J. Aravind
M. Kamaraj
M. Prashanthi Devi
S. Rajakumar *Editors*

Strategies and Tools for Pollutant Mitigation

Avenues to a Cleaner Environment

Springer

Strategies and Tools for Pollutant Mitigation

J. Aravind • M. Kamaraj • M. Prashanthi Devi
S. Rajakumar
Editors

Strategies and Tools for Pollutant Mitigation

Avenues to a Cleaner Environment

Springer

Editors
J. Aravind
Department of Civil Engineering-
Environmental Engineering
Dhirajlal Gandhi College of Technology
Omalur, Tamil Nadu, India

M. Prashanthi Devi
Department of Environmental
Management
Bharathidasan University
Tiruchirappalli, Tamil Nadu, India

M. Kamaraj
Department of Biotechnology
College of Biological and Chemical
Engineering
Addis Ababa Science and Technology
University
Addis Ababa, Ethiopia

S. Rajakumar
Department of Marine Biotechnology
Bharathidasan University
Tiruchirappalli, Tamil Nadu, India

ISBN 978-3-030-63574-9 ISBN 978-3-030-63575-6 (eBook)
https://doi.org/10.1007/978-3-030-63575-6

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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

Contents

Part I Reviews on Energy and Environment

Part II Cleaner Technologies on Mitigation of Organic and Inorganic Pollutants

Part III Impact and Risk Assessments

Part I
Reviews on Energy and Environment

Chapter 1
A Review on Production, Properties, and Applications of Microbial Surfactants as a Promising Biomolecule for Environmental Applications

Kidist Mulugeta, M. Kamaraj, Mesfin Tafesse, and J. Aravind

Abstract Biosurfactants are molecular compounds of the surface-active group-containing hydrophobic and hydrophilic moieties in one single molecule that dispenses themselves among two immiscible fluids, and these are usually harnessed with the aid of microorganisms and are considered environmentally friendly. The production of microbial biosurfactants is regarded as one of the key developments in the twenty-first century due to its importance and implementations in the industrial process. The biosurfactants have several benefits over chemical surfactants, such as a higher degree of biodegradability, foaming, selectivity, and specific activity. Microbial surfactants can be labeled as "green" because of their less toxicity, coupled with better environmental compatibility, working amicably on a wide range of physicochemical environments, and also contribution to limiting the greenhouse effect. Hence, biosurfactants are expected to be promising alternatives for synthetic surfactants for industrial, environmental, and domestic applications soon. Notably, the biosurfactants exhibit competent and feasible applications in different environmental avenue, such as bioremediation, oil, and petroleum recovery, expelling pollutants, wastewater treatment, etc. Although possessing potential applications with diverse characteristics and properties over synthetic surfactants, biosurfactants are not able to compete with synthetic molecules due to their limitations, such as high production cost, less production yield, bioreactor design and its related complications, inefficient downstream processes, limited availability of substrate, etc. These precincts can overcome with the development of better reactor design, product recovery technology, ecologically and environmentally favored media, and decreased

---

K. Mulugeta · M. Kamaraj (✉) · M. Tafesse
Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

Bioprocess and Biotechnology Center of Excellence, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

J. Aravind
Department of Civil Engineering- Environmental Engineering, Dhirajlal Gandhi College of Technology, Omalur, Tamil Nadu, India

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_1

production cost. The current review emphasizes the overall view of biosurfactants, their properties, classification, source, production, and characterization along with its environmental applications.

Keywords Biosurfactants · Environmental applications · Sustainable · Properties · Screening · Characterization

1 Introduction

Surfactants or surface-active agents are amphipathic molecules and can form micelles, as they are a group of molecular compounds with hydrophilic and hydrophobic moieties and have the potential to distribute themselves between two immiscible fluids. This property of surfactant tends to reduce surface tension or interfacial tension (ST/IFT) and cause the solubility of nonpolar compounds in polar solvents (Udoh and Vinogradov 2019). The nonpolar moiety is a hydrocarbon chain, whereas the polar moiety can be ionic (anionic or cationic), amphoteric, or nonionic (Pacwa-Płociniczak et al. 2011) as illustrated in the inset of Fig. 1.1.

Currently, as a result of synthetic surfactants nature, it can be a threat to the ecosystem. The biosurfactants can overcome this once it takes over a significant role

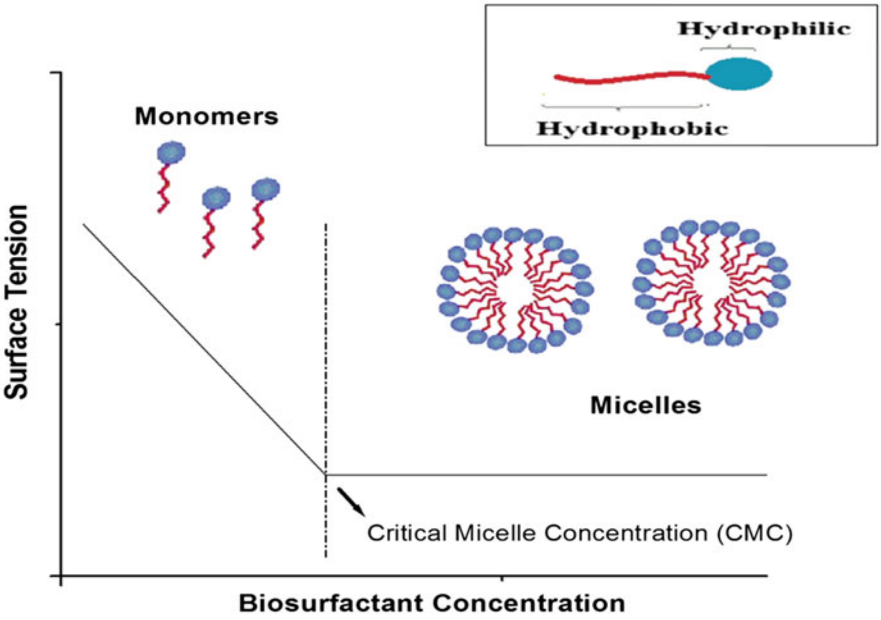


Fig. 1.1 Relationship between surface tension and biosurfactant concentration and formation of micelles. Inset shows the hydrophilic head and hydrophobic tail of surfactant monomer

in the surfactant creation. To replace synthetic surfactants by biodegradable, sustainable green energies and renewable surfactants, the development of new strategies is a unique obstacle for humans and environmental protection agencies (Ajala et al. 2015). This predicament triggers the discovery of an equally potential and effective but environmentally friendly alternative to synthetic surfactants: a class of surfactants derived from microorganisms, known as “biosurfactants.” They are similarly diverse within the structure and function and are gaining priority because of their eco-friendly and biodegradability attributes (Shekhar et al. 2015). Biosurfactants are secondary metabolites and produced in the stationary phase of the microbial growth curve (Yan et al. 2012). Like synthetic surfactants, biosurfactants have the ability to lowering interfacial and surface tension. The molecular mass of the biosurfactants ranges from 500 to 1500 daltons, and their critical micelle concentration (CMC) differs from 1 to 200 mgL^{-1}. Another crucial property of biosurfactants is the hydrophilic-lipophilic balance (HLB) that specifies the value of hydrophobic and hydrophilic compounds in surface-active substances and also has an effect on the stability of an emulsion. The hydrophobic state is indicated by low HLB value with more nonpolar groups forming water-in-oil (W/O) emulsions. In comparison, a hydrophilic state marked by a high HLB means more polar groups and prefer for oil-in-water (O/W) emulsion. HLB numbers are in range as follows for different applications (Tadros 2013): 3–6 for W/O emulsifier, 7–9 for a wetting agent, 12–15 for detergent, and 15–18 for solubilizer.

Many aerophilic microbes reported for their production of biosurfactants only in the presence of carbon sources such as fats, oil, and hydrocarbons in their media. The most known biosurfactant producer are bacteria genera (*Bacillus*, *Pseudomonas*, and *Acinetobacter*), fungi genera (*Fusarium* spp. and *Aspergillus* spp.), and yeast genera (*Pseudozyma* and *Candida*). These surface-active compounds have benefits for biosurfactant-producing microorganisms, such as ensure exponential biomass increase, grow on water-immiscible substrates, exhibit antimicrobial activities against possible predators, and make them survive harsh environmental conditions (Silva et al. 2014).

The price of chemical or synthetic surfactants, such as sodium lauryl sulfate, tends to fall in the range of \$1–2/kg, and biosurfactants, such as amino acid-based surfactants, were \$3–4/kg. Sophorolipid is the most widely available and the cheapest microbial biosurfactant, and the sales price has recently been published to be at \$3–4/kg. Based on a growth rate of 3.5% per year, the global biosurfactant market is expected to reach 2.2 billion \$. In addition to this, due to the increasing demand from Africa, Latin America, and Asia, the global production is estimated to reach 476,512 tons that account for around 21% of the total manufacture (Roelants et al. 2018).

Before 2011, more than 200 patents were registered about the production of biosurfactants, with 35% of them on sophorolipids, 10% on mannosylerythritol lipids, and 50% of these patents on rhamnolipids (Vecino et al. 2017). Biosurfactants have high potential for its applications in industrial scale; however, the production and commercialization have not been enough (Varjani and Upasani

2017). The costs of processing/purification, raw materials, and low yields negatively impacted the production process. The cost of the raw materials accounts for 30% of the total production cost (Ahmad et al. 2016).

Hence, the microbial biosurfactants are a better alternative for the synthetic surfactants; there is a need to understand them for the effective use in different applications. The review highlights the informations about the microbial-based biosurfactants. More specifically, an overview of the physical and chemical properties, classification, source, production, characterization, and its applications is summarized.

2 Physical and Chemical Properties of Biosurfactants

2.1 *Surface and Interface Activity*

Surfactant has the ability in reducing surface tension and the interfacial tension, i.e., surface tension of the water is reduced from 72.75 mNm^{-1} to 35 mNm^{-1} (at 20 °C) and interfacial tension of water from 40 to 1 mNm^{-1} with the added surfactant. Surfactin produced from *B. subtilis* decreased the surface tension of water to 25 mNm^{-1} and interfacial tension of water to less than 1 mNm^{-1}. *Pseudomonas aeruginosa* produces rhamnolipid biosurfactants that reduced the water surface tension more effectively than many other known surfactants (Kim et al. 2015). The relationship between surface tension, biosurfactant concentration, and formation of micelles is depicted in Fig. 1.1.

2.2 *Tolerance, Toxicity, and Biodegradability*

Most of the biosurfactants and their surface activity can resist environmental factors, such as temperature, pH, and salt. The biosurfactant produced by *Arthrobacter protophormiae* is stable at a temperature of (30–100 °C) and pH (2–12) (Singh and Cameotra 2004). Lipopeptides, which are produced by *Bacillus subtilis*, are stable at NaCl concentrations of >15% and pH range of 4–12 (Cheng et al. 2016). Mutagenicity and toxicity of biosurfactants (*Pseudomonas aeruginosa*), when contrasted with that of synthetic surfactants, advocated the biosurfactant's least effects on this context (Flasz et al. 1998; Shah et al. 2016). Synthetic surfactants take more time to degrade than biosurfactants (Mohan et al. 2006). Rhamnolipid biosurfactants are readily biodegradable in both aerobic and anaerobic conditions. Synthetic surfactant, for example, Triton X-100, is partially biodegradable under aerobic conditions, and it does not degrade under anaerobic conditions (Hirata et al. 2009).

2.3 Emulsification and De-emulsification

Biosurfactants have a property to stabilize (emulsify) or destabilize (de-emulsify) the emulsion. The feature of biosurfactants as an emulsifying agent depends on the hydrocarbon structure. Biosurfactants can emulsify many hydrocarbons, such as kerosene, tridecane, tetradecane, hexadecane, diesel, benzene, and other hydrocarbons. Peele et al. (2016) tested the emulsifying activity of biosurfactant produced by a marine bacterium with different hydrocarbons, such as xylene, benzene, diesel, kerosene, and petrol. Ahmad et al. (2016) used the ANN method to optimize the growth condition of *Klebsiella* sp. FKOD36 to enhance lipase, and the best outcome under the ANN model arrived at an enhanced value of biosurfactant yield 31.67% and 31.68% emulsification index. Rocha e Silva et al. (2017) explored the use of biosurfactants from *Candida guilliermondii*, *Candida lipolytica*, *Candida sphaerica, Pseudomonas aeruginosa*, *Pseudomonas cepacia*, and *Bacillus* sp. as de-emulsifiers of petroleum derivatives and obtained promising results for the bacterial strains where recovery was 65% higher when compared with that of yeast's (35–40%).

2.4 Foam and Wet Property of Biosurfactants

Foams are colloidal systems with liquid or solid as the continuous phase and gas as the dispersed phase. Foams have two different structures that may occur in foams containing polyhedral cells and in spherical bubbles (Drenckhan and Hutzler 2015). The presence of oils and other particles increases the instability of foam due to the stabilization effect of surfactants (Belhaij and Al-Mahdy 2015). Jian et al. (2011) investigated the foam facility and stability of a biosurfactant named tea saponin. Tea saponin was able to produce foam, but its durability and foamability was accelerated when the synthetic surfactant was added. Wetting is the process that another fluid displaces the phase of a liquid from the surface of a solid. The wetting property of the surfactant indicates the ability of a liquid to cover the solid surface. The balance between cohesive and adhesive forces specified the degree of wettability of a surfactant. Cohesive force is the force that prevents the liquid drop from making contact with the solid surface and causing the droplet to ball up. The force between a liquid and a solid that allows the liquid drop to spread across the surface is called adhesive force (Lee et al. 2017; Liu et al. 2017).

3 Classification and Source of Biosurfactants

Biosurfactant, unlike chemical surfactants which are categorized based on the polarity of the functional group, is mainly classified based on their chemical composition and the origin of the microbes (Sharma et al. 2016). The summary of its classification is illustrated in Fig. 1.2.

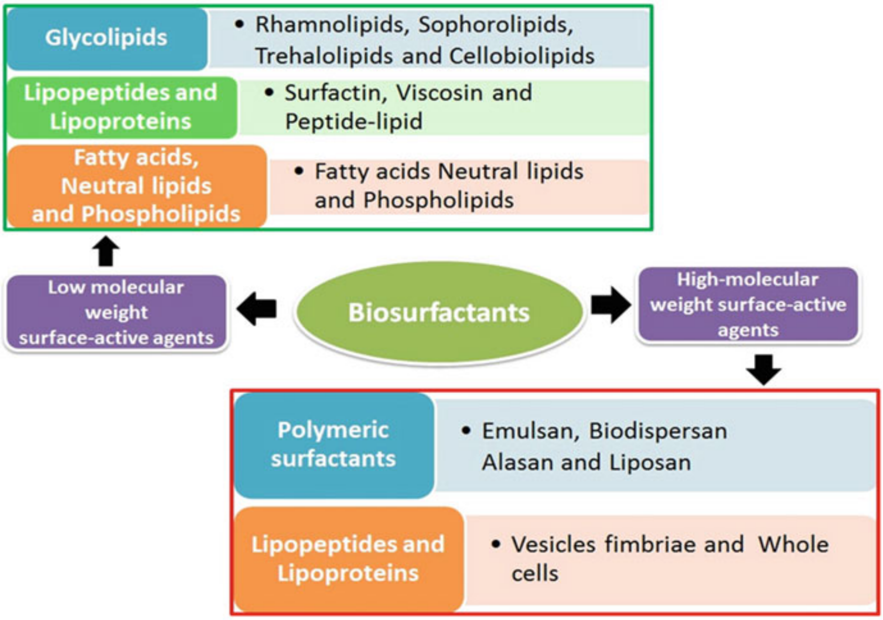


Fig. 1.2 Classification of biosurfactants

Glycolipids are made up of carbohydrate-lipid linkage, which forms a connection of either ether group or ester group. Sophorolipids, rhamnolipids, and trehalolipids are some examples of glycolipids (Rikalović et al. 2015). Sophorolipids are glycolipids that consist of a dimeric carbohydrate. A glycosidic bond is used to link sophorose with long-chain hydroxyl fatty acid. Yeasts produce them; i.e., *Candida bombicola* is the well-known type of yeast in the production of this kind of biosurfactant. A reduction in the surface tension of *n*-hexadecane and water from 40 to 5 mNm^{-1} was reported for these biosurfactants (Díaz de Rienzo et al. 2016; Jimenez-Penalver et al. 2016). Rhamnolipids are the glycolipids with rhamnose sugar linked to the units of hydroxydecanoic acid and known to be produced by *P. aeruginosa* (Rikalović et al. 2015). Trehalolipids are the glycolipids when disaccharide trehalose is connected at C-6 to mycolic. Trehalose lipids are produced by *Arthrobacter* sp. and *Rhodococcus erythropolis* (Sharma et al. 2016). Lipopeptides and lipoproteins are composed of the amino acid chain (peptide) linked to a fatty acid. The peptide may be either cyclic or linear. Surfactin is composed of unsaturated fat and a seven-amino-acid ring structure joined by lactone linkage. It reduces the surface tension of water from 72 to 27.9 mNm^{-1} (Nguyen and Gotz 2016). Fatty acids, neutral lipids, and phospholipids are components of cell structures and have surface activity (Satpute et al. 2010b). *Rhodococcus erythropolis* grown on n-alkane decreases the surface tension of water and produces phosphatidylethanolamine (Helfrich et al. 2015). The most known polymeric biosurfactants are liposan, emulsan, and alasan (Satpute et al. 2010b). Emulsan is an emulsifier for oil in water at concentrations as low as 0.001% to 0.01%. Liposan, synthesized by *C. lipolytica*, is an extracellular water-soluble emulsifier and composed of 17% proteins and 83% carbohydrates. Chakrabarti (2012) discusses the use of liposan as an emulsifier in the cosmetic industries and food. Particulate biosurfactants can form the extracellular membrane vesicles panel to produce a microemulsion, which plays a vital role in the alkane uptake by microbial cells.

3.1 Source of Biosurfactant

Biosurfactants are mainly produced by a diverse group of microorganisms such as bacteria, fungi, and yeast. Some of the biosurfactant-producing microorganisms are listed in Table 1.1**.**

4 Biosurfactant Production

In addition to the growth-dependent synthesis of biosurfactant, it may also occur by resting microbial cell systems. Various substrates, such as hydrophobic mixtures, hydrocarbons, solvents, chemicals, hydrophobic mixtures, waste products' oil wastes, vegetable oils, dairy products, and so on, are used for biosurfactant production (Bezza and Chirwa 2017; Ferreira et al. 2017; Gudina et al. 2015; Jimoh and Lin 2019a, b) (Figs. 1.3 and 1.4).

4.1 Sampling

Biosurfactant-producing microbes were isolated predominantly from water or soil samples contaminated with hydrophobic organic compounds such as refinery wastes (Batista et al. 2006). The marine environment is also considered a good source for sampling, and uncontaminated soils are less yielding than contaminated soils for the biosurfactant-producing microbes (Bodour et al. 2003).

4.2 Isolation

A pure culture is required for studying the properties of interest organism out of a mixed population; microbes, which show promise, are selected and enriched. Enrichment cultures that contain hydrophobic compounds (sole carbon source)

Table 1.1 List of biosurfactant-producing bacteria, fungi, and yeasts

Microorganisms		Biosurfactants	References
Bacteria	*Pseudomonas aeruginosa*	Rhamnolipids	Jadhav et al. (2011)
	Pseudomonas chlororaphis	Rhamnolipids	
	Pseudomonas fluorescens	Carbohydrate-lipid complex	Nerurkar et al. (2009)
	Acinetobacter spp.	Phospholipids	Kosaric (2001)
	Acinetobacter radioresistens	Alasan	de Lima et al. (2009)
	Bacillus subtilis	Surfactin	Arguelles-Arias et al. (2009)
	Bacillus amyloliquefaciens	Surfactin	
	Bacillus subtilis	Subtilisin	Sutyak et al. (2008)
	Bacillus subtilis	Lipopeptide	Felix et al. (2019)
	Bacillus spp.	Amino acids-lipids	Sutyak et al. (2008)
	Bacillus licheniformis	Peptide lipids	Begley et al. (2009)
	Debaryomyces polymorphus	Carbohydrate-lipid complex	Nerurkar et al. (2009)
	Leuconostoc mesenteroides	Viscosin	Banat et al. (2010)
	Serratia rubidaea	Rhamnolipids	Jadhav et al. (2011)
	Rhodotorula glutinis	Carbohydrate-protein complex	Oloke and Glick (2005)
	Halomonas eurihalina	Sulfated heteropolysaccharide	Gutierrez et al. (2007)
	Myroides	L-ornithine lipids	Maneera and Dikit (2007)
	Serratia marcescens	Serrawettin	Lai et al. (2009)
	R. graminis	Polyol lipids	Amaral et al. (2006)
	Rhodococcus erythropolis	Trehalose lipids	Muthusamy et al. (2008)
Fungi	*Candida apicola*	Sophorolipids	Felse et al. (2007)
	Candida bombicola	Sophorolipids	
	Candida antarctica	Sophorolipids	
	Candida botistae	Sophorolipids	
	Candida stellata	Sophorolipids	
	Candida riodocensis	Sophorolipids	
	Penicillium chrysogenum	Polyketide derivative	Gao et al. (2011)
	Penicillium chrysogenum	Monoketide derivative	Gao et al. (2011)
	Torulopsis bombicola	Sophorose lipid	Kim et al. (1997)
	Aspergillus ustus	Glycolipoprotein	Alejandro et al. (2011)
	Trichosporon ashii	Sophorolipid	Chandran and Das (2010)
Yeast	*Pseudozyma fusifornata*	Mannosylerythritol lipids	Morita et al. (2007)
	Pseudozyma parantarctica	Mannosylerythritol lipids	
	Pseudozyma tsukubaensis	Mannosylerythritol lipids	
	Saccharomyces cerevisiae	Mannano protein	Cameron et al. (1988)
	Kluyveromyces marxianus	Mannano protein	Lukondeh et al. (2003)

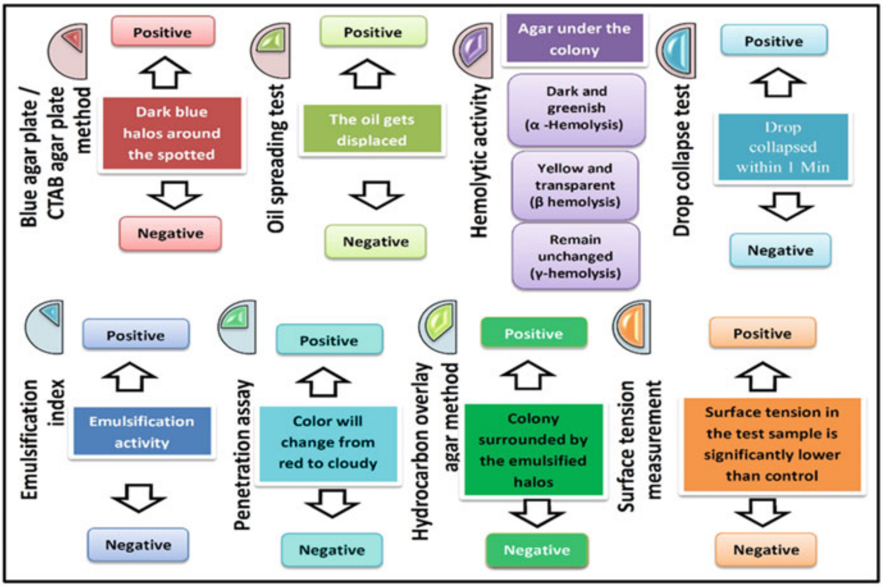


Fig. 1.3 Screening methods for biosurfactants

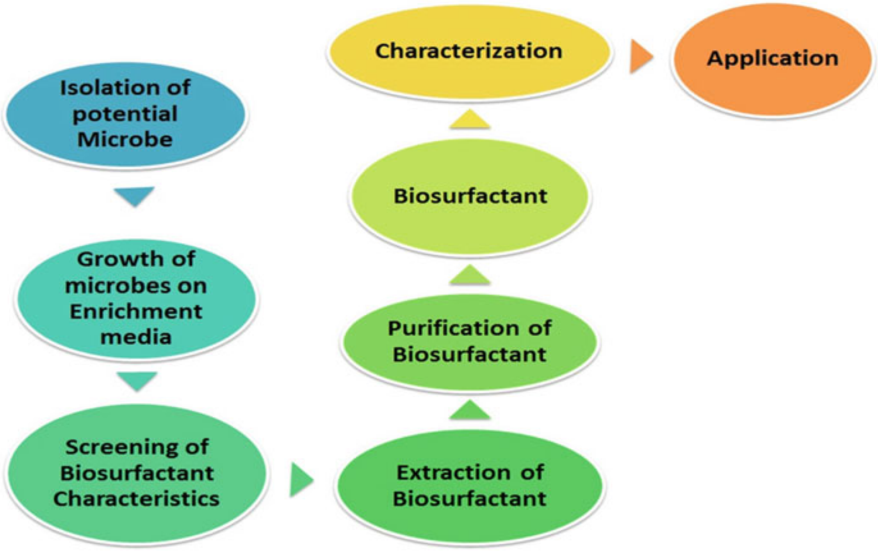


Fig. 1.4 Schematic presentation of a laboratory-limited biosurfactant production procedure: sampling to isolation to growth in MSM to the screening of extraction and purification of biosurfactants to the characterization of extracted biosurfactant

are generally used for the isolation of biosurfactant-producing microbes (Giani et al. 1997). It may be possible that the biosurfactant-producing microbial populations are present in the collected sample that is not enriched by the applied enrichment media (Bodour et al. 2003).

4.3 Screening Methods

Different methods are used for the screening of biosurfactant efficiency such as oil spreading test, hemolysis test, emulsification index, drop collapse method, blue agar plate method, and hydrocarbon overlay agar method (Samanta et al. 2012). Also, nowadays, computational modeling and structured screenings are used for the development of biosurfactants with high-value properties, and these factors play a crucial role in future research (Zhao et al. 2016).

4.4 Raw Materials for Biosurfactant Production

Several cheap waste materials have been used as substrates for biosurfactant production, thus bringing cost-effectiveness with much-needed waste management (Satpute et al. 2017). The use of industrial waste as a substrate for the production of biosurfactant in recent times paved the way not only for a cost-effective production process but also in establishing a sustainable and clean environment regarding the waste generated (Patil and Rao 2015).

Olive oil mill effluent (OOME) is a concentrated black liquor with a water-soluble portion of ripe olives and water used for the extraction of olive oil. OOME has polyphenols that cause adverse effects when disposed to the environment. However, it also contains sugars (20–80 gL^{-1}), nitrogen compounds (12–24 gL^{-1}), organic acids (5–15 gL^{-1}), and residual oil (0.3–5 gL^{-1}) (Santos et al. 2016a). Molasses is a by-product of beet and sugarcane processing, which are rich sole of carbon source. The high sugar content (48–56%) makes molasses the first choice for biosurfactant production. Joshi et al. (2008) used molasses to produce biosurfactants from strains of *Bacillus*. The dairy industry provides a different type of whey, such as whey waste, curd whey, lactic whey, and cheese whey. Lactic whey contains a high amount of lactose (approximately 75%). Whey disposal causes pollution problems, especially in countries that depend on a dairy economy (Helmy et al. 2011). Starchy waste was obtained from the potato processing industry. In addition to 80% water content, potato waste has fats (0.1%), carbohydrates (17%), and proteins (2%) and also trace elements, vitamins, and inorganic minerals (Helmy et al. 2011).

Meat processing industries are the major source of animal fat and tallow. Due to the lower degree of harm to health, vegetable oils gain a large part of the market than animal fat (Banat et al. 2014). Soap stocks or oil cakes are obtained from oilseed processing involving the use of chemicals with the refining of seed-based edible oils. Biosurfactant production achieves a greater yield when *C. apicola or C. antarctica* grow on soap stock and oil refinery wastes (Bednarski et al. 2004). Corn steep liquor is obtained from washing water and soaking of kernels and also fractioning into germen (oil) and starch, which contains 40% solid matter. It contains a low-fat content (0.9% to 1.2%), 21% to 45% proteins, approximately 8% ash (containing K^+, Ca^{2+}, Mg^{2+}, etc.), 20% to 26% lactic acid, and approximately 3% carbohydrates (Helmy et al. 2011).

4.5 Media Formulation for Biosurfactant Production

Statistical methods such as Plackett-Burman, Taguchi designs and response surface methodology (RSM) are frequently used for media optimization to optimize biosurfactant production due to the capacity of handling multiple data at the same time (Eswari et al. 2016; Hassan et al. 2016; Santos et al. 2016b). RSM was used to optimize media for rhamnolipid production by *Pseudomonas aeruginosa* using blackstrap molasses as the sole carbon source (Raza et al. 2016). The factors monitored were carbon:nitrogen (C:N) ratio, duration of incubation (time), and total sugar.

In RSM, the relationship between the dependent and independent variables is represented as follows:

$$Y = f\ (\mathrm{TS; C:N; Time})$$

The rhamnolipid yield was found to be affected by all the factors and observed to be maximum at C:N = 20, $T = 5$ days, and total sugar 2%. A rich mineral salt medium with non-limiting nitrogen and carbon source, neutral pH, and availability of critical metal cations are found to be most favorable for the production of lipopeptides (Biniarz et al. 2017). A work carried out by Cruz et al. (2018) on fortifying agricultural feedstock with crude glycerol and Mn yielded an enhanced growth and 793 mg $L^{-1}$of the crude lipopeptide.

4.6 Factors Affecting Biosurfactant Production

A variety of microorganisms produce biosurfactants that are either extracellular predominantly or attached intracellularly during growth (Santos et al. 2016a). The production of biosurfactants by microorganisms occurs most of the time during the exponential or stationary growth phase when a shortage of nutrients appears.

Factors affecting biosurfactant production are divided into two: (a) environmental and (b) nutritional (Moya et al. 2015; Gudina et al. 2015; Varjani and Upasani 2017). Different element ratios, such as carbon:phosphorus, carbon:nitrogen, carbon:iron, and carbon:magnesium, and optimization of these rates should be necessary to achieve high production yield of biosurfactants (Gudina et al. 2015).

4.6.1 Carbon Sources

There are different carbon sources, such as starchy substrates, oils, animal fat, petroleum effluents, lactic whey, olive oil mill effluent, plant-derived oils, distillery wastes, molasses, vegetable oils and oil wastes, soap stock, and hydrophobic mixtures (motor oil, crude oil, kerosene, diesel, paraffin), with great potential in optimizing biosurfactant production (Abouseoud et al. 2008; Bezza and Chirwa 2015, 2017; Lee et al. 2018; Patowary et al. 2017). *Pseudomonas aeruginosa* yields a better biosurfactant of 4.99 gL^{-1} by utilizing the motor oil, 4.76 gL^{-1} on *n*-hexadecane (Joice and Parthasarathi 2014), and 4.11 gL^{-1} on diesel fuel (Jimoh and Lin 2019a).

4.6.2 Nitrogen Sources

A variety of inorganic and organic nitrogen sources, such as yeast extract, $NaNO_3$, KNO_3, NH_4NO_3, and urea, are used in the production of biosurfactants. The choosing organic over inorganic nitrogen source or the inverse depends on the microbial strain or medium composition (Elazzazy et al. 2015; Ghribi and Ellouze-Chaabouni 2011). Maximum production of biosurfactants from *Virgibacillus salarius* was reported when yeast extract was used as a nitrogen source (Hu et al. 2015).

4.6.3 Carbon:Nitrogen Ratio

Excessive nitrogen limits the buildup of products and leads to the synthesis of cellular material. In contrast, high C:N ratios (i.e., low nitrogen levels), favoring cell metabolism toward the production of metabolites, limit bacterial growth (Xia et al. 2012). The presence of diesel fuel:$(NH4)_2SO_4$ ratio of 3:1 resulted in a high yield of biosurfactant production (3.79 gL^{-1}) with surface tension reduction (32.0 mNm^{-1}) and highest dry cell weight (0.35 gL^{-1}) (Jimoh and Lin 2019a).

4.6.4 Phosphate and Salinity Source

Phosphate is usually provided in the form of triphosphates to harness biosurfactants. Maqsood and Jamal (2011) reported that the maximum concentration of rhamnolipids is yielded when the gram-negative bacterium was cultivated under lower phosphate concentration along with the addition of ethanol. Additionally, the salinity of a specific medium is a critical part of biosurfactant production. For some biosurfactant items, opposite perceptions are seen, which means not influenced by fixations up to 10% (weight/volume) even if slight diminishments in the CMC were distinguished (Kugler et al. 2015).

4.6.5 Growth Conditions

There are different growth conditions, such as pH, temperature, incubation time, aeration, and agitation speed, that affect the biosurfactant production output. *Pseudomonas putida* MTCC produced a maximum of 2.5 gL^{-1} of lipopeptide biosurfactant at pH 8.0 (Kanna et al. 2014). At pH 6.8, biosynthesis from *Actinomycetes nocardiopsis* A17 was observed to be the highest, but the activity of biosurfactant was still maintained at lower and higher pH (Chakraborty et al. 2015). Aeration and agitation speed also affect the production of biosurfactants. Agitation speed affects oxygen molecules and the mass transfer efficiency of the medium components, and incubation periods also affect biosurfactant production (Yao et al. 2015). Thavasi et al. (2009) demonstrated that 4 days is the optimal incubation period for *Azotobacter chroococcum.* The size of the inoculum is also another critical factor that has a significant consequence in biosurfactant synthesis (Nalini and Parthasarathi 2017).

4.7 Biosurfactant Extraction, Purification, and Characterization

4.7.1 Biosurfactant Extraction

Downstream processing accounts for 70–80% of total production costs in many biotechnological processes (Varjani et al. 2014). Different methods, such as adsorption-desorption, solvent extractions, ion exchange, centrifugation, acid precipitation, and filtration, are known for their extraction. Several solvents, such as chloroform, hexane, pentane, ethyl acetate, methanol, etc., are used for the extraction of biosurfactants (Fig. 1.5).

4.7.2 Biosurfactant Purification

There are different biosurfactant purification techniques available, including thin-layer chromatography (TLC), dialysis, lyophilization, and isoelectric focusing (IEF). In the TLC method by using chloroform:methanol:water (10:10:0.5 v/v/v) mixture, a part of the crude biosurfactant is separated on a silica gel plate. Utilizing a developing solvent system with a different color developing reagent, such as ninhydrin, is

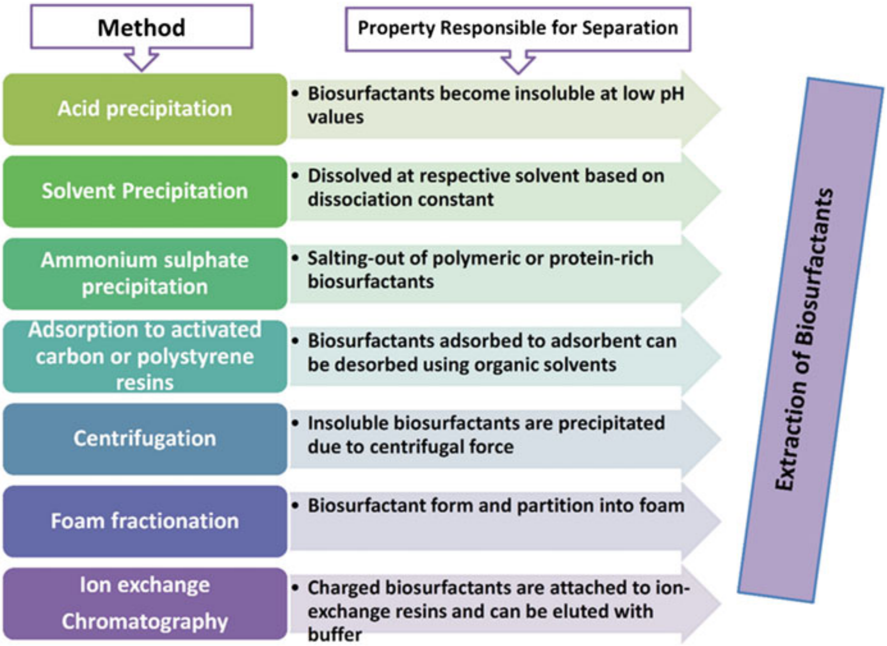


Fig. 1.5 Various available extraction methods for biosurfactant from microbes

used to characterize biosurfactants. The collected precipitate containing the biosurfactant is dissolved in 10 ml of sterile distilled water and dialyzed further with double distilled water for 48 h at 10 °C, in dialysis and lyophilization method, at 4 °C in an airtight container; the dialysate is stored for further use (Satpute et al. 2010a).

4.7.3 Biosurfactant Characterization

There are several spectroscopic and chromatography methods used for the characterization of biosurfactants, i.e., Fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR), thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC), etc. (Fig. 1.6).

FT-IR and NMR spectroscopic methods. Based on functional groups, FT-IR can explain some components of an unknown mixture (Elazzazy et al. 2015; Antoniou et al. 2015). NMR gives information related to the functional groups about the position of linkages within the carbohydrate molecules and lipids due to transitions in atoms with a magnetic moment when an external magnetic field is applied.

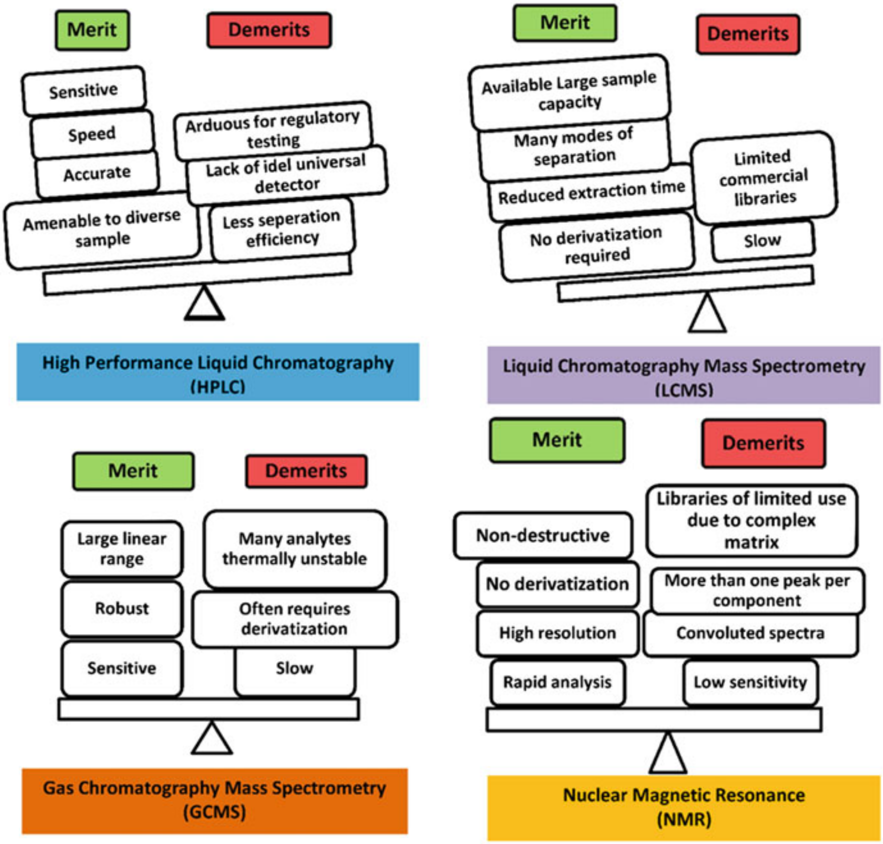


Fig. 1.6 Merits and demerits of methods prescribed for biosurfactant characterization

An initial purification by removing the worst interferences and also to concentrate the sample to a significant quantity requires for liquid chromatography-mass spectroscopy (LC-MS) analysis of biosurfactants (Biniarz et al. 2017). It utilizes differences in hydrophobicity to achieve partitioning between a polar mobile phase and a nonpolar stationary phase. The use of high-performance liquid chromatography (HPLC) was reported in the quantification, characterization, and purification of biosurfactant (Pathak and Keharia 2014). Gas chromatography-mass spectroscopy (GCMS) is used to characterize the biosurfactants by measuring the molecular weight of the compound. The sample needs hydrolytic cleavage between the carbohydrate/lipid and peptide/protein portions present in the biosurfactant for HPLC analysis. The GC-MS results are analyzed by fatty acid derivatization to fatty acid methyl esters (FAME) and further conversion to trimethylsilyl (TMS) derivatives (Vandana and Singh 2018).

5 Environmental Application of Biosurfactants

5.1 Use of Biosurfactant in Contaminated Soils

Majorly, common heavy metals found in contaminated soils are mercury (Hg), lead (Pb), arsenic (As), chromium (Cr), cadmium (Cd), zinc (Zn), nickel (Ni), and copper (Cu), which can cause many health issues and environmental hazards (Adamu et al. 2015; Hu et al. 2017; Li and Qian 2017; Liu et al. 2015, 2017; Tang et al. 2015). In the removal of heavy metals from contaminated soil by using a biosurfactant solution, three main steps are involved. First, the heavy metals adsorbed on the surface of contaminated soil particles separate through the sorption of biosurfactant molecules at the interfaces between metal in aqueous solution and wet soil (sludge). Then, the metal will be absorbed by biosurfactants and trapped within the micelle through electrostatic interactions. Finally, through the method of membrane separation, the biosurfactant can be recovered (Guan et al. 2017; Ibrahim et al. 2016).

Biosurfactants were also found to be a good agent to improve and augment soil properties, including via their bioremediation capacities. In situ bioremediation application of biosurfactants were influenced by soil properties, C:N ratio, and other factors, such as inherent temperature, pH, etc. Vecino Bello et al. (2012) postulated on the influence of pH, salinity, and temperature of soil on the bioremediation capabilities of *L. pentosus* biosurfactant, and also these parameters impacted the surface tension of the biosurfactant; besides these soil parameters, the emulsifying capacity of this biosurfactant was impacted by operation time (Vecino et al. 2014).

Biosurfactants produced by microorganisms are reported to modify the physical properties of soil. Lin et al. (2017) noted the effect of the rhamnolipid (125 and 625 $\mathrm{mgL^{-1}}$) in lowering flow rates ($\mathrm{cmh^{-1}}$) in soil when packed with it than rather with $CaCl_2$ and $FeCl_3$; thus, the biosurfactant limited the soil permeability. In another study, Rufino et al. (2011) gauged the role of *Candida lipolytica* biosurfactant in hydraulic conductivity; these attributes of biosurfactants varied on the soil texture, long compounding with the dosage involved and other features of biosurfactants (Abu-Zreig et al. 2003). Another insight is the ability of plant roots to hold the biosurfactant in the rhizosphere, impacting the retention and enhancing plant's capability to siphon the moisture and nutrients from the soil micropores. Soil hydrophobicity is a significant factor in land management, and it is accessed by wettability (measured by wettability angle). Surfactants were noted for their impact to alter this wettability of soils (Akbulut et al. 2012). Hallmann and Mędrzycka (2015) assayed the performance and power of rhamnolipid on contact angle on soil surfaces and proved that, at a minimal load of rhamnolipid, it impacted the contact angle. Al-Wahaibi et al. (2014) published a study wherein *B. subtilis* B30 accomplished lipopeptide biosurfactant 58.7° in the wettability of soils. As with the case of other applications of biosurfactants, the wettability effects of biosurfactants also hinged on the type and nature of biosurfactants harnessed via microorganisms (Sarafzadeh et al. 2014). Augmentation with the aid of biosurfactants to plant protection fabrications and/or fertilizers can minimize the interphase tensions and generate heightened wettability of soil and further aid the alienation of hydrophobic pollutants from these soil constituents (Mulligan et al. 2001a, b).

Biosurfactants have been widely investigated for its role in the remediation of soils contaminated with pesticides, heavy metals, and hydrocarbons to enhance the soil quality, thus maximizing the crop yield (Kumar et al. 2006; Ochoa-Loza et al. 2007). Bioremediation of biosurfactant is usually achieved via minimizing the water repellence of these hydrophobic pollutants. *L. pentosus* biosurfactant was noted for its role in repelling natural pollutants, such as humic acids (Paradelo et al. 2009), and biodegradation of octane in the soil (Moldes et al. 2013; Vecino et al. 2013). In the past decade, notable publications were made on biosurfactant's role as emulsifying agents for remediation of pesticide-contaminated soils (Guo et al. 2016; Mani et al. 2011; Singh et al. 2016; Wan et al. 2014). A team under Singh et al. (2016) proved that the solubility of chlorpyrifos increased up to 87% in the medium augmented with biosurfactant of *Pseudomonas* sp. Wan et al. (2014) established that maximizing the dosage of rhamnolipids impacted positively on the solubility of lindane, and Manickam et al. (2012) published a work wherein rhamnolipids harnessed via *Pseudomonas aeruginosa* heightened the solubilization of hexachlorocyclohexane (HCH) by nine times. The use of *Burkholderia cenocepacia* biosurfactant magnified the solubility of three pesticides (methyl parathion, ethyl parathion, and trifluralin) (Wattanaphon et al. 2008). Zhang et al. (2013) proved that the exile of triclosan from soil sediments accelerated with the aid of rhamnolipids.

5.2 *Applications of Biosurfactants in Oil Recovery*

In the microbial-enhanced oil recovery (MEOR) technique, different microbial species are applied to generate biosurfactants for oil recovery enhancement such as *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis* (Dhanarajan et al. 2017; Fernandes et al. 2016; Zhao et al. 2018). The injection of microbes causes the reduction of interfacial tension and oil viscosity between the surface of the rock matrix and oil hydrocarbons that can facilitate the mobilization of oil, and, further, increment of oil recovery is the principle of this technique (Hosseininoosheri et al. 2016) as shown in Fig. 1.7.

Rocha e Silva et al. (2017) explored the use of biosurfactants from *Candida guilliermondii*, *Candida lipolytica*, *Candida sphaerica*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, and *Bacillus* sp. as de-emulsifiers of petroleum derivatives and obtained promising results for the bacterial strains where recovery was 65% higher when compared with that of yeast's (35–40%) (Rocha e Silva et al. 2017).

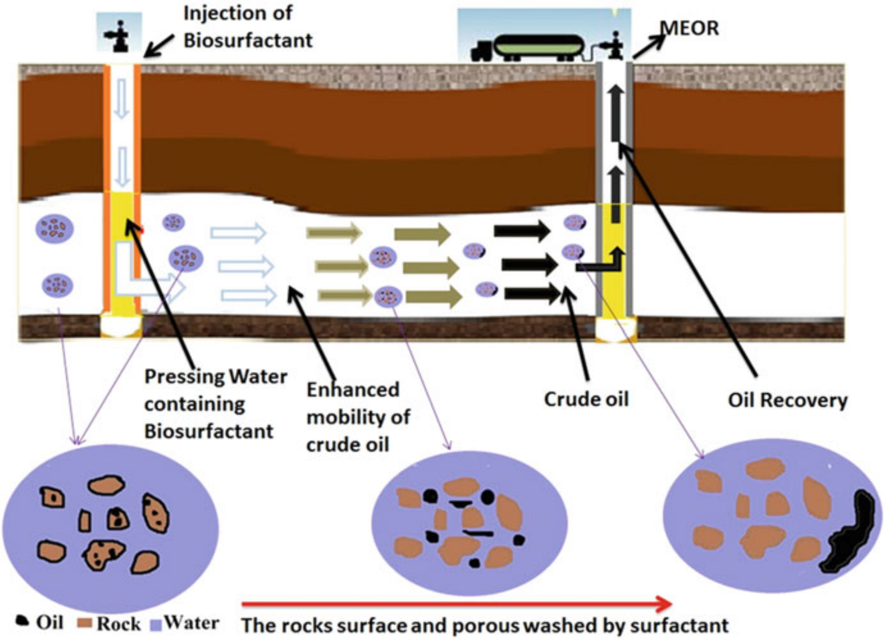


Fig. 1.7 MEOR of crude oil

Sharma et al. (2018) established the advantage of biosurfactants in enhanced oil recovery when they produced them from two isolates *Bacillus amyloliquefaciens* SAS-1 and *Bacillus subtilis* BR-15, also confirming the other strong attributes such as emulsification (60–78%) at a higher temperature and under flexible pH condition. They also reported better oil recovery of 56.91 ± 1.52 and 66.31 ± 2.32%, respectively, for the two reported strains, which was 75–94% higher to that of conventional ways (Sharma et al. 2018).

Many studies are reported on the role of biosurfactant on various environmental applications, mainly on its role in oil recovery to minimize the pollution at the site. El-Sheshtawya et al. (2016) employed *Bacillus licheniformis* and *Candida albicans* for this purpose and recorded the recovery as 16.6 and 8.6 wt%, respectively. In the similar context, Ivshina et al. (2016) postulated the exile of PAHs from soil using *Rhodococcus ruber* biosurfactants and found out that the application of biosurfactant assisted greatly the bio-treatability of the contaminated soils. In yet another investigation, Xia et al. (2011) evaluated the performance of biosurfactants from the three bacteria: *P. aeruginosa, B. subtilis*, and *Rhodococcus erythropolis*. The results revealed that *P. aeruginosa* exhibited a maximal emulsification index at 80%, and the isolates also proved to be used in the elimination of heavy metals.

6 Conclusion

This review gives information on biosurfactant properties, classification, source, production, and application. Biosurfactants are greener when compared to chemical or synthetic surfactants, but the harnessing of biosurfactant is expensive. Generally, biosurfactants have a unique character as a result of this, and they gain priority for different use, mainly for environmental applications. As a recommendation for future research, it is better to work on it to make it readily available.

References

Abouseoud M, Yataghene A, Amrane A, Maachi (2008) Biosurfactant production by free and alginate entrapped cells of *Pseudomonas fluorescens*. J Ind Microbiol Biotechnol 35:1303–1308

Abu-Zreig M, Rudra RP, Dickinson WT (2003) Effect of application of surfactants on hydraulic properties of soils. Biosyst Eng 84:363–372

Adamu C, Nganje T, Edet A (2015) Heavy metal contamination and health risk assessment associated with abandoned barite mines in Cross River State, Southeastern Nigeria. Environ Nanotechnol Monit Manag 3:10–21

Ahmad Z, Crowley D, Marina N, Jha S (2016) Estimation of biosurfactant yield produced by *Klebsiella* sp. FKOD36 bacteria using artificial neural network approach. Meas 81:163–173

Ajala OE, Aberuagba F, Odetoye A (2015) Biodiesel: sustainable energy replacement to petroleum-based diesel fuel – a review. Chem Bio Eng Rev 2(3):145–156. https://doi.org/10.1002/cben.201400024

Akbulut S, Nese Kurt Z, Arasan S (2012) Surfactant modified clays consistency limits and contact angles. Earth Sci Res J 16:95–101

Alejandro CS, Hernantez H, Maria JF (2011) Production of glycolipids with antimicrobial activity by Ustilago maydis FBD12 in submerged culture. Afr J Microbiol Res 5(17):2512–2523

Al-Wahaibi Y, Joshi S, Al-Bahry S, Elshafie A, Al-Bemani A, Shibulal B (2014) Biosurfactant production by *Bacillus subtilis* B30 and its application in enhancing oil recovery. Colloid Surf B Biointerface 114:324–333

Amaral PFF, da Silva JM, Lehocky M, Barros-Timmons AMV, Coelho MAZ, Marrucho IM, Coutinho JAP (2006) Production and characterization of a bioemulsifier from *Yarrowia lipolytica*. Process Biochem 41:1894–1898

Antoniou E, Fodelianakis S, Korkakaki E, Kalogerakis N (2015) Biosurfactant production from marine hydrocarbon-degrading consortia and pure bacterial strains using crude oil as carbon source. Front Microbiol 6:274–287

Arguelles-Arias A, Ongena M, Halimi B, Lara Y, Brans A, Joris B, Fickers P (2009) *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. Microb Cell Factories 8:63

Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L et al (2010) Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol 87:427–444

Banat IM, Satpute SK, Cameotra SS, Pati R, Nyayanit NV (2014) Cost effective technologies and renewable substrates for biosurfactants' production. J Front Microbiol 12(5):697

Batista SB, Mounteer AH, Amorim FM, Totola MR (2006) Isolation and characterization of biosurfactant/bioemulsifier producing bacteria from petroleum contaminated sites. Bioresour Technol 97(6):868–875

Bednarski W, Adamczak M, Tomasik J, Plaszvzyk M (2004) Application of oil refinery waste in the biosynthesis of glycolipids by yeast. J Bioresour Technol 95:15–18

Begley M, Cotter PD, Hill C, Ross RP (2009) Identification of a novel two-peptide lantibiotic, lichenicidin, following rational genome mining for Lan M proteins. Appl Environ Microbiol 75:5451–5460

Belhaij A, Al-Mahdy O (2015) Foamability and foam stability of several surfactants solutions: the role of screening and flooding. J Pet Environ Biotechnol 6:4. https://doi.org/10.4172/2157-7463.1000227

Bezza FA, Chirwa EMN (2015) Biosurfactant from *Paenibacillus dendritiformis* and its application in assisting polycyclic aromatic hydrocarbon (PAH) and motor oil sludge removal from contaminated soil and sand media. Process Saf Environ Prot 98:354–364

Bezza FA, Chirwa EMN (2017) Pyrene biodegradation enhancement potential of lipopeptidebiosurfactant produced by *Paenibacillus dendritiformis* CN5 strain. J Hazard Mater 321:218–227

Biniarz P, Łukaszewicz M, Janek T (2017) Screening concepts, characterization and structural analysis of microbial-derived bioactive lipopeptides: a review. Crit Rev Biotechnol 37:393–410

Bodour A, Drees K, Maier R (2003) Distribution of biosurfactant producing bacteria in undisturbed and contaminated arid southwestern soils. Appl Environ Microbiol 69(6):3280–3287

Cameron DR, Cooper DG, Neufeld RJ (1988) The mannoprotein of *Saccharomyces cerevisiae* is an effective bioemulsifier. Appl Environ Microbiol 54(6):1420–1425

Chakrabarti S (2012) Bacterial biosurfactant characterization, antimicrobial and metal remediation properties. Ph.D. thesis, National Institute of Technology, Surat, India

Chakraborty S, Ghosh M, Chakraborti S, Jana S, Sen KK, Kokare C, Zhang L (2015) Biosurfactant produced from *Actinomycetes nocardiopsis* A17: characterization and its biological evaluation. Int J Biol Macromol 79:405–412.

Chandran P, Das N (2010) Biosurfactant production and diesel oil degradation by yeast species *Trichosporon asahii* isolated from petroleum hydrocarbon contaminated soil. Int J Eng Sci Technol 2:6942–6953

Cheng Y, He HJ, Yang CP, Zeng GM, Li X, Chen H, Yu GL (2016) Challenges and solutions for biofiltration of hydrophobic volatile organic compounds. Biotechnol Adv 34(6):1091–1102

Cruz JM, Hughes C, Quilty B, Montagnolli RN, Bidoia ED (2018) Agricultural feedstock supplemented with manganese for biosurfactant production by *Bacillus subtilis*. Waste Biomass Valoriz 9:613–618

de Lima JCB, Ribeiro EJ, Servulo EFC, Resende MM, Cardoso VL (2009) Biosurfactant production by *Pseudomonas aeruginosa* grown in residual soybean oil. Appl Biochem Biotechnol 152:156–168

Dhanarajan G, Rangarajan V, Bandi C, Dixit A, Das S, Ale K, Sen R (2017) Biosurfactant-biopolymer driven microbial enhanced oil recovery (MEOR) and its optimization by an ANN-GA hybrid technique. J Biotechnol 256:46–56

Díaz de Rienzo MA, Stevenson P, Marchant R, Banat IM (2016) Antibacterial properties of biosurfactants against selected Gram-positive and negative bacteria. FEMS Microbiol Lett 363:fnv224. https://doi.org/10.1093/femsle/fnv224

Drenckhan W, Hutzler S (2015) Structure and energy of liquid foams. Adv Colloid Interf Sci 224:1–16. https://doi.org/10.1016/j.cis.2015.05.004

Elazzazy AM, Abdelmoneim TS, Almaghrabi OA (2015) Isolation and characterization of biosurfactant production under extreme environmental conditions by alkali-halo-thermophilic bacteria from Saudi Arabia. Saudi J Biol Sci 22(4):466–475

El-Sheshtawya HS, Aiada I, Osman ME, Abo-ELnasrb A, Kobisya AS (2016) Production of biosurfactants by *Bacillus licheniformis* and *Candida albicans* for application in microbial enhanced oil recovery. Egypt J Pet 25:293–298

Eswari JS, Anand M, Venkateswarlu C (2016) Optimum culture medium composition for lipopeptide production by *Bacillus subtilis* using response surface model-based ant colony optimization. Sadhana 41:55–65

Felix NAK, Martins JJL, Lima JG, Giro MEA, Cavalcante KF, Melo VMM et al (2019) Purification and characterization of a biosurfactant produced by *Bacillus subtilis* in cashew, apple juice and its application in the remediation of oil-contaminated soil. Colloid Surf B 175:256–263

Felse PA, Shah V, Chan J, Rao KJ, Gross RA (2007) Sophorolipid biosynthesis by *Candida bombicola* from industrial fatty acid residues. Enzyme Microb Technol 40(2):316–323

Fernandes PL, Rodrigues EM, Paiva FR, Ayupe BAL, McInerney MJ, Tótola MR (2016) Biosurfactant, solvents and polymer production by *Bacillus subtilis* RI4914 and their application for enhanced oil recovery. Fuel 180:551–557. https://doi.org/10.1016/j.fuel.2016.04.080

Ferreira A, Vecino X, Ferreira D, Cruz JM, Moldes AB, Rodrigues LR (2017) Novel cosmetic formulations containing a biosurfactant from *Lactobacillus paracasei*. Colloid Surf Biointerface 155(4):522–529. https://doi.org/10.1016/j.colsurfb.2017.04.026

Flasz A, Rocha CA, Mosquera B, Sajo C (1998) A comparative study of the toxicity of a synthetic surfactant and one produced by *Pseudomonas aeruginosa* ATCC 55925. Med Sci Res 26:181–185

Gao Y, Li Q, Ling W, Zhu X (2011) Arbuscular mycorrhizal phytoremediation of soils contaminated with phenanthrene and pyrene. J Hazard Mater 185:703–709

Ghribi D, Ellouze-Chaabouni S (2011) Enhancement of *Bacillus subtilis* lipopeptide biosurfactants production through optimization of medium composition and adequate control of aeration. Biotechnol Res Int 2011:653654. https://doi.org/10.4061/2011/653654

Giani C, Wullbrandt D, Rothert R, Meiwes J (1997) *Pseudomonas aeruginosa* and its use in a process for the biotechnological preparation of Rhamnose. US Patent 5658793A

Guan R, Yuan X, Wu Z, Wang H, Jiang L, Li Y, Zeng G (2017) Functionality of surfactants in waste-activated sludge treatment: a review. Sci Total Environ 609:1433–1442. https://doi.org/10.1016/j.scitotenv.2017.07.189

Gudina EJ, Fernandes EC, Rodrigues AI, Teixeira JA, Rodrigues LR (2015) Biosurfactant production by *Bacillus subtilis* using corn steep liquor as culture medium. Front Microbiol 6:59

Guo Q, Yan J, Wen J, Hu Y, Chen Y, Wu W (2016) Rhamnolipid-enhanced aerobic biodegradation of triclosan (TCS) by indigenous microorganisms in water-sediment systems. Sci Total Environ 571:1304–1311

Gutierrez T, Mulloy B, Bavington C, Black K, Green DH (2007) Partial purification a chemical characterization of a glycoprotein (putative hydrocolloid) emulsifier produced by a marine bacterium antarctobacter. J Appl Microbiol Biotechnol 76:1017–1026

Hallmann E, Mędrzycka K (2015) Wetting properties of biosurfactant (rhamnolipid) withsynthetic surfactants mixtures in the context of soil remediation. Ann UMCS Chemia 70:29–39

Hassan M, Essam T, Yassin AS, Salama A (2016) Optimization of rhamnolipid production by biodegrading bacterial isolates using Plackett-Burman design. Int J Biol Macromol 8:573–579

Helfrich M, Ludwig B, Thoms C, Gleixner G, Flessa H (2015) The role of soil fungi and bacteria in plant litter decomposition and macroaggregate formation determined using phospholipid fatty acids. Appl Soil Ecol 96:261–264

Helmy Q, Kardena E, Funamizu N, Wisjnuprapto (2011) Strategies toward commercial scale of biosurfactant production as potential substitute for its chemically counter parts. Int J Biotechnol 12:66–86

Hirata M, Ryu Y, Oda K, Igarashi A, Nagatsuka T, Furuta T, Sugiura M (2009) Novel characteristics of sophorolipids, yeast glycolipid biosurfactants, as biodegradable low-foaming surfactants. J Biosci Bioeng 108:142–146

Hosseininoosheri P, Lashgari HR, Sepehrnoori K (2016) A novel method to model and characterize in-situ bio-surfactant production in microbial enhanced oil recovery. Fuel 183:501–511. https://doi.org/10.1016/j.fuel.2016.06.035

Hu X, Wang C, Wang P (2015) Optimization and characterization of biosurfactant production from marine *Vibrio* sp. strain 3B-2. J Front Microbiol 6:976

Hu B, Jia X, Hu J, Xu D, Xia F, Li Y (2017) Assessment of heavy metal pollution and health risks in the soil-plant-human system in the Yangtze river delta China. Int J Environ Res Public Health 19 (9):1042. https://doi.org/10.3390/ijerph14091042

Ibrahim WM, Hassan AF, Azab YA (2016) Biosorption of toxic heavy metals from aqueous solution by Ulva lactuca activated carbon. Egypt J Basic Appl Sci 3(3):241–249. https://doi.org/10.1016/j.ejbas.2016.07.005

Ivshina I, Kostina L, Krivoruchko A, Kuyukina M, Peshkur T, Anderson P, Cunningham C (2016) Removal of polycyclic aromatic hydrocarbons in soil spiked with model mixtures of petroleum hydrocarbons and heterocycles using biosurfactants from *Rhodococcus ruber* IEGM 231. J Hazard Mater 312:8–17

Jadhav M, Kalme S, Tamboli D, Govindwar S (2011) Rhamnolipid from *Pseudomonas desmolyticum* NCIM-2112 and its role in the degradation of Brown 3REL. J Basic Microbiol 51:1–12

Jian XX, Liao LW, Zhu WM, Zhang WM, Jiang JX (2011) Synergism and foaming properties in binary mixtures of a biosurfactant derived from *Camellia oleifera* Abel and synthetic surfactants. J Colloid Interface Sci 359:487–492. https://doi.org/10.1016/j.jcis.2011.04.038

Jimenez-Penalver T, Gea A, Sanchez A, Font X (2016) Production of sophorolipids from winterization oil cake by solid-state fermentation: optimization, monitoring and effect of mixing. Biochem Eng J 115:93–100

Jimoh AA, Lin J (2019a) Enhancement of *Paenibacillus* sp. D9 lipopeptide biosurfactant production through the optimization of medium composition and its application for biodegradation of hydrophobic pollutants. Appl Biochem Biotechnol 187:724–743

Jimoh AA, Lin J (2019b) Production and characterization of lipopeptide biosurfactant producing *Paenibacillus* sp. D9 and its biodegradation of diesel fuel. Int J Environ Sci Technol 16:4143–4158

Joice PA, Parthasarathi R (2014) Optimization of biosurfactant production from *Pseudomonas aeruginosa* PBSC1. Int J Curr Microbiol Appl Sci 3:140–151

Joshi S, Bharucha C, Jha S, Yadav S, Nerurkar A, Desai AJ (2008) Biosurfactant production using molasses and whey under thermophilic conditions. Bioresour Technol 99:195–199

Kanna R, Gummadi SN, Kumar GS (2014) Production and characterization of biosurfactant by *Pseudomonas putida* MTCC 2467. J Biol Sci 14:436–445

Kim SY, Oh DK, Lee KH, Kim JH (1997) Effect of soybean oil and glucose on sophorose lipid fermentation by *Torulopsis bombicola* in continuous culture. Appl Microbiol Biotechnol 48:23–26

Kim LH, Jung Y, Yu H-W, Chae K-J, Kim S (2015) Physicochemical interactions between rhamnolipids and *Pseudomonas aeruginosa* biofilm layers. Environ Sci Technol 49 (6):3718–3726

Kosaric N (2001) Biosurfactants and their application for soil bioremediation. Food Technol Biotechnol 39:295–304

Kugler JH, Le Roes-Hill M, Syldatk C, Hausmann R (2015) Surfactants tailored by the class Actinobacteria. Front Microbiol 6:212

Kumar M, Leon V, De Sisto Materano A, Ilzins OA (2006) Enhancement of oil degradation by co-culture of hydrocarbon degrading and biosurfactant producing bacteria. Pol J Microbiol 55:139–146

Lai CC, Huang YC, Wei WH, Chang JS (2009) Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil. J Hazard Mater 167:609–614

Lee SM, Lee JY, Yu HP, Lim JC (2017) Synthesis of environment friendly biosurfactants and characterization of interfacial properties for cosmetic and household products formulations. Colloids Surf A Physicochem Eng Asp 536:224–233

Lee DW, Lee H, Kwon BO, Khim JS, Yim UH, Kim BS, Kim JJ (2018) Biosurfactant-assisted bioremediation of crude oil by indigenous bacteria isolated from Taean beach sediment. Environ Pollut 241:254–264

Li X, Qian P (2017) Identification of an exposure risk to heavy metals from pharmaceutical-grade rubber stoppers. Food Drug Anal 25(3):723–730

Lin Z, Zhang R, Huang S, Wang K (2017) Impact of chemical leaching on permeability and cadmium removal from fine-grained soils. Environ Sci Pollut Res 24:18229–18239. https://doi.org/10.1007/s11356-017-9523-2

Liu Y, Wang H, Li X, Li J (2015) Heavy metal contamination of agricultural soils in Taiyuan, China. Pedosphere 25(6):901–909. https://doi.org/10.1016/S1002-0160(15)30070-9

Liu Z, Li Z, Zhong H, Zeng G, Liang Y, Chen M et al (2017) Recent advances in the environmental applications of biosurfactant saponins: a review. J Environ Chem Eng 5:6030–6038. https://doi.org/10.1016/j.jece.2017.11.021

Lukondeh T, Ashbolt NJ, Rogers PL (2003) Evaluation of *Kluyveromyces marxianus* FII 510700 grown on a lactose-based medium as a source of a natural bioemulsifier. J Ind Microbiol Biotechnol 30(12):715–720

Maneera S, Dikit P (2007) Characterization of cell-associated bioemulsifier from Myroides sp. SM1, a marine bacterium. Songklanakarin J Sci Technol 29: 769–779

Mani A, Hamid SS, Ramalingam S, Kaliamoorthi R (2011) Effect of rhamnolipid potential on biodegradation of endosulfan by *Pseudomonas aeruginosa* in batch studies. J Biosci Technol 2:268–278

Manickam N, Bajaj A, Saini HS, Shanker R (2012) Surfactant mediated enhanced biodegradation of hexachlorocyclohexane (HCH) isomers by *Sphingomonas* sp. NM05. Biodegradation 23:673–682

Maqsood MI, Jamal A (2011) Factors affecting the rhamnolipid biosurfactant production. Pak J Biotechol 8(1):1–5

Mohan PK, Nakhla G, Yanful EK (2006) Biokinetics of biodegradation of surfactants under aerobic, anoxic and anaerobic conditions. Water Res 40(3):533–540

Moldes AB, Paradelo R, Vecino X, Cruz JM, Gudina E, Rodrigues L et al (2013) Partial characterization of biosurfactant from *Lactobacillus pentosus* and comparison with sodium dodecyl sulphate for the bioremediation of hydrocarbon contaminated soil. Biomed Res Int 2013:961842

Morita T, Konishi M, Fukuoka T, Imura T, Kitamoto D (2007) Physiological differences in the formation of the glycolipid biosurfactants, mannosylerythritol lipids, between *Pseudozyma antarctica* and *Pseudozyma aphidis*. Appl Microbiol Biotechnol 74(2):307–315

Moya RI, Tsaousi K, Rudden M, Marchant R, Alameda EJ, Garcia RM, Banat IM (2015) Rhamnolipid and surfactin production from olive oil mill waste as sole carbon source. Bioresour Technol 198:231–236

Mulligan CN, Yong RN, Gibbs BF (2001a) Heavy metal removal from sediments by biosurfactants. J Hazard Mater 85:111–125

Mulligan CN, Yong RN, Gibbs BF (2001b) Remediation technologies for metal contaminated soils and groundwater: an evaluation. Eng Geol 60:193–207

Muthusamy K, Gopalakrishnan S, Ravi T, Sivachidambaram P (2008) Biosurfactants: properties, commercial production and application. Curr Sci 94:736–747

Nalini S, Parthasarathi R (2017) Optimization of rhamnolipid biosurfactant production from *Serratia rubidaea* SNAU02 under solid-state fermentation and its biocontrol efficacy against Fusarium wilt of eggplant. Ann Agrar Sci 16(2):108–115

Nerurkar AS, Hingurao KS, Suthar HG (2009) Bioemulsifiers from marine microorganisms. J Sci Ind Res 68:273–277

Nguyen MT, Gotz F (2016) Lipoproteins of Gram-positive bacteria: key players in the immune response and virulence. Microbiol Mol Biol Rev 80(3):891–903

Ochoa-Loza FJ, Noordman WH, Jannsen DB, Brusseau ML, Maier RM (2007) Effect of clays, metal oxides, and organic matter on rhamnolipid biosurfactant sorption by soil. Chemosphere 66:1634–1642

Oloke JK, Glick BR (2005) Production of Bioemulsifier by an unusual isolate of salmon red melanin containing *Rhodotorula glutinis*. Afr J Biotechnol 4(2):164–171

Pacwa-Płociniczak M, Płaza GA, Piotrowska-Seget Z, Cameotra SS (2011) Environmental applications of biosurfactants: recent advances. Int J Mol Sci 12: 633-654

Paradelo R, Moldes AB, Dominguez JM, Barral MT (2009) Reduction of water repellence of hydrophobic plant substrates using biosurfactant produced from hydrolyzed grape marc. J Agric Food Chem 57:4895–4899

Pathak KV, Keharia H (2014) Application of extracellular produced by endophytic *Bacillus subtilis* K1 isolated from aerial roots of banyan (*Ficus benghalensis*) in microbially enhanced oil recovery (MEOR). 3Biotech 4(1):41–48

Patil Y, Rao P (2015) Industrial waste management in the era of climate change – a smart sustainable model based on utilization of active and passive biomass. In: Walter Leal F (ed) Handbook on climate change adaptation. Springer, Berlin/Heidelberg, pp 2079–2092

Patowary K, Patowary R, Kalita MC, Deka S (2017) Characterization of biosurfactant produced during degradation of hydrocarbons using crude oil as sole source of carbon. Front Microbiol 8: 279. https://doi.org/10.3389/fmicb.2017.00279

Peele KA, Ch VR, Kodali VP (2016) Emulsifying activity of a biosurfactant produced by a marine bacterium. 3 Biotech 6(2):177. https://doi.org/10.1007/s13205-016-0494-7

Raza ZA, Ahmad N, Khalid ZM, Ahmad N (2016) Response surface optimization in biosurfactant production by using a renewable growth substrate. Indian J Biotechnol 15:217–229

Rikalović MG, Vrvić MM. Karadžić IM (2015) Rhamnolipid biosurfactant from *Pseudomonas aeruginosa*: from discovery to application in contemporary technology. J Serb Chem Soc 80(3):279–304

Rocha e Silva FCP, Roque BAC, Rocha e Silva NMP et al (2017) Yeasts and bacterial biosurfactants as demulsifiers for petroleum derivative in seawater emulsions. AMB Express 7:202. https://doi.org/10.1186/s13568-017-0499-6

Roelants SLKW, Van Renterghem L, Maes K, Everaert B, Vanlerberghe B, Demaeseneire S, Soetaert WI (2018) Microbial biosurfactants: from lab to market. In: Banat IM, Thavasi R (eds) Microbial biosurfactants and their environmental and industrial applications. CRC Press, Boca Raton, pp 340–362

Rufino RD, Rodrigues GIB, Campos-Takaki GM, Sarubbo LA, Ferreira SRM (2011) Application of a yeast biosurfactant in the removal of heavy metals and hydrophobic contaminant in a soil used as slurry barrier. Appl Environ Soil Sci 2011:939648

Samanta A, Pal P, Mandal A, Sinha C, Lalee A, Das M et al (2012) Estimation of biosurfactants activity of Alkaline protease producing bacteria isolated from municipal solid waste. Cent Eur J Exp Biol 1:26–35

Santos DKF, Rufino RD, Luna JM, Santos VA, Sarubbo LA (2016a) Biosurfactants: multifunctional biomolecules of the 21st century. Int J Mol Sci 17(3):401

Santos BF, Ponezi AN, Fileti AMF (2016b) Strategy for waste management in the production and application of biosurfactant through surface response methodology. Clean Techn Environ Policy 18:787–795

Sarafzadeh P, Hezave AZ, Mohammadi S, Niazi A, Ayatollahi S (2014) Modification of rock/fluid and fluid/fluid interfaces during MEOR processes, using two biosurfactant producing strains of *Bacillus stearothermophilus* SUCPM# 14 and *Enterobacter cloacae*: a mechanistic study. Colloid Surf B 117:457–465

Satpute SK, Banat IM, Dhakephalkar PK, Banpurkar G, Chopade BA (2010a) Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. Biotechnol Adv 28 (4):436–450

Satpute SK, Banpurkar AG, Dhakephalkar PK, Banat IM, Chopade BA (2010b) Methods for investigating biosurfactants and bioemulsifiers: a review. Crit Rev Biotechnol 30(2):127–144

Satpute SK, Płaza GA, Banpurkar AG (2017) Biosurfactants' production from renewable natural resources: example of innovative and smart technology in circular bioeconomy. Manag Syst Prod Eng 25:46–54

Shah A, Shahzad S, Munir A, Nadagouda MN, Khan GS, Shams DF et al (2016) Micelles as soil and water decontamination agents. Chem Rev 116(10):6042–6074

Sharma D, Saharan BS, Kapil S (2016) Structural properties of biosurfactants of lab. In: Biosurfactants of lactic acid bacteria. Springer, pp 47–60

Sharma R, Singh J, Verma N (2018) Production, characterization and environmental applications of biosurfactants from *Bacillus amyloliquefaciens* and *Bacillus subtilis*. Biocatal Agric Biotechnol 16:132–139

Shekhar S, Sundaramanickam A, Balasubramanian T (2015) Biosurfactant producing microbes and their potential applications: a review. Crit Rev Environ Sci Technol 45:1522–1554

Silva NMPR, Rufino RD, Luna JM, Santos VA, Sarubbo LA (2014) Screening of *Pseudomonas* species for biosurfactant production using low-cost substrates. Biocatal Agric Biotechnol 3 (2):132–113

Singh P, Cameotra SS (2004) Enhancement of metal bioremediation by use of microbial surfactants. Biochem Biophys Res Comm 319:291–297

Singh P, Saini HS, Raj M (2016) Rhamnolipid mediated enhanced degradation of chlorpyrifos by bacterial consortium in soil-water system. Ecotoxicol Environ Saf 134:156–162

Sutyak KE, Wirawan RE, Aroutcheva AA, Chiindas ML (2008) Isolation of the *Bacillus subtilis* antimicrobial peptide subtilosin from the dairy product derived *Bacillus amyliquefaceiens*. Appl Microbiol 104:1067–1074

Tadros TF (2013) Emulsion formation, stability, and rheology. In: Tadros TF (ed) Emulsion formation and stability. https://doi.org/10.1002/9783527647941.ch1

Tang Z, Zhang L, Huang Q, Yang Y, Nie Z, Cheng J, Chai M (2015) Contamination and risk of heavy metals in soils and sediments from a typical plastic waste recycling area in North China. Ecotoxicol Environ Saf 122:343–351. https://doi.org/10.1016/j.ecoenv.2015.08.006

Thavasi R, Subramanyam Nambaru VRM, Jayalakshmi S et al (2009) Biosurfactant production by *Azotobacter chroococcum* isolated from the marine environment. Mar Biotechnol 11:551. https://doi.org/10.1007/s10126-008-9162-1

Udoh T, Vinogradov J (2019) Experimental investigations of behaviour of biosurfactants in brine solutions relevant to hydrocarbon reservoirs. Colloid Interface 3(1):24

Vandana P, Singh D (2018) Review on biosurfactant production and its application. Int J Curr Microbiol Appl Sci 7(8):4228–4241

Varjani SJ, Upasani VN (2017) Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant. Bioresour Technol 232:389–397

Varjani SJ, Rana DP, Bateja S, Sharma MC, Upasani VN (2014) Screening and identification of biosurfactant (bioemulsifier) producing bacteria from crude oil contaminated sites of Gujarat, India. IJIRSET 3(2):9205–9213

Vecino Bello X, Devesa-Rey R, Cruz JM, Moldes AB (2012) Study of the synergistic effects of salinity, pH, and temperature on the surface-active properties of biosurfactants produced by *Lactobacillus pentosus*. Agric Food Chem 60:1258–1265

Vecino X, Devesa-Rey R, Cruz JM, Moldes AB (2013) Evaluation of biosurfactant obtained from *Lactobacillus pentosus* as foaming agent in froth flotation. Environ Manag 128:655–660

Vecino X, Barbosa-Pereira L, Devesa-Rey R, Cruz JM, Moldes AB (2014) Study of the surfactant properties of aqueous stream from the corn milling industry. Agric Food Chem 62:5451–5457

Vecino X, Cruz JM, Moldes AB, Rodrigues LR (2017) Biosurfactants in cosmetic formulations: trends and challenges. Crit Rev Biotechnol 37:911–923

Wan Z-L, Wang L-Y, Wang J-M, Yuan Y, Yang X-Q (2014) Synergistic foaming and surface properties of a weakly interacting mixture of soy glycinin and biosurfactants tevioside. Agric Food Chem 62:6834–6843

Wattanaphon HT, Kerdsin A, Thammacharoen C, Sangvanich P, Vangnai AS (2008) A biosurfactant from Burkholderia cenocepacia BSP3 and its enhancement of pesticide solubilization. J Appl Microbiol 105:416–423

Xia W, Dong H, Yu L, Yu D (2011) Comparative study of biosurfactant produced by microorganisms isolated from formation water of petroleum reservoir. Colloid Surf A 392:124–130

Xia WJ, Luo Zb., Dong, HP, Yu L, Cui QF, Bi YQ (2012) Synthesis, characterization, and oil recovery application of biosurfactant produced by indigenous *Pseudomonas aeruginosa* WJ-1 using waste vegetable oils. Appl Biochem Biotechnol 166:1148–1166. https://doi.org/10.1007/s12010-011-9501-y

Yan P, Lu M, Yang Q, Zhang H, Zhang Z, Chen R (2012) Oil recovery from refinery oily sludge using a rhamnolipid biosurfactant-producing Pseudomonas. Bioresour Technol 116:24–28

Yao S, Zhao S, Lu Z, Gao Y, Lv F, Bie X (2015) Control of agitation and aeration rates in the production of surfactin in foam overflowing fed-batch culture with industrial fermentation. Rev Argent Microbiol 47:344–349

Zhang X, Guo Q, Hu Y, Lin H (2013) Effects of monorhamnolipid and dirhamnolipid on sorption and desorption of triclosan in sediment-water system. Chemosphere 90:581–587

Zhao F, Zhou J-D, Ma F, Shi R-J, Han S-Q, Zhang J, Zhang Y (2016) Simultaneous inhibition of sulfate-reducing bacteria, removal of H_2S and production of rhamnolipid by recombinant *Pseudomonas stutzeri* Rhl: applications for microbial enhanced oil recovery. Bioresour Technol 207:24–30

Zhao F, Li P, Guo C, Shi R-J, Zhang Y (2018) Bioaugmentation of oil reservoir indigenous *Pseudomonas aeruginosa* to enhance oil recovery through in-situ biosurfactant production without air injection. Bioresour Technol 251:295–302

Chapter 2
Hairy Roots as a Source for Phytoremediation

Anil Kumar Moola, P. Balasubramanian, Lakkakula Satish, Sasanala Shamili, Manikandan Ramesh, T. Senthil Kumar, and B. D. Ranjitha Kumari

Abstract "Phytoremediation," application of green plants to process and regulate the waste materials in soil, water, and the air is an important part of the new field of ecological engineering. Phytoremediation has been addressed as among the promising and eco-friendly processes for the decontamination of several environmental pollutants. In recent years, in vitro plant cultures play an important role in the phytoremediation process. This chapter focuses on the development of hairy root clones by using *Agrobacterium rhizogenes* in various plant species and their application in the remediation process. It is well-known that enzymes that are expected to be necessitated in the detoxifying process of lethal compounds. In view of the ease of employing this in vitro culture method as a transgenic arena, and interdependent the huge progress in functional genomics studies, it is required to develop novel hairy root cultures that capable to express, provoke, and metabolize additional genes in a greater efficiently under in vitro and in vivo. The present chapter summarized the differences between how hairy roots are more helpful in converting toxic to nontoxic forms comparatively with normal roots. This chapter also reports the most up-to-date achievements of using hairy root cultures in the phytoremediation process. This information is essential for assessing the feasibility of a remediation process prior to its filed uptake.

Keywords Hairy roots · Enzymes · Phytoremediation · *Agrobacterium rhizogenes*

A. K. Moola (✉) · T. S. Kumar · B. D. R. Kumari
Department of Botany, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

P. Balasubramanian
Department of Biotechnology & Medical Engineering, National Institute of Technology, Rourkela, Odisha, India

L. Satish · S. Shamili
The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of Negev, Beer Sheva, Israel

M. Ramesh
Department of Biotechnology, Alagappa University, Karaikudi, Tamil Nadu, India

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_2

1 Introduction

The stages of environmental contamination by the use of pesticides, heavy metals (HMs), leather tannery effluents, phenols, organic matter, explosives, azo dyes have increased significantly over the last few decades, mostly due to industries and tanneries (Perotti et al. 2020). Tannery effluents contain large quantities of both inorganic and organic contaminants, such as phenols and hexavalent chromium (Cr (VI)). It is well known that toxicity depends upon the redox state. In the case of chromium, for example, Cr (VI) is highly toxic compared to Cr (III) due to its high solubility, availability, and mobility in the soil as well as through biological membranes. Based on the facts, the removal of toxic compounds from the environment is of high relevance for a safe environment. In this sense, many biological methods have been proposed to remove these harmful substances from the water bodies and soil (Chen et al. 2012). Phytoremediation, using plant vegetation to degrade the contaminated environments, is a green and eco-friendly technology that has gained importance concerning traditional decontamination methods (Flocco and Giulietti 2007).

In recent decades, a research area in the area of phytoremediation experienced increasing interest, mainly in Europe and the United States, but also in developing countries. Even though it was primarily implemented for the degradation of different kinds of inorganic impurities from soil samples, the phytoremediation approach has increasingly proving to be proficient for the processing of organic contaminants additionally. These facts are making it necessary to acquire discernment into the machinery essential for the decontamination process. Prior to the implementation of a phytoremediation protocol to field conditions, it is necessary to conduct laboratory-scale studies, for which the plant experimental models systems are essential. These model systems permit the control and reproducibility in experimental conditions necessary for conducting basic phytoremediation research.

In vitro plant cultures especially hairy root cultures (HRCs) are a useful alternative methodology for decontamination of PCBs, trinitrotoluene (TNT), textile dyes, phenolics, HMs, and radioactive nuclides (Agostini et al. 2013). Hairy roots (HRs) are originated by the infection of explants with *Agrobacterium rhizogenes* strain, gram-negative soil bacteria by transfers of the transfer DNA comprising the loci between the T_R and T_L region of the root-inducing plasmid (Chandra 2012; Lal 2020; Sujatha et al. 2013). Concomitantly, as the genetic and biochemical properties of HRs were exposed, scientific community started focussing on its exploitation for research benefits. The main reason behind this is the major property that has attracted scientists the most was the equal or sometimes higher potential of HRs to produce important bioactive compounds, and the HRs biotechnology podium has proved to be valuable systems for studying key aspects of pollutants phytoremediation (Georgiev et al. 2012). Even plant root cultures also provoke the breakdown of harmful substances in the soils through the secretion of root exudates and oxido-reductive enzymes that are mainly involved in the degradation of organic pollutants (Jha et al. 2020).

Though the research on *A. rhizogenes* transformed HRs is available for several years for enhancing the secondary metabolites (SMs) but during the past few years' research on HRCs is increasing. Mainly, during the last decade research on HRs podium is helping in understanding the mechanism of the phytoremediation process. In the present chapter, we focus on the source of HRs from different medicinal plants used to remediate the contaminants and tabulated in Table. 2.1. It is therefore unsurprising that the major objective of current research is to diversify the research to understand the plausible reason how HRs are superficial in comparison with normal roots.

2 Hairy Roots in Phytoremediation

Plant cell cultures have been employed for the biotransformation of various organochlorine, organophosphorus pesticides, phenols, PCBs, and explosives like GTN (Anees et al. 2020; Doran 2009; Scheel and Sandermann 1977). Over the last 30 years, HRs have been exploited for different kinds of beneficial intents, distinguishing from metabolic engineering, and industrially important recombinant protein synthesis to conduct an assessment of phytoremediation (Praveen et al. 2014; Wilson and Roberts 2012). The advantage of HRs system could allow variations to carry out the organization of phyto-molecules that cannot realistically be generated by chemical synthesis. The molecular basis of genetic transformation of HRCs by using *A. rhizogenes* strains is well known and recently detailed in many studies (Gutierrez-Valdes et al. 2020; Mehrotra et al. 2020; Rency et al. 2019; Satish et al. 2019). More recently, HRs technology has been established as a biotechnological concept in a majority of plant root systems. HRs, fine fibrous structures can be grown in large mass in culture media in a controlled environment and can, therefore, be subjected to various physiological assays such as phytoremediation that are formed on plant tissues infected by *A. rhizogenes* (Georgiev et al. 2007). Plants do not necessarily cause the same types of metabolic reactions that bacteria or fungi do, rather than mineralizing a contaminant the way a bacterium would, plants typically interact with a xenobiotic compound in three phases. The process of phytoremediation encompasses three detoxification phases (Abhilash et al. 2009; Betts 1998; Schröder et al. 2007).

Level – I: Transformation, including oxidation, reduction and hydrolysis, and the catalysis to speedup the ratio of virtually all the chemical reactions through enzymes for example P450 monooxygenases, reductases, peroxidases, dehydrogenases, and esterases.

Level – II: For better solubility, the process initiated with the conjugation of contaminants with endogenous compounds such as mono-, oligo- and polysaccharides, peptides, proteins, amino acids.

Table 2.1 Phytoremediation of environment pollutants by hairy root culture of different plant species

Heavy metals	Hairy roots	References
Cadmium	*Alyssum bertoloni*	Boominathan and Doran (2003)
Nickel	*Thlaspi caerulescens*	Boominathan and Doran (2003)
	Alyssum murale	Vinterhalter et al. (2008)
	A. bertolonii	Ibañez et al. (2016)
Copper	*Rubia tinctorum*	Maitani et al. (1996)
	Hyptis capitata	Malik et al. (2016)
Zinc	*Solanum nigrum*	Subroto et al. (2007)
Uranium	*Daucus carota*	Straczek et al. (2009)
	Armoracia rusticana	Soudek et al. (2011)
	Brassica juncea	Flocco and Giulietti (2007)
	Chenopodium amaranticolor	
Arsenic	*N. tabacum*	Talano et al. (2014)
	V. zizanioides	Moogouei (2018)
Chromium	*B. napus* HR and *Pantoea* sp. FC 1	Ontanon et al. (2014)
Cadmium and lead	*B. juncea*	Eapen et al. (2007)
Cadmium	*(M. oleifera T. latifolia C. proxmus)1*	Ghada et al. (2017)), Flocco and Giulietti (2007), Malik et al. (2016)
	(Beta vulgaris Nicotiana tabacum Solanum nigrum Thlaspi caerulescens)2	
	Adenophora lobophylla	
	A. potaninii	
Cr(VI)	*Lemna minuta Kunth*	Paisio et al. (2018)
Nitrate	*V. zizanioides*	Moogouei (2018)
Metformin and cesium	*A. chlorostachys*	Moogouei (2018)
Zinc and nickel	*B. juncea*	Ismail and Theodor (2012)
Copper	*N. tabacum*	Perez-Palacios (2015)
Chromium	*B. napus*	Perotti et al. (2020)
Phenol and its derivatives		
Phenol	*B. juncea*	Singh et al. (2006)
	Raphanus sativus	
	Azadirachta indica	
	Beta vulgaris	
	B. napus	Coniglio et al. (2008)
	Lycopersiconesculentum	Gonzalez et al. (2006); Oller et al. (2005)
	Armoracia lapathifolia A. lapathifolia	Flocco and Giulietti (2007)
	Tomato hairy roots Dacus carota	González et al. (2006)

(continued)

Table 2.1 (continued)

Heavy metals	Hairy roots	References
2,4-Dichlorophenol	*B. napus*	Agostini et al. (2003)
	Nicotiana tabacum	Angelini et al. (2014)
N-acetyl-4-aminophenol	*Armoracia rusticana*	Huber et al. (2009)
Polychlorinated biphenyls	*Solanum nigrum*	Mackova et al. (1997)
Tetracycline and oxytetracycline	*Helianthus annuus*	Gujarathi et al. (2005)
Dichloro-diphenyl-trichloroethane	*B. juncea*	Suresh et al. (2005)
	Cichorium intybus	
Guaiacol, catechol, phenol, 2-chlorophenol, and 2,6-dichlorophenol	*Daucus carota*	De Araujo et al. (2002), De Araujo et al. (2006)
	Ipomoea batatas	
	Solanum aviculare	
TNT	*M. aquaticum*	Hughes et al. (1996)
	M. spicatum	
	Catharanthus roseus	
TNT	*Catharanthus roseus*	Bhadra et al. (1999)
Pesticides		
Dichloro-diphenyl-trichloroethane (DDT)	*B. juncea*	Suresh et al. (2005)
	Cichorium intybus	
Explosives		
Tri-nitro-toluene (TNT)	*Catharanthus roseus*	Hughes et al. (1996)
Explosives (DNT, TNT; ADNTs; DANTs)	*A. rusticana*	Nepovím et al. (2004)
RDX and HMX	*Catharanthus roseus*	Malik et al. (2016)
TCE	*Atropa belladonna*	Malik et al. (2016)
Azo dyes		
Reactive red 198 (RR198)	*Tagetes patula*	Patil et al. (2009)
Reactive green 19 A HE4BD	*Sesuvium portulacastrum*	Lokhande et al. (2015)
Reactive black 8	*Physalis minima*	Jha et al. (2014)
Acid red 114 (AR114)	*Ipomoea carnea*	Jha et al. (2016)
Reactive red 120	*Helianthus annuus*	Srikantan et al. (2018)
Methyl Orange	*B. juncea*	Telke et al. (2011)
Green 19A- HE4BD	Sesuvium *portulacastrum*	Lokhande et al. (2015)

Abbreviations from the table: *TNT* Trinitrotoluene, *TCE* Trichloroethylene, *RDX* Royal demolition explosive, *HMX* High melting explosive

Level – III: In this phase, transportation and accumulation of soluble contaminations will be done into vacuole organelle or they can bound to cell wall and diminished.

The HRCs perhaps also be deliberated as prescreening for plants with improved potential for phytoremediation (Suza et al. 2008). So many applications including the capability of rapidly growing under microbe-free culture conditions, with an increased surface area of contact in the middle of contaminant and plant tissues, and genetically and metabolic consistency as compared to wild type have been provided for HRs applications in phytoremediation process (Gujarathi et al. 2005; Georgiev et al. 2007). Transformed roots are amenable to genetic modifications and may advance the characterization of genes that regulate the phytoremediation potentials of plants. In addition, the expression of appropriate genes in plant root system improves the rhizo-degradation of extremely recalcitrant compounds, for example, PAHs and PCBs, etc. (Abhilash et al. 2009; Gerhardt et al. 2009).

Another advantage of using HRs for studying about the phytoremediation system is their capability to develop large quantities of root exudates that are consisting of enzymes and few kinds of metal-chelating substances that may detoxify or cutoff the adverse organic and inorganic pollutants in the soil (Bais et al. 2006; Doty 2008; Gujarathi et al. 2005). Flocco et al. (1998) reported in *A. lapathifolia* and concluded that roots comprise major levels of peroxidase enzymes (E.C. 1.11.1.7) that are recognized to be concerned with the detoxification process of phenols and some other aromatic molecules. Among all the toxic compounds that contaminate the water and agricultural soils, polychlorinated biphenyls (organic chlorine molecules) and some inorganics, in particular, HMs and radionuclides (an atom that has excess nuclear energy) have been the important issues in phytoremediation investigation. These important characteristics made HRCs have been referred to as an exceptional experimental model to remediate organic and inorganic pollutants because of their biochemical and genetic stability through cost-effective phytoremediation treatments as phytostabilization, phytovolatilization, rhizofiltration, phytodegradation, and phytoextraction (Upadhyay et al. 2019; Majumder and Jha 2012). Several reports have concluded and reported that HRs originated from various kinds of plant species can be exploited for the treatment of different organic and inorganic pollutants including heavy metals, dyes, ions, pesticides, excess nutrients, and solvents (Fig. 2.1).

2.1 Phytoremediation for Heavy Metal Degradation

HMs are characterized as metallic components that have comparatively highly thick contrary to water (Tchounwou et al. 2012). There are 59 elements categorized as HMs on the terrestrial crust, which are discharged into the environment through man-made activities or by natural constituents. Among those five HMs viz., copper, chromium, cadmium, zinc, and lead are believed to be extremely harmful (Shaban et al. 2016). HMs may cause the brain to injure and also creates several other disorders in living beings. These HMs cannot be humiliated easily and their remediation from the environment is very essential. In this situation, the application of advanced biological methods especially HRCs is the only alternative and gives more attention since they are believed to be renewable, eco-friendly, and produce valuable credentials for enzymatic reactions (Jadhav et al. 2009).

HMs contamination of soils is one of the particularly relevant environmental issues throughout the world (Doumett et al. 2008; Nouri et al. 2006). Consequently,

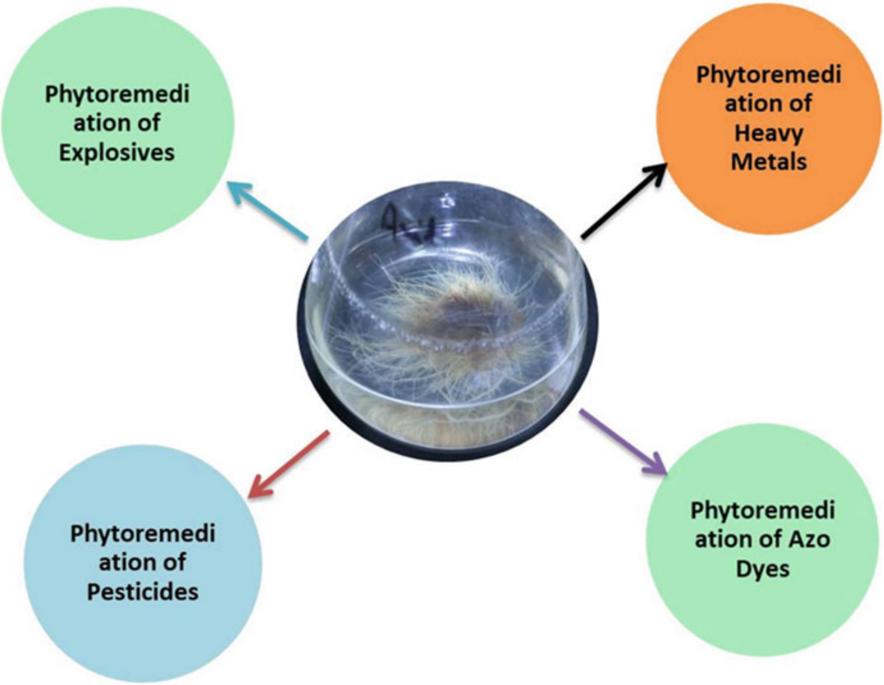


Fig. 2.1 Source of hairy roots for Phytoremediation of various organic and inorganic pollutants

the direct discharge of recycling wastewater for horticultural and agricultural usage and is considered as an impersonating potential threat to the health of the population (Yapoga et al. 2013). Reverse osmosis, chemical oxidation, coagulation-flocculation, filtration, adsorption, photodegradation, and advanced oxidation methods have been reported for dye decolorization and degradation, but high cost, generation of high-risk by-products, and high amounts of energy demand, have restricted the application of these approaches (Joo et al. 2007; Bizani et al. 2006; Crini 2006). In order to study the physiological mechanisms, research on different plant species has been carried out on the toxicity of cadmium to HRs. In all these research studies, HRCs demonstrate an admirable ability to absorb HM cadmium and to eradicate the contagiousness of this compound (Shi et al. 2012; Subroto et al. 2007; Vinterhalter et al. 2008).

2.2 *Phytoremediation of Phenols*

HRCs have been investigated for phytoremediation of different kinds of pollutants like dichlorophenol (a chlorinated by-product of phenol) by *Brassica napus* (Agostini et al. 2003), dichloro-diphenyl-trichloroethane (DDT) by *B. juncea* and

Cichorium intybus (Suresh et al. 2005), phenol by *Helianthus annuus* (Jha et al. 2013). Phenol is the most important natural contaminant in environment through coal conversion process, petroleum refineries, pesticides, and petrochemical products (Jha et al. 2020). As individuals learn about the dangers of the contaminants, they can share their knowledge with others. However, the lack of resources available to people in less developed countries can impact significantly how much of a difference education can make. Often, individuals in less developed areas are more focused on surviving day-to-day than on the possible long-term effects of hazardous chemicals (Russell 2005). Moreover, the proficiency of plants to metabolize harmful substances will rely on the biochemical properties of metabolizing enzymes and different defensive mechanisms that may extend the plant tissue survival rate. In fact, the results from a comparative study of peroxidase enzymes from HRs of *Daucus carota*, *Ipomoea batatas*, and *Solanum aviculare* evidenced an interspecific divergence in the priority for chlorophenol and phenol among the peroxidases (de Araujo et al. 2004). Also, peroxidase isozymes engaged in the degradation of phenol compounds within a species may indicate the difference in substrate selection and the efficiency catalytic activity of phenol metabolism (Coniglio et al. 2008). This is considering that these investigations are essential in creating awareness about the enzymatic mechanisms of pollutant remediation for choosing selective enzymes that might be developed in large quantities and used as catalysts for breakdown of the contaminants (Gonzalez et al. 2006).

2.3 Phytoremediation of Xenobiotic Compounds

The HRCs are known for their fast growth, high metabolic activity, and genetic as well as biochemical stability have been exploited for studies on biotransformation of various xenobiotics and were proven to be very effective (Giri and Lakshmi Narasu 2000). The detonating materials viz., TNT and hexahydro-1,3,5-trinitro-1,3,5-triazine are extensive environmental pollutants frequently identified as sub-contaminants in the army training fields (Rylott et al. 2011). Degradation of the exploding TNT to further components through *Catharanthus roseus* HRCs was reported by Hughes et al. (1996). Similarly, the biotransformation of anthracene by HRCs of *Medicago sativa* and found that the root concentration factors were higher than that of whole plants (Paul and Campanella 2000). The well-organized nature of HRCs offers additional benefits, making them further susceptible for growing in large-scale levels using bioreactors to understand the mechanisms in detail. Phytoremediation of DDT to DDD and DDE has been claimed using the cell-suspension cultures of *Glycine max* and *Triticum aestivum* (Arjmand and Sandermann Jr 1985; Scheel and Sandermann 1977). The common remediation pathway of DDT involves subtractive dechlorination to DDD followed by dihydrochlorination to DDE. The DDE was evidenced to be further humiliated to DDMU through a dechlorination reaction (Hay and Focht 1998; Quensen et al. 1998). According to Suresh et al. (2005), *Cichorium intybus* and *B. juncea* HRCs are stable for the phytoremediation of insecticide DDT and concluded that DDT did not cause growth inhibition in HRCs of *C. intybus and B. juncea* at the added concentration. These cultures confirmed that the growth pattern, biomass, and differentiation are comparable to those of nontreated controls. Similarly, Scheel and Sandermann (1977) reported in parsley and soybean and observed rapid uptake of DDT. It has been suggested that the transformation of examined pesticides in the plants mostly occurs via direct oxidation and subsequent conjugation pathways (Kurashvili et al. 2016). It is estimated and reported by the US department of defense that, between 16 billion to 160 billion needed for clean-up of unexploded munitions (U.S. GAO 2014).

2.4 Phytoremediation of Azo Dyes

Rapid industrial improvement particularly from pharmaceutical, fabric, food processing units, leather, and agricultural-based industries is a matter of serious concern for causing environmental pollution. Almost 10,000 different textile dyes such as reactive, disperse, basic, etc. are used for coloring and printing purposes by processing industries (Jha et al. 2020). Mainly, 90% of the dyeing process involves from the overall textile-released liquid effluents, which consists of a mixture of various pollutants viz., organochlorines, surfactants acids or bases, HMs lead, salts, phthalates, suspended solids, dyes, and several other chemicals (Zaharia and Suteu 2013). The main dye used frequently in textile is azo dye as a result of their superior features, durability against microbial decomposition and higher photolytic strength, and risk to the environment and human health in view of their renowned issues such as mutagenicity, carcinogenic effects, and toxicity (Forss and Welander 2011; Mansour et al. 2011). Taking all of this into account, HRs technology is an alternative to physical and conventional methods for detoxification (Golob et al. 2005). Textile dyes degradation was successfully reported with *Tagetes patula* and *Physalis minima* HRCs by inducting the enzymes DCIP reductase and azo reductase (Jha et al. 2015; Patil et al. 2009). The phytotoxicity experiments demonstrated that the nonhazardous quality of degraded dyes by using HRs of *P. minima* and *Sesuvium portulacastrum* (Jha et al. 2014; Lokhande et al. 2015). Jha et al. (2016) reported in *Ipomoea carnea* that synergetic activity of oxidative and reductive enzymes discharged from the HRCs may be the reason for the phytoremediation of the dyes. In another dye degradation report, the dye remediation was very effective with reactive red 120 as inducer in HRCs of *H. annuus* with unique attention on the effect of light on adsorption equilibrium and dye-degradation kinetics (Srikantan et al. 2018). It has been reported that *B. juncea* HRCs increase the decolonization of methyl orange to 92% within 4 days of the incubation period. The enzyme assay of HRs obtained after the decolorization of methyl orange indicated considerable intracellular laccase activity.

2.5 Hairy Roots in Other Environmental Applications

In view of the increased demand for SMs, particularly as drug precursors, there is a need to develop new strategies for production. Production of genetically stable HRCs from many plant species has opened up the possibilities for fundamental studies and a lot of feasible applications of biotechnology. Since the main obstacle with in vitro cell cultures is that the transformants are genetically unstable from generation to generation, tend to produce low yields of SM and time-consuming process (Giri and Narasu 2000). HRCs importance for SMs in pharmaceutical, cosmetics, and various fields is increased. In this respect, hairy or transformed root cultures have plenty of advantages compared to the normal ones such as genetic and biochemical stability and need only regular media (Pitta–Alvarez et al. 2000). The SMs formed by HRCs are the same as those usually synthesized in mother plants with a higher yield (Karuppusamy 2009). Many reports are available on the importance of HRCs in SMs production and other applications (Boobalan and Kamalanathan 2020; Makhzoum et al. 2013; Moola and Diana 2019; Shi et al. 2020). The plausible mechanism of HRs to study uptake, metabolism, and removal of organic pollutants was described in Figs. 2.2 and 2.3.

