungi for PAH degradation are different and depend on the particular fungal species and on the environmental conditions. The chief fungi known for PAH degradation are divided into two groups. The first group is the white-rot fungi that have been thoroughly investigated by the researchers (Cajthaml et al. 1997). These ligninolytic fungi produce non-specific extracellular enzymes with very low substrate specificity, which are mainly lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), phenoloxidases (lacases, tyrosinases), and H_2O_2-producing enzymes that are able to degrade the irregular structure of lignin present in wood and other organic compounds. These ligninolytic enzymes have been found to carry out a one electron radical oxidation, producing cation radicals from pollutants through appearance of quinines (Vyas et al. 1994).

The second group is the non-ligninolytic fungi. They produce cytochrome P450 monooxygenase (Cerniglia and Sutherland 2001; Marco et al. 2015; Reyes-Cesar et al. 2014). Among numerous white-rot fungi, *Phanerochaete chrysosporium*, *Bjerkandera adusta*, and *Pleurotus ostreatus* have been extensively studied. These fungi can produce both ligninolytic and non-ligninolytic enzymes; however, it is still unclear to what degree each enzyme is involved in PAH degradation (Bezalel et al. 1997). On the other hand, another type of ligninolytic fungi, known as brown-rot fungi, represent another class of ligninolytic fungi known to degrade hemicellulose and cellulose by producing hydrogen peroxide. Very few studies have investigated degradation of PAHs, such as phenanthrene, fluoranthene, and fluorine with some brown-rot fungi species, such as *Sulphureus* sp. and *Flammulina* sp. (Sack and Gunther 1993; Cerniglia and Sutherland 2010).

The most abundant fungi in polluted environments are yeasts (Haritash et al. 2009), and they can oxidize PAH with alternative carbon sources. In fact, the degradation rate of phenanthrene by yeast *Rhodotorula glutinis* was studied and was found to be nearly equal to the degradation rate by the bacteria *Pseudomonas aeruginosa* (Romero et al. 1998). Figure 5.1 shows the pathways used by non-ligninolytic fungi and ligninolytic fungi to metabolize PAHs. The non-ligninolytic fungi oxidize aromatic ring using cytochrome P450 monooxygenase enzyme to produce an arene oxide (Sutherland et al. 1995). The arene oxide is later hydrated to a *trans*-dihydrodiol through an epoxide-hydrolase (Bamforth and Singleton 2005). Phenol derivatives may also be produced via nonenzymatic rearrangement of the compound; despite the incomplete PAH mineralization by non-ligninolytic fungi, PAH conjugates that are produced usually have less toxicity and are more soluble.

In fact, Pothuluri et al. (1992) studied the degradation of fluoranthene using *Cunninghamella elegans*, which is a non-ligninolytic organism, and reported that the produced metabolites such as 3-fluoranthene-β-glucopyranoside, 3-(8-hydroxy-fluoranthene)-β-glucopyranoside, fluoranthene trans-2,3-dihydrodiol, and 8-hydroxy-fluoranthene-trans-2,3-dihydrodiol had no mutagenic effect when applied on a rat liver homogenate fraction (Pothuluri et al. 1992). Figure 5.1 also shows the degradation pathway of PAHs using white-rot fungi. As mentioned earlier, these microorganisms oxidize PAH ring by generating free radicals such as hydroxyl free radicals by donating one electron. This generates a selection of PAH quinones and acids. Degradation studies of the ligninolytic fungi have shown that PAHs may be degraded by combining ligninolytic enzymes, cytochrome P450 monooxygenases, and epoxy dehydrolases. The compound can be completely mineralized (Bamforth and Singleton 2005).

5.5 Anaerobic Degradation

The microbial communities in contaminated environments, i.e., soils, aquifers, sediments, and groundwater, may exist under the anaerobic conditions. Relatively recently, it was discovered that PAHs undergo biotransformation in such conditions (Bamforth and Singleton 2005). The detailed data about the exact degradation pathways involved enzymes and/or genes of catabolic mechanisms, are still very scarce, and were reviewed by Widdel and Rabus 2001, Meckenstock et al. 2004, Foght 2008, and Carmona et al. 2009. In the aerobic environment, the oxygen is final electron acceptor and a co-substrate that is involved in the cleavage of aromatic ring. However, when the molecular oxygen is not present, there is a need for alternative electron acceptors, i.e., manganese, nitrate, sulfate, ferrous iron, or carbon dioxide, to oxidize the aromatic molecules (Foght 2008). However, each electron acceptor conserves the different yields of energy. Nitrate, followed by ferric ion, exhibits the greatest energy conservation (Carmona et al. 2009). The sulfate as final electron acceptor has limited ability to conserve the energy (Widdel and Rabus 2001; Carmona et al. 2009).

The PAHs that contain polar substituent, e.g., naphthol, can be degraded under the methanogenic conditions (Mihelcic and Luthy 1988). The biodegradation of aromatic compounds depends on the redox potential of electron-accepting system; thus there is a vast diversity of biochemical strategies among anaerobic microorganisms. For instance, Rockne et al. (2000) isolated three bacterial cultures from a highly enriched denitrifying consortium (Rockne et al. 2000). Two of the obtained strains transformed 70 to 90% of naphthalene, wherein the process was nitrate dependent (Rockne et al. 2000). The first strain was related to *Pseudomonas stutzeri* and was able to oxidize naphthalene by coupling it with denitrification. The second strain was related to *Vibrio pelagius* and reduced NO_3^- to NO_2^-. Moreover, these cultures led to partial mineralization of naphthalene (17% transformed to CO_2) compared to nearly complete mineralization of phenanthrene (96%) (Rockne and Strand 2001). Langenhoff et al. (1996) investigated the degradation of hydrocarbons, including naphthalene under different anaerobic conditions (Langenhoff et al. 1996). The most efficient degradation was under sulfate-reducing conditions, where the complete removal occurred after 100 days of the experiment. In the manganese-reducing column, the degradation varied between 10% and 60% during first 300 days. The analogous situation occurred under nitrate-reducing conditions, where the removal efficiency ranged between 10% and 50% during first 200 days. As mentioned earlier, the studies on anaerobic degradation pathways of PAHs are scarce. However, some information has been presented about naphthalene and 2-methylnaphthalene biotransformation, which has been summarized in Fig. 5.2.

First report of experiments carried out with sulfate-reducing freshwater culture suggested that naphthalene was transformed to 2-naphthoic acid via carboxylation (Fig. 5.1) (Meckenstock et al. 2000). However, further studies on N47 culture implied that naphthalene undergoes methylation to 2-methylnaphthalene, where methyl group is a product of acetyl-CoA pathway (carbon dioxide reduction) (Fig. 5.1). Then, 2-methylnaphthalene is oxidized to 2-naphthoic acid or naphthoyl-CoA via addition of fumarate (Safinowski et al. 2006). Moreover, the enzymes responsible for 2-methylnaphthalene degradation to 2-naphthoic acid, i.e., naphthyl-2-methyl-succinate synthase, succinyl-CoA:naphthyl-2-methyl-succinate CoA-transferase, and naphthyl-2-methyl-succinyl-CoA dehydrogenase, were detected in vitro. The genes encoding the responsible enzymes and their regulation mechanism are still to discover.

The other sulfate-reducing marine cultures from *Desulfobacteraceae* family (NaphS2, NaphS3, and NaphS6) were able to degrade naphthalene, 2-methylnaphthalene, and 2-naphthoate to CO_2 (Carmona et al. 2009). The *nmsA* gene encoding 2-naphthylmethyl-succinate synthase from all three strains was sequenced. The protein exhibited 50% identity with the large subunit of benzyl succinate synthase (enzyme activating toluene degradation). It also includes the consensus radical-bearing glycine (Musat et al. 2009). The absence of nmsA in naphthalene-grown cells indicates that degradation via 2-methylnaphthalene does not occur in the marine strains. The genome sequencing of NaphS2 and gene

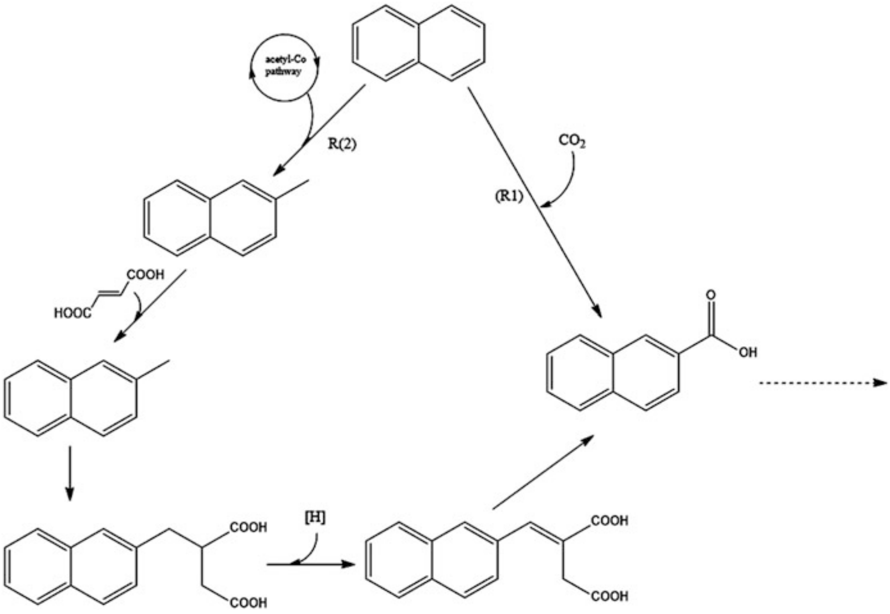


Fig. 5.2 Schematic pathway for anaerobic naphthalene degradation. (R1) naphthalene transformation to 2-naphthoic acid via carboxylation, (R2) naphthalene methylation to 2-methylnaphthalene (carbon dioxide reduction), then it is oxidized to 2-naphthoic acid. Then the ring cleavage occurs. Based on (Safinowski and Meckenstock 2006; Meckenstock et al. 2000)

expression/proteomic analysis showed that it transforms naphthoyl-CoA in a manner analogous to benzoyl-CoA degradation (DiDonato et al. 2010). The *nmsA* genes were not upregulated during naphthalene-grown cells of NaphS2, when three genes encoding possible aromatic carboxylases were regulated. It indicated that naphthalene degradation might occur via carboxylation.

5.6 Factors Influencing the Biodegradation of PAHs

The aim of bioremediation is to apply it to the real environment. However, these conditions cannot be regulated as they are constantly changing. This can affect the microbial communities and alter their efficiency to degrade PAHs in soil and water. The most common factors influencing the microorganisms and their ability to transform PAHs are presented henceforth.

5.6.1 Temperature

Temperature is a crucial factor influencing the growth and metabolism of microorganisms. Most of the PAH degradation studies were conducted in ambient temperature. However, PAHs can be detected in aquatic or soil samples throughout the world (Huntley et al. 1993; Masih and Taneja 2006), where temperature changes according to atmospheric or climatic conditions. Psychrophilic organisms live in low temperatures, where thermophiles exist in higher temperatures. In moderate temperature, the mesophilic organisms grow. Hence, it must be taken under consideration, which kind of microorganism has to be applied.

The optimal temperature must be also attained for the enzymatic reactions. The most common enzymes degrading PAHs are oxygenases, dehydrogenases, and ligninolytic enzymes, i.e., lignin peroxidase, laccase, and manganese peroxidase (Bharagava et al. 2009; Haritash et al. 2009). However, they are active in different temperature range. Most of the enzymes are active in moderate temperatures, but they lose their activity with increase and/or decrease of temperature. For instance, laccase exhibits 30% its activity at 5 °C and 31% at 75 °C, when its optimum is at 45 °C. The enzymes can also be active in extreme temperatures. The fungal laccase from *Marasmius quercophilus* has its optimum at 80 °C, when Mn-dependent peroxidase is active even at 75 °C (Haritash et al. 2009).

With the temperature increase, the PAH solubility also increases due to partition coefficient decrease (20–30% decrease per 10 °C rise between 5 and 45 °C) (Lüers and Hulscher 1996). Hence, there are more easily accessible for microorganisms with the temperature increase (Ghosal et al. 2016). Furthermore, the dissolved oxygen concentration decreases with the temperature decrease, which can affect the activity of aerobic organisms. However, at high temperature, some of the PAHs may undergo transformation, which may form more toxic or persistent by-products than the parent compound (Müller et al. 1998). Furthermore, with the increase of the temperature, the enhanced mass transfer is observed in soils due to proportional dependence of diffusion coefficient and inversely proportional dependence of the partition coefficient (Farrell and Reinhard 1994). Studies on the effect of the short-term heating on following PAH biodegradation showed that heating at 120 °C, 1 h, improved their degradation efficiency after 21 days from 9.5% to 27% (Bonten et al. 1999).

5.6.2 pH

Similar to temperature effects, the microorganisms are pH-dependent. Most of them favor circumneutral conditions. However, due to PAH contamination, the pH may change. For instance, the presence of coal spoil in soil induces the acidification of the environment due to sulfide oxidation (Ghosal et al. 2016). The leakage from demolition waste may lead to pH increase of the soil. Thus, the pH adjustment of treated soil is recommended; the alkaline soil may be treated with ammonium nitrate or sulfate, when the application of calcium or magnesium carbonate may be a solution for acidic soils.

5.6.3 Oxygen

The presence of oxygen significantly influences the biodegradation. PAH removal may be proceeded under aerobic and anaerobic conditions. The anaerobic degradation is slower than the aerobic degradation, and its exact pathway is not as well studied. As mentioned earlier in Sect. 4.1., with decrease in oxygen concentration, the activity of aerobic microorganisms decreases as well (Liu et al. 2014). The mono- and dioxygenase, the crucial enzymes in aerobic degradation, require oxygen for the initial oxidation of PAH rings. Moreover, it was reported that some of the sediments, such as mangrove environment, may have low oxygen concentration (around 1 mg l^{-1} dissolved oxygen) due to tidal cycle (Li et al. 2011). The degradation under such conditions has the same pathway as under anaerobic conditions. With such low oxygen level and in the presence of manganese (IV), oxygen consumption was lower due to the influence of the inoculation of the enriched bacterial consortia and the amendment of manganese (IV). Another study by Hurst et al. (1996) investigated the influence of oxygen tension on the biodegradation of PAHs in contaminated soil (Hurst et al. 1996). Even at low oxygen level (2%), the transformation of ^{14}C-pyrene led to mineralization. Moreover, there was no significant difference in the degradation level between the lowest (2%) and the highest (21%) oxygen concentration. However, under anaerobic conditions, the degradation of ^{14}C-pyrene could be neglected.

5.6.4 Bioavailability

The effectiveness of bioremediation is affected by the ability of microbes to access the compounds. Their bioavailability depends on mass transfer parameters, which is controlled by various physicochemical factors, i.e., diffusion, sorption, desorption, dissolution, and biological factors like metabolism and uptake (Bosma et al. 1997). Mass transfer is usually described as desorption kinetics. In case of PAH desorption, it is considered as biphasic, where a rapid release of labile fraction is followed by slow release of resistant fraction (Chai et al. 2006). PAHs tend to sorb onto various surfaces and materials, i.e., clays, humic acids, and black carbon, which significantly reduce their availability (Benhabib et al. 2010; Rein et al. 2016; Liu et al. 2009). The decrease of aquatic solubility of PAHs, with their increase of molecular weight, also hinders their bioavailability. Moreover, the process of aging of contaminants also reduces the bioremediation rate. The long sorption onto soil particles results in lowering PAH chemical and biological extractability (Ghosal et al. 2016).

On other hand, the addition of bacteria-producing biosurfactants such as *Pseudomonas aeruginosa* can enhance the PAH bioavailability. Surfactants may increase PAH concentration in system's aqueous phase (Haritash et al. 2009). Furthermore, the addition of light oils also enhances PAH degradation. For instance, *Sphingomonas paucimobilis* is able to co-metabolically degrade PAH due to inorganic nutrients obtained from these oils (Haritash et al. 2009).

5.6.5 Nutrients, Substrates, and End Products

Each microorganism needs the suitable nutrients for cellular growth and metabolism. The highest requirement is for compounds containing carbon. The study of Lee et al. (2003) showed that the addition of pyruvate as a second carbon source stimulated *Pseudomonas putida* G7 growth and enhanced the adaptation of bacteria to degrade naphthalene (Lee et al. 2003). The PAHs in the environment are present as a mixture. The presence of different substrates determines the degradation pathway. Hence, there is a possibility that the other carbon source may inhibit the PAH degradation due to diauxic growth. For instance, it was noticed that the degradation of PAHs with low molecular weight occurs after the degradation of PAHs with higher molecular weight (Ghosal et al. 2016). The competitive mechanism of PAH degradation between naphthalene/phenanthrene and fluorene/phenanthrene was also observed (Stringfellow and Aitken 1995). The degradation in binary and tertiary mixtures as well as in sole-substrate system of naphthalene, phenanthrene, and pyrene was also investigated (Rodríguez and Bishop 2005). The PAH (3,4-rings) removal efficiency of binary systems was 87.9% and 70.1% with acetate and naphthalene as supplements.

On the other hand, the biodegradation of pyrene was hindered by phenanthrene presence in tertiary system. In sole-substrate system, the phenanthrene and pyrene were not degraded. Other than the carbon source, microorganisms need other minerals such as phosphorous, nitrogen, or iron, which are essential to grow and metabolize. Hence, in the contaminated sites, which exhibit poor nutrient content, the biodegradation is limited. For instance, the marine systems have low level of phosphorous and nitrogen. The supplementation of such sites is required to enhance the degradation. However, there are some studies showing that the high nutrient level also may hinder the bioremediation of PAHs (Ghosal et al. 2016). In addition, it should be also considered that transformation products may be more toxic than the parent compounds. It is known that some of the oxygenated PAHs are as toxic as their parent compounds or even more toxic (Chibwe et al. 2017). The biodegradation of targeted PAH may not be complete, which results in accumulation of toxic compounds.

5.7 Conclusion

Usage of microorganism to degrade the toxic and persistent compounds in environment is an eco-friendly and cost-effective manner of dealing with those contaminants. Multifarious methods have been tested for their efficiency of PAH biodegradation. The bacterial and fungal cultures were studied for their degradation pathway, enzyme usage, and even gene regulation. In recent years, the potential of anaerobic degradation was also investigated to increase the potential of green methods in different environmental compartments, where the oxygen concentration is insignificant, i.e., soils or sediments. Thus, the adaptation of microorganisms to contaminated sites has a crucial part in increasing biodegradation efficiency. The use of suitable microorganisms regarding pH, temperature, nutrient content, or PAHs present in the environment is also the important part of degradation system designing. The possibility of toxic by-product formation also should be taken under consideration. To decrease the accumulation of biotransformation products and to avoid their management, the most efficient biodegradation is the one that leads to mineralization of the compound.

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Chapter 6
Splendid Role of Nanoparticles as Antimicrobial Agents in Wastewater Treatment

Viabhav Kumar Upadhayay, Amir Khan, Jyoti Singh, and Ajay Veer Singh

Abstract In modern era, the treatment of wastewater requires other advanced technologies for offering clean water supply. Treatment of wastewater is requisite, as the untreated wastewater is a common source of chemical pollutants and microbial pathogens and causes contamination of surrounding water and land sources. The science of 'nanotechnology' can present excellent potential in advancement of wastewater treatment methods for safer disposal or further utilization of treated wastewater. Development and synthesis of nanoparticles and other nanomaterials and determining their efficacy for removal of aquatic pollutants are burning areas of research in nanoscience world. The unique physiochemical properties of nanoparticles present wide-spectrum antimicrobial activities for eliminating risks of waterborne diseases. These particles inactivate microbial pathogens through showing broad arrays of mechanisms of antimicrobial actions. Nanoparticles also remove other inorganic and organic sources of contaminants from wastewater and exhibit their potential use in wastewater management. This chapter focuses on basic introduction of nanoparticles and their possible application as disinfectants in wastewater treatment processes.

Keywords Nanoparticles · Antimicrobial agents · Wastewater · Microbial pathogens · Treatment

6.1 Introduction

Water is an immense gift from nature on planet earth which fulfilled the basic need of daily lives from smallest entities such as microorganisms to higher animals and plants. It provides the basic foundation for food, nutritional and environmental

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security and secures the sustainability of plants and regulates diverse processes of life. Therefore it becomes more important to improve and preserve water quality in a better way, as more global water resources are experiencing arrival of contaminated water from several anthropogenic sources and commencing the problem of water pollution. Effluents from industrial, domestic and other activities create the resources of wastewater comprising of several organic and inorganic compounds, especially heavy metals, and living entities in the form of pathogenic microorganisms engender the risk of human health hazards. If untreated wastewater discharged into streams or river or the surrounding environment, then it may cause contamination of surrounding surface water and groundwater resources.

The water for drinking purposes should be free from faecal and total coliform as suggested by the World Health Organization (WHO) (WHO 2012); otherwise the risk of waterborne disease outbreaks can set forth various kinds of illnesses globally. For providing clean and affordable water, it is required to treat the wastewater for safe discharge or its further use; to overcome this issue, advanced and innovative technologies are needed to maintain healthy life. Global natural water resources are not available for longer time, so conservation of natural resources by means of the remediation of contaminated water and recycling methods is the alternative way of receiving fresh water in the coming periods. Therefore, there is a necessity for the development of an appropriate, low-cost and rapid wastewater treatment technique.

In modern time a number of techniques such as chlorination, distillation, boiling, activated carbon treatment, low-frequency ultrasonic irradiation, water sediment filters, reverse osmosis, etc. are used for water treatment (Samanta et al. 2016). There are certain advanced levelled technologies being used for wastewater treatment such as chemical oxidation, Fenton and photoprocesses, supercritical water oxidation, ultraviolet (UV)-based processes, photocatalytic redox processes, sonolysis, and electron beams and γ-ray irradiation (Choi et al. 2010). One more effective and extraordinary advanced technology includes the nanotechnology, which provides an opportunity of an effective exclusion of contaminants and offers opportunities to develop next-generation water supply systems. This prominent technology uses several types of nanoparticles, nanomembrane and nano-powder for treatment of surface water, groundwater detection and removal of chemical substances such as toxic heavy metals (e.g. Cd, Cu, Pb, Hg, Ni, Zn), biological substances including certain germs (viruses and bacteria) and organic and inorganic materials (Theron et al. 2008).

Nanoparticles are described to have high specific surface area and additionally contain unique adsorption capacity because of various types of distribution of disordered surface sites and reactive surface sites (Samanta et al. 2016). Besides possessing large surface area, nanoparticles exhibit unique characteristics including catalytic potential and high reactivity potential, and this trait makes them as potential adsorbing materials over conventional materials (Santhosh et al. 2016). Nanomaterials can be employed in water and wastewater treatment facility in both cost-effective and energy-effective manner (Hossain et al. 2014). Application of nanomaterials as adsorbent, photocatalyst and antimicrobial agents in wastewater treatment is useful as they remove the aquatic pollutants (Santhosh et al. 2016). Wastewater is a hub for various pathogenic microorganisms such as bacteria (e.g. *Salmonella* spp., *Escherichia* spp., *Shigella* spp., *Pseudomonas* spp., *Listeria* spp., etc.), virus (norovirus, hepatitis A, rotavirus, etc.) and protozoans (*Cryptosporidium parvum*, *Cryptosporidium hominis* and *Giardia duodenalis*), and wastewater-associated microorganisms cause gastrointestinal infection (Paillard et al. 2005; Li et al. 2009; Cai and Zhang 2013; Chahal et al. 2016).

Therefore, the application of nanoparticles (e.g. nano metal oxides, carbon nanotubes (CNTs), fullerene nanoparticles, dendrimers, zerovalent iron (FeO/ZVINP)) is increased to inactivate microorganisms for disinfecting the water. The present article outlined the brief introduction of nanoparticles and their application for pathogen inactivation in wastewater and removal of a number of organic and inorganic contaminants to deal with wastewater treatment challenges.

6.2 Types and Sources of Wastewater

Wastewater can be explained briefly as ‘any storm water runoff, or sewage of different systems (industrial, domestic or commercial) or any combination of these carried by water’. Anthropogenic and industrial activities determine the type and volume of wastewater, discharge pattern and chemical status of treated waste matter. However, the wastewater has been broadly categorized into four main types such as agricultural, domestic, industrial and urban. Many agricultural activities from the surrounding farm’s processes generate wastewater which can contaminate groundwater, while the urban wastewater is considered as the combination of wastewater from industrial, domestic and surrounding sewage and rainwater too (Naidoo and Olaniran 2013). More emphasis has been given to domestic and industrial sewage as a source of pollutants, but the attention on agricultural runoff has been increased, as it is expected to contain high level of residues of chemical fertilizers and pesticides, which can cause threat of health hazards and problem of eutrophication (Department of Water Affairs; Pretoria, South Africa 2011).

Domestic wastewater originated from household activities and is a mixture of grey and black water sources. However, the industrial wastewater is consisting of industrial wastes including runoff of pulp, petrochemical, pharmaceuticals and food industry as well as different chemicals, salts and acids. Moreover, the industrial wastewater contains wide arrays of heavy metals which are responsible for causing threat to environment and specially show toxicity towards human and animals. The runoff of surrounding industries together determines the composition of industrial wastewater encompassing broad range of inorganic and organic sources of chemical pollutants or contaminants (Naidoo and Olaniran 2013).

6.3 Microbial Pathogens in Wastewater

Wastewater originated from different sources contains a variety of pathogenic microorganisms and causes health-associated risks. Both cellular microorganisms (bacteria, protozoans) and noncellular microorganisms (viruses) present in wastewater are also the human pathogens. Predominately, the enteric pathogens enter into wastewater through faecal materials and pose threat of various ailments in humans. *Escherichia* spp., *Salmonella* spp., *Shigella* spp., *Vibrio cholera* and *Yersinia* spp. are important organisms which come into the category of enteric pathogens and are found in wastewater and may cause gastrointestinal infections such as dysentery, diarrhoea and others including typhoid, enteritis, legionellosis, melioidosis and stomach ulcer. Moreover, other ailments caused by bacteria in wastewater include respiratory infections (*Legionella pneumophila* and *Mycobacterium avium*), leptospirosis (*Leptospira*) and wound infections (*Pseudomonas aeruginosa*) (Chahal et al. 2016). *Listeria monocytogenes* became the emerging wastewater pathogen which caused ‘listeriosis’ outbreaks associated with wastewater globally (Paillard et al. 2005).

Other noncellular organisms are viruses which are abundantly found in wastewater and are associated with several diseases. Important viral pathogens present in wastewater are enteric viruses (norovirus, hepatitis A, rotavirus, astroviruses and adenoviruses) and the different enteroviruses (Cai and Zhang 2013). Enteric viruses can be responsible for a variety of ailments in humans including gastroenteritis, hepatitis, myocarditis and meningitis (Chahal et al. 2016). Protozoans, namely, *Cryptosporidium parvum*, *Cryptosporidium hominis* and *Giardia duodenalis*, are commonly associated with wastewater and are considered as intestinal parasites infecting both animals and humans (Li et al. 2009). The elimination of above-mentioned pathogens with other toxic materials during treatment process is necessitated before discharging the treated effluent in rivers or stream or reusing the treated water.

6.4 Modern Approaches in Wastewater Treatment

Reclamation of wastewater is essential for elimination of several hazardous compounds and microbial pathogens and exploitation of treated wastewater for further use. For removal of hazardous and toxic materials, biomolecules, chemical compounds and microorganisms from wastewater, a number of physical, chemical and biological strategies are employed. However, the development of high-throughput research gave other advanced approaches for wastewater treatment with higher efficiency. Numerous massive approaches such as membrane bioreactors, micro- and ultrafiltration, nanofiltration and nanotechnology are common in modern time for providing clean water. Membrane bioreactor is an integrated system for the biodegradation of waste materials with membrane filtration and shows the traits of effective removal of organic, inorganic and biological materials from wastewater. It is an efficient and reliable technology and an alternative practice over the conventional activated sludge processes and also considered as better option for industrial and domestic wastewater treatment (Le-Clech et al. 2006).

Technologies encompassing with microfiltration and ultrafiltration provide advanced level of water treatment facility. Both microfiltration and ultrafiltration are helpful in elimination of a broad range of contaminants including suspended solids and microorganisms (Waeger et al. 2010). Moreover, the technique of membrane filtration as a replacement process is employed for gravitational sedimentation primarily in conventional biological process and as well in advanced processes for secondary treated wastewater. Using of nanofiltration has been increased in modern time, and it became a more advanced approach for treating wastewater over the reverse osmosis (RO) as it shows the traits of lower-energy consumption and high flux rate (Shon et al. 2013). Nanofiltration and reverse osmosis are profoundly used in eradication of minute-sized organic molecules and ions (such as ammonia) too (Abdel-Fatah 2018). Furthermore, nanotechnology made more advancement in treatment efficacies of wastewater through the application of nanomaterials, and it has been fruitful in supplying satisfactory amount of water from safe use of various nonconventional water sources.

6.5 Nanotechnology: An Advanced Prospective in Wastewater Treatment

For maintaining and providing better quality of water, various approaches are employed. Among traditional approaches, activated carbon adsorption, solvent extraction and common chemical oxidation are very common, and moreover these techniques are not economically reasonable, and therefore cannot be implicated in routine way. On the other hand, biological ways for wastewater treatment are very common and environment-friendly practice for the wastewater treatment plants, but they are costlier and laborious approach (Ahluwalia and Goyal 2007). Drawbacks to being cost-effective and time-consuming, the biological ways for wastewater treatment cannot be the foremost option that could reduce the concentration of environmental pollutants rapidly.

Therefore, to eradicate the environmental toxic contaminants, an efficient and rapid practice is required that could remove toxicants to a safer level within a feasible cost. A special branch of science, ‘nanotechnology’ is an emerging area primarily focused on production and development of novel ‘nanomaterials’ having increased affinity, high capacity and improved selectivity for several heavy metals and other environmental contaminants. The following important features including enhanced reactivity, high surface area to volume ratio and unique physicochemical properties augment the applicability of nanomaterials (Kim et al. 2007). Escalating disease burdens due to microbial pathogens worldwide, different tactics have been explored for challenging the waterborne diseases and elimination of microbial pathogens during wastewater treatment. In modern scenario using nanoscale materials as 'potential antimicrobial agents' is (Morones et al. 2005; Kim et al. 2007) increased, and in the near future, this approach could be a potential strategy over other classical antimicrobial drugs.

Considering water treatment the modern researches have been focused on the following four stipulated areas such as (a) adsorptive exclusion of pollutants or toxicants; (b) degradation of pollutants; (c) disinfection and microbial control; and (d) membrane filtration (Li et al. 2008). High surface area and increased specificity are the important characteristics which make nanomaterials as 'superabsorbent'. For instance, the magnetic nano-adsorbents show high adsorptive efficiency for eliminating heavy metals (e.g. arsenic) and radionuclides from water. Moreover, the superparamagnetic traits of nano-magnetite permit separation in low magnetic fields to facilitate recycling and reuse (Mayo et al. 2007). Catalytic and photocatalytic properties are also present in numerous nanomaterials, and such properties impart benefits for nanomaterials to carry out oxidative or reductive degradation of chemical pollutants such as degradation of pesticides, antibiotics, pharmaceutical compounds and other recalcitrant pollutants (Lee et al. 2009).

Moreover, current approaches of using nanotechnology are highly delightful over chemical disinfection processes without releasing harmful by-products. Implication of 'functionalized fullerenes' and 'TiO_2-based nanocomposites' in the presence of visible and UV light presented antibacterial and viral inactivation activity, and this approach was also successful against disinfectant-resistant pathogens such as *Cryptosporidium* and *Giardia* (Dunlop et al. 2002; Hossain et al. 2014). The development of 'superior catalysts' comprising of palladium-coated nanoparticles has been promoted to quick dechlorination of organic solvents such as trichloroethylene (Nutt et al. 2005).

6.6 Nanoparticles: Types and Synthesis

Nanotechnology is plentifully connected with all aspects of science including physics, chemistry, biology, material science and medicine. Moreover, this leading field of science showed various applications in numerous aspects of research and in routine developing areas such as new material design and electronics. The use of nanotechnology in medical research is the fastest area of research where development and exploration of nanoparticles or metal-based nanoparticles are in infancy. There are numerous ways for synthesizing nanoparticles, and these ways are classically categorized into three groups: (a) chemical, (b) physical and (c) biological. Among these chemical ways are rapid for making high amounts of nanoparticles, but releasing certain toxic chemicals depicts non-eco-friendly behaviour. On the other hand, physical method employing nanoparticle synthesis is usually costly and requires complex equipment. Therefore, the biological ways are another important route for preparation of nanoparticles. Such kind of green synthesis of nanoparticles is a valuable approach due to low-cost preparation and less toxicity.

6.6.1 Classification of Nanoparticles

Nanoparticles are categorized into three groups such as inorganic nanoparticles, organic nanoparticles and carbon-based nanoparticles (Ealia and Saravanakumar 2017) (Fig. 6.1).

Inorganic nanoparticles are mainly metal and metal oxide based and find their applications in various aspects of scientific research. Almost all the metals can be processed into their nanosized materials through either destructive or constructive methods (Salavati-niasari et al. 2008). Aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag) and zinc (Zn) are the common examples of metals frequently employed in synthesis of nanoparticles (Ealia and Saravanakumar 2017; Salavati-niasari et al. 2008). On the contrary, the metal oxide-based nanoparticles are prepared to change or modify the activities of their respective metal-based nanoparticles. For instance, in the presence of oxygen, iron (Fe) nanoparticles instantly oxidize to iron oxide (Fe_2O_3) at room temperature; thus modified trait amplifies its reactivity and efficiency compared to iron nanoparticles (Tai et al. 2007). Other commonly synthesized metal oxide-based nanoparticles are aluminium oxide (Al_2O_3), cerium oxide (CeO_2), magnetite (Fe_3O_4), silicon dioxide (SiO_2), titanium oxide (TiO_2) and zinc oxide (ZnO) (Ealia and Saravanakumar 2017).

Organic nanoparticles are another type of nanoparticles and showed tremendous development in the area of research development. Dendrimers, micelles, liposomes and ferritin are generally referred as the organic nanoparticles or polymer, which exhibited eco-friendly traits such as biodegradable and non-toxic. Dendrimers are designed as modified adsorbents showing the capacity of removing both organics and heavy metals. Moreover, the interior shells of dendrimers can be hydrophobic

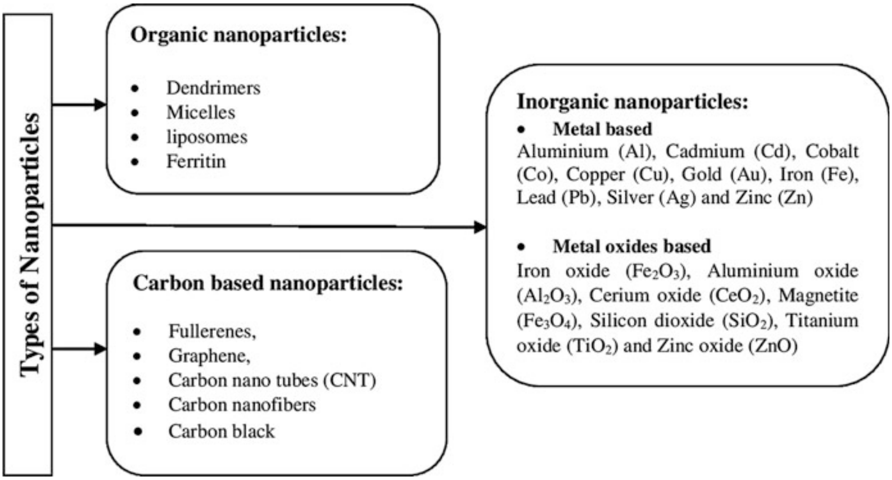

Fig. 6.1 Graphic representation of types of nanoparticles

for sorption of organic compounds, or the exterior branches can be modified (e.g. hydroxyl- or amine-terminated) for adsorption of heavy metals (Crooks et al. 2001). Moreover, several particles such as micelles and liposomes possess a hollow core also called as 'nanocapsules' and show sensitivity towards thermal and electromagnetic radiations such as heat and light (Tiwari et al. 2008). In different sectors of industries such as cosmetics and pharmaceuticals, these molecules act as a carrier, and furthermore in the area of food and farming industries, liposomes are efficiently used for encapsulation and delivering of entrapped unstable compounds (Akbarzadeh et al. 2013).

One more important class of nanoparticles is 'carbon based' which can be further subclassified into fullerenes, graphene, carbon nanotubes (CNT), carbon nanofibers and carbon black and sometimes 'activated carbon in nanosize' (Ealia and Saravanakumar 2017). Fullerenes (buckyballs) are allotropes of carbon existing in various forms such as C_{70}, C_{76}, C_{84} and C_{540} (Powell and Kanarek 2006). Such forms of carbon contain unique properties and wide industrial applications (antimicrobial agent in water treatment, stored hydrogen in fuel cell-powered car and gas adsorption) and thus attract the attention of worldwide researchers (Hossain et al. 2014). 'Graphene' is a hexagonal network comprised of carbon atoms in a two-dimensional planar surface, and the thickness of the graphene sheet is approximately 1 nm.

Carbon nanotubes (CNTs) are important nanomaterials categorized into two types such as 'multiwalled carbon nanotubes' and 'single-walled carbon nanotubes' having diameters of 100 nm and 0.7 nm, respectively (Ealia and Saravanakumar 2017). CNTs have unique physical, chemical and mechanical properties and present massive industrial applications such as its passive or active implication in concrete technology, textiles, conductor and semiconductor technology, solar cell, water treatment, air purification and biotechnology and in area of mechanical engineering and medicine (Hossain et al. 2014). CNTs show high adsorption ability that mainly stems from the huge specific surface region and the varied contaminant-CNT interactions. And the external surface of individual CNTs is the available surface area for the adsorption purpose (Yang and Xing 2010). 'Nanofibers' with core-shell structure show the tailored shell surface chemistry for precise adsorption and reactive core for degradation (Ealia and Saravanakumar 2017).

6.6.2 Synthesis of Nanoparticles

Two important approaches have been suggested for the nanoparticles synthesis such as 'bottom-up' approach and 'top-down' approach. Bottom-up approach is also known as 'constructive method' where the building-up of a structure from atom to clusters to nanoparticles takes place. Several methods including sol-gel, spinning, chemical vapour deposition (CVD), pyrolysis and biosynthesis are used as bottom-up methods for synthesis of nanoparticles. Sol-gel is the most preferred bottom-up method and a wet-chemical process employing chemical solution, which act as precursor for an integrated system of distinct particles. Typically, metal oxides and chlorides are preferred precursors in sol-gel method (Ramesh 2013).

Preparation of nanoparticles by spinning is performed by a spinning disc reactor (SDR), where a rotating disc is dwelled inside the reactor. Nitrogen and other inert gases are filled into the reactor to remove the oxygen (Tai et al. 2007). As the disc rotates at various speeds, it allows precursors and water to pump in, and spinning commences the atoms or molecules to fuse together which are precipitated, collected and dried (Mohammadi et al. 2014). ‘Chemical vapour deposition (CVD)’ is the method for the deposition of a thin film of gaseous reactants onto a substrate, and this process is performed in a reaction chamber at ambient temperature through combining gas molecules. A chemical reaction occurs as a heated substrate comes in contact with the combined gas, and this reaction engenders a thin film of product on the substrate surface that is recovered and used (Ealia and Saravanakumar 2017).

‘Pyrolysis’ is a common practice in industries for large-scale production of nanoparticles. It involves burning a ‘precursor’ either liquid or vapour that is fed into the furnace at high pressure (Kammler et al. 2001). ‘Biosynthesis’ is a green and environment-friendly approach for the preparation of nanoparticles (Kuppusamy et al. 2016), and this approach uses bacteria, plant extracts and fungi along with the precursors for the synthesis of nanoparticles. The second crucial approach of nanoparticle synthesis is ‘top-down method’ or ‘destructive method’ where the reduction of a bulk material to nanometric scale particles takes place. This approach includes mechanical milling, nanolithography, laser ablation, sputtering and thermal decomposition, and these mentioned methods are widely used for the synthesis of nanoparticles. Among these, mechanical milling is utilized for pursuing the purpose of milling and post annealing of nanoparticles during synthesis where various elements are pulverized in an inert atmosphere (Yadav et al. 2012).

‘Nanolithography’ method of ‘top-up approach’ is concerned with the study of fabricating nanometric scale structures with a minimum of one dimension in the size range of 1–100 nm. Optical, electron-beam, multiphoton, nanoimprint and scanning probe lithography are the various aspects of nanolithographic processes (Pimpin and Srituravanich 2012). The selective benefit of nanolithography is to create a cluster with desired shape and size from a single nanoparticle while being a costlier approach, and the need of complex experimental instruments shows its disadvantages (Ealia and Saravanakumar 2017).

Moreover, laser ablation synthesis in solution (LASiS) is a general method for nanoparticle synthesis from various solvents, where laser beam-mediated irradiation of a metal submerged in a liquid solution condenses a plasma plume that formed nanoparticles (Amendola and Meneghetti 2009). ‘Sputtering’ is the deposition of nanoparticles on a surface by ejecting particles from it by colliding with ions (Shah and Gavrin 2006), followed by annealing (Ealia and Saravanakumar 2017). The following parameters such as thickness of the layer, temperature and period of annealing and type of substrate determine the shape and size of the nanoparticles

(Lugscheider et al. 1998). Furthermore, ‘thermal decomposition’ is considered as an endothermic chemical decomposition commenced by heat which breaks the chemical bonds in the compound and produced nanoparticles at specific temperatures (Salavati-niasari et al. 2008).

6.7 Application of Nanoparticles in Pathogen Elimination

Due to rampant escalation of human population, a number of anthropogenic activities and poor water management practices have led to increase chance of waterborne diseases. Wastewater contains a variety of pathogenic microorganisms, and to prevent the disease burden provoked by such pathogens required several other disinfection practices. Multiple types of disinfectants are used widely, but their harmful effects for environment and quick degradation properties make most of them unsuitable for disinfection purpose. An ideal disinfectant must carry the following properties: (a) broad-spectrum antimicrobial activity; (b) no harmful by-products during and after use; (c) no harmful effect on human health; (d) economically feasible and easily applicable; (e) easy to store; (f) highly soluble in water; and (g) amenable to safe disposal (Rutala et al. 2008). Conventional disinfectants, such as chlorine and ozone, can result in the formation of toxic disinfection by-products (DBPs) (such as halogenated disinfection by-products, carcinogenic nitrosamines and bromate) and thus pose a threat to environment and human health.

UV-mediated disinfection aroused as another prominent option for oxidative disinfection because it produces minimum DBPs, though it requires elevated dosage for killing certain viruses such as ‘adenoviruses’. Such limitations provoked for the development of alternative approaches that can improve the strength of disinfection while avoiding DBP production (Qu et al. 2013). Nanotechnology is a promising field of applied science that utilizes the physicochemical properties of nanomaterials as a means to regulate their size, surface area and shape in turn to produce various nanoscale-sized materials. A number of nanomaterials, such as nano-Ag, nano-ZnO, nano-TiO_2, nano-Ce_2O_4, CNTs and fullerenes, depict antimicrobial activities without strong oxidation and thus have minor propensity to produce DBPs (Qu et al. 2013; Hossain et al. 2014). The appropriate application of any nanoparticles should also contain the following features: (1) physical and chemical stability in aqueous phase, i.e., the nanoparticles should not be aggregated and settled, and they must be highly effective and reactive, and (2) if photoexcitation is needed, the nanoparticles must be active in solar light illumination (Hossain et al. 2014).

A remarkable number of nanoparticles such as photocatalytic titanium dioxide (TiO2) (Simon-Deckers et al. 2009), magnesium oxide (MgO) (Huang et al. 2005), calcium oxide (CaO), zinc oxide (ZnO) (Jones et al. 2008), aqueous fullerene nanoparticles (nC_{60}), chitosan (Hossain et al. 2014), nanosilver (nAg) (Sondi and Salopek-Sondi 2004), carbon nanotubes (CNT) (Vecitis et al. 2010), dendrimer

(Tiwari et al. 2008), aluminium oxide (Al_2O_3) (Simon-Deckers et al. 2009) and zerovalent iron (Fe^0/ZVINP) (You et al. 2005) have been proposed to inactivate microorganisms found in drinking water, wastewater, surface water and other different sources (Li et al. 2008). Candidate nanoparticles have a broad range of industrial uses such as water disinfection, food preservation, foods, textiles and medicine. In water, TiO_2 is the most common type of nanoparticle that is used to inactivate pathogens (Hossain et al. 2014). Another very important nanoparticle is 'silver nanoparticles (AgNPs)' having a number of applications, such as sensors, catalysts, anticancer and antimicrobial agents. AgNPs have shown prominent antimicrobial activities against bacteria, fungi and viruses (Kashyap et al. 2012). On the other hand, AgNP synthesis produces toxic waste, such as ammonia, which is harmful for the human health (Shenashen et al. 2014).

Therefore, green synthesis of AgNPs is an environment-friendly approach, and this strategy uses various routes such as plants, microorganisms and other non-toxic materials (Iravani 2011). Silver nanoparticles can improve water quality for high-end use and have also been used in ceramic microfilters where these act as a barrier for waterborne diseases (Peter-Varbanets et al. 2009). ZnO nanoparticles show antimicrobial properties and become fatal to microbial activity into the water after producing ROS (Jones et al. 2008; Hossain et al. 2014). Immobilized ZnO nanoparticles are considered as suitable water disinfectant over the released ZnO/Zn ions, as the Zn ions present toxicity to humans (Hossain et al. 2014).

In water treatment technology, alumina (Al_2O_3) acquired noteworthy attention because of its coagulating property and suitable disinfectant. According to the study of Simon-Deckers et al. (2009), Al_2O_3 was found to inactivate *E. coli* (70% at particle concentration of 500 mg/l). Several traits of nanoparticles such as antimicrobial activities, fibrous shape and high conductivity of carbon nanotubes (CNTs) facilitate novel CNT filters for the eradication of both bacteria and virus. The thin layer of CNTs efficiently eliminates bacteria and virus by size exclusion and depth filtration, respectively (Brady-Estevez et al. 2010). By a tiny intermittent voltage (2e3 V), multiwalled nanotubes (MWNTs) can directly oxidize attached microbial pathogens such as bacteria and viruses and lead to their inactivation just in seconds (Rahaman et al. 2012). Chitosan, a deacetylation derivative of chitin and a degradable biopolymer, showed well-known antimicrobial properties (Hossain et al. 2014).

Rabea et al. (2003) evaluated the minimum inhibitory concentration (MIC) of chitosan against fungi and bacteria and determined that *Corynebacterium michiganense* was most sensitive to chitosan (Rabea et al. 2003). Fe^0 or zerovalent iron nanoparticles (ZVINPs) also showed numerous promising uses such as (a) permeable reactive barrier for groundwater remediation, (b) water and wastewater treatment and (c) hazardous waste remediation and pollution control (Hossain et al. 2014). ZVINPs inhibit bacterial cells through production of oxidants either on surface or intracellular and thus block the activation of microbial cells (Lee et al. 2008). ZVINPs were also found to inactivate waterborne viruses such as MS2 and ϕX174 with higher efficiency (You et al. 2005).

6.8 Mechanism of Nanoparticles for Killing the Microbial Pathogens

It is widely accepted that most of the nanoparticles have shown broad-spectrum antimicrobial activity with lower or no toxicity towards humans, and in modern days the application of nanoparticles has been the promising choice for water disinfection. Various mechanisms are adapted by nanoparticles for demolishing or deactivating both cellular (bacteria, fungi and protozoa) and noncellular organisms (viruses). The mechanisms of antimicrobial actions of nanoparticles are schematically represented in Fig. 6.2.

Microbial inactivation depends on the number of factors such as amount/concentration of nanoparticles, light wavelength and its intensity, pH, degree of hydroxylation, temperature, oxygen availability and ROS (Markowska-Szczupak et al. 2011). TiO_2 particle can inactivate the cellular architecture of microorganisms under UV/solar irradiation by forming few reactive oxygen species (ROS) namely 'hydroxyl radical ($^{\bullet}OH$)', 'superoxide radical ($O_2^{\bullet -}$)' and 'hydrogen peroxide (H_2O_2)' (Huang et al. 2005; Li et al. 2008). Virus inactivation was depending on free •OH on surface, while ROS was a secondary path. Hydroxyl radicals and ROS inactivate the capsid proteins of virus and disintegrate the DNA or RNA through fragmentation (Hossain et al. 2014). MgO can inactivate microorganisms by the production of O2•− on its surface, and the inactivation of microbial pathogen might depend on a number of factors such as contact of MgO particles with microorganisms, alkaline condition of the particle surface, particle specific surface area, duration of contact and accessibility of oxygen on surface. MgO nanoparticles produce 'ROS' which generally attacked carbonyl group of peptide linkages and ultimately promoted the process of protein degradation.

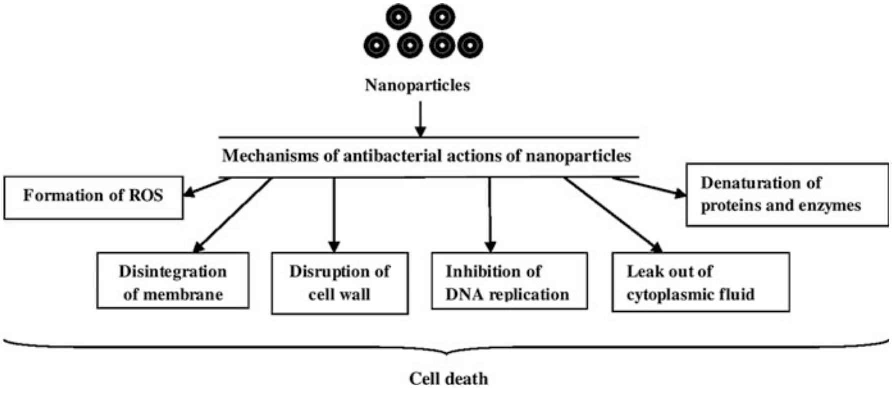

Fig. 6.2 Schematic representation of mechanisms of antimicrobial actions of nanoparticles for killing bacterial pathogens

Moreover, smaller particle size might form more superoxide ions and enhance the chance of bacterial destruction (Huang et al. 2005). ZnO nanoparticles exhibit antimicrobial activity through the release of reactive oxygen species (ROS), where the released ROS cause oxidative stress through demolishing nucleic acids (such as DNA), rupturing of cellular membrane and degradation of cellular proteins (Qu et al. 2013). Increased antimicrobial effects have been reported under high concentration of small-sized ZnO nanoparticles (Jones et al. 2008). Another promising example of nanoparticle is ‘silver nanoparticles’ which attracted the attention of worldwide research due to its excellent potential as antimicrobial agent. Since historically, the silver is always considered as a strong antimicrobial agent and showed broad range of industrial applications such as in healthcare and external medicine (Huh and Kwon 2011). It also has been reported that silver can generally bind with thiol group in enzymes/proteins (such as NADH dehydrogenase II) and results in the inactivation of enzymes (Li et al. 2008).

Moreover, silver ions can avert the process of DNA replication and make structural changes in the cell envelope and cause irreversible cell damage through accumulation of silver nanoparticles in the cytoplasm (Sondi and Salopek-Sondi 2004; Qu et al. 2013). Silver nanoparticles also exhibited antiviral mechanisms against several enveloped viruses by binding with a viral envelope glycoprotein (Elechiguerra et al. 2005). Carbon nanotubes (CNTs) are one of the most important nanomaterials showing potential antibacterial activity through a wide array of mechanisms such as physical perturbation of the cell membrane, oxidative stress and releasing out of cytoplasmic fluid (Vecitis et al. 2010). The antimicrobial effects of CNTs depend on their unique physicochemical properties, and the purified single-walled nanotubes (SWNTs) could be strong antimicrobial agent as they potentially damaged the cell membrane of microorganisms (Kang et al. 2007).

Additionally, the ‘graphene’ and ‘graphite’ materials also exhibited antimicrobial actions through the similar mechanisms (Liu et al. 2011). Chitosan presents antimicrobial activity against a wide range of pathogens including bacteria and fungi. However, antimicrobial activity of chitosan relies on several traits including molecular weight, availability of lipids and proteins as interferences, degree of deacetylation or increased ionized amino groups, the host, acid solvents, pH, pKa, surface charge, concentration of chitosan, ionic strength of matrix, reaction time, chelating capacity and types of bacteria (Rabea et al. 2003; Holappa et al. 2006; Hossain et al. 2014).

Bacterial cell surface possesses negative charge which allows positively charged chitosan and promotes cellular leakage (Holappa et al. 2006). Chitosan restrains the microbial growth and can interfere with the formation of mRNA and proteins after penetration of cellular nuclei (Rabea et al. 2003; Hossain et al. 2014). It can also hamper the bacteriophage infection and bacteriophage multiplication depending on the concentration of chitosan in the medium (Rabea et al. 2003).

6.9 Miscellaneous Application of Nanoparticles in Wastewater Treatment

Application of nanoparticles in wastewater treatment not only addressed the challenges which incapacitated existing treatment technology but also gave new competence for treatment facility. Thus, a revolutionary approach of 'nanoparticle-based wastewater treatment' has been established in recent times that can be efficient in expanded supply of water from unconventional water sources. Nanotechnology has been resulted in a highly efficient wastewater treatment practice without reliance on high infrastructures (Qu et al. 2013). Wastewater from different sources is rich in chemicals substances (heavy metals, organic compounds) and biological substances (algae, microbial pathogens, cyanobacterial toxins and antibiotics).

Therefore, application of nanoparticles can be an alternative strategy over other conventional wastewater treatment strategies, as the nanoparticles have essential features for removing chemical and biological substances from wastewater. Nanoparticles carry out different mechanisms such as adsorption, photocatalysis and oxidation for removal of certain chemical compounds and heavy metals for achieving the motto of wastewater treatment (Santhosh et al. 2016). Metal oxide nanomaterials, particularly TiO_2 and CeO_2 and carbon nanotubes (CNTs), have been proposed as 'catalysts' in heterogeneous catalytic ozonation processes, and it offered a way for quick and relatively complete degradation of organic contaminants. The increasing approach of using titanium dioxide (TiO_2) as a photocatalyst positively influenced wastewater treatment through organic dye degradation, and it is a highly oxidizing agent showing various other promising qualities such as long-term photostability, low cost and low toxicity (Qu et al. 2013).

Exploitation of nanoparticles such as ZVINP is efficient in elimination of existed pollutants such as heavy metals, organic dyes, trihalomethanes (THM), antibiotics, chlorinated ethane and methane and inorganic anions (Hossain et al. 2014). High affinity of nanoparticles towards heavy metals resulted in reduced effect of numbers of heavy metals and other halogenated organics. Gold nanoparticles were also found to be highly effective in masking the toxic effect of halogenated organics such pesticides and heavy metals (Pradeep and Anshup 2009). The development of other noble nanoparticles is potentially required that may be the strong agents for improving the tactics used in wastewater treatment. Zinc oxide (ZnO) nanoparticles were efficient in removal of arsenic contaminants and also exhibited the ability to remove arsenic from water (Singh et al. 2013). The role of carbon nanotubes (CNTs) cannot be forgotten as it played an important role in heavy metal removal. Oxidized carbon nanotubes (CNTs) also showed elevated capacity of adsorption for numerous metal ions (Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}) with fast kinetics (Qu et al. 2013). Iron nanoparticles also play crucial role in wastewater treatment, particularly in the elimination of heavy metal contaminants, and these nanoparticles use two paths for water treatment: (a) first the use of iron nanoparticles as nanosorbent or immobilization carrier for removal of harmful pollutants and (b) second the use of iron nanoparticles as photocatalysts to disintegrate or to convert harmful pollutants into a less toxic form (Xu et al. 2012).

