Maulin P. Shah *Editor*

Microbial Bioremediation & Biodegradation

Springer

Microbial Bioremediation & Biodegradation

Maulin P. Shah
Editor

Microbial Bioremediation & Biodegradation

Springer

Editor
Maulin P. Shah
Environmental Microbiology Lab
Bharuch, Gujarat, India

ISBN 978-981-15-1811-9 ISBN 978-981-15-1812-6 (eBook)
https://doi.org/10.1007/978-981-15-1812-6

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

Preface

The scientific interest in the fate of environmental pollutants generated by industrial and urban activities does not only refer to the search for ways to favor mitigation or, ideally, the complete elimination of the affected sites. It also provides information on how new environmental microorganisms evolve for first molecular devices to tolerate and then catabolize many of these toxic molecules. Along with resistance to antibiotics, the emergence of new biodegradative routes for new compounds is one of the most outstanding cases of contemporary biological evolution in real time. Understanding the rules of this evolution thus provides new principles for predicting and, if accelerated, biochemical adaptation to the new chemical structures. These phenomena occur in space and time and also at very different scales depending on the nature and size of the pollutants at stake. The impact of pollutants that received considerable attention decades ago is diminishing in many cases due to better industrial procedures along with environmental awareness and growing legal regulations. Unfortunately, the last decade has witnessed the appearance of other types of pollutants (particularly greenhouse gases, plastics, and micropollutants) that threaten not only specific sites but also the functioning of the environment. This state of affairs calls for new bioremediation strategies that take into account the multinational complexity involved in possible interventions far beyond the focus on specific biodegradation pathways. Fortunately, the environmental microbiome and the possibilities to engage it with the tools of Systems and Synthetic Biology are the best resource to face the phenomenal challenge of preserving the biosphere in a good way for future generations. The growing industrialization and urbanization of our societies over the last century has left us a heritage of emissions that, whether they are natural or synthetic molecules, have had an impact on virtually all Earth's ecosystems. This combination of circumstances has paved the way for the science of biodegradation (that is, understanding—and ultimately refactoring—how microorganisms catabolize otherwise unpleasant environmental chemicals) and the technology of bioremediation (using biological agents to eliminate or at least mitigate pollution at given sites).

Bharuch, Gujarat
India

Maulin P. Shah

Contents

About the Editor

Maulin P. Shah received his Ph.D. (2002–2005) in Environmental Microbiology from Sardar Patel University, Vallabh Vidyanagar, Gujarat. He has served as an Assistant Professor at Godhra, Gujarat University, in 2001. He has edited 25 books in the area of wastewater microbiology.

Bioremediation Approaches for Treatment of Pulp and Paper Industry Wastewater: Recent Advances and Challenges

1

Vineet Kumar, Indu Shekhar Thakur, and Maulin P. Shah

Abstract

Pulp and papermaking industry is a large consumer of fresh water and also an important source of dark-brown-colored wastewater, generated during various stages of pulping and papermaking activities. The colored wastewater discharged from pulp and paper industry even after secondary treatment remains toxic and complex in nature and retains high amount of lignin, lignin residues, resins, acids, chlorinated phenols, and various persistent organic pollutants (POPs) including the adsorbable organic halides (AOXs; halogenated or organochlorine). The existing various conventional methods along with integrated processes (aerated lagoons and activated sludge plants) cannot efficiently treat pulp and paper industry wastewater due to its complex and recalcitrant nature. Hence, the discharged partially treated/or untreated wastewater are contributing to deteriorating water quality due to increasing biological oxygen deman and chemical oxygen demand and decrease of dissolved oxygen.

In a terrestrial ecosystem, the wastewater irrigated soil showed decrease of moisture content and increase of pH and toxic heavy metals content. To tackle this problem associated with hazardous waste disposal, the existing pulp and paper industry wastewater treatment process needs to be improved with better treatment outcomes. Although, several physicochemical methods are available for the treatment of such wastewater, they are more energy intensive and suffer from residual effect. In addition, they are very expensive, inefficient, and produce a huge amount of toxic sludge which is difficult to handle and also produces volatile organic compounds on burning. To combat these challenges, biological

V. Kumar (✉) · I. S. Thakur
Environmental Microbiology and Biotechnology Laboratory, School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, India

M. P. Shah
Environmental Microbiology Lab, Bharuch, Gujarat, India

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_1

treatment using bacteria, fungi, yeasts, and algae has evolved as a preferred means to treat and reduce the toxic organic compounds loaded in generated pulp and paper industry wastewater.

Keywords
Adsorbable organic halides · Persistent organic pollutants · Phytoremediation · Chlorinated lignin · Bleaching

1.1 Introduction

Pulp and paper industry are among the most important industries in the world not only for an economical purpose but also for a social purpose. Besides, it is also one of the major polluting industry discharging a variety of organic and inorgaic pollutants such as gaseous, liquid, and solid into the environment (Ali and Sreekrishnan 2001; Lacorte et al. 2003; Singh and Chandra 2019). Consider that manufacture of paper consumes significant quantities of wastewater, as high as 200–350 m^3 $tonne^{-1}$ of paper produced, of which nearly 75% is discharged as wastewater (Nagarathnamma et al. 1999). Currently, there are 759 paper mills operating in India, out of which 30 are wood-based large-scale mills, 150 are agro-based medium-scale mills, and 579 are recycled fiber-based medium and small-scale mills, producing 3.40, 2.42, and 5.10 Mtpa paper, respectively (Rajwar et al. 2017). As per the Ministry of Environment, Forest and Climate Change (MOEF&CC), Government of India, the pulp and paper sector is in the Red Category list of 17 industries having a high pollution potential owing to its serious environmental threat. Most significant sources of pollutants in pulp and paper mills are pulping of raw materials (wood chips), pulp bleaching, and paper coating processes (Bajpai et al. 1993; Nagarathnamma et al. 1999; Yadav et al. 2010; Rocha-Santos et al. 2010; Singh and Chandra 2019). Pulping process results in dissolved forms of lignin and other wood components called black liquor (BL) due to its black color, whereas bleaching produces the mono-aromatic compounds like chlorophenols, catechols, and guaiacols and numerous high-molecular-weight organic compounds like phenols, chlorolignins, chlorophenols, adsorbable organic halides (AOXs), extractable organic halides (EOXs), and plasticizers (Mishra and Thakur 2010; Mishra et al. 2014; Chandra et al. 2011a, b). Besides, bleached effluent is heavily loaded with organic matter, having high suspended solids (SS), color, biological oxygen demand (BOD), chemical oxygen demand (COD), total organic chlorides (TOC), chlorinated resin acids, phenols, dioxins, and furans (Larsson et al. 1988; van Driessel and Christov 2001; Leadbitter 2009; Yadav et al. 2010; Malaviya and Rathore 2007). These parameters have discharge limits, laid down by various environmental regulatory authorities around the globe. The high values of COD in wastewater also indicate the recalcitrance of chemicals that have escaped biodegradation processes (Mahesh et al. 2006; Gommers et al. 2007; Chen et al. 2012a, b, c). These chemicals may be persistent in nature and may cause several problems to animals, plants,

microorganisms and human healt (Singh and Chandra 2019). According to the United States Environmental Protection Agency (USEPA), 27% (wt %) of municipal solid waste is composed of paper waste, and about 100 million kg of toxic pollutants are released every year from the paper industries.

Some large-scale pulp and paper mills have recovery boilers to burn much of the BL they produce, generating steam and recovering the cooking chemicals, viz., sodium hydroxide (NaOH) and sodium sulfide (Na_2S_2) which are used to separate lignin from the cellulose fibers of wood chips needed for papermaking (Pokhrel and Viraraghavan 2004; Mishra and Thakur 2010). This chemical reaction and burning of organic materials release a considerable amount of heat energy which is recovered by transferring it through water-filled tubes in walls of the recovery boiler. However, they are inefficient, costly, and produce a huge amount of toxic sludge which is difficult to handle (Thompson et al. 2001; Mishra and Thakur 2010; Qadir and Chhipa 2015). On burning, volatile organic compounds (VOCs) like dioxins and furans are formed which are more toxic than the parental compounds. Small-scale pulp and paper mills often lack such installations due to their high operational costs and do not have satisfactory and adequate wastewater treatment facilities; as a result, unrecovered wastewater amplify the pollutant toxicity and are a cause of serious environmental concern (Oke et al. 2017; Singh and Thakur 2006; Medhi et al. 2011; Ojunga et al. 2010; Tyor et al. 2012). In aquatic system, it blocks the photosynthesis reaction processes and decreases the dissolved oxygen (DO) level which adversely affects the flora and fauna and causes toxicity to aquatic ecosystem (Poole et al. 1977; Leadbitter 2009; Hou et al. 2018), whereas in the contaminated soil, it showed the accumulation of toxic recalcitrant organic pollutants and heavy metals (Kumar and Chopra 2011; Pradhan and Behera 2011; Roy et al. 2008; Medhi et al. 2011). Several pollutants that discharged in pulp and paper mill are also reported as carcinogenic, mutagenic, clastogenic, and endocrine-disrupting in nature (Haq et al. 2017; Mishra et al. 2014). Therefore, it is mandatory for pulp and paper mills to comply with the appropriate standards set by Central Pollution Control Board (CPCB), New Delhi, India. However, potential advanced processes that are used for wastewater treatment discharged from pulp and paper industry includes chemical coagulation, flocculation, precipitation, ion exchange, advanced oxidation processes, ozone treatment, electrochemical degradation, membrane processes (especially reverse osmosis, nanofiltration, and ultrafiltration), photocatalytic degradation, and adsorption on activated carbon, as a means of removing color and turbidity from wastewater (Subramonian et al. 2017; Gonder et al. 2012; Birjandi et al. 2016; Mahesh et al. 2016; Yeber et al. 1999; Stephenson and Duff 1996; Pihlajamäki and Nyström 2002; Mahesh et al. 2006; Rodrigues et al. 2008).

However, these treatment approaches offer an economic nonviability, limited versatility, operational constraints, partial treatment, and plausible formation of secondary hazardous by-products and also generate a huge amounts of toxic sludge that limit their industrial applicability (Pokhrel and Viraraghavan 2004; Thompson et al. 2001; Zhang et al. 2009). Researchers across the globe have tried to devise innovative methods for achieving maximum reduction in the color, BOD, and COD loadings of pulp and paper mill wastewater (Gommers et al. 2007; Singh and Thakur

2006; Singhal and Thakur 2012). The conventional biological treatment methods, such as activated sludge (AS) and aerated lagoons (extended aeration methods), are ineffective in removing color and phenolics and also do not decolorize wastewater very effectively (Lerner et al. 2007; Qadir and Chhipa 2015; Erkan and Engin 2017). However, certain advanced biotechnological treatment methods, such as biodegradation using potent microorganisms, can prove to be effective for further treatment of toxic organic pollutants and decolorization of such wastewater compared to chemical treatment and conventional aerobic–anaerobic treatment, as lesser sludge would be produced, with an additional low-cost benefit (Ragunathan and Swaminathan 2004; Abira et al. 2005; Dias et al. 2005; Chandra and Singh 2012; Chandra and Kumar 2015b, 2017b). The use of microbes for biodegradation of refractory organic compounds is an efficient, relatively cost-effective, and environment-friendly tool for the treatment of industrial wastewater (Kumar et al. 2018; Kumar and Chandra 2018a, b). However, biotechnological methods using fungi, bacteria, and actinomycetes are less effective for complete decolorization and detoxification of pulp and paper industry wastewater (Latorre et al. 2007; Raj et al. 2005; Raj et al. 2014a, b; Singhal and Thakur (2009a, b). Although a plethora of information is available on biological treatment methods for BL, there is an acute shortage of efforts to make the process being implemented effective on a large scale application.

1.2 Pulp and Paper Industry Wastewater Generation and its Characteristics

Paper manufacturing process involves three steps: pulping (also called delignification), bleaching, and finally papermaking. The purpose of pulping is to extract cellulosic content from plant materials obtained from hardwood or softwood trees. Generally, three approaches like mechanical pulping, chemical pulping, and a combination of both mechanical and chemical pulping are known to produce pulp from wood (Sandstrom et al. 1988; Esposito et al. 1991; Martin and Manzanares 1994; Thompson et al. 2001). However, the main drawback of mechanical pulping is yielded low-quality pulps, unsuitable for high-strength fiber products, and high energy requirements (Stephenson and Duff 1996). Mechanical pulping causes less pollution than chemical pulping. The most important delignification (chemical pulping) processes are kraft, sulfite, and soda pulping (Abdelaziz et al. 2016; Becker and Wittmann 2019; Wong 2009). Kraft pulping is a process in which wood chips are cooked in a large pressure vessel called a digester at 155–175 °C in an aqueous solution NaOH and Na_2S_2, also known as white liquor, to dissolve lignin from cellulose and hemicellulose fibers of the wood chips. The thus formed hydroxide (OH-) and hydrosulfide (HS-) anions crack the aromatic ether bonds within the lignin structure and release low-molecular-weight thiolignin oligomers (Abdelaziz et al. 2016). Sulfite pulping is a process of cooking of wood chips at 140–170 °C in alkaline, a pH neutral or an acidic environment, depending on the added sulfite salt (Abdelaziz et al. 2016; Schutyser et al., 2018). The ether bonds within the lignin structure are thereby hydrolyzed and subsequently sulfonated by the sulfite ions

(SO_3^{-2}) in the liquor. Sulfite pulping produces fully water-soluble, highly degraded lignosulfonates with a sulfur content of 4–7 wt% (Abdelaziz et al. 2016; Schutyser et al., 2018; Van den Bosch et al., 2018). Established in 1874, sulfite pulping became the dominant process for wood delignification until kraft pulping was established in the 1930s. Similar to kraft and sulfite pulping, soda pulping involves cooking biomass at 160–170 °C in presence of soda (NaOH) and—optionally—anthraquinone, the latter increasing the efficiency by promoting reductive ether bond cleavage (Abdelaziz et al. 2016; Schutyser et al., 2018; Van den Bosch et al., 2018). The wastewater generated at the end of pulping stage called BL is a dark brown in color due to dissolved lignin and its degradation products, hemicelluloses, resins, acids, and phenols (Hermosilla et al. 2015). The BL has high COD, BOD, and TSS (Pokhrel and Viraraghavan 2004). In the pulping process, less than 50% yields are achieved, and the pulp requires further extensive bleaching. During the bleaching process, wood components such as lignin and some carbohydrates are structurally modified, oxidized, degraded and chlorinated (Thompson et al. 2001; Leadbitter 2009; Oke et al. 2017). This is followed by an alkaline extraction phase using high temperature, pH, and consistency, which transforms the oxidized products into a soluble form. In the extraction stage, chlorinated oxidized lignins, not soluble in the acidic chlorination stage, are solubilized and dissolved into the spent liquor. The final bleaching is performed by oxidizing agents: chlorine dioxide and hydrogen peroxide. In India, bleaching is still being done with chlorine. Chlorine dioxide is used by very few mills for viscosity protection in the first bleaching stage (10–15% substitution) and for brightening in the final bleaching stages (Nagarathnamma et al. 1999). The use of chlorine-based bleaching chemicals results in the generation of a large number of toxic chlorinated organic compounds. The wastewater generated at bleaching stage has toxic colored compounds, including chlorophenols, EOXs, AOXs, and a small proportion of extremely toxic DDT, polychlorinated biphenyls (PCBs), and polychlorinated dibenzodioxins (PCDDs) (Savant et al. 2006; Chandra and Kumar 2015b; Lacorte et al. 2003; Rocha-Santos et al. 2010; Singh and Chandra 2019). In addition, chromophoric and highly oxidized polymeric lignin/chlorolignin derivatives are formed giving rise to the characteristic dark color to BL (Fig. 1.1; Nagarathnamma et al. 1999; Esposito et al. 1991; Chedchant et al. 2009; Chandra et al. 2011a, b; Mishra et al. 2014). A large number of pulp and paper mills are reluctant to recycle bleach plant wastewater to the chemical recovery system due to the corrosive nature of chloride ion and the substantial dilution of the chemicals to be recycled. Acid precipitation of lignin is a commonly applied treatment to BL after precipitation of more than 90% of lignin is removed from the solution as solid material. In addition, the precipitated lignin generates large volumes of sludge, which requires further treatment and disposal (Thompson et al. 2001; Pokhrel and Viraraghavan 2004). Nevertheless, the remaining soluble percentage is composed of oxidized and partially degraded lignin (predominantly composed of oligomeric lignin compounds) chlorinated organics responsible for the mutagenicity of the effluent. The high-molecular-weight persistent chlorinated organic compounds along with residual lignin generated during pulp bleaching are the major contributor to effluent color, COD, and chronic toxicity (Ali and Sreekrishnan 2001; Pandey

Fig. 1.1 Pulp and paper industry wastewater. (**a**–**c**) the huge volume of complex brown color wastewater generated during pulping and bleaching process and discharged into the environment after secondary treatment; (**c**, **d**) a large view of the collected pulp and paper industry wastewater

et al. 2012; Verma 2008; Maheshwari et al. 2012; Thompson et al. 2001). Finally, the brown/black color effluent generated during pulping and bleaching processes is a complex mixture of hundreds of compounds like lignin, tannin, chlorinated phenol compounds, suspended solids, diterpene alcohols, waxes, fatty acids, resin acids, fatty acids and their degraded products, phenols, dioxins, furans, chlorinated resin acids, chlorinated phenol, chlorinated hydrocarbons, various surfactants, dibenzo-p-dioxins, and dibenzofurans (Fig. 1.1; Ali and Sreekrishnan 2001; Rocha-Santos et al. 2010; Savant et al. 2006; Lacorte et al. 2003). While some of these pollutants are naturally occurring wood extractives (e.g., tannins, resin acids, stilbenes, lignin), others are xenobiotic compounds that are unintentionally generated formed during the process of pulping and papermaking processes (Thompson et al. 2001; Lacorte et al. 2003).

Thus, effluents discharged from industries are heavily loaded with organic matter containing 200 organics and 700 kinds of inorganic compounds (Table 1.2; Chandra and Singh 2012; Chandra and Abhishek 2011; Chandra et al. 2011a, b; Haq et al. 2016; Haq et al. 2017; Karrascha et al. 2006). Table 1.1 summarizes the physico-chemical characteristics of different kinds of influent generated during pulp and paper making process in pulp and paper industry. Some of the pollutants notably polychlorinated dibenzodioxins and dibenzofurans (dioxins and furans) are

Table 1.1 Physicochemical characteristics of wastewater discharged from various industries (Chandra and Abhishek 2011; Chandra et al. 2011a, b; Arivoli et al. 2015)

Parameters	BL	RGPPME	PPME	PPME
pH	8.8 ± 0.2	9.0 ± 0.2	8117.50 ± 185	7.80 ± 0.012
Color (Pt/co)	3100 ± 22.32	6100 ± 3.5	–	877.29 ± 4.65
EC (μS cm^{-1})	–	–	–	3.87 ± 0.06
Turbidity (NTU)	–	–	–	274.36 ± 1.04
BOD (mg L^{-1})	5100 ± 167.6	7360 ± 153	5850 ± 50.12	230.18 ± 2.75
COD (mg L^{-1})	12,245 ± 439.5	18,700 ± 440	16,400 ± 120	981.75 ± 4.29
TDS (mg L^{-1})	402.68 ± 53.92	1402 ± 1.5	840 ± 32.45	2129.17 ± 37.16
TSS (mg L^{-1})	–	–	100 ± 4.00	1179.17 ± 30.43
TS (mg L^{-1})	–	–	940 ± 2.71	3316.67 ± 39.56
Total phenol (mg L^{-1})	38.54 ± 2.61	38.5 ± 2.8	1272 ± 30.45	4.93 ± 0.07
AOX (mg L^{-1})	4.7 ± 0.2	–	–	–
Total nitrogen	–	–	571 ± 25.12	–
Lignin (mg L^{-1})	663 ± 4.23	1000 ± 1.1	614 ± 8.13	–
Sulphate (mg L^{-1})	1762 ± 41.11	1800 ± 14	–	73.67 ± 1.43
Tannin (mg L^{-1})	–	–	–	42.38 ± 0.49
PCP (mg L^{-1})	–	–	145.11 ± 4.56	
Phosphate (mg L^{-1})	BDL	BDL		2.65 ± 0.05
K+ (mg L^{-1})	–	12.2 ± 1.33	86.52 ± 2.58	–
Na + (mg L^{-1})	–	102 ± 11	136.56 ± 4.56	–
Cl^- (mg L^{-1})	–	–	31.42 ± 0.86	–
Nitrate (mg L^{-1})	–	3 ± 4.5	41.52 ± 3.56	–
Heavy metals				
Cd (mg L^{-1})	0.06 ± 0.03	BDL	0.2078 ± 0.09	–
Cr (mg L^{-1})	0.255 ± 0.04	BDL	0.2020 ± 0.01	–
Cu (mg L^{-1})	0.105 ± 0.05	0.105 ± 0.013	0.5110 ± 0.10	–
Fe (mg L^{-1})	3.99 ± 0.91	3.990 ± 0.47	1.203 ± 0.04	–
Ni (mg L^{-1})	2.84 ± 0.06	2.840 ± 0.38	0.1500 ± 0.02	–
Zn (mg L^{-1})	1.5 ± 0.30	1.500 ± 0.17	0.3330 ± 0.01	–
Hg (mg L^{-1})	–	–	0.8750 ± 0.03	–
Pb (mg L^{-1})	–	–	0.0148 ± 0.00	–

BL black liquor, *RGPPME* rayon grade pulp paper mill effluent, *PPME* pulp paper mill effluent, *BDL* below detection limit, *Cd* cadmium, *Cr* chromium, *Cu* copper, *Fe* iron, *Ni* nickel, *Zn* zinc, *Hg* mercury, *Pb* lead, *BOD* biological oxygen demand, *COD* chemical oxygen demand, *TSS* total suspended solid, *TS* total solid, *TDS* total dissolved solid, *TOC* total organic carbon, *TVS* total volatile solids, *EC* electrical conductivity, *PCP* pentachlorophenol, K^+ potassium, Na^+ sodium, Cl^- chloride, *AOX* adsorbable organic halides

recalcitrant to degradation and tend to persist in nature (Mishra and Thakur 2010). They are thus known as POPs and have been classified as "priority pollutants" by the USEPA as well as the "dirty dozen" group of POPs identified by the United Nations Environment Program. It is well-established that many of these contaminants are

Fig. 1.2 Wastewater discharged from the pulp and paper industry after secondary treatment. (**a**, **b**) deposition of sludge in aquatic bodies

acute and/or chronic toxins (Nestmanna and Lee 1985; Costigan et al. 2012). This has resulted in a growing concern about the potential adverse effects of genotoxicants on aquatic biota and public health through the contamination of drinking water supplies, recreational waters, or edible organic species.

Eventually, pulp is used to produce paper, but the short fibers are not retained within the paper production and are returned to the wastewater (Jenkins et al. 2003). These residual sludge fibers and other materials detrimental to paper production (e.g. filler, ink) are separated from the wastewater by decantation in the clarifier. Then, the sedimentation material is directed to the press where it becomes the sludge. The sludge is called primary sludge when it originates from the production of virgin wood fiber or deinked paper sludge when it is produced by removing inks from postconsumer fiber. The secondary sludge formed after treatment of wastewater by activated sludge process (Fig. 1.2). Paper production generates around 45% of wastewater sludge. The wastewater sludge is enriched with various fiber wood compounds such as lignin, carbohydrate polymers (cellulose and hemicellulose), and other extractives (lipids and others) in addition to some potentially toxic compounds such as chlorinated organics, resin acids, and heavy metals (Raj et al. 2007a). The heavy metals (HMs) in wastewater sludge are of major concern from the ecotoxicological risk perspectives. A variety of odorous compounds generated by secondary treatment units have also been reported, including sulfur compounds, wood-derived terpenes, and organic acids. These compounds contribute to the pungent stack emissions of total reduced sulfur and other compounds from pulp and paper mills (Watson et al. 2003).

1.3 Distribution and Structural Components of Lignin

Lignin is a major component of lignocellulosic biomass, and processed in enormous amounts in the pulp and paper industry worldwide. It is a complex heteropolymer, of para-hydroxyphenyl propane units linked together via a variety of ether and C–C bonds. Lignin is basically formed by the random coupling of radical species arising

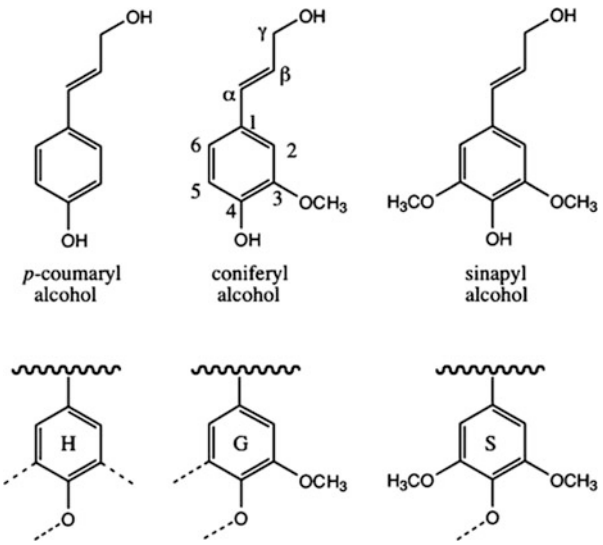


Fig. 1.3 Primary lignin monomers and corresponding unit (Wong 2009)

from the peroxidase-mediated dehydrogenation of three cinnamyl alcohol derivatives: p-coumaryl, coniferyl, and sinapyl alcohols. The corresponding phenylpropanoid units in the lignin polymer (known as lignin polymer units) are denoted as p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, respectively, based on the methoxy substitution on the aromatic rings (Fig. 1.3). The content of these three immediate biosynthetic precursor alcohols varies not only in different plant species but also in the different tissues of the same plant. In gymnosperms, the primary lignin precursors are the two monolignol conifery and p-coumaryl alcohols, while in angiosperms, sinapyl alcohol is also present (Garg and Modi 1999). It is closely associated with cellulose and covalently attached to hemicelluloses. The ether and C–C linkages present in lignin are not susceptible to hydrolytic attack, and therefore, lignin is highly resistant to breakdown (Bugg et al. 2011). Approximately 50–80% of all interunit bonds are β-O-4 ether bonds. In addition, subunits are connected by α-O-4 linkages, β-5 linkages, β-β linkages, 5–5 linkages, and biphenyl and diaryl ether structures. The double bond, conjugated with the aromatic ring, quinone methides, and quinone groups, is responsible for the color of their solution (van Driessel and Christov 2001). These chemicals are responsible for the dark color and toxicity of the wastewater discharged from the pulp and paper industry (Fig. 1.3). Lignin present in wood is converted to thio-lignin and alkali lignin in the kraft pulping. Chlorophenols from the pulp bleaching process are found both in free and bound forms in dissolved organic matter and particles; high- and low-molecular-weight chlorinated compounds are produced by complex reactions between chlorine and lignin in the wood pulp. Under natural conditions, these compounds are slowly degraded to various chlorinated phenolics which may be methylated under aerobic conditions. The low-molecular-weight phenolics and their methylated counterparts (which are more lipophilic) cause toxicity and

bioaccumulate in fish. The dark brown color not only is aesthetically unacceptable but also could inhibit the process of photosynthesis in natural aquatic environments due to the barrier effect of sunlight. To minimize the impact of effluents on the environment, several treatment technologies have been employed, although little is known on their efficiency to eliminate the toxicity attributed to the presence of organic compounds.

1.4 Environmental Fate of Pulp and Paper Industry Wastewater

The wastewater discharged from pulp and paper industry remains toxic and complex due to retaining of high color, BOD, COD, TDS, TSS, and also consisting of potentially toxic chlorinated compounds even after conventional secondary wastewater treatment processes (Raj and Chandra 2004; Emeka et al. 2011; Mishra et al. 2013; Wu et al. 2005). Due to high pollution load and color-contributing substances, pulp and paper wastewater poses a serious aquatic and soil pollution (Fig. 1.4). In aquatic ecosystem, the dense brown color of this wastewater inhibits the natural process of photosynthesis due to reduced penetration of solar radiation and decreases the dissolved oxygen level, which adversely affects flora and fauna and causes toxicity (Hall et al. 2009; Ojunga et al. 2010; Hewitt et al. 2008; Swamy et al. 2011; Ali and Sreekrishnan 2001). The toxicity assessment of pulp and paper wastewater on fish reproductive system has been reported by various workers (Parks et al. 2001; Orlando et al. 2002; Oakes et al. 2005; Wartman et al. 2009; Orrego et al. 2011; Martel et al. 2017; Hou et al. 2018). The short-term exposure of pulp paper mill wastewater to the flora and fauna of aquatic and terrestrial ecosystem has been observed by Verma (2008). Pathan et al. (2009) also showed the behavioral changes in freshwater fish *Rasbora daniconius* exposed to paper mill wastewater and further higher concentration created an adverse effect on fish. Tyor et al. (2012) also tested the toxicity of pulp and paper industry wastewater by using the *Daphnia* test model. Similarly, Pandey et al. (2012) showed the comparison of fish toxicity and Microtox toxicity of luminescence bacteria due to bleach plant effluent released from agro- and wood-based pulp and paper mills and also showed the impact of pulp paper mill wastewater on survival and hatchability of *Cyprinus carpio.* The result showed that paper mill effluent treated eggs hatch susceptibility and adverse effect and development stages are badly affected and effluent showed ultimately lethal effect. The color-causing organic compounds have also been implicated in the appearance of algal blooms (Dileká et al. 1999). The physicochemical properties of river water were analyzed by Emeka et al. (2011); Lacorte et al. (2003) attempted an overview of organic compounds that contribute to the toxicity of pulp and paper industry wastewater. Presence of organic compounds in the wastewater has contributed to deterioration of water quality due to the mixing of organic compounds in the recipient ecosystem, i.e., aquatic and terrestrial ecosystem. The effect of different pollutants present in pulp paper mill wastewater in long-term study at a multi-tropic level in aquatic communities receiving water bodies in the United States

Fig. 1.4 Environmental impact of secondary treated wastewater discharged from pulp and paper industry. (**a**–**c**) A view of the contaminated site showing aquatic pollution due to discharging of colored complex wastewater. (**d**, **e**) Irrigation of agricultural field through discharged effluent affecting the crop as well as soil microflora and texture

has been also evaluated. The study has shown the toxic effect on fish macrovertebrate, phytoplankton, and other flora and fauna (Hall et al. 2009). The primary reproductive effects in fish due to being exposed to pulp and paper wastewater were reported by Hewitt et al. (2008). The toxic effect of pulp and paper mill wastewater on phytoplankton and macroinvertebrates in River Nzoia, Kenya was studied by Ojunga et al. (2010). This study has concluded that the wastewater produce changes in both physicochemical parameters of the receiving water and contribute to nutrient loading, especially phosphorus and nitrate, on the deteriorating water quality and

eutrophication eliminates some taxa of both phytoplankton and macroinvertebrates, whereas others such as *Microcystis* sp. and *Chironomus* sp. appear to thrive in contaminated environment due to their tolerance to changing water quality. The genetic disturbance by pulp paper mill wastewater on large mouth bass (*Micropterus salmoides*) was reported by Denslow et al. (2004).

In a terrestrial ecosystem, the wastewater irrigated soil showed the decrease of moisture content and increase of pH as well as accumulation of heavy metals, i.e., Zn, Cu, Cd, Cr, and Pb in soil. The studies revealed that mill effluent has a deleterious effect on seed germination and growth parameter of rice and mustard and pea. It also has been noted that the effluent concentration above 50% was found inhibitory for plant growth parameter. Accumulation of contaminants into the terrestrial ecosystem is due to gradual percolation of contaminants which in turn changes the soil texture (Roy et al. 2008; Pradhan and Behera 2011; Kumar and Chopra 2011). In many developing countries, farmers irrigate their crop plants with water bodies which might be severely exposed to industrial effluents. This leads to risks of bioaccumulation of toxicants in the food chain. Thus, it is important to treat the industrial effluents before their final discharge. This continuous practice of irrigation of agricultural field through discharged effluent affecting the crop as well as soil texture (Medhi et al. 2008, 2011; Devkumari and Selvaseelan 2008). Pathan et al. (2009) reported that the toxicity of paper mill wastewater to fish *Rasbora daniconius* and its LC_{50} values were assessed for different concentration of effluent for 24–96 h exposure periods. In addition, the impact of paper mill wastewater on the survival and hatchability of eggs of *Cyprinus carpio* was reported by Tyor et al. (2012). However, the health hazards of polluted underground water due to pulp paper mill effluent in the vicinity of the pulp paper industry are not known so far.

These compounds, mostly complex aromatic in nature, also impart heavy toxicity to the aquatic systems, thus entering the food chain. Many researchers have reported that the mixing or direct entry of pulp paper mill effluent into the recipient ecosystem (aquatic and terrestrial ecosystem) is responsible for potential health hazards as mill wastewater mixing consequently increases the organic or inorganic compounds, i.e., enhancing or supporting the growth of numerous *total coliform*, *fecal coliform*, *Klebsiella* spp., *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *Enterobacter* spp., *Salmonella*, *Vibrio cholerae*, *Shigella* spp., *Citrobacter* sp., etc. (Huntley et al. 1766; Clark et al. 1992; Liss and Allen 1992; Megraw and Farkas 1993; Gauthier and Archibald 2001; Chandra et al. 2006). Beauchamp et al. (2006) investigated the thermotolerant coliform population of one paper mill effluent and two paper mill sludges and wood chips screening rejects using chromogenic media. Large numbers of thermotolerant coliforms, i.e., 7,000,000 MPN g^{-1} sludge (dry weight; d.w.), were found in combined sludges. From this first series of isolations, bacteria were purified on the MacConkey medium and identified as *Citrobacter freundii, Enterobacter* sp., *E. sakazakii, E. cloacae, Escherichia coli, K. pneumoniae, K. pneumoniae* subsp. *rhinoscleromatis, K. pneumoniae* subsp. *ozaenae, K. pneumoniae* subsp. *pneumoniae, Pantoea* sp., *Raoultella terrigena,* and

R. planticola. Second, the presence of thermotolerant coliforms was measured at more than 3700–6000 MPN g^{-1} (d.w.) sludge, whereas *E. coli* was detected from 730 to more than 3300 MPN g^{-1} (d.w.) sludge. The presence of thermotolerant coliform bacteria and *E. coli* was sometimes detected from wood chips screening rejects in large quantities. Also, indigenous *E. coli* were able to multiply into the combined sludge, and inoculated *E. coli* isolates were often able to multiply in wood chips and combined sludge media. This study points out that the coliform bacteria are introduced by the wood chips in the wastewater, where they can survive through the primary clarifier and regrow in combined sludges. Furthermore, Emeka et al. (2011) reported the variation in physicochemical dynamics due to the impact of paper mill wastewater that discharge into the Owerrinta River, Eastern Nigeria. Long et al. in 2012 from the United States have reported the characterizing paper mill wastewater using indicators and source-tracking methods. This study examined potential public health implications of *E. coli* in a Wisconsin river that receives paper mill wastewater upstream of a public beach. Furthermore, the effects of solid wood waste discharge on the physicochemical and microbial identification of the Warri River were reported by Idise et al. in 2012 from Nigeria. Lee et al. (2012) carried out some significant work where they have conducted a survey and reported the effect on the skin and health of children living in upstream and downstream villages from a pulp and paper mill. This study has reported that the ill effect on children who drank water directly from the river was compared with those who never did. River water analysis has shown physicochemical variation within the acceptable range except for fecal coliform (6 MPN/100 mL). Moreover, Lee et al. (2012) surveyed and observed that the pulp and paper mill wastewater has created health-related problems to the downstream population of the river.

1.5 Biological Treatment Methods of Pulp and Paper Industry Wastewater

Pulp and paper industry is a very water-intensive industry in terms of freshwater use. Currently, the increasing needs to reduce water consumption and to satisfy tightened discharge standards in stringent environmental regulations have forced pulp and paper industries to treat their effluent for safe disposal in environment using advanced treatment processes. Most wastewater treatment processes (WWTPs) use aerobic and/or anaerobic biological processes to remove organic contaminants in wastewaters (Singh and Thakur 2006). The commonly available biological treatment methods adopted in the pulp and paper industry to lower the pollution load indices like BOD and COD include anaerobic lagoon, stabilization pond, aerated lagoon, activated sludge process, or its modification depending on the local conditions. Aerobic processes are preferably used in most pulp and paper mills because of their ease of operation as well as the relatively low capital and operating costs.

1.5.1 Aerobic Treatment Process

Among aerobic technologies, AS and aerated lagoons are commonly used wastewater treatment approach applied in pulp and paper industry (Fig. 1.5; van Ginkel et al. 1999; Erkan and Engin 2017; Pokhrel and Viraraghavan 2004; Lerner et al. 2007). Despite the widespread usage, these technologies still suffers from instability, high sludge production, and high operating cost. The most important operational difficulty associated with activated sludge is the separation of sludge from the clarified wastewater. Implications of conventional AS process used for pulp and paper industry wastewater with modification to a low sludge production (LSP) process have been studied for treating (Talat Mahmood et al. 2006). The LSP system produced 36% less sludge than the base case system, while both systems removed 96% BOD, 73% COD, and 56% AOXs from a bleach kraft mill wastewater. The LSP system required approximately 25% higher aeration than the conventional activated sludge system. The LSP sludge settled much better than the conventional activated sludge and had superior dewatering properties. This could lead to settling and dewatering chemical cost savings. The odorous compound released from pulp and paper mill wastewater and their reduction were also investigated by Watson et al. (2003). They reported that the AS may be helpful for reduction of odorous gases. However, the AS and aerated lagoons are not able to effectively mitigate the pollution load of pulp and paper mill wastewater. Because the microorganisms present in the conventional activated sludge system are not effective in degrading compounds like lignin, therefore, complete treatment of such wastes remains elusive.

Fig. 1.5 A view of the aerated activated sludge treatment of pulp and paper industry wastewater

1.5.1.1 Bioaugmentation/Biostimulation Process for Efficient Treatment of Pulp Paper Effluent

Bacterial Bioaugmentation/Biostimulation

In the recent past, biotechnological approaches for the remediation of contaminated environment have gained worldwide attention (Chandra et al. 2018a, b, c, d, e; Kumar and Chandra 2020a, b; Perestelo et al. 1989; Morii et al. 1995; Thakur 2004). Bioremediation is considered a cost-effective and environment-friendly technology with great potential to remove target compounds from contaminated sites or for treatment of wastewater (Malaviya and Rathore 2007; Raj et al. 2014a, b; Kumar et al. 2018; Kumar and Chandra 2020a, b; Chandra and Kumar 2017a, b). It is a set of techniques that improve the degradation capacity of contaminated areas (Chandra and Kumar 2015b). They use bioaugmentation strategy (introduction of specific degradable strains or consortia of microorganisms) (Yu and Mohn 2002; Yadav et al. 2016) or biostimulation strategy (introduction of nutrients, inducers, and oxygen) (Chandra et al. 2018a). Bioaugmentation is the introduction of a group of natural or genetically engineered microorganisms to decontaminate soil and water (Chen et al. 2012b). Comparing with the common biotreatment process, the inoculated indigenous or allochthonous microbial strains can enhance the biodegradation of target pollutants, serving to strengthen or complement the metabolic capabilities of the indigenous microbial community (Mishra et al. 2014). An important factor for successful bioaugmentation is the selection of potential bacteria that can not only degrade contaminants but can also adapt to an adverse environment, usually higher toxicity of the contaminated area (Dudášová et al. 2014). The major bacterial species successfully used in bioaugmentation and biostimulation processes for kraft lignin degradation and decolorization of pulp and paper industry wastewater are *Paenibacillus* sp., *Aneurinibacillus aneurinilyticus, Bacillus* sp. (Raj et al. 2007a, b, 2014a), *Serratia marcescens*, *Citrobacter* sp., *Klebsiella pneumonia* (Raj et al. 2007a, b; Chandra and Abhishek 2011), *Pseudomonas, Bacillus, Pannonibacter, Ochrobactrum* (2011), *Bacillus megaterium*, *Pseudomonas aeruginosa* (Tiku et al. 2010), *Novosphingobium* sp., *Aeromonas formicans* (Gupta et al. 2001), *Pseudomonas fluorescens* (Chauhan and Thakur 2002), *Comamonas* sp. B-9 (Chen et al. 2012a) *Pseudomonas, Ancylobacter,* and *Methylobacterium* (Keharia and Madamwar 2003). Yu and Mohn (2002) successfully used the *Zoogloea resiniphila* DhA-35 in the bioaugmentation treatment of resin acid containing pulp and paper mill wastewater. Similarly, Muttray et al. (2001) used *Pseudomonas abietaniphila* BKME-9 for the treatment of resin acid in the pulp and paper mill wastewater. Chauhan and Thakur (2002) treated pulp and paper mill wastewater in a fixed-film bioreactor by *P. fluorescens* and noted reductions of 45% lignin, 75% color, 79% COD, and 66% phenol within 15 days of incubation. Removal of organochlorine from bleached kraft pulp and paper mill wastewater by dehalogenating indigenously grown *Pseudomonas*, *Ancylobacter*, and *Methylobacterium* strains was reported by Fulthorpe and Allen (1995). Keharia and Madamwar (2003) compared the degradation potential of *Pseudomonas, Ancylobacter*, and *Methylobacterium* strains for organochlorine from bleached

kraft pulp and paper mill wastewater. They observed that *Ancylobacter* showed the broad substrate range but could significantly reduce the AOXs from softwood wastewater only, whereas *Methylobacterium* with limited substrate range was capable of degrading AOXs from both hardwood and softwood effluents. Singhal and Thakur (2009a, b) reported the decolorization and detoxification of pulp paper mill wastewater under un-optimized and optimized conditions by *Cryptococcus* sp. This bacterial isolate reduced the 27% color and 24% lignin content of the wastewater in 15 days under un-optimized conditions. However, enhanced reduction in color (50–53%) and lignin (35–40%) was noted to occur after optimum treatment conditions were reached during the 24 h incubation: pH 5.0, temperature 35–40 °C, shaking speed 125 rpm, dextrose 1.0% w/v, tryptone 0.1% w/v, and inoculum size 7.5% v/v. Recently, *Halomonas* sp. and *Bacillus* sp. have been used for BL degradation and decolorization at high pollution load (Yang et al. 2008). Singh et al. (2011) reported bioremediation of pulp and paper mill wastewater by a tannic acid-degrading bacterium *Enterobacter* sp. Prior to the bioremediation of wastewater, authors optimized various parameters, viz., inoculum size, agitation, temperature, and treatment duration by using Qualitek-4 software. In batch culture experiment, the reduction of lignin up to 73% and color up to 82% along with COD and BOD with 16 h retention time was observed. Mishra and Thakur (2010) isolated *Bacillus* sp. from pulp and paper mill sludge and used this isolate in degradation and decolorization of BL. They noted that maximum color was removed at pH 8, temperature 35 °C, shaking speed 200 rpm, sucrose 2.5%, and inoculum size 5% (w/v) within 48 h from 10% BL. However, after optimization of various nutritional and environmental parameters by using the Taguchi approach, twofold increase in the removal of color and lignin from 25–69% and 28–53%, respectively, was noted. This study indicated the significance of Taguchi's approach in decolorization and delignification of lignin in pulp and paper mill wastewater. Chandra and Abhishek (2011) studied the decolorization of BL in axenic and mixed condition by isolated bacterial strains, i.e., *Citrobacter freundii* and *Citrobacter* sp., and characterized their metabolites. Under mixed culture condition, the aerobic treatment could reduce 79% AOX, 79% color, 82% COD, and 60% lignin after 144 h of the incubation period. It was also observed that mixed bacterial culture produced the optimum level of peroxidase enzyme compared to axenic bacterial strain. The comparative GC–MS analysis of control and degraded BL revealed that along with lignin fragment, some chlorophenolic compounds, 2,4,6-trichlorophenol, 2,3,4,5-tetrachlorophenol, and pentachlorophenol, were detected in BL degraded by axenic culture, whereas these chlorophenolic compounds were completely absent in BL degraded by mixed bacterial culture (Table 1.2). Similarly, the decolorization of BL by a potential bacterial consortium consisting of *S. marcescens*, *Citrobacter* sp., and *Klebsiella pneumoniae* under optimized environmental and nutritional conditions has been reported by Chandra et al. (2011a). The study has shown that bacterial growth and BL degradation were associated with ligninolytic enzyme production and numerous metabolites were also detected in bacterial degraded BL (Table 1.2; Chandra et al. 2011a, b). The pulp and paper mill wastewater decolorization and detoxification by using the different inoculums ratio in mixed bacterial culture have been evaluated at laboratory scale (Chandra et al. 2011b). This study deals with the degradation and

detoxification of pulp paper mill wastewater by three bacterial strains, i.e., *S. marcescens*, *S. liquefaciens*, and *Bacillus cereus* in different ratios, and found that two ratios, 4:1:1 and 1:4:1, were effective for the degradation of pulp and paper mill wastewater. These ratios reduced the various pollution parameters from pulp and paper mill wastewater. HPLC and GC–MS analysis also showed that the mixed bacterial culture in 4:1:1 ratio degraded 95% of lignin and 98% of chlorophenols, and several other related compounds, whereas ratio 1:4:1 reduced lignin and chlorophenols up to 84% and 58%, respectively, after 7 days of incubation (Table 1.2). Chandra and Singh (2012) also studied the decolorization and detoxification of rayon grade pulp paper mill wastewater in different nutritional as well as environmental parameters by a developed bacterial consortium comprising *S. marcescens*, *Citrobacter* sp., and *K. pneumoniae* strains. The degradation study result showed that the ligninolytic activities were found to be growth associated and the developed bacterial consortium was efficient for the reduction of color, BOD, and COD up to 85%, 74%, and 83%, respectively. The GC–MS analysis also showed that most of the compounds detected in untreated wastewater were diminished after bacterial treatment, while formic acid hydrazide, 4-cyclohexane-1,2-dicarboxylic acid, carbamic acid, 1,2-benzenedicarboxylic acid, and erythro pentanoic acid were found as new metabolites. Simultaneously, Chandra and Singh (2012) also reported the decolorization and detoxification of rayon grade (RG) pulp paper mill effluent by mixed bacterial culture comprising *Pseudochrobactrum glaciale, Providencia rettgeri,* and *Pantoea* sp. The results showed that mixed culture effectively reduced color, COD, and BOD up to 96.02%, 91%, and 92.59%, respectively, from pulp paper mill effluent within 216 h of the incubation period. During degradation and decolorization, maximum enzyme activity for lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase was recorded at 48, 72, and 144 h of the incubation period, respectively. Further, GC–MS analysis revealed that majority of the compounds present in the untreated sample were completely removed and only a few metabolites were generated after bacterial treatment (Table 1.2). A mammalian cell line-based toxicological evaluation of pulp and paper mill BL biodegraded in a soil microcosm by indigenous alkalotolerant *Bacillus* sp. was reported by Mishra et al. (2014). GC–MS analysis performed after biodegradation showed the formation of simpler compounds like *p*-hydroxyhydrocinnamic acid, homovanillic acid methyl ester, and 3,5-dimethoxy-*p*-coumaric alcohol. The methyltetrazolium assay for cytotoxicity, 7-ethoxyresorufin-O-deethylase assay for dioxin-like behavior, and alkaline comet assay for genotoxicity evaluation were carried out with the human hepatocarcinoma cell line HuH-7 before and after bacterial treatment. The result revealed that bioremediation for 15 days reduced toxicity, as shown by a 139-fold increase in BL LC_{50} value, a 343-fold reduction in benzo(a)pyrene equivalent value, and a fivefold reduction in the olive tail moment. Similarly, Haq et al. (2016, 2017) evaluated the bioremediation potentiality of ligninolytic enzyme producing *S. liquefaciens* for detoxification of wastewater discharged from pulp and paper industry after secondary treatment and characterized their metabolic products. The bacterium *S. liquefaciens* effectively reduced color, lignin, COD, and phenol of real

Table 1.2 Identified organic compounds present in pulp and paper industry wastewater and their degradation and characterized metabolic products after bacterial treatment (Chandra and Singh 2012; Chandra and Abhishek 2011; Chandra et al. 2011a, b; Haq et al. 2016, 2017)

Effluent	S. no.	Name of identified compound	UW	BTW
Rayon grade pulp paper mill wastewater	1.	4-Isopropoxy-butyric acid	+	–
	2.	3,7,11,15,18-pentaoxa-2,19-disilaneicosane	+	–
	3.	Butane-1-ol	+	–
	4.	Propane	+	–
	5.	2-methyl-2,4-dimethoxy butane	–	+
	6.	4,5-octanediol,3,6-dimethyl	–	+
	7.	Diphenylthiocarbazide	+	–
	8.	Propane,1-(1-Ethoxyethoxy)	+	–
	9.	Cyclohexanecarboxylic acid	–	+
	10.	6-Oxabicyclo9,3,1,0,0hexan-3-one	+	–
	11.	Pyrrolo(1,2A)pyrazine-1,4-dione, hexahydro	+	–
	12.	Pyrrolo(1,2A)pyrazine-1,4-dione, hexahydro	+	–
	13.	Trichloroacetyl isocyanate	+	–
	14.	1-Phenyl-1-nonyne	–	+
	15.	Tetradecanoic acid	+	–
	16.	6-Chlorohexanoic acid	+	–
	17.	2,5-Piperazinedione,3,6-bis(2-methyl propyl)	+	+
	18.	Pyrrolo(1,4-dione,hexahydro-3-(phenyl methyl)	+	–
	19.	1-Chloro Octadecane	+	–
	20.	1,2 Benzene carboxylic acid	+	–
	21.	4,8-Dimethyl undecane	+	–
	22.	3-Trifluoroacetoxydodecane	+	–
	23.	Benzeneacetic acid,3-tetradecyl ester	+	–
	24.	Cyclo-(L-leucyl-l-phenylalanyl)	+	–
	25.	Butanoic acid	+	–
Black liquor	1.	Propanoic acid	–	+
	2.	Acetic acid	–	+
	3.	Butanoic acid	–	–
	4.	Benzoic acid	–	+
	5.	2,4,6 trichloro phenol	–	–
	6.	2,3,4,5 tetrachloro phenol	–	–
	7.	Tetradecanoic acid	–	+
	8.	Pentachlorophenol	–	–
	9.	Dibutyl phthalate	–	+
	10.	Hexadecanoic acid	–	–
	11.	Octadecanoic acid	–	+
	12.	Bis(2-ethylhexyl) phthalate	+	+
Black liquor	1.	Propanoic acid	+	–
	2.	Formic acid hydrazide	–	
	3.	4-Cyclohexane-1,2-dicarboxylic acid	–	

(continued)

Table 1.2 (continued)

Effluent	S. no.	Name of identified compound	UW	BTW
	4.	1,2 Butanediol	+	–
	5.	Carbamic acid	–	
	6.	3-Cyclohexane 1-methanol	+	–
	7.	2-Methoxy phenol (Guaiacol)	+	–
	8.	4-Methyl benzaldehyde	+	–
	9.	Benzoic acid	+	–
	10.	Benzene acetic acid	+	–
	11.	Benzylemalonic acid	+	–
	12.	3-Hydroxy-4-methoxymandilic acid	+	–
	13.	Butylated hydroxytoluene	+	–
	14.	2,4-Bis (1,1-dietyl)-phenol	+	–
	15.	Heptadecanoic acid	+	–
	16.	2-Methoxy propanoyl chloride	+	–
	17.	4-Hydroxy-3,5-dimethoxy benzaldehyde	+	–
	18.	Tetradecanoic acid	+	–
	19.	1,2-benzenedicarboxylic acid	–	+
	20.	Dibutyle phthalate	+	–
	21.	Erythropentanoic acid	–	+
	22.	Ricinoleic acid	+	–
	23.	Phthalate	+	+
	24.	Cholesterol trimethylsilyl ether	+	+
	25.	1,1-(1,2-ethanediyl) bis[4-methoxy] benzene	+	–
	26.	2,4-Bis (1-phenylethyl)-pheno	+	–
	27.	Bis (2-ethylhexyl) phthalate	+	–
Pulp and paper mill wastewater	1.	Propanoic acid	+	+
	2.	Benzeneacetonitrile	–	–
	3.	Pyridine	–	–
	4.	Phosphoric acid	+	–
	5.	1[(Formyl)oxymethyl]benzene	–	+
	6.	(+)-5-Hydroxy-6-(1-hydroxyethyl)-2,7-dimethoxynapthoquinone	+	–
	7.	l-(+)-Tartaric acid, bis(trimethyl silyl) ether, bis(trimethyl silyl)ester	+	–
	8.	3-Octadecene, (E)-	+	–
	9.	Uric acid	–	+
	10.	D-Fructose, 1,3,4,5,6-pentakis-O-(trimethyl silyl)-O, methyloxime	+	–
	11.	(2 R,3 S)-2-[(E)-2-(Ethoxycarbonyl)ethenyl]-2, 3-dimethylaziridine	–	+
	12.	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	–	+
	13.	1,4-Diazo-2,5-dioxo-3-isobutyl bicyclo (4.3.0) nonane	–	+
	14.	1-Octadecene	+	–

(continued)

Table 1.2 (continued)

Effluent	S. no.	Name of identified compound	UW	BTW
	15.	Hexadecanoic acid	+	–
	16.	1-Monolinoleoyl glycerol trimethyl silyl ether	+	–
	17.	1-Heneicosanol	+	–
	18.	Octadecanoic acid	+	–
	19.	Tetracosanic acid	+	–
	20.	A-D-Galactopyranoside, methyl 2,3-bis-o-(trimethyl silyl)-, cyclic methylbronate	+	–
	21.	2′-4′-6′-Trinitro-5′-phenyl-1,1′:3′,1″-terphenyl	+	–
	22.	N,N′-Dicyclohexyl-1-cyano-7-pyrrolidinylperylene-3,4:9,10-tetracarboxylic acid	+	–
Pulp paper mill wastewater	1.	1-O-Pentadecylglycerol	+	–
	2.	Glycerol	–	+
	3.	L-Glutamic acid	–	+
	4.	Iron,tricarbonyl(N-(phenyl-2-yridinylmethylene) benzenamine)	+	–
	5.	Butanal	–	+
	6.	Hexanedioic acid	+	–
	7.	D-galactofuranose	+	–
	8.	D-Fructose	–	+
	9.	D-Glucose	+	–
	10.	D-Gluconic acid	–	–
	11.	D-Mannitol	+	–
	12.	Glucopyranose	+	–
	13.	1,6,8-trihydroxy-2-isopropyl-3-methoxy-9,10-anthraquinone	–	+
	14.	2,4-dimethoxyphenyl	+	–
	15.	2,6-Dinitro-4,40-di-tert-butylbiphenyl	–	+
	16.	Diethyl 3,4-dihydro-2-nepthyl-phosphonate	+	–
	17.	4,6-dimethoxy-2,3-dimethyl	+	–
	18.	2,4,6-trinitro-5-phenyl	+	–
Pulp paper mill wastewater	1.	2-Ethoxyethoxy-Trimethylsilane	–	+
	2.	Propylene carbonate	–	+
	3.	Butanoic acid,2-oxo (acid)	–	+
	4.	Methanediamine,N,N,N,N-tetramethyl	–	+
	5.	2-Ethoxyethoxy-trimethylsilane	–	+
	6.	Butane,2Ethoxy-	–	+
	7.	Diphenylthiocarbazide	–	+
	8.	1-(2,4-Diethoxy-phenyl)Ethanone	–	+
	9.	1,4-Dimethoxy-2-Phenylbutane(phenol)	–	+
	10.	Oxalic acid,Cyclobutyl heptadecylester (cyclo)	–	+
	11.	8-Pentadecanone(ketone)	+	–

(continued)

Table 1.2 (continued)

Effluent	S. no.	Name of identified compound	UW	BTW
	12.	1,2Benzenedicarboxylic acid,Bis (2-Methylpropyl) Ester	+	−
	13.	1-Phenyl-1-nonyne(surfactant)	−	+
	14.	Sulphurousacid,Octadecyl 2-Propylester	+	−
	15.	Benzene,1,3-Bis(1-methylethenyl)	−	+
	16.	3-Ethenyl-6-Dimethylaminomethyleneaminobenzonitrile	−	+
	17.	N-(3-Bromo-1-Methyloxycarbonyl-1H-Indol-2-YLmethyl)-N-(1-Methoxycarbonyl-2-methybutyl	−	+
	18.	Proponoic acid,2-(Benzoylamino)-333 Trifluro-2-[(Triflurомethyl)phenyl]amino-ethyl	−	+
	19.	Butane,2-phenyl-3-(trimethylsilyloxy)	−	+
	20.	2-Propanoic acid,3(4-Methylphenyl)-, ethylester	−	+
	21.	2-Propanoic acid,3-(MethylPhenyl), Ethylester	−	+
	22.	Phthalicacid,Dodecyl 2-Ethylhexylester	−	+

+ present, − absent, *UE* untreated effluent, *BTE* bacterial treated effluent

wastewater after 144 h of treatment at 30 °C, pH 7.6, and 120 rpm. Further, the bacterium-treated effluent was evaluated for residual toxicity assessment by alkaline single-cell (comet) gel electrophoresis (SCGE) assay using *Saccharomyces cerevisiae* MTCC 36 as a model organism. The toxicity reduction to treated effluent was found up to 49.4%. They also characterized the major metabolic products during bacterial treatment of pulp paper mill wastewater as shown in Table 1.2. Tiku et al. (2010) also reported the holistic bioremediation of pulp mill wastewater using three autochthonous bacteria strains, *P. aeruginosa*, *and B. megaterium*, to reduce the BOD and COD level of such wastewater up to permissible level, i.e., 30 mg L^{-1} and 250 mg L^{-1}, respectively, within a retention time of 24 h in batch culture. However, the continuous mode of treatment may further decrease the retention time. A concomitant reduction in TDS, AOXs, and the color was also observed. The bacterial degradation of lignin is limited compared to fungi.

Fungal Bioaugmentation/Biostimulation

Fungi are the only microorganisms studied extensively for the degradation and decolorization of lignin and its related monomers (Hofrichter 2002). The use of fungi has a great potential for tertiary treatment and removal of residual organic compounds in wastewater discharged from pulp and paper industries (Wu et al. 2005; Apiwattanapiwat et al. 2006; Da Re and Papinutti 2011; Rajwar et al. 2017). White-rot fungi, such as *Phanerochaete chrysosporium* (Zouari et al. 2002; Mittar et al. 1992; Wu et al. 2005), *Trametes* (*Coriolus*) *versicolor*

(Martin and Manzanares 1994; Manzanares et al. 1995; Modi et al. 1998; Garg and Modi 1999; Bajpai et al. 19,993; Mehna et al. 1995; Archibald et al. 1990), *P. radiata* (Lankinen et al. 1991; Hatakka 2001), *Marulius tremellosus* (Lankinen et al. 1991), *Rhizomucor pusillus* (van Driessel and Christov 2001), *Lentinus edodes* (Esposito et al. 1991; Wu et al. 2005), *Pleurotus* spp., *P. sajor-caju*, *P. platypus*, *P. citrinopileatus* (Ragunathan and Swaminathan 2004), *Steccherinum* sp. (Da Re and Papinutti 2011), *Datronia* sp. (Chedchant et al. 2009), and *Trichaptum* (Apiwattanapiwat et al. 2006), have been reported to be effective in reducing the various pollution parameters of pulp and paper industry wastewater. Decolorization and detoxification of extraction-stage effluent from chlorine bleaching of kraft pulp by *Rhizopus oryzae* have been investigated by Nagarathnamma and Bajpai (1999). Table 1.3 shows the analytical results for the effluent sample. A total of 37 standards of chlorophenols and chloroaldehydes were run, and 13 types of chlorophenols and three types of chloroaldehydes were found in the extraction-stage effluent (Table 1.3). *R. oryzae* was found to decolorize, dechlorinate, and detoxify bleach plant effluent at lower co-substrate concentrations. With glucose at 1 g L^{-1}, this fungus removed 92–95% color, 50% COD, 72% AOXs, and 37% EOXs in 24 h at pH of 3–5 and temperatures of 25–45 °C, although the fungus removed up to 78% of the color without added co-substrate.

Bioremediation of pulp and paper industry wastewater by a novel fungal consortium, comprising two basidiomycetous fungi (*Merulius aureus* syn. *Phlebia* sp. and an unidentified genus) and a deuteromycetous fungus (*Fusarium sambucinum* Fuckel MTCC 3788), isolated from pulp and paper mill wastewater-affected soils in immobilized condition was assessed by Malaviya and Rathore (2007). First, these fungus isolates were immobilized on nylon mesh, and the developed consortium was further used for the treatment of pulp and paper mill wastewater in a continuously aerated benchtop bioreactor. The treatment resulted in the reduction of lignin, color, and COD of the wastewater in the order of 79.0%, 78.6%, and 89.4% in 4-day incubation period. A major part of reductions in lignin, color, and COD of the wastewater occurred within the first 24 h of the treatment, which was also characterized by a steep decline in the pH of the wastewater. Singhal and Thakur (2009a) evaluated the efficiency of the biological treatment process for the decolorization and detoxification of pulp and paper mill wastewater for its safe disposal in the environment. In this study, they used *Emericella nidulans* var. *nidulans* for the treatment process. The process parameters for optimum decolorization of pulp and paper wastewater were optimized by the Taguchi approach. Decolorization of wastewater was improved by 31% with reduction in 66.66% color and 37% lignin after treatment by *E. nidulans* var. *nidulans* in batch culture. Variation in pH from 6.0 to 5.0 had the most significant effect on decolorization (71%), while variation in temperature from 30 to 35 °C had no effect on the process. Later, treated effluent was evaluated for genotoxicity by alkaline single-cell gel electrophoresis assay using *Saccharomyces cerevisiae* MTCC 36 as a model organism, indicating a 60% reduction in toxicity. Rocha-Santos et al. (2010) also evaluated the effects of a tertiary treatment by fungi (*Pleurotus sajor-caju*, *T. versicolor*, *P. chrysosporium*, and *R. oryzae*) on individual organic compounds of a *Eucalyptus globulus* bleached

Table 1.3 Identified organic pollutants from pulp and paper industry wastewater and their degradation by fungi (Nagarathnamma and Bajpai 1999; Rajwar et al. 2017)

S. no.	Identified compounds	UW	TW	S. no.	Identified compounds	UW	TW
1.	1–3-Dimethyl benzene	−	+	1.	2-Chlorophenol	+	−
2.	Acetic acid	−	+	2.	4-Chlorophenol	+	+
3.	Phenol	−	−	3.	2,6-Dichlorophenol	+	−
4.	1-Methyl-4-(1-methylethenyl)-cyclohexene	+	−	4.	5-Chloroguaiacol	+	−
5.	Diethylene glycol monoacetate	−	−	5.	4-Chlorocatechol	+	−
6.	3,7-Dimethyl-1,6-octadien-3-ol	+	−	6.	4,6-Dichloroguaiacol	+	+
7.	4-Tert-butyl-2-methylphenol	−	−	7.	4,5-Dichloroguaiacol	+	−
8.	Diethylene glycol diacetate	−	−	8.	3,5-Dichlorocatechol	+	+
9.	(−)-β-Caryophyllene	+	−	9.	3,4,6-Trichloroguaiacol	+	−
10.	4-Hexen-2-one,5-phenyl	+	−	10.	4,5-Dichlorocatechol	+	−
11.	Hexadecane	−	+	11.	3,4,5-Trichloroguaiacol	+	−
12.	Nonadecane	−	−	12.	4,5,6-Trichloroguaiacol	+	+
13.	Phytane	−	+	13.	Tetrachloroguaiacol	+	+
14.	Diisobutyl phthalate	+	+	14.	2-Chlorosyringaldehyde	+	+
15.	Pentadecanoic acid	−	+	15.	Trichlorosyringaldehyde	+	−
16.	Butyl phthalyl butyl glycolate	+	−	16.	2,6-Dichlorosyringaldehyde	+	−
17.	Eicosane	−	+	17.			
18.	Benzene-1,2-dicarboxylic acid	+	−	18.			
19.	Octyl phthalate	+	−	19.			
20.	Butyl-octyl-diphenylamine	+	−	20.			
21.	Tetracontane	−	+	21.			

+ present, − absent, *UW* untreated wastewater, *TW* fungi treated wastewater

kraft pulp and paper mill wastewater discharged after secondary treatment. A total of 38 compounds (carboxylic acids, fatty alcohols, phenolic compounds, and sterols) were detected and quantified in the *E. globulus* bleached kraft pulp mill final effluent discharged after secondary treatment. The four fungus species showed an adequate capacity to eradicate organic compounds and color from wastewater. Biodegradation of pulp and paper mill wastewater by co-culturing ascomycetous fungi in the repeated batch process has also been studied by Rajwar et al. (2017). A fungal consortium (consisting *Nigrospora* sp. and *Curvularia lunata*) exhibited enhanced biomass production under optimized medium conditions and significantly reduced color (82.3%), BOD (85.6%), COD (80%), and lignin concentration (76.1%) under catalytic enzyme activity; however, unutilized Lac, MnP, and LiP activities were observed to be 13.5, 11.4, and 9.4 U mL^{-1} after the third cycle of wastewater treatment in repeated batch process. The GC–MS analysis also showed the reduction of complex organic compounds and the formation of numerous low-molecular-weight metabolites. This indicated the massive potential of the novel fungal consortium to degrade recalcitrant organic pollutants. Biological treatment of pulp and paper industry wastewater by oleaginous yeast *Rhodosporidium kratochvilovae* was integrated with production of biodiesel as reported by Patel et al. (2017). *R. kratochvilovae* has the remarkable efficiency to reduce the toxicity of phenols (99.60%) and lignin (94.27%), respectively, from the wastewater with a high reduction in COD (94.22%), BOD (77.36%), and TDS (84.59%). The integrated process establishes toxic removal from pulp and paper industry wastewater along with sustainable biodiesel production for transportation fuels.

Algal Treatment (Phycoremediation)

Microalgal culture offers a cost-effective approach to remove nutrients from wastewater discharged from pulp and paper industry after secondary treatment (Saikia et al. 2010, 2011) . Microalgae have a high capacity for inorganic nutrient uptake, and they can be grown in mass culture in outdoor solar bioreactors. Dileká et al. (1999) reported the removal of color and AOXs from pulping effluent by mixed culture of algae obtained from the oxidation pond of the wastewater treatment plant. The mixed culture of algae was composed mainly of *Chlorella*, *Chlorococcum*, and *Chlamydomonas* species. Besides these, *Microcystis* and *Anabaena* species were present to a somewhat lesser extent, and a few species of *Euglena, Phacus, Nitzschia, Cyclotella, Pandorina, Eudorina, Gonium,* and *Prymnesium* were also observed. For the total mill effluent (composed of both pulping and bleaching effluents), AOX removal was found to be independent of initial color value and was around 70%. Up to 80% removal of color from pulping effluent was achieved within 30 days under continuous lighting conditions. It was found that algae reduced the color of pulping of relatively low initial color more efficiently than that of high initial color. Under simulated field lighting conditions, up to 60% color removal from pulping effluent was observed after 60 days of exposure, whereas for the total mill effluent, it was up to 64% after 45 days of incubation. Tarlan et al. (2002a) reported 58% of COD, 84% of color, and 80% of AOXs removal from pulp and

paper industry wastewaters by using some green algae (*Chlorella*), and diatom species were dominant in the treatment. This study also showed that algae grew mixotrophically, and the main mechanism of color and organics removal from pulping effluents was partly metabolism and partly metabolic conversion of colored and chlorinated molecules to noncolored and non-chlorinated molecules. In a separate study, Tarlan et al. (2002b) treated highly polluted pulp and paper industry wastewaters in sequential batch reactors (SBR) by using algae and found up to 74% COD and 74% color removal in about 40 days of incubation batch studies. From the preliminary SBR experiments, filling period was found to be a critical step affecting the overall efficiency when mixing and aeration are applied during filling. For all filling periods, COD, color, and AOXs removal efficiencies increased with increasing filling time. Maximum removal efficiencies achieved were 60–85% for COD, 42–75% for color, and 82–93% for AOXs for the filling periods of 4–12 days. Authors stated that organics in the contaminated wastewater were both chlorinated and non-chlorinated; algae removed these contaminants mainly by metabolism, and chlorine cleavage from chlorinated organic molecules was more rapid than the degradation of non-chlorinated and colored organics. Adsorbed lignin on algal biomass was found to be varying between 10 and 20% depending on filling period applied. Most recently, removal of nutrients and organic pollution load from pulp and paper industry wastewater by microalgae in outdoor open pond has been reported by Usha et al. (2016). In this lab study, a mixed culture of microalgae, containing two *Scenedesmus* species, was used for pulp and paper mill wastewater treatment and microalgal cultivation, and result showed a maximum of 82% and 75% removal of BOD and COD, respectively. The author recommended that pulp and paper mill wastewater could be used effectively for cultivation of microalgae to minimize the freshwater and nutrient requirements.

1.5.2 Anaerobic Treatment

Many highly chlorinated compounds are known to be quite stable and difficult to degrade. However, anaerobes can sometimes catalyze biotransformation reactions in which chloride ions of the chlorinated compounds are displaced by protons (Chandra and Kumar 2015b). The more chloride ions are thus removed, the more reactive the resultant compounds become, thereby rendering them susceptible to conventional AS treatment. The anaerobic treatment provides advantages of pollution decreasing with energy production. Anaerobic digestion is a process frequently employed for the secondary treatment of industrial wastewater. It has many potential advantages in comparison with aerobic treatment such as lower sludge production, lower chemical consumption, smaller land requirements due to smaller reactors, and energy production in the form of methane. Anaerobic technologies provide good treatment efficiencies at low hydraulic retention times. However, it was reported that anaerobic microorganisms are more sensitive to toxic substances than aerobic microorganisms when anaerobic treatment is utilized for bleached kraft wastewaters (Johansson

2012; Lin et al. 2012). For this reason, several authors investigated aerobic membrane bioreactor (MBR) for pulp and paper mill wastewater treatment, and it was reported that the COD removal efficiencies were found to be between 86% and 99.5% (Bérubé and Hall 2000; Galil et al. 2003; Dias et al. 2005; Gommers et al. 2007; Lerner et al. 2007; Zhang et al. 2009; Savant et al. 2006). Lafond and Ferguson (1991) reported that anaerobic treatment in an upflow hybrid reactor removed 17–40% of AOXs. Similarly, Mishra et al. (2016) compared the effluent treatment efficiency of a hybrid unit of upflow fixed-bed anaerobic bioreactor (UFBAB) along with slow sand filter (SSF) with the single-unit UFBAB for paper and pulp mill wastewater. The hybrid system showed better treatment efficiency as the SSF provides a polishing effect to the effluent generated after the UFBAB treatment. Erkan and Engin (2017) used a submerged membrane bioreactor (sMBR) to eliminate dissolved substances present in paper mill wastewater. In this study, an sMBR was operated for the treatment of paper mill industry wastewater at 35 h of HRT and 40 days of SRT. The COD, ammonical nitrogen (NH_3–N), and total phosphorous (TP) removal efficiencies were found to be 98%, 92.99%, and 96.36%. The results demonstrated that sMBR was a suitable treatment for the removal of organic matter and nutrients for treating paper mill wastewater except for the problem of calcium accumulation.

1.6 Ligninolytic Enzymes in Degradation and Decolorization of Pulp and Paper Industry Wastewater

Lignin is difficult to biodegrade; white-rot fungi are the most widely unique organisms able to degrade lignin efficiently to complete mineralization (Chandra et al. 2015b; Glenn and Gold 1985; Kirk et al. 1984; Tien and Kirk 1984; Ahmad et al. 2010). However, several bacterial species recently reported as lignin degraders of genera *Bacillus pumilus* and *Bacillus atrophaeus* (Huang et al. 2013), *Aneurinibacillus aneurinilyticus* (Raj et al. 2007a), *Bacillus* sp., (Raj et al. 2007b), *Novosphingobium* sp. B-7 (Chen et al. 2012a, b, c), *Pandoraea* sp. B-6 (Shi et al. 2013), *Comamonas* sp. B-9 (Chen et al. 2012a), *Dysgonomonas* sp. WJDL-Y1 (Duan et al. 2016a), *Acetoanaerobium* sp. WJDL-Y2 (Duan et al. 2016b), *Xanthomonas* sp. (Archana and Mahadevan 2002), *Paenibacillus* sp., *A. aneurinilyticus* (Raj et al. 2007a, b), *Gordonia* strin JW8 (Chen et al. 2012b), *Citrobacter freundii*, and *Serratia marcescens* (Abhishek et al. 2017). The major extracellular ligninolytic enzymes involved in lignin biodegradation by fungi as well as bacteria are lignin peroxidase (LiP; EC 1.11.1.14) and manganese peroxidase (MnP; EC 1.11.1.13) and laccase (Lac; EC 1.10.3.2) (Ahmad et al. 2010; Abdelaziz et al. 2016; D'Souza et al. 2006; Chandra et al. 2017a). White-rot fungi produce various isoforms of extracellular enzymes that give these fungi the ability to degrade lignin and also allow them to grow in presence of a wide range of recalcitrant organic pollutants. In fact, these enzymes have demonstrated to be capable of degrading a vast number of environmental contaminants, including dyes, polychlorinated biphenyls, melanoidins, and pesticides, making ligninolytic

enzymes as a potential efficient tools for biotechnological processes of wastewater pollutants (Kumar and Chandra 2018a; Kumari et al. 2002). MnP is an extracellular oxidoreductase enzyme that belongs to class II fungal haem-containing peroxidases produced by almost all wood-colonizing white root and several litter-decomposing basidiomycetes during secondary metabolism in response to nitrogen or carbon starvation (Hofrichter 2002; Chen and Wan 2017). It has also been produced by some indigenous bacterial strains (Kumar and Chandra 2018a; Xu et al. 2018; Huang et al. 2013). During the catalytic process, the MnP system generates highly reactive and nonspecific free radicals that cleave carbon–carbon and ether interunit bonds of various phenolics and non-phenolic compounds (Hofrichter 2002). Generally, MnP catalyzes the oxidation of Mn^{2+} to Mn^{3+} chelate Mn^{3+} to form stable complexes that diffuse freely and oxidized phenolic substrate (e.g., simple phenol, amines, dyes, phenolic lignin substructure, and dimers) by one-electron oxidation of the substrate, yielding phenoxy radical intermediate, which undergoes rearrangement, bond cleavage, and nonenzymatic degradation to yield several breakdown products. Figure 1.6 illustrates the catalytic cycle of MnP enzyme.

Similarly to MnP, LiP is also an extracellular H_2O_2-dependent heme-containing glycoprotein produced by white-rot fungi as well as some bacterial species (Kumari et al. 2002; Ahmad et al. 2010; Abdelaziz et al. 2016; Xu et al. 2018). Among them, LiP was first discovered in nitrogen and carbon-limited cultures of *P. chrysosporium* and since then has become one of the most studied peroxidases. It catalyzes the oxidative cleavage of C_α–C_β linkages, β-O-4 linkages, and other bonds present in lignin and its model compounds (Chandra et al. 2017a, b). The enzyme also catalyzes side-chain cleavages, benzyl alcohol oxidations, demethoxylation, ring-opening reactions, and oxidative dechlorination. The immobilization of LiP and MnP produced by *P. chrysosporium* on Amberlite IRA-400 resin and its utilization on the remediation of effluent from pulp and paper industry were evaluated by Peralta-Zamora et al. (1998). They reported that immobilized enzyme was very effective in removing color and phenolics species from kraft effluent with insignificant adsorption of colored species by the support. Decolorization of kraft effluent by free and immobilized lignin peroxidases and horseradish peroxidase was studied by Ferrer et al. (1991). The free lignin peroxidase and horseradish peroxidase removed color from kraft effluent. Laccases are multi-copper-containing polyphenol oxidases that are widely distributed in microorganisms, insects, and plants, showing a specific function in each of them. From this group, white-rot fungi are the most studied organism to produced laccases. Laccase catalyzes the oxidation of various aromatic compounds, particularly those with electron-donating groups such as phenols (-OH) and anilines ($-NH_2$), by using molecular oxygen as an electron acceptor. Laccases use molecular oxygen to oxidize a variety of aromatic and nonaromatic hydrogen donors via a mechanism involving radicals. These radicals can undergo further laccase catalyzation of the reaction and/or nonenzymatic reaction such as polymerization and hydrogen abstraction. Therefore, laccase has also the ability to oxidize a wide range of phenolic and non-phenolic substrates (Wong 2009). This enzyme has attracted wide attention because of their number of diverse applications, namely, delignification of lignocellulosic, cross-linking of polysaccharides, detoxification of

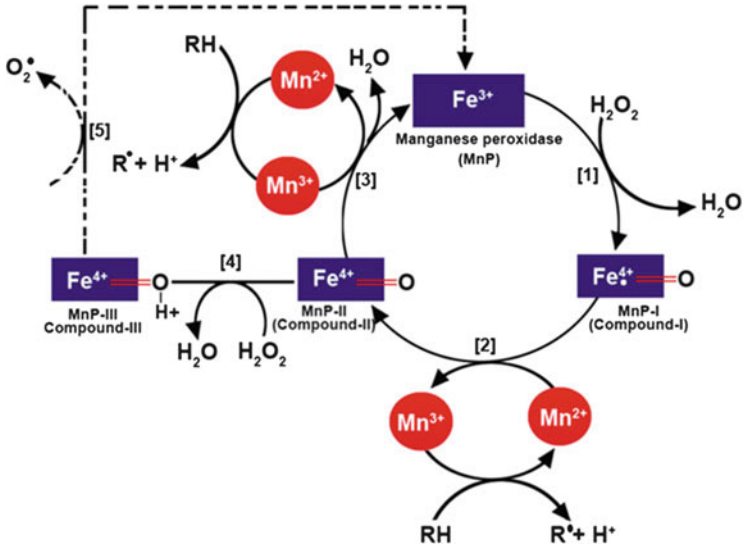


Fig. 1.6 Catalytic cycle of manganese peroxidase (Chandra et al. 2017a)

waste, and transformation of dye (Manzanares et al. 1995). A wide variety of microorganisms—bacteria, yeasts, molds, and algae—have been implicated in lignin biodegradation as well as decolorization of pulping effluents (Garg and Modi 1999). Lignin degradation by fungi is essentially a secondary metabolic process, as fungi and bacteria do not utilize lignin as a carbon source for their growth. This unique feature makes fungi suitable for their application in pulp pretreatment, which can reduce the energy requirement during the mechanical pulping and also will increase the efficiency of bioconversion (Kirk et al. 1992; Kang et al. 2007). Several fungus species such as *Aspergillus niger* (Kannan and Oblisami 1990), *Bjerkandera adusta*, *Phanerochaete chrysosporium* (Costa et al. 2017), *Fibrodontia* sp. (Kreetachat et al. 2016), *Cryptococcus* sp. (Singhal and Thakur 2009b), *Paecilomyces* sp. (Chuphal et al. 2005), *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Lentinus edodes*, *Trametes versicolor* (Wu et al. 2005), and *Emericella nidulans* var. *nidulans* (Singhal and Thakur 2009a) have been reported to decolorize and detoxify the paper and pulp industry wastewater and remove lignin efficiently from the wastewater. Michel Jr et al. (1991) investigated the role of MnP and LiP of *P. chrysosporium* in the decolorization of kraft bleach plant effluent (KBPE). They observed when *P. chrysosporium* was grown in a medium with no Mn^{2+} and high levels of LiPs, but negligible levels of MnP were produced, and the rate and extent of KBPE decolorization by such cultures were quite low. This indicated that LiP plays a relatively minor role in KBPE decolorization. Further, high rates of KBPE decolorization were seen on 3 and 4 days of incubation, when the cultures exhibit high levels of MnP activity but little or no LiP activity. The results of this study indicated that MnP plays a relatively major role than LiPs in KBPE decolorization by *P. chrysosporium.* Yadav et al. (2010) treated the kraft pulp of mixed hardwood with lignin-degrading

fungi *Ceriporiopsis subvermispora* during the bleaching pretreatment. They observed that the fungal treatment made the bleaching process energy efficient and reduced the chlorine consumption up to 4.8%, lignin content 4.7%, and pollution load in terms of COD and BOD by 32.6% and 41.5%, respectively. In bacterial bioremediation of pulp and paper industry wastewater, the role of lignin-degrading enzymes (Lac, LiP, and MnP) is already documented. A diverse spectrum of ligninolytic bacteria capable of degrading lignin and other organic pollutants present in pulp and paper industry wastewater has been isolated and identified over the years (Kumar et al. 2012; Ojha and Markandeya 2016; Raj et al. 2007a, b; Chandra et al. 2011a, b; Chandra and Singh 2012; Raj et al. 2007a, b). Arica et al. (2009) used immobilization of laccase onto nonporous poly(GMA/EGDMA) beads for degradation of industrial effluent. In addition, Sharma et al. (2008) immobilized the enzyme tannase (E.C.3.1.1.20) to possess desirable properties such as stability at extreme pH and temperature and board substrate specificity for industrial applications. The studies by Chandra and Singh (2012) showed induction of Lac, LiP, and MnP during bioremediation of rayon grade pulp and paper mill wastewater by a developed bacterial consortium of *Pseudochrobactrum glaciale*, *Providencia rettgeri*, and *Pantoea* sp. They reported a 90% reduction of lignin and chlorophenol within 216 h of treatment. The studies by Raj et al. (2014a, b) also confirmed the induction of laccase enzyme during bioremediation of paper mill wastewater by *Paenibacillus* sp. Hooda et al. (2015) conducted a study to explore the degradation of pulp and paper mill wastewater by *Brevibacillus agri* strain RJH-1, a rod-shaped gram-positive bacterium isolated from sludge, based on its efficiency to reduce COD, color, AOX, and lignin content under batch and semicontinuous reactor processes. In the batch study, the isolate reduced 47% color, 69% COD, 39% AOX, and 37% lignin after 5 days, whereas in control flask, 26% color, 40% COD, 22% AOX, and 19% lignin reduction were observed by the indigenous bacterial communities present in such wastewater. During semicontinuous reactor study, it reduced 62% COD, 37% color, 30% lignin, and 40% AOX of wastewater at a retention time of only 32 h, whereas the reduction in 21% color, 36% COD, 29% AOX, and 18% lignin was reported in control reactor. This study confirmed that the *B. agri* has the potential to degrade the lignin and reduce the color and COD of the pulp and paper mill wastewater. Lignin decolorization and degradation of pulp and paper mill effluent by ligninolytic bacteria *Bacillus subtilis*, *B. endophyticus*, and *Bacillus* sp. have been reported by Ojha and Markandeya (2016). A LiP-producing *Serratia liquefaciens* was used for bioremediation of pulp and paper mill effluent. The treatment led to toxicity as well as pollution parameter reduction (Haq et al. 2016). Gaur et al. (2018) also investigated that *Klebsiella pneumoniae* strain NITW715076_2 was capable of 74.5% decolorization of pulp and paper industry at the optimized condition. They observed that Lac and MnP activity was increased at the optimum value of pH 6.5, temperature 35 °C, agitation speed 130 rpm, inoculum size 4 mL, carbon source (1%), sucrose and nitrogen source (0.5%), and yeast extract. MnP, LiP, and laccase are the most important ligninolytic enzymes involved in biomechanical pulping and kraft pulp bleaching. In the laboratory scale, consumption of refining energy in mechanical pulping was reduced with MnP

pretreatment. However, MnP degraded residual lignin of kraft pulp and enhanced the pulp bleaching effect. The laccases have also attracted considerable interest for pulp biobleaching. During lignin degradation, laccases are thought to act on small phenolic lignin fragments.

1.7 Emerging Approaches for Pulp and Paper Industry Waste Treatment

1.7.1 Phytoremediation Approaches

Phytoremediation is an emerging, cost-effective, eco-friendly, in situ technology that uses plants to remediate pollutants from the soil, sludge, sediments, and water contaminated with organic and inorganic contaminants (Garbisu and Alkorta 2001; Chandra et al. 2015a, 2018b, c, d; Chandra and Kumar 2017c, 2018). Phytoremediation utilizes plants and their associated microorganisms to reduce, remove, degrade, and/or immobilize harmful environmental pollutants (Chandra and Kumar 2015a). This can reduce risk from contaminated soil, sludges, sediments, and water through contaminant removal or degradation (Alkorta et al. 2004; Rajkumar and Freitas 2008; Chandra and Kumar 2017c). Generally, phytoremediation technology is focused on the ability of plants to accumulate higher concentrations of HMs (up to 100 times the normal concentration) in their shoot and leaves (i.e., they are hyperaccumulator plants as defined by Baker (1981)) (Chandra et al. 2018c, d). Plants have been found to remediate paper mill wastewater, containing multiple contaminants including HMs, viz., Fe, Zn, Cu, Ni, Mn, Hg, and Pb, with variable success (Kumar and Chopra 2016). Several potential native plants that grow on pulp and paper industry waste-contaminated sites under natural conditions have indicated the phytoremediation potential (Fig. 1.7; Chandra et al. 2018b, c, d).

Mishra et al. (2013) assessed the phytoremediation potentials for remediation of HMs by six aquatic macrophytes plants, including *Eichhornia crassipes, Hydrilla verticillata, Jussiaea repens, Lemna minor, Pistia stratiotes,* and *Trapa natans*, grown in paper mill effluent. They found that all the plants caused decreased levels of Cu and Hg in the effluent. Among the six tested plants, *L. minor* and *E. crassipes* were showed high tolerance to Cu and Hg with increased hyperaccumulation. Similarly, a study was conducted by Mazumdar and Das (2015) in Northeast India, to assess the phytoremediation potential of Pb, Zn, Fe, and Mg by 25 wetland plants grown on paper mill wastewater-contaminated sites. Out of 25 species, 10 species were excluders, and the rest were accumulators for different HMs. All the plant species thrived in high Fe, Mg, Pb, and Zn in soil and water, which indicated promise for phytoremediation. Further, the same authors conducted a separate study in 2016 to assess the potential of an aquatic fern, *Salvinia cucullata*, to remediate high BOD, COD, TS, TSS, TDS, P, hardness, and chloride and several HMs (Cd, Cu, Cr, Ni, Pb, Mg, Mn, Fe, and Zn) containing pulp and paper mill wastewater after treating it for 28 days (Das and Mazumdar 2016). They demonstrated that *S. cucullata* thrives in different concentrations of pulp and paper

Fig. 1.7 Some native plants grown on pulp and paper industry wastewater-discharged site showing in situ phytoremediation of hazardous pollutants. (**a**) *Commelina benghalensis*; (**b**) *Phragmites australis* L.; (**c**) *Argemone mexicana*; (**d**) *Alternanthera* sp.

mill wastewater and was capable of accumulation of HMs in different parts, beyond the permissible limits. The fact that this plant survived a wide range of wastewater concentrations and flourished well, particularly at 25% (v/v) treatment, shows better growth, augmentation of all the major antioxidant enzymes, and its capacity to resist membrane injury and attacks of H_2O_2 and O_2 reflected its potential as a phytoremediator. Nevertheless, from the biochemical and anatomical perspective, beyond 25% (v/v) wastewater, the plants suffered stress. Simultaneously, Kumar and Chopra (2016) conducted a laboratory experiment to investigate the reduction of pollution load of paper mill wastewater through phytoremediation technique using water caltrop (*Trapa natans*). *Trapa natans* significantly removed TDS, BOD, COD, TKN, PO_3^{-4}, Ca^{2+}, Mg^{2+}, K^+, Cd, Cu, Fe, Ni, Pb, and Zn of the paper mill wastewater. They recommended that *T. natans* can be used for the treatment of paper mill wastewater up to 50% concentration for 60 days using phytoremediation technique. Chandra et al. (2017b) investigated the HMs phytoextraction potential of native wetland plants growing on organic pollutant-rich pulp paper sludge. They selected 12 representative native plants based on their luxuriant growth on the pulp paper sludge and evaluated the plants for their phytoextraction potential of HM

removal. The metal accumulation pattern revealed that all the native plants growing on sludge sediments have accumulated tested metals in root and shoot. Thus, it was observed that all the growing plants had HM phytoextraction efficiencies in the organic pollutant-rich environment. The HMs (Cd, Cu, Fe, Pb, Mn, and Zn) uptake by water lettuce (*Pistia stratiotes* L.) from paper mill effluent (PME) with its prediction modeling has been studied by Kumar et al. (2019). Lab-scale phytoremediation experiments were performed in glass aquariums to grow *P. stratiotes* in 0% (bore well water as a control), 25%, 50%, 75%, and 100% concentrations of PME. The results showed that *P. stratiotes* was capable of uptaking maximum contents of all heavy metals in its roots, leaves, and the whole plant when grown in 75% PME concentration. This work represents an effective method to model heavy metal uptake by *P. stratiotes* from PME. The author recommended that this methodology can also be adopted for predicting effective metal uptake by plant species being used for the phytoremediation of heavy metals from industrial effluents. The potential prospect of wetland plant for bioremediation of different pollutants from pulp paper mill effluent has also been reported through the constructed wetlands (CWs) treatment system (Kumar and Chopra 2016; Arivoli et al. 2015; Rani et al. 2011). Phytoremediation of HMs and organic pollutants using CWs offer effective, reliable treatment to pulp and paper industry wastewater in a simple and inexpensive manner. CWs are engineered systems that have been designed and constructed to utilize the natural processes, involving wetland vegetation, soils, and their associated microbial assemblages to assist in treating wastewater (Kadlec and Wallace 2009; Vymazal 2014). Macrophytes are the main biological component of wetland ecosystems; they contribute directly to pollution reduction through uptake and assimilation and indirectly by facilitating the growth of important pollutant-degrading microorganisms through complex interactions in the rhizosphere (Guan et al. 2015; Kumar and Chandra 2018b). They not only assimilate pollutants directly into their tissues but also act as catalysts for purification reactions by increasing the microbial diversity in the root zone through the release of oxygen and exudates and promotion of a variety of chemical and biochemical reactions that enhance purification (Stottmeister et al. 2003; Chandra et al. 2018e). Several experiments with the use of CWs to treat wastewaters discharged from pulp and paper industries were carried out by various workers (Knight et al. 1994; Tettleton et al. 1993; Hatano et al. 1994; Moore et al. 1994). Thut (1990, 1993) studied a 3750 m^2 horizontal flow-constructed wetlands (HF-CWs) planted with *P. australis* and *S. californicus* to treat pulp mill wastewater. The system was very effective in removing BOD with removal being consistently between 80% and 90%. Hammer et al. (1993) reported on the use of HF-CWs for removal of color from pulp mill wastewater. The early color removal results were encouraging despite the concomitant export of BOD_5. The authors suggested that a treatment system for tannins and lignins should be designed to optimize environmental conditions and retention times to enhance fungal decomposition of complex organics and incorporate similar components for further decomposition by bacterial populations. Since fungal populations require an attachment substrate, vegetated sand or porous soil substrate is likely to simulate natural soil conditions and provide an aerobic environment and

hydraulic conductivity needed to enhance fungal growth. Knight et al. (1994) reported the use of free water surface (FWS)-CWs consisting of six cells receiving secondary-treated effluent indicated that the cells with the longest length/width ratio (10:1) performed better than cells with a lower aspect ratio (5:1 and 2.5:1). Removal of phenol from pulp and paper mill wastewaters was studied by Abira et al. (2005) in Webuye, Kenya. The HF-CWs with an area of 30.7 m^2 was filled with gravel to a depth of 0.3 m and planted with *Cyperus immensus*, *C. papyrus*, *P. mauritianus*, and *Typha domingensis*. The inflow phenol concentration varied between 0.43 and 1.7 mg L^{-1}, while the outflow phenol concentrations ranged from 0.18 to 0.23 mg L^{-1} and from 0.1 to 0.13 mg L^{-1} for the HRT of 5 and 3 days, respectively. In India, Choudhary et al. (2010) used an HF-CWs to remove chlorinated resin and fatty acids from a paper mill wastewater. The experimental wetlands with a total area of 5.25 m^2 were filled with gravel and planted with *Canna indica*. At an HRT of 5.9 days, the removal efficiency varied between 92% for 9,10,12,13-tetrachlorostearic acid and 96% for 9,10-dichlorostearic acid. The authors concluded that the most probable mechanisms for the removal of chlorinated resin and fatty acids were adsorption/absorption and microbial degradation in the root zone of the plants. Arivoli et al. (2015) demonstrated the feasibility of CWs to treat the heavy metals from pulp and paper industry wastewater by using vertical flow constructed wetlands (VF-CWs) planted with commonly available macrophytes such *as T. angustifolia, Erianthus arundinaceus,* and *Phragmites australis.* The results indicate that the removal efficiencies of VF-CWs for Fe, Cu, Mn, Zi, Ni, and Cd were 74, 80, 60, 70, 71, and 70%, respectively. On the contrary, the removal efficiency of the unplanted system was significantly lower ranging between 31% and 55%. Among the macrophytes, *T. angustifolia* and *E. arundinaceus* exhibited comparatively higher bioconcentration factor (102–103) than *P. australis.* Rani et al. (2011) carried out a pilot-scale study to examine the feasibility of a CWs system for treatment of pulp and paper mill wastewater during summers as well as winters at different HRT, viz., 1.5, 3.5, and 6.5 day. Wetland beds were prepared with easily available plants such as *T. angustifolia* and *Canna indica*. Comparison of mean inlet and outlet concentrations showed that the CWs system could effectively reduce the output of color (89.4%), BOD_5 (80.01%), COD (86.6%), and TS (87.6%) during summer and color (74.90%), BOD_5 (72.07%), COD (70.94%), and TS (72.15%) during winter at 3.5 day HRT.

1.7.2 Vermiremediation

Vermitechnology is an appropriate technique to reduce the level of hazardous substances from wastewater sludge solids. Vermicomposting involves combined interaction between earthworms and microbes for faster mineralization of organic wastes to produce a mature and stable final product known as vermicompost (Sonowal et al. 2013; Bhat et al. 2017). Earthworms carry out toxicity reduction of industrial wastes very efficiently during vermicomposting process (Bhat et al. 2018).

The chlorogocyte cells and the intestinal microorganisms in earthworms can detoxify most of the wastes/sludges (Srivastava et al. 2005). Co-composting with and without *Eisenia fetida* for the conversion of toxic paper mill sludge to a soil conditioner was studied by Kaur et al. (2010). It was observed that mixing cattle dung with the sludge improved physicochemical characteristics (with transition metals in the permissible range for manures) of the products of both the processes and enhanced its acceptability for worms. A higher decline in organic carbon and higher content of nitrogen and phosphorous along with lower electrical conductivity and higher pH of the products of vermicomposting indicated that *E. fetida* helped in the fast conversion of toxic paper mill sludge into a soil conditioner in 100 day. Vermistabilization of paper mill wastewater sludge using *E. fetida* has been carried out by several researchers (Sutharn et al. 2014). Gupta and Garg (2009) reported the vermiremediation and nutrient recovery of nonrecyclable paper waste employing *E. fetida.* In this study, an attempt has been made to vermicompost nonrecyclable postconsumer paper waste amended with cow dung (CD), employing *E. fetida* earthworm in order to transform it into a value-added product, i.e., vermicompost. Vermicomposting of paper waste resulted in a net reduction in ash content and total organic carbon (42.5–56.8%), but increment in total Kjeldahl nitrogen (2.0–2.4-fold), total potassium (2.0-fold), and total phosphorous (1.4–1.8-fold) was achieved after 91 day of worms' activity. The C/N ratio decreased with time in all the worm-worked vermireactors in the range of 71.9–82.0%, depicting an advanced degree of organic matter stabilization.

1.8 Two-Stage Sequential/Phase Separation/Sequential/Combined Approaches for Pulp and Paper Industry Wastewater Treatment

The establishment of sequential anaerobic–aerobic/two-step wastewater treatment facilities is a promising approach to reduce color and toxic contaminants from pulp and paper industry wastewater. The sequential anaerobic and aerobic treatment in two-step bioreactor was evaluated for removal of color in the pulp and paper mill wastewater (Singh and Thakur 2006). In anaerobic treatment, lignin (25%), color (70%), AOX (15%), COD (42%), and phenol (39%) were reduced in 15 days of incubation. Further, the anaerobically treated wastewater was separately applied in a bioreactor in the presence of fungal strain (*Paecilomyces* sp.) and bacterial strain (*Microbrevis luteum*). This study showed reduction in lignin (86%), color (95%), AOX (67%), COD (88%), and phenol (63%) by *Paecilomyces* sp., whereas *M. luteum* showed reduction in lignin (69%), color (76%), COD (75%), AOX (82%), and phenol (93%) by day third when 7-day anaerobically treated wastewater was further treated by aerobic microorganisms. The two or more types of microbes may be attempted sequentially, in which one organism may transform the original organic pollutant by initial catabolic reactions to products that are then mineralized by another organism(s). The potential fungal and bacterial strains (*Paecilomyces* sp. and *Pseudomonas syringae* PV myricae) isolated from pulp and paper mill

wastewater were applied for the treatment of pulp and paper industry wastewater in a two-step and three-step fixed-film sequential bioreactor containing sand and gravel at the bottom of the reactor for immobilization of microbial cells. The result revealed that microbes exhibited significant reduction in lignin (79.5%), color (88.5%), phenol (87.7%), and COD (87.2%) in two-step aerobic sequential bioreactor and lignin (76.5%), color (87.7%), phenol (87.2%), and COD (83.9%) in three-step anaerobic–aerobic sequential bioreactor. The concept of sequential treatment is very important because both anaerobic and aerobic fungi and bacteria can be used to treat effluent at different stages in the bioreactor (Chuphal et al. 2005). Similarly, two-step sequential treatment of pulp and paper industry wastewater by *C. albidus* and *E. nidulans* var. *nidulans* in a sequential manner in 2 L bioreactor was reported by Singhal and Thakur (2012). In treatment (I), the wastewater was first treated by *C. albidus* (stage A), and this treated wastewater was further treated by *E. nidulans* var. *nidulans* (stage B). In treatment (II), wastewater was first treated by *E. nidulans* var. *nidulans* (stage C), and this treated wastewater was further treated by *C. albidus* (stage D). Treatment (I) was more efficient than treatment (II) with 71%, 51%, 44%, and 70% reduction in color, lignin, COD, and genotoxicity, respectively. Class distribution of comets also showed that treatment (I) was more efficient than treatment (II). The author recommended that the effluent treatment process (I) can be scaled up for industrial use.

1.9 Challenges and Future Prospects

The detoxification of pulp and paper wastewater after secondary treatment, prior to discharge in the environment, is a thrust need of the country for sustainable development of industry and the environment. During the papermaking process, pulping is the most important polluting step. Pulping technologies have undergone constant improvements due to market demands and new developments in research. Enzymatic processes are being developed to increase pulp brightness, to reduce troublesome pith, to improve paper quality, and to purify the effluent. Efforts have been made to improve the pulp-producing process by using isolated enzymes. These efforts have limited success as lignin, which is the major problem, and lack the regular and ordered repeating units found in other natural polymers.

Several techno-economic analyses have been carried out recently in order to optimize the production processes to have less environmental drawbacks while providing more quality for the products from an economic perspective. However, the industry is not yet able enough to minimize the pollution load in final wastewater, and it is expected for wastewater from pulp and paper industry to remain as one of the most polluted industrial wastewaters through the world containing recalcitrant and complex organic compounds. The conventional biological treatments have shown a limited efficiency for the treatment of recalcitrant and complex pollutants such as AOXs which can remain in the treated wastewater, causing several environmental and health problems. Hence, there is also a need to perform further life cycle assessment studies for the conventional treatment methods applied to this type

of wastewater. Certain biological treatments offer opportunities to reduce cost (both capital and operating), reduce energy consumption, and minimize environmental impact. The application of such versatile biological agents for the treatment of industrial wastewater on large scale is still limited due to several factors, such as limited amount and sources of biocatalysts, lack of optimum substrate specificities, environmental and cultural conditions required for the growth of microorganisms, competition from native microbes, lack of efficient microbial expression, and appropriate treatment reactor vessels, that make the biological treatment processes slow compared to the conventional processes. However, the necessity of allocating a relatively large area for biological treatments, relatively long-time treatment requirements as well as uncompleted treatment, and local issues such as the bad smell resulting from the bacterial activities are the main drawbacks of such systems. Further, it appears that enzymes such as laccase and peroxidase also have great potential for decolorization of phenolic effluents. However, the activities of the bacterial and fungal enzyme system can be enhanced by the utilization of innovative advanced techniques such as cell/enzyme immobilization and nanotechnology. Protein engineering can be exploited to improve enzyme stability, substrate specificity, and kinetic properties. However, further detailed studies are required to be carried out for selected conditions, which allow more efficient decolorization process from technical and economic viewpoints suitable for commercialization.

A large amount of enzymes required for the effluent treatment and presence of all the enzymes in a single microorganism is difficult, thus becoming a bottleneck for industrial application of microbes and their enzyme system in paper and pulp mill wastewater treatment. However, the application of two or more microbes in combinations can solve the problem of limited resource availability of enzymes. The biodegradation efficiency can further be enhanced by exploring the microbial expression for a specific enzyme. Furthermore, isolation, characterization of new microbial strains, immobilization, and genetics of lignin-degrading microorganisms are the area of future research required to make the direct use of biological agents in wastewater treatment processes.

1.10 Conclusion

Safe disposal of wastewater from pulp and paper industry is a matter of debate which is continuously tainting the environment as treated or raw wastewater is discharged back into the receiving ecosystem, resulting in negative environmental impacts. Several physicochemical methods have been attempted for the removal of color and toxicity from discharged wastewater, but often are not implemented, because of the high costs involved. More recently, the paper and pulp industry has been investigating the use of biological remediation steps to replace or augment current treatment strategies. Although, biological treatments offer opportunities to reduce cost (both capital and operating), reduce energy consumption, and minimize environmental impact, these methods are comparatively slow, and available natural enzyme sources (microorganisms) cannot meet the market demand due to low yields

and their incompatibility toward standard industrial processes. Therefore, to achieve the desired standard norms for discharging of wastewater, successful implementation of microbes in biological treatment processes of paper and pulp mill effluent requires the identification of optimum application conditions such as pH, temperature, substrate specificities, and reaction media. However, pulp and paper industry wastewater containing different types of pollutants does not easily degrade by the single-step treatment process. Therefore, treatment of wastewater by a novel two-step treatment/phase separation method might be a novel and more promising approach for the bioremediation of pulp and paper industry wastewater. The use of hybrid systems, a combination of either biological and physicochemical processes or two biological processes, for wastewater treatment under optimized operating conditions is the most appropriate option for pulp and paper industry to obtain a satisfactory contaminant removal performance with higher efficiencies, especially for color removal; reduce GHG emission and energy costs; and meet environmental regulations.

References

Abdelaziz OY, Brink DP, Prothmann J, Ravi K, Sun M, Garcia-Hidalgo J, Sandahl M, Hulteberg CP, Turner C, Liden G, Gorwa-Grauslund MF (2016) Biological valorization of low molecular weight lignin. Biotechnol Adv 34(8):1318–1346

Abhishek A, Dwivedi A, Tandan N, Kumar U (2017) Comparative bacterial degradation and detoxification of model and Kraft lignin from pulp paper wastewater and its metabolites. Appl Water Sci 7(2):757–767

Abira MA, van Bruggen JJA, Denny P (2005) Potential of a tropical subsurface constructed wetland to remove phenol from pre-treated pulp and paper mill wastewater. Water Sci Technol 51:173–176

Ahmad M, Taylor CR, Pink D, Burton K, Eastwood D, Bending GD, Bugg TD (2010) Development of novel assays for lignin degradation: comparative analysis of bacterial and fungal lignin degraders. Mol BioSyst 6(5):815–821

Ali M, Sreekrishnan TR (2001) Aquatic toxicity from pulp and paper mill effluents: a review. Adv Environ Res 5:175–196

Alkorta I, Hernandez-Allica J, Becerril J, Amezaga I, Albizu I, Garbiscu C (2004) Recent finding on the phytoremediation of soil contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. Rev Environ Sci Biotechnol 3:71–90

Apiwattanapiwat W, Siriacha P, Vaithanomsat P (2006) Screening of fungi for decolorization of wastewater from pulp and paper industry. Kasetsart Journal (Nat Sci) 40:215–221

Archana PI, Mahadevan A (2002) Lignin degradation by bacteria. Prog Ind Microbiol 36:311–330

Archibald F, Paice MG, Jurasek L (1990) Decolorization of Kraft bleachery effluent chromophores by *Coriolus (Trametes) versicolor*. Enzym Microb Technol 12:846–853

Arica MY, Altintas B, Bayramoglu G (2009) Immobilization of laccase onto spacer–arm attached non–porous poly (GMA/EGDMA) beads: application for textile dye degradation. Bioresour Technol 100:665–669

Arivoli A, Mohanraj R, Seenivasan R (2015) Application of vertical flow constructed wetland in treatment of heavy metals from pulp and paper industry wastewater. Environ Sci Pollut Res 22:13336–13343

Bajpai P, Mehna A, Bajpai PK (1993) Decolorization of Kraft bleach plant effluent with the white rot fungus Trametes versicolor. Process Biochem 28:377–384384

Baker AJM (1981) Accumulator and excluder-strategies in the response of plant to heavy metals. J Plant Nutr 3:643–654

Beauchamp CJ, Simao-Beaunoir A, Beaulieu C, Chalifour F (2006) Confirmation of *E. coli* among other thermotolerant coliform bacteria in paper mill effluents, wood chips screening rejects and paper sludges. Water Res 40:2452–2462

Becker J, Wittmann C (2019) A field of dreams: lignin valorization into chemicals, materials, fuels, and health-care products. Biotechnol Adv. S0734-9750(19)30035-7

Bérubé PR, Hall ER (2000) Effects of elevated operating temperatures on methanol removal kinetics from synthetic Kraft pulp mill condensate using a membrane bioreactor. Water Res 34(18):4359–4366

Bhat SA, Singh J, Vig AP (2017) Instrumental characterization of organic wastes for evaluation of vermicompost maturity. J Anal Sci Technol 8:2

Bhat SA, Singh S, Singh J, Kumar S, Bhawana VAP (2018) Bioremediation and detoxification of industrial wastes by earthworms: vermicompost as powerful crop nutrient in sustainable agriculture. Bioresour Technol 252:172–179

Birjandi N, Younesi H, Bahramifar N (2016) Treatment of wastewater effluents from paper-recycling plants by coagulation process and optimization of treatment conditions with response surface methodology. Appl Water Sci 6:339–348

Bugg TDH, Ahmad M, Hardiman EM, Rahmanpour R (2011) Pathways for degradation of lignin in bacteria and fungi. Nat Prod Rep 28:1883–1896

Chandra R, Abhishek A (2011) Bacterial decolorization of black liquor in axenic and mixed condition and characterization of metabolites. Biodegradation 22:603–611

Chandra R, Kumar V (2015a) Mechanism of wetland plant rhizosphere bacteria for bioremediation of pollutants in an aquatic ecosystem. In: Chandra R (ed) Advances in *Biodegradation and Bioremediation of Industrial Waste*. CRC Press, Boca Raton, pp 329–379

Chandra R, Kumar V (2015b) Biotransformation and biodegradation of organophosphates and organohalides. In: Chandra R (ed) Environmental Waste Management. CRC Press, pp 475–524

Chandra R, Kumar V (2017a) Detection of androgenic-mutagenic compounds and potential autochthonous bacterial communities during in situ bioremediation of post methanated distillery sludge. Front Microbiol 8:887

Chandra R, Kumar V (2017b) Detection of *Bacillus* and *Stenotrophomonas* species growing in an organic acid and endocrine-disrupting chemicals rich environment of distillery spent wash and its phytotoxicity. Environ Monit Assess 189:26

Chandra R, Kumar V (2017c) Phytoextraction of heavy metals by potential native plants and their microscopic observation of root growing on stabilized distillery sludge as a prospective tool for in-situ phytoremediation of industrial waste. Environ Sci Pollut Res 24:2605–2619

Chandra R, Kumar V (2018) Phytoremediation: a green sustainable technology for industrial waste management. In: Chandra R, Dubey NK, Kumar V (eds) Phytoremediation of environmental pollutants. CRC Press, Boca Raton

Chandra R, Singh R (2012) Decolourisation and detoxification of rayon grade pulp paper mill effluent by mixed bacterial culture isolated from pulp paper mill effluent polluted site. Biochem Eng J 61:49–58

Chandra R, Singh S, Raj A (2006) Seasonal bacteriological analysis of gola river water contaminated with pulp paper mill waste in Uttaranchal, India. Environ Monit Assess 118:393–406

Chandra R, Abhishek A, Sankhwar M (2011a) Bacterial decolorization and detoxification of black liquor from rayon grade pulp manufacturing paper industry and detection of their metabolic products. Bioresour Technol 102:6429–6436

Chandra R, Singh R, Yadav S (2011b) Effect of bacterial inoculum ratio in mixed culture for decolourization and detoxification of pulp paper mill effluent. J Chem Technol Biotechnol 87:436–444

Chandra R, Saxena G, Kumar V (2015a) Phytoremediation of environmental pollutants: an eco-sustainable green technology to environmental management. In: Chandra R

(ed) Advances in biodegradation and bioremediation of industrial waste. CRC Press, Boca Raton, pp 1–29

Chandra R, Kumar V, Yadav S (2015b) Microbial degradation of lignocellulosic waste and its metabolic products. In: Chandra R (ed) Environmental waste management. CRC Press, pp 249–298

Chandra R, Kumar V, Yadav S (2017a) Extremophilic ligninolytic enzymes. In: Sani R, Krishnaraj R (eds) Extremophilic enzymatic processing of lignocellulosic feedstocks to bioenergy. Springer, Cham

Chandra R, Yadav S, Yadav S (2017b) Phytoextraction potential of heavy metals by native wetland plants growing on chlorolignin containing sludge of pulp and paper industry. Ecol Eng 98:134–145

Chandra R, Kumar V, Tripathi S (2018a) Evaluation of molasses-melanoidin decolourisation by potential bacterial consortium discharged in distillery effluent. 3 Biotech 8:187

Chandra R, Kumar V, Tripathi S, Sharma P (2018b) Heavy metal phytoextraction potential of native weeds and grasses from endocrine-disrupting chemicals rich complex distillery sludge and their histological observations during in situ phytoremediation. Ecol Eng 111:143–156

Chandra R, Dubey NK, Kumar V (2018c) Phytoremediation of environmental pollutants. CRC Press, Boca Raton, FL

Chandra R, Kumar V, Singh K (2018d) Hyperaccumulator versus nonhyperaccumulator plants for environmental waste management. In: Chandra R, Dubey NK, Kumar V (eds) Phytoremediation of environmental pollutants. CRC Press, Boca Raton, FL

Chandra R, Kumar V, Tripathi S, Sharma P (2018e) Phytoremediation of industrial pollutants and life cycle assessment. In: Chandra R, Dubey NK, Kumar V (eds) Phytoremediation of environmental pollutants. CRC Press, Boca Raton, FL

Chauhan N, Thakur IS (2002) Treatment of pulp and paper mill effluent by *Pseudomonas* fluorescens in fixed film bioreactor. Pollut Res 4(4):429–434

Chedchant J, Petchoy O, Vaithanomsat P, Apiwatanapiwat W, Kreetachat T, Chantranurak S (2009) Decolorization of lignin containing effluent by white–rot fungus Datronia sp. KAPI0039. In: Proceedings of the 47th Kasetsart University Annual Conference, Bangkok

Chen Z, Wan CX (2017) Biological valorization strategies for converting lignin into fuels and chemicals. Renew Sust Energ Rev 73:610–621

Chen YH, Chai LY, Zhu YH, Yang ZH, Zheng Y, Zhang H (2012a) Biodegradation of Kraft lignin by a bacterial strain *Comamonas* sp. B-9 isolated from eroded bamboo slips. J Appl Microbiol 125:900–906

Chen J, Zhan P, Koopman B, Fang G, Shi Y (2012b) Bioaugmentation with *Gordonia* strain JW8 in treatment of pulp and paper wastewater. Clean Technol Environ Policy 14:899–904

Chen Y, Chai L, Tang C, Yang Z, Zheng Y, Shi Y, Zhang H et al (2012c) Kraft lignin biodegradation by *Novosphingobium* sp. B-7 and analysis of the degradation process. Bioresour Technol 123:682–685

Choudhary AK, Kumar S, Sharma C (2010) Removal of chlorinated resin and fatty acids from paper mill wastewater through constructed wetland. *World Academy of Science*. Eng Technol 80:67–71

Chuphal Y, Kumar V, Thakur IS (2005) Biodegradation and decolorization of pulp and paper mill effluent by anaerobic and aerobic microorganisms in a sequential bioreactor. World J Microb Biot 21:1439–1445

Clark T, Mitchell C, Donnison A (1992) Bacteriological water quality of pulp and paper mill effluent: the problem of *Klebsiella pneumoniae*. In Proceedings of the 1992 TAPPI International Environmental Conference, pp 171–180

Costa S, Dedola DG, Pellizzari S, Blo R, Rugiero I, Pedrini P, Tamburini E (2017) Lignin biodegradation in pulp-and-paper mill wastewater by selected white rot fungi water. Water 9 (12):935

Costigan SL, Werner J, Ouellet JD, Hill LG, Law RD (2012) Expression profiling and gene ontology analysis in fathead minnow (*Pimephales promelas*) liver following exposure to pulp and paper mill effluents. Aquat Toxicol (122–123):44–55

D'Souza DT, Tiwari R, Sah AK, Raghukumar C (2006) Enhanced production of laccase by a marine fungus during treatment of colored effluents and synthetic dyes. Enzym Microb Technol 38:504–511

Da Re V, Papinutti L (2011) Black liquor decolorization by selected white-rot fungi. Appl Biochem Biotechnol 165:406–415

Das S, Mazumdar K (2016) Phytoremediation potential of a novel fern, *Salvinia cucullata*, Roxb. Ex Bory, to pulp and paper mill effluent: physiological and anatomical response. Chemosphere 163:62–72

Denslow ND, Kocerha J, Sepúlveda MS, Gross T, Holm SE (2004) Gene expression fingerprints of largemouth bass (Micropterus salmoides) exposed to pulp and paper mill effluents. Mutat Res 552(1–2):19–34

Devkumari MS, Selvaseelan DA (2008) Impact of paper mill treated effluent irrigation and solid wastes amendment on the productivity of *Cumbu napier* (CO-3) – a field study. Asian J Exp Sci 22(3):285–293

Dias JCT, Rezende RP, Silva CM, Linardi VR (2005) Biological treatment of Kraft pulp mill foul condensates at high temperatures using a membrane bioreactor. Process Biochem 40 (3–4):1125–1129

Dileká FB, Taplamacioglu HM, Tarlan E (1999) Colour and AOX removal from pulping effluents by algae. Appl Microbiol Biotechnol 52:585–591

van Driessel B, Christov L (2001) Decolorization of bleach plant effluent by mucoralean and white-rot fungi in a rotating biological contactor reactor. J Biosci Bioeng 92(3):271–276

Duan J, Liang J, Wang Y, W D, Wang D (2016a) Kraft lignin biodegradation by *Dysgonomonas* sp. WJDL-Y1, a new anaerobic bacterial strain isolated from sludge of a pulp and paper mill. J Microbiol Biotechnol 26(10):1765–1773

Duan J, Huo X, Du WJ, Liang JD, Wang DQ, Yang SC (2016b) Biodegradation of Kraft lignin by a newly isolated anaerobic bacterial strain, *Acetoanaerobium* sp. WJDL-Y2. Lett Appl Microbiol 62:55–56

Dudášová H, Lukáčová L, Murínová S, Puškárová A, Pangallo D, Dercová K (2014) Bacterial strains isolated from PCB-contaminated sediments and their use for bioaugmentation strategy in microcosms. J Basic Microbiol 54(4):253–260

Emeka CI, Ihediohamma EE, Linus N, Ndubuisi UC, Ebele AG (2011) Physicochemical dynamics of the impact of paper mill effluents on Owerrinta river, eastern Nigeria. J Env Chem Ecotox 3 (11):298–303

Erkan HS, Engin GO (2017) The investigation of paper mill industry wastewater treatment and activated sludge properties in a submerged membrane bioreactor. Water Sci Technol 76 (7–8):1715–1725

Esposito E, Canhos VP, Nelson D (1991) Screening of lignin-degrading fungi for removal of color from Kraft mill wastewater with no additional extra carbon-source. Biotechnol Lett 13:571–576

Ferrer I, Dezotti M, Durdn N, de Qufmica F (1991) Decolorization of Kraft effluent by free and immobilized lignin peroxidases and horseradish peroxidase. Biotechnol Lett 13(3):577–532

Fulthorpe RR, Allen DG (1995) A comparison of organochlorine removal from bleached kraft pulp and paper-mill effluents by dehalogenating *Pseudomonas*, *Ancylobacter* and *Methylobacterium* strains. Appl Microbiol Biotechnol 42:782–789

Galil NI, Sheindorf C, Stahl N, Tenenbaum A, Levinsky A (2003) Membrane bioreactors for final treatment of wastewater. Water Sci Technol 48(8):103–110

Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77(3):229–236

Garg SK, Modi DR (1999) Decolorization of pulp-paper mill effluents by white-rot fungi. Crit Rev Biotechnol 19(2):85–112

Gauthier F, Archibald F (2001) The ecology of "fecal indicator" bacteria commonly found in pulp and paper mill water systems. Water Resour 35(9):2207–2218

Gaur N, Narasimhulu K, Pydi Setty Y (2018) Extraction of ligninolytic enzymes from novel Klebsiella pneumoniae strains and its application in wastewater treatment. Appl Water Sci 8:111

van Ginkel CG, Kester H, Stroo CA, van Haperen AM (1999) Biodegradation of EDTA in pulp and paper mill effluent by activated sludge. Water Sci Technol 40(11–12):259–265

Glenn JK, Gold MH (1985) Purification and characterization of an extracellular Mn(II)-dependent peroxidase from the lignin-degrading basidiomycete, *Phanerochaete chrysosporium*. Arch Biochem Biophys 242(2):329–341

Gommers K, De Wever H, Brauns E, Peys K (2007) Recalcitrant COD degradation by an integrated system of ozonation and membrane bioreactor. Water Sci Technol 55(12):245–251

Gonder ZB, Arayici S, Barlas H (2012) Treatment of pulp and paper mill wastewater using ultrafiltration process: optimization of the fouling and rejections. Indian Eng Chem Res 51:6184–6195

Guan W, Yin M, He T, Xie S (2015) Influence of substrate type on microbial community structure in vertical-flow constructed wetland treating polluted river water. Environ Sci Pollut Res Int 22:16202–16209

Gupta R, Garg VK (2009) Vermiremediation and nutrient recovery of non-recyclable paper waste employing *Eisenia fetida*. J Hazard Mater 162(1):430–439

Gupta V, Minocha AK, Jain N (2001) Batch and continuous studies on treatment of pulp mill wastewater by *Aeromonas formicans*. J Chem Technol Biotechnol 76(6):547–552

Hall TJ, Fisher RP, Rodgers JH, Minshall GW, Landis WG, Kovacs TG, Firth BK, Dube MG, Deardorff TL, Borton DL (2009) A long-term, multitrophic level study to assess pulp and paper mill effluent effects on aquatic communities in four us receiving waters: background and status. Integr Environ Assess Manag 5(2):189–198

Hammer DA, Pullin BP, McMurry DK, Lee JW (1993) Testing color removal from pulp mill wastewaters with constructed wetlands. In: Moshiri AG (ed) Constructed wetlands for water pollution improvement. CRC Press/Lewis Publishers, Boca Raton, Florida, pp 449–452

Haq I, Kumar S, Kumari V, Singh SK, Raj A (2016) Evaluation of bioremediation potentiality of ligninolytic *Serratia liquefaciens* for detoxification of pulp and paper mill effluent. J Hazard Mater 305:190–199

Haq I, Kumar S, Raj A, Lohani M, Satyanarayana GNV (2017) Genotoxicity assessment of pulp and paper mill effluent before and after bacterial degradation using *Allium cepa* test. Chemosphere 169:642–650

Hatakka A (2001) Biodegradation of lignin. In: Hofrichter M, Steinbuchel A (eds) Lignin, humic substances and coal. Wiley-VCH, Weinheim, pp 129–180

Hatano K, Frederick DJ, Moore JA (1994) Microbial ecology of constructed wetland used for treating pulp mill wastewater. Water Sci Technol 29(4):233–239

Hermosilla D, Merayo N, Gasco A, Blanco A (2015) The application of advanced oxidation technologies to the treatment of effluents from pulp and paper industry: a review. Environ Sci Pollut Res 22:168–191

Hewitt LM, Kovacs TG, Dube MG, MacLatchy DL, Martel PH, McMaster ME, Paice MG, Parrott JL, van Den Heuvel MR, van Der Kraak GJ (2008) Altered reproduction in fish exposed to pulp and paper mill effluents: roles of individual compounds and mill operating conditions. Environ Toxicol Chem 27(3):682–697

Hofrichter M (2002) A review: lignin conversion by manganese peroxidase (MnP). Enzyme Microb Tech 30:454–466

Hooda R, Bhardwaj NK, Singh P (2015) Screening and identification of ligninolytic bacteria for the treatment of pulp and paper mill effluent. Water Air Soil Poll 226:305

Hou LP, Yang Y, Shu H, Ying GG, Zhao JL, Fang GZ, Xin L, Shi WJ, Yao L, Cheng XM (2018) Masculinization and reproductive effects in western mosquito fish (*Gambusia affinis*) after long-term exposure to androstenedione. Ecotoxicol Environ Saf 147:509–515

Huang XF, Santhanam N, Badri DV, Hunter WJ, Manter DK, Decker SR, Vivanco JM, Reardon KF (2013) Isolation and characterization of lignin-degrading bacteria from rainforest soils. Biotechnol Bioeng 110(6):1616–1626

Huntley BE, Jones AC, Cabelli VJ (1766) *Klebsiella* densities in waters receiving wood pulp effluents. J Water Pollut Control Fed 48(7):1766–1771

Idise OE, Okoko FJ, Ogar O, Egbah A (2012) The effects of solid wood waste discharge on the physico-chemical and microbial characteristics of the Warri river. Afr J Microbiol Res 6 (20):4302–4314

Jenkins RL, Wilson EM, Angus RA, Howell WM, Kirk M (2003) Androstenedione and progesterone in the sediment of a river receiving paper mill effluent. Toxicol Sci 73:53–59

Johansson T (2012) Application of membrane bioreactors in the pulp and paper industry. Master's Thesis, Uppsala University, Environmental and Aquatic Civil Engineering Program.

Kadlec RH, Wallace SD (2009) Treatment wetlands, 2nd edn. CRC Press, Boca Raton, FL

Kang K, Sung J, Kim D (2007) Evaluation of white-rot fungi for biopulping of wood. Mycobiology 35(4):205–209

Kannan K, Oblisami G (1990) Decolorization of pulp and paper mill effluent by growth of *Aspergillus niger*. World J Microbiol Biotechnol 6:114–116

Karrascha B, Parra O, Cid H, Mehrens M, Pacheco P, Urrutia R, Valdovinos C, Zaror C (2006) Effects of pulp and paper mill effluents on the microplankton and microbial self-purification capabilities of the Biobıo River, Chile. Sci Total Environ 359:194–208

Kaur A, Singh J, Vig AP, Dhaliwal SS, Rup PJ (2010) Cocomposting with and without *Eisenia fetida* for conversion of toxic paper mill sludge to a soil conditioner. Bioresour Technol 101 (21):8192–8198

Keharia H, Madamwar D (2003) Bioremediation concepts for treatment of dye containing wastewater: a review. Indian J Exp Biol 41:1068–1075

Kirk TK, Tien M, Faison BD (1984) Biochemistry of the oxidation of lignin by *Phanerochaete chrysosporium*. Biotechnol Adv 2(2):183–199

Kirk TK, Burgess RR, Koning JW (1992) Use of fungi in pulping wood: an overview of biopulping research. In: Leatham GF (ed) Frontiers in industrial mycology. Springer, Boston, pp 99–111

Knight RL, Hilleke J, Grayson S (1994) Design and performance of the champion pilot-constructed wetland treatment system. TAPPI J 77:240–245

Kreetachat T, Chaisan O, Vaithanomsat P (2016) Decolorization of pulp and paper mill effluents using wood rotting fungus *Fibrodontia* sp. RCK783S. Int J Environ Sci Dev 7(5). https://doi.org/10.7763/IJESD.2016.V7.792

Kumar V, Chandra R (2018a) Characterisation of manganese peroxidase and laccase producing bacteria capable for degradation of sucrose glutamic acid-maillard products at different nutritional and environmental conditions. World J Microbiol Biotechnol 34:32

Kumar V, Chandra R (2018b) Bacterial assisted phytoremediation of industrial waste pollutants and eco-restoration. In: Chandra R, Dubey NK, Kumar V (eds) Phytoremediation of environmental pollutants. CRC Press, Boca Raton, FL

Kumar V, Chandra R (2020a) Bacterial-assisted phytoextraction mechanism of heavy metals by native hyperaccumulator plants from distillery waste contaminated site for eco-restoration. In: Microbes for sustainable development and bioremediation. CRC Press, Boca Raton, FL

Kumar V, Chandra R (2020b) Bioremediation of melanoidins containing distillery waste for environmental safety. In: Saxena G, Bharagava RN (eds) Bioremediation of industrial waste for environmental safety, Microbes and methods for industrial waste management, vol II. Springer, Singapore

Kumar V, Chopra AK (2011) Alterations in physico-chemical characteristics of soil after irrigation with paper mill effluent. J Chem Pharm Res 3(6):7–22

Kumar V, Chopra AK (2016) Reduction of pollution load of paper mill effluent by phytoremediation technique using water caltrop (*Trapa natans* L.). Cogent Environ Sci 2(1). https://doi.org/10.1080/23311843.2016.1153216

Kumar V, Dhall P, Kumar R, Singh YP, Kumar A (2012) Bioremediation of agro-based pulp mill effluent by microbial consortium comprising autochthonous bacteria. Sci World J:7, 127014

Kumar V, Shahi SK, Singh S (2018) Bioremediation: an eco-sustainable approach for restoration of contaminated sites. In: Singh J, Sharma D, Kumar G, Sharma NR (eds) Microbial bioprospecting for sustainable development. Springer

Kumar V, Singh J, Kumar P (2019) Heavy metal uptake by water lettuce (*Pistia stratiotes* L.) from paper mill effluent (PME): experimental and prediction modeling studies. Environ Sci Pollut Res 26(14):14400–14413

Kumari M, Yadav RS, Yadav KD (2002) Secretion of lignin peroxidase by *Penicillium citrinum, Fusarium oxysporum* and *Aspergillus terreus*. Indian J Exp Biol 40(7):802–880

Lacorte S, Latorre A, Barcelo D, Rigol A, Malmqvist A, Welander T (2003) Organic compounds in paper mill process waters and effluents. Trends Anal Chem 22(10):725–737

Lafond RA, Ferguson JF (1991) Anaerobic and aerobic biological treatment processes for removal of chlorinated organics from Kraft bleaching wastes. In: Tappi proceeding of the environmental conference. Tappi Press, Atlanta, pp 797–812

Lankinen VP, Inkeröinen MM, Pellinen J, Hatakka AI (1991) The onset of lignin-modifying enzymes, decrease of AOX and color removal by white-rot fungi grown on bleach plant effluents. Water Sci Technol 24(3–4):189–198

Larsson L, Andersson T, Forlin L, Hardig J (1988) Physiological disturbances in fish exposed to bleached Kraft mill effluents. Water Sci Technol 20:67–76

Latorre A, Malmqvist A, Lacorte S, Welander T, Barcelo D (2007) Evaluation of the treatment efficiencies of paper mill white waters in terms of organic composition and toxicity. Environ Pollut 147:648–655

Leadbitter D (2009) Some effects of bleached Kraft pulp mill effluents on aquatic organisms – a review. Wetlands 8(3):28–35

Lee J, Koh D, Andijani M, Saw SM, Munoz C, Chia SE, Wong ML, Hong CY, Ong CN (2012) Effluents from a pulp and paper mill: a skin and health surveys of children living in upstream and downstream villages. Occup Environ Med 59:373–379

Lerner M, Stahl N, Galil NI (2007) Comparative study of MBR and activated sludge in the treatment of paper mill wastewater. Water Sci Technol 55:23–29

Lin H, Gao W, Meng F, Liao BQ, Leung KT, Zhao L, Chen J, Hong H (2012) Membrane bioreactors for industrial wastewater treatment: a critical review. Crit Rev Environ Sci Technol 42:677–740

Liss SN, Allen DG (1992) Microbiological study of a bleached Kraft pulp mill aerated lagoon. J Pulp Paper Sci 18(6):216–220

Long SC, Stietz JR, Olstadt J, Hedman CJ, Plummer JD (2012) Characterizing paper mill effluent using indicators and source tracking methods. J Am Water Works Assoc 104(3):E150–E161

Mahesh S, Prasad B, Mall ID, Mishra IM (2006) Electrochemical degradation of pulp and paper mill wastewater. Part 1. COD and color removal. Ind Eng Chem Res 45:2830–2839

Mahesh S, Garg KK, Srivastava VC, Mishra IM, Prasad B, Mall ID (2016) Continuous electrocoagulation treatment of pulp and paper mill wastewater: operating cost and sludge study. RSC Adv 6:16223–16233

Maheshwari R, Rani B, Saxena A, Prasad M, Singh U (2012) Analysis of effluents released from recycled paper industry. Science Sage 3(1):82–85

Malaviya P, Rathore VS (2007) Bioremediation of pulp and paper mill effluent by a novel fungal consortium isolated from polluted soil. Bioresour Technol 98:3647–3651

Manzanares P, Fajardo S, Martin C (1995) Production of ligninolytic activities when treating paper pulp effluents by *Trametes versicolor*. J Biotechnol 43:125–132

Martel PH, O'Connor B, Kovacs TG, van den Heuvel MR, Parrott JL, McMaster ME, MacLatchy DL, van Der Kraak GJ, Hewitt LM (2017) The relationship between organic loading and effects on fish reproduction for pulp mill effluents across Canada. Environ Sci Technol 51 (6):3499–3507

Martin C, Manzanares P (1994) A study of the decolorization of straw soda pulping effluents by *Trametes versicolor*. Bioresour Technol 47:209–214

Mazumdar K, Das S (2015) Phytoremediation of Pb, Zn, Fe, and Mg with 25 wetland plant species from a paper mill contaminated site in North East India. Environ Sci Pollut Res 22:701–710

Medhi UJ, Talukdar AK, Deka S (2008) Effect of pulp and paper mill effluent on seed germination and seedling growth of mustard (*Brassica campestris*), pea (*Pisum sativum*) and rice (*Oryza sativa*) seeds. Pollut Res 27(3):437–442

Medhi UJ, Talukdar AK, Deka S (2011) Impact of paper mill effluent on growth and development of certain agricultural crops. J Environ Biol 32:185–188

Megraw SR, Farkas MO (1993) *E. Coli*: a potential source of native fecal coliforms in pulp paper mill effluent. Pulp Paper Canada 94(6):39–41

Mehna A, Bajpai P, Bajpai PK (1995) Studies on decolorization of effluent from a small pulp mill utilizing agriresidues with *Trametes versicolor*. Enzym Microb Technol 17:18–22

Michel FC Jr, Balachandra Dass S, Grulke EA, Adinarayana Reddy C (1991) Role of manganese peroxidases and lignin peroxidases of *Phanerochaete chrysosporium* in the decolorization of Kraft bleach plant effluent. Appl Environ Microbiol 57(8):2368–2375

Mishra M, Thakur IS (2010) Isolation and characterization of alkalotolerant bacteria and optimization of process parameters for decolorization and detoxification of pulp and paper mill effluent by Taguchi approach. Biodegradation 21:967

Mishra S, Mohanty M, Pradhan C, Patra HK, Das R, Sahoo S (2013) Physicochemical assessment of paper mill effluent and its heavy metal remediation sing aquatic macrophytes – a case study at JK paper mill, Rayagada, India. Environ Monit Assess 185(5):4347–4359

Mishra M, Das MT, Thakur IS (2014) Mammalian cell-line based toxicological evaluation of paper mill black liquor treated in a soil microcosm by indigenous alkalo-tolerant *Bacillus* sp. Environ Sci Pollut Res 21:2966–2976

Mishra T, Ramola S, Shankhwar AK, Rabha AK, Srivastava RK (2016) Pulp and paper mill effluent treatment by hybrid anaerobic upflow fixed-bed bioreactor combined with slow sand filter. Desalin Water Treat 57:10528–10536

Mittar D, Khanna PK, Marwaha SS, Kennedy JF (1992) Biobleaching of pulp and paper mill effluents by *Phanerochaete chrysosporium*. J Chem Technol Biotechnol 53:81–92

Modi DR, Chandra H, Garg SK (1998) Decolorization of bagasse based paper mill effluent by white-rot fungus, *Trametes versicolor*. Bioresource Technol 66:79–81

Moore JA, Skarda SM, Sherwood R (1994) Wetland treatment of pulp mill wastewater effluent. Water Sci Technol 29(4):241–247

Morii H, Nakamiya K, Kinoshita S (1995) Isolation of a lignin-decolorizing bacterium. J Ferment Bioeng 80:296–299

Muttray AF, Yu Z, Mohn WW (2001) Population dynamics and metabolic activity of *Pseudomonas abietaniphila* BKME-9 within pulp mill wastewater microbial communities assayed by competitive PCR and RT-PCR. FEMS Microbiol Ecol 38(1):21–31

Nagarathnamma R, Bajpai P (1999) Decolorization and detoxification of extraction-stage effluent from chlorine bleaching of Kraft pulp by *Rhizopus oryzae*. Appl Environ Microbiol 65 (3):1078–1082

Nagarathnamma R, Bajpai P, Bajpai PK (1999) Studies on decolourization, degradation and detoxification of chlorinated lignin compounds in Kraft bleaching effluents by *Ceriporiopsis subvermispora*. Process Biochem 34:939–948

Nestmanna ER, Lee EG-H (1985) Genetic activity in *Saccharomyces cerevisiae* of compounds found in effluents of pulp and paper mills. Mutat Res/Genet Toxicol 155(1–2):53–60

Oakes KD, Tremblay LA, van der Kraak GJ (2005) Short-term lab exposures of immature rainbow trout (*Oncorhynchus mykiss*) to sulfite and Kraft pulp-mill effluents: effects on oxidative stress and circulating sex steroids. Environ Toxicol Chem 24(6):1451–1461

Ojha AK, Markandeya (2016) Lignin decolorization and degradation of pulp and paper mill effluent by ligninolytic bacteria. Iranica J Energ Environ 7(3):282–293

Ojunga S, Masese FO, Manyala JO, Etiegni L, Onkware AO, Senelwa K, Raburu PO, Balozi BK, Omutange ES (2010) Impact of a Kraft pulp and paper mill effluent on phytoplankton & macroinvertebrates in river Nzoia, Kenya. Water Qual Res J Canada 45(2):235–250

Oke N, Singh S, Garg A (2017) A comparative treatment of bleaching wastewater by physicochemical processes. Water Sci Technol 76(9–10):2367–2379

Orlando EF, Davis WP, Guillette LJ Jr (2002) Aromatase activity in the ovary and brain of the eastern mosquitofish (*Gambusia holbrooki*) exposed to paper mill effluent. Environ Health Perspect 110:429–433

Orrego R, Pandelides Z, Guchardi J, Holdway D (2011) Effects of pulp and paper mill effluent extracts on liver anaerobic and aerobic metabolic enzymes in rainbow trout. Ecotoxicol Environ Saf 74(4):761–768

Pandey A, Panwar S, Mishra S, Siddiqui NA (2012) Comparison of fish toxicity & microtox toxicity of luminescent bacteria due to bleach plant effluent released from agro & wood based pulp and paper mills. Environ Anal Toxicol 2(1):1–4

Parks LG, Lambright CS, Orlando EF, Guillette LJ Jr, Ankley GT, Gray LE Jr (2001) Masculinization of female mosquitofish in Kraft mill effluent-contaminated Fenholloway River water is associated with androgen receptor agonist activity. Toxicol Sci 62(2):257–267

Patel A, Arora N, Pruthi V, Pruthi PA (2017) Biological treatment of pulp and paper industry effluent by oleaginous yeast integrated with production of biodiesel as sustainable transportation fuel. J Clean Prod. https://doi.org/10.1016/j.jclepro.2016.10.184

Pathan TS, Sonawane DL, Khillare YK (2009) Toxicity and behavioural changes in freshwater fish *Rasbora daniconius* exposed to paper mill effluent. Bot Res Int 2(4):263–266

Peralta-Zamora P, Gomes De Morases S, Esposito E, Antunes R, Reyes J, Duran N (1998) Decolourisation of pulp mill effluents with immobilised lignin and manganese peroxidase from *Phanerochaete chrysosporium*. Int Technol 19:521–528

Perestelo F, Falcon MA, Perez ML, Roig EC, de la Fuente Martin G (1989) Bioalteration of Kraft pine lignin by *Bacillus megaterium* isolated from compost piles. J Ferment Bioeng 68:151–153

Pihlajamäki A, Nyström M (2002) Comparison of nanofiltration and tight ultrafiltration membranes in the filtration of paper mill process water. J Desal 149:131–136

Pokhrel D, Viraraghavan T (2004) Treatment of pulp and paper mill wastewater – a review. Sci Total Environ 333:37–58

Poole NJ, Wildish DJ, Kristmanson DD, Waldichuk M (1977) The effects of the pulp and paper industry on the aquatic environment. Environ Sci Technol 8(1–4):153–195

Pradhan SC, Behera P (2011) Changes in some physical properties of soil amended with effluent of emami paper mills located at Balgopalpur, Balasore, Orissa. The Bioscan 6(1):153–155

Qadir I, Chhipa RC (2015) Critical evaluation of some available treatment techniques for textile and paper industry effluents: a review. Am Chem Sci J 6(2):77–90

Ragunathan R, Swaminathan K (2004) Biological treatment of a pulp and paper industry effluent by *Pleurotus* spp. World J Microbiol Biotechnol 20:389–393

Raj A, Chandra R (2004) Comparative analysis of physico-chemical and bacteriological parameters of Kraft and pulp paper mill effluents. Indian J Environ Protect 24(7):481–489

Raj A, Chandra R, Patel DK (2005) Physico-chemical characterization of pulp and paper mill effluent and toxicity assessment by a tubificid worm, *Tubifex tubifex*. Toxicol Int 12(2):109–118

Raj A, Chandra R, Reddy MMK, Purohit HJ, Kapley A (2007a) Biodegradation of Kraft lignin by a newly isolated bacterial strain, *Aneurinibacillus aneurinilyticus* from the sludge of a pulp paper mill. World J Microbiol Biotechnol 23(6):793–799

Raj A, Reddy MMK, Chandra R (2007b) Decolorisation and treatment of pulp and paper mill effluent by lignin-degrading *Bacillus* sp. J Chem Technol Biotechnol. https://doi.org/10.1002/jctb.1683

Raj A, Kumara S, Haq I, Singh SK (2014a) Bioremediation and toxicity reduction in pulp and paper mill effluent by newly isolated ligninolytic *Paenibacillus* sp. Ecol Eng 71:355–362

Raj A, Kumar S, Haq I, Singh SK (2014b) Bioremediation and toxicity reduction in pulp and paper mill effluent by newly isolated ligninolytic *Paenibacillus* sp. Ecol Eng 71:355–362

Rajkumar M, Freitas H (2008) Influence of metal resistant-plant growth-promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. Chemosphere 71:834–842
Rajwar D, Paliwal R, Rai JPN (2017) Biodegradation of pulp and paper mill effluent by co-culturing ascomycetous fungi in repeated batch process. Environ Monit Assess 189:482
Rani N, Maheshwari RC, Kumar V, Vijay VK (2011) Purification of pulp and paper mill effluent through *Typha* and *Canna* using constructed wetlands technology. J Water Reuse Desalin 1 (4):237–242
Rocha-Santos T, Ferreira F, Silva L, Freitas AC, Pereira R, Diniz M, Castro L, Peres I, Duarte AC (2010) Effects of tertiary treatment by fungi on organic compounds in a Kraft pulp mill effluent. Environ Sci Pollut Res 17:866–874
Rodrigues AC, Boroski M, Shimada NS, Garcia JC, Nozaki J, Hioka N (2008) Treatment of paper pulp and paper mill wastewater by coagulation–flocculation followed by heterogeneous photocatalysis. J Photochem Photobiol A Chem 194(1):1–10
Roy RP, Prasad J, Joshi AP (2008) Changes in soil properties due to irrigation with paper industry wastewater. J Environ Sci Eng 50(4):277–282
Saikia MK, Kalita S, Sarma GC (2010) Algal indices to predict pulp and paper mill pollution load of Elenga Beel (Wetland) Assam, India. Soc Appl Sci 1(4):815–821
Saikia MK, Kalita S, Sarma GC (2011) An experimental investigation on growth stimulation (+) and inhibition (−) of algae (*Oscillatoria chlorina* and *Scenedesmus quadricauda*) treated with pulp and paper mill effluents. Int J Appl Biol Pharm Technol 2(4):87–94
Sandstrom O, Neuman E, Karas P (1988) Effects of bleached pulp mill effluent on growth and gonad function in Baltic coastal fish. Water Sci Technol 20:107–118
Savant DV, Abdul-Rahman R, Ranade DR (2006) Anaerobic degradation of adsorbable organic halides (AOX) from pulp and paper industry wastewater. Bioresour Technol 97:1092–1104
Schutyser W, Renders T, Van den Bosch S, Koelewijn S-F, Beckham GT, Sels BF (2018) Chemicals from lignin: an interplay of lignocellulose fractionation, depolymerisation, and upgrading. Chem Soc Rev 47:852–908
Sharma S, Agarwal L, Kumar R (2008) Purification, immobilization and characterization of tannase from *Penicillium* variable. Bioresour Technol 99:2544–2551
Shi Y, Chai L, Tang C, Yang Z, Zheng Y, Chen Y, Jing Q (2013) Biochemical investigation of Kraft lignin degradation by *Pandoraea* sp. B-6 isolated from bamboo slips. Bioprocess Biosyst Eng 36:1957–1965
Singh AK, Chandra R (2019) Pollutants released from the pulp paper industry: aquatic toxicity and their health hazards. Aquat Toxicol. https://doi.org/10.1016/j.aquatox.2019.04.007
Singh P, Thakur IS (2006) Colour removal of anaerobically treated pulp and paper mill effluent by microorganisms in two steps bioreactor. Bioresour Technol 97:218–223
Singh YP, Dhall P, Mathur RM, Jain RK, Thakur V, Kumar V, Kumar R, Kumar A (2011) Bioremediation of pulp and paper mill effluent by tannic acid degrading *Enterobacter* sp. Water Air Soil Pollut 218:693–701
Singhal A, Thakur IS (2009a) Decolourisation and detoxification of pulp and paper mill effluent by *Emericella nidulans* var. nidulans. J Hazard Mat 171:619–625
Singhal A, Thakur IS (2009b) Decolourisation and detoxification of pulp and paper mill effluent by *Cryptococcus* sp. Biochem Eng J 46:21–27
Singhal A, Thakur IS (2012) Two step sequential treatment of pulp and paper mill effluent by *Cryptococcus albidus* and *Emericella nidulans* var. nidulans in 2 L bioreactor. Can J Chem Eng. https://doi.org/10.1002/cjce.20547
Sonowal PJ, Dhamodharan K, Khwairkpam M, Kalamdhad AS (2013) Feasibility of vermicomposting dewatered sludge from paper mills using *Perionyx excavatus*. Eur J Environ Sci 3:6–23
Srivastava R, Kumar D, Gupta SK (2005) Bioremediation of municipal sludge by vermitechnology and toxicity assessment by *Allium cepa*. Bioresour Technol 96:1867–1871
Stephenson R, Duff S (1996) Coagulation and precipitation of a mechanical pulping effluent—II. Toxicity removal and metal salt recovery. J Water Res 30:793–798

Stottmeister U, Wiessner A, Kuschk P, Kappelmeyer MK, Bederski RA, Muller H, Moormann H (2003) Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnol Adv 22:93–117

Subramonian W, Wu TY, Chai S (2017) Photocatalytic degradation of industrial pulp and paper mill effluent using synthesized magnetic Fe_2O_3-TiO_2: treatment efficiency and characterizations of reused photocatalyst. J Environ Manag 187:298–310

Sutharn S, Sajwan P, Kumar K (2014) Vermiremediation of heavy metals in wastewater sludge from paper and pulp industry using earthworm *Eisenia fetida*. Ecotoxicol Environ Saf 109:177–184

Swamy NK, Singh P, Sarathy IP (2011) Precipitation of phenols from paper industry waste paper using ferric chloride. RASÁYAN J Chem 4:452–456

Talat Mahmood PE, Asce M, Elliott A (2006) Activated sludge process modification for sludge yield reduction using pulp and paper wastewater. J Environ Eng 132:1019–1027

Tarlan E, Dilek FB, Yetis U (2002a) Effectiveness of algae in the treatment of a wood-based pulp and paper industry wastewater. Bioresour Technol 84(1):1–5

Tarlan E, Yetis Ü, Dilek FB (2002b) Algal treatment of pulp and paper industry wastewaters in SBR systems. Water Sci Technol 45(12):151–158

Tettleton RP, Howell FG, Reaves RP (1993) Performance of a constructed marsh in the tertiary treatment of bleach Kraft pulp mill effluent: results of a 2-year pilot project. In: Moshiri AG (ed) Constructed wetlands for water quality improvement. Lewis Publishers, Boca Raton, FL, pp 437–440

Thakur IS (2004) Screening and identification of microbial strains for removal of colour and adsorbable organic halogens in pulp and paper mill effluent. Process Biochem 39 (11):1693–1699

Thompson G, Swai J, Kay M, Forster C (2001) The treatment of pulp and paper mill effluent: a review. Bioresour Technol 77:275–286

Thut RN (1990) Treatment of pulp mill effluent by an artificial marsh. Large-scale pilot study. Proceeding of the TAPPI Environmental Conference, TAPPI, TAPPI, Washington, DC, pp. 121–127

Thut RN (1993) Feasibility of treating pulp mill effluent with a constructed wetland. In: Moshiri AG (ed) Constructed wetlands for water quality improvement. Lewis Publishers, Boca Raton, FL, pp 441–447

Tien M, Kirk TK (1984) Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization, and catalytic properties of a unique H_2O_2-requiring oxygenase. Proc Natl Acad Sci U S A 81(8):2280–2284

Tiku DK, Kumar A, Chaturvedi R, Makhijani SD, Manoharan A, Kumar R (2010) Holistic bioremediation of pulp mill effluents using autochthonous bacteria. Int Biodeterior Biodegradation 64:173–183

Tyor AK, Fulia A, Sharma RK (2012) Impact of paper mill effluent on the survival and hatchability of *Cyprinus carpio*. Res J Environ Toxicol 6(2):33–41

Usha MT, Sarat Chandra T, Sarada R, Chauhan VS (2016) Removal of nutrients and organic pollution load from pulp and paper mill effluent by microalgae in outdoor open pond. Bioresour Technol 214:856–860

Van den Bosch S, Koelewijn SF, Renders T, Van den Bossche G, Vangeel T, Schutyser W, Sels BF (2018) Catalytic strategies towards lignin-derived chemicals. Top Curr Chem (Cham) 376(5):36

Verma Y (2008) Toxicity assessment of pulp- paper mill effluents employing *Daphnia* bioassay. Jpn J Environ Toxicol 11(2):151–156

Vymazal J (2014) Constructed wetlands for treatment of industrial wastewaters: a review. Ecol Eng 73:724–751

Wartman CA, Hogana NS, Hewitt LM, McMaster ME, Landman MJ, Taylor S, Kovacs TG, van den Heuvel MR (2009) Androgenic effects of a Canadian bleached kraft pulp and paper effluent as assessed using three spine stickle back (*Gasterosteus aculeatus*). Aquat Toxicol 92(3):131–139

Watson SB, Ridal J, Zaitlin B, Lo A (2003) Odours from pulp mill effluent treatment ponds: the origin of significant levels of geosmin and 2-methylisoborneol (MIB). Chemosphere 51(8):765–773

Wong DW (2009) Structure and action mechanism of ligninolytic enzymes. Appl Biochem Biotechnol 157(2):174–209

Wu J, Xiao Y, Yu H (2005) Degradation of lignin in pulp mill wastewaters by white-rot fungi on biofilm. Bioresour Technol 96:1357–1363

Xu Z, Qin L, Cai M, Hua W, Jin M (2018) Biodegradation of Kraft lignin by newly isolated *Klebsiella pneumoniae, Pseudomonas putida,* and *Ochrobactrum tritici* strains. Environ Sci Pollut Res Int 25(14):14171–14181

Yadav RD, Chaudhry S, Dhiman SS (2010) Biopulping and its potential to reduce effluent loads from bleaching of hardwood Kraft pulp. Bioresources 5:159–171

Yadav D, Pruthi V, Kumar P (2016) Enhanced biological phosphorus removal in aerated stirred tank reactor using aerobic bacterial consortium. J Water Process Eng 13:61–69

Yang C, Cao G, Li Y, Zhang X, Ren H et al (2008) Correction: a constructed alkaline consortium and its dynamics in treating alkaline black liquor with very high pollution load. PLOS ONE 3 (12). https://doi.org/10.1371/annotation/2cc5374b-b2cf-4f78-9952-0343272bfe2f

Yeber MC, Rodrlguez J, Freer J, Baeza J, Durdn N, Mansilla HD (1999) Advanced oxidation of a pulp mill bleaching wastewater. Chemosphere 39(10):1679–1688

Yu Z, Mohn WW (2002) Bioaugmentation with the resin acid-degrading bacterium *Zoogloea resiniphila* DhA-35 to counteract pH stress in an aerated lagoon treating pulp and paper mill effluent. Water Res 36:2793–2801

Zhang Y, Ma C, Ye F, Kong Y, Li H (2009) The treatment of wastewater of paper mill with integrated membrane process. Desalination 236(1–3):349–356

Zouari H, Labat M, Sayadi S (2002) Degradation of 4-chlorophenol by the white rot fungus *Phanerochaete chrysosporium* in free and immobilized cultures. Bioresour Technol 84:145–150

Microbial Remediation of Heavy Metals

2

Lakhan Kumar and Navneeta Bharadvaja

Abstract

Background: The indiscriminate use of heavy metals for anthropogenic purposes such as in color pigments, batteries, fertilizers, or other industrial products has brought a significant change in their presence and concentration in the environment. This alteration results in accumulation of one or more heavy metals at a place surpassing the natural admissible limits causing pollution in the air, water, and soil. Most of heavy metals, even at very low concentrations, are toxic, carcinogenic, and mutagenic in nature. Humans and animals contract various diseases when they are in prolonged exposure to heavy metals through dermal contact, inhalation, and consumption of foodstuffs having heavy metals in it. As per the reports published by many public health organizations, several million human populations throughout the world are suffering from heavy metal-associated diseases.

Methods: Various approaches used to degrade heavy metals include physical, chemical, biological, or a combination of these methods, but many of these methods are not environment-friendly and economically viable. Not a single method claims complete degradation of heavy metals. Salts of heavy metals, in general, are water-soluble and cannot be separated through physical means. Physiochemical methods bring secondary pollution at the site of treatment. The application of microbes in heavy metal remediation and degradation has been under investigation for decades as they transform them into a less or nontoxic form. It is comparatively more effective, economic, and environment-friendly. Microbial metabolic secretions, such as low-molecular-weight organic acids, can dissolve heavy metals and soil particles containing heavy metal minerals. Microbes use various processes such as precipitation, biosorption, and enzymatic

L. Kumar · N. Bharadvaja (✉)
Plant Biotechnology Laboratory, Department of Biotechnology, Delhi Technological University, Delhi, India
e-mail: navneetab@dce.ac.in

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_2

transformation to degrade or reduce heavy metals into innocuous or less toxic form that are more stable, less mobile, or inert.

Conclusion: In view of this, the present chapter investigates the abilities of microbes in terms of tolerance and degradation of heavy metals. Further, this study undertakes an assessment of human health risks associated with presence of heavy metals in water and microbial bioremediation as a tool to reduce the ill effect of these heavy metals on human health and environment. Also, recent advances in biotechnological tools and techniques to explore microbial population for heavy metal bioremediation and biodegradation have been discussed.

Keywords
Bioremediation · Heavy metals · Human health risks · Microbial degradation · Genetic engineering

2.1 Introduction

The rapid population growth, associated development and globalization for anthropogenic purposes have increased the indiscriminate use of heavy metals. Metal smelters, mining, coloring agents, batteries, fertilizers, or other compulsory industrial/household products have brought a significant change in their presence and concentration in the environment (Anyanwu et al. 2018). This alteration results in amassing of one or several heavy metals at a place surpassing the natural admissible limits causing pollution in the air, water, and soil. Most of heavy metals, even at very low concentrations, are toxic, carcinogenic, and mutagenic in nature (Tchounwou et al. 2012; Jaishankar et al. 2014). Heavy metals, particularly the nonessential elements, when present into the soil, air, or water cause overwhelming effects on the biological diversity of the recipient environment (Ali et al. 2019). Since metals, due to their inherent nature, cannot be destroyed but can be transformed into one form to another, they are highly persistent and remain in the environment and tend to accumulate and magnify in the food chain (Li et al. 2015; Wuana and Okieimen 2011). Some metals are essential for proper growth and development (nutrition) but may prove hazardous when consumption exceeds the required limit (Singh et al. 2011; Hejna et al. 2018). Humans and other life forms contract various diseases when they are in prolonged exposure to heavy metals through dermal contact, inhalation, and consumption of foodstuffs having heavy metals in it (Anyanwu et al. 2018; Jaishankar et al. 2014; Sharma et al. 2014; Alissa and Ferns 2011). Ali and Khan have explained bioaccumulation of heavy metals and its transfer and associated health risks to humans in detail (Ali and Khan 2018). Effluents loaded with heavy metals discharged from domestic, agricultural, and industrial sources when mixed with surface water, river, and soil pollutes them (Anyanwu et al. 2018). Galvanized metal pipes, plated plumbing fittings, etc. contaminate drinking water in piped distribution system. As per the reports published by many public health organizations and research articles, several million human populations throughout

the world are suffering from heavy metal-associated diseases (Järup 2003; WHO 2011; Mamtani et al. 2011). Minamata disease (mercury poisoning) and itai-itai (cadmium poisoning) are world famous heavy metal-associated human health hazards (Harada 1995; Masanori Kaji 2012).

Various approaches are used to degrade heavy metals that include chemical, physical, biological, or a combination of these methods, but many of these methods are not environment-friendly and economically viable. Not a single method claims complete degradation of heavy metals (Emenike et al. 2018). Salts of heavy metals, in general, are water-soluble and cannot be separated through physical means. Physiochemical methods such as filtration, reverse osmosis, chemical precipitation, membrane technology, oxidation or reduction, evaporation, and ion exchange bring secondary pollution at the site of treatment (Barakat 2011; Gunatilake 2015). The application of microbes and plants in heavy metal remediation and degradation has been under investigation for decades as they transform them into a less or nontoxic form. It is comparatively more effective, economic, and environment-friendly (Rajendran et al. 2003; Ayangbenro and Babalola 2017). Microbial metabolic secretions such as organic acids are able to dissolve heavy metals or soil particles containing heavy metals in them (Wuana and Okieimen 2011). Microbes use different mechanisms such as precipitation, biosorption, and enzymatic transformation to degrade or reduce heavy metals into a less toxic form that are more stable, less mobile, or inert (Rajendran et al. 2003; Ojuederie and Babalola 2017). In view of this, this chapter investigates the capabilities of microbial diversity in terms of tolerance and degradation of heavy metals. Further, this study undertakes an assessment of human health risks associated with presence of heavy metals in water and microbial bioremediation as a tool to reduce the ill effect of these heavy metals on human health and the environment. Also, recent advances in biotechnological tools and techniques to explore microbial population for heavy metal bioremediation and biodegradation have been discussed.

2.2 Heavy Metals

Heavy metals, now a very common known term for its obvious harmful impacts on the environment, refer to metals or a metalloid element having relatively high density ranging from 3 to 7.5 gram per cubic centimeters (Alissa and Ferns 2011; Appenroth 2009). For example, arsenic, antimony, cadmium, chromium, lead, mercury, and selenium are metals with a high atomic weight and a density at least five times greater as compared to water. Most of heavy metals, even at very low concentrations, are toxic, carcinogenic, and mutagenic in nature (Tchounwou et al. 2012; Jaishankar et al. 2014). Among 35 elements considered dangerous for human population, 23 (in box) are termed as heavy metals. They are nonbiodegradable in nature. Heavy metals are prominent out of many causes of environmental pollution (air, water, and the soil). Increasing level of these metals in the environment has attracted worldwide concern for their mitigation owing to the toxicity shown by most of them. Heavy metal compounds are often used in metal smelters, mining, coloring

agents, fertilizers, batteries, or other compulsory industrialized/household products. Gradually, these metals enter into the environment through evaporation or the soil as sediment. Then, these deposited elements are absorbed and accumulated into the biosphere (Appenroth 2009; Sahni 2011).

Arsenic (As), antimony (Sb), bismuth (Bi), cerium (Ce), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), gold (Au), gallium (Ga), iron (Fe), lead (Pb), mercury (Hg), manganese (Mn), nickel (Ni), platinum (Pt), silver (Ag), thallium (Tl), tellurium (Te), tin (Sn), uranium (U), vanadium (V), and zinc (Zn)

Sources of heavy metals in the environment

Most significant sources of heavy metals in the environment are, first, pedogenetic process, i.e., the weathering and erosion of parent materials such as minerals; second, volcanic eruptions; and, lastly, forest fires and biogenic source. They are widely found in the earth's crust. Apart from it, anthropogenic activity excessively adds in its wide circulation in the environment (Wuana and Okieimen 2011; Dixit et al. 2015). The indiscriminate use for anthropogenic purposes (Table 2.1) has brought a significant change in the presence and concentration of heavy metals in the environment, altering the geochemical cycle and biochemical balance. Other sources of heavy metal contamination in the environment are wastewaters coming from domestic households, industrial wastewater and waste discharges, agricultural wash-off, combustion of conventional fossil fuels, sanitary landfills, biomedical equipment and implants, etc. (Kumar et al. 2019; Elumalai et al. 2017). Industrial effluents as well as domestic wastewater carry significant amount of heavy metals such as cadmium, chromium, copper, lead, and mercury with them in metabolic waste products, utensils, corrosion of water distribution and supply pipes, detergents, etc. (Sahni 2011). Wastewater treatment does not facilitate complete removal of pollutants from wastewater and usually removes less than 50% of the metal content. Due to this, effluent even after treatment leaves the treatment plant with significant metal loadings. The sludge generated in treatment plant is also found to be rich in metal content (Akpor et al. 2014; Sharma and Bhattacharya 2017; Arjoon et al. 2013). Domestically used detergent is a source of many heavy metals like arsenic, chromium, cobalt, iron, manganese, nickel, and zinc (Jenkins 1998; Aonghusa and Gray 2002a, b). Similarly, mining, milling, plating and surface finishing, fire crackers, vehicular pollution, biocides and fertilizers, paints, cosmetics, pharmaceutical, computer, and communications technology-based industries are contributing to increasing heavy metal concentration in the environment in an uncontrolled manner (Sahni 2011).

2.3 Environmental Impact

Heavy metals, due to their higher solubility in water, easily get absorbed by biological systems. They cannot be easily separated from water. Heavy metals cannot be broken into further smaller forms, thus having long-lasting existences in

Table 2.1 Anthropogenic sources of heavy metals in the environment (Sahni 2011; Aonghusa and Gray 2002a; Sundar and Chakravarty 2010; Hutton and Hutton 1987; Tangahu et al. 2011; Jan et al. 2015; Bradl 2005; Alloway 2013; Mohammed et al. 2011; Mohammadi Roozbahani et al. 2015; Web-5 n.d.; Web-6 n.d.; Belzile et al. 2011)

Heavy metals	Anthropogenic sources
Barium	Computer hardware industries such as computer screen manufacturing; drilling muds used by oil and gas industries; paints; bricks; tiles; glass; rubber; ceramics; emissions from mining, refineries, or processing units of barium minerals; manufacturing of barium products, etc.
Beryllium	Beryllium production units, ceramic industries, motherboards and finger clips manufacturing plants and wastes, coal, nuclear power production plants, space-based industries, etc.
Antimony	Alloys and organic catalytic materials, coal-fired power plants, smelting lead and other metals, burning of fossil fuels, vehicle exhaust, plastics, textiles, rubber, adhesives, pigments, paper, etc.
Arsenic	Fungicide, pesticide, and herbicide production and application; metal smelters; mining activities; and chemical wastes
Cadmium	Fertilizer industry; nuclear fission plants; by-products from refining of copper, lead, and zinc; pesticide manufacturers; cadmium–nickel batteries; petroleum additives such as TEL; plating industries; cathode ray tubes; semiconductor chips; welding; cigarette smoke; etc.
Copper	Iron and steel industry, fertilizer industry, burning of wood, discharge of mine tailings, disposal of fly ash, municipal and industrial wastes without proper treatment, etc.
Cobalt	High-temperature alloys, permanent magnets, hard metal tools, etc.
Chromium	Asbestos, cement, and steel industries; metallurgical and chemical industry processes using chromium compounds; etc.
Iron	Cast and wrought iron; rusting; steel and alloys; construction, transportation, and Machine manufacturing; etc.
Lead	Lead smelters, automobile emissions, glass panels, computer monitors, burning of coal and oil, lead arsenate pesticides, smoking, paints and chemical industries, mining and plumbing, etc.
Mercury	Major electrical and electronics equipment and parts like sensors, thermostats, switches, relays, mobile phones, batteries, and flat panel displays (replaces the cathode ray tubes' use); mining and refining of mercury; pesticides; laboratories using mercury; etc.
Nickel	Burning of coal, oil, and other fossil fuels; mining and refining industries; nickel–alloy manufacturing/nickel-based metallurgical industries; surgical instruments; nickel refining; electroplating; chemical reagents; incineration of municipal wastes; etc.
Zinc	Artillery and smoke grenades, pesticides, fertilizers, coal and fuel consumption, iron and steel production plants, zinc refinery plants, galvanizing processes, corrosion of galvanized structures, etc.
Vanadium	From cleaning oil tanks, metallurgy, etc.
Selenium	Coal mining, oil refineries, burning of fossil fuels, glass manufacturing industry, chemical synthesis, etc.

the environment. Arsenic, cadmium, copper, lead, nickel, chromium, and zinc cause reduction in species diversity in an ecosystem (Appenroth 2009).

2.3.1 Effect of Heavy Metal Contamination

Soil: Heavy metal contamination affects soil respiration. Heavy metals exert lethal effects toward composition of soil microflora by disturbing key microbial processes and enzyme activity. Heavy metals bind with active groups of the enzyme meant to bind with essential nutrients. It causes reduction in the number and activity of microbes and thus soil microbial biomass. Microbial metabolic entropy increases, and carbon fixation from organic to bio-carbon decreases in soil polluted with heavy metals. Accumulation of heavy metals reduces the crop yield and fertility and productivity of the soil. Vegetables and fruits grown on heavy metal-contaminated soils are more prone to insect attacks (Singh and Kalamdhad 2011; Rzymski et al. 2015).

Microbial Population: Microbes play major roles in nutrient recycling in the environment. Heavy metal contamination affects the respiration and metabolism of microorganisms and affects their community structure. Thus, ecosystem functioning can be dangerously disturbed, and long-term soil activity and productiveness may be threatened. Metals exert a range of detrimental effects on microbial cells. Heavy metal exposure hinders cellular processes and limits growth. For example, arsenic causes enzyme deactivation (Singh and Kalamdhad 2011; Igiri et al. 2018). Cadmium, mercury, and lead are responsible for denaturing protein, destroying nucleic acids, altering transcription process, hindering cell division, etc. Chromium reduces oxygen uptake, elongates lag phase, and thus inhibits growth. Copper inhibits enzymatic activities, thus disrupting cellular processes. Nickel and mercury disturb cell membrane structure. Selenium, silver, and zinc affects microbial growth rate. Zinc causes reduction in biomass and sometimes death to microbial system. Chromium and cadmium are responsible for major bacterial community structure changes (Hodson 2013; Ding et al. 2017; Xie et al. 2016; Sobolev and Begonia 2008; Giller et al. 2009; Abdu et al. 2017; Hattori 1992; Oliveira and Pampulha 2006).

Plants: Plants normally absorb heavy metals and utilize them for their metabolism. But when present in excess of required concentration, heavy metals impart physiological dysfunction, biochemical processes, and malnutrition in plants and, at higher concentration, it can be deadly to the plants. For example, hexavalent chromium causes yellowing of wheat and paddy leaves. Generally, immediate adverse hazardous effects on plants are not observed on heavy metal contamination. The reason for that lies in accumulation of these metals in vacuoles, cell walls, and barks of plants. Arsenic is toxic to legumes, onions, and rice (Singh and Kalamdhad 2011). Cadmium, lead, nickel, and chromium cause growth inhibition. Lead enters into plant through stomata. Lead contamination is toxic to leafy vegetables at low pH. Selenium causes mutation, and mercury affects plant in various ways. Heavy metal uptake in plants can hinder natural uptake of nutrients, homeostasis, and ultimately their growth and development. Their uptake can also cause delayed

germination, induced genotoxicity, reduction in photosynthesis efficiency and rate of respiration, loss of enzymatic activities, oxidative stress, premature leaf fall, reduction in biomass, and stunted growth (Yadav 2010; Hegedüs et al. 2001; Wojcik and Tukiendorf 2004; Mohanpuria et al. 2007; Li et al. 2009; Fryzova et al. 2018; Viehweger 2014).

Heavy Metal-Associated Diseases in Animals/Livestock: Heavy metals when consumed or accumulated in concentration more than permitted disturb biochemical and physiological functions in animals. Various studies on effects of heavy metals on animals have demonstrated reproductive and teratogenic effects. The toxic effects usually associated with chronic exposure to pollutant heavy metals are mutagenicity, carcinogenicity, teratogenicity, immunosuppression, poor body condition, and impaired reproduction. Chromium poisoning causes irritation and ulcers in stomach and small intestines, anemia, and damages to reproduction system; mercury causes adverse neurological and behavioral changes, cellular degeneration, and brain necrosis; cadmium poisoning results into osteoporosis (skeletal damage), disturbances in calcium metabolism, renal stones formation, etc. in animals (Pandey and Madhuri 2014; Ayangbenro and Babalola 2017; Juwarkar and Yadav 2010; Rzymski et al. 2015).

Humans: Heavy metals enter into the human body via air, water, food, and body implants and start accumulating in the human body, causing great and irreversible harm to human health (Anyanwu et al. 2018; Ngole-Jeme and Fantke 2017). Heavy metals may cause steady decline in sperm count, troubled ovulation cycles, increasing menstrual disorders, infertility, spontaneous abortions, premature births, and birth defects (Tchounwou et al. 2012). Children, in particular, are more vulnerable to heavy metal-exposure-associated diseases (Jaishankar et al. 2014). Human health hazards associated with heavy metals have been discussed in detail in later section.

2.4 Human Health Hazards

Humans and other life forms contract various diseases (Table 2.2) when they are in prolonged exposure to heavy metals through dermal contact, inhalation, and consumption of foodstuffs having heavy metals in it (Anyanwu et al. 2018; Ngole-Jeme and Fantke 2017). Owing to their physiochemical characteristics, heavy metals persist in the environment. In many cases, only change occurs in chemical state from one to another one and ultimately accumulating in the food chain (Gall et al. 2015). As per the reports published by many public health organizations, several million human populations throughout the world are suffering from heavy metal-associated diseases. Long-term heavy metal exposure may result in gradually developing muscular, physical, and neurological degenerative processes that mimic Parkinson's disease, Alzheimer's disease, multiple sclerosis, and muscular dystrophy (Anyanwu et al. 2018). It may cause various forms of cancer. Major health hazards are produced with exposure to arsenic, cadmium, lead, mercury, etc. (Mamtani et al. 2011; Hutton and Hutton 1987). Heavy metals, if present in more than permissible limit, may result in acute or chronic toxicity (poisoning), consequentially damaged or reduced mental and central nervous functions, altered blood

Table 2.2 Heavy metal-associated health hazards (Järup 2003; Harada 1995; Barakat 2011; Sahni 2011; Sundar and Chakravarty 2010; Hutton and Hutton 1987; Jan et al. 2015; Mohammed et al. 2011; Singh and Kalamdhad 2011; Huff et al. 2007; WHO 2010, 2011; Rafati Rahimzadeh et al. 2017)

Heavy metals	Associated health hazards/health effects	Other remarks
Arsenic	Cardiovascular diseases, skin cancer, leucomelanosis, arsenicosis, gangrene also known as "black foot," disturbance in the peripheral vascular and nervous systems, diabetes, enlargement of the spleen and lever, high blood pressure, stillbirths, spontaneous abortions, etc. malnutrition may aggravate the effects of arsenic in blood vessels	Major routes of arsenic absorption in human body are inhalation, ingestion, and skin contact. Consequently, it enters into the gastrointestinal tract and lung and then into the bloodstream. Arsenic-contaminated water is a major threat to consumers' health
Mercury	Nervous disorders, insomnia, memory loss, excitability, irritation, tremor, gingivitis, and Minamata diseases. Fetus is more vulnerable to mercury exposure	Autoimmune diseases, depression, disturbance of vision, hair loss, etc.
Lead	Cause damage to many organs such as the brain, kidneys, endocrine system, reproduction system; inhibits blood cell formation which results into anemia; affects the central nervous system; abnormality in pregnancy and fertility; young children more vulnerable toward mental retardation, semipermanent brain damage, etc.	Pb interferes with heme synthesis and thus creates a barrier in blood formation. Pb induces renal tubular dysfunction and thus brings anomalies in kidney's function. Pb poisoning compromises with immune system, interfering with cell maturation and skeletal growth. Lead can cross the placental barrier and may reach the fetus, causing miscarriage, abortions, and stillbirths
Cadmium	Affects the heart; accumulation in kidneys causes renal tubular dysfunction, salivation, difficulty in breathing, nausea, vomiting, pain, anemia, kidney failure, and diarrhea; causes high blood pressure and damage and cancer to lungs. Cadmium fumes cause cardiovascular diseases and interferes with zinc and copper metabolism	The gastrointestinal tract is the major route of cd uptake in both humans and animals. Inhalation of cadmium dust or smoke results into dryness of the throat, pain to the chest and stomach, coughing, increased uneasiness, and bronchial complications
Chromium	Causes damage to DNA, ulcerations, dermatitis and allergic skin reactions, asthmatic bronchitis, bronchospasms, edema, etc.	Oxidation state of chromium decides its toxicity level; respiratory symptoms may include coughing and wheezing, shortness of breath, and nasal itch
Copper	Causes demyelination, kidney diseases, headaches, stomachaches, dizziness, vomiting and diarrhea, liver damage, death if consumed in high amount, *Wilson's disease* characterized by a hepatic cirrhosis, brain damage, etc.	Demyelination refers to damage to the defensive or protective covering (myelin sheath) that surrounds nerve fibers in the brain, optic nerves, and spinal cord

(continued)

Table 2.2 (continued)

Heavy metals	Associated health hazards/health effects	Other remarks
Iron	Haemochromatosis	When present in more than the required amount in humans, it results in haemochromatosis
Barium	Cancer; birth defects; brain swelling; damage to the heart, liver, and spleen; paralysis in humans; fluctuations in blood pressure; etc.	Symptoms include vomiting, diarrhea, muscle weakness, abdominal cramps, difficulties in breathing, numbness around the face, etc.
Beryllium	Lung cancer, skin diseases, etc.	Symptoms include shortness of breath, weight loss, cough, heart and lung damages, lung cancer on inhalation
Antimony	Pneumonitis, non-cardiogenic pulmonary edema, etc.	Symptoms include irritation in the eyes and lungs, stomach pain, vomiting, diarrhea, stomach ulcers, etc.
Cobalt	Reduction in white blood cell number, lung fibrosis, pneumonitis, etc.	Symptoms include hair loss, skin burn on exposed areas, vomiting, diarrhea, coma, mutation, etc.
Nickel	Lower respiratory irritation, pneumonitis, delayed systemic toxic effects, dermatitis also known as "nickel itch," etc.	Symptoms include dizziness, weakness, headaches, vomiting, nausea, and epigastric pain. Nickel fumes cause irritation to respiratory tracts.
Zinc	Food poisoning, upper and lower irritation, fever, diarrhea, abdominal pain, delayed onset pneumonitis, lack of coordination in muscles, acute renal failure, etc.	Symptoms of zinc poisoning include dizziness, nausea, dehydration, stomach ache, electrolyte imbalance, vomiting, lethargies, and sometimes bleeding

composition, and damage to the lung, liver, kidney, and other vital organs. Symptoms associated with heavy metal poisoning are sometimes vague and difficult to diagnose at early state. In general, heavy metal toxicity can cause chronic degenerative diseases, gastrointestinal disorders, chronic fatigue, vision problems, and susceptibility to fungal infections. Genotoxicity and cancers can also occur (Tchounwou et al. 2012; Järup 2003; Harada 1995; Sahni 2011; Singh and Kalamdhad 2011; Huff et al. 2007). According to the World Health Organization (WHO 2011), the common toxic "heavy metals" that can be of public health concerns include beryllium (Be), aluminum (Al), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo), silver (Ag), cadmium (Ca), tin (Sn), antimony (Sb), barium (Ba), mercury (Hg), thallium (Tl), and lead (Pb) (WHO 2010, 2011).

Toxic effects of heavy metals on cellular processes and components (Igiri et al. 2018; Fashola et al. 2016):

1. Cell membrane disruption (Cd, Cu, Ni, Hg, Pb, Zn).
2. Inhibition of enzymatic activities (As, Cd, Cu, Hg, Pb).

3. DNA damage (As, Cd, Hg, Pb), inhibition of transcription (Hg), and translation (Cd, Hg, Pb).
4. Protein denaturation (Cd, Hg, Pb).
5. Inhibits cell division (Cd, Hg, Ni, Pb).

2.4.1 Acceptance Limits of Various Heavy Metals

In general, an elemental concentration in the environment, air, water, and soil, arises due to natural as well as anthropogenic activities. This alteration results in accumulation of one or more heavy metals at a place surpassing the natural admissible limits causing pollution in the air, water, and soil. Living organisms utilize some metals such as calcium, chromium, cobalt, copper, iron, magnesium, manganese, molybdenum, nickel, potassium, selenium, sodium, and zinc as essential nutrients for their growth and development. Increased concentration or certain oxidation states of some metals, however, cause detrimental effects toward growth and development of living organisms. The permissible limits of various metals are given in Table 2.3.

2.4.2 Indian Rivers Polluted with Heavy Metals

From time immemorial, human civilizations have discharged their wastes either solid or liquid into running water sources or rivers. Now, due to rapid industrialization and globalization, many contaminants including most dangerous and nonbiodegradable heavy metals from industries, households, and agricultural fields are finding their ways to the rivers and polluting them. To prevent heavy metal contamination, water quality monitoring to reduce adverse effect on river ecosystem in our country and at global platform has emerged as a critical challenge today. According to a report published by Central Water Commission (CWC), 42 Indian rivers possess

Table 2.3 Permissible limits of heavy metals in soil/water/foodstuffs/air (Elumalai et al. 2017; Yadav et al. 2019; Sharma et al. 2018)

Heavy metals	Permissible limits as BIS[a] 10500: 2012 in river water (μg/L)	EPA regulatory limit (PPM)[b]
Arsenic	10.0	0.01
Cadmium	3.0	5.0
Copper	50.0	–
Chromium	50.0	0.1
Iron	300.0	–
Lead	10.0	15.0
Mercury	–	2.0
Nickel	20.0	0.2 (WHO limit)
Zinc	5000.0	0.5

[a]Bureau of Indian Standards
[b]Environmental Protection Agency

extremely high concentration of neurotoxic heavy metals. These rivers have at least two heavy metals beyond the acceptable limits. Most of the Indian rivers are having copper (10), chromium (21), cadmium (25), nickel (25), lead (69 rivers), and iron (137) beyond the permissible limits. The most revered river, the Ganga, has been found to be contaminated with five heavy metals, namely, chromium, copper, nickel, lead, and iron. Unavailability of safe and sound water for drinking and farming is transforming to become a major public health issue in India today (Pandey et al. 2018).

2.4.3 Indian Scenario

2.4.3.1 Delhi

A recent study conducted by the Centre for Occupational and Environmental Health in 2017 in association with Central Pollution Control Board (CPCB) reported that there were excessive watering, redness, and burning sensation in the eyes post Diwali in some parts of Delhi. Urine sample collected from the some population (sample size 787) when investigated was found to have increased level of heavy metals including lead, barium, and strontium. The main reason behind this was the direct or indirect exposure to firecrackers. Four hospitals under observation were found to have admitted 20% more patients with stroke-related symptoms. There was a 40% surge in admission due to cardiac problems at eight hospitals and 45% increase due to respiratory problems post Diwali in nine hospitals (Web-1 2018).

2.4.3.2 Bangalore (Karnataka)

Researchers from Vijaya College, Bangalore, found accumulation of various heavy metals on fur, feathers, and human hair across the city. At least seven different heavy metals, most of them toxic, were absorbed from air. Six different heavy metals including mercury were found to be present on human hair. Birds like hens were found carrying seven heavy metals on their feathers and crows five heavy metals including cadmium, iron, nickel, and zinc. Various heavy metals were found in sheep, goats, cats, and dogs, some even carrying molybdenum. Under long-term exposure, it facilitates easy absorption of these heavy metals into bloodstream and poses higher risks of allergies and adversities in metabolism. Arsenic, a gaseous by-product of industries, was found in higher concentration in industrial areas in Rajajinagar, Bangalore. Similarly, the amount of lead and cadmium (as a result of battery production or disposal) was also higher in industrial areas (Web-2 2018).

2.4.3.3 Karnataka, Kerala, and Tamil Nadu

Banana cultivation field soils were found to have higher amount of Cu, Mg, Cr, and Co than normal limits. An extensive study of over 250 soil samples from three Indian states, Karnataka, Kerala, and Tamil Nadu, has revealed that the majority of the banana cultivation fields are contaminated with copper, magnesium, chromium, and cobalt at concentration higher than the threshold levels for normal soils (Pacha 2018).

2.4.3.4 Coimbatore (Tamil Nadu)

Untreated sewage water which irrigated some leafy vegetables investigated for presence of heavy metals was found to be contaminated with high level of cadmium, copper, lead, manganese, and zinc. Long-term application of raw untreated sewage water, which contains heavy metals, attributed the presence and concentration of heavy metals in the area under irrigation (Web-3 2003).

2.4.3.5 Malwa Region (Punjab)

Indiscriminate use of chemical fertilizers, pesticides, insecticides, and herbicides has changed the subsoil of this region to an extent that many newborns have developed limb deformities and many have attracted neuronal disorders. Heart ailments and cancer are rampant among adult population. The reason behind this is that the level of many heavy metals in soils in Punjab is beyond their permissible limit (Web-4 2018).

2.5 Remediation Techniques of Heavy Metal Degradation and Removal from Contaminated Sites

Various approaches used to degrade heavy metals include physical, chemical, biological, or a combination of these methods, but many of these methods are not environment-friendly and economically viable (Emenike et al. 2018). In addition to this, many of these methods do not ensure restoration without residual effects. Not a single method claims complete degradation or removal of heavy metals. Salts of heavy metals, in general, are water-soluble and cannot be separated through physical means. Physiochemical methods bring secondary pollution at the site of treatment (Sharma et al. 2018). Conventional physicochemical methods are costly and not efficient and successful in areas with low metal toxicity (Barakat 2011; Gunatilake 2015). A comparison between biological and physiochemical methods of heavy metal removal or degradation has been provided in Table 2.4. Biological remediation is based on application of microbes and plants and their derivatives for metal removal from the environment (Rajendran et al. 2003; Ayangbenro and Babalola 2017; Gadd 2004). Several plants such as *Spartina maritima* (arsenic, lead, copper, and zinc), *Arundo donax* (cadmium and zinc), *Eichhornia crassipes* (water hyacinth) (cadmium, copper, chromium, iron, and zinc), *Plectranthus amboinicus* (lead), *Amaranthus paniculatus* (nickel), *Carex pendula* (lead), and genetically engineered *Arabidopsis thaliana* (arsenic) have been investigated for their heavy metal removal from soils, known as phytoremediation (Tangahu et al. 2011). Different methods based on the removal or degradation mechanism of phytoremediation can be categorized into phytoextraction (extraction of accumulated metal ions in aerial plants by disposing or burning), phytofiltration (removal of metals from aqueous waters by roots or seeds of plants), phytostabilization (plant roots absorb the heavy metals and maintain them in rhizosphere), phytovolatilization (volatilization of heavy metals from plant's foliage), and phytodegradation (degradation of heavy metals through plant materials and associated microorganisms) (Ojuederie and

Table 2.4 Biological methods vs. physiochemical methods (Kumar and Bharadvaja 2019)

Biological methods	Physiochemical methods
1. It includes methods based on biosorption, bio-immobilization, bioleaching, biomineralization, phytoremediation, plant–microbe interaction, rhizoremediation, hyper-accumulators, designer microbe approach, etc. 2. It utilizes plants, microbes, and components derived from them like enzymes or any biopolymer for heavy metal degradation and remediation 3. Natural process with a scope to increase its efficiency and rate of removal by introducing modern tool and techniques of biotechnology 4. Eco-friendly and efficient methods as it possesses the ability to remove even trace amount of heavy metals from the polluted site 5. Minimal exposure of on-site workers to the contaminants and any ill effects of them 6. Eliminates the need of transport, thus less expensive to physiochemical methods 7. Adaptability of microbes and other biological systems in non-native environment decreases their metal removal efficiency 8. No universal application. Different biological systems are required for different metals depending upon the nature of the metal to be removed. Also, biological processes are slow as compared to physiochemical processes	1. It includes methods based on electrochemical treatment, oxidation or reduction, membrane technology, reverse osmosis, chemical precipitation, ion exchange, evaporation and filtration, etc. 2. Expensive method, as it needs an institutional setup comprising dedicated skilled workers and specialized instruments, continuous power supply, chemicals, etc. 3. Lesser impact of abiotic factors such as relative humidity, temperature, light availability as compared to bioremediation. Quicker in comparison to bioremediation. 4. Synthesis of toxic gases, thus detrimental impact on human and environment. Posttreatment monitoring of effluents is highly required. 5. Ineffective in areas with low heavy metal toxicity or concentration. 6. Physiochemical methods bring secondary pollution at the site of treatment. 7. Expensive method, as it needs an institutional setup comprising dedicated workers and specialized instruments, continuous power supply, chemicals, etc. 8. Transportation of waste materials or contaminants from human settlements to predecided site of treatment or otherwise transport, installation, and operation of equipment/chemicals/power supply at the heavy metal-contaminated site

Babalola 2017; Arjoon et al. 2013). Microbes, for example, *Acinetobacter calcoaceticus, Aureobacterium esteroaromaticum, Achromobacter* sp., *Aeromonas* sp., *Bacillus licheniformis, Enterobacter* sp., *Escherichia* sp., *Micrococcus* sp., *Klebsiella oxytoca*, and *Pseudomonas putida*, have been reported in heavy metal removal and degradation (Rajendran et al. 2003; Igiri et al. 2018; Gadd 2004; Tabak et al. 2005). Some cyanobacteria and algae like *Chlorella* sp. and *Neochloris* sp. have been reported to remove chromium from wastewater (Igiri et al. 2018; Kaplan 2013). There is a vast literature dealing with diverse use of plants and microbes in biotransformation and removal of heavy metals from contaminated soil. Bioremediation is therefore an environment-friendly, green, and proficient way of reclaiming environments polluted with heavy metals by making use of the intrinsic as well as induced biological mechanisms of biological systems to get rid of harmful contaminants.

2.6 Microbial Bioremediation of Heavy Metals

Bioremediation of heavy metal pollutants is a sustainable approach that utilizes the metabolic activity of microbes to remove, reduce or transform, or degrade them. It facilitates heavy metal decomposition or immobilization by exercising the existing metabolic potential of microbes with new catabolic functions resulting from selection or by introduction of genes encoding such functions. The application of microbes in heavy metal remediation and degradation has been under investigation for decades as they transform, degrade, or chelate various toxic compounds into a less or nontoxic form. Change in oxidation state of metals alters their water solubility and toxicity (Rajendran et al. 2003; Ayangbenro and Babalola 2017). *Bacillus* sp., *Pseudomonas* sp., *Streptomyces* sp., *Aspergillus* sp., *Rhizopus* sp., *Penicillium* sp., etc. have significant heavy metal removal ability. It is comparatively more effective, economic, and environment-friendly. Microbial metabolic secretions such as organic acids are able to dissolve heavy metals or soil particles containing heavy metals in them. Microbes use different mechanisms such as precipitation, biosorption, and enzymatic transformation to degrade or reduce heavy metals into a less toxic form that are more stable, less mobile, or inert (Arjoon et al. 2013; Tabak et al. 2005). Microbes use two processes, namely, aerobic and anaerobic, depending upon use and not use of oxygen for cellular respiration to generate energy-utilizing chemical energy stored in the bonds of organic molecules. Difference between these two processes has been summarized by Kumar and Bharadvaja (2019). Different microbes have different adaptability to be an inhabitant of a heavy metal-contaminated environment. Microbes have a limited range of tolerance toward heavy metals. It also depends upon the toxicity of heavy metals. Their degradation ability mainly depends on degradative plasmids and spores. pH, temperature, etc. affects the degradation efficiency. Several factors which determine microbial degradation have been elaborated in later sections. The mechanisms of microbial degradation of heavy metals are mainly biosorption, bioaccumulation, biotransformation, bioleaching, biomineralization, and co-metabolism (Igiri et al. 2018; Tabak et al. 2005). Biosorption, which solely depends on cell surface structure, is established to be the prime mechanism. Biomass, in both living and dead conditions, can be used for heavy metal removal. Application of dead biomass/biological system is more feasible than using living biological system and bioaccumulation for metal removal at large scale. It avoids the requirement of continuous nutrient supply and maintenance of proper growth conditions and operation of a complicated bioreactor system (White et al. 1995).

Advantage of microbe-assisted heavy metal remediation (Jin et al. 2018; Kumar and Bharadvaja 2019):

1. Both microbes and pollutant heavy metals can be completely removed from soils; thus, soil structure remains unaltered. It facilitates a low-cost treatment and maintenance of soil structure.
2. Secondary pollution can be avoided at treatment sites. Thus, no any further harmful effect on the population in direct or indirect contact or exposure.
3. Native microbial population can be used for remediation purposes which do not need any effort or manipulation in the environment for microbial growth.
4. Microorganisms can be trained to degrade and remediate the target heavy metals through natural selection or by means of insertion and expression of functional genes of the desired purpose.

2.6.1 Mechanisms of Microbial Heavy Metal Bioremediation

Microbial remediation occurs in two ways—either microbes secrete enzymes like oxidases and reductase having capable of metal degradation or they develop resistance against the harmful effects of heavy metals present in their internal or external environment. Microbial cell surface due to having negatively charged surface becomes the active site for metal cations binding. The cell wall of microbes contains hydroxyl, alcohol, carboxylate, amine, phosphoryl, carbonyl, ester, sulfhydryls, thiol, and thioether like functional groups which facilitate metal binding through different interactions including covalent bonding, ionic interactions, or van der Wall forces of attraction, and thus, removal of metals from the environment is exercised. Major microbial mechanisms of heavy metal removal from the environment are biosorption and bioaccumulation. Biosorption is metabolism-independent mechanism, while bioaccumulation is metabolism-dependent. Biosorption is a passive uptake of heavy metals, while bioaccumulation involves active uptakes of heavy metals from the environment. In bioaccumulation, metal ions under cellular metabolic processes pass across cell membrane and enter into cytoplasm. Bioaccumulation, also known as active uptake, is dependent on a range of physicochemical and biological mechanisms. Also, accumulating microbes should have adaptability and tolerance toward a wide range of heavy metals at varying concentrations. It must include the abilities to simultaneously transform heavy metals from one to another less toxic and less harmful form and keep the metal contained. Bioaccumulation includes sequestration, redox reactions, species transformation, etc. Biosorption process utilizes living as well as dead biomass, carried out as passive uptake of metals through surface complexion onto the cell wall and surface layers. It includes ion exchange, precipitation reaction, redox process, and surface complexation. Efficiency of biosorption mechanism depends upon the pH and temperature of the surrounding environment, biological system, nature and concentration of heavy metals, etc. Among all, pH exerts maximum effect on biosorption as it causes alteration in metal surface properties, functional properties,

and competition of metallic ions with hydrogen ions present in the system. Another mechanism, extracellular sequestration, is accumulation of heavy metals in the periplasm in the form an insoluble complex. For example, sulfate-reducing bacteria generate large amount of hydrogen sulfide that causes precipitation of heavy metal cations. Transfer of some heavy metals from cytoplasm to periplasm also occurs. In intracellular sequestration, complexion of heavy metals which occurs by various cellular compounds is termed as fourth mechanism. Remediations of heavy metals which interact with surface ligands and gradually transported into the cell cytoplasm go through this channel. Some other mechanisms include reduction of heavy metal ions by microbial cell which involves change in oxidation states of metals or metalloids in order to reduce toxicity toward their own, that is, known as biotransformation. Microbial plasma membrane, cell wall, or capsule prohibits the entry of metals into cell cytoplasm which is termed as exclusion by permeability membrane or barrier. In extrusion mechanism, metals are pushed out through the cell under plasmid or chromosomal mediated events.

2.6.2 Factors that Affect Microbial Heavy Metal Degradation Capacity

Microbes due to their smaller size easily get into contact with contaminants and facilitate quick and effective treatment or deduction of pollutants to admissible or less hazardous state. The success of microbial remediation depends upon the nature of microbes involved, characteristics of contaminants, and chemical and geological conditions at the contaminated sites. Other critical factors that affect bioremediation efficiency of microorganisms include temperature, pH, soil structure, air/oxygen availability, dissolved oxygen, redox potentials, availability and solubility of nutritional contents, nutrient diffusion, mass transfer, moisture content, water solubility, chemical composition, and concentrations of heavy metals (Igiri et al. 2018; Kumar and Bharadvaja 2019).

2.7 Advancements in Microbial Technologies for Promising Heavy Metal Removal from the Environment

1. Integration of immobilization techniques (adsorption, electrostatic and/or covalent binding on a surface, flocculation, entrapment, encapsulation) for better adsorption and absorption of heavy metals from polluted environmental sample (Macaskie 2007). Immobilization helps in fixing the microbial cells or enzymes, limiting their mobility, and washing off at their places and increases the retention time, their reusability, viability, and catalytic activity. It increases the efficiency of bioremediation process and makes them economically competitive (Dzionek et al. 2016). Adsorption of heavy metals on cell surface takes place by weak forces of interaction. Because of weak forces of interaction, there is high probability of leakage of cells from the adsorbed surface. Covalent and/or electrostatic

binding methods are similar but comparatively more stable to physical adsorption immobilization; thus, it provides a lesser degree of cell leakage from the bioremediation setup (Bayat et al. 2015). Entrapments provide a limited mobility to microbial cells or derivatives inside a protected and manipulatable environment of heterogeneous matrix. They cannot leak out from the matrix but can move freely inside the matrix. It increases the exchange of nutrients and metabolites into a limited space. Thus, it increases the bioremediation efficiency of microbial cells. The ratio of pore size of the carrier to the cell size is a critical determinant of successful entrapment technique. Encapsulation refers to use of some semipermeable coating materials over the cell to protect it from the harsh environmental conditions. Limited permeability of the membrane and its degradation by the growing cells make this technique undesirable for microbial bioremediation (Dzionek et al. 2016; Datta et al. 2013; Mohamad et al. 2015).

2. Application of modern biotechnological tools such as genetic engineering or recombinant DNA technology has been exercised to increase heavy metal bioremediation and biodegradation (Timmis and Pieper 1999; Xu et al. 2010). Synthetic biology and system biology approach can facilitate a microbial consortia having ability to transform, degrade, leachate, or remove heavy metals from environmental sample of concern. Metagenomics and metabolic studies provide information on microbial diversity and population and functional composition of diverse microbial population for metal resistance genes which can be used for improving the ability of microbial strains for heavy metal removal or degradation. Genetic engineering has provided ability to transfer one or more desired traits from one species to another to establish a particular strain for bioremediation of soil, sludge, or contaminated water (Diep et al. 2018; Pandey et al. 2018; Lv et al. 2018). Researchers have listed many microbes, plants in individual capacity as well as microbes–plants, and genetically modified microbes assisting plants in heavy metal bioremediation (Sharma et al. 2018; Tiwari and Lata 2018; Gupta and Diwan 2017; Igiri et al. 2018). Programmed death of the synthetic microorganisms soon after the heavy metal bioremediation could minimize the risks of horizontal gene transfer and any possible ill effects like uncontrolled growth and proliferation.
3. Application of naturally occurring biofilms for heavy metal removal from the environment can be a better strategy (Teitzel and Parsek 2003; Meliani and Bensoltane 2016). Biofilms bind substantial quantities of heavy metals under pristine conditions and serve as a medium for the precipitation of insoluble mineral phases. Biofilms contain diverse range of microbial population having exopolymeric substances which exhibits excellent surfactant or emulsifier properties. It increases the bioavailability of heavy metal contaminants. Microalgae–bacteria aggregates can be also used for heavy metal removal from wastewater water (Igiri et al. 2018).
4. New omics-based approaches include proteomics, genomics, transcriptomics, and metatranscriptomics which provide extraordinary information starting from genes, to proteins, to metabolites that can be used for effective bioremediation (Singh et al. 2015). Genes involved in microbe responsible for any heavy metal degradation or reduction to an innocuous state can be determined; genome

sequence of that involved microbe can be analyzed (genomics), and further, the whole microbial population and their genome can be used to construct a library for identification and utilization of microbes for heavy metal remediation (Malla et al. 2018). It can be helpful in establishment of microbial consortia capable of efficiently and effectively removing diverse range of heavy metals from the site of pollution.