Another important aspect of using HRs is biofuel, it was the renewable energy produced from animal fats and vegetable oils. Biofuels and bio-based chemicals produced from renewable resources have come into existence as a reliable technology for the degradation of fossil-fuel-dependent resources, which report on concerns about their global environment pollution (Habibi et al. 2017). It helped to decrease environmental pollution than fossil-fuel. To improve the content of the oil and

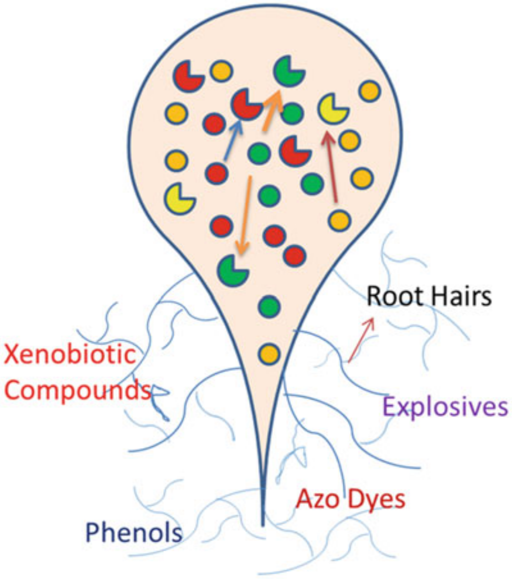


Fig. 2.2 The hypothesis of environmental contaminants (red, green, and yellow) may initiate the reactive oxygen species in hairy roots; it will help in the enhanced production of metabolizing enzymes that can be used for decontamination

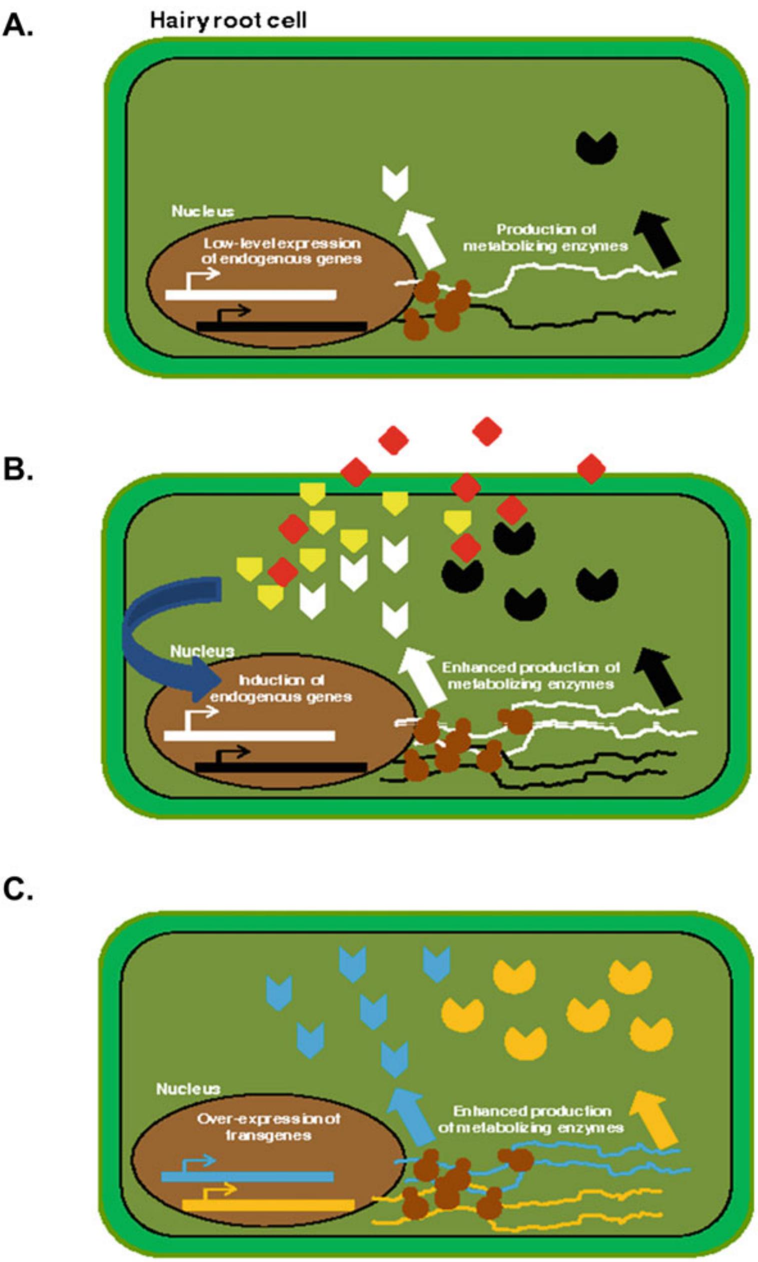


Fig. 2.3 Metabolism of environmental contaminants by hairy root cells
(**a**) a cartoon depiction of a hairy root cell expressing contaminant metabolizing enzymes (white chevron and black pie) at basal levels; (**b**) environmental contaminants (red diamonds) may promote the production of reactive oxygen species (yellow pentagon), the enhanced production of ROS scavenging enzymes and antioxidants (white chevron), and/or contaminant metabolizing enzymes (black pie); (**c**) the expression of transgenes of animal or plant origin may also result in the enhanced production of contaminant metabolizing enzymes (blue chevron and orange pie) and phytoremediation capacity of plants (Suza et al. 2008)

economic properties of plants, genetic engineering is the most powerful tool to develop plants for achieving this purpose. *Jatropha curcas* is a potential biodiesel crop that contains toxic compounds such as curcin, phorbol esters, trypsin inhibitors, lectin, and phytate (Annarao et al. 2008). The explants of *J. curcas* (leaf, cotyledons, and embryonic axes) were treated with *A. rhizogenes* and gene transformed cultures were undergoing to direct or indirect organogenesis. After the organogenesis, the foreign genes were integrated into transformed plant shoots (Habibi et al. 2017). Only a few reports are focused on developing plant varieties for biofuel production, researchers may focus on HRs for increasing the biodiesel production.

3 Hairy Roots Versus Normal Roots

A. rhizogenes is established for the HRs induction of plants that helps to produce plentiful adventitious roots (Largia et al. 2016). The HRs can be developed in vitro, without the rhizogenic bacterium, and they usually grow greater than regular roots of the identical plant. Plant roots help mainly in phytoextraction process by acting as a channel for the absorption of contaminants, which is then translocated through the vascular system and concentrated in plant harvestable tissues (Doty 2008). Since, biotransformation is a technique of altering the functional groups of xenobiotics to produce specific products, the use of enzymes to remediate the contaminants was reported by Krings and Berger (1998). Prior to entering the pollutants and at the later stage to the root system, the contaminants may turn into the target for decontamination through internal plant metabolites in a method known as phytodegradation (Boominathan et al. 2004). Since the root system is the key source for up taking the contaminants either in the soil or water, it may provide a key point for the assessment of the phytoremediation potential (Suza et al. 2008). Investigations by many researchers effectively investigated the mechanism (Table 2.1) for accumulation, absorption, distribution, tolerance, and detoxification of lethal compounds through HRCs. Talano et al. (2020) reported that bioremediation study using HR system will be beneficial to measure the potential of certain plant species and to estimate the outcome of natural roots that are indirectly attached to ground soil and its hazardous pollutants. They have advantages like a wider area of exterior contact between pollutants and tissues, rapid in vitro growth, and greater genetic as well as metabolic balance with respect to its wild type roots. In addition, HRs have the ability to take up a high concentration of toxins without any adverse effects in comparison with normal roots. More differences between wild type and HRs were tabulated in Table 2.2. Most of the reports about HRs induction through *A. rhizogenes* proved that the transformed HRCs showed higher root biomass contrary to normal root biomass, and the ratio of lateral root formation is very rapid in HRCs than normal cultures on hormone-free culture media (Rency et al. 2019). Normal roots also can induce SMs, however, the HRCs show much more growth kinetics concerning its

Table 2.2 Difference between tap, adventitious, and hairy root system

Tap root system	Adventitious root system	Hairy root system
It develops from the radical of the embryo	It develops from part of the plant other than the radical or not derivatives	It can be developed by using *A. rhizogenes* transformation
Genetically metabolically less stable and not comparative	Genetically metabolically not stable	Genetically metabolically high stable and cellular differentiation
It has a persistent primary root known as the taproot	The primary root is short-lived	Root has the ability to grow rapidly
The system grows deep into the soil	If underground, do not grow into the soil	Root system does not follow the geotropism
The tap root system is always underground	The adventitious root system may be underground or aerial	Hairy root system never be underground
The main root called as taproot and lateral branches called as secondary roots this in-turn produce tertiary roots and so on	A number of main roots developed at one spot and all roots are similar thicknesses	A number of main roots developed at different spots where the bacteria were wounded and all roots are similar thicknesses.
Not able to produce a large number of exudates/enzymatic machinery	Ability to produce a minimum quantity of exudates/enzymatic machinery but there is a variation	Ability to produce a large number of exudates/enzymatic machinery but there is a variation

rapid growth, root biomass generation, higher lateral root forming, and improved accumulation of bioactive compounds (Chandra and Chandra 2011; Mukundan and Hjortso 1990).

4 Conclusion and Way Forward

Considering these backgrounds, it is concluded that, HRs, enzymes, or metabolites of HRCs provides complementary information to understand the industrial cleanup process. However, the broad research on HRs, and the complete transformation of toxic to nontoxic is still unknown. The use of HRs extracts or enzymes definitely can be a substitute to pure enzymes since these are highly expensive to acquire and might be deactivated more promptly in the course of reaction with a subsequent decrease of its activity. These results will not only help in understanding about the enzymatic process that involved in phytoremediation of phenols, HMs, explosives, and dyes but also helps in the understanding of the complex interaction between toxic chemicals, plant cells, and microorganisms to clean up the environment and to design the novel transgenic plants with improved remediation traits.

In order for their future application, it is important and necessary to select an optimal condition to obtain the most effective removal by considering the economy of the process. In addition, transgenic plants also help in mineralize major complex compounds and also, it is expected that considering the recent advances in genetics, proteomics, and metabolomics and novel detoxifying enzymes could be identified and expressed into plants allowing the host plant to have a wider range of phytoremediation capabilities. Even, phyto-technologies have contributed significantly to mitigate and control environmental pollution. Still, more research has to be done and even many challenges have to overcome on the enhancement of pollution uptake and decontamination process which is an important challenge for current research.

References

Abhilash PC, Jamil S, Singh N (2009) Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics. Biotechnol Adv 27(4):474–488

Agostini E, Coniglio MS, Milrad SR, Tigier HA, Giulietti AM (2003) Phytoremediation of 2, 4-dichlorophenol by Brassica napus hairy root cultures. Biotechnol Appl Biochem 37 (2):139–144

Agostini E, Talano MA, González PS, Oller ALW, Medina MI (2013) Application of hairy roots for phytoremediation: what makes them an interesting tool for this purpose? Appl Microbiol Biotechnol 97(3):1017–1030

Anees M, Qayyum A, Jamil M, Rehman FU, Abid M, Malik MS, Yunas M, Ullah K (2020) Role of halotolerant and chitinolytic bacteria in phytoremediation of saline soil using spinach plant. Int J Phytoremediation 22(6):653–661

Angelini VA, Agostini E, Medina MI, González PS (2014) Use of hairy roots extracts for 2, 4-DCP removal and toxicity evaluation by Lactuca sativa test. Environ Sci Pollut Res 21(4):2531–2539

Annarao S, Sidhu OP, Roy R, Tuli R, Khetrapal CL (2008) Lipid profiling of developing Jatropha curcas L. seeds using 1H NMR spectroscopy. Bioresour Technol 99(18):9032–9035

Arjmand M, Sandermann H Jr (1985) Metabolism of DDT and related compounds in cell suspension cultures of soybean (Glycine max L.) and wheat (Triticum aestivum L.). Pestic Biochem Physiol 23(3):389–397

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Betts KS (1998) Technology update: getting to the root phytoremediation. Environ Sci Technol 32 (1):22A–22A

Bhadra R, Wayment DG, Hughes JB, Shanks JV (1999) Confirmation of conjugation processes during TNT metabolism by axenic plant roots. Environ Sci Technol 33(3):446–452

Bizani E, Fytianos K, Poulios I, Tsiridis V (2006) Photocatalytic decolorization and degradation of dye solutions and wastewaters in the presence of titanium dioxide. J Hazard Mater 136(1):85–94

Boobalan S, Kamalanathan D (2020) Tailoring enhanced production of aervine in Aerva lanata (L.) Juss. Ex Schult by *Agrobacterium rhizogenes*-mediated hairy root cultures. Indus Crops Prod 155:112814

Boominathan R, Doran PM (2003) Cadmium tolerance and antioxidative defenses in hairy roots of the cadmium hyperaccumulator, Thlaspi caerulescens. Biotechnol Bioeng 83(2):158–167

Boominathan R, Saha Chaudhury NM, Sahajwalla V, Pauline MD (2004) Production of nickel bio-ore from hyperaccumulator plant biomass: applications in phytomining. 86:243–250

Chandra S (2012) Natural plant genetic engineer *Agrobacterium rhizogenes*: role of T-DNA in plant secondary metabolism. Biotechnol Lett 34(3):407–415

Chandra S, Chandra S (2011) Engineering secondary metabolite production in hairy roots. Phytochem Rev 10:371–395

Chen Z, Huang Z, Cheng Y, Pan D, Pan X, Yu M, Pan Z, Lin Z, Guan X, Wu Z (2012) Cr (VI) uptake mechanism of Bacillus cereus. Chemosphere 87(3):211–216

Coniglio MS, Busto VD, González PS, Medina MI, Milrad S, Agostini E (2008) Application of Brassica napus hairy root cultures for phenol removal from aqueous solutions. Chemosphere 72 (7):1035–1042

Crini G (2006) Non-conventional low-cost adsorbents for dye removal: a review. Bioresour Technol 97(9):1061–1085

De Araujo BS, Charlwood BV, Pletsch M (2002) Tolerance and metabolism of phenol and chloroderivatives by hairy root cultures of Daucus carota L. Environ Pollut 117(2):329–335

de Araujo BS, de Oliveira JO, Machado SS, Pletsch M (2004) Comparative studies of the peroxidases from hairy roots of Daucus carota, Ipomoea batatas and Solanum aviculare. Plant Sci 167(5):1151–1157

De Araujo BS, Dec J, Bollag JM, Pletsch M (2006) Uptake and transformation of phenol and chlorophenols by hairy root cultures of Daucus carota, Ipomoea batatas and Solanum aviculare. Chemosphere 63(4):642–651

Doran PM (2009) Application of plant tissue cultures in phytoremediation research: incentives and limitations. Biotechnol Bioeng 103(1):60–76

Doty SL (2008) Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179(2):318–333

Doumett S, Lamperi L, Checchini L, Azzarello E, Mugnai S, Mancuso S, Petruzzelli G, Del Bubba M (2008) Heavy metal distribution between contaminated soil and Paulownia tomentosa, in a pilot-scale assisted phytoremediation study: influence of different complexing agents. Chemosphere 72(10):1481–1490

Eapen S, Singh S, D'souza SF (2007) Advances in development of transgenic plants for remediation of xenobiotic pollutants. Biotechnol Adv 25(5):442–451

Flocco CG, Giulietti AM (2007) In vitro hairy root cultures as a tool for phytoremediation research. In: Phytoremediation. Humana Press, pp 161–173

Flocco CG, Alvarez MA, Guilietti AM (1998) Peroxidase production in vitro by Armoracia lapathifolia (horseradish)-transformed root cultures: effect of elicitation on level and profile of isoenzymes. Biotechnol Appl Biochem 28:33–38

Forss J, Welander U (2011) Biodegradation of azo and anthraquinone dyes in continuous systems. Int Biodeterior Biodegradation 65(1):227–237

Georgiev MI, Pavlov AI, Bley T (2007) Hairy root type plant in vitro systems as sources of bioactive substances. Appl Microbiol Biotechnol 74(6):1175

Georgiev MI, Agostini E, Ludwig-Müller J, Xu J (2012) Genetically transformed roots: from plant disease to biotechnological resource. Trends Biotechnol 30(10):528–537

Gerhardt KE, Huang XD, Glick BR, Greenberg BM (2009) Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. Plant Sci 176(1):20–30

Ghada M, Najla AI, Sajad MH, Fida A, Eltohami MS, Abdurahman HN, Elhassan HE, Elsaman T (2017) Efficacy of some sudanese medicinal plants extracts to remove heavy metals from water. Aust J Basic Appl Sci 11(3):51–55

Giri A, Lakshmi Narasu M (2000) Production of podophyllotoxin from Podophyllum hexandrum: a potential natural product for clinically useful anticancer drugs. Cytotechnology 34:17–26

Giri A, Narasu ML (2000) Transgenic hairy roots: recent trends and applications. Biotechnol Adv 18(1):1–22

Golob V, Vinder A, Simonič M (2005) Efficiency of the coagulation/flocculation method for the treatment of dyebath effluents. Dyes Pigments 67(2):93–97

Gonzalez PS, Capozucca CE, Tigier HA, Milrad SR, Agostini E (2006) Phytoremediation of phenol from wastewater, by peroxidases of tomato hairy root cultures. Enzym Microb Technol 39 (4):647–653

González PS, Capozucca CE, Tigier HA, Milrad SR, Agostini E (2006) Phytoremediation of phenol from wastewater, by peroxidases of tomato hairy root cultures. Enzym Microb Technol 39 (4):647–653

Gujarathi NP, Haney BJ, Park HJ, Wickramasinghe SR, Linden JC (2005) Hairy roots of Helianthus annuus: a model system to study phytoremediation of tetracycline and oxytetracycline. Biotechnol Prog 21(3):775–780

Gutierrez-Valdes N, Häkkinen ST, Lemasson C, Guillet M, Oksman-Caldentey KM, Ritala A, Cardon F (2020) Hairy root cultures—A versatile tool with multiple applications. Front Plant Sci 11

Habibi P, De Sa MFG, Makhzoum A, Malik S, da Silva ALL, Hefferon K, Soccol CR (2017) Bioengineering hairy roots: phytoremediation, secondary metabolism, molecular pharming, plant-plant interactions and biofuels. In: Sustainable agriculture reviews. Springer, Cham, pp 213–251

Hay AG, Focht DD (1998) Cometabolism of 1, 1-dichloro-2, 2-bis (4-chlorophenyl) ethylene by Pseudomonas acidovorans M3GY grown on biphenyl. Appl Environ Microbiol 64 (6):2141–2146

Huber C, Bartha B, Harpaintner R, Schröder P (2009) Metabolism of acetaminophen (paracetamol) in plants—two independent pathways result in the formation of a glutathione and a glucose conjugate. Environ Sci Pollut Res 16(2)

Hughes JB, Shanks J, Vanderford M, Lauritzen J, Bhadra R (1996) Transformation of TNT by aquatic plants and plant tissue cultures. Environ Sci Technol 31(1):266–271

Ibañez S, Talano M, Ontañon O, Suman J, Medina MI, Macek T, Agostini E (2016) Transgenic plants and hairy roots: exploiting the potential of plant species to remediate contaminants. New Biotechnol 33(5):625–635

Ismail AM, Theodor PA (2012) The effect of heavy metals Zn and Ni on growth of in vitro hairy root cultures of Indian mustard Brassica juncea L. Int J Adv Biotechnol Res 3:688–697

Jadhav SU, Ghodake GS, Telke AA, Tamboli DP, Govindwar SP (2009) Degradation and detoxification of disperse dye scarlet RR by Galactomyces geotrichum MTCC 1360. J Microbiol Biotechnol 19:409–415

Jha P, Jobby R, Kudale S, Modi N, Dhaneshwar A, Desai N (2013) Biodegradation of phenol using hairy roots of Helianthus annuus L. Int Biodeterior Biodegradation 77:106–113

Jha P, Modi N, Jobby R, Desai N (2014) Differential expression of antioxidant enzymes during degradation of azo dye reactive black 8 in hairy roots of L. Int J Phytoremediation 17(4):305–312

Jha P, Modi N, Jobby R, Desai N (2015) Differential expression of antioxidant enzymes during degradation of azo dye reactive black 8 in hairy roots of Physalis minima L. Int J Phytoremediation 17(4):305–312

Jha P, Jobby R, Desai NS (2016) Remediation of textile azo dye acid red 114 by hairy roots of Ipomoea carnea Jacq. and assessment of degraded dye toxicity with human keratinocyte cell line. J Hazard Mater 311:158–167

Jha P, Sen R, Jobby R, Sachar S, Bhatkalkar S, Desai N (2020) Biotransformation of xenobiotics by hairy roots. Phytochemistry 176:112421

Joo DJ, Shin WS, Choi JH, Choi SJ, Kim MC, Han MH, Ha TW, Kim YH (2007) Decolorization of reactive dyes using inorganic coagulants and synthetic polymer. Dyes Pigments 73(1):59–64

Karuppusamy S (2009) A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures. J Med Plants Res 3(13):1222–1239

Krings U, Berger RG (1998) Biotechnological production of flavours and fragrances. Appl Microbiol Biotechnol 49:1–8. https://doi.org/10.1007/s002530051129

Kurashvili MV, Adamia GS, Amiranashvili LL, Ananiasvili TI, Varazi TG, Pruidze MV, Gordeziani MS, Khatisashvili GA (2016) Targeting of detoxification potential of microorganisms and plants for cleaning environment polluted by organochlorine pesticides. Annal Agrarian Sci 14(3):222–226

Lal R (2020) Genetic engineering of plants for crop improvement. CRC Press

Largia MJV, Satish L, Johnsi R, Shilpha J, Ramesh M (2016) Analysis of propagation of Bacopa monnieri (L.) from hairy roots, elicitation and Bacoside A contents of Ri transformed plants. World J Microbiol Biotechnol 32(8)

Lokhande VH, Kudale S, Nikalje G, Desai N, Suprasanna P (2015) Hairy root induction and phytoremediation of textile dye, reactive green 19A-HE4BD, in a halophyte, Sesuvium portulacastrum (L.) L. Biotechnol Rep 8:56–63

Mackova M, Macek T, Kucerova P, Burkhard J, Pazlarova J, Demnerova K (1997) Degradation of polychlorinated biphenyls by hairy root culture of Solanum nigrum. Biotechnol Lett 19 (8):787–790

Maitani T, Kubota H, Sato K, Takeda M, Yoshihira K (1996) Induction of phytochelatin.(class III metal loth ionein) and incorporation of copper in transformed hairy roots of Rubia tinctorum exposed to cadmium. J Plant Physiol 147(6):743–748

Majumder A, Jha S (2012) Hairy roots: a promising tool for phytoremediation. In: Microorganisms in environmental management. Springer, Dordrecht, pp 607–629

Makhzoum AB, Sharma P, Bernards MA, Trémouillaux-Guiller J (2013) Hairy roots: an ideal platform for transgenic plant production and other promising applications. In: Phytochemicals, plant growth, and the environment. Springer, New York, pp 95–142

Malik S, Andrade SA, Mirjalili MH, Arroo RR, Bonfill M, Mazzafera P (2016) Biotechnological approaches for bioremediation: in vitro hairy root culture. In: Transgenesis and secondary metabolism, vol 1

Mansour HB, Mosrati R, Ghedira K, Chekir-Ghedira L (2011) Decolorization of textile wastewater by Pseudomonas putida: toxicity assessment. Environ Eng Sci 28(7):489–495

Mehrotra S, Mishra S, Srivastava V (2020) Hairy roots biotechnology unzipped: a journey of reality and promises. In: Hairy root cultures based applications. Springer, Singapore, pp 1–10

Moogouei R (2018) Use of terrestrial plants for phytoremediation of pollutants from solutions. Iran J Sci Technol Trans A: Sci 42(4):1753–1759

Moola AK, Diana RKB (2019) Elicitation as a means for enhanced plant secondary metabolites through hairy root system. J Adv Sci Res 10(3)

Mukundan U, Hjortso MA (1990) Thiophene content in normal and transformed root cultures of tagetes erects: A comparison with Thiophene content in roots of intact plants. J Exp Bot 41:1497–1501

Nepovím A, Podlipná R, Soudek P, Schröder P, Vaněk T (2004) Effects of heavy metals and nitroaromatic compounds on horseradish glutathione S-transferase and peroxidase. Chemosphere 57(8):1007–1015

Nouri J, Mahvi AH, Babaei A, Ahmadpour E (2006) Regional pattern distribution of groundwater fluoride in the shush aquifer of Khuzestan County, Iran. Fluoride 39(4):321

Oller ALW, Agostini E, Talano MA, Capozucca C, Milrad SR, Tigier HA, Medina MI (2005) Overexpression of a basic peroxidase in transgenic tomato (Lycopersicon esculentum mill. cv. Pera) hairy roots increases phytoremediation of phenol. Plant Sci 169(6):1102–1111

Ontanon OM, González PS, Ambrosio LF, Paisio CE, Agostini E (2014) Rhizoremediation of phenol and chromium by the synergistic combination of a native bacterial strain and Brassica napus hairy roots. Int Biodeterior Biodegradation 88:192–198

Paisio CE, Fernandez M, González PS, Talano MA, Medina MI, Agostini E (2018) Simultaneous phytoremediation of chromium and phenol by Lemna minuta Kunth: a promising biotechnological tool. Int J Environ Sci Technol 15(1):37–48

Patil P, Desai N, Govindwar S, Jadhav JP, Bapat V (2009) Degradation analysis of reactive red 198 by hairy roots of tagetes patula L.(Marigold). Planta 230(4):725–735

Paul R, Campanella B (2000) Use of Alfalfa (Medicago sativa L.) to stimulate biodegradation of anthracene in dredging sludges. In: Inter-COST workshop on bioremediation, COST Action, vol 831

Perez-Palacios P (2015) Environmental Biotechnology, development of biotechnological strategies for the bioremediation of heavy metals. Spain*:* Doctoral thesis

Perotti R, Paisio CE, Agostini E, Fernandez MI, González PS (2020) CR (VI) phytoremediation by hairy roots of Brassica napus: assessing efficiency, mechanisms involved, and post-removal toxicity. Environ Sci Pollut Res:1–10

Pitta–Alvarez SI, Spollansky TC, Giulietti AM (2000) The influence of different biotic and abiotic elicitors on the production and profile of tropane alkaloids in hairy root cultures of Brugmansia candida. Enzym Microb Technol 26(2–4):252–258

Praveen N, Thiruvengadam M, Yang YS, Kim SH, Murthy HN, Chung IM (2014) Production of gymnemic acid from hairy root cultures of Gymnema sylvestre R. Br. as influenced by polyunsaturated fatty acids (PUFAs) and their antioxidant activity. Ind Crop Prod 54:54–61

Quensen JF, Mueller SA, Jain MK, Tiedje JM (1998) Reductive dechlorination of DDE to DDMU in marine sediment microcosms. Science 280(5364):722–724

Rency AS, Pandian S, Kasinathan R, Satish L, Swamy MK, Ramesh M (2019) Hairy root cultures as an alternative source for the production of high-value secondary metabolites. In: Natural bio-active compounds. Springer, Singapore, pp 237–264. https://doi.org/10.1007/978-981-13-7438-8_10

Russell K (2005) The use and effectiveness of phytoremediation to treat persistent organic pollutants. US Environmental Protection Agency Office of Solid Waste and Emergency Response Technology Innovation and Field Services Division

Rylott EL, Budarina MV, Barker A, Lorenz A, Strand SE, Bruce NC (2011) Engineering plants for the phytoremediation of RDX in the presence of the co-contaminating explosive TNT. New Phytol 192(2):405–413

Satish L, Rency AS, Muthubharathi BC, Shamili S, Rameshkumar R, Swamy MK, Ramesh M (2019) Transgenic plant cell cultures: a promising approach for secondary metabolite production. In: Natural bio-active compounds. Springer, Singapore, pp 79–122

Scheel D, Sandermann H (1977) Metabolism of DDT and Kelthane in cell suspension cultures of parsley (*Petroselinum hortense*, Hoffm.) and soybean (Glycine max L.). Planta 133(3):315–320

Schröder P, Scheer CE, Diekmann F, Stampfl A (2007) How plants cope with foreign compounds. Translocation of xenobiotic glutathione conjugates in roots of barley (*Hordeum vulgare*) (9 pp). Environmental Science and Pollution Research-International 14(2):114–122

Shaban NS, Abdou KA, Hassan NEHY (2016) Impact of toxic heavy metals and pesticide residues in herbal products. Beni-Suef Univ J Basic Appl Sci 5(1):102–106

Shi H, Wang Y, Tsang PE, Chan LA (2012) Alleviated affect of exogenous CaCl2 on the growth, antioxidative enzyme activities and cadmium absorption efficiency of Wedelia trilobata hairy roots under cadmium stress. Sheng wu gong cheng xue bao= Chinese J Biotechnol 28 (6):747–762

Shi M, Wang Y, Wang X, Deng C, Cao W, Hua Q, Kai G (2020) Simultaneous promotion of tanshinone and phenolic acid biosynthesis in Salvia miltiorrhiza hairy roots by overexpressing Arabidopsis MYC2. Ind Crop Prod 155:112826

Singh S, Melo JS, Eapen S, D'souza SF (2006) Phenol removal using Brassica juncea hairy roots: role of inherent peroxidase and H2O2. J Biotechnol 123(1):43–49

Soudek P, Petrová Š, Benešová D, Vaněk T (2011) Uranium uptake and stress responses of in vitro cultivated hairy root culture of Armoracia rusticana. Agrochimica 55(1):15–28

Srikantan C, Suraishkumar GK, Srivastava S (2018) Effect of light on the kinetics and equilibrium of the textile dye (reactive red 120) adsorption by Helianthus annuus hairy roots. Bioresour Technol 257:84–91

Straczek A, Wannijn J, Van Hees M, Thijs H, Thiry Y (2009) Tolerance of hairy roots of carrots to U chronic exposure in a standardized in vitro device. Environ Exp Bot 65(1):82–89

Subroto, M.A., Priambodo, S. and Indrasti, N.S., 2007. Accumulation of zinc by hairy root cultures of Solanum nigrum

Sujatha G, Zdravković-Korać S, Ćalić D, Flamini G, Kumari BR (2013) High-efficiency *Agrobacterium rhizogenes*-mediated genetic transformation in Artemisia vulgaris: hairy root production and essential oil analysis. Ind Crop Prod 44:643–652

Suresh B, Sherkhane PD, Kale S, Eapen S, Ravishankar GA (2005) Uptake and degradation of DDT by hairy root cultures of Cichorium intybus and Brassica juncea. Chemosphere 61 (9):1288–1292

Suza W, Harris RS, Lorence A (2008) Hairy roots: from high-value metabolite production to phytoremediation. Electron J Integr Biosci 3(1):57–65

Talano MA, Oller ALW, González P, González SO, Agostini E (2014) Effects of arsenate on tobacco hairy root and seedling growth, and its removal. Vitro Cellular & Developmental Biology-Plant 50(2):217–225

Talano MA, Ibañez S, Sosa Alderete LG, Wevar Oller AL, Vezza ME, Medina MI, Agostini E (2020) Increasing knowledge about phytoremediation through a practical approach for undergraduate students. J Biol Educ:1–7

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

Telke AA, Kagalkar AN, Jagtap UB, Desai NS, Bapat VA, Govindwar SP (2011) Biochemical characterization of laccase from hairy root culture of Brassica juncea L. and role of redox mediators to enhance its potential for the decolorization of textile dyes. Planta 234 (6):1137–1149

U.S. GAO – DOD operational ranges: more reliable cleanup cost estimates and a proactive approach to identifying contamination are needed. [WWW Document], 2014. https://www.gao.gov/products/GAO-04-6014.28.20

Upadhyay, A.K., Singh, D.P., Singh, N.K., Pandey, V.C. and Rai, U.N., 2019. Sustainable phytoremediation strategies for river water rejuvenation . In Phytomanagement of polluted sites (pp. 301-311). Elsevier

Vinterhalter B, Savić J, Platiša J, Raspor M, Ninković S, Mitić N, Vinterhalter D (2008) Nickel tolerance and hyperaccumulation in shoot cultures regenerated from hairy root cultures of Alyssum murale Waldst et Kit. Plant Cell Tissue Organ Cult 94(3):299–303

Wilson SA, Roberts SC (2012) Recent advances towards development and commercialization of plant cell culture processes for the synthesis of biomolecules. Plant Biotechnol J 10(3):249–268

Yapoga S, Ossey YB, Kouame V (2013) Phytoremediation of zinc, cadmium, copper and chrome from industrial wastewater by eichhornia crassipes. Int J Conserv Sci 4(1)

Zaharia C, Suteu D (2013) Coal fly ash as adsorptive material for treatment of a real textile effluent: operating parameters and treatment efficiency. Environ Sci Pollut Res 20(4):2226–2235

Chapter 3
Pretreatment of Wheat Straw Using Ionic Liquids for Bioethanol Production: A Review

Ibsa Neme and Chandran Masi

Abstract Ethanol production from sustainable and inexpensive farming residues decreases greenhouse gas emissions, for instance, carbon dioxide, nitrogen dioxide, sulfur oxide, and eliminate smog from the atmosphere. A certain number of agricultural residues and plentifully misused materials are oat straw, rice straw, and wheat straw; waste potato, switchgrass, corn steep liquor, sweet sorghum, bagasse, sugar beet tubers, etc. are used as potential material for bioethanol making in different countries. Wheat straw deposits are one among the residues produced in substantial amounts globally, including Ethiopia. However, the elimination of lignin out of lignocelluloses untreated residues is the most critical step. Various pretreatment methods are explained before by numerous researchers with chemical, physical, biological, and ionic liquid pretreatment. This review carefully evaluates the performance of highly capable and recent techniques of hydrolysis and discusses the potential of wheat straw residue for bioethanol production, using less energy-demanding and more eco-friendlier techniques than the recent commercial pretreatment techniques. The most popular of these is the ionic liquids (ILs). Important pretreatment steps by ionic liquids, such as *1-allyl-3-methylimidazolium chloride,1-ethyl-3-methylimidazolium acetate, 1-butyl-3methylimiazolium,1-ethyl-3-methylimidazolium acetate, 1-butyl-3-methylimidazolium hydrogen sulfate, 1-butyl-3-methylimidazolium thiocyanate, 1-ethyl-3-methylimidazolium diethyl phosphate, 1-methyl-3-(4-sulfobutyl) imidazolium bisulfate,* and *cholinium taurate*, will be evaluated. Additionally, critical parameters affecting the treatment process, such as temperature, the ratio of ionic liquids to residue dose, concentration of ionic liquids, and time taken, would also be discussed.

Keywords Lignocellulosic materials · Pretreatment · Ionic liquids · Bioethanol

---

I. Neme
Department of Chemical Engineering, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

C. Masi (✉)
Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia
e-mail: chandran.chandran@aastu.edu.et

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_3

1 Introduction

Bioethanol can be prepared from renewable biomass materials because renewable resources have positive environmental impacts and also possess important economic potential over fossil fuel. The significance of bioethanol is increasing due to various reasons, such as global warming and climate change (Swain et al. 2019). Bioethanol has been used for vehicular use as part of the internal combustion engine of the vehicle. Due to several properties of bioethanol, such as higher flame speeds, broad flammability limits, higher octane number, and high heat of vaporization, it allows for shorter burn time, leaner burn engine, and high compression ratio. This proved that bioethanol has high advantages in terms of efficiency when compared to that of gasoline. Bioethanol can be used in combination with gasoline or as a separate entity. Bioethanol as stated helps in reducing air pollution, as it has low reactivity and higher oxygen content and it also helps to break the supply and demand chain of the foreign oil fields from where gasoline is bought, thereby initiating a self-sufficient transport fuel for each country, all with the help of biomass. Bioethanol is available and can be used as two types based on its mixture with gasoline, such as low-level blend and high-level blend. The low-level blend, or the E10 blend, consists of 10% bioethanol and 90% gasoline, whereas the high-level blend consists of either 85% bioethanol and 15% gasoline, known as E85 blend, or 95% bioethanol and 5% gasoline, known as E95 blend (Balat 2009). These blending and usage of bioethanol as fuels in vehicles can be achieved without any modifications to the present internal combustion engine of the vehicle (Loppacher 2005). As bioethanol production can reduce the usage of petroleum greenhouse gas emissions, it can also promote the farming industry, by being the supplier of biomass required for bioethanol production (Bull 1994). An overview of the changes in emission, by both high blend and low blend bioethanol, is tabulated in Table 3.1 (Kadam 2000). Lignocellulosic biomass appears to be a potentially renewable resource that can be used for the production of biofuels and bioproducts. Lignocellulose is basically represented as grasses, hardwood, softwood, domestic solid waste, food industry residue, and agricultural and forest residues. These resources include sugars, polymerized in the form of cellulose, hemicellulose, and lignin to form a complex structure. Ionic liquids have the potential to dissolve biomass by effectively disrupting the dynamic network of non-covalent carbohydrate-lignin interactions (André et al. 2013). In the abovementioned lignocellulose materials, agricultural and forest remains are largely unused and abundantly available, which can be used as competitive feedstock for the production of bioethanol. Specifically, “wheat straw” residue is the most useful biomass in terms of its availability. Europe, East Asia, and America are the most wheat producers in the world. Internationally, eight hundred and fifty (850) million metric tons of “wheat straw” is produced per annum, which can be measured as a huge agricultural residue (Swain et al. 2019). Similar to other agricultural residues, wheat straw is made up of 33%–40% (w/w) cellulose, 20%–25% (w/w) hemicellulose, and 15%–20% (w/w) lignin (Swain et al. 2019). A comparative study on the proximate analysis and the presence of major elements, such as wheat straw, rice

Table 3.1 Changes in emission due to the usage of bioethanol as vehicular fuel (Binod et al. 2010)

Emission	Low-level blend (E10)	High-level blend (E85, E95, and E100)
Carbon monoxide	Decrease – 25% to 35%	Decrease – 25% to 25%
Carbon dioxide	Decrease – 10%	Decrease – 100%
Nitrogen oxides	Increase or decrease – 5%	Decrease – 20%
Volatile organic compound exhaust	Decrease – 7%	Decrease – 30%
Volatile organic compound evaporative	No change	Decrease
Sulfur dioxide and particulate matter	Decrease	Decrease
Aldehydes	Increase – 30% to 50%	Data not available
Benzene, butadiene, and derivatives	Decrease	Decrease – 50%

Table 3.2 Proximate analysis and elemental analysis of wheat straw, rice straw, and rice husk (Binod et al. 2010)

	Wheat straw	Rice straw	Rice husk
Percentage of dry fuel: Proximate analysis			
Fixed carbon	17.71	15.86	16.22
Volatile matter	75.27	65.47	63.52
Ash	7.02	18.67	20.26
Percentage elemental analysis in ash			
SiO_2	55.32	74.67	91.42
CaO	6.14	3.01	3.21
MgO	1.06	1.75	<0.01
Na_2O	1.71	0.96	0.21
K_2O	25.60	12.30	3.71

husk, and rice straw, is tabulated in Table 3.2, which very well denotes that wheat straw consists of most of these elements in a higher percentage than that of the rice straw and rice husk (Binod et al. 2010). Due to its high cellulose and hemicelluloses composition, it can be hydrolyzed into bioethanol making in the world.

Pretreatment is intended to enhance the digestibility of polysaccharides in lignocellulosic biomass to maximize their release of fermentable sugars without their substantial destruction. A competent deconstruction of biomass is the path toward the complete utilization of lignocellulosic biomass. However, the multipart and rigid structures of lignocellulose biomass make it highly resistant to biological, physical, and chemical degradation. The pretreatment method is crucial to deconstruct the recalcitrance of lignocellulosic biomass, for hydrolysis and downstream fermentation of released sugars (Fu and Mazza 2011). Various pretreatment methods (i.e., chemical, biological, physical) are present. However, each of these techniques has their drawbacks: for instance, chemical methods are expensive and most appropriate for high-value paper products; biological methods require an extensive residence time; the physical and irradiation pretreatments are power demanding and do not eliminate the lignin; hot water and steam explosion pretreatment methods are measured as very positive; however, they need a high amount of pressure/temperature and use of catalyst. A researcher discovered that new and nonvolatile solvents called ionic liquids (ILs) were able to dissolve large amounts of lignocellulosic materials (Reddy 2015; Guan et al. 2018). Generally, ionic liquids are defined as salts with a low melting point and possess an organic cation and inorganic anion (Galbe and Wallberg 2019). Ionic liquids (ILs) are the potential solvents for the pretreatment of lignocelluloses materials as they are thermally constant, are eco-friendly, and have low volatility. Ionic liquids have the potential to dissolve biomass by effectively disrupting the dynamic network of non-covalent carbohydrate-lignin interactions (Yang et al. 2011). Nowadays, the usage of ionic liquids in the extraction of carbohydrates from the lignocellulosic materials is studied extensively (André et al. 2013). This review article compares the extractive capacity of various types of ionic liquids, discussing the most favorable situation for the pretreatment of wheat straw residue separately for bioethanol production.

2 Availability of Wheat Straw

Wheat straw is considered as a potential feedstock for ethanol production. Europe, East Asia (i.e., China, India), the United States of America, and Canada are the most wheat producers in the world. Globally, around 850 million metric tons of wheat straw per annum is produced, which can be considered as a big farming residue. Wheat straw can produce about 120 billion liters of bioethanol per annum, which can substitute 93 billion liters of gasoline (Fu and Mazza 2011). Ethiopia is the top fourth cereal-producing country in Africa. Wheat, maize, teff, sorghum, and barley are the main cereal crops, covering more than 80% of the croplands (André et. al. 2013; Tucho and Nonhebel 2015). The annual yields of crops directly determine their corresponding quantity of residues. Wheat straw is one of the most abundant lignocellulosic crop residues in Ethiopia. Its annual production is about 2.1 tons per hectare crop yield, and its residue is 2.7 tons per hectare annually (Wei et al. 2012; Tucho and Nonhebel 2015). Losing this huge quantity of residues for a country, like Ethiopia, is an enormous energy/fuel loss.

2.1 Potential and Properties of Wheat Straw as a Precursor for Bioethanol Production

Wheat straw is one of the lignocellulosic biomass, constituting of 33%–40% cellulose, 20%–25% hemicelluloses, and 15%–20% lignin. Figure 3.1 provides a general

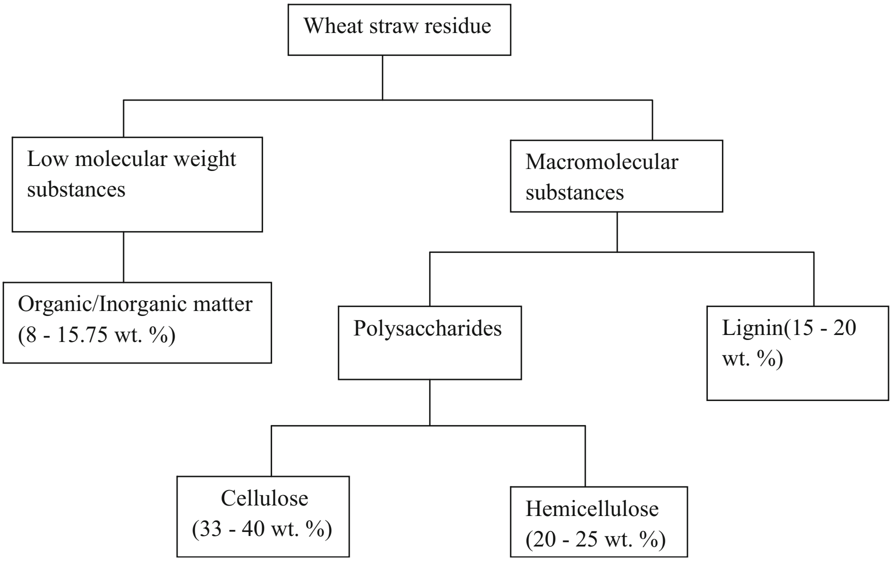


Fig. 3.1 General chemical composition of wheat straw residue (Swain et al. 2019)

overview of the composition of wheat straw residue. Out of these, cellulose and hemicelluloses are the main components for second-generation bioethanol production. Lignin is the component that is removed during the pretreatment of wheat straw. This unreacted component is used for porous material production, energy sources for ethanol, and current production (Swain et al. 2019). In the situation of biorefining lignocellulosic residue, mineral elements can accumulate in conveyor streams, which can challenge the processing and can promote the tearing of equipment. Comprehensive information about the mineral content of the lignocellulosic biomass in the biorefining plant is an essential requirement for designing the optimal biorefinery process. Thus, the preparation method requires to be modified to specify wheat straw residues based on their properties and future purpose.

2.2 *Pretreatment of Wheat Straw*

The most important processing challenge in the production of biofuel is the hydrolysis of the biomass. Pretreatment refers to the solubilization and separation of one or further of the biomass constituents. Lignocellulosic biomass is made up of a matrix of cellulose and lignin confined by hemicellulose chains (Angell et al. 2007). Figure 3.2 explains the process flow diagram (PFD) of wheat straw pretreatments by ionic liquids. Hence, pretreatment is performed to break the matrix to decrease the quantity of cellulose crystallinity. Generally, different pretreatment methods, chemical (dilute acid, alkali, oxidizing agent, organic solvents), biological (enzymatic,

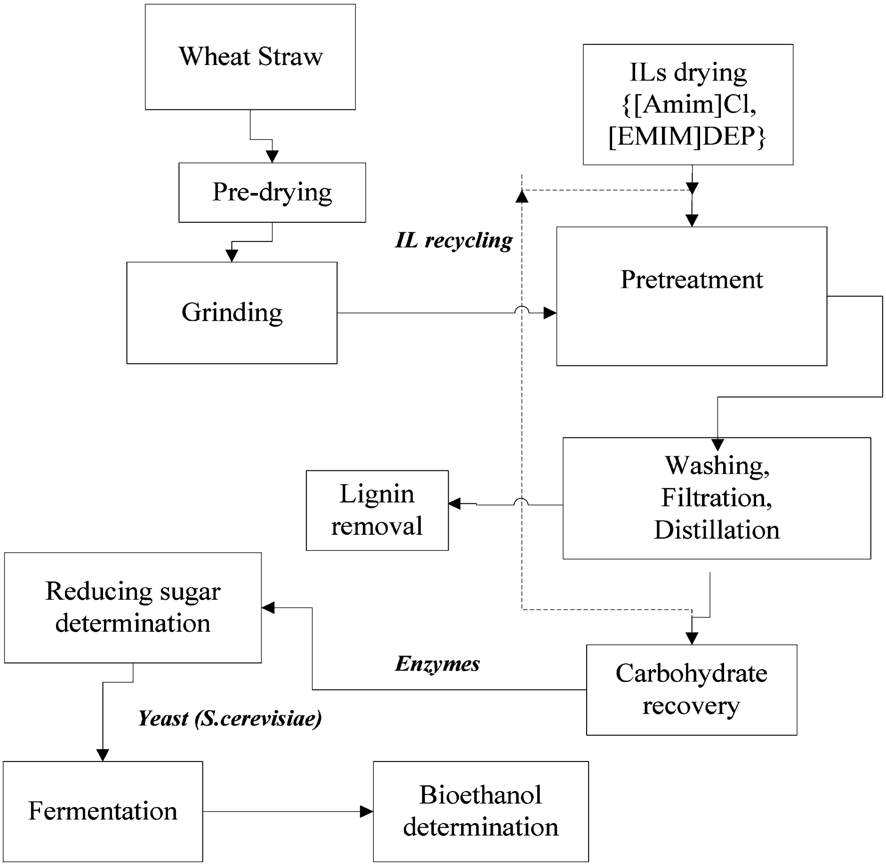


Fig. 3.2 Process flow diagram of bioethanol production from wheat straw using ionic liquid solvents (Swain et al. 2019)

microbial), physical (wet oxidation, irradiation, milling), and thermal (steam explosion, hydrothermal), are present. However, each of these pretreatment techniques has their drawbacks: for instance, chemical methods are expensive and most appropriate for high-value paper products; biological methods require an extensive residence time; the physical and irradiation pretreatments are power demanding and do not eliminate the lignin; hot water and steam explosion pretreatment methods are measured as very positive; however, they need a high amount of pressure/temperature and use of a catalyst (Bernardo et al. 2019).

3 Impacts of Wheat Straw Pretreatment Using Ionic Liquids on a Sustainable and Cleaner Environment

When we think of "sustainable energy," the energy resource that regularly appears to the mind is, naturally, biomass. As a potential energy source, biomass is moderately plentiful (ranked third, next to oil and coal). Biomass to energy conversions is possibly the most sustainable and is considerably cleaner than coal: for instance, in biomass-derived energy, nitrogen compound (NO_x) and sulfur compound (SO_x) emissions are very low. An additional benefit of biomass is being agreeable to small-scale installations with the guarantee of energy accessibility and economic growth in rural and developing areas. Accordingly, a biomass processing capacity must be able to convert various feedstocks without major penalties in overall performance, sugar yield, and fuel production. Ionic liquid pretreatments are less energy demanding, easier to handle, and more environmentally friendlier than the other pretreatment methods like chemical and physical methods (Zhao et al. 2009). Chemicals used for pretreatment are generally toxic to the microbes and enzymes used in the downstream processing to complete the biomass conversion process; thus, these reaction agents can only be used once, making it more expensive (Hu and Ragauskas 2012). For instance, sulfuric acid used in biomass pretreatment is not cost-effective for reuse; it should be removed and disposed of using strategies that produce huge amounts of solid wastes or wastewater. Additionally, in some cases, this results in improper sugar losses or additional energy requirement and greenhouse gas exhaustions like ammonia (Humbird et al. 2011). Physical pretreatment of biomass conversion to ethanol is very energy extensive and is highly polluted (Sikarwar et al. 2016). Ionic liquids have also been largely considered for green chemistry with many emerging kinds of research being conducted in the usage of ILs as a matter of important subject in Green Chemistry (Welton 2011). Another study tells us that the ionic liquids are known for their low volatility than that of the conventional pretreatment chemicals used, and this proves that these ILs can proceed with the pretreatment of the biomass at atmospheric pressure at high temperature. ILs are also non-odorous and are relatively safer liquor, which can be easily handled (Brandt et al. 2013). ILs have attractive characteristics as a possible replacement for conventional solvents in the separation process (Gonfa et al. 2011). Ionic liquids have gained new attention to chemists as a green solvent for various purposes. The toxicity analysis of 1-alkyl-3-methylimidazolium chloride-based ionic liquids was assessed using the nematode *Caenorhabditis elegans*. In this toxicity analysis, where various concentrations of the selected IL were preceded, their result is interpreted as to understand the LC_{50}/mg mL^{-1}, which is represented in Table 3.3. Thus, after the exposure of IL to the nematode, the initial chemical exposure was not fatal to the worm, as it survived the exposure; moreover, there was successful reproduction by the nematode, stating that the IL has a very low impact on the environmental sustainability, especially to that of the life present on the ground (Swatloski et al. 2004). A specific ionic liquid 1-ethyl-3-methylimidazolium acetate, or known as EMIMAc, is considered as a nontoxic, noncorrosive, and also biodegradable ionic

Table 3.3 Toxicity analysis of 1-alkyl-3-methylimidazolium chloride-based ionic liquid on *Caenorhabditis elegans* (Swatloski et al. 2004)

Toxicity	LC_{50}/mg mL^{-1}
Not acutely toxic	$>10^{-1}$
Slightly toxic	10^{-2}–10^{-1}
Moderately toxic	10^{-3}–10^{-2}
Highly toxic	10^{-4}–10^{-3}
Very highly toxic	$<10^{-4}$

liquid due to the absence of a reactive side group, and they react with the cellodextrin reducing end groups (Liebert 2010). Ionic liquids are well-known for their minimal air emissions, as they are stable up to 300 °C and have low volatility and fluidity (Dadi et al. 2007). Along with the rising attention in lignocellulosic biomass, the function of ionic liquids was expanded to the conversion and characterization of biomass (da Costa Lopes and Bogel-Łukasik 2015). ILs can efficiently eliminate lignin and hemicellulose under mild conditions in various biomass-related applications, including pretreatment and characterization (Yoo et al. 2017).

4 Ionic Liquids (ILs)

Ionic liquids are salts with melting temperatures below 100 °C, characterized by particularly low vapor pressure, high thermal stability, and low flammability (Galbe and Wallberg 2019). IL anions play an important function in the dissolution of cellulose and lignocellulosic biomass (Ghandi 2014). These are being widely presented as "green solvents" for numerous industrial applications, mostly in the area of the catalyst, chemical production, and taking apart of cellulose-based biomass (Article 2015). The chance of recovering almost 100% of the used ILs to their original state makes ionic liquids smart (Heinze et al. 2005). The most common forms of ILs contain the imidazolium cation, which can pair with anions, for example, acetate, sulfate, nitrate, chloride, bromide, and triflate. ILs are prepared and developed to pretreat definite biomass under the most favorable situation, by combining anions and cations, which can result in an estimated formulation of 10^9 ILs (Menon and Rao 2012). The major obstacle in lignocellulosic biomass enzymatic hydrolysis of cellulose is lignin. This is because lignin prevents access for the enzymes to degrade the lignocellulosic biomass. However, this lignin was very well removed by the ionic liquid pretreatment and also maximized the accessibility for the enzymes to the lignocellulosic biomass. The pretreatment also helped in depleting the crystallinity of the cellulose present in the biomass. Thus, efficient enzymatic hydrolysis was conducted as a result of ionic liquid pretreatment (Fu and Mazza 2011).

4.1 General Types of Ionic Liquids

4.1.1 Protic Ionic Liquids (*PILs*)

PILs are a typical group of ionic liquids that are produced by an easy acid-base neutralization, for instance, the combination of organic acids and amine bases; additionally, they are moderately cost-competitive chemicals (Angell et al. 2007). The interactions in ion pairs of PILs [diethylmethylammonium (dema) and dimethylpropylammonium (dmpa) based ionic liquids] are stronger (Tsuzuki et al. 2013). Pyridinium, 1 methylimidazolium, pyrrolidinium, and acetate are combined as the most competent for lignin removal, because of their high iconicity (Docampo-Álvarez et al. 2016, Achinivu 2018).

4.1.2 Aprotic Ionic Liquids

Aprotic ionic liquids are consisting of the combinations of Bronsted acids and bases, including ethyltrimethylammonium-based ionic liquids. Aprotic ionic liquid-based ILs are less compared to protic ionic liquids (Tsuzuki et al. 2013). Among them, the most successful and largely used ILs in native wheat straw pretreatment are 1-ethyl-3-methylimidazolium chloride (EMIM-Cl), BMIM-Cl, and 1-ethyl-3-methylimidazolium acetate (EMIM-OAc). In different publications, “acronyms” of ILs are not constantly the same. The number of possible ILs is very bulky; as a result, only a little of the commonly utilized ionic liquids are present (Fu and Mazza 2011).

4.2 Different Types of ILs for Pretreatment of Wheat Straw

4.2.1 1-Allyl-3-Methylimidazolium Chloride

1-Allyl-3-methylimidazolium chloride (Amim-Cl) has been designed for the dissolution and regeneration of cellulose. Temperature is a significant issue in the pretreatment involving Amim-Cl. High temperature induces important changes in the composition of wheat straw (Zhi-guo and Hong-zhang 2012). Amim-Cl is sticky with reactive side chains; hence, when Amim-Cl was used to treat wheat straw, high temperature ($\geq$150 °C) was more efficient than low temperature. It is found that Amim-Cl can be dissolved by 14.8% weight of cellulose as in dissolved pulp or 8% weight of cotton linter. However, dissolving Amim-Cl in wild lignocellulose is comparatively difficult than that of cellulose (Zhang and Chen 2012). The wheat straw’s compact structure doesn’t allow the ionic liquid to be penetrated much deeper, which might be the reason that dissolving Amim-Cl in wild lignocellulose is difficult. The pretreated wheat straw using this IL contained 38.4% cellulose, 24.2%hemicellulose, and 18.3% lignin for 2 h of heating (Zhi-guo and Hong-zhang 2012).

4.2.2 1-Ethyl-3-Methylimidazolium Acetate (Emim-CH_3COO)

Emim-CH_3COO is an aprotic imidazolium and in-room temperature ionic liquid normally reported due to its high solubility properties for biomass pretreatment. It is helpful for in situ enzymatic saccharifications because of its biocompatibility and enzymatic activity (André et al. 2013). High-purity cellulose and 52.7% of lignin were obtained previously by pretreatment of wheat straw using Emim-CH_3COO (Zhu et al. 2017).

4.2.3 1-Butyl-3-Methylimidazolium Hydrogen Sulfate (Bmim-HSO_4)

Acid-based ILs can catalyze hemicellulose, cellulose, or lignin. Bmim-HSO_4 was reported as being capable of hydrolyzing and converting the hemicellulose fraction of wheat straw without additional usage of catalyst (Bernardo et al. 2019). As a consequence of the acidic property of the Bmim-HSO_4, increased lignin content can be observed in the regenerated material. This also helps to avoid lignin extraction to the liquid stream and also promotes the hydrolysis process. The hydrolysis of wheat straw using Bmim-HSO_4 at 140 °C for 90 min resulted in 75% lignin release (André et al. 2013).

4.2.4 1-Butyl-3-Methylimidazolium Thiocyanate (Bmim-SCN)

Bmim-SCN is the most studied IL as a result of its excellent solubility properties for biomass pretreatment. The use of Bmim-SCN and Bmim-$N(CN)_2$ did not permit an absolute dissolution of wheat straw, opposing to Bmim-HSO_4 with which a microscopic absolute dissolution was determined. Bmim-SCN when used for wheat straw pretreatment, the purity of the fractionated sample, and the recovery in biomass can be tuned, providing flexibility in the process. The usage of Bmim-SCN also helped to obtain high-purity lignin. Bmim-SCN is a more competent IL for the residue fractionation, as a larger quantity of each fraction (cellulose, hemicellulose, and lignin-rich material) was recovered (André et al. 2013).

4.2.5 1-Ethyl-3-Methylimidazolium Diethyl Phosphate (EMIM-DEP)

EMIM-DEP is advantageous, as it is simple to be synthesized on a commercial scale with high yield. Phosphate-based ILs are less viscous than the chloride type and are more thermally stable than format ones. The yield of reducing sugars from wheat straw pretreated with EMIM-DEP IL at 130 °C for 30 min was 54.8% after being enzymatically hydrolyzed for 12 h, and the glucose obtained was 2.51 mg/10 mg regenerated wheat straw (Galbe and Wallberg 2019).

4.2.6 1-Methyl-3-(4-Sulfobutyl)Imidazolium Bisulfate (HSO_3-BMIM-HSO_4)

It has the highest catalytic performance and was used to further evaluate the effects of ionic liquid concentration, reaction time, and temperature on the conversion rate of wheat straw when compared with IL of BMIM-Cl and BMIM-HSO_4. This indicates that the HSO_4 anion is more efficient than the Cl anion at dissolving and converting wheat straw. The conversion rates of wheat straw catalyzed by BMIM-HSO_4 was 85.8% (Guan et al. 2018).

4.2.7 Cholinium Taurate (Ch-Tau) ILs

Taurine is one of the sulfur-containing amino acids but does not form proteins. It is produced from renewable materials and, in natural form, can be found in high concentrations in skeletal and cardiac muscle and brain tissue. The acids intended for this IL production contains the alkylated derivatives of glycine (sarcosine and N, N-dimethyl glycine), nutritional ingredient (taurine), important bio-based platform compounds (levulinic acid and 2-furoic acid), and inorganic acids (H_2SO_4 and H_3PO_4). The pretreatment of wheat straw with Ch-Tau ILs at 90 °C obtained 68.8% of lignin, which was separated from wheat straw (Ren et al. 2016) (Table 3.4).

5 Conclusion

Ionic liquid pretreatment provides a potentially cost-effective way designed for bioethanol production from agricultural residue, due to less usage and easy recycling of ILs. Out of the various types of ionic liquids, 1-butyl-3-methylimidazolium chloride (Bmim-Cl) and 1-butyl-3-methylimidazolium thiocyanate (Bmim-SCN) have better cellulose solubility characteristic. However, the ionic liquids involving Cl- and SCN- are corrosive and toxic and did not permit an absolute dissolution of wheat straw. EMIM-DEP and BMIM-HSO_4 are among the best cellulose solvents in terms of solubility characterization and compatibility with hydrolytic enzymes. Additionally, EMIM-DEP is easy to synthesize than BMIM-Cl ILs. Furthermore, they are an environment-friendly ionic liquid. In terms of experiment conditions, for all of IL variants, 150 °C or more temperature is suitable for the pretreatment of wheat straw. Regenerated cellulose of wheat straw hydrolyzed more easily than being treated with water, and their enzymatic hydrolysis rates were improved excellently.

Table 3.4 Different types of ionic liquids and summary of their performance for wheat straw pretreatment are listed below

Ionic liquids	Experimental conditions	Target wheat straw composition	Composition treated yield	Ethanol production	Reference
Amim-Cl	(1) ____				Zhi-guo and Hong-zhang (2012)
	(2) ____	Glucose	46.7%		
	(3) 150 °C	Hemicellulose	10.8%	55.2%	
	(4) 6 h		24.2%		
	(5) 0.5 g	Lignin			
Emim-CH_3COO	(1) 150 mg				Fu and Mazza (2011), Zhu et al. (2017)
	(2) 0.5 mm	Glucose	19.7(%w/w		
	(3)150 °C	Cellulose	biomass)		
	(4) 24 h	Lignin	29.5 mg	________	
	(5) 2 g		52.7%		
Bmim-SCN	(1)				Da Costa Lopes et al. (2013)
	(2)	Cellulose	83.4% (w/w)		
	(3)120 °C	Hemicellulose	11.7% (w/w)	________	
	(4) 6 h	Lignin	25.3 mg		
	(5) 5% w/w				
Bmim-HSO_4	(1) 0.5 mm				André et al. (2013)
	(2)_____	Glucose	93.1 ± 4.1 Mol %		
	(3) 120 °C	Reducing sugars		62.3 Mol%	
	(4) 6 h	Lignin	62%wt.		
	(5)5% (w/w biomass)				
EMIM-DEP	(1) ____				Li et al. (2009)
	(2) ≤ 0.5 cm	Glucose	2.51 mg/ 10 mg RWS		
	(3) 130 °C	Reducing sugars	54.8% RWS	0.43 g/g of glucose in 26 h	
	(4) 12 h				
	(5) 120 g	Lignin	____		
Ch-Tau	(1) ≤ 2% wt.				
	(2) 250–420 μm	Glucose	____		
	(3) 90 °C	Reducing sugars	74.2%	____	Ren et al. (2016)
	(4) 48 h	Lignin	68.8%		
	(5)150 g				

(1) ILs concentration, (2) particle size, (3) effect of temperature, (4) effect of contact times, (5) wheat straw dosage

Note: *RWS* regenerated wheat straw

References

Achinivu EC (2018) Protic ionic liquids for lignin extraction—a lignin characterization study. Int J Mol Sci 19(2):428. https://doi.org/10.3390/ijms19020428

André M, Lopes C, João KG, Rubik DF, Bogel-łukasik E, Duarte LC, Andreaus J, Bogel-łukasik R (2013) Bioresource technology pre-treatment of lignocellulosic biomass using ionic liquids: wheat straw fractionation. Bioresour Technol, 142:198–208. https://doi.org/10.1016/j.biortech.2013.05.032

Angell CA, Byrne N, Belieres JP (2007) Parallel developments in aprotic and protic ionic liquids: physical chemistry and applications. Acc Chem Res 40(11):1228–1236. https://doi.org/10.1021/ar7001842

Balat M (2009) Bioethanol as a vehicular fuel: a critical review. Energy Sources, Part A 31 (14):1242–1255. https://doi.org/10.1080/15567030801952334

Bernardo JR, Gírio FM, Łukasik RM (2019) The effect of the chemical character of ionic liquids on biomass pre-treatment and posterior enzymatic hydrolysis. Molecules 24(4):808. https://doi.org/10.3390/molecules24040808

Binod P, Sindhu R, Singhania RR, Vikram S, Devi L, Nagalakshmi S et al (2010) Bioethanol production from rice straw: an overview. Bioresour Technol 101(13):4767–4774. https://doi.org/10.1016/j.biortech.2009.10.079

Brandt A, Gräsvik J, Hallett JP, Welton T (2013) Deconstruction of lignocellulosic biomass with ionic liquids. Green Chem 15(3):550–583. https://doi.org/10.1039/C2GC36364J

Bull SR (1994) Renewable alternative fuels: alcohol production from lignocellulosic biomass. Renew Energy 5(5–8):799–806. https://doi.org/10.1016/0960-1481(94)90091-4

da Costa Lopes AM, Bogel-Łukasik R (2015) Acidic ionic liquids as sustainable approach of cellulose and lignocellulosic biomass conversion without additional catalysts. ChemSusChem 8 (6):947–965. https://doi.org/10.1002/cssc.201402950

da Costa Lopes AM, João KG, Bogel-Łukasik E, Roseiro LB, Bogel-Łukasik R (2013) Pretreatment and fractionation of wheat straw using various ionic liquids. J Agric Food Chem 61(33):7874–7882. https://doi.org/10.1021/jf401980p

Dadi AP, Schall CA, Varanasi S (2007) Mitigation of cellulose recalcitrance to enzymatic hydrolysis by ionic liquid pretreatment. Appl Biochem Biotechnol 137(1–12):407–421. https://doi.org/10.1007/s12010-007-9068-9

Docampo-Álvarez B, Gómez-González V, Méndez-Morales T, Rodríguez JR, López-Lago E, Cabeza O et al (2016) Molecular dynamics simulations of mixtures of protic and aprotic ionic liquids. Phys Chem Chem Phys 18(34):23932–23943. https://doi.org/10.1039/C6CP03700C

Fu D, Mazza G (2011) Optimization of processing conditions for the pretreatment of wheat straw using aqueous ionic liquid. Bioresour Technol 102(17):8003–8010. https://doi.org/10.1016/j.biortech.2011.06.023

Galbe M, Wallberg O (2019) Pretreatment for biorefineries: a review of common methods for efficient utilisation of lignocellulosic materials. Biotechnol Biofuels 12(1):1–26. https://doi.org/10.1186/s13068-019-1634-1

Ghandi K (2014) A review of ionic liquids, their limits and applications. Green Sustain Chem 2014. https://doi.org/10.4236/gsc.2014.41008

Gonfa G, Bustam MA, Man Z, Mutalib MA (2011) Unique structure and solute–solvent interaction in imidazolium based ionic liquids: a review. Asian Trans Eng 1:24–34. https://doi.org/10.1002/chin.201341261

Guan Q, Lei T, Wang Z, Xu H, Lin L, Chen G et al (2018) Preparation of ethyl levulinate from wheat straw catalysed by sulfonate ionic liquid. Ind Crop Prod 113:150–156. https://doi.org/10.1016/j.indcrop.2018.01.030

Heinze T, Schwikal K, Barthel S (2005) Ionic liquids as reaction medium in cellulose functionalization. Macromol Biosci 5(6):520–525. https://doi.org/10.1002/mabi.200500039

Hu F, Ragauskas A (2012) Pretreatment and lignocellulosic chemistry. Bioenergy Res 5 (4):1043–1066. https://doi.org/10.1007/s12155-012-9208-0

Humbird D, Davis R, Tao L, Kinchin C, Hsu D, Aden A, Schoen P, Lukas J, Olthof B, Worley M, Sexton D, Dudgeon D (2011) Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol: dilute-acid pretreatment and enzymatic hydrolysis of corn stover. National Renewable Energy Laboratory, May, 1–147. https://doi.org/10.2172/1107470

Kadam K (2000) Environmental life cycle implications of using bagasse-derived ethanol as a gasoline oxygenate in Mumbai (Bombay). No. NREL/TP-580-28705). National Renewable Energy Lab, Golden. https://doi.org/10.2172/772426

Li Q, He YC, Xian M, Jun G, Xu X, Yang JM, Li LZ (2009) Improving enzymatic hydrolysis of wheat straw using ionic liquid 1-ethyl-3-methyl imidazolium diethyl phosphate pretreatment. Bioresour Technol 100(14):3570–3575. https://doi.org/10.1016/j.biortech.2009.02.040

Liebert T (2010) Cellulose solvents–remarkable history, bright future. In: Cellulose solvents: for analysis, shaping and chemical modification. American Chemical Society, pp 3–54. https://doi.org/10.1021/bk-2010-1033.ch001

Loppacher LJ (2005) The conflicting international trade law governing products of biotechnology: the case of biofuels. J Int Biotechnol Law 2(2):54–61. https://doi.org/10.1515/jibl.2005.2.2.54

Menon V, Rao M (2012) Trends in bioconversion of lignocellulose: biofuels, platform chemicals & biorefinery concept. Prog Energy Combust Sci 38(4):522–550. https://doi.org/10.1016/j.pecs.2012.02.002

Reddy, P. (2015). A critical review of ionic liquids for the pretreatment of lignocellulosic biomass. S Afr J Sci, 111(11–12), 1–9. doi: https://doi.org/10.17159/sajs.2015/20150083

Ren H, Zong MH, Wu H, Li N (2016) Efficient pretreatment of wheat straw using novel renewable cholinium ionic liquids to improve enzymatic saccharification. Ind Eng Chem Res 55 (6):1788–1795. https://doi.org/10.1021/acs.iecr.5b03729

Sikarwar VS, Zhao M, Clough P, Yao J, Zhong X, Memon MZ et al (2016) An overview of advances in biomass gasification. Energy Environ Sci 9(10):2939–2977. https://doi.org/10.1039/C6EE00935B

Smuga-Kogut M, Wnuk AD, Zgórska K, Kubiak MS (2015) Production of ethanol from wheat straw. Pol J Chem Technol 17(3):89-94. https://doi.org/10.1515/pjct-2015-0055.

Swain MR, Singh A, Sharma AK, Tuli DK (2019) Bioethanol production from rice-and wheat straw: an overview. In: Bioethanol production from food crops. Academic, pp 213–231. https://doi.org/10.1016/B978-0-12-813766-6.00011-4

Swatloski RP, Holbrey JD, Memon SB, Caldwell GA, Caldwell KA, Rogers RD (2004) Using Caenorhabditis elegans to probe toxicity of 1-alkyl-3-methylimidazolium chloride based ionic liquids. Chem Commun (6):668–669. https://doi.org/10.1039/B316491H

Tsuzuki S, Shinoda W, Miran MS, Kinoshita H, Yasuda T, Watanabe M (2013) Interactions in ion pairs of protic ionic liquids: comparison with aprotic ionic liquids. J Chem Phys 139 (17):174504. https://doi.org/10.1063/1.4827519

Tucho GT, and Nonhebel S (2015) Bio-wastes as an alternative household cooking energy source in Ethiopia. 9565–9583. https://doi.org/10.3390/en8099565

Wei L, Li K, Ma Y Hou X (2012) Dissolving lignocellulosic biomass in a 1-butyl-3-methylimidazolium chloride-water mixture. Ind Crop Prod 37(1):227–234. http://dx.doi.org/10.1016/j.indcrop.2011.12.012

Welton T (2011) Ionic liquids in green chemistry. Green Chem 13(2):225–225. https://doi.org/10.1039/C0GC90047H

Yang Q, Wu S, Lou R, Gaojin LV (2011) Structural characterization of lignin from wheat straw. Wood Sci Technol 45(3):419–431. https://doi.org/10.1007/s00226-010-0339-1

Yoo CG, Pu Y, Ragauskas AJ (2017) Ionic liquids: promising green solvents for lignocellulosic biomass utilization. Curr Opin Green Sustain Chem 5:5–11. https://doi.org/10.1016/j.cogsc.2017.03.003

Zhang Z, Chen H (2012) Enhancement of the enzymatic hydrolysis of wheat straw by pretreatment with 1-allyl-3-methylimidazolium chloride ([Amim] Cl). Afr J Biotechnol 11(31):8032–8037. https://doi.org/10.5897/AJB11.3583

Zhao H, Jones CL, Baker GA, Xia S, Olubajo O, Person VN (2009) Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis. J Biotechnol 139(1):47–54. https://doi.org/10.1016/j.jbiotec.2008.08.009

Zhi-guo Z, Hong-zhang C (2012). Enhancement of the enzymatic hydrolysis of wheat straw by pretreatment with 1-allyl-3-methylimidazolium chloride ([Amim]Cl). 11(31); 8032–8037. https://doi.org/10.5897/AJB11.3583

Zhu S, Luo F, Huang W, Huang W, Wu Y (2017) Comparison of three fermentation strategies for alleviating the negative effect of the ionic liquid 1-ethyl-3-methylimidazolium acetate on lignocellulosic ethanol production. Appl Energy 197:124–131. https://doi.org/10.1016/j.apenergy.2017.04.011

Chapter 4
Harnessing the Sustainable Bioresource, Cellulose at the Nanoscale for Multifarious Environmental Applications

Ebrahim M. Abda and Rocktotpal Konwarh

Abstract Recent years have stood in testimony to the tremendous research impetus garnered by cellulosic nanomaterials, particularly in the realm of environmental biotechnology. Renewability and eco-friendly attributes, complemented by outstanding biophysicochemical features, such as large surface area, lightweight, desirable tensile strength, and stiffness, besides surface tailorability, endow special niche to cellulosic nanomaterials in the domain of material technology. The write-up in the chapter gears off with *au courant* extraction (primarily derived from plants, bacteria, algae, and tunicate) and fabrication approaches of cellulosic nanomaterials and nanocomposites, respectively, *vis-à-vis* processing and surface modification-structure-property perspective to produce cutting-edge materials with anticipated properties and effectiveness. The content is then streamlined toward a comprehensive discussion on the latest advances and developments in the application of cellulose-based nanomaterials/nanocomposites in the areas of wastewater treatment, water and air purification, heavy metal mitigation, biosensing, carbon sequestration, oil and gas field drilling, and so on. An attempt is also made to highlight the practical snags and commercial prospects of resorting to cellulosic nanomaterials while seeking sustainable environmental solutions.

Keywords Nanocellulose · Cellulose nanocrystal · Cellulose nanofiber · Water treatment · Environmental applications

---

E. M. Abda (✉)
Department of Biotechnology, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

R. Konwarh
Department of Biotechnology, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

Centre of Excellence- Nanotechnology, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_4

1 Introduction

Our planet – earth seems to have entered into a new period of geologic history due to the unprecedented effects of human activities on the environment. Ironically, attempts to mount a unanimous global response to major environmental problems have failed to reach a consensus among the governments of different countries. Several global organizations, including the WHO, FAO, and World Bank have promoted and encouraged the sustainable use of natural resources. Albeit (fragmented) measures have been adopted across the globe, pollution of the various ecosystems is on an increasing trend as a result of urbanization, deforestation, industrialization, land degradation, etc. This has negatively impacted the realization of basic human rights, such as those concerning universal access to safe drinking water and the right to live in a clean environment (Singh and Singh 2017). Multiple exciting technologies, developed over the decades and used in environmental remediation, are largely unable to cope up with the extent of pollutants released from various sources (Paul and Jons 2016; Yang et al. 2019). Moreover, many of these technologies are based on synthetic materials, leading to issues of recycling and safe disposal (Unuabonah and Taubert 2014; Shemshadi 2012). Over the years, increasing attention has been paid to bio-based materials to address major environmental problems, with special thrust on bioresources that are abundant, environmentally benign, recyclable, and efficient. Among others, cellulose, chitosan, starch, and lignin have been widely employed as alternatives to synthetic polymers. In this context, besides the traditional use in the paper and pulp industry, biofuel, and so on, cellulose has garnered considerable popularity in material technology due to its abundance, recyclability, eco-friendly attribute, and amenability for surface functionalization, paving the way toward a wide gamut of applications (Mokhena and John 2020; Abitbol et al. 2016).

On a pertinent note, among others, nanocellulose (NC) (cellulosic structure in the nanoscale) has gained tremendous research impetus for a plethora of uses. The constantly growing popularity of NC, both in the academia and the industry, may be bracketed together with the outstanding application of NC in medicine, tissue engineering, material reinforcement, etc., as vouched by the increasing number of articles in the scientific literature repositories. There have been constant efforts to harness the remarkable properties of NC, including large surface area, desirable tensile strength and stiffness, and abundance of –OH groups, which offer numerous sites for surface modification and functionalization (Trache et al. 2020; Dufresne 2019). Interestingly, NC has opened up a number of plausible avenues to address complex environmental challenges, such as wastewater decontamination, encompassing the removal of various chemical and biological pollutants. Howbeit, NC has only recently expanded its applications in the realm of environmental remediation and captured spotlight use in wastewater treatment, water and air purification, soil biosensing, heavy metal abatement, carbon sequestration, oil and gas field drilling applications, etc. Thus, the content in this chapter is streamlined to provide an insight into the basic understanding of the structure-function accord of nanocellulose and the prospective applications in the environmental realm.

2 ABCs of Cellulose and Nanocellulose

Cellulose is the most abundant renewable polymer (Mokhena and John 2020) produced by several organisms for a variety of functions, including structural support, source of energy, and cell connection to form tissues. Globally, one billion tons of cellulose are produced annually, of which 6×10^9 tons are mainly used in the paper, fiber, and material industry as well as in the chemical industry (Habibi et al. 2010). Plants contribute to the lion's share of cellulose but can also be extracted from sea animals, algae, fungi, amoebae, cellular slime molds, and certain bacteria. Regardless of its origin, cellulose is a high-molecular-weight polysaccharide, composed of β-D-glucopyranose units linked together by β-1, 4 glucosidic bonds. Plant cellulose is hierarchically assembled from elementary fibrils, which are in turn generally made of 18–24 or more cellulose macromolecules, associated through intra- and inter hydrogen bonding and van der Waals forces (Nishiyama 2009; Fernandes et al. 2011; Oehme et al. 2015; Okita et al. 2010). The elementary fibrils are stacked together to form nanofibrils, which link together to form cellulosic fibers, typically observed in the vascular systems of woody plants (Fig. 4.1). Cellulose contains a highly disordered or amorphous zone and ordered crystalline regions. Cellulose exists in six types of polymorphs, namely, cellulose I, II, $\mathrm{III_I}$, $\mathrm{III_{II}}$, $\mathrm{IV_I}$, and $\mathrm{IV_{II}}$, as confirmed with nuclear magnetic resonance (NMR), infrared, and diffraction studies (O'Sullivan 1997; Wada et al. 2004). While cellulose I is the native cellulose from plant or microbial origin (Vander Hart and Atalla 1984; Sugiyama et al. 1991), other polymorphs are produced either from native or various derivatives by chemical and thermal treatments (Mahmud et al. 2019).

Nanocellulose, on the other hand, generally refers to *nanosized cellulosic fibers* or *cellulosic crystallites* that could be extracted from multiple sources, such as plants, bacteria, algae, and some sea animals, following top-down or bottom-up strategies of nanomaterial synthesis. The nanoscale attributes of high surface area and aspect ratio, quantum size effects, special morphology, amenability for a plethora of chemical functionalization, and notable biocompatibility have conferred a special niche to nanocellulosic materials in a wide gamut of applications. Based on the recommendations of the Technical Association of the Pulp and Paper Industry (TAPPI) and the relevant international organizations, nanocellulose has been classified into two categories: *crystalline nanocellulose* (CNCs) and *cellulosic nanofiber* (CNFs) (Carpenter et al. 2015). Multiple nomenclatures exist for both crystalline nanocellulose (also called nanocrystalline cellulose (NCC) and cellulose nanowhisker (CNW)) and cellulose nanofiber (also called nanofibrillated cellulose (NFC), nanofibrillar cellulose, and nanofibrous cellulose). The two types vary based on the extraction process, cellulose source, and physico-mechanical properties, especially with regard to dimensions, purity, and crystallinity. In addition, nanocellulose can also be obtained from bacteria (bottom-up method), in particular from *Komagataeibacter xylinus* (previously classified as *Acetobacter* or *Gluconacetobacter*), with the ability to synthesize NC from low-molecular-weight sugars and alcohols as starting materials (Vigentini et al. 2019; Gopu and Govindan 2018). Although bacterial nanocellulose has a chemical formula similar to plant-

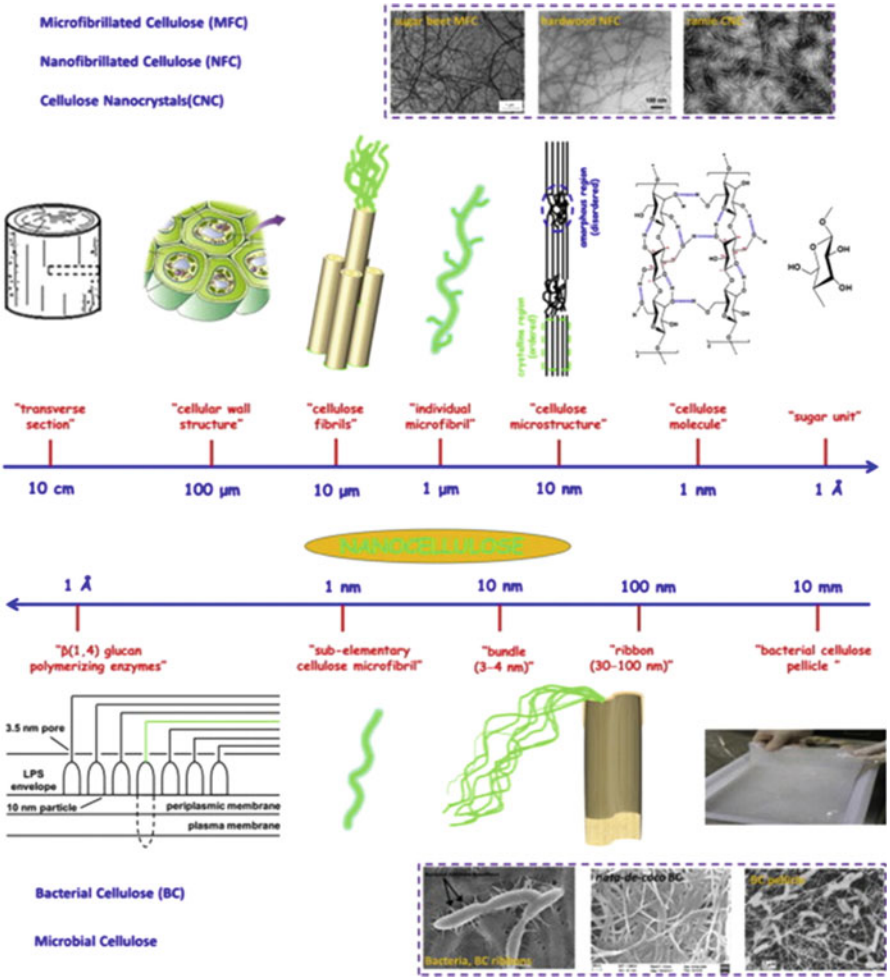


Fig. 4.1 Hierarchical structure of cellulose; top image (from large unit to small unit): cellulose nanocrystals (CNC). Micro-/nanofibrillated cellulose (MFC and NFC); bottom image (from tiny unit to small unit): bacterial cellulose (BC). Transmission electron micrographs of sugar beet MFC; and scanning electron micrographs of BC ribbons, *nata-de-coco* BC, BC pellicle. (*Reproduced with permission from* Lin and Dufresne 2014, *Copyright © 2014 Published by Elsevier Ltd.*)

based NC, it has unique properties, such as high purity, high degree of polymerization, high crystallinity (70–80%), high water content (90%), and high heat and mechanical stability; however, expensive fermentation processes, involved in its production have limited its widespread use (Torres et al. 2019; Chen et al. 2016; da Gama and Dourado 2018). Other forms of nanocellulose include amorphous nanocellulose and cellulose nanoyarn (CNY). Amorphous nanocellulose is mainly prepared from regenerated cellulose via acid hydrolysis followed by ultrasound disintegration (Ioelovich 2008). An electrospinning technique is applied to solutions containing cellulose to generate cellulose nanoyarn (Quan et al. 2010; Stylianopoulos et al. 2012; Araki and Miyayama 2020).

3 Extraction of NC

Nanocellulose can be extracted from plant-based feedstocks (An et al. 2020; Gea et al. 2020; Trilokesh and Uppuluri 2019), certain aerobic bacteria (Vigentini et al. 2019), algae (Zhou et al. 2019), and marine animals (Iwamoto et al. 2011). The green alga, *Cladophora* in particular, has become popular for the production of highly crystalline nanocellulose (Zhou et al. 2019). Its product shows a remarkable inertness toward chemical treatments and therefore finds promising applications in filters (Metreveli et al. 2014). Indeed, cheaper and more abundant resources, such as lignocellulose feedstock, are considered the most promising source. However, the bulk cellulose in plants (stems, leaves, roots, and fruit) and agricultural residues, such as rice straw, wheat bran, bagasse, fruit peel, coconut husk, etc., exist together with other polymers and therefore requires chemical hydrolysis or mechanical defibrillation to produce crystalline nanocellulose and nanocellulosic fibers, respectively. On the other hand, many research laboratories mostly use pure cellulose (that may be obtained from cotton fibers or other sources) or microcrystalline cellulose or wood pulp (delignified wood that contains almost pure cellulose); however, the large-scale production of nanocellulose is based on the continuous supply of raw materials and consequently uses lignocellulosic starting material, with the extraction and production of NC, executed in three prime steps: *pre-treatment, NC conversion,* and *post-treatment.*

Pre-treatment, aimed at releasing cellulose from lignin, hemicellulose, wax, or any other impurities, can be carried out using chemical, mechanical, or biological methods or a combination of these methods (Baruah et al. 2018; Wan and Li 2012; Putro et al. 2016; Mahmood et al. 2019). Typically, dozens of pre-treatment methods are available, and the choice of method depends on the type of NC, scalability, efficiency, and the cost of the process. Furthermore, pre-treatment of lignocellulosic feedstocks is often projected as a serious bottleneck in commercialization, and readers can consult excellent reviews to get a compressive insight into these aspects (Kumar and Sharma 2017; Ahorsu et al. 2018). Alkali- or mechanically pre-treated feedstock is usually converted to cellulose at nanoscale by applying strong acids (Kandhola et al. 2020), oxidative reagents (Isogai and Zhou 2019), ionic acids (Babicka et al. 2020), or physical (Cherian et al. 2010) or mechanical forces (Piras et al. 2019). Mineral acid hydrolysis, which is preceded by alkali treatment (NaOH 4%, 80 °C) and a bleaching process (usually with $NaClO_2$), is a frequently used method for the extraction of nanocellulose crystals. The approach mainly cleaves the glycosidic bonds in noncrystalline regions of cellulose fibrils, yielding needlelike cellulose crystals with higher crystallinity (over 60%), shorter length (10–100 nm), and a lower degree of polymerization than CNFs (Mokhena and John 2020). On the other hand, various mechanical methods are employed for nanocellulose fiber extraction, including high-pressure homogenization (HPH) or microfluidization, grinding, ball milling, steam explosion, ultrasonication, cryocrushing, high-speed blending, and so on (Xie et al. 2018). The mechanical approaches normally produce long fiber networks with a typical diameter of less than 100 nm and lengths of 0.1 to 2 mm. The final step is usually concerned with the recuperation of NC and neutralization of toxic chemicals generated thereof, especially for acid treatments, and thereby incurring additional costs. Other drawbacks, often cited with the mechanical methods, include the requirement of bulky equipment, clogging, and the energy-intensive nature of the process. As a result, these difficulties limit large-scale commercialization of NC and often lead to the search for a greener way of nanocellulose extraction. It should also be clear that some of the chemical or mechanical methods have been developed primarily for cellulose extraction used in the food, pharmaceutical, and biofuels industries, as evinced by research on NC in the past two decades. For these and other methods, the readers are suggested to refer to recently published excellent reviews (Xie et al. 2018; Phanthong et al. 2018). In the context of "going green," it is also pertinent to mention that research has been directed toward exploring a number of enzymes (Michelin et al. 2020) for the production of NC (Fig. 4.2).

Enzymatic hydrolysis of lignocellulose with cellulase releases the cellulose in combination with pre-treatment methods that are used to delignify the feedstock (Karim et al. 2017; Ribeiro et al. 2019; Squinca et al. 2020). However, large-scale production of NC suffers from practical snags due to processing costs, scale-up problems, and a lack of efficient enzymes. Moving ahead, as exemplary approaches, we have presented our discussion on two NC-synthesis approaches, based on *ammonium persulfate (APS)* and *ionic liquids* in the subsequent section.

Sulfuric acid hydrolysis of lignocellulosic feedstock has a long application history in the delignification and production of cellulose. In fact, other acids, including hydrochloric acid, phosphoric acid, hydrobromic acid, and formic acid, have also been used, but the agglomeration of the resulting product tends to limit their use (Yu et al. 2013). The use of sulfuric acid also generates sulfonated NC with a stable dispersion but with poor thermal stability, which constrains their use in composite materials. Additionally, the corrosion of equipment and the large amount of toxic chemicals generated by the process have raised environmental concerns, therefore, requiring a toxicity neutralization step that further increases the cost of the manufacturing process (Leung et al. 2011). Recently, the use of *ammonium persulfate* (APS) as an oxidant has been viewed as an eco-friendly method of producing CNC (Zhang et al. 2016). The method had attracted considerable attention due to its low long-term toxicity, high water solubility, low cost, and lesser use of harsh extractive agents. In addition, directly carboxylated cellulose nanocrystals are produced from lignocellulose by concomitant removal of lignin, hemicellulose, and other plant contents (Zhang et al. 2016; Oun and Rhim 2017, 2018; Liu et al. 2020). The free radicals generated in the oxidation process are responsible for removing the noncellulosic components as well as breaking down the amorphous region, thereby assisting the extraction of CNCs (Mascheroni et al. 2016). The resulting carboxylated CNCs are amenable for additional surface modification and tailoring with

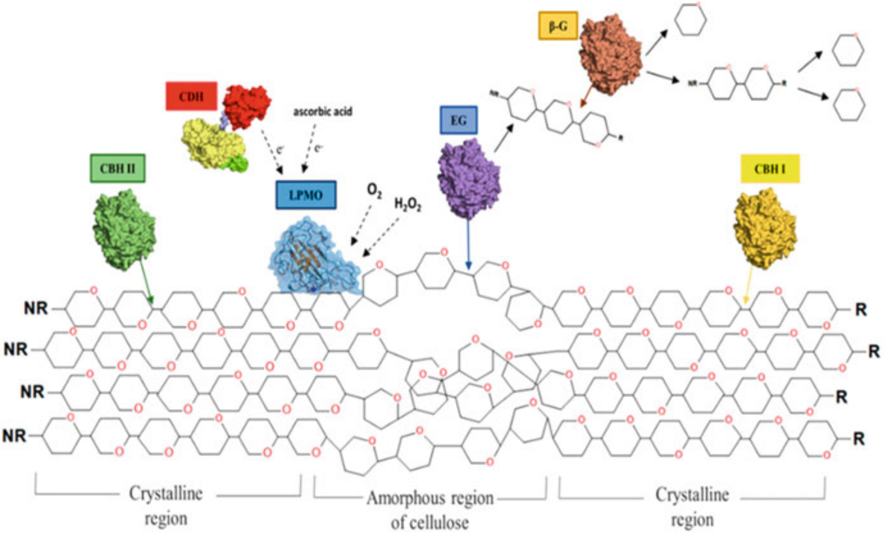


Fig. 4.2 Overall schematic representation of the mechanisms involved in NC production by distinct classes of enzymes. *R* reducing cellulose end, *NR* nonreducing cellulose end, *EG* endoglucanase, *CBH I* exoglucanase removing cellobiose from R end, *CBH II* exoglucanase removing cellobiose from NR end, *β-G* β-glucosidase, *CDH* cellobiose dehydrogenase, *LPMO* lytic polysaccharide monooxygenases. (*Reproduced from* Michelin et al. 2020, *© 2020 by the authors. Licensee MDPI, Basel, Switzerland, under the provision of the Creative Commons Attribution (CC BY) license (*http://creativecommons.org/licenses/by/4.0/)

various chemical units via hydrogen bonding (Chakrabarty and Teramoto 2018), electrostatic interactions (Lombardo and Thielemans 2018), as well as coordination complexation (Janicki et al. 2017). The APS method has been used to generate CNCs with a crystallinity index of 93.5% using cotton linters (Oun and Rhim 2017). Additionally, the method has also been shown to be effective with other cellulosic raw materials, including microcrystalline cellulose and straw, for the production of CNCs (Oun and Rhim 2017, 2018). The strategy can also be combined with other NC extractions approaches in order to increase its efficiency, since the NC yield is considered to be less satisfactory compared to other methods, such as 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-mediated oxidation and periodate-chlorite oxidation. Liu et al. (2020) developed a modified APS method that was used to produce CNCs from cotton pulp. It was faster and resulted in higher yield due to the generation of more than two free radicals compared to the conventional APS oxidation, which generates only a single type of free radical. The researchers used N, N, N´, N´-tetramethylethylenediamine (TMEDA or TEMED), which formed a redox-initiating system with APS and served as an electron donor to catalyze the decomposition of $S_2O_8^{2-}$, generating free radicals and hydrogen peroxide within a relatively short time. The developed process was projected suitable for the production of CNCs on a commercial scale. Amoroso et al. (2020) reported an alternative microwave-assisted ammonium persulfate process for the production of cellulose nanocrystals. Herein, the cotton powder was introduced into microwave pressure vessels and 1 M APS solution was added to fabricate CNCs. By varying the reaction conditions with the microwave, high-quality CNCs with a defined hydrodynamic diameter (150 nm) and zeta potential (−0.040 V) were produced in just 90 minutes. The APS process has also been modified to produce CNFs. Filipova et al. (2018) generated CNFs from bleached birch kraft pulp by resorting to combined APS oxidation and mechanical treatment, based on a high shear laboratory mixer and ultrasonication. The CNFs had a diameter of 20–300 nm, a crystallinity index of 74.3%, and a zeta potential −26.9 ± 1.8 mV.

Ionic liquids (ILs) are liquid salt compounds that form both anionic and cationic species at room temperature or near room temperature. They are considered a green solvent due to several salient features, including very low or even negligible vapor pressure, high thermal stability, and chemical stability, good solubility in polar and nonpolar solvents, easy recovery, and nonflammability, among others (Usmani et al. 2020). Notably, their attractive applications in cellulose hydrolysis include homogenous catalysis, executed under mild conditions, with the possibility of re-extraction of the solvent. Studies have been undertaken to facilitate NC generation from microcrystalline cellulose (MCC), wood pulp, wax, etc., using ionic liquids as solvent and catalyst, most commonly, 1-butyl-3-methylimidazolium hydrogen sulfate ([Bmim][HSO_4]), 1-ethyl-3-methylimidazolium acetate (EMIMAc), and 1-ethyl-3-methylimidazole chloride ([EMIM][Cl]) (Vinogradova and Chen 2016). Tan et al. (2015) produced rod-shaped CNCs (diameter, 15 to 20 nm, and length, 70 to 80 nm) by dissolving MCC with [Bmim][HSO_4]. The CNCs were small in size and high in crystallinity at the optimal reaction temperature of 90 °C. Additionally, mixed solvent systems were chosen by Miao et al. (2016) to prepare hydrophobic CNCs from wood pulpboard. In their work, they used tetrabutylammonium acetate-dimethylacetamide mixed solvent system in combination with acetic anhydride to cleave the amorphous cellulose and to acetylate the hydroxyl groups. The method has been shown to be very effective in generating nanocrystals, compatible with polymer materials. Al Hakkak et al. (2019) attempted nonsolvent addition and drying to produce rather rare spherical cellulose nanoparticles by dissolving MCC with EMIMAc. The anhydrous environment assisted in preventing the aggregation of the generated nanocellulose. Ionic liquids-assisted fabrication of NC can also be combined with enzymatic or mechanical methods to yield a product with desired properties. An important consideration in this aspect is the selection of enzymes, where their activity and stability remain acceptable in the presence of ionic liquids. In the presence of [Bmim][HSO_4], Zhao et al. (2017) resorted to enzymatic esterification for the production of cellulose nanocrystals. Phanthong et al. (2017) applied ball milling in the presence of ionic liquid and produced cellulose nanocrystals (Phanthong et al. 2017). Ball milling is considered a fast and a green way for milling cellulose, which effectively affects the microscopic and macroscopic properties. As a result, the combined approach achieved a yield of 93.1%, and multiple rounds of reuse of the ILs were also possible. Prior to moving ahead, readers may peruse the following table (Table 4.1) to have a contrasting account of the various methods used for the preparation of NC.

Table 4.1 Advantages and disadvantages of various approaches for the preparation of NC (*reproduced with permission from* Wang et al. 2019*, Copyright © 2019 Informa UK Limited, trading as Taylor & Francis Group*)

Type	Method	Advantages	Disadvantages
CNC, CNW	Acid hydrolysis method	Well-rounded technology, uniform particle size	Difficult recovery of residue, troublesome post-treatment
	Enzymatic hydrolysis method	Pollution-free, better specificity	Low efficiency, high cost
	Ionic liquid method	Environment friendly, easy recovery, high efficiency	High cost, few varieties of solvents
	TEMPO oxidation	Mild reaction conditions, simple operation, and low energy consumption	Harmful to the environment
CNF, MFC	TEMPO oxidation	Mild reaction conditions, simple operation, and low energy consumption	Harmful to the environment
	APS oxidation	Low cost, environment friendly	Wide particle size distribution
	Physical methods	Simple process, well-developed technology	High energy consumption, wide particle size distribution
	Electrospinning method	High aspect ratio	Limited types of solvents
BNC	Biological method	Low energy consumption, pollution-free	Long time, high cost, complicated preparation process

Abbreviation: *CNC* cellulose nanocrystals, *CNF* cellulose nanofibers, *CNW* cellulose nanowhiskers, *MFC* microfibrillated cellulose, *BNC* bacterial nanocellulose, *TEMPO* 2,2,6,6,-tetramethylpiperidine-1-oxyl

4 Surface Modification

In the previous sections, we have highlighted the outstanding features of cellulose and NC. Another feature that has actually catapulted the applications of NC in environmental remediation by multifold is the amenability for a diverse gamut of surface modifications. Environmental remediation in the perspective of removal/treatment of pollutants in wastewater, soil, air, and drinking water requires an understanding of the behavior of the pollutants: they may be anionic, cationic, polar, nonpolar, organic or inorganic substances, etc. So, the effective removal of these pollutants depends on the interaction of these molecules with NC. NC is rich in hydrophilic OH-group, and thereby, it opens up enough scope for surface modification to facilitate optimal interactions with foreign substances via electrostatic interaction, ion-exchange reaction, hydrophobic interaction, π-π interactions, and hydrogen bonding (Hokkanen et al. 2016; Zhu et al. 2020; Chakrabarty and Teramoto 2018; Lombardo and Thielemans 2018). Besides imparting ionic charges to the surface of NC, surface modification overcomes the problem of aggregation and enhances compatibility with other polymers. Furthermore, the surface engineered materials thus produced should also be nontoxic, recyclable, and biodegradable. Albeit NC materials meet these criteria, howbeit, few reports second a low level of cellulose toxicity under certain experimental conditions, while surface modification for environmental applications should be addressed with adequate deliberation, especially if intended for use in drinking water treatment and filtration (Ogonowski et al. 2018). Tailoring of the surface of NC can be achieved via physical, chemical, or enzymatic methods. Hydrolase and oxidoreductase enzymes are often used in the enzymatic surface modification, and this is an environmentally benign approach (Araújo et al. 2008; Afrin and Karim 2017).

Moreover, the surface can be cationized, anionized, or hydrophobicized depending on the pollutants in consideration by employing multiple methods, including sulfonation, polymer grafting, oxidation, esterification, nucleophilic substitution, etherification, silylation, and carbamation (Trache et al. 2020; Sharma et al. 2019) (Fig. 4.3). NC extraction methods, such as sulfuric acid hydrolysis and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) oxidation, usually result in the introduction of negatively charged functional groups, and such type of nanocellulose-

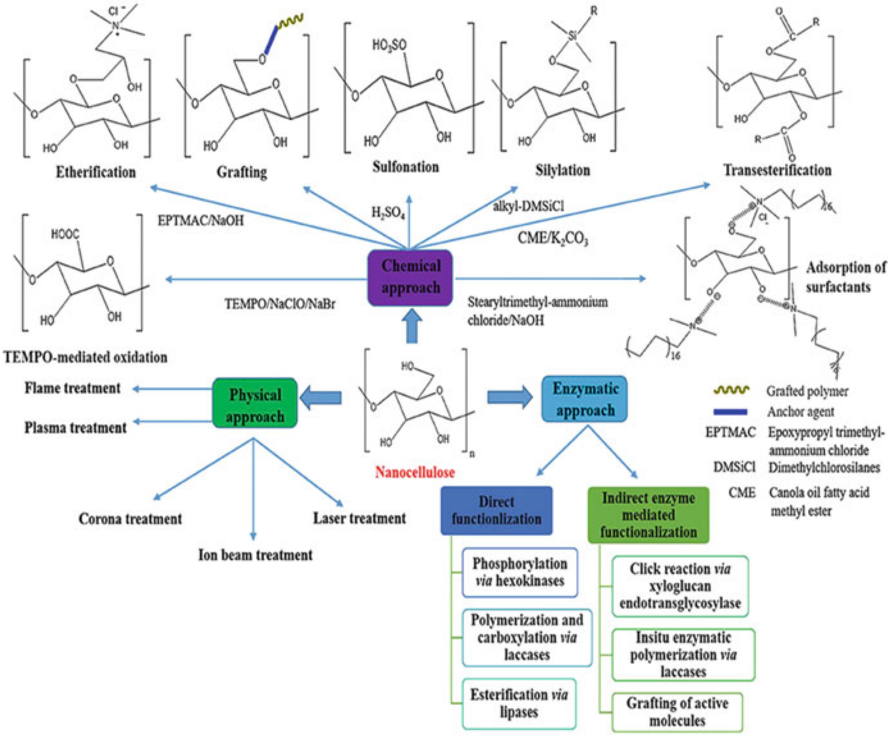


Fig. 4.3 Schematic representation of the most commonly used surface modification routes of nanocellulose. (*Reproduced from* Trache et al. 2020*; Copyright © 2020 Trache, Tarchoun, Derradji, Hamidon, Masruchin, Brosse and Hussin, under the provisions of the* Creative Commons Attribution License (CC BY))

based materials has been proven to be very effective in the removal of heavy metals (Zhu et al. 2020; Khoathane et al. 2015). On the other hand, amination of surface hydroxyls of nanocellulose via grafting and quaternation with various alkyl chains (methylamine, butylamine, and hexylamine) by mechanical homogenization could introduce a positive charge at the surface and hence be used for the removal of anionic pollutants (Wang et al. 2016; Yue et al. 2019).

5 Spectrum of Neoteric NC-Based Materials and Exploratory Endeavors for Environmental Applications

For environmental applications, nanocellulose may be employed as filtration membranes, adsorbent, flocculent, catalyst, and so on (Fig. 4.4a). In fact, many of the exceptional properties of NC, such as high porosity, high surface area, and tunable surface chemistries, have increased the application of NC in water treatment aimed at removing pollutants (Derami et al. 2020; Xu et al. 2018a; Varghese et al. 2019). Among others, the prominent materials that have captured immense research impetus over the recent decades for pollutant capturing/mitigation and other allied applications are the ***hydrogels*** – the three-dimensional colloidal gels of cross-linked polymers that can absorb and retain water and aqueous substances (Wichterle and Lim 1960; Klein and Poverenov 2020; Nam et al. 2018; Sinha and Chakma 2019). Although many hydrogels with demonstrable economic importance have been synthesized from polyethylene glycol (PEG), polyvinyl alcohol (PVA), and polyvinyl pyrrolidone (PVP), environmental concerns have driven the use of natural polymers, such as nanocellulose. CNCs- or CNFs-based hydrogels offer a variety of practical advantages, including biodegradability, nontoxicity, high water content, and biocompatibility. In fact, the mechanical strength of cellulose hydrogels during the swollen state is considered to be problematic, but this has been improved with composite fabrication, cross-linking, and other approaches. For example, Xu et al. (2019) produced TEMPO-oxidized individualized cellulose nanofibrils cross-linked physically and chemically, having a compressive strength of 450 kPa and a compressive strain of 90% without cracking. The process for the production of hydrogels based on CNCs includes either physical or chemical stabilization of an aqueous solution of a certain concentration. Depending on the shape, manufacturing process, type of cross-linking, and application, CNC-based hydrogels are classified as traditional bulk hydrogels and injectable hydrogels (Shojaeiarani et al. 2019). Pertinently, dual cross-linked hydrogels appear to be very efficient in solving the mechanical strength problems associated with nanocellulose-based hydrogels. The method uses both physical and chemical cross-linking approaches, and the resulting hydrogels with their highly porous structure, similarity to the natural extracellular matrix (ECM), the capability of enclosing cells within their matrix, and the ability to access deep-seated areas merit special mention, both in biomedical and environmental applications.