Moreover, there is a need for development of membranes modified with nanofibres for the removal of micro-sized particles with less fouling tendency. The addition of several nanoparticles (TiO_2, alumina, silica and zeolite) to polymeric ultrafiltration membranes has resulted in the augmented membrane surface hydrophilicity, increased water permeability and fouling resistance (Ramakrishna et al. 2006). The effect of nanoparticles on membrane selectivity and permeability relies on the types, dimension and amount of nanoparticles (Qu et al. 2013).

6.10 Conclusion

Reclamation of wastewater is a prime need for reducing the risk of potentially hazard materials (inorganic and organic contaminants, heavy metals and pathogenic microorganisms), before discharging treated water into the river or further reutilization. However, advance in nanotechnology has revolutionized the treatment procedure for wastewater with high efficacy. Nanoparticles of various kinds showed their efficiency in removal of contaminants and inactivation of human pathogens. Due to its high surface area and higher catalytic efficiency, and a wide range of mechanisms (photocatalysis, oxidation and adsorption), it makes ‘nano metals’ capable for eliminating both organic and inorganic pollutants and treating wastewater with better efficiency. Wide-spectrum antimicrobial mechanisms are exhibited by a number of nanoparticles including membrane disruption, production of reactive oxygen species (ROS) and disintegration of cellular macromolecules for killing microbial pathogens. These strategies conclude the multifarious and splendid role of nanoparticles in water and wastewater treatment of new era.

6.11 Future Prospects

Application of nanoparticles in area of water treatment is wider, but current challenges are still to be addressed in further advancement in nanotechnology. The future research is required to employing nanoparticles for removal of pollutants with extra efficiency and lower toxicity/side effects. To make nanotechnology implications cost-effective and easy to operate, it is required for the scientific world to accept the challenges of wastewater problems. The current research is also directing the future for using nanomaterials in huge amounts as powerful tools for solving the purpose of wastewater treatment.

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Chapter 7
Metagenomics for Novel Enzymes: A Current Perspective

Dinesh Yadav, Aiman Tanveer, and Sangeeta Yadav

Abstract Microbial enzymes are considered to be the backbone of most of the industrial bioprocesses and are substantially influencing all spheres of life. The research trends in microbial enzymes have witnessed several innovations over the years in the form of different state-of-the-art technologies, namely, directed evolution, metagenomics, recombinant DNA technology, bioinformatics and relevant science of omics. The major constraint of microbial enzymes lies on the source itself as only less than 1% of the existing microbial diversity is being utilized based on its ease of cultivation while the remaining 99% still needs to be explored. The metagenomic approach has been established as a potential tool to access the untapped microbial diversity. It involves isolation of metagenomic DNA from diverse environmental samples directly, followed by extracting the genetic information either by function- or sequence-driven high-throughput approaches. Several novel metabolites and biocatalysts have been isolated using this approach. The metagenome screening, mining of sequenced microbial genomes, and exploring diversity of extremophiles could be utilized to expand the list of industrially important microbial enzymes. This chapter provides an insight into different aspects of metagenomics and its immense potential application exclusively for characterization of novel microbial enzymes.

Keywords Metagenomics · Microbial diversity · Recombinant DNA technology · Enzymes · Bioinformatics

7.1 Introduction

Enzymes derived from microbial system are widely used in the industrial processes. The various factors that make enzymes a promising tool in the catalytic reaction are costs, enhanced efficiency, better recovery of the products, and reduced use of toxic

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compounds. Microorganisms occupy a major portion of the earth biomass accounting to approximately 4–6 × 10^{30} cells (Bunge et al. 2014). These wide population of microorganisms are present in varied habitats ranging from hot springs, salt brines, acid mine waters, deep-sea hydrothermal vents, and Antarctic ices (Mirete et al. 2016). Although large numbers of culture media and culture conditions have been established, only around 1% of the microbes could be cultured in laboratory using traditional microbiological methods (Culligan et al. 2014). Introduction of metagenomics and its allied fields, metatranscriptomics and metaproteomics, have proved very helpful in exploring the uncultured microbial population. These can be a promising source of many commercially important enzymes. Keeping in view the recent advances in identifying new biocatalysts from environmental DNA, metagenomics is generally considered the most promising methodology (Lorenz and Eck 2005). Metagenomics provides comprehensive view of the genome derived directly from the microorganisms. Different environmental niches have been explored, and new sites are currently being investigated for the discovery of novel biocatalysts. So, metagenomics can be used for exploring the microbial diversity as well as finding the novel enzymes with better catalytic properties. New better high-throughput sequencing techniques have come up that help in efficient and accurate identification of genes encoding suitable biocatalysts from complex metagenomes. In this chapter, an overview of the basic metagenomic methodologies and discovery of novel enzymes through the metagenomic approach is discussed.

7.2 Metagenomics: An Introduction

The term metagenomics was introduced by **Jo Handelsman**. With the introduction of metagenomics, scientists have realized the immense potential of the microbial population present in the diverse environmental habitats. Metagenomics emphasizes on the complete genetic composition of the microbes present in any habitat or niche. It focuses on the isolation of total community DNA and comprehensive genomic analysis of the data achieved (Schmeisser et al. 2007). The fast rate of discovery in this field has helped in comprehensive understanding of the microbial population present in a given habitat (Riesenfeld et al. 2004). It has also assisted in better understanding of the microbial life and discovery of new biocatalysts (Banik and Brady 2008; Edwards et al. 2006; Frias-Lopez et al. 2008; Venter et al. 2004). The advent of low-cost and high-throughput sequencing methodologies has greatly boosted up the number and size of metagenomic sequencing projects, such as the Sorcerer II Global Ocean Sampling (GOS) (Biers et al. 2009; Rusch et al. 2007) or the metagenomic comparison of 45 distinct microbiomes and 42 viromes (Dinsdale et al. 2008). Since the metagenomic-based enzyme discovery methods are now being assisted by the newer sequencing technologies, it is gradually replacing and complementing the traditional culture-dependent methodologies. The immense potential of the metagenomic-based technologies provides wide volume of data and information.

The number of metagenomic sequences obtained by high-throughput metagenomic projects is organized for better understanding of the sequence information through computational and bioinformatics methods. Again, bioinformatics software are used for establishment of the phylogenetic relationship between the sequences. If any novel hit is obtained, then biotechnological methods are required for the cloning, expression, and characterization of genes and their products. This integrated approach of the metagenomic-based study has led to the exponential increase in the rate of gene discovery. Novel enzymes have been isolated from different metagenomes like marine environments (Biller et al. 2018), gastrointestinal microbiomes of different vertebrate and insect species (particularly for the plant-degrading activities) (Li et al. 2005; Wang et al. 2009), different soils (Lim et al. 2005; Yun et al. 2004), and "extreme" environments, including acid mine drainage (Tyson et al. 2004), alkaline and haloalkaline lakes (Rees et al. 2004), Antarctic mineral soils (Heath et al. 2009), and many more sites.

On the basis of targeted objective, there are two basic strategies taken up in metagenomic-based study, functional screening, and homology screening. For carrying out any of the above analysis, library has to be prepared. For preparation of the library, entire metagenomic DNA is cloned and subsequently screened to look for new classes of genes for new or known functions, including genes encoding, i.e., lipases, antibiotics, antibiotic resistance genes, oxidoreductases, catabolic enzymes, and biotin synthesis.

7.2.1 Sequence Homology-Based Enzyme Discovery

Basic techniques used for the identification of novel enzyme by sequence homology are PCR and colony hybridization. But for identification by these methods, prior knowledge of the sequence is needed for designing the primer or probe. But by this method, only variants of the known enzymes can be identified. This method has been used for the identification of genes for several novel enzymes such as dioxygenases, nitrite reductases, [Fe-Fe]-hydrogenases, [NiFe] hydrogenases, hydrazine oxidoreductases, chitinases, proteases, herbicide-degrading genes and copper resistance enzymes, and glycerol dehydratases (Simon and Daniel. 2011; Niehaus et al. 2011; Zaprasis et al. 2010; De la Iglesia et al. 2010). The traditional PCR and hybridization techniques are gradually been taken over by the high-throughput next-generation sequencing techniques. The sequencing data thus obtained can be easily processed through bioinformatics-based tools, and many information like the enzymatic sequence motifs can be easily derived. Moreover, the high-throughput analysis provides more flexible homology searches than PCR. The existing metagenomic data can be further analyzed for functional analysis whereby the gene of interest is optimized for heterologous expression. This is also known as synthetic metagenomics.

Sequence-based approach has been used to discover novel hydrolases from hot environments (Wohlgemuth et al. 2018). Together with the help of functional and sequence-based screening of 15 hot spring metagenomes, around 100 potentially new hydrolases have been obtained. Out of these, many have been structurally and functionally characterized. Among the characterized hydrolases are thermostable carboxylesterases, enol lactonases, quorum sensing lactonases, gluconolactonases, epoxide hydrolases, and cellulases. Through this strategy, hyperthermostable carbonic anhydrase has been identified from an active hydrothermal vent chimney (Fredslund et al. 2018). DNA was sequenced from the sample collected from active hydrothermal vent chimney through Illumina sequencing (300 bp PE run) with read count of 12,141,468 with 2,888,817,382 bp. An α-type carbonic anhydrase gene was isolated and cloned. The recombinant gene has been biochemically and structurally characterized.

Another instance of use of sequence-based metagenomics for the isolation of gene is discovery of novel acetyl xylan esterase from hot desert hypolith metagenomic DNA (Adesioye et al. 2018). The metagenomic DNA was sequenced and annotated for acetyl xylan esterases (AcXEs) by bioinformatics-based tools. A ~36-kDa protein ($\mathrm{Axe1}_{\mathrm{NaM1}}$) was cloned, expressed, purified, and characterized. Moreover, the sequence-based data provided informational background for carrying out the site-directed mutagenesis-based analysis. Point mutation (N96S) of this protein improved both thermal stability and catalytic efficiency.

7.2.2 Function-Based Enzyme Discovery

This method is commonly used for the discovery of enzymes. The screening of enzymes depends on the metabolic activities of metagenomic-library-containing clones. With the help of this strategy, novel enzymes bearing unique functions can be identified as no sequence information is required (Daniel 2005; Ferrer et al. 2009; Gloux et al. 2010; Handelsman 2004; Riesenfeld et al. 2004). Functional metagenomics can be carried out by three different approaches, through phenotypic detection of the biomolecules, through heterologous complementation of host strains or mutants, and by induced gene expression (Simon and Daniel 2011).

In the simple readout assays based on the phenotypic detection, libraries are plated on media containing a substrate for an enzyme of interest. The active enzyme acts on the substrate to form a halo or appearance of color. The positive clones capable of acting on the enzyme are identified. Through this method Popovic et al. have identified 714 clones showing positive activity for carboxylesterase (Popovic et al. 2017). Eighty clones have been confirmed to have esterase activity belonging to 17 different families. Three metagenomic enzymes were displayed, while seven proteins were positive for polyester depolymerization activity against polylactic acid and polycaprolactone. Enzymes active against bacteria have been reported from the Arizona soil DNA and metagenomic DNA library (Iqbal et al. 2014). A total of 700,000 cosmid clones of the library prepared in *Ralstonia metallidurans* were screened through growth inhibition in a bacterial overlay assay. The positive colonies were analyzed for the presence of peptidase, lipase, and glycolytic activities.

The second strategy for function-driven screening is heterologous complementation of host strains or mutants of host strains for which the targeted gene is essential for growth under selective conditions. With the help of this technique, large number of metagenomic libraries can be screened easily in comparatively less time. This method is highly accurate and sensitive as no false positives are detected (Simon et al. 2009). Novel β-galactosidases have been detected and isolated through functional complementation by cloning of the metagenomic DNA in a surrogate host. Soil metagenomic library carried in an IncP cosmid was used for functional complementation for β-galactosidase activity in both *Sinorhizobium meliloti* (*Alphaproteobacteria*) and *Escherichia coli* (*Gammaproteobacteria*). One β-galactosidase was selected which was encoded by seven overlapping clones. It was identified to be belonging to glycoside hydrolase family 2.

Through biochemical analysis three other ORFs were confirmed to have β-galactosidase activity. Out of these three ORFs, Lac36W_ORF11 and Lac161_ORF7 possessed conserved domains and did not show similarities to known glycoside hydrolases. ORF Lac161_ORF10 had no conserved domain and did not show similarity to known glycoside hydrolases. Bioinformatics and structural analysis showed its affinity with novel enzyme family with a five-bladed propeller glycoside hydrolase domain. Through complementation-based homology screening, an exonuclease ExoMeg1 has been identified from metagenomic library (Silva-Portela et al. 2016). This is the first exonuclease gene identified through the metagenomic approach. The hypothetical protein has been annotated as a member of the exo-endo-phos superfamily. This enzyme exhibited 3′–5′ exonuclease activity on both single- and double-stranded DNA. It was also divalent metal-dependent, EDTA-sensitive, and salt-resistant. Clone bearing the ORF of this gene could complement strains deficient in recombination or base excision repair.

Substrate-induced gene expression screening (SIGEX) is another type of function-based screening given by Uchiyama et al. in 2005. It is a high-throughput screening which uses GFP expression system in combination with fluorescence-activated cell sorting. The mechanism underlying SIGEX is that catabolic-gene expression is induced mainly by specific substrates and is often controlled by regulatory elements located close to catabolic genes (Uchiyama et al. 2005). To perform SIGEX-based screening, the metagenomic DNA is cloned in vector upstream of the *gfp* gene and influences the expression of *gfp*. When the substrate is added, the clones showing positive GFP expression are selected by fluorescence-activated cell sorting. But the drawback of this method is that the expression may be governed by some other transcriptional regulators.

A similar technique is metabolite-regulated expression (METREX), devised by Williamson et al. (2005). To identify metagenomic clones producing small molecules, a biosensor that can perform quorum sensing is placed inside the host cell along with the metagenomic DNA clone. The biosensor controls the expression of gfp gene. When the expression of metagenomic DNA exceeds the threshold level, green fluorescent protein (GFP) is produced which is detected by fluorescence microscopy.

Another technique used is product-induced gene expression (PIGEX) (Uchiyama and Miyazaki 2010). The enzymatic activities are detected by the expression of *gfp*, which is triggered by product formation. PIGEX has been used for mining of amidases from metagenomic sample derived from activated sludge by using the benzoate-responsive transcriptional regulator BenR sensor. Recombinant *E. coli* strains bearing the sensor molecule were cultured along with 96,000 metagenomic clones in the presence of the substrate benzamide. The sensor cell fluoresced in response to the benzoate production by the metagenomic clones.

These recent techniques have given great boost to the identification of novel enzymes by functional metagenomic-based approach.

7.3 Metagenomic Workflow

The metagenomic workflow involves several steps, namely, selection of sample source, metagenomic DNA isolation, preparation of libraries, and screening of targeted clones from the library. The steps involved in metagenomics for isolating novel enzymes are shown in Fig. 7.1.

7.3.1 Sample Source

Metagenomics deals with the culture-independent analysis of the microbial genomes from diverse habitats. It can be soil samples, sedimentation from aquatic habitats or samples from gut, oral cavity, and excreta. Soil samples are most routinely used in metagenomics, based on the fact that they are a rich source of microbes belonging to archaea, bacteria, and protists. It may range from alluvial soil, mountain soil, forest soil, desert soil, to soil contaminated with different pollutants like oil and alkaline bases.

7.3.2 Metagenomic DNA Isolation

The quality and quantity of isolated metagenomic DNA define the representativeness of the prepared metagenomic library. It is relatively simple to isolate DNA from water samples as dissolved impurities are easily removed. Humic acid, nucleic acids, and other charged particles present in the soil make isolation of DNA from soil sample an exigent task. The contaminants have to be removed for effective downstream processing like library preparation. Since large quantity of highly purified DNA is required for library preparation, it makes DNA isolation a challenging task (Wilkinson et al. 2002). The availability of commercial kits for isolation of good

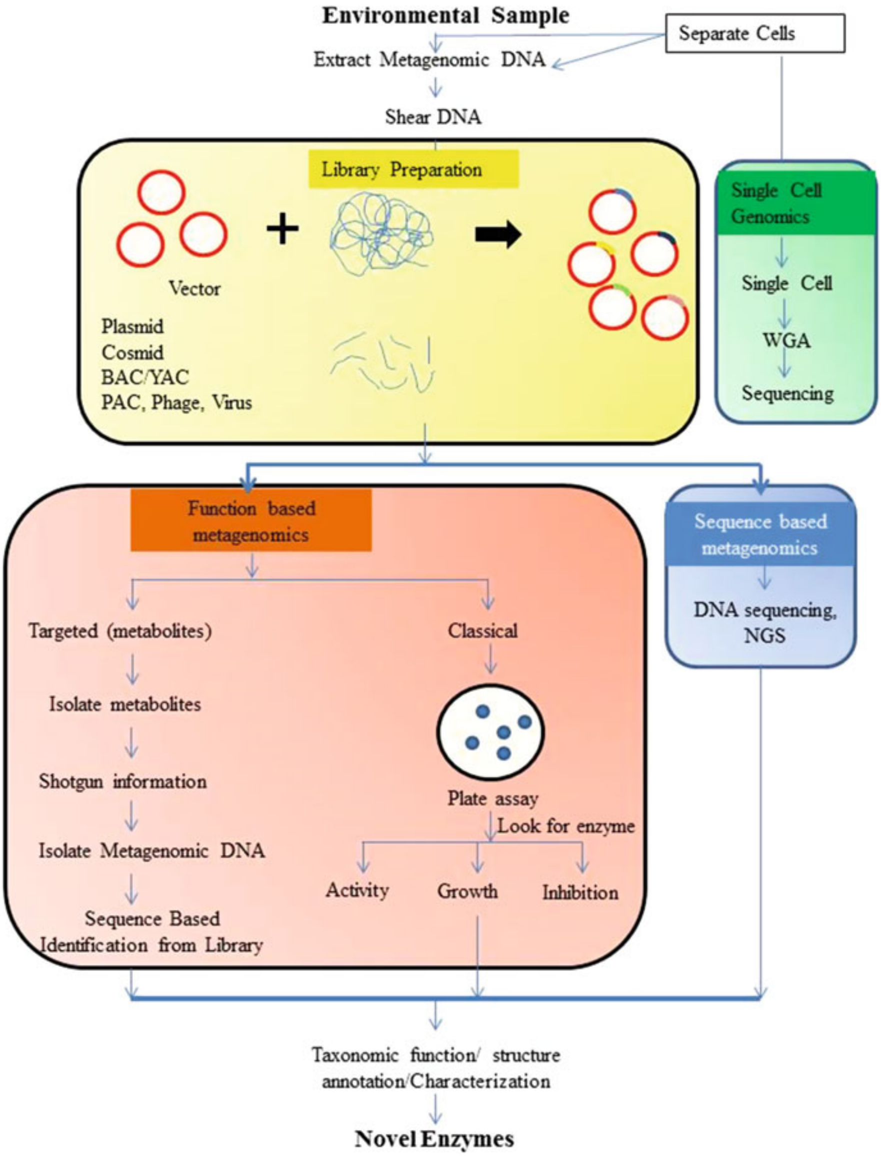

Fig. 7.1 Metagenomics workflow for isolation of novel enzymes

quality of DNA from diverse types of soil has substantially influenced the metagenomic approach in search of novel genes.

For isolation of metagenomic DNA, either the whole soil samples can directly be applied for isolation of DNA (direct method) or only the cells separated from soil matrix are subjected to DNA isolation (indirect method). In indirect method, the cells are recovered by size exclusion chromatography, followed by cell lysis for extraction of DNA. By this technique, large fragments of DNA are obtained, though with lower yields. The selective isolation of cells with reference to bacterial cells has been reviewed recently (Nnadozie et al. 2015).

In the direct method, cells are subjected to both shear forces and detergent containing lysis buffers to break the cell wall. However, optimal shear forces must be applied to avoid breakage of DNA strands. The lysed samples are then subjected to phenol: chloroform treatment and DNA precipitation. Although this method results in higher yield, the DNA fragment size is comparatively smaller, ranging from 1 to 50 kb and hence not preferred for the preparation of large insert libraries (Desai and Madamwar 2007).

7.3.3 Library Preparation

Preparation of suitable library is a key step in metagenomics. The size of metagenomic DNA fragments, vector, and the host cell defines the size and number of clones of a representative library. The library preparation involves fragmentation of the genomic DNA either by shearing or restriction digestion. The generated fragments are then cloned into a suitable vector and transformed in an appropriate host. The clones of library are then screened by either sequence- or function-based approach.

The DNA isolated by direct method is frequently used for library preparation (Henne et al. 1999; Rondon et al. 2000). Depending upon the size of insert, library is defined as small insert and large insert library. For small-size inserts, plasmid vectors are used that can incorporate not more than 15 kb of the insert. For larger inserts up to 40 kb cosmid or phasmid libraries are prepared. For inserts exceeding 40 kb, BAC-based library is prepared. Generally, the host preferred in library preparation is predominantly *Escherichia coli*. The vector used defines the size of library, copy number, and the method of library screening.

The small insert libraries are preferred for fishing out single genes and operons, while the large insert libraries are more commonly used to explore larger genes or gene clusters of a metabolic pathway. A homologue of stress-induced protein encoding gene, Water Hypersensitivity domain (WHy), has been recently detected from the phasmid clone of Antarctic desert soil metagenomic library using sequence-based approach. The NPN motif at the N terminus of the protein showed identity with the homologue from *Pseudomonas* genome. This newly identified protein could be a good candidate for developing cold and freeze tolerance (Anderson et al. 2015). Apart from exploring genes, the diversity studies can also be performed by preparing a library of 16S rRNA genes. This library is a representative of the diverse microbial population in the soil metagenome.

7.3.4 Screening

The library driven from complex environmental sample needs to be examined by highly sensitive and high-throughput methods. PCR is the most frequently used tool for sequence-driven screening (Quaiser et al. 2002, 2003; Ginolhac et al. 2004). Probe-based hybridization is also used for the screening of desired clones. Both PCR- and hybridization-based methods need prior sequence information for designing of suitable primers or probes, respectively. These methods are useful in identification of new members of the known gene families as the primers or probes are designed against the conserved regions of desired genes. A cytochrome P450 monooxygenase gene, *syk181*, has been identified from the soil metagenomic library from Kyeryong Mountains in Korea (Kim et al. 2007). Similarly, polyketide synthases (Seow et al. 1997; Ginolhac et al. 2004) and nitrile hydratases (Precigou et al. 2001) has also been identified by sequence-based approach.

Random sequencing of the environmental-derived metagenomic library can provide a rough overview of the microbial habitat in a given ecosystem, but the fine-level analysis by enormous sequencing needs to be done for precise species composition. Microarray is a high-throughput technique for sequence-based screening of clones harboring target genes of interest. The DNA is extracted from BAC or phasmid clone and then subjected to spotting on the DNA microarray. Positive clones containing the target gene are detected by fluorescent-based probes (Park et al. 2010). This technique serves as an important tool in analysis and cataloging of the metagenomic clones. It has also proven to be an effective means for profiling of the microbial communities present in diverse habitats (Cho et al. 2002; Zhou et al. 2002).

Recently, a metagenomic tool GeoChip 4.0 has been developed for microarray-based metagenomic analysis. It has been used to type the vegetation diversity in the Tibetan mountainous grassland (Gao et al. 2014). However, the sensitivity of microarray is much lower than the PCR-based screening (Zhou et al. 2002). This may lead to incomplete representation of the genetic information in the metagenomic library.

The constraint of library preparation can be overcome by NGS strategy. The Roche 454 pyrosequencing (Edwards et al. 2006) and Illumina sequencing (Lazarevic et al. 2009; Qin et al. 2010) are preferred for metagenomics. The Illumina platform is preferred to decipher complete genomic information of the complex metagenomes (Hess et al. 2011). Ion torrent personal genome machine has also been applied to the metagenomic samples (Whiteley et al. 2012; Solonenko et al. 2013).

In contrast to the sequence-based metagenomics, the function-based screening is done on the basis of enzyme activity. This is helpful in discovering new protein families showing catalytic activity toward a substrate (Reyes-Duarte et al. 2012). This function-based screening has the potential to identify novel enzymes even though they do not exhibit sequence homology with the known enzymes of the same family that are present in the databases. The newly discovered enzymes may have different catalytic residues at their active site. Hence this can serve as a tool in assigning functions to the proteins referred to as hypothetical in the databases.

There have been several reports of identifying novel enzymes through the function-based approach. Antimicrobial activity has been detected in the BAC-derived metagenomic library prepared from human plaque and saliva DNA. They were identified to encode homologues of glutamyl-tRNA reductase (GluTR) enzyme which plays role in the C5 pathway of tetrapyrrole synthesis (Arivaradarajan et al. 2015). Seven putative β-glycosidases and two putative glycosyl transferases have been identified by functional screening of two metagenomic DNA libraries derived from agricultural soil (Stroobants et al. 2014). Although functional screening has resulted in deciphering several novel enzymes from diverse sources, the heterologous expression of the identified gene(s) in a different host is always a challenging task (Gabor et al. 2004). Attempts have been made to express the clone in an alternative host for optimal expression of the cloned library like the use of *Bacillus subtilis* as host for functional screening (Biver et al. 2013a, b). Another promising host is *Rhizobium leguminosarum* (Li et al. 2005), and an alternative host for functional metagenomic screening has been reviewed recently (Liebl et al. 2014).

7.4 Novel Microbial Enzymes by Metagenomic Approach

Microbial enzymes are of great importance in the development of industrial bioprocesses. A number of industrial products are made using enzymes. Microbes have served and continue to serve as the backbone for most of the enzymes. Industrial applications of these enzymes include pulp and paper, leather, detergents and textiles, pharmaceuticals, chemical, food and beverages, biofuels, animal feed, and personal care (Anbu et al. 2015; Choi et al. 2015).

Metagenomics is being used to discover new microbial enzymes whose catalytic properties can further be improved or modified by different strategies based on rational-, semi rational-, and random-directed evolution (Adrio and Demain 2014; Baweja et al. 2016). Several novel enzymes have been discovered through metagenomic approach as listed in Table 7.1. Important group of enzymes includes lipolytic enzymes, polysaccharide-degrading enzymes, proteolytic enzymes, and few oxidoreductases.

Among lipolytic enzymes are esterases or lipases. The discovery of these enzymes has received much attention as these have some interesting features like non-requirement of cofactors, remarkable stability in organic solvents, broad substrate specificity, stereoselectivity, and positional selectivity (Lee et al. 2006c). The features of lipolytic enzymes are especially attractive for organic synthesis if the enzymes with specific chiral resolution could be retrieved from the soil metagenome. Among the characterized metagenomic esterases found so far are two largest esterases known, a 325-kDa esterase from a deep-sea hypersaline anoxic basin and a 336-kDa octameric esterase from a drinking water biofilm (Elend et al. 2006). The deep-sea hypersaline basin displays habitat-related properties as they were more active at alkaline pH and also displays higher activities under high-pressure conditions (Ferrer et al. 2005a). Esterases from the soil and drinking water biofilm

Table 7.1 List of some novel enzymes deciphered through the metagenomic approach in recent years

Enzyme	Approaches used	Source	Reference
Hydrolases	Sequence-based metagenomics	Hot and other extreme environments	Wohlgemuth et al. (2018)
Bifunctional cellulase/hemicellulase	Sequence-based metagenomics	Black goat rumen	Lee et al. (2018)
Esterases	Functional metagenomics	Sea sediment and hot spring microbial mat	Ranjan et al. (2018)
Carbonic anhydrases	Sequence-based metagenomics	Active hydrothermal vent chimney	Fredslund et al. (2018)
Acetyl xylan esterase	Sequence-based metagenomics	Hot desert hypolith	Adesioye et al. (2018)
Esterases	Functional metagenomics	Deep-sea sediment	Huo et al. (2018)
L-Asparaginase	Functional metagenomics	Forest soil	Arjun et al. (2018)
Lignin-transforming enzymes	Functional metagenomics	Coal beds	Ho et al. (2018)
Carbamate-degrading enzymes	Functional metagenomics	Bovine rumen microbiome	Ufarté et al. (2017)
Pectinases	Sequence-guided strategy coupled with enrichment culture technique	Apple pomace-adapted compost	Zhou et al. (2017)
Lignocellulose-degrading enzymes	Sequence-based metagenomics	Gut microbiome of the common black slug *Arion ater*	Joynson et al. (2017)
β-Xylosidases	Sequence-based metagenomics	Hot spring soil	Sato et al. (2017)
Cellulases	Sequence-based metagenomics	Oil reservoir	Lewin et al. (2017)
Alkaline protease	Functional metagenomics	Mangrove sediment	Pessoa et al. (2017)
Lignocellulases	Functional metagenomics	Landfill sites	Ransom-Jones et al. (2017)
Hydrolases	Functional metagenomics	Oil-impacted mangrove sediments	Ottoni et al. (2017)
Laccase or laccase-like enzymes	Functional metagenomics	Acidic bog soil	Ausec et al. (2017)
Tauroursodeoxycholic acid biotransformation enzymes	Sequence-based metagenomics	Gut microbiome of black bears	Song et al. (2017)
Thermostable amine transferases	Sequence-based metagenomics	Hot spring	Ferrandi et al. (2017)
Salt-tolerant esterase	Functional metagenomics	Deep-sea sediment of the South China Sea	Zhang et al. (2017)
Fibrolytic enzymes	Functional metagenomics	Indian crossbred cattle fed finger millet straw	Jose et al. (2017)

(continued)

Table 7.1 (continued)

Enzyme	Approaches used	Source	Reference
Toluene monooxygenase	Sequence function-based metagenomics	Hydrocarbon-polluted sediment	Bouhajja et al. (2017)
Antibiotic resistance enzymes	Sequence-based metagenomics	Atlantis II Deep Red Sea brine pool	Elbehery et al. (2017)
Bilirubin-oxidizing enzyme	Sequence-based metagenomics	Wastewater treatment-activated sludge	Kimura and Kamagata (2016)
Cellulases	Functional metagenomics	Microorganisms associated with coral	Sousa et al. (2016)
Glycosyl hydrolase family 16 β-agarase	Functional metagenomics	Mangrove soil	Mai et al. (2017)
Peptidases	Functional metagenomics	Yucatán underground water	Apolinar-Hernández et al. (2016)
β-Lactamase	Functional metagenomics	Environment with high levels of biocide exposure	Salimraj et al. (2016)
Lignocellulase	Functional metagenomics	Sugarcane bagasse and cow manure	Colombo et al. (2016)
Esterase	Functional metagenomics	Marine mud	Gao et al. (2016)
Lipolytic biocatalysts	Functional metagenomics	Global Ocean sampling dataset	Masuch et al. (2015)
Glycoside hydrolase GH16 SCLam	Sequence-guided strategy coupled with enrichment culture technique	Sugarcane soil metagenome	Alvarez et al. (2015)
Epoxide hydrolases (EHs) Tomsk-LEH, CH55-LEH	Sequence-based metagenomics	Hot spring metagenomic library	Ferrandi et al. (2015)
Esterase-active enzyme	Sequence-based metagenomics	Heated compost and hot spring	Leis et al. (2015)
Carboxyl esterase	Sequence-based metagenomics	*R. exoculata* gill metagenome	Alcaide et al. (2015)
Lipase esterase		Bovine rumen metagenome	Prive et al. (2015)
Fe-Fe hydrogenase genes	Bioinformatics and functional metagenomics	Municipal wastewater treatment plant	Tomazetto et al. (2015)
Glycosyl hydrolase	Functional metagenomics	Industrial soil bagasse collection site	Kanokratana et al. (2015)
Rubisco	Functional metagenomics Fosmid clone	Hydrothermal vent fluid	Bohnke and Perner (2015)
Lichenase gene	Functional metagenomics	Soil metagenome	Kim et al. (2014)

(continued)

Table 7.1 (continued)

Enzyme	Approaches used	Source	Reference
Cellulase	Functional metagenomics Fosmid clone	Gut microflora of *Hermetia illucens*	Lee et al. (2014)
GH44 family endoglucanase gene mgcel44	Functional metagenomics	Mangrove forest	Mai et al. (2014)
Lipase	Functional metagenomics	Soil metagenome	Pindi et al. (2014)
Oxygenase	Sequence-based metagenomics	Wastewater	Jadeja et al. (2014)
Lipolytic enzyme gene, est9x	Sequence-based metagenomics	Marine microbial metagenome of the South China Sea	Fang et al. (2014)
Mercuric reductase	Functional metagenomics	Unique deep brine environment of Atlantis II in the Red Sea	Sayed et al. (2014)
Two β-glucosidases	Functional metagenomics	Amazon soil metagenomic library	Bergmann et al. (2014)
Cold-active phthalate esters hydrolase	Functional metagenomics	Biofilms of a wastewater treatment plant	Jiao et al. (2013)
Organic solvent-tolerant lipolytic enzyme	Functional metagenomics	Activated sludge	Roh and Schmid (2013)
Family A DNA polymerases	Shotgun metagenomics	Marine virioplankton	Schmidt et al. (2014)
Thermostable esterase	Functional metagenomics	Activated sludge	Shao et al. (2013)
Novel endoxylanase family GH10	Functional metagenomics	Sugarcane soil metagenome	Alvarez et al. (2013)
Active cellulases	Functional metagenomics	Mining bioremediation system	Mewis et al. (2013)
Novel esterase subfamily	Sequence based	Terrestrial microenvironments of suburban and countryside soil from across China	Zhang et al. (2013)
Endo-β-1,4-glucanase gene	Functional metagenomics	Paper mill sludge and dairy sludge	Yasir et al. (2013)
Hemicellulases	Functional metagenomics	Comb and gut microbiomes of fungus-growing termite *Pseudacanthotermes militaris*	Bastien et al. (2013)
β-Glucosidase	Functional metagenomics	Compost	Uchiyama et al. (2013)
(Hemi)cellulase genes	Sequence based	Biogas digester	Yan et al. (2013)
Non-ribosomal peptide synthetases and polyketide synthases	Sequence based	Australian marine sponges	Woodhouse et al. (2013)

(continued)

Table 7.1 (continued)

Enzyme	Approaches used	Source	Reference
Novel organic solvent-tolerant esterase	Functional metagenomics	Temperate forest soil	Berlemont et al. (2013b)
Thermo-alkali-stable lipase	Functional metagenomics	Oil-contaminated soil	Zheng et al. (2013)
Cold-active, acidic endocellulase	Functional metagenomics	Cold desert of Ladakh	Bhat et al. (2013)
Novel thermostable cellulolytic genes	Sequence based	Cellulose-degrading sludge	Xia et al. (2013)
Novel cold-adapted esterase	Functional metagenomics	Antarctic soil metagenome	Berlemont et al. (2013a)
Alkaline protease	Functional metagenomics	Saline habitat	Purohit and Singh (2013)
New carboxylic ester hydrolases	Functional metagenomics	Forest soil metagenomic library	Biver and Vandenbol (2013)
Novel xylanase	Sequence based	Holstein cattle rumen	Cheng et al. (2012)
True lipase	Functional metagenomics	Soil	Zheng et al. (2012)
Cold-adapted esterase	Sequence based	Arctic intertidal metagenomic library	Fu et al. (2013)
Organic solvent stable lipase	Functional metagenomics	Soil	Khan and Jithesh (2012)
Cellulase-encoding gene	Sequence based	Buffalo rumen	Nguyen et al. (2012)
Multifunctional glycosyl hydrolase	Functional metagenomics	Calf rumen	Ferrer et al. (2012)
Glycoside hydrolases	Functional metagenomics	Compost	Dougherty et al. (2012)
Novel lignocellulose-degrading enzymes	Functional metagenomics	Higher termite guts inhabiting microbes	Nimchua et al. (2012)
Novel feruloyl esterases	Sequence based	Fecal samples of *Rusa unicolor* and *Equus burchelli*	Chandrasekharaiah et al. (2012)
Thermostable pyrethroid-hydrolyzing enzyme	Functional metagenomics	Turban Basin metagenomic library	Fan et al. (2012)
Cold-adapted esterase	Functional metagenomics	Mountain soil	Ko et al. (2012)
Xylanases	Sequence based	Yak rumen	Ko et al. (2012)
A novel family 10 xylanase	Sequence based	Compost	Jeong et al. (2012)
New lipase family	Functional metagenomics	Brazilian Atlantic Forest	Faoro et al. (2011)
Alginate lyase	Functional metagenomics	Gut microflora of abalone	Sim et al. (2012)
Novel esterase	Functional metagenomics	Neritic sediments of the South China Sea	Peng et al. (2011)

(continued)

Table 7.1 (continued)

Enzyme	Approaches used	Source	Reference
Extremely alkaliphilic and cold-active esterase	Functional metagenomics	Antarctic desert soil	Hu et al. (2012)
Novel β-lactamase gene	Functional metagenomics	Wastewater treatment plants	Uyaguari et al. (2011)
Acetylhydrolase	Functional metagenomics	Earthworm-associated microbial community	Navarro-Fernandez et al. (2011)
Novel lipases	Functional metagenomics	Microbial community in the pitcher fluid of the carnivorous plant Nepenthes hybrida	Morohoshi et al. (2011)
Novel feruloyl esterase	Functional metagenomics	Soil	Sang et al. (2011)
Aliphatic amidase	Functional metagenomics	Psychrotrophic and haloalkaliphilic nesterenkonia isolate	Nel et al. (2011)

displayed unusual properties. Both were, however, highly stable at alkaline pH and displayed unique substrate spectra with Est A3 being able to hydrolyze substrate such as 7-[3-octylcarboxy-(3-hydroxy-3-methyl-butyloxy)]-coumarin, a normally unreactive secondary ester (Elend et al. 2006).

Agarases are enzymes which can liquefy agar, and they can be divided into A and B agarases, depending on whether they cleave the a-L-(1, 3) or the B-D-(1,4) linkage of the polymer. In general, agarases have been produced by bacterial sources isolated from marine environments (Michel et al. 2006). However, screening of soil metagenomic library revealed a total of four agarolytic clones containing 12 agarases genes (Voget et al. 2003).

Amylases have enormous industrial application, especially in the detergent and food industries. Amylases particularly stable over a wide range of pH and temperature are preferred in industries, and hence efforts are made to isolate extremophiles producing amylases (Rothschild and Mancinelli 2001). The detection and characterization of novel amylolytic enzymes from metagenomic DNA libraries have also been reported (Rondon et al. 2000; Richardson et al. 2002; Voget et al. 2003; Ferrer et al. 2005b). One of the characterized amylases, from a soil metagenome, displayed an interesting characteristic in that it is stable and active under alkaline conditions, with a pH optimum at pH 9.0, a feature highly preferred in detergent industry (Rothschild and Mancinelli 2001; Yun et al. 2004). The amylases active under acidic pH and high-temperature conditions have also been isolated by metagenomics.

Celluloses are the most abundant biomass in nature, which provides a great potential for a number of applications, most notably biofuel production. Complete degradation of cellulose involves action of three types of cellulases including endoglucanases (EC3.2.1.4), exoglucanases (EC3.2.1.91), and β-glucosidases (EC3.2.1.21) acting synergistically to convert cellulose to glucose (Lynd et al. 2002). A broad range of microorganisms exhibiting these activities either in separate enzymes or in multi-protein complexes have been described, and several glycosyl hydrolases have been isolated, characterized, and classified directly from pure cultures (Davies and Henrissat 2002). Many novel cellulases with unique properties have been screened from different metagenomic libraries. One novel endoglucanase screened from ruminal metagenomic library revealed optimal activity at low pH (4.5) and stability for a broad pH range (3.5–10.5) (Duan et al. 2009). Another endoglucanase (CelA) was reported with high tolerance in ionic liquids, having sufficient stability and activity to create novel options for cellulose degradation in homogeneous catalysis. From marine metagenome libraries using functional screening and recombinant expression, a β-glucosidase (Bgl1A) with excellent glucose tolerance was identified, having great potential for industrial applications due to its low sensitivity to product inhibition (Fang et al. 2010). Another β-glucosidase (Bgl1B) was also obtained from the same library, exhibiting relatively higher activity and stability at pH values between 7.0 and 9.0 (Fang et al. 2009).

Chitinases are second most abundant polymer in nature after cellulose responsible for breakdown and recycling of chitin (Howard et al. 2003). These enzymes are mainly used in plant resistance against fungal diseases (Carstens et al. 2003). Attempt has been made to isolate novel chitinases from marine environment using metagenomic approach (Cottrell et al. 1999).

Proteases are used in diverse industrial applications. This largest group of industrial enzymes is mostly obtained from bacteria (Orhan et al. 2005). Its applications are wide, ranging from protein stain removal, food preparation, biofilm removal, to leather preparation (Bashir et al. 2014). Owing to its wide range of applications, researchers have isolated several novel proteases from metagenomic sources. Alkaline protease possessing oxidant stability has been isolated from forest-soil metagenomic library (Biver et al. 2013a, b). The purified and overexpressed protein showed maximal activity between pH 9 and 10 and at temperature of 50 °C. This enzyme displayed high resistance to oxidizing agents, hence useful in many biotechnological applications. Several proteases along with lipases and two clones (SA343 and SA354) have been obtained from the metagenomic library prepared from slaughterhouse drain biofilm DNA (Thies et al. 2016). These enzymes displayed with biosurfactant and hemolytic activities.

Hemicelluloses are important renewable polymer in lignocellulosic material. It is a complex polymer constructed of xylan (β-1, 4 linked xylose) and mannan (β-1, 4 linked mannose), which is rapidly hydrolyzed into monosaccharide under mild catalytic conditions. β-1, 4 xylanase (E.C 3.1.2.8) is the main enzyme responsible for the breakdown of non-hydrolyzed polymer (Brennan et al. 2004). Several novel xylanases have been screened from environmental DNA libraries. Cloning of a cold active xylanase Xyn8 has been reported (Lee et al. 2006b). A xylanase displaying high activity at reduced temperature and weakly alkaline conditions isolated from a soil-derived metagenome has been reported (Hue et al. 1995). Complete hydrolysis of xylan to xylose is also mediated by xylosidases and has been reported from metagenomes of diverse environments like an insect gut, a thermophilic anaerobic digester, and waste lagoon of a dairy farm (Brennan et al. 2004; Lee et al. 2006b).

Similarly xylanase active at lower temperature has been screened from the lagoon metagenome (Lee et al. 2006a). The microorganisms present in insect gut are difficult to cultivate, and metagenomics is the only approach to explore such microbial diversity. The metagenomic surveys of the insect gut resulted into four novel xylanases showing activity at acidic pH with a temperature optimum of 50 °C and also display unique hydrolysis products (Brennan et al. 2004).

Pectin is an important component of the cell wall of plant and fungi. Degradation of the pectin polymer is essential for many biological processes like fruit ripening and fruit abscission. This vital degradation process is carried out by pectinase group of enzymes. Few novel pectinases have also been investigated by metagenomic intervention. A novel pectinase capable of working under extreme temperature conditions, i.e., both thermostable and thermoactive, has been revealed by metagenomics (Singh et al. 2012). A GH28 family pectinase has also been identified from the metagenomic library prepared from Western Ghats, India, soil samples (Sathya et al. 2014). It has been reported to be catalytically active in broad range of temperature and pH. A novel alkaline pectate lyase of high catalytic activity has also been isolated from the alkaline environment soil (Wang et al. 2013).

7.5 Conclusion

A metagenomic approach has been substantially applied for the search novel microbial enzymes, antibiotics, transporters, antibiotic resistance genes, etc. The steps in metagenomics, namely, isolation of metagenomic DNA from different environmental samples directly and preparation of libraries and screening, have been drastically improved with the availability of efficient commercial kits. Further, several bioinformatics tools and software exclusively for metagenomic analyses have been developed. The deciphering of microbial genome sequences has provided opportunity for searching novel genes for industrially important enzymes by performing extensive genome mining.

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Chapter 8
Cyanobacteria: The Eco-friendly Tool for the Treatment of Industrial Wastewater

Suraj Sen and Santosh Kumar Karn

Abstract Cyanobacteria have been regarded as green eco-friendly bioremediation tools in recent years because of their potential applications in wastewater treatment. Several strains of cyanobacteria were reported to accumulate and detoxify different types of organic and inorganic pollutants. Studies also confirm that cyanobacterial consortia are capable of degrading oil components. There are still many unknown cyanobacteria that have tremendous degradation capacity for organic compounds present in nature, and it is important to assess the potential of cyanobacteria isolates indigenous to sites contaminated with organic and inorganic pollutants. Cyanobacteria are one of the potential organisms useful to mankind in various ways. Cyanobacteria constitute a vast potential resource in varied applications such as combating pollution, agriculture, food, feed, fuel, fertilizer, medicine, and industry.

Keywords Cyanobacteria · Wastewater · Organic and inorganic pollutants · Bioremediation · Degradation

8.1 Introduction

Cyanobacteria (blue-green algae) comprise a unique group of organisms with worldwide distribution. These organisms are considered as algae because of their microscopic morphology, pigmentation, and oxygen-evolving photosynthesis. Cyanobacteria are the largest group of photosynthetic prokaryotes, as judged by their widespread occurrence, frequency, abundance, and morphological diversity. Cyanobacteria are among the potential candidates because of being the earliest of the inhabitants of this planet: their existence can be traced back to 3.8 billion years. In

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contrast to other bacteria or algae, the cyanobacteria are one of the very few groups of organisms that can convert inert atmospheric nitrogen into an organic form, such as nitrate or ammonia.

Phycoremediation (remediation by means of cyanobacteria) is a valuable technique for improving the physiochemical properties of wastewater because it does not involve harmful chemicals and huge amounts of energy, in contrast to other conventional methods (Brar et al. 2017). Phycoremediation has been successfully applied for wastewater treatment for its eco-friendly characteristics. A few algal species such as *Scenedesmus*, *Chlamydomonas*, *Chlorella*, *Pediastrum*, *Nitzschia*, *Cosmarium*, and *Botryococcus* are used for phycoremediation for wastewater and for biofuel production as well (Hoh et al. 2016; Sun and Simsek 2017). Nitrogen and phosphorus, essential components for the growth of algae, are commonly present in wastewater. Kesaano and Sims (2014) suggest that algae can be beneficial in removing the excess nitrogen and phosphorus present in wastewater through absorption and sequestration mechanisms. Another application of algae can be the removal of toxic metals and organic compounds from industrial wastewater. A variety of morphological details might be seen; however, the usual variety is sensibly unpretentious contrasted with different life forms.

Cyanobacteria might be unicellular or frontier and the two renditions may demonstrate a covering of mucilage. Pioneer cyanobacteria might be filamentous (straightforward, blended, and spread) and in uncommon events frame unpredictable or cuboidal cell totals and sometimes plates or sheets. The individual cells are prokaryotic in nature. Cutting-edge cyanobacteria incorporate about 2000 species in 150 genera and 5 orders with an extraordinary assortment of shapes and sizes. Ultra-auxiliary examinations plainly show that the cyanobacteria are prokaryotic; that is, they need cores and different organelles and they have a peptidoglycan cell divider that is the average of gram-negative Eubacteria. They additionally have a few highlights that set them apart from other microorganisms, particularly their photosynthetic mechanical assembly and oxygen generation. Cyanobacteria are among potential candidates because the cyanobacteria are ubiquitous in distribution, found in all latitudes. The ability of Cyanobacteria to fix atmospheric N_2 independently or in association with another organism contributes a fixed form of nitrogen to natural ecosystems.

Cyanobacteria are the most efficient among all living organisms in the harvesting of solar energy and currently appear highly useful for bioremediation and biofuel production. Various vital advances have happened in cyanobacterial biotechnology in the current years. Their capacity to develop in exceptionally dirty situations makes these a potential contender for treatment of sewage and modern effluents (Karn 2016). Even in soil bioremediation, microalgae when added result in improving the physicochemical properties such as pH, electrical conductivity, and the important elemental content in the soil such as potassium, phosphorus, and nitrogen (Renuka et al. 2016). Blue-green algae such as *Nostoc*, *Anabaena*, *Gloeotrichia*, *Aulosira*, and *Spirulina* have been used as biofertilizers to fix atmospheric nitrogen in the soil (Priyadarshani and Rath 2012). Microalgae have been found very important in rice cultivation in paddy fields for their intrinsic ability of nitrogen fixation, high yield, eco-friendliness, and low cost (Dineshkumar et al. 2017). *Spirulina platensis* was able to absorb ammonia and nitrate from freshwater and was used as biofertilizer to promote the growth of leafy vegetables such as *Eruca sativa*, *Amaranthus gangeticus*, and *Brassica rapa*. The performance of *Spirulina platensis* as fertilizer was found to be comparable to and even better than that of chemical-based fertilizers (Wuang et al. 2016).

Microalga–bacterial aggregates have been successfully used for wastewater treatment for a long time for their shorter reaction time, easy nutrient uptake ability, and easy resource recovery potential. However, the system is struggling with respect to the poor settlement of the algal biomass and the harvesting problem. Thus, more emphasis should be provided to improve the harvesting potential and resource recovery. Moreover, the microbiological aspects and the methanogenic potential of the microalgal–bacterial aggregates should also be considered (Quijano et al. 2017). Proper schematic culture, establishment, production, and application of cyanobacteria will prove an advantage for environmental biotechnology. As we all know, the rapidly increasing population, the increasing demand for industrial establishments, a development of a luxury lifestyle, and overexploitation of available resources to meet our requirements has created problems such as pollution of the land, air, and water. Therefore, we need to either reduce the pollutant creation or to find simultaneous alternative solutions for remediation. Cyanobacteria are a potential candidate for such problems.

8.2 Industrial Wastewaters: An Overview

Industrial wastewaters contain suspended solid, colloidal, and broken-down (mineral and natural) solids. They might be either too corrosive or soluble and may contain high or low groupings of shaded issue. These squanders may contain dormant, natural, or poisonous materials and conceivably pathogenic microbes. Modern wastewater is released into waterways, lakes, and beachfront areas. This practice has brought about genuine contamination issues in water and caused negative impacts on the ecological framework and human life. Wastewater produced from modern uses can contain toxins, for example, biochemical oxygen demand (BOD), suspended solids, supplements, overwhelming metals, oils, and other dangerous natural and inorganic chemicals (Karn and Chakrabarti 2015). If left untreated, these pollutants can cause serious harm to the environment. Wastewater treatment systems typically employ multi-stage water treatment operations to remove suspended solids, BOD, chemical oxygen demand (COD), coloring agents, heavy metals, greases, and oils. The major pollutants of industrial wastewater are listed in Table 8.1.

Natural modern wastewater primarily contains natural mechanical waste spilled from concoction ventures and vast-scale compound works, which essentially utilize natural substances in synthetic responses. Most natural modern wastewaters are delivered by the accompanying enterprises and plants, for example, pharmaceuticals,

Table 8.1 Most common pollutants present in industrial effluents

Pollutant	Sources
Arsenic	Sheep dipping
Cadmium	Plating
Chromium	Plating, chrome-tanning, alum anodizing
Citric acid	Soft drinks and citrus fruit processing
Copper	Copper plating, copper pickling
Cyanides	Gas manufacture, plating, metal cleaning
Nickel	Plating
Zinc	Galvanizing zinc plating, rubber process
Free chlorine	Laundries, paper mill, textile bleaching
Acetic acid	Acetate rayon, beet root manufacture
Acids	Chemical manufacture, mines, textiles manufacture
Alkalies	Cotton and straw kiering, wool scouring
Ammonia	Gas and coke and chem. Manufacture
Fats, oils, grease	Wool scouring, laundries, textile industries
Fluorides	Scrubbing of flue gases, glass etching
Formaldehyde	Synthetic resins and penicillin manufacture
Hydrocarbons	Petrochemical and rubber factories
Mercaptans mills	Oil refining, pulp
Nitro compounds	Explosive and chemical works
Organic acids	Distilleries and fermentation plants
Phenols	Gas and coke manufacture, chemical plants
Starch	Food processing, textile industries
Sugars	Dairies, breweries, sweet industries
Sulfides	Textile industry, tanneries, gas manufacture
Sulfites	Pulp processing, viscose film manufacture
Tannic acid	Tanning, sawmills
Tartaric acid	Dyeing, wine, leather, chemistry manufacture

beautifying agents, natural dyestuffs, paste and cements, cleansers, engineered cleansers, pesticides and herbicides; tanneries and calfskin processing plants; textile production lines; cellulose and paper fabricating plants; oil refining industry; breweries; and aging industrial facilities (Karn et al. 2011).

8.3 Cyanobacteria: In Degradation of Industrial Pollutants

The organic pollutants that are commonly used in industry and agriculture and are potential sources of degradation by cyanobacteria include phenols, pesticides, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). Using cyanobacteria, even highly toxic compounds such as 2,4,6-trinitrotoluene, nitrophenol, and *p*-nitrophenol, the highly chlorinated aliphatic pesticide lindane, organophosphorus compounds, phthalate esters, and DDT are being removed (Cepoi et al. 2016). Numerous cyanobacterial strains are utilized by different creators in squander water treatment and these under research center conditions (Noue and Proulx 1988; Lee et al. 1995; Rai and Mallick 1992). A few investigations showed that cyanobacterial strains have potential in treating effluents from essential settled swine (Canizares et al. 1991), paper mills, sewage (Manoharan and Subramanian 1992a, b), phenolic compounds (Klekner and Kosaric 1992), dairy (Boominathan and Doran 2003), dye (Vijayakumar et al. 2005), and sago industries (Kasthuri 2008). Several species of microalgae, particularly cyanobacteria such as *Oscillatoria* (Vijayakumar 2005; Manoharan and Subramanian 1992a, b; Boominathan and Doran 2003; Fogg and Thake 1987; Hashimato and Furukawa 1989; Manoharan and Subramanian 1992a, b), *Phormidium* (Blier et al. 1995; Noue and Basseres 1989; Pouliot et al. 1989), *Aphanocapsa* (Boominathan and Doran 2003), and *Westiellopsis* (Vijayakumar 2005) have been successfully used for the treatment of effluents from various industries.

To treat a number of aromatic pollutants and structurally related compounds, recent advances in research have focused on the treatment of distillery wastewaters. The capacity to metabolize phenolics such as lignin or tannins and their ability to degrade melanoidins, the main colored compounds of slops, is also considered. Some enzymatic aspects of phenol and melanoidin biodegradation are also considered (Amores-Sanchez et al. 2015).

Kuritz (1998) observed 15 strains of cyanobacteria from three taxonomic groups that are able to degrade lindane (λ-hexachlorocyclohexane, a recalcitrant pesticide). The degradation pathway was observed from strains of *Anabaena* sp. PCC 7120 and *Nostoc ellipsosporum*. These cyanobacteria dechlorinated lindane first to pentachlorocyclohexene and then into a mixture of trichlorobenzenes. Lindane dechlorination by these organisms occurred only in the presence of nitrate in the medium. Both ammonium and darkness inhibited the process. This combination of observations led us to the hypothesis that the nitrate-reduction system of cyanobacteria may be involved in dechlorination. Ibrahim et al. (2014) reported evidence of the ability of *Aspergillus oryzae*, *Nostoc muscorum*, and *Streptomyces platensis* to biodegrade and utilize malathion (*O*,*O*-dimethyl-*S*-[1,2-di (ethoxycarbonyl) ethyl] phosphorodithioate) as a source of phosphorus. Additionally featured was the productivity of algal strains to develop under high groupings of malathion with improvement of biomass sugar and protein content. Further, *N. muscorum* overtopped alternate strains in expelling more than 90% of malathion.