5. Nanotechnological approach for heavy metal removal: Nanotechnology has provided the manipulation of material's properties for removal of pollutants including heavy metals from wastewater (Kahrizi et al. 2016; Mitra et al. 2017). The dimension of material reduces to nanoscale which imparts it excellent surface properties and quantum effects (Parvin et al. 2019). It contributes an extraordinary adsorption capacity and reactivity to the material which favors the removal of heavy metals (Zhao et al. 2016). Several nanomaterials including carbon, silicon, zinc, grapheme-based nanomaterials and nanostructure or their composites, zero-valent metals, and several metal-oxide-based nanocomposites and nanomaterials have been investigated for heavy metal removal from wastewater (Sheet et al. 2014; Gangadhar et al. 2012; Le et al. 2019). Carbon nanotubes (CNTs) have excellent adsorption capacity toward manganese, thallium, copper, lead, chromium, etc. owing to possible adsorption active sites like interstitial channels, internal sites, and external groove sites. High production cost, difficulty in separation of CNTs from wastewater which further increases the treatment cost, risk of secondary pollution, etc. limit the suitability of large-scale application of CNTs for heavy metal removal from wastewater (Parvin et al. 2019; Yang et al. 2019). Grapheme is another carbon-based nanomaterials which can be used for heavy metal removal from wastewater. Grapheme-based nanoscale materials show high affinity toward adsorption of lead, mercury, cadmium, etc. Silica-based nanomaterials possess excellent surface characteristics and have been tested for heavy metal adsorption from heavy metal-polluted water (Liu et al. 2019). Nanosilica can be surface modified by groups like $–NH_2$ and –SH or serve as the support of nanocomposites. Zero-valent metal nanoparticles have shown a great potential for wastewater remediation in recent times (Arjoon et al. 2013). Zero-valent iron nanoparticles have been found to have a potential to remove mercury, chromium, copper, nickel, and cadmium from polluted water. Metal-oxide-based nanomaterials of iron, manganese, zinc, titanium, aluminum, magnesium, cerium, zirconium, etc. have been reported to be efficient in treating wastewater contaminated with heavy metals such as cadmium, copper, lead, zinc, and mercury along with other pollutants (Le et al. 2019; Yang et al. 2019; Parvin et al. 2019; Sheet et al. 2014; Singh et al. 2013).
6. Others: Microbial bioremediation efficiency can be increased by amending the reaction environment by adding inorganic nutrients, biosurfactants, bulking agents, composts, and biochar (Igiri et al. 2018). Modifications in microbes have been tried in order to achieve high metal uptake over broad range of pH, temperature, and prevailing atmospheric conditions. Microbial fuel cell-based techniques are also in recent trends and have been extensively studied (Mathuriya and Yakhmi 2014; Gajda et al. 2017; Ezziat et al. 2019) for heavy metal removal from wastewater or bioremediation (Igiri et al. 2018; Wu et al. 2017).

2.8 Challenges

1. Reliable and inexpensive biological mass/system for effective heavy metal removal from the site of interest.
2. Assessment of future health hazards associated with genetically modified organisms.
3. Prevention of horizontal gene transfer, uncontrolled growth, and expansion of genetically modified organisms.
4. Prevention of air, water, and soil contamination with heavy metals.
5. Prevention of heavy metal-associated diseases to living beings.
6. Conservation of biodiversity.

2.9 Control Measures

The unwanted chemical nature (toxicity) and bioavailability of heavy metals can be altered through redox reactions; however, the elemental property remains unchanged. Therefore, removal of heavy metals from the environment in entirety is an impossible task. The following measures can be adopted to prevent the environment from hazardous effects of heavy metal contamination:

1. To control the dust emissions through chimneys, and use electrostatic precipitator units.
2. Establishment of effluent treatment plants and sewage treatment plants to treat the wastewater before their release into the environment. Frequent monitoring of quality of discharge water. Use of chemical, biological, or combination of both in order to complete removal of heavy metals from the discharged water.
3. Promotion of effective and efficient implementation of air, water, and soil pollution control laws and regulations. Enforcement and monitoring are critical to its success.
4. Prevent or reduce the use of fertilizers and biocides containing heavy metals and promotion of organic farming.
5. Awareness programs on heavy metal-contamination-associated hazards, prevention, and mitigation strategies.

References

Abdu N, Abdullahi AA, Abdulkadir A (2017) Heavy metals and soil microbes. Environ Chem Lett 15(1):65–84

Akpor OB, Ohiobor GO, Olaolu TD (2014) Heavy metal pollutants in wastewater effluents: sources, effects and remediation. Adv Biosci Bioeng 2(4):37

Ali H, Khan E (2018) Bioaccumulation of non-essential hazardous heavy metals and metalloids in freshwater fish. Risk to human health. Environ Chem Lett 16:903–917

Ali H, Khan E, Ilahi I (2019) Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. J Chem 2019:1–14

Alissa EM, Ferns GA (2011) Heavy metal poisoning and cardiovascular disease. J Toxicol 2011:870125

Alloway BJ (2013) Sources of heavy metals and metalloids in soils. In: Alloway B (ed) Heavy metals in soils. Environmental pollution, vol 22. Springer, Dordrecht, pp 11–50

Anyanwu B, Ezejiofor A, Igweze Z, Orisakwe O, Anyanwu BO, Ezejiofor AN, Igweze ZN et al (2018) Heavy metal mixture exposure and effects in developing nations: an update. Toxics 6 (4):65

Aonghusa CN, Gray NF (2002a) Laundry detergents as a source of heavy metals in Irish domestic wastewater. J Environ Sci Health A 37(1):1–6

Aonghusa CN, Gray NF (2002b) Laundry detergents as a source of heavy metals in Irish domestic wastewater. *J Environ Sci Health A* 37(1):1–6

Appenroth KJ (2009) Definition of "heavy metals" and their role in biological systems. In: Soil heavy metals. Soil biology, vol 19. Springer, Berlin, Heidelberg, pp 19–29

Arjoon A, Olaniran AO, Pillay B (2013) Co-contamination of water with chlorinated hydrocarbons and heavy metals: challenges and current bioremediation strategies. Int J Environ Sci Technol 10(2):395–412

Ayangbenro AS, Babalola OO (2017) A new strategy for heavy metal polluted environments: a review of microbial biosorbents. Int J Environ Res Public Health 14(1):94

Barakat MA (2011) New trends in removing heavy metals from industrial wastewater. Arab J Chem 4(4):361–377

Bayat Z, Hassanshahian M, Cappello S (2015) Immobilization of microbes for bioremediation of crude oil polluted environments: a mini review. Open Microbiol J 9:48–54

Belzile N, Chen Y-W, Filella M (2011) Human exposure to antimony: I. Sources and intake. Crit Rev Environ Sci Technol 41(14):1309–1373

Bradl HB (2005) Chapter 1 sources and origins of heavy metals. Interface Sci Technol 6(C):1–27

Datta S, Christena LR, Rajaram YRS (2013) Enzyme immobilization: an overview on techniques and support materials. 3 Biotech 3(1):1–9

Diep P, Mahadevan R, Yakunin AF (2018) Heavy metal removal by bioaccumulation using genetically engineered microorganisms. Front Bioeng Biotechnol 6:157

Ding Z, Wu J, You A, Huang B, Cao C (2017) Effects of heavy metals on soil microbial community structure and diversity in the rice (*Oryza sativa* L. subsp. Japonica, food crops Institute of Jiangsu Academy of Agricultural Sciences) rhizosphere. Soil Sci Plant Nutr 63(1):75–83

Dixit R, Wasiullah, Malaviya D, Pandiyan K, Singh U, Sahu A, Shukla R et al (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability 7(2):2189–2212

Dzionek A, Wojcieszyńska D, Guzik U (2016) Natural carriers in bioremediation: a review. Electron J Biotechnol 23:28–36

Elumalai V, Brindha K, Lakshmanan E, Elumalai V, Brindha K, Lakshmanan E (2017) Human exposure risk assessment due to heavy metals in groundwater by pollution index and multivariate statistical methods: a case study from South Africa. Water 9(4):234

Emenike CU, Jayanthi B, Agamuthu P, Fauziah SH (2018) Biotransformation and removal of heavy metals: a review of phytoremediation and microbial remediation assessment on contaminated soil. Environ Rev 26(2):156–168

Ezziat L, Elabed A, Ibnsouda S, El Abed S (2019) Challenges of microbial fuel cell architecture on heavy metal recovery and removal from wastewater. Front Energy Res 7:1

Fashola M, Ngole-Jeme V, Babalola O, Fashola MO, Ngole-Jeme VM, Babalola OO (2016) Heavy metal pollution from gold mines: environmental effects and bacterial strategies for resistance. Int J Environ Res Public Health 13(11):1047

Fryzova R, Pohanka M, Martinkova P, Cihlarova H, Brtnicky M, Hladky J, Kynicky J (2018) Oxidative stress and heavy metals in plants. Rev Environ Contam Toxicol 245:129–156

Gadd GM (2004) Microbial influence on metal mobility and application for bioremediation. Geoderma 122:109–119

Gajda I, Stinchcombe A, Greenman J, Melhuish C, Ieropoulos I (2017) Microbial fuel cell—a novel self-powered wastewater electrolyser for electrocoagulation of heavy metals. Int J Hydrogen Energy 42(3):1813–1819
Gall JE, Boyd RS, Rajakaruna N (2015) Transfer of heavy metals through terrestrial food webs: a review. Environ Monit Assess 187(4):201
Gangadhar G, Maheshwari U, Gupta S (2012) Application of Nanomaterials for the removal of pollutants from effluent streams. Nanosci Nanotechnol-Asia 2:140–150
Giller KE, Witter E, McGrath SP (2009) Heavy metals and soil microbes. Soil Biol Biochem 41 (10):2031–2037
Gunatilake SK (2015) Methods of removing heavy metals from industrial wastewater. J Multidiscip Eng Sci Stud (JMESS) 1:12–18
Gupta P, Diwan B (2017) Bacterial exopolysaccharide mediated heavy metal removal: a review on biosynthesis, mechanism and remediation strategies. Biotechnol Rep 13:58–71
Harada M (1995) Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit Rev Toxicol 25(1):1–24
Hattori H (1992) Influence of heavy metals on soil microbial activities. Soil Sci Plant Nutr 38 (1):93–100
Hegedüs A, Erdei S, Horváth G (2001) Comparative studies of H2O2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. Plant Sci 160(6):1085–1093
Hejna M, Gottardo D, Baldi A, Dell'Orto V, Cheli F, Zaninelli M, Rossi L (2018) Review: nutritional ecology of heavy metals. Animal 12(10):2156–2170
Hodson ME (2013) Effects of heavy metals and metalloid/s on soil organisms. Environ Pollut 22:141–160
Huff J, Lunn RM, Waalkes MP, Tomatis L, Infante PF (2007) Cadmium-induced cancers in animals and in humans. Int J Occup Environ Health 13(2):202–212
Hutton M and Hutton, M. (1987) Human health concerns of lead, mercury, cadmium and arsenic. Lead, mercury, cadmium and arsenic in the environment. Wiley, pp 53–68
Igiri BE, Okoduwa SIR, Idoko GO, Akabuogu EP, Adeyi AO, Ejiogu IK (2018) Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review. J Toxicol 2018:1–16
Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN (2014) Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol 7(2):60–72
Jan AT, Azam M, Siddiqui K, Ali A, Choi I, Haq QMR (2015) Heavy metals and human health: mechanistic insight into toxicity and counter defense system of antioxidants. Int J Mol Sci 16 (12):29592–29630
Järup L (2003) Hazards of heavy metal contamination. Br Med Bull 68(1):167–182
Jenkins D (1998) The effect of reformulation of household powder laundry detergents on their contribution to heavy metals levels in wastewater. Water Environ Res 70(5):980–983
Jin Y, Luan Y, Ning Y, Wang L, Jin Y, Luan Y, Ning Y et al (2018) Effects and mechanisms of microbial remediation of heavy metals in soil: a critical review. Appl Sci 8(8):1336
Juwarkar AA, Yadav SK (2010) Bioaccumulation and biotransformation of heavy metals. Bioremed Technol:266–284
Kahrizi H, Bafkar A, Farasati M (2016) Effect of nanotechnology on heavy metal removal from aqueous solution. J Cent South Univ 23(10):2526–2535
Kaji M (2012) Role of experts and public participation in pollution control: the case of Itai-itai disease in Japan. Ethics Sci Environ Politics 12:99–111
Kaplan D (2013) Absorption and adsorption of heavy metals by microalgae. Handbook of microalgal culture. Wiley, Oxford, pp 602–611
Kumar L, Bharadvaja N (2019) Enzymatic bioremediation: a smart tool to fight environmental pollutants. In: Bhatt P (ed) Smart bioremediation technologies: microbial enzymes, 1st edn. Elsevier, pp 99–118
Kumar L, Bidlan R, Sharma J, Bharadvaja N (2019) Biotechnological management of water quality: a mini review. Biosci Biotech Res Comm 12(1):140–146

Le AT, Pung S-Y, Sreekantan S, Matsuda A, Huynh DP (2019) Mechanisms of removal of heavy metal ions by ZnO particles. Heliyon 5(4):e01440
Li H-F, Gray C, Mico C, Zhao F-J, McGrath SP (2009) Phytotoxicity and bioavailability of cobalt to plants in a range of soils. Chemosphere 75(7):979–986
Li Q, Liu H, Alattar M, Jiang S, Han J, Ma Y, Jiang C (2015) The preferential accumulation of heavy metals in different tissues following frequent respiratory exposure to PM 2. 5 in rats. Sci Rep 5(5):16936
Liu L, Luo X-B, Ding L and Luo S-L (2019) Application of nanotechnology in the removal of heavy metal from water. Nanomaterials for the removal of pollutants and resource reutilization, Elsevier, pp 83–147
Lv D, Zhu T, Liu R, Li X, Zhao Y, Sun Y, Wang H et al (2018) Effects of co-processing sewage sludge in the cement kiln on PAHs, heavy metals emissions and the surrounding environment. Int J Environ Res Public Health 15(4):698
Macaskie LE (2007) An immobilized cell bioprocess for the removal of heavy metals from aqueous flows. J Chem Technol Biotechnol 49(4):357–379
Malla MA, Dubey A, Yadav S, Kumar A, Hashem A, Abd-Allah EF (2018) Understanding and designing the strategies for the microbe-mediated remediation of environmental contaminants using omics approaches. Front Microbiol 9:1132
Mamtani R, Stern P, Dawood I, Cheema S (2011) Metals and disease: a global primary health care perspective. J Toxicol 2011:319136
Mathuriya AS, Yakhmi JV (2014) Microbial fuel cells to recover heavy metals. Environ Chem Lett 12(4):483–494
Meliani A, Bensoltane A (2016) Biofilm-mediated heavy metals bioremediation in PGPR pseudomonas. J Bioremed Biodegr 7(5):1–9
Mitra S, Sarkar A, Sen S (2017) Removal of chromium from industrial effluents using nanotechnology: a review. Nanotechnol Environ Eng 2(1):11
Mohamad NR, Marzuki NHC, Buang NA, Huyop F, Wahab RA (2015) An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes. Biotechnol Biotechnol Equip 29(2):205–220
Mohammadi Roozbahani M, Sobhan Ardakani S, Karimi H, Sorooshnia R (2015) Natural and anthropogenic source of heavy metals pollution in the soil samples of an industrial complex a case study. Iranian J Toxicol 9(29):1336–1341
Mohammed AS, Kapri A, Goel R (2011) Heavy metal pollution: source, impact, and remedies. In: Biomanagement of metal-contaminated soils. Springer, Dordrecht, pp 1–28
Mohanpuria P, Rana NK, Yadav SK (2007) Cadmium induced oxidative stress influence on glutathione metabolic genes of Camellia sinensis (L.) O. Kuntze. Environ Toxicol 22 (4):368–374
Ngole-Jeme VM, Fantke P (2017) Ecological and human health risks associated with abandoned gold mine tailings contaminated soil. PLoS One 12(2):e0172517
Ojuederie OB, Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int J Environ Res Public Health 14(12). https://doi.org/10.3390/ijerph14121504
Oliveira A, Pampulha ME (2006) Effects of long-term heavy metal contamination on soil microbial characteristics. J Biosci Bioeng 102(3):157–161
Pacha A (2018) Heavy metal contamination in south Indian banana fields. The Hindu, Available at: https://www.thehindu.com/sci-tech/science/heavy-metal-contamination-in-south-indian-banana-fields/article24843048.ece. Accessed 15 July 2019
Pandey G, Madhuri S (2014) Heavy metals causing toxicity in animals and fishes. Res J Anim Vet Fish Sci 2(2):17–23
Pandey K, Sengupta R and Bajpai I (2018) 42 rivers have extremely high concentration of neurotoxic heavy metals. DownToEarth, Available at: https://www.downtoearth.org.in/news/water/huge-amounts-of-toxic-heavy-metals-swim-in-indian-rivers-60545 (accessed 14 July 2019)

Parvin F, Rikta SY and Tareq SM (2019) Application of nanomaterials for the removal of heavy metal from wastewater. Nanotechnology in water and wastewater treatment. Elsevier, p. 137–157
Rafati Rahimzadeh M, Rafati Rahimzadeh M, Kazemi S, Moghadamnia A-A (2017) Cadmium toxicity and treatment: an update. Caspian J Intern Med 8(3):135–145
Rajendran P, Muthukrishnan J, Gunasekaran P (2003) Microbes in heavy metal remediation. Indian J Exp Biol 41:935–944
Rzymski P, Tomczyk K, Rzymski P, Poniedziałek B, Opala T, Wilczak M (2015) Impact of heavy metals on the female reproductive system. Ann Agric Environ Med 22(2):259–264
Sahni SK (2011) Hazardous metals and minerals pollution in India: sources, toxicity and management. In: Indian national science academy. Angkor Publishers (P) Ltd., pp 1–29
Sharma B, Singh S, Siddiqi NJ (2014) Biomedical implications of heavy metals induced imbalances in redox systems. Biomed Res Int 2014:1–26
Sharma S, Bhattacharya A (2017) Drinking water contamination and treatment techniques. Appl Water Sci 7(3):1043–1067
Sharma S, Tiwari S, Hasan A, Saxena V, Pandey LM (2018) Recent advances in conventional and contemporary methods for remediation of heavy metal-contaminated soils. 3 Biotech 8(4):216
Sheet I, Kabbani A, Holail H (2014) Removal of heavy metals using nanostructured graphite oxide, silica nanoparticles and silica/graphite oxide composite. Energy Procedia 50:130–138
Singh J, Kalamdhad AS (2011) Effects of heavy metals on soil, plants, human health and aquatic life. Int J Res Chem Environ 1(2):15–21
Singh R, Gautam N, Mishra A, Gupta R (2011) Heavy metals and living systems: an overview. Indian J Pharmacol 43(3):246–253
Singh S, Barick KC, Bahadur D (2013) Functional oxide nanomaterials and nanocomposites for the removal of heavy metals and dyes. Nanomater Nanotechnol 3:20
Singh S, Parihar P, Singh R, Singh VP, Prasad SM (2015) Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front Plant Sci 6:1143
Sobolev D, Begonia MFT (2008) Effects of heavy metal contamination upon soil microbes: lead-induced changes in general and denitrifying microbial communities as evidenced by molecular markers. Int J Environ Res Public Health 5(5):450–456
Sundar S, Chakravarty J (2010) Antimony toxicity. Int J Environ Res Public Health 7 (12):4267–4277
Tabak HH, Lens P, Van Hullebusch ED, Dejonghe W (2005) Developments in bioremediation of soils and sediments polluted with metals and radionuclides-1. Microbial processes and mechanisms affecting bioremediation of metal contamination and influencing metal toxicity and transport. Rev Environ Sci Biotechnol 4:115–156
Tangahu BV, Sheikh Abdullah SR, Basri H, Idris M, Anuar N, Mukhlisin M (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng 2011:1–31
Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy metal toxicity and the environment. Exp Suppl 2012 101:133–164
Teitzel GM, Parsek MR (2003) Heavy metal resistance of biofilm and planktonic Pseudomonas aeruginosa. Appl Environ Microbiol 69(4):2313–2320
Timmis KN, Pieper DH (1999) Bacteria designed for bioremediation. Trends Biotechnol 17 (5):201–204
Tiwari S, Lata C (2018) Heavy metal stress, signaling, and tolerance due to plant-associated microbes: an overview. Front Plant Sci 9:452
Viehweger K (2014) How plants cope with heavy metals. Bot Stud 55(1):35
Web-1 (2018) Heavy metals in urine samples post-Diwali. The Hindu, Available at: https://www.thehindu.com/news/cities/Delhi/heavy-metals-in-urine-samples-post-diwali/article25793218.ece. Accessed 15 July 2019

Web-2 (2018) Heavy metals adding to air pollution woes in Bengaluru. The Hindu, Available at: https://www.thehindu.com/news/national/karnataka/heavy-metals-adding-to-air-pollution-woes-in-bengaluru/article24302136.ece. Accessed 15 July 2019

Web-3 (2003), Imbibing toxic/heavy metals through leafy vegetables. The Hindu, Available at: https://www.thehindu.com/seta/2003/11/06/stories/2003110600110300.htm. Accessed 15 July 2019

Web-4 (2018), Heavy metals, deep impact. The Tribune, Available at: https://www.tribuneindia.com/news/sunday-special/perspective/heavy-metals-deep-impact/534755.html. Accessed 15 July 2019

Web-5 (n.d.) Cobalt—Sources, Available at: https://www.mineravita.com/eng/cobalt_sources.php. Accessed 14 July 2019

Web-6 (n.d.). Cobalt—Uses, Benefits, Sources and Dosage, Available at: https://www.planetayurveda.com/library/cobalt/. Accessed 14 July 2019

White C, Wilkinson SC, Gadd GM (1995) The role of microorganisms in biosorption of heavy metals and radionuclides G. M. Gadd. Int Biodeterior Biodegradation 34(1):17–40

WHO (2010) Background document for development of WHO Guidelines for Drinking-Water Quality

WHO (2011) Adverse health effects of heavy metals in children

Wojcik M, Tukiendorf A (2004) Phytochelatin synthesis and cadmium localization in wild type of Arabidopsis thaliana. Plant Growth Regul 44(1):71–80

Wu MS, Xu X, Zhao Q, Wang ZY (2017) Simultaneous removal of heavy metals and biodegradation of organic matter with sediment microbial fuel cells. RSC Adv 7(84):53433–53438

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol 2011:1–20

Xie Y, Fan J, Zhu W, Amombo E, Lou Y, Chen L, Fu J (2016) Effect of heavy metals pollution on soil microbial diversity and Bermudagrass genetic variation. Front Plant Sci 7:755

Xu Z, Lei Y, Patel J (2010) Bioremediation of soluble heavy metals with recombinant Caulobacter crescentus. Bioeng Bugs 1(3):207–212

Yadav SK (2010) Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S Afr J Bot 76(2):167–179

Yadav M, Gupta R, Sharma RK (2019) Green and sustainable pathways for wastewater purification. In: Ahuja S (ed) Advances in water purification techniques. Elsevier, New York, pp 355–383

Yang J, Hou B, Wang J, Tian B, Bi J, Wang N, Li X et al (2019) Nanomaterials for the removal of heavy metals from wastewater. Nano 9(3):424

Zhao M, Xu Y, Zhang C, Rong H, Zeng G (2016) New trends in removing heavy metals from wastewater. Appl Microbiol Biotechnol 100(15):6509–6518

Dyes: Effect on the Environment and Biosphere and Their Remediation Constraints

3

Kunal Jain, Chirayu Desai, Onkar Tiwari, and Datta Madamwar

Abstract

The early excitement of industrialization during the twentieth century and unprecedented population rise have now compelled us to think about developing environmental remediation strategies on a priority basis to save the basic essential components of life. Understanding the impact of dyes and dye intermediates which have been the major component of industrial pollutants in the environment is the prime need, to reclaim the pristine environment. Physical and chemical environmental cleanup technologies developed for dye and textile effluents are proven to be expensive and energy consuming, often generate toxic by-products, and more importantly are faced with limited success in a narrower scope. Consequently, the need for an alternate approach has led to the development of self-sustainable, greener biological methods (i.e., bioremediation). It offers a great advantage of astonishing catabolic diversity of the innate microbial population inhabiting the polluted environment. Factors like geological aspects, climate, soil and water characteristics, waste and disposal facilities, etc. play a vital role in the success of different technologies (including bioremediation). Besides chemical structure, degree of recalcitrance, toxicity, and bioavailability of dye molecules are considered significant parameters for their treatments. In this review, an attempt has been made to understand the complexities and constraints of existing technologies and few optimistic scenarios to improve and develop new methodologies for treatment of industrial effluents from dye and textile industries.

K. Jain · D. Madamwar (✉)
Environmental Genomics and Proteomics Lab, UGC-Centre of Advanced Study, Post Graduate Department of Biosciences, Sardar Patel University, Satellite Campus, Anand, Gujarat, India

C. Desai
PD Patel Institute of Applied Science, Charotar Institute of Science and Technology, Changa, Gujarat, India

O. Tiwari
Department of Biotechnology, Ministry of Science & Technology, New Delhi, India

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_3

Keywords
Industrial effluent · Xenobiotic(s) · Remediation technologies · Physicochemical · Bioremediation

3.1 Introduction

Waste generation is an inevitable side effect of production and consumption activities which tends to increase along with socioeconomic advancement of the human society. The concern is the presence of compounds such as aliphatics, aromatics, polychlorinated biphenyls, halogen, and other organic and inorganic pollutants in the open environment. Not only their presence but their concentrations also greatly affect the ecosystem functioning in several ways. And once into the environment, with due course of time, these xenobiotic compounds become threatening to the self-regulating capacity of the biosphere (Sen and Chakrabarti 2009; Beltrame et al. 2010; Prasad et al. 2010). The human perturbation in the environment can be classified into two main categories – (a) waste generation (food waste, kitchen waste, sewage, wastewater, agricultural waste, etc.) and (b) use of chemicals (in the form of chemical fertilizers, pesticides, insecticides, toxic products and by-products from chemical industries, industrial waste, effluents, etc.) – since most of the problems can be traced to either of them directly or indirectly.

The approaches for restoring or cleaning of any contaminated sites generally include (a) identification of the problem (i.e., whether it is soil or water or air pollution and what type of contaminant is present) and (b) assessment of the nature and degree of the hazard (i.e., level and strength of pollution). The recognition and identification of the problem and its characteristics is highly imperative for selection of best methods available for remedial action. Over the years, the need to remediate the perturbed sites has led to the development of diverse technologies (over the conventional approach of disposal at an isolated region) which emphasizes the molecular breakdown (i.e., degradation) and detoxification of the contaminants (Wang and Chen 2007; Weber 2007; Kulkarni et al. 2008; Busca et al. 2008).

Dye and dye intermediates are among the few xenobiotic and recalcitrant compounds which persist longer in the environment and often found to be harmful to the biosphere. Therefore, along with the other xenobiotic compounds, studies have been directed toward exploiting the potential sources to remove dye compounds from the contaminated environment. The methods, systems, technologies, and strategies developed, applied, and improvised are well discussed. However, nearly after four decades of widespread study on developing remediation strategies, we are still searching for an optimistic, comprehensive, and universal solution to reclaim the contaminated environment across the globe.

In this chapter, an attempt has been made to understand the complexities and constraints of existing technologies which led to their limited use in already established setups. Further, this chapter focuses on few optimistic scenarios to

improve and develop new methodologies for treatment of industrial effluents from dye and textile industries.

3.2 Effect of Dyes on the Environment and Biosphere

Considering both the volume and composition of the waste effluents, the dye industry is rated as one of the foremost industrial sectors that pollute the environment (Cristovao et al. 2008). Many organized and non-organized small, medium, and large industrial sectors are involved in dye production and its applications. Therefore, estimation of the correct amount of dye produced globally would be difficult. However, for an approximation, the annual production of various classes of dyes has reached up to $>7.5 \times 10^5$ metric tons annually (McMullan et al. 2001; Mate and Pathade 2012). Of this, nearly 280,000 tons (i.e., 2–50%) from textile dyes are discharged as effluents (Mass and Chaudhari 2005; Jegatheesan et al. 2016). To dye 1 kg of cotton, it requires 70–150 L water, 30–60 g dyestuff, and 0.6–0.8 kg NaCl. Therefore, at the end of the process, it generates nearly 20–30% of applied unfixed dyes at a concentration of ~2000 ppm along with high inorganic salts, few acids and bases, and other auxiliary compounds (Solis et al. 2012; Dasgupta et al. 2015). Thus, the textile industrial wastewaters always contain pollutants of multicomponent origin, making them highly heterogeneous and thereby extremely difficult to remediate by available technologies. Therefore, no single treatment process can treat these effluents effectively adequately (Pang and Abdullah 2013).

Among the different dyes used globally, azo dyes represent nearly 60% of total dyes (Fu and Viraraghavan 2001). In aquatic environments, many dyes including azo compounds exert an inhibitory effect on photosynthesis, increase the rate of dissolved oxygen depletion, and are toxic to flora and fauna (Solis et al. 2012). The acute toxicity of dyes is generally low; however, few purified azo dyes are directly mutagenic and carcinogenic (van der Zeer 2002). Moreover, in many cases, the intermediates generated during molecular breakdown of dye compounds are reported to be more toxic than parent molecules. In the case of azo dyes, in the environment, azo cleavage is purely a chemical reaction. Under oxygen-deficient conditions, redox mediators of abiotic or biotic origin catalyze the transfer of redox equivalent from donor to azo bond, and dyes are reduced (Hong et al. 2007). Therefore, upon entering the environment, the reduction of native compounds results in formation of constituent aromatic amines which have been recognized as more toxic/carcinogenic than their parent compound and toxic to cellular mechanisms (Jadhav et al. 2010; Forss et al. 2013).

According to Saratale et al. (2011), bacterial toxicity of azo dyes depends on the mechanisms of their degradation. First, several azo dyes are toxic only after the reduction of azo bonds and generation of aromatic amines, mostly through anaerobic mode. These aromatic amines are further oxidized to reactive electrophilic species that covalently bind to DNA. In a second mechanism, oxidation of azo dyes without cleavage of azo bonds generates free aromatic amines, while in the last mechanism, azo bonds are directly oxidized to form highly reactive electrophilic diazonium salts

of azo dyes. All the three mechanisms are found to be compound (and/or bacteria) specific. Therefore, the toxicity because of azo dyes might be caused by a single or combinatorial effect of different degradation mechanisms. The toxicity or mutagenicity of reduced dye or aromatic amines is strongly correlated to its three-dimensional molecular structure. The nature and position of substitutions (like amino, carboxy, halogen, methyl, methoxy, nitro, or sulfonate groups) on aromatic rings greatly influences the toxicity of the dye molecules with various degrees of intensity (Chung and Cerniglia 1992). It was found that Acid Red 18 and Acid Red 27 are non-mutagenic, while structurally similar Acid Red 26 is carcinogenic due to the presence of a methyl group and the substitution position of the sulfonate group (Solis et al. 2012).

However, the results of several studies on dye toxicity revealed contradicting observations. Ferraz et al. (2011) observed that 3-methoxy-4-aminoazobenzene is a strong mutagen in a bacterial system and a potent hepatocarcinogen in a mammalian system (in rat studies), whereas 2-methoxy-4-aminoazobenzene is an extremely weak mutagen (in bacteria) and apparently noncarcinogenic. The azo toxicity is well established in a bacterial system, but for animal models, more studies are required to correlate mutagenicity and carcinogenicity. The lack of correlation is probably due to existence of complex metabolic pathways, in a mammalian system (Brown and DeVito 1993). Furthermore, the physicochemical treatment also leads to the production of toxic intermediates. The products generated during oxidation of indigo blue through coagulation with $Al_2(SO_4)_3$, electroincineration, or the use of laccase proved to be more toxic than the dye itself (Solis-Oba et al. 2009). Thus, the treatment (or restoration) of an environment contaminated with dyes and dye intermediates is highly important.

3.3 Technologies for Dye Removal

Before we discuss about the technologies and their limitations, we need to understand what makes it so difficult for the technologies to remove or to remediate the wastewaters containing dyes. For metabolism and degradation of any compounds (either natural or synthetic), their chemical and molecular structures play a significant role, which is equally true for dyes. The basic raw materials used for the manufacturing of dye and dye intermediates are benzene, toluene, xylene, and naphthalene along with certain heavy metals. To increase the solubility of dyes, polar groups like sulfonic acids are substituted on the aromatic nucleus; and they are subtly designed to make chemically, photolytically, and biologically very stable molecules. All these properties provide highly persistent characteristics to dye compounds (in the environment), and even at a concentration of 1 mg/L (ppm), they can be visible in the aquatic ecosystems (Nigam et al. 2000; Rieger et al. 2002; Savin and Butnaru 2008). Moreover, benzene rings are thermodynamically very stable, which contributes to their persistent nature in the environment (Diaz 2004).

The initial results of numerous experimental observations revealed that dyes with simple chemical structures and low molecular weights are degraded faster. Dyes

with complex chemical structures and higher substitutions and molecular weights require more energy and time and are difficult to degrade (Sani and Banerjee 1999). In other studies, the decolorization and degradation of dyes were found to be dependent on dye class rather than chemical and molecular structures (Greaves et al. 1999).

Hitz and his co-workers had few interesting but prominent observations: (1) the rate of decolorization and degradation of *reactive dyes* is lower; (2) for *acidic dyes*, the number of sulfonate groups reduces the rate of decolorization; and (3) *direct dyes* are decolorized at a faster rate, irrespective of the number of sulfonate substitutions (Hitz et al. 1978). With the above observations, it could be concluded that the rate of dye decolorization and degradation is directly related to sulfonate substitutions. However, its effect is related to mechanisms by which dyes are being decolorized. In biological methods, if dyes are being decolorized through an enzymatic process, which is cytoplasmic bound, the presence of sulfonate groups would decrease the rate of dye decolorization. The sulfonate group would hinder the transfer of dye molecules through the cell membrane. Moreover, with the increase in the number of sulfonate groups, the rate of decolorization would decrease. But when decolorization takes place outside the cells, the sulfonate group has little effect on dye decolorization (Pearce et al. 2003).

For azo dyes, along with the number of azo bonds, the nature and position of substitutions on the aromatic nucleus are directly correlated and play an important role for dye decolorization and degradation. The monoazo compounds are decolorized at a higher/faster rate when compared to diazo or triazo compounds (Pearce et al. 2003). Dyes with carboxyl substitution are difficult to decolorize, as compared to sulfonated dyes (Kulla 1981). Dyes with hydroxyl and amino groups have more chances to be decolorized and degraded at higher rates, than compounds with nitro, methyl, or methoxy groups (Nigam et al. 1996). Zimmerman and co-workers, after their study on Orange II dye, have made the following generalizations: (1) substitutions with charged groups in the proximity of azo bonds/group would decrease the rate of decolorization; (2) a hydroxyl group at the ortho position of dye containing the naphthol ring is a prerequisite for initiation of decolorization reaction; (3) another (second) polar substituent inhibits the reaction, by lowering the dye affinity for enzymes; and (4) the rate of decolorization will increase with electron-withdrawing substituents on the phenyl ring of the dye compound (Zimmerman et al. 1982). Moreover, Walker and Ryan (1971) also noticed that substitutions with electron-withdrawing groups ($–SO_3H$), ($–SO_2H$) at the para position relative to azo bonds in the phenyl ring may increase the rate of decolorization.

The major route by which dyes enter into the environment is through industrial effluents (either from production or textile and finishing industries) (Easton 1995). Industrial effluents from the textile industry are a complex mixture of many substances ranging from easily degradable starch molecules to recalcitrant polluting compounds including unused dyes and their intermediates and organochlorine pesticide-like compounds and to heavy metals associated with dyes and the dyeing process, besides high concentration of different salts. The unused proportion of dye

compounds forms a large proportion of the effluents. Depending upon the usage, wastewater contains different dye classes, which in turn depend on dye consumption, along with degree of fixation of different dye compounds on various fabrics (Easton 1995). The dye fixation for reactive dyes is lowest, while basic dyes have higher affinity for their substrates (Easton 1995). It is estimated that about 800 mg/L of hydrolyzed reactive dye remains in the dye bath after the dyeing process (Steenken-Richter and Kermer 1992). Several modifications in the dyeing process are being made to increase the rate of fixation of reactive dyes. However, degree of fixation varies and ranges between 50 and 90% depending upon the dye and substrate used and may increase with dyes containing two reactive groups (Carr 1995).

Textile effluents are typically characterized by residual color (as high as 50% of unused dyes), pH ranging from 5 to 12, excess TDS content, high chemical oxygen demand (COD) and relatively low biological oxygen demand (BOD) values, BOD/COD ratio ranging between 0.2 and 0.5 (which indicates that effluent contains a large amount of organic matter which is nonbiodegradable), and a host of other auxiliary chemicals (Yusuff and Sonibare 2004; Faryal and Hameed 2005; Savin and Butnaru 2008; Akan et al. 2009; Kuberan et al. 2011). Consequently, due to release of these hazardous dyes as effluents, it also increases their potential ecotoxic risk to environmental flora and fauna, and through biomagnification it enters in the food chain and may affect humans (van der Zeer 2002). Thus, the wastewater, upon discharging into the water bodies without any adequate treatment, affects the multi-segments of the environment leading to irreversible persistent changes.

3.3.1 Physicochemical Technologies

Technologies available for dye removal can broadly be classified based on their mode of action into physical, chemical, and biological methods, which are either pretreatment, main treatment, or posttreatment procedures. These technologies can be applied either at a dye house or at a common treatment plant (or can be at a sewage plant) (Southern 1995; Pearce et al. 2003). Treating the dye effluent at a sewage plant has cost impediment, while at a dye house or common treatment plant (i.e., single universally applicable), the solution is unrealistic due to color and chemical composition of the effluent which changes at every production cycle along with other accessory chemicals (Pearce et al. 2003).

The initial conventional wastewater treatment technologies have gradually evolved into diverse physicochemical methods. The methods were either used as standalone or in combination or in sequential manner depending on the type of effluents to be treated. The major physical/chemical methods for dye/textile effluent treatment involve membrane filtration, coagulation/flocculation, precipitation, flotation, adsorption, ion exchange, ion pair extraction, ultrasonic mineralization, electrolysis, advanced oxidation (chlorination, bleaching, ozonation, Fenton's oxidation, and photocatalytic oxidation), chemical reduction, use of nanoparticles, carbon nanotubes, etc. (Slokar and Le Marechal 1998; Robinson et al. 2001; Pizzolato

et al. 2002; Alaton and Ferry 2003; Kusvuran et al. 2004; Gogate and Pandit 2004a, b; Gupta et al. 2013). These technologies are not recently developed. An ample amount of literature and information is available discussing their principal mode of action, type of application, merits and limitations, etc. As mentioned above, here we would highlight constraints and limitations of various existing methods and technologies for wastewater treatment. Though through steady research the other newer technologies have been developed, no technologies have been replaced with another. Instead, they have been combined and integrated to form hybrid or sequential systems.

3.3.1.1 Limitations of Physicochemical Technologies

Among the different technologies used, *coagulation* is very simple and widely used for a long time. Though it is economical, it produces a large quantity of sludge, and the process is pH dependent. Coagulation is inefficient in removing highly soluble dyes including azo, reactive, acid, and basic dyes, but easily removes disperse, sulfur, and vat dyes (Hao et al. 2000; Fu and Viraraghavan 2001; Robinson et al. 2001). Like coagulation, *activated carbon adsorption* (ACA) is pH dependent, but efficient in removing azo and reactive dyes and more suitable for basic dyes and inefficient for disperse, sulfur, and vat dyes. The major limitation of ACA is its regeneration. It involves adsorbent loss, and its disposal is economically not feasible (Hao et al. 2000; Fu and Viraraghavan 2001; Robinson et al. 2001; Fernandez et al. 2014). In the *ion exchange* technique, the resins are dye specific and economically not feasible to use for a large volume of wastewater, and resin regeneration is cost intensive. The only limited advantage of this technology is adsorbent used for dye recovery can be regenerated without any loss (Slokar and Le Marechal 1998; Robinson et al. 2001; Yagub et al. 2014).

With more advance technologies like *chemical oxidation*, the generation of secondary pollution persists, along with thermodynamic and kinetic limitations. One of the major limitations of the process is release of aromatic amines; hence, complete mineralization (breakdown) of the dye compound is not possible (Slokar and Le Marechal 1998; Robinson et al. 2001). In *advanced oxidation processes* (AOPs), a large quantity of highly reactive free radicals are produced, which aid in achieving better decolorization than the conventional oxidation process. However, generation of secondary pollution is also a major problem along with cost consideration, while the efficiency is also reduced by the presence of radical scavengers, some of which are pH dependent (Slokar and Le Marechal 1998; Robinson et al. 2001). In an advance form of AOPs, i.e., *electrochemical advanced oxidation processes*, the advantages are their high energy efficiency, easy handling and simple requirement for operation, amenability to automation, and application to remove COD in the range from 0.1 to 100 g/L. However, the main drawbacks of some of these technologies are high cost of electrical supply, low conductance in many wastewaters (thus requiring supplementing extra electrolytes), and loss of activity and limited life span of electrode because of fouling and deposition of organic matter of the surface (Sirés et al. 2014).

With the development of few more chemical processes, such as application of *Fenton's reagent*, the efficiency of treatment technologies also enhanced. Fenton's reagent is simple to use and easy to apply, effectively removes soluble and insoluble dyes from the effluents even from high suspended solids of wastewater, and even reduces certain levels of COD. However, Fenton's reagent is effective in the very narrow pH range (<3.5), and the reaction time is comparatively longer, with obvious inherent drawback of secondary sludge generation (Marechal et al. 1997; Hao et al. 2000; Robinson et al. 2001; Wang et al. 2011).

There is no sludge formation when *ultraviolet (UV)/ozone (O_3)* is used for remediation of dye-containing wastewater. The reaction time is comparatively shorter, and different dyes (except disperse dyes) can be removed, but the treatment shows better results for reactive dyes. The treatment is again pH dependent, with negligible removal of COD. UV/O_3 has a short half-life and requires special/dedicated handling, its generation is economically not feasible during large-scale application, and one of the major constraints is gas-liquid mass transfer (Marechal et al. 1997; Hao et al. 2000; Robinson et al. 2001; Fu and Viraraghavan 2001; Gogate and Pandit 2004a, b). Another physical method involving *UV/H_2O_2* also suffers the limitation of penetration of light, and it requires primary separation of solids before the application of UV. The treatment is dye specific (i.e., it cannot be used for all dyes) and pH dependent (preferably lower pH to nullify the scavenging effect of the radical). The only advantage of applying *UV/H_2O_2* is it does not produce sludge and treatment time is relatively shorter (Marechal et al. 1997; Gogate and Pandit 2004a, b).

In *electrochemical* methods, COD reduction is an added advantage which can be effectively applied for both soluble and insoluble dyes. The inherent limitation of these methods is high cost of electricity. Different variants have different drawbacks such as high amount of sludge formation posttreatment and generation of secondary pollution during treatment of chlorinated organics and heavy metals. The treatment efficiency depends on type and nature of dye to be treated (Robinson et al. 2001; Chen 2004). In *photocatalysis* methods, light penetration is a major issue along with the fouling effect of catalysts, and they require posttreatment separation of (fine) catalysts. The added advantage of these methods is reduction of COD with no sludge formation (Konstantinou and Albanis 2004).

The observations from above methods and technologies can be summarized as follows: Many of the technologies are economically not feasible at large scale, generation of secondary pollution normally is toxic, and accumulation of sludge due to excessive chemical treatment or secondary by-products in turn requires disposal and further treatment. Some technologies are dye specific and therefore cannot be used for complex dye effluents. Textile effluents contain a spectrum of dyes, natural impurities extracted from the fibers, and other accessory products like dispersants, leveling agents, acids, alkalis, salts, and heavy metals (Laing 1991). Consequently, the effluent is highly colored with high biological oxygen demand (BOD) and chemical oxygen demand (COD). It has a high conductivity and is alkaline in nature. Thus, such technologies are complicated and cost restrictive.

3.3.2 Biological Technology

As noted above, conventional wastewater treatment involving physicochemical processes tasted initial success, but sooner it was realized that such technologies have inherent drawbacks. The limitations of physicochemical technologies have forced us to explore for alternative methods which led to the development of biological techniques (i.e., bioremediation) for treatment of dye and textile effluents. Biological methods are natural processes, comparatively simpler, and economically feasible; require less water and energy consumption as compared to other methods; and generally mineralize the whole compound, a therefore environmentally greener technology (Banat et al. 1996; Saratale et al. 2011). However, biological treatment procedures are ambiguous, different, and divergent when employing microorganisms for remediation of dye-containing effluents (Forgacs et al. 2004).

In *bioremediation* (different variants are developed over time), microorganisms (bacteria, fungi, algae), plants (phytoremediation), and enzymatic degradation are the major driving forces which have an immense potential to restore the pristine environment by binding, immobilizing, oxidizing, volatilizing, or other means of transforming xenobiotic compounds (Lovely 2003). The simplest and basic mechanism of color (dye) removal from industrial effluents by a microbial source (whole bacterial/fungal cell) is adsorption of dye on the biomass (live/dead). But it is similar to many other physical adsorption mechanisms for color removal and is not suitable for long-term treatment. During adsorption, dye is concentrated onto the biomass, which in turn becomes saturated with time, and the dye-adsorbent complex needs to be disposed at a safer place (Southern 1995). Another mechanism of bioremediation involves enzymatic cleavage of chromophore groups (viz., azo bonds –N=N– of azo dyes) and sequential mineralization of intermediates, termed as biodegradation (Jain et al. 2012; Shah et al. 2016). It may be either anaerobic or aerobic and involves a combination of both the conditions. While considering the reaction between azo dyes and microbial cells (especially bacterial and fungal), there are significant differences between the physiology of microorganisms grown under aerobic and anaerobic conditions (Stolz 2001). Reduction of azo bonds (chromophore) under aerobic conditions is generally considered as a specific reaction, and microorganisms need to be specifically adapted (Nakanishi et al. 2001; Blumel et al. 2002; Pearce et al. 2003; Blumel and Stolz 2003; Bin et al. 2004; Chen et al. 2004; Hong et al. 2007).

Dye degradation studies in the authors' laboratory have revealed that dyes are a poor source of carbon and energy, due to their synthetic origin and structural complexity, and generally regarded as recalcitrant compounds for microorganisms (Khan et al. 2014). They require an additional source of easily metabolizable carbon compound(s) (viz., glucose, yeast extract, etc.) for their growth and subsequent metabolism of dye molecules (Jain et al. 2012; Balapure et al. 2014; Shah et al. 2016). Most of the traditional methods employing microorganisms for dye decolorization involve molecular oxygen (i.e., under aerobic conditions) as final electron acceptor for microbial respiration. But wastewater effluents and/or dye-contaminated sites (sediments or aquifers) are often anoxic or hypoxic. We

have frequently observed better dye-degrading efficiency by bacterial consortia under anoxic conditions (Jain et al. 2012; Balapure et al. 2014; Shah et al. 2016). Therefore, in the presence of co-metabolites and under oxygen-limiting conditions, better dye decolorization was observed (Jain et al. 2012; Balapure et al. 2015).

Depending upon their molecular structures, three-dimensional orientation of a functional group, they persist in the natural environment. Some of the dye molecules are simpler in structure and consumed early without affecting the native microbial community at the disposal or polluted sites, while some dyes are highly recalcitrant and persist longer in the environment, which might exert selective pressure on microbial communities, eliminating the sensitive species, resulting in the perpetuation of adapted communities (Nojiri et al. 2004). The flexibility in adaptation is accelerated during accidental releases and discharge of industrial effluents at higher concentrations. The degree of adaptation in communities is due to their versatile catabolic diversity which is often shared by phylogenetically unrelated strains of either same or different geographic regions (Nojiri et al. 2004).

3.3.2.1 Bioremediation Is Still an Empirical Science!

Bioremediation has an immense potential to reinstate the pristine environments in an ecologically accepted manner. Microbially mediated bioremediation has generated significant interest because of its minimalism, cost-effectiveness, and promises to deliver environmentally friendly outcome than nonbiological approaches (Lovely 2003). However, bioremediation is still far behind to reach its true potential as anticipated from its initial success. It was generally observed that strategies which are successful at one site might not work in another location. Further, a microbial system that demonstrates good remediation potential under laboratory conditions might not function equally in an open environment. On the other hand, though bioremediation appears to be simple, its implementation (unlike other physicochemical methods whose parameters are known and controlled) and the mechanisms of controlling the growth and functioning of microorganisms at a contaminated environment are still poorly understood (Lovely 2003).

Since bioremediation is a natural process and dye degradation is a biochemical mechanism, besides microbial community involved in bioremediation, certain vital parameters are critical and need to be recognized, characterized, and understood. Factors like temperature, pH, availability of co-substrates, dissolved oxygen concentration, salt concentration, type and source of reduction equivalents, etc. affect the biodegradation of dyes. As noted above, dye-related parameters like class and type of dyes (viz., azo, anthraquinone, azine, lactone, reactive, acidic, direct, disperse, vat, monoazo, diazo, etc.), dye concentration, dye side groups (i.e., substitutions), degradation intermediates and reduction metabolites, and organic dye additives also affect their biodegradability. Therefore, the factors mentioned above and also described in Table 3.1 need to be studied before developing any technology. In the authors' laboratory, they often study (major) factors which are found to influence dye degradation under environmental conditions (Moosvi et al. 2007; Jain et al. 2012; Shah et al. 2016; Balapure et al. 2014).

Table 3.1 Factors affecting the bioremediation process in the natural environment with respect to dyes

No.	Factors	Characteristics
1	Microbial source	Bacteria and fungi proved to be efficient dye degraders
		Requires adaptation, acclimatization, and enrichment of potential microbes
		Minimum cell numbers are required for efficient treatment (i.e., biomass concentration is critical)
		Production of enzymes either constitutive or inductive
		Chances of mutation and horizontal gene transfer of genetically modified strain
		Generation of toxic intermediates/metabolites in pure culture system
2	Aerobic vs. anaerobic process	Oxidation and reduction potential
		Type of microbial population at the site
		Availability of electron acceptors
3	Growth substrate vs. co-metabolism	Presence of alternate carbon sources and their concentration
		Degree of recalcitrance (i.e., type of dyes)
		Microbial interaction within the community (competition, succession, predation, etc.)
4	Environmental condition	Major and foremost is lack of nutrients in natural habitat
		Variation in temperature and pH
		Presence of growth inhibitory compounds
		Availability of redox mediators
5	Dye	Chemical structure and type and position of substitutions
		Concentration of dyes
		Solubility and toxicity at high concentration
6	Bioavailability of dyes (physicochemical)	Equilibrium sorption
		Irreversible sorption
		Incorporation into humic matters
7	Mass transfer limitations	Oxygen diffusion and solubility concentration (either for aerobic or anaerobic processes)
		Diffusion of nutrients
		Solubility/miscibility of dyes in/with water (e.g., disperse dyes are insoluble in water)

Modified from Boopathy (2000)

It has now been understood nearly after five decades of studies that a successful implementation of biological remediation techniques for restoring the sites contaminated with dyes and dye intermediates requires a multidisciplinary approach from expertise of various fields including but not limited to microbiology, chemistry, chemical engineering, soil science, geology, and environmental engineering (Juwarkar et al. 2010). In order to use bioremediation more effectively, comprehensive understanding of the matrix characteristics of the ecosystem to be treated and the properties of the dyes is needed. The performance monitoring of any applied

remediation technologies is always a significant part for site remediation (Lai et al. 2007). This usually involves periodic measurement of site parameters (viz., physical, chemical, and microbiological) to assess whether the applied remediation technologies are performing as anticipated, including well-defined and measurable remediation objectives, such as reduction in mass discharge rate from contaminant sources and so on (Juwarkar et al. 2010).

More precisely, microbial communities need to be characterized in terms of their structure, phenotypic potential (what are their biocatalytic capabilities?), function (which ones among those biocatalytic capabilities are known?), and interactions with the environment. Therefore, knowing the absolute phylogenetic diversity of the contaminated environment would readily aid in understanding the type of community involved in sustaining the perturbed ecology which in turn helps in the bioremediation process. Unfortunately, in actual practices and in majority of the studies, much of the obligatory information is not readily available, and the application of microorganisms in the bioremediation approach is highly empirical rather than knowledge based (Lovely 2003). Traditionally, microbes (majority of them are culturable—which represent merely $<2\%$ of the total community) involved in the bioremediation were characterized taxonomically rather than phylogenetically. This predictable limitation has hindered the understanding of the interacting community at the contaminated environment, and complete advantage of bioremediation has not been realized. Thus, bioremediation as a science is still far from its target.

Nevertheless, through the recent advances made in gene technology, bioremediation is now succeeding to take the real advantage of genomic-driven strategies for analyzing, monitoring, and assessing its course by considering multiple microorganisms simultaneously with various genomes, expressed transcripts, and proteins depending upon the environmental conditions (Stenuit et al. 2009; Juwarkar et al. 2010). High-throughput methodologies, including microarrays and fingerprinting (Karpouzas and Singh 2010), real-time polymerase chain reaction (Baek et al. 2009), genotypic profiling, ultrafast genome sequencing (i.e., using different next-generation sequencing platforms), and multiomics technologies (viz., metagenomics, metatranscriptomics, metaproteomics, and metabolomics) (Jerez 2009; Desai et al. 2010), show huge promise for developing effective technologies against recalcitrant dye molecules (Juwarkar et al. 2010). The emerging genomic and multiomics methodologies are now providing us better design to promote and restore environmental health at contaminated sites. Monitoring of remediation activities, identifying the key microbial players and their metabolic processes under specific xenobiotic compounds, and finally assembling an intelligent, site-specific, and dye-specific database of genes used for targeted bioremediation are becoming imperative (Stenuit et al. 2009; Juwarkar et al. 2010).

Another biological method which is comparatively newly developed is phytotechnology or phytoremediation. It is solar energy driven, ecologically greener, and carbon neutral for wastewater treatment (Ma et al. 2011; Khandare and Govindwar 2015). Various phytoremediation methods and technologies were developed in the last two decades and are now frequently being used in many laboratory studies and at few large-scale applications. Khandare and Govindwar

(2015) have recently discussed the different types of plants used in the textile effluent remediation. They have also described the various technologies like constructed wetlands, lagoons, soil-bed and pack-bed systems, and floating wetlands and few integrated methods like constructed wetland microbial fuel cells and so on. Phytotechnology is autotrophic; therefore, the cost consideration is less, and it is an economically more viable approach. It requires less nutrient supply and less maintenance, has theoretically no generation of secondary pollutants, and is self-regulating and a real practical tool for on-site and in situ applications.

Though numerous promising results were obtained, looking at its application and current state of research, phytoremediation is still an emerging technology. Most importantly, we lack the transition of lab-driven results and observations into filed-scale on-site application especially for the treatment of textile effluents. Another important limitation is availability of the main driving force of this technology, which is *plants*. Plants which are routinely used are seasonal which may further limit the application all over the years. Moreover, phytoremediation cannot be considered as primary or secondary treatment technology, unlike other physical/chemical/biological methods. The time and cost consideration is the biggest limitation in phytotechnology. Since it is mainly dependent on plants, the time required for a batch treatment is much longer as compared to conventional or even few biological methods.

3.4 Evolved Integrated Technologies

Along with the individual physicochemical and biological technologies, integrated processes (combination of physicochemical and/or biological) have been also used for treatment of dye effluents. These methods are employed as pretreatment or posttreatment to main processes. Conventionally, *coagulation* coupled with biological methods is widely used, which may be coagulation-biological or biological-coagulation; the choice depends on the type of dye effluent (depending upon the amount of nonbiodegradable substances and other toxic compounds), sludge quantity, and amount of coagulant required for treatment (Shah 2014). In the authors' laboratory, they have used a three-stage integrated system involving coagulation, anaerobic process, and aerobic process for the treatment of CETP wastewater (of equalization tank, where wastewater was collected from 525 small-scale industrial units manufacturing dyes and dye intermediates, pigments, textiles, and pharmaceuticals) (Moosvi and Madamwar 2007). The integrated method improved the treatment efficiency of the then existing CETP process by the combined use of ferric chloride and lime for coagulation, bringing the BOD_5/COD ratio of the wastewater between 0.4 and 0.6, to increase the efficiency of the subsequent biological treatment. In a secondary step, mixture of bacterial consortium DMAB and cow dung slurry was used in an anaerobic upflow fixed film bioreactor (for reduction of COD and color from the effluent) followed by aerobic treatment using *Pseudomonas aeruginosa* for degradation of aromatic amines and further removal of remaining COD and color. This combined process has removed 94% of COD and

89% of color from the effluent, thereby increasing the treatment efficiency from COD removal of 40–45% and color removal of 35–40% after aerobic treatment at CETP (Moosvi and Madamwar 2007).

In an advance form of integrated processes, treatment strategies are designed such that to make the technology more synergetic rather than additive as a conventional approach (Sarria et al. 2003). In this process, advanced oxidation is coupled with an activated sludge treatment, where pretreatment of chemical oxidation would partially degrade more complex compounds (dye structure) to simpler intermediates to enhance the remediation potential of biological processes. Such combination not only improves the overall efficiency of the process but also simultaneously reduces the cost of any individual process (Shah 2014).

In another process, to make effluent readily reusable for industrial use, *low-pressure reverse osmosis* frequently referred to as *nanofiltration* is employed after biological treatment. The method was found to produce less fouling smell as compared to direct nanofiltration of dye baths. The sensitivity of the process can be enhanced by using a specific molecular cutoff-size membrane in a segregated dye bath, where the membrane retains dye molecules and allows passage of salts from the effluent. The filtrate containing salts can be reused for dye bath reconstruction, and the retained concentrated dye sludge may be degraded by any suitable biological processes (Shah 2014).

In certain cases, different adsorbents and chemicals (generally activated carbon) are directly added during the activated sludge process. A fine balance between adsorbent and biomass has to be maintained, because under relatively higher biomass concentration, the adsorbents might lose their properties of adsorption by entrapping floc matrix and gradually impair microbial growth and decrease dye reduction efficiency of the system (Bes-Piá et al. 2003). For example, during oxidative degradation, subsequent adsorption on dye on the adsorption material gradually declines. It has been observed that dye removal by such process is more additive rather than synergistic (as the basic properties of integrated technologies) (Kim et al. 2004). Nevertheless, such processes are economically more sustainable as color and COD removal can be obtained in a single step, without any further requirement of physicochemical step (Shah 2014). Few of the initial successes of integrated or hybrid treatment systems are listed in Table 3.2.

A large number of studies (for treatment of dye-containing wastewater based on a physicochemical, biological, or integrated approach) are reported either under controlled laboratory conditions or at pilot scale, but very selective technologies are being commercialized at industrial level. One of such technologies (for textile wastewater) has been successfully applied at Northern Ireland, in United Kingdom, based on bioadsorption mechanisms; commercially the technology is known as "BIOCOL." The process involves immobilization of whole cells of *Shewanella putrefaciens* on activated carbon support material. This carbon biocatalyst (bacterial cells) is enclosed in an adsorption cartridge through which the textile effluent is being pumped for removal of color from the effluent. Activated carbon adsorbs dye molecules by providing large surface area for decolorization of dyes by cells of *Shewanella putrefaciens* (Pearce et al. 2003).

Table 3.2 Few typical examples of initial success of integrated/hybrid technology used in dye degradation

No.	Combinatorial technology	Applied to	Remediation efficiency	References
1	Anaerobic/ aerobic stage I/aerobic stage II (MBR)/ ozonation	Wastewater containing reactive azo dyes [stream A (40%): Dyeing/color preparation/ printing Stream B (60%): Fiber pretreatment, washing]	Stream A: After anaerobic/aerobic pretreatment, was diverted into municipal wastewater Stream B: Mean combined DOC and color removal after anaerobic, aerobic stage I, MBR, and ozonation (1 g O_3/m^3) were 53%, 65%, 87%, and 87%; DOC and color removal after anaerobic, aerobic 1, MBR, and ozonation [67%, 65%, 72%, 87%]	Krull and Döpkens (2004)
2	Membrane filtration (NF)/ UV-H_2O_2/wet air oxidation (WAO) / Biological (immobilization)	Textile wastewater	After membrane filtration and advanced oxidation process, wastewater was reused along with reuse of membrane concentrate treatment by WAO and biological treatment	Lee et al. (2001)
3	Biological/ flocculation/O_3+ H_2O_2	Textile wastewater	Treatment process begins with activated sludge removal followed by flocculation. Removal of 85, 99.5, and 85% DOC, BOD, and CODcr. Concurrent subsequent O_3+ H_2O_2 treatment completely removed BOD and 50% of remaining DOC and CODcr	Lim et al. (2004)
4	Biological (anaerobic/ aerobic)/O_3/ biological (aerobic)	Concentrated dye bath containing azo dye C.I. reactive black 5 and High salt concentrations	Pretreatment by biological method removed >70% color from dye bath, and ozonation further increased its biodegradability in following aerobic reactor. Ozonation at 6gO_3/gDOC was	Libra and Sosath (2003)

(continued)

Table 3.2 (continued)

No.	Combinatorial technology	Applied to	Remediation efficiency	References
			applied to achieve combined removal of >95% decolorization and 80% DOC	
5	Coagulation by sodium bentonite/ activated sludge	Wastewater from dyeing industry and finishing natural/ synthetic fiber industry	Pretreatment by sodium bentonite (2 g/L) prior to activated sludge treatment reduced 40% of initial biodegradable and inert soluble COD, while sodium bentonite treatment (posttreatment) followed by activated sludge treatment did enhance decolorization but only 20% residual soluble COD removal achieved	Dulkadiroglu et al. (2002)

Modified from Hai et al. (2007)

3.5 Perspective

The expansion of modern chemistry, advancement in structural dynamics, and development of more environmentally resistant dye compounds have released indefinite structurally diverse dyes into the environment that have never been present in the biosphere in such significant amounts. After more than four decades of study, we are still finding a promising answer to how to deal with increasing evidence of the deteriorative effect of dyes and dye intermediates on the environment which has been significantly perturbed since industrialization from the twentieth century.

Before realizing the massive role of microorganisms in sustaining the active biosphere, various physical, chemical, and physicochemical environmental cleanup technologies (for dye removal) have been developed and universally applied, but they have gradually succumbed to their inherent limitations. Economic constraints, greater energy consumption, and generation of sludge and secondary pollution are few of the major limitations; and more importantly, these technologies were unable to treat wide varieties of dye-containing effluents.

However, bioremediation proved to be a promising technology. Many of the dye-degrading microorganisms isolated and characterized under laboratory conditions are now recognized to make a minor contribution to bioremediation in an open environment (Watanabe 2001). Therefore, total field bioremediation is becoming a difficult task, using both native microflora and genetically engineered microorganisms. The strategies need to be refined in that continued study would aid

in discovering the metabolic route followed by the dye molecules during their metabolism (i.e., which microbial physiological machinery contributes to the catabolism), mechanistic factors governing the degradation system, and types of metabolites generated though different routes of breakdown (i.e., environmental and enzymatic chemical transpiration).

3.6 Conclusion

One of the major shortcomings of any environmental cleanup technologies (including biological processes) is that no two environmental problems occur completely under identical conditions. The variation has always been observed in the type, class, and concentration of dyes and accessory elements found in effluent, climatic conditions and hydrogeodynamics of the polluted sites; and most importantly, it is unrealistic to mimic completely the natural contaminated ecology in the laboratory (Watanabe 2001). Thus, looking at the magnitude of such complexities, current knowledge about remediation of dye and textile effluents has lagged behind to provide universal technologies that are governed by common rationales. Considering the immediate need for a technically, scientifically, and economically satisfying treatment technology, a flurry of combination of basic and advance technologies are being studied and developed at different stages of commercialization. More validations and integrations of various methodologies or processes into the current technologies are required. There should be a common underlying principle for the combination/s of technologies so as to provide a comprehensive system eliminating the limitations of individual processes which can render both an efficient and economically viable solution.

Acknowledgment The authors acknowledge the Department of Biotechnology, Ministry of Science and Technology, New Delhi, for financial support (BT/Env/BC/01/2014).

References

Akan JC, Abdulrahman FI, Ayodele JT, Ogugbuaja VO (2009) Impact of tannery and textile effluent on the chemical characteristics of Challawa River, Kano state Nigeria. Aust J Basic Appl Sci 3:1933–1947

Alaton IA, Ferry JL (2003) Merits of polyoxotungstates as environmental remediation catalysts: a novel wet oxidation technology for refractory industrial pollutants. J Environ Sci Health A Tox Hazard Subst Environ Eng 38:2435–2445

Baek KH, Yoon BD, Cho DH, Kim BH, Oh HM, Kim HS (2009) Monitoring bacterial population dynamics using real-time PCR during the bioremediation of crude-oil contaminated soil. J Microbiol Biotechnol 19:339–345

Balapure KH, Jain K, Chattaraj S, Bhatt NS, Madamwar D (2014) Co-metabolic degradation of diazo dye—reactive blue 160 by enriched mixed cultures BDN. J Hazard Mater 279:85–95

Balapure K, Bhatt N, Madamwar D (2015) Mineralization of reactive azo dyes present in simulated textile waste water using down flow microaerophilic fixed film bioreactor. Bioresour Technol 175:1–7

Banat MI, Nigam P, Singh D, Marchant R (1996) Microbial decolorization of textile-dye-containing effluents: a review. Bioresour Technol 58:217–227

Beltrame MO, De Marco SG, Marcovecchio JE (2010) Effects of zinc on molting and body weight of the estuarine crab *Neohelice granulata* (*Brachyura*: *Varunidae*). Sci Total Environ 408:531–536

Bes-Piá A, Mendoza-Roca JA, Alcaina-Miranda MI, Iborra-Clar A, Iborra-Clar MI (2003) Combination of physico-chemical treatment and nanofiltration to reuse wastewater of a printing, dyeing and finishing textile industry. Desalination 157:73

Bin Y, Jiti Z, Jing W, Cuihong D, Hongman H, Zhiyong S, Yongming B (2004) Expression and characteristics of the gene encoding azoreductase from *Rhodobacter sphaeroides* AS1.1737. FEMS Microbiol Lett 236:129–136

Blumel S, Stolz A (2003) Cloning and characterization of the gene coding for the aerobic azoreductase from *Pigmentiphaga kullae* K24. Appl Microbiol Biotechnol 62:186–190

Blumel S, Knackmuss HJ, Stolz A (2002) Molecular cloning and characterization of the gene coding for the aerobic azoreductase from *Xenophilus azovorans* KF46F. Appl Environ Microbiol 68:3948–3955

Boopathy R (2000) Factors limiting bioremediation technologies. Bioresour Technol 74:63–67

Brown MA, DeVito SC (1993) Predicting azo dye toxicity. Crit Rev Env Sci Technol 23:249–324

Busca G, Berardinelli S, Resini C, Arrighi L (2008) Technologies for the removal of phenol from fluid streams: a short review of recent developments. J Hazard Mater 160:265–288

Carr CM (1995) Chemistry of the textile industry. Blackie Academic and Professional, Glasgow, p 276

Chen G (2004) Electrochemical technologies in wastewater treatment. Sep Purif Technol 38(1):11

Chen H, Wang RF, Cerniglia CE (2004) Molecular cloning, over expression, purification, and characterization of an aerobic FMN-dependent azoreductase from *Enterococcus faecalis*. Protein Expr Purif 34:302–310

Chung KT, Cerniglia CE (1992) Mutagenicity of azo dyes: structure-activity relationships. Mutat Res 277:201–220

Cristovao RO, Tavares APM, Ribeiro AS, Loureiro JM, Boaventura RAR, Macedo EA (2008) Kinetic modelling and simulation of laccase catalyzed degradation of reactive textile dyes. Bioresour Technol 99:4768–4774

Dasgupta J, Sikder J, Chakraborty S, Curcio S, Drioli E (2015) Remediation of textile effluents by membrane based treatment techniques: a state of the art review. J Environ Manag 147:55–72

Desai C, Pathak H, Madamwar D (2010) Advances in molecular and "-omics" technologies to gauge microbial communities and bioremediation at xenobiotic/anthropogen contaminated sites. Bioresour Technol 101:1558–1569

Diaz E (2004) Bacterial degradation of aromatic pollutants: a paradigm of metabolic versatility. Int Microbiol 7:173–180

Dulkadiroglu H, Dogruel S, Okutman D, Kabdasli I, Sozen S, Orhon D (2002) Effect of chemical treatment on soluble residual COD in textile wastewaters. Water Sci Techol 45:251–259

Easton JR (1995) The dye maker's view. In: Cooper P (ed) Colour in dye house effluent. Society of Dyers and Colourists, Bradford, pp 9–21

Faryal R, Hameed A (2005) Isolation and characterization of various fungal strains from textile effluent for their use in bioremediation. Pak J Bot 37:1003–1008

Fernandez ME, Nunell GV, Bonelli PR, Cukiermana AL (2014) Activated carbon developed from orange peels: batch and dynamic competitive adsorption of basic dyes. Ind Crop Prod 62:437–445

Ferraz ERA, Umbuzerio GA, de-Almeida G, Caloto-Oliveria A, Chequer FMD, Zanomi MVB (2011) Differential toxicity of disperse red 1 and disperse red 13 in the Ames test, HepG2, cytotoxicity assay and daphnia acute toxicity test. Environ Toxicol 26:489–497

Forgacs E, Cserhati T, Oros G (2004) Removal of synthetic dyes from wastewaters: a review. Environ Int 30:953–971

Forss J, Pinhassi J, Lindh M, Welander U (2013) Microbial diversity in a continuous system based on rice husks for biodegradation of the azo dyes reactive red 2 and reactive red 5. Bioresour Technol 130:681–688

Fu Y, Viraraghavan T (2001) Fungal decolourization of dye wastewaters: a review. Bioresour Technol 79:251–262

Gogate PR, Pandit AB (2004a) A review of imperative technologies for wastewater treatment I: oxidation technologies at ambient conditions. Adv Environ Res 8:501

Gogate PR, Pandit AB (2004b) A review of imperative technologies for wastewater treatment II: hybrid methods. Adv Environ Res 8:553–597

Greaves AJ, Phillips DAS, Taylor JA (1999) Correlation between the bioelimination of anionic dyes by an activated sewage with molecular structure. Part 1: literature review. J Soc Dyers Color 115:363–365

Gupta VK, Kumar R, Nayak A, Saleh TA, Barakat MA (2013) Adsorptive removal of dyes from aqueous solution onto carbon nanotubes: a review. Adv Colloid Interf Sci 193–194:24–34

Hai IF, Yamamoto K, Fukushi K (2007) Hybrid treatment systems for dye wastewater. Crit Rev Environ Sci Technol 37(4):315–377

Hao OJ, Kim H, Chaing PC (2000) Decolourization of wastewater. Cri Rev Environ Sci Technol 30:449–505

Hitz HR, Huber W, Reed RH (1978) The absorption of dyes on activated sludge. J Soc Dyers Color 94(2):71–76

Hong YG, Xu M, Guo J, Xu Z, Chen X, Sun G (2007) Respiration and growth of *Shewanella decolorationis* S12 with an azo compound as the sole electron acceptor. Appl Environ Microbiol 73(1):64–72

Jadhav JP, Kalyani DC, Telke AA, Phugare SS, Govindwar SP (2010) Evaluation of the efficacy of a bacterial consortium for the removal of color, reduction of heavy metals and toxicity from textile dye effluent. Bioresour Technol 101:165–173

Jain K, Shah V, Chapla D, Madamwar D (2012) Decolorization and degradation of azo dye – reactive violet 5R by an acclimatized indigenous bacterial mixed cultures-SB4 isolated from anthropogenic dye contaminated soil. J Hazard Mater 213–214:378–386

Jegatheesan V, Pramanik BK, Chen J, Navaratna D, Chang CY, Shu L (2016) Treatment of textile wastewater with membrane bioreactor: a critical review. Bioresour Technol 204:202–212

Jerez CA (2009) Biomining microorganisms: molecular aspects and applications in biotechnology and bioremediation. In: Advances in applied bioremediation. Springer, Berlin, pp 239–256

Juwarkar AA, Singh SK, Mudhoo A (2010) Comprehensive overview of elements in bioremediation. Rev Environ Sci Biotechnol 9:215–288

Karpouzas DG, Singh BK (2010) Application of fingerprinting molecular methods in bioremediation studies. Methods Mol Biol 599:69–88

Khan Z, Jain K, Soni A, Madamwar D (2014) Microaerophilic degradation of sulphonated azo dye—reactive red 195 by bacterial consortium AR1 through co-metabolism. Int Biodeterior Biodegrad 94:167–175

Khandare RV, Govindwar SP (2015) Phytoremediation of textile dyes and effluents: current scenario and future prospects. Biotechnol Adv 33:1697–1714

Kim TH, Park C, Yang J, Kim S (2004) Comparison of disperse and reactive dye removals by chemical coagulation and Fenton oxidation. J Hazard Mater 112:95

Konstantinou IK, Albanis TA (2004) TiO_2-assitated photocatalytic degradation of azo dyes in aqueous solution: kinetic and mechanistic investigations: a review. Appl Catal B Environ 49:1–14

Krull R, Döpkens E (2004) Recycling of dyehouse effluents by biological and chemical treatment. Water Sci Technol 49(4):311

Kuberan T, Anburaj J, Sundaravadivelan C, Kumar P (2011) Biodegradation of azo dye by *Listeria* sp. Int J Environ Sci 1:1760–1770

Kulkarni PS, Crespo JG, Afonso CAM (2008) Dioxins sources and current remediation technologies - a review. Environ Int 34:139–153

Kulla HG (1981) Biodegradation of synthetic organic colorants. In: Leisinger T, Hutter R, Cook AM, Nuesch J (eds) Microbial degradation of xenobiotics and recalcitrant compounds: FEMS symposium no. 12. Academic Press for the Swiss Academy of Sciences and the Swiss Society of Microbiology on behalf of the Federation of European Microbiological Societies, London

Kusvuran E, Gulnaz O, Irmak S, Atanur OM, Yavuz HI, Erbatur O (2004) Comparison of several advanced oxidation processes for the decolorization of reactive red 120 azo dye in aqueous solution. J Hazard Mater 109:85–93

Lai KCK, Surampalli RY, Tyagi RD, Lo IMC, Yan S (2007) Performance monitoring of remediation technologies for soil and groundwater contamination: review. ASCE practical periodical of hazardous, toxic and radioactive. Waste Manag 11(3):132–157

Laing IG (1991) The impact of effluent regulations on the dyeing industry. Rev Prog Color Relat Top 2:56–70

Lee HHW, Chen G, Yue PL (2001) Integration of chemical and biological treatments for textile industry wastewater: a possible zero-discharge system. Water Sci Technol 44(5):75

Libra JA, Sosath F (2003) Combination of biological and chemical processes for the treatment of textile wastewater containing reactive dyes. J Chem Technol Biotechnol 78:1149

Lim BR, Hu HY, Ahn KH, Fujie K (2004) Oxidative treatment characteristics of biotreated textile-dyeing wastewater and chemical agents used in a textile-dyeing process by advanced oxidation process. Wa. Sci Tech 49(5–6):137

Lovely DR (2003) Cleaning up with genomics: applying molecular biology to bioremediation. Nat Rev Microbiol 1:35–44

Ma Y, Prasad M, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Advanc 29:248–258

Marechal AM, Slokar YM, Taufer (1997) Decolourization of chlorotriazine reactive azo dyes with H_2O_2/UV. Dyes Pigments 33:281–298

Mass R, Chaudhari S (2005) Adsorption and biological decolourization of azo dye reactive red 2 in semicontinuous anaerobic reactors. Process Biochem 40:699–705

Mate MS, Pathade G (2012) Biodegradation of C.I. reactive red 195 *by Enterococcus faecalis* strain YZ66. World J Microbiol Biotechnol 28:815–826

McMullan G, Meehan C, Conneely A, Kirby N, Robinson T, Nigam P, Banat IM, Marchant R, Smyth WF (2001) Microbial decolourisation and degradation of textile dyes. Appl Microbiol Biotechnol 56:81–87

Moosvi S, Madamwar D (2007) An integrated process for the treatment of CETP wastewater using coagulation, anaerobic and aerobic process. Bioresour Technol 98:3384–3392

Moosvi S, Kher X, Madamwar D (2007) Isolation, characterization and decolorization of textile dyes by a mixed bacterial consortium JW-2. Dyes Pigments 74:723–729

Nakanishi M, Yatome C, Ishida N, Kitade Y (2001) Putative ACP phosphodiesterase gene (acpD) encodes an azoreductase. J Biol Chem 276:46394–46399

Nigam P, Banat IM, Singh D, Marchant R (1996) Microbial process for the decolorization of textile effluent containing azo, diazo and reactive dyes. Process Biochem 31(5):435–442

Nigam P, Armour G, Banat IM, Singh D, Marchant R (2000) Physical removal of textile dyes from effluents and solid-state fermentation of dye-adsorbed agricultural residues. Bioresour Technol 72:219–226

Nojiri H, Shintani M, Omori T (2004) Divergence of mobile genetic elements involved in the distribution of xenobiotic-catabolic capacity. Appl Microbiol Biotechnol 64:154–174

Pang YL, Abdullah AZ (2013) Current status of textile industry wastewater management and research Progress in Malaysia: a review. Clean (Weinh) 41(8):751–764

Pearce CI, Lloyd JR, Guthrie JT (2003) The removal of colour from textile wastewater using whole bacterial cell: a review. Dyes Pigments 58:179–196

Pizzolato TM, Carissimi E, Machado EL, Schneider IAH (2002) Colour removal with NaClO of dye wastewater from an agate-processing plant in Rio Grande do Sul, Brazil. Int J Miner Process 65:203–211

Prasad MNV, Freitas H, Fraenzle S, Wuenschmann S, Markert B (2010) Knowledge explosion in phytotechnologies for environmental solutions. Environ Pollut 158:18–23

Rieger PG, Meier HM, Gerle M, Vogt U, Groth T, Knackmuss HJ (2002) Xenobiotics in the environment: present and future strategies to obviate the problem of biological persistence. J Biotechnol 94:101–123

Robinson T, McMullan G, Marchant R, Nigam P (2001) Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresour Technol 77:247–255

Sani RK, Banerjee UC (1999) Decolorization of triphenylmethane dyes and textile and dye-stuff effluent by *Kurthia* sp. Enzym Microb Technol 24:433–437

Saratale RG, Saratale GD, Chang JS, Govindwar SP (2011) Bacterial decolourization and degradation of azo dyes: a review. J Taiwan Inst Chem Eng 42:138–157

Sarria V, Deront M, Péringer P, Pulgarin C (2003) Degradation of a biorecalcitrant dye precursor present in industrial wastewaters by a new integrated iron (III) photoassisted-biological treatment. Appl Catal B Environ 40:231

Savin II, Butnaru R (2008) Wastewater characteristics in textile finishing mills. Environ Eng Manag J 7:859–864

Sen R, Chakrabarti S (2009) Biotechnology-applications to environmental remediation in resource exploitation. Curr Sci 97:768–775

Shah M (2014) Effective treatment systems for azo dye degradation: a joint venture between physico-chemical and microbiological process. Int J Environ Biorem Biodegrad 2(5):231–242

Shah B, Mohan V, Jain K, Madamwar D (2016) Microaerophilic symmetric reductive cleavage of reactive azo dye—Remazol brilliant violet 5R by developed consortium VIE6: community synergism. Appl Biochem Biotechnol 180:1029–1042

Sirés I, Brillas E, Oturan MA, Rodrigo MA, Panizza M (2014) Electrochemical advanced oxidation processes: today and tomorrow. A review. Environ Sci Pollut Res 21:8336–8367

Slokar YM, Le Marechal AM (1998) Methods of decolourization of textiles. Dyes Pigments 37:335–356

Solis M, Solis A, Perezb HI, Manjarrez N, Floresa M (2012) Microbial decolouration of azo dyes: a review. Process Biochem 47:1723–1748

Solis-Oba M, Eloy-Juarez M, Teutli M, Nava JL, Gonzalez I (2009) Comparison of advanced techniques for the treatment of an indigo model solution: electro incineration, chemical coagulation and enzymatic. Rev Mex Ing Quim 8:275–282

Southern TG (1995) Technical solutions to the colour problem: a critical review. In: Cooper P (ed) Colour in dyehouse effluent. Society of Dyers and Colourists, Bradford, pp 73–91

Steenken-Richter I, Kermer WD (1992) Decolourizing textile effluents. Color Technol 108 (4):182–186

Stenuit B, Eyers L, Schuler L, George I, Agathos SN (2009) Molecular tools for monitoring and validating bioremediation. In: Advances in applied bioremediation, vol 17. Springer, Berlin, pp 339–353

Stolz A (2001) Basic and applied aspects in the microbial degradation of azo dyes. Appl Microbiol Biotechnol 56:69–80

Walker R, Ryan AJ (1971) Some molecular parameters influencing rate of reduction of azocompounds by intestinal microflora. Xenobiotica 1(4/5):483–486

Wang GD, Chen XY (2007) Detoxification of soil phenolic pollutants by plant secretory enzyme. In: Willey N (ed) Phytoremediation. Methods in bioremediation, vol 23. Humana, Totowa, pp 49–57

Wang Z, Xue M, Huang K, Liu Z (2011) Textile dyeing wastewater treatment. In: Hauser P (ed) Advances in treating textile effluent. InTech, pp 91–116

Watanabe T (2001) Microorganisms relevant to bioremediation. Curr Opin Biotechnol 12(3):237–241

Weber R (2007) Relevance of PCDD/PCDF formation for the evaluation of POPs destruction technologies-review on current status and assessment gaps. Chemosphere 67:109–117

Yagub MT, Sen TK, Afroze S, Ang HM (2014) Dye and its removal from aqueous solution by adsorption: a review. Adv Colloid Interf Sci 209:172–184

Yusuff RO, Sonibare JA (2004) Characterization of textile industries' effluents in Kaduna Nigeria and pollution implications. Global Nest: Int J 6:212–221

van der Zeer FP (2002) Anaerobic azo dye reduction. Ph.D. thesis. Wageningen University, Wageningen

Zimmerman T, Kulla HG, Leisinger T (1982) Properties of purified Orange II azoreductase, the enzyme initiating azo dye degradation by pseudomonas KF46. Eur J Biochem 129:197–203

4 Microbial Bioremediation and Biodegradation of Hydrocarbons, Heavy Metals, and Radioactive Wastes in Solids and Wastewaters

Rahul Kumar and Subir Kundu

Abstract

In this era of rapid industrialization, there is an increase in humongous use of nonrenewable resources which generate wastes in terms of hydrocarbons, heavy metals, and radioactive wastes. Increase in the wastes generated is a great threat to the environment as well as human life. The hydrocarbon industry, mining industry, and increase in the use of radioactive sources of energy created enormous toxic heaps, which are not degradable for years to come. No novel technology has been developed to rapidly tackle these critical masses of toxic wastes. Underground dumping and deep-sea disposal are the only treatment methods are being carried out. The great marine oil spill in the Mexican Gulf and wastes of nuclear warheads are a critical threat to humankind. The primary focus of the chapter is the treatment of these wastes through different biological processes and routes. A huge number of treatment studies on different wastes utilizaing biological methods are being done. Less heed is given to wastes such as hydrocarbon wastes, heavy metals, and more specifically radioactive wastes. Major challenges in the treatment of these wastes are that they have slower rate of degradation and handling of these kinds of wastes becomes a major roadblock. Chemolithotrophs and chemoorganotrophs are the major microorganisms which utilize chemical energy for their functioning. Facultative anaerobes and obligate anaerobes are known for their high COD treatment efficiency which is highly effective in treatment of such category of wastes. This chapter focuses on the use of these microorganisms for the treatment of the three main kinds of wastes, i.e., hydrocarbons, heavy metals, and radioactive wastes from solids and wastewaters.

Keywords

Hydrocarbon · Heavy metals · Radioactive wastes · Solid waste · Wastewater · Biological treatment

R. Kumar · S. Kundu (✉)
Bioreactor Design and Cell Processing Laboratory, School of Biochemical Engineering, Indian Institute of Technology, Banaras Hindu University, Varanasi, UP, India
e-mail: rahulk.bce17@itbhu.ac.in; skundu.bce@itbhu.ac.in

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_4

4.1 Introduction

According to the WHO, around 1 billion across the globe are deprived of healthy air and water. Approximately around three million die due to bad air and water quality. September 2003–November 2003 is the warmest quarter recorded. Across the globe, around 1 million seabirds die due to pollution and global warming every year. A colossal amount of untreated sewage water is released (approximately 1.2 trillion gallons) dumped in natural water bodies. More than 3 million children <5 years of age die annually (WHO). The major causes for this are the rate at which industrialization is occurring, increased growth rate of the population, and complete negligence toward environmental conditions and health, which results in global crisis in terms of environmental pollution. Anthropogenic activities release pollutants into the environment at different levels such as in air, water, and soil which leads to air, water, and soil pollution and respective health issues (Bannister 1992). A vast array of chemicals, heavy metals, nonrenewable fuels such as polycyclic aromatic hydrocarbon (PAHs), and radioactive wastes are the major environmental toxic pollutants. These pollutants enter the food chain and become the very part of the toxic intake of food, which imparts various hazards to human health and is toxic to the environment. These chemicals tend to accumulate and are serially transferred from one food chain to another creating a toxic network of multiple food chains. Soil, water, and air get contaminated with these toxic components such as chemicals, heavy metals, PAHs, and radioactive wastes raising health concerns over the past decade as they are mutagenic and carcinogenic. From the mentioned components, PAHs are the most lethal and chronic disease-causing component. Radioactive wastes tend to be bound in particular zones and are harmful when released untreated and uncontrolled in the environment (Dash et al. 2012; Mangwani et al. 2014). As for heavy metals, they are present in minimum concentration as a part of the environment in natural form. Increase in the concentration of heavy metals is lethal for environmental health, and it is increasing due to anthropogenic activities. The increase in concentration of heavy metals such as arsenic, cadmium, lead, mercury, chromium, and selenium is found in soil as well as water. Soil pollution with any of the mentioned components is the most critical problem. Toxic components such as heavy metals, radioactive wastes and hydrocarbons will penetrate the soil deep into the bottom layers, contaminate groundwater, and again enter the food chain (Perfus-Barbeoch et al. 2002; Vinodhini and Narayanan 2008; Duruibe et al. 2007). Major sources of pollutants, their level of contamination, and respective health hazards are shown in Table 4.1.

Bioremediation utilizes living microorganisms, i.e., microbes and plants, for the treatment of pollutants or contaminants affecting the environment. Contaminants are transformed from harmful and toxic to degradable and less toxic form. The bioremediation process is classified into two broad categories, i.e., in situ and ex situ bioremediation. In situ remediation demands the treatment of contaminated soil and marine life at the site of the source of the contaminated waste. The advantage of this process is being less expensive, and the transportation factor is absent. Furthermore, in situ remediation can be classified again into intrinsic and engineered. Intrinsic bioremediation requires substrates in the form of nutrients,

Table 4.1 Major components of environmental pollution, their source, their minimum level of contamination, and respective health hazards

Contaminant	Exposure source	Permissible level	Health effects
Arsenic	Food, air, and drinking water	Air, 1–3 ng/m^3; drinking water, 2 μg/L; and soil, <97 mg/kg	Carcinogenic, cardiovascular effects
Cadmium	Cigarette smoking, food, air, and water	Air, <5 ng/m^3; drinking water, <5 μg/L; soil, <0.27 mg/kg	Bone mineralization
Lead	Lead-based paint, food, drinking water	Air, <0.05 ng/m^3; drinking water, <10 μg/L; soil, <30 g/kg	High blood pressure, decrease in fertility
Mercury	Dental fillings, incinerators	Air, <1.5 ng/m^3; drinking water, <100 ng/L; soil, <17 mg/kg	Diarrhea, kidney damage
PAHs	Air and food	Air, <1 ng/m^3; drinking water, <0.01 μg/L; soil, <0.01 μg/g	Cancer, mutations, skin irritation

Source: WHO (2000)

and other required gases are supplied to the site contaminated via aqueous systems. In the case of engineered bioremediation, engineered or specific types of microbes along with the nutrients are added to enhance the degradation efficiency (Chowdhury and Chandra 1986).

Ex situ remediation requires the contaminated unit, whether soil or water, to be treated at a different location from that of the site of origin. The process becomes expensive. Depending upon the type of contaminants, ex situ bioremediation is classified into two categories, i.e., solid-phase treatment and aqueous-phase or slurry-phase treatment. In case of solid-phase treatment, wastes such as agricultural wastes, wastes from industries, biodegradable wastes from animals, plant wastes, and domestic wastes are treated. In case of slurry-phase treatment, a slurry-phase reactor which involves the aqueous systems is used for maintenance of culture or microbial growth conditions which in turn increases the metabolic activity of microbes and the efficiency of the treatment process. Biomass generated is reused to treat another batch of contaminated units to decrease the overall cost of the process (Halder 2014; Evans and Furlong 2003; Ike et al. 2007; Cunningham and Philp 2000).

4.2 Heavy Metals

4.2.1 Introduction

Revolution of the modern industrial era in order to satisfy the requirements of the growing population resulted in the release of the exceedingly hazardous and dangerous chemicals into the environment which in turn created an imbalance in the environment and human health as well. Lethal heavy metals are the chemicals which result from such anthropogenic activities which get accumulated in our

environment; and their lethal effects are clearly visible on human health, flora and fauna, and marine life as well. Heavy metals usually are the metals or the metalloids having high atomic mass and specific gravity five times more than water (Girma 2015). The main reason behind the accumulation of heavy metals and their high concentration in the environment is due to the various anthropogenic activities such as mining, smelting, and various chemical pesticides used for various agricultural purposes. Effluents from various industries are releasing a huge amount of heavy metals into the environment, primarily aquatic bodies. A wide array of applications of heavy metals in medical, agricultural, and industrial settings make them more available and increase their exposure to humans as well as the environment. Heavy metals such as Cu, Fe, Co, Mn, and Se are required for human growth in traces but within certain limits. In excess, these heavy metals are lethal in nature. On the other hand, heavy metals such as As, Hg, Pb, and Cd are effective carcinogens as per the WHO and United States Environmental Protection Agency (USEPA) (Saraswat 2014; Olaniran et al. 2013).

Multiple methods and techniques have been developed to get rid of this increasing heavy metal concentration in the environment. Physical and chemical methods are being employed such as adsorption, ultrafiltration, reverse osmosis, ion exchange, chemical precipitation, etc. All these chemical techniques are much expensive and lead to the generation of secondary wastes and are unable to achieve the desired level of treatment efficiency at low heavy metal concentration (Verma and Kaur 2016). The need is the kind of treatment technique that does not compromise or put external secondary load on ecological health and human health across the globe and goes along with industrial growth as well. In this regard, bioremediation is considered as a useful approach; and being eco-friendly in nature, it also does not create a secondary load on the environment. The microbes metabolize the deadly heavy metals and release important components, and bio-products, which are less toxic and easily degradable. Different microbes such as bacteria, fungi, and algae have cellular machinery to metabolize and remove heavy metals from the environment, i.e., from different sources such as marine (aquatic systems), which is contaminated with industrial effluents, and soil, which is contaminated due to different anthropogenic activities (Gawali Ashruta et al. 2014).

4.2.2 Toxicity of Heavy Metals

Heavy metals are also an important part in the growth of the human body, and deficiency of such heavy metals leads to various serious disorders and diseases. For example, copper acts as an important co-factor of different metalloenzymes such as cytochrome oxidase, catalase, and ferroxidase; therefore, it is a crucial factor in different metabolic synthesis (Tchounwou et al. 2012). However, increase in copper concentration leads to lethal effects such as Wilson's disease. Arsenic, cadmium, chromium, copper, mercury, lead, and nickel are considered as heavy metals. Their permissible limits and lethal effects are shown in Table 4.1. Metals dissolved in water are usually utilized or consumed by marine or aquatic life which leads to

initiation of a chain reaction and begins this vicious cycle in the aquatic life where these heavy metals get accumulated at each stage of the cycle. Mercury in aquatic systems leads to formation of methylmercury, the most lethal and toxic compound occurring via a process known as methylation of mercury (Olukanni et al. 2014). This state of mercury becomes efficient enough to cross cellular membranes and gets accumulated in fatty tissues of different species of aquatic or marine life. High concentration of methylmercury also leads to the decrease in rate of hatching as well as decreased growth rate among marine flora and fauna (Yadav et al. 2016). Toxicity due to mercury also leads to neurological disorders such as partial blindness, weakening of muscles, and body spasms. High concentration of cadmium causes *itai-itai* disease as well as dysfunction of the kidneys, soft bone, and muscle tissues. Low concentration of Cr(VI) induces the inhibition of the natural growth of marine flora and fauna. Concentration greater than 60 ppb of Cr(VI) leads to inhibition of algal growth. Toxicity due to lead majorly affects the skeleton structure of fish. Fish in comparison to microalgae are more sensitive to lead contamination. Concentration higher than 550 ppb of lead in microalgae inhibits the photosynthesis process and the enzymes involved in it, while only 100 ppb of lead concentration inhibits the functioning of fish's gills (Rajendran et al. 2003; Kumar et al. 2010).

Exposure of high concentration of heavy metals to plants effects different metabolic activities in plants. According to WHO guidelines maximum threshold level of heavy metals in soli is 08 mg/kg for cadmium, 35 mg/kg nickel, 36 mg/kg of copper, 50 mg/kg of zinc, 85 mg/kg and for cadmium it is 10 mg/kg (Rieuwerts et al. 1998). High heavy metal concentration leads to the decrease in stomatal conductance and reduced concentration of photosynthetic pigments such as chlorophyll and phycocyanin. Toxic effects of heavy metals were also reported to affect the cell wall and plasma membrane of cells. Alteration of lipid content and concentration was observed due to toxic effects of heavy metals. High concentration of aluminum leads to the lipid peroxidation process by generating free radicals. In the case of microbes, heavy metals such as cadmium, chromium, and lead when present in soil in different ratios have lethal effects on soil microbes. Acid phosphatase and urease activity is affected by soil microbes. Phospholipids and other fatty acid compositions are altered in different soil microbes. Effects of heavy metals on microbes depend on various factors such as solubility, bioavailability, and toxicity of the metal (Stern 2010; Harvey and McArdle 2008; Tchounwou et al. 2008).

4.2.3 Bioremediation: Heavy Metal Removal

Numerous microbes have established efficiency for the removal of heavy metals and are resistant to heavy metals. Microbes usually survive heavy metal contamination via two ways: (1) Microbes have the capacity to bind the metal to the cell wall or EPS (extrapolymeric substances) or induce metal precipitation inside or outside the cell. (2) Microbes adopt specific metabolic modifications to tackle high-metal concentration surroundings and aid in the detoxification of the contaminant.

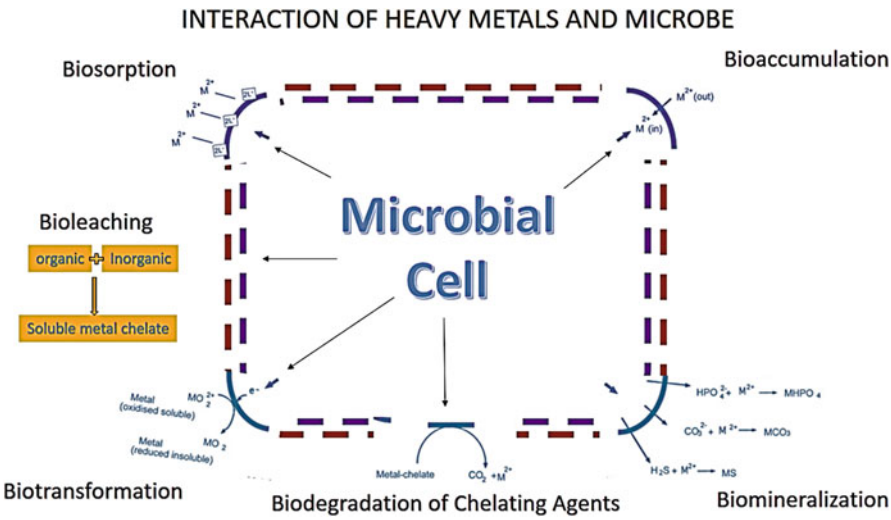


Fig. 4.1 Mechanisms involved in removing heavy metals from different contaminated sites

Different mechanisms are involved and developed as shown in Fig. 4.1 to remove and tackle toxicity of heavy metals (Bradl 2005).

A process known as metal efflux pumping is similar to various bacteria. Bacteria which is resistance to Arsenic possesses arsenic efflux mechanism of ATPase and also arsenate reductase which reduces As(V) to As(III). Another bacterial system has shown resistance to mercury due to the presence of mercury reductase which reduces Hg(II) to elemental mercury. Different mechanisms and techniques to detoxify heavy metals are developed by microbes such as biotransformation, bio-leaching, biosorption, biomineralization, and bioaccumulation. All the mechanisms and techniques are discussed below (Wayne et al. 1999; Wright and Welbourn 2002; Taub Frieda 2004).

4.2.3.1 Biotransformation

In microbial systems, biotransformation means the heavy metal is converted to a less toxic form or a form from which it can be easily recovered. There are two major types of microbial biotransformation, i.e., the redox reaction conversion process of inorganic forms and conversion from inorganic to organic form and vice versa. The process is basically methylation and demethylation. Source of energy for microbes is the oxidation of metals like Fe, S, Mn, and As (Ogundele et al. 2015). While in reduction of metals such as As, Se, Cr, and Ur are reduction process where microbes use metals as a terminal electron acceptor for anaerobic respiration. Coupling of reduction and respiration reactions might take place or not depending upon the microbe used. Examples of aerobic and anaerobic reduction are Cr(VI) to Cr(III), Se(VI) to elemental Se, U(VI) to U(IV), and Hg(II) to elemental Hg. These examples of biotransformation are major components of biological geochemical cycles of heavy metals, and these properties of microbes are utilized in microbial

bioremediation of soil and aqueous systems (Srivastava et al. 2012; Bibi and Hussain 2005; Shanker et al. 2005).

4.2.3.2 Biosorption

The process is related to nonspecific binding of heavy metals to the surface of the cells or the extrapolymeric substances known as exopolysaccharides. Dead cells also contribute in providing surface for heavy metal binding, and the process does not involve any enzymes. The process is capable of adsorbing heavy metals even from diluted solutions. Two different types of biosorption are observed, i.e., active and passive biosorption. Passive biosorption does not involve formation of any chemical bonds, and the energy for interaction is low; therefore, the process is reversible in nature. The process also does not depend upon the external environmental conditions such as pH, temperature, and concentration of ions. In the case of the active biosorption process, it is slow and is dependent on cell metabolism (Barajas-Aceves 2005; Chander et al. 2002; Misra 1992). Specific proteins such as metallothioneins form complexes with heavy metals. This process is controlled by external environmental conditions such as temperature, pH, and effects of different inhibitors. Cell walls of different microbial species such as microalgae, yeast, bacteria, and fungi are efficient biosorbents. Regeneration of biosorbents is easy with techniques such as the action of acid or the use of a chelating agent. EPS are the polymers microbes produce and release outside the cell wall, which comprise polysaccharides, proteins, and other small moieties. The EPS have characteristic property to adsorb heavy metals when cations of metals are interacting with oppositely charged regions of EPS. For this the reason, EPS are generally used for the treatment of aquatic systems such as wastewater (Huckle et al. 1993). Exopolysaccharides from active sludge adsorbed 45 mg/g Cd(II) and 80 mg/g Zn (II). *Rhodobium marinum* species are a kind of non-sulfur bacterial species found to have heavy metal removal efficiency of 90–97% for different heavy metal compositions such as Cd, Cu, Pb, and Zn using the EPS they produce. *Gloeothece* sp. removed 25 mg/g of Cu(II) and 44 mg/g of Pb(II) by the EPS it produced. EPS of Cu-tolerant *Sinorhizobium meliloti* were found to tolerate Cu concentration of up to 1.4 mM. Variations in binding efficiencies of different EPS from different species are due to the presence of different functional groups on the protein moieties on the polymers of EPS. EPS from microbes have the great potential to remove heavy metals from soil as well as aqueous systems and play a critical role in bioremediation of heavy metals (Morby et al. 1993; Turner et al. 1995; Choudhury and Srivastava 2001).

4.2.3.3 Bioaccumulation

The process is considered to have very high energy requirement and to be energy dependent. The process is similar as of heavy metal transportation system. The reason why the process is highly energy dependent due to the influx of heavy metals across bacterial membrane via different mechanisms, which requires energy to occur. Mechanisms such as ion pump channels, endocytosis channels, carrier mediated transport channels, complex and lipid permeation requires high energy to

Table 4.2 Bioaccumulation of different heavy metals by different microbes at an initial concentration and single solution (Issazadeh et al. 2013)

Species	Cu^{2+} (mg/g)	Cd^{2+} (mg/g)	Co^{2+} (mg/g)	Zn^{2+} (mg/g)
Aspergillus niger	3.96	7.52	4.34	2.41
Aspergillus awamori	4.32	8.15	5.17	2.51
Aspergillus ussamii	4.50	7.95	5.03	2.56
Rhizopus delemar	17.16	33.12	18.82	10.31
Candida blankii	6.61	16.54	9.54	5.35
Saccharomyces cerevisiae	7.44	18.60	10.73	6.12
Debaryomyces senii	3.72	9.25	4.31	3.27

occur resulting in the process being high ATP requirement dependent. Numerous active transport channels were reported for different bacterial and fungal species. Cadmium intake by *Pseudomonas putida,* silver influx by *Thiobacillus ferrooxidans,* lead and cadmium uptake by *Citrobacter* sp., and Cr influx by *Bacillus subtilis* are few examples of heavy metal uptake by different microbial species (Vasudevan et al. 2001; Issazadeh et al. 2013; Lei et al. 2008).

Bioaccumulation of heavy metals such as Cu, Cd, Co, and Zn is shown in Table 4.2 for an initial metal concentration of 1 mM and for a mixed solution of metals.

4.2.3.4 Bioleaching

Bioleaching is a bio-hydrometallurgical process where the heavy metals contained inside the sulfur fraction of the sediments are solubilized. In the case of treatments of sediments, bioleaching is preferred, also known as bioaugmentation. The process is carried out using chemolithoautotrophic bacteria such as *Acidothiobacillus* sp., capable enough to oxidize sulfur elemental forms and Fe ions as well in an acidic environment and also having the capacity to solubilize heavy metals. Extraction of Cd, Pb, Hg, Cu, and other heavy metals was studied using mixed cultures of acidophilic Fe or S oxidizing bacteria via bioleaching from a sediment. Biomining is a broad term that covers bioleaching and bio-oxidation (Panwichian et al. 2011; Pereira et al. 2011; Hou et al. 2013). Metabolic activity of microorganisms secretes low-molecular-weight organic acids, which are capable of dissolving heavy metals and small particles of soil containing heavy metals. Microbes are efficient enough to use the substrates, i.e., the nutrients, and energy to produce organic acids which encourage leaching of cadmium. In the presence of additional nutrients for the metabolic activity of microbes, the leaching rate increased to 36% as compared to the leaching rate in the absence of nutrients, which was 9%. Microbes which are majorly prokaryotic readily undergo redox reactions and alter the valence of the heavy metals which in turn alters the property of the heavy metals that can be utilized in their elemental forms and are much less toxic than their previous forms (Beolchini et al. 2007; Li et al. 2014).

4.3 Radioactive Wastes

4.3.1 Introduction

Uranium (U), technetium (Tc), thorium (Th), neptunium (Np), and plutonium (Pu) are the major and most common wastes generated due to anthropogenic activities such as coal mining, burning of coal in thermal power plants, deep-sea oil exploration, nuclear power generation, medical wastes from radiotherapy of cancer patients, etc. The mining industry is a major producer and generator of radioactive wastes into the environment. A maximum number of ores contain limited yet non-extractable radioactive minerals. The industries and companies extract the required concentration from the ore; and the remaining amount of the mineral in the ore, which is not extractable or requires higher downstream costs, is dumped (Coates et al. 2001). This series of extraction and dumping leads to increase in concentration of radioactive wastes in the environment leading to lethal and carcinogenic results in plants, marine life, as well as humans. Higher oxidation state of these compounds or minerals is highly toxic and is treated and reduced to low oxidation state and dumped. After long-term accumulation, these wastes tend to oxidize for higher oxidation states. Furthermore, groundwater is affected, contaminating the area for a longer period. Increased solubility of radioactive waste contaminants renders the toxic and lethal effect in groundwater for a longer time. Some of the suggestion is the anaerobic reduction of the radioactive wastes to render them insoluble in water. Three different major techniques as discussed are generally utilized in the treatment of radionuclides, namely, bioaccumulation, biosorption, and biotransformation. Biotransformation is a majorly used method as compared to other methods, which are ineffective (Nelson and Lovett 1978).

4.3.2 Microorganisms and Treatment of Radioactive Wastes

Treatment of radionuclides is done using anaerobic microorganisms, which act on the radionuclides enzymatically resulting in a reduction reaction from higher-state radionuclides to lower-state radionuclides of uranium and neptunium. There are few groups of microorganisms, which are responsible for anaerobic reduction of radionuclides: (1) mesophilic sulfate-reducing bacteria, (2) thermophilic bacteria, and (3) mesophilic Fe(III)-reducing microorganisms and acid-tolerant microorganisms. Radioactive wastes due to elements present in actinide series can be treated based on enzymatic or chemical treatments. The following are the different processes of treatments, which can be and are being utilized for the removal of radionuclides (Karmakar et al. 2012). Reactions involved in the removal of radioactive contaminants are shown in Fig. 4.2:

$$UO_{2(soluble)}^{2+} + H_2 \rightarrow UO_{2(insoluble)} + 2H^+$$
$$UO_{2(soluble)}^{2+} + CH_2O + H_2O \rightarrow UO_{2(insoluble)} + 2H^+$$
$$TcCO_{4(soluble)}^{-} + 3e^- + 4H^+ \rightarrow TcO_2 \cdot nH_2O_{(insoluble)} + (2-n)H_2O$$
$$NpO_{(soluble)}^{2+} + 1e^- + 4H^+ \rightarrow Np_{(insoluble)}^{4+} + (2-n)H_2O$$
$$Pu_{(soluble)}^{5+\text{ or }6+} \rightarrow Pu_{(insoluble)}^{4+}$$

Fig. 4.2 Reactions involved in the removal of radionuclides

1. Using oxidoreductase enzyme for oxidation-reduction reactions (enzymatic modes of treatment).
2. Prevention of the solubility of the actinides altering the pH of the contaminated aqueous system.
3. Use of a chelation agent.
4. Removal using biomass and exopolysaccharides via biosorption.
5. Process of biomineralization and creating a stable mineral form.
6. Biodegradation of actinides with the aid of organic components.

Uranium reductase reduces U(VI) to uraninite, i.e., UO_2, in the presence of electron donors (organic compounds) or hydrogen. Uranium reductase is the enzyme used in the process and is obtained from *Geobacter*, *Desulfovibrio*, and *S. putrefaciens.* The periplasmic space of *Geobacter* is reported to have the reductase enzyme. In the case of *Desulfovibrio vulgaris*, the enzyme is present in soluble fraction which requires the presence of cytochrome and hydrogenase. *S. putrefaciens* have the capability to reduce U(VI) with the aid of nitrite-reducing enzymes (Lovley et al. 1993a). *Saccharomyces cerevisiae* is known to accumulate uranium externally on the surface subject to the external conditions such as pH, temperature, and interference by the presence of charge on the cell, which alters due to the interference of anions and cations. Reduction in positive charge on the external surface of the cells of *Saccharomyces cerevisiae* results in the increased uptake of the metal uranium (Lovley et al. 1993b). The uranium gets accumulated in the sites of phosphate groups and the carboxyl group of the cell wall. In the case of *Rhizopus arrhizus*, the primary binding site for uranium and thorium is a chitin component. Jilsk et al. reported the removal of 95% of radium (^{226}Ra) from waste stream using the mycelia of *Penicillium chrysogenum* within 3 months of growth. Plutonium is found lethal for microorganisms due to its very high radiation rather than its effects due to toxicity. Still, very highly resistant bacteria and fungal strains are capable of integrating the Pt in the cell walls, which results in the change of form in the cell, and variation in mobility was observed in the solution (Lovley 2003). Filamentous algae *Cladophora*, *Oedogonium*, and *Rhizoclonium* amass uranium on the cellular surfaces, and in the case of *Chlorella* species, absorption takes places intracellularly. The absorption of uranium in *Chlorella* species is independent of whether metabolism can be easily enhanced using chelating agents such as EDTA. Dead biomass of *Chlorella* species killed with the application of heat was observed to amass large

concentration of uranium intracellularly. In the case of technetium (Tc), reduction can take place using any microorganism. Species such as *Geobacter*, *Desulfovibrio*, *and S. putrefaciens* reduce Tc at neutral pH. *Acidithiobacillus ferrooxidans* reduces Tc at acidic conditions, and other halophilic bacterial species reduce Tc at alkaline conditions. In the case of neptunium (Np), the reduction is done using ascorbic acid produced by *S. putrefaciens.* Microbes mentioned in the discussion can be utilized to treat mine wastes and unutilized fuel wastes ensuring that no radionuclide escapes into groundwater. Reduction is also useful in the prevention of radioactive disintegration by reducing the radionuclide to a lower state. After the reduction, solubility of radionuclide is reduced which induces precipitation in the case of water bodies. Precipitated radionuclide must be handled with utmost caution and safety and must be dumped/stored in a leak-proof container (Macaskie et al. 1996; Lloyd et al. 2000).

4.4 Hydrocarbon Wastes

4.4.1 Introduction

Carbon and hydrogen are the major components of the hydrocarbons. In this century, hydrocarbons are the major source of energy. A tenfold increase in hydrocarbon consumption in different industries and in daily utilization of automobiles leads to contamination of soil as well as aquatic systems. Implications of hydrocarbon pollution are far worse in terms of terrestrial, marine, and air pollution. Oil spills, leakage of pipelines, production, refining, and other processes are increasing the concentration of pollution exponentially in the ecosystem. This threatens the life of the humans and other life forms present on the planet. In 2010, an oil leakage took place in Mumbai, which polluted the Arabian Sea at a very vast extent. Accumulation of these pollutants in plants and animals leads to mutation and proves lethal in majority of cases (Macaulay 2015).

Numerous physiochemical methods have been developed to treat and decontaminate the site. The major disadvantage of such methods is that they are labor intensive and capital cost intensive and generate secondary wastes due to the involvement of chemicals and other raw materials, which are not ecofriendly. Other nontoxic techniques such as evaporation, burying, dispersion, and washing are available; but the efficiency of treatment is considerably low. A simple yet cost-effective method is required in order to limit the pollution (Thapa et al. 2012).

4.4.2 Bioremediation: Hydrocarbon Waste

Bioremediation is such method, which harnesses the capability of microorganisms to utilize hydrocarbons as substrate and convert them into useful products. Hydrocarbons contain different categories of wastes such as alkanes, aromatic hydrocarbons, phenols, and polycyclic aromatic hydrocarbons.

4.4.2.1 Alkanes: Bioremediation and Biodegradation

Air Pollution due to alkane-based hydrocarbons is major in percentage and is related to petroleum pollution. Alkanes having shorter carbon chain are degraded easily as compared to other hydrocarbon components, which in turn decreases their hydrophobicity. Straight-chain alkanes (C10–C24) are the most rapidly degrading component. Strains of *A. calcoaceticus* and nocardioforms are among the microorganisms that grow easily on n-alkane having C30–C40 carbon atoms. Increase in chain length also decreases the degradation efficiency of alkanes using microorganisms, also reducing the solubility of n-alkanes in aqueous systems (Jain and Bajpai 2012).

Pseudomonas sp. and *Acetobacter* sp. have been observed to degrade alkane, and biodegradation begins by oxidizing the methyl group and results in alcohol production. Alcohol is then dehydrogenated to form aldehyde and further oxidized to carboxylic acid. The resulting carboxylic acid further goes in the metabolic pathway and gets metabolized in the β-oxidation pathway of fatty acids. *Rhodococcus* sp. is known to reduce terminal as well as subterminal methyl units. This oxidation is carried out by an enzyme known as monooxygenase and results in the production of secondary alcohol, which is then converted to ketone followed by conversion into fatty acids. *A. calcoaceticus S19* genetically modified species was able to convert octadecane to octadecanol, and the final product was octadecanoic acid (Salleh et al. 2003; Das and Chandran 2011; de la Cueva et al. 2016).

4.4.2.2 Aromatic Hydrocarbons: Bioremediation and Biodegradation

Toluene, benzenes, and ethyl benzenes are among the high-percentage pollutants in the aromatic hydrocarbon category. Together, the compounds are known as BTEX. BTEX compounds are toxic when released into groundwater. These compounds are monoaromatic in nature which increases their solubility in water and contaminate water at high concentration. Increase in mobility makes the BTEX contaminate surface and subsurface water bodies. For BTEX biodegradation, microbial activity was observed at different temperature zones, i.e., cold and temperate zones; and it was found that the BTEX compounds are getting degraded at both temperatures without lowering the microbial activity. In case of BTEX degradation, it was found to degrade in shallow aquifers at natural gas producing sites (Franchi et al. 2016). Toluene gets reduced in methanogenic conditions. Regions were identified such as sulfate reduction zones and found out that the maximum degradation of BTEX was in region of sulfate-reducing bacteria. Thermophilic bacteria were found to biodegrade the aromatic hydrocarbons. Certain species of *Pseudomonas* were found to degrade a variety of aromatic hydrocarbons having aromatic side chains such as phenols, anilines, and other combinations. *Thermus aquaticus* and *Thermus* sp. isolated by Chen and Taylor are two thermophilic bacteria found to degrade BTEX co-metabolically (May and Katopodis 1990; Bajpai et al. 1998; Whyte et al. 1998).

Oxidation of benzene is a result of three-enzyme system where two hydroxyl groups are attached to the benzene ring resulting in the formation of cis-dihydrodriol. After the formation of dihydrodriol, it is then dehydrogenated to catechol. Catechol

formed is cleaved using two different oxidative methods, i.e., cleavage at different site meta-cleavage and ortho-cleavage to generate semi-aldehyde and further oxidation leads to formation of muconic acid. Toluene degrades at a faster rate as compared to other BTEX compounds and is biodegraded easily in aerobic conditions and is studied with *Pseudomonas* sp. Apart from *Pseudomonas*, other microorganisms that show the ability to degrade toluene are *Mycobacterium*, *Rhodococcus*, and *Acinetobacter*. Toluene is degraded in different manners by different microorganisms such as formation of benzoic acid when degraded at the methyl group by *Pseudomonas sp.* In the case of xylenes, biodegradation occurs via oxidation at the methyl group resulting in the formation of toluic acid, tolualdehydes, and methyl-catechol (Bradley and Chapelle 1995; Gieg et al. 1999; Lugowski et al. 1997).

4.4.2.3 Phenols: Bioremediation and Biodegradation

Phenol is a by-product of oil industries and is among the most common components of wastewaters. Treatment of wastewater containing phenol is affected by variation in temperature. Onysko et al. (2000) found out that the most appropriate temperature range when using psychrotrophic *Pseudomonas putida* is 10–25 °C for treatment of wastewater containing phenol. In a similar case, Pills and Davis concluded and found out that another species of psychrotrophic *Pseudomonas putida* was capable of removing a variety of phenolic compounds. This species of psychrotrophic *Pseudomonas putida* can be integrated and used in different processes such as active sludge treatment processes and trickling filters. The strain could work in combination with other strains for bioremediation of a combination of hydrocarbons (Chen and Taylor 1995, 1997; Müller et al. 1998).

4.4.2.4 Polycyclic Aromatic Hydrocarbons (PAHs): Bioremediation and Biodegradation

As per the United States Environmental Protection Agency (USEPA), there are about 16 different types of PAHs which are responsible for pollution. Out of the 16 types of PAHs, naphthalene, phenanthrene, and pyrene are among the major ones responsible for the contamination of soil. Species of *Sphingomonas* and *Pseudomonas* have been isolated which can degrade naphthalene, phenanthrene, and fluorene (Hubert et al. 2001). It was also observed that the species isolated are capable of degrading BTEX compounds. Degradation of PAHs due to thermophilic bacteria still requires major experimental studies to establish significant results. Thermophilic bacteria isolated by Muller in 1998 were capable of degrading anthracene. Metabolites were different for these microorganisms and have different pathways for degradation of hydrocarbons, which are very little known as compared to mesophilic bacteria. *Bacillus thermoleovorans* was found to degrade naphthalene at 60 °C (Juteau et al. 1999).

Naphthalene is among the simplest forms of PAHs and is easily degraded by the microorganisms. Catabolism of PAHs starts with the oxidation of PAHs to a dihydrodriol by multiple enzymes acting on the PAHs. Similarly, two different types of cleavage occur, i.e., meta cleavage and ortho cleavage of the dihydroxylated

intermediates of PAHs resulting in the formation of catechol followed by the conversion of this catechol into acids such as tricarboxylic acid. In the case of naphthalene, it is first degraded via oxidation to cis-1,2-dihydroxy-1,2-dihydronapthalene. After another round of oxidation of this compound, this compound is converted into 1,2-dihydroxynapthalene. After the ortho or meta cleavage, this compound is converted into salicylaldehyde and pyruvate (Onysko et al. 2000; Margesin and Schinner 2001; Pillis and Davis 1985). Salicylaldehyde is then further oxidized to salicylate and then to catechol. Cerniglia and Heitcamp in 1989 concluded that biodegradation of PAHs is inversely related to the number of benzene rings fused together. PAHs have low water solubility, and low energy of their resonance structure makes them persistent to low molecular weight. Co-metabolism is the process that has been used to biotransform high-molecular-weight PAHs to lower-molecular-weight PAHs using different microorganisms when testing their growth on fluoranthene. Pyrene also has been found to be degraded by different microorganisms using different metabolic pathways and co-metabolic pathways (Aislabie et al. 2000; Cerniglia 1993; Jain et al. 2010).

4.5 Conclusion and Future Prospects

Removal of hydrocarbons, heavy metals, and radioactive wastes is still a major challenge. The rate at which the wastes are generated in any of the category is far higher than the rate of treatment of these wastes even with the right techniques. This creates imbalance in the production to treatment ratio in terms of time. The focus must be on the efficiency in terms of time after selecting an optimum technique. Engineering methods to minimize the time for treatment should be the major focus after defining the treatment technique. Still, bioremediation faces challenges such as chlorinated hydrocarbons and higher aromatic hydrocarbons still resistant to degradation by microbes. Genetically modified microorganisms will improve the efficiency of biodegradation but possess disadvantages of mutation and other side effects, but bioremediation techniques are economical as compared to their chemical counterparts and can be implemented at large scale. Use of bioreactors must be put into action in order to increase the removal efficiency and decrease treatment time. Majority of the techniques are aerobic in nature which makes the process in bioremediation limited. More anaerobic processes are required to be developed to biodegrade the contaminants in anaerobic regions.

Acknowledgment We would like to thank the School of Biochemical Engineering, Indian Institute of Technology (Banaras Hindu University), and MHRD, India, for providing the resources required for the completion of this project. The authors would also like to thank the Institute's main library for providing tools for proofreading to improve and enhance the manuscript.

References

Aislabie J, Foght J, Saul D (2000) Aromatic hydrocarbon-degrading bacteria from soil near Scott Base, Antarctica. Polar Biol 23(3):183–188

Bajpai U, Kuhad RC, Khanna S (1998) Mineralization of [14C] octadecane by Acinetobacter calcoaceticus S19. Can J Microbiol 44(7):681–686

Bannister W (1992) The biological chemistry of the elements: the inorganic chemistry of life. Biochem Educ 20:62–63

Barajas-Aceves M (2005) Comparison of different microbial biomass and activity measurement methods in metal-contaminated soils. Bioresour Technol 96(12):1405–1414

Beolchini F, Ubaldini S, Passariello B, Gül N, Türe D, Vegliò F, Danovaro R, Dell'Anno A (2007) Bioremediation of dredged sediments polluted by heavy metals. In: Advanced materials research, vol 20. Trans Tech, pp 307–310

Bibi M, Hussain M (2005) Effect of copper and lead on photosynthesis and plant pigments in black gram [Vigna mungo (L.) Hepper]. Bull Environ Contam Toxicol 74(6):1126–1133

Bradl H (2005) Heavy metals in the environment: origin, interaction and remediation, vol 6. Elsevier, Amsterdam

Bradley PM, Chapelle FH (1995) Rapid toluene mineralization by aquifer microorganisms at Adak, Alaska: implications for intrinsic bioremediation in cold environments. Environ Sci Technol 29 (11):2778–2781

Cerniglia CE (1993) Biodegradation of polycyclic aromatic hydrocarbons. Curr Opin Biotechnol 4 (3):331–338

Chander K, Klein T, Eberhardt U, Joergensen R (2002) Decomposition of carbon-14-labelled wheat straw in repeatedly fumigated and non-fumigated soils with different levels of heavy metal contamination. Biol Fertil Soils 35(2):86–91

Chen CI, Taylor RT (1995) Thermophilic biodegradation of BTEX by two Thermus species. Biotechnol Bioeng 48(6):614–624

Chen CI, Taylor RT (1997) Thermophilic biodegradation of BTEX by two consortia of anaerobic bacteria. Appl Microbiol Biotechnol 48(1):121–128

Choudhury R, Srivastava S (2001) Zinc resistance mechanisms in bacteria. Curr Sci 81:768–775

Chowdhury BA, Chandra RK (1986) Biological and health implications of toxic heavy metal and essential trace element interactions. Prog Food Nutr Sci 11(1):55–113

Coates JD, Bhupathiraju VK, Achenbach LA, Mclnerney MJ, Lovley DR (2001) Geobacter hydrogenophilus, Geobacter chapellei and Geobacter grbiciae, three new, strictly anaerobic, dissimilatory Fe (III)-reducers. Int J Syst Evol Microbiol 51(2):581–588

Cunningham CJ, Philp JC (2000) Comparison of bioaugmentation and biostimulation in ex situ treatment of diesel contaminated soil. Land Contam Reclam 8(4):261–269

Das N, Chandran P (2011) Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol Res Int 2011:941810

Dash HR, Mangwani N, Chakraborty J, Kumari S, Das S (2012) Marine bacteria: potential candidates for enhanced bioremediation. Appl Microbiol Biotechnol 97:561–571

de la Cueva SC, Rodríguez CH, Cruz NOS, Contreras JAR, Miranda JL (2016) Changes in bacterial populations during bioremediation of soil contaminated with petroleum hydrocarbons. Water Air Soil Pollut 227(3):91

Duruibe JO, Ogwuegbu MOC, Egwurugwu JN (2007) Heavy metal pollution and human biotoxic effects. Int J Phys Sci 2(5):112–118

Evans GM, Furlong JC (2003) Environmental biotechnology: theory and application. J Air Waste Manage Assoc 53(11):1418–1419

Franchi E, Agazzi G, Rolli E, Borin S, Marasco R, Chiaberge S, Conte A, Filtri P, Pedron F, Rosellini I, Barbafieri M (2016) Exploiting hydrocarbon-degrading indigenous bacteria for bioremediation and phytoremediation of a multicontaminated soil. Chem Eng Technol 39 (9):1676–1684

Gawali Ashruta A, Nanoty V, Bhalekar U (2014) Biosorption of heavy metals from aqueous solution using bacterial EPS. Int J Life Sci 2:373–377

Gieg LM, Kolhatkar RV, McInerney MJ, Tanner RS, Harris SH, Sublette KL, Suflita JM (1999) Intrinsic bioremediation of petroleum hydrocarbons in a gas condensate-contaminated aquifer. Environ Sci Technol 33(15):2550–2560

Girma G (2015) Microbial bioremediation of some heavy metals in soils: an updated review. Indian J Sci Res 6(1):147

Halder S (2014) Bioremediation of heavy metals through fresh water microalgae: a review. Scholars Acad J Biosci 2(11):825–830

Harvey LJ, McArdle HJ (2008) Biomarkers of copper status: a brief update. Br J Nutr 99(S3):S10–S13

Hou W, Ma Z, Sun L, Han M, Lu J, Li Z, Mohamad OA, Wei G (2013) Extracellular polymeric substances from copper-tolerance Sinorhizobium meliloti immobilize Cu2+. J Hazard Mater 261:614–620

Hubert A, Wenzel KD, Engewald W, Schüürmann G (2001) Accelerated solvent extraction-more efficient extraction of POPs and PAHs from real contaminated plant and soil samples. Rev Anal Chem 20(2):101–144

Huckle JW, Morby AP, Turner JS, Robinson NJ (1993) Isolation of a prokaryotic metallothionein locus and analysis of transcriptional control by trace metal ions. Mol Microbiol 7(2):177–187

Ike A, Sriprang R, Ono H, Murooka Y, Yamashita M (2007) Bioremediation of cadmium contaminated soil using symbiosis between leguminous plant and recombinant rhizobia with the MTL4 and the PCS genes. Chemosphere 66(9):1670–1676

Issazadeh K, Jahanpour N, Pourghorbanali F, Raeisi G, Faekhondeh J (2013) Heavy metals resistance by bacterial strains. Ann Biol Res 4(2):60–63

Jain PK, Bajpai V (2012) Biotechnology of bioremediation-a review. Int J Environ Sci 3 (1):535–549

Jain PK, Gupta VK, Gaur RK, Bajpai V, Gautam N, Modi DR (2010) Fungal enzymes: potential tools of environmental processes. Lambert Academic, Saarbrücken, pp 44–56

Juteau P, Rho D, Larocque R, LeDuy A (1999) Analysis of the relative abundance of different types of bacteria capable of toluene degradation in a compost biofilter. Appl Microbiol Biotechnol 52 (6):863–868

Karmakar S, Kundu AP, Kundu K, Kundu S (2012) Microbial treatment of heavy metals, oil, and radioactive contamination in wastewaters, wastewater treatment: advanced processes and technologies. CRC Press (Taylor & Francis Group), Boca Raton, FL, p 185

Kumar A, Bisht BS, Joshi VD (2010) Biosorption of heavy metals by four acclimated microbial species. Bacillus spp., pseudomonas spp., staphylococcus spp. and Aspergillus Niger. J Biol Environ Sci 4:97–108

Lei Z, Yu T, Ai-Zhong D, Jin-Sheng W (2008) Adsorption of cd (II), Zn (II) by extracellular polymeric substances extracted from waste activated sludge. Water Sci Technol 58(1):195–200

Li Q, Csetenyi L, Gadd GM (2014) Biomineralization of metal carbonates by Neurospora crassa. Environ Sci Technol 48(24):14409–14416

Lloyd JR, Yong P, Macaskie LE (2000) Biological reduction and removal of Np (V) by two microorganisms. Environ Sci Technol 34(7):1297–1301

Lovley DR (2003) Cleaning up with genomics: applying molecular biology to bioremediation. Nat Rev Microbiol 1(1):35

Lovley DR, Roden EE, Phillips EJP, Woodward JC (1993a) Enzymatic iron and uranium reduction by sulfate-reducing bacteria. Mar Geol 113(1–2):41–53

Lovley DR, Widman PK, Woodward JC, Phillips EJ (1993b) Reduction of uranium by cytochrome c3 of Desulfovibrio vulgaris. Appl Environ Microbiol 59(11):3572–3576

Lugowski AJ, Palmateer GA, Boose TR and Merriman JE, Uniroyal Chemical Ltd (1997) Biodegradation process for de-toxifying liquid streams. U.S. Patent 5,656,169

Macaskie LE, Lloyd JR, Thomas RAP, Tolley MR (1996) The use of micro-organisms for the remediation of solutions contaminated with actinide elements, other radionuclides, and organic contaminants generated by nuclear fuel cycle activities. Nucl Energy 35(4):257–271

Macaulay BM (2015) Understanding the behaviour of oil-degrading micro-organisms to enhance the microbial remediation of spilled petroleum. Appl Ecol Environ Res 13(1):247–262

Mangwani N, Shukla SK, Rao TS, Das S (2014) Calcium-mediated modulation of Pseudomonas mendocina NR802 biofilm influences the phenanthrene degradation. Colloids Surf B: Biointerfaces 114:301–309

Margesin R, Schinner F (2001) Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles 5(2):73–83

May SW, Katopodis AG (1990) Hydrocarbon monooxygenase system of pseudomonas oleovorans. In: Methods in enzymology, vol 188. Academic Press, pp 3–9

Misra TK (1992) Bacterial resistances to inorganic mercury salts and organomercurials. Plasmid 27 (1):4–16

Morby AP, Turner JS, Huckle JW, Robinson NJ (1993) SmtB is a metal-dependent repressor of the cyanobacterial metallothionein gene smtA: identification of a Zn inhibited DNA-protein complex. Nucleic Acids Res 21(4):921–925

Müller PJ, Kirst G, Ruhland G, Von Storch I, Rosell-Melé A (1998) Calibration of the alkenone paleotemperature index $U_{37}K'$ based on core-tops from the eastern South Atlantic and the global ocean (60° N-60° S). Geochim Cosmochim Acta 62(10):1757–1772

Nelson DM, Lovett MB (1978) Oxidation state of plutonium in the Irish Sea. Nature 276(5688):599

Ogundele DT, Adio AA, Oludele OE (2015) Heavy metal concentrations in plants and soil along heavy traffic roads in north Central Nigeria. J Environ Anal Toxicol 5(6):1

Olaniran A, Balgobind A, Pillay B (2013) Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. Int J Mol Sci 14(5):10197–10228

Olukanni DO, Agunwamba JC, Ugwu EI (2014) Biosorption of heavy metals in industrial wastewater using micro-organisms (Pseudomonas aeruginosa). Am J Sci Ind Res 5(2):81–87

Onysko KA, Budman HM, Robinson CW (2000) Effect of temperature on the inhibition kinetics of phenol biodegradation by pseudomonas putida Q5. Biotechnol Bioeng 70(3):291–299

Panwichian S, Kantachote D, Wittayaweerasak B, Mallavarapu M (2011) Removal of heavy metals by exopolymeric substances produced by resistant purple nonsulfur bacteria isolated from contaminated shrimp ponds. Electron J Biotechnol 14(4):2–2

Pereira S, Micheletti E, Zille A, Santos A, Moradas-Ferreira P, Tamagnini P, De Philippis R (2011) Using extracellular polymeric substances (EPS)-producing cyanobacteria for the bioremediation of heavy metals: do cations compete for the EPS functional groups and also accumulate inside the cell? Microbiology 157(2):451–458

Perfus-Barbeoch L, Leonhardt N, Vavasseur A, Forestier C (2002) Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. Plant J 32:539–548

Pillis LJ and Davis LT (1985) Microorganism capable of degrading phenolics. Patent Number US Patent 4, 556, 638

Rajendran P, Muthukrishnan J, Gunasekaran P (2003) Microbes in heavy metal remediation. Indian J Exp Biol 41:935–944

Rieuwerts JS, Thornton I, Farago ME, Ashmore MR (1998) Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. Chem Spec Bioavailab 10(2):61–75

Salleh AB, Ghazali FM, Rahman RNZA, Basri M (2003) Bioremediation of petroleum hydrocarbon pollution. Indian J Biotechnol 2:411–425

Saraswat S (2014) Patent analysis on bioremediation of environmental pollutants. J Bioremed Biodegr 5(251):1–6

Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31(5):739–753

Srivastava G, Kumar S, Dubey G, Mishra V, Prasad SM (2012) Nickel and ultraviolet-B stresses induce differential growth and photosynthetic responses in Pisum sativum L. seedlings. Biol Trace Elem Res 149(1):86–96

Stern BR (2010) Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations. J Toxic Environ Health A 73(2–3):114–127

Taub Frieda B (2004) Fish 430 lectures (biological impacts of pollutants on aquatic organisms). University of Washington College of Ocean and Fishery Sciences, Seattle

Tchounwou PB, Newsome C, Williams J and Glass K (2008) Copper-induced cytotoxicity and transcriptional activation of stress genes in human liver carcinoma (HepG2) cells. In: Metal ions in biology and medicine: proceedings of the... International Symposium on Metal Ions in Biology and Medicine held...= Les ions metalliques en biologie et en medecine:... Symposium international sur les ions metalliques (Vol. 10, p. 285). NIH Public Access

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

Thapa B, Kc AK, Ghimire A (2012) A review on bioremediation of petroleum hydrocarbon contaminants in soil. Kathmandu Univ J Sci Eng Technol 8(1):164–170

Turner JS, Robinson NJ, Gupta A (1995) Construction of Zn 2+/cd 2+−tolerant cynadobacteria with a modified metallothionein divergon: further analysis of the function and regulation ofsmt. J Ind Microbiol 14(3–4):259–264

Vasudevan P, Padmavathy V, Tewari N, Dhingra SC (2001) Biosorption of heavy metal ions. J Sci Indust Res 60(2):112–120

Verma N, Kaur G (2016) Trends on biosensing systems for heavy metal detection. In: Comprehensive analytical chemistry, vol 74. Elsevier, pp 33–71

Vinodhini R, Narayanan M (2008) Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (common carp). Int J Environ Sci Technol 5:179–182

Wayne GL, Ming-Ho Y, Raton CPB (1999) Introduction to environmental toxicology: impacts of chemicals upon ecological systems. J Ecotoxicol 9(3):231–232

WHO (2000) Adverse health effects of heavy metals in children. In: Children's health and the environment WHO training package for the health sector world. World Health Organization, Geneva

Whyte LG, Hawari J, Zhou E, Bourbonnière L, Inniss WE, Greer CW (1998) Biodegradation of variable-chain-length alkanes at low temperatures by a Psychrotrophic Rhodococcussp. Appl Environ Microbiol 64(7):2578–2584

Wright DA, Welbourn P (2002) Environmental toxicology, vol 11. Cambridge University Press, Cambridge

Yadav A, Batra NG, Sharma A (2016) Phytoremediation and phytotechnologies. International journal of pure and applied. Bioscience 4(2):327–331

Advancement of Omics: Prospects for Bioremediation of Contaminated Soils

5

Kriti Gupta, Rimi Biswas, and Angana Sarkar

Abstract

The soil is a complex mixture of organic matter and minerals, supporting a discrete array of life. Severely polluted soils have been detoxified using a variety of microorganisms. Bioremediation is a process of removal of environmental contaminants utilizing microbes through a variety of enzymatic processes. In situ processing, high public acceptance, and a comparatively lower cost hasten the overall process of bioremediation. However, it is not always effective due to its relatively long time scales and the variable range of contaminants. Varying degrees of success rate have been noticed at different sites worldwide. This chapter attempts to link the traditional and cutting edge technologies such as metagenomics, metatranscriptomics, metaproteomics, and metabolomics to numerous bioremediation techniques as they play a symbolic role in the study of the regulation of numerous mineralization pathways. Extensive data are being generated using these techniques, but their application is still in the infant stage. A stepwise organization of data is needed within the instructive databases. Microbial-assisted contaminant attenuation and in-depth analysis of the organism's metabolism will accelerate the overall process of bioremediation. Thereafter, the next decade will going to decipher the cellular mechanisms and molecular manipulations using an integrated omic tool approach.

Keywords

Soil · Contaminants · Bioremediation · Mineralization · Omic tool

K. Gupta · R. Biswas · A. Sarkar (✉)
Department of Biotechnology and Medical Engineering, National Institute of Technology Rourkela, Rourkela, Odisha, India
e-mail: sarkara@nitrkl.ac.in

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_5

5.1 Introduction

The soil is the most common home of more than 10^{16} diverse microbes/ton due to its heterogeneity, favoring the formation of micro-niches (Olaniran et al. 2013). Soil contamination is a change in the biological and physiochemical nature of the soil which has a detrimental effect on the living organisms residing in it. The different types of soil contamination (Fig. 5.1) are agricultural waste, industrial waste, urban waste, and radioactive waste. The fertilizers, pesticides, industrial effluents, and radionuclides flow down to the nearby water bodies or any other soil location resulting in biomagnification. This creates an interruption in the biochemical pathways and leads to harmful diseases. Improper dumping of waste in landfills and public places results in erroneous disintegration of the waste and deposition of contaminants in the soil. Limitless deposition of waste results in increased bacterial growth that causes a rise in the generation of methane gas which eventually leads to global warming. Nuclear power plants and nuclear testing add wavering amount of radioactive material to the soil (Mishra et al. 2015).

Earlier, the disposal of waste was done by throwing the waste in a hole, but due to the lack of new areas, the practice was difficult. With the emerging techniques like chemical decomposition and incineration at high temperature, the disposal of waste became effective, but they came with several disadvantages like obscure methods, expensive, and others. Alternative techniques like bioremediation were hence implemented (Karigar and Rao 2011).

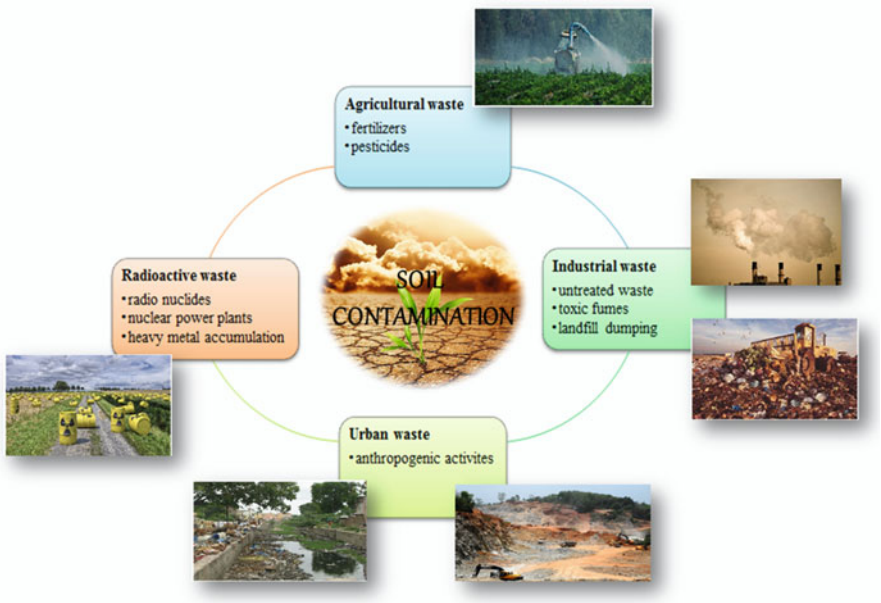


Fig. 5.1 Classification of soil contamination pertaining to their source

Bioremediation is the process of speeding up the process of natural biodegradation in the contaminated areas by the application of microbes (Calvo et al. 2009). The various strategies that are being used to remediate the soil are either removing the pollutant present in the soil or reducing its effect by stabilizing it (containment) (Cunningham and Berti 1993). In general, bioremediation strategies can be classified into the following three processes:

1. Biodegradation: Organic compounds are fragmented down into reduced inorganic or organic compounds.
2. Biotransformation: The hazardous molecules are reformed into a reduced or nonhazardous molecule.
3. Biomineralization: The organic compounds are entirely degraded into inorganic compounds like carbon dioxide or H_2O_4.

The type of contaminant determines the remediation process that will be implemented. The soil remediation cost depends on soil properties, site conditions, volume to be remediated, and type of contaminant. The increasing urbanization and industrialization lead to contamination of soil by organic and inorganic pollutants and, hence, have led to the deterioration of the environment and the human health (Dong et al. 2013).

5.2 Traditional Technologies for Soil Remediation

Along with biological, physical, and chemical concerns, remediation strategy depends upon the legitimate and economic considerations as well. Such strategies are preferred that result in minimum adulteration to the soil.

Different strategies for bioremediation of contaminated soil (Fig. 5.2) are:

5.2.1 Physical Remediation

It includes predominantly thermal desorption and soil replacement. It can be further done by replacing clean soil with contaminated soil, followed by its treatment, soil spading (the contaminated area is dug deep to spread the contaminants into deep sites and naturally degrading the pollutant), and soil importing (clean soil is added to the affected site on the surface, and mixing is done to decrease the concentration of the pollutant).

Thermal desorption is the removal of the pollutant by its volatility.

5.2.2 Chemical Remediation

1. *Chemical leaching*: Soil washing/flushing is done with reagents, surfactants, water, and chelating agents. Soil washing is a strategy where liquids like aqueous

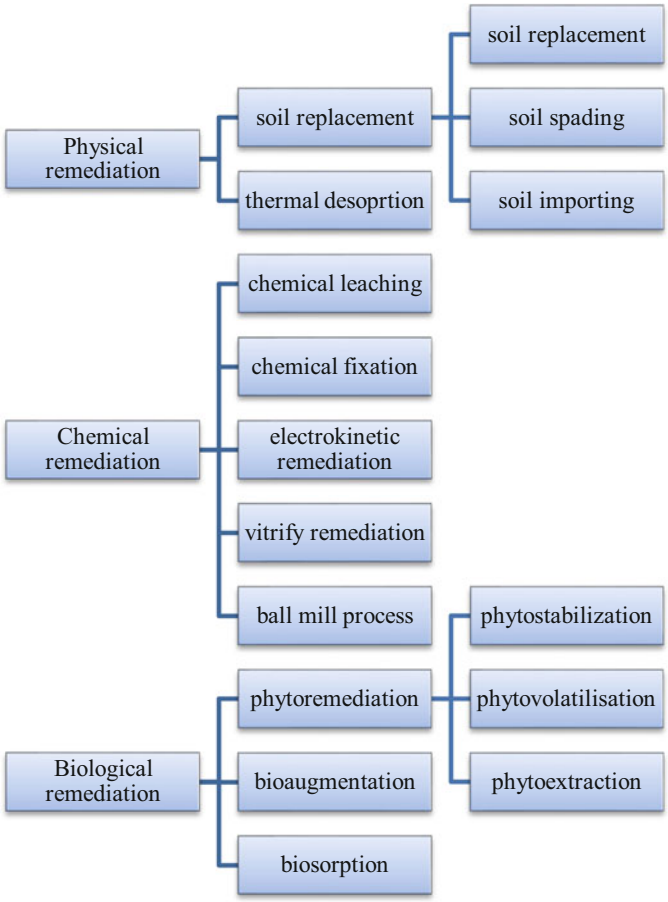


Fig. 5.2 Conventional methods for treatment of soil contamination

solutions are used to separate the pollutants from the soil. The contaminants adhere to the soil particles, but they have low water solubility. To increase the solubility, additives like surfactants and chelating agents are applied along with the process (Mao et al. 2015).

2. *Chemical fixation*: The movement of heavy metals is decreased by adding reagents. The reagents make the heavy metals insoluble in soil, thereby decreasing its toxicity (Yao et al. 2012).
3. *Electrokinetic remediation*: In this strategy, voltage is applied at the two sides of the soil to create an electric field gradient. This technique provides minimum disturbance to the topsoil and treats the lower surface contaminants (in situ) (Gan et al. 2009).
4. *Vitrify remediation*: The organic matter present in the soil is heated at 1400–2000 °C, and it is volatized. The end products (after pyrolysis and steam)

are collected and cooled to form a rock-type substance that creates hindrance in the movement of the heavy metals. This technique can be applied in situ as well as ex situ (Yao et al. 2012).

5. *Ball mill process*: Soil sample along with grinding media is added to the reactor (i.e., mill pot). In the absence of any chemical agents, the grinding process removes the contaminants and maintains the soil property as well (Shin et al. 2016).
6. *Subcritical water extraction process*: Instead of organic solvent, superheated water is used as a solvent. The water is heated at a pressure less than 22.1 MPa and temperature range of 100–374 °C. It follows the principle of pressurized liquid extraction (PLE) (Islam et al. 2013).

5.2.3 Biological Remediation

1. *Phytoremediation* is containment or removal of the contaminants by the use of green plants. Phytoremediation generally includes three processes: phytostabilization (adsorption, reduction, and precipitation of the pollutants at the roots of the plants), phytovolatilization (converting pollutant to a gaseous state), and phytoextraction (tolerant and accumulating plants are used) (Yao et al. 2012).
2. *Bio-augmentation* is the introduction of microorganisms at the contaminated site. The microbes are generally added to such areas where the microbes that can degrade the pollutants are in a low amount (autochthonous microorganisms) or the population present at the site does not have the catabolic pathway to degrade the pollutants (Yao et al. 2012).
3. *Biosorption*: Biomass (containing inactive, dead microbes) can bind to heavy metals and concentrate them. First, physical adsorption at the cell surface occurs, and then the metal ions gain access to the cytoplasm via the cell membrane. The type of microorganisms used defines the type and quantity of metal binding on them (Yao et al. 2012).

5.3 Traditional Tools of Omics

Specific genes (often 16s rRNA gene) were cloned in early environmental gene sequencing to produce a microbial diversity profile unlike microbial genome sequencing and traditional microbiology which depends upon cultivated cultures. The inherent soil microbial functions are nutrient recycling along with essential elements, the formation of organic matter, and decomposition aiding the natural process of soil bioremediation (Garbeva et al. 2004). The microbial world primarily constitutes of the organisms that are already cultured consisting of 1% of the overall soil microbial community. Using culture-based techniques, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Firmicutes* are the most governing phyla isolated from soil (Schloss and Handelsman 2004). The viable and nonculturable

microorganisms inherently propagate in habitual environments, but then they are dormant in the laboratory or artificial surroundings. Standard culture-based approaches cannot culture these organisms, but they embrace a copious standing in the ecosystem. Hence lipids, nucleic acids, or proteins were used from the soil samples for direct assessment of their function to overcome this problem. Familiar genes such as ITS, 18S rRNA, and 16S rRNA are used as a biomarker for identification of microbial community population in the culture-independent techniques. Hence for an enhanced phylogenetic and functional categorization of the microbial community in the soil, amalgamation of specific molecular tools such as genetic fingerprinting, quantitative PCR, fluorescence in situ hybridization technique (FISH), microbial lipid analysis, stable isotope probing, microradiography, clone library method, and DNA microarray has been developed to understand the interaction of the microorganisms with various natural factors in the soil microenvironment.

Genetic fingerprinting techniques perform the direct analysis of specific molecular biomarker genes using their amplified PCR products. The relationship between diverse communities of microbes is studied using cluster-assisted analysis which compares fingerprints from various samples using software such as GelCompar. Temperature gradient gel electrophoresis and denaturing gradient gel electrophoresis (TGGE/DGGE), single-stranded conformation polymorphism (SSCP), random amplified polymeric DNA (RAPD), terminal restriction fragment length polymorphism (T-RFLP), ribosomal intergenic spacer analysis (RISA), amplified ribosomal DNA restriction analysis (ARDRA), and length heterogeneity PCR (LH-PCR) are the most prominent techniques used in genetic fingerprinting. Multiple samples are evaluated at a glance through a generated community fingerprint based on sequence polymorphism.

Amplified ribosomal DNA fragments get separated using DGGE and TGGE. Identical length DNA fragments get separated based on their variable and nucleotide composition. At the 5′ end, a GC-rich primer prevents the thorough alienation of the PCR fragments. The illustration of a solo species by several bands is the constraint associated with this method (Dowd et al. 2008). The solicitation of traditional omic tools along with their sampling source is presented in Table 5.1. Using this technique, analysis of rhizospheric bacterial populations and assessment of the microbial soil community have been done in paddy agricultural soils in recent times (Srivastava et al. 2016; Schloter et al. 2018). RAPD, due to its high speed and ease of use, is considered a simpler technique for the assessment of inherently allied bacterial species, functional and structural interpretation of the microbial communities in the soil, and genetic fingerprinting. Synthetic oligonucleotide primers having random nucleotide sequences are annealed at multiple locations on the genomes at low temperature. Assessment of laboratory-scale biodegradation of fuel oil-contaminated soil has been studied using this technique (Piñón-Castillo et al. 2017). SSCP uses the principle of separation of a single-stranded DNA by electrophoresis. T-RFLP uses fluorescently labeled 5′ primers. Advanced throughput analysis and evaluation of numerous assorted samples at a lone time is the major advantage of this technique. Using bacterial 16S rRNA or 18S rRNA gene amplicon and fungal communities to breed restriction fragment contours, ARDRA serves as

Table 5.1 Application of traditional omic approaches in soil bioremediation

Traditional tools	Application	Sample	References
DGGE/TGGE	Rhizoremediation using GMO and its effect on different bacterial communities	PCB-contaminated soils	De Cárcer et al. (2007)
	Identification of compositional and structural changes in fungal and bacterial communities under different autotoxin concentrations	Cucumber seedling from agricultural soils	Zhou and Wu (2012)
	Evaluation of change in environmental conditions in carbon dioxide-rich volcanic vents	High CO_2 concentrated sites at Laacher See	Frerichs et al. (2013)
	Analysis and diversity assessment of rhizospheric bacterial population	Paddy soil	Srivastava et al. (2016)
	Assessment of soil quality using microbial indicators	Agricultural soil	Schloter et al. (2018)
	Analysis of degradation assessment of polycyclic aromatic compounds	Agricultural land around gas plants	Shahsavari et al. (2019)
RAPD	Evaluation of the soil microbial genetic structure by soil microbial community analysis	Multisampling points of rhizospheric and nonrhizospheric soils where *Panax ginseng* had been grown for 3 years	Li et al. (2010)
	To determine the genetic fidelity of micro-propagated plants	*Eclipta alba*, a medicinally important plant	Singh et al. (2012)
	Analysis of induced heavy metal *Hibiscus rosa-sinensis*	*Hibiscus rosa-sinensis* plant	Bhaduri and Fulekar (2015)
	To determine the laboratory-scale biodegradation by autochthonous bacteria	Fuel oil-contaminated soil	Piñón-Castillo et al. (2017)
	Viral and bacterial community responses to subsurface Fe (III) reduction	Subsurface soil	Liang et al. (2019)
SSCP	Distribution and diversity of polyhydroxyalkanoate-producing bacteria	Agricultural soil	Gasser et al. (2009)
	To analyze rhizosphere and fungal diversity	Canary Islands	Zachow et al. (2009)
	Phylogenetic studies of soil microbial communities	Urban storm water sedimentary layer	Badin et al. (2012)

(continued)

Table 5.1 (continued)

Traditional tools	Application	Sample	References
	Rapid profiling of soil microbial communities	Agricultural topsoil	Stefanis et al. (2013)
	Analysis of microbial diversity	Landslide soil	Guida et al. (2014)
	Assessment of soil microbial community and impact on agricultural land management	Agricultural soil, Mediterranean region	Bevivino and Dalmastri (2017)
	Hybridization in the *Thaumetopoea pityocampa-wilkinsoni* complex	Pine soil samples	Petrucco-Toffolo et al. (2018)
	Analysis of microbial community diversity in contaminated soil	Agricultural soil	Panigrahi et al. (2019)
T-RFLP	Evaluation of bacterial microbial diversity between two pinyon rhizosphere soils samples and two tree interspace soil samples	Pinyon rhizospheric soil and interspace soil samples	Dunbar et al. (1999)
	Compositional change in response within bacterial communities to AM extraradical mycelia in artificial conditions	Arbuscular mycorrhizal infected plants on bulk soil samples	Toljander et al. (2007)
	Yield determination of oilseed rape monocultures	Rhizospheric and bulk agricultural soils	Hilton et al. (2013)
	Myxomycetes' characterization in two different soil samples	Agricultural soil	Hoppe and Schnittler (2015)
	Comparison of microbial communities under Cacao agroforestry, Peru	Tropical soil, Peru	Buyer et al. (2017)
	Acetogenic contribution to anaerobic degradation in rice field soils	Rice field soils	Fu et al. (2018)
	Assessment of microbial diversity	Rhizospheric soil, Trindade Island, Brazil	Camacho-Montealegre et al. (2019)
ARDRA	Inoculation of PGPB strain between mother and daughter strawberry plants via stolon	Bulk soils growing strawberry plants	Guerrero-Molina et al. (2012)
	Assessment of microbial diversity in different soils	Pepper-grown field	Lee et al. (2006)

(continued)

Table 5.1 (continued)

Traditional tools	Application	Sample	References
	Isolation of clusters of soil and identification of isolates having antagonistic properties against *Phytophthora capsici*	Soils from glacial area	Yang et al. (2012a, b)
	Assessment, development, and evaluation of a biopile for remediation of soil	Hydrocarbon-contaminated soil	Baldan et al. (2015)
	Comparison of bacterial diversity for forensic applications	Agricultural soil	Habtom et al. (2017)
	Analysis of nitrogen-fixing bacterial community during the rice-growing season	Agricultural soil	Chakraborty and Islam (2018)
LH-PCR	Differentiation of soil bacterial community structure	Two different soil types with conventional and continuous grass plots	Ritchie et al. (2000)
	Characterization of phylotypes in soil fungal communities	Tomato-cultivated agricultural soils	Wu et al. (2008)
	Assessment of soil microbial properties by fecal detritus interaction	Pasture soil	Slade et al. (2016)
	Analysis of nitrogen cycling during decomposition by vertebrates	Forest soil	Keenan et al. (2018)
	Assessment of bioremediation potential of contaminated soil	Municipal waste-dumped soil	Awasthi et al. (2019)
RISA	To determine the effect on the structure of the rhizobacterial community in field-grown maize by PGPR *Azospirillum lipoferum* CRT1	Maize-grown agricultural topsoil	Baudoin et al. (2009)
	Estimation of species richness from multiple samples in different environments	Decatur silt loam soil	Mathew et al. (2012)
	Analysis of soil microbial communities	Agricultural soil	Navarro et al. (2015)
	Analysis of microbial community structure and diazotrophic abundance	Paddy soil	Srivastava and Mishra (2018)

(continued)

Table 5.1 (continued)

Traditional tools	Application	Sample	References
	Characterization of microbial communities in industrial soil	Industrial soil	Shekhar et al. (2020)
Quantitative PCR	Evaluation of bacterial diversity in an acidified soil by 16s rDNA analysis	Acid forest soil, Mt. Coot-Tha, Brisbane, Australia	Stackebrandt et al. (1993)
	Determination of abundant population of bacteria and specific methanotrophic groups	Flooded rice fields	Kolb et al. (2003)
	Evaluation of common microbial taxonomic groups using taxon-specific real-time primers	Agricultural soil	Fierer et al. (2005)
	Quantitative detection of sulfate reducers, methane oxidizers, and ammonia oxidizers by targeting dsrA, pmoA, and amoA genes	Saline and hypersaline soda lakes	Foti et al. (2007)
	Effect on the ammonia-oxidizing activity by bacterial communities in the rhizosphere of a fluvo-aquic soil by long-term fertilization	Fluvo-aquic soil	Ai et al. (2013)
	Assessment of soil acidobacterial communities	Amazon forest soil and soybean croplands	Navarrete et al. (2013)
	Analysis of soil microbial diversity and abundance of relic DNA	Agricultural soil	Carini et al. (2017)
	Quantification of *Fusarium* species in the root rot complex	Field pea soil	Zitnick-Anderson et al. (2018)
	Assessment of microbial respiration by digestate application	Weathered petroleum hydrocarbon-contaminated soil	Gielnik et al. (2019)
FISH	Evaluation of different microbial communities and its phylogenetics and diagnostics	Bulk agricultural soils	Moter and Göbel (2000)
	Detection of live bacteria in *Arabidopsis thaliana*'s root segments by CARD-FISH	*Arabidopsis thaliana* plants grown in natural soil conditions	Lundberg et al. (2012)
	Attenuation of complex tar oil in soil	Tar oil-laden soil	Ivanov et al. (2017)

(continued)

Table 5.1 (continued)

Traditional tools	Application	Sample	References
	Physiochemical and microbial analysis of dumpsite soil	Dumpsite soil	Oshoma et al. (2017)
	Analysis of microbial bioremediation of pollutants	Agricultural soil	Bharagava et al. (2019)
Microbial lipid analysis	Assessment of soil microbes using lipid extraction	Agricultural soil	Oates et al. (2017)
	Analysis of microbial nematodes in aggregates of soil	Red soil	Jiang et al. (2018)
Stable isotopic probing	Investigation of groundwater to characterize *Pseudomonas* species which degrade naphthalene	High pseudomonas population in groundwater microcosms	Huang et al. (2007)
	Detection of spatial variation within active microorganisms in relation to rhizospheric carbon flow	Rice microcosms in rhizospheric soils	Lu et al. (2007)
	Identification and assessment of *Planctomycetes* of a complex heteropolysaccharides	Agricultural soil	Wang et al. (2015)
	Analysis of active diazotrophs	Agricultural soil	Angel et al. (2018)
Microradiography	Characterization of autotrophic nitrifying bacteria in biofilms	Nitrogen-rich soils	Okabe et al. (2004)
Clone library	Characterization of microbial diversity in subsurface mining-affected soils	South Dakota, USA	Rastogi et al. (2009)
	Assessment of carbon fixation rates and bacterial diversity	Agricultural soil, China	Lynn et al. (2017)
	Identification of *Bacillus* community in Ararat plain, Armenia	Saline alkaline soil	Panosyan et al. (2018)
	Identification of heavy metal hyper-tolerant eukaryotic aldehyde dehydrogenase	Metal-contaminated soil	Mukherjee et al. (2019)

the most crucial tool in the process of unique clone identification obtained from the environment. Analysis of nitrogen-fixing bacterial communities in peak rice-growing season has been done using this technique (Chakraborty and Islam 2018). RISA analyzes the phylogenetic diversity of the microbes built on the intergenic length adjustment in the transcribed spacer region within the 23S and 16S genes for prokaryotes and 23S and 18S encoding rRNA genes for eukaryotes (Fuhrman et al. 2008). Quantitative PCR determines the manifestation and plethora of operative and taxonomic gene markers in the exploration of soil microbial communities (Bustin et al. 2005). SYBR green fluorescent dyes or fluorescent probes measure the accumulated amplicons in each cycle of PCR. Quantification of *Fusarium* species in root rot complex in field pea soil has been studied using quantitative PCR (Zitnick-Anderson et al. 2018). The conjugation of a fluorochrome with an oligonucleotide probe is the basic principle behind FISH. Due to its immense genetic stability and high copy number, probes of 16S rRNA are conventionally exploited in this technique. On hybridizing the homologous sequence to its fluorescent probe, the fluorescent intensity is measured using a fluorescent microscope. Lipids as opposed to nucleic acids are used in microbial lipid analysis for the assessment of soil microbial communities (Banowetz et al. 2006). The biomass of a cell has a constant amount of fatty acids which is stable in nature and gives a clear differentiating picture between the different taxonomic populations of microbes. Extraction of fatty acids is done using saponification and derivatization, generating FAMEs which are further analyzed through gas chromatography. The radiolabelled substrate is used in microradiography by metabolic active cells. Combining microradiography with FISH identifies the phylogenetic active cells which are radioactive in nature and consume the substrate (Rogers et al. 2007). The individual gene fragments are sequenced following cloning in the clone library method. The PCR-obtained sequences are compared to green gene, ribosomal data projects, and gene banks (DeSantis et al. 2007). Characterization of the microorganisms in environmental samples is also done using DNA microarrays. DNA from the environmental samples generates fluorescently labeled amplified PCR products which are directly hybridized to the microarrays having known sequence molecular probes (Gentry et al. 2006). The overall evaluation process is enhanced due to the rapid replication by the DNA microarray, aiding as a significant advantage. The intensity of the signal on the microarray is directly related to the ampleness of the target organism in the sample. Besides having enhanced responses, these conventional techniques also have their specific limitations. However, with the integration of advanced omic tools, the process of microbial community analysis has been undergoing an enhanced revolution with improved understanding and application to decipher the role of microbial communities in soil bioremediation.

5.4 Advanced Omic Tools

The DNA-based molecular techniques do not provide detailed information about gene expressions under in situ conditions. Hence sequences within the metagenomic databases from uncultured microbes provide fruitful insight into the functional microbial diversity. Therefore, post-genomic methodologies such as metagenomics, metatranscriptomics, proteogenomics, and metaproteomics provide a potential connection among the genetic and functional resemblances between numerous communities of microbes, hastening the process of soil bioremediation.

The direct collection of microbial genomes from ecological samples is known as metagenomics, community genomics, or environmental genomics (Riesenfeld et al. 2004). The communication of uncultured organisms with altered environmental factors and their biochemical role can hence be studied using metagenomics. Using functional metagenomic libraries, various functional molecules such as microbial enzymes (lipase, amylase, cellulase) and antibiotics, by companies such as Terragen are derived (Rondon et al. 2000). In the aerobic conditions, the acid sulfate soil microbial community was characterized to study the structural and functional genes, using the tools of metagenomics. Both the topsoil and parent materials underwent significant changes on incubating in aerobic conditions. The archaeal community significantly decreased, whereas the sulfur-cycling genes enhanced in the parent material (Su et al. 2017). However, at the genetic level, the relationship between community composition and taxonomic diversity remains to be determined.

Metatranscriptomics or environmental transcriptomics is the study of the variations in the microbial expression of genes under specific conditions by capturing the total mRNA (Moran 2009). Recently, the overall phylogenetic pool of the functionally and taxonomically appropriate microbial communities has been analyzed using the double-RNA method or both the rRNA and mRNA, noticing a considerable diversity among the microbial communities. Recently, through direct species electron transfer, the interaction between *Methanothrix* and *Geobacter* was studied in paddy soils under methanogenic terrestrial environments. *Methanothrix* is the prominent microbial contributor in the global methane production, but very little is known about its physiology and ecology. The transformation of methane from acetate serves as an important contribution by *Methanothrix* in terrestrial ecosystems (Holmes et al. 2017). Nitrogen-transforming reductive gene transcripts were identified through metatranscriptomics in waterlogged paddy soils. Reductive nitrogen transformation was actively induced due to the presence of anoxic environments (Masuda et al. 2017). Using rice straw as a source of carbon, the severity of seawater salinity on paddy fields was studied to observe the significant changes in mRNA expression throughout the whole community (Peng et al. 2017). The diversity of microorganisms is crucially analyzed using metatranscriptomics, elucidating the community composition and deciphering their potential in soil bioremediation.

Metaproteomics or environmental proteomics is the qualitative and quantitative study of proteins on a large scale of diverse microbial species (Wilmes and Bond 2006). Under stressful conditions, proteofingerprints are generated to indicate the functional status of microbial societies (Keller and Hettich 2009). In recent times,

metaproteomics has been exercised in various environments such as sediments, soil, freshwater, and marine systems. However incorrect metagenomic information, flush variety of microbial organisms, and soil heterogeneity have a negative influence on the process described (Wang et al. 2016). The bacterial metabolic functions correlated with a plant in serpentine soil contaminated with nickel, cobalt, and chromium were also interpreted using metaproteomic approach. (Mattarozzi et al. 2017). A bacterial protein database was constructed through the genera identified using 16S DNA profiling. A continuum of bacteria is revealed by the proteins involved in response to stimulus and transportation of nutrients. The bacterial biocatalysts play a vital part in the evolution of a post-petroleum bio-based economy, but the difficulty in analyzing the genetic information limits the biocatalyst's capability (Sukul et al. 2017). The bioactivity from the proteome of an environmental sample can hence be efficiently analyzed using functional metaproteomics. Hence biomass quantification in metaproteomics serves as an important tool to interpret the correlation between various soil microbial communities in a sustainable environment.

Metabolomics characterizes the response of soil microbial communities to specific biological factors, abiotic pressures, and its immediate environment. Organic, low molecular weight biomolecules occurring naturally in a cell, tissue, or biofluid are known as metabolites. A comprehensive study of metabolomics is the production of a range of metabolites or metabolomes in communication with a natural environmental stimulation (Miller 2007). Recently, metabolomics has had numerous prominent applications in the field of environmental sciences such as the development of biomarkers, feedback to altered levels of environmental stress, assessment of risks on toxicant exposure, and disease monitoring and diagnosis (Viant 2009). Prominent deviations were observed in the metabolic pathways of *S. meliloti.* Simultaneously this could be related to other bacteria experiencing a series of abiotic pressure. Some of the noticeable modification changes were the presence of intermediates in myo-inositol degradation and changes in the biosynthesis of exopolysaccharides and pentose phosphate pathway (PPP). In accordance to metabolic adaptation, enhanced acid tolerance phenotypes and improved competitiveness in nodulation are associated with the same in rhizobia (Draghi et al. 2017). Osmotic adjustment and osmoprotection were calculated in the classical species of cowpea (Goufo et al. 2017). The organic solutes in plants uphold an optimal turgor by enacting as osmolytes or as radical scavengers to protect the metabolic functions and hence survive the extreme drought conditions. By analyzing the salt-tolerant mechanisms and the metabolic profile of plants, enhanced sustainable crop production can be achieved worldwide, leading to the development and protection of natural plant reserves and hence aiding the bioremediation potential of the soil microbial community.

5.5 Application of Omic Tools in Bioremediation

Microorganism utilizes organic compounds as a sole carbon source and to manage their biomass and assemble suitable enzymes and cofactors for their oxidation/reduction. Hence, the organic compounds should be nontoxic or less damaging to microbial growth. The microorganisms participating in the metabolic degradation of organic compounds are heterotrophic. Molecular methods like cloning, fingerprinting, ARISA, RFLP, etc. are used to study microbial diversity (Fig. 5.3). These techniques yield information on how environmental factors change the microbial community structure. More advanced techniques like Illumina and 454 sequencing are also being used to study the microbial diversity of the polluted areas. Different approaches are used to remediate contaminated soils (Yergeau et al. 2014). The present scenario includes the implementation of various omic tools (Table 5.2) to study the microbial diversity of the contaminated soil with that of uncontaminated soil, thus providing better insight for the development of the new remediation technique or improving the already existing methods.

The uptake of heavy metals like mercury can lead to biomagnification. The heavy metals interrupt with the energy metabolism of the plants. Transcriptomics helps in the early detection at molecular levels. The changes in the genes in the presence of a low and high concentration of metals can also be studied (Villiers et al. 2012;

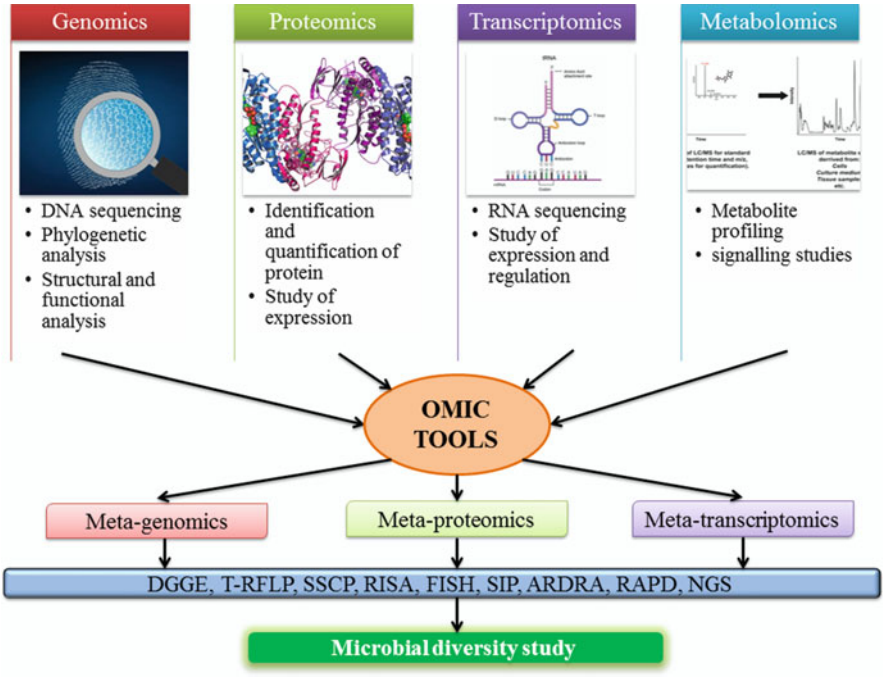


Fig. 5.3 Application of omic tools in soil bioremediation: a conceptual framework

Table 5.2 Application of advanced omic approaches in soil bioremediation

	Contaminants	Omic tools	Applications	References
Biotransformation	Mercury	Transcriptomics	Analyzing metabolic pathway and tolerance response	Beauvais-Flück et al. (2017)
	Arsenic	Transcriptomics, proteomics, metabolomics	Analysis of transport and accumulation as in plant	Tripathi et al. (2012)
	Zinc	Proteomics	Differentially expressed proteins in *Arabis paniculata*	Zeng et al. (2011)
	Cadmium	Proteomics, transcriptomics	The interaction between *Arabidopsis halleri* and selected bacterial strains	Farinati et al. (2011)
			Study the response of the plant to cadmium, viz., high-throughput techniques	Villiers et al. (2012)
		Metabolomics	Analysis of metabolic and growth profile on cadmium-contaminated tomato plants	Hediji et al. (2010)
			Study of the response of cadmium exposure by *Arabidopsis thaliana*	Sun et al. (2010)
	Nickel	Proteomics	Phenotype analysis after Ni hyperaccumulator In *Noccaea caerulescens* subsp. *caerulescens*	Visioli et al. (2012)
	Trace metals	Metagenomics	The decrease in microbial diversity of arbuscular mycorrhizal fungi found in contaminated soil	Hassan et al. (2011)
	Cadmium and zinc	NSG	Pyrosequencing revealed the interaction between *Arabidopsis halleri* and the microbial community	Muehe et al. (2015)
	Copper	Genomics	DGGE analysis to study the microbial diversity of contaminated and uncontaminated soil	Altimira et al. (2012)
	Uranium, nickel, cobalt, cadmium	Genomics	To determine the genomic sequence of *Caulobacter* sp. strain OR37	Utturkar et al. (2013)
Biodegradation	Hydrocarbon, pesticides, herbicides	Metagenomics	Transgenic plants that contain transgenes which either metabolize the xenobiotic or increase the resistance toward the pollutant	Abhilash et al. (2009)

			Analysis of anaerobic degradation of quinoline by denitrifying bacteria using metagenomics	Wang et al. (2017)
			Anaerobic degradation of hydrocarbons using metagenomic analysis	Espínola et al. (2018)
			To test the PAH removal potential by indigenous bacteria from Taean coast, Korea	Lee et al. (2018)
			Degradation of herbicides and pesticides by identifying a novel gene	Jayaraman et al. (2019)
	Fertilizers	Metagenomics	Shotgun metagenomic sequencing reveals a shift in the pathways of the microbes	Fierer et al. (2012)
			Analysis of organically fertilized zoo soil using metagenomics	Meneghine et al. (2017)
	Hydrocarbons	Bacterial modifications	Saline environment results in biodegradation	Le Borgne et al. (2008)
		Proteomics	Partially explains the changes that occur in the soil microbial diversity	Bastida et al. (2010)
			Proteomic characterization of plasmid PLA1 and its biodegradation potential of degrading polycyclic aromatic compounds	Yun et al. (2014)
			Proteomic analysis of the biodegradation of cyanide wastes	Luque-Almagro et al. (2016)
			Analysis of pyrene degradation by *Brevibacillus brevis*	Wei et al. (2017)
			Biodegradation and structural analysis of aniline degrading bacteria	Hou et al. (2018)
			Assessment of microbial function and diversity in petroleum-associated environments	Pal et al. (2019)
		Genomics	Using DGGE and 16S rRNA analysis, the microbial diversity of the forest soil was studied	Ahn et al. (2006)
			16S rRNA study and phylogenetic analysis were used to study the microbial community	Hamamura et al. (2006)

(continued)

Table 5.2 (continued)

	Contaminants	Omic tools	Applications	References
			Genomic tools were used to study the remediation effect of different plants was assayed via Phytoremediation	Phillips et al. (2006)
			DGGE and phylogenetic analysis revealed a shift in the microbial diversity	Labbé et al. (2007)
			The phylogenetic and biogeographic diversity of thermoacidophilic cyanidiales were studied	Toplin et al. 2008
			The contaminated and uncontaminated soils were assayed for the microbial differences	Vivas et al. (2008)
			DNA probe labeling and pyrosequencing were incorporated to study the pathway of hydrocarbon-degrading genes	Bell et al. (2011)
			The PCR-DGGE analysis was used to assess the total petroleum hydrocarbon (TPH)	Maqbool et al. (2012)
			Using RT-qPCR, the hydrocarbon degrading genes were studied	Yergeau et al. (2012)
			Monitoring and detection of genes that lead to aromatic and aliphatic degradation by oligonucleotide microarray method	Kim et al. (2014)
			Subtraction of cDNA revealed presence of zinc finger motifs in the high accumulators of organic pollutants	Inui et al. (2015)
			PCR-DGGE was used to assay the better of the two bioremediation techniques applied	Khudur et al. (2015)
			Genomic analysis and enrichment of root endophytic bacteria from *Populus deltoides*	Utturkar et al. (2016)
			Analysis of pyrene degradation by bacterial consortia	Wanapaisan et al. (2018)

			Analysis of biodegradation potential of *Rhodococcus* strain	Zampolli et al. (2019)
		Functional Metaproteome	Analysis of the biochemical pathways of the microbial communities	Benndorf et al. (2007)
			The analysis of PAH degradation by bacteria in terrestrial and marine habitats	Grube et al. (2015)
			Characterization of phenanthrene-degrading bacterial consortia and assignment of their ecological roles	Festa et al. (2017)
			Analysis of microbial community structure using rhizoremediation	Kotoky et al. (2018)
		Next-generation sequencing	Pyrosequencing helped to study the relationships among soil properties, pollution rate, and microbial diversity	Sutton et al. (2012)
			454 sequencing evaluates the microbial community with the oil spill	Yang et al. (2012a, b)
			Ion torrent and Illumina sequencing revealed high expression of hydrocarbon-degrading genes in the contaminated soil	Yergeau et al. (2014)
			Analysis of marine microbial communities in crude oil-contaminated water	Krolicka et al. (2017)
			Bacterial diversity analysis in heavy oil well reservoir	Shibulal et al. (2018)
			Microbial distribution analysis in PAH-contaminated landfill soil	Koshlaf et al. (2019)
		Transcriptomics	Analysis of biomass degradation by anaerobic fungal isolate	Couger et al. (2015)
			Analysis of polysaccharide-degrading potential by bacteria in arctic sea sediments	Rapp et al. (2016)
			Analysis of alkane degradation by *Pseudomonas extremaustralis*	Tribelli et al. (2018)

(continued)

Table 5.2 (continued)

	Contaminants	Omic tools	Applications	References
			Analysis of drainage effect on paddy soil microbiome	Abdallah et al. (2019)
		Metabolomics	Analysis of biodegradation potential of the bacterial population in contaminated crude oil	Bargiela et al. (2015)
			Study of biodegradation of azo dye by bacterial consortia	Shanmugam et al. (2017)
			Analysis of biodegradation of hydrocarbons anaerobically	Gieg and Toth (2018)
			Analysis of nanomaterials in agricultural soil	Zhao et al. (2019)
	Herbicide	Metabolomics	Study of alachlor biodegradation by *Paecilomyces marquandii*	Szewczyk et al. (2015)

Beauvais-Flück et al. 2017). The tools of genomics like DGGE (denaturing gradient gel electrophoresis) of 16S rRNA enhance the study of several communities of microbes in non-polluted and polluted soils and, therefore, help in the isolation of the heavy metal-resistant bacterial strains (Altimira et al. 2012; Utturkar et al. 2013). The adaptation of any organisms to the surroundings is reflected in their biological activities which can be calculated by doing transcriptomics, proteomics, and metabolomics analysis. Thus, the techniques of the bioremediation can be enhanced, and the scope of remediation technique can be improved (Hediji et al. 2010; Tripathi et al. 2012). The bacterial soil community affects the uptake of metals by plants by either stimulating the plant growth or by metabolizing the heavy metals. Pyrosequencing, a next-generation tool, of 16S rRNA provides a better picture of plant-metal-microbe interaction in the soil (Muehe et al. 2015).

Phytoremediation is one of the cost-effective remediation techniques in use for years now. The purpose of plants to attenuate the xenobiotics makes them more feasible method than the physical and chemical processes. The transgenic plants result in either degradation of the xenobiotics or increased resistance of the plant to the pollutant (Abhilash et al. 2009). The industrial effluents are estimated to be 5% saline and hypersaline. Microbial diversity is less as compared to non-extreme environments. Thus degradation of the pollutant becomes a significant problem in such regions. The halophilic microorganisms are proposed to be a favorable applicant for the remediation of the hypersaline environments. Though the metabolization of hydrocarbon reduces at high salt concentration, a lengthy exposition period has shown a significant amount of degradation and metabolized hydrocarbons (Le Borgne et al. 2008). The genomic sequence analysis discloses the genes that might be involved in the degradation. Further, proteomic analysis of the microbe in the presence of different concentrations of hydrocarbons affirms the genes involved in the degradation (Yun et al. 2014; Wei et al. 2017). Application of techniques like RT-qPCR quantifies the expression of various hydrocarbon-degrading genes and thus provides an insight into the shift in the microbial communities (Yergeau et al. 2012).

5.6 Future Prospects

There have been unprecedented changes in the field of microbial ecology with the augmentation and advancement of numerous molecular-based omic tools. The inherent functional and taxonomical diversity of the innate communities of microbes present in the soil has been investigated using diverse post-genomic approaches, revealing the superficial knowledge about the metabolic and genetic heterogeneity present in the most copious organisms in the planet, known as the prokaryotes. Implicit questions, for instance, “How the physical, chemical, and biological factors regulate the microbial communities?” and “How many bacterial species are currently present in the planet?”, and the broad knowledge about the metabolic diversity in the elementally present microorganisms still remain to be understood. As most of the classified genes have no autologous sequences in the present databases, deciphering

the utilitarian roles of uncultured organisms has become an appalling task. Numerous technical challenges still remain to be overcome although there has been immense progress in the field of identification and classification of the intrinsic microbial communities in the soil by the application of proteogenomic, transcriptomic, and metagenomic approaches. New insights into the soil microbiology can be provided using interdisciplinary omic system technologies, revealing the interaction between proteins, genes, and environmental factors. Hence the upcoming years will see a prioritized area of research in the development of consequential multi-omics approaches.

References

Abdallah RZ, Wegner CE, Liesack W (2019) Community transcriptomics reveals drainage effects on paddy soil microbiome across all three domains of life. Soil Biol Biochem 132:131–142

Abhilash PC, Jamil S, Singh N (2009) Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics. Biotechnol Adv 27:474–488

Ahn JH, Kim MS, Kim MC, Lim JS, Lee GT, Yun JK, Kim TS, Kim TS, Ka JO (2006) Analysis of bacterial diversity and community structure in forest soils contaminated with fuel hydrocarbon. J Microbiol Biotechnol 16:704–715

Ai C, Liang G, Sun J, Wang X, He P, Zhou W (2013) Different roles of rhizosphere effect and long-term fertilization in the activity and community structure of ammonia oxidizers in a calcareous fluvo-aquic soil. Soil Biol Biochem 57:30–42

Altimira F, Yáñez C, Bravo G, González M, Rojas LA, Seeger M (2012) Characterization of copper-resistant bacteria and bacterial communities from copper-polluted agricultural soils of Central Chile. BMC Microbiol 12:193

Angel R, Panhölzl C, Gabriel R, Herbold C, Wanek W, Richter A, Eichorst SA, Woebken D (2018) Application of stable-isotope labelling techniques for the detection of active diazotrophs. Environ Microbiol 20:44–61

Awasthi AK, Li J, Pandey AK, Khan J (2019) An overview of the potential of bioremediation for contaminated soil from municipal solid waste site. In: Emerging and eco-friendly approaches for waste management. Springer, Singapore, pp 59–68

Badin AL, Mustafa T, Bertrand C, Monier A, Delolme C, Geremia RA, Bedell JP (2012) Microbial communities of urban stormwater sediments: the phylogenetic structure of bacterial communities varies with porosity. FEMS Microbiol Ecol 81:324–338

Baldan E, Basaglia M, Fontana F, Shapleigh JP, Casella S (2015) Development, assessment and evaluation of a biopile for hydrocarbons soil remediation. Int Biodeterior Biodegradation 98:66–72

Banowetz GM, Whittaker GW, Dierksen KP, Azevedo MD, Kennedy AC, Griffith SM, Steiner JJ (2006) Fatty acid methyl ester analysis to identify sources of soil in surface water. J Environ Qual 35:133–140

Bargiela R, Mapelli F, Rojo D, Chouaia B, Tornés J, Borin S, Richter M, Del Pozo MV, Cappello S, Gertler C, Genovese M (2015) Bacterial population and biodegradation potential in chronically crude oil-contaminated marine sediments are strongly linked to temperature. Sci Rep 5:11651

Bastida F, Nicolás C, Moreno JL, Hernández T, Garcia C (2010) Tracing changes in the microbial community of a hydrocarbon-polluted soil by culture-dependent proteomics. Pedosphere 20:479–485

Baudoin E, Nazaret S, Mougel C, Ranjard L, Moënne-Loccoz Y (2009) Impact of inoculation with the phytostimulatory PGPR Azospirillum lipoferum CRT1 on the genetic structure of the rhizobacterial community of field-grown maize. Soil Biol Biochem 41:409–413

Beauvais-Flück R, Slaveykova VI, Cosio C (2017) Cellular toxicity pathways of inorganic and methyl mercury in the green microalga Chlamydomonas reinhardtii. Sci Rep 7:8034

Bell TH, Yergeau E, Martineau C, Juck D, Whyte LG, Greer CW (2011) Identification of nitrogen-incorporating bacteria in petroleum-contaminated arctic soils by using [15N] DNA-based stable isotope probing and pyrosequencing. Appl Environ Microbiol 77:4163–4171

Benndorf D, Balcke GU, Harms H, Von Bergen M (2007) Functional metaproteome analysis of protein extracts from contaminated soil and groundwater. ISME J 1:224

Bevivino A, Dalmastri C (2017) Impact of agricultural land management on soil bacterial community: a case study in the Mediterranean area. In: Soil biological communities and ecosystem resilience. Springer, Cham, pp 77–95

Bhaduri AM, Fulekar MH (2015) Biochemical and RAPD analysis of Hibiscus rosa sinensis induced by heavy metals. Soil Sediment Contam 24:411–422

Bharagava RN, Purchase D, Saxena G, Mulla SI (2019) Applications of metagenomics in microbial bioremediation of pollutants: from genomics to environmental cleanup. In: Microbial diversity in the genomic era. Academic, Oxford, pp 459–477

Bustin SA, Benes V, Nolan T, Pfaffl MW (2005) Quantitative real-time RT-PCR–a perspective. J Mol Endocrinol 34:597–601

Buyer JS, Baligar VC, He Z, Arévalo-Gardini E (2017) Soil microbial communities under cacao agroforestry and cover crop systems in Peru. Appl Soil Ecol 120:273–280

Calvo C, Manzanera M, Silva-Castro GA, Uad I, González-López J (2009) Application of bioemulsifiers in soil oil bioremediation processes. Sci Total Environ 407:3634–3640

Camacho-Montealegre CM, Rodrigues EM, Tótola MR (2019) Microbial diversity and bioremediation of rhizospheric soils from Trindade Island-Brazil. J Environ Manag 236:358–364

Carini P, Marsden PJ, Leff JW, Morgan EE, Strickland MS, Fierer N (2017) Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nat Microbiol 2:16242

Chakraborty A, Islam E (2018) Temporal dynamics of total and free-living nitrogen-fixing bacterial community abundance and structure in soil with and without history of arsenic contamination during a rice growing season. Environ Sci Pollut Res 25:4951–4962

Couger MB, Youssef NH, Struchtemeyer CG, Liggenstoffer AS, Elshahed MS (2015) Transcriptomic analysis of lignocellulosic biomass degradation by the anaerobic fungal isolate Orpinomyces sp. strain C1A. Biotechnol Biofuels 8:208

Cunningham SD, Berti WR (1993) Remediation of contaminated soils with green plants: an overview. In Vitro Cell Dev Plant 29:207–212

De Cárcer DA, Martín M, Mackova M, Macek T, Karlson U, Rivilla R (2007) The introduction of genetically modified microorganisms designed for rhizoremediation induces changes on native bacteria in the rhizosphere but not in the surrounding soil. ISME J 1:215

DeSantis TZ, Brodie EL, Moberg JP, Zubieta IX, Piceno YM, Andersen GL (2007) High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. Microb Ecol 53:371–383

Dong ZY, Huang WH, Xing DF, Zhang HF (2013) Remediation of soil co-contaminated with petroleum and heavy metals by the integration of electrokinetics and biostimulation. J Hazard Mater 260:399–408

Dowd SE, Sun Y, Secor PR, Rhoads DD, Wolcott BM, James GA, Wolcott RD (2008) Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. BMC Microbiol 8:43

Draghi WO, Del Papa MF, Barsch A, Albicoro FJ, Lozano MJ, Pühler A, Niehaus K, Lagares A (2017) A metabolomic approach to characterize the acid-tolerance response in Sinorhizobium meliloti. Metabolomics 13:71

Dunbar J, Takala S, Barns SM, Davis JA, Kuske CR (1999) Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. Appl Environ Microbiol 65:1662–1669

Espínola F, Dionisi HM, Borglin S, Brislawn CJ, Jansson JK, Mac Cormack WP, Carroll J, Sjöling S, Lozada M (2018) Metagenomic analysis of subtidal sediments from polar and

subpolar coastal environments highlights the relevance of anaerobic hydrocarbon degradation processes. Microb Ecol 75:123–139
Farinati S, DalCorso G, Panigati M, Furini A (2011) Interaction between selected bacterial strains and Arabidopsis halleri modulates shoot proteome and cadmium and zinc accumulation. J Exp Bot 62:3433–3447
Festa S, Coppotelli BM, Madueño L, Loviso CL, Macchi M, Tauil RM, Valacco MP, Morelli IS (2017) Assigning ecological roles to the populations belonging to a phenanthrene-degrading bacterial consortium using omic approaches. PLoS One 12:e0184505
Fierer N, Jackson JA, Vilgalys R, Jackson RB (2005) Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl Environ Microbiol 71:4117–4120
Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J 6:1007
Foti M, Sorokin DY, Lomans B, Mussman M, Zacharova EE, Pimenov NV, Kuenen JG, Muyzer G (2007) Diversity, activity, and abundance of sulfate-reducing bacteria in saline and hypersaline soda lakes. Appl Environ Microbiol 73:2093–2100
Frerichs J, Oppermann BI, Gwosdz S, Möller I, Herrmann M, Krüger M (2013) Microbial community changes at a terrestrial volcanic CO2 vent induced by soil acidification and anaerobic microhabitats within the soil column. FEMS Microbiol Ecol 84:60–74
Fu B, Conrad R, Blaser M (2018) Potential contribution of acetogenesis to anaerobic degradation in methanogenic rice field soils. Soil Biol Biochem 119:1–10
Fuhrman JA, Steele JA, Hewson I, Schwalbach MS, Brown MV, Green JL, Brown JH (2008) A latitudinal diversity gradient in planktonic marine bacteria. Proc Natl Acad Sci 105:7774–7778
Gan S, Lau EV, Ng HK (2009) Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). J Hazard Mater 172:532–549
Garbeva PV, Van Veen JA, Van Elsas JD (2004) Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu Rev Phytopathol 42:243–270
Gasser I, Müller H, Berg G (2009) Ecology and characterization of polyhydroxyalkanoate-producing microorganisms on and in plants. FEMS Microbiol Ecol 70:142–150
Gentry TJ, Wickham GS, Schadt CW, He Z, Zhou J (2006) Microarray applications in microbial ecology research. Microb Ecol 52:159–175
Gieg LM, Toth CR (2018) Anaerobic biodegradation of hydrocarbons: metagenomics and metabolomics. In: Consequences of microbial interactions with hydrocarbons, oils, and lipids: biodegradation and bioremediation. Springer, Cham, pp 1–42
Gielnik A, Pechaud Y, Huguenot D, Cébron A, Riom JM, Guibaud G, Esposito G, van Hullebusch ED (2019) Effect of digestate application on microbial respiration and bacterial communities' diversity during bioremediation of weathered petroleum hydrocarbons contaminated soils. Sci Total Environ 670:271–281
Goufo P, Moutinho-Pereira JM, Jorge TF, Correia CM, Oliveira MR, Rosa EA, António C, Trindade H (2017) Cowpea (Vigna unguiculata L. Walp.) metabolomics: osmoprotection as a physiological strategy for drought stress resistance and improved yield. Front Plant Sci 8:586
Grube M, Cernava T, Soh J, Fuchs S, Aschenbrenner I, Lassek C, Wegner U, Becher D, Riedel K, Sensen CW, Berg G (2015) Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative omics. ISME J 9:412
Guerrero-Molina MF, Winik BC, Pedraza RO (2012) More than rhizosphere colonization of strawberry plants by Azospirillum brasilense. Appl Soil Ecol 61:205–212
Guida M, Cannavacciuolo PL, Cesarano M, Borra M, Biffali E, D'Alessandro R, De Felice B (2014) Microbial diversity of landslide soils assessed by RFLP and SSCP fingerprints. J Appl Genet 55:403–415
Habtom H, Demanèche S, Dawson L, Azulay C, Matan O, Robe P, Gafny R, Simonet P, Jurkevitch E, Pasternak Z (2017) Soil characterisation by bacterial community analysis for

forensic applications: a quantitative comparison of environmental technologies. Forensic Sci Int Genet 26:21–29
Hamamura N, Olson SH, Ward DM, Inskeep WP (2006) Microbial population dynamics associated with crude-oil biodegradation in diverse soils. Appl Environ Microbiol 72:6316–6324
Hassan SE, Boon E, Ma S-A, Hijri M (2011) Molecular biodiversity of arbuscular mycorrhizal fungi in trace metal-polluted soils. Mol Ecol 20:3469–3483
Hediji H, Djebali W, Cabasson C, Maucourt M, Baldet P, Bertrand A, Zoghlami LB, Deborde C, Moing A, Brouquisse R, Chaïbi W (2010) Effects of long-term cadmium exposure on growth and metabolomic profile of tomato plants. Ecotoxicol Environ Saf 73:1965–1974
Hilton S, Bennett AJ, Keane G, Bending GD, Chandler D, Stobart R, Mills P (2013) Impact of shortened crop rotation of oilseed rape on soil and rhizosphere microbial diversity in relation to yield decline. PLoS One 8:e59859
Holmes DE, Shrestha PM, Walker DJ, Dang Y, Nevin KP, Woodard TL, Lovley DR (2017) Metatranscriptomic evidence for direct interspecies electron transfer between Geobacter and Methanothrix species in methanogenic rice paddy soils. Appl Environ Microbiol 83:e00223–e00217
Hoppe T, Schnittler M (2015) Characterization of myxomycetes in two different soils by TRFLP-analysis of partial 18S rRNA gene sequences. Mycosphere 6:216–227
Hou L, Wu Q, Gu Q, Zhou Q, Zhang J (2018) Community structure analysis and biodegradation potential of aniline-degrading bacteria in biofilters. Curr Microbiol 75:918–924
Huang WE, Stoecker K, Griffiths R, Newbold L, Daims H, Whiteley AS, Wagner M (2007) Raman-FISH: combining stable-isotope Raman spectroscopy and fluorescence in situ hybridization for the single cell analysis of identity and function. Environ Microbiol 9:1878–1889
Inui H, Hirota M, Goto J, Yoshihara R, Kodama N, Matsui T, Yamazaki K, Eun H (2015) Zinc finger protein genes from Cucurbita pepo are promising tools for conferring non-Cucurbitaceae plants with ability to accumulate persistent organic pollutants. Chemosphere 123:48–54
Islam MN, Jo YT, Jung SK, Park JH (2013) Evaluation of subcritical water extraction process for remediation of pesticide-contaminated soil. Water Air Soil Pollut 224:1652
Ivanov P, Eickhorst T, Wehrer M, Georgiadis A, Rennert T, Eusterhues K, Totsche KU (2017) Natural attenuation of aged tar-oil in soils: a case study from a former gas production site. InEGU General Assembly Conference Abstracts 19:8586
Jayaraman S, Thangaiyan S, Mani K, Nagarajan K, Muthukalingan K (2019) Identification of a novel gene through the metagenomic approach to degrade the targeted pollutant. Microb Biodegrad Xeno Comp 30:204
Jiang Y, Qian H, Wang X, Chen L, Liu M, Li H, Sun B (2018) Nematodes and microbial community affect the sizes and turnover rates of organic carbon pools in soil aggregates. Soil Biol Biochem 119:22–31
Karigar CS, Rao SS (2011) Role of microbial enzymes in the bioremediation of pollutants: a review. Enzyme Res. https://doi.org/10.4061/2011/805187
Keenan SW, Schaeffer SM, Jin VL, DeBruyn JM (2018) Mortality hotspots: nitrogen cycling in forest soils during vertebrate decomposition. Soil Biol Biochem 121:165–176
Keller M, Hettich R (2009) Environmental proteomics: a paradigm shift in characterizing microbial activities at the molecular level. Microbiol Mol Biol Rev 73:62–70
Khudur LS, Shahsavari E, Miranda AF, Morrison PD, Nugegoda D, Ball AS (2015) Evaluating the efficacy of bioremediating a diesel-contaminated soil using ecotoxicological and bacterial community indices. Environ Sci Pollut Res 22:14809–14819
Kim S, Krajmalnik-Brown R, Kim JO, Chung J (2014) Remediation of petroleum hydrocarbon-contaminated sites by DNA diagnosis-based bioslurping technology. Sci Total Environ 497:250–259
Kolb S, Knief C, Stubner S, Conrad R (2003) Quantitative detection of methanotrophs in soil by novel pmoA-targeted real-time PCR assays. Appl Environ Microbiol 69:2423–2429

Koshlaf E, Shahsavari E, Haleyur N, Osborn AM, Ball AS (2019) Effect of biostimulation on the distribution and composition of the microbial community of a polycyclic aromatic hydrocarbon-contaminated landfill soil during bioremediation. Geoderma 338:216–225

Kotoky R, Rajkumari J, Pandey P (2018) The rhizosphere microbiome: significance in rhizoremediation of polyaromatic hydrocarbon contaminated soil. J Environ Manag 217:858–870

Krolicka A, Boccadoro C, Nilsen MM, Baussant T (2017) Capturing early changes in the marine bacterial community as a result of crude oil pollution in a Mesocosm experiment. Microbes Environ 2:358–366

Labbé D, Margesin R, Schinner F, Whyte LG, Greer CW (2007) Comparative phylogenetic analysis of microbial communities in pristine and hydrocarbon-contaminated alpine soils. FEMS Microbiol Ecol 59:466–475

Le Borgne S, Paniagua D, Vazquez-Duhalt R (2008) Biodegradation of organic pollutants by halophilic bacteria and archaea. J Mol Microbiol Biotechnol 5:74–92

Lee DW, Lee H, Lee AH, Kwon BO, Khim JS, Yim UH, Kim BS, Kim JJ (2018) Microbial community composition and PAHs removal potential of indigenous bacteria in oil contaminated sediment of Taean coast, Korea. Environ Pollut 234:503–512

Lee MS, Do JO, Park MS, Jung S, Lee KH, Bae KS, Park SJ, Kim SB (2006) Dominance of Lysobacter sp. in the rhizosphere of two coastal sand dune plant species, Calystegia soldanella and Elymus mollis. Anton Leeuw Int J G 90:19–27

Li Y, Ying Y, Zhao D, Jin S, Ding W (2010) Genetic diversity analysis on rhizosphere soil microbial population of Panax ginseng and Panax quinquefolium by RAPD. Zhongcaoyao = Chin Tradit Herb Drug 41:1871–1875

Liang X, Zhuang J, Löffler FE, Zhang Y, DeBruyn JM, Wilhelm SW, Schaeffer SM, Radosevich M (2019) Viral and bacterial community responses to stimulated Fe (III)—bioreduction during simulated subsurface bioremediation. Environ Microbiol 21:2043–2055

Lu Y, Abraham WR, Conrad R (2007) Spatial variation of active microbiota in the rice rhizosphere revealed by in situ stable isotope probing of phospholipid fatty acids. Environ Microbiol 9:474–481

Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, Del Rio TG, Edgar RC (2012) Defining the core Arabidopsis thaliana root microbiome. Nature 488:86

Luque-Almagro VM, Moreno-Vivián C, Roldán MD (2016) Biodegradation of cyanide wastes from mining and jewellery industries. Curr Opin Biotechnol 38:9–13

Lynn TM, Ge T, Yuan H, Wei X, Wu X, Xiao K, Kumaresan D, Wu J, Whiteley AS (2017) Soil carbon-fixation rates and associated bacterial diversity and abundance in three natural ecosystems. Microb Ecol 73:645–657

Mao X, Jiang R, Xiao W, Yu J (2015) Use of surfactants for the remediation of contaminated soils: a review. J Hazard Mater 285:419–435

Maqbool F, Wang Z, Xu Y, Zhao J, Gao D, Zhao YG, Bhatti ZA, Xing B (2012) Rhizodegradation of petroleum hydrocarbons by Sesbania cannabina in bioaugmented soil with free and immobilized consortium. J Hazard Mater 237:262–269

Masuda Y, Itoh H, Shiratori Y, Isobe K, Otsuka S, Senoo K (2017) Predominant but previously-overlooked prokaryotic drivers of reductive nitrogen transformation in paddy soils, revealed by metatranscriptomics. Microbes Environ 32:180–183

Mathew RP, Feng Y, Githinji L, Ankumah R, Balkcom KS (2012) Impact of no-tillage and conventional tillage systems on soil microbial communities. Appl Environ Soil Sci. https://doi.org/10.1155/2012/548620

Mattarozzi M, Manfredi M, Montanini B, Gosetti F, Sanangelantoni AM, Marengo E, Careri M, Visioli G (2017) A metaproteomic approach dissecting major bacterial functions in the rhizosphere of plants living in serpentine soil. Anal Bioanal Chem 409:2327–2339

Meneghine AK, Nielsen S, Varani AM, Thomas T, Alves LM (2017) Metagenomic analysis of soil and freshwater from zoo agricultural area with organic fertilization. PLoS One 12:e0190178

Miller MG (2007) Environmental metabolomics: a SWOT analysis (strengths, weaknesses, opportunities, and threats). J Proteome Res 6:540–545
Mishra RK, Mohammad N, Roychoudhury N (2015) Soil pollution: causes, effects and control. Tropical Forest Research Institute 3:20–30
Moran MA (2009) Metatranscriptomics: eavesdropping on complex microbial communities-large-scale sequencing of mRNAs retrieved from natural communities provides insights into microbial activities and how they are regulated. Microbe 4:329
Moter A, Göbel UB (2000) Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. J Microbiol Methods 41:85–112
Muehe EM, Weigold P, Adaktylou IJ, Planer-Friedrich B, Kraemer U, Kappler A, Behrens S (2015) Rhizosphere microbial community composition affects cadmium and zinc uptake by the metal-hyperaccumulating plant Arabidopsis halleri. Appl Environ Microbiol 81:2173–2181
Mukherjee A, Yadav R, Marmeisse R, Fraissinet-Tachet L, Reddy MS (2019) Heavy metal hypertolerant eukaryotic aldehyde dehydrogenase isolated from metal contaminated soil by metatranscriptomics approach. Biochimie 160:183–192
Navarrete AA, Kuramae EE, de Hollander M, Pijl AS, van Veen JA, Tsai SM (2013) Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. FEMS Microbiol Ecol 83:607–621
Navarro E, Fabrègue O, Scorretti R, Reboulet J, Simonet P, Dawson L, Demanèche S (2015) RisaAligner software for aligning fluorescence data between Agilent 2100 Bioanalyzer chips: application to soil microbial community analysis. BioTechniques 59:347–349
Oates LG, Read HW, Gutknecht JL, Duncan DS, Balser TB, Jackson RD (2017) A lipid extraction and analysis method for characterizing soil microbes in experiments with many samples. J Vis Exp 16:e55310
Okabe S, Kindaichi T, Ito T (2004) MAR-FISH—An ecophysiological approach to link phylogenetic affiliation and in situ metabolic activity of microorganisms at a single-cell resolution. Microbes Environ 19:83–98
Olaniran AO, Balgobind A, Pillay B (2013) Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. Int J Mol Sci 14:10197–101228
Oshoma CE, Igbeta B, Omonigho SE (2017) Analysis of microbiological and physiochemical properties of top soil from municipal dumpsites in Benin City. J Appl Sci Environ Manage 21:985–990
Pal S, Roy A, Kazy SK (2019) Exploring microbial diversity and function in petroleum hydrocarbon associated environments through Omics approaches. In: Microbial diversity in the genomic era. Academic Press, Cambridge, pp 171–194
Panigrahi S, Velraj P, Rao TS (2019) Functional microbial diversity in contaminated environment and application in bioremediation. In: Microbial diversity in the genomic era. Academic Press, pp 359–385
Panosyan H, Hakobyan A, Birkeland NK, Trchounian A (2018) Bacilli community of saline-alkaline soils from the Ararat plain (Armenia) assessed by molecular and culture-based methods. Syst Appl Microbiol 41:232–240
Peng J, Wegner CE, Liesack W (2017) Short-term exposure of paddy soil microbial communities to salt stress triggers different transcriptional responses of key taxonomic groups. Front Microbiol 8:400
Petrucco-Toffolo E, Basso A, Kerdelhué C, Ipekdal K, Mendel Z, Simonato M, Battisti A (2018) Evidence of potential hybridization in the Thaumetopoea pityocampa-wilkinsoni complex. Agric For Entomol 20:9–17
Phillips LA, Greer CW, Germida JJ (2006) Culture-based and culture-independent assessment of the impact of mixed and single plant treatments on rhizosphere microbial communities in hydrocarbon contaminated flare-pit soil. Soil Biol Biochem 38:2823–2833
Piñón-Castillo HA, Gutiérrez DL, Hernández-Castillo D, Muñoz-Castellanos LN, Rivera-Chavira BE, Nevárez-Moorillón GV (2017) Laboratory-scale biodegradation of fuel oil no. 6 in

contaminated soils by Autochthonous bacteria. In: Advances in bioremediation and phytoremediation. IntechOpen
Rapp JZ, Bienhold C, Offre P, Boetius A (2016) Polysaccharide degradation potential of bacterial communities in Arctic deep-sea sediments (1200–5500 m water depth). In: 16th international symposium on microbial ecology, Montréal, Canada, 21 August 2016–26 August
Rastogi G, Stetler LD, Peyton BM, Sani RK (2009) Molecular analysis of prokaryotic diversity in the deep subsurface of the former Homestake gold mine, South Dakota, USA. J Microbiol 47:371–384
Riesenfeld CS, Schloss PD, Handelsman J (2004) Metagenomics: genomic analysis of microbial communities. Annu Rev Genet 38:525–552
Ritchie NJ, Schutter ME, Dick RP, Myrold DD (2000) Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. Appl Environ Microbiol 66:1668–1675
Rogers SW, Moorman TB, Ong SK (2007) Fluorescent in situ hybridization and micro-autoradiography applied to ecophysiology in soil. Soil Sci Soc Am J 71:620–631
Rondon MR, August PR, Bettermann AD, Brady SF, Grossman TH, Liles MR, Loiacono KA, Lynch BA, MacNeil IA, Minor C, Tiong CL (2000) Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl Environ Microbiol 66:2541–2547
Schloss PD, Handelsman J (2004) Status of the microbial census. Microbiol Mol Biol Rev 68:686–691
Schloter M, Nannipieri P, Sørensen SJ, van Elsas JD (2018) Microbial indicators for soil quality. Biol Fertil Soils 54:1–10
Shahsavari E, Schwarz A, Aburto-Medina A, Ball AS (2019) Biological degradation of polycyclic aromatic compounds (PAHs) in soil: a current perspective. Curr Pollut Rep 5:1–9
Shanmugam BK, Easwaran SN, Lakra R, Deepa PR, Mahadevan S (2017) Metabolic pathway and role of individual species in the bacterial consortium for biodegradation of azo dye: a biocalorimetric investigation. Chemosphere 188:81–89
Shekhar SK, Godheja J, Modi DR (2020) Molecular Technologies for Assessment of bioremediation and characterization of microbial communities at pollutant-contaminated sites. In: Bioremediation of industrial waste for environmental safety. Springer, Singapore, pp 437–474
Shibulal B, Al-Bahry SN, Al-Wahaibi YM, Elshafie AE, Al-Bemani AS, Joshi SJ (2018) Analysis of bacterial diversity in different heavy oil Wells of a reservoir in South Oman with alkaline pH. Scientifica 2018:10
Shin YJ, Park SM, Yoo JC, Jeon CS, Lee SW, Baek K (2016) A new approach for remediation of as-contaminated soil: ball mill-based technique. Environ Sci Pollut Res 23:3963–3970
Singh SK, Rai MK, Sahoo L (2012) An improved and efficient micropropagation of Eclipta alba through transverse thin cell layer culture and assessment of clonal fidelity using RAPD analysis. Ind Crop Prod 37:328–333
Slade EM, Roslin T, Santalahti M, Bell T (2016) Disentangling the 'brown world' faecal–detritus interaction web: dung beetle effects on soil microbial properties. Oikos 125:629–635
Srivastava M, Kaushik MS, Mishra AK (2016) Linking the physicochemical properties with the abundance and diversity of Rhizospheric bacterial population inhabiting Paddy soil based on a concerted multivariate analysis of PCR-DGGE and RISA. Geomicrobiol J 33:894–905
Srivastava M, Mishra AK (2018) Comparative responses of diazotrophic abundance and community structure to the chemical composition of paddy soil. Environ Sci Pollut Res 25:399–412
Stackebrandt E, Liesack W, Goebel BM (1993) Bacterial diversity in a soil sample from a subtropical Australian environment as determined by 16S rDNA analysis. FASEB J 7:232–236
Stefanis C, Alexopoulos A, Voidarou C, Vavias S, Bezirtzoglou E (2013) Principal methods for isolation and identification of soil microbial communities. Folia Microbiol 58:61–68
Su JQ, Xia Y, Yao HY, Li YY, An XL, Singh BK, Zhang T, Zhu YG (2017) Metagenomic assembly unravel microbial response to redox fluctuation in acid sulfate soil. Soil Biol Biochem 105:244–252

Sukul P, Schäkermann S, Bandow JE, Kusnezowa A, Nowrousian M, Leichert LI (2017) Simple discovery of bacterial biocatalysts from environmental samples through functional metaproteomics. Microbiome 5:28

Sun X, Zhang J, Zhang H, Ni Y, Zhang Q, Chen J, Guan Y (2010) The responses of Arabidopsis thaliana to cadmium exposure explored via metabolite profiling. Chemosphere 78:840–845

Sutton NB, Maphosa F, Morillo JA, Al-Soud WA, Langenhoff AA, Grotenhuis T, Rijnaarts HH, Smidt H (2012) Impact of long term diesel contamination on soil microbial community structure. Appl Environ Microbiol 79:619–630

Szewczyk R, Soboń A, Słaba M, Długoński J (2015) Mechanism study of alachlor biodegradation by Paecilomyces marquandii with proteomic and metabolomic methods. J Hazard Mater 291:52–64

Toljander JF, Lindahl BD, Paul LR, Elfstrand M, Finlay RD (2007) Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. FEMS Microbiol Ecol 61:295–304

Toplin JA, Norris TB, Lehr CR, McDermott TR, Castenholz RW (2008) Biogeographic and phylogenetic diversity of thermoacidophilic cyanidiales in Yellowstone National Park, Japan, and New Zealand. Appl Environ Microbiol 74:2822–2833

Tribelli PM, Rossi L, Ricardi MM, Gomez-Lozano M, Molin S, Iustman LJ, Lopez NI (2018) Microaerophilic alkane degradation in pseudomonas extremaustralis: a transcriptomic and physiological approach. J Ind Microbiol Biotechnol 45:15–23

Tripathi RD, Tripathi P, Dwivedi S, Dubey S, Chakrabarty D (2012) Arsenomics: omics of arsenic metabolism in plants. Front Physiol 3:275

Utturkar SM, Bollmann A, Brzoska RM, Klingeman DM, Epstein SE, Palumbo AV, Brown SD (2013) Draft genome sequence for Caulobacter sp. strain OR37, a bacterium tolerant to heavy metals. Genome Announc 1:e00322–e00313

Utturkar SM, Cude WN, Robeson MS, Yang ZK, Klingeman DM, Land ML, Allman SL, Lu TY, Brown SD, Schadt CW, Podar M (2016) Enrichment of root endophytic bacteria from populus deltoides and single-cell-genomics analysis. Appl Environ Microbiol 82:5698–5708

Viant MR (2009) Applications of metabolomics to the environmental sciences. Metabolomics 5:1–2

Villiers F, Hugouvieux V, Leonhardt N, Vavasseur A, Junot C, Vandenbrouck Y, Bourguignon J (2012) Exploring the plant response to cadmium exposure by transcriptomic, proteomic and metabolomic approaches: potentiality of high-throughput methods, promises of integrative biology. In: Metal toxicity in plants: perception, signaling and remediation. Springer, Berlin, pp 119–142

Visioli G, Vincenzi S, Marmiroli M, Marmiroli N (2012) Correlation between phenotype and proteome in the Ni hyperaccumulator Noccaea caerulescens subsp. caerulescens. Environ Exp Bot 77:156–164

Vivas A, Moreno B, del Val C, Macci C, Masciandaro G, Benitez E (2008) Metabolic and bacterial diversity in soils historically contaminated by heavy metals and hydrocarbons. J Environ Monit 10:1287–1296

Wanapaisan P, Laothamteep N, Vejarano F, Chakraborty J, Shintani M, Muangchinda C, Morita T, Suzuki-Minakuchi C, Inoue K, Nojiri H, Pinyakong O (2018) Synergistic degradation of pyrene by five culturable bacteria in a mangrove sediment-derived bacterial consortium. J Hazard Mater 342:561–570

Wang DZ, Kong LF, Li YY, Xie ZX (2016) Environmental microbial community proteomics: status, challenges and perspectives. Int J Mol Sci 17:1275

Wang X, Sharp CE, Jones GM, Grasby SE, Brady AL, Dunfield PF (2015) Stable-isotope probing identifies uncultured Planctomycetes as primary degraders of a complex heteropolysaccharide in soil. Appl Environ Microbiol 81:4607–4615

Wang Y, Tian H, Huang F, Long W, Zhang Q, Wang J, Zhu Y, Wu X, Chen G, Zhao L, Bakken LR (2017) Time-resolved analysis of a denitrifying bacterial community revealed a core microbiome responsible for the anaerobic degradation of quinoline. Sci Rep 7:14778

Wei K, Yin H, Peng H, Liu Z, Lu G, Dang Z (2017) Characteristics and proteomic analysis of pyrene degradation by Brevibacillus brevis in liquid medium. Chemosphere 178:80–87
Wilmes P, Bond PL (2006) Metaproteomics: studying functional gene expression in microbial ecosystems. Trends Microbiol 14:92–97
Wu T, Chellemi DO, Graham JH, Rosskopf EN (2008) Assessment of fungal communities in soil and tomato roots subjected to diverse land and crop management systems. Soil Biol Biochem 40:1967–1970
Yang MM, Xu LP, Xue QY, Yang JH, Xu Q, Liu HX, Guo JH (2012a) Screening potential bacterial biocontrol agents towards Phytophthora capsici in pepper. Eur J Plant Pathol 134:811–820
Yang S, Wen X, Jin H, Wu Q (2012b) Pyrosequencing investigation into the bacterial community in permafrost soils along the China-Russia crude oil pipeline (CRCOP). PLoS One 7:e52730
Yao Z, Li J, Xie H, Yu C (2012) Review on remediation technologies of soil contaminated by heavy metals. Procedia Environ Sci 16:722–729
Yergeau E, Sanschagrin S, Beaumier D, Greer CW (2012) Metagenomic analysis of the bioremediation of diesel-contaminated Canadian high arctic soils. PLoS One 7:e30058
Yergeau E, Sanschagrin S, Maynard C, St-Arnaud M, Greer CW (2014) Microbial expression profiles in the rhizosphere of willows depend on soil contamination. ISME J 8:344
Yun SH, Choi CW, Lee SY, Lee YG, Kwon J, Leem SH, Chung YH, Kahng HY, Kim SJ, Kwon KK, Kim SI (2014) Proteomic characterization of plasmid pLA1 for biodegradation of polycyclic aromatic hydrocarbons in the marine bacterium, Novosphingobium pentaromativorans US6-1. PLoS One 9:e90812
Zachow C, Berg C, Müller H, Meincke R, Komon-Zelazowska M, Druzhinina IS, Kubicek CP, Berg G (2009) Fungal diversity in the rhizosphere of endemic plant species of Tenerife (Canary Islands): relationship to vegetation zones and environmental factors. ISME J 3:79
Zampolli J, Zeaiter Z, Di Canito A, Di Gennaro P (2019) Genome analysis and-omics approaches provide new insights into the biodegradation potential of Rhodococcus. Appl Environ Microbiol 103:1069–1080
Zeng XW, Qiu RL, Ying RR, Tang YT, Tang L, Fang XH (2011) The differentially-expressed proteome in Zn/cd hyperaccumulator Arabis paniculata Franch. In response to Zn and cd. Chemosphere 82:321–328
Zhao L, Zhang H, White JC, Chen X, Li H, Qu X, Ji R (2019) Metabolomics reveals that engineered nanomaterial exposure in soil alters both soil rhizosphere metabolite profiles and maize metabolic pathways. Environ Sci Nano 6:1716–1727
Zhou X, Wu F (2012) P-Coumaric acid influenced cucumber rhizosphere soil microbial communities and the growth of Fusarium oxysporum f. sp. cucumerinum Owen. PLoS One 7: e48288
Zitnick-Anderson K, Simons K, Pasche JS (2018) Detection and qPCR quantification of seven Fusarium species associated with the root rot complex in field pea. Can J Plant Pathol 40:261–271

Microbial Biotransformation of Hexavalent Chromium [Cr(VI)] in Tannery Wastewater

6

Arukula Deepa and Brijesh Kumar Mishra

Abstract

Tannery wastewater contains hexavalent chromium [Cr(VI)], which is one of the most prevalent contaminants due to its essential applications in tannery industries. Tannery wastewater also contains a complex of organic, inorganic, and dissolved solids which are more carcinogenic to human and aquatic life and result in adverse effects on the environment; hence treatment of these pollutants is a significant concern to the society. The removal of Cr(VI) by microorganisms predominantly occurs through biotransformation and adsorption, where the mechanism mainly depends on the surface nature of the biosorbent and the availability of the reductants. Hexavalent chromium removal using relevant methods such as biodegradation and microbial bioremediation, are carried out proficiently by the addition of indigenous or exogenous microbes. This elevates the degradation rate of Cr(VI) naturally to achieve remarkably higher removal effeciency in tannery wastewater and bring it to the permissible limits prior to discharge. This chapter mainly focuses on bioremediation and the mechanism of a microbial cell, sources, as well as its effects in a detailed manner for the emerging technologies in industrial applications.