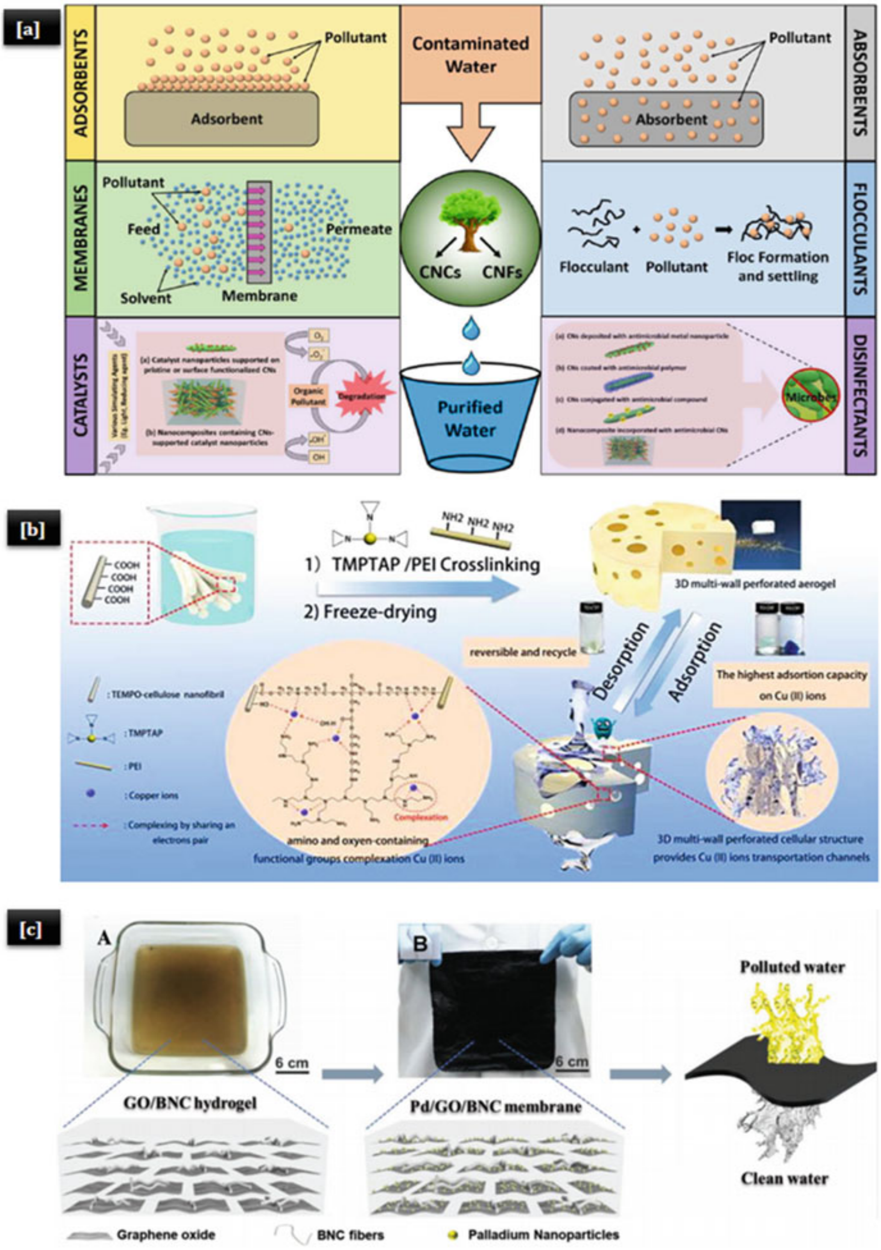


Fig. 4.4 (**a**) Schematic diagram describing the various water/wastewater treatment processes in which CN-based systems can be utilized. (*Reproduced with permission from Mohamaad* et al. *2018, Copyright © 2018, Royal Society of Chemistry*) (**b**) 3D multiwall perforated nanocellulose-based polyethylenimine aerogels for ultrahigh efficient and reversible removal of Cu(II) ions from water. (*Reproduced with permission from* Mo et al. 2019, *Copyright © Elsevier, 2019*) (**c**) Schematic illustration and photographs showing the various steps including A) GO/BNC hydrogel and B) Pd/GO/BNC membrane involved in the fabrication of Pd/GO/BNC membrane. (*Reproduced with permission from Xu* et al. *2018; Copyright © 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim*)

Aerogels, on the other hand, are ultralightweight materials with three-dimensional porous networks. They are increasingly valuable in the environmental remediation of heavy metals and dyes and oil removal from water as well as in water purification, due to their high porosity, large specific surface area, and ease of separation from aqueous solutions (Sharma et al. 2020b). The nanocellulosic aerogels can be produced in three steps: *dissolving/dispersing cellulose or cellulose derivatives*, *forming cellulose gel according to the sol-gel process,* and *drying cellulose gel while maintaining the porous 3D structure*. Various methods are available for drying the cellulose gel, in particular oven-drying, freeze-drying, and supercritical drying, which are preceded by solvent exchange. The NC-based aerogels usually have a pore size in the range of 2–50 nm with high porosity (generally>90%). To improve their function, NC-based aerogels can be cross-linked with other materials, including 3-mercaptopropyltrimethoxysilane, trimethylolpropane tris (2-methyl-1-aziridine) propionate (TMPTAP), and others. Mo et al. (2019) demonstrated the formation of 3D multiwall perforated nanocellulose-based polyethylenimine aerogels (TO-CNF/TMPTAP/PEI) using 0.6 wt% TEMPO-oxidized CNF. The CNF suspension was untrasonicated for 30 min and cross-linked with trimethylolpropane-tris-(2-methyl-1-aziridine) propionate (TMPTAP) and polyethyleneimine (PEI) under adjusted pH and concentrations of the cross-linking agents. The resulting recyclable aerogels obtained post freeze-drying were rich in amino-functionalized surfaces and showed an ultrahigh adsorption capacity for removing heavy metal ions from aqueous environments (Fig 4.4b). Similarly, Xu et al. (2018b) developed an innovative approach for the production of cellulose nanofibers (CNFs)/multiwalled carbon nanotubes (MWCNTs) and carbon aerogels, using bamboo powder as the initial bioresource, through a simple dipping and carbonization process. The pertinent properties of the synthesized aerogels included low density (0.056 g cm^{-3}), high porosity (95%), and an efficient ability to separate oily droplets from the water beside a high adsorption efficacy for a wide range of oils and organic solvents. Another porous structure based on NC are the ***xerogels***, fabricated via evaporative drying of wet gels. However, their manufacturing process has been a challenge due to the shrinking of the gels that ultimately reduce porosity. Nevertheless, several approaches, such as solvent exchange and the use of very fine type CNFs with a width of ~3 nm have expanded the domains NC as materials for xerogel synthesis (Yamasaki et al. 2019).

Concert of various organic or inorganic materials with nanocellulose yields products of superior quality. Nanocarbon, nanographene, organic polymer matrices, as well as various inorganic nanoparticles have been adroitly employed to fabricate *avant-garde* ***NC-based nanocomposites*** (Jodeh et al. 2018; Tshikovhi et al. 2020; Lebogang et al. 2019). As an exemplary endeavor, Bossa et al. (2017) reported the fabrication of cellulose nanocrystal-zero valent iron nanocomposites (CNC-nano-ZVI) for prospective in situ groundwater remediation. Their study attested that NC improved the mobility of nano-ZVI particles. Additional attractive applications of NC pertain to the preparation of nanocellulose-based composite membranes and highly porous adsorption composites (Sharma et al. 2020a; Patel et al. 2019). In this regard, functionalized bacterial nanocellulose (BNC) composites have also fetched a commendable focus for wastewater treatment. To cite for evidence, palladium/graphene oxide/BNC-based hybrid, robust, and reusable membrane was reported to be effective enough in treating a contaminant cocktail of methylene blue, 4-nitrophenol, and rhodamine 6G, besides demonstrating a high efficiency for methyl orange degradation during filtration (Fig. 4.4c) (Xu et al. 2018a, b). NC- and NC-based composites have been explored across a wide spectrum of environmental applications, including toxicant-sensing, air purification, and mitigation of toxic materials, including heavy metals and industrial dyes, water purification, fabrication of antimicrobial membranes, catalysis, and energy storage, among others. The applications of most of these materials rely on harnessing the specific pros, emanating as a result of the interactions between diverse nanomaterials and nanocellulose (Wei et al. 2014), as exemplified in a couple of studies cited underneath.

Successful deployment of ***nanocellulosic sensors for detection and monitoring of contaminants*** in the environment, food, cosmetics, and other stuffs has opened new portals of research and commercial plausibility. The following examples stand in testimony to this aspect. In recent years, albeit, silver nanoparticles (Ag NPs), known for their antimicrobial attributes, have fetched their inclusion in a number of consumer products, such as socks, deodorants etc., although their entry into the environment and human body has been an issue of apprehension. In this regard, a luminescent hydrogel with an orange/red emission at 617 nm was fabricated through the incorporation of photosensitive bis(2,2-bipyridine)-[4-(4-methyl-2,2-bipyridin-4-yl) proplylamine] ruthenium(II) dihexafluorophosphate complex in to negatively charged carboxylated NC (Ruiz-Palomero et al. 2016). With a commendable sensitivity to the presence of AgNPs, the luminescent gels were established as apt sensors for silver nanoparticles in real samples with recoveries in the range of 84–94%. The pertinence of the study could be easily traced in the context of nanotoxicology, and as such, designing nanocellulosic sensors for other nanomaterials (e.g., carbon nanotubes, etc.) could be an interesting proposition. In a similar vein, a recent study evinced the relevance of aerogels in concomitant monitoring and remediation of environmental hazardous substances like organic dyes. Chook et al. (2015) had exploited a porous aerogel based on AgNPs-functionalized cellulosic nanofibrils for SERS (Raman spectroscopy-based) detection (at concentrations ranging from 5×10^{-3} M to 5×10^{-7} M) as well as catalytic degradation of rhodamine B.

Among the endeavors to develop a ***green air purification approach***, the use of films, foams, as well as sponges based on nanocellulose, exhibiting porous morphologies, seems quite attractive. In this regard, NC film-based technology for gas separation has been reported. As a representative example, besides exhibiting selective permeability for hydrogen gas, carboxylated NC membranes, post subjecting to a soaking treatment with 0.1 M HCl, were found to display higher H_2 permeation rates (Fukuzumi et al. 2013). By the same token, assessment of CO_2 capture (under 40% humidity) by aerogels (fabricated through freeze-drying of dispersions of cellulosic nanofibrils and aminosilanes) registered a mark of 0.695 mmol CO_2/g, while desorption was documented at 90 °C under argon ambiance (Gebald et al. 2011). The researchers had reported no negative trade-off with respect to the adsorption potency as well as the structural integrity up to 20 adsorption/desorption cycles. Alternatively, NC foams with polyethylenimine (in lieu of aminosilanes) have also been exploited for CO_2 trapping (Sehaqui et al. 2015). Herein, the deposition of urea in the sorbent was counterchecked due to the presence of water, thereby providing an additional edge to the system in CO_2 adsorption.

In a similar vein, applications as ***filtration membranes, absorbents, adsorbents, catalysts, as well as flocculants in the realm of water treatment*** have garnered tremendous research impetus in the recent years (Mahfoudhi and Boufi 2017; Mohammed et al. 2018; Voisin et al. 2017). ***CNC-based nanocomposite membranes*** (with a large surface area and wide range of pore size) have been fabricated using polymer matrices, such as chitosan, gelatin, poly(vinylidene fluoride) (PVDF), and poly(acrylonitrile) (PAN), for the ***removal of dyes, heavy metal ions, bacteria, viruses, and oil*** (Mohammed et al. 2018). In a similar vein, ***CNF-nanopaper/nanocomposite membranes***, based on poly(acrylonitrile) (PAN), poly(ether sulfone), poly(vinylidene fluoride) (PVDF), cellulose triacetate, chitosan, and silk fibroin, find application in ***ultrafiltration, forward osmosis, as well as nanofiltration*** for a wide range of materials, including ***viruses, oil, heavy metal ions, and organic solvents*** (Mohammed et al. 2018). Similarly, silver nanoparticles and chitosan (an antimicrobial biopolymer) have been adeptly employed to harness NC for ***antimicrobial applications,*** while the applications of CN as ***catalysts*** for the degradation of various organic pollutants are noteworthy (Fig. 4.5) (Mohammed et al. 2018). To mention about the application of NC in ***flocculation***, the functionalization with amines, acidic groups, and pyridinium, on one hand, and specific uses of CNF for separating solid particles from public wastewater and that of CNC with microbial cells of bacteria and microalgae as well as surfactants, on the other, merit special mention (Vandamme et al. 2015; Mohammed et al. 2018).

CNCs have been used in concert with nanoparticles or polymers to develop efficient ***adsorbent platforms for dye mitigation in aqueous media*** (Table 4.2). Amino-functionalization, carboxylation, imidazolium grafting, and maleic anhydride conjugation are the commonly adopted strategies for chemical modification of CNCs to facilitate adsorption of various pollutants, under a wide range of pH and, preferably, at room temperature (Mohammed et al. 2018). Interestingly, reports on fabrication of CNC nanocomposites based on alginate, dispersion of magnetic beads, use of hydrolyzed poly(acrylamide) (HPAM), poly(vinyl amine) beads, or electrospun fibers have widened the scope (Chen et al. 2014; Zhou et al. 2013, Jin et al. 2015b, Nypelö et al. 2014). In a similar vein, a plethora of chemically functionalized CNF aerogels (e.g., MnO_2-coated CNF and GTMAC-grafted CNF) have been employed as adsorbents besides other polymer nanocomposites, fabricated using high-intensity ultrasonication and TEMPO-mediated oxidation, for effective dye removal (Wei et al. 2014; Mohammed et al. 2018). The ***removal of heavy metal ions*** (Table 4.2) (e.g., cadmium, copper, iron, lead, nickel, silver, and vanadium) in the context of water treatment using pristine, acrylamide-grafted, anhydride-modified, carboxylic acid conjugated, phosphorylated, succinic bisphosphonate-modified CNCs has been reviewed extensively (Mohammed et al.

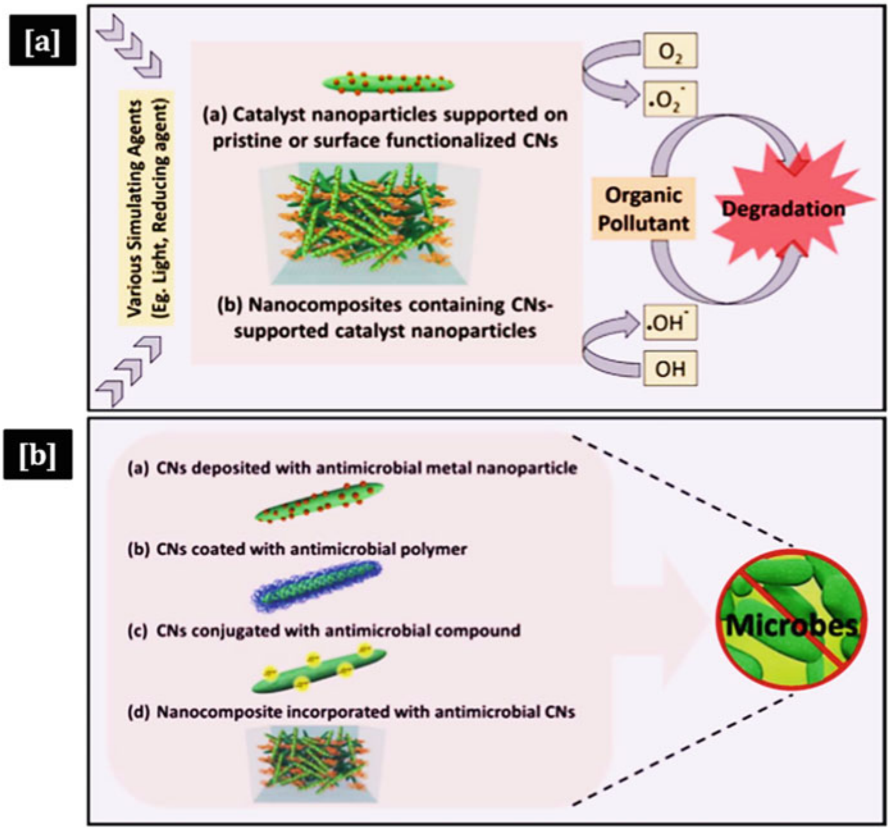


Fig. 4.5 Schematic diagram showing the various types of CN-based systems having catalytic and disinfectant properties. (*Reproduced with permission from* Mohammed et al. 2018*, Copyright © 2018, Royal Society of Chemistry*)

2018; Shak et al. 2018; Sheikhi 2019). The time required may vary from a few minutes to a couple of hours. On a similar note, cadmium, cobalt, copper, chromium, iron, nickel, silver, uranium oxide, and zinc have been the targets for pristine CNF, aminopropyltriethoxysilane (APTES)-modified, carboxylated, carbonated hydroxyapatite (CHA)-modified, phosphorylated, succinic anhydride-grafted and sulfonated CNFs, as well as CNFs-filled magnetic chitosan hydrogel beads (Mohammed et al. 2018; Zhou et al. 2014).

Mohammed et al. (2018) had also highlighted that CNC and its various nanocomposites find applications in ***addressing contamination*** and ensuring removal of sulfamethoxazole (an antibiotic); acetaminophen (an analgesic and antipyretic); biomolecules like hemoglobin, creatinine, trypsin, and immunoglobulin G; as well as substances like N,N-diethyl-meta-toluamide (a prime component in insect repellents) and chlorpyrifos (an organophosphate pesticide). The use of APTES, CHA, epoxy, hydroxycarbonate apatite (HAP) and modified CNF has

Table 4.2 A representative studies on nanocellulose-based adsorbents

Cellulosic nanomaterial-based adsorbent	Target pollutant	Adsorption capacity (mg/g)	Time (min)	References
Poly(itaconic acid)–poly(methacrylic acid)-grafted-nanocellulose/nanobentonite composite	Co (II)	350.8	120	Anirudhan et al. (2016)
	U (VI)	121.02	120	Anirudhan et al. (2015)
Sulphonated nanofibrillar cellulose	Pb(II)	248.6	1200	Suopajärvi et al. (2015)
Cationic cellulose nanofibers functionalized with glycidyltrimethylammonium chloride	PO_4^{3-}	55		Sehaqui et al. (2016)
	SO_4^{2-}	50		
	F^-	10.6		
	NO_3^-	44		
Nanocellulose-amino linked by maleic acid supported on magnetite	Arsenic	85.3	90	Taleb et al. (2019)
Amino-functionalized nanocrystalline cellulose	Acid red GR	134.7	300	Jin et al. (2015a)
	Congo red 4BS	199.5		
	Reactive light yellow K-4G	183.0		
Cellulose nanocrystal-alginate hydrogel beads	Methylene blue	255.5	210	Mohammed et al. (2016)
Cellulose aerogel based on cationic cellulose nanofibrils	Blue dye CR19	230	80	Maatar and Boufi, (2017)
	Red dye 180	160		
	Orange dye 142	560		

been reported for the mitigation of nitrates, hydrogen sulfide, and phosphates (Mohammed et al. 2018). As exemplary evidence, Luo et al. (2019) manufactured CNFs, by applying mechanical shearing to a pristine bamboo pulp. Predetermined doses of ferric chloride solution and sodium hydroxide solution were added to the CNFs suspension. The entire suspension was stirred and centrifuged before freeze-drying. The nanocellulose /$Fe(OH)_3$ composites were shown to be very effective for addressing low-concentration phosphorus-containing wastewater (Fig. 4.6a). On the other hand, the scaling phenomenon is a serious concern, particularly in water-based industries. The conventionally used anti-scaling materials based on nitrogen, phosphorous, and sulfur are interlaced with potential hazardous ecological consequences. In this regard, hairy nanocellulose (characterized by dicarboxylated amorphous cellulosic chains) coating onto planar silica surfaces could inhibit the nucleation and subsequent growth of $CaCO_3$, a common industrial scale (Sheikhi et al. 2018), thereby establishing the potential of NC in developing ***green antiscalant.***

The readers may also easily perceive the impetus received ***by green and renewable electronics*** as well as ***green energy technology*** (Resch et al. 2008) in the

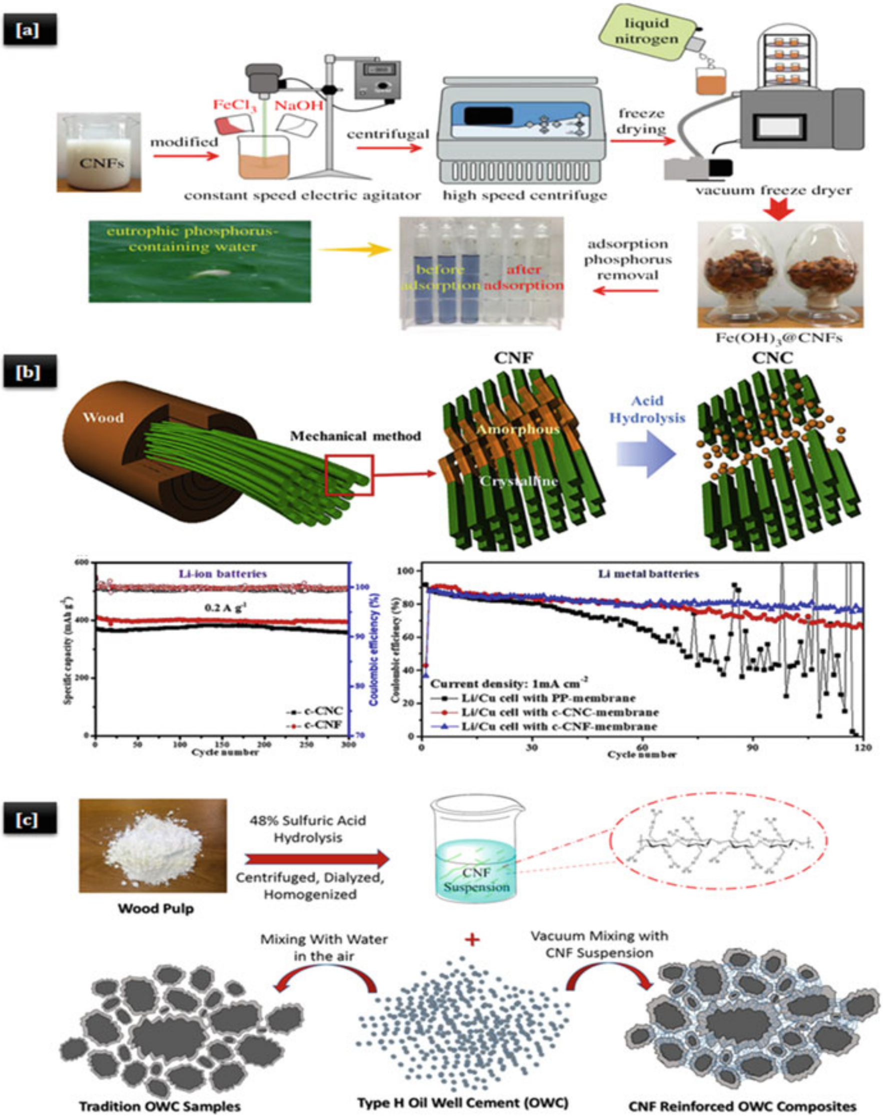


Fig. 4.6 (**a**) Schematic diagram of the optimized materials experimental program. Iron-modified nanofibers for low-concentration phosphorus-containing wastewater treatment. (*Reproduced from* Luo et al. 2019, *Copyright © 2019 The Authors (Published by the Royal Society), under the terms of the Creative Commons Attribution License,* http://creativecommons.org/licenses/by/4.0/) (**b**) Cellulose derived structured carbons on the electrochemical behavior of lithium metal-based batteries. (*Reproduced with permission from* Kim et al. 2019. *Copyright © 2019 Elsevier*) (**c**) Schematic of the preparation method of cellulose nanofiber reinforced OWC composites. (*Reproduced from* Sun et al. 2016 *under the provisions of Creative Commons Attribution 4.0 International License.* http://creativecommons.org/licenses/by/4.0/)

context of the incessant fossil fuel consumption and the various ecological issues including climate change. In this regard, nanocellulose-based aerogel, flexible film, as well as the three-dimensional structures have been adroitly employed to power portable electronics and electric vehicles as well as fabricate energy capture devices (Lasrado et al. 2020). The authors have reviewed that matrix-reinforcement with nanocellulose augments the power conversion efficacy of solar cells and the piezoelectric performance of piezoelectric materials. By the same token, sustainability, biodegradability, and surface tailorability of nanocellulose project it to be a substrate of choice in fabricating flexible electrodes as well as separators in lithium-ion batteries (LIBs) and supercapacitors. To cite a recent endeavor, the effect of morphology and crystallinity of carbonized CNF and CNC (c-CNF and c-CNC) on the overall electrochemical reactions in Li metal-based batteries were assessed (Kim et al. 2019). Interestingly, the cell's augmented specific capacity (and downscaling of the overall electrical conductivity of the electrode) was bracketed together with carbon derived from the amorphous region of CNF. Contrastingly, the high capacity of 412 mAh g^{-1} at a current density of 0.2 A g^{-1} was recorded for the c-CNF electrode with respect to 370 mAh g^{-1} for the corresponding c-CNC counterpart, although a better rate capacity was recorded for the latter in comparison to the former. Pertinently, a commendable improvement in Coulombic efficiency and cycle stability (over 120 cycles) was documented on the application of polypropylene (PP) separators modified with c-CNF and c-CNC in Li/Cu cells (Fig. 4.6b).

On the other hand, various CNF aerogel nanocomposites (exhibiting ~20–350 g oil removal efficacy per g of the absorbent) based on the use of silane agents, PVA and TiO_2, have been ably employed for the ***removal of oil*** (Korhonen et al. 2011; Zheng et al. 2014; Zhang et al. 2014). Similarly, the selective absorption of various organic solvents, oils, milk fat, and hydrophobic proteins was documented for cellulosic scaffold, fabricated via freeze-drying ice-templating of chemically modified (Abraham et al. 2017). The report by Yang and Cranston (2014) merits special mention here. An ultralightweight CNC aerogel, with bimodal (mesopores <50 nm and macropores >1 μm) pore distribution, was fabricated by freeze-drying of a hydrogel, obtained as a consequence of hydrazone cross-linking of hydrazide-modified CNCs with aldehyde-modified CNCs. Pertinently, the system could absorb both dodecane (72 ± 5 g/g of aerogel) and water (160 ± 10 g/g of aerogel), thereby attesting its prospective application as superabsorbent material besides oil/water separations.

We would also like to bring to the attention of the readers that excellent thermal and physicochemical attributes of nanocellulose, attributable to their crystalline structure, could be exploited in ***designing high-performance oil and gas drilling systems*** (Ramasamya and Amanullaha 2020). NC has been found to endow desirable mud attributes, including viscous properties, augmented fluid loss nature, and generation of thin mudcake in drilling fluids. The application of ***NC in oil well cementing*** **(OWC)** (Fig. 4.6c) has been marked with the betterment of the properties of cement-like structural rigidity and yield stress, assisting to resist unfavorable action of any extrinsic factors. NC's use has been documented to improve the mechanical, flexural, and rheological properties of cement (Sun et al. 2016), thereby attesting the prospects of nanocellulosic materials in cement-related drilling applications.

6 Challenges, Future-Direction of Research, and Concluding Remarks

Among others, the following hallmarks with respect to cellulosic nanomaterials merit special mention:

a) Greater sustainability and environmental friendly attribute of NC in contrast to petro-based polymers and other materials used for environmental applications
b) Lower carbon footprint associated with their production in comparison to activated carbon (used widely for bioremediation purposes), produced via thermal strategies
c) Large surface-area-to-volume ratio and quantum size consequences, offering a wide scope for surface molecular engineering and augmenting the number of effective adsorption sites
d) Feasibility to use in its pristine form and amenability for the fabrication of various nanocomposites with advanced biophysicochemical features, including desirable mechanical strength and dispersibility in water, providing avenues for their use in diverse formats, including filtration membranes, adsorption platforms, flocculants, aerogels, sensors, catalysts, etc.

Pertinently, retrieval of more than 550 granted patents on cellulosic nanomaterials in Google Patents database as well as projected turnover for ~700 million USD by 2024 attest to the escalating interest that NCs have garnered amidst the academic and industrial fraternities (Mokhena and John 2020). Post perusal of the various literature reports, cited in this chapter, one may easily perceive the immense prospects of NC for environmental applications. As noted in the write-up, NCs could provide green alternatives for addressing industrial dye- and metal-related pollution. Moreover, CNs could serve as befitting materials for water treatment operations, in particular, pertaining to catalysis for pollutant degradation as well as pathogenic microbial disinfection. In this context, CNs could open new portals of devising apt stratagems for addressing various environmental issues across multiple industries (e.g., pulp and paper, leather tanning, textile, fertilizer, paints etc.). Furthermore, there lies the scope for developing NC-based green energy technology.

At this juncture, it is prudent to mention, however, that the cost of production of NC might be an issue to ponder on. In fact, highly purified CNCs may cost around USD 10–25 per kg (Mohammed et al. 2018). Nevertheless, commercial large-scale production may be envisaged to cut the costs, and moreover, low-purity CNCs could be effectively employed for wastewater treatment protocols. On the other hand, not many studies have vouched for the effective regeneration of NC-based adsorbents, and this could be a plausible future avenue to delve into. By the same token, cost-effective, safe, and easy functionalization strategies would be critical to improving the dispersibility of NCs within hydrophobic polymeric supports, employed in commercial filtration membranes. Although nanocellulosic materials are considered "green," howbeit, an all-inclusive life-cycle assessment, starting from extraction to the fabrication of NC-based products, biodegradation profiling, as well as in vitro and in vivo toxicity assessments, to gauge the biointerfacial actions would be indispensable to bring out the real worth of cellulosic nanomaterials for environmental applications. Nevertheless, on a concluding note, we would like to state that cellulosic nanomaterials could serve as a befitting testimony to the concept of "the power of the small to bring mammoth changes for a better tomorrow."

References

Abitbol T, Rivkin A, Cao Y, Nevo Y, Abraham E, Ben-Shalom T, Lapidot S, Shoseyov O (2016) Nanocellulose, a tiny fiber with huge applications. Curr Opin Biotechnol 39:76–88

Abraham E, Weber DE, Sharon S, Lapidot S, Shoseyov O (2017) Multifunctional cellulosic scaffolds from modified cellulose nanocrystals. ACS Appl Mater Interfaces 9(3):2010–2015

Afrin S, Karim Z (2017) Isolation and surface modification of nanocellulose: necessity of enzymes over chemicals. Chem Bio Eng Rev 4(5):289–303

Ahorsu R, Medina F, Constantí M (2018) Significance and challenges of biomass as a suitable feedstock for bioenergy and biochemical production: A review. Energies 11(12):3366

Al Hakkak J, Grigsby WJ, Kathirgamanathan K, Edmonds NR (2019) Generation of spherical cellulose nanoparticles from ionic liquid processing via novel nonsolvent addition and drying. Adv Material Sci Eng 2019:2081027

Amoroso L, Muratore G, Ortenzi MA, Gazzotti S, Limbo S, Piergiovanni L (2020) Fast production of cellulose nanocrystals by hydrolytic-oxidative microwave-assisted treatment. Polymers 12 (1):68

An VN, Nhan HTC, Tap TD, Van TTT, Van Viet P (2020) Extraction of high crystalline nanocellulose from biorenewable sources of Vietnamese agricultural wastes. J Polym Environ 28:1–10

Anirudhan TS, Deepa JR, Binusreejayan S (2015) Characterization of multicarboxyl-functionalized nanocellulose/nanobentonite composite for the adsorption of uranium (VI) from aqueous solutions: kinetic and equilibrium profiles. Chem Eng J 273:390–400

Anirudhan TS, Deepa JR, Christa J (2016) Nanocellulose/nanobentonite composite anchored with multi-carboxyl functional groups as an adsorbent for the effective removal of Cobalt(II) from nuclear industry wastewater samples. J Colloid Interface Sci 467:307–320

Araki J, Miyayama M (2020) Wet spinning of cellulose nanowhiskers; fiber yarns obtained only from colloidal cellulose crystals. Polymer 188:122116

Araújo R, Casal M, Cavaco-Paulo A (2008) Application of enzymes for textile fibres processing. Biocatal Biotransformation 26(5):332–349

Babicka M, Woźniak M, Dwiecki K, Borysiak S, Ratajczak I (2020) Preparation of nanocellulose using ionic liquids: 1-Propyl-3-Methylimidazolium chloride and 1-Ethyl-3-Methylimidazolium chloride. Molecules 25(7):1544

Baruah J, Nath BK, Sharma R, Kumar S, Deka RC, Baruah DC, Kalita E (2018) Recent trends in the pretreatment of lignocellulosic biomass for value-added products. Front Energ Res 6:141

Bossa N, Carpenter AW, Kumar N, de Lannoy CF, Wiesner M (2017) Cellulose nanocrystal zero-valent iron nanocomposites for groundwater remediation. Environ Sci Nano 4(6):1294–1303

Carpenter AW, de Lannoy CF, Wiesner MR (2015) Cellulose nanomaterials in water treatment technologies. Environ Sci Technol 49(9):5277–5287

Chakrabarty A, Teramoto Y (2018) Recent advances in nanocellulose composites with polymers: A guide for choosing partners and how to incorporate them. Polymers 10(5):517

Chen L, Berry RM, Tam KC (2014) Synthesis of β-cyclodextrin-modified cellulose nanocrystals (CNCs)@ Fe3O4@ SiO2 superparamagnetic nanorods. ACS Sustain Chem Eng 2(4):951–958

Chen X, Yuan F, Zhang H, Huang Y, Yang J, Sun D (2016) Recent approaches and future prospects of bacterial cellulose-based electroconductive materials. J Mater Sci 51(12):5573–5588

Cherian BM, Leão AL, De Souza SF, Thomas S, Pothan LA, Kottaisamy M (2010) Isolation of nanocellulose from pineapple leaf fibres by steam explosion. Carbohydr Polym 81(3):720–725

Chook SW, Chia CH, Chan CH, Chin SX, Zakaria S, Sajab MS, Huang NM (2015) A porous aerogel nanocomposite of silver nanoparticles-functionalized cellulose nanofibrils for SERS detection and catalytic degradation of rhodamine B. RSC Adv 5(108):88915–88920

da Gama FMP, Dourado F (2018) Bacterial NanoCellulose: what future? Bioimpacts 8(1):1

Derami HG, Gupta P, Gupta R, Rathi P, Morrissey JJ, Singamaneni S (2020) Palladium nanoparticle-decorated mesoporous polydopamine/bacterial nanocellulose as a catalytically active universal dye removal ultrafiltration membrane. ACS Applied Nano Material 3 (6):5437–5448

Dufresne A (2019) Nanocellulose processing properties and potential applications. Curr Forest Report 5(2):76–89

Fernandes AN, Thomas LH, Altaner CM, Callow P, Forsyth VT, Apperley DC, Kennedy CJ, Jarvis MC (2011) Nanostructure of cellulose microfibrils in spruce wood. Proc Natl Acad Sci 108(47): E1195–E1203

Filipova I, Fridrihsone V, Cabulis U, Berzins A (2018) Synthesis of nanofibrillated cellulose by combined ammonium persulphate treatment with ultrasound and mechanical processing. Nanomaterials 8(9):640

Fukuzumi H, Fujisawa S, Saito T, Isogai A (2013) Selective permeation of hydrogen gas using cellulose nanofibril film. Biomacromolecules 14(5):1705–1709

Gea S, Siregar AH, Zaidar E, Harahap M, Indrawan DP, Perangin-Angin YA (2020) Isolation and characterisation of cellulose nanofibre and lignin from oil palm empty fruit bunches. Dent Mater 13(10):2290

Gebald C, Wurzbacher JA, Tingaut P, Zimmermann T, Steinfeld A (2011) Amine-based nanofibrillated cellulose as adsorbent for CO_2 capture from air. Environ Sci Technol 45 (20):9101–9108

Gopu G, Govindan S (2018) Production of bacterial cellulose from Komagataeibacter saccharivorans strain BC1 isolated from rotten green grapes. Prep Biochem Biotechnol 48 (9):842–852

Habibi Y, Lucia LA, Rojas OJ (2010) Cellulose nanocrystals: chemistry, self-assembly, and applications. Chem Rev 110(6):3479–3500

Hokkanen S, Bhatnagar A, Repo E, Lou S, Sillanpää M (2016) Calcium hydroxyapatite microfibrillated cellulose composite as a potential adsorbent for the removal of Cr (VI) from aqueous solution. Chem Eng J 283:445–452

Ioelovich M (2008) Cellulose as a nanostructured polymer: a short review. Bioresources 3 (4):1403–1418

Isogai A, Zhou Y (2019) Diverse nanocelluloses prepared from TEMPO-oxidized wood cellulose fibers: Nanonetworks, nanofibers, and nanocrystals. Curr Opin Solid State Mater Sci 23 (2):101–106

Iwamoto S, Isogai A, Iwata T (2011) Structure and mechanical properties of wet-spun fibers made from natural cellulose nanofibers. Biomacromolecules 12(3):831–836

Janicki R, Mondry A, Starynowicz P (2017) Carboxylates of rare earth elements. Coord Chem Rev 340:98–133

Jin L, Li W, Xu Q, Sun Q (2015a) Amino-functionalized nanocrystalline cellulose as an adsorbent for anionic dyes. Cellul 22(4):2443–2456

Jin L, Sun Q, Xu Q, Xu Y (2015b) Adsorptive removal of anionic dyes from aqueous solutions using microgel based on nanocellulose and polyvinylamine. Bioresour Technol 197:348–355

Jodeh S, Hamed O, Melhem A, Salghi R, Jodeh D, Azzaoui K, Benmassaoud Y, Murtada K (2018) Magnetic nanocellulose from olive industry solid waste for the effective removal of methylene blue from wastewater. Environ Sci Pollut Res 25(22):22060–22074

Kandhola G, Djioleu A, Rajan K, Labbé N, Sakon J, Carrier DJ, Kim JW (2020) Maximizing production of cellulose nanocrystals and nanofibers from pre-extracted loblolly pine kraft pulp: a response surface approach. Bioresource Bioprocess 7:1–16

Karim Z, Afrin S, Husain Q, Danish R (2017) Necessity of enzymatic hydrolysis for production and functionalization of nanocelluloses. Crit Rev Biotechnol 37(3):355–370

Khoathane MC, Sadiku ER, Agwuncha CS (2015) Surface modification of natural fiber composites and their potential applications. Surface Modification of Biopolymers; John Wiley & Sons, Inc., Hoboken, pp 370–400

Kim PJ, Kim K, Pol VG (2019) A comparative study of cellulose derived structured carbons on the electrochemical behavior of lithium metal-based batteries. Energ Storage Mater 19:179–185

Klein M, Poverenov E (2020) Natural biopolymer-based hydrogels for use in food and agriculture. J Sci Food Agric 100(6):2337–2347

Korhonen JT, Kettunen M, Ras RH, Ikkala O (2011) Hydrophobic nanocellulose aerogels as floating, sustainable, reusable, and recyclable oil absorbents. ACS Appl Mater Interfaces 3 (6):1813–1816

Kumar AK, Sharma S (2017) Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. Bioresource Bioprocess 4(1):7

Lasrado D, Ahankari S, Kar K (2020) Nanocellulose-based polymer composites for energy applications—a review. J Appl Polym Sci 137(27):48959

Lebogang L, Bosigo R, Lefatshe K, Muiva C (2019) Ag_3PO_4/nanocellulose composite for effective sunlight driven photodegradation of organic dyes in wastewater. Mater Chem Phys 236:121756

Leung AC, Hrapovic S, Lam E, Liu Y, Male KB, Mahmoud KA, Luong JH (2011) Characteristics and properties of carboxylated cellulose nanocrystals prepared from a novel one-step procedure. Small 7(3):302–305

Lin N, Dufresne A (2014) Nanocellulose in biomedicine: current status and future prospect. Eur Polym J 59:302–325

Liu Y, Liu L, Wang K, Zhang H, Yuan Y, Wei H, Wang X, Duan Y, Zhou L, Zhang J (2020) Modified ammonium persulfate oxidations for efficient preparation of carboxylated cellulose nanocrystals. Carbohydr Polym 229:115572

Lombardo S, Thielemans W (2018) Thermodynamics of the interactions of positively charged cellulose nanocrystals with molecules bearing different amounts of carboxylate anions. Phys Chem Chem Phys 20(26):17637–17647

Luo Y, Liu M, Chen Y, Wang T, Zhang W (2019) Preparation and regeneration of iron-modified nanofibres for low-concentration phosphorus-containing wastewater treatment. R Soc Open Sci 6(9):190764

Maatar W, Boufi S (2017) Microporous cationic nanofibrillar cellulose aerogel as promising adsorbent of acid dyes. Cellul 24(2):1001–1015

Mahfoudhi N, Boufi S (2017) Nanocellulose as a novel nanostructured adsorbent for environmental remediation: a review. Cellul 24(3):1171–1197

Mahmood H, Moniruzzaman M, Iqbal T, Khan MJ (2019) Recent advances in the pretreatment of lignocellulosic biomass for biofuels and value-added products. Curr Opinion Green & Sustainab Chem 20:18–24

Mahmud MM, Perveen A, Jahan RA, Matin MA, Wong SY, Li X, Arafat MT (2019) Preparation of different polymorphs of cellulose from different acid hydrolysis medium. Int J Biol Macromol 130:969–976

Mascheroni E, Rampazzo R, Ortenzi MA, Piva G, Bonetti S, Piergiovanni L (2016) Comparison of cellulose nanocrystals obtained by sulfuric acid hydrolysis and ammonium persulfate, to be used as coating on flexible food-packaging materials. Cellul 23(1):779–793

Metreveli G, Wågberg L, Emmoth E, Belák S, Strømme M, Mihranyan A (2014) A size-exclusion nanocellulose filter paper for virus removal. Adv Healthc Mater 3(10):1546–1550

Miao J, Yu Y, Jiang Z, Zhang L (2016) One-pot preparation of hydrophobic cellulose nanocrystals in an ionic liquid. Cellul 23(2):1209–1219

Michelin M, Gomes DG, Romaní A, Polizeli MDL, Teixeira JA (2020) Nanocellulose production: exploring the enzymatic route and residues of pulp and paper industry. Molecules 25(15):3411

Mo L, Pang H, Tan Y, Zhang S, Li J (2019) 3D multi-wall perforated nanocellulose-based polyethylenimine aerogels for ultrahigh efficient and reversible removal of Cu (II) ions from water. Chem Eng J 378:122157

Mohammed N, Grishkewich N, Waeijen HA, Berry RM, Tam KC (2016) Continuous flow adsorption of methylene blue by cellulose nanocrystal-alginate hydrogel beads in fixed bed columns. Carbohydr Polym 136:1194–1202

Mohammed N, Grishkewich N, Tam KC (2018) Cellulose nanomaterials: promising sustainable nanomaterials for application in water/wastewater treatment processes. Environ Sci Nano 5 (3):623–658

Mokhena TC, John MJ (2020) Cellulose nanomaterials: new generation materials for solving global issues. Cellul 27(3):1149–1194

Nam J, Jung IB, Kim B, Lee SM, Kim SE, Lee KN, Shin DS (2018) A colorimetric hydrogel biosensor for rapid detection of nitrite ions. Sens Actuators B 270:112–118

Nishiyama Y (2009) Structure and properties of the cellulose microfibril. J Wood Sci 55 (4):241–249

Nypelö T, Abreu CR, Kolen'ko YV, Rivas J, Rojas OJ (2014) Microbeads and hollow microcapsules obtained by self-assembly of pickering magneto-responsive cellulose nanocrystals. ACS Appl Mater Interfaces 6:16851–16858

O'sullivan AC (1997) Cellulose: the structure slowly unravels. Cellul 4(3):173–207

Oehme DP, Downton MT, Doblin MS, Wagner J, Gidley MJ, Bacic A (2015) Unique aspects of the structure and dynamics of elementary Iβ cellulose microfibrils revealed by computational simulations. Plant Physiol 168(1):3–17

Ogonowski M, Edlund U, Gorokhova E, Linde M, Ek K, Liewenborg B, Könnecke O, Navarro JR, Breitholtz M (2018) Multi-level toxicity assessment of engineered cellulose nanofibrils in Daphnia magna. Nanotoxicology 12(6):509–521

Okita Y, Saito T, Isogai A (2010) Entire surface oxidation of various cellulose microfibrils by TEMPO-mediated oxidation. Biomacromolecules 11(6):1696–1700

Oun AA, Rhim JW (2017) Characterization of carboxymethyl cellulose-based nanocomposite films reinforced with oxidized nanocellulose isolated using ammonium persulfate method. Carbohydr Polym 174:484–492

Oun AA, Rhim JW (2018) Isolation of oxidized nanocellulose from rice straw using the ammonium persulfate method. Cellul 25(4):2143–2149

Patel DK, Dutta SD, Lim KT (2019) Nanocellulose-based polymer hybrids and their emerging applications in biomedical engineering and water purification. RSC Adv 9(33):19143–19162

Paul M, Jons SD (2016) Chemistry and fabrication of polymeric nanofiltration membranes: a review. Polymer 103:417–456

Phanthong P, Karnjanakom S, Reubroycharoen P, Hao X, Abudula A, Guan G (2017) A facile one-step way for extraction of nanocellulose with high yield by ball milling with ionic liquid. Cellul 24(5):2083–2093

Phanthong P, Reubroycharoen P, Hao X, Xu G, Abudula A, Guan G (2018) Nanocellulose: extraction and application. Carbon Res Convers 1(1):32–43

Piras CC, Fernández-Prieto S, De Borggraeve WM (2019) Ball milling: a green technology for the preparation and functionalisation of nanocellulose derivatives. Nanoscale Adv 1(3):937–947

Putro JN, Soetaredjo FE, Lin SY, Ju YH, Ismadji S (2016) Pretreatment and conversion of lignocellulose biomass into valuable chemicals. RSC Adv 6(52):46834–46852

Quan SL, Kang SG, Chin IJ (2010) Characterization of cellulose fibers electrospun using ionic liquid. Cellul 17(2):223–230

Ramasamy J, Amanullah M (2020) Nanocellulose for oil and gas field drilling and cementing applications. J Petrol Sci Eng 184:106292

Resch G, Held A, Faber T, Panzer C, Toro F, Haas R (2008) Potentials and prospects for renewable energies at global scale. Energy Policy 36(11):4048–4056

Ribeiro RS, Pohlmann BC, Calado V, Bojorge N, Pereira N Jr (2019) Production of nanocellulose by enzymatic hydrolysis: trends and challenges. Eng Life Sci 19(4):279–291

Ruiz-Palomero C, Soriano ML, Valcárcel M (2016) Gels based on nanocellulose with photosensitive ruthenium bipyridine moieties as sensors for silver nanoparticles in real samples. Sens Actuators B 229:31–37

Sehaqui H, Gálvez ME, Becatinni V, Cheng Ng Y, Steinfeld A, Zimmermann T, Tingaut P (2015) Fast and reversible direct CO_2 capture from air onto all-polymer nanofibrillated cellulose – polyethylenimine foams. Environ Sci Technol 49(5):3167–3174

Sehaqui H, Mautner A, de Larraya UP, Pfenninger N, Tingaut P, Zimmermann T (2016) Cationic cellulose nanofibers from waste pulp residues and their nitrate, fluoride, sulphate and phosphate adsorption properties. Carbohydr Polym 135:334–340

Shak KPY, Pang YL, Mah SK (2018) Nanocellulose: recent advances and its prospects in environmental remediation. Beilstein J Nanotechnol 9(1):2479–2498

Sharma A, Thakur M, Bhattacharya M, Mandal T, Goswami S (2019) Commercial application of cellulose nano-composites – a review. Biotechnol Report 21:e00316

Sharma PR, Sharma SK, Lindström T, Hsiao BS (2020a) Nanocellulose-enabled membranes for water purification: perspectives. Adv Sustainable Syst 4(5):1900114

Sharma V, Shahnaz T, Subbiah S, Narayanasamy S (2020b) New insights into the remediation of water pollutants using nanobentonite incorporated nanocellulose chitosan based aerogel. J Polym Environ 28:2008–2009

Sheikhi A (2019) Emerging cellulose-based nanomaterials and nanocomposites. In: Nanomaterials and polymer nanocomposites. Elsevier, Amsterdam, pp 307–351

Sheikhi A, Olsson AL, Tufenkji N, Kakkar A, Van De Ven TG (2018) Overcoming interfacial scaling using engineered nanocelluloses: A QCM-D study. ACS Appl Mater Interfaces 10 (40):34553–34560

Shemshadi R (2012) Application of synthetic polymers as adsorbents for the removal of cadmium from aqueous solutions: batch experimental studies. Caspian J Environ Sci 10(1):1–8

Shojaeiarani J, Bajwa D, Shirzadifar A (2019) A review on cellulose nanocrystals as promising biocompounds for the synthesis of nanocomposite hydrogels. Carbohydr Polym 216:247–259

Singh RL, Singh PK (2017) Global environmental problems. In: Principles and applications of environmental biotechnology for a sustainable future. Springer, Singapore, pp 13–41

Sinha V, Chakma S (2019) Advances in the preparation of hydrogel for wastewater treatment: a concise review. J Environ Chem Eng 7(5):103295

Squinca P, Bilatto S, Badino AC, Farinas CS (2020) Nanocellulose production in future biorefineries: an integrated approach using tailor-made enzymes. ACS Sustain Chem Eng 8 (5):2277–2286

Stylianopoulos T, Kokonou M, Michael S, Tryfonos A, Rebholz C, Odysseos AD, Doumanidis C (2012) Tensile mechanical properties and hydraulic permeabilities of electrospun cellulose acetate fiber meshes. J Biomed Mater Res B Appl Biomater 100(8):2222–2230

Sugiyama J, Persson J, Chanzy H (1991) Combined infrared and electron diffraction study of the polymorphism of native celluloses. Macromolecules 24(9):2461–2466

Sun X, Wu Q, Lee S, Qing Y, Wu Y (2016) Cellulose nanofibers as a modifier for rheology, curing and mechanical performance of oil well cement. Sci Rep 6:31654

Suopajärvi T, Liimatainen H, Karjalainen M, Upola H, Niinimäki J (2015) Lead adsorption with sulfonated wheat pulp nanocelluloses. J Water Process Eng 5:136–142

Taleb K, Markovski J, Veličković Z, Rusmirović J, Rančić M, Pavlović V, Marinković A (2019) Arsenic removal by magnetite-loaded amino modified nano/microcellulose adsorbents: effect of functionalization and media size. Arab J Chem 12(8):4675–4693

Tan XY, Abd Hamid SB, Lai CW (2015) Preparation of high crystallinity cellulose nanocrystals (CNCs) by ionic liquid solvolysis. Biomass Bioenergy 81:584–591

Torres FG, Arroyo JJ, Troncoso OP (2019) Bacterial cellulose nanocomposites: An all-nano type of material. Mater Sci Eng C 98:1277–1293

Trache D, Tarchoun AF, Derradji M, Hamidon TS, Masruchin N, Brosse N, Hussin MH (2020) Nanocellulose: from fundamentals to advanced applications. Front Chem 8:392

Trilokesh C, Uppuluri KB (2019) Isolation and characterization of cellulose nanocrystals from jackfruit peel. Sci Rep 9(1):1–8

Tshikovhi A, Mishra SB, Mishra AK (2020) Nanocellulose-based composites for the removal of contaminants from wastewater. Int J Biol Macromol 152:616–632

Unuabonah EI, Taubert A (2014) Clay–polymer nanocomposites (CPNs): adsorbents of the future for water treatment. Appl Clay Sci 99:83–92

Usmani Z, Sharma M, Gupta P, Karpichev Y, Gathergood N, Bhat R, Gupta VK (2020) Ionic liquid based pretreatment of lignocellulosic biomass for enhanced bioconversion. Bioresour Technol 304:123003

Vandamme D, Eyley S, Van den Mooter G, Muylaert K, Thielemans W (2015) Highly charged cellulose-based nanocrystals as flocculants for harvesting Chlorella vulgaris. Bioresour Technol 194:270–275

Vander Hart DL, Atalla RH (1984) Studies of microstructure in native celluloses using solid-state ^{13}C NMR. Macromolecules 17(8):1465–1472

Varghese AG, Paul SA, Latha MS (2019) Remediation of heavy metals and dyes from wastewater using cellulose-based adsorbents. Environ Chem Lett 17(2):867–877

Vigentini I, Fabrizio V, Dellacà F, Rossi S, Azario I, Mondin C, Benaglia M, Foschino R (2019) Set-up of bacterial cellulose production from the genus Komagataeibacter and its use in a gluten-free bakery product as a case study. Front Microbiol 10:1953

Vinogradova YS, Chen JY (2016) Micron-and nano-cellulose fiber regenerated from ionic liquids. J Textile Instit 107(4):472–476

Voisin H, Bergström L, Liu P, Mathew AP (2017) Nanocellulose-based materials for water purification. Nanomaterials 7(3):57

Wada M, Heux L, Sugiyama J (2004) Polymorphism of cellulose I family: reinvestigation of cellulose IVI. Biomacromolecules 5(4):1385–1391

Wan C, Li Y (2012) Fungal pretreatment of lignocellulosic biomass. Biotechnol Adv 30 (6):1447–1457

Wang Y, Zhao L, Peng H, Wu J, Liu Z, Guo X (2016) Removal of anionic dyes from aqueous solutions by cellulose-based adsorbents: equilibrium, kinetics, and thermodynamics. J Chem Eng Data 61(9):3266–3276

Wang J, Liu X, Jin T, He H, Liu L (2019) Preparation of nanocellulose and its potential in reinforced composites: a review. J Biomater Sci Polym Ed 30(11):919–946

Wei H, Rodriguez K, Renneckar S, Vikesland PJ (2014) Environmental science and engineering applications of nanocellulose-based nanocomposites. Environ Sci Nano 1(4):302–316

Wichterle O, Lim D (1960) Hydrophilic gels for biological use. Nature 185(4706):117–118

Xie H, Du H, Yang X, Si C (2018) Recent strategies in preparation of cellulose nanocrystals and cellulose nanofibrils derived from raw cellulose materials. Int J Polymer Sci 2018:7923068

Xu T, Jiang Q, Ghim D, Liu KK, Sun H, Derami HG, Wang Z, Tadepalli S, Jun YS, Zhang Q, Singamaneni S (2018a) Catalytically active bacterial nanocellulose-based ultrafiltration membrane. Small 14(15):1704006

Xu Z, Jiang X, Tan S, Wu W, Shi J, Zhou H, Chen P (2018b) Preparation and characterisation of CNF/MWCNT carbon aerogel as efficient adsorbents. IET Nanobiotechnol 12(4):500–504

Xu H, Liu Y, Xie Y, Zhu E, Shi Z, Yang Q, Xiong C (2019) Doubly cross-linked nanocellulose hydrogels with excellent mechanical properties. Cellul 26(16):8645–8654

Yamasaki S, Sakuma W, Yasui H, Daicho K, Saito T, Fujisawa S, Isogai A, Kanamori K (2019) Nanocellulose Xerogels with high porosities and large specific surface areas. Front Chem 7:316

Yang X, Cranston ED (2014) Chemically cross-linked cellulose nanocrystal aerogels with shape recovery and superabsorbent properties. Chem Mater 26(20):6016–6025

Yang Z, Zhou Y, Feng Z, Rui X, Zhang T, Zhang Z (2019) A review on reverse osmosis and nanofiltration membranes for water purification. Polymers 11(8):1252

Yu H, Qin Z, Liang B, Liu N, Zhou Z, Chen L (2013) Facile extraction of thermally stable cellulose nanocrystals with a high yield of 93% through hydrochloric acid hydrolysis under hydrothermal conditions. J Mater Chem A 1(12):3938–3944

Yue X, Huang J, Jiang F, Lin H, Chen Y (2019) Synthesis and characterization of cellulose-based adsorbent for removal of anionic and cationic dyes. J Eng Fiber Fabric 14:1558925019828194

Zhang Z, Sèbe G, Rentsch D, Zimmermann T, Tingaut P (2014) Ultralightweight and flexible silylated nanocellulose sponges for the selective removal of oil from water. Chem Mater 26 (8):2659–2668

Zhang K, Sun P, Liu H, Shang S, Song J, Wang D (2016) Extraction and comparison of carboxylated cellulose nanocrystals from bleached sugarcane bagasse pulp using two different oxidation methods. Carbohydr Polym 138:237–243

Zhao G, Wang F, Lang X, He B, Li J, Li X (2017) Facile one-pot fabrication of cellulose nanocrystals and enzymatic synthesis of its esterified derivative in mixed ionic liquids. RSC Adv 7(43):27017–27023

Zheng Q, Cai Z, Gong S (2014) Green synthesis of polyvinyl alcohol (PVA)–cellulose nanofibril (CNF) hybrid aerogels and their use as superabsorbents. J Mater Chem A 2(9):3110–3118

Zhou C, Lee S, Dooley K, Wu Q (2013) A facile approach to fabricate porous nanocomposite gels based on partially hydrolyzed polyacrylamide and cellulose nanocrystals for adsorbing methylene blue at low concentrations. J Hazard Mater 263:334–341

Zhou Y, Fu S, Zhang L, Zhan H, Levit MV (2014) Use of carboxylated cellulose nanofibrils-filled magnetic chitosan hydrogel beads as adsorbents for Pb (II). Carbohydr Polym 101:75–82

Zhou S, Nyholm L, Strømme M, Wang Z (2019) Cladophora cellulose: unique biopolymer nanofibrils for emerging energy, environmental, and life science applications. Acc Chem Res 52(8):2232–2243

Zhu C, Monti S, Mathew AP (2020) Evaluation of nanocellulose interaction with water pollutants using nanocellulose colloidal probes and molecular dynamic simulations. Carbohydr Polym 229:115510

Chapter 5
Allelochemicals as Natural Herbicides for Sustainable Agriculture to Promote a Cleaner Environment

Hemalatha Palanivel, Gebiyaw Tilaye, Sathish Kumar Belliathan, Solomon Benor, Solomon Abera, and M. Kamaraj

Abstract Allelopathy interaction initiates communication between plants and microorganisms, by liberating secondary metabolites as signaling molecules that have the potential to impact the growth and development of neighboring plants in both natural and agricultural ecosystems. Herbicide residues pollute soil and groundwater, the hasty spread of invasive weed populations, growing resistant weed species, and interrelated health hazards have directed the research focus to detect and set up new alternative weed management approaches. Allelopathic strategies are being a key phenomenon for weed control by breeding allelopathic varieties of crops, allelopathic crops as cover crops, and incorporating them in crop rotational cycle, and identification of allelochemicals from various sources, such as plants and microorganisms, could proceed toward sustainable agriculture with a greener and cleaner environment. Genetic mapping of QTLs linked to allelochemicals,

---

H. Palanivel (✉) · S. Abera
Department of Biotechnology, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

Centre of Excellence of Bioprocess and Biotechnology, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia
e-mail: hemalatha.palanivel@aastu.edu.et

G. Tilaye
Centre of Excellence of Bioprocess and Biotechnology, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

S. K. Belliathan
Centre for Environmental Sciences, Addis Ababa University, Addis Ababa, Ethiopia

S. Benor
Department of Biotechnology, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

Ministry of Science and Higher Education (MoSHE), Addis Ababa, Ethiopia

M. Kamaraj
Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_5

understanding the modes of action, molecular mechanisms of allelopathy, and soil dynamics in crop weed completion are advancing rapidly in relevance to weed management as a bioherbicide. The adaptive plasticity of plants will enable them to change their morphological and physiological traits after their exposure to allelochemicals in the weed crop competition scenario. The existence of genetic variation correlated to the allelopathic potential for weed suppression could be further exploited for looming organic plant breeding approaches.

Keywords Allelopathy · Allelochemicals · Bioherbicide · Organic agriculture · Plant breeding · QTLs · Weed management

1 Introduction

Weeds overlap spatially with beneficial agricultural crops. They divest the crops with restricted nutritional elements, land, sunlight, and water. Weeds stubbornly compete with crops, affecting a significant loss in their yield productivity (Mushtaq et al. 2020). Crop and yield losses produced by weeds are far high and above the damages from any category of agronomic pests, such as insects, diseases, nematodes, rodents, etc. (Abouziena and Haggag 2016). Since the dawn of agriculture, hand weeding, mechanical weeding, and weedicide applications were the conventional weed control approaches that have been practiced (Jabran et al. 2015). However, extensive use of herbicides to control the weeds has led to serious ecological and environmental complications such as herbicide resistance and residual effect, a shift in weed flora, and environmental pollution and health hazards due to their noxious residual responses in the land, water, and food chain. The harmful effect of commercial herbicides makes it appropriate to reconnoiter various new weed management alternatives (Vyvyan 2002). Eco-friendly preferences in weed management force researches for identifying novel sources and tools. Natural compounds hold a great promise for the detection of innovative and environmentally nontoxic natural herbicides. The International Allelopathy Society defined the term in 1996 as “any process involving secondary metabolites produced by plants, algae, bacteria, and fungi that influences the growth and development of agriculture and biological systems.” The allelopathic plants discharging allelochemicals into the environment are biodegradable and, thus, cause less pollution (Duke et al. 2002). They are an applicable alternate tool for synthetic herbicides since allelochemicals do not have residual or toxic effects. This is an appropriate application of allelopathy toward the enhancement of crop productivity and environmental protection by an eco-friendly approach to the control of weeds. Allelopathy is a natural ecological phenomenon that may be a beneficial or detrimental interaction between the organisms; it may be planted to plant to insect or plant to microorganisms that are triggered by the action of chemicals referred to as allelochemicals (Rice 1984). Allelopathy is a significant ecological tool in natural and managed ecosystems. These non-nutritional chemicals are mainly produced through secondary metabolisms; mostly, they are by-products of several physiological processes in the plant system. The allelochemicals have the potential to influence the growth and development of neighboring plants in both natural and agricultural ecosystems (Korres et al. 2019). An allelopathic effect is generally referred to as a type of adverse synergy (Meiners et al. 2012), but useful interactions have also been described, relying on the allelochemicals considered, the plant studied, and the concentration tested (Hill et al. 2006). Chemicals with allelopathic potential are generally in the conjugated state in all plants and many tissues, including leaves, rhizomes, pollen, inflorescences, seeds and fruits, stems, and roots (Favaretto et al. 2018). Under specific conditions, these allelochemicals may be permanently liberated into the environment (atmosphere or rhizosphere) in enough quantities and with adequate perseverance to affect the neighboring or successional plant. Allelochemicals have immense potential to be used for herbicide synthesis, facilitating the detection of a new mechanism of action. Genetic and molecular studies are acutely useful to perceive better knowledge for decoding the accurate function of allelochemicals in plant-plant interactions (Duke et al. 2001). Weed infestation represents a major constraint to agricultural production. Herbicide application is the most important weed control measure used in regular agricultural practices to reduce the labor cost. However, alternate strategies to herbicides are rising significantly to minimize the reliance on chemical inputs and to alleviate the toxic impacts that these compounds impose on the environment. The deep knowledge of allelopathy might establish an important association to increase the acceptance of agricultural products through organic agriculture in current challenging consumer markets. Allelopathic compounds can be exploited as a very robust tool in organic farming by understanding the synergetic, additive, and antagonistic effects between the species and identifying the novel natural substances with herbicidal potential and their environmental fate. The objective of this chapter was to discuss the contemporary advances in scientific understanding about allelopathy for sustainable agriculture, cleaner, and greener environment.

2 Allopathic Interactions and Allelopathic Compounds

Allelopathy is a biochemical and physiological process but still not comprehensively studied in the plant world when we refer to the range of biochemical compounds through which plants can communicate with each other and with microbiota. Biochemical dialogue among the organisms may have positive (stimulatory) or negative (inhibitory) effects, and the synthesis and release of allelochemicals are triggered by many environmental and genetic characteristics to the natural and anthropogenic ecosystems. The plants are sessile and cannot move from their possible enemies and also toward favorable individuals. So, the plant kingdom has different interactions between the plant communities and soil rhizospheres, such as crop-crop, crop-weed, and weed-weed, for resource competition and for the defense that impacts on plant diverse community (Bernards 2010). It has been approximated that over 10.000 allelochemicals are synthesized by higher plants, with substantial variability in their function and mechanism of action in target plants (Weston et al.

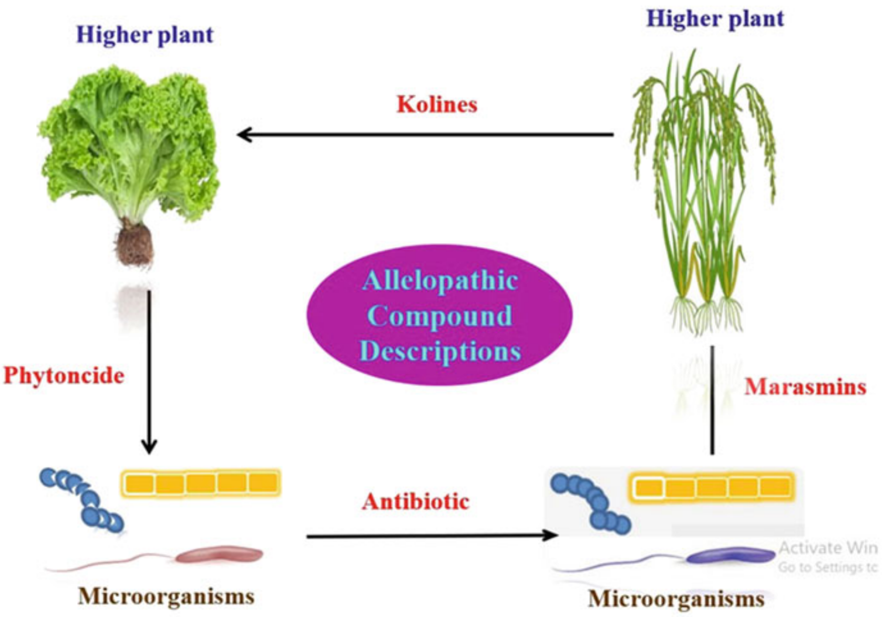


Fig. 5.1 Special terminologies for allelopathic compounds involvement in the plants

2012). The first validated allelochemical was juglone (5,hydroxyl-1,4-naphthoquinone), which emanates from black walnut (*Juglans nigra*) (Williamson and Weidenhamer 1990). As illustrated in Fig. 5.1, (Grummer 1955) special terminologies are used for the chemicals involved in allelopathy based on the kind of plant producing the compound and the type of plant affected. They are as follows:

1. Antibiotic – a chemical substance produced by a microorganism that inhibits and is effective against a microorganism.
2. Phytoncide – an inhibitor compound produced by higher plants that affect the microorganism.
3. Marasmins – the biochemical compounds produced by microorganisms and detrimental to the higher plants.
4. Kolines—the biochemical compounds produced by the higher plants and effective against higher plants.

However, with the exemption of antibiotics, these descriptions have received narrow usage.

Most researchers have selected to use the broad terminology of allelopathic chemicals or allelochemicals. Allelochemicals can be classified into ten categories (Li et al. 2010a, b), conferring to their structural relationship in R moiety and characters:

1. Water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes, and ketones.
2. Simple lactones.

3. Long-chain fatty acids and polyacetylenes.
4. Quinines (benzoquinone, anthraquinone, and complex quinines).
5. Phenolic.
6. Cinnamic acid and its derivatives.
7. Coumarins.
8. Flavonoids.
9. Tannins.
10. Steroids and terpenoids (sesquiterpene lactones, diterpenes, and triterpenoids).

2.1 Biosynthetic Pathways of Allelochemicals

Allelochemicals, such as polyphenols, include several classes of structurally different natural products and biogenetically originating from the shikimate-phenylpropanoids-flavonoids pathways. In comparison with animals, plants produce a vast spectrum of plant secondary metabolites because of their immobile nature which made them impossible to get away from its enemies or competitors; thus, they developed such a biochemical-based defense mechanism to protect them from their predators. There are divergent principles to classify allelochemicals. Some of them are based on the carbon skeleton structure and functional groups. Some allelochemicals are classified based on the biogenetic origin of metabolites (Latif et al. 2017). Based on the carbon skeleton and functional groups, allelochemicals can be further divided into alkaloids, fatty acids, flavonoids, phenolic compounds, quinones, and more common terpenoids (Latif et al. 2017). Quinones and phenolics are the maximum frequently characterized classes of allelopathic phytotoxins (Inderjit 1996). Quinones are oxidized phenols, and phenols are produced from the redox reaction of quinones, and changes between these chemical states have physiological and biological connotations (Harborne 1989). The group of allelochemicals and their ecological role were tabulated in Table 5.1.

Allelochemicals are commonly called "secondary metabolites" synthesized by organisms, such as plants, animals, or microorganisms, and are not needed for basic (primary) metabolism. Allelochemicals are produced by using three different biosynthetic pathways, viz., shikimic acid, acetate-malonate, and mevalonic acid, which are the main precursors to biosynthesize the allelopathic compounds. So far, various allelochemicals have been recognized and analyzed from a range of plant species. These allelochemicals are chemically diverse, being represented by phenolic compounds (simple phenolics, flavonoids, coumarins, and quinones), terpenoids (monoterpenes, sesquiterpenes, diterpenes, triterpenes, and steroids), alkaloids and nitrogen-containing chemicals (nonprotein amino acids, benzoxazinoids, cyanogenic glycosides), and many other chemical families. It is holding the researcher's attention that secondary allelochemicals are biosynthesized substantially from a few primary metabolites: α-amino acids, acetyl-coenzyme A, mevalonic acid, and intermediates of the shikimic acid pathway (Hebert 1981). The biosynthetic pathways of

Table 5.1 Allelochemicals and their mechanism of action with ecological role

No	Class of the allelochemicals	Description of the biosynthetic pathways	Agroecological role and mechanism of action	References
1.	**Terpenoids**	From mevalonic acid and isopentenyl pyrophosphate pathways	Signaling molecules, protective agents against the light, as reproductive hormones	Scavo et al. (2019), Latif et al. (2017), Aslam et al. (2017)
		Composed of from 5 carbon rings	An inhibitory effect on the growth and seedling germination of plants	
		Their common biosynthetic origin is from acetyl-CoA or glycolytic intermediates 24,000 naturally occurring terpenoids volatile nature	Affects cell division and also causes shoot growth abnormality	
2	**Phenolic Compounds**	Aromatic ring with one hydroxyl group and possibly other substituents;	Defense mechanisms in higher plants	Hameed et al. (2019), Latif et al. (2017)
			It is used for a defense system against different biotic factors	
		It is benzene rings with hydroxyl group/groups attached to one or more ringed carbon/s easily soluble in water due to their unsaturated structure; 8000 naturally occurring phenol compounds	It has a great contribution to the color of the plant	Ashraf et al. (2017)
		Biosynthetically phenolic compounds are originated from the phenylpropanoid-acetate biosynthetic pathway		
3.	**Flavonoids**	Are the main groups of phenolic allelochemicals; are polyphenols with two aromatic rings (rings A and B) and an oxygenated heterocycle (ring C); 4000 flavonoid compounds	Pigmentation and defense	Ashraf et al. (2017)
			They are also used to protect cells from UVB radiation	
		Synthesized by plants in response to microbial infection		
		Have two important groups: Flavones and flavonols		

(continued)

Table 5.1 (continued)

No	Class of the allelochemicals	Description of the biosynthetic pathways	Agroecological role and mechanism of action	References
4.	**Alkaloids**	They are heterocyclic nitrogen-containing basic compounds and have alkaline chemical nature	Defensive roles in plant interactions against herbivores, microorganisms, fungi; deter herbivory	Hameed et al. (2019)
		20,000 compounds identified to date, representing great structural biosynthetic diversity	Allelopathic activity against common annual weeds	

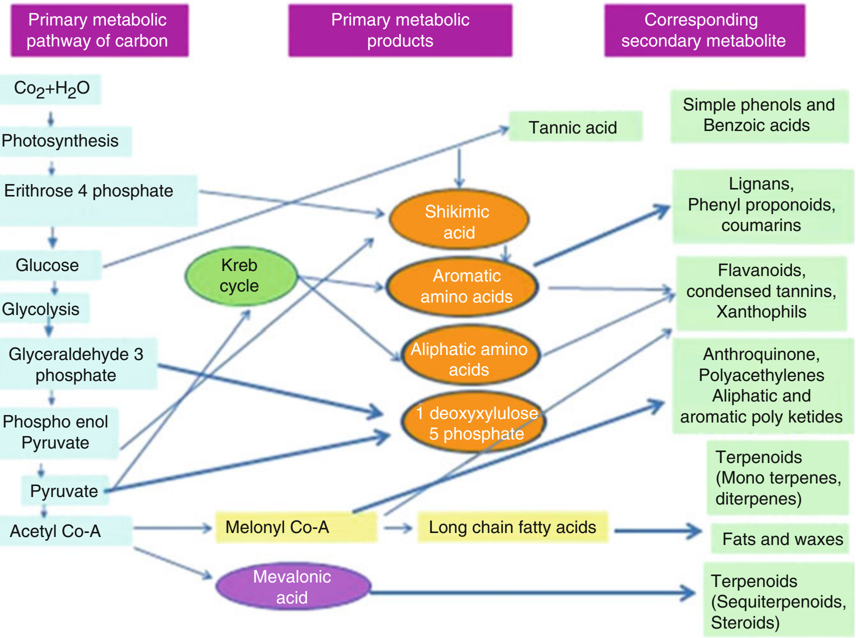


Fig. 5.2 The biosynthetic pathways of common allelochemicals

common allelochemicals were described in Fig. 5.2. The origination of these compounds leans on the presence of precursor molecules and by triggering the specific functional gene cascade. The stimulation of genes is mandatory for the biosynthesis of allelochemicals is often reliant on environmental stimuli (Croteau et al. 2000).

2.2 Release of Allelochemicals

Plants use different methods to release allelochemicals by exudation, through crop residue decomposition in the soil, through leaching from aerial parts, volatilization, and pollen spreading in some plants (Hameed et al. 2019). Volatilization and leaching (Bertin et al. 2003) are the two main processes of the release of allelochemicals into the atmosphere or rhizosphere. Recently, Pereira et al. (2016) reported that the volatile mode of release of allelopathic compounds (allelopathins) would broadly reign in plants thriving in hot, dry climates, whereas water-soluble allelopathins would preponderate in wetter climates (Pereira et al. 2016). Different methods for the release of allelochemicals into the atmosphere or rhizosphere under the suitable physiological, environmental, and ecological conditions are listed hereunder and described in Fig. 5.3.

1. Leaching – The allelochemicals leached through precipitation, due to the moisture content in the air through dewdrops, rain, or flooding, which may ooze out the allelopathic chemicals from the aboveground plant parts and consequently deposit them on other plants or into the soil. Leaching may likewise happen through plant residues (Bertin et al. 2003; Cruz-Ortega et al. 2002).
2. Volatilization – The allelopathic compounds are discharged into the atmospheric air. It is culpable only if the atmosphere was in dry, hot, or semiarid conditions. The allelochemicals might be engrossed as vapors by neighboring or surrounding plants, by the condensation form of these vapors as dew, or the condensate may reach the rhizosphere and absorbed by the roots (Putnam and Tang 1986).

Fig. 5.3 Release and mode of action of allelochemicals in different environmental and ecological conditions

3. Decomposition of residues – It is challenging to determine if these toxic entities are encompassed in residues and released upon decomposition or microorganisms transform the simple residues into these toxic elements as an outcome of the existence of microbial enzymes (Mushtaq and Siddiqui 2018; Kohli et al. 2001).
4. Root exudation – Root perspiration of allelochemicals from plant roots bequeaths one of the major direct allelochemicals into the rhizosphere zone in the soil, regardless of whether these compounds are effectively exuded, leaked, or arise from dead cells (Bertin et al. 2003).
5. Allelochemicals released from pollen – Pollen allelopathy occurs when pollen grains are transmitted either inter- or intraspecific transfer. The pollen allelopathy occured either pollen transmitted either inter oe intraspecific transfer due to pre and post zygotic barriers (Murphy 2001).

There are wide variations in the natural and agroecosystems as the mode of release pattern of allelochemical discharge into the atmosphere is contingent on the physicochemical traits and the plant organ involved in allelopathic interaction.

2.3 Factors Affecting the Production and Release of Allelochemicals

The biosynthesis and release of allelochemicals are controlled by various factors, such as genetic, environmental, ecological, and physiological factors. Nevertheless, allelochemical type and concentration released into the environment determined the communal effects of the plant itself and environmental factors.

2.3.1 Genetic Factors

The plant features include the species, variety, plant and its development stages, the organs and tissues, which are interrelated to allelochemical production and release (Iannucci et al. 2013). The production of allelochemicals through secondary metabolism in plants is dependent on the genetic element responsible. Numerous studies have been conducted to know the molecular and genetic basis for abiotic stress or herbivores and pathogen infection signaling in plants, but less effort has been devoted to allelopathy responses. Research has validated that genetic variation of the allelopathic traits is in a wide range in within crop species (Dilday et al. 1991; Olofsdotter et al. 1999; Jensen et al. 2001). Allelopathic properties differ between varieties or genotypes (Li and Shen 2006): as an example, a rice (*Oryza sativa* L.) breeding program that undertaken the evolution of cultivars with increased level allelochemical synthesis has advanced from the elucidation of the enzymes and genes involved in momilactone B synthesis (Toyomasu et al. 2008). Momilactone B is a diterpene compound that suppresses weed at a maximum degree. By using the reverse genetics technique, it became possible to identify the genes encoding diterpene synthesis in methylerythritol phosphate (MEP) pathway (Dudareva et al. 2013). Research is in the field of genetics on the allelopathic traits are not fully explored by the plant breeders as the inheritance pattern of allelopathic characters are very much complex, and to isolate and quantification of the allelochemicals in a diverse germplasm collection, the task is limited because of the availability of necessary high-throughput analytical techniques.

Olofsdotter et al. (1995) hypothesized that allelopathy may be polygenic in trait and weakly associated with yield and yield-related characters in crops. The allelopathic chemicals are released in higher quantity to the environment that is stimulated by stress conditions. The stressors may regulate the genes for the biosynthesis of allelochemicals and release them to the environment (Einhellig 1996). If we acquire more genetic information, the inheritance pattern of regulatory genes that are responsible for the synthesis and expression of allelochemicals that pave way to identify the novel secondary metabolites to initiate breeding programs for developing allelopathic cultivars. Although genetic dissection of allelopathic traits is still in the juvenile stage and will emerge into new frontiers of research to develop potential bioherbicides, as an innovative outlook, molecular and bioinformatics-based advanced research will shed light on the activation of gene and its transcriptional and translational changes associated with stressors.

2.3.2 Environmental Factors

Environmental factors can precisely or obliquely affect the allelopathic pursuance of a plant with an adaptive response to the environment. The plant functional characters have been largely applied to analyses of species-specific traits, and the significance of the terminology holds different dimensions when the plant is exposed to environmental stresses. Plants can receive and realize a range of external and internal stimuli from their neighborhood settings, while environmental changes can influence crop allelopathy and its functional characters (Rivoal et al. 2011). The environment can alter allelopathy in the following means, viz., generating of allelopathic compounds (GxE interactions), their bioavailability, and their influence on target species.

Several studies have shown that the amount of allelochemicals produced and released by plants is correlated to environmental factors (Paré and Tumlinson 1999, Agrawal 2001). The key important environmental factors that can impact crop allelopathy include UV radiation, temperature, water, nutrient availability, and competition stress. For example, one of the valuable adaptive processes to enhance UVB radiation is the augmented production of water-soluble phenolics in foliage tissues under an elevated level of UVB radiation in *herbaceous plants* (Li et al. 2010a, b). The UVB radiation is transmitted by the reflective mechanism through the epicuticular wax layer, and the UV rays reach the epidermal tissues and protect the underlying tissues against harmful UVB radiation. Various factors, such as temperature extremes, deficiency of nutrients, and heavy metals, enhance the allelochemical production in many plant species (Maqbool et al. 2013). Several sources of rain leachates range significantly in their thwarting effects, and rain leachates from plant debris and litter are probably to be a key pathway of allelopathy (Song et al. 2019). Allelochemicals in dry environments bivouac in the soil for a protracted period in comparison with humid environments (Einhellig 1999). The scientific pieces of evidence showed that higher plants growing with minimal nutrients, moisture stress, and temperature conditions are hypersensitive to allelochemicals than those grown in normal environments (Rivoal et al. 2011). The soil system is an active and continually changing and dynamic environment that influences the fate and functions of allelochemicals concerning time and space. The ecological conditions which foist water and nutrient deficiency stress and severe temperatures would increase allelopathy.

3 Mechanism of Allelochemical Action

Allelopathy has been well recognized for many years ago (Rice 1984), despite it decoding the mechanisms of action for the allelochemicals is not documented (Mohamadi and Rajaie 2009). The allelochemicals usually affect the nutrient uptake and mineral absorption, cell division, membrane permeability, photosynthesis, respiration, growth and development, phytotoxicity, enzyme activity, and protein synthesis, which are all influenced by allelochemicals. Sorgoleone, a lipophilic benzoquinone, is a well-recognized compound for its allelopathic nature that is synthesized by *Sorghum bicolor*, which acts as a potential allelochemical by inhibiting the photosystem II (Weir et al. 2004). Many secondary metabolites including allelochemicals will modify membrane permeability in suitable concentrations that caused leakage of cell contents, which leads to programmed cell death (apoptosis) and necrosis (Li et al. 2010a, b; Chai et al. 2013).

Higher concentrations of rutin and quercetin have been exhibited to hinder the seed germination of plant species by impairing the respiration process and ATP levels in embryogenic callus through substrate oxidation or restricting phosphate ion uptake that may unfasten the oxidative phosphorylation mechanism (Takahashi et al. 1998). Allelochemicals disturb plant growth by persuading different phases of respiration, such as electron transfer in the mitochondria, oxidative phosphorylation, CO_2 generation, and ATP enzyme activity. Hejl and Koster (2004) witnessed that juglone could reach mitochondrial root cells of corn, thereby restricting root oxygen uptake. Allelochemical monoterpenoids, viz., camphor, 1,8-cineole, beta-pinene, alpha-pinene, and camphene, reserved cell proliferation and DNA synthesis in plant meristems (Nishida et al. 2005). All phenolic acids can influence the structure of DNA and RNA. Ferulic acid and cinnamic acid as well as many phenols and alkaloids can also inhibit protein synthesis (Li et al. 2010a, b). The plant response to allelochemicals is identical to biotic and abiotic stress. This signposted that allelochemicals might have pertinent functions in the cross talk flanked by biotic and abiotic stress signaling molecules as allelochemicals generate reactive oxygen species (ROS). None of the allelochemicals is generic; they are specific in their mechanism of action. Additionally, it should be reconnoitered in future research to discover their respective molecular target sites and to understand their gene regulation and protein synthesis process (Kato-Noguchi et al. 2013; Weston and Mathesius 2013).

4 Strategies for the Use of Allelochemicals as a Bioherbicide

Viable weed control is demanding to ensure food security for the growing population of the globe. Chemical weed control is a paramount practice among the various weed management methods that have been operated to control weeds in different crops and ecological circumstances. However, the weed control by weedicides is fading due to the progression of herbicide resistance in weeds and environmental concerns. Several natural allelopathic compounds have been identified from plants (Jabran 2017). These innate phytotoxins could be tested directly as natural herbicides or might be explored to develop novel bioherbicides (Dayan and Duke 2014). Zohaib et al. (2016) studied more than 30 weed species including phytotoxic compounds that displayed robust reticence against different crops and weeds; the phytotoxic compounds can be explored to manage weeds. Most of the allelochemicals are water-soluble compounds, so the application of allelochemicals on plants is easier; there is no need for additional surfactants (Dayan et al. 2009; Vyvyan 2002). The chemical structures of allelochemicals are very much companionable to the environment than synthetic ones. They consist of higher oxygen- and nitrogen-rich molecules with a comparably few so-called “heavy atoms,” a halogen substitute, and are categorized by the lack of “unnatural” rings. These characteristic features decrease allelochemical’s environmental half-life and forbid accumulation of the allelopathic compound in soil. Structure complexity creates more chiro-centers, converting them into more reactive and unstable substances. The versatility of allelopathins brands them assuring tools, retaining specific properties in identifying unique, specific target sites in receiver plants. While relatively 70% of all recently registered active pesticide components have their origins from a natural product (Cantrell et al. 2012), hence, the natural allelochemicals may serve as next-generation herbicides due to their safer toxicological and environmental profiles for a sustainable cleaner environment.

The allelochemicals inhibit photosynthesis and respiration and bind in different target sites than synthetic herbicides. Allelochemicals are also portrayed with multisite action in plants with low specificity. Therefore, this peculiar attribute exempts the application of an allelopathic compound as a selective herbicide, but at the same time, the effects of allelopathins in acceptor plants are highly dose dependent (Belz et al. 2005). This allows the opportunity to search out compounds exhibiting selectivity. Generally, monocotyledonous plants are more resistant to allelochemicals than dicotyledonous ones. Therefore, the usage of a compound as a potential herbicide is possible but rather restricted to the cultivation of exact crops with a defined weed composition.

4.1 Plant Allelopathins as a Source of Bioherbicides

Plant allelopathins can be positively used in integrated weed management. The plant allelochemicals are not suitable under field conditions because the mode of action is different and profitable also. To evade masking impacts of one allelopathin by another plant allelochemical, researchers are interested in the isolation and application of a single, specific compound for weed elimination. The list of allelochemicals isolated from various plants that may act as inhibitors of weed seed germination and/or weed growth is summarized in Table 5.2.

4.2 Allelopathic Crops in Weed Suppression through Intercropping and Crop Rotation Methods

4.2.1 Intercropping

Handling croplands bestowing to natural agroecological principles will restrain weed infestations in all crops, and crop rotation has long been documented for its competence to thwart weeds from evolving to cause severe yield reduction. Combining crops with allelopathic features into the rotation complements another component of weed control. The practice of allelopathic crops in agriculture is currently being realized as a mechanism of crop rotation and intercropping, as cover crops or as green manure (Farooq et al. 2014; Haider et al. 2015). Population density of weeds and biomass production was significantly reduced using crop rotation and intercropping systems (Jabran et al. 2015). Intercropping of sorghum (*Sorghum bicolor* L.), sesame (*Sesamum indicum* L.), and soybean (*Glycine max* L.) as intercrops in cotton (*Gossypium hirsutum* L.) crop showed a greater net profit and a substantial reduction on purple nutsedge (*Cyperus rotundus* L.) growth when compared with the cotton alone in a 2-year experiment (Iqbal et al. 2007). Several allelopathic crop plants intercropped with maize tested the growth and development of several narrow- and broad-leaved weed species (Nawaz et al. 2014).

4.2.2 Cover Crops

The cover crops with allelopathic features having added advantages, such as safeguarding plants from soil erosion, maintaining soil fertility and structure, and improving nitrogen fixation, are also effectively employed in weed control (Tursun et al. 2018; Altieri et al. 2011). Some of the important cover crops comprise canola, rapeseed, cereal rye, crimson clover, wheat, red clover, brown mustard, oats, cowpea, fodder radish, annual ryegrass, mustards, buckwheat, hairy vetch, and black mustard. The strong allelopathic cover crops suppress weeds by physically suppressing through shade effect, fall of temperature, and competition of cover

Table 5.2 Allelochemicals sequestered from plants that exhibit an inhibitory effect on weeds

Crop	Scientific name	Allelochemical	Sensitive plants/ weeds	References
Rice	*Oryza sativa L*	Salicylic, p-coumaric, o-hydroxyphenyl acetic, syringic, ferulic, benzoic, p-hydroxybenzoic, octacosanoic, m-coumaric, and o-coumaric acids	Barnyard grass, *(Echinochloa colonum L.), Orobanche cumana Wallr. Orobanche minor* *Lactuca sativa. L*	Rimando et al. (1998), Ma et al. (2014), Jung et al. (2004), Chung et al. (2006)
Wheat	*Triticum aestivum L.*	Hydroxamic acids and its derivative compounds, phenolic acids, and short-chain fatty acids	*Lolium rigidum*	Bertin et al. (2003), Wu et al. (2004)
Cucumber	*Cucumis sativus L*	Benzoic and cinnamic acids	*Avena sativa*, *Secale cereale*, autotoxic	Yu et al. (2003), Tawaha and Turk (2003), Wu et al. (2004), Burgos et al. (2004), Ding et al. (2007)
Mustard	*Brassica species*	Allyl isothiocyanate	*Amaranthus blitoides Lolium rigidum*	Weston (1996), Alcantara et al. (2011)
Buckwheat	*Fagopyrum esculentum L*	Fatty acids	*Arabidopsis thaliana*	Weston et al. (2012), Golisz et al. (2008)
Clovers	*Trifolium* spp.	Isoflavonoids	*A. retroflexus* and *C. album*	Weston et al. (2012), Campiglia et al. (2012)
Canola	*Brassica napus*	Sinapic, gentisic, coumaric, syringic, ferulic, salicylic caffeic and p-hydroxybenzoic acid	*Lolium rigidum*	Asaduzzaman et al. (2014), Haddadchi and Gerivani (2009), Olkowski et al. (2003)
Sorghum	*Sorghum bicolor*	Sorgoleone	*L. rigidum Sorghum halepense L., Cyperus rotundus L., Echinocloa Colona L. (link), Convolvulus arvensis L., Portulaca oleracea L.*	Wu et al. (2003), Ben-Hammouda et al. (1995), Cheema et al. (2003)
Sunflower	*Helianthus annum*	Heliannone, guaianolides	*Triticum aestivum Avena fatua L., Sinapis alba*	Kamal (2011), Bogatek et al. (2006), Kupidłowska et al. (2006)

crops with weeds for inputs. Furthermore, the releases of allelochemicals from cover crops especially leachate from rain will help to decay the weed seed bank in conservation tillage system or no-tillage circumstances. The weed control potential of cover crops also relied on specific weed species, e.g., sorghum as a cover crop controls effectively on broad-leaved weeds, but narrow-leaved weeds were not controlled (Einhellig and Leather 1988). The organic farming system is heavily dependent on allelopathic cover crops for weed control (Mirsky et al. 2013).

It was described that cover crop combinations of *Sinapis alba* L., (*Raphanus sativus* var. *niger J*. Kern) and *Vicia sativa* inhibited weeds. These authors suggested that biochemical synthesis is liable for weed suppression of cover crops. Allelopathy is a vital substitute for overcoming herbicide-related complications in weed management for sustainable agriculture (Narwal 2000).

4.3 Plant Breeding and Biotechnology for Bioherbicide Development

Allelopathy has been debated past due to the plethora of less than strong research about this focus and the often tentative claims about the role of allelochemicals in plant-plant interactions (Duke 2010). For decades, efforts have been made to improve the allelopathic properties of crops by conventional breeding and varietal selection programs (Batish et al. 2011; Bertholdsson et al. 2012; Worthington and Reberg-Horton 2013). Reports on genetic diversity in weed control among the cultivars of *Cucumis sativus* L. (Putnam and Duke 1974) and *Oryza sativa* L. (Dilday et al. 1991) appointed breeding as a possible strategy to improve the ability to protect against weeds and thus enable using crop allelopathy for weed management with improved allelopathic traits. In this focus, huge screenings were commenced to detect highly efficient allelopathic donors that could be utilized in breeding programs. The study of the genetic process involved in the allelopathy of crop plants is a new challenge for weed control through biological measure, but very limited work has been done till present.

4.3.1 Germplasm Screening for Allelopathic Cultivars

Improvement in crop cultivars is the merely unexplored field of research to any great extent as a weed management strategy (Khush 1996). The prospect of integrating the allelopathic characters into improved crop cultivars, which would decline the necessity for herbicidal application to the crop, is a worthy investigation (Khush 1996). No commercial cultivars acquiring allelopathic traits have been evolved (Duke et al. 2001). Morphological characteristics, such as early seedling emergence, seedling vigor, fast growth rates that produce a dense canopy, greater plant height, greater root volume, and longer growth duration, are known to increase the ability of cultivars to compete with weeds (Berkowitz 1988) that are sources of organic plant breeding approaches for a pollution-free and cleaner environment. Plant height is often labeled as one of the best important traits in the overall competitive capability of a crop and accounts for a similar percentage of total competitive ability (Gaudet and Keddy 1988). Practically, weed control will be a challenging task because of a wide range of susceptible weed species, and, thus, incorporation of allelopathic traits appeared to depend on combining genes for diverse allelochemicals in one cultivar, or choosing cultivars that already have such genes will be an appropriate approach. In the recent past, the rice germplasm screening bestowed valuable insights into crop allelopathy and its application in weed management and identified elite allelopathic genotypes, predominantly Asian rice lines, which have been utilized to commence traditional breeding programs to improve allelopathy in rice (Gealy et al. 2005). In wheat, screening of allelopathic varieties and further improving the allelopathic potential at the genetic level are studied to identify new potent wheat varieties, to analyze the allelopathic impact of wheat on weeds, and to isolate and identify the allelopathic compounds and their collegial and combined effects on physiological mechanisms of plants ((Bertholdsson 2010).

4.3.2 Molecular Breeding for Improving Allelopathic Traits

The allelopathic genetic traits are knitted with environmental and ecological conditions expressed in genotype by environmental interactions, causing different expressions in a different environment. The allelopathic potential of crops is weakly associated to yield and yield-related characters due to its polygenic nature (Olofsdotter et al. 1999). In the identification of genes regulating allelopathy, without insight knowledge about the genetics, breeding allelopathic cultivars is practically impossible. Generally, allelopathy is a quantitative trait because of the genetic variability in allelopathic strength within a plant species (Dilday et al. 1998). Recently, DNA marker-assisted selection has been understood to detect QTLs responsible for the synthesis of allelochemicals in various crops. Identification of allelic loci governing the production of allelochemicals would assist in improving cereals for augmenting the release of these biochemical compounds. Successful genetic manipulation of allelopathic traits has been validated for crops, such as rice (Olofsdotter et al. 1999, 2002; Amb and Ahluwalia 2016), sorghum (Shehzad and Okuno 2020), and wheat (Wu et al. 2000). In sorghum, QTL mapping found nine QTLs (three for deterring germination and six for the length of roots in lettuce), and a total of 17 QTLs (seven for inhibiting germination and ten for the length of roots in lettuce) were identified in a linkage study.

The previous scientific reports have shown the existence of several allelochemicals and the multigenic nature of allelopathic traits in sorghum (Shehzad and Okuno 2020). The quantitative trait loci (QTL) regulating allelopathic effects in rice have been identified (Xu et al. 2002; Xu et al. 2003). The ISSR (inter-simple sequence repeat) molecular marker technique was used by Lin et al. (2005) to evaluate the genetic diversity in rice and barley allelopathy. There are several scientific reports on the successful usage of the RFLP technique to identify the QTLs conferring the synthesis of phytochemicals in several crops. The similarities among the allelopathic agents and these phytochemical-based features make it probable to exploit the same research methodology to study the genetic control of the allelochemical production in crops (Wu et al. 1999). The allelopathic research in crops is still in its infancy. Further research along this line will offer a great prospect for more understanding of the genetic control of allelopathy.

4.3.3 Genetic Engineering Approaches for Improving Allelopathic Traits

Crop allelopathy has hardly ever been used effectually by farmers in weed management. Conventional breeding techniques have not been successful in developing highly allelopathic crops coupled with higher yield potential. Genetic engineering may perhaps have the potential for overcoming this bottleneck. Crops have been developed resistant to pests, pathogens, and herbicides with transgenic technology, but it has not evolved crops that hamper weeds with allelochemicals (Duke et al. 2003). The strategies for developing allelopathic crops by genetic engineering are a quite complex process, usually involving multiple genes. One can select to enhance the production of allelochemicals already present in a crop or to impart the synthesis of new compounds. Several putative allelochemicals have been identified by observing for recognized phytotoxic chemicals in plant species; if a novel compound is responsible for allelopathy, field screening will not be productive. The best accurate method for the determination of prospective allelochemicals synthesized by a plant is that of bioassay-directed isolation.

A molecular biology strategy for enhancement of prevailing biochemical pathways for allelochemical synthesis is restricted in several crops due to the dearth of information or fragmentary knowledge of the biochemical pathways related to it. An arduous strategy is the detection of genes encoding major and crucial enzymes for allelochemical synthesis and to edulcorate the enzymes for elucidating the responsible genes (Canel 1999). Mutagenesis using transposable elements has been a valuable technique for gene isolation for identifying the biochemical pathways responsible for allelochemical production. In addition to this, an effective and delineated transposable element is obligatory in the concerned plant species to utilize this technique (Duke et al. 2001). Another potential genetic engineering-based bioherbicidal weed management strategy is the improvement of cover crops that are autotoxic after they have inhibited weeds, but already they start competing with the crop. Stanislaus and Cheng (2002) established the proof of concept of this strategy by expressing the *Barnase* gene cassette (which encodes a highly toxic ribonuclease) in *Nicotiana tabacum* (tobacco) that is regulated by HSP81 heat-shock promoter of *Arabidopsis thaliana.* The plant-incorporated protectants (PIPs) can also be used for developing bioherbicides, but there were no successful examples of bioherbicide PIPs, which should be improved by transgenic research. The PIPs will be a promising biotechnology strategy, so further study is needed to identify their mechanism of action (Duke 2003; Bertin et al. 2008). All conferred techniques offer a strong knowledge of allelopathy. Nonetheless, a thorough understanding of such complex communications among this phenomenon leads us one step forward for the progression of novel strategies for finding innovative bioherbicide for a sustainable and cleaner environment.

5 Conclusion

The exploitation of allelopathic weed control through rotational crops, intercrops, cover crops, mulches, crop residues, and allelochemical water extracts will offer sustainable agriculture and a cleaner environment and, moreover, viable weed control because of the useful impacts of these techniques on soil fertility status, organic matter, and ecosystem biodiversity. Furthermore, endeavors to create responsiveness to industries for formulating allelochemical-based natural herbicides, researching the allelopathy of unexplored fields, applying the hormesis knowledge for dose-response for allelopathic interactions among species, and conception of the allelochemical mechanism of action and their fate in different environment and ecosystem will advance the competence of allelopathic weed control. Scanty understanding of the transportation and biological degradation of allelochemicals in soil or the population genetics of allelopathic plant species, elucidating signal transduction pathways related to allelochemical synthesis and mode of action makes the allelopathic technique as complex for imeediate practical utility. There is an adequate scope to research donor and recipient plants. Various scientific research fields, such as molecular breeding, transgenic technology, plant tissue culture, stress physiology, plant source-sink relationship, allelopathy hormesis, ecobiology, and identifying novel allelochemicals to be intergrated for exploring the potential of allelopathy for cleaner environment. High-throughput analytical techniques in allelopathy studies could be utilized for investigating and describing distinctive agrochemicals that are derived from characteristic natural sources. Allelopathy grasps out promise for improvements in crop production through discovering novel eco-friendly bioherbicides with new modes of action, beneficial to crops but noxious to weeds, and without the formation of dangerous residues, less pollution, and restricted land degradation that leads toward the cleaner environment and green cover. Finally, the genetic manipulation of crops through various biotechnological strategies to evolve crops that carry genes and traits for the desirable attributes of a successful and strong competitor to their weeds. Allopathic research for a sustainable cleaner, pollution-free environment would be a strong motivating scientific target that is currently being pursued.