It was observed that oil-polluted sites are rich in cyanobacterial consortia capable of degrading oil components. Cyanobacteria within these consortia facilitated the degradation processes by providing the associated oil-degrading bacteria with the necessary oxygen, organics, and fixed nitrogen. Cyanobacterial hydrogen has been considered as an extremely encouraging wellspring of elective vitality and has now been made financially accessible. Notwithstanding these applications, cyanobacteria are likewise utilized for aquaculture, wastewater treatment, nourishment, manures, and creation of auxiliary metabolites including exo-polysaccharides, vitamins, poisons, proteins, and pharmaceuticals. Future research should center on confirming new cyanobacterial strains for creating high esteem items and altering genetically existing strains to guarantee the greatest possible generation of the coveted items

(Ferris et al. 1996; Ward et al. 1997; Nubel et al. 1999; Abed and Garcia-Pichel 2001; Garcia-Pichel and Pringault 2001). Several strains of cyanobacteria were found to accumulate polyhydroxyalkanoates, which can be used as a substitute for nonbiodegradable petrochemical-based plastics.

Uma and Subramanian (1990) treated ossein factory effluent that had caused reduced calcium and chloride levels, enabling 100% survival and multiplication of tilapia fish with only cyanobacteria as feed source. Shashirekha et al. (1997) found that *Phormidium valderianum* BDU 30501 was able to tolerate and grow at a phenol concentration of 50 mg/l and removed 38 mg/l within a retention period of 7 days. These results provide evidence of the possibility of treating a variety of phenol-containing effluents (Karna et al. 1999). Microalgae such as *Oscillatoria* have been noted to diminish azo colors by the soluble cytoplasmic reductases known as azo-reductase (Noel and Rajan 2014). These chemicals change azo colors into sweet-smelling amines, and in this manner expulsion is accomplished (Robinson et al. 2001). Cyanobacteria, being a photosynthetic green growth, might be presented as an optional stage for mechanical material-emanating treatment in the material units. EI-Sheekh et al. (2012) observed that the oxidation of phenolic mixes was joined by a shift in wavelength and change in the shading, for example, the oxidation of phenol to catechol by *Volvox aureus*, *Nostoc linckia*, and *Oscillatoria rubescens*. They found that the most noteworthy level of remediation of naphthalene by *N. linckia* was 47.71% following 7 days of hatching. The marine cyanobacteria *Phormidium* sp. expel hexadecane (45%) and diesel oil (37%) from the fluid stage when developed in genuine seawater enhanced with supplements and within the site of these hydrocarbons within 10 days.

The in-part sanitized surface-dynamic operators were created by a *Phormidium* biodisk added to upgrade the potential evacuation of this strain for hexadecane and diesel (Morales and Michel 2014). Cyanobacteria preferred different microorganisms so far as flexibility and development modes (autotrophic, heterotrophic, or mixotrophic). Because of these preferences cyanobacteria are outstanding among other alternatives to corrupt natural contaminations in the marine environment. Cyanobacteria have been tested with various phenolic compounds, with contradictory results; that is, some studies found that these species efficiently remove alkanes and polycyclic aromatic hydrocarbons (PAHs) (Cerniglia et al. 1980; Al-Hasan et al. 1998; Kumar et al. 2001), although other studies found that they do not have a direct role in the removal of phenolic compounds (Chavan and Mukherji 2010). *Microcystis novacekii* is fit for evacuating methyl parathion, an organophosphorus pesticide, from the way of life medium with an extraction rate higher than 90%. The unconstrained altered form was not very large, which demonstrates a high productivity level of organic evacuation. No metabolites of methyl parathion were recognized in the way of life medium at the fixation levels assessed (0.10–2.00 mg/dm^3). The mechanisms proposed to explain pesticide removal are bioaccumulation and mineralization. EC_{50} for 72 h was 17.60 mg/dm^3, much higher than the usual concentrations in surface waters. This finding indicates that *M. novacekii* is highly tolerant of this pesticide and may represent an important bioremediation agent of contaminated environments (Fioravante et al. 2000).

Algae are presently in control of approximately 50% of the origin of O_2 and CO_2 sequestration everywhere. Algae fulfill a critical function in the carbon budget for the entire world. Only a few types of animals can debase polycyclic sweet-smelling hydrocarbons (PAHs) and different constituents of unrefined petroleum and refined oil-based commodities (Cerniglia 1992; Cheung and Kinkle 2001). Others have potential applications in CO_2 sequestration and remediation (Stephens et al. 2010). Algae form an imperative segment of soil microflora and are omnipresent, presumably representing up to 27% of the aggregate microbial biomass in the soil (McCann and Cullimore 1979). They are more far reaching than the other free-living microorganisms equipped for di-nitrogen acquisition and therefore are essential for the nitrogen economy of soils. Noel and Rajan (2014) connected four local types of cyanobacteria, for example, *Nostoc muscorum*, *Anabaena variabilis*, *Lyngbya majuscula*, and *Oscillatoria salina* for bio-treatment, which were refined in BG11 media and titrated into the untreated effluent. Following 25 days of treatment the shading was observed, and a noteworthy increase in pH level and decreases in COD, BOD, calcium, magnesium, sulfate, zinc, and nickel. From this result, it is evident that cyanobacteria can effectively remediate the pollutants from textile industrial effluent. Hydrocarbons can be shown as noticeably unsafe, particularly on the off chance that they enter the natural order, because a few of the more industrious mixes, such as PAH and PCB, are cancer causing (International Agency for Research on Cancer 1983). As per the Stockholm Convention on Persistent Organic Pollutants, 9 of the 21 persistent natural chemicals are pesticides. Classes of natural pesticides incorporate organochlorines, organophosphates, organometallics, pyrethroids, and carbamates, among others (Gilden et al. 2010; Stockholm Tradition 2011). The algae were seen to be powerful in hyper-accumulation of excessive metals and also in debasement of xenobiotics (Suresh and Ravishankar 2004). As of late, the utilization of microalgae in bioremediation of shaded wastewater has attracted awesome enthusiasm because of their focal part in the carbon dioxide obsession. In addition, the algae biomass generated has great potential as feedstock for biofuel production (Huang et al. 2010).

These bioremediation capacities of microalgae are helpful for natural maintainability (Ellis et al. 2012; Lim et al. 2010). The asphaltenes, the most complex hydrocarbons of oil, contain nitrogen, sulfur, and oxygen (Mitra-Kirtley et al. 1993), and are extremely impervious to microbial corruption (Guiliano et al. 2000). Pineda-Flores et al. (2001) found that a microbial consortium segregated from raw petroleum could take quicker and more noteworthy oxygen utilization when it was blended to phaltenes as the main wellspring of carbon and vitality, and can corrupt the unrefined petroleum as well. Some microalgae create proteins equipped for debasing unsafe natural mixes to change the oil hydrocarbons into less lethal mixes (Davies and Westlake 1979).

Hong et al. (2008) observed the accumulation and degradation abilities of the polycyclic aromatic hydrocarbon (PAH) compound fluoranthene (FLA) by the two algal species *Nitzschia* sp. and *Skeletonema costatum*. He observed *Nitzschia* sp. degraded FLA more than did *S. costatum*, indicating that FLA was a more recalcitrant PAH compound. The microalgal species additionally demonstrated practically identical or higher productivity in the evacuation of the PAH–FLA blend contrasted with PAH or FLA alone, suggesting that the propinquity of one PAH fortified the altered form of the other.

Munoz and Guieysse (2006) proposed that it is conceivable to utilize microalgae to deliver the O_2 required by acclimatized microbes to biodegrade risky poisons, for example, polycyclic aromatic hydrocarbons, phenolics, and natural solvents. At the point when PAH are taken up by microorganisms, they initiate vigorous digestion by addition of two oxygen particles by microbes and green growth to deliver either *cis*-dihydrodiols or phenols (Al-Turki 2009). Cerniglia et al. (1980) studied the oxidation of naphthalene by cyanobacteria and microalgae.

Further, nine cyanobacteria, five green algae, one red alga, one brown alga, and two diatoms could oxidize naphthalene under photoautotrophic conditions, suggesting that the ability to oxidize naphthalene is widely distributed among the algae. The PAH phenanthrene (PHEN) is an exceptionally poisonous contaminant, usually found in oceanic situations, the impacts of which on sea-dwelling plants have not been examined thoroughly (Burritt 2008). The green alga *Chlamydomonas reinhardtii* demonstrated an awesome capacity to gather and debase the herbicide prometryne (Jin et al. 2012). Moro et al. (2012) contemplated the impact of the herbicides chlortoluron and mesotrione on three microalgae species: two species of Chlorophyceae (*Pediastrum tetras*, *Ankistrodesmus fusiformis*) and one diatom (*Amphora coffeaeformis*). Chlortoluron demonstrated a huge inhibitory impact on the development of *A. coffeaeformis*, although mesotrione instigated an expansion in cell thickness in *A. fusiformis*. The role of the algae *Chlorococcum* sp. and *Scenedesmus* sp. in degradation of α-endosulfan (a cyclodiene insecticide) was convincingly demonstrated when these algae degraded α-endosulfan to endosulfan sulfate, the major metabolite, and endosulfan ether, a minor metabolite, in a defined liquid medium (Sethunathan et al. 2004). At the point when a very thick algal inoculum was utilized, the two metabolites seemed to experience or promote corruption as evident from their collection of just small amounts and the presence of an endosulfan-determined aldehyde.

Hussein et al. (2016) observed, in long-term study, the nearness of developing green growth brought about expulsion rates of pesticides from 87% to 96.5%, whereas in short-term examination, the nearness of live green growth cells prompted expulsion rates from 86% to 89%, and dead green growth biomass accomplished expulsion extended from 96% to 99%. The main mechanism behind the removal of pesticides in the water phase is proposed to be absorption into the algal cells. The disengaged microalga species *Oscillatoria limnetica* was developed in BG-11 media for biomass creation and to test their capacity to degrade glyphosate, which is the generally utilized organophosphorus herbicide by superior fluid chromatography investigation. There were noteworthy contrasts in the rate of glyphosate expulsion of the medium after 3, 5, 7, 14, and 35 days after all medicines. It was 97.5% after 7 days at 5 mg/l treatment, 57.9% and 37.1% after 14 days at 10 mg/l and 15 mg/l treatments, respectively, and 99.9% after 35 days at 20 mg/l treatment. Maximum removal efficiency percentages of isolated species were increased to reach their maximum in all treatments after 35 days (Salman and Abdul-Adel 2015).

Glyphosate-based herbicides are the world's leading broad-spectrum, post-emergent, and nonselective herbicides for the control of annual and perennial weeds in agricultural lands, ornamental and residential gardens, and aquatic systems (Lipok et al. 2010). Nitrate might be taken up successfully by photosynthetic microorganisms, for example, cyanobacteria, which require generally settled nitrogen, inorganic carbon, and light for development; the utilization of photosynthetic life forms would limit the need of chemicals and nonrenewable energy sources for nitrate expulsion, subsequently prompting effective asset recuperation and reuse. *Synechococcus* sp. strain PCC 7942 showed the most noteworthy nitrate take-up rate, yet all species indicated fast expulsion of nitrate from groundwater. Nitrate (NO_3) fixations in groundwater have expanded comprehensively (Kapoor and Viraghavan 1997). Groundwater in numerous areas is utilized as a supply for drinking water, and high nitrate fixation is a potential hazard to general well-being, especially to newborn children (Gangolli et al. 1994). The cyanobacteria played an important role in their removal. Several investigations have shown the accomplishment of utilizing the algal societies to expel supplements from wastewater rich in nitrogenous and phosphorous mixes (Neos and Varma 1966), and consequently they have been utilized widely in adjustment lakes and in tertiary treatment of sewage for the expulsion of toxins from the wastewater.

Nitrate might be taken up successfully by photosynthetic microorganisms, for example, cyanobacteria, which generally require settled nitrogen, inorganic carbon, and light for development; the utilization of photosynthetic life forms would limit the need of chemicals and nonrenewable energy sources for nitrate expulsion, subsequently prompting effective asset recuperation and reuse. *Synechococcus* sp. strain PCC 7942 showed the most noteworthy nitrate take-up rate, yet all species indicated fast expulsion of nitrate from groundwater. Nitrate (NO_3) fixations in groundwater have expanded comprehensively (Kapoor and Viraghavan 1997). Wastewater, manures, and domesticated animals are real wellsprings of nitrate in groundwater supplies (Hem 1992). Groundwater in numerous areas is utilized as a supply for drinking water, and high nitrate fixations display a potential hazard to general well-being, especially to newborn children (Gangolli et al. 1994). There, cyanobacteria had an important role in their removal.

Halophilic cyanobacteria produce many exo-polysaccharides (EPS), which can be significant to oil recuperation by diminishing its surface strain and expanding its solvency and portability. EPS, when gelated under antacid conditions, was utilized to expel colors emanating from materials. The halophilic cyanobacterium *Aphanocapsa halophytica* was utilized for the creation of EPS, and its yield was expanded tenfold by immobilizing the cells on light-diffusing optical filaments (Matsunaga et al. 1996). The cyanobacterium *Cyanothece* sp. ATCC 51142 had maximum EPS production at 45% (w/v) NaCl and pH 7 (Shah et al. 1999). Other EPS-producing cyanobacteria are the halotolerant *Anabaena* sp. ATCC 33047 (Moreno et al. 1998) and *Synechococcus* sp. (Matsunaga et al. 1991). It is necessary in future research to investigate the potential of additional cyanobacteria in EPS production. The roles of different cyanobacteria for the removal of organic pollutants are also illustrated in Table 8.2.

Table 8.2 Role of cyanobacteria in removal of organic compounds

Species	Organic pollutant	References
Agmenellum quadruplicatum	Bisphenol	Cerniglia et al. (1979)
Amphora coffeaeformis	Herbicide (mesotrione)	Moro et al. (2012)
Ankistrodesmus fusiformis	Mesotrine	Moro et al. (2012)
Chlamydomonas reinhardtii	Herbicide (fluroxypyr)	Zhang et al. (2011)
Chlamydomonas reinhardtii	Herbicide (prometryne)	Jin et al. (2012)
Chlorococcum	Alpha-endosufan	Sethunathan et al. (2004)
Monoraphidium braunii	Bisphenol	Gattullo et al. (2012)
Microcystic novacekii	Methyl parathion	Fioravante et al. (2000)
Nostoc linckia	Napthalene	El-Sheekh et al. (2012)
Oscillatoria	Azo dyes	Noel and Rajan (2014)
Oscillatoria limnetica	Glyphosate	Salman and Abdul-Adel (2015)
Oscillatoria rubescens	Napthalene	El-Sheekh et al. (2012)
Pediastrum tetras	Chlortoluron	Moro et al. (2012)
Phormidium	Hexadecane, diesel oil	Morales and Michel (2014)
Phormidium valderianum	Phenol	Shashirekha et al. (1997)
Phytoplankton	Chlorinated hydrocarbons	Harding and Phillips (1978)
Scenedesmus obliquus GH2	Crude oil degradation	Tang et al. (2010)
Selenastrum capricornutum	Benzene, toluene, naphthalene	Gavrilescu (2010)
Scenedesmus	Alpha-endosufan	Sethunathan et al. (2004)
Spirulina platensis	Malathion	Ibrahim et al. (2014)

(continued)

Table 8.2 (continued)

Species	Organic pollutant	References
Cyanobacteria	Phenols, pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCB)	Cepoi et al. (2016)
Cyanobacteria	2,4,6-Trinitrotoluene, nitrophenol, *p*-nitrophenol	Cepoi et al. (2016)
Cyanobacteria	Chlorinated aliphatic pesticides, lindanes, organophosphorus compounds, phthalate esters, DDT	Cepoi et al. (2016)
Nostoc ellipsosporum	λ-Hexachlorocyclohexane	Kuritz (1998)
Aspergillus oryzae, *Nostoc muscorum*, *Streptomyces platensis*	Malathion (*O*,*O*-dimethyl-*S*-[1,2-di (ethoxycarbonyl)ethyl]phosphorodithioate)	Ibrahim et al. (2014)
Phormidium valderianum BDU	Phenol	Shashirekha et al. (1997)
Nitzschia, *Skeletonema. costatum*	Polycyclic aromatic hydrocarbons (PAH), fluoranthene (FLA)	Hong et al. (2008)
Synechococcus	Nitrate	Kapoor and Viraghavan (1997)
Volvox aureus, *Nostoc linckia*, *Oscillatoria rubescens*	Naphthalene	EI-Sheekh et al. (2012)
Algae	Xenobiotics	Suresh and Ravishankar (2004)

8.4 Removal of Heavy Metals from Industrial Wastewaters Using Cyanobacteria

Substantial metals are components having nuclear weights in the vicinity of 63.5 and 200.6, and a specific gravity greater than 5.0 more than water. Living creatures require the following measures of some substantial metals, including cobalt, copper, press, manganese, molybdenum, vanadium, strontium, and zinc. Intemperate levels of fundamental metals, in any case, can impede living beings. Substantial metals that are generally abundant in the Earth's outside layer and occasionally utilized as a part of mechanical procedures or horticulture are poisonous to people; these can make significant alterations in the biochemical cycles of living things (Srivastava and Majumder 2008). Most of the point sources of heavy metal pollutants are industrial wastewater from mining, metal processing, tanneries, pharmaceuticals, pesticides, organic chemicals, rubber and plastics, lumber and wood products, etc. (Bailey et al. 1999; Khraisheh et al. 2005; Sekhar et al. 2004; Mohammadi et al. 2005). Heavy metals can bind to the surface of microorganisms and may even penetrate inside the cell.

Inside the microorganism, the excess metals can be synthetically changed as the microorganism utilizes compound responses to process sewage water, and residues demonstrate shocking levels of natural issue. Pathogens, composts, pesticides, and substantial metals utilize an organic framework as a modest and proficient method for supplements and expulsion of excess metals from wastewaters. Mechanical effluents are the real wellsprings of copper in nature. It is imperative to detect copper levels in water because abundant amounts of copper in the human body are related to unfriendly well-being impacts, for example, liver and kidney harm, weakness, schizophrenia, and Alzheimer's disease. Heavy metals even show poisonous impacts toward soil biota by influencing key microbial procedures and reducing the number and movement of soil microorganisms. Substantial metals cause harmful consequences for soil microorganisms, henceforth bringing about the difference in the assorted variety, populace size, and general action of the soil microbial groups (Ashraf and Ali 2007). Increased Pb in soils may diminish soil efficiency and a low Pb level may repress some imperative plant actions, that is, photosynthesis, mitosis, and water retention with poisonous side effects of dim green leaves, withering of more established leaves, distressed foliage, and dark-colored short leaves and roots (Bhattacharyya et al. 2008).

Heavy metal contamination is not only an outcome with unfriendly consequences for different parameters identifying with plant quality and yield, but also causes changes in the size, structure, and movement of the microbial group (Yao et al. 2003). Substantial metal contamination of the soil is caused by different metals, particularly Cu, Ni, Cd, Zn, Cr, and Pb (Hinojosa et al. 2004). Cr(VI) is a solid oxidizing operator and is very harmful, whereas Cr(III) is a micronutrient and a non-damaging animal variety 10–100 times less lethal than Cr(VI). Cr(VI) has been reported to cause shifts in the composition of soil microbial populations and is known to cause detrimental effects on microbial cell metabolism at high concentrations (Shun-Hong et al. 2009). All in all, an expansion of metal focus antagonistically influences soil microbial properties, such as breath rate, and chemical action, which seem, by all accounts, to be exceptionally valuable pointers of soil contamination. Retention by plant roots is one of the primary courses of passage of substantial metals in the natural pecking order. Seed germination was gradually delayed in the presence of increasing concentration of lead (Singh and Kalamdhad 2011). Heavy metals such as mercury, lead, tin, cadmium, selenium, and arsenic are introduced to nature by various human activities and are stored gradually in the surrounding water and soil (Babel and Kurniawan 2003; Sergeev et al. 1996; Sorme and Lagerkvist 2002; Gupta et al. 2015).

In many developing countries, little attention is paid to environmental issues so that the drainage of wastewater into lakes and rivers is very common. These uncontrolled activities cause poisoning of freshwater resources, which affects the entire ecosystem. Industrial wastewater has a much greater potential for environmental damage than their relative part in municipal wastewater. Industrial wastewater may contain different pollutants with a wide range of effects. These effects may include damage to transmission and treatment systems of municipal wastewater, damage to the quality of municipal-treated wastewater, or the contamination of soil and groundwater.

Cyanothece sp. CCY 0110, a unicellular and marine cyanobacterium, beforehand appeared to be a very proficient discharged polysaccharide (RPS) maker engaged with the expulsion of copper, lead, and cadmium, excessive metals ordinarily found in dirtied waters. RPS is a key player in metal evacuation, revealing that bioremediation for the most part happens by biosorption. Some copper was also accumulated in the cells as this is an essential metal for cell metabolism. Extracellular polymeric substances (EPS)-producing cyanobacteria or their isolated polymers can be useful to remove heavy metals from polluted waters. The effects of the presence and concentration of copper, lead, cadmium, and lithium, heavy metals commonly found in polluted water bodies, were evaluated in the cells of *Cyanothece* sp. CCY 0110. The results revealed that each heavy metal affected the cells in a different manner, copper being the more deleterious, followed by lead, cadmium, and lithium. In the presence of copper, the cells lower their metabolic rate to invest energy in the activation of detoxification mechanisms, resulting in a remarkable recovery.

In contrast, the toxic effects of cadmium were cumulative, revealing lower efficiency of the mechanisms of a nonessential metal detoxification compared to those of an essential one. The knowledge generated by this study can contribute to the implementation of heavy metal removal systems based on cyanobacterial EPS or their isolated polymers (Mota et al. 2016). The *Oscillatoria* sp. culture used as a low-cost biomass sorbent for the removal of Cd(II) heavy metal ions from aqueous solutions in batch experiments showed that *Oscillatoria* sp. has a remarkable ability to take up the Cd(II) heavy metal ion. Biosorption capacities for Cd(II) were strongly dependent on the pH of the solution. The dried biomass of *Oscillatoria* sp. showed a higher biosorption capacity than the native one. Heavy metal-resistant *Oscillatoria* sp. has a significant role in bioremediation of heavy metals in wastewater.

Anabaena variabilis showed a good capacity of Zn^{2+} ion biosorption, highlighting its potential for the effluent treatment process. It was demonstrated in free and immobilized *A. variabilis* embedded in calcium alginate has a strong effect on biosorption capacity that is maximum at pH 8. A desorption study showed that 85–95% of Zn^{2+} ions can be desorbed from the biosorbent using 0.1 M EDTA. Qualitative analysis of the functional groups such as carboxyl, hydroxyl, amino, amide, and amine groups was responsible for absorption of Zn^{2+} ions. The study indicated that algae biomass can be used to develop high-capacity biosorbent materials for the removal and recovery of Zn^{2+} ions from dilute industrial wastewater (Gaur and Dhankhar 2009). Beta strains of *Actinomycetes* and *Streptomyces* sp. 19H for biosorption of cadmium, chromium, silver, and gold form watery arrangements.

The examination of these outcomes affirmed that the chosen life forms were productive in retention of overwhelming metals. The proficiency of biosorption of Cr(III), Cr(VI), Cd(II), Au(III), and Ag(I) particles likely relies upon the species utilized and in addition on the conditions under which the take-up forms continued. Study of three metals (Cd, Hg, Pb) using cells of *Oscillatoria limosa* indicate that the order of uptake of Cd, Hg, and Pb was 82%, 78%, and 72%, respectively. The study also indicated that the metal uptake appeared to be a concentration-independent phenomenon wherein an increase in metal concentration resulted in an increased uptake of metal (Sivakami et al. 2015). Many cyanobacteria produce extracellular polymeric substances (EPS) mainly of polysaccharidic nature. The ability of the EPS-producing cyanobacteria to remove heavy metals from aqueous solutions has been widely reported in recent years. Further, the effect of different concentrations of Cu^{2+} and/or Pb^{2+} was evaluated on the growth/survival of *Gloeothece* sp. PCC 6909 and its sheath, the less mutant *Gloeothece* sp. CCY 9612.

The outcomes acquired obviously demonstrate that the two phenotypes are more seriously influenced by Cu^{2+} than by Pb^{2+}, and that the mutant is more sensitive to the previous metal than the wild type. Apparent ultra-auxiliary changes were similarly seen in the wild and mutant cells presented to abnormal states (10 mg/l) of Cu^{2} $^{+}$. Also, in bi-metal frameworks, Pb^{2+} was specially evacuated in contrast with Cu^{2+}, being the RPS of the mutant that is the most effective polysaccharide division in metal expulsion (Pereira et al. 2011). The microalga *Spirulina platensis* (cyanobacterium) has been accounted for as a biosorbent for expulsion of a few excessive metals from mechanical effluents. The cyanobacterium was presented to obscure centralizations of zinc. Adsorption of substantial metal, in particular Zn, by the biomass was assessed under various conditions that included pH, contact time, temperature, centralization of adsorbate, and the grouping of dry biomass. The adsorption of heavy metals was found to increase gradually along with a decrease in biomass concentration. The biosorption of zinc was estimated as the maximum in effluent containing 100 mg of dry biomass for 1 l (0.1 g/l) at pH 8 at 35 °C with the optimal contact time of 60 min. The present examination portrays Zn retention by biomass of *Spirulina platensis* that has been utilized to expel numerous toxins from effluents by assimilation (Palaniswamy and Veluchamy 2017).

A blue-green growth, *Oscilfaloria* sp., and the green growth *Chara* sp. have been utilized as a part of the present examination to survey their capacity to expel Ni (II) particles from the effluents having a high centralization of Ni and the impact of this metal on the dry issue substance of the green growth. *Oscillatoria*, being blue-green growth, proficiently evacuated more Ni(II) particles than *Chara* from the mechanical wastewater (Parikh and Rao 2005). Cyanobacterial strains such as *Oscillatoria* sp., *Nostoc* sp., *Anabaena* sp., *Gloeocapsa* sp., *Plectonema* sp., and *Gloeothece* sp. were used for bioremediation of wastewater and chromium sorption The observed results show that all the algal isolates show good chromium biosorption efficiency. All the algal isolates showed biosorption efficiency in the range of 50–80%. Among various isolate algal strains, *Gloeothece* sp. PL-5 shows maximum biosorption efficiency of chromium, that is, 81.7% at 30 °C after 72 h of incubation under shaking conditions (Gahlout et al. 2017).

Shashirekha et al. (1997) found that *Phormidium valderianum* BDU 30501 was able to tolerate and be effective for optimal sorption/desorption of heavy metal ions (Cd^{2-}, $Co^{2-)}$ (Karna et al. 1999). Cyanobacteria, as one of the micro-living materials, have good potential for eliminating heavy metals from freshwater (Inthorn et al. 2002). Anjana et al. (2007) studied the bioabsorption of chromium from metal-contaminated soil in the premises of a textile mill by using the cyanobacteria strains *Nostoc calcicola* and *Chroococcus* sp. Raungsomboon et al. (2008) tried to remove lead from an aquatic environment using the cyanobacterium *Gloeocapsa* sp. Biosorption of Cd (Borisev et al. 2009), Ag (Qureshi et al. 1996), and Au (Ali and Saleh 2012) was determined by the cyanobacteria *Spirulina platensis*; the optimum pH value was 7.0 for the removal of Ag and Cd by *Spirulina platensis*. The results depend on the species used as well as on the conditions under which the uptake processes proceeded. It was shown that metal removal by microorganisms was influenced by physicochemical parameters.

Microcystis, an abundantly occurring nuisance cyanobacterium in many eutrophic ponds and reservoirs of India and other tropical countries, is responsible for unpleasant odor, fish kill caused by the sharp decrease in oxygen content, and the death of wild birds and cattle from ingestion of the toxins produced by certain strains. However, it appears to be a potential candidate for use as a metal biosorbent (Pradhan and Rai 2000). This cyanobacterium occurs in the natural immobilized state because it has a capsule that has tremendous potential for interaction with cations (Singh et al. 2000, 2001). *Microcystis* was able to remove all the tested metals to some extent (24–76%), but bivalent metals were not removed as efficiently as reported in batch cultures. *Microcystis* showed efficiency in metal removal from single bimetallic as well as multi-metallic conditions. Because *Microcystis* is a nuisance and bloom-forming cyanobacteria, its biotechnological exploitation in metal removal at the field scale should follow an eco-sustainable approach. Kumar et al. (2013) observed the efficiency of *Dolichospermum flos-aquae* NTMS07 in the removal of Cr(VI) from exposed water at various concentrations (2.5, 5, 7.5, 10 mg/l) over different time intervals of contact (1–5 days). Further observed chromium removal was maximum at 2.5 mg/l, and decreased with increased concentration. Chromium (Cr) is an important heavy metal often critically contaminating soil and water. Its contamination in the environment has increased as a result of such activities as metal plating, anodizing, ink manufacture, improper disposal of dyes, pigments, glass, ceramics, glues, tanning, wood preserving, textiles, and corrosion inhibitors in cooling water.

Kiran and Thanasekaran (2012) demonstrated Cu(II) removal by biosorption using acid-treated *Nostoc muscorum* and confirmed that Cu(II) removal by *N. muscorum* is the result of its surface binding, showing excellent potential for heavy metal removal from wastewater. For instance, copper at a concentration higher than 1 mg/l can cause several liver-related ailments in humans. *Synechococcus* sp. PCC 7942 exhibited extraordinary resistance to arsenate.

Conceivable systems incorporate avoidance from the cell, intra- and additional cell authoritative, and potential cooperation with protein, for example, metallothioneins, which tie and detoxify such divalent metal cations as Cd^{2+}, Cu^{2+}, and Zn^{2+}. *Synechococcus* sp. PCC 7942 communicates flag-bearer RNA for metallothioneins because of these divalent metal particles at different fixations. Be that as it may, the reaction to arsenate happens just when cells are refined in phosphate-constrained media. This result proposes that under phosphate-adequate conditions arsenate is particularly avoided by the cell (Ybarra and Webb 1998). Overall consideration is drawn toward cyanobacteria for their conceivable utilization of sustenance, manure, fuel, bolstering and shading generation, separated from different auxiliary metabolites including vitamins. Cyanobacteria are the selectors, potential organisms that are valuable for us from numerous aspects.

8.5 Conclusion

Cyanobacteria have gained much attention in recent years because of their potential applications in wastewater treatment. Several strains of cyanobacteria were found to accumulate and detoxify different organic and inorganic pollutants. Studies also confirm that cyanobacterial consortia are capable of degrading the components of oil. There are still many unknown cyanobacteria that have tremendous degradation capacity for organic compounds present in nature, and it is important to assess the potential of cyanobacteria isolates indigenous to sites contaminated with both organic and inorganic pollutants. Cyanobacteria are one of the potential organisms useful to mankind in various ways. Cyanobacteria constitute a vast potential resource in varied applications such as combating pollution, marine culture, food, feed, fuel, fertilizer, medicine, and industry.

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Chapter 9
Constructed Wetlands: An Emerging Green Technology for Phosphorus Treatment in Industrial Wastewater

Valentina Carrillo, Ana María Leiva, and Gladys Vidal

Abstract In recent decades, constructed wetlands (CWs) have been used successfully for the treatment of industrial wastewater. This work reviewed the phosphorus (P) removal of industrial wastewater using an emerging green technology such as CWs. The removal of P in the CWs is generated through different transformations of P in the soil and water column, which includes processes such as sorption, desorption, precipitation, decomposition, fragmentation, leaching, mineralization, sedimentation, and assimilation of plants and microorganisms. The total phosphorus (TP) removal efficiencies of different industrial wastewaters in CWs showed values from 26% to 68%. The highest efficiencies (55–68%) were shown in the wastewater of industries from animal and agricultural origin, except for the wastewater of the swine industry that had a removal value of 37%. It should be noted that there is lack information about the P removal from industrial effluents in CWs. This situation can be explained because the objective of different researches is focused on the organic matter and nitrogen removal which makes it difficult to compare the performance for removing P in industrial wastewater.

Keywords Constructed wetlands · Industrial wastewater · Phosphorus removal · Leaching · Mineralization · Sedimentation

9.1 Introduction

Today's society has seen human civilizations emerge, develop, and prosper. Human actions such as the intensification of industrialization, the depletion of resources, and the inefficient use of nutrients for food production have altered the global biogeochemical cycles reaching a level that damages the systems that maintain the Earth

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(Rockström et al. 2009). Phosphorus (P) is a fundamental nutrient that increases productivity in agriculture and sustains the food security of a growing world population (Cordell et al. 2009).

Currently, modern agriculture is increasingly dependent on the use of chemical fertilizers of P. However, these fertilizers are produced from a nonrenewable resource such as mineral deposits of phosphate rock, which could be depleted within the next decades. On the other hand, P is one of the main pollutants, which produces the growth of algae and combined with nitrogen (N) causes serious problems of eutrophication on water resources and ecosystem services that they provide (Falahati et al. 2018). Global anthropogenic P loads are distributed around the world by 52% in Asia (30% in China). Secondly, Europe is contributing approximately 19%, followed by Latin America and the Caribbean with 13% and North America (7%). Moreover, the largest contribution of global anthropogenic P comes from the domestic sector (54%), agriculture (38%), and industry (8%) (Mekonnen and Hoekstra 2018).

Industrialization and effluent production have potentially significant impacts on water resources, human health, and the environment (WWAP 2017). Social pressures have led to a continuous movement that pressures the industry to reduce the amount of wastewater and treat it. Many industries have opted for sustainable industrial development through ecology, green or non-conventional technologies (Wu et al. 2015b). Constructed wetlands (CWs) are technologies that provide a natural, environmental, and sustainable treatment of wastewater. They also stand out for having an effective cost due to low operational and maintenance costs (Auvinen et al. 2016; Saeed et al. 2018).

CWs have been denominated biologically by treatments designed to mimic the physical, chemical, and biological processes of natural wetlands and remove excess nutrients such as nitrates, phosphates, pollutants, toxic substances, and pathogens from industrial and municipal wastewater. The treatment is based on the interactions between substrates, plants, and microorganisms (Vymazal 2007, 2009).

This chapter proposes to review the use and the removal efficiencies of P in different CWs treating various industrial wastewaters, such as industries of animal, agricultural, and other types of industries. It has been shown that CWs are not only effective for domestic wastewater but also, they have been tested in different industrial effluents (Saeed et al. 2018; Vymazal 2009, 2014). However, the knowledge on the use of CWs for P removal in several industrial wastewaters is insufficient, because they do not consider the P as the main objective of treatment.

9.2 The Presence of Phosphorus in Industrial Wastewater

With the development of industrial activities, the consumption of fresh water has increased. Volumes of wastewater generated will be doubled by 2025 (UNEP FI 2007). The industrial wastewater consumption varies according to their production capacity and processing technologies. For example, it is estimated that pulp and paper industry produces 178–303 m^3/d of wastewater (Chandra and Singh 2012), while the industries of animal origin generate approximately 50–120 m^3/d (Cooman et al. 2003; Gutiérrez-Sarabia et al. 2004; Villamar et al. 2013).

The potential impact of industrial wastewater does not depend directly on the amount of discharged volume but rather on the quality of the wastewater. Pollutants from industrial wastewater differ substantially in their composition. These vary according to the sources of raw material and manufacturing processes that are carried out. Different types of pollutants can be found such as sulfides, organic matter, solids, inks, dyes, phenols, heavy metals, herbicides, insecticides, nutrients, proteins, fatty acids, salts, oils, hydrocarbons, surfactants, biocides, organic pollutants, sterols, phenols, polyphenols, triterpenes, and pathogenic microorganisms, among others (Saeed et al. 2018; Cai et al. 2013).

Table 9.1 shows some characteristics of industrial wastewater, such as pH values, conductivity, organic matter, and nutrients. According to their origin, the industries can be divided into animals such as dairy manure, swine, abattoir, poultry, and aquaculture. In the case of the agriculture industry, the production of winery and olive mill has been included. Finally, there are other industries such as textile, tannery, and pulp and paper that have been evaluated.

Livestock wastewaters from abattoir, swine, poultry, and dairy manure industries, among others, have organic loads greater than 1 g/L of chemical oxygen demand (COD) that include proteins, fats, and oils. In the same way, agricultural industries such as the production of winery and olive mill do not have the reputation of being a polluting industry. However, its effluents achieve COD concentrations above 10 g/L and with low pH values of 4.6–5.5. These values indicate that this type of wastewater represents a potential risk to the environment if they are discharged without any type of treatment (Mosse et al. 2011; Coskun et al. 2010).

Commonly, industrial wastewaters suffer from a nutrient deficit, which can generate a bulking effect and a reduction in treatment efficiency. For an effective treatment, the COD/N/P ratio should be close to 100/2.5/0.5 (Serrano 2005). For example, tannery wastewaters have an average COD/N/P ratio of 200/22/2 and showed that the soaking liquor contained a greater amount of N but lower amounts of P. In this case, the effluents have a lower biodegradability (Lefebvre et al. 2005).

Regarding the physicochemical characteristics of these effluents, the agricultural industries that include high livestock production, and the agriculture of vegetal origin, present the higher concentration of nutrients (N and P). On the one hand, in livestock, factors such as diet, age, growth phase, productivity, and management of animals modify the nutrient content in animal wastewater (Villamar et al. 2013). In this type of industry, PT and total nitrogen (TN) concentrations vary between 0.4 and 446 mg/L and 98–3570 mg/L, respectively (Calheiros et al. 2009; Yetilmezsoy and Sakar 2008; Finlayson and Chick 1983; Neubauer et al. 2012).

The higher content of P stands out mostly in manure and wastewater runoff contaminated with manure. Table 9.1 shows that TP concentrations reach values in the industry of animal origin that vary between 226 and 446 mg/L (Yetilmezsoy and Sakar 2008; Wang et al. 2010). Successively, agricultural runoff shows TP

Table 9.1 Physicochemical characteristics of different industrial wastewaters

Type of water	Physicochemical characteristics								
	COD (mg/L)	pH	Conductivity (mS/m)	TN (mg/L)	NH_4^+ (mg/L)	NO_3^--N (mg/L)	TP (mg/L)	PO_4^{-3}-P (mg/L)	References
Olive mill	40,300–153,000	4.6–7.4	425–530	131–532	24.3	194	64–182	–	Coskun et al. (2010), Ammary (2004), Yalcuk et al. (2010), Del Bubba et al. (2004)
Winery	15,553–49,105	5.0–5.5	190–193	35–110[a]	–	–	35–52	–	Mosse et al. (2011) and Bustamante et al. (2005)
Abattoir	3633–6100	6.8–7.4	198–290	530–810[a]	57	1.6	15–50	28	Gutiérrez-Sarabia et al. (2004) and Gannoun et al. (2009)
Poultry	12,052	6.9–7.3	86–449	98–1825	34–987	0.08	15–446	8	Finlayson and Chick (1983) and Yetilmezsoy and Sakar (2008)
Swine	1400–28,271	7.2–8.1	460–2020	453–3570	410–2540	0.5–1.5	13.8–193.6	–	Plaza de los Reyes and Vidal (2015), Villamar et al. (2015), Plaza de los Reyes et al. (2014) and Neubauer et al. (2012)
Dairy manure	1219–38,230	7.0–7.6	260	65–3305	22–200	0.3	12.8–266	–	Falahati et al. (2018), Mantovi et al. (2003), Villamar et al. (2013) and Wang et al. (2010)
Pulp and paper	136–22,189	6.8–8.1	281–384	0.53–24	0.4	–	–	24	Lefebvre et al. (2005), Calheiros et al. (2009) and Cooman et al. (2003)

Aquaculture	30–96	7.3–7.5	11.2–68	–	0.2–5	0.7–40	0.29–0.35	0.1	Chandra and Singh (2012), Jarpa et al. (2012), Arivoli et al. (2015) and Chamorro et al. (2010)
Tannery	2200–2840	7.7–8.4	1005–5750	160[a]–390	98–255[b]	0.3–59	0.4–21	7	Comeau et al. (2001), Ansari et al. (2017) and Schulz et al. (2003)
Textile	326–1714	7.0–10	1250	62	1.7	3.6	–	17	Ong et al. (2009) and Punzi et al. (2015)

[a] Values in TKN in this range

[b] Values in ammonia in this range

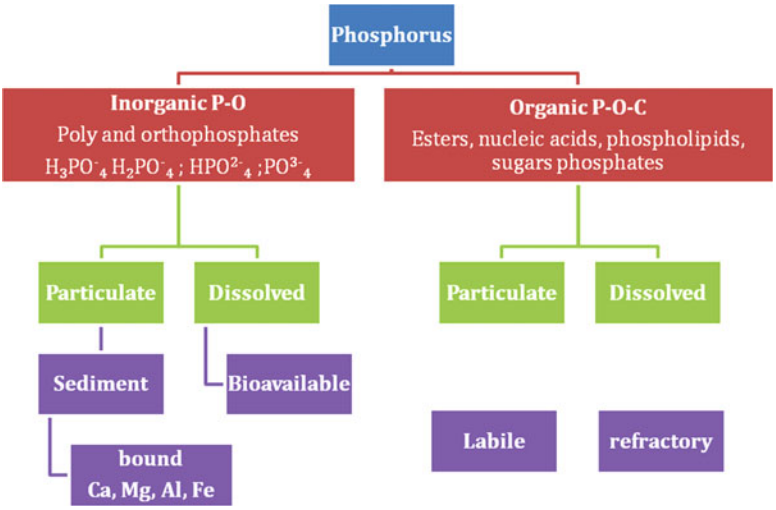

Fig. 9.1 Different forms of P in water. (Adapted from Vymazal and Kröpfelová 2008; DeLaune and Reddy 2008)

concentrations ranging from 52 to 182 mg/L (Coskun et al. 2010; Ammary 2004; Del Bubba et al. 2004; Mosse et al. 2011; Bustamante et al. 2005). These values are considered high concentrations of TP compared to domestic wastewater which vary between 5 and 30 mg/L (Vidal and Hormazábal 2018; Cai et al. 2013). The lowest concentrations of TP were reported in industries as aquaculture with values that fluctuated between 0.29 and 0.35 mg/L. In this case, large volumes of wastewater (10,000 m^3/d) play a fundamental role in diluting concentrations (Comeau et al. 2001; Schulz et al. 2003).

TP is an environmental indicator for water, where approximately 70% is found as inorganic P and 30% as organic P in domestic wastewater (Valsami-jones 2004). Figure 9.1 shows the different forms of P. Organic P is poorly characterized, and it is diverse with respect to size and structure, ranging from simple organic phosphorus compounds such as sugar phosphates, phospholipids, and nucleic acids to phosphate esters. The particulate and soluble organic P can be divided into refractory and labile. The last one is generally transformable to inorganic P (DeLaune and Reddy 2008).

Likewise, inorganic P is the natural form of P, as orthophosphate ions. Orthophosphate is the most readily available form of phosphate for biological absorption. However, it is generally found in poorly soluble forms, which can be bound to Ca, Mg, Fe, and Al depending on the pH range (DeLaune and Reddy 2008).

The specific characteristics of several industrial wastewaters make them difficult to treat and often cause serious treatment limitations. Tannery wastewater is characterized by saline effluents with a conductivity of 1005–5780 mS/m (Cooman et al. 2003). The biggest conflict of the abattoir industry in water treatment is the high content of total solids that vary between 5060 and 5400 mg/L (Gannoun et al. 2009). The presence of high content of salts and solids affects adversely the biological processes in wastewater treatment systems (Lefebvre et al. 2005). Therefore, it becomes necessary stages of pretreatments to reduce the risk in the following stages of treatment.

The conflict in agricultural wastewater is related to the content of herbicides, fungicides, insecticides, and nitrate and phosphate components of agricultural operations. They can contain particles, ions, dissolved molecules, phytotoxic phenolic substances, antibacterial, and microorganisms which are discharged into the receiving water reducing significantly the water quality (Del Bubba et al. 2004). Dissolved organic components and colloidal materials are removed during secondary treatment by biological or chemical treatments, while the removal of dissolved inorganic components, such as N and P, takes place in the process of advanced treatment or biological treatment (Cai et al. 2013).

9.3 Treatment Technology: Constructed Wetlands

9.3.1 Basic Concepts of Constructed Wetlands

The treatments based on CWs have turned out to be an attractive solution for the treatment of domestic wastewater (Vymazal 2010). They have been developed as a natural biological treatment and a non-conventional technology (Wu et al. 2015b). The CWs have been highlighted as a low-cost technology, with designs that are easy to operate and maintain, with low energy consumption and a good integration into the environment (Vymazal 2007; Wu et al. 2015b).

From the 1990s decade, the applications of CWs have increased significantly, with more than 50,000 systems in operation around the world (Vymazal 2011; Wallace and Knight 2006). This increase has been due to the need to treat effluents in remotely located places (Auvinen et al. 2016). For decades, CWs have been mainly used for the treatment of domestic and municipal wastewater. However, the application of this technology for treating agricultural and industrial wastewater, mine wastewater, runoff agriculture, and food processing, among others, has increased (Saeed et al. 2018; Vymazal 2009, 2010, 2014).

CWs are natural systems which treat wastewater under different operating conditions taking advantage of the processes that involve different components such as vegetation, soil, and microorganism. The main function of these systems is the removal of the organic load. However, the nutrient and pathogen removal have been also studied in this type of systems (Vymazal 2010). The variability of the CW performance is influenced by various factors such as hydraulics, flow direction, and vegetation (Kadlec and Wallace 2009).

Figure 9.2 shows the classification of CWs. According to the hydraulics, the CWs are classified in superficial flow, or also called free water flow (FWS), and in subsurface flow. Subsurface flow wetlands can be classified according to the direction of water flow in horizontal (HSSF) or vertical (VSSF). Another classification is the type of vegetation to be used; they can be floating, emergent, or submerged

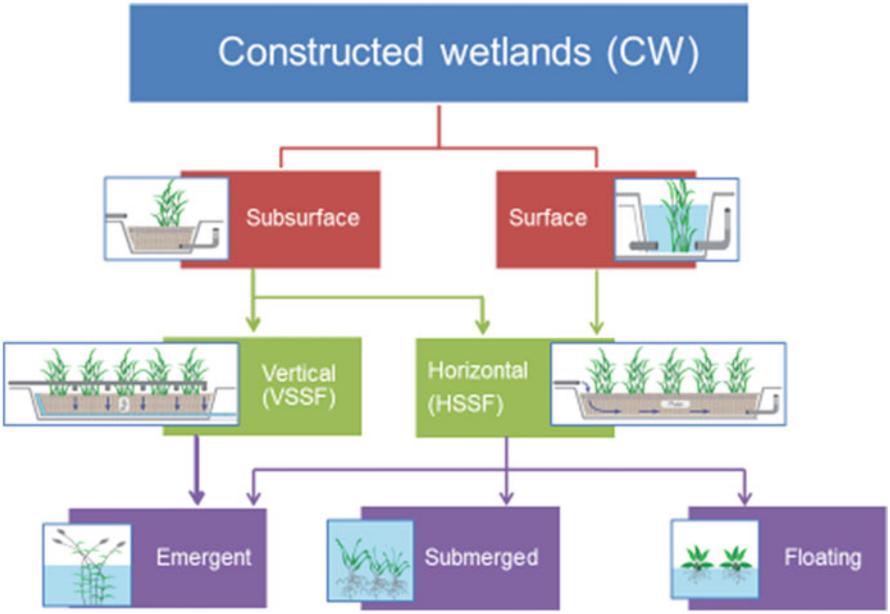

Fig. 9.2 Classification of constructed wetlands for effluent treatment. (Adapted from Kadlec and Wallace 2009; Vymazal 2007)

macrophytes. In addition, several types of CW configurations can be combined to achieve a greater treatment effect. These systems are called hybrid systems. The combination is usually done to achieve greater removal efficiency in a more complex or specific treatment (Kadlec and Wallace 2009; Vymazal 2005, 2010).

CWs have shown to achieve removal efficiencies of organic matter and nutrients between 55% and 95% and 30–60%, respectively (López et al. 2015; Vymazal 2007, 2010). It has been reported that CWs have been successful for a different type of industrial wastewater showing performance of organic matter between 64 and 73%, 74%, and 78–86% for aquaculture olive mill and textile industry effluents, respectively (Schulz et al. 2003; Del Bubba et al. 2004; Ong et al. 2009). For industries such as tannery**,** winery, abattoir, and dairy manure, the removal efficiencies are above 90% (Calheiros et al. 2009; Kim et al. 2014; Gutiérrez-Sarabia et al. 2004; Mantovi et al. 2003).

In the case of nutrient performance, Vymazal (2007) showed that N and P had removal efficiencies of 40–55% and 40–60%, respectively. Regarding P removal, the loading rate removed varied between 45 and 73 gP/m^2 years depending on the type of CWs and the input load. The wastewater biodegradability and the inlet loading rates are critical factors to achieve higher removal rates using CWs treatments (Saeed and Sun 2017).

9.3.2 Removal Mechanisms of Phosphorus in Constructed Wetlands

The removal of P in CWs is generated through processes that are based on biological principles and biogeochemical cycles. The contribution of these processes depends on the type of CWs and its biotic and abiotic components (Vymazal 2007). The different transformations of P in the soil and in the water column of CWs are shown in Fig. 9.3, which includes processes such as sorption, desorption, precipitation, decomposition, fragmentation, leaching, mineralization, sedimentation, and assimilation of plants and microorganisms (Vymazal and Kröpfelová 2008).

The P enters to the water column of the CWs as particle or soluble where it is quickly absorbed by the biofilm that grows adhered to the support medium, the roots, and the rhizomes of the plants (Kadlec and Wallace 2009). In the biofilm occurs the biological removal of P in the form of phosphate through the assimilation of plants and microorganisms and through the mineralization of the vegetation remains and soluble organic phosphorus (Vymazal and Kröpfelová 2008).

Part of the particulate P not absorbed by plants or microorganisms tends to precipitate. The precipitation and subsequent sorption of P are closely related by the physicochemical and hydrological properties of the support material (Vohla et al. 2011). The sorption of P in CWs is given by different elements such as Fe, Al, Ca, and Mg forming part of different components, particularly hydroxyapatite, ferric iron, and aluminum oxyhydroxides (DeLaune and Reddy 2008). In this case, precipitation and sorption are responsible for the removal of all forms of P (Kadlec and Wallace 2009).

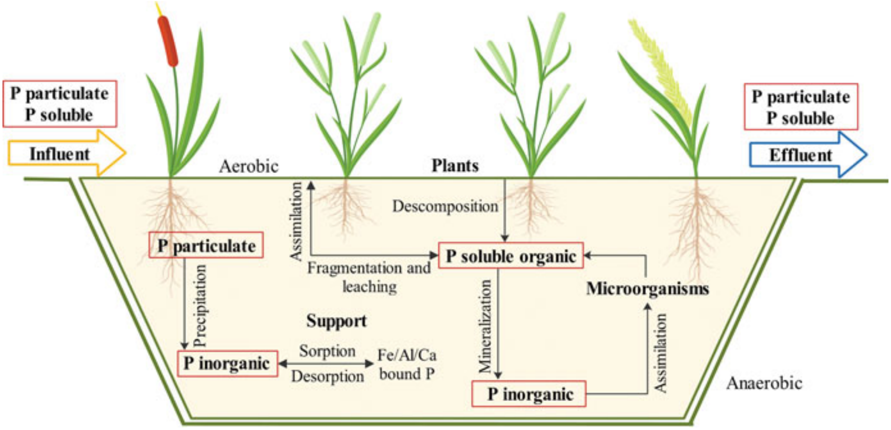

Fig. 9.3 Transformations of P in constructed wetlands in subsurface wetland. (Adapted from López et al. 2016; Vymazal and Kröpfelová 2008)

Despite plants and biofilm provide short-term retention and facilitate the long-term storage of P through the accumulation of organic matter, long-term global retention is determined by sediments and support medium characteristics (Vymazal and Kröpfelová 2008). These two processes are saturables, which means that they have finite capacity. The saturation leads the advance of P during the transition toward a stable and sustainable state. This process of stabilization is one of the disadvantages of retention of P in CWs, since the early temporal capacity to sequester P is lost (Kadlec 2005).

9.3.2.1 Support Medium

The selection of the support medium is the process of greater relevance in CWs for the P removal. It is in the support medium where the wastewater makes contact with the filtration substrate and the sorption and precipitation of P occur (Vymazal 2007). The removal of P in CWs is controlled by the interaction of the oxide-reduction potential (ORP), pH values, and the content of Fe, Mg, Al, and Ca in minerals that are interlinked with each other. With a positive ORP and pH with alkaline tendency, the P adsorption occurs with Ca and to a lesser extent with Mg. While with Al and Fe, P adsorption only occurs at negative ORP and acid neutral pH (Vohla et al. 2011; Vymazal and Kröpfelová 2008).

OPR is a factor that affects the removal capacity P depending on the selected support medium. Andrés et al. (2018) found that ORP> +50 mV enhance the P removal efficiency with zeolite. On the other hand, ORP <−0 mV can cause the transformation of crystalline minerals of Al and Fe to the amorphous form, which has a greater capacity of P sorption than the crystalline oxides due to their greater number of surface hydroxyl groups (Vymazal and Kröpfelová 2008).

However, the potential for P removal in CW depends to a large extent on the materials used to overcome the limited P removal capacity of CWs support. Several alternatives have been suggested and tested (Vohla et al. 2005; Arias et al. 2001; Vera et al. 2014; Brix et al. 2001). The support materials can be classified into natural, industrial by-products, and man-made artificial products. Many of them have been tested as a substrate in CWs (Arias et al. 2001; Vohla et al. 2011; Saeed et al. 2018). According to Vohla et al. (2011), industrial by-products have shown high P removal capacities (up to 420 g·P/kg for some furnace slags), followed by natural materials (maximum 40 g·P/kg for heated opoka) and finally artificial filter supports (maximum 12 g·P/kg for Filtralite).

A study evaluated the removal of P from a mixture of substrate (sand and dolomite) in VSSF where the wetlands were able to remove more than 45% of the initial P (Prochaska and Zouboulis 2006). Likewise, mixed supports not only have reactive surfaces for microbial fixation but also can provide high hydraulic conductivity to avoid short circuit in CWs (Wu et al. 2015b).

Other factors to be taken into account in the applicability of the support materials are the saturation time, the local availability, the heavy metal content, and the recycling capacity of the saturated filter media as fertilizer (Vohla et al. 2011). Likewise, another important factor is the longevity of the sorption capacity and the recycling of the saturated material, because the sorption and precipitation are saturable and decrease with time (Vymazal 2007).

9.3.2.2 Plants

The P of the wastewater in a CW is absorbed by the plants through their leaves and their roots (Vymazal 2007). The functions of the plants in the CWs are the provision of substrate (roots and rhizomes) for the growth of attached bacteria, radial loss of oxygen (diffusion of oxygen from the roots to the rhizosphere), and absorption and assimilation of nutrients. Free orthophosphate is the only form of P that is believed to be used directly by algae and macrophytes. Therefore, it represents an important link between the organic and inorganic P cycle in wetlands (Vymazal 2007).

The efficiency of the plant in CWs depends on several factors: type of CWs (e.g., HSSF or VSSF, with or without recirculation), quality and quantity of the wastewater, species of plants and its combinations, seasons and climate, the type of support, and the plant management, such as the harvest regime (Shelef et al. 2013).