Keywords

Bioremediation · Chromium toxicity · Hexavalent chromium · Biosorption · Bioaccumulation · Tannery wastewater

A. Deepa · B. K. Mishra (✉)
Department of Environmental science and Engineering, Indian Institute of Technology (Indian School of Mines), Dhanbad, Jharkhand, India
e-mail: brijesh@iitism.ac.in

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_6

6.1 Introduction

Environmental pollution due to anthropogenic and industrial activities has led to unfavorable damage to the ecosystem. The discharge of heavy metal pollution from industries has enduring lethal effects to humans and the environment. Heavy metal pollution, such as Cr, Cd, Zn, Hg, etc., which are nonbiodegradable in nature, its contamination, and problems caused to ecosystem were well documented (Raskin and Ensley 2000). The accumulation of heavy metals in humans has several consequences such as carcinogenesis, neuromuscular control defects, skin irritation, respiratory problems, mental retardation and abnormalities, as well as a wide range of other effects (Thiele 1995). Among these metals, hexavalent chromium [Cr(VI)] is extensively used in many industrial processes such as leather industries, electroplating, metallurgies, pigment synthesis, petroleum products, wood preservation, etc. Out of these industries, leather tanning industries are one of the most important and old expanding industries in India. The effluent generated by these tanning industries contains a huge amount of organic and inorganic load rich in chromates and sulfates (Durai and Rajasimman 2011). The tannery effluent from these industries generates more than 17,000 tons of Cr wastes into the environment (Kamaludeen et al. 2003). Cr(VI) compound is more lethal than Cr(III), which precipitates when pH is more than 5.5 because of the formation of insoluble hydroxides in water bodies. The World Health Organization (WHO) fixed the maximum tolerance level of the total chromium at 0.05 and 0.1 mg/L in drinking water and inland surface water. But in the case of industrial wastewater, the discharge ranged from 0.1 to 400 mg/L (World Health Organization 2004). The treatment of tannery wastewater through different physicochemical and biological process was commonly employed prior to their discharge into the environment. The physicochemical treatment methods such as coagulation-flocculation, sedimentation, electro-flotation, electro-chemical treatment, ion exchange, and filtration are more cost-effective and not environmentally friendly (Song et al. 2003). In order to overcome these problems, biological methods such as bioremediation process play a crucial role in the removal of heavy metals which are more economically feasible option as well as environmentally sustainable. Biological approach by the use of microbes and other microbial-integrated process in remediation of Cr(VI) from tannery effluents is more popular in the current days (Jeyasingh and Philip 2005). The aim of this chapter is mainly focused on different microbial-integrated processes such as bioremediation and biodegradation and also the mechanism of a microbial cell as well as its effects in a detailed manner for the emerging technologies in industrial applications.

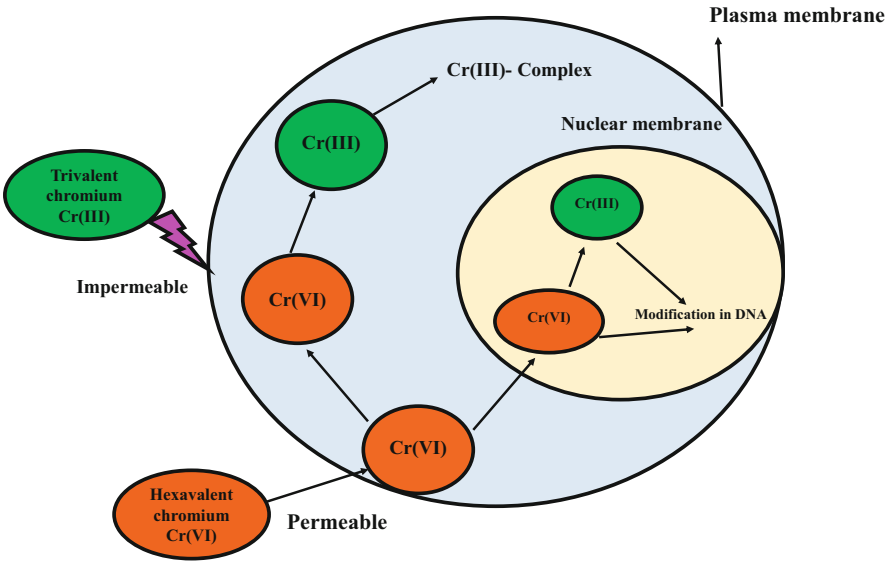


Fig. 6.1 Mechanism of Cr(VI) toxicity and mutagenicity of a microbial cell. (Espouse and modified by Narayani and Shetty (2013))

6.2 Toxicity of Cr(VI) to the Environment and its Mechanism in Microbial Cell

Chromium is one of the most copious elements which is extensively used in the tannery industries. Chromium in the environment mainly exists in two oxidation states, i.e., Cr(VI) and Cr(III). Cr(VI) is remarkably toxic and highly soluble compound that can cross the membrane of living organisms, whereas Cr(III) is less toxic, insoluble with low mobility, and an essential trace metal which forms strong binding complexes with oxygen (Gupta et al. 2019). In certain conditions, Cr (III) can be oxidized to Cr(VI) which is limited by the concentration of dissolved Cr (III), pH, and ionic strength (Fig. 6.1) (Apte et al. 2006).

6.3 Bioremediation of Cr(VI)

Bioremediation is a new technology for eco-friendly refinement of the polluted environment with the use of living microorganisms which degrades the environmental pollutants. This in situ treatment has several advantages over conventional treatment techniques; bioremediation has received the attention in the recent times because of the process in which the microbes are involved to detoxify and degrade the hazardous environmental pollutants (Kaksonen et al. 2003; Malik 2004; Eccles 1995).

6.3.1 Biosorption of Chromium by Microorganisms

Biosorption can be defined as the ability of the biological materials in which soluble chemicals interact with the materials of biological cell surfaces (Kaduková and Virčíková 2005). Various biosorbents such as microbial-based biomass (bacteria and fungi) and plant-based biomass algae are considered in terms of cost-effectiveness and environmentally friendly for the removal of pollutants (Hlihor et al. 2017). The use of microorganisms as biosorbents mainly absorbs the metal from the tannery effluent, and also it converts the lethal metals into less toxic through oxidation-reduction mechanism (Gupta et al. 2019). Researchers reported that, with the help of chromium resistant bacterial strains like *Pseudomonas* sp., *Microbacterium* sp., *Desulfovibrio* sp., and *Rhizopus sexualis* and the fermented bagasse by *Enterobacter* sp., *Escherichia coli*, *Shewanella algae*, *Rhizopus sexualis* or *Aspergillus terreus*, etc. can be used to remove chromium from the tannery wastewater (Saranraj and Sujitha 2013). Srinath et al. (2002) reported that the biosorption of chromium by *Bacillus coagulans* (*live cells*), *Bacillus circulans*, *Bacillus megaterium*, *and Bacillus coagulans* (*dead cells*) showed the removal efficiency of Cr(VI) by 47.6%, 64%, 69%, and 79.8%, respectively, with a retention time of 24 h. Whereas in the case of *Brevibacterium* sp. *CrT-12* (*live cells*) showed 100% removal efficiency with a retention time of 72 h (Faisal and Hasnain 2004). Researchers also reported that *Acinetobacter* sp. *and Cellulosimicrobium funkei strain AR6* species showed the removal efficiency of Cr(VI) of 100% and 80.43% with a retention time of 72 hand 120 h, respectively (Srivastava et al. 2007; Karthik et al. 2017).

6.3.1.1 Biosorption Mechanisms

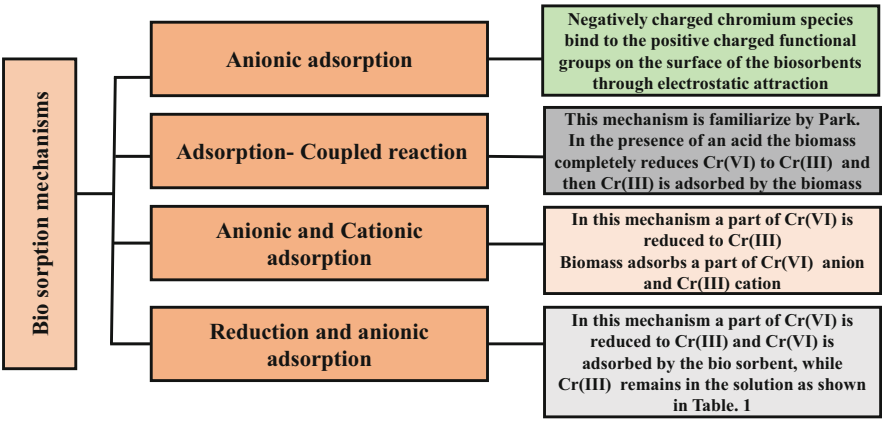


Fig. 6.2 Biosorption mechanisms of hexavalent chromium

6.3.2 Bioaccumulation of Chromium

Chromium (CrO_4^{2-}) is a mutagen as well as carcinogen which overcomes the cell permeability barrier via transport pathways for the structurally similar anion So_4^{2-}. Cr(VI) enters into the cell via facilitated transport through nonselective anion channel and sulfate transporters (Joutey et al. 2015). Inside the cell, the reduced CrO_4^{2-} under oxidation damages DNA via one-electron reduction, which gives transient Cr(V) and Cr(VI) species by continuously producing free radicals like RS^- and OH^-. As a result, it alters the DNA by changing the bond cleavage with the change in gene expression as shown in Fig. 6.1. Cr(VI) is quickly reduced to Cr(III), and therefore the concentration of Cr(VI) will never equal on the both sides of the plasma membrane; Cr(VI) is bioaccumulated through the reduction capacity of the cell which will be the main power (Pattanapipitpaisal et al. 2002; Joutey et al. 2015). Trivalent [Cr(III)] is a stable and noncarcinogen which is impermeable to the biological membranes; Trivalent [Cr(III)] promptly forms complex with the biological pertinent ligand molecules which are taken up by the cells (Ksheminska et al. 2005).

6.4 Microbial Mechanism of Cr(VI) Reduction to Cr(III)

6.4.1 Reduction of Cr(VI) by Microbes Under Aerobic Condition

Cr(VI) reduction by bacteria generally eventuates in two- or three-step process in aerobic condition. Cr(VI) is reduced to Cr(V) which is a short-term, intermediate Cr (IV) before the final conversion to stable end product Cr(III). But, it is uncertain whether the reduction of Cr(VI) to Cr(III) is an enzyme-mediated or spontaneous process (Joutey et al. 2015). NADH and NADPH are the common electron donors in Cr(VI) reduction process. Cr(VI) reduction is predominantly associated with the soluble proteins which utilizes NADH as an electron donor to enhance the reduction efficiency of chromium in the aerobic condition (Elangovan et al. 2010). Several researchers have reported the chromate reductase activity in cell-free extracts during aerobic Cr(VI) reduction (Tripathi and Garg 2013).

6.4.2 Reduction of Cr(VI) by Microbes Under Anaerobic Condition

In anaerobic conditions, Cr(VI) reduction is mediated by both the soluble and membrane-associated enzymes (Cheung and Gu 2007). In the respiratory chain, Cr (VI) assists as an electron acceptor; on the contrary, carbohydrates, proteins, fats, hydrogen, and NADPH serve as electron donors. The Cr(VI)-reducing activities of anaerobes are associated with their electron transfer system in catalyzing the electron shuttle along with the respiratory chain (Mangaiyarkarasi et al. 2011).

6.4.3 Enzyme-Mediated Cr(VI) Reduction

Reduction of Cr(VI) mostly occurs in two mechanisms: (1) *extracellular Cr (VI) reduction* and (2) *intracellular Cr(VI) reduction.*

6.4.3.1 Extracellular Cr(VI) Reduction

In order to reduce Cr(VI), extracellular enzymes are produced by bacteria and transported into the media. Many researchers have reported that for the remediation of Cr(VI), the extracellular chromate reductase is produced by *Pseudomonas putida* in Cr(VI) reduction (Priester et al. 2006; Gupta et al. 2019). Extracellular Cr (VI) reduction is favorable to the organism in which the cell does not require transport mechanisms to carry the chromate and dichromate into the cell. Therefore, extracellular reduction of Cr(VI) protects the cell from the DNA-damaging effects of Cr(VI). Researchers also reported that the production of extracellular chromate reductases by *Bacillus amyloliquefaciens* and the bacteria with membrane-bound reductases can also reduce Cr(VI) to Cr(III) by extracellular processes. For the survival in Cr(VI)-contaminated environment, certain bacterial species have adopted the extracellular Cr(VI) reduction process.

6.4.3.2 Intracellular Cr(VI) Reduction

The Cr(VI) reduction is mediated by the enzymes, which involve the cytoplasmic-soluble chromate enzymes. In this intracellular process, the use of NADH, NADPH, flavoproteins, and other hemeproteins readily reduces Cr(VI) to Cr(III) (Ackerley et al. 2004). Most of the intracellular proteins catalyze one-electron reduction of Cr (VI) to Cr(V). Hence, the harmful reactive oxygen species (ROS) are generated which provokes DNA damage (Joutey et al. 2015). Many studies have reported that Cr(VI) reductase was found to be in the cytoplasmic fraction of several chromium-resistant bacteria such as *Streptomyces* sp., *Bacillus subtilis*, *Rhodobacter*, *Pseudomonas aeruginosa*, etc. (Fig. 6.2., Table 6.1) (Joutey et al. 2015, Fig. 6.3).

Table 6.1 Microbial-mediated Cr(VI) reduction from the tannery effluent

Name of strain	% of Cr(VI) removal	References
Cellulosimicrobium sp.	98.6	Naeem et al. (2013)
Pseudomonas aeruginosa	57.7	Ganguli and Tripathi (1999)
Staphylococcus aureus	90	Ilias et al. (2011)
Pseudomonas aeruginosa	94	Munawaroh et al. (2017)
Pediococcus pentosaceus	90	Ilias et al. (2011)
Ochrobactrum intermedium	97.1	Batool et al. (2012)

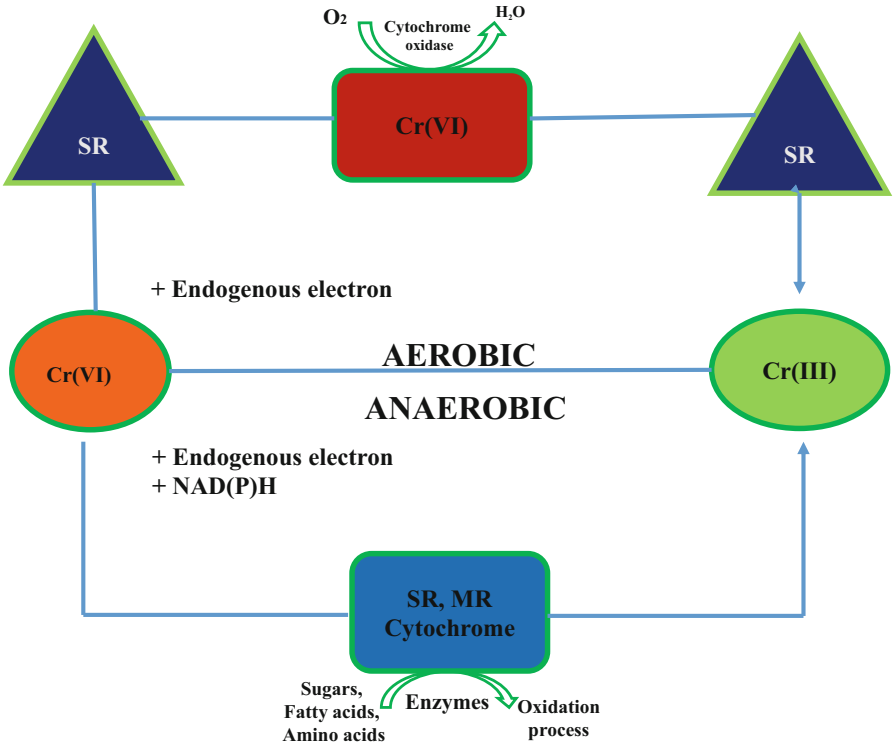


Fig. 6.3 Microbial mechanisms of aerobic and anaerobic Cr(VI) reduction (Adopted and modified from Joutey et al. (2015))

6.5 Role of Microbial Consortium in Cr(VI) Remediation from the Tannery Effluent

To remove Cr(VI) by biosorption, *cyanobacterial mat* comprising a consortium of blue-green algae such as *Chlorella* sp., *Oscillatoria*, *and Phormidium* sp. was formulated by Shukla et al. (2012). In this experiment, different concentrations of chromium were carried out in batches with 15–30, 2–110, and 300 mg, respectively, at a pH in the range of 5.5–6.2. The results revealed that the 96% removal of metal was observed within 3.5 h of the treatment process and also found that the best adsorption was at 4 ppm and at 25 ppm in the selected concentration range.

The remediation of Cr(VI) by *Saccharomyces cerevisiae, Bacillus subtilis,* and *Pseudomonas aeruginosa* was studied by Benazir et al. (2010) in consortia and in immobilized forms, and their efficiencies were compared in remediation of Cr(VI) and clearly documented that, the initial concentration of Cr(VI) in the effluent was around 770 mg/L before the remediation, but after remediation, it has been reduced to 5.2–5.7 mg/L. *Saccharomyces cerevisiae-Pseudomonas* consortia followed by the

immobilized beads of *S. cerevisiae and S. cerevisiae-B. subtilis* consortia showed the maximum remediation ability.

Pinon-Castillo et al. (2010) reported that the bacterial consortium imparted the conspicuous hexavalent chromium removal after 15 days of incubation in a medium M9 at a pH 6.5 and 8.0 from industrial wastewater. The bacterial consortia (T-RFLP) exhibited a highest number of operational taxonomic units in an alkaline carbonate medium. Genomic libraries were obtained for the consortia exhibiting optimal Cr (VI) removal. They revealed that the dominated genera in bacterial consortium were *Pseudomonas/Stenotrophomonas* and *Enterobacter/Halomonas,* respectively. Pinon-Castillo et al. (2010) concluded that *Pseudomonas fluorescens* and *Enterobacter aerogenes* were efficient in Cr(VI) reduction and adsorption to the biomass.

Biosorption is considered as a cost-effective technology worldwide; and the potential of blue–green algae (BGA), for treating metal-bearing effluents. Efficient biosorption capacity of Spirulina, Oscillatoria, and Synechocystis individually and as a consortium, as bisorbents to remove Cr3þ from a segregated stream, viz., exhaust chrome liquor (ECL) and synthetic BCS solution, was studied and found that these are more efficient in reducing sulfates, BOD, COD, etc. Hence, the results revealed that algal consortia could be a good alternative to the conventional treatment methods for leather and other industrial wastewater containing chromium.

Bhattacharya et al. (2015) studied a consortium of four naturally isolated strains for the remediation of Cr(VI) from the tannery effluent. This study concluded that the bacterial consortia resulted in 78% removal of Cr(VI) with initial concentration of Cr(VI) at 16 mg/L at 96 h of treatment. Hence, he concluded that the formulated bacterial consortium could be effectively used for the removal of Cr(VI) from the tannery wastewater.

References

Ackerley DF, Gonzalez CF, Park CH, Blake R, Keyhan M, Matin A (2004) Chromate-reducing properties of soluble flavoproteins from pseudomonas putida and Escherichia coli. Appl Environ Microbiol 70(2):873–882

Apte AD, Tare V, Bose P (2006) Extent of oxidation of Cr (III) to Cr (VI) under various conditions pertaining to natural environment. J Hazard Mater 128(2-3):164–174

Batool R, Yrjala K, Hasnain S (2012) Hexavalent chromium reduction by bacteria from tannery effluent. J Microbiol Biotechnol 22(4):547–554

Benazir JF, Suganthi R, Rajvel D, Pooja MP, Mathithumilan B (2010) Bioremediation of chromium in tannery effluent by microbial consortia. Afr J Biotechnol 9(21):3140–3143

Bhattacharya A, Gupta A, Kaur A, Malik D (2015) Simultaneous bioremediation of phenol and Cr (VI) from tannery wastewater using bacterial consortium. Int J Appl Sci Biotechnol 3(1):50–55

Cheung KH, Gu JD (2007) Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: a review. Int Biodeterior Biodegradation 59(1):8–15

Durai G, Rajasimman M (2011) Biological treatment of tannery wastewater—a review. J Environ Sci Technol 4(1):1–17

Eccles H (1995) Removal of heavy metals from effluent streams—why select a biological process? Int Biodeterior Biodegradation 35(1–3):5–16

Elangovan R, Philip L, Chandraraj K (2010) Hexavalent chromium reduction by free and immobilized cell-free extract of Arthrobacter rhombi-RE. Appl Biochem Biotechnol 160(1):81
Faisal M, Hasnain S (2004) Microbial conversion of Cr (VI) in to Cr (III) in industrial effluent. Afr J Biotechnol 3(11):610–617
Ganguli A, Tripathi AK (1999) Survival and chromate reducing ability of Pseudomonas aeruginosa in industrial effluents. Lett Appl Microbiol 28(1):76–80
Gupta P, Rani R, Chandra A, Varjani S, Kumar V (2019) The role of microbes in chromium bioremediation of tannery effluent. In: Water and wastewater treatment technologies. Springer, Singapore, pp 369–377
Hlihor RM, Apostol LC, Gavrilescu M (2017) Environmental bioremediation by biosorption and bioaccumulation: principles and applications. In: Enhancing Cleanup of environmental pollutants. Springer, Cham, pp 289–315
Ilias M, Rafiqullah IM, Debnath BC, Mannan KSB, Hoq MM (2011) Isolation and characterization of chromium (VI)-reducing bacteria from tannery effluents. Indian J Microbiol 51(1):76–81
Jeyasingh J, Philip L (2005) Bioremediation of chromium contaminated soil: optimization of operating parameters under laboratory conditions. J Hazard Mater 118(1-3):113–120
Joutey NT, Sayel H, Bahafid W, El Ghachtouli N (2015) Mechanisms of hexavalent chromium resistance and removal by microorganisms. In: Reviews of environmental contamination and toxicology, vol 233. Springer, Cham, pp 45–69
Kaduková J, Virčíková E (2005) Comparison of differences between copper bioaccumulation and biosorption. Environ Int 31(2):227–232
Kaksonen AH, Riekkola-Vanhanen ML, Puhakka JA (2003) Optimization of metal sulphide precipitation in fluidized-bed treatment of acidic wastewater. Water Res 37(2):255–266
Kamaludeen SPB, Arunkumar KR, Ramasamy K (2003) Bioremediation of chromium contaminated environments. Indian J Exp Biol 41:972–985
Karthik C, Ramkumar VS, Pugazhendhi A, Gopalakrishnan K, Arulselvi PI (2017) Biosorption and biotransformation of Cr (VI) by novel Cellulosimicrobium funkei strain AR6. J Taiwan Inst Chem Eng 70:282–290
Ksheminska H, Fedorovych D, Babyak L, Yanovych D, Kaszycki P, Koloczek H (2005) Chromium (III) and (VI) tolerance and bioaccumulation in yeast: a survey of cellular chromium content in selected strains of representative genera. Process Biochem 40(5):1565–1572
Malik A (2004) Metal bioremediation through growing cells. Environ Int 30(2):261–278
Mangaiyarkarasi MM, Vincent S, Janarthanan S, Rao TS, Tata BVR (2011) Bioreduction of Cr (VI) by alkaliphilic Bacillus subtilis and interaction of the membrane groups. Saudi J Biol Sci 18 (2):157–167
Munawaroh HSH, Gumilar GG, Kartikasari S, Kusumawaty D (2017) Microbial reduction of Cr (VI) into Cr (III) by locally isolated Pseudomonas aeruginosa. IOP Conf Ser Mater Sci Eng 180 (1):012279
Naeem A, Batool R, Jamil N (2013) Cr (VI) reduction by Cellulosimicrobium sp. isolated from tannery effluent. Turk J Biol 37(3):315–322
Narayani M, Shetty KV (2013) Chromium-resistant bacteria and their environmental condition for hexavalent chromium removal: a review. Crit Rev Environ Sci Technol 43(9):955–1009
Pattanapipitpaisal P, Mabbett AN, Finlay JA, Beswick AJ, Paterson-Beedle M, Essa A et al (2002) Reduction of Cr (VI) and bioaccumulation of chromium by gram positive and gram negative microorganisms not previously exposed to Cr-stress. Environ Technol 23(7):731–745
Pinon-Castillo HA, Brito EMS, Goñi-Urriza M, Guyoneaud R, Duran R, Nevarez-Moorillon GV et al (2010) Hexavalent chromium reduction by bacterial consortia and pure strains from an alkaline industrial effluent. J Appl Microbiol 109(6):2173–2182
Priester JH, Olson SG, Webb SM, Neu MP, Hersman LE, Holden PA (2006) Enhanced exopolymer production and chromium stabilization in pseudomonas putida unsaturated biofilms. Appl Environ Microbiol 72(3):1988–1996
Raskin I, Ensley BD (2000) Phytoremediation of toxic metals. John Wiley and Sons, New York

Saranraj P, Sujitha D (2013) Microbial bioremediation of chromium in tannery effluent: a review. Int J Microbiol Res 4(3):305–306

Shukla D, Vankar PS, Srivastava SK (2012) Bioremediation of hexavalent chromium by a cyanobacterial mat. Appl Water Sci 2(4):245–251

Song Z, Williams CJ, Edyvean RGJ (2003) Tannery wastewater treatment using an upflow anaerobic fixed biofilm reactor (UAFBR). Environ Eng Sci 20(6):587–599

Srinath T, Verma T, Ramteke PW, Garg SK (2002) Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria. Chemosphere 48(4):427–435

Srivastava S, Ahmad AH, Thakur IS (2007) Removal of chromium and pentachlorophenol from tannery effluents. Bioresour Technol 98(5):1128–1132

Thiele DJ (1995) Metal detoxification in eukaryotic cells. Crisp Data Base of National Institute of Health, Washington, DC

Tripathi M, Garg SK (2013) Co-remediation of pentachlorophenol and Cr6+ by free and immobilized cells of native Bacillus cereus isolate: spectrometric characterization of PCP dechlorination products, bioreactor trial and chromate reductase activity. Process Biochem 48 (3):496–509

World Health Organization (2004) Guidelines for drinking-water quality, vol 1. WHO, Geneva

Bioremediation: A Low-Cost and Clean-Green Technology for Environmental Management

7

Daniela Landa-Acuña, Richard Andi Solórzano Acosta, Edwin Hualpa Cutipa, Celia Vargas de la Cruz, and Bernabe Luis Alaya

Abstract

As the industry advances and the world population increases, the planet has accumulated the waste generated by human activity. Many of them are nondegradable and others of slow degradation that favor their accumulation in nature without adequate treatment. Although oil spills are the most notorious episodes, there is a range of pollutants derived from all types of industry such as pesticides, refrigerants, solvents, detergents, heavy metals, and the already abundant plastics. Faced with this problem, the use of microorganisms is a valuable tool in the remediation of soils, taking advantage of its metabolic potential, adaptability insurmountable to different environments, and the symbiotic behavior that can establish with plants. Genetic engineering has also given way to the study of genetically modified microorganisms as bioremediation agents, which express specific genes in the presence of pollutants. The bacterial species mostly used in bioremediation are *Acinetobacter* sp., *Burkholderia cepacia*, *Deinococcus radiodurans*, *Dehalococcoides ethenogenes*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, and some fungi.

Keywords

Bioremediation · Contaminants · Genetic engineering

D. Landa-Acuña · R. A. S. Acosta · E. Hualpa Cutipa · B. Luis Alaya
Laboratory of Microbial Ecology and Biotechnology, Department of Biology, Faculty of Sciences, National Agrarian University La Molina (UNALM), Lima, Peru

C. Vargas de la Cruz (✉)
Latin American Center for Teaching and Research on food bacteriology (CLEIBA), Faculty of Pharmacy and Biochemistry, Major National University of San Marcos, Lima, Peru
e-mail: cvargasd@unmsm.edu.pe

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_7

7.1 Introduction

In the present scenario, humanity has become aware of the need to preserve the environment and everything that inhabits it, the reason: pollution, the effects on life on the planet are felt directly or indirectly. They are the most important factors of the industry, the manufacturing industry (Benitez-Campo 2011) and mining (Beltrán-Pineda and Gómez-Rodríguez 2016). The use of toxic substances in their manufacturing processes and the use of technologies promote the generation of pollutants, which usually accumulate and/or transform in the environment, giving way to the publication of other highly toxic compounds. It should be mentioned that the industry has gained momentum due to the increase in the world population in the latest trends (Herrera 2017), the demand for goods and services that come hand in hand with the consumption, and exploitation of natural resources (Garzón et al. 2017). On this situation, there is a need to generate clean production processes in the use of clean technologies with low impact on the environment.

Therefore, the concern to generate technologies appeared (Cabrera 2014), in which its use causes minimal impacts on the environment, which promotes a more efficient use of energy, as well as the application of strategies that promote the use of biotechnology to generate clean production processes without having to ignore economic growth, preventing and mitigating maximum environmental impacts on ecosystems and thus preserving their functions, in order to guarantee future generations the satisfaction of their needs (Xercavins et al. 2005).

One of the fields in which biotechnology is very much important, as a useful tool that promotes the application of techniques for the benefit of the environment to recover contaminated environments, one of these strategies is bioremediation, with the potential to contribute to sustainable development. Since it involves the use of mechanisms that have proven to be cheaper than chemical methods and up to 80% cheaper (Benitez-Campo 2011). Bioremediation is a set of procedures for the biological treatment of environmental contaminants that require living organisms such as plants and microorganisms (especially bacteria and fungi) that degrade and/or transform dangerous contaminants (Maeir et al. 2009).

7.2 Arbuscular Mycorrhizal Fungi and the Remediation of Soils Contaminated with Heavy Metals

7.2.1 Arbuscular Mycorrhizal Fungi

The arbuscular mycorrhizal fungi (AMF) belong to the division *Glomeromycota*. They are mutualist symbiote microorganisms that live in association with the roots of more than 80% of terrestrial plants (Smith and Read 2008) for millions of years (Honrubia 2009). They are called arbuscular as they form inside the cortical cells of the root some structures of tree form. In these occurs an exchange of nutrients between the two microorganisms (Brundrett 2004).

Arbuscular mycorrhizae are known for their important role in the nutrition of plants, soil stabilization, and presence in different conditions of deficiency or toxicity of elements. Reports such as those described by showed that mycorrhization decreased toxicity in several species in contaminated soils, and suggest that the vesicles of arbuscular mycorrhizal fungi play a role in detoxifying the plant by storing toxic compounds.

Arbuscular mycorrhizae affect the structure of the microbial community of the rhizosphere, which will be different given the metabolic interaction with mycorrhizal hyphae or spores with respect to a non-mycorrhized root, determining the mycorrhizosphere. These populations have capacities (solubilize phosphates, production of hormones, siderophores, etc.) that when interacting with arbuscular mycorrhizae can have synergistic effects on the plant. Others favor mycorrhizal colonization, being considered as facilitators of mycorrhization or mycorrhiza helpers.

7.2.2 Importance of Arbuscular Mycorrhizal Fungi in Environments Contaminated with Heavy Metals

HMS can establish mycorrhizae with most terrestrial plants even though their soils are highly contaminated (Cabral et al. 2015). Among the effects of mycorrhizal fungi on the growth and development of plants under stress conditions with heavy metals, we highlight the ability of these fungi to improve physiological and morphological mechanisms, increase the biomass of plants and the absorption of nutrients immobile as phosphorus, and decrease the toxicity of metals in plants (Kanwal et al. 2015).

The presence of plants colonized with arbuscular mycorrhizal fungi has been reported in soils contaminated with heavy metals, which suggests that the mycorrhizal symbiosis would have acquired tolerance to live in these soils and that it may have an important role in phytoremediation.

Arbuscular mycorrhizas contribute to reducing the accumulation of heavy metals in the plant through mechanisms such as phytostabilization and/or phytoextraction (Toro et al. 2017). Phytoextraction involves the use of hyperaccumulative plants for the absorption of heavy metals and their transport and concentration in the aerial biomass (stems, leaves, flowers, fruits, and seeds), so that they can be eliminated from the environment (Pajević et al. 2016). On the contrary, phytostabilization does not remove heavy metals from the environment but immobilizes them in the roots of plants (Abdelhameed and Metwally 2019).

7.2.3 Glomalins

Unlike phytoextraction, where studies have shown the largest accumulation of heavy metals such as lead in mycorrhized roots compared to non-mycorrhized (Upadhyaya et al. 2010). In the rhizosphere of plants with arbuscular mycorrhizae, heavy metals

are retained by the mechanism known as phytostabilization by soil proteins called glomalins (Gonzalez-Chavez et al. 2004).

Glomalin described as a glycoprotein found in the soil specifically associated with the mycorrhizal fungi of phylum *Glomeromycota* (Wright and Upadhyaya 1998) and associated with thermal shock proteins (Malekzadeh et al. 2016) and is generated by degradation of HMA hyphae associated with the root of the plant (Driver et al. 2005) is specifically located in the inner cell wall layers (L2 and L3) of mycelium and AMF spores, in greater percentage, and it is less abundant in the outer layer (L1) (Cabral et al. 2015).

Mycorrhization between plants subjected to stress conditions induced by heavy metals could increase the production of glomalin by mycorrhizal fungi (Purin and Rillig 2007). Some studies have been reported in which the role of glomalins in the reduction of the toxicity of some heavy metals is demonstrated by the accumulation of these in the roots of the plants (Gonzalez-Chavez et al. 2004, Cornejo et al. 2008). It seems that the higher the pollutant concentration, the greater the capacity of GRSP to bind contaminants and make them unavailable (Cornejo et al. 2008).

7.2.4 Mycorrhizae and their Role in Decreasing Heavy Metals

One of the most common heavy metals present in altered ecosystems is copper (Cu), which is very toxic to plants that grow in these environments. This toxicity can cause losses in the diversity and functionality of native plant species, which leads to extensive damage to local vegetation, a strong change in soil characteristics and limitation of vegetation (Ginocchio 2000; Adriano 2001). The glomalins act directly in the sequestration of Cu in a soil highly contaminated with, specifically in the zone of the external mucilaginous hyphal wall, the cell wall and within the hyphal cytoplasm (Gonzalez-Chavez et al. 2002; Cornejo et al. 2008).

Lead (Pb) is a heavy metal present in soils due to anthropomorphic activities, representing a serious risk to the environment (Cecchi et al. 2008). Research has shown a wide diversity of mycorrhizal fungi where the genera *Acaulospora* and *Glomus* stand out in soils with high concentrations of Pb; these fungi would be responsible for conferring the necessary tolerance so that plants can be established in these contaminated environments, (Schneider et al. 2016).

Cadmium (Cd) is a nonessential, potentially dangerous heavy metal that can be phytotoxic when its concentration has exceeded the limit (0.5 μg Cd/g soil) (Aibibu et al. 2010; Yuan et al. 2014; Zafarzadeh et al. 2018). In nature it is present in the earth's crust; however, as a result of the use of pesticides and fertilizers such as phosphate rock, its disposition has increased and can be linked to the trophic chain through the absorption by plants up to the human being, what constitutes a potential risk to human health (Upadhyaya et al. 2010).

Reports such as those described by Abdelhameed and Metwally (2019) demonstrate the potential of mycorrhizal fungi to alleviate the effects produced by Cd in plants of *Trigonella foenumgraecum* compared to the non-mycorrhizal ones, by means of phytostabilization processes. Other reports such as those described by

found that *S. photeinocarpum* inoculated with *G. versiforme* showed an increase in antioxidant parameters such as phytochelatins, in addition to increasing the P concentration and the accumulation of Cd in the stem. On the contrary, reported a considerable reduction of Cd and Pb and a greater activity of antioxidant enzymes in the fruits of *P. peruviana* inoculated with two *Glomeromycota* fungi.

7.3 Microbial Biotechnology and its Application in the Bioremediation of Contaminated Soils

Global environmental pollution is a problem that humanity faces today. The generation of waste or polluting agents resulting from anthropogenic activities is causing the problem of soil contamination. Many strategies have been planted to solve this problem; however, the use of microorganisms in the bioremediation of soils is presented as a low-cost alternative and friendly to the environment. Many reports on the successful removal and degradation of soil contaminants using only microorganisms have been developed. Contaminants such as crude oil, hydrocarbons, lead, and mercury, among others, have been removed from contaminated environments using microorganisms. However, this success is basically due to the fact that these studies were conducted in contracted environments. One of the disadvantages of bioremediation is when trying to apply this process on a large scale in field conditions. Because there are limiting factors such as the availability and specific interaction between the contaminant and the microorganism, in addition to the impossibility of isolating microorganisms with high degradation potential using conventional techniques. To overcome this problem, a metagenomic approach can be applied using massive high-throughput sequencing tools that allow us to know the structure of communities in contaminated environments; it also allows monitoring the behavior of microorganisms throughout the process of bioremediation. The search for new microorganisms in unexplored environments could offer an advantage in obtaining bacteria with a high potential for biodegradation of pollutants from soil, water, and air. All this will lead to improved bioremediation processes.

Next, we review the potential of microorganisms to remove and degrade soil contaminants and the main difficulties and shortcomings of bioremediation processes. The application of metagenomic approaches to monitor the behavior of microorganisms during the bioremediation process is also evaluated.

7.3.1 Microbial Biotechnology and Pollution

Pollution of the environment is a problem that humanity is facing today. This contamination can be physical or chemical and can occur naturally in the environment; the overaccumulation of pollutants that exceed the natural capacity of environmental degradation triggers processes of environmental damage that put at risk the health of living beings. Pollution has existed since the beginning of the earth;

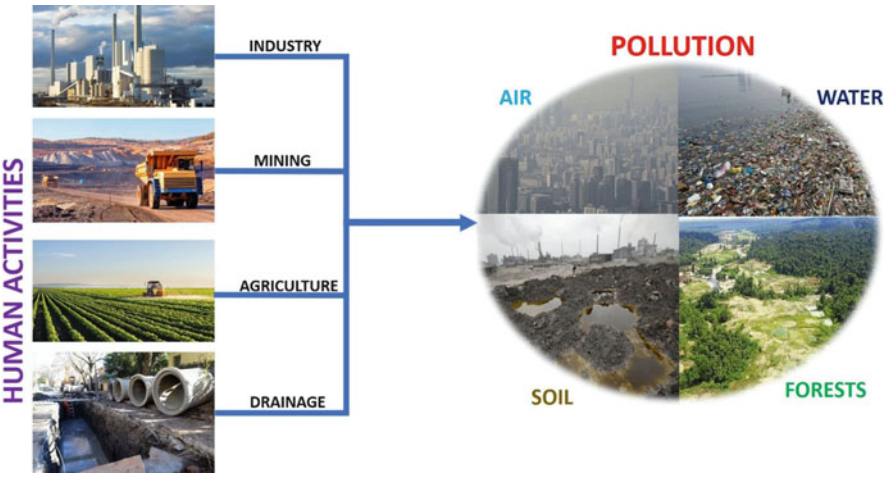


Fig. 7.1 Main human activities and their effect on the pollution of terrestrial ecosystems

however, since the industrial revolution, its presence has been notorious and harmful. All countries, both developed and those in development, present this pollution problem in their urban areas, due in principle to technological progress, population growth, and overexploitation of resources. A general outline of the main sources of pollution of terrestrial ecosystems is presented in Fig. 7.1. The diverse ecosystems present contamination of air, water, and soil due to the generation of toxic gases and dumping of substances and activities such as agriculture and mining (Muralikrishna and Manickam 2017; Rai 2016; Ahmad 2016).

Anthropogenic activities such as intensive agriculture, mining, industry, and the generation of household waste contribute in a harmful way to soil pollution. Some of the pollutants are hydrocarbons, solvents, pesticides, and heavy metals, among others. Heavy metal contamination is a common event in both industrialized and developing countries. Household waste generates a large amount of solid pollutants that are deposited in municipal dumps or are incinerated and will eventually generate soil and air pollution. The presence of contaminants in the soil alters the metabolism of plants that reduce crop yields. In addition, plants have the capacity to absorb these contaminants and can easily include them in the food chain (Muralikrishna and Manickam 2017).

Several studies have been carried out on the negative effects of oil and heavy metals on the invertebrates that live in the soils, since they interact directly with these contaminating agents (Access 2018). The presence of heavy metals in soils generates high toxicity and is considered one of the most serious pollution problems. There are in situ and ex situ technologies that are used with the purpose of reducing, removing, and degrading heavy metals present in soils. However, chelating agents and surfactants that are used in this technology end up being leached into groundwater

and pollute groundwater. Therefore, the study and application of new technologies that overcome these technical deficiencies are required (Singh Sidhu 2018).

An alternative strategy for the chelation, removal, and degradation of pollutants in soils is the use of microorganisms, because they have been shown to be very effective biological agents in bioremediation. Thanks to their metabolic and enzymatic plasticity, they have the ability to degrade a large amount of contaminating substances. Many microbial consortia have been identified from contaminated environments, and the main microbial genera that compose it are *Acinethobacter*, *Actinobacter*, *Acaligenes*, *Arthrobacter*, *Bacillins*, *Berijerinckia*, *Flavobacterium*, *Methylosinus*, *Mycrobacterium*, *Mycococcus*, *Nitrosomonas*, *Nocardia*, *Penicillium*, *Phanerochaete*, *Pseudomonas*, *Rhizoctomia*, *Serratio*, *Trametes*, and *Xanthobacter* (Singh 2014).

7.3.2 Microorganisms Present in Contaminated Soils

The presence and activity of microorganisms in contaminated soils can be closely linked to the quantity or mixture of contaminants. Initially it was believed that the pollutants acted in an individual way on the microorganisms and that multiple chemical substances did not interact or their toxicology was irrelevant at low concentrations. So much so that most of the research articles published in toxicology journals described a polluting agent as the most relevant. However, the study and knowledge of the mixtures of contaminants in soils can be beneficial, neutral, or adverse and will depend on the composition of the contaminant, the microorganism, or the environment studied. Based on these studies, it was concluded that the mixture of contaminants could have a harmless, additive, synergistic, or inhibitory effect on the activity of microorganisms in contaminated soils (Ramakrishnan et al. 2012). Therefore, there is a complex interaction between the contaminants present in an environment and the microorganisms that colonize it (Fig. 7.2).

Studies carried out on soils with presence of mine tailings showed that there is a significant correlation between the physicochemical properties of the soil, the structure, and the biological diversity of the community, which was composed of some dominant bacterial groups (*Firmicutes*, *Proteobacteria*, and *Actinobacteria*) and that in addition they were highly tolerant to the presence of Hg. The species *Pseudomonas plecoglossicida* turned out to be the strain with the highest degree of tolerance toward Hg, which would indicate its potential use in the bioremediation of soils with the presence of this contaminant (Ji et al. 2018). The response of microbial communities to environmental stress is a critical issue in soil ecology. Studies conducted by Ventorino et al. (2018) show that the composition of a microbial population can vary significantly in a polluted environment, basically due to the presence of contaminants in the soil which influence the establishment of new communities. These established communities could be used as biomarkers to assess the quality and health of soils (Bosse et al. 2018; Mansouri et al. 2019; Occhipinti et al. 2018). Therefore, the use of biomarkers in bioremediation is a technology that has already been applied; in addition it would help in the identification of native

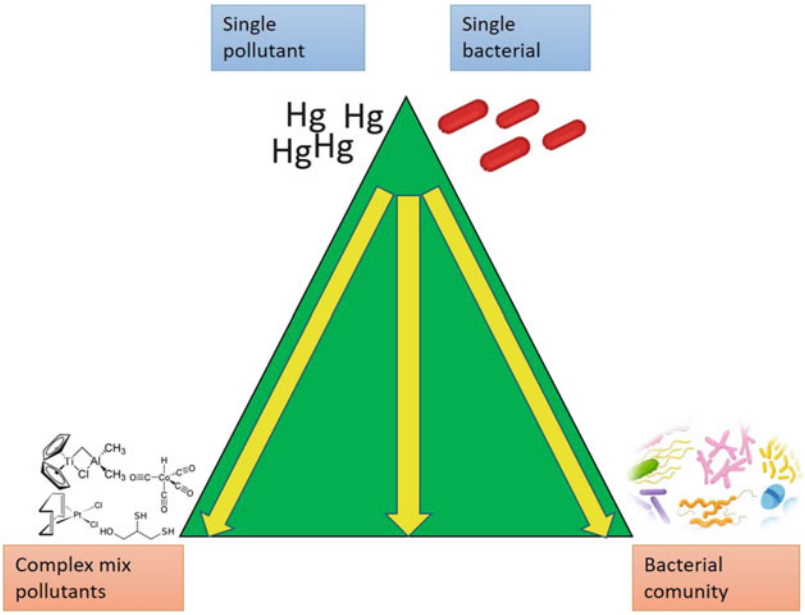


Fig. 7.2 Diagram of the interaction of chemical pollutants and microorganisms

populations of microorganisms with biodegradable potential of specific xenobiotic agents in contaminated soils.

A great diversity of gram-negative and gram-positive bacteria can be found in soils with different levels of contamination. Studies carried out by Ajayi and Abiola (2018) report a high survival capacity to crude oil of bacteria isolated from soils contaminated with crude oil. Species such as *Micrococcus luteus*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Acinetobacter lwoffi* proved to have a high potential to degrade oil crude. The tolerance and degradation potential of these microorganisms make them ideal for the treatment of soils contaminated with oil. This microbial potential can be used to propose bioremediation strategies that help mitigate the harmful effects of pollutants in ecosystems.

Soils with a high concentration of lead (57 × 101 mg/kg) in different areas of Nigeria have presented a high population of predominant bacterial genera such as *Bacillus, Proteus*, *Achromobacter*, *Citrobacter*, *Corynebacerium*, *Alcaligenes*, *Pseudomonas*, *Staphylococcus*, *Klebsiella*, *Escherichia*, *Agrobacterium*, *Enterobacter*, and *Diplococcus*, which reveals a distribution of microorganisms in contaminated environments. These toxicant-tolerant microorganisms (Pb) are considered as potential bioremediation agents in contaminated sites; they also provide a basis for the implementation of eco-friendly bioremediation strategies (Kazaure 2018).

7.3.3 Use and Application of Microorganisms in Soil Bioremediation

The use of microorganisms for the bioremediation of soils contaminated with petroleum hydrocarbons in controlled environments has been investigated in different latitudes (Abatenh et al. 2017). However, the application of this strategy in real field conditions is limited by several factors. Some factors such as toxic effects of petroleum hydrocarbons, the bioavailability of the pollutant, and environmental and metabolic restrictions reduce the effectiveness of microorganisms in the bioremediation of these contaminated environments. In this regard, there is an urgent need to improve these strategies by (i) deepening the basic mechanisms of interaction between bacteria and petroleum hydrocarbons to overcome the limits of microbial absorption of hydrocarbons, (ii) improving contact between bacteria and the contaminating agent through the development of compatible biosurfactants, and (iii) using high-throughput massive sequencing technologies for the search for petroleum hydrocarbon-degrading bacteria in environments not yet explored (Xu et al. 2018).

The search for microorganisms tolerant to low temperatures with potential to degrade hydrocarbons in regions with cold climates has been developed. Strains of the genres *Chryseobacterium*, *Bacillus*, and *Pseudomonas* showed a high biodegradation capacity of crude oil in ranges of 10 °C, pH 7, and salinity of 10 g/L. These microorganisms reached a removal efficiency of up to 50–60% in soils containing 5.8–10.6 g of oils/kg of soil. The high removal efficiency of these microorganisms is a potential application in the decontamination of soils found in latitudes with cold climates (Wang et al. 2016).

The bioremediation of soils with high levels of petroleum hydrocarbons (3700 mg/L) was developed by microbial consortiums composed of strains of *Pseudomonas* sp., *Stenotrophomonas* sp., *Achromobacter* sp., *Brevibacillus* sp., and *Staphylococcus*. These bacterial strains were isolated from soils highly contaminated with petroleum hydrocarbons located near refineries dedicated to the extraction of crude oil in Iran. About 6% of the pollutant was degraded by the microbial consortiums adapted probably due to the ability of microorganisms to adapt. These results demonstrate and support the use of microorganisms as a potential for bioremediation that can be coupled with other technologies (biopiles, biomagnification, etc.) already existing to enhance their effect (Samarghandi et al. 2018).

Potential candidates for the biodegradation of used lubricating oils present in soils of tropical regions of Thailand have been investigated. Species of phylum *Proteobacteria* were found to be predominant in the soils analyzed (Jamarillo 2011). The structure of the bacterial communities was related to the physicochemical parameters of each soil in addition to the presence of the contaminant. The main species widely distributed and with tolerant characteristics in the contaminated soils of this Thai region was *Enterobacter* sp., which suggests that this strain is potentially related to the biodegradation of used lubricating oils and could also be applied to

monitor natural attenuation and develop strategies to accelerate bioremediation (Meeboon et al. 2017).

The combination of chemical fertilizers and microorganisms of the species *Rhizobium* sp.I3 associated with mendong plants (*Fimbristylis globulosa*) presents a characteristic tool with potential for the bioremediation of leaded crops. This pollution is generated by the development of the industry that generates an increase of lead (Pb) in the fields due to the use of industrial wastewater for the irrigation of crops (Khan et al. 2018). The absorption of lead by the plants studied is related to characteristics such as soil pH, cation exchange capacity, organic matter, and total amount of microorganisms present. The area of the plant with the greatest absorption characteristic is the root, due to the direct contact with the contaminant and the colonizing capacity of the bacteria. This combination represents a technology with potential for bioremediation of agricultural soils contaminated with lead. Alternative of this.

7.3.4 Metagenomic Approaches Applied to Soil Bioremediation

For a better understanding and development of bioremediation processes, a metagenomic field approach can be applied, which consists in analyzing the variation of bacterial communities throughout the period of degradation of crude oil present in soils, demonstrating that there is a dominant group (proteobacteria) that behaves as a key organism in remediation processes immediately after the contaminant has been added (Ezekoye et al. 2018a, b). A large amount of metagenomic data was obtained after studying the changes in the composition and diversity of microbial communities present in mining soils where iron is extracted. These data provide an interesting point of view that allow comparing the dynamics of communities in soils that are in the process of rehabilitation with those that are not yet. This set of data generated provides valuable information for the rehabilitation and ecological restoration of soils after the closure of mining activities (Gastauer et al. 2019).

The role of microorganisms in biogeochemical cycles can be studied through a metagenomic approach. This approach allows us to have an understanding of the processes of functional adaptation of bacteria. Metagenomic analysis of soils belonging to manganese mines confirms the predominance of the group of proteobacteria as key agents in the bioremediation processes of these pollutants. However, very little has been studied of its role in the processes of biogeochemical cycling of Mn. Using metagenomics, it has been shown that there is a close relationship of microbial activity with the biogeochemical cycle of Mn. In addition, this type of approach takes advantage of traditional crop-dependent techniques and generates a deeper insight into the function of bacterial genes possibly associated with Mn cycling in the ecosystem (Ghosh and Das 2018).

The monitoring of the bioremediation processes is vital for the treatment of a contaminated environment; also the use of conventional techniques and chemical and metagenomic approach should be taken into account in order to improve the said process. This combination would help in the management and design of more

accurate and effective bioremediation technologies in addition to allowing their monitoring. Therefore, the government agencies involved must implement environmental policies considering the adoption of high-throughput mass sequencing technologies as a reference framework for monitoring the progress of bioremediation (Ezekoye et al. 2018a, b).

7.3.5 Conclusions and Future Perspectives

Microorganisms offer great potential in the degradation, removal, and restoration of pollutants in soils. Some microbial strains have shown excellent performance in the removal of a variety of pollutants such as crude oil, mercury, manganese, lead, and petroleum hydrocarbons, among others. The role of microorganisms in the bioremediation of soils is a key factor in the success of the restoration of contaminated environments using environment-friendly technologies. Bioremediation based on the use of microorganisms also offers a low-cost alternative.

Despite its potential as an eco-friendly and low-cost treatment, the practical application of microorganisms in a real field treatment scenario remains limited to date. One of the limiting factors is the impossibility of isolating and cultivating microorganisms with potential for degradation of contaminating agents by means of conventional techniques; another factor is the availability and interaction of the contaminant with the microorganism degrader. A potential solution to overcome this issue could be based on the use of high-performance mass sequencing techniques (metagenomic approach).

The search for new microorganisms in unexplored environments could offer an advantage in obtaining bacteria with a high potential for biodegradation of pollutants from soil, water, and air. All this will lead to improved bioremediation processes.

7.4 Phytoremediation

At present there are several proposed techniques for the rehabilitation of pollutants (soil, sediment, water, atmosphere, etc.). These remediation technologies are highly variable, depending on the contaminated matrix, the nature of the contaminants, the level of contamination, and the availability of resources. Phytoremediation is the decontamination of soils, the purification of wastewater, or the cleaning of indoor air, using vegetables, whether vascular plants, algae, and, by extension, the ecosystems that these plants contain. It is an emerging technology with great potential for efficient and low-cost cleaning for a wide range of organic and inorganic contaminants.

7.4.1 Definition and Scope of Phytoremediation

Plants used in phytoremediation can tolerate, absorb, accumulate, degrade, destroy, or inhibit compounds, contaminants, or microorganisms. Phytoremediation as a biological technique allows the decontamination of soils or the purification of wastewater through in situ treatments. Plants also help prevent wind, rain, and groundwater from spreading pollution to other areas.

Phytoremediation is used to treat pollutants such as heavy metals (Volke-Sepulveda et al. 2005) and wastewater with biological contaminants such as coliforms (Arias et al. 2010) and hydrocarbons.

The most general criterion for its classification is based on the specific density, according to which elements with a density greater than 5 g/cm^3 are included in this group. However, some authors consider this definition as inappropriate, since the specific density is not a reference in terms of the reactivity or toxicity of a metal (Duffus 2002; Volke-Sepulveda et al. 2005). Currently, the term "heavy metal" is used to refer in a broad way to those metals or metalloids with potential to cause toxicity problems (Alloway 2013).

7.4.1.1 Mercury

Unlike other metals, mercury is present in the atmosphere in a gaseous state, which facilitates its transport through the biosphere. Mercury contamination does not only affect the soil; this problem also occurs in aquatic environments due to anthropogenic discharges.

7.4.1.2 Arsenic

Arsenic can occur in organic variants, produced by the action of the metabolism of microorganisms, plants, and mammals. Forms such as monomethylarsenic acid, dimethylarsen, arsenobetaine, and arsenosugars are less toxic than inorganic ones.

7.4.1.3 Lead

The main sources of lead emissions to the environment are the smelting and processing of metals, the recycling of lead acid batteries, mining through the disposal of mine tailings, and the contamination of the atmosphere due to the use of gasoline with lead (Volke-Sepulveda et al. 2005).

7.4.1.4 Chromium

Chromium can exist in different forms, depending on its oxidation state; it can be in liquid, solid, or gaseous state. The most common chemical forms are Cr (0), Cr (III), and Cr (VI), the latter being the most toxic form.

7.4.1.5 Hydrocarbons

The main factors that show the contamination of soils and bodies of water by hydrocarbons are accidental spills during the exploration, extraction, and transport of the same. As the exploitation, extraction, and transport of hydrocarbons cause pollution to the environment, it is important to note that phytoremediation is a

promising biotechnological strategy when recovering contaminated environments. As an alternative to recovering from soils contaminated with hydrocarbons, the use of biological elements that contribute to the oxidation, degradation, transformation, and complete mineralization of these contaminants has been established (Ferrera-Cerrato et al. 2006). Phytoremediation as a biological technique allows us to carry out the decontamination of soils or the purification of wastewater, due to the restorative capacity of some plants.

7.4.2 Removal of Enterobacteria

For the treatment of these waters contaminated by coliforms, there are traditional treatments such as UV filtration, chlorination, ozone, titanium dioxide, and others, which due to their high cost are not viable in rural populations, so we are looking for cheaper alternatives that are eco-friendly. Phytoremediation represents an alternative, sustainable, environment-friendly, and low-cost technology for the restoration of contaminated environments and effluents (Lenntech 2016).

7.4.3 Types of Plants According to their Phytoremediation Capacity

Plants exposed to heavy metals can present different physiological responses, which vary depending on the species of the plant, the specific metal to which it is exposed, and the concentration in the soil of it. Based on these responses, the plants can be classified into three types: exclusive, indicators, and accumulators of heavy metals (Baker 1981).

Exclusive: plants where the accumulation of metals in the aerial part is much lower compared to the concentration of metals in the soil.
Indicators: plants where the accumulation of metals in the aerial tissue keeps a linear relationship with respect to the concentration of the soil.
Accumulators: plants where the accumulation of metals in their aerial part is much greater than the concentration of metals in the soil.

7.4.3.1 Species Used in Phytoremediation

Around 400 species of hyperaccumulative metal plants are known worldwide, with the Brassicaceae family standing out, since there are several species that can accumulate more than one type of metal (Gratao et al. 2005). From Table 7.1 to 7.4

Herbaceous, shrub, and tree species can be used for the phytoremediation of soils contaminated by heavy metals; in addition, 400 hyperaccumulatory species of the families Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae have been identified. Among the plants used for phytoremediation of lead are

Table 7.1 Main species used in phytoremediation

Scientific name	Common name
Anthoxanthum odoratum	Grama de olor
Agrostis canina	Heno gris
Agrostis capillaris	Pasto quila
Dactylopteryx flexuosa	Algarroba
Festuca ovina	Pasto
Holcus lanatus	Heno Blanco
Silene vulgaris	Colleja
Medicago sativa	Alfalfa
Zea mays	Maíz
Nicotiana tabacum	Tabaco
Sorghum bicolor	Sorgo
Amaranthus hybridus	Amaranto

Fuente: Volke-Sepulveda et al. (2005)

Table 7.2 Main species used in phytoremediation

Nombre científico	Nombre común
Spirodela intermedia	Lenteja de agua
Salvinia auriculata	Helecho africano
Pistia stratiotes	Lechuga de agua
Eichornia crassipes	Jacinto de agua
Ceratopteris thalictroides	Helecho de agua
Azolla caroliniana	Helecho de agua
Lemna minor	Lenteja de agua

Fuente: Arias et al. (2010)

sunflower (Helianthus annuus) and Indian mustard (Brassica juncea) (Volke-Sepulveda et al. 2005) (Tables 7.1 and 7.2).

7.4.4 Characteristics of a Phytoremediator Species

The main characteristics that macrophytes should have that can be used for a phytoremediation process are having a rapid growth rate, high productivity, preferably being local species, and being easy to manage, among others (Poveda and Velasteguí 2013).

7.4.5 Parameters to Determine the Phytoremediation Aptitude of a Plant

To determine if a plant species could be used in a phytoremediation treatment, the following parameters have to be taken into account: the bioconcentration factor (BCF) and the translocation factor (FT) (Ali et al. 2013). The FBC determines the

efficiency of the plant to accumulate the metal coming from the soil in its tissue and is calculated as follows:

$$\text{BCF} = \text{concentration of metal in tissue}/\text{concentration of metal in the soil}$$

While the FT indicates the efficiency to transport the metal from the root to its aerial part, it is calculated as follows:

$$\text{FT} = \text{metal concentration in the aerial part}/\text{concentration of metal in the root}$$

A plant can be considered an accumulator if its FT is equal to or greater than 1, while those that have the capacity to accumulate from 5 to 500 times more than the average are called "hyperaccumulators" (Rascio and Navari-Izzo 2011).

7.4.6 Mechanisms for Elimination of Pollutants by the Plant (Table 7.3)

Table 7.3 Breve descripción de los mecanismos de eliminación de contaminantes por la planta

Method	Description
Phytoextraction	The plants are harvested for further incineration, because their use can be dangerous. This mechanism includes both organic and inorganic pollutants that do not have the capacity to transform into nontoxic substances
Phytovolatilization	It occurs as growing plants absorb water along with various types of contaminants, some of which can reach the leaves and evaporate or volatilize in the atmosphere
Phytodegradation	It is responsible for transforming a pollutant into one less "harmful" to the environment, which can only occur with organic pollutants
Phyto-immobilization	It causes the subjection and reduction of the bioavailability of contaminants through the production of chemical compounds in the interface-soil-root which inactivate the toxic substances, either by adsorption or precipitation processes
Phytostabilization	Contaminants of soil or water are immobilized by adsorption, precipitation, and accumulation of substances in the roots of plants. In the same way, this process reduces the mobility of pollutants and prevents their migration to groundwater or air

Fuente: Velásquez (2017)

Table 7.4 Advantages and disadvantages of phytoremediation

Advantages	Disadvantages
The plants can be used as extractor pumps to purify contaminated soils and water	The process is limited to the penetration depth of the roots or shallow water
Phytoremediation is an appropriate method to decontaminate large areas or to finalize the decontamination of restricted areas in long terms	Phytotoxicity is a limiting factor in heavily contaminated areas. Risk for the food chain, if species used as source of food are chosen
Phytoremediation is a methodology with good public acceptance	The times of the process can be very long
Phytoremediation generates less secondary waste	It is necessary to better understand the nature of degradation products (phytodegradation)
The plants use solar energy. The treatment is in situ	Not all plants are resistant to grow in the presence of contaminants

Fuente: Velásquez (2017)

7.4.6.1 Advantages and Disadvantages of Phytoremediation

Although phytoremediation is an economic and in many ways beneficial technique for the recovery of soils, it may contain some disadvantages that must be known before starting a recovery project based on this technique. Phytoremediation among its advantages stands out as an appropriate method to decontaminate soil and water naturally, with the help of the biological processes of ecosystems and solar energy, without the need to add chemical substances that can become more dangerous than the same pollutants. However, phytoremediation is characterized by being a process that can take months or even years, which manifests itself as a disadvantage when choosing it, but as a viable alternative and friendly to the environment (Table 7.4).

References

Abatenh E, Gizaw B, Tsegaye Z, Wassie M (2017) Open journal of environmental biology the role of microorganisms in bioremediation-a review (en línea). Open J Environ Biol 2(1):38–46. https://doi.org/10.17352/ojeb.000007

Abdelhameed RE, Metwally RA (2019) Alleviation of cadmium stress by arbuscular mycorrhizal symbiosis. Int J Phytoremediation 28:1–9

Access, O. 2018 We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists TOP 1%. Long-Haul Travel Motivation by International Tourist to Penang i(tourism):13

Adriano DC (2001) Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risk of metals. Springer, New York, p 867

Ahmad P (2016) Plant metal interaction: emerging remediation techniques. s.l., s.e. 619 p. doi: https://doi.org/10.1016/B978-0-12-803158-2.00002-3

Aibibu N et al (2010) Cadmium accumulation in Vetiveria zizanioides and its effects on growth, physiological and biochemical parameters. Bioresour Technol 101:6297–6303

Ajayi AO, Abiola AK (2018) Microbial diversity of petroleum polluted soil at Ayetoro community in Ilaje Riverine oil producing areas of Ondo state, Nigeria. Prog Petrochem Sci 1(5):1–7. https://doi.org/10.31031/pps.2018.01.000525

Ali H, Khan E, Sjad MA (2013) Phytoremediation of heavy metals-concepts and applications. Chemosphere 91:869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075

Alloway BJ (2013) Heavy metals in soils, trace metals and metalloids in soils and their bioavailability, 3rd edn. Springer, Reading, p 587. https://doi.org/10.1007/978-94-007-4470-7

Arias S, Betancur F, Gómez G, Salazar J, Hernández M (2010) Fitorremediación con humedales artificiales para el tratamiento de aguas residuales porcinas. Informador Técnico (Colombia) 74:12–22

Baker AJM (1981) Accumulators and excluders -strategies in the response of plants to heavy metals. J Plant Nutr 3:643–654. https://doi.org/10.1080/01904168109362867

Beltrán-Pineda ME, Gómez-Rodríguez AM (2016) Biorremediación de Metales Pesados Cadmio (Cd), Cromo (Cr) y Mercurio (Hg), Mecanismos Bioquímicos e Ingeniería Genética: Una Revisión. Revista Facultad De Ciencias Básicas 12(2):172–197. https://doi.org/10.18359/rfcb.2027

Benitez-Campo N. (2011). Cleaner production and bioremediation for reduction of pollution in the industry of chrome tannery. Universidad del Valle, Cali, Colombia. Ambiente y Sostenibilidad (1):25–31. doi:https://doi.org/10.25100/ays.v1i1.4335

Bosse M, Heuwieser A, Heinzel A, Lukas A, Oliveira G, Mayer B (2018) Biomarker panels for characterizing microbial community biofilm formation as composite molecular process. PLoS One 13(8):1–20. https://doi.org/10.1371/journal.pone.0202032

Brundrett M (2004) Diversity and classification of mycorrhizal associations. Biol Rev Camb Philos Soc 79(3):473–495. Review

Cabral L, Soares CRFS, Giachini AJ, Siqueira JO (2015) Arbuscular mycorrhizal fungi in phytoremediation of contaminated areas by trace elements: mechanisms and major benefits of their applications. World J Microbiol Biotechnol 31(11):1655–1664

Cabrera C (2014) The concept and vision of development as a basis for evaluation. Econ Soc 18 (30):47–65

Cecchi M, Dumat C, Alric A, Felix-Faure B, Pradere P, Guiresse M (2008) Multi-metal contamination of a calcic cambisol by fallout from a lead-recycling plant. Geoderma 144(1–2):287–298

Cornejo P, Meier S, Borie G, Rillig MC, Borie F (2008) Glomalin-related soil protein in a Mediterranean ecosystem affected by a copper smelter and its contribution to cu and Zn sequestration. Sci Total Environ 406:154–160

Driver JD, Holben WE, Rillig MC (2005) Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. Soil Biol Biochem 37:101–106

Duffus JH (2002) Heavy metals – a meaningless term? (IUPAC technical report). Pure Appl Chem 74:793–807. https://doi.org/10.1351/pac200274050793

Ezekoye CC et al (2018a) Framework for monitoring bioremediation projects by regulatory agencies. Clin Biotechnol Microbiol 2.5:457–471

Ezekoye CC, Chikere CB, Okpokwasili GC (2018b) Field metagenomics of bacterial community involved in bioremediation of crude oil-polluted soil. J Bioremed Biodegr 09(05). https://doi.org/10.4172/2155-6199.1000449

Ferrera-Cerrato R, Rojas-Avelizapa N, Poggi-Varaldo HM, Alarcón A, Cañizares-Villanueva RO (2006) Procesos de biorremediación de suelo y agua contaminados por hidrocarburos del petróleo y otros compuestos orgánicos. Rev Latinoam Microbiol 48(2):179–187

Garzón JM, Rodríguez-Miranda JP, Hernández-Gómez C (2017) Aporte de la biorremediación para solucionar problemas de contaminación y su relación con el desarrollo sostenible. Universidad y Salud 19(2):309–318. https://doi.org/10.22267/rus.171902.93

Gastauer M, Vera MPO, de Souza KP, Pires ES, Alves R, Caldeira CF, Ramos SJ, Oliveira G (2019) Data descriptor: a metagenomic survey of soil microbial communities along a rehabilitation chronosequence after iron ore mining (en línea). Scientific Data 6:1–10. https://doi.org/10.1038/sdata.2019.8

Ghosh S, Das AP (2018) Metagenomic insights into the microbial diversity in manganese-contaminated mine tailings and their role in biogeochemical cycling of manganese (en línea). Sci Rep 8(1):1–12. https://doi.org/10.1038/s41598-018-26311-w

Ginocchio R (2000) Effects of a copper smelter on a grassland community in the Puchuncavi Valley, Chile. Chemosphere 41:15–23

Gonzalez-Chavez C, D'Haen J, Vangronsveld J, Dodd JC (2002) Copper sorption and accumulation by the extraradical mycelium of different Glomus spp. (arbuscular mycorrhizal fungi) isolated from the same polluted soil. Plant Soil 240:287–297

Gonzalez-Chavez MC, Carrillo-Gonzalez R, Wright SF, Nichols KA (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut 130:317–323

Gratao PL, Prasad MNV, Cardoso PF, Lea PJ, Azevedo RA (2005) Phytoremediation: green technology for the clean-up of toxic metals in the environment. Braz J Plant Physiol 17:53–64. https://doi.org/10.1590/S1677-04202005000100005

Herrera L (2017) New technologies for cleaner production: a state of the art. Polytechnic University Salesiana of Ecuador. Academic article. Available at: https://dspace.ups.edu.ec/bitstream/123456789/14334/1/UPS-GT001912.pdf

Honrubia M (2009) Las micorrizas: una relación planta-hongo que dura más de 400 millones de años. Anales Jard Bot Madrid 66S1:133–144

Jamarillo IR (2011) La micorriza arbuscular (MA) centro de la rizosfera: comunidad microbiologica dinamica del suelo. Contactos 81:17–23

Ji H, Zhang Y, Bararunyeretse P, Li H (2018) Characterization of microbial communities of soils from gold mine tailings and identification of mercury-resistant strain (en línea). Ecotoxicol Environ Saf 165(30):182–193. https://doi.org/10.1016/j.ecoenv.2018.09.011

Kanwal S, Bano A, Malik AN (2015) Effects of arbuscular mycorrhizal fungi on wheat growth, physiology, nutrition and cadmium uptake under increasing cadmium stress. IJAAR 7:30–42

Kazaure MB (2018) Distribution of bacteria in lead contaminated soil in Anka local government area, Zamfara state, Nigeria (en línea). Acta Sci Microbiol 1(7):2581–3226. Disponible en https://actascientific.com/ASMI/pdf/ASMI-01-0086.pdf

Khan NT, Jameel N, Khan MJ (2018) A brief overview of contaminated soil remediation methods. BioTechnol Ind J Res 14(4):168

Lenntech. (2016) Enfermedades transmitidas por el agua. Retrieved February 16, 2016, from http://www.lenntech.es/procesos/desinfeccion/deseases/enfermedadestransmitidas-por-el-agua.htm

Maeir RM, Pepper IL, Gerba CP (2009) Environmental microbiology, 2nd edn. Academic Press. Elsevier, San Diego California, p 585

Malekzadeh E, Aliasgharzad N, Majidi J, Aghebati-Maleki L, Abdolalizadeh J (2016) Cd-induced production of glomalin by arbuscular mycorrhizal fungus (Rhizophagus irregularis) as estimated by monoclonal antibody assay. Environ Sci Pollut Res Int 23(20):20711–20718. Epub 2016 Jul 29

Mansouri A, Abbes C, Ben Mouhoub R, Hassine SB, Landoulsi A (2019) Enhancement of mixture pollutant biodegradation efficiency using a bacterial consortium under static magnetic field. PLoS One 14(1). https://doi.org/10.1371/journal.pone.0208431

Meeboon N, Leewis MC, Kaewsuwan S, Maneerat S, Leigh MB (2017) Changes in bacterial diversity associated with bioremediation of used lubricating oil in tropical soils. Arch Microbiol 199(6):839–851. https://doi.org/10.1007/s00203-017-1356-3

Muralikrishna, I V.; Manickam, V. (2017) Environmental management: science and engineering for industry (en línea). s.l., s.e. 1–664 p. DOI: https://doi.org/10.1016/B978-0-12-811989-1.00001-4

Occhipinti A, Eyassu F, Rahman TJ, Rahman PKSM, Angione C (2018) In silico engineering of pseudomonas metabolism reveals new biomarkers for increased biosurfactant production. PeerJ 6:e6046. https://doi.org/10.7717/peerj.6046

Pajević S, Borišev M, Nikolić N, Arsenov DD, Župunski M (2016) Phytoextraction of heavy metals by fast-growing trees: a review. In: Ansari AA (ed) Phytoremediation. Springer International, Cham

Poveda R y Velasteguí R (2013). Evaluación de especies acuáticas flotantes para la fitorremediación de aguas residuales industrial y de uso agrícola previamente caracterizadas en el cantón ambato, provincia de tungurahua. Carrera ingeniería bioquímica, Universidad Técnica de Ambato, Ambato, Ecuador, URL http://repositorio.uta.edu.ec/jspui/handle/123456789/8455i

Purin S, Rillig MC (2007) The arbuscular mycorrhizal fungal protein glomalin: limitations, progress, and a new hypothesis for its function. Pedobiologia 51:123–130

Rai PK (2016) Biomagnetic Monitoring of Particulate Matter: In the Indo-Burma Hotspot Region (en línea). s.l., s.e. 1–198 p. Disponible en https://www.scopus.com/inward/record.uri?eid=2-s2.0-85014489011&partnerID=40&md5=ca1fcd5eaa99424badbba5f56ebaebf2

Ramakrishnan B, Megharaj M, Venkateswarlu K, Sethunathan N, Naidu R (2012) Reviews of Environmental Contamination and Toxicology Volume 218 (en línea). s.l., s.e., vol. 218. doi: https://doi.org/10.1007/978-1-4614-3137-4

Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumalating plants: how and why do they do it? And what makes them so interesting? Plant Sci 180:169–181. https://doi.org/10.1016/j.plantsci.2010.08.016

Samarghandi MR, Arabestani MR, Zafari D, Rahmani AR, Afkhami A, Godini K (2018) Bioremediation of actual soil samples with high levels of crude oil using a bacterial consortium isolated from two polluted sites: investigation of the survival of the bacteria. Global NEST J 20 (2):432–438

Schneider J, Bundschuh J, do Nascimento CWA (2016) Arbuscular mycorrhizal fungi-assisted phytoremediation of a lead-contaminated site. Sci Total Environ 572:86–97

Singh R (2014) Microorganism as a tool of bioremediation technology for cleaning environment: a review. Proc Int Acad Ecol Environ Sci 4(1):1

Singh Sidhu GP (2018) Heavy metal toxicity in soils: sources, remediation technologies and challenges. Adv Plants Agric Res 5(1):445–446. https://doi.org/10.15406/apar.2016.05.00166

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press, Cambridge

Toro M, Gamarra R, López L, Infante C (2017) Arbuscular mycorrhizal fungi and the remediation of soils contaminated with hydrocarbons. In: Anjum N (ed) Chemical pollution control via microorganisms. Nova Science, New York, pp 79–96

Upadhyaya H, Panda SK, Bhattacharjee MK, Dutta S (2010) Role of arbuscular mycorrhiza in heavy metal tolerance in plants: prospects for phytoremediation. J Phytology 2(7):16–27

Velásquez A (2017) Contaminación de suelos y aguas por hidrocarburos en Colombia. Análisis de la fitorremediación como estrategia biotecnológica de recuperación. Revista de Investigación Agraria y Ambiental – Volumen 8 Número 1 – enero - junio de 2017 – ISSN 2145–6097

Ventorino V, Pascale A, Adamo P, Rocco C, Fiorentino N, Mori M, Faraco V, Pepe O, Fagnano M (2018) Comparative assessment of autochthonous bacterial and fungal communities and microbial biomarkers of polluted agricultural soils of the terra dei Fuochi (en línea). Sci Rep 8 (1):1–13. https://doi.org/10.1038/s41598-018-32688-5

Volke-Sepulveda T, Velasco-Trejo JA, de la Rosa Pérez DA (2005) Suelos contaminados por metales y metaloides: muestreo y alternativas para su remediación. Secretaría de Medio Ambiente y Recursos Naturales. Instituto Nacional de Ecología, Ciudad de México, México, p 144

Wang J, Wang J, Zhang Z, Li Y, Zhang B, Zhang Z, Zhang G (2016) Cold-adapted bacteria for bioremediation of crude oil-contaminated soil. J Chem Technol Biotechnol 91(8):2286–2297. https://doi.org/10.1002/jctb.4814

Wright SF, Upadhyaya A (1998) A survey of soils for agrgregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil 198:97–107

Xercavins J, Cayuela D, Cervantes G, Sabater A (2005) Sustainable development. Editions UPC, Catalunya

Xu X, Liu W, Tian S, Wang W, Qi Q, Jiang P, Gao X, Li F, Li H, Yu H (2018) Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: a perspective analysis. Front Microbiol 9:1–11. https://doi.org/10.3389/fmicb.2018.02885

Yuan M et al (2014) Enhancement of Cd phytoextraction by two Amaranthus species with endophytic Rahnella sp. JN27. Chemosphere 103:99–104

Zafarzadeh A, Rahimzadeh H, Mahvi AH (2018) Health risk assessment of heavy metals in vegetables in an endemic esophageal cancer region in Iran. Health Scope 7(3):e12340

Microbial Degradation of Pharmaceuticals and Personal Care Products from Wastewater

8

Sudeeptha Girijan and Mathava Kumar

Abstract

Pharmaceuticals and personal care products (PPCPs) are a matter of emerging concern. Their concentrations have been detected worldwide in aqueous and soil matrices. PPCPs are present more frequently in aqueous matrices and are found to be highly toxic to aquatic organisms. The partial treatment and subsequent discharge of PPCPs from wastewater treatment plants make them a major source of PPCP contamination of water bodies. The extent of PPCP removal depends on the type of treatment, and the efficiency of both physicochemical and biological methods is introduced. However, biological methods offer a much cheaper, less energy-intensive, and sustainable option when compared to the other methods. The removal efficiencies under different biological environments show that aerobic conditions are most sited for PPCP removal. Moreover, the literature also conveys that for most of the compounds, biodegradation was the major pathway except for some highly hydrophobic compounds. The major factors affecting PPCP removal are also discussed in detail. A case study with metronidazole as the target compound and a suspended mixed culture demonstrates the biodegradation process in conventional biological systems under varying PPCP, MLSS concentrations, and C/N ratios. However, the problem associated with proliferation of antibiotic-resistant bacteria is a major concern and needs to be resolved.

Keywords

Biodegradation · Pharmaceuticals · Metronidazole · Design of experiments · Wastewater treatment

S. Girijan · M. Kumar (✉)
Environmental and Water Resources Engineering Division, Department of Civil Engineering, IIT Madras, Chennai, India
e-mail: mathav@iitm.ac.in

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_8

8.1 Introduction

The term PPCP stands for pharmaceuticals and personal care products. These compounds attend to various personal needs of humans, including health benefits, agriculture, livestock management, etc., and have been in use for a very long time. However, they have started to emerge as a new class of contaminants due to their apparent deteriorative effect on the aquatic ecosystem. These compounds are administered directly to humans or animals, and their occurrence in the environment has been limited to very low concentrations usually ranging from a few ng/L to μg/L (Carballa et al. 2007). This is one of the major reasons that they have gone undetected till date. The advent of new analytical techniques capable of detecting even trace concentrations of compounds has led to the discovery of the widespread occurrence of these compounds, especially in the aquatic ecosystem. Since then, their occurrence and fate in various environmental systems have been studied fervently. Even so, analysis techniques are not fully efficient in predicting PPCP concentrations due to lack of knowledge of transformation products.

Rapid industrialization, globalization, and growth in the health industry have increased the overall usage rate of PPCPs (Fent et al. 2006). After use, these compounds end up in the waste stream through sewage, runoff, etc. One of the major reasons of concern toward these pollutants includes lack of any proper quality standards regarding PPCP discharge, which means there is an unregulated discharge of these pollutants into the environment. Standards for PPCP discharge cover a few limited compounds and have been adopted by only a few countries. In India, IS 10500:2012 specifies limits for 18 pesticides. Thus a variety of different compounds are introduced into the environment. These waste streams act as a sink for the PPCPs, and the synergistic and cumulative effects of these compounds are not known well.

In addition, the conventional biological-based treatment systems are not efficient in removing these compounds. The compounds, especially pharmaceuticals, are made resistant to biodegradation so that they serve their intended purpose. For this reason, these compounds released as such or in their metabolized forms end up in surface water and other drinking water sources. This growing gap between usage and removal leads to accumulation of these compounds in the aquatic systems. PPCPs have been reported to cause adverse effects even at trace concentrations, especially hormone-mimicking and hormonal preparations (Fent et al. 2006). The effects of these compounds at relatively high concentrations on the aquatic life and subsequently on humans are largely unknown. The metabolic pathway of these compounds during biodegradation is also not understood well. Many compounds, such as carbamazepine, diclofenac, etc., are found to show negative removal (Blair et al. 2015) in activated sludge processes which are attributed to the conjugation of their metabolized forms to produce the parent compound. In addition, knowledge of the toxicity of PPCPs on aquatic life is limited to acute data for single compounds without much regard to cumulative effects of multiple compounds and transformation products.

Another major concern related to the occurrence of PPCPs in aquatic systems is the possible occurrence of antibiotic-resistant genes. The presence of relatively high concentrations of antibiotics over prolonged periods can act as a stress and lead to the development of resistant genes in the microbes naturally occurring in that ecology. Since there may be more than one antibiotic, the strains developed may be multidrug resistant. Although naturally occurring microflora are largely non-pathogenic, genetic material can transfer between microbes through exchangeable genetic components such as plasmids.

In summary, we can say that wide and high level usage of these compounds, recalcitrant and bioaccumalative nature of some of them, inability of conventional treatment processes to remove PPCPs, possible health effects on humans and aquatic life, response generated from microbial communities like origination of antibiotic resistance genes, poor understanding of the metabolic pathways, synergistic effects of these pollutants, etc. make these contaminants a serious concern to the ecosystem. Although the direct effects of these trace organic contaminants are not apparent, the contamination is bound to increase with the years. One of the key focus areas to mitigate this problem is improving the existing treatment systems to effect PPCP removal. As part of this, studies have explored various methods like advanced oxidation processes, adsorption, etc. However, biodegradation is a more sustainable option and also convenient for a retrofitting of the existing wastewater treatment system. Therefore, it is essential to divert some focus to understanding the biodegradation of PPCPs to effectively mitigate the problem of PPCP contamination.

This chapter tries to introduce to the reader the significance and the current state of PPCP contamination. Further on, the fate of PPCPs in various biological treatment systems and the mechanism of their removal are discussed. Their behavior with variations in the wastewater characteristics and reactor environment is also discussed. This is followed up with a case study on the removal of a model PPCP compound under varying parameters. Finally, the future direction and scope of the topic is elaborated.

8.2 PPCPs

Pharmaceuticals can be classified under the various therapeutic classes which include antibiotics, analgesics, lipid regulators, steroids, hormones, psychiatric drugs, etc., whereas personal care products include fragrances, sunscreen agents, insect repellants, etc. (Ellis 2006). They are organic in nature with functional groups such as amides, azoles, carboxyl, amines, etc. attached to them. After consumption, most of these compounds are excreted as such or in their metabolized forms. These compounds eventually end up in the environment since further degradation of PPCPs within the environment is less or nonexistent. The occurrence of PPCPs has been reported majorly in aqueous matrices and to a lesser degree in solid matrix. The reported occurrences and the pathway of PPCPs within the environment are explained in the following sections.

8.2.1 Exposure Route of PPCPs in the Environment

The major sources of PPCPs are from domestic households, industrial wastewaters, hospital effluents, and runoff from animal farming, agriculture, and aquaculture (Ellis 2006). The concentrated effect of PPCP contamination can be observed in the waste effluent waters of domestic households, industries, and hospitals, whereas the discharges of the other sources are usually direct without treatment. Thus we can consider the original source of PPCPs to be the manufacturing industries from where they are distributed for human and animal consumption. PPCPs are then excreted in their metabolized forms or otherwise and end up in the waste stream. The exposure route of PPCPs starting from manufacturing units to its occurrence in surface and drinking waters is shown in Fig. 8.1.

While most of the PPCPs consumed by humans ideally enter the waste treatment units, some amounts are also indiscreetly introduced directly into the environment during recreation, through soak pits, etc. The PPCPs such as veterinary antibiotics and others meant for animals usually come out as nonpoint discharges into the aqueous or soil matrix without any treatment. Runoff from fields and application of manure are the major pathways for occurrence of veterinary antibiotics in the environment (Kemper 2008).

The fate of PPCPs in the environment as well as the treatment plants is greatly affected by the properties of the compound such as the K_d value, Henrys constant, pK_a value, etc. PPCPs entering the treatment plants undergo partial degradation resulting in residual concentrations and possible degradation intermediates in the discharge. This causes the occurrence of PPCPs in surface water sources. Subsurface water flows may lead to interaction and transfer of these contaminants to groundwater as well. Solid wastes generated in the hospitals, households, as well as the treatment plants with or without further treatment are disposed directly to the soil or in landfills. These PPCPs can spread into groundwater or surface water through leachate and runoff. Groundwater and surface water reservoirs acting as raw water sources introduce these PPCPs into drinking water which is the main point of exposure to humans. Other organisms are exposed to PPCP contamination mainly via surface water contamination.

8.2.2 Occurrence of PPCPs

PPCP contamination is widespread occurring all across the globe, and the conventional treatment process adopted in WWTPs is efficient in removing only a few easily degradable compounds such as salicylic acid, ibuprofen, acetaminophen, etc. (Kimura et al. 2005; Sipma et al. 2010). This is reflected in the positive findings of PPCPs in wastewaters, surface waters, groundwaters, and even drinking waters. However, PPCPs may also be introduced into the environment exclusive of WWTPs such as through runoffs from organic farms, poultry farms, landfills, etc. The concentrations of PPCPs recorded are usually highest in wastewater influents and effluents followed by surface waters probably due to dilution. Groundwaters and

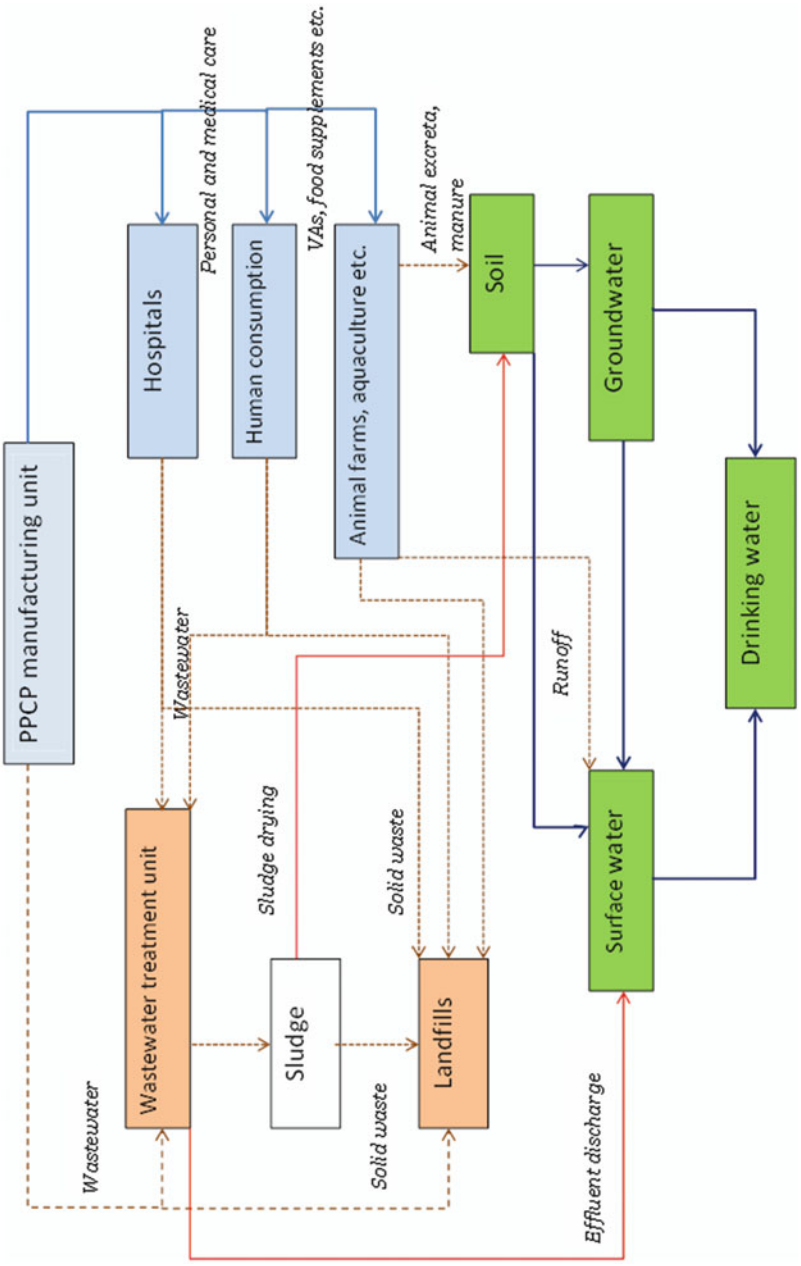


Fig. 8.1 Exposure route of PPCPs in the environment

drinking waters show still lower concentrations of PPCPs. However, their concentrations in these matrices can potentially go up in the coming years. A comparison of the PPCP contamination in different wastewater matrices is shown in Fig. 8.2.

8.2.2.1 Occurrence of PPCPs in Wastewaters

As a consequence of treatment inefficiency of PPCPs, WWTPs are one of the major sources of PPCP contamination. PPCP occurrence has been reported in WWTPs of China, Korea, Greece, Spain, etc. The influent concentration of PPCPs is found to be affected mainly by usage (Choi et al. 2008; Luo et al. 2014), and the effluent concentration is dependent on factors such as treatment type, operating parameters, and biodegradability of the compound. For example, caffeine was found to be the most abundant compound in WWTPs in Greece which could be attributed to their higher usage (Kosma et al. 2014); lincomycin and oxytetracycline, having very high degradability, showed low concentration even at higher usage value (Hu et al. 2010). Type of wastewater can also influence the influent concentrations. A comparison of urban and hospital wastewater in Ioannina, Greece indicates higher concentrations of antibiotics for hospital wastewaters but no significant difference in concentration of other compounds (Kosma et al. 2014). However, a more comprehensive review shows average concentrations of PPCPs much higher in hospital wastewaters (ranging from 4 to 150 times) than in urban wastewaters (Verlicchi et al. 2012). Livestock wastewater is also suggested as a major source of antibiotics, showing a maximum concentration of 211 μg/L in China (Liu et al. 2013).

PPCP concentrations are detected in influent as well as effluent of WWTPs and vary depending on the removal efficiency of WWTP for that particular compound. Study of five WWTPs in Ulsan, Korea showed high influent concentrations of acetaminophen, atenolol, and lincomycin. While acetaminophen was almost completely removed, atenolol was removed with 64% efficiency, and lincomycin was removed with an efficiency of −11%. This indicates a higher concentration in the effluent than at the influent (Kumar et al. 2011). Negative removal has also been reported for carbamazepine (Sipma et al. 2010) and has been explained as transformation of metabolites into parental form. Other possible explanations include desorption from particulate matter, atmospheric deposition, hydrolysis, and errors in estimation.

8.2.2.2 Occurrence of PPCPs in Surface Waters

One of the major causes of surface water contamination is discharge from WWTPs. A study conducted in Korea showed higher PPCP concentrations at sampling points downstream of WWTPs indicating the role of wastewater discharge in surface water contamination (Choi et al. 2008). The usually observed concentrations in surface waters are much lesser than in wastewaters which could be attributed to dilution, sorption, biodegradation, photodegradation, etc. (Buser et al. 1998; Luo et al. 2014).

Carbamazepine, triclosan, and parabens were investigated in surface waters of India. Concentration of carbamazepine ranged from 5 to 20 ng/L which was similar to studies in Korea. Triclosan was found to be the most abundant with maximum

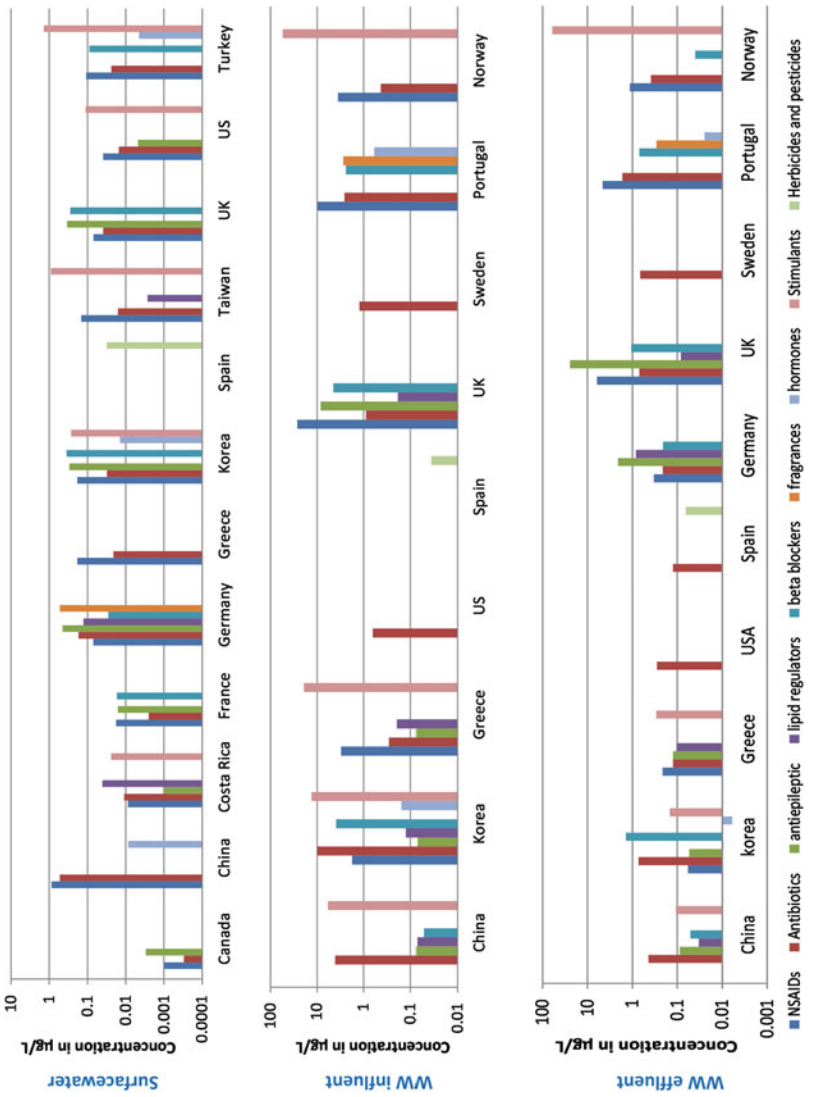


Fig. 8.2 Comparison of PPCP concentrations reported in different aqueous matrices across the globe

concentrations reaching 5 μg/L. Their concentrations in aqueous and sediment phase indicated that these concentrations were independent parameters with the sediment phase concentrations showing low variations (Ramaswamy et al. 2011). The distribution of PPCPs in surface waters can also be related to usage, dosage, excretion rate, etc. similar to wastewater trends. Pharmaceutical compounds in surface waters of France showed low values within 25 ng/L except for metformin (antidiabetic drug) which showed a maximum value of 735 ng/L which has a high usage and dosage (Vulliet and Cren-Olivé 2011). A nationwide survey of surface waters of the USA indicated a very high detection frequency (DF) for the PPCP compounds with steroids showing the highest frequency of about 90%. However, the highest concentration percentage was observed for detergent metabolites followed by steroids and plasticizers showing that higher DF need not necessarily translate to higher concentration and vice versa. Although nonprescription drugs had a high DF, their concentration percentages were less. However, highest concentration of caffeine observed was around 6 μg/L which is much higher than most of the other observations within 1 μg/L (Kolpin et al. 2002).

8.2.2.3 Occurrence of PPCPs in Groundwater

Groundwater may get contaminated with PPCPs through septic tank seepage, landfill leaching, contaminated recharge water, contaminated soil, etc. (Hu et al. 2010; Teijon et al. 2010). Study of seasonal variations and PPCP concentrations in groundwater was found to have no correlation (Peng et al. 2014) and is suggested to be more or less a function of subsurface transport processes including retention time (Teijon et al. 2010). In the same study, landfill leachates, as a source of PPCP contamination of groundwater, were studied. The results indicated that groundwater, though contaminated with PPCPs, did not show any strong correlation with the distance from landfill site. PPCP concentrations in groundwater were found to be generally lower than nearby surface water or wastewater concentrations (Teijon et al. 2010; Peng et al. 2014). PPCP concentrations detected in groundwater across different countries usually fell within 100 ng/L (Peng et al. 2014).

8.2.2.4 Occurrence of PPCPs in Other Sources

Apart from aqueous phase, PPCPs have also been detected in sediments and soil (Ramaswamy et al. 2011). One of the causes of soil contamination is through manure application (Hu et al. 2010). Study of transport of contaminants in soil-aqueous systems shows that contaminants with higher water solubility end up in aqueous systems and those with higher K_d values end up in soil systems. One of the most common cases of occurrence of PPCPs in the solid phase is in the sewage sludge. The presence of PPPCPs in sewage sludge affects their treatability, disposal, and reuse/recovery applications (Narumiya et al. 2013). A study conducted in four STPs in Japan found 45 PPCP compounds in the secondary sludge of an activated sludge process with concentrations ranging from 0.1 to 10^4 μg/kg (Narumiya et al. 2013). PPCPs have also been detected in drinking water across the globe at comparatively lower concentrations ranging from 0 to 100 ng/L (Yang et al. 2017). PPCPs like

carbamazepine, ibuprofen, gemfibrozil, and bisphenol-A were detected in drinking waters in Canada (Kleywegt et al. 2011).

8.2.3 Removal in Physicochemical and Biological Systems

The widespread occurrence of PPCPs and their presence even in drinking waters is alarming. The intervention to this problem needs to happen at the treatment plants receiving wastewaters. This has fuelled a lot of research into the treatment of PPCP-contaminated wastewater. Various biological and physicochemical processes have been researched for PPCP removal. Removal efficiencies vary with the type of process investigated (Table 8.1). One of the major setbacks encountered is the trace amounts of the pollutant present which increases their chances of bypassing the treatment especially when present in complex wastewaters along with gross organic contaminants.

8.2.3.1 Advanced Oxidation Processes

Among the physicochemical processes, advanced oxidation processes (AOPs) are one of the most commonly investigated techniques for PPCP removal (Luo et al. 2014). They include photocatalytic processes (solar or UV catalyzed), ozonation, electrolysis, ultrasonication, microwave, pulsed plasma, etc. Advanced oxidation processes rely on the high oxidative capacity of free radicals such as hydroxyl radicals for the degradation of trace contaminants (Mohapatra et al. 2014; Carra et al. 2015). They are effective in the removal of even persistent PPCPs such as carbamazepine (Mohapatra et al. 2014). AOPs have a high degradation rate and are devoid of a secondary waste stream (Mohapatra et al. 2014). However, one of the limitations associated with these systems are their energy efficiencies. Many researches are being conducted to improve the energy efficiency and reduce the cost of treatment. UV-LEDs were studied as a potential measure in reducing the energy input and successfully employed for the degradation of acetamiprid in a photo-Fenton process (Carra et al. 2015).

8.2.3.2 Membrane Separation Processes

Membrane separation processes help in PPCP removal by a purely physical process of size exclusion, filtration, and electrostatic repulsion. They include ultrafiltration, nanofiltration, and reverse osmosis. Reverse osmosis was found to remove 41 investigated PPCPs at removal efficiencies greater than 97%. However, the comparison of RO with an ozone/biofilter treatment revealed that the energy consumption was about five times higher for an RO process (Lee et al. 2012). Moreover, waste streams generated need further treatment.

8.2.3.3 Biological Processes

Biological processes such as activated sludge process (ASP), sequencing batch reactors (SBRs), upflow anaerobic sludge blanket (UASB), and biofilters and fixed and moving bed reactors have been studied for PPCP removal. The PPCP removal

Table 8.1 Removal efficiencies of PPCPs under different physicochemical and biological treatment methods

Process	Operating conditions	Compound	Removal efficiency (in %)	References
Photo-Fenton process	UVC-LED illumination at 20 W/m^2; 12 mg/L H_2O_2 and 3 mg/L Fe; 20 min run; 100 µg/L initial concentration	Acetamiprid	100	Carra et al. (2015)
Ozone-biofiltration	4–8 mg/L initial concentration	Amoxicillin, ATL, butalbital, caffeine, CBZ, DEET, dilantin, NPX, sucralose, SMX, TCEP	100	Lee et al. (2012)
		Iohexal, iopromide, meprobamate, primidone	60, 80, >70, >80	
Reverse osmosis	12 LMH; 7 bar operating pressure; 2% recovery	41 PPCPs	>97%	Lee et al. (2012)
Constructed wetland	Hybrid full scale; 317 m^2 vertical flow; 229 m^2 horizontal flow; 240 m^2 free water surface; *Phragmites australis* and mixed species	IBP, DCF, ACE, tonalide, TCS, bisphenol A, oxybenzene, ethinyl estradiol	>99, >89, 99, 90, 79, >99, 100, 100	Ávila et al. (2014)
Anoxic-aerobic photobioreactor	2 d HRT; 90–200 mg/L carbon loading	IBP, NPX, SA, TCS, propylparaben	91, 28.7, 83, 85, 87	López-Serna et al. (2019)
Anaerobic-anoxic-aerobic photobioreactor	3–4 d HRT; 200 mg/L carbon loading	IBP, NPX, SA, TCS, propylparaben	94, 52, 97, 100, 100	López-Serna et al. (2019)
Upflow anaerobic sludge blanket (UASB)	0.5 to1 d-HRT; 0.5 to 1 m/h upflow velocity; SRT > 100 d	SMX, TMP, and NPX	>80	Alvarino et al. (2014)
		Fragrances and hormones	40–60	
		CBZ, IBP, ERY, RXM, DZP, FLX, DCF	<20	
AnMBR (UASB + MBR)	HRT 6 h; SRT 30 d; 30 °C; pH 7.5; HF membrane module of 0.02 m^2 and 0.04 µm	TCS, SMX, androsterone, Nonylphenol, androstenedione, testosterone	>90	Monsalvo et al. (2014)
		DCF, IBP, CBZ, meprobamate, atrazine, estriol, estrone, DEET, and primidone	<10	

(continued)

Table 8.1 (continued)

Process	Operating conditions	Compound	Removal efficiency (in %)	References
Granular SBR	2.25 g/L MLSS; 22 °C; pH of 7–7.5; 2.52 kg COD/m^3 d; 8 cycles per day	Prednisolone, NPX, SMX, NFX	70–85	Zhao et al. (2014)
		IBP	45	
Aerobic SBR	HRT, 1 d; SRT, 10 d; 8 h cycles, DO > 2 mg/L	ATL, TMP, SMX	89, 21, 38	Stadler et al. (2015)
Anoxic/aerobic SBR	DO > 2 mg/L for half the reaction time		63, 3.4, 35	
Micro aerobic SBR	DO 0.3 mg/L		73, 5.8, 62	
MBBR	Biomass attached on plastic carriers; three-stage MBBR treating hospital effluent, 15–18 °C, flowrate 0.5 L/h, 50% filling ratio	B-blockers, X-ray contrast media, sulfonamides, IBP, clarithromycin other analgesics, CBZ, ERY TMP	5–40, 60–80 <20, >90, 0.90,<20, <20, <20, 30	Casas et al. (2015)
FBBR	HRT, 4 d; no biomass removal; pelletised *Trametes versicolor*	Clofibric acid	80	Cruz-Morató et al. (2013)
Oxidation ditch	1800 m^3 reactors with surface aeration; HRT, 1 d	18 PPCPs	17 out of 18 > 75, DCF < 25	Salgado et al. (2012)
CAS	HRT, 13 h; MLSS, 1.7 g/L	IBP; ketoprofen, mefenamic acid, NPX; clofibric acid, dichlorprop, DCF	>90, medium removal, <50	Kimura et al. (2005)
MBR	0.04 μm pore size; 0.09 m^2 nominal surface area; 2.5 g/L to 12 g/L MLVSS; temperature − 18 to 24 °C; pH −6.5 to 8.5; DO, 2–3 mg/L; HRT, 0.5–1 d; SRT, 72 d	NPX and IBP; ERY and ROX; fragrances; SMX; TMP; CBZ, diazepam, and DCF	>85, >75, >45, 52, 36, <25	Reif et al. (2008)

ATL atenolol, *CBZ* carbamazepine, *DEET* N,N-diethyl-m-toluamide, *NPX* naproxen, *SMX* sulfamethoxazole, *TCEP* tris(2-carboxyethyl)phosphine, *IBP* ibuprofen, *DCF* diclofenac, *ACE* acetaminophen, *TCS* triclosan, *SA* salicylic acid, *ROX* roxithromycin

efficiencies observed in the most commonly employed biological processes are given in Table 8.1.

In addition to heterotrophic bacteria, other agents such as symbiotic cultures of bacteria and algae have also been studied for PPCP removal. The high HRTs in these

systems favor PPCP removal. However, compounds such as naproxen, which were well removed by bacterial systems, were removed only by 52% in an algal-bacterial photobioreactor (López-Serna et al. 2019). Moreover, white rot fungi, *Phanerochaete chrysosporium*, immobilized on wood chips was found to remove carbamazepine (CBZ) and naproxen (NPX) by 61 and 90.35%, respectively (Li et al. 2015). These organisms are known for their nonspecific oxidative enzymes and represent an attractive means of removal for persistent compounds such as PPCPs.

Constructed wetlands are natural systems that have been successfully employed for PPCP removal. Although phytoremediation is the major removal pathway, adsorption onto soil, photodegradation, etc. also play a prominent role in the removal. Various flow configurations like vertical flow, horizontal flow, and free water systems have varying effect on PPCP removal. A hybrid wetland consisting of all three configurations in series was found to remove all the investigated PPCPs by more than 80% (Ávila et al. 2014). However, the large space requirement makes them unsuitable for high-throughput systems.

Among the biological-based processes, MBR technology is very promising for PPCP removal. MBR is an advanced biological treatment method, which combines activated sludge process with membrane technology for solid separation. The ability of MBR to retain a high biomass concentration and high SRT is an advantageous trait to treat persistent compounds such as PPCPs (Kimura et al. 2005; Sipma et al. 2010). In addition, the fouling layers on the membrane are also found to aid in PPCP removal (Monsalvo et al. 2014). MBRs have been constantly demonstrated to perform better than conventional activated sludge systems (Radjenović et al. 2009; Sipma et al. 2010). MBRs are more efficient in treating compounds with intermediate degradability. However, compounds with low degradability (such as carbamazepine and diclofenac) are untreated or ineffectively treated in both systems (Kimura et al. 2005; Radjenović et al. 2009).

8.2.4 Effects of PPCPs on the Ecosystem

The presence of PPCPs is found in both aqueous and soil matrices. However, the abundance and the exposure to aquatic sources make the effects of PPCPs in aquatic systems more important. One of the major and direct effects of PPCPs is the toxicity to the aquatic life. PPCPs, especially pharmaceuticals, are potentially very toxic not only to lower organisms such as the microbes but also to other aquatic organisms such as crustaceans, fishes, etc. Pharmaceuticals are manufactured to act on specific enzymes or other biomolecules to manipulate or aid various functions of the body. However, these compounds are administered at regulated dosages. The adverse effect of PPCPs on higher organisms can be explained by the unregulated exposure and similarity in the biomolecules performing important bodily functions such as reproductive, neural, and gastric functions (Fent et al. 2006). These biomolecules act as receptors for the compounds which interrupts the metabolic pathways of the organism. The toxicity studies carried out for PPCPs mainly focus on acute toxicity and also are simplified to the context of a single compound. The real scenario is quite

different since the pharmaceuticals are quite widespread. The toxicity of the metabolites of the compounds is also not well investigated (Fent et al. 2006). Acute toxicity results are usually carried out within 48 h and the toxicity is represented in terms of EC_{50} values. EC_{50} values may vary for a single compound depending on the type of target organism. Results for the toxicity studies of PPCPs on different trophic level organisms are shown in Table 8.2. In general, the values are lower for lower trophic level organisms. Propanolol, fluoxetine, and diclofenac are some of the most toxic pharmaceuticals investigated (Cleuvers 2003; Fent et al. 2006).

Most of the pharmaceuticals have EC_{50} values higher than 1 mg/L which is higher than their usual environmental concentrations (Fent et al. 2006). However, the chronic toxicity values are lower than acute toxicity, sometimes even by three orders of magnitude (Fent et al. 2006) and may not necessarily follow the same order as acute toxicity values (Wollenberger et al. 2000). It was observed that veterinary antibiotics, oxolinic acid and tiamulin, were toxic to *Daphnia magna* at environmentally relevant concentrations (4.6 and 40 mg/L, respectively) (Wollenberger et al. 2000). Estrogen, EE_2 (17α-ethinylestradiol), was also found to impair the male sexual development in fat head minnows at concentrations as low as 4 ng/L (Länge et al. 2001). Toxicity studies have also been carried out for aquatic plants. *Lemna minor* (duckweed) was found to be more sensitive to six of the nine pharmaceuticals investigated when compared with *Daphnia magna* and *Desmodesmus subspicatus* (Cleuvers 2003). Moreover, acute toxicity studies conducted for mixtures of compounds showed that the mixture toxicity varied with the mode of action of each pharmaceutical and followed the theories of similar or dissimilar action (Cleuvers 2003). In addition, the toxicity values of clarithromycin and its major metabolite, 14-hydroxy(R)-clarithromycin, were found to be similar (Baumann et al. 2014). This stresses the importance of studying the effluent quality not only in terms of PPCP removal but also in terms of toxicity.

In addition to the direct toxic effects on aquatic organisms, they can also affect the ecosystem by encouraging the development of antibiotic-resistant traits in microbes. The presence of tetracycline- and sulfonamide-resistant genes was detected in the agricultural wastewaters and sediments in Colorado, USA. The quantity of antibiotic-resistant genes (ARGs) could be positively correlated to the agricultural/urban activity (Pruden et al. 2006).

8.3 Fate of PPCPs in Biological Systems

Domestic and industrial wastewaters are conventionally treated through a common schematic involving a primary treatment like coagulation and sedimentation, followed by a secondary biological treatment. This could then be followed by a tertiary treatment like chlorination, UV, ultrafiltration, etc. Most conventional wastewater treatment systems employ an activated sludge process for organic matter removal. In addition, nitrification and denitrification units may also be installed for nutrient removal. Moreover, sludge obtained from the primary and secondary

Table 8.2 Toxicity effects of various PPCPs on different trophic level organisms

Target organism	Compound	Toxicity test	Toxicity unit	Toxicity value	References
Daphnia magna	CA, CBZ, IBP-Na, DCF-Na, NPX-Na, captopril, metformin, propanolol, metoprolol, oxilinic acid, tiamulin, SDZ, streptomycin, tylosin, oxytetracycline,	Acute toxicity 48 h	EC_{50} (mg/L)	72, >100, 108, 68, 174, >100, 64, 7.5, >100, 4.6, 40, 221, 487, 680, >1000	Cleuvers (2003), Wollenberger et al. (2000)
	TET, MNZ, olaquindox	Acute toxicity 48 h	NOEC (mg/L)	340, 1000, 1000	Wollenberger et al. (2000)
	Tiamulin, SDZ, TET, oxytetracycline	Chronic 21 d test	EC_{50} (mg/L)	5.4, 13.7, 44.8, 46.2	Wollenberger et al. (2000)
	Oxilinic acid, streptomycin, tylosin, MNZ	Chronic 21 d test	NOEC (mg/L)	0.38, 32, 45, 250	Wollenberger et al. (2000)
	Clarithromycin, 14-hydroxy(R)-clarithromycin, N-desmethyl clarithromycin	Acute 48 h immobilization test	EC_{50} (mg/L)	>2, >2, >0.7	Baumann et al. (2014)
Desmodesmus subspicatus	CA, CBZ, IBP-Na, DCF-Na, NPX-Na, captopril, metformin, propanolol, metoprolol	Acute toxicity 48 h	EC_{50} (mg/L)	115, 74, 315, 72, >320, 168, >320, 5.8, 7.3	Cleuvers (2003)
	Clarithromycin, 14-hydroxy(R)-clarithromycin, N-desmethyl clarithromycin	Acute 72 h growth	EC_{50} (μg/L)	37.1, 46.3, 575.2	Baumann et al. (2014)
Lemna minor	CA, CBZ, IBP-Na, DCF-Na, NPX-Na, captopril, metformin, propanolol, metoprolol	Acute toxicity 48 h	EC_{50} (mg/L)	12.5, 25.5, 22, 7.5, 24.2, 25, 110, 114, >320	Cleuvers (2003)
	Clarithromycin	7 d growth	NOEC (mg/L)	0.8	Baumann et al. (2014)
Anabaena flos-aquae	Clarithromycin	Acute 72 h growth	EC_{50} (mg/L)	0.0121	Baumann et al. (2014)

Danio rerio	Clarithromycin, 14-hydroxy(R)-clarithromycin, N-desmethyl clarithromycin	Acute 48 h embryo lethality	EC_{50} (mg/L)	>2	Baumann et al. (2014)
Pimephales promelas (fathead minnow)	17α-ethinylestradiol	Chronic 305 d test	NOEC (ng/L)	1	Länge et al. (2001)

CA clofibric acid, *CBZ* carbamazepine, *IBP* ibuprofen, *DCF* diclofenac, *NPX* naproxen, *SDZ* sulfadiazine, *TET* tetracycline, *MNZ* metronidazole

settling is usually digested anaerobically before disposing them to drying beds or for reuse and resource recovery. Moreover, anaerobic systems may also be employed for organic matter removal in the secondary treatment, for example UASB and anaerobic MBRs. However, as evidenced by the presence of PPCPs in surface waters, conventional treatment systems are not very efficient in removing these contaminants. Many full-scale, pilot-scale, and lab-scale biological treatment systems have been investigated to observe removal efficiencies of a variety of different PPCP compounds (Onesios et al. 2009).

8.3.1 Removal Efficiencies in Conventional Biological Systems

Biological systems include aerobic systems such as activated sludge, MBRs, etc. where oxidation is the major pathway of degradation with oxygen as the electron acceptor. Aerobic systems cause the conversion of organic matter to carbon dioxide and water. In addition, ammonia and other nitrogen-containing compounds are converted to nitrates. Anoxic systems, where nitrate is the electron acceptor, also favor oxidative degradation. However, the product formed is nitrogen gas. Many systems such as attached growth systems, SBRs, etc. have combinations of aerobic/anoxic environments within the reactor (Münch et al. 1996; Guo et al. 2010) for simultaneous removal of C, N, and P. On the contrary, anaerobic systems essentially utilize reductive pathways where the organic matter acts as the electron acceptor and reduces to form methane, hydrogen gas, etc. The removal efficiencies of PPCPs under different redox environment were found to be different. This can be attributed to the degradation pathways and microbial community under different redox environments. Biodegradation rates of different PPCPs under different microbial communities are given in Table 8.3.

A study on PPCP removal by nitrifier-rich sludge showed that 8 of the 11 investigated compounds showed removals greater than 75%. The PPCP removals could be positively correlated to the nitrification rate of the biomass (Alvarino et al. 2014). It was hypothesized that the ammonia monooxygenase enzyme had a positive effect on PPCP removal and the metabolism was assumed to happen by hydroxylation or O-dealkylation (Fernandez-Fontaina et al. 2012; Alvarino et al. 2014).

The anaerobic systems were observed to be less efficient in the removal of most of the compounds except for specific compounds such as sulfamethoxazole (SMX) and trimethoprim (TMP) (Alvarino et al. 2014; Monsalvo et al. 2014; Su et al. 2014). A study on the removal of PPCPs in a UASB observed that the biodegradation constants for the compounds (except for SMX, TMP, and NPX) were 2–4 orders of magnitude lesser than a comparable ASP system (Alvarino et al. 2014). This shows that most of the degradation pathways are oxidative. However, removal efficiencies of SMX, TMP, caffeine, acetaminophen (ACE), and diltiazem were greater than 80% in an anaerobic sludge digester, whereas the rest of investigated PPCPs showed removals less than or equal to 50% (Narumiya et al. 2013).

Table 8.3 Biodegradation constants of PPCPs under different microbial communities

Reactor type	Compound	Pseudo-first-order-specific biodegradation constant (L/g/d)	References
Activated sludge process	SMX, SDZ	0.12, 0.174	Li and Zhang (2010)
	ACE, azithromycin, caffeine, estriol, estrone, GMF, IBP, NPX, ranitidine	53.52, 0.24, 50.88, 42.72, 54.96, 0.72, 40.32, 25.68, 1.44	Blair et al. (2015)
	Celestolide, estrone, IBP, estradiol, galaxolide, NPX, ROX, ethinyl estradiol, tonalide, diazepam, ERY, SMX, TMP, DCF	18, 14, 16, 11, 8, 7, 2.2, 2, 2, 2, 1, 0.7, 0.6, 0.1	Alvarino et al. (2014)
Upflow anaerobic sludge blanket	TMP, NPX, SMX, estradiol, celestolide, estrone, IBP, DCF, ROX, fluoxetine, CBZ, ERY	1.8, 1.11, 0.36, 0.07, 0.05, 0.04, 0.007, 0.007, 0.006, 0.003, 0.003, 0.001	Alvarino et al. (2014)
Nitrifier sludge	Galaxolide, IBP, tonalide, NPX, ERY, TMP, ROX, fluoxetine	(in μg/g/d)	Fernandez-Fontaina et al. (2012)
		39, 29.8, 26.5, 26.3, 19.8, 18.6, 15.7, 12	

SMX sulfamethoxazole, *SDZ* sulfadiazine, *ACE* acetaminophen, *GMF* gemfibrozil, *IBP* ibuprofen, *NPX* naproxen, *TMP* trimethoprim, *DCF* diclofenac, *ROX* roxithromycin, *ERY* erythromycin

8.3.2 Removal Mechanisms

The removal of PPCPs during wastewater treatment may occur due to a variety of processes such as biodegradation, adsorption, volatilization, and hydrolysis (Li and Zhang 2010). In addition to this, light-sensitive compounds such as acetaminophen are also found to undergo photodegradation (Li et al. 2017). While most studies focus on the total removal of PPCPs from the wastewater, distinction between the removal mechanisms is seldom studied. A study on 11 pharmaceuticals using freshwater and saline wastewater sludge concluded that the major removal mechanisms were biodegradation and adsorption (Li and Zhang 2010). While biodegradation causes complete or partial mineralization of the contaminants, adsorption brings about a mere phase change. Therefore, an ideal system will have a major percentage of removal through biodegradation. However, adsorption is not unfavorable since adsorption onto the cell surfaces is the preliminary step in biodegradation. However, it was observed that the sorbed compounds did not show any desorption from the sludge even when the supernatant concentrations reduced (Blair et al. 2015). In general, removal of PPCPs is mainly attributed to biodegradation, while adsorption is a minor removal pathway (Salgado et al. 2012). A study on the presence and degradation of in situ PPPCPs observed that out of the 57 pharmaceuticals studied, 48 were detected in the supernatant, while only 29 were found sorbed onto the sludge (Blair et al. 2015).

Removal by biodegradation varied visibly within various classes of PPCPs and even for the same compound under different conditions (Onesios et al. 2009; Li and Zhang 2010; Blair et al. 2015). Many PPCPs such as acetaminophen, ibuprofen, naproxen, metformin, etc. are consistently reported to have removal percentages higher than 90 (Salgado et al. 2012; Blair et al. 2015), whereas compounds such as galaxolide, tonalide, celestolide, cashmeran, traseolide, and diclofenac are shown to have low removals in a conventional activated sludge process (Salgado et al. 2012). Moreover, negative removal efficiencies have also been reported in treatment units. Carbamazepine, sulfadiazine, ciprofloxacin, clarithromycin, trimethoprim, etc. are some examples of compounds showing negative removal (Blair et al. 2015).

The amount of PPCPs biosorbed on the sludge is a function of K_d (solid-water partitioning coefficient) or K_{ow} (octanol-water partitioning coefficient) values. A minimum K_d value of 500 L/kg$_{SS}$ is recommended for appreciable removal through sorption (Ternes et al. 2004; Zhang et al. 2008). Musk fragrances have high K_{ow} values and are found to be removed majorly by adsorption in an ASP (Salgado et al. 2012). However, these values described only the hydrophobic interactions and could not apply to electrostatic interactions. The sorption mechanism could be better understood considering the pK_a values of the compounds and the reactor pH (Fernandez-Fontaina et al. 2012). In general, compounds with basic functional groups such as amino acids are sorbed well in high pHs, whereas acidic functional groups are sorbed in lower pHs (Narumiya et al. 2013). Fluoroquinolones like norfloxacin, ofloxacin, and ciprofloxacin showed adsorption of 60.5%, 42.3%, and 52.8%, respectively, in spite of their low K_d values (Li and Zhang 2010). These compounds along with tetracycline, triclosan, and triclocarban are constantly reported to have high sorption onto the sludge (Narumiya et al. 2013; Blair et al. 2015). Sorption of PPCPs onto a nitrifying activated sludge was found to be comparable to that of activated sludges and remained constant under different operating parameters (Fernandez-Fontaina et al. 2012). In addition, the sorption of PPCPs onto anaerobic sludge showed that the sorbed concentrations were lower than aerobic sludges which was attributed to lower surface area of anaerobic sludge flocs (Alvarino et al. 2014). However, the trend of sorption was similar to that of aerobic sludge with fluoxetine and musk fragrances showing the highest sorption. This shows the strong dependence of the sorbed values to the pH, pK_a, and K_d values than on the redox activity or type of biomass.

Similarly, the volatilization rate could be correlated to the Henry's constant value of the compounds. Studies indicate that most of the PPCPs, other than certain musk fragrances, do not undergo volatilization in treatment plants (Alvarino et al. 2014).

8.3.3 Factors Affecting PPCP Removal

The various factors that affect biodegradation of PPCPs include microbial acclimatization to PPCPs, nature and concentrations of PPCP compounds, and operating parameters such as SRT, HRT, C/N ratio, MLVSS concentration, membrane flux, etc.

Pharmaceutical concentrations play an important role in their removal by microbes. Most biodegradation studies of PPCPs follow a first-order or pseudo-first-order kinetics (Li and Zhang 2010; Blair et al. 2015) which by itself shows the dependence of the rate of removal on the pharmaceutical concentration. This shows that the rate of degradation is better with a higher concentration of PPCPs. This observation was indicative of the co-metabolic nature of the degradation. In addition, it was observed that acetaminophen, caffeine, metformin, triclosan, and triclocarban did not show any degradation when the concentrations dropped to 90, 40, 1000, 60, and 50 ng/L, respectively (Blair et al. 2015). This shows that the microbes resort to degrading the compound only when their concentrations reach a threshold limit. This could limit the removal efficiencies of PPCPs present in low concentrations and could be solved by concentrating PPCPs near the proximity of microbes with the help of adsorbents.

The increase in SRT in MBR was found to improve the removal efficiency of PPCPs. The effect of SRT on PPCP removal was not prominent till an SRT of about 33 d, and during this phase, the PPCP removal was found to depend more on the substrate concentration. However, an increase in SRT to above 60 d showed a remarkable difference in PPCP removal efficiencies (Göbel et al. 2007). An investigation of PPCP removal by nitrifiers observed increase in diclofenac removal from 15% to 70% when SRT was increased to 150 d (Fernandez-Fontaina et al. 2012). This was attributed to an increased biodiversity and lower F/M ratios, which influences co-metabolism. However, the effect of SRT was not apparent for a majority of the compounds. Thus, a critical SRT could be prescribed for each PPCP compound beyond which the increase in SRT did not have any effect on removal efficiency. Another major operational factor affecting PPCP removal is HRT. The effect of HRT is found to be more pronounced than that of SRT. In general, PPCP removal efficiencies decreased with decrease in HRT (Fernandez-Fontaina et al. 2012; Ávila et al. 2014) probably due to the higher substrate loading and shorter contact times. However, the degradation rates were found to increase with lower HRTs indicating a co-metabolic pathway for removal (Fernandez-Fontaina et al. 2012). HRTs affected removal efficiencies of different PPCP compounds in a different manner. Well-removed and scarcely removed substances showed much lesser dependence on HRT (Fernandez-Fontaina et al. 2012; Ejhed et al. 2018). Ejhed et al. (2018) correlated the dependence on HRT to the extent of biodegradation.

On the contrary, the application of acclimated microbes enhanced the PPCP removal even at lower SRTs (Clara et al. 2005; Reif et al. 2008). The chemical structure of compounds is also a key factor in affecting their removal efficiencies. In general, compounds with simple structure are degraded easily in treatment systems, whereas the presence of chlorine and/or two or more aromatic rings makes the compounds resistant to degradation (Kimura et al. 2005). Therefore, the characteristics of the influent wastewater (including C/N and inert solids) also have an impact on the removal efficiency of PPCPs. Kimura et al. (2005) investigated the effect of pretreatment on removal efficiency of PPCPs in MBR, which did not show any significant difference. However, the NH_4-N content was

demonstrated to have an effect on removal of PPCPs such as atenolol, IBP, GMF, NPX, caffeine, estrone, diuron, and N,N-diethyl-m-toluamide (DEET) (Phan et al. 2015). C/N ratios have an impact on the type of microbial community which in turn leads to a difference in the SMP and EPS components in the bioreactor (Wang et al. 2014; Han et al. 2015). This could further affect the type of degradation favored and can impact PPCP removal efficiency.

The mobility or state of biomass in the reactor also has an impact on PPCP removal. Comparing the suspended and attached biomass, micropollutants were found to be better degraded by the attached biomass (Luo et al. 2015). Attached growth systems have higher SRT and higher biomass concentration, which can lead to better removal efficiency. Generally, it has been observed that the diversity of microbes is more in an attached growth systems (Yang et al. 2009). In addition, the introduction of carriers also helps in the growth of slow-growing microbes, which can potentially degrade PPCPs. Immobilization of biomass on carriers can either be on the surface or within a matrix. Higher microbial activity was observed in attached growth MBRs (AG-MBRs) as indicated by the higher specific oxygen uptake rate (SOUR) (Yang et al. 2009; Jamal Khan et al. 2011). This was thought to be due to the higher specific surface area owing to the smaller size of flocs. The addition of granular materials like powdered and granular activated carbon (PAC and GAC) and clay (Serrano et al. 2011; Nguyen et al. 2014) has been researched as a means of enhancing removal efficiencies of MBRs. The difference in BOD/COD removal efficiencies comparing suspended and attached growth systems under similar conditions is very small (Yang et al. 2009, Leyva-Díaz et al. 2016). However, attached growth systems remove total nitrogen and phosphorous in a better manner (Jamal Khan et al. 2011), which is attributed to the DO gradient along the biofilm resulting in an oxic zone at the surface and a deeper anoxic zone. As a result, it helps in simultaneous nitrification and denitrification and also promotes the growth of phosphate-accumulating organisms (PAOs).

8.4 Selected Pharmaceutical Removal in Suspended Biomass System

Antibiotics are one of the most commonly reported and extensively used therapeutic classes in pharmaceuticals (Verlicchi et al. 2010). Metronidazole ($C_6H_9N_3O_3$, MNZ) belonging to the group of antibiotics and acetaminophen ($C_8H_9NO_2$, ACE) belonging to NSAIDs are frequently used for antibacterial treatment and as an analgesic, respectively. MNZ and ACE also have very high water solubility, i.e., 9.5 and 14 g/L, respectively. MNZ is usually removed with low efficiencies in treatment units and ACE is one of the most ubiquitous PPCPs observed in the environment. These properties pave the way for their entry into wastewater treatment plants (WWTPs) and other aquatic systems. Thus, it is necessary to understand the biodegradation of these compounds in WWTPs. Batch studies using a suspended biomass culture and MNZ and ACE as the target compounds were conducted under different conditions for this purpose. Biomass concentration, C/N ratio, MNZ, and ACE concentrations

were identified as the major parameters affecting PPCP degradation, and the variation of these parameters resulted in different conditions. There have been instances where nonfunctional effluent treatment plants are discharging pharmaceuticals into the environment in the range of several mg/L (Larsson et al. 2007). Therefore, to apply the outcome of the investigation to both surface water and industrial wastewater, the concentrations of MNZ and ACE were selected in the range of 0.01 to 5 mg/L.

8.4.1 Application of CCD in Batch Biomass Systems

Central composite design (CCD) is a commonly used method for optimization studies. It can be carried out for any number of parameters. Previously, the central composite design (CCD) and analysis of variance (ANOVA) were used to explore the effect of independent variables and their interactions. Moreover, the CCD was successfully applied in predicting removal efficiencies of pollutants and determining the maximum removal conditions with good accuracy (Rigas et al. 2005). In this method, the response is measured at three kinds of points, namely, edge points, star points, and center points. The numbers of these points vary based on the number of parameters and the type of design chosen. We have selected a 2k design. The concept can be best understood if we take the simple case of two variables. As per CCD, we assume a square within a circle whose circumference decides the range of experiments and the optimum value is assumed to lie within the square. The edge points are denoted as −1 and 1; the star points are denoted as $-\alpha$ and α; and 0 represents the center point. For any "n" number of parameters, the extreme points are taken as the $-\alpha$ and – values; the – values are given by the formula 2^(n/4); and the center point is the midpoint of the range. In the current study, the four parameters – MLSS concentration (A), C/N ratio (B), MET conc. (C), and ACE conc. (D) – are the independent variables. The – value is 2. The points of concern are calculated as shown in Table 8.4.

A four parameter full factorial design (FFD) with five levels with the independent variables was adopted. The FFD yielded a total of 31 runs, which included seven runs at the center point with the following conditions: MLSS, 2750 mg/L; C/N ratio, 15; MNZ concentration, 2.505 mg/L; and ACE concentration, 2.5 mg/L. Experiments were conducted using an acclimatized suspended culture at all the CCD generated conditions under temperature- and pH-controlled environments. The experiments were continued for 7 d and at regular intervals; samples were

Table 8.4 Calculation of α value for the independent parameters

Parameters		Range	−α	−1	0	1	α
Initial MLSS	A	500–5000 mg/L	500	1625	2750	3875	5000
C/N ratio	B	5–25	5	10	15	20	25
MET concentration	C	0.01–5 mg/L	0.01	1.2575	2.505	3.7525	5
ACE concentration	D	0–5 mg/L	0	1.25	2.5	3.75	5

collected from the flasks and analyzed for MLSS, MNZ, and ACE (Table 8.5). These values were used as dependent variables for exploring the effect of independent variables under various experimental conditions. The ANOVA technique helped to identify the interaction effect between the parameters (to ensure the fit and reliability based on the R^2 and p values) and to construct a quadratic regression model.

Table 8.5 MLSS, MNZ, and ACE concentrations measured at the various experimental conditions

Run	iMLSS (mg/L)	C/N	MNZ (mg/L)	ACE (mg/L)	MNZ removal (%)	ACE removal (%)	fMLSS (mg/L)	Specific MNZ removal rate (mg/kg d)
1	1625	10	3.7525	1.25	100.0	100.0	2079	397.55
2	2750	25	2.505	2.50	100.0	100.0	5632	149.48
3	1625	20	3.7525	3.75	100.0	097.2	2204	359.24
4	3875	10	3.7525	3.75	100.0	100.0	4296	197.55
5	2750	15	2.5050	5.00	098.8	100.0	3259	219.50
6	3875	20	3.7525	3.75	100.0	100.0	6027	220.52
7	2750	15	2.5050	2.50	090.1	100.0	3526	206.72
8	3875	10	1.2575	1.25	100.0	100.0	4337	069.59
9	3875	20	1.2575	1.25	100.0	100.0	6099	068.92
10	1625	10	1.2575	1.25	100.0	097.1	1748	173.05
11	1625	20	3.7525	1.25	100.0	100.0	2074	440.60
12	2750	05	2.5050	2.50	100.0	100.0	2132	264.42
13	2750	15	2.5050	2.50	100.0	100.0	2847	194.55
14	2750	15	5.0000	2.50	099.9	100.0	4287	291.33
15	0500	15	2.5050	2.50	100.0	100.0	896	168.90
16	2750	15	2.5050	2.50	100.0	100.0	2445	188.12
17	1625	20	1.2575	3.75	100.0	100.0	2141	120.51
18	3875	10	3.7525	1.25	100.0	100.0	4159	218.67
19	2750	15	2.5050	2.50	100.0	100.0	2810	197.96
20	3875	20	1.2575	3.75	100.0	100.0	6361	066.74
21	2750	15	2.5050	0.00	100.0	100.0	2043	252.80
22	5000	15	2.5050	2.50	100.0	100.0	7090	118.70
23	2750	15	2.5050	2.50	100.0	100.0	2471	226.10
24	3875	20	3.7525	1.25	100.0	100.0	6495	212.07
25	3875	10	1.2575	3.75	100.0	100.0	4401	082.44
26	2750	15	0.0100	2.5	100.0	100.0	3070	001.15
27	2750	15	2.5050	2.5	100.0	100.0	2765	223.49
28	1625	10	1.2575	3.75	100.0	099.0	1454	147.78
29	1625	20	1.2575	1.25	100.0	100.0	2650	132.80
30	1625	10	3.7525	3.75	100.0	100.0	2135	456.72
31	2750	15	2.5050	2.5	100.0	100.0	2916	213.02

8.4.2 MNZ and ACE Removal in Batch Biosystems

The experimental results confirmed that bacteria acclimatized to MNZ could effectively degrade both compounds within 7 d. However, complete removal of ACE was observed within 3 d, which could be attributed to the easily degradable nature of ACE (Behera et al. 2011). On the contrary, 3 d MNZ removal ranged from 14.1% to 100% corresponding to the runs conducted at minimum iMLSS (500 mg/L) and minimum MNZ concentration (0.01 mg/L), respectively.

However, it was observed that the MNZ removal efficiency increased over time. The removal occurred by both biodegradation and adsorption. The percentage contribution of biodegradation and adsorption in the total removal for MNZ shows that most of the removal was by biodegradation (Fig. 8.3). The results conform to earlier studies that also observed low biosorption for hydrophilic compounds such as MNZ (Nguyen et al. 2014). It can be noticed that 7 d MNZ removal was higher than 90% under all experimental conditions. The higher level of MNZ removal observed in the present study as compared to earlier investigations (Kamińska et al. 2015, Luo et al. 2015, Nguyen et al. 2013, Verlicchi et al. 2012) could be due to (a) higher hydraulic retention time (HRT) provided in all experiments, (b) acclimatization of the microbes to MNZ, and (c) larger iMLSS concentrations. While the removal efficiency obtained was comparable to a similar batch reactor (Kamińska et al., 2015), the removal rates obtained in the current study were significantly higher than earlier investigations. The high initial concentration of pharmaceuticals adopted could be the reason for this observed difference. The above observations indicate that the acclimatized biomass can effectively degrade MNZ and ACE.

Moreover, it was observed from the MNZ removal profiles that MNZ removal was insignificantly affected under identical iMLSS (1625 mg/L) and MNZ concentrations. On the contrary, a significant effect on MNZ removal efficiency was observed when the conditions were vice versa. Comparing the specific removal rates of MNZ under different run conditions, it was observed that increase in ACE concentration, C/N ratio, and MLSS led to reduced removal rates. However, it was

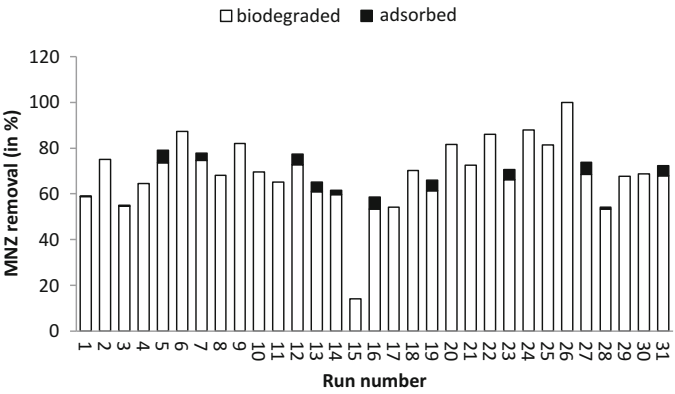


Fig. 8.3 Contribution of adsorption and biodegradation in 3 d removal of MNZ

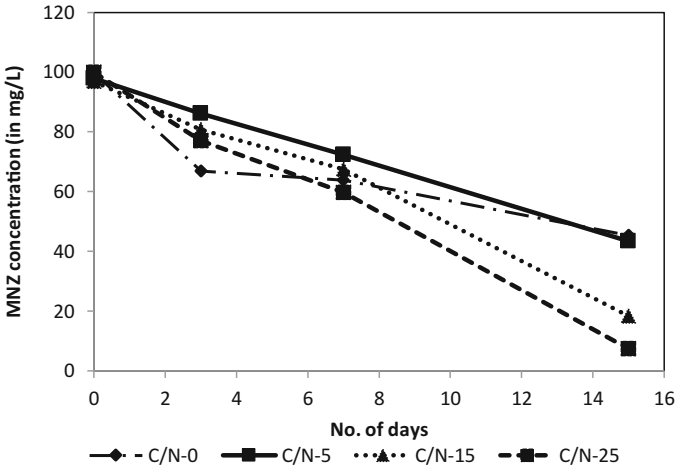
observed that the specific removal rate increased with increase in influent MNZ concentration. These results are in good agreement with previous investigations (Joss et al. 2006). Based on the values of fMLSS and iMLSS (Table 8.5), the percentage increase in bacterial growth was calculated, and it was observed to be positive when the C/N ratio was greater than 15. It is therefore recommended to have a C/N ratio of 20 or more for efficient MNZ and ACE removals.

8.4.3 Effect of C/N Ratio on MNZ Removal

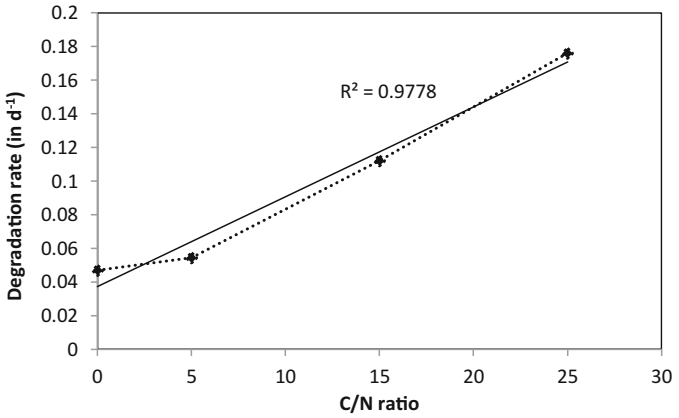
A set of batch experiments were conducted to ascertain the effect of C/N ratio on MNZ degradation. The C/N ratios were maintained at 0, 5, 15, and 25 using dextrose and ammonium chloride. In all these experiments, the initial MNZ and iMLSS concentrations were maintained at 100 and 2750 mg/L, respectively. Higher MNZ concentration was used to evaluate the behavior of the acclimatized bacteria at higher concentrations of MNZ in the absence of other carbon sources. All other conditions were maintained similar to the CCD batch experiments and the experiments were conducted for 15 d. The MNZ removal at the end of 7 d ranged between 26 and 40% which is much lesser compared to the removal obtained (90–100%) at lower MNZ concentrations (<5 mg/L). The experiment conducted at C/N ratio of 0, i.e., MNZ as the sole carbon source, showed 36% MNZ removal, which indicates that MNZ could be utilized as a carbon source by the biomass. Figure 8.4a shows the variation of MNZ concentration under various C/N ratios. The MNZ removal ranged from 56 to 93% after 15 d; however, the maximum removal efficiency was obtained for the experimental run with highest C/N ratio (25). This observation is comparable with the results obtained in the CCD experiments after 7 d. This could be attributed to the requirement of higher SRT for the removal of higher initial MNZ concentration. On the contrary, the first-order degradation rate constant corresponding to each C/N ratio was calculated from the MNZ concentration data over a period of 15 d (Fig. 8.4b). The first-order degradation rate constants ranged from 0.05 to 0.18 d^{-1} for C/N ratio 0–25, respectively. The higher value of degradation rate constant indicates a better MNZ utilization per day. The observed increase in MNZ degradation rate constant values with C/N ratios maybe attributed to better MLSS growth and more efficient utilization of MNZ as a co-substrate. Moreover, a linear correlation with R^2 value of 0.98 was observed for MNZ rate constants and C/N ratio (Fig. 8.4b), showing a significant influence of C/N ratio on MNZ removal efficiency. In summary, a higher biomass concentration and C/N ratio favor better removal of MNZ.

8.5 Summary and Future Direction

One of the major limitations associated with application of biological processes for PPCP removal is the persistence and even selection of antibiotic-resistant genes (ARGs) and antibiotic-resistant bacteria (ARB). Therefore, in addition to acting as a point source of pharmaceutical contamination, wastewater treatment plants are also



(a)



(b)

Fig. 8.4 Variation of (**a**) MNZ concentration at different C/N ratios and (**b**) MNZ degradation rate with C/N ratio

acting as hubs of antibiotic-resistant genes and unscrupulously introducing them to the environment (Rizzo et al. 2013). Conventional disinfection techniques at the normally used contact times and dosages were found to have no significant effect on ARBs and ARGs, although the total amount of microbes was reduced (Rizzo et al. 2013). In addition, the transferability of resistant genes through transferable genetic materials such as plasmids and integrons is also not well investigated. A better understanding of the horizontal gene transfer between species and how they are affected under the treatment provided will help in curbing the spread on resistant pathogens. These studies also need to be extended to the treatment and management of the biosolids generated.

References

Alvarino T et al (2014) Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors. J Hazard Mater 278:506–513. https://doi.org/10.1016/j.jhazmat.2014.06.031

Ávila C et al (2014) Constructed wetland system for wastewater treatment and reuse. Ecol Eng 80:108–116. https://doi.org/10.1016/j.ecoleng.2014.07.056

Baumann M et al (2014) Aquatic toxicity of the macrolide antibiotic clarithromycin and its metabolites. Chemosphere 120:192–198. https://doi.org/10.1016/j.chemosphere.2014.05.089

Behera SK, Kim HW, Oh J-E, Park H-S (2011) Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. Sci Total Environ 409(20):4351–4360

Blair B et al (2015) Evaluating the degradation, sorption, and negative mass balances of pharmaceuticals and personal care products during wastewater treatment. Chemosphere 134:395–401. https://doi.org/10.1016/j.chemosphere.2015.04.078

Buser HR, Poiger T, Müller MD (1998) Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: rapid photodegradation in a lake. Environ Sci Technol 32(22):3449–3456. https://doi.org/10.1021/es980301x

Carballa M et al (2007) Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. Water Res 41(10):2139–2150. https://doi.org/10.1016/j.watres.2007.02.012

Carra I et al (2015) Application of high intensity UVC-LED for the removal of acetamiprid with the photo-Fenton process. Chem Eng J 264:690–696. https://doi.org/10.1016/j.cej.2014.11.142

Casas ME et al (2015) Biodegradation of pharmaceuticals in hospital wastewater by staged moving bed biofilm reactors (MBBR). Water Res 83:293–302. https://doi.org/10.1016/j.watres.2015.06.042

Choi K et al (2008) Seasonal variations of several pharmaceutical residues in surface water and sewage treatment plants of Han River, Korea. Sci Total Environ 405(1–3):120–128. https://doi.org/10.1016/j.scitotenv.2008.06.038

Clara M et al (2005) Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. Water Res 39(19):4797–4807. https://doi.org/10.1016/j.watres.2005.09.015

Cleuvers M (2003) Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicol Lett 142(3):185–194. https://doi.org/10.1016/S0378-4274(03)00068-7

Cruz-Morató C et al (2013) Continuous treatment of clofibric acid by Trametes versicolor in a fluidized bed bioreactor: identification of transformation products and toxicity assessment. Biochem Eng J 75:79–85. https://doi.org/10.1016/j.bej.2013.03.020

Ejhed H et al (2018) The effect of hydraulic retention time in onsite wastewater treatment and removal of pharmaceuticals, hormones and phenolic utility substances. Sci Total Environ 618:250–261. https://doi.org/10.1016/j.scitotenv.2017.11.011

Ellis JB (2006) Pharmaceutical and personal care products (PPCPs) in urban receiving waters. Environ Pollut 144(1):184–189. https://doi.org/10.1016/j.envpol.2005.12.018

Fent K, Weston AA, Caminada D (2006) Ecotoxicology of human pharmaceuticals. Aquat Toxicol 76(2):122–159. https://doi.org/10.1016/j.aquatox.2005.09.009

Fernandez-Fontaina E et al (2012) Influence of nitrifying conditions on the biodegradation and sorption of emerging micropollutants. Water Res 46(16):5434–5444. https://doi.org/10.1016/j.watres.2012.07.037

Göbel A, Mcardell C, Joss A, Siegrist H, Giger W (2007) Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. Sci Total Environ 372(2–3):361–371

Joss et al (2006) Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. Water Res 40(8):1686–1696. https://doi.org/10.1016/j.watres.2006.02.014

Guo W et al (2010) Roles of polyurethane foam in aerobic moving and fixed bed bioreactors. Bioresour Technol 101(5):1435–1439. https://doi.org/10.1016/j.biortech.2009.05.062

Han R et al (2015) Micro-polluted surface water treatment and trace-organics removal pathway in a PAC-MBR system. Process Biochem 50(9):1422–1428. https://doi.org/10.1016/j.procbio.2015.05.021

Hu X, Zhou Q, Luo Y (2010) Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. Environ Pollut 158(9):2992–2998. https://doi.org/10.1016/j.envpol.2010.05.023

Jamal Khan S et al (2011) Performance of suspended and attached growth MBR systems in treating high strength synthetic wastewater. Bioresour Technol 102(9):5331–5336. https://doi.org/10.1016/j.biortech.2010.09.100

Kamińska B, Majewska K, Skwierawska A, Łukasik N, Kozłowska-Tylingo K (2015) Removal of pharmaceutically active compounds in sequencing batch reactor. J Environ Sci Eng A 4(9):484–489

Kemper N (2008) Veterinary antibiotics in the aquatic and terrestrial environment. Ecol Indic 8 (1):1–13. https://doi.org/10.1016/j.ecolind.2007.06.002

Kimura K, Hara H, Watanabe Y (2005) Removal of pharmaceutical compounds by submerged membrane bioreactors (MBRs). Desalination 178(1–3 SPEC. ISS):135–140. https://doi.org/10.1016/j.desal.2004.11.033

Kleywegt S et al (2011) Pharmaceuticals, hormones and bisphenol a in untreated source and finished drinking water in Ontario, Canada — occurrence and treatment efficiency. Sci Total Environ 409(8):1481–1488. https://doi.org/10.1016/j.scitotenv.2011.01.010

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. Streams, 1999−2000: a national reconnaissance. Environ Sci Technol 36(6):1202–1211

Kosma CI, Lambropoulou DA, Albanis TA (2014) Investigation of PPCPs in wastewater treatment plants in Greece: occurrence, removal and environmental risk assessment. Sci Total Environ 466–467:421–438. https://doi.org/10.1016/j.scitotenv.2013.07.044

Kumar BS et al (2011) Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. Sci Total Environ 409(20):4351–4360. https://doi.org/10.1016/j.scitotenv.2011.07.015

Länge R et al (2001) Effects of the synthetic estrogen 17α-ethinylestradiol on the life-cycle of the fathead minnow (Pimephales promelas). Environ Toxicol Chem 20(6):1216–1227. https://doi.org/10.1002/etc.5620200610

Larsson DGJ, de Pedro C, Paxeus N (2007) Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J Hazard Mater 148(3):751–755

Lee CO, Howe KJ, Thomson BM (2012) Ozone and biofiltration as an alternative to reverse osmosis for removing PPCPs and micropollutants from treated wastewater. Water Res 46 (4):1005–1014. https://doi.org/10.1016/j.watres.2011.11.069

Leyva-Díaz JC, Muñío MM, González-López J, Poyatos JM (2016) Anaerobic/anoxic/oxic configuration in hybrid moving bed biofilm reactor-membrane bioreactor for nutrient removal from municipal wastewater. Ecol Eng 91:449–458

Li B, Zhang T (2010) Biodegradation and adsorption of antibiotics in the activated sludge process. Environ Sci Technol 44(9):3468–3473

Li X et al (2015) Removal of carbamazepine and naproxen by immobilized Phanerochaete chrysosporium under non-sterile condition. New Biotechnol 32(2):282–289. https://doi.org/10.1016/j.nbt.2015.01.003

Li Y et al (2017) Photosensitized degradation of acetaminophen in natural organic matter solutions: the role of triplet states and oxygen. Water Res 109:266–273. https://doi.org/10.1016/j.watres.2016.11.049

Liu L et al (2013) Chemosphere elimination of veterinary antibiotics and antibiotic resistance genes from swine wastewater in the vertical flow constructed wetlands. Chemosphere 91 (8):1088–1093. https://doi.org/10.1016/j.chemosphere.2013.01.007

López-Serna R et al (2019) Removal of contaminants of emerging concern from urban wastewater in novel algal-bacterial photobioreactors. Sci Total Environ 662:32–40. https://doi.org/10.1016/j.scitotenv.2019.01.206

Luo Y et al (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci Total Environ 473–474:619–641. https://doi.org/10.1016/j.scitotenv.2013.12.065

Luo Y et al (2015) Evaluation of micropollutant removal and fouling reduction in a hybrid moving bed biofilm reactor-membrane bioreactor system. Bioresour Technol 191:355–359. https://doi.org/10.1016/j.biortech.2015.05.073

Mohapatra DP et al (2014) Analysis and advanced oxidation treatment of a persistent pharmaceutical compound in wastewater and wastewater sludge-carbamazepine. Sci Total Environ 470–471:58–75. https://doi.org/10.1016/j.scitotenv.2013.09.034

Monsalvo VM et al (2014) Removal of trace organics by anaerobic membrane bioreactors. Water Res 49:103–112. https://doi.org/10.1016/j.watres.2013.11.026

Münch EV, Lant P, Keller J (1996) Simultaneous nitrification and denitrification in bench-scale sequencing batch reactors. Water Res 30(2):277–284. https://doi.org/10.1016/0043-1354(95)00174-3

Narumiya M et al (2013) Phase distribution and removal of pharmaceuticals and personal care products during anaerobic sludge digestion. J Hazard Mater 260:305–312. https://doi.org/10.1016/j.jhazmat.2013.05.032

Nguyen LN, Hai FI, Kang J, Price WE, Nghiem LD (2013) Removal of emerging trace organic contaminants by MBR-based hybrid treatment processes. Int Biodeterior Biodegradation 85:474–482

Nguyen LN et al (2014) The effects of mediator and granular activated carbon addition on degradation of trace organic contaminants by an enzymatic membrane reactor. Bioresour Technol 167:169–177. https://doi.org/10.1016/j.biortech.2014.05.125

Onesios KM, Yu JT, Bouwer EJ (2009) Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. Biodegradation 20(4):441–466. https://doi.org/10.1007/s10532-008-9237-8

Peng X et al (2014) Occurrence and ecological potential of pharmaceuticals and personal care products in groundwater and reservoirs in the vicinity of municipal landfills in China. Sci Total Environ 490:889–898. https://doi.org/10.1016/j.scitotenv.2014.05.068

Phan HV et al (2015) Nutrient and trace organic contaminant removal from wastewater of a resort town: comparison between a pilot and a full scale membrane bioreactor. Int Biodeterior Biodegrad 102:40–48. https://doi.org/10.1016/j.ibiod.2015.02.010

Pruden A et al (2006) Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environ Sci Technol 40(23):7445–7450. https://doi.org/10.1021/es060413l

Radjenović J, Petrović M, Barceló D (2009) Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res 43(3):831–841. https://doi.org/10.1016/j.watres.2008.11.043

Ramaswamy BR et al (2011) GC-MS analysis and ecotoxicological risk assessment of triclosan, carbamazepine and parabens in Indian rivers. J Hazard Mater 186(2–3):1586–1593. https://doi.org/10.1016/j.jhazmat.2010.12.037

Reif R et al (2008) Fate of pharmaceuticals and cosmetic ingredients during the operation of a MBR treating sewage. Desalination 221(1–3):511–517. https://doi.org/10.1016/j.desal.2007.01.111

Rigas F, Dritsa V, Marchant R, Papadopoulou K, Avramides EJ, Hatzianestis I (2005) Biodegradation of lindane by Pleurotus ostreatus via central composite design. Environ Int 31(2):191–196

Rizzo L et al (2013) Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Sci Total Environ 447:345–360. https://doi.org/10.1016/j.scitotenv.2013.01.032

Salgado R et al (2012) Assessing the removal of pharmaceuticals and personal care products in a full-scale activated sludge plant. Environ Sci Pollut Res 19(5):1818–1827. https://doi.org/10.1007/s11356-011-0693-z

Serrano D et al (2011) Removal of persistent pharmaceutical micropollutants from sewage by addition of PAC in a sequential membrane bioreactor. Water Res 45(16):5323–5333. https://doi.org/10.1016/j.watres.2011.07.037

Sipma J et al (2010) Comparison of removal of pharmaceuticals in MBR and activated sludge systems. Desalination 250(2):653–659. https://doi.org/10.1016/j.desal.2009.06.073

Stadler LB et al (2015) Effect of redox conditions on pharmaceutical loss during biological wastewater treatment using sequencing batch reactors. J Hazard Mater 282:106–115. https://doi.org/10.1016/j.jhazmat.2014.08.002

Su L et al (2014) Effect of redox conditions on pharmaceutical loss during biological wastewater treatment using sequencing batch reactors. J Hazard Mater 282:106–115. https://doi.org/10.1016/j.jhazmat.2014.08.002

Teijon G et al (2010) Occurrence of emerging contaminants, priority substances (2008/105/CE) and heavy metals in treated wastewater and groundwater at Depurbaix facility (Barcelona, Spain). Sci Total Environ 408(17):3584–3595. https://doi.org/10.1016/j.scitotenv.2010.04.041

Ternes TA, Herrmann N, Bonerz M, Knacker T, Siegrist H, Joss A (2004) A rapid method to measure the solid–water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge. Water Res 38(19):4075–4084

Verlicchi P, Galletti A, Petrovic M, Barceló D (2010) Hospital effluents as a source of emerging pollutants: an overview of micropollutants and sustainable treatment options. J Hydrol 389(3–4):416–428

Verlicchi P et al (2012) Hospital effluent: investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. Sci Total Environ 430:109–118. https://doi.org/10.1016/j.scitotenv.2012.04.055

Vulliet E, Cren-Olivé C (2011) Screening of pharmaceuticals and hormones at the regional scale, in surface and groundwaters intended to human consumption. Environ Pollut 159(10):2929–2934. https://doi.org/10.1016/j.envpol.2011.04.033

Wang L et al (2014) Removal of polycyclic musks by anaerobic membrane bioreactor: biodegradation, biosorption, and enantioselectivity. Chemosphere 117(1):722–729. https://doi.org/10.1016/j.chemosphere.2014.10.004

Wollenberger L, Halling-Sørensen B, Kusk KO (2000) Acute and chronic toxicity of veterinary antibiotics to Daphnia magna. Chemosphere 40(7):723–730. https://doi.org/10.1016/S0045-6535(99)00443-9

Yang S et al (2009) Comparison between a moving bed membrane bioreactor and a conventional membrane bioreactor on organic carbon and nitrogen removal. Bioresour Technol 100(8):2369–2374. https://doi.org/10.1016/j.biortech.2008.11.022

Yang Y et al (2017) Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: a review. Sci Total Environ 596–597:303–320. https://doi.org/10.1016/j.scitotenv.2017.04.102

Zhang Y, Geißen SU, Gal C (2008) Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies. Chemosphere 73(8):1151–1161. https://doi.org/10.1016/j.chemosphere.2008.07.086

Zhao X et al (2014) PPCPs removal by aerobic granular sludge membrane bioreactor. Appl Microbiol Biotechnol 98(23):9843–9848. https://doi.org/10.1007/s00253-014-5923-0

Extremophiles: A Powerful Choice for Bioremediation of Toxic Oxyanions

9

Sana Alavi, Shokufeh Rafieyan, Maryam Yavari-Bafghi, and Mohammad Ali Amoozegar

Abstract

By having toxic effects on human health, oxyanion contamination in wastewater is considered a priority among environmental concerns to be resolved. Widespread applications of the oxyanions in various industries and metal mining activities in certain environments resulted in high concentrations of these materials especially effluent in their toxic forms. One of the acceptable methods considered by a number of researchers to reduce their toxicity is bioreduction of oxyanions by microorganisms which is considered as one of the bioremediation methods. However, the major problem for bioremediation by conventional methods is the extreme conditions of industrial effluents, such as high salinity, high or low pH, and high temperature. Nature itself has given the solution to this problem, i.e., *extremophiles*, microorganisms that survive in these harsh conditions. Due to their ability in reducing metalloids and detoxifying them, they are suitable candidates for biological treatments. Here, the efficacy of various extremophile groups in bioremediation of major oxyanions including arsenic, selenium, chromium, and tellurium is reviewed.

Keywords

Toxicity · Oxyanion contamination · Metalloid · Arsenic · Selenium · Chromium · Tellurium · Bioremediation · Extremophiles

S. Alavi
Department of Nanobiotechnology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

S. Rafieyan
Department of Cell and Molecular Biology and Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran

M. Yavari-Bafghi · M. A. Amoozegar (✉)
Extremophiles Laboratory, Department of Microbiology, Faculty of Biology, College of Science, University of Tehran, Tehran, Iran
e-mail: amoozegar@ut.ac.ir

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_9

9.1 Extremophiles

The term extremophile was first used in 1974 (Horikoshi et al. 2011). Extremophiles are organisms that are able to adapt and survive in habitats that may be fatal to other forms of life. Extremophiles can be divided into two broad categories: microorganisms that survive in very harsh conditions from the human perspective and require one or more extreme factors to grow, called the "extremophiles," and a larger group of organisms able to withstand the conditions in the extreme environments and have the ability to grow, but their growth will not be optimal, which are called the "extremotrophs" (Orellana et al. 2018). Extremophiles include the members of all three domains of life: archaea, bacteria, and eukaryotes. Most extremophiles are prokaryotes, of which archaea has a high proportion. The group also includes eukaryotes such as protista, including algae, fungi, and protozoa (Rampelotto 2013).

Extremophiles are able to grow in various environments with extreme conditions on the planet. Extremely acidic, basic, salty, hot, and ice niches, environments rich in organic solvents and toxic metals, deep oceans, and high-pressure areas are among these environments (Rampelotto 2013). Areas such as hypersaline environments and deserts, hot springs, geysers, and deep seas are some examples of extreme areas around the world (Rothschild and Mancinelli 2001). Based on the growth potential in each of the various environmental conditions, extremophiles constitute various groups including psychrophiles, thermophiles, acidophiles, basophiles, halophiles, heavy metal tolerants, oligotrophs, piezophiles, radiation tolerants, toxic substance tolerants, xerophiles, and endolithic organisms. The organisms that can survive in habitats with several extreme physicochemical factors are called "polyextremophiles" (Horikoshi et al. 2011). In order to survive and grow in extreme environments, extremophiles had achieved certain adaptations and metabolic abilities that are not present in mesophilic microorganisms; hence, they have a high potential for environmental applications, biotechnology, and industrial processes (Rampelotto 2013).

One of the major capabilities found in extremophiles is their potential role in elimination of the environmental pollutants either organic or inorganic. There are many investigations being carried out in this regard, one of which is the toxic metalloids (all exist as oxyanions). Metalloids and their toxicity and how a microbial entity is overcoming their toxicity while interacting with them is under focus in this chapter.

9.2 Metalloid Oxyanions and their Toxicity

The word "metalloid" derived from the Latin word "metallum" and the Greek word "oeides" is attributed to a group of elements that possess physicochemical properties between metals and nonmetals (Gordh and Headrick 2001). All the metalloids are in their oxyanionic form, and they can be found in multiple oxidation states (e.g., As (III) and As (V)) to have the most diverse behavior in the environment.

This group includes arsenic (As), chromium (Cr), antimony (Sb), boron (B), germanium (Ge), silicon (Si), and tellurium (Te). Some other elements, such as astatine (At) and selenium (Se), are also sometimes added to this list. In addition to their industrial applications (as a constituent of solar batteries, semiconductor devices, ceramics, polymers, etc.), metalloids have also been used in medicine and agriculture. Metalloids affect living organisms in multiple ways. Although they have essential and important functions in many organisms like humans, they are toxic at high concentrations and may cause different illnesses (Bienert and Tamás 2018). Arsenic (As), selenium (Se), chromium (Cr), and tellurium (Te) are the four metalloids studied in this chapter.

9.2.1 Arsenoxyanions

Arsenic (As) with atomic number 33 is the twentieth most abundant element in the Earth's crust. Arsenic is cycling in the atmosphere, biosphere (humans, plants, animals, and microorganisms), lithosphere (soil, earth, crust, rocks, minerals, and sediments), and hydrosphere (seawater and freshwater) through mining and smelting, industrial wastewater and sewage, agricultural applications, and biological and anthropogenic activities. Application of insecticides and herbicides, cotton and wool processing, food supplements and other industrial activities in which arsenic is implemented, are amongst human activities contributing to arsenic pollution (Fatoki et al. 2013; Jebelli et al. 2018). Arsenic has four oxidation states −3, 0, −3, and 5. Arsenite [As (III)] and arsenate [As (V)] are two common inorganic forms of arsenic in water, soil, and underground water (Strawn 2018). The existence of these two oxidation states depends on the physicochemical conditions of the location (redox potential (Eh) and pH) and the biological activities of the microorganisms (reduction of As (V), oxidation of As (III), and methylation reactions). AsO_4^{3-}, $HAsO_4^{2-}$, and $H_2AsO_4^-$ are species of arsenate, and As $(OH)_3$, As $(OH)_4^-$, $AsO_2OH_2^-$, and AsO_3^{3-} are species of arsenite. Naturally, arsenic is in connection with other minerals, and the most abundant arsenic ore minerals are arsenopyrite (FeAsS) and arsenian pyrite (Fe (S, As)$_2$), realgar (AsS), and orpiment (As_2S_3) (Fatoki et al. 2013). Organic and inorganic forms of arsenic have many applications in different industries and are usually used as a poison. As (v) is used for producing insecticides and pesticides. They are employed in the wood industry, leather preservatives, glass-making, and have military and commercial applications. As (v) is used for producing insecticides and pesticides too. The organic forms of arsenic like p-arsanilic acid are used as animal (pigs and poultry) feed to improve their weight gain and prevent diseases. Also, medicines that contain arsenic like Fowler's solution, Asiatic pills, arsphenamine, etc. are used for respiratory diseases, head lice, and plague (Fatoki et al. 2013; Upadhyay et al. 2019). Some forms of arsenic are toxic for most of the organisms, except some bacterial species which are able to acquire energy from it. Arsenic enters the water cycle and underground water from natural and anthropogenic sources and enters the food cycle through drinking and plant irrigation (Upadhyay et al. 2019). Toxicity, bioavailability, and the mobility of arsenic depend on its solubility and binding capacity

(Jebelli et al. 2018). Arsenite has higher mobility and toxicity than arsenate. Arsenite binds to sulfhydryl groups in enzymes and affects their function (Lièvremont et al. 2009). Consumption of drinking water with an arsenic concentration of more than 50 microgram causes diseases such as melanosis, gangrene, lung cancer, and death. Therefore, the existence of arsenic in drinking water may threaten the life of human beings (Jebelli et al. 2018).

9.2.2 Selenoxyanions

Selenium is derived from the Greek word "selene," which means the moon. Its atomic number is 34 and belongs to the sixth group of the periodic table and was discovered in 1817 by Jöns Jacob Berzelius. The element has oxidation states of −2, +2, +4, and +6, and selenium is its elemental form with an oxidation level of zero (Khurana et al. 2019). Selenite (Se^{4+}) and selenate (Se^{6+}) oxyanions are the most abundant inorganic species of selenium that enter the soil from different natural and artificial sources and will become available to the plants (Ali et al. 2017; Guan et al. 2018). These two species are soluble and are absorbed, metabolized, and excreted by organisms (Zhou et al. 2019). At high concentrations, selenite oxyanion, the most toxic form of selenium, should be reduced to elemental selenium by redox reactions (Khurana et al. 2019). Selenium, on the contrary, is a micronutrient and is essential for human health in trace amounts. This element is part of the selenoproteins and acts in processes including thyroid hormone regulation, carbohydrate metabolism, redox homeostasis, inflammatory and immunological responses, and brain function maintenance (Tan et al. 2018). Selenium has antitumor, antioxidant, anti-stress, and fertility enhancement properties. It also affects growth, metabolism, the skeletal system, and hormonal balance (Guan et al. 2018). Diabetes, Keshan disease, thyroid dysfunction, and arthrophyma are diseases caused by selenium deficiency. Therefore, nowadays selenium is used both as food supplements and in the form of selenium nanoparticles (SeNPs), in order to prevent diseases and for treatment and drug delivery. Various therapeutic benefits are addressed for SeNPs, such as antitumor, anti-inflammatory, antioxidant, and anti-diabetes properties (Guan et al. 2018; Khurana et al. 2019).

Typically, young people are allowed to use selenium 55 μg/day. Dosage of lower than 15 μg/day and higher than 400 μg/day leads to selenium deficiency and selenium poisoning, respectively. Selenium poisoning causes selenosis, with symptoms such as nausea, vomiting, fatigue, garlic breath, irritability, hair loss, and nails fragility, colorlessness, and falling (MacFarquhar et al. 2010; Tan et al. 2018). Air pollution caused by traffic, food supplements, cigarette smoking, and coal combustion are among the top sources of selenium (Vinceti et al. 2018).

9.2.3 Chromoxyanions

Chromium is the 17th element in the Earth's crust with atomic number 24. This element is one of the largest inorganic pollutants in the environment that originates

from various natural resources such as weathering of rocks, volcanoes, and rock outcroppings and anthropogenic sources, including wastewater produced by the textile, electroplating, and tanning industries. Chromium species are extensively found in groundwater, soils, rocks, and food (such as bananas, beef, fish, tea, wheat flour, fruits, vegetables, etc.). It has various oxidation states between −2 and +6 (Chen et al. 2018; Hamilton et al. 2018; Rodríguez and Mandalunis 2018). The most common forms of chromium are Cr (III) and Cr (VI). Cr (III) is more stable than others and has the ability to form stable complexes with organic and inorganic ligands in the environment, while Cr (VI) is present in the form of chromite and dichromate anions, especially in the aquatic environment. Oxygen concentration, pH, and redox potential of the environment are the determinant factors for chromium speciation, in which dichromate is dominant at pH < 6.8 (Gutiérrez-Corona et al. 2016; Chen et al. 2018; Zhao et al. 2018). The negative charge of chromate and dichromate causes their poor absorption in the soil and hence high mobility of Cr (VI). On the contrary, Cr (III) has less toxicity than Cr (VI) by forming stable complexes and being less mobile (Figueiredo and Quintelas 2014; Gutiérrez-Corona et al. 2016; Zhao et al. 2018). Chromium is an important industrial compound that is widely used in metallic plating, industrial catalyst fabrication, insecticide manufacturing, refining, dying, and tanning industries (Figueiredo and Quintelas 2014; Thorgersen et al. 2017; Hamilton et al. 2018). This widespread use of chromium in the industry has made it one of the 16 most toxic pollutants. Chromium has teratogenic and carcinogenic effects, causing skin ulcers; digestive, respiratory, neurological, and reproductive disorders; and allergies in humans (Thorgersen et al. 2017; Rodríguez and Mandalunis 2018; Zhao et al. 2018). Excessive accumulation of this element in plants causes damage to the roots and shoot growth and photosynthesis process (Pratush et al. 2018). Although a daily intake of 200–500 μg of Cr (III) is essential for glucose and lipid metabolism (Rodríguez and Mandalunis 2018), at high concentrations it is toxic and harmful and may damage DNA. On the contrary, Cr (VI) is highly reactive and 1000 times more toxic than Cr (III) and is known as a lung cancer agent (Chen et al. 2018; Rager et al. 2019).

9.2.4 Telluroxyanions

Tellurium is a crystalline semimetal and a silvery-white element, discovered in 1782 by Franz Joseph Müller von Reichenstein. Tellurium is derived from the Latin word tellus meaning Earth and is the heaviest nonradioactive member of the chalcogen family as the 72nd element of the Earth's crust (Chivers and Laitinen 2015; Zare et al. 2017). This metalloid is found in four relatively stable oxidation levels in nature: telluride [Te (-II)], elemental tellurium [Te (0)], tellurite [Te (+IV)], and tellurate [Te (+VI)]. Tellurite is the most abundant oxyanion, and tellurate is limited to the biosphere due to its limited solubility. Elemental tellurium is also found in small amounts in the Earth's crust and is usually combined with other metals such as gold (calaverite) and silver (sylvanite) (Chivers and Laitinen 2015; Zare et al. 2017).

Tellurium is an unnecessary and harmful ingredient for animals and plants, but fungi can use tellurium in place of sulfur and selenium in their amino acids (Najimi et al. 2017). Recently, the telluru-methionine has been discovered in bacteria and yeasts (Zare et al. 2017). Tellurium can be accumulated within herbs too. Humans and animals are exposed to this element through the use of the herbs that have accumulated tellurium (Ogra 2017). Tellurium is the fourth trace element necessary for the human body. Moreover, it has commercial applications in various fields, including copper refining, rubber manufacturing, pharmacotherapy, metal-oxidizing solutions, imaging and diagnosis, and solar panels. Tellurite has antibacterial properties, and cadmium telluride nanoparticles also have cytotoxic and anticancer properties (Najimi et al. 2017; Zare et al. 2017). However, certain species of tellurium are highly toxic to most organisms. This toxicity depends on several factors, including the applied dose, route of administration, and the oxidation state (Zare et al. 2017). In fact, tellurium toxicity is due to the reaction with the SH group and the formation of telenotrisulfide toxic compounds (Safhi et al. 2018). The molecular mechanism of tellurium toxicity has not yet been fully elaborated. However, it has been found that tellurium compounds cause oxidative stress, resulting in cell apoptosis and DNA damage. Neurotoxicity and necrosis are also induced by diphenylditelluride and tellurium tetrachloride ($TeCl_4$), respectively (Najimi et al. 2017). Usually, the toxicity of tellurium is associated with symptoms such as garlic breath odor, metallic taste in the mouth, vomiting, nausea, and general fatigue (Zare et al. 2017; Safhi et al. 2018). In conclusion, heavy metal/metalloid pollution is one of the major environmental problems that need special attention. This contamination can have natural or human-made origins. Studies, especially after the 1950s, showed that most contaminants from metal release to the environment are related to anthropogenic actions (Han et al. 2002; Jian et al. 2011).

Unlike organic pollutants, most metals cannot be biodegradable (Kirpichtchikova et al. 2006), and their sustainability is very high, so there is a demand for new approaches to clean them out from the environment, as they are threatening to human health and ecosystem (Wuana and Okieimen 2011).

9.3 Remediation Techniques

In general, there are two classifications in remediation methods. The first method which is based on treatment place is divided into in situ and ex situ treatment methods. In in situ treatment, the soil is treated in the original location, while the ex situ treatment is the excavation of contaminated soil and removing it from the original place. The second is based on the mechanism of treatment, which has been categorized as physical, chemical, and biological remediation (bioremediation) processes (Sidhu 2016). Below, the physical, chemical, and biological remediation methods are described, and Fig. 9.1 represents the pollution routes and the possible remediation techniques to overcome them.

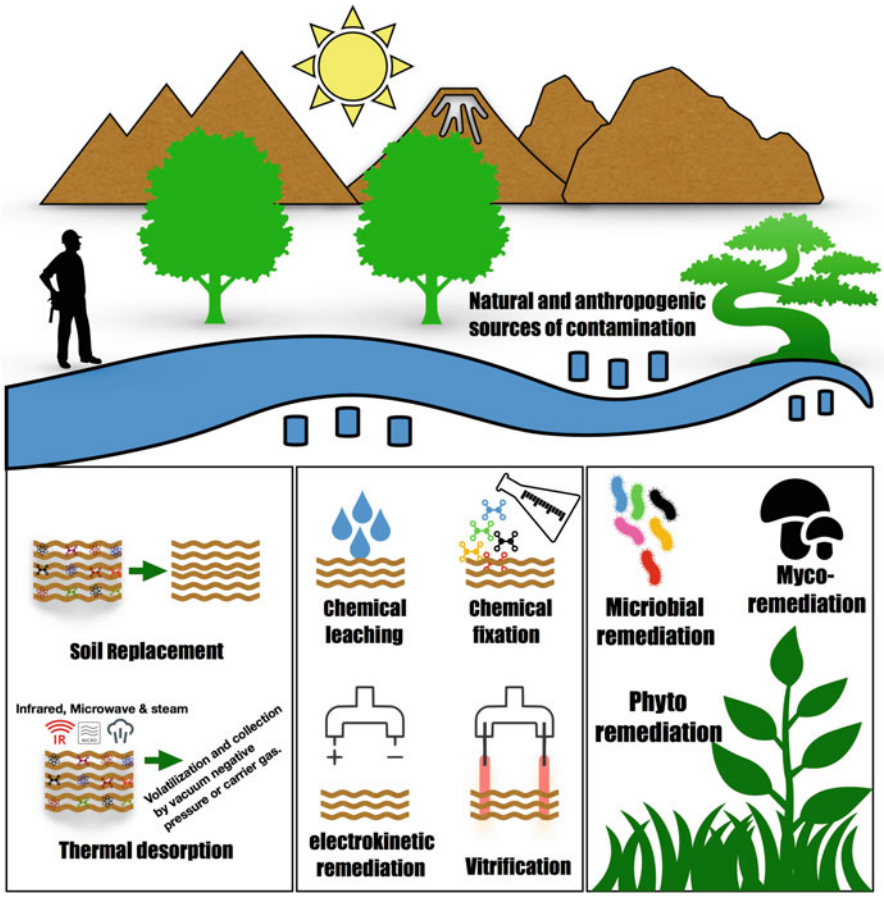


Fig. 9.1 Natural and anthropogenic origins are the main source for contamination stream into the environment. Remediation techniques used for elimination of these pollutants are *physical* (**a**), *chemical* (**b**), and *biological* (**c**). In *physical remediation* either the soil is replaced with the clean soil or thermal desorption for volatilization of the contaminants is used, and it is collected by vacuum negative pressure or a carrier gas. In *chemical techniques*: *leaching*, washing the soil by running water; *fixation*, adding chemical materials to react with the contaminants; *electrokinetic*, using high voltage; or *vitrification*, using high temperature is implemented to remove the pollutants. In *biological techniques*: Biological matters including plants, fungi, and microorganisms are used to absorb the contamination

9.3.1 Physical Remediation

The physical remediation involves two methods, replacement of soil and thermal desorption. In the soil replacement method, clean soil is used to completely or partly replace the contaminated soil, in order to reduce the pollutant's concentration in the soil. This method can be used for a small contaminated area. In the thermal desorption method, steam, microwave, and infrared radiation are used to volatilize

the pollutant, and the volatile heavy metals will be collected by vacuum negative pressure or carrier gas (Yao et al. 2012; Sidhu 2016).

9.3.2 Chemical Remediation

Chemical remediation includes chemical leaching, chemical fixation, electrokinetic remediation, and the vitrification technologies (Yao et al. 2012).

In chemical leaching method, contaminated soil is washed with freshwater, specific reagents, and other fluids or gases. After the heavy metals are transferred from soil to the liquid phase, the recovery from leachate is done (Yao et al. 2012).

During chemical fixation method, some reagents or materials are added to contaminated soil to decrease its mobility and so decrease its migration to the environment (Yao et al. 2012; Sidhu 2016). Organic and inorganic soil conditioning materials such as clay, cement, zeolite, phosphate, minerals, microbes, and organic materials are used for immobilization of heavy metals in the soil (Khalid et al. 2017).

For electrokinetic remediation, removal of heavy metals from the soil is done using electric currents. In this method, electric field gradient is formed on both sides of the contaminated soil. Then, the soil contaminants are carried to two polar sides of the electrolytic cell through electromigration, electroosmotic flow, or electrophoresis (Acar and Alshawabkeh 1993; Yao et al. 2012; Su 2014).

The vitrification technology is a process in which the soil is heated at a high temperature (1400–2000 °C) so that the contaminants get volatilized or decomposed (Yao et al. 2012).

Among all the aforementioned approaches described above, the most appropriate seems to be bioremediation which will be discussed more in the next section.

9.3.3 Bioremediation

Bioremediation is the implementation of living organisms for cleanup of contaminated environment to return it to its normal state. Various biological treatments are used as they are efficient, low-energy-consuming, environmental friendly with no secondary pollution, and cost-effective with long-term viability, have more tendency to be publicly accepted, and no intricacy is present in their technical process (Stocking et al. 2000). Moreover, it is possible to run this process both in anaerobic and aerobic environments (Vidali 2001).

Bioremediation utilizes microbes, fungi, or plants. Some are from the same habitat and nutrients, and other factors are provided to enhance the environment for them (bio-enhancement); some are introduced to augment the pollutant treatment (bio-augmentation) (Kharayat 2012; Qiu et al. 2012).

However, there are some shortcomings in using biological machinery as often remediation is elaborate and the desired aim will not be accomplished, there are many compounds that remediation has not yet been investigated for them (Wackett

and Hershberger 2001), and in many occasions good biological entity fails to depollute the environment (Ramos et al. 2010).

Due to the importance of microbial remediation and the purpose of this chapter, the microbial bioremediation is described in more details in the upcoming discussions.

9.4 Metalloid Oxyanion Detoxification Mechanisms

Different mechanisms are adopted by microorganisms to survive in the presence of toxic metalloid oxyanion while interacting with them. Till now, different metabolic functions have been proposed for oxyanion removal including energy conservation, metal assimilation, and metal detoxification. For halophiles, dissimilatory reduction (energy conservation) is the most reported way in which halophiles can overcome oxyanions' toxicity. They respire them and use the energy released to grow in anaerobic conditions. Selenite-respiring bacterium, *Selenihalanaerobacter shriftii* strain DSSe-1, which has been discussed previously is an example. Regarding assimilation of these metalloids (especially selenium) by halophilic organisms, no report has been published.