References

Abouziena HF, Haggag WM (2016) Weed control in clean agriculture: a review. PlantaDaninha 34 (2):377–392

Agrawal A (2001) Phenotypic plasticity in the interactions and evolution of species. Science 294:321–326

Alcantara C, Pujadas A, Saavedra M (2011) Management of *Sinapis alba* subsp. *mairei* winter cover crop residues for summer weed control in southern Spain. Crop Prot 30:1239–1244

Altieri MA, Lana MA, Bittencourt HV, Kieling AS, Comin JJ, Lovato PE (2011) Enhancing crop productivity via weed suppression in organic no-till cropping systems in Santa Catarina. Brazil J Sustain Agric 35:855–869. https://doi.org/10.1080/10440046.2011.588998

Amb MK, Ahluwalia AS (2016) Allelopathy: potential role to achieve new milestones in rice. Rice Sci 23:165–183. https://doi.org/10.1016/j.rsci.2016.06.001

Asaduzzaman M, An M, Pratley JE, Luckett DJ, Lemerle D (2014) Canola (*Brassica napus*) germplasm shows variable allelopathic effects against annual ryegrass (*Lolium rigidum*). Plant Soil 380:47–56

Ashraf A, AdilSarfraz R, Mahmood A (2017) Phenolic compounds' characterization of *Artemisia rutifolia spreng* from Pakistani flora and their relationships with antioxidant and antimicrobial attributes. Int J Food Prop 20(11):2538–2549. https://doi.org/10.1080/10942912.2016.1243556

Aslam F, Khaliq A, Matloob A, Tanveer A, Hussain S, Zahir ZA (2017) Allelopathy in agro-ecosystems: a critical review of wheat allelopathy-concepts and implications. Chemoecology 27:1–24. https://doi.org/10.1007/s00049-016-0225-x

Batish DR, Kohli RK, Singh HP (2011) Allelopathy: concept, chemicals involved, and possible utilization for weed management. J Plant Biol 38:33–42

Belz RG, Hurle K, Duke SO (2005) Dose-response- a challenge for allelopathy? Nonlinear Biol Toxicol Med 3(2):173–211. https://doi.org/10.2201/nonlin.003.02.002

Ben-Hammouda M, Kremer RJ, Minor HC, Sarwar M (1995) A chemical basis for the differential allelopathic potential of sorghum hybrids on wheat. J Chem Ecol 21:775–786. https://doi.org/10.1007/BF02033460

Berkowitz AR (1988) Competition for resources in weed-crop mixtures. In: Altieri MA, Liebman M (eds) Weed management in agrosystems: ecological approaches. CRC Press, Boca Raton, pp 89–119

Bernards MA (2010) Plant natural products: a primer. Can J Zool 88(7):601–614. https://doi.org/10.1139/Z10-035

Bertholdsson NO (2010) Breeding spring wheat for improved allelopathic potential. Weed Res 50:49–57. https://doi.org/10.1111/j.1365-3180.2009.00754.x

Bertholdsson NO, Andersson SC, Merker A (2012) Allelopathic potential of *Triticum spp*., *Secale spp*. and *Triticosecale spp*. and use of chromosome substitutions and translocations to improve weed suppression ability in winter wheat. Plant Breed 131:75–80. https://doi.org/10.1111/j.1439-0523.2011.01895.x

Bertin C, Weston LA, Kaur H (2008) Allelopathic crop development: molecular and traditional plant breeding approaches. Plant Breed Rev 30:231–258

Bertin C, Paul RN, Duke SO, Weston LA (2003) Laboratory Assessment of the Allelopathic Effects of Fine Leaf Fescues. J Chem Ecol 29:1919–1937. https://doi.org/10.1023/A:1024810630275

Bogatek R, Gniazdowska A, Zakrzewska W, Oracz K, Gawronski S (2006) Allelopathic effects of sunflower extracts on mustard seed germination and seedling growth. BiologiaPlantarum 50, 156-158. https://doi.org/10.1007/s10535-005-0094-6

Burgos NR, Talbert RE, Kim KS, Kuk YI (2004) Growth inhibition and root ultrastructure of cucumber seedlings exposed to allelochemicals from rye (*Secale cereale*). J Chem Ecol 30:671–689. https://doi.org/10.1023/B:JOEC.0000018637.94002.ba

Campiglia E, Radicetti E, Mancinelli R (2012) Weed control strategies and yield response in a pepper crop (*Capsicum annum* L.) mulched with hairy vetch (*Vicia villosa* Roth.) and oat (*Avena sativa* L.) residues. Crop Prot 33:65–73. https://doi.org/10.1016/j.cropro.2011.09.016

Canel C (1999) From genes to phytochemicals: the genomics approach to the characterization and utilization of plant secondary metabolism. ActaHortic 500:51–57

Cantrell CL, Dayan FE, Duke SO (2012) Natural products as sources for new pesticides. J Nat Prod 75:1231–1242. https://doi.org/10.1021/np300024u

Chai TT, Ooh KF, Chue PS, Wong FC (2013) *Leucaena leucocephala* leachate compromised membrane integrity, respiration and ant oxidative defense of water hyacinth leaf tissues. Bot Stud 54(1):1–7. https://doi.org/10.1186/1999-3110-54-8

Cheema Z, Jaffer I, Khaliq A (2003) Reducing isoproturon dose in combination with sorgaab for weed control in wheat. Pak J Weed Sci Res 9:60

Chung IM, Ali M, Ahmad A, Lim JD, Yu CY, Kim JS (2006) Chemical constituents of rice (*Oryza sativa*) hulls and their herbicidal activity against duckweed (*Lemna paucicostata*). Phytochem Anal 17:36–45. https://doi.org/10.1002/pca.879

Croteau R, Kutchan TM, Lewis NG (2000) Natural products (secondary metabolites). In: Buchanan B, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants. American Society of Plant Biologists, Rockville, pp 1250–1268

Cruz-Ortega R, Ayala-Cordero GA, AL. (2002) Allelochemical stress produced by the aqueous leachate of *Callicarpa acuminata*: effects on roots of bean, maize, and tomato. Physiol Plantarum 116:20–27. https://doi.org/10.1034/j.1399-3054.2002.1160103.x

Dayan FE, Duke SO (2014) Natural compounds as next-generation herbicides. Plant Physiol 166:1090–1105. https://doi.org/10.1104/pp.114.239061

Dayan FE, Cantrell CL, Duke SO (2009) Natural products in crop protection. Bioorg Med Chem 17 (12):4022–4034. https://doi.org/10.1016/j.bmc.2009.01.046

Dilday RH, Nastasi P, Lin J, Smith RJ (1991) Allelopathic activity in rice (*Oryza sativa* L.) against duck salad (*Heteranthera limosa* (Sw.) will.). In: Hansen JD, Shaffer MJ, Ball DA, Cole CV (eds) Symposium proceedings 'Sustainable agriculture for the Great Plain'. USDA, Agricultural Research Services, ARS-89, pp 193–201

Dilday RH, Yan WG, Moldenhauer KAK, Gravois KA (1998) Allelopathic activity in rice for controlling major aquatic weeds. In: Olofsdotter M (ed) Allelopathy in rice. Rice Research Institute, Manila, pp 7–26

Ding J, Sun Y, Xiao CL, Shi K, Zhou YH, Yu JQ (2007) Physiological basis of different allelopathic reactions of cucumber and figleaf gourd plants to cinnamic acid. J Exp Bot 58:3765–3773. https://doi.org/10.1093/jxb/erm227

Dudareva N, Klempien A, Muhlemann JK, Kaplan I (2013) Biosynthesis, function, and metabolic engineering of plant volatile organic compounds. New Phytol 198:16–32. https://doi.org/10.1111/nph.12145

Duke SO (2003) Weeding with transgenes. Trends Biotechnol 21:192–195. https://doi.org/10.1016/s0167-7799(03)00056-8

Duke SO (2010) Allelopathy: current status of research and future of the discipline. A commentary. Allelo J 25:17–30

Duke S, Scheffler B, Dayan F, Weston L, Ota E (2001) Strategies for using transgenes to produce Allelopathic crops. Weed Technol 15(4):826–834. https://doi.org/10.1614/0890-037X(2001)015[0826:SFUTTP]2.0.CO;2

Duke SO, Dayan FE, Rimando AM, Schrader KK, Aliotta G, Oliva A, Romagni JG (2002) Chemicals from nature for weed management. Weed Sci 50(2):138–151. https://doi.org/10.1614/0890-037X(2001)015[0826:SFUTTP]2.0.CO;2

Duke SO, Dayan EE, Baerson SE, Romagni JG, Agarwal A, Oliva A (2003) Natural phytotoxins with potential for development in weed management strategies. In: Ramos G, Voss G (eds) Chemistry of crop protection. Wiley-VCH Verlag, Weinheim, pp 143–154

Einhellig FA (1996) Interactions involving allelopathy in the cropping system. Agron J 88:886–893. https://doi.org/10.2134/agronj1996.00021962003600060007x

Einhellig FA (1999) An integrated view of allelochemicals amid multiple stresses. In: Inderjit, Dakshini KMM, Foy CL (eds) Principles and practices in plant ecology. CRC Press, Boca Raton, pp 479–494

Einhellig FA, Leather GR (1988) Potentials for exploiting allelopathy to enhance crop production. J ChemEcol 14:1829–1844. https://doi.org/10.1007/BF01013480

Farooq M, Hussain T, Wakeel A, Cheema ZA (2014) Differential response of maize and mungbean to tobacco allelopathy. Exp Agric 50:611–624. https://doi.org/10.1017/S0014479714000106

Favaretto A, Scheffer-Basso SM, Perez NB (2018) Allelopathy in Poaceae species present in Brazil. A review. Agron Sustain Dev. https://doi.org/10.1007/s13593-018-0495-5

Gaudet CL, Keddy PA (1988) A comparative approach to predicting competitive ability from plant traits. Nature 334:242–243

Gealy D, Ottis B, Talbert R, Moldenhauer K, Yang W (2005) Evaluation and improvement of allelopathic rice germplasm at Stuttgart, Arkansas, USA. In: JDI H, An M, Wu H, Kent JH (eds) Proceedings fourth world congress on allelopathy, Charles Sturt University, WaggaWagga, NSW, Australia, 21–26 August 2005. International Allelopathy Society, pp 157–163

Golisz A, Sugano M, Fujii Y (2008) Microarray expression profiling of Arabidopsis thaliana L. in response to allelochemicals identified in buckwheat. J Exp Bot 59:3099–3109. https://doi.org/10.1093/jxb/ern168

Grummer G (1955) Die gegenseitigeBeeinflussunghohererPflanzen-Allelopathie. Fischer, Jena

Haddadchi GR, Gerivani Z (2009) Effects of phenolic extracts of canola (*Brassica napus* L.) on 444 germination and physiological responses of soybean (*Glycine max* L.) seedlings. Int J Plant 445 Prod 3:63–74

Haider G, Cheema ZA, Farooq M, Wahid A (2015) Performance and nitrogen use of wheat cultivars in response to the application of allelopathic crop residuesand3,4-dimethyl pyrazole phosphate. Int J Agric Biol 17:261–270

Hameed A, Hussain SA, Ansar H, Suleria R (2019) Co-evolution of secondary metabolites. December. https://doi.org/10.1007/978-3-319-76887-8

Harborne JB (ed) (1989) Plant phenolics, Methods in plant biochemistry no. 1, vol 1. Academic, London

Hebert RB (1981) The biosynthesis of secondary metabolites. Chapman and Hall Ltd, New York, pp 1–179

Hejl AM, Koster KL (2004) Juglone disrupts root plasma membrane H+-*ATP ase* activity and impairs water uptake, root respiration, and growth in soybean (*Glycine max*) and corn (*Zea mays*). J Chem Ecol 30:453–471. https://doi.org/10.1023/B:JOEC.0000017988.20530.d5

Hill EC, Ngouajiio, Nair MG (2006) Differential response of weeds and vegetable crops to aqueous extracts of hairy vetch and cowpea. Hortic Sci 41(3):695–700. https://www.sciencedirect.com/bookseries/advances-in-botanical-research/vol/82

Iannucci A, Fragasso M, Platani C, Papa R (2013) Plant growth and phenolic compounds in the rhizosphere soil of wild oat (Avena fatua L.). Front Plant Sci 4:509. https://doi.org/10.3389/fpls.2013.00509

Inderjit (1996) Plant phenolics in allelopathy. Bot Rev 62(2):186–202. https://doi.org/10.1007/BF02857921

Iqbal J, Cheema ZA, An M (2007) Intercropping of field crops in cotton for the management of purple nutsedge (*Cyperus rotundus* L.). Plant Soil 300:163–171. https://doi.org/10.1007/s11104-007-9400-8

Jabran K (2017) Manipulation of allelopathic crops for weed control, 1st edn. Springer. https://doi.org/10.1007/978-3-319-53186-1

Jabran K, Mahajan G, Sardana V, Chauhan BS (2015) Allelopathy for weed control in agricultural systems. Crop Prot 72:57–65

Jensen LB, Courtois B, Shen L, Li Z, Olofsdotter M, Mauleon RP (2001) Locating genes controlling allelopathic effects against *Echinochloa crus-galli* (L.) in upland rice. Agric J 93:21Ð26

Jung WS, Kim KH, Ahn JK, Hahn SJ, Chung IM (2004) Allelopathic potential of rice (*Oryza sativa* L.) residues against Echinochloa crus-galli. Crop Prot 23:211–218. https://doi.org/10.1016/j.cropro.2003.08.019

Kamal J (2011) Impact of allelopathy of sunflower (*Helianthus annuus* L.) roots extract on the physiology of wheat (*Triticum aestivum* L.). Afr J Biotechnol 10(65):14465–14477. https://doi.org/10.5897/AJB11.988

Kato-Noguchi H, Ota K, Kujime H, Ogawa M (2013) Effects of momilactone on the protein expression in Arabidopsis germination. Weed Biol Manag 13:19–23. https://doi.org/10.1111/wbm.12005

Khush GS (1996) Genetic improvement of rice for weed management. In: Naylor R (ed) Herbicides in Asian rice: transitions in weed management. Institute for Int. Studies, Stanford University, and Manila (Philippines): Int. Rice Research Institute, Palo Alto, pp 201–207

Kohli RK, Singh HP, Batish DR (2001) Allelopathy in agroecosystems. Food Products Press, New York. https://doi.org/10.1300/J144v04n02_01

Korres NE, Burgos NR, Duke SO, Fujii Y, SarpongAppiah K (2019) Allelopathy for sustainable weed management. Weed Control, pp 166–190. https://doi.org/10.1201/9781315155913-9

Kupidłowska E, Gniazdowska A, Stepien J, Corbineau F, Vinel D, Skoczowski A, Janeczko A, Bogatek R (2006) Impact of sunflower (*Helianthus annuus* L.) extracts upon reserve mobilization and energy metabolism in germinating mustard (*Sinapis alba* L.) seeds. J Chem Ecol 32:2569–2583

Latif S, Chiapusio G, Weston LA (2017) Chapter two – Allelopathy and the role in plant defense. https://doi.org/10.1016/bs.abr.2016.12.001

Li ZH, Shen YX (2006) Allelopathic effects of different varieties of *Medicago sativa* weed regrowth in winter. Acta Pratac Sin 15:36–42

Li F-R, Peng S-L, Chen B-M, Hou Y-P (2010a) A meta-analysis of the responses of woody and herbaceous plants to elevated ultraviolet-B radiation. ActaOecologica 36(1):1–9. https://doi.org/10.1016/j.actao.2009.09.002

Li ZH, Wang Q, Ruan X, Pan CD, Jiang JD (2010b) Phenolics and plant allelopathy. Molecules: A J Synth Chem Natl Prod Chem 15(12):8933–8952. https://doi.org/10.3390/molecules15128933

Lin WX, He HQ, Chen XX, Xiong J, Song BQ, Liang YY, Liang KJ (2005) Use of ISSR molecular marker approach to estimate genetic diversity in rice and barley allelopathy. In: Haper JDI, An M, Wu H, Kent JH (eds) Proceedings of the fourth world congress on allelopathy "Establishing the Scientific Base". WaggaWagga, Australia, pp 168–174

Ma Y, Zhang M, Li Y, Shui J, Zhou Y (2014) Allelopathy of rice (Oryza sativa L.) root exudates and its relations with *Orobanche cumana* Wallr. and *Orobanche minor* Sm. germination. J Plant Interact 9(1):722–730. https://doi.org/10.1080/17429145.2014.912358

Maqbool N, Wahid A, Farooq M, Cheema ZA, Siddique KHM (2013) Allelopathy and abiotic stress interaction in crop plants. In: Cheema ZA, Farooq M, Wahid A (eds) Allelopathy. Springer, Berlin, pp 451–468. https://doi.org/10.1007/978-3-642-30595-5_19

Meiners SJ, Kong CH, Ladwig LM, Pisula NL, Lang KA (2012) Developing an ecological context for allelopathy. Plant Ecol 213:1221–1227. https://doi.org/10.2307/23267444

Mirsky SB, Ryan MR, Teasdale JR, Curran WS, Reberg-Horton CS, Spargo JT, Wells MS, Keene CL, Moyer JW (2013) Overcoming weed management challenges in cover crop-based organic rotational no-till soybean production in the eastern United States. Weed Technol 27:193–203

Mohamadi N, Rajaie P (2009) Effects of aqueous eucalyptus (*E. camadulensis Labill*) extracts on seed germination, seedling growth, and physiological responses of *Phaseolus vulgaris* and *Sorghum bicolor*. Res J Biol Sci 4(12):1292–1296

Murphy SD (2001) The role of pollen allelopathy in weed ecology. Weed Technol 15(4):867–872

Mushtaq W, Siddiqui MB (2018) Allelopathy in Solanaceae plants. J Plant Protect Res 58(1):1–7. https://doi.org/10.24425/119113

Mushtaq W, Siddiqui MB, Hakeem KR (2020) Allelopathy: potential for green agriculture, Springer briefs in agriculture. Springer. https://doi.org/10.24425/119113

Narwal SS (2000) Allelopathy in ecological agriculture, allelopathy in ecological agriculture and forestry. Springer, pp 11–32. https://doi.org/10.1007/978-94-011-4173-4

Nawaz A, Farooq M, Cheema SA, Cheema ZA (2014) Role of allelopathy in weed management. Recent advances in weed management. Springer, New York, pp 39–61

Nishida N, Tamotsu S, Nagata N, Saito C, Sakai A (2005) Allelopathic effects of volatile monoterpenoids produced by Salvia leucophylla: inhibition of cell proliferation and DNA synthesis in the root apical meristem of Brassica campestris seedlings. J Chem Ecol 31:1187–1203. https://doi.org/10.1007/s10886-0054256-y

Olkowski AA, Amarowicz R, Peiquiang Y, Mckinnon JJ, Maenz DD (2003) A rapid HPLC 558 method for determination of major phenolic acids in plant material. Polish J Food Nutri Sci 559(12):53–57

Olofsdotter M, Navarez D, Moody K (1995) Allelopathic potential in rice (*Oryza sativa* L.) germplasm. Ann Appl Biol 127:543–560. https://doi.org/10.1111/j.1744-7348.1995.tb07611.x

Olofsdotter M, Navarez D, Rebulanan M, Streibig JC (1999) Weed suppressing rice cultivars–does allelopathy play a role? Weed Res 39:441–454. https://doi.org/10.1046/j.1365-3180.1999.00159.x

Olofsdotter M, Rebulanan M, Madrid A, Dali W, Navarez D, Olk DC. (2002). Why phenolic acids are unlikely primary allelochemicals in rice. J Chem Ecol 28(1):229–242. https://doi.org/10.1023/a:1013531306670.

Paré PW, Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. Plant Physiol 121(2):325–332. https://doi.org/10.1104/pp.121.2.325

Pereira IP, Dias AS, Dias LS (2016) Defensive role of allelopathic secondary compounds in plants I: testing two independent general hypotheses. J Negative Results Ecol Evol Biol 11:10–24

Putnam AR, Duke WB (1974) Biological suppression of weeds: evidence for allelopathy in accessions of cucumber. Science 185:370–371

Putnam AR, Tang CS (1986) Allelopathy: state of the science. In: Putnam AR, Tang CS (eds) The science of allelopathy. Wiley, New York, pp 1–19

Rice EL (1984) Allelopathy, 2nd edn, Rice EL (ed). Academic, Orlando, p 422

Rimando AM, Dayan FE, Czarnota MA, Weston LA, Duke SO (1998) A new photosystem II electron transfer inhibitor from Sorghum bicolor. J Nat Prod 61:927–930. https://doi.org/10.1021/np980325h

Rivoal A, Fernandez C, Greff S, Montes N, Vila B (2011) Does competition stress decrease allelopathic potential? Biochem Syst Ecol 39:401–407. https://doi.org/10.1016/j.bse.2011.05.017ff.ffhal-01764833f

Scavo A, Abbate C, Mauromicale G (2019) Plant allelochemicals: agronomic, nutritional, and ecological relevance in the soil system. Plant Soil 442:23–48. https://doi.org/10.1007/s11104-019-04190-y

Shehzad T, Okuno K (2020) Genetic analysis of QTLs controlling allelopathic characteristics in sorghum. PLoS One 15(7):e0235896. https://doi.org/10.1371/journal.pone.0235896

Song Q, Qin F, He H et al (2019) Allelopathic potential of rain leachates from Eucalyptus urophylla on four tree species. Agroforest Syst 93:1307–1318. https://doi.org/10.1007/s10457-018-0240-8

Stanislaus MA, Cheng CL (2002) Genetically engineered self-destruction: an alternative to herbicides for cover crop systems. Weed Sci 50:794–801.

Takahashi L, Sert MA, Kelmer-Bracht AM, Bracht A, Ishii-Iwamoto EL (1998) Effects of rutin and quercetin on mitochondrial metabolism and ATP levels in germinating tissues of *Glycine max*. Plant Physiol Biochem 36:495–501

Tawaha AM, Turk MA (2003) Allelopathic effects of black mustard (*Brassica nigra*) on 597 germinations and growth of wild barley (*Hordeum spontaneum*). J Agron Crop Sci 5:298–306. https://doi.org/10.1046/j.1439-037X.2003.00047.x

Toyomasu T, Kagahara T, Okada K, Koga J, Hasegawa M, Mitsuhashi W, Sassa T, Yamane H (2008) Diterpene phytoalexins are biosynthesized in and exuded from the roots of rice seedlings. Biosci Biotech Bioch 72:562–567

Tursun N, Işık D, Demir Z, Jabran K (2018) Use of living, mowed, and soil-incorporated cover crops for weed control in apricot orchards. Agronomy 8(8):150

Vyvyan WR (2002) Allelochemicals as leads to herbicides and agrochemicals. Tetrahedron 58:1631–1646

Weir TL, Park SW, Vivanco JM (2004) Biochemical and physiological mechanisms mediated by allelochemicals. Curr Opin Plant Biol 7:472–479. https://doi.org/10.1016/j.pbi.2004.05.007

Weston LA (1996) Utilization of allelopathy for weed management in agroecosystems. Agron J 88:860–866. https://doi.org/10.2134/agronj1996.00021962003600060004x

Weston LA, Mathesius U (2013) Flavonoids: their structure, biosynthesis and role in the rhizosphere, including allelopathy. J Chem Ecol 39(2):283–297. https://doi.org/10.1007/s10886-013-0248-5.

Weston LA, Ryan PR, Watt M (2012) Mechanisms for cellular transport and release of allelochemicals from plant roots into the rhizosphere. J Exp Bot 63:3445–3454. https://doi.org/10.1093/jxb/ers054

Williamson GB, Weidenhamer JD (1990) Bacterial degradation of juglone: evidence against allelopathy. J Chem Ecol 16(5):1739–1742. https://doi.org/10.1007/BF01014105

Worthington M, Reberg-Horton C (2013) Breeding cereal crops for enhanced weed suppression: optimizing allelopathy and competitive ability. J Chem Ecol 39:213–231. https://doi.org/10.1007/s10886-013-0247-6

Wu, Pratley, Lemerle, Haig (1999) Crop cultivars with allelopathic capability. Weed Research 39:171–180. https://doi.org/10.1046/j.1365-3180.1999.00136.x

Wu H, Pratley J, Lemerle D, Haig T (2000) Evaluation of seedling allelopathy in 453 wheat (Triticum aestivum) accessions against annual ryegrass (Lolium rigidum) by the equalcompartment-agar method. Aust J Agric Res 51:937–944. https://doi.org/10.1071/AR00017

Wu H, Pratley J, Ma W, Haig T (2003) Quantitative trait loci and molecular markers associated with wheat allelopathy. Theor Appl Genet 107:1477–1481. https://doi.org/10.1007/s00122-003-1394-x

Wu FZ, Pan K, Ma FM, andan, X.D. (2004) Effects of cinnamic acid on photosynthesis and cell ultrastructure of cucumber seedlings. ActaHortic Sin 31:183–188

Xu ZH, Yu LQ, Zhao M, Zhang X, Wei XH, Guo LB, Ye YL (2002) Studies on the quantitative trait and gene location of rice (*Oryza sativa* L.) allelopathy against barnyard grass (*Echinochloa crus-galli* (L.) Bureau.). Plant Prot 28:40–42

Xu ZH, Yu LQ, Zhao M (2003) Rice allelopathy to barnyard grass. Chin J Appl Ecol 14:737–740

Yu JQ, Ye SF, Zhang MF, Hu WH (2003) Effects of root exudates and aqueous root extracts of cucumber (*Cucumis sativus*) and allelochemicals, on photosynthesis and antioxidant enzymes in cucumber. Biochem Syst Ecol 31:129–139. https://doi.org/10.1016/S0305-1978(02)00150-3

Zohaib A, Abbas T, Tabassum T (2016) Weeds cause losses in field crops through allelopathy. Nat Sci Biol 8:47–56

Chapter 6
Strategies and Limitations of Water Treatment Methods for Point-of-Use Application

N. R. Srinivasan, M. Kamaraj, and S. Venkatesa Prabhu

Abstract Water-related diseases threaten human survival across the globe. This is because of the severe contamination of water all over the world. Water is being contaminated due to the rapid growth of population and industrialization. As a result, the demand for potable water is increased in many developing countries. The surface sources for potable water are rivers, lakes, and aquifers. Particularly, to ensure its quality, water obtained from these sources should be free from chemical and biological contaminants. In developing countries, most of the people (60%) depend on the groundwater source. However, groundwater needs to be treated as the soil does not remove all the contaminants. Moreover, in various parts of the world, water supply is done through the network of pipelines to the individual consumers from water treatment plants. The quality of water may get degraded due to the formation of rust while flowing through pipes. Chlorination is a widely adopted method in many countries for water disinfection. It exhibits low disinfection efficiency and results in the generation of toxic by-products. This chapter describes the strategies and limitations of water treatment methods to remove both chemical and biological contaminants for point-of-use applications.

Keywords Chemical disinfectants · Physical disinfectants · Filtration methods · Comparison of treatment technologies

---

N. R. Srinivasan (✉) · S. V. Prabhu
Department of Chemical Engineering, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

Nanotechnology Center of Excellence, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

M. Kamaraj
Nanotechnology Center of Excellence, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_6

1 Introduction

On the earth's surface, approximately two-thirds of the portion is full of water in the form of oceans and freshwater. The contribution from the oceans is around 97.2% of its availability, whereas the freshwater source contributes about 2.7%, of which 0.35% is contaminated due to various anthropogenic activities (Akshay et al. 2020). In general, water contamination occurs due to the presence of undesirable chemicals, microbes, and suspended solids. The presence of these contaminants in water could lead to waterborne diseases such as dysentery and cholera. Water scarcity and contamination are major problems in most of the places across the world. According to the World Health Organization report (WHO), 844 million people do not have safe water all over the world (WHO/UNICEF 2017).

The main reasons for water contamination are climate change, population, and industrialization (Okello et al. 2015). It is necessary to provide cost-effective water treatment technologies for low-income countries. To have safe and drinkable water, the WHO set guidelines for centralized and decentralized water treatment systems. Figure 6.1 shows the water treatment process involved in a centralized system to provide safe water. In most of the countries, water treatment plant undergoes various physicochemical processes such as sedimentation, coagulation/flocculation, etc. (Matsumoto et al. 1995). This chapter discusses the strategies and limitations involved in both chemical and physical disinfectants.

2 Chemical Disinfectants

2.1 Chlorination

The pathogenic microorganisms (viruses, bacteria, and protozoa) are of utmost concern in water treatment. Over the years, chemical control of microorganisms is the most important and widely accepted method for a water treatment plant. Chlorine compounds such as chlorine gas, chlorine dioxide, chloramines, and hypochlorite are widely used to treat water (Zhang et al. 2012). Particularly, the addition of chlorine in water reacts with organic matter, reducing agents and ammonia. Chlorination is preferred not only for killing microbes and also for odor removal in water. One of the main drawbacks of chlorination is toxic to humans if the concentration exceeds above the permissible limit. During chlorination, chlorine reacts with natural organic matter (NOM), iodide (I^-), and bromide (Br^-) to generate various harmful disinfection by-products (DBPs), including trihalomethanes (THM), haloacetic acids, etc. (Richardson et al. 2007). The predominant organic matters in water are humic and fulvic acids. The formation of DBPs is influenced by chlorine dosage, contact time, organic matter, pH, temperature, etc. Based on the animal and epidemiological studies, these by-products may produce adverse effects on humans (carcinogenicity or cytotoxic). The required concentration of chlorine for bacteria,

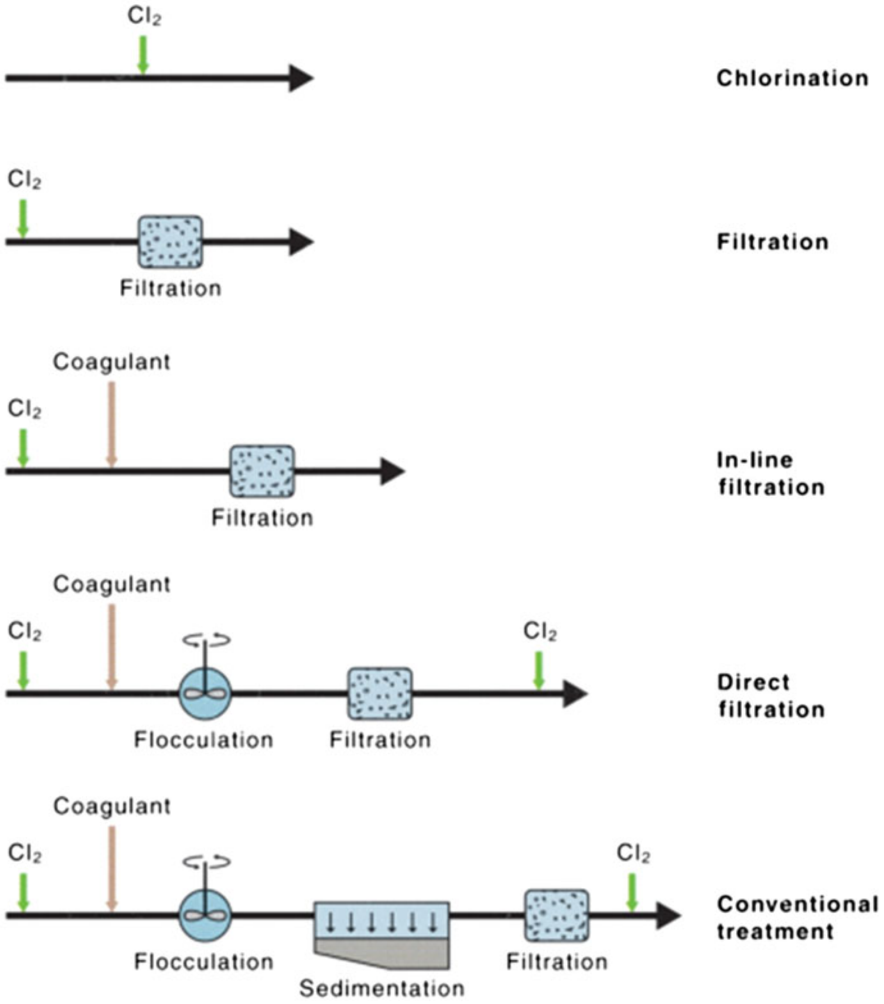


Fig. 6.1 Water treatment process (Adapted from Environmental Microbiology, Academic Press, San Diego, CA, 2000)

virus (poliovirus), and parasites are 0.1, 1, and 5 ppm, respectively (Bitton 2014). Moreover, the microbicide occurs when chlorine interacts with the surface of the pathogen. The mechanisms by which the disinfection occurs are the damage of cell surface, oxygen uptake, and oxidative phosphorylation, inhibiting the enzyme activity and physical damage to DNA. According to the WHO, the maximum value for chlorite in drinking water should not exceed 0.2 mg/L (Twort et al. 2000).

Generally, chlorine is added as a gas (Cl_2) and salt (sodium hypochlorite). Chlorine gas is rapidly hydrolyzed in water to form hypochlorous acid (HOCl), which further dissociates and generates hypochlorite ion (OCl^-) according to

Eqs. (6.1) and (6.2). These products form at different pH (particularly, HOCl is at pH – 5.0 and OCl^- is at pH –10.0). These free chlorine-based compounds react with both inorganic and organic matter present in water to form chlorinated compounds.

$$Cl_2 + H_2O \leftrightarrow HOCl + H^+ + Cl^- \tag{6.1}$$

$$HOCl \leftrightarrow H^+ + OCl^- \tag{6.2}$$

Hypochlorous acid reacts with ammonium ion to produce monochloramine, dichloramine, and trichloramine according to Eqs. (6.3), (6.4) and (6.5). The formation of different types of chloramine depends on the pH value and the concentration of NH_3 in water.

$$NH_3 + HOCl \rightarrow NH_2Cl + H_2O \tag{6.3}$$

$$NH_2Cl + HOCl \rightarrow NHCl_2 + H_2O \tag{6.4}$$

$$NHCl_2 + HOCl \rightarrow NCl_3 + H_2O \tag{6.5}$$

The term "free chlorine" is the combination of hypochlorous acid and the hypochlorite, whereas chloramines are called combined chlorine. Moreover, these chlorinated compounds have disinfection ability to oxidize sulfides (S^-), ferrous (Fe^{2+}), and manganese (Mn^{2+}) ions. As a result of impurities in water, chlorine demand is assessed based on the concentration of residual chlorine in the water. The breakeven point occurs when the amount of chlorine increases beyond the point where the residual chlorine is detected. It generally occurs in the pH range of 7.0–7.5.

The effective dosage of chlorine concentration is at the breakpoint or slightly higher. The concentration should be enough to break chemical bonds of contaminants as well as destroy pathogens. It is important to keep free available chlorine residual by supplying an excess quantity of chlorine for disinfection. Figure 6.2. shows the breakpoint chlorination curve in different stages. In the initial stage, there is no free residual chlorine as complete oxidation occurs (Point A). Next, the curve is linear until point B as the formation of chloramines and chloro-organic occurs at this stage. Furthermore, oxidative destruction occurs with an increase in the concentration of chlorine. After that, the number of residual chlorine decreases and reaches a point called breakpoint (Point C). Then, the residual chlorine keeps on increasing as the concentration increases (Point D). The breakpoint concentration varies with the quality of raw water (Al-Abri et al. 2019).

Table 6.1 shows the concentration of chlorine-based disinfectants and contact time required for the inactivation of *E. coli*. In the water treatment plant, chlorine is added in the form of elemental chlorine (chlorine gas), sodium hypochlorite, and calcium hypochlorite. Each form has distinct advantages and disadvantages of water disinfection (Table 6.2).

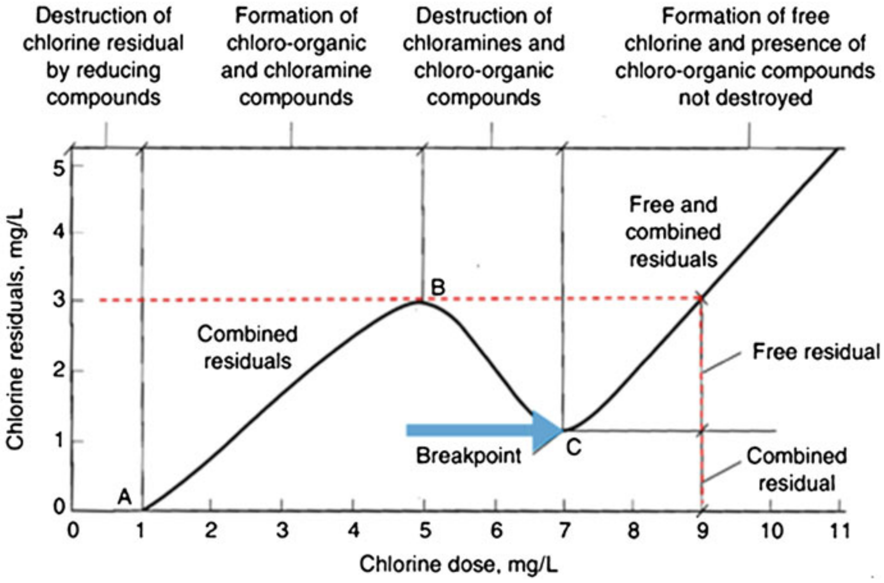


Fig. 6.2 Breakpoint chlorination curve (https://naturalpoolproducts.com/category/main/)

Table 6.1 Contact time required to inactivate *E. coli* (Hall and Hyde 1992)

	Concentration (ppm)	Contact time, min	pH	Temperature, °C
Free chlorine	0.1	0.4	6	5
	0.1	6	8.5	20–29
	0.4	1	8.5	20–29
Monochloramine	0.1	60	4.5	15
	1	6	4.5	15
Dichloramine	1	64	9	15

Although chlorination remains the most widely accepted method, the water treatment system may choose either one of the chlorine-based disinfection or their combination.

2.2 *Iodine*

Iodine is a strong oxidant, which is more effective at a lower dosage and less sensitive to pH and organic matter present in water. The active disinfectants are elemental iodine and hypoiodous acid. The main disadvantage is that it could create allergic reactions to any individual. The recommended concentration is 150ug/day for adults. The concentration below 1 mg/l is effective for bacteria. However, the same concentration will take a few hours to kill *Giardia cysts*. Iodine has more

Table 6.2 The advantages and disadvantages of chlorine-based chemical disinfectant

S. No	Chlorine-based disinfectants	Advantages	Disadvantages
1.	Elemental chlorine	Cost-effective	Hazardous and highly corrosive
2.	Sodium hypochlorite	Less hazardous	High cost, formation of chlorite and chlorate, storage difficulties, limited shelf life
3.	Calcium hypochlorite	More stable than sodium hypochlorite	Formation of by-products like chlorite and chlorate, higher cost than elemental chlorine
4.	Chloramines	Formation of trihalomethanes and brominated products, more stable than free chlorine	Weak oxidant, hazardous to humans and aquatic life
5.	Chlorine dioxide	Effective method as compared to chlorine, no formation of by-products like trihalomethanes	Formation of by-products like chlorite and chlorate, may exhibit taste and odor problems, high operating cost

advantages, such as chemical stability and less reactivity with organic nitrogenous contaminants. The required concentration shoots up when the turbidity increases. It is considered to be more effective for water containing sludges. The usage of iodine as a disinfectant is restricted because of the cost, and it cannot be applied to a large system (White 1992; Howard et al. 2000).

2.3 Silver

Recently, silver has been proven to be an effective antibacterial agent against microbes since the times of ancient Greeks. It can be added in the form of salt (silver nitrate), colloidal suspension, and a small bed of silver (Srinivasan et al. 2013). For effective usage, silver can be deposited on the surface of porous carbon to inactivate microbes. Mostly, the support could be either ceramic or polymeric materials. These kinds of systems have been established against both gram-positive and gram-negative bacteria, which shows an almost 100% reduction in bacterial colonies. In this case, several mechanisms have been proposed in the literature to inactivate microbes such as cell wall rupture, generation of reactive oxygen species, and deactivation of proteins (Klueh et al. 2000). The use of silver as a disinfectant has become popular in most of European countries and the USA, and it is accepted for the bacteriological quality of stored water.

2.4 Potassium Permanganate, Ferrates, and Ozone

Potassium permanganate is also a strong oxidizing agent that oxidizes iron and manganese ions and removes odor and taste. It is reported that it has antibacterial properties against *Legionella pneumophila* (Yahya et al. 1989). Moreover, it is also considered as a poor disinfectant because the concentration required to inactivate microbes is too high (1 g/L). Bromine has the potential to control microbes in places where a small area is to be disinfected (e.g., swimming pool). It works well by forming hypobromous acid (HBrO) and other compounds like bromamines, which can be potent antimicrobial products. The addition of bromine in water disinfection is costlier than chlorine-based compounds. The main disadvantage is its reactivity with ammonia or other amines that may hinder its effectiveness. The mechanism behind the inactivation of microbes is oxidative stress (Taylor and Butler 1982). Ferrates react with reducing agents and various compounds that can effectively inactivate microbes (Audette et al. 1971). It is reported that ferrates can kill *Pseudomonas* over a range of concentrations (0–50 ppm) (Murmann 1975). Interestingly, zero-valent iron nanoparticles are considered as an alternative to permeable reactive barriers. It is reported that nanostructured iron adsorbs on the viral capsid, leading to inactivation (Ryan et al. 2002).

Ozone is one of the strongest disinfectants and oxidants that can remove color and turbidity and inactivates microorganisms. It is generated by passing dry oxygen or air through high voltage electrodes. In literature, the inactivation of *Salmonella* is reported with a 5 and 6 log removal at a concentration (2 mg/L) after the interaction time of 45 and 60s, respectively (Jamil et al. 2017).

This process does not produce chlorinated THMs or HAAs, and it reacts with bromine-containing compounds and forms toxic contaminants like bromate and brominated organics. Ozone breaks down complex organic molecules, and smaller size molecules can increase the growth of microbes and disinfection by-products during the processes. The mechanism behind the inactivation of microbes by ozone is the attack on the double bonds of the lipid layers in the cell membrane (Smith 1967).

2.5 Coagulation

Coagulation involves the addition of chemicals to remove dissolved and suspended solids by filtration and sedimentation. The addition of coagulating chemicals (e.g., alum) will increase the settling rate of particles by combining smaller particles into flocs. The required concentration ranges from 10 to 30 mg/L of water. After the addition of chemicals, at least 30 min is required to settle at the bottom, and then the clear water above the flocs can be decanted off.

3 Physical Disinfectants

Chemical disinfectants protect humans against waterborne microbial diseases. However, it has several disadvantages, including the formation of toxic by-products, taste, and odor problems. Moreover, in the longer term, there is pressure on the industries to reduce the production of chlorine-based disinfectants for environmental issues. In a commercial scale, membrane-based processes are available to remove microbes as well as chemical contaminants. It operates without chemical disinfection, or at least to reduce the number of chemicals used for disinfection. Further, pressure-driven membrane processes (microfiltration, ultrafiltration, nanofiltration, and reverse osmosis) can remove chemical contaminants and microbes based on their pore size. From the point of toxicological issues, this membrane process would prevent the formation of disinfection by-products and undesirable chemicals. The main issues in the membrane-based processes are the removal efficiency of microbes and biofouling. As an alternative to chemical disinfection, UV radiation is also capable of removing bacteria and viruses. This part describes the principles involved in the physical processes.

3.1 Ultraviolet Radiation

UV radiation energy waves ranges from 100 to 400 nm. The optimum UV range is between 245 and 285 nm for germicidal effects. This process does not produce any toxic by-products or taste and odor problems. It has several demerits such as high cost as compared to the chemical disinfectant, UV lamps maintenance, and photoreactivation of enteric bacteria. The effectiveness is decreased in the effluents by substances like phenolic compounds, humic substances, lignin sulfonates, and ferric iron. The presence of suspended matter may protect microbes from UV light, leading to the implementation of pretreatment techniques (filtration) in water treatment.

UV light damages microbial RNA or DNA at a wavelength of 260 nm. This causes thymine dimerization, which blocks the replication of nucleic acid and effectively inactivates microbes. In the case of viruses, UV light attacks the genome, followed by a virus protein coat. Figure 6.3 shows the UV treatment of drinking water. A minimum dose (16,000 μW s/cm^2) is recommended for drinking water. This leads to a 99.9% reduction of bacterial coliforms (DeMers and Renner 1992). The factors that affect the performance are biological films, reactor geometry, short circuiting, microorganism clumping, and turbidity (Sawyer 1992). The continuous exposure to UV radiation does not change the water chemistry. As a result, there is no formation of THM or other disinfection by-products.

Fig. 6.3 UV treatment for drinking water (https://www.gigahertz-optik.de/en-us/basics-light-measurement/apps/uv-disinfection-lamp-control/)

3.2 Solar Disinfection

The use of sunlight is an ancient practice for many centuries. In developing countries, it is considered as a low-cost, simple technique for safe drinking water. The water is to be filled in a transparent glass or plastic bottle and is exposed to sunlight for a few hours. It is reported that many microbes are eliminated by solar disinfection. There are two pathways in solar disinfection to inactivate microbes. Figure 6.4 shows the direct and indirect effects of sunlight exposure.

The efficacy of this process depends on several factors. The factors that affect the performance are organic compounds (humic acids), inorganic salt, dissolved oxygen, light intensity, temperature, and type of container (Davies and Evison 1991). In literature, *Entamoeba histolytica* and cysts of *Giardia* spp. are inactivated by sunlight exposure (e.g., within 10 min at 56 °C) (Ciochetti and Metcalf 1984). The temperature is achieved with a solar hot box cooker. Thus, solar disinfection is not applicable all over the world, but it may be appropriate for sunny places in which

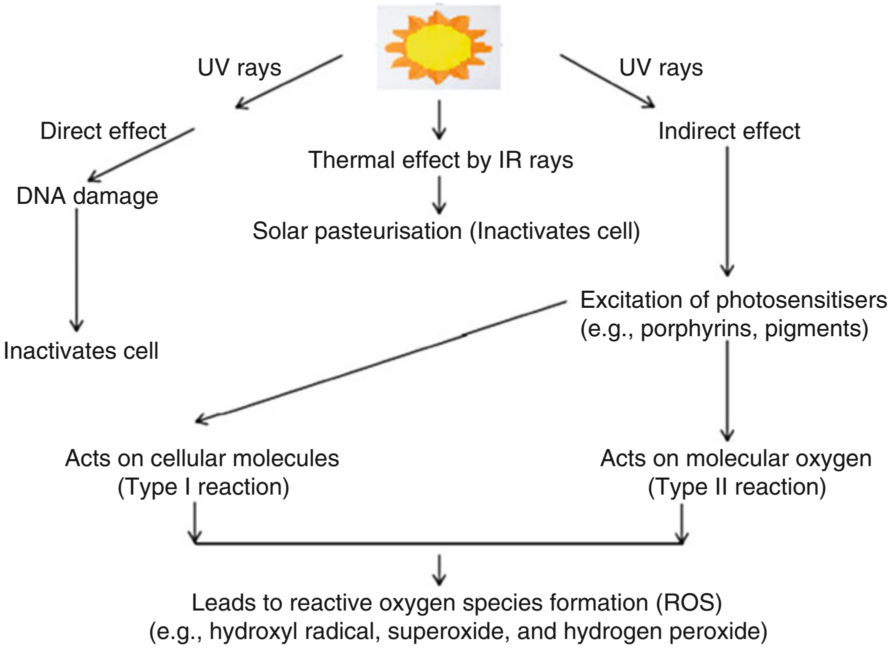


Fig. 6.4 Water disinfection by solar radiation

there is no realistic alternative treatment process. Boiling is another way of inactivating microbes. To achieve this, water is to be maintained in a boiling state for 5–10 min.

3.3 Filtration Methods

3.3.1 Membrane-Based Techniques

Membrane-based techniques are used to remove suspended solids, microbes, and ions from water. The principle behind these filters is physical separation. The separation is dependent on the size of the pores in the membrane. Substances that are larger than the size of the pores are completely removed, whereas the smaller size substances are partially removed, depending on the structure of a refuse layer. Figure 6.5 shows membrane-based techniques for water purification. Microfiltration (01.–2 bar) and ultrafiltration (2–10 bar) filters are low pressure-dependent processes that can remove microbes and suspended solids. The other substances (monovalent species and small colloids) in water can be effectively removed by nanofiltration (8–10 bar) and reverse osmosis (10–80 bar).

Osmosis is the flow of small size organic molecules through a semipermeable membrane from one side to another due to the difference in concentration across the

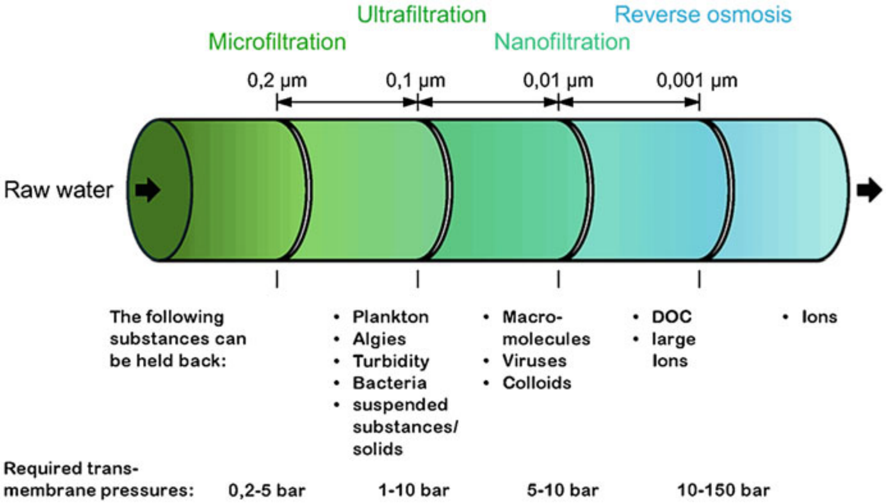


Fig. 6.5 Membrane-based water treatment (https://hbsciu.com/2015/10/12/modifying-water-purification-membranes-with-nanomaterials/)

membrane. At equilibrium, the concentration is balanced on both sides by the osmotic pressure. If the applied pressure is greater than the osmotic pressure, the solvent will flow to the side of the solution through the membrane (Fig. 6.6). This phenomenon is called reverse osmosis (RO) (Kim et al. 2005). Nanofiltration is effective for the removal of Mg^{2+} and Ca^{2+} ions and small size organic compounds. This method could be used to remove disinfection by-products (trihalomethanes) formed during the process of chlorination. RO is known for desalting brackish water and seawater. This process can be used to remove low-molecular-weight organic compounds from water.

The main issues in membrane-based technique are fouling and scaling issues. Fouling issues are due to organic or inorganic colloids and biological contaminants, while scaling is due to the presence of high levels of salt (calcium sulfate fluoride or carbonate or magnesium hydroxide) (Cheryan 1998; Baker 2004).

3.3.2 Sand Filtration

Slow sand filtration (SSF) is a simple process that allows water to pass through the bed of sand, and it can remove solids, microbes, and heavy metals in water. SSF is filled with a combination of coarse and fine sand as a filter medium, as shown in Fig. 6.7. To support this medium, pebbles and gravels are used. It consists of a layer of sand with a height of 60–120 cm and a gravel layer of 30–50 cm. The combination of coarse and fine sand helps in the removal of organic matter in water. Moreover, the flow rate is low in the case of SSF and maturation period (up to 40 days). The water is fed into the column from the top, and treated water is to be collected at the

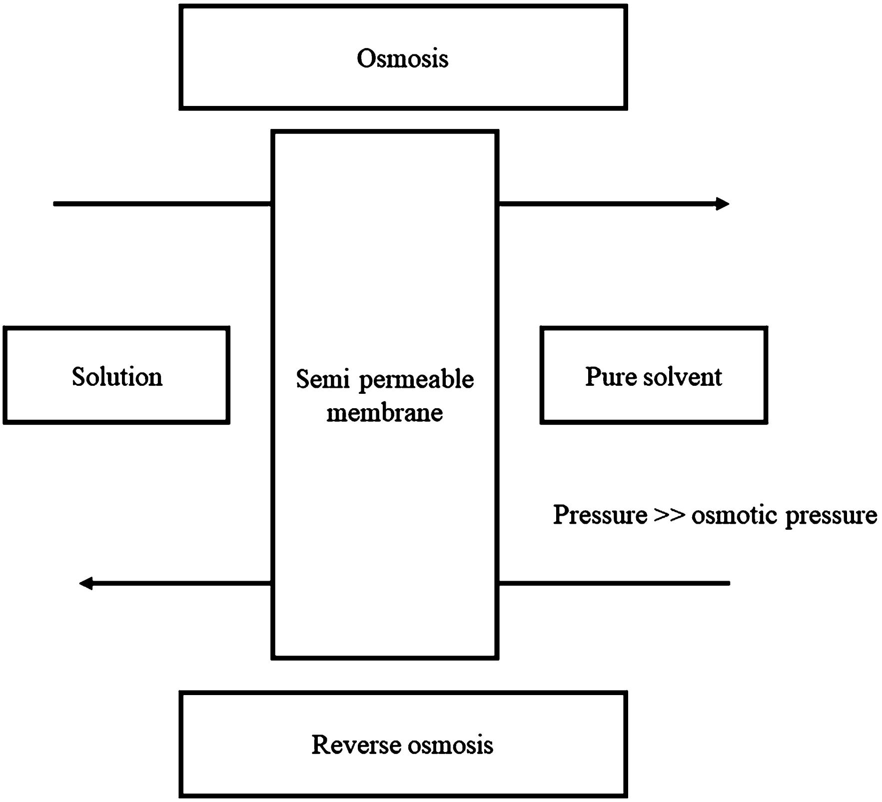


Fig. 6.6 Water disinfection by reverse osmosis

bottom of SSF. In the SSF process, biological filtration takes place in parallel with the physical filtration. Further, bacterial colonies form a slimy layer (schmutzdecke) during the operation of SSF. This layer hosts bacteria, protozoans, diatoms, and metazoan. The biological activity is enhanced by the accumulation of inorganic and organic debris on the surface of the layer. It helps in the removal of NOM, transforms synthetic organic compounds, retains microbes, and purifies water without any biological contaminants. The formation of this biolayer (schmutzdecke) increases head loss, thereby increasing SSF performance during the start-up phase. The quality of water remains poor in the initial period of operation, and it can be improved after the ripening process. The main operational problem in SSF is clogging. It is caused by the suspended solids and biofilm formation on the surface of media. During the process of clogging, head loss increases in the filter, and beyond a point the filter is aborted. To overcome this issue, the removal of the top layer of the sand particle is the most preferred method. In remote areas, SSF performance can be improved by reducing the loading rate, pretreatment of water, and increasing the frequency of dosage. However, SSF demands large areas per unit volume of water to be treated,

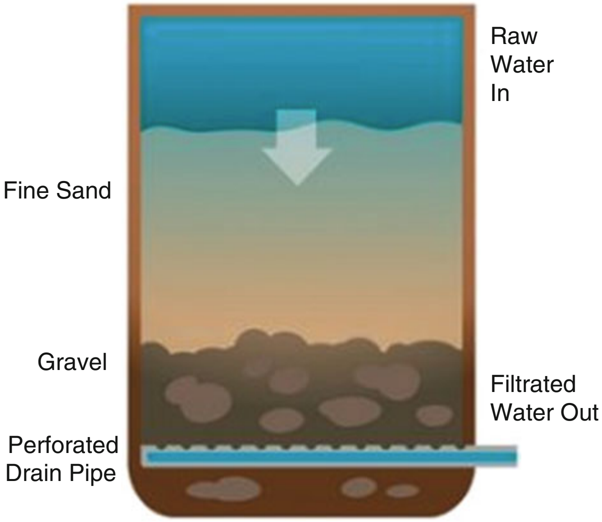


Fig. 6.7 Slow sand filter (https://www.fertinnowa.com/technologies/slow-sand-filtration-removal-algae-diseases-horticulture/)

which in turn suits for the rural community. SSF is known for the removal of turbidity, waterborne pathogens, and suspended solids. The factors that affect the performance of the filter are temperature, sand size, filter depth, and flow rate. Particularly, some of the microbes are small enough to pass through filters as individual particles. Further, the turbidity in water can affect the removal efficiencies of microbes due to the presence of particulate matter, which can block the filters and results in bypasses. Therefore, the combination of pretreatment, sand filtration, and chemical disinfection methods are possible ways to reduce microbial contaminants in drinking water (Alvarez et al. 2008; Hoslett et al. 2018).

3.3.3 Rapid Sand Filtration

RSF is an important physical process in drinking water treatment plants that involves the removal of large suspended solids. It requires prior and post-treatment stages. Typically, RSF is made of a sand bed with a height ranging from 1.5 to 2.5 m, and it is operated in the downward direction at a filtration velocity of 3–8 m/h (Fig. 6.8). The filter medium (usually sand) size ranges from 0.4 to 1.5 mm. These filters perform well when the turbidity of the incoming water should be below 20 NTU. RSF involves solid-liquid separation with an objective to reduce turbidity (0.3–1.0 NTU). Recently, the removal of *Giardia* cysts and *Cryptosporidium* oocysts with a turbidity of 0.1–0.3 NTU. It requires preparatory treatment (coagulation and flocculation) to have a higher throughput of water. The hydraulic loading rate depends on the incoming water quality, temperature, and the media that we used in the filter. Typically, the filter with sand exhibits around 6 $m^3/m^2/h$. It is reported that a clean filter will possess a head loss of around 0.3 m. Once the filter reaches the head loss of

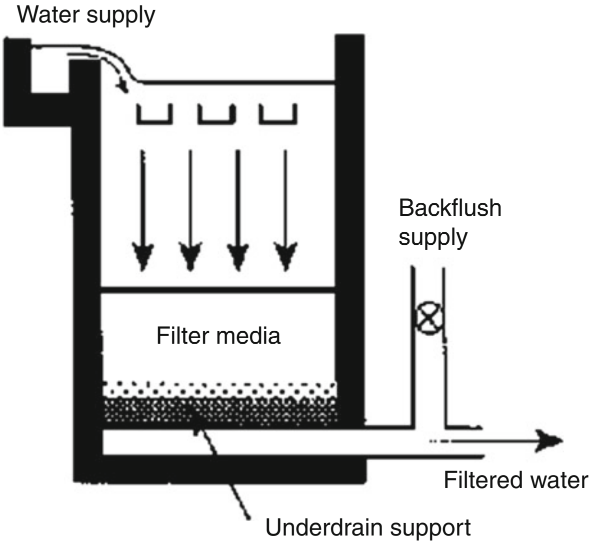


Fig. 6.8 Rapid sand filtration (http://www.ce.memphis.edu/1101/notes/filtration/filtration-1.html)

around 1.5–2 m, the filter needs to be cleaned by backwashing. A critical stage is reached when the frictional resistance increase in the top layer of the sand bed exceeds the static head of water above the bed of sand. The bottom sand layer behaves like a vacuum, and water just passed through the media rather than getting filtered through them. As a result, the developed negative pressure tends to release the gases present in water. The gas bubbles prefers to stick with the surface of the sand. This phenomenon is called as air binding. Hence, the filter needs to be cleaned as soon as it reaches the optimum value. Interestingly, mud accumulates on the surface of sand particles. Over a period of time, mud becomes like a ball when there is an inadequate washing. Once mud balls are formed in a filter, it is difficult to clear. The fine sand particle in the top layer of the bed shrinks and causes cracks in the bed. The crack becomes large with the increase in loss of head.

In RSF, backwashing is to be done periodically to avoid clogging. During the backwash, water is fed into the filter in the upward direction at a velocity to expand the filter media. The mechanism of cleaning is based on the hydraulic shear forces on the media. After backwashing, larger particles tend to settle at the bottom of the filter, and fine particles will settle at the top of the filter. As a result, large particle creates void spaces at the bottom, and clogging will start to occur at the top of the filter, which leads to incomplete use of bed that is restricted. The ripening period of RSF is 1–4 days which is very low as compared to SSF. This process requires preparatory treatment (coagulation and flocculation) to have a higher throughput of water (Han et al. 2009; Management SS and W 2012).

3.3.4 Granular Activated Carbon

Activated carbon used in water purification is produced from different materials such as coconut shells, wood, and coal. Particularly, it has high potential in water treatment due to its characteristics such as porous texture, high surface area functional groups, etc. Granular activated carbon (GAC) is used in both filtration and post-filtration methods to capture organic and odor compound. Generally, GAC is replacing RSF in most of the places, thereby reducing the necessity of further filtration. The filters that are based on GAC can operate at a high flow rate than SSF. Therefore, it is predominantly used in water treatment plants where the space is limited. Furthermore, the life cycle of GAC depends on how long the bed gets saturated with the targeted pollutants. As far as the biological activity is concerned, it can generate issues like clogging, dead zones, and detachment of microbes from the surface of GAC.

Nowadays, silver impregnation is being done to the surface of carbon to remove chemicals and inactivate microbes. Further, activated carbon in combination with a chemical disinfectant is also employed to remove both chemical and biological contaminants. To obtain maximum efficiency, it is desirable to have a maximum specific surface area in the smallest volume. Generally, GAC exhibits a specific surface area ranging from 300 to 1500 m^2/g and an abundant quantity of polar functional groups. As a result of functional groups, microbes are trapped on the carbon surface. It is reported that GAC removes microbes (*E.coli*, MS2, and spores of *Cryptosporidium parvum* and *Giardia lamblia*) by the process of adsorption. The centralized system involves various treatment stages such as sedimentation, coagulation/flocculation, filtration, and disinfection. On the other hand, the decentralized system consists of MF/UF and GAC filters.

4 Future Perspectives on Water Disinfection

The quest for safe water has been the main priority across the globe. The necessity increases for humans due to population growth, natural resources depletion, and ill-managed treatment methods for water, especially the lack of disinfection and treatment methods, specifically in small villages. We need to create awareness about the existing systems and motivate people to have clean and safe drinking water. This chapter describes a variety of disinfection techniques for safe water, such as chemical and physical methods, including traditional systems such as chlorination and sand filtration. Each technique has merits and demerits for having safe water. Therefore, the hybrid process can be accepted as a solution to have safe water. The factors that decide the economics of the process are the physical, chemical, and biological quality of water and environmental factors like pH, temperature, etc.

References

Akshay P, Shreekanth S, Rajesh R, Prasad G (2020) Portable and efficient graphene-oxide based multistage filtration unit for water purification. Mater Today Proc:2344–2350. https://doi.org/10.1016/j.matpr.2020.02.504

Al-Abri M, Al-Ghafri B, Bora T et al (2019) Chlorination disadvantages and alternative routes for biofouling control in reverse osmosis desalination. npj Clean Water:2. https://doi.org/10.1038/s41545-018-0024-8

Alvarez C, Bland L, Kim YG, Mitchell TJ, Ruiz J, Soberanis L, Preston Y (2008) Sustainable community development – water slow-sand filtration. John Brown University. https://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.highlight/abstract/8625/report/

Audette RJ, Quail JW, Smith PJ (1971) Ferrate (VI) ion, a novel oxidizing agent. Tetrahedron Lett 12:279–282

Baker RW (2004) Membrane technology and application. Wiley, New York

Bitton G (2014) Microbiology of drinking water: production and distribution, 1st edn. John Wiley & Sons, Inc, Hoboken

Cheryan M (1998) Ultrafiltration and microfiltration handbook. Technomic Pub. Co. Inc., Lancaster

Ciochetti DA, Metcalf RH (1984) Pasteurization of naturally contaminated water with solar energy. Appl Environ Microbiol 47:223–228. https://doi.org/10.1128/aem.47.2.223-228.1984

Davies CM, Evison LM (1991) Sunlight and the survival of enteric bacteria in natural waters. J Appl Bacteriol 70:265–274. https://doi.org/10.1111/j.1365-2672.1991.tb02935.x

DeMers LD, Renner RC (1992) Alternative disinfection technologies for small drinking water systems. AWWA and AWWARF, Denver

Hall T, Hyde RA (1992) Water treatment processes and practice. WRc, Swindon

Han S, Fitzpatrick CSB, Wetherill A (2009) The impact of flow surges on rapid gravity filtration. Water Res 43:1171–1178. https://doi.org/10.1016/j.watres.2008.12.003

Hoslett J, Massara TM, Malamis S et al (2018) Surface water filtration using granular media and membranes: a review. Sci Total Environ 639:1268–1282. https://doi.org/10.1016/j.scitotenv.2018.05.247

Howard, Backer, Joe H (2000) Use of iodine for water disinfection: iodine toxicity and maximum recommended dose. Environ Health Perspect 108:679–684

Jamil A, Farooq S, Hashmi I (2017) Ozone disinfection efficiency for indicator microorganisms at different pH values and temperatures. Ozone Sci Eng 39:407–416. https://doi.org/10.1080/01919512.2017.1322489

Kim TU, Amy G, Drewes JE (2005) Rejection of trace organic compounds by high-pressure membranes. Water Sci Technol 51:335–344. https://doi.org/10.2166/wst.2005.0654

Klueh U, Wagner V, Kelly S et al (2000) Efficacy of silver-coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation. J Biomed Mater Res 53:621–631. https://doi.org/10.1002/1097-4636(2000)53:6<621::AID-JBM2>3.0.CO;2-Q

Management SS and W (2012) Rapid sand filtration. https://sswm.info/water-nutrient-cycle/water-purification/hardwares/semi-centralised-drinking-water-treatments/rapid-sand-filtration

Matsumoto MR, Jensen JN, Reed BE (1995) Physicochemical processes. Water Environ Res 67:419–432. https://doi.org/10.2175/106143095x133437

Murmann k RRPR (1975) [(OC)sWP(C6Hs)zCH2CH2P(C6H5)2]2PtC12. Inorg Chem 14:203–205

Okello C, Tomasello B, Greggio N et al (2015) Impact of population growth and climate change on the freshwater resources of Lamu Island, Kenya. Water (Switzerland) 7:1264–1290. https://doi.org/10.3390/w7031264

Richardson SD, Plewa MJ, Wagner ED et al (2007) Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. Mutat Res - Rev Mutat Res 636:178–242. https://doi.org/10.1016/j.mrrev.2007.09.001

Ryan JN, Harvey RW, Metge D et al (2002) Field and laboratory investigations of inactivation of viruses (PRD1 and MS2) attached to iron oxide-coated quartz sand. Environ Sci Technol 36:2403–2413. https://doi.org/10.1021/es011285y

Sawyer H (1992) Environmental engineers & scientists. Nostrand, disinfection alternatives for safe drinking water. Van Nostrand Reinhold, New York

Smith D (1967) Disinfection and sterilization of polluted water with ozone. In: Proceedings of 2nd annual symposium on water research, McMasters University. Hamilton, Ontario, p 52

Srinivasan NR, Shankar PA, Bandyopadhyaya R (2013) Plasma treated activated carbon impregnated with silver nanoparticles for improved antibacterial effect in water disinfection. Carbon N Y 57:1–10. https://doi.org/10.1016/j.carbon.2013.01.008

Taylor GR, Butler M (1982) A comparison of the virucidal properties of chlorine, chlorine dioxide, bromine chloride and iodine. J Hyg (Lond) 89:321–328. https://doi.org/10.1017/S0022172400070856

Twort AC, Ratnayaka DD, Brandt MJ (2000) Water supply, 5th edn. Elsevier, London

White G (1992) Handbook of chlorination. Van Nostrand Reinhold, New York

WHO/UNICEF (2017) Progress on drinking water, Sanitation and hygiene: 2017 update and SDG baselines. Geneva

Yahya MT, Gerba CP, Landeen LK et al (1989) Evaluation of potassium permanganate for inactivation of bacteriophage ms-2 in water systems. J Environ Sci Heal Part A Environ Sci Eng 24:979–989. https://doi.org/10.1080/10934528909375530

Zhang H, Zhang Y, Shi Q et al (2012) Study on transformation of natural organic matter in source water during chlorination and its chlorinated products using ultrahigh resolution mass spectrometry state key laboratory of environmental aquatic chemistry, research center for eco-environme. Environ Sci Technol 46:4396–4402

Part II

Cleaner Technologies on Mitigation of Organic and Inorganic Pollutants

Chapter 7
Recent Trends in Application of Bacterial Polymers to Mitigate Organic and Inorganic Pollutants

P. Muthukumaran, J. Aravind, M. Kamaraj, and K. K. Ramachandran

Abstract Bacteria are adapted in harnessing an array of polymers to perform assorted roles, as a stored reserve, protection matrixes, etc., and these have conducive attributes that can be tailored for industrial and environmental applications, via serving as a renewable, biodegradable, and environmentally benign ingredients. Emerging contaminants pose a huge environmental concern, and the conventional methods are proving ineffective, hence the need for alternatives, such as the role of extracellular microbial biopolymers, whose anionic architecture allows the sequestration of positively charged heavy metal ions, which bring about bio-detoxification of these xenobiotics. Microbial polymer also promises as an alternative of conventional plastics. Bacterial polymers can be classified as polysaccharides, polyamides, polyesters, and the inorganic polyanhydrides. Aside the scope of production of commercially viable polymers, enzymatic and chemical path offers the arena to tailor these bacterial polymers. The current work explores the recent trends and advances in microbial polymers, especially its role in environmental applications.

Keywords Oil recovery · Heavy meatal sorption · Dye removal · Wastewater flocculation · Soil remediation

---

P. Muthukumaran
Kumaraguru College of Technology-India, Coimbatore, Tamil Nadu, India

J. Aravind (✉)
Department of Civil Engineering- Environmental Engineering, Dhirajlal Gandhi College of Technology, Omalur, Tamil Nadu, India

M. Kamaraj
Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

K. K. Ramachandran
Dr GRD Institutions-India, Coimbatore, Tamil Nadu, India

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_7

1 Introduction

Globalization has escalated the existing environmental issues, triggering suitable research to mitigate these issues. Biopolymers with their biodegradability, environmentally benign mode of processing, and flexible applications are distinctive replacement to non-sustainable products and harnessed via biorefineries with integrated bioprocesses. Biopolymers are generated by an array of microorganisms; these when tailored make it more biocompatible and non-detrimental on living systems. Considering the biochemical receptivity, microbial biopolymers can be categorized as capsular, repertory, and exopolysaccharides (Schmid and Sieber 2015; Sukan et al. 2015).

Biopolymer from bacteria has created unparalleled acceptance for a range of environmental applications, as it possesses vacillating biological functions with versatile properties; this understanding has paved way for tailored biopolymers with modified properties to serve as a renewable source for an array of environmental applications (Rehm 2010). Microbial polymers are essentially propagated by fermentation or by the polymerization. Synthetic, nonbiodegradable plastic materials could be displaced by these environmentally benign, biodegradable, renewable sources tailored to suit particular needs (Liguori et al. 2016). Microorganisms produce biopolymers by amassing extracellular materials such as exopolysaccharides (EPSs) and are engaged in green chemical synthesis and to handle diverse environmental issues (Pepe et al. 2013). These exopolysaccharides are composed of carbs as well as non-carbs moieties (pyruvate, acetate, succinate, and phosphate) (Llamas et al. 2012).

Microbial polymers have an extensive range of applications and can likely to replace synthetic materials and can be harnessed from renewable source under flexible conditions. This reduces costs related to their biosynthesis (Huang et al. 2018) and can mitigate waste management, as many agricultural residues can be used as a substrate to cultivate the microbes capable of producing these polymers of specific choice and interest. Having such desirable functionalities and properties, biopolymers find many applications in various sectors mainly in the environmental domain to mitigate environmental issues (Kumar et al. 2020; Tang et al. 2012). This chapter discusses such microbial biopolymers for assorted environmental applications (Fig. 7.1 and 7.2).

2 Environmental Application of Microbial Biopolymers

2.1 Oil Recovery

A wide variety of microbial polymers are used in recovery of petroleum hydrocarbons from contaminated site. The basic mechanism is these biopolymers expose hydroxyl group, which directly makes it to dipole, ion-dipole, hydrogen bonds with

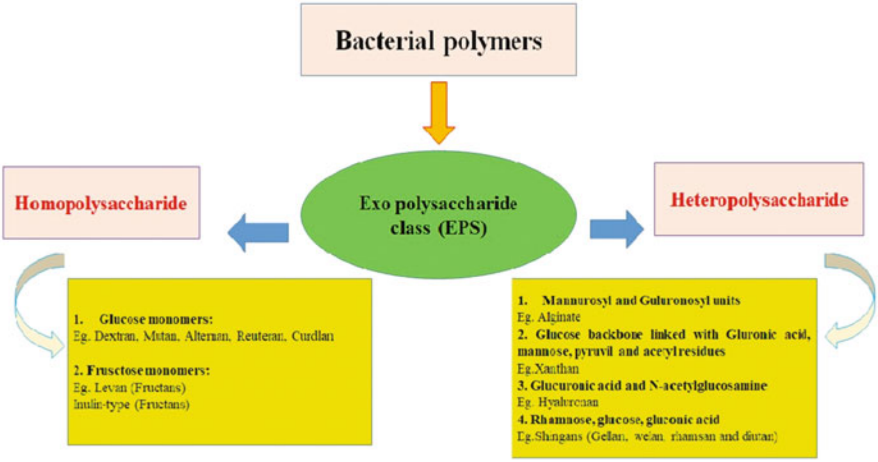


Fig. 7.1 Bacterial EPS classification with few examples

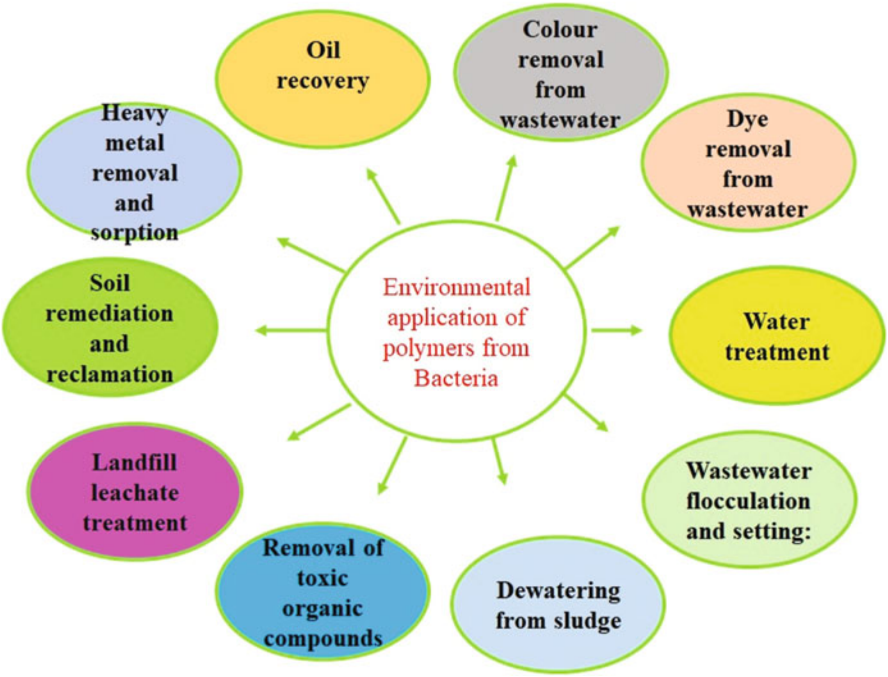


Fig. 7.2 Environmental applications of polymers from bacteria

polymer structure as well as other substances (Xu et al. 2013). Several scientific studies showed that a variety of biopolymers from bacterial origin bolstered microbial enhanced oil recovery (MEOR) as identified and studied. Few notable examples are xanthan gum (*Xanthomonas* sp.), glucan (*Lactobacillus suebicus, Pediococcus parvulus*), levan (*Bacillus* sp.), pullulan (*Aureobasidium* sp.), dextran (*Leuconostoc* sp.), and welan gums (*Alcaligenes* sp.) (Becker et al. 1998; Kim and Fogler 1999; Singh et al. 2009; Liu et al. 2010; Xu et al. 2013).

2.2 Heavy Metal Removal and Sorption

When we think about usage of biopolymers from microorganism, we should understand the mechanism of interaction of extracellular polysaccharide with any external pollutants, particularly heavy metals. Microbial cells and metal ion interaction mechanisms are classified based on involvement of metabolism (active and passive uptake of metal ions). These kinds of metabolism always depend upon the interaction of heavy metals (Alluri et al. 2006). Similarly, other reports showed that intact microbial cells and cell-bound EPS showed potential applications for metal remediation, particularly in industrial and environmental wastewater remediation (Lakzian 2008). Microbial polymers, possess enough number of active and ionizable functional chains, particularly with noncarbohydrate substituents such as acetamido assembly of chitins, anatomical polysaccharides of fungi, amine, sulfhydryl and carboxyl assembly in proteins, phosphodiester (teichoic acid), phosphate, hydroxyl links in polysaccharide imparts overall negative charge to the polymer (Wang et al. 2010). Several studies reported that cellulose beads show significant efficiency removal of heavy metals compared to cellulose alone. Arsenate and Cu (II) show 83.56 mgg^{-1} and 33.2 mgg^{-1}, respectively (Barakat and Schmidt 2010; Guo and Chen 2005) (Fig. 7.3).

Similarly, variety of metal ions present in medium and that are bound to bacterial exopolysaccharides, owing to the synergy between EPS and divalent cations, particularly Ca^{2+} and Mg^{2+}. These divalent cations scope the pivotal role in preserving the microbial assemble during metal ion removal study (Mayer et al. 1999) (Table 7.1 and 7.2).

2.3 Dye Removal from Wastewater

Dye is one of the most dominant pollutants in both terrestrial and aquatic ecosystem. Still now, several conventional and advance techniques are practiced to remove dye from contaminated site. In general, methods applied for treatment/removal of pollutants are dependent on the contaminants and pollutants, such as organic pollutants, heavy metals, dyes, etc. (Arfin et al. 2019). For dye removal studies, microbial polymers as adsorbents are most widely used. In such polymers, cellulose is the most

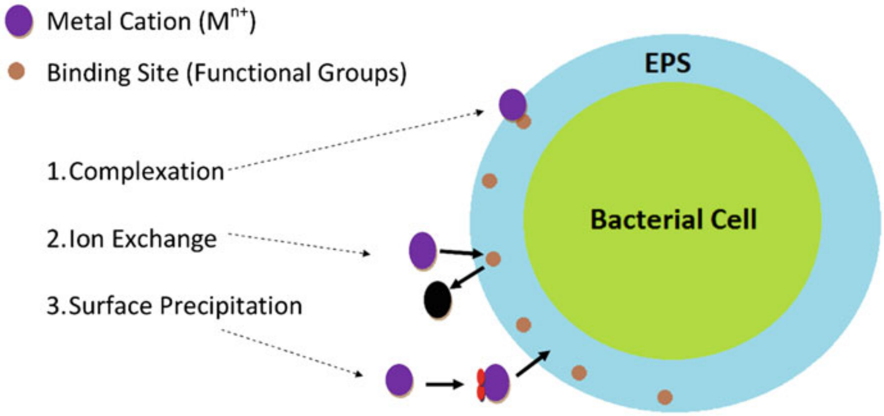


Fig. 7.3 Schematic diagram of mechanism of metal-EPS interactions

Table 7.1 Functionality of natural polymers and its properties

Sl. No.	Natural polymers	Functional group involved	Properties
1	Chitosan	Amino groups, hydroxyl groups	Biocompatible, galvanized tissues, antitumor
2	Cellulose	Hydroxyl groups	Biodegradable, abundant
3	Alginate	Carboxylate side groups	Degradable, biocompatible
4	Peptide	Carbonyl groups	Oxidative, brief half-life, least toxic
5	Starch	Ether groups, hydroxyl groups	Biodegradable, economical, versatile
6	Chitin	Amino functionality, hydroxyl functionalities	Biodegradation, biocompatibility
7	Collagen	Hydrophilic and/or hydrophobic amino acids	Biocompatible, hydrophilicity, permeability
8	β-cyclodextrin	Ether groups, hydroxyl groups	Not toxic, biodegradable, biocompatible, hydrophobic interior, and hydrophilic exterior

abundantly used. Nowadays, several reports particularly on modified polymers, such as cellulose nanofibrils and nanocrystals, showed 212.7 mgg^{-1} of malachite green (Jiang et al. 2017) and 106 mgg^{-1} of methylene blue dye adsorption capacity (Nekouei et al. 2017).

2.4 Raw and Drinking Water Treatment

One of the main and important application of exopolysaccharides (EPSs) is producing bacterial isolates in wastewater treatment. This treatment process may

Table 7.2 Bacterial polymers and their metal ion removal efficiency

Sl. No	Heavy metals	Bacterial isolates	Type of polymers	Metal removal efficiency	References
1	Copper	*Paenibacillus polymyxa*	Homogeneous Consortial EPS	1602 mg $Cu^{2+}g^{-1}$ EPS	Acosta et al. (2005)
2	Lead	*Lactobacillus plantarum*	Homogeneous Consortial EPS	276.44 mg $Pb^{2+}g^{-1}$ EPS, at 1000 ppm initial metal load	Feng et al. (2012)
3	Arsenic	*Herminiimonas arsenicoxydans*	Homogeneous Consortial EPS	Up to 5 $mmolL^{-1}$ metal ion uptake	Marchal et al. (2010)
4	Chromium	*Ochrobactrum anthropi*	Heterogeneous Consortial EPS	57.8 mg $Cr^{6+}g^{-1}$ EPS at initial metal load of 280 ppm	Ozdemir et al. (2003)
5	Cadmium	*Shewanella oneidensis*	Heterogeneous Consortial EPS	80% Cd^{2+} removal	Ha et al. (2010)
6	Manganese	*Acetobacter sp.*	Phosphorylated bacterial EPS (cellulose)		Oshima et al. (2008)
7	Lead	*Methylobacterium organophilum*	Homogeneous Consortial EPS	18% Pb^{2+} removal from 0.04 ppm initial metal load	Kim et al. (1996)
8	Zinc	*Bacillus firmus*	Homogeneous Consortial EPS	722 mg $Zn^{2+}g^{-1}$ EPS (61.8%)	Salehizadeh and Shojaosadati (2003)
9	Mercury	*Azotobacter chroococcum*	Homogeneous Consortial EPS	47.87% Hg^{2+} (38.9 $mgHgg^{-1}$ EPS)	Rasulov et al. (2013)
10	Nickel	*Chryseomonas luteola*	Alginate bead-immobilized EPS	1.989 mmol $Cu^{2+}g^{-1}$ EPS	Ozdemir et al. (2005)
11	Cobalt	*Acetobacter sp.*	Phosphorylated bacterial EPS (cellulose)	Up to 90% reduction from initial metal load 0.1 $mmoldm^3$	Oshima et al. (2008)

or may not contain cations (Buthelezi et al. 2009; Li et al. 2009; Ma et al. 2008; Nontembiso et al. 2011). Bacterial isolates, which include *Bacillus subtilis*, *Exiguobacterium acetylicum*, *Klebsiella terrigena*, *Staphylococcus aureus*, *Pseudomonas pseudoalcaligenes*, and *Pseudomonas plecoglossicida*, were capable of production of EPS, and EPS from these bacteria at the concentration of 10 mgL^{-1} increases turbidity from 84.1 to 93.6% of river water (Buthelezi et al. 2009).

Similarly, EPS from *Bacillus* sp. was studied for the jar tests to compare the kaolin turbidity removal potential (Ma et al. 2008). This experimental study reveals efficiency of turbidity on bar with other polymers: EPS (86%), $Al_2(SO_4)_3$ (95%), and $Fe_2(SO_4)_3$ (96%). When EPS was mixed with $Fe_2(SO_4)_3$, it showed that there is significant improvement in raw water treatment. Another notable report is there is no residual ferric or aluminum ion accumulation noticed when it was mixed with EPS after treatment of raw water. Li et al. (2009) reported that EPS from *Bacillus* *licheniformis* and mixed with $CaCl_2$ showed significant improvement in drinking water treatment. At variable temperature, from 4 to 25 °C, the effectiveness of EPS on drinking water treatment was also reported. The drinking water COD and turbidity removal from the water were noticed as 61.2% and 95.6%, respectively. Based on the experimental study by Li et al. (2009), the EPSs synthesized by *Bacillus licheniformis* were safe, and it is recommended for drinking water treatment. It can remove both gram-positive (*S. aureus* and *Streptococcus faecalis*) and gram-negative (*Escherichia coli* and *Klebsiella oxytoca*) bacteria from river water up to 98.3%. In substitution of alum study, Buthelezi et al. (2009) reported that bacterial EPS is a potential alternative to alum and it acts as the best organic coagulant in the river water treatment.

2.5 Wastewater Flocculation and Setting

Flocculation and setting is the main unit operation process still practiced in wastewater treatment process. Still, both conventional and advanced oxidation processes are also implemented to speed up flocculation and settling process. One of the emerging areas is usage of microbes and/or microbial products as flocculants in wastewater treatments. Exopolysaccharides from *Serratia ficaria* were showed to be potentially used in wastewater treatments and industries such as brewery, soy sauce brewing, meat processing, pulp and paper, etc. (Gong et al. 2008). For example, EPS is used to treat swine wastewater with turbidity (91%) and COD (42%) of removal efficiency, and these percentages of removal are better than the polyaluminum chloride (Zhang et al. 2012). Lian et al. (2008) reported that the EPS from *Bacillus mucilaginosus* is most commonly used to treat municipal, brewage, and pharmaceutical wastewater. Consortium and their effectiveness are also studied to check their potential removal efficiency. The EPS from *Staphylococcus* sp. and *Pseudomonas* sp. applied in treatment of indigotin printing and dyeing wastewater, and it was reported that COD removal percentage was around 80 (Zhang et al. 2007). Similarly, polysaccharide-based EPS from *Paenibacillus elgii* (B69) showed the wastewater COD (68%) and turbidity (83%) removal potential (Li et al. 2013). Based on literature survey, majority of the reports indicated that the application of EPS in wastewater treatment demonstrated at laboratory scale.

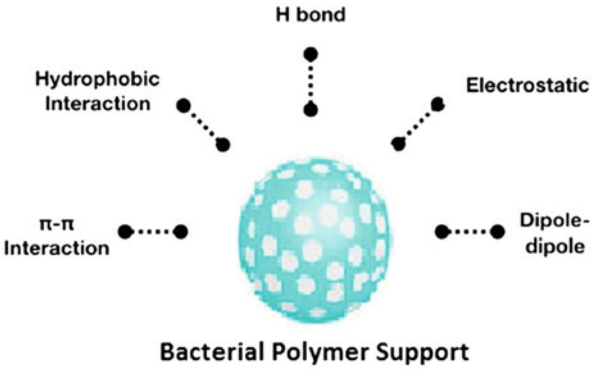
2.6 Dewatering from Sludge

In the area of sludge dewatering process, particularly in mechanical sludge dewatering process, huge quantities of chemically derived polymers are mostly used. In wastewater treatment process, it will become highly expensive (More et al. 2010). When we are using exopolysaccharides (EPS) in sludge dewatering process, the concentration, type, and characteristics of EPS are very important factors, which directly affect the dewatering efficacy in sludge (Houghton et al. 2001; Houghton and Stephenson 2002; More et al. 2010). Similarly, these EPSs are used as bioflocculant materials in dewatering of sludge. One of the studies reveals that EPS obtained from the bacteria *Klebsiella* sp. showed sludge dewatering efficiency similar to chemical flocculant such as alum at particular concentrations (Yang et al. 2012). Similarly, Zhang et al. (2010) reported that at 0.17% (w/w) concentration of *P. mirabilis* with the combination of 1.3% (w/w), $CaCl_2$ will increase the sludge dewatering efficacy. These combination and conditions worked out well at neutral pH (7–7.5). As of now, very few scientific reports are available, and it needs to explore detailed investigations on EPS in sludge dewatering process in the near future.

2.7 Removal of Toxic Organic Compounds

Several studies and scientific reports show the role of exopolysaccharides (EPS) in removal of various organic pollutants from wastewater, sludge, and contaminated site. In particular, degradation of polycyclic aromatic hydrocarbons (PAHs), bioremediation by using microorganisms (Zhang et al. 2011). Several EPS-producing bacterial species were applied to remove PAHs from contaminated soils (Jia et al. 2011; Zhang et al. 2011). When we applied EPS to the contaminated site, the interaction of EPS and PAHs, mostly exothermic hydrophobic interactions, take place. In situ application of PAHs, degrading bacteria species with EPS-producing potential will increase their degradation efficacy of PAHs (Zhang et al. 2011). Similarly, Liu et al. (2001) studied EPS-producing bacteria species that, increase the release of soil-bound phenanthrene. Jia et al. (2011) reported that *Zoogloea* sp. and *Aspergillus niger* showed more than 30% of pyrene degradation potential after 35 days, the rate of pyrene degradation increased with increase in EPS concentration as well as increased specific surface area of the EPS (Fig. 7.4).

Fig. 7.4 Interaction mechanisms for the processes of elimination of organic pollutants



2.8 Landfill Leachate Treatment

In the era of landfill leachate treatment, bacterial polymers play a vital role in the removal of humic acids from landfill. *Rhizomonas* sp. was used to extract exopolysaccharides (EPS) instead of alum for the removal of humic acids in synthetic solutions. Apart from these EPS showed significant reduction of chemical oxygen demand (COD) up to 45% during landfill leachate treatment (Zouboulis et al. 2004). Similarly, other reports showed that optimum EPS dosage of per liter was 20 mg and pH range from 7−7.5 gives better result and it will go up to 85% of humic acid removal by EPS-producing bacterial species. Newly established (<5-year-old) landfill's leachate are abundant source of organic matter; these can be harnessed (as raw materials) by bacteria for EPS generation (Fusconi et al. 2006; Zouboulis et al. 2004).

2.9 Soil Remediation and Reclamation

Since the past two decades, several studies were reported particularly in the remediation of xenobiotic by the development of biofilm reactors (Singh et al. 2006). The formation of biofilms take place by the action of microbial communities and exopolysaccharides from microbes. This kind of microbial biofilms has shown higher rate of degradation of recalcitrant compounds as well as these microbial biofilms have shown the immobilization of these recalcitrant compounds via EPS matrix (Singh et al. 2006). Similarly, the EPS matrix is used to reduce migration of solids in soil during runoff water; it reduces heavy metal transport, and particularly it stabilizes soil structure and texture and reduces formation of dust during soil pollution process (Gerbersdorf et al. 2008).

EPS are obtained from different single pure and/or mixed cultures, and these exopolysaccharides are obtained via aerobic fermentation process. After production, EPS are directly separated from the production media and extracted as non-cross-linking material. These nonreactive (non-cross-linking) EPS, directly applied to the soil, (mix the soil with dry EPS), and, water added to form a gel within the soil matrix, (soil molecules bind with EPS and form a soil-EPS intermediates). In particular, bacterial EPS directly acts as a cohesive force in the formation of surface erosion resistance in soil and their sediments (Droppo 2009). For example, cyanobacteria are previously used and applied for promoting this kind of soil adhesion studies in arid regions (Prasanna et al. 2008). Soil modification using these EPS-rich natural products gives pathway to understand the concept of bio-geo-civil engineering (Ivanov and Chu 2008).

2.10 Challenges and Prospects

Bacterial polymers have been deeply scrutinized for the sake of substituting the current available chemical technologies with environmental remediation strategy, and exploring its literature shows much promise due to its flexible and attractive attributes (Fig. 7.5). Prudent governing of experimental conditions can support enhanced production and recovery of these microbial polymers for suitable environmental application. On the context of reusability and its specificity as an adsorbent; suitable tailoring and/or immobilization can enhance the performance of a specific polymer in use. An assortment of published studies has established various avenues and conducive agents for immobilization; yet, there is still a vacuum on other uncharted agents to be engaged for immobilization as well as for tailoring hybrid composites of these microbial polymers. The major impediment is the fabrication of the technology to push these research works to commercial scale for real-time applications; the next credibility hinges on the endeavor for optimization and formulation of a continuous operational mode, culminating in the advancement of an exceedingly sustainable, economic, and environmentally recuperative technology for the mitigation of environmental pollution. However, owing to the inherent limits of microorganism's ability to generate higher yields of multiple products also proves as another impediment. Therefore, multiple product processes with tailor-made approach tuned to specific metabolic engineering combined with genetic engineering strategies can enhance production potential. Overall, limited investigation has been established (when compared to macro-sourced biopolymer) in the application of microbial polymer for specific environmental application, but with dedicated research and proper investment, multiple microbial polymer production, via fermentation, can be the future bioprocessing approach.

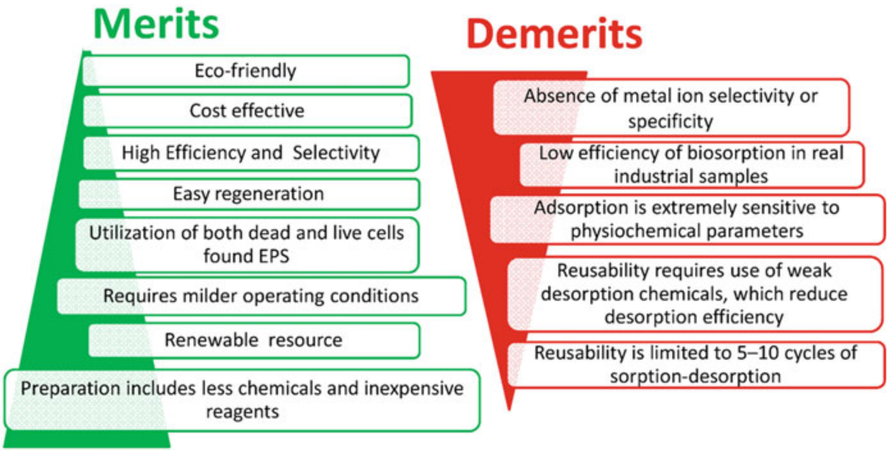


Fig. 7.5 Major merits and demerits of microbial polymers

References

Acosta MP, Valdman E, Leite S, Battaglini F, Ruzal S (2005) Biosorption of copper by *Paenibacillus polymyxa* cells and their exopolysaccharide. World J Microbiol Biotechnol 21:1157–1163

Alluri HK, Ronda SR, Settalluri VS, Bondili JS, Suryanarayana V, Venkateshwar P (2006) Biosorption: an eco-friendly alternative for heavy metal removal. Afr J Biotechnol 6 (25):2924–2931

Arfin T, Sonawane K, Saidankar P, Sharma S (2019) Ch. 14: role of microbes in the bioremediation of toxic dyes. In: Shahid-ul-Islam (ed) Integrating green chemistry and sustainable engineering. Scrivener Publishing, Beverly, pp 443–472

Barakat M, Schmidt E (2010) Polymer-enhanced ultrafiltration process for heavy metals removal from industrial wastewater. Desalination 256:90–93

Becker A, Katzen F, Puhler A, Ielpi L (1998) Xanthan gum biosynthesis and application: a biochemical/genetic perspective. Appl Microbiol Biotechnol 50(2):145–152

Buthelezi SP, Olaniran AO, Pillay B (2009) Turbidity and microbial load removal from river water using bioflocculants from indigenous bacteria isolated from wastewater in South Africa. Afr J Biotechnol 8(14):3261–3266

Droppo IG (2009) Biofilm structure and bed stability of five contrasting freshwater sediments. Mar Freshw Res 60(7):690–699

Feng M, Chen X, Li C, Nurgul R, Dong M (2012) Isolation and identification of an exopolysaccharide-producing lactic acid bacterium strain from Chinese paocai and biosorption of Pb (II) by its exopolysaccharide. J Food Sci 77:T111–T117

Fusconi R, Godinho MJL, Hernández ILC, Bossolan NRS (2006) *Gordonia polyisoprenivorans* from groundwater contaminated with landfill leachate in a subtropical area: characterization of the isolate and exopolysaccharide production. Braz J Microbiol 37(2):168–174

Gerbersdorf SU, Jancke T, Westrich B, Paterson DM (2008) Microbial stabilization of riverine sediments by extracellular polymeric substances. Geobiology 6(1):57–69

Gong WX, Wang SG, Sun XF, Liu XW, Yue QY, Gao BY (2008) Bioflocculant production by culture of *Serratia ficaria* and its application in wastewater treatment. Bioresour Technol 99 (11):4668–4674

Guo X, Chen F (2005) Removal of arsenic by bead cellulose loaded with iron oxyhydroxide from groundwater. Environ Sci Technol 39:6808–6818

Ha J, Gélabert A, Spormann AM, Brown GE (2010) Role of extracellular polymeric substances in metal ion complexation on *Shewanella oneidensis*: batch uptake thermodynamic modeling, ATR-FTIR, and EXAFS study. Geochim Cosmochim Acta 74:1–15

Houghton JI, Stephenson T (2002) Effect of influent organic content on digested sludge extracellular polymer content and dewaterability. Water Res 36(14):3620–3628

Houghton JI, Quarmby J, Stephenson T (2001) Municipal wastewater sludge dewaterability and the presence of microbial extracellular polymer. Water Sci Technol 44(2-3):373–379

Huang L, Chen Z, Wen Q, Zhao L, Lee DJ, Yang L, Wang Y (2018) Insights into feast-famine polyhydroxyalkanoate (pha)-producer selection: microbial community succession, relationships with system function and underlying driving forces. Water Res 131:167–176

Ivanov V, Chu J (2008) Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. Rev Environ Sci Biotechnol 7(2):139–153

Jia C, Li P, Li X, Tai P, Liu W, Gong Z (2011) Degradation of pyrene in soils by extracellular polymeric substances (EPS) extracted from liquid cultures. Process Biochem 46(8):1627–1631

Jiang F, Dinh DM, Hseih Y (2017) Adsorption and desorption of cationic malachite green dye on cellulose nanofibril aerogels. Carbohydr Polym 173:286–294

Kim DS, Fogler HS (1999) The effects of exopolymers on cell morphology and culturability of *Leuconostoc mesenteroides* during starvation. Appl Microbiol Biotechnol 52(6):839–844

Kim S, Kim J, Kim C, Oh D (1996) Metal adsorption of the polysaccharide produced from *Methylobacterium organophilum*. Biotechnol Lett 18:1161–1164

Kumar A, Sharma G, Naushad M, Al- Muhtaseb AH, Garcia-Penas A, Mola GT et al (2020) Bio-inspired and biomaterials-based hybrid photocatalysts for environmental detoxification: a review. Chem Eng J 382:122937

Lakzian A (2008) Adsorption capability of lead: nickel and zinc by exopolysaccharide and dried cell of *Ensifer meliloti*. Asian J Chem 20:6075–6080

Li Z, Zhong S, Lei HY, Chen RW, Yu Q, Li HL (2009) Production of a novel bioflocculant by *Bacillus licheniformis* X14 and its application to low temperature drinking water treatment. Bioresour Technol 100(14):3650–3656

Li O, Lu C, Liu A, Zhu L, Wang PM, Qian CD et al (2013) Optimization and characterization of polysaccharide-based bioflocculant produced by *Paenibacillus elgii* B69 and its application in wastewater treatment. Bioresour Technol 134:87–93

Lian B, Chen Y, Zhao J, Teng HH, Zhu L, Yuan S (2008) Microbial flocculation by *Bacillus mucilaginosus*: applications and mechanisms. Bioresour Technol 99(11):4825–4831

Liguori R, Ventorino V, Pepe O, Faraco V (2016) Bioreactors for lignocelluloses conversion into fermentable sugars for production of high added value products. Appl Microbiol Biotechnol 100:597–611

Liu A, Ahn IS, Mansfield C, Lion LW, Shuler ML, Ghiorse WC (2001) Phenanthrene desorption from soil in the presence of bacterial extracellular polymer: observations and model predictions of dynamic behavior. Water Res 35(3):835–843

Liu C, Lu J, Lu L, Liu Y, Wang F, Xiao M (2010) Isolation, structural characterization and immunological activity of an exopolysaccharide produced by Bacillus licheniformis 8-37-0-1. Bioresour Technol 101(14):5528–5533

Llamas I, Amjres H, Mata JA, Quesada E, Bejar V (2012) The potential biotechnological applications of the exopolysaccharide produced by the halophilic bacterium *Halomonas almeriensis*. Molecules 17:7103–7120

Ma F, Zheng L, Chi Y (2008) Applications of biological flocculants (BFs) for coagulation treatment in water purification: turbidity elimination. Chem Biochem Eng Q 22(3):321–326

Marchal M, Briandet R, Koechler S, Kammerer B, Bertin PN (2010) Effect of arsenite on swimming motility delays surface colonization in *Herminiimonas arsenicoxydans*. Microbiology 156(2010):2336–2342

Mayer C, Moritz R, Kirschner C, Borchard W, Maibaum R, Wingender J, Flemming H-C (1999) The role of intermolecular interactions: studies on model systems for bacterial biofilms. Int J Biol Macromol 26:3–16

More TT, Yan S, Tyagi RD, Surampalli RY (2010) Potential use of filamentous fungi for wastewater sludge treatment. Bioresour Technol 101(20):7691–7700

Nekouei F, Nekouei S, Keshtpour F, Noorizadeh H, Wang S (2017) Cr(OH)3-NPs-CNC hybrid nanocomposite: a sorbent for adsorptive removal of methylene blue and malachite green from solutions. Environ Sci Pollut Res 24:25291–252308

Nontembiso P, Sekelwa C, Leonard MV, Anthony OI (2011) Assessment of bioflocculant production by *Bacillus* sp. Gilbert, a marine bacterium isolated from the bottom sediment of Algoa Bay. Mar Drugs 9(7):1232–1242

Oshima TK, Kondo K, Ohto K, Inoue K, Baba Y (2008) Preparation of phosphorylated bacterial cellulose as an adsorbent for metal ions. React Funct Polym 68:376–383

Ozdemir G, Ozturk T, Ceyhan N, Isler R, Cosar T (2003) Heavy metal biosorption by biomass of *Ochrobactrum anthropi* producing exopolysaccharide in activated sludge. Bioresour Technol 90:71–74

Ozdemir G, Ceyhan N, Manav E (2005) Utilization in alginate beads for Cu (II) and Ni (II) adsorption of an exopolysaccharide produced by *Chryseomonas luteola* TEM05. World J Microbiol Biotechnol 21:163–167

Pepe O, Ventorino V, Cavella S, Fagnano M, Brugno R (2013) Prebiotic content of bread prepared with flour from immature wheat grain and selected dextran-producing lactic acid bacteria. Appl Environ Microbiol 79:3779–3785

Prasanna R, Jaiswal P, Kaushik BD (2008) Cyanobacteria as potential options for environmental sustainability – promises and challenges. Indian J Microbiol 48(1):89–94

Rasulov BA, Yili A, Aisa HA (2013) Biosorption of metal ions by exopolysaccharide produced by *Azotobacter chroococcum* XU1. J Environ Prot 4:989–993

Rehm BHA (2010) Bacterial polymers: biosynthesis, modifications and applications. Nat Rev Microbiol 8:578–592

Salehizadeh H, Shojaosadati SA (2003) Removal of metal ions from aqueous solution by polysaccharide produced from *Bacillus firmus*. Water Res 37:4231–4235

Schmid J, Sieber V (2015) Enzymatic transformations involved in the biosynthesis of microbial exo-polysaccharides based on the assembly of repeat units. Chembiochem 16:1141–1147

Singh R, Paul D, Jain RK (2006) Biofilms: implications in bioremediation. Trends Microbiol 14 (9):389–397

Singh RS, Saini GK, Kennedy JF (2009) Downstream processing and characterization of pullulan from a novel colour variant strain of *Aureobasidium pullulans* FB-1. Carbohydr Polym 78 (1):89–94

Sukan A, Roy I, Keshavarz T (2015) Dual production of biopolymers from bacteria. Carbohydr Polym 126(1):47–51

Tang XZ, Kumar P, Alavi S, Sandeep KP (2012) Recent advances in biopolymers and biopolymer-based nanocomposites for food packaging materials. Crit Rev Food Sci Nutr 52(5):426–442

Wang S, Teng S, Fan M (2010) Interaction between heavy metals and aerobic granular sludge. In: Kumar SS (ed) Environmental management. IntechOpen, pp 173–188. https://doi.org/10.5772/10106

Xu L, Xu G, Liu T, Chen Y, Gong H (2013) The comparison of rheological properties of aqueous welan gum and xanthan gum solutions. Carbohydr Polym 92(1):516–522

Yang Q, Luo K, Liao DX, Li XM, Wang DB, Liu X et al (2012) A novel bioflocculant produced by *Klebsiella* sp. and its application to sludge dewatering. Water Environ J 26(4):560–566

Zhang ZQ, Bo L, Xia SQ, Wang XJ, Yang AM (2007) Production and application of a novel bioflocculant by multiple-microorganism consortia using brewery wastewater as carbon source. J Environ Sci 19(6):667–673

Zhang Z, Xia S, Zhang J (2010) Enhanced dewatering of waste sludge with microbial flocculant TJ-F1 as a novel conditioner. Water Res 44(10):3087–3092

Zhang Y, Wang F, Yang X, Gu C, Kengara FO, Hong Q et al (2011) Extracellular polymeric substances enhanced mass transfer of polycyclic aromatic hydrocarbons in the two-liquid-phase system for biodegradation. Appl Microbiol Biotechnol 90(3):1063–1071

Zhang CL, Cui YN, Wang Y (2012) Bioflocculant produced from bacteria for decolorization, Cr removal and swine wastewater application. Sustain Environ Res 22(2):129–134

Zouboulis AI, Chai XL, Katsoyiannis IA (2004) The application of bioflocculant for the removal of humic acids from stabilized landfill leachates. J Environ Manag 70(1):35–41

Chapter 8
Bioremediation: Efficient Technology to Combat Pesticide Pollutants in Environment

Raman Kumar Ravi and R. Y. Hiranmai

Abstract Pesticides are chemical substances applied to manage the agricultural and domestic pests, but indiscriminate and unsafe use leads to their accumulation in the environment. Bioaccumulation of pesticides is of great public health concern due to their toxicity. Therefore, there is a need to develop assays and methods to easily access and degrade pesticide pollutants in nature. Bioremediation is a key technology that has been proven to be of inestimable value for controlling and managing the pesticides contamination with the help of microorganisms. It includes various strategies that are gaining more scientific and public interest as it is economic and eco-friendly. The microorganisms existing in contaminated environment are explored to degrade the pesticides. Recent advancement in science and technology has provided facts about genetic structure and metabolic activities of microorganisms, which is helpful in the process of bioremediation. In this chapter, we have highlighted the advancements and efficient bioremediation practices to remove pesticide pollutants in the environment.