The accumulation of nutrients in the plants is limited. For this reason, it represents only a small proportion (2.3–9%) of the loading rates due to the saturation capacity of the plant (Luo et al. 2018; Vohla et al. 2005; López et al. 2015). This P storage in the aerial biomass of the emergent macrophytes is usually short-term, because the absorption of the plant represents only the temporary storage. The nutrients are released into the water after the decomposition of the plant; therefore, the portions of macrophytes on the ground P are returned to the water in the case of FWS, while in the HSSF and VSSF, P is returned underground (Kadlec and Wallace 2009; Vymazal 2007).

Macrophytes that have shown efficiencies in P retention are *Typha* spp., *Phragmites* spp., and *Schoenoplectus* spp. The P concentration in different tissues varies between 0.15% and 0.24% of dry weight (DW) of the plant, removing between 5% and 10% of P in the wastewater (Vera et al. 2014). Other species that stand out in the retention of P are the water hyacinth (*E. crassipes*) reaching up to 45 g TP/m^2 (Vymazal 2007) and the duckweed (*L. minor*) removing an annual average of 13 g TP/m^2 (Adhikari et al. 2015).

The species of plants commonly used in CWs are the common reed (*Phragmites* spp.) and the bulrush or tule (*Schoenoplectus* spp.), due to their tolerance to substantial changes in pH (4–10), salinity (20–45 mg Cl/L), temperature (10–32 °C), and assimilation of nutrients (López et al. 2016). The last one has the potential to treat various effluents due to its ability to adapt to different variables.

In industrial wastewater, with high organic loads and higher amounts of solids and salts, the survival of macrophytes is crucial. Therefore, the negative effect of this type of wastewater should be considered (Wu et al. 2015a). The choice of species should be made considering the characteristics of the effluents. For example, in the case of tannery wastewater, salt-tolerant plants such as halophytes should be considered (Calheiros et al. 2009). High pH conditions (>8) are considered a limiting environment for plant growth, mainly due to the limited availability of P as a consequence of coprecipitation with Ca and Mg (Wu et al. 2015a).

9.3.2.3 Microorganisms

The contribution of microorganisms on P consumption in CWs is quick as those organisms grow and multiply rapidly. The amount of retention varies between 12% and 13% (Vymazal and Kröpfelová 2008). Its action depends on the polyphosphate-accumulating organisms (PAO) to absorb P of the wastewater, concentrating phosphorus in biomass (Yuan et al. 2012).

In biomass highly enriched in PAO (e.g., 60–90%) obtained with synthetic water, it has been shown that P can form 15–20% of the DW of the cell (Yuan et al. 2012). Although bacteria are generally considered decomposers that simply mineralize organic P, they have also been shown to regulate the flow of P through the sediment-water interface (Vymazal 2007). In comparison with other components, the microbial storage (0.5–1.0 g·P/m^2·year) is lower than the storage of macrophytes (P, 1.0–2.5 g·P/m^2·year, and soil adsorption, 1.5–3.8 g·P/m^2·year) with a yield that varies between 25% and 30% for plants and 38–46% for soil (Vidal and Hormazábal 2018).

Research on the recovery of phosphate from sludge is limited and minimal. Some studies show the application of biotechnology for recovery P (Blank 2012). The biosolids enriched with P contain 5–7% of DW and can be applied directly on the soil or solubilized and recover P as a mineral product. The direct application has been effective, but the product is bulky and carries pollution risks that must be managed (Yuan et al. 2012).

9.3.3 Operational Factors That Influence the Removal of Phosphorus in Constructed Wetlands

9.3.3.1 Hydraulic Loading Rates and Retention Time

Hydrology is one of the main factors to control the CWs performance, and the flow rate must also be regulated to achieve a satisfactory treatment depending on the objective to be eliminated. The optimal design of the hydraulic load rate (HLR) and the hydraulic retention time (HRT) is relevant. Because of the higher HLR, the faster is the passage of wastewater through the support, thus reducing the optimum contact time between the substrate of wastewater and the microorganisms associated with the support medium (Dotro et al. 2015). Therefore, for an appropriate microbial community to establish itself in CWs and have an adequate contact time to eliminate contaminants, a longer HRT and consequently a slower HLR are necessary (Wu et al. 2015b).

In relation to the retention capacity of P (g·P/kg) and the HRT of various support materials, there is no significant correlation. This behavior can be explained by the different characteristics of the support material. Vohla et al. (2005) studied different support materials in a time of 24 h, where the sand and gravel had a P retention capacity of 2.45 g·P/kg and 3–3.6 g·P/kg, respectively. At the same time, Arias et al.

(2001) reported that the P retention capacity of some sands would be exhausted after a few months, while in others it would persist up to several years.

It should be noted that very prolonged HRT can generate desorption of P from the support due to exceeding the maximum retention capacity (Vohla et al. 2011). This is why short-term storage is higher (4.0–8.3 g·P/m^2·year) compared to long-term storage (5 years 0.92 g·P/m^2·year) (Vymazal and Kröpfelová 2008). Finally, the general relationship between the hydraulic parameters, the retention of P for each support, and the optimum range can be different in each type of support medium and type of wetland (Vohla et al. 2011).

9.3.3.2 Feeding Mode of Influent

The feeding mode can also be considered a factor in the removal of P. According to Jia et al. (2010), intermittently operated systems remove more TP (>90%) than the continuous flood system (87%). The increase of effluent P in the flood system may reflect the possible saturation of the filter media and the decrease of the sorption capacity of the support medium.

9.3.3.3 Temperature and Aeration

The removal of P also depends on the temperature. This is related to the seasonal variations because during the winter seasons the plants and the microbial biomass decompose and P releases the precipitates to the water column. The temperature limit value is estimated at 15 °C, where the removal efficiencies of TP vary between 19% and 68.8%. On the other hand, over 15 °C, the efficiencies increase from 30% to 92% (Akratos and Tsihrintzis 2007). The same temperature can be related to the HRT. Akratos and Tsihrintzis (2007) compared different temperatures, and determined at temperatures above 15 °C, the HRT of 8 days was adequate, but for temperatures below 15 °C, HRT greater than 14 or 20 days was needed.

For aerated or non-aerated CWs, nonsignificant differences in the P removal were observed. However, in aerated CWs, the removals of P were more stable, ranging from 30% to 60%. These efficiency improvements are due to the level of dissolved oxygen that could accelerate P precipitation and adsorption to the substrate (Zhang et al. 2010).

9.3.3.4 Types of Constructed Wetlands

Depending on the type of CW, the main mechanism for removing P varied. The retention of P in CWs for the treatment of wastewater is usually higher (approximately 75 g/m^2·years) than in natural wetlands (Vymazal 2007). HSSF and VSSF have the greatest potential for removing P by sorption mechanism according to the support medium used. In contrast, HSSF has greater potential because the substrate is constantly flooded and there is not much fluctuation in the ORP of the support medium. In the case of VSSF systems, the influent is fed intermittently and this oxygenation may cause desorption and later P release (Vymazal 2007).

Another difference is the useful life in the types of CWs; HSSF and VSSF can have a life significantly shorter than the FWS (up to 10 years) due to the effect of clogging (Wu et al. 2015b).

9.4 Removal of Phosphorus from Industrial Wastewater in Constructed Wetlands

Since the 1950s, CWs have been used for domestic wastewater treatments. However, since the 2000s, they have been used successfully for many industrial wastewaters with different types of combinations (Vymazal 2014). The use of CWs for industrial wastewater has been reviewed by Vymazal and Kröpfelová (2008), Kadlec and Wallace (2009), and Vymazal (2014). However, there is not very precise information regarding the removal of P from industrial wastewater in CWs, because its approach is not the primary one. Table 9.2 shows a review of the removal efficiencies of P in CWs of different types of industrial wastewater, highlighting the type of CWs, the pretreatments, the support used, the loads of inputs, and finally the removal efficiencies of P.

Traditionally, treatments with subsurface flows need pretreatment stages. The primary treatment consists to remove solids, while later stages use CWs and/or natural technologies (Vymazal 2005). As mentioned in Sect. 9.2, pollutant concentrations in industrial effluents vary widely according to their production. Therefore, the treatment of industrial effluents often requires one or more pretreatment stages as shown in Table 9.2. The pretreatments described are settler tank, septic tank, trickling filter, and anaerobic lagoon which use physicochemical processes such as particle sedimentation, flotation, and coagulation-flocculation. These processes promote the sedimentation and precipitation of P due to its form as particulate P.

The removal efficiencies of TP in different industrial wastewaters show values from 26% to 68%. These values are similar to those found by Vymazal (2007), where the TP removal varied between 40% and 60%. The variability on the removal efficiency of P is related with the origin of raw materials in industrial processes. The highest efficiencies (55–68%) were shown in the wastewater of industries of animal and agricultural origin, except for the wastewater of the swine industry that had a removal value of 37%. These removal values of P are within a range according to Vymazal (2014), which show percentages that fluctuated from 30% to 87%.

Comparing with other industries, such as textiles, tannery, and abattoir, the removal efficiencies are lower (5–38%) compared to other industries. Generally, these effluents are difficult to treat due to the specific characteristics. They contain high salinity, total solids, and dyes, among others (Table 9.1). In the CWs, the treatment performance can be affected by these industrial wastewaters because they

Table 9.2 Summary of phosphorus removal efficiencies in constructed wetlands of different types of industrial wastewater

Type of water	Type of wetland	Pretreatment	Support	HRT (d)	Influent (g·P/m^2·d)	Efficiency P (%)	References
Aquaculture	HSSF	–	Gravel	0.31	0.4	68	Schulz et al. (2003)
Textile	VSSF	–	Gravel	3	0.03	26–37	Ong et al. (2009)
Poultry	HSSF	Aerated storage tank	Red gravel	3	0.3	61	Finlayson and Chick (1983)
Dairy manure	HSSF	Septic tank	Fine and medium gravel	10	1.1	61	Mantovi et al. (2003)
Wine	VS∗	Trickling filter with FeCl3	Fine gravel and sand	1	1.3	60	Kim et al. (2014)
Olive mill	HSSF	Tank settler with calcium hydroxide	–	3	1.8	55	Del Bubba et al. (2004)
Pulp and paper mill	VSSF	Primary treatment tank	Gravel – sand – red soil-clay	1	1.67[a]	52	Arivoli et al. (2015)
Tannery	HSSF	Settler tank	Filtralite ®	7	0.009	38	Calheiros et al. (2009)
Swine	FWS	Lagoon anaerobic	Gravel	–	0.16–0.3	37	Villamar et al. (2015)
Abattoir	HSSF	Lagoon anaerobic	Gravel	10.6	1.3[a]	5	Gutiérrez-Sarabia et al. (2004)

[a] PO_4^{3}-P∗ partially saturated

can affect the growth of plants and microorganisms (Wu et al. 2015a). The lower removal efficiency of P (5%) was reflected in the effluent coming from abattoir. This behavior is justified by the short-term sorption capacity since P is reduced when reaching the saturation of removal of P (Gutiérrez-Sarabia et al. 2004).

Regarding TP input loading rates, these values ranged from 0.03 to 1.8 g·P/m^2·d, with the lowest P loads in the textile and tannery industry and higher loads in the olive mill industry. Other high values of P loading are expressed in PO_4^{3-}-P with values of 1.3 and 1.67 g·P/m^2·d for the abattoir and the pulp and paper industry, respectively. It should be noted that the P removal depends on the input load and the type of CWs to be used. With low P loading rates (0.03–0.05 g·P/m^2·d), the removal by vegetation could have a greater contribution in FWS (Vymazal 2007). Making a comparison between the types of wetlands, it can be seen that there are no important differences between removal efficiencies of HSSF and VSSF. In both cases, percentage varied between 38% and 68% and 26–60%, respectively. It should be noted that of the treatments reviewed in Table 9.2, unusually CWs are operated with P as the main treatment objective. Therefore, it shows traditional materials such as gravel, with a relatively low sorption capacity (3–3.6 g·P/kg) (Vymazal 2007). However, an alternative to increase the removal of P in CW is to perform a mixture of different support materials obtaining values from 52% to 61% (Mantovi et al. 2003; Kim et al. 2014; Arivoli et al. 2015).

9.5 Conclusions and Perspectives

In the recent decades, CWs have been used successfully for the treatment of industrial wastewater. However, this alternative treatment to remove P is difficult to apply due to the low P concentration of the wastewater. P is found in most of the agricultural industry (cattle of animal origin and agriculture of vegetable origin). The highest removal efficiencies (55–68%) of P in CWs were shown in such effluents. On the other hand, industrial wastewater with higher concentrations of salts and total solids, such as the textile industry, tannery, and abattoir, can generate negative effects not only on the performance of the treatment and the hydraulics but also on the growth of the plants of the CW. For these reasons, the use of previous aerobic or anaerobic treatments and/or coagulant addiction is recommended.

It should be noted that there is little information about the removal of P from industrial effluents in CWs. This approach is not the main one, which makes it difficult to compare or revise P in industrial wastewater. During the last years, P has taken great importance due to its great need as a basic chemical element for living beings. Moreover, it is fundamental to increase productivity in agriculture and sustain the food security of a growing world population. For this reason, it is necessary to research new sources of P. In the coming decades, this element will suffer from scarcity, and the industrial wastewater will be an interesting alternative. In this context, there is a need to improve P security and sustainable management of P. To promote more efficient use of this nutrient, improving its recovery and reuse and at the same time decreasing P discharges to continental waters to reduce pollution and possible eutrophication of waters are the new challenges to achieve a balance of nutrient P on Earth.

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Chapter 10
Megaplastics to Nanoplastics: Emerging Environmental Pollutants and Their Environmental Impacts

Sayali S. Patil, Rohit V. Bhagwat, Vinay Kumar, and Trupti Durugkar

Abstract Pollution of pristine environmental resources such as water, air, and soil by various anthropogenic activities is becoming a serious cause of concern, worldwide. Besides many other sources of pollution, plastics have emerged as a rapidly spreading environmental pollutant. Excessive use of plastics in various forms in industrial and/or household applications and their further improper disposal have led to water, soil, and air pollution. This has disturbed the ecological balance of marine, freshwater, and terrestrial ecosystems largely. Throughout the history of mankind predicted that by 2050, the ocean will see more plastic in marine water than aquatic species, which highlights an alarming situation if the plastic pollution has not been controlled. Considering this severity, in this book chapter, authors shed a light on the current burning issue of plastic pollution by addressing various aspects such as sources of plastics, their applications, pathways of entering plastic debris in environment, and their deteriorating impacts on ecology and human health. Moreover, preventative and control measures are recommended to avoid the environmental damage caused by various forms of plastics. Case studies specific to Asia-Pacific and European countries are also discussed to highlight the global issue of plastic pollution. In short, this chapter will give readers an insight into global status of plastic pollution, political resolutions, upcoming research trends, and environmental policies to help maintain environmental sustainability by handling the danger of plastic pollution in a rational manner.

Keywords Megaplastics · Nanoplastics · Plastic pollution · Global issue · Environmental policies

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10.1 Introduction

The current applications of plastics and their benefits were predicted by Yarsley and Couzens in 1940 with much optimism:

> It is world free from moth and rust and full of color, a world largely built up of synthetic materials made from the most universally distributed substances, a world in which nations are more and more independent of localized naturalized resources, a world in which a man like a magician, makes what he wants for almost every need out of what is beneath and around him. (Yarsley and Couzens 1945)

Today plastics have become an important part of human daily activities. Plastics have a variety of applications in packaging, transport, healthcare, construction, electronics, and many more other industries due to their significant properties like strength, weight, durability, and economical viability (Fig. 10.1a).

As a result of its versatility, global plastic production has undergone rapid and consistent growth (Fig. 10.1b).

However, the problems associated with waste management and plastic debris are more prominent now than 70 years ago when predications were "how much brighter and cleaner a world (it would be) than that which preceded this plastic age" (Yarsley and Couzens 1945).

The term "plastic" defines a subcategory of the larger class of materials called polymers. Polymers are very large molecules that have characteristically long chain-like molecular architecture and therefore very high average molecular weights. They may consist of repeating identical units (homopolymers) or different subunits in various possible sequences (copolymers). Those polymers that soften on heating, and can be molded, are generally referred to as "plastic" materials. These include both virgin plastic resin pellets (easily transported prior to manufacture of plastic objects) and the resins mixed (or blended) with numerous additives to enhance the performance of the material (Reisser et al. 2013). A more scientifically rigorous definition of plastic pieces might refer to nano (<1 nm), micro (<5 mm), meso (<2.5 cm), macro (<1 m), and mega (>1 m) size ranges (GESAMP 2015). Many different types of plastic are produced globally, but the market is dominated by six classes of plastics: polyethylene (PE, high and low density), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS, including expanded EPS), polyurethane (PUR), and polyethylene terephthalate (PET) (Lithner et al. 2011) (Fig. 10.2). Plastics are usually synthesized from fossil fuels, but biomass can also be used as feedstock. The production chain for the most common artificial and natural polymers is illustrated in Fig. 10.3. The figure includes some examples of common applications for particular industrial or commercial applications.

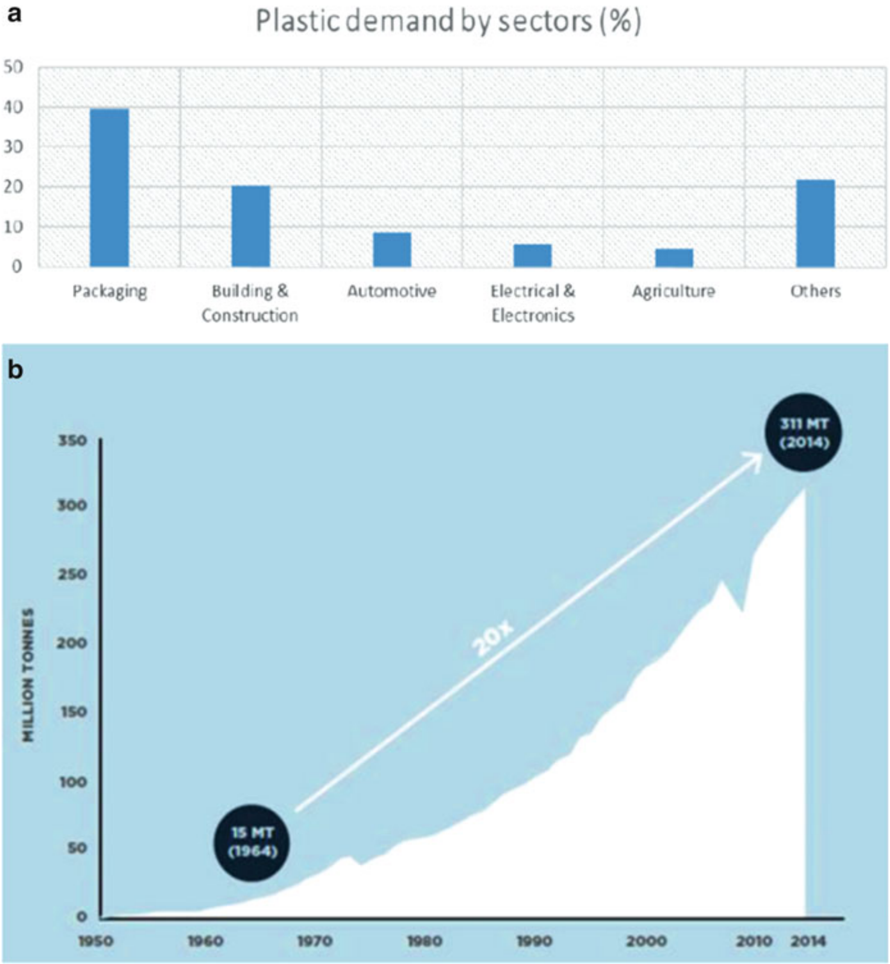

Fig. 10.1 Sector-wise production of plastic in the world (**a**); global production of plastic (**b**). (Adapted from Plastics Europe, Plastics – the Facts 2015)

10.2 Sources and Potential Entry Pathways of Plastics into the Environment

There are primary and secondary sources of plastics present in the environment. The distinction is based on whether the particles were originally manufactured to be that size (primary) or whether they have resulted from the breakdown of larger items (secondary). Fragmentation and degradation play an essential role in the formation of secondary microplastics, but the processes are not very well understood. There is evidence that microplastics are littered into the environment at all steps in the life cycle of a plastic product from producers to waste management. Micro- and

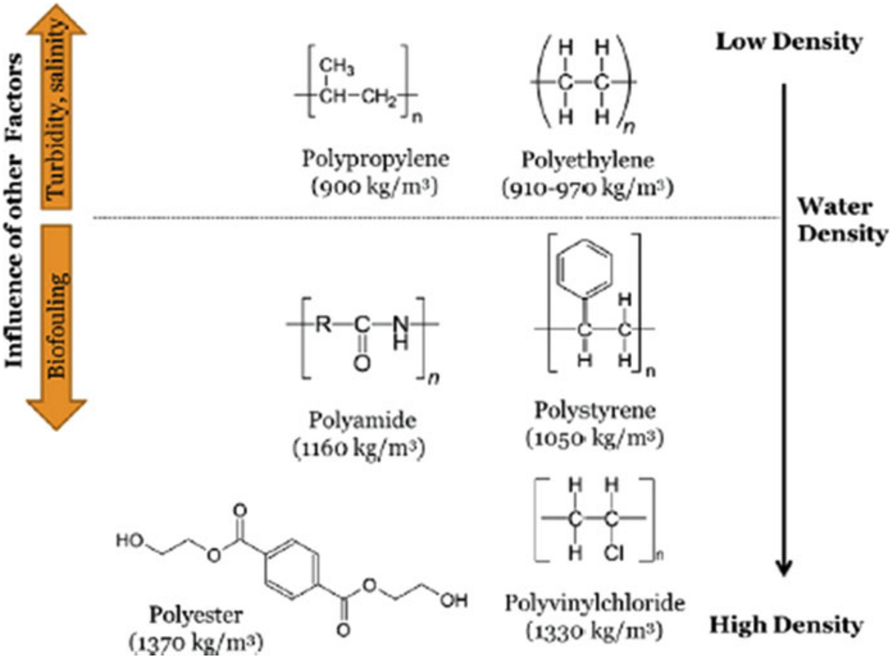

Fig. 10.2 Densities, structures, and expected distributions of different plastic polymers observed in water column with factors affecting buoyancy, and the direction of the change, are indicated with the arrows on the left. (Adapted from Anderson et al. 2016)

Fig. 10.3 Production of the most common artificial (plastic) and natural polymers, including some typical applications. (Adapted from Plastics Europe 2016)

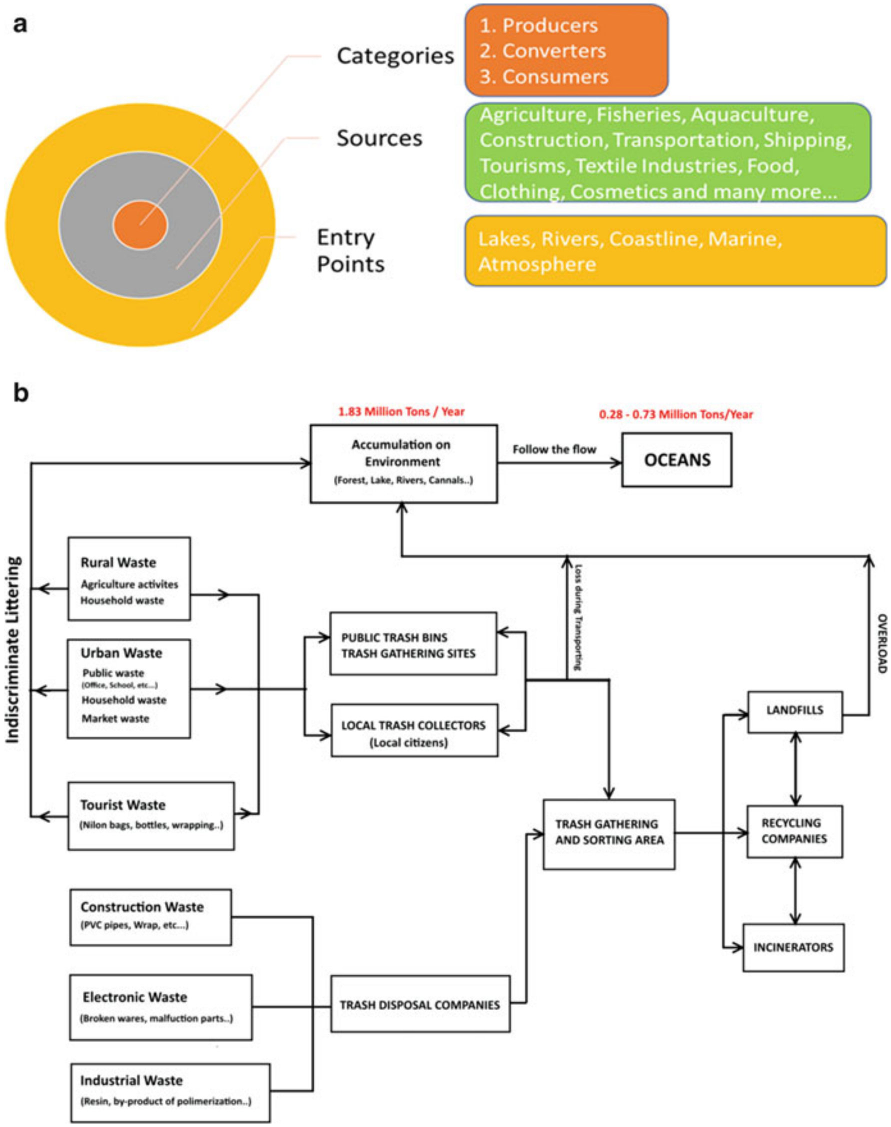

Fig. 10.4 **(a)** Sources and potential entry points of mega- to nanoplastics and **(b)** potential routes of plastic wastes into an environment in detail

nanoplastics can enter the marine environment via riverine systems and coastlines, directly at sea from vessels and platforms, or by wind-induced transport in the atmosphere. The category, sources, and entry points, through which plastic enters the environment and causes severe pollution, is illustrated in Fig. 10.4.

10.2.1 Plastics Accumulation

When the plastic waste spreads on the surface of the sea and from the sea to the coast, it can also move vertically and is termed as biofouling or accumulation of microorganisms, plants, or algae onto the plastic debris which makes them heavier, and this makes the debris eventually sink at the base of the sea. Most of the plastics are subjected to fouling.

Lobelle and Cunliffe (2011) observed the development of films of microorganisms on plastic debris in the sea, and it developed rapidly on the debris, and the plastic starts to sink at the bottom of the sea surface. This data helped to identify the types of plastics which were afloat and dipping and which are possible hazards for surface-feeding or seafloor-feeding wildlife. The size, mass, and composition of plastics that are present in the ocean are important to know and understand the impacts of plastics (Moret-Ferguson et al. 2010).

Plastics, when accumulated in the marine or the land environment, affect both habitats on land and in the sea. The plastic is buoyant in the marine environment; it can settle to the bottom of the sea surface, which can be pulled by the oceanic currents or due to fouling. Sediments also keep the plastics on the sea floor. Once the plastics are accumulated in the sea floor, it changes the working of the flora and fauna. Goldberg (1997) suggested that the plastic sheets act like mantle, blocking the gas exchange and leading to low oxygen levels called as anoxia or hypoxia. Plastic waste accumulated in the sea can create artificial hard grounds and causes problems for the burying creatures (Gregory 2009).

10.2.1.1 Microplastics Accumulation

Microplastics are broadly disseminated and accumulated in the marine environment. Millions of plastic debris are lost or discarded into the oceans. Microplastics which are <5 mm in size form a principal component to this debris. These may get accumulated in the seabed either as primary microplastics or secondary microplastics as a result of the breaking down of larger particles. These microplastics penetrate into the marine environment by different pathways like sewage systems, low disposal management, breaking down of larger plastic materials due to photochemical activities, river inputs, and stormwater outflows (Cole et al. 2011; Sadri and Thompson 2014; Dris et al. 2016).

When the plastics reach the ocean bed, their density is modified due to biofouling, leaching, and summing up with marine aggregates. The low energy environment, depleting oxygen levels, cold temperature, and lack of sunlight result in the slow breakdown of the debris (Andrady 2011). Microplastics get accumulated even to the most distant deep-sea habitats and have been recovered from a minimum of 2 cm depth from the abyssal sediments. Plankton records show a significant increase in microplastic pollution from the 1960s to 1990s in the North Atlantic surface waters.

A record shows the accumulation of microplastics in the Belgian beach of increasing pollution from 1993 to 2008.

Long rivers are also accountable for accumulation of plastic remains to the seabed. They carry the waste plastic debris to the sea due to high flow rate and the strength of the bottom currents. In small rivers, the rate of accumulation is slightly lower than that in larger rivers as the plastic debris and the waste get accumulated in the zones adjacent to the sites closer to the estuaries. Deep submarine movements also transport plastics to the seabed. Human activities also contribute to the patterns of seabed debris accumulation such as urban development, fishing activities, littering in the beach areas, and tourism. The fish industry is the main component for debris in the fishing areas. For example, in Eastern China Sea, 72% of debris accounts for marine pollution and 65% from the Celtic Sea (Barnes et al. 2009).

10.2.2 Translocation of Plastics

Throughout the history of mankind, the conquest related to the marine environment as the disposal of the waste was not a major concern. This is mainly because, marine ecosystem was regarded as unlimited sinks able to disperse, dilute and redistribute synthetic and natural substances. But, in the last few decades, the disposal caused much hazardous destruction to the marine habitat. Approximately 6.4 million tons of litter is deposited in the seas and ocean with around 13,000 plastic debris floating per square kilometer in the ocean.

Plastic debris poses a threat to wildlife; the main dangers involve the entanglement in and ingestion of plastic debris. Improper hndling and disposal of plastic waste can cause serious injuries to animals like movement restrictions, feeding problems, breathing difficulty and many more. Plastic when consumed by animals, it may remain undigested in their system can cause decrrease stimuli for feeding, blockage of gastrointestinal tract etc. Accumulation of platics in their body system also inhibit secretion of gastric enzymes and hormones which may eventually lead to reproduction abnormalities.

Plastic contamination in the oceans poses to contribute toward increased organic pollutants; hence, polychlorinated biphenyls (PCBs) a component of plastic along with other hazardous chemical including dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), and bisphenol A (BPA) are found as major contaminants in oceanic plastic debris. These compounds pose hazardous effects on wildlife health and further undergo biomagnification causing direct risk to human health. These toxic compounds induce health problems like cancer, diabetes, arthritis, endocrine disruption, developmental impairment, and hypomethylation (Diamanti-Kandarakis et al. 2009).

Plastic debris has potentially invaded and destructed fauna and induces forced migration to new environments. Plastic debris of marine environment promote colonization and subsequent dispersal of marine species where in barnacles, bryozoans, polychaetes, dinoflagellates, algae, and molluscs are found to adhere to plastic debris. One of the common ways of marine debris translocation to new areas is through terrestrial animals; one such example is the ants that carry debris from Brazilian mainland to San Sebastian Island. Other examples include iguanas taking flotsam to Caribbean islands. Most of plastic debris is found in the deposition on beaches. This is due to the littering activities, insufficient recycling facilities, and discarded fishing nets and as plastics can float easily and therefore tend to accumulate on the beaches. This marine debris is an internationally recognized world problem in all the oceans. This floating plastics support colonization of many marine organisms and since plastic debris can remain undissolved or degraded on the water surface for substantial periods.

UNEP (2016) claims that millions of vertebrates and invertebrates including fish, turtles, seabirds, and sea mammals are known to either be trapped or entangled or may ingest the plastic debris, which eventually lead in impaired movement, reduced in reproductive output, lacerations, ulcers, and death. Such ingestion can be high in marine animals due to mixing of plastics in their food. For example, 95% of fulmars washed ashore were found dead in the North Sea as they had plastics in their guts. There are reports showing the debris ingested by the seabirds like albatross and prions. Small plastic fragments mainly formed due to mechanical and chemical breakdown of larger plastic items which are also called as microplastics impose a major concern. These are reported to contaminate the marine habitat massively. Such microplastics enter into the marine ecosystem through domestic cleaning applications and industrial applications like shot blasting or scrubbers used in proprietary hand cleansers and due to the spillage of plastic pellets and powders which are used as a feedstock for manufacturing of most of the plastic products. This microplastic debris strongly affects the health of ecosystems and humans.

Recent studies have shown that the effects of microplastics or microplastic particles on food webs of marine ecosystem. More than half of the modern plastics contains at least one hazardous ingredient and due to various low disposal activities enter into the marine ecosystem and increases the toxicity by absorbing the persistent organic pollutants on the surface. These concentrated toxins will be delivered to the marine animals through ingestion of plastics or through the process of endocytosis and will be transferred through their food webs. This process of biomagnification happens when these microplastics are sufficiently small enough to get ingested even by the small organisms like planktivorous fish and zooplanktons that are involved in the marine food web. For example, in Tasmania and Australia, microplastics are reported to be found in the stomach of the southern bluefin tuna which were coming out from the guts of their prey, myctophid fish. This shows that plastic debris can be transferred to the affected organism, and then it is biomagnified in the process of the food chain. If this process continues, plastics can certainly impose hazardous effect on the food web and virtually the heath of the organisms including humans.

In Australia, the plastic threats to marine ecosystem are also impacted through relatively large debris of abandoned fishing nets, plastic bags, etc. (Keep Australia Beautiful 2016). In marine creatures, especially in fish, the first organ that is exposed to the plastic debris is the gills which eventually gets entrapped within the gill filaments; the gills are the first organ which are exposed to debris or anthropogenic particles during respiration which increases the possibility of these particles to get stuck on the gill filaments leading to abnormal respiration. Studies have shown that the polyethylene fragments were found trapped in the gill filaments of the blue mussel (von Moos et al. 2012). Most of the field studies have shown the presence of microplastic accumulation in the gastrointestinal tract of the fish.

The presence of microplastics has also been found in dried fish in the eviscerated flesh and excised organs of Indian mackerel, spotty-face anchovy, greenback mullet, and Belanger's croaker. These fish are dried up for human consumption and are mainly caught along the coastal line waters (Abdussamad et al. 2010; Agatis and Dramaga 2011; Saha and Kabir 2015). The presence of different types of microplastics was found in these four fish identified under micro-Raman spectroscopy as shown in the Fig. 10.5.

10.3 Microplastics Assessment Methodologies

10.3.1 Sampling Methods

Sampling, processing, and extraction methods generally involve combination of basic methods as selective sampling of visible pieces, sieving, flotation, filtration, density separation, etc. (Blair et al. 2017). Depending upon the source, methods sampling and extraction can be selected. Sources such as freshwater and waste waters would involve different methods from that of food materials such as salts, fish, and other sea organisms. Similarly sampling of microplastics differs in the case of sampling from agricultural lands.

In the case of freshwater, sampling of microplastics is undertaken using neuston or plankton nets or manta trawls (Fig. 10.5), whereas sampling and extraction from waste waters are done using filters and other techniques such as "flotation, centrifugation, density separation and/or digestion with acids, bases or enzymes from natural inorganic and organic matrices" (Blair et al. 2017; Miller et al. 2017; GWRC 2015). For lake sediments grab sampling equipments such as Van Veen, Ekman, Peterson, and Ponar grabs (Fig. 10.6) are used, while for shoreline sampling, bulk sampling methods such as steel trowels and box corers are used (Blair et al. 2017; Miller et al. 2017).

Sea organisms tend to ingest plastic particles which accumulate within these organisms resulting in physical injury or even translocation to other tissues or organs (Cauwenberghe et al. 2015). Collection of sea organisms is generally undertaken using various types of nets, e.g., beach seine net and another grab sampling equipment, as shown in Fig. 10.7 (Vendel et al. 2017).

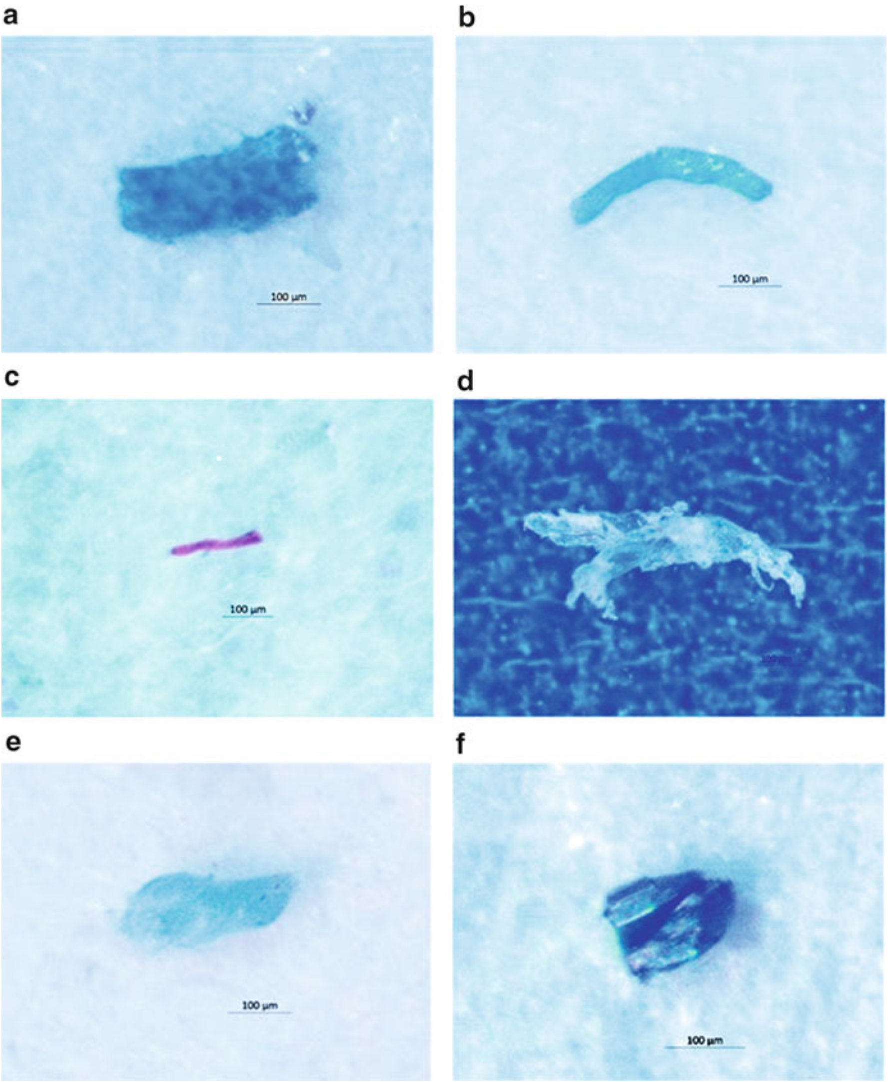

Fig. 10.5 Isolated particles from dried fish. These particles were identified using micro-Raman spectroscopy as (**a**) phthalocyanine, (**b**) polypropylene + phthalocyanine, (**c**) polyethylene terephthalate, (**d**) polyethylene, (**e**) Hostasol Green, and (**f**) actinolite. (Source: https://www.nature.com/articles/s)

10.3.2 Detection and Analytical Methods

Detection of plastic particles depends on the dynamics of type of the sample, chemical composition of plastic material, and the particle size itself. Many techniques, viz., stereo microscopy, Raman spectroscopy, Fourier transform infrared

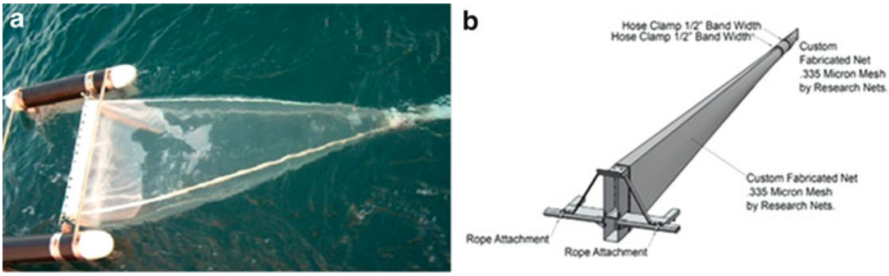

Fig. 10.6 (**a**) Neuston Net. (Adapted from Frias et al. 2011). (**b**) Manta Trawls. (Adapted from Eriksen et al. 2018)

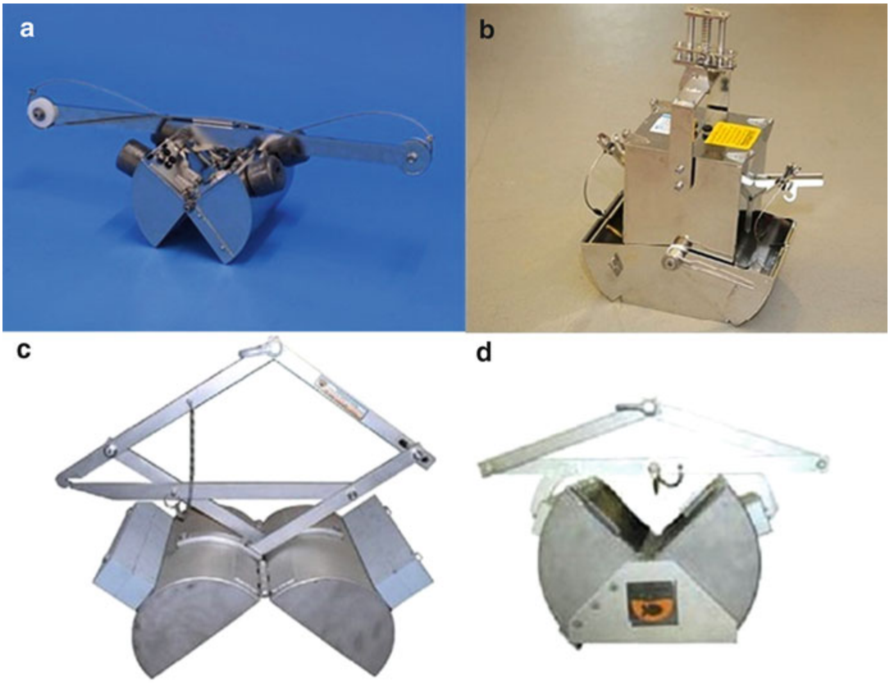

Fig. 10.7 (**a**) Van Veen Grab and (**b**) Ekman Grab. (Source: http://www.kc-denmark.dk/products/sediment-samplers.aspx), (**c**) Peterson Grab, and (**d**) Ponar Grab. (Source: https://shop.sciencefirst.com/wildco/461-benthic-grabs)

spectroscopy (FTIR), micro-FT-IR and molecular mapping by focal plane array (FPA), pyrolysis-gas chromatography mass spectrometry (Py-GC-MS), electron microscopy (EM), scanning electron microscopy (SEM), SEM coupled with energy-dispersive X-ray (EDS), microanalysis, thermal desorption gas chromatography mass spectrometry (TDS-GC-MS), thermogravimetry (TG), and differential thermogravimetry (DTG), are reported for detection and analysis of plastic particles

(Blair et al. 2017; Wagner and Lambert 2017; Dümichen et al. 2015; GWRC 2015). However, no studies using TEM have been reported as of now (Blair et al. 2017). Depending upon the sample, estimations of the plastic particles are done using various statistical methods such as normal distribution pattern (KS test), one-way analysis of variance (ANOVA), nonparametric Kruskal-Wallis H-test for multiple comparisons, Tukey's Honest Significant Difference (HSD), Mann-Whitney U test, Kruskal-Wallis H-test, t-test, Shapiro-Wilk test, and softwares, e.g., Statistica and Genstat (Seth and Shriwastav 2018; Lwanga et al. 2017; Kühn et al. 2017).

10.4 Potential Impacts of Plastic Pollution on Environment

Over the next 20 years, plastic production is expected to double and almost quadruple resulting in having more plastic in ocean than fish by 2050 (World Economic Forum 2016). Globally, it is estimated that at least 8 million tons of plastic will enter the world's oceans each year equivalent to dumping the contents of one garbage truck into the ocean every minute. At current rates, this is expected to increase to two per minute by 2030 and four per minute by 2050. The plastic waste generated by 192 coastal countries in 2010 was calculated to be 275 million tons, out of which 2–5% were assumed to be mismanaged, thus ending up in the oceans (Jambeck et al. 2015). This section will discuss the impacts of severe plastic pollution on well-being of marine, freshwater, terrestrial ecosystem as well as human health.

10.4.1 Impacts on Freshwater Ecosystem

Currently, it is estimated that between 1.15 and 2.41 million tons of plastic waste enters the ocean every year from rivers (Lebreton et al. 2017). Microplastics in freshwater have been severely understudied in general, compared to marine systems, and therefore, the presence and effects of mega- and nanoplastics in freshwater ecosystems remains largely unknown (Anderson et al. 2016). Only a few local studies have reported levels of plastic contamination in freshwater systems worldwide (Wagner et al. 2014). Such freshwater studies generally focus on microplastics contaminating sediment and waters of lakes and rivers. A few studies have demonstrated the presence of microplastics in freshwater systems which might be a reason to raise concern about the presence of microplastic in drinking water since human population is highly dependent on freshwater systems for drinking water supply and food resources (Eerkes-Medrano et al. 2015). For instance, a study on beach sediments from Lake Garda, Italy, revealed an abundance of 1108 ± 983 microplastic particles/m^2 at the north shore of the lake used for drinking water supply (Imhof et al. 2013) most likely originating from postconsumer products and lakeside sources such as fishing gear and ropes.

10.4.2 Impacts on Marine Ecosystem

Land-based sources, as opposed to marine-based sources, are considered the dominant input of plastics into oceans (GESAMP 2016). In aquatic systems, microplastics have become a floating mobile habitat for algae, bryozoans, dinoflagellates, isopods, marine worms, and microbes. Numerous aquatic species are reported to be exposed to mega- and microplastic via ingestion, entanglement, and so-called ghost fishing. Over 180 species of animals including turtles, marine mammals, fish, and birds were first reported to ingest plastic particles (Laist 1997). A more recent study states that 663 species are affected by ingestion and entanglement by marine debris of which >80% is attributed to plastic debris. Out of this fraction, 11% was microplastic, 24% rope and netting, 20% plastic fragments, 17% packaging, and 16% other fishing debris (CBD 2012). Impacts of entanglement include drowning, reduced ability to catch food or escape predators, and wounds. Ingestion can block the ingestion tract, causing suffocation and damaging stomach linings (Fig. 10.8). Plastic debris, when bulky or tangled, is difficult to pass and may become permanently lodged in the digestive tracts of these animals. Especially evolutionary adaption makes it impossible for the likes of turtles to reject plastic bags, which resemble jellyfish when immersed in water, as they have a system in their throat to stop slippery foods from otherwise escaping. Thereby, blocking the passage of food and causing death through starvation or infection. The occurrence of plastic litter in the marine environment poses not only a physical threat to organisms; it might also pose a toxicological threat to wildlife.

Plastics, especially in the marine environment, might also act as a vector for nonindigenous species and therefore represent a threat to domestic species (Cózar et al. 2014). Plastic particles in the environment can also carry chemicals of two different origins: chemicals that were added or used during manufacturing such as additives, monomers, and by-products and environmental pollutants sorbed to the plastic surface. Microplastics have been found in sea ice, fin whales, and seal scat,

Fig. 10.8 Plastic debris threatening the life of marine animals. (Adapted from Plastics Europe 2016)

suggesting that Arctic organisms will not be spared from exposure to microplastics and their associated contaminants (Anderson et al. 2016). This region is of cultural and ecological importance to the world, and organisms such as polar bears and cetacean species are long-lived and feed at high trophic levels, potentially increasing their susceptibility to accumulated microplastics and contaminants such as persistent organic pollutants (POPs). The hazards with mega- and nanoplastics in the environment are:

- Physical effects
- Bioaccumulation
- Desorption and toxicity of pollutants
- Leaching and toxicity of additives and monomers
- Transport of invasive species

A summary of selected studies demonstrating effects from plastic ingestion for different species is given in Table 10.1.

10.4.3 Biodiversity/Ecological Impacts

Population effect studies and risk assessments of plastics are lacking in the scientific literature. Several reports state that wildlife in the areas of the world's largest accumulation zones are heavily affected, for example, albatrosses in the North Pacific Ocean. Laysan albatrosses ingest large quantities of mostly macroplastics, but population effects are poorly understood. A correlation between ingested plastic load and decreased body condition in albatross chicks has been reported (Auman et al. 1997). The occurrence of a high ratio of plastic fragments relative to zooplankton is also of concern and might cause population effects due to disturbed energy balance in zooplankton feeders (Panti et al. 2015). Six times higher mass of plastic particles compared to plankton was found at the North Pacific gyre (Moore et al. 2001). There are most likely other organisms in areas heavily polluted by microplastics that are affected but have not been studied yet. Few studies have addressed the risk for the entire populations. One study reports a risk model for turtles using probability of debris ingestion as end point (Schuyler et al. 2016). The results show that up to 52% of sea turtles globally may have ingested debris. Olive ridley turtles are the most at-risk, and the highest-risk regions for turtle populations are the east coasts of the USA, Australia, and South Africa and the East Indian Ocean and Southeast Asia. Another potential oceanic impact from microplastics affecting an entire population is the enhanced oviposition in the endemic pelagic insect *Halobates sericeus* (Goldstein et al. 2012).

An increase in microplastic abundance could be associated with increase in *H. sericeus* which was interpreted as a substantial expansion of hard substrate available to *H. sericeus* and other substrate-associated organisms. So far it remains unclear if chemicals that are adsorbed to plastic or chemicals originating from plastic itself pose a considerable risk to wildlife via the uptake of these substances that are

Table 10.1 Summary of some selected studies showing the effects of plastics on different species

Species	Exposure method and concentration	Particle type	Effects	References
Scenedesmus sp.	1.6–40 mg/mL	20 nm PS	ROS increased, absorption, photosynthesis affected	Bhattacharya et al. (2010)
Lugworm (*Arenicola marina*)	5% of PVC pre-spiked with chemicals and clean PVC	PVC	Uptake of chemicals from plastic, decreased ability to engineer sediments, >30% more susceptible to oxidative stress by clean PVC	Browne et al. (2013)
Arenicola marina	0–5% by weight	130 μm unplasticized PVC	Ingestion, reduced feeding, increased phagocytic activity, reduced available energy reserves, lower lipid reserves	Wright et al. (2013)
Blue mussel (*Mytilus edulis*)	0, 0.1, 0.2, 0.3 g/L	30 nm PS	Ingestion, particles in pseudofeces, reduced filtering	Wegner et al. (2012)
Mytilus edulis	2.5 g/L	0–80 μm HDPE	Retention in digestive tract, transfer to lymph system, strong inflammatory response	Von Moos et al. (2012)
Pacific oyster (*Crassostrea gigas*)	0.023 mg/L	2, 6 μm PS	Increased filtration and assimilation, decreased oocyte number, diameter and sperm velocity, decreased larval yield and larval development of MP-exposed parents, significant shift of energy allocation from reproduction to structural growth	Sussarellu et al. (2016)
Shore crab (*Carcinus maenas*)	50 μL of suspended 0.5 μm PS particles	0.5 μm PS	Translocation into the hemolymph and tissue of crabs	Farrell and Nelson (2013)
Carcinus maenas	1.5 × 105 PS/g of mussel	8–10 μm PS	Retention of particles up to 14 days in the foregut; ingestion via gills and retention of up to 21 days	Watts et al. (2014)

(continued)

Table 10.1 (continued)

Species	Exposure method and concentration	Particle type	Effects	References
Tigriopus japonicus	5.25 × 105 PS/mL	6 μm PS	Ingestion, egestion, mortality, decreased fecundity	Lee et al. (2013)
Common goby, juveniles (*Pomatoschistus microp*)	18.4, 184 μg/L	1–5 μm PE	Modulation of bio-availability and bio-transformation of pyrene, decreased energy in simultaneous exposure of PE and pyrene, reduced acetylcholinesterase (Lind et al.) activity	Oliveira et al. (2013)
Japanese medaka (*Oryzias latipes*)	Ground up as 10% of diet	3 mm LDPE preexposed in marine environment	Liver toxicity, liver pathology, hepatic stress	Rochman et al. (2013)
Oryzias latipes	Ground up as 10% of diet	3 mm virgin PE pellets and preexposed marine PE pellets were ground to <0.5 mm	Decreased choriogenin expression (Chg H) in males exposed to marine plastic and vitellogenin, and increased estrogen receptor alpha expression in females exposed to virgin and marine plastic	Rochman et al. (2014)
European seabass larvae (*Dicentrarchus labrax*)	0.1 and 10 microbeads/g diet	10–45 μm PE	Ingestion, egestion, slight impact on mortality rate only with highest concentration of PE, cytochrome-P450-1A1 expression significantly positively correlated to the number of microbeads scored per larvae	Mazurais et al. (2015)

PS polystyrene, *PVC* polyvinylchloride, *PE* polyethylene, *PP* polypropylene, *HD* high density, *LD* low density, *Dw* dry weight

often classified as persistent, bioaccumulating, and toxic (PBT). There is evidence that the ingestion of plastic debris by seabirds is linked to dimethyl sulfide, a chemical cue released by phytoplankton in response to foraging activity (Savoca et al. 2016). The research team demonstrated experimentally that PE and PP microplastics exposed in the ocean for 3 weeks produce dimethyl sulfide. The migration facilitated by microplastics can affect population and ecosystem dynamics.

Plastics can add significant effects to an already stressed ecosystem. Some of the species that are affected by plastic pollution in the marine environment are endangered. In fact 15% of the species that are affected by entanglement and ingestion are on the IUCN Red List (CBD 2012). In particular the critically endangered Hawaiian monk seal *Monachus schauinslandi*, the endangered loggerhead turtle *Caretta caretta*, northern fur seal *Callorhinus ursinus*, and white-chinned petrel *Procellaria aequinoctialis* are of concern. Effects on the population level can be seen in species like the northern fulmar *Fulmarus glacialis* (van Franeker et al. 2011) and in lobster *Nephrops norvegicus*, which are commercially important species (Murray and Cowie 2011). The northern fulmar represents a very good species for the ecological monitoring of marine litter due to its high abundance and wide distribution in the environment. In the time period of 2003–2007, 95% of 1295 fulmars sampled in the North Sea had plastic in their stomach, and 58% of the birds exceeded the critical level of 0.1 g plastic in the stomach. The average amount of plastic in the stomachs was found to be 35 pieces weighing 0.31 g, and higher loads of plastic were found in juvenile birds compared to adults (van Franeker et al. 2011).

10.4.4 Impacts on Terrestrial Ecosystem

Understanding the impacts of plastic pollution for terrestrial ecosystem is still in infancy stage (Ng et al. 2018). Plastic loading rates in the agroecosystem could be high due to primary (manufactured) micro- and nanoplastics (e.g., waterborne paints, medical applications, electronics, coatings, adhesives) or indirectly as secondary microplastics or nanoplastics generated as breakdown of larger plastic debris (Duis and Coors 2016). Microplastics that pass through wastewater treatment plants, 95% of microplastics are estimated to be retained in the biosolids (Ziajahromi et al. 2016). As both treated wastewater and biosolids are getting used in agriculture for irrigation and fertilizers, the microplastic loading on agriculture land is likely to be high (Mohapatra et al. 2016). In Europe, it is estimated that approximately 63,000–430,000 tons of microplastic enter the agroecosystem annually through biosolids alone, while estimates for North America range from 44,000 to 300,000 tons annually (Nizzetto et al. 2016). Australian and New Zealand Biosolids Partnership (2016) have estimated that between 2800 and 19,000 tons of microplastics are applied to Australian agroecosystem each year through biosolids. Besides biosolids, composts derived from non-source-separated residential waste or mixed municipal solid waste and source-separated garden organic waste are also sources of plastic pollution in agroecosystem.