Among the studied microorganisms, extremophiles seem to be more efficient and promising due to their innate characteristics. Below, investigations done for four categories of extremophiles including halophiles, alkaliphiles, acidophiles, and thermophiles are reviewed. These organisms use different mechanisms in overcoming the toxic metalloids they encounter. Next discussions are focused on the mechanisms especially put into action by the extremophiles discussed. Figure 9.2 summarizes the mechanisms proposed by researchers in this field.

9.4.1 Halophiles and Toxic Oxyanions Bioremediation

Different concentrations of salt from freshwater environments to saturated ones exist in natural habitats. Halophilic, salt-loving, organisms are those who prevalently inhabit in these environments (Oren 2002). They belong to all three domains of life. Formally they are divided into slight, moderate, or extreme halophiles. Their salt (NaCl) requirements differ, and best growth occurs at 2–5% for "slight," 5–20% for "moderate," and 20–30% for "extreme" halophilic organisms. Halotolerant means that their best growth occurs in 0–5% of NaCl concentrations, and NaCl concentration of up to 3% can be tolerated depending on environmental and nutritional factors (DasSarma and Arora 2002).

In comparison to non-halophilic organisms, there are few reports on halophilic organisms' capability in oxyanion tolerance and removal. As it was shown, resistance toward oxyanions was much higher for the halophilic organism than the previously reported non-halophiles.

Nieto et al. had investigated the tolerance pattern of moderately halophilic bacteria including *Deleya halophila*, *Acinetobacter* sp., *Flavobacterium* sp.,

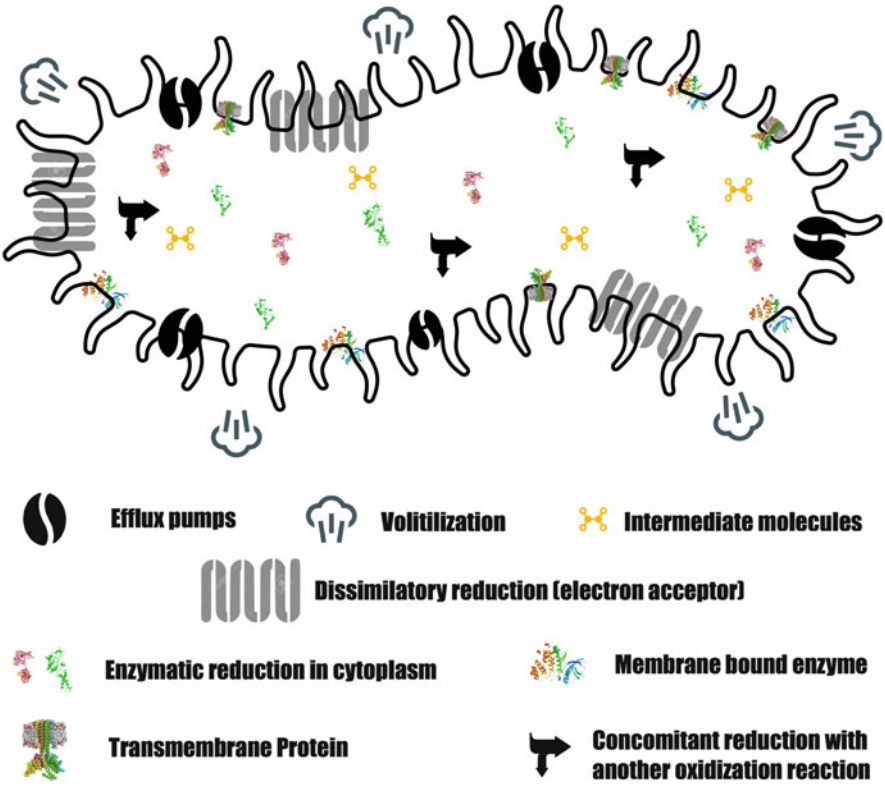


Fig. 9.2 Different mechanisms reported by extremophiles to overcome the toxicity of metalloid oxyanions, including: *Efflux pumps:* which transport the oxyanions out of the cell to reduce its cytoplasmic concentration. *Volatilization:* the toxic oxyanion is converted to its volatile species, e.g., methylation. *Intermediate molecules:* molecules which are generated during a reaction is cross reacting with the oxyanion and convert the oxyanion to its less toxic form e.g. monothioarsenate. *Dissimilatory reduction* in which the oxyanion is used as the final electron acceptor e.g. arsenate, Enzymatic reduction in the cytoplasm e.g. arsenate, chromate and tellurite reductase, *Membrane-bound enzymes* reduce the toxic material, e.g., copper-dependent membrane-associated Cr (VI) reductase. *Transmembrane proteins* which transport the pollutants to the cell so that they get reduced, e.g., chromate reduction. *Concomitant reduction with another oxidization reaction*, e.g., oxidized glycerol or glucose to acetate and CO_2 with concomitant reduction of selenate to selenite or selenium is reported (Please note that it may not be considered as a mechanism as it may be finally realized that these simultaneous reactions are due to an intermediate being exchanged in the reactions and not the oxyanions' direct involvement in the first reaction)

Marinococcus, *Sporosarcina*, *Micrococcus*, and *Staphylococcus* toward ten heavy metals including arsenate and chromium. The most metal tolerant of all among the 238 strains tested were *Acinetobacter* sp. strains. Natural susceptibility levels of the organisms studied in this report were heterogeneous, and strains of *Deleya halophila* were the most homogenous among them (Nieto et al. 1989).

Nesterenkonia sp. strain MF2, a Gram-positive moderately halophilic chromate-reducing bacterial strain, was isolated from effluents of tanneries. This strain was capable of tolerating up to 600 mM of chromate and complete reduction of 0.2 mM highly toxic and soluble CrO_4^{2-} into almost nontoxic and insoluble Cr (III) in 24 h under aerobic condition (Amoozegar et al. 2007).

There are some reports on the isolation of halophiles capable of withstanding these oxyanions. The selenite-respiring bacterium, *Selenihalanaerobacter shriftii* strain DSSe-1, is an obligately anaerobic halophilic bacterium from the Dead Sea that grew by concomitant reduction of selenate to selenite plus elemental selenium (Switzer Blum et al. 2001). In an investigation at hydrothermal vents of the Juan de Fuca Ridge in the Pacific Ocean aiming for isolation of tellurite- and selenite-resistant bacteria, all strains grew over a wide range of NaCl concentrations (up to 10%), and they required NaCl concentrations of 0.5% for growth. Growth capability for strain Se-1-1 was at 15% (w/v) NaCl, for strains Se-1-3-red and Se-6-2-red it was at 20% (w/v) NaCl, and strain Se-1-2-or showed the strongest requirement for salt, not growing below 1.5% (w/v) NaCl. These strains are closely related to genus *Pseudoalteromonas* (Rathgeber et al. 2002).

In another research, Kabiri et al. (2009) have isolated *Halomonas* strain MAM from a hypersaline soil in Garmsar, Iran, which has a high level of resistance toward tellurite and selenite. The same group has isolated and investigated tellurite and selenite removal by a moderately halophilic bacterium, *Salinicoccus iranensis* strain QW6, and has reported a high capacity of 0.5–1 mM for tellurite removal and 2–10 mM for selenite removal by this organism (Amoozegar et al. 2008). Moreover the synergistic effect of selenite presence in tellurite removal was reported. It seems that an intermediate is formed in one oxyanion detoxification mechanism that can react with the second oxyanion to remove or facilitate its removal, but the exact mechanism is not fully proven.

Halomonas sp. ZM3 was isolated from Zelazny, the largest mineral waste repository in Poland with MIC 9 and 100 mM toward As (III) and As (V), respectively (Dziewit et al. 2013). Another *Halomonas species*, *Halomonas smyrnensis* KS802, a moderately halophilic bacterium was isolated from multi-pond solar salterns of Gujarat, India, and complete reduction of 2 mM Cr (VI) was achieved in 12 h when the strain was grown in medium for halophiles (MH medium) supplemented with 4% galactose and 5% NaCl at pH 7 and 32 °C (Biswas et al. 2018). Characterization of a bacterial strain Cr (VI) reduction both in immobilized and cell-free extract status was carried out by Focardi et al. A new moderately halophilic Cr (VI)-resistant bacterial strain TA-04 was isolated from polluted marine sediments near a stainless steel plant in southern Italy. This bacterium was able to reduce Cr (VI) in the presence of 80 g/L NaCl, and the growth was inhibited by 4.0 mM Cr (VI) (Focardi et al. 2012). The first report on the purification of the enzyme responsible for tellurite reduction in a halophilic organism was reported by Alavi et al. (2014) which seems to be a nitrate reductase with tellurite reduction activity.

Halophilic organisms capable of metal/metalloid detoxification identified till now are summarized in Table 9.1.

Table 9.1 Halophilic organisms and metalloid detoxification

Halophilic bacteria

Metalloid	Organism	Description and morphological characteristics	Mechanism	References
Selenite-respiring bacterium	*Selenihalanaerobacter shriftii DSSe-1*	Gram-negative, nonmotile rod, anaerobic bacterium	Oxidized glycerol or glucose to acetate + CO_2 with concomitant reduction of selenate to selenite plus elemental selenium, and the other electron acceptor is arsenate	Switzer Blum et al. (2001)
Tellurite, selenite	*Halomonas* strain MAM	Gram-negative, motile, curved rod, nonspore-forming, facultatively anaerobic bacterium	Methylation (garlic odor due to dimethyl telluride production), alkylation and volatilization of Te. Local deposition of metallic selenium particles was near cytoplasmic membrane and also outside the cells probably due to selenium efflux or secretion of an extracellular enzyme implicating in Se reduction	Kabiri et al. (2009)
Tellurite, selenooxyanions	*Salinicoccus iranensis* strain QW6	Gram-positive, non-sporulating, nonmotile, strictly aerobic coccus bacterium	Transformation of tellurite and selenite to elemental tellurium and selenium, respectively. Synergetic effect of selenite on higher removal of tellurite was reported	Amoozegar et al. (2008)
As (III), As (V)	*Halomonas* sp. ZM3	Gram-negative, motile, rod bacterium	Resistance (MICs for As (III) and As (V) of 9 mM and 700 mM, respectively)	Dziewit et al. (2013)
Arsenate, chromium	*Deleya halophila*	Gram-negative, nonmotile, aerobic, rod bacterium	Resistance (MIC for As 10 mM and for Cr 2 mM)	Nieto et al. (1989); Valderrama et al. (1991)
	Acinetobacter sp.	Gram-negative bacteria	Resistance (MIC for As 10 mM and for Cr 2 mM)	Nieto et al. (1989)
	Flavobacterium sp.	Gram-negative, rod-shaped, aerobic bacteria	Resistance (MIC for As 10 mM and for Cr 2 mM)	Nieto et al. (1989)

	Marinococcus	Gram-positive, strictly aerobic, chemolithoautotrophic, and nonspore-forming bacteria	Resistance (MIC for As 1 mM and for Cr 0.5–1 mM in different species)	Nieto et al. (1989)
	Sporosarcina	Gram-positive, endospore-forming	Resistance (MIC for As 5 mM and for Cr 5 mM)	Nieto et al. (1989)
	Micrococcus	Gram-positive and strictly aerobic coccus bacterium	Resistance (MIC for As 5 mM and for Cr 5 mM)	Nieto et al. (1989)
	Staphylococcus	Gram-positive grape-like cluster bacteria	Resistance (MIC not reported)	Nieto et al. (1989)
	Nesterenkonia sp. strain MF2	Gram-positive, non-sporulating, nonmotile, strictly aerobic coccus bacterium	Reduction of soluble Cr (VI) (as CrO_4^{2-}) into almost nontoxic and insoluble Cr (III) under aerobic condition	Amoozegar et al. (2007)
Selenooxyanion, chromate, arsenate, tellurite	10 different *Bacillus* species	Gram-positive spore-forming aerobic bacteria	Resistance (MIC for selenooxyanion: Between 10 and 40 mM)	Amoozegar et al. (2005)
Chromate	*Halomonas* sp. TA-04	Gram-negative heterotrophic bacterium	Cr (VI) reduction both by whole cells and cell-free extract	Focardi et al. (2012)
	Halomonas smyrnensis KS802	Gram-negative, non-sporulating, motile rod bacterium	Cr (VI) and deposition of Cr (III) on bacterial cells along with distinct changes in cellular morphology Possible formation and complexation of chromium hydrogen phosphate and chromium hydroxide with cells	Biswas et al. (2018)
Arsenate-respiring	*Halarsenatibacter silvermanii* strain SLAS-1^T	Gram-negative, motile, curved rod bacterium	Facultative chemoautotrophic arsenate respirer (reduction of As (V) to As (III))	Blum et al. (2009)

(continued)

Table 9.1 (continued)

Halophilic bacteria				
Metalloid	Organism	Description and morphological characteristics	Mechanism	References
Halophilic archaea				
Arsenate	*Halococcus hamelinensis*	Nonmotile, strictly aerobic archaea	Presence of genes encoding arsenate reductase enzymes	Gudhka et al. (2015)
Selenite	*Halorubrum xinjiangense* strain 106	Non-sporulating, aerobic rod archaea	Selenite reduction and presence of high amounts of selenium-containing particles in the culture medium indicating the efficient transport of elemental selenium out of the cell	Güven et al. (2012)
Arsenic	*Haloarcula* sp. IRU1	Aerobic archaea	Arsenic bioaccumulation	Taran et al. (2013)
Arsenic resistance	*Halobacterium* sp. strain NRC-1	Aerobic chemoorganotrophic archaea	Arsenic resistance through arsenite (III) methyltransferase (approved by arsM gene knockout), finding an arsenite extrusion system with significant differences from bacterial counterparts	Wang et al. (2004)

9.4.2 Halotolerant and Toxic Oxyanions Bioremediation

There are some reports on halotolerant organisms. *Paenibacillus* sp. strain TeW, isolated from heavy metal-contaminated sediment, was highly resistant to tellurite (Chien and Han 2009). Leitão (2009) has reviewed the capability of *Penicillium* species in the bioremediation field. The first detailed report on effective reduction of Cr (VI) was for the halotolerant bacteria, *Planococcus maritimus* VITP2l, which was isolated from Kumta coastal region (Sangeetha et al. 2012; Zhao et al. 2018). The halotolerant bacteria exhibited complete reduction of 100 and 200 mg/L of Cr (VI) within 24 and 28 h, respectively, and greater than 90% reduction was observed for higher concentrations of Cr (VI) in the range of 300–500 mg/L (pH 7, 35 °C, and 4% (w/v) NaCl) (Sangeetha et al. 2012).

Reduction of selenite to red elemental selenium by moderately halotolerant *Bacillus megaterium* strains BSB6 and BSB12 isolated from Bhitarkanika mangrove soil able to completely reduce selenite (up to 0.25 mM) within 40 h under optimum growth condition at 37 °C, pH 7.5, and 7% (w/v) salt (NaCl) was analyzed (Mishra et al. 2011).

Biosorption and detoxification of Cr (VI) by the tannery effluent acclimatized halotolerant *Bacillus* sp. strain pv26 (designated as TVU-K1) was investigated by Vijayanand and Hemapriya (2014). Strain TVU-K1 showed remarkable tolerance (up to 400 mg/L) toward Cr (VI). Atomic absorption spectroscopy revealed that TVU-K1 showed 81% Cr (VI) reduction ability (Vijayanand and Hemapriya 2014).

Chromium-resistant and chromium-reducing bacteria, belonging to *Arthrobacter*, *Pseudomonas*, and *Corynebacterium* isolated from chromite mine overburden and seepage samples of Orissa (renamed as Odisha), India, were found to tolerate 12–18 mM Cr (VI) during growth. Viable cells of these isolates were also capable of growing and reducing 100 mM Cr (VI) quite efficiently in Vogel Bonner (VB) broth under batch cultivation (Dey et al. 2014).

Bacillus sp. strain QW90, a bacterial strain with MIC of 550 mM for selenite and the capability to reduce selenite, was characterized. This strain was able to grow in NaCl range of 0–30%, and the optimum growth was seen at 30 °C, pH 7.0, and 3% (w/v) NaCl (Khalilian et al. 2014). It seems that this bacterium is halotolerant like other *Bacillus* species even though it was not mentioned by them.

A chromate-reducing actinomycete, *Arthrobacter* sp. SUK 1205, isolated from chromite mine overburden of Odisha, India, exhibited significant chromate reduction during growth. Chromate-reducing efficiency was promoted when glycerol and glucose were used as electron donors and pH and temperature were maintained at 7.0 and 35 °C, respectively (Dey and Paul 2015).

In 2016, Dey et al. isolated two halotolerant arsenite and arsenate resistance bacteria which were identified as a *Bacillus* sp. (SW2) and *Aneurinibacillus aneurinilyticus* (SW4), respectively. SW2 and SW4 capability for bioremediation were analyzed by using 100 ppm concentration of these toxic species, and it was revealed that 51.45% and 51.99% for arsenite and 53.29% and 50.37% for arsenate, respectively, were removed by these strains (Dey et al. 2016).

In another study, a selenate-reducing bacterium (strain 9a) and a tellurite-reducing bacterium (strain Taa) were isolated from brackish areas in Osaka, Japan. Both bacterial strains isolated belonged to *Shewanella* species, and by contrast, they were halotolerant. Under anaerobic conditions strain 9a could remove 45–70% of 1.0 mM selenate and selenite from water containing up to 3% (w/v) NaCl within 4 days, while strain Taa within 3 days could anaerobically and aerobically remove 70–90% of 0.4 mM tellurite from water containing up to 6% (w/v) NaCl. Under microaerobic conditions insoluble tellurium formed by strain Taa was globular, but it was nanorod under aerobic conditions (Soda et al. 2018).

Psychrobacter glacincola BNF20, isolated from a sediment sample from King George Island, Antarctica, was the first genome sequence reported for this species. Previously reported as a halotolerant bacteria, this species showed high tellurite (MIC 2.3 mM) and chromate (MIC 6.0 mM) resistance (Muñoz-Villagrán et al. 2018). Other than the mentioned investigations, more studies are reviewed and summarized in Table 9.2.

9.4.3 Halophilic Archaea and Toxic Oxyanions Bioremediation

Arsenic resistance in *Halobacterium* sp. strain NRC-1 which is an excellent model for post-genomic analyses of heavy metal resistance was examined by an improved gene knockout system (Wang et al. 2004). Using genetic analyses for metal detoxification investigations, Wang et al. proposed different mechanisms for arsenic tolerance in *Halobacterium*. One was the alkylation and exportation of the product outside the cell. The other mechanism was the volatilization of arsenite by its alkylation, i.e., methylation (Wang et al. 2004).

The first reported example of an arsenate-respiring extreme halophile is *Halarsenatibacter silvermanii* strain SLAS-1 that lives in the Searles lake brine. The potential substrate interactions of strain SLAS-1 with other diverse extremophiles present in the same habitat have been investigated too (Blum et al. 2009).

Among halophilic archaea which are supposed to show high resistance toward salts, there are some reports on their tolerance and resistance. *Halorubrum xinjiangense* strain 106, a selenite-reducing archaea, with 25 mM MIC toward selenite was isolated from Tuz (salt) Lake in Turkey (Güven et al. 2012).

In a study by Taran et al., the ability of *Haloarcula* sp. IRU1, a novel halophilic archaeon isolated from Urmia Lake, Iran, for arsenic bioaccumulation was investigated and optimized by Taguchi experimental design. Under optimum conditions (temperature 40 °C, pH 8, and $NaAsO_2$ at 90 mg/L), the microorganism was able to perform their desired function with a 60.89 percent removal of arsenic (Taran et al. 2013). Also, for halophilic stromatolite archaeon, *Halococcus hamelinensis*, through genome analyses, it was inferred that arsenical pump-driving ATPase, arsenate reductase, and arsenical-resistance protein ACR3 are present (Gudhka et al. 2015).

Table 9.2 Halotolerant organisms and metalloid detoxification

Halotolerant bacteria				
Metalloid	Organism	Description and morphological characteristics	Mechanism	References
Arsenic-resistant	*Bacillus* sp. KM02	Gram-positive rod aerobic bacterium	Resistance (MIC of 4500 ppm for arsenate, 600 ppm for arsenite)	Dey et al. (2016)
	Aneurinibacillus aneurinilyticus strain BS-1	Gram-positive rod aerobic bacterium	Resistance (MIC of 4500 ppm for arsenate, 600 ppm for arsenite)	Dey et al. (2016)
Tellurite	*Paenibacillus* sp.	Gram-positive, endospore-forming, rod-shaped aerobic bacterium	Aerobic reduction	Chien and Han (2009)
	Shewanella species strain Taa	Gram-negative, rod-shaped aerobic, anaerobic, or microaerophilic bacterium	Aerobically or anaerobically reduce selenite and tellurite to their elemental forms	Soda et al. (2018)
Detoxification of chromate Cr (VI)	Halotolerant *Bacillus* strain pv26	Gram-positive rod aerobic bacterium	Reduce Cr (VI) under aerobic or anaerobic conditions through electron transport systems containing cytochromes	Vijayanand and Hemapriya (2014)
	Planococcus maritimus VITP21	Gram-positive, circular, optically transparent filiform aerobic bacterium	Chromate reduction mainly associated with the soluble cytosolic fraction of the cell	Sangeetha et al. (2012)
Selenite, tellurite	Four strains of *Pseudoalteromonas, Se-1-1, Se-1-3-red, Se-6-2-red, and Se-1-2-or*	Gram-negative straight or curved rod-shaped obligately aerobic bacterium	Metalloid resistance (MIC of K_2TeO_3 ranged from 1500 to greater than 2500 μg/mL, and the MIC of Na_2SeO_3 ranged from	Rathgeber et al. (2002)

(continued)

Table 9.2 (continued)

Halotolerant bacteria				
Metalloid	Organism	Description and morphological characteristics	Mechanism	References
			6000 to greater than 7000 μg/mL for 10 strains), accumulation of metallic tellurium or selenium.	
Selenite	*Bacillus megaterium* strains BSB6 and BSB12	Gram-positive motile rod aerobic bacterium	Reduction of Se (IV) up to 0.25 mM and formation of crystalline spherical selenium particles	Mishra et al. (2011)
Selenate, selenite	*Shewanella* species strain 9a	Gram-negative, rod-shaped aerobic, anaerobic, or microaerophilic bacterium	Reducing selenate and selenite into elemental selenium under anaerobic conditions rather than aerobic conditions, suggesting that this occurred via dissimilatory reduction	Soda et al. (2018)
Tellurite (MIC 2.3 mM) and chromate (MIC 6.0 mM) resistance	*Psychrobacter glacincola*	Gram-negative, nonmotile, rods or coccobacilli aerotolerant bacterium	Resistance (tellurite (MIC 2.3 mM) and chromate (MIC 6.0 mM))	Muñoz-Villagrán et al. (2018)

9.4.4 Alkaliphiles and Toxic Oxyanions Bioremediation

Optimum pH for microorganism's growth depends on the conditions of growth, especially nutrients, metal ions, and temperature. Accordingly, several microorganisms have more than one optimum pH for growth. The microorganisms with the optimal growth at pH 9 and above, often between 10 and 12 which are unable to grow or have slow growth in natural pH, are called "alkaliphiles" (Horikoshi 1999; Horikoshi et al. 2011). In general, there are few articles about oxyanions bioremediation by alkaliphiles, and among them, the most attention has been given to chromium. Until 2004, when Ye et al. introduced a new bacterium called *Alkaliphilus metalliredigens* as a novel alkaliphilic and metal-reducing

bacterium, bioremediation of metalloids by alkaliphiles was not reported. *Alkaliphilus metalliredigens* is a strict anaerobe bacterium with the optimum growth at pH 9.5 and obtained from leachate ponds. This bacterium uses Cr (VI), Fe (III)-citrate, Fe (III)-EDTA, or Co (III)-EDTA as the electron acceptors and can reduce metals at pHs of up to 11 (Ye et al. 2004).

The other report on bioreduction of Cr (VI) by alkaliphilic microorganisms is related to an alkaliphilic *Bacillus subtilis*, isolated from tannery effluent-contaminated soil by Mary Mangaiyarkarasi et al. (2011). This strain could grow at pH 9 and reduce 100% of Cr (VI) in alkaline conditions. The ability to reduce Cr (VI) is associated with the membrane-bound proteins (Mary Mangaiyarkarasi et al. 2011). Alkaliphilic *Amphibacillus* sp. KSUCr3 isolated from hypersaline soda lake is another strain with high Cr (VI)-reducing capability under alkaline conditions, introduced by Ibrahim et al. (2011). This strain rapidly reduced 5 mM of Cr (VI) over 24 h showing a high efficiency in detoxification of chromate. This strain not only could tolerate high concentration of Cr (VI), 75 mM, but also could tolerate high concentrations of other heavy metals including Mn^{2+} (100 mM), Ni^{2+} (100 mM), Mo^{2+} (75 mM), Pb (75 mM), Co^{2+} (5 mM), Zn^{2+} (2 mM), and Cu^{2+} (2 mM) (Ibrahim et al. 2011). In 2012, subcellular fractions of *Amphibacillus* sp. KSUCr3 were investigated to determine the placement of the chromate reductase enzyme. This research showed that the enzyme was a membrane-bound enzyme, with copper ion requirement for its activity (Ibrahim et al. 2012a).

Bacillus sp. KSUCr9a, an alkaliphilic bacterium isolated from soda lakes in northern Egypt, showed Cr (VI) resistance up to 75 mM, which was reported by Ibrahim et al. in 2012. This bacterium also showed halotolerance nature (reduction of Cr (VI) in 0–20% NaCl) and resistance to Cd^{2+} (50 mM), Mo^{2+} (75 mM), Mn^{2+} (100 mM), Cu^{2+} (2 mM), Ni^{2+} (100 mM), Pb^{2+} (75 mM), Co^{2+} (5 mM), and Zn^{2+} (2 mM) (Ibrahim et al. 2012b).

The other study on Cr (VI) reduction by alkaliphiles was reported by Watts et al. (2015). This group introduced an alkaliphilic bacterium belonging to *Halomonas* genus from a chromite ore processing residue (COPR) extract that could rapidly reduce significant concentrations of aqueous Cr (VI), 2.5 mM to predominantly Cr (III) minerals, under anaerobic and alkaline conditions up to pH 10.5 (Watts et al. 2015).

Citricoccus alkalitolerans CSB1 is another alkaliphilic strain with the ability of chromate removal, introduced by Abhay et al. (2016). This bacterium could tolerate 210 μg/mL of Cr (VI) and up to 25% NaCl (w/v) at pH 8.0–10.0 and remove nearly 98% of 120 μg/mL within 72 h at pH 9 (Abhay et al. 2016).

Literature review shows that bioremediation of other oxyanions by alkaliphilic bacteria has been less investigated. Roh et al. (2007) examined metal reduction and mineral formation using *Alkaliphilus metalliredigens* (QYMF), an alkaliphilic bacterium isolated from leachate pond. This bacterium used selenate (SeO_4^{2-}), chromate (CrO_4^{2-}), Co (III)-EDTA, Fe (III)-citrate, and Fe (III)-EDTA as electron acceptors at pH 9.5 (Roh et al. 2007). In 2008, Fisher et al. reported the *Alkaliphilus oremlandii* strain OhiLAs as a newly isolated strain with the ability of inorganic and organic arsenic transformation. Table 9.3 summarizes the alkaliphiles involved in bioremediation of metalloids.

Table 9.3 Alkaliphilic organisms and metalloid detoxification

Alkaliphilic bacteria				
Oxyanion	Organism	Description and morphological characteristics	Mechanism	References
Chromium	*Alkaliphilus metalliredigens* isolate QYMF	Gram-positive, straight cells, some slightly curved, spore-forming (terminal endospores), motile, strict anaerobe	Cr (VI) as electron acceptors, reduction	Ye et al. (2004)
	Bacillus subtilis	Gram-positive, facultative anaerobe	Reduction by chromate reductase (membrane-bound protein)	Mary Mangaiyarkarasi et al. (2011)
	Amphibacillus sp. KSUCr3	Gram-positive, facultative anaerobe	Reduction	Ibrahim et al. (2011)
	Bacillus sp. KSUCr9a	Gram-positive	Reduction	Ibrahim et al. (2012b)
	Halomonas sp. Mono Lake isolate	Gram-negative, obligate heterotroph, facultative anaerobe	Direct, anaerobic, enzymatic Cr (VI) reduction	Watts et al. (2015)
	Citricoccus alkalitolerans CSB1	Gram-positive, alkaliphile and salt-tolerant	Tolerance and removal (surface binding as well as intracellular uptake of chromium by the bacterial cell)	Abhay et al. (2016)
Chromium and selenium	*Alkaliphilus metalliredigens* QYMF	Gram-positive, anaerobe	Chromate and selenate as electron acceptors	Roh et al. (2007)
Arsenic	*Alkaliphilus oremlandii* OhiLAs	Gram-positive, spore-forming, motile, low mole %GC, strict anaerobe	Reduction of arsenate by using it as terminal electron acceptor. Expression of a respiratory arsenate reductase	Fisher et al. (2008)

9.4.5 Haloalkaliphiles and Toxic Oxyanions Bioremediation

The group of bacteria able to grow under the combination of extremities (high salts and alkaline pH) are referred to as haloalkaliphiles. They have special mechanisms to adapt themselves so that they can survive and grow under high salt and pH conditions (Chela-Flores 2013).

Bacillus arsenicoselenatis sp. nov. and *Bacillus selenitireducens* sp. nov. are two anaerobic haloalkaliphilic bacteria reported to respire oxyanions of selenium and arsenic (Switzer Blum et al. 1998). For tellurite which is the most toxic metalloid among them, maximum MIC reported was by a haloalkaliphilic archaeon *Natronococcus occultus* (Pearion and Jablonski 1999).

A haloalkaliphilic deltaproteobacterium designated as strain MLMS-1 isolated from anoxic bottom water from Mono Lake, California, USA, was a Gram-negative, motile curved rod that grew by oxidizing sulfide while reducing arsenate (Hoeft et al. 2004). It was the first obligate arsenate-respiring chemoautotroph reported confirmed by the incorporation of $H_{14}CO_3$ into dark-incubated cells (Hoeft et al. 2004) which couples arsenate reduction to arsenite with the oxidation of sulfide to sulfate (Planer-Friedrich et al. 2015). Reduction of arsenate occurred concomitantly with the removal of sulfide, and no loss of sulfide occurred in control cultures without arsenate or in sterilized samples containing both arsenate and sulfide (Hoeft et al. 2004).

Selenihalanaerobacter shriftii strain SLAS-1, an extremely haloalkaliphilic anaerobic bacterium, which showed dissimilatory reduction of selenite and arsenite was isolated from the sediments and could grow via As (V) respiration (Oremland et al. 2005). Arsenite can serve as the electron donor for a facultative chemoautotrophic bacterium *Alkalilimnicola ehrlichii* sp. nov. from Mono Lake, California (Hoeft et al. 2007).

Genome analyses indicate that a conventional arsenite oxidase (Aox) is absent; instead it has two operons encoding respiratory arsenate reductase (Arr). Under chemolithoautotrophic conditions, one operon is expressed, and it exhibits both arsenite oxidase and arsenate reductase activity. It was shown that depending on the electron potentials of the molybdenum center and [Fe–S] clusters, additional subunits, or constitution of the electron transfer chain, Arr can be a reductase as well as an oxidase (Richey et al. 2009).

In another research, light-dependent oxidation of arsenite to arsenate, coupled with autotrophic growth, by an *Ectothiorhodospira*-dominated consortium of bacteria in Mono Lake, California, was reported for the first time (Budinoff and Hollibaugh 2008). Most of the haloalkaliphilic species reported are from Mono Lake, California, USA. *Bacillus beveridgei* strain MLTeJB, a facultative anaerobic haloalkaliphile from the same area, was reported to respire oxyanions of tellurium, selenium, and arsenic (Baesman et al. 2009). Another haloalkaliphilic sulfate-respiring bacterium, *Desulfohalophilus alkaliarsenatis* gen. Nov. strain SLSR-1, was isolated from another extreme environment of California, Searles Lake (Blum et al. 2012).

Sorokin et al. inoculated an anaerobic enrichment culture with a sediment sample from soda lakes of the Kulunda Steppe. The enrichment culture conditions were pH 10, moderate salinity, elemental sulfur as electron acceptor, and formate as electron donor. This resulted in domination of a new haloalkaliphilic, obligate anaerobe, Gram-positive and spore-forming bacterium, *Desulfuribacillus alkaliarsenatis*, designated as strain AHT28 (Sorokin et al. 2012).

Pseudochrobactrum saccharolyticum LY10, a potent novel alkaliphilic halotolerant strain, was isolated and characterized for its high Cr (VI)-reducing ability. Maximum reduction rate was achieved under optimum conditions with initial pH 8.3, 20 g/L NaCl, 55 mg/L Cr (VI), and 1.47×10^9 cells/mL of cell concentration through metabolism-dependent bioreduction process (Long et al. 2013).

The first report on haloalkaliphilic *Halomonas* from tannery effluent was reported by Mabrouk et al. (2014). Hexavalent chromium reduction by newly isolated chromate-resistant haloalkaliphilic *Halomonas* sp. M-Cr which was the most potent strain among other strains showed an 82% reduction of 50 mg/L Cr (VI) in 48 h, concomitant with discoloring of the medium yellow color and the formation of white insoluble precipitates of Cr (III). It exhibited growth up to 3500 mg/L of Cr (VI) and 20% (w/v) NaCl and showed strong Cr (VI) reduction under alkaline condition, pH 10 (Mabrouk et al. 2014).

In another study recently by Verma and Agarwal (2016), a chromate-resistant haloalkaliphilic strain of *Bacillus circulans* TVD-5 was isolated from tannery solid waste.

Cellulosimicrobium funkei strain AR8 is a novel haloalkaliphilic bacterium which was reported by Karthik et al. (2017), and reduction of Cr (VI) by this bacterium was evaluated under different conditions by the researchers. They proved the bioconversion of Cr (VI) to Cr (III) and the involvement of extra-/intracellular- reducing machinery in its reduction (Karthik et al. 2017). For more information on the alkaliphilic microorganism's strategies for overcoming toxic metalloids, please refer to Table 9.4.

9.4.6 Acidophiles and Toxic Oxyanions Bioremediation

"Acidophile" is the group of extremophiles which shows the ability to survive in low-pH environments (<3) and can be found in all three domains of organisms (Hallberg and Johnson 2001). According to the generally accepted categorization, "extreme acidophiles" refer to microorganisms with the optimum pH for growth of 3 or less (Johnson 1998), while the microorganisms with optimum pH between 3 and 5 are categorized in "moderate acidophile" group. Furthermore, the microorganisms that have a pH optimum above 5 but are active in low-pH environments are referred to as "acid-tolerant" microorganisms (Johnson 2008). The acidophiles include a number of autotrophic and heterotrophic organisms, which can thrive in natural and man-made acidic environments (Sharma et al. 2016), and therefore, they can be an important choice for bioremediation of acidic effluents that may contain oxyanions.

Table 9.4 Haloalkaliphilic organisms and metalloid detoxification

Halophilic organisms				
Metalloid	Organism	Description and morphological characteristics	Mechanism	References
Tellurium oxyanions (Te (VI), Te (IV)), selenium oxyanions (Se (VI), Se (IV)), and arsenate (As (V))	*Bacillus beveridgei* Strain MLTeJB	Gram-positive, motile rod with a peritrichous flagellum, facultative anaerobic bacterium	Respiration using Te (VI), Te (IV), Se (VI), Se (IV), As (V) as electron acceptors	Baesman et al. (2009)
Arsenite	*Alkalilimnicola ehrlichii* sp. nov.	Gram-negative, short motile rod facultative anaerobic and facultative chemoautotrophic bacterium	Chemoautotrophic growth with either As (III), H2, or sulfide serving as the electron donor and with NO_3^- as the electron acceptor	Oremland et al. (2002); Hoeft et al. (2007)
	Ectothiorhodospira-dominated consortium of bacteria	Arsenite-dependent photoautotroph bacterium	Light-dependent oxidation of arsenite to arsenate, coupled with autotrophic growth	Budinoff and Hollibaugh (2008)
Arsenate	*Halarsenatibacter silvermanii*, strain SLAS-1	Gram-negative curved rod, motile, strictly anaerobic, bacterium	Dissimilatory (respiratory) reduction of arsenate to arsenite	Oremland et al. (2005); Blum et al. (2009)
	Deltaproteobacterium strain MLMS-1	Gram-negative, motile curved rod, anaerobic chemolithotroph bacterium	Obligate arsenate-respiring chemoautotroph which grows by coupling arsenate reduction to arsenite with the oxidation of sulfide to sulfate, the intermediate molecule is discovered to be monothioarsenate	Hoeft et al. (2004); Planer-Friedrich et al. (2015)
	Desulfohalophilus alkaliarsenatis gen. Nov. strain SLSR-1	Gram-negative motile vibrio obligate anaerobic bacterium	Respiration (dissimilatory arsenate and sulfate reduction)	Blum et al. (2012)

(continued)

Table 9.4 (continued)

Halophilic organisms				
Metalloid	Organism	Description and morphological characteristics	Mechanism	References
Chromate	*Pseudochrobactrum saccharolyticum LY10*	Gram-negative short rod obligate aerobe bacterium	Reduction	Long et al. (2013)
	Bacillus circulans TVD-5	Gram-positive, rod-shaped aerobic bacterium	Resistance by reducing Cr (VI) aerobically	Verma and Agarwal (2016)
	Halomonas sp. *M-Cr*	Aerobic bacterium	Resistance by reducing Cr (VI) into Cr (III)	Mabrouk et al. (2014)
	Cellulosimicrobium funkei strain AR8	Gram-positive rod, aerobic bacterium	Bioconversion of Cr (VI) to Cr (III)	Karthik et al. (2017)
Selenite and arsenate	*Bacillus arsenicoselenatis bacillus selenitireducens*	Gram-positive rod, anaerobic bacterium	Respiration (dissimilatory reduction of As (V) to As (III) with concomitant oxidation of lactate to acetate plus CO2)	Switzer Blum et al. (1998)
Tellurite	*Natronococcus occultus*	Heterotrophic aerobic archaeon	Resistance with MIC 10 mM (maybe methylation of tellurium due to garlic odor produced)	Pearion and Jablonski (1999)

In the literature review, the first report on bioremediation of toxic oxyanions by acidophilic microorganisms is related to Torma and Habashi which has been conducted in 1972. They reported the copper (II) selenide oxidation by *Thiobacillus ferrooxidans* (Torma and Habashi 1972). Another report on *Thiobacillus ferrooxidans* showed its ability to tolerate 75 mM chromium. Furthermore, in this study, using electron microscopy it was revealed that the chromium uptake by this bacterium occurred through precipitation by a chromium-rich compound on the surface of the bacterial cells (Baillet et al. 1998). Also, according to a report conducted by Zhou et al. (2004), 100% Cr removal by acidophilic *Thiobacillus* was reported after 8 days of bioleaching.

Acidiphilium rubrum is another acidophilic bacterium which has a high tolerance to Fe, Cr, and Ni by accumulating them inside the cell (Itoh et al. 1998).

On chromium removal by acidophiles, another bacterium reported was *Acidocella aromatica* strain PFBC, an extremely acidophilic Fe (III)-reducing bacterium, which was tested for chromate removal. This bacterium could readily reduce 20 μM Cr (VI) to Cr (III) at pH 2.5 (the final concentration of Cr (VI) 0.4 μM (0.02 mg/L) (Masaki et al. 2015).

Gan et al. (2018) investigated chromium reduction capacity in *Acidithiobacillus thiooxidans*, *At. Ferrooxidans*, and *Leptospirillum ferrooxidans*. They showed that the interaction between these bacteria and pyrite significantly promote Cr (VI) reduction. In this research *Acidithiobacillus thiooxidans* showed maximum reduction efficiency (Gan et al. 2018). In another research, executed by Gan et al., sulfide mineral-based Cr (VI) reduction was examined. They reported *Acidithiobacillus ferrooxidans* could enhance Cr (VI) reduction by the acceleration of acid nonsoluble sulfide mineral dissolution (Gan et al. 2019).

In a report by Zeng et al. in 2016, enrichment of a sulfur-oxidizing community from tannery sludge was performed, and bioleaching of tannery wastewater containing 0.9–1.2% chromium by the enriched community was evaluated. At maximum, up to 96.8% Cr bioleaching efficiency was reported, and *Acidithiobacillus thiooxidans* was introduced as the main bacterial strain in this community (Zeng et al. 2016).

In other research by Zeng et al. which was conducted in 2019, chromium bioleaching by an enriched bacterial community was reported. In this study the maximum rate of sludge treatment was 4.60 g/L/day with over 90% chromium removal. *Acidithiobacillus bacterium* was reported to be critical for bioleaching of tannery sludge. The other genera which showed important roles in this process were *Sulfobacillus*, *Alicyclobacillus*, *Mycobacterium*, and *Acidiphilium* (Zeng et al. 2019).

In the report on the evaluation of iron, copper, and chromium transformation by extreme acidophiles, Johnson et al. (2017) showed that *Acidithiobacillus ferridurans* and *Acidiphilium cryptum* could reduce chromium (VI) only in the presence of iron (III).

There are many researches about arsenic removal by acidophiles. Among them, *Acidithiobacillus ferrooxidans* is an acidophilic strain by which arsenic removal was considered by some researchers. According to research done in 2003,

Acidithiobacillus ferrooxidans strain CC1 showed the ability of arsenic removal. This strain precipitated arsenic as arsenite only when grown on ferrous iron. By the results obtained from this research, it was deduced that removal of arsenite by co-precipitation with ferric iron is a general property of *At. ferrooxidans* species (Duquesne et al. 2003). On the same note, arsenic tolerance of *At. ferrooxidans* BYQ-12 was examined, and this bacterium was used for bioleaching arsenic from realgar. The inhibitory concentrations of arsenite and arsenate for this strain was 32 and 64 mM, respectively, and the maximum rate of arsenic bioleaching was 73.97% (Yan et al. 2017). Also, Gao et al. (2018) showed the ability of *At. ferrooxidans* BY3 in bio-adsorption and biotransformation of arsenic. Furthermore, Kamde et al. reported arsenic removal mediated by iron oxidation using *At. ferrooxidans* in 2018. In this research, they showed that using a combination of bio-oxidation and filtration, arsenic removal efficiency was improved (Kamde et al. 2018).

In the other research, performed by Bruneel et al. (2003), the newly acidophilic *Thiomonas* strains with the ability of growth near neutral pH was isolated which has demonstrated the capability of oxidizing As (III) to As (V) in vitro. Also, Duquesne et al. (2008) introduced another bacterium belonging to genus *Thiomonas* with arsenite oxidation ability. This bacterium catalyzed the oxidation of arsenite by arsenite oxidase, which is a membrane-bound enzyme (Duquesne et al. 2008).

Another bacterium with the capability of arsenic resistance is a moderately thermophilic acidophilic bacterium, *Acidithiobacillus caldus* KU. This bacterium showed inducible chromosomal encoded resistance to arsenate, arsenite, and antimony. In this study, Dopson et al. found that in the presence of energy source, the induced bacterium could transport arsenate and arsenite out of the cell. Furthermore, in this research by southern hybridization analyses, it was revealed that induced *At. caldus* expressed arsenate reductase. By this analysis, it was shown that on the chromosome of *At. caldus* and other Gram-negative acidophilic bacteria, there is a homolog of *Escherichia coli arsB*, indicating that probably resistance to arsenic is a highly conserved property in these microorganisms (Dopson et al. 2001).

Some researchers focused on mixed cultures for oxyanions removal investigations. Sulfate-reducing bacteria (SRB) are acidophilic bacteria that can remove oxyanions, as suggested by some researchers. Le Pape et al. (2017) observed complete removal of arsenic and zinc from the solution of 1.06 and 0.23 mmol/L, respectively, of each oxyanion by indigenous SRB consortium in acid mine drainage water. They proposed that the reduction of As_2S_3 to AsS relies on the biogenic H_2S, which enhanced the As removal efficiency (Le Pape et al. 2017). Also, Serrano and Leiva reported the removal of As from acid mine drainage (AMD) mediated by acid/metal-tolerant SRB. They showed that SRB can tolerate high concentrations of As (~3.6 mg/L) and accomplish As removal up to 73% (Serrano and Leiva 2017). In the other research, de Matos et al. (2018) reported simultaneous bio-precipitation of sulfate and trivalent arsenic by immobilized acidophilic SRB mixed culture. They showed that this consortium is resistant to up to 8.0 mg/L of arsenite. *Pantoea agglomerans*, *Enterobacter* sp., *Citrobacter* sp., *Cupriavidus metallidurans*, *Ralstonia* sp., and *Burkholderia cepacia* were the main bacterial species in this

consortium, and the efficiency of SO_4^{2-} and As (III) removal were 74.8% and 80%, respectively (de Matos et al. 2018).

Hong et al. (2016) conducted a study on oxidation of arsenic by mixed cultures and pure cultures of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. In this research refractory gold concentrates which contain a high amount of arsenic, approximately 139.67 g/kg of total As, were used in the reaction. Oxidation percentages during 358 h for adapted mixed culture, non-adapted mixed culture, and pure cultures of *At. ferrooxidans* and *At. thiooxidans* were 72.20%, 38.20%, 27.70% and 11.45%, respectively. In this study, the maximum oxidation of As was 77% at 120 h of reaction, achieved by adapted culture. The adaptation of each bacterium was done by cultivating them separately in the presence of gold concentrates as the source of arsenic (Hong et al. 2016).

Sulfolobus acidocaldarius strain BC is a thermoacidophilic archaeon with the ability of arsenite oxidation to arsenate that was introduced in 1992 (Sehlin and Lindström 1992).

In addition to the cases mentioned, in 2004, Casiot et al. reported arsenic oxidation and bioaccumulation by *Euglena mutabilis*, an acidophilic protozoan. This protozoan can oxidate arsenite to arsenate in few days and bioaccumulate As in the cell as inorganic arsenite and arsenate (Casiot et al. 2004). All researches about metalloid bioremediation by acidophiles are summarized in Table 9.5.

9.4.7 Thermophiles and Toxic Oxyanions Bioremediation

The organisms with the ability of growth in temperatures between 60 and 85 °C are called "thermophile." "Hyperthermophiles" are organisms with the optimum growth temperature at or higher than 80 °C (Horikoshi et al. 2011).

In the context of toxic oxyanions' bioremediation by thermophiles, there are reports since 1988. *Thermus scotoductus* strain SA-01 is a thermoacidophilic strain, which showed the ability of Cr (VI) reduction under the aerobic condition and at the optimum temperature of 80 °C (Opperman and van Heerden 2007). Furthermore, in 2008 a membrane-bound chromate reductase from this bacterium was purified (Opperman and Van Heerden 2008). Purification and characterization of this enzyme showed that it is active at pH 6.3 and temperature of 65 °C. Further studies showed that the enzyme activity depends on Ca^{2+} or Mg^{2+} ions and NADH or NADPH, with a preference for NADPH. Sequence analyses determined that chromate reductase is related to the old yellow enzyme family, particularly xenobiotic reductases participant in the oxidative stress response (Opperman et al. 2008).

In another research Bhowmick et al. (2009) reported an anaerobic thermophilic bacterium, with 86–96% similarity to *Thermoanaerobacter* strains. This *Thermoanaerobacter* BSB-33 strain could reduce Cr (VI) at an optimum temperature of 60 °C and pH 6.5. Fractionation of cell-free extracts demonstrated the reduction of Cr (VI) both in cytoplasm and membrane (Bhowmick et al. 2009). In the other research performed by Bhattacharya et al. 2015, complete genome sequencing of the BSB-33 strain was performed, and based on 16S and cpn60 UT

Table 9.5 Acidophilic organisms and metalloid detoxification

Acidophilic bacteria				
Oxyanion	Organism	Description and morphological characteristics	Mechanism	References
Selenium	*Thiobacillus ferrooxidans*	Gram-negative, chemolithotroph, aerobic (capable of anaerobic growth on sulfur substrates under certain conditions)	Oxidation of copper selenide	Torma and Habashi (1972); Leduc (2002)
Chromium	*Thiobacillus ferrooxidans*	Gram-negative, chemolithotroph, aerobic (capable of anaerobic growth on sulfur substrates under certain conditions)	Tolerance of high concentration of Cr^{3+} Uptake of Cr^{6+} by precipitation on the surface of bacterial cells	Baillet et al. (1998); Leduc (2002)
	Acidophilic *Thiobacillus*	Gram-negative, sulfur-oxidizing, nonphototrophic rods	Chromium solubilization	Zhou et al. (2004); Robertson and Kuenen (2006)
	Acidiphilium rubrum	Gram-negative, purple photosynthetic, chemoheterotrophic aerobe	Tolerance by accumulating of Fe, Cr, and Ni metals inside cells	Itoh et al. (1998); Matsuzawa et al. (2000)
	Acidocella aromatica PFBC	Gram-negative, motile rod, extreme acidophilic, mesophilic, obligately heterotroph Aerobic, but capable of Fe reduction under microaerobic and anaerobic conditions	Reduction and immobilization of Cr (VI)	Jones et al. (2013); Masaki et al. (2015)
	Acidithiobacillus thiooxidans	Gram-negative, rod, motile, obligate chemolithotroph and autotroph, strictly aerobic	Cr (VI) reduction	Kelly and Wood (2015); Gan et al. (2018)
	Acidithiobacillus ferrooxidans	Gram-negative, rod, motile, obligate chemolithotroph and autotroph, strictly aerobic	Cr (VI) reduction	Kelly and Wood (2015); Gan et al. (2019)
	Acidithiobacillus thiooxidans	Gram-negative, rod, motile, obligate	Bioleaching of chromium	Kelly and Wood

(continued)

Table 9.5 (continued)

Acidophilic bacteria				
Oxyanion	Organism	Description and morphological characteristics	Mechanism	References
		chemolithotroph and autotroph, strictly aerobic		(2015); Zeng et al. (2016)
	Acidithiobacillus	Gram-negative, rod, motile, obligate chemolithotroph and autotroph, strictly aerobic	Bioleaching of chromium	Kelly and Wood (2015); Zeng et al. (2019)
	Sulfobacillus	Gram-positive, mesophilic or slightly thermophilic, aerobic, mixotrophic with limited autotrophic and chemoorganotrophic growth	Bioleaching of chromium	da Costa et al. (2015); Zeng et al. (2019)
	Alicyclobacillus	Gram-positive; one species gram-negative, aerobic, many nonmotile, but some motile	Bioleaching of chromium	Da Costa et al. (2015); Zeng et al. (2019)
	Mycobacterium	Gram-positive, pleomorphic, coccoid form to long slender rods	Bioleaching of chromium	Percival and Williams (2014); Zeng et al. (2019)
	Acidiphilium	Gram-negative, strictly aerobic, chemoorganotrophic and chemolithotrophic, containing photosynthetic pigments	Bioleaching of chromium	Zeng et al. (2019) Hiraishi and Imhoff (2015)
	Acidithiobacillus ferridurans	Gram-negative, straight rod, obligate chemolithoautotroph, facultative anaerobe, mesophile	Cr (VI) reduction	Hedrich and Johnson (2013); Johnson et al. (2017)
	Acidiphilium cryptum	Gram-negative, rod, mesophile, aerobic, heterotroph	Cr (VI) reduction	Harrison Jr (1981); Johnson et al. (2017)

(continued)

Table 9.5 (continued)

Acidophilic bacteria				
Oxyanion	Organism	Description and morphological characteristics	Mechanism	References
Arsenic	*Acidithiobacillus ferrooxidans* CC1	Gram-negative, rod, motile, obligate chemolithotroph and autotroph, strictly aerobic	Precipitated arsenic as arsenite	Duquesne et al. (2003); Kelly and Wood (2015)
	Acidithiobacillus ferrooxidans BYQ-12	Gram-negative, rod, motile, obligate chemolithotroph and autotroph, strictly aerobic	Bioleaching	Kelly and Wood (2015); Yan et al. (2017)
	Acidithiobacillus ferrooxidans BY3	Gram-negative, rod, motile, obligate chemolithotroph and autotroph, strictly aerobic	Bio-adsorption and biotransformation	Kelly and Wood (2015); Gao et al. (2018)
	Acidithiobacillus ferrooxidans	Gram-negative, rod, motile, obligate chemolithotroph and autotroph, strictly aerobic	Oxidation	Kelly and Wood (2015); Kamde et al. (2018)
	Thiomonas sp. (B1, B2, B3) More than 99% homology with the group Ynys1	Gram-negative rods Heterotrophic and non-obligatory acidophiles B1 and B3: Nonmotile B2: Motile	More than 90% oxidation of the As (III) by B2 and B3 strains (B1: No effect on arsenic speciation)	Bruneel et al. (2003)
	Thiomonas sp. Isolate 3As	Gram-negative, short rod, motile, mesophile, moderate acidophile, facultative chemolithoautotroph, obligate aerobes	Arsenite oxidation by membrane-bound enzyme (arsenite oxidase)	Moreira and Ricardo (1997); Duquesne et al. (2008)
	Acidithiobacillus caldus KU	Gram-negative, moderately thermophilic	Resistant to arsenate, arsenite, and antimony by inducible, chromosomally resistance mechanism Capable of efflux arsenite and arsenate in the presence of an energy source Also expression of arsenate reductase activity	Dopson et al. (2001)

(continued)

Table 9.5 (continued)

Acidophilic bacteria				
Oxyanion	Organism	Description and morphological characteristics	Mechanism	References
	Sulfate-reducing bacteria (SRB) consortium	Chemolithotrophic bacteria that use sulfate as terminal electron acceptor Anaerobic, capable of tolerating the transient presence of oxygen	Complete removal of arsenic (1.06 mmol/L) and zinc (0.23 mmol/L) from solution Precipitation of As, Zn, and Fe as biogenic sulfides Reduction of As_2S_3 to AsS by biogenic H_2S	Le Pape et al. (2017)
	Sulfate-reducing bacteria (SRB)	Chemolithotrophic bacteria that use sulfate as terminal electron acceptor Anaerobic, capable of tolerating the transient presence of oxygen	Tolerance of high concentration of As (~3.6 mg/L) Up to 73% removal of As (initial concentration of As: 3.6 mg/L)	Serrano and Leiva (2017)
	Sulfate-reducing bacteria (SRB) (*Pantoea agglomerans*, *Enterobacter* sp., *Citrobacter* sp., *Cupriavidus metallidurans*, *Ralstonia* sp., and *Burkholderia cepacia*)	Chemolithotrophic bacteria that use sulfate as terminal electron acceptor Anaerobic, capable of tolerating the transient presence of oxygen	Resistant to arsenite up to 8.0 mg/L, 80% removal of As (III) (precipitation as arsenic sulfide or adsorption on calcium alginate beads)	Barton and Fauque (2009); de Matos et al. (2018)
	Acidithiobacillus ferrooxidans and *Acidithiobacillus thiooxidans*	Gram-negative, rod, motile, obligate chemolithotroph and autotroph, strictly aerobic	More than 70% oxidation of refractory gold concentrates with approximately 139.67 g/kg As by mixed culture	Kelly and Wood (2015); Hong et al. (2016)
Acidophilic archaea				
Arsenic	*Sulfolobus acidocaldarius*	Spherical cells, producing frequent lobes Facultative autotroph, thermophilic acidophile	Arsenite oxidation Arsenate reduction	Brock et al. (1972); Sehlin and Lindström (1992)

(continued)

Table 9.5 (continued)

Acidophilic bacteria				
Oxyanion	Organism	Description and morphological characteristics	Mechanism	References
Acidophilic protozoa				
Arsenic	*Euglena mutabilis*	Benthic photosynthetic protozoan	Oxidation of arsenite to arsenate Bioaccumulation of As in the cell (336±112 μg As/g dry wt.) as inorganic arsenite (105±52 μg As/g dry wt.) and arsenate (231±112 μg As/g dry wt.) Adsorption of As at the cell surface (57 mg/g dry wt.)	Casiot et al. (2004)

region sequence identity, this strain was classified as *Thermoanaerobacter thermohydrosulfuricus.* They showed that unlike other *T. thermohydrosulfuricus* strains, BSB-33 strain is able to anaerobically reduce Fe (III) and Cr (VI) optimally at 60 °C. In this report, the researchers revealed that several oxidoreductases are involved in the chromate reduction. Among them, top candidate genes were nitrite reductase, dihydrolipoamide dehydrogenase, and NADH:flavin oxidoreductase (Bhattacharya et al. 2015).

In 2016, removal of hexavalent chromium by a thermophilic denitrifying bacterium, *Chelatococcus daeguensis* TAD1, was reported. This strain could simultaneously remove Cr (VI) and NO_3^--N at 50 °C, in presence of 15 mg/L Cr (VI) as the initial concentration. Removal of chromium by this bacterium was inhibited in the presence of Cu, Zn, and Ni (Li et al. 2016).

Another research on Cr (VI) reduction was done for a thermophilic bacterium by Singh et al. (2015). In this report Cr (VI) reduction by an obligate thermophile methanogen, *Methanothermobacter thermautotrophicus*, was investigated. Complete reduction of hexavalent chromium at concentrations of 0.2 and 0.4 mM was reported. But the decrease in reduction at higher concentrations of Cr (VI) was observed (for concentrations of 1, 3, and 5 mM of Cr^{6+}, 43.6%, 13.0% and 3.7% reduction was reported, respectively) (Singh et al. 2015).

Investigation of hexavalent chromium reduction by thermophilic bacteria was done by Bai et al. (2018). In this study Cr (VI) reduction by *Caldicellulosiruptor saccharolyticus*, an extremely thermophilic bacterium, during glucose fermentation at 70 °C was examined. They showed that this bacterium has the ability of complete reduction of Cr (VI) at the concentration of 40 mg/L within 12 h and precipitation of

reduced product Cr (III) on the cell surface. They showed that the addition of neutral red as an electron mediator could shorten reduction time to 1 h (Bai et al. 2018). Chen et al. (2019) investigated isotope fractionation of Cr (VI) during reduction by *Caldicellulosiruptor saccharolyticus* under different metabolic pathways and environmental parameters. This research showed that Cr isotope enrichment factors did not change by chromium reduction pathways and temperature, but it was sensitive to the electron donor type (Chen et al. 2019).

Aeribacillus pallidus BK1 is another novel thermophilic strain with the optimum growth temperature of 60 °C and the ability of chromium reduction. The minimal inhibition concentration for chromium by this strain was reported to be 400 mg/L. The BK1 strain was able to remove 98.34% and 86.89% of Cr (VI) after 36 h with initial hexavalent chromium concentrations of 20 and 100 mg/L, respectively. When the initial concentration of Cr (VI) was 300 mg/L, 33.65% removal was observed. This study showed that Cr (VI) was transported to the cytoplasm by transmembrane transportation and reduction was done in the cytoplasm by the reductase (Ma et al. 2019).

Tellurite is another oxyanion which its reduction by thermophiles has been studied by few researchers. In a report published in 1988, Moscoso et al. studied the ability of *Bacillus stearothermophilus* in tellurite reduction and demonstrated the resistance of this bacterium to potassium tellurite (Moscoso et al. 1998). In another research, resistance to potassium tellurite and sodium selenite by *Thermus thermophilus* HB8 and *Thermus flavus* AT-62 was proved (Chiong et al. 1988a). Furthermore, they have demonstrated the tellurite-reducing ability of *Thermus thermophilus* HB8 and showed that the presence of tellurite reductase in cell-free extract could be the reason of this bacterium's ability to resist and grow in the presence of ($>10^{-4}$ M) $K_2TeO_3^-$ (Chiong et al. 1988b).

About arsenic reduction by thermophiles, Gihring and Banfield (2001) isolated a new strain in *Thermus* genus from the arsenic-rich environment. They showed that *Thermus* HRI3 has the ability of rapid oxidization of inorganic As (III) to As (V) and it also used As (V) for respiration (Gihring and Banfield 2001). In another study, Gihring et al. reported rapid oxidization of arsenite to arsenate by *Thermus aquaticus* YT1 and *Thermus thermophilus* HB8 (Gihring et al. 2001). Other strains belonging to *Thermus* genus with the ability to reduce arsenate are *Thermus sediminis* L198 and L423, two yellow pigmented strains, which were isolated from a geothermal spring, introduced by Zhou et al. (2018). These strains showed growth at temperatures from 45 to 75 °C, oxidizing thiosulfate to sulfate, and growth by using arsenate as the terminal electron acceptor for aerobic or anaerobic respiration (Zhou et al. 2018).

In the other study in 2003, Takai et al. isolated a new strain of *Deferribacter desulfuricans*, a thermophilic bacterium from a deep-sea hydrothermal vent. This strain required the elemental sulfur (S^0), nitrate, or arsenate for growth as the electron acceptors (Takai et al. 2003).

The other report was published by Cuebas et al. (2011). This group reported the *Geobacillus kaustophilus* strain isolated from geothermal soils and showed its capability in resistance toward arsenic (Cuebas et al. 2011).

Attempts have also been made to find mechanisms of resistance to arsenate in bacteria. In 2013, Heath presented the investigation of arsenite oxidase from *Thermus thermophilus* HB8 strain (Heath 2013), and Del Giudice et al. (2013) reported studies in the field of arsenate detoxification mechanism(s) in resistant bacteria. Giudice et al. introduced a novel arsenate reductase from *Thermus thermophilus* HB27. Also *TtarsC* gene, a putative chromosomal arsenate reductase gene, on the genome of *T. thermophilus* HB27 was found (Del Giudice et al. 2013). Moreover, Antonucci et al. worked on arsenic resistance mechanism(s) in *Thermus thermophilus* HB27 and reported two adjacent genes involved in arsenic resistance, *TtsmtB* and *TTC0354*, encoding an ArsR/SmtB transcriptional repressor and a Zn^{2+}/Cd^{2+}-dependent membrane ATPase, respectively. They showed that mutants for these genes are more sensitive to arsenic (Antonucci et al. 2017). In another research on arsenic resistance mechanism(s) in *Thermus thermophilus* HB27 in 2018, Antonucci et al. reported that this bacterium has a set of genes encoding proteins involved in arsenic resistance. These proteins are arsenate reductase, *Tt*ArsC; arsenic efflux protein, *Tt*ArsX; and arsenic responsive transcriptional repressor, *Tt*SmtB. They showed that *Tt*ArsX and *Tt*SmtB are also required for cadmium resistance (Antonucci et al. 2018).

Some strains had the ability to reduce different oxyanions. Podosokorskaya et al. (2016) introduced a novel moderate thermophile bacterium, *Tepidibacillus infernus*, with the optimum growth at 50 °C temperature. They reported that this bacterium can respire selenite and arsenate. In this research, the reduction of arsenate (10 mM) to arsenite and selenate (10 mM) or selenite (5 mM) to elemental selenium was reported (Podosokorskaya et al. 2016). *Bacillus thermoamylovorans* SKC1, a moderately thermophilic bacterium, could reduce chromate (at initial concentrations of up to 150 mg/L), selenite, tellurite, and soluble Fe (III) (Slobodkina et al. 2007).

Respiration of arsenate and selenate by *Pyrobaculum arsenaticum*, a hyperthermophilic archaeon that is isolated from a hot spring, was reported in 2000. This archaeon used arsenate, thiosulfate, or elemental sulfur as electron acceptors (Huber et al. 2000). Another thermophilic archaeon with the ability of arsenate detoxification is *Sulfolobus acidocaldarius* strain BC that was mentioned in the previous section (Sehlin and Lindström 1992). Table 9.6 provides further information on metalloid detoxification by thermophiles.

9.5 Concluding Remarks

In summary, the concern for metalloid oxyanions' toxicity and their excess release to the environment is still a hot topic that needs immediate investigations resulting in an effective solution. As the most probable origin of these contaminations in the environment is anthropogenic, the first solution would be to reduce their release to the environment and in the next step eliminate their toxicity to reduce their detrimental effects on the living organisms.

Even though many remediation techniques have been developed till now, the biological remediation seems to be more promising as it implements a natural

Table 9.6 Thermophilic organisms and metalloid detoxification

Oxyanion	Organism	Description and morphological characteristics	Mechanism	References
Thermophilic bacteria				
Chromium	*Thermus scotoductus* SA-01	Gram-negative, short rods, aerobic, nonmotile heterotrophic	Reduction of chromium (chromate reductase)	Kristjánsson et al. (1994); Opperman and van Heerden (2007, 2008); Opperman et al. (2008)
	Thermoanaerobacter thermohydrosulfuricus BSB-33	Gram-positive, straight to curved rod, slight tumbling motility, obligate anaerobe	Reduction of both Cr (VI) and Fe (III) anaerobically at 60 °C	Bhattacharya et al. (2015); Bhowmick et al. (2009)
	Chelatococcus daeguensis TAD1	Gram-negative, rod, aerobic, anaerobic growth in the presence of nitrate	Reduction of Cr (VI) (initial concentration of 10 mg/L, at 30–50 °C) Simultaneous removal of both Cr (VI) and NO_3^--N (Cr initial concentration of 15 mg/L at 50 °C)	Yoon et al. (2008); Li et al. (2016)
	Methanothermobacter thermautotrophicus	Obligate thermophilic methanogen	Complete reduction of Cr (VI) (0.2 and 0.4 mM)	Singh et al. (2015)
	Caldicellulosiruptor saccharolyticus	Extremely thermophilic, anaerobic fermentative	Complete reduction of 40 mg/L Cr (VI) within 12 h, and 80 mg/L and 160 mg/L of Cr (VI) to 65% and 44%, respectively	Bai et al. (2018)
	Aeribacillus pallidus BK1	Gram-positive, small rod, motile, aerobic, alkali-tolerant	Tolerance of 20–600 mg/L Cr (VI) with extreme tolerance of 400 mg/L 98.34%, 86.87%, and 33.65% Cr (VI) removal at initial concentration of 20 mg/L, 100 mg/L, and 300 mg/L of Cr (VI) respectively	Ma et al. (2019)

(continued)

Table 9.6 (continued)

Oxyanion	Organism	Description and morphological characteristics	Mechanism	References
Tellurium	*Bacillus stearothermophilus*	Gram-positive rod, strict aerobe	Resistance to potassium tellurite	Moscoso et al. (1998)
Tellurium and selenium	*Thermus thermophilus* HB8	Gram-negative rod, containing yellow pigment, nonmotile, aerobic	NADH-dependent reduction of potassium tellurite (K_2TeO_3) and reduction of sodium selenite by reductase enzyme	Oshima and Imahori (1974); Chiong et al. (1988a, b)
	Thermus flavus AT-62	Gram-negative, rod, obligate aerobic	NADH-dependent reduction of potassium tellurite (K_2TeO_3) and reduction of sodium selenite	Saiki et al. (1972); Chiong et al. (1988a)
Arsenic	*Thermus* HRI3	Gram-negative, rod or filamentous cells, many are strictly aerobic, some strains capable of anaerobic growth by using nitrate as electron acceptor	Arsenite oxidation and arsenate respiration	Gihring and Banfield (2001); Da Costa et al. (2006)
	Thermus aquaticus YT1	Gram-negative, rod, nonmotile, obligate aerobe	Oxidation of arsenite to arsenate	Brock and Freeze (1969); Gihring et al. (2001)
	Thermus thermophilus HB8	Gram-negative rod, containing yellow pigment, nonmotile, aerobic	Oxidation of arsenite to arsenate	Oshima and Imahori (1974); Gihring et al. (2001)
	Thermus thermophilus HB8	Gram-negative rod, containing yellow pigment, nonmotile, aerobic	Two-electron oxidation of arsenite to arsenate by arsenite oxidase	Oshima and Imahori (1974); Heath (2013)
	Thermus thermophilus HB27	Gram-negative rod, containing yellow pigment, nonmotile, aerobic	Tolerance to arsenate and arsenite up to 20 mM and 15 mM respectively Encoding chromosomal arsenate reductase (TtarsC) gene	Oshima and Imahori (1974); Del Giudice et al. (2013)
	Thermus sediminis L198	Gram-negative, rod, nonmotile, aerobic; salt tolerant (0–2% (w/v) NaCl), heterotrophic or	Arsenate as terminal electron acceptor	Zhou et al. (2018)

	Thermus sediminis L423	chemolithotrophic growth by oxidation of thiosulfate to sulfate		
	Deferribacter desulfuricans	Gram-negative, bent, flexible rod with polar flagellum but no motility, strictly anaerobic and obligate heterotroph	Arsenate as electron acceptor	Takai et al. (2003)
	Geobacillus kaustophilus A1	Gram-positive, rod-shaped, spore-forming, aerobic or facultative anaerobic bacteria in the genus	Resistance to high levels of arsenate (MIC = 80 mM) Growth not inhibited by antimonite (5 mM) and arsenite (15 mM)	Cuebas et al. (2011)
Chromium, selenium, tellurium	*Bacillus thermoamylovorans* SKC1	Gram-positive, straight rod, slightly motile, facultative anaerobe, chemoorganotroph, moderately thermophile, neutrophile	Reduction of chromate (at initial concentrations of up to 150 mg/L), selenite (MIC = 8 mM), tellurite (MIC = 4 mM)	Slobodkina et al. (2007)
Arsenic, selenium	*Tepidibacillus infernus* MBL-TLP	Gram-positive, motile, straight or slightly curved rods, aerotolerant anaerobe, organotroph, moderately thermophile	Respiration of arsenate and selenate arsenate (10 mM), selenate (10 mM), and selenite (5 mM) as electron acceptors	Podosokorskaya et al. (2016)
Thermophilic archaea				
Arsenic, selenium	*Pyrobaculum arsenaticum* PZ6	Strictly anaerobe, facultative organotroph, cylinder-shaped rods	Respiration of arsenate and selenate arsenate or selenate as electron acceptors, produce arsenite from arsenate, elemental selenium from selenate	Huber et al. (2000)
Arsenic	*Sulfolobus acidocaldarius*	Spherical cells, producing frequent lobes Facultative autotroph, thermophilic acidophile	Arsenite oxidation (enzymatic) Arsenate reduction	Brock et al. (1972); Sehlin and Lindström (1992)

solution. Among all the bioremediation techniques, microbial entities have attracted more attention. With introduction of extremophiles and the similarity of their natural habitat to the contaminated areas, the idea of their capability in this field was further strengthened. For example, halophilic organisms are naturally not only withstanding the high salt concentrations but also love the salts and require them for growth and viability. As all the metalloids are in their oxyanionic form, their presence may resemble the salt's presence in the halophilic organism's habitat, and these organisms have previously developed the mechanism to overcome high salts' toxicity or their application in their own favor to acquire energy. Or having high concentrations of the metalloid in an acidic or alkaline effluent or in high-temperature areas requires the application of an organism acclimated to live in these environments normally defined as harsh for other living organisms.

Moreover, the synergistic effect of simultaneous presence of the oxyanions on their removal by halophilic organisms has not yet been investigated for other extremophiles and the mechanism(s) behind this phenomenon is yet to be investigated. On the contrary, these toxic metalloids once neutralized can be useful in different industries especially in nanoscale. Nature has provided a way to manufacture these nanoparticles using a green way, i.e., microorganisms. They can be a living factory not only reducing the toxicity of the oxyanions but also manufacturing something useful out of these contaminants.

References

Abhay PC, Rawat P, Singh DP (2016) Isolation of Alkaliphilic bacterium *Citricoccus alkalitolerans* CSB1: an efficient biosorbent for bioremediation of tannery wastewater. Cell Mol Biol 62:135. https://doi.org/10.4172/1165-158X.1000135

Acar YB, Alshawabkeh AN (1993) Principles of electrokinetic remediation. Environ Sci Technol 27:2638–2647. https://doi.org/10.1021/es00049a002

Alavi S, Amoozegar MA, Khajeh K (2014) Enzyme (s) responsible for tellurite reducing activity in a moderately halophilic bacterium, *Salinicoccus iranensis* strain QW6. Extremophiles 18:953–961. https://doi.org/10.1007/s00792-014-0665-6

Ali F, Peng Q, Wang D et al (2017) Effects of selenite and selenate application on distribution and transformation of selenium fractions in soil and its bioavailability for wheat (Triticum aestivum L.). Environ Sci Pollut Res 24:8315–8325. https://doi.org/10.1007/s11356-017-8512-9

Amoozegar MA, Ghasemi A, Reza MR, Naddaf S (2007) Evaluation of hexavalent chromium reduction by chromate-resistant moderately halophile, *Nesterenkonia* sp. strain MF2. Process Biochem 42:1475–1477. https://doi.org/10.1016/j.procbio.2007.07.001

Amoozegar MA, Ashengroph M, Malekzadeh F et al (2008) Isolation and initial characterization of the tellurite reducing moderately halophilic bacterium, Salinicoccus sp. strain QW6. Microbiol Res 163:456–465. https://doi.org/10.1016/j.micres.2006.07.010

Amoozegar MA, Hamedi J, Dadashipour M, Shariatpanahi S (2005) Effect of salinity on the tolerance to toxic metals and oxyanions in native moderately halophilic spore-forming bacilli. World J Microbiol Biotechnol 21:1237–1243. https://doi.org/10.1007/s11274-005-1804-0

Antonucci I, Gallo G, Limauro D et al (2017) An ArsR/SmtB family member regulates arsenic resistance genes unusually arranged in *Thermus thermophilus* HB27. Microb Biotechnol 10:1690–1701. https://doi.org/10.1111/1751-7915.12761

Antonucci I, Gallo G, Limauro D et al (2018) Characterization of a promiscuous cadmium and arsenic resistance mechanism in *Thermus thermophilus* HB27 and potential application of a

novel bioreporter system. Microb Cell Factories 17(78). https://doi.org/10.1186/s12934-018-0918-7

Baesman SM, Stolz JF, Kulp TR, Oremland RS (2009) Enrichment and isolation of *Bacillus beveridgei* sp. nov., a facultative anaerobic haloalkaliphile from Mono Lake, California, that respires oxyanions of tellurium, selenium, and arsenic. Extremophiles 13:695–705

Bai Y-N, Lu Y-Z, Shen N et al (2018) Investigation of Cr (VI) reduction potential and mechanism by *Caldicellulosiruptor saccharolyticus* under glucose fermentation condition. J Hazard Mater 344:585–592. https://doi.org/10.1016/j.jhazmat.2017.10.059

Baillet F, Magnin JP, Cheruy A, Ozil P (1998) Chromium precipitation by the acidophilic bacterium *Thiobacillus ferrooxidans*. Biotechnol Lett 20:95–99. https://doi.org/10.1023/A:1005351718705

Barton LL, Fauque GD (2009) Biochemistry, physiology and biotechnology of Sulfate-reducing bacteria. Adv Appl Microbiol 68:41–98. https://doi.org/10.1016/S0065-2164(09)01202-7

Bhattacharya P, Barnebey A, Zemla M et al (2015) Complete genome sequence of the chromate-reducing bacterium *Thermoanaerobacter thermohydrosulfuricus* strain BSB-33. Stand Genomic Sci 10(74). https://doi.org/10.1186/s40793-015-0028-7

Bhowmick DC, Bal B, Chatterjee NS et al (2009) A low-GC Gram-positive *Thermoanaerobacter*-like bacterium isolated from an Indian hot spring contains Cr (VI) reduction activity both in the membrane and cytoplasm. J Appl Microbiol 106:2006–2016. https://doi.org/10.1111/j.1365-2672.2009.04159.x

Bienert GP, Tamás MJ (2018) Editorial: molecular mechanisms of metalloid transport, toxicity and tolerance. Front Cell Dev Biol 6(99). https://doi.org/10.3389/fcell.2018.00099

Biswas J, Bose P, Mandal S, Paul AK (2018) Reduction of hexavalent chromium by a moderately halophilic bacterium, *Halomonas smyrnensis* KS802 under saline environment. Environ Sustain 1:411–423. https://doi.org/10.1007/s42398-018-00037-x

Blum JS, Han S, Lanoil B et al (2009) Ecophysiology of "*Halarsenatibacter silvermanii*" strain SLAS-1T, gen. Nov., sp. nov., a facultative chemoautotrophic arsenate respirer from salt-saturated Searles Lake, California. Appl Environ Microbiol 75:1950–1960. https://doi.org/10.1128/AEM.02614-08

Blum JS, Kulp TR, Han S et al (2012) *Desulfohalophilus alkaliarsenatis* gen. nov., sp. nov., an extremely halophilic sulfate- and arsenate-respiring bacterium from Searles Lake, California. Extremophiles 16:727–742. https://doi.org/10.1007/s00792-012-0468-6

Brock TD, Brock KM, Belly RT, Weiss RL (1972) Sulfolobus: a new genus of sulfur-oxidizing bacteria living at low pH and high temperature. Arch Mikrobiol 84:54–68. https://doi.org/10.1007/BF00408082

Brock TD, Freeze H (1969) *Thermus aquaticus* gen. n. and sp. n., a nonsporulating extreme thermophile. J Bacteriol 98:289–297

Bruneel O, Personné JC, Casiot C et al (2003) Mediation of arsenic oxidation by *Thiomonas* sp. in acid-mine drainage (Carnoulès, France). J Appl Microbiol 95:492–499. https://doi.org/10.1046/j.1365-2672.2003.02004.x

Budinoff CR, Hollibaugh JT (2008) Arsenite-dependent photoautotrophy by an *Ectothiorhodospira*-dominated consortium. ISME J 2:340–343. https://doi.org/10.1038/ismej.2007.115

Casiot C, Bruneel O, Personné J-C et al (2004) Arsenic oxidation and bioaccumulation by the acidophilic protozoan, *Euglena mutabilis*, in acid mine drainage (Carnoulès, France). Sci Total Environ 320:259–267. https://doi.org/10.1016/j.scitotenv.2003.08.004

Chela-Flores J (2013) Polyextremophiles: summary and conclusions. In: Seckbach J, Oren A, Stan-Lotter H (eds) Polyextremophiles. Cellular origin, life in extreme habitats and astrobiology, vol 27. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-6488-0_29

Chen G, Bai Y, Zeng RJ, Qin L (2019) Effects of different metabolic pathways and environmental parameters on Cr isotope fractionation during Cr (VI) reduction by extremely thermophilic bacteria. Geochim Cosmochim Acta 256:135–146. https://doi.org/10.1016/j.gca.2019.01.002

Chen L, Zhang J, Zhu Y, Zhang Y (2018) Interaction of chromium(III) or chromium(VI) with catalase and its effect on the structure and function of catalase: an *in vitro* study. Food Chem 244:378–385. https://doi.org/10.1016/j.foodchem.2017.10.062

Chien C-C, Han C-T (2009) Tellurite resistance and reduction by a *paenibacillus* sp. isolated from heavy metal–contaminated sediment. Environ Toxicol Chem 28:1627-1632. https://doi.org/10.1897/08-521.1

Chiong M, Barra R, González E, Vásquez C (1988a) Resistance of *Thermus* spp. to potassium tellurite. Appl Environ Microbiol 54:610–612

Chiong M, González E, Barra R, Vásquez C (1988b) Purification and biochemical characterization of tellurite-reducing activities from *Thermus thermophilus* HB8. J Bacteriol 170:3269–3273. https://doi.org/10.1128/jb.170.7.3269-3273.1988

Chivers T, Laitinen RS (2015) Tellurium: a maverick among the chalcogens. Chem Soc Rev 44:1725–1739. https://doi.org/10.1039/c4cs00434e

Cuebas M, Sannino D, Bini E (2011) Isolation and characterization of arsenic resistant *Geobacillus kaustophilus* strain from geothermal soils. J Basic Microbiol 51:364–371. https://doi.org/10.1002/jobm.201000314

da Costa MS, Rainey FA, Albuquerque L (2015) Sulfobacillus. In: Whitman WB, Rainey F, Kämpfer P et al (eds) Bergey's manual of systematics of Archaea and Bacteria. Wiley, Chichester, pp 1–6

Da Costa MS, Rainey FA, Nobre MF (2006) The genus Thermus and relatives. In: Dworkin M, Falkow S, Rosenberg E et al (eds) The prokaryotes. Springer, New York, pp 797–812

DasSarma S, Arora P (2002) Halophiles. In: Encyclopedia of life sciences, vol 8. Nature, London, pp 458–466

de Matos LP, Costa PF, Moreira M et al (2018) Simultaneous removal of sulfate and arsenic using immobilized non-traditional SRB mixed culture and alternative low-cost carbon sources. Chem Eng J 334:1630–1641. https://doi.org/10.1016/j.cej.2017.11.035

Del Giudice I, Limauro D, Pedone E et al (2013) A novel arsenate reductase from the bacterium *Thermus thermophilus* HB27: its role in arsenic detoxification. Biochim Biophys Acta 1834:2071–2079. https://doi.org/10.1016/j.bbapap.2013.06.007

Dey S, Pandit B, Paul AK (2014) Reduction of hexavalent chromium by viable cells of chromium resistant bacteria isolated from chromite mining environment. J Min 2014:1–8. https://doi.org/10.1155/2014/941341

Dey S, Paul AK (2015) Hexavalent chromate reduction during growth and by immobilized cells of *Arthrobacter* sp. SUK 1205. Sci Technol Dev 34:158–168. https://doi.org/10.3923/std.2015.158.168

Dey U, Chatterjee S, Mondal NK (2016) Isolation and characterization of arsenic-resistant bacteria and possible application in bioremediation. Biotechnol Rep 10:1–7. https://doi.org/10.1016/j.btre.2016.02.002

Dopson M, Lindström BE, Hallberg KB (2001) Chromosomally encoded arsenical resistance of the moderately thermophilic acidophile *Acidithiobacillus caldus*. Extremophiles 5:247–255. https://doi.org/10.1007/s007920100196

Duquesne K, Lebrun S, Casiot C et al (2003) Immobilization of Arsenite and ferric iron by *Acidithiobacillus ferrooxidans* and its relevance to acid mine drainage. Appl Environ Microbiol 69:6165–6173. https://doi.org/10.1128/AEM.69.10.6165-6173.2003

Duquesne K, Lieutaud A, Ratouchniak J et al (2008) Arsenite oxidation by a chemoautotrophic moderately *acidophilic Thiomonas* sp.: from the strain isolation to the gene study. Environ Microbiol 10:228–237. https://doi.org/10.1111/j.1462-2920.2007.01447.x

Dziewit L, Pyzik A, Matlakowska R et al (2013) Characterization of *Halomonas* sp. ZM3 isolated from the Zelazny Most post-flotation waste reservoir, with a special focus on its mobile DNA. BMC Microbiol 13(59). https://doi.org/10.1186/1471-2180-13-59

Fatoki OS, Akinsoji OS, Ximba BJ et al (2013) Arsenic contamination: Africa the missing gap. Asian J Chem 25:9263–9268. https://doi.org/10.14233/ajchem.2013.15360

Figueiredo H, Quintelas C (2014) Tailored zeolites for the removal of metal oxyanions: overcoming intrinsic limitations of zeolites. J Hazard Mater 274:287–299. https://doi.org/10.1016/j.jhazmat.2014.04.012

Fisher E, Dawson AM, Polshyna G et al (2008) Transformation of inorganic and organic arsenic by *Alkaliphilus oremlandii* sp. nov. strain OhILAs. Ann N Y Acad Sci 1125:230–241. https://doi.org/10.1196/annals.1419.006

Focardi S, Pepi M, Landi G et al (2012) International Biodeterioration & biodegradation hexavalent chromium reduction by whole cells and cell free extract of the moderate halophilic bacterial strain *Halomonas* sp. TA-04. Int Biodeterior Biodegradation 66:63–70. https://doi.org/10.1016/j.ibiod.2011.11.003

Gan M, Gu C, Ding J et al (2019) Hexavalent chromium remediation based on the synergistic effect between chemoautotrophic bacteria and sulfide minerals. Ecotoxicol Environ Saf 173:118–130. https://doi.org/10.1016/j.ecoenv.2019.01.079

Gan M, Li J, Sun S et al (2018) Synergistic effect between sulfide mineral and acidophilic bacteria significantly promoted Cr (VI) reduction. J Environ Manag 219:84–94. https://doi.org/10.1016/j.jenvman.2018.04.118

Gao Q, Tang D, Song P et al (2018) Bio-adsorption and bio-transformation of arsenic by *Acidithiobacillus ferrooxidans* BY3. Int Microbiol 21:207–214. https://doi.org/10.1007/s10123-018-0017-y

Gihring TM, Banfield JF (2001) Arsenite oxidation and arsenate respiration by a new *Thermus* isolate. FEMS Microbiol Lett 204:335–340. https://doi.org/10.1111/j.1574-6968.2001.tb10907.x

Gihring TM, Druschel GK, McCleskey RB et al (2001) Rapid arsenite oxidation by *Thermus aquaticus* and *Thermus thermophilus*: field and laboratory investigations. Environ Sci Technol 35:3857–3862. https://doi.org/10.1021/es010816f

Gordh G, Headrick DH (eds) (2001) A dictionary of entomology. CABI publishing, New York

Guan B, Yan R, Li R, Zhang X (2018) Selenium as a pleiotropic agent for medical discovery and drug delivery. Int J Nanomedicine 13:7473–7490. https://doi.org/10.2147/IJN.S181343

Gudhka RK, Neilan BA, Burns BP (2015) Adaptation, ecology, and evolution of the halophilic stromatolite archaeon *Halococcus hamelinensis* inferred through genome analyses. Archaea 2015:1–11. https://doi.org/10.1155/2015/241608

Gutiérrez-Corona JF, Romo-Rodríguez P, Santos-Escobar F et al (2016) Microbial interactions with chromium: basic biological processes and applications in environmental biotechnology. World J Microbiol Biotechnol 32:191. https://doi.org/10.1007/s11274-016-2150-0

Güven K, Mutlu MB, Ceyhun Ç, Kutlu HM (2012) Isolation and identification of selenite reducing archaea from Tuz (salt) Lake in Turkey. J Basic Microbiol 53:397-401. https://doi.org/10.1002/jobm.201200008

Hallberg KB, Johnson DB (2001) Biodiversity of acidophilic prokaryotes. Adv Appl Microbiol 49:37–84. https://doi.org/10.1016/S0065-2164(01)49009-5

Hamilton EM, Young SD, Bailey EH, Watts MJ (2018) Chromium speciation in foodstuffs: a review. Food Chem 250:105–112. https://doi.org/10.1016/j.foodchem.2018.01.016

Han FX, Banin A, Su Y et al (2002) Industrial age anthropogenic inputs of heavy metals into the pedosphere. Naturwissenschaften 89:497–504. https://doi.org/10.1007/s00114-002-0373-4

Harrison AP Jr (1981) *Acidiphilium cryptum* gen. Nov., sp. nov., heterotrophic bacterium from acidic mineral environments. Int J Syst Evol Microbiol 31:327–332. https://doi.org/10.1099/00207713-31-3-327

Heath MD (2013) Investigations of the arsenite oxidase from a model thermophile and characterisation of the enzyme from its mesophilic counterparts. UCL (University College London)

Hedrich S, Johnson DB (2013) *Acidithiobacillus ferridurans* sp. nov., an acidophilic iron-, sulfur- and hydrogen-metabolizing chemolithotrophic gammaproteobacterium. Int J Syst Evol Microbiol 63:4018–4025. https://doi.org/10.1099/ijs.0.049759-0

Hiraishi A, Imhoff JF (2015) Acidiphilium. In: Bergey's manual of systematics of Archaea and Bacteria. American Cancer Society, New York, pp 1–14

Hoeft SE, Blum JS, Stolz JF et al (2007) *Alkalilimnicola ehrlichii* sp. nov., a novel, arsenite-oxidizing haloalkaliphilic gammaproteobacterium capable of chemoautotrophic or heterotrophic growth with nitrate or oxygen as the electron acceptor. Int J Syst Evol Microbiol 57:504–512. https://doi.org/10.1099/ijs.0.64576-0

Hoeft SE, Kulp TR, Stolz JF et al (2004) Dissimilatory arsenate reduction with sulfide as electron donor: experiments with Mono Lake water and isolation of strain MLMS-1, a chemoautotrophic arsenate respirer. Appl Environ Microbiol 70:2741–2747. https://doi.org/10.1128/AEM.70.5.2741-2747.2004

Hong J, Silva RA, Park J et al (2016) Adaptation of a mixed culture of acidophiles for a tank biooxidation of refractory gold concentrates containing a high concentration of arsenic. J Biosci Bioeng 121:536–542. https://doi.org/10.1016/j.jbiosc.2015.09.009

Horikoshi K (1999) Alkaliphiles: some applications of their products for biotechnology. Microbiol Mol Biol Rev 63:735–750

Horikoshi K, Antranikian G, Bull AT et al (eds) (2011) Extremophiles handbook. Springer Science & Business Media, Berlin

Huber R, Sacher M, Vollmann A et al (2000) Respiration of arsenate and selenate by hyperthermophilic archaea. Syst Appl Microbiol 23:305–314. https://doi.org/10.1016/S0723-2020(00)80058-2

Ibrahim ASS, El-Tayeb MA, Elbadawi YB, Al-Salamah AA (2011) Isolation and characterization of novel potent Cr (VI) reducing alkaliphilic *Amphibacillus* sp. KSUCr3 from hypersaline soda lakes. Electron J Biotechnol 14(4). https://doi.org/10.2225/vol14-issue4-fulltext-4

Ibrahim ASS, El-Tayeb MA, Elbadawi YB et al (2012a) Hexavalent chromate reduction by alkaliphilic *Amphibacillus* sp. KSUCr3 is mediated by copper-dependent membrane-associated Cr (VI) reductase. Extremophiles 16:659–668. https://doi.org/10.1007/s00792-012-0464-x

Ibrahim ASS, Elbadawi YB, El-Tayeb MA, Al-Salamah AA (2012b) Hexavalent chromium reduction by novel chromate resistant alkaliphilic *Bacillus* sp. strain KSUCr9a. Afr J Biotechnol 11:3832–3841. https://doi.org/10.5897/AJB11.3026

Itoh S, Iwaki M, Wakao N et al (1998) Accumulation of Fe, Cr and Ni metals inside cells of acidophilic bacterium *Acidiphilium rubrum* that produces Zn-containing bacteriochlorophyll a. Plant Cell Physiol 39:740–744. https://doi.org/10.1093/oxfordjournals.pcp.a029428

Jebelli MA, Maleki A, Amoozegar MA et al (2018) Isolation and identification of the native population bacteria for bioremediation of high levels of arsenic from water resources. J Environ Manag 212:39–45. https://doi.org/10.1016/j.jenvman.2018.01.075

Jian C, Yang Z, Su Y et al (2011) Phytoremediation of heavy metal/metalloid-contaminated soils. In: Steinberg RV (ed) Contaminated soils: environmental impact, disposal and treatment. ISBN 978-1-60741-791-0

Johnson DB (1998) Biodiversity and ecology of acidophilic microorganisms. FEMS Microbiol Ecol 27:307–317. https://doi.org/10.1111/j.1574-6941.1998.tb00547.x

Johnson DB (2008) Biodiversity and interactions of acidophiles: key to understanding and optimizing microbial processing of ores and concentrates. Trans Nonferrous Met Soc China 18:1367–1373. https://doi.org/10.1016/S1003-6326(09)60010-8

Johnson DB, Hedrich S, Pakostova E (2017) Indirect redox transformations of iron, copper, and chromium catalyzed by extremely acidophilic bacteria. Front Microbiol 8:211. https://doi.org/10.3389/fmicb.2017.00211

Jones RM, Hedrich S, Johnson DB (2013) Acidocella aromatica sp. nov.: an acidophilic heterotrophic alphaproteobacterium with unusual phenotypic traits. Extremophiles 17:841–850. https://doi.org/10.1007/s00792-013-0566-0

Kabiri M, Amoozegar MA, Tabebordbar M et al (2009) Effects of selenite and Tellurite on growth, physiology, and proteome of a moderately Halophilic bacterium. J Proteome Res 8:3098–3108. https://doi.org/10.1021/pr900005h

Kamde K, Pandey RA, Thul ST et al (2018) Microbially assisted arsenic removal using *Acidothiobacillus ferrooxidans* mediated by iron oxidation. Environ Technol Innov 10:78–90. https://doi.org/10.1016/j.eti.2018.01.010

Karthik C, Barathi S, Pugazhendhi A et al (2017) Evaluation of Cr (VI) reduction mechanism and removal by *Cellulosimicrobium funkei* strain AR8, a novel haloalkaliphilic bacterium. J Hazard Mater 333:42–53. https://doi.org/10.1016/j.jhazmat.2017.03.037

Kelly DP, Wood AP (2015) Acidithiobacillus. In: Bergey's manual of systematics of Archaea and Bacteria. American Cancer Society, New York, pp 1–5

Khalid S, Shahid M, Niazi NK et al (2017) A comparison of technologies for remediation of heavy metal contaminated soils. J Geochem Explor 182:247–268. https://doi.org/10.1016/j.gexplo.2016.11.021

Khalilian M, Zolfaghari MR, Soleimani M, Zand Monfared MR (2014) *Bacillus* sp. strain QW90, a bacterial strain with a high potential application in bioremediation of selenite. Rep Health Care 1:7–11

Kharayat Y (2012) Distillery wastewater: bioremediation approaches. J Integr Environ Sci 9:69–91. https://doi.org/10.1080/1943815X.2012.688056

Khurana A, Tekula S, Saifi MA et al (2019) Therapeutic applications of selenium nanoparticles. Biomed Pharmacother 111:802–812. https://doi.org/10.1016/j.biopha.2018.12.146

Kirpichtchikova TA, Manceau A, Spadini L et al (2006) Speciation and solubility of heavy metals in contaminated soil using X-ray microfluorescence, EXAFS spectroscopy, chemical extraction, and thermodynamic modeling. Geochim Cosmochim Acta 70:2163–2190. https://doi.org/10.1016/j.gca.2006.02.006

Kristjánsson JK, Hjörleifsdóttir S, Marteinsson VT, Alfredsson GA (1994) *Thermus scotoductus*, sp. nov., a pigment-producing thermophilic bacterium from hot tap water in Iceland and including Thermus sp. X-1. Syst Appl Microbiol 17:44–50. https://doi.org/10.1016/S0723-2020(11)80030-5

Le Pape P, Battaglia-Brunet F, Parmentier M et al (2017) Complete removal of arsenic and zinc from a heavily contaminated acid mine drainage via an indigenous SRB consortium. J Hazard Mater 321:764–772. https://doi.org/10.1016/j.jhazmat.2016.09.060

Leduc L (2002) The chemolithotrophic bacterium Thiobacillus ferrooxidans. FEMS Microbiol Rev 14:103–119. https://doi.org/10.1016/0168-6445(94)90001-9

Leitão AL (2009) Potential of *Penicillium* species in the bioremediation field. Int J Environ Res Public Health 6:1393–1417. https://doi.org/10.3390/ijerph6041393

Li H, Huang S, Zhang Y (2016) Cr (VI) removal from aqueous solution by thermophilic denitrifying bacterium *Chelatococcus daeguensis* TAD1 in the presence of single and multiple heavy metals. J Microbiol 54:602–610. https://doi.org/10.1007/s12275-016-5295-5

Lièvremont D, Bertin PN, Lett M-C (2009) Arsenic in contaminated waters: biogeochemical cycle, microbial metabolism and biotreatment processes. Biochimie 91:1229–1237. https://doi.org/10.1016/j.biochi.2009.06.016

Long D, Tang X, Cai K et al (2013) Cr (VI) reduction by a potent novel alkaliphilic halotolerant strain *Pseudochrobactrum saccharolyticum* LY10. J Hazard Mater 256–257:24–32. https://doi.org/10.1016/j.jhazmat.2013.04.020

Ma Y, Zhong H, He Z (2019) Cr (VI) reductase activity locates in the cytoplasm of *Aeribacillus pallidus* BK1, a novel Cr (VI)-reducing thermophile isolated from Tengchong geothermal region, China. Chem Eng J 371:524–534. https://doi.org/10.1016/j.cej.2019.04.085

Mabrouk MEM, Arayes MA, Sabry SA (2014) Hexavalent chromium reduction by chromate-resistant haloalkaliphilic *Halomonas* sp. M-Cr newly isolated from tannery effluent. Biotechnol Biotechnol Equip 28:659–667. https://doi.org/10.1080/13102818.2014.937092

MacFarquhar JK, Martin C, Burk RF et al (2010) Acute selenium toxicity associated with a dietary supplement. Arch Intern Med 170:256-261. https://doi.org/10.1001/archinternmed.2009.495

Mary Mangaiyarkarasi MS, Vincent S, Janarthanan S et al (2011) Bioreduction of Cr (VI) by alkaliphilic *Bacillus subtilis* and interaction of the membrane groups. Saudi J Biol Sci 18:157–167. https://doi.org/10.1016/j.sjbs.2010.12.003

Masaki Y, Hirajima T, Sasaki K, Okibe N (2015) Bioreduction and immobilization of hexavalent chromium by the extremely acidophilic Fe (III)-reducing bacterium *Acidocella aromatica* strain PFBC. Extremophiles 19:495–503. https://doi.org/10.1007/s00792-015-0733-6

Matsuzawa Y, Kanbe T, Suzuki J, Hiraishi A (2000) Ultrastructure of the acidophilic aerobic photosynthetic bacterium Acidiphilium rubrum. Curr Microbiol 40(6):398–401. https://doi.org/10.1007/s002840010077

Mishra RR, Prajapati S, Das J et al (2011) Reduction of selenite to red elemental selenium by moderately halotolerant *Bacillus megaterium* strains isolated from Bhitarkanika mangrove soil and characterization of reduced product. Chemosphere 84:1231–1237. https://doi.org/10.1016/j.chemosphere.2011.05.025

Moreira D, Ricardo A (1997) Phylogeny of *Thiobacillus cuprinus* and other mixotrophic Thiobacilli: proposal for *Thiomonas* gen. nov. Int J Syst Bacteriol 47:522–528. https://doi.org/10.1099/00207713-47-2-522

Moscoso H, Saavedra C, Loyola C et al (1998) Biochemical characterization of tellurite-reducing activities of *Bacillus stearothermophilus* V. Res Microbiol 149:389–397. https://doi.org/10.1016/S0923-2508(98)80321-5

Muñoz-Villagrán CM, Mendez KN, Cornejo F et al (2018) Comparative genomic analysis of a new tellurite-resistant *Psychrobacter* strain isolated from the Antarctic peninsula. PeerJ 6:4402. https://doi.org/10.7717/peerj.4402

Najimi S, Shakibaie M, Jafari E et al (2017) Acute and subacute toxicities of biogenic tellurium nanorods in mice. Regul Toxicol Pharmacol 90:222–230. https://doi.org/10.1016/j.yrtph.2017.09.014

Nieto JJ, Fernandez-Castillo R, Marquez MC et al (1989) Survey of metal tolerance in moderately halophilic eubacteria. Appl Environ Microbiol 55:2385–2390

Ogra Y (2017) Biology and toxicology of tellurium explored by speciation analysis. Metallomics 9:435–441. https://doi.org/10.1039/C7MT00022G

Opperman DJ, Piater LA, van Heerden E (2008) A novel chromate reductase from *Thermus scotoductus* SA-01 related to old yellow enzyme. J Bacteriol 190:3076–3082. https://doi.org/10.1128/JB.01766-07

Opperman DJ, van Heerden E (2007) Aerobic Cr (VI) reduction by *Thermus scotoductus* strain SA-01. J Appl Microbiol 103:1907–1913. https://doi.org/10.1111/j.1365-2672.2007.03429.x

Opperman DJ, Van Heerden E (2008) A membrane-associated protein with Cr (VI)-reducing activity from *Thermus scotoductus* SA-01. FEMS Microbiol Lett 280:210–218. https://doi.org/10.1111/j.1574-6968.2007.01063.x

Orellana R, Macaya C, Bravo G et al (2018) Living at the frontiers of life: extremophiles in Chile and their potential for bioremediation. Front Microbiol 9:2309. https://doi.org/10.3389/fmicb.2018.02309

Oremland RS, Hoeft SE, Santini JM et al (2002) Anaerobic oxidation of Arsenite in Mono Lake water and by a facultative, Arsenite-oxidizing chemoautotroph, strain MLHE-1. Appl Environ Microbiol 68:4795–4802. https://doi.org/10.1128/AEM.68.10.4795-4802.2002

Oremland RS, Kulp TR, Blum JS et al (2005) A microbial arsenic cycle in a salt-saturated, extreme environment. Science (80-) 308:1305–1308. https://doi.org/10.1126/science.1110832

Oren A (2002) Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. J Ind Microbiol Biotechnol 28:56–63. https://doi.org/10.1038/sj/jim/70001

Oshima T, Imahori K (1974) Description of *Thermus thermophilus* (Yoshida and Oshima) comb. nov., a nonsporulating thermophilic bacterium from a Japanese thermal spa. Int J Syst Evol Microbiol 24:102–112. https://doi.org/10.1099/00207713-24-1-102

Pearion CT, Jablonski PE (1999) High level, intrinsic resistance of *Natronococcus occultus* to potassium tellurite. FEMS Microbiol Lett 174:19–23. https://doi.org/10.1111/j.1574-6968.1999.tb13544.x

Percival SL, Williams DW (2014) Mycobacterium. In: Microbiology of waterborne diseases. Elsevier, pp 177–207

Planer-Friedrich B, Härtig C, Lohmayer R et al (2015) Anaerobic Chemolithotrophic growth of the Haloalkaliphilic bacterium strain MLMS-1 by disproportionation of Monothioarsenate. Environ Sci Technol 49:6554–6563. https://doi.org/10.1021/acs.est.5b01165

Podosokorskaya OA, Merkel AY, Gavrilov SN et al (2016) *Tepidibacillus infernus* sp. nov., a moderately thermophilic, selenate-and arsenate-respiring hydrolytic bacterium isolated from a gold mine, and emended description of the genus Tepidibacillus. Int J Syst Evol Microbiol 66:3189–3194. https://doi.org/10.1099/ijsem.0.001166

Pratush A, Kumar A, Hu Z (2018) Adverse effect of heavy metals (As, Pb, Hg, and Cr) on health and their bioremediation strategies: a review. Int Microbiol 21:97–106. https://doi.org/10.1007/s10123-018-0012-3

Qiu R-L, Guerif J, ZHANG R-D et al (2012) Bioremediation of contaminated soil and water. Pedosphere 22:425. https://doi.org/10.1016/S1002-0160(12)60028-9

Rager JE, Suh M, Chappell GA et al (2019) Review of transcriptomic responses to hexavalent chromium exposure in lung cells supports a role of epigenetic mediators in carcinogenesis. Toxicol Lett 305:40–50. https://doi.org/10.1016/j.toxlet.2019.01.011

Ramos JL, Duque E, van Dillewjin P et al (2010) Removal of hydrocarbons and other related chemicals via the Rhizosphere of plants. In: Timmis KN (eds) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 2575–2581

Rampelotto PH (2013) Extremophiles and extreme environments. Life 3:482–485. https://doi.org/10.3390/life3030482

Rathgeber C, Yurkova N, Stackebrandt E et al (2002) Isolation of tellurite- and selenite-resistant bacteria from hydrothermal vents of the Juan de Fuca Ridge in the Pacific Ocean. Appl Environ Microbiol 68:4613–4622. https://doi.org/10.1128/AEM.68.9.4613-4622.2002

Richey C, Chovanec P, Hoeft SE et al (2009) Respiratory arsenate reductase as a bidirectional enzyme. Biochem Biophys Res Commun 382:298–302. https://doi.org/10.1016/j.bbrc.2009.03.045

Robertson LA, Kuenen JG (2006) The genus *Thiobacillus*. Prokaryotes vol 5 Proteobacteria Alpha Beta Subclasses 5:812–827. doi: https://doi.org/10.1007/0–387-30745-1_37

Rodríguez J, Mandalunis PM (2018) A review of metal exposure and its effects on bone health. J Toxicol 2018:1–11. https://doi.org/10.1155/2018/4854152

Roh Y, Chon C-M, Moon J-W (2007) Metal reduction and biomineralization by an alkaliphilic metal-reducing bacterium, *Alkaliphilus metalliredigens* (QYMF). Geosci J 11:415–423. https://doi.org/10.1007/BF02857056

Rothschild LJ, Mancinelli RL (2001) Life in extreme environments. Nature 409:1092–1101. https://doi.org/10.1038/35059215

Safhi MM, Alam MF, Khuwaja G et al (2018) Selenium in combination with tellurium protects the toxicity of tellurium in the liver mitochondria of rats. Bull Environ Pharmacol Life Sci 7:90–95

Saiki T, Kimura R, Arima K (1972) Isolation and characterization of extremely Thermophilic bacteria from Hot Springs. Agric Biol Chem 36:2357–2366. https://doi.org/10.1080/00021369.1972.10860589

Sangeetha S, Silviya S, Gurunathan J (2012) Hexavalent chromium reduction by metal resistant and halotolerant *Planococcus maritimus* VITP21. Afr J Microbiol Res 6:7339–7349. https://doi.org/10.5897/AJMR12.143

Sehlin HM, Lindström EB (1992) Oxidation and reduction of arsenic by *Sulfolobus acidocaldarius* strain BC. FEMS Microbiol Lett 93:87–92. https://doi.org/10.1111/j.1574-6968.1992.tb05045.x

Serrano J, Leiva E (2017) Removal of arsenic using acid/metal-tolerant sulfate reducing bacteria: a new approach for bioremediation of high-arsenic acid mine waters. Water 9:994. https://doi.org/10.3390/w9120994

Sharma A, Parashar D, Satyanarayana T (2016) Acidophilic microbes: biology and applications. In: Rampelotto P (ed) Biotechnology of extremophiles. Grand challenges in biology and biotechnology, vol 1. Springer, Cham. https://doi.org/10.1007/978-3-319-13521-2_7

Sidhu GPS (2016) Heavy metal toxicity in soils: sources, remediation technologies and challenges. Adv Plants Agric Res 5:00166. https://doi.org/10.15406/apar.2016.05.00166

Singh R, Dong H, Liu D et al (2015) Reduction of hexavalent chromium by the thermophilic methanogen *Methanothermobacter thermautotrophicus*. Geochim Cosmochim Acta 148:442–456. https://doi.org/10.1016/j.gca.2014.10.012

Slobodkina GB, Bonch-Osmolovskaya EA, Slobodkin AI (2007) Reduction of chromate, selenite, tellurite, and iron (III) by the moderately thermophilic bacterium *Bacillus thermoamylovorans* SKC1. Microbiology 76:530–534. https://doi.org/10.1134/S0026261707050037

Soda S, Ma W, Kuroda M et al (2018) Characterization of moderately halotolerant selenate- and tellurite-reducing bacteria isolated from brackish areas in Osaka. Biosci Biotechnol Biochem 82:173–181. https://doi.org/10.1080/09168451.2017.1406794

Sorokin DY, Tourova TP, Sukhacheva MV, Muyzer G (2012) *Desulfuribacillus alkaliarsenatis* gen. nov. sp. nov., a deep-lineage, obligately anaerobic, dissimilatory sulfur and arsenate-reducing, haloalkaliphilic representative of the order *Bacillales* from soda lakes. Extremophiles 16:597–605. https://doi.org/10.1007/s00792-012-0459-7

Stocking AJ, Deeb RA, Flores AE et al (2000) Bioremediation of MTBE: a review from a practical perspective. Biodegradation 11:187–201. https://doi.org/10.1023/A:1011126414642

Strawn DG (2018) Review of interactions between phosphorus and arsenic in soils from four case studies. Geochem Trans 19:10. https://doi.org/10.1186/s12932-018-0055-6

Su C (2014) A review on heavy metal contamination in the soil worldwide: situation, impact and remediation techniques. Environ Skept Critics 3:24-38.

Switzer Blum J, Burns Bindi A, Buzzelli J et al (1998) *Bacillus arsenicoselenatis* , sp. nov., and *Bacillus selenitireducens*, sp. nov.: two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. Arch Microbiol 171:19–30. https://doi.org/10.1007/s002030050673

Switzer Blum J, Stolz JF, Oren A et al (2001) *Selenihalanaerobacter shriftii* gen. nov., sp. nov., a halophilic anaerobe from Dead Sea sediments that respires selenate. Arch Microbiol 175:208–219. https://doi.org/10.1007/s002030100257

Takai K, Kobayashi H, Nealson KH, Horikoshi K (2003) *Deferribacter desulfuricans* sp. nov., a novel sulfur-, nitrate- and arsenate-reducing thermophile isolated from a deep-sea hydrothermal vent. Int J Syst Evol Microbiol 53:839–846. https://doi.org/10.1099/ijs.0.02479-0

Tan H, Mo H-Y, Lau A, Xu Y-M (2018) Selenium species: current status and potentials in cancer prevention and therapy. Int J Mol Sci 20:75. https://doi.org/10.3390/ijms20010075

Taran M, Safari M, Monaza A et al (2013) Optimal conditions for the biological removal of arsenic by a novel halophilic archaea in different conditions and its process optimization. Polish J Chem Technol 15:7–9. https://doi.org/10.2478/pjct-2013-0017

Thorgersen MP, Lancaster WA, Ge X et al (2017) Mechanisms of chromium and uranium toxicity in *Pseudomonas stutzeri* RCH2 grown under anaerobic nitrate-reducing conditions. Front Microbiol 8:1529. https://doi.org/10.3389/fmicb.2017.01529

Torma AE, Habashi F (1972) Oxidation of copper (II) selenide by *Thiobacillus ferrooxidans*. Can J Microbiol 18:1780–1781. https://doi.org/10.1139/m72-278

Upadhyay MK, Shukla A, Yadav P, Srivastava S (2019) A review of arsenic in crops, vegetables, animals and food products. Food Chem 276:608–618. https://doi.org/10.1016/j.foodchem.2018.10.069

Valderrama MJ, Quesada E, Bejar V et al (1991) *Deleya salina* sp. nov., a moderately halophilic gram-negative bacterium. Int J Syst Bacteriol 41:377–384. https://doi.org/10.1099/00207713-41-3-377

Verma T, Agarwal S (2016) Production and optimization of extracellular alkaline protease from halotolerant chromate resistant *Bacillus circulans* isolated from tannery solid waste. Int J Plant Prot 9:211–218. https://doi.org/10.15740/has/ijpp/9.1/211-218

Vidali M (2001) Bioremediation. An overview. Pure Appl Chem 73:1163–1172. https://doi.org/10.1351/pac200173071163

Vijayanand S, Hemapriya J (2014) Biosorption and detoxification of Cr (VI) by tannery effluent acclimatized halotolerant bacterial strain pv26. Int J Curr Microbiol Appl Sci 3:971–982

Vinceti M, Filippini T, Wise LA (2018) Environmental selenium and human health: an update. Curr Environ Health Rep 5:464–485. https://doi.org/10.1007/s40572-018-0213-0

Wackett LP, Hershberger CD (2001) Biocatalysis and biodegradation: microbial transformation of organic compounds. ASM, Washington, DC

Wang G, Kennedy SP, Fasiludeen S et al (2004) Arsenic resistance in *Halobacterium* sp. strain NRC-1 examined by using an improved gene knockout system. J Bacteriol 186:3187–3194. https://doi.org/10.1128/JB.186.10.3187-3194.2004

Watts MP, Khijniak TV, Boothman C, Lloyd JR (2015) Treatment of alkaline Cr (VI)-contaminated leachate with an alkaliphilic metal-reducing bacterium. Appl Environ Microbiol 81:5511–5518. https://doi.org/10.1128/AEM.00853-15

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol 2011:402647:1–20. https://doi.org/10.5402/2011/402647

Yan L, Hu H, Zhang S et al (2017) Arsenic tolerance and bioleaching from realgar based on response surface methodology by *Acidithiobacillus ferrooxidans* isolated from Wudalianchi volcanic lake, Northeast China. Electron J Biotechnol 25:50–57. https://doi.org/10.1016/j.ejbt.2016.11.007

Yao Z, Li J, Xie H, Yu C (2012) Review on remediation technologies of soil contaminated by heavy metals. Procedia Environ Sci 16:722–729. https://doi.org/10.1016/j.proenv.2012.10.099

Ye Q, Roh Y, Carroll SL et al (2004) Alkaline anaerobic respiration: isolation and characterization of a novel alkaliphilic and metal-reducing bacterium. Appl Environ Microbiol 70:5595–5602. https://doi.org/10.1128/AEM.70.9.5595–5602.2004

Yoon J-H, Kang S-J, Im W-T et al (2008) *Chelatococcus daeguensis* sp. nov., isolated from wastewater of a textile dye works, and emended description of the genus Chelatococcus. Int J Syst Evol Microbiol 58:2224–2228. https://doi.org/10.1099/ijs.0.65291-0

Zare B, Nami M, Shahverdi A-R (2017) Tracing tellurium and its nanostructures in biology. Biol Trace Elem Res 180:171–181. https://doi.org/10.1007/s12011-017-1006-2

Zeng J, Gou M, Tang Y-Q et al (2016) Effective bioleaching of chromium in tannery sludge with an enriched sulfur-oxidizing bacterial community. Bioresour Technol 218:859–866. https://doi.org/10.1016/j.biortech.2016.07.051

Zeng J, Li J, Gou M et al (2019) Effective strategy for improving sludge treatment rate and microbial mechanisms during chromium bioleaching of tannery sludge. Process Biochem 83:159–167. https://doi.org/10.1016/j.procbio.2019.05.019

Zhao Z, An H, Lin J et al (2018) Progress on the photocatalytic reduction removal of chromium contamination. Chem Rec 19:873–882. https://doi.org/10.1002/tcr.201800153

Zhou C, Huang J-C, Liu F et al (2019) Selenium removal and biotransformation in a floating-leaved macrophyte system. Environ Pollut 245:941–949. https://doi.org/10.1016/j.envpol.2018.11.096

Zhou E-M, Xian W-D, Mefferd CC et al (2018) *Thermus sediminis* sp. nov., a thiosulfate-oxidizing and arsenate-reducing organism isolated from Little Hot Creek in the Long Valley caldera, California. Extremophiles 22:983–991. https://doi.org/10.1007/s00792-018-1055-2

Zhou LX, Fang D, Zhou SG et al (2004) Removal of Cr from tannery sludge by acidophilic *Thiobacilli*. Huan Jing Ke Xue 25:62–66

Conventional and Nonconventional Biodegradation Technologies for Agro-Industrial Liquid Waste Management

10

Sebastián Pineda Pineda and Juan Carlos Higuita Vásquez

Abstract

Nowadays, the world is in a period of waste accumulation in large quantities, which strongly influences the health of ecosystems and ultimately the human community. Many companies worldwide produce thousands of effluents whose alternative uses are still unclear. Therefore, every agro-industrial sector has greater responsibility toward safe utilization of agro-materials, either through waste recycling or the correct treatment of waste to reduce their toxicity and the environmental burden.

Many biodegradation technologies have been developed in order to address this problem such as vermicomposting, unicellular protein production, energy generation (e.g., biogas, biofuels, and hydrogen) and waste utilization as co-substrate in fermentation processes. Thus, new studies and green nonconventional methods have been settled aiming at waste recycling like biohythane production, dark fermentation, and bioremediation. Other alternatives are based on physicochemical processes such as fertigation, livestock feed production, combustion, gasification, membrane operations, and electrochemical processes. Many treatments consist of a combination of both technologies: biological and physicochemical processes.

Agro-industrial liquid wastes (ALW) contain a considerable amount of organic matter, which can be decomposed by biodegradation reactions. Biodegradation is the process by which organic compounds are fragmented into smaller substances. Organic material can be degraded aerobically, with oxygen, or anaerobically, without oxygen. These reactions occur in order to obtain energy for microbial growth, and it is commonly associated with the production of value-

S. P. Pineda · J. C. H. Vásquez (✉)
Research group in Chemical, Catalytic and Biotechnological Processes, The Chemical Engineering Department, Universidad Nacional de Colombia sede Manizales, Campus La Nubia, Manizales, Caldas, Colombia
e-mail: jchiguitav@unal.edu.co

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_10

added products such as enzymes, pigments, biopolymers, biofuels, food flavoring compounds and bioactive compounds, among others.

However, ALW are characterized by high values of biochemical and chemical oxygen demands (BOD, COD), suspended solids, high electrical conductivity, phenols, aromatic compounds, low pH values, and the prohibition of disposal on soil or rivers. Many conventional technologies increase the emissions of CO_2, CH_4, N_2O, and other volatile greenhouse gases emissions. In addition, the increase of compounds like NO_3^-, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, Na^+, and other metals in soil might contaminate groundwater when leached out. Wrong treatment also promotes eutrophication and undesirable changes in ecosystems and their proper functioning.

Keywords

Agro-industrial liquid wastes · Waste management · Physicochemical technologies · Biological technologies

10.1 Introduction

Today, waste management is of great importance since many wastes are produced at large scale and commonly generate several negative factors, such as high levels of pollution (Arm et al. 2011). ALW are the residues obtained after chemical and biochemical processes in the industry. Their composition varies considerably depending on several factors such as raw materials and the different operation processes (Krzywonos et al. 2009).

ALW contain high loads of dissolved solids and recalcitrant organic matter (e.g., nitrogen-colored polymers of brown color, phenols, etc.), ash, and usually have low pH (3–5). Biochemical and Chemical Oxygen Demand (BOD and COD, respectively) are indicators of the contamination potential which vary in ranges between 35,000–50,000 and 100,000–150,000 mg O_2 L^{-1} respectively (Bustamante et al. 2005). These characteristics of ALW, combined with their high production volume, necessarily require an adequate treatment and final management. It is common that governments stipulate a maximum limit for BOD and COD of agro-industrial wastewater discharge to body waters in a range of 50–400 mg O_2 L^{-1} and 150–650 mg O_2 L^{-1}, respectively. ALW are commonly used as culturing media in order to achieve the bioconversion from sugars or simple organic acids to high value-added products. Furthermore, the organic load is reduced and therefore its toxicity.

In many countries, the application of ALW as fertilizer is the most commonly used treatment due to its composition and easy disposal. This alternative is the simplest and cheapest solution for management of effluents. Utilization of ALW as fertilizer demands a small initial investment, low maintenance costs, has no technological complexity, and usually increases crop yields. However, adverse environmental impacts, such as salinization and leaching of soil nitrates, should be considered (Parnaudeau et al. 2008). Also, contamination of aquifers and even the

worsening of global warming by releasing nitrous oxide (N_2O) in soil are other problems when ALW are used as fertilizers.

The aim of this chapter is to show useful information about conventional and nonconventional biodegradation technologies to ALW management. This report was focused on a global situation since many producing countries have the same issues with their AWL final disposal. Additionally, this work was performed facing ALW management using physicochemical technologies and biological processes.

10.2 Issues Associated with ALW

ALW usually contain high amounts of phenol compounds (34–542 mg L^{-1}), organic matter, carbohydrates, aromatic compounds, and other carbon-rich compounds. Irrigation with ALW in agricultural soil tripled CO_2 emissions under flooding conditions (200 m^3 ha^{-1}). Nevertheless, many authors reported that the addition of ALW to soil generates CH_4 fluxes from −64.4 to 3.1 μg m^{-2} h^{-1} for the control soil and from −42.0 to 44.3 μg m^{-2} h^{-1} in the soil added with ALW. Additionally, the annual application of 46 kg of N ha^{-1} in the form of ALW rendered a N_2O emission from 0.31 to 0.52 kg ha^{-1}. CO_2, CH_4, and N_2O increased emissions are also problems associated with ALW irrigation. Total volatile solid contents in ALW are high, between 79,000 and 82,222 mg L^{-1} depending on their origin. Applied to crops, the amount of organic materials like nitrate (NO_3^-), ammonium (NH_4^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), and metals will increase in soil and when leached out might contaminate groundwater. ALW also promote eutrophication and undesirable changes in ecosystems and their functioning (Moran-Salazar et al. 2016).

Eutrophication is the process through which lakes, streams, or bays become overloaded with excess of nutrients such as nitrogen and phosphorus. When the aquatic life dies, microorganisms feed on the remains as part of the decomposition process and consequently consume the available oxygen in water. This leaves little dissolved oxygen for fish and other aquatic animals thus resulting in the suffocation of aquatic life (SSSA 2018). Different technologies have developed for ALW management and are divided into the following two main categories: biological and physicochemical processes. In turn, each of these two categories is divided into conventional and nonconventional approaches.

10.3 Biological Technologies for ALW Management

The environmental damage caused by discarding ALW into the soil or water bodies was an incentive for research aiming to find alternative economic applications for this agro-industrial waste. Results from such studies indicate that, when properly used, ALW could contribute to improve soil quality (Silva et al. 2006), agricultural productivity, clean energy generation and bioremediation, among others.

10.4 Conventional Technologies

10.4.1 Vermicomposting

Composting is considered as a technique in which aerobic mesophilic and thermophilic microorganisms consume organic matter as a substrate under controlled conditions. This biological process produces a stabilized, mature, deodorized, and hygienic material free of pathogens and plant seeds and rich in humic substances that can be used as soil conditioner (Lim et al. 2015).

Usually this technology is used in combination with solid wastes. If earthworms are applied in this method, it can be known as an integrated composting–vermicomposting process. Earthworms can transform the organic fraction of the mixture between solid and liquid wastes into a nutrient-rich fertilizer under aerobic conditions. *Eisenia fetida* is a species of earthworms which under suitable pH, temperature, and moisture has the potential to convert organic waste into products with a high nutritious value that can be used as a biofertilizer (Meunchang et al. 2005; Amouei et al. 2010; Lim and Wu 2016).

For example, co-composting followed by vermicomposting of the ALW mixtures, cow manure, and chopped bagasse was performed for 60 days using earthworms of *Eisenia fetida* species by Alavi et al. (2017). The results showed that the trend of changes in C/N was decreasing. The pH of the final fertilizer was in alkaline range (8.1–8.4) and the total potassium decreased during the process ranging from 0.062% to 0.15%, while the total phosphorus increased from 0.06% to 0.10%. The germination index (GI) for all samples was 100%, while the cellular respiration maturity index was <2 mg C-CO_2 g^{-1} organic carbon day^{-1}, thus confirming a very stable compost. The results of this study indicate that the compost obtained from the co-composting/vermicomposting process could be used as a soil amendment.

10.4.2 Biogas Production

Anaerobic digestion of ALW can be regarded as a favorable strategy, since the digestate could still be used to partially substitute the mineral fertilizers on the crops and the produced biogas could be improved to biomethane and sold as a new energy product (Beil and Beyrich 2013; Janke et al. 2014). However, before being applied on a large scale, the anaerobic digestion process should be carefully assessed, especially concerning the characteristics of substrates such as organic matter and nutritional value, macronutrients, trace elements, and specific biogas production.

In order to obtain an appropriate anaerobic digestion process, a balance among the main nutrients such as carbon, nitrogen, phosphorus, and sulfur is necessary. If a substrate has a too high C/N ratio, or in other words, has a lack of nitrogen, it may negatively influence on the functioning microbial community. According to a study performed by Janke et al. (2015), the lowest methane yield for some ALW after

35 days was 246 ± 15 N m L $gCOD^{-1}$, and the maximum yield was 302 ± 06 N mL g COD^{-1}.

España-Gamboa et al. (2012) modified a laboratory-scale up flow anaerobic sludge blanket (UASB) reactor to obtain methane by treating ALW. The report showed that the COD removal efficiency was 69% at an optimum organic loading rate (OLR) of 17.05 kg COD m^{-3} day^{-1}, achieving a methane yield of 0.263 m^3 kg^{-1} COD and an 84% biogas methane content. Effluent characterization presented lower values than the ALW, except for potassium, sulfide, and ammonia nitrogen.

10.4.3 Utilization of ALW as Co-Substrate in Fermentation Processes

ALW can replace certain percentages of the raw materials used in fermentation processes since these wastes have fermentable sugars and many nutrients in its composition. According to Fadel and Abdel-Naser (2014), ethanol yields in a fermentation using *Saccharomyces cerevisiae* can vary depending on the volume proportion of ALW used as shown in Table 10.1.

There was a slight change in the efficiency when 40% v/v ALW were used instead of water and a relative decrease is observed in the ethanol yield and fermentation efficiency when ALW were introduced above 50% v/v instead of water in the fermentation medium.

In addition, the ALW organic load decreases because this waste is recycled in another bioprocess. This means that the alternative for ALW management also reduces the environmental impact.

Table 10.1 Effect of recycling varying ALW amounts on ethanolic fermentation

ALW (% v/v)	EY (%)	RS (%)	FE (%)
0	11.0	1.8	100.0
10	11.0	1.9	100.0
20	11.1	1.9	100.9
30	11.0	2.1	100.0
40	10.7	2.2	97.3
50	10.5	2.3	95.5
60	10.2	2.5	92.7
70	9.6	3.0	87.3
80	9.3	3.8	84.6
90	8.9	4.4	80.9
100	8.5	4.9	77.3

EY ethanol yield, *RS* residual sugars, *FE* fermentation efficiency
Taken from Fadel and Abdel-Naser (2014)

10.4.4 Unicellular Protein Production

Bioconversion of agricultural and industrial liquid wastes into microbial protein has been receiving increasing attention from the 1970s. The reduction of organic loads and at the same time the production of a valuable commodity is the greatest advantage of such a process. Among the yeast species that can be used for such purposes, *Candida utilis* is particularly attractive taking into account its high protein content, good amino acid profile, and the possibility of growth from different substrates (Nasseri et al. 2011).

Through this technology, the ALW organic load can be reduced by 75% or 60% when the process was carried out in batches or in continuous regimes, respectively. Cell concentration reaches values around 8.0 g L^{-1}.

The yeasts have big advantages such as their size (easier to harvest), lower nucleic acid content, high amino acids contents (as lysine), and the ability to grow at low pH. Some of the disadvantages are the lower growth rates, relative lower protein content (between 45% and 65%), and lower content in methionine than in bacteria. Filamentous fungi have harvesting advantages, but they have some limitations such as lower growth rates as that of yeasts, lower protein content, and acceptability. Other microorganism used for unicellular protein is the algae, but it has disadvantages because it has cellulosic cell walls which are not digested by human beings. In addition, they also frequently concentrate heavy metals. In the case of algae, it has to be stressed that, due to technical and economic reasons, it is not the general intention to isolate and utilize the sole protein, but to propagate the whole algal biomass. To date, various new technologies have been employed worldwide for mass production and processing of photoautotrophic microalgae.

Microalgae, cyanobacteria, and aquatic plants are renewable source of nutrients and high-value compounds that could be used as feedstock for the production of many products (Appenroth et al. 2017; Laurens et al. 2017). They contain proteins, fatty acids, pigments, antioxidants, etc., that are an interesting source to be utilized as food and feed supplements. Cultivation of microalgae and duckweed (aquatic plant) offers some interesting advantages, such as continuous harvesting, use of nonarable land, and use of ALW or seawater as the basis of the cultivation medium. Furthermore, they can face the increasing global food demand. Some microalgal species, such as *Arthrospira* (*Spirulina*), *Chlorella*, and *Dunaliella* are rich in proteins (>50%dw) and have the ability to be grown in polluting liquid effluents. Sugar and organic acid biodegradation from AWL by microalgae show productivities in a range of 20–30 t_{DM} ha^{-1} $year^{-1}$ (Walsh et al. 2015).

10.5 Nonconventional Technologies

10.5.1 Soil Bioremediation

The use of biomass to clean up polluted soils is an effective approach because of the critical role that microorganisms play in biodegradation of organic pollutants and removal/stabilization of heavy metals (Mrozik and Piotrowska-Seget 2010; Polti et al. 2014).

Among environmental microorganisms, actinobacteria have been extensively reported as potential bioremediation agents (Alvarez et al. 2017). They may be well suited for inoculation in soil because of their mycelial growth, their relatively rapid growth rates and capability to colonize substrates.

Aparicio et al. (2017), used actinobacteria biomass to clean up contaminated soils as an attractive biotechnology approach. The authors report the ability of four actinobacteria, *Streptomyces sp.* M7, MC1, A5, and *Amycolatopsis tucumanensis*, to generate biomass from ALW. In all cases, the decrease in pesticides present in the studied soils was about 50% after 14 days of incubation. However, chromium removal was statistically different depending on the preparation methodology of the inoculum. While the combined actinobacteria biomass recovered from their respective single cultures removed about 85% of the chromium, the mixed culture biomass removed more than 95%.

10.5.2 Dark Fermentation

The gradual introduction of fuels with progressively lower carbon content per unit of energy (wood—coal—oil—natural gas) results in a constant decarbonization of the global fuel mix. This chain of lower carbon content fuel ends in Hydrogen (H_2). Currently, the cost of H_2 produced from biological processes is very high, especially due to medium cost and process sensitivity. In some bioprocesses for biohydrogen production (such as dark fermentation of organic matter), volatile fatty acids, which are platform molecules that can be used as raw material for green chemistry, are produced and accumulated in the liquid phase. The production and commercialization of these platform molecules will have a great impact on the economics of technologies for biohydrogen production.

The use of microbial consortia and liquid industrial wastes for biohydrogen production by dark fermentation is seen as a crucial strategy in an effort to overcome the economic and technical disadvantages of this potential technology. Sydney et al. (2018) carried out fermentation in an ALW-based medium supplemented with pure or complex carbon sources. Authors demonstrated that consortia LPBAH1 and LPBAH2 were predominantly composed of *Oxalobacteraceae* and *Lactobacillaceae*, while LPBAH3 was rich in sporulating *Lactobacillaceae* (> 96%). The most relevant results related to biohydrogen and volatile fatty acids production were as follows:

1. The highest biohydrogen yield was achieved with LPBAH1 (>50% *Oxalobacteraceae*) in an ALW medium supplemented with sugarcane juice (1.59 ± 0.21 mol H_2 mol^{-1} glucose).
2. The lower H_2 yields were achieved with LPBAH3, which otherwise produced the highest amount of butyric acid (up to 10 gL^{-1}).

10.5.3 Biohythane Production

Anaerobic digestion consists fundamentally on a sequence of metabolic reactions taking place through hydrolysis, acidogenesis, acetogenesis, and finally methanogenesis phases. These stages aim to stimulate the microbial degradation of organic matter to obtain different compounds and bioenergy precursors.

Biohythane is a mixture of biohydrogen and biomethane. Most of the studies performed in this area report a two-stage biohythane generation technique, where the first stage consists principally of a hydrogen fermentation followed by a methane fermentation reaction in a specific bioreactor (Liu et al. 2014; Kongjan et al. 2013). Lab-scale bioreactors have been developed and evaluated with different kinds of substrates in order to produce biohythane (Farghaly and Tawfik 2017; Lin et al. 2015).

Different microorganisms have been used for biohythane production. Costa et al. (2015) reported biohythane production from *Sargassum* sp.; Jariyaboon et al. (2015) used *Thermoanaerobacterium* sp., *Clostrdium* sp., *Methanosarcina mazei,* and *Methanothermobacter defluvii* to synthesize biohythane, and Elreedy et al. (2015) evaluated biohythane production using *Proteobacteria* and *Firmicutes.*

Pinto et al. (2018) submitted a study about the co-digestion of three abundant solid coffee residues (green coffee powder, parchment, and defatted cake) and ALW under thermophilic anaerobic conditions. They proposed a pilot plant under optimized conditions using a mesophilic sludge seed to produce biohydrogen. The initial conditions were acidogenic regimes (pH 5.0–6.5) followed by methanogenic conditions (pH 6.5–8.0). The bioreactor produced a hydrogen-rich biohythane for the first 15 days with a maximum yield on day four (31.45% hydrogen). For the co-digestion of the defatted cake and ALW, the only gas of interest produced was biohydrogen 32% v/v between the ninth and 32nd day.

10.6 Physicochemical Degradation Processes for ALW

Among the different treatment technologies, biological processes are commonly the preferred option based on the economy of the treatment. However, when recalcitrant compounds are involved, biological processes may not be totally effective since the inhibitory effects associated with the presence of such recalcitrant organic compounds may cause low yields and process instabilities (Ioannou et al. 2015). Many treatments consist on a combination of both, biological and physicochemical processes.

10.7 Conventional

10.7.1 Fertigation

ALW have many components such as organic matter, K, N, Ca, Mg, and K being the most relevant mineral element for the agricultural use of the wastes. Therefore, ALW utilization can contribute to enhancing crops productivity with effects on the physicochemical and biological soil qualities (Jiang et al. 2012).

However, ALW flows applied in crops must follow appropriate guidelines, which fluctuate according to soil characteristics. Specific recommendations must be followed for each area to prevent excessive use and subsequent mineral lixiviation, for example, of nitrates and potassium, and contamination of underground waters. In addition, the high content of organic matter in ALW can increase pollution.

It is evident that there is no unanimity about the polluting capacity of ALW. Different points of view indicate, on the one hand, toxic effects on ground and surface waters and, on the other hand, that rational use of this waste does not result in environmental risk. Nevertheless, it should be emphasized that ALW can behave as a pollutant or beneficial fertilizer depending on the amount applied. In this context, the appropriate application of ALW must consider soil chemical and physical characteristics, besides aspects like the history of residue application, the intensity of cultivation in the agricultural area, and the proximity of water springs.

10.7.2 Concentration by Evaporation

ALW *in natura* are diluted solutions and their application to soil is carried out in large quantities. The distribution and treatment of ALW frequently involve three different stages: (a) the primary transport from industry to the storage tanks, (b) the secondary transport from the tanks to the treatment industries, and in some cases (c) the distribution to final destination. Each phase has logistic costs represented mainly on equipment, infrastructure, power, and management techniques hence making its use problematic in areas distant from the production places. However, ALW can be concentrated by evaporation, resulting in a product with higher economic viability that can be transported to distant locations. In many liquid wastes, it is possible to recover up to 78% of the water, thus reducing its volume by evaporation processes.

10.7.3 Animal Feedstock

Some ALWs are considered as nonconventional feedings, but are not universally used in animal feedstock. Hence, if they are properly used, they could be an important element in the sustainable animal feed production systems (Iranmehr et al. 2011). ALW may be used as an economical animal feed due to their low

cost and the presence of organic compounds (i.e., acids, alcohols, and sugars), minerals, and nitrogen compounds (amino acids and peptides).

Determination of the protein fraction is one of the most relevant indicators for using ALW as animal feeding and as raw material for elaborating new feedstocks. This aspect is important due to the benefits of its functional diversity for different animal species (Silva et al. 2011). Protein quality is a key concept in protein nutrition, mainly determined by the profile and proportion of amino acids, although other structural characteristics and solubility may affect its digestibility and in turn, its nutritional value.

10.7.4 Combustion

Low calorific fuels such as ALW have calorific values that are too low to be burnt with conventional burners (between 10.45 and 7.47 MJ kg^{-1}). However, it is possible to burn such fuels in a SSB-LCL (SAACKE Swirl Burner for Low Calorific Liquids) firing system and to feed the resulting exhaust gases to a boiler or a combustor. The system consists of a SAACKE swirl burner with a special burner throat. The SSB is a well-proven gun-type burner for industrial and power station plants. This burner was developed for the combustion of natural gas and fuel oil and operates according to the "mixing at the burner mouth" principle. This burner results in low CO and NO_x emissions. It is meanwhile also used in many applications for low calorific gases with even below 3.0 MJ m^{-3} LHV (lower heating value) (Schopf and Erbino 2010).

10.7.5 Gasification

The main objective of gasification is to convert a lignocellulosic matrix in volatile gases and char that can be used in other processes to generate different kinds of energy. In case of ALW, the major problem is the high water content and the minimum percentage of biomass. However, good yields can be achieved with the adequate technology (Dirbeba et al. 2016).

Gasification is a widely explored technology for its potential in providing higher efficiency cycles. Extensive ranges of biomass and waste products have been studied as potential fuels. Patel and Nikhil (2000) studied gasification of concentrated ALW using a spray-type air-blown laboratory reactor at a temperature range of 677–727 °C. Gasification experiments frequently are conducted in an inclined plate reactor with rectangular cross section (80 mm × 160 mm) and 3000 mm long. A support flame was found necessary in the injection zone in addition to the regenerative heat transfer. Effluent with 60% solids was injected as film on the reactor bed. The typical gas fractions obtained during gasification condition (air ratio = 0.3) are 10.0–11.5% of CO_2, 10.0–12.0% of CO, 6.7–8.0% of H_2, 1.75% of CH_4, 0.2–0.4% of H_2S, and about 2% of saturated moisture. The carbon conversion obtained was in the range of 95–96%.

10.8 Nonconventional

10.8.1 Membranes

Membrane technology has achieved over the last years a great commercial and strategic importance. The increasing interest in this technology is mainly associated with its relative simplicity, ease of use, low energy consumption, and application in the separation of liquid and gas mixtures (van de Water and Maschmeyer 2004).

ALW can be concentrated through membranes. These waste management processes are a promising approach for improving its use. Moreover, concentrating ALW may increase fertilization quality and may both reduce transport costs and broaden the application range. This approach may represent an alternative method to evaporation (Amaral et al. 2016). Thus, the permeate stream arising from the membrane module could be recycled, whereas the retentate stream could be used in fertigation.

10.8.2 Electrochemical Process

An interesting alternative for ALW treatment is the use of electrochemical processes to achieve a partial oxidation of the organic compounds before the biological step. The electrochemical oxidation process is expected to decrease the toxicity of some organic materials and allow subsequent degradation of the substrate by an anaerobic consortium (Tröster et al. 2002).

Electrochemical oxidation is an attractive technology due to its capability to treat (under moderate conditions, ambient temperature and pressure) toxic and/or complex organic contaminants present in industrial or domestic wastewaters. Organic pollutants can be reduced by either direct or indirect oxidation (Prabhakaran et al. 2009; Flores et al. 2017).

Anodic oxidation of organic materials takes place in association with the oxygen transfer from water to the reaction products. In other words, water is the source of oxygen atoms for the whole oxidation. These reactions are intensely related to the anode surface. Therefore, if the anode contacts are weak, the electrochemical activity on the way to oxygen evolution will be lower and the chemical reactivity toward organic oxidation will be higher (Kapałka et al. 2010).

10.9 Conclusion

There are many conventional technological alternatives for ALW treatment, like concentration by evaporation, combustion, livestock feed production, gasification, vermicomposting, yeast production, biogas production, and recycling in fermentation. Based on the foregoing, many efforts have been directed to allocate, properly, the large volume of these wastes. Hence, new studies and green nonconventional methods have been developed aiming at recycling and disposing ALW. Most of the

nonconventional technologies were emerging for improving the environmental and economics sustainability. Some of them are biohythane production, dark fermentation, and bioremediation as biological alternatives and membrane and electrochemical processes from a physicochemical point of view. Those technologies are not completely developed, but the studies about these processes are increasing exponentially. These emerging technologies reduce the pollution load through the biotransformation of complex organic compounds into value-added products or clean energy.

References

Alavi N, Daneshpajou M, Shirmardi M, Goudarzi G, Neisi A, Babaei A (2017) Investigating the efficiency of co-composting and vermicomposting of vinasse with the mixture of cow manure wastes, bagasse, and natural zeolite. Waste Manag 69:117–126. https://doi.org/10.1016/J.WASMAN.2017.07.039

Alvarez A, Saez J, Davila Costa J, Colin V, Fuentes M, Cuozzo S, Amoroso M (2017) Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. Chemosphere 166:41–62. https://doi.org/10.1016/j.chemosphere.2016.09.070

Amaral M, Andrade L, Neta L, Magalhaes N, Santos F, Mota G, Carvalho R (2016) Microfiltration of vinasse: sustainable strategy to improve its nutritive potential. Water Sci Technol 73 (6):1434–1441. https://doi.org/10.2166/wst.2015.606

Amouei A, Asgharnia H, Khodadi A (2010) Study of compost quality from rural solid wastes (Babol, Iran). J Mazandaran Univ Med Sci 19(74):55–61. Retrieved from https://jmums.mazums.ac.ir/browse.php?a_id=600&sid=1&slc_lang=en

Aparicio J, Benimeli C, Almeida C, Polti M, Colin V (2017) Integral use of sugarcane vinasse for biomass production of actinobacteria: potential application in soil remediation. Chemosphere 181:478–484. https://doi.org/10.1016/J.CHEMOSPHERE.2017.04.107

Appenroth K, Sree K, Böhm V, Hammann S, Vetter W, Leiterer M, Jahreis G (2017) Nutritional value of duckweeds (Lemnaceae) as human food. Food Chem 217:266–273. https://doi.org/10.1016/J.FOODCHEM.2016.08.116

Arm M, Suer P, Arvidsson H, Lindqvist J (2011) Technical and environmental long-term properties of industrial residues—summary of field and laboratory investigations. Waste Manag 31 (1):101–107. https://doi.org/10.1016/j.wasman.2010.09.004

Beil M, Beyrich W (2013) Biogas upgrading to biomethane. In: The biogas handbook, pp 342–377. https://doi.org/10.1533/9780857097415.3.342

Bustamante M, Paredes C, Moral R, Moreno-Caselles J, Pérez-Espinosa A, Pérez-Murcia M (2005) Uses of winery and distillery effluents in agriculture: characterisation of nutrient and hazardous components. Water Sci Technol 51(1):145–151. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15771110

Costa J, Oliveira J, Pereira M, Alves MM, Abreu A (2015) Biohythane production from marine macroalgae Sargassum sp coupling dark fermentation and anaerobic digestion. Biores Technol 190:251–256. https://doi.org/10.1016/j.biortech.2015.04.052

Dirbeba M, Brink A, DeMartini N, Lindberg D, Hupa M (2016) Sugarcane vinasse CO_2 gasification and release of ash-forming matters in CO_2 and N_2 atmospheres. Bioresour Technol 218:606–614. https://doi.org/10.1016/J.BIORTECH.2016.07.004

Elreedy A, Tawfik A, Kubota K, Shimada Y, Harada H (2015) Hythane (H_2 + CH_4) production from petrochemical wastewater containing mono-ethylene glycol via stepped anaerobic baffled reactor. Int Biodeterior Biodegradation 105:252–261. https://doi.org/10.1016/J.IBIOD.2015.09.015

España-Gamboa E, Mijangos-Cortés J, Hernández-Zárate G, Maldonado J, Alzate-Gaviria L (2012) Methane production by treating vinasses from hydrous ethanol using a modified UASB reactor. Biotechnol Biofuels 5(1):82. https://doi.org/10.1186/1754-6834-5-82

Fadel M, Abdel-Naser (2014) Recycling of vinasse in ethanol fermentation and application in Egyptian distillery factories. Afr J Biotechnol 13(47):4390–4398. https://doi.org/10.5897/AJB2014.14083

Farghaly A, Tawfik A (2017) Simultaneous hydrogen and methane production through multi-phase anaerobic digestion of paperboard mill wastewater under different operating conditions. Appl Biochem Biotechnol 181(1):142–156. https://doi.org/10.1007/s12010-016-2204-7

Flores N, Thiam A, Rodríguez R, Centellas F, Cabot P, Garrido J, Sirés I (2017) Electrochemical destruction of trans-cinnamic acid by advanced oxidation processes: kinetics, mineralization, and degradation route. Environ Sci Pollut Res 24(7):6071–6082. https://doi.org/10.1007/s11356-015-6035-9

Ioannou L, Puma G, Fatta-Kassinos D (2015) Treatment of winery wastewater by physicochemical, biological and advanced processes: a review. J Hazard Mater 286:343–368. https://doi.org/10.1016/J.JHAZMAT.2014.12.043

Iranmehr M, Khadem A, Rezaeian M, Afzalzadeh A, & Pourabedin, M. (2011). Krmiva. Krmiva: Časopis o hranidbi životinja, proizvodnji i tehnologiji krme (Vol. 53). Hrvatsko agronomsko društvo. Retrieved from https://hrcak.srce.hr/index.php?show=clanak&id_clanak_jezik=106269

Janke L, Leite A, Nikolausz M, Schmidt T, Liebetrau J, Nelles M, Stinner W (2015) Biogas production from sugarcane waste: assessment on kinetic challenges for process designing. Int J Mol Sci 16(9):20685–20703. https://doi.org/10.3390/ijms160920685

Janke L, Leite A, Wedwitschka H, Schmidt T, Nikolausz M, & Stinner W (2014) Biomethane production integrated to the Brazilian Sugarcane Industry: The case study of São Paulo State. European Biomass Conference and Exhibition Proceedings, 1295–1299. doi:https://doi.org/10.5071/22ndeubce2014-3dv.2.4

Jariyaboon R, O-Thong S, Kongjan P (2015) Bio-hydrogen and bio-methane potentials of skim latex serum in batch thermophilic two-stage anaerobic digestion. Bioresour Technol 198:198–206. https://doi.org/10.1016/j.biortech.2015.09.006

Jiang Z, Li Y, Wei G, Liao Q, Su T, Meng Y, Lu C (2012) Effect of long-term vinasse application on physico-chemical properties of sugarcane field soils. Sugar Tech 14(4):412–417. https://doi.org/10.1007/s12355-012-0174-9

Kapałka A, Fóti G, Comninellis C (2010) Basic principles of the electrochemical mineralization of organic pollutants for wastewater treatment. In: Electrochemistry for the environment. Springer New York, New York, NY, pp 1–23. https://doi.org/10.1007/978-0-387-68318-8_1

Kongjan P, O-Thong S, Angelidaki I (2013) Hydrogen and methane production from desugared molasses using a two-stage thermophilic anaerobic process. Eng Life Sci 13(2):118–125. https://doi.org/10.1002/elsc.201100191

Krzywonos M, Cibis E, Miskiewicz T, Ryznar-Luty A (2009) Utilization and biodegradation of starch stillage (distillery wastewater). Electron J Biotechnol 12(1):6–7. https://doi.org/10.4067/s0717-34582009000100006

Laurens L, Markham J, Templeton D, Christensen E, Van Wychen S, Vadelius E, Pienkos P (2017) Development of algae biorefinery concepts for biofuels and bioproducts; a perspective on process-compatible products and their impact on cost-reduction. Energy Environ Sci 10 (8):1716–1738. https://doi.org/10.1039/C7EE01306J

Lim S, Wu T (2016) Characterization of matured vermicompost derived from valorization of palm oil mill byproduct. J Agric Food Chem 64(8):1761–1769. https://doi.org/10.1021/acs.jafc.6b00531

Lim S, Wu T, Lim P, Shak K (2015) The use of vermicompost in organic farming: overview, effects on soil and economics. J Sci Food Agric 95(6):1143–1156. https://doi.org/10.1002/jsfa.6849

Lin C, Chu C, Lay C, Leu H (2015) Fermentative hydrogen and methane productions from organic wastes: a review. Curr Biochem Eng 3(1):16–23. https://doi.org/10.2174/2212711902666150519231824

Liu Z, Li Q, Zhang C, Wang L, Han B, Li B, Xing X (2014) Effects of operating parameters on hydrogen production from raw wet steam-exploded cornstalk and two-stage fermentation potential for biohythane production. Biochem Eng J 90:234–238. https://doi.org/10.1016/J.BEJ.2014.06.013

Meunchang S, Panichsakpatana S, Weaver R (2005) Co-composting of filter cake and bagasse; by-products from a sugar mill. Bioresour Technol 96(4):437–442. https://doi.org/10.1016/J.BIORTECH.2004.05.024

Moran-Salazar R, Sanchez-Lizarraga A, Rodriguez-Campos J, Davila-Vazquez G, Marino-Marmolejo E, Dendooven L, Contreras-Ramos S (2016) Utilization of vinasses as soil amendment: consequences and perspectives. Springerplus 5(1). https://doi.org/10.1186/s40064-016-2410-3

Mrozik A, Piotrowska-Seget Z (2010) Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. Microbiol Res 165(5):363–375. https://doi.org/10.1016/j.micres.2009.08.001

Nasseri A, Rasoul-Ami S, Morowvat M, Ghasemi Y (2011) Single cell protein: production and process. Am J Food Technol 6(2):103–116. https://doi.org/10.3923/ajft.2011.103.116

Parnaudeau V, Condom N, Oliver R, Cazevieille P, Recous S (2008) Vinasse organic matter quality and mineralization potential, as influenced by raw material, fermentation and concentration processes. Bioresour Technol 99(6):1553–1562. https://doi.org/10.1016/J.BIORTECH.2007.04.012

Patel NM, & Nikhil (2000) Studies on the combustion and gasification of concentrated distillery effluent. Indian Institute of Science, 1–23. Retrieved from https://www.sciencedirect.com/science/article/pii/S0082078496800798

Pinto M, Mudhoo A, de Alencar Neves T, Berni M, Forster-Carneiro T (2018) Co-digestion of coffee residues and sugarcane vinasse for biohythane generation. J Environ Chem Eng 6(1):146–155. https://doi.org/10.1016/J.JECE.2017.11.064

Polti M, Aparicio J, Benimeli C, Amoroso M (2014) Simultaneous bioremediation of Cr(VI) and Lindane in soil by Actinobacteria. Int Biodeterior Biodegradation 88:48–55. https://doi.org/10.1016/j.ibiod.2013.12.004

Prabhakaran D, Kannadasan T, Basha C (2009) Treatability of resin effluents by electrochemical oxidation using batch recirculation reactor. Int J Environ Sci Technol 6(3):491–498. https://doi.org/10.1007/BF03326088

Schopf N, Erbino P (2010) Thermal utilisation of vinasse as alternative fuel. Proc Int Soc Sugar Cane Technol 27. Retrieved from https://www.atamexico.com.mx/wp-content/uploads/2017/11/COPRODUCTS-33-Schopf.pdf

Silva A, Cabeda M, Carvalho F, Lima J (2006) Alterações físicas e químicas de um Argissolo amarelo sob diferentes sistemas de uso e manejo. Revista Brasileira de Engenharia Agrícola E Ambiental 10(1):76–83. https://doi.org/10.1590/S1415-43662006000100012

Silva C, Arcuri S, Campos C, Vilela D, Alves J, Schwan R (2011) Using the residue of spirit production and bio-ethanol for protein production by yeasts. Waste Manag 31(1):108–114. https://doi.org/10.1016/j.wasman.2010.08.015

SSSA (2018) Home | Soil Science Society of America. Retrieved March 23, 2018, from https://www.soils.org/

Sydney E, Novak A, Rosa D, Pedroni Medeiros A, Brar S, Larroche C, Soccol C (2018) Screening and bioprospecting of anaerobic consortia for biohydrogen and volatile fatty acid production in a vinasse based medium through dark fermentation. Process Biochem 67:1–7. https://doi.org/10.1016/J.PROCBIO.2018.01.012

Tröster I, Fryda M, Herrmann D, Schäfer L, Hänni W, Perret A, Stadelmann M (2002) Electrochemical advanced oxidation process for water treatment using DiaChem® electrodes. Diam Relat Mater 11(3–6):640–645. https://doi.org/10.1016/S0925-9635(01)00706-3

van de Water L, Maschmeyer T (2004) Mesoporous membranes: a brief overview of recent developments. Top Catal 29(1/2):67–77. https://doi.org/10.1023/B:TOCA.0000024929.79470.7f

Walsh B, Rydzak F, Palazzo A, Kraxner F, Herrero M, Schenk P, Obersteiner M (2015) New feed sources key to ambitious climate targets. Carbon Balance Manag 10(1):26. https://doi.org/10.1186/s13021-015-0040-7

White Rot Fungi: Nature's Scavenger

11

Ajit Patel, Vanita Patel, Harsh Patel, Ujjval Trivedi, and Kamlesh Patel

Abstract

The world's worst offenders in terms of pollution are related to the xenobiotic organopollutants, often toxic and recalcitrant in nature. They have complex aromatic structures which are persistent and recalcitrant, for example, xenobiotics such as phenols, plastics, hydrocarbons, paints, synthetic dyes, pesticides, insecticides, paper and pulp mill effluents, and pharmaceuticals. Applications of physicochemical methods are quite expensive to operate; moreover, they introduce secondary pollutants during the "remediation" process. The white rot fungi technology is eco-friendly and cost-effective and thus has emerged as a viable method for bioremediation in a wide range of synthetic dyes. Moreover, they do not require preconditioning to a particular pollutant, tolerate high concentrations of pollutants and nutrient limitation induces the production of extracellular ligninolytic enzymes with broad substrate specificity which includes several kinds of laccases, peroxidases, and oxidases producing H_2O_2. They utilize soluble as well as insoluble hazardous compounds as a nutrient source and convert them to simple fragmented forms. White rot fungi also remove the pollutants from the effluents by absorption, adsorption, and accumulation. Recently, many researchers have successfully exploited white rot fungi, for example, *Pleurotus ostreatus, Phanerochaete chrysosporium, Tramates hirsute, T. versicolor, Lentinus edodes,* and *Trichoderma longibrachiatum* for bioremediation of xenobiotics. The aim of this chapter is to address the present status and development of bioremediation strategies for synthetic textile dyes using white rot fungi and their enzymes.

A. Patel (✉)
J. & J. College of Science, Sardar Patel University, Nadiad, Gujarat, India

V. Patel
V.P & R.P.T.P. Science College, Vallabh Vidyanagar, Gujarat, India

H. Patel · U. Trivedi · K. Patel
P. G. Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_11

Keywords
Bioremediation · Organopollutants · White rot fungus · Ligninolytic enzymes · Xenobiotic

11.1 Introduction

The unsustainable development of industries in this modern world has led to incredible increase in environmental pollution. Large amount of pollutants are produced by these industries resulting in a considerable fall in the levels and quality of the ecosystem. The "World's Worst Pollution Problems 2016: The Toxics Beneath Our Feet" published by Green Cross Switzerland and Pure Earth (Earth and Cross 2016) presents an update of the top ten polluting industries based on the global burden of disease caused by each source. They include used lead acid battery (ULAB) recycling, mining and ore processing, tanneries, dumpsites, industrial estates, smelting, artisanal small-scale gold mining (ASGM), product manufacturing, chemical manufacturing, and the dye industry. These industries collectively put over 32 million people at risk and account for 7–17 million Disability-Adjusted Life Years (DALYs) in low- and middle-income countries.

One of the major environmental problems facing by the world today is the contamination of soil, water, and air by toxic chemicals. White rot fungi are extraordinary in their versatility to degrade a large variety of complex and recalcitrant environmental pollutants that contaminate soil and groundwater ecosystems, constituting a potential danger to human and animal health. It is fairly well proven now that white rot (as well as some nonwhite rot fungi) have the ecological and biochemical capability to degrade important categories of toxic chloro-organic pollutants. Certain hazardous compounds, such as synthetic textile dyes, polycyclic aromatic hydrocarbons (PAH), pentachlorophenols (PCP), polychlorinated biphenyls (PCB), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), 2 benzene, toluene, ethylbenzene, and xylene (BTEX), and trinitrotoluene (TNT) are persistent in the environment and are known to have carcinogenic and/or mutanogenic effects. Due to the magnitude of this problem and the lack of a reasonable solution, a rapid, cost-effective, ecologically responsible method of clean-up is the need of hour. One growing mechanism of decontamination that may fit these requirements is bioremediation, that is, application of microorganisms to degrade toxic organopollutants is an efficient, economical approach that has been successful in laboratory studies.

White rot fungi are a group of fungi comprising some 1600–1700 species characterized by the ability to depolymerize and mineralize lignin using a set of extracellular ligninolytic enzymes (Gilbertson 1980; Alexandropoulos et al. 1996). Most known white rot fungi are Basidiomycetes, although a few Ascomycetes genera within the *Xylariaceae* are also capable of white rot decay (Eaton and Hale 1993). White rot fungi can be exploited in bioremediation system as they possess an extracellular lignin-degrading system which can degrade insoluble chemicals such as lignin (a heterogeneous polyphenolic polymer) or an extremely diverse range of

very persistent or toxic environmental pollutants (Barr and Aust 1994). The mycelial growth allows rapid colonization of substrates and hyphal extension enables penetration of soil, thus making it advantageous to reach the pollutants (Reddy and Mathew 2001). These fungi use abundant, economical lignocellulosic materials as a nutrient source and can tolerate a wide range of environmental conditions like temperature, pH, and moisture levels (Maloney 2001).

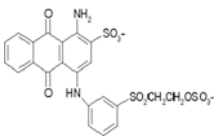
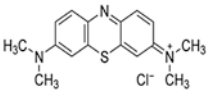
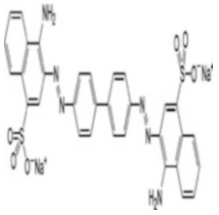
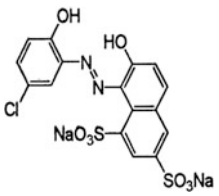
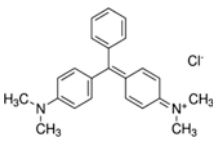
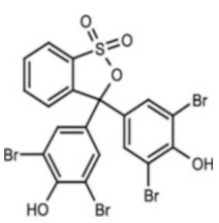
The three major families of lignin-modifying enzymes believed to be involved in lignin degradation are laccases, lignin peroxidases, and manganese peroxidases (Reddy and Mathew 2001). The key step in lignin degradation by ligninolytic enzymes involves the formation of highly reactive free radical intermediates, which are formed when one electron is removed or added to the ground state of a chemical (Reddy and Mathew 2001), thereby triggering oxidation or reduction of "neighboring" compounds. Variety of reactions carried out by these radicals includes benzylic alcohol oxidation, carbon–carbon bond cleavage, hydroxylation, phenol dimerization/polymerization, and demethylation (Pointing 2001). Ligninolytic enzymes are not substrate specific and they are not induced by either lignin or other related compounds (Cancel et al. 1993). As ligninolytic mechanism is the most widely studied and used in bioremediation, it has been shown that this system is also involved in the oxidation of a significant number of xenobiotics as polycyclic aromatic hydrocarbons, synthetic and natural dyes, and some pesticides such as pentachlorophenol. Compounds that have an aromatic structure, such as the recalcitrant xenobiotics, are also highly susceptible to degradation by ligninolytic enzymes (Field et al. 1993; Barr and Aust 1994). To date, the fungi including *Phanerochaete chrysosporium*, *Trametes versicolor*, *Bjerkandere adusta,* and *Pleurotus ostreatu*s have been studied for the bioremediation of xenobiotic organic pollutants and waste treatment.

11.2 Synthetic Dyes and Their Applications

Dyes are used to color fabrics; bright colorful textiles around us make the world a very charming place. Synthetic dyes are primarily used in the production of consumables and are commonly found in paints, textiles, printing inks, paper, and plastics—adding to the color and patterns of materials (Table 11.1).

Natural dyes have increasingly been replaced by chemical dyes that provide and retain richer color throughout wash and exposure (Kant 2012). The chemical property of dye is modified when the fabric is soaked in it, so the resulting color stays permanently even after repeated use. Dyes are classified into acid, reactive, direct, basic, vat, disperse, metal complex, mordant, and sulfur types. Most of the dyes have their own production process but sulfuric acid, chromium, copper, other metals, additives, solvents, and chemicals are added as a catalyst for synthesis. As a result, the wastewater effluents of dye factories can pose a complex threat to the health and environment. Two-third of the total dyestuff market is occupied by the textile industry (Riu et al. 1998), which consumes large volumes of water and chemicals for wet processing of the textiles. Azo dyes contain N=N double bonds

Table 11.1 Examples of synthetic dye and their applications in various industries

Group	Structure	Example of dye	λ_{max}	Application
Anthrapyridone		Poly R-481	515	Wool, polyamide, silk, nylon, leather
Anthraquinone		Poly B-411	597	
Anthraquinone		RBBR	590	
Cationic thiazine		Methylene blue	600	Wool, leather, silk, modified cellulose fibers
Di azo		Acid Red-97	530	Cellulose fibers, cotton, viscose, leather, nylon, synthetic fibers, paper, inks, paints, plastics
Di azo		Congo red	680	
Di azo		Direct red-81	562	
Di azo		Reactive red	521	
Di azo		Reactive black-39	580	
Mono azo		Eriochrome black-T	551	
Mono azo		Reactive violet 5R	559	
Mono azo		Reactive blue-28	573	
Multi azo		Acid brown	600	
Tri aryl methane		Phenol red	424	Synthetic fibers, paper, inks
Tri aryl methane		Malachite green	615	
Tri aryl methane		Crystal violet	532	
Tri phenyl methane		Aniline blue	600	Wool, leather, silk, modified cellulose fibers
Tri phenyl methane		Bromo phenol blue	591	
Tri phenyl methane		Bromo thymol blue	439	
Tri phenyl methane		Coomassie brilliant blue R-250	602	
Tri phenyl methane		Basic Fuchsin	532	

and account for about 70% of all textile dyestuffs produced. Anthraquinone dyes are toxic, carcinogenic, mutagenic, and resistant to microbial degradation (Itoh et al. 1996). Triphenylmethane dyes are characterized by the presence of chromogen

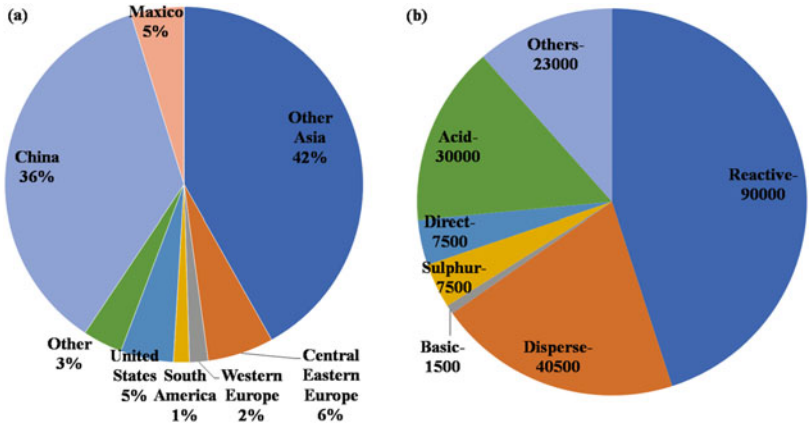


Fig. 11.1 (**a**) World consumption of synthetic dyes in 2017 (Chemical Economics Handbook 2018). (**b**) Annual production of various dyes in India (Dyestuff Industry in India and China by Angela T. Forrester 2019 (Beebe 2019))

containing three phenyl groups attached to the central carbon atom. A major cause of color discharge pollution into the environment is incomplete absorption of dyes onto the textile fiber during the dyeing process. Various industries use up to 40,000 different types of synthetic dyes and pigments, and the annual production of dyestuff is up to 450,000 tons globally. Textile manufacturing process uses more than 10,000 different types of dyes. Figure 11.1a represents the consumption of synthetic dyes throughout the world, and Fig. 11.1b shows production of different type of dyes in India. Among them, the major and more versatile class of dye is azo dye, and the annual production accounts for up to 50.0% of the total dyes produced (Zollinger 2003). Azo dyes are largely used in several industries such as textile, paper and printing, leather and tanning, food, color photography, pharmaceuticals and medicine, cosmetic and hair coloring, wood staining, agricultural, biological and chemical research, light-harvesting arrays, and photoelectrochemical cells (Kuhad et al. 2004; Couto 2009).

11.2.1 Production of Textile Effluents Containing Synthetic Dyes

The global annual production of synthetic dyes is nearly 800,000 tons. During dyeing process, 10–25% of synthetic dyes are lost, and 2–20% is directly discharged as aqueous effluents in the environment by textile industry. During the dyeing and finishing operations, up to 200,000 tons of these dyes are lost to effluents every year. It can be estimated that approximately 75% of the dyes, belonging to the classes of the reactive (~36%), acid (~25%), and direct dyes (~15%) were discharged by Western European textile processing industries (Couto and Toca-Herrera 2006). Synthetic dyes have been widely used to determine the specific surface area of

Table 11.2 Types of textile finishing processes and their water consumption (adapted from Bertea and Bertea 2008)

Types of finishing process	Water consumption, 10^{-3} m^3 kg^{-1} textile product		
	Minimum	Medium	Maximum
Raw wool washing	4.2	11.7	77.6
Wool finishing	110.9	283.6	657.2
Fabric finishing			
Short process	12.5	78.4	275.2
Complex processing	10.8	86.7	276.9
Cloth finishing			
Simplified processing	8.3	135.9	392.8
Complex process	20	83.4	377.8
Panty processing	5.8	69.2	289.4
Carpet finishing	8.3	46.7	162.6
Fiber finishing	3.3	100.1	557.1
Non-fabrics finishing	2.5	40	82.6
Yarn finishing	33.4	212.7	930.7

activated sludge process for groundwater tracing, to ensure successful management of sewage and wastewater treatment (Forgacs et al. 2004). Textile industry is the largest consumer of these dyes which cover 65.0% of the dye market. Selections of different classes of dyes depend on the types of fibers to which they can be applied. The most commonly used dyes are reactive dyes as they can be applied to both natural (silk, wool, cotton) and synthetic (modified acrylics, polyesters) fibers (O'Neill et al. 1999).

Reactive dyes have one or more reactive groups and are capable of forming a covalent bond with a compatible group of synthetic fiber. They are very popular due to their high wet-fastness, brilliance, and range of hues (Hao et al. 2000). Acid and basic dyes are commonly used for dyeing natural fibers (wool, cotton, and silk) and some synthetics (polyesters, acrylic, and rayon) fibers. Direct dyes as the name indicates are directly applied to cellulose fibers, and they are used for dyeing rayon, paper, and leather and to a small extent to nylon. Mordant dyes have limited applications and they are used for dyeing wool, leather, furs, and anodized aluminum, while solvent dyes are used for dyeing inks, plastics, wax material, fat, and mineral oil products. Unfortunately, the textile industry consumes enormous amounts of water (up to 200 L kg^{-1} of textile fabricated) and chemicals in its operation (Table 11.2 and Fig. 11.2) (Bertea and Bertea 2008). Due to the higher level of contamination in dyeing and finishing processes (i.e., dyes and their breakdown products, pigments, dye intermediates, auxiliary chemicals and heavy metals, etc.), recycling of treated wastewater has been recommended (Table 11.3).

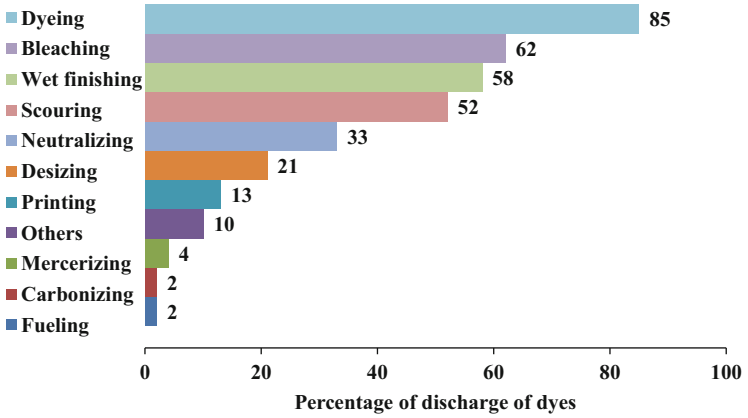


Fig. 11.2 Percentage of dyes discharged by various units of textile industry (adapted from Bertea and Bertea 2008)

Table 11.3 Types of pollutants and their concentration in textile waste water (EWA 2005)

Type of finishedtextile	Dyes, g kg^{-1} textile product	Auxiliaries, g kg^{-1} textile product	Basic chemical compounds, g kg^{-1} product
Polyester fibers	18	129	126
Fabrics from synthetic fibers	52	113	280
Fabrics from cotton	18	100	570
Dyed fabrics from cellulose fibers	11	183	20,088
Printed fabrics from cellulose fibers	88	180	807

11.2.2 Environmental Impact of Textile Dye Effluents

Color is the first contaminant to be recognized in wastewater. Synthetic dyes in water (<1 mg L^{-1}) are highly visible, so they are usually the first undesirable change to be recognized in wastewater, and they also affect the aesthetic merit, transparency, and gas solubility in water bodies. In addition, textile dyes at high concentrations inhibit sunlight penetration, respiration activities, consequently upsetting the biological and photosynthetic processes in the aquatic environment. The dyes which are synthesized using benzidine and other aromatic compounds are generally carcinogenic and are a major problem, because such anthraquinone-based dyes are resistant to degradation due to their fused aromatic ring structure. Some disperse dyes have good ability to bio accumulate, and the azo and nitro compounds are reduced in sediments to toxic amines (e.g., $R_1\text{-}N{=}N\text{-}R_2 + 4H^+ + 4e^- \rightarrow R_1\text{-}NH_2 + R_2\text{-}NH_2$). Many of the textile chemicals degrade slowly and continue to consume oxygen even after 30 days and produce potentially mutagenic, carcinogenic, and harmful effluents

Table 11.4 Examples of synthetic dye and their toxicity in human being

Name of the dye	Effects on human being
Reactive Brilliant Red	Inhibits function of human serum albumin
Acid Violet-7	Induce chromosomal aberration, lipid peroxidation, acetyl cholinesterase in mice
Disperse Red-1 & Disperse Orange-1	Increase the frequency of micronuclei in human lymphocytes
Reactive Black-5	Decrease urease activity, arginine ammonification rate in terrestrial ecosystem
Disperse Blue-291	Mutagenic, cytotoxic, genotypic effects, formation of micronuclei, DNA fragmentation in human hepatoma cells
Direct Black-38	Urinary bladder cancer in humans
Direct Blue-15	Mutagenic

(Hao et al. 2000; Pinheiro et al. 2004) for life forms mainly because of the carcinogens, such as benzidine, naphthalene, and other aromatic compounds (Suteu et al. 2009a, b; Zaharia et al. 2009). Furthermore, the presence of these dyes for a long time in watercourses leads to bioaccumulation of dyes in fishes and other organisms.

Dyes are persistent in nature, for example, the half-life time of hydrolyzed Reactive Blue 19 is about 46 years at pH 7 and 25 °C (Hao et al. 2000). Some dyes decompose in nature but the degradation products may also have a toxic impact on the aquatic environment (Carmen and Daniela 2012). Azo dyes and their degradation products (aromatic amine) can cause allergies, dermatitis, skin irritation, carcinogenic and mutagenic actions, as well as acute and chronic toxicity (Yaseen and Scholz 2017; Carmen and Daniela 2012). Different synthetic dyes exhibit different biological activities, hence our knowledge concerning their behavior in the environment and health hazards remain incomplete (Forgacs et al. 2004). Azo dyes constitute the largest group of synthetic colorants and thus they are the most common synthetic dyes released into the environment (Zhou and He 2007; Ali 2010). Some azo dyes are toxic to mammalian system and have been linked to bladder cancer, spleen sarcomas, and hepatocarcinomas (Table 11.4). They produce nuclear anomalies and chromosomal aberrations in experimental animals and in cultured mammalian cells, respectively. Assessment of the toxicity of dyes is important because increased incidences of bladder cancer have been reported in workers exposed to huge quantities of azo dyes (Puvaneswari et al. 2006). Many screening methods have been developed to detect mutagenic/carcinogenic substances. These have played important roles in screening of suspected chemicals and also in studying the mechanisms of mutagenesis and carcinogenesis, thus they provide valuable information to assess the genetic effects of chemicals in human being. Microorganisms have been extensively used for quick screening of effluents and chemicals for toxicity. Hao et al. (2000), in their review, tabled the results on the toxicity assessment for the single cell alga, *Selenastrum capricornutum,* and for the

fathead minnow, *Pimephales promela*. Pereira et al. (2009a, b) successfully used yeast *Saccharomyces cerevisiae* to study the effect of azo dye Sudan Orange-G and the anthraquinone dye Acid Blue-62 before and after an enzymatic treatment. The Ames test is commonly used to access the mutagenic potential of many compounds (Ames et al. 1975). Mathur et al. (2006) evaluated the mutagenic potential of commonly used textile dyes in India by Ames test using *Salmonella typhimurium* TA 100 strain. Seven dyes were tested by him, and the result showed that six dyes had mutagenic activity. Nath et al. (2016) observed the harmful impact of synthetic dyes in the oestrous cycle and in the reproductive system of rats. Amin et al. (2010) reported negative impact of dyes in biochemical markers of vital organs, such as the liver and kidney.

Besides this, dyes in water bodies can also affect the health of human beings by various ways. They can also have acute and/or chronic effects on living systems depending on their concentration and length of exposure. The first and major concern is to remove color and to eliminate or significantly decrease the toxicity (i.e., detoxification) of dye-containing wastewater. Both the developed and developing countries impose stringent standards day by day for the elimination of dyes from industrial effluents (Robinson et al. 2001).

11.3 Wastewater Remediation

Due to the globalization of world market, a number of challenges are being faced by the textile industries, for example, maintaining the quality and productivity, highly competitive atmosphere, more stringent ecological parameters, and legislation on the limits of color discharge. This in turn directs the industry for innovations and changes in the present technology as well as to develop environmental protective treatment strategies that effectively eliminate the dyes from the waste. Many physicochemical and biological methods have been studied and applied for this purpose, but the choice of method is based on the characteristics of wastewater and economic factors.

11.3.1 Physicochemical Methods for Remediation of Textile Effluents

Synthetic dyes are recalcitrant in nature, it is nearly impossible to remove them by conventional wastewater treatments such as adsorption, photo-oxidation, coagulation, flocculation, photo and chemical degradation. Chemical methods cleave the chromophoric group present in the dye and produce toxic compounds, which are the major disadvantage of this method (Robinson et al. 2001). Textile wastewaters are characterized on the basis of chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH, color, and salinity. The final composition of the wastewater usually depends on the type of chemicals and dyes used during dry and wet-processing steps (Dos Santos et al. 2007; Chequr et al. 2013). The main

Table 11.5 Color concentrations limits and quantum of water generated from industries (adapted from Anjaneyulu et al. 2005)

Industry	Quantum of water generated (m^3 ton^{-1})	Color concentration standards (hazen units)	Color limits (hazen units) USPHS	BIS
Textile	120 m^3 ton fiber	1100–1300	0–25	20
Pulp and paper				
Large	175 m^3 ton $paper^{-1}$	100–600	0–10	5–101
Small	150 m^3 ton $paper^{-1}$			
Tannery	28 m^3 ton raw $hide^{-1}$	400–500	10–50	25
Kraft mill	40 m^3 ton^{-1}	2100–2300	10–40	20
Sugar	0.4 m^3 ton $cane^{-1}$	150–200	5–10	20

pollutants present in textile effluents are recalcitrant organics, color, toxicants, surfactants, and chlorinated compounds (Mansour et al. 2012). Biological systems are the most viable choice available for effluent treatment/decolorization considering the manpower requirements, running expenses, and ease in developing and adopting the technology. Due to the complex composition of wastewater, it is difficult to select best treatment option for the bioremediation of a specific type of industrial waste water. The selection of such processes depends on the effluent composition, characteristics of the dyes, toxicity of the degradation products, and cost as well as the future use of the treated water.

Table 11.5 presents the concentration of colors, their standard limits, and the amount of wastewater produced by textile and other industries in India and the United States (Anjaneyulu et al. 2005). European Community (EC) regulations are also becoming more stringent with increasing awareness about wastewater pollution (O'Neill et al. 1999). Pollution prevention programs also need to focus on introducing new technologies which reduce water and energy consumption as well as make the effluent water reusable. Another case of water pollution is so-called "red-water" pollution which occurs during the last stage of purification of trinitrotoluene (TNT) (Hao et al. 1994). Bleaching of pulp, paper, and textile fibers are also the major sources of colored wastewaters.

Various technologies, which include physical, chemical, and biological methods, have been developed for the removal of synthetic dyes from waters and wastewaters to reduce their environmental impact (Fig. 11.3). Physical methods such as sorption techniques, irradiation, and membrane-filtration processes (micro, ultra, nanofiltration, reverse osmosis); chemical methods such as coagulation or flocculation combined with flotation and filtration, precipitation, or flocculation with Fe(II)/$Ca(OH)_2$, electroflotation, electrochemical processes (electrocoagulation and electro-oxidation), ion exchange, conventional oxidation methods (e.g., with ozone), irradiation, and biological methods such as biosorption, aerobic, and anaerobic microbial degradation, and the use of pure enzymes have normally been applied. All the methods have their own advantages and disadvantages (Table 11.6).

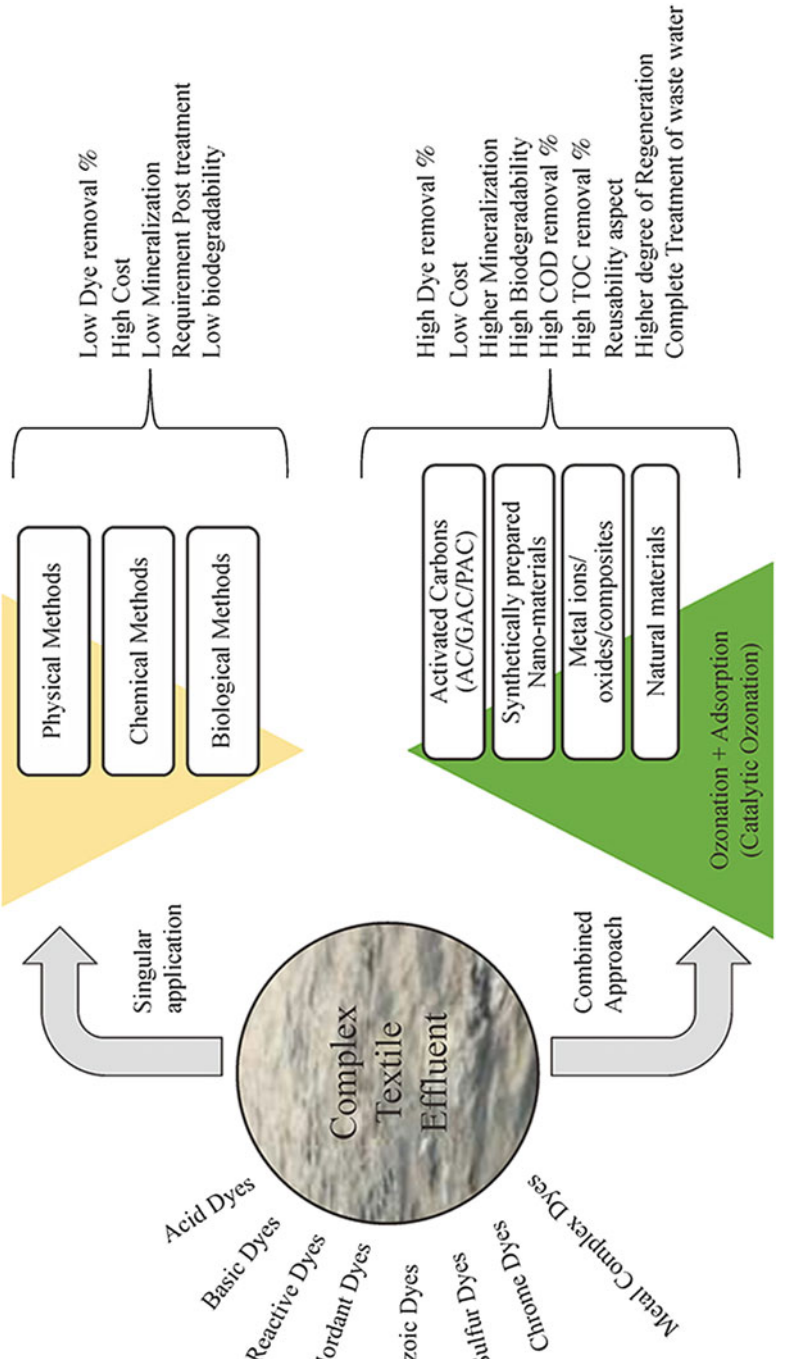


Fig. 11.3 Treatment methods used for the removal of dyes from wastewater effluent (Figure obtained from Khamparia et al. 2017)

Table 11.6 Physicochemical methods applied for wastewater treatment with their advantages and limitations (Table adopted from Zaharia et al. 2012)

Treatment methodologies	Treatment stages	Advantages	Limitations
Physicochemical treatments			
Precipitation Coagulation flocculation	Pre/main treatment	Short detention time and low capital costs Relatively good removal efficiencies	Agglomerate separation and treatment Selected operating condition
Electrokinetic coagulation	Pre/main treatment	Economically feasible	High sludge production
Irradiation	Posttreatment	Effective oxidation at lab scale	Requires a lot of dissolved oxygen (O_2)
Electrochemical oxidation	Pretreatment	No additional chemicals required and the end- products are non- dangerous/hazardous	Cost-intensive process; mainly high cost of electricity
Ion exchange	Main treatment	Regeneration with low loss of adsorbents	Specific application; not effective for all dyes
Membrane filtration	Main treatment	Removes all dye types; recovery and reuse of chemicals and water	High running cost concentrated sludge production Dissolved solids are not separated in this process
Photocatalysis	Posttreatment process	Carried out at ambient conditions Inputs are not toxic and expensive Complete mineralization with shorter detention times	Effective for small amount of colored compounds Expensive process
Adsorption with solid adsorbents such as			
Activated carbon	Pre/post treatment	Economically attractive Good removal efficiency of wide variety of dyes	Very expensive; cost intensive regeneration process
Peat pretreatment	Effective adsorbent due to the cellular structure	No activation required	Surface area is lower than activated carbon
Coal ashes	Pretreatment	Economically attractive Good removal efficiency	Larger contact times and huge quantities are required Specific surface area for adsorption are lower than activated carbon

The major disadvantages of physicochemical methods are that they are highly expensive, less efficient, less versatile, need specialized equipment, interfered by other wastewater constituents and moreover they are not capable of handling the generated waste (Van and Villaverde 2005; Kaushik and Malik 2009). It is very important to develop efficient, economic, and environment-friendly technologies for reduction of dye content in the wastewater to acceptable levels at affordable cost (Couto 2009). None of the technology in use today has universal application because the removal and degradation of dyes depend on their physical and chemical attributes, as well as the selection of treatment method. The technologies used and those in development for the removal of dyes have been discussed in several reports (Vandevivere et al. 1998; Robinson et al. 2001; Forgacs et al. 2004; Anjaneyulu et al. 2005; Hai et al. 2007; Hao et al. 2007; Mondal 2008). Some of the processes do not satisfactorily remove the color of dye, while others are expensive (Mondal 2008).

11.3.2 Biological Treatments

Biological treatment of wastewater is usually termed as bioremediation. Bioremediation is the use of biological system such as microorganisms (bacteria, yeast, filamentous fungi, Actinomycetes, and algae) or their enzymes for the removal and/or breakdown of xenobiotics (synthetic organic chemical compounds).

These processes are energy dependent and take advantage of the biochemical reactions taking place in living system and their enzymes for the breakdown of polluting agents (organopollutants) into different products. Biological methods can lead to complete mineralization of organic pollutants at low cost, and above all they are considered environmentally friendly (Pandey et al. 2007). They also get rid of BOD, COD, and suspended solids. The main limitation with some dyes and/or their degradation products may be toxicity to the organisms used in the process. Yaseen and Scholz (2017) recommended shallow wetland systems planted with aquatic plants for the treatment of wastewater containing dyes. However, use of such treatments is limited and was in use for a short time period (Muthunarayanan et al. 2011; Sivakumar et al. 2013). Percentage and type of dyes in the textile effluents depends on their degree of fixation with the fabric (Carmen and Daniela 2012). Treatment performances of textile dye effluents by electrochemical oxidation (Randazzo et al. 2011), ozonation (Wijannarong et al. 2013), biosorption (Guendouz et al. 2016), bacterial degradation (Kolekar et al. 2013), and fungal degradation (Taha et al. 2014) have been reported.

Kagalkar et al. (2010), Khandare et al. (2011), and Kabra et al. (2012) have reported the application of Aracheae, Portulacaceae, and *Glandularia pulchella* for phytoremediation, respectively. The microbial degradation and biosorption of dyes are often economical and eco-friendly as compared to physical and chemical processes as they (biodegradation) may lead to complete mineralization of dyes (Pandey et al. 2007) (Table 11.7).

Table 11.7 Biological treatment methods used for wastewater treatment with their advantages and limitations (Table adopted from Zaharia et al. 2012)

Treatment methodologies	Treatment stages	Advantages of biological treatments	Limitations
Aerobic process	Posttreatment	Partial or complete Decolorization for all classes of dyes	Expensive treatment
Anaerobic	Process main treatment	Resistant to wide variety of complex colored compounds Biogas produced is used for stream generation	Longer adaptation phase
Single cell (fungal, algal, and bacterial)	Posttreatment	Good removal efficiency for low volumes and concentrations	Very effective for specific color removal Culture maintenance is cost intensive and difficult Cannot cope up with large volumes of wastewater
Enzymatic treatment	Posttreatment	Effective for specifically selected compounds unaffected by shock loadings and shorter contact times required	Enzyme isolation and purification is tiresome Partial efficiency due to the presence of interferences
Redox mediators	Pre/ supportive treatment	Easily available and enhances the process by increasing electron transfer efficiency	Concentration of redox mediator may give antagonistic effect Also depends on biological activity of the system
Engineered wetland systems	Pre/post treatment	Cost-effective technology and can be operated with huge volumes of wastewater	Requires high capital for initial installation and expertise Management during monsoon becomes difficult

11.4 Role of White Rot Fungi for Bioremediation of Synthetic Textile Dyes

White rot fungi play a crucial role in the global carbon cycle. On the basis of type of rot cause by wood-degrading fungi, they are divided into three groups: white-rot, brown-rot, and soft-rot fungi. White rot fungi (WRF) can degrade lignin efficiently under natural conditions; they mostly colonize dead or living wood (Eriksson et al. 1990). Lignin degradation by these fungi is thought to occur during secondary metabolism and typically under nitrogen starvation. A wide variety of lignin

degradation efficiency and selectivity abilities, enzyme patterns, and substrates enhancing lignin degradation are reported from these fungi (Hofrichter 2002). WRF have sustained our interest because several studies have reported their ability to decolorize and detoxify wide range of synthetic dyes (Fu and Viraraghavan 2001).

Advantages of white rot fungi (WRF) for bioremediation of dyes:

1. They use cheap and easily available agriculture waste as a source of nutrient.
2. Due to their extracellular degradation system, they can tolerate relatively high concentration of pollutants.
3. As compared to other microorganisms they are able to survive in the presence of several toxic xenobiotics.
4. They have a nonspecific, free-radical-based degradation mechanism that is able to degrade a mixture of toxic xenobiotic compounds.
5. They do not require preconditioning for particular toxic chemicals.
6. They can withstand a wide range of adverse environmental conditions.
7. Solubility of the pollutant is not important for the degradation or biotransformation of a pollutant and degradation is proportional to its concentration.

Based on their ligninolytic enzyme production pattern (i.e., Lignin Peroxidasep (LiP), Manganese ProxidaseP (MnP), lacasse, Versatile Peroxidase (VP), or their combination), wood-rotting fungi can be divided into three groups (Hatakka 2001):

1. LiP-, MnP-, and laccase-producing.
2. MnP- and laccase-producing.
3. LiP- and laccase-producing fungi.

The most common group among the white rot fungi is the MnP- and laccase-producing group (Hatakka 1994). The search for ligninolytic enzymes with different properties and potential applications is still ongoing. It is expected that new isolated fungi and their enzymes may catalyze the chemical reactions more effectively helping in the successful remediation processes. An enzyme-based method uses very less energy and also poses minimal adverse effect on the ecosystems. Among extracellular enzymes, laccases are oxidoreductive ligninolytic enzymes having broad substrate specificity (Baldrian 2006). Laccases are also known as "green enzymes" because they use oxygen present in air as an electron acceptor. They have broad substrate specificity. Laccases- and laccase- mediated system can degrade and detoxify a wide range of phenolic and non-phenolic recalcitrant environmental pollutants. They have been used by many industries including paper, pulp, textile, petrochemical, food processing, medical and health care, and in designing of biosensors and nanotechnology (Upadhyay et al. 2016). Large-scale operation requires higher amount of crude and purified laccases at low cost. White rot fungi generally produce higher amount of laccases (Shekher et al. 2011), but the physiological requirements for laccase production vary with different white rot fungi (Wesenberg et al. 2003). Biodecolorization of wastewater involves two methods:

(1) biosorption and (2) biodegradation (Fu and Viraraghavan 2001; Padmesh et al. 2005; Prigione et al. 2008).

11.4.1 Removal of Dyes by Biosorption Using White Rot Fungi

Biosorption is a method of accumulating and concentrating the pollutants from wastewater using biological systems (live or dead microorganisms or biomaterials) for their recovery and disposal. Because of easy availability of biomass and the flexibility of using microbes like bacteria, yeast, fungi, and algae, biosorption has been widely employed for adsorption of a wide range of dyes from textile effluents (Fu and Viraraghavan 2001; Padmesh et al. 2005; Prigione et al. 2008). Textile dyes have different chemistry and thus their interaction with microorganisms or biomaterials for biosorption depends on the chemistry of both (Robinson et al. 2001; Erdem et al. 2005). The process is affected by biofunctional groups in fungal biomass, specific surface properties, initial pH of the dye solution, and presence of factors like salts and ions which compete with dyes for binding. The advantages of using biomass for wastewater treatment are that it is cheap and readily available in bulk, and requires simple and cheap growth media (Fu and Viraraghavan 2002). Besides this, it effectively reduces the concentration of dye to very low levels. It is far more superior to the conventional absorption processes. The problem of toxic waste and nutrient requirement with the use of living biomass is eliminated by using dead ones.

The major disadvantage is that the process is slow, and some bioreactors have been reported to be clogged. Bioadsorption of dyes does not destroy them and thus remain on the microbial biomass in its intact form. An alternative method for removing dyes is adsorption on various lignocelluloses but it may cause pollution. Nigam et al. (2000) showed that the dye-adsorbed lignocelluloses (wheat straw, corncobs, and wood chips) are suitable substrates for solid-state fermentation by *T. versicolor* and *P. chrysosporium,* and the resulting fermented substrate could be used as fertilizer or soil conditioner which is an excellent and easy way for removing and bioremediation of dyes from wastewaters. Barley husks were used to adsorb and decolorize (53.0%) mixed dyes (cibacron yellow C-2R, cibacron red C-2G, cibacron blue C-R, remazol black B, and remazol red RB) under solid-state fermentation condition by *B. adusta*.

11.4.2 Removal of Dyes by Biodegradation Using White Rot Fungi

Biodegradation implies destruction of the parental pollutant into fragments by microbes or by their enzymes. Many a time, complete mineralization is achieved by conversion of xenobiotics into CO_2, H_2O, and inorganic salts. Few organic molecules are recalcitrant and are not degraded because of their synthetic origin and complex chemical structure. White rot fungi have been exploited since many years as a promising biotechnology for the dye decolorization from textile effluents.

This emerging technology attempts to overcome the selective disadvantage of other physicochemical methods of dye degradation, that is, >70.0% of organic material can be converted to biosolids (Forgacs et al. 2004). Moreover, these processes are far more economic. An efficient dye degradation biotechnology development requires selection of a suitable strain and optimization of favorable conditions to achieve maximum degradation of dyes (Novotny et al. 2004; Lucas et al. 2008). Strains of white rot fungi vary in their physiological requirements which provide them the potentials to be utilized for decolorization of different dyes. The efficacy of bioremediation of dyes can be increased by isolating new strains or by the adaptation of existing ones. Both liquid and solid medium can be used to study decolorization of dyes present in the media by white rot fungi (Table 11.8) as well as their extracellular enzymes.

11.4.2.1 Study of Dye Decolorization on Solid Agar Medium

The agar plate screening method can be used to study the decolorization of dyes present in solid media. The decolorization of dye on solid agar medium could be observed by visual removal of color during active growth of fungi. Eichlerová et al. (2006) selected Dichomitus squalens to decolorize eight structurally different dyes [(Orange G (azo dye), Amaranth (monoazo dye), Orange I (monoazo dye), Remazol Brilliant Blue R (reactive anthraquinone dye), Cu-phthalocyanine dye, Poly R-478 (polyaromatic anthraquinone dye), Malachite Green and Crystal Violet (Tri aryl methane)]. He reported that there was a reduction in the mycelial growth of Dichomitus squalens in presence of malachite green and crystal violet even at a low concentration (0.05 g L^{-1}), while at 0.1 g L^{-1} concentration of malachite green, the growth was entirely inhibited suggesting negative impact of these dyes.

11.4.2.2 Study of Dye Decolorization Using Active Growth of Fungi in Liquid Medium

Decolorization of textile dyes by growing cells of white rot fungi in liquid media has been reported. The most effective carbon and nitrogen sources for fungal (*Ganoderma* sp.WR-1) and laccase-mediated decolorization of Amaranth and textile dyes were starch and yeast extract, respectively. Agitation gave better results. It also decolorized other dyes [Reactive Orange 16 (monoazo), Orange II, Acid Red 106 (monoazo), Cibacron Brilliant Red 3B-A (reactive monoazo)] partially after 8 h. RBBR and industrial effluents having reactive dyes were effectively decolorized but individual dyes were decolorized faster. Sometimes as reported by Couto et al. (2002), addition of activators like Tween 80, veratrylalcohol, manganese(IV) oxide to the medium for enzyme (ligninolytic) production by microorganisms (*Phanerochaete chrysosporium*) increases the degradation rate of Poly R-478 dye.

Similarly, Kiran et al. (2012) reported that highest decolorization ratios for *P. ostreatus* IBL-02 and *P. chrysosporium* IBL-03 using reactive dye 222 (diazo dye) in an agitated liquid batch cultures under the optimum fermentation conditions were 92.0% and 86.0%, respectively. Rice bran (2.0 g%), ammonium oxalate (0.1 g %), $MnSO_4$ (1.0 mM), and $CuSO_4$ (1.0 mM) were the most effective carbon source, nitrogen source, mediator, and metal ion responsible for dye decolorization and

Table 11.8 Methods used for dye decolorization by white rot fungi

Incubation mode	Microorganism	Dye	Reference
Dye decolorization by submerged growth of white rot fungi			
Static batch	*F. trogii* ATCC 200800 *T. versicolor* ATCC 200801 *P. chrysosporium*	Crystal Violet	Yesilada et al. (1995)
Static batch, shaking batch	*Dichomitus squalens*	Orange G, RBBR	Eichlerová et al. (2007)
Shaking batch	*T. versicolor* strain 1	Reactive blue 4	Yemendzhiev et al. (2009)
Shaking batch	*P. ostreatus* MTCC 142	Crystal violet	Kunjadia et al. (2012)
Shaking batch	*Coprinus plicatilis*	Turquoise blue HFG	Akdogan and Topuz (2014)
Shaking batch	*Coriolopsis* sp. (1c3)	Crystal violet, methyl Violet, cotton blue Malachite green	Chen and Ting (2015)
Shaking batch	*Curvularia* sp.	Congo red	Senthilkumar et al. (2015)
Shaking batch	*P. ostreatus,* *P. sapidus,* *P. forida*	Coralene Golden Yellow, Coralene navy blue, Coralene dark red	Kunjadia et al. (2016)
Shaking batch	*Ganoderma* sp. En3	RBBR, indigo carmine, Methyl green	Lu et al. (2016)
Dye decolorization in liquid media by immobilized white rot fungi			
Attached on pine wood chips, attached on palm oil fiber	*T. versicolor,* *P. chrysosporium*	Levafx blue, Remazol Brilliant red	Boehmer et al. (2006)
Entrapped in alginate beads	*C. gallica,* *B. adusta,* *T. versicolor,* *T. Trogii*	Lanaset Grey G	Daâssi et al. (2013)
Entrapped in alginate beads	*T. versicolor* U97	Reactive green 19	Sari et al. (2016)
Dye decolorization in liquid media by white rot fungal pellets (whole cells)			
Shaking batch, repeated batch	*T. trogii* ATCC 200800	Reactive blue 19, reactive blue 49, acid violet 43, reactive black 5, reactive Orange 16, acid black 52	Park et al. (2007a, b)
Shaking batch	*P. sanguineus*	Crystal violet	Sulaiman et al. (2013)
Repeated batch	Ganoderma weberianum TZC1	Indigo dye, indigo dye-containing textile wastewater	Tian et al. (2013)
Shaking batch	*Ganoderma* sp. En3	Reactive Orange 16, indigo Jean dyeing real wastewater	Ma et al. (2014)

enzyme activities. At 1.0 mM $CuSO_4$, these organisms showed highest decolorization which was 96.0%. When *Pleurotus ostreatus* MTCC 142 strain was analyzed for extracellular enzyme production and decolorization potential using increased concentration of crystal violet under submerged conditions, there was an inhibitory effect of higher concentration of crystal violet. At 20 mg L^{-1} crystal violet concentration, 92.0% decolorization was attained but it was reduced to 32.0% after 10 days at 50 mg L^{-1} of the dye and again less than 10% decolorization was obtained after 10 days at concentration of 100 mg L^{-1} and above. Moreover, laccase and manganese peroxidase production was induced in presence of the dye, whereas the production of lignin peroxidase was not triggered by the dye indicating laccase and manganese peroxidase dependent decolorization of crystal violet (Kunjadia et al. 2012).