Keywords Pesticides · Bioremediation · Microorganisms · Environment · Pollution

---

R. K. Ravi (✉)
Department of Agriculture, Uttaranchal College of Science and Technology, Dehradun, Uttarakhand, India

School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar, Gujarat, India

R. Y. Hiranmai
School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar, Gujarat, India

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_8

1 Introduction

Pesticides are the diverse group of chemicals being used globally and categorized as herbicides, insecticides, fungicides, rodenticides, molluscicides, and nematicides, depending on the target pests. In agricultural sector, these pesticides are applied to prevent or control pest diseases, weeds, and other plant pathogens in order to maintain high agricultural productivity or reduce yield losses (Damalas and Eleftherohorinos 2011). Approximately 45% of food productivity is lost due to pests every year; thus, there is a need to increase the world food production capacity for the rapidly growing population (Schreinemachers and Tipraqsa 2012; Abhilash and Singh 2009). The pesticides are the only toxic chemicals used intentionally into the environment to kill pests. But its uncontrolled utilization has created a lot of health-associated problems. It is estimated that nearly 300,000 deaths account globally every year due to pesticides poisoning (Sabarwal et al. 2018). Therefore, its detection and degradation becomes a necessity and an inevitable area of research. Bioremediation is considered as the removal of unwanted toxic compounds from the contaminated site or environment using biological activities, i.e., elimination of undesirable materials from environment applying biological sources like plants and microbial diversity or the substances obtained from them. Though it is a natural ongoing process, various interventions can be introduced to improve its efficiency as well as make it faster and specific to decontaminate the polluted sites (Hlihor et al. 2017; Shankar et al. 2011; Iwamoto and Nasu 2001; Vidali 2001). Bioremediation can be defined as the application of microbial metabolism to remove or decompose persistent pollutants, such as pesticides from the environment (Huang et al. 2018). Several microbes have been found with efficient metabolic activities that are able to convert pesticides into less toxic metabolites (Hussaini et al. 2013; Gupta et al. 2019). Several microorganisms have been reported having immense potential to deal with xenobiotic compounds (Gupta et al. 2017; Rathour et al. 2018).

Although the biological methods of pesticide removal have encountered, some limitations and challenges, such as screening and identification of potential microorganisms, complex pesticide structures, and delicate environmental conditions (Vikrant et al. 2018; Liu et al. 2019a, b), advancement in technology leads these techniques far above the conventional bioremediation methods. This chapter is mainly focused on the application of microbial metabolisms for bioremediation of pesticides. This also deals with the degradation or removal of pesticides by advanced bioremediation technology. This chapter will provide clues for finding new research approaches in the highly challenging bioremediation field through microbial methods and advanced tools and technologies.

2 Different Sources of Pesticide Contamination

Due to regular anthropogenic activities, the global environmental pollution has increased rapidly. Several persistent toxic compounds, like synthetic pesticides, industrial toxicants, organic solvents, and detergents, are contaminating the environment. Among these pesticides are of great concern (Ollera et al. 2011) due to its toxicity and persistency. The pesticides are released into the environment by several sources, i.e., emissions from agricultural activities, manufacturing and formulation industries, municipal waste, and soil infiltration. Recently, due to the development of agriculture and industry, large amounts of pesticides and other chemicals have been released into the environment (Morillo and Villaverde 2017; Varjani et al. 2018). Agriculture and forestry are the primary source of pesticide contamination in the environment. The increased food demand due to the rapidly growing population has promoted the use of pesticides in different agricultural activities with an increasing rate (Morillo and Villaverde 2017). Agricultural runoff, industrial wastewater, and chemical spills can greatly promote the pollution of pesticides in the environment through biological amplification (Duirk and Collette 2005). Pesticides may pollute the environment through two sources, point sources and nonpoint sources. The point source is a recognizable source of pollution, while a nonpoint source is a source from a wide area. It is impossible to distinguish pollution sources in a large area, such as the transfer of pesticides to surface water from multiple sources that pollute aquatic and terrestrial ecosystems. The common point sources of pesticide pollution in the environment are as follows: agricultural discharge, municipal waste, pesticides application in the agricultural field, wastewater discharge from the agricultural industry, plant residues contaminated by pesticides, improper treatment, and disposal of organic waste near water sources. The most common source of soil penetration, wastewater from spraying containers, and pesticide pollution is cleaning used containers (Neumann et al. 2002; Spanoghe et al. 2004). Opposite to this, nonpoint source pollution of pesticides has no specific discharge point, which is also called diffusion source. Agriculture is the main source of diffuse pollution, and its management is very complex, requiring careful analysis and understanding of many natural and man-made processes (Müller et al. 2002). So far, many treatment technologies have been practiced, but most of them cannot achieve satisfactory degradation effects (Gaya and Abdullah 2008).

3 Bioremediation of Pesticide

Bioremediation is considered as the treatment of polluted environmental medium, such as soil, water, and subsurface material, by altering the environmental condition that supports the microbial growth and development for degrading the target compounds. However, biodegradation is the process in which microbial biomass can effectively break down organic substances into its metabolites. Due to the long life

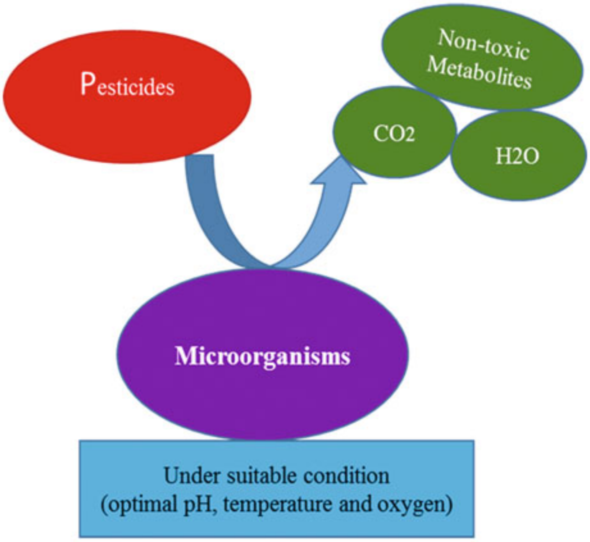


Fig. 8.1 Degradation of pesticides into its metabolites

span, the term biodegradation is often used in ecology, waste management, and environmental remediation. The global annual use of pesticides is about 2 million tons, of which 24% are used in the United States, 45% are used in Europe, and the remaining 25% are used in other parts of the world. According to reports, India consumes 3.8% of the world's total pesticide production. The consumption of agricultural pesticides in India, South Korea, and Japan is 0.5, 6.6, and 12.0 kg ha-1, respectively (Gupta 2004). Earlier, the application of pesticides was relatively helpful in reducing crop yield losses caused by pests, thus opening the way for increasing crop productivity. However, the widespread use of pesticides has caused pollution of the natural environment and has caused some long-term effects on human society (Bhanti and Taneja 2007). Due to the persistence nature, lack of selectivity, biomagnification, and accumulation under different nutrient levels, it is reported that organochlorine is the cause of pest resistance development and harmful effects on nontarget organisms (Carson 1962). The potential threat to all living things caused by the abuse of pesticides has now become one of the most serious environmental or health problems in the world. People mainly complain about the availability of pesticides in drinking water, vegetables, mammalian blood, human food, dairy products, fat samples, and other foods. The pesticide residues are present in different environmental samples through the contaminated food chain, which directly indicates the acute or chronic exposure of human beings to pesticides.

Biodegradation involves the ability of microorganisms to remove contaminants. It is the most promising, relatively effective, and cost-effective technology. This process involves the complete decomposition of the organic compounds into its metabolites (Fig. 8.1). Microbial transformation is an energy-driven process required to detoxify pollutants. Microorganisms have ubiquitous properties. Compared with other living organisms on the earth, they have a large amount of biomass and have a wider variety and catalytic ability (Paul et al. 2005). It can function even in the absence of oxygen and other extreme conditions. Searching for potential microorganisms that can degrade pollutants, understanding their genetic and biochemical composition, and developing methods for their application in this field have become important tasks for mankind (Megharaj et al. 2011).

3.1 Chemistry Behind Pesticide Degradation

The chemical structure of pesticides strongly influences the susceptibility of pesticides and so their biodegradation. Many studies have shown the relationship between the chemical structure of pesticides and their biodegradability. Some pesticides are easily degraded, but the degradation of some of them are hindered due to the presence of anionic species (Julia et al. 2001). Qualitative analysis of the chemical structure of pesticides can be used to develop bioremediation strategies. Compounds with groups carboxylate (-C(O)OR), amide ($-C(O)NR_2$), phosphate, hydroxyl (-OH), formyl (-CHO), and carboxyl (-COOH)) are prone to biodegradation due to anoxic conditions. These structural groups are common in nature, and microorganisms may have developed the enzymes needed to deal with them. On the other hand, the presence of certain structural groups reduces the chance of biodegradation. These groups are chlorine and nitro groups, especially those on aromatic rings, quaternary carbon ($CR_1R_2R_3R_4$, no $R = H$), and tertiary nitrogen ($NR_1R_2R_3$, no $R = H$), reducing the chance of bioremediation (Schwarzenbach et al. 2003). The enzymes play as a central role in biodegradation (Riya and Jagatpati 2012). Microbial enzymes can convert or degrade pesticides and are an innovative treatment technology used to remove chemicals from polluted environments. The ability of microorganisms to interact chemically and physically makes them an effective degrading agent, leading to structural changes or complete degradation of the target compound. In the microbial community, bacteria, fungi, and actinomycetes are the main pesticide degrading agents (Briceño et al. 2007). Enzyme-catalyzed degradation of pesticides is more effective than the existing chemical methods. Among microorganisms, fungi and bacteria are considered to be excellent microorganisms that produce extracellular enzymes. White rot fungi have been found to be promising bioremediation agents, especially for compounds that are not easily degraded by bacteria. This ability stems from the production of extracellular enzymes and acts on a broad spectrum of organic compounds. Some of these extracellular enzymes are involved in lignin degradation, such as lignin peroxidase, manganese peroxidase, laccase, and oxidase.

4 Microbial Diversity Involved in Degradation of Pesticides

Bioremediation is considered a natural and environmentally friendly technology that can remove various toxic pollutants (Gupta et al. 2016). Microorganisms can metabolize various chemical substances and their derived materials, such as organic complexes, heavy metals, and chlorinated and non-chlorinated pesticides. Microorganisms, such as bacteria, fungi, or algae, can be used to remove organic or inorganic chemicals from the soil environment (Megharaj and Naidu 2017). These potential organisms can degrade toxic chemicals. They may be wild type or genetically modified. Microbes can completely metabolize pollutants or change the chemical structure, thereby promoting their degradation and effectively eliminating pollutants from the environment (Abatenh et al. 2017; Rathour et al. 2018; Gupta et al. 2019). Microorganisms use pesticides as carbon and nutrient sources through their own multiple enzymatic mechanisms (Huang et al. 2018). Several bacteria that degrade pesticides have been isolated, and the list is rapidly expanding (Bass and Field 2011); some of them are listed in the Table. 8.1.

Table 8.1 Microorganisms involved in pesticide degradation

Pesticides	Degrading microorganisms	References
Chlorpyrifos	*Pseudomonas* sp.	Chauhan and Jha (2018)
	Microbial consortium	Singh et al. (2016)
	Bacterial consortium	Ravi et al. (2015)
	Pseudomonas fluorescens	Lakshmi et al. (2008)
	Bacillus subtilis	Lakshmi et al. (2008)
Dichlorvos	*Pseudomonas* sp.	Chauhan and Jha (2018)
Endosulfan	*Bacillus subtilis*	Kumar et al. (2014)
	Pseudomonas sp.	Harikumar et al. (2013)
	Staphylococcus equorum	Bhattacharjee et al. (2014)
	Aspergillus niger	Bhalerao and Puranik (2007)
	Alcaligenes faecalis JBW4	Kong et al. (2013)
Atrazine	*Citricoccus*	Yang et al. (2018)
	Pseudomonas sp.	Lima et al. (2009)
DDT	*Chryseobacterium* sp.	Qu et al. (2015)
	Bacillus sp.	Pant et al. (2013)
Diazinon	*Pseudomonas* sp.	Cycon et al. (2009)
Cypermethrin	*Streptomyces aureus* HP-S-01	Chen et al. (2012)
Methyl parathion	*Pseudomonas* sp. WBC-3	Wang et al. (2014)
	Bacterial consortium	Ravi et al. (2015)
Parathion	*Serratia marcescens*	Cycon et al. (2013)
Glyphosate	*Agrobacterium* strain T10	Ortiz-Hernández et al. (2013)
Lindane	*Pseudomonas paucimobilis*	Rehmat (2012)

4.1 Bacterial Degradation of Pesticides

The biodegradation of pesticides involves the oxidation of parent compounds to CO_2, H_2O, or less toxic compounds (Abatenh et al. 2017; Doolotkeldieva et al. 2018). Bacterial strains belonging to *Burkholderia, Flavobacterium, Arthrobacter, Azotobacter*, and *Pseudomonas* are known to degrade pesticides (Glazer and Nikaido 2007). The pesticide degradation depends not only on the enzyme system of the microorganisms but also on available conditions, such as pH, temperature, and nutrients (Doolotkeldieva et al. 2018). The methods and processes of pesticide degradation vary from compound to compounds and the types of bacteria involved in the process. Both *Pseudomonas* sp. and *Klebsiella pneumoniae* have hydrolases that can degrade organophosphate pesticides and neonicotinoids (Pathak 2018). Sometimes, environmental factors can cause partial degradation of pesticides, leading to accumulation of pesticide residue in the environment. This may cause inhibition of microbial populations. Dichlorodiphenyltrichloroethane (DDT), an organochlorine pesticide, is partially degraded into metabolites of dichlorodiphenyltrichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE), and their toxicity is even higher than the parent compound (Foght et al. 2001). Before introducing microorganisms for pesticide degradation, optimization of the environmental condition becomes necessary and thus inhibits accumulation of metabolites in the soil.

4.2 Fungal Degradation of Pesticides

Although both bacterial and fungal species play a vital role in pesticide degradation, fungi introduce structural changes and then release them into the soil, making them susceptible to further degradation by bacteria and finally turning pesticides into nontoxic substances (Gianfreda and Rao 2004). The degradation of pesticides follows different pathways, depending on its nature, environmental conditions, and microbe type (Ortiz-Hernández et al. 2013). *Phanerochaete chrysosporium* are able to degrade a wide range of pesticides (Singh 2017). The white rot fungi are able to degrade several types of pesticides, such as lindens, atrazine, durone, tert-butazine, metalaxyl, DDT, γ-hexachlorocyclohexane, dieldrin, aldrin, heptachlor, chlordane, and mirex (Pathak 2018). Similarly, fungal species, like *Auricularia auricula, Coriolus versicolor, Agrocybe semiorbicularis, Dichomitus squalens, Stereum hirsutum, Flammulina velutipes, Pleurotus ostreatus, Avatha discolor,* and *Hypholoma fasciculare*, have been shown to degrade different types of pesticides triazines, phenylamide, phenylurea, dicarboximide, chlorinated, and organophosphorus compounds (Bending et al. 2002).

4.3 Enzymes Involved in Pesticide Degradation

Enzymes produced by microorganisms in the soil in various metabolic processes are the key to pesticide bioremediation. Enzymes play a major role in the biodegradation of pesticides, at a significant rate, and can help in restoring the polluted environment in the future (Rao et al. 2010). These enzymes also promote the degradation of pesticides in target organisms, through inherent detoxification processes and developed metabolic resistance. The fungal enzymes oxidoreductase, laccase, and peroxidase are mainly used to remove polyaromatic hydrocarbon compounds from fresh water, seawater, or terrestrial water (Balaji et al. 2014). Over the years, organophosphorus-degrading enzymes have been studied in detail, and several bacteria, fungi, and cyanobacteria that use organophosphorus compounds as carbon and nutrient sources have also been isolated (Ghosal et al. 2016; Kandpal 2014).

5 Bioremediation Strategies and Its Application

Depending on the treatment site, there are two basic strategies for removing contaminants, i.e., in situ and ex situ. In situ techniques involve on-site removal of pollutants, while ex situ methods are used for off-site management of pollutants, i.e., in laboratory-scale experiments. The consistency and kinetics of biodegradation of pesticides depends on the microorganism's activities (Jorgensen 2007). The different strategies for both the technique of bioremediation are summarized in Fig. 8.2**.**

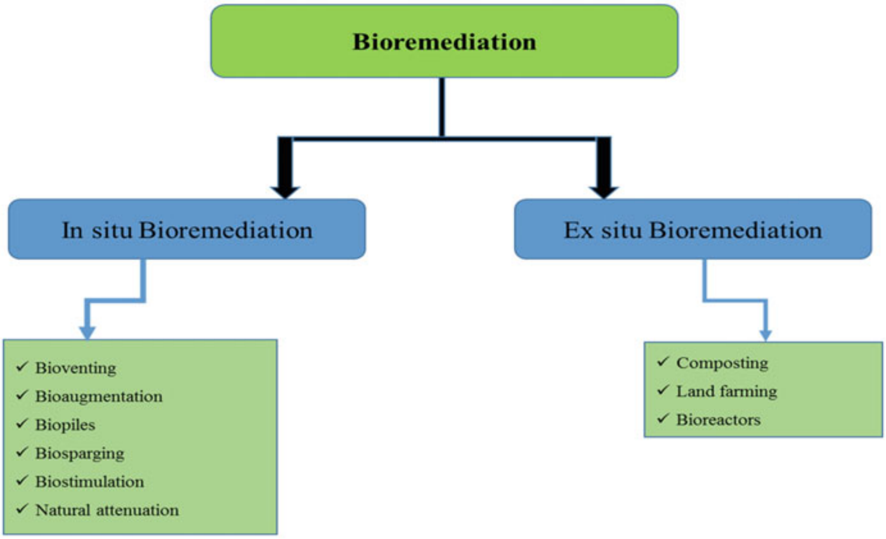


Fig. 8.2 Flow diagram of different bioremediation strategies

5.1 In Situ Techniques

In the in situ technology, the pollutants are treated at the contaminated site by simulating the microorganism's metabolism as well as nutrients and optimized environmental conditions (Ahmad et al. 2018). The pollutants are effectively treated on site, thereby avoiding any site excavation or damage to the ecological structure. In this process, the pollutants undergo microbial decomposition under natural conditions and produce carbon dioxide, water, and other less toxic by-products (Doolotkeldieva et al. 2018; Abatenh et al. 2017). The method is conducive to feasible, economical, environmentally friendly, and sustainable to achieve detoxification of contaminated sites. The most common in situ strategies are bioventing, bioaugmentation, biopiles, biosparging, biostimulation, and natural attenuation.

5.1.1 Bioventing

Bioventing involves the supply of nutrients and air through wells to stimulate the native bacteria of the contaminated soil. Bioventing provides low oxygen flow necessary for the biodegradation and accelerates the elimination and degradation of toxic compounds.

5.1.2 Bioaugmentation

Bioaugmentation is used to biodegrade specific soil and groundwater pollutants. It involves adding cultured microbial population into the particular subsurface environment to biodegrade the desired pollutants.

5.1.3 Biopiles

Biopiles provide a favorable environmental conditions for endemic aerobic and anaerobic microbial diversity to remove pollutants by controlling losses of the pollutants by volatilization and leaching.

5.1.4 Biosparging

Biosparging is the process in which air (oxygen) and nutrients are injected to increase groundwater oxygen concentrations and biological activity of naturally occurring microorganisms. This technique accelerates the biodegradation of the toxic organic compounds.

5.1.5 Biostimulation

Biostimulation involves the alteration in the environmental condition to stimulate the native bacterial population capable to perform bioremediation. The process can be done by adding various forms of rate-limiting nutrients and electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon.

5.1.6 Natural Attenuation

Natural attenuation comprises the processes that leads to reduction of the mass, toxicity, mobility, or volume of pollutants without human intervention.

5.2 Ex Situ Techniques

The ex situ techniques involve excavating contaminated samples from selected locations and transporting them to another location for processing (Ahmad et al. 2018). The factors that control off-site techniques include treatment costs, geographic location, type and depth of pollutants, and pollution levels (Azubuike et al. 2016). Due to other parameters, i.e., soil excavation, the processing cost of the ex situ technique may be much higher than the in situ technique.

5.2.1 Composting

Composting is the technique, in which nonhazardous organic materials such as manure are added to contaminated soil. These organic materials support in developing the microbial population growth, thereby improving the temperature for composting. The prepared compost bed has a variety of possibilities for bioremediation of pollutants using organic chelating agents, changing pH, redox potential, and surfactant production.

5.2.2 Land Farming

Land farming technology is used to decontaminate the contaminated site by excavating the contaminate soil and spread on prepared beds, and farming is carried out regularly until the pollutants are degraded. The main purpose of this technology is to motivate the naturally degraded microbial diversity and promote the aerobic degradation of pollutants.

5.2.3 Bioreactors

The reactor is used for bioremediation in which contaminated solid material (soil, sediment, sludge) or water is processed in a designed controlled system. The bioreactors are used for ex situ bioremediation of contaminated soil and water pumped up from a contaminated plume.

6 Approaches for Enhancing the Bioremediation of Pesticides

Bioremediation is a process that is carried out to remove the toxic compounds from the contaminated site using microbial biomass. Although bioremediation may be carried out by several ways, bioaugmentation, biostimulation, and cell immobilization are found very effective for enhanced degradation of pesticides.

6.1 Bioaugmentation

Bioaugmentation is the repair process in which exogenous microbial populations with specific catabolic activities have been applied in biodegradation process at contaminated sites or in the bioreactors. This process is performed either on-site or off-site for the removal of harmful contaminants (Head and Oleszkiewicz 2004; Perelo 2010) and is widely regarded as an effective microbiological method that promotes the degradation of pesticides (Dams et al. 2007; Plangklang and Reungsang 2010). This process is able to eliminate a wide range of contaminants, such as NH_3, H_2S, and petroleum by-products, along with organic and inorganic contaminants (Khan et al. 2005; Park et al. 2008; Xu et al. 2008).

6.2 Biostimulation

In order to improve the microbial efficiency for pesticide degradation, different external stimuli, such as carbon sources, vitamins, oxygen, and other nutrients, have been applied, called biostimulation (Kanissery and Sims 2011). The addition of stimulating nutrients (carbon sources) can promote a quick depletion of available stocks of major nutrients (phosphorus and nitrogen) (Huang et al. 2018; Plangklang and Reungsang 2010). Nutritional supplements are used as inducers to enhance the enzymatic activity and metabolite generation during pesticide biodegradation (Plangklang and Reungsang 2010; Robles-Gonzalez et al. 2008). In order to enhance the microbial degradation caused by stimulation, water-soluble nutrients, such as

NH_3NO_3, $NaNO_3$, KNO_3, K_2HPO_4, and $MgNH_4PO_4$, are applied after mixing with fertilizer (Lima et al. 2009).

6.3 Cell Immobilization

These days, cell immobilization has been used for the biological removal of pesticides, and it is found that it is much efficient than the other types of degradation techniques, because it can maintain catalytic activities for a longer period of time (Richins et al. 2000; Chen and Georgiou 2002; Martin et al. 2000). Compared to conventional biological systems using free cells, whole-cell immobilization has shown significant advantages, such as the possibility of using high cell density, avoiding cell washing even at high dilution rates, being easy to separate from the reaction cell system, repeated use of cells, and better protection of cells from harsh environments. Previous reports indicate that this higher productivity is caused by immobilization-induced cell or genetic modification. There is evidence that immobilized cells have greater tolerance to the perturbation of the reaction environment and lower sensitivity to toxic substances, which makes immobilized cell systems particularly attractive for handling toxic substances such as pesticides (Ha et al. 2008). In addition, the enhancement of the degradability of immobilized cells is mainly due to the protection of cells from the inhibitory substances present in the environment. It was observed that the degradation rate of repeated operations increased in successive batches, indicating that as time passed, the cells became better adapted to the reaction conditions (Ha et al. 2009).

7 Technological Advancement in Bioremediation of Pesticides

With the development of science and technology, different techniques, such as genetic engineering, metagenomics, genomics and proteomics, nanotechnological approaches, etc., are in use for enhanced degradation of pesticides (Fig. 8.3).

7.1 Genetic Engineering for Biodegradation of Pesticides

Microorganisms respond distinctly to different types of stresses and adapt to the contaminated environment condition. This process can be enhanced by using genetic engineering technology. Recombinant DNA and other molecular biology techniques have enabled (a) targeted genes encoding enzymes in metabolic pathways to be amplified, destroyed, and/or modified; (b) pathway bottlenecks to be minimized,

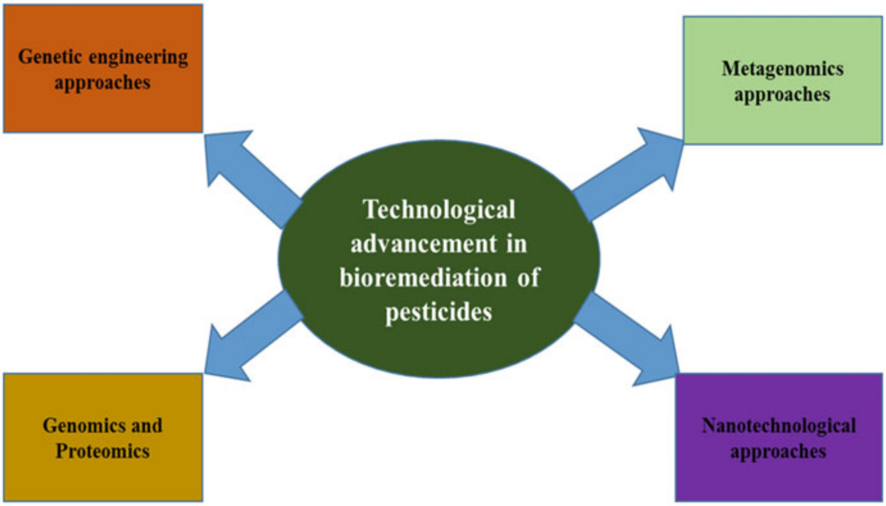


Fig. 8.3 Advanced techniques for bioremediation of pesticides

(c) increase redox and energy generation; and (d) recruiting heterologous genes to give new characteristics (Shimizu 2002; Megharaj et al. 2011). Various genetic approaches have been developed and used to optimize the enzymes, metabolic pathways, and organisms relevant for biodegradation (Pieper and Reineke 2000). New information on metabolic pathways and degradation bottlenecks are still accumulating, and the available molecular toolbox needs to be strengthened. However, even in a single modified organism, the introduced gene or enzyme needs to be integrated into the regulatory and metabolic network to be correctly expressed (Shimizu 2002; Pieper and Reineke 2000; Cases and de Lorenzo 2005). Genetically modified microorganisms have demonstrated the detoxification of organophosphate pesticides for the first time, and they have been cloned and expressed the gene encoding hydrolase in *P. pseudoalcaligenes*, *Yarrowia lipolytica, Escherichia coli*, *Streptomyces lividans*, and *Pichia pastoris* (Wu et al. 2004; Fu et al. 2004; Yu et al. 2009; Shen et al. 2010; Wang et al. 2012).

7.2 Metagenomics Approaches for Biodegradation of Pesticides

The complexity of microbial diversity depends on various interacting parameters, including pH, moisture, soil structure, climate change, and biological activity (Liu et al. 2019a, b). More than 99% of the microorganisms in the natural environment are difficult to cultivate under laboratory conditions, so they are not friendly to basic research and biotechnological applications (Zhou et al. 2010). In the past two decades, the development of technologies, capable of directly isolating nucleic acids from environmental sources, has allowed the study of diversity among microorganisms. By performing nucleic acid analysis on environmental samples, scientists can actively study the microbial communities present in the soil without cultivation (Jeffries et al. 2018; Zhou et al. 2010). Every organism in the surrounding environment has a unique set of genomes, and all community members with mixed genomes form a "metagenome." Metagenome technology (metagenomics) has led to the accumulation of DNA sequences, which are now used to develop new biotechnological applications (Kumar et al. 2015). Due to the limitation of being able to cultivate every kind of microorganism present in the environment, genomics search has appeared and will always lead to the dissection of previously unknown genes and proteins, which can be used more effectively to degrade chemical pesticides (Jeffries et al. 2018; Rayu et al. 2012).

7.3 Genomics and Proteomics Role in Biodegradation of Pesticides

The traditional molecular biology and genetics approaches are part of functional genomics methods, which can analyze the phenotypic modifications caused by mutagenesis or gene knockout in the organism's genome. Currently, functional genomics has received widespread attention due to the use of important innovative technologies for whole-genome assessment using bioinformatics (Jaiswal et al. 2019).

The new approaches include proteomics for microarrays and metabolic engineering for identifying proteins, characterizing, and expressing them to study their interactions and transcriptome profiles (Zhao and Poh 2008). The implementation of proteomics in environmental bioremediation research provides a global view of the biological composition of microbial cells and provides a promising strategy for the use of molecular methods of bioremediation (Jaiswal et al. 2019). Proteomics study shows the changes in protein patterns, as well as the functions and interactions between proteins (Kellner 2000; Chandrasekhar et al. 2014). It can also show the gene expression and regulation and protein-protein interaction of comprehensive analysis (Baginsky et al. 2010; Aslam et al. 2017). The mixed use of proteomics and genomics allows us to gain insight into the regulatory checkpoints of various metabolic pathways, thereby improving the understanding of gene functions (Manzoni et al. 2016).

Due to exposure to xenobiotic compounds in biological samples, proteomics has allowed extensive evaluation of the overall changes in protein profiles. The identification of key proteins involved in microbial responses in a given physiological state can promote the understanding of which genes are involved in the bioremediation process and how to regulate them (Singh 2017). Recently, proteomics research has been used to identify the degradation pathways of various xenobiotic compounds and their adaptive responses to their toxic effects in different microorganisms (Liu et al. 2017; Seo et al. 2013; Vandera et al. 2015; Festa et al. 2017; Wei et al. 2017). Several studies regarding microbial biotransformation and their specific effects on biological systems have been carried out, but extensive effects on the global metabolism of microorganisms with the ability to degrade these molecules have not yet been discovered (Bardot et al. 2015). Microbial degradation of pesticides using proteomics methods can determine the relevant enzymes, catalyzing the process, and the main metabolic pathways, utilizing carbon as energy, and the proteins related to damage caused by pesticide exposure. The application of proteomics methods in the field of pesticide biodegradation research has recently become more and more important.

The prime objective of the functional genomics technique is to use basic strategies to expand the scope of biological research, from the analysis of the function of a single gene and protein to the simultaneous and systematic analysis of all genes and proteins in the system (Rayu et al. 2012). The direct method to assess the biological function of genes is to determine the specific conditions under which the gene is disrupted or complementary to other genes. The combined use of transcriptomics and proteomics with traditional genetic methods is helpful to understand the gene function (Rayu et al. 2012; Zhao and Poh 2008). Metabolic engineering evaluates molecular biology approaches to improve cell characteristics, by applying the rational genetic alterations (Santos-Merino et al. 2019). A precise knowledge about microbial physiology and host cells will promote an effective bioremediation process (Rayu et al. 2012). With the development of new genomics tools, problems, such as oxygen stress, availability of nutrients, or high concentrations of pollutants along different geochemical gradients, can be solved (Ruuskanen et al. 2019). Finally, by monitoring the overall microbial community structure and function, strong decision can be made on how to control the field environment conditions to achieve effective bioremediation of pesticides (Maphosa et al. 2012).

7.4 Nanotechnological Approaches for Biodegradation of Pesticides

Nanotechnology has emerged as a promising technology with increasing applications in all applied fields, including the field of bioremediation, where it can be applied to remove the soil contamination (Guerra et al. 2018). Due to increase in pollution and its toxic effects, the use of this technologies to remove pesticides has attracted the attention of the scientific community. The size and unique properties of nanomaterials have become the point of interest for remediation of various environmental pollutants, including organic pesticides (Maeda 2011). Nowadays, nanocomposites, bio-nanopolymers, nanoparticles, and bimetallic nanoparticles have been widely used for detection and remediation of pesticide (Garcia and Takashima 2003). The metal oxide nanoparticles are effective photocatalysts that can degrade various toxic pollutants (Panigrahi et al. 2004; Shah et al. 2003).

The advancement in nanotechnological approaches can utilize both the phases, i.e., solid phase and solution phase, to decontaminate the soil under aerobic and anaerobic conditions (Medina-Pérez et al. 2019). For example, iron-based nanoparticles can effectively convert chlorinated organic compounds. The interaction of bacteria and zero-valent iron [Fe(0)] produces biological iron oxide, which can catalyze the dehalogenation of organic pollutants, chlorinated solvents, and pesticides (Quinn et al. 2005). Similarly, silver and gold nanopreparations can efficiently remove chlorpyrifos and malathion pesticides, either in solution phase or coated over alumina (Momic et al. 2016). In the solid phase, the long-term interaction between nanoparticles and pesticides causes them to adsorb on the surface and eventually lead to precipitation. The alumina-loaded nanoparticles are more effective in removing pesticides. Nanoparticles are firmly attached on an inert surface that absorbs pesticides and then are catalytically destroyed. At room temperature, the CCl bonds of hydrocarbons are broken, forming amorphous carbon as a by-product of the reaction (Nair and Pradeep 2007). In addition to single-nanoparticle formulations, stable Fe-Pd bimetallic nanoparticles have been studied, under aerobic and anaerobic conditions, for the degradation of chlorinated pesticides (lindane and atrazine) (Kumar and Pannu 2018). Nanoparticles behave as strong electron donors under anaerobic conditions, leading to the complete reduction of lindane and atrazine in the presence of Fe and Pd as catalysts. In contrast, nanoparticles under aerobic conditions will undergo a Fenton-like reaction. The hydroxyl radicals and active oxygen in the iron nanoparticles promote the oxidation of lindane, by dehalogenation or dehydrohalogenation, whereas dealkylation of alkyl amino side chain is required for atrazine removal (Joo and Zhao 2007).

8 Conclusions and Future Prospects

Due to human activities, huge amounts of pesticides are released into the environment, causing adverse effect on living beings. The microbial assisted pesticide degradation is found as the efficient, ideal, and most sustainable approaches. The microbial communities play an important role in the decomposition of pesticides, nutrient recycling, and biodegradation to repair polluted environments. Pesticides in water and soil can change the diversity and integrity of microbial communities. However, genetic flexibility and metabolic versatility enable microorganisms to resist the presence of pollutants in the environment, including pesticides. These microorganisms have developed strong strategies to degrade pesticides that pollute their environment. This versatile characteristic stands them in the row of potential candidate for pesticide bioremediation. The development of technologies, like genetic engineering, metagenomics, genomics and proteomics, and nanotechnology, has provide the crucial role in biodegradation of pesticides. The advancement in biotechnology and nanotechnology has started a new era of innovations that has the potential to revolutionize the bioremediation sector as biological, efficient, and clean remedial solutions to ensure the sustainability of our planet in the coming future.

References

Abatenh E, Gizaw B, Tsegaye Z, Wassie M (2017) Application of microorganisms in bioremediation-review. J Environ Microbiol 1:02–09

Abhilash PC, Singh N (2009) Pesticide use and application: an Indian scenario. J Hazard Mater 165 (13):1–12

Ahmad M, Pataczek L, Hilger TH, Zahir ZA, Hussain A, Rasche F, Schafleitner R, Solberg SO (2018) Perspectives of microbial inoculation for sustainable development and environmental management. Front Microbiol 9:29–92. https://doi.org/10.3389/fmicb.2018.02992

Aslam B, Basit M, Nisar MA, Khurshid M, Rasool MH (2017) Proteomics: technologies and their applications. J Chromatogr Sci 55:182–196. https://doi.org/10.1093/chromsci/bmw167

Azubuike CC, Chikere CB, Okpokwasili GC (2016) Bioremediation techniques classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol 32:180

Baginsky S, Hennig L, Zimmermann P, Gruissem W (2010) Gene expression analysis, proteomics, and network discovery. Plant Physiol 152:402–410. https://doi.org/10.1104/pp.109.150433135

Balaji V, Arulazhagan P, Ebenezer P (2014) Enzymatic bioremediation of polyaromatic hydrocarbons by fungal consortia enriched from petroleum contaminated soil and oil seeds. J Environ Biol 35:521–529

Bardot C, Besse-Hoggan P, Carles L, Le Gall M, Clary G, Chafey P, Federici C, Broussard C, Batisson I (2015) How the edaphic *Bacillus megaterium* strain Mes11 adapts its metabolism to the herbicide mesotrione pressure. Environ Pollut 199:198–208. https://doi.org/10.1016/j.envpol.2015.01.029

Bass C, Field LM (2011) Gene amplification and insecticide resistance. Pest Manag Sci 67 (8):886–890

Bending G, Friloux DM, Walker A (2002) Degradation of contrasting pesticides by white rot fungi and its relationship with ligninolytic potential. FEMS Microbiol Lett 212:59–63

Bhalerao TS, Puranik PR (2007) Biodegradation of organochlorine pesticide, endosulfan, by a fungal soil isolate, Aspergillus niger. Int Biodeterior Biodegrad 59(4):315–321

Bhanti M, Taneja A (2007) Contamination of vegetables of different seasons with organophosphorous pesticides and related health risk assessment in northern India. Chemosphere 69:63–68

Bhattacharjee K, Banerjee S, Bawitlung L, Krishnappa D, Joshi SR (2014) A study on parameters optimization for degradation of endosulfan by bacterial consortia isolated from contaminated soil. Proc Natl Acad Sci India B 84(3):657–667

Briceño G, Palma G, Duran N (2007) Influence of organic amendment on the biodegradation and movement of pesticides. Crit Rev Environ Sci Technol 37:233–271. https://doi.org/10.1080/10643380600987406

Carson R (1962) The silent spring. Houghton Mifflin, New York

Cases I, de Lorenzo V (2005) Promoters in the environment: transcriptional regulation in its natural context. Nat Rev Microbiol 3:105–118

Chandrasekhar K, Dileep A, Lebonah DE (2014) A short review on proteomics and its applications. Int Lett Nat Sci 12:77–84

Chauhan PS, Jha B (2018) Pilot scale production of extracellular thermo-alkali stable laccase from *Pseudomonas* sp. S2 using agro waste and its application in organophosphorus pesticides degradation. J Chem Technol Biotechnol 93(4):1022–1030

Chen W, Georgiou G (2002) Cell-surface display of heterologous proteins: from high throughput screening to environmental applications. Biotechnol Bioeng 5:496–503. https://doi.org/10.1002/bit.10407

Chen S, Geng P, Xiao Y, Hu M (2012) Bioremediation of β-cypermethrin and 3-phenoxybenzaldehyde contaminated soils using *Streptomyces aureus* HP-S-01. Appl Microbiol Biot 94:505–515

Cycon M, Wojcik M, Piotrowska-Seget Z (2009) Biodegradation of the organophosphorus insecticide diazinon by *Serratia* sp. and *Pseudomonas* sp. and their use in bioremediation of contaminated soil. Chemosphere 76:494–501

Cycon M, Zmijowska A, Wojcik M, Piotrowska-Seget Z (2013) Biodegradation and bioremediation potential of diazinon-degrading *Serratia marcescens* to remove other organophosphorus pesticides from soils. J Environ Manag 117:7–16

Damalas CA, Eleftherohorinos IG (2011) Pesticide exposure, safety issues, and risk assessment indicators. Int J Environ Res Public Health 8(5):1402–1419

Dams RI, Paton GI, Killham K (2007) Rhizoremediation of pentachlorophenol by *Sphingobium chlorophenolicum* ATCC 39723. Chemosphere 68:864–870

Doolotkeldieva T, Konurbaeva M, Bobusheva S (2018) Microbial communities in pesticide-contaminated soils in Kyrgyzstan and bioremediation possibilities. Environ Sci Pollut Res 25:31848–31862

Duirk SE, Collette TW (2005) Organophosphate pesticide degradation under drinking water treatment conditions. EPA/600/R-05/103. National Exposure, and US Environmental Protection Agency

Festa S, Coppotelli BM, Madueño L, Loviso CL, Macchi M, Tauil RMN, 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. https://doi.org/10.1371/journal.pone.0184505

Foght J, April T, Biggar K, Aislabie J (2001) Bioremediation of DDT contaminated soils: a review. Biorem J 225:1058–1137

Fu GP, Cui Z, Huang T, Li SP (2004) Expression, purification, and characterization of a novel methyl parathion hydrolase. Protein Expr Purif 36:170–176

Garcia JC, Takashima K (2003) Photocatalytic degradation of imazaquin in an aqueous suspension of titanium dioxide. J Photochem Photobiol A Chem 155:215–222

Gaya UI, Abdullah AH (2008) Heterogeneous photocatalytic degradation of organic contaminants over titanium dioxide: a review of fundamentals, progress and problems. J Photochem Photobiol C: Photochem Rev 9(1):1–12

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

Gianfreda L, Rao MA (2004) Potential of extra cellular enzymes in remediation of polluted soils: a review. Enzym Microb Technol 35:339–354

Glazer AN, Nikaido H (2007) Microbial biotechnology: fundamentals of applied microbiology. Cambridge University Press, Cambridge/New York, pp 510–528

Guerra FD, Attia MF, Whitehead DC, Alexis F (2018) Nanotechnology for environmental remediation: materials and applications. Molecules 23:17–60

Gupta PK (2004) Pesticide exposure-Indian scene. Toxicology 198(1–3):83–90

Gupta M, Mathur S, Sharma TK, Rana M, Gairola A, Navani NK et al (2016) A study on metabolic prowess of *Pseudomonas* sp. RPT 52 to degrade imidacloprid, endosulfan and coragen. J Hazard Mater 301:250–258

Gupta J, Rathour R, Kumar M, Thakur IS (2017) Metagenomic analysis of microbial diversity in landfill lysimeter soil of Ghazipur landfill site, New Delhi. India Genome Announc 5:01104–01117

Gupta J, Rathour R, Singh R, Thakur IS (2019) Production and characterization of extracellular polymeric substances (EPS) generated by a carbofuran degrading strain *Cupriavidus* sp. ISTL7. Bioresour Technol 282:417–424

Ha J, Engler CR, Lee SJ (2008) Determination of diffusion coefficients and diffusion characteristics for chlorferon and diethylthiophosphate in Ca-alginate gel beads. Biotechnol Bioeng 100 (4):698–706. https://doi.org/10.1002/bit.21761

Ha J, Engler CR, Wild J (2009) Biodegradation of coumaphos, chlorferon, and diethylthiophosphate using bacteria immobilized in Ca-alginate gel beads. Biores Technol 100:1138–1142

Harikumar PS, Jesitha K, Sreechithra M (2013) Remediation of endosulfan by biotic and abiotic methods. J Environ Prot 4(05):418

Head M, Oleszkiewicz J (2004) Bioaugmentation for nitrification at cold temperatures. Water Res 38:523–530

Hlihor RM, Gavrilescu M, Tavares T, Favier L, Olivieri G (2017) Bioremediation: an overview on current practices, advances, and new perspectives in environmental pollution treatment. Hindawi 34

Huang Y, Xiao L, Li F, Xiao M, Lin D, Long X et al (2018) Microbial degradation of pesticide residues and an emphasis on the degradation of cypermethrin and 3-phenoxy benzoic acid: a review. Molecules 23:2313

Hussaini SZ, Shaker M, Iqbal MA (2013) Isolation of bacterial for degradation of selected pesticides. Adv Biores 4:8285

Iwamoto T, Nasu M (2001) Current bioremediation practice and perspective. Biosci Bioeng 92 (1):18

Jaiswal S, Singh DK, Shukla P (2019) Gene editing and systems biology tools for pesticide bioremediation: a review. Front Microbiol 10:87

Jeffries TC, Rayu S, Nielsen UN, Lai K, Ijaz A, Nazaries L et al (2018) Metagenomic functional potential predicts degradation rates of a model organophosphorus xenobiotic in pesticide contaminated soils. Front Microbiol 9:147

Joo SH, Zhao D (2007) Destruction of lindane and atrazine using stabilized iron nanoparticles under aerobic and anaerobic conditions. Chemosphere 70:418425

Jorgensen KS (2007) In situ bioremediation. Adv Appl Microbiol 61:285305

Julia F, Trevor A, Kevin B, Jackie A (2001) Bioremediation of DDT contaminated soils: a review. Biorem J 5:225–246

Kandpal V (2014) Biopesticides. Int J Environ Res Dev 4:191–196

Kanissery RG, Sims GK (2011) Biostimulation for the enhanced degradation of herbicides in soil. Appl Environ Soil Sci 10. https://doi.org/10.1155/2011/843450

Kellner R (2000) Proteomics. Concepts and perspectives. Fresenius J Anal Chem 366:517–524

Khan E, Huang C, Reed BE (2005) Hazardous waste treatment technologies (JSTOR)

Kong L, Zhu S, Zhu L, Xie H, Su K, Yan T, Wang J, Wang J, Wang F, Sun F (2013) Biodegradation of organochlorine pesticide endosulfan by bacterial strain Alcaligenes faecalis JBW4. J Environ Sci 25(11):2257–2264

Kumar D, Pannu R (2018) Perspectives of lindane (γ-hexachlorocyclohexane) biodegradation from the environment: a review. Bioresour Biopro 5:29

Kumar A, Bhoot N, Soni I, John PJ (2014) Isolation and characterization of a *Bacillus subtilis* strain that degrades endosulfan and endosulfan sulphate. 3. Biotech 4(5):467–475

Kumar S, Krishnani KK, Bhushan B, Brahmane MP (2015) Metagenomics: retrospect and prospects in high throughput age. Biotechnol Res Int 13. https://doi.org/10.1155/2015/121735

Lakshmi CV, Kumar M, Khanna S (2008) Biotransformation of chlorpyrifos and bioremediation of contaminated soil. Int Biodeterior Biodegradation 62:204–209

Lima LD, Viana P, Andre S, Chelinho S, Costa C, Ribeiro R et al (2009) Evaluating a bioremediation tool for atrazine contaminated soils in open soil microcosms: the effectiveness of bioaugmentation and biostimulation approaches. Chemosphere 74:187–192

Liu S, Gu C, Dang Z, Liang X (2017) Comparative proteomics reveal the mechanism of tween 80 enhanced phenanthrene biodegradation by *Sphingomonas* sp. GY2B. Ecotoxicol Environ Saf 137:256–264. https://doi.org/10.1016/j.ecoenv.2016.12.015

Liu J, Yin M, Zhang W, Tsang DCW, Wei X, Zhou Y, Xiao T, Wang J, Dong X, Sun Y, Chen Y, Li H, Hou L (2019a) Response of microbial communities and interactions to thallium in contaminated sediments near a pyrite mining area. Environ Pollut 248:916–928

Liu M, Sui X, Hu Y, Feng F (2019b) Microbial community structure and the relationship with soil carbon and nitrogen in an original Korean pine forest of Changbai Mountain, China. BMC Microbiol 19:218

Maeda K (2011) Photocatalytic water splitting using semiconductor particles: history and recent developments. J Photochem Photobiol C: Photochem Rev 12(4):237–268. https://doi.org/10.1016/j.jphotochemrev.2011.07.001

Manzoni C, Kia DA, Vandrovcova J, Hardy J, Wood NW, Lewis PA et al (2016) Genome, transcriptome and proteome: the rise of omics data and their integration in biomedical sciences. Brief Bioinf 19:286302

Maphosa F, Lieten SH, Dinkla I, Stams AJ, Smidt H, Fennell DE (2012) Ecogenomics of microbial communities in bioremediation of chlorinated contaminated sites. Front Microbiol 3:351

Martin M, Mengs G, Plaza E, Garbi C, Sánchez A, Gibello A, Gutierrez F, Ferrer E (2000) Propachlor removal by Pseudomonas strain GCH1 in a immobilized-cell system. Appl Environ Microbiol 66(3):1190–1194

Medina-Pérez G, Fernández-Luqueño F, Núñez EV, López-Valdez F, Prieto Mendez J, Madariaga-Navarrete A et al (2019) Remediating polluted soils using nanotechnologies: environmental benefits and risks. Pol J Environ Stud 28:1013–1030

Megharaj M, Naidu R (2017) Soil and brownfield bioremediation. Microb Biotechnol 10:1244–1249

Megharaj M, Ramakrishnan B, Venkateswarlu K, Sethunathan N, Naidu R (2011) Bioremediation approaches for organic pollutants: a critical perspective. Environ Int 37:1362–1375

Momic T, Pasti TL, Bogdanovic U, Vodnik V, Mrakovic A, Rakocevic Z et al (2016) Adsorption of organophosphate pesticide dimethoate on gold nanospheres and nanorods. J Nanomater 11. https://doi.org/10.1155/2016/8910271

Morillo E, Villaverde J (2017) Advanced technologies for the remediation of pesticide contaminated soils. Sci Total Environ 586:576–597

Müller K, Bach M, Hartmann H, Spiteller M, Frede HG (2002) Point-and nonpoint source pesticide contamination in the Zwester Ohm catchment, Germany. J Environ Qual 31(1):309–318

Nair S, Pradeep T (2007) Extraction of chlorpyrifos and malathion from water by metal nanoparticles. J Nanosci Nanotechnol 7:17

Neumann M, Schulz R, Schäfer K, Müller W, Mannheller W, Liess M (2002) The significance of entry routes as point and non-point sources of pesticides in small streams. Water Res 36 (4):835–842

Ollera S, Malatoa J, Sánchez-Pérezb A (2011) Combination of advanced oxidation processes and biological treatments for wastewater decontamination – a review. Sci Total Environ 409 (20):4141–4166. https://doi.org/10.1016/j.scitotenv.2010.08.061.15

Ortiz-Hernández ML, Sánchez-Salinas E, Dantán-González E, Castrejón-Godínez ML (2013) Pesticide biodegradation: mechanisms, genetics and strategies to enhance the process. In: Chamy R, Rosenkranz F (eds) Biodegradation-life of science. Intech, China, pp 251–287

Panigrahi S et al (2004) General method of synthesis for metal nanoparticles. J Nanopart Res 6:411–414

Pant G, Mistry SK, Sibi G (2013) Isolation, identification and characterization of p, p0-DDT degrading bacteria from soil. J Environ Sci Technol 6(3):130–137

Park D, Lee DS, Kim YM, Park JM (2008) Bioaugmentation of cyanide-degrading microorganisms in a full scale cokes wastewater treatment facility. Bioresour Technol 99:2092–2096

Pathak VM (ed) (2018) Handbook of research on microbial tools for environmental waste management. IGI Global

Paul D, Pandey G, Pandey J, Jain RK (2005) Accessing microbial diversity for bioremediation and environmental restoration. Trends Biotechnol 23:135–142

Perelo LW (2010) Review: in situ and bioremediation of organic pollutants in aquatic sediments. J Hazard Mater 177:81–89

Pieper DH, Reineke W (2000) Engineering bacteria for bioremediation. Curr Opin Biotechnol 11:262–270

Plangklang P, Reungsang A (2010) Bioaugmentation of carbofuran by *Burkholderia cepacia* PCL3 in a bioslurry phase sequencing batch reactor. Process Biochem 45:230–238

Qu J, Xu Y, Ai GM, Liu Y, Liu ZP (2015) Novel Chryseobacterium sp. PYR2 degrades various organochlorine pesticides (OCPs) and achieves enhancing removal and complete degradation of DDT in highly contaminated soil. J Environ Manag 161:350–357

Quinn J, Geiger C, Clausen C, Brooks K, Coon C, O'Hara S et al (2005) Field demonstration of DNAPL dehalogenation using emulsified zerovalent iron. Environ Sci Technol 39:1309–1318

Rao MA, Scelza Scotti R, Gianfreda L (2010) Role of enzymes in the remediation of polluted environments. J Soil Sci Plant Nutr 10:333–353

Rathour R, Gupta J, Tyagi B, Kumari T, Thakur IS (2018) Biodegradation of pyrene in soil microcosm by *Shewanella* sp. ISTPL2, a psychrophilic, alkalophilic and halophilic bacterium. Bioresour Technol Rep 4:129–136

Ravi RK, Pathak B, Fulekar MH (2015) Bioremediation of persistent pesticides in rice field soil environment using surface soil treatment reactor. Int J Curr Microbiol App Sci 4(2):359–369

Rayu S, Karpouzas DG, Singh BK (2012) Emerging technologies in bioremediation: constraints and opportunities. Biodegradation 23:917–926

Rehmat H (2012) Enzyme based removal of pesticides from soil and water: a review, https://pdfs.semanticscholar.org/2fc9/7968c6787b3c792283672ca79e3c1b91bc35.pdf

Richins R, Mulchandani A, Chen W (2000) Expression, immobilization, and enzymatic characterization of cellulose-binding domain-organophosphorus hydrolase fusion enzymes. Biotechnol Bioeng 69:591–596

Riya P, Jagatpati T (2012) Biodegradation and bioremediation of pesticides in soil: its objectives, classification of pesticides, factors and recent developments. World J Sci Technol 2(7):36–41

Robles-Gonzalez IV, Fava F, Poggi-Varaldo HM (2008) A review on slurry bioreactors for bioremediation of soils and sediments. Microb Cell Factories 7:1

Ruuskanen MO, Colby St G, Pierre St KA, Louis VL, Aris-Brosou S, Poulain AJ (2019) Microbial genomes retrieved from high Arctic lake sediments encode for adaptation to cold and oligotrophic environments. Limnol Oceanogr 9999:115

Sabarwal A, Kumar K, Singh RP (2018) Hazardous effects of chemical pesticides on human health–cancer and other associated disorders. Environ Toxicol Pharmacol 63:103–114. https://doi.org/10.1016/j.etap.2018.08.018

Santos-Merino M, Singh AK, Ducat DC (2019) New applications of synthetic biology tools for cyanobacterial metabolic engineering. Front Bioeng Biotechnol 7

Schreinemachers P, Tipraqsa P (2012) Agricultural pesticides and land use intensification in high, middle and low income countries. Food Policy 37(6):616–626

Schwarzenbach RP, Gschwend PM, Imboden DM (2003) Environmental organic chemistry. Wiley, Hoboken

Seo JS, Keum YS, Li QX (2013) Metabolomic and proteomic insights into carbaryl catabolism by *Burkholderia* sp. C3 and degradation of ten N-methyl carbamates. Biodegradation 24:795–811. https://doi.org/10.1007/s10532-013-9629-2

Shah SI et al (2003) Semiconductor metal oxide nanoparticles for visible light photocatalysis, (0210284). NSF nanoscale science and engineering grantees conference Dec 16–18

Shankar J, Abhilash PC, Singh HB, Singh RP, Singh DP (2011) Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. Gene 480:19

Shen YJ, Lu P, Mei H, Yu HJ, Hong Q, Li SP (2010) Isolation of a methyl parathion-degrading strain *Stenotrophomonas* sp. SMSP-1 and cloning of the ophc 2 gene. Biodegradation 21:785–792. https://doi.org/10.1007/s10532-010-9343-2

Shimizu H (2002) Metabolic engineering–integrating methodologies of molecular breeding and bioprocess systems engineering. J Biosci Bioeng 94:563–573

Singh RL (ed) (2017) Principles and applications of environmental biotechnology for a sustainable future. Springer, Singapore

Singh P, Saini HS, Raj M (2016) Rhamnolipid mediated enhanced degradation of chlorpyrifos by bacterial consortium in soil-water system. Ecotoxicol Environ Saf 134:156–162

Spanoghe P, Maes A, Steurbaut W (2004) Limitation of point source pesticide pollution: results of bioremediation system. Commun Agric Appl Biol Sci 69(4):719–732

Vandera E, Samiotaki M, Parapouli M, Panayotou G, Koukkou AI (2015) Comparative proteomic analysis of *Arthrobacter phenanthrenivorans* Sphe3 on phenanthrene, phthalate and glucose. J Proteome 113:73–89. https://doi.org/10.1016/j.jprot.2014.08.018

Varjani SJ, Joshi RR, Kumar PS, Srivastava VK, Kumar V, Banerjee C, Kumar RP (2018) Polycyclic aromatic hydrocarbons from petroleum oil industry activities: effect on human health and their biodegradation. In: Varjani SJ, Gnansounou E, Gurunathan B, Pant D, Zakaria ZA (eds) Waste bioremediation. Springer, Singapore, pp 185–199

Vidali M (2001) Bioremediation: an overview. Pure Appl Chem 73(7):1163–1172

Vikrant K, Giri BS, Raza N, Roy K, Kim KH, Rai BN et al (2018) Recent advancements in bioremediation of dye: current status and challenges. Bioresour Technol 253:355–367

Wang XX, Chi Z, Ru SG, Chi ZM (2012) Genetic surface-display of methyl parathion hydrolase on Yarrowia lipolytica for removal of methyl parathion in water. Biodegradation 23:763–774. https://doi.org/10.1007/s10532-012-9551-z

Wang L, Chi XQ, Zhang JJ, Sun DL, Zhou NY (2014) Bioaugmentation of a methyl parathion contaminated soil with *Pseudomonas* sp. strain WBC-3. Inter Biodeter Biodegr 87:116–121

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. https://doi.org/10.1016/j.chemosphere.2017.03.049

Wu NF, Deng MJ, Liang GY, Chu XY, Yao B, Fan YL (2004) Cloning and expression of ophc2, a new organophosphorus hydrolase gene. Chin Sci Bull 49:1245–1249

Xu G, Zheng W, Li Y, Wang S, Zhang J, Yan Y (2008) Biodegradation of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol by a newly isolated *Paracoccus* sp. strain TRP. Int Biodeterior Biodegrad 62:51–56

Yang X, Wei H, Zhu C, Geng B (2018) Biodegradation of atrazine by the novel *Citricoccus* sp. strain TT3. Ecotoxicol Environ Saf 147:144–150

Yu H, Yan X, Shen W, Hong Q, Zhang J, Shen Y, Li S (2009) Expression of methyl parathion hydrolase in *Pichia pastoris*. Curr Microbiol 59:573–578

Zhao B, Poh LC (2008) Insights into environmental bioremediation by microorganisms through functional genomics and proteomics. Proteomics 8:874–881

Zhou J, He Z, Van Nostrand JD, Wu L, Deng Y (2010) Applying GeoChip analysis to disparate microbial communities. Microbe 5:60–65

Chapter 9
Removal of Dyes From Industrial Effluents Using Bioremediation Technique

Narayana Saibaba KV

Abstract Rapid growth in unstrategic development and industrialization over the few decades triggered the environmental problems worldwide. Synthetic dyes are one of the major pollutants discharged from industries such as textile, dying, printing, tannery, etc. Commonly used physicochemical techniques for the recovery of metals and dyes are electrodialysis, ozonization, flocculation, coagulation, adsorption, etc. Procurement and regenerative costs associated with these methods provided the opportunity for the scientists to search for the novel, simple, effective, ecofriendly, and cost-efficient methods. This search has given a wide scope for the use of bioremediation technique for the treatment of metals and dyes from industrial effluents. Bioremediation involves the use of natural organism for the management of industrial effluents which break down the substances aerobically and anaerobically and transform them into other components or eliminates/minimize the adverse effects of pollutants. The advantages of these methods are that these can be used in situ or ex-situ. These bioremediation methods include processes such as biosorption, bioaugmentation, bioleaching, biostimulation, etc. The present chapter highlights the application of bioremediation methods for the removal of dyes from industrial effluents. Bioremediation abilities of various biomasses in the removal of dyes are emphasized in this chapter. In the present chapter, a detailed discussion is presented on various bioremediation methods, microorganism used in bioremediation of dyes, and their advantages and disadvantages. Additionally, various mechanisms involved in bioremediation of dyes have been explained. The technical and economic aspects of the process for large-scale applications have been discussed.

Keywords Bioremediation · Biodegradation · Biosorption · Dyes · Adsorption · Textile effluents

---

N. S. KV (✉)
GITAM University, Visakhapatnam, India
e-mail: skvn@gitam.edu

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_9

1 Introduction

Rapid urbanization and industrialization during past few decades resulted the global economic growth by providing food security and job security to mankind. But at the same time, nonstrategic urbanization and industrialization led to pollution due to discharge of the large quantities of pollutants such as organic and inorganic chemicals, metals, dyes, etc. into the environment. Textile, dying, printing, leather, and tannery industries use various synthetic dyes for imparting color and aesthetic value to their products and discharge large quantities of pollutants into the environment. Effluents from these industries are highly colored, contain toxic dyes and metals, and have a deleterious effect on the environment (Saibaba et al. 2012). These metal dyes contaminate the soil and enter into the food chain through accumulation in plants, vegetables, grains, and fruits. Consumption of these food products create many health hazards to humans and other animals living on the earth and water. Thus, these pollutants need to be treated before they are discharged into the water bodies.

Textile industry is not only one of the principal users of synthetic dyes but also the largest generator of effluent containing hazardous dyes. Textile, dyeing, and printing industries generate large quantities of dye effluents from processes such as textile wet processing, dyeing, washing, etc. More than 8000 chemical produces are associated with dyes and dying processes. Over 7×10^5 metric tons of dyes are being produced annually from over 10^5 types of commercial dyes. Most of these dyes are wasted during the textile dying and printing processes; this wastage could be in the range of 2% for basic dyes to 50% for reactive dyes. On an average, approximately 15% of the dyes used in the textile industry are reaching the environment through effluents and are causing severe land and water contamination. Most of these dyes are physically, chemically, and photolytically stable, which persist in the environment for longer periods. Bioaccumulation of these dyes in the food chain causes serious health hazards to humans and other living animals. Long-term exposure of these dyes is carcinogenic and mutagenic to humans and causes skin irritation, eye blindness, nausea, cancer, etc. (Saibaba et al. 2011, 2012; Saibaba and King 2013a; Vital et al. 2016).

Various types of synthetic dyes such as acidic dyes, basic dyes, reactive dyes, metal complex dyes, sulfur dyes, and fluorescent dyes, are being used for various applications. Textile industries also use dichromates or chromium complexes as mordents (Aksu and Karabayır 2008; Arpita et al. 2015). Most of the synthetic dyes used in the textile industry are metal complexes. Metal complex dyes are made up of dyes and heavy metals such as Cr, Co, Cu, Pb, etc. Long-term contact of chromium causes health problems such as irritation in the lungs, stomach ache, lowered immunity, and also damage to the health hormones. Accumulation of chromium in plants inhibits the photosynthesis, damages the tissue, and decreases the growth (Shanker et al. 2005; Li et al. 2013; Arpita et al. 2015). Cobalt exposure for longer times may create asthma, dermatitis, cancer, etc. (Min et al. 2013). Long-term exposure of copper may result to kidney and liver failures and brain damage and sometimes leads to death (Saha et al. 2010; Ghosh and Das 2014). Lead exposure may lead to severe kidney failure and nervous system failure and may damage the reproductive system (Wu et al. 2014).

Synthetic dyes used in the textile industry are highly colored, stable, and designed to resist the effects of laundering and sunshine. Dye-containing effluents are generally treated using conventional physicochemical methods such as ion exchange, membrane separation, osmosis, adsorption, coagulation, flocculation, etc. Most of these methods involve high cost of operation, low efficiency, and limited versatility and produce large quantities of sludge and create disposal problems (Saibaba and King 2013a; Priya and Nagan 2015). Thus, the development of efficient, simple, easy to use, economical, and environmental-friendly technologies for dye removal is of utmost importance. Over the past few decades, dye removal using bioremediation has been gaining importance. In bioremediation microorganisms remove the dyes from effluents through various processes such as biotransformation, biodegradation, biosorption, etc. Bioremediation methods are relatively inexpensive and efficient and offer a wide choice in the use of microorganisms. Bioremediation can be used for complete removal of dyes; these methods produce less sludge, and more importantly, these methods are ecofriendly.

2 Bioremediation

Textile dyes are one of the major pollutants discharged into the water bodies. These dyes are very harmful to humans and other flora and fauna living on the earth. The presence of dyes, even in very small concentrations, gives bright color to the water and makes them unusable (Saibaba and King 2013b). Even as low as 1 ppm concentration of synthetic dye gives a bright color and makes the water unaesthetic. But the textile industry effluents contain very high concentrations of dyes ranging 10 ppm to 200 ppm. Industrial dye effluents contain significant amounts of suspended solids, BOD/COD ratio from 0.2 to 0.5, and high fluctuations in pH, temperature, and salinity and affect the environment adversely. Hence, these effluents need to be treated before they are discharged into the environment for environmental protection and sustainability. Most of the dyes are brightly colored, soluble in water, reactive, and very difficult to treat by conventional water treatment methods. However, most of the dyes, metals, and other organic materials can be degraded by biological methods. Biological methods are ecofriendly and produce good results for dye removal processes. Large number of bacteria, fungi, and algae are found to be effective for the removal of dyes by bioremediation methods.

Bioremediation is a process in which organic and inorganic pollutants are degraded or transformed into less toxic components through biological processes (Mueller et al. 1996). In bioremediation, toxic components are detoxified or degraded to less toxic components by intracellular accumulation or by enzymatic reactions. In bioremediation, both living and nonliving biomass can be used. Bioremediation is very effective for the removal of pollutants from the different contaminated areas such as soil and solid waste materials such as sludge, industrial effluents, etc. According to Huang et al. (1991), in bioremediation, organic and inorganic pollutants are biologically degraded or transformed into innocuous materials naturally or by adding bioremediation-promoting components such as electron acceptor, nutrients, etc.

Bioremediation techniques are more advantageous compared to conventional methods as it can be implemented on site, therefore, minimizing personnel risks, and sludge disposal problems due to the generation of less effluent volume when compared to traditional approaches (Yadav et al. 2017). It is reported in the literature that many organisms such as bacteria, fungi, and algae have the ability to degrade, transform, or chelate the dye pollutants into harmless or less toxic components either naturally or by genetic modification (Mosa et al. 2016). Bioremediation process is a very effective approach due to the ability of microorganisms to interact with various types of pollutants. Microorganisms used in bioremediation can transform dye pollutants from one oxidative state to another; thus, it reduces the toxic effects of the pollutants. Bioremediation through microbial metabolism offers a viable, safer, efficient, and less expensive method for the removal of pollutants.

3 Microorganisms Used in Bioremediation

In recent years, research on the utilization of microorganisms in bioremediation for the treatment of textile dye effluents has been in focus. Various studies reported the mechanisms of bioremediation of dye effluents using microorganisms such as bacteria, fungi, and algae. Studies were also conducted on the ability of living and nonliving organisms, chemically and physically treated organisms, and genetically modified organisms in bioremediation of dyes. In bioremediation, both microorganisms present in the contaminated area and microorganisms isolated from the other sites can be used. This allows the use of a wide variety of microbes for the dye removal process. However, studies reported that microorganisms existing in the contaminated area showed high tolerance towards environmental fluctuations and high ability in removing dyes from the effluents. Several studies showed that many microorganisms could tolerate the heavy metals present in dye effluents and are able to degrade them into less toxic components or completely eliminate them by utilizing them for growth metabolic processes (Qazilbash 2004).

Microbial resistance and tolerance towards metal ions in dye effluents are very important parameters in the success of bioremediation process since these microbes have close contact with the pollutants for long durations during the degradation of pollutants. The heavy metal resistance of microorganisms helps in the bioremediation process in two ways; first is by reducing the concentration of metal ions through the elimination of metal-chelating substances or by the breakdown of a specific transport mechanism. Second is by the binding of ions on the intracellular microbial cells (Joho et al. 1995).

Many bacteria such as *Staphylococcus arlettae* (Elisangela et al. 2009); fungi such as *Trametes pubescens, Pleurotus ostreatus* (Casieri et al. 2008), *Aspergillus niger, Fusarium oxysporum*, and *Fusarium oxysporum* (Shahid et al. 2013); and algae such as *Chlorella vulgaris*, *Elkatothrix viridis*, and *Volvox aureus* (El-Sheekh et al. 2009) were isolated and studied by many researchers for their potential in the bioremediation of dyes. These studies reinforced the potential of microorganisms as efficient and ecofriendly organisms for the removal of dyes from industrial effluents.

3.1 Bacteria

Bioremediation of industrial dye effluents is carried out using various microorganisms such as bacteria, fungi, and algae. Different organisms follow different mechanisms in dye removal. Bacteria are found to be very efficient in the removal of dyes from industrial effluents due to their size and their ability to grow in different environmental conditions. The bacterial surface is made up of various functional groups such as carboxyl, phosphate, amine, hydroxyl, etc. These groups facilitate the biosorption of metal complex dyes from the industrial effluents. For example, the negative charge of carboxyl groups present on the bacterial cell wall can bind positive dye ions through electrostatic interactions. In general increasing the pH increases the negative charge on the cell wall due to deprotonation and favors the adsorption of positive metals and other dye complexes. Similarly decreasing pH below 7 protonates the cell wall surface and increases the positive charge on the cell wall and favors the adsorption of negative metals and other dye complexes. Solution pH may also alter the dyes, and hydrolysis reactions can take place.

Biological techniques are simple to design and economical to operate; thus, they have become one of the highly focused technologies for dye removal. Bioremediation using microorganisms involves the microbial degradation and enzymatic removal of dyes. Many bacteria were identified as potential and economic dye removal components. Some of the important bacteria used in the bioremediation of textile dyes are tabulated in Table 9.1.

3.2 Fungi

Fungi are available in high quantities due to its ability to adapt to any type of ecosystem and grow easily under various environments. It is recognized as potential microorganism for dye removal from industrial effluents. Both naturally occurring fungi and genetically modified fungi are reported to be efficient in bioremediation process. Fungi contain various extracellular proteins, organic acids, and other metabolites. Fungal surface primarily consists of carboxyl, amino, phosphate, hydroxyl, and sulfhydryl groups on the cell wall surface. These functional groups are responsible for the binding of various dyes on the cell wall through electrostatic

Table 9.1 Studies on bioremediation of dyes using bacteria

S. No.	Microorganism name	Dyes studied	References
1	*Lactobacillus casei, L. paracasei, L.rhamnosus*	Reactive Lanasol black B (RLB), Eriochrome red B (RN), I. yellow (SGL)	Khaled et al. (2010)
2	*Pseudomonas putida*	Golden yellow	Leebana et al. (2012)
3	*Micrococcus luteus, Listeria denitrificans, Nocardia atlantica*	Orange W3R, red FNR, yellow FN2R, blue FNR or navy WB	Hassan et al. (2013)
4	*Bacillus megaterium*	Turquoise blue	Joshi et al. (2013)
5	*Bacillus* sp.	Reactive red 239 (RR239)	Guadie et al. (2017)
6	*Pandoraea pulmonicola YC32*	Malachite green	Chen et al. (2009)
7	*Acinetobacter calcoaceticus YC210*	Victoria blue R (VBR)	Chen et al. (2011)
8	*Comamonas* sp.	Direct red 5B	Jadhav et al. (2008)
9	*Basillus* sp.	Brown 3REL	Dawkar et al. (2008)
10	*Lyngbya lagerlerimi, Oscillatoria rubescens, Nostoc linckia*	Methyl red, orange II, G-red (FN-3G), basic cationic, basic fuchsin	El-Sheekh et al. (2009)
11	*Proteus mirabilis*	Red RBN	Chen et al. (1999)
12	*Aeromonas hydrophila*	Crystal violet, basic fuchsin, brilliant green, malachite green	Ren et al. (2006)
13	*Pseudomonas* sp.	Reactive black-5	Hussain et al. (2013)
14	*Pseudomonas aeruginosa*	Reactive red-120, reactive black-5, reactive yellow-2, and reactive orange-16	Maqbool et al. (2016)
15	*Proteus* sp. *NA6*	Reactive yellow-2 (RY2)	Abbas et al. (2016)
16	*Psychrobacter alimentarius, Staphylococcus equorum*	Reactive black 5, reactive golden ovifix, reactive blue BRS	Khalid et al. (2012)
17	*Neisseria* sp., *Vibrio* sp., *Bacillus* sp., *Bacillus* sp., *Aeromonas* sp.	Novacron orange FN-R, Novacron brilliant blue FN-R, Novacron super black G, Bezema yellow S8-G, Bezema red S2-B	Karim et al. (2018)
18	*Lactobacillus delbrueckii*	Reactive orange 16(RO 16) and reactive black 5(RB 5)	Siti et al. (2013)
19	*Enterobacter* sp. CV–S1	Crystal violet	Roy et al. (2018)
20	*Staphylococcus hominis*	Acid orange	Singh et al. (2014)

attractions and complexation reactions. In addition, during the biosorption process, chitin present on the cell wall forms complexation reactions with dyes and facilitates dye removal.

Fungal bioremediation was found to be more attractive than bacterial bioremediation due to availability of higher amounts of biomass at a cheaper cost (Shahid et al. 2013). The excellent functions of these fungi in biodegradation of textile dyes suggest the use of these microorganisms in the bioremediation process. Fungi can be used as an alternative for efficient treatment of wastewaters contaminated with textile dye effluents. Many researchers studied the efficiency of fungal biomass in the removal of dyes from effluents. The white rot fungi proved to be very efficient for the removal of azo dyes from the effluents. *Phanerochaete chrysosporium* is capable of metabolizing various types of azo dyes. Studies on fungi (*Aspergillus niger* and *Phanerochaete chrysosporium*) isolated from soil contaminated with the dye effluents (malachite green, nigrosin, and basic fuchsin) showed excellent bioremediation properties (Rani et al. 2014). Some of the important organisms studied for the removal of dyes are highlighted in Table 9.2.

3.3 Algae

The utilization of algal biomass has been in focus since the realization of its potentials in bioremediation of textile dye effluents. Algae are found to be potential organisms for bioremediation of dyes due to their ability to grow both in fresh water and saltwater. Algae are photoautotroph organisms; they can synthesize food by absorbing CO_2 directly from the air and light energy from the sun. Immobilized algae cells were reported to be efficient in the removal of metal dye complexes. Both living and nonliving algae were reported to have high potential in the removal of dyes especially in biosorption process. High surface area and cell wall properties of the algae favor the biosorption of dyes. Functional groups such as carboxyl, amino, sulphonic, phosphorous, hydroxyl, carbonyl, etc. present on the algae cell wall surface enable the biosorption of various types of dyes from the industrial effluents through electrostatic attractions and complexation reactions. Microalgae and macroalgae were found to remove dyes by various processes such as biosorption, biodegradation, and biotransformation. Microalgae degrade dyes by removing nitrogen, phosphorous, and carbon from water, thus reduces the eutrophication in the water. Algae's tolerance ability to waste waters and its rapid growth makes it available in large quantities for dye removal process. Table 9.3 depicts the important dye removal studies carried out using algae.

Many organisms isolated from the environment were found to be useful in the bioremediation process. However, their efficiency depends on the physicochemical conditions present in the water. Wide ranges of organisms are available with different metabolic activities and hence suitable for bioremediation of different dyes. The success of the organisms depends on their survival capacity to such

Table 9.2 Studies on bioremediation of dyes using fungi

S. No.	Microorganism name	Dyes studied	References
1	*Trametes villosa, Pycnoporus sanguineus*	Drimaren brilliant blue dye	Machado et al. (2006)
2	*Pleurotus ostreatus Gloeophyllum odoratum, Polyporus picipes, Fusarium oxysporum*	Triphenylmethane brilliant green, azo Evans blue	Przystas et al. (2015)
3	*Aspergillus tamarii, Penicillium purpurogenum*	Brilliant blue, Bromophenol blue, malachite green	Ramalingam et al. (2010)
4	*Aspergillus niger*	Congo red	Asses et al. (2018)
5	*Pycnoporus sanguineus*	Remazol brilliant blue R, reactive black 5, reactive red 195, and reactive yellow 145	Marim et al. (2016)
6	*Pleurotus ostreatus*	Remazol RG, Livafix red CA, Prucion navy PXG and Prucian blue PX5R	Siddique et al. (2012)
7	*Aspergillus niger, Phanerochaete chrysosporium*	Malachite green, Nigrosin and basic fuchsin	Rani et al. (2014)
8	*Aspergillus terreus SA3, Aspergillus flavus SA2, Alternaria* sp. *SA4, Penicillium* sp.	Acid red (AR) 151, orange II	Ali et al. (2010)
9	*Issatchenkia occidentalis*	Methyl orange, acid orange 7	Ramalho et al. (2004)
10	*Phanerochaete chrysosporium*	Direct violet 51 (DV), reactive black 5 (RB), Ponceau Xylidine (PX), and Bismarck brown R (BB)	Enayatzamir et al. (2010)
11	*Phanerochaete chrysosporium*	Acid red −18	Vasudevan and Kanimozhi (2011)
12	*Micoteca da Universidade do Minho*	Reactive black 5, poly R-478	Ottoni et al. (2013)
13	*Penicillium ochrochloron*	Malachite green	Shedbalkar and Jadhav (2011)
14	*Phanerochaete chrysosporium*	Reactive black 5, poly R-478.	Ottoni et al. (2013)
15	*Candida tropicalis*	Remazole blue, Remazole black B	Aksu and Donmez (2005)
16	*Aspergillus Niger, Aspergillus terreus*	Procion red MX-5B	Almeida and Corso (2014)
17	*Pleurotus ostreatus, Gloeophyllum odoratum, Polyporus picipes*	Brilliant green, Evans blue	Przystaś et al. (2018)
18	*Aspergillus Niger*	Direct violet	El-Rahim et al. (2008)
19	*Lentinus crinitus*	Reactive blue 220 (RB220) and reactive black 5 (RB5), and anthraquinone dye, Remazol brilliant blue R (RBBR)	Tavares et al. (2020)
20	*Lentinus crinitus*	Remazol brilliant blue R	Almeida et al. (2018)

Table 9.3 Studies on bioremediation of dyes using algae

S. No.	Microorganism name	Dyes studied	References
1	*Cosmarium* species	Malachite green	Daneshvar et al. (2006)
2	*Chara* sp.	Malachite green	Khataee et al. (2010a, b)
3	*Chlorella vulgaris*, *Elkatothrix viridis*, *Volvox aureus*	Methyl red, orange II, G-red (FN-3G), basic cationic, basic fuchsin	El-Sheekh et al. (2009)
4	*Sargassum horneri*	Malachite green	Angelova et al. (2016)
5	*Cladophora* sp.	Malachite green	Khataee and Dehghan (2011)
6	*Chlorella, Cosmarium, Euglena* species	Malachite green (MG)	Khataee et al. (2010a, b)
7	*Caulerpa scalpelliformis*	Basic yellow	Aravindhan et al. (2007)
8	*Pithophora* sp.	Malachite green	Kumar et al. (2006)
9	*Daphnia magna*	Cibacron blue 3GA	Bayramoglu et al. (2019)
10	*Chlorella vulgaris*	Congo red (CR)	Hernández-Zamora et al. (2015)
11	*Filamentous algae (AAC)*	Basic blue 41 (BB 41)	Afshin et al. (2018)
12	*Spirulina platensis microalgae (SP)*	Reactive red 120 (RR-120)	Cardoso et al. (2012)
13	*Ulva lactuca*	Basic blue 9	El Sikaily et al. (2006)
14	*U. lactuca*	Direct yellow 12	El Nemr et al. (2006)
15	*Spirogyra*	Direct Brown 2-Diazo	Mohan and Karthikeyan (2000)
16	*Sargassum wightii*	Methylene blue (MB)	Kumar et al. (2015)
17	Sargassum oligocystum	Methylene blue (MB) and methyl violet (MV)	Foroutan et al. (2019)
18	*Ulva intestinalis*	Methyl orange	Poorni et al. (2020)
19	*Pithophora sp*	Malachite green	Kumar et al. (2006),
20	*Nitzschia perminuta*	Tartrazine, Ponceau ss	Omar (2008).

extremely harsh conditions and their ability to metabolize and detoxify or degrade or biotransform the pollutants.

4 Types of Bioremediation

Biological methods are found to be efficient for the removal of dyes from the effluents. Bioremediation of pollutants involves one or a combination of many processes such as diffusion of material between cell wall and liquid phase, biosorption of dye particles onto the cell wall, precipitation, complexation, and oxidation-reduction reactions (Joutey et al. 2015; Avery and Tobin 1993; Brady and Duncan 1994; Krauter et al. 1996; Veglio and Beolchini 1997). Bioremediation

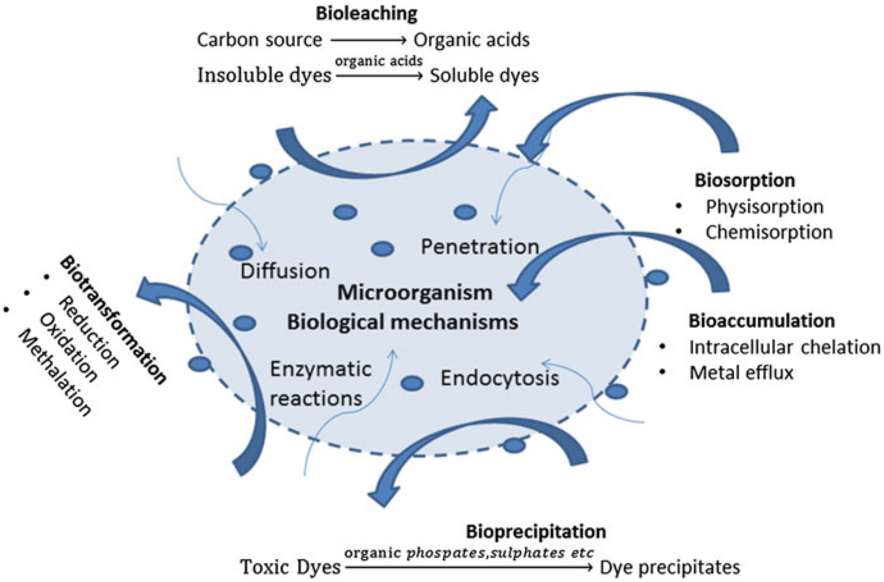


Fig. 9.1 Basic bioremediation mechanisms for dye removal

of dyes involve processes such as biosorption, bioaccumulation, bioaugmentation, biostimulation, bioleaching, oxidation, reductions, etc. Figure 9.1 shows the basic mechanisms involved in the bioremediation of dyes (Jin et al. 2018).

4.1 Biosorption

Biosorption process is a physicochemical interaction between the pollutant and cellular compounds of biomass. It involves the accumulation of contaminants such as dyes, metals, etc. onto the biosorbent surface through metabolically mediated or physicochemical pathways (Abbas et al. 2014). Biosorption methods are very efficient for the separation of challenging biodegradable pollutants such as dyes and metals from the liquid phase. In biosorption, pollutants transfer from fluid to solid phase and accumulate on the surface of the biosorbent surface through ion exchange, precipitation, complexation, etc. mechanisms and reduce the pollutant concentration in water. Biosorption of dyes may also involve the formation of stable complexes with organic ligands and redox reactions from which pollutants can move into the cell. Figure 9.2 pictorially shows the basic mechanisms involved in the biosorption of dyes from the textile dye effluents (Bahafid et al. 2017).

Biosorption is a surface phenomenon in which cell surface plays a vital role in the biosorption process. Microorganisms cell surface is principally made up of cell wall; the mucus layer around the cell wall facilitates the easy adsorption of dye pollutants.

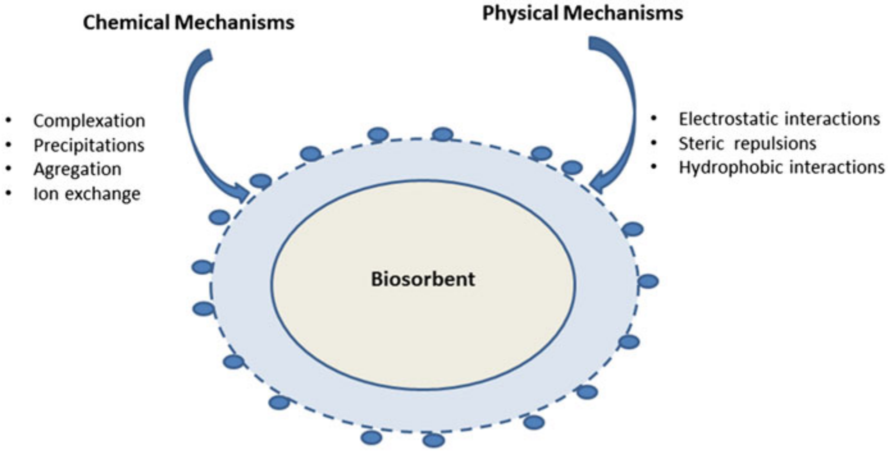


Fig. 9.2 Biosorption mechanisms of dyes

The cell surface contains many functional groups such as nitrogen, oxygen, sulpur, phosphorous, etc. These functional groups are responsible for the bonding of dye particles on the cell surface. Alive or dead biomass can be used as biosorbent in biosorption technique. Biosorbent can be regenerated for the recovery of pollutants and can be used again for the biosorption process (Lima et al. 2008).

4.2 Bioaccumulation

Bioaccumulation is defined as the process in which toxic pollutants are transported into the cell of a living organism. It is an intracellular process, and hence, a very complex mechanism is involved in bioaccumulation. The success of the bioaccumulation process depends on the growth of the organism; hence, suitable physicochemical conditions are required for this process. Bioaccumulation of dye particles is achieved in two steps (Chojnacka 2009). In the first step, dye pollutants are rapidly adsorbed on to the living cell wall by one or a combination of physisorption, i.e., electrostatic attraction, complexation, chelation, and ion exchange processes. This process is passive and rapid and completes in a very short time. The second step is the intracellular process, which is a very complex and slow process. In this step, the dye pollutants slowly penetrate into the cell membrane and reach into the cells. Dye pollutants transferred through the cell membrane get converted to other species or precipitated within the cell by the living organisms. The penetration of dye and other contaminated particles into the cell membrane and transport into the cells is an active process, which requires respiration of organisms. Bioaccumulation is more complex than the biosorption process; the complete mechanism of this process is not yet fully known. Cytoplasmic detoxification is one of the major strategies in the bioaccumulation of metal ions from the dye effluents. This cytoplasmic detoxification is achieved by carrying the dye pollutants external to the cell or to less subtle cellular compartments, thus making the pollutants not available for stimulating the cytotoxic effects. Living biomass may develop strategies such as metal efflux, intracellular chelation by metallothionein protein and phytochelation, and metal compartmentalization in vacuoles for the detoxification, but the exact mechanism of intracellular accumulation is still not explained (Tamás and Wysocki 2010; Kamizono et al. 1989; Kneer et al. 1992; Presta and Stillman 1997).

Studies conducted on the investigation of the mechanism involved in metal detoxification process confirm the importance of vacuole in the detoxification of pollutants from the industrial effluents. The detoxification process is achieved by one or a combination of the processes such as biological degradation, storing of metabolites, and control of cytosolic concentrations of pollutants. Studies showed that the cells having lower vacuoles were highly sensitive to environmental conditions and had less metal removal capacities (Ramsay and Gadd 1997; Thorsen et al. 2012). Bioaccumulation process is governed by the structural, physical, and biological properties, genetic mutations, population of the living organisms, and also concentration of pollutants. The growth of living cells depends on the composition of the growth medium, pH, temperature, and presence of other pollutants, inhibitors, surfactants, etc. The mechanism involved in the bioaccumulation process is also influenced by various metabolic activities of the organisms such as respiration, metabolite release, nutrient intake, etc. (Gadd 2009; Kujan et al. 1995).

4.3 Biosorption and Bioaccumulation

In both biosorption and bioaccumulation methods, pollutants or contaminants transfer from the environment to the surface of the microorganism or biomass. In biosorption, contaminants adsorb onto the biomass cell wall surface. The rate of adsorption depends on the cell composition, cell wall structure, and kinetics of the process. Biosorption is a passive process and does not require any energy/respiration, whereas bioaccumulation is an active metabolic process and requires energy and respiration. In biosorption, pollutants adsorb on the cellular surface mostly by a physical process; hence, it may be reversible. On the other hand, bioaccumulation is partially reversible; moreover, biosorption process is fast compared to the bioaccumulation (Vijayaraghavan and Yun 2008; Velásquez and Dussan 2009).

Several studies reported that bioaccumulation process is more effective in the removal of metal ions present in the dyes compared to biosorption. However, the biosorption process is very attractive and economically feasible in large-scale applications compared to bioaccumulation process since the bioaccumulation process requires the close control of the growth of living organisms that are very complex, costly, and difficult. Moreover, wide varieties of biosorbents are able to adsorb these pollutants, including bacterial, fungi, algae, yeast, industrial waste, and agricultural waste; therefore, biosorption finds more attraction in industrial use.

4.4 Bio-oxidation/Bioreduction

Bio-oxidation/bioreduction and bioleaching processes are the two main categories of biomining process. Bioreduction is the process in which a reduction of metal ions to the lower redox state takes place and is responsible for the detoxification process. In bioreduction, removal of pollutants can be achieved either by oxidation or reduction reactions (Siddique et al. 2012). The detoxification mechanism involved in the bioreduction process is not clear. The information available in the literature regarding eukaryotic cells and yeast cells related to bioreduction is limited, and its exact mechanism is not known yet. The type of mechanism, such as enzymatic or nonenzymatic and intracellular or extracellular, responsible for the removal of metal dyes is not known completely.

Microorganism participates in the oxidation reactions change the valence of pollutants present in the effluents and thereby enhances mobility and reduces the toxic effect of the pollutants. Bio-oxidation studies reported that microorganisms could reduce the valence of mercury from Hg^{2-} to easily evaporated Hg^0 by aerobic bacteria; water-soluble and toxic chromium can be reduced from Cr^{6-} to less toxic and water-insoluble Cr^{3-} by the *Corynebacterium* (Chanmugathas and Bollag 1988; Marchenko et al. 2015).

4.5 Bioleaching

Bioleaching is the process in which pollutants are removed from the contaminant source by microorganisms. Bioleaching is also defined as an alteration of solid pollutants into water-soluble forms with the help of microorganisms. Pollutant solubility in the microorganism stains is the key factor in bioleaching. In contrast to bioleaching, immobilized bioremediation methods such as biosorption, bioaccumulation, and precipitation methods involve the transformation of pollutants into an insoluble form.

In the bioleaching process, metal dye ions are mobilized from contaminated soil or effluents by biological dissolution and complexation mechanisms. Secretions resulted from the microbial metabolism are responsible for the bioleaching process. Low-molecular-weight organic acids (such as oxalic acid, citric acid, etc.) produced by the microorganisms can dissolve heavy metal dye ions and other pollutants present in the contaminant site. It is reported in the literature that pollutants concentrate on solid waste through sorption, ion exchange, or complexation reactions. However, in acidic environments, most metals show high mobility, and it was reported that fungi could predominantly influence the solubility of metals under acidic conditions (Gadd 1993). Gadd (1993) proved that microbial interaction with metal pollutants is significant in both natural and synthetic environments in altering the physical and chemical states of the pollutants. He further reported that the biochemical activity of fungi and other microorganisms significantly influences the mobility characteristics and modifies the biogeochemical cycles. Acydolysis and complexolysis were reported to be important reactions responsible for the solubilization of metal dyes. Metabolites having metal-complexing properties such as phenolic compounds and organic acids are also found to be involved in metal solubilization processes. Organic acids having low molecular weight are found to be good leaching agents. It was reported in the literature that low-molecular-weight organic acids provide protons required for solubilization process and metal chelating anion to participate in the metal complexation reaction with the metal cations. Organic acids are useful in two ways: first, they acidify the substrate which in turn enhances the mobility characteristics of metal ions, and the second one is they form complex reaction responsible for the solubility of metal ions (Gadd 1999; 2004). Bioleaching process also depends on the nutrient availability; studies showed that bioleaching rate significantly improved with nutrient uptake. Some microorganisms such as *Citrobacter* may also form insoluble phosphate coat on the cell surface that can entrap dye pollutants during bioleaching.

4.6 Bioaugmentation

Bioaugmentation is the process of adding specific pure microbial strains or mixed strains to the contaminated sites for the removal of pollutants. Added microorganisms alter the physicochemical characteristics of the pollutants and detoxify the site. Indigenous microorganism, i.e., microorganisms already present in the contaminated site, may not be available in sufficient quantity or may not be efficient in removing mixed pollutants; hence, adding additional amounts of the required type of microorganisms enhances the detoxification process (Leahy and Colwell 1990; Herrero and Stuckey 2015). Studies on the application of various bacteria, yeast, and fungi in bioaugmentation of dyes were conducted worldwide. Studies conducted on microorganisms such as *C. fabianii, C. tropicalis, and Candida digboiensis* in the bioaugmentation of pollutant removal showed the efficiency of these microorganisms in pollutant removal from the site. It is reported that the application of mixed microbial cultures rather than the pure cultures is more efficient in bioaugmentation process. Addition of mixed microbial cultures follows different metabolic pathways and creates suitable environments required for bioremediation of pollutants (He et al. 2014).

4.7 Biostimulation

Biostimulation and bioaugmentation are very important strategies in executing the bioremediation process. Biostimulation is the process in which microbial growth-stimulating agents such as electron acceptors, nutrients, etc. are added to the contaminated site to enhance the growth of existing microbial population, whereas in bioaugmentation microorganisms are added to the contaminated site to enhance microbial degradation process (Dedyukhina and Eroshin 1991). In biostimulation, the addition of nutrients such as glucose as carbon and energy source for the removal of dyes stimulates the production of enzymes for the co-metabolism of glucose and enhances the dye removal process (Semrany et al. 2012).

5 Issues Related to Bioremediation

In spite of bioremediation is simple, easy, efficient, and economical technique for the removal of dyes from industrial effluents, it has not been used widely in large-scale industrial applications. The constraints associated with bioremediation technique in dye removal need to be addressed for its use in large-scale applications. One of the main problems restricting the use of bioremediation in large-scale applications is the use of live microorganisms. Living organisms require nutrients for their continuous growth and function. Microorganisms need both micro- and macronutrients such as carbon (C), hydrogen (H), nitrogen (N), sulfur (S), phosphorous (P), potassium (K), copper, cobalt, zinc, iron, etc. A phototropic alga gets CO_2 directly from the air and does not require any additional supply of nutrients. Various types of microorganisms, such as bacteria, fungi, and algae, are used in bioremediation, and they require different nutrients for their growth. The use of large quantities of nutrients and various types of nutrients restrict the use in large-scale applications due to economic constraints. Economical and nutrient-rich medium is to be developed for its use in large-scale applications. Abundantly and cheaply available plant, animal, and waste medium are a good option for this purpose.

Continuous monitoring and controlling of bioremediation process in large-scale industrial applications is very difficult and depends on many factors such as soil type, temperature and pH of the medium, oxygen availability, nutrient availability, microbial population, electron availability, etc. (Khan et al. 1997). Furthermore, most of the studies conducted on bioremediation were carried in a laboratory under sterile conditions. But industrial effluents are highly complex and contain a mixture of various pollutants like metals, dyes, phenols, etc., and physical conditions maintained in mixed cultures may not be suitable for all organisms. Hence, highly resistant microorganisms are needed to be isolated and can be used for bioremediation of industrial effluents. Isolated microorganisms are expected to give better results compared to mixed cultures. However, using pure cultures for bioremediation may not be economical; therefore, there is a need to develop and use the indigenous organisms to make the process economically viable. Moreover, nutrient cost and aeration and agitation costs add extra economic burden in large-scale applications. Development of new bioremediation methods for the removal of dyes has been in intense research. However, there is a significant gap between laboratory experiments and industrial application. Efforts should be made to minimize this gap so that experiments can be conducted on large-scale applications. Hence, new technologies and strategies need to be developed for the wide application of bioremediation in large-scale industries.

6 Conclusions

Bioremediation is one of the promising technologies used in the treatment of industrial dye effluents. Biological materials possess the required genetic, biochemical, and physiological characteristics for the efficient removal of dyes from the effluents, and these characteristics establish them as the ultimate choice in the decontamination of soil and water. Several research studies demonstrate that bioremediation of dyes present in textile industry effluents is simple, efficient, and economical. However, broad screening of organisms should be undertaken for the effective removal of dyes.

Utilization of industrial waste biomass for dye removal has gained momentum in recent years. Strategies must be developed for the utilization of highly growing plants and living organisms for bioremediation as well as for the production of valuable products such as biofuels, alcohol, organic acids, etc. Technological advances and a better understanding of structural and functional behavior of microbes establish bioremediation as an alternative to conventional physicochemical dye removal methods.