Plant-microbe-plastic response was studied for agroecosystem where organismal-level response to micro- and nanoplastics was reported on earthworms *Lumbricus* *terrestris* exposed to 28% PE microplastics (w/w in dry plant litter) and above showed growth inhibition (Huerta Lwanga et al. 2016). Studies on algae showed that nanoplastics adsorbed on the cell wall of microalgae such as *Scenedesmus* and *Chlorella* indicated that nanopolyesternes interfered with algal photosynthesis due to increased water turbidity and light scattering, coverage of algal cell surface with microplastics, or immobilization of algae at concentration of around 1.5 mgl^{-1} and above (Nolte et al. 2017). To assess the soil microbiome response to micro- and nanoplastics, a pot trial experiment with 67.5 and 337.5 $kgha^{-1}$ plastic mulch residue (20×20 mm) was conducted (Wang et al. 2016) at constant moisture content; soil microbial biomass, enzyme activities (dehydrogenase and fluorescein diacetate hydrolysis), and functional diversity (community level physiological profile) tended to decrease with increasing concentrations of plastic mulch residue. Uptake of microplastics by plants is not expected. The high molecular weight or large size of plastic particles prevents their penetration through the cellulose-rich plant cell wall. In contrast, nanoplastics have been shown to enter plant cells (Ng et al. 2018). There are no studies on translocation and storage of nanoplastics in plants. Similarly, there is no data on the toxicity of nanoplastics on plants (Ng et al. 2018).

General observations on toxicity of carbon nanoparticles that may have relevance to future studies using nanoplastics are the following: (1) phytotoxicity tests such as germination, root elongation, and growth measures across studies indicate that the sensitivity depends on plant species and the physiochemical properties of the engineered carbon nanoparticles; (2) cell damage occurs through genotoxicity and cytotoxicity (Shen et al. 2010); and (3) interactions between different types of engineered carbon nanoparticles with pesticides can increase or decrease the uptake of pesticides by different crops (Torre-Roche et al. 2013). So far, studies on the ecological impact of plastic in soil are mostly at organismal level or on the soil microbiome. But, impact studies at higher biological organization are difficult (Browne et al. 2015), suggesting that existing knowledge of ecological linkages, where known, and population models, where the linkages are unknown, can be used to deduce such impacts. Currently, only one laboratory study explored such ecological linkage. The study showed that the *L. terrestris* had lower biomass under the exposure of 7% microplastic (w/w in dry plant litter), while the burrows occurred in significantly higher numbers, and burrow walls were denser compared to the control without exposure to microplastics; however, the burrow length was similar across all treatments during the 14-day experiment (Huerta Lwanga et al. 2017). These results indicate soil porosity may increase as a result of earthworm-microplastic interaction. Additionally, microplastics may also have direct effect on soil porosity, as both synthetic water soluble and gel-forming polymers are used as soil conditioners to improve water infiltration, water retention, and soil stabilization.

Millions of plastics are being disposed in the marine and the terrestrial environment. Plastic pollution poses threat to planta and animals including humans. In landfills, the decomposition of plastics takes up to 1000 years, leaching potentially toxic substances into the soil and water. The impact of microplastics in the soil sediments could cause a negative effect to the ecosystems. Most of the plastics end up in soil and in water where it disintegrates into microplastics, and these microplastics can further break into smaller particles, referred to as nanoparticles or nanoplastics (<0.1 μm). These particles can potentially enter the food chain and produce hazardous health effects.

Sewage is an important factor which causes the distribution of microplastics. Eighty percent to 90% of plastic particles are contained in the sewage. Sewage in the form of sludge is applied to the fields as fertilizers; due to this thousands of microplastics end up each year in the soil. These microplastics can be found even in the tap water. Microplastics interact with soil fauna which affects the soil functions. For example, earthworms make their burrows differently due to the presence of microplastics in the soil, which affects the earthworms functioning and lowers the soil condition (de Souza Machado et al. 2017).

Chlorinated plastics can release harmful chemicals in the soil which can be moved into the underground water or any other surrounding water sources and to the ecosystem. This causes potentially harmful effects on the species that drink water. When plastic particles break down, they put on new physical and chemical properties which elevate the risk of noxious effect on organisms. Additives like phthalates and bisphenol A (BPA) leach out of plastic particles. These additives cause hormonal effects and disrupt the hormone system of vertebrates and invertebrates. The minute particles cause skin irritation and cross the blood barrier or the placenta.

Plastic Bags Harm to Soil Ecosystem

- When the plastic bags are thrown to the soil, it has impacts on the fertility of the soil.
- Plastic bags do not dissolve, but they break into smaller pieces into microplastics or nanoparticles and remain for many years contaminating the soil, water, and the oceans.
- Plastic bags gradually release toxic chemicals that affect animals when they are consumed.
- When plastics are burned, they discharge toxic chemicals which get deposited in the soil surface, water, and on plants.

10.4.5 Impacts on Human Health

There is mounting evidence of the occurrence of plastic particles in marine organism that are part of the human food chain, and this might also represent a potential threat to human health via biomagnification. A possible exposure pathway of humans to microplastic is represented by the diet, especially since there are studies available that demonstrate the presence of microplastic in commercially important fishes, shrimps, and mussels (Devriese et al. 2015; Romeo et al. 2015; Van Cauwenberghe and Janssen 2014). Microscopic fibers ranging from 200 to 1500 μm have been found in mussels (average 3.5 fibers/10 g mussel) from Belgian stores which was in the same range as wild-caught mussels in the same study (De Witte et al. 2014). Furthermore, synthetic fibers were reported in 63% of commercially important brown shrimp caught in the Southern North Sea and Channel area (Devriese et al. 2015). There are also studies that reported nonmarine sources of microplastic in the food chain. For example, 19 honey samples were analyzed for colored fibers and fragments, and colored material was found in all of the samples (Liebezeit and Liebezeit 2013). Fiber counts ranged from 40/kg to 660/kg of honey, and fragments ranged from 0 to 38/kg of honey. Sources were identified as introduction of particles during the processing of honey and/or particles were introduced by the bees into the hive. Honey samples originated mostly from Germany but also from France, Italy, Spain, and Mexico. Five commercial sugars were analyzed as well, and in all refined samples, fibers (mean 217 ± 123/kg of sugar) and fragments (32 ± 7/kg of sugar) were found. Unrefined cane sugar was found to contain 560 fibers and 540 fragments per kilogram of honey. Furthermore, a total of 24 German beer brands were analyzed in a study for microplastic fibers, fragments, and granular material (Liebezeit and Liebezeit 2014). Contamination was found in all samples with fiber counts ranging from 2 to 79 fibers/L, 12–109 fragments/L, and 2–66 granules/L.

As potential sources of the contamination of natural and synthetic fibers in clothing that become airborne, materials that were used during the production process and bottles that might have been already contaminated or became contaminated during the cleaning process were pointed out. A study on 15 different table salts in China demonstrated the presence of microplastics in the samples (Yang et al. 2015). The amount of microplastics ranged from 550 to 681 particles/kg in sea salts, 43–364 particles/kg in lake salts, and 7–204 particles/kg in rock/well salts. Sea salts were found to be significantly higher contaminated with microplastics than other salts which underline the contamination of marine products. In sea salts particles measuring less than 200 μm were detected to be the predominant type of microplastic, accounting for 55% of the particles with PET as the most abundant polymer type followed by PE and cellophane. The risk of microplastic transfer from the gastrointestinal tract in humans and other mammals to other tissues is real. Hussain et al. (2001) showed that PE particles could transfer from the gut to lymph and circulatory system in humans. PS particles up to 240 nm were shown to be taken up by the placenta in a human ex vivo study (Wick et al. 2010). Nanosize particles of PS also decreased cell contractility of human muscle cells and resulted in cellular damage of human blood vessels (Berntsen et al. 2010; Fröhlich et al. 2010). Further effects related to the plastic polymer itself are not described; however, knowledge can probably be extracted from the field of medical transplants using polymer materials of different types.

Another concern in regard to the exposure of plastics (mainly macro and microfibers) in humans is the plastic-associated chemicals (PACs) such as bisphenol A and phthalates. These compounds are well-known as endocrine disruptors and interfere with the hormone system. In one population-based human study, levels of BPA and several phthalate metabolites were associated with lipid infiltration of the vascular wall and therefore suggesting that these chemicals play a role in atherosclerosis (Lind and Lind 2011). Furthermore, BPA was reported to be positively associated with cardiovascular disease and prevalent myocardial infarction in a cross-sectional analysis of 1455 adults (Lind and Lind 2012).

10.5 Preventative and Control Measures

Preventive and control measures to plastic pollution mainly involve physical, chemical, biological, technological, and regulatory interventions. Physical interventions mainly involve development of appropriate local-, regional-, and national-level infrastructure (EMUN 2017) to manage the plastic waste using of various technologies, viz., Waste to Energy and others. Further, increased awareness about plastic consumption and handling and managing the plastic waste from unit level such as household can also have high positive impact on preventing and controlling the plastic pollution, and other physical methods such as recovery, recycling, and reusing (3Rs) of various plastic forms would reduce the plastic contamination in the environment.

Biological and chemical methods deal with the decomposition of the discarded plastic. The existing chemical decomposition methods are not economically viable at industrial scale (Koushal et al. 2014) and therefore require more research on scaling up the technology. Enormous work is being undertaken on biological decomposition of the plastic material. Many researchers have been using various biological forms such as bacteria, fungi, worms, insects, etc. where various forms of plastics have been subjected to biodegradation. Degradation of polyethylene using *Bacillus subtilis* (Vimala and Mathew 2015) and wax moth *Galleria mellonella* (Bombelli et al. 2017), application of mixed culture of *Lysinibacillus xylanilyticus* and *Aspergillus niger* for degradation of low-density polyethylene (LDPE) (Esmaeili et al. 2013), use of *Pseudomonas* species for LDPE (Kyaw et al. 2012), and ability of *Penicillium oxalicum* and *Penicillium chrysogenum* (Ojha et al. 2017) to degrade plastic pave the way for the application of biological material for plastic degradation.

Technological interventions can be applied by developing alternative material to plastics and/or developing plastics with more biodegradable components which eventually would help in faster degradation of the plastic in an environmentally sound manner. Use of commercialized biopolymers can be one of the alternatives to reduce synthetic components in plastics. Materials including starch, soy, cellulose, or lignin could provide viable alternatives as biopolymers. Similarly, plastics based on bacteria or natural fibers have been reported as alternatives for plastics (Faris et al. 2014). Other technological interventions include use of polymer in bitumen roads, pavements, etc. (Appiah et al. 2017; Manju et al. 2017; Jafar 2016; Koushal et al. 2014) and use of techniques such as incineration (Huang 1995), mechanical recycling, and chemical recycling for production of new petrochemical product and plastics. Pyrolysis, gasification and hydrogenation for production of gases and pyrolytic oils can be considered as an alternative ways (Awasthi et al. 2017).

10.6 Evidence from Asia-Pacific-European Countries: Case Studies

Plastic is known to be ubiquitous pollutant which remains in the environment for longer period of time. Eriksen et al. (2014) reported the presence of plastic pollution across the five subtropical gyres, costal Australia, Bay of Bengal, and Mediterranean Sea. Evidences of plastic pollution have been reported from estuarine waters of Western Australia (Hajbane and Pattiaratchi 2017); inland freshwaters of Wuhan, China (Wang et al. 2017); along the Belgian coast (Claessensa et al. 2011); lagoons of Venice, Italy (Vianello et al. 2013); beaches in Mumbai, India (Jayasiri et al. 2013); Swiss lakes in Switzerland; Lake Garda, Lake Bolsena, and Lake Chiusi in Italy; Rhine-Main area in Germany; Seine River in France; Danube River in Austria (Wu et al. 2018); Levantine coast of Turkey (Gündoğdu and Çevik 2017); and coastal areas of Brunei Darussalam, Cambodia, East and West Malaysia, Indonesia, Myanmar, the Philippines, Singapore, Thailand, Timor-Leste, and Vietnam (Todd et al. 2010).

10.7 Socioeconomical Aspects of Plastic Pollution

Plastic pollution is a global concern where presence of various forms of plastics ranging from macro fractions to nanoplastics has been reported in various ecosystems and environmental regimes. The socioeconomic status of the region can severely be affected by the pollution, e.g., fisheries have been one of major livelihood business in the coastal regions of various parts in the world, but majority of the coastal areas have been reported of severe pollution. Plastics contribute a significant part in the coastal pollution due dumping of solid wastes. This has considerably damaged the marine ecosystem, thus affecting commercial coastal and marine fisheries (Islam and Tanaka 2004) and coastal tourism (Watkins et al. 2015), thus impacting livelihoods of the local communities. Microplastic ingestion has been reported by many laboratory and field studies, but its movement across the food chain and its toxic impacts on the entire ecosystem are still being investigated to a great extent and pose a significant concern (Cole et al. 2011). Horton et al. (2017) reported that terrestrial pollution poses greater threat to environment and human life than that of marine pollution as annual disposal or dumping of plastic on the land "is estimated at 4–23 times that released to oceans."

Presence of plastics in urban and peri-urban environments poses a serious threat of contaminating soils, groundwater aquifers (Hurley and Nizzetto 2018), inland waterways, etc. This contamination exhibits potential pathway of exposing biotic components including human health to toxic components of plastic. In urban and peri-urban areas, plastic pollution is known to cause blockage of sewers and drainages creating unhygienic conditions, breeding grounds for mosquitoes causing various diseases (Wachira et al. 2014). It has become a global practice to undertake high-intensity agriculture to feed the population and the livestock. Intense agriculture makes use of various plastic products for activities like mulching, etc. Liu et al. (2014) report that "Large amounts of residual plastic film have detrimental effects on soil structure, water and nutrient transport and crop growth, thereby disrupting the agricultural environment and reducing crop production." Loss of grazing areas of livestock and deteriorating soil health due to plastic pollution (Brodhagen et al. 2017; UN Environment 2017) would result in loss of agricultural productivity and associated revenue.

10.8 Path Toward Plastic-Free Environment

Plastic pollution is a shared responsibility and we can all do our bit to reduce its impacts. Worldwide, the impact of plastic shopping bags has received significant attention, and there have been many attempts to reduce their use. Policy intervention plays a very important and necessary role in achieving targets. Over the years many, international agencies, viz., the United Nations Environment Program (UNEP); United Nations Educational, Scientific and Cultural Organization (UNESCO); Oceans Compact; United Nations-Oceans (UN-Oceans); EPA (Environmental Protection Agency); Global Partnership on Marine Litter; Online Marine Litter Network; Global Programme of Action for the Protection of the Marine Environment from Land-based Activities (GPA); Global Partnership on Waste Management and Environment Management Group, have played a very important role in combating plastic pollution. Since 1972, major global players have been formulating and implementing various regulations and treaties such as the London Convention (1972) on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter, International Convention for the Prevention of Pollution from Ships (1973/1978), United Nations Convention on the Law of the Sea/MARPOL (Part VIII, Section 2, Articles 117–120), Convention for the Protection of the Marine Environment of the North-East Atlantic (the OSPAR Convention) (1992), Marine Strategy Framework Directive (Directive 2008/56/EC), EU Strategy (2018) for Plastics in the Circular Economy, and UN Sustainable Development goals (2030) (EC 2018; EMUN 2017 and Eunomia 2016).

Figure 10.9 explains the strategic plan that needs to be followed to work together at national and international level in curbing global plastic pollution. More than 30 countries have implemented voluntary or regulatory approaches to reduce the use of lightweight plastic bags. Countries such as Bangladesh, South Africa, China, Ethiopia, Eritrea, France, Italy, Kenya, Morocco, and Tanzania have banned plastic bag use. England, Ireland, Wales, Denmark, and Germany have used point-of-sale charges to reduce plastic bag use. Though there is no national plastic bag ban or charge in the USA, over 100 local counties and municipalities have plastic bag bans or charges, and California has a state ban on plastic bags. Internationally, France passed a legislation in 2016 to reduce the environmental impacts of single-use plastic tableware (plates, cups, and cutlery). The legislation comes into effect in 2020 and

Fig. 10.9 Strategic action plan toward plastic-free environment

will require disposable tableware to be compostable at home and composed of at least 50% biologically sourced material (The State of Victoria Department of Environment, Land, Water and Planning 2017).

At the Fifth International Marine Debris Conference in 2011, delegates came together to develop the "Honolulu Strategy." The strategy provides a framework for global efforts to reduce the impacts of marine debris. In 2012, the United Nations Environment Programme (UNEP 2016) launched the Global Partnership on Marine Litter, a voluntary partnership for international governments, other organizations, and individuals to reduce the impacts of marine litter.

10.9 Areas of Future Research

Adverse effect concentrations of various plastic types, particle forms, and sizes are lacking for all species. It is known that plastics are ingested, that chemicals can sorb to particles and desorb in species, and that biological effects can occur. However, it is currently not possible to assess the risk of macro- and nanoplastics since the reported studies do not present dose-response relationships. The most prevalent polymer types found in the environment is PP, PE, and different polymers in the shape of fibers, and it is therefore recommended to prioritize these in effect studies. There are indications that smaller size particles are more hazardous; they can easily enter the food web, they have a larger capacity to sorb or incorporate pollutants, and larger particles will eventually fragment to smaller particles. The goals must therefore be to generate relevant dose-response relationships and adverse effect concentrations with emphasis on exposure to the dominant size fractions and forms of PP, PE, and PS particles and synthetic fibers from clothes (polyesters, polyamide). Tests should be done on ecologically and economically relevant marine and freshwater species (invertebrates and fish) under standardized conditions. In such tests it is also critical to develop standardized methods for expressing plastics doses, taking particle form, polymer type, and size distribution into consideration. Preferably, the doses should be expressed in the same way as in field measurements, and it would be helpful if field surveys report abundance, weight, and type of plastics. This would greatly facilitate the possibility to perform actual risk assessments, putting critical effect levels in relation to actual exposure levels. The toxicity testing should be designed so effects of plastics and effects of plastic-associated chemicals can be distinguished. The testing would generate new knowledge regarding mechanisms of toxicity of plastics and associated additives and adsorbed chemicals. Crucial in this research is the issue of bioavailability of plastic-associated chemicals in living organism. Therefore, mechanisms that influence desorption in the gastrointestinal tract of sorbed and incorporated chemicals and the extent of chemical uptake should be studied. The relative role of organ accumulation of plastic-associated chemicals to the total load of chemicals from the surrounding environment including diet should also be quantified.

Besides effect testing, a standardized global qualitative and quantitative biomonitoring program focusing on temporal and geographical variation of occurrence of microplastics in freshwater and marine ecosystems on all trophic levels should be implemented. These data will be crucial in understanding the point sources, trends, microplastic dynamics regarding fate and behavior, and development of potential risks to guide plastic management strategies. Another area of focus is studies on occurrence of microplastics in the terrestrial environment with emphasis on synthetic fibers and agricultural areas using sewage sludge as fertilizers.

The question whether microplastics will fragment to nanoplastics in significant amounts remains to be answered. Therefore, it is crucial to direct research resources toward elucidation of occurrence of nanoplastics in freshwater and marine ecosystems. Further, laboratory studies on fragmentation processes from micro- to nanoplastics should be performed, as should uptake and toxicity studies of nanoplastics in relevant organisms.

Human exposure to microplastics is not well studied. Putative exposure pathways include microplastics entering the lungs via air or entering the gastrointestinal tract via water and food. None of these pathways have been quantified. Therefore, it is crucial to assess and quantify the exposure pathways of microplastics to humans. The effects of microplastics on humans are to a great extent unknown although knowledge from the pharmaceutical field should be carefully reviewed. Therefore, a systematic assessment of microplastics toxicity on human should be carried out. Typically, for effect assessment this would entail extrapolation of effects found in experimental animal studies and from occupational settings. Exposure scenarios and actual measurements of microplastics exposure are also needed. Put together, generation of this kind of research data should facilitate human risk assessment of microplastics of different size, form, and polymer type. The risk assessment should also include effects of microplastic-associated additives and also effects of nanoplastics if there is a potential human exposure of this particle size class.

10.10 Concluding Remarks

Plastics make an important part of human lives and well-beings. Owing to their unmatchable properties including strength, low weight, durability, and cost-effectiveness, plastics and their products are vastly used for packaging, transport, healthcare, construction, electronics, and several other industries. Consequently, the global plastic production has seen rapid growths in the last few decades. Unfortunately, it has led to the generations of huge amounts of plastic wastes and debris, serious threat to the biosphere. Plastics get accumulated through various ways, biofouling or accumulation of microorganisms, plants or algae onto the plastic debris, which makes them heavier, and this makes the debris eventually sink at the base of the sea. Various types of plastics and plastic wastes are known to cause serious negative implications on marine, freshwater, and terrestrial ecosystems as well as on human health. Plastic waste management has become a serious challenge in contemporary times and necessitates effective approaches to tackle this problem; one potent way might be via integrating the physical, chemical, biological, and technological approaches coupled with regulatory interventions. Through this chapter, we have proposed some promising approaches to make our planet plastic-free, including mainly through innovation, technological advents, and collaborative efforts for providing better alternatives, creating public awareness, and developing governmental policies to name a few. However, more comprehensive and in-depth studies are needed not only for detailed assessment of hazardous effects each type of plastics exert on life forms but also for developing better environment-friendly but cost-effective alternatives.

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Chapter 11
Biosorption of Heavy Metals: Potential and Applications of Yeast Cells for Cadmium Removal

Bashar Hadi and Muftah H. El-Naas

Abstract Biosorption is a physicochemical process that uses nonliving biomass to remove metal ions. The process is promising and innovative biotechnology, which has excellent economic feasibility for metal removal and/or recovery due to its efficiency, simplicity, cost-effectiveness, and abundance of biomass when compared to industrial synthetic adsorbents. Practically, most biomaterials have an affinity for metal species. By using a low-value biosorbents such as a waste stream, two sustainable goals can be achieved in a single process: reducing the waste stream and removing/recovering metals from industrial effluents. In literature, several studies have investigated the efficiency of various biosorbents to remove/recover metals from solution. This chapter critically reviews different aspects of biosorption research such as the characteristics of an ideal biosorbents, the key main parameters affecting the metal uptake performance, different metal uptake mechanisms, the process advantages and drawbacks, and potential of biosorption as a possible industrial process. It also summarizes key recent developments in these areas and the significance of biosorption in wastewater treatment processes and in the environment.

Keywords Cadmium · Heavy metal · Industrial effluents · Treatment and biosorption

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11.1 Introduction

Metals tend to maintain indefinitely, circulate, and finally accumulate throughout the food chain, which increases the serious health hazard threat involved and eventually jeopardizes human life. In many cases, the technologically and economically important heavy metals are the same as those considered a real threat to the environment because of their accumulation in the environment caused by man's activities (Al-Saydeh et al. 2017). High levels of contaminated heavy metal wastewater are produced by metal plating operations, mining and metallurgy industries, metal processing, battery or galvanizing and accumulator manufacturing operations, thermal power generation (especially coal-fired plants), as well as nuclear power generation (Abbas et al. 2014). The effluents from these industries and mine tailings might be the major discharge source of various combinations of toxic heavy metals. Therefore, it would be ideal to have an on-site treatment facility for the elimination of heavy metals from wastewater. It is practical from a policy standpoint that those who are responsible for metal pollution should be in charge of its remediation. Such a treatment facility that can reduce the heavy metals waste streams would be more efficient than treating large volumes of a mixture of heavy metals in a general sewage plant.

The impact of heavy metals on the environment is well known, where the big three metals, mercury, lead, and cadmium, are in the limelight (Sulaymon et al. 2010). The significant use of mercury and lead in various industries has been significantly reduced. In contrast to mercury and lead, cadmium is toxic and abundant in its distribution throughout the world. The use of cadmium in different industries has been steadily increasing in the past few years. It is widely used in many industrial processes such as metallurgical alloying, mining, smelter operations, metal plating, electroplating, paints, pigments, electronics, and nickel-cadmium batteries. The annual production of cadmium was 24,900 mt for the whole world in 2015 (Brown et al. 2017).

Recently, cadmium has attracted much attention because of its extremely serious health hazard. Its toxic effects are particularly prominent in higher trophic level animals, mainly humans. It tends to accumulate in the human body where it has a half-life of 10–30 years. The toxicity of cadmium is responsible for kidney damage, bone disease, as well as cancer. Cadmium tends to accumulate in the human body at an average of 30 mg for an American male. It is mainly deposited in the kidneys, 33%, and in the liver, 14% (Naja and Volesky 2009). Therefore, the study of cadmium uptake and recovery from metal-bearing effluent by an innovative metal uptake method is of high potential interest. Since metals are recycling in the environment, the only effective way of preventing heavy metals poisoning is metal removal and/or recovery. Conventional treatment methods are expensive and ineffective in certain cases. An alternative efficient and environment-friendly method in detoxification of waste effluent should be studied extensively due to the demand of metal species. An effective and economical process to remove/recover toxic heavy metals from industrial wastewater is in extreme demand, which can substantially decrease the overall process cost. Ideally, metal recovery should be investigated in parallel with the removal process.

The use of a biosorption method, using microorganisms or their products to remove toxic heavy metals, represents a promising technology. It can be a strong and inexpensive candidate for remediation of heavy metal-contaminated wastewater. The biosorption key process parameters should be studied extensively to improve the performance of the process.

11.2 Metal Uptake Processes

11.2.1 Conventional Metal Uptake Methods

Physicochemical processes are used for heavy metal uptake from metal-contaminated wastewater effluent. Conventional technologies, such as neutralization, precipitation, chemical oxidation or reduction, ion exchange, activated carbon adsorption, filtration, electrochemical treatment, solvent extraction, and evaporation recovery, and membrane technologies, such as electrodialysis and reverse osmosis, have been used to treat metal-bearing solutions disposed from contaminated effluent. However, these technologies have significant disadvantages including low efficiency, high energy requirements, high cost, and generation of other toxic waste products (Al-Saydeh et al. 2017; Abdi and Kazemi 2015). These treatment processes may become extremely expensive or inefficient especially in dilute metal solution, i.e., less than 100 mg/L (Abbas et al. 2014). Major disadvantages of most common processes can be summarized as follows (Gadd 2009).

(a) Evaporation method can be economically feasible with a highly concentrated metal solution. Evaporation lagoons are an inexpensive and an easy way to treat wastewater effluent, where the main cost of lagoons is the capital cost for suitable land. The operational cost of this technique is high, because the process is ineffective and time consuming. Also, metals cannot be selectively separated from the effluent, where a sludge of a mixture of waste metals is obtained. Consequently, another downstream sequestering method such as ion exchange or biosorption needs to be applied to recover certain metal species.
(b) Membrane technology is usually applied in desalting seawater and specific food processes. The concentration of metal ion in the feed should be considerably low for a successful membrane operation. Increasing the metal concentration may lead to not only a decrease in the separation efficiency but also to membrane scaling. The higher the metal concentration, the higher the required energy for separation, and the lower the observed efficiency. The membranes are expensive materials that have a short operation time and cannot operate with various types of chemicals and solution pHs. Also, they lose their productivity with time and are subjected to decomposition in the presence of microorganisms, compaction,

and scaling. All these major cost drawbacks make the membrane technology less favored for the use in wastewater treatment.

(c) Precipitation and clarification technologies require a large volume of chemicals to treat wastewater effluent, which make it an expensive technology. The metal concentration after the precipitation process is often higher than the acceptable discharging limits. Moreover, metal cyanide complexes cannot be treated by this method, thus they must be separated by other methods prior to the precipitation process. In this process, one type of wastewater is converted to another, i.e., sludge with metal hydroxides. Although the waste volume is reduced, the impurities concentration can still be unacceptably high. Other drawback of the process is the poor settling and filtration properties of the resulting sludge.

(d) Activated carbon is the most widely used commercial adsorbent, since it has a good adsorptive capacity for organic compounds. However, the activation and regeneration process of the used carbon are a costly step of the carbon adsorption process. The activation process must be carried out after each regeneration process of the saturated carbon. The uptake capacity decreases after each activation/regeneration cycle.

(e) Ion-exchange resins have been used extensively in wastewater treatment; however, few factors still limit their application. They are often cannot tolerate thermal and chemical changes such as the effects of oxidation, where organic materials can cause fouling of the resins. Chemical decomposition can take place when metallic species in the solution break the polymer chains or modify the functional groups. The metal uptake capacity of resins is affected by the presence of calcium or magnesium ions in the effluent stream. Moreover, ion-exchange resins are usually more expensive than biosorbent materials. It is well known that the most expensive biosorbent, e.g., biosorbent derived from freshwater alga, *Chlorella vulgaris*, is still less expensive than the cheapest ion-exchange resins. A quantitative comparison between metal uptake capacity by biosorbent materials and ion-exchange resins shows that biosorbents can reach similar or even higher uptake capacities than synthetic ion-exchange resins (Wang and Chen 2009).

Water quality standards in many countries clearly emphasize on controlling or reducing the level of heavy metals in discharge effluent to a certain limit. Although industrial processes have certain standard limits, drinking water quality has a much lower limit that may significantly increase the cost of treatment. The acceptable safety limits for cadmium intake have little general agreement among different health organizations. In the United States, the water standard level of cadmium in drinking and waste discharge has been set at 5 and 100 μg/L, respectively (Zhao et al. 2002; Bazrafshan et al. 2015).

11.2.2 Biological Treatment Method

The main concerns of early studies of metal uptake have been solely on nutritional and toxicological aspects accompanied by the presence of metal. Alternative metal uptake and/or recovery methods have been investigated, which are based on metal sequestering properties of natural biomaterials. The potential use of certain types of microorganism in contaminated wastewater treatment has being studied intensively (Al-Saydeh et al. 2017; Abbas et al. 2014; Abdel-Ghani and El-Chaghaby 2014; Mehta and Gaur 2005; Fiset et al. 2008; Abdi and Kazemi 2015; Ayangbenro and Babalola 2017; De Gisi et al. 2016). The use of various microorganisms or their products to remove toxic heavy metals has given promising results as an inexpensive remediation method for heavy metal-contaminated wastewater. In literature, biosorbent originating from fungal biomass or yeast has been shown to be effective in heavy metal uptake. A variety of yeast species show the ability to remove cadmium from solution. Preliminary screening of different biomass types for their affinity of cadmium removal showed few dissimilarities and an interesting potential regarding the biomass of a common yeast *Saccharomyces cerevisiae*.

11.2.2.1 Biosorption and Bioaccumulation

It is important to define an appropriate terminology related to the metal uptake by various types of microorganisms. The metal accumulation by active cells is usually referred to as bioaccumulation. This process depends on the metabolic activity of the cell, which in sequence can be significantly affected by the presence of metallic ions. Many studies on metal uptake by biomass have been conducted with living organisms for environmental, toxicological, and pharmaceutical purposes rather than in terms of industrial application. Bioaccumulation, which has been mainly studied by microbiologists for its toxicological importance, is less desirable for metal removal and/or recovery design process (Abbas et al. 2014). This can be due to the requirement of the cells to be active which eventually means a special culture environment.

The metal transfer from the surrounding environment into the cell may be achieved by active transport accompanied with metabolic activities. However, it is possible that a passive metal uptake by the cell can be a part of the process of metal transportation throughout the cell wall, membrane, and into the cell interior, where it becomes practically a part of the cell structure or a metabolic product (Dhankhar and Hooda 2011). Recently, attention has turned to the usage of inactive, dead microorganisms as potential industrial tools for metal uptake and/or recovery. Biosorption is a property of certain types of inactive, nonliving, microorganism to uptake heavy metal ions from contaminated solutions even at low concentrations. Biosorbent, derived from biomass, can show chemical substance properties, i.e., an ion exchange of biological origin.

The term biosorption is generally used for the metabolically independent passive metal ion uptake by dead biomass. It should be distinguished from bioaccumulation, which is mainly an active metabolically dependent process that takes place in living microorganisms. Metabolically active cells are used in bioaccumulation, which is opposite to biosorption and requires a different approach for investigation (Abdi and Kazemi 2015). Frequently, biosorption and bioaccumulation terms are used as an exchangeable term for the metal uptake process since the main mechanism is not known. The use of inactivated microorganisms is not subjected to physiological constraints. It eliminates some undesirable issues related to the maintenance of an active microbial culture such as nutrient supply, toxicity thresholds, and thermal inactivation of the microorganism and cross contamination by other microorganisms. Living cells cannot resist the toxic effect of a heavy metal effluent, which may result in cell death. Therefore, passive biosorption may be ideal for such a case. Moreover, inactive brewer's yeast cells of *S. cerevisiae* were significantly more efficient than those of active cells in metal uptake capacity of copper, zinc, and nickel for 1.3, 14, and 16 times, respectively (Machado et al. 2009). Also, cadmium uptake by inactive biomass of *Bacillus cereus* was found to be more than those of living yeast cells (Ganguly et al. 2011).

The major challenges in the biosorption field are to select the potential types of biomass from a very large pool of available and inexpensive biomaterials and to define the predominant mechanism that controls the biosorption operation. Therefore, biosorption studies have been scattered in several different research contributions. Few extensive reviews are available in the literature dealing with large number of biomaterials that have been tested for their metal uptake capacity under various conditions.

11.2.2.2 Biosorption Advantages

The biosorption process has many advantages including low amount of biological sludge for disposal, high efficiency in the treatment of low concentration effluent, and no nutrients required as for the case of bioaccumulation. The use of dead biomass in wastewater applications shows some advantages since the biosorption application and the production of the microbial cells can be a separate process, which can be optimized to increase the metal uptake. Furthermore, the biomass is an active chemical formulation, which is the main raw source to produce efficient biosorbent material. These biosorbents can be greatly efficient, selective, and inexpensive. These properties give biosorbents the advantages over commercial ion-exchange resins and activated carbons. Several potential properties of biosorbents make metal uptake by biosorption an economical and feasible treatment process. The major advantages of this innovative treatment technology are (Abbas et al. 2014):

- Dilute metal solution can be selectively treated.
- Biosorbent has very low affinity for calcium and magnesium ion species and other light metals in general.
- Effluent discharge concentrations meet the environmental limits.
- Biosorption systems present low capital cost and very low operational costs.

- Biosorption systems can operate over a wide range of pH values (pH 3–9).
- Biosorption systems are effective over a temperature range of 4–90 °C.
- The cost of toxic sludge disposal is reduced substantially by converting pollutant metals to a metal product solution.

Conversely, the main disadvantages of biosorption process can be its ineffectiveness at highly concentrated metal-bearing solutions as well as being undefined technology when compared with well-established technologies, e.g., ion exchange.

11.2.2.3 Comparison Between Biosorption and Conventional Methods

The commercial feasibility of biosorption may be improved by a combination of different approaches including the use of waste industrial biomass, regeneration of the biomass after each biosorption cycle, and recycle and sale of the eluted metal species. Biosorption is eventually suitable as a polishing process in wastewater treatment. By applying biosorption, it is possible to obtain high standard drinking water quality, e.g., initial metal concentrations of 1–100 mg/L can be reduced to final metal concentrations of <0.01–0.1 mg/L (Abbas et al. 2014). A higher uptake affinity for the metal at low residual concentration is obtained in the biosorption process. It is recommended to use another pretreatment technique such as precipitation, for concentrated metal solutions, when the metal concentration is higher than 100 mg/L. This is to avoid unreasonably fast exhaustion of the sorption capacity. However, this approach has the main drawback that is represented by generation of toxic hazard sludges from which it may not be feasible to recover the metals.

11.2.2.4 Economic Feasibility of Biosorption

The main advantages of biosorption when compared to the conventional methods are wide range of environmental operating conditions and strong economical alternative due to the inexpensive abundant raw biosorbent materials. These materials include waste products from other industries, e.g., fermentation by-product, or naturally abundant biomass such as marine algae. The cost of the biosorption process strongly depends on the cost of the biosorbent material itself that can remove metal ions. The capacity of the biosorbent to remove metals plays an important role in efficiency and the feasibility of the process. Also, selectivity of the process for a certain metal species is an important issue for selecting the most appropriate technique. The cost of raw biomass varies from no cost, i.e., just the collecting cost, to USD $16/kg (Ngo et al. 2015). Eventually, harvesting and drying practices are the major costs of marine algal biomass types. The cost is much lower than that of commercial activated carbon of USD $20/kg (Rafatullah et al. 2010).

The overall feasibility of the biosorption process is determined by several factors such as biosorbent uptake capacity, process kinetics, the effect of other metal ion presence, the performance of the biosorbent, regeneration ability, and the possibility of metal recovery. The biosorption process is an economically feasible and technically effective technology for metal uptake and recovery. The capital and operational cost of the biosorption process is very competitive with existing treatment technologies.

Recovery of metals from solution may be beneficial since the metal may be subsequently desorbed from the biomass and recovered for reuse. The biosorbent can be regenerated, incinerated, or stored in landfills. The reuse of the biosorbent can significantly increase the economic advantages of the process. The regeneration of biosorbent after each metal sequestering cycle is as important as the metal uptake capacity. Investigations on metal elution indicate that it is possible to obtain a significant amount of the sorbed metal species when it is released from the biosorbent material in a much smaller volume of eluting solution.

Some types of biosorbent can be reused efficiently for many cycles. This simply can be achieved by metal desorption by washing the biomass with an appropriate acid, base, or salt solution. The resulting highly concentrated metal solution can be recovered, recycled, and sold. The overall goal of the biosorption process is to concentrate the metal solution by a factor of 100 or more (Mishra 2014). Consequently, the process cost can be reduced significantly, which increases the economic advantage of the biosorption process.

11.2.2.5 Biosorption Contacting Systems

The lack of detailed design information for biosorption can be because the metal sequestering process by various biomaterials is still not fully understood. The biosorbent particles should be immobilized and processed prior to their use in the operation to provide good flow conditions and suitable pressure drop in column operation. These conditions must be optimized to maximize sorption performance (Michalak et al. 2013; Vijayaraghavan et al. 2005). Due to the fast kinetics of the sorption processes, mass transfer of the sorbed metal to the available active site can control the overall sorption rate.

The biosorption column can be operated on two main parallel cycles consisting of loading and regeneration. The column operation can be illustrated as loading the biomass, passing the metal-containing effluent through the biosorbent where the removal of the heavy metals occurs. The biosorbent should be regenerated once it reaches the breakthrough point, i.e., when it is saturated with heavy metals. The regenerations process produces small volumes of concentrated solution of heavy metals that can be easily recovered by conventional methods. The cycle ends by backwashing of the bed with water to remove any suspended solids or trace of regeneration agents (Mishra 2014). A pair of biosorption columns as shown in Fig. 11.1 should be used in parallel to maintain a continuous biosorption process; therefore, while the biosorbent is being regenerated, the other column is undergoing the metal biosorption process. Generally, application tools for biosorption techniques are like those of ion exchange and activated carbon adsorption (Sulaymon et al. 2013a).

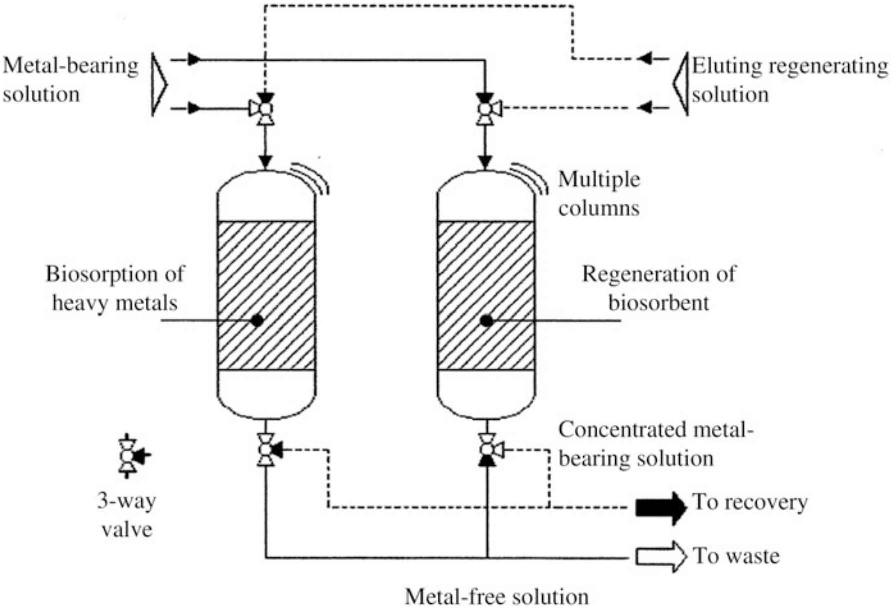

Fig. 11.1 A schematic diagram of a biosorption process (Volesky 1990a)

The most widely used contacting configuration for sorption processes is the fixed bed reactor system and its modifications, which makes the most effective use of reactor volume. The fundamentals of deriving and evaluating the key fixed bed sorption process parameters have been studied extensively in the chemical engineering literature. The operational saturation metal uptake, the minimum theoretical bed depth, and the bed service time represent the key parameters characterizing biosorbent performance in a fixed bed column (Muhamad et al. 2010). The concentration difference, known to be the driving force in the sorption process, can be optimized. In general, the metal uptake process in a fixed bed system is substantially affected by three main regimes: the sorption equilibrium, the sorption particle mass transfer, and the flow pattern through the packed bed. The overall sorption performance of the column is determined by all these three factors, which are in turn controlled by the service time of the bed (Sulaymon et al. 2013a; Muhamad et al. 2010; Naja and Volesky 2006). At the breakthrough point, a regeneration step is required for biosorbent materials.

11.3 Selection of Biosorbent Material

Many biological materials and metabolically inactive cells, such as bacteria, fungi, and algae, have shown potential for heavy metal uptake. They can serve as the basis for producing innovative and potent biosorbent materials. Several studies have shown that different types of waste biomass have been found to be good biosorbents with a high metal uptake capacity. However, only those that have a low cost and good efficiency and selectivity for heavy metals are suitable for use in a full-scale biosorption process. An extensive search for new promising types of biomass is still proceeding in both the academic and industrial research fields.

Despite the search for the innovative technology of biosorption is mainly concerned with metal uptake capacity, the interaction between the cells and the heavy metal species, the kinetics of the metal removal process, and other essential properties of the biosorption process are still not fully understood (Abbas et al. 2014). This may serve as a guide in the search for an effective biosorbent. In the current state of the art, tedious experimental assessment of selected available types of biomass is still the main base for discovering potential biosorbents. However, it has been roughly concluded throughout the literature that certain types of biomass are more appropriate in biosorption processes than others.

Ideally, either an abundant natural material or a waste biomass is the source of raw biosorbent material where potential biosorbent can be renewable and economically feasible. Natural marine algae present enormous amounts of inexpensive biomass capable of being the raw material for providing biosorbents. Another continuous source of biosorbent is specifically cultured biomass produced by simple fermentation. Although the cost of this type of biomass may be high compared to the natural one, the cost is still considered to be competitive with those of conventional technologies such as ion-exchange resins. Alternatively, waste biomass that is generated in large quantities as waste from large-scale industrial fermentation processes, e.g., pharmaceutical, biochemical, and enzyme production, can be used as biosorbent. This biomass can create a possible disposal problem. For example, large quantities of microbial biomass have been periodically disposed into the oceans after being a by-product from enzyme production in Japan. The use of waste biomass as a source of biosorbent material can strongly increase the economic competitiveness of the biosorption technology since the biomass is inexpensive, recoverable at the end of fermentation, and produced in large amounts.

An ideal biosorbent material, which is used in the removal and/or recovery of metals from contaminated solution, can be characterized as follows (Gadd 2009):

1. Cost-effective, inexpensive waste or abundant raw biomass material, serving as the source for a new category of biosorbent.
2. High metal uptake and rapid kinetics of the biosorption process.
3. Ability to be regenerated: usually it is achieved in a two-step process consisting of a metal elution from the biosorbent stage, which gives a high concentrated metal solution, and a simple washing stage to eliminate any trace of eluting reagent and complete the regeneration stage of biosorbent to be used in another cycle with minimal loss of the biosorbent.
4. Metal selectivity: if desirable, a wide range of heavy metals can be removed without interference from common light metals. Some types of biosorbents can remove most of heavy metals without specific priority, while others can be specific for certain types of metals only.

5. High adaptability, operated under a wide range of pH values, temperatures, and other process conditions and arrangements.
6. Concentration independence, good heavy metal uptakes for low concentrations, less than 10 mg/L, as well as for high concentrations, higher than 100 mg/L.
7. Suitable mechanical properties, particle size, hardness, density, and shape that can be used in different continuous flow configurations.
8. High resistance for organics: low levels (less than 5000 mg/L) do not affect metal uptake.

11.4 Biomass Cell Wall

The cell wall is the external cellular skeleton of the microorganism cell, which is in a direct contact with the external environment especially with soluble liquid medium materials. Because of the negatively charged cell walls, this interaction is very important in the case of metallic species. The cell wall surface consists of a mixture of monovalent and divalent groups that can exchange a cation by another, e.g., H^+, which in turn depends on the strength of the individual ligand complex.

11.4.1 Cell Wall Composition

Little progress has been done in identifying the exact role of several cell wall components in metal binding. Understanding of the chemistry of metal-ligand interactions could clarify the preferential impact of various ligands on metal species. Park et al. (2010) related the nature of binding and strength of interaction of individual metal ion species to the different types of functional groups located at the cell wall surface. The behavior of metal biosorption by different types of microorganisms depends on the chemical composition of the cell. The cell walls can be considered as a complex ion-exchange material like a commercial resin. However, the ion binding capacity totally depends on the presence of active functional groups and the structure of the cell wall. The number and type of binding sites depends on the chemical composition of the cell wall membrane. Different microorganisms present great varieties of cell wall types that may have different metal uptake affinities (Sulaymon et al. 2013b). Eventually, most of the sequestered metals are found on the cell walls.

The structure of algal cell wall is complex since more than ten layers can be found in some algal cells. Most of the algal cells are covered by mucilaginous layer that has a significant metal sorption capacity because it contains uronic acid. Alginate is a common term for a family of linear polysaccharides consisting of 1,4-linked β-D-mannuronic and α-glucuronic acid component randomly configured in a block-wise way along the chain (Wang et al. 2014; Davis et al. 2003). Using formic acid, ethanol, and triethylphosphite to modify amino, carboxyl, phosphate groups, Velkova et al. (2015) studied the binding of zinc by *S. cerevisiae* brewer's yeast cells. They found the biosorption process depends on the presence of carboxyl, phosphate, amino, sulfhydryl, and hydroxyl groups, where carboxylic groups are the most important followed by amino and phosphate groups. The green freshwater alga *Chlorella* was found to contain uronic acids that may be the reason behind its chelating ability of polysaccharides. In addition, the brown alga *Sargassum fluitans* contains carboxyl groups of alginates and the sulfate groups of fucoidan. It plays a critical role in the complexation of heavy metals (Mehta and Gaur 2005; Davis et al. 2003).

The structure of the cell wall in most fungal cells has two main phases: (**a**) an outer layer containing glucans, mannans, or galactans and (**b**) an inner microfibrillar layer that shows crystalline characteristics, which are presented by the parallel configuration of chitin chains, cellulose chains, or in some yeast of non-cellulosic glucan. A continuous transition between both layers exists (Gow et al. 2017). The microfibrils of phycomycetes consist of cellulose, which also contains uronic acids, while in the higher fungi, they are made up of chitin. Chitin consists of a polymer of *N*-acetyl-ᴅ-glucosamine, which has lost a minority of the acetyl groups. An extensive review of chitin chemistry can be found in the literature (Zargar et al. 2015). These chains are connected by proteins, lipids, and polysaccharides that can act as active sites in binding metal ions (Vianna et al. 2000; Pagnanelli et al. 2000). In addition, the fungal cell walls contain pigments, polyphosphates, and inorganic ions. The formation of a complex between the metal species and the chitin has been proposed.

Chitin and chitosan have been suggested as the main components in the sequestering of metals since they are found in different quantities in the fungal cell wall. Generally, they are natural polysaccharides that are widely distributed in fungal biomass (Gow et al. 2017). The active part of the cell wall in binding metal species consists of polysaccharide. Therefore, the capability of the biomass in sequestering metal ion species depends on the polysaccharide content in the biomass (Davis et al. 2003). Even in the absence of biomass, polysaccharides have been shown to have the ability of sequestering metals (Gupta and Diwan 2017). Stereochemical variation in the polysaccharide structures can make significant difference in the affinity for the metallic species. Consequently, it substantially affects the biosorption performance by biosorbent materials. The chemical composition of three different common fungal cells is shown in Table 11.1.

The polyphosphate and carboxyl groups on the cell wall surface of *S. cerevisiae* are active in the metal sequestering. This view explains the improved metal uptake by microorganisms grown in synthetic medium since they have higher phosphate content in the cell wall. The phosphoryl groups form stable complexes, while carboxyl groups are involved in metal binding only after phosphoryl groups are saturated. A strong relationship was found between the decrease in metal uptake by fungal and algal biomass and the degree of blocking of carboxyl groups by esterification in the pretreated biomass (Velkova et al. 2015). Similarly, the heavy metal uptake capacities by seaweed algae were found to be proportional to the carboxyl group's content and related to the electronegativity of studied metal species (Sulaymon et al. 2013b; Davis et al. 2003). In addition, the selectivity of

Table 11.1 Chemical composition of various fungi (Volesky 1990b)

Component	*Saccharomyces cerevisiae*	*Aspergillus*	*Mucor rouxii*
Nitrogen	2.10	–	–
Phosphate	0.31	11.5	23.3
Lipid	8.50	–	6.8
Extracted lipid	–	7.4	–
Protein	13.0	8.3	6.3
Chitin	1.0	8.3	6.3
Chitosan	1.0	18.2	9.4
Glucan	28.8	–	–
Mannan	31.0	45.5	3.8
Other carbohydrates	–	51.3	9.0
Ash	–	4.0	2.0

Values presented as percentages of dry weight of walls

ion-exchange resins such can be due to the presence of carboxyl groups in their synthetic matrix as it has been attributed by their manufacturer.

Several active negatively charged chemical groups can attract the metal species and sequester them such as acetamido groups of chitins; structural polysaccharides of fungi; amino and phosphate groups in nucleic acids; amino, amido, sulfhydryl, and carboxyl groups in proteins; hydroxyls in polysaccharides; and carboxyls and sulfates in the polysaccharides of marine algae. Nevertheless, research concerning using yeast as biosorbent of heavy metals indicated the importance of cell wall protein in binding cobalt, copper, and cadmium. Therefore, the more protein and/or sulfhydryl group content in the yeast, the more metal binding capacity may be obtained in the biosorption process (Michalak et al. 2013; Davis et al. 2003).

11.4.2 Location of Metal Bound

The place where metal uptake is occurring is not completely defined. The metal sequestering may take place on sites on the cell lipopolysaccharides or by certain chelates, siderophores, in the cell walls. Another proposal is that the uptake process strongly depends on the protein existence in the cell wall. Other researchers refer it to the presence of polysaccharides. Knowledge of metal uptake location in the cell can provide efficient evident direction about the significance of binding groups if it is related to data about which biomolecules build up the different parts of the cell. Electro-micrographs of bacterial, fungal, and algal cells in the literature indicate that the cell wall plays a critical role in metal ion (Dhankhar and Hooda 2011). Biomass residue resulting from the cell breaking may still be of interest in the biosorption process since the active binding sites are presented at cell walls (Volesky 1990a).

Furthermore, metal species are found to deposit all around the budding cells and inside the cell itself. The slow penetration by small ionic species throughout very porous membrane into the cell interior has been observed (Davis et al. 2000). This indicates that some intracellular components participate in the biosorption process. Biochemical or other physicochemical mechanisms may be involved in metal binding on or in the cell walls.

11.5 Metal Uptake Mechanism

Despite the extensive literature available on metal-biomass interaction, few studies have been conducted on examination of well-defined metal sequestering by a specific mechanism as opposed to the overall uptake where several types of removal mechanisms may occur simultaneously. For a better understanding of the dominant mechanisms in metal sequestering by biomass, more detailed and accurate knowledge about biomolecule structure and binding sites represented by different types of cell is needed. By revealing the different mechanisms behind biosorption phenomena by various biosorbent materials, the effect of different influencing parameters can be better estimated. Furthermore, determining the controlling mechanisms in metal biosorption allows one to scale-up the process that can be used in application fields (Abbas et al. 2014).

11.5.1 Classification of Binding Mechanisms

Biosorption may involve many different physicochemical mechanisms that are affected by the surrounding environmental conditions, the type of a metal, its ionic form, and the active sites responsible for metal binding. Several possible physicochemical mechanisms including adsorption, complexation, coordination, chelation, microprecipitation, ion exchange, and ion entrapment, because of the concentration gradient and diffusion through cell walls and membranes, may take place as shown in Fig. 11.2. A single combination of different mechanisms can contribute to the overall metal uptake process by biosorption.

An understanding of the metal-ligands interaction mechanism may substantially clarify the current expansive definition of biosorption mechanism in the literature. The proposal of ion exchange as the main mechanism in the metal uptake for a certain process may not eventually contradict the claim that chelation is the relevant mechanism. In addition, the overall mechanism of ion exchange can be related to the sub-mechanisms of complexation or adsorption and vice versa (Limousin et al. 2007).

Complexation plays an important role in metal-ligand and sorbate-sorbent interactions. A complex is a polyatomic molecule that consists of one or more central atoms surrounded by ligands, which are attached to it. Complexes can be neutral, positively charged, or negatively charged. Microprecipitation is the deposition of electrically neutral material at the surface of the biomass. It does not essentially involve a type of bond between the biomass surface and the deposited material. However, it may be assisted by the binding of metal species to different active binding sites that may play a

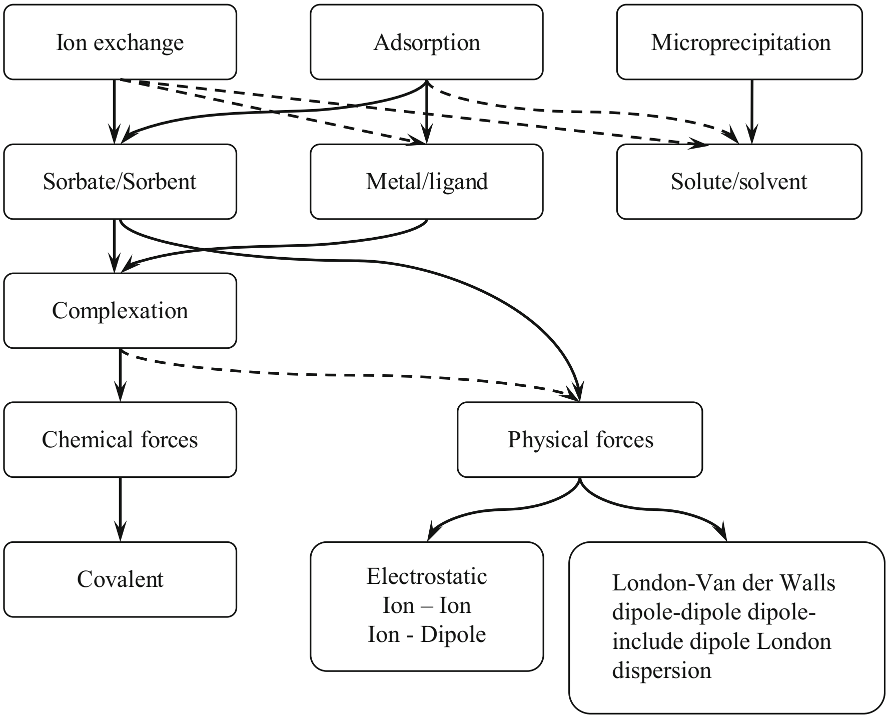

Fig. 11.2 Metal biosorption mechanisms: bold line, mechanism probably significant in biosorption by *Sargassum* biomass; dashed line, secondary importance binding relation (Volesky and Schiewer 1999)

role as nucleation sites for further precipitation. Microprecipitation depends on interactions between the solute and the solvent and occurs when the local solubility is exceeded. In general, metal ions precipitate as salts in the form of phosphates, sulfides, carbonates, oxides, etc. This can play a vital role in the metal uptake process that should be given a special concern (Davis et al. 2003).