Decolorization activity of the azo dye congo red was analyzed using 42 white rot fungi. Out of which, *T. pubescens* Cui 7571 exhibited the highest performance with direct red 28 (diazo dye) (Si et al. 2013), *Pleurotus eryngii* F032 showed the highest decolorization activity (94.0%) with reactive black 5 (diazo dye). The decolorization activity decreased with an increase in the initial dye concentration due to the toxicity of the dye on fungal growth. High color removal was attributed to the solubility of the dye in Tween 80 (Hadibarata et al. 2013). Saratale et al. (2009) demonstrated the degradation of the dye "Navy blue HER" by fungus *Trichosporon beigelii* NCIM-3326 using HPLC analysis. Enayatizamir et al. (2011) reported that *P. chrysosporium* degrade 92.0% azo black reactive five dyes after 3 days of treatment. *P. chrysosporium* URM6181 and *Curvularia lunata* URM6179 strains decolorized 95.0% of indigo dye from textile effluent within 10 days of treatment (Miranda et al. 2013).

Uses of immobilized growing cells or immobilized enzymes are more promising and an economical alternative as compared to free cells, since they are not lost, thereby allowing their repeated use. The factors that affect biodegradation of dyes are variable abiotic conditions, for example, pH, temperature, DO, metals, nitrate concentration, and salts, as the changes in these parameters affect the microorganisms and their decomposition activities. Textile wastewater contains different classes of dyes and thus varies in their composition and pH. Thus, proper choice of organisms or their enzymes capable of working under the available conditions is very important, for example, fungi and their enzymes work better in acidic or neutral media (Abdulla et al. 2000; Kandelbauer et al. 2004; Almansa et al. 2004; Zille et al. 2005), while bacteria and their enzymes prefer alkaline conditions (Pereria et al. 2009a, b).

Senthilkumar et al. (2015) studied synthetic dye bath effluent containing amido black 10 B (diazo dye) decolorization using *P. chrysosporium* under the varying concentrations of azo dye, glucose, and manganese sulfate. Fungal growth was adversely affected, and decrease in decolorization was noticed by an increase in dye concentration of the effluent. Glucose favored the process of decolorization, because when glucose concentration was increased, the fungal growth also increased. Manganese sulfate induced the production of manganese peroxidase. Concentration above 0.5 g% led to a decrease in the rate of decolorization. Laccase

production along with lignin peroxidase and manganese peroxidase was induced by addition of 0.5 g starch or lignin to dye bath effluents which enhanced the rate of color removal.

Şaşmaz et al. (2011) used crude laccase obtained from the submerged culture of *Trametes versicolor* ATCC 200801 for the decolorization of reactive red 198, rem blue RR, dylon navy 17, rem red RR, and rem yellow RR dyes and reported that the types of mediator and dye structure have significant role in decolorization. *Trametes hirsuta* Bm2 produced three laccase isoenzymes (Lac I, II, and III) in liquid media containing 2.0 g% wheat bran (Zapata-Castillo et al. 2015). The isoenzymes decolorized indigo carmine (100%) in the presence of syringaldehyde (0.2 mM). As compared to the purified one, crude laccase decolorized the dye more efficiently and hence the application of crude enzyme was more economic. Shankar and Nill (2015) used crude laccase from *Peniophora* sp. (NFCCI-2131) produced in liquid static culture for the decolorization of amido black, crystal violet, brilliant green, methyl orange along with methylene blue and reported that in presence of 1.0 mM ABTS or 0.1 mM HBT, it efficiently decolorized crystal violet (96.0%).

11.4.2.3 Study of Dye Decolorization Using Immobilized Fungal Biomass

Generally, fungal growth has been found to be inhibited by textile dyes at high concentration. At 64.0 μM concentration of malachite green on solid media, *P. chrysosporium* was inhibited, whereas *F. sclerodermeus* was more resistant (Papinutti and Forchiassin 2004). Both fungi were inhibited in liquid media. Hence, an alternative approach to overcome this problem of toxicity or growth inhibition is the use of whole pellets or immobilized cells (attachment on support material such as polymers, activated carbon, or lignocellulosic materials and entrapment in a matrix, such as agar, gel, or other synthetic polymers) (Papinutti and Martínez 2006). Dye decolorization in liquid media by immobilized white rot fungi is presented in Table 11.8.

As compared to free pellets, the pellets of T. versicolor immobilized on activated carbon showed higher stability and decolorization efficiency of acid violet 7 (monoazo dye). Decolorization efficiency of the immobilized pellets in a fluidized bed reactor with a repeated batch feeding was much higher and stable than the continuous flow feeding. Boehmer et al. (2006) studied decolorization activities of reactive dyes (levafix blue and remazol brilliant red) by immobilized (pine wood chips or palm oil fiber) cultures of *T. versicolor* and *P. chrysosporium*. The color of levafix blue was removed more than 80.0% by both fungi after 24 h of incubation. The maximum decolorization of remazol brilliant red by *T. versicolor* was about 50.0% within 4 days. *T. pubescens* and *P. ostreatus* were successfully immobilized on polyurethane foam cubes which detoxified reactive dyes [B49 dye (industrial anthraquinone), R423 (industrial azo) and RBBR] in a bioreactor. About 97.0% of the anthraquinone dyes (RBBR and B49) and 65.0% of the azo dye (R243) were removed by these fungi during five sequential cycles (Casieri et al. 2008).

Birhanli et al. (2013) reported that the crude laccase produced by immobilized culture of *Funalia trogii* ATCC 200800 on copper-impregnated apricot stone-based

activated carbon decolorized reactive blue 171, reactive black 5, and indigo carmine without mediator. They also reported that the temperature for dye decolorization was significantly related to pH of the medium. Metal complex dye lanaset gray G was decolorized (75.0%, 70.0%, 60.0%, and 68.0%) using immobilized (Ca-alginate beads) pellets of *C. gallica*, *B. adusta*, *T. versicolor,* and *T. trogii,* respectively, after three cycles. Daâssi et al. (2013) reported the involvement of laccase as main enzyme in decolorization. Sari et al. (2016) reported decolorization (44.0 and 70.0%) of Reactive Green 19 using *T. versicolor* U97 free cells in flasks and also immobilized cells (alginate beads) in a bioreactor within 72 h.

11.4.2.4 Study of Dye Decolorization Using Metabolically Active Fungal Cell (Pellet)

Immobilization methods are mostly costly. Hence, whole fungal pellets which are in self-immobilized forms can be used as an alternative to immobilized forms (Wang et al. 2005). Pellets are more advantageous than growing cell because of their economical and practical process, reusability, long-term storability, maintenance of high and long-term decolorization abilities, and tolerance to high concentrations of dyes. Moreover, they can be easily separated from the liquid medium and effectively decolorize various dyes (Table 11.8). Knapp et al. (1997) showed the biodegradation and decolorization of orange II dye (up to 98.0%) with repeated use of fungal pellets. Swamy and Ramsay 1999 reported that agitated cultures showed higher decolorization than static cultures which was attributed to physiological state as pellets have increased mass and oxygen transfer.

The pellets of *C. versicolor* could also decolorize the wastewaters from printing and dyeing industry (Lin et al. 2003). Various white rot fungal pellets (*T. versicolor* ATCC 200801, *T. trogii* ATCC 200800, *P. chrysosporium* ME446, *P. florida*, *P. ostreatus*, and *Pleurotus sajor-caju*) were reported to show high astrazon dye decolorization activity under repeated-batch mode (Yesilada et al. 2003). Small differences in dye structures including steric effect and redox potential affect the decolorization rate of *T. trogii* ATCC 200800 cultivated on either solid (yeast-malt-peptone-glucose agar medium) or in a liquid phase (batch and repeated-batch) which decolorized the blue dyes more rapidly than the black dyes. Solid culture took 10 days whereas batch liquid culture took 3 days for complete decolorization.

Decolorization of anthraquinone-based dyes (reactive blue 19, reactive blue 49, and acid violet 43) occurs faster than the azo-based dyes (reactive black 5, reactive orange 16, and acid black 52) in liquid batch cultures (Park et al. 2007a, b). Whole cells of *P. ostreatus* and *P. chrysosporium* showed different specificities in decolorizing the dye wastewaters mainly due to the laccases which reduced the toxicity of the acid dye wastewater both in the presence and absence of nutrients. The differences in activity could be due to the differences in the enzyme activity profiles of fungi, mainly laccases in *P. ostreatus* and manganese peroxidase in *P. chrysosporium* (Faraco et al. 2009). The pre-grown pellets showed higher decolorization (85.0%) efficiency on mixed dye, characteristic of industrial effluents than growing cells (32.0%) within 24 h in batch culture. Hence, the pellet has more efficacies in decolorization of mixed dyes (Yesilada et al. 2010). An actively

growing 5-day-old submerged agitated cultures *Ganoderma* sp. En3 not only showed high adaptation and tolerance toward the sulfonated azo dye reactive orange 16 but also decolorized and detoxified it even at a high concentration efficiently (Ma et al. 2014).

11.4.2.5 Decolorization Dyes by Semisolid-State and Solid-State Fermentation

Dye decolorization activity of white rot fungi was also investigated under solid-state fermentation conditions wherein a moistened solid is the substrate which is also an attachment place and a source of nutrient for fungi (Couto et al. 2002; Boran and Yesilada 2011). Murugesan et al. (2007) reported that the maximum laccase and MnP activities were obtained from 7-day while studying RBBR and remazol black 5 decolorization activity by white rot fungus *Ganoderma lucidum* KMK2 under solid-state fermentation using wheat bran, laccase being the major ligninolytic enzyme responsible for color removal of both the dyes. This fact was also supported by the results of Polyacrylamide gel electrophoresis (PAGE) (Murugesan et al. 2007). Reports on RBBR decolorization activity of *T. pubescens* grown on sunflower seed shells under solid-state fermentation conditions in temporary immersion bioreactors during five successive batches are available. Rodriguez-Couto (2011, 2012) studied decolorization of textile wastewater containing RBBR by *T. pubescens* grown on various supports under semisolid-state fermentation conditions. Dye-adsorbed sunflower seed shells could be used for production of high titers of laccase by *T. pubescens* under semisolid-state conditions (Rodriguez-Couto et al. 2009). This system was efficiently scaled up to static tray reactor. Ozmen and Yesilada (2012) demonstrated the laccase production and astrazon dye decolorization activity of *T. versicolor* ATCC 200801 and *T. trogii* ATCC 200800. *T. trogii* showed 80.0% and 69.0% decolorization activity, respectively, against astrazon black and astrazon blue dye adsorbed onto wheat bran, whereas *T. versicolor* showed 86.0% and 84.0% astrazon black and astrazon blue dye decolorization, respectively, under same conditions.

Polycyclic aromatic hydrocarbons (PAHs) comprise of various prime organic pollutants which are toxic, mutagenic, and/or carcinogenic in nature. Besides this, they are most ubiquitous, refractory, and unmanageable. Owing to these properties, PAHs have created an alarming situation as far as public health and environmental health is concerned (Ghosal et al. 2016). During past few years, fungi have been studied comprehensively for the biodegradation of PAHs and reports of various fungal species degrading different PAHs are now available (Cerniglia 1992; Cerniglia and Sutherland 2010). Lee et al. (2014) reported that *Peniophora incarnate* KUC8836 and *Phlebia brevispora* KUC9033 degrade phenanthrene as well as pyrene at 25 mg L^{-1} concentration. Lee et al. (2013) reported that *Merulius tremellosus* KUC9161 showed a higher rate (83.6%) of pyrene degradation than *P. chrysosporium* (68.5%) which is a well-known PAHs degrader.

Generally, these organic dye molecules are too large to penetrate into the microbial cells to be degraded. Thus, it becomes necessary that the degradative enzymes be extracted or they produce extracellular enzymes for dye decolorization.

11.4.3 Bioremediation of Dyes by Ligninolytic Enzymes

White rot fungi secrete several extracellular enzymes which have broad substrate specificity to facilitate the remarkable biotransformation or biodegradation of a wide variety of synthetic dyes (Wesenberg et al. 2003). Ligninolytic enzymes have been studied from a large number of fungi for bioremediation of xenobiotics. These enzymes offer great variability in terms of induction mechanisms, degree of polymorphism, expression of different isoenzymes, and physicochemical and catalytic properties. This variability determines the potential application of the isolated fungi and their enzymes to oxidize and thereby degrade variety of selected chemical substrates.

11.4.3.1 Production of Ligninolytic Enzymes

Ligninolytic fungi have usually been grown in defined liquid media low in N (Keyser et al. 1978) or in C (Haapala and Linko 1993) and containing supplements such as Mn, veratryl alcohol, and Tween 80 (Hatakka 2001). Lignin model compounds such as dimeric β-O-4 model compounds and synthetic lignin (DHP) have been used in liquid cultures to study lignin mineralization or enzyme activities. The ligninolytic enzyme profile produced by the lignin degrading fungi in defined culture media is different from that produced in media where lignocellulosic substrates such as wood, sawdust, straw, or other natural substrates are used (Orth et al. 1993). The production of MnP and LiP isoenzymes by *P. chrysosporium* is strongly affected by medium composition (Cullen 1997).

A lignocellulosic substrate (wheat straw and hemp woody core) promotes the production of MnP and LiP of *P. chrysosporium* under culture conditions in which N and C is non-limiting (Kapich et al. 2004). *Pleurotus ostreatus* produces different MnP isoenzymes when grown on sawdust and in defined culture media (Kamitsuji et al. 2004) and *Pleurotus eryngii* VPs was first reported from straw-based cultures (Camarero et al. 1999). The ecological role for the large variety of MnP, LiP, or laccase isoenzymes in ligninolytic fungi is not yet clear (Table 11.9). It is assumed that the varying enzyme profile/production results from adaptation to different natural culture conditions and substrates (Conesa et al. 2002).

Laccases

Laccase is one of the enzymes that has been studied since the nineteenth century. In 1883, Yoshida was the first to extract it from the exudates of *Rhus vernicifera* (Japanese lacquer tree), from which the designation laccase was derived. In 1896, Bertrand and Laborde for the first time demonstrated the presence of laccase in fungi (Desai and Nityanand 2011). Fungal laccases are more attractive due to their ability to catalyze wide variety of organic compounds which include polymeric lignin and humic substances (Baldrian 2006). Generally, ligninolytic fungi produce at least one laccase isoenzyme, and laccases are the principal ligninolytic enzymes present in the soil. In addition, laccase-mediated delignification of agroindustrial products increases the nutritional value of animal feed and soil fertilizer (Gonzalez et al. 2013). Laccases require only molecular oxygen for catalysis so they are suitable for

Table 11.9 Ligninolytic enzymes and their mode of action

Enzyme	Cofactor	Catalyzed reactions	Fungi	References
Laccase	O_2	Phenol oxidation	*Trametes versicolor*	Yaropolov et al. (1994)
Lignin peroxidise	H_2O_2	Phenol polymerization	*Phanerochaete chrysosporium*	Gold and Alic (1993), Haglund (1999), Piontek et al. (2001), Erden et al. (2009)
Manganese peroxidise	H_2O_2	Phenol oxidation; Oxidize Mn^{2+} to Mn^{3+}	*Phanerochaete chrysosporium*	Hofrichter (2002)
Cellobiose-quinone Oxireductase		Quinone reduction; Celobiose degradation	*Phanerochaete chrysosporium*	Soares (1998)
Aryl alcohol oxidase		H_2O_2 production	*Pleurotus sabor-caju*	Martínez et al. (2009)
Glyoxal Oxidase		H_2O_2 production	*Phanerochaete chrysosporium*	Martínez et al. (2009)
Manganese-independent peroxidise		Activity on aromatic substrates	*Phanerochaete chrysosporium*	Wyatt and Broda (1995), Ruiz and Martínez (2009)
Versatile peroxidase	H_2O_2	Oxidizes Mn^{2+}; high redox-potential aromatic compounds	*Pleurotus* sp.	Ruiz and Martínez (2009)
Cellobiose dehydrogenase		Lignin degradation degradation; unites the hydrolytic and oxidative systems; dispose manganese (Mn^{2+}) for MnP through precipitate, reduction from manganese oxide (MnO_2)	*Phanerochaete chrysosporium*	Henriksson et al. (2000), Kersten and Cullen (2007), Carvalho et al. (2009)

biotechnological applications like transformation or immobilization of the xenobiotic compounds in environment (Couto and Herrera 2006). Laccases have been evaluated for a large number of biotechnological applications like dye degradation, bioremediation of some toxic industrial wastes (Chlorinated aromatic compounds, polycyclic aromatic hydrocarbons, nitroaromatics, and pesticides), and development of biosensors (Gonzalez et al. 2013, Couto and Herrera 2006; Sanchez et al. 2010).

Laccases (benzenediol: oxygen oxidoreductases) are copper containing polyphenol oxidases (blue oxidases) which act on diphenols, polyphenols, substituted phenols, diamines, aromatic amines, benzene thiols, inorganic compounds like iodine and allied substances having oxygen as electron acceptor (Fig. 11.4). In active holoenzyme form, they are monomeric, dimeric, or tetrameric glycoprotein having four copper atoms per monomer. These four copper atoms in their protein structure

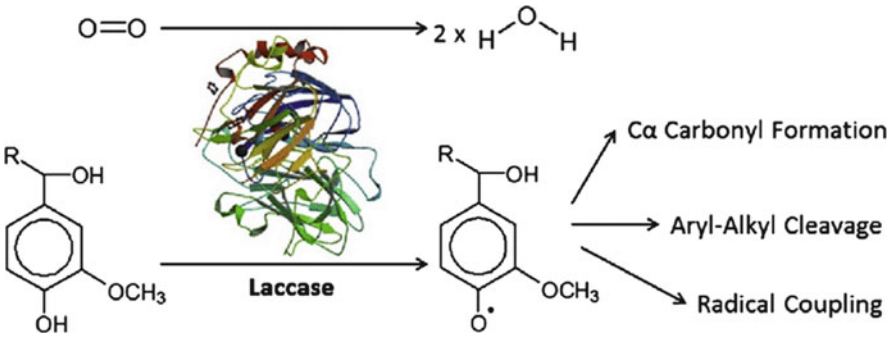


Fig. 11.4 Laccase-mediated oxidation of phenolic subunits of lignin [Modified from (Kunamneni et al. 2007)] [The structure of laccase used was obtained from *Coprinopsis cinerea* (1A65)]

have been categorized in three groups on the basis of data obtained using UV-visible and electron paramagnetic resonance (EPR) spectroscopy (Palmieri et al. 2000).

Laccases catalyze four $1e^-$ oxidation of a reducing substrate with concomitant two e^- reduction of dioxygen to water. The stochiometry includes four molecules of reducing substrate for each molecule of oxygen, involving a total transfer of four electrons [$4RH + O_2 \rightarrow 4R + 2H_2O$]. The first step of catalysis is reduction of the reducing substrate by copper (Cu^{2+} to Cu^+) at the T1 site, which is the primary electron acceptor. Laccase can only act on the phenolic subunits of lignin which leads to Cα oxidation, Cα-Cβ cleavage, and aryl-alkyl cleavage. Laccases can catalyze one-electron oxidation of a wide range of aromatic compounds (Thurston 1994) like polyphenols (Archibald et al. 1997), methoxy-substituted monophenols, and aromatic amines (Bourbonnais et al. 1995) to the corresponding quinones. Phenol-oxidizing enzymes (laccases) preferably polymerize lignin by coupling the phenoxy radicals which are produced during oxidation of lignin phenolic groups (Campos et al. 2001). Majority of the laccases have copper molecules in their active centre and they are classified as blue copper oxidases but some laccases do not show these typical characteristics.

Laccases isolated from solid-state fungal cultures were yellow brown and did not show typical blue laccase spectrum. The remarkable property of yellow laccase is that they oxidize non-phenolic lignin models and veratryl alcohol in the presence of oxygen (Rogalski and Leonowicz 2004). One of the laccases from *P. ostreatus* does not show blue color and has been described as white colored by the author (Palmieri et al. 1997). The result of atomic adsorption of this laccase showed that it has one copper atom, two iron atoms, and one zinc atom as compared to four coppers in typical laccase.

Laccase Mediator System (LMS)

The conditions which limit the laccase catalyzed oxidation of substrates are steric hindrance (for macromolecular compounds), very low affinity between the compound and the active site of enzyme, as well as high redox potential of the putative

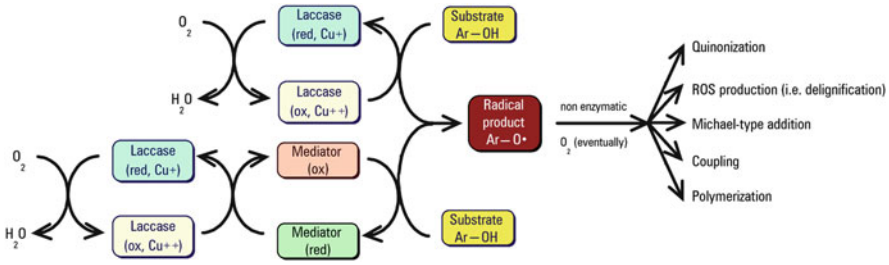


Fig. 11.5 Mechanism of laccase action in the presence and absence of redox mediators (Figure obtained from Zucca et al. 2015)

substrates. Due to lower redox potential, laccases can oxidize only phenolic compounds. This obstacle can be overcome by using a laccase mediator system (LMS) (Strebotnik and Hammel 2000). Laccase mediators are usually low molecular weight substrate, water-soluble molecules, having high redox potential (>900 mV), capable of behaving as one-electron shuttles between the enzyme and the to-be oxidized compounds (Kawai et al. 1989; Bourbonnais and Paice 1990) (Fig. 11.5). Laccase mediator compounds are generally laccase substrates. Laccase enzyme removes one electron from the substrate and produce free radicals which live long enough to diffuse away from the enzyme active site and capable of oxidizing other substrate present in the environment, thus restoring their stable electronic configurations at the expense of other substrates which are not directly oxidized by laccase (Call and Mücke 1997).

Lignin Peroxidases

The peroxidases are ferric-iron containing heme oxidases requiring peroxides for function. These enzymes have common ability to catalyze one electron oxidations, resulting in formation of free radical species inside the lignin polymer. Afterwards, the radical undergoes spontaneous reaction leading to the incorporation of oxygen, bond cleavages, and finally to the breakdown of the lignin molecule. Lignin peroxidases (LiPs) are capable of mineralizing a variety of recalcitrant aromatic compounds (Shrivastava et al. 2005). LiP has a typical peroxidase catalytic cycle similar to horseradish peroxidase in many aspects (Wong 2009). The general mechanism of LiP catalyzed reaction consists of two steps:

1. A $2e^-$ oxidation of the native ferric enzyme [Fe(III)] to yield compound I intermediate that exists as a ferric iron porphyrin radical cation [Fe(IV) = O^+, LiP-I], with the peroxide substrate (H_2O_2) cleaved at the O–O bond (Fig. 11.6).
2. A two consecutive $1e^-$ reduction of ferric ion porphyrin radical cation [Fe (IV) = O^+, LiP-I] by electron donor substrates like veratryl alcohol (VA) returns enzyme in its native state.

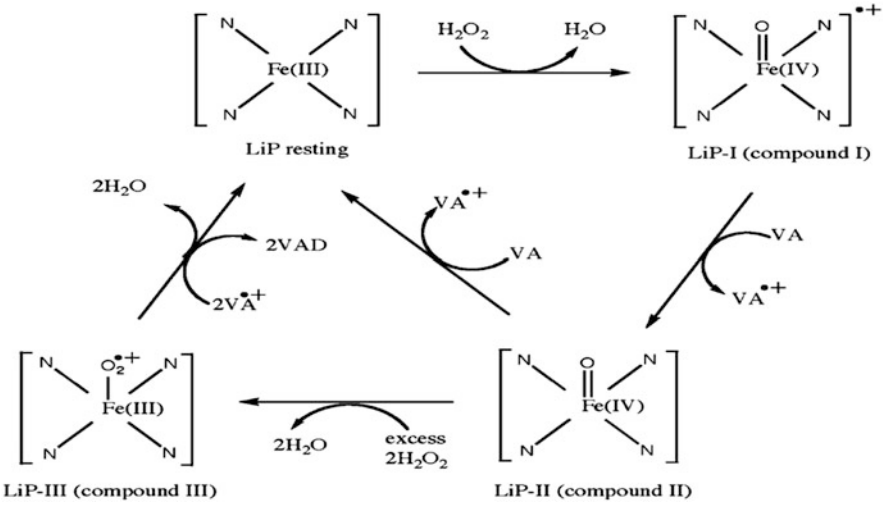


Fig. 11.6 Mechanism of action of lignin peroxidise (Figure adopted from Wong 2009)

The enzyme convert ferric ion porphyrin to the oxidation state using VA, yielding compound II [Fe(IV) = O, LiP-II] and a VA radical cation (VA^+). In some cases, LiP-I can also return to the native (resting) enzyme by a direct $2e^-$ reduction.

$$\text{LiP}[\text{Fe(III)}] + H_2O_2 \rightarrow \text{LiP-I}[\text{Fe(IV)} = O^+] + H_2O$$

The natural fungal secondary metabolites veratryl alcohol (VA) and 2, chloro 1, 4, dimethoxybenzene act as redox mediators to stimulate the LiP catalyzed oxidation of a wide range of recalcitrant substrates (Christian et al. 2005). Oxidation of VA by YK-LiP2 from *P. chrysosporium* and other LiPs reveal ordered bi–bi ping–pong mechanisms (Hirai et al. 2005).

Manganese Peroxidases

Manganese peroxidases (MnPs) are extracellular glycoproteins with an iron protoporphyrin IX (heme) prosthetic group. MnPs are secreted in multiple isoforms in carbon and nitrogen limited media supplemented with Mn^{2+} and VA. Mn-dependent peroxidases are unique in utilizing Mn^{2+} as the reducing substrate. MnP oxidizes Mn^{2+} to Mn^{3+}, which in turn oxidizes a variety of monomeric phenols including dyes as well as phenolic lignin model compounds. The catalytic cycle thus entails the oxidation of Mn^{2+} by compound I (MnP^{1+}) and compound II (MnP^{2+}) to yield Mn^{3+}. The characteristics of the cycle are very similar to that of LiP. Addition of one $1e^-$ equivalent of H_2O_2 to the native enzyme yields MnP-I, which is a Fe(IV)-oxo-porphyrin radical cation [Fe(IV) = O^+]. The peroxide bond of H_2O_2 is cleaved subsequent to a $2e^-$ transfer from the enzyme heme-porphyrin.

$$\mathrm{MnP} + \mathrm{H_2O_2} \rightarrow \mathrm{MnP\text{-}I} + \mathrm{H_2O_2} \quad (11.1)$$

$$\mathrm{MnP\text{-}I} + \mathrm{Mn^{2+}} \rightarrow \mathrm{MnP\text{-}II} + \mathrm{Mn^{3+}} \quad (11.2)$$

$$\mathrm{MnP\text{-}II} + \mathrm{Mn^{2+}} \rightarrow \mathrm{MnP} + \mathrm{Mn^{3+}} + \mathrm{H_2O} \quad (11.3)$$

Mn(III) in turn mediates the oxidation of organic substrates.

$$\mathrm{Mn^{3+}} + \mathrm{RH} \rightarrow \mathrm{Mn^{2+}} + \mathrm{R} + \mathrm{H^{+}} \quad (11.4)$$

The conversion of MnP-I to MnP-II can also be achieved by the addition of other electron donors, such as ferrocyanide and a variety of phenolic compounds (Wariishi et al. 1988). In the reduction of compound II to generate the native enzyme, however, Mn(II) is an obligatory redox coupler for the enzyme to complete its catalytic cycle. Mn^{2+} performs the role of mediator for MnP. High resolution crystal structure has revealed that MnP catalyzes the peroxide-dependent oxidation of Mn^{2+} to Mn^{3+} and Mn^{3+} is released from the enzyme in complex with oxalate or with other chelator (Sundramoorthy et al. 2005).

Versatile Peroxidases

Versatile peroxidases (VPs) are glycoproteins with hybrid properties capable of oxidizing typical substrates of other Basidiomycete peroxidases including Mn (II) and also veratryl alcohol (VA). VPs form an attractive ligninolytic enzyme group due to their dual oxidative ability to oxidize Mn(II) and also phenolic and non-phenolic aromatic compounds (Wesenberg et al. 2003). It has been found that VPs can also efficiently oxidize high redox-potential compounds such as dye reactive black 5 (RB5) as well as a wide variety of phenols, including hydroquinones (Gomez-Toribio et al. 2001). It has been suggested that VPs can oxidize substrates spanning a wide range of potentials, including low and high redox potentials. Similar to the MnP mechanism, Mn(III) is released from VPs and acts as a diffusible oxidizer of phenolic lignin and free phenol substrates. Among Basidiomycete peroxidases, VPs have attracted the greatest biotechnological attention due to their catalytic versatility, which includes the degradation of compounds that other peroxidases are not able to oxidize directly.

Other Lignin Degrading Enzymes and Accessory Enzymes

In addition to ligninases, other fungal extracellular enzymes which act as accessory enzymes have been found to be involved in lignin degradation. These include oxidases generating H_2O_2, which provide the hydrogen peroxide required by peroxidases and mycelium-associated dehydrogenases, which reduce lignin-derived compounds (Martinez et al. 2005). Oxidases generating H_2O_2 include aryl-alcohol oxidase (AAO) (EC1.1.3.7) found in various fungi, such as *P. eryngii* and glyoxal oxidase found in *P. chrysosporium* (Kersten 1990). In addition, aryl-alcohol dehydrogenases (AAD) (a flavoprotein) and quinone reductases (QR) are also

Table 11.10 Biological applications of ligninolytic enzymes

Enzyme	Applications
Laccase	Spore resistance, Rhizomorph formation, pathogenesis, fruiting body formation, pigment synthesis, lignin degradation
Lignin peroxidase	Biodegradation of lignin, defense of fungi against pathogens
Manganese peroxidase	Degradation of lignin, interspecific fungal interactions

involved in lignin degradation by fungi (Guillen et al. 1992). Moreover, it has been shown that cellobiose dehydrogenase (CDH), which is produced by many fungi under cellulolytic conditions, is also involved in lignin degradation in the presence of H_2O_2 and chelated Fe ions (Henriksson et al. 1995).

Ligninolytic fungi and their enzymes are used for the biological functions as well as for degradation of many compounds as shown in Table 11.10. Majority of earlier studies have been done on lignin-degrading enzymes of organisms *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, and *Trametes versicolor*. There has been a growing interest in studying ligninolytic enzymes of wider array of white rot fungi. These fungi can withstand high pH range and produce accessory enzymes such as H_2O_2 forming glyoxal oxidase, aryl alcohol oxidase, oxalate producing oxalate decarboxylase (ODC), NAD-dependent formate dehydrogenase (FDH), and P_{450} mono-oxygenase. These are versatile and robust organisms having enormous potential for oxidative bioremediation of a variety of toxic chemical pollutants due to their high tolerance to toxic substances in the environment. WRF are capable of mineralizing a wide variety of toxic xenobiotics due to nonspecific nature of their extracellular lignin mineralizing enzymes (LMEs).

11.4.4 Bioremediation by Ligninolytic Enzymes

Oxidative degradation of dyes is mediated by white rot fungi (WRF) through their ligninolytic enzymes. WRF and their enzymes have found profound applications in the bioremediation of dye effluents because of their ability to degrade a diverse range of organic pollutants including azo, heterocyclic, and polymerize dyes (Abdulla et al. 2000). Ligninolytic fungi and their enzymes are involved in the degradation of the complex and recalcitrant polymer lignin. Ligninolytic enzymes are highly versatile in nature. The demand for application of ligninolytic enzyme complexes in industry and biotechnology is increasing due to their use in a variety of processes. Ligninolytic enzymes have potential applications in a large number of fields, including the chemical, fuel, food, agricultural, paper, textile, cosmetic, and many other industrial sectors. Their capacities to remove xenobiotic substances and dyes make them a useful tool for bioremediation purposes. Ligninolytic enzymes are promising and can replace the conventional chemical processes of several industries. Thus, there is a broad field of investigation that is almost entirely open to new findings, and

it is quite reasonable to propose that many new applications will be found in the near future.

Chhabra et al. (2015) immobilized laccase obtained from *Cyathus bulleri* in poly (vinyl alcohol)-boric acid or polyvinyl alcohol-nitrate beads and it was used for the decolorization of simulated effluent containing acid red 27 (acidic monoazo dye) or basic green 4 (malachite green) dyes. The simulated effluent containing 100 μM acid violet 17 was decolorized 90.0% in the presence of 100 μM ABTS by laccase entrapped in PVA-nitrate up to 10 cycles. It also decolorized simulated effluent containing 100 μM basic green 4 (95.0%) in the presence of 100 μM ABTS up to 20 cycles under batch mode. Adak et al. (2016) reported that the laccase produced by *Pseudolagarobasidium acaciicola* LA 1 under solid-state fermentation on parthenium biomass was a thermostable enzyme and it functioned optimally at pH 4.5 and temperature 60 °C. It decolorized RBBR (90.0%) and RB5 (33.0%) without mediator after 4 h and 48 h, respectively.

Sayahi et al. (2016) used purified laccase from *T. trogii* to decolorize reactive black 5 (RB5), reactive violet 5 (RV5), and also the mixture of RB5 and RV5 and RBBR. The highest decolorization of 25 mg L^{-1} dye RB5 (93.0%) was achieved at 1.0 U ml^{-1} laccase and 1.0 mM HBT concentration while the maximum decolorization of 25 mg L^{-1} dye RV5 (100%) was obtained at 0.5 U mL^{-1} enzyme and 0.5 mM HBT concentration. RBBR also acted as a mediator and increased the decolorization of these two dyes. The purified laccase also decolorized mixed dyes (RBBR, RB5, and RV5) 55.0% without HBT after 24 h. The laccase of *Trametes versicolor* ATCC 200801 was immobilized by Ilk et al. (2016) on the poly (MA-alt-MVE)-g-PLA/ODA-MMT nanocomposite by adsorption or covalent coupling and used for the decolorization of reactive red 3 (monoazo dye). He reported that under the optimum conditions, the dye decolorization potential of the immobilized laccase (65%) was much higher than free laccase (33%) and even after 10 cycles, the activity of immobilized laccase was retained by 77.0%.

The purified laccase enzyme of *Trametes versicolor* IBL-04 immobilized onto chitosan microspheres by Asgher et al. (2017) showed higher catalytic efficiency and higher thermal as well as storage stability. The immobilized enzyme decolorized reactive red 195A (100.0%), reactive violet 1(99.0%), reactive yellow 145A (98.0%), reactive black 5 (97.0%), and reactive blue 21 (89.0%) after 4 h incubation in the presence of 1 mM ABTS as a redox mediator and retained its 80.0% activity after 10 cycles (Asgher et al. 2017). Ajit et al. (2017) applied RSM to optimize RBBR (1000 mg L^{-1}) decolorization using *C. caperata* DN laccase and achieved 542 mg L^{-1} of decolorization within 1.0 h by 1.0 U ml^{-1} of laccase without application of mediator. After laccase treatment, the toxicity of RBBR dye was significantly reduced.

Enzymes produced by white rot fungi are involved in green biodegradation due to its catalytic properties. The xenobiotic compounds are a major source of contamination in soil, and laccase can efficiently degrade them (Couto and Herrera 2006). Moreover, polycyclic aromatic hydrocarbons (PAHs), which arise from natural oil deposits and utilization of fossil fuels, are also degraded by laccases (Anastasi et al. 2009). Many PAHs have been found to exhibit cytotoxic, mutagenic, and

carcinogenic properties that represent serious risk to human health (Bamforth and Singleton 2005). Lignin peroxidases (LiP) present a non-specific biocatalyst mechanism and hence can also act on PAHs. LiP from *P. chrysosporium* was one of the first enzymes from Basidiomycete reported for PAH degradation. MnP has been used for mineralization of many environmental contaminants and are useful for bioremediation process. Due to their ability to degrade azo, heterocyclic, reactive, and polymeric dyes, it degrades trichloro bis (4, chlorophenyl) ethane (DDT), trinitrotoluene (TNT) and polycyclic aromatic hydrocarbons (PAHs) too (Gomes et al. 2009; Wen et al. 2009).

11.5 Product Identification and Mechanism of Dye Degradation

It is necessary to identify the metabolites produced after biodegradation of pollutants to determine the metabolic pathways. This is important to gain the knowledge about the fate of organic pollutants and also for the evaluation of the toxicity of the intermediates and main products as well as to describe the microbial system and/or enzymatic activities. According to the structure of dye, different microorganisms/enzymes may have different pathways for degradation. Dyes absorb light in the visible region of the spectra, and each one has a maximal wavelength so the application of spectrophotometry is the easiest way to monitor dye degradation. Intermediates and degradation products also contribute to the absorbance spectra, thus all the present molecules are quantified by this technique. For the isolation and characterization of the intermediates and products of dye degradation, advanced techniques of chromatography such as gas chromatography (GC), high performance liquid chromatography (HPLC), nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (Ion-trap, MALDI), and capillary electrophoresis (CE) are available. Extraction of the aqueous sample with an organic solvent or filtration are used when a heterogeneous catalyst or solid substrate is used or when a prior separation is required. Estimation of CO_2 and NH_3 produced during the microbial growth in culture media can also provide valuable information. Recent studies have been published on the mechanisms and pathways using the abovementioned techniques by Lopez et al. (2004), Vanhulle et al. (2008), Bafana et al. (2009), and Pereira et al. (2009).

11.6 Future Perspectives

Textile and dyeing wastewaters, two important environmental pollutants, contain various dyes. Physical and chemical methods for dye removal are common methods. However, they pose various disadvantages. Conventional biological treatment systems such as activated sludge are not efficient for bioremediation and decolorization. Furthermore, anaerobic treatment can produce toxic aromatic amines. White rot fungi are a group of the most efficient microorganisms in decolorization and degradation of textile dyes. Dyes exist in variety of different structural forms making

them more complex for being degraded, and this complexity of degradation of dyes demand more intense research. Moreover, most of the available studies on dye degradation have been limited to azo dyes. Other classes of dyes viz. anthraquinone, indigoid, xanthene, arylmethane, and phthalocyanine derivatives also seek further attention. The dye degradation pathways, being more complex, are not yet thoroughly understood and the thrust in that direction has high scope for the development of future modern technology. The on-going research in the degradation technology associated with the field of microbiology, molecular biology, chemistry, and genetics are fundamental for that knowledge. Moreover, the better evaluation and understanding of the effect of colored substances and their products in the environment or during the treatment processes will be possible. This understanding, in turn, will lead to improvisation in the technology and more efficient application from existing treatment processes. Isolation and studies of novel microorganisms and enzymes having broader substrate specificity and higher activity for their ability and capacity as potential agents in pollution remediation will be carried out. Random or selective modification of the microorganisms and enzymes using genetic engineering can greatly facilitate in designing microbes with higher catalytic efficiency for a broader range of compounds. Optimization of the remediation process in terms of time, efficiency, and stabilization will be more profitable. The understanding and progress in biochemical, biological, and process engineering is also important for its implementation at industrial scale. The most promising process for complete mineralization and detoxification of the colored effluent seem to be the combination of more than one treatment (biologic and/or chemical) application. The study and execution of novel treatments shall not only be focused on pollution diminution but also in the recycling of water and appropriate utilization of the final byproducts.

References

Abdullah N, Nawawi A, Othman I (2000) Fungal spoilage of starch-based foods in relation to its water activity (aw). J Stored Prod Res 36(1):47–54

Adak A, Tiwari R, Singh S, Sharma S, Nain L (2016) Laccase production by a novel white-rot fungus *Pseudolagarobasidium acaciicola* LA 1 through solid-state fermentation of Parthenium biomass and its application in dyes decolorization. Waste Biomass Valori 7(6):1427–1435

Ajit P, Vanita P, Juhi P, Ujjval T, Kamlesh P (2017) Evaluation of catalytic efficiency of *Coriolopsis caperata* DN Laccase to decolorize and detoxify RBBR dye. Water Conserv Sci Eng 2(3):85–98

Alexandropoulos CJ, Mims CW, Blackwell M (1996) Introductory mycology, 4th edn. Wiley, New York

Ali H (2010) Biodegradation of synthetic dyes: a review. Water Air Soil Pollut 213(1–4):251–273

Almansa E, Kandelbauer A, Pereira L, Cavaco-Paulo A, Guebitz GM (2004) Influence of structure on dye degradation with laccase mediator systems. Biocatal Biotransformation 22 (5–6):315–324

Ames BN, McCann J, Yamasaki E (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat Res 31(6):347–363

Amin KA, Hameid HA II, Elsttar AA (2010) Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food Chem Toxicol 48(10):2994–2999

Anastasi A, Coppola T, Prigione V, Varese GC (2009) Pyrene degradation and detoxification in soil by a consortium of basidiomycetes isolated from compost: role of laccases and peroxidases. J Hazard Mater 165(1–3):1229–1233

Anjaneyulu Y, Chary NS, Raj DSS (2005) Decolourization of industrial effluents–available methods and emerging technologies–a review. Rev Environ Sci Biotechnol 4(4):245–273

Archibald FS, Bourbonnais R, Jurasek L, Paice MG, Reid ID (1997) Kraft pulp bleaching and delignification by *Trametes versicolor*. J Biotechnol 53(2–3):215–236

Ardag Akdogan H, Canpolat Topuz M (2014) Decolorization of turquoise blue HFG by *Coprinus plicatilis* for water bioremediation. Biorem J 18(4):287–294

Asgher M, Noreen S, Bilal M (2017) Enhancing catalytic functionality of *Trametes versicolor* IBL-04 laccase by immobilization on chitosan microspheres. Chem Eng Res Des 119:1–11

Bafana A, Chakrabarti T, Muthal P, Kanade G (2009) Detoxification of benzidine-based azo dye by *E. gallinarum*: time-course study. Ecotoxicol Environ Saf 72(3):960–964

Baldrian P (2006) Fungal laccases—occurrence and properties. FEMS Microbiol Rev 30 (2):215–242

Bamforth SM, Singleton I (2005) Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. J Chem Technol Biotechnol 80(7):723–736

Barr D, Aust S (1994) Mechanisms white rot fungi use to degrade pollutants. Environ Sci Technol 28:78–87

Beebe NHF (2019) A complete bibliography of publications in Chymia: annual studies in the history of chemistry: 1948–1967; historical studies in the physical sciences: 1969–1971; historical studies in the physical and biological sciences: 1986–2007; and historical studies in the natural sciences: 2008–date

Bertea A, Bertea AP (2008) Decolorisation and recycling of textile wastewater. Performantica Ed, Iasi

Birhanli E, Erdogan S, Yesilada O, Onal Y (2013) Laccase production by newly isolated white rot fungus *Funalia trogii*: effect of immobilization matrix on laccase production. Biochem Eng J 71:134–139

Boehmer U, Suhardi SH, Bley T (2006) Decolorizing reactive textile dyes with white-rot Fungi by temporary immersion cultivation. Eng Life Sci 6(4):417–420

Boran F, Yesilada O (2011) Enhanced production of laccase by fungi under solid substrate fermentation condition. Bioresources 6(4):4404–4416

Bourbonnais R, Paice MG (1990) Oxidation of non-phenolic substrates. FEBS Lett 267(1):99–102

Bourbonnais R, Paice MG, Reid ID, Lanthier P, Yaguchi M (1995) Lignin oxidation by laccase isozymes from *Trametes versicolor* and role of the mediator 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonate) in Kraft lignin depolymerization. Appl Environ Microbiol 61(5):1876–1880

Call HP, Mücke I (1997) History, overview and applications of mediated lignolytic systems, especially laccase-mediator-systems (Lignozym®-process). J Biotechnol 53(2–3):163–202

Camarero S, Sarkar S, Ruiz-Dueñas FJ, Martínez MJ, Martínez AT (1999) Description of a versatile peroxidase involved in the natural degradation of lignin that has both manganese peroxidase and lignin peroxidase substrate interaction sites. J Biol Chem 274(15):10324–10330

Campos R, Kandelbauer A, Robra KH, Cavaco-Paulo A, Gübitz GM (2001) Indigo degradation with purified laccases from *Trametes hirsuta* and *Sclerotium rolfsii*. J Biotechnol 89 (2–3):131–139

Cancel A, Orth A, Tien M (1993) Lignin and veratryl alcohol are not inducers of the ligninolytic system of *Phanerochaete chrysosporium*. Appl Environ Microbiol 59:2909–2913

Carmen Z, Daniela S (2012) Textile organic dyes—characteristics, polluting effects and separation/elimination procedures from industrial effluents—a critical overview. In: Organic pollutants ten years after the Stockholm convention-environmental and analytical update. IntechOpen

Carvalho MB, Martins I, Leitão MC, Garcia H, Rodrigues C, San Romão V, Pereira CS (2009) Screening pentachlorophenol degradation ability by environmental fungal strains belonging to the phyla Ascomycota and Zygomycota. J Ind Microbiol Biotechnol 36(10):1249–1256

Casieri L, Varese GC, Anastasi A, Prigione V, Svobodova K, Marchisio VF, Novotný Č (2008) Decolorization and detoxication of reactive industrial dyes by immobilized fungi *Trametes pubescens* and *Pleurotus ostreatus*. Folia Microbiol 53(1):44

Cerniglia CE (1992) Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3 (2–3):351–368

Cerniglia CE, Sutherland JB (2010) Degradation of polycyclic aromatic hydrocarbons by fungi. In: Handbook of hydrocarbon and lipid microbiology, pp 2079–2110

Chen SH, Yien Ting AS (2015) Biodecolorization and biodegradation potential of recalcitrant triphenylmethane dyes by *Coriolopsis* sp. isolated from compost. J Environ Manag 150:274–280

Chequer FMD, de Oliveira GAR, Ferraz ERA, Cardoso JC, Zanoni MVB, de Oliveira DP (2013) Textile dyes: dyeing process and environmental impact. In: Eco-friendly textile dyeing and finishing. IntechOpen

Chhabra M, Mishra S, Sreekrishnan TR (2015) Immobilized laccase mediated dye decolorization and transformation pathway of azo dye acid red 27. J Environ Health Sci Eng 13(1):38

Christian V, Shrivastava R, Shukla D, Modi H, Vyas BRM (2005) Mediator role of veratryl alcohol in the lignin peroxidase-catalyzed oxidative decolorization of Remazol brilliant blue R. Enzym Microb Technol 36(4):426–431

Conesa A, Punt PJ, van den Hondel CA (2002) Fungal peroxidases: molecular aspects and applications. J Biotechnol 93(2):143–158

Couto SR (2009) Dye removal by immobilised fungi. Biotechnol Adv 27(3):227–235

Couto SR, Herrera LT (2006) Inhibitors of laccases: a review. Curr Enzym Inhib 2(4):343–352

Couto SR, Toca-Herrera JL (2006) Lacasses in the textile industry. Biotechnol Mol Biol Rev 1 (4):115–120

Couto SR, Gundín M, Lorenzo M, Sanromán MÁ (2002) Screening of supports and inducers for laccase production by *Trametes versicolor* in semi-solid-state conditions. Process Biochem 38 (2):249–255

Cullen D (1997) Recent advances on the molecular genetics of ligninolytic fungi. J Biotechnol 53 (2–3):273–289

Daassi D, Mechichi T, Nasri M, Rodriguez-Couto S (2013) Decolorization of the metal textile dye Lanaset Grey G by immobilized white-rot fungi. J Environ Manag 129:324–332

Desai SS, Nityanand C (2011) Microbial laccases and their applications: a review. Asian J Biotechnol 3(2):98–124

Dos Santos AB, Cervantes FJ, van Lier JB (2007) Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology. Bioresour Technol 98(12):2369–2385

Earth P, Cross G (2016) The World's worst pollution problems 2016: the toxics beneath our feet. Zurich, 53p

Eaton RA, Hale MD (1993) Wood: decay, pests and protection. Chapman and Hall Ltd. New York

Eichlerova I, Homolka L, Benada O, Kofronova O, Hubalek T, Nerud F (2007) Decolorization of Orange G and Remazol brilliant blue R by the white rot fungus *Dichomitus squalens*: toxicological evaluation and morphological study. Chemosphere 69(5):795–802

Eichlerová I, Homolka L, Nerud F (2006) Synthetic dye decolorization capacity of white rot fungus Dichomitus squalens. Bioresour Technol 97(16):2153–2159

Enayatizamir N, Tabandeh F, Rodríguez-ir S, Yakhchali B, Alikhani HA, Mohammadi L (2011) Biodegradation pathway and detoxification of the diazo dye reactive black 5 by *Phanerochaete chrysosporium*. Bioresour Technol 102(22):10359–10362

Erdem E, Çölgeçen G, Donat R (2005) The removal of textile dyes by diatomite earth. J Colloid Interface Sci 282(2):314–319

Erden E, Ucar MC, Gezer T, Pazarlioglu NK (2009) Screening for ligninolytic enzymes from autochthonous fungi and applications for decolorization of Remazole marine blue. Braz J Microbiol 40(2):346–353

Eriksson KEL, Blanchette RA Ander P (1990) Morphological aspects of wood degradation by fungi and bacteria. In: Microbial and enzymatic degradation of wood and wood components. Springer, Berlin, pp 1–87

EWA (2005) Efficient use of water in the textile finishing industry, official publication of the European water association (EWA). Brussels, Belgium

Faraco V, Pezzella C, Miele A, Giardina P, Sannia G (2009) Bio-remediation of colored industrial wastewaters by the white-rot fungi *Phanerochaete chrysosporium* and *Pleurotus ostreatus* and their enzymes. Biodegradation 20(2):209–220

Field J, Jong E, Feijo-Costa G, Bont J (1993) Screening for ligninolytic fungi applicable to the biodegradation of xenobiotics. Trends Biotechnol 11:44–49

Forgacs E, Cserhati T, Oros G (2004) Removal of synthetic dyes from wastewaters: a review. Environ Int 30(7):953–971

Fu Y, Viraraghavan T (2001) Fungal decolorization of dye wastewaters: a review. Bioresour Technol 79(3):251–262

Fu Y, Viraraghavan T (2002) Removal of Congo red from an aqueous solution by fungus *Aspergillus niger*. Adv Environ Res 7(1):239–247

Ghosal D, Ghosh S, Dutta TK, Ahn Y (2016) Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. Front Microbiol 7:1369

Gilbertson RL (1980) Wood-rotting fungi of North America. Mycologia 72(1):1

Gold MH, Alic M (1993) Molecular biology of the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Microbiol Rev 57(3):605–622

Gomes E, Aguiar AP, Carvalho CC, Bonfá MRB, Silva RD, Boscolo M (2009) Ligninases production by Basidiomycetes strains on lignocellulosic agricultural residues and their application in the decolorization of synthetic dyes. Braz J Microbiol 40(1):31–39

Gómez-Toribio V, Martínez AT, Martínez MJ, Guillén F (2001) Oxidation of hydroquinones by the versatile ligninolytic peroxidase from *Pleurotus eryngii*: H_2O_2 generation and the influence of Mn^{2+}. Eur J Biochem 268(17):4787–4793

Gonzalez JC, Medina SC, Rodriguez A, Osma JF, Alméciga-Díaz CJ, Sánchez OF (2013) Production of *Trametes pubescens* laccase under submerged and semi-solid culture conditions on agro-industrial wastes. PLoS One 8(9):e73721

Guendouz S, Khellaf N, Djelal H, Ouchefoun M (2016) Simultaneous biosorption of the two synthetic dyes, direct red 89 and reactive green 12 using nonliving macrophyte *L. gibba* L. Desalin Water Treat 57(10):4624–4632

Guillén F, Martínez AT, Martínez MJ (1992) Substrate specificity and properties of the aryl-alcohol oxidase from the ligninolytic fungus *Pleurotus eryngii*. Eur J Biochem 209(2):603–611

Haapala R, Linko S (1993) Production of *Phanerochaete chrysosporium* lignin peroxidase under various culture conditions. Appl Microbiol Biotechnol 40(4):494–498

Hadibarata T, Zubir MMFA, Chuang TZ, Yusoff ARM, Salim MR, Fulazzaky MA, Nugroho AE (2013) Degradation and transformation of anthracene by white-rot fungus Armillaria sp. F022. Folia Microbiol 58(5):385–391

Haglund C (1999) Biodegradation of xenobiotic compounds by the white-rot fungus *Trametes trogii*. Molecular Biotechnology Programme, Uppsala University School of Engineering, Uppsala, 30 p

Hai FI, Yamamoto K, Fukushi K (2007) Hybrid treatment systems for dye wastewater. Crit Rev Environ Sci Technol 37(4):315–377

Hao C, Shepson PB, Drummond JW, Muthuramu K (1994) Gas chromatographic detector for selective and sensitive detection of atmospheric organic nitrates. Anal Chem 66(21):3737–3743

Hao OJ, Kim H, Chiang PC (2000) Decolorization of wastewater. Crit Rev Environ Sci Technol 30 (4):449–505

Hao J, Song F, Huang F, Yang C, Zhang Z, Zheng Y, Tian X (2007) Production of laccase by a newly isolated Deuteromycete fungus Pestalotiopsis sp. and its decolorization of azo dye. J Ind Microbiol Biotechnol 34(3):233

Hatakka A (1994) Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. FEMS Microbiol Rev 13(2–3):125–135

Hatakka A (2001) Biodegradation of lignin. In: Hofrichter M, Steinbüchel A (eds) Lignin, humic substances and coal, vol 1. Wiley-VCH, Weinheim, pp 129–180

Henriksson G, Ander P, Pettersson B, Pettersson G (1995) Cellobiose dehydrogenase (cellobiose oxidase) from Phanerochaete chrysosporium as a wood-degrading enzyme. Studies on cellulose, xylan and synthetic lignin. Appl Microbiol Biotechnol 42(5):790–796

Henriksson G, Zhang L, Li J, Ljungquist P, Reitberger T, Pettersson G, Johansson G (2000) Is cellobiose dehydrogenase from *Phanerochaete chrysosporium* a lignin degrading enzyme? Biochim Biophys Acta Protein Struct Mol Enzymol 1480(1–2):83–91

Hirai H, Sugiura M, Kawai S, Nishida T (2005) Characteristics of novel lignin peroxidases produced by white-rot fungus *Phanerochaete sordida* YK-624. FEMS Microbiol Lett 246 (1):19–24

Hofrichter M (2002) Lignin conversion by manganese peroxidase (MnP). Enzym Microb Technol 30(4):454–466

Ilk S, Demircan D, SAĞLAM S, SAĞLAM N, Rzayev ZM (2016) Immobilization of laccase onto a porous nanocomposite: application for textile dye degradation. Turk J Chem 40(2):262–276

Itoh K, Kitade Y, Yatome C (1996) A pathway for biodegradation of an anthraquinone dye, CI disperse red 15, by a yeast strain *Pichia anomala*. Bull Environ Contam Toxicol 56(3):413–418

Kabra AN, Khandare RV, Waghmode TR, Govindwar SP (2012) Phytoremediation of textile effluent and mixture of structurally different dyes by *Glandularia pulchella* (sweet) Tronc. Chemosphere 87(3):265–272

Kagalkar AN, Jagtap UB, Jadhav JP, Govindwar SP, Bapat VA (2010) Studies on phytoremediation potentiality of *Typhonium flagelliforme* for the degradation of brilliant blue R. Planta 232 (1):271–285

Kamitsuji H, Honda Y, Watanabe T, Kuwahara M (2004) Production and induction of manganese peroxidase isozymes in a white-rot fungus *Pleurotus ostreatus*. Appl Microbiol Biotechnol 65 (3):287–294

Kandelbauer A, Maute O, Kessler RW, Erlacher A, Gübitz GM (2004) Study of dye decolorization in an immobilized laccase enzyme-reactor using online spectroscopy. Biotechnol Bioeng 87 (4):552–563

Kant R (2012) Textiledyeingindustryandenvironmentalhazard. Nat Sci 4(1):22–26

Kapich AN, Prior BA, Botha A, Galkin S, Lundell T, Hatakka A (2004) Effect of lignocellulose-containing substrates on production of ligninolytic peroxidases in submerged cultures of *Phanerochaete chrysosporium* ME-446. Enzym Microb Technol 34(2):187–195

Kaushik P, Malik A (2009) Fungal dye decolourization: recent advances and future potential. Environ Int 35(1):127–141

Kawai S, Umezawa T, Higuchi T (1989) < original> Oxidation of methoxylated benzyl alcohols by laccase of *Coriolus versicolor* in the presence of syringaldehyde. Wood Res 76:10–16

Kersten PJ (1990) Glyoxal oxidase of *Phanerochaete chrysosporium*: its characterization and activation by lignin peroxidase. Proc Natl Acad Sci 87(8):2936–2940

Kersten P, Cullen D (2007) Extracellular oxidative systems of the lignin-degrading Basidiomycete *Phanerochaete chrysosporium*. Fungal Genet Biol 44(2):77–87

Keyser P, Kirk TK, Zeikus JG (1978) Ligninolytic enzyme system of *Phanerochaete chrysosporium:* synthesized in the absence of lignin in response to nitrogen starvation. J Bacteriol 135:790–797

Khamparia S, Jaspal DK (2017) Adsorption in combination with ozonation for the treatment of textile waste water: a critical review. Front Environ Sci Eng 11(1):8

Khandare RV, Kabra AN, Kurade MB, Govindwar SP (2011) Phytoremediation potential of *Portulaca grandiflora* hook. (Moss-rose) in degrading a sulfonated diazo reactive dye navy blue HE2R (reactive blue 172). Bioresour Technol 102(12):6774–6777

Kiran S, Ali S, Asgher M, Anwar F (2012) Comparative study on decolorization of reactive dye 222 by white rot fungi *Pleurotus ostreatus* IBL-02 and *Phanerochaete chrysosporium* IBL-03. Afr J Microbiol Res 6(15):3639–3650

Knapp JS, Zhang FM, Tapley KN (1997) Decolourisation of Orange II by a wood-rotting fungus. J Chem Technol Biotechnol Int Res Process Environ Clean Technol 69(3):289–296

Kolekar YM, Konde PD, Markad VL, Kulkarni SV, Chaudhari AU, Kodam KM (2013) Effective bioremoval and detoxification of textile dye mixture by Alishewanella sp. KMK6. Appl Microbiol Biotechnol 97(2):881–889

Kuhad RC, Sood N, Tripathi KK, Singh A, Ward OP (2004) Developments in microbial methods for the treatment of dye effluents. Adv Appl Microbiol 56:185

Kunamneni A, Ballesteros A, Plou FJ, Alcalde M (2007) Fungal laccase—a versatile enzyme for biotechnological applications. In: Communicating current research and educational topics trends in applied microbiology, vol 1, pp 233–245

Kunjadia PD, Patel FD, Nagee A, Mukhopadhyaya PN, Dave GS (2012) Crystal violet (Triphenylmethane dye) decolorization potential of *Pleurotus ostreatus* (MTCC 142). Bioresources 7 (1):1189–1199

Kunjadia PD, Sanghvi GV, Kunjadia AP, Mukhopadhyay PN, Dave GS (2016) Role of ligninolytic enzymes of white rot fungi (Pleurotus spp.) grown with azo dyes. SpringerPlus 5(1):1487

Lee H, Jang Y, Kim J-M, Kim G-H, Kim J-J (2013) White-rot fungus *Merulius tremellosus* KUC9161 identified as an effective degrader of polycyclic aromatic hydrocarbons. J Basic Microbiol 53(2):195–199

Lee H, Jang Y, Choi YS, Kim MJ, Lee J, Lee H, Kim JJ (2014) Biotechnological procedures to select white rot fungi for the degradation of PAHs. J Microbiol Methods 97:56–62

Lin JP, Lian W, Xia LM, Cen PL (2003) Production of laccase by *Coriolus versicolor* and its application in decolorization of dyestuffs:(I) production of laccase by batch and repeated-batch processes. J Environ Sci 15(1):1–4

Lopez C, Valade AG, Combourieu B, Mielgo I, Bouchon B, Lema JM (2004) Mechanism of enzymatic degradation of the azo dye Orange II determined by ex situ 1H nuclear magnetic resonance and electrospray ionization-ion trap mass spectrometry. Anal Biochem 335 (1):135–149

Lu Y, Jiang Z, Aydin K, Verlinde J, Clothiaux EE, Botta G (2016) A polarimetric scattering database for non-spherical ice particles at microwave wavelengths. Atmos Meas Tech 9 (10):5119–5134

Lucas M, Mertens V, Corbisier AM, Vanhulle S (2008) Synthetic dyes decolourisation by white-rot fungi: development of original microtitre plate method and screening. Enzym Microb Technol 42(2):97–106

Ma L, Zhuo R, Liu H, Yu D, Jiang M, Zhang X, Yang Y (2014) Efficient decolorization and detoxification of the sulfonated azo dye reactive Orange 16 and simulated textile wastewater containing reactive Orange 16 by the white-rot fungus Ganoderma sp. En3 isolated from the forest of Tzu-Chin Mountain in China. Biochem Eng J 82:1–9

Maloney S (2001) Pesticide degradation. In: Gadd G (ed) Fungi in bioremediation. Cambridge University Press, Cambridge

Mansour HB, Houas I, Montassar F, Ghedira K, Barillier D, Mosrati R, Chekir-Ghedira L (2012) Alteration of in vitro and acute in vivo toxicity of textile dyeing wastewater after chemical and biological remediation. Environ Sci Pollut Res 19(7):2634–2643

Martínez ÁT, Speranza M, Ruiz-Dueñas FJ, Ferreira P, Camarero S, Guillén F et al (2005) Biodegradation of lignocellu- losics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin Lignocellulosic materials. Int Microbiol 8:195–204

Martinez AT, Ruiz-Duenas FJ, Martinez MJ, del Rio JC, Gutierrez A (2009) Enzymatic delignification of plant cell wall: from nature to mill. Curr Opin Biotechnol 20(3):348–357

Mathur N, Bhatnagar P, Bakre P (2006) Assessing mutagenicity of textile dyes from Pali (Rajasthan) using Ames bioassay. Appl Ecol Environ Res 4(1):111–118

Miranda R d CM d, Gomes E d B, Pereira N, Marin-Morales MA, Machado KMG, Gusmao NB d (2013) Biotreatment of textile effluent in static bioreactor by *Curvularia lunata* URM 6179 and *Phanerochaete chrysosporium* URM 6181. Bioresour Technol 142:361–367

Mondal S (2008) Methods of dye removal from dye house effluent-an overview. Environ Eng Sci 25(3):383–396

Murugesan K, Nam IH, Kim YM, Chang YS (2007) Decolorization of reactive dyes by a thermostable laccase produced by *Ganoderma lucidum* in solid state culture. Enzym Microb Technol 40(7):1662–1672

Muthunarayanan V, Santhiya M, Swabna V, Geetha A (2011) Phytodegradation of textile dyes by water hyacinth (*Eichhornia crassipes*) from aqueous dye solutions. Int J Environ Sci 1(7):1702

Nath PP, Sarkar K, Mondal M, Paul G (2016) Metanil yellow impairs the estrous cycle physiology and ovarian folliculogenesis in female rats. Environ Toxicol 31(12):2057–2067

Nigam P, Armour G, Banat IM, Singh D, Marchant R (2000) Physical removal of textile dyes from effluents and solid-state fermentation of dye-adsorbed agricultural residues. Bioresour Technol 72(3):219–226

Novotný Č, Svobodová K, Kasinath A, Erbanová P (2004) Biodegradation of synthetic dyes by *Irpex lacteus* under various growth conditions. Int Biodeterior Biodegradation 54(2–3):215–223

O'Neill C, Hawkes FR, Hawkes DL, Lourenço ND, Pinheiro HM, Delée W (1999) Colour in textile effluents–sources, measurement, discharge consents and simulation: a review. J Chem Technol Biotechnol Int Res Process Environ Clean Technol 74(11):1009–1018

Orth AB, Royse DJ, Tien MING (1993) Ubiquity of lignin-degrading peroxidases among various wood-degrading fungi. Appl Environ Microbiol 59(12):4017–4023

Ozmen N, Yesilada O (2012) Valorization and biodecolorization of dye adsorbed on lignocellulosics using white rot fungi. Bioresources 7(2):1656–1665

Padmesh TVN, Vijayaraghavan K, Sekaran G, Velan M (2005) Batch and column studies on biosorption of acid dyes on fresh water macro alga *Azolla filiculoides*. J Hazard Mater 125 (1–3):121–129

Palmieri G, Giardina P, Bianco C, Scaloni A, Capasso A, Sannia G (1997) A novel white laccase from *Pleurotus ostreatus*. J Biol Chem 272(50):31301–31307

Palmieri G, Giardina P, Bianco C, Fontanella B, Sannia G (2000) Copper induction of laccase isoenzymes in the ligninolytic fungus *Pleurotus ostreatus*. Appl Environ Microbiol 66 (3):920–924

Pandey A, Singh P, Iyengar L (2007) Bacterial decolorization and degradation of azo dyes. Int Biodeterior Biodegradation 59(2):73–84

Papinutti VL, Forchiassin F (2004) Modification of malachite green by *Fomes sclerodermeus* and reduction of toxicity to *Phanerochaete chrysosporium*. FEMS Microbiol Lett 231:205–209

Papinutti L, Martínez MJ (2006) Production and characterization of laccase and manganese peroxidase from the ligninolytic fungus *Fomes sclerodermeus*. J Chem Technol Biotechnol Int Res Process Environ Clean Technol 81(6):1064–1070

Park C, Lee M, Lee B, Kim SW, Chase HA, Lee J, Kim S (2007a) Biodegradation and biosorption for decolorization of synthetic dyes by *Funalia trogii*. Biochem Eng J 36(1):59–65

Park C, Lim J-S, Lee Y, Lee B, Kim S-W, Lee J, Kim S (2007b) Optimization and morphology for decolorization of reactive black 5 by *Funalia trogii*. Enzym Microb Technol 40(7):1758–1764

Pereira EL, Campos CMM, Moterani F (2009) Effects of pH, acidity and alkalinity on the microbiota activity of an anaerobic sludge blanket reactor (UASB) treating pigmanure effluents. Ambiente Agua 4(3):157–168

Pereira L, Coelho AV, Viegas CA, dos Santos MMC, Robalo MP, Martins LO (2009a) Enzymatic biotransformation of the azo dye Sudan Orange G with bacterial CotA-laccase. J Biotechnol 139 (1):68–77

Pereira L, Coelho AV, Viegas CA, Ganachaud C, Iacazio G, Tron T, Martins LO (2009b) On the mechanism of biotransformation of the anthraquinonic dye acid blue 62 by laccases. Adv Synth Catal 351(11–12):1857–1865

Pinheiro HM, Touraud E, Thomas O (2004) Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters. Dyes Pigments 61(2):121–139

Piontek K, Smith AT, Blodig W (2001) Lignin peroxidase structure and function. Biochem Soc Trans 29(Pt 2):111–116

Pointing S (2001) Feasibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol 57:20–33

Prigione V, Varese GC, Casieri L, Marchisio VF (2008) Biosorption of simulated dyed effluents by inactivated fungal biomasses. Bioresour Technol 99(9):3559–3567

Puvaneswari N, Muthukrishnan J, Gunasekaran P (2006) Toxicity assessment and microbial degradation of azo dyes. Indian J Exp Biol 44:218–226

Randazzo S, Scialdone O, Brillas E, Sirés I (2011) Comparative electrochemical treatments of two chlorinated aliphatic hydrocarbons. Time course of the main reaction by-products. J Hazard Mater 192(3):1555–1564

Reddy C, Mathew Z (2001) Bioremediation potential of white rot fungi. In: Gadd G (ed) Fungi in bioremediation. Cambridge University Press, Cambridge

Riu J, Schönsee I, Barcelo D (1998) Determination of sulfonated azo dyes in ground water and industrial effluents by automated solid-phase extraction followed by capillary electrophoresis/mass spectrometry. J Mass Spectrom 33(7):653–663

Robinson T, McMullan G, Marchant R, Nigam P (2001) Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresour Technol 77(3):247–255

Rodríguez-Couto S (2011) Production of laccase and decolouration of the textile dye Remazol brilliant blue R in temporary immersion bioreactors. J Hazard Mater 194:297–302

Rodríguez-Couto S (2012) A promising inert support for laccase production and decolouration of textile wastewater by the white-rot fungus *Trametes pubescesns*. J Hazard Mater 233:158–162

Rodríguez-Couto S, Osma JF, Toca-Herrera JL (2009) Removal of synthetic dyes by an eco-friendly strategy. Eng Life Sci 9(2):116–123

Rogalski J, Leonowicz A (2004) Production and application of laccase. In: Pandey A (ed) Encyclopedia on bioresource technology. Haworth Press, New York, pp 1–736

Ruiz-Dueñas FJ, Martínez ÁT (2009) Microbial degradation of lignin: how a bulky recalcitrant polymer is efficiently recycled in nature and how we can take advantage of this. Microb Biotechnol 2(2):164–177

Sánchez-Martín J, González-Velasco M, Beltrán-Heredia J (2010) Surface water treatment with tannin-based coagulants from Quebracho (*Schinopsis balansae*). Chem Eng J 165(3):851–858

Saratale RG, Saratale GD, Kalyani DC, Chang JS, Govindwar SP (2009) Enhanced decolorization and biodegradation of textile azo dye scarlet R by using developed microbial consortium-GR. Bioresour Technol 100(9):2493–2500

Sari AA, Tachibana S, Muryanto, Hadibarata T (2016) Development of bioreactor systems for decolorization of Reactive Green 19 using white rot fungus. Desalin Water Treat 57 (15):7029–7039

Şaşmaz S, Gedikli S, Aytar P, Güngörmedi G, Çabuk A, Hür E, Kolankaya N (2011) Decolorization potential of some reactive dyes with crude laccase and laccase-mediated system. Appl Biochem Biotechnol 163(3):346–361

Sayahi E, Ladhari N, Mechichi T, Sakli F (2016) Azo dyes decolourization by the laccase from *Trametes trogii*. J Textile Inst 107(11):1478–1482

Senthilkumar S, Perumalsamy M, Prabhu HJ, Thajudin N (2015) Optimization of process parameters for efficient decolorization of Congo red by novel fungus Curvularia sp. using box–Behnken design. Desalin Water Treat 54(6):1708–1716

Shankar S, Nill S (2015) Effect of metal ions and redox mediators on decolorization of synthetic dyes by crude laccase from a novel white rot fungus Peniophora sp. (NFCCI-2131). Appl Biochem Biotechnol 175(1):635–647

Shekher R, Sehgal S, Kamthania M, Kumar A (2011) Laccase: microbial sources, production, purification, and potential biotechnological applications. Enzyme Res 2011:217861
Shrivastava R, Christian V, Vyas BRM (2005) Enzymatic decolorization of sulfonphthalein dyes. Enzym Microb Technol 36(2–3):333–337
Si J, Cui BK, Dai YC (2013) Decolorization of chemically different dyes by white-rot fungi in submerged cultures. Ann Microbiol 63(3):1099–1108
Sivakumar D, Shankar D, Prathima AV, Valarmathi M (2013) Constructed wetland treatment of textile industry wastewater using aquatic macrophytes. Int J Environ Sci 3(4):1223
Soares CHL (1998) Estudos mecanisticos da degradação de efluentes de indústrias de papel e celulose por fungos Basidiomicetos degradadores de madeira
Srebotnik E, Hammel KE (2000) Degradation of nonphenolic lignin by the laccase/1-hydroxybenzotriazole system. J Biotechnol 81(2–3):179–188
Sulaiman SS, Annuar MSM, Abdul Razak NN, Ibrahim S, Bakar B (2013) Triarylmethane dye decolorization by pellets of *Pycnoporus sanguineus*: statistical optimization and effects of novel impeller geometry. Biorem J 17(4):305–315
Sundaramoorthy M, Youngs HL, Gold MH, Poulos TL (2005) High-resolution crystal structure of manganese peroxidase: substrate and inhibitor complexes. Biochemistry 44(17):6463–6470
Suteu D, Zaharia C, Bilba D, Muresan R, Popescu A, Muresan A (2009a) Decolorization waste waters from the textile industry-physical methods, chemical methods. Ind Textila 60 (5):254–263
Suteu D, Zaharia C, Muresan A, Muresan R, Popescu A (2009b) Using of industrial waste materials for textile wastewater treatment. Environ Eng Manag J 8(5):1097–1102
Swamy J, Ramsay JA (1999) The evaluation of white rot fungi in the decoloration of textile dyes. Enzym Microb Technol 24(3–4):130–137
Taha M, Adetutu EM, Shahsavari E, Smith AT, Ball AS (2014) Azo and anthraquinone dye mixture decolourization at elevated temperature and concentration by a newly isolated thermophilic fungus, Thermomucor indicae-seudaticae. J Environ Chem Eng 2(1):415–423
Thurston CF (1994) The structure and function of fungal laccases. Microbiology 140(1):19–26
Tian CE, Tian R, Zhou Y, Chen Q, Cheng H (2013) Decolorization of indigo dye and indigo dye-containing textile effluent by *Ganoderma weberianum*. Afr J Microbiol Res 7(11):941–947
Upadhyay P, Shrivastava R, Agrawal PK (2016) Bioprospecting and biotechnological applications of fungal laccase. 3 Biotech 6(1):15
Van der Zee FP, Villaverde S (2005) Combined anaerobic–aerobic treatment of azo dyes—a short review of bioreactor studies. Water Res 39(8):1425–1440
Vandevivere PC, Bianchi R, Verstraete W (1998) Treatment and reuse of wastewater from the textile wet-processing industry: review of emerging technologies. J Chem Technol Biotechnol Int Res Process Environ Clean Technol 72(4):289–302
Vanhulle S, Enaud E, Trovaslet M, Billottet L, Kneipe L, Jiwan JLH, Marchand-Brynaert J (2008) Coupling occurs before breakdown during biotransformation of acid blue 62 by white rot fungi. Chemosphere 70(6):1097–1107
Wang L, Ridgway D, Gu T, Moo-Young M (2005) Bioprocessing strategies to improve heterologous protein production in filamentous fungal fermentations. Biotechnol Adv 23(2):115–129
Wariishi H, Akileswaran L, Gold MH (1988) Manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium*: spectral characterization of the oxidized states and the catalytic cycle. Biochemistry 27(14):5365–5370
Wen X, Jia Y, Li J (2009) Degradation of tetracycline and oxytetracycline by crude lignin peroxidase prepared from *Phanerochaete chrysosporium*—a white rot fungus. Chemosphere 75(8):1003–1007
Wesenberg D, Kyriakides I, Agathos SN (2003) White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol Adv 22(1–2):161–187
Wijannarong S, Aroonsrimorakot S, Thavipoke P, Kumsopa C, Sangjan S (2013) Removal of reactive dyes from textile dyeing industrial effluent by ozonation process. APCBEE Procedia 5:279–282

Wong DW (2009) Structure and action mechanism of ligninolytic enzymes. Appl Biochem Biotechnol 157(2):174–209

Wyatt AM, Broda P (1995) Informed strain improvement for lignin degradation by *Phanerochaete chrysosporium*. Microbiology 141(11):2811–2822

Yaropolov AI, Skorobogat'Ko OV, Vartanov SS, Varfolomeyev SD (1994) Laccase. Appl Biochem Biotechnol 49(3):257–280

Yaseen DA, Scholz M (2017) Treatment of synthetic textile wastewater containing dye mixtures with microcosms. Environ Sci Pollut Res 25(2):1980–1997

Yemendzhiev H, Alexieva Z, Krastanov A (2009) Decolorization of synthetic dye reactive blue 4 by Mycelial culture of white-rot Fungi *Trametes Versicolor* 1. Biotechnol Biotechnol Equip 23(Suppl 1):230–232

Yesilada O (1995) Short communication: decolourization of crystal violet by fungi. World J Microbiol Biotechnol 11(5):601–602

Yesilada O, Asma D, Cing S (2003) Decolorization of textile dyes by fungal pellets. Process Biochem 38(6):933–938

Yesilada O, Yildirim SC, Birhanli E, Apohan E, Asma D, Kuru F (2010) The evaluation of pre-grown mycelial pellets in decolorization of textile dyes during repeated batch process. World J Microbiol Biotechnol 26(1):33

Zaharia C, Suteu D, Muresan A, Muresan R, Popescu A (2009) Textile wastewater treatment by homogenous oxidation with hydrogen peroxide. Environ Eng Manag J 8(6):1359–1369

Zaharia C, Suteu D, Muresan A (2012) Options and solutions for textile effluent decolorization using some specific physico-chemical treatment steps. Environ Eng Manag J 11(2):493–509

Zapata-Castillo P, Villalonga-Santana L, Islas-Flores I, Rivera-Muñoz G, Ancona-Escalante W, Solís-Pereira S (2015) Synergistic action of laccases from *Trametes hirsuta* Bm2 improves decolourization of indigo carmine. Lett Appl Microbiol 61(3):252–258

Zhou M, He J (2007) Degradation of azo dye by three clean advanced oxidation processes: wet oxidation, electrochemical oxidation and wet electrochemical oxidation—a comparative study. Electrochim Acta 53(4):1902–1910

Zille A, Gornacka B, Rehorek A, Cavaco-Paulo A (2005) Degradation of azo dyes by *Trametes villosa* laccase over long periods of oxidative conditions. Appl Environ Microbiol 71 (11):6711–6718

Zollinger H (2003) Color chemistry: synthesis, properties and applications of organic dyes and pigments, 3rd edn. Wiley-VCH, Cambridge

Zucca P, Cocco G, Sollai F, Sanjust E (2015) Fungal laccases as tools for biodegradation of industrial dyes. Biocatalysis 1(1):82–108

Nanobioremediation: An Emerging Approach for a Cleaner Environment

12

Ekta Singh, Riyaz Ali M. Osmani, and Rinti Banerjee

Abstract

Global environmental issues have emerged owing to rapid industrialization and urbanization. Ecological imbalance and pollution have raised serious concerns and, thus, led to adoption of better anthropogenic practices and environmental cleanup technologies including physical, chemical, and biological methods. Current treatment practices, although efficient, have made remediation processes complex. Among existing technologies, bioremediation and biotransformation are prominently being used for heavy metal remediation of soil and water, whereas biodegradation is used for toxic pollutants like polyaromatic insecticides, pesticides, plasticizers, and petroleum hydrocarbons. This chapter overviews nanotechnology-based alternative treatment strategies for efficient and sustainable bioremediation and biodegradation. It discusses the advantages and disadvantages of current technologies as well as comments on the future directions in this field.

Keywords

Nanobioremediation · Degradation · Pollutants · Nanoparticles · Environment

12.1 Introduction

The literal meaning of word "remediate" is "to solve," and "bioremediation" can be defined as a process in which diverse biological agents, like fungi, bacteria, protists, and/or their secretions or enzymes, are utilized for completely degrading environmental pollutants or for conversion into much less toxic forms of the same (van Dillewijn et al. 2007).

E. Singh · R. A. M. Osmani · R. Banerjee (✉)
Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, Maharashtra, India
e-mail: rinti@iitb.ac.in

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_12

12.2 Health and Environmental Issues of Pollution

Our environment is pollutants including polyaromatic hydrocarbons (PAHs), organic substituted and halogenated pesticides/dyes, toxic heavy metals, etc., and exposure with an organism can occur via various sources and routes. Mostly, industrial workers are a higher risk of direct exposure. Nowadays, water remediation is of key concern as the spread is rapid. They also get converted into epoxides and form adducts with DNA which act as potential mutagens (Cookson Jr 1995; Vasudevan et al. 2018). The potential of animal and human fetal tissues to metabolize PAHs is higher as compared to adults (Cecchin et al. 2017a). This may explain higher risks of exposure in pregnancy and birth defects that has been seen in animals. With respect to carcinogenicity of PAH in humans, it is yet unclear. In context of risk to human health, exposure to heavy metals like lead, cadmium, mercury, arsenic, etc. damages vital organs and causes toxicity and hormonal impairment (Tripathi et al. 2018).

12.3 Conventional Methods for Remediation

12.3.1 Physical Methods

Precipitation: This approach involves use of suitable anionic sulfate salts of manganese, copper, ammonium, iron, alum, etc. for precipitating the metallic salts. Since this process involves acidic pH and chemicals, it is not very specific, efficient for removing metal contaminants at trace concentrations, and is very expensive (Mondal et al. 2006).

Ion exchange: This is a common technique mostly employed in industries for separating out heavy metals from industrial effluents by using solid-phase anion and cation exchange resins. However, this technique is also very expensive, pH sensitive, concentration-limited efficiency, and not very selective (Ahluwalia and Goyal 2007).

Electrowinning and electrocoagulation: These are electrochemical approaches which are also used in industries for leaching of metals and wastewater treatment. However, these techniques have very limited efficiency and are again expensive (Kumar and Gopinath 2017).

Cementation: Another approach that combines precipitation and electrochemistry for separation of heavy metals like copper, lead, gold, silver, cadmium, tin, gallium, arsenic, etc.

Adsorption: This process involves physic- and chemisorption over adsorbent surfaces like activated carbon or alumina, iron oxide-coated sand, copper-zinc granules, etc. over which metals are attached and thus metal ion removal is achieved. This process is affected by pH, surface area, and surface energy (Ahluwalia and Goyal 2007; Kumar and Gopinath 2017).

Membrane filtration: This process involves metal separation from water by filtration through a semipermeable membrane along a pressure gradient. This is

however an ineffective technique due to coprecipitation of ferrous and manganous irons and overall expensive (Kumar and Gopinath 2017).

Electrodialysis: This technique involves reverse osmosis under an applied electric field via semipermeable membrane. It is an efficient technique for groundwater remediation and bioaugmentation, however, being expensive and complex for bulk treatments (Mondal et al. 2006; Kumar and Gopinath 2017).

12.3.2 Chemical Treatment Methods

Reduction: It involves treatment of polluted soils that are permeable at an alkaline pH by injecting H_2S and diothionates acting as reductants and help to immobilize and degrade pollutants. However, since most of these chemical reactions such as in case of colloidal form of zerovalent ion when injected leads to toxicity due to products formed in the process, it is not a very suitable technique.

Chemical washing: It involves direct extraction of heavy metals by acids which is a complex process that damages the soil quality and can lead to hazards.

Chelate flushing: As the name suggests, a huge amount of heavy metals can be extracted by chelating agents like ethylenediaminetetraacetic acid (EDTA) which are regenerated and recyclable when used in the form of solvent-loaded resins and display an efficiency of 100% (Gray 1999; Amonette et al. 1994; Fruchter et al. 1997; Sevougian et al. 1994).

12.3.3 Biological Treatment Methods

Bioremediation refers to the application of biological agents like plants, fungi, microbes, etc. to remove pollutants from air, water, or soil by either degradation, removal, or conversion of toxicants into nontoxicants resulting into a permissible acceptable concentration. For remediation of heavy metals in soil and water, this approach is highly suitable as it is ecologically safe and economically feasible and hence a sustainable approach. However, the efficiency of bioremediation becomes limited when polluting agents become highly toxic for microbes and plants that are used as microbial and phytoremedial agents, respectively. Some of the commonly used microorganisms popularly include genera like *E. coli*, *Bacillus*, *Klebsiella*, *Alcaligenes*, *Rhodococcus*, and *Pseudomonas* (Cantrell et al. 1995; Manning et al. 2002).

Bioremediation consists of various remediation strategies, such as bioaugmentation, that is, natural attenuation process by using indigenous microorganisms, stimulated process by adding nutrients (biostimulation), use of genetically modified organisms, phytoremediation involving the use of some plants, and biomineralization involving the thorough biodegradation of organic substances into inorganic components (Li and Li 2011; Lacina et al. 2015; Wei et al. 2012a; Joutey et al. 2013).

12.3.3.1 Biofiltration

This technique involves an aqueous and porous microbial surface acting as a biological filter to remove contaminants by adsorbing them onto the surface and eventually incorporating them into complexes with organic constituents of water. Biotransformation occurs and results into biomass, CO_2, and water as metabolic by-products (Devinny et al. 1999; Srivastava and Majumdar 2008).

12.3.3.2 Biosorption

As the name suggests, it is a very similar technique to biofiltration. The main difference between both techniques is that in biofiltration, water passing through biofilter has attachment and microbial growth happening gradually and simultaneously, followed by degradation and detachment of microbes. The process is passive and utilizes microbial biomass either live or dead for bioremediation including cell wall components of algal, fungal, or bacterial origin. It provides advantages like regeneration of biosorbents, metal recovery, minimizing sludge, and affordable capital and operational cost (Hashim et al. 2011). The biomass bed acts as an ion exchange matrix and can remediate water with very low levels of heavy metal pollutants (Volesky and Holan 1995). Biosorbents can be surface immobilized for scale-up applications; however, analysis of efficacy, reproducibility, and affordability attributes need to be assessed. Various approaches like complex formation, chelation, electrostatic interactions, and ion exchange have been used for heavy metal removal using agricultural materials. The efficiency and stability of sorption process needs prior chemical treatment of pollutants. In a nutshell, biosorption process is a promising approach due to high rate of sorption, easily and naturally available sorbents, inexpensiveness, and nontoxicity.

12.3.3.3 Biophysiochemical Method

As the name suggests, this process involves physical methods, i.e., adsorption or coagulation, coupled with biochemical phenomenon. It is a widely used alternative for conventional physiochemical process for remediation of arsenic. Chemolithotroph strain *A. ferrooxidans* BY-3 isolated from mining regions has been used for biosorption of arsenic from polluted water (Wang and Chen 2009; Jain and Singh 2012; Yana et al. 2010). Not only bacteria but at least five distinct fungal isolates have also been used for arsenic bioremediation (Srivastava et al. 2011a). Apart from arsenic, microbial oxidation of iron using *G. ferruginea* and *L. ochraceus* was also used. In situ production of iron oxides forms a surface or platform for metal absorption from aqueous solution when passed over it. Such a combination of biological oxidation-filtration sorption process bypasses the use of chemical additives and hence is more effective in achieving simultaneous removal of multiple heavy metal groundwater pollutants like Mn, Fe, and As (Pokhrel and Viraraghavan 2009).

12.3.3.4 Novel Biosorbents

Genetically engineered biosorbents have enhanced selective and specific adsorption potential for toxic metal pollutants. These have been tested for their compatibility for

bioremediation of industrial effluents. This technique holds promise as an effective alternative; however, it is a relatively expensive approach (Vijayaraghavan and Yun 2008).

12.3.3.5 Bioaugmentation

This is an in situ process wherein genetically modified microbes with enhanced metabolic capacity are employed for bioremediation of specific pollutants. A very good example is a genetically modified *Escherichia* strain in which arsenic accumulation is enhanced by overexpressing arsenical resistance operon repressor gene (Kostal et al. 2004). Both genomic and metagenomic studies have identified newer genes encoding higher level of arsenic resistance. Newer or modified pathways for arsenic resistance can be orchestrated through directed evolution, metagenomics, and genome shuffling (Chauhan et al. 2009; Dai and Copley 2004).

12.3.3.6 Bacterial Sulfate Reduction (BSR)

Anaerobic packed-bed reactor system can be used for arsenic, chromium, nickel, and other heavy metal pollutants in acidic conditions from water and acid mine drainages using sulfate-reducing bacterial strains like *Desulfovibrio desulfuricans* (Jong and Parry 2003; Simonton et al. 2000; Steed et al. 2000). An efficiency up to 60–77% can be achieved. An alternate approach can be to remove arsenic by sequestration into insoluble sulfides in the presence of sulfate-reducing bacteria under anaerobic conditions. Apart from this, different organic substrates with sulfur oxide act as the terminal electron acceptor. Mobility can be increased by converting As(III) into As(V) and toxicity is reduced (Fukushi et al. 2003).

12.3.3.7 Phytoremediation

It is a process in which plants which possess tolerance toward certain metals are used for remediation of pollutants like arsenic. These plants accumulate pollutants intracellularly rendering them nontoxic. Hydrilla is a commonly used aquatic plant used in phytoremediation by phytofiltration. Phytoextraction involves uptake and translocation of pollutants in vascular system. Contaminant phytodegradation, also known as phytotransformation, involves uptake of pollutants by plants intracellularly or secretion of enzymes extracellularly for degradation. Formation of toxic intermediates is a bottleneck in this process. Phytovolatilization is another process in which uptake of metal pollutants occurs and released via transpiration into the atmosphere (Jadia and Fulekar 2009; Pivetz 2001; Ali et al. 2013a; Srivastava et al. 2011b).

The advantages of bioremediation over conventional treatment methods include competency, cost-effectiveness, minimal sludge formation, selectivity, lack of additives or nutrient requirement, regeneration of the biosorbent, and higher possibilities of metal recovery (Kratochvil and Volesky 1998). When the bioremediation occurs by its own self, it is termed as intrinsic bioremediation or natural attenuation, and when it is intentionally incited with fertilizer addition for enhancing bioavailability within the medium, then it is known as biostimulated bioremediation. Few most frequently used bioremediation technologies include bioleaching,

bioreactor, bioventing, composting, bioaugmentation, biostimulation, composting, land farming, rhizofiltration, phytoremediation, etc. (Li and Li 2011).

Bioremediation of polluted and contaminated site usually works in either one way of the two possibilities: (1) diverse substances and parameters including right nutrient, temperature, oxygen amount, etc. are utilized to augment the growth of pollutant-degrading microbes (indigenous microbes) present at the contaminated sites. (2) Specialized microbes (exogenous microbes) are directly introduced to degrade the pollutants. But in bioremediation by both means one gets rid of harmful chemicals and other pollutants. Thus, bioremediation application broadly comprises and falls under two main categories. In situ bioremediation is comparatively preferable strategy as it involves treatment of toxic materials directly at the site of contamination and hence less expensive and minimal discharge of pollutants occurs in the environment. Also due to confinement of pollutants and toxins and other pollutants, much lesser amount of nanomaterials (NMs) can clean larger volumes or area of polluted things. However, this process is significantly slower and sometimes poses difficulties in managing it. On the contrary, ex situ bioremediation relies on excavation of toxic substances and contaminated materials before actual treatment. The boons offered by ex situ bioremediation technique are faster processing, easier control, can be applied to a broad range of contaminants and soil types, etc. (Prokop et al. 2000; Dasand and Ansari 2009).

12.4 Nanobioremediation: Need for an Alternative Technology

12.4.1 Historical Perspective

The term "nanotechnology" was coined by Norio Taniguchi, in 1974 (Taniguchi 1974). Accordingly, "nanotechnology" was then broadly defined as the synthesis and application of structure of particles with dimensions less than 100 nm (Sadowski 2010; Rajendran and Gunasekaran 2007; Aagosh et al. 2013). In general, nanoparticles (NPs) can be defined as sub-nanosized colloidal structures, which can be synthesized by either employing synthetic or semisynthetic polymers (Vyas and Khar 2004). The synthesis of these nanosized sub-colloidal structures forms the fundamental and vital part of research owing to their countless applications in the field of information storage, electronics, optoelectronics, materials science, sensing devices, catalysis, recording media, environmental cleaning, drug delivery and medicine, etc. (Mallikarjuna et al. 2011).

It puts an ease on the researcher as it permits engineering of material properties through size control that further drives the research toward potential applications of these nanomaterials (NMs) in bioremediation (Shourian et al. 2009). The reduction in dimension of the materials to nanoscale level has distinct effects on assorted properties which may significantly differ from its counterpart bulk material (Duncan 2006).

12.4.2 Science of Bioremediation with Nanomaterials

Recently, a range of NMs have been explored in wastewater, soil, and air treatment (Fig. 12.1). For removal of respective pollutants here, the size-dependent unique properties of NMs (like higher surface area, strong capability of sorption, high reactivity, and faster dissolution) play a major role. Although several nanotechnological approaches that have been put forth are proven to be successful at lab scale, very few have been utilized at small-scale testing or commercialized to date. However, nanotechnological approaches like nanotech-associated membranes, nano-photocatalysts, and nanoadsorbents are most popular and even products based on these are commercialized.

Different properties exhibited by diverse NMs are beneficial for their use in bioremediation, for example, materials at nanoscale have a higher surface area, hence higher contact volume of material for interaction thereby enhancing its reactivity. Additionally, NMs have quantum effect, thus lowering the required activation energy and making chemical reactions feasible. Another phenomenon

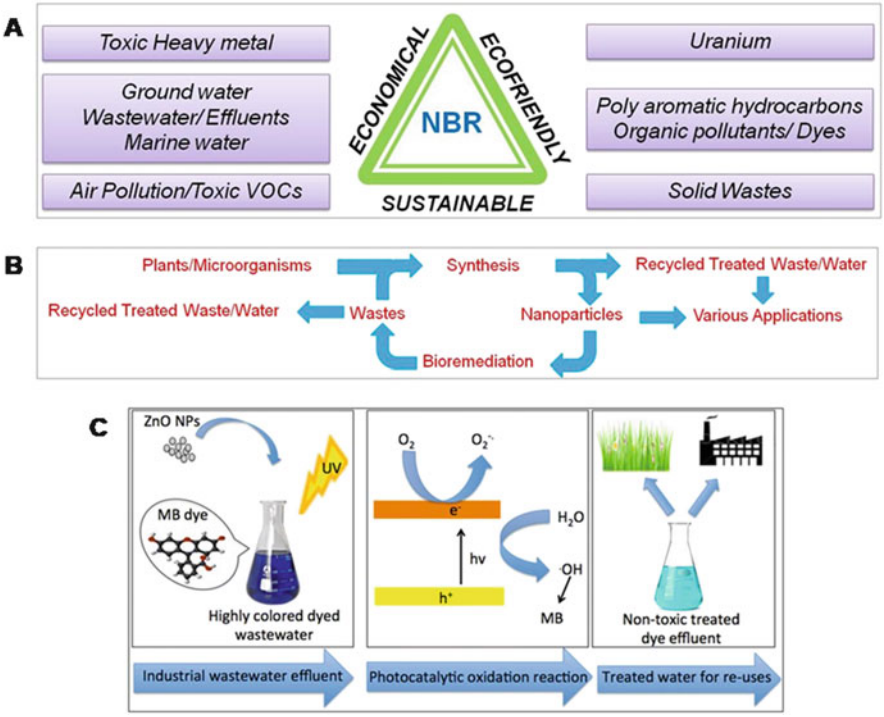


Fig. 12.1 (**a**) Schematic illustration showing various pollutant targets and the three most important attributes of NBR (nanobioremediation). (**b**) Flowchart depicting the cycling of nanomaterials and wastes in the environment through green synthesis and remediation process using nanotechnology. (**c**) Illustrative setup for wastewater treatment and recycling by photocatalytic ZnO NPs (Figure reproduced with copyrights permission from Tayeb et al. 2019)

displayed by NPs is surface plasmon resonance which can be used for toxicity detection. On the basis of shape and size, numerous metallic and nonmetallic NMs can be used for environmental cleanup. This is because NPs have the ability to diffuse or infiltrate into a contamination zone where microparticles are unable to reach besides their reactivity toward redox-sensitive pollutants is much higher. It is experiential that nanosized Fe^0 coated with oxide has the ability to form weak complexes with carbon tetrachloride (CT) and other similar contaminants, thereby increasing its reactivity. CT reacts with Fe^0 using electron transfer and is converted into either formate, CH_4, or CO_2; however in batch experiments and field assessment, trichloroethene, benzoquinone, and halogenated aliphatic hydrocarbons can be degraded into chemical by-products with comparatively quite lower toxicities (Nurmi et al. 2005). Furthermore, pentachlorophenol (PCP) degradation was achieved in a laboratory setting using TiO2 nanotubes through a photoelectrocatalytic reaction (Quan et al. 2005). Single-metal NPs can also be used as biocatalysts for reducing and removing chlorine.

A bioreductive assay was conducted in which it was observed that Pd(0) NPs were deposited on the cell wall and inside the cytoplasm of *Shewanella oneidensis*. Addition of electron donors such as hydrogen, acetate, and formate resulted in charging Pd(0) with H^* radicals. When a chlorinated contaminant such as PCP was brought in contact with these Pd(0)-coated, charged *S. oneidensis*, the H^* radical reacts on the Pd (0) resulting in removal of chlorine (de Windt et al. 2005). Microbial cells capable of degrading or biorecovering specific chemicals can be immobilized using NPs. Magnetic Fe_3O_4 nanoparticles modified with ammonium oleate were coated on cell surface of *Pseudomonas delafieldii*. Upon application of an external magnetic field, cells were separated from bulk solution and recycled for the treatment again. Microbial cells coated with NPs have been demonstrated to desulfurize organic sulfur from fossil fuel in a bioreactor system (Shan et al. 2005).

12.4.3 Nanosensors and Purifiers

Biosynthesis of NMs/NPs along with their applications in the field of sensors is inspiring for researchers as far as the nanotechnology research is concerned. In environment, nanosensors have been broadly used to monitor the environmental pollutants (inorganic as well as organic) that are prevalent in the atmosphere, in soils, and also in wastewater. Nano-detection sensors are used to detect and trace heavy metals and the persistent organic pollutants, and these sensors can be commercialized for application in real environmental systems (Ion et al. 2010).

Among all the diverse varieties of sensors, viz., biosensors, optical sensors, and electrochemical sensors, the usefulness as well as efficacy of the biosensors is highly encouraged to sense the contaminants within the environment. Functional components of a biosensor include the recognition element (for its binding to target component) and the transducer element (for signaling the downstream event). The sensor can bind the recognition element differently depending upon the factors such

as response time, signal to noise ratio, selectivity, and the limits of detection (Shipway et al. 2000; Anker et al. 2008).

Various NMs/NPs synthesized from various sources impart unique physicochemical properties that can be used to create new recognition and transduction processes forthe biological sensors (Pandey et al. 2008; Asefa et al. 2009; Agasti et al. 2010; Welser et al. 2011). Karuppiah et al. (2014) have reported the synthesis of AgNPs by wild twig bark (*Acacia nilotica*) and used it to detect 4-nitrophenol. AgNP-modified electrode had shown good electrocatalytic ability toward the 4-nitrophenol detection, owing to its commendable conductivity as well as compatibility.

Au and AgNPs were synthesized using extracts from stem of *B. rhamnoides* along with reduction of Au3+ ions to AuNPs consequently resulting into reduction of 4-nitrophenol (Gangula et al. 2011). The potential of Au as well as AgNPs for 4-nitrophenol reduction was tested in the presence of NaBH4. Owing to a large potential difference, a kinetic barrier consequently decreases the viability of this reduction. However, the kinetic barrier was overcome in the presence of NPs, by assisting the electron relay from donor (BH4-) to acceptor (4-nitrophenol). The detection of reaction could be accomplished by calorimetry by measuring the absorbance of 4-nitrophenolate ion at 400 nm.

Furthermore, a number of heavy metals present, such as Cr^{3+}, Cr^{6+}, Cd^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, and Hg^{2+}, act as toxic inorganic pollutants in environment. Lately, NMs/NPs have been used to detect the heavy metals and are also used widely as the colorimetric sensors possessing the high sensitivity. This approach is simple and cost-effective in order to track the toxic metallic pollutants. The applications of Ag and AuNPs for metal sensing have been documented in a very well manner, owing to the tunable size and distance-dependent optical properties of NPs with high extinction coefficients at a visible region (Lee et al. 2007; Nolan and Lippard 2008; Ray 2010; Aragay et al. 2011).

Incorporation of chelating agent on the surface of biosynthesized AuNPs is required for colorimetric sensing of metal ions. The metal ions in the environment induce aggregation of NMs/NPs and form a multi-dentate interparticle complex with chelating ligand, thereby causing a color change leading to the metal biosensing (Saha et al. 2012).

Silva-De Hoyos et al. have developed the speedy and efficient biogenic route by using *Citrus paradisi* extract as both reducing and capping agents, to synthesize the AuNPs having tunable size and optical properties (Silva-De Hoyos et al. 2018). These biosynthesized AuNPs have demonstrated metal sensing ability for Cu^{2+}, Zn^{2+}, Pb^{2+}, Ca^{2+}, and Hg^{2+} via both the plasmonic and fluorescent sensing techniques.

In addition, evaluation for the catalytic activity of biosynthesized AuNPs using IPE of bacterial strain *Staphylococcus warneri* was carried out by Nag et al. (2018). These biosynthesized AuNPs have shown greater surface-enhanced Raman scattering (SERS) activity in order to detect toxic compounds. Also, these NPs have shown an ability to degrade completely the toxic nitro aromatic pollutants such as 4-nitrophenol, 4-nitroaniline, 2-nitrophenol, and 2-nitroaniline with three times recyclability of their catalytic activity.

Wang et al. have detected Hg^{2+} with the help of unmodified AgNPs and mercury-specific oligonucleotides by using them both as the sensors (Wang et al. 2010). Additionally, 11-mercaptoundecanoic acid-functionalized nanoparticles (MUA-AuNPs) (13 nm) were used for the sensing of aqueous heavy metals like Cd, Pb, Zn, and Hg. The end point is a color change from red to blue obtained through heavy metal ion chelation process with the surface carboxylates, thereby proving successful application in metal sensing (Kim et al. 2001a).

Farhadi et al. have quoted the green synthesis of unmodified AgNPs for detection of Hg^{2+} (Farhadi et al. 2012). On the contrary, other study have reported the colorimetric detection of Mn2+ by AgNPs, in which AgNPs were cofunctionalized with 4-mercapto benzoic acid and melamine and used as a probe. Using L-tyrosine as both the capping and reducing agents has demonstrated a green synthesis-mediated metal biosensor for detecting Pb^{2+}, Mn^{2+}, and Hg^{2+} ions in an aqueous medium (Zhou et al. 2012).

Synthesis of nitrogen-doped carbon nanodots (CNDs) was carried out by Gu et al. (2016), and for this to achieve, the microwave treatment of lotus root (LR) was implemented. The synthesized LR-CNDs have shown selective sensitivity toward the Hg^{2+} with a detection limit of 18.7 nM. A variety of NMs/NPs are not only being biosynthesized but also functionalized for sensing several organic as well as inorganic environmental pollutants.

12.5 Green Synthesis of NPs for Nanobioremediation

Nanobioremediation (NBR) is the use of nanoparticles/nanomaterials produced by plant, fungi, and/or bacteria by means of nanotechnology, for elimination of environmental contaminants such as heavy metals, organic and nonorganic waste. Different bioremediation approaches are schematically shown in Fig. 12.2. In the past two decades, nanoscale materials owing to its efficacy, cost-effectiveness, and eco-friendly nature have replaced the existing treatment materials (Emmanuel et al. 2014). NBR is an emerging technique for environmental cleanup by removal of pollutants. Chemical and physical remediation, incineration, and bioremediation are the presently available technologies for remediation of the contaminated sites. With advancement in technology, an environmentally friendly and economically feasible alternative of contaminant removal from environment is obtainable by bioremediation (Dastjerdi and Montazer 2010). There are three key approaches of bioremediation which include use of microorganisms, use of plants, and enzymatic remediation.

Remediation of soil and water contaminated with heavy metals and organic and inorganic pollutants can be achieved using nanoparticles. In a recent study, it was observed that nanosized zerovalent ions can be used to degrade organic contaminants such as atrazine, molinate, and chlorpyrifos (Dimitrov 2006; Kavitha et al. 2013). The efficacy of phytoremediation can be increased using nanotechnology. Phytoremediation can be combined with nanoparticles in enzyme-based bioremediation (Ingale and Chaudhari 2013; Okhovat et al. 2015). For example, some complex organic compounds such as long-chain hydrocarbons and organochlorides

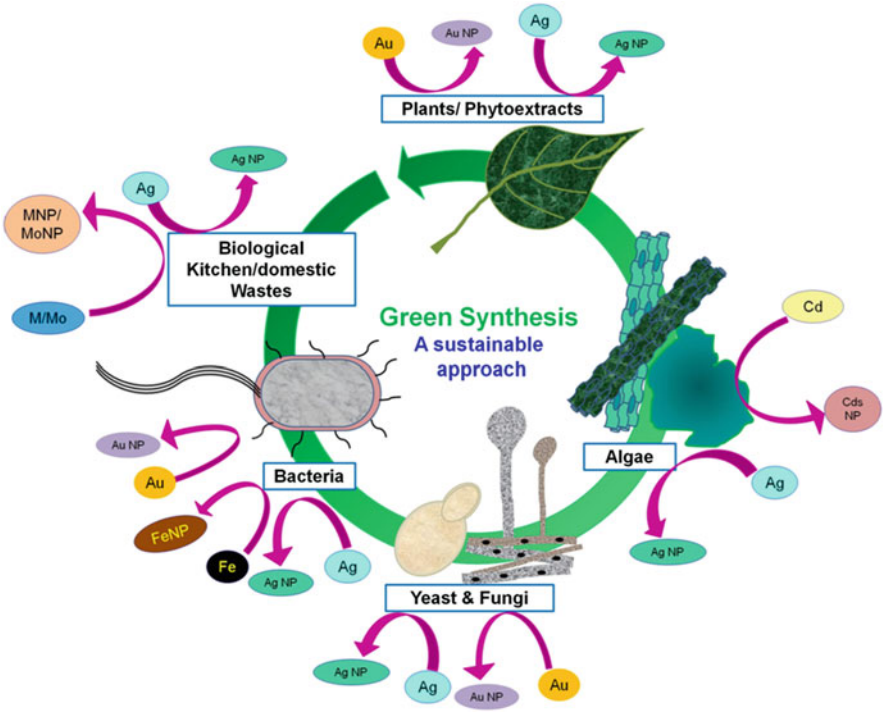


Fig. 12.2 Diagrammatic illustration showing green synthesis of nanoparticles as a sustainable approach (*NPs* nanoparticles, *M/Mo* metal/metal oxides; abbreviated elements as in periodic table)

are particularly resistant to degradation by microbes as well as plants. A combination of nanotechnology and biotechnology could be used to overcome this drawback: using nano-encapsulated enzymes, complex organic compounds would be degraded into simpler compounds, which can now be rapidly degraded by microbes and plants.

Nanoscale materials can be synthesized using microbial potential to reduce metal ions. Extracellular enzymes secreted by microorganisms can be used to synthesize relatively pure nanoparticles (Kumar et al. 2011; Tripathi et al. 2015; Alani et al. 2012; Durán et al. 2011; Krishnaswamy et al. 2014; Nanda and Saravanan 2009; Kalishwaralal et al. 2010; Calderon and Fullana 2015). Bacteria are valuable for NBR due to its unique metal binding property. Not only bacteria but fungi and yeast are also being used for nanoparticle biosynthesis (Yadav 2017; Balaji et al. 2009; Park et al. 2011; Ahmad et al. 2003a; Balagurunathan et al. 2011; Kuppusamy et al. 2014; Ahmad et al. 2003b; Malarkodi et al. 2013; Narayanan and Sakthivel 2013; He et al. 2007; Castro-Longoria et al. 2011). Fungi are used for bulk synthesis of nanoparticles because of its characteristic property of fungi of large protein volumes. However, synthesis of nanoparticles using plant extract methods is faster as compared to the microbial methods (Mishra et al. 2011; Saravanan and Nanda 2010). A

major drawback of NP synthesis by fungus is the production of catalytic enzymes which reduces salts to their corresponding solid NPs. This needs to be rectified to achieve a wide range of application of this method (Narayanan and Sakthivel 2011; Binupriya et al. 2010; Elcey et al. 2014; Arcon et al. 2012; Kaul et al. 2012; Zhang 2003a; Lee et al. 2008; Raliya and Tarafdar 2014; Raliya and Tarafdar 2013; Xu et al. 2005; Mazumdar and Haloi 2011). Advantages of microbes over other biological methods include the ease of handling, high growth rates, low cost requirement, easy culture methods, and less hazards caused to the environment (Kim et al. 2018; Varshney et al. 2010; Parveen et al. 2016; Umer et al. 2012; Majumder 2012; Salvadori et al. 2014; Honary et al. 2012; Cuevas et al. 2015; Abboud et al. 2014). Yeast threads and many fungi are being used for the synthesis of nanoparticles. Use of fungi has an advantage over use of bacteria for production of large amounts of nanoparticles (VishnuKirthi et al. 2011; Khan and Fuleka 2016; Jayaseelan et al. 2013a; Tarafdar et al. 2013; Subramanyam and Siva 2016; Raliya et al. 2013; Gurunathan et al. 2015; Yadav et al. 2018; Zhang 2009). Using fungi for synthesizing nanoparticles is considered as an eco-friendly route.

Environmental pollution is a major concern globally. Since the last two decades, crucial efforts have been put forth to minimize the extent of pollution, to manage diverse sources of pollutants, and to remediate the polluted soil, water, and air. Recently, a dried waste pool of Pb mine was used to conduct a field study for finding different native accumulator species of plants. The heavy metal contents in the dried pool soil and in the plants grown there were determined by flame atomic absorption spectrophotometry. As expected the heavy metal (Pb, Zn, Ni, Cu) contents of the soil were noted to be much higher than that of the natural soil. Also, the study findings revealed that few dominant vegetations, viz., *Scariola orientalis, Centaurea virgata, Gundelia tournefortii, Reseda lutea, Noaea mucronata*, and *Elaeagnus angustifolia*, have majorly accumulated toxic metals. To be more specific, the researchers concluded from the study results that *Noaea mucronata* (F. Chenopodiaceae) accumulated Pb majorly in addition to other heavy metals Cu, Ni, and Zn, whereas *Reseda lutea* and *Marrubium vulgare* have shown major accumulation of Fe and Cd, respectively. The researchers have evaluated bioaccumulation potential of NPs prepared using *N. mucronata* extracts using polluted water in containers. Experimental outcomes have depicted a manyfold reduction in heavy metal content over a bioremediation span of 3 days (Mohsenzadeh and Chehregani Rad 2012).

12.6 Generalized Mechanisms

More recently emphasis is on developing eco-friendly and sustainable nanotechnology-based platforms for environmental cleanup and remediation. A repertoire of nanomaterials or nanoparticles of organic or inorganic nature have been synthesized using microbial machineries. These have different ranges of sizes, shapes, and functions. Overall, nanomaterials and nanoparticles which are biologically produced employ three basic mechanisms for remediation of toxic metals and

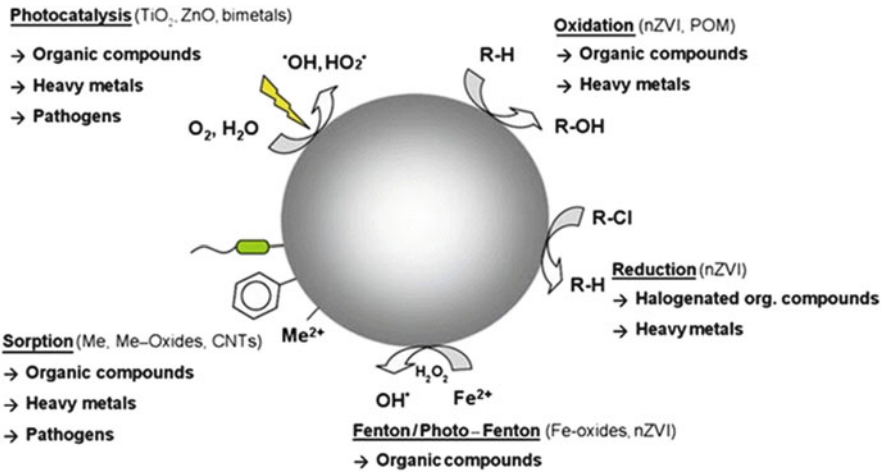


Fig. 12.3 Different mechanisms for nanobioremediation (R, compound; Me, metal; nZVI, nanoscale zerovalent iron; CNTs, carbon nanotubes) (Figure reproduced with copyrights permission from Prasse and Ternes 2010)

compounds, i.e., adsorption, transformation, and catalysis/photocatalysis (Fig. 12.3). In the following section, these mechanisms have been discussed in details.

12.6.1 Adsorption

Adsorption is a physical process wherein contaminants are adsorbed over an adsorbent surface due to ionic interactions. This process is exothermic in nature leading to negative value of enthalpy and decreased entropy. The Gibbs free energy decreases when the temperature and pressure is constant and the process occurs spontaneously. Nanoscale bioadsorbents can be an excellent choice of alternate technology for environmental pollution due to their economic and ecologic feasibility (Ruthven 1984). Various nanoscale adsorbents which are prokaryotic or eukaryotic in origin are used for remediating organic as well as inorganic pollutants.

12.6.1.1 Metal/Metal Oxide Nanoparticles (me/MeONPs)

These are used specifically for adsorption of heavy metal pollutants or more recently have been shown to have implications in organic pollutant remediation. Magnetic metal/metal oxide nanoparticles (Me/MeONPs) are used for large-scale and effective heavy metal remediation in wastewater treatment. Among all such particles, iron oxide NPs are the most effective in terms of adsorption and cost (Hu et al. 2010). It has been reported that significantly higher arsenic adsorption efficiency can be achieved by iron oxide nanoparticles as compared to conventional method. These nanoparticles had been prepared from tea extract and thus were eco-friendly and affordable as well for effective and faster wastewater treatment (Lunge et al. 2014).

Apart from this, iron oxide-based hollow nanospheres were shown to be as effective as magnetic separation for adsorption of dyes and other organic pollutants, thus making them a promising and novel approach. Yeast strains, for instance, *R. mucilaginosa*, have been used to synthesize copper nanoparticles using copper ions consequently leading to remediation of copper contaminated wastewater. Copper oxide-based nanomaterials have been used for adsorption of lead and organic dye suggesting the versatility of metal oxide-based nanoparticles (Salvadori et al. 2014). Silver and gold metallic NPs have also been reported to have applications in remediation. Zerovalent silver-based nanoscale adsorbents which are produced biosynthetically by using phytoextract from *Phyllanthus emblica* have been shown to be effective in remediation of mercury (Siva et al. 2013). Silver colloids have also been explored for the adsorption of organic compounds. Green synthesis of silver nanoparticles has also been one of the approaches with great potential for nanobioremediation.

12.6.1.2 Bimetallic Nanoparticles (BNPs)

Microorganisms can synthesize bimetallic nanoparticles both intracellularly and extracellularly. It has been shown that these can be applied for removal of both organic and inorganic pollutants in an effective and inexpensive way. A very good example is bimetallic gold-silver nanoparticles produced by using SCPs of BGA *Spirulina* (Chang et al. 2011; Govindaraju et al. 2008). Apart from this, these phyto-bimetallic nanoparticles can act as reductants which are safer than chemical reductants. Phenol and flavonoid groups in these phyto reductants assist in reduction and thereby imparts stability (Kumari et al. 2015). The other bimetallic nanoparticles synthesized by biological reduction process include silver-selenium, titanium-nickel, and gold-palladium, to name a few. Owing to a high catalytic efficiency, they have been suggested as potential tools for environmental remediation (Litter et al. 2014). Lastly, it needs to be mentioned that green synthesis of bimetallic nanoparticles for nanobioremediation is a promising approach, but extensive research needs to be carried out in this direction.

12.6.1.3 Modified Nanoparticles

As discussed above, metallic, metallic oxide, and bimetallic nanoparticles have been used for remediation. However, there are limitations in terms of attributes like extremely high reactivity, surface energy and magnetic properties, easy oxidation by dissolved air in water, and nonspecific competition for adsorption by molecules other than target pollutants. To ensure a longer residence and prevent aggregation of these nanoparticles in remediation systems, surface modifications can be done using polymers, silica, or other metals. Stability is achieved by coating their surface with organic polymers or surfactants and inorganic silica, oxides, or sulfides of metals. Surface-modified nanomaterials and nanoparticles achieve wastewater decontamination by binding the contaminants over their surface, magnetic selective adsorption, electrostatic interactions, and ligand binding (Jeevanandam et al. 2016). These surface modifications provide additional binding sites and thus increase efficacy of heavy metal and contaminant removal, e.g., surface modification of metallic and

metal oxide nanoparticles by organic materials leads to extra binding sites and facilitates higher cadmium adsorption. A combined removal of trichloroethylene (organic) and chromium (inorganic) pollutants has been demonstrated using agarose-iron nanoparticle hydrogel based on green synthesis (Nehru and Sivakumar 2012). Biologically synthesized smart nanoparticles and nanomaterials have been fabricated using extracts of *Amaranthus* plant to form PVA-coated silver nanoparticles. Nanocomposites consisting of silver, copper,and zerovalent iron nanoparticles have been used for multiple remediation purposes in treating effluents from pharmaceutical, tannery, and textile industries. In one of the reports, a nanocomposite composed of chitosan/silver NPs, copper NPs, and carbon nanotubes was shown to be highly effective for remediation of copper, cadmium, and lead toxic contaminants in water in a short time of 10 min and moreover possessed regeneration capacity making this process overall feasible (Suman et al. 2015). In another study, a novel, multipurpose, and recyclable nanocomposite was fabricated for targeting organic dyes, inorganic heavy metals, and microbial contaminants in water treatment. This nanocomposite was composed of nanocellulose and silver nano-embedded pebbles.

12.6.1.4 Other Nanosorbents

Chitosan methacrylic acid (MAA) nanoparticles have also been explored for adsorption of lead, cadmium, and nickel ions from water (Suvith and Philip 2014). Akaganeite NMs have also been tested for the sorption of antimony and arsenic derivatives (Shi et al. 2015). Nanocrystalline akaganeite (-FeOOH)-coated quartz sand (CACQS) can be used for bromate ion adsorption in wastewater (Tang and An 1995). ArsenXnp is hybrid ion exchange material consisting of polymers and oxidized nanoparticles which is commercially used in arsenic remediation. Similarly, nanocrystalline titanium dioxide bead with trade name ADSORBSIA is effective in arsenic remediation (Jayaseelan et al. 2013b). Both ArsenXnp and ADSORBSIA are economically feasible for small- to medium-scale remediation systems for drinking water.

12.6.2 Transformation

The mechanism of transformation involves oxidation and reduction chemistry for remediation of both organic and inorganic pollutants. This process is effective in reducing metal toxicity by stabilizing oxidation states of these metals. Zerovalent iron nanoparticles have been explored extensively in research for transformation of heavy metal pollutants including cadmium, chromium, nickel, zinc, and lead. The application of nanoscale zerovalent iron (nZVI) particles for transformation of chromium has been explored at bench and field scale as well. These particles reduce chromium by donating electrons and releasing ferrous ions, H_2 molecules, active H^+, and solid minerals with iron as a result of corrosion during the process (Singh et al. 2012). In one of the reports, these nZVI particles were shown to be effective in removing up to 85% of zinc ions in water. The dissolved oxygen in water leads to

corrosion, hence resulting into a coating of iron oxides over the surface of these nanoparticles. This increases the adsorption and coprecipitates the zinc ions effectively (Liang et al. 2014). Various organic pollutants such as polyaromatics, pesticides, nitrosubstituted amine, and aromatics, halogenated solvents and pesticides, organophosphates, etc. have been reduced using nanoscale zerovalent iron particles. A redox potential cycling occurs between different oxidation states of nanoparticles and metal ions (Rychoudhury and Scheytt 2013).

12.6.3 Catalysis

The process of catalysis plays a key role in energy production and environmental pollution control and remediation. Catalytic efficiency determines the resultant in above applications and is governed by number of active sites, larger surface area, and hence smaller size of the catalytic particle (Chaturvedi et al. 2012). Nanoscale catalysts of biological origin possess all these properties and are ecologically and economically feasible (Neyaz et al. 2014). Photocatalysis is the extended term used for light- or photon-based reaction catalysis. This approach can be used for biodegradation and remediation of organic and inorganic pollutants. The mechanism involves activation of semiconductor materials by irradiation in visible or ultraviolet range which leads to electron transfer between valence and conduction band, thereby generating holes. These holes are responsible for splitting of water into protons and hydroxyl ions which consequently oxidize and degrade pollutants. Superoxide anions are also generated in the process due to interaction of electrons in conductive band with the dissolved oxygen. The redox reaction with holes and electrons causes gradual degradation of organic compounds (Khan et al. 2015a).

Green synthesis of metal oxide-based nanoparticles, for instance, silver, titanium, and zinc, has been suggested to have implications in photocatalytic remediation. The advantages include cost-effectiveness, higher stability, and rate of reaction (Bhakya et al. 2015a). Silver nanoparticles from stem bark and root extracts of *Helicteres isora* have been studied for their role in reducing and degrading organic dyes such as methylene blue, safranin, eosin methylene blue, crystal violet, methyl orange, malachite green, etc. (Suvith and Philip 2014). Gold nanoparticles produced using intracellular proteins extracted from *Pycnoporus sanguineus* have been shown to photocatalytically degrade 4-nitroaniline. Titanium oxide-based nanoparticles of bacterial origin have photocatalytic applications in water disinfection (Tang and An 1995).

12.6.4 Fenton Reaction

Fenton reaction involves catalysis of hydrogen peroxide into a highly toxic hydroxyl free radical in an advanced oxidation process for remediation of water containing inorganic and recalcitrant organic pollutants. This approach is an alternative used in wastewater polluted with phenolics, pharmaceuticals, oil refinery products, etc. In

the conventional method, mostly iron oxides which are easily available and affordable are used as heterogeneous Fenton's catalysts. In this reaction, H_2O_2 splits into hydroxyl ions and hydroxyl radical and oxidizes ferrous into ferric ions. A second molecule of H_2O_2 subsequently reduces ferric into ferrous ions and produces proton and hydroperoxyl radicals which act as strong oxidizing agents for degradation of pollutants. Fenton's reaction like catalysis using hematite nanoparticles in conjuction with white rot fungi has been reported for degradation of bisphenol A which is a plasticizer and pollutant. The green synthesis of iron nanpoarticles in the Fenton reaction has been used for removal of nitrate from wastewater (Wang et al. 2014). In a recent review, the utility of Fenton's reaction and fluidized bed Fenton's process for assisting iron nanoparticle-based bioremediation has been emphasized. Its application has been demonstrated in synthetic wastewater treatment and has been tested in actual wastewater effluents (Tisa et al. 2014). A comprehensive list of examples of NMs used in bioremediation is quoted in Table 12.1.

12.7 Types of Nanomaterials and their Applications in Bioremediation and Biodegradation

A variety of NMs having different shapes and morphologies are used (Fig. 12.4). As compared to other conventional tools, NMs are superior adsorbents owing to their high surface area and reactivity, thus, beneficial for treatment of drinking water and industrial wastewater (Khin et al. 2012).

Adsorption, photocatalysis, or reduction of contaminants attributed by NMs can be used for treatment of wastewater (Das et al. 2015). Over the years, novel nanoscale materials such as nanoscale zeolites, metal oxides, Pd/Fe0 bimetallic NPs, zinc oxide, carbon nanotubes, chitosan, and graphene nanosheets have been investigated for bioremediation. Additionally, a number of organic NMs, including dendrimers, modified dendrimers, carbon nanotubes, calcium alginate, and multiwalled carbon nanotubes, have been developed for removal of metals, radionucleotide remediations, hydrogenation of toluene, and degradation of crude oil and phenol. Likewise, TiO_2 NPs, polysulfone-zVI, Ag-iron oxide/fly ash, poly (acrylic acid)-coated Fe oxide, and chitosan-gelatin/graphene oxide have been used for remediation of As, U, nitrate, and PCBs.

12.7.1 Metallic Nanoparticles

Iron NPs are considered as one of the first nanoparticles to be used in environmental cleanup (Tratnyek and Johnson 2006). For remediation of contaminated land or groundwater, the iron-based technologies used are broadly divided into two groups on the basis of chemistry involved in the process of remediation: adsorptive/immobilization technology, which uses iron as a sorbent, and reductive technology, which uses iron as an electron donor to degrade or convert contaminants into less toxic or mobile form (Cundy et al. 2008). However, many technologies make use of both

Table 12.1 Examples of use of nanomaterials in remediation

Process exploited	Target compounds	Nanomaterials used	Some of novel properties	Reference
Photocatalysis	Organic pollutants, VOCs, azo dye, Congo red dye, 4-chlorophenol and orange II, PAHs	TiO_2, ZnO, species of iron oxides (Fe III, Fe_2O_3, Fe_3O_4)	Photocatalytic activity in solar spectrum, low human toxicity, high stability and selectivity, low cost, and so forth	Khedr et al. (2009), Wang (2007), Bandara et al. (2007), Bahnemann (2004), Kim et al. (2001b), Wu et al. (2000)
Adsorption	Heavy metals, organic compounds, arsenic, phosphate, Cr(IV), mercury, PAHs, DDT, dioxin	Iron oxides, carbon-based nanomaterials such as dendrimersand polymers, carbon nanotubes (CNTs)	High specific surface area and assessable adsorption sites, selective and more adsorption sites, short intra-particle diffusion distance, tunable surface chemistry, easy reuse, and so forth	Bhaumik et al. (2012), Pan et al. (2010), Mueller and Nowack (2009, Mueller and Nowack 2010) Rickerby and Morrison (2007), Stafiej and Pyrzynska (2007)
Disinfection	Diamines, phenols, formaldehyde, hydrogen peroxide, silver ions, halogens, glutaraldehyde, acridines	Nanosilver/titanium dioxide (Ag/TiO_2) and CNTs	Strong antimicrobial activity, low toxicity and cost, high chemical stability, ease of use, and so forth	Amin et al. (2014), Donnell and Russell (1999)
Redox reactions	Halogenated organic compounds, metals, nitrate, arsenate, oil, PAH, PCB	Nanoscale zerovalent iron (nZVI), nanoscale calcium peroxide	Electron transfers such as photosynthesis, respiration, metabolism, and molecular signaling, nature of their redox centers	Zhang (2003b), Tratnyek and Johnson (2006), Nowack (2008), Klimkova et al. (2008), Chang et al. (2005, Chang et al. 2007), Varanasi et al. (2007)
Membranes	Chlorinated compounds, polyethylene, 1,2-dichlorobenzene, organic and inorganic solutes, halogenated organic solvents	NanoAg/TiO_2/zeolites/ magnetite and CNTs	Strong antimicrobial activity, hydrophilicity, low toxicity to humans, high mechanical and chemical stability, high permeability and selectivity, photocatalytic activity, and so forth	Donnell and Russell (1999)

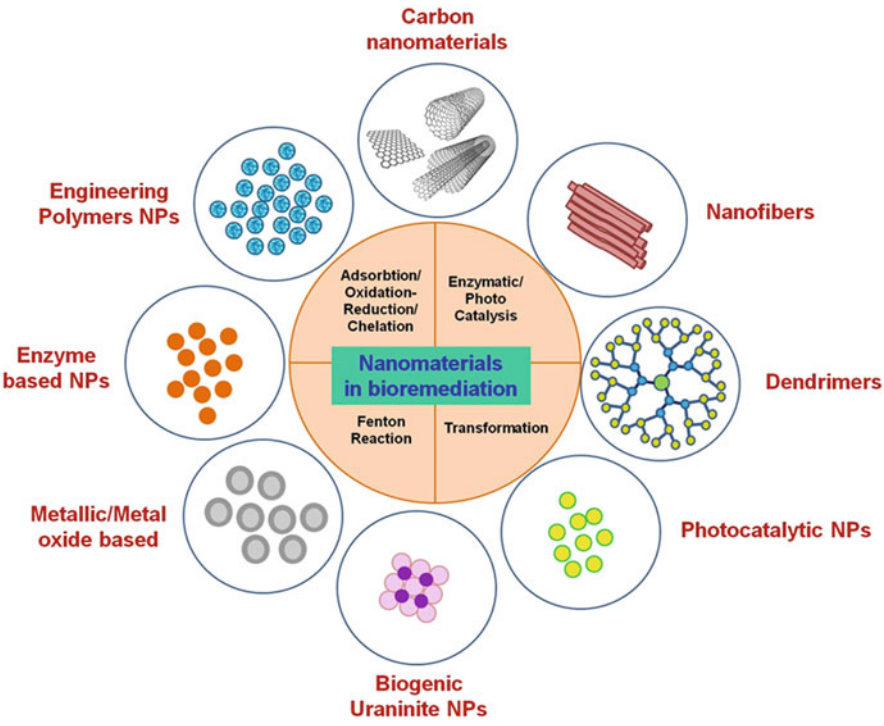


Fig. 12.4 Diagrammatic illustration showing different types of nanomaterials used in bioremediation. Inset depicting the mechanisms for nanobioremediation (*NPs* nanoparticles)

processes. Another extensively studied metallic NPs are Zn NPs, a semiconductor photocatalyst, which are capable of degrading organic dyes. A wide variety of compounds from dyes to phenols as well as pharmaceutical drugs can be completely degraded by Zn NPs using photocatalysis process (El-Kemary et al. 2010). Noble metal NPs like gold and silver have tremendous applications in diverse areas. Lately, the potential of Au and AgNPs in degradation of organic dyes has been analyzed. Lastly, copper NPs can exceptionally degrade organic dyes.

As(III), highly toxic, mobile, and predominant arsenic species found in anoxic groundwater, can be removed by nanoscale zerovalent iron (nZVI) (Kanel et al. 2005). Highly poisonous groundwater pollutant, As(V), can also be removed using nZVI as a colloidal reactive barrier material (Kanel et al. 2006). From an aqueous solution, Cr(VI) and Pb(II) can be rapidly separated and immobilized using zerovalent iron NPs "ferragels," which reduces the chromium to Cr(III) and the Pb to Pb(0), whereas Fe is oxidized to goethite (-FeOOH) (Ponder et al. 2000). In another study, anionic, hydrophilic carbon (Fe/C) and poly (acrylic acid)-supported (Fe/PAA) zerovalent Fe-NP were used for dehalogenation of chlorinated hydrocarbons in groundwater as well as in soils (Schrick et al. 2004). A reactive wall can be constructed in the path of a contaminated groundwater plume using iron

to degrade halogenated organic compounds (Wang and Zhang 1997). High surface-area nickel-iron NPs (1:3 Ni/Fe) have been studied as a reagent for the dehalogenation of trichloroethylene (TCE) (Schrick et al. 2002). PCP from aqueous solutions can be vanished because of dechlorination by zerovalent metals (ZVMs) or sorption to ZVM-related surfaces (Kim and Carraway 2000). Studies have been carried out to determine capability of powdered zerovalent iron for dechlorination of DDT and other related compounds at room temperature. In particular, DDT, DDD [1,1-dichloro-2,2-bis(p-chlorophenyl)ethane], and DDE [2,2-bis(p-chlorophenyl)-1, 1-dichloroethylene] can be successfully dechlorinated using powdered zerovalent iron in a buffered anaerobic aqueous solution at 20 °C, with and without the presence of nonionic surfactant Triton X-114 (Sayles et al. 1997) .

Metal-based nanoscale adsorbents can be used for removing heavy metals, such as Hg, Ni, Cu, Cd, As, Pd, Cr, etc. Calcium-doped zinc oxide NPs can serve like a selective adsorbent for removal of lead (Khan et al. 2015b). NMs composed of metallic oxide particles and magnetite and titanium oxide can adsorb heavy metals more effectively even when compared to activated carbon which is the most primitive and popularly used adsorbent (Mayo et al. 2007). Alternatively, metallic hydroxide particles can be coupled with activated carbon for arsenic and organic pollutant removal in wastewater treatment (Hristovski et al. 2009a; Hristovski et al. 2009b). Some of the most frequently used NPs of metallic oxide nature used in wastewater remediation include oxides of Mn, Cu, Ce, Mg, Ti, Fe, AgNPs, and titanate nanoflowers (Gupta et al. 2011a; Goswami et al. 2012; Cao et al. 2010; Gao et al. 2008; Luo et al. 2010; Fabrega et al. 2011; Feng et al. 2012; Huang et al. 2012a). Titanate nanoflowers display higher adsorption capacity as well as selectivity as compared to titanate nanotubes and nanowires. Further, Zhang et al. prepared arrays of magnesium hydroxide nanotubes to form $Mg(OH)_2/Al_2O_3$ composites in order to remove nickel ions from contaminated water (Zhang et al. 2006). A detailed list of metallic NPs used for bioremediation is covered in Table 12.2.

12.7.2 Enzyme NPs in Bioremediation

Enzymes are biological catalysts and play an important role in bioremediation. However, there are limitations such as short half-life and stability as well as manufacturing and purification cost which restrict their use in remediation. These limitations can be overcome by coupling them with magnetic nanoparticles. These make the process of separation of reactants and products from their sites easy under the influence of magnetic force. In a study, trypsinase and peroxidase enzymes have been attached to core-shell magnetic nanoparticles, and it was observed that these modified enzymes had a significant increase in half-life, activity, and stability. The magnetic nanoparticles conferred higher lifetime to these enzymes by shielding them from oxidation process. Also, magnetic properties enhance efficiency and post-catalysis productivity (Qiang et al. 2007).

Table 12.2 Metallic NPs used for bioremediation

Metallic NPs	Remediating moiety	Reference
AuNPs	4-Nitrophenol	Huang et al. (2009)
	Methylene blue	Gupta et al. (2010a)
	Tertiary dye effluent (methyl orange, acid orange 10, acid red 88)	Sathishkumar et al. (2013)
	Methylene blue	Suvith and Philip (2014)
AgNPs	Methylene blue	Morones et al. (2005)
	Textile effluent	Corso and De Almeida (2009)
	4-Nitrophenol	Gangula et al. (2011)
	Coomassie brilliant blue G-250	Arunachalam et al. (2012)
	Congo red	Modi et al. (2015)
	Organic dyes (methyl violet, eosin, safranin, methyl orange, methylene blue)	Bhakya et al. (2015b)
Cu NPs	Dichloromethane	Huang et al. (2012b)
	Methylene blue	Sinha and Ahmaruzzaman (2015)
	Methyl orange	Soomro and Nafady (2015)
Zn NPs	4-Chlorocatechol	Kamat (2002)
	Resorcinol	Pardeshi and Patil (2009)
	Fuchsine	Zhou et al. (2009)
	Congo red and benzopurpurine 4B	Elaziouti and Ahmed (2011)
	Rhodamine B	Zhao and Wang (2011)
	Phenol	Kruefu et al. (2012)
	Cd(II)	Srivastava et al. (2013)
	Rhodamine B	Ali et al. (2013b)
	Methylene blue	Srivastava and Thakur (2006)
	Methylene blue	Jain et al. (2014)
	Direct red 23	Kumar et al. (2014)
	Eriochrome black-T dye	Kazeminezhad and Sadollahkhani (2014)
	Brown CGG dye	Islam et al. (2015)
	Formaldehyde	Darvishi Cheshmeh Soltani et al. (2015)
	Malachite green	Khezami et al. (2016)
	Organic dyes	Sanna et al. (2016)
FeNPs	Pentachlorophenol	Kim and Carraway (2000)
	Perchlorate	Moore et al. (2003)
	Chlorinated ethanes	Song and Carraway (2005)
	Alachlor, pretilachlor	Kim et al. (2006)
	Ni(II)	Li and Zhang (2006)
	Brominated methanes	Lim et al. (2007)

(continued)

Table 12.2 (continued)

Metallic NPs	Remediating moiety	Reference
	4,4′-Dinitrostilbene-2,2′-disulfonic acid	Fan et al. (2007)
	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)	Naja et al. (2008)
	Dibenzo-p-dioxins and furans	Kim et al. (2008)
	Lindane, atrazine	Joo and Zhao (2008)
	Polychlorinated biphenyls (PCBs)	Choi et al. (2008)
	Atrazine	Satapanajaru et al. (2008)
	Alachlor and atrazine	Bezbaruah et al. (2009)
	Pyrene	Chang and Kang (2009)
	Cu(II), Cr(VI)	Huang and Chen (2009)
	As(V), Cr(VI)	Pradeep (2009)
	Cr(VI)	Cutting et al. (2010)
	Pb(II), hg(II)	Ambashta and Sillanpää (2010)
	Metolachlor	Santornchot et al. (2010)
	Cu(II), Pb(II)	Mahdavian and Mirrahimi (2010))
	Trichloroethylene	Smuleac et al. (2011)
	Cationic and anionic dyes	Shahwan et al. (2011)
	Nitrate	Ryu et al. (2011)
	Chlorobenzene	Lee et al. (2011)
	Uranium	Fan et al. (2012a)
	Dichloroethane	Wei et al. (2012b)
	Cr(VI) and cd(II)	Li et al. (2013a)
	Cd2+	Boparai et al. (2013)
	Dissolved sulfides	Chaung et al. (2014)

12.7.3 Engineered Polymeric NPs

Polycyclic aromatic hydrocarbons (PAHs) are a major pollutant in environment. Hydrophobic nature of these compounds makes them recalcitrant and insoluble and immobilizes them within soil. These compounds get sequestered and become unavailable by partitioning in nonaqueous phase. Modified polymeric nanoparticles are being used effectively to increase their solubility and availability making them accessible for biodegradation. An example is poly(ethylene)glycol-modified urethane acrylate (PMUA) precursor chain which helps in increasing bioavailability of sorbed and NAPL-sequestered phenanthrene and thus enhances effective rate of in situ mineralization of phenanthrene. In pumping and treatment-based water remediation process, bioreactors can recycle the extracted nanoparticles. These PMUA NPs are stable in a heterogeneous microbial population and thus recyclable after phenanthrene or other PAH is bound and subsequently biodegraded (Tungittiplakorn et al. 2005). Amphiphilic polyurethane (APU) NPs composed of polyurethane acrylate

anionomer (UAA) have also been used for remediation of PAHs locked up in soil by enhancing their desorption and transport in a similar way as surfactant micelles but have greater stability. APU particles have hydrophobic interior with a high affinity for phenanthrene (PHEN) and hydrophilic surfaces that promote particle mobility in soil. Size of the hydrophobic segment in chain synthesis influences affinity of APU particles toward pollutants and charge density over the surface influences its mobility within soil. The ability to control particle properties offers the potential to produce different NPs optimized for varying contaminant type and soil condition (Tungittiplakorn et al. 2004).

In a case study, chitosan-grafted biopolymer was studied for adsorption of metal ions. The underlying mechanisms involved are speculated to be chelation, electrostatic interactions, and ion pair formation (Guibal 2004). Other than these, complexation and diffusion might occur due to van der Waals and hydrophobic interactions, H bond, and ion exchange (Varma et al. 2004; Saha and Sarkar 2012). Apart from these, iron-oxide-coated sand (Devi et al. 2014) and magnetic nanoparticles (Ekramul Mahmud et al. 2016) encapsulated with polymer exhibit very high potential for heavy metal remediation.

12.7.4 Carbon Nanomaterials

Initially, activated carbon was used as an adsorbent for metal ion pollutants in remediation process; however, it was not effective at levels as low as in parts per billion. With technological development and research, new generation of carbon-based materials such as fullerenes, graphenes, and carbon nanotubes (Fig. 12.5) replaced activated carbon (Mauter and Elimelech 2008). Although activated carbon is a better adsorbent, it has limitations in remediation of heavy metals like a (Daus et al. 2004). Carbon-based nanomaterials such as nanocrystals and carbon nanotube (s) (CNT(s)) have extraordinary features which can have a broad range of environmental applications, namely, as sorbents, high-flux membranes, depth filters, antimicrobial agents, environmental sensors, renewable energy sources, and pollution prevention (Mauter and Elimelech 2008). Single-walled, multiwalled, and hybrid carbon nanotubes (SWCNTs, MWCNTs, and HCNTs) have tested for remediation of ethylbenzene-contaminated water. It was observed that SWCNTs were more efficient. Thus, it could be used for water remediation and prevent environmental and health hazards of ethylbenzene (Bina et al. 2012). Recently, cyclodextrins (CD) and CNT(s) have been used for detecting and treating water pollutants. CDco-hexamethylene/toluene-diisocyanate polyurethanes and CNT-modified equivalents have been used in reducing the level of organic contaminants in water up to very low levels. An eco-friendly adsorbent, CNTs immobilized by calcium alginate (CNTs/CA) have been used for remediation of copper up to 69.9% even in acidic pH conditions (Li et al. 2010a). CNTs can be modified according to the target-specific pollutants. MWCNT(s) (Kandah and Meunier 2007a) and magnetic MWCNT nanocomposites have been used for remediation of water polluted with nickel and cationic dye, respectively (Gong et al. 2009a).

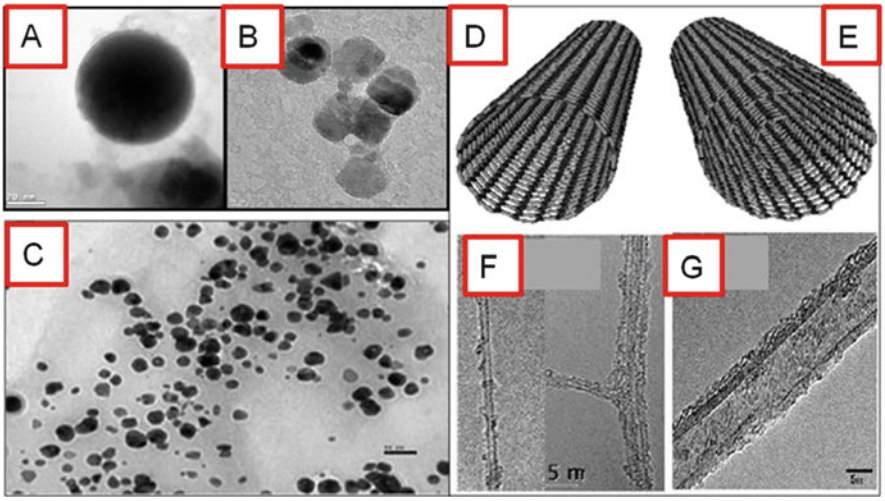


Fig. 12.5 Nanomaterials with different types of morphologies for various applications. (**a**, **b**) TEM image of nano-Fe^0 particle in the suspension. (**c**) TEM image of synthesized biogenic AgNPs. (**d**, **e**) Schematic structures of SWCNT and MWCNT. (**f**, **g**) TEM image of SWCNT and MWCNT (Figure reproduced with copyrights permission from Kumar et al. 2017; Gowramma et al. 2015; Eatemadi et al. 2014)

12.7.5 Nanofibers

Nanofibers are ultrafine nanomaterials made of metals, polymers, or ceramics by a simple and low-cost process called electrospinning (Cloete et al. 2010; Malwal and Gopinath 2016). The design of nanofibers, i.e., its composition, spatial organization morphology, and diameter, is influenced by their applications. They are porous and have large surface area and thus can form mats with complex pore structures. These have been utilized in air filtration on a commercial scale; however, their application in water remediation is still elusive. Nanofibers can remove microparticles at a high rate from water and are therefore used in preliminary treatment followed by ultrafiltration or reverse osmosis (Peng et al. 2015). Nanoparticles with specific functional groups or materials like titanium dioxide are incorporated into nanofibers during electrospinning, and then these are used in production of membrane filters for multipurpose applications. Electrospun polyacrylonitrile nanofiber mats are used as potential adsorbents for heavy metal ion remediation (Kampalanonwat and Supaphol 2010). Carbon nanofibers grown on iron (Fe) are being used for remediation of wastewater polluted with arsenic (V) (Gupta et al. 2010b). Nickel oxide nanofibers which have been fabricated using poly(ethylene oxide) template have been studied for their use in degradation of dyes in water effluents (Malwal and Gopinath 2015).

12.7.6 Dendrimers

These nanoscale polymers are highly branched monodispersed macromolecules with potential applications first reported by Buhleier et al. (Buhleier et al. 1978), Tomalia et al. (Tomalia et al. 1984), and Newkome et al. (Newkome et al. 1985). The dendrimer structure has specific composition and has three components, namely, a centralized core, interior, and terminal branch cell (Undre et al. 2013a). The presence of voids in the structure facilitates interaction with other substance (Undre et al. 2013b). Dendrimers can therefore make composites with nanoparticles for enhancing catalytic activity and can be effectively used for water and dye remediation. The advantages include increased reactivity, surface area, and decreased toxicity making them ideal for use in clean water recovery units, e.g., PAMAM dendrimers. Apart from this, filtration units with titanium oxide porous ceramic filters with pores impregnated with an alkylated poly(propylene imine) dendrimer or poly (ethyleneimine) hyperbranched polymer, etc. has been designed for organic pollutant remediation. Dendrimers possess hydrophobic interior for adsorbing organic pollutants and possess a hydroxyl or amine group on the surface exterior to capture heavy metals. The dendrimer in an ultrafiltration system can thus recover copper ions (Rao Kotte et al. 2015). After the adsorption process, the dendrimers along with heavy metal ions can be regenerated in acidic conditions as well.

12.7.7 Photocatalytic

Photocatalysis as described in the mechanisms section is one of the most important and effective pretreatment approaches for remediation of nonbiodegradable and toxic pollutants. However, decreased activity and slow kinetic reaction are major obstacles in their use (Qu et al. 2013). Nanoscale semiconductor materials like oxides of zinc, titanium, and tungsten and cadmium sulfide have been used for photocatalytic remediation applications. These are easily available, affordable, and very less toxic in nature (Lin et al. 2005; Jing et al. 2006; Nakataa and Fujishima 2012).

12.7.8 Biogenic Uraninite NPs

Biogenic uraninite nanoparticles have a considerable small size and biological origin, making them interesting option for uranium remediation. Irrespective of its very small size, the molecular-scale structure, surface area, energy, and dissolution rates of hydrated biogenic uraninite are similar to coarse abiotic uranium oxide. These observations have suggested nanoparticle size to be a factor determining aqueous reactivity and bioremediation of subsurface uranium iron pollution (Bargar et al. 2008). An inclusive list of NMs used in the remediation of organic and inorganic pollutants is covered in Table 12.3.

Table 12.3 Nanomaterials used in the remediation of organic and inorganic pollutants

Nanoscale materials (NMs/NPs)	Pollutants	Reference
Organic NMs		
Dendrimers (PAMAM)	Binding of Cu(II), iron chelators, radionuclide remediation, metal ion remediation	Ottaviani et al. (2000), Diallo et al. (2005), Mankbadi et al. (2011), Guo et al. (2012), Barakat et al. (2013)
Modified dendrimers (PAMAM/TiO_2)	Remediation of Cu(II), Ni(II), and Cr(III)	Crump et al. (2008)
Dendrimer-encapsulated nanoparticles (DENs)	Cobalt (Co) oxidation and toluene hydrogenation	Xia et al. (2019)
Carbon nanotubes (CNTs)	Ethylbenzene	Bina et al. 2012
CNTs/Ca-alginate	Cu(II) removal	Li et al. (2010b)
CeO_2-CNTs	Heavy metal ions	Peng et al. (2005), Di et al. (2006)
Multiwalled CNTs (MWCNTs)	Cationic dyes, crude oil degradation, Ni(II), naphthalene	Kandah and Meunier (2007b), Cho et al. (2008), Gong et al. (2009b), Abbasian et al. (2016)
Hybrid CNTs (HCNTs)	Ethylbenzene	Sharma et al. (2018)
CNT/Ce-TiO_2	Phenol degradation	Shaari et al. (2012)
CNTs/Fe-Ni/TiO_2	Degradation of methylene blue	Ma et al. (2014)
MWCNT/ZnO	Acetaldehyde degradation	Saleh et al. (2011)
CS-MWCNT	Degradation of Congo red	Chatterjee et al. (2010)
MWCNTs/PAAM	Pb(II) removal, Pb and organic compound removal	Gupta et al. (2011b), Yang et al. (2011)
Chitosan nanoparticles	Pb(II) removal	Qi and Xu (2004)
Magnetic chitosan/graphene oxide (MCGO)	Pb(II) removal, methylene blue	Fan et al. (2013), Ramesha et al. (2011)
Modified magnetic chitosan chelating resin (CSIS)	Adsorption of Cu(II), Co(II), Ni(II)	Monier et al. (2010)
Chitosan derivatives (chitosan-GLA)	Pb(II) biosorption	Ngah and Fatinathan (2010)
Ethylenediamine-modified magnetic chitosan nanoparticles (EMCN)	Adsorption of dyes	Castillo et al. (2014)
Graphene nanosheets (GNSs)	Heavy metal removal	Huang et al. (2011), Fan et al. (2012b)
Exfoliated graphene oxide and reduced graphene oxide (EGO and RGO)	Adsorption of anionic and cationic dyes	Zhou et al. (2011)
Polypyrrole-reduced graphene oxide (PPy-RGO)	Adsorption of Hg(II)	Chandra and Kim (2011)
Chitosan-gelatin/graphene oxide (CGGO)	Removal of arsenic (As)	Chandra et al. (2010)

(continued)

Table 12.3 (continued)

Nanoscale materials (NMs/NPs)	Pollutants	Reference
Self-assembled perylene-3,4,9,10-tetracarboxylic diimide-NH(PDINH) supramolecular system	Degradation of phenol, methylene blue (MB), rhodamine B (RhB), methyl orange (MO), bisphenol A (BPA)	Liu et al. (2016)
7,7,8,8-Tetracyanoquinodimethane-perylene tetracarboxylic diimide (TCNQ-PTCDI)	Phenol degradation and oxidation of water	Zhang et al. (2016)
NH2-MIL-68@TPA-covalent organic framework hybridmaterial (NH2-MIL-68@TPA-COF)	Organic pollutant degradation	Peng et al. (2018)
Graphene quantum dots/Mn-N-TiO_2/g-C3N4 (GQDs/TCN)	Photodegradation of organic pollutants, like p-nitrophenol, diethyl phthalate, and ciprofloxacin	Nie et al. (2018)
PTCDI/P25 TiO_2 nanoparticles	Decomposition of MO, phenol, and formic acid	Wei et al. (2016)
Zeolites	Heavy metals	Oliveira et al. (2004), Kocaoba et al. (2007), Guan et al. (2010), Mallard et al. (2015)
β-Cyclodextrin-zeolites	Organics	Jia et al. (2009)
Inorganic NMs		
TiO_2	Organic, arsenic, γ-HCH	Varshney et al. (2016), Wang et al. (2016), Jing et al. (2009)
3D MnWO4-TiO_2	Degradation of dye	Xu et al. (2007)
Ti3+ self-doped mesoporous TiO_2	Organics	Hassan et al. (2015)
TiO_2/LDH	Dye removal	Wen et al. (2015)
Ni/Pt-doped TiO_2	Degradation of rhodamine B dye	Shao et al. (2014)
Titanium phosphate	Heavy metal removal	Pol et al. (2016)
Polyacrylic acid-modified nZVI (PAA-nZVI)	Heavy metal arsenate removal	Laumann et al. (2013)
3MPA-SPION	Uranium, ammonium, bisphenol A	Morillo et al. (2015), Zare et al. (2016)
Nano-Fe_3O_4	Uranium, plutonium, nitrate, cadmium, chromium, antimony	Crane et al. (2011), Li et al. (2011a), Dorjee et al. (2014)
Iron NPs-Ca alginate	Inorganic nitrate removal	Georgiou et al. (2015)
Polysulfone-zerovalent iron	As(III) remediation	Fresnais et al. (2013)
Polyacrylic acid-coated iron oxide	Dye removal	Shi et al. (2013))

(continued)

Table 12.3 (continued)

Nanoscale materials (NMs/NPs)	Pollutants	Reference
Kaolinite-supported Fe/Ni nanoparticles Ag-iron oxide/ fly ash	Simultaneous catalytic remediation of lead and nitrate	Joshi et al. (2015)
G-nZVI	Polychlorinated biphenyls	Jabeen et al. (2013)
IONPs immobilized in PEG/nylon membrane	Pb(II) absorption, treatment of Pb(II)-contaminated water, nitrobenzene	Tong et al. (2011)
MNHFO	Adsorption of as(III)	Gupta et al. (2010c)
IONPs embedded in orange peel pith	Cr(VI) removal	López-Téllez et al. (2011)
Fe(II)-montmorillonite	Cr(VI) removal	Vinuth et al. (2015)
FeS	Metals (Hg) and halogenated organics	Xiong et al. (2009); Henderson and Demond 2013)
Copper oxide	Removal of methylene blue, dechlorination of organic dichloromethane	Mustafa et al. (2013)
CdSe	Photocatalytic degradation of dye	Harris and Kamat (2009)
CdS	Cd(II) removal, organic dye	Raj et al. (2016), Pant et al. (2014)
Zinc oxide	Photo remediation of heavy metals, pharmaceuticals, toxic dyes and chemicals, thorium	Banerjee et al. (2012), Choina et al. (2015)
Hydrous cerium oxide (HCO)	Cr(VI) removal	Albadarin et al. (2014)
Bimetal iron(III)-titanium(IV) oxide (NHITO)	Arsenic removal	Gupta and Ghosh (2009)
Fe/Pd nanoparticles	Dye removal	Luo et al. (2016)
CMC-stabilized Pd/FeNPs	Pentachlorophenol remediation	Yuan et al. (2012)
Fe-Mn oxide nanoparticles	As(III) immobilization	An and Zhao (2012)
Pd/Fe0 bimetallic NPs	Degradation of γ-HCH	Singh et al. (2012)
Ni/Fe nanoparticles	Dechlorination of PCB	Seteni et al. (2013)
N-F-TiO_2	Bisphenol A	He et al. (2016)
Au/CdS nanoparticles	Cyanide solution	Aazam (2014)

12.8 Nanobioremediation in Marine Ecosystems

In the recent past, diverse technologies (to be specific physical, chemical, biological) have been proposed and adopted for protecting and restoring marine environment from various pollutants. Most of these technologies have established their significance in retort contingency plans set for marine oil spillage. In a broad sense,

biostimulation (stimulating the growth of indigenous microorganism) and bioaugmentation (inoculating external degrading bacteria) are recognized as promising approaches for decontaminating/detoxifying a polluted site with minimal degree of impact on ecological system (Hassanshahian et al. 2012). Though bioremediation offers versatility in application and is seen as a flexible and excellent strategy for recovering pollutants, it is practically ineffective in case of higher concentrations of pollutants, xenobiotics, refractory compounds, etc., resulting in untenable recovery time and treatment efficiencies (Mapelli et al. 2017). Considering these things, nanotechnological developments and integration of NMs represent a potential solution to deal with pollutants to facilitate bioremediation and thereby surpass the allied limitations for ex situ or in situ applications. Moreover, a combined approach emerges to embrace a quite wider range of crucial applications with greater cost cutting and control over formation of undesired by-products (Cecchin et al. 2017b; Yadav et al. 2017).

In context of all these facts, it seems fascinating to make use of NPs and other NMs in optimization of varied recovery strategies implied in harsh environmental conditions as like the Antarctic and Arctic areas (Cappello et al. 2014). The traditional bioremediation strategies (bioaugmentation and/or biostimulation) are minimally effective under the odd polar environmental conditions corresponding to reduced bacterial catalytic rate, and thus NPs and NMs can offer numerous efficient ways for dealing with this problem.

12.9 Nanobioremediation in Air Pollution

Frequent exposure to sulfur oxides, nitrogen oxides, and volatile organic compounds (VOCs) can lead to potential human health damages and could also result in severe symptoms (Husken et al. 2009; Li et al. 2011b; Zhu et al. 2017). To be more particular, the VOC concentration indoor always supersedes the outdoor one. The most widely adopted approaches for removing VOCs are enhancing ventilation rate, pollutant source control, and cleaning of air (Okachi and Onaka 2004; Yu and Jeong Tai 2010). Nowadays most advanced air purifying or cleaning systems are usually based on adsorbents like activated carbon, photocatalysis, or ozonolysis (Guerra et al. 2018a; Huang et al. 2016; Jeong et al. 2005; Liu et al. 2017; Lofrano et al. 2017; Solsona et al. 2016; Wang et al. 2018; Yu and Kim 2012; Zou et al. 2006). Currently, the most effective method to regulate VOCs and gaseous oxides in ambient air purification is by photocatalytic oxidation (Boulamanti and Philippopoulos 2009; Farhanian et al. 2013; Ifang et al. 2014). Activated carbon or natural coal (e.g., wood or coconut shells) can be used for adsorption of VOCs. But this process is expensive and cumbersome as the adsorbent cannot be regenerated and hence nonreusable (Khan and Ghoshal 2000). Zeolite (Vidal et al. 2012), mesoporous organosilica (Moura et al. 2011), and low-cost materials (e.g., green coconut shells, etc.) (Crisafully et al. 2008) are being explored for adsorption of VOCs.

Nanotechnological developments and advanced NMs are not only having promising applicability in water and soil treatment for removing respective pollutants but also are of great significance in getting rid of pollutants from air (Guerra et al. 2018b; Masciangioli and Zhang 2003; Vaseashta et al. 2007; Wang et al. 2018; Xiong et al. 2018; Yunus et al. 2012). In the upcoming lines we are briefing a few prominent examples of applications of NM-based approaches in cleaning air. Many reports cover such implications involving volatile organic compounds (VOCs) and toxic gases such as metal oxides and metals (Sinha and Suzuki 2007), polymers (Guerra et al. 2017), silica (Nomura and Jones 2013), plasmonic (Wang et al. 2013), and carbon (Lithoxoos et al. 2010; Ong et al. 2010).

The potential use of NMs for cleaning air basically depends on several factors like air type, recycling processes and recovery processes of NMs, etc. Metal oxide and metal-based NMs have further widened the scope of nanometallic component applications in removal of VOCs and gases. Few selective examples of such attempts are use of mesoporous Fe oxide for VOC (toluene and propane) oxidation (Som et al. 2010), use of titanate nanotubes for specific catalytic reduction of gases (NH_3, NO_2) (Camposeco et al. 2014; Chen et al. 2013; Lee et al. 2017), and AuNPs with highly porous Mn oxide for removing organic pollutants from indoor air (hexane, toluene, acetaldehyde), etc. (Sinha and Suzuki 2007). According to the researchers, the said pollutants from the air were efficiently removed and degraded in a much better way than that of the conventional catalytic systems. Silica NMs exhibit high adsorption capacity and have been deeply studied and utilized for gaseous contaminant remediation. For example, silica NMs have been developed in form of amine-modified xerogels and were used for removing H_2S and CO_2 from natural gas (Huang et al. 2003), and porous silica and amine-modified aluminosilicate were used for selectively removing ketones, aldehydes, and CO_2 (Bollini et al. 2011; Drese et al. 2011; Hazen 2018). All these silica-based NMs work on basis of covalent or ionic bonding among amine functional group of NP surface and molecule of interest, which offers rapid capture of materials of interest with greater selectivity (Guerra et al. 2018a, 2018b). In a research attempt, Guerra et al. have developed polymeric NPs followed by surface activation via incorporation of amine functional groups onto the surface using poly(ethyleneimine). The surface-activated NPs exhibited higher VOCs capturing capacity with greater efficiency. In a similar fashion, functionalized polymeric NPs were also prepared for capturing aldehyde and carboxylic acid gaseous pollutants (Gethard et al. 2011; Campbell et al. 2015). Apart from these, plasmonic NMs have also depicted promising potential in this meadow owing to their customized properties (Wang et al. 2013, 2018).

12.10 Bioremediation of Electronic Waste

Electronic waste usually referred as “e-waste” is obtained from integrated circuits of discarded and obsolete electronic goods such as laptops, computers, mobile phones, radios, iPods, printers, batteries, etc. to list a few. Almost all components (>90%) of integrated circuits can be reused and recycled. Furthermore, many heavy metals

forming part of such circuits (e.g., As, Cu, Si, Fe, Pb, Hg, Zn, Cd, Ba, and Cr) are valuable and reasonable for recycling. Recycling of these e-wastes via chemical and/or physical treatments using varied reagents and strong acids is prone to be hazardous and non-economical and further enhances burden on the environment. On the contrary, use of biological methods for synthesizing NPs and NMs via microorganisms and plant extracts can be an effective way for recycling, which is quite safe and economical too. In a scientific report, Majumder et al. have used *Lantana camara* weed leaf extract and microbes (*Pseudomonas* and *Fusarium*) for extraction of Cu as NPs from integrated circuits of e-wastes. The efficiency of extraction reported using the biological tool was much higher, and this research thereby demonstrated application of biological methods in bioremediation of metals from e-wastes, which is economical as well as eco-friendly (Majumder 2012).

12.11 Advances in Nanobioremediation Technology

One of the key aspects of nanobioremediation is implication of biotechnological tools for environmental cleanup. It mainly encompasses three noteworthy attributes, viz., (a) use of clean, benign, green NMs; (b) provision of solution for removing harmful materials from polluted sites; and (c) utilization as sensor for environmental variability (Tratnyek and Johnson 2006).

To be particular, "environmental improvements" can be achieved by putting efforts in the nanobioremediation and its prominent phenomenon recognized as reactive or adsorptive techniques which can be applied on-site (known as in situ) or off-site (known as ex situ) for removal of pollutants. The former technique works on the basis of sequestration for heavy metal contaminant removal, while the later technique works by degrading organic contaminants.

For a long-term mitigation of environmental pollutants, implication of these nanobiotechnological strategies is the need of time. Diverse biogenic NMs developed for the remediation purpose and explored for the same include metal oxide variants and noble metals particularly titanium dioxides and bimetallic forms. Among these all, nanoscale zerovalent iron (nZVI) and nZVI derivates are noted to be more significant in nanoremediation. Researchers have reported encouraging applicability of these NMs in degradation of unrelenting organic compounds and removal of toxic metals such as arsenic and chromium (Thome et al. 2015a).

Owing to its cost-effectiveness, higher efficiency, and application at large scale, in situ treatment of contaminants is most feasible and usually preferred approach. The principle involves injection of nZVI into porous contaminated sites such as sediments, aquifers, and soil. The accelerated degradability of pollutants in turn of nZVI's higher reactivity has been well established in many reports of literature. However, sequential reduction and loss of reactivity with aging and pollutant agglomeration are major hurdles in abundant use of nZVI in bioremediation (Reddy and Adams 2010; Yan et al. 2013). Considering these facts, attempts have been made to improve the reactivity, stability, and mobility of nZVI via surface coating with organic polymers, e.g., lactate, guar gum, etc. These attempts to modify

nZVI properties and action potential were a big success, and post-coating nZVI depicted improved aforementioned characteristics (Reddy et al. 2014). In this fashion, augmented site decontamination has been achieved via a simple strategy.

The general mechanism of bioremediation process starts with reduction of contaminant level followed by biodegradation of the same, till the contaminant level go down than risk level. Along with the water and/or wastewater and soil treatment, nowadays air pollution monitoring and air pollutant control are also in progression with advancements in nanotechnological approaches. Carbon nanotubes have shown manyfold absorption of dioxin than that of activated carbon owing to its higher surface area in nanoform. Nanobiotechnological approaches in nanobioremediation are still in progressive stage, since advancements allied to their shape, size, and surface chemistry are yet to be optimal. Moreover, making use of these NMs for nanobioremediation will certainly cause release of these NMs in different strata of environment, and mankind would be surely exposed to them via ingestion, inhalation, injection, topical contact, etc. Thus, to assess toxicity on biological systems and to confirm the degree of safety of these fabricated NMs, a thorough in-depth research is the need of the hour.

12.12 Pros and Cons of Nanomaterials in Bioremediation

Though NPs offer promising outcomes in contaminated site treatment and removal of pollutants, a few tribulations are associated with their reactivity loss with passing time, transportation, and effect on microbes (Bhakyaraj et al. 2017; Li et al. 2017). FeNPs exhibit a loss in reactivity post certain time period and also depict a blocking effect in soil via blocking the soil pores, which prevents passage of liquids through soil. Researchers proposed use of stabilizers like lactate to enhance mobility of FeNPs in soil consequently facilitating their transport in soil (Soni and Prakash 2012a, b).

Another major concern with the NPs is the toxicity of metallic and nondegradable nanomaterials on microbial strains and communities. Till date numerous experimental assessments have been performed to determine effects of NPs on microbes, and results of these studies are conflicting (Soni and Prakash 2012c). Few studies reported inhibitory effects on microbes like *Escherichia coli* and *Staphylococcus aureus* (Soni and Prakash 2014, 2015; Morrison et al. 2002). On the contrary, in few other research studies, clear stimulation of NPs as electron donors on microbes has been reported (Zhang 2009; Naraginti and Sivakumar 2014).

As we all know, soil microorganisms are enormously imperative for the natural cycle of nutrients in the environment, and they possess the ability of naturally degrading organic contaminants and/or of reducing and immobilizing the heavy metals. Hence, a radical reduction in these microflora could result in weakening of contamination resistance of soil (Prasad and Jha 2009; Mashrai et al. 2013). Apart from this, toxic effect of nano-iron oxide-based nanoparticles has tendency to disrupt the cell membrane by generating reactive oxygen species which ultimately lead to cell death. In addition, absorption of nutrients via cell membrane is hindered by

nano-iron oxide compounds, thereby inhibiting their growth (Chong et al. 2010; Turner 2017). However, nano-iron oxide-based compounds have not been reported to show any adverse effects on the growth of fungal colonies (Thome et al. 2015b; Tratnyek and Johnson 2006). To reduce the toxicity level of iron NPs, many researchers have even proposed coating of these particles with organic polymers. Furthermore, several studies have proved that for resisting and countering toxicity of NPs, sometimes even microbes produce array of specific degrading enzymes (Zhang et al. 2018; Zeng et al. 2015).

In case of Cu NPs, it has been established that their toxicity varies with respect to change in their size, and they have depicted varied effects during in vivo toxicity assessment based on particle size (Chen et al. 2006). In addition, excessive Cu intake leads to jaundice, hemolysis, hepatocirrhosis, lipid profile imbalance, renal dysfunction, oxidative stress, etc. and could be fatal as well (Bertinato and L'Abbé 2004; Zietz et al. 2003; Galhardi et al. 2004). Cu NPs' potential to cause nephrotoxicity and hepatotoxicity along with severe spleen injuries has been confirmed in experimental rat and mouse models. Moreover, they have also induced gill toxicity when assessed using zebrafish model. Few researchers have even reported DNA damaging capability of Cu NPs (Li et al. 2013b).

The emergence of Ag and AuNPs in bioremediation holds promising future applications in diverse areas. Despite their numerous plus points in terms of synthesis, degrading efficiency, and long-term reactivity, toxicity of Ag and AuNPs remains a crucial concern. In fact there is lack of plenty information about the short-term and long-term effects on these NPs on health and environment. As like Cu NPs, even AuNPs exhibit varied properties and toxicity profile based on their shape and sizes (Chen et al. 2009). Rod-shaped AuNPs were assessed for toxicity on human keratinocyte cells (HaCaT) and noted to cause significant toxic effects on the same. In addition AuNPs have depicted potential of impairing mitochondrial integrity of human adenocarcinoma breast cells (Chithrani et al. 2006; Qiu et al. 2010). Furthermore, AgNPs have also reflected genotoxic and cytotoxic responses on human cell lines along with ROS induction and mitochondrial dysfunction consequently leading to damaged DNA and chromosomal anomaly (Sambale et al. 2015).

12.13 Conclusion

Green synthesis of nanoparticles and recycling the synthesized particles for subsequent remediation process can help us to attain a sustainable and circular resource-waste economy. Biosynthesis gives an alternative to minimize chemical synthesis and is simple, cost-effective, and time saving. Zn, Ag, Au, Fe, Cu, and other nanoparticles have been synthesized by this process. On the contrary, biosynthesis of FeNPs using microorganisms needs to be studied in detail as it is significantly involved in degradation of many organic compounds and dyes. Green synthesis of metal NPs is one of the exciting domains in the current scenario. Not only have nanomaterials been utilized for the treatment of wastewater, groundwater,

and soil, but the technology of nanobioremediation has also been well studied and extended to air pollution detection and control, managing uranium, and e-wastes.

12.14 Nanobioremediation: Way Forward

Nanotechnology has potential applications in the environment remediation in aspects like sensor fabrication, effluent treatment, toxic pollutant remediation, soil reclamation, and green synthesis. The demand for nanoscale materials, tools, and devices has increased globally. However, the effects of exposure in the long run, details of pathways, and actual fate and bioaccumulation are naive areas which need to be studied thoroughly. The green manufacturing aspects of nanotechnology could help in in situ or pollutant remediation at the source. This has great impact in lowering the energy input and costs of process and logistics. Taking into consideration all the above factors, process for production and application of these nanomaterials needs to be designed for monitoring and cleanup of pollutants in environment.

Acknowledgments The authors would like to express gratitude to Indian Institute of Technology Bombay for infrastructural and academic support.

Conflict of Interests: The authors declare no conflict of interests.

References

Aagosh V, Anuradha S, Nupur M (2013) Bioremediation of environmental and agricultural lignocellulosic waste through mushroom cultivation. Int J Chem Sci 11(1):399–409

Aazam ES (2014) Environmental remediation of cyanide solutions by photocatalytic oxidation using Au/CdS nanoparticles. J Ind Eng Chem 20:2870–2875

Abbasian F, Lockington R, Palanisami T, Megharaj M, Naidu R (2016) Multiwall carbon nanotubes increase the microbial community in crude oil contaminated fresh water sediments. Sci Total Environ 539:370–380

Abboud Y, Saffaj T, Chagraoui A, Bouari E, Brouzi K, Tanane O, Ihssane B (2014) Biosynthesis, characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract (Bifurcaria bifurcata). Appl Nanosci 4:571–576

Agasti SS, Subinoy R, Myoung-Hwan P, Chae Kyu K, Chang-Cheng Y, Vincent MR (2010) Nanoparticles for detection and diagnosis. Adv Drug Deliv Rev 62:316–328

Ahluwalia SS, Goyal D (2007) Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour Technol 98:2243–2257

Ahmad A, Senapati S, Khan MI, Kumar R, Ramani R, Srinivas V, Sastry M (2003a) Nanotechnology 14:824. https://doi.org/10.1088/0957-4484/14/7/323

Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M (2003b) Extracellular biosynthesis of monodisperse gold nanoparticles by a novel Extremophilic actinomycete, Thermomonospora sp. Langmuir 19(135):3550–3553

Alani F, Moo-Young M, Anderson W (2012) Biosynthesis of silver nanoparticles by a new strain of Streptomyces sp. compared with Aspergillusfumigatus. World J Microbiol Biotechnol 28 (3):1081–1086

Albadarin AB, Yang Z, Mangwandi C, Glocheux Y, Walker G, Ahmad MNM (2014) Experimental design and batch experiments for optimization of Cr(VI) removal from aqueous solutions by hydrous cerium oxide nanoparticles. Chem Eng Res Des 92:1354–1362

Ali H, Khan E, Sajad MA (2013a) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91:869–888

Ali MA, Idris MR, Quayum ME (2013b) Fabrication of ZnO nanoparticles by solution-combustion method for the photocatalytic degradation of organic dye. J Nanostruct Chem 3(1):1–6

Ambashta RD, Sillanpää M (2010) Water purification using magnetic assistance: a review. J Hazard Mater 180(1):38–49

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

Amonette JE, Szecsody JE, Schaef HT, Templeton JC, Gorby YA, Fruchter JS (1994) Abiotic reduction of aquifer materials by dithionite: A promising in situ remediation technology. In: Proceedings of the 33rd Hanford symposium on health and the environment, vol 2. Battelle Press, Columbus, OH, Pasco, Forum (Wash), pp 851–881

An B, Zhao D (2012) Immobilization of As (III) in soil and groundwater using a new class of polysaccharide stabilized Fe–Mn oxide nanoparticles. J Hazard Mater 211:332–341

Anker JN, Hall WP, Lyandres O, Shah NC, Zhao J, Van Duyne RP (2008) Biosensing with plasmonic nanosensors. Nat Mater 7:442–453

Aragay G, Pons J, Merkoci A (2011) Recent trends in macro-, micro-, and nanomaterial-based tools and strategies for heavy-metal detection. Chem Rev 111:3433–3458

Arcon I, Piccolo O, Paganelli S, Baldi F (2012) XAS analysis of a nanostructured iron polysaccharide produced anaerobically by a strain of Klebsiella oxytoca. Biometals 25(5):875–881

Arunachalam R, Dhanasingh S, Kalimuthu B, Uthirappan M, Rose C, Mandal AB (2012) Phytosynthesis of silver nanoparticles using *Coccinia grandis* leaf extract and its application in the photocatalytic degradation. Colloids Surf B Biointerfaces 94:226–230

Asefa T, Duncan CT, Sharma KK (2009) Recent advances in nanostructured chemosensors and biosensors. Analyst 134:1980–1990

Bahnemann D (2004) Photocatalytic water treatment: solar energy applications. Sol Energy 77 (5):445–459

Balagurunathan R, Radhakrishnan M, Rajendran RB, Velmurugan D (2011) Biosynthesis of gold nanoparticles by actinomycete Streptomyces viridogens strain HM10. Indian J Biochem Biophys 48:331

Balaji DS, Basavaraja S, Deshpande R, Mahesh DB, Prabhakar BK, Venkataraman A (2009) Extracellular biosynthesis of functionalized silver nanoparticles by strains of Cladosporium cladosporioides fungus. Colloids Surf B Biointerfaces 68(1):88–92

Bandara J, Klehm U, Kiwi J (2007) Raschig rings-Fe2O3 composite photocatalyst activate in the degradation of 4-chlorophenol and Orange II under daylight irradiation. Appl Catal B Environ 76(1–2):73–81

Banerjee P, Chakrabarti S, Maitra S, Dutta BK (2012) Zinc oxide nano-particles-sonochemical synthesis, characterization and application for photo-remediation of heavy metal. Ultrason Sonochem 1:85–93

Barakat MA, Ramadan MH, Alghamdi MA, Algarny SS, Woodcock HL, Kuhn JN (2013) Remediation of Cu(II), Ni(II), and Cr(III) ions from simulated wastewater by dendrimer/titania composites. J Environ Manage 117:50–57

Bargar JR, Bernier-Latmani R, Giammar DE, Tebo BM (2008) Biogenic uraninite nanoparticles and their importance foruraniumremediation. Elements 4(6):407–412

Bertinato J, L'Abbé MR (2004) Maintaining copper homeostasis: regulation of copper-trafficking proteins in response to copper deficiency or overload. J Nutr Biochem 15:316–322

Bezbaruah AN, Thompson JM, Chisholm BJ (2009) Remediation of alachlor and atrazine contaminated water with zero-valent iron nanoparticles. J Environ Sci Health B 44(6):518–524

Bhakya S, Muthukrishnan S, Sukumaran M, Muthukumar M, Kumar S, Rao MV (2015a) Catalytic degradation of organic dyes using synthesized silver nanoparticles: a green approach. J Bioremed Biodegr 6:1

Bhakya S, Muthukrishnan S, Sukumaran M, Muthukumar M, Kumar S, Rao MV (2015b) Catalytic degradation of organic dyes using synthesized silver nanoparticles: a green approach. J Bioremed Biodeg 6:312

Bhakyaraj K, Kumaraguru S, Gopinath K, Sabitha V, Kaleeswarran PR, Karthika V, Sudha A, Muthukumaran U, Jayakumar K, Mohan S, Arumugam A (2017) J Cluster Sci 28:463

Bhaumik M, Maity A, Srinivasuc VV, Onyango MS (2012) Chem Eng J 181–182:323–333

Bina B, Pourzamani H, Rashidi A, Amin MM (2012) Ethylbenzene removal by carbonnanotubes from aqueous solution. J Environ Public Health 2012, ArticleID 817187, 8p

Binupriya AR, Sathishkumar M, Vijayaraghavan K, Yun SI (2010) Bioreduction of trivalent aurum to nano-crystalline gold particles by active and inactive cells and cell-free extract of Aspergillus oryzae var. viridis. J Hazard Mater 177(1–3):539–545

Bollini P, Didas SA, Jones CW (2011) Amine-oxide hybrid materials for acid gas separations. J Mater Chem 21(39):15100–15120. https://doi.org/10.1039/C1JM12522B.

Boparai HK, Joseph M, O'Carroll DM (2013) Cadmium (Cd2+) removal by nano zerovalent iron: surface analysis, effects of solution chemistry and surface complexation modeling. Environ Sci Pollut Res 20(9):6210–6221

Boulamanti AK, Philippopoulos CJ (2009) Photocatalytic degradation of C5C7 alkanes in the gas-phase. Atmos Environ 43(20):3168–3174. https://doi.org/10.1016/j.atmosenv.2009.03.036

Buhleier E, Wehner W, Vögtle F (1978) 'Cascade'-and "nonskidchain-like" synthesesofmolecularcavitytopologies. Synthesis 2:155–158

Calderon B, Fullana A (2015) Heavy metal release due to aging effect during zero-valent iron nanoparticles remediation. Water Res 83:1–9

Campbell ML, Guerra FD, Dhulekar J, Alexis F, Whitehead DC (2015) Target-specific capture of environmentally relevant gaseous aldehydes and carboxylic acids with functional nanoparticles. Chem A Eur J 21(42):14834–14842. https://doi.org/10.1002/chem.201502021

Camposeco R, Castillo S, Mugica V, Mejia-Centeno I, Marín J (2014) Novel V_2O_5/$NTiO_2Al_2O_3$ nanostructured catalysts for enhanced catalytic activity in NO reduction by NH_3. Catal Commun 45:5458. https://doi.org/10.1016/j.catcom.2013.10.025

Cantrell KJ, Kaplan DI, Wietsma TW (1995) Zero-valent iron for the in situ remediation of selected metals in groundwater. J Hazard Mater 42:201–212

Cao CY, Cui ZM, Chen CQ, Song WG, Cai W (2010) Ceria hollow nanospheres produced by a template-free microwave-assisted hydrothermal method for heavy metal ion removal and catalysis. J Phys Chem 114:9865–9870

Cappello S, Mancini G, Pistone A, Azzaro M, Bottino F, Genovese L et al (2014) STRANgE, integrated physical–biological–mechanical system for recovery in of the "oil spill" in Antarctic environment. Rev Environ Sci Bio/Technol 3(4):369–337

Castillo VA, Barakat MA, Ramadan MH, Woodcock HL, Kuhn JN (2014) Metal ion remediation by polyamidoamine dendrimers: a comparison of metal ion, oxidation state, and titania immobilization. Int J Environ Sci Technol 11:1497–1502

Castro-Longoria E, Vilchis-Nestor AR, Avalos-Borja M (2011) Biosynthesis of silver, gold and bimetallic nanoparticles using the filamentous fungus Neurospora crassa. Colloids Surf B Biointerfaces 83(1):42–48

Cecchin I, Reddy KR, Thomé A, Tessaro EF, Schnaid F (2017a) Nanobioremediation: Integration of nanoparticles and bioremediation for sustainable remediation of chlorinated organic contaminants in soils. Int Biodeter Biodegr 119:419–428

Cecchin I, Reddy KR, Thorme A, Tessaro EF, Schnaid F (2017b) Nanobioremediation: Integration of nanoparticles and bioremediation for sustainable remediation of chlorinated organic contaminants in soils. Int Biodeteriorat Bioremed 119:419–428

Chandra V, Kim KS (2011) Highly selective adsorption of Hg2+ by a polypyrrole-reduced graphene oxide composite. Chem Commun 47:3942–3944

Chandra V, Park J, Chun Y, Lee JW, Hwang IC, Kim KS (2010) Water-dispersible magnetite-reduced graphene oxide composites for arsenic removal. ACS Nano 4:3979–3986
Chang MC, Kang HY (2009) Remediation of pyrene-contaminated soil by synthesized nanoscale zero-valent iron particles. J Environ Sci Health A 44(6):576–582
Chang M, Shu H, Hsieh W, Wang M (2005) Using nanoscale zero-valent iron for the remediation of polycyclic aromatic hydrocarbons contaminated soil. J Air Waste Manag Assoc 55(8):1200–1207
Chang M-C, Shu H-Y, Hsieh W-P, Wang M-C (2007) Remediation of soil contaminated with pyrene using ground nanoscale zero-valent iron. J Air Waste Manag Assoc 57(2):221–227
Chang C, Lian F, Zhu L (2011) Simultaneous adsorption and degradation of γ-HCH by nZVI/Cu bimetallic nanoparticles with activated carbon support. Environ Pollut 159:2507–2514
Chatterjee S, Lee MW, Woo SH (2010) Adsorption of congo red by chitosan hydrogel beads impregnated with carbon nanotubes. Bioresour Technol 101:1800–1806
Chaturvedi S, Dave PN, Shah NK (2012) Applications of nano-catalyst in new era. J Saudi Chem Soc 16:307–325
Chauhan NS, Ranjan R, Purohit HJ, Kalia VC, Sharma R (2009) Identification of genes conferring arsenic resistance to *Escherichia coli* from an effluent treatment plant sludge metagenomic library. FEMS Microbiol Ecol 67:130–139
Chaung SH, Wu PF, Kao YL, Yan W, Lien HL (2014) Nanoscale zero-valent iron for sulfide removal from digested piggery wastewater. J Nanomater 2014:518242
Chen Z, Meng H, Xing G, Chen C, Zhao Y, Jia G, Chai Z (2006) Acute toxicological effects of copper nanoparticles in vivo. Toxicol Lett 163:109–120
Chen YS, Hung YC, Liau I, Huang GS (2009) Assessment of the in vivo toxicity of gold nanoparticles. Nanoscale Res Lett 4:858–864
Chen X, Cen C, Tang Z, Zeng W, Chen D, Fang P et al (2013) The key role of pH value in the synthesis of titanate nanotubes-loaded manganese oxides as a superior catalyst for the selective catalytic reduction of NO with NH3. J Nanomater 7:871528. https://doi.org/10.1155/2013/871528
Chithrani BD, Ghazani AA, Chan WC (2006) Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano Lett 6:662–668
Cho HH, Smith BA, Wnuk JD, Fairbrother DH, Ball WP (2008) Influence of surface oxides on the adsorption of naphthalene onto multiwalled carbon nanotubes. Environ Sci Technol 42:2899–2905
Choi H, Al-Abed SR, Agarwal S, Dionysiou DD (2008) Synthesis of reactive nano-Fe/Pd bimetallic system-impregnated activated carbon for the simultaneous adsorption and dechlorination of PCBs. Chem Mater 20(11):3649–3655
Choina J, Bagabas A, Fischer C, Flechsig GU, Kosslick H, Alshammari A, Schulz A (2015) The influence of the textural properties of ZnO nanoparticles on adsorption and photocatalytic remediation of water from pharmaceuticals. Catal Today 241:47–54
Chong MN, Jin B, Chow CW, Saint C (2010) Recent developments in photocatalytic water treatment technology: a review. Water Res 44(10):2997–3027
Cloete TE, Kwaadsteniet MD, Botes M, Lopez-Romero JM (2010) Nanotechnology in water treatment applications. Caister Academic Press, Wymondham
Cookson JT Jr (1995) Bioremediation engineering: Design and application. McGraw-Hill, Inc, New York
Corso CR, De Almeida ACM (2009) Bioremediation of dyes in textile effluents by Aspergillus oryzae. Microb Ecol 57(2):384–390
Crane RA, Dickinson M, Popescu IC, Scott TB (2011) Magnetite and zero-valent iron nanoparticles for the remediation of uranium contaminated environmental water. Water Res 45:2931–2942
Crisafully R, Milhome MAL, Cavalcante RM, Silveira ER, De Keukeleire D, Nascimento RF (2008) Removal of some polycyclic aromatic hydrocarbons from petrochemical wastewater using low-cost adsorbents of natural origin. Bioresour Technol 99(10):4515–4519. https://doi.org/10.1016/j.biortech.2007.08.041

Crump CJ, Gilbertson JD, Chandler BD (2008) CO oxidation and toluene hydrogenation by Pt/TiO_2 catalysts prepared from dendrimer encapsulated nanoparticle precursors. Top Catal 49:233–240
Cuevas R, Durán N, Diez MC, Tortella GRO, Rubilar O (2015) J Nanomater 2015:1
Cundy AB, Hopkinson L, Whitby RL (2008) Use of iron-based technologies in contaminated land and groundwater remediation: a review. Sci Total Environ 400(1):42–51
Cutting RS, Coker VS, Telling ND, Kimber RL, Pearce CI, Ellis BL, Arenholz E (2010) Optimizing Cr (VI) and Tc (VII) remediation through nanoscale biomineral engineering. Environ Sci Technol 44(7):2577–2584
Dai MH, Copley SD (2004) Genome shuffling improves degradation of the anthropogenic pesticide pentachlorophenol by Sphingobium chlorophenolicum ATCC 39723. Appl Environ Microbiol 70:2391–2397
Darvishi Cheshmeh Soltani R, Rezaee A, Safari M, Khataee AR, Karimi B (2015) Photocatalytic degradation of formaldehyde in aqueous solution using ZnO nanoparticles immobilized on glass plates. Desalin Water Treat 53(6):1613–1620
Das S, Sen B, Debnath N (2015) Recent trends in nanomaterials applications in environmental monitoring and remediation. Environ Sci Pollut Res 22:18333–18344
Dasand I, Ansari SA (2009) Nanomaterials in science and technology. J Sci Ind Res 68(8):657–667
Dastjerdi R, Montazer M (2010) A review on the application of inorganic nano-structured materials in the modification of textiles: focus on anti-microbial properties. Colloid Surf B 79(1):5–18
Daus B, Wennrich R, Weiss H (2004) Sorption materials for arsenic removal from water: a comparative study. Water Res 38(12):2948–2954
Devi RR, Umlong IM, Das B, Borah K, Thakur AJ, Raul PK, Banerjee S, Singh L (2014) Removal of iron and arsenic (III) from drinking water using iron oxide-coated sand and limestone. Appl Water Sci 4:175–182
Devinny JS, Deshusses MA, Webster TS (1999) Biofiltration for air pollution control. CRC Press, Boca Raton, pp 1–320
Di ZC, Ding J, Peng XJ, Li YH, Luan ZK, Liang J (2006) Chromium adsorption by aligned carbon nanotubes supported ceria nanoparticles. Chemosphere 62:861–865
Diallo MS, Christie S, Swaminathan P, Johnson JH, Goddard WA (2005) Dendrimer enhanced ultrafiltration. 1. Recovery of Cu (II) from aqueous solutions using PAMAM dendrimers with ethylene diamine core and terminal NH2 groups. Environ Sci Technol 39:1366–1377
van Dillewijn P, Caballero A, Paz JA, Gonzalez-Perez MM, Oliva JM, Ramos JL (2007) Bioremediation of 2,4,6-trinitrotoluene under field conditions. Environ Sci Technol 41(4):1378–1383
Dimitrov D (2006) Interactions of antibody-conjugated nanoparticles with biological surfaces. Colloids Surf A Physicochem Eng Asp 8:282–283
Donnell GM, Russell AD (1999) Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 12(1):147–179
Dorjee P, Amarasiriwardena D, Xing B (2014) Antimony adsorption by zero-valent iron nanoparticles (nZVI): Ion chromatography–inductively coupled plasma mass spectrometry (IC–ICP-MS) study. Microchem J 116:15–23
Drese JH, Talley AD, Jones CW (2011) Aminosilica materials as adsorbents for the selective removal of aldehydes and ketones from simulated bio-oil. ChemSusChem 4(3):379–385. https://doi.org/10.1002/cssc.201000347
Duncan R (2006) Polymer conjugates as anticancer nanomedicines. Nat Rev Cancer 6:688–701. https://doi.org/10.1038/nrc1958
Durán N, Marcato PD, Durán M, Yadav A, Gade A, Rai M (2011) Mechanistic aspects in the biogenic synthesis of extracellular metal nanoparticles by peptides, bacteria, fungi, and plants. Appl Microbiol Biotechnol 90(5):1609–1624
Eatemadi A, Daraee H, Karimkhanloo H et al (2014) Carbon nanotubes: properties, synthesis, purification, and medical applications. Nanoscale Res Lett 9(1):393. https://doi.org/10.1186/1556-276X-9-3934

Ekramul Mahmud HNM, Obidul Huq AK, Yahya RB (2016) The removal of heavy metal ions from wastewater/aqueous solution using polypyrrole-based adsorbents: a review. RSC Adv 6:14778–14791

Elaziouti A, Ahmed B (2011) ZnO-assisted photocatalytic degradation of congo Red and benzopurpurine 4B in aqueous solution. J Chem Eng Process Technol 2:1–9

Elcey C, Kuruvilla AT, Thomas D (2014) Synthesis of magnetite nanoparticles from optimized iron reducing bacteria isolated from iron ore mining sites. Int J Curr Microbiol Appl Sci 3:408–417

El-Kemary M, El-Shamy H, El-Mehasseb I (2010) Photocatalytic degradation of ciprofloxacin drug in water using ZnO nanoparticles. JOL 130(12):2327–2331

Emmanuel R, Karuppiah C, Chen SM, Palanisamy S, Padmavathy S, Prakash P (2014) Green synthesis of gold nanoparticles for trace level detection of a hazardous pollutant (nitrobenzene) causing Methemoglobinaemia. J Hazard Mater 279:117–124

Fabrega J, Luoma SN, Tyler CR, Galloway TS, Lead JR (2011) Silver nanoparticles: behaviour and effects in the aquatic environment. Environ Int 37:517–531

Fan X, Zhang F, Zhang G, Du J (2007) Mechanism of 5-amino-2-formylbenzene sulfonic acid formation during reduction of 4,4′-dinitrostilbene-2,2′-disulfonic acid by Zero-Valent iron. Dyes Pigm 75(1):189–193

Fan FL, Qin Z, Bai J, Rong WD, Fan FY, Tian W, Zhao L (2012a) Rapid removal of uranium from aqueous solutions using magnetic Fe_3O_4@ SiO_2 composite particles. J Environ Radioact 106:40–46

Fan L, Luo C, Li X, Lu F, Qiu H, Sun M (2012b) Fabrication of novel magnetic chitosan grafted with graphene oxide to enhance adsorption properties for methyl blue. J Hazard Mater 215:272–279

Fan L, Luo C, Sun M, Li X, Qiu H (2013) Highly selective adsorption of lead ions by water-dispersible magnetic chitosan/graphene oxide composites. Colloids Surf B Biointerfaces 103:523–529

Farhadi K, Forough M, Molaei R, Hajizadeh S, Rafipour A (2012) Highly selective Hg2+ colorimetric sensor using green synthesized and unmodified silver nanoparticles. Sens Actuators B 161:880–885

Farhanian D, Haghighat F, Lee C-S, Lakdawala N (2013) Impact of design parameters on the performance of ultraviolet photocatalytic oxidation air cleaner. Build Environ 66:148–157. https://doi.org/10.1016/j.buildenv.2013.04.010

Feng L, Cao M, Ma X, Zhu Y, Hu C (2012) Super paramagnetic high-surface area Fe_3O_4 nanoparticles as adsorbents for arsenic removal. J Hazard Mater 217–218:439–446

Fresnais J, Yan M, Courtois J, Bostelmann T, Bée A, Berret JF (2013) Poly (acrylic acid)-coated iron oxide nanoparticles: quantitative evaluation of the coating properties and applications for the removal of a pollutant dye. J Colloid Interface Sci 395:24–30

Fruchter JS, Cole CR, Williams MD, Vermeul VR, Teel SS, Amonette JE, Szecsody JE, Yabusaki SB (1997) Creation of a subsurface permeable treatment barrier using in situ redox manipulation. Pacific Northwest National Laboratory, Richland

Fukushi K, Sasaki M, Sato T, Yanase N, Amano H, Ikeda H (2003) A natural attenuation of arsenic in drainage from an abandoned arsenic mine dump. Appl Geochem 18:1267–1278

Galhardi CM, Diniz YS, Faine LA, Rodrigues HG, Burneiko RC, Ribas BO, Novelli EL (2004) Toxicity of copper intake: lipid profile, oxidative stress and susceptibility to renal dysfunction. Food Chem Toxicol 42:2053–2060

Gangula A, Podila R, Karanam L, Janardhana C, Rao AM (2011) Catalytic reduction of 4-nitrophenol using biogenic gold and silver nanoparticles derived from Breynia rhamnoides. Langmuir 27:15268–15274

Gao C, Zhang W, Li H, Lang L, Xu Z (2008) Controllable fabrication of mesoporous MgO with various morphologies and their absorption performance for toxic pollutants in water. Crystal Growth and Design 8:3785–3790

Georgiou Y, Dimos K, Beltsios K, Karakassides MA, Deligiannakis Y (2015) Hybrid [polysulfone–zero valent iron] membranes: synthesis, characterization and application for As III remediation. Chem Eng J 281:651–660

Gethard K, Sae-Khow O, Mitra S (2011) Water desalination using carbon-nanotube-enhanced membrane distillation. ACS Appl Mater Interfaces 3(2):110–114. https://doi.org/10.1021/am100981s

Gong J-L, Wang B, Zeng G-M et al (2009a) Removal of cationic dyes from aqueous solution using magnetic multi-wall carbon nanotube nanocomposite as adsorbent. J Hazard Mater 164 (2-3):1517–1522

Gong JL, Wang B, Zeng GM, Yang CP, Niu CG, Niu QY, Liang Y (2009b) Removal of cationic dyes from aqueous solution using magnetic multi-wall carbon nanotube nanocomposite as adsorbent. J Hazard Mater 164:1517–1522

Goswami A, Raul PK, Purkait MK (2012) Arsenic adsorption using copper (II) oxide nanoparticles. Chem Eng Res Des 90(9):1387–1396

Govindaraju K, Basha SK, Kumar VG, Singaravelu G (2008) Silver, gold and bimetallic nanoparticles production using single-cell protein (Spirulina platensis) Geitler. J Mater Sci 43:5115–5122

Gowramma B, Keerthi U, Rafi M et al (2015) Biogenic silver nanoparticles production and characterization from native stain of Corynebacterium species and its antimicrobial activity. 3 Biotech 5(2):195–201. https://doi.org/10.1007/s13205-014-0210-4

Gray NF (1999) Water technology. Wiley, New York, pp 473–474

Gu D, Shang S, Yu Q, Shen J (2016) Green synthesis of nitrogen-doped carbon dots from lotus root for Hg (II) ions detection and cell imaging. Appl Surf Sci 390:38–42

Guan H, Bestland E, Zhu C, Zhu H, Albertsdottir D, Hutson J, Ellis AV (2010) Variation in performance of surfactant loading and resulting nitrate removal among four selected natural zeolites. J Hazard Mater 183:616–621

Guerra FD, Campbell ML, Whitehead DC, Alexis F (2017) Tunable properties of functional nanoparticles for efficient capture of VOCs. Chem Select 2(31):9889–9894. https://doi.org/10.1002/slct.201701736

Guerra FD, Attia M, Whitehead D, Alexis F (2018a) Nanotechnology for environmental remediation: materials and applications. Molecules 23(7):1760

Guerra FD, Campbell ML, Attia MF, Whitehead DC, Alexis F (2018b) Capture of aldehyde VOCs using a series of amine-functionalized cellulose nanocrystals. Chem Select 3(20):54955501. https://doi.org/10.1002/slct.201703149

Guibal E (2004) Interactions of metal ions with chitosan-based sorbents. Sep Purif Technol 38:43–74

Guo R, Guo X, Yu D, Hu J (2012) Application research in water treatment of PAMAM dendrimer. Huagong Jinzhan 3:039

Gupta K, Ghosh UC (2009) Arsenic removal using hydrous nanostructure iron (III)–titanium (IV) binary mixed oxide from aqueous solution. J Hazard Mater 161:884–892

Gupta N, Singh HP, Sharma RK (2010a) Single-pot synthesis: plant mediated gold nanoparticles catalyzed reduction of methylene blue in presence of stannous chloride. Colloids Surf A 367 (1):102–107

Gupta AK, Deva D, Sharma A, Verma N (2010b) Fe-grown carbon nanofibers for removal of arsenic (V) in wastewater. Ind Eng Chem Res 49(15):7074–7084

Gupta K, Maity A, Ghosh UC (2010c) Manganese associated nanoparticles agglomerate of iron (III) oxide: synthesis, characterization and arsenic (III) sorption behavior with mechanism. J Hazard Mater 184:832–842

Gupta K, Bhattacharya S, Chattopadhyay D, Mukhopadhyay A, Biswas H, Dutta J, Ray NR, Ghosh UC (2011a) Ceria associated manganese oxide nanoparticles: synthesis, characterization and arsenic (V) sorption behavior. Chem Eng J 172:219–229

Gupta VK, Agarwal S, Saleh TA (2011b) Synthesis and characterization of alumina-coated carbon nanotubes and their application for lead removal. J Hazard Mater 185:17–23

Gurunathan S, Kim E, Han JW, Park JH, Kim JH (2015) Green chemistry approach for synthesis of effective anticancer palladium nanoparticles. Molecules 20(12):22476–22498
Harris C, Kamat PV (2009) Photocatalysis with CdSe nanoparticles in confined media: mapping charge transfer events in the subpicosecond to second timescales. ACS Nano 3:682–690
Hashim MA, Mukhopadhyay S, Sahu JN, Sengupta B (2011) Remediation technologies for heavy metal contaminated groundwater. J Environ Manage 92:2355–2388
Hassan MS, Amna T, Al-Deyab SS, Kim HC, Khil MS (2015) Monodispersed 3D MnWO4–TiO_2 composite nanoflowers photocatalysts for environmental remediation. Curr Appl Phys 15:753–758
Hassanshahian M, Emtiazi G, Cappello S (2012) Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea. Mar Pollut Bull 64:7–12
Hazen TC (2018) Bioremediation. In: Microbiology of the terrestrial deep subsurface 2018 Jan 10. CRC Press, pp 247–266
He S, Guo Z, Zhang Y, Zhang S, Wang J, Gu N (2007) Biosynthesis of gold nanoparticles using the bacteria Rhodopseudomonas capsulata. Mater Lett 61(18):3984–3987
He X, Aker WG, Pelaez M, Lin Y, Dionysiou DD, Hwang HM (2016) Assessment of nitrogen–fluorine-codoped TiO_2 under visible light for degradation of BPA: implication for field remediation. J Photochem Photobiol A 314:81–92
Henderson AD, Demond AH (2013) Permeability of iron sulfide (FeS)-based materials for groundwater remediation. Water Res 47:1267–1276
Honary S, Barabadi H, Fathabad EG, Naghibi F (2012) Dig J Nanomater Biostruct 7:999
Hristovski KD, Nguyen H, Westerhoff PK (2009a) Removal of arsenate and 17-ethinyl estradiol (EE2) by iron (hydr) oxide modified activated carbon fibers. J Environ Sci Health A Tox Hazard Subst Environ Eng 44(4):354–361
Hristovski KD, Westerhoff PK, Moller T, Sylvester P (2009b) Effect of synthesis conditions on nano-iron (hydr) oxide impregnated granulated activated carbon. Chem Eng J 146(2):237–243
Hu H, Wang Z, Pan L (2010) Synthesis of monodisperse Fe_3O_4@ silica core–shell microspheres and their application for removal of heavy metal ions from water. J Alloys Compd 492:656–661
Huang SH, Chen DH (2009) Rapid removal of heavy metal cations and anions from aqueous solutions by an amino-functionalized magnetic nano-adsorbent. J Hazard Mater 163 (1):174–179
Huang HY, Yang RT, Chinn D, Munson CL (2003) Amine-grafted MCM-48 and silica xerogel as superior sorbents for acidic gas removal from natural gas. Ind Eng Chem Res 42 (12):2427–2433. https://doi.org/10.1021/ie020440u.
Huang J, Wang W, Lin L, Li Q, Lin W, Li M, Mann S (2009) A general strategy for the biosynthesis of gold nanoparticles by traditional Chinese medicines and their potential application as catalysts. Chem Asian J 4(7):1050–1054
Huang ZH, Zheng X, Lv W, Wang M, Yang QH, Kang F (2011) Adsorption of lead (II) ions from aqueous solution on low-temperature exfoliated graphene nanosheets. Langmuir 27:7558–7562
Huang J, Cao Y, Liu Z, Deng Z, Tang F, Wang W (2012a) Efficient removal of heavy metal ions from water system by titanate nanoflowers. Chem Eng J 180:75–80
Huang CC, Lo SL, Lien HL (2012b) Zero-valent copper nanoparticles for effective dechlorination of dichloromethane using sodium borohydride as a reductant. Chem Eng J 203:95–100
Huang Y, Ho SS, Lu Y, Niu R, Xu L, Cao J et al (2016) Removal of indoor volatile organic compounds via photocatalytic oxidation: a short review and prospect. Molecules 21(1). https://doi.org/10.3390/molecules21010056
Husken G, Hunger M, Brouwers HJH (2009) Experimental study of photocatalytic concrete products for air purification. Build Environ 44(12):2463–2474. https://doi.org/10.1016/j.buildenv.2009.04.010
Ifang S, Gallus M, Liedtke S, Kurtenbach R, Wiesen P, Kleffmann J (2014) Standardization methods for testing photo-catalytic air remediation materials: problems and solution. Atmos Environ 91:154161. https://doi.org/10.1016/j.atmosenv.2014.04.001

Ingale AG, Chaudhari AN (2013) Biogenic synthesis of nanoparticles and potential applications: an eco-friendly approach. J Nanomed Nanotechol 4(165):1–7

Ion AC, Ion I, Culetu, D. Gherase, Carbonbased nanomaterials. In: Environmental applications, Romania, 2010.