References

Abbas SH, Ismail IM, Mostafa TM, Sulaymon AH (2014) Biosorption of heavy metals: a review. J Chem Sci & Technol 3:74–102

Abbas N, Hussain S, Azeem F et al (2016) Characterization of a salt resistant bacterial strain Proteus sp. NA6 capable of decolorizing reactive dyes in presence of multi-metal stress. World J Microbiol Biotechnol 32(11):181. https://doi.org/10.1007/s11274-016-2141-1

Afshin S, Mokhtari SA, Vosoughi M, Sadeghi H, Rashtbari Y (2018) Data of adsorption of Basic Blue 41 dye from aqueous solutions by activated carbon prepared from filamentous algae. Data Brief 21:1008–1013. https://doi.org/10.1016/j.dib.2018.10.023

Aksu Z, Donmez G (2005) Studied the combine effect of molasses sucrose and Remazole Blue or Remazole Black B reactive dye on the growth and bioaccumulation properties of adapted Candida tropicalis. Process Biochem 40:2443–2453

Aksu Z, Karabayir G (2008) Comparison of biosorption properties of different kinds of fungi for the removal of Gryfalan Black RL metal-complex dye. Bioresour Technol 99:7730–7741. https://doi.org/10.1016/j.biortech.2008.01.056

Ali N, Hameed A, Ahmed S (2010) Role of brown-rot fungi in the bioremoval of azo dyes under different conditions. Braz J Microbiol 41(4):907–915. https://doi.org/10.1590/S1517-83822010000400009

Almeida EJ, Corso CR (2014) Comparative study of toxicity of azo dye Procion Red MX-5B following biosorption and biodegradation treatments with the fungi Aspergillus niger and Aspergillus terreus. Chemosphere 112:317–322. https://doi.org/10.1016/j.chemosphere.2014.04.060

Almeida PH, Oliveira ACC, Souza GPN et al (2018) Decolorization of remazol brilliant blue R with laccase from Lentinus crinitus grown in agro-industrial by-products. An Acad Bras Cienc 90 (4):3463–3473. https://doi.org/10.1590/0001-3765201820170458

Angelova R, Baldikova E, Pospiskova K, Maderova Z, Safarikova M, Safarik I (2016) Magnetically modified Sargassum horneri biomass as an adsorbent for organic dye removal. J Clean Prod 137:189–194

Aravindhan R, Rao JR, Nair BU (2007) Removal of basic yellow dye from aqueous solution by sorption on green alga Caulerpa scalpelliformis. J Hazard Mater 142(1–2):68–76

Arpita G, Manisha GD, Sreekrishnan TR (2015) Recent advances in bioremediation of heavy metals and metal complex dyes: review. J Environ Eng C4015003:1–14. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000965

Asses N, Lamia A, Neila H, Moktar H (2018) Congo red decolorization and detoxification by aspergillus niger: removal mechanisms and dye degradation pathway. Biomed Res Int 3049686 (9):1. https://doi.org/10.1155/2018/3049686

Avery SV, Tobin JM (1993) Mechanism of adsorption of hard and soft metal ions to Saccharomyces cerevisiae and influence of hard and soft anions. Appl Environ Microbiol 59:2851–2856

Bahafid W, Joutey NT, Asri M, Sayel H, Tirry N, El Ghachtouli N (2017) Yeast biomass: an alternative for bioremediation of heavy metals, yeast – industrial applications, Antonio Morata and Iris Loira, Intech Open https://doi.org/10.5772/intechopen.70559

Bayramoglu G, Salih B, Akbulut A, Arica MY (2019) Biodegradation of Cibacron Blue 3GA by insolubilized laccase and identification of enzymatic byproduct using MALDI-ToF-MS: toxicity assessment studies by Daphnia magna and Chlorella vulgaris. Ecotoxicol Environ Saf 170:453–460. https://doi.org/10.1016/j.ecoenv.2018.12.014

Brady D, Duncan JR (1994) Bioaccumulation of metal cations by Saccharomyces cerevisiae. Appl Microbiol Biotechnol 41:149–154

Cardoso NF, Lima EC, Royer B et al (2012) Comparison of Spirulina platensis microalgae and commercial activated carbon as adsorbents for the removal of Reactive Red 120 dye from aqueous effluents. J Hazard Mater 241-242:146–153. https://doi.org/10.1016/j.jhazmat.2012.09.026

Casieri L, Varese GC, Anastasi A et al (2008) Decolorization and detoxication of reactive industrial dyes by immobilized fungi Trametes pubescens and Pleurotus ostreatus. Folia Microbiol 53:44. https://doi.org/10.1007/s12223-008-0006-1

Chanmugathas P, Bollag JM (1988) A column study of the biological mobilization and speciation of cadmium in soil. Arch Environ Contam Toxicol 17:229–237

Chen KC, Huang WT, Wu JY, Houng JY (1999) Microbial decolorization of azo dyes by Proteus mirabilis. J Ind Microbiol Biotechnol 23(1):686–690. https://doi.org/10.1038/sj.jim.2900689

Chen CY, Kuo JT, Cheng CY, Huang YT, Ho IH, Chung YC (2009) Biological decolorization of dye solution containing malachite green by Pandoraea pulmonicola YC32 using a batch and continuous system. J Hazard Mater 172(2–3):1439–1445. https://doi.org/10.1016/j.jhazmat.2009.08.009

Chen CC, Chen CY, Cheng CY, Teng PY, Chung YC (2011) Decolorization characteristics and mechanism of Victoria Blue R removal by Acinetobacter calcoaceticus YC210. J Hazard Mater 196:166–172. https://doi.org/10.1016/j.jhazmat.2011.09.015

Chojnacka K (2009) Biosorption and bioaccumulation in practice. Nova Science Publishers, New York, p 137

Daneshvar N, Ayazloo M, Khataee A, Pourhassan M (2006) Biological decolorization of dye solution containing malachite green by microalgae Cosmarium sp. Bioresour Technol 12:121–128

Dawkar VV, Jadhav UU, Jadhav SU, Govindwar SP (2008) Biodegradation of disperse textile dye Brown 3REL by newly isolated Bacillus sp. VUS. J Appl Microbiol 105(1):14–24. https://doi.org/10.1111/j.1365-2672.2008.03738.x

Dedyukhina EG, Eroshin VK (1991) Essential metal ions in the control of microbial metabolism. Process Biochem 26:31–37

El Nemr A, Abdelwahab O, Khaled A, El Sikaily A (2006) Biosorption of Direct Yellow 12 from aqueous solution using green alga Ulva lactuca. Chem Ecol 22(4):253–266. https://doi.org/10.1080/02757540600812875

El Sikaily A, Khaled A, El Nemr A, Abdelwahab O (2006) Removal of Methylene Blue from aqueous solution by marine green alga Ulva lactuca. Chem Ecol 22(2):149–157. https://doi.org/10.1080/02757540600579607

Elisangela F, Andrea Z, Fabio DG, Cristiano RM, Regina DL, Artur CP (2009) Biodegradation of textile azo dyes by a facultative Staphylococcus arlettae strain VN-11 using a sequential microaerophilic/aerobic process. Int Biodeter Biodegr 63:280–288

El-Rahim WM, Khalil WK, Eshak MG (2008) Genotoxicity studies on the removal of a direct textile dye by a fungal strain, in vivo, using micronucleus and RAPD-PCR techniques on male rats [published correction appears in J Appl Toxicol. 2008 Aug;28(6):814]. J Appl Toxicol 28(4):484–490. https://doi.org/10.1002/jat.1299

El-Sheekh MM, Gharieb MM, Abou-El-Souod GW (2009) Biodegradation of dyes by some green algae and cyanobacteria. Int Biodeter Biodegr 63:699–704

Enayatzamir K, Alikhani HA, Yakhchali B, Tabandeh F, Rodríguez-Couto S (2010) Decolouration of azo dyes by Phanerochaete chrysosporium immobilised into alginate beads. Environ Sci Pollut Res Int 17(1):145–153. https://doi.org/10.1007/s11356-009-0109-5

Foroutan R, Mohammadi R, Razeghi J, Ramavandi B (2019) Performance of algal activated carbon/Fe3O4 magnetic composite for cationic dyes removal from aqueous solutions. Algal Res 40:101509

Gadd GM (1993) Interactions of fungi with toxic metals. New Phytol 124:25–60

Gadd GM (1999) Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes. Adv Microb Physiol 41:47–92

Gadd GM (2004) Mycotransformation of organic and inorganic substrates. Mycologist 18:60–70. https://doi.org/10.1017/S0269915XO4002022

Gadd GM (2009) Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. J Chem Technol Biotechnol 84:13–28

Ghosh A, Das P (2014) Optimization of copper adsorption by soil of polluted wasteland using response surface methodology. Indian Chem Eng 56(1):29–42

Guadie A, Tizazu S, Melese M, Guo W, Ngo HH, Xia S (2017) Biodecolorization of textile azo dye using Bacillus sp. strain CH12 isolated from alkaline lake. Biotechnol Rep (Amst) 15:92–100. https://doi.org/10.1016/j.btre.2017.06.007

Hassan MM, Alam MZ, Anwar MN (2013) Biodegradation of textile azo dyes by bacteria isolated from dyeing industry effluent. Int Res J Biol Sci 2(8):27–31

He Z, Yao Y, Lu Z, Ye Y (2014) Dynamic metabolic and transcriptional profiling of Rhodococcus sp. strain YYL during the degradation of tetrahydrofuran. Appl Environ Microbiol 80:2656–2664. https://doi.org/10.1128/AEM.04131-13

Hernández-Zamora M, Cristiani-Urbina E, Martínez-Jerónimo F et al (2015) Bioremoval of the azo dye Congo Red by the microalga Chlorella vulgaris. Environ Sci Pollut Res Int 22(14):10811–10823. https://doi.org/10.1007/s11356-015-4277-1

Herrero M, Stuckey DC (2015) Bioaugmentation and its application in wastewater treatment: a review. Chemosphere 140:119–128

Huang JP, Huang CP, Morehart AL (1991) Removal of heavy metals by fungal (Aspergillus oryzae) adsorption. In: Vernet JP, heavy metals in the environment. Elsevier, London

Hussain S, Maqbool Z, Ali S et al (2013) Biodecolorization of Reactive Black-5 by a metal and salt tolerant bacterial strain Pseudomonas sp. RA20 isolated from Paharang drain effluents in Pakistan. Ecotoxicol Environ Saf 98:331–338. https://doi.org/10.1016/j.ecoenv.2013.09.018

Jadhav UU, Dawkar VV, Ghodake GS, Govindwar SP (2008) Biodegradation of Direct Red 5B, a textile dye by newly isolated Comamonas sp. UVS. J Hazard Mater 158(2–3):507–516. https://doi.org/10.1016/j.jhazmat.2008.01.099

Jin Y, Luan Y, Ning Y, Wang L (2018) Effects and mechanisms of microbial remediation of heavy metals in soil: a critical review. Appl Sci 8:1336. https://doi.org/10.3390/app8081336

Joho M, Inouhe M, Tohoyama H, Murayama T (1995) Nickel resistance mechanisms in yeasts and other fungi. J Ind Microbiol 14:164–168

Joshi B, Kabariya K, Nakrani S, Khan A, Parabia FM, Doshi HV, Thakur MC (2013) Biodegradation of turquoise blue dye by Bacillus megaterium isolated from industrial effluent. Am J Environ Protec 1(2):41–46

Joutey NT, Sayel H, Bahafid W, El Ghachtouli N (2015) Mechanisms of hexavalent chromium resistance and removal by microorganisms. Rev Environ Contam Toxicol 233:45–69. https://doi.org/10.1007/978-3-319-10479-9_2

Kamizono A, Nishizawa M, Teranishi Y, Murata K, Kimura A (1989) Identification of a gene conferring resistance to zinc and cadmium ions in the yeast Saccharomyces cerevisiae. Mol Gen Genet 219:161–167

Karim ME, Dhar K, Hossain MT (2018) Decolorization of textile reactive dyes by bacterial monoculture and consortium screened from textile dyeing effluent. J Genet Eng Biotechnol 16(2):375–380

Khaled E, Hassan G, Khider M, Mandour R (2010) Safe biodegradation of textile azo dyes by newly isolated lactic acid bacteria and detection of plasmids associated with degradation. J Biored Biodegrd 1(3):1–6

Khalid A, Kausar F, Arshad M, Mahmood T, Ahmed I (2012) Accelerated decolorization of reactive azo dyes under saline conditions by bacteria isolated from Arabian seawater sediment. Appl Microbiol Biotechnol 96(6):1599–1606. https://doi.org/10.1007/s00253-012-3877-7

Khan AG, Bari A, Chaudhry TM, Qazilbash AA (1997) Phytoremediation- strategy to decontaminate heavy metal polluted soils and to conserve the biodiversity of Pakistan soils. In: Mufti SA, Woods CA, Hasan SA (eds) . Biodiversity of Pakistan Museum of Natural History, Islamabad and Florida Museum of Natural History, Gainesville

Khataee AR, Dehghan G (2011) Optimization of biological treatment of a dye solution by macroalgae Cladophora sp. using response surface methodology. J Taiwan Inst Chem Eng 42 (1):26–33

Khataee AR, Dehghan G, Ebadi A, Zarei M, Pourhassan M (2010a) Biological treatment of a dye solution by Macroalgae Chara sp.: effect of operational parameters, intermediates identification and artificial neural network modeling. Bioresour Technol 101(7):2252–2258. https://doi.org/10.1016/j.biortech.2009.11.079

Khataee AR, Zarei M, Pourhassan M (2010b) Bioremediation of malachite green from contaminated water by three microalgae: neural network Modeling. Clean Soil Air Water 38(1):96–103. https://doi.org/10.1002/clen.200900233

Kneer R, Kutchan TM, Hochberger A, Zenk MH (1992) Saccharomyces cerevisiae and Neurospora crassa contain heavy metal sequestering phytochelatin. Arch Microbiol 157:305–310

Krauter P, Martinelli R, William K, Martins S (1996) Removal of Cr4+ from ground water by S. cervisiae. Biodegradation 7:277–286

Kujan P, Votruba J, Kamenik V (1995) Substrate-dependent bioaccumulation of cadmium by growing yeast Candida utilis. Folia Microbiol 40(3):288–292

Kumar KV, Ramamurthi V, Sivanesan S (2006) Biosorption of malachite green, a cationic dye onto Pithophora sp., a fresh water algae. Dyes Pigments 69:102–107

Kumar PS, Pavithra J, Suriya S, Ramesh M, Kumar AN (2015) Sargassum wightii, a marine alga is the source for the production of algal oil, bio-oil, and application in the dye wastewater treatment. Desalin Water Treat 55(5):1342–1358. https://doi.org/10.1080/19443994.2014.924032

Leahy JG, Colwell RR (1990) Microbial degradation of hydrocarbons in the environment. Microbial Rev 53(3):305–315

Leebana VJ, Santhanum H, Geetha K, Raj SA (2012) Biodegrdation of direct golden yellow, a textile dye by Pseudomonas putida. Desalin Water Treat 39:1–9

Li C, Xu Y, Jiang W, Dong X, Wang D, Liu B (2013) Effect of NaCl on the heavy metal tolerance and bioaccumulation of Zygosaccharomyces rouxii and Saccharomyces cerevisiae. Bioresour Technol 143:46–52

Lima EC, Royer B, Vaghetti JCP, Simon NM, Cunha BM, Pavan FA, Benvenutti EV, Cataluna-Veses R, Airoldi C (2008) Application of Brazilian pine-fruit shell as a biosorbent to removal of reactive red 194 textile dye from aqueous solution: kinetics and equilibrium study. J Haz Mat 155:536–550

Machado KMG, Luciana CA, Compart LCA, Morais RO, Luiz H, Rosa LH, Santos MH (2006) Biodegradation of reactive textile dyes by Basidiomycetous fungi from Brazilian ecosystems. Braz J Microbiol 37:481–487

Maqbool Z, Hussain S, Ahmad T et al (2016) Use of RSM modeling for optimizing decolorization of simulated textile wastewater by Pseudomonas aeruginosa strain ZM130 capable of simultaneous removal of reactive dyes and hexavalent chromium. Environ Sci Pollut Res Int 23 (11):11224–11239. https://doi.org/10.1007/s11356-016-6275-3

Marchenko AM, Pshinko GN, Demchenko VY, Goncharuk VV (2015) Leaching heavy metal from deposits of heavy metals with bacteria oxidizing elemental sulphur. J Water Chem Technol 37:311–316

Marim RA, Oliveira AC, Marquezoni RS et al (2016) Use of sugarcane molasses by Pycnoporus sanguineus for the production of laccase for dye decolorization. Genet Mol Res 15(4) Published 2016 Oct 17. https://doi.org/10.4238/gmr15048972

Min SW, Hasnat MA, Rahim AA, Mohamed N (2013) Optimisation of the batch reactor for the removal of cobalt ions from chloride media. Chemosphere 90(2):674–682

Mohan SV, Karthikeyan J (2000) Removal of Diazo dye from aqueous phase by algae Spirogyra species. Toxicol Environ Chem 74(3–4):147–154. https://doi.org/10.1080/02772240009358877

Mosa KA, Saadoun I, Kumar K, Helmy M, Dhankher OP (2016) Potential biotechnological strategies for the cleanup of heavy metals and metalloids. Front Plant Sci 7:1–22

Mueller JG, Cerniglia CE, Pritchard PH (1996) Bioremediation of environments contaminated by polycyclic aromatic hydrocarbons. In: Bioremediation: principles and applications. Cambridge University Press, Cambridge, pp 125–194

Omar HH (2008) Algal decolorization and degradation of monoazo and diazo dyes. Pak J Biol Sci 11:1310–1316

Ottoni CA, Santos C, Kozakiewicz Z, Lima N (2013) White-rot fungi capable of decolourising textile dyes under alkaline conditions. Folia Microbiol (Praha) 58(3):187–193. https://doi.org/10.1007/s12223-012-0196-4

Poorni S, Priya M, Sharmila S (2020) Adsorption of dyes by marine algae. Plant Cell Biotechnol & Molecul Biol 20(23–24):1238–1241

Presta A, Stillman MJ (1997) Incorporation of copper into the yeast Saccharomyces cerevisiae. Identification of cu(I)-metallothionein in intact yeast cells. J Inorg Biochem 66:231–240

Priya AK, Nagan S (2015) Bioremediation of dye effluent and metal contaminated soil: low-cost method for environmental cleanup by microbes. J Environ Sci Eng 57(2):109–119

Przystas W, Zablocka-Godlewska E, Grabinska-Sota E (2015) Efficacy of fungal decolorization of a mixture of dyes belonging to different classes. Braz J Microbiol 46(2):415–424. https://doi.org/10.1590/S1517-838246246220140167

Przystaś W, Zabłocka-Godlewska E, Grabińska-Sota E (2018) Efficiency of decolorization of different dyes using fungal biomass immobilized on different solid supports. Braz J Microbiol:

[Publication of the Brazilian Society for Microbiology] 49(2):285–295. https://doi.org/10.1016/j.bjm.2017.06.010

Qazilbash AA (2004) Isolation and characterization of heavy metal tolerant biota from industrially polluted soils and their role in bioremediation. Biological Sci 41:210–256

Ramalho PA, Cardoso MH, Cavaco-Paulo A, Ramalho MT (2004) Characterization of azo reduction activity in a novel ascomycete yeast strain. Appl Environ Microbiol 70:2279–2288

Ramalingam S, Saraswathy N, Shanmugapriya S, Shakthipriyadarshani S, Sadasivam S, Sanmugaprakash M (2010) Decolorization of textile dyes by Aspergillus tamari, mixed fungal culture and Peniceillium purpurogenum. J Sci Ind Res 69:151–153

Ramsay LM, Gadd GM (1997) Mutants of Saccharomyces cerevisiae defective invacuolar function confirm a role for the vacuole intoxic metalion detoxification. FEMS Microbiol Lett 152 (2):293–298

Rani B, Vivek K, Jagvijay S, Sandeep B, Priyanku T, Shivesh S, Ritu K (2014) Bioremediation of dyes by fungi isolated from contaminated dye effluent sites for bio-usability. Braz J Microbiol 45(3):1055–1063

Ren S, Guo J, Zeng G, Sun G (2006) Decolorization of triphenylmethane, azo, and anthraquinone dyes by a newly isolated Aeromonas hydrophila strain. Appl Microbiol Biotechnol 72 (6):1316–1321. https://doi.org/10.1007/s00253-006-0418-2

Roy DC, Biswas SK, Saha AK, Sikdar B, Rahman M, Roy AK, Prodhan ZH, Tang SS (2018) Biodegradation of Crystal Violet dye by bacteria isolated from textile industry effluents. PeerJ 6: e5015. https://doi.org/10.7717/peerj.5015

Saha P, Datta S, Sanyal SK (2010) Application of natural clayey soil as adsorbent for the removal of copper from wastewater. J Environ Eng 136:1409–1417. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000289

Saibaba KVN, King P (2013a) Equilibrium and thermodynamic studies for dye removal using biosorption. Int J Res Eng & Technol 1(3):17–24

Saibaba KVN, King P (2013b) Modelling and optimization of dye removal process using hybrid response surface methodology and genetic algorithm approach. J Fundam Renew Energy Appl 4 (1). https://doi.org/10.4172/2090-4541.1000126

Saibaba KVN, King P, Gopinadh R, Sreelakshmi V (2011) Response surface optimization of dye removal by using Waste prawn shells. Int J Chem Sci Appl 2(3):186–193

Saibaba KVN, King P, Gopinadh R, Lakshmi DKN (2012) Response surface optimization for the decolorization of crystal violet dye from aqueous solutions by waste crab shells. Int J App Environ Sci 7(2):149–154

Semrany S, Favier L, Djelal H, Taha S, Amrane A (2012) Bioaugmentation: possible solution in the treatment of bio-refractory organic compounds (BioROCs). Biochem Eng J 69:75–86

Shahid A, Singh J, Bisht S, Teotia P, Kumar V (2013) Biodegradation of textile dyes by fungi isolated from North Indian field soil. Env Asia 6(2):51–57

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

Shedbalkar U, Jadhav JP (2011) Detoxification of malachite green and textile industrial effluent by Penicillium ochrochloron. Biotechnol Bioproc E 16:196. https://doi.org/10.1007/s12257-010-0069-0

Siddique M, Mahmmod A, Sheikh M, Gafoor A, Khaliq S, Bukhai M, Yousaf K, Rehman K, Andleeb S, Naeem MM (2012) A study on the biodegradation of some reactive textile dyes by white rot fungus (Pleurotus ostreatus). World Appl Sci J 18(2):181–185

Singh RP, Singh PK, Singh RL (2014) Bacterial decolorization of textile azo dye acid orange by Staphylococcus hominis RMLRT03. Toxicol Int 21(2):160–166. https://doi.org/10.4103/0971-6580.139797

Siti ZM, Nurhaslina CR, Ku HKH (2013) Removal of synthetic dyes from wastewater by using Bacteria, Lactobacillus delbruckii. Int Refereed J Eng & Sci (IRJES) 2(5):01–07

Tamás MJ, Wysocki R (2010) How Saccharomyces cerevisiae copes with toxic metals and metalloids. FEMS Microbiol Rev 34:925–951

Tavares MF, Avelino KV, Araújo NL et al (2020) Decolorization of azo and anthraquinone dyes by crude laccase produced by Lentinus crinitus in solid state cultivation. Braz J Microbiol 51 (1):99–106. https://doi.org/10.1007/s42770-019-00189-w

Thorsen M, Jacobson T, Vooijs R, Navarrete C, Bliek T, Schat H, Tamás MJ (2012) Glutathione serves an extracellular defence function to decrease arsenite accumulation and toxicity in yeast. Mol Microbiol 84(6):1177–1188

Vasudevan N, Kanimozhi R (2011) Decolourisation of azo dye, Acid Red-18 by Phanerochaete chrysosporium. J Environ Sci Eng 53(3):349–354

Veglio F, Beolchini F (1997) Removal of metals by biosorption: a review. Hydrometallurgy 44:301–316

Velásquez L, Dussan J (2009) Biosorption and bioaccumulation of heavy metals on dead and living biomass of Bacillus sphaericus. J Hazard Mater 167:713–716. https://doi.org/10.1016/j.jhazmat.2009.01.044

Vijayaraghavan K, Yun YS (2008) Bacterial biosorbents and biosorption. Biotechnol Adv 26:266–291. https://doi.org/10.1016/j.biotechadv.2008.02.002

Vital KR, Saibaba KVN, Beebi SK, Gopinadh R (2016) Dye removal by adsorption: a review. J Bioremed Biodegr 7(6). https://doi.org/10.4172/2155-6199.1000371

Wu Q, You R, Clark M, Yu Y (2014) Pb(II) removal from aqueous solution by a low-cost adsorbent dry desulfurization slag. Appl Surf Sci 314:129–137

Yadav KK, Gupta N, Kumar V, Singh JK (2017) Bioremediation of heavy metals from contaminated sites using potential species : a review. Indian J Environ Prot 37:65–84

Chapter 10
A Harmless Approach on Textile Effluent Detoxification: Bioremediation and Recent Strategies

Kavitha Rajan, Venkatesa Prabhu Sundramurthy, and Melkamu Kifetew Merine

Abstract Wastewater effluents, particularly, from textile industries deepened with high level of organic and inorganic pollutants, such as binders, surfactants, dyes, acids, salts, thickeners, reducing agents, etc. Since the uptake of some kind of synthetic dyes by the fibers is quite low, the presence of dyes in the wastewaters is immensely high. Such kind of extremely colored wastewaters harshly affects the photosynthetic function of receiving water bodies and makes a severe negative impact on aquatic life because of oxygen depletion and diminishing penetration of light. Due to their toxicity, it may be lethal to certain forms of marine life. Therefore, such wastewaters must be subjected to treat before discharge. Different researchers have suggested various cutting-edge innovations using physicochemical and biochemical methods to treat the effluents from dye manufacturing and textile industries. Though the traditional treatment techniques, physical and chemical treatments, are feasible for decolorization, they yet require more energy and chemicals. In addition, these techniques result the accumulated contaminants into liquid or solid side streams that necessitate extra-treatment strategies for anticipated disposal. On other hand, the biological approaches are proven to be less expensive and harmless to the environment. In this process, different microbes including fungi, bacteria, and algae are used, which can completely mineralize pollutants. Keeping in the view, this chapter focuses to discuss on the different factors effecting the biological removal of dye, microorganisms involved and mechanism of biodegradation of dyes, application of enzymes. Additionally, the genetically engineered cultures in biological dye removal have also been discussed.

K. Rajan
Department of Textile Technology, Federal Technical and Vocational Education and Training Institute, Addis Ababa, Ethiopia

V. P. Sundramurthy (✉)
Department of Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

M. K. Merine
Department of Environmental Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_10

Keywords Textile effluent · Biodegradation · Synthetic dye · Wastewater treatment

1 Introduction

Recently, the textile industries are gaining a remarkable economic significance globally. The textile industry sector is diverse bodies which involve different manufacturing elements with different kind of fibers for manufacturing various fabrics having attractive properties. In the textile industries, more than 8000 chemical compounds are used annually for preparing 400 billion m^2 of fabric around the world. During the manufacturing process of textile fabrics, different chemical treatments such as sizing, scoring, mercerizing, coloring, printing, and finishing are carried out (Alkaya et al. 2012). Wet processing is a common method which utilizes huge quantities of water and various chemical compounds for ameliorating the properties of auxiliary textiles, such as fabric texture, serviceability, and durability.

As a primary wet processing step, starch content is removed from the cloth by “desizing.” After desizing, the fabric may contain oils, fats, waxes, seed pieces, leaf particles, natural coloring matters, etc. The presence of oils, fats, and waxes is considerably influencing for determining the hydrophobic characteristics of the cloth. So, these compounds are liable to affect the cloth absorbance that leads to the subsequent process of improper dyeing, printing, and finishing. Hence, eradication of these impurities from the fabric is much important and is called scouring (or) Kier boiling. The removal of natural coloring material is carried out by the technique called bleaching. Luster of the fabric can be boosted through mercerization. Dyeing is a very important process for adding colors to the fabric. During the dyeing process, different synthetic dyes, curing agents, bleaches, solvents, and heavy metal enriched detergents are used.

The surplus hazardous chemicals, such as polyvinyl chloride, chlorine, benzidine, and toluidine, involved in the different steps on textile processing are the key factor for toxic emissions from the textile mills (Choudhury 2017). Additionally, formaldehyde, lead, and mercury are other harmful and cancer-causing agents, which are used in routine textile operations. The release of these untreated toxics would contaminate rivers and groundwater reservoirs. Over 10,000 different dyes and pigments are expected to be used in textile industry. Based on the current statistics, more than 7105 tons of synthetic colors are produced annually (Hasanbeigi and Price 2015). Approximately 40% of these dyes use organic volatile chlorine, carcinogen. Such organic matter present in the textile effluents needs a great concern of wastewater treatment, since they respond with many disinfectants, in specific chlorine (Brüschweiler and Merlot 2017).

The chemical pollutants from the textile wet processing can also pose a serious safety risk to the environment. There are different schematic approaches that have been proposed for controlling the textile wet processes to meet a better environment and to protect the ecosystem. Modern management for the treatment of waste effluent from the textile industries can be processed through physical, chemical, or biological methods. Trapping of contaminants to avoid spreading and polluting the uncontaminated areas through different strategies, like encapsulation, filtration, settling by gravity, adsorption, and stabilization, is known as physical method (Katheresan et al. 2018). Chemical methods for waste management can be done through the use of chemical reactions, in specific, chemical oxidation, that converts toxic compounds to harmless compounds (Sivaram et al. 2018). Biological treatment is the method that uses different microbes or plants to degrade or at least hold the waste in place so that they do not travel in the atmosphere. Selective microorganisms, including bacteria (single or mixed culture) and algae, may degrade a broad range of dyes; they may biologically degrade under specific circumstances (pH, temperature, and required nutrient) and even completely mineralize them (Enayatzamir et al. 2010). So far, different bacterial strains (*Pseudomonas putida*, *Agrobacterium radiobacter*, *Bacillus* sp., *Sphingomonas paucimobilis*, and *Aeromonas hydrophila*), fungi (*Mycobacterium avium*, *Mycobacterium intracellular*, *Mycobacterium scrofulaceum*, *Mycobacterium marinum*, and *Mycobacterium chelonae*), yeast, and *actinomycetes* have been documented as successful decoloring biological agent for dye. On another hand, biological approaches do have some drawbacks in their implementation and also endure due to the toxicity of coloring materials (Daneshvar et al. 2007). Therefore, the current researches need to consider for emerging technologies to point out the right approach for a specific job. Waste transport, recycling, engineered processing, biological land conservation, and environmental laws are played a key role in the evolution of a waste treatment and management before it reaches to the final destination. However, the physical, chemical, and biological methods differ in terms of performance, expense, and their outcome effect on the environment (Liu et al. 2007). This chapter intends to present a brief discussion about various treatments of textile effluent, specifically, biological remediation methods.

2 Wet Processing in Textile Industry and Effluents

Textile industries entail a lot of manufacturing operations, which comprise several steps and mechanisms. Textile wet processing (Fig. 10.1) is the methods that are used to ameliorate the desirable properties of textiles. Wet processing in textile industries comprises pretreatment (or) preparation, coloring, and finishing. The pretreatment consists of a sequence of chemical and other procedures that are applied in the gray stage to textiles. The textiles cannot be colored or engraved at this point. Pretreatment processes strengthen the textiles, so that in the later stages of textile wet processing can use dyes and chemicals. Coloring is nothing but dyeing that helps to add colors to textiles. Printing that offers unique design to fulfill the customer needs (Hussain and Wahab 2018). There will be no space for textile designers without the coloring. Furthermore, the customer also anticipates textiles to fulfill certain end-use requirements. For instance, an umbrella should at least be waterproofed, and

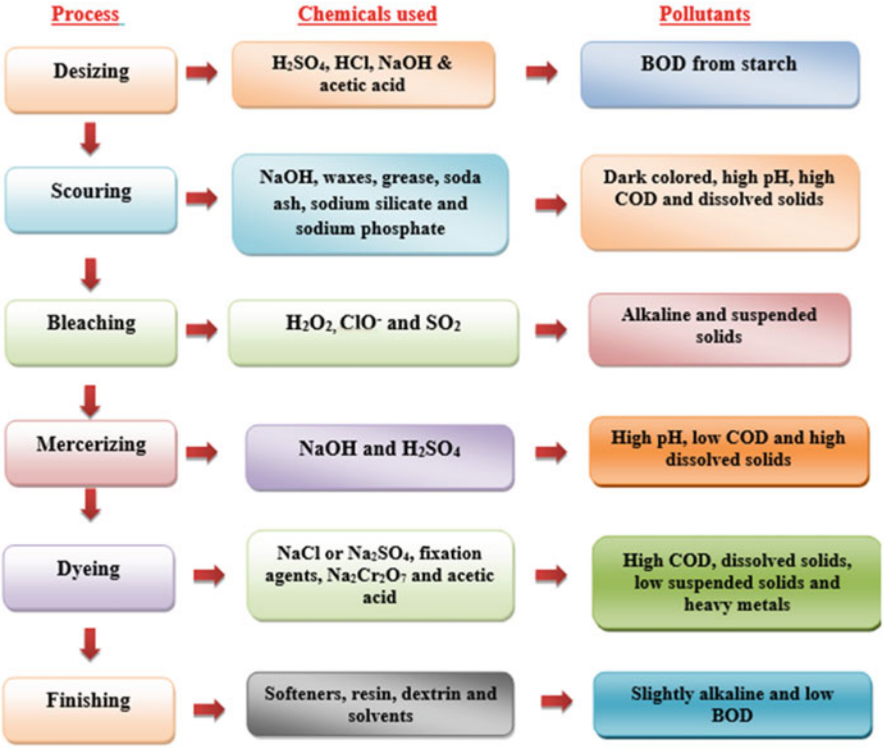


Fig. 10.1 Common textile processing and its pollutants

bandages should undergo microbial finish. Keeping this view, textiles undergo numerous finishing processes to reach those standards. In addition, various chemicals and chemical reactions are involved in textile wet processing for efficient textile products (Gulzar et al. 2018).

2.1 *Pretreatment Process*

The pretreatment includes desizing, scouring, bleaching, and mercerization. Desizing constitutes more than 50% of the preparation's emission load, while scouring is around 10–25%. For the subsequent process, efficient preparation is a must, as the finishing and tinting process is affected by any residual impurities on the fabric (Yaseen and Scholz 2019). The pretreatment process is also expensive, with excessive use of large quantities of chemicals, auxiliaries, and resources, such as heat, electricity, and water. The residues, cotton impurities and auxiliary precipitations from pretreatment, that have a detrimental impact on the subsequent coloring operations, which can require lengthy multistage intermediate washing operations, can also affect the environment. Consequently, the method for pretreatment must meet the coloring and finishing stage specifications.

Desizing It is a process to treat the fabric for hydrolyzing the excess starch, using synthetic detergents and sodium hydroxide and/or acids or enzymes. "Size" is an adhesive material used to make weaving easier. Size generally has a high demand for biological oxygen and significantly contributes at waste effluent. Generally, there are three methods that are adopted for desizing. They are acid desizing, oxidative desizing, and enzyme desizing. Desizing techniques are tending to hydrolyze the starch. However, synthetic starches are differing from natural starches that remain intact during desizing process. It can be retrieved and recycled (Kavitha et al. 2020). The characteristics of textile wastewater depend on the sizes used in the desizing process. Normally, the total BOD and total solids load of textile wastewater seem to be high. Far from the desizing stage, almost 50% of the total BOD in fabric treatment is derived when starch is used as a size (Araújo et al. 2008).

Scouring Natural fibers have impurities, such as oils, fats, waxes, minerals, leafy matter, and motes, that mess during dyeing and printing. During the scouring, detergents, soaps, and other supporting agents, such as alkaline solutions, surfactants, defoamers, and emollients, are exploited (Rahman Bhuiyan et al. 2016). Scouring effluents have been characterized as high concentrations of BOD and COD, high temperatures (70 °C–80 °C), pH value as high as 10–12, enriched with sodium cations, and small quantities of TDS. Dyeing effluents have elevated temperature, excessive concentrations of solids, high COD, alkaline pH, and a considerable proportion of augmented sodium ions (Unido 1992).

Bleaching It is a process to remove the natural coloring matter by using calcium hypochlorite, sodium hypochlorite, and hydrogen peroxide. The bleaching unit effluents have low level of BOD. However, the nonvolatile constituents present in the wastewater were observed to be high (Tzanov et al. 2001).

Mercerizing In this process, fabrics are permeated with concentrated sodium hydroxide solution aimed to attain enhanced luster, strength, and dye affinity to fabrics. A cold sodium hydroxide solution is applied, which enables the fibers to swell and follow a circular cross section. Most mercerization units have their own caustic recovery platform to ensure the waste minimization. Wastewaters from mercerized unit is characterized as typically low BOD and enriched with suspended solids (Orhon et al. 2001).

2.2 Coloration Process

The dyeing unit in the textile industries has a great impact for polluting the water bodies and surrounding environment. Dye(s) plays a vital role in adding value, beauty, and satisfying the customer's anticipation. In the dying unit, the use of synthetic dye(s) is found to be increased rapidly due to its cost-effectiveness and excellent durability. Despite this, a large discharge of colored contaminated effluent has been found in different textile industries. Such effluents have notable color concentration greater than 1 mg/L (Hassan and Carr 2018). The presence of ammonia, aniline, vat dyes, nitroglycerin, sodium bicarbonate, soaps, chloride salts, and heavy metals, such as bronze, arsenic, gold, zinc carbonate, Hg, nickel, and cobalt chloride hexahydrate, and other auxiliary chemicals, which together present in the effluents from coloration process, makes it as extremely toxic. Methanol-based dye fixing agents, cationic-based softeners, and nonrecyclable coloring substances may also be the other dangerous chemicals found in that wastewaters (Khatri et al. 2015). Besides dyes, diverse auxiliary chemical compounds are used, which additionally end up within the waste water. Color and numerous auxiliaries, including organic acids, fixing agents, defoamers, oxidizing/reducing agents, and diluents, are regular pollution that are generated in the course of the dyeing step. Quite a big amount of dyes leaves the system in an unfixed state. The exact amount and the form of pollution rely on the dyes and the procedure used (Broadbent 2001).

The different steps in printing and coloring unit include pretreatment, coloring, printing, and finishing. These processes release huge quantities of starch, waxes, carboxyl methyl cellulose (CMC), polyvinyl alcohol, wetting agents, hydrochloric acid, Cl_2, NaOH, H_2O_2, emulsifiers, sodium silicate, potassium sulfate, and linters. These contaminants are unique outcome from the printing and dyeing unit effluent. They contain high starch, elevated dissolved solids (DS), high BOD, low suspended solids, and moderate level of heavy metals and seem to be oily and slightly alkaline (Hardin 2010). In some dyeing processes, around 75% of the total used salts end up in the generated wastewaters. Printing is a coloring operation, as is dyeing, but it is a "localized" coloring that typically covers only a specific section of the textile portion. Printing is normally achieved by using thickened dyes or pigment pastes on the surface, in accordance with the color design (Hashemi Sanatgar et al. 2017). During this process, some unused printing paste is discarded that can lead for water contamination. In order to minimize this risk, the printing paste can also be quickly extracted with smaller volume lost. The printed paste absorption can be increased using additional energy that can minimize the discarded pollutants. Recently, researchers show great interest in color control due to their toxic and genotoxic effects on living organisms as they have been recognized as carcinogenic substances, such as benzidines and other aromatic compounds (Bisschops and Henri 2003).

2.3 Finishing Process

Finishing involves one or more processes performed on fiber, yarn, or textile to enhance their behavioral quality, in specific, durability. For this, many chemicals are used in padding machines that move the substance under a roller guide and between two padding sheets. Before the textiles are transferred to a steaming or washing and drying machine, excess liquid is drained from the sheets (Savin and Butnaru 2008). This process is achieved by the fabric with a certain amount of UV-protective substances, which protect the cotton fabric from the UV rays. Though not all materials require the same finishing processes, different types of chemicals and chemical reactions will lead to the final effect. Effluents from this unit contain low levels of BOD and COD and reasonable suspended solids. When such kind of discharges from finishing processes pose to be toxic, that may cause skin issues (Tables 10.1 and 10.2).

Table 10.1 Characteristics of wet processing textile effluent

Parameter	Desizing	Scouring	Bleaching	Mercerizing	Dyeing	Reference
pH	5.83–6	10–13	8.5–9.6	8–10	7–10	Ghaly et al. (2014)
BOD (mg/L)	1700–5200	260–400	50–100	20–50	400–1200	Ghaly et al. (2014)
COD (mg/L)	10000–15000	1200–3300	150–500	100–200	1000–3000	Ghaly et al. (2014)
Color (ADMI)	16000–32000	694	153	600–1900	1450–4750	Carmen and Daniela (2012)
TS (mg/L)		7600–17400	2300–14400		500–14100	Carmen and Daniela (2012)
Chlorides (mg/L)			40–175		48–601	Savin and Butnaru (2008)

ADMI the American Dye Manufactures Institute unit, *TS* total solids, *COD* chemical oxygen demand, *BOD* biochemical oxygen

Table 10.2 Characteristics of typical untreated textile wastewater reference (Mostafa 2015)

Parameter	Range
pH	6–10
Temperature (°C)	35–45
Total d solids (mg/L)	8000–12,000
BOD (mg/L)	80–6000
COD (mg/L)	150–12,000
Total suspended solids (mg/L)	15–8000
Total dissolved solids (mg/L)	2900–3100
Chlorine (mg/L)	1000–6000
Free chlorine (mg/L)	<10
Sodium (mg/L)	<10
Fe, Zn, Cu, As, Ni, B, F, Mn, Hg, PO_4, and Co	<10
Oil and grease (mg/L)	10–30
TNK (mg/L)	10–30
NO_3–N (mg/L)	<15
Sulphate (mg/L)	600–1000
Silica (mg/L)	<15
Color (Pt–Co)	50–2500

3 Wastewater Treatment in Textile Processing

Wastewater treatment process is an overall water treatment system consisting primary treatment, secondary treatment, and tertiary treatment (Wang et al. 2011). Figure 10.2 depicts the outlines of the mordent wastewater treatment process. A number of effluent treatment plants have been originated in the last decade as a cooperative venture to treat small- and medium-sized industrial clusters of wastewaters. In the pretreatment phase, insoluble particles are removed so that they are restricted from reaching the treatment area, which may hinder the treatment operation. Primary treatment removes suspended solids, excessive quantities of the effluent oil, lubricant, and granular materials (Eswaramoorthi et al. 2008). During secondary treatment, BOD, formaldehyde, oil, and color(s) contents, present in the effluents, are significantly reduced under aerobic or anaerobic conditions. A few additional sophistic techniques are employed in tertiary treatments, such as electrodialysis, reverse osmosis, and ion exchange process (Holkar et al. 2016). In general, the textile effluents are treated by physical, chemical, and biological treatments.

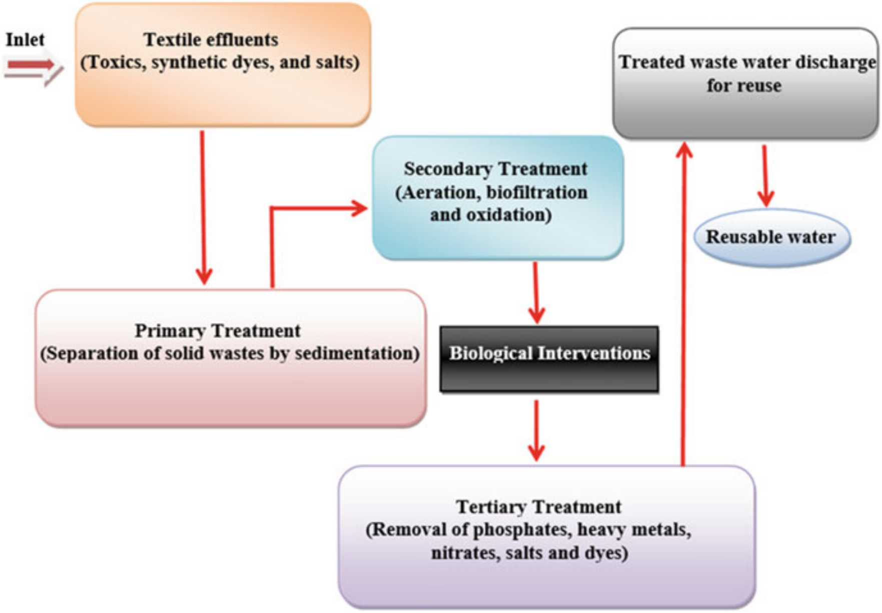


Fig. 10.2 Outlines of textile wastewater treatment process

3.1 Physical Treatment Methods

In the physical treatment methods, separation of particles is achieved through electrical attraction, gravity sedimentation, dispersion, or physical barriers forces. These methods do not hinder the chemical structure of the pollutants present in the wastewaters but only effect changes in the physical state (Crini et al. 2019). Adsorption is one of the efficient physical methods to treat wastewaters. In the adsorption, activated carbon, silicon polymers, and kaolin are the most studied and proven to be potential adsorbents that have appreciable ability to adsorb various dyes (Bizuneh 2012). Physical adsorption occurs when the adsorbent has an unstable interspecies bond, while the chemical adsorption occurs when the electron exchanges are observed to be strong between the interspecies (Ahmad and Mondal 2009). Recently, adsorption has been modernized using novel adsorbents, such as eggshells, sugar cane bagasse, silica gel, almond peel, etc. (Ellis et al. 2012, Dotto et al. 2019). Coagulation and flocculation are the treatments that are normally used to remove the organic matter, but there are no effects on the removal of soluble dyes. However, this method effectively removes insoluble colors (Lin and Lin 1993). The applicability of chemical methods is doubtful because of its high operating cost and restrictions on disposal of endpoint sludge. Nowadays, ozone wastewater treatment using ozone oxidization is proven to be an effective methodology for textile wastewater treatment (Langlais et al. 1991). During this treatment, ozone specifically attacks the bestow color double bonds to decolorize the effluents. However, several reports documented that the substantial reduction in COD could not be resulted by ozonation (Coste et al. 1996, Adams et al. 1995). Moreover, additional costs may apply for installation of ozonation unit (Kurniawan et al. 2006). Another salient approach, membrane filtration, is widely studied method and well developed for an efficient removal of dyes. This approach has been proven to be cost-effective and less water consumption (Chang 2014). In addition to the elimination of BOD and COD, it has unique features, such as appreciable tolerance against temperature and harmful toxic gases. However, it's expensiveness, risk on clogging, and membrane replacement hinder its practical applicability (Srivastava et al. 2015).

3.2 Chemical Treatment Methods

Chemicals play a very important role in accelerating wastewater disinfection and treatment process. Some organic compounds are used together with physical and biological processes. They involve appropriate methods, such as chemical precipitation, coagulation and flocculation, and chemical oxidation. Chemical precipitation is one of the most extensively used methods for textile wastewater treatment. Dissolved toxic metals can be removed successfully through this method. Precipitation reaction can be triggered by adding precipitation reagents that make solubilized metals to form agglomerated solid particles and are subsequently removed by

Table 10.3 The main advantages and disadvantages of physiochemical methods in textile wastewater treatments

Treatment method	Advantages	Disadvantages	References
Adsorption	Easy to operate, low cost, applicability in wide range of pH, high metal binding capacity	Mass production of waste products	Krzysztof Piaskowski et al. (2018)
Nano-filtration	Separation of minerals hydrolyzation of dyes and auxiliaries	Treatment for complex and high-concentration pollutant is difficult	Yasir Aljaberi and Mohammed (2018)
Oxidation	Rapid and proficient	Chemicals and high energy requirements	Srivastava et al. (2015)
Coagulant	Simple and economically feasible	High amount outcome sludge	Kasperchik et al. (2012)
Ozonation	Highly efficient	Short half-life, unstable stability, comparatively expensive.	Yasir Aljaberi and Mohammed (2018)
Chemical precipitation	Low capital cost and simple to operate	Generation of sludge that needs extra operational cost for handling and disposal	Yasir Aljaberi and Mohammed (2018)

filtration. This method depends on the type and concentration of the metals present in the wastewater. Sodium or calcium hydroxides are extensively applied for hydroxide precipitation to convert dissolved metals into solid particles (Holkar et al. 2016). Oxidation can be successfully exploited for color degradation. In this process, biologically produced oxygen and ozone, sodium hypochlorite, hydrogen peroxide, and strong acids are used as oxidation agents. The mechanism of degradation through oxidation is known to create smaller breakable molecules by oxidation. However, conventional oxidation reaction could not oxidize toxic organic dyes completely. This drawback could be overcome using the advanced oxidation processes (AOPs) with some additional cost expenses. Nano-filtration can be used for complete eradication of color discharges present in the textile effluents. In this process, the membranes consisting of low-molecular-weight organic complexes, divalent ions, and wide monovalent ions are employed that can hydrolyze the reactive dyes and auxiliary dyes (Ellouze et al. 2012). However, high expensive operative cost limits its success on practical applicability (Table 10.3).

3.3 Biological Methods for Textile Effluent Treatment

Compared to physical and chemical methods, biological methods are proven to be a cost-effective and environmentally friendly. Different researchers have studied the different possibilities of biological approaches, and various reports have been documented for several microorganisms applicable to textile effluent treatment

Table 10.4 Various biological method of textile industry effluent and their merits and demerits

Biological method	Merits	Demerits	Reference
Biodegradation	Comparatively economic and simultaneous production of biogas	Consortiums of mixed cultures with combination of anaerobic and aerobic system have shown effective decolorization	Neha and Remya (2019)
Biosorption	Metabolism is independent; live or/and dead biomass can be employed and economically feasible and can have high selectivity, low operating cost, and no toxic effect on microorganisms	Large-scale treatment still not explored, disposal of the dye accumulated biomass needs chemical modification and further treatment	Asha and Viraraghavan (2010)
Enzymatic	Effective for selective compounds, and immobilized enzymes have been observed as more cost effective	Studies on scale-up and complete analysis for byproducts are needed; cost of enzymes, stability, and enzyme inhibition also limit the enzyme process	Chacko and Kalidass (2011)
Conventional biological treatment with aeration	More efficient on BOD, COD, and TSS reduction with appreciable color removal	Not suitable for toxic heavy metal reduction. Ineffective for nonbiodegradable dye stuff reduction	Vo et al. (2020)

(Brahmbhatt and Jasrai 2016). Interestingly, a recent finding has shown that the use of natural-born algae and duckweed plants for the treatment of textile wastewaters is quite successful (Sekomo et al. 2014). From the review of short literature survey, different biological approaches for textile waste treatment are presented with its merits and demerits in Table 10.4. The use of microorganisms to eradicate the toxics from the environment is typically known as bioremediation. Using this emerging environmental engineering field, the microorganisms are adapted to toxic waste and can grow spontaneously into new-resistant strains (Saratale et al. 2001). This kind of toxic acclimatized strains can convert toxic chemicals to less harm substances. The microbial systems can degrade the recalcitrant compounds using bio-mechanisms of enzymes. Research communities have developed many bioremediation techniques for textile effluents treatment, including the degradation of complex organic substances induced with different enzymes such as laccases (Hatvani and Mécs 2001), diarylpropane oxygenase (Duran and Esposito 2000), NADH-DCIP reductase (Bhosale et al. 2006), and aminopyrine N-demethylase (Owas 2017). Such an enzymological treatment exhibits the highest possible results for detoxifying textile wastewaters toward the degradation of organic pollutants (Frank et al. 2001).

3.3.1 Microbial Treatments for Bioremediation

Microorganisms account for half of our planet's biomass; however, very little (5%) is known about the microbial diversity in the biosphere (Curtis and Reinhard 1994). Microbes have a high capability of multiplication that offers a more economical and eco-friendly strategy for the reduction of environmental pollutants; also, it keeps a simpler way to biodegrade many xenobiotic compounds. The present microbial remediation researches focus on degrading persistent organic compounds by isolating and identifying specific microorganism or consortium of microorganism which can breakdown the substances, such as benzene, phenol, toluene, atrazine, etc. Even complex compounds, such as polychlorinated biphenyl (PCBs), dichlorodiphenyltrichloroethane (DDT), and hexachlorocyclohexane (HCH), can also be degradable by appropriate microbes (Saleh et al. 1980; Yagi and Sudo 1980; Dmochewitz and Ballschmiter 1988; Solis et al. 2012). According to the literature, the microbial strains used to degrade different chemicals are typically extracted from environmental samples (wastewaters, sludge, manure, and polluted soil) and developed with traditional enrichment techniques. Several documentations have been reported about the microbial sources, including fungi, bacteria, yeasts, algae, and actinomycetes (Lim et al. 2010).

The effluents from textile industries contain a variety of organic and inorganic compounds, as it relies on its source for its particular existence and concentrations. Fatty acids, carbohydrates, and proteins are the common organic matters present in the textile effluents. Heavy metals, salts, sulfides, sulfates, and nitrates are also observed extensively as inorganic pollutants. Although microorganisms may express enzymes that are capable of degrading the most recalcitrant pollutants, still issues do remain. The application of enzyme technology is well-known not only in textile production industry, as a way of destiny, but also in bioremediation processes. Therefore, enzymology can be considered as a key stone for environmental biotechnology, in specific, biological remediation, be it aerobic or anaerobic. For bioremediation, the most studied enzymes are lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13), and laccases (EC 1.10.3.2). Among these, laccases possess tremendous potential for bioremediation, because of their remarkable hydrolyzing ability to allow a wider spectrum of applications (Ellis et al. 2012).

Enzymes are versatile biocatalysts, with an increasing number of applications in biotechnology. Fascinatingly, its properties allow them to attract and get advantage for conventional treatments against pollutants. The key challenges of the bioengineering strategy include the development of protocol for the production of precious enzymes, using recombinant DNA techniques and deployment of such techniques, in order for a desired lacquer to be manufactured under large-scale yield and robust industrial applications. This strategy can be a valuable scope to push the enzyme ideally suited for industrial applications (Rodriguez-Couto 2013).

The efficacy of dyes decolorization is stated to have enzymes from both anaerobic and aerobic systems, typically from white-rot fungus, *Phanerochaete chrysosporium*. The laccases and manganese/lignin peroxidases that are capable of oxidative free radical azo cleavage are secreted by this species (Saravanan 2013). Due to the variability in the industrial effluent composition as well as the structural diversity of the dyes, biodegradation of dyes is more recalcitrant. Enzymes have a range of characteristics that make them more competitive as compared with conventional catalysts. They are degradable key ingredients, allow activity at different concentration levels of substrates, and enable activity over a broad spectrum of pH, temperature, and salinity. It can acquire self-induced particle agglomeration and is quick to manipulate. These advantages are incorporated to their high specific characteristics and catalytic actions with the possibility of designing enzymes accompanying desirable characteristics through genetic manipulation and parallel computing layout for potential treatment of wastewater. Table 10.5 presents some selective microbial culture applied for removal of dye degradation.

Yeast Culture

Yeast is commonly available culture that has different advantages, such as low cost, easily available, and better resistance to unusual conditions. Up to date, several yeasts were observed to be capable of treating textile wastewaters. Various reports are documented about the applicability of these cultures for dye decolorization. Table 10.5 presents a condensed overview on recent reports about the different cultures used for dye degradation. Decolorization is carried out by the mechanisms, biosorption, biodegradation, and bioaugmentation. From the recent observations, the genus *Candida* is the most widely studied culture for decolorization of dyes. A thermotolerant yeast, *Kluyveromyces marxianus*, show remarkable ability for removing Remazol Black-B (Singh 2014).

Bacterial Culture

Compared to the fungal system, bacterial decolorization is quicker with respect to the nature of dyes. Hence, among the different isolated microorganisms, bacteria are frequently used. The bacterial strains, *Bacillus, Pseudomonas, Serratia liquefaciens,* Halobacterium, and *Staphylococcus* sp., are the most studied for decolorization and detoxification. Different studies found that the isolated and established bacterial strains are more effective and have tremendous potential for textile degradation under flexible environmental conditions.

Fungal Cultures

Fungi can be employed for biodegrading and transforming the toxic substances into safe, tolerable, or useful products. Specific fungal strains with their extracellular enzymes are well-known to degrade a wide range of recalcitrant compounds, such as xenobiotics, lignin, and dyes. Several studies have shown that certain fungal strains

Table 10.5 Selective microbial cultures applicable for removal of dye degradation

Strain	Dye	Concentration (mg L^{-1})	Decolorization (%)	Time	References
Yeast culture					
Trichosporon beigelii	Navy Blue HER	50	95%	24 h	Dafale et al. (2010)
Candida krusei	Basic Violet 3	10	100%	24 h	Deivasigamani and Das (2011)
Candida zeylanoides	Azo dye	20	90%	24 h	Jafari et al. (2014)
Sterigmatomyces halophilus SSA-1575	Reactive Black 5	50	98%	24 h	Rani et al. (2014)
Fungal culture					
Aspergillus niger	Malachite green	85.5	93.33%	14 days	Haq et al. (2018)
White-rot fungus *Cyathus bulleri*	Kiton blue A	50	88%	6 h	Vats and Mishra (2017)
Bjerkandera adusta OBR105	Reactive and acid dyes	200	99%	3 days	Singh et al. (2010)
Marasmius cladophyllus UMAS	Remazol Brilliant Blue R	200	100%	15 days	Singh et al. (2010)
Bacterial culture					
B. megaterium	Synozol red 6HBN	20	98%	96 h	Ajaz et al. (2020)
Pseudomonas extremorientalis BU118	Congo red	100	75%	24 h	Neifar et al. (2016)
Serratia liquefaciens	Azure-B	100	90%	48 h	Haq et al. (2018)
Staphylococcus sp.	Remazol Brilliant Blue R	100	100%	12 h	Karthikeyan et al. (2017)
Bacillus aryabhattai DC100	Coomassie Brilliant Blue G-250	150	100%	72 h	Paz et al. (2017)

can degrade various types of synthetic colors, such as azo, triphenyl, methane, silicone, phthalocyanine, and heterocyclic compounds. The lignolytic and non-lignolytic fungi were used by many researchers to decolorize the dyes present in the wastewater (Singh et al. 2010). Among them, the lignolytic white-rot fungi are observed to be potential microorganisms for dye degradation. The fungi-like *Aspergillus niger, white-rot fungus, Cyathus bulleri,* and *Marasmius cladophyllus* have shown an efficient decolorization (Sumandono et al. 2015). Especially, *white-rot fungi* produce ligninolytic enzymes, such as peroxidase, manganese peroxidase, and laccase, which involved in the degradation of several dyes (Dafale et al. 2010).

Microalgae

Recently, the use of microalgae for bioremediation textile wastewaters has drawn a huge interest due to their excellent role in carbon dioxide fixation. The culture of vulgaris in the textile waste effluent showed that these microalgae can be used to extract color and reduce COD. Additionally, the biomass from algae has great value as feedstock for the production of biofuel (Huang et al. 2010). Such kind of bioremediation with microalgae can be an ideal for environmental sustainability (Ellis et al. 2012, Chandran et al. 2002). Bioremediation using microalgae shows several advantages in terms of cost-effectiveness and easy scale-up. Using microalgae, bioremediation, and CO_2 mitigation can be carried out simultaneously; also, high-added-value molecules can be biosynthesized.

Aerobic Biodegradation

The toxic compounds present in the textile wastewaters can be decomposed into harmless inorganic solids by the bacterial catalysis being involved during the biodegradation treatment. Generally, bacteria can be categorized into aerobic, anaerobic, and facultative bacteria based on the oxygen requirement. The aerobic bacteria purify wastewaters, by decomposing it, and reduce unpleasant odors with the help of aerobic and facultative bacteria. The aerobic treatment process is performed by an activated sludge and biofilm process (Robinson et al. 2001). Activated sludge process (ASP) is a widely used biological process to treat textile wastewater effluent treatment. In this process, a kind of colony comprising deferent microorganisms that have high level of biosorption and decay capacity, colloquially called as “activated sludge.” Such kind of biological treatment eliminates the organic matter, residual solids, and non-settable solids. Here, microorganisms, particularly bacteria, are employed. ASP works based on the principle of microorganisms that forms a colony by growing and clumping together. During ASP, the microorganisms form agglomerated organic materials and settle down to the bottom of the tank, which creates a clear liquid free from suspended solids. The most commonly used activated sludge methods are the oxidation ditch and the sequenced batch reactor (SBR) process (Mani and Bharagava 2018). A biofilm is a biological effluent treatment process that involves microorganisms, which are attaching to the outer edge of the fixed object to develop a film, which allows purifying the swirling effluent. The biofilm mechanisms are mainly carried out at biological contact oxidation, revolving biological contractors, and fluidized bioreactor (Dos Santos et al. 2007).

Anaerobic Biodegradation

Anaerobic degradation occurs when the medium contains no oxygen or very low oxygen. This procedure also degrades some sort of waste in the effluents. So, the substance can be forecasted with low water solubility, which may be subject to anaerobic degradation. Anaerobic degradation is accomplished by the anaerobic bacteria (Seshadri et al. 1994). In this case, acidic medium-acting bacteria act on organic substances, such as carbohydrates and fats. Such organic materials can be converted into alcohols and other basic compounds through the catalysis of acidic bacteria. These outcome products stimulate the acetogenic bacteria, which then transform them into carbon dioxide and molecular hydrogen. Anaerobic reduction of azo dyes can be an effective and economic treatment process for color removal from textile wastewaters (Strotmann et al. 1995).

3.3.2 Agricultural Residues for Color Removal

Agricultural wastes are nonconventional low-cost adsorbents for metal adsorption, being porous, and lightweight due to their fibrous existence. There are many sustainable natural adsorbents that are commonly available globally, either at no cost or at very low cost, as crop residues. Corncob shreds, wood chips and wheat straw, cassava waste, coconut wastes, coir pith, and oil palm waste can be used for their capacity to absorb individual colors and color mixtures. The retained agricultural substances were used instead of using chemicals to extract color from dye-contaminated water to examine them as an economical and eco-friendly bioremediation process. Studies showed that a color removal can be achieved up to 70–75% using agro-waste in the removal of dye decolorization (Wong et al. 2004)

3.3.3 Chitin and Chitosan

Chitin is known as one of the important natural polymers. The two marine crustaceans, crabs and shrimp, are the principle sources for chitin. Chitosan is the derivative of chitin. While deacetylation of chitin is carried out to reach about 50%, chitin becomes soluble in aqueous acidic media and is called as chitosan. Chitosan, cationic polyelectrolyte, has been investigated for different industrial effluent treatment. It can act in the agglutination of colloidal particles due to its efficient electrostatic action. Apart from acting as an auxiliary agglutinating polyelectrolyte, chitin and chitosan are good adsorbents, able to efficiently remove azo dyes from textile effluents (Mahajan 2004).

3.4 Integrated Approaches with Biotechnological Interventions

3.4.1 Cell Factories and Whole-Cell Biocatalysts (WCBs)

The use of plants, fungi, microbes, etc. is primarily considered in bioremediation to remove the contaminants. In recent bioremediation techniques, the enzymes and whole-cell-based biocatalysts show a quite better functional specificity with a broad range of substrates. Metabolic engineering provides the classical way for the different unique strategies, powered by the computational biology-based genome-level reconstructions. These strategies under the protein engineering seem to be beneficial to develop the efficacy of enzyme catalyzation. Keeping the view, aiming to different bio-products and industrial applications, a complex buildup on the pathways of enzyme-catalyzed reaction can be designed as whole-cell biocatalysts (WCB), through metabolic engineering (Han et al. 2018). Therefore, in microbial engineering, several opportunities are open up with novel frontiers toward high-valued biomolecules. Some specific microorganisms can cultivate even existence of inhibitors in the fermentation media. Moreover, they can accrue improved concentrations of intracellular lipids with specific optimal conditions. Such kind of genomic features of the microbes can be a leading biological agent for sustainable cell factories, directed at production of high-valued industrially applicable products and enzymes (Almyasheva et al. 2018). As mentioned earlier, the laccases have a good ability of detoxifying different phenolic and aromatic organic substances to less harmful fragments. Using diffuse adhesion technique, one of the metabolic engineering techniques, the laccases, can be attached on the surface of *E. coli*. These engineered *E. coli* cells can be applied for bioremediation of organic phenolic compounds after immobilization (Vikrant et al. 2018). The effluents from textile industries are associated with heavy metals and dyes that need effective remedial treatments to meet the required task with respect to legislation of pollution control. For this case, specific microbial cell factories having the characteristics of intrinsic tolerance against toxic compounds, like solvent-tolerant microorganisms, are highly considered. Another sophisticated approach, protein engineering, can be applied for ameliorating the desirable properties of enzymes. However, immobilization to appropriate carriers, stabilization, and implementation are observed as critical steps. In situ immobilization is a promising approach in bioengineering that produces highly active enzymes and carrier materials, using synthetic biology way. It is proven to be cost-effective for producing biocatalyst for the use of bioremediation (Potter 1911).

3.4.2 Microbial Fuel Cell Technology

An interesting idea for generating electricity, accompanying decaying organic wastes with the fundamental principle of bioenergy production using microbes, was examined in 1911 (Liu et al. 2004). Even though the proof of concept and devices with different types were already reported elsewhere, wide investigations are needed to further explore the method for low cost and robustness (Karube et al. 1976). In this method, continuous production of hydrogen is achieved by immobilized whole cells under the aerobic environment at optimal pH and temperature (Santoro et al. 2017). The microbial fuel cell (MFC) is cathode and anode chambers, as depicted in Fig. 10.3, that produce protons and electrons. In the process, a steady flow of electrons is generated in the wire connecting anode and

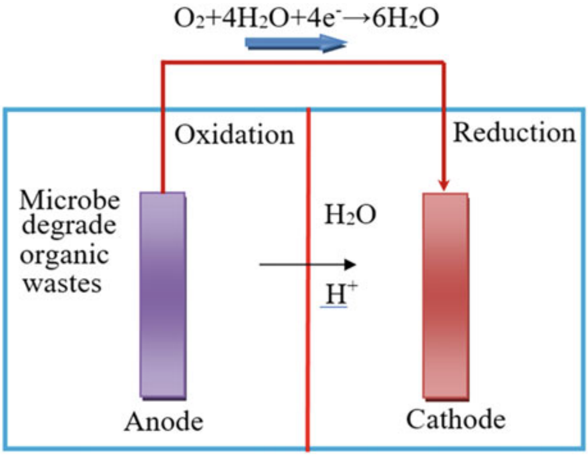


Fig. 10.3 Mechanism of microbial fuel cell

cathode chambers. Outcome results of MFCs are highly affected by different parameters, such as permeability, biological factors, density of the medium, and oxidation and reduction potential. During the process, oxygen serves as the electron acceptor in the cathode chamber. Bio-catalyzation improves the rate of reactions and reduces the activation energy (Rahimnejad et al. 2015). Hence, in the overprocess, potential hazardous wastes are not generated in large scale. This value enables the favorable exploitation of MFC technique for in situ bioremediation of contaminated sites (Chandrasekhar et al. 2018). Recently, waste management specialists are focusing in the recovery of useful resources from the textile wastewaters using microbes. Aiming to production of bioenergy from textile effluents, the implementation of the MFC technique can be utilized as an appropriate tool. The investigations on this thematic have demonstrated that the *Klebsiella* species have shown quite substantial ability on generation of electricity from the biodegradation of dyes such as Reactive Blue-19 (Holkar et al. 2018). Some researchers have used hybrid anoxygenic photosynthetic bacteria for anaerobic biodegradation of poly(lactic) acid textiles (Qi et al. 2018). In another study, *desulfovibrio* and *proteobacteria* have been employed for degradation of dyes, reduction of sulfate, and recalcitrant substances removal along with simultaneous production of electricity (Miran et al. 2018). Therefore, the use of microbes is uniquely several beneficial for the bioremediation of textile effluent treatment, through an environmentally friendly with sustainable style.

3.4.3 Nanotechnological Applications

Carbon nanotubes (CNTs) are well-known nanomaterials used for the sensitive identification and detection. So far, in order to ameliorate the performance of enzyme immobilization, such as loading, stability, activity, and cost reduction, various novel technologies have been examined toward the applications for the textile waste treatment. Most recently, the application of nanotechnology in enzymes immobilization has gained a huge interest, because this approach offers more benefits with respect to a broader spectrum of pH, homogeneous catalysis, metabolic systems engineering, high surface-volume ratio, applicability to wide temperature range, synthetic biology of multienzyme cascades, and improved thermal stability (Lorenzo et al. 2018). Recent developments in synthetic biology, systems biology, metabolic engineering, and microfluidic techniques provide tools for transforming through integration of novel nanobiological components to attain sophisticated sensing, regulation, and metabolic function for environmental remedial applications. Thus, the integrated approaches delivered comparatively better outcome for making specific and sensitive bio-remedial systems with high efficiency and wide applications (Vigneshvar et al. 2016).

3.5 Appropriation of Best Practices

3.5.1 Eco-Friendly Alternatives to Conventional Manufacturing

Many textile companies are using innovative and eco-friendly technologies to resolve environmental problems created by traditional production processes. Some of these are air dyeing technology (ADT), enzymatic one-bath pretreatment, laser bleaching and washing, digital printing, etc. Natural dyeing methods are useful in minimizing the effect of the dyeing processes on the ecosystem, although they require twice the amount of dyeing materials (such as wild plants and lichens) and mordant to the weight of the fiber (Fitzpatrick et al. 2010).

3.5.2 Substituted Textile Process Products

The high-polluting and nondegradable chemicals can be replaced by less polluted chemicals. Similarly, textile companies can choose treatment plants that cause less harmful emissions, such as replacing chemicals with mechanical ones (Tsai and Chou 2004).

3.5.3 Less Polluting Substances

The choosing of less polluting raw material is an initial screening procedure, and by following this approach, textile companies can mitigate the generation of waste from the initial stage, such as using vat dyes rather than azo dyes where appropriate, and reuse dye and wash wastewater for the preceding process.

4 Conclusions and Perspectives

Waste minimization plays a major role in reducing the pollution load and the cost. The chapter reviewed sustainable concerns to treat textile effluent through some common methods, including flocculation, coagulation, adsorption, ozonation, and biological method. Compared with physiochemical methods, biological methods can be an appropriate method in terms of cost efficiency and be a better alternative for textile wastewater treatment. All the other techniques are expensive and have significant drawbacks when applied to the textile effluent treatment.

References

Adams CD, Fusco W, Kanzelmeyer T (1995) Ozone, hydrogen peroxide/ozone and UV/ozone treatment of chromium and copper complex dyes: decolorization and metal release. Ozone Sci Eng 17:149–161

Ahmad R, Mondal PK (2009) Application of acid treated almond peel for removal and recovery of brilliant green from industrial wastewater by column operation. Sep Sci Technol 44 (7):1638–1655

Ajaz M, Shakeel S, Rehman A (2020) Microbial use for azo dye degradation – a strategy for dye bioremediation. Int Microbiol 23:149–159. https://doi.org/10.1007/s10123-019-00103-2

Alkaya E, Bögürcü M, Ulutaş F, Demirer GN (2012) Cleaner (sustainable) production in textile wet processing. In: Non-conventional textile waste water treatment, Nova Science Publishers, Inc, pp 205–229

Almyasheva NR, Shuktueva MI, Petrova DA, Kopitsyn DS, Kotelev MS, Vinokurov VA, Novikov AA (2018) Biodiesel fuel production by Aspergillus niger whole-cell biocatalyst in optimized medium. Mycoscience 59(2):147e152

Araújo R, Casal M, Cavaco-Paulo A (2008) Application of enzymes for textile fibres processing. Biocatal Biotransform. https://doi.org/10.1080/10242420802390457

Asha S, Viraraghavan T (2010) Decolorization of dye wastewaters by biosorbents: a review. J Environ Manag 91:1915–1929. https://doi.org/10.1016/j.jenvman.2010.05.003

Bhosale S, Saratale G, Govindwar S (2006) Biotransformation enzymes. J Bas Microbiol 46:444–448

Bisschops I, Henri S (2003) Literature review on textile wastewater characterisation. Environ Technol 24:1399–1411. https://doi.org/10.1080/09593330309385684

Bizuneh A (2012) Textile effluent treatment & decolorization techniques. Chem Bulgarian J Sci Edu 21:434–456

Brahmbhatt NH, Jasrai RT (2016) The role of algae in bioremediation of textile effluent. Int J Eng Res Gen Sci 4(1):443–453

Broadbent AD (2001) Basic principles of textile coloration. Society of Dyers and Colourists, England

Brüschweiler BJ, Merlot C (2017) Azo dyes in clothing textiles can be cleaved into a series of mutagenic aromatic amines which are not regulated yet. Regulat Toxicol Pharmacol. https://doi.org/10.1016/j.yrtph.2017.06.012

Carmen Z, Daniela S (2012) Textile organic dyes – characteristics, polluting effects and separation/elimination procedures from industrial effluents – a critical overview. In: Puzyn T, Mostrag-Szlichtyng A (eds) Organic pollutants ten years after the Stockholm convention – environmental and analytical update. IntechOpen. https://doi.org/10.5772/32373

Chacko J, Kalidass S (2011) Enzymatic degradation of azo dyes – a review. Int J Environ Sci 1:1250–1255

Chandran C, Singh D, Nigam P (2002) Remediation of textile effluent using agricultural residues. Appl Biochem Biotechnol 102–103:1–6

Chandrasekhar K, Kadier A, Kumar G, Nastro RA, Jeevitha V (2018) Challenges in microbial fuel cell and future scope. In: Microbial fuel cell. Springer, Cham, p 483e499

Chang S (2014) Anaerobic membrane bioreactors (AnMBR) for wastewater treatment. Adv Chem Eng Sci. https://doi.org/10.4236/aces.2014.41008

Choudhury AKR (2017) Sustainable chemical technologies for textile production. Sustain Fib Text Elsevier Inc. https://doi.org/10.1016/B978-0-08-102041-8.00010-X

Coste CO, Marmagne, Jacquart JC (1996) Effluents decoloration. L'Industrie Text 1278:46–51

Crini G, Lichtfouse E, Wilson LD, Morin-Crini N (2019) Conventional and non-conventional adsorbents for wastewater treatment. Environ Chem Lett. Springer. https://doi.org/10.1007/s10311-018-0786-8

Curtis GP, Reinhard M (1994) Reductive dehalogenation of hexachloroethane, carbon tetrachloride and bromoform by anthrax hydroquinone disulfonate and humic acid. Environ Sci Technol Co New York 28:2393–2401

Dafale N, Wate S, Meshram S et al (2010) Bioremediation of wastewater containing azo dyes through sequential anaerobic–aerobic bioreactor system and its biodiversity. Environ Rev 18:21–36

Daneshvar N, Ayazloo M, Khataee AR, Pourhassan M (2007) Biological decolorization of dye solution containing malachite green by microalgae Cosmarium sp. Biores Technol. https://doi.org/10.1016/j.biortech.2006.05.025

Deivasigamani C, Das N (2011) Biodegradation of basic violet 3 by Candida krusei isolated from textile wastewater. Biodegradation 22(6):1169–1180

Dmochewitz S, Ballschmiter K (1988) Microbial transformation of technical mixtures of polychlorinated biphenyls (PCB) by the fungus *Aspergillus niger*. Chemosphere 17:111–121

Dos Santos AB, Cervantes FJ, van Lier JB (2007) Review paper on current technologies for decolorization of textile wastewaters: perspectives for anaerobic biotechnology. Biores Technol 98(12):2369–2385

Dotto J, Fagundes-Klen MR, Veit MT, Palácio SM, Bergamasco R (2019) Performance of different coagulants in the coagulation/flocculation process of textile wastewater. J Clean Prod. https://doi.org/10.1016/j.jclepro.2018.10.112

Duran N, Esposito E (2000) Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review. Appl Catal B Environ 28:83–99

Ellis JT, Hengge RC, Sims MCD (2012) Acetone, butanol and ethanol production from wastewater algae. Biore Technol 111:491–495

Ellouze E, Tahri N, Amar RB (2012) Enhancement of textile wastewater treatment process using nanofiltration. Desalinization 286:16–23

Enayatzamir K, Alikhani HA, Yakhchali B, Tabandeh Rodríguez-Couto S (2010) Decolouration of azo dyes by Phanerochaete chrysosporium immobilised into alginate beads. Environ Sci Poll Res. https://doi.org/10.1007/s11356-009-0109-5

Eswaramoorthi S, Dhanapal K, Chauhan D (2008) Advanced in textile waste water treatment: the case for UV-Ozonation and membrane bioreactor for common effluent treatment plants in Tirupur, Tamil Nadu, India. Environment with people's involvement Adin A. and Asano T. 1998. The role of physicochemical treatment in wastewater reclamation and reuse. Wat Sci Tech, 37:79–90 & Co-ordination in India. Coimbatore, India

Fitzpatrick B, Johnson J, Kump D, Smith J, Voss S, Shaffer H (2010) Rapid spread of invasive genes into a threatened native species. Proc Natl Acad Sci USA 107:3606–3610

Frank PVZ, Lettinga G, Field JA (2001) Azo dye decolourization by anaerobic granular sludge. Chemosphere 44:1169–1176

Ghaly A, Ananthashankar R, Alhattab M, Ramakrishnan V, Ghaly A (2014) Chemical engineering & process technology production, characterization and treatment of textile effluents: a critical review. J Chem Eng Process Technol. https://doi.org/10.4172/2157-7048.1000182

Gulzar T, Farooq T, Kiran S, Ahmad I, Hameed A (2018) Green chemistry in the wet processing of textiles. In: The impact and prospects of green chemistry for textile technology. Elsevier. https://doi.org/10.1016/B978-0-08-102491-1.00001-0

Han L, Zhao Y, Cui S, Liang B (2018) Redesigning of microbial cell surface and its application to whole cell biocatalysis and biosensors. App Biochem Biotechnol 185(2):396e418

Haq I, Raj A, Markandeya (2018) Biodegradation of Azure-B dye by Serratia liquefaciens and its validation by phytotoxicity, genotoxicity and cytotoxicity studies. Chemosphere 196:58–68

Hardin IR (2010) Enzymatic treatment versus conventional chemical processing of cotton. In: Advances in textile biotechnology. Elsevier Inc. https://doi.org/10.1533/9780857090232.2.132

Hasanbeigi A, Price L (2015) A technical review of emerging technologies for energy and water efficiency and pollution reduction in the textile industry. J Clean Prod. Elsevier Ltd. https://doi.org/10.1016/j.jclepro.2015.02.079

Hashemi Sanatgar R, Campagne C, Nierstrasz V (2017) Investigation of the adhesion properties of direct 3D printing of polymers and nanocomposites on textiles: effect of FDM printing process parameters. Appl Surf Sci. https://doi.org/10.1016/j.apsusc.2017.01.112

Hassan MM, Carr CM (2018) A critical review on recent advancements of the removal of reactive dyes from dyehouse effluent by ion-exchange adsorbents. Chemos Elsevier Ltd. https://doi.org/10.1016/j.chemosphere.2018.06.043

Hatvani N, Mécs I (2001) Production of laccase and manganese peroxidase by Lentinus edodes on malt-containing by-product of the brewing process. Proc Biochem 37:491–496

Holkar CR, Jadhav AJ, Pinjari DV, Mahamuni NM, Pandit AB (2016) A critical review on textile wastewater treatments: possible approaches. J Environ Manage. https://doi.org/10.1016/j.jenvman.2016.07.090

Holkar CR, Arora H, Halder D, Pinjari DV (2018) Biodegradation of reactive blue 19 with simultaneous electricity generation by the newly isolated electrogenic Klebsiella sp. C NCIM 5546 bacterium in a microbial fuel cell. Int Biodeterior Biodegrad 133:194e201

Huang GF, Chen D, Wei ZX, Chen G (2010) Biodiesel production by microalgal biotechnology. Appl Energ 87:38–46

Hussain T, Wahab A (2018) A critical review of the current water conservation practices in textile wet processing. J Clean Prod. https://doi.org/10.1016/j.jclepro.2018.07.051

Jafari N, Soudi M, Kasra-Kermanshahi R (2014) Biodecolorization of textile azo dyes by isolated yeast from activated sludge: Issatchenkia orientalis JKS6. Ann Microbiol 64. https://doi.org/10.1007/s13213-013-0677-y

Karthikeyan V, Kumar MA, Mohanapriya P et al (2017) Biodegradation of Remazol Brilliant Blue R using isolated bacterial culture (Staphylococcus sp. K2204). Environ Technol 39(22):1–8

Karube I, Matsunaga T, Tsuru S, Suzuki S (1976) Continuous hydrogen production by immobilized whole cells of Clostridium butyricum. Biochim Biophys Acta Gen Sub 444(2):338e343

Kasperchik V, Yaskevich A, Bil'dyukevich A (2012) Wastewater treatment for removal of dyes by coagulation and membrane processes. Petrol Chem 52. https://doi.org/10.1134/S0965544112070079

Katheresan V, Kansedo J, Lau SY (2018) Efficiency of various recent wastewater dye removal methods: a review. J Environ Chem Eng. https://doi.org/10.1016/j.jece.2018.06.060

Kavitha R, Venkatesa Prabhu S, Belew BM, Solomon E, Mekonen SW (2020) RSM based process modeling and optimization for amylase enzyme production and its desizing potential. Int J Innov Technol Exp Eng 9(7):382–391. https://doi.org/10.35940/ijitee.F4149.059720

Khatri A, Peerzada MH, Mohsin M, White M (2015) A review on developments in dyeing cotton fabrics with reactive dyes for reducing effluent pollution. J Clean Prod. https://doi.org/10.1016/j.jclepro.2014.09.01

Kurniawan TA, Chan GY, Babel S (2006) Physico-chemical treatment techniques for wastewater laden with heavy metals. Chem Eng J. https://doi.org/10.1016/j.cej.2006.01.015

Langlais B, Reckhow DA, Brink DR (1991) Ozone in water treatment: application and engineering. Lewis Publishers, London

Lim SL, Chu WL, Phang SM (2010) Use of Chlorella vulgaris for bioremediation of textile wastewater. Bioresource Technol 101:7314–7322

Lin S, Lin CM (1993) Treatment of textile waste effluents by ozonation and chemical coagulation. Water Res 27:1743–1748

Liu H, Ramnarayanan R, Logan BE (2004) Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environ Sci Technol 38(7):2281e2285

Liu ZT, Yang Y, Zhang L, Liu ZW, Xiong H (2007) Study on the cationic modification and dyeing of ramie fiber. Cellulose. https://doi.org/10.1007/s10570-007-9117-0

Lorenzo V, Prather KL, Chen GQ, O'Day E, von Kameke C, Oyarzun DA, Okano H (2018) The power of synthetic biology for bioproduction, remediation and pollution control: the UN's Sustainable Development Goals will inevitably require the application of molecular biology and biotechnology on a global scale. EMBO reports e45658

Mahajan SP (2004) Pollution control in process Industries. Summer report, IIT, Bombay, 5

Mani S, Bharagava R (2018) Textile industry wastewater: environmental and health hazards and treatment approaches, pp 59–62

Miran W, Jang J, Nawaz M, Shahzad A, Lee DS (2018) Sulfate-reducing mixed communities with the ability to generate bioelectricity ands degrade textile diazo dye in microbial fuel cells. J Hazard Mater 352:70e79

Mostafa M (2015) Waste water treatment in textile industries – the concept and current removal technologies. J Biodiv Environ Sci 7:501–525

Neha S, Remya N (2019) Microwave photo-oxidation with diverse oxidants for Congo red degradation: effect of oxidants, degradation pathway and economic analysis. Environ Technol 2019:1–11

Neifar M, Chouchane H, Mahjoubi M et al (2016) Pseudomonas extremorientalis BU118: a new salt-tolerant laccase-secreting bacterium with biotechnological potential in textile azo dye decolourization. 3Biotech, pp 6107

Orhon D, Germirli Babuna F, Insel G (2001) Characterization and modelling of denim-processing wastewaters for activated sludge. J Chem Technol Biotechnol 76:919–931

Owas SZ (2017) Application of natural coagulant for removal of dye from synthetic textile wastewater. Int J Adv Res Dev 2(4):2455–4030

Paz A, Carballo J, Pérez MJ et al (2017) Biological treatment of model dyes and textile wastewaters. Chemosphere 181:168–177

Piaskowski K, Świderska-Dąbrowska R, Zarzycki PK (2018) Dye removal from water and wastewater using various physical, chemical, and biological processes. J AOAC Int 101 (5):1371–1384. https://doi.org/10.5740/jaoacint.18-0051

Potter MC (1911) Electrical effects accompanying the decomposition of organic compounds. Proceed R Soc London B 84(571):260e276

Qi X, Bo Y, Ren Y, Wang X (2018) The anaerobic biodegradation of poly (lactic) acid textiles in photosynthetic microbial fuel cells: self-sustained bioelectricity generation. Poly Degrad Stab 148:42e49

Rahimnejad M, Adhami A, Darvari S, Zirepour A, Oh SE (2015) Microbial fuel cell as new technology for bioelectricity generation: a review. Alexandria Eng J 54(3):745e756

Rahman Bhuiyan MA, Mizanur Rahman M, Shaid A, Bashar MM, Khan M (2016) Scope of reusing and recycling the textile wastewater after treatment with gamma radiation. J Clean Prod. https://doi.org/10.1016/j.jclepro.201510.029

Rani B, Kumar V, Singh J et al (2014) Bioremediation of dyes by fungi isolated from contaminated dye effluent sites for bio-usability. Braz J Microbiol 45(3):1055–1063. https://doi.org/10.1590/s1517-83822014000300039

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. Biores Technol. https://doi.org/10.1016/S0960-8524(00)00080-8

Rodriguez-Couto S (2013) Treatment of textile wastewater by white-rot fungi: still a faraway reality? Text. Text Light Indus Sci Technol 2:113–119

Saleh FY, Lee GF, Wolf HW (1980) Selected organic pesticides, the occurrence, transformation and removal from domestic wastewater. J WPCF 52(1):19–28

Santoro C, Arbizzani C, Erable B, Ieropoulos I (2017) Microbial fuel cells: from fundamentals to applications. A review. J Power Sources 356:225e244

Saratale R, Saratale G, Chang J, Govindwar S (2001) Bacterial decolorization and degradation of azo dyes: a review. J Taiwan Inst Chem Eng 42:138–157

Saravanan, P (2013) Studies on treatment of textile dyes using yeast cultures. Dissertation, Anna University

Savin I, Butnaru R (2008) Wastewater characteristics in textile finishing mills. Environ Eng Manag J 7(6):859–864. https://doi.org/10.30638/eemj.2008.113

Sekomo CB, Rousseau DPL, Saleh AS, Lens PNL (2014) Heavy metal removal in duckweed and algae ponds as a polishing step for textile wastewater treatment. Ecol Eng 44:102–110

Seshadri S, Bishop PL, Agha AM (1994) Anaerobic/Aerobic treatment of selected azo dyes in waste water. Waste Manage 14:127–137

Singh SN (2014) Microbial degradation of synthetic dyes in wastewaters. Springer; ISBN: 978-3-319-10942-8, p 367

Singh S, Pakshirajan K, Daverey A (2010) Enhanced decolourization of Direct Red-80 dye by the white rot fungus Phanerochaete chrysosporium employing sequential design of experiments. Biodegradation 21(4):501–511

Sivaram NM, Gopal PM, Barik D (2018) Toxic waste from textile industries. In: Energy from toxic organic waste for heat and power generation. Elsevier. https://doi.org/10.1016/B978-0-08-102528-4.00004-3

Solis M, Solis A, Pérez H, Manjarrez N, Flores M (2012) Microbial decolouration of azo dyes: a review. Process Biochem:47.1723–47.1748

Srivastava S, Agrawal S, Mondal M (2015) Characterization, isotherm and kinetic study of Phaseolus vulgaris husk as an innovative adsorbent for Cr (VI) removal. Kor J Chem Eng. https://doi.org/10.1007/s11814-015-0165-0

Strotmann UJ, Schwartz H, Pagga U (1995) the combined CO2/DOC test a new method to determine the biodegradability of organic compounds. Chemosphere 30:525–538

Sumandono T, Saragih H, Migirin Watanabe Tet al. (2015) Decolorization of Remazol Brilliant Blue R by new isolated white rot fungus collected from tropical rain forest in East Kalimantan and its ligninolytic enzymes activity. Procedia Environ Sci 28:45–51

Tsai WT, Chou YH (2004) Government policies for encouraging industrial waste reuse and pollution prevention in Taiwan. J Clean Prod 12:725–736

Tzanov T, Calafell M, Guebitz GM, Cavaco-Paulo A (2001) Bio-preparation of cotton fabrics. Enzy Microb Technol. https://doi.org/10.1016/S0141-0229(01)00388-X

UNIDO & Ministry of International Trade and Industry Japan (MITI) (1992) Handy manual textile industry – output of a seminar on energy conservation in textile industry, Organised by the Energy Conservation Center (ECC) Japan, Malaysia & Indonesia

Vats A, Mishra S (2017) Decolorization of complex dyes and textile effluent by extracellular enzymes of Cyathus bulleri cultivated on agro-residues/domestic wastes and proposed pathway of degradation of Kiton blue A and reactive orange 16. Environ Sci Pollut Res 24:1–13

Vigneshvar S, Sudhakumari CC, Senthilkumaran B, Prakash H (2016) Recent advances in biosensor technology for potential applications – an overview. Front Bioeng Biotechnol 4:11

Vikrant K, Giri BS, Raza N, Roy K, Kim KH, Rai BN, Singh RS (2018) Recent advancements in bioremediation of dye: current status and challenges. Biores Technol 253:355e367

Vo HNP, Ngo HH, Guo W, Chang SW, Nguyen DD, Chen Z, Xiaochang C, Wang RC, Zhang X (2020) Microalgae for saline wastewater treatment: a critical review. Crit Rev Environ Sci Technol 50(12):1224–1265

Wang Z, Xue M, Huang K, Liu Z (2011) Textile dyeing wastewater treatment. In: Advances in treating textile effluent. InTech. https://doi.org/10.5772/22670

Wong YC, Szeto YS, Cheung WH, McKay G (2004) Adsorption of acid dyes on chitosan equilibrium isotherm analyses. Process Biochem 39:693–702

Yagi O, Sudo R (1980) Degradation of polychlorinated biphenyls (PCBs) by microorganisms. J WPCF 52(5)

Yaseen DA, Scholz M (2019) Textile dye wastewater characteristics and constituents of synthetic effluents: a critical review. Int J Environ Sci Technol. https://doi.org/10.1007/s13762-018-2130-z

Yasir Aljaberi F, Mohammed W (2018) The most practical treatment methods for wastewaters: a systematic review. Mesopot Environ J 5:1–28

Chapter 11
Modern Bioremediation Approaches for Clean and Green Environment

Ankita Murmu and Murugan Sevanan

Abstract Environmental problems are grave concern to humans and other living organisms. These problems include contamination of soil, water, and air with harmful chemicals. Due to undesirable human activities, the environment is deteriorating, thereby imposing risk on natural resources. The planet Earth is believed to be rich in its natural resources, which are depleting due to toxic contaminants. In the recent past, the fight against environmental pollution has become a crucial challenge worldwide. In this modern world, a cost-effective and environment friendly approach towards sustainability remains the need of the hour. Bioremediation has proved to be one of the most promising technologies for the elimination of harmful pollutants. Chemical and physical methods of remediation have not been proved to be much effective like the bioremediation technique. Bioremediation has been proved to be safer, cleaner, and economical, which makes it the most desirable solution for a clean environment. To date, researchers around the world have looked upon various approaches to enhance bioremediation techniques. This chapter discusses the modern bioremediation approaches which have been used to deal with environmental issues.

Keywords Environmental pollution · Bioremediation · Biological methods · Green environment

1 Introduction

One of the major issues from the past few decades is the environmental pollution, which has affected the overall ecosystem creating imbalance. According to the World Health Organization (WHO), nine out of ten people breath air having very harmful pollutants, leading to 4.2 million deaths every year (Samuel et al. 2019,

---

A. Murmu · M. Sevanan (✉)
Department of Biotechnology, School of Agriculture and Biosciences, Karunya Institute of Technology and Sciences (Deemed to be University), Coimbatore, India

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_11

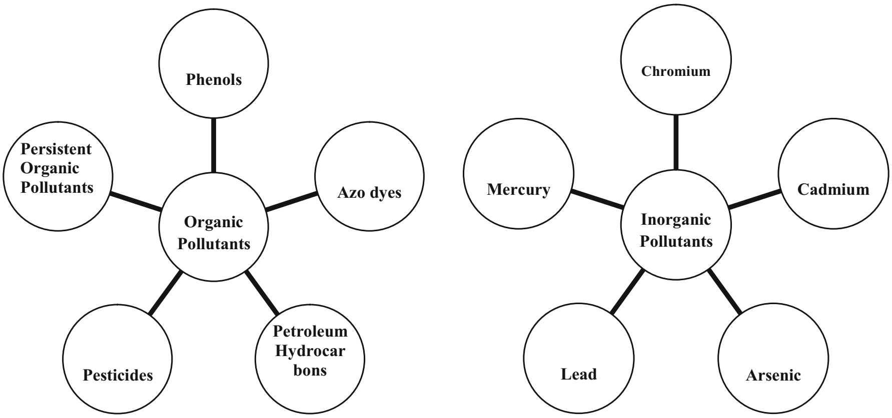


Fig. 11.1 Types of pollutants (Saxena and Bharagava 2017)

2020). There are various causes for environmental pollution, such as emission of fossil fuels, exhaust gases and chemical effluents from industries, pollution from vehicles, and hazardous agricultural practices. The harmful pollutants can be divided into organic and inorganic pollutants (Fig. 11.1), such as heavy metals, dyes, pesticides, and crude oil, which are of an environmental and public health concern due to their toxicities. The major source of pollution containing heavy metals is mining activities, which have impacted the ecosystem causing alterations and loss of biodiversity. Various dyes are also utilized in textile processing industries, which are not susceptible to degradation by physicochemical methods. The health of human beings is compromised when they are exposed to pesticides or chemicals in drinking water and food products from agricultural lands. Apart from this, oil spills due to leakage from industries can form sludge and pollute the soil and water. Moreover, industrial waste disposal is causing serious human health hazards due to their improper management. There has been a spread of emerging infectious diseases causing multiple other complications in human beings due to lack of proper sanitation and clean water sources (Tijani et al. 2016; Alrumman et al. 2016; Ali and Khan 2017). Various organic and inorganic pollutants, like feces, heavy metals, pesticides, dyes, hydrocarbons, etc., have polluted the soil (Datta et al. 2020). Researchers around the world are still finding effective ways to monitor and combat environmental threats through bioremediation strategies in order to mitigate associated risks.

1.1 Types of Bioremediation

Bioremediation is classified majorly into two types – ex situ and in situ bioremediation. Ex situ bioremediation technique eliminates contaminants or pollutants from polluted sites, such as soil and water, and subsequently transports them to another site for treatment. While in the case of in situ bioremediation technique, the pollutant

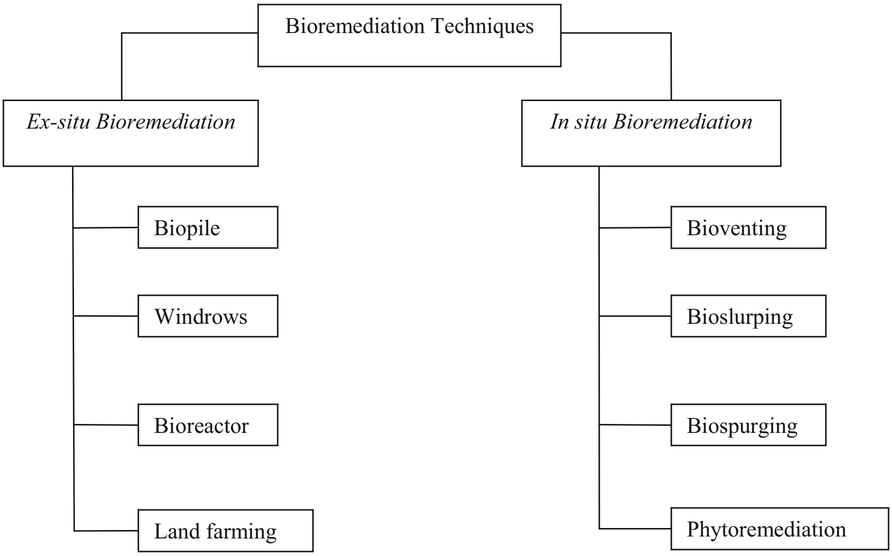


Fig. 11.2 Types of bioremediation techniques (Azubuike et al. 2016)

is treated at the site of pollution. Compared to ex situ bioremediation, in situ bioremediation is considered as less expensive. The ex situ and in situ bioremediation techniques are further divided into subtypes, which are depicted in Fig. 11.2. The selection of appropriate bioremediation technique depends on the nature of the contaminant, environment type and location, and associated environmental policies (Frutos et al. 2012; Smith et al. 2015). Apart from these, some other influencing biotic and abiotic factors are also considered.

1.2 *Role of Bioremediation in Remediating Environmental Pollutants*

Bioremediation has been considered as a sustainable technique for many advantageous reasons to achieve a clean and green environment. The use of microorganisms has been proficient to degrade dissolved and suspended metals (Dundar et al. 2015). Several biological agents are employed in the removal of heavy metals, such as arsenic (Akhter et al. 2017) and inorganic mercury (Dash and Das 2015). More than 95% removal of hydrocarbon from groundwater has been reported by Beškoski et al. (2017) using zymogenous microorganisms. The remediation of crude oil using bioremediation approaches has also been possible (Wu et al. 2017). Toxic dyes discharged from the textile processing industries harm the flora and fauna, changing the normal functioning of the ecosystem. In regard to the toxicity generated due to textile effluents, bioremediation using potential microorganisms has been proved to be efficient. Sinha et al. (2016) reported 96% decolorization of direct red −31 dye using *Chlorella pyrenoidosa* strain NCIM 2738. Microorganisms such as *Trichoderma asperellum* and DDB I strain of *Enterobacter cloacae* have been used in the degradation of crystal violet and hair dye, respectively (Shanmugam et al. 2017; Maiti et al. 2017).

Pesticides that are generally used to control pests or insects to protect the crops and improve the yield of agricultural products has become a threat to the environment. The nearby surface and groundwater bodies are also affected due to contamination with pesticides. Moreover, the management of the contaminants through the remediation process is difficult, as they are known to be persistent in the environment (Nisha et al. 2015). Hence, the degradation of pesticides from different sources with potential microorganisms has been implemented. *Aspergillus niger* AN 400 was used in degrading atrazine from wastewater, which showed 70% degradation efficiency (Marinho et al. 2017). Similarly, 46% degradation of isoproturon was achieved in agricultural soil using the AK1 strain of *Sphingomonas* sp. (Li et al. 2017). The use of both pure and mixed culture of *Streptomyces* spp. has shown >70% removal of organophosphate-based pesticide (chlorpyrifos) and 50% removal of its intermediate 3,5,6-trichloro-2-pyridinol (TCP) (Briceño et al. 2016).

2 Modern Approaches in Bioremediation

2.1 Electrokinetic Bioremediation

Electrokinetic (EK) technology is used for the efficient removal of contaminants of different types, using both in situ and ex situ processes under an electrical potential gradient. Electrokinetic processes depend on various factors, such as the composition of the electrolyte, ionic strength, pH, electrical conductivity and field strength, zeta potential, types and chemistry of soil, permeability and content of water, nature of arrangement, and electrode materials (Tummala and Tewari 2018). EK involves three phenomena, viz., electroosmosis, electromigration, and electrophoresis. Electroosmosis is the transport of liquid in a porous material from anode to cathode under the influence of an applied electric field. It helps in the removal of uncharged organic molecules in the matrix due to a net flux of water created in the soil pore during the process. Electromigration involves the movement of cations toward the cathode and the anions toward the anode. Electrophoresis is the separation or movement of charged or dispersed particles under the influence of an electric field. EK-remediation has been applied for the in situ treatment of less permeable soils, and many successful works have been reported for effective removal of various contaminants from such soils (Rodrigo et al. 2014). The EK technique has been further enhanced, by combining with bioremediation (Table 11.1), and is considered as a prominent approach for the treatment of contaminants in the heterogeneous matrix. An electric field gradient is formed, when the voltage is applied to both sides of the soil. Electrolysis in the samples produces hydrogen (H^+) and hydroxyl (OH^-)

Table 11.1 Recent remediation's using EK-bioremediation techniques

Techniques	Contaminants	Removal/degradation	References
EK-bioremediation	Perchloroethylene (PCE)	98.5%	Chang et al. (2018)
EK-bioremediation	Phthalate esters (PAEs)	68% for di-n-butyl phthalate (DnBP), 42% for diisodecyl phthalate (DiDP)	Yang et al. (2016)
EK-bioremediation	Resins	23.6%	Ma et al. (2020)
EK-bioremediation	Polyaromatic hydrocarbons (PHAs)	55.9%	Li et al. (2016)
	Pyrene		
Phytoremediation-assisted EK-bioremediation	*n*-hexadecane	107.23 ± 4.62 mg $\text{kg}^{-1}\cdot\text{d}^{-1}$	Wu et al. (2020)

ions by the oxidation and reduction process simultaneously in the anode and cathode chambers.

$$\text{Anode(Oxidation)} : 2\text{H}_2\text{O} \rightarrow 4\text{H}^+ + \text{O}_2 + 4\text{e}^- \tag{11.1}$$

$$\text{Cathode(Reduction)} : 2\text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{H}_2 + 2\text{OH}^- \tag{11.2}$$

The process of EK-bioremediation showed promising results in the removal of oxyfluorfen from 0.11 up to 0.17 mg/kg/day than the single process of bioremediation (Barba et al. 2018). EK-remediation has also been combined with phytoremediation for the removal of contaminants from contaminated soil. Feasible removal of lead, arsenic, and cesium from contaminated paddy soil was evaluated by Mao et al. (2016), coupling EK-remediation with phytoremediation. The germination and growth of plant, which is hindered due to the contaminated soil, was favored through EK-phytoremediation, using ryegrass with significant removal of heavy metals and polycyclic aromatic hydrocarbons (Acosta-Santoyo et al. 2017). However, the EK process can also pose a limitation in treating soil contaminants. Deposition of hardness in the electrode and reduction in the motility of bacterial cells can occur, if a high amount of calcium and magnesium are present in the contaminated soil, leading to inefficient removal (Annamalai and Sundaram 2020).

2.2 *Nanobioremediation*

Various nanoparticles have played a significant role in the treatment of pollutants in the environment. The different properties of nanoparticles are considered to be useful in the mechanism of bioremediation. The large surface area enhances reactivity, quantum effects make chemical reactions feasible, and the exhibited surface plasmon resonance helps detect toxicity (Singh et al. 2020). Both in situ and ex situ bioremediation make use of nanoparticles. According to shape, size, structure, and composition, there are different types of nanoparticles, such as nanotubes, nanofibers, nanoshells, nanoclusters, nanocomposites, dendrimers, and nanosheets. The main advantage of using nanoparticles for a clean and green environment lies in its shape and size, as they can diffuse into a contamination zone, which is not possible with the microparticles. Moreover, different types of nanoparticles have been utilized for eliminating contaminants from the environment (Goutam et al. 2018). Using magnesium oxide (MgO) nanoparticles, 100% degradation of the textile effluent Acid Red 73 was achieved (Jorfi et al. 2016). Similarly, removal of methyl orange dye was possible using nano-zinc oxide particles (Hemapriyamvadha and Sivasankar 2015). Nearly, 100% photodegradation of the dye methylene blue was obtained using copper sulfide (CuS) and graphene oxide (GO) nanocomposites, prepared through hydrothermal method (Saranya et al. 2014). Dong et al. (2018) reported tetracycline degradation using iron or nickel bimetallic nanoparticles, in which a decreasing trend of the removal efficiency was visualized. Though nanoparticles have been useful in remediating the contaminants, several problems have been reported due to its highly reactive nature and in situ transport processes (Cundy et al. 2008; Tosco et al. 2014).

The nanobioremediation approach came into effect to overcome the challenges associated with the use of nanoparticles alone. Nanobioremediation uses biosynthetic nanoparticles from microorganisms as well as from plants for the degradation of contaminants (Yadav et al. 2017). There have been several successful nanobioremediation, which were reported to eliminate various contaminants (Table 11.2). The removal of radioactive iodine by *Deinococcus radiodurans* R1 (Au-DR) was successful by adding biogenic gold (Au) nanomaterials into Au-DR, which is radiation-resistant through biomineralization (Choi et al. 2017). The combination of biotic and abiotic degradation was used for degrading Aroclor 1248 (Le et al. 2015). In a similar study, nano zerovalent iron (nZVI) and *Burkholderia xenovorans* were used subsequently, which resulted in 89% degradation of the congeners and 90% biodegradation in the biphenyls, respectively. Titanium dioxide nanoparticles synthesized by rhizospheric microorganisms, such as *Micrococcus lylae* (MF1), *Micrococcus aloeverae* (MF2), *Cellulosimicrobium* sp. (MF3), their consortium, and the root extracts, were found to be successful in degrading toxic methyl orange dye (Fulekar et al. 2018). The combined effect of MgO nanoparticles and yeast *Candida* sp. SMN04 was also studied for degrading pharmaceutical pollutant cefdinir (Adikesavan and Nilanjana 2016).

Though nanobioremediation provides several advantages, it has few limitations too. Encapsulating microorganisms in monolithic nanofibers has shown to decrease the activity and lifespan of microorganisms (Letnik et al. 2015). Leaching has been one of the limitations, as during the adsorption process it leads to penetration of nanoparticles into the food chain or food web, by getting stored in plants, animals, and human beings (Singh and Khan 2018).

Table 11.2 Nanobioremediation of environmental pollutants

Nanoparticles	Microorganisms used	Pollutant removed	References
Palladium/nanoscale iron (Pd/nFe)	*Sphingomonas wittichii* RW1 (DSM 6014)	2,3,7,8-Tetrachlorodibenzop-dioxin (2,3,7,8-TeCDD)	Bokare et al. (2012)
nZVI (nano zerovalent iron)-immobilized alginate beads	*Bacillus subtilis*, *Escherichia coli*, and *Acinetobacter junii*	Chromium (VI)	Ravikumar et al. (2016)
Carbon nanotubes	*Shewanella oneidensis* MR-1	Chromium (VI)	Yan et al. (2013)
Iron(II, III) oxide (Fe_3O_4) nanoparticles	*Sphingomonas* sp. XLDN2-5 cells	Carbazole	Li et al. (2013)
Carboxymethyl cellulose (CMC)-Pd/Fe	*Sphingomonas* sp.	Lindane	Singh et al. (2013).
nZVI	*Sphingomonas* sp. PH-07	Polybrominated diphenyl ethers (PBDEs)	Kim et al. (2012)
Nanoscale zinc oxide (n-ZnO)	*Candida* VITJzN04	Lindane	Salam and Das (2015)

2.3 Microbial Fuel Cells

Microbial fuel cells (MFCs) have been focused due to its several advantages over conventional treatment systems (Li et al. 2014; Gude 2016). It uses microbes that catabolize organic biodegradable substrates to generate bioelectricity. The designs of MFCs are similar to traditional fuel cells containing anode and cathode chambers physically separated by a proton exchange membrane (Fig. 11.3). The anode chamber consists of microorganisms that play an essential role in generating electrons and protons. Moreover, it provides all the required conditions for biomass degradation. The external circuit allows the generated electrons to pass through, which are then reduced to electron acceptors in the cathode. The proton exchange membrane passes the protons generated in the anode chamber to the cathode chamber completing the electrical circuit. The reduction reactions, generating electrons and protons and electron acceptor conditions, develop a biopotential, which leads to the generation of bioelectricity in MFCs (Rahimnejad et al. 2011).

MFCs have been used for the treatment of wastewater containing nonbiodegradable metals. Some of the heavy metal-containing groups, with high redox potentials, can serve as electron acceptors (Wang and Ren 2014), equipping MFCs for both degradation and recovery of heavy metals (Mathuriya and Yakhmi 2014). Microorganisms also have the potential to facilitate bioremediation in areas containing heavy metals. Both single-chamber and dual-chamber MFCs have used microorganisms for the effective removal of metal from wastewater. Complete reduction of chromium (VI) was achieved using *Shewanella decolorationis* S12, *Klebsiella pneumoniae* L17, and mixed culture in a dual-chamber MFC (Liu et al.

Fig. 11.3 Schematic representation of an MFC separated by proton exchange membrane

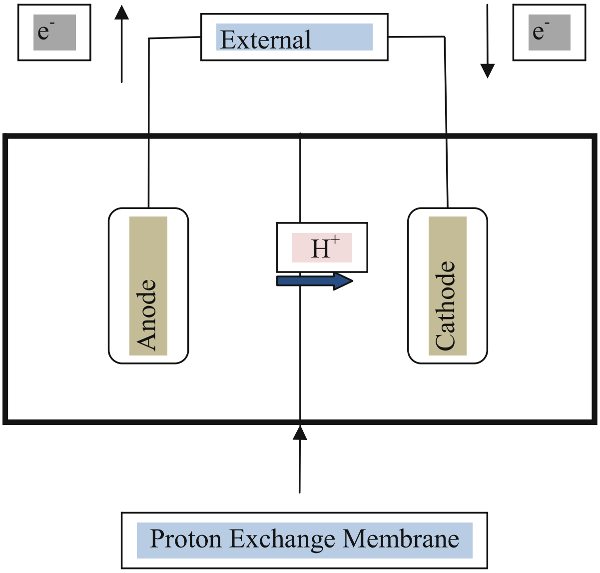


Table 11.3 Energy output generated by MFCs

Composition of microorganisms	Energy generated	References
P. aeruginosa	1530 mWm^{-2}	He et al. (2012)
Mixed community of microbes	3.6 Wm^{-3}	Xiao et al. (2012)
Shewanella oneidensis MR-1	661 mWm^{-3}	Wang et al. (2013)
Mixed community of anaerobic microbes	7.07 mWm^{-2}	Chen et al. (2014)
E. coli	1624 mWm^{-2}	Mehdinia et al. (2014)
S. oneidensis	1060 mWm^{-2}	Zhao et al. (2014)
E. coli	434 mWm^{-2}	Kumar et al. (2014)
Mixed community of microbes	1018 mWm^{-2}	Hou et al. (2015)
Mixed community of *Geobacter* spp.	11, 2000 Wm^{-3}	Ren et al. (2016)
Mixed community of aerobic microbes	750 ± 70 mWm^{-2}	Kim et al. (2016)
S. cerevisiae	304 mWm^{-2}	Parkash (2016)
Bacteria from dairy wastewater	161 mWm^{-2}	Sekar et al. (2019)

2012). In another study, microorganisms have removed 90% cadmium and 97% zinc, using single-chamber MFCs by electricity simultaneously (Abourached et al. 2014). Iron(III) was removed using single-chamber MFC with >89% conversion efficiency at 150 h (Li et al. 2014). Gold cations (Au^{3+}) were removed using *Shewanella putrefaciens*, which showed electrodeposition of gold on grade G-10 graphite electrodes (Varia et al. 2014). Cobalt was reported to be recovered at a rate of 0.079 ± 0.001 $mmolL^{-1}$ h^{-1} (Huang et al. 2015). Hence, large amount of energy output have been generated using microorganisms in MFCs (Table 11.3).

The uses of microalgae in MFC have also been investigated (Rajesh and Ghangrekar 2016). Microalgae such as *Golenkinia* sp. produced a maximum energy of 6.3 W/m^3 (Hou et al. 2016). Similarly, Cui et al. (2014) used microalgae as a substrate in the anodic chamber of MFC, which achieved a maximum power output of 1.9 W/m^2. Luimstra et al. (2014) investigated photosynthetic electrogenic activity in algae and cyanobacteria by incorporating photosynthetic species in the anodic chamber of MFC, producing a power output of 6.2 mW/m^2. Rapid degradation of dyes, which has high redox potential such as azo dyes, was possible in the anodic chamber of MFC (Fernando et al. 2014). Khan et al. (2015) reported the effective degradation of azo dye, using an anaerobic-aerobic process in a single-chamber MFC. Degradation of xenobiotic compounds, like refractory organic pesticides (Cao et al. 2015), and phenanthrene and benzene (Adelaja et al. 2017) have also been successful by MFC.