11.5.2 Ion Exchange

Although different uptake mechanisms can be involved in the biosorption process, substantial evidence suggests ion exchange is the main mechanism of metal biosorption. Recent studies revealed that uptakes of divalent metals were accompanied by the release of ionic species from the biosorbents (Michalak et al. 2013).

Freshwater alga biomass pretreated with a solution of Ca^{2+} and Na^{+} has been found to release cations of these light metals into the solution along with the ability to remove the heavy metal cations. The relative quantities of heavy metals adsorbed by the biomass are approximately equal to the amount of light metals released into the solution (Sulaymon et al. 2013b). Similar results are obtained with fungal biomass (Dhankhar and Hooda 2011). Many studies clearly show that the ion-exchange mechanism is strongly involved in the binding of metal species on the surface of cell walls that contains alginate. Chemical analysis such as electron microscopy, infrared spectroscopy, X-ray dispersion, and diffraction analysis can be used to show that ion exchange is the main mechanism in the biosorption phenomenon. The IR spectra of original and used biomass samples indicate no shifts in the major bands. However, the change in intensities may be explained as changes in concentrations rather than structural changes. These results can be another evidence of the ion-exchange phenomenon in the biosorption process (Michalak et al. 2013).

Sulaymon et al. (2013b) found that the amount of released light ions (K, Na, Ca, Mg) from the native alga was higher in cadmium and copper metal solutions than in lead and arsenic metal solutions. Insignificant change in potassium release was found in the four investigated metals. It was concluded that ion exchange is responsible for the sorption process. In addition, Singleton and Simmons (1996) found that the binding of Ag by *S. cerevisiae* causes ions to be released into the solution with a stoichiometry of 1.5:1 silver adsorbed to ion released. This stoichiometry does not change strongly with biomass concentration. The main ions released are hydrogen followed by magnesium and calcium.

Ion exchange, rather than sorption to free sites, is the main relevant predominant mechanism in biosorption (Davis et al. 2003). Since the overall charge of the biomass material must be neutral, any binding of one cation must be accompanied by either a stoichiometric release of other cations or by the binding of anions. Therefore, it is unlikely that free anions will be bound to the biomass since the biomass itself is a negatively charged surface. The natural selectivity of biosorbents, which has been observed but never clarified, can now be better understood. The well-established and developed principle of ion exchange can be applied now in biosorption. This can be used as a good tool by researchers in this field.

11.5.3 Binding Forces

The forces between atoms or molecules can be categorized into two main classifications: chemical and physical. Chemical forces occur over short distances (0.1–0.2 nm). Chemical bonds are strong with energy values reported in literature ranges between 40 and 400 kJ/mol. Covalent bonds are formed by the sharing of electron clouds between nonionic molecules. Physical forces can be classified into two main types: electrostatic and London-van der Waals forces. The energy of physical sorption is reported as 2–40 kJ/mol in the literature. In such a bond, the electrons remain in their original situations. Electrostatic forces extend over a long range and are the strongest among various physical bonds. The force magnitude is proportional to each ion charge and inversely proportional to the square of the distance between the ions. The mechanisms and forces that are probably the most important in the case of metal binding are already illustrated in Fig. 11.2. The driving force of this bonding is the affinity of the sorbate species to the sorbent, supported by physical electrostatic attraction between metal ions and biomass ligands (Volesky and Schiewer 1999).

The amount of released protons increased with an increase in the binding strength of the metal ion species. However, proton release did not indicate covalent binding since part of the protons may have also been bound by electrostatic attraction forces and thus may be easily replaceable by electrostatically bound ions (Abbas et al. 2014). It can be concluded that in cases where no protons were involved, e.g., most alkaline and alkaline earth metals, electrostatic attraction was the dominant binding mechanism, while covalent binding took place accompanied by proton release (Naja and Volesky 2011). They showed that the ionic and covalent metal binding can be characterized by cation displacement experiments. In addition, covalent bonding was observed at low concentrations, whereas electrostatic interactions become significantly important at higher concentrations. The researchers concluded that the ability of the cell to form covalent bonds was probably the main limiting factor of metal uptake. Despite electrostatic interaction of metal species and wall ligands of *S. cerevisiae*, these bonds show an increased covalent character.

The electrostatic force binding alone is weaker than the binding force involving covalent bonds, where it occurs between positively charged ions and negatively charged surface sites. Sulfate groups can assist in forming covalent bonds between adjacent chains of fucoidans and other sulfated polysaccharides. Copper was adsorbed not only by ion exchange but also by additional covalent bonding with the carboxyl groups of *Vaucheria pectins* (Naja and Volesky 2011).

Furthermore, the metal sequestering mechanism by algae varies with different metals from relatively weak electrostatic bonding observed in alkali and alkaline earth (Ca, Na) to stronger bonding for certain metals with oxygen, nitrogen, and sulfur containing ligands forming a covalent bond (Cu). A complex redox reaction may take place with some noble metals (Davis et al. 2003; Naja and Volesky 2011). To better understand the reasons why some ions bind stronger than others, it is essential to consider the properties of different metals. Such knowledge can be used as the basis to predict how strongly an ion may bind to a different biomass. Also, it can be used to have a better understanding of the metal sequestering mechanism.

11.6 Pretreatment, Recovery, and Regeneration

The affinity of different sorbate species for the sorbent is a very critical principle in the biosorption process. For the case of a sorbate “A” being sorbed by a “B”-saturated biosorbent, the theory of ion exchange indicates that there are two possibilities depending on the respective affinities of A and B for the biosorbent. These two possibilities can reflect the performance of the biosorbent. In favorable operation, when species A is more strongly bound to the surface than B, i.e., affinity of A > affinity of B, then the required contact period is small. However, if the affinity of B is greater than that of A, this period can be extended further, and it is obviously an unfavorable operation. Biosorption can be defined in the light of this effect as a process where toxic heavy metals may be exchanged for nontoxic species. The positions of protons, heavy metals, and light metals in the affinity series are the main guide to determine whether the ionic forms and regenerates are suitable or not.

The biomass can be regenerated and reused in several sorption-desorption cycles in the biosorption process. The number of these cycles strongly related to the physical properties of the biosorbent, where biomass immobilization may improve its properties such as particle size, particle strength, and chemicals resistance. Well-prepared biosorbents are like ion-exchange resins in many features, such as their convenient application in metal removal/recovery processes (Park et al. 2010). Thus, using the raw biomass as biosorbent may not be practical in biosorption processes since it may not have the required characteristics for the process.

A good eluting agent must be effective, inexpensive, non-damaging to the biosorbent, and environmentally friendly. For a biosorbent, the smallest possible elutant (liquid) volume is desired. The elution efficiency depends on the binding strength of the desorbed ion to the biosorbent surface and is determined by the ratio of the metal desorbed into the solution after the elution process to the metal initially bound to the biomass. The elution efficiency decreases with an increase in pH. Not all sites created by desorbing protons are effective for metal uptake. This can be because the binding of metal ions can significantly reduce the surface charge density, hence reducing the effectiveness of the residual sites.

The biosorbent can be prepared in different ionic forms such as protonated (H-form) or saturated with Ca^{2+}, Mg^{2+}, Na^{+}, etc. by washing it with mineral acids, salts, and/or bases. The capacity of the biosorbent was found to increase more than three times by converting the biomass *Rhizopus arrhizus* from H- to Na- or Ca/Mg forms by washing it with a solution of $NaHCO_3$ and hard water, respectively (Michalak et al. 2013). Different mineral acids and few salt solutions, e.g., $CaCl_2$, showed a metal elution, or desorption, efficiency close to 100%. An insignificant difference between the elution efficiency of $CaCl_2$ and that of $Ca(NO_3)_2$ was found. The cost of $CaCl_2$ is almost half of $Ca(NO_3)_2$. In addition, the nitrate residuals in the environment are considered a potential risk, while this is not the case for chlorides since a higher level of chlorides can be tolerated (Davis et al. 2000).

Desorption can be also achieved by acid wash, e.g., HCl or H_2SO_4. The use of acid-washed biosorbent may have a negative effect on metals with low affinities for the carboxyl groups, which cannot replace the strongly bound proton from the H-saturated biosorbent. Neutralization of these groups may be required before the loading stage to obtain an effective sorption of heavy metals (Park et al. 2010). Concentrated acids may have a pronounced negative effect on the structure of alginate chains and the hydrogen bonding capacity of the biomass by destroying them, as well as by simultaneous hydrolysis of polysaccharides. This may explain the lowered metal uptake capacity in certain processes (Horsfall et al. 2006). However, relatively diluted acid did not cause any significant damage to the cross-linked biomass in two operating cycles. The HCl, 0.1 and 1.0 N, wash of the biomass can release the entire bound metal (Park et al. 2010). Hydrochloric acid, 0.1 N HCl, can effectively remove cadmium, copper, and zinc from the biomass. This desorption process was completed without damaging the structure of the biomass. Various studies show that acid wash pretreatment improves metal uptake capacity of various biomasses. Protonation process ensures homogeneity of biomass surface, which leads to uniform surface behavior. Protonation increases the metal uptake of copper and cadmium using *B. lentus* (about 30 mg/g), while it has insignificant effect on *S. cerevisiae* (<5 mg/g). This can be due to the higher lipid content in *B. lentus*, which may be involved in metal binding (Vianna et al. 2000). In addition, the acid pretreatment can lead to a release of large amounts of intracellular components, which can bind metal species due to the presence of different functional groups and may reduce the amount of metal bound by the biomass. Non-protonated biomass of *B. lentus* and *A. oryzae* shows a higher metal uptake capacity than those of treated biomass at a pH value of 4.5 (Vianna et al. 2000). This can be related to the differences among the original cell wall polymers in various microorganisms. Therefore, the dilute acid, HCl, has been established as an efficient elutant.

The treatment of biomass with alkaline acetone shows an increase in the overall negative charge of the cell walls. This can be due to the dissociation of ionic groups thus increasing the total number of active sites suitable for binding of metal species. Pretreated biosorbent by NaOH or other alkaline solution shows an increase in the metals uptake capacity by different biomass (Vianna et al. 2000). Similar observations were confirmed by Velkova et al. (2015), where NaOH-treated *S. cerevisiae* yeast cell gave the highest zinc uptake when compared with other methods that have an order of untreated biomass $CaCl_2$, H_3PO_4, and H_2O_2, which gave the lowest metal uptake.

Therefore, not all chemical pretreatment methods improve the metal uptake capacity. Few chemical modifications are known to remove proteins, which contains several components that are capable of binding metals such as amino, thiol, and phosphate groups. Removal of proteins may decrease the ability to bind metals. Chemical modifications are likely to change the composition of biomass by introducing different new chemical groups to the biomass. The degree of chemical modification for a given biomass cannot be predicted. Therefore, the best modification configuration and experimental conditions must be evaluated for each type of biomass due to the difference in the chemical composition.

11.7 Parameters Affecting the Biosorption Process

The amount of metal bound on the biosorbent depends on many environmental factors and especially the selected biosorbent. The physiological state of the organism, its nutrients status, growing medium conditions, the type of chemical modification, and the specific surface properties of the microorganism, e.g., particle size and the type of active binding site responsible for the metal uptake, are all essential parameters influencing the performance of a biosorbent. Environmental conditions during the biosorption process such as solution pH, temperature, biosorbent concentration, the metal of interest, its ionic form, its initial concentration, the presence of other cations and/or anions, and concentration of other impurities are the main environmental factors that play an important role in the biosorption process (Abbas et al. 2014; Pagnanelli et al. 2000). Practically, solution pH and the presence of other cations have a significant effect on the efficiency of biosorption.

11.7.1 Solution pH

Many studies have shown the important role of the solution pH in the biosorption process. Changes in the initial pH values have a great effect on the metal uptake that may indicate different possible binding mechanisms. The pH value of the metal-bearing solution can affect the biosorption process in three different ways. Firstly, the binding active sites may be altered, where the availability of free binding sites depends on the pH. At a low pH, all the active sites are protonated, and complete desorption of the bound metal ion species can be obtained. Therefore, acid treatment is a method for regeneration of the sorbent material or recovery of the bound metal species. A two-unit decrease in the pH value can in some cases result in an approximately 90% reduction of metal binding. Secondly, extremely low pH values, which are used in desorption processes, i.e., regeneration, may have a negative effect by damaging the structure of the biosorbent material. Thirdly, the dissociation of the metal ion in the aqueous solution is pH dependent. The presence of metals in aqueous solutions is typically in the form of hydrate cations in solvation shells at low pH, while hydroxides may form at a higher pH. The formation of metal oxide and hydroxide complexes and/or precipitates is frequently referred to as hydrolysis, i.e., decomposition by water (Farhan and Khadom 2015; Schiewer and Patil 2008).

Few biosorption studies thought that at higher pH values, a combined sorption-microprecipitation mechanism might be responsible for the accumulation of heavy metals inside the cells and/or on the cell walls; however most heavy metals start to precipitate at pH >5.5 (Schiewer and Patil 2008). The study of the biosorption phenomena becomes more complicated when metals tend to form insoluble microprecipitates, because the predominant mechanism will not be defined accurately. However, from the process application viewpoint, it may desirably increase the metal uptake, thus increasing the apparent overall uptake capacity of biosorbent materials. The pH range of metal precipitation varies with respect to the metal and its salts. Typically, investigation of the pH effects over a value range of more than 7.0 is impossible because metal precipitation takes place and interferes with the process (Hadi et al. 2003). For example, addition of sodium hydroxide (NaOH), which may be used to adjust pH, to a lead nitrate solution can result in the formation of insoluble complexes of $Pb(NO_3)_2Pb(OH)_2$ and $Pb(NO_3)_25Pb(OH)_2$. The precipitation of these salts starts at pH values above 5.0 and 6.7 for lead and nickel, respectively (Davis et al. 2000). However, this effect was neglected in many studies. Increasing the pH to more than 9.0, like in research work described by Zouboulis et al. (1999), Al-Homaidan et al. (2014), and Fadel et al. (2017), can precipitate the metal where the biosorption is not the technology responsible for metal uptake. Therefore, the pH dependence of biosorption represents a limitation of the biosorption process for the

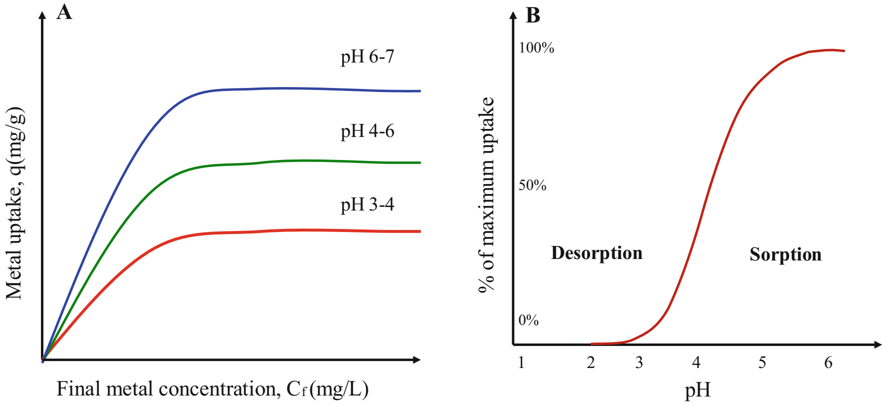

Fig. 11.3 (**a**) Effect of pH on the metal uptake isotherm (Volesky 2003); (**b**) Metal biosorption equilibrium uptake as a function of solution pH (Kratochvil and Volesky 1998)

treatment of wastewaters since the solution pH should be kept below the value of precipitation.

The uptake capacity by different types of biomass increases significantly with pH increases from 2 to 6 and levels off at a pH greater than 6.0 (Sulaiman 2015). In acidic solutions with a pH of 2.0, protonation of the fungal cell wall takes place. Increasing the pH, the negative charge density may increase on the cell wall surface because of the deprotonation of the metal sequestering sites and hence increase biosorption (Say et al. 2001). The low pH value of 3.5 was unfavorable, whereas the maximum uptake capacity decreases significantly when compared to that at pH 6.0 (Farhan and Khadom 2015). In addition, Vianna et al. (2000) found that metal sequestering was low for *B. lentus*, *A. oryzae*, and *S. cerevisiae* under acidic conditions at pH values of 2.5 and 3.5. The metal uptake was much higher at a pH value of 4.5 than those at pH of 2.5 and 3.5. The metal uptake substantially depends on pH, and the predominant uptake mechanism most likely involves electrostatic attraction to negatively charged binding sites on the cell walls. Figure 11.3a, b shows the effect of solution pH on the metal uptake.

Metal uptake depends on the attraction of the sorbate species to the solid surface and its lyophobic behavior, which indicates that sorption increases with a decrease in solubility. Since the solubility of many metal complexes in aqueous solution decreases with an increase of pH, this may give another possible explanation to the increasing of metal sorption as pH values increase. Moreover, hydrolyzed ion species have less hydration, i.e., less energy is required for the removal of the hydrated species upon binding (Davis et al. 2000). A further increase of pH leads to a decrease of the metal complexes solubility to a level of precipitation.

The theory of acid-base equilibria can be applied to biosorption, which states that in the pH range of 2.5–5.0, the binding of heavy metals is determined mainly by the state of dissociation of the weakly acidic groups. The most significant functional group at pH 4.5 may be the phosphate, carboxyl, and sulfate and amino groups, at the fungal cell wall surface (Say et al. 2001). Low pH values indicate high H^+ ion concentrations, which may have a competition effect between the protons and the metal ions. At high pH values, a lowered competition by protons for the same active binding sites takes place, since most of the biomass cell will be in the unprotonated state. The rate of proton uptake was found to depend on solution pH, where faster proton uptake rates were observed in more acidic solutions (Sulaymon et al. 2013b). A rapid drop in pH may occur during the first hour in non-adjusted pH metal-bearing solutions (Hadi et al. 2003). This can be because small quantities of polysaccharides may leach into the solution from the biomass. Thus, it can interrupt the kinetics of metal uptake by interfering with the solution pH (Davis et al. 2000).

Consequently, the pH must be controlled during the whole period of contact until the sorption equilibrium is obtained. The pH can be adjusted with acid and/or base to keep its value close to the optimum pH during the first few hours of the sorption process. Although pH control or buffering of the solution is essential in biosorption studies, it may strongly affect the metal uptake process. The additive for pH control should be selected carefully to avoid any interference with the biosorption process such as reduction of the metal uptake capacity (Sulaiman 2015).

The optimum pH for metal uptake varies with different biomass types and metals of interest. For example, Hadi et al. (2003) found that the optimum pH for the removal of cadmium by *S. cerevisiae* and *K. fragilis* is 5.0, which was close to the cadmium precipitation limit, while this value was found within a range of 5.0–6.0 for lead, zinc, chromium, cobalt, cadmium, and copper using the same yeast cell *S. cerevisiae* cells (Sulaiman 2015). Similarly, the optimum pH was found between 5.0 and 6.0 for copper metal uptake using red algae (*Palmaria palmata*) and beer draff (Li et al. 2011). The optimum solution pH was found to be 6.0 to achieve maximum metal uptake of different ion species by fungal biomass (Say et al. 2001). The optimal pH values were found within a range of 4 and 5 for uptake of lead, copper, cadmium, and arsenic using algae (Sulaymon et al. 2013b). Finally, a pH value of 4.5 was found to be the optimum in another study (Davis et al. 2000). These results indicate a competition between heavy metal ions and protons for the available cell wall binding sites. At pH values higher than 5.0, the cadmium hydroxides may start to precipitate.

11.7.2 Solution Chemistry

The solution chemistry of metals plays a great role in the biosorption investigation since it is related to their hydration and hydrolysis reaction. The large charge-to-size ratio of cations results in an increase in hydration energy if no reaction beyond the coordination of water molecules to the cation occurs:

$$\mathrm{M}^{+} + n\mathrm{H_2O} \rightarrow \left[\mathrm{M}(\mathrm{H_2O})_n\right]^{+}$$

In aqueous systems, metal or metalloid oxides are generally covered with surface hydroxyl groups, so that hydroxylated oxide particles may be considered as polymeric oxo acids or oxo bases. The charge of the metal or metalloid hydrated oxides depends on the solution pH with proton transfers at the amphoteric surface. Therefore, the surface chemistry of metal oxides and hydroxides should be taken into consideration (Abbas et al. 2014; Sulaiman 2015).

11.7.2.1 Cations

Most industrial effluents are likely to contain several different toxic heavy metals. In this case, biosorption involves a competitive ion exchange where several toxic heavy metals compete for a limited number of active binding sites available on the biosorbent. However, many biosorption studies are carried out only on single metal solution. Therefore, the results obtained from these studies may not be valid when applied to industrial effluent treatment. Ion species competition on available different binding site has been shown to affect biosorption by several different biomass types and metals (Sulaymon et al. 2013b; Sulaiman 2015).

The chemical form of metal ions, e.g., cationic or hydrolyzed neutral/anionic species, plays an important role in the biosorption process. The effect of other metallic ions (or even anionic co-ions) on the performance of single metal biosorption has been investigated. It gave different results from the original (single metal) sorption when a known concentration of a mixture was introduced. The metal uptake of a single metal decreases with the presence of other competing ion species. However, the overall metal uptake of different ion species has been reported to increase slightly (Say et al. 2001). The amount of reduction depends on the binding strength of various ions to the biomass. The inhibition of heavy metals binding to biomass by light or alkali metals, e.g., K and Na, is insignificant in comparison with that by heavy metals, e.g., Zn, Cu, Fe, and U. It can be generally concluded that the light metals such as alkaline and alkaline earth metals have less binding strength than heavy metal ions or radioactive elements. Therefore, light metals may be less likely to interfere with the biosorption of heavy metals (Sulaymon et al. 2013b).

The metal competition effect on biosorption is as important as the metal concentration of the metal of interest in the solution. For example, cadmium is found to have a significant effect on decreasing the biosorption of silver when compared to the presence of sodium (Volesky and Schiewer 1999). In fungal biomass of *R. arrhizus*, the inhibition effect of the presence of uranium is much higher on the binding of cadmium, zinc, and silver than that of these elements on the binding of uranium. In addition, zinc shows a weak binding among the heavy metals; hence it is more significantly affected by other ions. Conversely, in few cases, the presence of metals in the solution may have an influence on the biomass structural composition. For instance, copper was responsible for protein removal from *S. cerevisiae*, which is related to the metal concentration in the solution. The released protein may bind to the copper and prevent the biosorption process (Singliton and Simmons 1996).

11.7.2.2 Anions

An increase of anion concentration has inhibitory effects on metal uptake by *S. cerevisiae*, probably by forming less cationic, neutral, or anionic metal complexes. At higher anion concentrations, there is no evidence of metal uptake (Fadel et al. 2017). Hypothetically, the presence of anionic ligands can lead to one of the following:

1. Formation of complexes that have higher affinity for the sorbent than the free metals, i.e., improved sorption process.
2. Formation of complexes that have lower affinity for the sorbent than the free metals, i.e., reduced sorption process.
3. Interaction of these anions with the biomass wall surface, which may change the texture of the active sites. This can either improve or reduce the sorption process.

The third type of interaction is less likely to occur especially with biosorbent materials that have negatively charged carboxyl and sulfate groups as the probable binding sites on their cell walls surface. This is because these functional groups are not expected to interact with anions such as sulfate, nitrate, etc. (Michalak et al. 2013).

The presence of other ligands may have a negative effect on metal binding, except OH. This indicates that the biosorbent has less affinity for the metal-ligand complexes than for the free metal ion. The effect of ligands in solution can be described as competitive with the biomass for sequestering the metal ion species. This influence may be insignificant in few cases unless these anions form strong complexes with the metal ion, e.g., EDTA, which may cause an inhibition of binding. In addition, the influence of the ligand presence is of secondary importance since considerable concentrations of strong complex are not expected in typical metal-containing industrial effluents (Michalak et al. 2013).

11.7.3 Temperature

The effect of temperature on the biosorption process is relatively small, where the temperature is critical in the energy-dependent mechanism such as metal bioaccumulation. However, energy-independent mechanisms are less influenced by solution temperature changes, since the biosorption process is a physicochemical interaction (Bazrafshan et al. 2015). In addition, its influence in the lower temperature range is slightly more prominent. Biosorption processes are typically exothermic, where the equilibrium constant decreases with a temperature increase. For most metals, the heat of reaction is found to be constant, regardless of the degree of site occupation. However, for copper the heat of reaction decreases with an increase of the site occupation degree. This may indicate that there are either different binding sites involved in the process or different types of copper complexes with biomass may be formed. The heat of reaction was in a range of 7 and 11 kJ/mol for heavy metals, while it was about 2.5 and 6 kJ/mol for light metals (Sag and Kutsal 2000).

The binding of cobalt by the brown alga *Ascophyllum nodosum* increases from 50% to 70% with increasing temperature within a range of 4–23 °C. The increase becomes insignificant until the temperature reaches 40 °C, whereas the temperature of 60 °C can change the structure of the sorbent and consequently reduce its uptake capacity. A 20% increase was obtained when the temperature increased from 4 to 55 °C for *Spirulina* algae (Volesky and Schiewer 1999). The metal uptake of lead and cadmium by the brown seaweed *Cystoseira baccata* in significantly changed with increasing temperature in the range of 15–55 °C (Lodeiro et al. 2006). The initial biosorption rates of *Z. ramigera* for Fe and Pb were found to increase with temperature in a range of 35–45 °C. Moreover, the Fe and Cr uptake by *R. arrhizus* was greater at higher temperatures. However, the uptake rate constants of lead by *Z. ramigera* and Nickel by *R. arrhizus* were insignificantly affected by temperature (Sag and Kutsal 2000). Using *S. cerevisiae*, Ting and Sun (2000) found that copper uptake was temperature independent between 10 and 50 °C. Generally, to avoid decomposition of the biosorbent materials, biosorption application processes must take place at a narrow range of temperatures, i.e., 5–50 °C. Warm water might extract polysaccharides and other cell components that have low molecular weight. It may denature some polypeptides, which may provide some amino acid metal coordination sites for metal species.

11.7.4 Initial Metal Concentration

At sufficiently high metal concentration, more active sites can be occupied when compared to low metal concentration (Mukhopadhyay et al. 2007). However, the driving force alone is not enough to replace all protons from all sites. Proton removal by a basic medium provides anion functional group sites that represent an attractive force for metal binding.

Metal uptake per unit mass weight increases with an increase in metal concentration, even though some saturation situations of few hard ions were observed at higher concentrations (Hadi et al. 2003). The initial biosorption rate of Pb, Fe, Cr, and Ni by *Z. ramigera* increased with increasing initial metal ion concentration, C_i, up to 300 mg/L. The same trend was observed with *R. arrhizus* up to 200 mg/L (Sag and Kutsal 2000). In addition, the metal uptake capacity by *P. chrysosporium* increased with an increase in initial metal concentration until it reached the saturation level, which was around 300 mg/L (Say et al. 2001).

11.7.5 Biosorbent Concentration

Increasing the biomass concentrations increases the biosorbed metal, because of an increase in the surface area and subsequently the available binding sites. However, the metal uptake per unit biosorbent weight decreases with increasing biosorbent concentration, which was observed by several studies (Farhan and Khadom 2015; Hadi et al. 2003; Malkoc and Nuhoglu 2005; Mukhopadhyay et al. 2007). At high biosorbent concentration, the interference between the binding sites may increase, and the driving force of metal concentration may become insufficient to completely cover the available binding sites, which result in low metal uptake per unit mass. Different hypotheses have been proposed to explain this effect such as reduced effective mixing at high biomass densities, interference between various binding sites, limited availability of metal species in the solution, and electrostatic interactions (Ting and Sun 2000).

These results show that the hypothesis of electrostatic interaction between cells as a significant factor in the biomass dependence is invalid. Therefore, it is useless to increase biomass by more than 10 g/L to purify a 100 mg/L contaminated solution. A reduction in the biomass concentration increases the metal to biomass ratio at a given metal concentration. Therefore, metal uptake capacity increases per gram of biosorbent, if it is not saturated. The same maximum uptake can be achieved with a low biomass concentration when it is saturated and with an unsaturated biomass concentration.

11.7.6 Particle Size

Ideally, decreasing the particle size increases the metal uptake due to an increase of the surface area. However, this has a negative impact on the biosorption process due the difficulty of separation and possible clogging of the process. The size distribution of biosorbent particles had insignificant effect on the cadmium uptake (Davis et al. 2000). This agrees with the hypothesis that the metal uptake does not occur exclusively on the biosorbent particle surface. Apparently, the cationic metals diffuse through the particle, and the metal uptake rate can be dominated by interparticle metal ion mass transfer.

Conversely, Leusch et al. (1995) found that the particle size and chemical modification affected the biosorption performance. They were found to have a significant but unpredictable influence on the process. The metal uptake was higher with larger particles (0.84–1.00 mm) than that of smaller particles (0.105–0.295 mm) of *Sargassum fluitans* and *Ascophyllum nodosum*. They concluded that the larger the particles, the higher metal uptake obtained. This observation was more obvious at low concentrations than at high concentrations where the differences between large and small particles were 93% and 48% for low and high concentrations, respectively. In addition, the bigger the particle, the better the equilibrium metal sorption.

11.7.7 Morphological Conditions and Age of the Cells

The morphological differences within the same type of cells can influence the sorption process. The sorption efficiency and the capacity of a biomass depend not only on the biomass species itself but also on the growth conditions, physiological state, age of the microorganism, and the carbon source that is used in the cultivation process. Different preparations of the yeast biomass have significant effect on the uptake of uranyl ion. In addition, there is strong evidence that different media and growth conditions produce different microbial culture growth characteristics and hence metal uptake capacities (Broach 2012).

Younger *S. cerevisiae* and *K. fragilis* yeast cells (24 h) were found to bind cadmium slightly more than the older one (72 h). Thus, younger yeast cells were more effective in sequestering cadmium ions (Hadi et al. 2003). This can be due to the decrease of protein in the cell wall with age and increase in carbohydrate content with respect to the same period. In addition, the structure of alginates differs between young and old tissues as well as between different parts of the same plant. Also, the sulfate groups and uronic acids content in algal biomass differ with respect to season and geographical area even within the same species (Wang et al. 2014). Table 11.2 shows the chemical structure for young and old cells for the whole cells and isolated cell walls of the *S. cerevisiae* yeast. Protein represents higher concentration in young cells and the whole cells than that of old cells and isolated cell walls. This may indicate that protein is involved in the metal uptake by *S. cerevisiae.*

Table 11.2 Chemical analysis of whole cells and isolated cell walls of *S. cerevisiae* at 24 h and 96 h. CHO carbohydrate (Singleto and Simmons 1996)

Preparation	Content (% dry mass w/w)	
	24 h old	96 h old
Cell walls		
Protein	15.7 ± 1.7	10.9 ± 2.1
Total CHO	82.6 ± 4.5	90.9 ± 1.8
Mannan	38.6 ± 2.1	39.8 ± 0.8
Glucan	43.0 ± 2.3	49.9 ± 1.1
Chitin	1.0 ± 0.1	1.3 ± 0.1
Protein: total CHO	0.19	0.12
Whole cells		
Protein	36.6 ± 2.0	28.7 ± 1.3
Total CHO	40.7 ± 0.1	56.3 ± 1.9
Protein: total CHO	0.90	0.51

11.8 Kinetics and Modeling of Biosorption

11.8.1 Kinetics of Biosorption

The kinetics of the biosorption process is very important to identify the required contact time between the biosorbent materials and the metal-bearing solution to achieve a desirable metal uptake. The faster the metal uptake by the biosorbent, the more desirable the biosorption since shorter contact time is needed. This means that smaller equipment is required which directly reduces not only the capital cost but also the operating cost. Both equilibrium and kinetics studies of the process are important for evaluation of biosorption performance and the process design. Furthermore, the desorption process, which can determine the ability of biosorbents to be reused, is another important feature.

Biosorption involves essentially very rapid sorption reaction mechanisms. Typically, the biosorption of heavy metals has two main phases: an initial fast (<4 s) metal uptake related to surface binding on the cell walls, followed by a much slower uptake. This may be an indication that a different secondary metal binding mechanism occurs in the second slow phase, which represents the diffusion of ions into the cell structures (Park et al. 2010).

In addition, in many biosorption systems, there are many indications that most of the metal biosorption occurs in a matter of 5–15 min after solid-liquid contact (Abbas et al. 2014). The metal uptake kinetics is usually very fast, in the order of minutes or even of seconds. The copper uptake by *Aspergillus niger* occurred very fast with 70% of the final uptake taking place within 30 min. Additional metal uptake takes place slowly over several hours. Also, the maximum cadmium uptake by *S. cerevisiae* and *K. fragilis* yeast cells was achieved within 5 min. Insignificant levels of metal uptake were observed after this period, i.e., less than 10% (Hadi et al. 2003). Consequently, the rapid kinetics enhances the use of biosorbents in continuous mode contactors.

11.8.2 Modeling of Biosorption

Models are essential tools for scale-up from laboratory scale to an application process scale. Accurate models can assist in interpreting experimental data and predicting the change in the system under different conditions to assess the efficiency and feasibility of a biosorption process. It is a relatively simple task to obtain a single equilibrium sorption isotherm in a laboratory. A small amount of the required sorbent is supplied to a solution of the sorbate. The amount of metal, M, bound per mass of biosorbent is called metal uptake, q. The uptake depends not only on the sorbent material, X, but also on the metal concentration, C_f, of all sorbates in addition to other parameters such as solution pH. The sorption isotherm is a plot of the metal uptake, q, and the residual equilibrium concentration of remaining sorbate in the solution, C_f. Increasing the metal concentration in the solution, the binding increases from zero until it reaches its maximum as shown in Fig. 11.3b. The environmental conditions in the sorption system should be controlled especially the pH. The time required to attain the sorption equilibrium can be determined by a preliminary sorption kinetics test. Ideally, the sorption equilibrium is established once the sorbate concentration becomes unchanged in the solution. In a few cases, the equilibrium is established after 6 h of metal-biomass contact (Say et al. 2001). It is favorable that a sorbent material possesses a high metal uptake capacity and a high affinity for the metal ion species, which is indicated by a steep slope of the isotherm curve at low residual metal concentrations. Although many experimental studies have been published on metal biosorption, a much greater effort should be made to develop models that can predict the metal uptake phenomenon (Davis et al. 2003). Conventional models cannot effectively describe the metal uptake. More studies are required to develop and modify the existing mathematical models.

In the literature, the most common sorption isotherm models used are a simple Langmuir's model or Freundlich's model, where the metal uptake is obtained as a function of the final equilibrium metal concentration without indicating the pH of the solution or the presence of other ions. A recent extensive review of most used isotherm models can be found in Abbas et al. (2014), Park et al. (2010), and Limousin et al. (2007).

11.8.3 Limitations of Conventional Models

Conventional models are widely applied due to their simplicity and sufficiency of describing experimental data at various operating conditions. However, they are unable to predict the effect of critical parameters such as pH, ionic strength, metal competition, and more complex uptake behavior (Abbas et al. 2014). They do not take into consideration the electrostatic interaction since they assume that the cell wall consists of a homogenous network of functional groups (Limousin et al. 2007). Do not have a meaningful physical interpretation of the biosorption process. Not only the results cannot be extrapolated, but also no conclusions can be drawn for systems operating under various conditions. These simple conventional models do not consider the effects of any external environmental factors. In addition, an irregular pattern may be represented by the biosorption isotherms because of the complex structure of the biosorbent, its multiple heterogeneous active sites, and the complex solution chemistry of some metallic species. Different researchers of the biosorption phenomenon rarely have considered this issue. They regularly force smooth isotherm curves through scattered experimental points and/or use the earlier simplistic sorption models to fit those experimental data points.

Despite several indications of ion exchange as the main mechanism of biosorption by nonliving biomass, most studies of biosorption continue to analyze experimental data obtained by means of models originally developed for sorption on granular activated carbon. The models for activated carbon cannot identify if biosorbents may operate on different ionic cycles, which are determined by the types of acids and/or bases used for pretreating the biomass in the regeneration step. Therefore, the elementary difference between ion exchange and sorption on granulated activated carbon limits the use of these models in biosorption.

Despite the critical role of solution pH in sorption processes, it is usually neglected in most mathematical descriptions of the process. The complex structure of biosorbent materials, which may consist of multiple active binding sites, makes the modeling of the pH effect in the biosorption process difficult (Abbas et al. 2014). Consequently, isotherms have been studied separately for each pH value. Such a step is essential since both Langmuir and Freundlich isotherm models do not consider the effect of pH. Several attempts have been made in literature to lump the effects of several parameters including the pH into one empirical parameter.

11.8.4 Ion-Exchange Isotherm

Ion-exchange isotherm model is difficult to apply due to the complex structure of the biomass when compared to a synthetic ion-exchange resin. Volesky and Schiewer (1999) proposed a model based on the ion exchange of two sites for metal uptake by *Sargassum fluitans*, which consists of two main active groups mainly carboxyl and sulfate. It can predict the equilibrium binding of cadmium, copper, and zinc by seaweed biomass as a function of pH and the final metal concentration. The proton concentration is integrated into the model independently as a variable so that the prediction of metal uptake by biosorption at different solution pH values becomes an easy calculation. However, this model neglects the effect of metal ion hydrolysis in aqueous solution on biosorption performance. Yang and Volesky (1999) proposed a model that considers ion exchange between protons in the same biomass and hydrolyzed uranium ion as a simple competition for available binding sites where no reverse reactions take place. It assumed that due to the uranium hydrolysis in aqueous solution, the total uranium ion concentration is not necessarily the same as the free uranium ion concentration. The model can predict the uranium and proton binding based on the pH value and total uranium concentration. Sulaymon et al. (2013a) applied a general ion-exchange equilibrium model to describe lead, copper, cadmium, and arsenic uptake by algae.

The Langmuir equation and the ion exchange constant for the uptake of a metal ion, M, replacing a proton, H, on an active site, B, can be explained as follows:

$$\begin{gathered}\text{Langmuir}: \quad B + M \leftrightarrows BM \\ {}^{BM}K* = \frac{BM}{B[M]};\ [B]_t = [B] + [BM]\end{gathered} \tag{11.1}$$

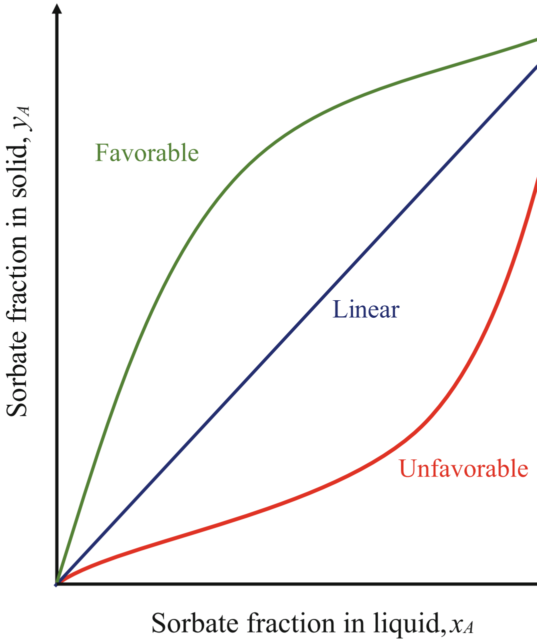

Fig. 11.4 Typical ion-exchange equilibrium isotherm (Leusch et al. 1995)

$$\text{Ion exchange}: BH + M \leftrightarrows BM + H$$
$$^{BM}K = \frac{BM[H]}{BH[M]};\ [B]_t = [BH] + [BM] \tag{11.2}$$

Therefore,

$$^{BM}K* = {}^{BM}K/[H] \tag{11.3}$$

The main difference between the two methodologies is that the Langmuir isotherm assumes that all sites are initially unoccupied and neglects any possibility of a reverse reaction of the displaced ion, i.e., proton. The ion-exchange approach assumes that all active binding sites are initially occupied and the number of free sites remains constant. The difference between the two approaches was especially obvious at low metal concentrations, since the effect of the reverse reaction involving the displaced ions is pronounced at these concentrations (Limousin et al. 2007). Figure 11.4 represents the most generalized description of the ion-exchange sorption equilibrium isotherm for binary systems.

Although the ion-exchange model is probably closer to reality than that of the Langmuir model, it is not very satisfactory. Even though constant many free sites may be reasonable as an assumption for a constant pH system, it may be invalid for system with varying pH. The modeling of commercial ion-exchange resin can be simply achieved, since these materials contain only one active group. However, biosorbents are complex materials that contain several heterogeneous active groups.

In addition, since the metal uptake increases with an increase in pH, modeling the competitive binding of metals and protons by using only a metal-proton-ion-exchange constant is not appropriate (Abbas et al. 2014).

11.9 Conclusions

Heavy metals are environmentally toxic but can be precious materials at the same time in many cases. Conventional removal/recovery methods are usually expensive and ineffective at low metal concentrations. Biosorption has evolved as a cost-effective and environment-friendly polishing process, which has been studied extensively by different research areas. A wide range of publications is available in the literature, which has investigated the various factors affecting the biosorption technology, including operational and environmental parameters, where solution pH was found to play the most critical role. Most studies were focused on the kinetics and equilibrium of biosorption, the uptake capacity of different sorbate using various biosorbents, recovery of biosorbed metals, and modeling of the essential parameters. Batch biosorption process has been studied extensively with few continuous biosorption attempts and biosorption reactor. Despite many experimental studies investigated the biosorption process, several technical issues need to be addressed to commercialize the technology, which is required to meet the industrial demands. Detailed economic analyses are required for large-scale industrial wastewater, taking into consideration the recovery, separation, and purification issues. Simplified mathematical models to describe real-life cases of multi-metal systems are required, where different biosorption mechanisms should be tested to reveal the most dominant one(s). Finally, efficiency and selectivity of biomaterials should be the main criteria to examine the new biosorbents. Ideal biosorbents should be abundantly available and/or waste material to decrease the overall cost of the process, which is the key element that kept the biosorption process an attractive option for the last decade.

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Chapter 12
Toxic Elements in Bangladesh's Drinking Water

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Abstract Increasing exposure to heavy metal contamination in aquatic environment can have serious health consequences in Bangladesh. Although a shift from using surface water to groundwater significantly reduced waterborne diseases in Bangladesh, vast areas of Bangladesh are affected by groundwater arsenic contamination above WHO drinking water guideline. There are also some other emerging contaminants in drinking water which have not historically been considered as pollutants but are now being more widely detected. Millions of Bangladeshis are drinking water with unsafe levels of arsenic (As), manganese (Mn), boron (B), barium (Ba), chromium (Cr), molybdenum (Mo), nickel (Ni), lead (Pb), or uranium (Ur). Approximately, 45% area of Bangladesh contains groundwater with As concentration greater than the standard limit for Bangladesh drinking water. Manganese, Pb, Ni, and Cr are also found at significant concentrations in groundwater. About 50%, 3%, <1%, and <1% of Bangladesh's area exceeds WHO guidelines for Mn, Pb, Ni, and Cr, respectively. Besides these, concentrations of Cd and B are higher than the safe value in river water which might create an adverse effect on riparian ecosystem. Most groundwater contamination originates either as point source or nonpoint source. Urban areas contribute more pollutants to groundwater than non-urban areas. Industrial areas are more adversely affected by heavy metals compared to non-industrial areas. These emerging contaminants in the form of heavy metals pose significant human health risks. In order to understand the significance of public health strategy of safe drinking water, a thorough review of emerging contaminants in drinking water, its toxicity, and health hazards, as well as future directions toward sustainable development goals (SDGs), is necessary.

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12.1 Introduction

Contamination of heavy metals is considered as a global crisis, especially in developing countries such as Bangladesh due to its toxicity in aquatic environment (Ali et al. 2016; Islam et al. 2015a; Ahmed et al. 2015a, b). Abundant intrusions of heavy metals are reported in the environment due to natural and anthropogenic activities (Wilson and Pyatt 2005; Khan et al. 2008). The increasing exposure to heavy metals has serious health consequences (Islam et al. 2014, 2015a, b; Yi et al. 2011; Martín et al. 2015). Heavy metal pollution in water is a major problem in Bangladesh (Islam et al. 2015b) due to unplanned urbanization and industrialization. The discharge of untreated effluents from various industries into environment has become a major public concern in Bangladesh due to its detrimental effects on water quality (Khadse et al. 2008; Venugopal et al. 2009; Islam et al. 2015a, b).

Exposure to groundwater arsenic is a serious concern in public health standpoint and the affected countries are the USA, Taiwan, Mexico, Mongolia, Argentina, India, Chile, and Bangladesh (Argos et al. 2010). The situation is shocking in Bangladesh. The substantial transition from surface water to groundwater as the main source of drinking water may have significantly reduced waterborne diseases in Bangladesh; however, large areas of Bangladesh are affected by disease and death from arsenic and other toxic elements in groundwater (Frisbie et al. 2002). In Bangladesh, about half of the hand-pumped tube wells supply groundwater with arsenic level above 50 mg/L which is the acceptable level in Bangladesh (Smith et al. 2000). According to the Bangladesh Atomic Energy Commission, the arsenic level is between 150 and 200 ppb (parts per billion) in tube well in the districts boarding West Bengal.

In an estimation, about 80 million people of the country are arsenic affected, and one in ten is prone to developing cancer from the poisoning (Uddin et al. 2004). Two national-scale surveys of tube well for other toxic elements revealed higher concentrations of some toxic metals exceeding WHO drinking water guidelines (Frisbie et al. 2002; BGS and DPHE 2001b) raising concerns of possible synergistic effects (Frisbie et al. 2009) on human health. Arsenic in Bangladesh's tube well water is considered to be the most significant health risk. Thus, routine testing of drinking water for arsenic is considered crucial and is institutionalized. Evidence showed that much of the researches focused on arsenic distribution and its health impacts. Other toxic elements in drinking water received far less attention. In terms of public health policy, testing water only for arsenic is not enough to ensure safe drinking water. Evaluating the correlations between arsenic and other commonly occurring toxic elements is essential to ensure safe drinking water supply. In order to understand the significance of public health strategy of safe drinking water, a thorough review of emerging contaminants in drinking water, their toxicity, and health hazards, as well as future directions toward SDGs, is necessary.

12.2 Emerging Contaminants in Groundwater: Global and Bangladesh Context

The term "emerging contaminants" is generally used to refer to compounds previously not considered or known to be significant to groundwater but are now being more widely detected. There is a wide variety of sources and pathways for these compounds to enter the environment. So, the number of regulated contaminants will continue to grow slowly over the next several decades (Stuart et al. 2011). As analytical methods continue to improve, recent studies have revealed that emerging contaminants, which have not historically been considered pollutants, have now raised significant concerns to public health professionals and environmental engineers and scientists (Xagoraraki and Kuo 2008). The criteria for environmental quality are intimately linked to emerging contaminants. As a new compound begins to cause concerns, data accumulate on its environmental chemistry, its ecotoxicological and human toxicities, as well as its epidemiology. This eventually results in government actions to establish environmental guidelines or criteria to ensure adequate protection. In a similar sequence, compounds that are already regulated are often re-evaluated with the addition of new data (Sauvé and Desrosiers 2014).

Most groundwater contamination originates at the land surface and is classified as either point source, for example, from spills or an industrial site, or nonpoint source, for example, from agriculture-related compounds. Probably the greatest threat is exposure to undetected contamination (Beckie 2013). Most water pollutants originate from human activity. Primary anthropogenic sources of water pollution include poorly treated or untreated municipal sewage, individual septic system discharge, agricultural livestock wastes, fertilizers, pesticides, industrial chemical wastes, spilled petroleum products, spent solvents, etc. Once pollutants are introduced into receiving surface water and groundwater bodies via discharge processes, surface runoff, subsurface infiltration, or atmosphere precipitation, they are transported within the water cycle in a global context (Xagoraraki and Kuo 2008). However, in some instances natural processes also contribute to pollutants. Urban areas compared to the non-urban areas contribute more pollutants to groundwater due to high density of on-site wastewater treatment systems (Kurwadkar 2014).

The unplanned urbanization and industrialization of Bangladesh have detrimental effects on the quality of water and different aquatic fauna. The disposal of urban wastes, untreated effluents from various industries, and agrochemicals to open water bodies and rivers has reached an alarming level in Bangladesh, continually increasing the metal level and deteriorating water quality (Khadse et al. 2008; Venugopal et al. 2009; Islam et al. 2015a, b).

Groundwater arsenic contamination is a global phenomenon, while the most affected regions are the South and East Asia. In Southeast Asia, an elevated level of arsenic in groundwater is threatening to public health of about 40–60 million people (McCarty et al. 2011). Evidence based on available data shows that more than 700,000 cases of reported arsenicosis and additional 12.5 million cases of predicted arsenicosis are documented in this region. The history of finding arsenic crisis in

Bangladesh and West Bangla is quite old (the early 1990s) compared to Vietnam and Cambodia (last 7–10 years). Jointly, about 150 million people of Bangladesh and India (Bacquart et al. 2012) as well as over 296 million of global population are at the risk of As contamination in groundwater (Chakraborti 2013). Higher level of arsenic in groundwater is well reported in Chile, Mexico, China, Argentina, the USA, and Hungary (Smedley and Kinniburgh 2002; Bhattacharya et al. 2002).

West Bengal, Bangladesh, and Vietnam are also among the countries with elevated level of arsenic (Bhattacharya et al. 1997, 2002, 2007; Bundschuh et al. 2009). It is reported from multiple metal analyses that the existence of other toxic elements has been found above the WHO drinking water guidelines and thus the number of people at risk is increasing from drinking contaminated groundwater (Bacquart et al. 2012). Half of their sampled tube wells were detected to have arsenic above WHO guidelines, whereas Mn and B were also found at significant concentrations, respectively. Their report suggested that 75% of this area has no safe water indicating maps of As, Mn, and B. Thus, the distribution of other metals besides As needs to be taken into account as a public health challenge.

A recent study found that the concentrations of As, Cr, Cd, and Pb were higher than the safe value which indicated that the rivers are polluted by these heavy metals and might create an adverse effect on the riparian ecosystem (Ali et al. 2016). The industrially developed area beside the Karnaphuli River is increasing the heavy metal pollution day by day. Lead in gasoline has been phased out in Bangladesh in 1999. However, blood lead levels (BLLs) above 5 μg/dL still persist across the country (Mitra et al. 2012; Gleason et al. 2014). In spite of having higher BLLs in urban areas compared to the rural areas, BLLs are higher than expected in non-industrial rural areas. Several studies conducted in Munshiganj, Dinajpur, and Narayanganj showed higher BLLs among the children (≥5 μg/dL) living in rural areas (Mitra et al. 2009; Gleason et al. 2014; Rodrigues et al. 2016). Lead is considered to be a potential neurotoxin which poses a serious threat to public health and human intellectual (Tong et al. 2000). Rural pregnant women in Bangladesh are exposed to lead poisoning from different sources found in a study conducted jointly by ICDDRB and Stanford University (Maswood 2018).

Cadmium is a common environmental pollutant and is a global concern for increasing neurodevelopmental disorders (Grandjean and Landrigan 2014). According to Kippler et al. (2016) and Sanders et al. (2015), in the last decade, some observational studies found that prenatal and childhood exposure to cadmium may affect cognitive function; however, the evidence is insufficient. A number of studies conducted in Bangladesh showed association between pregnant mother's exposures to cadmium and children's IQ performance (Jeong et al. 2015), risk of learning disabilities (Ciesielski et al. 2012).

Problems in the groundwater also exist with high concentrations of iron and manganese. Boron is problematic in some higher salinity areas in southern Bangladesh. Limited analyses of uranium also suggest that this may be a problem in some groundwater, though the health consequences of uranium are poorly understood. Concentrations of ammonium are found to be very high in many samples, and these may cause some acceptability problems. Similarly, high iron in drinking water is not a health problem; however, it may be unacceptable to users at high concentrations. Manganese, on the other hand, is regarded as a potential health problem in water, and WHO has set a guideline value of 0.5 mg/l for this element (BGS and DPHE 2001a). High concentrations of manganese are found in current Brahmaputra and Ganges floodplains. Rare surpassing the limits above WHO guideline values were also observed for nickel, chromium, lead, and barium, although these were usually not significantly above the guideline values. A limited number of samples analyzed for selenium found none above the analytical detection limit of 0.5 μg/l. It is possible that some selenium is present in the more aerated shallow groundwater (BGS and DPHE 2001a).

No data are available for mercury in Bangladesh groundwater. Apart from groundwater affected by industrial pollution, concentrations of mercury are expected to be low and significantly less than the WHO guideline value of 1 μg/l. However, in the anaerobic environment of the groundwater, there is a possibility of the presence of some mercury (BGS and DPHE 2001a).

It is evident that rapid economic development as a new challenge in Asian countries creates exposure to toxic substances through various pathways. The living environment is affected by climate change which is the reason of various infectious diseases. Over half of the world's population lives in Asia, and global health of this region is greatly affected by changes in nutrition condition, environmental health, and infectious diseases (McCarty et al. 2011).

12.3 Extent of Contamination in Bangladesh's Drinking Water

Globally millions of people have exposure to highly arsenic-contaminated drinking water. Due to its significant toxicity, As is a global concern as a pollutant of drinking water and groundwater, which has been reported in the USA, China, Chile, Bangladesh, Nepal, Vietnam, Taiwan, Mexico, Argentina, Poland, Italy, Finland, Spain, Canada, Hungary, New Zealand, Japan, and India (Jain and Singh 2012; Mohan and Pittman Jr 2007). In Bangladesh, estimations reveal that more than 35 million people are exposed to arsenic-contaminated drinking water at levels exceeding WHO drinking water guidelines of 10 μg/L (Flanagan et al. 2012). In Taiwan the first incident of a large-scale health problem caused by naturally occurring arsenic was identified and recorded in 1968. Afterward, Chile's contamination case was largely documented in the 1970s. In the 1980s, the problems in West Bengal, India, as well as in Ghana, Mexico, and several other countries were brought into attention. Bangladesh was known to be the largest contamination case to date. In the early 1990s, patients from western districts in Bangladesh started to cross the border to visit hospitals in Calcutta, but the official exploration of the problem was initiated in 1995. After 1997, a number of studies and initiatives started to discover that most of the country should be considered at serious risk (Alaerts et al. 2001).

According to Jiang et al. (2012), Bangladesh is the most affected country among the arsenic-contaminated countries. In Bangladesh, about 77 million people are exposed to arsenic toxicity in drinking water. Tens of millions of Bangladeshis are drinking water with unsafe levels of As, Mn, B, Ba, Cr, Mo, Ni, Pb, or U. Arsenic in Bangladesh's tube well water was found to be the most significant health risk (Frisbie et al. 2002). Approximately 45% of Bangladesh's area contains groundwater with As concentrations greater than the 0.05 mg/L Bangladesh national drinking water standard. Similarly, 50%, 3%, <1%, and <1% of Bangladesh's area exceeds WHO guidelines for Mn, Pb, Ni, and Cr, respectively (Frisbie et al. 2002). Similarly, B, Ba, Cr, Mo, Ni, Pb, and U were discovered at concentrations above WHO health-based guidelines in relatively small areas of Bangladesh.