Islam AA, Ferdous T, Das AK (2015) Photodegradation of brown CGG dye using ZnO nanoparticles synthesized by ionic template method. ICMEIE-2015 Proceedings, pp 1–5

Jabeen H, Kemp KC, Chandra V (2013) Synthesis of nano zerovalent iron nanoparticles–graphene composite for the treatment of lead contaminated water. J Environ Manage 130:429–435

Jadia CD, Fulekar MH (2009) Phytoremediation of heavy metals: recent techniques. Afr J Biotechnol 8(6):921–928

Jain CK, Singh RD (2012) Technological options for the removal of arsenic with special reference to South East Asia. J Environ Manage 107:1–18

Jain N, Bhargava A, Panwar J (2014) Enhanced photocatalytic degradation of methylene blue using biologically synthesized "protein-capped" ZnO nanoparticles. Chem Eng J 243:549–555

Jayaseelan C, Rahuman AA, Roopan SM, Kirthia AV, Se-K K, Iyappan M, Siva C (2013a) Spectrochim Acta A Mol Biomol Spect 107:82

Jayaseelan C, Rahuman AA, Roopan SM, Kirthi AV, Venkatesan J, Kim SK, Iyappan M, Siva C (2013b) Biological approach to synthesize TiO_2 nanoparticles using *Aeromonas hydrophila* and its antibacterial activity. Spectrochim Acta A 107:82–89

Jeevanandam J, Chan YS, Danquah MK (2016) Biosynthesis of metal and metal oxide nanoparticles. ChemBioEng Rev 3:55–67

Jeong J, Sekiguchi K, Lee W, Sakamoto K (2005) Photodegradation of gaseous volatile organic compounds (VOCs) using TiO_2 photoirradiated by an ozone-producing UV lamp: decomposition characteristics, identification of by-products and water-soluble organic intermediates. J Photochem Photobiol A Chem 169(3):279–287. https://doi.org/10.1016/j.jphotochem.2004.07.014

Jia K, Pan B, Lv L, Zhang Q, Wang X, Pan B, Zhang W (2009) Impregnating titanium phosphate nanoparticles onto a porous cation exchanger for enhanced lead removal from waters. J Colloid Interface Sci 331:453–457

Jing L, Yichun Q, Baiqi W, Shudan L, Baojiang J, Libin Y, Wei F, Honggang F, Jiazhong S (2006) Review of photoluminescence performance of nano-sized semiconductor materials and its relationships with photocatalytic activity. Solar Energy Mater Solar Cells 90(12):1773–1787

Jing C, Meng X, Calvache E, Jiang G (2009) Remediation of organic and inorganic arsenic contaminated groundwater using a nanocrystalline TiO_2-based adsorbent. Environ Pollut 157:2514–2519

Jong T, Parry DL (2003) Removal of sulphate and heavy metals by sulphate reducing bacteria in short-term bench scale up flow anaerobic packed bed reactor runs. Water Res 37:3379–3389

Joo SH, Zhao D (2008) Destruction of lindane and atrazine using stabilized iron nanoparticles under aerobic and anaerobic conditions: effects of catalyst and stabilizer. Chemosphere 70(3):418–425

Joshi MK, Pant HR, Liao N, Kim JH, Kim HJ, Park CH, Kim CS (2015) In-situ deposition of silver-iron oxide nanoparticles on the surface of fly ash for water purification. J Colloid Interface Sci 453:159–168

Joutey NT, Bahafid W, Sayel H, Ghachtouli NE (2013) Biodegradation: involved microorganisms and genetically engineered microorganisms. In: Chamy R, Rosenkranz F, editors. Biodegradation-life of sciences, pp 290–320. ISBN 978-953-51-1154-2.

Kalishwaralal K, Deepak V, Pandian SRK, Kottaisamy M, Mani Kanth S, Kartikeyan B, Gurunathan S (2010) Colloids Surf B Biointerfaces 77:257

Kamat PV (2002) Photophysical, photochemical and photocatalytic aspects of metal nanoparticles. J Phys Chem 106(32):7729–7744

Kampalanonwat P, Supaphol P (2010) Preparation and adsorption behavior of aminated electro spun polyacrylonitrile nanofibre mats for heavy metal ion removal. Appl Mater Interfaces 2 (2):3619–3627

Kandah MI, Meunier J-L (2007a) Removalof nickel ionsfrom water by multi-walled carbonnanotubes. J Hazard Mater 146(1–2):283–288
Kandah MI, Meunier JL (2007b) Removal of nickel ions from water by multi-walled carbon nanotubes. J Hazard Mater 146:283–288
Kanel SR, Manning B, Charlet L, Choi H (2005) Removalof arsenic(III) from groundwater by nanoscale zero-valent iron. Environ Sci Technol 39(5):1291–1298
Kanel SR, Greneche J-M, Choi H (2006) Arsenic(V)removal from groundwater using nano scale zero-valent iron as a colloidal reactive barrier material. Environ Sci Technol 40(6):2045–2050
Karuppiah C, Palanisamy S, Chen SM, Emmanuel R, Ali MA, Muthukrishnan P, Al-Hemaid FM (2014) Green biosynthesis of silver nanoparticles and nanomolar detection of p-nitrophenol. J Solid State Electrochem 18:1847–1854
Kaul R, Kumar P, Burman U, Joshi P, Agrawal A, Raliya R, Tarafdar J (2012) Magnesium and iron nanoparticles production using microorganism and various salts. Mater Sci Poland 30:254–258
Kavitha KS, Baker S, Rakshith D, Kavitha HU, Yashwantha Rao HC, Harini BP, Satish S (2013) Int Res J Bio Sci 2:66–76
Kazeminezhad I, Sadollahkhani A (2014) Photocatalytic degradation of Eriochrome black-T dye using ZnO nanoparticles. Mater Lett 120:267–270
Khan R, Fuleka MH (2016) Biosynthesis of titanium dioxide nanoparticles using Bacillus amyloliquefaciens culture and enhancement of its photocatalytic activity for the degradation of a sulfonated textile dye Reactive Red 31. J Colloid Interface Sci 475:184–191
Khan FI, Ghoshal AK (2000) Removal of volatile organic compounds from polluted air. J Loss Prev Process Ind 13(6):527–545. https://doi.org/10.1016/S0950-4230(00)00007-3
Khan MM, Adil SF, Al-Mayouf A (2015a) Metal oxides as photocatalysts. J Saudi Chem Soc 19:462–464
Khan SB, Marwani HM, Asiri AM, Bakhsh EM (2015b) Exploration of calcium doped zinc oxide nanoparticles as selective adsorbent for extraction of lead ion. Desalin Water Treat:1–10. https://doi.org/10.1080/19443994.2015.1109560
Khedr M, Abdelhalim K, Soliman N (2009) Synthesis and photocatalytic activity of nano-sized iron oxides. Mater Lett 63:598–601
Khezami L, Taha KK, Ghiloufi I, El Mir L (2016) Adsorption and photocatalytic degradation of malachite green by vanadium doped zinc oxide nanoparticles. Water Sci Technol 73 (4):881–889
Khin MM, Nair AS, Babu VJ, Murugan R, Ramakrishna S (2012) A review on nanomaterials for environmental remediation. Energ Environ Sci 5:8075–8109
Kim Y-H, Carraway ER (2000) Dechlorination of pentachlorophenol by zero valent iron and modified zero valent irons. Environ Sci Technol 34(10):2014–2017
Kim Y, Johnson RC, Hupp JT (2001a) Gold nanoparticlebased sensing of "spectroscopically silent" heavy metal ions. Nano Lett 1:165–167
Kim YC, Sasaki S, Yano K, Ikebukuro K, Haphimoto K, Karube I (2001b) Photocatalytic sensor for the determination of chemical oxygen demand using flow injection analysis. Anal Chem Acta 59:432
Kim HY, Kim IK, Shim JH, Kim YC, Han TH, Chung KC, Kim IS (2006) Removal of alachlor and pretilachlor by laboratory-synthesized zerovalent iron in pesticide formulation solution. Bull Environ Contam Toxicol 77:826
Kim JH, Tratnyek PG, Chang YS (2008) Rapid dechlorination of polychlorinated dibenzo-p-dioxins by bimetallic and nanosized zerovalent iron. Environ Sci Technol 42(11):4106–4112
Kim A, Muthuchamy N, Yoon C, Joo S, Park K (2018) MOF-derived Cu@ Cu2O nanocatalyst for oxygen reduction reaction and cycloaddition reaction. Nano 8:138
Klimkova S, Cernik M, Lacinova L, Nosek J (2008) Application of nanoscale zero-valent iron for groundwater remediation: laboratory and pilot experiments. Nano 3:287–289
Kocaoba S, Orhan Y, Akyüz T (2007) Kinetics and equilibrium studies of heavy metal ions removal by use of natural zeolite. Desalination 214:1–10

Kostal JRY, Wu CH, Mulchandani A, Chen W (2004) Enhanced arsenic accumulation in engineered bacterial cells expressing ArsR. Appl Environ Microbiol 70:4582–4587
Kratochvil D, Volesky B (1998) Advances in the biosorption of heavy metals. Trends Biotechnol 16(7):291–300
Krishnaswamy K, Vali H, Orsat VJ (2014) Value-adding to grape waste: Green synthesis of gold nanoparticles. J Food Eng 142:210–220
Kruefu V, Ninsonti H, Wetchakun N, Inceesungvorn B, Pookmanee P, Phanichphant S (2012) Photocatalytic degradation of phenol using Nb-loaded ZnO nanoparticles. Eng J-CANDA 16 (3):91–100
Kumar SR, Gopinath P (2017) Nano-bioremediation applications of nanotechnology for bioremediation. In: Handbook of advanced industrial and hazardous wastes management. CRC Press, Boca Raton, pp 27–48
Kumar D, Karthik L, Kumar G, Roa K (2011) B Pharmacology 3:31100
Kumar G, Kumar R, Hwang SW, Umar A (2014) Photocatalytic degradation of direct red-23 dye with ZnO nanoparticles. J Nanosci Nanotechnol 14(9):7161–7166
Kumar N, Labille J, Bossa N et al (2017) Enhanced transportability of zero valent iron nanoparticles in aquifer sediments: surface modifications, reactivity, and particle traveling distances. Environ Sci Pollut Res 24:9269–9277
Kumari MM, Jacob J, Philip D (2015) Green synthesis and applications of Au–Ag bimetallic nanoparticles. Spectrochim Acta, Part A 137:185–192
Kuppusamy P, Yousoff MM, Manian GP, Govindan N (2014) Saudi Pharm J 24:473. https://doi.org/10.1016/j.jsps.2014.11.013
Lacina P, Dvorak V, Vodickova E, Barson P, Kalivoda J, Goold S (2015) The application of nano-sized zero-valent iron for in situ remediation of chlorinated ethylenes in groundwater: a field case study. Water Environ Res 87(4):326
Laumann S, Micić V, Lowry GV, Hofmann T (2013) Carbonate minerals in porous media decrease mobility of polyacrylic acid modified zero-valent iron nanoparticles used for groundwater remediation. Environ Pollut 179:53–60
Lee JS, Han MS, Mirkin CA (2007) Colorimetric detection of mercuric ion (Hg2+) in aqueous media using DNA-functionalized gold nanoparticles. Angew Chem 119:4171–4174
Lee C, Kim JY, Lee WI, Nelson KL, Yoon J, Sedlak DL (2008) Bactericidal effect of zero-valent iron nanoparticles on Escherichia coli. Environ Sci Technol 42:4927
Lee CL, Lee HY, Tseng KH, Hong PA, Jou CJG (2011) Enhanced dechlorination of chlorobenzene by microwave-induced zero-valent iron: particle effects and activation energy. Environ Chem Lett 9(3):355–359
Lee T, Liou S, Bai H (2017) Comparison of titania nanotubes and titanium dioxide as supports of lowtemperature selective catalytic reduction catalysts under sulfur dioxide poisoning. J Air Waste Manage Assoc 67(3):292305. https://doi.org/10.1080/10962247.2016.1231144
Li Y, Li B (2011) Study on fungi-bacteria consortium bioremediation of petroleum contaminated mangrove sediments amended with mixed biosurfactants. Adv Mat Res 183–185:1163–1167
Li XQ, Zhang WX (2006) Iron nanoparticles: the core-shell structure and unique properties for Ni (II) sequestration. Langmuir 22(10):4638–4642
Li Y, Liu F, Xia B et al (2010a) Removal of copper from aqueous solution by carbon nanotube/calcium alginate composites. J Hazard Mater 177(1–3):876–880
Li Y, Liu F, Xia B, Du Q, Zhang P, Wang D, Xia Y (2010b) Removal of copper from aqueous solution by carbon nanotube/calcium alginate composites. J Hazard Mater 177:876–880
Li Y, Li T, Jin Z (2011a) Stabilization of Fe0 nanoparticles with silica fume for enhanced transport and remediation of hexavalent chromium in water and soil. J Environ Sci 23:1211–1218
Li X, Zhu Z, Zhao Q, Wang L (2011b) Photocatalytic degradation of gaseous toluene over $ZnAl_2O_4$ prepared by different methods: a comparative study. J Hazard Mater 186(23):2089–2096. https://doi.org/10.1016/j.jhazmat.2010.12.111

Li Y, Ma H, Ren B, Li T (2013a) Simultaneous adsorption and degradation of Cr (VI) and Cd (II) ions from aqueous solution by silica-coated Fe0 nanoparticles. J Anal Methods Chem 2013:649503

Li F, Lei C, Shen Q, Li L, Wang M, Guo M, Yao S (2013b) Analysis of copper nanoparticles toxicity based on a stressresponsive bacterial biosensor array. Nanoscale 5:653–662

Li G, Li Y, Wang Z, Liu H (2017) Mater Chem Phys 187:1330. https://doi.org/10.1016/j.matchemphys.2016.11.057

Liang W, Dai C, Zhou X, Zhang Y (2014) Application of zero-valent iron nanoparticles for the removal of aqueous zinc ions under various experimental conditions. PLoS One 9:e85686

Lim TT, Feng J, Zhu BW (2007) Kinetic and mechanistic examinations of reductive transformation pathways of brominated methanes with nano-scale Fe and Ni/Fe particles. Water Res 41 (4):875–883

Lin HF, Liao SC, Hung SW (2005) The dc thermal plasma synthesis of ZnO nanoparticles for visible-light photocatalyst. J Photochem Photobiol A 174(1):82–87

Lithoxoos GP, Labropoulos A, Peristeras LD, Kanellopoulos N, Samios J, Economou IG (2010) Adsorption of N_2, CH_4, CO and CO_2 gases in single walled carbon nanotubes: a combined experimental and Monte Carlo molecular simulation study. J Supercrit Fluids 55(2):510–523. https://doi.org/10.1016/j.supflu.2010.09.017

Litter MI, Cortina JL, Fluza AMA, Futuro A, Tsakiroglou C (2014) In-situ technologies for groundwater treatment: the case of arsenic. In: Bundschuh J, Holländer HM, Ma LQ (eds) In-situ remediation of arsenic contaminated sites. CRC Press, Boca Raton

Liu D, Wang J, Bai X, Zong R, Zhu Y (2016) Self-assembled PDINH supramolecular system for photocatalysis under visible light. Adv Mater 28:7284–7290

Liu P, Yu X, Wang F, Zhang W, Yang L, Liu Y (2017) Degradation of formaldehyde and benzene by TiO_2 photocatalytic cement based materials. J Wuhan Univ Technol Mater Sci Ed 32 (2):391396. https://doi.org/10.1007/s11595-017-1608-8

Lofrano G, Libralato G, Brown JJ (2017) Nanotechnologies for environmental remediation—applications and implications. Springer, New York

López-Téllez G, Barrera-Díaz CE, Balderas-Hernández P, Roa-Morales G, Bilyeu B (2011) Removal of hexavalent chromium in aquatic solutions by iron nanoparticles embedded in orange peel pith. Chem Eng J 173:480–485

Lunge S, Singh S, Sinha A (2014) Magnetic iron oxide (Fe_3O_4) nanoparticles from tea waste for arsenic removal. J Magn Magn Mater 356:21–31

Luo T, Cui J, Hu S, Huang Y, Jing C (2010) Arsenic removal and recovery from copper smelting wastewater using TiO_2. Environ Sci Technol 44:9094–9098

Luo F, Yang D, Chen Z, Megharaj M, Naidu R (2016) Onestep green synthesis of bimetallic Fe/Pd nanoparticles used to degrade Orange II. J Hazard Mater 303:145–153

Ma L, Chen A, Lu J, Zhang Z, He H, Li C (2014) In situ synthesis of CNTs/Fe–Ni/TiO_2 nanocomposite by fluidized bed chemical vapour deposition and the synergistic effect in photocatalysis. Particuology 14:24–32

Mahdavian AR, Mirrahimi MAS (2010) Efficient separation of heavy metal cations by anchoring polyacrylic acid on superparamagnetic magnetite nanoparticles through surface modification. Chem Eng J 159(1):264–271

Majumder BR (2012) Bioremediation: copper nanoparticles from electronic-waste. Int J Eng Sci Technol 4:4380

Malarkodi C, Rajeshkumar S, Vanaja M, Paulkumar K, Gnanajobitha G, Annadurai G (2013) Seaweed-mediated synthesis of gold nanoparticles using Turbinaria conoides and its characterization. J Nanostruct Chem 3(1):44

Mallard I, Städe LW, Ruellan S, Jacobsen PAL, Larsen KL, Fourmentin S (2015) Synthesis, characterization and sorption capacities toward organic pollutants of new β-cyclodextrin modified zeolite derivatives. Colloids Surf A 482:50–57

Mallikarjuna K, Narasimha G, Dillip GR, Praveen B, Shreedhar B, Shreelakshmi C, Reddy VS, Devaprasad RB (2011) Green synthesis of silver nanoparticles using Ocimum leaf extract and their characterization. Dig J Nanomater Biostruct 6:181–186
Malwal D, Gopinath P (2015) Fabrication and characterization of poly (ethylene oxide) templated nickel oxide nanofibers for dye degradation. Environ Sci Nano 2:78–85
Malwal D, Gopinath P (2016) Fabrication and applications of ceramic nanofibers in water remediation: a review. Crit Rev Environ Sci Technol 46(5):500–534
Mankbadi MR, Barakat MA, Ramadan MH, Woodcock HL, Kuhn JN (2011) Iron chelation by polyamidoamine dendrimers: a second-order kinetic model for metal–amine complexation. J Phys Chem B 115:13534–13540
Manning BA, Hunt ML, Amrhein C, Yarmoff JA (2002) Arsenic (III) and Arsenic (V) reactions with zerovalent iron corrosion products. Environ Sci Technol 36:5455–5461
Mapelli F, Scoma A, Michaoud G, Aulenta F, Boon N, Borin S et al (2017) Biotechnologies for marine oil spill cleanup: indissoluble ties with microorganisms. Trends Biotechnol 35:860–870
Masciangioli T, Zhang W-X (2003) Peer reviewed: environmental technologies at the nanoscale. Environ Sci Technol 37(5):102A–108A. https://doi.org/10.1021/es0323998.
Mashrai A, Khanam H, Aljawfi RN (2013) Arabian J Chem 10:S1530. https://doi.org/10.1016/j.arabjc.2013.05.004
Mauter MS, Elimelech M (2008) Environmental applications of carbon-based nanomaterials. Environ Sci Technol 42:5843–5859
Mayo JT, Yavuz C, Yean S, Cong L, Shipley H, Yu W, Falkner J, Kan A, Tomson M, Colvin VL (2007) The effect of nanocrystalline magnetite size on arsenic removal. Sci Technol Adv Mater 8(1–2):71–75
Mazumdar H, Haloi N (2011) J Microbiol Biotechnol Res 1:39
Mishra A, Tripathy S, Wahab R, Jeong SH, Hwang I, Yang YB, Kim YS, Shin HS, Yun SI (2011) Appl Microbiol Biotechnol 92:617
Modi S, Pathak B, Fulekar MH (2015) Microbial synthesized silver nanoparticles for decolorization and biodegradation of azo dye compound. J Environ Nanotechnol 4(2):37–46
Mohsenzadeh F, Chehregani Rad A (2012) Bioremediation of heavy metal pollution by nanoparticles of noaea mucronata. Int J Biosci Biochem Bioinformatics 2:85–89
Mondal P, Majumdar CB, Mohanty B (2006) Laboratory based approaches for arsenic remediation from contaminated water: recent developments. J Hazard Mater B137:464–479
Monier M, Ayad DM, Wei Y, Sarhan AA (2010) Adsorption of Cu(II), Co(II), and Ni(II) ions by modified magnetic chitosan chelating resin. J Hazard Mater 177:962–970
Moore AM, De Leon CH, Young TM (2003) Rate and extent of aqueous perchlorate removal by iron surfaces. Environ Sci Technol 37(14):3189–3198
Morillo D, Uheida A, Pérez G, Muhammed M, Valiente M (2015) Arsenate removal with 3-mercaptopropanoic acidcoated superparamagnetic iron oxide nanoparticles. J Colloid Interface Sci 438:227–234
Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, Yacaman MJ (2005) The bactericidal effect of silver nanoparticles. Nanotechnology 16(10):2346
Morrison SJ, Metzler DR, Dwyer BP (2002) Removal of As, Mn, Mo, Se, U, V and Zn from groundwater by zero-valent iron in a passive treatment cell: reaction progress modeling. J Contam Hydrol 56:99
Moura CP, Vidal CB, Barros AL, Costa LS, Vasconcellos LCG, Dias FS et al (2011) Adsorption of BTX (benzene, toluene, o-xylene, and p-xylene) from aqueous solutions by modified periodic mesoporous organosilica. J Colloid Interface Sci 363(2):626–634. https://doi.org/10.1016/j.jcis.2011.07.054
Mueller NC, Nowack B (2009) Nanotechnology developments for the environment sector—report of the Observatory NANO EU FP7 project
Mueller NC, Nowack B (2010) Nanoparticles for remediation: solving big problems with little particles. Elements 6:395–400

Mustafa G, Tahir H, Sultan M, Akhtar N (2013) Synthesis and characterization of cupric oxide (CuO) nanoparticles and their application for the removal of dyes. Afr J Biotechnol 12:6650–6660

Nag S, Pramanik A, Chattopadhyay D, Bhattacharyya M (2018) Green-fabrication of gold nanomaterials using Staphylococcus warneri from Sundarbans estuary: an effective recyclable nanocatalyst for degrading nitro aromatic pollutants. Environ Sci Pollut Res 25:2331–2349

Naja G, Halasz A, Thiboutot S, Ampleman G, Hawari J (2008) Degradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) using zerovalent iron nanoparticles. Environ Sci Technol 42 (12):4364–4370

Nakataa K, Fujishima A (2012) TiO_2 photocatalysis: design and applications. J Photochem Photobiol C Photchem Rev 13:169–189

Nanda A, Saravanan M (2009) Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE. Nanomed Nanotechnol Biol Med 5:452

Naraginti S, Sivakumar A (2014) Spectrochim Acta A Mol Biomol Spectrosc:128. https://doi.org/10.1016/j.saa.2014.02.083

Narayanan KB, Sakthivel N (2011) Synthesis and characterization of nano-gold composite using Cylindrocladium floridanum and its heterogeneous catalysis in the degradation of 4-nitrophenol. J Hazard Mater 189:519–525

Narayanan K, Sakthivel N (2013) Mycocrystallization of gold ions by the fungus Cylindrocladium floridanum. World J Microbiol Biotechnol 29:2207

Nehru K, Sivakumar M (2012) Biosynthesis of Ag nanoparticles using Amaranthus tristis extract for the fabrication of nanoparticle embedded PVA membrane. Curr Nanosci 8:703–708

Newkome GR, Yao Z-Q, Baker GR, Gupta VK (1985) Cascade molecules: a new approach to micelles. J Org Chem 50(11):2003–2004

Neyaz N, Siddiqui WA, Nair KK (2014) Application of surface functionalized iron oxide nanomaterials as a nanosorbents in extraction of toxic heavy metals from ground water: a review. Int J Environ Sci 4:472–483

Ngah WW, Fatinathan S (2010) Pb(II) biosorption using chitosan and chitosan derivatives beads: equilibrium, ion exchange and mechanism studies. J Environ Sci 22:338–346

Nie YC, Yu F, Wang LC, Xing QJ, Liu X, Pei Y, Zou JP, Dai WL, Li Y, Suib SL (2018) Photocatalytic degradation of organic pollutants coupled with simultaneous photocatalytic H2 evolution over graphene quantum dots/Mn-N-TiO_2/g-C3N4 composite catalysts: performance and mechanism. Appl Catal B 227:312–321

Nolan EM, Lippard SJ (2008) Tools and tactics for the optical detection of mercuric ion. Chem Rev 108:3443–3480

Nomura A, Jones CW (2013) Amine-functionalized porous silicas as adsorbents for aldehyde abatement. ACS Appl Mater Interfaces 5(12):5569–5577. https://doi.org/10.1021/am400810s

Nowack B (2008) In: Krug H (ed) Nanotechnology. Wiley-VCS Verlag GmbH & Co, Weinheim, pp 1–15

Nurmi JT, Tratnyek PG, Sarathy V et al (2005) Characterization and properties of metallic iron nanoparticles: spectroscopy, electrochemistry, and kinetics. Environ Sci Tech 39(5):1221–1230

Okachi T, Onaka M (2004) Formaldehyde encapsulated in zeolite: a long-lived, highly activated one-carbon electrophile to carbonyl-ene reactions. J Am Chem Soc 126(8):23062307. https://doi.org/10.1021/ja039737p

Okhovat N, Hashemi M, Golpayegani AA (2015) Photocatalytic decomposition of Metronidazolein aqueous solutions using titanium dioxide nanoparticles. J Mater Environ Sci 6(3):792–799

Oliveira LC, Petkowicz DI, Smaniotto A, Pergher SB (2004) Magnetic zeolites: a new adsorbent for removal of metallic contaminants from water. Water Res 38:3699–3704

Ong YT, Ahmad AL, Zein SHS, Tan SH (2010) A review on carbon nanotubes in an environmental protection and green engineering perspective. Braz J Chem Eng 27:227–242

Ottaviani MF, Favuzza P, Bigazzi M, Turro NJ, Jockusch S, Tomalia DA (2000) A TEM and EPR investigation of the competitive binding of uranyl ions to starburst dendrimers and liposomes: potential use of dendrimers as uranyl ion sponges. Langmuir 16:7368–7372

Pan G, Li L, Zhao D, Chen H (2010) Immobilization of non-point phosphorus using stabilized magnetite nanoparticles with enhanced transportability and reactivity in soils. Environ Pollut 158:35–40

Pandey P, Datta M, Malhotra BD (2008) Prospects of nanomaterials in biosensors. Anal Lett 41:159–209

Pant B, Pant HR, Barakat NA, Park M, Han TH, Lim BH, Kim HY (2014) Incorporation of cadmium sulfide nanoparticles on the cadmium titanate nanofibers for enhanced organic dye degradation and hydrogen release. Ceram Int 40:1553–1559

Pardeshi SK, Patil AB (2009) Solar photocatalytic degradation of resorcinol a model endocrine disrupter in water using zinc oxide. J Hazard Mater 163(1):403–409

Park Y, Hong YN, Weyers A, Kim YS, Linhardt RJ (2011) Polysaccharides and phytochemicals: a natural reservoir for the green synthesis of gold and silver nanoparticles. IET Nanobiotechnol 5:69

Parveen F, Sannakki B, Mandke MV, Pathan HM (2016) Solar Energy Mater Solar Cells 144:371. https://doi.org/10.1016/j.solmat.2015.08.033188

Peng X, Luan Z, Ding J, Di Z, Li Y, Tian B (2005) Ceria nanoparticles supported on carbon nanotubes for the removal of arsenate from water. Mater Lett 59:399–403

Peng C, Zhang J, Xiong Z, Zhao B, Liu P (2015) Fabrication of porous hollow g-Al_2O_3 nanofibers by facile electrospinning and its application for water remediation. Micropor Mesopor Mater 215:133–142

Peng Y, Zhao M, Chen B, Zhang Z, Huang Y, Dai F, Zhuangchai L, Xiaoya C, Chaoliang T, Zhang H (2018) Hybridization of MOFs and COFs: a new strategy for construction of MOF@ COF core–shell hybrid materials. Adv Mater 30:1705454

Pivetz BE (2001) Phytoremediation of contaminated soil and ground water at hazardous waste sites. Environmental Research Services Corporation. EPA/540/S 01/500

Pokhrel D, Viraraghavan T (2009) Biological filtration for removal of arsenic from drinking water. J Environ Manage 90:1956–1961

Pol R, Guerrero M, García-Lecina A, Altube A, Rossinyol E, Garroni S, Pellicer E (2016) Ni-, Pt-and (Ni/Pt)doped TiO_2 nanophotocatalysts: a smart approach for sustainable degradation of Rhodamine B dye. Appl Catal B 181:270–278

Ponder SM, Darab JG, Mallouk TE (2000) Remediation of Cr(VI) and Pb(II) aqueous solutions using supported, nanoscalezero-valentiron. Environ Sci Technol 34(12):2564–2569

Pradeep T (2009) Noble metal nanoparticles for water purification: a critical review. Thin Solid Films 517(24):6441–6478

Prasad K, Jha AK (2009) Nat Sci 1:129

Prasse C, Ternes T (2010) Removal of organic and inorganic pollutants and pathogens from wastewater and drinking water using nanoparticles—a review. Nanoparticles in the water cycle. Springer, Berlin

Prokop G, Schamann M, Edelgaard I (2000) Management of contaminated sites in Western Europe. European Environment Agency, Copenhagen

Qi L, Xu Z (2004) Lead sorption from aqueous solutions on chitosan nanoparticles. Colloids Surf A 251:183–190

Qiang Y, Sharma A, Paszczynski A, Meyer D (2007) Conjugates of magnetic nanoparticle-enzyme for bioremediation. In: Proceedings of the 2007 NSTI Nanotechnology Conference and Trade Show, vol 4, pp 656–659

Qiu Y, Liu Y, Wang L, Xu L, Bai R, Ji Y, Chen C (2010) Surface chemistry and aspect ratio mediated cellular uptake of Au nanorods. Biomaterials 31:7606–7619

Qu X, Alvarez PJJ, Li Q (2013) Applications of nanotechnology in water and wastewater treatment. Water Res 47:3931–3946

Quan X, Yang S, Ruan X, Zhao H (2005) Preparation of titania nanotubes and their environmental applications as electrode. Environ Sci Technol 39(10):3770–3775

Raj R, Dalei K, Chakraborty J, Das S (2016) Extracellular polymeric substances of a marine bacterium mediated synthesis of CdS nanoparticles for removal of cadmium from aqueous solution. J Colloid Interface Sci 462:166–175

Rajendran P, Gunasekaran P (2007) Nanotechnology for bioremediation of heavy metals. In: Environmental bioremediation technologies 2007. Springer, Berlin, pp 211–221

Raliya R, Tarafdar JC (2013) ZnO nanoparticle biosynthesis and its effect on phosphorous-mobilizing enzyme secretion and gum contents in Clusterbean (Cyamopsis tetragonoloba L.). Agribiol Res 2:48–57

Raliya R, Tarafdar JC (2014) Int Nano Lett 4:1

Raliya R, Rathore I, Tarafdar JC (2013) Development of microbial nanofactory for zinc, magnesium, and titanium nanoparticles production using soil fungi. J Bionanosci 7:59

Ramesha GK, Kumara AV, Muralidhara HB, Sampath S (2011) Graphene and graphene oxide as effective adsorbents toward anionic and cationic dyes. J Colloid Interface Sci 361:270–277

Rao Kotte M, Kuvarega AT, Cho M, Mamba BB, Diallo MS (2015) Mixed matrix PVDF membranes with in situ synthesized PAMAM dendrimer-like particles: a new class of sorbents for Cu(II) recovery from aqueous solutions by ultrafiltration. Environ Sci Technol 49:9431–9442

Ray PC (2010) Size and shape dependent second order nonlinear optical properties of nanomaterials and their application in biological and chemical sensing. Chem Rev 110:5332–5365

Reddy KR, Adams JA (2010) Sustainable remediation of contaminated sites. Momentum Press, New York, NY

Reddy KR, Khodadoust AP, Darko-Kagya K (2014) Transport and reactivity of lactate-modified nanoscale iron particles for remediation of DNT in subsurface soils. J Environ Eng 140 (12):04014042. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000870

Rickerby D, Morrison M (2007) Workshop on Nanotechnologies for Environmental Remediation, JRC Ispra

Ruthven DM (1984) Principles of adsorption and adsorption processes. Wiley, New York

Rychoudhury T, Scheytt T (2013) Potential of zerovalent iron nanoparticles for remediation of environmental organic contaminants in water: a review. Water Sci Technol 68:1425–1439

Ryu A, Jeong SW, Jang A, Choi H (2011) Reduction of highly concentrated nitrate using nanoscale zero-valent iron: effects of aggregation and catalyst on reactivity. Appl Catal B 105(1):128–135

Sadowski Z (2010) Biosynthesis and applications of silver and gold nanoparticles. In: Perez DP (ed) Silver nanoparticles. InTech, Croatia, pp 257–276. https://doi.org/10.5772/8508

Saha S, Sarkar P (2012) Arsenic remediation from drinking water by synthesized nano-alumina dispersed in chitosan-grafted polyacrylamide. J Hazard Mater 227–228:68–78

Saha K, Agasti SS, Kim C, Li X, Rotello VM (2012) Gold nanoparticles in chemical and biological sensing. Chem Rev 112:2739–2779

Saleh TA, Gondal MA, Drmosh QA, Yamani ZH, Al-Yamani A (2011) Enhancement in photocatalytic activity for acetaldehyde removal by embedding ZnO nanoparticles on multiwall carbon nanotubes. Chem Eng J 166:407–412

Salvadori MR, Ando RA, Nascimento CAO, Corrêa B (2014) PLos One 9:e87968

Sambale F, Wagner S, Stahl F, Khaydarov RR, Scheper T, Bahnemann D (2015) Investigations of the toxic effect of silver nanoparticles on mammalian cell lines. J Nanomater. https://doi.org/10.1155/2015/136765

Sanna V, Pala N, Alzari V, Nuvoli D, Carcelli M (2016) ZnO nanoparticles with high degradation efficiency of organic dyes under sunlight irradiation. Mater Lett 162:257–260

Santornchot P, Satapanajaru T, Comfort SD (2010) Application of nano-zero valent iron for treating metolachlor in aqueous solution. World Acad Sci Eng Technol 48:625–628

Saravanan M, Nanda A (2010) Extracellular synthesis of silver bionanoparticles from Aspergillus clavatus and its antimicrobial activity against MRSA and MRSE. Colloids Surf B Biointerfaces 77:214

Satapanajaru T, Anurakpongsatorn P, Pengthamkeerati P, Boparai H (2008) Remediation of atrazine-contaminated soil and water by nano zerovalent iron. Water Air Soil Pollut 192(1–4):349–359

Sathishkumar P, Mangalaraja RV, Anandan S, Ashokkumar M (2013) Photocatalytic degradation of ternary dye mixture in aqueous environment using gold nanoparticles loaded amino and mercapto functionalized TiMCM-41 nanocatalysts in the presence of visible light. Sep Purif Technol 102:67–74

Sayles GD, You G, Wang M, Kupferle MJ (1997) DDT, DDD, and DDE dechlorination by zero-valent iron. Environ Sci Technol 31(12):3448–3454

Schrick B, Blough JL, Jones AD, Mallouk TE (2002) Hydrodechlorination of trichloroethylene to hydrocarbons using bimetallic nickel-iron nanoparticles. Chem Mater 14(12):5140–5147

Schrick B, Hydutsky BW, Blough JL, Mallouk TE (2004) Deliveryvehiclesforzerovalentmetalnanoparticlesinsoiland groundwater. Chem Mater 16 (11):2187–2193

Seteni B, Ngila JC, Sikhwivhilu K, Moutloali RM, Mamba B (2013) Dechlorination of 3, 3′, 4, 4 ′-tetrachlorobiphenyl (PCB77) in water, by nickel/iron nanoparticles immobilized on L-lysine/PAA/PVDF membrane. Phys Chem Earth 66:60–67

Sevougian SD, Steefel CI, Yabusaki SB (1994) Enhancing the design of in situ chemical barriers with multicomponent reactive transport modeling, in situ remediation: scientific basis for current and future technologies. In: Proceedings of the 33rd Hanford symposium on health and the environment. Battelle Press, Columbus, OH, Pasco, Forum (Wash), pp 1–28

Shaari N, Tan SH, Mohamed AR (2012) Synthesis and characterization of CNT/Ce-TiO2 nanocomposite for phenol degradation. J Rare Earths 30:651–658

Shahwan T, Sirriah SA, Nairat M, Boyacı E, Eroğlu AE, Scott TB, Hallam KR (2011) Green synthesis of iron nanoparticles and their application as a Fenton-like catalyst for the degradation of aqueous cationic and anionic dyes. Chem Eng J 172(1):258–266

Shan G, Xing J, Zhang H, Liu H (2005) Biodesulfurization of dibenzothiophene by microbial cells coated with magnetite nanoparticles. Appl Environ Microbiol 71(8):4497–4502

Shao L, Yao Y, Quan S, Wei H, Wang R, Guo Z (2014) Onepot in situ synthesized TiO_2/layered double hydroxides (LDHs) composites toward environmental remediation. Mater Lett 114:111–114

Sharma B, Dangi AK, Shukla P (2018) Contemporary enzyme based technologies for bioremediation: a review. J Environ Manage 210:10–22

Shi L, Du J, Chen Z, Megharaj M, Naidu R (2013) Functional kaolinite supported Fe/Ni nanoparticles for simultaneous catalytic remediation of mixed contaminants (lead and nitrate) from wastewater. J Colloid Interface Sci 428:302–307

Shi C, Zhu N, Cao Y, Wu P (2015) Biosynthesis of gold nanoparticles assisted by the intracellular protein extract of Pycnoporus sanguineus and its catalysis in degradation of 4-nitroaniline. Nanoscale Res Lett 10:1–8

Shipway AN, Katz E, Willner I (2000) Nanoparticle arrays on surfaces for electronic, optical, and sensor applications. ChemPhysChem 1:18–52

Shourian M, Noghabi KA, Zahiri HS, Bagheri T, Karbalaei R, Mollaei M, Rad I, Ahadi S, Raheb J, Abbasi H (2009) Efficient phenol degradation by a newly characterized Pseudomonas sp. SA01 isolated from pharmaceutical wastewaters. Desalination 246(1-3):577–594

Silva-De Hoyos LE, Sánchez-Mendieta V, CamachoLópez MA, Trujillo-Reyes J, Vilchis-Nestor AR (2018) Plasmonic and fluorescent sensors of metal ions in water based on biogenic gold nanoparticles. Arabian J Chem. https://doi.org/10.1016/j.arabjc.2018.02.016

Simonton S, Dimsha M, Thomson B, Barton LL, Cathey G (2000) Long-term stability of metals immobilized by microbial reduction. In: Proceedings of the 2000 conference on hazardous waste research: environmental challenges and solutions to resource development, production and use, Southeast Denver, CO, pp 394–403

Singh R, Misra V, Singh RP (2012) Removal of hexavalent chromium from contaminated ground water using zerovalent iron nanoparticles. Environ Monit Assess 184:3643–3651

Sinha T, Ahmaruzzaman M (2015) Green synthesis of copper nanoparticles for the efficient removal (degradation) of dye from aqueous phase. Environ Sci Pollut Res 22(24):20092–20100
Sinha AK, Suzuki K (2007) Novel mesoporous chromium oxide for VOCs elimination. Appl Catal B Environ 70(1):417–422. https://doi.org/10.1016/j.apcatb.2005.10.035
Siva S, Sameem SM, Sudharsan S, Kannan RS (2013) Synthesis, characterization and application of zero-valent silver nano adsorbents. Int J Innov Res Sci Eng Technol 2:8023–8037
Smuleac V, Varma R, Sikdar S, Bhattacharyya D (2011) Green synthesis of Fe and Fe/Pd bimetallic nanoparticles in membranes for reductive degradation of chlorinated organics. J Membr Sci 379 (1):131–137
Solsona B, García T, Sanchis R, Soriano MD, Moreno M, Rodríguez-Castellón E et al (2016) Total oxidation of VOCs on mesoporous iron oxide catalysts: soft chemistry route versus hard template method. Chem Eng J 290:273281. https://doi.org/10.1016/j.cej.2015.12.109
Som C, Berges M, Chaudhry Q, Dusinska M, Fernandes TF, Olsen SI et al (2010) The importance of life cycle concepts for the development of safe nanoproducts. Toxicology 269(2):160–169. https://doi.org/10.1016/j.tox.2009.12.012
Song H, Carraway ER (2005) Reduction of chlorinated ethanes by nanosized zero-valent iron: kinetics, pathways, and effects of reaction conditions. Environ Sci Technol 39(16):6237–6245
Soni N, Prakash S (2012a) Efficacy of fungus mediated silver and gold nanoparticles against Aedes aegypti larvae. Parasitol Res 110(1):175–184
Soni N, Prakash S (2012b) Synthesis of gold nanoparticles by the fungus Aspergillus niger and its efficacy against mosquito larvae. Rep Parasitol 2:1–7
Soni N, Prakash S (2012c) Fungal-mediated nano silver: an effective adulticide against mosquito. Parasitol Res 111:2091
Soni N, Prakash S (2014) Microbial synthesis of spherical nanosilver and nanogold for mosquito control. Ann Microbiol 64(3):1099–1111
Soni N, Prakash S (2015) Antimicrobial and mosquitocidal activity of microbial synthesized silver nanoparticles. Parasitol Res 114(3):1023–1030
Soomro RA, Nafady A (2015) Catalytic reductive degradation of methyl orange using air resilient copper nanostructures. J Nanomater 2015:120
Srivastava NK, Majumdar CB (2008) Novel biofiltration methods for the treatment of heavy metals from industrial wastewater. J Hazard Mater 151:1–8
Srivastava S, Thakur IS (2006) Evaluation of bioremediation and detoxification potentiality of Aspergillus niger for removal of hexavalent chromium in soil microcosm. Soil Biol Biochem 38 (7):1904–1911
Srivastava PK, Vaish A, Dwivedi S, Chakrabarty D, Singh N, Tripathi RD (2011a) Biological removal of arsenic pollution by soil fungi. Sci Total Environ 409:2430–2442
Srivastava S, Shrivastava M, Suprasanna P, D'Souza SF (2011b) Phytofiltration of arsenic from simulated contaminated water using *Hydrilla verticillata* in field conditions. Ecol Eng 37:1937–1194
Srivastava V, Gusain D, Sharma YC (2013) Synthesis, characterization and application of zinc oxide nanoparticles (n-ZnO). Ceram Int 39(8):9803–9808
Stafiej A, Pyrzynska K (2007) Adsorption of heavy metal ions with carbon nanotubes. Sep Purif Technol 58:49–52
Steed VS, Suidan MT, Gupta M, Miyahara T, Acheson CM, Sayles GD (2000) Development of a sulfate-reducing biological process to remove heavy metals from acid mine drainage. Water Environ Res 72:530–535
Subramanyam SG, Siva K (2016) Int J Life Sci Res 4:69
Suman AK, Gera M, Jain VK (2015) A novel reusable nanocomposite for complete removal of dyes, heavy metals and microbial load from water based on nanocellulose and silver nano-embedded pebbles. Environ Technol 36:706–714
Suvith VS, Philip D (2014) Catalytic degradation of methylene blue using biosynthesized gold and silver nanoparticles. Spectrochim Acta A 118:526–532

Tang WZ, An H (1995) UV/TiO_2 photocatalytic oxidation of commercial dyes in aqueous solutions. Chemosphere 31:4157–4170

Taniguchi N (1974) On the basic concept of nanotechnology. In: Proceedings of international conference on precision engineering (ICPE), Tokyo, Japan, pp 18–23

Tarafdar A, Raliya R, Wang W-N, Biswas P, Tarafdar J (2013) Green synthesis of TiO2 nanoparticle using Aspergillus tubingensis. Adv Sci Eng Med 5:943

Tayeb AM, Tony MA, Ismaeel EK (2019) Engineered nanostructured ZnO for water remediation: operational parameters effect, Box–Behnken design optimization and kinetic determinations. Appl Water Sci 9:43. https://doi.org/10.1007/s13201-019-0921-0

Thome A, Reddy KR, Reginatto C, Cecchin I (2015a) Review of nanotechnology forsoil and groundwater remediation: Brazilian perspectives. Water Air Soil Pollut 226(4):1–20. https://doi.org/10.1007/s11270-014-2243-z

Thome A, Reddy KR, Reginatto C, Cecchin I (2015b) Water Air Soil Pollut 226:1e20

Tisa F, Raman AAA, Daud WMAW (2014) Applicability of fluidized bed reactor in recalcitrant compound degradation through advanced oxidation processes: a review. J Environ Manage 146:260–275

Tomalia DA, Baker H, Dewald J et al (1984) A new class of polymers: starburst-dendritic macromolecules. Polym J 17(1):117–132

Tong M, Yuan S, Long H, Zheng M, Wang L, Chen J (2011) Reduction of nitrobenzene in groundwater by iron nanoparticles immobilized in PEG/nylon membrane. J Contam Hydrol 122:16–25

Tratnyek PG, Johnson RL (2006) Nanotechnologies for environmental cleanup. Nano Today 1:44–48

Tripathi V, Fraceto LF, Abhilas PC (2015) Sustainable clean-up technologies for soils contaminated with multiple pollutants: plant-microbe-pollutant and climate nexus. Ecol Eng 82:330

Tripathi S, Sanjeevi R, Anuradha J, Chauhan DS, Rathoure AK (2018) Nano-bioremediation: nanotechnology and bioremediation. In: Biostimulation remediation technologies for groundwater contaminants. IGI Global, pp 202–219

Tungittiplakorn W, Lion LW, Cohen C, Kim J-Y (2004) Engineered polymeric nanoparticles for soil remediation. Environ Sci Technol 38(5):1605–1610

Tungittiplakorn W, Cohen C, Lion LW (2005) Engineered polymeric nanoparticles for bioremediation of hydrophobic contaminants. Environ Sci Technol 39(5):1354–1358

Turner RJ (2017) Metal-based antimicrobial strategies. J Microbial Biotechnol 10(5):1062–1065

Umer A, Naveed S, Ramzan N (2012) World Sci Publ Company 7:18

Undre SB, Singh M, Kale RK (2013a) Interaction behaviour of trimesoylchloride derived 1st tier dendrimers determined with structural and physicochemical properties required for drug designing. J Mol Liq 182:106–120

Undre SB, Singh M, Kale RK, Rizwan M (2013b) Silibinin binding and release activities moderated by interstices of trimesoyl, tridimethyl, and tridiethyl malonate first-tier dendrimers. J Appl Polym Sci 130:3537–3554

Varanasi P, Fullana A, Sidhu S (2007) Remediation of PCB contaminated soils using iron nanoparticles. Chemosphere 66:1031–1038

Varma AJ, Deshpande SV, Kennedy JF (2004) Metal complexation by chitosan and its derivatives: a review. Carbohydr Polym 55:77–93

Varshney R, Seema B, Gaur MS, Pasricha R (2010) J Metals 62(102):187

Varshney G, Kanel SR, Kempisty DM, Varshney V, Agrawal A, Sahle-Demessie E, Nadagouda MN (2016) Nanoscale TiO_2 films and their application in remediation of organic pollutants. Coord Chem Rev 306:43–64

Vaseashta A, Vaclavikova M, Vaseashta S, Gallios G, Roy P, Pummakarnchana O (eds) (2007) Nanostructures in environmental pollution detection, monitoring, and remediation, Vol. 8. Taylor & Francis, Boca Raton, FL

Vasudevan V, Gayathri KV, Krishnan ME (2018) Bioremediation of a pentacyclic PAH, Dibenz (a, h) Anthracene—a long road to trip with bacteria, fungi, autotrophic eukaryotes and surprises. Chemosphere 202:387–399

Vidal CB, Raulino GSC, Barros AL, Lima ACA, Ribeiro JP, Pires MJR et al (2012) BTEX removal from aqueous solutions by HDTMA-modified Y zeolite. J Environ Manage 112:178–185. https://doi.org/10.1016/j.jenvman.2012.07.026

Vijayaraghavan K, Yun YS (2008) Bacterial biosorbents and biosorption. Biotechnol Adv 26:266–291

Vinuth M, Naik HSB, Manjanna J (2015) Remediation of hexavalent chromium from aqueous solution using clay mineral Fe (II)–montmorillonite: encompassing anion exclusion impact. Appl Surf Sci 357:1244–1250

VishnuKirthi A, AbdulRahuman G, Rajakumar S, Marimuthu T, Santhoshkumar C, Jayaseelan G, Elango A, AbduzZahir C, Kamarajbagawan A (2011) Mater Lett 65:2745

Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol Prog 11:235–250

Vyas SP, Khar RK (2004) Targeted and controlled drug delivery—novel carrier systems, 1st edn. CBS Publication, New Delhi, p 331

Wang CT (2007) Photocatalytic activity of nanoparticle gold/iron oxide aerogels for azo dye degradation. J Non-Cryst Solids 353:1126–1133

Wang J, Chen C (2009) Biosorbents for heavy metals removal and their future. Biotechnol Adv 27:195–226

Wang C-B, Zhang W-X (1997) Synthesizing nanoscale iron particles for rapid and complete dechlorination of TCE and PCBs. Environ Sci Technol 31(7):2154–2156

Wang Y, Yang F, Yang X (2010) Colorimetric detection of mercury (II) ion using unmodified silver nanoparticles and mercury-specific oligonucleotides. ACS Appl Mater Interfaces 2:339–342

Wang S, Sun H, Ang HM, Tade´ MO (2013) Adsorptive remediation of environmental pollutants using novel graphene-based nanomaterials. Chem Eng J 226:336–347. https://doi.org/10.1016/j.cej.2013.04.070

Wang T, Jin X, Chen Z, Megharaj M, Naidu R (2014) Green synthesis of Fe nanoparticles using eucalyptus leaf extracts for treatment of eutrophic wastewater. Sci Total Environ 466–467:210–213

Wang AN, Teng Y, Hu XF, Wu LH, Huang YJ, Luo YM, Christie P (2016) Diphenylarsinic acid contaminated soil remediation by titanium dioxide (P25) photocatalysis: degradation pathway, optimization of operating parameters and effects of soil properties. Sci Total Environ 541:348–355

Wang D, Pillai SC, Ho S-H, Zeng J, Li Y, Dionysiou DD (2018) Plasmonic-based nanomaterials for environmental remediation. Appl Catal Environ 237:721–741. https://doi.org/10.1016/j.apcatb.2018.05.094

Wei YT, Wu SC, Yang SW, Che CH, Lien HL, Huang D (2012a) Biodegradable surfactant stabilized nanoscale zero-valent iron for in situ treatment of vinyl chloride and 1,2-dichloroethane. J Hazard Mater 211–212(211–212):373–380

Wei YT, Wu SC, Yang SW, Che CH, Lien HL, Huang DH (2012b) Biodegradable surfactant stabilized nanoscale zero-valent iron for in situ treatment of vinyl chloride and 1,2-dichloroethane. J Hazard Mater 211:373–380

Wei W, Liu D, Wei Z, Zhu Y (2016) Short-range π–π stacking assembly on P25 TiO_2 nanoparticles for enhanced visible-light photocatalysis. ACS Catal 7:652–663

Welser K, Adsley R, Moore BM, Chan WC, Aylott JW (2011) Protease sensing with nanoparticle based platforms. Analyst 136:29–41

Wen M, Zhang S, Dai W, Li G, Zhang D (2015) In situ synthesis of Ti3+ self-doped mesoporous TiO_2 as a durable photocatalyst for environmental remediation. Chin J Catal 36:2095–2102

de Windt W, Aelterman P, Verstraete W (2005) Bioreductive depositionofpalladium(0) nanoparticlesonShewanellaoneidensis with catalytic activity towards reductive dechlorination ofpolychlorinatedbiphenyls. Environ Microbiol 7(3):314–325

Wu F, Sheng DN, Hua HL (2000) Chemosphere 41:1233–1238

Xia S, Song Z, Jeyakumar P, Shaheen SM, Rinklebe J, Ok YS, Bolan N, Wang H (2019) A critical review on bioremediation technologies for Cr(VI)-contaminated soils and wastewater. Crit Rev Environ Sci Technol 49(12):1027–1078

Xiong Z, He F, Zhao D, Barnett MO (2009) Immobilization of mercury in sediment using stabilized iron sulfide nanoparticles. Water Res 43:5171–5179

Xiong T, Yuan X, Wang H, Leng L, Li H, Wu Z et al (2018) Implication of graphene oxide in Cdcontaminated soil: a case study of bacterial communities. J Environ Manage 205:99106. https://doi.org/10.1016/j.jenvman.2017.09.067.

Xu J-C, Mei L, Guo X-Y, Li H-U (2005) Zinc ions surface-doped titanium dioxide nanotubes and its photocatalysis activity for degradation of methyl orange in water. J Mol Catal A Chem 226:123–127

Xu Z, Xie Q, Shuo C, Zhao H, Yu L (2007) Photocatalytic remediation of γ-hexachlorocyclohexane contaminated soils using TiO_2 and montmorillonite composite photocatalyst. J Environ Sci 19:358–361

Yadav KK (2017) J Mater Environ Sci 8:740

Yadav KK, Singh JK, Gupta N, Kumar V (2017) A review of nanobioremediation technologies for environmental cleanup: a novel biological approach. J Mater Environ Sci 8:740–757

Yadav D, Kumar P, Kapur M, Mondal MK (2018) Environ Prog Sustain Energy. https://doi.org/10.1002/ep.12920

Yan W, Lien H-L, Koel BE, Zhang W-X (2013) Iron nanoparticles for environmental clean-up: Recent developments and future outlook. Environ Sci Process Impacts 15(1):63–77. https://doi.org/10.1039/C2EM30691C

Yana L, Yina H, Zhang S, Lenga F, Nana W, Li H (2010) Biosorption of inorganic and organic arsenic from aqueous solution by Acidithiobacillus ferrooxidans BY-3. J Hazard Mater 178:209–217

Yang S, Hu J, Chen C, Shao D, Wang X (2011) Mutual effects of Pb (II) and humic acid adsorption on multiwalled carbon nanotubes/polyacrylamide composites from aqueous solutions. Environ Sci Technol 45:3621–3627

Yu CWF, Jeong Tai K (2010) Building pathology, investigation of sick buildings—VOC emissions. Indoor Built Environ 19(1):3039. https://doi.org/10.1177/1420326X09358799

Yu CWF, Kim JT (2012) Photocatalytic oxidation for maintenance of indoor environmental quality. Indoor Built Environ 22(1):3951. https://doi.org/10.1177/1420326X12470282

Yuan S, Long H, Xie W, Liao P, Tong M (2012) Electrokinetic transport of CMC-stabilized Pd/Fe nanoparticles for the remediation of PCP-contaminated soil. Geoderma 185:18–25

Yunus IS, Harwin, Kurniawan A, Adityawarman D, Indarto A (2012) Nanotechnologies in water and air pollution treatment. Environ Technol Rev 1(1):136148. https://doi.org/10.1080/21622515.2012.733966

Zare K, Sadegh H, Shahryari-ghoshekandi R, Asif M, Tyagi I, Agarwal S, Gupta VK (2016) Equilibrium and kinetic study of ammonium ion adsorption by Fe_3O_4 nanoparticles from aqueous solutions. J Mol Liq 213:345–350

Zeng S, Weng X, Tong Y, Lin W, Chen Z (2015) Simultaneous removal of Pb (II) and Cd (II) from aqueous solution by green synthesized iron nanoparticles. Acta Sci Circumst 35:3538

Zhang W-X (2003a) Nanoscale iron particles for environmental remediation: an overview. J Nanopart Res 5:323

Zhang W (2003b) Nanoscale iron particles for environmental remediation: an overview. J Nanopart Res 5:323–332

Zhang W-X (2009) Soy protein and/or soy derivatives with zero-valent iron compositions and use for environmental remediation. U.S. Patent # US, 7(507) 345 B2

Zhang S, Cheng F, Tao Z, Gao F, Chen J (2006) Removal of nickel ions from wastewater by Mg $(OH)_2$/MgO nanostructures embedded in Al_2O_3 membranes. J Alloys Compd 426:281–285

Zhang Z, Wang J, Liu D, Luo W, Zhang M, Jiang W, Zhu Y (2016) Highly efficient organic photocatalyst with full visible light spectrum through π–π stacking of TCNQ–PTCDI. ACS Appl Mater Interfaces 8:30225–30231

Zhang W, Lo Irene MC, Hu L, Voon CP, Lim BL, Versaw WK (2018) Environ Sci Technol 52:4385. https://doi.org/10.1021/acs.est.7b06697

Zhao J, Wang L (2011) Degradation of Rhodamine B in aqueous solution by the UV/ZnO photocatalytic process. In: 2011 international conference on materials for renewable energy and environment

Zhou H, Fan T, Han T, Li X, Ding J, Zhang D, Ogawa H (2009) Bacteria-based controlled assembly of metal chalcogenide hollow nanostructures with enhanced light-harvesting and photocatalytic properties. Nanotechnology 20(8):085603

Zhou L, Jin J, Liu Z, Liang X, Shang C (2011) Adsorption of acid dyes from aqueous solutions by the ethylenediaminemodified magnetic chitosan nanoparticles. J Hazard Mater 185:1045–1052

Zhou Y, Zhao H, Li C, He P, Peng W, Yuan L, Zeng L, He Y (2012) Colorimetric detection of Mn2+ using silver nanoparticles cofunctionalized with 4-mercaptobenzoic acid and melamine as a probe. Talanta 97:331–335

Zhu L, Jacob DJ, Keutsch FN, Mickley LJ, Scheffe R, Strum M et al (2017) Formaldehyde (HCHO) as a hazardous air pollutant: mapping surface air concentrations from satellite and inferring cancer risks in the United States. Environ Sci Technol 51(10):5650–5657. https://doi.org/10.1021/acs.est.7b01356

Zietz BP, Dieter HH, Lakomek M, Schneider H, Keßler-Gaedtke B, Dunkelberg H (2003) Epidemiological investigation on chronic copper toxicity to children exposed via the public drinking water supply. Sci Total Environ 302:127–144

Zou L, Luo Y, Hooper M, Hu E (2006) Removal of VOCs by photocatalysis process using adsorption enhanced TiO2SiO2 catalyst. Chem Eng Process Process Intensif 45(11):959–964. https://doi.org/10.1016/j.cep.2006.01.014

Bioelectrochemical System for Bioremediation and Energy Generation

13

M. M. Ghangrekar and B. Neethu

Abstract

Bioelectrochemical systems (BESs) are one of the rising technologies capable of converting chemical energy into electric energy and vice versa by achieving simultaneous wastewater bioremediation. The microorganisms involved in the process are the core of BESs as they catalyse the oxidation of organic matter present in wastewater to produce electrons. While the chemical energy present in wastewater is converted into electrical energy in a microbial fuel cell (MFC), the electrical energy is being used to produce chemicals in a microbial electrolysis cell (MEC). Similarly, appropriate use of ion exchange membranes makes MFC capable of desalinating saline water and also facilitates recovery of nutrients from wastewater. The BESs have also proved its efficiency in utilising the solar energy for application in photosynthetic MFC employing microalgae as well as higher plants. Moreover, wastewater bioremediation in MFCs has extended its applicability in treating diverse waste streams starting from industrial and domestic to wastewater containing dye, organo-chloride, nitrate, ammonia, etc. Most remarkable advancement in BES research started with the recovery of value-added products including heavy metals, apart from generation of power. Even though innovative designs and low-cost efficient materials for electrodes, catalysts and proton exchange membranes (PEMs) for application in BESs have been developed, there are quite a few challenges of BESs that need to be addressed to take this technology forward. This chapter showers light on the microbial aspects of BES, with special focus on MFC, along with a thorough discussion on the recent developments in BES research emphasising its bottlenecks and challenges.

M. M. Ghangrekar (✉) · B. Neethu
Department of Civil Engineering, Indian Institute of Technology, Kharagpur, India
e-mail: ghangrekar@civil.iitkgp.ac.in

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_13

Keywords
Exo-electrogens · Desalination · Heavy metal recovery · Microalgae · Recalcitrant compound removal · Wastewater treatment

13.1 Introduction

Water is one of nature's precious gifts to mankind that is a renewable resource, however at the same time a finite resource too. Apart from the fact that every living organism needs water for survival, water plays a crucial role in the sustainability of industrial, agricultural and production sectors. Water use has more than tripled globally since the 1950s, and one out of every six persons does not have regular access to safe drinking water. Lack of access to a safe water supply and sanitation affects the health of 1.2 billion people annually (WHO and UNICEF 2014). Water quality has been degraded by domestic and industrial pollution sources as well as non-point sources. Growing population, rising industrialisation and expanding agriculture have pushed up the water demand. The CPHEEO estimates about 70–80% of total water supplied for domestic use gets converted to sanitary wastewater and in addition to this industries too contribute a remarkable quantity of wastewater. Thus an increasing use of water indirectly increases the wastewater generation. As it is always recommended to use water wisely, same is applicable for wastewater, i.e. using wastewater wisely can make it a valuable resource. Thus, wastewater from households, industries and agriculture should not be seen as a problem but as a valuable resource, which could meet the demands for water, energy and nutrients.

A microbial fuel cell (MFC) is relatively new and emerging technology, which produces electrical energy from the chemical energy stored in the organic molecules present in wastewater or any aqueous solution (Logan et al. 2006). The working principle of an MFC is similar to that of a fuel cell with a difference that microbes act as a catalyst in anoxic anodic chamber and organic matter present in wastewater acts as fuel. Assuming a 100% conversion efficiency, theoretically 1 kg of organic matter (glucose as substrate) removed can produce 1 kWh of energy (Aelterman et al. 2006). A typical two-chambered MFC consists of an anaerobic anodic chamber containing the anode and bacterial consortia (electrogenic bacteria), a cathodic chamber containing the cathode and the terminal electron acceptor (TEA), a proton exchange membrane (PEM) separating these two chambers and the electrical circuit that allows the electron transport from anode to cathode. A dominant challenge during the commercialisation of this technology lies in the initial cost. A major part of the fabrication cost is attributed to the PEM, electrodes and energy supplied in providing oxygen. Hence, there is a need for low-cost and effective alternatives for each of these. Even though the energy consumed by providing aeration in the cathodic chamber can be eliminated by providing air cathodes, it does not provide any value addition to the technology in terms of products.

The application of microorganisms for bioremediation and biodegradation is gaining much importance in today's world owing to the fact that these

eco-friendly microorganisms do not produce any environmentally toxic by-products on biodegrading the wastewater. An MFC is a promising technology that utilises these eco-friendly microorganisms; however it offers a step forward to normal anaerobic treatment systems as it has the potential to generate direct electric power along with wastewater treatment. This chapter throws light on the basic microbial electron transfer mechanisms involved in an MFC with a detailing of techniques to confirm the same including electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), etc. Also, a short review on different applications of MFC including microbial desalination cell (MDC), microbial carbon capture cell (MCC) and sediment microbial fuel cell (SMFC) are being covered. A special focus was given to the bioremediation of wastewater, including domestic and industrial, and emphasis was laid on nitrate removal, recalcitrant removal and heavy metal recovery using MFC. The bottlenecks and future perspectives are being discussed at the end of the chapter as a guidance to take the research on MFC forward.

13.2 Electrochemically Active Biofilms

Bacteria have great adaptability and can survive in coldest to warmest condition and also eat a wide range of organic matter as well as some inorganic matters. Electrochemically active bacteria are those groups of microorganisms that can transfer electrons to external electron acceptors, which means they can give way to electricity generation. One of the major hurdles is in harnessing the power from these tiny bacterial cells. Bioelectrochemical systems (BES) can efficiently harness electrical energy out of electrochemically active bacteria. In BES, these bacteria are employed in the anaerobic anodic chamber with a purpose to harvest electrical energy from wastewater, and these are referred to as electrochemically active biofilms when developed on the electrodes. Bacteria produce electricity by extracellular electron transfer (EET), as they generate electrons while oxidation of substrate and transfer them across their cell membrane through tiny channels. The existing techniques to measure this are time-consuming and involve large sample size and complicated extraction protocol, and these techniques rupture the cell and denature the proteins.

13.2.1 Mechanisms of Electron Transfer

The electrochemically active bacterial species that exhibit the ability to directly transfer the electrons exogenously outside the cells are called electrogens (Kumar et al. 2015). Various mechanisms have been proposed for extracellular transport of electrons by electrogenic bacteria to the anodic surface. The electron transfer mechanisms can be direct electron transfer and mediated electron transfer (Fig. 13.1). Direct transfer can take place via membrane-bound cytochromes or via electrically conductive nanowires (pili), whereas, electron transfer is mediated by redox mediators or oxidation of reduced secondary metabolites in mediated electron transfer.

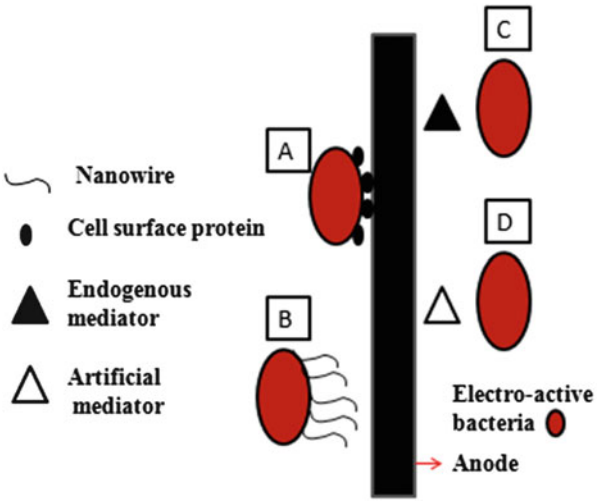


Fig. 13.1 Different mechanisms of electron transfers from microorganisms to electrode through (*A*) cell surface proteins, (*B*) nanowires, (*C*) endogenous mediator produced by microorganisms and (*D*) presence of exogenous mediator

Direct reduction of an exogenous acceptor occurs by direct contact between the cell's oxidoreductases and the terminal electron acceptor (electrode). *Rhodoferax ferrireducens*, which can quantitatively transfer electrons to graphite electrodes without the need for an electron-shuttling mediator, uses the direct transfer mechanism (Chaudhuri and Lovley 2003, b). Also, iron-reducing bacteria, *Klebsiella* sp. *IR21*, isolated from the anode biofilm of an MFC, gave a power density of 8.9 ± 3.65 mW/m^2 (Lee et al. 2016). The electrons also get transferred via electrically conductive proteinaceous filaments, i.e. nanowires, produced by the bacteria. Pili of *G. sulfurreducens* was reported to serve as biological nanowires, transferring electrons from the cell surface to the surface of Fe(III) oxides (Reguera et al. 2005).

Some bacteria transfer electrons with the help of mediators, which are redox compounds. Mediators that transfer electrons can be secreted by bacteria as in *Shewanella*, which secretes flavins that mediate extracellular electron transfer (Marsili et al. 2008). Also, *Citrobacter freundii Z7,* isolated from the anodic biofilm of MFC inoculated with aerobic sewage sludge, gave a maximum power density of 204.5 mW/m^2, and experiments indicated that the strain Z7 transferred electrons via secreted mediators (Huang et al. 2014). In some other cases, the redox compounds include artificial mediators, which are chemicals that facilitate the shuttling of electrons from inside of cell to electrodes outside the cell. For developing a novel cost-effective electrode material and power production from domestic wastewater using three different mediators, methylene blue, neutral red and 2-hydroxy-1,4-naphthoquinone were selected as electrode mediators with different concentrations, where methylene blue is reported to give a power density of 636 mW/m^2 (Taskan et al. 2015). Some other artificial mediators reported so far include thionine, humic acid, potassium ferricyanide (Rahimnejad et al. 2013), anthraquinone-2-6 and others (Kumar et al. 2015).

Table 13.1 Electroactive bacteria used in microbial fuel cells

Microbes	Power density/current density	References
Geobacter sulfurreducens	3147 mA/m^2	Bond and Lovley (2003)
Geobacter metallireducens	40 mW/m^2	Min et al. (2005)
Shewanella oneidensis	3700 mA/m^2	Hasan et al. (2017)
Shewanella putrefaciens	4.92 mW/m^2	Pandit et al. (2014)
Rhodopseudomonas palustris	3510 mW/m^3	Call et al. (2017)
Thermincola ferriacetica	11,200 mA/m^2	Lusk et al. (2016)
Pseudomonas aeruginosa	316 mW/m^2	Ali et al. (2017)
Desulfovibrio desulfuricans	233 mA/m^2	Kang et al. (2014)
Klebsiella pneumoniae	12.87 W/m^3	Islam et al. (2018)

13.2.2 Application of Electrogens in MFC

A phototrophic purple non-sulphur bacterium *Rhodopseudomonas palustris* DX-1 was reported to produce power density of 2720 mW/m^2. This DX-1 also utilised a wide variety of substrates (volatile acids, yeast extract, and thiosulphate) for power production in different metabolic modes, proving its activity from a range of simple to complex sources of organic matter (Xing et al. 2008). A bacterial strain *Ochrobactrum anthropi* YZ-1 as isolated from MFC was capable of producing a power density of 89 mW/m^2 using acetate as the electron donor in the U-tube MFC (Table 13.1). This strain was also capable of producing current using a wide range of substrates, including acetate, lactate, propionate, butyrate, glucose, sucrose, cellobiose, glycerol and ethanol (Zuo et al. 2008). Similarly, *Acidiphilium* sp. strain *3.2 Sup 5* cells that were isolated from an extreme acidic environment were able to produce high-density electrocatalytic currents, up to 3 A/m^2 at a poised potential in the absence of redox mediators (Malki et al. 2008). Direct electron transfer from different anaerobically grown *Shewanella putrefaciens* strains without any electrochemical mediators showed electrochemical activities; however, no activities were observed in aerobically grown *Shewanella putrefaciens* (Kim et al. 2002).

Shewanella oneidensis DSP10 grown on graphite felt under minimal nutrient conditions gave power density of 1500 mW/m^2 from the mini-MFC (Biffinger et al. 2007). Also, higher concentrations of DSP10 were sustained at pH of 7, whereas this trend was reversed at pH of 5, which is not favourable for DSP10, and this pH is not suitable for MFCs because of elevated acidity levels in anolyte (Biffinger et al. 2007). *Propionibacterium freudenreichii* used as biocatalyst in a glycerol-oxidising MFC gave a maximum open circuit voltage of 485 mV and a maximum power density of 14.9 mW/m^2 (Reiche et al. 2016). *Klebsiella pneumoniae* strain L17 used as biocatalyst in MFCs achieved the maximum voltage outputs of 426.2 mV and showed the presence of an electrochemically active compound that could transfer electrons between *K. pneumoniae L17* and the anode (Deng et al. 2010).

13.2.3 Biofilm Electrochemistry

An electrochemically active biofilm can interact with metal electrode, and hence it can be diagnosed through electrochemical analysis techniques including cyclic voltammetry and electrochemical impedance spectroscopy. Anodic behaviour can be diagnosed by investigating the characteristics of electron transferred to the electrode, whereas the electrons getting removed signify the cathodic behaviour. Biofilm formed by a mixed bacterial culture contains species with different metabolic activities, and the electrochemical behaviour depends on the concentration of the electrochemical bacterial species present. Even in case of a single pure culture species, concentration gradient plays a major role along with the mediator characteristics. The pH, redox potential of the anolyte as well as biofilm, availability of oxygen, etc. also play a significant role in governing the electrochemical nature of biofilm. In order to analyse the electrochemical behaviour of the bacterial species, a potential is applied with respect to a known reference potential, which may or may not cause a flow of current. The produced current is measured to relate it back to the electrode potential. The possible mechanism that controls the electrochemical reaction in the cell is assessed based on the observed current-potential relationship.

13.2.3.1 Cyclic Voltammetry: A Tool to Analyse the Biofilm Electrochemical Phenomenon

A CV analysis is absolutely necessary to analyse an electrochemical reaction occurring in any electrochemical cell. The electrogenic bacterial species forms a biofilm that can interact with the electrode material by transferring electrons produced during the bacterial metabolism to the electrode. The CV can be used to characterise the electrochemical behaviour of this biofilm by applying a potential with respect to a known reference potential. A linear polarization potential scan starting from initial potential to reach a final potential is done, and this causes flow of current, which is measured. The results of this wide range of potential give rise to the voltammogram, which is further used to investigate the possible electron transfer mechanisms. The electrogenic bacterial community, which is associated with certain redox couples, generates a steady-state current in MFC. Reduction and oxidation peaks are formed during the forward and reverse scans around the formal reduction potential of the redox couple, which can be detected by the CV. On the usage of a mixed culture inoculum, the biofilm on the electrode will contain multiple species having different metabolic activities. Hence, the electrochemical behaviour of the electrode containing biofilm will depend on electrochemical or concentration gradients formed within the biofilm. In short, the electron transfer in biofilms is associated with complete acetate oxidation to electrons, protons and CO_2, where transfer of electron outside the cell is considered to be the rate limiting step. Thus, cyclic voltammetry is a promising analysis tool, which by employing of electrochemical theories explores the electron transfer mechanism in the BES.

13.2.3.2 Electrochemical Impedance Spectroscopy

The overpotentials associated with BES are normally investigated with the help of voltammetry studies. The BES are associated with several losses including ohmic loss, losses due to microbial kinetics, solution resistance losses, etc. However, voltammetric studies are not capable of identifying the contribution of each of these in the performance of BES. These individual contributions can be identified and quantified by electrochemical techniques including EIS. While the voltammetry techniques consider direct current, the EIS method involves alternating current. During EIS, voltage is applied at small sinusoidal amplitude, and the magnitude and shift in the response current is measured and analysed. The experiment is carried out at a range of frequencies as different process involved different i-v response at different frequencies. This makes it easy to understand the individual contributions, thus making it possible to quantify and identify the individual resistance as well as the overpotentials.

13.3 Introduction to Microbial Fuel Cell

In an MFC, the chemical energy present in the wastewater is converted into electrical energy by bacterial catalysis (Logan et al. 2006). An MFC consists of basically two processes, oxidation of organic matter in an anaerobic condition and reduction of a terminal electron acceptor. However to assist the completion of the overall process in an efficient way, it is necessary to accommodate several components into an MFC (Fig. 13.2). These include a proton exchange membrane, electrodes, catalysts, circuit, external resistance, etc. Each of the components used in an MFC should satisfy a specific set of properties to get maximum output out of it, which are described in brief here.

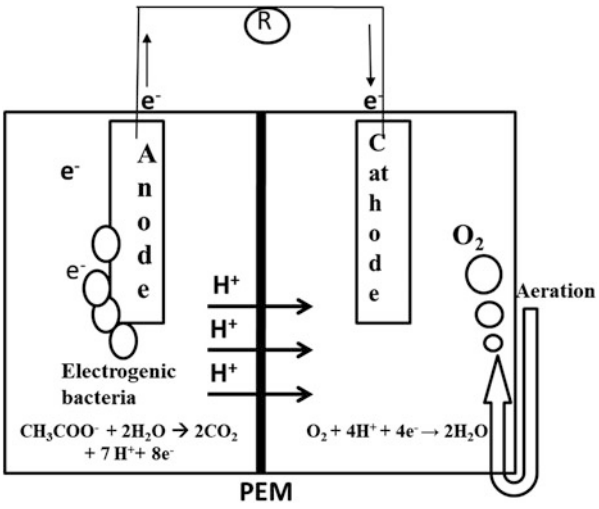


Fig. 13.2 Schematic diagram of a typical two-chambered microbial fuel cell

Table 13.2 Review of different methane suppression techniques employed in MFC

Techniques	Power density	References
Hexadecatrienoic acid from marine algae *Chaetoceros*	21.43 W/m^3	Rajesh et al. (2015)
Lauric acid	4.8 W/m^3	Rajesh et al. (2014)
2-Bromoethanesulfonate (concentration of 0.1–0.27 mM)	124 mW/cm^2	Chae et al. (2010)
Heat treatment	454 mW/m^2	Tiwari and Ghangrekar (2015)
Ultra-sonication treatment	65.5 mW/cm^2	More and Ghangrekar (2010)

13.3.1 Electrogenic Bacteria

The electrogenic bacteria are the core of an MFC. Earlier in 1911, when Potter first came out with the concept of producing electricity out of bacterial cells, the scope to take the technology to the field scale was very little (Potter 1911). The main challenge was in using this phenomenon in wastewater. Wastewater contains complex substrates, and hence culturing of a pure bacterial species and avoiding contamination of species in waste stream are major challenges. However, the mixed culture bacteria are not substrate specific, and hence, the efficiency on using the mixed culture in removing organic matter from wastewater is much higher as compared to the usage of pure culture for the same purpose. This challenge has led scientists to explore the possibilities to efficiently utilize the mixed bacterial culture by specifically enhancing the activity of electrogenic bacterial community (Tiwari and Ghangrekar 2015). Most of the investigations and techniques used for suppressing the methanogens were adopted based on either the techniques employed in suppressing the methanogens in the digestive tract of ruminants or those employed in enhancing the hydrogen production (Table 13.2).

13.3.2 Terminal Electron Acceptor

Oxygen upon reduction in the presence of proton yields water, which makes it the most suitable electron acceptor in an MFC. Oxygen can be either provided through aeration of catholyte or in the form of photosynthetic oxygen produced by microalgae, or even air cathode can be employed in MFC. However, the sluggish reduction kinetics of oxygen has led to the use of expensive catalysts, which forced the need of alternate terminal electron acceptor in MFC. Nitrate and heavy metals, including copper, iron, chromium, etc., have also been used as electron acceptor, with a dual purpose of treating the waste stream along with supply of electron acceptor in the cathodic chamber. Apart from this, ferricyanide, hydrogen peroxide, perchlorate, persulphate, etc. have proved to be highly efficient electron acceptors in an MFC (Table 13.3). The key point that should be kept in mind while selecting the

Table 13.3 Comparative evaluation of performance of MFCs operated with different TEA

Terminal electron acceptor	Substrate	Power density (mW/m^2)	Reference
Permanganate	Glucose	115.6	You et al. (2006)
Potassium ferricyanide	Acetate	166.7	Li et al. (2009)
Nitrate	Domestic wastewater	117.7	Fang et al. (2011)
H_2O_2	Glucose	22	Tartakovsky and Guiot (2006)
$CuSO_4$	Glucose	314 mW/m^3	Tao et al. (2011)
Vanadium	Glucose	614	Zhang et al. (2010)

electron acceptor is the challenges to be faced during the disposal; in other words the end product on reduction should be a valuable recovery rather than an add-on to environmental pollution. Hence, the wastewater having the potential to be an efficient electron acceptor should be targeted with an aim to treat the same without employing separate costly treatment technologies.

13.3.3 Electrode Material

High electrical conductivity, better biocompatibility, hydrophilicity, anti-corrosiveness, efficient electron transfer, cost-effectiveness, etc. are some of the factors that should be satisfied while selecting the electrode material for an MFC. The anode used in an MFC should allow efficient bacterial attachment and should have high microbe accessible surface area, such as electrode with macroscale pores that assist in internal colonisation of microbes. The electrodes used so far include carbon-based electrodes as well as non-carbonaceous electrodes like stainless steel. Carbon felt, paper, mesh, foam, etc. as well as graphite rod are some of the commonly used carbon-based electrodes, which are highly biocompatible and inert. Researchers have been exploring different forms of carbon-based electrode owing to its low cost and ease of synthesis.

An ideal anode for an MFC should favour proper microbial attachment on the electrode surface, should assist in high electron transfer and should have minimum electrode resistance (Mustakeem 2015). The living biofilm on the anode acts as biocatalyst in the anodic chamber, and hence bacterial-electrode interaction is one of the critical parameters that determines the efficiency of MFC (Franks et al. 2010). The interaction is very much dependent on the nature of anode material including its surface roughness, porosity, biocompatibility, etc. (Canuto de Almeida e et al. 2019). Apart from this, the electrode material should be biocompatible, should have high electrical conductivity, should not decompose in wastewater, and should be hydrophilic and anti-corrosive (Wei et al. 2011). Hence, an investigation on low-cost long-lasting anode material, which can effectively transfer the electrons

produced by electrogenic bacteria, is of high priority for effective wastewater treatment and power generation in MFC.

Graphite rod, graphite fibre, carbon felt, carbon cloth and carbon paper are some of the most extensively used carbon-based anode materials in MFC. Even though graphite rods have good conductivity and chemical stability as compared to other forms of carbon-based electrodes, Chaudhuri and Lovley observed a reduced bacterial-electrode interaction on the usage of graphite rod anode as compared to carbon felt, which was evident from the better power generation and bacterial biofilm formation in MFC with carbon felt as anode (Chaudhuri and Lovley 2003). However, the hydrophobic nature of untreated carbon felt restricts the development of biofilm and hence demands pretreatments including nitric acid pretreatment, UV/O_3 pretreatment, etc. for effective biofilm growth, which otherwise adds up to the overall cost (Cornejo et al. 2015; Hidalgo et al. 2016; Neethu et al. 2018). Similarly, the short life span, cost and clogging nature reduce the scope of usage of carbon paper, cloth and fibre as electrode material (Zhou et al. 2011).

13.3.4 Proton Exchange Membrane

The protons are produced during the oxidation of organic matter in the anodic chamber by electrogenic bacteria. In addition to the completion of the electrochemical cycle and circuit, the transfer of proton to the cathodic chamber also helps in balancing the pH of the anolyte so as to provide an ambient environment for bacterial survival. Therefore, the PEM developed should be efficient enough to transfer a major portion of the protons produced in the anodic chamber. Even though proton conductivity is the primary function of a PEM, the membrane developed should also satisfy several other characteristics. As the electrogenic bacteria require an anaerobic environment for its growth and activity, the membrane separator should be able to maintain the anaerobicity of the anodic chamber by allowing minimum or no oxygen transfer from the cathodic side to the anodic side. Similarly, transfer of the substrate from the anodic chamber to cathodic chamber should not occur, which will otherwise cause substrate loss for the bacteria in the anodic chamber. One of the major challenges associated with the scaling up of MFC is the cost and stability of the electrode and separator material used. The PEM has an equal role on both these factors, and this has led to researches in developing low-cost PEMs that are stable enough and have the capacity to handle the hydraulic pressure developed (Table 13.4). The cost associated with the operation and fabrication of an MFC should be kept to minimum so as to be a low-cost alternative to the existing wastewater treatment technologies. In view of enhancing the performance of MFC, it is always recommended to use low-cost materials with high performance efficiency for fabrication.

Table 13.4 Comparative evaluation of performance of MFCs using different proton exchange membrane materials

Separator material	Thickness (mm)	Power density (mW/m^2)	Internal resistance (Ω)	References
Nylon	0.17	443 ± 27	84.6	Zhang et al. (2010)
Nafion 117	0.183	57.5 ± 3.9	93.0	Choi and Hu (2013)
Earthenware	3	1042 mW/m^3	–	Behera and Ghangrekar (2011)
SPEEK		77.30	811	Ghasemi et al. (2013)
Sulphonated polystyrene-ethylene-butylene-polystyrene (SPSEBS)	0.18	600 ± 14	70	Ghasemi et al. (2013)
Polyvinylchloride/4A zeolite	–	250 ± 5	57	Nagar et al. (2019)
Polyvinylchloride	–	92	193	Nagar et al. (2019)

13.3.5 Oxygen Reduction Catalyst

Oxygen is the most reliable and easily available terminal electron acceptor, which does not deliver any toxic product on reduction. However, one of the performance-hindering factors for an MFC is the sluggish oxygen reduction kinetics at the cathode, which can be resolved by the use of an oxygen reduction reaction (ORR) catalyst. High catalytic activity, high specific surface area, higher stability, non-toxicity and ease of synthesis are the major factors that are to be considered while selecting a catalyst. However, along with these properties, an ideal ORR catalyst should be cost-effective for application in an MFC, which is predominantly engineered for wastewater bioremediation. Considering the binding energy, platinum is considered to be the most efficient cathode catalyst for enhancing the ORR kinetics in an MFC. The cost associated with the same has led researchers to optimise the Pt dosing as well as to alloy it with other transition metals including Ni. Still research is progressing to explore low-cost efficient catalyst including the biochar-based catalyst as well as non-metallic catalysts.

13.3.5.1 Biomass-Derived Cathode Catalyst for Application in MFCs

A low-cost electrode as well as catalyst with high activity and durability is the need of the hour for the MFC. The reduction of oxygen in the cathodic chamber occurs either through a direct four-electron pathway or a two-step peroxide pathway. The most efficient ORR catalysts drive the reaction to a better involvement of the four-electron pathway, which leads to higher power output as well as lower production of peroxide intermediates, because the peroxide formation adversely affects the

electrode and PEM. One of the major drawbacks in MFC is the sluggish ORR at the cathode, which was resolved by the usage of Pt-based catalyst. However, apart from high cost, Pt-based catalyst is associated with CO poisoning, methanol crossover and long-term instability due to particle aggregation and dissolution. Metal nanoparticle-based catalysts with a good support material have been used in MFC to achieve higher electrochemically active surface area.

A good support material should have sufficient electrical conductivity and higher surface area, which can be attained on the use of porous carbon materials. The properties of carbon-based materials including its higher electrical conductivity, stability and functionality have increased the interest of researchers in developing low-cost carbon-based electrode materials and catalysts. Hence, sustainable and ample biomass reserves can be an alternative option for the production of the same. Waste to wealth can be achieved if it is possible to convert the huge tons of agricultural as well as other biomass waste generated globally into novel catalytic or electrode materials. The selection of appropriate synthesis methods, with respect to the source, is crucial in order to obtain high surface area and reactive sites with high stability (Borghei et al. 2018). The ORR catalysts including carbon supported on Pt, N-doped carbon, heteroatom-doped carbon, Fe/Co N-doped carbon, etc. have been produced from biomass so far (Chen et al., 2011).

13.4 Applications of MFCs

13.4.1 Microbial Desalination Cell

The demand for fresh water and clean energy is driving the need for converting an MFC into an MDC, wherein desalination of saline water is attained along with wastewater treatment. Ion exchange membranes (IEMs) are most commonly used for desalination of saline water, and it is a promising tool, which when oriented properly can be applied in MDC for value-added product recovery. In an MDC, mainly the potential gradient created due to the transfer of electrons from the anodic chamber to the cathodic chamber and the concentration gradient between the desalinating chamber and its adjacent chamber are responsible for the desalination. In an investigation using a three-chambered MDC, a maximum power density of 2 W/m^2 alongside removal of 90% salt from water, present in desalination chamber, was attained (Cao et al. 2009). However, three-chambered MDC is associated with certain challenges including the hindrance for passage of H^+ ions from the anodic chamber to the cathodic chamber, as well as the accumulation of chloride ions in the anolyte. This decreases the anolyte pH, which poses threat to bacterial community in the anodic chamber, which can reduce the efficiency of MDC.

In order to overcome this challenge, Pradhan and Ghangrekar modified the three-chambered MDC into multi-chambered MDC, wherein the issue of pH imbalance was solved by the usage of a cation exchange membrane (CEM) adjacent to the anodic chamber, which transferred the H^+ from the anolyte to the adjacent concentrate chamber (Pradhan and Ghangrekar 2014). Thus, MDC exploits wastewater as a

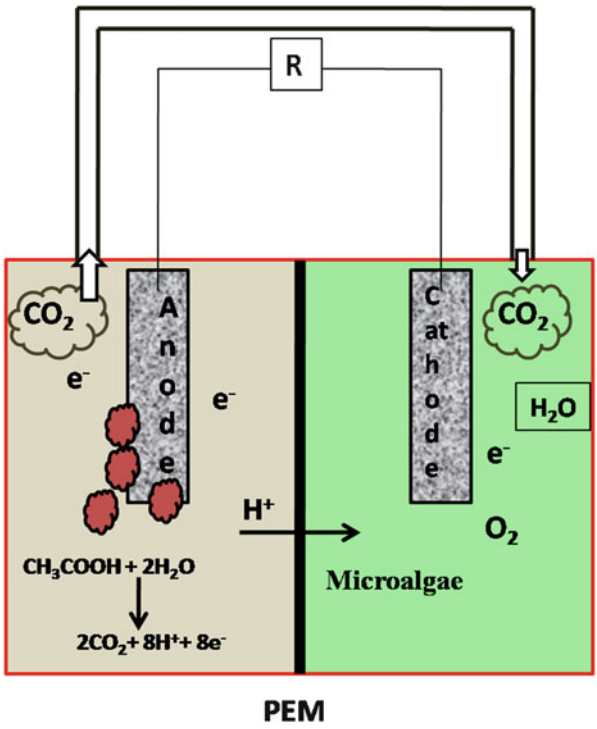


Fig. 13.3 Schematic representation of a microbial carbon-capture cell

viable substrate to yield electricity, which also has been exploited for desalination. This technology shows promising approach by offering low-cost solution for desalination of saline and brackish water (Neethu et al. 2019b). However, the use of chemical catholyte and costly cathode catalyst makes MDC unsustainable for future field-scale applications, which need to be overcome by the use of low-cost terminal electron acceptors. The dependence of the performance of MDCs on the salt concentration and MDC configuration is yet to be investigated to draw a final conclusion on it.

13.4.2 Microbial Carbon-Capture Cell

As discussed in the above section regarding the supply of low-cost electron acceptor (O_2) with additional benefit of value addition, cultivation of microalgae in the catholyte is one of the alternatives (Fig. 13.3). Microalgae can provide an attractive solution for providing the photosynthetic oxygen as TEA by utilising the nutrients in wastewater along with sequestering CO_2 from anodic off gas, and further it can be an excellent feedstock for biodiesel production upon harvesting. Hence, MCC is a sustainable technology that uses oxygen produced by algal biomass as electron acceptor for accomplishing concurrent electricity generation, CO_2 sequestration,

wastewater treatment and algal biomass production (Neethu et al. 2018). Different applications of microalgae in MFC have been investigated so far (Rajesh and Ghangrekar 2016).

In the catholyte, microalgae *Golenkinia* sp. proved to be a source of oxygen and achieved a maximum power density of 6.3 W/m^3 (Hou et al. 2016). A maximum power output of 1.9 W/m^2 was achieved by using microalgae as substrate in the anodic chamber of MFC (Cui et al. 2014). The marine algae *Chaetoceros* is reported to inhibit the growth of methanogenic archaea in anodic chamber, due to the presence of long-chain saturated fatty acid, and this MFC attained a power density of 21.43 W/m^3 (Rajesh et al. 2015). Apart from improving the power generation, microalgae have the potential to remediate wastewater rich in nutrients and heavy metals (Huang et al. 2017; Logroño et al. 2017). Investigations were also done to explore the photosynthetic electrogenic activity in algae and cyanobacteria, wherein incorporating photosynthetic species in the anodic chamber of MFC gave a power density of 6.2 mW/m^2 (Luimstra et al. 2014).

13.4.3 Sediment Microbial Fuel Cell

The SMFC is a modification of MFC, where oxygen is available in the overlying water, and on cathode, oxygen reduction occurs to complete the circuit by reducing it to water (Wang et al. 2014). Thus, oxygen availability is one of the major factors that govern the performance of SMFC (Fig. 13.4). Unlike most MFCs, which contain a membrane to separate the compartments containing the anode (where oxidation takes place) and the cathode (where reduction takes place), SMFC functions without membranes. A SMFC can have better application in natural water bodies, if it could power small autonomous devices; however here the low power generation has become a major challenge. A previous investigation on the effect of using different electrode materials in SMFC has reported a maximum power density of 16 mW/m^2 using graphite felt electrode and 38 mW/m^2 using graphite felt multiwalled carbon nanotubes (GF-MWNT) (Wang et al. 2014). On the contrary, an investigation performed using a rotating cathode for increasing oxygen availability gave a

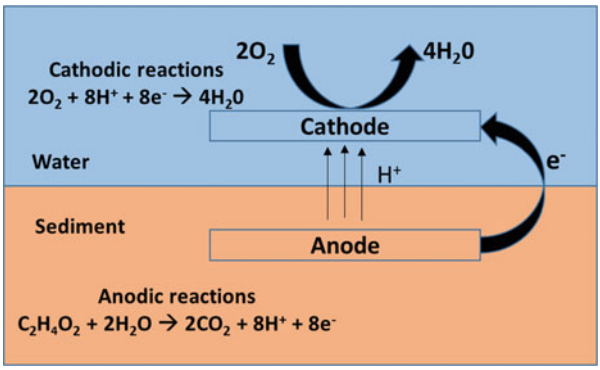


Fig. 13.4 Schematic diagram of sediment microbial fuel cell

power density of 49 mW/m^2 (He et al. 2007). Hence, the performance of a SMFC is dependent on several factors including the electrode material, sediment characteristics, oxygen availability in catholyte, etc. Sediment remediation, mitigation of the aquatic water pollution, algae cultivation, etc. are some of the major applications of SMFC. Incorporation of microalgae in the cathodic side of SMFC makes it a sediment microbial carbon-capture cell (SMCC) with a multiple advantage of algae cultivation and nutrient removal from the overlying pond water (Neethu and Ghangrekar 2017).

13.5 Bioremediation and Biodegradation in MFC

An MFC converts the chemical energy present in wastewater to electrical energy via the bacterial catalysis. The nature of bacterial community present in the anodic compartment is of ultimate importance in determining the efficiency of the system. Equally important is the substrate that is to be provided for the bacteria in the anodic chamber. In 1911, when Potter explored the capacity of electrogenic bacteria in producing electricity, the media used for bacterial metabolism was synthetic substrate. However, later on researchers have taken the technology to a level that even the domestic wastewater and industrial wastewater were able to replace the synthetic media for bacterial culture. This has expanded the possible applications of a BES including wastewater treatment and value-added product recovery. Apart from the normal wastewater treatment technologies practised today, a BES has an added advantage that it does not require separate units for targeting treatment of different pollutants present in wastewater. On the contrary, bioremediation can be carried out in a single bioreactor, wherein apart from removal of carbonaceous compounds, nitrification, denitrification, heavy metal removal and removal of sulphate compounds can be accomplished. The application of BES technologies in treating different wastewater streams including domestic, industrial and wastewater containing recalcitrant compounds is reviewed below.

13.5.1 Bioremediation of Domestic and Industrial Wastewater

The core of MFCs is the electrons generated in the anodic chamber, which are produced on substrate oxidation by electrogenic bacteria, and hence the type of substrate fed into the MFC is of ultimate importance. The substrate that is fed into the MFC can range from pure organic substrates including acetate, glucose, etc. to the complex substrates including cellulose, protein, fatty acids, etc. The concentration and components present in the substrate depend on the source of wastewater fed into the MFC. Apart from the domestic wastewater, which mainly contains organic compounds, industrial wastewater including agro-based industries, fertilizer industry, distilleries, dairy, etc. also have proven to be promising source of substrate for anodic bacteria in MFC. A promising organic matter removal can be achieved on usage of easily biodegradable organic compounds present in wastewater as substrate

in MFC. This was much evident from an investigation carried out with different substrates, namely, glucose, fructose and sucrose, wherein the highest power density and COD removal efficiency, respectively, were achieved in MFC using glucose (136 mW/m^2 and 88.5%) as substrate as compared to MFC operated with sucrose (8.8 mW/m^2 and 54.2%) and fructose (3.6 mW/m^2 and 67.5%).

The excellent biodegradability of organic matter by bacteria in an MFC was also experimented on the liquid fraction of pressed municipality solid waste, wherein a 94% COD removal efficiency was achieved in the anodic chamber (Koók et al. 2016). Further the better performance of MFC fed with pure substrate was evident when MFC fed with glucose gave almost three times higher power density as compared with the one having domestic wastewater as substrate to bacteria (Liu and Logan 2004). However, in addition to a better power generation, equally important is to take advantage of MFC as an environmental and energy-friendly solution to treat the wastewater generated from different sources. With this focus, research was carried out in utilising the MFC in treating different forms of wastewaters (Table 13.5).

The performance of an MFC depends on several factors including substrate characteristics, electrode, PEM, bacteria, TEA, etc. Hence, for a single substrate, the performance exhibited by MFC will vary with other parameters. For example, distillery wastewater when treated in single-chamber and double-chambered MFC gave a respective power density of 28.15 mW/m^2 and 17.7 mW/m^2; however, with similar COD removal efficiencies (60%), surprisingly, the same substrate (distillery wastewater) gave a power density of 1000 mW/m^2 using a thermophilic MFC (Ha et al. 2012). Hence, it is difficult to judge the performance of an MFC based on the type of substrate by ignoring other dependable factors/parameters that affect the performance of MFC. A detailed performance evaluation of MFC operated with different substrates along with their operating condition is furnished below (Table.13.5).

13.5.2 Bioremediation of Nitrogen-Rich Wastewater

One of the major focuses of MFC was bioremediation of wastewater, that is, the removal of organic matter in wastewater; however equally important is the removal of nitrogen present in the wastewater, which will otherwise lead to eutrophication of receiving water body. Removal of nitrate in an MFC can be achieved either in the cathodic chamber or in the anodic chamber. In the cathodic chamber, nitrate removal can be achieved with the help of bio-cathode (algae) or by nitrate reduction to nitrogen. A total nitrogen removal of 81.6% was achieved in the cathodic side of a photosynthetic microbial fuel cell using algae in the cathodic zone (Neethu and Ghangrekar 2017). Also, a 90% total nitrogen removal was attained in a planted constructed wetland MFC, wherein root exudates of *Ipomoea aquatica* were utilised (Liu et al. 2013).

Nitrate can be a potential electron acceptor in the cathodic side of an MFC, on reduction of which it gets converted to nitrogen. Thus, for the first time in the

Table 13.5 Comparative evaluation of MFCs using different substrates in the anodic chamber

Substrate (COD)	Configuration	Inoculum	TEA/catalyst	Power density (mW/m^2)	COD removal (%)	Reference
Dairy (COD 4.5–5.0 g/L)	Dual chamber	Bacteria from dairy wastewater	Cu-doped FeO as anode catalyst	161	75	Sekar et al. (2019)
Distillery (4.67 g/L)	Dual chamber	Yeast	$C_6N_6FeK_3$	304	69.3	Parkash (2016)
Food processing (1.9 g/L)	Dual chamber	Mixed anaerobic sludge	–	230	86	Mansoorian et al. (2013)
Seafood processing	Upflow MFC	Mixed anaerobic sludge	–	105	83	Jayashree et al. (2016)
Meat packing wastewater (MPW) (6.0 g/L)	Single chamber	Bacteria in MPW	Pt as cathode catalyst	139	87	Heilmann and Logan (2006)
Winery (6.8 g/L)	Dual chamber	Mixed anaerobic sludge	HCl solution (pH 3.5)	105	17	Penteado et al. (2016)
Swine wastewater (8.32 g/L)	Dual chamber	Bacteria present in swine wastewater	Pt as cathode catalyst	110	92	Ogugbue et al. (2015)

literature, Clauwaert et al. (2007) reported nitrate reduction without hydrogen production in the cathodic chamber by achieving simultaneous organic matter removal in the anodic chamber. Here, the capability of *Geobacter* species in directly accepting electrons from the graphite felt and reducing nitrate to nitrite was best utilised for remediation of nitrate-rich wastewater, by simultaneously achieving a power density of 8 W/m^3 (Clauwaert et al. 2007). Further investigation on this concept was carried out by directing the anolyte to an aerobic chamber for oxidation of ammonium to nitrate, which is again fed back to the cathodic chamber of MFC for denitrification, hence achieving a complete treatment of single wastewater stream in MFC (Virdis et al. 2008).

Apart from cathodic chamber, nitrate removal can also be achieved in the anodic chamber of an MFC. About 85% of nitrate removal was achieved in a single-chamber air cathode MFC, where the nitrate-reducing bacteria present in the anodic chamber assisted in denitrification (Sukkasem et al. 2008). Also, investigations were carried out by employing pure culture autotrophic denitrifiers, *Pseudomonas* sp. *C27,* where a power density of 40 mW/m^2 was achieved (Lee et al. 2012). However, it was reported that even though the denitrifying bacterial concentration increased with an increase in the concentration of nitrate in the anodic chamber, a decrease occurred to the proportion of electrogenic bacteria in the anodic chamber (Liu and Logan 2004). This challenge was overcome by using a novel denitrifying electrogenic strain EB-1, isolated from anodic biofilm capable of giving a power density of 840 mW/m^2 by achieving simultaneous denitrification (Jin et al. 2018).

Recently, Jin et al. focused on anodic denitrifying dual-chamber MFCs, which achieved a maximum simultaneous heterotrophic denitrification and electricity generation at a COD/N ratio of 5:1 in the anodic chamber; however, the electrogenic bacterial population in the anodic chamber of MFC operated with denitrifying bacteria was low as compared to the control experiment operated without denitrifying bacteria in the anodic chamber (Jin et al. 2019). Hence, the main challenge of using denitrifying bacteria in the anodic chamber is observed to be the suppression of electrogenic bacteria, which needs to be overcome by proper optimisation of operating parameters.

13.5.3 Microbial Fuel Cell for Recalcitrant Remediation

Xenobiotic compounds are those compounds which are man-made chemicals that are present in the environment at a concentration higher than their natural concentration. Even though bacterial community is capable of degrading most of the xenobiotics, there are certain compounds that are exceptional. These synthetic compounds whose biodegradability is very slow or which are non-biodegradable and exists in environment for long are classified as recalcitrant (Faber 1979). This reluctance of microorganism in degrading the recalcitrant has been differently explained in the literature as it might be due to a large molecular size or due to difficulties in penetration or due to low solubility in water, etc. (Faber 1979). Recalcitrant compounds can range from halogenated compounds like halocarbons

Table 13.6 Classification and sources of recalcitrant compounds

Classification	Compounds	Source
Halocarbon	$CHCl_3$	Solvents and propellants in spray cans of cosmetics, paints, etc.
	CF_4, Freons, CCl_2F_2, CC_lF_3, CCI_3F	Condenser units of cooling systems
	Lindane, DDT, BHC,	Insecticides
	Dalapon, 2, 4-D, 2, 4, 5-T	Herbicides
Polychlorinated biphenyls (PCB's)	Covalently linked benzene rings with halogens in place of hydrogen	Plasticisers, insulator coolants in transformers and as heat exchange fluid
Synthetic polymers	Polyethylene, polystyrene, polyvinyl chloride, etc. and nylons	Wrapping, garments, materials, etc.
Alkylbenzyl sulphonates	Surface-active detergents superior to soaps	Cleaning
Oil mixtures	Recalcitrant due to non-solubility	Large oil spills

and polychlorinated biphenyls to complex synthetic polymers (Table 13.6). Hence, the more complex is the structure of the compound, the more difficult is its biodegradation. Genetic engineering tools have been applied to a much greater extent to modify the microorganism to make them capable of degrading the recalcitrant compounds.

Even though BESs have been mainly focused on power generation, recently the application of BES for bioremediation is gaining priority. The remediation of xenobiotic compounds using BES is a recent and upcoming promising treatment technique. Xenobiotic compounds cannot be directly degraded by microorganisms owing to its complexity and hence are not readily used for bacterial metabolism. Therefore, in most of the cases the xenobiotic compounds are treated outside the cell rather than inside the cell. Several investigations have been carried out, wherein the xenobiotic compounds act as an electron acceptor.

An anaerobic-aerobic process using single-chamber MFC has led to effective degradation of azo dye when used as substrate (Danish Khan et al. 2015). Even though the biodegradation mechanism of non-recalcitrant compounds has been widely discussed in the literature, it is important to know the mechanism of recalcitrant degradation by electrogenic bacteria. To state a few, say for azo dye degradation mechanism, the high redox potential of azo dye makes it a good electron accepting candidate, and hence higher electron transfer rates can lead to rapid reductive degradation of azo dyes in the anodic compartment of MFC (Fernando et al. 2014). Similarly, chloronitrobenzene compounds are known to have highly electron withdrawing nature and can be efficiently reduced to much lower toxic forms. However, in a different investigation, the aerobic treatment of pentachlorophenol in cathodic compartment of dual-chambered MFC also has proven to be better than its anaerobic treatment in single-chambered MFC (Khan et al. 2018). Degradation of xenobiotic compounds in the anodic chamber has been widely investigated, which includes the degradation of trichloroethane (Aulenta et al.

2011), polychlorinated biphenyls (Chun et al. 2013), refractory organic pesticide (Cao et al. 2015), hydrocarbons (Morris and Jin 2008), phenanthrene and benzene (Adelaja et al. 2017), phenanthrene (Adelaja et al. 2014), etc. Hence, the non-biodegradability nature of certain xenobiotics that poses restrictions in its biodegradability can be overcome by utilising their electron transfer mechanism and subsequent degradation in BES.

13.5.4 Value-Added Product Recovery in Microbial Fuel Cell: Heavy Metal Recovery

Even though heavy metals are inevitable part of various industrial, medical and several other applications, their presence in the wastewater causes heavy threat to the environment. The non-biodegradability of the heavy metals opens up the scope to recover heavy metal from the wastewater streams. Even though several conventional methods including precipitation, coagulation, ion exchange, etc. have been used for heavy metal removal, the MFC can come out as an emerging technology, where simultaneous removal as well as recovery of heavy metal is possible. In an MFC, the cathodic chamber, which is a destination for the electrons and protons produced in the anodic chamber, can be efficiently used for reduction of heavy metals having a higher redox potential to get reduced on accepting electrons and precipitate. For example, the introduction of Cr(VI), which is highly soluble and harmful, into the cathodic chamber of MFC gets reduced to less toxic Cr(III) (Wang et al. 2008). Similarly, Li et al. added vanadium oxide ($NaVO_3$) to the anodic chamber, and the action of *Rhodoferax ferrireducens* assisted in removal of 75.8% $NaVO_3$ in the anodic chamber while achieving a 64% electron recovery. Also, a follow-up investigation was carried out to investigate the fate of MFC on using the V in the cathodic side as electron acceptor, wherein only 26% removal of V was achieved (Zhang et al. 2012). In addition, tetrachloroaurate was used as an electron acceptor in order to recover gold efficiently. Surprisingly, a 99.98% recovery of Au was achieved for an Au(III) concentration of 200 ppm (Choi and Hu 2013). Likewise investigations have reported recovery of several other heavy metals including copper (Wang et al. 2010), silver (Yun-Hai et al. 2013), selenite (Chellamuthu et al. 2011), arsenic (Xue et al. 2013), zinc (Fradler et al. 2014), cadmium (Abourached et al. 2014), etc. Therefore, the recovery of these value-added heavy metals from the wastewater streams with simultaneous power generation takes the MFC technology a step ahead of other conventional wastewater treatment technologies.

13.6 Bottlenecks and Future Perspective

The major areas in the research on MFC that need to be focused are the reduction in fabrication cost and enhancement in performance using low-cost material for different components. The PEM, electrode and catalyst used in fabrication of MFC account for the major cost and are also the performance-determining components

in MFC. Optimisation of operating conditions is equally important in enhancing the performance of MFC. Equally important is the enrichment of electrogenic bacteria on the anode, which is an inevitable element in an MFC. Rather than going for pure electrogenic species in MFC, it is always recommended to use mixed culture bacterial species considering the issue of substrate specificity and feasibility while dealing with real wastewater. Hence, there is a need to explore biological and natural techniques to suppress the methanogenic archea present in the mixed culture in order to enhance the activity of electrogenic bacteria. In MFCs, power generation is one of the major goals, and hence, microorganisms capable of generating electricity in MFCs have gained increasing research interest. Until now, experimentations have been done to understand the microbial electrogenic consortia responsible for electricity generation in MFC. Still there is a need to understand the optimum conditions for maximum bacterial activity so that it can be exploited in such a way that the electrons are diverted from natural electron acceptors to the electrode effectively.

As a key component of MFC, the PEMs are gaining extensive attention in recent years because of its selective permeability towards protons to run the MFC in highly efficient way. Protons exhibit excess mobility in aqueous system than other ions; hence in other biological systems and materials, the proton conductivity as well as water mobility increases with water uptake (Neethu et al. 2019a). However, the Nafion membrane, which is most commonly used in MFC, is associated with several limitations such as oxygen diffusion, cation accumulation, substrate crossover, durability due to fouling, high cost, etc. (Hasani-Sadrabadi et al. 2010). Biological membranes have very high water permeability and selectivity, which can improve the performance of membrane in terms of proton conductivity (Qu et al. 2013). Also, the ceramic membranes with cation exchangers proved to be a low-cost alternative to the costly Nafion membrane, however with a far low proton mass transfer coefficient than Nafion (Ghadge and Ghangrekar 2015). Hence, there is a need to explore the scope to improve the performance of the ceramic membranes as well as the use of easily available biological membranes. Also, as most of the catalysts are expensive and toxic, there is a need to explore a low-cost catalyst, which can increase the oxygen reduction reaction. Rather than synthesising or procuring catalyst, the possibility of making catalyst out of waste stream has not been experimented so far.

13.7 Summary

A microbial fuel cell is a promising low energy-consuming technology, which converts organic matter present in wastewater to electrical energy. The multiple applications of MFC make it unique as compared to other technologies used for wastewater treatment. The MFC technology still needs further development in order to harvest maximum possible electricity and attain high level of bioremediation. There are several factors, which are discussed in detailed in this chapter, that significantly affect the performance of MFCs and are required to be modified for more flexibility for its practical field-scale applications. Also, organised

multidisciplinary efforts are further required for scaling up of MFC to enhance power production as well as wastewater treatability.

Acknowledgements The research project was supported by the Department of Biotechnology, Ministry of Science and Technology, Government of India (BT/EB/PAN IIT/2012), providing the financial assistance.

References

Abourached C, Catal T, Liu H (2014) Efficacy of single-chamber microbial fuel cells for removal of cadmium and zinc with simultaneous electricity production. Water Res 51:228–233. https://doi.org/10.1016/j.watres.2013.10.062

Adelaja O, Keshavarz T, Kyazze G (2014) Enhanced biodegradation of phenanthrene using different inoculum types in a microbial fuel cell. Eng Life Sci 14:218. https://doi.org/10.1002/elsc.201300089

Adelaja O, Keshavarz T, Kyazze G (2017) Treatment of phenanthrene and benzene using microbial fuel cells operated continuously for possible in situ and ex situ applications. Int Biodeterior Biodegrad 116:91. https://doi.org/10.1016/j.ibiod.2016.10.021

Aelterman P, Rabaey K, Pham HT, Boon N, Verstraete W (2006) Continuous electricity generation at high voltages and currents using stacked microbial fuel cells. Environ Sci Technol 40:3388. https://doi.org/10.1021/es0525511

Ali N, Anam M, Yousaf S, Maleeha S, Bangash Z (2017) Characterization of the electric current generation potential of the pseudomonas aeruginosa using glucose, fructose, and sucrose in double chamber microbial fuel cell. Iran J Biotechnol 15:216. https://doi.org/10.15171/ijb.1608

Aulenta F, Tocca L, Verdini R, Reale P, Majone M (2011) Dechlorination of trichloroethene in a continuous-flow bioelectrochemical reactor: effect of cathode potential on rate, selectivity, and electron transfer mechanisms. Environ Sci Technol 45:8444. https://doi.org/10.1021/es202262y

Behera M, Ghangrekar MM (2011) Electricity generation in low cost microbial fuel cell made up of earthenware of different thickness. Water Sci Technol J Int Assoc Water Pollut Res 64 (12):2468–2473. https://doi.org/10.2166/wst.2011.822

Biffinger JC, Pietron J, Ray R, Little B, Ringeisen BR (2007) A biofilm enhanced miniature microbial fuel cell using Shewanella oneidensis DSP10 and oxygen reduction cathodes. Biosens Bioelectron 22:1672–1679. https://doi.org/10.1016/j.bios.2006.07.027

Bond DR, Lovley DR (2003) Electricity production by Geobacter sulfurreducens attached to electrodes. Appl Environ Microbiol 69:1548–1555. https://doi.org/10.1128/AEM.69.3.1548-1555.2003

Borghei M, Lehtonen J, Liu L, Rojas OJ (2018) Advanced biomass-derived electrocatalysts for the oxygen reduction reaction. Adv Mater 30. https://doi.org/10.1002/adma.201703691

Call TP, Carey T, Bombelli P, Lea-Smith DJ, Hooper P, Howe CJ, Torrisi F (2017) Platinum-free, graphene based anodes and air cathodes for single chamber microbial fuel cells. J Mater Chem A 5:23872. https://doi.org/10.1039/C7TA06895F

Canuto de Almeida e Silva T, Bhowmick GD, Ghangrekar MM, Wilhelm M, Rezwan K (2019) SiOC-based polymer derived-ceramic porous anodes for microbial fuel cells. In: Biochem Eng J, vol 148, p 29. https://doi.org/10.1016/j.bej.2019.04.004

Cao X, Huang X, Liang P, Xiao K, Zhou Y, Zhang X, Logan BE (2009) A new method for water desalination using microbial desalination cells. Environ Sci Technol 43:7148. https://doi.org/10.1021/es901950j

Cao X, Song HL, Yu CY, Li XN (2015) Simultaneous degradation of toxic refractory organic pesticide and bioelectricity generation using a soil microbial fuel cell. Bioresour Technol 189:87. https://doi.org/10.1016/j.biortech.2015.03.148

Chae PS, Rasmussen SG, Rana RR, Gotfryd K, Chandra R, Goren MA, Kruse AC, Nurva S, Loland CJ, Pierre Y, Drew D (2010) Maltose–neopentyl glycol (MNG) amphiphiles for solubilization, stabilization and crystallization of membrane proteins. Nat Methods 7(12):1003

Chaudhuri SK, Lovley DR (2003) Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. Nat Biotechnol 21(10):1229–1232. https://doi.org/10.1038/nbt867

Chellamuthu P, Ward M, Nealson K, Kronen M (2011) Removal of selenite with microbial fuel cells utilizing shewanella oneidensis MR-1. In: Fuels and petrochemicals division—core programming topic at the 2011 AIChE annual meeting

Chen Z, Higgins D, Yu A, Zhang L, Zhang J (2011) A review on non-precious metal electrocatalysts for PEM fuel cells. Energy Environ Sci 4(9):3167–3192. https://doi.org/10.1039/c0ee00558d

Choi C, Hu N (2013) The modeling of gold recovery from tetrachloroaurate wastewater using a microbial fuel cell. Bioresour Technol 133:589. https://doi.org/10.1016/j.biortech.2013.01.143

Chun CL, Payne RB, Sowers KR, May HD (2013) Electrical stimulation of microbial PCB degradation in sediment. Water Res 47:141. https://doi.org/10.1016/j.watres.2012.09.038

Clauwaert P, Rabaey K, Aelterman P, De Schamphelaire L, Pham TH, Boeckx P, Boon N, Verstraete W (2007) Biological denitrification in microbial fuel cells. Environ Sci Technol 41:3354–3360. https://doi.org/10.1021/es062580r

Cornejo JA, Lopez C, Babanova S, Santoro C, Artyushkova K, Ista L et al (2015) Surface modification for enhanced biofilm formation and electron transport in Shewanella anodes. J Electrochem Soc 162:H597. https://doi.org/10.1149/2.0271509jes

Cui Y, Rashid N, Hu N, Rehman MSU, Han JI (2014) Electricity generation and microalgae cultivation in microbial fuel cell using microalgae-enriched anode and bio-cathode. Energy Convers Manag 79:674–680. https://doi.org/10.1016/j.enconman.2013.12.032

Danish Khan M, Abdulateif H, Ismail IM, Sabir S, Zain Khan M (2015) Bioelectricity generation and bioremediation of an azo-dye in a microbial fuel cell coupled activated sludge process. PLoS One 10:e0138448. https://doi.org/10.1371/journal.pone.0138448

Deng LF, Li FB, Zhou SG, Huang DY, Ni JR (2010) A study of electron-shuttle mechanism in Klebsiella pneumoniae based-microbial fuel cells. Chin Sci Bull 55:99–104. https://doi.org/10.1007/s11434-009-0563-y

Faber MD (1979) Microbial degradation of recalcitrant compounds and synthetic aromatic polymers. Enzym Microb Technol 1:226. https://doi.org/10.1016/0141-0229(79)90041-3

Fang C, Min B, Angelidaki I (2011) Nitrate as an oxidant in the cathode chamber of a microbial fuel cell for both power generation and nutrient removal purposes. Appl Biochem Biotechnol 164:464. https://doi.org/10.1007/s12010-010-9148-0

Fernando E, Keshavarz T, Kyazze G (2014) External resistance as a potential tool for influencing azo dye reductive decolourisation kinetics in microbial fuel cells. Int Biodeterior Biodegrad 89:7. https://doi.org/10.1016/j.ibiod.2013.12.011

Fradler KR, Michie I, Dinsdale RM, Guwy AJ, Premier GC (2014) Augmenting microbial fuel cell power by coupling with supported liquid membrane permeation for zinc recovery. Water Res 55:115. https://doi.org/10.1016/j.watres.2014.02.026

Franks AE, Malvankar N, Nevin KP (2010) Bacterial biofilms: the powerhouse of a microbial fuel cell. Biofuels 1:589. https://doi.org/10.4155/bfs.10.25

Ghadge AN, Ghangrekar MM (2015) Development of low cost ceramic separator using mineral cation exchanger to enhance performance of microbial fuel cells. Electrochim Acta 166:320. https://doi.org/10.1016/j.electacta.2015.03.105

Ghasemi M, Daud WRW, Ismail AF, Jafari Y, Ismail M, Mayahi A, Othman J (2013) Simultaneous wastewater treatment and electricity generation by microbial fuel cell: Performance comparison and cost investigation of using Nafion 117 and SPEEK as separators. Desalination 325:1–6. https://doi.org/10.1016/j.desal.2013.06.013

Ha PT, Lee TK, Rittmann BE, Park J, Chang IS (2012) Treatment of alcohol distillery wastewater using a Bacteroidetes-dominant thermophilic microbial fuel cell. Environ Sci Technol 46:3022–3030

Hasan K, Grattieri M, Wang T, Milton RD, Minteer SD (2017) Enhanced bioelectrocatalysis of Shewanella oneidensis MR-1 by a naphthoquinone redox polymer. ACS Energy Lett 2:1947–1951. https://doi.org/10.1021/acsenergylett.7b00585

Hasani-Sadrabadi MM, Dashtimoghadam E, Majedi FS, Kabiri K, Solati-Hashjin M, Moaddel H (2010) Novel nanocomposite proton exchange membranes based on Nafion® and AMPS-modified montmorillonite for fuel cell applications. J Membr Sci 365:286–293. https://doi.org/10.1016/j.memsci.2010.09.014

He Z, Shao H, Angenent LT (2007) Increased power production from a sediment microbial fuel cell with a rotating cathode. Biosens Bioelectron 22(12):3252–3255

Heilmann J, Logan BE (2006) Production of electricity from proteins using a microbial fuel cell. Water Environ Res 78:531. https://doi.org/10.2175/106143005x73046

Hidalgo D, Tommasi T, Bocchini S, Chiolerio A, Chiodoni A, Mazzarino I, Ruggeri B (2016) Surface modification of commercial carbon felt used as anode for Microbial Fuel Cells. Energy 99:193. https://doi.org/10.1016/j.energy.2016.01.039

Hou Q, Nie C, Pei H, Hu W, Jiang L, Yang Z (2016) The effect of algae species on the bioelectricity and biodiesel generation through open-air cathode microbial fuel cell with kitchen waste anaerobically digested effluent as substrate. Bioresour Technol 218:902–908. https://doi.org/10.1016/j.biortech.2016.07.035

Huang J, Zhu N, Cao Y, Peng Y, Wu P, Dong W (2014) Exoelectrogenic bacterium phylogenetically related to citrobacter freundii, isolated from anodic biofilm of a microbial fuel cell. Appl Biochem Biotechnol 175:1879–1891. https://doi.org/10.1007/s12010-014-1418-9

Huang D, Song BY, He YL, Ren Q, Yao S (2017) Cations diffusion in Nafion117 membrane of microbial fuel cells. Electrochim Acta 245:654–663. https://doi.org/10.1016/j.electacta.2017.06.004

Islam MA, Ethiraj B, Cheng CK, Yousuf A, Thiruvenkadam S, Prasad R, Rahman Khan MM (2018) Enhanced current generation using mutualistic interaction of yeast-bacterial coculture in dual chamber microbial fuel cell. Ind Eng Chem Res 57:813–821. https://doi.org/10.1021/acs.iecr.7b01855

Jayashree C, Tamilarasan K, Rajkumar M, Arulazhagan P, Yogalakshmi KN, Srikanth M, Banu JR (2016) Treatment of seafood processing wastewater using upflow microbial fuel cell for power generation and identification of bacterial community in anodic biofilm. J Environ Manag 180:351. https://doi.org/10.1016/j.jenvman.2016.05.050

Jin X, Guo F, Liu Z, Liu Y, Liu H (2018) Enhancing the electricity generation and nitrate removal of microbial fuel cells with a novel denitrifying exoelectrogenic strain EB-1. Front Microbiol 9. https://doi.org/10.3389/fmicb.2018.02633

Jin X, Guo F, Ma W, Liu Y, Liu H (2019) Heterotrophic anodic denitrification improves carbon removal and electricity recovery efficiency in microbial fuel cells. Chem Eng J 370:527. https://doi.org/10.1016/j.cej.2019.03.023

Kang CS, Eaktasang N, Kwon DY, Kim HS (2014) Enhanced current production by Desulfovibrio desulfuricans biofilm in a mediator-less microbial fuel cell. Bioresour Technol 165:27–30. https://doi.org/10.1016/j.biortech.2014.03.148

Khan N, Khan MD, Nizami AS, Rehan M, Shaida A, Ahmad A, Khan MZ (2018) Energy generation through bioelectrochemical degradation of pentachlorophenol in microbial fuel cell. RSC Adv 8:20726. https://doi.org/10.1039/c8ra01643g

Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim BH (2002) A mediator-less microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens. Enzym Microb Technol 30:145–152. https://doi.org/10.1016/S0141-0229(01)00478-1

Koók L, Rózsenberszki T, Nemestóthy N, Bélafi-Bakó K, Bakonyi P (2016) Bioelectrochemical treatment of municipal waste liquor in microbial fuel cells for energy valorization. J Clean Prod 112:4406. https://doi.org/10.1016/j.jclepro.2015.06.116

Kumar R, Singh L, Wahid ZA, Din MFM (2015) Exoelectrogens in microbial fuel cells toward bioelectricity generation: a review. Int J Energy Res 39:1048. https://doi.org/10.1002/er.3305

Lee CY, Ho KL, Lee DJ, Su A, Chang JS (2012) Electricity harvest from nitrate/sulfide-containing wastewaters using microbial fuel cell with autotrophic denitrifier, Pseudomonas sp. C27. Int J Hydrog Energy 37:15827. https://doi.org/10.1016/j.ijhydene.2012.01.092

Lee YY, Kim TG, Cho K s (2016) Enhancement of electricity production in a mediatorless air–cathode microbial fuel cell using Klebsiella sp. IR21. Bioprocess Biosyst Eng 39:1005–1014. https://doi.org/10.1007/s00449-016-1579-8

Li J, Fu Q, Liao Q, Zhu X, Ye D d, Tian X (2009) Persulfate: a self-activated cathodic electron acceptor for microbial fuel cells. J Power Sources 194:269. https://doi.org/10.1016/j.jpowsour.2009.04.055

Liu H, Logan BE (2004) Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environ Sci Technol 38:4040. https://doi.org/10.1021/es0499344

Liu S, Song H, Li X, Yang F (2013) Power generation enhancement by utilizing plant photosynthate in microbial fuel cell coupled constructed wetland system. Int J Photoenergy 2013:1. https://doi.org/10.1155/2013/172010

Logan BE, Hamelers B, Rozendal R, Schröder U, Keller J, Freguia S, Aelterman P, Verstraete W, Rabaey K (2006) Microbial fuel cells: methodology and technology. Environ Sci Technol 40:5181–5192. https://doi.org/10.1021/es0605016

Logroño W, Pérez M, Urquizo G, Kadier A, Echeverría M, Recalde C, Rákhely G (2017) Single chamber microbial fuel cell (SCMFC) with a cathodic microalgal biofilm: a preliminary assessment of the generation of bioelectricity and biodegradation of real dye textile wastewater. Chemosphere 176:378–388. https://doi.org/10.1016/j.chemosphere.2017.02.099

Luimstra VM, Kennedy SJ, Güttler J, Wood SA, Williams DE, Packer MA (2014) A cost-effective microbial fuel cell to detect and select for photosynthetic electrogenic activity in algae and cyanobacteria. J Appl Phycol 26:15. https://doi.org/10.1007/s10811-013-0051-2

Lusk BG, Parameswaran P, Popat SC, Rittmann BE, Torres CI (2016) The effect of pH and buffer concentration on anode biofilms of Thermincola ferriacetica. Bioelectrochemistry 112:47–52. https://doi.org/10.1016/j.bioelechem.2016.07.007

Malki M, De Lacey AL, Rodríguez N, Amils R, Fernandez VM (2008) Preferential use of an anode as an electron acceptor by an acidophilic bacterium in the presence of oxygen. Appl Environ Microbiol 74:4472–4476. https://doi.org/10.1128/AEM.00209-08

Mansoorian HJ, Mahvi AH, Jafari AJ, Amin MM, Rajabizadeh A, Khanjani N (2013) Bioelectricity generation using two chamber microbial fuel cell treating wastewater from food processing. Enzym Microb Technol 52:352. https://doi.org/10.1016/j.enzmictec.2013.03.004

Marsili E, Baron DB, Shikhare ID, Coursolle D, Gralnick JA, Bond DR (2008) Shewanella secretes flavins that mediate extracellular electron transfer. Proc Natl Acad Sci 105:3968–3973. https://doi.org/10.1073/pnas.0710525105

Min B, Cheng S, Logan BE (2005) Electricity generation using membrane and salt bridge microbial fuel cells. Water Res 39:1675–1686. https://doi.org/10.1016/j.watres.2005.02.002

More TT, Ghangrekar MM (2010) Improving performance of microbial fuel cell with ultrasonication pre-treatment of mixed anaerobic inoculum sludge. Bioresour Technol 101 (2):562–567

Morris JM, Jin S (2008) Feasibility of using microbial fuel cell technology for bioremediation of hydrocarbons in groundwater. J Environ Sci Heal A Tox Hazard Subst Environ Eng 43:18. https://doi.org/10.1080/10934520701750389

Mustakeem (2015) Electrode materials for microbial fuel cells: nanomaterial approach. Mater Renew Sustain Energy 4. https://doi.org/10.1007/s40243-015-0063-8

Nagar H, Badhrachalam N, Rao VVB, Sridhar S (2019) A novel microbial fuel cell incorporated with polyvinylchloride/4A zeolite composite membrane for kitchen wastewater reclamation and power generation. Mater Chem Phys 224:175–185. https://doi.org/10.1016/j.matchemphys.2018.12.023

Neethu B, Ghangrekar MM (2017) Electricity generation through a photo sediment microbial fuel cell using algae at the cathode. Water Sci Technol 76:3269–3277. https://doi.org/10.2166/wst.2017.485

Neethu B, Bhowmick GD, Ghangrekar MM (2018) Enhancement of bioelectricity generation and algal productivity in microbial carbon-capture cell using low cost coconut shell as membrane separator. Biochem Eng J 133:205. https://doi.org/10.1016/j.bej.2018.02.014

Neethu B, Bhowmick GD, Ghangrekar MM (2019a) A novel proton exchange membrane developed from clay and activated carbon derived from coconut shell for application in microbial fuel cell. Biochem Eng J 148:170. https://doi.org/10.1016/j.bej.2019.05.011

Neethu B, Pradhan H, Sarkar P, Ghangrekar MM (2019b) Application of ion exchange membranes in enhancing algal production alongside desalination of saline water in microbial fuel cell. MRS Adv 4:1077. https://doi.org/10.1557/adv.2019.170

Ogugbue CJ, Ebode EE, Leera S (2015) Electricity generation from swine wastewater using microbial fuel cell. J Ecol Eng 16:26. https://doi.org/10.12911/22998993/60450

Pandit S, Khilari S, Roy S, Pradhan D, Das D (2014) Improvement of power generation using Shewanella putrefaciens mediated bioanode in a single chambered microbial fuel cell: effect of different anodic operating conditions. Bioresour Technol 166:451–457. https://doi.org/10.1016/j.biortech.2014.05.075

Parkash A (2016) Utilization of distillery wastewater for electricity generation using microbial fuel cell. J Appl Emerg Sci 6(2):79–86

Penteado ED, Fernandez-Marchante CM, Zaiat M, Cañizares P, Gonzalez ER, Rodrigo MAR (2016) Energy recovery from winery wastewater using a dual chamber microbial fuel cell. J Chem Technol Biotechnol 91:1802. https://doi.org/10.1002/jctb.4771

Potter MC (1911) Electrical effects accompanying the decomposition of organic compounds. Proc R Soc B Biol Sci 84:260–276. https://doi.org/10.1098/rspb.1911.0073

Pradhan H, Ghangrekar MM (2014) Multi-chamber microbial desalination cell for improved organic matter and dissolved solids removal from wastewater. Water Sci Technol 70:1948. https://doi.org/10.2166/wst.2014.438

Qu X, Alvarez PJJ, Li Q (2013) Applications of nanotechnology in water and wastewater treatment. Water Res 47:3931–3946. https://doi.org/10.1016/j.watres.2012.09.058

Rahimnejad M, Najafpour GD, Ghoreyshi AA, Bakeri G, Talebnia F, Oh SE (2013) Investigation of different mediators in microbial fuel cell with cyclic voltammeter. Pakistan. J Biotechnol 10:37–51

Rajesh PP, Ghangrekar MM (2016) Bioelectricity generation from marine algae chaetoceros using microbial fuel cell. https://doi.org/10.1007/978-81-322-2773-1_22

Rajesh PP, Noori MT, Ghangrekar MM (2014) Controlling methanogenesis and improving power production of microbial fuel cell by lauric acid dosing. Water Sci Technol 70(8):1363–1369. https://doi.org/10.2166/wst.2014.386

Rajesh PP, Jadhav DA, Ghangrekar MM (2015) Improving performance of microbial fuel cell while controlling methanogenesis by Chaetoceros pretreatment of anodic inoculum. Bioresour Technol 180:66–71. https://doi.org/10.1016/j.biortech.2014.12.095

Reguera G, McCarthy KD, Mehta T, Nicoll JS, Tuominen MT, Lovley DR (2005) Extracellular electron transfer via microbial nanowires. Nature 435:1098–1101. https://doi.org/10.1038/nature03661

Reiche A, Sivell J l, Kirkwood KM (2016) Electricity generation by Propionibacterium freudenreichii in a mediatorless microbial fuel cell. Biotechnol Lett 38:51–55. https://doi.org/10.1007/s10529-015-1944-8

Sekar AD, Jayabalan T, Muthukumar H, Chandrasekaran NI, Mohamed SN, Matheswaran M (2019) Enhancing power generation and treatment of dairy waste water in microbial fuel cell using cu-doped iron oxide nanoparticles decorated anode. Energy 172:173. https://doi.org/10.1016/j.energy.2019.01.102

Sukkasem C, Xu S, Park S, Boonsawang P, Liu H (2008) Effect of nitrate on the performance of single chamber air cathode microbial fuel cells. Water Res 42:4743. https://doi.org/10.1016/j.watres.2008.08.029

Tao HC, Li W, Liang M, Xu N, Ni JR, Wu WM (2011) A membrane-free baffled microbial fuel cell for cathodic reduction of cu(II) with electricity generation. Bioresour Technol 102:4774. https://doi.org/10.1016/j.biortech.2011.01.057

Tartakovsky B, Guiot SR (2006) A comparison of air and hydrogen peroxide oxygenated microbial fuel cell reactors. Biotechnol Prog 22:241–246. https://doi.org/10.1021/bp050225j

Taskan E, Ozkaya B, Hasar H (2015) Combination of a novel electrode material and artificial mediators to enhance power generation in an MFC. Water Sci Technol 71:320–328. https://doi.org/10.2166/wst.2014.487

Tiwari BR, Ghangrekar MM (2015) Enhancing electrogenesis by pretreatment of mixed anaerobic sludge to be used as inoculum in microbial fuel cells. Energy Fuels 29(5):3518–3524. https://doi.org/10.1021/ef5028197

Virdis B, Rabaey K, Yuan Z, Keller J (2008) Microbial fuel cells for simultaneous carbon and nitrogen removal. Water Res 42:3013. https://doi.org/10.1016/j.watres.2008.03.017

Wang G, Huang L, Zhang Y (2008) Cathodic reduction of hexavalent chromium [Cr(VI)] coupled with electricity generation in microbial fuel cells. Biotechnol Lett 30:1959–1966. https://doi.org/10.1007/s10529-008-9792-4

Wang Z, Lim B, Lu H, Fan J, Choi C (2010) Cathodic reduction of Cu^{2+} and electric power generation using a microbial fuel cell. Bull Kor Chem Soc 31:2025. https://doi.org/10.5012/bkcs.2010.31.7.2025

Wang D-B, Song T-S, Guo T, Zeng Q, Xie J (2014) Electricity generation from sediment microbial fuel cells with algae-assisted cathodes. Int J Hydrog Energy 39(25):13224–13230. https://doi.org/10.1016/j.ijhydene.2014.06.141

Wei J, Liang P, Huang X (2011) Recent progress in electrodes for microbial fuel cells. Bioresour Technol 102:9335. https://doi.org/10.1016/j.biortech.2011.07.019

WHO & UNICEF (2014) Progress on sanitation and drinking-water—2014 update. The Joint Monitoring Programme for Water Supply and Sanitation . . . http://apps.who.int/iris/bitstream/10665/81245/1/9789241505390_eng.pdf?ua=1

Xing D, Zuo Y, Cheng S, Regan JM, Logan BE (2008) Electricity generation by Rhodopseudomonas palustris DX-1. Environ Sci Technol 42:4146–4151. https://doi.org/10.1021/es800312v

Xue A, Shen ZZ, Zhao B, Zhao HZ (2013) Arsenite removal from aqueous solution by a microbial fuel cell-zerovalent iron hybrid process. J Hazard Mater 261:621. https://doi.org/10.1016/j.jhazmat.2013.07.072

You S, Zhao Q, Zhang J, Jiang J, Zhao S (2006) A microbial fuel cell using permanganate as the cathodic electron acceptor. J Power Sources 162:1409. https://doi.org/10.1016/j.jpowsour.2006.07.063

Yun-Hai W, Bai-Shi W, Bin P, Qing-Yun C, Wei Y (2013) Electricity production from a bio-electrochemical cell for silver recovery in alkaline media. Appl Energy 112:1337. https://doi.org/10.1016/j.apenergy.2013.01.012

Zhang BG, Zhou SG, Zhao HZ, Shi CH, Kong LC, Sun JJ, Yang Y, Ni JR (2010) Factors affecting the performance of microbial fuel cells for sulfide and vanadium (V) treatment. Bioprocess Biosyst Eng 33:187. https://doi.org/10.1007/s00449-009-0312-2

Zhang B, Feng C, Ni J, Zhang J, Huang W (2012) Simultaneous reduction of vanadium (V) and chromium (VI) with enhanced energy recovery based on microbial fuel cell technology. J Power Sources 204:34. https://doi.org/10.1016/j.jpowsour.2012.01.013

Zhou M, Chi M, Luo J, He H, Jin T (2011) An overview of electrode materials in microbial fuel cells. J Power Sources 196:4427. https://doi.org/10.1016/j.jpowsour.2011.01.012

Zuo Y, Xing D, Regan JM, Logan BE (2008) Isolation of the exoelectrogenic bacterium Ochrobactrum anthropi YZ-1 by using a U-tube microbial fuel cell. Appl Environ Microbiol 74:3130–3137. https://doi.org/10.1128/AEM.02732-07

Ligninolysis: Roles of Microbes and Their Extracellular Enzymes

14

Ira Chaudhary and Smita Rastogi Verma

Abstract

Biodegradation of lignin, one of the most abundant components of lignocellulosic plant biomass, represents a key step for carbon recycling. The structure of lignin, however, makes it recalcitrant to degradation. This correlates both to environmental issues and agroindustrial utilization of lignocellulosic plant biomass. By cross-linking to both cellulose and hemicellulose, lignin forms a barrier that prevents the accessibility of chemicals or lignocellulolytic enzymes into the interior of lignocellulosic structure. The presence of lignin negatively affects the utility of cellulose in pulp and paper industry, textile industry, biofuel production, as well as animal feed. To improve the bioprocessing of lignocellulosic feedstocks in various industries, more effective degradation methods of lignin are in high demand. Some microbes are able to efficiently degrade lignin using a combination of extracellular ligninolytic enzymes, organic acids, mediators, and several accessory enzymes. Exploring the range of ligninolytic microbial biodiversity is the key to developing effective and eco-friendly strategies for environmental restoration or optimal and sustainable agroindustrial utilization of plant biomass. This study gives an insight of ligninolytic microbes and the extracellular enzymes implicated in lignin degradation.

Keywords

Laccase · Lignin biodegradation · Ligninolytic microbes · Lignin peroxidase · Manganese peroxidase

I. Chaudhary
Department of Biosciences, Integral University, Lucknow, India

S. R. Verma (✉)
Department of Biotechnology, Delhi Technological University, Delhi, India

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_14

14.1 Introduction

Lignocellulose, the major structural component of all plants, consists of three major components, viz., cellulose, hemicellulose, and lignin (Sanchez 2009). Cellulose is the major constituent of plant cell wall and is the most abundant organic molecule on earth. It is a linear polymer of glucose, and several such chains are arranged in parallel to form microfibrils. Hemicelluloses are heterogeneous polymers of pentoses (e.g., xylose, arabinose), hexoses (e.g., mannose, galactose, rhamnose), and sugar acids (e.g., glucuronic acid, galacturonic acid). Lignin is a phenolic heteropolymer, which is formed by oxidative polymerization of plant *p*-hydroxycinnamyl alcohols, namely, coniferyl alcohol, sinapyl alcohol, and *p*-coumaryl alcohol (Rastogi and Dwivedi 2008). Lignin and hemicellulose polymers act as recalcitrant adhesive for cellulose microfibrils. The recalcitrant nature of lignin is due to its heterogeneous aromatic structure, several stable linkages, and highly branched polymer network and is therefore very difficult to degrade (Abdel-Hamid et al. 2013; Ruiz-Dueñas and Martínez 2009).

Lignin, though crucial for the adaptive strategies of vascular plants, exerts negative consequences on the lignocellulosic biodegradation. The biodegradation of lignin embodies a crucial step for carbon recycling on earth because forest ecosystems contain enormous amount of wood. Lignocellulosic wastes are produced in huge amounts by industries including those of forestry, pulp and paper, agriculture, food, and in municipal solid wastes and animal wastes. Lignocellulosic biomass-based industries focus mainly on cellulose, whereas lignin is discarded mainly as waste or with very limited usage. In order to improve the sustainability and success of these plant polysaccharide-based industries, lignin should be utilized in improved ways and with minimal waste generation.

Currently employed chemical approaches for industrial waste treatment are expensive and toxic to environment. Hence these wastes are mostly left untreated, which raises many environmental concerns (Palacios-Orueta et al. 2005). To reduce or overcome these adverse effects, it becomes indispensable to search inexpensive, eco-friendly, and sustainable approaches for lignin degradation.

Besides having environmental impact, lignin has agroindustrial consequences as well (Schmidt 2006). The presence of lignin prevents the accessibility of enzymes for cellulose degradation. It hampers the optimal utilization of cellulose in pulp and paper industry, textile industry, biorefinery, and forage digestibility (Rastogi and Dwivedi 2014; Santos et al. 2013).

Though lignin resists attack by most microbes, nature has evolved catabolic pathways to completely degrade lignin through the production of devoted ligninolytic enzyme systems (Nelsen et al. 2016). Lignin biodegradation depends both on the degradative ability of the microbial population and environmental conditions (Waldrop et al. 2000). The focus thus shifted from chemical-based degradation of industrial effluents to eco-friendly approach using lignin-degrading microbes and the extracellular enzymes secreted by them. However, so far, very few ligninolytic microbes have been identified, which do not meet the industrial demands and are a challenge for researchers globally. Hence, discovering new microbial

strains and understanding their enzyme system for lignin degradation become essential. The current chapter focuses on succinctly describing the known ligninolytic microbes and the major enzymes secreted by them that are involved in decomposing lignin in the lignocellulosic biomass. Furthermore, the screening of microbes with high lignin-degrading potential is discussed.

14.2 Chemical Basis of Recalcitrant Nature of Lignin

The three *p*-hydroxycinnamyl alcohols form monolignols through successive side-chain reduction and ring hydroxylation/methylation reactions occurring at several different levels (Rastogi and Dwivedi 2008). In the last step of lignin biosynthesis, plant peroxidases/laccases oxidize monolignol to their phenoxy radicals (Higuchi 1997). Chemical coupling of the resonant forms of these radicals results in the formation of a three-dimensional network of lignin polymer (Gellerstedt and Henriksson 2008). Due to prevalence of the corresponding radical forms and higher stability of the coupling products, ether linkages between the phenolic position C4 and a side-chain (or aromatic ring) carbon of the *p*-hydroxy phenylpropanoid precursors are strongly predominant in the growing polymer. Due to the high frequency of these ether linkages, the aromatic lignin units are basically nonphenolic; hence lignin units cannot be oxidized by low-redox-potential oxidoreductases, such as the plant peroxidases. The bulky nature of the lignin polymer forming a complex 3-D network represents an additional limitation for biodegradation since the enzyme accessibility is strongly reduced.

14.3 Ligninolytic Microbes

Certain fungal and bacterial species have been demonstrated to degrade lignin (Hatakka and Hammel 2011; Huang et al. 2013). Ligninolytic ability of these microbes owes to the presence of various lignin-degrading enzymes. These microbes have developed two main strategies, viz., presence of catalytic residues widely exposed at the surface of ligninolytic enzymes and use of redox mediators participating in the enzymatic attack. Due to the presence of various enzymes and low molecular weight compounds, these microbes have developed various competition- and mutualism-based strategies that confer them the ability to decompose wood, to flourish in different ecological niches, and to utilize lignocellulosic plant biomass (Janusz et al. 2013). On the basis of their action on lignin and other lignocellulosic materials, microbes are grouped as simultaneous and selective lignin degraders. Microbes that selectively degrade lignin may find significant potential in the processes where the main objective is to get lignin-free cellulose for subsequent use, for example, in biopulping, biofuel production, textile industry, and animal feed (Anderson and Akin 2008).

Fungi are more efficient in lignin breakdown, whereas delignification by bacteria is slower and more limited (Sigoillot et al. 2012). Fungi are thought to be the most

substantial contributors to lignin degradation in natural systems due to their ability to produce enormous amounts of extracellular ligninolytic enzymes and hence have attracted a great deal of interest as potential biomass degraders for large-scale agroindustrial applications. Hence our main focus is on lignin-degrading fungi.

Lignin-degrading bacteria mainly belong to three classes, viz., actinomycetes, α-proteobacteria, and γ-proteobacteria (Huang et al. 2013; Kumar et al. 2018; Li et al. 2009; Wang et al. 2016; Wilhelm et al. 2019). Bacteria of several genera such as *Alcaligenes, Arthrobacter, Pseudomonas, Streptomyces*, and *Nocardia* readily degrade single-ring aromatic substrates.

Wood-degrading fungi mostly live as saprotrophs or weak parasites in natural and human-affected forest ecosystems. Generally, wood-rotting fungi are the only group of microbes capable of mineralization of lignin and are considered as primary lignin degraders. Depending on the morphology of wood decomposition, the saprophytic fungi are divided into three main groups, namely, white-rot fungi, brown-rot fungi, and soft-rot fungi (Liers et al. 2011). Among all these, white-rot fungi have an extraordinary capability for oxidative depolymerization and consequent mineralization of lignin. Although lignin resists attack by most microbes, basidiomycetes white-rot fungi can efficiently degrade lignin completely to CO2 and H2O (Aarti et al. 2015; Dashtban et al. 2010). Representatives of wood-decaying fungi are those belonging to *Basidiomycota*, *Polyporales*, and *Agaricales* (Rytioja et al. 2014). Some common examples are *Phanerochaete chrysosporium, Ceriporiopsis subvermispora, Trametes versicolor, Phlebia radiata, Pleurotus ostreatus*, and *Pleurotus eryngii*. Brown-rot fungi colonize wood by degrading cellulose and can degrade lignin only partially. Among most-studied wood-decaying brown-rot fungi that selectively degrade lignin are *Gloeophyllum trabeum, Serpula lacrymans*, *Coniophora puteana, Physisporinus rivulosus*, and *Dichomitus squalens*, whereas *Trametes versicolor, Heterobasidion annosum, P. chrysosporium*, and *Irpex lacteus* simultaneously degrade all cell wall components. White-rot basidiomycetes mostly affect hardwood of angiosperms, whereas brown-rot fungi mostly attack softwoods of gymnosperms (Hatakka 2005; Sigoillot et al. 2012). Fungi belonging to *Ascomycota* mostly cause soft-rot decay. The ability to degrade lignin by these fungi is limited (Cragg et al. 2015).

14.4 Ezymes Implicated in Lignin Degradation

The importance of certain wood-degrading enzymes produced by different microbes has been highlighted through analysis of genomics and secretomic data (Janusz et al. 2013). Genome sequence analysis of ligninolytic fungi has revealed that there is not one defined set of enzymes for lignin degradation (Floudas et al. 2012). The composition of the set of oxidative enzymes being produced depends on the microbes, and different microbes differ with respect to the production of the type of ligninolytic enzyme(s) and its isozyme(s).

Microbial enzymes implicated in lignin degradation are divided into two main groups of extracellular oxidative enzymes, viz., lignin-modifying enzymes (LME)

and lignin-degrading auxiliary (LDA) enzymes (Hammel and Cullen 2008). These lignin-degrading secreted enzymes are collectively termed ligninases.

Microbial LMEs are classified into heme-containing peroxidases and phenol oxidase. Phenol oxidase includes laccase (Lac), whereas heme-containing peroxidases are lignin peroxidase (LiP), manganese peroxidase (MnP), multifunctional (versatile) peroxidase (VP), and dye-decolorizing peroxidase (DyP) (Lambertz et al. 2016). Some of these peroxidases attack lignin or lignin fragments from a distance. These can oxidize mediators, e.g., manganese ions and lignin-derived aromatic compounds (e.g., formation of veratryl alcohol cation radical), and generate small oxidizing agents that penetrate the lignin polymer and trigger depolymerization via radical chemistry (Hunt et al., 2013; Nousiainen et al. 2009). LMEs are summarized in Table 14.1. LDAs are oxidative enzymes and do not catalyze lignin degradation on their own. These act in concert with LMEs and facilitate the degradation process by producing the required H_2O_2 (da Silva Coelho-Moreira et al. 2013). LDA enzymes include glyoxal oxidase, aryl alcohol oxidase, heme-thiolate haloperoxidases, glucose dehydrogenase, pyranose 2-oxidase, cellobiose dehydrogenase, etc. These enzymes are frequently found in white-rot fungi secretomes (Levasseur et al. 2008).

Laccase (benzenediol: oxygen oxidoreductases; E.C 1.10.3.2), also called blue multicopper oxidase, is a copper-containing polyphenol oxidase (Arora and Sharma 2010). It catalyzes the oxidation of mono-, di-, and polyphenols, aromatic amines, and nonphenolic compounds to free radicals and the four-electron reduction of oxygen directly to water. These free radicals undergo spontaneous reactions resulting in the cleavage of various bonds (Leonowicz et al. 2001). Certain chemical mediators act as intermediate substrates for laccase activity. These mediators form oxidized radicals, which then react with the high redox potential substrates (Arora and Sharma 2010). Thus, laccase catalyzes the oxidation of nonphenolic substructures in the presence of mediators such as 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonate) (ABTS) derived naturally from the substrates or produced by the fungus (Christopher et al. 2014).

Lignin peroxidase (E.C 1.11.1.14), an oxidoreductase, is an extracellular hemeprotein. Its action depends on H_2O_2 and has an unusually high redox potential and low optimum pH (Erden et al. 2009; Hammel and Cullen 2008). It can act both on phenolic and nonphenolic aromatic compounds. LiP, like classical peroxidases, oxidizes phenolic substrates, while the oxidation of the nonphenolic methoxybenzenes is unique to LiP (Oyadomari et al. 2003). It preferentially cleaves the Cα-Cβ bonds in lignin molecule but is also capable of ring opening and other reactions.

Manganese peroxidase (E.C 1.1 1.1.13), an oxidoreductase, catalyzes the H_2O_2-dependent oxidation of lignin and lignin derivatives (Hammel and Cullen 2008). Mn (II) is the preferred substrate for MnP. The enzyme catalyzes oxidation of Mn(II) to Mn(III), which in turn oxidizes the phenolic substrate (Hofrichter 2002). Organic acids produced by microbes, for example, oxalate and malonate, chelate Mn (III) to form stable complexes with high redox potentials. This facilitates the dissociation of Mn (III) from the enzyme, thereby activating the MnP system (Mäkelä et al. 2002;

Table 14.1. Major microbial enzymes implicated in lignin degradation

Enzyme-cofactor	Substrate	Metals or ions	Mediators	General reaction	Major reaction
LiP-heme	H_2O_2	Iron	Veratryl alcohol	1,2-Bis (3,4-dimethoxyphenyl) propane-1,3-diol + H_2O_2 ⇌ 3,4-dimethoxybenzaldehyde + 1-(3,4-dimethoxyphenyl)ethane-1,2-diol +H_2O	Aromatic ring oxidized to cation radical
MnP-heme	H_2O_2, Mn	Ca^{2+} Cd^{2+} Mn^{2+} Sm^{3+}	Organic acid as chelators, thiols, unsaturated fatty acids	$2Mn(II) + 2H^+ + H_2O_2 \rightleftharpoons 2Mn(III) + 2H_2O$	Mn(II) oxidized to Mn(III); chelated Mn(III) oxidizes phenolic compounds to phenoxyl radicals
VP-heme	H_2O_2	Mn^{2+} Ca^{2+} Cu^{2+} Iron	Compounds similar to LiP and MnP mediators	Donor + H_2O_2 ⇌ oxidized donor + $2H_2O$	Dual oxidative ability to oxidize Mn(II) and phenolic and nonphenolic aromatic compounds similar to MnP and LiP, respectively
Lac	O_2	Ca^{2+}	Phenols, aniline, 3-hydroxyanthranilic acid, *N*-hydroxyacetanilide, syringaldehyde, hydroxybenzotriazole, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)	4 Benzenediol + O_2 ⇌ 4 Benzosemiquinone + $2H_2O$	Phenols are oxidized to phenoxyl radicals
		Cd^{2+}			
		Cu^{2+}			
		Mn^{2+}			
		K^+			
		H_2O_2			
		Imidazole			
		K_2SO_4			
		Na_2SO_4			
		$(NH_4)_2SO_4$			

Schmidt 2006). Mn ion, thus, acts as a diffusible redox couple in the reaction rather than as an enzyme-binding activator.

Versatile peroxidase (EC 1.11.1.16) is also known as hybrid peroxidase or lignin-manganese peroxidase, since VP combines LiP and MnP catalytic properties. VPs exhibit dual oxidative ability to oxidize Mn(II) and also phenolic and nonphenolic aromatic compounds. VP oxidizes typical LiP substrates, e.g., veratryl alcohol, methoxybenzenes, nonphenolic model lignin compounds, and Mn^{2+}. The substrate specificity of VPs is similar to that of LiPs, including oxidation of high and medium redox potential compounds. VP also oxidizes azo dyes and other nonphenolic compounds with high redox potential in the absence of mediators (Garcia-Ruiz et al. 2014). Due to their catalytic versatility, which includes the degradation of compounds that other peroxidases are unable to oxidize directly, VPs have attracted the greatest biotechnological attention.

Dye-decolorizing peroxidases (EC 1.11.1.19) are a member of the novel heme peroxidase family (DyP-type peroxidase superfamily), showing no homology to classic fungal heme peroxidases including LiP, MnP, and VP. DyPs are active at low pH values and are able to degrade different dyes, particularly anthraquinone dyes (Zamocky et al. 2015). DyP oxidizes phenolic compounds, e.g., 2,6-dimethoxyphenol and guaiacol. Induced transcription of genes encoding DyPs is accompanied by the increased expression of transcripts for H_2O_2-generating enzymes such as glyoxal oxidase, alcohol oxidase, and pyranose 2-oxidase (Qin et al. 2018).

In addition to LMEs, other extracellular enzymes also act as accessory enzymes for catalysis of lignin degradation. These accessory enzymes are called lignin-degrading auxiliary (LDA) enzymes. In order to degrade lignin, microbes require sources of extracellular H_2O_2, to support the oxidative turnover of LMEs responsible for ligninolysis. Examples of LDA enzymes include extracellular oxidases that reduce molecular O2 to H_2O_2, with the synergistic oxidation of a cosubstrate. Microbes catalyze the Fenton reaction for the conversion of H_2O_2 to OH. These free radicals nonspecifically attack polysaccharides as well as lignin in plant cell walls, thereby creating some cleavage sites that permit easy penetration of lignocellulolytic enzymes.

The most characterized oxidases generating H_2O_2 are glyoxal oxidase and aryl alcohol oxidase. Glyoxal oxidase (GLOX; EC 1.2.3.5), a copper radical protein, generates extracellular H_2O_2 by oxidizing dicarbonyl and hydroxycarbonyl compounds, such as glyoxal and methylglyoxal (Yamada et al. 2014). Aryl alcohol oxidase (AAO; EC 1.1.3.7) belongs to glucose-methanol-choline oxidase/dehydrogenase family (GMC). It produces H_2O_2 by oxidative dehydrogenation of phenolic and nonphenolic aryl alcohols, polyunsaturated (aliphatic) primary alcohols, or aromatic secondary alcohols (Ferreira-Neila et al. 2010). In addition, aryl alcohol dehydrogenases (ADH) and quinone reductases (QR) are also involved in lignin degradation by fungi. It has been suggested that the H_2O_2 required in ligninolysis is formed during the cycling reaction of AAO and ADH, which converts alcohol to aldehyde in an NADP-dependent reaction (Hernandez-Ortega et al. 2012).

Two types of heme-thiolate haloperoxidases implicated in lignin degradation are chloroperoxidases (CPO, EC 1.11.1.10) and aromatic peroxygenases (APO, unspecific peroxygenases, EC 1.11.2.1). CPO catalyzes epoxidation and hydroxylation of organic sulfides, olefins, and aromatic rings. It chlorinates as well as cleaves the major structures of lignin (Hofrichter and Ullrich 2006; Ortiz-Bermudez et al. 2003). APO transfers oxygen to aromatic and aliphatic substrates similar to cytochrome P450. It utilizes H_2O_2 rather than directly transferring molecular oxygen. It has basic ferryl environment of their active center, due to which it has high reactivity toward benzylic C-H and phenolic substrates compared to typical peroxidases or model compounds (Piontek et al. 2010; Wang et al. 2015).

Glucose dehydrogenase (GDH; EC 1.1.99.10) is flavin adenine dinucleotide (FAD)-dependent extracellular enzyme. In comparison with GOX, it does not use oxygen as an external electron acceptor but instead uses phenoxy radicals, quinones, redox dyes, and iron complexes such as ferricyanide and ferrocenium hexafluorophosphate (Piumi et al. 2014). Pyranose 2-oxidase (POX; EC 1.1.3.10) catalyzes the C-2 oxidation of aldopyranoses with the reduction of O2 to H_2O_2 (de Koker et al. 2004).

Moreover, cellobiose dehydrogenase (CDH; EC 1.1.99.18), which is secreted mainly by many white-rot fungi under cellulolytic conditions, is also involved in lignin degradation in the presence of H_2O_2 and chelated Fe ions. It reduces Fe^{3+} to Fe^{2+} and O2 to H_2O_2 (Beeson et al. 2015). The brown-rot fungi, except *Coniophora puteana*, do not produce CDH (Kajisa et al. 2004). It is proposed that the effect of CDH on lignin degradation is through the reduction of quinones, which can be used by ligninolytic enzymes or the support of a Mn-peroxidase reaction.

14.5 Radical Chemistry in Lignin Degradation

Oxidation of lignin polymer by LMEs generates aromatic radicals. These evolve through C4-ether breakdown, aromatic ring cleavage, Cα-Cβ breakdown, and demethoxylation. Breakdown of Cα-Cβ in lignin releases aromatic aldehydes, or these may be synthesized de novo by microbes. These aromatic aldehydes are the substrates for AAO-catalyzed generation of H_2O_2 in cyclic redox reactions also involving AAD (Hernandez-Ortega et al. 2012). C4-ether breakdown generates phenoxy radicals. If not first reduced by oxidases to phenolic compounds, these phenolic radicals may repolymerize on the lignin polymer, which is acted upon again by LMEs. Phenoxy radicals when subjected to Cα-Cβ breakdown generate *p*-quinones. Quinones participate in oxygen activation in redox cycling reactions involving QR and LMEs (Rastogi and Chaudhary 2011). This results in reduction of the ferric iron present in wood, either by superoxide cation radical or directly by the semiquinone radicals and its reoxidation with concomitant reduction of H_2O_2 to hydroxyl free radical ($OH^{\bullet}$). Hydroxyl free radical is a very strong oxidizing agent that can initiate the attack on lignin in the initial stages of wood decay, when the penetration of LMEs is prevented by the small pores in the intact cell wall. Lignin degradation then proceeds by oxidative attack of the enzymes as described above. In

the final steps, simple products resulting from lignin degradation are incorporated into intracellular catabolic pathways.

14.6 Lignin-Degrading Enzyme Activity Is Indicative of Lignin Degradation Capability

Bioligninolytic system exploiting lignin-degrading microbes with high lignin-degrading capability can be a promising strategy in improving lignin biodegradation. Screening of lignin-degrading microbial species can be easily done on the basis of these ligninolytic enzymes (Herpoël et al. 2000; Santhanam et al. 2012). The screening strategy should be easy, inexpensive, rapid, and sensitive and applicable under industrial conditions. Thus, plate assay method for ligninolytic enzymes is the best choice.

LiP plate assay is based on oxidative decolorization of azure B, a lignin-like recalcitrant heterocyclic thiazine dye. Oxidation of azure B in the presence of high concentration of H_2O_2 involves production of two water molecules, abstraction of two electrons per unit of azure B, and ultimately aromatic ring cleavage. This results in the formation of clear zones on agar plates (Aguiar and Ferraz 2007; Arantes and Milagres 2007; Schmidt 2006). LiP enzyme activity is determined spectrophotometrically in the enzyme extract according to the protocol described by Archibald (1992), which is based on analysis of blue coloration resulting from the oxidation of azure B. The increase in absorbance at 651 nm (millimolar extinction coefficient 48.8 mM) is recorded for 1 min against reagent blank. One unit (U) of LiP activity is defined as activity of enzyme that catalyzes the conversion of 1 μmol of azure B per minute.

The plate assay of MnP activity is easily done in the presence of pH indicator dye phenol red. MnP enzyme activity in enzyme extract is analyzed spectrophotometrically according to the protocol described by Kuwahara et al. (1984), which is based on the oxidation of phenol red to give red coloration. The increase in absorbance is observed for 1 min at 520 nm (extinction coefficient 22 mM^{-1} cm^{-1}) against reagent blank. Mn-dependent activity is calculated by subtracting the absorbance in the absence of MnSO4. The absorbance is measured in 1 min intervals after addition of H_2O_2. One unit of MnP activity is defined as activity of enzyme that catalyzes the conversion of 1 μmol of phenol red per minute.

Laccase-catalyzed reaction in the presence of non-catalytic cooxidant ABTS is conveniently used for plate assay of lignin degraders. The formation of bluish-green halos around the microbial colonies is considered as a positive test for Lac activity. Laccase activity is determined spectrophotometrically by monitoring the oxidation of ABTS (Poppius-Levlin et al. 1999), which is based on the oxidation of ABTS to yield green coloration. It is measured by an increase in absorbance at 420 nm (extinction coefficient 36×10^4 mM^{-1} cm^{-1}) for 1 min against reagent blank. One unit of Lac activity is defined as activity of enzyme that catalyzes the conversion of 1 mol of ABTS per minute.

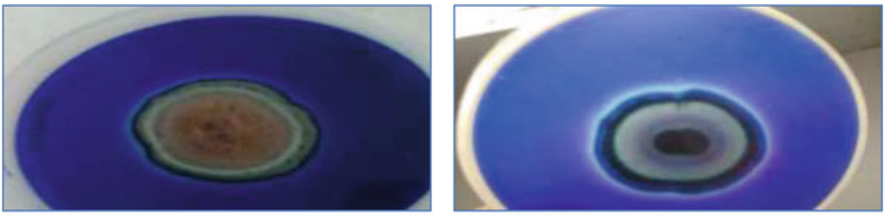


Fig. 14.1 LiP-positive fungal isolate showing white colony with clear zone in the presence of azure B. (**a**) Front view. (**b**) Back view

In our lab, the main focus was on lignin-degrading fungi. Screening of fungi from deteriorated wood samples with high lignin degradation potential was performed on the basis of ligninolytic enzymes plate assay. Based on LiP plate assay, a total of 20 LiP-positive fungal cultures were screened from deteriorated wood samples on Olga et al. medium [peptone 0.3 g, glucose 1.0 g, KH_2PO_4 0.03 g, $ZnSO_4$ 0.0001 g, K_2HPO_4 0.004 g, $FeSO_4$ 0.00005 g, $MnSO_4$ 0.0005 g, $MgSO_4$ 0.0005 g, agar 2.0 g, azure B 0.05%, pH 6.0], incubated for 7–10 days at 30 °C (Olga et al. 1998). LiP-positive fungal cultures were screened by their ability to decolorize azure B, thereby producing clear zones (Fig. 14.1), and were considered as putative lignin degraders.

After screening the cultures by LiP plate assay technique, quantitative analyses of LiP, MnP, and Lac were done by spectrophotometric method for the 20 putative lignin degraders. Inoculum from each fungal colony tested positive in LiP plate assay was separately added to LMM broth medium [glucose 10 g, sodium tartrate 2 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KCl 0.5 g, yeast extract 1 g, $CuSO_4$ 150 μM, Na_2EDTA 0.5 g, $FeSO_4$ 0.2 g, $ZnSO_4 \cdot 7H_2O$ 0.01 g, $MnCl_2 \cdot 4H_2O$ 0.003 g, H_3BO_4 0.003 g, $CoCl_2 \cdot 6H_2O$ 0.02 g, $CuCl_2 \cdot 2H_2O$ 0.001 g, $Na_2MoO_4 \cdot 2H_2O$ 0.003 g, pH 6.0] and incubated at 120 rpm, 25 °C for 1 week (Dhouib et al. 2005). For each isolate, the enzyme extract obtained after homogenization and filtration was subjected to LiP, MnP, and Lac enzymatic analysis procedures as described earlier. It was determined from spectrophotometric analyses that LiP, MnP, and Lac enzyme activities were present in all the 20 fungal isolates and ranged between 53.06 ± 0.01 and 182.78 ± 0.15 U/mg protein, 9.24 ± 0.05 and 139.56 ± 0.02 U/mg protein, and 10.68 ± 0.02 and 166.59 ± 0.01 U/mg protein, respectively. The results are summarized in Table 14.2.

14.7 Molecular Characterization of Ligninolytic Microbes

Currently, very little is known about the diversity of lignin-degrading microbes and their role in lignin degradation and nutrient cycling. Discovery of novel ligninolytic microbes, enzymes, and their biochemical characterization is essential for the deconstruction of biomass for its application in industries. A polyphasic strategy involving

Table 14.2 Quantitative analysis of lignin peroxidase, manganese peroxidase and laccase enzymatic activities in fungal isolates screened on the basis of LiP plate assay method

Fungal isolate number	LiP-specific activity (U/mg protein)	MnP-specific activity (U/mg protein)	Lac-specific activity (U/mg protein)
1	69.78 ± 0.15	12.09 ± 0.01	28.2 ± 0.06
2	74.78 ± 0.1	20.15 ± 0.02	12.87 ± 0.04
3	182.78 ± 0.15	112.09 ± 0.01	50.2 ± 0.06
4	62.93 ± 0.05	13.41 ± 0.09	23.57 ± 0.05
5	173.22 ± 0.01	139.56 ± 0.02	40.36 ± 0.09
6	67.77 ± 0.09	10.04 ± 0.01	36.37 ± 0.03
7	63.38 ± 0.01	19.08 ± 0.05	20.44 ± 0.02
8	79.06 ± 0.03	15.35 ± 0.05	28.24 ± 0.01
9	180.75 ± 0.01	125.21 ± 0.03	42.59 ± 0.08
10	60.70 ± 0.04	18.10 ± 0.1	26.29 ± 0.04
11	65.11 ± 0.08	11.16 ± 0.04	23.6 ± 0.08
12	87.69 ± 0.01	25.61 ± 0.04	19.56 ± 0.01
13	72.13 ± 0.02	10.24 ± 0.03	16.06 ± 0.01
14	78.56 ± 0.01	21.09 ± 0.04	17.18 ± 0.09
15	63.73 ± 0.09	9.24 ± 0.05	10.68 ± 0.02
16	53.06 ± 0.01	29.35 ± 0.06	137.22 ± 0.03
17	131.72 ± 0.06	49.21 ± 0.01	166.59 ± 0.01
18	76.57 ± 0.01	21.1 ± 0.01	32.14 ± 0.01
19	78.77 ± 0.03	14.04 ± 0.08	22.37 ± 0.08
20	93.93 ± 0.01	10.41 ± 0.01	38.57 ± 0.03

morphological, biochemical, and molecular biological techniques is essential to obtain an improved understanding of the interaction between the microbes and environment. The foremost incentive of the research should be on the development of PCR-based techniques for the identification of microbial community structure in environmental samples. These methods include sequence comparisons of conserved genomic regions such as 16S rRNA (for bacteria)/18S rRNA (for fungi) or comparisons of random amplified polymorphic DNA (RAPD) in the genomic DNA with corresponding data on known organisms. Molecular genetic techniques using DNA polymorphism have been increasingly used to identify a novel genus (O'Neill et al. 2003). Morphological and biochemical markers may be affected by environmental factors and growth practices, whereas DNA-based markers provide efficient and reliable tools for measuring genetic diversity in microbes and studying evolutionary relationships. Among the molecular markers, amplification using random primers and gene-specific primers is employed in genetic research due to their speed and simplicity. In our lab, among all the LiP-positive fungal isolates, five most promising fungal isolates (isolate numbers 3, 9, 5, 17, 20) were identified by 18S rRNA universal primer sequencing as *Agaricomycetes* sp., *Fusarium oxysporum*, *Schizophyllum commune*, *Bipolaris tetramera*, and *Basidiomycota* sp., respectively. RAPD analysis using random decameric primers revealed polymorphic and distinguishable banding patterns, indicative of genetic diversity of fungal isolates.

14.8 Conclusion

Isolation and characterization of lignin-degrading microbes with high activity or strain improvement to get maximum yield of such enzymes is the need of the future so as to facilitate efficient carbon cycling by catalyzing lignin degradation and also to exploit the plant biomass to its full capacity in an environment-friendly way. Selective lignin degraders are most attractive in removing lignin from the perspective of biotechnological applications. Further, investigation is needed to identify the novel proteins involved in lignin degradation and their mechanisms of action. Future strategies should aim at engineering ligninolytic enzyme consortium so as to ensure that these enzymes meet industry standards and requirements. Engineering goals may include directed evolution and hybrid approaches for these enzymes that exceed the limits imposed by nature, engineering ligninolytic secretomes to create consolidated bioprocessing microbes with synthetic biology applications, synthetic secretomes, and evolved ancestral ligninases (Alcalde 2015; Presley et al. 2018). In addition, the suppression of lignin biosynthesis enzymes via plant genetic engineering may be of potential use in overcoming some of the problems related to the recalcitrance of lignin (Rastogi and Dwivedi 2006, 2008).

References

Aarti C, Arasu MV, Agastian P (2015) Lignin degradation: a microbial approach. South Indian J Biol Sci 1:119–127

Abdel-Hamid AM, Solbiati JO, Cann IKO (2013) Insights into lignin degradation and its potential industrial applications. Adv Appl Microbiol 82:1–28

Aguiar A, Ferraz A (2007) Fe^{3+}- and Cu^{2+}-reduction by phenol derivatives associated with Azure B degradation in Fenton-like reactions. Chemosphere 66:947–954

Alcalde M (2015) Engineering the ligninolytic enzyme consortium. Trends Biotechnol 33:155–162

Anderson WF, Akin DE (2008) Structural and chemical properties of grass lignocelluloses related to conversion for biofuels. J Ind Microbiol Biotechnol 35:355–366

Arantes V, Milagres AMF (2007) The synergistic action of ligninolytic enzymes (MnP and Laccase) and Fe^{3+}-reducing activity from white-rot fungi for degradation of Azure B. Enzym Microb Technol 42:17–22

Archibald FS (1992) A new assay for lignin-type peroxidases employing the dye azure B. Appl Environ Microbiol 58:3110–3116

Arora DS, Sharma RK (2010) Ligninolytic fungal laccases and their biotechnological applications. Appl Biochem Biotechnol 160:1760–1788

Beeson WT, Vu VV, Span EA, Phillips CM, Marletta MA (2015) Cellulose degradation by polysaccharide monooxygenases. Annu Rev Biochem 84:923–946

Christopher LP, Yao B, Ji Y (2014) Lignin biodegradation with laccase-mediator systems. Front Energy Res 2:1–13

Cragg SM, Beckham GT, Bruce NC, Bugg TD, Distel DL, Dupree P, Etxabe AG, Goodell BS, Jellison J, McGeehan JE, McQueen-Mason SJ, Schnorr K, Walton PH, Watts JE, Zimmer M (2015) Lignocellulose degradation mechanisms across the Tree of Life. Curr Opin Chem Biol 29:108–119

da Silva C-MJ, Maciel GM, Castoldi R, da Silva MS, Dorneles Inácio F, Bracht A, Peralta RM (2013) Involvement of lignin-modifying enzymes in the degradation of herbicides. In: Price A (ed) Herbicides–advances in research. In Tech, Rijeka

Dashtban M, Schraft H, Syed TA, Qin W (2010) Fungal biodegradation and enzymatic modification of lignin. Int J Biochem Mol Biol 1:36–50

de Koker TH, Mozuch MD, Cullen D, Gaskell J, Kersten PJ (2004) Isolation and purification of pyranose 2-oxidase from *Phanerochaete chrysosporium* and characterization of gene structure and regulation. Appl Environ Microbiol 70:5794–5800

Dhouib A, Hamza M, Zouari H, Mechichi T, Hmidi R, Labat M, Martinez MJ, Sayadi S (2005) Screening for ligninolytic enzyme production by diverse fungi from Tunisia. World J Microbiol Biotechnol 21:1415–1423

Erden E, Ucar CM, Gezer T, Pazarlioglu NK (2009) Screening for ligninolytic enzymes from autochthonous fungi and applications for decolorization of Remazole Marine Blue. Braz J Microbiol 40:346–353

Ferreira-Neila P, Hernandez-Ortega A, Herguedas B, Rencoret J, Gutiérrez A, Martínez MJ, Jiménez-Barbero J, Medina M, Martínez AT (2010) Kinetic and chemical characterization of aldehyde oxidation by fungal aryl-alcohol oxidase. Biochem J 425:585–593

Floudas D, Binder M, Riley R, Barry K, Blanchette RA, Henrissat B, Martínez AT, Otillar R, Spatafora JW, Yadav JS, Aerts A, Benoit I, Boyd A, Carlson A, Copeland A, Coutinho PM, de Vries RP, Ferreira-Neila P, Findley K, Foster B, Gaskell J, Glotzer D, Górecki P, Heitman J, Hesse C, Hori C, Igarashi K, Jurgens JA, Kallen N, Kersten P, Kohler A, Kües U, Kumar TKA, Kuo A, La Butti K, Larrondo LF, Lindquist E, Ling A, Lombard V, Lucas S, Lundell T, Martin R, DJ ML, Morgenstern I, Morin E, Murat C, Nagy LG, Nolan M, Ohm RA, Patyshakuliyeva A, Rokas A, Ruiz-Dueñas FJ, Sabat G, Salamov A, Samejima M, Schmutz J, Slot JC, St. John F, Stenlid J, Sun H, Sun S, Syed K, Tsang A, Wiebenga A, Young D, Pisabarro A, Eastwood DC, Martin F, Cullen D, Grigoriev IV, Hibbett DS (2012) The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336:1715–1719

Garcia-Ruiz E, Mate DM, Gonzalez-Perez D, Molina-Espeja P, Camarero S, Martınez AT, Ballesteros AO, Alcalde M (2014) Directed evolution of ligninolytic oxidoreductases: from functional expression to stabilization and beyond. In: Fessner W (ed) Cascade biocatalysis. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 1–22

Gellerstedt G, Henriksson G (2008) In: Belgacem M, Gandini A (eds) Lignins: major sources, structure and properties. Elsevier, Amsterdam, pp 201–224

Hammel KE, Cullen D (2008) Role of fungal peroxidases in biological ligninolysis. Curr Opin Plant Biol 11:349–355

Hatakka A (2005) Biodegradation of lignin. In: Alexander S (ed) Biopolymers [online]. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Hatakka A, Hammel KE (2011) Fungal biodegradation of lignocelluloses. In: Hofrichter M (ed) Industrial applications. Springer, Berlin, pp 319–340

Hernandez-Ortega A, Ferreira-Neila P, Martinez AT (2012) Fungal aryl-alcohol oxidase: a peroxide-producing flavoenzyme involved in lignin degradation. Appl Microbiol Biotechnol 93:1395–1410

Herpoël I, Moukha S, Lesage-Meessen L, Sigoillot JC, Asther M (2000) Selection of *Pycnoporus cinnabarinus* strains for laccase production. FEMS Microbiol Lett 183:301–306

Higuchi T (1997) Biochemistry and molecular biology of wood. Springer, London

Hofrichter M (2002) Lignin conversion by manganese peroxidase (MnP). Enz Microb Technol 30:454–466

Hofrichter M, Ullrich R (2006) Heme-thiolate haloperoxidases: versatile biocatalysts with biotechnological and environmental significance. Appl Microbiol Biotechnol 71:276–288

Huang XF, Santhanam N, Badri DV, Hunter WJ (2013) Isolation and characterization of lignin-degrading bacteria from rainforest soils. Biotechnol Bioeng 110:1616–1626

Hunt CG, Houtman CJ, Jones DC, Kitin P, Korripally P, Hammel KE (2013) Spatial mapping of extracellular oxidant production by a white rot basidiomycete on wood reveals details of ligninolytic mechanism. Environ Microbiol 15:956–966

Janusz G, Kucharzyk KH, Pawlik A, Staszczaka M, Paszczynskic AJ (2013) Fungal laccase, manganese peroxidase and lignin peroxidase: gene expression and regulation. Enzym Microb Technol 52:1–12

Kajisa T, Yoshida M, Igarashi K, Katayamab A, Nishino T, Samejimaa M (2004) Characterization and molecular cloning of cellobiose dehydrogenase from the brown- rot fungus *Coniophora puteana*. J Biosci Bioeng 98:57–63

Kumar M, Verma S, Gazara RK, Kumar M, Pandey A, Verma PK, Thakur IS (2018) Genomic and proteomic analysis of lignin degrading and polyhydroxyalkanoate accumulating β-proteobacterium *Pandoraea* sp. ISTKB Biotechnol Biofuels 11:154–176

Kuwahara M, Glenn JK, Morgan MA, Gold MH (1984) Separation and characterization of two extracellular H_2O_2 dependent oxidases from ligninolytic cultures of *Phanerochaete chrysosporium*. FEBS Lett 169:247–250

Lambertz C, Ece S, Fischer R, Commandeur U (2016) Progress and obstacles in the production and application of recombinant lignin-degrading peroxidases. Bioengineered 7:145–154

Leonowicz A, Cho NS, Luterek J, Wilkolazka A, Wojtas-Wasilewska M, Matuszewska A, Hofrichter M, Wesenberg D, Rogalski J (2001) Fungal laccase: properties and activity on lignin. J Basic Microbiol 41:185–227

Levasseur A, Piumi F, Coutinho PM, Rancurel C, Asther M, Delattre M, Henrissat B, Pontarotti P, Asther M, Record E (2008) FOLy: an integrated database for the classification and functional annotation of fungal oxidoreductases potentially involved in the degradation of lignin and related aromatic compounds. Fungal Genet Biol 45:638–645

Li J, Yuan H, Yang J (2009) Bacteria and lignin degradation. Front Biol China 4:29–38

Liers C, Arnstadt T, Ullrich R, Hofrichter M (2011) Patterns of lignin degradation and oxidative enzyme secretion by different wood- and litter-colonizing basidiomycetes and ascomycetes grown on beech-wood. FEMS Microbiol Ecol 78:91–102

Mäkelä M, Galkin S, Hatakka A, Lundell T (2002) Production of organic acids and oxalate decarboxylase in lignin-degrading white rot fungi. Enzyme Microb Technol 30:542–549

Nelsen MP, DiMichele WA, Peters SE, Boycea CK (2016) Delayed fungal evolution did not cause the Paleozoic peak in coal production. Proc Natl Acad Sci U S A 113:2442–2447

Nousiainen P, Maijala P, Hatakka A, Martinez AT (2009) Syringyl-type simple plant phenolics as mediating oxidants in laccase catalyzed degradation of lignocellulosic materials: model compound studies. Holzforschung 63:699–704

O'Neill R, Snowdon RJ, Kohler W (2003) Population genetics aspects of biodiversity. Prog Bot 64:115–137

Olga VKS, Elena VS, Valeria PG, Olga VM, Natalia VL, Aida ND, Alexander IJ, Alexander M (1998) Purification and characterization of the constitutive form of laccase from basidiomycete *Coriolus hirsutus* and effect of inducers on laccase synthesis. Biotechnol Appl Biochem 28:47–54

Ortiz-Bermudez P, Srebotnik E, Hammel KE (2003) Chlorination and cleavage of lignin structures by fungal chloroperoxidases. Appl Environ Microb 69:5015–5018

Oyadomari M, Shinohara H, Johjima T, Wariishi H, Tanaka H (2003) Electrochemical characterization of lignin peroxidase from the white-rot basidiomycete *Phanerochaete chrysosporium*. J Mol Catal B Enzymatic 21:291–297

Palacios-Orueta A, Chuvieco E, Parra A, Carmona-Moreno C (2005) Biomass burning emissions: a review of models using remote-sensing data. Environ Monit Assess 104:189–209

Piontek K, Ullrich R, Liers C, Diederichs K, Plattner DA, Hofrichter M (2010) Crystallization of a 45 kDa peroxygenase/ peroxidase from the mushroom *Agrocybe aegerita* and structure determination by SAD utilizing only the haem iron. Acta Crystallogr F 66:693–698

Piumi F, Levasseur A, Navarro D, Zhou S, Mathieu Y, Ropartz D, Ludwig D, Faulds CB, Record E (2014) A novel glucose dehydrogenase from the white-rot fungus *Pycnoporus cinnabarinus*: production in *Aspergillus niger* and physicochemical characterization of the recombinant enzyme. Appl Microbiol Biotechnol 98:10105–10118

Poppius-Levlin K, Wang M, Tamminen T, Hortling B, Viikari L, Niku-Paavola ML (1999) Effects of laccase/HBT treatment on pulp and lignin structures. J Pulp Paper Sci 25:90–94

Presley GN, Panisko E, Purvine SO, Schilling JS (2018) Coupling secretomics with enzyme activities to compare the temporal processes of wood metabolism among white and brown rot fungi. Appl Environ Microbiol 84:1–12

Qin X, Luo H, Zhang X, Yao B, Ma F, Su X (2018) Dye-decolorizing peroxidases in Irpex lacteus combining the catalytic properties of heme peroxidases and laccase play important roles in ligninolytic system. Biotechnol Biofuels 11:302–312

Rastogi S, Chaudhary I (2011) Microbial degradation of lignin for enhancing the agroindustrial utility of plant biomass. In: Paterson RJ (ed) Lignin: properties and applications in biotechnology and bioenergy. Nova Science Publishers Inc., New York, pp 307–330

Rastogi S, Dwivedi UN (2006) Down-regulation of lignin biosynthesis in transgenic *Leucaena leucocephala* harboring O-methyltransferase gene. Biotechnol Prog 22:609–616

Rastogi S, Dwivedi UN (2008) Manipulation of lignin in plants with special reference to *O*-methyltransferase. Plant Sci 174:264–277

Rastogi Verma S, Dwivedi UN (2014) Lignin genetic engineering for improvement of wood quality: applications in paper and textile industries, fodder and bioenergy production. South Afr J Bot 91:107–125

Ruiz-Dueñas FJ, Martínez AT (2009) Microbial degradation of lignin: how a bulky recalcitrant polymer is efficiently recycled in nature and how we can take advantage of this. Microb Biotechnol 2:164–177

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

Sanchez C (2009) Lignocellulosic residues: biodegradation and bioconversion by fungi. Biotechnol Adv 27:185–194

Santhanam N, Badri DV, Decker SR, Manter DK (2012) Lignocellulose decomposition by microbial secretions. In: Vivanco MJ, Baluska F (eds) Secretions and exudates in biological systems. Springer, Berlin, pp 125–153

Santos RB, Hart PW, Jameel H, Chang H (2013) Wood based lignin reactions important to the biorefinery and pulp and paper industries. Bioresources 8:1456–1477

Schmidt O (2006) Wood and tree fungi: biology, damage, protection and use. Springer, Berlin

Sigoillot JC, Berrin JG, Bey Lesage-Meessen L, Levasseur A, Lomascolo A, Record E, Uzan-Boukhris E (2012) Fungal strategies for lignin degradation. In: Jouanin L, Lapierre C (eds) Lignins: biosynthesis, biodegradation and bioengineering, vol 61. Academic Press, Elsevier, London, pp 263–308

Waldrop MP, Balser TC, Firestone MK (2000) Linking microbial community composition to function in a tropical soil. Soil Biol Biochem 32:1837–1846

Wang L, Nie Y, Tang Y-Q, Song X-M, Cao K, Sun L-Z, Wang Z-J, Wu X-L (2016) Diverse Bacteria with lignin degrading potentials isolated from two ranks of coal. Front Microbiol 7:1428–1441

Wang X, Ullrich R, Hofrichter M, Groves JT (2015) Heme-thiolate ferryl of aromatic peroxygenase is basic and reactive. Proc Natl Acad Sci U S A 112:3686–3691

Wilhelm RC, Singh R, Eltis LD, Mohn WW (2019) Bacterial contributions to delignification and lignocellulose degradation in forest soils with metagenomic and quantitative stable isotope probing. ISME J 13:413–429

Yamada Y, Wang J, Kawagishi H, Hirai H (2014) Improvement of ligninolytic properties by recombinant expression of glyoxal oxidase gene in hyper lignin-degrading fungus *Phanerochaete sordida* YK-624. Biosci Biotechnol Biochem 78:2128–2133

Zamocky M, Hofbauer S, Schaffner I, Gasselhuber B, Nicolussi A, Soudi M, Pirker KF, Furtmüller PG, Obingera C (2015) Independent evolution of four heme peroxidase superfamilies. Arch Biochem Biophys 574:108–119

Biosorption of Heavy Metals by Cyanobacteria: Potential of Live and Dead Cells in Bioremediation

15

Shachi Singh

Abstract

Biosorption is a physicochemical process that allows certain microorganisms to passively bind contaminants onto their cellular structures. Mostly this process has been applied in the removal of heavy metals from contaminated water bodies. The technique is highly efficient and cost-effective and allows recovery of metal as well as the biomass. Among various organisms used for biosorption of heavy metals, such as bacteria, fungi, algae, higher plants, etc., cyanobacteria hold special position due to their unique structural properties and high metal sorption capabilities. Several cyanobacterial strains as live or dead cells have been used to remove heavy metals from contaminated sites. In view of its importance, this chapter discusses how cyanobacteria have been used in bioremediation of heavy metals. Attempts will be made to highlight the properties of cyanobacteria useful for biosorption and the process parameters responsible for enhancing the metal removal capability. In addition, mechanisms underlying sorption process are discussed.

Keywords

Biosorption · Physicochemical · Heavy metal · Cyanobacteria · Bioremediation

15.1 Introduction

Heavy metal pollution has become one of the most serious concerns in many countries. It arises due to disposal of heavy-metal-containing wastes into the environment, particularly in water bodies (Verma and Dwivedi 2013). Heavy metal wastes are generated by various activities, such as application of pesticides and fertilizers, mining and smelting industries, leather industry, electroplating, chemical

S. Singh (✉)
Botany Section, MMV, Banaras Hindu University, Varanasi, India

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_15

industry, automobile exhausts, etc. (Mohammed et al. 2011). Heavy metals such as lead, copper, cadmium, zinc, chromium, nickel, and mercury are highly toxic to living organisms. They are nonbiodegradable and hence remain in the atmosphere for longer period of time causing higher toxicity. They accumulate inside living organisms through food chain and affect various metabolic processes (Mohammed et al. 2011). All groups of living organisms including human beings, animals, aquatic organisms, plants, and microbes are affected by heavy metal pollution.

Many techniques have been developed to remove heavy metal from the environment, such as reverse osmosis, electrophoresis, ultra-ion exchange, chemical precipitation, membrane filtration, etc. (Barkat 2011). Although these conventional methods are successful in removing heavy metal contamination, a more environment-friendly technique needs to be developed which can generate least toxic wastes. Biosorption seems to be an eco-friendly and cost-effective alternative for removing heavy metal. It is a physicochemical process that uses biological material for removing contaminants. In biosorption, metal ions passively bind to the surface of the biological material through various physicochemical processes, such as adsorption, precipitation, ion exchange, etc. (Gadd 2009; Michalak et al. 2013; He and Chen 2014).

Wide varieties of material are available in nature which had been used for the removal of heavy metals from contaminated sites, such as bacteria, fungi, algae, agricultural wastes, etc. (Wang and Chen 2009). However, in recent years, cyanobacterial-based bioremediation has been in focus for cleanup operations of contaminated sites. Metal removal by these organisms involves a rapid phase of passive surface adsorption followed by energy-dependent slower phase of metal ion transport across cell membrane. Large number of data is available in literatures describing their role in bioremediation; however, it is not possible to discuss each and every aspect of the process. Therefore, in this review, attempts will be made to focus on the surface phenomenon and the factors influencing biosorption by cyanobacteria.

15.2 Potential of Cyanobacteria as Biosorbent

Cyanobacteria are a diverse group of nitrogen fixing, oxygenic photosynthetic prokaryotes that are widely distributed in freshwater, marine, and terrestrial environments (Singh 2014). A significant contribution to metal sorption has been attributed to these organisms since they are abundant in natural environments. Cyanobacteria have some advantages over other microorganisms including their unique cell wall compositions and greater mucilage volume with high binding affinity, large surface area, and simple nutrient requirements (Micheletti et al. 2008a, b). They can be easily cultivated on a large scale providing a low cost biomass for the biosorption process (Pathak et al. 2018). Several cyanobacterial strains as live and dead cells have been used to treat heavy metal pollution through biosorption. Their metal sorption capability was found to be better than many other natural materials used for the removal of heavy metal, such as bacterial or fungal

biomass, agricultural waste, and industrial by-products (Wang and Chen 2009; Kananarlapudi et al. 2018).