2.4 Metagenomics and Their Role in Bioremediation

Microorganisms play an important role in numerous processes, including functioning of the ecosystem. There remains a greater challenge in cultivating and understanding microorganisms' physiology for bioremediation (Overmann et al. 2017). By 2017, around 400,000 different bacterial and archaea species were predicted to be identified (Yarza et al. 2014). Yet, there is a lack of characterization of microbial communities in contaminated sites due to huge microbial diversity and the challenge in cultivating them. Approaches such as metagenomics in combination with high-throughput sequencing technologies have revealed the diversity, adaptation, and evolution of microorganisms prevailing in the contaminated sites. Metagenomics allows studying the genetic material of microbial populations, by isolating them directly from environmental samples, and aids in facilitating the process of bioremediation. Metagenomics targets the community profile of individual organisms at meta-level. As 99% of microbes are uncultivable, which poses a limitation to the culturable techniques (Dickson et al. 2014; Bursle and Robson 2016), metagenomics aims to overcome this limitation.

Meta-omics technologies, such as metaproteomics and metatranscriptomics, have developed over the years and play a pivotal role in diversified applications (Dubey et al. 2020). Metatranscriptomics involves the analysis of metagenomic mRNA, which provides information about gene expression profiles of complex microbial communities present in the environmental sample. There is a huge significance of metatranscriptomics studies, as it can strengthen the results obtained through metagenomics and investigate the gene activity within a specified environmental condition. Metaproteomics involves an analysis of all the protein samples from environmental sources. In a study, microbial diversity and metaproteomic analysis of activated sludge showed *Burkholderiales* populations have better degradation ability for polycyclic aromatic hydrocarbons and their increase in activated sludge helps improve the degradation performance of naphthalene (Li et al. 2019). Metabolomic approaches have helped to analyze the potential metabolite profiles of microorganisms. However, the data generated by metabolomics differs significantly from the data generated by metagenomics and metatranscriptomics (Aguiar-Pulido et al. 2016). Metabolomics identified more than 4776 metabolites for bioremediation of different sites contaminated with petroleum hydrocarbons (Bargiela et al. 2015a; Malla et al. 2018; Dong et al. 2019). Biodegradation potential of the bacterial population in contaminated crude oil (Bargiela et al. 2015b), and anaerobic biodegradation of hydrocarbons (Gieg and Toth 2016) were also studied and analyzed using metabolomics.

For accessing biodegradative genes from metagenome of polluted environment, various metagenomic strategies, such as function-based screening and sequence-based screening, have been implemented (Pushpanathan et al. 2014). Metagenomic approaches for bioremediation have identified hydrocarbon-degrading genes of *Pseudomonas* sp. and *Rhodococcus* sp. in Arctic soil of Canada (Yergeau et al. 2012). Williams et al. (2014) identified *Flavobacterium* sp., *Enterobacter cloacae*, and *Ralstonia* sp., through metagenomic techniques and, used them for bioremediation of chromium (VI) present in groundwater. Several eco-friendly and cost-effective methods have been explored for arsenic contaminated water. In a study, Ma et al. (2016) investigated the capacity of rhizosphere microorganisms to enhance phytoremediation of arsenic-contaminated environments. The scalability of rhizoremediation in arsenic contamination is known very little. Therefore, several environmental genomic studies have been carried out to study ecosystems contaminated with arsenic (Huang et al. 2016). Also, the involvement of molecular mechanisms has been investigated in detail (Andres and Bertin 2016). The metagenomic analysis identified HL18 as the highest occurring methanotroph in the community for reduction of mercury (Hg) and arsenic (As), revealing all the genes necessary for the reduction of Hg (II) and As (V).

Metagenomic approaches aided in the identification of multiple enzymes that have the capability to degrade pollutants, like insecticides, dyes, pesticides, and plastics, using their bioremediation properties (Ufarté et al. 2015). In the degradation of oil spills in marine environments, biosurfactants and biosurfactant-producing strains of microorganisms have been widely used. Metagenomic analysis has led to the discovery of palmitoyl putrescine and N-acyl amino acids; the two novel biosurfactants (Jackson et al. 2015; Williams and Trindade 2017). MetaBoot, a software using a machine learning framework, holds the potential to identify pollution biomarkers based on metagenomic datasets obtained from contaminated ecosystems (Wang et al. 2015). Portable sequencing platforms, like Oxford Nanopore sequencers, have been developed to overcome the tediousness of transportation of samples for pollutant and pathogen monitoring (Oulas et al. 2015). The sequencing data storage, processing, and technologies associated with metagenomics, and also the pipelines of metagenomics have been improved, which have made it cost-efficient (van Dijk et al. 2018).

3 Conclusion

It is no doubt that bioremediation is leading a way forward toward sustainable development. Bioremediation has emerged as a safe, cost-effective, and environmentally friendly method. The knowledge regarding the pollutants has enhanced research work in areas of bioremediation, by incorporating it with various other techniques. However, for successful bioremediation, different environmental factors and geological characteristics of a polluted site also need to be considered. There is an urgent need to bring out the lab-scale works to commercial scale for potential implementation. More data about unexplored microbial communities need to be available in the online databases for better understanding of microbial genetics and its efficacy in degrading pollutants.

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

Acosta-Santoyo G, Cameselle C, Bustos E (2017) Electrokinetic-enhanced ryegrass cultures in soils polluted with organic and inorganic compounds. Environ Res 158:118–125. https://doi.org/10.1016/j.envres.2017.06.004

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 Biodegradation 116:91–103. https://doi.org/10.1016/j.ibiod.2016.10.021

Adikesavan S, Nilanjana D (2016) Degradation of cefdinir by Candida sp. SMN 04 and MgO nanoparticles – an integrated (nano-bio) approach. Environ Prog Sustain 35(3):706–714. https://doi.org/10.1002/ep.12279

Aguiar-Pulido V, Huang W, Suarez-Ulloa V et al (2016) Metagenomics, metatranscriptomics, and metabolomics approaches for microbiome analysis: supplementary issue: bioinformatics methods and applications for big metagenomics data. Evol Bioinform 12:EBO-S36436. https://doi.org/10.4137/EBO.S36436

Akhter M, Tasleem M, Alam MM, Ali S (2017) In silico approach for bioremediation of arsenic by structure prediction and docking studies of arsenite oxidase from *Pseudomonas stutzeri* TS44. Int Biodeterior Biodegradation 122:82–91. https://doi.org/10.1016/j.ibiod.2017.04.021

Ali H, Khan E (2017) Environmental chemistry in the twenty-first century. Environ Chem Lett 15 (2):329–346. https://doi.org/10.1007/s10311-016-0601-3

Alrumman SA, El-kott AF, Keshk SMAS (2016) Water pollution: source and treatment. Am J Environ Eng 6(3):88–98

Andres J, Bertin PN (2016) The microbial genomics of arsenic. FEMS Microbiol Rev 40 (2):299–322. https://doi.org/10.1093/femsre/fuv050

Annamalai S, Sundaram M (2020) Electro-bioremediation: an advanced remediation technology for the treatment and management of contaminated soil. In: Bharagava RN, Saxena G (eds) Bioremediation of industrial waste for environmental safety, 1st edn. Springer, Singapore, pp 183–214. https://doi.org/10.1007/978-981-13-3426-9_8

Azubuike CC, Chikere CB, Okpokwasili GC (2016) Bioremediation techniques-classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol 32(11):180. https://doi.org/10.1007/s11274-016-2137-x

Barba S, Villaseñor J, Rodrigo MA, Cañizares P (2018) Can electro-bioremediation of polluted soils perform as a self-sustainable process? J Appl Electrochem 48(6):579–588. https://doi.org/10.1007/s10800-018-1172-8

Bargiela R, Herbst FA, Martínez Martínez M et al (2015a) Metaproteomics and metabolomics analyses of chronically petroleum polluted sites reveal the importance of general anaerobic processes uncoupled with degradation. Proteomics 15(20):3508–3520. https://doi.org/10.1002/pmic.201404

Bargiela R, Mapelli F, Rojo D et al (2015b) Bacterial population and biodegradation potential in chronically crude oil-contaminated marine sediments are strongly linked to temperature. Sci Rep 5:11651. https://doi.org/10.1038/srep11651

Beškoski VP, Miletić S, Ilić M et al (2017) Biodegradation of isoprenoids, steranes, terpanes, and phenanthrenes during *in situ* bioremediation of petroleum contaminated groundwater. CLEAN-Soil Air Water 45(2):1600023. https://doi.org/10.1002/clen.201600023

Bokare V, Murugesan K, Kim JH et al (2012) Integrated hybrid treatment for the remediation of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. Sci Total Environ 435:563–566. https://doi.org/10.1016/j.scitotenv.2012.07.079

Briceño G, Schalchli H, Mutis A et al (2016) Use of pure and mixed culture of diazinon-degrading Streptomyces to remove other organophosphorus pesticides. Int Biodeterior Biodegradation 114:193–201. https://doi.org/10.1016/j.ibiod.2016.06.018

Bursle E, Robson J (2016) Non-culture methods for detecting infection. Aust Prescr 39(5):171. https://doi.org/10.18773/austprescr.2016.059

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 189:87–93. https://doi.org/10.1016/j.biortech.2015.03.148

Chang CH, Yang HY, Chen SK et al (2018) Electrokinetic-enhanced bioremediation of tetrachloroethylene. Int Biodeterior Biodegradation 132:251–258. https://doi.org/10.1016/j.ibiod.2018.04.013

Chen CY, Chen TY, Chung YC (2014) A comparison of bioelectricity in microbial fuel cells with aerobic and anaerobic anodes. Environ Technol 35(3):286–293. https://doi.org/10.1080/09593330.2013.826254

Choi MH, Jeong SW, Shim HE et al (2017) Efficient bioremediation of radioactive iodine using biogenic gold nanomaterial-containing radiation-resistant bacterium, *Deinococcus radiodurans* R1. Chem Commun 53(28):3937–3940. https://doi.org/10.1039/C7CC00720E

Cui Y, Rashid N, Hu N et al (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

Cundy AB, Hopkinson L, Whitby RL (2008) Use of iron-based technologies in contaminated land and groundwater remediation: a review. Sci Total Environ 400(1–3):42–51. https://doi.org/10.1016/j.scitotenv.2008.07.002

Dash HR, Das S (2015) Bioremediation of inorganic mercury through volatilization and biosorption by transgenic *Bacillus cereus* BW-03 (pPW-05). Int Biodeterior Biodegradation 103:179–185. https://doi.org/10.1016/j.ibiod.2015.04.022

Datta S, Rajnish KN, Samuel MS et al (2020) Metagenomic applications in microbial diversity, bioremediation, pollution monitoring, enzyme and drug discovery. A review. Environ Chem Lett:1–13. https://doi.org/10.1007/s10311-020-01010-z

Dickson RP, Erb-Downward JR, Prescott HC et al (2014) Analysis of culture-dependent versus culture-independent techniques for identification of bacteria in clinically obtained bronchoalveolar lavage fluid. J Clin Microbiol 52(10):3605–3613. https://doi.org/10.1128/JCM.01028-14

Dong H, Jiang Z, Deng J et al (2018) Physicochemical transformation of Fe/Ni bimetallic nanoparticles during aging in simulated groundwater and the consequent effect on contaminant removal. Water Res 129:51–57. https://doi.org/10.1016/j.watres.2017.11.002

Dong X, Greening C, Rattray JE et al (2019) Metabolic potential of uncultured bacteria and archaea associated with petroleum seepage in deep-sea sediments. Nat Commun 10(1):1–12. https://doi.org/10.1038/s41467-019-09747-0

Dubey RK, Tripathi V, Prabha R et al (2020) Metatranscriptomics and metaproteomics for microbial communities profiling. In: Unraveling the soil microbiome. Springer, Cham, pp 51–60. https://doi.org/10.1007/978-3-030-15516-2_5

Dundar E, Sonmez GD, Unver T (2015) Isolation, molecular characterization and functional analysis of $OeMT_2$, an olive metallothionein with a bioremediation potential. Mol Gen Genomics 290(1):187–199. https://doi.org/10.1007/s00438-014-0908-3

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 Biodegradation 89:7–14. https://doi.org/10.1016/j.ibiod.2013.12.011

Frutos FG, Pérez R, Escolano O et al (2012) Remediation trials for hydrocarbon-contaminated sludge from a soil washing process: evaluation of bioremediation technologies. J Hazard Mater 199:262–271. https://doi.org/10.1016/j.jhazmat.2011.11.017

Fulekar J, Dutta DP, Pathak B, Fulekar MH (2018) Novel microbial and root mediated green synthesis of TiO_2 nanoparticles and its application in wastewater remediation. J Chem Technol Biotechnol 93(3):736–743. https://doi.org/10.1002/jctb.5423

Gieg LM, Toth CRA (2016) Anaerobic biodegradation of hydrocarbons: metagenomics and metabolomics. In: Steffan RJ (ed) Consequences of microbial interactions with hydrocarbons, oils, and lipids: biodegradation and bioremediation. Handbook of hydrocarbon and lipid microbiology. Springer, Cham, pp 1–42. https://doi.org/10.1007/978-3-319-44535-9_16-1

Goutam SP, Saxena G, Singh V et al (2018) Green synthesis of TiO_2 nanoparticles using leaf extract of Jatropha curcas L. for photocatalytic degradation of tannery wastewater. Chem Eng J 336:386–396. https://doi.org/10.1016/j.cej.2017.12.029

Gude VG (2016) Wastewater treatment in microbial fuel cells-an overview. J Clean Prod 122:287–307. https://doi.org/10.1016/j.jclepro.2016.02.022

He Z, Liu J, Qiao Y et al (2012) Architecture engineering of hierarchically porous chitosan/vacuum-stripped graphene scaffold as bioanode for high performance microbial fuel cell. Nano Lett 12 (9):4738–4741. https://doi.org/10.1021/nl302175j

Hemapriyamvadha R, Sivasankar T (2015) Sonophotocatalytic treatment of methyl orange dye and real textile effluent using synthesised nano zinc oxide. Color Technol 131(2):110–119. https://doi.org/10.1111/cote.12139

Hou J, Liu Z, Li Y, Yang S, Zhou Y (2015) A comparative study of graphene-coated stainless steel fiber felt and carbon cloth as anodes in MFCs. Bioprocess Biosyst Eng 38(5):881–888. https://doi.org/10.1007/s00449-014-1332-0

Hou Q, Nie C, Pei H et al (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 L, Liu Y, Yu L et al (2015) A new clean approach for production of cobalt dihydroxide from aqueous Co (II) using oxygen-reducing biocathode microbial fuel cells. J Clean Prod 86:441–446. https://doi.org/10.1016/j.jclepro.2014.08.018

Huang LN, Kuang JL, Shu WS (2016) Microbial ecology and evolution in the acid mine drainage model system. Trends Microbiol 24(7):581–593. https://doi.org/10.1016/j.tim.2016.03.004

Jackson SA, Borchert E, O'Gara F, Dobson AD (2015) Metagenomics for the discovery of novel biosurfactants of environmental interest from marine ecosystems. Curr Opin Biotechnol 33:176–182. https://doi.org/10.1016/j.copbio.2015.03.004

Jorfi S, Barzegar G, Ahmadi M et al (2016) Enhanced coagulation-photocatalytic treatment of Acid red 73 dye and real textile wastewater using UVA/synthesized MgO nanoparticles. J Environ Manag 177:111–118. https://doi.org/10.1016/j.jenvman.2016.04.005

Khan MD, Abdulateif H, Ismail IM et al (2015) Bioelectricity generation and bioremediation of an azo-dye in a microbial fuel cell coupled activated sludge process. PLoS One 10(10):e0138448. https://doi.org/10.1371/journal.pone.0138448

Kim YM, Murugesan K, Chang YY et al (2012) Degradation of polybrominated diphenyl ethers by a sequential treatment with nanoscale zero valent iron and aerobic biodegradation. J Chem Technol Biotechnol 87(2):216–224. https://doi.org/10.1002/jctb.2699

Kim KY, Yang W, Evans PJ, Logan BE (2016) Continuous treatment of high strength wastewaters using air-cathode microbial fuel cells. Bioresour Technol 221:96–101. https://doi.org/10.1016/j.biortech.2016.09.031

Kumar GG, Hashmi S, Karthikeyan C et al (2014) Graphene oxide/carbon nanotube composite hydrogels-versatile materials for microbial fuel cell applications. Macromol Rapid Commun 35 (21):1861–1865. https://doi.org/10.1002/marc.201400332

Le TT, Nguyen KH, Jeon JR et al (2015) Nano/bio treatment of polychlorinated biphenyls with evaluation of comparative toxicity. J Hazard Mater 287:335–341. https://doi.org/10.1016/j.jhazmat.2015.02.001

Letnik I, Avrahami R, Rokem JS et al (2015) Living composites of electrospun yeast cells for bioremediation and ethanol production. Biomacromolecules 16(10):3322–3328. https://doi.org/10.1021/acs.biomac.5b00970

Li Y, Du X, Wu C et al (2013) An efficient magnetically modified microbial cell biocomposite for carbazole biodegradation. Nanoscale Res Lett 8(1):1–5. https://doi.org/10.1186/1556-276X-8-522

Li WW, Yu HQ, He Z (2014) Towards sustainable wastewater treatment by using microbial fuel cells-centered technologies. Energy Environ Sci 7(3):911–924. https://doi.org/10.1039/C3EE43106A

Li T, Wang Y, Guo S et al (2016) Effect of polarity-reversal on electrokinetic enhanced bioremediation of Pyrene contaminated soil. Electrochim Acta 187:567–575. https://doi.org/10.1016/j.electacta.2015.11.097

Li R, Dörfler U, Munch JC, Schroll R (2017) Enhanced degradation of isoproturon in an agricultural soil by a *Sphingomonas sp*. strain and a microbial consortium. Chemosphere 168:1169–1176. https://doi.org/10.1016/j.chemosphere.2016.10.084

Li S, Hu S, Shi S et al (2019) Microbial diversity and metaproteomic analysis of activated sludge responses to naphthalene and anthracene exposure. RSC Adv 9(40):22841–22852. https://doi.org/10.1039/C9RA04674G

Liu L, Yang Y, Li DL (2012) Accelerated hexavalent chromium [Cr (VI)] reduction with electrogenerated hydrogen peroxide in microbial fuel cells. Adv Mater Res 512:1525–1528. https://doi.org/10.4028/www.scientific.net/AMR.512-515.1525

Luimstra VM, Kennedy SJ, Güttler J et al (2014) A cost-effective microbial fuel cell to detect and select for photosynthetic electrogenic activity in algae and cyanobacteria. J Appl Phycol 26 (1):15–23. https://doi.org/10.1007/s10811-013-0051-2

Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. J Environ Manag 174:14–25. https://doi.org/10.1016/j.jenvman.2016.02.047

Ma J, Zhang Q, Chen F et al (2020) Remediation of resins-contaminated soil by the combination of electrokinetic and bioremediation processes. Environ Pollut 260:114047. https://doi.org/10.1016/j.envpol.2020.114047

Maiti S, Sinha SS, Singh M (2017) Microbial decolorization and detoxification of emerging environmental pollutant: cosmetic hair dyes. J Hazard Mater 338:356–363. https://doi.org/10.1016/j.jhazmat.2017.05.034

Malla MA, Dubey A, Yadav S et al (2018) Understanding and designing the strategies for the microbe-mediated remediation of environmental contaminants using omics approaches. Front Microbiol 9:1132. https://doi.org/10.3389/fmicb.2018.01132

Mao X, Han FX, Shao X et al (2016) Electro-kinetic remediation coupled with phytoremediation to remove lead, arsenic and cesium from contaminated paddy soil. Ecotoxicol Environ Safe 125:16–24. https://doi.org/10.1016/j.ecoenv.2015.11.021

Marinho G, Barbosa BCA, Rodrigues K et al (2017) Potential of the filamentous fungus *Aspergillus niger* AN 400 to degrade Atrazine in wastewaters. Biocatal Agric Biotechnol 9:162–167. https://doi.org/10.1016/j.bcab.2016.12.013

Mathuriya AS, Yakhmi JV (2014) Microbial fuel cells to recover heavy metals. Environ Chem Lett 12(4):483–494. https://doi.org/10.1007/s10311-014-0474-2

Mehdinia A, Ziaei E, Jabbari A (2014) Facile microwave-assisted synthesized reduced graphene oxide/tin oxide nanocomposite and using as anode material of microbial fuel cell to improve power generation. Int J Hydrog Energy 39(20):10724–10730. https://doi.org/10.1016/j.ijhydene.2014.05.008

Nisha KN, Devi V, Varalakshmi P, Ashokkumar B (2015) Biodegradation and utilization of dimethylformamide by biofilm forming *Paracoccus sp*. strains MKU1 and MKU2. Bioresour Technol 188:9–13. https://doi.org/10.1016/j.biortech.2015.02.042

Oulas A, Pavloudi C, Polymenakou P et al (2015) Metagenomics: tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. Bioinform Biol Insight. https://doi.org/10.4137/BBI.S12462

Overmann J, Abt B, Sikorski J (2017) Present and future of culturing bacteria. Annu Rev Microbiol 71:711–730. https://doi.org/10.1146/annurev-micro-090816-093449

Parkash A (2016) Utilization of distillery wastewater for electricity generation using microbial fuel cell. J Appl Emerg Sci 6(2):79–86

Pushpanathan M, Jayashree S, Gunasekaran P, Rajendhran J (2014) Microbial bioremediation: a metagenomic approach. In: Das S (ed) Microbial biodegradation and bioremediation, 1st edn. Elsevier, pp 407–419. https://doi.org/10.1016/B978-0-12-800021-2.00017-0

Rahimnejad M, Jafary T, Haghparast F et al (2011) Nafion as a nanoproton conductor in microbial fuel cells. Turk J Eng Environ Sci 34(4):289–292

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

Ravikumar KVG, Kumar D, Kumar G et al (2016) Enhanced Cr (VI) removal by nanozerovalent iron-immobilized alginate beads in the presence of a biofilm in a continuous-flow reactor. Ind Eng Chem Res 55(20):5973–5982. https://doi.org/10.1021/acs.iecr.6b01006

Ren H, Tian H, Gardner CL et al (2016) A miniaturized microbial fuel cell with three-dimensional graphene macroporous scaffold anode demonstrating a record power density of over 10000 Wm^{-3}. Nanoscale 8(6):3539–3547. https://doi.org/10.1039/C5NR07267K

Rodrigo MA, Oturan N, Oturan MA (2014) Electrochemically assisted remediation of pesticides in soils and water: a review. Chem Rev 114(17):8720–8745. https://doi.org/10.1021/cr500077e

Salam JA, Das N (2015) Degradation of lindane by a novel embedded bio-nano hybrid system in aqueous environment. Appl Microbiol Biotechnol 99(5):2351–2360. https://doi.org/10.1007/s00253-014-6112-x

Samuel MS, Jose S, Selvarajan E et al (2019) Biosynthesized silver nanoparticles using *Bacillus amyloliquefaciens*; application for cytotoxicity effect on A549 cell line and photocatalytic degradation of p-nitrophenol. J Photochem Photobiol B 202:111642. https://doi.org/10.1016/j.jphotobiol.2019.111642

Samuel MS, Suman S, Selvarajan E et al (2020) Immobilization of Cu_3 (btc) 2 on graphene oxide-chitosan hybrid composite for the adsorption and photocatalytic degradation of methylene blue. J Photochem Photobiol B 204:111809. https://doi.org/10.1016/j.jphotobiol.2020.111809

Saranya M, Santhosh C, Ramachandran R et al (2014) Hydrothermal growth of CuS nanostructures and its photocatalytic properties. Powder Technol 252:25–32. https://doi.org/10.1016/j.powtec.2013.10.031

Saxena G, Bharagava RN (2017) Organic and inorganic pollutants in industrial wastes: ecotoxicological effects, health hazards, and bioremediation approaches. In: Bharagava RN (ed) Environmental pollutants and their bioremediation approaches, 1st edn. CRC Press/Taylor & Francis Group, Boca Raton, pp 23–56. https://doi.org/10.1201/9781315173351-3

Sekar AD, Jayabalan T, Muthukumar H et al (2019) Enhancing power generation and treatment of dairy waste water in microbial fuel cell using Cu-doped iron oxide nanoparticles decorated anode. Energy 172:173–180. https://doi.org/10.1016/j.energy.2019.01.102

Shanmugam S, Ulaganathan P, Sivasubramanian S et al (2017) *Trichoderma asperellum* laccase mediated crystal violet degradation-optimization of experimental conditions and characterization. J Environ Chem Eng 5(1):222–231. https://doi.org/10.1016/j.jece.2016.11.044

Singh N, Khan S (2018) Nanobioremediation: an innovative approach to fluoride (F) contamination. In: Prasad R, Aranda E (eds) Approaches in bioremediation, 1st edn. Springer, Cham, pp 343–353. https://doi.org/10.1007/978-3-030-02369-0_15

Singh R, Manickam N, Mudiam MKR et al (2013) An integrated (nano-bio) technique for degradation of γ-HCH contaminated soil. J Hazard Mater 258:35–41. https://doi.org/10.1016/j.jhazmat.2013.04.016

Singh E, Osmani RAM, Banerjee R (2020) Nanobioremediation: an emerging approach for a cleaner environment. In: Shah MP (ed) Microbial bioremediation & biodegradation, 1st edn. Springer, Singapore, pp 309–363. https://doi.org/10.1007/978-981-15-1812-6_12

Sinha S, Singh R, Chaurasia AK, Nigam S (2016) Self-sustainable Chlorella pyrenoidosa strain NCIM 2738 based photobioreactor for removal of Direct Red-31 dye along with other industrial pollutants to improve the water-quality. J Hazard Mater 306:386–394. https://doi.org/10.1016/j.jhazmat.2015.12.011

Smith E, Thavamani P, Ramadass K et al (2015) Remediation trials for hydrocarbon-contaminated soils in arid environments: evaluation of bioslurry and biopiling techniques. Int Biodeterior Biodegradation 101:56–65. https://doi.org/10.1016/j.ibiod.2015.03.029

Tijani JO, Fatoba OO, Babajide OO, Petrik LF (2016) Pharmaceuticals, endocrine disruptors, personal care products, nanomaterials and perfluorinated pollutants: a review. Environ Chem Lett 14(1):27–49. https://doi.org/10.1007/s10311-015-0537-z

Tosco T, Papini MP, Viggi CC, Sethi R (2014) Nanoscale zerovalent iron particles for groundwater remediation: a review. J Clean Prod 77:10–21. https://doi.org/10.1016/j.jclepro.2013.12.026

Tummala CM, Tewari S (2018) Electro-kinetic remediation processes – a brief overview and selected applications. MOJ Civil Eng 4(1):57–58. https://doi.org/10.15406/mojce.2018.04.00097

Ufarté L, Laville É, Duquesne S, Potocki-Veronese G (2015) Metagenomics for the discovery of pollutant degrading enzymes. Biotechnol Adv 33(8):1845–1854. https://doi.org/10.1016/j.biotechadv.2015.10.009

van Dijk EL, Jaszczyszyn Y, Naquin D, Thermes C (2018) The third revolution in sequencing technology. Trends Genet 34(9):666–681. https://doi.org/10.1016/j.tig.2018.05.008

Varia JC, Martinez SS, Velasquez-Orta S, Bull S (2014) Microbiological influence of metal ion electrodeposition: studies using graphite electrodes, $[AuCl_4]-$ and *Shewanella putrefaciens*. Electrochim Acta 115:344–351. https://doi.org/10.1016/j.electacta.2013.10.166

Wang H, Ren ZJ (2014) Bioelectrochemical metal recovery from wastewater: a review. Water Res 66:219–232. https://doi.org/10.1016/j.watres.2014.08.013

Wang H, Wang G, Ling Y et al (2013) High power density microbial fuel cell with flexible 3D graphene-nickel foam as anode. Nanoscale 5(21):10283–10290. https://doi.org/10.1039/c3nr03487a

Wang X, Su X, Cui X, Ning K (2015) MetaBoot: a machine learning framework of taxonomical biomarker discovery for different microbial communities based on metagenomic data. Peer J 3:e993. https://doi.org/10.7717/peerj.993

Williams W, Trindade M (2017) Metagenomics for the discovery of novel biosurfactants. In: Charles TC, Liles MR, Sessitsch A (eds) Functional metagenomics: tools and applications, 1st edn. Springer, Cham, pp 95–117. https://doi.org/10.1007/978-3-319-61510-3_6

Williams PJ, Botes E, Maleke MM et al (2014) Effective bioreduction of hexavalent chromium-contaminated water in fixed-film bioreactors. Water SA 40(3):549–554. https://doi.org/10.4314/wsa.v40i3.19

Wu M, Li W, Dick WA et al (2017) Bioremediation of hydrocarbon degradation in a petroleum-contaminated soil and microbial population and activity determination. Chemosphere 169:124–130. https://doi.org/10.1016/j.chemosphere.2016.11.059

Wu Y, Wang S, Cheng F et al (2020) Enhancement of electrokinetic-bioremediation by ryegrass: sustainability of electrokinetic effect and improvement of n-hexadecane degradation. Environ Res 109717. https://doi.org/10.1016/j.envres.2020.109717

Xiao L, Damien J, Luo J et al (2012) Crumpled graphene particles for microbial fuel cell electrodes. J Power Source 208:187–192. https://doi.org/10.1016/j.jpowsour.2012.02.036

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(2):740–757

Yan FF, Wu C, Cheng YY et al (2013) Carbon nanotubes promote Cr (VI) reduction by alginate-immobilized Shewanella oneidensis MR-1. Biochem Eng J 77:183–189. https://doi.org/10.1016/j.bej.2013.06.009

Yang GC, Huang SC, Jen YS, Tsai PS (2016) Remediation of phthalates in river sediment by integrated enhanced bioremediation and electrokinetic process. Chemosphere 150:576–585. https://doi.org/10.1016/j.chemosphere.2015.12.044

Yarza P, Yilmaz P, Pruesse E et al (2014) Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nat Rev Microbiol 12(9):635–645. https://doi.org/10.1038/nrmicro3330

Yergeau E, Sanschagrin S, Beaumier D, Greer CW (2012) Metagenomic analysis of the bioremediation of diesel-contaminated Canadian high arctic soils. PLoS One 7(1):e30058. https://doi.org/10.1371/journal.pone.0030058

Zhao CE, Wang WJ, Sun D et al (2014) Nanostructured graphene/TiO_2 hybrids as high performance anodes for microbial fuel cells. Chem Eur J 20(23):7091–7097. https://doi.org/10.1002/chem.201400272

Chapter 12
Arsenic-Transforming Bacteria: A Potential Weapon for Arsenic-Contaminated Soil

Prabhakaran Narayanasamy and Radhesh Krishnan Subramanian

Abstract Arsenic (As) is one of the ten major poisonous metals occurring on earth crust or environment originated from rocks, minerals, and anthropogenic sources. This toxic metalloid occurs as insoluble trivalent arsenite (As III) and pentavalent arsenate (As V) which are fatal to human health as recognized by the World Health Organization (WHO). The insoluble form of arsenic occurs in trivalent arsenite (As III) and pentavalent arsenate (As V) forms. As V is a structural equivalent of phosphate and breaks oxidative phosphorylation, which is a vital reaction of energy uptake in many beings including humans. Processing a phosphate structure analogue which could enter any bacterial cell through phosphate transport system and phosphorylates replacing phosphate thereby inhibits the metabolism. Arsenic is uptaken by aquaglyceroporins at neutral pH in bacteria, yeasts, and mammals by binding to sulfhydryl groups of cysteine residues in proteins leading to an increased cytotoxicity. The soil microbes play a substantial role in its biogeochemical processing, by directly taking part in As speciation or doing so circuitously through redox interactions with other metals and nutrients, e.g., iron and nitrogen. The development of descriptive and comparative approaches with the cumulative number of genomes available was conceived not only to identify several genetic elements of the arsenic metabolism but also to explicate their phylogenetic circulation and its regulation. Numerous mechanisms are used by these microbes such as avoiding/reducing the toxicity due to arsenic by a phosphate uptake, by lipid peroxidation in the cell membranes, and by modifying the *ars* gene which controls the arsenic detoxification pathway. In this context, we will discuss in detail on the importance of arsenic-transforming bacteria and its potential application in bioremediation of contaminated soil.

Keywords Arsenic · Arsenate · Arsenite · Arsenic-transforming bacteria · Bioremediation · Soil

---

P. Narayanasamy · R. K. Subramanian (✉)
T. Stanes and Company Limited, Coimbatore, India

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_12

Abbreviations

As	Arsenic
As III	Arsenite
As V	Arsenate

1 Introduction

Arsenic (As) pollution causes a high ecological risk that impends the well-being of human health. Industrialization, overexploitation, and urbanization have worsened the natural resources, and it is estimated that water quality would further worsen with the increased rate of arsenic contamination in the coming years. In some regions of China, India, Pakistan, and Bangladesh, numerous "cancer villages" are recorded (Alka et al. 2020). The water quality has become a serious threat (Mohapatra et al. 2013) in the past two decades. Intrusion of saline water and indiscreet use of fertilizers and pesticides are the foremost factors which worsened the quality of groundwater with high arsenic (As), iron (Fe), and fluoride (F) content. As is one of the most predominant lethal metalloid in the environment, restricted not only to geochemical (minerals and rocks) origin in an insoluble form but also from anthropogenic sources (Ji and Silver 1992, 1995). Trivalent arsenite (As III) and pentavalent arsenate (As V) are insoluble forms of As. According to the Comprehensive Environmental Response (Compensation and Liability Act) As is categorized as the No. 1 carcinogenic substance; it is ranked No. 5 among the potentially toxic elements (PTEs). Arsenic has been called "essential toxins" because it is required in trace amounts for growth and metabolism but is toxic at high concentration (Stolz et al. 2002). Being the 33rd atomic number, this heavy metal is the twentieth highest ubiquitous and naturally occurring metalloid in the earth's crust. Arsenite is reportedly more toxic than arsenate (nearly 100 times) and can be oxidized to arsenate microbiologically or chemically (Ehrlich 1996; Muller et al. 2003). Nearly one-third of the periodic table elements are responsible for the transformation, degradation, or adsorption mediated by microbial activity (Stolz et al. 2002). Microbes have developed a diverse and distinct mechanisms to avoid the toxicity of arsenic: (A) by membrane lipids – peroxidation reactions (Ahmann et al. 1994; Ji and Silver 1995), (B) reducing the uptake of As V by phosphate uptake channel (Holt 1994; Ji and Silver 1995), and (C) through the ars operon that regulates arsenic detoxification pathway (Ji and Silver 1995; Mukhopadhyay et al. 2002). In recent years, the understanding on arsenic degradation or transformation has been significantly advanced. A steady progress in recent researches on the physiological processes of degradation or transformation delineating some of the biochemical mechanisms has paved the way for better understanding of pathway. The understanding of Arsenic as an inducer of oxidative stress is a good example of such a biochemical mechanism. Numerous intuitions have been gained on identifying the gene expression changes that accompany As stress in recent findings. As-induced cellular metabolic processes and the resultant damage is a prerequisite to research. Phytobial remediation to study As (III or V) mitigation is an innovative tool using plants and microbes to study As contamination in the environment. Recently, plant growth-promoting bacteria (PGPB) that assists phytoremediation has been highly exhibited for both promoting plant metal resistance/tolerance and inducing plant growth by adherence/transformation or degradation from toxic to nontoxic forms of As (Alka et al. 2020). Being similar to a phosphate structure, Arsenate reportedly enters the bacterial cell mediated by phosphate transport system. Its toxicity is due to its interference in normal phosphorylation processes by replacing cellular phosphate. In the recent past, entry of arsenite in the cells through aquaglyceroporins (mammalian) at neutral pH in bacteria, yeasts, and mammals was well demonstrated. Furthermore, its toxicity depends on its binding to sulfhydryl clusters of cysteine deposits in proteins, thus deactivating them (Ji and Silver 1992).

2 Arsenic Concentration in Aquatic Environment

In the present time, the existence of arsenic in groundwater has become a substance of serious fear due to its adverse health effects. As groundwater contamination becomes prevalent in many countries around the world, including Argentina, Bangladesh, Canada, China, Chile, Hungary, India, Japan, Mexico, New Zealand, Pakistan, Poland, Taiwan, and the United States (Shahid et al. 2015, 2017; Shakoor et al. 2015, 2016; Naujokas et al. 2013; Bhowmick et al. 2018; Naidu et al. 2006). Severe arsenicosis cases like cancerous keratosis, black foot disease (BFD), and hyperpigmentation have been reported in Taiwan. A huge population in Bangladesh, many areas in Punjab and Sindh provinces in Pakistan, and nearly 59 districts in West Bengal, India (Chakraborti et al. 2010) are dependent on As-contaminated groundwater for consumption and irrigation needs (Bhowmick et al. 2018; Shakoor et al. 2015; Abedin et al. 2002; Farooqi et al. 2007; Shahid et al. 2017). The first 2 arsenic contaminations in India were reported from West Bengal in 1984, and since then many states in the floodplains of the Himalayan plateau has joined the list. Above 0.2 million people in West Bengal have been detected with clinical manifestation of As-related toxicity. The geogenic release of As in the groundwater in some countries is notably more than 3000 μg L^{-1} groundwater (Shahid et al. 2015; Niazi et al. 2017; Shakoor et al. 2015). Recent reports of As contamination in groundwater from large areas of the north eastern (NE) region of India have highlighted the propensity of the problem. Water consumption at the NE region is reliant on naturally occurring spring, dug well, and pond water. Conferring to recommendations of the World Health Organization (WHO), the safety limit of As in potable water is 10 μg L^{-1}, which is being followed throughout the world with exceptions in some South and Southeast Asian countries (Shahid et al. 2017; Abid et al. 2016; Pio et al. 2015; Ravenscroft et al. 2009). Remarkably, it has been projected that about 60% of the water resources that are polluted with arsenic levels are considerably above the BIS- (50 μg/l) and WHO (10 μg/l)-approved limits. Keeping the guidelines of the WHO for As content in potable water, nearly 100 million people in South and Southeast Asia alone are at arsenic-induced risk. Added to it, above 200 million people universally are at danger of As poisoning (Rahman et al. 2009; Roberge et al. 2009; Singh et al. 2015). Twenty districts of Assam have As content above 50 μg/l (Bordoloi 2012), in a study conducted by Northeastern Regional Institute of Water and Land Management (NERIWALM). Seawater usually has less than 2 μg L^{-1} of As (Seow et al. 2012). Different sources of freshwater, such as streams, rivers, and lakes have As concentrations ranging from 0.15 to 0.45 μg L^{-1} depending on the sources and geochemical characteristics of the district (Singh et al. 2015). Compared to river waters, the lake waters possess a lower As contamination (Smedley and Kinniburgh 2012). Most As-polluted areas are existing around great estuaries and the proximate areas of main rivers that instigate from the Himalayan mountain range. Reportedly being the highest As concentration at Bengal delta, nearly 45 million (88%) people are affected with high (>50 μg L^{-1}) levels of As poisoning (Ravenscroft et al. 2009; Singh et al. 2015).

3 Arsenic Contamination and Its Effects on Human Well-Being

Arsenic is a toxic pollutant that raises much concern from both the ecological and human health perspectives. Human being may get exposed to arsenic in water from wells bored into arsenic-rich ground layers or in water polluted by agrochemical and industrial waste (Hughes et al. 1988). They may come in contact with arsenic in contaminated mists, fumes, or dusts. There are possibilities of human consumption of food cultivated with arsenic-contaminated groundwater or in arsenic-rich soil or polluted with pesticides derived with arsenic (Nriagu and Azcue 1990). However, it is still unknown to the scientific community how the milk is free from As contamination from the milk of arsenic-affected cows. Similarly a food grown in arsenic-contaminated areas, arsenic is not found inside the food like cereals, pulses/grains, and fruits but may be found in the external layer of fruits or food due to spraying of arsenic-polluted water. A few stray reports of arsenic in cow's milk are most likely due to adulteration of milk by arsenic-polluted water. Acclimatization of low dose through food or water is the principal path of this metalloid into the organism, where absorption takes place in the gastrointestinal track, followed by its release into the bloodstream. Liver converts the toxic form to less toxic form and is eventually excreted in the urine. Only very high exposure can, in fact, lead to considerable accretion in the body. Acute levels of As entry are reportedly through dermal exposure and inhalation (Caroli et al. 1996). For many decades, severe/prolonged arsenic poisoning and its medical use are known. Arsenic was recommended orally as Fowler's solution in stimulant combinations against leukemia, asthma, and other malignancy treatment (Leslie and Smith 1978; Zachariac et al. 1974). Parentally, As was used in the past for the treatment of trypanosomiasis (Nash 1960), syphilis (Moore 1933), topical eosinophilia (Maegraith 1966), psoriasis, verruca planum, and Lichen planus (Goodman and Gilman 1942). Human interventions in the use of arsenic in the form of weedicides, insecticides, and rodenticides for their agricultural, industrial, and domestic purposes are getting reduced because of the replacement by pesticides with low toxicity (Chisholm 1970). Arsenic-contaminated beer could eventually lead to hepatic cirrhosis and chronic hepatitis (Reynold 1901; Wolf 1974). Thus, human activities with As-contaminated potable water have exposed us to As toxicity (Nordstrom 2002) through farming systems including As-based pesticides irrigation, fertilization, mining operations, and geochemical processes (Meharg et al. 2009; Smedley and Kinniburgh 2002).

4 Arsenic Translocation From Soil to Plant

It is a universal accord that As is not prerequisite for plants, while the researches are still out on whether or not it is a natural constituent of some plants. Conferring to Gulz et al. (Gulz et al. 2005), a very acute concentration of As could play catalytic effects in plants. The As concentration in plants is usually below 1.0 mg kg^{-1} dry weight (DW) (Adriano 2001). The accumulation of As by plants in the roots and its translocation to shoots can occur either by active or passive transport system. It is reported that As gets accumulated in plants on dry weight (<0.1%) basis in an As-polluted soil (Austruy et al. 2013). Some monocots like paddy are known to accumulate heavy metals in the grains (Sommella et al. 2013). Compared with US and Chinese rice samples, As values from Spanish rice were estimated to be lower (Carbonell-Barrachina et al. 2012). Numerous transporter proteins govern the As translocation as the latter has a concentration gradient affinity between the farmers' practice (source of soil or water) and the plant. Similarly, As concentrations in Italian rice may vary from about 0.1 mg/kg grains to 0.3 mg/kg grains depending on the sample site, variety, and region. Cadmium are usually lower and are detected at rates between 0.01 and 0.1 mg/kg, and those for chromium values range from 0.1 mg/kg to 1.0 mg/kg grains. Apart from being analogous to potassium (P), the As uses numerous Pi channels to translocate inside a cell using a chemical gradient (Lei et al. 2012). In plants, the main constituents of P channels involved in As V uptake (Nussaume et al. 2011; LeBlanc et al. 2013) are Pi transporter proteins (PHT) which contain both low- and high-affinity P transports that are governed by PHT1 proteins. Nevertheless, these PHTs are involved in high-affinity transport and low-affinity transport that are still unidentified. The unidirectional PHT1 proteins may induce low-affinity activity in plants; thereby, plants uptake As III via numerous bidirectional nodulin-26-like intrinsic proteins (NIPs), thus, allowing the As (III) to translocate in both pumps depending on its gradient. In florae, As III is also stated to use silicon (Si) transporters due to the analogy of Si and As (III); thus, deficiency in Si increases the Si transporter (Lsi 1) influx in them. Being localized at epidermal and endodermal cells, the transporters (Lsi1 and Lsi 2) govern the Si accumulation in plants by influx and efflux system, respectively, thus enhances the translocation across different tissues and cells (Bakhat et al. 2017; Ma et al. 2007; Ma and Yamaji 2006). Furthermore, the As speciation and its translocation/adsorption in plants are also controlled by physicochemical properties and plant physiological tolerance mechanisms under stress (Khalid et al. 2017). Plants are well equipped with tolerance mechanisms by biological and biochemical mechanisms that play a crucial role in the As speciation and translocation from roots to shoots (Pourrut et al. 2011). Thus, the physiological fluctuations within plants could also alter As translocation/uptake by plants.

5 Arsenic Toxicity: Effects on Plant Metabolism

Worldwide, Arsenic (As) contamination is an important concern in human food chain which is not restricted by any economic boundaries. Rice grain is one of the major sources of human As outside of contaminated drinking water (Meharg et al. 2009). Arsenic (As)-contaminated rice samples were collected from different parts of the world. As was found to be distributed normally in samples from developing economic countries, but in developed countries samples of As contents were high (Meharg et al. 2009). Therefore, eradication of the accumulation of As in rice grain is one of the important research objective. Arsenic is nonessential and primarily toxic to plants. In plants, the roots are the first tissue to be affected by As, where the metalloid prevents root growth and its proliferation. Upon translocation to the shoot, As can severely obstruct plant growth by slowing down or apprehend development and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield, and fruit production (reviewed by Garg and Singla 2011). High concentration of As plays a major role in metabolic processes, which will affect plant growth leading to death. The majority of plants will retail As content in their roots. Nevertheless, As translocation to the shoot and other parts of the plant is based on the genotype. Various physiological progressions are vulnerable to As toxicity. Plants which are exposed to As lead to cellular membrane damage and electrolyte leakage (Singh et al. 2006). In plants, membrane damage is regularly coincided by an increase in malondialdehyde, a product of lipid peroxidation, indicating to the role of oxidative stress in As toxicity. Arsenic exposure to plants induces antioxidant defense mechanisms. The synthesis of ascorbate, the γ-Glu-Cys-Gly tripeptide glutathione (GSH), and the GSH oligomer (γ-Glu-Cys)n-Gly) phytochelatin (PC) increases throughout the plant, but particularly in the roots (Schmöger et al. 2000; Li et al. 2004; Geng et al. 2006; Singh et al. 2006; Khan et al. 2009), whereas anthocyanin accumulates in the leaves (Catarecha et al. 2007). Low As burden causes the number of nitrogen-fixing root nodules to be repressed in soybean (Vázquez et al. 2008). The molecular mechanisms underlying above physiological responses to As exposure are not well studied, but have of late attracted increased attention. Paradoxes related to As toxicity will stimulate the plant growth in lower As concentrations (Miteva 2002; Garg and Singla 2011). In *Arabidopsis* *thaliana* plant, paradoxes related to As toxicity occurs under different conditions (Chen et al. 2010), which indicates that the particular trait is not based on As disruption in the interaction of plant and biotic stress condition. Instead of that, it will result in plant metabolism which directly interacts with As or plant nutrients which directly interact with As. When the proper mechanism is not known, the growth benefit comes from As stimulation of Pi uptake (Tu and Ma 2003). There are comparatively only limited species of plants that are As tolerant in nature. Among them are *Pteris vittata* and other members of the *Pteridaceae* family plants that accumulate more As (Zhao et al. 2009). The growth of the abovementioned plants is not affected when they are exposed to high concentration of As. An important aspect of the high accumulation of As in plant phenotype is that it restricts to shoot itself, instead of allowing toxicity transfer to shoots. Different plants are being studied to determine the mechanism behind the hyperaccumulation of As; unfortunately, it is not clear how to avoid As toxicity, while highly accumulating in the leaves of the plants (Ma et al. 2001; Pickering et al. 2006; Meharg and Hartley-Whitaker 2002). An improved understanding of the mechanisms responsible for As resistance and toxicity in plants needs to be studied further. For safe cropping, different phytoremediation processes are very important, and that need to be clearly understood. A different group of plants which is resistant to As is needed for soil remediation and rehabilitation of contaminated sites. For the area in which the land/groundwater is contaminated by As, more As-resistant plants need to be planted to overcome the As contamination.

6 Arsenic Effect on DNA Structure Modifications

Studies on As-induced genotoxic responses on plants and animals have been reported (Ahmad et al. 2012; Patra et al. 2004). In biotransformation, the production of ROS is initially linked with its genotoxicity (Dalle-Donne et al. 2006). Therefore, the production of ROS can generate DNA–protein aberrations and can cause DNA and oxidative base damage (Cadet and Wagner 2013), a breakdown of chromatid/chromosome or exchange (Patra et al. 2004), apyrimidinic/apurinic sites formation (Faita et al. 2013), DNA–protein cross-links (Woźniak and Blasiak 2003), aberrations in chromosome, exchange in sister chromatid, formation of micronuclei, and aneuploidy and deletion (Kitchin and Wallace 2008). ROS attack on plant DNA leads to its based modification, accordingly releasing 8-oxoguanosine (8-OHdG) from the DNA structure (Ziech et al. 2010). 8-OHdG release leads to transversion mutation (G:C to T:A) (Okamoto et al. 2008). In many plant tissues, the accumulation of 8-oxoguanine (8-OHdG) has been evaluated, in response to As exposure (Crohns 2010; De Vizcaya-Ruiz et al. 2009). Similarly, As will replace P in phosphate groups of DNA, which will affect the plant metabolism by organo-arsenic compounds (Shipton 2014; Tofan-Lazar and Al-Abadleh 2012). Additionally, ROS or indirectly at time of the base excision repair mechanism can cause single-strand breaks in DNA (Kligerman et al. 2010). *Zea mays* and *Vicia faba* will raise the frequency of the micronuclei in the peripheral root tip cells, because As is one of the well-known inducers of chromosomal and chromatid aberrations (Colognato et al. 2007; Duquesnoy et al. 2010). As-induced genotoxic effects in affected plant tissues were caused by reductions in telomere length and inhibition in DNA repair processes and nucleotide excision repair and base excision repair (Faita et al. 2013). Numerous researches on the denature of normal RAPD results might be linked to the events of DNA damage, e.g., point mutation or chromosomal reorganization induced by genotoxic elements (Atienzar et al. 1999, 2000). Similarly, Ahmad et al. (Schulz et al. 2008) has shown that the frequency of RAPD band loss increased with rising concentrations and durations of As exposure in *Oryza sativa* seedlings. The changes found in the RAPD banding patterns of As-affected plant tissues could be observed as changes in genomic template stability, possibly related to modifications in both physiological and biochemical characteristics (Cenkci et al. 2010; Adhikari and Pal 2015; Körpe and Aras 2011).

6.1 Mitigation of Arsenic Accumulation in Rice

Human exposure to As through rice intake is a global health concern. There is a crucial need to remediate As-contaminated paddy soils and to screen for low As accumulating rice varieties, so that As contamination can be limited. The above review explains about the number of agronomic, physicochemical, and different biological methods, which can lower the As contamination in paddy growing area. Different studies explained about other alternate water irrigation practice that will bring down the As contamination in rice grains. Silicon (Si) application in As-affected paddy growing soil will limit the uptake of AS III. Redox-sensitive element (i.e., Fe and Mn) supplementation and the biochar (BC) assimilation can immobilize As in the rice growing area (Fig. 12.1). Introduction of different microbes is another in-situ method to reduce As content in rice grains. Alteration of gene expression in rice plants will also reduce the accumulation of As in paddy grains. Biogeochemical cycle of the rice agroecosystem, availability of sources, water management policies, and cost involved in the practice will play a major role in As mitigation process (Kumarathilaka et al. 2019).

A multifaceted and interdisciplinary understanding of As biogeochemistry in paddy agroecosystems and the mechanisms in As metabolism in rice plants is important to ensure low As levels in the rice soil solution and rice tissues. Water management, physicochemical and biological methods, or combinations of these methods can be successfully adapted to decrease inorganic and methylated As species content in rice agroecosystems (Fig. 12.2). Even though each technique has its limitations, the advantages far outweigh the disadvantages (Kumarathilaka et al. 2019).

In terms of biological approaches, the rate of microbial-driven As III oxidation and As volatilization in As-contaminated paddy environments needs to be increased to utilize the eco-friendly approach in a sustainable manner. It is essential to identify

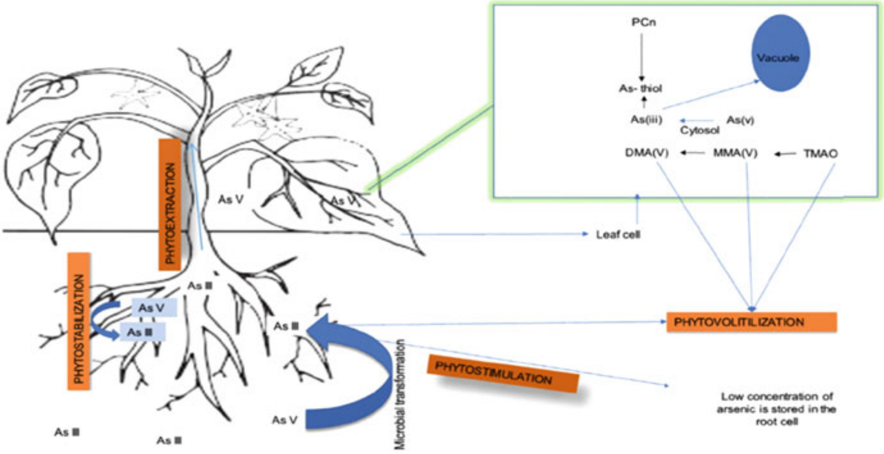


Fig. 12.1 Effects of nutrient supplementation on As bioavailability in rice soils and As uptake and translocation in rice plants (Kumarathilaka et al. 2019)

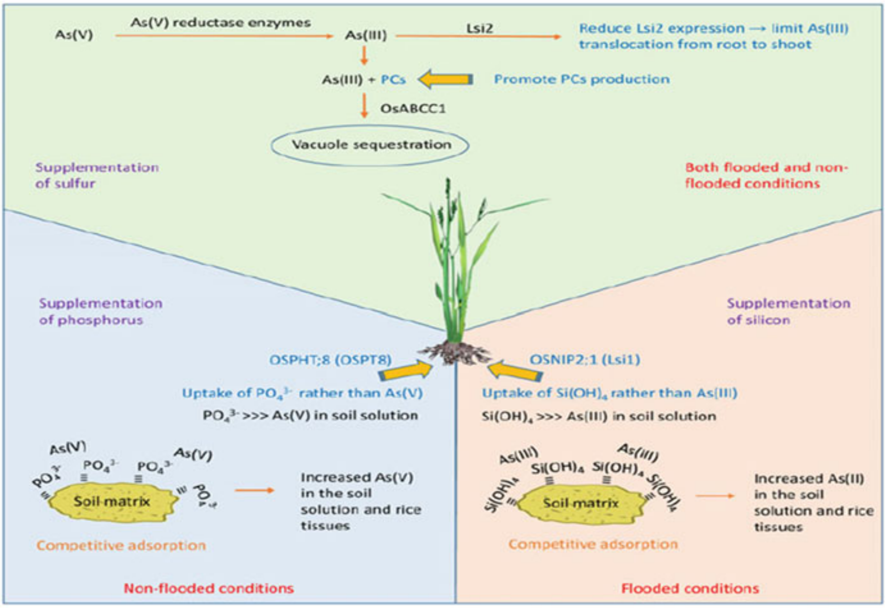


Fig. 12.2 Possible interactions between BC (pristine and modified) and As in the rice agroecosystem (Kumarathilaka et al. 2019)

rate-limiting factors in microbe-mediated As III oxidation and As volatilization processes in order to take measures to stimulate the reactions. Increased root porosity and high rate of ROL in rice roots may promote aerobic conditions in the rice rhizosphere and subsequently reduce As bioavailability. Studies related to the rice root anatomy would produce rice plants with increased root porosity and a high rate of ROL. It is also necessary to investigate how rice and other plant species metabolize As; thus, new endogenous and exogenous genes become available for the mitigation of As accumulation in rice grains (Kumarathilaka et al. 2019).

7 Bioremediation

Removal of arsenic from its contaminated soil incurs numerous methods under bioremediation. Bioremediation is the process of using microorganisms especially bacteria to degrade/remediate the arsenic contamination by means of its metabolic process particular its pathways (Das and Sarkar 2018; Hlihor et al. 2017). Different bacteria have been reported regarding the degradation of heavy metals, e.g., As, zinc (Zn), lead (Pb), and selenium (Se). Many studies reported about the about microorganisms' survival mechanism in the existence of As and its detoxification process by intracellular bioaccumulation, involving redox and methylation reactions. Both aerobic and anaerobic degradation pathways have been implicated for their existence with As. For degrading As contaminants, bioremediation process will introduce a higher number of chemoautotrophic bacteria (either aerobic or anaerobic (Alka et al. 2020). While As III passes through the aquaglyceroporin (GLpF) across the cell membrane, few As-resistant bacteria gain energy during detoxification, oxidation, or reduction of As, methylation of As V and As III, or demethylation of organic Asals, which use phosphate transporters to facilitate the uptake of As V. Different bacterial isolates are capable of solubilizing As through adsorption, organic ligand production, practice compartmentalization, biosorption, and mineral weathering caused by microbes.

7.1 Phytoremediation

Phytoremediation process is involved in the following mechanism: phytostabilization, phytoextraction, phytovolatilization, and phytofiltration (Fig. 12.3). Phytoextraction involves the absorption and movement of contaminants from the soil to the different parts of the plants through the root. Plants can absorb metal in huge amounts; that is, few of the plants did not show any toxicity symptoms. Some of the plants can absorb only very less toxic accumulation, which will not express any toxic symptoms (Alka et al. 2020). Microbes and plants are used in combination to reduce or remove the heavy metal toxicity which is known as phytobial remediation. Microorganisms play a vital role in biogeochemical process, they can form communities, and it will help the growth process of the plant and can reduce/remove the toxic heavy metal. In addition to above functions, it also plays an important role in forming mutual relationship in mangrove vegetation and occupies

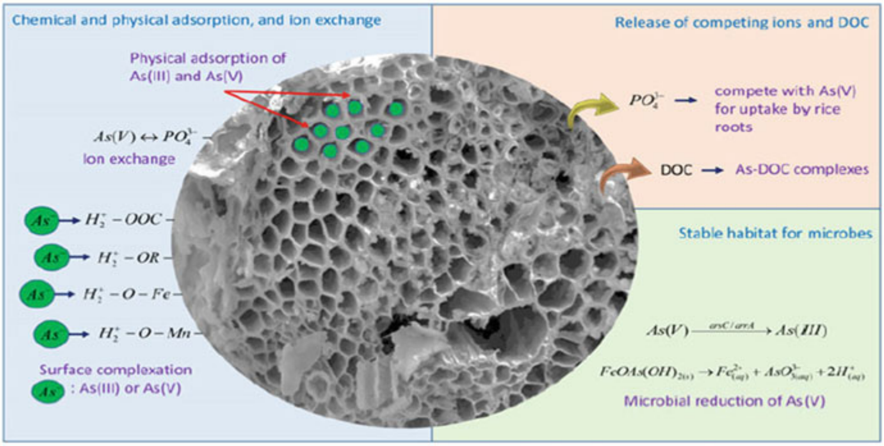


Fig. 12.3 Overview of phytoremediation processes that occur in plants. (Source: Modified from Kushwaha et al. 2015)

the rhizosphere, which is the symbiotic association in roots of the plants and endophytic nature in roots. Microbes produce metabolites which will help the plant to grow and are involved in the process of heavy metal removal. Before producing metabolites, microbes provide the nutrients; by using that, it protects the plants. Subsequently, rhizo microbes are involved in the bioremediation of metal-contaminated water areas (Alka et al. 2020).

7.2 Plant Growth-Promoting Bacteria

Plant growth-promoting (PGB) bacteria are also involved in the bioremediation process through their mutualistic relationship with plants; thus, it accelerates the plant efficiency (de Andrade et al. 2019). Naturally they can tolerate high As (heavy metal) contamination, and it will deliver benefits to both the soil and plants. Further study is needed to clarify changes that occur in bacteria, especially metabolic (the release of chelators and oxidation/reduction reactions) and physiological changes (soil pH alteration) which will enhance the phytoremediation progression by increasing the metal bioavailability (Tara et al. 2019). PGPB helps plant growth promotion, while high levels of metal contamination in soil change mobility and bioavailability (Rehman et al. 2019; Tara et al. 2019; Yahaghi et al. 2019). PGPB can be categorized in the following ways: 1. free-living bacteria, in mutual relations with the plants, and 2. endophytic bacteria which can colonize within plants (Soldan et al. 2019). Moreover, PGPB play an important role in improving plant growth under metal stress by producing substances such as siderophores 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase and phytohormones (Alka et al. 2020).

7.3 *Arsenic-Transforming Bacteria and Its Diversity with Genes Related to Arsenic and Enzymes Resulting From Arsenic-Contaminated Aquatic Sediment*

Bacteria are the most prerequisite in As cycling. However, only few studies have reported 16S rRNA and functional genes which is related to arsenic-contaminated tropical sediment.

Using culture-derived metagenomic and bioinformatic approaches, the variety in microorganism especially bacteria, As III and As V transformation in freshwater sediment process, was involved by bacterial genes and enzymes related and in anaerobic As III- and As V-enrichment cultures (ECs) are reported. The taxonomic profile reveals significant differences among these diverse communities. *Dechloromonas*, *Arcobacter*, *Clostridium thermopalmarium,* and *Sedimentibacter* were exclusively found in these contaminated sediments (EC); however, *Anaerobacillus* was limited to AsV-EC. Novel microorganisms that are both As V-reducers and As III-oxidizers were identified: *Acidovorax facilis*, *Dechloromonas*, *A. delafieldii*, *Shewanella*, *Aquabacterium*, *Macellibacteroides fermentans*, and *C. thermopalmarium.* Phylogenic variations were shown among the aioA, arsC, and arrA genes and those of different species, which indicates horizontal gene transfer. Structural and functional reliability and familial subgroups can be assessed using ArsC and AioA sets of amino acids. The situations necessary for As V reduction are conserved, proposing robust discriminating pressure for sustaining the functionality of ArsC. Altogether, these findings highlight the role of freshwater sediment bacteria in arsenic mobility and the untapped diversity of dissimilatory arsenate-resistant and arsenate-reducing bacteria contributing to arsenic toxicity in aquatic/marine environments (Suhadolnik et al. 2017) (Fig. 12.4).

The large set of effects reported on arsenic-exposed biofilms such as the reduction of biomass and algal growth (especially diatoms), the reduction of the proportion of autotrophic vs. heterotrophic organisms, or the lowering nutrient cycling and nitrogen content brings into question the arsenic concentration thresholds established by the US EPA for freshwater systems. The toxicity results described was under arsenic concentrations of 130 g L/1 and lower and concluded that recent studies do not support either the criterion maximum concentration of 340 _g L^{-1} (acute exposure), proposed by the US EPA in freshwaters, or the criterion continuous concentration (chronic exposure) of 150 _g L^{-1}, and it was suggested that these thresholds should be updated. It is also important to note that arsenic exposure thresholds for human health (10 g L^{-1}) are 15 times lower than those established for environmental health (Fig. 12.5 and Barral-Fraga et al. 2020) (Fig. 12.6).

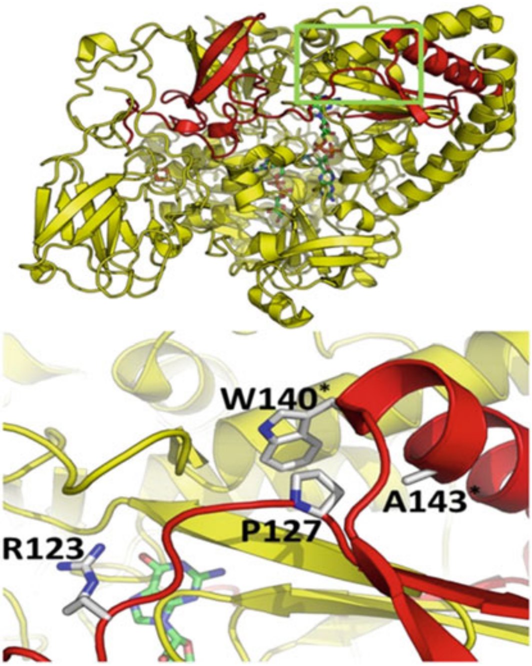


Fig. 12.4 Three-dimensional structure of the arsenite oxidase protein complex from *Rhizobium* species, complexed to molybdopterin guanosine dinucleotide (MGD). The region shown in red corresponds to the translation of amplified sequences in this study. The rectangle in green, zoomed in the bottom panel, shows the region containing the four conserved residues codified in the amplicons. The residues marked with an asterisk correspond to those not present in the translated amplicons found in this study (position 140 is dominated by glutamate, while position 143 is dominated by tryptophan). The ligand in the background, with carbon atoms shown in green, corresponds to MGD. (Source from – Suhadolnik et al. 2017)

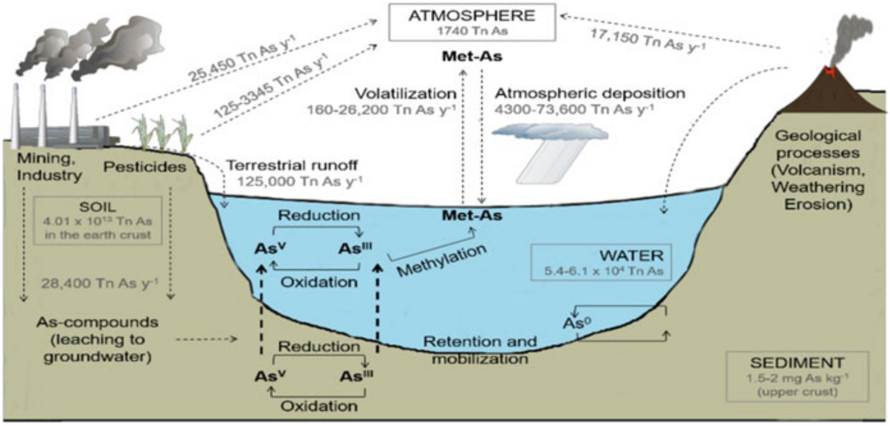


Fig. 12.5 Global arsenic (As) biogeocycle, with some estimated arsenic fluxes. Met-As = methylarsenicals; As V = arsenate; As III = arsenite; As^0 = arsenic; t = tons; t y^{-1} = tons per year (Barral-Fraga et al. 2020)

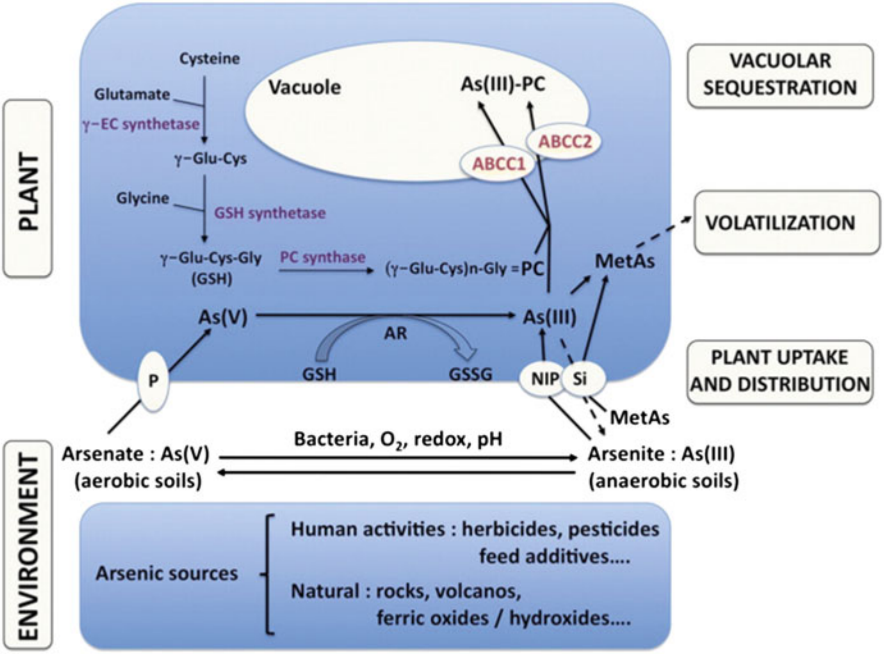


Fig. 12.6 Arsenic bioaccumulation in arsenic-contaminated soil (Al-Makishah et al. 2020)

7.4 Arsenic-Transforming Bacteria

Brevibacterium casei, a marine-based, gram-positive bacterial isolate which is heavy metal tolerant. A proteomic study of the isolate was carried out in the presence of chromium and arsenic which was revealed over all 2,549 proteins, in which 880 proteins found were commonly expressed in the absence of chromium and arsenic metals. In the absence of any metal like the concentration of chromium (100 mgL^{-1}) and arsenic (750 mgL^{-1}), 533, 212, and 270 proteins were unique. The following proteins are only expressed when chromium and arsenic are present: thioredoxin reductase, antibiotic biosynthesis monooxygenase, cytochrome C oxidase subunit II, and *ArsR* family transcriptional regulator. Other proteins such as thioredoxin–disulfide reductase, superoxide dismutase, and lipid hydroperoxide reductase were upregulated in the presence of chromium and arsenic. Cell functioning-related proteins were downregulated in response to chromium and arsenic. Major functions like energy metabolism, amino acid metabolism, carbohydrate metabolism, and translations were affected in response to chromium and arsenic. Peptide mass fingerprinting of *B. casei* exposed to arsenic and chromium metals, respectively, revealed the harmful effect of these metals on the bacteria and its approach to overcome the stress (Shah and Damare 2020).

Studies have unraveled the major means of regulating As contamination, which includes processes utilizing both methylation and redox reactions. As volatilization is a method that includes the reduction of As (V) to As (III) which is followed by a conversion to dimethyl As (V) DMA (V), TMA (III) (trimethylarsine), and TMA (III) oxide (TMAO) in the presence of glutathione (GSH) and SAM (S-adenosyl methionine). It was primarily established in fungi in the 1980s and afterward seen in bacteria, archaea, algae, plants, marine animals, and humans. *Aspergillus fumigatus* and *Pseudomonas* spp. (Shariatpanahi et al. 1981), *Tetrahymena pyriformis* (Chatterjee et al. 2017), methanogens (Michalke et al. 2000), and *Rhodopseudomonas palustris* (Ke et al. 2018) were also involved in biomethylation and volatilization. Bioremediation is the important process known for its advantages: 1. no secondary pollution, 2. no complexity in the technical process, 3.very low energy consumption, 4. very efficient method, 5. no additional construction required, and 6. Long-term viability (Shishir and Mahbub 2019). Therefore, it has been extensively and successfully implemented worldwide for several purposes. Shishir and Mahbub (2019) state that there are restrictions to bioremediation, in which it is restricted to biodegradable compounds only; therefore, all heavy metal contaminants may not be treated using this technology. Henceforth, biodegradation is still considered to be safe, as biodegradation residues can sometimes be more difficult and unsafe than the parent compound. In addition, these techniques are often very specific and depend on many parameters such as the concentration of pollutants, microbial population, site factors, environmental conditions, and nutrient levels that make the process difficult. Bioremediation is less efficient and unsuccessful in natural condition but serves as an environment conducive to microbial growth and activity (Shishir and Mahbub 2019).

8 Conclusion

Arsenic transformation and sequestration are important processes in toxicity reduction. A huge number of populations depend on water containing unacceptable levels of arsenic for drinking as well as economic activities. The investigation of microbial behavior and identifying microorganisms for bioremediation is vital in understanding arsenic-tolerant microbes in contaminated areas. Of the several possible methods, one highly efficient and eco-friendly as well as economically sound strategy is to consider biologically remediation processes. Several previous studies have shown that some microbes have the capacity of detoxifying and reducing toxicity by transforming one form of arsenic to another. Moreover, some microbes have immobilized toxic forms of arsenic in contaminated areas. In addition, different hyperaccumulator plants also have the capacity to remediate the toxicity of arsenic in different contaminated agroecosystems. Some microbes have also been found to contain certain genes that facilitate the oxidization and reduction in arsenic toxicity. This has led to the demand for greater exploration in order to identify microbes with the bioremediation capacity to immobilize and reduce the toxicity of arsenic contamination in the environment (Al-Makishah et al. 2020) (Tables 12.1 and 12.2).

Table 12.1 Arsenic-induced physiological and biochemical changes in plants

S. No.	Plant species	Substrate used	Type of As (V/III)	Cause or effect on plant	Cited references
1.1.	*Cicer arietinum L.*	Soil	As V	Overexpression of dehydration/desiccation responsive genes (MIPS, PGIP, and DRE).	Tripathi et al. (2017)
				Decrease in antioxidant enzyme activities (GR, CAT, SOD, APX, and GPX)	
2.	*Zea mays L.*	Soil	As III	Decrease in gas exchange attributes (photosynthetic rate, transpiration rate, stomatal conductance) and chlorophyll content	Anjum et al. (2017)
3.	*Zea mays L.*	Soil	As V	Increase in shoot As and P concentrations, reduction in pigment concentrations (chlorophyll A, chlorophyll B, and total chlorophyll), and gas exchange attributes	Mehmood et al. (2017)
4.	*Brassica napus* and *Brassica juncea*	Soil	As V	Reduction in growth attributes (leaf area, plant height, number of leaves, shoot and root dry biomass), gas exchange attributes (photosynthetic rate, transpiration rate, stomatal conductance), photosynthetic pigments and water use efficiency	Niazi et al. (2017)

(continued)

Table 12.1 (continued)

S. No.	Plant species	Substrate used	Type of As (V/III)	Cause or effect on plant	Cited references
5.	*Vigna mungo L.*	Soil	As V	Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids decreasedwith increasing As concentration. Lipid peroxidation was increased. The activities of antioxidative enzymes (SOD, POD, and APX) except CAT were increased.	Srivastava et al. (2017)
6.	*Glycine max*	Soil	As V and As III	Changes in the expression of a key messenger (phosphatidic acid) via phospholipase D and phospholipase C. Moreover, a rapid and significant stomatal closure	Armendariz et al. (2016a)
7.	*Glycine max*	Soil	As V and As III	Reduction in chlorophyll content and increase in lipid peroxidation. Reduction in root cortex area, broken cells in the outer cortical layer and cell death of root tips. Dark deposits in cortex cells and within phloem cell walls and xylem vessel elements	Armendariz et al. (2016b)

(continued)

Table 12.1 (continued)

S. No.	Plant species	Substrate used	Type of As (V/III)	Cause or effect on plant	Cited references
8.	*Oryza sativa L.*	Hydroponic	As V	Increased leakage of electrolytes andincreased root arsenate reductase activity along with relatively lower root-to-shoot As translocation in As-tolerant rice genotype BRRI 33 than in sensitive genotype BRRI 51. Decrease in Pi content and increase in PCs content in roots	Begum et al. (2016)
9.	*Boehmeria nivea L.*	Hydroponic	As III	Reduction in chlorophyll concentrations, relative water concentrations, SOD and CAT activities. Increase in H_2O_2, malondialdehyde (MDA) content, and electrolyte leakage	Mubarak et al. (2016)
10.	*Oryza sativa L.*	Hydroponic	As III	Carbohydrate metabolism and photosynthesis were greatly affected; however, As did not cause any significant oxidative damage to plants	Sanglard et al. (2016)
11.	*Oryza sativa L. var. Triguna*	Hydroponic	As III	Reduction in shoot and root growth, biomass production, and protein concentrations	Upadhyay et al. (2016)

(continued)

Table 12.1 (continued)

S. No.	Plant species	Substrate used	Type of As (V/III)	Cause or effect on plant	Cited references
12.	Aquatic plants species (*Vallisneria gigantea, Azolla filiculoides,* and *Lemna minor*)	Hydroponic	As V	Changes in fluorescence spectra and damage to photosystem II	Iriel et al. (2015)
13.	*Brassica juncea L.*	Soil	As V	Affected plant growth and biochemical stress indicators such as protein content, lipid peroxidation, and antioxidative enzymes (SOD, CAT, POD, APX, GR)	Kanwar and Bhardwaj (2015)
14.	*Pisum sativum L.*	Hydroponic containing NaHS (0, 100 μM)	As V	As uptake caused reduction in chlorophyll fluorescence, nitrogen content concentrations of H2S and nitric oxide (NO). The activities of cysteine desulfhydrase and nitrate reductase were also decreased. Increasing levels of ROS caused damage to lipids, proteins, and membranes.	Singh et al. (2015)
15.	*Oryza sativa L.*	Hydroponic	As V	Increases in hydrogen peroxide and lipid peroxidation	Nath et al. (2014)
16.	*Anadenanthera peregrina, Myracrodruon urundeuva*	Soil	As V	Increase in hydrogen peroxide and lipid peroxidation	Gomes et al. (2013)

(continued)

Table 12.1 (continued)

S. No.	Plant species	Substrate used	Type of As (V/III)	Cause or effect on plant	Cited references
17.	*Trigonella foe num_graecum L.*	Soil	As V	Reductions in radicle length, dry weight, and chlorophyll content	Talukdar (2013)
18.	*Hydrilla verticillata*	Hydroponic	As V	Decline in chlorophyll content and rate of photosynthesis	Srivastava et al. (2013)
19.	*Oryza sativa L.*	Hydroponic	As III	Reductions in seed germination, root and shoot length, chlorophyll and protein content, and genomic stability	Ahmad et al. (2012)
20.	*Oryza sativa L.*	Soil culture (field study)	Groundwater As concentrations (17, 27, and 53 μg L^{-1}) and soil As concentrations (10.4, 12.6, and 15.5 μg g^{-1})	Both essential and nonessential amino acids were decreased as the grain As concentration was increased in high As accumulating rice genotypes. Nonessential amino acids were increased in low As accumulating rice genotypes	Dwivedi et al. (2012)
21.	*Vigna mungo*	Soil	As V	Delayed nodule formation and reduction in nitrogenase activity	Mandal et al. (2011)
22.	*Cicer arietinum L.*		As V	Reduction in chlorophyll, relative leaf water, sucrose, proteins, starch, and sugar concentrations. Reduction in Ca, P, Fe, and amino acids like Lys, Met, Pro, Thr, Trp, and Val	Malik et al. (2011)

(continued)

Table 12.1 (continued)

S. No.	Plant species	Substrate used	Type of As (V/III)	Cause or effect on plant	Cited references
23.	*Lemna minor L.*	Hydroponic	As V and As III	Reduction in chlorophyll, and increase inelectrolyte leakage and lipid peroxidation	Duman et al. (2010)
24.	*Helianthus annuus*	Soil	As V	Reductions in plant growth and ionic concentrations (K, Ca, Mg, Si, Fe, Zn, Cu, Rb, and Sr)	Gunes et al. (2010)
25.	*Festuca arundinacea*	Hydroponic	As V	Excessive ROS accumulation, membrane perturbation, and lipid peroxidation	Jin et al. (2019)
26.	*Cicer arietinum L.*	Soil	As V	Increase in H_2O_2 content and lipid peroxidation. Reduction in SOD and nonenzymatic antioxidants activities. Increase in CAT and APX activities	Gunes et al. (2009)
27.	*Trifolium pratense*	Soil	As V	Increase in SOD, POD, and glutathione activities. Reduction in chlorophyll and carotenoid concentrations	Mascher et al. (2002)

Reproduced from Abbas et al. (2018)

Table 12.2 Reported organisms and the presence of arsenic metabolism genes

Genus and species	Arsenic/arsenate metabolizing genes	References
Chrysiogenes arsenatis	Arsenate respiratory reductase (*arr*)	Krafft and Macy (1998)
Bacillus selenitireducens	Arsenate respiratory reductase (*arr*)	Afkar et al. (2003)
Shewanella sp. Strain ANA-3	Arsenate respiratory reductase (*arr*)	Saltikov et al. (2005)
Corynebacterium glutamicum	arsB3 and arsC4 being constitutive and the expression of arsR1, arsB1, arsC1, arsC1′, arsR2, arsB2, and arsC2 being inducible by arsenite	Ordóñez et al. (2005)
Escherichia coli, *Pseudomonas aeruginosa*	Arsenic transcriptional repressor (arsR), arsenite permease (arsB), and arsenate reductase (arsC)	Cai et al. (1998), Cai and DuBow (1996), Ellis et al. (2006) and Rosen et al. (1992)
Enterobacteria	arsR, arsB, and arsC	Diorio et al. (1995)
Staphylococcus plasmids pI258 and pSX267	arsR, arsB, and arsC	Gotz et al. (1983) and Silver et al. (1993)
Bacillus sp. and *Aneurinibacillus aneurinilyticus*	Oxidizing As (III) due to an As (III)-resistant efflux membrane protein	Dey et al. (2016)
Flavobacterium sp., *Achromobacter* sp., and *Uncultured clones.*	*aioA* gene	Karn et al. (2017)
Geobacillus kaustophilus A1	*arsC1, arsC2, and arsC3*	Cuebas et al. (2011)
Aeromonas, *Exiguobacterium*, *Acinetobacter*, *Bacillus* and *Pseudomonas*	*arsB*	Anderson and Cook (2004)
Genera like *Acidithiobacillus*, *Bacillus*, *Deinococcus, Desulfitobacterium,* and *Pseudomonas*	*arsB*	Oremland et al. (2004) and Suresh et al. (2004)
Enterobacter sp. (MNZ1), *Klebsiellapneumoniae*1 (MNZ4), and *Klebsiella pneumonia* 2 (MNZ6)	*arsC*	Abbas et al. (2014)

References

Abbas SZ, Riaz M, Ramzan N, Zahid MT, Shakoori FR, Rafatullah M (2014) Isolation and characterization of arsenic resistant bacteria from wastewater. Braz J Microbiol 45 (4):1309–1315

Abbas G, Murtaza B, Bibi I (2018) Arsenic uptake, toxicity, detoxification, and speciation in plants: physiological, biochemical, and molecular aspects. Int J Environ Res Publ Health 15(1):59. https://doi.org/10.3390/ijerph15010059

Abedin MJ, Cotter-Howells J, Meharg AA (2002) Arsenic uptake and accumulation in rice (*Oryza sativa L.*) irrigated with contaminated water. Plant Soil 240:311–319

Abid M, Niazi NK, Bibi I, Farooqi A, Ok YS, Kunhikrishnan A, Ali F, Ali S, Igalavithana AD, Arshad M (2016) Arsenic(V) biosorption by charred orange peel in aqueous environments. Int J Phytoremediation 18:442e449

Adhikari D, Pal S (2015) Molecular markers in plant-based bioassays for the detection of molecular endpoints to probe of aquatic genotoxicity – an overview. J Environ Sociobiol 12:143–162

Adriano DC (2001) Arsenic. In: Trace elements in terrestrial environments. Springer, Berlin, pp 219–261

Afkar E, Lisak J, SaltikovCW BP, Oremland RS, Stolz JF (2003) The respiratory arsenate reductase from Bacillus selenitireducens strain MLS10. FEMS Microbiol Lett 226:107–112

Ahmad MA, Gaur R, Gupta M (2012) Comparative biochemical and RAPD analysis in two varieties of rice (Oryza sativa) under arsenic stress by using various biomarkers. J Hazard Mater 217:141–148

Ahmann D, Roberts AL, Krumholz LR, Morel FMM (1994) Microbe grows by reducing arsenic. Nature 71(6500):750. https://doi.org/10.1038/371750a0

Alka S, Shahir S, Ibrahim N, Chai T-T, Bahari ZM, Manan FA (2020) The role of plant growth promoting bacteria on arsenic removal: a review of existing perspectives. Environ Technol Innov 17

Al-Makishah NH, Taleb MA, Barakat MA (2020) Arsenic bioaccumulation in arsenic-contaminated soil: a review. Chem Pap 74:2743–2757. https://doi.org/10.1007/s11696-020-01122-4

Anderson CR, Cook GM (2004) Isolation and characterization of arsenate-reducing bacteria from arsenic-contaminated sites in New Zealand. Curr Microbiol 48:341–347

Anjum SA, Tanveer M, Hussain S, Ashraf U, Khan I, Wang L (2017) Alteration in growth, leaf gas exchange, and photosynthetic pigments of maize plants under combined cadmium and arsenic stress. Water Air Soil Pollut 228:13

Armendariz AL, Talano MA, Travaglia C, Reinoso H, Oller ALW, Agostini E (2016a) Arsenic toxicity in soybean seedlings and their attenuation mechanisms. Plant Physiol Biochem 98:119–127

Armendariz AL, Talano MA, Villasuso AL, Travaglia C, Racagni GE, Reinoso H, Agostini E (2016b) Arsenic stress induces changes in lipid signalling and evokes the stomata closure in soybean. Plant Physiol Biochem 103:45–52

Atienzar FA, Conradi M, Evenden AJ, Jha AN, Depledge MH (1999) Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in Daphnia magna exposed to benzo (a) pyrene. Environ Toxicol Chem 18:2275–2282

Atienzar FA, Cordi B, Donkin ME, Evenden AJ, Jha AN, Depledge MH (2000) Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, Palmaria palmata. Aquat Toxicol 50:1–12

Austruy A, Wanat N, Moussard C, Vernay P, Joussein E, Ledoigt G, Hitmi A (2013) Physiological impacts of soil pollution and arsenic uptake in three plant species: Agrostis capillaris, Solanum nigrum and Vicia faba. Ecotoxicol Environ Saf 90:28–34

Bakhat HF, Zia Z, Fahad S, Abbas S, Hammad HM, Shahzad AN, Abbas F, Alharby H, Shahid M (2017) Arsenic uptake, accumulation and toxicity in rice plants: possible remedies for its detoxification: a review. Environ Sci Pollut Res 24:9142–9158

Barral-Fraga L, Barral MT, MacNeill KL, Martiñá-Prieto D, Morin S, Rodríguez-Castro MC, Tuulaikhuu BA, Guasch H (2020) Biotic and abiotic factors influencing arsenic biogeochemistry and toxicity in fluvial ecosystems: a review. Int J Environ Res Public Health 17:2331

Begum MC, Islam MS, Islam M, Amin R, Parvez MS, Kabir AH (2016) Biochemical and molecular responses underlying differential arsenic tolerance in rice (*Oryza sativa* L.). Plant Physiol Biochem 104:266–277

Bhowmick S, Pramanik S, Singh P, Mondal P, Chatterjee D, Nriagu J (2018) Arsenic in groundwater of West Bengal, India: a review of human health risks andassessment of possible intervention options. Sci Total Environ 612:148–169

Bordoloi R (2012) Existence of arsenic in groundwater and its effect on health. Int J Comput Appl Eng Sci 2(3):270–272

Cadet J, Wagner JR (2013) DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. Cold Spring Harb Perspect Biol 5:a012559

Cai J, DuBow MS (1996) Expression of the *Escherichia coli* chromosomal ars operon. Can J Microbiol 42:662–671

Cai J, Salmon K, Du Bow MS (1998) A chromosomal ars operon homologue of Pseudomonas aeruginosa confers increased resistance to arsenic and antimony in *Escherichia coli*. Microbiology 144:2705–2713

Carbonell-Barrachina AA, Wu X, Ramírez-Gandolfo A, Norton GJ, Burló F, Deacon C, Meharg AA (2012) Inorganic arsenic contents in rice-based infant foods from Spain, UK, China and USA. Environ Pollut 163:77–83

Caroli F, Torre LA, Petrucci F, Violante N (1996) Element speciation in bioinorganic chemistry. In: Caroli S (ed) Chemical analysis series, vol 135. Wiley, Chichester, pp 445–463

Catarecha P, Segura MD, FrancoZorrilla JM, García-Ponce B, Lanza M, Solano R, Paz-Ares J, Leyva A (2007) A mutant of the Arabidopsis phosphate transporter PHT1;1 displays enhanced arsenic accumulation. Plant Cell 19:1123–1133

Cenkci S, Ciğerci IH, Yıldız M, Özay C, Bozdağ A, Terzi H (2010) Lead contamination reduces chlorophyll biosynthesis and genomic template stability in Brassica rapa L. Environ Exp Bot 67:467–473

Chakraborti D, Rahman MM, Das B, Murrill M, Dey S, Mukherjee SC, Dhar RK, Biswas BK, Chowdhury UK, Roy S (2010) Status of ground water arsenic contamination in Bangladesh: a 14-year study report. Water Res 44:5789–5802

Chatterjee S et al (2017) Arsenic: source, occurrence, cycle, and detection. In: Arsenic contamination in the environment. Springer, pp 13–35

Chen W, Chi Y, Taylor NL, Lambers H, Finnegan PM (2010) Disruption of ptLPD1 or ptLPD2, genes that encode isoforms of the plastidiallipoamide dehydrogenase, confers arsenate hypersensitivity in Arabidopsis. Plant Physiol 153:1385–1397

Chisholm JJ Jr (1970) Arsenic poisoning from rodenticides. Pediatr Clin North Am 17:591

Colognato R, Coppede F, Ponti J, Sabbioni E, Migliore L (2007) Genotoxicity induced by arsenic compounds in peripheral human lymphocytes analysed by cytokinesis-block micronucleus assay. Mutagenesis 22:255–261

Crohns M (2010) Antioxidants, cytokines and markers of oxidative stress in lung cancer: associations with adverse events, response and survival. Tampere University Press, University of Tampere, Tampere

Cuebas M, Villafane A, McBride M, Yee N, Bini E (2011) Arsenate reduction and expression of multiple chromosomal ars operons in Geobacillus kaustophilus A1. Microbiology 157:2004–2011

Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A (2006) Biomarkers of oxidative damage in human disease. Clin Chem 52:601–623

Das J, Sarkar P (2018) Remediation of arsenic in mung bean (Vigna radiata) with growth enhancement by unique arsenic-resistant bacterium *Acinetobacter lwoffii*. Sci Total Environ 624:1106–1118

De Andrade FM et al (2019) Beneficial effects of inoculation of growth-promoting bacteria in strawberry. Microbiol Res 223:120–128

De Vizcaya-Ruiz A, Barbier O, Ruiz-Ramos R, Cebrian ME (2009) Biomarkers of oxidative stress and damage in human populations exposed to arsenic. Mutat Res 674:85–92

Dey U, Chatterjee S, Mondal NK (2016) Isolation and characterization of arsenic-resistant bacteria and possible application in bioremediation. Biotechnol Rep 10:1–7

Diorio C, Cai J, Marmor J, Shinder R, Du Bow MS (1995) An Escherichia coli chromosomal ars operon homolog is functional in arsenic detoxification and is conserved in gram-negative bacteria. J Bacteriol 177:2050–2056

Duquesnoy I, Champeau GM, Evray G, Ledoigt G, Piquet-Pissaloux A (2010) Enzymatic adaptations to arsenic-induced oxidative stress in Zea mays and genotoxic effect of arsenic in root tips of Vicia faba and Zea mays. Comptes Rendus Biol 333:814–824

Duman F, Ozturk F, Aydin Z (2010) Biological responses of duckweed (*Lemna minor L.*) exposed to the inorganic arsenic species As (III) and As(V): Effects of concentration and duration of exposure. Ecotoxicology 19:983–993

Dwivedi S, Mishra A, Tripathi P, Dave R, Kumar A, Srivastava S, Chakrabarty D, Trivedi PK, Adhikari B, Norton GJ (2012) Arsenic affects essential and non-essential amino acids differentially in rice grains: inadequacy of amino acids in rice based diet. Environ Int 46:16–22

Ehrlich HL (1996) Geomicrobiology, vol 12, 3rd edn. Marcel Dekker Inc., New York, pp 276–293

Ellis DR, Gumaelius L, Indriolo E, Pickering IJ, Banks JA, Salt DE (2006) A novel arsenate reductase from the arsenic hyper accumulating fern Pteris vittata. Plant Physiol 141:1544–1554

Faita F, Cori L, Bianchi F, Andreassi MG (2013) Arsenic-induced genotoxicity and genetic susceptibility to arsenic-related pathologies. Int J Environ Res Public Health 10:1527–1546

Farooqi A, Masuda H, Firdous N (2007) Toxic fluoride and arsenic contaminated groundwater in the Lahore and Kasur districts, Punjab, Pakistan and possible contaminant sources. Environ Pollut 145:839–849

Garg N, Singla P (2011) Arsenic toxicity in crop plants: physiological effects and tolerance mechanisms. Environ Chem Lett 9:303–321

Geng CN, Zhu YG, Hu Y, Williams P, Meharg AA (2006) Arsenate causes differential acute toxicity to two P-deprived genotypes of rice seedlings (*Oryza sativa* L.). Plant Soil 279:297–306

Gomes M, Carneiro M, Nogueira C, Soares A, Garcia Q (2013) The system modulating ROS content in germinating seeds of two Brazilian savanna tree species exposed to As and Zn. Acta Physiol Plant 35:1011–1022

Goodman L, Gilman A (1942) The pharmacology basis of therapeutics. Macmian Company, New York. 4th printing

Gotz F, Zabielski J, Philipson L, Lindberg M (1983) DNA homology between the arsenate resistance plasmid pSX267 from Staphylococcus xylosus and the penicillinase plasmid pI258 from Staphylococcus aureus. Plasmid 9:126–137

Gulz PA, Gupta SK, Schulin R (2005) Arsenic accumulation of common plants from contaminated soils. Plant Soil 272:337–347

Gunes A, Pilbeam DJ, Inal A (2009) Effect of arsenic–phosphorus interaction on arsenic-induced oxidative stress in chickpea plants. Plant Soil 314:211–220

Gunes A, Inal A, Bagci EG, Kadioglu YK (2010) Combined effect of arsenic and phosphorus on mineral element concentrations of sunflower. Commun Soil Sci Plant Anal 41:361–372

Hlihor RM et al (2017) Bioremediation: an overview on current practices, advances, and new perspectives in environmental pollution treatment. BioMed Res Int

Holt JG (1994) Burgey's manual of systematic bacteriology, 9th edn

Hughes JP, Polissar L, Van Belle G (1988) Evaluation and synthesis of health effects studies of communities surrounding arsenic producing industries. Int J Epidemiol 17:407–413

Iriel A, Dundas G, Cirelli AF, Lagorio MG (2015) Effect of arsenic on reflectance spectra and chlorophyll fluorescence of aquatic plants. Chemosphere 119:697–703

Ji G, Silver S (1992) Reduction of arsenate to arsenite by the ArsC protein of the arsenic resistance operon of Staphylococcus aureus plasmid pI258. Proc Natl Acad Sci U S A 89:9474–9478

Ji G, Silver S (1995) Bacterial resistance mechanisms for heavy metals of environmental concern. Ind Microbiol 14:61–75

Jin W, Wang Z, Sun Y, Wang Y, Bi C, Zhou L, Zheng X (2019) Impacts of biochar and silicate fertilizer on arsenic accumulation in rice (*Oryza sativa* L.). Ecotoxicol Environ Saf 189:109928

Kanwar MK, Bhardwaj R (2015) Arsenic induced modulation of antioxidative defense system and brassinosteroids in Brassica juncea L. Ecotoxicol Environ Saf 115:119–125

Karn SK, Pan X, Jenkinson IR (2017) Bio-transformation and stabilization of arsenic (As) in contaminated soil using arsenic oxidizing bacteria and $FeCl_3$ amendment. 3 Biotech 7(1):50. https://doi.org/10.1007/s13205-017-0681-1

Ke C et al (2018) Characterization of recombinant *E. coli* expressing arsr from *Rhodopseudomonaspalustris* cga009 that displays highly selective arsenic adsorption. Appl Microbiol Biotechnol 102(14):6247–6255

Khalid S, Shahid M, Niazi NK, Rafiq M, Bakhat HF, Imran M, Abbas T, Bibi I, Dumat C (2017) Arsenic behaviour in soil-plant system: biogeochemical reactions and chemical speciation influences. In: Enhancing cleanup of environmental pollutants. Springer, Berlin, pp 97–140

Khan I, Ahmad A, Iqbal M (2009) Modulation of antioxidant defence system for arsenic detoxification in Indian mustard. Ecotoxicol Environ Saf 72:626–634

Kitchin KT, Wallace K (2008) Evidence against the nuclear in situ binding of arsenicals–oxidative stress theory of arsenic carcinogenesis. Toxicol Appl Pharmacol 232:252–257

Kligerman AD, Malik SI, Campbell JA (2010) Cytogenetic insights into DNA damage and repair of lesions induced by a monomethylated trivalent arsenical. Mutat Res 695:2–8

Körpe DA, Aras S (2011) Evaluation of copper-induced stress on eggplant (*Solanummelongena* L.) seedlings at the molecular and population levels by use of various biomarkers. Mutat Res 719:29–34

Krafft T, Macy JM (1998) Purification and characterization of the respiratory arsenate reductase of Chrysiogenes arsenatis. Eur J Biochem 255:647–653

Kumarathilaka P, Seneweera S, Ok YS, Meharg AA, Bundschuh J (2019) Mitigation of arsenic accumulation in rice: an agronomical, physico-chemical, and biological approach – a critical review. Crit Rev Environ Sci Technol 50(19):1984–2015. https://doi.org/10.1080/10643389.2019.1618691

Kushwaha A et al (2015) Heavy metal detoxification and tolerance mechanisms in plants: implications for phytoremediation. Environ Rev 24(1):39–51

LeBlanc MS, McKinney EC, Meagher RB, Smith AP (2013) Hijacking membrane transporters for arsenic phyto extraction. J Biotechnol 163:1–9

Lei M, Wan XM, Huang ZC, Chen TB, Li XW, Liu YR (2012) First evidence on different transportation modes of arsenic and phosphorus in arsenic hyper accumulator Pteris vittata. Environ Pollut 161:1–7

Leslie ACD, Smith H (1978) Self-poisoning by the abuse of arsenic containing tonics. Med Sci Law 18:159–162

Li Y, Dhankher OP, Carreira L, Lee D, Chen A, Schroeder JI, Balish RS, Meagher RB (2004) Overexpression of phytochelatin synthase in Arabidopsis leads to enhanced arsenic tolerance and cadmium hypersensitivity. Plant Cell Physiol 45:1787–1797

Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. Trends Plant Sci 11:392–397

Ma LQ, Komar KM, Tu C, Zhang WH, Cai Y, Kennelley ED (2001) A fern that hyperaccumulates arsenic. Nature 409:579

Ma JF, Yamaji N, Tamai K, Mitani N (2007) Genotypic difference in silicon uptake and expression of silicon transporter genes in rice. Plant Physiol 145:919–924

Malik JA, Goel S, Sandhir R, Nayyar H (2011) Uptake and distribution of arsenic in chickpea: Effects on seed yield and seed composition. Commun Soil Sci Plant Anal 42:1728–1738

Mandal SM, Gouri SS, De D, Das BK, Mondal KC, Pati BR (2011) Effect of arsenic on nodulation and nitrogen fixation of blackgram (Vigna mungo). Indian J Microbiol 51:44–47

Mascher R, Lippmann B, Holzinger S, Bergmann H (2002) Arsenate toxicity: Effects on oxidative stress response molecules and enzymes in red clover plants. Plant Sci 163:961–969

Maegraith B (1966) In tropical eosinophilia. Price's textbook of medicine. Oxford University Press, Bombay

Meharg AA, Hartley-Whitaker J (2002) Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytol 154:29–43

Meharg AA, Williams PN, Adomako A, Lawgali YY, Deacon C, Villada A, Cambell RCJ, Sun G, Zhu YG, Feldmann J, Rabb A, Zhao FJ, Islam R, Hossain S, Yanai J (2009) Geographical variation in total and inorganic arsenic content of polished (white) rice. Environ Sci Technol 43:1612–1617

Mehmood T, Bibi I, Shahid M, Niazi NK, Murtaza B, Wang H, Ok YS, Sarkar B, Javed MT, Murtaza G (2017) Effect of compost addition on arsenic uptake, morphological and physiological attributes of maize plants grown in contrasting soils. J Geochem Explor 2017(178):83–91

Michalke K et al (2000) Production of volatile derivatives of metal (Loid) S by microflora involved in anaerobic digestion of sewage sludge. Appl Environ Microbiol 66(7):2791–2796

Miteva E (2002) Accumulation and effect of arsenic on tomatoes. Commun Soil Sci Plant Anal 33:1917–1926

Mohapatra T, Nayak AK, Raja R, Shahid M (2013) Vision 2050. Central Rice Research Institute. Indian Council of Agricultural Research, Cuttack

Moore JE (1933) In modern treatment of syphilis. In: Thomas CC (ed) Springfield

Mubarak H, Mirza N, Chai LY, Yang ZH, Yong W, Tang CJ, Mahmood Q, Pervez A, Farooq U, Fahad S (2016) Biochemical and metabolic changes in arsenic contaminated Boehmeria nivea L. BioMed Res Int 2016:1423828

Mukhopadhyay R, Rosen BP, Phung LT, Silver S (2002) FEMS Microbiol Rev 26:311–325

Muller D, Lievremont D, Simeonova DD, Hubert JC, Lett MC (2003) Arsenite oxidase aox genes from a metal-resistant beta-proteobacterium. J Bacteriol 185:135–141

Naidu R, Smith E, Owens G, Bhattacharya P (2006) Managing Arsenic in the Environment: From Soil to Human Health; CSIRO Publishing: Clayton, Australia

Nash TA (1960) Review of the African trypanosomiasis problem. Trop Dis Bull 57:973–1013

Nath S, Panda P, Mishra S, Dey M, Choudhury S, Sahoo L, Panda SK (2014) Arsenic stress in rice: Redox consequences and regulation by iron. Plant Physiol Biochem 80:203–210

Naujokas MF, Anderson B, Ahsan H, Aposhian HV, Graziano JH, Thompson C, Suk WA (2013) The broad scope of health effects from chronic arsenic exposure: Update on a worldwide public health problem. Environ. Health Perspect 121:295.