Twenty-two million people in Bangladesh are consuming drinking water which does not meet the Bangladesh national standard for arsenic. Of those, 5.6 million are exposed to >0.2 milligrams per liter and are in extreme health danger. Ten contaminants such as arsenic, barium, boron, copper, fluoride, manganese, molybdenum, nickel, selenium, and uranium have health-based WHO guideline values. The WHO guideline value of 0.4 mg per liter is most frequently exceeded by manganese. The Bangladesh standard for manganese is stricter at 0.1 mg per liter. Another element, iron, is found to exceed the Bangladesh standard of 1.0 mg per liter. Although 93% of deep tube wells meet the Bangladesh standard for arsenic, only 60% of deep tube wells meet the Bangladesh standards for arsenic, manganese, and iron (UNICEF 2009).

The result from a study which was conducted in Narayanganj reported that certain trace elements like As, Pb, Cd, Cr, Ni, and Mn are of great concern, because these elements exceed the acceptable limit of WHO and Bangladesh standards for drinking water. Lead concentration in all the samples of the study area is higher than the WHO and Bangladesh standards. The minimum concentration in the study area is 0.1 mg/l, whereas the maximum admissible limit is 0.01 mg/L (WHO 2004). Manganese concentration in some samples are eight to ten times higher than the WHO and Bangladesh standards. The maximum concentration (2.80 mg/L) is found at shallow tube well water, whereas the maximum admissible limit is 0.5 mg/L (WHO 2004).

Most of the water samples show that Cd concentration is ten times higher than the Bangladesh drinking water standard. The minimum concentration is 0.01 mg/L, whereas the maximum admissible limit is 0.005 mg/L (DoE 1997). Nickel concentrations in all of the samples exceed the Bangladesh drinking water guideline. On the other hand, Ba concentration is ten times higher than the Bangladesh and WHO drinking water guidelines. The minimum concentration is 0.01 mg/L, whereas the maximum admissible limit is 0.003 mg/l (DoE 1997) and 0.01 mg/L (WHO 2004).

The heavy metal concentrations (Pb, Cr, Mn, Co, Ni, Cu, Zn, As, and Cd) in Buriganga water and sediment were very high and, in most cases, exceeded permissible limits recommended by the Bangladesh government and other international organizations (Bhuiyan et al. 2015), and concentrations of Al and Mn surpassed the limit set by the WHO (2004). The concentrations of Pb, Cu, and Zn were above the EPA guideline for heavily polluted sediment, and the amount of Cd and Cr falls in the criteria of moderately to highly polluted range (Saha and Hossain 2011). The concentrations of Cr, Pb, Cd, Zn, Cu, Ni, Co, and As in water significantly exceeded the admissible limit both in summer and winter season (Mohiuddin et al. 2011). Ship breaking area in Chittagong is also highly affected by heavy metal. The sediment samples were moderately to heavily polluted with Pb, Cu, Zn, and Cr, but the concentrations in water were found below the permissible limit except Cr (0.511 ± 0.284) mg/l (Aktaruzzaman et al. 2014).

Industrial area is adversely affected by heavy metals rather than non-industrial area. Being long persistent metals in nature, these substances are exacerbating health problems both in human being and fish. As a consequence, various fatal and chronic diseases find their permanent residency in living organisms through the process of bio magnifications.

12.4 Toxicity and Health Hazards

Toxicity of heavy metals depends on the dose, route of exposure, as well as the age, gender, genetics, and nutritional status of exposed persons. Its multiple uses in different sectors such as industrial, domestic, agricultural, and technological make environmental diffusion, increasing potential impacts on health and environment (Tchounwou et al. 2012). They also mentioned that arsenic, cadmium, chromium, lead, and mercury are high-ranked contaminants due to their greater degree of toxicity among the priority metals which have public health significance. According to the US Environmental Protection Agency (EPA), these toxicants are known as human carcinogens (known or probable). Thirty-five metals are threatening to human health, of which 23 metals are heavy metals (Mosby et al. 1996). Considering public health significance, As, Cd, and Pb are extensively studied among the other heavy metals (ATSDR 2015; EPA 2015).

A number of review papers well documented the consequences of arsenic toxicity in humans and animals (Shankar and Shanker 2014). Arsenic, a well-known carcinogen, is one of the most hazardous chemicals (EPA 2001). About 35–77 million people in Bangladesh are exposed to arsenic-contaminated drinking water (BGS and DPHE 2001a). The health consequence of excessive and long-term (5–10 years) intake of inorganic arsenic in drinking water and food is arsenicosis, generally used for arsenic-related health problems such as skin cancers, skin disorders (Fig. 12.1), internal cancers (bladder, kidney, and lung), diseases of the blood vessels of the legs and feet, diabetes, high blood pressure, and reproductive disorders (Santra et al. 2013; USEPA 2013). The inorganic forms of arsenic (trivalent arsenite (As-III) and pentavalent arsenate (As-V) in terrestrial environment are more dominant and toxic than the organic forms in general. Arsenic causes harmful effects on general protein metabolism with high toxicity (Rai et al. 2011).

According to WHO's report (1996a, b), drinking water with 10 μg/L is associated with 3 extra deaths per 5000 people from skin cancers and 10 extra deaths per 5000 people from bladder, liver, or lung cancer (Morales et al. 2000). Frisbie et al. (2005)

Fig. 12.1 Skin lesion due to arsenic toxicity

found those melanosis, leukomelanosis, keratosis, hyperkeratosis, and nonpitting edemas are associated with chronic arsenic poisoning in Bangladesh. When individuals fall victim of arsenicosis, they face dreadful effects on their livelihoods, family life, and earning capability. The physical appearance deteriorates making women socially excluded. Exposure to higher arsenic contamination may cause societal stress, disability, poverty, and reduced market value of affected agricultural products leading to low income of the farmers (Brinkel et al. 2009).

Next to As, Pb was the second most investigated toxicant considering its public health effects. Pb affects the central nervous, renal, hematopoietic, cardiovascular, gastrointestinal, musculoskeletal, endocrinological, reproductive, neurological, developmental, and immunological systems (ATSDR 2015). According to the US Environmental Protection Agency (2008), noncarcinogenic disorders in humans such as neurotoxicity, developmental delays, hypertension, impaired hearing acuity, impaired hemoglobin synthesis, male reproductive impairment, etc. are caused by Pb. The exposure to Pb is particularly serious to infants, children up to 6 years of age, and pregnant women (WHO 1996a, b). The third most frequently reported heavy metal in drinking water is Cd due to its public health significance (Fernandez-Luqueno et al. 2013; WB 2016). Exposure to Cd-contaminated drinking water leads to chronic renal failure (ATSDR 2015; Bawaskar et al. 2010), while long-term exposure to Cd results in kidney failure (Gobe and Crane 2010). Upon long exposure at low concentrations, it could get deposited in the kidney, ultimately leading to kidney disease, fragile bones, and lung damage (Bernard 2008). Chronic exposure to Cd could lead to anemia, anosmia (loss of sense of smell), cardiovascular diseases, renal problems, osteoporosis, and hypertension (ATSDR 2015). According to Chakrabarti et al. (2013), Cd can cause both acute and chronic intoxications. Henson and Chedrese (2004) found association between Cd exposure during pregnancy and premature birth and reduced birth weights.

In Bangladesh, both Mn and As in drinking water are reported to be emerging threats to rural public health (Khan et al. 2011). Akter et al. (2016) found that the median value of Mn concentrations exceeded Bangladesh standard at most of the study sites. Other Bengali studies have reported higher Mn levels in drinking water based on WHO standards (Khan et al. 2012). Approximately 8% of children were exposed to excess Mn concentrations that exceeded both WHO and Bangladesh standards (>0.4 and >0.1 mg/L, respectively). Children are reported to be particularly vulnerable to higher Mn concentrations due to their low protective mechanism. Several studies have reported that exposure to high Mn concentrations threatens children's cognitive (Roels et al. 2012), behavioral, and neuropsychological health (Wasserman et al. 2006). Infants and children are reported to be more susceptible to Mn toxicity than adults (Hafeman et al. 2007), and a number of Bangladesh studies have shown that children's intellectual functions, and consequently their academic achievements, were adversely affected by Mn exposure in drinking water (Khan et al. 2012; Wasserman et al. 2006; Hafeman et al. 2007).

Contradictory to these findings, a higher Mn level in drinking water was shown to be protect against fetal loss during pregnancy of undernourished women in Bangladesh (Rahman et al. 2013). Frisbie et al. (2009) reported in their study conducted in western Bangladesh that drinking water with unsafe level of As may also have unsafe level of Mn, U, Pb, Ni, Cr, or other toxic elements. A number of studies showed the effects of chronic U ingestion from drinking water ranged from adverse kidney function (Zamora et al. 1998) to nephrotoxic effects even at low concentrations (Kurttio et al. 2002). Like the kidneys, the bone may even be affected by the toxicity of U. Ni is known to be "carcinogenic to humans" by inhalation exposure. On the other hand, total Cr in drinking water is categorized as "inadequate or no human and animal evidence of carcinogenicity" by the (EPA 1996).

12.4.1 Public Health Strategies Addressing Toxic Elements in Drinking Water

Water quality and quantity in Bangladesh are under threat due to surface and groundwater pollution from various sources, urban runoff, climate and extreme weather events, etc. These challenges toward public health encourage adoption of a number of national and international strategies to facilitate sustainable development. Access to safe drinking water for healthy life is mentioned in national and international policies. Sustainable Development Goal 6 gives emphasis on access to safe and affordable drinking water for all by 2030. To achieve this target, some of the necessary actions in the 7th fifth five-year plan were as follows: (i) ensuring safe water facilities for all by developing different water supply options; (ii) providing water supply options in affected areas; (iii) ensuring safe water facilities in the hydro-geologically difficult and problematic areas; (iv) examining water quality, monitoring, and surveillance system; and (v) hydrological and hydro-geological investigation of ground and surface water.

Besides, improving water quality is targeted in SDG 6 by reducing pollution, removing dumping, and minimizing release of hazardous chemicals and materials into water. To achieve this goal, actions targeted in the 7th five-year plan were as follows: (i) integrated water treatment and efficient water supply facilities in urban areas; (ii) shifting the dependence of water supply from groundwater to surface water with improvement in surface water quality; and (iii) construction, operation, and maintenance of water treatment plants, water abstraction facilities, and water distribution system for providing drinking water to public, industrial, and commercial organizations. To achieve SDG 6, relevant policy instruments were as follows: (i) national policy for safe water supply and sanitation 1998, (ii) national water policy 1998, (iii) national water management plan 2004, (iv) national policy for arsenic mitigation and implementation plan 2004, (v) pro-poor strategy for water and sanitation sector 2005, (vi) national strategy for water and sanitation hard to reach areas of Bangladesh 2011, (vii) Bangladesh water act 2013, and (viii) participatory water management regulations 2014. Moreover, the Bangladesh Environment Conservation Act is in place to prevent water pollution.

The national water policy of Bangladesh 1998 is designed to enable the national goals of economic development, poverty reduction, food security, public health, and safety for people and protection of natural environment. Surface and groundwater pollution by industrial effluent discharge into water bodies is considered as significant water management issue. Poor water quality affects the availability of fresh water for different uses. Contamination of surface water and groundwater by agricultural pollutants, industrial discharge, domestic pollution, and urban runoff exacerbate water quality and endanger public health (GoB 1998). The national policy for arsenic mitigation (2004) complements the national water policy and the national policy for safe water supply and sanitation 1998 in order to support the national goals of poverty alleviation, public health, and food security (GoB 2004). Moreover, the national policy for safe water and sanitation (1998) aimed to ensure equitable access to safe water at affordable cost and to develop public health (GoB 1998).

It is reported that millions of Bangladeshis are exposed to arsenic contaminations exceeding WHO drinking water guideline of 10 μg/L (Ahmed et al. 2006). In the 1970s, the installation of deep tube wells was encouraged to reduce the occurrence of waterborne diseases from drinking contaminated surface water (BGS and DPHE 2001a). Rural people started showing evidence of health consequences from the early 1990s because of drinking high levels of As-contaminated water (Dhar et al. 1997). An arsenic testing campaign was initiated in 2001 and completed in 2004. By 2005, about 1.4 million tube wells were detected with arsenic concentrations exceeding Bangladesh drinking water standard of 50 μg/L. To identify arsenic safe tube well, the affected tube wells having As concentrations at above the Bangladesh standard were marked as red, and tube wells found to have arsenic at below the standard were marked as green (Ahmed et al. 2006). According to (Frisbie et al. 2009), much attention is given on studying arsenic distribution and its health effects without giving proper attention to other toxic elements in Bangladesh's drinking water. They have also suggested testing tube wells for multiple toxic elements, as testing of tube wells only for arsenic is not sufficient to ensure safe drinking water and thus public health.

To achieve SDG 6, establishment of water quality examination, monitoring, and surveillance system is taken into action. Integration of water quality and public health is necessary to better understand emerging contaminants, drinking water-related health risks, and public health interventions. According to the Drinking Water Action Plan by the Environmental Protection Agency (EPA), such integration helps advance detection of emerging drinking water-related public health risks as well as prioritization of mitigation measures of toxic elements in drinking water (EPA 2016).

12.4.2 Mitigation Strategies

The emerging importance of arsenic and other naturally occurring toxicants places a new burden on water supply authorities and policy makers (Alaerts et al. 2001). Knowledge and awareness on arsenic contamination have developed among the people over the years. About 80% of people now know that arsenic in drinking water is a problem. The most commonly taken action by households, familiar with arsenic contamination, is to avoid the affected tube well and collect water from the nearby safe tube well (Unicef 2008). To prevent exposure to arsenic contamination and to minimize health impact, the government's strategy is to screen all the tube wells to identify the affected tube wells and to measure the level of contamination. If the tube well is detected with higher concentration of arsenic than Bangladesh drinking water standard, then the tube well is marked as red, and arsenic safe tube well is marked as green. Compared to developed countries, the development of a sustainable mitigation strategy in developing countries requires consideration of additional factors particularly financial, technical, and administrative constraints (Alaerts et al. 2001). It is reported that the exposure to arsenic occurs through various media including food, soil, and air. In Bangladesh, arsenic is significantly found in water which is the principal contributor to daily human consumption of As. Therefore, reduction of such arsenic intake is attempted through the provision of arsenic-free water.

A lot of arguments are still ongoing about the technological advancements and drawbacks of various mitigations strategies in Bangladesh. After testing various mitigation options such as piped water supply, rainwater harvesting, pond sand filters, and dug wells (Berg et al. 2006), questions have been raised about their safety, affordability, and convenience (Ahmed et al. 2006). All of these mitigation options, which are different from currently much used shallow or deep tube wells, require substantial maintenance. Well-accepted existing technology is considered as emergency interventions (Smith et al. 2000) even if adjustment through behavioral change is required. A number of studies depicted that intervention programs including health education and labeling of tube wells increase awareness on As-related health consequences (Hadi 2003; Hanchett et al. 2002).

Naturally occurring heavy metals in groundwater raised public concerns because of their degree of toxicity and potential effects on human health and environment. These metallic elements are known to be systemic toxicants inducing multiple organ damage even at lower levels of exposure and are classified as human carcinogens (Tchounwou et al. 2012). Chakraborti et al. (2010) mentioned that about 80 million people were vulnerable to groundwater As contamination above the WHO drinking water standard (10 μg/L). Considering public health policy, providing As free safe drinking water for Bangladeshi households is an urgent need. Therefore various water treatment approaches were taken into account such as treatment of surface water (typically low in As), rainwater harvesting, treatment of pond waters, use of deeper (>150 m deep) wells to extract groundwater with low-As, and exploring low-technology, low-cost, locally fitted systems for arsenic removal from groundwater. However, these approaches have some practical problems in terms of applicability, economy, infrastructure requirements, generic dissemination, and future sustainability.

Treatment of surface water involves setting up industrial-scale water purification and distribution plants which are expensive, time-consuming, and investment-intensive, particularly for rural Bangladesh (Cheng et al. 2004; Sarkar et al. 2010). Water from deeper aquifer has low As concentrations (van Geen et al. 2003). Such aquifer in the Bengal basin as a source of arsenic safe water is used for domestic purpose on a limited basis (Stollenwerk et al. 2007; Islam 2011), although most of the basin is vulnerable to downward migration of high arsenic concentrated shallow groundwater caused by withdrawal of deeper groundwater significantly for irrigation (Radloff et al. 2011). Rainwater harvesting for clean water is particularly feasible in the case of individual household, but installing such system may not be financially viable for many rural households (Cheng et al. 2004; Visoottiviseth and Ahmed 2009).

Arsenic remediation technologies such as ion exchange, activated alumina, reverse osmosis, membrane filtration, modified coagulation/filtration, and enhanced lime softening were tested so far (EPA 2001; Berg et al. 2006). For any technology to be suitable and sustainable in Bangladesh, it should be simple, cost-effective, versatile, transferrable, and adaptable both at household and community levels (Visoottiviseth and Ahmed 2009). According to Berg et al. (2006) none of the technology recommended by US Environmental Protection Agency is applicable in low-income countries due to their sophisticated technical systems. The most widely used technologies, which are cost-effective for As removal in Bangladesh and in other developing countries, are oxidation, co-precipitation and adsorption onto coagulated flocs, and adsorption onto sorptive media (Ahmed 2001).

The water sector in Bangladesh needs to identify a sustainable means of supplying safe water in arsenic-affected areas and to build arsenic testing capacity locally for surveillance (George et al. 2012). It is reported that public health benefits can be maximized if highly contaminated areas (concentrations >200 μg/L) are targeted first for safe water supply. In the intervention area of Comilla, the proportion of population drinking safe water increased from 75% in 2007 to 81% in 2009 due to the provision of safe water at high-risk communities, public education, and social mobilization. It is reported that past achievements will be lost if arsenic mitigation measures are not sustained. Building testing capacity locally will lead to sustained awareness in areas with high arsenic exposure and give people more control over their water supply, although instilling a social norm of periodically testing well water is essential for sustainability (Madajewicz et al. 2007). Compliance with the national drinking water standard can be facilitated by informing people about which tube well is contaminated and which are not and by providing new water supply system to the most affected areas. The social acceptability and sustainability are crucial factors to be considered when choosing among arsenic mitigation strategies, in addition to the costs of the technologies involved (UNICEF and BBS 2011).

Frisbie et al. (2002) reported that millions of people in Bangladesh are being affected by drinking metal-contaminated groundwater which needs urgent attention. They mentioned that arsenic was found to be the most significant contaminant in terms of health risks. However, mitigation efforts should not be restricted to arsenic only; the health risks from other toxic elements need to be addressed. They also emphasized taking actions on supplying drinking water with safe levels of As, Mn, Pb, Ni, Cr, and other toxic elements and agents that increase arsenic poisoning. Frisbie et al. (2009) also mentioned in another paper that most of the attention are directed toward arsenic concentrations in groundwater; however, testing only arsenic is not enough to ensure safe drinking water and should be further evaluated for removal of multiple toxic elements such as Mn, U, Pb, Ni, Cr, etc. to address the threats to public health. A review matrix of researches on toxic elements in Bangladesh's drinking water is developed and shown in Table 12.1. Important policy implications suggested through the research findings are compiled to comprehend mitigation strategies of the impacts of heavy metals.

12.5 Future Directions Toward Sustainable Development Goals (SDGs)

Ensuring availability and sustainable management of good-quality water is set as one of the SDGs which is a challenge for policy makers and water, sanitation, and hygiene (WASH) practitioners, particularly in the face of changing climatic conditions, increasing populations, poverty, and negative effects of human development. To increase access to safe drinking water and improved water quality are targeted in SDG 6 by reducing pollution, eliminating dumping, and minimizing release of hazardous chemicals into water. Public health is at risk due to chemical contaminants in drinking water which may have immediate health consequences. Drinking water sources are susceptible to pollutants depending on geological conditions and

Table 12.1 Review matrix of researches on toxic elements in Bangladesh's drinking water

Author(s)	Year	Toxicants in Bangladesh's drinking water	Policy implications toward SDGs
Ahmed	2001	As	Adaptation of As removal technologies in rural context
Ahmed, Ahuja, Alauddin, Hug, Lloyd, Pfaff, Pichler, Saltikov, Stute, and van Geen	2006	As	Expansion of water testing, revision of national policy for arsenic mitigation 2004
Ahmed, Baki, Islam, Kundu, Habibullah-Al-Mamun, Sarkar, and Hossain	2015	Cu, Mn, Ni, Cr, Zn, As, Se, Mo, Ag, Cd, Sb, Ba, Pb	Health risk due to combined impacts of heavy metals and continuous and excess consumption targets to cancer risk
Ahmed, Shaheen, Islam, Habibullah-Al-Mamun, Islam, and Banu	2015	Cd, As, Pb, Cr, Ni, Zn, Se, Cu, Mo, Mn, Sb, Ba, V, Ag	Consumption of As and Pb are a matter of concern due to carcinogenic risk
Akter, Jhohura, Akter, Chowdhury, Mistry, Dey, Barua, Islam, and Rahman	2016	As, Mn,	Emphasis on awareness raising on chemical contents in drinking water to improve public health at household level
Ali, Ali, Islam, and Rahman	2016	Cr, As, Pb, Cd	Continuous monitoring of As, Cd, and Pb is necessary to assess the risk for ecological safety
Argos, Kalra, Rathouz, Chen, Pierce, Parvez, Islam, Ahmed, Rakibuz-Zaman, and Hasan	2010	As	Association of chronic Ar exposure through drinking water with increased mortality rate. Resources are needed to mitigate health effects of arsenic exposure
Bhuiyan, Dampare, Islam, and Suzuki	2015	Pb, Cr, Mn, Co, Ni, Cu, Zn, , As, Cd	Examining correlation of heavy metals to assess pollution levels. The major sources of metals are from tannery, paint, municipal sewage, textiles, and agricultural activities
Brinkel, Khan, and Kraemer	2009	As	Mental health problems are more common among the arsenic-affected people. More investigation in arsenic affected areas is required to understand underlying mechanisms of poor mental health
Chakraborti, Rahman, Das, Murrill, Dey, Mukherjee, Dhar, Biswas, Chowdhury, and Roy	2010	As	Managing available water resources is a challenge. Development of community-specific safe water sources coupled with local participation and education is required to slow the current effects of widespread As poisoning

(continued)

Table 12.1 (continued)

Author(s)	Year	Toxicants in Bangladesh's drinking water	Policy implications toward SDGs
Cheng, van Geen, Jing, Meng, Seddique, and Ahmed	2004	As, Fe, Mn, Cr, Ni, Cu, Se, Mo, Cd, Sb, Ba, Hg, Pb, U	The average concentrations of Cr, Ni, Cu, Se, Mo, Cd, Sb, Ba, Hg, Pb, U according to WHO guideline in arsenic treated water
Dhar, Biswas, Samanta, Mandal, Chakraborti, Roy, Jafar, Islam, Ara, and Kabir	1997	As	More than 50 million people would be at risk in Bangladesh. Proper utilization of its vast surface and rain water resources, proper watershed management is required urgently
Flanagan, Johnston and Zheng	2012	As	Arsenic mitigation approach: prioritizing provision of safe water for people exposed to >200 μg/L arsenic and building local arsenic testing capacity
Frisbie, Mitchell, Mastera, Maynard, Yusuf, Siddiq, Ortega, Dunn, Westerman, Bacquart, and Sarkar	2009	As, B, Ba, Cr, Fe, Mn, Mo, Ni, Pb, Sb, Se, U, Zn,	Testing tube well for As only doesn't ensure safe water and safe concentrations of Mn, U, Pb, Ni, and Cr. All tube wells should be tested for As, then for Mn and U, and then for B, Ba, Cr, Mo, Ni, and Pb
Frisbie, Mitchell, Yusuf, Siddiq, Sanchez, Ortega, Maynard, and Sarkar	2005	As	Drinking water tube well should be tested periodically for As. An inexpensive and highly sensitive laboratory method is suggested for measuring As<10 μg/L
Frisbie, Ortega, Maynard, and Sarkar	2002	As, Mn, Pb, Ni, Cr, Se, Zn	Apart from health risks from individual toxins (As), possible multimetal synergistic and inhibitory effects are discussed
George, Zheng, Graziano, Rasul, Hossain, Mey, and van Geen	2012	As	Rapid screening of well using arsenic test kit
Gleason, Shine, Shobnam, Rokoff, Suchanda, Hasan, Sharif, Mostofa, Amarasiriwardena, and Quamruzzaman	2014	Pb	Potentially important source of lead exposure
Hadi	2003	As	Raising awareness about arsenic poisoning in rural communities
Hafeman, Factor-Litvak, Cheng, van Geen, and Ahsan	2007	Mn	Association between manganese exposure and infant mortality is indicated

(continued)

Table 12.1 (continued)

Author(s)	Year	Toxicants in Bangladesh's drinking water	Policy implications toward SDGs
Hanchett, Nahar, van Agthoven, Geers, and Rezvi	2002	As	Public education by explaining water testing value, giving them opportunities to ask questions, repeating messages, continuing to educate children about the risk of consuming surface water, and conducting community-wide education program for people of all ages
Islam, Ahmed, Habibullah-Al-Mamun, and Hoque	2015	Cr, Ni, Cu, As, Cd, Pb	Comprehensive risk assessment of heavy metals of aquatic environment
Islam, Ahmed, Habibullah-Al-Mamun, Islam, Ibrahim, and Masunaga	2014	As, Pb	Carcinogenic risk of As and Pb for adult people is indicated
Jiang, Ashekuzzaman, Jiang, Sharifuzzaman, and Chowdhury	2012	As	Development of arsenic removal technologies should follow sources and quality of As-polluted water, socioeconomic and literacy of people, effectiveness of arsenic removal by reducing cost, making user-friendly technology, overcoming maintenance problems, resolving sludge management issues
Khan, Factor-Litvak, Wasserman, Liu, Ahmed, Parvez, Slavkovich, Levy, Mey, and van Geen	2011	Mn	Growing concern about neurotoxicologic effects of WMn in children
Khan, Wasserman, Liu, Ahmed, Parvez, Slavkovich, Levy, Mey, van Geen, and Graziano	2012	Mn	Children's academic achievement may be hampered due to high concentrations of Mn exposure
Maswood	2018	Pb	Raising public awareness about harmful effects of lead poisoning particularly for children and pregnant women
Mitra, Ahua, and Saha	2012	Pb	Risk factors for future educational interventions to prevent exposure to lead poisoning are identified
Mitra, Haque, Islam, and Bashar	2009	Pb	Universal lead screening for preschool and school-aged children and a lead education program for parents are recommended

(continued)

Table 12.1 (continued)

Author(s)	Year	Toxicants in Bangladesh's drinking water	Policy implications toward SDGs
Radloff, Zheng, Michael, Stute, Bostick, Mihajlov, Bounds, Huq, Choudhury, and Rahman	2011	As	Prioritizing monitoring of some areas vulnerable to arsenic intrusion
Rahman, Åkesson, Kippler, Grandér, Hamadani, Streatfield, Persson, El Arifeen, and Vahter	2013	Mn	Effects of drinking water Mn exposure during pregnancy upon fetal and infant survival
Rodrigues, Bellinger, Valeri, Hasan, Quamruzzaman, Golam, Kile, Christiani, Wright, and Mazumdar	2016	As, Pb	Adverse effects of arsenic and manganese on neurodevelopment among the children
Smith, Lingas, and Rahman	2000	As	Allocation of fund and prompt expansion of interventions are required to address the problem. Community education and participation are necessary for successful intervention
van Geen, Zheng, Versteeg, Stute, Horneman, Dhar, Steckler, Gelman, Small, and Ahsan	2003	As	The depth of drilling necessary to reach these low-As aquifers ranges from 30 to 120 m depth
Wasserman, Liu, Parvez, Ahsan, Levy, Factor-Litvak, Kline, van Geen, Slavkovich, and Loiacono	2006	Mn	Children are at risk of Mn-induced neurotoxicity

agricultural, industrial, and other man-made activities. Ensuring the safety of drinking water is, therefore, a growing problem. To ensure availability of safe drinking water and to improve public health interventions, assessment of drinking water quality based on multiple chemical parameters is essential for Bangladesh.

Among the other SDGs, poverty- and health-related goals such as goal 1 (ending poverty everywhere), goal 2 (ending hunger, achieving food security, improving nutrition, and promoting sustainable agriculture), and goal 3 (ensuring healthy lives and promoting well-being for all at all ages) are well associated with SDG 6. The SDG 13 targets battling climate change and its impacts, which pose serious threat to safe water availability and its quality (SDG 6). Sea level rise due to climate change presents severe challenge to water management in Bangladesh. Moreover, the country is facing safe water crisis due to wastage, river pollution, declining groundwater tables, and salinity intrusion. Therefore, the significance of protecting water bodies and rivers to facilitate sustainable management of water resources is targeted in SDG 6. Water management is a significant component to achieve in SDG 6, and there is also a link between poverty and water availability.

Ensuring access to safe water requires preservation of natural resources, investment in adequate infrastructure, and repair and maintenance of infrastructure. According to the UNDP, protecting and restoring water-related natural features such as forest, mountains, wetlands, and rivers are essential to overcome water scarcity. One of the targets aims to reduce water pollution by removing dumping and eradicating discharge of harmful chemicals into the water. Recycling and safe reuse of wastewater could be an important strategy. According to the UNDP, more international aid is required to improve water efficiency and support water treatment technologies in developing countries.

The underlying challenges to sustainable development of water resources are divided into two: transboundary and domestic (Nadira and Shixiang 2018). The authors reported some challenges as poor management, low investment, lack of political and administration policy, lack of technology, etc. The water balance on conflicting issues of too much water during monsoon and too little water during rainy season is necessary for tapping both surface and groundwater resources (Nadira and Shixiang 2018). Water management by using relevant policies and regulations can facilitate Sustainable Development Goal 6 as the government's policy deals with water reuse, water resource management, water rights, industrial water use, wetland restoration, domestic water supplies, water pollutions, etc.

The SDG 6 ensures availability and sustainable management of safe drinking water for all by 2030. The underlying challenges toward this goal include emerging contaminants in drinking water posing new challenges to water supply authorities and policy makers. Some reviewed papers reported that arsenic concentrations in drinking water attained much attention in spite of the prevalence of other toxic elements (e.g., Pd, Ni, Cr, Mn, U) in Bangladesh's drinking water. Thus, mitigation efforts should be improved by evaluating the health risks of other toxic elements in drinking water. In addition, the water treatment measures should not be limited to As only which does not ensure safe water in terms of public health policy but also need to be further evaluated for removal of Mn, U, Pb, Ni, Cr, etc.

12.6 Conclusions

It is evident that health consequences due to toxicity of heavy metals in drinking water affect millions of people and need urgent action. Public health is under serious threat due to widespread water poisoning in Bangladesh which is thought to be the worst contamination problem in the world. Ninety-seven percent of the country's population use groundwater for household purposes, as surface water is managed badly. Different research findings reported about the toxicity and its impact on health due to some emerging contaminants (Mn, Pb, Ni, Cr, U, etc.) along with As contamination in drinking water. The Government of Bangladesh has adopted some water management strategies to achieve national and international targets including safe water availability and its sustainable management for all. Supply of safe water that has acceptable levels of AS, Mn, Pd, Ni, Cr, and other toxic elements needs to be ensured to protect public health in Bangladesh. Existing water-related strategies need to be studied to identify policy gaps, and necessary actions need to be taken accordingly to meet sustainable development goals.

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Chapter 13
Bacterial Conversion of Waste Products into Degradable Plastics: An Inexpensive Yet Eco-friendly Approach

Naima Khan and Nazia Jamil

Abstract In a recent scenario bio-plastics are expected to change the image of the petroleum-based plastics as they are presenting solutions for waste problems and contributing the environment sustainability. Bio-plastics are intracellular polymers that are synthesized by the bacteria in the situations where there is nutrient depletion of some essential elements. Due to their biodegradability and environment-friendly nature, bio-plastics have outshined the conventional petrochemical-based plastics and, hence, have captured the attention of both the researchers and the industrialists since the last few decades. The only drawback of these bio-plastics is the cost of their production, but the researchers in the recent years have been successful in producing different bio-plastics from the different waste materials such as agro-industrial wastes and activated sludge. In this way the production of bio-plastics acts in two ways: firstly, they can terminate the use of non-biodegradable petrochemical plastics, and, secondly, by their production we can transform the different hazardous wastes into bio-plastics. Industrial, agriculture and dairy wastes are rich in carbon content. This chapter provides detailed information about classification waste materials to be used as carbon sources for different metabolic pathways. It also deals with different techniques to convert these wastes to produce bio-plastics using indigenous bacteria.

Keywords Plastics · Waste products · Bacterial conversion · Indigenous bacteria · Environment friendly approach

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13.1 Introduction

Petrochemical-based plastics are used widely since 1967 when chemical engineers developed cheap methods to convert petroleum to strands of plastics. Since then petrochemical industry has flourished making plastic bottles to Barbie dolls. Approximately 6.3 billion tons of waste plastic has been produced, and only 9% has been recycled and 12% incinerated; the rest has been dumped in landfills and natural environment since the 1950s. Ten percent of waste discarded each day – 3.6 million ton – include plastic. Polluted air because of this waste kills 7 million people each year. Different countries such as Bangladesh, France, and Rwanda have fully banned plastic bags, and others such as China, Britain, and Germany have minimized its production (Economist 2018; Jambeck et al. 2015). A great deal of research has emphasized on the minimization of plastic, and many policies have been made to address this environment-degrading issue (Wu et al. 2009).

An alternative to conventional plastics is polyhydroxyalkanoates (PHA) that are microbial polyesters which make them biocompatible within the living systems and nontoxic. PHAs are stiff (Zou et al. 2017), elastic (Wecker et al. 2015), crystalline (Zhila and Shishatskaya 2018), and biodegradable; these properties can be tuned by carefully selecting monomeric composition, bacterial production strain, substrates, and pre- and post-synthetic processing. These properties and many others make them suitable replacement for petrochemical polymers (Koller et al. 2017).

Hydroxybutyric acids were first discovered in 1923 by Lemoigne at the Pasteur Institute; he demonstrated formation of 3-hydroxybutyric acid by aerobic spore-forming *Bacillus* in anaerobic suspensions. He further investigated and successfully estimated the quantity of the polymer formed. Finally, in 1927, he extracted the polymer of 3-hydroxybutyric acid from *Bacillus* using chloroform (Lemoigne 1927). However, the commercial-scale production of poly(3-hydroxybutyrate), P(3HB), was not practiced until the early 1960s. Baptist and Werber at W.R. Grace and Company (USA) started producing it on commercial scale and earned many patents. They used this polymer to produce sutures and prosthetic devices for the first time. However, their efforts had to be terminated due to high fermentation and extraction cost (Baptist and Werber 1963; Baptist and Werber 1965). The advantage PHAs possess over other biodegradable polymers is their ability to degrade under both aerobic and anaerobic conditions.

The degradation can also be done by thermal or enzymatic hydrolysis. In a biological system such as animal tissues, PHAs can be hydrolyzed enzymatically or nonenzymatically as well as by microbial depolymerases (Philip et al. 2007). The biodegradability of a polymer depends on its physical and chemical properties. Low molecular weight PHAs are more susceptible to biodegradation. High melting point decreases their biodegradation potential (Philip et al. 2007). Mochizuki and Hirami (1997) suggested that polymer biodegradation depends on the chemical organization of such functional groups and hydrophilicity-hydrophobicity balance of the polymer and also on highly systematic structures mainly crystalline, orientation, and morphological properties (Mochizuki and Hirami 1997). The microbial population and the temperature also increase biodegradability in the given environment (Tokiwa and Calabia 2004).

13.2 Plastic Waste Generation and Contribution in Land Pollution

It wouldn't be wrong if we claim that sustainable environment is the key to a sustainable planet. Any development without considering its impact on the environment will be a house built on sand. With the development of cheap strategy to synthesize plastics in the early 1900s, the industry went overboard with its overuse. Plastics were supposed to be a boon to the mankind, but instead their overuse proved to be a havoc to the environment's sustainability. In 2016 global plastic production was 335 million metric tons (Portal 2018). According to an estimate, 8 million tons of plastic is dumped in oceans. East Asian countries are found to be producing this waste at a faster rate than any other country. Bottle caps, plastic bags, straws, containers, and bottles all ultimately end up into the oceans endangering the life of its inhibitors. Marine organisms have been found with stomach full of plastics including straws. These plastics are mostly non-biodegradable, and some plastics take up to 400 years to partly degrade (Acharya 2018). In 2017 the USA recycled only 9.5% of 33.6 million tons of plastic produced (Leung 2018). About 300 million tons of plastic are produced around the globe per year, while only 7% of it is recycled, and the rest is dumped into landfills and oceans which take decades to break down. Incineration of plastic waste produce escalated levels of carbon as it is derived from petroleum and natural gas (Pathak et al. 2014). To ensure a sustainable planet, we need to control the plastic consumption and look for alternative, and here bio-plastics come handy.

13.3 Bacterial Production of Plastics

PHAs were first observed by Beijerinck as refractile bodies inside bacterial cells in 1888 (Shrivastav et al. 2013). In 1926 Maurice Lemoigne discovered that the bacteria produce bio-plastics. He isolated polyhydroxybutyrate (PHB) from *Bacillus megaterium*. Bio-plastics are eco-friendly as they are biocompatible and biodegradable.

Efforts have been made to increase cost-effective production of polyhydroxyalkanoates including the use of cheap carbon sources, indigenous wild-type bacteria, genetically modified bacteria, and mixed microbial cultures. Nowadays extremophiles seem to be the ultimate choice as they particularly reduce sterilization cost and can be cultivated in open cultures.

A number of bacteria both Gram-negative and Gram-positive such as *Pseudomonas*, *Bacillus*, *Ralstonia*, *Aeromonas*, and *Rhodobacter* and certain Archaea, especially members of the *Halobacteriaceae* like *Haloferax sulfurifontis*, synthesize polyhydroxyalkanoates (Anderson and Dawes 1990). Marine environment is supposedly a "high-nutrient econiche" so marine bacteria and archaea synthesize PHAs that have remarkable commercial potential. These organisms accumulate up to 80% dry cell weight PHAs (Philip et al. 2007). This displays that PHA-producing microbes are widespread in the environment.

13.3.1 Characteristics of Bio-plastics

Polyhydroxyalkanoates (PHAs) belongs to a family of linear polyesters of three, four, five, and six hydroxy acids (Ibrahim et al. 2017), being synthesized by various bacteria (Marcos-García et al. 2017) through the fermentation of sugars (Albuquerque et al. 2007), lipids (Burniol-Figols et al. 2018), alkanes, alkenes (Jiang et al. 2016), and alkanoic acids (Lemos et al. 2006). Bio-plastics are found as intracellular granules. These inclusions can be extracted and exhibit a number of properties such as PHAs which are thermoplastic (Bugnicourt et al. 2014) and elastomeric (Larrañaga et al. 2016). PHAs can be recycled, are biomaterials, and are very easy to completely degrade into carbon dioxide and water (Sridewi et al. 2006). Bio-plastics are easy to process and are biodegradable. All these properties make bio-plastics an excellent replacement for petrochemical; in addition, the biocompatibility of biopolymers makes them very promising candidates for medical applications (Devaraj 2017). Hydrolysis of PHAs yields R-hydroxy alkanoic acids, which can be widely used as chiral starting materials in pharmaceutical, chemical, and medical industries (Pilla 2011).

Polyhydroxyalkanoates (PHAs) are often divided into three groups. This classification is based on the number of constituent carbon atoms in their monomers – short-chain-length (SCL) PHAs (3–5 carbon atoms) and medium-chain-length (MCL) PHAs (6–14 carbon atoms) and long chain polymers having more than 14 carbon atoms in their monomer units (Ong et al. 2018).

13.3.2 Metabolic Pathways Involved in Bio-plastic Production

The chemical structure and nature of the PHA can be controlled and manipulated by incorporating various monomers into the polymer, and this can be achieved by supplementing the growth media with related monomer precursor. These precursors

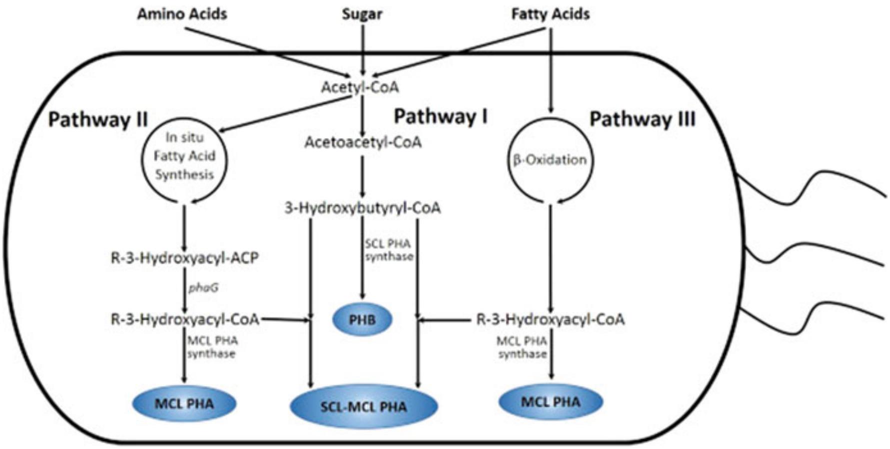

Fig. 13.1 General metabolic pathways for PHA synthesis. (Chen et al. 2016)

are various fatty acid derivatives that can be changed into PHA monomers through the organism's indigenous metabolic pathway such as *β*-oxidation. The *β*-oxidation pathway catabolizes fatty acids to produce energy from the respiratory electron transport chain. Besides *β*-oxidation there are various other metabolic pathways in an organism which can be diverted toward the synthesis of the PHA. The route of the PHA synthesis depends upon the substrate provided. In case of sugars, acetyl-CoA from pyruvic acid is converted into 3-hydroxybutyryl-CoA, which is then converted into SCL PHA. Sometimes bacteria go for in situ fatty acid synthesis which is more or less similar to the other pathways physiologically shown in Fig. 13.1.

13.3.2.1 Genetic Factors Controlling Bio-plastic Production

Poly(3-hydroxybutyrate) or PHB is the most common PHA; PHB synthesis operon phaCAB was first cloned from *Ralstonia eutropha* consisting of three genes including β-ketothiolase (PhaA), NADPH-dependent acetoacetyl-CoA reductase (PhaB), and PHB synthase (PhaC). PhaC is the most important enzyme in the PHB synthesis flux as shown in Fig. 13.2 (Li et al. 2017).

13.3.3 Halophiles Dominating the Bio-plastic Industry

Halophilic bacteria can be grown in extreme conditions including high concentration of salts which other bacteria are unable to withstand. Such organisms can even be cultivated in unsterile conditions under selective growth conditions. Due to high salt

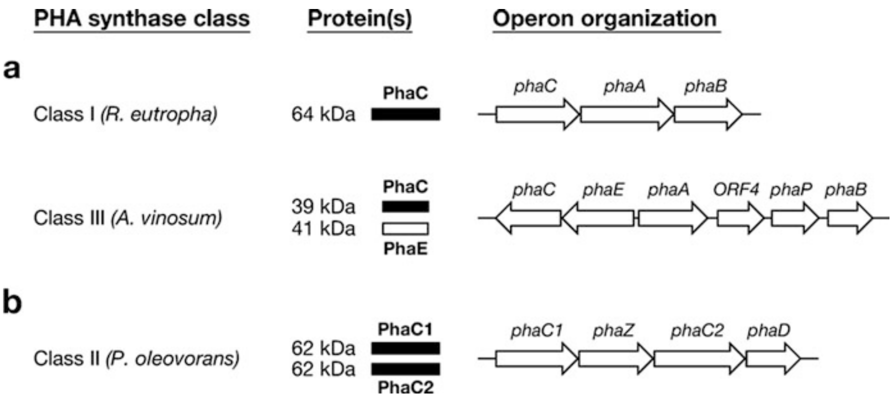

Fig. 13.2 Various operon systems involved in PHA synthesis in different organisms. (Stubbe et al. 2005)

concentration, the dominant population will be halophiles so there is no need to waste resources on providing sterile conditions. The continuous supply of carbon sources under other nutrient-nitrogen and phosphorous acts as a trigger for bacteria to accumulate PHA. The PHAs formed by halophilic bacteria are thought to be more heat resistant and thermoplastic. According to Quillaguaman a moderate halophile *Halomonas boliviensis* can accumulate PHA up to 80% of its dry cell weight when supplied with sodium acetate and butyric acid as carbon source (Quillaguaman et al. 2005). *Haloarcula* sp. was found to produce 63% PHB of cell dry weight (Taran and Amirkhani 2010). The genes involved in PHA synthesis are identified as *phaE* and *phaC* and many others. Legat et al. (2010) have identified almost 20 halophilic strains with the potential to produce PHA and related polymers. More recently halophiles are considered as choice organisms due to their low maintenance cost (Yin et al. 2015); in the future halophiles might take over the biotech industry due to this very significant property.

13.4 Waste Carbon Sources for Bio-plastic Production

Various waste carbon sources are being utilized for the production of PHA as bacteria and other microorganisms have the ability to break down these wastes into simple absorbable compounds. Every region produces its specific waste according to its staple food, climate, and other physical parameters. Some general waste includes fatty acid and non-fatty acid wastes. Luckily bacteria can degrade both types of compounds and use them as carbon sources. This approach leads to solve the problem of waste disposal as well as manufacturing of useful products.

13.4.1 Non-fatty Acid Waste Usage in Bio-plastic Production

Non-fatty acid waste usually is of carbohydrate nature and includes various sugars, molasses, food waste, and agricultural waste. All of this sugary waste is broken down to simple sugars and then shunted into the acetyl-CoA pathway where the excess of carbon is converted into useful by-products depending upon the nature of the organism. Various bacteria have the ability to utilize different sugars and convert them into PHA as shown in Table 13.1.

Table 13.1 Molasses, food waste, agricultural waste, and various sugars as carbon sources for PHA production

Bacteria	Carbon source	Polymer %	References
Bacillus spp.	Sucrose	59	Thirumala et al. (2010)
Bacillus spp.	Starch	51	Thirumala et al. (2010)
Enterobacteriaceae bacterium pfw1	Molasses	58	Naheed et al. (2012)
Bacillus cereus 64-ins	Potato starch	64	Ali and Jamil (2014)
Bacillus cereus	Sucrose	50	Naeem et al. (2018)`
Exiguobacterium	Potato starch	9	Iqbal et al. (2016)
Stenotrophomonas	Potato starch	10	Iqbal et al. (2016)
Halomonas boliviensis	*Wheat bran*	50	Van-Thuoc et al. (2008)
Bacillus megaterium	Molasses	46	Gouda et al. (2001)
Burkholderia cepacia ipt 048	*Sugar cane bagasse hydrolysate*	62	Silva et al. (2004)
Bacillus sphaericus	Agro-industrial residues	19	Ramadas et al. (2009)
Mixed microbial culture	Molasses	75	Albuquerque et al. (2010)
Bacillus species	Soy molasses	90	Full et al. (2006)

13.4.2 *Fatty Acid Waste Usage in Bio-plastic Production (Table 13.2)*

Table 13.2 Oil-contaminated water, crude glycerol, and mineral oils as carbon sources for PHA production

Bacteria	Carbon source	Polymer %	References
Azotobacter chroococcum strain h23	Wastewater from olive oil mills	80	Pozo et al. (2002)
Pseudomonas species	Corn oil	36	Chaudhry et al. (2011)
Paracoccus denitrificans	Crude glycerol	48	Mothes et al. (2007)
Cupriavidus necator dsm 545	Waste glycerol	62	Cavalheiro et al. (2009)
Burkholderia cepacia atcc 17759	Glycerol	31	Zhu et al. (2010)
Bacillus megaterium	Glycerol	60	Naranjo et al. (2013)
Zobellella denitrificans mw1	Glycerol	85	Ibrahim and Steinbüchel (2009)
Pseudomonas aeruginosa 47 t2	Waste cooking oil	36	Haba et al. (2007)

13.4.3 *Wastewater as Carbon Source in Bio-plastic Production (Table 13.3)*

Table 13.3 Wastewater and activated sludge as carbon source for PHA production

Organisms	Carbon source	PHA (%)	References
Pseudomonas sm01 (kf270348)	Industrial wastewater (100%)	45	Munir and Jamil (2015)
Mixed culture	Activated sludge	30	Chua et al. (2003)
Exiguobacterium	Wastewater	48	Iqbal et al. (2016)
Stenotrophomonas	Wastewater	36	Iqbal et al. (2016)
Mixed culture	Activated sludge	48	Bengtsson et al. (2008)
Mixed culture	Activated sludge	31	Dionisi et al. (2004)
Mixed culture	Activated sludge	78	Serafim et al. (2004)
Mixed culture	Activated sludge (aerobic)	29	Rodgers and Wu (2010)
Mixed culture	Activated sludge (anaerobic)	50	Rodgers and Wu (2010)
Mixed culture	Activated sludge	57	Mengmeng et al. (2009)
Enterobacter aerogenes	Sewerage water	96	Ceyhan and Ozdemir (2011)
Mixed culture	Dark fermented paperboard mill wastewater	67	Farghaly et al. (2017)

13.5 Biodegradation of Bio-plastics

Biodegradation have been tested in both natural and artificial environments such as soil (Boyandin et al. 2012) marine environment (Volova et al. 2010), fungal degradation (Matavulj and Molitoris 1992), in wastewater (Lee and Choi 1999), clay organomodifiers, surfactants (Bordes et al. 2009) catalytic and enzymatic degradation, etc. Degradation of PHA or related polymers is thought to be influenced by its chemical nature, temperature, moisture content, and microbial community of the soil (Boyandin et al. 2012). Prokaryotic and eukaryotic organisms can degrade PHAs as they produce extracellular PHA depolymerases that break down the polymer into water-soluble components. More than 80 depolymerases of prokaryotic and eukaryotic origin have been identified and purified (Kim et al. 2007). The dominant degrading microorganism identified genera are *Variovorax*, *Stenotrophomonas*, *Acinetobacter*, *Pseudomonas*, *Bacillus*, and *Xanthomonas* and as micromycetes from *Penicillium*, *Paecilomyces*, *Acremonium*, *Verticillium*, and *Zygosporium*. Boyandin et al. (2012) *Enterobacter* sp. (four strains), *Bacillus* sp., and *Gracilibacillus* sp. (Volova et al. 2010) genera *Burkholderia*, *Bacillus*, *Cupriavidus*, *Mycobacterium*, and *Nocardiopsis* and such micromycetes as *Acremonium*, *Gongronella*, *Paecilomyces*, and *Penicillium*, *Trichoderma* (Boyandin et al. 2013). The advantage PHAs possess over other biodegradable polymers is their ability to degrade under both aerobic and anaerobic conditions. The degradation can also be done by thermal or enzymatic hydrolysis. In biological systems such as animal tissues, PHAs can be hydrolyzed enzymatically or nonenzymatically as well as by microbial depolymerases (Philip et al. 2007). The biodegradability of a polymer depends on its physical and chemical properties. Low molecular weight PHAs are more susceptible to biodegradation. High melting point decreases their biodegradation potential (Philip et al. 2007). Mochizuki and Hirami explained that biodegradation of solid polymers is influenced by chemical structure (especially functional groups and hydrophilicity-hydrophobicity balance) and highly ordered structures (mainly crystallinity, orientation, and morphological properties) (Mochizuki and Hirami 1997). The microbial population and the temperature also increase biodegradability in the given environment (Tokiwa and Calabia 2004).

13.6 Medical and Industrial Importance of Bio-plastics

PHAs are biodegradable and can be inserted into the human body without any danger, and there is no need to remove them. Their biocompatible nature also makes them suitable for medical purposes as they elicit minimal foreign body response. PHAs are hydrophobic and can be converted into films, porous matrices, microcapsules, microspheres, and nanoparticles (Shrivastav et al. 2013). Extensive research is being done on PHAs to make them suitable for manufacturing of absorbable monofilaments sutures, multifilament sutures, and extrusion lines for fibers, film, and tubing. In 2007, TEPHA polymer was established, and they are already producing absorbable sutures with high tensile strength and strength retention (Inc 2018). PHA-based nano-vehicles as therapeutic delivery carriers, including nanoparticles, micelles, liposomes, and vesicles, have received considerable attention in recent years, and these sophisticated materials have demonstrated significant impact on the drug bioavailability, better encapsulation, and less toxic properties of biodegradable polymers (Li and Loh 2017).

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Chapter 14
Treatment of Leather Industry Wastewater and Recovery of Valuable Substances to Solve Waste Management Problem in Environment

Manish Chandra Kannaujiya, Tamal Mandal, Dalia Dasgupta Mandal, and Monoj Kumar Mondal

Abstract Every leather industry produces a large quantity of wastewater having enormous amount of pollutants which have a deleterious effect on the environment drastically if it is discharged without appropriate treatment. The tanning processes are responsible for the discharge of uncontrolled COD, BOD, TDS, Cr (III), chlorides, sulphates, and other heavy metals. This study provides a review of the treatment of leather wastewater involving physical treatment, electrocoagulation, adsorption, chemical precipitation, ozonation, electro-oxidation, photocatalytic ozonation, Fenton oxidation, biochemical/biological treatment, and hybrid treatment processes. It is clear from the study of some other papers that chemical or biological treatment followed by membrane separation has the potential capability to remove the harmful contaminants from tannery wastewater. Researchers also reported that the tannery industrial solid waste used after removal of chromium has a good amount of minerals (P and K) which can be further used for several purposes including as fertiliser.

Keywords Leather industry wastewater · Physico-chemical · Biological · Integrated treatment · Value-added products

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14.1 Introduction

Tannery wastewater is characterised by high contents of high organic and inorganic pollutants imposing high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) load together with high chromium and dark colour. Other parameters include total dissolved solid (TDS), total suspended solids (TSS), total Kjeldahl nitrogen (TKN), collagen content, oil and grease, etc. Characteristics of tannery wastewater vary depending on the tanning process and the amount of water used (Apaydin et al. 2009). Tannery wastewaters are basic in nature, due to the presence of high amount of organic substances and chemicals used (Kongjao et al. 2008). The high organic content of tannery wastewater can cause various environmental problems (Leta et al. 2004). The physico-chemical characteristic of the wastewater is shown in Table 14.1.