Excellent biosorption potential by metabolically active cells of several cyanobacterial strains had been reported. Live cells of *Oscillatoria anguistissima* showed very high capacity for the biosorption of zinc, measuring 641 mg/g of cell dry weight (Ahuja et al. 1999a, b). The biosorption of mercury by two strains of cyanobacteria, *Spirulina platensis* and *Aphanothece flocculosa*, was also very high, showing 456 and 428 mg/g biosorption capability, respectively (Cain et al. 2008). *Nostoc entophytum* ISC32 isolated from oil-contaminated regions in southern Iran was able to absorb 302.91 mg/g of cadmium from aqueous solution. Its metal removal capability increased up to 29% under microgravity conditions (Alidoust et al. 2016). A high copper removal ability through biosorption had been reported for many cyanobacterial strains; they include *Cyanospira capsulata*, 240 mg/g (Paperi et al. 2006); *Cyanothece* 16Som 2, 201 mg/g; *C. capsulata*, 143 mg/g; *Cyanothece* ET 5, 113 mg/g (Micheletti et al. 2008a, b); and *S. platensis*, 67.93 mg/g (Celekli et al. 2010), whereas *Gloeothece* sp. strain PCC 6909 wild type and its sheathless mutant showed 46.3 and 26.7 mg/g of copper removal, respectively (Micheletti et al. 2008a, b). Chromium removal was 196, 95, and 67 mg/g by *Cyanothece* strains16Som 2, CE 4, and TI 4, respectively (Micheletti et al. 2008a, b). *Aphanothece halophytica* could efficiently take up zinc from aqueous solution with a maximum capacity of 133 mg/g (Incharoensakdi and Kitjaharn 2002). *Nostoc commune* also showed good metal removal capability by removing 126.32 and 115.41 mg/g of Cd and Zn, respectively, from aqueous solution (Morsy et al. 2011). *Anabaena sphaerica* maximum biosorption capacities for live cells were 121.95 mg/g for Cd and 111.1 mg/g for Pb (Abdel -Aty et al. 2013), whereas *Fischerella ambigua* ISC67 removed 98.03 mg/g of Zn ions at optimum conditions (Safari, Ahmady-Asbchin 2018). The live cyanobacterium *Nostoc muscorum* Meg 1 isolated from a rice field of Meghalaya, India, receiving contaminated water from coal mine effluents, removed 71.4 mg/g of Cd (Ahad et al. 2017). *Microcystis* and *Synechocystis* cells were good adsorbents for antimony (Sb) in the form of anion Sb $(OH)_6^-$ and cation Sb(III) with a sorption capacity of 4.68 mg/g shown by *Synechocystis* (Sun et al. 2011; Zhang et al. 2011). Two marine cyanobacteria-unicellular Synechococcus elongatus BDU 75042 and filamentous Anabaena torulosa, were found to remove 53.5 and 77.35 mg/g of uranium respectively (Acharya and Apte 2012). The effectiveness of *Phormidium* as an adsorbent for rare earth metals had been tested. The rare earth elements (REEs) include 15 lanthanide elements ($Z = 57–71$) and yttrium ($Z = 39$). Their adsorption density was found to be La, 375 mg/kg; Pr, 57 mg/kg; Nd, 217 mg/kg; Sm, 35 mg/kg; Gd, 20 mg/kg; and Dy, 14 mg/kg (Kim et al. 2011). *Lyngbya taylorii* showed maximum metal removal capacities of 1.47 mmol Pb, 0.37 mmol Cd, 0.65 mmol Ni, and 0.49 mmol Zn per gram of dry biomass (Klimmek & Stan). Efficient removal of Cr from tannery effluent by live cells of *Arthrospira platensis* (Pandi et al. 2009) and from retan chrome liquor by *Spirulina fusiformis*, Zn and Ni removal by *S. maxima* (Balaji et al. 2015), and tolerance of Zn, Cd, and Pb by *Phormidium uncinatum* (Audiiolia et al. 1993) indicate their role in wastewater treatment.

Living cyanobacterial biomass had been effective in biosorption of heavy metals; however, in many cases, dead cells have shown to be equally important. Dead cells are less affected by the toxic ions; in addition, they require little care and maintenance. Maximum uptake of Cd was 44.56 mg/g in living cells of *Spirulina platensis* (Murugesan et al. 2008), whereas dry biomass of *Spirulina platensis* was able to remove 73.64 mg/g of Cd (Celekli and Bozkurt 2011) and 357 mg/g as reported by Solisio et al. (2008), indicating higher capability of dead cells to remove metals. Dried *Spirulina* biomass was also shown to remove 90.91 mg/g of Cr as reported by Rezaei (2016) and 41.12 mg/g of Cr as reported by WonKwak et al. (2015), 69.04 mg/g of Ni (Celekli and Bozkurt 2011), 48.21 mg/g of Pb, and 8.75 mg/g of Zn (Aneja et al. 2010). Adsorption capacities of dried biomass of *Nostoc sphaeroides* were greater for Cr than fresh macrocolonies (Jiang et al. 2016). Dried biomass of *Nostoc muscorum* removed 22.92 mg/g of Cr (Gupta and Rastogi 2008). *Nostoc linckia* also showed good biosorption of Cr from wastewater of galvanic industry (Zinicovscaia et al. 2014). When compared to live controls, autoclaving improved the binding capacities of *Anabaena* sp. (Coder and Reeves 1994).

Biosorption studies of immobilized cell have also shown good results. The biosorption of Cd by immobilized *Spirulina platensis* TISTR 8217 on alginate gel and silica gel showed maximum adsorption of 70.92 and 36.63 mg Cd/g biomass, respectively (Rangsayatorn et al. 2004). Biosorption capacity determined for the immobilized *Anabaena* sp. ATCC 33047 system was as high as 162 mg Cd per gram dry biomass, entrapped within the reticulate network of polyurethane foam disks (Clares and Guerrero 2015). Immobilized biomass of *Microcystis aeruginosa* showed good result with Cu (Pradhan and Rai 2001).

15.3 Cell Surface Chemistry and Metal Binding

The cyanobacterial surfaces are complex structures containing distinct surface layers, each with unique metal binding properties. A cell wall capable of passively adsorbing high amounts of solubilized metals is present; in addition to that, many cyanobacterial species surround their cell walls with a polysaccharide sheath, which adds on additional metal binding property (Fiore and Trevors 1994; Hoiczyk and Hansel 2000). The role of cyanobacterial surfaces in metal binding was demonstrated by various workers. El-Enany and Issa (2000) showed that about 65–60% of Cd or Zn was distributed on the cell surface of *Nostoc linckia* and *Nostoc rivularis*, whereas 91% of the total Cd removed *by Nostoc muscorum* was found to be adsorbed on the cell surface, describing their role in metal binding (Ahad et al. 2017). Similarly, when the cells of *Aphanothece halophytica* with the adsorbed zinc were treated with EDTA, it was found that less than 10% of zinc remained associated with the cells, suggesting that the uptake of zinc is ascribed to cell surface binding of the metal (Incharoensakdi and Kitjaharn 2002).

The cell surface chemistry plays an important role in the biosorption mechanism. The affinity for metal ions to bind onto cyanobacterial surfaces comes from the presence of proton-active surface enriched with various functional groups on the cell wall and exopolymer sheath (Fiore and Trevors 1994). These functional groups can deprotonate and bind metal ions to form stable ligand-metal surface complexes. Infrared (IR) spectra for intact *Calothrix* filaments and separated exopolymeric sheath material indicated that the reactive sites on *Calothrix* surfaces were heterogeneously distributed between the exopolymer sheath and cell wall (Yee et al. 2004). Concentration of proton-active surface sites was found to be higher on the cell wall as compared to the overlying sheath. Sorption experiments done with Cu, Cd, and Pb ions demonstrated that the carboxyl groups on the cyanobacterial cell wall were the dominant sink for metals (Yee et al. 2004). Peptidoglycan isolated from cell walls of the cyanobacterium *Anabaena flos-aquae* demonstrated binding of Cu(II) by amine and carboxyl ligands, whereas whole cell fractions containing the exopolymer sheath were shown to bind Cu(I) as well as Cu(II) by phosphate ligands (Kretschmer et al. 2004). IR analysis of *Microcystis* cells confirmed the presence of a large number of -COO(−) and some amino groups on the cell wall, where oxygen and nitrogen donor atoms were found to play a vital role in biosorption of Fe, Ni, and Cr (Prahan et al. 2007). Adsorption study for uranium with the marine unicellular cyanobacterium *Synechococcus elongatus* BDU130911 showed 92%, 85%, and 20% adsorption of uranium in the control, amine-blocked treatments, and carboxyl-blocked treatments, respectively, indicating the role of carboxylic group in uranium binding (Vijayaraghavan et al. 2018). The ATR-IR spectra confirmed the involvement of amino, carboxyl, and hydroxyl groups in the removal of $Sb(OH)_6^-$ by *Microcystis* (Sun et al. 2011). *Spirulina* sp. revealed the presence of hydroxyl, amino, carboxylic, and carbonyl groups involved in the biosorption of Cr(IV) ions (Rezaei 2016). The process of Cr(III) removal by *Spirulina* sp. was hindered when carboxyl and phosphate groups were esterified, pointing out the important role of these groups in the biosorption process (Chojnacka et al. 2005). Similarly, chemical modifications of functional groups after methanol esterification caused a decrease in adsorption of Cu and Cd by *S. platensis*, demonstrating the role of carboxyl groups in metal binding (Fang et al. 2011). Carboxylic acid was also shown to be the main functional group responsible for metal binding in *Phormidium valderianum* and *Synechococcus* sp. PCC 7942 (Gardea-Torresdey et al. 1998; Karna et al. 1999). Amino and hydroxyl groups were shown to play an important role in the binding of Pb by *Spirulina maxima* (Gong et al. 2005). EDX and FTIR analyses confirmed participation of hydroxyl, carbonyl, carboxyl, and phosphate groups in biosorption of Cd onto the cell surfaces of *Nostoc muscorum* (Ahad et al. 2017), whereas amide, hydroxyl, C=O, and C–O groups are involved in the sorption of Cr(VI) ions (Gupta and Rastogi 2008). Amino, carboxyl, hydroxyl, and carbonyl groups present on the cell surface were shown to participate in the biosorption of Cd and Zn by *Nostoc commune* (Morsy et al. 2011) and Cd and Pb by *Anabaena sphaerica* (Abdel-Aty et al. 2013), whereas carboxyl, hydroxyl, sulfite, and amino groups are likely responsible for the biosorption of Zn by *Fischerella ambigua* (Safari, Ahmady-Asbchin 2018).

Studies have shown that sheaths of cyanobacteria consist mainly of polysaccharides composed of at least one uronic acid and several neutral sugars with various negatively charged groups, such as sulfate, phosphate, and carboxylate, providing binding sites for cations (Fiore and Trevors 1994). In *Microcystis flos-aquae* slime, galacturonic acid was predominant, and it was suggested that charge attraction to carboxyl groups contributed to Fe binding (Plude et al. 1991). Isolated sheaths of two filamentous cyanobacteria, *Calothrix parietina* and *Calothrix scopulorum*, composed of neutral sugar, amino acids, and small amounts of glucosamine and galacturonic acid, showed binding of heavy metals, such as copper, cobalt, iron, zinc, nickel, manganese, and molybdenum (Weckesser et al. 1988). Galacturonic and glucuronic acids present in the exopolysaccharide sheath were involved in the removal of Cr by *Synechocystis* (Ozturk et al. 2009). The cyanobacterium *Gloeothece* sp. and its sheathless mutant when tested for their abilities to remove Cu ions revealed that the mutant was more effective in the removal of the heavy metal than the wild type (Micheletti et al. 2008a, b). Although the mutant does not possess a sheath, it released large amounts of polysaccharidic material into the culture medium. The released polysaccharides of the wild type and the mutant were composed of the same 11 sugars, although in different amounts, and the metal ions were preferably bound to the cell wall and to release polysaccharide functional groups (carboxyl and amide groups) rather than to the sheath (Micheletti et al. 2008a, b). Isolated sheath of Gloeothece sp. have also shown to bind metal (Tease and Walker 1987).

15.4 Mechanism of Biosorption

Binding of metal in biosorption is a passive process that does not involve any requirement of energy (Michalak et al. 2013). *N. muscorum* when incubated with Cd in light and dark demonstrated an equal metal removal capability (Ahad et al. 2017). Further when photosynthetic electron transport chain, a process that provides energy in the form of ATP, was blocked by DCMU, cells were again able to remove almost similar amount of Cd (Ahad et al. 2017). This process was further explained by using glucose as a source of energy; its addition did not appear to increase the amount of zinc taken up by *A. halophytica*; moreover, the presence of carbonyl cyanide *m*-chlorophenylhydrazone, an uncoupler, or *N*,*N*′-dicyclohexylcarbodiimide, an inhibitor of ATPase, hardly affected Zn uptake by the cells (Incharoensakdi and Kitjaharn 2002). *Oscillatoria* sp. H1 when used for the removal of Cd ions as its dry biomass, alive and heat-inactivated immobilized form showed almost similar absorption capability indicating removal as surface phenomenon (Katırcıoğlu et al. 2008).

Adsorption, chelation, ion exchange, and surface precipitation are different processes reportedly involved in biosorption (Bilal et al. 2018). Among them, ion exchange seems to be the principal mechanism of biosorption in cyanobacteria, involving different functional groups present on the cell surface. Biosorption studies of Cr, Cd, and Cu ions by the cyanobacteria *Spirulina* showed that the process

equilibrium was reached quickly in less than 5–10 min, suggesting that the mechanism of biosorption is rather chemisorption than physical adsorption and was further confirmed by the low surface area associated with physical adsorption and by the presence of cations that appeared in the solution after biosorption (Chojnacka et al. 2005). The biosorption of Co also showed that it is an ion-exchange process, as the Co binding was accompanied by the release of large amounts of Mg ions in the solution containing *O. anguistissima* cells (Ahuja et al. 1999a, b). Cell surface study by scanning electron microscopy and surface area measurement of the biomass of *Spirulina*, *Microcystis*, and several other cyanobacterial strains further demonstrated that physical adsorption was not the dominant mechanism of biosorption (Fang et al. 2011). As discussed above, cyanobacteria cell walls are polyanionic in nature and effectively act as cation exchangers due to the presence of various negatively charged functional groups present on the cell surface.

15.5 Factors Affecting Biosorption

15.5.1 Effect of pH

The pH of the solution is an important factor since it influences solubility, total charge of the biosorbent, as well as metal speciation (Bilal et al. 2018). At low pH, the H^+ ions are closely associated with the active ligands of the biosorbent, and therefore, there exists a competition between the protons and metal ions for the binding sites, whereas at higher pH, there exists lower number of H^+ ions, and the number of active sites of the negatively charged functional groups is free and exposed resulting in increased biosorption of positively charged metal ions. At a very high pH, the metal might begin to precipitate and can form hydroxides, thereby hindering the biosorption process (Bilal et al. 2018). The observed low Zn biosorption by *A. halophytica* at acidic pH was likely owing to the strong competition from hydrogen ions for binding sites on the cell surface; increasing the pH further to 6.5 and 7 resulted in a progressive linear increase in metal adsorption (Incharoensakdi and Kitjaharn 2002). Biosorption study of *Nostoc muscorum* revealed that an increase in the pH from 3 to 8 increased the metal removal capacity of the biomass and reached a plateau around pH 5 and 6 for both Pb and Cd, respectively (Dixit and Singh 2013). Gupta and Rastogi (2008) observed an increase in the biosorption of Pb in *Oedogonium* sp. by increasing pH. The Cu loading level was 30 times higher in the cell wall sample prepared at pH 5 than in the sample prepared at pH 2 in *Anabaena flos-aquae*, clearly indicating a pH-dependent ion-exchange process involved in the binding (Kretschmer et al. 2004). Optimum pH for Cd removal by *M. aeruginosa* was observed to be 7, and values below and above 7 were found to cause a decrease in metal binding property of the cell (Rzymski et al. 2014). The adsorption of Cu by *S. platensis* increased with an increase in pH from 5 to 7, whereas highly alkaline pH conditions (pH 9 and above) led to a decline in adsorption efficiency (Al-Homaidan et al. 2014). Similarly, adsorption of Cd by dry biomass of *S. platensis* also increased from pH 3 to pH 8,

and there was a decrease in the uptake of Cd at alkaline pH 9, which could be attributed to the formation of a metal complex such as Cd-OH that might have reduced the availability of Cd for adsorption (Rangsayatorn et al. 2004).

Optimum pH varies depending upon the chemical property of the metal. Maximum biosorption of Pb and Cd occurred at pH 5 and 6 by *Nostoc muscorum* (Dixit and Singh 2013) and at pH 3 and 5.5 by *Anabaena sphaerica* (Abdel -Aty et al. 2013). This difference occurred because Pb has higher electronegativity than Cd and hydronium ion; therefore, its affinity to the surface functional groups is higher at low pH value (Lou et al. 2018). A similar situation occurring in *Spirulina* sp. where the maximum biosorption of Pb was at pH 4 and 10 while Zn at pH 8 and 10 (Aneja et al. 2010) also reflects that the adsorption pH depends on the chemical nature of the metal.

15.5.2 Effect of Temperature

An increase or decrease in temperature causes a change in the biosorption capacity of the biosorbent. High temperatures enhance the biosorptive removal of metals by increasing their kinetic energy, but are also associated with structural damage of the biomass, thus limiting the process (Bilal et al. 2018). Gradual increase in temperature from 10 to 40 °C led to corresponding increase in the absorption of Pb and Cd by *N. muscorum*; however, further rise in the temperature above 40 °C resulted into decline in the absorption of both the metals (Dixit and Singh 2013). The findings of *Spirulina* indicate that the optimum temperature for Cr uptake was 40 °C (Rezaei 2016), for Cu, 37 °C (Al-Homaidan et al. 2014), and for Cd, 26 °C (Al-Homaidan et al. 2015). Similarly, the highest biosorption efficiency of Cd by *Aphanothece* sp. was attained at 30 °C, while the lowest efficiency was found at 47 °C, by either the damage of active binding sites in the biomass or desorption of metal ions by high temperature due to weakening of binding forces (Awalina et al. 2017).

15.5.3 Effect of Initial Metal Concentration

The mass transfer resistance between the liquid and solid phases can be overcome by the initial concentration of metal ion. It has been observed that the biosorption capacity of the biosorbent increases with the increase in metal ion concentration and then reaches a saturation value. This occurs because initially all the binding sites are free and completely available for interaction with the metal ions; however, when the metal concentration increases, most of binding sites are occupied and are not available for further binding, thereby halting the sorption process (Michalak et al. 2013). The amount of Cd removed was found to increase with an increase in the initial metal concentration from 0.5 to 3 ppm. Beyond 3 ppm, there was no further increase in the process because all the binding sites on the cell surface were occupied, reaching saturation limit (Ahad et al. 2017). With an increase in concentration of Cu from 10 to 100 mg/L, there was a gradual increase in adsorption from

47.65 to 90.61% by *S. platensis*; however, further increase above 150 mg/L led to a decline indicating saturation of all the binding sites (Al-Homaidan et al. 2014). A consistent decrease in metal removal was observed for Pb and Zn by *Spirulina* after 40 mg/L metal solution (Aneja et al. 2010). Optimum metal concentration for the maximum sorption of Cd and Pb was 60 and 80 μg/mL, respectively, by *Nostoc muscorum* (Dixit and Singh 2013). About 0.2 g Cd was retained per g dry weight of *Anabaena* biomass, regardless of the initial concentration of the ion (Clares and Guerrero 2015).

15.5.4 Effect of Biosorbent Concentration

It has been observed that an increase in cyanobacterial biomass is associated with an increase in the biosorption process. It occurs because larger surface area generates more available metal binding sites (Michalak et al. 2013). An increase in the dose of adsorbent from 0.7 to 2.8 g/L resulted into an increase in the Pb and Cd removal by *N. muscorum*; however, a further increase did not exhibit any significant effect on the metal removal (Dixit and Singh 2013). Biosorption efficiency of Cd and Pb ions by *A. sphaerica* was significantly increased with subsequent increase in the biosorbent dose and almost became constant at higher dosage than 0.1 g/100 mL and 0.2 g/100 mL for Pb and Cd, respectively (Abdel -Aty et al. 2013). The removal of Zn from aqueous solution by *A. halophytica* was increased as the cell concentration increased up to 0.2 g/L (Incharoensakdi and Kitjaharn 2002). Optimum biomass dose for removal of Cd by *Anabaena* was 0.445 g/L (Clares and Guerrero 2015), whereas 2 g of *S. platensis* was sufficient to get excellent Cd removal when the initial metal concentration was 100 mg/L (Al-Homaidan et al. 2015). The optimum dose of *S. platensis* for maximal removal of copper ions was found to be 0.050 g/100 mL, and adsorption declined with a further increase in biomass concentration from 0.05 g/100 mL (Al-Homaidan et al. 2014). It is likely that higher cell concentration might lead to the formation of cell aggregates, thereby reducing the effective biosorption area.

15.5.5 Effect of Contact Time

Initially, all the binding sites are vacant and hence freely available for the metals to bind; therefore, the rate of biosorption is very rapid initially. With an increase in time, the rate of biosorption decreases due to a decrease in the available free binding sites (Michalak et al. 2013). The uptake of Cd by immobilized *Anabaena* sp. ATCC 33047 was very fast in the beginning, with 65% of total Cd being removed from the solution within the first 5 min of incubation, followed by a slower phase (Clares and Guerrero 2015). *Spirulina* sp. exhibited rapid biosorption in first 15 min by removing 90% Pb and 89% of Zn from metal solutions; thereafter, increase in metal removal was marginal (Aneja et al. 2010). The biosorption study of Cd and Pb ions using *A. sphaerica* biomass indicated that the biosorption of both metals was

rapid in the first 20 min and then was gradually increased till the equilibrium was attained at 60 and 90 min for Cd and Pb, respectively (Abdel -Aty et al. 2013). It was noted that the rate of adsorption of Cd by *S. platensis* was very rapid during the first 30 min (Al-Homaidan et al. 2015); the maximum adsorbed amount of Cr ions by *Spirulina* was achieved within 60 min (Rezaei 2016) and Cu ions within the first 90 min (Al-Homaidan et al. 2014) and then was followed by a longer equilibrium period. The difference in the rate of metal sorption could be explained by the difference in the type of metal and variation in the cell surface chemistry of different cyanobacterial strains.

15.5.6 Effect of Cations and Anions on the Metal Removal

Studies conducted to demonstrate the effect of various cations and anions on adsorption of Pb and Cd by *Nostoc muscorum* showed that the cations Na^+, K^+, and Mg^{2+} had little effect; in contrast, the absorption percentage of both metals was significantly reduced in the presence of Ca^{2+} ion (Dixit and Singh 2013). Reduction might be due to its competition with these metals for the binding sites. The presence of Mg^{2+} and Ca^{2+} ions has shown to decline the Cu and Co adsorption capacity of *Oscillatoria anguistissima* cells (Ahuja et al. 1997, 1999b). Mg^{2+} or Ca^{2+} ions have also been observed to reduce copper removal capability of *Cyanospira capsulata* and *Nostoc* PCC7936 (De Philippis et al. 2003). The inhibitory effect of Ca on Zn biosorption has been reported for a freshwater cyanobacterium *Oscillatoria anguistissima* (Ahuja et al. 1999a). Calcium along with Hg and Pb also inhibited zinc adsorption by *Aphanothece halophytica* (Incharoensakdi and Kitjaharn 2002). Among the various anions tested, EDTA caused a significant decrease in the removal of Pb and Cd by *Nostoc muscorum* (Dixit and Singh 2013). The effect of EDTA could be interpreted as relatively stronger binding of Pb and Cd with EDTA than the metal binding sites on the cell surface. Similarly, sulfate and nitrate (0. 75–10 mM) were found to drastically reduce the extent of Co biosorption in *O. anguistissima* (Ahuja et al. 1999b).

15.5.7 Effect of Desorbing Agents on Metal Removal and Reusability of the Biomass

Maximum desorption of Pb and Cd was achieved in the presence of EDTA and HNO_3, respectively, by *N. muscorum* and could be repeatedly used up to six biosorption/desorption cycles without significant loss of its initial metal adsorption capacity (Dixit and Singh 2013). Similarly, HNO_3 and EDTA also caused 80% recovery of Cr from *N. muscorum* and were reused in five biosorption–desorption cycles (Gupta and Rastogi 2008). Chromium could also be recovered from *Spirulina* biomass using HNO_3 or NaOH (Rezaei 2016). The successive recycling of the adsorption–desorption process by *Spirulina* for Cd removal was stable for more than five cycles (Rangsayatorn et al. 2004). Copper was desorbed by distilled water

at pH 2 from *Microcystis* biomass (Pradhan and Rai 2000), and it was observed that immobilized *M. aeruginosa* could be used for 10 cycles of adsorption–desorption cycle (Pradhan and Rai 2001). Wang et al. (2010) demonstrated that HNO_3 was a better desorbing agent than HCl, EDTA, and citric acid to remove Cu from cyanobacterial bloom containing *Microcystis* as a major constituent. HCl and NaOH effectively removed copper ions trapped in the biomass of *Cyanospira capsulata* (Paperi et al. 2006). High acidic or alkaline conditions favor desorption by weakening the electrostatic force of attraction between metal and the functional groups (Bilal et al. 2018). Some salts, such as Na_2CO_3, have also shown to cause efficient desorption of Co from the biomass of *O. anguistissima* (Ahuja et al. 1999b).

15.5.8 Effect of Multi-metals on the Metal Removal Efficiency

In multi-metal systems, generally it has been observed that the interaction is competitive, where the metal ions already sorbed on the biomass exert a strong hindrance to the access of other ions at the adjacent adsorption sites. However, in some cases, a noninteractive or synergistic behavior could also be observed. The presence of binary metals, Pb and Cd, showed that one metal ion resulted into decreased sorption of other metal ion by *Nostoc muscorum*, and it was observed that Pb caused inhibition of Cd sorption (Dixit and Singh 2013). Studies with *Lyngbya taylorii* also indicated a preference for the uptake of lead over cadmium, nickel, and zinc in a four-metal solution (Klimmek and Stan 2001). Lead was also shown to be sequestered preferentially over Cd by *Microcystis aeruginosa* (Rzymski et al. 2014). Similarly, *Synechococcus* sp. PCC 7942 exhibited a nearly threefold higher bound rate for Pb than for Cd, requiring significantly shorter contact time for effective metal removal (Rzymski et al. 2014). Micheletti et al. (2008a, b) tested nine exopolysaccharide-producing cyanobacterial strains for their ability to remove Cr, Cu, and Ni in a multiple-metal solution. It was observed that in the strain *Cyanothece* VI 13, the presence of the three metals caused a drastic reduction in the sorption of Ni and all the binding sites were occupied by the two other metals. An antagonistic action was observed between metal ions in *C. capsulata* and *Cyanothece* strains CE 4, ET 5, and VI 22 indicating a competitive interaction among the metals for the binding sites on the biomass. *N. linckia* accumulated about 30-fold of Zn and tenfold of Cd, whereas *N. rivularis* accumulated about tenfold of Zn and twofold of Cd when added together showing preference of one metal over the other (El-Enany and Issa 2000). *Nostoc sphaeroides* adsorption properties of Cu, Cd, Cr, Pb, Ni, and Mn also showed that coexisting ions significantly decreased the metal adsorption capacity (Jiang et al. 2016). *Microcystis* when grown under continuous culture in the presence of Cr(VI), Cd, and Cu as mixtures of two or three metals showed that Cr and Cd had a positive interaction that increased the removal percentages of both these metals (Rai and Tripathi 2007). The difference in sorption dynamics and the biosorbent capacity for different metals has been explained in terms of the difference in the ionic size of metals, ionic radii, electrode potential, and affinity to the functional groups on the biosorbents.

15.6 Conclusion

The above review highlights the potential of cyanobacteria in removing heavy metal contamination. It can be clearly seen that a wide variety of cyanobacterial strains as live and dead cells have the capability to remove metal ions in high concentrations. Metal sorption efficiency varies depending upon the cell surface chemistry of the cyanobacterial strain and the chemical property of the metal ion chosen. The method is safe and more environment friendly as compared to the available chemical techniques and hence can easily be developed into industrial product for the treatment of contaminated sites. Process parameters such as pH, temperature, contact time, concentration of metal ions, biomass, etc., can be adjusted to achieve maximum metal removal.

References

Abdel -Aty AM, Ammar NS, Ghafar HHA, Ali RK (2013) Biosorption of cadmium and lead from aqueous solution by fresh water alga *Anabaena sphaerica* biomass. J Adv Res 4:367–374

Acharya C, Apte SK (2012) Marine cyanobacteria as suitable candidates for uranium recovery from aquatic environment. In: Indo-US Workshop on Cyanobacteria, Lonavala

Ahad RIA, Goswami S, Syiem MB (2017) Biosorption and equilibrium isotherms study of cadmium removal by *Nostoc muscorum* Meg 1: morphological, physiological and biochemical alterations. 3 Biotech 7:104–126

Ahmady-Asbchin S (2018) Biosorption of zinc from aqueous solution by cyanobacterium *Fischerella ambigua* ISC67: optimization, kinetic, isotherm and thermodynamic studies. Water Sci Technol 78:1525–1534

Ahuja P, Gupta R, Saxena RK (1997) *Oscillatoria anguistissima*: a promising Cu^{2+} biosorbent. Curr Microbiol 35:151–154

Ahuja P, Gupta R, Saxena RK (1999a) Sorption and desorption of cobalt by *Oscillatoria anguistissima*. Curr Microbiol 39:49–52

Ahuja P, Gupta R, Saxena RK (1999b) Zn^{2+} biosorption by *Oscillatoria anguistissima*. Process Biochem 34:77–85

Al-Homaidan A, Al-Houri HJ, Al-Hazzani AA, Elgaaly G, Moubayed NMS (2014) Biosorption of copper ions from aqueous solutions by *Spirulina platensis* biomass. Arab J Chem 7:57–62

Al-Homaidan AA, Alabdullatif JA, Al-Hazzani AA, Al-Ghanayem AA, Alabbad AF (2015) Adsorptive removal of cadmium ions by *Spirulina platensis* dry biomass. Saudi J Biol Sci 22:795–800

Alidoust L, Soltani N, Modiri S, Haghighi O, Azarivand A, Khajeh K, Zahiri HS, Vali H, Noghabi KA (2016) Cadmium uptake capacity of an indigenous cyanobacterial strain, *Nostoc entophytum* ISC32: new insight into metal uptake in microgravity-simulating conditions. Microbiology 162:246–255

Aneja RK, Chaudhary G, Ahluwalia SS, Goyal D (2010) Biosorption of Pb^{2+} and Zn^{2+} by non-living biomass of *Spirulina* sp. Indian J Microbiol 50:438–442

Audiiolia S, Goyal D, Saxena RK (1993) Zinc tolerance in *Phormidium uncinatum*. Folia Microbiol 38:341–344

Awalina HA, Haryani GS, Setiadi T (2017) Equilibrium and kinetic modelling of cadmium (II) biosorption by Dried Biomass Aphanothece sp. From aqueous phase. Conf Ser Earth Environ Sci 65:1–13

Balaji S, Kalaivani T, Rajasekaran C, Siva R, Shalini M, Das R, Madnokar V, Dhamorikar P (2015) Bioremediation potential of *Arthrospira platensis* (*Spirulina*) against chromium(VI). CLEAN Soil Air Water 43:1018–1024

Barakat MA (2011) New trends in removing heavy metals from industrial wastewater. Arab J Chem 4:361–377

Bilal M, Rasheed T, Sosa-Hernández JE, Raza A, Nabeel F, Iqbal HMN (2018) Biosorption: an interplay between marine algae and potentially toxic elements—a review. Mar Drugs 65:1–16

Cain A, Vannela R, Woo LK (2008) Cyanobacteria as a biosorbent for mercuric ion. Bioresour Technol 99:6578–6586

Celekli A, Bozkurt H (2011) Bio-sorption of cadmium and nickel ions using *Spirulina platensis*: kinetic and equilibrium studies. Desalination 275:141–147

Çelekli A, Yavuzatmaca M, Bozkurt H (2010) An eco-friendly process: predictive modelling of copper adsorption from aqueous solution on *Spirulina platensis*. J Hazard Mater 173:123–129

Chojnacka K, Chojnacki A, Górecka H (2005) Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue–green algae *Spirulina* sp.: kinetics, equilibrium and the mechanism of the process. Chemosphere 59:75–84

Clares ME, Guerrero MG (2015) Garcı'a-Gonza'lez M (2015). Cadmium removal by *Anabaena* sp. ATCC 33047 immobilized in polyurethane foam. Int J Environ Sci Technol 12:1793–1798

Corder SL, Reeves M (1994) Biosorption of nickel in complex aqueous waste streams by cyanobacteria. Appl Biochem Biotechnol 45:847–859

De Philippis R, Paperi R, Sili C, Vincenzini M (2003) Assessment of the metal removal capability of two capsulated cyanobacteria, *Cyanospira capsulata* and *Nostoc* PCC7936. J Appl Phycol 15:155–161

Dixit S, Singh DP (2013) Phycoremediation of lead and cadmium by employing *Nostoc muscorum* as biosorbent and optimization of its biosorption potential. Int J Phytoremediation 15:801–813

El-Enany AE, Issa AA (2000) Cyanobacteria as a biosorbent of heavy metals in sewage water. Environ Toxicol Pharmacol 8:95–101

Fang L, Zhou C, Cai P, Chen W, Rong X, Dai K, Liang W, Gu J, Huang Q (2011) Binding characteristics of copper and cadmium by cyanobacterium *Spirulina platensis*. J Hazard Mater 190:810–815

Fiore MF, Trevors JT (1994) Cell composition and metal tolerance in cyanobacteria. Biometals 7:83–103

Gadd GM (2009) Biosorption: critical review of scientific rationale environmental importance and significance for significance for pollution treatment. J Chem Biotechnol 84:13–28

Gardea-Torresdey JL, Arenas JL, Webb R, Tiemann KJ (1998) Effects of carboxyl group esterification on metal binding ability of *Synechococcus* sp. PCC 7942 (Cyanobacteria). In: Proceedings of the joint conference on the environment. Kansas State University, Manhattan, pp 105–109

Gong R, Ding Y, Liu H, Chen Q, Liu Z (2005) Lead biosorption and desorption by intact and pretreated *Spirulina maxima* biomass. Chemosphere 58:125–130

Gupta VK, Rastogi A (2008) Sorption and desorption studies of chromium(VI) from nonviable cyanobacterium *Nostoc muscorum* biomass. J Hazard Mater 154:347–354

He J, Chen JP (2014) A comprehensive review on biosorption of heavy metals by algal biomass: materials, performances, chemistry, and modeling simulation tools. Bioresour Technol 160:67–78

Hoiczyk E, Hansel A (2000) Cyanobacterial cell walls: news from an unusual prokaryotic envelope. J Bacteriol 182:1191–1199

Incharoensakdi A, Kitjaharn P (2002) Zinc biosorption from aqueous solution by a halotolerant cyanobacterium *Aphanothece halophytica*. Curr Microbiol 45:261–264

Jiang J, Zhang N, Yang X, Song L, Yang S (2016) Toxic metal biosorption by macrocolonies of cyanobacterium *Nostoc sphaeroides* Kützing. J Appl Phycol 28:2265–2277

Kananarlapudi SLRK, Chintalpudi VK, Muddada S (2018) Application of biosorption for removal of heavy metals from wastewater. In: Derco J, Varna B (eds) Biosorption, pp 69–116

Karna RR, Uma L, Subramanian G, Mohan PM (1999) Biosorption of toxic metal ions by alkali-extracted biomass of a marine cyanobacterium, *Phormidium valderianum* BDU 30501. World J Microbiol Biotechnol 15:729–732

Katırcıoğlu H, Aslım B, Türker AR, Atıc T, Beyat Y (2008) Removal of cadmium(II) ion from aqueous system by dry biomass, immobilized live and heat-inactivated *Oscillatoria* sp. H1 isolated from freshwater (Mogan Lake). Bioresour Technol 99:4185–4191

Kim J, Dodbiba G, Tanimura Y, Mitsuhashi K, Fukuda N, Okaya K, Matsuo S, Fujita T (2011) Leaching of rare-earth elements and their adsorption by using blue-green algae. Mater Trans 52:1799–1806

Klimmek S, Stan HJ (2001) Comparative analysis of the biosorption of cadmium, lead, nickel, and zinc by algae. Environ Sci Technol 35:4283–4288

Kretschmer XC, Meitzner G, Gardea-Torresdey JL, Webb R (2004) Determination of Cu environments in the cyanobacterium *Anabaena flos-aquae* by X-ray absorption spectroscopy. Appl Environ Microbiol 70:771–780

Lou H, Cao X, Yan X, Wang L, Chen Z (2018) Adsorption performance of Cd(II), Cr(III), Cu(II), Ni(II), Pb(II) and Zn(II) by aminated solution-blown polyacrylonitrile micro/nanofibers. Water Sci Technol 2017:378–389

Michalak I, Chojnacka K, Witek-Krowiak A (2013) State of the art for the biosorption process—a review. Appl Biochem Biotechnol 170:1389–1416

Micheletti E, Colica G, Viti C, Tamagnini P, De Philippis R (2008a) Selectivity in the heavy metal removal by exopolysaccharide-producing cyanobacteria. J Appl Microbiol 105:88–94

Micheletti E, Pereira S, Mannelli F, Moradas-Ferreira P, Tamagnini P, De Philippis R (2008b) Sheathless mutant of cyanobacterium *Gloeothece* sp. strain PCC 6909 with increased capacity to remove copper ions from aqueous solutions. Appl Environ Microbiol 74:2797–2804

Mohammed AS, Kapri A, Goe R (2011) Heavy metal pollution: source, impact, and remedies. In: Khan MS, Zaidi A, Goel R, Musarrat J (eds) Biomanagement of metal-contaminated soils, pp 1–28

Morsy FM, Hassan SHA, Koutb M (2011) Biosorption of cd(II) and Zn(II) by *Nostoc commune*: isotherm and kinetics studies. CLEAN-Soil, Air, Water 36:680–687

Murugesan AG, Maheswari S, Bagirath G (2008) Biosorption of cadmium by live and immobilized cells of *Spirulina Platensis*. Int J Environ Res 2:307–312

Ozturk S, Aslim B, Suludere Z (2009) Evaluation of chromium (VI) removal behavior by two isolates of Synechocystis sp. in terms of exopolysaccharide (EPS) production and monomer composition. Bioresour Technol 100:5588–5593

Pandi M, Shashirekha V, Swamy M (2009) Bioabsorption of chromium from retan chrome liquor by cyanobacteria. Microbiol Res 164:420–428

Paperi R, Micheletti E, De Philippis R (2006) Optimization of copper sorbing–desorbing cycles with confined cultures of the exopolysaccharide-producing cyanobacterium *Cyanospira capsulata*. J Appl Microbiol 101:1351–1356

Pathak J, Rajneesh MPK, Singh SP, Häder DP, Sinha RP (2018) Cyanobacterial farming for environment friendly sustainable agriculture practices: innovations and perspectives. Front Environ Sci 6:1–13

Plude JL, Parker DL, Schommer OJ, Timmerman RJ, Hagstrom SA, Joers JM, Hnasko R (1991) Chemical characterization of polysaccharide from the slime layer of the cyanobacterium *Microcystis flosaquae* C3-40. Appl Environ Microbiol 57:1696–1700

Pradhan S, Rai LC (2000) Optimization of flow rate, initial metal ion concentration and biomass density for maximum removal of Cu2+ by immobilized Microcystis. World J Microbiol Biotechnol 16:579–584

Pradhan S, Rai LC (2001) Copper removal by immobilized Microcystis aeruginosa in continuous flow columns at different bed heights: study of the adsorption/desorption cycle. World J Microbiol Biotechnol 17:829–832

Prahan S, Singh S, Rai LC (2007) Characterization of various functional groups present in the capsule of Microcystis and study of their role in biosorption of Fe, Ni and Cr. Bioresour Technol 98:595–601

Rai PK, Tripathi BD (2007) Removal of heavy metals by the nuisance cyanobacteria Microcystis in continuous cultures: an eco-sustainable. Technol Environ Sci 4:53–59

Rangsayatorn N, Pokethitiyook P, Upatham ES, Lanza GR (2004) Cadmium biosorption by cells of *Spirulina platensis* TISTR 8217 immobilized in alginate and silica gel. Environ Int 30:57–63

Rezaei H (2016) Biosorption of chromium by using *Spirulina* sp. Arab J Chem 9:846–853

Rzymski P, Niedzielski P, Karczewski J, Poniedziałek B (2014) Biosorption of toxic metals using freely suspended Microcystis *aeruginosa* biomass. Cent Eur J Chem 12:1232–1238

Singh S (2014) A review on possible elicitor molecules of cyanobacteria: their role in improving plant growth and providing tolerance against biotic or abiotic stress. J Appl Microbiol 117:1364–5072

Solisio C, Lodi A, Soletto D, Converti A (2008) Cadmium biosorption on *Spirulina platensis* biomass. Bioresour Technol 99:5933–5937

Sun F, Wu F, Liao H, Xing B (2011) Biosorption of antimony(V) by freshwater cyanobacteria *Microcystis* biomass: chemical modification and biosorption mechanisms. Chem Eng J 171:1082–1090

Tease BE, Walker RW (1987) Comparative composition of the sheath of the cyanobacterium Gloeothece ATCC 27152 cultured with and without combined nitrogen. J Gen Microbiol 133:3331–3339

Verma R, Dwivedi P (2013) Heavy metal water pollution-a case study. Recent Res Sci Technol 5:98–99

Vijayaraghavan R, Ellappan V, Dharmar P, Lakshmanan U (2018) Preferential adsorption of uranium by functional groups of the marine unicellular cyanobacterium *Synechococcus elongatus* BDU130911. 3 Biotech 8:170

Wang J, Chen C (2009) Biosorbents for heavy metals removal and their future. Biotechnol Adv 27:195–226

Wang K, Liu Y, Li D (2010) Biosorption of copper by cyanobacterial bloom-derived biomass harvested from the eutrophic Lake Dianchi in China. Curr Microbiol 61:340–345

Weckesser J, Hofmann K, Jürgens UJ, Whitton BA, Raffelsberger B (1988) Isolation and chemical analysis of the sheaths of the filamentous cyanobacteria *Calothrix parietina* and *C. scopulorum*. J Gen Microbiol 134:629–634

WonKwak H, KonKim M, YunLee J, Yun H, HwaKim M, Park YH, Lee KH (2015) Preparation of bead-type biosorbent from water-soluble *Spirulina platensis* extracts for chromium (VI) removal. Algal Res 7:92–99

Yee N, Benning LG, Phoenix VR, Ferris FG (2004) Characterization of metal-cyanobacteria sorption reactions: a combined macroscopic and infrared spectroscopic investigation. Environ Sci Technol 38:775–782

Zhang D, Pan X, Zhao L, Mu G (2011) Biosorption of antimony (Sb) by the cyanobacterium *Synechocystis* sp. Pol J Environ Stud 20:1353–1358

Zinicovscaia I, Cepoi L, Valuta A, Rudi L, Culicov OA, Frontasyeva MV, Kirkesali EI, Pavlov SS, Tatiana M (2014) Nostoc Linckia as biosorbent of chromium and nickel from electroplating industry wastewaters. J Mater Sci Eng B 4:242–247

Bioremediation of Pharmaceuticals in Water and Wastewater

16

Chhaya, Trishikhi Raychoudhury, and Sanjeev Kumar Prajapati

Abstract

Current demographic trends, the rise of chronic diseases, the accessibility of inexpensive generic treatments, and the emergence of "lifestyle" drugs have been the key to increased pharmaceutical medicine use throughout the world. These pharmaceuticals are now the group of emerging contaminants with rising concern in the scientific world due to their presence in surface water, such as lake and river, groundwater, soil, and even drinking water and their associated impact on invertebrates, vertebrates, and ecosystem structure and function. The two main routes of such contaminations are (1) when such drugs taken are excreted in faces and urine and (2) when unused drugs are thrown down. Research undertaken has found that 60–80% of these pharmaceutical medicines are flushed down the toilet or dumped as regular household waste that ends up in sewage treatment plants, which are generally not designed to remove such pollutants from wastewater. Bioremediation is a process where degradation of contamination is done with the help of different microorganisms, which is one of the cost-effective methods that has been used until now. Though there are some interesting reports on the bioremediation of pharmaceuticals from water, further research is crucial for the systematic development of novel technologies to deal with such emerging contaminants. This chapter, therefore, is focused on summarizing and consolidating findings from the current state of the art in the area of pharmaceutical bioremediation.

Chhaya · T. Raychoudhury
Department of Civil and Environmental Engineering (DCEE), Indian Institute of Technology (IIT) Patna, Patna, Bihar, India

S. K. Prajapati (✉)
Environmental and Biofuel Research Lab, Department of Hydro and Renewable Energy (HRED), Indian Institute of Technology (IIT) Roorkee, Roorkee, Uttarakhand, India
e-mail: sanjukfah@iitr.ac.in

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_16

Keywords
Bioremediation · Pharmaceuticals · Microorganism · Wastewater

16.1 Introduction

Pharmaceuticals are biologically active compounds that are designed to prevent and treat a variety of ailments in humans and other animals. Pharmaceuticals are crucial for the maintenance of good health and quality of life. Initially, the natural compounds such as plant crude extracts and shrubs were the principal source of therapeutics. In order to meet the needs of the intense requirement of medicines during the First World War, the focus shifted toward research on easy and fast-producing pharmaceuticals such as synthetic analogs. Since then, synthetic drugs and other pharmaceuticals are in use, and their consumption is increasing day by day. In the current scenario, we are living in a period where mortality has been converted into morbidity, and the average person gets more medical attention than in any previous period. According to the WHO, out of the 56.9 million deaths worldwide in 2016, more than 54% of death are due to ischemic heart disease; stroke; chronic obstructive pulmonary disease; lower respiratory infections; Alzheimer's disease and other dementias; tracheal, bronchial, and lung cancers; diabetes mellitus; road injury; diarrheal disease; and tuberculosis (WHO 2015). Until now, thousands of pharmaceuticals have been flourished from different raw materials such as plant derivatives, synthetic chemicals, and animal derivatives, and the numbers keep on increasing with the growing demand. The major elements of pharmaceutical consumed are antibiotics, analgesic, antipyretic, chemotherapy products, antidepressants, and hormones. Studies have reported the twofold amplification of defined daily intake of cholesterol-lowering, antidiabetic, antihypertensive, antidepressant drugs in OECD member countries (OECD Indicators 2017).

After consumption of pharmaceutical drugs by a consumer, they undergo various metabolic processes such as cleavage, hydroxylation, and glucuronidation (Beausse 2004). Since many of these pharmaceuticals cannot be assimilated and metabolized by the humans/animals, they are excreted after slight modification or in the unaltered form via urine and feces (Dębska et al. 2004). Moreover, unused or expired pharmaceuticals are also discharged to the environment without proper treatment. Consequently, the concentrations of several commonly used pharmaceuticals are increasing rapidly in water and wastewater leading to deleterious effects on the environment. Pharmaceuticals are presumed "pseudo-persistent" contaminants in aquatic environments due to their constant consumption and their polar and nonvolatile properties. These pharmaceuticals are now the group of emerging contaminants with rising concern in the scientific world due to their presence in surface water, such as lake and river, groundwater, and soil in the range from ng to μg/L. Richardson and Bowron (1985) were the first who introduced pharmaceuticals as environmental contaminants. In the late 1990s, their negative environmental effect has been acknowledged, when they were reported as "agents of subtle change" (Daughton

and Ternes 1999). These compounds generally accumulate in the system via the food chain and show their impact on invertebrates, vertebrates, and ecosystem structure and function. The two main routes of such contaminations are (1) when such drugs taken are excreted in feces and urine and (2) when unused drugs are thrown down.

Human excretes pharmaceuticals as a mixture of the parent compound and metabolites, which mainly consist of transformation products and conjugated glucuronides (Heberer 2002). These conjugated compounds are cleaved and release their parent compound into treated wastewater during wastewater treatment processes (Jelic et al. 2011). Sludge from wastewater treatment plants and human excreta are used as fertilizers and may be the major sources of pharmaceutical contamination. Leaching of pharmaceuticals from soil or direct discharge of industrial waste in the subsurface is the main source for groundwater contaminants by pharmaceuticals. At present, almost 3.4 million deaths were attributed to waterborne diseases (Rajaram and Das 2008). The existence of pharmaceuticals in the environment is mainly associated with the discharge of treated wastewater effluent from wastewater treatment plants. Different studies reported that 60–80% of these pharmaceuticals are flushed down in the toilet or dumped as regular household waste that ends up in sewage treatment plants, which are generally not designed to remove such pollutants from wastewater.

The pharmaceuticals are present in the environment as a complex structure, which could cause unwanted synergistic effects. The recent studies on some of the pharmaceuticals such as 17b-estradiol (E2), 17a-ethinylestradiol (EE2), and diclofenac reported them as priority hazardous substances (Petrie et al. 2015). Also, the propagation of antibiotic-resistant microbes in the environment due to the presence of antibacterial drugs is an emerging issue (Petrie et al. 2015). The ubiquity of a high number of potentially toxic pharmaceuticals in the water source underpins the need to better understand their existence, fate, and ecological impact. More than 200 pharmaceuticals alone have been accounted in river waters globally, with maximum concentrations up to 6.5 mg/L for the antibiotic ciprofloxacin (López-Serna et al. 2013).

A study conducted on the detection of the trace amount of pharmaceuticals in the influent and effluent of the wastewater treatment plant and raw drinking water samples has screened 37 pharmaceuticals, four hormones, and a number of other micropollutants (Morasch et al. 2010; Perazzolo et al. 2010). A national survey accomplished by the U.S. Geological Survey showed that trace amount of diverse classes of pharmaceuticals, including prescription drugs, nonprescription drugs, antibiotics, and other wastewater related drugs, was detected with maximum concentrations in the range from 0.019 to 0.30 μg/L (Sun et al. 2015). Several pharmaceuticals, such as lincomycin, ibuprofen, erythromycin, acetaminophen, carbamazepine, roxithromycin, and metoprolol, are detectable in drinking waters.

Overall, it is evident that the occurrence of pharmaceuticals as emerging contaminants in the water has become a matter of serious concern, and this is the high time to focus on the removal of these from the wastewater treatment plant effluent before it is released in the aquatic environment. In the past decades, there have been studies on removal of pharmaceuticals from water and wastewater using

various physicochemical methods such as solar photocatalysis (Bernabeu et al. 2011), membrane separation, advanced oxidation process, sand filtration under aerobic/anaerobic conditions, and flocculation using chemical flocculants (Petrović et al. 2003). Further, in recent years, biochar has emerged as a promising adsorbent for the removal of pharmaceuticals, given that it has the high specific surface area and density of functional groups (Chen et al. 2008). For instance, rice husk and wood chip biochar are found to be efficient to remove levofloxacin from aqueous solution (Yi et al. 2016). Likewise, bioremediation has also been reported to be efficient in the degradation and removal of pharmaceuticals from water and wastewater. For instance, filamentous bacteria *Streptomyces* spp. were found efficient for carbamazepine removal (Popa et al. 2014). Similarly, fungi and algae have also been tested for remediation of selected pharmaceuticals from water. However, the particular area of pharmaceutical remediation is currently in developing stage and needs more serious and systematic research attempts.

Hence, in light of this, this chapter is focused on summarizing the current state of the art related to bioremediation of pharmaceuticals from water and wastewater. Initially, some of the commonly used pharmaceuticals are listed along with their characteristics and associated environmental impacts. Subsequently, the technological interventions on remediation of pharmaceuticals are reviewed and summarized to get the insight into the overall status of current development in this particular area.

16.2 Commonly Used Pharmaceuticals as Emerging Contaminants

According to current demographic trends, nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, antidepressants, antihypertensives, lipid regulators, hormones, and anticonvulsants are some commonly used pharmaceuticals (Fig. 16.1). NSAIDs are one of the most frequently recommended pharmaceuticals in medical practice. These drugs have anti-inflammatory, analgesic, and antipyretic effects that make them useful for fever and acute and chronic pain. They show their action via inhibiting the cyclooxygenase isozymes (Brune and Patrignani 2015). They have a huge area of their application such as for the treatment of musculoskeletal disorders (acute like injuries and gout and chronic like rheumatoid arthritis and osteoarthritis), dental pain, postoperative pain, headaches, and dysmenorrhea (Tomić et al. 2017). Antibiotics are another group of pharmaceuticals that used to prevent and treat the bacterial infections related with chronic diseases, burn and cancer treatment, surgery, neonatal care, and transplantations (Ventola 2015). It was considered as one of the remarkable creations of the twentieth century. Prior to this era, the infectious disease leads to huge morbidity and mortality throughout the globe.

Antidepressant is the basic pharmacological medication indicated for depression-investigated patients. The group antidepressant is further classified as old and new antidepressants. Older antidepressants are generally tricyclic antidepressants, while newer antidepressants include serotonin-norepinephrine reuptake inhibitors,

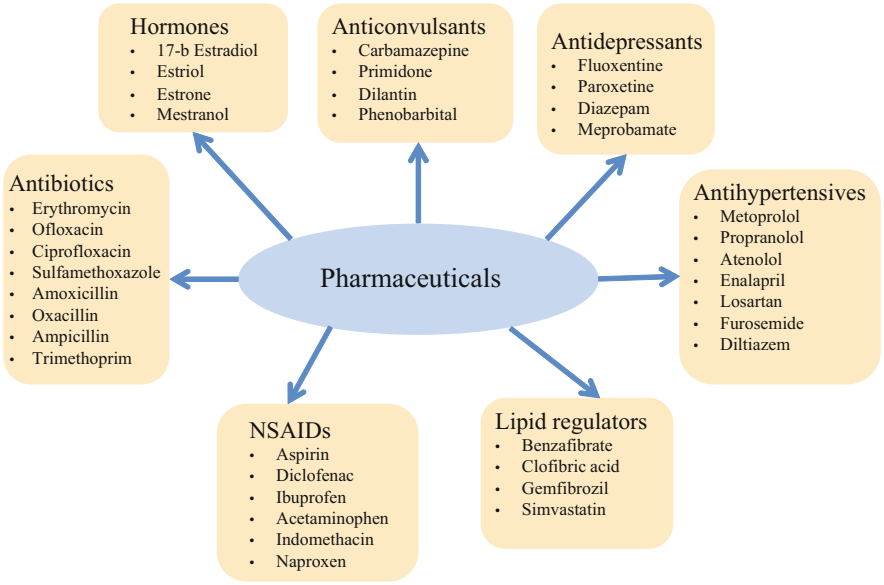


Fig. 16.1 Pharmaceuticals present in environments (Magureanu et al. 2015)

selective serotonin reuptake inhibitors, and monoamine oxidase inhibitors. Tricyclic antidepressant is supposed to inhibit the reuptake of acetylcholine, serotonin, and norepinephrine in the presynaptic membrane that further increases the serotonin amount in the synaptic cleft. Monoamine oxidase inhibitors inhibit the activity of the monoamine oxidase enzyme, which ends up to raise serotonin level (Sardar et al. 2016). Similarly, high blood pressure or hypertension is another serious cause of mortality and morbidity; hence, their management is quite important. Beta-blockers, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, calcium channel blockers, and diuretics are the five main groups that can be consumed for the introduction and maintenance of the treatment (Sarganas et al. 2016).

Anticonvulsants are pharmaceuticals that are consumed for the treatment of seizure. These anticonvulsants are differentiated as first- and second-generation anticonvulsants. First-generation anticonvulsants include benzodiazepine, phenobarbital, valproic acid, carbamazepine, and phenytoin that were introduced in the twentieth century, while second-generation anticonvulsants include oxcarbazepine, tiagabine, zonisamide, gabapentin, vigabatrin, topiramate, and pregabalin that were recently introduced. Most of the anticonvulsants reduce pain and anxiety and enhance the quality of sleep. Carbamazepine and gabapentin both act on sodium channels to inhibit their depolarization. Pregabalin and gabapentin act upon calcium channel in order to block it. A group of anticonvulsants, pregabalin, carbamazepine, and gabapentin, can suppress glutamate, an excitatory neurotransmitter lying in presynaptic level. Carbamazepine is potent to enhance an inhibitory neurotransmitter gamma-aminobutyric acid (Sardar et al. 2016).

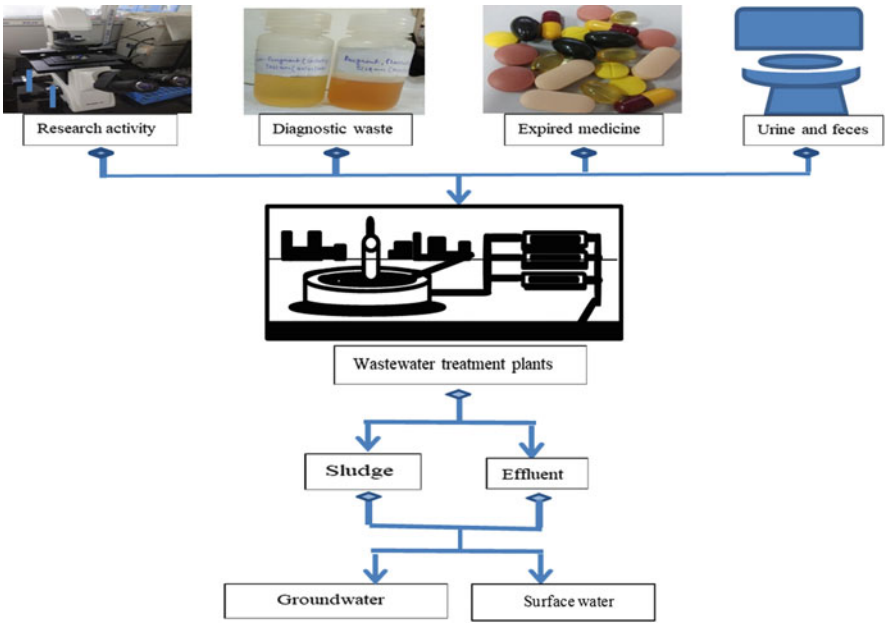


Fig. 16.2 Routes of pharmaceutical residue to the environment

These pharmaceuticals when released in the environment are directly or indirectly mixed to the surface water leading to contamination of various sources of water including groundwater (Fig. 16.2). Some of the pharmaceuticals, commonly found in wastewater, even after treatment through conventional sewage treatment plants, are summarized in Table 16.1.

In order to manage environmental pollution, several methods have been developed to remediate waste and reuse gray water containing pharmaceuticals. The ancient methods implemented for the remediation of wastewater include physical, chemical, and thermal methods that have their own disadvantages which include high maintenance cost, huge labor requirement, low efficiency, and huge equipment. In order to attain maximal efficiency for treatment processes, advanced technologies such as bioremediation have evolved, and research is ongoing for better outcomes.

16.3 Remediation of Pharmaceuticals

The pharmaceuticals that are present in municipal wastewater cannot be completely treated in wastewater treatment plants with conventional methods like physicochemical treatment processes including adsorption, coagulation, filtration, sedimentation, volatilization, and oxidation ponds. In several wastewater treatment plants, the final effluent is sent for disinfection process where UV irradiation and/or chlorination are applied, but both techniques have low oxidation capability and very few amounts of

Table 16.1 List of pharmaceuticals commonly found in wastewater influent, effluent, and surface waters (Petrie et al. 2015)

S. no	Name	Uses	Influent (ng/L)	Effluent (ng/L)	Surface water (ng/L)
1	Ibuprofen	Nonsteroidal anti-inflammatory	1681–33,764	143–4239	1–2370
2	Diclofenac	Nonsteroidal anti-inflammatory	69–1500	58–599	<0.5–154
3	Acetaminophen	Nonsteroidal anti-inflammatory	6924–492,340	<20–11,733	<1.5–1388
4	Naproxen	Nonsteroidal anti-inflammatory	838–1173	170–370	1–59
5.	Ketoprofen	Nonsteroidal anti-inflammatory	28–102	16–23	1–4
6	Estrone	Steroid estrogen	49	4.3–12	–
7	17β-estradiol	Steroid estrogen	20	0.4–1.3	–
8	17α-ethinylestradiol	Synthetic estrogen	1.0	0.20–0.47	–
9	Erythromycin	Antibacterial	71–2530	109–1385	<0.5–159
10	Ofloxacin	Antibacterial	180	10	–
11	Amoxicillin	Antibacterial	<87	31	<2.5–245
12	Metronidazole	Antibacterial	569–2608	265–373	<1.5–12
13	Chloramphenicol	Antibacterial	<4–248	<6–21	<10
14	Sulfamethoxazole	Antibacterial	<3–115	10–19	<0.5–2
15	Sulfapyridine	Antibacterial	914–4971	277–455	<2–28
16	Metoprolol	Beta-blocker	75–110	41–69	<0.5–10
17	Propranolol	Beta-blocker	60–638	93–388	<0.5–107
18	Atenolol	Beta-blocker	12,913–14,223	2123–2870	<1–487
19	Fluoxetine	Antidepressant	14–86	16–29	5.8–14
20	Venlafaxine	Antidepressant	120–249	95–188	1.1–35
21	Dosulepin	Antidepressant	21–228	57	0.5–25
22	Amitriptyline	Antidepressant	106–2092	66–207	<0.5–30
23	Nortriptyline	Antidepressant	5.1–114	7.6–33	0.8–6.8
24	Furosemide	Diuretic	1476–2789	629–1161	<6–129
25	Bezafibrate	Lipid regulator	420–971	177–418	<10–60
26	Simvastatin	Lipid regulator	<7–115	<3–5	<0.6

pharmaceutical are removed with their application. Recent research focused on the implementation of activated sludge, advanced oxidation processes and ozonation, membrane bioreactor, and membrane separation technology that are extensively executed for the removal of pharmaceutical residue from wastewater (Ahmed et al. 2017).

16.3.1 Physicochemical Methods for Pharmaceutical Remediation

In the past decades, several physicochemical approaches have been attempted for remediation of pharmaceuticals from water and wastewater. Advanced oxidation is one of the commonly used approaches for pharmaceutical remediation. Advanced oxidation process (AOP) is an eco-friendly electrochemical, chemical, or photochemical process, whose principal mechanism involves the synthesis of hydroxyl radical (the second strongest oxidizing agent after fluorine) as chief oxidant and other radicals. These radicals oxidize toxic, recalcitrant, and nonbiodegradable compound leading to degradation of target organic pollutants. The hydroxyl radical has potential to nonselectively react with various organics via dehydrogenation or hydroxylation until their complete mineralization. Huge research has been done with AOPs, and still, it is gaining research interest particularly due to two major reasons: (a) the area of potential application, and (b) the diverse technologies that are involved. AOPs include homogeneous and heterogeneous photocatalysis dependent on near solar visible irradiation or ultraviolet (UV), ozonation, electrolysis, wet air oxidation, ultrasound, and Fenton's reagent. Photolysis process involves the interaction of natural or artificial light with the organic pollutant molecule that causes induction of photochemical reactions which lead to direct degradation of their intermediate products whose further degradation eventually yields mineral end products (Doll and Frimmel 2003). Ultraviolet-C (UVC) radiation has the potential to degrade the micropollutant. They are applied directly and/or indirectly for the photolysis of various micropollutants. In indirect photolysis process, the light source emits the radiation which is absorbed by organic pollutants. These excited organic pollutants transfer their electron from their excited state to molecular oxygen in the ground state, or it corresponds to homolysis of organic micropollutant to form organic radicals to react with oxygen (Legrini et al. 1993). Indirect photolysis processes lead to the formation of various reactive species such as hydroxyl radicals, singlet oxygen, carbon-centered radicals, peroxyl radicals, and excited triplet state involving the irradiation of recalcitrant of organic pollutants (Wenk et al. 2011). In one study, free radicals were found to induce oxidative and reductive degradation of a class of broad-spectrum antibiotics, fluoroquinolones (Santoke et al. 2009), while in another study, these free radicals were evaluated for degradation of three beta-blockers: propranolol, metoprolol, and atenolol (Song et al. 2008)

In an advance H_2O_2/UVC method, the hydroxyl radicals are formed due to homolytic cleavage of peroxide by ultraviolet C light. The efficiency of hydroxyl radical formation increases when the irradiation is combined with hydrogen peroxide (Pereira et al. 2007). In one of the studies, three pharmaceuticals, ibuprofen, ciprofloxacin, and carbamazepine, were degraded with degradation range between 80 and 100% from wastewater with light-driven advanced oxidation processes (Monteoliva-García et al. 2019).

$$H_2O_2 + hv \rightarrow 2 \bullet OH \quad (\lambda < \text{ca.}280\,\text{nm}) \tag{16.1}$$

Studies conducted have found TiO_2-mediated solar photocatalysis effective in the removal of diclofenac and naproxen (Kanakaraju et al. 2016). In another study, immobilized TiO_2 photocatalysis was used for the removal of pharmaceutically active compounds under simulated solar irradiation. The process resulted in high removal efficiency for poorly degraded pharmaceutical contaminants such as 76 ± 3% for carbamazepine, 100% for propranolol, and 100% for diclofenac (He et al. 2016). Recently, magnetic carbon nanotube-TiO_2 was synthesized; its photocatalytic property was studied for the degradation of sulfamethoxazole and carbamazepine under solar irradiation (Awfa et al. 2019).

Fenton oxidation is a free radical chain reaction in which homogeneous oxidation with the Fenton reagent occurs in the presence of ferrous or ferric ions with hydrogen peroxide to produce hydroxyl radicals. Metal iron is considered to be a catalyst in this reaction (Saritha et al. 2007). In another photo-Fenton/UVC process, the additional hydroxyl radicals were produced in the presence of iron through Fenton's reaction. The efficiency of hydroxyl radical formation gets enhanced in the presence of UV irradiation, and the reaction is known as the photo-Fenton reaction (Shemer et al. 2006).

Furthermore, the photoactive species undergoes photoreduction process that leads to the formation of hydroxyl radicals and ferrous ions:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^- \tag{16.2}$$

$$FeOH^{2+} + hv \rightarrow Fe^{2+} + \bullet OH \quad (\lambda < \text{ca.}400\ \text{nm}) \tag{16.3}$$

$$Fe^{3+}(L)_n + hv \rightarrow Fe^{2+}(L)_{n-1} + Lox \bullet \quad (\lambda < \text{ca.}500\ \text{nm}) \tag{16.4}$$

In one of the studies, photo-Fenton reaction is found efficient for the degradation and mineralization (97%) of nystatin, an antifungal drug (Boucenna et al. 2019). The two pharmaceuticals fluoxetine and ciprofloxacin that are commonly present in hospital waste at low concentration (100 μg/L) were degraded with photo-Fenton reaction after anaerobic pretreatment of hospital effluent (Perini et al. 2017). Pharmaceutical contaminants such as amoxicillin, sulfamethazine, sulfathiazole, and ciprofloxacin were degraded with the photo-Fenton process under ultraviolet germicidal irradiation after biological treatment (Perini et al. 2018).

Electrochemical oxidation takes place over anode that is generally made up of Pt, graphite, PbO_2, IrO_2, boron-doped diamond, and several Ti-based alloys in the presence of appropriate electrolytes like NaCl and has been practiced for the degradation of various organic pollutants. Organic pollutants in electrochemical advanced oxidation processes are oxidized by hydroxyl radicals and are physisorbed at anode surface M, represented as M(•OH) (Brillas et al. 2009).

$$M + H_2O \rightarrow M(\bullet OH) + H^+ + e^- \tag{16.5}$$

In the solar photo-electro-Fenton process, both anodic oxidation and solar photo-Fenton reaction play a role in the production of H_2O_2 at the cathode (Moreira et al. 2016). In a pilot-scale study, the solar photo-electro-Fenton methods mineralize sulfamethoxazole with 83% of mineralization current efficiency (Murillo-Sierra et al. 2018). In another study, solar photo-electro-Fenton processes are applied for mineralization of amoxicillin. The mineralization percentage was found to be 85% (Garza-Campos et al. 2018).

From the current state of the art, it is evident that the physical-chemical methods are efficient for pharmaceutical remediation. However, the high cost of chemicals and operations, as well as associated hazardous effects of chemicals used and the by-products of the remediation reactions, makes these approaches non-feasible at large scale. Hence, there have been continuous efforts on the development of low-cost and environment-friendly techniques for pharmaceutical remediation. Fortunately, in recent years, bioremediation, involving the use of biomass as well as microorganisms, has emerged as an efficient alternative to the physical-chemical remediation technologies.

16.3.2 Bioremediation of Pharmaceuticals

Bioremediation is a natural process to decompose, detoxify, or eliminate hazardous wastes with the help of metabolic potential microorganisms and their enzymes that occur at several levels of the ecosystem. Bioremediation is a cost-effective process and is environment-friendly. Nowadays, bioremediation through green adsorbent is in practice. Green adsorbents are basically low-priced stuff developed from (1) agricultural waste or residue, (2) agricultural product and by-products (vegetables, fruits, etc.), and (3) other materials such as biochar, activated carbons, and nano-particles. Bioremediation with the help of microorganism is another most reliable process that has been practiced in the near past and is being explored at a fast rate. Bioremediation can be classified into two broad groups: (1) adsorption of pharmaceuticals on solid supports such as biochar, and (2) microorganism-mediated bioremediation.

16.3.2.1 Biochar-Based Adsorption of Pharmaceuticals

Adsorption is one of the well-established, convenient, and reliable techniques for the removal of various pollutants. Biochar is a low-cost carbonaceous sorbent that has gained an immense research interest. This is produced by thermal decomposition of organic materials under oxygen limit condition. Biochar can be generated from a wide variety of organic feedstock materials, such as sugarcane bagasse, rice husk, rice straw, bamboo, etc. Sorption capacity of biochar is defined as the maximum amount of sorbent adsorbed under equilibrium condition onto an adsorbent. The sorption capacity depends on various factors like specific surface area, source of origin, pore structure, surface properties, and type of solute. Chemical properties such as basic or acidic characters, functional groups, and point of zero charges are some influential factors. Some other parameters include the type of interaction between species whether electrostatic or physical and the size of sorbent molecules.

Biochar produced from different precursor biomass has different properties of porosity, surface area, and functional group that makes it a good adsorbent. It is an excellent sorbent for hydrophobic organic contaminants due to functionality and surface aromaticity (Vithanage et al. 2016). In the pyrolysis process, feedstock undergoes various chemical, molecular, and physical changes. Thermal decomposition causes loss of mass and hence volume reduction with least change in the parental structure of biomass. Additionally, pyrolysis causes alteration in H/C, O/C, and C/N ratios; cation exchange capacity; surface area; porosity; and functional groups. Biochar has been demonstrated to be effective in removing various water contaminants including pharmaceuticals. Several research reports have been done with raw as well as modified biochar that enhances the adsorption of pharmaceuticals (Table 16.2). For instance, studies with two different activated coconut shell biochars examined the removal of ibuprofen from water. The maximum removal efficiency was found to be 80.37% for *Cocos nucifera* shell biochar and 73.71% for *Cocos nucifera* shell biochar (Chakraborty et al. 2019). Another biochar synthesized from the olive stone was used for activation of persulfate to reactive radicals, which subsequently degrades sulfamethoxazole (Magioglou et al. 2019). Similarly, steam-activated mung bean husk biochar removes 99% ibuprofen from aqueous solution through biosorption process (Mondal et al. 2016). However, most of the studies reported in the recent years are based on the lab-scale setups. On the contrary, few reports are available on the utilization of biochar for removal of pollutants and nutrients from wastewater (Bock et al. 2016; Maurer et al. 2017). Hence, there is a need for serious effects toward utilization of biochar potential for pharmaceutical removal at the pilot and industrial scales.

16.3.2.2 Microbe-Based Remediation of Pharmaceuticals

It involves the use of microbes to eliminate or decompose the pollutants. In recent years, enormous microbes have been identified efficient in the removal of the compound formerly examined nondegradable. Microbes generally degrade or convert the pollutants into some other form that is not more toxic and problematic than the parent compound. Biodegradation of the waste depends on numerous factors, such as (1) concentration of the pollutant; (2) toxicity of the pollutant; (3) stereochemistry of the compound; (4) efficiency of the microbes; (5) conditions such as pH, temperature, and moisture; (6) retention time; and (7) the presence of other compounds, metals, and their concentration.

Processes with Indirect Involvement of Missed and Unknown Microbes

Activated Sludge Processes

The activated sludge is the most frequently practiced wastewater treatment technology. This is a biological process in which bacterial biomass suspension is used to degrade the pollutants under aerobic condition. This process is most commonly used for secondary wastewater treatment. The method is extensively used to attain stability in wastewater having an organic component in it. The process depends on establishing and sustaining a culture of degrading microbes and furnishing proximity

Table 16.2 Summary of recent reports utilizing agro-residue-based biochar for remediation of pharmaceuticals from aqueous medium

Agro-waste	Modification	Biochar property	Target pharmaceuticals	Removal efficiency	References
Sugarcane bagasse	Steam activated (SPAB), chemical activated (SCAB)	Surface area: 557 m^2/g, microporous volume: 0.273 cm^3/g, mesoporous: 0.310 cm^3/g	Ibuprofen	SCAB: 13.51 mg/g (91%), SPAB: 11.90 mg/g (82%)	Chakraborty et al. (2018)
Sugarcane bagasse	Anaerobic digested sugarcane bagasse	A small amount of Mg, Ca, Cu, Fe, and Al. Mg and Ca content increases with the pyrolysis	Sulfamethoxazole (SMX) and sulfapyridine (SPY)	SMX: 54.38 mg/g, SPY: 8.60 mg/g	Yao et al. (2018)
Sugarcane bagasse	Sodium dodecylbenzenesulfonate (SDBS) modified bagasse biomass in carbon nanotube (CNT)	Surface area: 336 m^2 /g, pore volume: 0.167 cm^3/g	Sulfapyridine	56%	Inyang et al. (2015)
Sugarcane bagasse	Pyrolyzed under oxygen-limited conditions from 100 to 600 °C		*p*-Benzoquinone, tetracycline, and polyvinyl alcohol		Li et al. (2016)
Rice husk	Pyrolyzed at 700 °C	Surface area: 211.7 m^2/g, pore diameter: 6.25 nm, pore volume: 0.12087 cm^3/g	Tetracycline hydrochloride (TC), doxycycline hydrochloride (DC), and ciprofloxacin (CF)	TC: 80.9 mg/g, DC: 85.2 mg/g, CF: 36.1 mg/g	Zeng et al. (2018b)
Rice straw	Pyrolyzed at 500 °C	BET surface area: 72.6 m^2/g, pore volume: 0.063 cm^3/g	Ibuprofen	56.0 mg/g	Salem and Yakoot (2016)
Rice straw	Pyrolyzed at 600 °C in oxygen-limiting condition	Surface area: 29.6 m^2/g, total pore volume: 0.069 cm^3/g	Sulfamethoxazole	3650 mg/kg	Li et al. (2015)
Rice straw	Pyrolyzed at 700 °C	BET surface area: 288.341 m^2/g	Tetracycline	80%	Fan et al. (2018)

Rice straw	Pyrolyzed at 700 °C	BET surface area: 20.55 m^2/g; pore volume: 0.0191 cm^3/g; average pore diameter: 6.42 nm	Doxycycline (DOX) and ciprofloxacin (CIP)	CIP: 60.18 mg/g; DOX: 108.42 mg/g	Zeng et al. (2018a)
Rice straw	H_3PO_4 modified biochar (700 °C)	Surface area: 372.21 m^2/g; total pore volume: 0.23	Tetracycline	552 mg/g	Chen et al. (2018)
Bamboo			Fluoroquinolone	45.88 ± 0.90 mg g^{-1} (99%)	Wang et al. (2015)

of degrading microbes and provides dissolved oxygen. The method depends on establishing and maintaining a population of degrading microorganisms and providing close contact of the degrading microorganisms and supply of dissolved oxygen. Here, microbes feed on an oxidizable organic compound in wastewater and form a biological floc. In one of the studies, bio-augmentation of municipal wastewater with activated sludge accelerates the degradation process for 14 pharmaceutical compounds (Muter et al. 2017). Similarly, various pharmaceuticals including cyclophosphamide, paracetamol, bezafibrate, carbamazepine, ciprofloxacin, and caffeine were removed with an efficiency of >80% using activated sludge process in the hospital wastewater treatment plants (Al Qarni et al. 2016). However, sometimes, the pharmaceuticals exhibit toxicity to the microbial population resulting in the failure of the entire treatment system (Wittebolle et al. 2005). Hence, proper attention shall be given to the selection as well as acclimatization of the selected microbes to be used in the activated sludge process for remediation of pharmaceuticals.

Membrane Bioreactor

The membrane filtration process provides various benefits, such as good disinfection capability, reduced footprint, sludge production, excellent effluent quality, higher volumetric loading, improved nitrification, process flexibility toward influent changes, etc., over the secondary settler in activated sludge processes (ASP). This technique is found effective in the removal of inorganic and organic contaminants as well as microbes from wastewater. It has acquired popularity in the latest years as a result of more stringent environmental regulations and increasing water reuse projects. In a study of hollow fiber and flat sheet, sponge membrane bioreactor was found efficient in the removal of several antibiotics such as ofloxacin, norfloxacin, tetracycline, trimethoprim, and ciprofloxacin (Nguyen et al., 2017). In another study, Wang et al. (2018) reported up to 95% removal of eight cytostatic drugs in an anaerobic osmotic membrane bioreactor with an extended sludge retention time of 60 days. Similarly, Prasertkulsak et al. (2019) observed up to 80% removal of 10 pharmaceutical compounds in a microbial sludge-based membrane bioreactor. However, as in the case of ASP, the main driving force in membrane bioreactor is a microbial community. Hence, the toxicity of the selected pharmaceuticals to the microorganism involved is of major concern.

Remediation Using the Pure Culture of Microorganisms

Bacteria

There have been several interesting reports on bioremediation of selected pharmaceuticals using bacterial cultures. A recent study reported that indigenous bacterial species *Comamonas aquatica* and *Bacillus* sp., isolated from La Noria Bridge, showed up to 92% degradation capacity of ibuprofen (Fortunato et al. 2016). The Gram-positive bacterial strain *Bacillus thuringiensis* B1(2015b) was found efficient in the removal of ibuprofen and naproxen (Marchlewicz et al. 2016). In another study, the bacterial strain *Serratia marcescens BL1* showed

93.47% ± 2.37% removal efficiency for ibuprofen (Xu et al. 2018). Bacterial strains *Starkeya* sp. C11, *Rhizobium* sp. C12, and *Brevibacterium* sp. D4, isolated from the municipal wastewater treatment plant, were found efficient in the removal of diclofenac and carbamazepine (Bessa et al., 2017a). Similarly, *Labrys portucalensis* F11 has been proved to degrade the two most prior emerging contaminants, i.e., carbamazepine and diclofenac (Bessa et al., 2017b). The group of endophytic bacteria (*Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*), isolated from plant *Phragmites australis*, was reported to show high removal efficiency for carbamazepine (Sauvêtre and Schröder, 2015). Hence, present studies evidence that the bacteria have the immense potential for bioremediation of pharmaceuticals. However, the field is still in the nascent stage and needs more systematic effects to convert the laboratory-based finding into useful technologies.

Fungi

Fungi have an amazing feature to decompose the waste. Mycoremediation for the treatment of pharmaceuticals and hospital effluent appears to be an attractive approach. In one study, the fungus *Mucor hiemalis* was concluded effective for the removal of acetaminophen (Esterhuizen-Londt et al. 2016). The advanced bio-oxidation process of white-rot fungi *Trametes versicolor* shows high pharmaceutical remediation from hospital wastewater (Vasiliadou et al. 2019). In another study, four pharmaceuticals, diclofenac, trimethoprim, carbamazepine, and sulfamethoxazole, were degraded with the fungus *Trametes versicolor*, and the treated effluent was demonstrated to be nondangerous (Alharbi et al. 2019). Similarly, white-rot fungi *Trichoderma harzianum* and *Pleurotus ostreatus* convert the carbamazepine and clarithromycin to nontoxic products (Buchicchio et al. 2016). *Trametes hirsuta* is another type of fungi that removes acetaminophen and carbamazepine with its immobilized laccase (Hachi et al. 2017). In addition, the fungus *Phanerochaete chrysosporium* is capable of removing ibuprofen and diclofenac (Rodarte-Morales et al. 2012).

Algae

Algae is a vast group of a productive eukaryotic photosynthetic aquatic organism with efficiency for converting nutrients and carbon dioxide to biomass. Algae are efficient in the removal of phosphorus and nitrogen from wastewater, and the biomass of algae is further used as raw matter for the production of bioenergy. Apart from nutrients, algae use the hazardous compound as their substrate to perform their metabolism that ends to harmless products. In the present day, there is an emerging research intrigue in using algal-based wastewater treatment technologies due to their efficient adaptability, rapid growth rate, huge photosynthetic efficiency, and their capacity to use waste as its substrate. Removal mechanism of pharmaceutical contaminants by microalgae includes intracellular and extracellular biodegradation, bioaccumulation, and adsorption. In intracellular degradation processes, the microalgae convert the complex contaminants in the simplest form with the help of catalytically degrading complex, while in extracellular processes, these microalgae excrete several extracellular polymeric substances that include protein,

polysaccharides, substituents (polysaccharide-link methyl and acetyl groups), lipids, and enzymes to their surrounding aqueous environment which acts as an external digestive system to metabolize the organic contaminants.

Bioaccumulation is the process by which microalgae take the organic contaminants along with their nutrients through an active metabolic process. In bioabsorption, the microalgae collect the soluble contaminants that are present in the aqueous phase. The microalgal strain *Scenedesmus obliquus RISE (UTEX417)* isolated from northern Sweden was tested for the removal of 19 pharmaceuticals (Gojkovic et al. 2019). Another study performed on the green alga *Nannochloris* sp. evaluated its efficiency for the removal of sulfamethoxazole, triclosan, and trimethoprim. The filamentous alga *Spirogyra* sp. was capable of removing six pharmaceutical compounds (carbamazepine, diclofenac, ibuprofen, acetaminophen, propranolol, and clofibric acid) and two endocrine-disrupting chemicals (bisphenol A and 17a-ethinylestradiol) (Garcia-Rodríguez et al. 2015). The genetically modified microalgae mutant *Chlorella PY-ZU1* has high removal efficiency of 94% for ethinylestradiol (Cheng et al. 2018). The antibiotic cefradine was removed with the help of the alga *Chlorella pyrenoidosa* combined to UV-algal technology in order to manage the toxicity of treated effluent (Du et al. 2015)

From the above discussion, it is clear that microbe-based bioremediation is a good option to deal with the pharmaceuticals in water and wastewater. However, using known culture instead of unknown mixed microbial community would be better as the toxicity and the tolerance levels of selected pharmaceuticals in the known microorganism can easily be assessed before their use in the actual treatment system.

16.4 Conclusion

Pharmaceutical compounds are recognized as the major emerging contaminants in water and wastewater effluent. Direct or indirect discharge of these compounds to the environment causes the contamination of surface and groundwater. In the last two decades, numerous studies have been carried out that explored the remedial efficiency of pharmaceuticals from water and wastewater using different processes This report gives an overview of both different physical-chemical and bioengineering processes used for removal of pharmaceuticals. Chemical oxidation processes such as advance H_2O_2/UVC method and photo-Fenton reaction were found to enhance the removal of various pharmaceuticals. Advance H_2O_2/UVC processes increase the hydroxyl radical formation that further enhances the degradation of pharmaceuticals. On the contrary, different biological methods such as biochar-based sorption process and microbe-based remediation processes have been found to be the best processes for degrading or eliminating various pharmaceutical contaminants that are present in wastewater and its effluent. Biochar, which is a low-cost carbonaceous sorbent, has shown better removal efficiency for various pharmaceutical contaminants such as ibuprofen, sulfamethoxazole, sulfapyridine, diclofenac, carbamazepine, etc. The performance of the conventional activated sludge process has been studied for the removal of various contaminants. Membrane

bioreactor processes have been found effective in the removal of various contaminants. Microbe-based remediation has the highest efficiency for the removal of several pharmaceutical contaminants. Furthermore, there is a scope of technological development in the area of microbe-based bioremediation at industrial scale.

References

Ahmed MB, Zhou JL, Hao Ngo H, Guo W, Thomaidis NS, Xu J (2017) Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: a critical review. J Hazard Mater 323:274–298. https://doi.org/10.1016/j.jhazmat.2016.04.045

Al Qarni H, Collier P, O'Keeffe J, Akunna J (2016) Investigating the removal of some pharmaceutical compounds in hospital wastewater treatment plants operating in Saudi Arabia. Environ Sci Pollut Res Int 23:13003–13014. https://doi.org/10.1007/s11356-016-6389-7

Alharbi SK, Nghiem LD, van de Merwe JP, Leusch FDL, Asif MB, Hai FI, Price WE (2019) Degradation of diclofenac, trimethoprim, carbamazepine, and sulfamethoxazole by laccase from *Trametes versicolor*: transformation products and toxicity of treated effluent. Biocatal Biotransformation:1–10. https://doi.org/10.1080/10242422.2019.1580268

Awfa D, Ateia M, Fujii M, Yoshimura C (2019) Novel magnetic carbon nanotube-TiO_2 composites for solar light photocatalytic degradation of pharmaceuticals in the presence of natural organic matter. J Water Process Eng 31:100836. https://doi.org/10.1016/J.JWPE.2019.100836

Beausse J (2004) Selected drugs in solid matrices: a review of environmental determination, occurrence and properties of principal substances. TrAC Trends Anal Chem 23:753–761. https://doi.org/10.1016/j.trac.2004.08.005

Bernabeu A, Vercher RF, Santos-Juanes L, Simón PJ, Lardín C, Martínez MA, Vicente JA, González R, Llosá C, Arques A, Amat AM (2011) Solar photocatalysis as a tertiary treatment to remove emerging pollutants from wastewater treatment plant effluents. Catal Today 161:235–240. https://doi.org/10.1016/J.CATTOD.2010.09.025

Bessa VS, Moreira I, Murgolo S, Mascolo G, Castro PML (2017a) Biodegradation of carbamazepine and diclofenac by bacterial strain Labrys portucalensis. In: ICEBB 2017 19th international conference on environmental biotechnology and bioremediation

Bessa VS, Moreira IS, Tiritan ME, Castro PML (2017b) Enrichment of bacterial strains for the biodegradation of diclofenac and carbamazepine from activated sludge. Int Biodeterior Biodegradation 120:135–142. https://doi.org/10.1016/J.IBIOD.2017.02.008

Bock EM, Coleman B, Easton ZM (2016) Effect of biochar on nitrate removal in a pilot-scale denitrifying bioreactor. J Environ Qual 45:762. https://doi.org/10.2134/jeq2015.04.0179

Boucenna A, Oturan N, Chabani M, Bouafia-Chergui S, Oturan MA (2019) Degradation of Nystatin in aqueous medium by coupling UV-C irradiation, H2O2 photolysis, and photo-Fenton processes. Environ Sci Pollut Res 26:1–13. https://doi.org/10.1007/s11356-019-05530-2

Brillas E, Sirés I, Oturan MA (2009) Electro-Fenton process and related electrochemical technologies based on Fenton's reaction chemistry. Chem Rev 109:6570–6631. https://doi.org/10.1021/cr900136g

Brune K, Patrignani P (2015) New insights into the use of currently available non-steroidal anti-inflammatory drugs. J Pain Res 8:105–118. https://doi.org/10.2147/JPR.S75160

Buchicchio A, Bianco G, Sofo A, Masi S, Caniani D (2016) Biodegradation of carbamazepine and clarithromycin by Trichoderma harzianum and Pleurotus ostreatus investigated by liquid chromatography—high-resolution tandem mass spectrometry (FTICR MS-IRMPD). Sci Total Environ 557–558:733–739. https://doi.org/10.1016/J.SCITOTENV.2016.03.119

Chakraborty P, Show S, Banerjee S, Halder G (2018) Mechanistic insight into sorptive elimination of ibuprofen employing bi-directional activated biochar from sugarcane bagasse: performance

evaluation and cost estimation. J Environ Chem Eng 6:5287–5300. https://doi.org/10.1016/j.jece.2018.08.017

Chakraborty P, Show S, Ur Rahman W, Halder G (2019) Linearity and non-linearity analysis of isotherms and kinetics for ibuprofen remotion using superheated steam and acid modified biochar. Process Saf Environ Prot 126:193–204. https://doi.org/10.1016/j.psep.2019.04.011

Chen B, Zhou D, Zhu L (2008) Transitional adsorption and partition of nonpolar and polar aromatic contaminants by biochars of pine needles with different pyrolytic temperatures. Environ Sci Technol 42:5137–5143. https://doi.org/10.1021/es8002684

Chen T, Luo L, Deng S, Shi G, Zhang S, Zhang Y, Deng O, Wang L, Zhang J, Wei L (2018) Sorption of tetracycline on H3PO4 modified biochar derived from rice straw and swine manure. Bioresour Technol 267:431–437. https://doi.org/10.1016/J.BIORTECH.2018.07.074

Cheng J, Ye Q, Li K, Liu J, Zhou J (2018) Removing ethinylestradiol from wastewater by microalgae mutant Chlorella PY-ZU1 with CO_2 fixation. Bioresour Technol 249:284–289. https://doi.org/10.1016/j.biortech.2017.10.036

Daughton CG, Ternes TA (1999) Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ Health Perspect 107:907–938. https://doi.org/10.1289/ehp.99107s6907

Dębska J, Kot-Wasik A, Namieśnik J (2004) Fate and analysis of pharmaceutical residues in the aquatic environment. Crit Rev Anal Chem 34:51–67. https://doi.org/10.1080/10408340490273753

Doll TE, Frimmel FH (2003) Fate of pharmaceuticals—photodegradation by simulated solar UV-light. Chemosphere 52:1757–1769. https://doi.org/10.1016/S0045-6535(03)00446-6

Du Y, Zhang S, Guo R, Chen J (2015) Understanding the algal contribution in combined UV-algae treatment to remove antibiotic cefradine. RSC Adv 5:59953–59959. https://doi.org/10.1039/C5RA10806C

Esterhuizen-Londt M, Schwartz K, Pflugmacher S (2016) Using aquatic fungi for pharmaceutical bioremediation: uptake of acetaminophen by Mucor hiemalis does not result in an enzymatic oxidative stress response. Fungal Biol 120:1249–1257. https://doi.org/10.1016/j.funbio.2016.07.009

Fan S, Wang Y, Li Y, Wang Z, Xie Z, Tang J (2018) Removal of tetracycline from aqueous solution by biochar derived from rice straw. Environ Sci Pollut Res 25:29529–29540. https://doi.org/10.1007/s11356-018-2976-0

Fortunato MS, Fuentes Abril NP, Martinefski M, Trípodi V, Papalia M, Rádice M, Gutkind G, Gallego A, Korol SE (2016) Aerobic degradation of ibuprofen in batch and continuous reactors by an indigenous bacterial community. Environ Technol 37:2617–2626. https://doi.org/10.1080/09593330.2016.1156773

Garcia-Rodríguez A, Matamoros V, Fontàs C, Salvadó V (2015) The influence of Lemna sp. and Spirogyra sp. on the removal of pharmaceuticals and endocrine disruptors in treated wastewaters. Int J Environ Sci Technol 12:2327–2338. https://doi.org/10.1007/s13762-014-0632-x

Garza-Campos B, Morales-Acosta D, Hernández-Ramírez A, Guzmán-Mar JL, Hinojosa-Reyes L, Manríquez J, Ruiz-Ruiz EJ (2018) Air diffusion electrodes based on synthetized mesoporous carbon for application in amoxicillin degradation by electro-Fenton and solar photo electro-Fenton. Electrochim Acta 269:232–240. https://doi.org/10.1016/j.electacta.2018.02.139

Gojkovic Z, Lindberg RH, Tysklind M, Funk C (2019) Northern green algae have the capacity to remove active pharmaceutical ingredients. Ecotoxicol Environ Saf 170:644–656. https://doi.org/10.1016/j.ecoenv.2018.12.032

Hachi M, Chergui A, Yeddou AR, Selatnia A, Cabana H (2017) Removal of acetaminophen and carbamazepine in single and binary systems with immobilized laccase from *Trametes hirsuta*. Biocatal Biotransformation 35:51–62. https://doi.org/10.1080/10242422.2017.1280032

He Y, Sutton NB, Rijnaarts HHH, Langenhoff AAM (2016) Degradation of pharmaceuticals in wastewater using immobilized TiO_2 photocatalysis under simulated solar irradiation. Appl Catal B Environ 182:132–141. https://doi.org/10.1016/J.APCATB.2015.09.015

Heberer T (2002) Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Lett 131:5–17. https://doi.org/10.1016/S0378-4274(02)00041-3

Inyang M, Gao B, Zimmerman A, Zhou Y, Cao X (2015) Sorption and cosorption of lead and sulfapyridine on carbon nanotube-modified biochars. Environ Sci Pollut Res 22:1868–1876. https://doi.org/10.1007/s11356-014-2740-z

Jelic A, Gros M, Ginebreda A, Cespedes-Sánchez R, Ventura F, Petrovic M, Barcelo D (2011) Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Res 45:1165–1176. https://doi.org/10.1016/J.WATRES.2010.11.010

Kanakaraju D, Motti CA, Glass BD, Oelgemöller M (2016) Solar photolysis versus TiO2-mediated solar photocatalysis: a kinetic study of the degradation of naproxen and diclofenac in various water matrices. Environ Sci Pollut Res 23:17437–17448. https://doi.org/10.1007/s11356-016-6906-8

Legrini O, Oliveros E, Braun AM (1993) Photochemical processes for water treatment. Chem Rev 93:671–698. https://doi.org/10.1021/cr00018a003

Li T, Han X, Liang C, Shohag MJI, Yang X (2015) Environmental technology sorption of sulphamethoxazole by the biochars derived from rice straw and alligator flag sorption of sulphamethoxazole by the biochars derived from rice straw and alligator flag. Environ Technol 36:245–253. https://doi.org/10.1080/09593330.2014.943299

Li G, Zhu W, Zhu L, Chai X (2016) Effect of pyrolytic temperature on the adsorptive removal of p-benzoquinone, tetracycline, and polyvinyl alcohol by the biochars from sugarcane bagasse. Korean J Chem Eng 33:2215–2221. https://doi.org/10.1007/s11814-016-0067-9

López-Serna R, Kasprzyk-Hordern B, Petrović M, Barceló D (2013) Multi-residue enantiomeric analysis of pharmaceuticals and their active metabolites in the Guadalquivir River basin (South Spain) by chiral liquid chromatography coupled with tandem mass spectrometry. Anal Bioanal Chem 405:5859–5873. https://doi.org/10.1007/s00216-013-6900-7

Magioglou E, Frontistis Z, Vakros J, Manariotis ID, Mantzavinos D, Magioglou E, Frontistis Z, Vakros J, Manariotis ID, Mantzavinos D (2019) Activation of Persulfate by biochars from valorized olive stones for the degradation of Sulfamethoxazole. Catalysts 9:419. https://doi.org/10.3390/catal9050419

Magureanu M, Mandache NB, Parvulescu VI (2015) Degradation of pharmaceutical compounds in water by non-thermal plasma treatment. Water Res 81:124–136. https://doi.org/10.1016/j.watres.2015.05.037

Marchlewicz A, Domaradzka D, Guzik U, Wojcieszyńska D (2016) Bacillus thuringiensis B1 (2015b) is a gram-positive Bacteria able to degrade naproxen and ibuprofen. Water Air Soil Pollut 227:197. https://doi.org/10.1007/s11270-016-2893-0

Maurer D, Koziel J, Kalus K, Andersen D, Opalinski S, Maurer DL, Koziel JA, Kalus K, Andersen DS, Opalinski S (2017) Pilot-scale testing of non-activated biochar for swine manure treatment and mitigation of Ammonia, hydrogen sulfide, odorous volatile organic compounds (VOCs), and greenhouse gas emissions. Sustainability 9:929. https://doi.org/10.3390/su9060929

Mondal S, Bobde K, Aikat K, Halder G (2016) Biosorptive uptake of ibuprofen by steam activated biochar derived from mung bean husk: equilibrium, kinetics, thermodynamics, modeling and eco-toxicological studies. J Environ Manag 182:581–594. https://doi.org/10.1016/J.JENVMAN.2016.08.018

Monteoliva-García A, Martín-Pascual J, Muñío MM, Poyatos JM (2019) Removal of carbamazepine, ciprofloxacin and ibuprofen in real urban wastewater by using light-driven advanced oxidation processes. Int J Environ Sci Technol 16:6005. https://doi.org/10.1007/s13762-019-02365-9

Morasch B, Bonvin F, Reiser H, Grandjean D, de Alencastro LF, Perazzolo C, Chèvre N, Kohn T (2010) Occurrence and fate of micropollutants in the Vidy Bay of Lake Geneva, Switzerland. Part II: Micropollutant removal between wastewater and raw drinking water. Environ Toxicol Chem 29. https://doi.org/10.1002/etc.222

Moreira FC, Soler J, Alpendurada MF, Boaventura RAR, Brillas E, Vilar VJP (2016) Tertiary treatment of a municipal wastewater toward pharmaceuticals removal by chemical and electrochemical advanced oxidation processes. Water Res 105:251–263. https://doi.org/10.1016/J.WATRES.2016.08.036

Murillo-Sierra JC, Ruiz-Ruiz E, Hinojosa-Reyes L, Guzmán-Mar JL, Machuca-Martínez F, Hernández-Ramírez A (2018) Sulfamethoxazole mineralization by solar photo electro-Fenton process in a pilot plant. Catal Today 313:175–181. https://doi.org/10.1016/j.cattod.2017.11.003

Muter O, P Erkons I, Selga T, Berzins A, Gudra D, Radovica-Spalvina I, Fridmanis D, Bartkevics V (2017) Removal of pharmaceuticals from municipal wastewaters at laboratory scale by treatment with activated sludge and biostimulation. Sci Total Environ 584–585:402–413. https://doi.org/10.1016/J.SCITOTENV.2017.01.023

Nguyen T-T, Bui X-T, Luu V-P, Nguyen P-D, Guo W, Ngo H-H (2017) Removal of antibiotics in sponge membrane bioreactors treating hospital wastewater: comparison between hollow fiber and flat sheet membrane systems. Bioresour Technol 240:42–49. https://doi.org/10.1016/J.BIORTECH.2017.02.118

OECD Indicators (2017) Health at a Glance 2017, OECD Publishing, Paris. https://doi.org/10.1787/health_glance-2017-en

Perazzolo C, Morasch B, Kohn T, Magnet A, Thonney D, Chèvre N (2010) Occurrence and fate of micropollutants in the Vidy Bay of Lake Geneva, Switzerland. Part I: Priority list for environmental risk assessment of pharmaceuticals. Environ Toxicol Chem 29. https://doi.org/10.1002/etc.221

Pereira VJ, Linden KG, Weinberg HS (2007) Evaluation of UV irradiation for photolytic and oxidative degradation of pharmaceutical compounds in water. Water Res 41:4413–4423. https://doi.org/10.1016/J.WATRES.2007.05.056

Perini JA d L, Silva BC e, Tonetti AL, Nogueira RFP (2017) Photo-Fenton degradation of the pharmaceuticals ciprofloxacin and fluoxetine after anaerobic pre-treatment of hospital effluent. Environ Sci Pollut Res 24:6233–6240. https://doi.org/10.1007/s11356-016-7416-4

Perini JAL, Tonetti AL, Vidal C, Montagner CC, Nogueira RFP (2018) Simultaneous degradation of ciprofloxacin, amoxicillin, sulfathiazole and sulfamethazine, and disinfection of hospital effluent after biological treatment via photo-Fenton process under ultraviolet germicidal irradiation. Appl Catal B Environ 224:761–771. https://doi.org/10.1016/j.apcatb.2017.11.021

Petrie B, Barden R, Kasprzyk-Hordern B (2015) A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. Water Res 72:3–27. https://doi.org/10.1016/j.watres.2014.08.053

Petrović M, Gonzalez S, Barceló D (2003) Analysis and removal of emerging contaminants in wastewater and drinking water. TrAC Trends Anal Chem 22:685–696. https://doi.org/10.1016/S0165-9936(03)01105-1

Popa C, Favier L, Dinica R, Semrany S, Djelal H, Amrane A, Bahrim G (2014) Potential of newly isolated wild *Streptomyces* strains as agents for the biodegradation of a recalcitrant pharmaceutical, carbamazepine. Environ Technol 35:3082–3091. https://doi.org/10.1080/09593330.2014.931468

Prasertkulsak S, Chiemchaisri C, Chiemchaisri W, Yamamoto K (2019) Removals of pharmaceutical compounds at different sludge particle size fractions in membrane bioreactors operated under different solid retention times. J Hazard Mater 368:124–132. https://doi.org/10.1016/J.JHAZMAT.2019.01.050

Rajaram T, Das A (2008) Water pollution by industrial effluents in India: discharge scenarios and case for participatory ecosystem specific local regulation. Futures 40:56–69. https://doi.org/10.1016/J.FUTURES.2007.06.002

Richardson ML, Bowron JM (1985) The fate of pharmaceutical chemicals in the aquatic environment. J Pharm Pharmacol 37(1):1–12. https://doi.org/10.1111/j.2042-7158.1985.tb04922.x

Rodarte-Morales AI, Feijoo G, Moreira MT, Lema JM (2012) Biotransformation of three pharmaceutical active compounds by the fungus Phanerochaete chrysosporium in a fed batch stirred

reactor under air and oxygen supply. Biodegradation 23:145–156. https://doi.org/10.1007/s10532-011-9494-9

Salem NA, Yakoot SM (2016) Non-steroidal anti-inflammatory drug, ibuprofen adsorption using Rice straw based biochar. Int J Pharmacol 12:729–736. https://doi.org/10.3923/ijp.2016.729.736

Santoke H, Song W, Cooper WJ, Greaves J, Miller GE (2009) Free-radical-induced oxidative and reductive degradation of fluoroquinolone pharmaceuticals: kinetic studies and degradation mechanism. J Phys Chem A 113:7846–7851. https://doi.org/10.1021/jp9029453

Sardar K, Rashid MA, Khandoker MR, Khan A (2016) Anticonvulsants and antidepressants in chronic pain management. J Recent Adv Pain 2:90–93. https://doi.org/10.5005/jp-journals-10046-0050

Sarganas G, Knopf H, Grams D, Neuhauser HK (2016) Trends in antihypertensive medication use and blood pressure control among adults with hypertension in Germany. Am J Hypertens 29:104–113. https://doi.org/10.1093/ajh/hpv067

Saritha P, Aparna C, Himabindu V, Anjaneyulu Y (2007) Comparison of various advanced oxidation processes for the degradation of 4-chloro-2 nitrophenol. J Hazard Mater 149:609–614. https://doi.org/10.1016/J.JHAZMAT.2007.06.111

Sauvêtre A, Schröder P (2015) Uptake of carbamazepine by rhizomes and endophytic bacteria of *Phragmites australis*. Front Plant Sci 6:83. https://doi.org/10.3389/fpls.2015.00083

Shemer H, Kunukcu YK, Linden KG (2006) Degradation of the pharmaceutical metronidazole via UV, Fenton and photo-Fenton processes. Chemosphere 63:269–276. https://doi.org/10.1016/J.CHEMOSPHERE.2005.07.029

Song W, Cooper WJ, Mezyk SP, Greaves J, Peake BM (2008) Free radical destruction of β-blockers in aqueous solution. Environ Sci Technol 42:1256–1261. https://doi.org/10.1021/es702245n

Sun J, Luo Q, Wang D, Wang Z (2015) Occurrences of pharmaceuticals in drinking water sources of major river watersheds, China. Ecotoxicol Environ Saf 117:132–140. https://doi.org/10.1016/j.ecoenv.2015.03.032

Tomić M, Micov A, Pecikoza U, Stepanović-Petrović R (2017) Clinical uses of nonsteroidal anti-inflammatory drugs (NSAIDs) and potential benefits of NSAIDs modified-release preparations. In: Microsized nanosized carriers nonsteroidal anti-inflammatory drugs, pp 1–29. https://doi.org/10.1016/B978-0-12-804017-1.00001-7

Vasiliadou IA, Molina R, Pariente MI, Christoforidis KC, Martinez F, Melero JA (2019) Understanding the role of mediators in the efficiency of advanced oxidation processes using white-rot fungi. Chem Eng J 359:1427–1435. https://doi.org/10.1016/J.CEJ.2018.11.035

Ventola CL (2015) The antibiotic resistance crisis: Part 1: causes and threats. P T 40:277–283

Vithanage M, Mayakaduwa SS, Herath I, Ok YS, Mohan D (2016) Kinetics, thermodynamics and mechanistic studies of carbofuran removal using biochars from tea waste and rice husks. Chemosphere 150:781–789. https://doi.org/10.1016/J.CHEMOSPHERE.2015.11.002

Wang Y, Lu J, Wu J, Liu Q, Zhang H, Jin S, Wang Y, Lu J, Wu J, Liu Q, Zhang H, Jin S (2015) Adsorptive removal of Fluoroquinolone antibiotics using bamboo biochar. Sustainability 7:12947–12957. https://doi.org/10.3390/su70912947

Wang X, Zhang J, Chang VWC, She Q, Tang CY (2018) Removal of cytostatic drugs from wastewater by an anaerobic osmotic membrane bioreactor. Chem Eng J 339:153–161. https://doi.org/10.1016/J.CEJ.2018.01.125

Wenk J, von Gunten U, Canonica S (2011) Effect of dissolved organic matter on the transformation of contaminants induced by excited triplet states and the hydroxyl radical. Environ Sci Technol 45:1334–1340. https://doi.org/10.1021/es102212t

WHO (2015) The top 10 causes of death [WWW document]. http://who.int/mediacentre/factsheets/fs310/en/

Wittebolle L, Boon N, Vanparys B, Heylen K, De Vos P, Verstraete W (2005) Failure of the ammonia oxidation process in two pharmaceutical wastewater treatment plants is linked to shifts in the bacterial communities. J Appl Microbiol 99:997–1006. https://doi.org/10.1111/j.1365-2672.2005.02731.x

Xu B, Xue G, Yang X (2018) Isolation and application of an ibuprofen-degrading bacterium to a biological aerated filter for the treatment of micro-polluted water. Front Environ Sci Eng 12:15. https://doi.org/10.1007/s11783-018-1080-5

Yao Y, Zhang Y, Gao B, Chen R, Wu F (2018) Removal of sulfamethoxazole (SMX) and sulfapyridine (SPY) from aqueous solutions by biochars derived from anaerobically digested bagasse. Environ Sci Pollut Res 25:25659–25667. https://doi.org/10.1007/s11356-017-8849-0

Yi S, Gao B, Sun Y, Wu J, Shi X, Wu B, Hu X (2016) Removal of levofloxacin from aqueous solution using rice-husk and wood-chip biochars. Chemosphere 150:694–701. https://doi.org/10.1016/J.CHEMOSPHERE.2015.12.112

Zeng Z, Tan X, Liu Y, Tian S, Zeng G, Jiang L, Liu S, Li J, Liu N, Yin Z (2018a) Comprehensive adsorption studies of doxycycline and ciprofloxacin antibiotics by biochars prepared at different temperatures. Front Chem 6:80. https://doi.org/10.3389/fchem.2018.00080

Zeng Z, Tian S, Liu Y, Tan X, Zeng G, Jiang L, Yin Z, Liu N, Liu S, Li J (2018b) Comparative study of rice husk biochars for aqueous antibiotics removal. J Chem Technol Biotechnol 93:1075–1084. https://doi.org/10.1002/jctb.5464

Bioremediation of Saline Soil by Cyanobacteria

17

Jay Bergi and Ratna Trivedi

Abstract

Cyanobacteria, of the domain bacteria, are one of the earliest monophyletic groups or molecular organs and are able to grow in all light-exposed habitats on the Earth. Cyanobacteria have developed mechanisms to adapt to a broad range of environmental factors during their long evolution. This chapter deals with cyanobacteria mechanisms in the soil nutrient cycle along with the phototrophic mechanisms that give them UV protection in various land-use patterns such as soil salinity stress and use of cyanobacteria in halophilic environments. Agricultural productivity is significantly increased by this cyanobacterial technology. The adverse effects of indiscriminate use of chemical fertilizers leads to diminished soil productivity and environmental quality. As a substitute for chemical fertilizer, cyanobacteria are economical, ecologically sustainable, and improve crop productivity and quality. The most preferable cyanobacterial biofertilizers are an effective consortium of *Azollas* spp. In an ecosystem, nitrogen fixation by free living cyanobacteria also significantly supplements soil nitrogen. Cyanobacterial species inoculate soil with a suspension of each species or a combination of species improving the germination percentage and influencing the other measured biochemical characters along with photosynthetic pigment segments of plants. This chapter also deals with phosphorus recycling by cyanobacteria, which is an important nutrient for saline soils and its crops. Consortium development in biofertilizers with cyanobacteria shows more potential, as it requires minimum dose and also increases nutrient transports in plants through bioaugmentation. Moreover, carbohydrates and

J. Bergi
Department of Biotechnology, Shree Ramkrishna Institute of Computer Education and Applied Sciences, Surat, Gujarat, India

R. Trivedi (✉)
Department of Environmental Science, Shree Ramkrishna Institute of Computer Education and Applied Sciences, Surat, Gujarat, India

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_17

proteins augmented with Na^+ are triggered with UV-A, UV-B, and MAA. The combination of cyanobacterial cells with half the suggested quantity of the chemical fertilizer was usually more active than adding the full dose of the plant growth promoting chemicals.

Keywords
Salinity stress · Halophilic microorganisms · Cyanobacteria · Nutrient cycling

17.1 Soil Salinity and Nutrient Cycling by Halophilic Microorganism

Soil salinization is a process of increasing the dissolved salts in the soil profile. Soil salinity has a significant role in soil health, which in turn affects soil productivity. Soil salinity values directly impact the physical and chemical properties along with growth and diversity of microorganisms, such as such as plants, microbes, protozoa, and nematodes, as well as the endo or exo rhizospheric nature of these microorganisms to survive. The soil salinity also influences the osmotic pressure. The function of various biochemical processes of the main macromolecules of life on Earth, namely carbohydrates, proteins, and nucleic acids, are based on carbon, water, and simple organic compounds such as CO_2, which are processed in complex organic structures based on carbon (Teal et al. 1979). Other than these elements—carbon, oxygen, and hydrogen—nitrogen is found in nucleic acids, amino acids, and proteins, while phosphorous is a major component of the backbone of DNA, RNA, lipids, and bioenergetic storage molecules, e.g., ATP. Other than organic phosphates, sulfur is also found as a major element in certain amino acids and proteins; all of these elements are immobilized and mineralized throughout biogeochemical cycles in the ecosystem (Fig. 17.1).

In various stages of natural biological life cycles, many geochemical cycling processes require different elements in diverse chemical states. Inorganic phosphorus occurs in living systems, but once it enters into living organisms, the

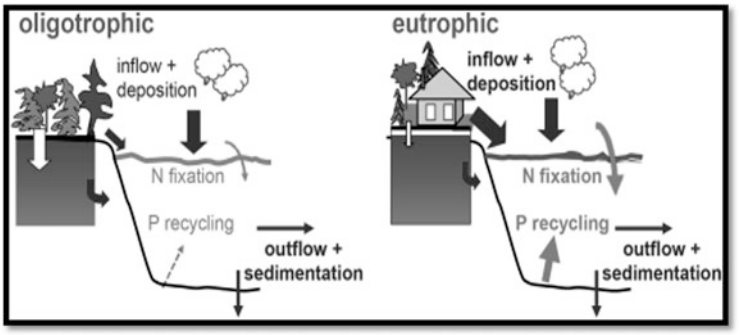


Fig. 17.1 Schematic of N and P fluxes in oligotrophic and eutrophic lakes

biotransformation process of the phosphorus renders it in simpler and more complex forms (Lipok et al. 2007). Inorganic phosphates are very widely distributed in nature, and are frequently present as insoluble salts, which alter the salinity of soils. Therefore, due to inadequate availability in surrounding habitat, phosphates have limited intervention in the cellular structure. The characteristics of phosphates prevent uncontrolled growth because it must be converted from the insoluble to soluble form (Zulpa et al. 2003). During this biochemical fermentation process, production of various acids is achieved that may be utilized by bacteria. Addition of soluble phosphates may influence land artificial fertility, in the form of either organophosphate pesticides or plant growth promoters that increase the crop yield. Many industries manufacturing detergents also use phosphates as one of the main components, which can add chemicals to rivers and lakes as industrial runoff (Ahmad and Winter 1968). Additionally, these phosphates influence the artificial growth of biological life that leads to over growth of algae in affected waters furthermore leading to algal blooms (Forlani et al. 2008).

Cyanobacteria, of the domain bacteria, are one of the earliest monophyletic groups or molecular organs and are able to grow in all light-exposed habitats on the Earth. Cyanobacteria have developed mechanisms to adapt to a broad range of environmental factors during their long evolution (Nisha et al. 2007).

The three main mechanisms through which the recycling of P is achieved by cyanobacteria are: (1) cyanobacteria that overwinter on or near lake sediments access sediment P and then transport it upward during seasonal recruitment; (2) in stratified lakes, cyanobacteria sink to the hypolimnion, acquire P, and then rise back to the surface during diel vertical migrations; and (3) benthic cyanobacteria enhance P release from sediments, increasing near-sediment P and also storing it in bodies (Fig. 17.2).

These can eliminate other plants of light; therefore, they can prevent development and metabolism, affecting both natural water and ecosystems. Certain algae behave as harmful algal blooms (HABs) that may also be toxic to animals. Various microorganisms are involved in the biological transformation of metal ions and cycling of non-metal elementals.

For the past two decades, biofertilizers, such as *Azotobacter, Azospirillium, Vesicular Arbuscular Mycorrhiza* (VAM), and cyanobacteria, were used extensively to minimize the frequent use of chemical fertilizers to improve the soil status and to be able to plant in a saline environment (Singh and Singh 1987; Singh et al. 2011).

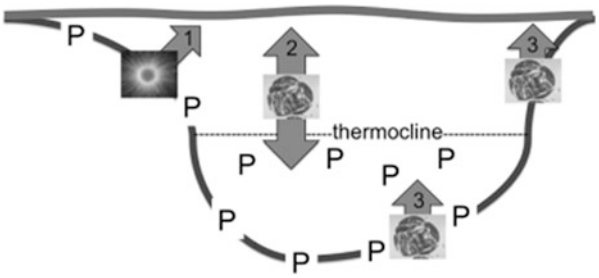


Fig. 17.2 Mechanism of P affected by cyanobacteria

These biofertilizers are testified to be eco-friendly, economical, and beneficial in saline soil. The potential of nitrogen-fixing (NF) bacteria including halophilic rhizobia to form a symbiotic relationship with leguminous plants and fix atmospheric nitrogen has been exploited in the field to meet the nitrogen requirement of the latter (Al-Hasan et al. 1998, 2001). This process provides an alternative to the use of nitrogenous fertilizer, whose excessive and imbalanced use over the decades has contributed to greenhouse gas emission (N_2) and underground water leaching, and it was shown to be very effective in both saline soils as well as saline resistant plants (Dalton and Kramer 2006).

About a 50% reduction of salt stress for corn and wheat can be obtained by involving halophilic microorganisms in salt leaching. Microorganisms play a significant role in alleviating salt stress in plants, resulting in increased crop yield. Plant growth-promoting (PGP) bacteria are a group of microorganisms that directly or indirectly enhance the growth of plants by colonization at their roots (Bohlool and Wiebe 1978). Halophilic microorganisms present in saline stressed soils indirectly influence the growth of plants by production of special compounds such as siderophore, indole acetic acid, and HCN that are furthermore used by plants for their own growth as well as to continue the solubilization of minerals and the breakdown of organic matter for easy uptake. Bioavailability of Fe^{2+} or Fe^{3+} synthesis and phytohormones, such as cytokinins, auxins, and gibberellins, can improve atmospheric nitrogen, increasing several stages of plant growth.

Certain iron dependent bacterial genus such as *Gallionella* and *Thiobacillus* have the potential for oxidizing ferrous (Fe^{2+}) ions into ferric (Fe^{3+}) ions. Moreover, many other halophilic bacteria can decrease a small amount of ferric iron to its ferrous state, and these microorganisms are included in a group of iron respiring microorganisms that obtain their energy by respiration where the final electron accepter is a ferric ion not an oxygen (Cohen 2002).

Several marine microorganisms' nuclei have been proposed for use in a biodesalination process that consists of the establishment of a free natural living basin of salt in seawater that can oblige as an ion exchanger. Most marine organisms already contain such a reservoir because they actively exclude and eliminate salt from their bodies. The mechanism of nutrient transport through the phototrophic marine microbes, cyanobacteria, utilizes the protein porins in their cell membrane (Fig. 17.3) that allows Na^+ movements within it. Some cell membrane proteins possess ATP as carrier molecules for nutrient transport systems. ATP powers Na^+ movement through ATPase either directly through Na^+-pumping or indirectly through H^+-pumping to the enzyme, which generates a proton motive force that drives H^+/Na^+ antiport.

During the growth and life phase, a saline environment has been generated by the cell with low salt reservoir through an active transportation mechanism in which transport proteins transport Na^+ across the plasma membrane. Here, during this transport system, ATPases acts as Na^+-ATPases or Na^+/H^+ antiportadors that establish pH gradients for ATPase. Na^+ export from the cytoplasm therefore relies on ATP and the proton motive force generated from light energy captured by photosystems and chemiosmosis in the thylakoid membrane. During the desalination

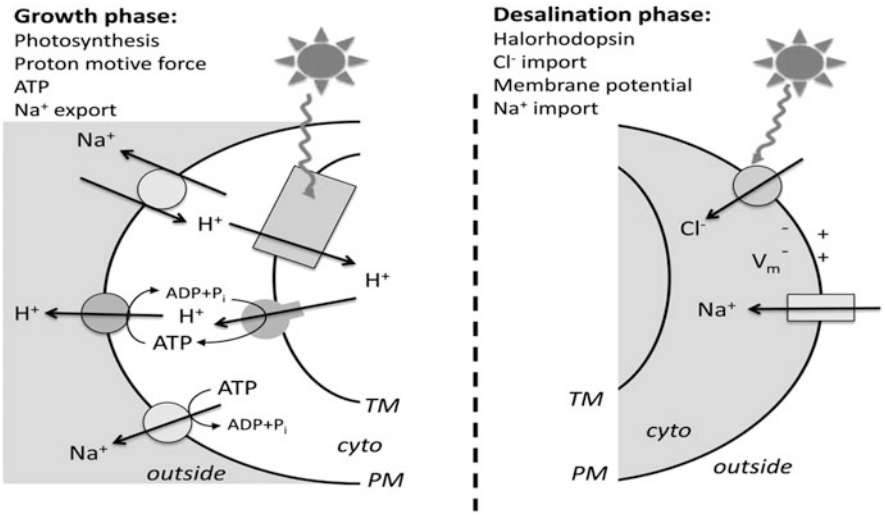


Fig. 17.3 Desalination process: transportation and energization of Na^+ in different phases

phase, Na^+ elution is halted through inhibition of photosynthetic ATP production where light energy is utilized directly by halothodopsin to pump chloride into the cells.

The requirement for ATP offers an opportunity to halt Na^+ export by reducing internal ATP stores using the environmental changes detailed above (e.g., omitting photosynthetically efficient wavelengths from the light spectrum, depleting phosphate, altering pH, or chelating Mg^{2+}, Fe^{2+}, or other essential metals) (El-Bestawy et al. 2007). Due to the change in the cell structural and metabolic development pattern, cultivation was achieved from an open to closed system after the high cell density led to a speedy decline in nutrient source and the ATP stores.

Magnetite metabolized in Magnetotactic bacteria, *Aquaspirillum magnetotactium,* requires conversion of the magnetic salt of magnetite from iron (Coutinho and Seeliger 1984). The mechanisms of biological transformation of manganese ions are similar to those of ions transformation. The bacteria involved in such biotransformations are known as biological magnets (El-Enany and Issa 2000). Life on Earth would not be possible without essential nutrients that are immobilized, mineralized via microorganisms in geochemical cycles, as well as entered into the food chain ending in a complex food web; all of these mechanisms are considered biotransformations (Cohen 2002; Converti et al. 2009).

The formation of microbial ecosystems with various communities of lithotrophic microorganism, such as phototrophs, chemotrophs, and chemoorganotrophs, have cyclic nutrient movement throughout these communities. Carbon dioxide is utilized as the supply to derive energy from for complex organic molecules, whereas lithotrophs obtain energy from inorganic compounds. Phototrophs utilize light as the sole source of energy, while chemotrophic microorganisms can survive in the dark, obtaining their energy from chemical compounds. All substances have diverse

modes of cycles with different rates, and it has been thought that it took two million years to separate a molecule of water on the planet, which is utilized for photosynthesis, resulting in the formation of life. The process of photosynthesis provides facilities to plants or photosynthetic microbes.

Atmospheric oxygen is released during the process of photosynthesis. According to scientific study, all oxygen present in the atmosphere is of biological origin, and its cycling is thought to take about 2000 years. During photosynthesis, CO_2 is produced from various organic compounds; furthermore, CO_2 is also released during various bioprocesses, such as respiration and fermentation. Atmospheric carbon dioxide takes about 300 years to cycle, and life without photosynthesis is difficult to imagine, because without photosynthesis, plants cannot survive. All these biochemical processes provide us with oxygen that we need to survive, and photosynthesis is also accountable for the manufacturing of molecular oxygen on the Earth. Oxygen can also be toxic to life, which is known as oxygen toxicity. According to evolution theory, formation of life on the Earth developed under the little or no oxygen condition. Adaptation mechanisms also developed in aerobic organisms so that they can protect themselves during oxygen toxicity. Other similar adaptations have been observed in the Atlantic and Pacific Oceans, where at certain dark depths no sunlight penetrates—hydrothermal vents—but life sustains. This indicates that life is not only dependent on light (Ali and Basit 1993). Thus, the chemolithotrophic bacteria present utilize heat and chemical energy as a source of nutrients; furthermore, these microbial communities or chemolithotrophic bacteria supply food for a diverse group of invertebrates.

Recently, it was observed that non-leguminous plants, such as rice, sugarcane, wheat, and maize, form an extended niche for various species of NF bacteria. Light is one of the most important factors determining cyanobacterial growth in their natural habitats since cyanobacteria are predominantly photoautotrophic microorganisms. In addition, light is an environmental factor controlling orientation and habitat selection. The cyanobacterial populations in rice paddy fields, particularly in the tropics, are often exposed to high white light and UV-B irradiances. Non-leguminous plants, such as rice, maize, and wheat, belonging to the Poaceae family, form staple foods for the approximately 7.8 billion people around the world. An exponential rise in world population indicates the need for increased crop production. Cyanobacteria are a diverse group of prokaryotes, widely distributed in fresh water, marine, and terrestrial environments. They are free living photosynthetic bacteria that exist singly or in colonies, and some of them are in filament forms (Dalton and Kramer 2006).

Cyanobacteria (blue-green algae) and eukaryote algae occur in freshwater, marine, and terrestrial (soil) habitats. In fact, these microorganisms comprise most of the world's biomass (Borowitzka 1999). Although most cyanobacteria are photoautotrophic, some are facultative heterotrophs, capable of growing on certain substrates in darkness. Also, some are non-phototrophic and hence are obligate heterotrophs. It is known that cyanobacteria supply more nitrogen in wetland rice fields in tropical regions than in dry-land fields, and this is attributable to the unique characteristics of wetland rice fields: along with water, there is a natural supply of plant nutrients, especially N, which encourages general plant growth, and the soil pH

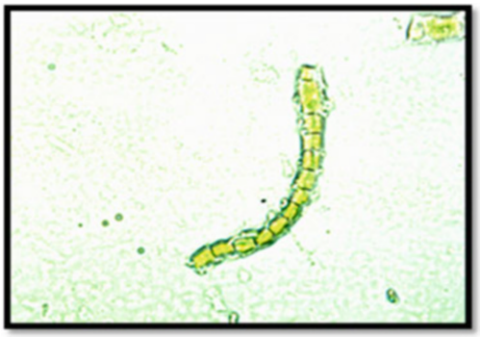


Fig. 17.4 Microscopic observation of cyanobacterial heterocyst (×45)

Fig. 17.5 Worldwide cultivation of *Azolla* spp.

is more neutral. Fixation of nitrogen has been done by free-living cyanobacteria and Azolla–Anabaena. These microorganisms perform this complex process of nitrogen development at the surface of soil as well as in floodwater. The majority of free-living cyanobacteria are heterocystous and nitrogen fixing (Fig. 17.4) and contribute an average of 20–30 kg N ha7', whereas the value is up to 600 kg ha1 for the *Azolla Anabaena* system (Fig. 17.5).

Cyanobacteria are able to synthesize and excrete organic/growth-promoting substances, which has great application in agro-industries. Over the past two or three decades, extensive studies have been done on cyanobacteria, along with various important fundamental and applied aspects of both kinds of cyanobacterial biofertilizers (the free-living cyanobacteria and the cyanobacterium Anabaena azollae in symbiotic association with the water fern Azolla), which include strain

identification, isolation, purification, and culture; laboratory analyses of their N_2-fixing activity and related physiology, biochemistry, and energetics; and identification of the structure and regulation of nitrogen fixing (nif) genes and the nitrogenase enzyme (Nozzi et al. 2013; Pandey et al. 2014). Certain free-living cyanobacterial strains possess a constitutive nitrogen fixation complex that has been improved through the technique of mutagenesis. Many scientist reported that certain symbiotic microorganisms such as Azolla–Anabaena have mutualistic nitrogen fixation processes. Some strains of cyanobacteria with respect to constitutive N_2 fixation and resistance to the non-congenital agronomic factors have been achieved. Some cyanobacteria produce halophillic extracellular enzymes under halophillic conditions, illustrating adaptation of organisms under some ecological conditions. Therefore, the cyanobacteria under investigation were screened for their enzymatic activity (Vaishampayan et al. 2001).

Due to high salt concentrations, two crucial problems occur: (a) lower water availability and (b) increased concentrations of ions. Cytoplasm has toxic effects on cellular metabolism in the presence of large concentrations of inorganic compounds, to ensure water uptake via osmosis active compounds concentrations must be higher than that of the surrounding medium. Two main strategies have been developed by microorganisms for the osmotic acclimation: (a) salt in strategy (b) salt out strategy, which create more nutrient transportation to microorganisms. Certain scientists studied salt strategy, in which higher concentrated salt ions (Mainly potassium chloride, 2–3 Molar) accumulated along with inorganic ions and entered in cytoplasm to confirm pressure and water uptake. Remarkably, in cyanobacteria, the chemical structure of the major compatible solute correlates with the final salt tolerance limit (Costa et al. 2008). Freshwater strains (resisting up to 600 mM NaCl equivalent to full seawater conditions) accumulate the sugars trehalose and/or sucrose. These compounds are also effective protectors against desiccation.

Morphological microbial diversity of cyanobacteria studied under unicellular, unbranched, colonial, pseudoparenchymatochstous, and heterocystous and heterotrichous forms (Fig. 17.6).

Researchers practically proved that heterocysts are the specialized cells that contain the fixing mechanism (Kumar and Singh 2016). Extensive studies have

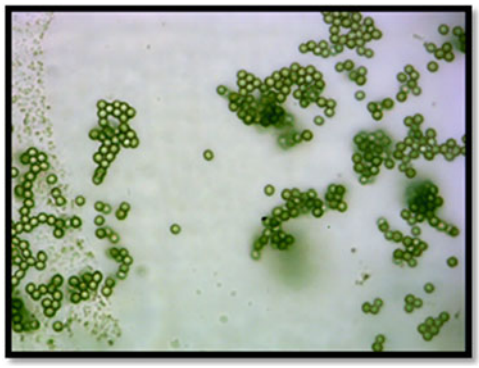


Fig. 17.6 Light micrograph of *Synechococcus* (×45)

Table 17.1 Important nitrogen-fixing Cyanobacterial genera

Unicellular filamentous	Non-heterocystous	Filamentous heterocystous
Aphanothece	*Lyngbya*	*Anabaenaa*
Chroococcidiopsis	*LPP group*	*Anabaenopsis*
DermocarpaMicrocoleu	*Microcoleuschthonoplastes*	*Aulosira*
Gloeocapsa (Gloeothece)	*Myxosarcina*	*Calothrixa*
Myxosarcina	*Oscillatoria*	*Camptylonema*
Pleurocapsa group	*Plectonemaboryanum*	*Chlorogloea*
Synechococcus	*Pseudoanabaena*	*Chlorogloeopsis*
Xenococcus	*Schizothrix*	*Cylindrospermum*
	Trichodesmium	*Fischerellaa*
		Gloeotrichia
		Haplosiphon
		Mastigocladus
		Nodularia
		Nostoca
		Nostochopsis
		Rivularia
		Scytonemaa
		Scytonematopsis
		Stigonema
		Tolypothrix
		Westiella
		Westiellopsis

been carried out on heterocyst, and it was proved by immunolabeli that heterocysts are the location of the enzyme nitrogenase. The methodology for quantification of nitrogen fixation was also developed; a mass spectrophotometric technique for isotopic nitrogen (N'5) and the acetylene-reduction technique have been in use worldwide for assaying the activity enzyme nitrogenase through estimation of total nitrogen over a period of time. On the basis of these techniques, the N_2-fixing cyanobacteria known so far have been classified into three groups: unicellular, filamentous nonheterocystous, and filamentous heterocystous (Table 17.1) (Vijayakumar 2012).

Other major rice cyanobacteria are very effective in saline soils: *Nostoc commune*, embeds in a dense matrix of mucilage and forms a ball like structure; *Scytonema* sp., showing hetero-cysts and characteristic typical false branching; *Calothix* sp., showing characteristic terminal heterocysts; *Nodularia* sp., with vegetative cells and heterocysts; *Gloeotrichia* sp., characterized by a ball-like circular assembly of filaments resembling radiating rays; and *Lyngbya* sp., having a typical yellow-brown coloration of the mucilage sheath due to the presence of scytonemin. These organisms also show beneficial impact in saline environment, including water, paddy, and soils. The larger population of N_2-fixing cyanobacteria comprises the filamentous and hetero-cystous forms (Table 17.1). *Nostoc commune* has been found

to be the most important source of biologically fixed N in the Arctic Spitsbergen. Activity of sheets of *Nostoc commune* assayed in situ showed linear relationships with their moisture content and assay temperature. Similarly, more than 100 strains of heterocystous cyanobacteria, belonging to the genera Anabaena, Nostoc, Nodularia, Cylindrospermum,Scytonema, Calothrix, Anabaenopsis, Mastigocladus, Fischerella, Tolypothrix, Aulosira, Stigonema, Haplosiphon, Chlorogloeopsis, Camptylonema, Gloeotrichia, Nostochopsis, Rivularia, Schytonematopsis, Westiella, Westiellopsis, Wollea, and Chlorogloea, have been found to be efficient N_2 fixers.

17.1.1 Photoprotective Mechanisms in Cyanobacteria in Saline Environment

Since it is clear that microorganisms evolved and microbial mats were well established early in the Precambrian, some mechanism(s) must have been functioning to protect these organisms from the deleterious effects of UV radiation. There are at least five adaptation strategies by which cyanobacteria try to avoid high white light and ultraviolet radiation stress (Fig. 17.7):

1. Production of ultraviolet-absorbing substances such as mycosporine-like amino acids (MAAs) and scytonemin.
2. Escape from ultraviolet radiation by migration into habitats with reduced light exposure. Such strategies include phototactic, photokinetic, and photophobic responses, vertical migration into deeper strata of mat communities and sinking and floating behavior by a combination of gas vacuoles and ballast. This allows them to change their position in the water column as environmental conditions change and thus to always ensure a nearly constant external environment.
3. Production of quenching agents such as carotenoids or systems such as superoxide dismutase react with and thereby neutralize the highly toxic reactive oxygen species produced by ultraviolet-B radiation.
4. Availability of a number of repair mechanisms such as photoreactivation and light-independent nucleotide excision repair of DNA and UV-A/blue-light mediated repair of the photosynthetic apparatus.
5. A number of cyanobacteria have the ability to vary their phycobiliprotein composition (phycocyanin/phycoerythrin ratio), which allows regulation of the balance of wavelengths of light absorbed, a phenomenon known as chromatic adaptation.

Cyanobacteria display a variety of strategies for protection against the detrimental effects of UV. Three general types of stress responses are found among cyanobacteria: stress avoidance, stress defence, and repair mechanisms.

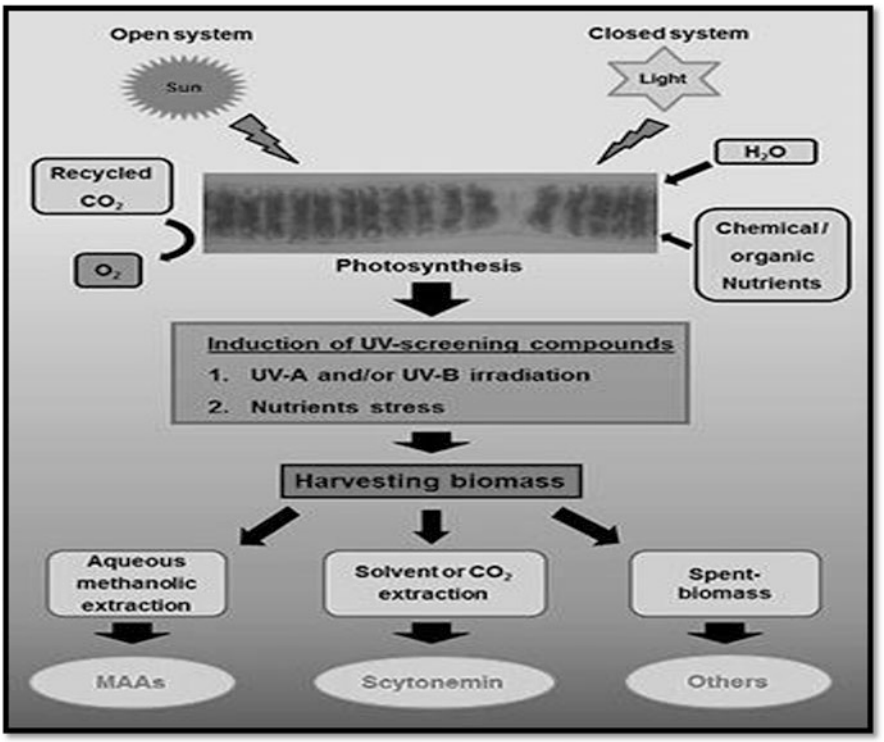


Fig. 17.7 Schematic diagram showing cyanobacterial bio-factory for ultraviolet-screening compounds production within bio-refinery concept. *UV* ultraviolet, *MAAs* mycosporine-like amino acids

17.1.2 UV-Stress Avoidance

Motile cyanobacteria can escape from high solar radiation by downward migration into mat communities or by sinking deeper into the water column. Although most of the filamentous cyanobacteria are motile by gliding, information on the influence of UV on vertical migration of cyanobacteria is limited. Daily vertical migration to avoid periods of incident high solar irradiance has been reported for *Oscillatoria* sp., and Spirulina cf. subsala. The vertical migration of Microcoleuschthonoplastes has been shown to be UV and PAR inducible. Since UV-B was by far the most effective waveband promoting migration, it has been suggested that M. chthonoplastescan sense UVB directly. UV-B-induced vertical migration may be an effective strategy to minimize UV-induced damage. On the other hand, migration led to a decreased overall productivity of the mat ecosystem (Bebout and GarciaPichel 1995). Further investigations are needed to assess the effect of migration on net primary productivity of mats.

17.1.3 UV-Stress Defence in Natural and Saline Environments

Synthesis of UV-absorbing compounds is an important mechanism preventing UV photodamage. Several studies provide evidence that mycosporine amino acids (MAA) protect cyanobacteria and other lower organisms by absorbing harmful UV radiation. Mycosporine amino acids are water-soluble, substituted cyclohexenones that are linked to amino acids and amino alcohols, and have absorption maxima between 310 and 360 nm. Their synthesis probably originates from the first part of the shikimate pathway. MAAs are widely distributed among cyanobacteria. However, the relative protection against UV-B damage provided by MAAs depends on the species and the location of the pigments therein. Significant, but limited, protection has been reported for various cyanobacteria with MAAs located in the cytoplasm. In these cases, only 10–26% of the photons are absorbed by the pigment. In *Nostoc commune*, MAAs are thought to play an important role in photoprotection because the MAAs are located in the extracellular glycan. Two out of three photons are absorbed by the pigment before cell membranes or targets within the cell are reached. Two UVA/B-absorbing pigments of *N. commune* with absorption maxima at 312 and 335 nm were found in colonies exposed to high solar radiation. One of them was the first mycosporine reported to be covalently linked to oligosaccharides (Fig. 17.8) and shown to be located in the extracellular glycan. The pigment provides protection, mainly by absorbing the harmful radiation.

Removal of toxic oxygen species can be another defense strategy. Carotenoids are well known for their antioxidant activity. They remove singlet oxygen and triplet chlorophyll and inhibit lipid peroxidation. Their photoprotective role against high intensity visible light is evident. UV-A and UV-B can cause oxidative stress by photodynamically generating reactive oxygen intermediates. An increase in the carotenoid Chl a ratio of cyanobacteria has been reported in response to UV-A and UV-B radiation. In the cyanobacterium *N. commune*, changes in the carotenoid pattern in response to UV-B irradiation have been observed and myxoxanthophyll and echinenone were suggested to act as outer membrane-bound UV-B photoprotectors.

In saline environment, mechanisms for UV defense require that active Na^+ export comes to a standstill, there will be net Na^+ influx into a cell until equilibrium with the external medium is reached. Further extraction of Na^+ from the medium will then require an energy source. To prevent renewal of Na^+ export, the energy-harvesting system employed during this phase should not use ATP as an intermediate. Good candidates for ATP-independent light-powered biological batteries are halorhodopsin (Hr) proteins. Hrs naturally occur in extremely salt-tolerant archaea (haloarchaea) and are membrane-integral proteins of the rhodopsin superfamily that form a covalent bond with the carotenoid-derived chromophore all-trans-retina.

Synthesis of extracellular polysaccharides may also help to limit UV damage. Bacterial extracellular polysaccharides (EPS) have been reported to provide protection against desiccation, phagocytosis, antibody recognition, and lysis by viruses. The EPS-containing sheath of cyanobacteria forms a buffer zone between the environment and the cell. Recently, it has been reported that UV-B irradiation

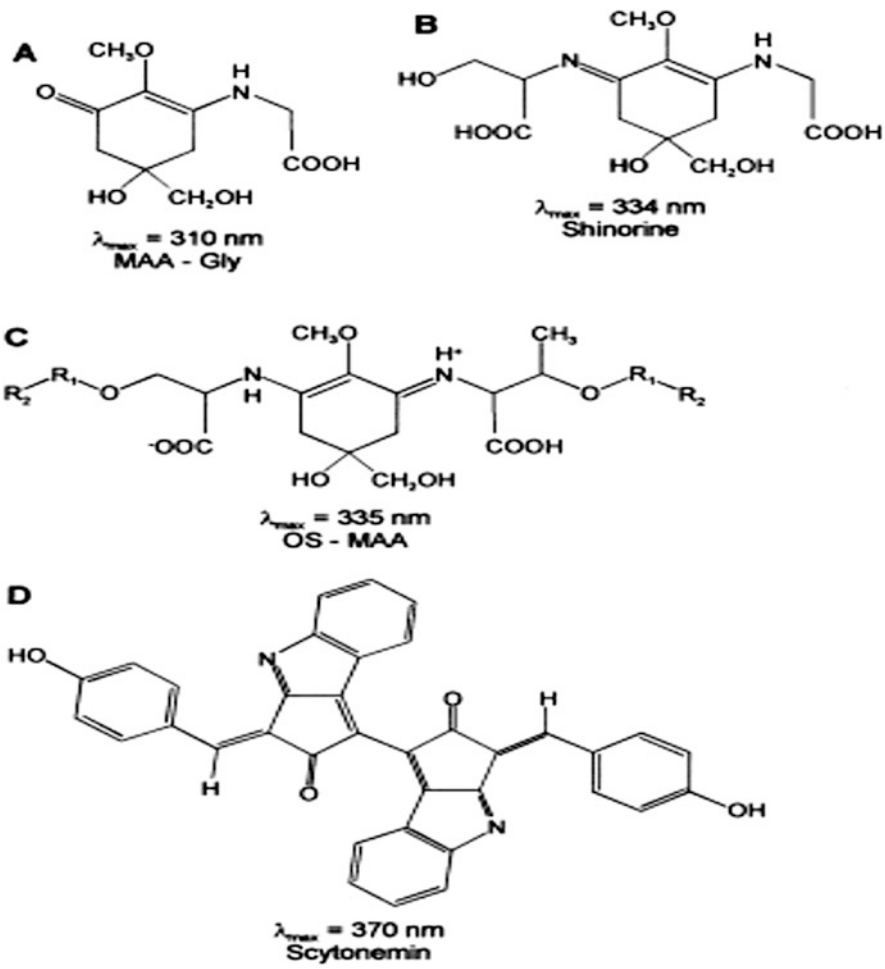


Fig. 17.8 Structures of mycosporine amino acids (MAA)

stimulates the extracellular glycan production of *N. commune*. The yield of EPS isolated from UV-B irradiated cultures was three times higher than that from control cultures. Leaf thickening in higher plants has been reported as a response to UV-B. With a thicker sheath, effective path lengths for the absorption of radiation are much longer and it has been suggested that EPS synthesis is stimulated to provide a matrix for the UV-A/B-absorbing oligosaccharides–mycosporines, which are located in the sheath of *N. commune*.

17.2 Active Repair Mechanisms

UV-damaged targets can be replaced by increased synthesis of the targets or by repair of damaged targets without de novo synthesis. DNA repair mechanisms are universal for all types of cells and have been studied extensively in *Escherichia coli.* UV-induced photoproducts can be recognized and repaired by several mechanisms in *E. coli*, including photoreactivation, excision repair and postreplication repair (SOS repair) (Walker and Marchant 1989). During photoreactivation, cyclobutane-type pyrimidine dimers are monomerized by the enzyme DNA photolyase, which is activated by UV-A and bluelight. Excision repair is light-independent and various enzymes are involved. First, damaged DNA is nicked, then short single-stranded segments spanning the base lesions are removed and the gaps are filled by resynthesis. Cyanobacteria have been found to exhibit both photoreactivation and excision repair. RecA-like genes from cyanobacteria have been shown to complement a RecA deletion in *E. coli*. The complemented RecA strains showed an increased UV-C resistance. The activation of the RecA protein by DNA damage is the first step of the SOS repair mechanism. The RecA protein cleaves the LexA repressor and the SOS genes are expressed (SOS regulon). In most studies related to DNA damage repair, UV-C irradiation has been used, and the induction of the expression of RecA by UV-A and UV-B irradiation has only recently been reported in *Pseudomonas aeruginosa*.

Increased protein degradation and resynthesis to replace UV-sensitive proteins as fast as they are damaged may help to counteract UV damage, which increased turnover of D1 and D2 proteins of the photosystem II reaction center in Synechocystis species, PCC6803, in response to UV-B irradiation. They suggested that UV-damaged D1 and D2 proteins are removed from the thylakoid and are replaced by newly synthesized D1 and D2 molecules. A specific cleavage site has been reported to be involved in the degradation of UV-B-damaged D1 protein. During recent years, the turnover of D1 protein has been studied in detail. It has been shown to be regulated by most stress conditions, and its turnover has been proposed to be a general adaptive response to environmental stresses.

17.3 Combinatory Strategies

A combination of several strategies may be used by photosynthetic organisms to acclimate to UV irradiation. In *N. commune* a cascade of physiological reactions was observed in response to UV-B irradiation: first a rapid increase in carotenoids, especially echinenone and myxoxanthophyll, and second, an increase in an extracellular UV-A/B-absorbing mycosporine, which was associated with extracellular glycan synthesis. Finally, scytonemin was induced slightly by UV-B and very strongly by UV-A irradiation. It has been proposed that the outer membrane-bound carotenoids provide a fast, active SOS response to counteract acute cell damage, whereas the glycan, with its UV-absorbing compounds, is a passive UV screen against long-term exposure.

17.3.1 Effect of Cyanobacterial Biofertilization in Saline Soils

Salinity and osmotic stresses induced many common proteins. In addition, unique salt stress- or osmotic stress-specific proteins were also induced in cyanobacteria, indicating differential regulation of protein synthesis by the two stresses. These show that cyanobacterial sensitivity and responses to salinity and osmotic stresses are distinct, independent phenomena. Nitrogen fixation in salt marsh sediments and saline soils has been attributed to sulfate reducing cyanobacteria, clostridia, cyanobacteria, and *Nostoc* and *Anabaena.*

Even though the criteria most used to evaluate the effect of cyanobacterial inocula require de novo studies by farming or addition in farming field to improve crop productivity, beneficial effects have been noted on plant size, on nitrogen content, and on the number of tillers, ears, spikelets, and filled grains per panicle. In in vitro study, relative gain increased up to 28%, while in in vivo study, gain yield increased up to 15% under Indian climatic conditions. With the pace of time microorganisms have continued to develop a complex stress tolerance system to survive with the changes in their external environment. As a result of alteration to their environment, many extremophilic microorganisms have evolved unique properties of considerable biotechnological and commercial significance. Halophilic or halotolerant eubacteria are characterized by a much greater metabolic diversity.

Cyanobacterial application to rice fields has been found to result in increased grain yield not only in India but also in China, Japan, the Philippines, and other rice-growing tropical countries. Beneficial effects of cyanobacterial biofertilization on paddy, grain, and straw yields have been shown in studies, including an increase in the N content, plant height, leaf length, number of tillers, cars, number of spikelets per panicle, number of filled grains per panicle, and amount of dry matter.

The cyanobacterial inoculation program is generally more successful in the dry season with mixed inocula with reduced or no inorganic nitrogenous fertilizers. These organisms released fixed nitrogen mainly in the form of polypeptides with fewer amino acids, vitamins, and auxin-like substances, either by exudation or by microbial degradation of dead cells. It has also been suggested that cyanobacteria can improve the bioavailability of phosphorus to plants by solubilizing and mobilizing the insoluble organic phosphates present in the soil with the help of phosphatase enzymes. Cyanobacteria have the ability to solubilize the insoluble form of $(Ca)_3(PO_4)_2$, $FePO_4$, $AlPO_4$, and hydroxyapatite $[Ca_5(PO_4)_3OH]$ in soils and sediments.

There is also the possibility to solubilize inorganic phosphate by growing a population of cyanobacteria for their own nutrition needs, and after their death, their cells are released in the soil, which are easily available to plants and other organisms. It is also observed that uptake of phosphorus by plants from algal materials was greater than that from the inorganic phosphates, when both were provided in equal amounts over a longer period of time. It may be because cyanobacteria could remove available phosphorus from the sphere of chemical fixation in soil by incorporating it into cell constituents or by absorbing it in excess

amounts, and then releasing it gradually for plants over a period of time through exudation, autolysis or microbial decomposition of dead cells.

The All India project on algae concluded that (1) by the use of cyanobacteria in unfertilized fields, a 10–15% increase in paddy yield is obtained, (2) in the presence of low doses of chemical fertilizer, nitrogen yield equivalent to 25 kg N/ha could be obtained, and (3) even at higher levels of nitrogen fertilizer, similar benefits could be obtained. Application of cyanobacterial biofertilizers in rice followed by wheat, along with other biofertilizers in consortia-phosphate solubilizing and mixed with vesicular arbuscular mycorrhiza (VAM) showed the beneficial effects of inoculation in both the crops. Application of bioinoculants alone (cyanobacteria, PSB or VAM) in the presence of 50% single super phosphate (SSP) plus 50% rock phosphate (RP) as a source of P were on par with P in paddy but not in wheat.

Cyanobacteria could be playing a potential role in the reclamation of salt affected (generally termed Usar land in some parts of India), arid or sub-arid soils. For amelioration of salt affected lands, chemical methods of using gypsum, sulfur or applying excessive irrigation, are not so cost-effective or environmentally friendly. Basically, salt affected soils (alfisol/sodic/alkaline/saline) are less productive, rigid soils impermeable to water due to the presence of excessive salts in the upper layers. They can be classified as alkaline and/or saline depending on the salt content. The alkaline soil is characterized by a high pH, high exchangeable Na, measurable amounts of carbonates, and it undergoes extensive clay dispersion (deflocculation due to the high zeta potential of active NaC). The poor hydraulic conductivity and reduced soil aeration make the soils infertile. The saline soil is characterized by a high amount of soluble salts (electrical conductivity more than 4 dS cm 1), imparting high osmotic tension to plant roots for absorption of water and nutrients. For the first time, cyanobacteria could be used as a tool for reclamation of Usar soils as they form a thick stratum on the soil surface and conserve the organic C, N, and P as well as moisture, and convert the NaC clay to Ca_2C clay.

Organic matter and N added by the cyanobacteria in such soils helps bind the soil particles and thus improves soil permeability and aeration. Since the cyanobacteria are capable of solubilizing nutrients from insoluble carbonate nodules through the secretion of oxalic acid; they improve the physicochemical quality of saline and alkali soils such as soil aggregation by lowering the pH, electrical conductivity, and hydraulic conductivity. There are certain physiological advantages associated with cyanobacteria which enable them to withstand these stresses: (a) Curtailment of NaC influx (b) Accumulation of inorganic (KC ion) or organic osmoregulators (sugars, quaternary amines, etc.)

Cyanobacterial application to organically poor semi-arid soils can play a significant role in their reclamation. The soils in these deserts or semi-arid regions are characterized by high compaction, low fertility, and water deficiency. They are also associated with problems of salinity and sodicity, which result in poor aeration and water infiltration, more soil erosion, and poor diversity of micro-flora. The poor physicochemical characteristics of soils ultimately have an adverse impact on plant growth and productivity. Cyanobacteria develop a superficial network of the trichomes/filaments on the soil, which not only binds the soil particles but also

results in enmeshing of the soil particles at depth. Cyanobacteria, as carbon and nitrogen fixers, can contribute to the improvement of soil nutrient status of organic carbon and nitrogen in arid soils. Cyanobacterial species such as Anabaena oscillarioides, A. aphanizomenoides, and Microcystis aeruginosa exhibited a salt tolerance ability ranging from 7 to 15 g/L. They are also known for the production of EPS, which help soil particles bind together (Mazor et al. 1996), and thus play a major role in improvement of soil moisture owing to their hygroscopic nature, and exopolysaccharides from cyanobacteria also contribute to reclamation of the desert soils.

17.4 Conclusion

The evolution of effective salt-acclimation mechanisms allowed cyanobacteria to colonize habitats that had low to high salt concentrations. For model cyanobacteria, the acclimation strategy is well characterized on the physiological and molecular levels. Three salt-tolerant groups, freshwater, moderately salt-tolerant, and halophilic cyanobacteria, have been defined, which differ not only in their ultimate salinity tolerance levels but also in the chemical nature of the major compatible solute. Nevertheless, the accumulated knowledge of salt acclimation from model cyanobacteria can be transferred to cyanobacteria that are not well studied. For example, many cyanobacterial genomes are available in public data bases and could be searched for key genes coding proteins for essential salt acclimation processes such as compatible solute biosynthesis and ion export.

Traditional conservation-based methods with modern technology can reduce farmers' dependence on chemical fertilizers and pesticides, as well as reduce the farming costs and environmental hazards. Emphasis is placed on an integrated plant nutrient supply concept involving regular use of organic resources such as organic manures/compost/green manure and biofertilizers integrated with low doses of chemical fertilizers in the cropping system for better results. A 10–15% increased grain yield along with a net saving is also accomplished. Depletion of soil fertility, low fertilizer-use efficiency, and growing environmental pollution are of major concern to agriculture, in terms of crop productivity. Biofertilizers such as cyanobacteria can provide a suitable supplement to the chemical fertilizers, and 'organic farming' can become a reality in the future. There is a definite need to deploy these biofertilizers in combination with organic composts and minimal doses of chemical fertilizers for reaping 'cleaner' and healthy harvests, securing food production and human health, protecting the environment, and saving scarce natural resources. Salt stress is a highly complex situation characterized by ionic and osmotic stresses and many secondary stresses, such as oxidative stress. Therefore, it is difficult to distinguish between the response to general stress or to the specific salt stress situation. Thus, species naturally competent to grow at high salinity that are also light and temperature tolerant will become the most promising starting points to develop cyanobacterial producer strains. Application of cyanobacterial biofertilizer to rice crop would bring an improvement in the soil physicochemical

properties and increased availability of phosphorus, when used along with rock phosphate and biologically fixed nitrogen under saline conditions. Some of the cyanobacteria would also provide growth-promoting substances and the quality of grain would be superior.

References

Ahmad MR, Winter A (1968) Studies on the hormonal relationships of algae in pure culture: I. The effect of indole-3-acetic acid on the growthof blue-green and green algae. Planta 78:277–286. https://doi.org/10.1007/BF00386428

Al-Hasan RH, Al-Bader DA, Sorkhoh NA, Radwan SS (1998) Evidence for n-alkane consumption and oxidation by filamentous cyanobacteria from oil-contaminated coasts of the Arabian Gulf. Mar Biol 130:521–527. https://doi.org/10.1007/s002270050272

Al-Hasan RH, Khanafer M, Eliyas M, Radwan SS (2001) Hydrocarbon accumulation by picocyanobacteria from the Arabian Gulf. J Appl Microbiol 91:533–540. https://doi.org/10.1046/j.1365-2672.2001.01414.x

Ali I, Basit MA (1993) Significance of hydrogen content in fuelcombustion. Int J Hydrogen Energy 18:1009–1011. https://doi.org/10.1016/0360-3199(93)90083-M

Bebout BM, GarciaPichel F (1995) UV B-induced vertical migrations of cyanobacteria in a microbial mat. Appl Environ Microbiol 61(12):4215–4222

Bohlool BB, Wiebe WJ (1978) Nitrogen-fixing communities in an intertidal ecosystem. Can 1 Microbiol 24:932–938

Borowitzka MA (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. J Biotechnol 70:313–321. https://doi.org/10.1016/S0168-1656(99)00083-8

Cohen Y (2002) Bioremediation of oil bymarinemicrobialmats. Inter Microbiol 5:189–193. https://doi.org/10.1007/s10123-002-0089-5

Converti A, Oliveira RP, Torres BR, Lodi A, Zilli M (2009) Biogas production and valorization by means of a two-step biological process. Bioresour Technol 100:5771–5776. https://doi.org/10.1016/j.biortech.2009.05.072

Costa JAV, Santana FB, Andrade MR, Lima MB, Frank DT (2008) Microalgae biomass and biomethane production in the south of Brazil. Biotechnol Lett 136:402–403

Coutinho R, Seeliger U (1984) The horizontal distribution of benthic algalflora in the Patos lagoon estuary, Brazil, in relation to salinity, substratum andwave. J Exp Marine Biol Ecol 80:247–257. https://doi.org/10.1016/0022-0981(84)90153-9

Dalton DA, Kramer S (2006) Plant-associated bacteria. Springer, Dordrecht, pp 105–130

El-Bestawy EA, Abd El-Salam AZ, Mansy AERH (2007) Potential use of environmental cyanobacterial species in bioremediation onlindane-contaminated effluents. Int Biodeterior Biodegrad 59:180–192. https://doi.org/10.1016/j.ibiod.2006.12.005

El-Enany AE, Issa AA (2000) Cyanobacteria as a biosorbent ofheavy metals in sewage water. Environ Toxicol Pharmacol 8:95–101. https://doi.org/10.1016/S1382-6689(99)00037-X

Forlani G, Pavan M, Gramek M, Kafarski P, Lipok J (2008) Biochemicalbasis for a wide spread tolerance of cyanobacteria to the phosphonateherbicide glyphosate. Plant Cell Physiol 49:443–456. https://doi.org/10.1093/pcp/pcn021

Kumar A, Singh JS (2016) Microalgae and cyanobacteria biofuels: asustainable alternate to crop-based fuels. In: Singh JS, Singh DP (eds) Microbes and environmental management. Studium Press Pvt. Ltd., New Delhi, pp 1–20

Lipok J, Owsiak T, Młynarz P, Forlani G, Kafarski P (2007) PhosphorusNMR as a tool to study mineralization of organophosphonates—the ability of *Spirulina* spp. to degrade glyphosate. Enzyme Microb Technol 41:286–291. https://doi.org/10.1016/j.enzmictec.2007.02.004

Mazor G, Kidron GJ, Vanshak A, Abeliovich A (1996) The role of cyanobacterial exopolysaccharides in structuring desert microbial crusts. FEMS Microbiol Ecol 21:121–130

Nisha R, Kaushik A, Kaushik CP (2007) Effect of indigenouscyanobacterial application on structural stability and productivityof an organically poor semiarid soil. Geoderma 138:49–56. https://doi.org/10.1016/j.geoderma.2006.10.007

Nozzi NE, Oliver JWK, Atsumi S (2013) Cyanobacteria asa platform for biofuel production. Front Bioeng Biotechnol 1:7. https://doi.org/10.3389/fbioe.2013.00007

Pandey VC, Singh JS, Singh DP, Singh RP (2014) Methanotrophs:promising bacteria for environmental remediation. Int J Environ Sci Technol 11:241–250. https://doi.org/10.2134/jeq2011.0179

Singh AL, Singh PK (1987) Influence of Azolla management on thegrowth, yield of rice and soil fertility. II. N and P contents of plants and soil. Plant Soil 102:49–54

Singh JS, Singh DP, Dixit S (2011) Cyanobacteria: an agent of heavymetal removal. In: Maheshwari DK, Dubey RC (eds) Bioremediation of pollutants. IK International Publisher Co, New Delhi, pp 223–243

Teal JM, Valiela T, Berlo D (1979) Nitrogen fixation by rhizosphere and free-living bacteria in salt marsh sediments. Limnol Oceanogr 24:126–132

Vaishampayan A, Sinha RP, Hader LD-P, Dey T, Gupta AK, Bhan U, Rao AL (2001) Bot Rev 67 (4):453–516

Vijayakumar S (2012) Potential applications of cyanobacteria in industrialeffluents—a review. J Bioremed Biodegr 3:1–6. https://doi.org/10.4172/2155-6199.1000154

Walker TD, Marchant HJ (1989) The seasonal occurrence of chroococcoid cyanobacteria at an Antarctic coastal site. Polar Biol 9:193–196

Zulpa G, Zaccaro MC, Boccazzi F, Parada JL, Storni M (2003) Bioactivity of intra and extracellular substances from cyanobacteria and lacticacid bacteria on “wood blue stain” fungi. Biol Control 27:345–348. https://doi.org/10.1016/S1049-9644(03)00015-X

Advancement in Treatment Technologies of Biopharmaceutical Industrial Effluents

18

Diptikanta Acharya, Sagarika Satapathy, D. Tapas Kumar Dora, Sushma Dave, and Jayashankar Das

Abstract

Biopharmaceuticals have set new standards for blockbuster drugs recently. A blockbuster drug is the one which has crossed $1 billion or more in annual sales. Top 15 biopharma products have annual revenue more than $2 billion, and anti-inflammatory drugs like humira are generating $10 billion in revenue per year. These bioproducts are mostly large organic molecules like enzymes, hormones, clotting factors, monoclonal antibodies, and peptide therapeutics compared to a low-molecular-weight pharmaceutical product. Around 1500 biomolecules are undergoing clinical trials, and success rate of a biomolecule to pass a clinical test is twice as compared to pharma molecule. These promising outputs and low side effects of biopharmaceutical products are attracting major pharmaceutical companies, and they are slowly shifting their research and development and sourcing toward these large molecular bioproducts. These molecules are produced mostly inside a bioreactor with upstream and downstream processes, and the effluent contents of these industries are a matter of concern because they contain various unorthodox organic components, i.e., recombinant fermentation broth, extremophiles, bacterial spores, and various antibiotics. The absence of an effective treatment methodology with various bio-industries to deal with this modern organic recombinant sludge is the major reason behind the increase in a number of untreatable superbugs in our ecosystem. There is an urgent need to

D. Acharya · S. Satapathy
Gandhi Institute of Engineering and Technology University, Gunupur, Rayagada, Odisha, India

D. Tapas Kumar Dora
DIT University, Dehradun, India

S. Dave (✉)
Jodhpur Institute of Engineering and Technology, Jodhpur, Rajasthan, India

J. Das
Gujarat Biotechnology Research Center (GBRC), Department of Science and Technology, Government of India, Gujarat, Gandhinagar, India

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_18

improve the traditional primary, secondary, and tertiary modes of wastewater treatment methodology to handle this new threat. This chapter provides an insight knowledge to traditional and modern methods implemented for effluent treatment for biopharmaceutical effluents and an advancement of treatment technologies like MODAR supercritical water oxidation, membrane biological reactor (MBR) technology, reverse osmosis technology, ozonation plant technology, and modular thermal plant treatment for effective treatment of biopharma effluent streams to achieve zero contaminant discharge strategy.

Keywords
Biopharmaceuticals · Traditional effluent treatment · MODAR supercritical water oxidation · Reverse osmosis technology · MBR technology

18.1 Introduction

Pharmaceutical industries particularly develop and produce value-added market products for human welfare. The pharmaceutical industries carry out research and development to make different types of market products for the improvement of millions of people all around the world. As far as the human life and health are concerned, the pharmaceutical industry has made great progress in recent years and made a great contribution toward the development of pharmaceuticals for different fields such as agriculture, poultry farming, fishery, and human health (Duca and Boldescu 2009).

Pharmaceuticals are present in wastewater since the human body does not degrade drugs completely, and many substances of pharmaceutical origin are not eliminated during wastewater treatment. Effluent from the pharmaceutical industry in different compartments of the environment is a new challenge not only for technologists of water but also for scientists involved in wastewater treatment (Gomes et al. 2017).

It has been proved in many studies that substances of pharmaceutical origin are not eliminated in the process of water treatment; their biodegradation in the environment is also difficult (Marketresearch.com 2018). Detailed chemical analysis of water is necessary for the safe use of water resources. It is important to identify all pollutants present in water and, hence, to fully evaluate water quality and predict effect on humans. The continuous improvement of analytical techniques makes it possible to identify a wider spectrum of components and improve detection limits. A brief review of input by different sources and fate and analysis of pharmaceuticals, parapharmaceuticals, and their metabolites in environment is presented.

In the past several years, one of the primary tasks of environmental chemistry has been detection, determination, and date studies of pharmaceuticals in different compartments of the environment, particularly in water ecosystems (Clara et al. 2005; Arnold et al. 2014). An important role is played in this area by analytical chemists who develop and introduce analytical practice procedures for determination

of traces of a wide spectrum of compound samples of complex matrices. The presence of different drugs in the environment results from manufacturing of medical formulations and the impact of the pharmaceutical industry on the environment (De Gusseme et al. 2009); discharge of large quantities of expired drugs (without treatment) by households (small scale) as well as hospital wastewater and wastes (much larger scale); and excretion of residues of drugs and their metabolites by animals and (Khetan and Collins 2007; Lacey et al. 2012; Gadipelly et al. 2014; Krzeminski et al. 2019) humans. Studies on metabolism and fate of pharmaceuticals in human and animal bodies have shown that a large fraction is excreted with feces and urine, and therefore, the drugs are present in municipal wastewater (Gros et al. 2010). Compounds of the group are biodegradation resistant and are not completely eliminated in the process of wastewater treatment (Quinn et al. 2008). They are present in the environment both in unchanged form and as metabolites. Pharmaceuticals enter the environment from a myriad of scattered points. The main sources of contamination include pharmaceutical production pollutants, wastewater treatment plants (WWTPs), hospitals, landfills, and even graveyards (Khetan and Collins 2007; Larsson 2014). The most investigated route of entry of pharmaceuticals into the environment is that from municipal WWTPs. Human excretion of unchanged or slightly transformed active pharmaceutical ingredients (APIs) conjugated to polar molecules such as glucuronide enters the WWTP where these conjugates may then be cleaved, releasing the original API into the environment (Snyder et al. 2007). Activated sludge WWTPs have received particular attention (Joss et al. 2004; Grandclément et al. 2017). A limited number of studies also found pharmaceuticals in drinking water and hospital wastewater (Shariati et al. 2010).

18.2 Types of Pharmaceuticals

The pharmaceutical industry is primarily driven by scientific discovery and development. It is an important component of the health-care system and mainly contributes via manufactured pharmaceutical active substances for human and animal welfare.

The pharmacological active substances may comprise of natural products or synthetic drugs. Natural products are those that are generally derived from microbial, plant, and animal resources, whereas synthetic drugs are those that are produced through chemical technologies. Some common types of naturally derived pharmaceuticals are antibiotics, steroids, peptide hormones, vitamins, enzymes, prostaglandins, and pheromones. Synthetic drugs are the recent achievements in the field of pharmacology and computer technology. Some common types of synthetic drugs are cardiovascular agents, CNS depressants, central nervous system (CNS) stimulants, hematological agents, steroids, etc. (Wang et al. 2018).

The pharmaceutical market's value is steeply climbing every day with a groundbreaking rate and is predicted to exceed $1.1 trillion globally by 2021 (Al-Farsi et al. 2018). Drug developers are now discovering an immeasurable range of novel,

potent, and beneficial life-improving pharmacy products that have an impact on the development and improvement of medical therapies. Currently, research and development initiatives are being taken in the pharmaceutical sectors like precision medicine and the creation of hyper-targeted drugs. Data preprocessing and analytical tools are also integrating different types of datasets with existing business processes providing a better picture of the scenario (Deegan et al. 2011). Different types of genetic research, recognition, and interpretation applications must be implemented in the pharmaceutical industry for the manufacturing of valuable products (Göbel et al. 2005). According to the view of Michael Elliott, CEO and chief analyst of the life sciences IT consulting firm Atrium Research, the virtualization of research has been the prime role driver to get people over some of the resistance and move to the cloud (Grandclément et al. 2017). Cloud technology is a recent trend, but its capacity to decrease the costs of pharmaceutical products is already proving to be principally beneficial for smaller organizations such that the budget-conscious companies are now able to execute best-practice business processes for the development of pharmaceutical products with stabilizing effects (Khetan and Collins 2007).

18.3 Application of Biopharmaceutical Products

Biopharmaceuticals are the kind of sophisticated products in the field of modern science. Generally, 3-D modeling systems are being used to design them (Larsson 2014). The important considerations of biopharmaceuticals are reductions in the operating costs, improvement in process technology, industry readiness to respond quickly, defining the manufacturing footprint, and an improvement in efficiency. Integration of science and deep operational excellence will make the biopharma industry of more potential to transform the health prospect of millions of people across the globe. In today's world, the deliberation of authentic health-care innovation is most challenging. It requires knowledge, development, and delivery system of new products toward the human and animal welfare. The most important development in the pharmaceutical industry is the discovery of novel biological output by implementing new innovative technologies (Oller et al. 2011). The pharmaceutical products' impact upon the public health generally comes after several rounds of R&D, testing, and final approval by appropriate regulatory bodies like FDA (Radjenović et al. 2009). Many factors are contributing to the challenges toward the success of biopharmaceutical potency like improvement in success rates and increase in the efficiency of clinical research, safety among large group of patients, information on potential interactions with other medicines, specific dosing instructions, discovery of drugs to treat serious conditions, filling an unmet medical need, provision of meaningful therapies for serious medical conditions, etc. As R&D challenges increase in the field of science, the researchers are continually adopting the new medical advances and are developing innovative medicines upon animal and human trials.

18.4 Environmental Impact

Pharmaceutical pollution is gradually being recognized as a major threat to ecosystems and human health globally. Pharmaceuticals can enter the environment at different stages of their life cycle, such as during production, use, and disposal. It means that they can ultimately wind up in our drinking water as well as accumulate in vegetables and aquatic animals. Nowadays, the main concern is about the occurrence of active pharmaceutical ingredients, solvents, intermediates, and raw materials in effluents from the pharmaceutical industry which has gained increasing attention. Environment and health are directly or indirectly affected by pharmaceutical effluents especially in the surrounding area of pharmaceutical industrial regions. Different classes of pharmaceutical compounds like analgesic, antidepressant, antihypertensive, contraceptive, antibiotic, steroids, hormones, etc., have been detected in water samples from mg/L to μg/L range. Although, at times, the detected amount is very small, it is highly potent and toxic for human, animal, and aquatic lives. In a recent study, extraordinarily high levels (mg/L) of several drugs were found in the effluents from local wastewater treatment plant. These drugs included non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, naproxen, and diclofenac, apart from some other solids, biodegradable and nondegradable organic compounds, etc. The long-term exposure of lower concentration to a higher concentration of complex pharmaceutical mixtures in effluents may cause acute and chronic damages (Oller et al. 2011), behavioral changes, accumulation in tissues, reproductive damage, and inhibition of cell proliferation. The existence of pharmaceutical compounds creates a major life-threatening problem; therefore, sincere efforts are essential to trim down the problem along with some adequate regulations for their monitoring and control. We must look not only at the effects of drugs in medical practice but also its environmental effects (Adishkumar et al. 2012). Another study shows that pharmaceutical production severely contaminates surface, ground, and drinking water as far as the environment is concern (Bernhard et al. 2006). Nowadays, ocean acidification and increasing discharges of pharmaceutical contaminants into aquatic systems are emerging trends and are perceived to be major catalysts of environmental change particularly affecting marine ecosystems adversely. Pharmaceuticals can go into the soil environment as well, and when animal slurries and sewage sludge are applied to the land as fertilizers, these pharmaceuticals may also be taken up by the soil organisms which may result in toxic effects upon organisms throughout the food chain. Also, the same study reveals that the high levels of broad-spectrum antibiotics as a pharma product could induce the development of antibiotic-resistant microorganisms. It has been established that such harmful effects may happen if the pharmaceutical compounds are transferred within the food web/chain. Pharmaceuticals in higher creatures are designed to target specific metabolic and molecular pathways and show possible side effects. *Much effluent treatment plants* are equipped with mechanical separation of solid particles through different size filters which separate finer particles either occurring in the incoming water or developing as a consequence of the chemical treatment of the water with flocculating agents. Pharmaceuticals are existing mainly in the dissolved phase; therefore,

biodegradation is suggested to be the most important elimination process in the effluent treatment. Many effluent treatment plants are following biological routes toward the eradication of organic content in the effluents. Depending on the characteristics of the specific compound of the pharmaceuticals and the characteristics of the surrounding environment, we can minimize the level of the undesirable substances from the biopharmaceutical effluents through aerobic and anaerobic biodegradation and abiotic revolution such as degradation of UV light, hydrolysis and sediment sorption, and direct and indirect photolysis; it means direct photolysis is the outcome of direct absorption of sunlight, while indirect photolysis includes natural photosensitizers (Clara et al. 2005). The exposed pharmaceutical substances are expected to be a threat to the entire life span of organisms, and it is possible that they may impact on nontarget organisms in the aquatic and terrestrial environments.

Biochemical oxygen demand (BOD5) and chemical oxygen demand (COD) are the most frequently used parameters for the description of biopharmaceutical effluents. Both of these parameters usually depend on many factors such as the extent of time required to determine each one of them. The production of antibiotics and vaccines by biopharma plants generates effluents containing very high BOD (biochemical oxygen demand), COD (chemical oxygen demand), TS (total solids), colloidal solids, toxicity, odor, etc. Industrial effluents are notable by their abnormal turbidity, conductivity, chemical oxygen demand (COD), total suspended solids (TSS), and total hardness. The biopharmaceutical effluents have a large number of organic compounds with a benzene ring and unsaturated bonds which results in low biodegradability and have less BOD value in spite of a high COD value.

Biopharma industry effluents pose potential hazards toward the natural system. Total suspended solids are solids in effluents that can be trapped by a filter including decaying plants, silt, animal matter, industrial wastes, and sewage. Particularly, biopharmaceutical effluents comprise of different types and sizes of solids which are harmful to the health status of aquatic life within a water body. The release of industrial effluents with surplus amount of suspended solids often shows the life-threatening problem toward the aquatic system and increase in the level of pathogens including bacteria, protozoa, etc. The aim of effluent treatment is to minimize and remove COD, BOD, suspended solids, and toxic compounds before they get released into the natural environment. Effluent, therefore, needs proper treatment to remove suspended solids, organic matter, and nutrients which is necessary before the effluent is discharged into aquatic bodies (Clara et al. 2005).

18.5 Technologies for Biopharmaceutical Wastewater Effluent

Traditional technologies dealing with effluents coming out of the production plant and to treat selected wastewater streams are the most effective and economically sound ways. Moreover, an additional goal is to reduce overall effluent emissions by reusing treated wastewater toward zero-discharge strategies. The removal efficiency of pharmaceuticals from effluents has been tested by many technologies; the main

objective is to maintain zero pollutants in wastewater. The removal efficiency of pharmaceuticals and pharmaceutical active components has been achieved by many different technologies such as active carbon filtration, biological degradation, membrane filtration technology (e.g., reverse osmosis, ultrafiltration at membrane biological reactors), and oxidation technologies combining different available advanced oxidation processes (AOP) like ozonation, hydrogen peroxide with radiation from UV light, etc. (Crane et al. 2006).

Biopharmaceutical effluents comprise a variety of agents, and their composition varies in response to a number of factors such as the production rate, the specific preparation, and the generating wastewater. All these variables mean that the pollution of the final effluent can be diversified and be changeable over time. Generally, these wastewater effluents contain a high amount of organic matter and slowly biodegradable organic compounds, such as aromatic compounds, inhibiting and toxic compounds, etc.

The pharmaceutical industry employs a wide array of wastewater treatment and disposal methods. Wastewater generated from this industry varies not only in composition but also in quantity by plant, season, and even time, depending upon the raw materials and the processes used in the manufacturing of various pharmaceuticals. Plant location also brings in a variable related to the quality of available water. Hence, it is very difficult to specify a particular treatment system for such a diversified pharmaceutical industry. Many alternative treatment processes are available to deal with the wide array of wastes produced from this industry, but they are specific to the type of industry and associated wastes. However, the analysis of published information in the public domain shows that there are many general approaches employed to treat pharmaceutical wastewaters which are moving bed biofilm reactor (MBBR) process, MODAR supercritical water oxidation process and MBR technology, mechanical steam water compression vacuum evaporators, reverse osmosis technology, ozonation plant technology, modular thermal plant treatment, advanced oxidation processes, and biological processes (Fig. 18.1) (Deegan et al. 2011). The best technology for treating the effluents generated by the biopharmaceutical industry will depend on each specific factor, such as significant variation and the wide range of possible compounds.

18.5.1 Activated Sludge Biological Process

This process is more efficient and is particularly used to eradicate the pollutants of biodegradable types. Although this is the most competitive process for wastewater treatment, there is a possibility of the occurrence of inhibiting and toxic compounds for the biomass and the low biodegradability of some effluents (some endocrine-disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) are known by their hydrophobicity). Thus, the traditional municipal wastewater treatment plants (MWWTP) based on activated sludge reactors tend to accumulate the bio-sludge (Göbel et al. 2005). Some works reveal that up to 45–90% of EDCs entering in MWWTP can be adsorbed and accumulated in the sludge and will

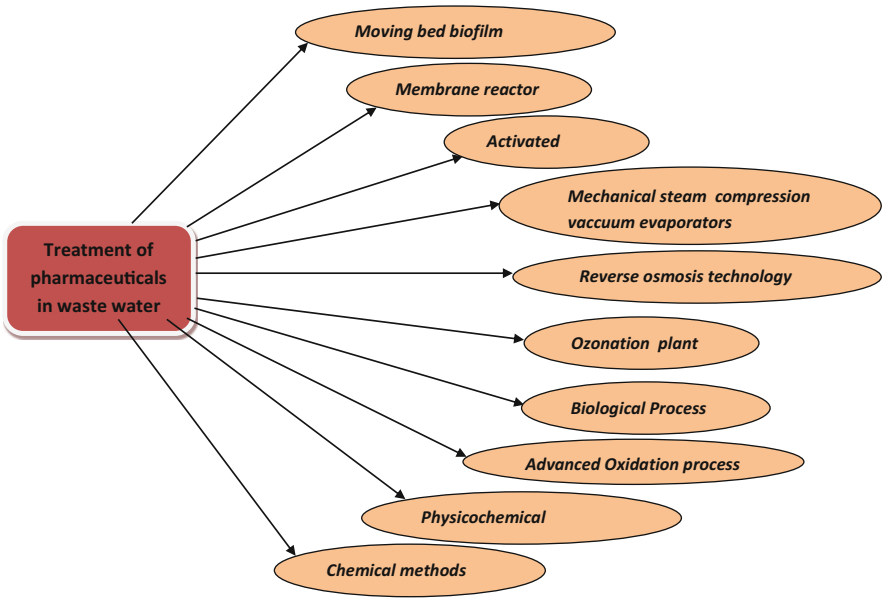


Fig. 18.1 Various technologies for the treatment of pharmaceutical in wastewater effluent

circulate along the treatment process (Tiwari et al. 2017). Moreover, some sludge management strategies involve the application of the sludge in farmland which will promote the spreading of the contaminants through the soils and aquifers. If ozonation is applied as a refining treatment after the biological effluent processing, it will not be able to avoid micropollutant deposition in the activated sludge. Thus, this chemical approach can be envisaged as a pretreatment before activated sludge. This way, it may promote persistent pollutant removal by avoiding their adsorption in the sludge. The main drawback of such procedure is related to the large volumes of municipal wastewaters that must be processed. Ozone in situ generation is energy consuming, and the operating costs can discourage the full-scale application of this methodology. However, the rising health problems and the growing reduction of water with quality that is fit for human application will surely be the driving forces for changing decision making and stakeholder priorities, thereby forcing policy to put environmental interests over the economics. Although some authors refer that ozonation improves MWW biodegradability, it should be considered that this wastewater is biodegradable and the removal of the PPCPs (which typically occur in streams at very low concentrations) will not substantially affect wastewater biodegradability. Thus, the main goal of ozonation will surely not be biodegradability enhancement but rather the removal of those persistent and harmful chemicals that are not degraded through the biological processes (Sipma et al. 2010).

18.5.2 Moving Bed Biofilm Reactor (MBBR) Process

MBBR technology is the efficient option of effluent treatment when the effluent is rich in organic matter. This technology consists of a biological reactor which supports the growth of biomass as a biofilm on plastic supports that are continuously moving within it. The bioreactor provides a specific area per unit of volume which allows more biomass to flow per unit of volume. MBBRs are much smaller than the activated sludge system. This technique is viable only when the pollution is biodegradable.

18.5.3 MBR Technology

Membrane bioreactor (MBR), a hybrid of ultrafiltration and biological remediation of wastewater, emerged as an alternative technology of activated sludge process. This technology overcomes the issues, viz., huge space requisite for secondary clarifiers, surplus sludge generation, liquid-solid separation, etc., related to activated sludge process. The advantages of MBR technology lie with inclusion of membranes and include elimination of secondary clarifiers and tertiary filtration; designed for prolonged sludge age, thus lower sludge generation; high effluent quality; higher loading rate capacity; and shorter hydraulic retention time. Moreover, the elimination of the secondary clarifiers results in lesser space requirement.

The sluggish-growing autotrophic bacteria in MBR result in nitrogen removal, thereby providing additional feature of high removal rate of biodegradable micropollutant. Simultaneously, the MBR also facilitates in the removal of COD, suspended solids, and phosphorus. The ratio of the suspended solids to total suspended solids in MBR is in the range of 0.46–0.55, which is lower than the permissible limit of 0.75–0.9. In 2004, Wen et al. reported the removal of NH_4^+, COD and turbidity by 93%, 80%, and 83%, respectively, from the hospital wastewater in the submerged membrane reactor. High COD removal in MBR is attributed to stable biomass concentration and retention of particulate matter that provides a stable condition for the growth of specialized microbial community efficient in micropollutant biodegradation. The application of membrane bioreactors (MBR) in hospital wastewater treatment has become a common exercise in the previous decades. De Gusseme et al. (2009) reported 99% removal of 17β-ethinylestradiol in nitrifier-enriched biomass of MBR (Dębska et al. 2004). Snyder et al. (2007) demonstrated that concentrations of caffeine, acetaminophen, sulfamethoxazole, carbamazepine, and gemfibrozil decreased as the compounds passed through the pilot MBR with removal efficiencies varying between 99.1% (sulfamethoxazole) and 99.9% (acetaminophen). Radjenović et al. (2009) found that the removal of acetaminophen from the aqueous phase by the MBR was greater than 99% (similar to the conventional activated sludge process). No elimination of gemfibrozil took place by conventional activated sludge treatment, whereas the MBR eliminated 30–40% of this compound. In the same study, carbamazepine remained untreated by both techniques. Removal efficiencies of sulfamethoxazole were higher by the

MBR technology (81%) than by the conventional activated sludge (75%). Kimura et al. (2005) reported high removal of ketoprofen and naproxen in MBR system, whereas the removal efficiency of clofibric acid, ibuprofen, diclofenac, and mefenamic acid was the same in CAS and MBR. The persistence and low removal of pharmaceutical residues in both systems could be due to the presence of the aromatic ring or chlorine group in their structure. MBR system is more efficient than CAS treatment for the removal of persistent micropollutant, especially for those compounds that are not readily degradable. Bernhard et al. (2006) observed that with high SRT, MBR process had a better removal of polar compounds like diclofenac, sulfophenyl carboxylate, and mecoprop. However, for the compounds such as sotalol and hydrochlorothiazide, removal efficiency was less compared to CAS process (Sipma et al., 2010). Studies revealed that increase in retention time in membrane bioreactor improved the degradation of estrogen (Joss et al., 2004). Radjenović et al. (2009) compared the degradation efficiency of pharmaceutical compounds in MBR with conventional activated sludge process. The degradation efficiency of compounds like diclofenac, metoprolol, and clofibric acid was 87.4%, 58.7%, and 71.8%, respectively, in MBR, whereas in CAS process, the degradation efficiency was only 50% for diclofenac and 27% for clofibric acid. No removal of metoprolol was observed in conventional activated sludge process. The removal rate of sulfamethoxazole was varied considerably maybe due to back conversion of N4-acetyl sulfamethoxazole to sulfamethoxazole during the degradation process. The removal efficiency of ibuprofen remains the same in both treatment processes. MBR treatment has a characteristic feature of retaining hydrophobic compounds, and the slow-growing nitrifying microorganism within the reactor with established biomass concentration makes MBR a better treatment technique than CAS (Huang and Lee 2015). Low sludge production and high removal of pharmaceutical residues in MBR treatment suggest that MBR technology could be an economical solution for the generation of clean water. MBR technology is more competent in the production of high-quality effluent than CAS; thus, MBR treated water is directly released into the environment. MBR is one of the powerful techniques to treat the emerging pollutants. However, the fouling of membrane and repeated washing are the factors that limit its application at large scale. Published investigation revealed that the presence of supporting medium for microbial growth in MBR would be a useful technique for decreasing membrane fouling rate and for removal of highly persistent micropollutant (Wei et al., 2012). Attached growth bioreactor provides a diverse microbial group of the aerobic, anoxic, and anaerobic zone, which offers high removal of persistent micropollutant. Arya et al. (2016) reported high removal of gemfibrozil and ciprofloxacin in submerged attached biofilter as compared to MBR. Enhanced pollutant removal in MBR could be achieved by the use of supporting medium to facilitate the biofilm growth and enhance the micropollutant retention. However, high operation and capital cost, membrane complexity, and fouling hinder energy costs; replacement cost of the membranes hinders its wide usage for industrial effluent treatment. The fouling, attributed to deposited microorganisms, sludge and solutes, etc., in the MBR results in reduced membrane performance and life cycle of the membrane, thereby enhancing the maintenance and operation cost.

Moreover, after prolonged use, the suspended particles deposited on the membrane surfaces and pores thereby clog the pores and thus diminish the permeability of the membrane. Furthermore, the membrane fouling owing to the heterogeneous nature of the solids and microorganisms deposited on the surface is an unavoidable challenge and hard to control in prolonged usage of the MBR.

Different forms of fouling, viz., pore thinning, pore blockage, and cake formation, occur in the MBRs. In pore thinning, the micropores of the membrane are clogged by the foulants, viz., biofoulants and organic and inorganic foulants, thereby decreasing the pore size. In pore blockage, the suspended particulates in the solution form owing to their sticky nature and adhere to the pore, thereby clogging the pore. The prolonged deposition and accretion of the bacterial masses, inorganic particulates, and biopolymers form a layer which leads to cake formation. The membrane resistance increases as the thickness of the cake increases.

18.5.4 Mechanical Steam Compression Vacuum Evaporators

The etched vacuum evaporation of the water with mechanical compression of steam becomes more proficient for the complex type of wastewater pollution owing to the existence of persistent, inhibitor, or toxic compounds and low biodegradability. In such technique, the wastewater is heated by steam as a heating medium in a heat exchanger operated under vacuum. Under vacuum conditions, the boiling point of water is brought down to 60 °C, thus reducing the energy cost. Under vacuum conditions, the water is evaporated, thereby leaving a concentrated pasty residue of solids, and the separated clean water can be further reused, thereby attaining the optimum conditions of sustainability with zero discharge (Iorhemen et al. 2016).

18.5.5 Reverse Osmosis Technology

Reverse osmosis is a water treatment technology that consists of a selective permeable membrane for the removal of ions, unwanted molecules, and larger particles from drinking water by applying an external pressure in a reverse way to the natural flow of solvent. The filter system of this technology has extremely tiny pores that help remove microscopic contaminants from the water. This technology is a widely used technology that works under a certain pressure which allows the source water through the selective membrane and removes the impurities present in water.

Numerous researchers have investigated on the different treatment methods to remove PPCPs from wastewater and receiving waters, including conventional activated sludge, soil aquifer treatment, advanced oxidation process, and biomembrane process (Joss et al. 2004; Göbel et al. 2005; Klavarioti et al. 2009; Deegan et al. 2011; Gadipelly et al. 2014; Miron et al. 2014). Among them, biomembrane process is one of the most effective treatment processes. The membrane processing technology, owing to its efficient, convenient, and economically viable characteristics, plays a prominent role in sewage water treatment. According

to Miron et al. (2014), membrane bioreactor (MBR) has a better effect (>80%) on most pharmaceuticals (naproxen, 99.3%; ofloxacin, 94.0%; bezafibrate, 95.8%; and paroxetine, 89.7%) than conventional activated sludge. Katsuki et al. have studied reverse osmosis (RO) membrane's effect on endocrine-disrupting chemicals (EDCs) and pharmaceutically active compounds (PhACs), and it shows that the polyamide membrane has achieved a good removal effect on 2-naphthol, 4-phenylphenol, caffeine, bisphenol A, sulfamethoxazole, and 17β-estradiol with a rate of 51–91%. Technologists have proven that ultrafiltration (UF)-RO double membrane process has good removal efficiency (90% on average) on PhACs and EDCs. Pomati et al. (2006) focused on the study of MBR-RO process's efficiency on 20 multiple-class pharmaceuticals (including psychiatric drugs, macrolide antibiotics, β-blockers, sulfonamide and fluoroquinolone antibiotics), and treatment has exhibited excellent overall removal of selected emerging contaminants with removal rates above 99%. Establishing MBR-NF (nanofiltration) treatment process, Larsson (2014) studied different nanofiltration membranes' efficiency to 40 trace organics, including pharmaceutically active compounds, steroid hormones, industrial compounds, and pesticides, and the removal rates are all above 95%.

18.5.6 Ozonation Plant Technology

The ozonation of biopharmaceutical effluents has several advantages such as an increase in dissolved oxygen, decrease in chemical oxygen demand, and also improvement in the aesthetic value of water due to a reduction in turbidity and color. The ozonation process is carried out for a short period of time, approximately 10–30 min. It is more effective than chlorine in destroying viruses and bacteria (Gomes et al. 2017).

18.5.7 Modular Thermal Plant Treatment

Thermal treatment is an essential process in all categories of production processes that have the main objective to destruct microorganisms by the application of heat. The main objective is to promote optimum performance downstream and filtration of boiler feed water streams which allow an industrial boiler or power plant to meet the strict requirements for more efficient boiler performance. Treatment of boiler feed water is essential for preventing excessive heat transfer equipment fouling and erosion of turbine blades (Iorhemen et al. 2016).

18.5.8 Advanced Oxidation Process

When the wastewater contains a high concentration of the chemically stable compound, toxic substances, with very low biodegradability, efficient processes are needed for destroying the pollutants. Advanced oxidation refers to a wide range of

technologies, most of which are based on generating hydroxyl radicals or the supply of energy required to destroy the pollutants. This technology is especially competitive for eliminating halogenated hydrocarbons, detergents, dyes, etc. A wide range of other available techniques like electrochemical oxidation, catalytic ozonation, anodic oxidation, the combination of ultraviolet radiation and hydrogen peroxide, Fenton's reagent, and photocatalysis are also showing fruitful results. All of these are techniques that can eliminate high loads of pollutants (Klavarioti et al. 2009).

AOPs can be broadly defined as aqueous phase oxidation methods based on the intermediacy of highly reactive species such as (primarily but not exclusively) hydroxyl radicals in the mechanisms leading to the destruction of the target pollutant. Over the past 30 years, research and development concerning AOPs has been immense particularly for two reasons, namely, (a) the diversity of technologies involved, and (b) the areas of potential application. Key AOPs include heterogeneous and homogeneous photocatalysis based on near ultraviolet (UV) or solar visible irradiation, electrolysis, ozonation, Fenton's reagent, ultrasound, and wet air oxidation, while less conventional but evolving processes include ionizing radiation, microwaves, pulsed plasma, and ferrate reagent. Although water and wastewater treatment is by far the most common area for research and development, AOPs have also found applications as diverse as groundwater treatment, soil remediation, municipal wastewater sludge conditioning, production of ultrapure water, and volatile organic compounds treatment and odor control. Depending on the properties of the waste stream to be treated and the treatment objective itself, AOPs can be employed either alone or coupled with other physicochemical and biological processes. Process coupling is conceptually beneficial usually leading to improved treatment efficiencies. For instance, AOPs may be employed as a pretreatment stage to convert initially bio-recalcitrant compounds to more readily biodegradable intermediates followed by biological posttreatment. On the contrary and for effluents containing biodegradable fractions, biological pretreatment followed by chemical posttreatment may be favorable as biodegradable compounds can be easily removed first and so subsequently do not compete for the chemical oxidant. Recent reviews on the applications of AOPs for water and wastewater treatment can be found elsewhere (Mantzavinos and Psillakis 2004).

18.5.9 Physicochemical Process

Physicochemical treatment of effluents focuses primarily on the separation of colloidal particles. This is achieved through the addition of coagulants and flocculants. These changes of the physical property of the colloids allow them to remain in an indefinitely stable form such as flocs with settling properties. This type of process used to remove 80 to 90% of total suspended solids (TSS), 40–70% of BOD5, 30–40% of COD, 17–100% of nutrients (N and P), and so on. This method has been successfully used for industrial effluents.

18.5.10 Chemical Process

The chemical treatment process consists of using some chemical reactions that improve the quality of water. The most commonly used chemical processes are chlorination, ozonation, and neutralization.

18.5.10.1 Chlorination

The chlorine compounds like chloramine or chlorine dioxide are mostly used in the chlorination process which is the common disinfection method. The harmful microorganisms can be killed rapidly by strong oxidant chlorine. Chlorine is dangerous to use because it is a toxic gas. The toxicity can be reduced by the use of sodium hypochlorite that can release free chlorine when dissolved in water.

18.5.10.2 Ozonation

Ozone is a very strong oxidizing agent and an unstable molecule which is most toxic to microorganisms present in water. The harmful cyst-forming protozoa and other pathogens can be killed using ozonation. Ozone can release reactive oxygen in exposure to ultraviolet light or a cold discharge. This reactive oxygen can kill the organisms.

Ozone is a strong oxidant that either decomposes in water to form hydroxyl radicals which are stronger oxidizing agents than ozone itself, thus inducing the so-called indirect oxidation, or attacks selectively certain functional groups of organic molecules through an electrophilic mechanism (Radjenović et al. 2009). Depending on the type of the substrate and the operating conditions in question, ozone oxidation is usually favored at increased pH values due to the increased production of hydroxyl radicals. Moreover, treatment performance is enhanced if ozone is combined with light irradiation and hydrogen peroxide or with iron or copper complexes that act as catalysts. Ozonation has been traditionally employed in drinking water treatment for odor and taste control and disinfection, as well as (in some cases) for wastewater disinfection. Therefore, it is not surprising that several studies have been carried out onsite in drinking water plants (Kimura et al. 2005; van Lier et al. 2008; De Gusseme et al. 2009; Gros et al. 2010; Gkotsis et al. 2014; Huang and Lee 2015).

18.5.10.3 Neutralization

Many industrial water treatment operations use a common chemical process called neutralization; it consists of the addition of acid or base to adjust the pH value back to its normal state. Lime is a base used for neutralization of acid wastes in industries (Arya et al. 2016).

18.5.10.4 Coagulation

Coagulation is a chemical process; through a chemical reaction, it forms insoluble end products used to remove substances from the wastewater. The commonly used coagulating chemicals are polyvalent metals; typical coagulants would be lime and iron-containing compounds such as ferric chloride or ferric sulfate and alum

(aluminum sulfate) (Wen et al. 2004; Sipma et al. 2010; Wei et al. 2012). The electrocoagulation performance evaluation was analyzed in terms of the main operational parameter influence: pH, current density, electrocoagulation time, stirring rate, and anode nature (Dębska et al. 2004; Csefalvay et al. 2007; Das et al. 2012). The optimum pH was 5 because it allowed us to obtain color, with organic matter reduction degrees of about 99% in a relatively short time. On the basis of the data, it can be concluded that the best results have been obtained at a current density of 13.94 mA/cm^2, the efficiency achieved being 92.8%. In terms of anode material, experimental data have shown that the best efficiency in the removal of color, namely, the organic matter expressed as COD, was obtained using sacrificial anode made of iron. With regard to the aluminum electrodes, results were low; the color removal degree achieved was 78.57% compared to 99% obtained in the case of iron anodes (Crane et al. 2006; Atun et al. 2007; Boroski et al. 2009; Adishkumar et al. 2012; Arnold et al. 2014; Arya et al. 2016).

18.5.11 Biological Process

The objective of the biological process is to remove or reduce the concentration of organic and inorganic compounds by using microorganisms. The biological treatment method is the eco-friendly mechanism which uses microorganisms such as bacteria for the complete biochemical decomposition of biopharmaceutical effluents into nontoxic stable end products. Biological methods are of two types such as aerobic and anaerobic based upon the availability of the dissolved form of oxygen. Biological treatment has a great potential to control odors and is also applicable to retard the growth of pathogenic microorganisms.

Biological treatment primarily uses aerobic and anaerobic microorganisms that biochemically decompose the organic solids to inorganic or stable end products. The commonly used devices in the biological treatment process are trickling filter with secondary settling tanks, activated sludge, and modifications with final settling tank (Göbel et al. 2005; Duca and Boldescu 2009; Gadipelly et al. 2014; Gkotsis et al. 2014).

18.6 Conclusion

Due to the high complexity nature, low degradability, high concentration deposition, and recalcitrant nature of pharmaceuticals in effluents, advanced treatment of effluents is essential. There are many kinds of advanced treatment methods; each method has its own texture. The future will bring out the development of growingly sophisticated effluent treatment solutions for a safer elimination of potent hazards and reaches toward the high-quality-level zero-discharge strategy.