Effluents are characterized by high amounts of pollutants as indicated by the values of pH, COD, etc. due to the chemicals used in leather processing. Today, in India, there are more than 2500 tanneries with 0.7 million tonnes of annual processing capacity of hides and skins, and the country produces the largest amount of leather in the world (Rajamani et al. 1995). About 80% of these tanneries use the chrome-tanning process. Indian tanneries discharge about 50,000 m^3/day of effluents which contain a high concentration of organic pollutants (Murugesan and Elangovan

Table 14.1 Physico-chemical characteristics of leather industry wastewater

pH	TDS^a	SS^a	COD^a	BOD$_5$^{a}	Total Cr^a	Sulphide^a	References
7.4	NA	2690	3700	1470	NA	440	Apaydin et al. (2009)
7–8.7	13,300; 19,700	600–955	4100–6700	630–975	11.5–14.3	NA	Kongjao et al. (2008)
10.7	6810	NA	11,153	2906	32.87	507	Leta et al. (2004)
6.8	6700	1300	3200	1250	40	NA	Babu et al. (2007)
7.2	NA	NA	2810	910	62	89	Kurt et al. (2007)
10.5	17,737	1147	3114	1126	83	55	Ram et al. (1999)
8–9	NA	2500–3000	5000–5500	3000–3500	80–100	NA	Roger et al. (2007)
NA	15,152	2004	8000	930	11.2	228	Koteswari and Ramanibai (2003)
7.5	NA	NA	5000–10,000	1500–2000	100	NA	Song et al. (2000)
7.9	21,620	1244	2533	977	258	860	Mandal et al. (2010a, b)
7.08	NA	2820 ± 350	4800	NA	90–100	NA	Ganesh et al. (2006)
8.5	8500	1690	5680	759	521	185	Bhattacharya et al. (2013)

^{a}(mg/L)

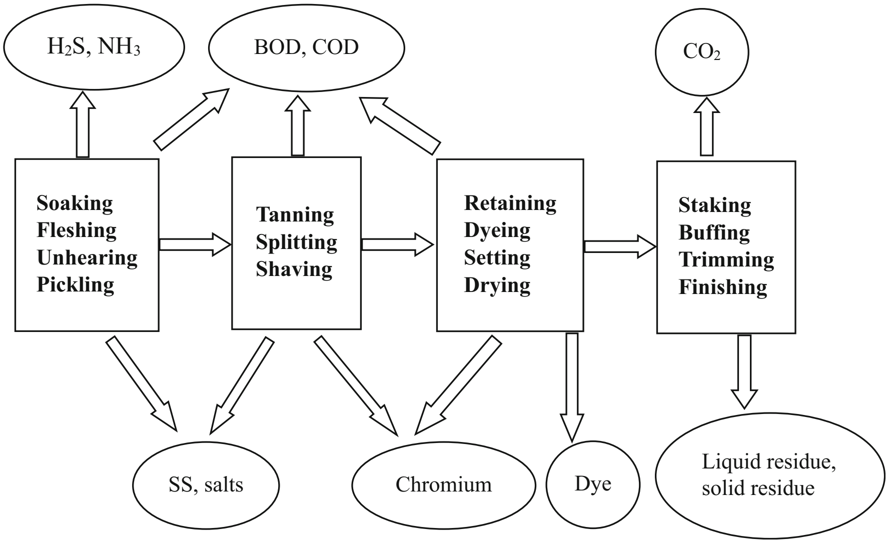

Fig. 14.1 Waste generation in leather industry from different processes

1994). Tannery wastewater is specially considered detrimental for its high amount of organic load and the presence of recalcitrant and poorly decomposable compounds such as tannins. Many techniques have been developed for the treatment of tannery wastewater, namely, physical, chemical, biochemical, membrane technology, or a combination of these technologies. The presence of sulphide in tannery effluent is attributed to beamhouse operations. Figure 14.1 clearly indicates the waste generation from the leather industry through different processes as (Sundar et al. 2011).

Chromium compounds are most commonly used for processing of hides in the tanning industry (Sreeram and Ramasami 2003). In this process, about 60–70% of chromium reacts with the hides. In leather processing, 850 kg of leather solid waste is generated, where per tonne of raw hide produces 150 kg of leather; the remaining waste can be utilised as some by-products such as in biodiesel and alkaline protease production (Ahmad and Ansari 2013). Chromium is found in nature in two major states [Cr (VI) and Cr (III)]; hexavalent chromium is more toxic than trivalent, and it induces acute and chronic toxicity, genotoxicity, neurotoxicity, immunotoxicity, and some other environmental toxicity and has shown mutagenic effects in the microbial system (Bagchi et al. 2002; Nishioka 1975; Petrilli and De 1977). The interchangeable form of chromium from one to another state depends on the pH and redox potential of the medium. Cr (VI) can be reduced biologically in the presence of iron and sulphides (Rai et al. 1989). Chromium is not biodegradable and tends to accumulate in living organisms with a potential carcinogenic effect, causing serious diseases and disorders (Rai et al. 1989; Bailey et al. 1999; Lee et al. 1995; Low et al. 1999). Figure 14.2 shows a typical flowsheet for the leather manufacturing process.

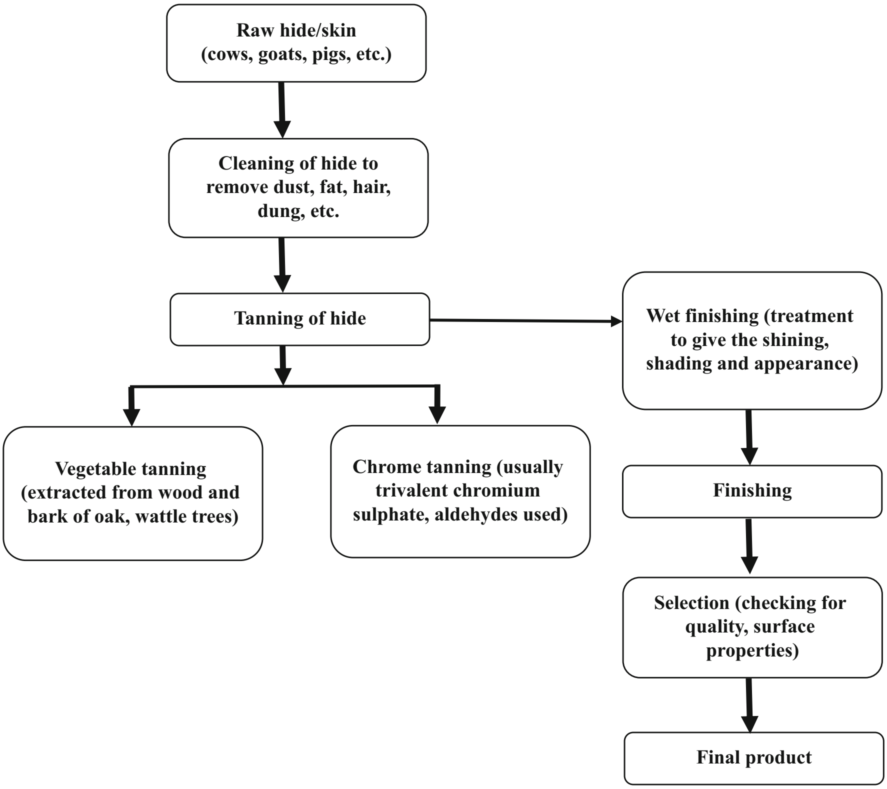

Fig. 14.2 Schematic of leather manufacturing process

14.2 Tannery Wastewater Treatment

Apart from the conventional treatment processes, there are some other technologies used, such as coagulation-flocculation (Ros and Gantar 1998; Ayoub et al. 2000; Haydar and Aziz 2009), ion exchange (Tiravanti et al. 1997), adsorption (Tahir and Naseem 2007), electrochemical treatment (Szpyrkowicz et al. 2005), biological treatment (Ros and Gantar 1998; Vijayraghavan and Murhy 1997), etc. Combined chemical/biological processes (Ros and Gantar 1998; Song et al. 2000, 2001, 2002, 2004; Iaconi et al. 2002; Ryu et al. 2007; Mandal et al. 2010a, b) are gaining interest to the industrialists for tannery wastewater treatment. The maximum permissible limit as prescribed by the Indian standard for inland surface water in the environment is given in Table 14.2.

Based on the physical, chemical, advanced oxidation, biological, and membrane processes, new concepts have been developed to treat effluents efficiently and economically.

Table 14.2 Indian standard for inland surface water in environment

Parameters	Unit	Standard
pH	NA	6–9
COD	$mg.L^{-1}$	250
BOD_3	$mg.L^{-1}$	30 (3 days, 27 °C)
TSS	$mg.L^{-1}$	100
Ammonia-N	$mg.L^{-1}$	15
Sulphide	$mg.L^{-1}$	2.0
Total chromium	$mg.L^{-1}$	2.0

14.2.1 Physical Treatment

Physical methods involve screening, sedimentation, aeration, filtration, etc., where no gross chemical or biological conversions are carried out, and strictly physical phenomena are imposed to treat wastewater or decrease the pollution load. Coarse screening is a typical phenomenon where larger objects are removed. In the sedimentation process, solids settle down by gravity. Usually, this consists of simply holding wastewater for a short period of time in a tank under suitable conditions, allowing the heavier solids to settle and separating it from the effluent (clarification). Sedimentation for solid separation is a very common unit operation and is routinely employed at the beginning and end of wastewater treatment operations. Other physical methods used in treatment consists of filtration. Oils and greases are removed by froth flotation methods by permitting them to float on the surface and removing them in the form of scums. The physical (primary) treatment methods are considered to be essential because of the huge amount of suspended solids coming from the leather processing step in tanning. Mixing the acid and alkaline waste at a controlled pH will result in coagulation of suspended solids.

14.2.2 Electrocoagulation Process

Electrocoagulation is a complex and interdependent process where a metal anode is used to produce a coagulating agent to treat the polluted water, subsequently producing electrolytic gases (mainly hydrogen at the cathode). Knowledge of electrochemistry, coagulation phenomena, and hydrodynamics are the bases of electrocoagulation (Holt et al. 2002). In the electrocoagulation process, aluminium and iron, sometimes steel electrode materials, are used. The metal ions, at an appropriate pH value, can widely range some of the coagulating agents and metal hydroxide that can be destabilized to aggregate the suspended particles which absorb dissolved contaminants (Daneshvar et al., 2006). The electrocoagulation process involves three stages: (1) formation of coagulants by electrolytic oxidation of the electrode, (2) destabilisation of contaminants, and (3) particulate suspension and breaking of emulsions.

Electrocoagulation was found to be effective to treat dye (Kim et al. 2002; Gurses et al. 2002; Kobya et al. 2006a), arsenic (Kumar et al. 2004), phosphate (Bektas et al. 2004), electroplating (Adhoum et al. 2004), metal finishing (Khelifa et al. 2005), poultry slaughterhouse (Kobya et al. 2006b), and textile industry wastewater (Kobya et al. 2003). Jing-wei et al. (2007) showed the removal rates of different pollutants as follows: COD 68%, NH_4-N^+ 43.1%, OC (organic carbon) 55.1%, sulphide 96.7%, and colour of wastewater 84.3%. Ayhan et al. (2009) have shown the removal efficiency of oil and grease (96%) and sulphide (82%) from tannery liming drum wastewater using direct current (DC) electrocoagulation (EC) at 35 mA/cm^2 at pH 3 for 10 min. For optimum treatment condition for tannery, wastewater was found at a current density 22.4 Am^{-2} with 3.67 L/min of flowrate in the reactor for 20 min electrolysis time at initial pH 7–9. In this condition, approximately 95% of COD, 96% BOD, 100% chromium, 96% TSS, 62% TKN, 50% TDS, and 99% oil-grease were removed (Sangkorn et al., 2007).

14.2.3 Adsorption

Adsorption is a process in which soluble substances adhere on suitable surfaces. Adsorbent-based treated wastewater was followed with biological treatment for soluble organic matters. Based on their size, activated carbon may be of two types, namely, granulated charcoal with a diameter greater than 0.1 mm and powder charcoal with the particle size less than 200 mesh (Metcalf and Eddy 1991). Further, the efficiency of adsorption process depends on the pH, particle size, and adsorbent dose (Fahim et al. 2006). Adsorption-based Cr (VI) removal using activated carbon has been done by different researchers (Huang and Wu 1975, 1977; Alaerts et al. 1989). The pH is the most influencing factor for adsorption of Cr (VI) using biomass-based cheap adsorbents like the bark of neem (*Azadirachta indica*) and subabul (*Leucaena leucocephala*).

The removal of Cr (VI) has been compared, showing a maximum removal at pH 2 using powdered activated charcoal and *Leucaena leucocephala* charcoal and pH 6 using *Azadirachta indica* charcoal (Kulkarni and Shrivastava 2002). Kumar et al. (2002) used GNSP in batch and anaerobic baffled reactor for reduction of chromium and COD and achieved 99.9 and 93%, respectively, at pH 7.7. Other biomass-based adsorbents such as wheat straw dust, sawdust, and coconut jute have been used for Cr (VI) removal and were compared with PAC, showing a maximum removal at pH 2.0 for PAC and pH 6.0 for other adsorbents (Rao and Bhola 2000). CTLSs have been used as low-cost adsorbents for the removal of Cr (VI), As (V), dyes, and surfactants from textiles effluents by many researchers (Na et al. 2006; Oliveira et al. 2007, 2008a; Sreeram et al. 2004). In 2016, Rosales et al. worked on the removal of leather dye and Cr (VI) with grapefruit peelings as a biosorbent, and achieved 45 and 55% removal of dyes and Cr (VI) mixture when the biosorbent was pretreated with 1 M H_2O_2 solution removal capacity of dyes (80%) and Cr (VI) (100%). Table 14.3 shows the physical properties of activated carbon used in the treatment process.

Table 14.3 Physical characteristics of activated carbon

BET surface area (m^2/g)	Total pore volume (cm^3/g)	References
793	0.49	Baccar et al. (2013)
1.18 ± 0.11	NA	Gomes et al. (2016)
677.9	NA	Amalraj and Pius (2014)
493.50	0.350	Kong et al. (2013a)
638.57	NA	Kong et al. (2013b)
355.9	NA	Marsal et al. (2012)
10.422	0.0508	Anand Kumar and Mandal (2011)
10.17	0.0167	Rosero et al. (2016)
491.0	0.440	Rosero et al. (2016)

Different studies have reported that CTLSs are effective in removing oils and hydrocarbon in contaminated wastewater due to high porosity and high buoyancy of fibres with low density (Sreeram et al. 2004; Tahiri et al. 2003; Saravanabhavan et al. 2004, 2007; Piccin et al. 2012; Gammoun et al. 2007b). Further, CTLS has been tested for vegetable tannin removal from mixed effluents and hazardous chromate and arsenate removal as ecological agents (Saravanabhavan et al. 2007; Przepiorkowska et al. 2003; Gammoun et al. 2007a, b). Huang et al. (2009) reported that CTLS is used as supporting matrix for Fe (III) and Al (III) to prepare low-cost adsorbent. Chestnut and mimosa tannins immobilised CTLS-based adsorbent used for Cr (VI) removal from wastewater (Chabaane et al. 2011). The efficiency of CTLS for the absorbance of VOC in LDPE tubes has been studied by Sanjuán et al. (2012). Further, Jiao et al. (2009) have studied the CTLS doped with Zr (VI)-based adsorbent for the removal of As (V) from aqueous solution. The use of CTLS as adsorbent and its potential application in the removal of phenol, methylene blue, and Cr (VI) from different wastewater have been studied by Kantarli and Yanikm (2010), Oliveira et al. (2008a, b, c), and Kong et al. (2013a, b), respectively.

The solid waste obtained after vegetable tanning process could be used for the preparation of low-cost adsorbents and for the successful removal of Cr (VI) and rhodamine B from simulated water and is a suitable alternative for commercial adsorbent for the removal of Cr (VI) and dyes from tannery wastewater (Anandkumar and Mandal 2011). Sometimes solid waste like chromium tanned leather waste (CTLW) was used for the removal of Acid Red 357 (AR 357) (148.2 mg/g) and dye (58%) (Piccin et al. 2016).

Chrome shavings (CS), a tannery waste which was used for the removal of AB 45 dye, showed the highest removal efficiency at 92.93%, with adsorbent dosage of 2.64 g/L, pH 0.98, and initial dye concentration 234.09 mg/L. Thus, low-cost CS can be considered as an adsorbent for AB 45 removal (Arthy and Saravanakumar 2013). Other solid wastes such as acid-treated microwave carbon tannery sludge (AMWCTS) are used as adsorbent for the removal of different leather dyes (Acid Black 210,1108 mg/g, and Acid Red 357589.5 mg/g) (Rosero et al. 2016). Adsorbent from olive-waste cake has the potential as the adsorbent to be 146.31 mg g^{-1} at 25 °C (Baccar et al. 2013).

Table 14.4 Isotherm parameters for adsorption onto leather industrial waste activated carbon

Pollutants	Temperature (K)	Isotherm parameter Langmuir K_L (L/mg)	 Freundlich K_F	References
AB 45	303	0.214	3.39	Arthy and Saravanakumar (2013)
Cr (VI)	323	0.1126	74.2489	Anand Kumar and Mandal (2011)
RB	323	0.150	87.4380	Anand Kumar and Mandal (2011)
B D TTO	298	0.025	NA	Baccar et al. (2013)
AB 210	298	0.01622	101.8	Rosero et al. (2016)
AR 357	298	0.06256	231.5	Rosero et al. (2016)
Cr (VI)	298	0.002	0.452	Oliveira et al. (2008b)
As (V)	298	0.253	8.722	Oliveira et al. (2008b)
RB	303	0.001931	31.960	Amalraj and Pius (2014)
MB	303	0.00008	11.931	Amalraj and Pius (2014)
MG	303	0.00004	8.806	Amalraj and Pius (2014)

Solid waste from leather industry can be used as an adsorbent for the removal of Cr (VI) (133 mg g^{-1}) and As (V) (26 mg g^{-1}) and could be a better replacement for commercial adsorbents in treating leather industry wastewater (Oliveira et al. 2007, 2008a, b, c, d). Solid waste generated from leather industry like chrome-tanned leather shavings could be used as an adsorbent (Pati et al. 2014) which has an adsorption ability on methylene blue (80 mg g^{-1}) and textile dyes (163 mg g^{-1}). Table 14.4 represents the adsorption kinetics; in most of the cases, adsorption onto leather industrial waste is represented by Langmuir and Freundlich isotherm. The adsorption of pollutants from leather industrial wastewater follows the Langmuir, Freundlich, and Temkin isotherm.

The Langmuir isotherm is given by Eq. (14.1):

$$\frac{C_e}{q_e} = \frac{1}{K_L q_m} + \frac{C_e}{q_m} \tag{14.1}$$

where K_L is the Langmuir constant (L/mg) and Ce concentration of adsorbate at equilibrium (mg/l) in the solution. Equations (14.2) and (14.3) represent the Freundlich and the Temkin isotherm of adsorption:

$$\log q_e = \log K_F + \left(\frac{1}{n}\right) \log C_e \tag{14.2}$$

$$q_e = \frac{\mathrm{RT}}{b} \ln\left(\mathrm{AC}_e\right) \tag{14.3}$$

where K_F is the Freundlich constant [(mg/g).(L/g)], R universal gas constant, b is constant, T temperature in Kelvin, ΔE (−ΔH) variation of adsorption energy (J mol^{-1}), and A Temkin constant (L mg^{-1}) corresponding to the maximum adsorption capacity (q_{max}) and maximum binding energy (Hamdaoui and Naffrechoux 2007; Kundu and Gupta 2006).

Equations (14.4) and (14.5) represent the linear form of pseudo-first-order and pseudo-second-order kinetics of adsorption:

$$\ln\left(q_e - q_t\right) = \ln q_e - k_1 t \tag{14.4}$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_{e2}} + \frac{1}{q_e} t \tag{14.5}$$

where k_1 and k_2 are the rate constants [$min^{-1}$and g(mg^{-1} min^{-1})] of pseudo-first order and pseudo-second order, respectively and qe and qt are the equilibrium and time-dependent capacity (mg g^{-1}) of adsorption. Table 14.5 shows the kinetic data available in the literature. Parameters such as Gibbs free energy, enthalpy, and entropy of pollutants in the adsorption have been shown in Table 14.6 and can be calculated by considering the equilibrium constants at different temperatures (Mall et al. 2006); free energy (ΔG°) can be calculated using the following Eqs. (14.6–14.8):

$$\Delta G^0 = -\mathrm{RT} \ln K \tag{14.6}$$

$$K = \frac{q_e}{C_e} \tag{14.7}$$

$$\Delta G^0 = \varDelta H^0 - T\varDelta S^0 \tag{14.8}$$

14.2.4 Chemical Treatment

Chemical processes involved in wastewater treatment are based on chemical reactions to bring about some change in the constituents of the pollutant by means of chemical reactions. In general, chemical methods are accompanied by an inherent disadvantage compared to physical methods. In other words, there is usually a net increase in the dissolved constituents due to sludge which can be a significant factor in the case of wastewater reuse. This section will deal with the main chemical unit processes, including chemical precipitation, advanced oxidation process (AOPs), disinfection, and dechlorination.

Table 14.5 Adsorption kinetic constants for uptake of various pollutants under different operating condition

Pollutants	Kinetic model	Kinetic constant		Initial Concentration (mg/L)	Temperature (K)	References
		q_e (mg/g)	K_2 (g/mg/min)			
B D TTO	Pseudo-second order	103.22	0.0007	NA	298	Baccar et al. (2013)
Cr (VI)	Pseudo-second order	96.15	5.16×10^{-4}	100	323	Anand Kumar and Mandal (2011)
RB	Pseudo-second order	93.34	1.25×10^{-4}	100	323	Anand Kumar and Mandal (2011)
AB 210	Pseudo-second order	167.9	2.184×10^{-4}	300	298	–
AB 210	Pseudo-second order	309.4	4.830×10^{-5}	600	298	–
AR 357	Pseudo-second order	189.5	0.001799	300	298	–
AR 357	Pseudo-second order	345.8	2.940×10^{-4}	600	298	–
Yellow 194	Pseudo-second order	174.7	6.42×10^{-4}	NA	NA	Piccin et al. (2012)
Red 357	Pseudo-second order	248.8	6.42×10^{-5}	NA	NA	Piccin et al. (2012)
Black 210	Pseudo-second order	34.6	1.30×10^{-3}	NA	NA	Piccin et al. (2012)
AB 45	Pseudo-second order	52.63	0.0021	NA	323	Arthy and Saravanakumar (2013)

Table 14.6 Thermodynamic parameters of adsorption process

Pollutants	Temperature (K)	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (kJ mol^{-1} K^{-1})	References
RB	303	−0.33	30.95	0.10	Amalraj and Pius (2014)
MB	303	−6.96	45.31	0.12	Amalraj and Pius (2014)
MG	303	−9.36	6.79	0.01	Amalraj and Pius (2014)
AB 45	303	−6.31	11.98	0.06	Arthy and Saravana Kumar (2013)
BD TTO	278	47.503	53.459	178.119	Baccar et al. (2013)

14.2.4.1 Chemical Precipitation

Chemical precipitation is a chemical treatment using coagulating reagent before untreated wastewater degradation, which increases the efficiency of the removal of suspended solids, BOD_5, and phosphorus, compared to sedimentation only without coagulation and flocculation of solids dissolved properly in settable flocs. $Al_2(SO_4)_3$ and $FeCl_3$ were used as a coagulant in the chemical-coagulation process. In the chemical coagulation process, 38–46% SS, 30–37% COD, and 74–99% chromium were removed when the initial concentration of coagulating agent is 12 mg/l. Ferric chloride was found to be more effective than aluminium sulphate as a coagulant. Chromium removal efficiency is greatly affected by wastewater pH and initial chromium concentration. Coagulation followed by centrifugation improves the removal efficiency of suspended solids to about 70%, with a high degree of clarification as indicated by 85–86% colour removal (Song et al. 2004). Ahmed et al. (2016) used *Kitasatospora* sp. combined with chemical-biological treatment system, and observed 98.4% Cr (VI), 77% COD and 81% turbidity, but Sharma and Malaviya (2014) have reported 62.33% total Cr, 71.80%, and 64.69% of turbidity using biological treatment combined with chemical precipitation with the help of *Fusarium chlamydosporium*.

14.2.4.2 Advanced Oxidation Processes (AOPs)

Since wastewater from leather industries are less biodegradable, advanced oxidation processes such as ozonation, electrochemical oxidation, photocatalytic oxidation and Fenton oxidation are used for treatment. AOPs are a very good technology to remove organic compounds from wastewater because the hydroxyl radicals have high capacity to destroy organic compounds. Ozonation, ultraviolet (UV) radiation, hydrogen peroxide (H_2O_2), and some of the catalytic reactions for reduction of residual organic compounds are measured in terms of COD, BOD or TOC (Sharma et al. 2011). The main reaction mechanism of oxidation process is the production of free radicals. Hydroxyl radicals are highly reactive free radicals and act as electrophiles; these properties rapidly react with all electron-rich organic compounds for destroying pollutants present in wastewater. The reduction potential of hydroxyl

Table 14.7 Different advanced oxidation process (AOPs) used for the treatment of leather industrial wastewater

COD (mg/l)	AOPs	Experimental conditions	Experimental output	Reference
2365	Ozonation	pH 3–11, time 60 min, O_3 flow rate 2.6 g O_3/h	17% COD removal, 24% BOD removal	Schrank et al. (2004)
30–360	Ozonation	pH 4–11, time 10–50 min, O_3 dose 1.6 mg/l	Greater than 97% decolourisation at 20 min after treatment	Srinivasan et al. (2009)
2900	Ozonation	O_3 dose 150 mg/l for 60 min	97% COD removal, 96% TSS removal, 91% TKN removal	Iaconi et al. (2010)
2177	Ozonation	pH 3–9, time 10–120 min,O_3 flow rate 1 and 8 g/h	30%–70% COD removal	Houshyar et al. (2012)
2365	Photocatalysis (UV/TiO_2)	pH 3–11, TiO_2 1 g/L, t = 120 min	6% COD removal, 15% BOD removal	Schrank et al. (2004)
200–800	Photocatalysis (UV/TiO_2)	pH 2.5, TiO_2 1 g/L, t = 180 min	66% COD removal, 92% BOD removal	Sauer et al. (2006)
1803	Fenton	$FeSO_4$. $7H_2O$ dose 50–240 (mg/l)	80% COD removal	Schrank et al. (2004)

radical is 2.80 Volt, and its oxidation reaction is faster than other conventional oxidants like H_2O_2, $K_2Cr_2O_7$, or $KMnO_4$. Various advanced oxidation processes to treat leather wastewater are shown in Table 14.7.

14.2.4.2.1 Iron-Catalysed Hydrogen Peroxide as Fenton Reagent

Many metals have ability to transfer oxygen, which increase the utility of hydrogen peroxide by forming more OH• radical, among these iron (e.g. Fe^{+2}) catalyst. Thankappan et al. (Fig. 14.3) observed more than 95% removal of DOC in fixed-bed column study of Fenton oxidation with a combination of granulated activated carbon.

Fenton reagents are very common for the treatment of various industrial effluents having a high range of toxic compounds (BTEX, phenols, petroleum wastes, pesticides, plastics, and rubber chemicals). This method is versatile and applied for the treatment of wastewater, sludge, or polluted soils by destroying organic pollutants, reducing toxicity and COD and BOD contents, and removing odour and colour. Mandal et al. (2010a, b) reported only 1.7 times enhancement in the % COD reduction by Fenton process when the dose of hydrogen peroxide increases about 2.3 times. Fenton system uses ferrous (Fe^{2+}) ions to react with H_2O_2 and produce hydroxyl radicals which have strong oxidation potential for the degradation of toxic

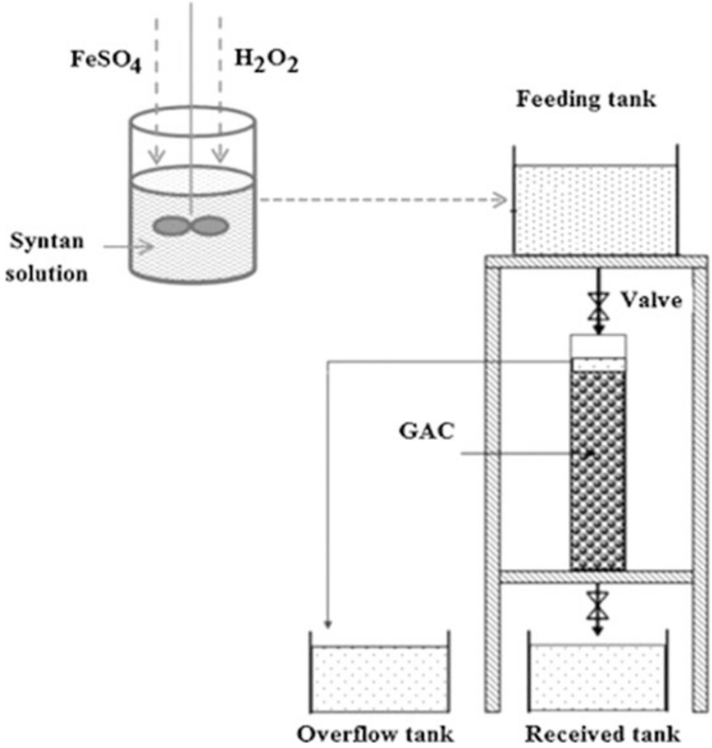

Fig. 14.3 Removal of leather tanning agent (syntan) by integrated treatment system of FO and adsorption

compounds present in effluents (Titus and Molina 2004). The reaction involved in the process are shown below.

Hydroxyl radicals react with Fe^{2+} ions and give Fe^{3+} ions, or they also react with organics and produce some products:

$$OH^{\bullet} + Fe^{2+} \rightarrow OH^{-} + Fe^{3+} \tag{14.9}$$

$$OH^{\bullet} + \text{Organics} \rightarrow \text{Products} \tag{14.10}$$

Hydrogen peroxide also reacts with hydroxyl radicals and produce other radicals, which in turn combine with each other for further production of hydrogen peroxide:

$$OH^{\bullet} + H_2O_2 \rightarrow H_2O + HO^{2\bullet} \tag{14.11}$$

$$OH^{\bullet} + OH^{\bullet} \rightarrow H_2O_2 \tag{14.12}$$

Fe^{3+} ions and radicals are produced during the reaction. The reactions are shown here:

$$\mathrm{H_2O_2 + Fe^{3+} \leftrightarrow H^+ + FeOOH^{2+}} \tag{14.13}$$

$$\mathrm{FeOOH^{2+} \rightarrow HO^{2\bullet} + Fe^{2+}} \tag{14.14}$$

$$\mathrm{HO^{2\bullet} \rightarrow Fe^{2+} \rightarrow HO^{2-} + Fe^{3+}} \tag{14.15}$$

$$\mathrm{HO^{2\bullet} + Fe^{3+}} \rightarrow O_2 + \mathrm{Fe^{2+}} + H^+ \tag{14.16}$$

14.2.4.2.2 Photo-Fenton Process (UV/Fe^{2+}/H_2O_2)

An improved version of the Fenton process is a photo-Fenton process. This process constitutes irradiation of the Fenton reagent with the help of UV light for hydroxyl radical production. Reactions of the photo-Fenton process are as follows:

$$\mathrm{H_2O_2 + UV \rightarrow OH^{\bullet} + OH^{\bullet}} \tag{14.17}$$

$$\mathrm{Fe^{3+} + H_2O + UV \rightarrow OH^{\bullet} + Fe^{2+} + H^+} \tag{14.18}$$

UV light not only forms hydroxyl radicals but recycles it by reducing Fe^{3+} catalyst to Fe^{2+}; when the concentration of ferrous (Fe^{2+}) is increased, the overall reaction mechanism is accelerated. Fenton and photo-Fenton reagents are found to be very effective in decolourisation and degradation of pollutants (Malik and Saha 2003).

14.2.4.2.3 Ozonation

It is a wastewater treatment process that destroys and removes synthetic organic pollutants, including antibacterial agents, through the infusion of ozone gas and production of very reactive oxygen species; thus they have the ability to remove organic compounds and a large number of microorganisms present in wastewater. Ozonation of wastewater treatment has a wide range of application, as it is efficient for disinfection as well as for the removal of both organic and inorganic pollutants. The use of ozone in a typical tannery effluent treatment plant in a strategic manner to maintain final BOD and COD levels up to desirable limits at all times/seasons is the theme of this paper. The different methods include pre-ozonation before the anaerobic stage and in the final aerator with augmented ozonation.

14.2.5 Membrane Technology

Membrane technologies are recently advanced methods to solve the problem of dissolved solids in effluents. Application of membrane system in tannery wastewater treatment together with MF/NF, UF/RO, and NF/RO is for the recovery of chromium in the presence of significant amount of BOD and proteins (Shaalan et al. 2001). Recovery of chromium and spent liquors in the leather industry (Ashraf et al. 1997; Cassano et al. 2001; Labanda et al. 2009; Pandey et al. 2003) decreases the amount of pollutants in unhairing and degreasing process (Cassano et al. 1997; Wang et al. 2011) in the biological treatment of tannery wastewater. Keerthi et al. (2013) reported that COD (90.2%) and colour (92.75%) removal in the HMBR and MBR process showed 72.69% and 75.82%, respectively. Microfiltration and reverse osmosis by a polymeric membrane in tannery waste effluent is more effective than the combined technology of ceramic membrane, and about 91% COD and BOD_5 was reduced in this process, but in the RO process, there is about 99% reduction of TOC and 82% reduction of sodium (Bhattacharya et al. 2013). The most significant disadvantage of membrane technology is fouling of membrane due to clogging and formation of cake layer by the adsorption of pollutants. However, proper selection of membranes and module like cross-flow mode avoids the fouling up to a large extent (Kumar and Pal 2012).

14.2.6 Biological Treatment

Biological treatment of wastewater is preferable than other treatment processes, wherein microbes are used for the reduction of pollutants; the decomposition rate of the aerobic process is faster than the anaerobic. Several problems arise during the biological treatments of tannery wastewater due to the presence of sulphide and chromium. The high concentration of some toxic compounds present in tannery wastewater causes eutrophication and other environmental problems (Durai and Rajasimman 2011). ASP is the most frequently used biological treatment of tannery wastewater. Table 14.8 shows some of the microorganisms used in leather industrial wastewater treatment.

14.2.6.1 Aerobic Biological Treatment

In aerobic biological treatment, microbes are used to convert organic carbon from the effluent into biomass and carbon dioxide; in this process, a large amount of sludge is generated. Ramteke et al. (2010) used some pathogenic bacterial species

Table 14.8 Microbes use in leather wastewater treatment

Microbes	BOD[a]	COD[a]	Chromium[a]	Sulphide[a]	References
T. ferrooxidans	93	98	72	62	Mandal et al. (2010a, b)
E. coli	98	NA	NA	NA	Ramteke et al. (2010)
Vibrio spp.	87	90	NA	NA	Ramteke et al. (2010)
Pseudomonas spp.	96	NA	NA	NA	Ramteke et al. (2010)
Halophytes	82	65	81	NA	Mandal et al. (2010a, b)
Halophytes	NA	95	NA	NA	Lefebvre et al. (2005)
Acidithiobacillus thiooxidans	NA	NA	99	NA	Wang et al. (2007)
Acinetobacter spp.	NA	NA	90	NA	Srivastava et al. (2007)

[a] shown removal in %

isolated from CETP for the reduction of COD and BOD levels during treatment of wastewater and achieved 98%, 87% and 96% removal by *Escherichia coli*, *Vibrio* species, and *Pseudomonas* species, respectively. From the wastewater generated by the oxidation of sulphide compounds having COD (550 mg/l) and ammonia concentration (90 mg/l), after 150 days of operation, 100% removal of ammonia, 90% COD, and 60–90% total nitrogen were removed (Goltara et al. 2003). Mandal in 2010 showed tannery wastewater treatment by three isolated halophytic bacteria individually, also in combination, and presented their efficiency.

The high salt-tolerant bacterial strain I has the highest Cr removal capability in comparison to strain II and III, where 65% COD and 82% BOD were removed in strain I, 58% COD, 76% BOD in strain II, and 36% COD and 48% BOD in strain III) (Mandal et al. 2010a, b). Another bacterial strain from genus *Planococcus* has been isolated which produces orange-red colour pigment in nutrient media in the presence of Cr^{6+}, tri-chlorophenol, and high concentration of salt as reported by Chitalya and Mandal in 2011. Behera et al. (2015) used *Planococcus* species for degradation of COD (80 ± 5%) from tannery wastewater in batch method. Salt-tolerant bacteria can be adopted in saline condition and degrade the toxic material present in saline wastewater.

The salinity of tannery wastewater makes it difficult for biological treatment. In CETP of tannery wastewater, four different types of bacterial strains are salt tolerant, namely, *Pseudomonas aeruginosa, Bacillus flexus, Exiguobacterium homiense*, and *Staphylococcus aureus*, are used in mixed and pure consortia, and are isolated from saline tannery wastewater. The salt-tolerant bacteria was identified and cultured for degradation of saline tannery wastewater (Sivaprakasam et al. 2008), but in hyper saline wastewater (Lefebvre et al. 2005), having rich organic matter and TDS there is difficulty created for conventional biological wastewater treatment, optimum removal of COD, $PO4^{3-}$, TKN and SS was 95%, 93%, 96% and 92%, respectively at 5 days HRT, with an organic loading rate (OLR) of 0.6 kg COD/m^3/day. Ganesh et al. showed that the removal of COD (80–82%), TKN (78–80%), and NH_3-N (83–99%) at low oxygen uptake rate (OUR) value and 1.9–2.1 kg/m^3/day of loading rate for 12 h in SBR are higher than the conventional aerobic process.

14.2.6.2 Anaerobic Biological Treatment

Anaerobic wastewater treatment produces small amount of sludge and biogas (CH_4 and CO_2), due to the conversion of organic waste. The anaerobic processes of tannery wastewater treatment are mainly operated by upflow anaerobic sludge blanket (UASB) reactors. Anaerobic digestion has been suggested as a potential treatment alternative by different workers (Vijayaraghavan and Ramanujam 1999; Tilche and Vieira 1991; Omil et al. 1999; Field and Lettinga 1987). Due to the negative impact of chromium on humans and living organisms, it is suggested that chromium metal be removed from tanning wastewater irrespective of its costs (Fabiani et al. 1997; Kocaoba and Akin 2002). A single UASB reactor is capable of treating both liquid and solid wastes present in tannery wastewater. The COD and BOD removal efficiency are about 46–85% and 65–93%, respectively, as reported by Rajasimman et al. (2007), and the gas production was up to 2–15 l/kg of waste. Calheiros et al. (2008) describe wetland plant *Typha latifolia* which is tolerant to high organic loadings and interruption in feed during biological treatment of wastewater in long-term operation. The system was operated at three hydraulic loading, 18, 8, and 6 cm/day, and obtained up to 652 kg BOD_5 $ha^{-1}day^{-1}$ and 1869 kg COD ha^{-1} day^{-1} removal from initial organic loadings of 1800 kg BOD_5 $ha^{-1}day^{-1}$ and 3849 kg COD $ha^{-1}day^{-1}$, respectively.

Mandal et al. and his team in 2010 observed the degradation of leather wastewater with the help of aerobic bacteria *Thiobacillus ferrooxidans*, Fenton reagents and its combination, and reported that the reduction of BOD5, COD, sulphide, total chromium and colour was happened with a contact time of 30 min in batch conditions. When *Thiobacillus ferrooxidans* were used solely, the maximum reduction extended up to 77, 80, 85, 52 and 89% in 21 days treatment, while in combination that menace chemical and Fenton's oxidation process, after the 72 h of biochemical treatment with *Thiobacillus ferrooxidans* in batch system gave 93, 98, 72, 62 and 100% removal of COD, BOD, sulphide, chromium and colour at pH 2.5 and temperature 30 °C (Mandal et al. 2010a, b). Wang et al. (2007) used the acidophilic sulphur-oxidising bacteria *Acidithiobacillus thiooxidans* which are able to degrade heavy metals from industrial and urban wastewater; when sludge is mixed with *Acidithiobacillus thiooxidans* culture, pH decreases up to 2 after 4 days, but after 6 days of incubation at 30 °C and 160 rpm, there is removal of chromium up to 99% from tannery sludge.

Srivastava et al. (2007) reported the use of bacterium *Acinetobacter* and one fungal species for the degradation of pentachlorophenol (PCP) and removal of chromium from tannery effluent. When they use bacterial consortium followed by a fungus, it could remove 90% chromium and 67% PCP in a sequential bioreactor. But in another bioreactor, the effluent was treated primarily by fungi followed by bacteria, which could only remove 64.7% chromium and 58% PCP. In Ayoub et al. (2011), the results show TSS, colour, phosphorus, chromium, COD, and BOD removal by 97, 99, 87, 99.7, 71, and 57%, respectively, through the use of lime and activated carbon. Conductivity and TDS increased due to the addition of lime up

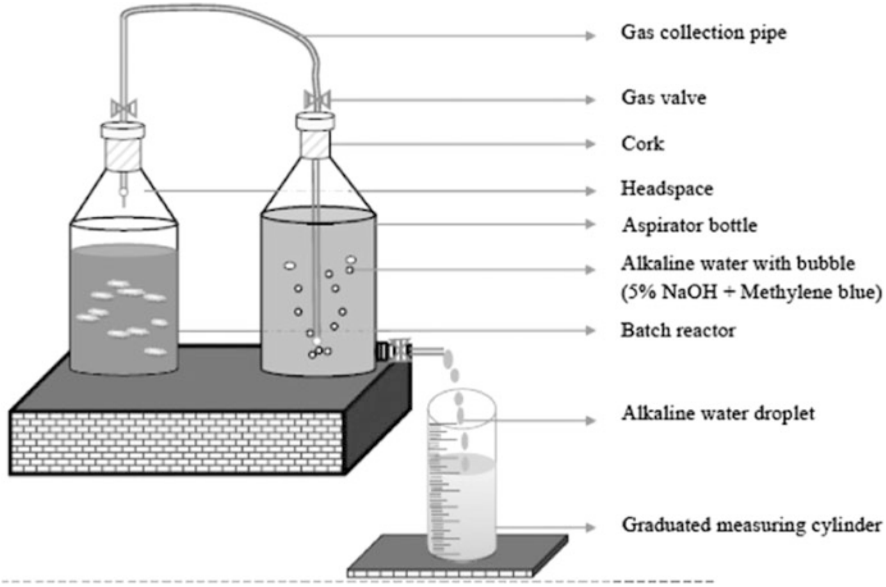

Fig. 14.4 Schematic diagram of biogas production in laboratory

to 36 and 30%, respectively, and arsenic removal of 56% was observed with the combination of coagulation with lime and activated carbon.

14.2.6.3 Biogas Production from Anaerobic Digestion of Leather Waste

Anaerobic digestion or biomethanation of leather waste is an alternative method of waste-to- energy production. Leather solid waste consists mostly of protein and lipids, with the lipid part of waste having a large renewable energy source (Cirne et al. 2007). Methane (55–70%), carbon dioxide (30–45%), and some other trace amounts of gases are present in biogas (Priebe et al. 2016). Anaerobic biomethanation is a complex process and consists of several phases (hydrolysis, acidogenesis, acetogenesis, and methanation) by using different groups of microbes (Deublein and Steinhauser 2008). The maximum rate of biogas production was found in chrome leather shaving waste but when collagen-based protein substrates were used it gave better results, during 3–36 days 162.2 mLg^{-1} of biogas 73.% methane obtained from the slaughterhouse (Priebe et al. 2016). Figure 14.4 shows the biomethylation process in lab scale (Helen and Shanmugam 2017).

14.2.7 Integrated/Hybrid Treatment System

An integrated/hybrid system (Fig. 14.5) is a process which combines a membrane filtration unit (MF/UF/NF) with other treatment processes such as adsorption,

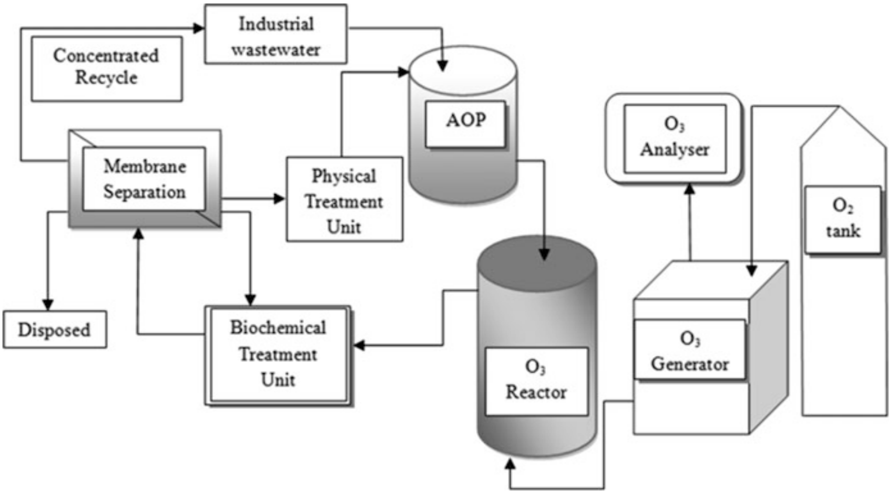

Fig. 14.5 Possible integrated scheme in leather wastewater treatment

coagulation, ion exchange, and the integrated membrane system (Ang et al. 2015). Nowadays, the biochemical treatment approaches are being used in several industries to detoxify or destroy pollutants aerobically (Ros and Gantar 1998; Vidal et al. 2004; Carucci et al. 1999; Ahn et al. 1996; Nandy et al. 1993). Tadesse et al. (2004) observed the effects of pH, temperature, and dissolved oxygen in advanced integrated wastewater pond system (AIWPS). They also showed that biological treatment was integrated with ozonation in tannery wastewater. Iaconi et al. (2010) observed that residual value of COD was lesser than the discharge limits of 160 mg/l. Suganthi et al. (2013) used a hybrid membrane bioreactor (HMBR) in combination with electrocoagulation and activated the sludge process used for the removal of colour and COD from tannery wastewater. Constructed wetlands (CWs) and integrated wastewater ponds are also utilised in tannery effluent treatment (Calheiros et al. 2008; Tadesse et al. 2004; Calheiros et al. 2012). Mandal et al. (2010a, b) studied leather wastewater treatment using aerobic bacteria with the help of *Thiobacillus ferrooxidans* and Fenton oxidation as a combination treatment.

14.3 Recovery of Value-Added Products from Tannery Waste

The recovery of certain valuable product from leather waste will be beneficial for small-scale tannery industries, thus are in demand, and studies are going on to use the treated tannery waste. Biogas was produced by the anaerobic digestion of solid waste from nutrient-rich effluents (Gnanamani and Bai 1992). Collagen is obtained after the removal of Cr from leather waste. It is a nitrogen-rich source which could be

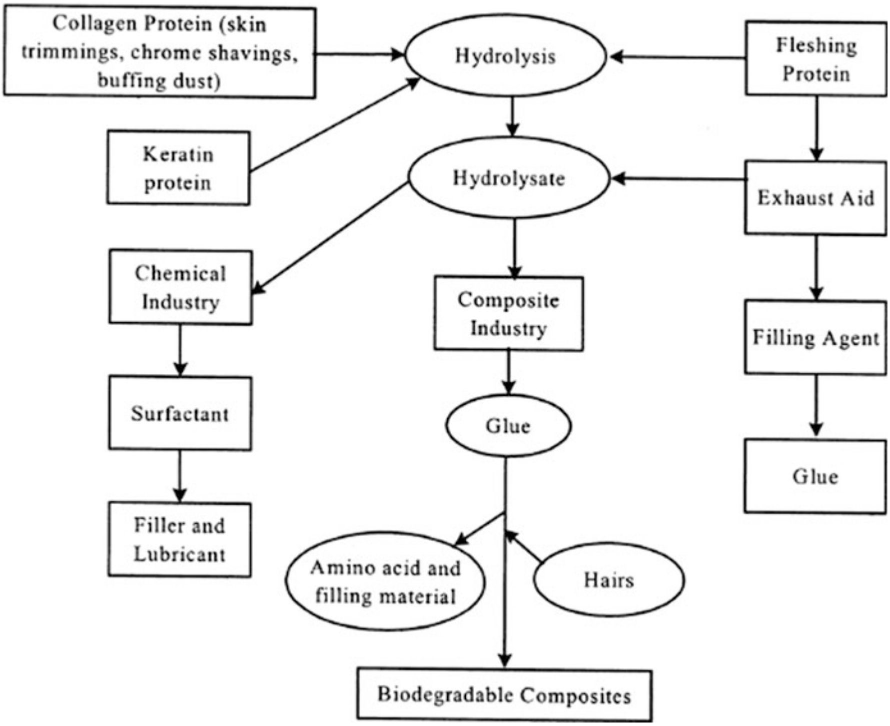

Fig. 14.6 Flow diagram of solid wastes utilisation (Kanagaraj et al. 2006)

used as a nutrient for collagenase-producing microbes. Collagenase is often used as a stabiliser and clarifier in the food industry. Jain and Jain (2010) isolated collagenase-producing microorganism *Streptomyces exfoliates* CFS 1068 from the soil.

It was reported that maximum collagenase (58 U) activity was found after 5days' incubation in simulated media where starch and soybean meals were used as carbon and nitrogen source, respectively. 850 kg of leather solid wastes are produced in leather processing, wherein when 1 tonne of raw hide is used, 150 kg of leather is formed. The remaining solid waste can be recycled and utilised as useful products such as in biodiesel and alkaline protease production (Ahmad and Ansari 2013). Utilisation of tannery waste for poultry feed as a protein source, fertiliser, and cosmetic is a step towards zero solid waste (Lima et al. 2010; Paul et al. 2013). Nowadays, due to industrialization, such types of solid wastes are increasing day by day, and use of these waste in the form of fertilisers has been an alternative option for environmental protection. The solid waste can be hydrolysed and used as a by-product in many ways (Kanagaraj et al. 2006) (Fig. 14.6).

Leather industrial solid waste was used as a nitrogen (N) source for growth of bean plants by Lima et al. (2010) and showed a clue to investigate the Cr-free nitrogen-rich solid waste as a source of bio-fertiliser when mixed with phosphorus (P) and potassium (K). In certain tropical areas, industrial/chemical fertilisers have high costs; to solve this problem of nitrogen availability for crops (Oliveira et al. 2008a, b, c, d), the solid waste from the leather industry is used after chromium extraction because of its high N content and incorporated with the minerals phosphorus and potassium (P and K), which could then be used as fertiliser. Minimisation of industrial waste could be feasible if the methodologies could be developed to add value to collagen materials, indigenous microbe's use for bio-colour generation using salts, organic and inorganic matter of the effluent.

A wide range of bacterial strains such as *Serratia marcescens*, *Pseudomonas magneslorubra*, *Vibrio psychroerythrous*, *S. rubidaea*, *V. gazogenes*, *Alteromonas rubra*, *Rugamonas rubra*, *Streptoverticillium rubrireticuli*, and *Streptomyces longisporus* have shown their potential in pigment production (Johnson and An 1991; Pandey et al. 2003; Tao et al. 2005). The wet blue leather yield sludge is produced from the tannery industries which contain approximately 30,000 mg of chromium/kg of wet blue leather waste (w/w). This sludge is considered the most dangerous and harmful waste due to the presence of chromium if discarded into the environment without proper treatment.

Expensive but cost-effective disposal system is therefore needed. Nowadays, several ways are used for disposing wastes, by making this sludge commercially valuable. Recycling this waste for production of eco-friendly materials having high-end value would be beneficial for the environment. Special attention has been given for the use of inexpensive precursors such as sludge to make the production commercial (Oliveira et al. 2008a, b, c, d; Nogueira et al. 2010, 2011; Dhayalan et al. 2007). Protein hydrolysates recovered from CTLSs were utilized as bio-fertiliser; many plants can also absorb amino acids, which sometimes serve as better nitrogen sources than ammonia or nitrate (Taylor et al. 1999; Ashmead et al. 1986). To increase the yield of crops, protein hydrolyses were recovered and channelled as an organic nitrogenous fertiliser (Kolomaznik et al. 1999). Protein hydrolysates obtained from natural plants or animals in fertiliser composition represent substances with chelated micronutrient properties; some of the micro-elements such as Fe, Co, Zn, Ca, Mg and Mn, when treated with hydrolysed proteins, can be easily absorbed by both plants and animals (Sirbu et al. 2008, 2009; Ashmed and Hsu 1985).

In 2004, Katsifas et al. reported the use of *Aspergillus carbonarius* for the degradation of CTLS wastes in fermentation and about 97% liquefaction of waste and liquid can be used for the recovery of chromium and liquid fertiliser and solid waste having the highest amount of nitrogen, nearly 64%. These shaving wastes can be treated for chrome with enzymes or alkali solutions (Taylor et al. 1990, 1992, 1997). The choice of the precursors and the optimisation of process condition determine the production quality of the final end products. The economy of the manufacturing process improves if any value-added by-products could be generated from the sludge, which may have a wide market, while taking care of sludge disposal.

In Indian scenario, the tannery wastewater treatments by conventional methods create lots of sludge having a hazardous impact on the environment. In recent years, this sludge has become a great environmental threat causing damage to the land and its surrounding areas where it is dumped. Still, there is no such technology for disposal by making any commercial use of this sludge. This could have improved the sludge management. The right investors need a critical and genuine evaluation of tannery process strategies at hand that should maintain equilibrium between leather sector utilities and community wellbeing.

14.4 Future Research Need and Challenges

In most of the developing countries, tanning operation is a family business and present in cluster form outside the residential areas. Workers in such industries are not very well educated, and they do not know the complexities of leather processing. The large amount of wastewater generated in the tannery industries is considered as one of the highly polluted and toxic wastewater discharged in natural water bodies indirectly or directly without any proper treatment. In summer season when the temperature is high, the rate of degradation of waste is high, and this causes air pollution in the environment by producing intolerable obnoxious smell. The presence of heavy metals such as Cr, Cu, Mn, Zn, Pb, Ni, Co, Ag, Fe, and Cd, dyes, BOD, COD, and odour is a major problem militating the recycling or reuse of wastewater due to their highly hazardous nature. Integrated treatment system to treat leather industrial wastewater may be the best choice for managing the hazardous waste from the industry.

Different technologies are available in the literature like adsorption (Tahir and Naseem 2007), Fenton treatment (Wang 2005), biological treatment (Ros and Gantar 1998; Vijayraghavan and Murthy 1997; Mandal et al. 2010a, b), ion exchange (Tiravanti et al. 1997), electrocoagulation (Gurses et al. 2002; Kobya et al. 2006a) which could be used for the treatment of tannery effluents, but no definitive treatment considered as an integral approach for recycling this wastewater has been reported in literature. Generally, there is insufficient management engagement in wastewater treatment plant as it is a nonrevenue-generating activity, which usually results in performance failure. Advanced treatment scheme like integration of slow biological remediation with fast chemical treatments like photo-Fenton and ozone treatments and finally polishing step by filtration with highly selective membranes may turn the highly polluted tannery wastewater into reclaimable water. Nanofiltration followed by microfiltration using some appropriate membranes has the capability to remove any microbes, solute particles, and ions present in the treated water. Thus a suitable treatment scheme for tannery wastewater can be projected which may integrate the biological and advanced photo-chemical treatment for decreasing the COD level and then microfiltration to remove suspended particles and finally nanofiltration to separate and concentrate the chromium, nitrate, phosphate, etc. to get reusable water. Value-added products may be recovered from the nanofiltration concentrated wastewater to add economy of the treatment process (Kumar and Pal 2013, 2015; Pal and Kumar 2014).

14.5 Conclusions

Physical, chemical, advanced oxidation processes, and biochemical/biological treatment of leather industrial wastewater have been reviewed in the present study. After reviewing the work on the treatment of leather industry waste, it clearly shows that the integrated treatment system may be the best option for managing this type of hazardous waste. The study indicates that all sorts of treatment are somehow effective to treat the leather industrial wastewater up to a certain extent, but no clear solution is available. To overcome this constraint for individual treatment system, probably the proper design of an integrated treatment system is more helpful for future work and implementation. Thereby, membrane technology may be added to the system for the final step of purification. For the final step, i.e. before disposing the wastewater, membrane separation is nationally accepted. But there is the absence of an integrated treatment system that can remove all the pollutants in a leather industry wastewater. It will be more effective if the solid waste of this industry can be utilized properly for the production and recovery of valuable products. The small-scale industries always face techno-economic constraints to handle their waste. Thus utilization for valuable product recovered from whole may relieve them from all constraints.

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