References

Adishkumar S, Sivajothi S, Rajesh Banu J (2012) Coupled solar photo-Fenton process with aerobic sequential batch reactor for treatment of pharmaceutical wastewater. Desalin Water Treat 48:89–95. https://doi.org/10.1080/19443994.2012.698799

Al-Farsi R, Ahmed M, Al-Busaidi A, Choudri BS (2018) Assessing the presence of pharmaceuticals in soil and plants irrigated with treated wastewater in Oman. Int J Recycl Org Waste Agric 7:165–172. https://doi.org/10.1007/s40093-018-0202-1

Arnold KE, Brown AR, Brown AR, Ankley GT, Sumpter JP (2014) Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. Philos Trans R Soc B Biol Sci 369:20130569. https://doi.org/10.1098/rstb.2013.0569

Arya V, Philip L, Murty Bhallamudi S (2016) Performance of suspended and attached growth bioreactors for the removal of cationic and anionic pharmaceuticals. Chem Eng J 284:1295–1307. https://doi.org/10.1016/j.cej.2015.09.070

Atun RA, Gurol-urganci I, Sheridan D (2007) Uptake and diffusion of pharmaceutical innovations in health systems. Int J Innov Manag 11:299–321. https://doi.org/10.1142/s1363919607001709

Bernhard M, Müller J, Knepper TP (2006) Biodegradation of persistent polar pollutants in wastewater: comparison of an optimised lab-scale membrane bioreactor and activated sludge treatment. Water Res 40:3419–3428. https://doi.org/10.1016/j.watres.2006.07.011

Boroski M, Rodrigues AC, Garcia JC, Sampaio LC, Nozaki J, Hioka N (2009) Combined electrocoagulation and TiO_2 photoassisted treatment applied to wastewater effluents from pharmaceutical and cosmetic industries. J Hazard Mater 162:448–454. https://doi.org/10.1016/j.jhazmat.2008.05.062

Clara M, Kreuzinger N, Strenn B, Gans O, Kroiss H (2005) The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Res 39:97–106. https://doi.org/10.1016/j.watres.2004.08.036

Crane M, Watts C, Boucard T (2006) Chronic aquatic environmental risks from exposure to human pharmaceuticals. Sci Total Environ 367:23–41. https://doi.org/10.1016/j.scitotenv.2006.04.010

Csefalvay E, Koczka K, Mizsey P (2007) Treatment of pharmaceutical waste water by hybrid separation processes. In: Proceedings of European Congress of Chemical Engineering (ECCE-6) Copenhagen, pp 1–4

Das MP, Bashwant M, Kumar K, Das J (2012) Control of pharmaceutical effluent parameters through bioremediation. J Chem Pharm Res 4:1061–1065

De Gusseme B, Pycke B, Hennebel T, Marcoen A, Vlaeminck SE, Noppe H, Boon N, Verstraete W (2009) Biological removal of 17α-ethinylestradiol by a nitrifier enrichment culture in a membrane bioreactor. Water Res 43:2493–2503. https://doi.org/10.1016/j.watres.2009.02.028

Dębska J, Kot-Wasik A, Namieśnik J (2004) Fate and analysis of pharmaceutical residues in the aquatic environment. Crit Rev Anal Chem 34:51–67. https://doi.org/10.1080/10408340490273753

Deegan AM, Shaik B, Nolan K, Urell K, Oelgemöller M, Tobin J, Morrissey A (2011) Treatment options for wastewater effluents from pharmaceutical companies. Int J Environ Sci Technol 8:649–666. https://doi.org/10.1007/BF03326250

Duca G, Boldescu V (2009) Pharmaceuticals and personal care products in the. Environment 28:27–35. https://doi.org/10.1007/978-90-481-2903-4_3

Gadipelly C, Rathod VK, Marathe KV (2014) Pharmaceutical industry wastewater: review of the technologies for water treatment. Ind Eng Chem Res 53(29):11571–11592. https://doi.org/10.1021/ie501210j

Gkotsis P, Banti D, Peleka E, Zouboulis A, Samaras P (2014) Fouling issues in membrane bioreactors (MBRs) for wastewater treatment: major mechanisms, prevention and control strategies. PRO 2:795–866. https://doi.org/10.3390/pr2040795

Göbel A, Thomsen A, McArdell CS, Joss A, Giger W (2005) Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. Environ Sci Technol 39:3981–3989. https://doi.org/10.1021/es048550a

Gomes J, Costa R, Quinta-Ferreira RM, Martins RC (2017) Application of ozonation for pharmaceuticals and personal care products removal from water. Sci Total Environ 586:265–283. https://doi.org/10.1016/j.scitotenv.2017.01.216

Grandclément C, Seyssiecq I, Piram A, Wong-Wah-Chung P, Vanot G, Tiliacos N, Roche N, Doumenq P (2017) From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: a review. Water Res 111:297–317. https://doi.org/10.1016/j.watres.2017.01.005

Gros M, Petrović M, Ginebreda A, Barceló D (2010) Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ Int 36:15–26. https://doi.org/10.1016/j.envint.2009.09.002

Huang L, Lee DJ (2015) Membrane bioreactor: a mini review on recent R&D works. Bioresour Technol 194:383–388. https://doi.org/10.1016/j.biortech.2015.07.013

Iorhemen OT, Hamza RA, Tay JH (2016) Membrane bioreactor (Mbr) technology for wastewater treatment and reclamation: membrane fouling. Membranes (Basel) 6:13–16. https://doi.org/10.3390/membranes6020033

Joss A, Andersen H, Ternes T, Richle PR, Siegrist H (2004) Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consequences for plant optimization. Environ Sci Technol 38:3047–3055. https://doi.org/10.1021/es0351488

Khetan SK, Collins TJ (2007) Human pharmaceuticals in the aquatic environment a review. Environ Technol 22:1383–1394. https://doi.org/10.1080/09593332208618186

Kimura K, Hara H, Watanabe Y (2005) Removal of pharmaceutical compounds by submerged membrane bioreactors (MBRs). Desalination 178:135–140. https://doi.org/10.1016/j.desal.2004.11.033

Klavarioti M, Mantzavinos D, Kassinos D (2009) Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. Environ Int 35:402–417. https://doi.org/10.1016/j.envint.2008.07.009

Krzeminski P, Tomei MC, Karaolia P, Langenhoff A, Almeida CMR, Felis E, Gritten F, Andersen HR, Fernandes T, Manaia CM, Rizzo L, Fatta-Kassinos D (2019) Performance of secondary wastewater treatment methods for the removal of contaminants of emerging concern implicated in crop uptake and antibiotic resistance spread: a review. Sci Total Environ 648:1052–1081. https://doi.org/10.1016/j.scitotenv.2018.08.130

Lacey C, Basha S, Morrissey A, Tobin JM (2012) Occurrence of pharmaceutical compounds in wastewater process streams in Dublin, Ireland. Environ Monit Assess 184:1049–1062. https://doi.org/10.1007/s10661-011-2020-z

Larsson DGJ (2014) Pollution from drug manufacturing: review and perspectives. Philos Trans R Soc Lond B Biol Sci 369:20130571. https://doi.org/10.1098/rstb.2013.0571

Mantzavinos D, Psillakis E (2004) Enhancement of biodegradability of industrial wastewaters by chemical oxidation pre-treatment. J Chem Technol Biotechnol 79:431–454. https://doi.org/10.1002/jctb.1020

Marketresearch.com (2018) The growing pharmaceuticals market: expert forecasts and analysis, pp 1–13

Miron AR, Chivu A-MA, Rikabi AAKK, Albu PC (2014) Pharmaceutical industry wastewater treatment through electrocoagulation. Rev Chim 65:1399–1406

Oller I, Malato S, Sánchez-Pérez JA (2011) Combination of advanced oxidation processes and biological treatments for wastewater decontamination—a review. Sci Total Environ 409:4141–4166. https://doi.org/10.1016/j.scitotenv.2010.08.061

Pomati F, Castiglioni S, Zuccato E, Fanelli R, Vigetti D, Rossetti C, Calamari D (2006) Effects of a complex mixture of therapeutic drugs at environmental levels on human embryonic cells. Environ Sci Technol 40:2442–2447. https://doi.org/10.1021/es051715a

Quinn B, Gagné F, Blaise C (2008) An investigation into the acute and chronic toxicity of eleven pharmaceuticals (and their solvents) found in wastewater effluent on the cnidarian, Hydra attenuata. Sci Total Environ 389:306–314. https://doi.org/10.1016/j.scitotenv.2007.08.038

Radjenović J, Petrović M, Barceló D (2009) Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res 43:831–841. https://doi.org/10.1016/j.watres.2008.11.043

Shariati FP, Mehrnia MR, Salmasi BM, Heran M, Wisniewski C, Sarrafzadeh MH (2010) Membrane bioreactor for treatment of pharmaceutical wastewater containing acetaminophen. Desalination 250:798–800. https://doi.org/10.1016/j.desal.2008.11.044

Sipma J, Osuna B, Collado N, Monclús H, Ferrero G, Comas J, Rodriguez-Roda I (2010) Comparison of removal of pharmaceuticals in MBR and activated sludge systems. Desalination 250:653–659. https://doi.org/10.1016/j.desal.2009.06.073

Snyder SA, Adham S, Redding AM, Cannon FS, DeCarolis J, Oppenheimer J, Wert EC, Yoon Y (2007) Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. Desalination 202:156–181. https://doi.org/10.1016/j.desal.2005.12.052

Tiwari B, Sellamuthu B, Ouarda Y, Drogui P, Tyagi RD, Buelna G (2017) Review on fate and mechanism of removal of pharmaceutical pollutants from wastewater using biological approach. Bioresour Technol 224:1–12. https://doi.org/10.1016/j.biortech.2016.11.042

Van Lier JB, Mahmoud N, Zeeman G (2008) Biological wastewater treatment: principles, moddeling and design—Chapter 16: anaerobic wastewater treatment

Wang Y, Wang X, Li M, Dong J, Sun C, Chen G (2018) Removal of pharmaceutical and personal care products (PPCPs) from municipalwaste water with integrated membrane systems, MBR-RO/NF. Int J Environ Res Public Health 15. https://doi.org/10.3390/ijerph15020269

Wei X, Li B, Zhao S, Wang L, Zhang H, Li C, Wang S (2012) Mixed pharmaceutical wastewater treatment by integrated membrane-aerated biofilm reactor (MABR) system—a pilot-scale study. Bioresour Technol 122:189–195. https://doi.org/10.1016/j.biortech.2012.06.041

Wen X, Ding H, Huang X, Liu R (2004) Treatment of hospital wastewater using a submerged membrane bioreactor. Process Biochem 39:1427–1431. https://doi.org/10.1016/S0032-9592(03)00277-2

Marine Bacteria: A Storehouse of Novel Compounds for Biodegradation

19

Neepa Pandhi and Shantkriti Shrinivasan

Abstract

Marine environments, which cover over the two-thirds of the earth's surface, constitute a great pool of diversified natural resources, as they comprise more than 95% of the biodiversity of the total environment. This broad biodiversity may be attributed to the broad spectrum of marine environments that are found on earth and can accommodate different types of life. Petroleum hydrocarbons are the most widespread contaminants within the marine environment. Pollution by hydrocarbons in marine environments may be the consequence of various natural (natural seepages) and/or anthropogenic activities (discharge during tanks and/or ships transportation and/or pipeline failures) as well as the chronic pollution (ships, harbors, oil terminals, freshwater run-off, rivers, and sewage systems). The increasing need to remedy adverse effects of anthropogenic activities on estuarine, coastal, and marine ecosystems has prompted the development of effective bioremediation strategies. In the natural environment, biodegradation of crude oil involves a succession of species within the consortia of the present. A consortium of many different bacterial species, with broad enzymatic capacities, is usually involved in oil degradation. The structurally diverse group of surface-active metabolites, synthesized by microorganisms, is classified as biosurfactants. The biosurfactants produced by some marine microorganisms are promising agents for bioremediation of hydrocarbons, particularly of oil pollution in marine environments. Because of the reduced surface and interfacial tensions exerted by these molecules, in both aqueous solutions and hydrocarbon mixtures,

N. Pandhi (✉)
Microbiology Department Shree M. & N. Virani Science College (Autonomous), Rajkot, Gujarat, India
e-mail: ndpandhi@vsc.edu.in

S. Shrinivasan
Department of Biotechnology, Kalasalingam Academy of Research and Education, Krishnankoil, Tamil Nadu, India

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_19

makes them potential candidates for enhancing oil recovery, and actually are under intense research, particularly for the bioremediation of the sea polluted by crude oil.

Keywords
Marine environment · Biodiversity · Bioremediation · Pollution · Hydrocarbon · Biosurfactants

Planet Earth is the only place in the universe where life is known to exist. Life forms are found in large varieties and diversity. Earth is a support system for plants, animals, and microbes as it provides land, water, air, mineral, and other resources for the sustenance of life. However, this planet is now becoming increasingly inhabitable and unsuitable for most of the species of living forms. This is due to the activities mankind has been doing since time immemorial for his own sustenance, comfort, ease, and luxury. These anthropogenic activities have created substantial scale depletion of natural resources and deterioration of environment. These have also resulted in climate change, land degradation, pollution of air, water, and soil, loss of biodiversity, accumulation of harmful recalcitrant chemicals, and many more related problems.

19.1 Environmental Pollution

Environmental pollution is a global problem affecting every corner of our planet, in the form of land, air, water, or noise pollution. Neither the ground, valley, mountains, oceans, nor rivers are spared. There are pollutants at the North Pole, South Pole and even in space. All these polluting agents have appeared in a magnanimous amount and at a rapid rate due to anthropogenic activities. Developing countries are more critically affected by pollution due to increasing industrialization, urbanization, high population growth, poverty, illiteracy, and unawareness. Biomes and ecosystems are deteriorating at a fast pace. Around 92% of deaths due to pollution are reported from the developing countries. Urban locations have become unsuitable for human habitation due to the quality of air, water, and land.

A wide range of pollutants are released every year in the environment through industrial processes. It is estimated that by 1950, more than 1,400,000 pounds of pesticides were in use, out of which, only a few, like polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), polyethylene, hydrochlorofluorocarbons (HCFCs), and chlorofluorocarbons (CFCs), have been studied for toxicity. Fossil fuel consumption for transportation, industry, and domestic purposes is one of the major reasons of environmental imbalance.

19.2 Hydrocarbon Pollutants

The pollution of soil and water by industrial chemicals is a serious problem afflicting the modern world. A substantial number of hydrophobic compounds enter the environment as natural products of animal, plant, or microbial origin like steroids, wax, terpenes, etc., and a great amount is generated due to anthropogenic activities, like hydrocarbon and its derivatives and petroleum. Petroleum hydrocarbons are the most frequently occurring environmental contaminants because of their extensive use in both aquatic and terrestrial ecosystems. Effective petroleum hydrocarbon remediation is challenging because petroleum is a complex mixture of aliphatic and aromatic hydrocarbons. Oil spills, leakage, and inadequate disposal of petroleum and petroleum products pose a serious threat to both aquatic and terrestrial ecosystems.

These pollutants can be degraded by some group of microorganisms that are capable of growth and survival in all ecological niches and have metabolic diversity which helps them in utilizing different carbon sources. Some of these substances have low aqueous solubility and high solid-water distribution ratios. This makes their interaction with and attack by microbial cells difficult. Thus, substrate bioavailability is an important factor, which is affected by both physical and chemical nature of the substrate. Other parameters of importance are the environment (water, soil, sediment, organic matter, etc.); the kinetic parameters like diffusion and flow rate, mass transfer, spatial separation between cells and substrate, etc.; and the physiological properties intrinsic to the cells.

19.3 Pollutant Processing in Marine Environment

Intensive studies have been carried out on the process and mechanism of pollutant removal in the marine environment. A variety of physical, chemical, and biological changes take place. Nonbiological processes are adsorption onto suspended particulate material, dispersion, dissolution, evaporation, photochemical oxidation, water-in-oil emulsification, sedimentation, and sinking. Biological processes include microbial degradation and ingestion by organisms. These three types of processes occur simultaneously and result into structural and chemical change in the pollutant, which in turn decides the rate and efficacy of biodegradation. A very important weathering process which takes place in the first 48 hours of a spill is generally evaporation, the process where in low to medium weight crude oil components having low boiling points get volatilized into the atmosphere. Evaporation can reduce one to two-thirds of an oil spill's mass. All other abiological processes of weathering do not account for any significant proportion.

19.4 Biodegradation of the Marine Pollutants

Biodegradation in nature by natural agents is the most important means to remove oil from the marine environment, especially the nonvolatile components of petroleum. Biodegradation, in general, is the process where microorganisms, mainly bacteria, fungi, yeast, etc., transform petroleum hydrocarbons into some simpler products. Many a times, some of these products are more complex, but are ideally converted to carbon dioxide, nontoxic hydrophilic products, and some microbial biomass. Technically, mere disappearance of oil is not biodegradation if it is not chemically transformed by microbial activities.

Marine environments have hydrocarbon-degrading microorganisms in very less abundance. The presence of petroleum hydrocarbons in the environment, however, may activate the organisms and stimulate their growth. This causes variations in the composition and structure of microbial communities in the contaminated area. Hassanshahian et al have shown that in the presence of pollutants, the number of crude oil-degrading bacteria increased significantly.

For understanding, evaluating, and developing the bioremediation strategies for a polluted site, identification of the organisms playing major roles in biodegradation is very important. Many marine biologists and microbiologists have made efforts to characterize these marine bacterial communities, to identify the degraders, and to study the catalytic potential of these organisms. Natural marine environments have very little concentrations of nitrogen and phosphorus to support the microbial growth, especially after the petroleum or hydrocarbon spill. Hence, these nutrients are added to a contaminated site for the stimulation of microbial growth and to increase biodegradation rate at the site.

19.5 Hydrocarbon-Degrading Marine Microorganism

Hydrocarbon-degrading bacteria were isolated in the early parts of the twentieth century, and around 79 bacterial genera are known that can use hydrocarbons as a sole carbon and energy source. Apart from this, 9 cyanobacterial genera, 103 fungal genera, and 14 algal genera are also known to degrade or transform hydrocarbons.

Hydrocarbon-degrading microorganisms are widely distributed in marine, freshwater, and soil habitats. Some of the well-established degraders of hydrocarbons are *Alcanivorax*, *Cellulomonas*, *Dietzia*, *Gordonia* groups, *Marinobacter*, *Microbulbifer*, *Micrococcus*, *Pseudomonas*, *and Sphingomonas*; molds of genera *Amorphoteca*, *Aspergillus*, *Fusarium*, *Graphium*, *Neosartorya*, *Penicillium*, *Paecilomyces*, and *Talaromyces*; and yeasts of genre *Candida*, *Pichia*, *and Yarrowia*. These microbial genera of hydrocarbon degraders are distributed worldwide. All aquatic ecosystems contain one or other type of oil-degrading bacteria. Hydrocarbon degradation is, in fact, not done by a single species of microorganism, as it may not be capable of degrading all the components of given oil. A consortium of microbes is usually required for significant overall degradation. Both the quantity

and the diversity of microbes vary in accordance with the quality and the quantity of the hydrocarbon pollutants.

19.6 Mechanism of Hydrocarbon Degradation by Microorganisms

Marine microorganisms as well as microbes of other habitat degrade hydrocarbon pollutants by mainly two pathways: aerobic and anaerobic.

19.6.1 Aerobic Degradation

19.6.1.1 Fundamental Reactions of Aerobic Degradation

The main step of aerobic degradation of hydrocarbon is the addition of one or two oxygen atoms, as per the case, to the hydrocarbon molecule. This converts it to an alkanol (if the pollutant is an aliphatic hydrocarbon) or to a phenol (if it is an aromatic molecule). Due to this, hydrocarbon becomes "active" and becomes more water soluble and introduces a reactive site for the next reactions. This requires energy, which is produced by the oxidation of a reduced intermediate like NADH. For the degradation of alkanes, different enzyme systems exist. Fatty acids are the main intermediates of the alkane degradation, which are produced from the alkanols via aldehydes. These acids are further acted upon by the pathway typical of physiological carboxylic acid degradation, in which the molecule is shortened stepwise. Fatty acids can also be excreted by the cells in the environment.

19.6.1.2 Complete Mineralization (Dioxygenase Pathway)

Bacteria generally prefer this pathway. A monoaromatic molecule or a single ring of polyaromatic compound is acted upon by an enzyme dioxygenase. This leads to stepwise oxidation of the molecule. One of the main intermediates of the pathway is pyruvate. Carbon dioxide is another main product along with production of biomass. This pathway can mineralize only up to four ring compounds.

19.6.1.3 Co-metabolic Transformation (Monooxygenase Pathway)

Mainly found in molds and yeasts, this pathway also occurs in some bacteria and algae. Degradation of the pollutant hydrocarbon is possible if a source of carbon and energy is available. The activity of the enzyme monooxygenase results in the formation of a highly reactive epoxide, which may be toxic or mutagenic. Trans-dihydrodiols are the end products that are not further metabolized under laboratory conditions.

19.6.2 Anaerobic Degradation

In 1988, Evans and Fuchs published their work on the anaerobic degradation of aromatic compounds. Till then, it was believed that hydrocarbons are degraded only by aerobic pathways. Since then, work has been done on the mechanism of anaerobic degradation of aliphatic and aromatic hydrocarbons. It has been shown that anaerobic hydrocarbon degradation does occur in nature, but it is considerably slower than aerobic degradation. Denitrifying bacteria, iron bacteria and sulfate reducers can metabolize hydrocarbons anaerobically. Some phototrophic bacteria and methanogenic consortia have also been found to degrade hydrocarbons anaerobically. The mechanism and the metabolic pathways are yet not completely understood.

19.6.2.1 Conditions and Factors That Affect Hydrocarbon Degradation

The most ideal conditions for biodegradation of marine pollutants rarely occur. There are various competing processes, mixture of pollutants, type and quantity of the pollutants, geographic location of the site, indigenous microbial flora, and the presence or absence of suitable physical and chemical conditions that govern the success of the process. Their removal by natural mechanisms is often very slow, expensive, and time consuming due to low oil surface to volume ratio which limits the bioavailability of the oil.

Biodegradation of oil works very effectively in the open sea. It, however, does not work with equal efficiency on beaches. Tides, wind, strong sun irradiation, and the nature of the pollutants decide the fate of biodegradation. Environmental variables can also greatly influence the rate and extent of biodegradation. There are various other factors that also affect biodegradation. Oxygen, nutrient availability, salinity, and lack of sufficient knowledge about factors are the major factors to be considered.

19.7 Biosurfactants

Probably the most effective method to treat oil-related contamination is the use of surfactants that disperse the oil and accelerate its mineralization. Synthetic surfactants have applications in the degradation processes of petroleum hydrocarbons, but they are environmentally hazardous. Biosurfactants have several advantages over them, such as lower toxicity, higher biodegradability, and effectiveness at extreme temperatures or pH values.

Biosurfactants, produced by microorganisms, are amphipathic surface active molecules containing hydrophilic and hydrophobic moieties that act by emulsifying hydrocarbons, increasing their solubilization, and subsequently rendering them available for microbial degradation. Bioemulsifier or bioemulsans are surface active compounds that do not necessarily reduce surface tension but form stable emulsions between liquid hydrocarbon and water mixtures and are hence also often referred to as biosurfactants. Apart from being used in bioremediation, these biological products

have potential uses in agriculture and cosmetic, pharmaceutical, detergent, food, textile, paper, and paint industries.

The hydrosphere marine environment represents the major component of the Earth's biosphere. It covers major (70%) part of the Earth's surface and makes up 90% of the volume of its crust. The maximum depth of 1000 m and 365 million km^2 is attributed to the hydrosphere oceanic system. Oceans represent a vast and exhaustive source of natural products in the globe, harboring the most diverse groups of flora and fauna. Marine microorganisms have, over the time, developed unique metabolic and physiological capabilities to flourish in extreme habitats and produce novel metabolites that are not often present in microbes of terrestrial origin. Therefore, this rich marine habitat provides a magnificent opportunity to discover newer compounds such as antibiotics, enzymes, vitamins, drugs, biosurfactants (BS), bioemulsifiers (BE), and other valuable compounds of commercial importance. Among these various marine bioactive compounds, BS/BE are of great importance due to their structural and functional diversity and industrial applications.

Biosurfactants are metabolites that exhibit surface activity and are synthesized by bacteria, yeast, and filamentous fungi grown on different carbon sources. These compounds consist of a hydrophobic moiety, usually a hydrocarbon chain of one or more fatty acids, which may be saturated, unsaturated, hydroxylated, or branched, attached to a hydrophilic moiety that may be an ester, a hydroxy group, phosphate, or carbohydrate. The hydrophilic and hydrophobic portions present in the surfactant molecule tend to distribute at the interface of fluid phases of different degrees of polarity (oil/water and air/water). This distribution of the molecules enables them to reduce surface and interfacial tensions. Rhamnolipids, which have these properties, are applied in different industrial processes, such as in the production of pharmaceuticals, chemicals, and cosmetics; in the synthesis of nanoparticles; in the bioremediation of environments polluted by oil and derivatives; and in the tertiary recovery of oil.

19.7.1 Types of Biosurfactants

19.7.1.1 Glycolipids

Most known biosurfactants are glycolipids. They are carbohydrates in combination with long-chain aliphatic acids or hydroxyl aliphatic acids. The linkage is by means of either ether or an ester group. Among the glycolipids, the best known are rhamnolipids, trehalolipids, and sophorolipids.

Rhamnolipids

These glycolipids, in which one or two molecules of rhamnose are linked to one or two molecules of β-hydroxydecanoic acid, are the best studied. While the OH group of one of the acids is involved in glycosidic linkage with the reducing end of the rhamnose disaccharide, the OH group of the second acid is involved in ester formation.

Production of rhamnose containing glycolipids was first described in *Pseudomonas aeruginosa* by Jarvis and Johnson (1949). L-rhamnosyl-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate and L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate, referred to as rhamnolipids 1and 2, respectively, are the principal glycolipids produced by *P. aeruginosa*.

Trehalolipids

Several structural types of microbial trehalolipid biosurfactants have been reported. Disaccharide trehalose linked at C-6 and C-6′ to mycolic acid is associated with most species of *Mycobacterium*, *Nocardia*, and *Corynebacterium*. Mycolic acids are long-chain, α-branched-β-hydroxy fatty acids. Trehalolipids from different organisms differ in the size and structure of mycolic acid, the number of carbon atoms, and the degree of unsaturation.

Sophorolipids

These glycolipids, which are produced mainly by yeasts such as *Torulopsis bombicola*, *T. petrophilum*, and *T. apicola*, consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid by glycosidic linkage. Generally, sophorolipids occur as a mixture of macrolactones and free acid form. It has been shown that the lactone form of the sophorolipid is necessary, or at least preferable, for many applications.

19.7.1.2 Lipopeptide and Lipoproteins

A large number of cyclic lipopeptides, including decapeptide antibiotics (gramicidins) and lipopeptide antibiotics (polymyxins), are produced. These consist of a lipid attached to a polypeptide chain.

Surfactin

The cyclic lipopeptide surfactin, produced by *Bacillus subtilis* ATCC21332, is one of the most powerful biosurfactants. It is composed of a seven-amino-acid ring structure coupled to a fatty-acid chain via lactone linkage. It lowers the surface tension from 72 to 27.9 mNm^{-1} at concentrations as low as 0.005%.

Lichenysin

Bacillus licheniformis produces several biosurfactants that act synergistically and exhibit excellent temperature, pH, and salt stability. These are also similar in structural and physiochemical properties to the surfactin. The surfactants produced by *B. licheniformis* are capable of lowering the surface tension of water to 27 mNm^{-1} and the interfacial tension between water and n-hexadecane to 0.36 mNm^{-1}.

19.7.1.3 Fatty Acids, Phospholipids, and Neutral Lipids

Several bacteria and yeasts produce large quantities of fatty acids and phospholipid surfactants during growth on *n*-alkanes. The hydrophilic and lipophilic balance (HLB) is directly related to the length of the hydrocarbon chain in their structures. In *Acinetobacter* sp. strain HO1-N, phosphatidylethanolamine-rich vesicles are

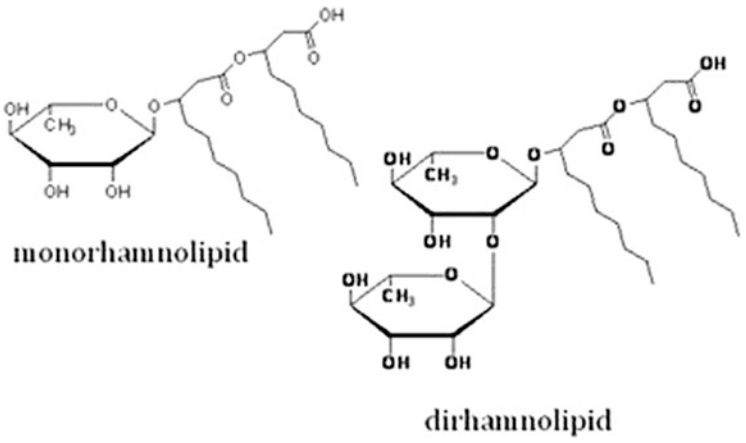
produced, which form optically clear microemulsions of alkanes in water. Phosphatidylethanolamine produced by R. erythropolis grown on *n*-alkane causes lowering of interfacial tension between water and hexadecane to less than 1 mNm^{-1} and a critical micelle concentration (CMC) of 30 mg L^{-1}.

19.7.1.4 Polymeric Biosurfactants

The best-studied polymeric biosurfactants are emulsan, liposan, alasan, lipomanan, and other polysaccharide–protein complexes. *Acinetobacter calcoaceticus* RAG-1 produces an extracellular potent polyanionic amphipathic heteropolysaccharide bioemulsifier. Emulsan is an effective emulsifying agent for hydrocarbons in water, even at a concentration as low as 0.001–0.01%. Liposan is an extracellular water-soluble emulsifier synthesized by *Candida lipolytica* and is composed of 83% carbohydrate and 17% protein.

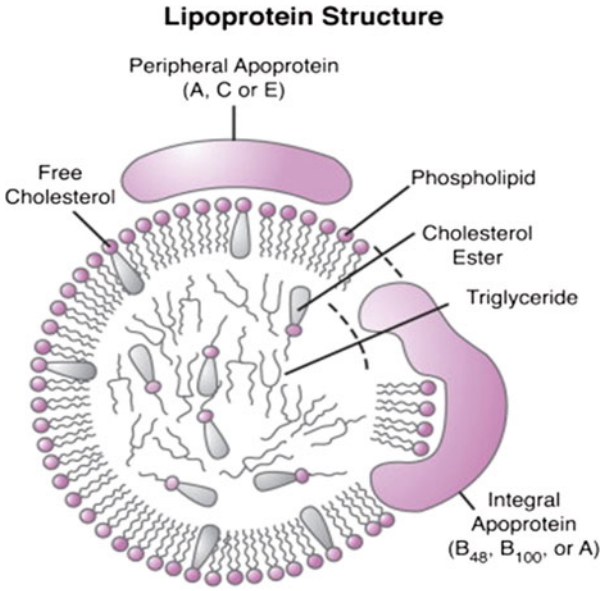
19.7.1.5 Particulate Biosurfactants

Extracellular membrane vesicles partition hydrocarbons to form a microemulsion, which plays an important role in alkane uptake by microbial cells. Vesicles of *Acinetobacter* sp. strain HO1-N with a diameter of 20–50 nm and a buoyant density of 1.158 cubicgcm^{-1} are composed of protein, phospholipids, and lipopolysaccharide.

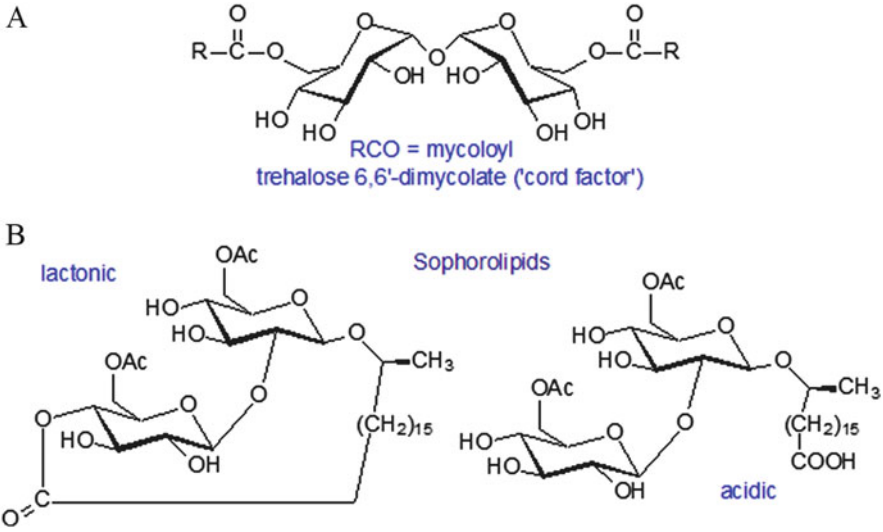


(Rhamnolipids—Courtesy: "Microbial Surfactants," Dr. Raina M. Maier, Uni. Of Arizona)

Biosurfactant-producing bacteria					
S. no.	Biosurfactant type	Source organism	Chemical composition	Applications	References
1	Glycolipids	*Alcaligenes*	Glucose and lipid	Inhibition of growth of microalgae	Poremba et al. (1991)
		Arthrobacter	Trehalose glycolipids	Emulsification activity	Schulz et al. (1991)
		Rhodococcus	Anionic glucose lipid	Surface activity	Abraham et al. (1998)
		Halomonas	Mannose, galactose, glucose, and lipids	Emulsifying properties	Pepi et al. (2005)
		Pseudomonas sp.	Rhamnolipid	Emulsifying properties	Desai and Banat (1997)
2	Lipopeptides	*Bacillus circulans*	Lipid and proteins	Antimicrobial activity	Das et al. (2008)
		Bacillus licheniformis	Lipid and proteins	Surface activity and antibacterial activity	Yakimov (1995)
		Azotobacter sp.	Lipid and protein	Emulsification crude oils	
		Bacillus subtilis	Surfactin	Surface activity	Desai and Banat (1997)
3	Phospholipids and fatty acids	*Myroides*	Cholic acid and deoxycholic acid	Good surface activity	Maneerat et al. (2005)
4	Glycolipopeptide	*Corynebacterium* sp.	Carbohydrate, lipid, and protein	Emulsification activity	



(Courtesy: Lipoprotein—An overview, Science Direct)



(A: Trehelolipids, B: Sophorolipids—Courtesy: LipidWeb—Rhamnolipids, Sophorolipids and Other Simple Glycolipids)

19.7.2 Properties of Biosurfactants

The main distinctive features of microbial surfactants that can be of interest for food processing are related to their surface activity; tolerance to pH, temperature, and ionic strength; biodegradability; low toxicity; emulsifying and demulsifying ability; and antimicrobial activity.

19.7.2.1 Surface and Interface Activity

A good surfactant can lower surface tension (ST) of water from 72 to 35 mNm^{-1} and interfacial tension (IT) of water/hexadecane from 40 to 1 mNm^{-1}. Surfactin from *B. subtilis* can reduce ST of water to 25 mNm^{-1} and IT of water/hexadecane to <1 mNm^{-1}. The rhamnolipids from *P. aeruginosa* decreased ST of water to 26 mNm^{-1} and IT of water/hexadecane to <1 mNm^{-1}; however, some rhamnolipid homologues have demonstrated lower values. The sophorolipids from *C. bombicola* were reported to reduce ST to 33 $mNm^{-1}$and IT to 5 mNm^{-1}. In general, bioemulsifiers are more effective and efficient, and their CMC (critical micelle concentration) is about 10–40 times lower than chemical surfactants, i.e., less surfactant is necessary to get a maximal decrease on ST.

19.7.2.2 Biodegradability

Unlike synthetic surfactants, microbial-produced compounds are easily degraded and particularly suited for environmental applications such as bioremediation. The increasing environmental concern among consumers and the regulatory rules imposed by governments have forced industry to search for alternative products such as biosurfactants.

19.7.2.3 Low Toxicity

Little data are available in literature regarding the toxicity of microbial surfactants. They are generally considered low or nontoxic products and therefore are found appropriate for pharmaceutical, cosmetic, and food uses. When comparing the toxicity of six biosurfactants, four synthetic surfactants, and two commercial dispersants, Poremba et al. (1991a, b) found that most biosurfactants were degraded faster, except for a synthetic sucrose stearate that showed structure homology to glycolipids and was degraded more rapidly than the biogenic glycolipids (rhamnolipids, trehalose lipids, sophorose lipids). These authors also reported that biosurfactants showed higher EC50 (effective concentration to decrease 50% of test population) values than synthetic dispersants.

Rhamnolipid surfactants are presently produced at commercial scale by Jeneil Biosurfactant Corp. (www.biosurfactant.com), which offers diverse formulations for different purposes. Recently, the greater consumer awareness of adverse allergic effects caused by artificial products stimulates the development of alternative ingredients, thus opening an excellent opportunity to expand the use of natural surfactants of microbial origin.

19.7.2.4 Emulsion Forming and Emulsion Breaking

An emulsion is a heterogeneous system, consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets, whose diameter in general exceeds 0.1 mm. Emulsions have an internal or dispersed phase and an external or continuous phase, so there are generally two types: oil-in-water (o/w) or water-in-oil (w/o) emulsions. Such systems possess a minimal stability, which may be accentuated by additives such as surface-active agents (surfactants). Thus, stable emulsions can be produced with a life span of months and years.

Biosurfactants may stabilize (emulsifiers) or destabilize (de-emulsifiers) the emulsion. High-molecular-mass biosurfactants are in general better emulsifiers than low-molecular-mass biosurfactants. Sophorolipids from *Torulopsis bombicola* have been shown to reduce surface and interfacial tension but not to be good emulsifiers. By contrast, liposan has been shown not to reduce surface tension but used successfully to emulsify edible oils. Polymeric surfactants offer additional advantages because they coat the droplets of oil, thereby forming very stable emulsions that never coalesce. This property is especially useful for making oil/water emulsions for cosmetics, food, and in dairy products.

Evaluation of emulsifying ability of biosurfactants is in general related to hydrocarbons such as kerosene because of their potential in environmental applications. Few attempts have been made to evaluate emulsion forming by biosurfactants with oils and fats used in food industry. A lipopeptide obtained from *B. subtilis* was able to form stable emulsions with soybean oil and coconut fat, suggesting its potential as emulsifying agent in foods. A mannoprotein from *Kluyveromyces marxianus* was able to form emulsions with corn oil that were stable for 3 months; the yeast was cultivated on whey-based medium suggesting potential application as food bioemulsifier. The extracellular carbohydrate-rich compound from *Candida utilis* was successfully used as an emulsifying agent in salad dressing formulations.

The use of yeast for production of biosurfactants is interesting because these organisms are generally recognized as safe and they are already present in many food manufacturing processes; on the contrary, products derived from bacteria such as the opportunistic *P. aeruginosa* still face some resistance concerning their use as food ingredients. In some cases, the emulsion, which is generated in one part of the process, may have to be destabilized in a subsequent operation to develop a certain functional property to the final product. De-emulsification can be of interest in food processing especially when related to fat and oil products as well as in waste treatment.

19.7.2.5 Antimicrobial Activity

Several biosurfactants have shown antimicrobial action against bacteria, fungi, algae, and viruses. The lipopeptide from *B. subtilis* showed potent antifungal activity. A significant reduction on the mycoflora present in stored grains of corn, peanuts, and cottonseeds was observed at concentration of 50–100 ppm.

Rhamnolipids inhibited the growth of harmful bloom algae species. A rhamnolipid mixture obtained from *P. aeruginosa* AT10 showed inhibitory activity

against the bacteria *Escherichia coli*, *Micrococcus luteus*, *Alcaligenes faecalis* (32 mg mL^{-1}), *Serratia marcescens*, *Mycobacterium phlei* (16 mg mL^{-1}), and *Staphylococcus epidermidis* (8 mg mL^{-1}) and excellent antifungal properties against *Aspergillus niger* (16 mg mL^{-1}), *Chaetomium globosum*, *Penicillium chrysogenum*, *Aureobasidium pullulans* (32 mg mL^{-1}), and the phytopathogenic *Botrytis cinerea* and *Rhizoctonia solani* (18 mg mL^{-1}).

Sophorolipids and rhamnolipids were found to be effective antifungal agents against plant and seed pathogenic fungi. Mycelial growth of *Phytophthora* sp. and *Pythium* sp. was 80% inhibited by 200 mg L^{-1} of rhamnolipids and 500 mg L^{-1} of sophorolipids. The mannosylerythritol lipid (MEL), a glycolipid surfactant from *Candida antarctica*, has demonstrated antimicrobial activity, particularly against Gram-positive bacteria. Besides their antimicrobial activity, new biological applications of biosurfactants have been found, and some reviews concerning the potential uses of microbial surfactants in biomedical sciences were recently published.

19.7.3 Applications of Biosurfactants

All surfactants are chemically synthesized. However, very recently, much attention has been given toward biosurfactants due to their broad range of functional properties and diverse synthetic capabilities of microorganisms. Most significant is their environmental acceptability, as they are easily biodegradable and have low toxicity than the synthetic surfactants. These unique natures of the biosurfactants allow their utilization and possible replacement of chemically synthesized surfactants in a large number of industrial operations. Furthermore, they are ecologically safe and can be applied in wastewater treatment and bioremediation. Some of the potential applications of biosurfactants in pollution and environmental control are microbial enhanced oil revival, hydrocarbon degradation in the soil environment and hexachlorocyclohexane degradation, and removal of heavy metal from contaminated soil and hydrocarbon in aquatic environment.

19.7.3.1 Potential Food Applications

Biosurfactants can be explored for several food-processing applications. They can be used as food-formulation ingredients.

Apart from their obvious role as agents that decrease surface and interfacial tension, thus facilitating the formation and stabilization of emulsions, the surfactants can have various other functions in food, for example, to control the aggregation of fat globules, stabilization of aerated systems, improvement of texture and shelf life of products containing starch, modification of rheological properties of wheat dough, and improvement of constancy and texture of fat-based products. In bakery and ice-cream formulations, biosurfactants act by controlling the consistency, slowing staling and solubilizing the flavor oils; they are added during cooking of fats and oil. Improvement in the stability of dough, volume, texture, and conservation of bakery products is obtained by addition of rhamnolipid surfactants. The study also

suggested the use of rhamnolipids to improve the properties of butter cream and frozen confectionery products. L-rhamnose has substantial potential as a forerunner for flavoring. It is already used industrially as a precursor of high-quality flavor components like furane.

19.7.3.2 Antiadhesive Agents

A biofilm is described as a group of bacteria that have formed a colony on a surface. The biofilm not only consists of bacteria but also includes all the extracellular material produced at the surface and any material trapped within the formed matrix. Bacterial biofilms that are present in the food industry surfaces are potential sources of contamination that may lead to food spoilage and transmission of disease. Thus, controlling the adherence of microorganisms to food-contact surfaces is an essential step in providing safe and quality products to consumers.

The involvement of biosurfactants in microbial adhesion and detachment from surfaces has been investigated. A surfactant produced by *Streptococcus thermophilus* has been used for fouling control of heat-exchanger plates in pasteurizers, as it slows down the colonization of other thermophilic organisms utilized as fat stabilizers and antispattering strains of *Streptococcus* that are responsible for fouling. The treatment of stainless steel surfaces with a biosurfactant obtained from *Pseudomonas fluorescens* inhibits the attachment of *L. monocytogenes*. The bioconditioning of surfaces through the use of microbial surfactants has been suggested as a new strategy to reduce adhesion.

19.7.3.3 Anticancer Activity

The biological activities of seven microbial extracellular glycolipids, together with mannosylerythritol lipids A, mannosylerythritol lipids B, rhamnolipid, polyol lipid, sophorose lipid, etc., have been studied. All these glycolipids, except rhamnolipid, were able to induce cell differentiation instead of cell proliferation in the human promyelocytic leukemia cell line HL60. STL and MEL noticeably increased common differentiation characteristics in monocytes and granulocytes, respectively. Exposure of B16 cells to MEL leads to the condensation of chromatin, DNA fragmentation, and sub-G1 arrest (the sequence of events in apoptosis). This is the first evidence that growth retards, and apoptosis and differentiation of the mouse malignant melanoma cells can be induced, by glycolipids. In addition, exposure of PC12 cells to MEL enhanced the activity of acetylcholinesterase and interrupted the cell cycle at the G1 phase, with resulting outgrowth of neurites and partial cellular differentiation lipid (MEL); a glycolipid surfactant from *Candida antarctica* has shown antimicrobial activity particularly against Gram-positive bacteria.

19.7.3.4 Antihuman Immunodeficiency Virus and Sperm-Immobilizing Activity

The increased incidence of human immunodeficiency virus (HIV)/AIDS in women aged 15–49 years has identified the urgent need for a female-controlled, effective, and safe vaginal topical microbicide. To overcome this challenge, sophorolipid synthesized by *C. bombicola* and its structural analogues have been studied for

their spermicidal, anti-HIV, and cytotoxic activities. The sophorolipid diacetate ethyl ester derivative is the most potent spermicidal and virucidal agent of the series of sophorolipids studied. The virucidal activity against HIV and sperm-immobilizing activity against human semen are similar to those of nonoxynol. Nevertheless, it also induced sufficient vaginal cell toxicity to raise concerns about its applicability for long-term microbicidal contraception.

19.7.3.5 Agents for Respiratory Failure

A deficiency of pulmonary surfactant which is a phospholipid protein complex is responsible for the failure of respiration in prematurely born infants. Isolation of the genes for protein molecules of this surfactant and cloning in bacteria has made possible its fermentative production for medical applications.

19.7.3.6 Agents for Stimulation of Skin Fibroblast Metabolism

The use of sophorolipids in lactone form comprises a major part of diacetyl lactones as agents for stimulating skin dermal fibroblast cell metabolism and mainly as agents for the stimulation of collagen neosynthesis, at a concentration of 0.01 ppm at 5% (p/p) of dry matter in formulation. This can be applied in cosmetology and also in dermatology. The purified lactone sophorolipid product is of importance in the formulation of dermis antiaging products because of its effect on the stimulation of cells of the dermis. By encouraging the production of new collagen fibers, purified lactone sophorolipids may be used both as a preventive measure against aging of the skin and used in creams for the body and in body milks, lotions, and gels that are used for the skin.

19.7.3.7 Pretreatment of Rubber Gloves Used for Surgery

Pretreatment of silicone rubber with surfactant produced by *S. thermophilus* inhibited by 85% the adhesion of *C. albicans*, whereas surfactants obtained from *L. fermentum* and *L. acidophilus* that adsorbed on glass reduced by 77% the number of adhering uropathogenic cells of *Enterococcus faecalis*. The biosurfactant obtained from *L. fermentum* inhibited *S. aureus* infection and adhered to surgical implants. Surfactin decreased the amount of biofilm formation by *Salmonella typhimurium*, *S. enterica*, *Escherichia coli*, and *Proteus mirabilis* in PVC plates and vinyl urethral catheters.

19.7.4 Countries Producing Biosurfactants

According to the report of *Clean India Journal*, May 16, 2014, the United States of America is a country producing the largest share of global market of biosurfactants. Leading companies in the market of biosurfactants include BASF Cognis, Ecover, Urumqi Unite, Saraya, and MG Intobio. The United States mostly use biosurfactants in food industries, agriculture, and textiles.

19.8 Summary

Environmental pollution is the most important issue for any nation in today's time. Various types of pollutants contaminate land, air, and water. Hydrocarbon is a major pollutant affecting these three. There are many physical, chemical, and biological methods for the mitigation of polluted sites. Biodegradation and bioremediation are effective eco-friendly treatment methods used for cleaning certain oil-contaminated water bodies. These methods have lower environmental impact compared to other methods for oil removal in the water.

Combined and integrated application of microbial systems and production of biosurfactants by them can be a key to effective biodegradation approaches hydrocarbon contamination of water bodies. This can also convert the toxic and hazardous pollutants into nonhazardous recyclable products like carbon dioxide, water, and biomass. A consortium of microbes can prove to be more effective than a single species. There is also a need for novel microorganisms, and they may be engineered to have better efficacy without causing adverse effects.

References

Abraham W-R, Meyer H, Yakimov M (1998) Novel glycine containing glucolipids from the alkane using bacterium Alcanivorax borkumensis. Biochim Biophys Acta – Lipids Lipid Metab 1393 (1):57–62

Abu-Ruwaida AS, Banat IM, Hadirto S, Saleem A, Kadri M (1991) Isolation of biosurfactant producing bacteria-product characterisation and evaluation. Acta Biotechnol 11:315–324

Arino S, Marchal R, Vandecasteele J (1996) Identification and production of rhamnolipidic biosurfactant by *Pseudomonas* sp. Applied Microbiol. Biotechnol. 45:162–168

Asmer HJ, Slegmund L, Fritz W, Wrey V (1988) Microbial production, structure elucidation and bioconversion of sophorose lipid. JAOCS 65:1460–1466

Asselineau C, Asselineau J (1978) Trehalose-containing glycolipids. Prog Chem Fats Lipids 16:59–99

Banat IM (1995) Characterisation of biosurfactants and their use in pollution removal-state of art (review). Acta Biotechnol 15:251–267

Banat IM, Makkar BS, Cameotra SS (2000) Potential commercial applications of microbial surfactants. Applied Microbiol Biotechnol 53:495–508

Beeba JL, Umbriet WW (1971) Extracellular lipids of *Thiobacillus thiooxidans*. J Bacteriol 108:612–615

Benincasa M (2007) Rhamnolipid produced from agroindustrial wastes enhances hydrocarbon biodegradation in contaminated soil. Curr Microbiol 54:445–449

Benincasa M, Contiero J, Manresa MA, Moraes IO (2002) Rhamnolipid production by *Pseudomonas aeruginosa* LBI growing on soap stock as the sole carbon source. J Food Eng 54:283–288

Bernheimer AW, Avigad LS (1970) Nature and properties of a cytological agent produced by *Bacillus subtilis*. J Gen Microbiol 61:361–369

Biermann M, Lange F, Piorr R, Ploog U, Rutzen H, Schindler J, Schmidt R (1987) Surfactants in consumer products. In: Falbe J (ed) Theory, technology and application. Springer, Heidelberg, pp 86–106

Bloomberg G (1991) Designing proteins as emulsifiers. Lebensmitte Technologie 24:130–131

Burger AE (1993) Estimating the mortality of seabirds following oil-spills-effects of spill volume. Mar Pollut Bull 26:140–143

Burns KA, Garrity SD, Levings SC (1993) How many years until mangrove ecosystems recover from catastrophic oil-spills of crude oil. J Exp Mar Biol Ecol 171:273–295

Caldini G, Cenci G, Manenti R, Morozzi G (1995) The ability of an environmental isolate of *Pseudomonas fluorescens* to utilise chrysene and other four-ring polynuclear aromatic hydrocarbons. Applied Microbiol. Biotechnol. 44:225–229

Cameotra SS, Makkar RS (2004) Recent applications of biosurfactants as biological and immunological molecules. Curr Opin Microbiol 7:262–266

Cameron DR, Cooper DG, Neufeld RJ (1988) The mannoprotein of *Saccharomyces cerevisiae* is an effective bioemulsifier. Applied Environ. Microbiol. 54:1420–1425

Chayabutra C, Wu J, Ju LK (2001) Rhamnolipid production by *Pseudomonas aeruginosa* under denitrification: effects of limiting nutrients and carbon substrates. Biotechnol Bioeng 72:25–33

Chen SY, Wei YH, Chang JS (2007) Repeated pH-stat fed-batch fermentation for rhamnolipid production with indigenous *Pseudomonas aeruginosa* S2. Applied Microbiol. Biotechnol. 76:67–74

Ciriglian MC, Carman GM (1984) Isolation of bioemulsifier from *Candida lipolytica*. Applied Environ Microbiol 48:747–750

Cohen R, Exerowa D (2007) Surface forces and properties of foam films from rhamnolipid biosurfactants. Adv Colloid Interfac 134:24–34

Das P, Mukherjee S, Sen R (2008) Antimicrobial potential of a lipopeptide biosurfactant derived from a marine Bacillus circulans. J Appl Microbiol 104(6):1675–1684

Davila AM, Marchel R, Vandecasteele JP (1997) Sophorose lipid fermentation with differentiated substrate supply for growth and production phases. Applied Microbiol Biotechnol 47:496–501

Davis DA, Lynch HC, Varley J (2001) The application of foaming for the recovery of surfactin from Bacillus subtilis ATCC 21332 cultures. Enzym Microb Technol 28:346–354

Desai JD, Banat IM (1997) Microbial production of surfactants and their commercial potential. Microbiol Mol Biol Rev 61:47–64

Deziel E, Leptine F, Milot S, Villemur R (2000) Mass spectrometry monitoring of rhamnolipids from growing culture of *Pseudomonas aeruginosa* strain from 57RP. Biochim Biophys Acta 1485:145–152

Edward A, Melchias G, Prabhu JA, Wilson A, Anbananthan V, Sivaperumal K (2011) Detection of Exopolysaccharides/Bioemulsifier Producing Bacterial Isolates from Petroleum Contaminated SoilSpectrum 2:1–7

Fiechter A (1992) Integrated systems for biosurfactant synthesis. Pure Applied Chem 64:1739–1743

Haba E, Espuny MJ, Busquets M, Manresa A (2000) Screening and production of rhamnolipids by *Pseudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oils. J Applied Microbiol 88:379–387

Hayes ME, Nestaas E, Hrebenar KR (1986) Microbial surfactants. ChemTech 16:239–243

Healy MG, Devine CM, Murphy R (1996) Microbial production of biosurfactants. ResourConservRecy 18:41–57

Hu Y, Ju LK (2001) Purification of lactonic sophorolipids by crystallization. J Biotechnol 87:263–272

Ilori MO, Amobi CJ, Odocha AC (2005) Factors affecting the production of oil degrading *Aeromonas* sp. isolated from a typical environment. Chemosphere 61:985–992

Jadeja PL, Pandhi ND (2016) Isolation and primary screening of biosurfactant producing marine Bacteria from hydrocarbon contaminated soil and water samples of Sikka coastal area, Gujarat, India. International Journal of Scientific Progress and Research (IJSPR) 24(01):89–93

Jadeja PL, Pandhi ND (2017) A comparative study of emulsion efficacy of biosurfactants produced from marine bacteria and chemical surfactants. Indian J Comp Microbiol Immunol Infect Dis 38 (02):122–129

Jarvis FG, Johnson MJ (1949) A Glyco-lipide produced by. J Am Chem Soc 71(12):4124–4126

Kaeppeli O, Finnerty WR (1979) Partition of alkane by an extracellular vesicle derived from hexadecane-grown Acinetobacter. J Bacteriol 140:707–712

Gnanamani A, Mandal AB (2011) Isolation, production and characterization of bioemulsifiers of marine bacteria of coastal Tamil Nadu. Sciences-New York 40(February):76–82

Maneerat S (2005) Biosurfactants from marine microorganisms. Songklanakarin J Sci Technol 27 (6):1263–1272

Mariashobana A, Nalini K, Thangapandian V (2014) Studies on biosurfactants produced by hydrocarbon degrading Bacteria. Journal of advanced botany and Zoology 2(2):1–6

Martínez-checa F, Toledo FL, El Mabrouki K, Quesada E, Calvo C (2007) Characteristics of bioemulsifier V2-7 synthesized in culture media added of hydrocarbons : chemical composition, emulsifying activity and rheological properties. Bioresour Technol 98:3130–3135

Nerurkar AS, Hingurao KS, Suthar HG (2009) Bioemulsifiers from marine microorganisms. Ind Res 68(April):273–277

Noudeh GD, Moshafi MH, Kazaeli P (2010) Studies on bioemulsifier production by Bacillus licheniformis PTCC 1595. J Biotechnol 9(January):352–356

Pekdemir T, Ishigami Y, Uchiyama H (1999) Characterization of aescin as a biosurfactant for environmental remediation. J Surfact Detergents 2(3):337–341

Pepi M, Cesàro A, Liut G, Baldi F (2005) An antarctic psychrotrophic bacterium Halomonas sp. ANT-3b, growing on n-hexadecane, produces a new emulsyfying glycolipid. FEMS Microbiol Ecol 53(1):157–166

Phetrong K, H-kittikun A, Maneerat S (2008) Production and characterization of bioemulsifier from a marine bacterium, Acinetobacter calcoaceticus subsp. anitratus SM7. Songklanakarin J Sci Technol 30(3):297–305

Poremba K, Gunkel W, Lang S, Wagner F (1991) Toxicity testing of synthetic and biogenic surfactants on marine microorganisms. Environ Toxicol Water Qual 6(2):157–163

Rikalović MG, Gojgić-cvijović G, Vrvić MM (2012) Production and characterization of rhamnolipids from *Pseudomonas aeruginosa* san-ai. J Serb Chem Soc 77(1):27–42

Schulz D, Passeri A, Schmidt M, Lang S, Wagner F, Wray V, Gunkel W (1991) Marine biosurfactants I. screening for biosurfactants among crude oil degrading marine microorganisms from the North Sea. Z Naturforsch 46c:197–203

Shoeb E, Ahmed N, Akhter J, Badar U, Siddiqui K, Ansari FA (2015) Screening and characterization of biosurfactant-producing bacteria isolated from the Arabian Sea coast of Karachi. Turk J Biol 39:1–7

Yakimov MM (1995) Oleispira antarctica gen. nov., sp. nov., a novel hydrocarbonoclastic marine bacterium isolated from Antarctic coastal sea water. Int J Syst Evol Microbiol 53(3):779–785

Energy-Efficient Anaerobic Ammonia Removal: From Laboratory to Full-Scale Application

20

Shou-Qing Ni, Hafiz Adeel Ahmad, Yiyi Zhao, Qianxia Li, Ying Dong, Shakeel Ahmad, Zhaojie Cui, and Zhuangming Qiao

Abstract

From discovery in the early 1990s to completion of full-scale anaerobic ammonium oxidation (anammox) reactor, it took almost two decades to uncover the secret veil of anammox bacteria. There were three milestones during the commercialization of anammox: the development of the first enrichment culture medium, completion of first commercial anammox reactor, and fast start-up of full-scale anammox plant. Till now, the culture of anammox bacteria experienced a big progress through two general strategies: (a) to start up a reactor from scratch, and (b) to seed the reactor with enriched anammox sludge. The first full-scale anammox reactor took 3.5 years to realize full operation using the first approach due to several reasons besides the lack of anammox sludge. On the contrary, the first Asian anammox reactor started up in 2 months, thanks to the availability of anammox seed. Along with the implementation of anammox plants, anammox eventually becomes the priority choice for ammonium wastewater treatment.

Keywords

Anammox · Full-scale · Laboratory · Medium · Milestone

S.-Q. Ni (✉) · H. A. Ahmad · Y. Zhao · Q. Li · Y. Dong · Z. Cui
Shandong Provincial Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, Qingdao, Shandong, PR China
e-mail: sqni@sdu.edu.cn

S. Ahmad
Department of Soil and Environmental Sciences, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

Z. Qiao
Shandong Meiquan Environmental Protection Technology Co., Ltd., Jinan, Shandong, PR China

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_20

20.1 Introduction

Conventional biological nitrogen removal from wastewater usually consists of two steps: nitrification and denitrification. During nitrification process, ammonium is biologically oxidized to nitrate, which is then reduced to nitrogen gas using organic matter as an electron donor during denitrification process. However, the application of conventional process of nitrogen removal increased the cost of treatment, when wastewater was loaded with lower organic carbon to nitrogen ratio. When BOD/TKN ratio is low as in many ammonium-rich wastewaters, biodegradable organic matter source must be added to achieve complete denitrification (Shiakowski and Mavinic 1989; Ahn 2006). Further, ammonium needs to be converted into nitrite or nitrate for denitrification with nitrifying bacteria on the expense of oxygen. The operations are rather cost-intensive for both oxygen demand for aerobic nitrification and organic substrate addition for denitrification (Jetten et al. 1997; Van Loosdrecht and Jetten 1998; Ahn 2006). Further, the surplus sludge generated in conventional biological nitrogen removal process also increases the treatment cost.

Anaerobic ammonium oxidation (anammox) is a novel, autotrophic, and cost-effective alternative to the traditional biological nitrogen removal process (Broda 1977; Pynaert et al. 2004; Strous and Jetten 2004). The existence of the bacteria was first predicted in the 1970s on the basis of thermodynamic calculations. Anammox bacteria oxidize ammonium to nitrogen gas using nitrite as an electron acceptor with the fixation of carbon dioxide under anoxic conditions (Table 20.1) (Strous et al. 1998).

The discovery of anammox process brought revolutionary changes to conventional biological nitrogen removal from wastewater. Some unique characteristics such as low biomass yield, no need for aeration, and no addition of external carbon sources (Chamchoi et al. 2008) make anammox process a promising and sustainable technique (Abma et al. 2007a), while the newly discovered anammox process opens up new possibilities for nitrogen removal from wastewater. The cost-energy benefits of anammox technology get the attention of the industries, municipalities, scientists, and engineers. The share of anammox research, when compared with conventional process, was much greater after their discovery. It has been reported that over 2200

Table 20.1 Reactions involved in the realization of anammox process

Reaction no.	Reaction	$\triangle G^{o\prime}$ (kJ/mol NH_4^+)	N_2 composition (%)	
			$^{14\text{-}15}N_2$	$^{15\text{-}15}N_2$
20.1[a]	$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+$	−297	75	25
20.2 [a]	$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$	−358	100	0
20.3[b]	$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$	−358	100	0

[a]Strous et al. (1998)
[b]van de Graaf et al. (1995)

research articles and about 450 patents were published from their discovery (Mao et al. 2017). In environmental aspects, anammox technology seems superior when compared with conventional process. The intermediate gases NO and N_2O were more pronounced from conventional process as compared to anammox (Fux and Siegrist 2004). Greenhouse gases including CO_2, NO, and N_2O from anammox process were lower (Hauck et al. 2016). Further, the impact of marine eutrophication from a two-step anammox process was 16% lower when compared with conventional process (Hauck et al. 2016). The odor from the transportation of extra sludge produced from conventional process was also the point of concern in terms of environment. However, the major obstacle for the implementation of anammox is the slow growth rate [$\mu_{max} = 0.065\ day^{-1}$, doubling time ($t_{1/2} = \ln 2/\mu_{max}$) of 11 days] of anammox microorganisms (Strous et al. 1998; Lopez et al. 2008), making this process difficult for practical wastewater treatments. Nevertheless, some studies reported a doubling time of 2–3.5 days (Zhang et al. 2017). The washout of anammox from the system further delays the start-up time of anammox. Meanwhile, anammox bacteria have been extremely difficult to cultivate in pure culture; even *Candidatus* Brocadia anammoxidans has only been purified to apparent homogeneity by Percoll density centrifugation (Strous et al. 1999). Therefore, lack of pure culture and slow growth nature of anammox make the study of cell biology, biochemistry, and physiology tiresome and challenging (Peeters and van Niftrik 2018). In order to fulfill practical application of anammox process to achieve the water quality standard, researchers focus on the enrichment of slow-growing anammox bacteria. The retention of biological activities of slow-growing anammox and enriched culture is the major concern to upgrade this technology to the industrial scale (Peeters and van Niftrik 2018). Many studies were carried out to enrich anammox organisms, either by different methods such as biofilm or granulation or by all types of reactors. Further, different pre- and posttreatment of activated as well anaerobic granular sludge had been practiced to shorten the start-up period of anammox from scratch. Autoclaving of the anaerobic granular sludge decreased the start-up time of anammox process by 12.5% when compared with control experiment (Wang et al. 2017). Further, the addition of nZVI has also been proven beneficial for the reduction of start-up time of anammox (Ren et al. 2015).

This chapter reviews the development of anammox process and relative studies in the laboratory, especially the discovery and biochemistry of the bacteria responsible for anaerobic ammonium oxidation. Special attention was paid on the commercialization and full-scale application of anammox technique.

20.2 Discovery and Phylogeny of Anammox

Already in 1932 it was reported that dinitrogen gas was generated via an unknown mechanism during fermentation in the sediments of Lake Mendota, Wisconsin, USA (Allgeier et al. 1932). More than 40 years ago, Richards (1965) noticed that most of the ammonium that should be produced during the anaerobic remineralization of organic matter was unaccounted for. As there was no known biological pathway for

this transformation, biological anaerobic oxidation of ammonium received little further attention (Arrigo 2005). Three decades ago, the existence of two chemolithoautotrophic microorganisms capable of oxidizing ammonium to dinitrogen gas was predicted on the basis of thermodynamic calculations (Arrigo 2005). It was thought that anaerobic oxidation of ammonium would not be feasible, assumed that the predecessors had tried and failed to establish a biological basis for those reactions (Kuenen 2008). By the 1990s, Arnold Mulder's fantastic observations were just consistent with Richards' suggestion (Kuenen 2008). In their anoxic denitrifying pilot reactor, ammonium disappeared at the expense of nitrite with a clear nitrogen production. The reactor used the effluent from a methanogenic pilot reactor, which contained ammonium, sulfide, and other compounds, and nitrate from a nitrifying plant as the influent. This process was named "anammox," and people realized that it had great significance in the removal of unwanted ammonium. Even without full understanding of anammox reaction, Arnold Mulder patented the process immediately (Mulder 1992, 1993). The discovery of anammox process was first publicly presented at the fifth European Congress on biotechnology (Van de Graaf et al. 1990). By the mid-1990s, the discovery of anammox in the fluidized bed reactor was published (Mulder et al. 1995). A maximum ammonium removal rate of 0.4 kg N/m^3/day was achieved. It was shown that for every mole of ammonium consumed, 0.6 mol of nitrate was required, resulting in the formation of 0.8 mol of N_2 gas (Eq. (20.1) in Table 20.1). In the same year, the biological nature of anammox was identified (Van de Graaf et al. 1995). Labeling experiments with $^{15}NH_4^+$ in combination with $^{14}NO_3^-$ showed that $^{14-15}N_2$ was the dominant product, making up 98.2% of the total labeled N_2. These findings conflicted with Reaction 1 in which the percentage of $^{14\text{-}15}N_2$ and $^{15\text{-}15}N_2$ in the formed dinitrogen gas would be 75% and 25%, respectively. It was realized that instead of nitrate, nitrite was assumed as the oxidizing agent of ammonium in anammox reaction (Eq. (20.2) in Table 20.1) (Van de Graaf et al. 1995). Based on previous study, Strous et al. (1998) calculated the stoichiometry of anammox process by mass balancing (Eq. (20.3) in Table 20.1), which is widely accepted by other groups. Later, anammox bacteria were identified as planctomycetes (Strous et al. 1999), and the first identified anammox organism was named *Candidatus* "Brocadia anammoxidans" (Kuenen and Jetten 2001). Before 2002, anammox was assumed to be a minor player in the N cycle within natural ecosystems (Francis et al. 2007). In 2002, anammox was found to play an important part in the biological nitrogen cycle, accounting for 24–67% of the total N_2 production in the continental shelf sediments that were studied (Thamdrup and Dalsgaard 2002). Globally, anammox may be responsible for 30–50% of N_2 production in the ocean (Devol 2003). Anammox is commonly present in the oxygen minimum zones (OMZs). The total nitrogen loss in the OMZs was reported about 100% in Chilean coast, 52–60% in Peruvian shelf, and 19–32% in Costa Rica (Dalsgaard et al. 2003; Lam et al. 2009). However, the nitrogen loss from the Arabian Sea was only 10% by anammox bacteria (Nicholls et al. 2007). The environmental factors, concentration of anammox bacteria, and anammox activities varied from region to region, which may be the reason of the

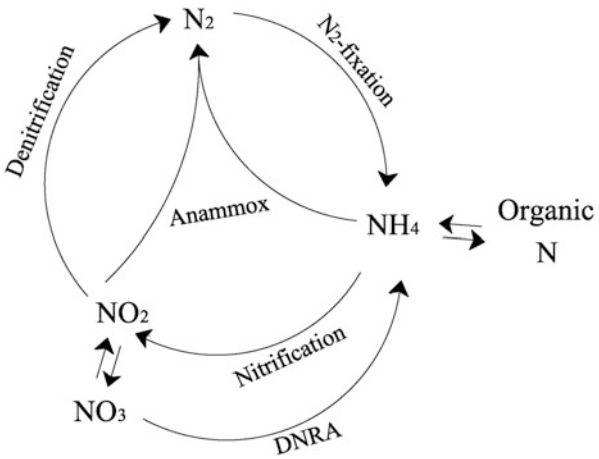


Fig. 20.1 The biological N cycle (based in part on Arrigo 2005). *DNRA* dissimilatory nitrate reduction to ammonium

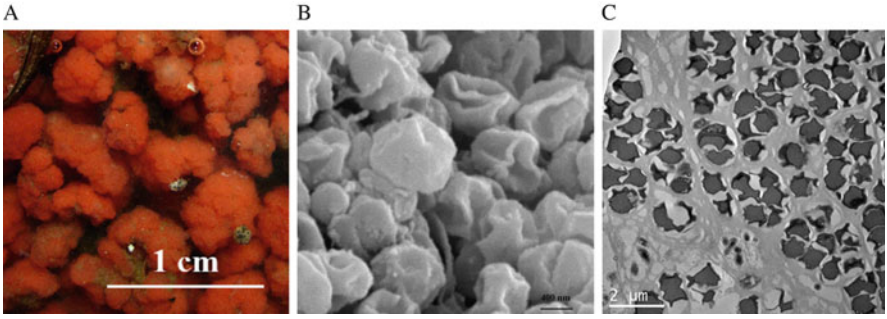


Fig. 20.2 The specific red color of anammox bacteria (**a**) and the typical irregular shapes of anammox bacteria displayed by scanning electron microscopy (**b**) and transmission electron microscopy images (**c**)

difference in the nitrogen loss at different places. The discovery of anammox process modified the concept of biological nitrogen cycle as depicted in Fig. 20.1.

The specific red color of anammox bacteria (Fig. 20.2a) is due to the heme c group of the protein cytochrome c that plays an important role in anammox metabolism (Jetten et al. 2009). The irregular shapes of anammox bacteria were displayed by both transmission electron microscopy and scanning electron microscopy images (Fig. 20.2b, c). The anammox species have a single membrane-bound anammoxosome and riboplasm with ribosome-like particles separated from paryphoplasm by an intracytoplasmic membrane. The cells contain three distinct membrane-bound compartments: the paryphoplasm, cytoplasm, and anammoxosome. The anammoxosome, center of energy metabolism, has no DNA

and ribosomes (Peeters and van Niftrik 2018). Further, the presence of ladderane lipid on the anammoxosome membrane serves as barrier against the transmembrane circulation of toxic as well as essential metabolites and is unique to anammox (Mercer et al. 2017).

Till now, six anammox genera have been discovered, with 16S rRNA gene sequence identities of the species ranging from 87 to 99% (Jetten et al. 2009; Khramenkov et al. 2013). It is well-known that all anammox bacteria belong to the same monophyletic order named Brocadiales and are related to Planctomycetales. Among them, five *Candidatus* anammox genera have been enriched from activated sludge and freshwater environments: "Kuenenia" (Schmid et al. 2000; Strous et al. 2006), "Brocadia" (Strous et al. 1999; Kuenen and Jetten 2001; Kartal et al. 2008), "Anammoxoglobus" (Kartal et al. 2007), "Anammoxomicrobium" (Khramenkov et al. 2013), and "Jettenia" (Quan et al. 2008). The sixth anammox genus, "*Candidatus* Scalindua" (Kuypers et al. 2003; Schmid et al. 2003; Van de Vossenberg et al. 2008), has often been detected in natural habitats, especially in marine sediments and oxygen minimum zones (Dalsgaard et al. 2005; Penton et al. 2006; Schmid et al. 2007; Woebken et al. 2008).

20.3 Possible Reaction Mechanisms for Anammox

To understand the possible metabolic pathway for anammox, ^{15}N-labeling experiments were first carried out in 1997 (Van de Graaf et al. 1997). These experiments showed that ammonium was biologically oxidized with hydroxylamine, most likely derived from nitrite, as the probable electron acceptor and converted into hydrazine. The conversion of hydrazine to dinitrogen gas is postulated as the reaction generating the electron equivalents for the reduction of nitrite to hydroxylamine. Generally, two possible reaction mechanisms were addressed (Jetten et al. 1999). A membrane-bound enzyme complex converts ammonium and hydroxylamine to hydrazine first, followed by the oxidation of hydrazine to dinitrogen gas in the periplasm. At the same time, nitrite is reduced to hydroxylamine at the cytoplasmic site of the same enzyme complex responsible for hydrazine oxidation with an internal electron transport (Fig. 20.3a). Another possible mechanism for anammox process is concluded as follows: ammonium and hydroxylamine are converted to hydrazine by a membrane-bound enzyme complex, hydrazine is oxidized in the periplasm to dinitrogen gas, and the generated electrons are transferred via an electron transport chain to nitrite reducing enzyme in the cytoplasm where nitrite is reduced to NH_2OH (Fig. 20.3b). Whether the reduction of nitrite and the oxidation of hydrazine occur at different sites of the same enzyme (Fig. 20.3a) or the reactions are catalyzed by different enzyme systems connected via an electron transport chain (Fig. 20.3b) remains to be investigated. The occurrence of hydrazine as an intermediate in microbial nitrogen metabolism is rare (Schalk et al. 1998). Hydrazine has been proposed as an enzyme-bound intermediate in the nitrogenase reaction (Dilworth and Eady 1991).

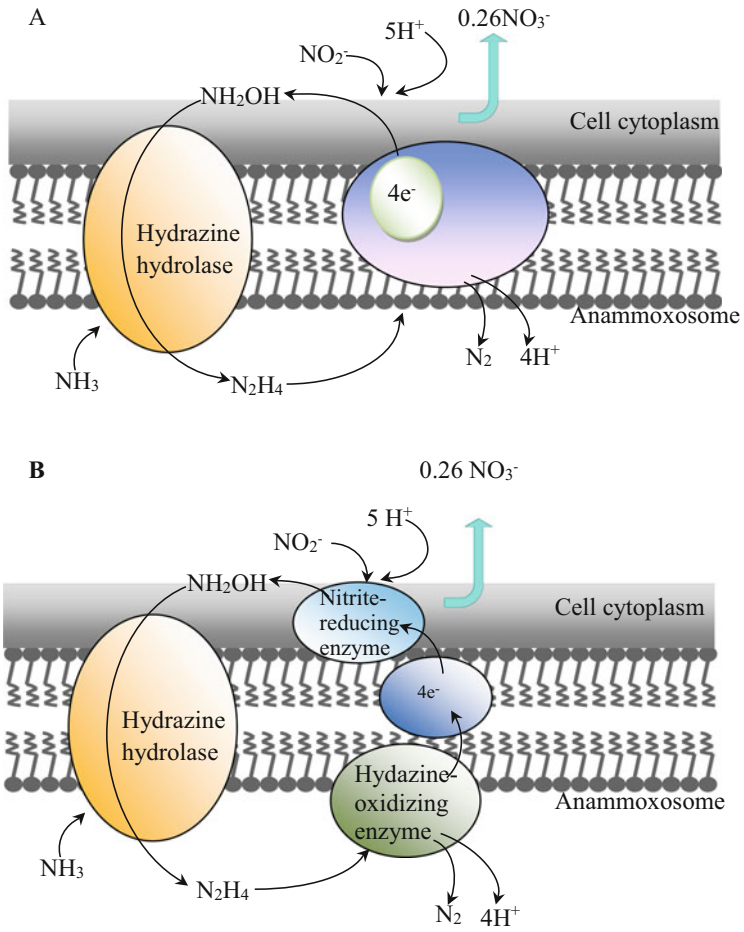


Fig. 20.3 Possible biochemical pathway and cellular localization of the enzyme systems involved in anammox reaction. Figure modified, with permission, from FEMS Microbiology Reviews (Jetten et al. 1999) and Process Biochemistry (Ahn 2006)

A possible role of NO or HNO in anammox was proposed by Hooper et al. (1997) by way of condensation of NO or HNO and ammonium on an enzyme related to the ammonium monooxygenase family, which results in the production of hydrazine. The formed hydrazine or imine could thereafter be converted by the enzyme hydroxylamine oxidoreductase to dinitrogen gas, and the reducing equivalents produced in the reaction are required to combine NO or HNO and ammonium or to reduce nitrite to NO. Environmental genomics analysis of the species *Candidatus* Kuenenia stuttgartiensis, through a slightly different and complementary metabolism mechanism, postulated NO to be the intermediate instead of hydroxylamine (Fig. 20.4) (Strous et al. 2006). But this hypothesis also agreed that hydrazine was

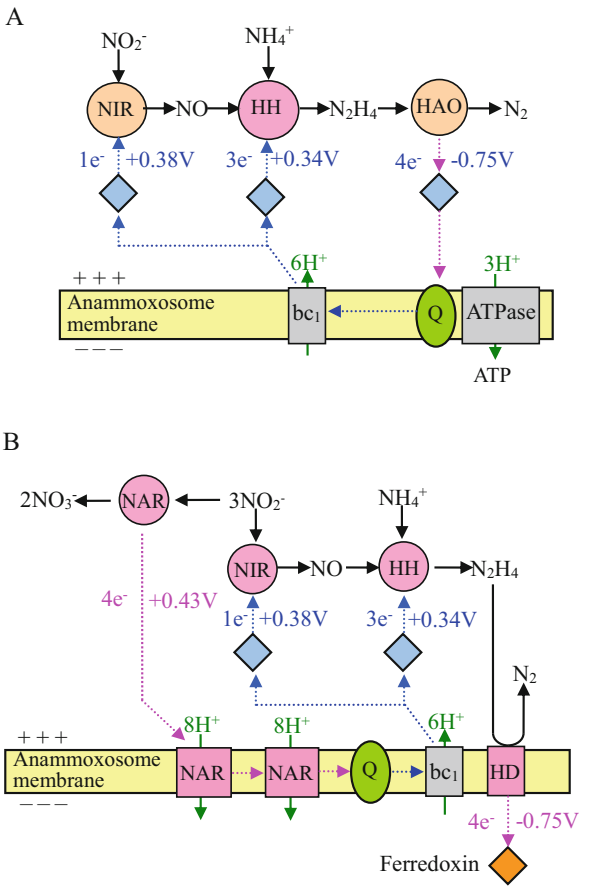


Fig. 20.4 Hypothetical metabolic pathways and reversed electron transport in the anammoxosome. (**a**) Anammox catabolism that uses nitrite as the electron acceptor for the creation of a proton motive force over the anammoxosomal membrane. (**b**) Proton motive force-driven reversed electron transport combines central catabolism with nitrate reductase (NAR) to generate ferredoxin for carbon dioxide reduction in the acetyl-CoA pathway. *HAO* hydrazine oxidoreductase, *HD* hydrazine dehydrogenase, *HH* hydrazine hydrolase, *NIR* nitrite oxidoreductase, *Q* quinine. Light blue diamonds, cytochromes; blue arrows, reductions; pink arrows, oxidations. Figure modified, with permission, from *Nature* (Strous et al. 2006)

an important intermediate in the process. In this pathway (Fig. 20.4), there are two enzymes unique to anammox bacteria: hydrazine hydrolase (HH) and hydrazine dehydrogenase (HD). The HH produces hydrazine from nitric oxide and ammonium, and HD transfers the electrons from hydrazine to ferredoxin. Few new genes such as some known fatty acid biosynthesis and S-adenosylmethionine radical enzyme genes (Strous et al. 2006) containing domains involved in electron transfer and catalysis were detected.

20.4 Basal and Designated Medium Development

Once nitrite was realized to be the electron acceptor with ammonium as electron donor, a basal medium containing ammonium, nitrite, bicarbonate, minerals, and trace elements was developed for the enrichment of anammox microorganisms (Van de Graaf et al. 1996). The medium contained ammonium (5–30 mM) and nitrite

(5–35 mM) as the only electron donor and electron acceptor, respectively, with bicarbonate (10 mM) as the only carbon source. Minerals and trace elements were also provided. Phosphate concentration of the medium was kept below 0.5 mM in order to avoid its possible inhibitory effect on the process, and medium was flushed with argon gas to achieve anaerobic conditions. Experiments which were carried out in a fluidized bed reactor with basal enrichment medium showed that the anaerobic ammonium removal rate increased from original 0.4 kg N/m^3/day to 2.4 kg N/m^3/day (Mulder et al. 1995). The maximum specific activity of the biomass in the fluidized bed reactor was 25 nmol NH_4^+/mg VS/min. For every mole of ammonium oxidized, 0.041 mol of CO_2 was incorporated into biomass. The estimated growth rate in the fluidized bed systems was 0.001/h, equivalent to a doubling time of about 29 days. The basal medium enhanced the activities of anammox bacteria.

The development of the basal medium, the milestone of anammox enrichment, turned on the fervent zeal for this infant investigation. Since then, a vast number of researchers flooded in this specific topic. As medium shows positive effects on anammox process, many studies focused their attention on this area. Unfortunately, there is no systemic medium development study like those for other bacteria (Fessehaie et al. 1999; Kridelbaugh and Doerner 2009).

In our lab, a study was conducted toward designing an appropriate medium by investigating growth requirement of anammox bacteria with respect to amino acids. A total of 20 L-amino acids were added to basal medium (Table 20.2). After experiment set I, set II was carried out to further evaluate the enhanced effects of the selective amino acids on microorganism growth. To quantify the growth of anammox bacteria, quantitative molecular techniques were employed. Preliminary experiments indicated that glycine, methionine, threonine, tryptophan, and tyrosine enhanced the growth of anammox bacteria. On the contrary, asparagine, aspartic acid, and histidine slightly decreased bacterial activities. Of the 20 L-amino acids, 12 L-amino acids (alanine, arginine, cysteine, glutamic acid, glutamine, isoleucine, leucine, lysine, phenylalanine, proline, serine, and valine) totally inhibited the growth of anammox bacteria, resulting in the sludge turning from reddish to blackish. Another three amino acids (asparagine, aspartic acid, histidine) slowed down the growth of anammox bacteria. This unpublished study would benefit anammox study and their application.

20.5 Anammox Culture in the Laboratory

Anammox process has been recognized as being difficult to apply for practical applications. Anammox bacteria grow in a mixture of bacterial populations, and they have not been isolated in a pure culture (Tsushima et al. 2007). Anammox bacteria, being strictly anaerobic and autotrophic, are difficult to enrich, which makes the application of this process limited due to unavailability of sufficient biomass required for the process. Different methods have been employed to culture and enrich anammox biomass from different types of seed sludge (Chamchoi and Nitisoravut 2007; Date et al. 2009). A relative population of 88% anammox bacteria

Table 20.2 Growth of anammox bacteria using basal medium with L-amino acids

Amino acid	Plate concentration (mmol/L)	OD_{600}[a]
Alanine	0.5	n.d.
Arginine	0.6	n.d.
Asparagine	0.3	−
Aspartic acid	0.3	−
Cysteine	0.3	n.d.
Glutamic acid	5.0	n.d.
Glutamine	5.0	n.d.
Glycine	0.1	+
Histidine	0.1	−
Isoleucine	0.3	n.d.
Leucine	0.3	n.d.
Lysine	0.3	n.d.
Methionine	0.3	+
Phenylalanine	0.3	n.d.
Proline	2.0	n.d.
Serine	4.0	n.d.
Threonine	0.3	+
Tryptophan	0.1	+
Tyrosine	0.1	+
Valine	0.3	n.d.

[a]Optical density (600 nm) after 7 days of incubation at 35 °C
+ means increase, − means decrease, n.d. means not detected because of the color change

was achieved in a batch study inoculated from a rotating biological contactor (RBC) treating a landfill leachate (Egli et al. 2001). Enrichment culture of anammox bacteria was also developed in lab-scale reactors inoculated with marine sediments (Van de Vossenberg et al. 2008) and paddy field soil samples and activated sludge from wastewater treatment plants (Keluskar et al. 2013).

The slow growth rate of anammox bacteria with the approximate doubling time of 11 days is the major obstacle for implementation of anammox process (Strous et al. 1998). A long start-up period is thus expected in anammox process. Shortening anammox process start-up period by reducing washout of anammox biomass becomes an important strategy for full-scale application. Different types of reactor design have been used to minimize the washout of anammox biomass including continuous stirred-tank reactor, anaerobic biological filtrated reactor, sequencing batch reactor (SBR), upflow reactor, and biofilm reactor (Imajo et al. 2004; Isaka et al. 2007; Strous et al. 1998; Van Dongen et al. 2001). The use of gel immobilization technology was also proved as an effective approach to avoiding the washout of anammox bacteria (Bae et al. 2015). Faster growth of anammox bacteria was achieved in a membrane bioreactor (MBR) (the doubling time was less than 10 days), resulting in an unprecedented purity of the enrichment of 97.6% (Van der Star et al. 2008). The immobilization of anammox with polyvinyl alcohol and

sodium alginate gel was reported to have higher maximum specific growth rate with 2–3 days of doubling time (Zhang et al. 2017). The formation of compact aggregates was reported to maintain a large amount of active anammox biomass in a reactor (Imajo et al. 2004). Therefore, granulation is also an alternative approach for anammox enrichment. In comparison with granulation and membrane bioreactor, SBR technology is also widely used for the enrichment of anammox.

In summary, there are two main approaches (strategies) to start up an anammox reactor: (a) to start a reactor from scratch, and (b) to inoculate it with highly enriched anammox sludge. For the first strategy, the reactor configuration is very important. The SBR technique ensured over 1-year reliable operation under stable conditions with efficient biomass retention (more than 90% of the biomass was maintained in the reactor) and homogeneous distribution of substrates, products, and biomass aggregates (Strous et al. 1998). The MBR was also applied successfully for cultivation of anammox bacteria with fast growth rate (the minimum doubling time for anammox bacteria was estimated to be 5.5–7.5 days) (Van der Star et al. 2008). Among different reactors, the anammox nonwoven membrane reactor (ANMR) is a novel reactor configuration to enrich anammox biomass (Ni et al. 2010a, 2010b). The reactor was developed by connecting a set of nonwoven membrane module, which also served as an effluent port, with an anaerobic reactor. The membrane module was installed outside the reactor, which is different from the immerged membrane reactors. Unlike conventional MBR, wastewater circulated in the membrane module, and the biofilms grew on the membrane interior surface. A large amount of the suspended biomass could remain in the reactor by filtration through the nonwoven membrane and biofilms, resulting in improvement of the effluent quality and enhancement of the solid retention in the reactor. After over 8 months of operation, the purity (percentage of anammox cells in the community) of anammox bacteria in the reactor was quantified to be 97.7% (Ni et al. 2010a). The cost-effective ANMR was shown to be suitable for the slowly growing anammox bacteria having the following advantages: (1) a large amount of the biomass could remain in the reactor by filtration through the nonwoven membrane and the formation of biofilm, (2) the formation of aggregates and biofilm enhanced the solid retention in the reactor, (3) the nonwoven membrane was cost-efficient, and (4) the design of the anaerobic reactor could dilute the influent medium and avoid inhibition from high nitrite concentrations, leading to high tolerance ability of substrates. Recently, the upflow anaerobic sludge blanket (UASB) reactor was highly recommended for the culture of slowly growing bacteria (Ni and Meng 2011; Ni et al. 2011; Ni et al. 2012a; b). This is because of not only the improvement of physiological conditions, making them favorable for bacteria and their interactions, especially syntrophisms in the anaerobic system, but also the formation of granular sludge, being the major reason of the successful introduction of the UASB reactor (Hulshoff Pol et al. 2004). Hence, granulation also improves anammox application. Surprisingly, Ni and his colleagues used inactive methanogenic granules as inoculum to realize fast granulation successfully (Ni et al. 2010c). The start-up nitrite concentration was significantly higher than the published toxic level for anammox bacteria and other lab-scale studies. The accommodations and proliferations of anammox bacteria in the inactive

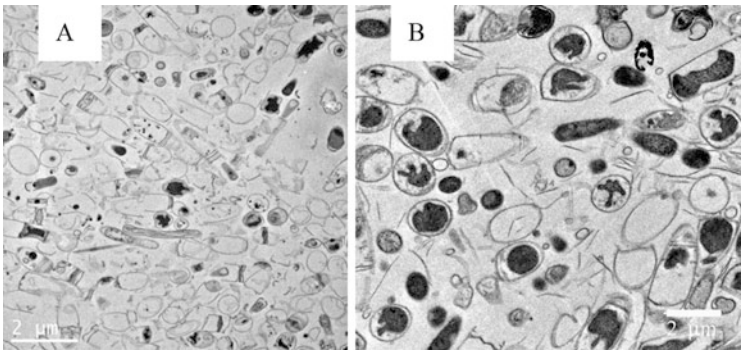


Fig. 20.5 (**a**) Transmission electron micrograph showing dormant cells in the seed granule (bar = 2 μm). (**b**) Transmission electron micrograph showing the anammox bacteria in the interior of granules (bar = 2 μm)

methanogenic granules might be the main reason for the high anammox purity in a short period. Anammox cells could use the skeleton of inactive methanogenic granules and proliferate from the interior as observed in TEM (Fig. 20.5). The second approach mentioned above significantly shortens the required time for anammox strat-up under the premise of large quantity of anammox sludge, but is usually limited by the lack of anammox sludge. The gradual construction of full-scale anammox plants increases the availability of anammox sludge. The introduction of the exotic anammox sludge to seed a granular reactor is a good choice (Ni et al. 2011). The reactor was started successfully in 2 weeks; in addition, high nitrogen removal was achieved for a long period, showing that the inoculation of mature anammox granules was ideal to start up a new reactor. Further, the start-up of anammox bacteria from activated sludge with immobilization was also an effective approach (Bae et al. 2015). The nitrogen removal rate of 1.12 kg N/m^3-day was achieved within 114 days. Therefore, the start-up of anammox is not an obstacle anymore on the laboratory-scale study.

20.6 Commercial Application of Anammox Process

The lack of pure cultures of anammox bacteria makes a genomic approach less straightforward. Combined with the low maximum specific growth rate of anammox bacteria and stringent operational conditions, the practical application of anammox fell far behind the research progress.

Many efforts have been made on the development of a marketable product. Here, we would like to mention Paques BV (Balk, the Netherlands) for its unremitting efforts on the practical application of anammox process. Early in 2001, van Dongen et al. (2001) scaled up lab-scale SHARON (single reactor system for high-activity ammonium removal over nitrite) reactor (Jetten et al. 1997) in collaboration with Paques BV. The effluent of the SHARON process was ideally suited as influent for

anammox process, for the ammonium was oxidized by 53% to nitrite, rather than nitrate in SHARON process at 1.2 kg N load per m^3 per day without pH control (Van Dongen et al. 2001). The combined SHARON-anammox system could work stably over long periods, and the authors predicted that the combination process was ready for full-scale implementation.

Based on constant and successful study, in 2007, the first full-scale granular anammox reactor was accomplished at the wastewater treatment plant of Waterboard Hollandse Delta in Rotterdam, the Netherlands (Abma et al. 2007a; Van der Star et al. 2007). This stands for the start of the commercial application of anammox process, exhibiting to be another milestone. The first full-scale 70 m^3 reactor was directly scaled up 7000-fold from 10 l lab-scale experiment. The reactor was initially inoculated with nitrifying sludge, and a total amount of 9.6 m^3, settled biomass from an anammox enrichment reactor, was added from day 622 to 1033 (Van der Star et al. 2007). Even with the addition of anammox sludge, the start-up took 3.5 years, 1.5 years longer than designed. Several reasons caused the long start-up time, besides the low growth rates of anammox microorganisms. Most important is that there was no anammox seed sludge available to inoculate the first full-scale reactor, and delay was caused by technical issues such as operational and temperature problems (Abma et al. 2007a), as the first full-scale reactor was directly scaled up from lab scale, skipping the pilot phase. This first full-scale reactor, on the contrary, had a pilot plant character. In September 2006, the reactor was in full operation, and the loading rate could be reached to a level of 750 kg/day, 50% higher than the design load.

Another four anammox plants were built before 2008, three in Europe and one in Asia (Table 20.3). The third reactor, part of a plant for the treatment of the effluent of a potato factory, exhibited a largest ammonium load rate. The capacity of the reactor is 1200 kg N/day, while only about 700 kg N/day is converted as no more nitrogen available in the wastewater. Japan built the first full-scale Asian anammox reactor at a semiconductor plant. In 2009, Paques Environmental Technology (Shanghai) released the news that an agreement had been reached to build the world's largest anammox-based wastewater treatment plant in China. Anammox process was designed to have a capacity for conversion of 11 tons of nitrogen per day, almost ten times larger than the largest plant built before 2008. The two-step combination of anammox and internal circulation (IC) reactors will be the sixth full-scale application of anammox. Since 2009, anammox experienced huge development. Another 11 anammox plants were implemented by Paques, seven of which are located in China. As the world's biggest developing market, China contributes significantly toward commercialization of anammox process.

Thanks to the experience from the established anammox plants, the start-up time of the marketable plant became shorter and shorter. This could be another milestone. The second reactor started up in 1 year, and it took 2 months for the start-up of the first Asian plant. Till now, more than 100 full-scale variant plants are in operation around the world (Lackner et al. 2014), mostly in Austria, China, Japan, the Netherlands, and the USA. All these emphasize on anammox process becoming a commercial technique.

Table 20.3 The brief description of worldwide full-scale anammox plants implemented by Paques[a]

Process	Place	Influent	Reactor volume (m^3)	Designed load (kgN/day)	Year
SHARON-anammox	Rotterdam, NL	Reject water	72	490 (750)[b]	2002
Nitrification-anammox	Lichtenvoorde, NL	Tannery	100	325 (150)[c]	2004
Anammox	Olburgen, NL	Potato processing	600	1200 (700)[c]	2006
Nitrification-anammox	Mie prefecture, JP	Semiconductor	50	220 (220)[b]	2006
Anammox	Niederglatt, Switzerland	Reject water	180	60 (60)[b]	2008
Anammox	Tongliao, China	Monosodium glutamate (MSG)	6600	11,000	2009
Anammox	Yichang, China	Yeast production	500	1000	2009
Anammox	Tongliao, China	MSG	4100	9000	2010
Anammox	The Netherlands	Reject water	425	600	2010
Anammox	Tai'an, China	Cornstarch and MSG	4300	6090	2011
Anammox	Poland	Distillery	900	1460	2011
Anammox	Wuxi, China	Sweetener	1600	2180	2011
Anammox	Wujiaqu, China	MSG	5400	10,710	2011
Anammox	Coventry, UK	Reject water	1760	4000	2011
Anammox	Shaoxing, China	Distillery	560	900	2011

[a]Abma et al. (2007b) and communication with Paques BV
[b]Values in parentheses mean achieved loads (kg N/day)
[c]No more nitrogen available

A variety of anammox-based systems were designed on the base of sludge distribution in the system. In the past, the majority of installations were based on a two-reactor system. However, the trend was shifted from two-reactor configuration into one-reactor system due to the easy operation and lower cost. Currently, the majority of the full-scale application comprised of moving bed biofilm reactor (Kowalski et al. 2019), granular sludge system (Speth et al. 2016), and SBR (Joss et al. 2009). The SBR configuration shared about 50% in the full-scale installation of anammox followed by granular and MBBR configurations (Lackner et al. 2014). The major installation of anammox technology comes from ANAMMOX®, ANITA™ Mox, DEMON®, DeAmmon®, and Cleargreen™. The biofilm technology was introduced in the full-scale application with brand names ANITA™ Mox and DeAmmon®. The biofilm technology is advantageous in terms of higher relative abundance and activity of anammox. Further, nitrite-oxidizing bacteria can be

suppressed easily and provide the immunity against the washing out of anammox (Kowalski et al. 2019). However, formation of anammox biofilm is a lengthy process which increases the start-up time up to several months (Morales et al. 2015). Another full-scale application consisted of granular sludge technology. ANAMMOX®, a brand name affiliated with Paques BV, was provided for the full-scale implementation of anammox-based treatment process with granular sludge system. Anammox granulation is an effective strategy to retain enough biomass in the treatment system and increase the process efficiency (Sobotka et al. 2017; Song et al. 2017). The increase of the granule size may decrease the process efficiency due to formation of gas pocket inside which causes the flotation of granules (Chen et al. 2010). The entrapment of gas inside of the granules decreased the density of granules which ultimately leads to the flotation of granules. Chen et al. (2010) calculated the density of floated and settled granules to about 979.02 ± 15.08 mg L^{-1} and 1036.4 ± 2.0 mg L^{-1}, respectively. Above all, recently, immobilization of anammox with polyethylene glycol gel was also reported on the full-scale anammox implementation (Isaka et al. 2017). Every technology including granulation, biofilm, as well as flocculent sludge has pros and cons. However, suspended sludge system showed better performance than biofilm for long-term process. Suspended sludge is classified into two systems including granulation and flocculation.

20.7 Cost and Energy Sustainability

The success or failure of any technology depends on its economic values. The estimated organic carbon (methanol) of 3 lb is required for the removal of 1 lb of nitrogen from wastewater, and continuous supply of organic carbon needs an extra tank for the storage of carbon source. The addition of carbon source increases the sludge production, and it was reported that the rate of sludge production with 1 lb removal of nitrogen is about 0.6 lb. The external carbon source cost, the carbon source storage tank cost, cost of on-site management of sludge or transportation to long distance, and space for extra sludge as well as carbon tank also added cost of treatment. In terms of energy, the highest share of energy used in the conventional process comes from aeration. Ammonium-rich wastewater should be completely oxidized into nitrite or nitrate for heterotrophic denitrification. In general, the conventional process is costly as well energy-exhaustive for the removal of nitrogen from wastewater.

The discovery of anammox changes the way to remove nitrogen from wastewater. Anammox process produces less sludge which can be managed on site. The anaerobic digestion of organic wastewater can generate energy, and the rejected wastewater treated with anammox technology may avoid the cost of organic carbon (Kwon et al. 2019). The required intensive aeration to produce nitrite and nitrate for subsequent denitrification process was also reduced by 63%, because anammox needs about 50% nitrite for ammonium oxidation. Otherwise, the treatment of organic-rich wastewater with conventional N/DN process wastes the potential organic energy source and costs more energy for aeration. The biogas generated

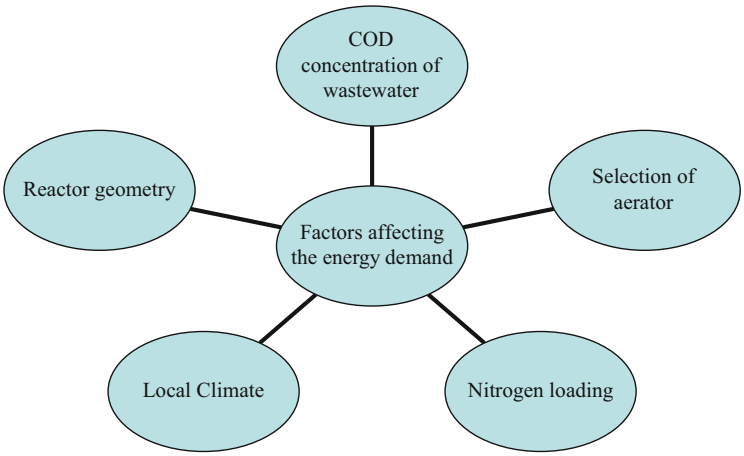


Fig. 20.6 Factor affecting the energy demand for nitrogen removal

from anaerobic digestion of organic materials in the wastewater can be used for the generation of heat and electricity (Gu et al. 2016). The heat would be used for wastewater temperature stability, and electricity can be used for aeration. However, the construction cost and operation cost varied from technology to technology. The construction cost for advanced wastewater treatment is higher than conventional system (Gu et al. 2016).

Different reports optimize the energy consumption between anammox-based technology and conventional system of wastewater treatment. Arias et al. (2018) compared the energy consumption between anammox-based technology named ELAN and conventional process. The sludge cost as well as cost of chemical has a brighter outlook due to their negligible values in ELAN technology (Vázquez-Padín et al. 2014). The electricity cost in the case of ELAN technology which was based on partial nitrification and anammox was lower than conventional process. The cost of energy was 0.27 €/m^3 for ELAN technology which was much lower than conventional system that accounted about 1.09 €/m^3.

As far as energy consumption is concerned, the anammox-based techniques have superiority over conventional N/DN. The energy requisite for the treatment of side stream with PN-Anammox SBR varied from 0.8 to 2 kWh kg N^{-1}. While the conventional process required about 4 kWh kg N^{-1}, which was 50% higher than PN-Anammox process (Lackner et al. 2014). The main energy consumption in the wastewater treatment comes from the aeration. Further, Fig. 20.6 presents the main factors affecting the energy requirement.

The efficient oxygen transfer in PN-Anammox process can further reduce the demand of energy. Wang et al. (2019) designed a novel system to remove nitrogen from wastewater with the reduction of wasted activated sludge at the lab scale and calculated energy demand for novel partial nitrification with simultaneous anammox-denitrification and sludge fermentation process (PN-SADF). Further, the

energy requirement was compared with the conventional N/DN. The annual energy consumption for PN-SADF was assumed 30% lower than the conventional process. The annual aeration energy for conventional process was about 1,368,750 kWh/year, which was higher than novel PN-SADF process (912,500 kWh/year). Similarly, the mixing energy of conventional process was higher than anammox-based process. Further, the energy for the transportation and disposal of extra sludge was 100% in the conventional N/DN process. However, the energy calculation was proposed on the base of laboratory-scale study. The construction cost and energy requirement varied from reactors to reactors and sludge distribution. Local climate also affects the cost of treatment and energy demand. It is proposed that clod area had higher demand of energy than hot area. However, energy and cost optimization needs further study to fully understand the technologies. Comparison of the cost and energy demand between different reactor systems needs time.

20.8 Conclusion

The discovery of the green process, anammox, brings revolutionary changes to conventional biological nitrogen removal. Playing an important part in the biological nitrogen cycle, this unique process makes great contribution to our environment and economy. Anammox development experienced several important points: laboratory culture based on basal medium, full-scale reactor system implementation, and extensive engineering applications. Although starting up the reactor from scratch is universal, inoculation with highly enriched anammox sludge is more feasible. Currently, more than 100 full-scale anammox systems are operational. Thus, application of anammox process offers an attractive alternative to current wastewater treatment systems for ammonia-nitrogen removal.

Acknowledgement The authors gratefully acknowledge the support from the National Natural Science Foundation of China (21777086), Taishan Scholar Youth Expert Program of Shandong Province (tsqn201909005), Key Research & Developmental Program of Shandong Province (2019JZZY020308), Natural Science Foundation for Distinguished Young Scholars of Shandong Province (JQ201809), Young Scholars Program of Shandong University (2016WLJH16, 2020QNQT012), and Shandong Provincial Water Conservancy Research and Technology Promotion Project (SDSLKY201802).

References

Abma WR, Schultz CE, Mulder JW, van der Star WRL, Strous M, Tokutomi T, van Loosdrecht MCM (2007a) Full-scale granular sludge Anammox process. Water Sci Technol 55(8–9):27–33

Abma W, Schultz C, Mulder JW, van Loosdrecht M, van der Star W, Strous M, Tokutomi T (2007b) The advance of Anammox. Water 21(February):36–37

Ahn YH (2006) Sustainable nitrogen elimination biotechnologies: a review. Process Biochem 41:1709–1721

Allgeier RJ, Peterson WH, Juday C, Birge EA (1932) The anaerobic fermentation of lake deposits. Int Rev Hydrobiol 26:444–461

Arias A, Salim I, Pedrouso A, Morales N, Mosquera-Corral A, Vázquez-Padín JR, Rogalla F, Feijoo G, Moreira MT (2018) Bottom-up approach in the assessment of environmental impacts and costs of an innovative anammox-based process for nitrogen removal. J Environ Manag 225:112–119

Arrigo KA (2005) Marine microorganisms and global nutrient cycles. Nature 437:349–455

Bae H, Choi M, Lee C, Chung YC, Yoo YJ, Lee S (2015) Enrichment of ANAMMOX bacteria from conventional activated sludge entrapped in poly(vinyl alcohol)/sodium alginate gel. Chem Eng J 281:531–540

Broda E (1977) Two kinds of lithotrophs missing in nature. Z Allg Mikrobiol 17:491–493

Chamchoi N, Nitisoravut S (2007) Anammox enrichment from different conventional sludges. Chemosphere 66:2225–2232

Chamchoi N, Nitisorvut S, Schmidt JE (2008) Inactivation of ANAMMOX communities under concurrent operation of anaerobic ammonium oxidation (ANAMMOX) and denitrification. Bioresour Technol 99:3331–3336

Chen J, Ji Q, Zheng P, Chen T, Wang C, Mahmood Q (2010) Floatation and control of granular sludge in a high-rate anammox reactor. Water Res 44:3321–3328

Dalsgaard T, Canfield DE, Petersen J, Thamdrup B, Lez JA-G (2003) N_2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. Nature 422:2–4

Dalsgaard T, Thamdrup B, Canfield DE (2005) Anaerobic ammonium oxidation (anammox) in the marine environment. Res Microbiol 156:457–464

Date Y, Isaka K, Ikuta H, Sumino T, Kaneko N, Yoshie S, Tsuneda S, Inamori Y (2009) Microbial diversity of anammox bacteria enriched from different types of seed sludge in an anaerobic continuous-feeding cultivation reactor. J Biosci Bioeng 107:281–286

Devol AH (2003) Solution to a marine mystery. Nature 422:575–576

Dilworth MJ, Eady RR (1991) Hydrazine is a product of dinitrogen reduction by the vanadium nitrogenase from *Azotobacter chroococcum*. Biochem J 277:465–468

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

Fessehaie A, Wydra K, Rudolph K (1999) Development of a new semiselective medium for isolating *Xanthomonas campestris* pv. *manihotis* from plant material and soil. Phytopathology 89:591–597

Francis CA, Beman JM, Kuypers MMM (2007) New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. ISME J 1:19–27

Fux C, Siegrist H (2004) Nitrogen removal from sludge digester liquids by nitrification/denitrification or partial nitritation/anammox: environmental and economical considerations. Water Sci Technol 50(10):19–26

Gu Y, Li Y, Li X, Luo P, Wang H, Robinson ZP, Wang X, Wu J, Li F (2016) The feasibility and challenges of energy self-sufficient wastewater treatment plants. Appl Energy 204:1463–1475

Hauck M, Maalcke-Luesken FA, Jetten MSM, Huijbregts MAJ (2016) Removing nitrogen from wastewater with side stream anammox: what are the trade-offs between environmental impacts? Resour Conserv Recycl 107:212–219

Hooper AB, Vannelli T, Bergmann DJ, Arciero DM (1997) Enzymology of the oxidation of ammonia to nitrite by bacteria. Antonie Van Leeuwenhoek 71:59–67

Hulshoff Pol LW, de Castro Lopes SI, Lettinga G, Lens PNL (2004) Anaerobic sludge granulation. Water Res 38:1376–1389

Imajo U, Tokutomi T, Furukawa K (2004) Granulation of Anammox microorganisms in up-flow reactors. Water Sci Technol 49(5–6):155–163

Isaka K, Sumino T, Tsuneda S (2007) High nitrogen removal performance at moderately low temperature utilizing anaerobic ammonium oxidation reactions. J Biosci Bioeng 103:486–490

Isaka K, Kimura Y, Matsuura M, Osaka T, Tsuneda S (2017) First full-scale nitritation-anammox plant using gel entrapment technology for ammonia plant effluent. Biochem Eng J 122:115–122

Jetten MSM, Horn SJ, MCM VL (1997) Towards a more sustainable municipal wastewater treatment system. Water Sci Technol 35:171–180

Jetten MSM, Strous M, van de Pas-Schoonen KT, Schalk J, Dongen UGJM, van de Graff AA, Logemann S, van Muyzer G, van Loosdrecht MCM, Kuenen JG (1999) The anaerobic oxidation of ammonium. FEMS Microbiol Rev 22:421–437

Jetten MSM, van Niftrik L, Strous M, Kartal B, Keltjens JT, Op den Camp HJM (2009) Biochemistry and molecular biology of anammox bacteria. Crit Rev Biochem Mol Biol 44:65–84

Joss A, Salzgeber D, Eugster J, König R, Rottermann K, Burger S, Fabijan P, Leumann S, Mohn J, Siegrist HR (2009) Full-scale nitrogen removal from digester liquid with partial nitritation and anammox in one SBR. Environ Sci Technol 43:5301–5306

Kartal B, Rattray J, van Niftrik L, van de Vossenberg J, Schmid M, Webb RI, Schouten S, Fuerst JA, Sinninghe Damste JS, Jetten MSM, Strous M (2007) *Candidatus* "Anammoxoglobus propionicus" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria. Syst Appl Microbiol 30:39–49

Kartal B, van Niftrik L, Rattray J, van de Vossenberg J, Schmid MC, Damste JSS, Jetten MSM, Strous M (2008) Candidatus "Brocadia fulgida": an autofluorescent anaerobic ammonium oxidizing bacterium. FEMS Microbiol Ecol 63:46–55

Keluskar R, Nerurkar A, Desai A (2013) Development of a simultaneous partial nitrification, anaerobic ammonia oxidation and denitrification (SNAD) bench scale process for removal of ammonia from effluent of a fertilizer industry. Bioresour Technol 130:390–397

Khramenkov SV, Kozlov MN, Kevbrina MV, Dorofeev AG, Kazakova EA, Grachev VA, Kuznetsov BB, Polyakov DY, Nikolaev YA (2013) A novel bacterium carrying out anaerobic ammonium oxidation in a reactor for biological treatment of the filtrate of wastewater fermented sludge. Microbiol 82(5):628–636

Kowalski MS, Devlin T, Basu S, Oleszkiewicz JA (2019) Accelerated start-up of a partial nitritation-anammox moving bed bio fi lm reactor. Biochem Eng J 145:83–89

Kridelbaugh D, Doerner KC (2009) Development of a defined medium for *Clostridium scatologenes* ATCC 25775. Lett Appl Microbiol 48:426–432

Kuenen JG (2008) Anammox bacteria: from discovery to application. Nat Rev Microbiol 6:320–326

Kuenen JG, Jetten MSM (2001) Extraordinary anaerobic ammonium oxidising bacteria. ASM News 67:456–463

Kuypers MMM, Sliekers AO, Lavik G, Schmid M, Jorgensen BB, Kuenen JG, Sinninghe Damste JS, Strous M, Jetten MSM (2003) Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature 422:608–611

Kwon K, Kim H, Kim W, Lee J (2019) Efficient nitrogen removal of reject water generated from anaerobic digester treating sewage sludge and livestock manure by combining anammox and autotrophic sulfur denitrification processes. Water 11:204. https://doi.org/10.3390/w11020204

Lackner S, Gilbert EM, Vlaeminck SE, Joss A, Horn H, Van Loosdrecht MCM (2014) Full-scale partial nitritation/anammox experiences—an application survey. Water Res 55:292–303

Lam P, Lavik G, Jensen MM, Van De Vossenberg J, Schmid M, Woebken D, Amann R, Jetten MSM, Kuypers MMM (2009) Revising the nitrogen cycle in the Peruvian oxygen minimum zone. Proc Natl Acad Sci U S A 106:4752–4757

Lopez H, Puig S, Ganigue R, Ruscalleda M, Balaguer MD, Colprim J (2008) Start-up and enrichment of a granular Anammox SBR to treat high nitrogen load wastewaters. J Chem Technol Biotechnol 83:233–241

Mao N, Ren H, Geng J, Ding L, Xu K (2017) Engineering application of anaerobic ammonium oxidation process in wastewater treatment. World J Microbiol Biotechnol 33:153

Mercer JAM, Cohen CM, Shuken SR, Wagner AM, Myles W, Iii FRM, Smith MD, Vahala R, Gonzalez- A, Boxer SG, Burns NZ (2017) Chemical synthesis and self-assembly of a Ladderane phospholipid. J Am Chem Soc 138:15845–15848

Morales N, Val del Río Á, Vázquez-Padín JR, Méndez R, Mosquera-Corral A, Campos JL (2015) Integration of the Anammox process to the rejection water and main stream lines of WWTPs. Chemosphere 140:99–105

Mulder A (1992) Anoxic ammonia oxidation. US Patent 5078884

Mulder A (1993) Anoxic ammonia oxidation. US Patent 5259959

Mulder A, van de Graaf AA, Roberton LA, Kuenen JG (1995) Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. FEMS Microbiol Ecol 16:177–184

Ni SQ, Meng J (2011) Performance and inhibition recovery of anammox reactors seeded with different types of sludge. Water Sci Technol 63:710–718

Ni SQ, Lee PH, Fessehaie A, Gao BY, Sung S (2010a) Enrichment and biofilm formation of Anammox bacteria in a non-woven membrane reactor. Bioresour Technol 101:1792–1799

Ni SQ, Lee PH, Sung S (2010b) The kinetics of nitrogen removal and biogas production in an anammox non-woven membrane reactor. Bioresour Technol 101:5767–5773

Ni SQ, Fessehaie A, Lee PH, Gao BY, Xu X, Sung S (2010c) Interaction of anammox bacteria and inactive methanogenic granules under high nitrogen selective pressure. Bioresour Technol 101:6910–6915

Ni SQ, Gao BY, Wang CC, Lin JG, Sung S (2011) Fast start-up, performance and microbial community in a pilot-scale anammox reactor seeded with exotic mature granules. Bioresour Technol 102:2448–2454

Ni SQ, Ni JY, Hu DL, Sung S (2012a) Effect of organic matter on the performance of granular anammox process. Bioresour Technol 110:701–705

Ni SQ, Sung S, Yue QY, Gao BY (2012b) Substrate removal evaluation of granular anammox process in a pilot-scale upflow anaerobic sludge blanket reactor. Ecol Eng 38:30–36

Nicholls JC, Davies CA, Trimmer M (2007) High-resolution profiles and nitrogen isotope tracing reveal a dominant source of nitrous oxide and multiple pathways of nitrogen gas formation in the central Arabian. Limnol Oceanogr 52:156–168

Peeters SH, van Niftrik L (2018) Trending topics and open questions in anaerobic ammonium oxidation. Curr Opin Chem Biol 49:45–52

Penton CR, Devol AH, Tiedje JM (2006) Molecular evidence for the broad distribution of anaerobic ammonium-oxidizing bacteria in freshwater and marine sediments. Appl Environ Microbiol 72:6829–6832

Pynaert K, Smets BF, Beheydt D, Verstraete W (2004) Start-up of autotrophic nitrogen removal reactors via sequential biocatalyst addition. Environ Sci Technol 38:1228–1235

Quan ZX, Rhee SK, Zuo JE, Yang Y, Bae JW, Park JR, Lee ST, Park YH (2008) Diversity of ammonium-oxidizing bacteria in a granular sludge anaerobic ammonium-oxidizing (anammox) reactor. Environ Microbiol 10:3130–3139

Ren L, Ni S, Liu C, Liang S, Zhang B, Kong Q, Guo N (2015) Effect of zero-valent iron on the start-up performance of anaerobic ammonium oxidation (anammox) process. Environ Sci Pollut Res 22:2925–2934

Richards FA (1965) Anoxic basins and fjords. In: Ripley JP, Skirrow G (eds) Chemical oceanography. Academic, London, pp 611–645

Schalk J, Oustad H, Kuenen JG, Jetten MSM (1998) The anaerobic oxidation of hydrazine—a novel reaction in microbial nitrogen metabolism. FEMS Microbiol Lett 158:61–67

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

Schmid M, Walsh K, Webb R, Rijpstra WIC, van de Pas-Schoonen K, Verbruggen MJ, Hill T, Moffett B, Fuerst J, Schouten S, Damsté JS, Harris J, Shaw P, Jetten MSM, Strous M (2003) Candidatus "Scalindua brodae", sp. nov., Candidatus "Scalindua wagneri", sp. nov., two new species of anaerobic ammonium oxidizing bacteria. Syst Appl Microbiol 26:529–538

Schmid MC, Risgaard-Petersen N, van de Vossenberg J, Kuypers MM, Lavik G, Petersen J, Hulth S, Thamdrup B, Canfield D, Dalsgaard T, Rysgaard S, Sejr MK, Strous M, op den Camp HJM, Jetten MSM (2007) Anammox bacteria in marine environments: widespread occurrence but low diversity. Environ Microbiol 9:1476–1484

Shiakowski DM, Mavinic DS (1989) Biological treatment of a high ammonia leachate: influence of external carbon during initial startup. Water Res 32:2533–2541

Sobotka D, Tuszynska A, Kowal P, Ciesielski S, Czerwionka K, Makinia J (2017) Long-term performance and microbial characteristics of the anammox-enriched granular sludge cultivated in a bench-scale sequencing batch reactor. Biochem Eng J 120:125–135

Song YX, Liao Q, Yu C, Xiao R, Tang CJ, Chai LY, Duan CS (2017) Physicochemical and microbial properties of settled and floating anammox granules in upflow reactor. Biochem Eng J 123:75–85

Speth DR, in 't Zandt MH, Guerrero-Cruz S, Dutilh BE, Jetten MSM (2016) Genome-based microbial ecology of anammox granules in a full-scale wastewater treatment system. Nat Commun 7:11172. https://doi.org/10.1038/ncomms11172

Strous M, Jetten MSM (2004) Anaerobic oxidation of methane and ammonium. Annu Rev Microbiol 58:99–117

Strous M, Heijnen JJ, Kuenen JG, Jetten MSM (1998) The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Appl Microbiol Biotechnol 50:589–596

Strous M, Fuerst JA, Kramer EHM, Logemann S, Muyzer G, van de Pas-Schoonen KT, Webb R, Kuenen JG, Jetten MSM (1999) Missing lithotroph identified as new planctomycete. Nature 400:446–449

Strous M, Pelletier E, Mangenot S, Rattei T, Lehner A, Taylor MW, Horn M, Daims H, Bartol-Mavel D, Wincker P, Barbe V, Fonknechten N, Vallenet D, Segurens B, Schenowitz-Truong C, Medigue C, Collingro A, Snel B, Dutilh BE, Op den Camp HJM, van der Drift C, Cirpus I, van de Pas-Schoonen KT, Harhangi HR, van Niftrik L, Schmid M, Keltjens J, van de Vossenberg J, Kartal B, Meier H, Frishman D, Huynen MA, Mewes HW, Weissenbach J, Jetten MSM, Wagner M, Le Paslier D (2006) Deciphering the evolution and metabolism of an anammox bacterium from a community genome. Nature 440:790–794

Thamdrup B, Dalsgaard T (2002) Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. Appl Environ Microbiol 68:1312–1318

Tsushima I, Kindaichi T, Okabe S (2007) Quantification of anaerobic ammonium-oxidizing bacteria in enrichment cultures by real-time PCR. Water Res 41:785–794

Van de Graaf AA, Mulder A, Slijkhuis H, Robertson LA, Kuenen JG (1990) Anoxic ammonium oxidation. In: Christiansen C, Munck L, Villadsen J (eds) Proceedings of the 5th European congress on biotechnology, vol I, pp 338–391. Munksgaard, Copenhagen

Van de Graaf AA, Mulder A, de Bruijn P, Jetten MSM, Robertson LA, Kuenen JG (1995) Anaerobic oxidation of ammonium is a biologically mediated process. Appl Environ Microbiol 61:1246–1251

Van de Graaf AA, de Bruijn P, Robertson LA, Jetten MSM, Kuenen JG (1996) Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. Microbiology 142:2187–2196

Van de Graaf AA, de Bruijn P, Robertson LA, Jetten MSM, Kuenen JG (1997) Metabolic pathway of anaerobic ammonium oxidation on the basis of ^{15}N studies in a fuidized bed reactor. Microbiology 143:2415–2421

Van de Vossenberg J, Rattray JE, Geerts W, Kartal B, van Niftrik L, van Donselaar EG, Damsté JSS, Strous M, Jetten MSM (2008) Enrichment and characterization of marine anammox bacteria associated with global nitrogen gas production. Environ Microbiol 10:3120–3129

Van der Star WRL, Abma WR, Blommers D, Mulder JW, Tokutomi T, Strous M, Picioreanu C, van Loosdrecht MCM (2007) Startup of reactors for anoxic ammonium oxidation: experiences from the first full-scale anammox reactor in Rotterdam. Water Res 41:4149–4163

Van der Star WRL, Miclea AI, van Dongen UGJM, Muyzer G, Picioreanu C, van Loosdrecht MCM (2008) The membrane bioreactor: a novel tool to grow anammox bacteria as free cells. Biotechnol Bioeng 101:286–294

Van Dongen U, Jetten MSM, van Loosdrecht MCM (2001) The SHARON®-Anammox® process for treatment of ammonium rich wastewater. Water Sci Technol 44:153–160

Van Loosdrecht MCM, Jetten MSM (1998) Microbiological conversions in nitrogen removal. Water Sci Technol 38:1–7

Vázquez-Padín JR, Morales N, Gutiérrez R, Fernández R, Rogalla F, Barrio JP, Campos JL, Mosquera-Corral A, Méndez R (2014) Implications of full-scale implementation of an anammox- based process as post-treatment of a municipal anaerobic sludge digester operated with co-digestion. Water Sci Technol 69(6):1151–1158

Wang Y, Bu C-N, Kang Q, Ahmad HA, Zhang J, Gao B, Ni SQ (2017) Autoclaved sludge as the ideal seed to culture anammox bacteria: reactor performance and microbial community diversity. Bioresour Technol 244:391–399

Wang B, Guo Y, Zhao M, Li B, Peng Y (2019) Achieving energy-efficient nitrogen removal and excess sludge reutilization by partial nitritation and simultaneous anammox denitrification and sludge fermentation process. Chemosphere 218:705–714

Woebken D, Lam P, Kuypers MM, Naqvi SW, Kartal B, Strous M, Jetten MS, Fuchs BM, Amann R (2008) A microdiversity study of anammox bacteria reveals a novel Candidatus Scalindua phylotype in marine oxygen minimum zones. Environ Microbiol 10:3106–3119

Zhang L, Narita Y, Gao L, Ali M, Oshiki M, Okabe S (2017) Maximum specific growth rate of anammox bacteria revisited. Water Res 116:296–303

Microbial Degradation of Natural and Synthetic Rubbers

21

Biraj Sarkar and Sukhendu Mandal

Abstract

In today's world, rubber has become one of the indispensable commodities due to its outstanding properties like elasticity, longevity, and wide range of applications. Natural rubber (NR) and synthetic rubber (SR) are widely used in everyday life. The staggering amount of rubber wastes generated each year and their disposal pose a harrowing threat for the environment and its ecosystem. Concerns regarding the disposal of solid polymeric wastes are expected to increase as the landfill capacity decreases. A huge fraction of solid polymeric wastes are rubber wastes, which is no more advised to be buried in land or incinerated due to the secondary pollution of soil or ambient air, respectively. These issues make the study on biodegradation of rubbers so relevant for sustainable environment. This chapter provides a review on the microbial degradation of natural rubber (NR), synthetic rubber (SR), and gutta-percha (GP) rubbers by different microbes and also in vitro disintegration of the rubbers by different enzymes.

Keywords

Poly-isoprene · Bioremediation · Biodegradation of rubber · Solid waste management

21.1 Introduction

According to the report from the Association of Natural Rubber Producing Countries (ANRPC), natural rubber production during 2017 was 13.34 million tonnes, and natural rubber consumption is estimated to be 13.04 million tonnes. The

B. Sarkar · S. Mandal (✉)
Laboratory of Molecular Bacteriology, Department of Microbiology, University of Calcutta, Kolkata, India

M. P. Shah (ed.), *Microbial Bioremediation & Biodegradation*,
https://doi.org/10.1007/978-981-15-1812-6_21

International Rubber Study Group (IRSG) reported that the world production of natural rubber increased by 7.5% and consumption increased by 4% during 2017 with a surplus of 0.29 million tonnes. The IRSG stipulated that synthetic rubber (SR) production and consumption during 2017 had increased by 1.5% and 2.1%, respectively. In today's world, rubber has become one of the indispensable commodities due to its outstanding properties like elasticity, longevity, and wide range of applications. Natural rubber (NR) and synthetic rubber (SR) are widely used in everyday life. The staggering amount of rubber wastes generated each year and their disposal pose a harrowing threat for the environment and its ecosystem. Concerns regarding the disposal of solid polymeric wastes are expected to increase as the landfill capacity decreases. A huge fraction of solid polymeric wastes are rubber wastes, which is no more advised to be buried in land or incinerated due to secondary pollution (CO_2, NO_x, and SO_2) of ambient air. These issues make the study on biodegradation of rubbers so relevant today.

This chapter provides a review on the microbial degradation of natural rubber (NR), synthetic rubber (SR), and gutta-percha (GP) rubbers by different microbes and also in vitro disintegration of the rubbers by different enzymes. Analysis of the biodegradation of rubber can be checked and estimated by the results of plate assay, decrease of rubber mass with respect to increasing protein concentration or wet weight of microbes, scanning electron microscopy (SEM) to visualize the colonization of rubber-degrading microbes on the rubber surface, and DNPH and Schiff's test for the detection of degradation by product compounds with aldehyde and ketone groups. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and measurement of CO_2 that evolved (Sturm's test) due to the growth of microbes using rubber as the sole carbon source revealed that both natural and synthetic rubbers can be degraded by microorganisms. Extracellular enzymes isolated from the different reported rubber-degrading bacteria like latex clearing protein (Lcp), rubber oxygenase A (RoxA), and rubber oxygenase B (RoxB) are shown to be the key enzymes which are involved in the initial step of cleaving the poly-isoprene backbone in rubber biodegradation process by different rubber-degrading bacteria. The degradation products and their analysis by selective inhibition of the degradation step steered to the inference that intermediates formed after the initial and extracellular cleaving of poly-isoprene backbone are degraded via β-oxidation pathway inside the bacterial cell (Bode et al. 2001). On the contrary, in fungal biodegradation of rubber, enzymes like laccase and manganese peroxidase are reported to be responsible (Fig. 21.1).

21.2 Solid Waste of Rubber in Environment

The staggering amount of rubber wastes generated each year and their disposal pose a harrowing threat for the environment and its flora and fauna. Concerns regarding the disposal of solid polymeric wastes are expected to increase as landfill capacity decreases. Solid rubber wastes if buried in land lead to soil infertility and if incinerated cause secondary pollution (CO_2, NO_x, and SO_2) of ambient air. Thus,

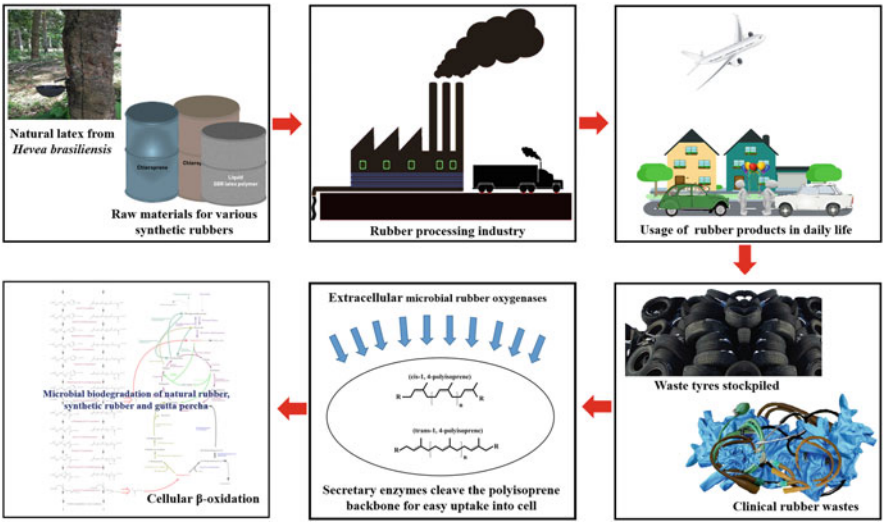


Fig. 21.1 A schematic representation of natural and synthetic rubber production, industrial processing, use of rubber in daily life, scrapping of rubber wastes, and finally an alternative process of bioremediation through microbial biodegradation

it might not be the best way to maximize the economic potential of the rubber (Zabaniotou and Stavropoulos 2003), and recycling may be a better option. Another option to avoid rubber-mediated environmental pollution would be its biodegradation.

A huge fraction of the world's solid waste is comprised of rubber wastes. Most of this waste comprises of worn-out tires. Because of increasing production of vehicles and a limited life span of the tires, each year about one billion tires are discarded around the globe (Kasai et al. 2017). Some of the tires generated in the world are used for recycling, with the rest being stockpiled or landfilled (Airey et al. 2003), which creates environmental problems and health hazards. It has been estimated that in 2011 among total waste tires generated globally, only 5% were exported for further processing, 7% were recycled on site, and 11% were burned for fuel, while the remaining 77% per year were illegally dumped, accumulated, or discarded in landfills (The Hindu 2016).

21.3 Recent Problems with Polymeric Rubber Waste Management

Waste tires stored in a dump or landfill generates a proliferation of undesired insects and rodents (Waste Management World 2010). The environmental hazard also includes a great fire risk. Because of their shape and longevity, the dumped waste tires occupy a large area and should be put to a new productive use (Gawel et al. 2011). Due to the various additives used in processing of rubbers such as

accelerators, retarders, and various antioxidants, the biodegradation of rubbers by normal means is a hurdle yet to overcome (Bredberg et al. 2000; Christinasson et al. 2000; Shah et al. 2013). Incineration of these solid rubber wastes generates particulate matters and also releases huge amount of carbon dioxide and other toxic gases, sulfur compounds, hydrocarbons, and oxides of carbon and nitrogen which would in turn cause environmental pollution and lead to global warming. This incineration of such rubber wastes also releases poisonous pyrolytic oil in environment. A single commercial car tire releases almost 7.5 L of pyrolytic oil when incinerated. There are chances that this oil may lead to soil and water contamination. Outdoor dumping of tires and other solid rubber wastes provides an appropriate condition for the breeding of undesired insects and rodents which may lead to different endemic diseases (Anonymous 2013; Nayanashree and Thippeswamy 2015b; Tsuchii et al. 1996). Discarding of various rubber products like balloons, bags, tubes, bands, and gloves in open spaces brings threat to animals too (Anonymous 2011; Nayanashree and Thippeswamy 2015b). It has been reported that almost all different types of rubbers contain fillers like carbon black, clays, calcium carbonates, or hydrated silica compounds, and when incinerated, they produce ash which may contain heavy metals. This implies that burning of scrap rubbers is not a good option to treat rubber wastes (Nayanashree and Thippeswamy 2015b; Tsuchii and Tokiwa 2001).

21.4 Decomposition and Disintegration of Rubber

21.4.1 Decomposition of Natural Rubber (NR) by Bacteria

Over the last 100 years, there have been serious researches for isolation and characterization of different efficient rubber-degrading bacteria and to understand the biochemical basis for the degradation of natural rubber (NR) and synthetic rubber (SR). To date, many rubber-degrading bacterial species have been identified (Table 21.1) which are capable of degrading cis-(1,4)-polyisoprene (both NR and SR) and trans-(1,4)-polyisoprene (also called gutta-percha or GP). Rubber-degrading bacteria can be divided into two groups, depending on their approaches to degrade the rubber (Linos et al. 2000a, b). The first group is capable of forming translucent halo zones secreting extracellular polyisoprene-cleaving enzymes, when cultivated on a solid media containing dispersed rubber latex particles. The most effective degraders in this group belong to the actinomycetes from genera *Actinoplanes*, *Streptomyces*, and *Micromonospora* (Imai et al. 2011; Jendrossek et al. 1997; Linos et al. 2000b; Rose and Steinbuchel 2002). The second group of rubber degraders does not form translucent halo zones when grown on solid media with dispersed latex particles. They require direct contact with the rubber and show adhesive growth on the rubber surface in liquid cultures using it as a substrate for carbon and energy. This group is comprised of the mycolic acid-containing actinobacteria belonging to the genera *Gordonia*, *Mycobacterium*, and *Nocardia*. To date, the complete NR degradation pathway has been well studied in two gram-positive actinomycetes, *Gordonia* (Hiessl et al. 2012) and *Nocardia* (Luo et al.

Table 21.1 Different rubber degraders and their rubber oxygenases

Rubber degraders	Type of rubber degraded	Degradation strategy	Rubber oxygenases involved	References
Gram negative				
Steroidobacter cummioxidans 35Y	NR, SR	B	RoxA, RoxB	Birke et al. (2017), Braaz et al. (2005), Braaz et al. (2004), Seidel et al. (2013), Sharma et al. (2018), Tsuchii and Takeda (1990)
Rhizobacter gummiphilus NS21	Latex dispersed in agar	B	RoxA, RoxB	Imai et al. (2013), Kasai et al. (2017)
Pseudomonas aeruginosa AL98	NR, vulcanized NR, natural rubber latex concentrate, SR	A	Unknown	Linos et al. (2000b)
Pseudomonas citronellolis	Vulcanized NR, SR	A	Unknown	Bode et al. (2000)
Acinetobacter calcoaceticus	Cross-linked NR latex gloves, SR	A	Unknown	Bode et al. (2001)
Gram positive				
Streptomyces sp. strain K30	NR, SR, NR latex	B	Lcp	Birke et al. (2015), Rose et al. (2005), Rother et al. (2016), Yikmis et al. (2008)
Streptomyces sp. strain CFMR 7	NR, vulcanized NR, NR latex	B	Lcp	Nanthini et al. (2017), Nanthini and Sudesh (2017)
Streptomyces griseus 1D	NR, SR, cross-linked NR	B	Lcp	Bode et al. (2001), Jendrossek et al. (1997)
Streptomyces coelicolor 1A	NR, SR, cross-linked NR	B	Lcp	Bode et al. (2000), Jendrossek et al. (1997)
Rhodococcus rhodochrous RPK1	NR, SR	A	Lcp	Watcharakul et al. (2016)
Gordonia westfalica Kb2	NR, SR	A	Lcp	Berekaa et al. (2000), Linos et al. (2002)
Gordonia polyisoprenivorans VH2	NR, SR	A	Lcp	Hiessl et al. (2012), Oetermann et al. (2018)
Gordonia polyisoprenivorans Kd2	NR, SR	A	Lcp	Berekaa et al. (2000); Linos et al. (1999)

(continued)

Table 21.1 (continued)

Rubber degraders	Type of rubber degraded	Degradation strategy	Rubber oxygenases involved	References
Nocardia nova SH22a	NR, SR	A	Lcp	Luo et al. (2014)
Nocardia farcinica E3	SR, NR	A	Lcp	Ibrahim et al. (2006)
Nocardia farcinica NVL3	SR, NR	A	Lcp	Linh et al. (2017)

2014). Two efficient gram-negative bacteria have been isolated and well-characterized so far: *Xanthomonas* sp. strain 35Y (now reclassified as *Steroidobacter cummioxidans* strain 35Y) (Sharma et al. 2018) and *Rhizobacter gummiphilus* NS21 (Birke et al. 2018). All the gram-positive rubber-degrading bacteria reported to date secrete the Lcp protein to oxidatively cleave the polyisoprene backbone in an *endo*-fashion and harbor one or more *lcp* gene homologues (Broker et al. 2008; Hiessl et al. 2012, 2014; Ibrahim et al. 2006; Imai et al. 2011; Rose et al. 2005), whereas the gram-negative bacteria are reported to harbor genes for RoxA and RoxB which cleave the polyisoprene in an *exo*- and *endo*-fashion, respectively (Birke et al. 2017, 2018; Jendrossek and Birke 2019; Sharma et al. 2018).

21.4.2 Decomposition of Synthetic Rubber (SR) by Bacteria

Actinobacteria like *Gordonia polyisoprenivorans* VH2, *Nocardia* sp. strain 835A, *N. nova* SH22a, and *Streptomyces* sp. K30 have been found to degrade synthetic poly(cis-1,4-isoprene) rubber (SR). *N. nova* SH22a is also reported to degrade poly-trans-1,4-isoprene rubber (gutta-percha or GP) efficiently. Being a gram-negative organism, *Xanthomonas* sp. strain 35Y showed impressive degradation of synthetic rubber (Sharma et al. 2018). The biological pathway of degradation of rubber (NR/SR or GP) is well demonstrated using *N. nova* SH22a (Luo et al. 2014). Apart from *N. nova* SH22a, there are other three known GP-degrading strains of *N. nova*, i.e., *N. nova* L1b, SEI2b, and SEII5a, and two different species of *Nocardia*: *N. jiangxiensis* SM1 and *N. takedensis* WE30 (Warneke et al. 2007). There also have been trials to heterologously express rubber-degrading genes in fast-growing organisms for efficient rubber degradation. *Nocardia* sp. 835A mutant strains Wh, Rw, and Rc showed degradation of vulcanized rubber tires. The heterologous expression of the NVL3 *lcp* from *Nocardia* in *Escherichia coli* BL21(DE3) allowed degradation of synthetic rubber by the *E. coli* BL21(DE3) (Linh et al. 2017). The different strains of *Gordonia* (like *Gordonia* sp. kb2 and kd2), *Micromonospora aurantiaca* W2b, and *Pseudomonas aeruginosa* AL98 showed growth on the synthetic rubber after removal of the antioxidants from the synthetic rubbers. There are reports of bacteria like *Mycobacterium fortuitum* NF4 growing on the synthetic rubbers (Berekaa et al. 2000).

21.4.3 Decomposition of Natural Rubber (NR) by Fungi

The biodegradation of rubber by fungi was first investigated by De Vries. The researcher observed the biodegradation of rubber using different strains of *Penicillium* and *Aspergillus* in a 10% (wt/vol) NaCl liquid medium with natural rubber as the substrate. A 6% increase in the biomass with 15.5–30.9% decrease in the weight of the rubber material after incubation of 19 months to 5 years was recorded (De Vries 1928). Schade reported a decent growth of fungi *Monascus ruber* and *Monascus purpureus* using purified natural rubber as a substrate but unable to use synthetic polychloroprene rubber as a substrate (Schade 1937). Kalinenko reported strains from *Aspergillus* and *Penicillium* capable of degrading rubbers (Kalinenko 1938). Kwiatkowska et al. used soil burial experiment to check the degradation of NR vulcanized sheets of certain composition and recorded weight losses of about 40% of the initial weight after 91 days and held an isolated fungal strain of *Fusarium solani* from the rubber surface responsible for the degradation (Kwiatkowska et al. 1980). Another report by Borel showed *Fusarium solani* capable of growing on NR. In fact, based on GPC curve obtained after 10 days of culture, *F. solani* was found to be a more rapid degrader compared to other rubber degraders used in the study like *Paecilomyces lilacinus*, *Cladosporium cladosporioides*, and *Phoma eupyrena* (Borel et al. 1982). Employing solution viscosity measurement as investigative tool, a reduction of 15% in the molecular weight of polyisoprene because of degradation by *Penicillium variabile* was recorded after 70 days by Williams (Williams 1982). In an attempt to isolate rubber-degrading microorganism from natural rubbers dumped in soil, two fungal isolates, *Aspergillus niger* and *Penicillium* species, were found to degrade the natural rubber sample by 28.3% and 25.9% of the initial weight, respectively, in an interval of 2 months (Nayanashree and Thippeswamy 2013). A strain of *Rhodotorula mucilaginosa* from liquid culture with NR and a filamentous fungus, *Alternaria alternata*, from the NR surface using the polyisoprene as the sole source of carbon were isolated and found to be capable of degrading the rubber (Bosco et al. 2018).

21.4.4 Decomposition of Synthetic Rubber (SR) by Fungi

It has been reported that the enzyme manganese peroxidase (MnP) produced by *Ceriporiopsis subvermispora* strain FP-90031 if incubated with non-vulcanized synthetic poly(cis-1,4-isoprene) as a substrate yielded low-molecular-weight products. Laccase is another enzyme isolated from *Coriolus* sp., which when incubated with rubber sheets gave similar molecular weight reductions for the incubated sheets and gel permeation chromatography profiles of the degradation products.

21.4.5 In Vitro Disintegration of Rubber

Various enzyme mediator systems comprising of radical-producing enzymes and their substrates serving as radical precursors were investigated in consideration to biological degradation of polyisoprene and rubber materials. Oxidative degradation of cis- and trans-1,4-polyisoprenes by two kinds of enzyme-mediator systems, lipoxygenase/linoleic acid and horseradish peroxidase/1-hydroxy-benzotriazole, was inspected at 37 °C in aqueous media and analyzed by gel permeation chromatography. Lipoxygenase and horseradish peroxidase activate their substrates, linoleic acid and 1-hydroxybenzotriazole, respectively, for cleaving of polyisoprene backbone of both kinds of isoprenes. Molecular weights of 1,4-polyisoprenes showed consequent decrease during the action under both mentioned enzyme-mediator systems, and the depolymerization was completely inhibited when added with butylated hydroxyl toluene. When the enzyme or the mediator from a reaction system was taken out, depolymerization did not progress, indicating that the scission of polymer chain is made by the radicals produced only in the presence of both enzyme and mediator. Fenton reagent with linoleic acid was also effective against the degradation of both 1,4-polyisoprenes. Vulcanized natural rubber latex gloves were treated under these three methods, and surface degradation with hole formation was observed with a scanning electron micrograph (Enoki et al. 2003).

Manganese peroxidase (MnP) isolated from *C. subvermispora* strain FP-90031 was incubated with non-vulcanized synthetic poly(cis-1,4-isoprene) for 48–96 h at 35 °C (Sato et al. 2003).

21.5 Recent Techniques to Analyze Rubber Degradation

There are various approaches to analyze the degradation of rubber. Different qualitative experiments like plate assay, scanning electron microscopy (SEM), attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), and Sturm's test are few of the preliminary tests used to screen the growth of microorganisms on rubber as a sole source of carbon and energy.

21.5.1 Growth of Rubber-Degrading Bacteria on Polyisoprene

The first test to screen rubber-degrading organisms isolated was to observe growth on the surface of rubber. Those able to degrade rubber or utilize polyisoprene as the sole source of carbon and energy for growth are supposed to be potent rubber degraders. In this context, there are two types rubber degraders found to date. The first group, when grown on latex-overlay minimal salt agar plate, was able to form translucent halos indicating secretion of some kind of extracellular enzyme which cleaves the rubber to form small oligo-isoprenes enabling uptake of the isoprenoids by the cells as observed for rubber-degrading *Streptomyces* sp. K30 (Rose et al. 2005) or *Micromonospora aurantiaca* W2b (Linos et al. 2000a, b). However, the

second group is incapable of producing halo zone on latex-overlay minimal salt agar plates but forms visible colonies directly on the rubber surface when suspended in minimal salt broth. They are adhesive in growth and form biofilms directly on the surface of the rubber to utilize the polyisoprene for sole source of carbon. Bacteria showing such adhesive growth are the most dominant degraders of rubber and represented by bacteria like *Gordonia polyisoprenivorans* VH2 (Hiessl et al. 2012) and *Nocardia nova* SH22a (Luo et al. 2014).

21.5.2 Detection of Aldehyde and Ketones Formed by Staining Methods

Two different qualitative staining tests were performed to identify aldehyde and ketone groups. Poly(cis-1,4-isoprene) degradation products were mixed with Schiff's reagent to detect aldehyde groups, whereas for the detection of carbonyl groups, 2,4-dinitrophenylhydrazine (DNPH) was employed (Rose et al. 2005). Both reactions were done at room temperature for 5 min. Schiff's staining is one of the most widely used staining approaches in this regard. Staining of the degraded rubber with Schiff's reagent showed the presence of aldehyde groups and gave rise to the formation of a purple color indicating the presence of degradation products containing aldehyde groups.

21.5.3 Scanning Electron Microscopy

Scanning electron microscopy is one of the different approaches to monitor the changes on the rubber surface due to colonization and biofilm production by the adhesively growing bacteria or the changes on the smoothness of surface due to the degradation of rubber by the extracellular enzymes secreted, where the rubber is the sole source of carbon. Scanning electron microscopy has always been one of the preliminary approaches for confirmation of biodegradation of rubber. Microorganisms belonging to the genera *Gordonia* (Berekaa et al. 2000, 2005), *Nocardia* (Linos et al. 2000a, b), *Streptomyces* (Gallert 2000), *Pseudomonas* (Bosco et al. 2018), *Micromonospora* (Berekaa et al. 2000), and *Paenibacillus lautus* (Hapuarachchi et al. 2016) are some of the rubber-metabolizing bacteria which have been checked under scanning electron microscopy for their growth and degradation of the rubber (Fig. 21.2).

21.5.4 ATR-FTIR Analysis

Attenuated total reflectance-Fourier transform infrared spectroscopy allows a non-destructive in situ analysis of various polyisoprene surfaces which are coated by overgrown microbial biofilms formed by the rubber-degrading bacteria, capable of utilizing the carbon from the polyisoprene backbone as a sole source. The decrease

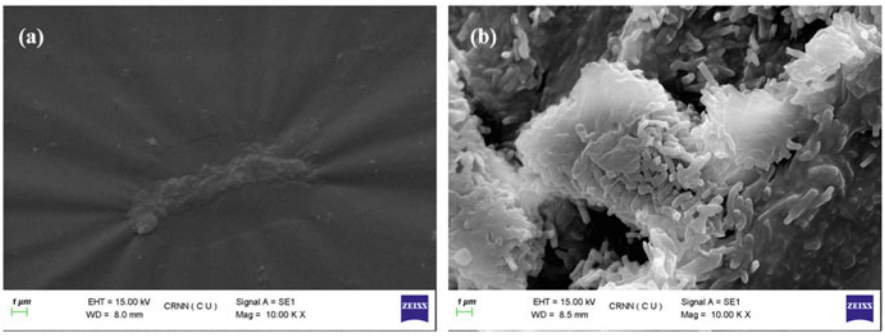


Fig. 21.2 Scanning electron microscopy of a *Gordonia polyisoprenivorans* colonizing on synthetic rubber (SR) surface after 14 days of incubation (**b**) and the uninoculated SR control (**a**)

in the number of double bonds in the polyisoprene chain, increase in the bands due to the increase of carbonyl groups most likely as a "keto" functional group, and broadening of bands because of aldehyde formation due to the oxidative cleavage of the polyisoprene backbone all can be measured by the ATR-FTIR spectroscopy.

21.5.5 Sturm's Test

Sturm's test is one of the conventional approaches used in laboratories to derive information on the biological conversion of carbon backbone of polymers like rubber to different products, as a result of the metabolic processes (Pagga et al. 2001). The quantity of carbon dioxide released can be measured during the cultivation of cells in a minimal salt medium with polyisoprene as the sole source of carbon provided. The carbon dioxide generated due to the cellular respiration gets trapped in a solution of 1 N NaOH and can be measured by titrating the remaining NaOH with 0.1 N HCl. Carbon dioxide liberation due to biodegradation of polyisoprene by *Bacillus* sp. AF-666 was measured by Sturm's test (Shah et al. 2012). The increased CFU/ml in the culture also verified the ability of microorganisms to utilize polyisoprene. Sturm's test (Sturm 1973) was commonly employed for evaluation of the biodegradability of polymer materials (Calmon et al. 2000).

21.5.6 Increase in Protein Concentration with Respect to Weight Loss of Rubber

It is quite conceivable that if there is any rubber-metabolizing bacteria growing in a medium where rubber is the sole source of carbon and energy, the microorganism is certainly going to use that polymer for its growth and will increase the total cell mass in the medium after certain period of time. As it has been predicted that approximately 50% of the bacterial dry cell mass is protein (Gallert 2000), one can monitor

the increase in the concentration of protein in medium with respect to the decrease in weight of the rubber. In 1995, Heisey et al. showed weight loss of the rubber with respect to the increase in protein concentration. Weight losses of the rubber strips used as a sole source for carbon and protein production due to the bacterial growth were determined after 6 weeks. Prior to protein measurement, cells attached loosely to the rubber were dislodged into the broth by boiling the cultures for 15 min, and this was followed by sonication for 15 minutes and vigorous shaking (300 reciprocations per minute) for 10 min. The rubber strips were then removed. Protein was extracted from the cells by adding sufficient NaOH to the culture broth to bring its NaOH concentration to 1 N and then by boiling the broth for 5 min. Any protein in cells remaining attached to the rubber was extracted by boiling the strips for 5 min in 3 mL of 1 N NaOH. Protein in the extracts was determined by a modified Lowry method. The rubber strips were dried at 52 °C and weighed after extraction.

21.5.7 Viscosity Determination Tests

This method is a conventional procedure generally used for molecular-weight determination. This procedure is based upon the calculation of the intrinsic viscosity. Rubber-degrading strains use the polyisoprene as their sole source of carbon for energy and growth. With the growth of the microbes due to the degradation of polyisoprene, there is subsequent decrease in the intrinsic viscosity values of rubbers used. Interestingly, it has been found that waste rubber tires produce higher intrinsic viscosity values compared to the intrinsic viscosity of natural rubbers incubated for same period of time with same rubber degrader; it is because of the inhibitory effects of the various additives present in the waste rubbers to microbial degradation.

21.6 Genomics and Proteomics of Rubber Degraders

To understand the cleaving of polyisoprene into oligo-isoprene and the uptake of the resultants by the bacterial cells, it is necessary to observe the roles of the key enzymes involved in the process of initial biodegradation of rubbers.

21.6.1 Latex Clearing Protein (Lcp)

For the ease of uptake and metabolism of high-molecular-weight rubber by bacterial cells, the polymers are first cleaved to low-molecular-weight oligomers outside the cells. Lcps are extracellular rubber oxygenases that catalyze the cleavage of poly-isoprene. Lcp in fact is involved in the initial cleavage step of poly-isoprene. Lcp is mostly distributed in gram-positive rubber-degrading actinobacteria, such as *Streptomyces* sp. K30 (Rose et al. 2005), *Gordonia westfalica*, *G. polyisoprenivorans* (Broker et al. 2008; Hiessl et al. 2012), *Actinoplanes missouriensis* (Jendrossek et al. 1997), *Nocardia farcinica* (Ibrahim et al. 2006), *N. nova* (Luo et al. 2014), and

Rhodococcus rhodochrous RPK1 (Watcharakul et al. 2016). There is no report of any gram-positive rubber-degrading bacterium without Lcp. Lcp is also found essential for the growth of adhesively growing group of bacteria on the rubber surface (Hiessl et al. 2012; Luo et al. 2014). Extracellular secretion of Lcp was first observed in *Streptomyces* sp. strain K30. According to the published reports, Lcp is a crucial constituent of the protein complex responsible for the clear zone formation on the latex overlay plates by the cleavage of polyisoprene and was confirmed by the heterologous expression of Lcp from *Streptomyces* sp. strain K30 in *S. lividans* strains TK23 and TK24 and a strain of *Saccharopolyspora erythraea* with the help of a plasmid pIJ6021::*lcp* which made the strains acquire the ability to cleave polyisoprene backbone. The Lcp secretion in the extracellular medium was confirmed by the detection of Lcp-eGFP fusion proteins in the cell-free supernatant employing anti-eGFP and anti-Lcp antibodies (Yikmis and Steinbuchel 2012).

The Lcps possess a monomeric quaternary structure and catalyze the oxidative cleavage of the double bonds in polyisoprene to yield products with terminal keto and aldehyde groups (Ilcu et al. 2017). Lcps produce a mixture of resultant products after cleavage of the polyisoprene that vary in the number of central isoprene units (Birke and Jendrossek 2014; Ibrahim et al. 2006; Ilcu et al. 2017). To date, only three Lcps ($Lcp1_{VH1}$ from *G. polyisoprenivorans* VH2, Lcp_{K30} from *Streptomyces* sp. strain K30, and Lcp_{Rr} from *Rhodococcus rhodochrous* RPK1) have been purified and biochemically well characterized (Hiessl et al. 2014); Birke and Jendrossek 2014, 2015; Rother et al. 2016). Lcp is a mono-heme cytochrome-*b* protein. It has been found that the Lcps (Lcp_{K30}, Lcp_{RPK1} and Lcp_{VH2}) are secreted outside the cell by twin-arginine translocation (Tat) pathway (Jendrossek and Birke 2019). A Tat signal peptide is found at the N-terminus of the Lcp gene products (Hiessl et al. 2012). The molecular weights for mature Lcp_{K30}, Lcp_{RPK1}, and Lcp_{VH2} proteins are 41 kDa, 42.2 kDa, and 41.7 kDa, respectively, which are much smaller compared to the molecular masses of RoxA and RoxB. The highest specific activity among the Lcps is found to be observed in Lcp_{K30} (4.7 U/mg in 37 °C) followed by Lcp_{RPK1} (3.1 U/mg in 30 °C) and Lcp_{VH2} (1.3 U/mg in 23 °C) (Jendrossek and Birke 2019). To date, from *Rhodococcus rhodochrous* RPK1 and *Streptomyces* sp. strain K30, only one *lcp* homologue each has been reported to be found, Lcp_{Rr} and Lcp_{K30}, respectively, which is also true for other reported rubber-degrading bacteria like *Micromonospora*, *Nocardia*, *Actinosynnema*, and *Thermomonospora*, whereas there are two *lcp* homologues identified in *G. polyisoprenivorans* VH2, one from the chromosome $lcp1_{VH2}$ (GPOL_c48310) with a gene length of 1.22 kb and the other from its p174 plasmid $lcp2_{VH2}$ (GPOL_174p00150) with a gene length of 1.17 kb. The highest number of *lcp* homologues among the other rubber-degrading bacteria is reported to be found in *Streptomyces* sp. strain CFMR, harboring three lcp homologues (Nanthini et al. 2017). It is claimed that any actinomycetes possessing one or more *lcp* homologues can use polyisoprene as the sole source of carbon and energy for its growth (Hiessl et al. 2012).

21.6.2 Rubber Oxygenase A (RoxA)

RoxA is another enzyme found to degrade both NR and SR by oxidative cleavage of the double bonds of poly(cis-1,4-isoprene) (Braaz et al. 2005; Hambsch et al. 2010). RoxA is an extracellular enzyme isolated from cell-free culture supernatant of latex-grown *Xanthomonas* sp. strain 35Y (Sharma et al. 2018). RoxA is secreted outside the cell by Sec pathway (Jendrossek and Birke 2019). To date, no RoxA homologues have been so far detected in gram-positive species or in *Archaea* (Jendrossek and Birke 2019). RoxA is found to be responsible for *endo*-type cleavage of double bonds in poly(cis-1,4-isoprene) to give single type of end product (ODTD, C_{15} oligo-isoprenoid) using a dioxygenase mechanism (Braaz et al. 2004; Shah et al. 2013). ODTD can be easily taken up by the cells as a sole carbon source. RoxA is a diheme cytochrome-*c* dioxygenase protein, which has been confirmed by biophysical characterization and structure determination (Schmitt et al. 2010; Seidel et al. 2013; Sharma et al. 2018). Compared to Lcp, the molecular mass of RoxA is much larger, ≈70 kDa ($RoxA_{35Y}$, 71.5 kDa; $RoxA_{NS21}$, 71.5 kDa) (Jendrossek and Birke 2019). The $RoxA_{35Y}$ three-dimensional structure (Seidel et al. 2013) and the cleavage mechanism (Schmitt et al. 2010; Birke et al. 2012) are clearly different from that of Lcps. RoxA cleaves poly(cis-1,4-isoprene) as well as the oligo-isoprenoids derived from cleavage of RoxB (another rubber oxygenase in gram-negative bacteria) in an *exo*-type manner. The RoxA protein is stable even at room temperature and does not require any additional cofactors. The highest specific activity of $RoxA_{35Y}$ was observed to be 2.6 U/mg, at 37 °C (Jendrossek and Birke 2019).

21.6.3 Rubber Oxygenase B (RoxB)

Another rubber oxygenase enzyme is found to be produced by gram-negative rubber-degrading bacteria harboring genes for RoxA. RoxB, too, is an extracellular enzyme and reported to be found in *S. cummioxidans* sp. nov. strain 35Y and *Rhizobacter gummiphilus* NS21. This enzyme too, like RoxA, is secreted by Sec pathway (Jendrossek and Birke 2019). While studying RoxB in *S. cummioxidans* 35Y, it has been observed that $RoxB_{35Y}$ cleaves the isoprene-polymer to a mixture of C_{20} and higher oligo-isoprenoids. The resultant oligo-isoprenoids obtained by action of $RoxB_{35Y}$ then get further cleaved by $RoxA_{35Y}$ to form the C15-oligo-isoprenoid 12-oxo-4,8-dimethyltrideca-4,8-diene-1-al (ODTD). ODTD can then be imported into the bacterial cells and used as a source for carbon and energy (Birke et al. 2018). $RoxB_{35Y}$ shares the presence of two *c*-type heme groups with $RoxA_{35Y}$ and revealed 38% amino acid similarity to $RoxA_{35Y}$. The oxidative cleavage of isoprene-polymer to a mixture of C_{20} and higher oligo-isoprenoids (Birke et al. 2017) is the only feature where $RoxB_{35Y}$ resembles Lcps. RoxB, too, is larger than Lcp with a molecular mass of ≈70kDa ($RoxB_{35Y}$, 70.3 kDa; $RoxB_{NS21}$, 70.8 kDa) (Jendrossek and Birke 2019). RoxB showed oxidative cleavage of isoprene-polymers at a specific activity of ≈6 U/mg. Among all well-studied rubber oxygenases, purified RoxB showed the highest specific activity of 4.5 U/mg (at 23 °C) and also found to

Table. 21.2 Few important characteristic features of the well-characterized rubber oxygenases

Protein attribute	Gene length (bp)	Secretion system	Cleavage type	Cleavage product(s)
$RoxA_{35Y}$	2037	Sec	Exo	ODTD
$RoxA_{NS21}$	2022	Sec	Exo	ODTD
$RoxB_{35Y}$	2046	Sec	Endo	V.L.
$RoxB_{NS21}$	2040	Sec	Endo	V.L.
Lcp_{K30}	1224	Tat	Endo	V.L.
Lcp_{RPK1}	1227	Tat	Endo	V.L.
Lcp_{VH2}	1224	Tat	Endo	V.L.

ODTD means 12-oxo-4,8-dimethyltrideca-4,8-diene-1-al. V.L. means cleavage products of variable lengths

exert a synergistic effect on the efficiency of RoxA by enhancing the production of ODTD molecules cleaving polyisoprene backbone, as observed in *Xanthomonas* sp. (Birke et al. 2017) (Table 21.2).

21.6.4 Superoxide Dismutase (SodA) and Oxidative Stress Response by Gram-Positive Bacteria

An extracellular superoxide dismutase is found to be formed during poly(*cis*-1,4-isoprene) degradation by *Gordonia westfalica* Kb1 and *G. polyisoprenivorans* VH2. This extracellular enzyme functions as a radical scavenger enzyme and performs the cleavage of poly(*cis*-1,4-isoprene) when harmful reactive oxygen species of superoxide anions are present in increased concentrations. The formation of SodA is then induced while growing on rubber. Moreover, it has been found that a suspension of *sodA gene* in strain *G. polyisoprenivorans* VH2 results in retarded growth on rubber but not if the substrate is succinate. But no secretory signal peptide was found to be present in the amino acid sequence. Polyisoprene materials have a tendency to get auto-oxidized in atmospheric oxygen and ozone, generating ROS. In addition, the scission of the double bonds in polymeric isoprene backbone by rubber-degrading bacteria was reported, and a cleavage of rubber involving radicals is possible. The involvement of a secreted extracellular superoxide dismutase in biodegradation of rubber was reported in *G. polyisoprenivorans* strain VH2 (SodA; GPOL_c03380) (Hiessl et al. 2012).

21.6.5 Laccase and Manganese Peroxidase

Both these enzymes are primarily reported to be found in different fungi species capable of degrading rubber, like white-rot fungi, *Penicillium chrysogenum*, *Rhodotorula mucilaginosa*, *Alternaria alternata*, *Fusarium solani*, etc. (Nayanashree and Thippeswamy 2015a).

There are also reports of *Bacillus subtilis* and *B. pumilus* to degrade rubber and show laccase and manganese peroxidase activity. But little advancement on these discoveries has been gone through to explore the features and the other biochemical processes (Nayanashree and Thippeswamy 2015b; Sato et al. 2001).

21.6.6 Different Pathways of Rubber Degradation

The NR-degradation process can be categorized into five major phases:

1. Rubber oxygenase biosynthesis by rubber-degrading bacteria growing on rubber and extracellular oxidative cleavage of polyisoprene chains
2. Uptake of the resultant oligo-isoprenes into the bacterial cells
3. Intracellular β-oxidation of the imported oligo-isoprenes
4. Metabolism of acetyl-CoA and propionyl-CoA
5. Anaplerotic reactions and gluconeogenesis

21.6.6.1 Rubber Oxygenase Biosynthesis by Rubber-Degrading Bacteria Growing on Rubber and Extracellular Oxidative Cleavage of Polyisoprene Chains

Rubber Oxidation by Gram-Negative Bacteria

For the sake of metabolism and easy uptake by the bacteria using rubber as the sole source of carbon and energy for growth, the water-insoluble high-molecular-weight polyisoprene molecules are required to be extracellularly cleaved into low-molecular-weight products. Studies show that gram-negative organisms like *Steroidobacter cummioxidans* strain 35Y secrete two extracellular rubber-degrading enzymes, i.e., rubber oxygenases, RoxA and RoxB (Sharma et al. 2018). The rubber gets cleaved into a mixture of C_{20} and higher oligo-isoprenoids in an *endo*-type fashion by the RoxB, resulting in higher number of chain ends. On the contrary, the oligo-isoprenoids derived from RoxB cleavage and the intact polyisoprene backbone of rubber get cleaved in an *exo*-type manner to the tri-isoprenoid ODTD by the RoxA. Being comparatively smaller than polyisoprene, ODTD can be easily taken up by the rubber-degrading bacterial cells directly or after oxidation of the terminal aldehyde group to a carboxylic acid group (Sharma et al. 2018).

Rubber Oxidation by Gram-Positive Bacteria

Gram-positive rubber-degrading actinomycetes like *G. polyisoprenivorans*, *N. nova*, *Streptomyces*, and *Rhodococcus rhodochrous* secrete Latex clearing protein (Lcp) (see Table 21.1). Lcps produce a heterogenous end product of C_{20}, C_{25}, C_{30}, and higher oligo-isoprenoids (Birke et al. 2017). It has been found that this protein is not only required for latex-clear-zone formation but also essential for the growth of adhesive bacteria on rubber (Hiessl et al. 2012). After the first oxidative cleavage of rubber, the resultant low-molecular-weight aldehyde intermediates need to get further extracellularly oxidized to the corresponding acids before they are taken up

by the bacterial cells, because aldehydes are generally volatile toward oxidation reactions and are harmful to bacterial cells (Luo et al. 2014). A similar step is observed in *Streptomyces* sp. strain K30, and it is believed to be catalyzed via a heterodimeric molybdenum-dependent hydroxylase encoded by oxiAB, which is found to be located downstream of lcp. This oxiAB enables *Streptomyces* sp. strain K30 to oxidize aldehyde intermediates (Rose et al. 2005), and it is transcribed during rubber degradation but not during growth on glucose (Yikmis et al. 2008). Recently, in another study, *Streptomyces* sp. strain CFMR 7 was found to harbor genes for *oxiA* and *oxiB* (Nanthini et al. 2017). Other than the above-mentioned two *Streptomyces* sp. strains, K30 and CFMR 7, this heterodimeric hydroxylase is missing in genomes of other known rubber-degrading strains, for instance, in adhesively growing *G. polyisoprenivorans* VH2 and *Nocardia farcinica* strain IFM 10152 or clear-zone-forming *S. coelicolor* strain A3 (Hiessl et al. 2012). Genes encoding the required enzyme(s) for this step in the other known rubber-degrading strains are yet to be explored.

21.6.6.2 Uptake of the Resultant Oligo-Isoprenes into the Bacterial Cells

After the extracellular cleavage of polyisoprene by latex clearing protein (Lcp) or RoxA and RoxB, the formed oligo-isoprene derivatives have to be imported into the rubber-degrading bacterial cells. It is believed that for the import of extracellular cleavage products, the gram-positive and gram-negative bacteria employ different transport proteins because their cell wall and membranes largely differ and the Lcp-derived extracellular degraded products of polyisoprene by gram-positive bacteria are comparatively larger (C_{20}, C_{25}, and higher oligo-isoprenoids) than the ODTD formed by RoxA and RoxB of gram-negative bacteria (Sharma et al. 2018).

It has been found that both the genomes of extensively studied gram-positive rubber-degrading species *G. polyisoprenivorans* VH2 (Hiessl et al. 2012) and *N. nova* SH22a (Luo et al. 2014) harbor genes for multiple YrbE/Mce clusters which may serve the purpose of import of primary degradation products of rubber (Sharma et al. 2018). In *G. polyisoprenivorans* M71, a mutant was found with rubber-negative phenotype after insertion of a transposon in the gene encoding an Mce protein (GPOL_c27400) which is one of the five Mce clusters located in the genome. All these Mce clusters consist of two genes encoding YrbE proteins, followed by six genes encoding Mce proteins (MceA-F). They are reported to be found in all mycobacteria and in various other actinobacteria and are almost generally clustered in the same fashion. Bioinformatic studies revealed that Mce clusters encode a novel subfamily of ABC uptake transporters, wherein the *yrbE* gene products function as permeases and the *mce* gene products as substrate binding proteins. For the *mce4* locus of *R. jostii* strain RHA1 that encodes an ATP-dependent steroid uptake system, it was validated that these clusters encode importing transporters. The ATPase, necessary for providing the required energy for this system, was predicted through in silico studies. A homologue to this predicted ATPase is also present in the genome of *G. polyisoprenivorans* (GPOL_c37790). All these evidences steered to the proposal that the import of the low-molecular-weight intermediate products resulting from the extracellular primary degradation into cell is an ATP-dependent, Mce

protein-driven mechanism. Mce proteins are predicted to be extra-cytoplasmatic or tethered to the membrane, and they could function as substrate-binding proteins and may mediate the movement of the intermediates across the cell wall (Hiessl et al. 2012). The YrbE/Mce type was not found to be present in the gram-negative rubber degraders. Gram-negative bacteria like *S. cummioxidans* strain 35Y, therefore, have adopted a different transport mechanism (Sharma et al. 2018). A novel ABC transport system for intermembrane phospholipid trafficking, which is known as the maintenance of bacterial outer membrane lipid asymmetry (Mla) pathway responsible for maintaining lipid asymmetry, has been described for gram-negative bacteria. The presence of the six genes, i.e., *mlaA*, *mlaB*, *mlaC*, *mlaD*, *mlaE*, and *mlaF*, encoding for this transport system has confirmed the same assumption. Comparative genomics has shown the complete Mla pathway to be conserved in *S. rubberoxidans* strain 35Y (Sharma et al. 2018). Moreover, two families of substrate-specific outer membrane transporters, i.e., TonB-dependent outer membrane receptors (TBDRs) and long-chain fatty acid transporters (FadL family), which are involved in import of fatty acid are also possessed by gram-negative bacteria. Gram-negative bacteria are also found to harbor *fadD* encoding fatty acyl-coenzyme A (CoA) synthetase (FACS) for the uptake of exogenous fatty acids. This FadL and FACS are predicted to perform together as fatty acid transport machinery in gram-negative bacteria. Interestingly, the gram-negative rubber degrader *S. cummioxidans* strain 35Y was reported to harbor a single copy of putative *fadL* family transporter gene and 11 copies of putative *FACS* genes (Sharma et al. 2018).

21.6.6.3 β-Oxidation

β-Oxidation is an iterative biological process that is responsible for the breakdown of fatty acids as a part of lipid metabolism. The involvement of enzymes required in the process of β-oxidation was reported to be found in gram-positive rubber-degrading species like *S. coelicolor* 1A (Bode et al. 2001), *G. polyisoprenivorans* VH2 (Hiessl et al. 2012), and *N. nova* SH22a (Luo et al. 2014) involved in catabolism of rubber. Comparative genomics showed that the complete set of genes employed in the β-oxidation pathway is conserved even in gram-negative rubber degraders like *S. cummioxidans* strain 35Y (Sharma et al. 2018). There are several explanations why the organic acids formed after primary degradation of rubber might be metabolized via β-oxidation only, like inhibition of the degradation by acrylic acid, which is a β-oxidation-specific inhibitor, and occurrence of various intermediates that are possibly results of β-oxidation only, as observed in *S. coelicolor* A1; β-oxidation is also reported to be responsible for degradation of squalene and similar branched-chain alkanes, and coincidently, it has been found that squalene is also metabolized by *G. polyisoprenivorans* VH2 (Hiessl et al. 2012).

In the proposed rubber degradation pathway, an acyl-CoA synthetase converts the acid to an acyl-CoA thioester (37 candidate genes identified). The resulting product is further catabolized by an acyl-CoA dehydrogenase, where 47 homologous ORFs in the genome sequence were found. Next, two steps analogous to the dehydration of polyunsaturated fatty acids in the rubber degradation pathway are proposed. First, the 2,4-dienoyl-CoA reductase (GPOL_c19120) catalyzes the reduction of double

bonds at even-numbered positions, followed by an isomerization step. The latter reaction could be catalyzed by an enoyl-CoA isomerase. The last step of the first β-oxidation cycle is then catalyzed by the thiolase, for which 12 homologous genes in the genome have been identified. In addition, the FadA/FadB β-oxidation complex (GPOL_c05460/GPOL_c05470) is encoded in the genome. The next step is most likely catalyzed by one of the two α-methylacyl-CoA racemases (GPOL_c25180, GPOL_c36450) located in the genome. One Mcr (GPOL_c36450) was found to be essential for strain VH2, since a disruption led to a total loss of the ability to utilize rubber for growth. This Mcr catalyzes the conversion of (R)- into the (S)-stereoisomer. Only the (S)-isomer can serve as a substrate for the acyl-CoA dehydrogenase in the next β-oxidation cycle. Such an involvement of Mcr was also suggested for the β-oxidation of methyl-branched alkanes in *Mycobacterium* sp. strain P101. Successive cycles of β-oxidation have been proposed, with propionyl-CoA and acetyl-CoA being consecutively released, as was also suggested, for example, pristine (Hiessl et al. 2012). The considerable set of genes, which are putatively involved in β-oxidation, could be an example for why transposon-induced mutants, which are totally defective in rubber utilization and show transposon insertion in genes belonging to this pathway at the same time, did not occur (Hiessl et al. 2012). Other paralogous genes might be able to partially or even fully restore the function of a disrupted gene.

21.6.6.4 Metabolism of Acetyl-CoA and Propionyl-CoA

In order to stimulate the respiratory chain, the primary degradation products of the rubber are channeled into the citric acid cycle. The predicted rubber biodegradation pathway resembles the pathway of biological degradation of polyunsaturated fatty acid and generates two products, acetyl-CoA and propionyl-CoA. As predicted for *G. polyisoprenivorans*VH2, *N. nova* SH22a, and *S. cummioxidans* 35Y, acetyl-CoA enters directly into the citric acid cycle and/or the glyoxylate bypass, while propionyl-CoA is connected with the central metabolic processes via two pathways, the methylcitrate pathway and the methylmalonyl-CoA pathway, by a series of biochemical reactions to convert it into succinic acid (Luo et al. 2014). Being one of the most extensively studied rubber-degrading bacteria, *G. polyisoprenivorans* VH2 genome is reported to possess genes encoding a methylcitrate synthase (GPOL_c17040), an aconitate hydratase (GPOL_c23340), a methylcitrate dehydratase (GPOL_c17020), and a methylisocitrate lyase (GPOL_c17030), which are the main enzymes known to play role in the mentioned two pathways. The strain VH2 is found to show genetic loci coding for biotin-dependent carboxylases. Two such carboxylase systems were detected where the α- and β-subunits are located next to each other (GPOL_c11960/GPOL_c11970 and GPOL_c36900/GPOL_c36910), one α-subunit (GPOL_c17230) and four β-subunits (GPOL_c04190, GPOL_c17300, GPOL_c28880, GPOL_c38300) (Hiessl et al. 2012). Some of the gene products are predicted to play a role in fatty acid synthesis precursor generation, and some are putatively involved in the methylmalonyl pathway. Furthermore, methylmalonyl-CoA epimerase (GPOL_c18400) and methylmalonyl-CoA mutase (GPOL_c23510/GPOL_c23520)

encoding genes are located in the genome. The methylmalonyl-CoA mutase-catalyzed step of the methylmalonyl pathway is vitamin B12 dependent (Hiessl et al. 2012). Moreover, it has been found that transposon-induced mutants due to disruption of the neighborhood of genes putatively involved in cobalamin biosynthesis showed impaired growth on polyisoprene substrates, implying to the possibility that the methylmalonyl pathway serves an important role in rubber degradation. The methylmalonyl pathway is also reported to be found in other rubber-degrading strains of *N. farcinica*, *S. coelicolor*, *A. mirum*, and *S. flavogriseus*, but the methylcitrate cycle is found to be missing in these bacteria.

21.6.6.5 Anaplerotic Reactions and Gluconeogenesis

When there is scarcity or absence of sugars as the carbon and energy source, rubber-degrading bacteria employ the conversion of intermediates of TCA cycle or the glyoxylate bypass to phosphoenol pyruvate (PEP). Based on genome analysis, the entire sets of genes involved in Krebs cycle and glyoxylate bypass, orthologs of enzymes, including malic enzyme and PEP synthase were found to be conserved in rubber-degrading bacteria like *S. cummioxidans* 35Y. Moreover, phosphoenol pyruvate serves as a substrate for several enzymatic reactions like those taking part in significant metabolic processes, such as gluconeogenesis, glyceroneogenesis, amino acid synthesis, and anaplerosis of the Krebs cycle or glyoxylate cycle. Phosphoenolpyruvate carboxykinase (PEPCK) is the major enzyme of this step and catalyzes the guanosine or adenosine mononucleotide-dependent reversible conversion of phosphoenol pyruvate and oxaloacetic acid. In many bacteria, phosphoenolpyruvate carboxykinase plays an essential role for their growth or survival when grown on organic acids as substrate for sole source of carbon and energy. The gram-negative bacterium *S. cummioxidans* strain 35Y is reported to harbor one gene coding for a putative PEP carboxykinase (Sharma et al. 2018) (Fig. 21.3).

21.7 Conclusion

With the increase in usage of rubber around the world, there has been consequent increase in the amount of rubber wastes. The discovery of different rubber-degrading microorganisms and their genes responsible for the enzymes to degrade different forms of rubber has lead scientists to explore different strategies to efficiently degrade rubber and rubber-generated wastes. Degradation by cultivation of microbial cells on the rubber as a source of carbon, energy, and/or substrate and direct employment of the enzymes in vitro have proved to be successful in numerous cases. The by-products generated have been found useful and are used commercially. Alternative measures will certainly take the dimension of dealing with bioremediation of rubber wastes into new heights. Oxidative cleavage of rubbers by various actinobacteria or by their secreted extracellular enzymes has been studied, and the mode of biodegradation has been explored to develop the current strategies being employed.

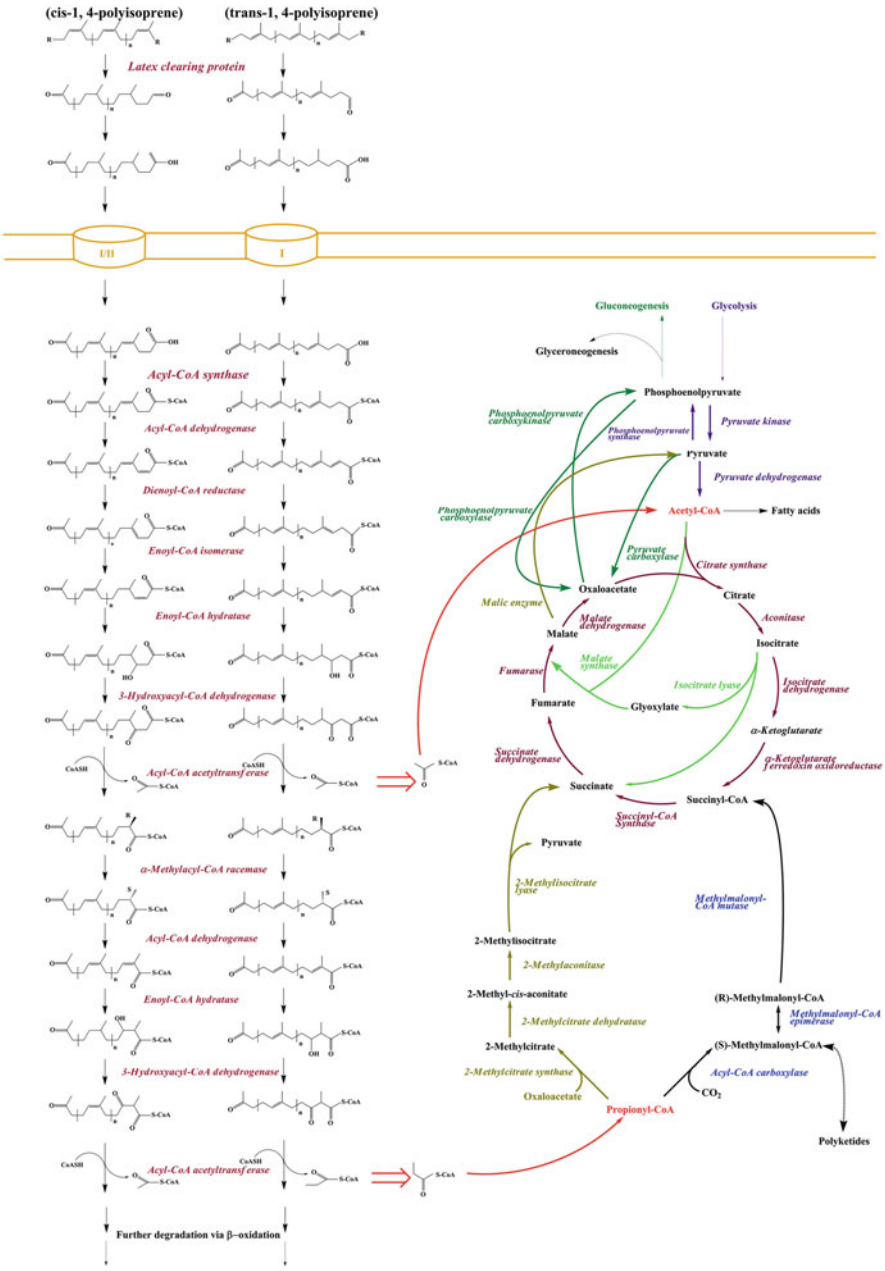


Fig. 21.3 The process of biodegradation of polyisoprene starting from extracellular cleavage to intracellular metabolic process. (*I*) represents the gram-positive machinery to import the oligo-isoprenoids formed, while (*II*) represents gram negative

References

Airey GD, Rahman MM, Collop AC (2003) Absorption of bitumen into crumb rubber using the basket drainage method. Int J Pavement Eng 4:105–119. https://doi.org/10.1080/1029843032000158879

Anonymous (2011) Malaysian rubber board. Sungei Buloh, Selangor, p 47000

Anonymous (2013) Complete information on area and production of rubber in India. In: Essay on Science Laboratory

Berekaa MM, Linos A, Reichelt R, Keller U, Steinbüchel A (2000) Effect of pretreatment of rubber material on its biodegradability by various rubber degrading bacteria. FEMS Microbiol Lett 184 (2):199–206. https://doi.org/10.1016/S0378-1097(00)00048-3

Birke J, Jendrossek D (2014) Rubber oxygenase and latex clearing protein cleave rubber to different products and use different cleavage mechanisms. Appl Environ Microbiol 80:5012–5020. https://doi.org/10.1128/AEM.01271-14

Birke J, Röther W, Jendrossek D (2015) Latex clearing protein (Lcp) of *Streptomyces* sp. strain K30 is a b-type cytochrome and differs from rubber oxygenase A (RoxA) in its biophysical properties. Appl Environ Microbiol 81:3793–3799. https://doi.org/10.1128/AEM.00275-15

Birke J, Hambsch N, Schmitt G, Altenbuchner J, Jendrossek D (2012) Phe317 is essential for rubber Oxygenase RoxA activity. Appl Environ Microbiol 78(22):7876–7883. https://doi.org/10.1128/AEM.02385-12

Birke J, Röther W, Jendrossek D (2017) RoxB is a novel type of rubber oxygenase that combines properties of rubber oxygenase RoxA and latex clearing protein (Lcp). Appl Environ Microbiol 83:e00721–e00717. https://doi.org/10.1128/AEM.00721-17

Birke J, Rother W, Jendrossek D (2018) *Rhizobacter gummiphilus* NS21 has two rubber oxygenases (RoxA and RoxB) acting synergistically in rubber utilization. Appl Microbiol Biotechnol 102(23):10245–10257. https://doi.org/10.1007/s00253-018-9341-6

Bode HB, Kerkhoff K, Jendrossek D (2001) Bacterial degradation of natural and synthetic rubber. Biomacromolecules 2(1):295–303. https://doi.org/10.1021/bm005638h

Bode HB, Zeeck A, Pluckhahn K, Jendrossek D (2000) Physiological and chemical investigations into microbial degradation of synthetic poly (cis-1,4-isoprene). Appl Environ Microbiol 66:3680–3685. https://doi.org/10.1128/AEM.66.9.3680-3685.2000

Borel M, Kergomard A, Renard MF (1982) Degradation of natural rubber by Fungi imperfecti. Agric Biol Chem 46:877–8781. https://doi.org/10.1080/00021369.1982.10865189

Bosco F, Antonioli D, Casale A, Gianotti V, Mollea C, Laus M, Malucelli G (2018) Biodegradation of unvulcanized natural rubber by microorganisms isolated from soil and rubber surface: a preliminary study. Biorem J 22:1–10. https://doi.org/10.1080/10889868.2018.1476454

Braaz R, Armbruster W, Jendrossek D (2005) Heme-dependent rubber oxygenase RoxA of *Xanthomonas* sp. cleaves the carbon backbone of poly(cis-1,4-isoprene) by a dioxygenase mechanism. Appl Environ Microbiol 71(5):2473–2478. https://doi.org/10.1128/AEM.71.5.2473-2478.2005

Braaz R, Fischer P, Jendrossek D (2004) Novel type of heme-dependent oxygenase catalyzes oxidative cleavage of rubber (poly-cis-1,4-isoprene). Appl Environ Microbiol 70 (12):7388–7395. https://doi.org/10.1128/AEM.70.12.7388-7395.2004

Bredberg K, Christiansson M, Stenberg B, Holst O (2000) Biotechnological processes for recycling of rubber products. In: Koyama T, Steinbüchel A (eds) Biopolymers, vol 2. Wiley-VCH, Verlag GmbH, Weinheim, pp 361–375

Broker D, Dietz D, Matthias Arenskotter M, Steinbüchel A (2008) The genomes of the non-clear-zone-forming and natural-rubber-degrading species *Gordonia polyisoprenivorans* and *Gordonia westfalica* harbour genes expressing Lcp activity in *Streptomyces* strains. Appl Environ Microbiol 74:2288–2297. https://doi.org/10.1128/AEM.02145-07

Calmon A, Dusserre-Bresson L, Bellon-Maurel V, Feuilloley P, Silvestre F (2000) An automated test for measuring polymer biodegradation. Chemosphere 41:645–651

Christinasson M, Stenberg B, Wallenberg LR, Holst O (2000) Toxic additives- a problem for microbial waste rubber desulphurization. Res Environ Biotechnol 3(1):11–21

De Vries O (1928) Zersetzung von Kautschuk-Kohlenwasserstoff durch Pilze. Zentralbl. Bakteriol. Parasitenkd. Inf Dent 74:22–24

Enoki M, Doi Y, Iwata T (2003) Oxidative degradation of *cis*- and *trans*-1,4-polyisoprenes and vulcanized natural rubber with enzyme-mediator systems. Biomacromolecules 4(2):314–320. https://doi.org/10.1021/bm025678g

Gallert C (2000) Degradation of latex and of natural rubber by *Streptomyces* strain La 7. Syst Appl Microbiol 23(3):433–441. https://doi.org/10.1016/s0723-2020(00)80075-2

Gawel I, Pilat J, Radziszewski P, Kowalski KJ, Krol JB (2011) Rubber modified bitumen. In: Polymer modified bitumen, pp 72–97. https://doi.org/10.1533/9780857093721.1.72

Hambsch N, Schmitt G, Jendrossek D (2010) Development of a homologous expression system for rubber oxygenase RoxA from *Xanthomonas* sp. J Appl Microbiol 109(3):1067–1075. https://doi.org/10.1111/j.1365-2672.2010.04732.x

Hapuarachchi SNS, Kariyapper SR, Gunawardana MBDMD, Egodage S, Ariyadasa TU (2016) Biodegradation of natural rubber latex by a novel bacterial species isolated from soil. In: Moratuwa engineering research conference (MerCon). https://doi.org/10.1109/mercon.2016.7480156

Hiessl S, Böse D, Oetermann S, Eggers J, Pietruszka J, Steinbüchel A (2014) Latex clearing protein—an oxygenase cleaving poly (cis-1,4-isoprene) rubber at the cis double bonds. Appl Environ Microbiol 80(17):5231–5240. https://doi.org/10.1128/AEM.01502-14

Hiessl S, Schuldes J, Thurmer A, Halbsguth T, Broker D, Angelov A, Liebl W, Daniel R, Steinbuchel A (2012) Involvement of two latex-clearing proteins during rubber degradation and insights into the subsequent degradation pathway revealed by the genome sequence of *Gordonia polyisoprenivorans* strain VH2. Appl Environ Microbiol 78(8):2874–2887. https://doi.org/10.1128/AEM.07969-11

Ibrahim E, Arenskotter M, Luftmann H, Steinbuchel A (2006) Identification of poly (cis-1,4-isoprene) degradation intermediates during growth of moderately thermophilic actinomycetes on rubber and cloning of a functional *lcp* homologue from *Nocardia farcinica* strain E1. Appl Environ Microbiol 72:3375–3382. https://doi.org/10.1128/AEM.72.5.3375-3382.2006

Ilcu L, Röther W, Birke J, Brausemann A, Einsle O, Jendrossek D (2017) Structural and functional analysis of latex clearing protein (Lcp) provides insight into the enzymatic cleavage of rubber. Sci Rep 7:6179. https://doi.org/10.1038/s41598-017-05268-2

Imai S, Ichikawa K, Muramatsu Y, Kasai D, Masai E, Fukuda M (2011) Isolation and characterization of *Streptomyces*, *Actinoplanes*, and *Methylibium* strains that are involved in degradation of natural rubber and synthetic poly (cis-1,4-isoprene). Enzym Microb Technol 49:526–531. https://doi.org/10.1016/j.enzmictec.2011.05.014

Imai S, Yoshida R, Endo Y, Fukunaga Y, Yamazoe A, Kasai D, Masai E, Fukuda M (2013) *Rhizobacter gummiphilus* sp. nov., a rubber degrading bacterium isolated from the soil of a botanical garden in Japan. J Gen Appl Microbiol 59(3):199–205. https://doi.org/10.2323/jgam.59.199

Jendrossek D, Birke J (2019) Rubber Oxygenases. Appl Microbiol Biotechnol 103(1):125–142. https://doi.org/10.1007/s00253-018-9453-z

Jendrossek D, Tomasi G, Kroppenstedt RM (1997) Bacterial degradation of natural rubber: a privilege of actinomycetes? FEMS Microbiol Lett 150(2):179–188. https://doi.org/10.1016/S0378-1097(97)00072-4

Kalinenko VO (1938) The role of actinomyces and bacteria in decomposing rubber. Mikrobiologiia (USSR) 17:119–128

Kasai D, Imai S, Asano S, Tabata M, Iijima S, Kamimura N, Masai E, Fukuda M (2017) Identification of natural rubber degradation gene in *Rhizobacter gummiphilus* NS21. Biosci Biotechnol Biochem 81(3):614–620. https://doi.org/10.1080/09168451.2016.1263147

Kwiatkowska D, Zyska BJ, Zankowicz LP (1980) Microbiological deterioration of natural rubber sheet by soil microorganisms. Biodeterioration 4:135–141

Linh DV, Huong NL, Tabata M, Imai S, Iijima S, Kasai D, Anh TK, Fukuda M (2017) Characterization and functional expression of a rubber degradation gene of a *Nocardia* degrader from a rubber processing factory. J Biosci Bioeng 123(4):412–418. https://doi.org/10.1016/j.jbiosc.2016.11.012

Linos A, Berekaa MM, Reichelt R, Keller U, Schmitt J, Flemming HC, Kroppenstedt RM, Steinbuchel A (2000a) Biodegradation of poly(cis-1,4-polyisoprene) rubbers by distinct actinomycetes: microbial strategies and detailed surface analysis. Appl Environ Microbiol 66 (4):1639–1645. https://doi.org/10.1128/aem.66.4.1639-1645.2000

Linos A, Berekaa MM, Steinbuchel A, Kim KK, Sproer C, Kroppenstedt RM (2002) *Gordonia westfalica* sp. nov., a novel rubber-degrading actinomycete. Int J Syst Evol Microbiol 52 (Pt 4):1133–1139. https://doi.org/10.1099/00207713-52-4-1133

Linos A, Reichelt R, Keller U, Steinbuchel A (2000b) A gram-negative bacterium, identified as *Pseudomonas aeruginosa* AL98, is a potent degrader of natural rubber and synthetic cis-1, 4-polyisoprene. FEMS Microbiol Lett 182(1):155–161. https://doi.org/10.1111/j.1574-6968.2000.tb08890.x

Linos A, Steinbuchel A, Sproer C, Kroppenstedt RM (1999) *Gordonia polyisoprenivorans* sp. nov., a rubber-degrading actinomycete isolated from an automobile tyre. Int J Syst Bacteriol 49 (Pt 4):1785–1791. https://doi.org/10.1099/00207713-49-4-1785

Luo Q, Hiessl S, Poehlein A, Daniel R, Steinbuchel A (2014) Insights into the microbial degradation of rubber and gutta-percha by analysis of the complete genome of *Nocardia nova* SH22a. Appl Environ Microbiol 80(13):3895–3907. https://doi.org/10.1128/AEM.00473-14

Nanthini J, Ong SY, Sudesh K (2017) Identification of three homologous latex-clearing protein (*lcp*) genes from the genome of *Streptomyces* sp. CFMR 7. Gene 628:146–155

Nanthini J, Sudesh K (2017) Biodegradation of natural rubber and natural rubber products by *Streptomyces* sp. strain CFMR 7. J Polym Environ 25(3):606–616. https://doi.org/10.1007/s10924-016-0840-1

Nayanashree G, Thippeswamy B (2013) Natural rubber degradation by *Aspergillus niger* and *Penicillium* sp. Int J Recent Sci Res 4(9):1337–1341

Nayanashree G, Thippeswamy B (2015a) Natural rubber degradation by laccase and manganese peroxidase enzymes of *Penicillium chrysogenum*. Int J Environ Sci Technol 12(8):2665–2672

Nayanashree G, Thippeswamy B (2015b) Biodegradation of natural rubber by Laccase and manganese peroxidase enzyme of *Bacillus subtilis*. Environ Process 2:761. https://doi.org/10.1007/s40710-015-0118-y

Oetermann S, Vivod R, Hiessl S, Hogeback J, Holtkamp M, Karst U, Steinbuchel A (2018) Histidine at position-195 is essential for association of heme-b in Lcp1VH2. Earth Syst Environ 2:5–14. https://doi.org/10.1007/s41748-018-0041-2

Pagga U, Schefer A, Muller RJ, Pantke M (2001) Determination of the aerobic biodegradability of polymeric material in aquatic batch tests. Chemosphere 42:319–331

Rose K, Steinbuchel A (2002) Construction and intergeneric conjugative transfer of a pSG5-based cosmid vector from *Escherichia coli* to the polyisoprene rubber degrading strain *Micromonospora aurantiaca* W2b. FEMS Microbiol Lett 211:129–132. https://doi.org/10.1111/j.1574-6968.2002.tb11214.x

Rose K, Tenberge KB, Steinbüchel A (2005) Identification and characterization of genes from Streptomyces sp. strain K30 responsible for clear zone formation on natural rubber latex and poly(cis-1,4-isoprene) rubber degradation. Biomacromolecules 6(1):180–188. https://doi.org/10.1021/bm0496110

Rother W, Austen S, Birke J, Jendrossek D (2016) Molecular insights in the cleavage of rubber by the latex-clearing-protein (Lcp) of *Streptomyces* sp. strain K30. Appl Environ Microbiol 82:6593–6602. https://doi.org/10.1128/AEM.02176-16

Sato S, Watanabe T, Honda Y, Kuwahara M (2001) Degradability of natural and synthetic polymers by the white rot fungus, *Ceriporiopsis subvermispora*. https://repository.kulib.kyoto-u.ac.jp/dspace/handle/2433/53129

Sato S, Honda Y, Kuwahara M, Watanabe T (2003) Degradation of vulcanized and nonvulcanized polyisoprene rubbers by lipid peroxidation catalyzed by oxidative enzymes and transition metals. Biomacromolecules 4:321–329

Schade AL (1937) Observations on a *Monascus* isolated from rubber. Mycologia 29:295–302

Schmitt G, Seiffert G, Kroneck PMH, Braaz R, Jendrossek D (2010) Spectroscopic properties of rubber oxygenase RoxA from *Xanthomonas* sp., a new type of dihaem dioxygenase. Microbiology (Reading, England) 156:2537–2548. https://doi.org/10.1099/mic.0.038992-0

Seidel J, Schmitt G, Hoffmann M, Jendrossek D, Einsle O (2013) Structure of the processive rubber oxygenase RoxA from *Xanthomonas* sp. Proc Natl Acad Sci U S A 110:13833–13838. https://doi.org/10.1073/pnas.1305560110

Shah AA, Hasan F, Shah Z, Kanwal N (2013) Biodegradation of natural and synthetic rubbers: a review. Int Biodeterior Biodegrad 83:145–157. https://doi.org/10.1016/j.ibiod.2013.05.004

Shah AA, Hasan F, Shah Z, Mutiullah Z, Hameed A (2012) Degradation of polyisoprene rubber by newly isolated *Bacillus* sp. AF-666 from soil. Appl Biochem Microbiol 48:37–42

Sharma V, Siedenburg G, Birke J, Mobeen F, Jendrossek D, Srivastava TP (2018) Metabolic and taxonomic insights into the Gram-negative natural rubber degrading bacterium *Steroidobacter cummioxidans* sp. nov., strain 35Y. PLoS One 13(5):e0197448. https://doi.org/10.1371/journal.pone.0197448

Sturm RNJ (1973) Biodegradability of non ionic surfactants: screening test for predicting rate and ultimate biodegradation. J Am Oil Chem Soc 50:159–167

Tsuchii A, Takeda K (1990) Rubber-degrading enzyme from a bacterial culture. Appl Environ Microbiol 56:269–274

Tsuchii A, Tokiwa Y (2001) Microbial degradation of tire rubber particles. Biotechnol Lett 23:963–969

Tsuchii A, Takeda K, Suzuki T, Tokiwa Y (1996) Colonization and degradation of rubber pieces by *Nocardia* sp. Biodegradation 7(1):41–48

The Hindu (2016) Turning waste tyre into 'green steel'. https://www.thehindu.com/business/Turning-waste-tyre-into-%E2%80%98green-steel%E2%80%99/article14518524.ece

Warneke S, Arenskötter M, Tenberge KB, Steinbüchel A (2007) Bacterial degradation of poly (trans-1,4-isoprene) (gutta percha). Microbiology 153:347–356. https://doi.org/10.1099/mic.0.2006/000109-0

Waste Management World (2010) Tyre recycling joint venture announced'. http://www.waste-management-world.com/index/display/article-display

Watcharakul S, Röther W, Birke J, Umsakul K, Hodgson B, Jendrossek D (2016) Biochemical and spectroscopic characterization of purified latex clearing protein (Lcp) from newly isolated rubber degrading *Rhodococcus rhodochrous* strain RPK1 reveals novel properties of Lcp. BMC Microbiol 16:92. https://doi.org/10.1186/s12866-016-0703-x

Williams GR (1982) The breakdown of rubber polymers by microorganisms. Int Biodeterioration Bull 18:31–36

Yikmis M, Arenskotter M, Rose K, Lange N, Wernsmann H, Wiefel L, Steinbuchel A (2008) Secretion & transcriptional regulation of the latex-clearing protein, Lcp, by the rubber-degrading bacterium *Streptomyces* strain K30. Appl Environ Microbiol 74(17):5373–5382. https://doi.org/10.1128/AEM.01001-08

Yikmis M, Steinbuchel A (2012) Historical and recent achievements in the field of microbial degradation of natural and synthetic rubber. Appl Environ Microbiol 78(13):4543–4551. https://doi.org/10.1128/AEM.00001-12

Zabaniotou AA, Stavropoulos G (2003) Pyrolysis of used automobile tires and residual char utilization. J Anal Appl Pyrolysis 70(2):711–722. https://doi.org/10.1016/S0165-2370(03)00042-1