Niazi NK, Bibi I, Fatimah A, Shahid M, Javed MT, Wang H, Ok YS, Bashir S, Murtaza B, Saqib ZA (2017) Phosphate-assisted phytoremediation of arsenic by Brassica napus and Brassica juncea: morphological and physiological response. Int J Phytoremediation 19:670–678

Nordstrom DK (2002) Public health – worldwide occurrences of arsenic in ground water. Science 296:2143–2145

Nriagu JO, Azcue JM (1990) Food contamination with arsenic in the environment. Adv Environ Sci Technol 23:121–143

Nussaume L, Kanno S, Javot H, Marin E, Pochon N, Ayadi A, Nakanishi TM, Thibaud M-C (2011) Phosphate import in plants: focus on the PHT1 transporters. Front Plant Sci 2

Okamoto Y, Chou PH, Kim SY, Suzuki N, Laxmi YS, Okamoto K, Liu X, Matsuda T, Shibutani S (2008) Oxidative DNA damage in XPC-knockout and its wild mice treated with equine estrogen. Chem Res Toxicol 21:1120–1124

Ordóñez E, Letek M, Valbuena N, Gil JA, Mateos LM (2005) Analysis of genes involved in arsenic resistance in Corynebacterium glutamicum ATCC 13032. Appl Environ Microbiol 71 (10):6206–6215. https://doi.org/10.1128/AEM.71.10.6206-6215.2005

Oremland RS, Stolz JF, Hollibaugh JT (2004) The microbial arsenic cycle in Mono Lake, California. FEMS Microbiol Ecol 48:15–27

Patra M, Bhowmik N, Bandopadhyay B, Sharma A (2004) Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environ Exp Bot 52:199–223

Pickering IJ, Gumaelius L, Harris HH, Prince RC, Hirsch G, Banks JA, Salt DE, George GN (2006) Localizing the biochemical transformations of arsenate in a hyper accumulating fern. Environ Sci Technol 40:5010–5014

Pio I, Scarlino A, Bloise E, Mele G, Santoro O, Pastore T, Santoro D (2015) Efficient removal of low-arsenic concentrations from drinking water by combined coagulation and adsorption processes. Sep Purif Technol 147:284–291

Pourrut B, Shahid M, Dumat C, Winterton P, Pinelli E (2011) Lead uptake, toxicity, and detoxification in plants. Rev Environ Contam Toxicol 213:113–136

Rahman MM, NG JC, Naidu R (2009) Chronic exposure of arsenic via drinking water and its adverse health impacts on humans. Environ Geochem Health 31(1):189–200

Ravenscroft P, Brammer H, Richards K (2009) Arsenic pollution: A global synthesis (Vol. 28). John Wiley & Sons: Hoboken, NJ, USA

Rehman K et al (2019) Enhancement of oil field-produced wastewater remediation by bacterially-augmented floating treatment wetlands. Chemosphere 217:576–583

Reynold ES (1901) In account of epidemic outbreak of arsenical poisons occurring in Beer drinkers in north of England and the Midland Countries in 1900. Lanccl 1:166–170

Roberge J, Abalos AT, Skinner JM, Kopplin M, Harris RB (2009) Presence of arsenic in commercial beverages. Am J Environ Sci 5:688–694

Rosen BP, Dey S, Dou D, Ji G, Kaur P, Ksenzenko M, Yu SS, Wu J (1992) Evolution of an ion-translocating ATPase. Ann N Y Acad Sci 671:257–272

Saltikov CW, Wildman RA Jr, Newman DK (2005) Expression dynamics of arsenic respiration and detoxification in Shewanella sp. Strain ANA-3. J Bacteriol 187:7390–7396

Sanglard LM, Detmann KC, Martins SC, Teixeira RA, Pereira LF, Sanglard ML, Fernie AR, Araújo WL, DaMatta FM (2016) The role of silicon in metabolic acclimation of rice plants challenged with arsenic. Environ Exp Bot 123:22–36

Schmöger MEV, Oven M, Grill E (2000) Detoxification of arsenic by phytochelatins in plants. Plant Physiol 122:793–802

Schulz H, Härtling S, Tanneberg H (2008) The identification and quantification of arsenic-induced phytochelatins—Comparison between plants with varying As sensitivities. Plant Soil 303:275–287

Seow WJ, Pan WC, Kile ML, Baccarelli AA, Quamruzzaman Q, Rahman M, Mahiuddin G, Mostofa G, Lin X, Christiani DC (2012) Arsenic reduction in drinking water and improvement in skin lesions: A follow-up study in Bangladesh. Environ Health Perspect 120:1733

Shahid M, Dumat C, Pourrut B, Abbas G, Shahid N, Pinelli E (2015) Role of metal speciation in lead-induced oxidative stress to Vicia faba roots. Russ J Plant Physiol 62:448–454

Shahid M, Dumat C, Khalid S, Niazi NK, Antunes PM (2017) Cadmium bioavailability, uptake, toxicity and detoxification in soil-plant system. Rev Environ Contam Toxicol 241:73–137

Shakoor MB, Niazi NK, Bibi I, Rahman MM, Naidu R, Dong Z, Shahid M, Arshad M (2015) Unraveling health risk and speciation of arsenic from groundwater in rural areas of Punjab, Pakistan. Int J Environ Res Public Health 12:12371–12390

Shakoor MB, Niazi NK, Bibi I, Murtaza G, Kunhikrishnan A, Seshadri B, Shahid M, Ali S, Bolan NS, Ok YS (2016) Remediation of arsenic-contaminated water using agricultural wastes as biosorbents. Crit Rev Environ Sci Technol 46: 467–499

Shah S, Damare S (2020) Cellular response of Brevibacterium casei #NIOSBA88 to arsenic and chromium – a proteomic approach. Braz J Microbiol. https://doi.org/10.1007/s42770-020-00353-7

Shariatpanahi M et al (1981) Biotransformation of the pesticide sodium arsenate. J Environ Sci Health B 16(1):35–47

Shipton WA (2014) The biology of fungi impacting human health. Gurgaon, PartridgeIndia

Shishir T, Mahbub N (2019) Review on bioremediation: a tool to resurrect the polluted rivers. Pollution 5(3):555–568

Silver S, Ji G, Broer S, Dey S, Dou D, Rosen BP (1993) Orphan enzyme or patriarch of a new tribe: the arsenic resistance ATPase of bacterial plasmids. Mol Microbiol 8:637–642

Singh N, Ma LQ, Srivastava M, Rathinasabapathi B (2006) Metabolic adaptations to arsenic induced oxidative stress in Pteris vittata L. and Pteris ensiformis L. Plant Sci 170:274–282

Singh VP, Singh S, Kumar J, Prasad SM (2015) Hydrogen sulfide alleviates toxic effects of arsenate in pea seedlings through up-regulation of the ascorbate–glutathione cycle: possible involvement of nitric oxide. J Plant Physiol 181:20–29

Smedley PL, Kinniburgh DG (2002) A review of the source, behaviour and distribution of arsenic in natural waters. Appl Geochem 17:517–568

Smedley P, Kinniburgh D (2012) A review of the source, behaviour and distribution of arsenic in natural waters. Appl Geochem 17:517–568

Soldan R et al (2019) Bacterial endophytes of mangrove propagules elicit early establishment of the natural host and promote growth of cereal crops under salt stress. Microbiol Res 223:33–43

Sommella A, Deacon C, Norton G, Pigna M, Violante A, Meharg AA (2013) Total arsenic, inorganic arsenic, and other elements concentrations in Italian rice grain varies with origin and type. Environ Pollut 181:38–43

Srivastava S, Srivastava A, Singh B, Suprasanna P, D'souza S (2013) The effect of arsenic on pigment composition and photosynthesis in Hydrilla verticillata. Biol Plant 57:385–389

Srivastava S, Sinha P, Sharma YK (2017) Status of photosynthetic pigments, lipid peroxidation and anti-oxidative enzymes in Vigna mungo in presence of arsenic. J Plant Nutr 40:298–306

Stolz JF, Basu P, Oremland RS (2002) Microbial transformation of elements: the case of arsenic and selenium. Int Microbiol 5:201–207

Suhadolnik MLS, Salgado APC, Scholte LLS et al (2017) Novel arsenic-transforming bacteria and the diversity of their arsenic-related genes and enzymes arising from arsenic-polluted freshwater sediment. Sci Rep 7(1):11231. https://doi.org/10.1038/s41598-017-11548-8

Suresh K, Reddy GSN, Sengupta S, Shivaji S (2004) Deinococcus indicus sp. nov., an arsenic-resistant bacterium from an aquifer in West Bengal, India. Int J Syst Evol Microbiol 54(2):457–461

Talukdar D (2013) Arsenic-induced changes in growth and antioxidant metabolism of fenugreek. Russ J Plant Physiol 60:652–660

Tara N et al (2019) On-site performance of floating treatment wetland macrocosms augmented with dye-degrading bacteria for the remediation of textile industry wastewater. J Clean Prod

Tofan-Lazar J, Al-Abadleh HA (2012) ATR-FTIR studies on the adsorption/desorption kinetics of dimethylarsinic acid on iron–(oxyhydr) oxides. J Phys Chem A 116:1596–1604

Tripathi P, Singh PC, Mishra A, Srivastava S, Chauhan R, Awasthi S, Mishra S, Dwivedi S, Tripathi P, Kalra A (2017) Arsenic tolerant *Trichoderma* sp. reduces arsenic induced stress in chickpea (Cicer arietinum). Environ Pollut 223:137–145

Tu C, Ma LQ (2003) Interactive effects of pH, arsenic and phosphorus on uptake of As and P and growth of the arsenic hyper accumulator Pteris vittata L. under hydroponic conditions. Environ Exp Bot 50:243–251

Upadhyay A, Singh N, Singh R, Rai U (2016) Amelioration of arsenic toxicity in rice: comparative effect of inoculation of Chlorella vulgaris and Nannochloropsis sp. on growth, biochemical changes and arsenic uptake. Ecotoxicol Environ Saf 124:68–73

Vázquez S, Esteban E, Carpena RO (2008) Evolution of arsenate toxicity in nodulated white lupine in a long-term culture. J Agric Food Chem 56:8580–8587

Wolf R (1974) On the question of occupational arsenic poisoning in vineyard workers. Berufsdermatosen 22(1):34–37

Woźniak K, Blasiak J (2003) In vitro genotoxicity of lead acetate: induction of single and double DNA strand breaks and DNA–protein cross-links. Mutat Res 535:127–139

Yahaghi Z et al (2019) Uptake and effects of lead and zinc on alfalfa (*Medicago Sativa* L.) seed germination and seedling growth: role of plant growth promoting bacteria. South Afr J Bot

Zachariac H, Sogard H, Nyfors A (1974) Liver biopsy in psoriatics previously treated with potassium arsenate. ActoDermVenereol (Stock) 54:235–236

Zhao FJ, Ma JF, Meharg AA, McGrath SP (2009) Arsenic uptake and metabolism in plants. New Phytol 181:777–794

Ziech D, Franco R, Georgakilas AG, Georgakila S, Malamou-Mitsi V, Schoneveld O, Pappa A, Panayiotidis MI (2010) The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development. Chem Biol Interact 188:334–339

Chapter 13

Phycoremediation of Heavy Metals, Factors Involved and Mechanisms Related to Functional Groups in the Algae Cell Surface – A Review

Abate Ayele, Arumuganainar Suresh, and Solomon Benor

Abstract The exorbitant usage of heavy metal in industries leads to accumulation in the ecosystem and causes various disorders. Many physico-chemical methods have developed for the removal of heavy metals from industrial wastewaters as well as the polluted environments. However, these conventional technologies are providing expensive, generate toxic secondary sludge, and not complete abatement. An alternative, the biological methods especially using algae for the treatment of metal-contaminated environments provides a cost-effective, rapid, and eco-friendly approach. Using algae (macro or micro) for removing pollutants (phycoremediation) is a versatile approach since algae can adapt easily with any environmental conditions and recognized as a sustainable technology for removing noxious heavy metals. Algal biomass contains a polyanionic cell wall that can bind heavy metals through the principle of adsorption (passive) onto the cell surface and absorption (active) into the cell with metal-binding peptides or contained in the vacuole. Moreover, various factors (pH, temperature, metal concentration, contact time, agitation speed) affect the biosorption of heavy metal. Thus, this review article deals with the abatement of heavy metals using macro and microalgae, factors

---

A. Ayele
Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

A. Suresh (✉)
Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

Department of Biological Sciences and Biotechnology, Institute of Advanced Research – The University for Innovation, Gujarat, India
e-mail: suresh.suresh@aastu.edu.et

S. Benor
Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

Office of Science and Research Affair Director General, Ministry of Science and Higher Education, Addis Ababa, Ethiopia

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_13

involved and mechanisms, and functional groups involved in the cell surface. Besides, suggested that the industrial-scale data's are required for the real application.

Keywords Adsorption · Functional groups · Heavy metals · Macroalgae · Microalgae · Phycoremediation

1 Introduction

Rapid industrialization and urbanization in the last century have led to ecological damage and cause problems of living and nonliving things. Among many industrial pollutants, heavy metal release into the water and the soil creates problems in the ecosystems. Even at a low concentration of heavy metal (example 0.00001 mg Hg/L) can cause a major threat to both terrestrial and aquatic environments and there are coupled several health risks (Jaishankar et al. 2014). Therefore, it is important to remove heavy metals such as Mercury (Hg), Chromium (Cr), Arsenic (As), Lead (Pb), and Cadmium (Cd) which are common heavy metals contaminants that present in wastewater discharge. Among the heavy metals, Pb has become a cosmopolitan environmental pollutant (Brahmbhatt et al. 2013). The heavy metals can accumulate in the food chain and food web of the aquatic and terrestrial ecosystem, magnify, and cause illness (Mann et al. 2011).

Once heavy metals get into the water through various means, the first target affected is algae. Algae have a long-exposure state with heavy metals stress or physico-chemical effects in the water bodies, hence the algae may produce a series of adaptive or tolerance mechanisms to adapt or tolerate the negative effects of toxic heavy metals (Monteiro et al. 2012). Algae survive in heavy metal contaminated medium within a certain range of levels by absorption, modification, accumulation, transfer, and discharge of the heavy metals. Therefore, the algae can reduce the negative effects of heavy metals, ensuring its sustainable growth for a long time. As a result, the application of algae for phycoremediation of heavy metal contaminated water and any environment offers greater advantages over other conventional methods. For instance, phycoremediation is cost-effective, eco-friendly, efficient even at low concentrations, oxygen production, carbon dioxide sequestration, use wastewater (Suresh et al. 2019), removal of air pollutants (Suresh and Benor 2020), biofuel production, and food production. However, hitherto this technology is not popular due to ignorance, needs to give much attention and extent. Therefore, in this review discussed various aspects of heavy metal removal using macro and microalgae, factors affecting, and its mechanisms involved.

2 Heavy Metals and Its Toxicity

Heavy metals belong to high atomic weight chemicals that have a density (5 g per cubic centimeter) approximately, five times greater than water (Duffus 2002). Accumulation of metals into organisms poses a dangerous health risk via bioconcentration, and biomagnifications mainly through the food chain (Mann et al. 2011). However, living organisms require different amounts of specific heavy metals for the regulation process in the living body. Namely, Cu, Fe, Co, Mn, Mo, and Zn are required in small amounts for growth and effective regulation. In other words, all metals are toxic and harm both terrestrial and aquatic ecosystems at some concentrations, and it comes from different industries (Fig. 13.1). Any, organisms exposed to heavy metals incline to have a defensive mechanism against its toxicity and it varies among living beings. There are much disorder has been accounted for the metals, for instance, Hg affects the nervous system and kidney in mammals, (Minamata disease), Ar cause cancer, Cr trigger allergic dermatitis, Pb affects mental development. Those toxic metals affect plants and microbes in terms of affecting photosynthesis in plants and enzymes systems in microbes including algae. The maximum contaminant level (MCL) allowed in drinking water varies according to its toxic potentials. For example, 0.002 mg/L for Hg, 0.005 mg/L for Cd, 0.015 mg/L for Pb, and 0.1 mg/L for Cr. (USEPA 2020).

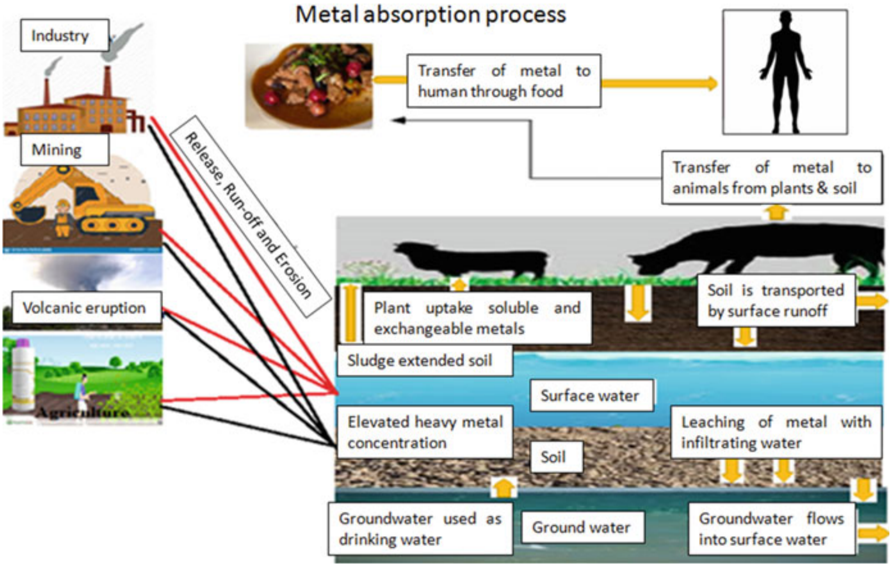


Fig. 13.1 Metal absorption process from water, soil, and air to food chain and finally to human beings

3 Physico-chemical Methods for Heavy Metal Removal

There are many physico-chemical methods such as chemical precipitation, flotation, coagulation and flocculation, ion exchange, membrane filtration, ultra-filtration, nano-filtration, reverse osmosis, electrolysis, electro-coagulation, and photocatalysis used to remove heavy metals from the contaminated medium (Saravanan et al. 2017). However, every process has its pros and cons. All the above-mentioned technologies are crucial for the removal of toxic heavy metals from contaminated environments. Nevertheless, these above-mentioned technologies have some disadvantages such as incomplete removal, produce a large amount of toxic secondary sludge, expensive, high-energy requirements, and eco-unfriendly. Besides, the above techniques may be ineffective when the high concentration of metal in wastewater (10–100 mg/L) (Mehta and Gaur 2005), therefore, the urgent need for alternative methods.

4 Algae and Its Uses in Bioremediation

Phycology is the scientific study of algae ("phyco" Greek for "alga") which are photosynthetic organisms that vary from single cells to multi-cellular. Algae is a primary producer, use Sunlight as an energy source and carbon dioxide as a carbon source and make food and release oxygen into the atmosphere (Bansal et al. 2018). Algae are defined as "thallophytes (plants lacking roots, stems, and leaves) that have chlorophyll as their primary photosynthetic pigment and distributed in many regions of the world, from being airborne or sub-aerial to being terrestrial or aquatic, either in freshwater or marine habitats. Many are eukaryotic organism but the term used to include cyanobacteria (blue-green algae), which are prokaryotic. Algae can be divided into two categories depending on its size, macroalgae (can be seen without the aid of a microscope) and microalgae (can be seen using only with the aid of a microscope). Moreover, the number of algae estimated is to be one to ten million, with a high percentage of the microalgae (Chu 2012). According to Packer (2009), algae have very high carbon capturing, photosynthetic efficiencies, and metabolism when compared to terrestrial plants (Suresh and Benor 2020). Using algae for the elimination or biotransformation of contaminants from any environment called phycoremediation and this technology is rising lately (Fig. 13.2).

5 Microalgae

Many algae are microscopic, unicellular, and exist single (*Chlorella vulgaris*), or in chains (*Anabaena*), or groups (*Volvox*), and can be motile (*Diatoms*), or non-motile (*Coccoid*). Depending on the species, their sizes can range from a few micrometers

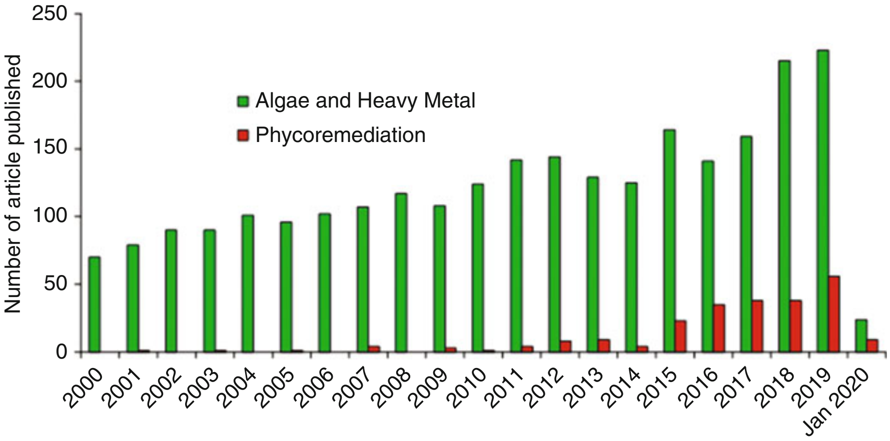


Fig. 13.2 The number of documents retrieved on algae and heavy metals related topics from the world from 2000- Jan 2020. The number of documents was determined by searching the Scopus database (https://www.scopus.com) on 31st January 2020 using the search terms "algae and heavy metals (green bar), phycoremediation (red bar)

(1 µm, *Nannochloropsis*) to a few hundred micrometers (400 µm, (*Oscillatoria*). In terms of biomass, the world's largest primary producers, responsible for at least 32% of global photosynthesis, and oxygen production as well as the best-known CO_2 sequestration (Kumar et al. 2015). Microalgae have unique character by being able to distinguish between the essential heavy metals needed by the organisms from their other counterparts and an important role in the removal of toxic heavy metals (Table 13.1).

6 Macroalgae

Seaweed is another name for macroalgae that are multi-cellular and macroscopic (Example, *Ulva lattuca, Sargassum*). Unlike microalgae, they have defined tissues containing specialized cells similar to terrestrial vascular plants. In general, macroalgae is preferred than microalgae for a removal for pollutant purposes (Table 13.1) because it can be easily harvested than microalgae from the water, but efficiency is less than the microalgae due to its less surface are and growth rate.

Table 13.1 Algal biomass used for removal of heavy metals

Microalgae	Heavy metals	Removal (%)	References
Scenedesmus obliquus	Cd	100	Chen et al. (2012)
Botryococcus sp.	Cr	94	Onalo et al. (2014)
	As	9	
Lyngbya	Pb	80	
Pseudopediastrum boryanum	Cr	70	Sutkowy and Kłosowski (2018)
Desmodesmus multivariabilis	Tl	94.04	Birungi and Chirwa (2018)
Macroalgae			
Ulva lactuca	Cd	99.2	Ghoneim et al. (2014)
	Pb, Cd	85	Ibrahim et al. (2018)
Ulva lactuca	Cd, Pb & Cr	83	Ibrahim et al. (2016)
Jania rubens	Hg	54–71	Anastopoulos and Kyzas (2015)
Sphaerococcus coronopifolius	Hg	70–90	
Jania rubens	Pb, Cd & Ni	91	Ibrahim et al. (2018)
Palmaria palmate	Pb	100	Prasher et al. (2004)
Pelvetia canaliculata	Cr	100	Hackbarth et al. (2016)
Sargassum myriocystum	Pb	86.67	Anastopoulos and Kyzas (2015)
Azolla filiculoides	Cr	83.34	

7 Heavy Metal Toxicity to Algae

The heavy metal concentration beyond a threshold limit causes direct toxicity to the algae moreover algae are highly sensitive to environmental changes or fluctuation as a result changes take place in their metabolism (Kaplan 2013). Toxic effects of heavy metals on algae caused by many ways such as the displacement or removing of essential metal ions from biomolecules, functional cellular units, inhibiting functional groups of biologically useful molecules, induction of cellular generation of reactive oxygen species, and inactivation of enzymes as well as disruption the integrity of cellular and organelle membrane (Fig. 13.3) (Naorbe and Serrano 2018). Also, found that some heavy metals blocking or inhibiting cell division, inhibit or block the DNA repair mechanisms, attack nucleic acid molecules, promote free radical chain reaction, disturbing thylakoid membrane, inhibit or block pigments synthesis reduction in photosynthetic electron transport, inhibit CO_2 fixation, blocking of respiratory oxygen consumption, inhibition of enzyme activity, blocking of protein synthesis, abnormal morphological development, impairment of motility in certain microalgae and an irreversible increase in plasmalemma permeability, leading to the loss of cell and changes in cell volume (Liu et al. 2016; Shilpi et al. 2014; Sziválk et al. 2009).

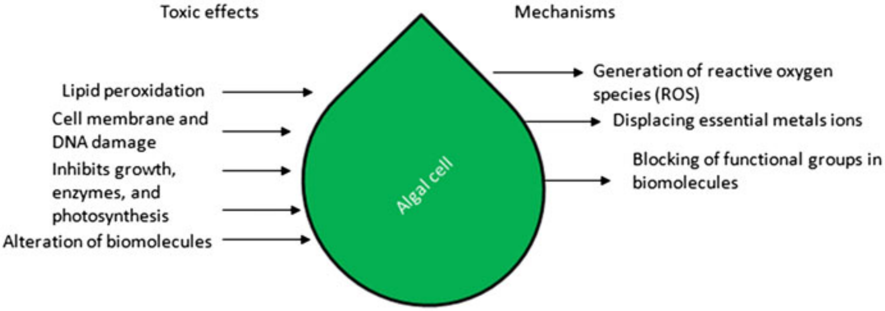


Fig. 13.3 Toxicity of heavy metals on algae

8 Phycoremediation of Heavy Metals

Heavy metals are a stable contaminant as it cannot be degraded or destroyed by any chemical or biological process. It can only be able to removed and contained using physico-chemicals and biological methods. Therefore, to adapt or survive to the toxic heavy metals, algae have developed extensive adaptation mechanisms (extracellular and intercellular) (Kumar et al. 2015). Moreover, it performs the specific mechanism to uptake the essential heavy metals required for their cell growth. Algae can bind metal ions, thus it can be used effectively to clean the metal contaminated medium especially in industrial effluent. Tables 13.1 and 13.2 indicate some microalgae and macroalgae used for the removal of heavy metals from the previous studies. Most of the studies were evidence in aqueous solutions and synthetic wastewater while real industrial effluent is limited. The efficiency of metal absorption was found to be different in macro and microalgae strains (Tables 13.1 and 13.2). The 100% removal efficiency was achieved using microalgae of *Scenedesmus obliquus* against cadmium (Chen et al. 2012), and macroalgae of *Palmaria palmate* and *Pelvetia canaliculata* against lead and chromium, respectively (Prasher et al. 2004; Hackbarth et al. 2016). A minimum of 2% removal was observed using microalgae of *Botryococcus* sp., against Cd (Onalo et al. 2014), whereas in the case of macroalgae (*Jania rubens*) showed a minimum of 54% Hg removal (Anastopoulos and Kyzas 2015).

Besides, metal biosorption via live cells and dead cells has been differing mainly in duration of adsorption however; ion exchange is the mutual process responsible in both biosorbents largely. Most of the previous literature also preferred dead biomass for the biosorption process due to its reduced time (a maximum of 30 min is enough to absorb 90% heavy metal) possibility in recycling and reuse of the biomass and does not require nutrients and will work in extreme environments. Whereas in living algal cell, sorption potential is limited, needs to provide nutrients, and depends on growth capacity and conditions of algal strain.

9 Factors Influencing Phycoremediation of Heavy Metals

Adsorption has been regarded as the main mechanism for metal ion removal via algal sorbent, nevertheless, various factors are influencing the biosorption potential of heavy metal phycoremediation, such as pH, algae dose, contact time, temperature, agitation speed, and metal concentration (Table 13.2).

9.1 Influence of pH

Environmental pH is one of the most important factors that directly influence the mechanism of biosorption to remove heavy metal from the aqueous solution. pH affects the association and dissociation of the functional groups of the biosorbent active sites as well as the ion solution chemistry. It was well known that the biosorption of cations and anions depend on the pH of the environment. The pH optimum for biosorption via algae varies from metal to other metals and algae to

Table 13.2 Factors influencing phycoremediation of heavy metals and its maximum biosorption capacity (Q_m)

Alage biomass	Biosorbents dose (g/L)	Time (min)	pH	Q_m (mg/g)	References
Microalgae					
Spirogyra sp.	1	120	4	265	Yaqub et al. (2012)
Oscillatoria laetevirens	0.1	60–75	5	103.1	Suman (2012)
Planothidium lanceolatum	0.4	30	1	93.45	Sbihi et al. (2012)
Chlorella vulgaris	1.2	60	3	83	Indhumathi et al. (2014)
Spirulina platensis	2	60	6	33	Lodi et al. (2008)
Nostoc muscorum	1	120	5	22.92	Gupta and Rastogi (2009)
Chlamydomonas reinhardtii	0.6	120	2	21.2	Arıca et al. (2005)
Pseudopediastrum boryanum	1	180	2	16.68	Sutkowy and Kłosowski (2018)
Scenedesmus obliquus	1	–	2	15.6	Dönmez et al. (1999)
Macroalgae					
Sargassum muticum	5	10	2	143	El Atouani et al. (2016)
Sargassum sp.	–	–	2	66.4	Cabatingan et al. (2001)
Padina sp.	–	60	2	54.6	Sheng et al. (2005)
Gracilaria salicornia	10	15	1	21.78	Belattmania et al. (2015)
Gracilaria corticata	20	240	4	5	Kavitha et al. (2016)

algae. In acidic pH, the positive metal ions that can able to bind with negatively charged algal biomass and increase the adsorption between the sorbents and the sorbate. It is worth to mention that, in case of more acidic pH (<3), the excess H+ ions neutralize the negatively charged biosorbent surface, and thereby, reduce the adsorption of metal ions (Kumar et al. 2008) therefore moderate acidic pH (3–5) is preferred and suggested. In the case of alkaline pH (>8), a decrease in biosorption may be due to the formation of soluble hydroxylated complexes of the metal ions and their competition with the active sites, and eventually, the biosorption would decrease (Saurav and Kannabiran 2011). Matheickal and Yu (1999) proposed another concept, that was precipitation reaction occurs in alkaline pH, and the formation of insoluble hydroxides of metal ion might reduce the concentration of free sorbate ion available for biosorption. In contrast to the above concept, Sen et al. (2018) reported that the alkaline pH of 11 was maximized the biosorption of chromium when compare with acidic pH of 5 in cyanobacterial biomass. Believed that the net negative charge present in the cyanobacterial biomass was comparatively higher in alkaline pH, that facilitates the binding of positive hexavalent Cr ion onto the cell surface. Therefore, it is clear that metal ion uptake is depended on the pH of the solution, and the behavior of many different functional groups present in the surface of algal cells as well as to complex formation constants (Zeraatkar et al. 2016).

9.2 Influence of Metal Concentration

Biosorption of heavy metals using algal biomass is largely dependent on the initial concentration of the metal ions in the solution phase. Originally, biosorption increases as the initial concentration of metal ion increases. Subsequently, no significant increase in metal sorption was observed by a tandem increase in the concentration of metal ions (Zeraatkar et al. 2016). For instance, the chromium uptake increased from 0.2234 to 4.2172 mg/g, as the initial chromium concentration increased from 5 to 150 mg/L. Meantime the percentage biosorption of chromium decreased from 89.37% to 56.23% as the initial chromium concentration increased from 5 to 150 mg/L (Kavitha et al. 2016). The decrease in percentage biosorption possibly attributed due to a lack of sufficient surface area to accumulate much more metal available in the solution (Ucun et al. 2002).

9.3 Influence of Biosorbent Concentration and Its Size

The increase in the dosage of biosorbent will increases the biosorption of metal ions due to the larger surface area that in effect increases the number of available binding sites. At lower concentrations of the biosorbent the amount of biosorbed metal per unit weight of the biosorbent is high (Kanamarlapudi et al. 2018). For example, Fe removal efficiency increased as the *Chlorella coloniales* dose increased at all applied initial Chromium concentrations, reaching their maximum values of 86.7% in 2.6 g/L of algae dose (Jaafari and Yaghmaeian 2019). At lower concentrations of the biosorbent, the Kavitha et al. (2016) study showed that the percentage biosorption of chromium was increased from 70.09% (1.7522 mg/g) to 75.59% (1.8897 mg/g) as the biosorbent size decreases from 104 to 44 μm with an increase in biosorbent dosage from 10 g/L to 25 g/L, at 303 K for 50 mg/L of chromium initial concentration of *Gracilaria corticata* biomass. While decrease in biosorbent sizes, the surface area of the biosorbent increases, thereby the number of active sites available on the biosorbent is better exposed to the sorbate. Similar results were noticed by other researchers.

9.4 Influence of Contact Time

Algae adsorbent adsorbs heavy metals passively on the surface of the cell wall quickly within a few minutes and reaches saturation at a maximum of 30 min, making biosorption highly dependent on contact time (Zeraatkar et al. 2016). As time increases, the rate of biosorption decreases due to an increase in the percentage of saturation of metal ions remaining in the solution (Kanamarlapudi et al. 2018; Jaafari and Yaghmaeian 2019). The rapid initial adsorption perhaps attributed to the accumulation of metals on to the surface of biosorbent, due to its large surface area and later the process becomes slower. For instance, Sbihi et al. (2012) observed that 93.45 mg Cr g^{-1} in 30 min of contact time in the microalgae of Diatoms sorbent and stated that the live biomass was taking more than a day to reach 60% removal. The same was evidenced by Das et al. (2018) found 20 days to remove >50% of total dissolved solids in tannery effluent using live microalgae. Moreover, suggested that the desorption process is starts when the contact time is raised. By this fact, the previous researchers have also reported that less time was preferred when using more biosorbents and stated that the biosorption process took place in two stages. The first stage was rapid and achieved the equilibrium and the second stage represented slower progressive adsorption and later the desorption progress (Kavitha et al. 2016; Sbihi et al. 2012; Tüzün et al. 2005). Besides, Sen et al. (2018), recently stated that the wide variation in incubation time was not that important factor since equilibrium was obtain within 5 min and did not consider as input variables.

9.5 Influence of Temperature

Adsorption is an exothermic process and therefore, by the Le Chatelier's principles, the amount of adsorption should increase with a decrease in the temperature of the physical adsorption. Whereas, chemical adsorption first increases with an increase in temperature and then starts to decrease. Increased biosorption of heavy metal at increasing temperature was attribute to bond rupture, which may increase the number of active sites involved in metal sorption or increase the affinity of metal sites at the initial rise (Mehta and Gaur 2005). At certain times, the temperature can have a positive or negative effect on biosorption. An increase or decrease in temperature induces a difference in the biosorption potential of the biosorbent (Kanamarlapudi et al. 2018). For example, according to Soleymani et al. (2014), temperature variation between 15 and 45 °C slightly increases the Cu, metal uptake capacity from 38.63 to 42.24 mg/g, respectively. The same effects were also observed by Kavitha et al. (2016), where the percentage biosorption of Cr increased from 79.31% to 91.44% as temperature increased from 283 to 323 K using a microalgae (*Gracillaria corticata*) biosorbent.

9.6 Influence of Agitation Speed

Mixing the solution also has a considerable impact on biosorption. The increased speed of agitation increases the biosorption ability of the biosorbent by decreasing its resistance to mass transfer (Kanamarlapudi et al. 2018). For instance, Mahmoud et al. (2017) found that the adsorption of Zn increased rapidly as the agitation speed increased from 50 to 200 rpm and a stage of equilibrium was attained at this speed with 76.7% and 90.5% removal efficiencies in case of both natural and modified adsorbents, respectively.

10 Mechanism of Heavy Metal Uptake by Algae

The uptake of metals from wastewater by algae can be passive or active, or both. Passive uptake is independent of cellular metabolisms and involves the binding of metals into polyanionic cell wall or ion exchange between protons and heavy metal ions at the binding site, adsorption by physical forces (van der Waals forces, covalent bonding, redox interaction, biomineralization), electrostatic interactions, surface complexation, micro-precipitation and bioaccumulation (Brinza et al. 2007). The cell wall composition of algae is playing a major role in binding with metal ions, which are either polysaccharides or different glycoproteins groups, specifically, glycan, mannan, alginic acid, proteins, and xylans. Additionally, the cell wall of cyanobacteria is composed of peptidoglycan, and produce sheaths with extracellular polymeric substances, which are utilize for sorption (Zeraatkar et al. 2016). The cell wall provides functional groups (carboxyl, phosphate, hydroxyl, amino, sulfur, sulfide, and thiol), oxygen, and nitrogen-based moieties as well as form coordinate bonds with heavy metal ions. Passive uptake is rapid, reaching maximum adsorption in 5–10 min and is unaffected by metabolic inhibitors and affected by environmental conditions as mentioned above. Passive binding is reversible and can occur in both living and dead biomass. The Fig. 13.4 depicts the additional mechanisms such as entrapment of metal both in the form of insoluble micro-deposits at inter and intra fibrillate capillaries and paracrystalline regions of polysaccharides and the binding to other biopolymers (RNA, polyphosphates) can contribute to the metal-binding (Bilal et al. 2018). The metal ions are adsorbed to the surface of the cells by interactions between the metal cation and the algal anions, functional groups and penetrate the cell membrane and enter to the cells by introducing molecular mimicry mechanisms whereby metal ions either compete for binding to many valent ion metals or, after binding to low molecular weight thiols, enter the cell of microalgae by active transport. The active process is slower than passive, dependents on cellular metabolism, affected by metabolic inhibitors and temperature.

Besides, algae produce extracellular and intracellular peptides that are capable to bind heavy metals (Fig. 13.4). Those peptides were classified as short-chain polypeptides named phytochelatins and metallothioneins found in algae (Peña-Salamanca et al. 2011). These peptides involve organic complexation of trace metals to maintain their concentrations at non-toxic levels. Metallothioneins are gene-encoded cysteine-rich polypeptides, but phytochelatins (PCs) are enzymatically synthesized with low molecular weight cysteine-rich along with glutamic acid and glycine amino acid. The primary property of these peptides through the thiol group of cysteine residues is their metal-binding ability. These groups of thiol enable PCs for binding to metal ions with elevated affinity and thus forming a powerful PC-metal complex (Mera et al. 2014; Liu et al. 2016). In addition, some extracellular macromolecules of polysaccharides such as alginates and fucoidan and intracellular polyphosphates have the chelating mechanisms with heavy metal in some macroalgae (García-García et al. 2016). The negatively charged macromolecules play the main role in the chemical coagulation of heavy metal for algal tolerance against toxic heavy metals (Wu et al. 2018; Liu et al. 2016).

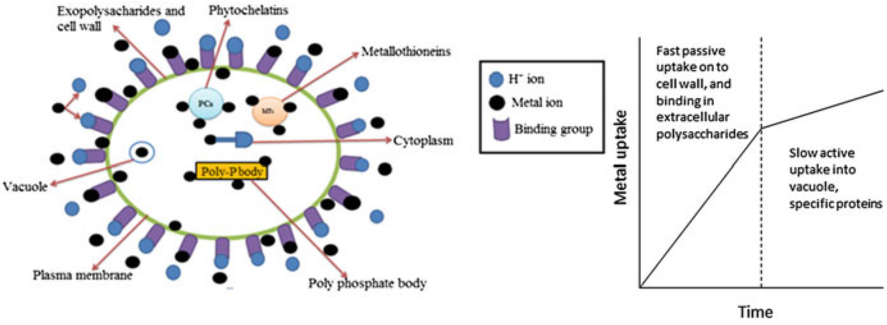


Fig. 13.4 The uptake of metals by algae biomass using passive (cell surface) and active process (Taken into the cell)

11 The Functional Groups of Algae for Heavy Metal Uptake

As mentioned, that the algal cell wall plays a vital role in heavy metal uptake as it contains macromolecules of polysaccharides, proteins, lipids, and lignin. It is pertinent to note that the glucosamine group is an important surface sugar constituent of various algal species (Takeda 1996). Furthermore, protonation of an amino group under low pH, could be associated with metal adsorption (Boddu et al. 2003; Bai and Abraham 2002). These contribute to various anionic functional groups (carboxyl, hydroxyl, phosphate, amino, sulfhydryl, amide, alkyl, and aromatic compound) and conserve an overall negative charge to the cell surface (Raize et al. 2004; Javanbakht et al. 2014). This anionic functional group (Fig. 13.5 and Table 13.3) has a strong binding affinity towards metal cations and is involved in metal adsorption through the ion exchange, physical adsorption, and complex formation.

Table 13.3 indicates that the common anionic functional groups such as O–H, N–H group, amino group, amine, and carboxyl groups are present in both macro and microalgae, respectively. However, specific groups also can be present. For instance, Matei et al. (2015) found some new functional groups called halides groups from *Spirogyra* sp. Some rare algal species that contain the functional group, acetyl, and ester groups (Utomo et al. 2016). The region between 3200 and 3600 cm^{-1} was indicative of the overlapping peaks, attributed to the stretching vibration of O–H and N–H (Deng and Ting 2005). The C–H stretching vibrations of $–CH_3$ and CH_2 functional groups were observed in the region between 3000 and 2800 cm^{-1} while the deformation stretching of C–H, $–CH_3$, and CH_2 functional groups was noted in the region 1300–1470 cm^{-1} (Yee et al. 2004). At 1411 cm^{-1}, a characteristic peak of

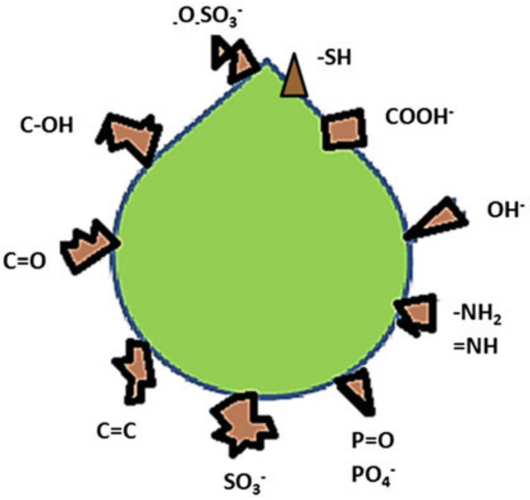


Fig. 13.5 Some functional groups present in algae surface for heavy metal adsorption

Table 13.3 Different functional groups identified in the algal biomass for the biosorption of heavy metals

Algae biomass	Heavy metal	Functional groups	References
Macroalgae			
Sargassum filipendula	Cu & Ni	–NH_2, –OH, C–H, O–C–O, C–OH, –SO_3	Kleinübing et al. (2010)
Ulva lactuca	Cd	–OH, –NH_2, O–CH_3 CH_2, & –$CONH_2$, C–OH or –COOH	Ghoneim et al. (2014)
Ceramium rubrum	Cu	–OH, –NH_2, –COOH, & –O–SO_3^-	Ege and Doner (2013)
Sargassum latifolium	Cu, Pb & Cd	–NO_2^+, –COOH, –OH, C–H & C–OH	Ibrahim (2016)
Cladophora glomerata	Cr, Mn & Mg	–OH, –NH, P=O or –SO_3^-	Michalak et al. (2018)
Merismopedia tenuissima	Cd & Cu	–OH, –NH_2, –CH, C=O, COO–, C– O, –COO, –COOH, C=O of polysaccharides	Fawzy (2016)
Acanthophora spicifera	Cr, Hg, Cd & Pb	–NH_2, –COOH, –O–SO_3^- & –OH	Tamilselvan et al. (2011)
Padina tetrastromatica	Cd	–NH_2, C–H, C–O, C–O–P, C–C, C–O–C, P=O	D'Souza et al. (2008)
Ceramium virgatum	Pb, Cd	–OH, –NH_2, –CH, C–OH & –COOH, P=O	Hannachi (2012)
Cheatomorpha antennina	Cu	–NH_2, –OH, –NO2, CO_3^{2-}	Lahari et al. (2013)
Ulva lactuca	Cd	CH3–, NH2$^+$–CH_2CH_3, C_6H_6, –$CONH_2$, –C=C–, C–OH & C_6H_5–OH	Saleh (2018)
Padina pavonica	Cd	–COOH, –$CONH_2$, C–OH & C_6H_5OH	
Sargassum fluitans	Cd	–C=O, –COOH & –SO_3^-	Fourest and Volesky (1996)
Ulva lactuca, Jania rubens, Pterocladia capillacea a, Colpomenia sinosa	Pb, Cd & Ni	–OH, –NH_2, P=O, C–O & C=O	Ibrahim et al. (2018)
Marine algae and freshwater algae biomass	Cu, Pb, Zn & Cd	–OH, $(C_{31}H_{34}O_{11})_n$, CH_3CO, C–H, C–C, C–O, and C–N	Utomo et al. (2016)
Microalgae			
Chlorella vulgaris	Tl & Cd	–COOH & –NH_2	Birungi and Chirwa (2017)
Scenedesmus acuminutus	Tl & Cd	–NH_2^+ &– NH_2^+	

(continued)

Table 13.3 (continued)

Algae biomass	Heavy metal	Functional groups	References
Chlorella sp.	Pb	–OH, –C=O, –COOH, –P=O	Li et al. (2017)
Chlorella sorokiniana	Pb	–OH, –COOH, –NH_2, P=O	Liang et al. (2017)
Mixed microalgae adsorbents Oscillatoria princeps (92%), *Spirogyra aequinoctialis* (3%), *Oscillatoria subbrevis* (2%), *Oscillatoria formosa* (1%), and others (1%).	Pb, Cd, As & Cu	–OH, –NH_2, –COOH or–COO–, –COO^-, –SO_3^-, COO– & P–O–C	Sulaymon et al. (2013)
Oscillatoria, Botryococcus, Scenedesmus and *Spirulina platensis*	Zn, Cu & Ni	–OH, –NH_2, –COOH, P=O & –SO_2^-	Chen et al. (2016)
Spirogyra sp.	Cd, Cr, Cu & Pb	O–H, C–OH/C_6H_5–OH, N–H, –COOH, C=N, C=O, C=C, S=O, CH_3, N–O, C–N, $NH2^+$–CH_2CH_3, –C≡C–H: C–H, –C≡C– & CH3–	Matei et al. (2015)
Spirogyra sp.	Cu	O–H, N–H, C=O, –NH_2, –COO, S=O	Al-Qodah et al. (2017)

symmetric vibrational COO– frequencies (plausibly attributed to that of terminal amino acid) was noted (Lin et al. 2005). Also Han et al. (2007, 2008) had proposed the participation of the carboxylate group in complexation and sequestration of Cr (III), generated post bio-reduction of Cr(VI) by the microalgal isolate, *Chlorella miniata*. The peaks at 1260 and 1034 cm^{-1} corresponded to P=O and C–O bonds of polysaccharides, respectively (Benning et al. 2004). The role of C–O bond in microalgal polysaccharides for biosorption of chromium (Han et al. 2007, 2008) and grape-stalk lignin for adsorption of copper and nickel ions (Villaescusa et al. 2004) have proposed previously. Post Cr(VI) treatment, the intensity of the peak in the region 3200–3500 cm^{-1} appeared augmented, suggesting a rise in the number of the free hydroxyl groups on the microalgal biomass. This perhaps bracketed together with the possible hydrolysis of the microalgal polysaccharides to smaller saccharides (oligo-, di-, mono-saccharides) due to the low pH used in the protocol (Han et al. 2007). Furthermore, the appearance of a new peak at 944 cm^{-1} in the spectrum for metal treated biomass was indicative of the presence of metal–O bond (Holman et al. 1999). The FTIR spectra were indicative of the complexation of the heavy metal with electronegative functional groups of amino, carboxyl, hydroxyl, and carbonyl groups in the microalgal biosorbents.

12 Future Aspects

So far, most of the research has been done using the batch experiment but with limited application in pilot-scale studies in the phycoremediation of heavy metals. The pilot-scale data will be important data at the real application in industrial scale. Different types of algae biosorbents with or without modification procedures have tested for the effective removal of heavy metal ions from the contaminated environment. It provides higher adsorption capacity, low cost but low regeneration ability. Therefore, more attention should give to improving regeneration ability. This attempt has been prepared to immobilize algae biomass to ensure their potential to reuse. However, need more studies on a large scale. Further research needs to be done in the future on the development and enhancement of the adsorption capacity of the algae sorbent material through selective surface modification procedures.

13 Conclusion

This review concluded that the importance of algal biomass for heavy metal removal from the contaminated environment. The phycoremediation has advantages over conventional and physio chemical methods in terms of cost, efficiency, hazards nature, versatility, scale, appealing, and sustainability. Moreover, algae possess numerous mechanisms (adsorption, absorption, precipitation, complex formation, coagulation), and anionic functional molecules to remove heavy metal ions and hence viewed as promising biosorbents. Algae biomass used in live or dead form for the process, while dead biomass is superior over live in terms of time, recovery, and reuse. Different algae have different potential in removing heavy metal; therefore need to select the most appropriate algal biosorbents and favorable physical conditions for the specific heavy metal and the mixture depends on the medium contamination. Further development of innovative and applicable techniques is required to overcome existing problems related to the phycoremdaition such as large-scale data, strain improvement (molecular level), reuse, and disposal of used biomass.

Acknowledgments The support of the Directorate of Research and Technology Transfer, Addis Ababa Science and Technology University, in funding Internal Research Grant (Code No. ICA 04/2011) to Dr. A. Suresh is gratefully acknowledged.

References

Al-Qodah Z, Al-Shannag M, Amro A, Bob M, Bani-Melhem K, Alkasrawi M (2017) Impact of surface modification of green algal biomass by phosphorylation on the removal of copper (II) ions from water. Turk J Chem 41:190–208. https://doi.org/10.3906/kim-1605-38

Anastopoulos I, Kyzas GZ (2015) Progress in batch biosorption of heavy metals onto algae. J Mol Liq 209:77–86. https://doi.org/10.1016/j.molliq.2015.05.023

Arıca MY, Tüzün İ, Yalçın E, İnce Ö, Bayramoğlu G (2005) Utilisation of native, heat and acid treated microalgae Chlamydomonas reinhardtii preparations for biosorption of Cr (VI) ions. Process Biochem 40:2351–2358. https://doi.org/10.1016/j.procbio.2004.09.008

Bai RS, Abraham TE (2002) Studies on enhancement of Cr (VI) biosorption by chemically modified biomass of Rhizopus nigricans. Water Res 36(5):1224–1236. https://doi.org/10.1016/s0043-1354(01)00330-x

Bansal A, Shinde O, Sarkar S (2018) Industrial wastewater treatment using phycoremediation technologies and co-production of value-added products. J Bioremed Biodegr 9:01. https://doi.org/10.4172/2155-6199.1000428

Belattmania Z, Tahiri S, Zrid R, Reani A, Elatouani S, Loukili H, Hassouani M, El Krati M, Bentiss F, Sabour B (2015) Bioremoval of hexavalent chromium from aqueous solutions by the brown seaweed *Dictyopteris polypodioides*. Res J Environ Toxicol 9:218–230. https://doi.org/10.3923/rjet.2015

Benning LG, Phoenix VR, Yee N, Tobin MJ (2004) Molecular characterization of cyanobacterial silicification using synchrotron infrared micro-spectroscopy. Geochim Cosmochim Acta 68 (4):729–741. https://doi.org/10.1016/S0016-7037(03)00489-7

Bilal M, Rasheed T, Sosa-Hernández JE, Raza A, Nabeel F, Iqbal H (2018) Biosorption: an interplay between marine algae and potentially toxic elements – a review. Mar Drug 16:65. https://doi.org/10.3390/md16020065

Birungi ZS, Chirwa EMN (2017) Bioreduction of thallium and cadmium toxicity from industrial wastewater using microalgae. Chem Eng Trans 57:1183–1188. http://hdl.handle.net/2263/63986

Birungi ZS, Chirwa E (2018) Bioremediation of toxic metals and recovery of target metals from actual wastewater using algal sorbents. Chem Eng Trans 64:535–540. https://doi.org/10.3303/CET1864090

Boddu VM, Abburi K, Talbott JL, Smith ED (2003) Removal of hexavalent chromium from wastewater using a new composite chitosan biosorbent. Environ Sci Technol 37 (19):4449–4456. https://doi.org/10.1021/es021013a

Brahmbhatt N, Patel R, Jasrai RT (2013) Heavy metal accumulation in *Oscillatoria sp.* induced a biochemical response. Adv Appl Sci Res 4:182–185. https://pdfs.semanticscholar.org/7bd4/27a7f94eb9759a542644139353838a2c2e0d.pdf

Brinza L, Dring MJ, Gavrilescu M (2007) Marine micro and macroalgal species as biosorbents for heavy metals. Environ Eng Manag J 6:237–251

Cabatingan LK, Agapay RC, Rakels JL, Ottens M, van der Wielen LA (2001) Potential of biosorption for the recovery of chromate in industrial wastewaters. Ind Eng Chem Res 40 (2302):2309. https://doi.org/10.1021/ie0008575

Chen CY, Chang HW, Kao PC, Pan JL, Chang JS (2012) Biosorption of cadmium by CO2 fixing microalga *Scenedesmus obliquus* CNW-N. Bioresour Technol 105:74–80. https://doi.org/10.1016/j.biortech.2011.11.124

Chen F, Chen BD, Gan GJ, Zhang L, Liang Y, Wu SK (2016) Biosorption of Zn2+ and Ni2+, Cu2+ by four kinds of active microalgae. J Mater Sci Chem Eng 4:12–19. https://doi.org/10.4236/msce.2016.411002

Chu WL (2012) Biotechnological applications of microalgae. IeJSME 6:24–37. https://core.ac.uk/download/pdf/25811683.pdf

Das C, Ramaiah N, Pereira E, Naseera K (2018) Efficient bioremediation of tannery wastewater by monostrains and consortium of marine Chlorella sp. and Phormidium sp. Int J Phytoremediation 20(3):284–292. https://doi.org/10.1080/15226514.2017.1374338

D'Souza L, Devi P, Shridhar PDM, Naik CG (2008) Use of Fourier transform infrared (FTIR) spectroscopy to study cadmium-induced changes in Padina. Anal Chem Insight 91:135–143. https://doi.org/10.4137/117739010800300001

Deng S, Ting YP (2005) Characterization of PEI-modified biomass and biosorption of Cu (II), Pb (II) and Ni (II). Water Res 39(10):2167–2177. https://doi.org/10.1016/j.watres.2005.03.033

Dönmez GC, Aksu Z, Öztürk A, Kutsal T (1999) A comparative study on heavy metal biosorption characteristics of some algae. Process Biochem 34:885–892. https://doi.org/10.1016/S0032-9592(99)00005-9

Duffus JH (2002) Heavy metals – a meaningless term? Pure Appl Chem 74:793–807. https://doi.org/10.1351/pac200274050793

Ege A, Doner G (2013) A new biosorbent for the removal of Cu (II) from aqueous solution; red marine alga, *Ceramium rubrum*. In: E3S Web of conferences, 1, 25009. EDP Sciences. https://doi.org/10.1051/e3sconf/20130125009

El Atouani S, Tahiri S, Reani A, Bentiss F, Krati ME, Sahibed-Dine A, Schamel A, Aarfane A, Sabour B (2016) Hexavalent chromium uptake from aqueous solutions using raw biomass of the invasive brown seaweed *Sargassum muticum* from the Moroccan shorelines: kinetics and isotherms. Eur Sci J 12:243–262. http://hdl.handle.net/20.500.12210/8897

Fawzy MA (2016) Phycoremediation and adsorption isotherms of cadmium and copper ions by *Merismopedia tenuissima* and their effect on growth and metabolism. Environ Toxicol Pharm 46:116–121. https://doi.org/10.1016/j.etap.2016.07.008

Fourest E, Volesky B (1996) Contribution of sulfonate groups and alginate to heavy metal biosorption by the dry biomass of *Sargassum fluitans*. Environ Sci Technol 30:277–282. https://doi.org/10.1021/es950315s

García-García JD, Sánchez-Thomas R, Moreno-Sánchez R (2016) Bio-recovery of non-essential heavy metals by intra- and extracellular mechanisms in free-living microorganisms. Biotechnol Adv 34:859–873. https://doi.org/10.1016/j.biotechadv.2016.05.003

Ghoneim MM, El-Desoky HS, El-Moselhy KM, Amer A, Abou El-Naga EH, Mohamedein LI, Al-Prol AE (2014) Removal of cadmium from aqueous solution using marine green algae, *Ulva lactuca*. Egypt J Aquat Res 40:235–242. https://doi.org/10.1016/j.ejar.2014.08.005

Gupta VK, Rastogi A (2009) Biosorption of hexavalent chromium by raw and acid-treated green alga *Oedogonium hatei* from aqueous solutions. J Hazard Mater 163:396–402. https://doi.org/10.1016/j.jhazmat.2008.06.104

Hackbarth FV, Maass D, de Souza AAU, Vilar VJP, de Souza SMAGU (2016) Removal of hexavalent chromium from electroplating wastewaters using marine macroalga *Pelvetia canaliculata* as the natural electron donor. Chem Eng J 290:477–489. https://doi.org/10.1016/j.cej.2016.01.070

Han X, Wong YS, Wong MH, Tam NFY (2007) Biosorption and bioreduction of Cr(VI) by a microalgal isolate, *Chlorella miniata*. J Hazard Mater 146:65–72. https://doi.org/10.1016/j.jhazmat.2006.11.053

Han X, Wong YS, Wong MH, Tam NFY (2008) Feasibility of using microalgal biomass cultured in domestic wastewater for the removal of chromium pollutants. Water Environ Res 80 (7):647–653. https://doi.org/10.1002/j.1554-7531.2008.tb00351.x

Hannachi Y (2012) Characterization of the biosorption of lead and cadmium with the red alga (*Ceramium virgatum*). Holist Approach Environ 2(3):93–109. https://hrcak.srce.hr/86217

Holman HYN, Perry DL, Martin MC, Lamble GM, McKinney WR, Hunter-Cevera JC (1999) Real-time characterization of biogeochemical reduction of Cr (VI) on basalt surfaces by SR FTIR imaging. Geomicrobiol J 16(4):307–324. https://doi.org/10.1080/014904599270569

Ibrahim WM, Hassan AF, Azab YA (2016) Biosorption of toxic heavy metals from aqueous solution by *Ulva lactuca* activated carbon. Egypt J Basic Appl Sci 3:241–249. https://doi.org/10.1016/j.ejbas.2016.07.005

Ibrahim WM, Abdel AY, Hamdy SM, Gad NS (2018) Comparative study for biosorption of heavy metals from synthetic wastewater by different types of marine algae. J Bioremed Biodegr 9:01. https://doi.org/10.4172/2155-6199.1000425

Indhumathi P, Syed Shabudeen PS, Shoba US, Saraswathy CP (2014) The removal of chromium from aqueous solution by using green microalgae. J Chem Pharm Res 6:799–808

Jaafari J, Yaghmaeian K (2019) Optimization of heavy metal biosorption onto freshwater algae (*Chlorella coloniales*) using response surface methodology (RSM). Chemosphere 217:447–455. https://doi.org/10.1016/j.chemosphere.2018.10.205

Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN (2014) Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol 7:60–72. https://doi.org/10.2478/intox-2014-0009

Javanbakht V, Alavi SM, Zilouei H (2014) Mechanisms of heavy metal removal using microorganisms as biosorbent. Water Sci Technol 69:1775–1787. https://doi.org/10.2166/wst.2013.718

Kanamarlapudi SLRK, Chintalpudi VK, Muddada S (2018) Application of biosorption for removal of heavy metals from wastewater. Biosorption 18:69. https://doi.org/10.5772/intechopen.77315

Kaplan D (2013) Absorption and adsorption of heavy metals by microalgae. Handbook of microalgal culture. Appl Phyco Biotech 2:602–611. http://www.bashanfoundation.org/contributors/retired/Kaplan-D/droraabsorption.pdf

Kavitha G, Sridevi V, Venkateswarlu P, Babu NC (2016) Biosorption of chromium from aqueous solution by *Gracilaria corticata* (red algae) and it's statistical analysis using response surface methodology. OALib 3:1–32. https://doi.org/10.4236/oalib.1102968

Kleinübing SJ, Vieira RS, Beppu MM, Guibal E, Silva MGCD (2010) Characterization and evaluation of copper and nickel biosorption on acidic algae *Sargassum filipendula*. Mater Res 13:541–550. https://doi.org/10.1590/S1516-14392010000400018

Kumar R, Bishnoi NR, Garima N, Bishnoi K (2008) Biosorption of chromium(VI) from aqueous solution and electroplating wastewater using fungal biomass. Chem Eng J 135:202–208. https://doi.org/10.1016/j.cej.2007.03.004

Kumar KS, Dahms H, Won E, Lee J, Shin K (2015) Microalgae – a promising tool for heavy metal remediation. Ecotoxicol Environ Saf 113:329–352. https://doi.org/10.1016/j.ecoenv.2014.12.019

Lahari SB, King P, Prasad VSRK (2013) Biosorption of copper by *Chaetomorpha Antennina* algae biomass thermodynamic studies. Chem Tech 8:109–114

Li Y, Xia L, Huang R, Xia C, Song S (2017) Algal biomass from the stable growth phase as a potential biosorbent for Pb (I) removal from water. RSC Adv 7:34600–34608. https://doi.org/10.1039/C7RA06749F

Liang S, Kang Y, Zeng L, Qin Y, Wang L, Zhang Q, Luo J (2017) How *Chlorella sorokiniana* and its high tolerance to Pb might be a potential Pb biosorbent. Pol J Environ Stud 26:1139–1146. https://doi.org/10.15244/pjoes/67532

Lin K, Simossis VA, Taylor WR, Heringa J (2005) A simple and fast secondary structure prediction method using hidden neural networks. Bioinformatics 21(2):152–159. https://doi.org/10.1093/bioinformatics/bth487

Liu D, Fu Y, Sun H (2016) A review on researches of interaction between heavy metals and microalgae in water. In: 2016 international conference on Civil, Transportation and Environment. Atlantis Press. https://doi.org/10.2991/iccte-16.2016.113

Lodi A, Soletto D, Solisio C, Converti A (2008) Chromium (III) removal by *Spirulina platensis* biomass. Chem Eng J 136:151–155. https://doi.org/10.1016/j.cej.2007.03.032

Mahmood Z, Zahra S, Iqbal M, Raza MA, Nasir S (2017) Comparative study of natural and modified biomass of Sargassum sp. for removal of Cd 2+ and Zn 2+ from wastewater. Appl Water Sci 7(7):3469–3481. https://doi.org/10.1007/s13201-017-0624-3

Mann RM, Vijver MG, Peijnenburg WJGM (2011) Metals and metalloids in terrestrial systems: bioaccumulation, biomagnification and subsequent adverse effects. In: Ecological impacts of toxic chemicals. Bentham Science Publishers, pp 43–62. https://doi.org/10.2174/978160805121211101010043

Matei GM, Kiptoo JK, Oyaro N, Onditi AO (2015) Biosorption of selected heavy metals by green algae, *Spirogyra* species and its potential as a pollution biomonitor. Chem Mater Res 7:42–52. https://pdfs.semanticscholar.org/410e/7a910ae3c7e0901e5b0d1c5730e009909496.pdf

Matheickal JT, Yu Q (1999) Biosorption of lead(II) and copper(II) from aqueous solutions by pre-treated biomass of Australian marine algae. Bioresour Technol 69:223–229. https://doi.org/10.1016/S0960-8524(98)00196-5

Mehta SK, Gaur JP (2005) Use of algae for removing heavy metal ions from wastewater: progress and prospects. Crit Rev Biotechnol 25:113–152. https://doi.org/10.1080/07388550500248571

Mera R, Torres E, Abalde J (2014) Sulfate, more than a nutrient protects the microalga *Chlamydomonas moewusii* from cadmium toxicity. Aqua Toxicol 148:92–103. https://doi.org/10.1016/j.aquatox.2013.12.034

Michalak I, Mironiuk M, Marycz K (2018) A comprehensive analysis of biosorption of metal ions by macroalgae using ICP-OES, SEM-EDX, and FTIR techniques. PLoS One 13:1–20. https://doi.org/10.1371/journal.pone.0205590

Monteiro CM, Castro PML, Malcata FX (2012) Metal uptake by microalgae: underlying mechanisms and practical applications. Biotechnol Prog 28:299–311. https://doi.org/10.1002/btpr.1504

Naorbe MC, Serrano AE Jr (2018) Effects of heavy metals on cell density, size, specific growth rate and chlorophyll-a of *Tetraselmis tetrathele* under controlled laboratory conditions. AACL Bioflux 11:589–597. http://www.bioflux.com.ro/docs/2018.589-597.pdf

Onalo JI, Matias-Peralta HM, Sunar NM (2014) Growth of freshwater microalga, *Botryococcus* sp. in heavy metal contaminated industrial wastewater. J Sci Technol 6:29–40. http://eprints.uthm.edu.my/id/eprint/8232

Packer M (2009) Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy. Energy Policy 37 (3428):3437. https://doi.org/10.1016/j.enpol.2008.12.025

Peña-Salamanca EJ, Rengifo-Gallego AL, Benitez-Campo N (2011) Detoxification mechanisms of heavy metals by algal-bacteria consortia. In: Handbook of marine macroalgae, pp 441–450

Prasher SO, Beaugeard M, Hawari J, Bera P, Patel RM, Kim SH (2004) Biosorption of heavy metals by red algae (*Palmaria palmata*). Environ Technol 25:1097–1106. https://doi.org/10.1080/09593332508618378

Raize O, Argaman Y, Yannai S (2004) Mechanisms of biosorption of different heavy metals by brown marine macroalgae. Biotechnol Bioeng 87:451–458. https://doi.org/10.1002/bit.20136

Saleh B (2018) Recovery of cadmium (Cd) biosorption by *Ulva lactuca* (Chlorophyceae) and *Padina pavonica* (Phaeophyceae) biomass. J Bio Innov 7(5):678–688. https://pdfs.semanticscholar.org/3dfb/427fbb4578f4a417c8cd57f46f08864a1f3e.pdf

Saravanan A, Kumar PS, Yashwanthraj M (2017) Sequestration of toxic Cr (VI) ions from industrial wastewater using waste biomass: a review. Desalin Water Treat 68:245–266

Saurav K, Kannabiran K (2011) Biosorption of Cr(III) and Cr(VI) by *Streptomyces* VITSVK9 spp. Ann Microbiol 61:833–841. https://doi.org/10.1007/s13213-011-0204-y

Sbihi K, Cherifi O, El Gharmali A, Oudra AB, Aziz F (2012) Accumulation and toxicological effects of cadmium, copper, and zinc on the growth and photosynthesis of the freshwater diatom *Planothidium lanceolatum* (Brébisson) Lange-Bertalot: a laboratory study. J Mater Environ Sci 3:497–506. https://www.jmaterenvironsci.com/Document/vol3/vol3_N3/49-JMES-178-2012-Sbihi.pdf

Sen S, Nandi S, Dutta S (2018) Application of RSM and ANN for optimization and modeling of biosorption of chromium(VI) using cyanobacterial biomass. Appl Water Sci 8:148. https://doi.org/10.1007/s13201-018-0790-y

Sheng PX, Tan LH, Chen JP, Ting Y (2005) Biosorption performance of two brown marine algae for removal of Chromium and Cadmium. J Dispers Sci Technol 25:679–686. https://doi.org/10.1081/DIS-200027327

Shilpi G, Shilpi S, Sharma S (2014) Tolerance against heavy metal toxicity in cyanobacteria: role of antioxidant defense system. Int J Pharm Pharm Sci 7:9–16. https://pdfs.semanticscholar.org/85e4/7b8671c05b077306c9a563dbae2de56e0df7.pdf

Soleymani F, Pahlevanzadeh H, Khani MH, Manteghian M (2014) Biosorption of cobalt (II) by intact and chemically modified brown algae: optimization using response surface methodology and equilibrium, dynamics and thermodynamics studies. Iran J Chem Eng 11(56):77. http://www.ijche.com/article_10203_098516bff78f41cf789b37942401f547.pdf

Sulaymon AH, Mohammed AA, Al-Musawi TJ (2013) Column biosorption of lead, cadmium, copper, and arsenic ions onto algae. J Bioproces Biotech 3:128. https://doi.org/10.4172/2155-9821.1000128

Suman D (2012) Biosorption of chromium and nickel by dried biomass of cyanobacterium *Oscillatoria laete-virens*. Int J Environ Sci 3:341–352. https://doi.org/10.6088/ijes.2012030131032

Suresh A, Benor S 2020 Microalgae-based biomass production for control of air pollutants. In: From biofiltration to promising options in gaseous fluxes biotreatment, 2020 January 1. Elsevier, pp 345–372

Suresh A, Tamilvanan S, Harini K et al (2019) Feasibility of cattle urine as nutrient medium for the microalgal biomass production. Globl J Environ Sci Manag 5(4):441–448. https://doi.org/10.22034/GJESM.2019.04.04

Sutkowy M, Kłosowski G (2018) Use of the coenobial green algae Pseudopediastrum boryanum (Chlorophyceae) to remove hexavalent chromium from contaminated aquatic ecosystems and industrial wastewaters. Water 10(6):712. https://doi.org/10.3390/w10060712

Sziválk I, Behra R, Sigg L (2009) Metal-induced reactive oxygen species production in *Chlamydomonas reinhardtii* (Chlorophyceae). J Phycol 45:427–435. https://doi.org/10.1111/j.1529-8817.2009.00663.x

Takeda H (1996) Cell wall sugars of some Scenedesmus species. Phytochemistry 42(3):673–675. https://doi.org/10.1016/0031-9422(95)00952-3

Tamilselvan N, Saurav K, Kannabiran K (2011) Biosorption of selected toxic heavy metals using algal species *Acathopora spicefera*. Pharmacology 1:518–528. https://www.researchgate.net/publication/207043961

Tüzün İ, Bayramoğlu G, Yalçın E, Başaran G, Çelik G, Arıca MY (2005) Equilibrium and kinetic studies on biosorption of Hg(II), Cd(II) and Pb(II) ions onto microalgae *Chlamydomonas reinhardtii*. J Environ Manag 77:85–92. https://doi.org/10.1016/j.jenvman.2005.01.028

Ucun H, Bayhan YK, Kaya Y, Cakici A, Algur OF (2002) Biosorption of chromium (VI) from aqueous solution by cone biomass of *Pinus sylvestris*. Bioresour Technol 85(2):155–158. https://doi.org/10.1016/S0960-8524(02)00086-X

United State Environmental Protection Agency (USEPA) (2020). https://www.epa.gov/groundwater-and-drinking-water/national-primary-drinking-water-regulations

Utomo HD, Tan KXD, Choong ZYD, Yu JJ, Ong JJ, Lim ZB (2016) Biosorption of heavy metal by algae biomass in surface water. J Environ Prot 7(11):1547–1560. https://doi.org/10.4236/jep.2016.711128

Villaescusa I, Fiol N, Martinez M, Miralles N, Poch J, Serarols J (2004) Removal of copper and nickel ions from aqueous solutions by grape stalks wastes. Water Res 38(4):992–1002. https://doi.org/10.1016/j.watres.2003.10.040

Wu J, Ma LL, Zeng RJ (2018) Role of extracellular polymeric substances in efficient chromium (VI) removal by algae-based Fe/C nano-composite. Chemosphere 211:608–616. https://doi.org/10.1016/j.chemosphere.2018.07.186

Yaqub A, Mughal MS, Adnan A, Khan WA, Anjum KM (2012) Biosorption of hexavalent chromium by *Spirogyra* spp.: equilibrium, kinetics, and thermodynamics. J Anim Plant Sci 22:408–415

Yee N, Benning LG, Phoenix R, Ferris FG (2004) Characterization of metal− cyanobacteria sorption reactions: a combined macroscopic and infrared spectroscopic investigation. Environ Sci Technol 38(3):775–782. https://doi.org/10.1021/es0346680

Zeraatkar AK, Ahmadzadeh H, Talebi AF, Moheimani NR, McHenry MP (2016) The potential use of algae for heavy metal bioremediation, a critical review. J Environ Manag 181:817–831. https://doi.org/10.1016/j.jenvman.2016.06.059

Chapter 14
Heavy Metal Extraction from E-Waste Through Bioleaching: A Promising Ecofriendly Approach

S. Venkatesa Prabhu, N. R. Srinivasan, and H. Sintayehu Mekuria

Abstract E-waste refers to waste from electronic devices and electrical appliances that are widely discarded. It includes televisions, washing machines, refrigerators, air conditioners, printers, cell phones, computers, and monitors. Currently, increasing technological developments reduce the lifespan of electronic and electrical equipment. Hence, much more e-waste is being produced. Based on recent statistics, the worldwide production of e-waste is increasing at a rate of 4.6% annually, and it is expected to reach about 52.2 metric tons in 2021. Its processing produces a sludge that is a great threat to the environment since it contains numerous toxic heavy metals. For environmental protection, physical methods have been extensively implemented by some enterprises that dismantle e-waste. The alkali and acid reagents used to extract metals from e-waste are quite costly. However, research communities globally are focusing on bio-hydrometallurgical techniques to treat e-waste for ecofriendly treatment and safe disposal. Although several treatment techniques are applied to leach heavy metals from e-wastes, this chapter focuses on bacterial leaching, colloquially called bioleaching. This method is proven and can be effectively applied for leaching heavy metals from e-waste. Three sections are developed in this chapter. First, the sources of various e-wastes and their heavy metal contents are examined. Second, the specific microorganisms that can be applied for bioleaching processes are discussed. Last, the optimal conditions, bioleaching mechanisms, and influences are presented.

Keywords Acidophilic bacteria · Bioleaching · Bio-solubilization · Detoxification · E-wastes · Heavy metals

---

S. Venkatesa Prabhu (✉) · N. R. Srinivasan · H. Sintayehu Mekuria
Department of Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_14

1 Introduction

Discarded electronics and electrical appliances are generally known as e-waste. They are mostly comprised of printers, refrigerators, washing machines, air conditioners, computers, televisions, copiers, and cell phones (Ilyas et al. 2007). Recently, rapid advances in science and technology have significantly shortened the lifespan of electronic and electrical equipment since consumers are eager to replace their current equipment with newer models having improved features (Okibe and Johnson 2004). Hence, huge quantities of electronics and electrical appliances are being discarded. Global e-waste production was 47 million tons in 2016, and it is increasing by 4.6% annually. It is estimated that discarded e-waste may reach 52.2 million tons in 2021. Owing to vast production of e-waste, various methodologies are being developed to address this issue (Bhattacharyya et al. 2013).

Generally, in hydrometallurgical processes, waste water is concurrently produced, which becomes e-waste sludge (Natarajan 1992). This kind of e-waste sludge is comprised of various toxic heavy metals. So far, based on ecofriendly environmental approaches, physical methods have been extensively utilized by enterprises that dismantle discarded electronics. However, researchers are globally concentrating on hydrometallurgical techniques for safe disposal of e-waste that eventually create e-waste sludge (Cao et al. 2012).

Improper treatment of e-waste sludge in turn generates secondary pollutants that contaminate groundwater and soil and even risk human health through enrichment in the food chain (Gu et al. 2017). In general, because of the heavy metal composition in the e-waste sludge, it is often identical to that of sewage sludge; some of the techniques that are applied to dispose of sewage sludge can also be employed to treat e-waste sludge (Kimura et al. 2011). Typically, e-waste sludge disposal is done through recycling, producing harmless effluents. Figure 14.1 shows the common technologies used for e-waste sludge treatment. These treatments include stabilization, incineration, solidification, and disposal in landfills (Gehrke et al. 1998). Recycling techniques used are hydrometallurgy, pyrometallurgy, and materialization (Hong and Valix 2014). Curing agents are used in stabilization and solidification of e-waste sludge containing heavy metals in a mixture substance, eventually producing non-toxic materials (Druschel et al. 2004).

Oxidation and fusion processes are carried out during incineration. In this method, the toxic elements present inside the e-waste sludge will be destroyed during thermal treatment (Roberts et al. 2002). Generally, to mitigate emissions of toxic elements, stabilization and solidification methods are used for treating e-waste sludge. The heavy metals present in e-waste sludge make it inappropriate for application to farm land. Hence, landfill methods are extensively implemented, in China, for example (Gurung and Chakraborty 2009). Owing to its lower secondary pollution and cost, hydrometallurgy techniques are more widely employed than pyrometallurgical methods.

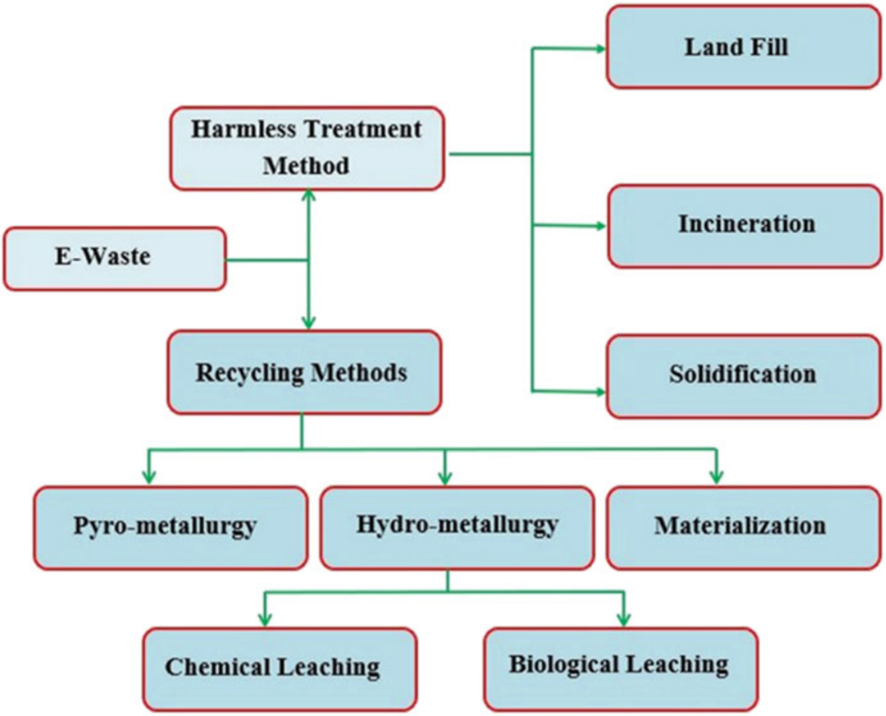


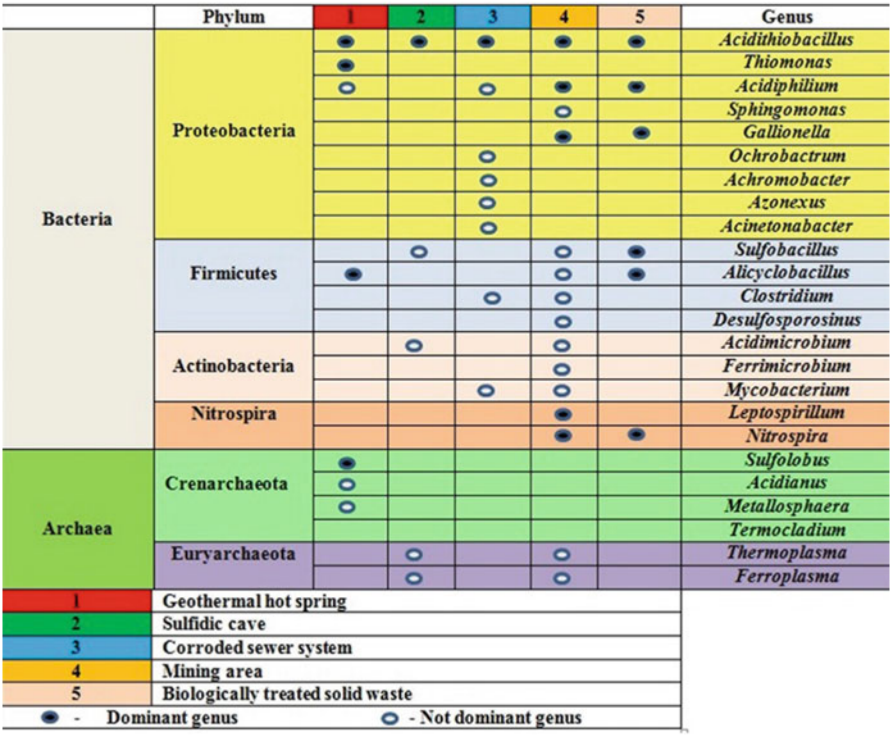
Fig. 14.1 Common technologies for e-waste sludge treatment

Microbial and chemical leaching processes are the most widely adopted leaching techniques in hydrometallurgy. However, owing to its formation of toxic elements, chemical leaching is not recommended (Sun et al. 2013). Furthermore, chemical leaching generates huge amounts of sludge. Microbial leaching, colloquially called bioleaching, is a group of techniques that refer to the use of direct or/and indirect influences of various microorganisms to remove metals from waste sludge (Cui and Zhang 2008). The metals present in this sludge can be extracted by reactions that include adsorption, exchange, oxidation, reduction, and dissolution. Microbial leaching methods are emerging technologies. They are extensively adopted for treatment of mining wastes, e-waste, and sewage sludge (Quatrini and Johnson 2018). The US Environmental Protection Agency suggested its own guideline process for disposing the harmful sludge. In China, widespread application of this sludge technology is not seen. Generally, solidification and landfill disposal are commonly employed. Recently, various investigators have done research on the materialization and thermal treatment of different types of sludge (Gu and Wong 2004). The German government encourages sludge recycling and minimization, and combinations of the above methods are implemented. However, there is not enough information focusing on the e-waste sludge treatment.

This chapter describes bioleaching techniques for removing heavy metals from the sludge generated by e-waste treatment. Specifically, it considers the sources of various e-waste sludges and their heavy metal contents, the microbial species involved in bioleaching, the conditions and optimization for bioleaching, and mechanisms of bioleaching. Related effects are discussed.

2 Bioleaching and Extremophiles

Both pyrometallurgy and hydrometallurgy are favorable for efficient and rapid and treatment of metal-bearing sludge. These processes use highly acidic environments and are done at elevated temperatures (Ilyas et al. 2013). The natural habitats of acidophilic microorganisms that may be used in such processes are given in Fig. 14.2. However, these methods have various environmental issues. Some produce toxic pollutants such as dioxins, highly acidic water, and furans (Grengg et al. 2015). Extremophile microorganisms have been widely used to extract the various valuable metals from e-waste through an economical and ecofriendly approach



	Phylum	1	2	3	4	5	Genus
Bacteria	Proteobacteria	●	●	●	●	●	*Acidithiobacillus*
		●					*Thiomonas*
		○		○	●	●	*Acidiphilium*
					○		*Sphingomonas*
					●	●	*Gallionella*
				○			*Ochrobactrum*
				○			*Achromobacter*
				○			*Azonexus*
				○			*Acinetonabacter*
	Firmicutes		○		○	●	*Sulfobacillus*
		●			○	●	*Alicyclobacillus*
				○	○		*Clostridium*
					○		*Desulfosporosinus*
	Actinobacteria		○		○		*Acidimicrobium*
					○		*Ferrimicrobium*
				○	○		*Mycobacterium*
	Nitrospira				●		*Leptospirillum*
					●	●	*Nitrospira*
Archaea	Crenarchaeota	●					*Sulfolobus*
		○					*Acidianus*
		○					*Metallosphaera*
							Termocladium
	Euryarchaeota		○		○		*Thermoplasma*
			○		○		*Ferroplasma*

1	Geothermal hot spring
2	Sulfidic cave
3	Corroded sewer system
4	Mining area
5	Biologically treated solid waste

● - Dominant genus ○ - Not dominant genus

Fig. 14.2 The typical distribution of microorganisms at the phylum and genus levels in various acidic environments

(Aarabi et al. 2010). These microorganisms have a peculiar characteristic of oxidizing elemental sulfur (S°) or reduced-sulfur elements, ferrous iron (Fe^{2+}), and pyrite to generate H_2SO_4 and ferric iron (Fe^{3+}) as leaching agents. Using these leaching agents, solid metals can be converted into solubilized ions in an aqueous reaction by the actions of bioleaching microbes (Rawlings 2005). These processes are known as bioleaching (Marra et al. 2018).

Acidithiobacillus ferrooxidans is a potential bioleaching bacterium that was isolated from the drainage of acid mine waste by Colmer et al. in 1947 (Colmer and Hinkle 1947). *A. ferrooxidans* was the first documented bioleaching microbe that could oxidize ferrous iron into ferric iron to extract copper from pyrite. Biological extraction of sulfide minerals was soon established (Zhu et al. 2011). These techniques are sustainable methods that require low cost and little energy while remaining ecofriendly. Presently, bioleaching is not only the only way to extract metals from sulfide minerals (Peng et al. 2012). Instead, it is extensively applied in the recovery of metals from various solid wastes, such as e-wastes, batteries, and catalysts. Recently, several investigations have furthered process development for bioleaching of many valuable metals such as cobalt, vanadium, and indium. A summary of selected extremophile microorganisms for bioleaching applications is given in Table 14.1.

Even though bioleaching has been successful for extracting various sulfide minerals, recovery of valuable metals from e-waste is still challenging (Vera et al. 2013). This is because there are significant differences between e-waste and sulfide minerals based on their chemical and physical properties, as well as their molecular structures (Jowkar et al. 2018). Moreover, e-waste contains harmful elements that may inactivate bioleaching microorganisms. Hence, bioleaching processes applicable to sulfide minerals cannot be simply applied to e-waste. A set of pretreatments are required including sieving, crushing, shredding, and sorting before any bioleaching process (Zhu et al. 2011).

3 Pathways of Bioleaching Processes

From previous research, bioleaching of e-waste using extreme acidophiles can be predominantly divided into pathways mediated by iron (Fe) and reduced sulfur (S), as well as a mixed pathway that is S- and Fe-mediated (Watanabe et al. 2009). Bioleaching mechanisms are unclear and still under investigation (Pathak et al. 2017; Pradhan et al. 2008). For each bioleaching pathway, there are two types of mechanisms, "direct" and "indirect," for treating sulfide minerals (Ziegler et al. 2009). Each bioleaching pathway may have a direct and/or indirect mechanism, but each microorganism will at least have an indirect mechanism (Liu et al. 2011). In the direct mechanism, transfer of electrons occurs directly between the sulfide minerals and the cells of microorganisms. In this case, the cells should be attached at the surface of the minerals (Havlik et al. 2010). Various microbes have been recognized that contribute in direct mechanisms, for example, *A. ferrooxidans*

Table 14.1 Typical acidophilic microorganisms for bioleaching and their energy sources

Microorganism	Electron donor					Optimum temperature range (°C)
	Reduced sulfur compound	S°	Fe^{2+}	Organic mater	pH range	
Iron-oxidizing autotrophs (Sun et al. 2013; Peng et al. 2012; Colmer and Hinkle 1947)						
Leptospirillum ferrooxidans	–	–	+	–	1.5–4.0	25–30
Leptospirillum ferriphilum	–	–	+	–	NA	NA
Ferroplasma acidiphilum	–	–	+	–	1.3–2.2	15–45
Leptospirillum ferrodiazotrophum	–	–	+	–	NA	NA
Ferrovum myxofaciens	–	–	+	–	2.0–3.4	4–37
Sulfur-oxidizing autotrophs (Zhu et al. 2011)						
Acidithiobacillus albertensis	+	+	–	–	2.5–4.5	25–30
Acidithiobacillus thiooxidans	+	+	–	–	0.5–5.5	10–37
Iron- and sulfur-oxidizing autotrophs (Ilyas et al. 2013; Jowkar et al. 2018)						
Acidithiobacillus ferrivorans	+	–	+	–	NA	4.0–5.0
Acidithiobacillus ferrooxidans	+	+	+	–	1.5–4.5	10–37
Acidithiobacillus ferridurans	+	+	+	–	2–3.5	25–29
Acidophilic heterotrophs (Pathak et al. 2017; Baker and Dopson 2007)						
Caldisphaera lagunensis	–	–	–	+	2.3–5.4	45–80
Caldivirga maquilingensis	–	–	–	+	2.3–5.9	60–92
Thermoplasma acidophilum	–	–	–	+	1.9–5.9	20–47
Thermocladium modestius	–	–	–	+	2.6–5.9	45–82
Mixotrophs (Watanabe et al. 2009; Appia et al. 1999)						
Acidimicrobium ferrooxidans	–	–	+	+	2–3	48–50
Acidithiobacillus caldus	+	+	–	+	1.0–3.5	32–52
Acidiphilium acidophilum	+	+	–	+	1.5–6.0	10–35
Ferrimicrobium acidiphilum	–	–	+	+	2–4	34–36
Alicyclobacillus ferrooxydans	+	–	+	+	2.0–6.0	17–60

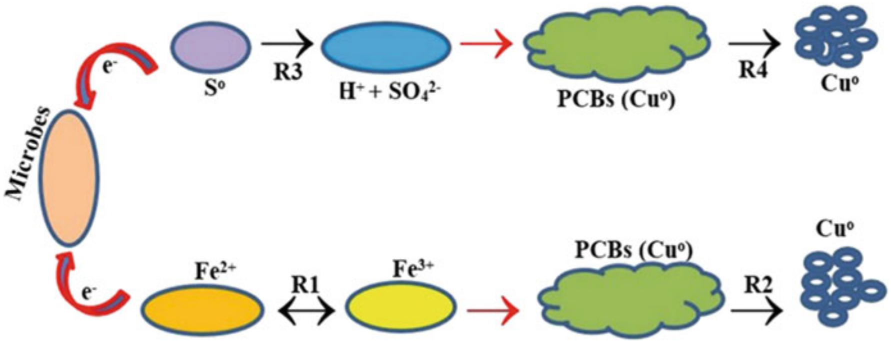
(continued)

Table 14.1 (continued)

	Electron donor					Optimum temperature range (°C)
Microorganism	Reduced sulfur compound	S°	Fe^{2+}	Organic mater	pH range	
Alicyclobacillus tolerans	+	+	+	+	1.5–5.0	20–50
Sulfolobus solfataricus	+	+	–	+	0.9–5.8	55–85
Sulfobacillus acidophilus	+	+	+	+	2.0–6.0	17–40
Acidianusbrierleyi	–	+	+	+	1.0–6.0	45–75
Metallosphaera sedula	+	+	–	+	1.0–4.5	50–80

(Marhual et al. 2008). Formation of an organic film of extracellular polymeric substances (EPS) has a significant influence on mineral-microbe interactions in mediating the reaction between the microorganisms and the surfaces of minerals (Bryner and Anderson 1957). The primary constituents in the EPS are proteins, sugars, redox-active components, and lipids (Elbehti et al. 2000). However, additional research is still required to better understand the direct mechanism for bioleaching (Huo et al. 2015).

In indirect mechanisms of bioleaching, three bioleaching pathways are considered. Indirect bioleaching toward a S-mediated reaction can be accomplished by the oxidation of reduced sulfur catalyzed using sulfur-oxidizing autotrophs or S- and Fe-oxidizing autotrophs to produce sulfuric acid. This results in media acidification producing solubilization of the metals present in e-waste (Gan et al. 2015; Appia et al. 1999). The indirect mechanism, mediated by Fe, includes two steps. In the first step, soluble ferrous iron (Fe^{2+}) present in the reaction medium serves as an electron donor by oxidizing it to ferric iron (Fe^{3+}). This is catalyzed by Fe-oxidizing autotrophs or Fe- and S-oxidizing autotrophs. In the second step, the solid metals are then oxidized by liberated electrons that leads to solubilization. In a mixed bioleaching mechanism, ferrous iron and reduced sulfur act as electron donors. Wang et al. (2009) reported that the synergistic influence of Fe-oxidizing and S-oxidizing autotrophs together has been found quite effective compared to that of the Fe- or S-oxidizing autotrophs alone in bioleaching process. This approach focused on leaching elemental copper (Cu°) from printed circuit boards (PCBs). Various metals exist in different forms in numerous types of e-waste. For instance, copper is present in PCBs in the form of Cu°, and indium is found in liquid crystal displays (LCD) as metal oxides (Vlasceanu et al. 2000; Baker and Dopson 2007). Some researchers speculated that the metals in various forms should have different ideal bioleaching pathways. Cu° recovery from PCBs is the best example (Fig. 14.3). In the case of chalcopyrite bioleaching, chalcopyrite is partially dissolved by chemical means at a low pH. Acidophilic autotrophs generate Fe^{3+} and H_2SO_4 by



$$(R1)\ 4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \text{ (Microbial Catalysis)}$$

$$(R2)\ Fe^{3+} + Cu^{\circ}_{(s)} \rightarrow Fe^{2+} + Cu^{2+} \text{ (Chemical Reaction)}$$

$$(R3)\ 2S^{\circ} + 3O_2 + 2H_2O \rightarrow 4H^+ + 2SO_4^{2-} \text{ (Microbial Catalysis)}$$

$$(R4)\ Cu^{\circ}_{(s)} + 4H^+ + O_2 \rightarrow Cu^{2+} + 2H_2O \text{ (Chemical Reaction)}$$

Fig. 14.3 An indirect bioleaching process for printed circuit boards. The black arrow indicates the product of the reaction. The red arrow refers to the reaction between the leaching agent and the solid waste. The red arced arrow refers the direction of electron transfer between the electron donor and the microbe

oxidizing Fe^{2+} and S°. Furthermore, Fe^{3+} and H_2SO_4 are used to dissolve Cu^{2+} from the chalcopyrite. No additional electron donors are required. Also, these processes can be mediated using a pure or mixed culture of microbes that can exploit both S° and Fe^{2+}. In the bioleaching of vanadium from spent catalyst, vanadium is in the form of a metal oxide. Wang et al. (2018) observed that the influence of the iron-mediated mechanism was pointedly low in vanadium bioleaching. Due to the possibility of thermodynamic interaction, Cu° may react with H^+ and Fe^{3+}. However, the efficiency of Cu bioleaching under an iron-mediated pathway was much higher than that of the sulfur-mediated pathway. Another study, carried out by Wang et al. (2016), showed that a mixed pathway was not particularly effective for the dissolution of Cu°.

Consequently, prior to metal bioleaching from e-waste, the properties and composition of each type of e-waste and the target metals to be leached should be investigated (Haanela and Johnson 2014). These prerequisite studies help to understand the process and choose appropriate bioleaching pathway (Urbieta et al. 2015; Liang et al. 2012). This is especially true of non-acidophilic microorganisms which utilize organic carbon as an energy source but can mobilize some metals from e-waste. For an instance, HCN-forming microbes can mobilize Ag as $[Ag(CN)_2]$ from PCB (Chen et al. 2011).

4 Influencing Factors for Bioleaching Processes

Several investigations have documented that the biomass and activity of the microorganisms associated with bioleaching are two potential factors that significantly influence the rate of oxidation of reduced sulfur substances such as pyrite and Fe^{2+}. The temperature, microbial species, pH, and type of e-waste are the four principal factors that can affect the biomass and microbial activity. Bioleaching is predominantly dominated by microbial communities or functional microbes. Wang et al. (2009) studied bioleaching of Cu from PCBs (with 7.8 g/L of solids loading) using two different microbial species. They observed that *A. ferrooxidans* showed a 99.0% Cu bioleaching efficiency using Fe^{2+} as the electron donor. However, *A. thiooxidans* showed only a 74.9% bioleaching of Cu using S° as the energy source (Baker and Banfield 2003; Macalady et al. 2007). Various experiments on bioleaching showed that these two acidophilic communities had apparent bioleaching capabilities to extract Cu from chalcopyrite (Gonzalez-Toril et al. 2003; Liang et al. 2010). Using pyrite (FeS_2) and S° as the energy sources, a mixed culture of *Sulfobacillus acidophilus* and *Sulfobacillus thermosulfidooxidans* showed bioleaching efficiencies 80%, 82%, 90%, and 85% for Al, Ni, Cu, and Zn, respectively, after 12 days. Another experiment carried out by Ilyas et al. (2013) using a mixed culture of *Thermoplasma acidophilum* and *S. thermosulfidooxidans* showed bioleaching efficiencies of 80% for Zn, 80% for Ni, 85% for Cu, and 75% for Al after 18 days.

Another important parameter, temperature, influences the activities of psychrotolerant, mesophilic, thermophilic, and extreme thermophilic microorganisms (Zhou et al. 2015). Various researchers showed that the efficiency of metal bioleaching was considerably improved using thermophilic microorganisms due to their higher growth temperatures and metal tolerance, as well as their remarkable metabolic characteristics (Hose et al. 2000). Additionally, a high temperature destabilizes the passivation layer caused by the precipitation of S° or Fe^{3+} that coats the surfaces of e-waste and limits the rate of bioleaching (Li and Li 2014; Zhang and Katayama 2012). Various studies have shown that a high pH hinders the growth of acidophilic microorganisms and stimulates Fe^{3+} precipitation. A lower initial pH can produce a more favorable environment for chemical leaching, even though it is not conducive to the metabolism of microbes (Isıldar et al. 2016).

Since e-waste contains hazardous substances, it adversely impacts microbial growth. Hence, the high dosage of the e-waste leads to the lower efficiency in metal bioleaching. Also, a high dosage of solid (e-waste) powder negatively influences the distribution of air and oxygen solubility (Okabe et al. 2007). Xiang et al. (2010) pointed out that the rate of Cu bioleaching from PCBs decreased with an increase in the solid-to-liquid ratio. The toxicity of heavy metal ions increases with the dosage of these toxic solids. This could be an important reason for the reduced efficiency of bioleaching. Since e-waste inhibits the activity and growth of microbes, the bioleaching initiation time, that is, when e-waste is added, could be imperative.

Various studies suggest that when growth of microorganism was separated from the bioleaching process, the time of targeted leaching was reduced (Mishra et al. 2008a). Zhu et al. (2011) observed that the removal of non-metallic elements can alleviate the toxic effects of e-waste on microbial growth. Other influencing factors such as the type of e-waste, initial concentration of electron donors, and inoculum size are not discussed in detail here.

5 Developing Efficient Methods for Bioleaching

5.1 Non-electrochemical Methods

The crucial bottlenecks in bioleaching processes are long reaction times and low efficiency (Wang et al. 2018). These problems hinder practical success. Recently, researchers have suggested some novel methods to improve bioleaching efficiency. Isıldar et al. (2016) suggested a process using a two-stage bioleaching. In this approach, acidophilic bioleaching microorganisms are cultivated in the absence of e-waste (Vincke et al. 2001). Then, the e-waste is added during the exponential growth phase of the microorganisms, and the culture is further incubated. In this method, the toxic impact of e-waste on microbes can be avoided, and the microbes can develop well (Okabe et al. 2007). Okibe and Johnson (2004) suggested a method based on the reduction of the negative impacts of excessive organics in the growth environment of the autotrophs. This method proposed development of a microbial ecosystem with the synergistic effects of acidophilic heterotrophs and autotrophs. Panda et al. (2017) observed that the microbial community leans toward better performance when mixed cultures of microorganisms are used in bioleaching rather than pure cultures. Peng et al. (2012) studied the influence of surfactants on bioleaching. They observed that supplementation of the media with surfactants increased the surface contact between electron donors and microbes' cells. Tween-80 is a good surfactant for stimulating microorganisms to metabolize elemental sulfur (Xiang et al. 2010; Yang et al. 2009). During the bioleaching process, a passivation layer is generally formed by precipitation of Fe^{3+} (such as jarosite) and S°. This passivation layer coats the e-waste and inhibits the free movement of electrons. As a result, metal bioleaching is suppressed, and the capital costs increase significantly (Mishra et al. 2008b; Zeng et al. 2010). The passivation layer should be destabilized to overcome this issue. In this case, generation of a Fe(III)-precipitate can be inhibited by controlling the pH of solution or by employing thermophilic or extremely thermophilic autotrophs for oxidation of S°, which can considerably accelerate the process (Aditiawati et al. 2009; Priya and Hait 2018).

5.2 Electrochemical Methods

In bioleaching processes, the dissolution of elemental metal (E°) or metal oxides (EO) from e-waste is predominantly based on the following reactions (Brandl et al. 2008):

$$\mathrm{Fe^{2+} + H^+ + O_2 \rightarrow Fe^{3+} + H_2O} \quad \text{(by microbial catalysis)}$$
$$\mathrm{S^{\circ} + H_2O + O_2 \rightarrow H^+ + SO_4{}^{2-}} \quad \text{(by microbial catalysis)}$$
$$\mathrm{Fe^{3+} + E^{\circ}_{(s)} \rightarrow Fe^{2+} + E^{n+}} \quad \text{(by chemical reaction)}$$
$$\mathrm{E^{\circ}_{(s)} + H^+ + O_2 \rightarrow E^{n+} + H_2O} \quad \text{(by chemical reaction)}$$
$$\mathrm{EO_{(s)} + H^+ \rightarrow E^{n+} + H_2O} \quad \text{(by chemical reaction)}$$

From these mechanisms, electrons play a vital part in bioleaching reactions. So, electrochemical influences alter the efficiency of bioleaching (Wang et al. 2016). In this sense, bioleaching can be promoted by accelerating extracellular electron transmission (EET). Several research studies have proposed that the bacterium *A. ferrooxidans* has remarkable EET activity. Through *A. ferrooxidans* catalysis, electrons are transferred for the oxidation of ferrous iron and reduce sulfur substances to oxygen that acts as electron acceptor. In the EET pathway, surface-exposed fibers, rusticyanin, type IV pili, and cytochrome c play an important role. Better e-waste bioleaching can be expected by promoting EET and electron transfer mediators (ETMs) (Cao et al. 2011).

The extraordinary redox-potential performance of ETMs to improve EET is done via donating and accepting electrons. Some distinctive exogenous ETMs are given in Table 14.2.

Table 14.2 Typical exogenous electron transfer mediators

Name of the chemical	Source	References
Activated carbon	Pyrolysis of carbon-rich organic materials at high temperature and pressure in furnace	Peng et al. (2012)
Biochar	Thermal disintegration of biomass with controlled oxygen supply (at<700 °C)	Yang et al. (2009)
Anthraquinone-2,6-disulfonate (2,6-AQDS)	Chemical synthesis	Cao et al. (2011)
Humic substance	It is a natural organic macromolecule from sediments, soils, etc.	Mishra et al. (2008b)
Methylene blue	Chemical synthesis	Brandl et al. (2001)
Cysteine	Chemical synthesis	Zhang and Forssberg (1997)
Neutral red	Chemical synthesis	Choi et al. (2004)

On this the list, biochar attained from biomass via pyrolysis process can be used as a potential exogenous mediator to participate in PCB bioleaching (Wang et al. 2016). Wang et al. (2016) observed a better efficiency in Cu bioleaching using biochar participation compared to bioleaching without biochar. They also obtained a leaching time that was reduced by one-third. A modified cathode using N_2-doped carbon nanotubes can be used for bioleaching of copper from PCBs to accelerate EET (Brandl et al. 2001). To acquire excellent bioleaching, electrochemical potential can be exposed. During sulfide mineral bioleaching, input of negative and positive potentials can be done to promote the growth and activity of *A. ferrooxidans*. When applying positive potential, a significant amount of oxygen is produced due to the electrolytic conditions and insufficient ferrous iron caused by the presence of stable ferric iron, which inhibits microbial activity (Vincke et al. 2001). Alternatively, negative potential enhances biomass production and activity because it is conducive to the reduction of ferric iron to ferrous iron that is needed for microbial catalysis (Zhang and Forssberg 1997). Also, it can be expected that the bioleaching microorganisms multiply rapidly in the exponential growth stage. Another appreciable benefit is that the precipitation of ferric iron can be suppressed under conditions of negative potential.

Additionally, bioleaching can be enhanced by the presence of metal ions. In this case, the adsorption of metal ions on mineral surfaces changes metabolic behavior through modification of electrochemical properties (Garcia-Moyano et al. 2007; Van der Zee et al. 2003). Various studies have documented that the presence of various metal ions, such as Hg^{2+}, Ag^{+}, Cu^{2+}, and Bi^{+}, has great influence on the electrochemical mechanisms of bioleaching. These metal ions help in the generation of metal sulfides that dissolve sulfides either by galvanic action or replacement in a crystal lattice. Miller et al. (1981) showed that Ag^{+} ions can improve Cu bioleaching from chalcopyrite, which resulted from the formation of a porous Ag_2S with very low adhesion. Thermodynamically, Ag_2S is more stable than chalcopyrite. Additionally, chalcopyrite can form electrochemical pairs and act as an anode to dissolve Cu. However, the effect of catalytic activity of specific metal ions on e-waste bioleaching will be different than for sulfides. This is because sulfides and e-waste have different potentials, which will affect the galvanic interaction between the metal ions and e-waste. The effect of metal ions on the bioleaching of e-waste needs further investigation (Choi et al. 2004).

6 Future Perspectives on Bioleaching

In this chapter, the diversity of acidophiles in acidic environments and their applications in e-waste bioleaching are concisely discussed. Extreme acidophilic microorganisms show great potential in bioleaching applications. Therefore, the use of acidophiles and extreme acidophiles in bioleaching should be one of the most important research areas in the future. From the current reports, various aspects need to be developed for bioleaching of e-wastes. They are:

(i) Formation of acidic liquids during bioleaching should be minimized or avoided. This is because such acidic wastes pollute the environment and harm ecosystems.

(ii) A crucial challenge in bioleaching processes is that they are time-consuming, which hinders their practical success. Therefore, it is required to improve the efficiency of bioleaching to the maximum extent. Additionally, combining multiple effective techniques is an attractive choice.

(iii) When using extreme acidophiles, further investigation of EET pathways is needed. Additional studies are required to understand whether or not specific microorganisms have EET pathways. This is still very unclear.

(iv) Further studies are needed when exogenous ETMs participate in bioleaching. This is to determine whether or not they can be reused or recycled.

(v) It is unknown whether or not specific acidophilic microorganisms can secrete endogenous ETMs. If so, it may be possible to produce endogenous ETMs using genetically engineered bacteria, thereby promoting bioleaching efficiency.

References

Aarabi KM, Rashchi F, Mostoufi N, Vahidi E (2010) Leaching of vanadium from LD converter slag using sulfuric acid. Hydrometallurgy 102:14–21. https://doi.org/10.1016/j.hydromet.2010.01.006

Aditiawati P, Yohandini H, Madayanti F, Akhmaloka (2009) Microbial diversity of acidic hot spring (Kawah Hujan B) in geothermal field of Kamojang Area, West Java-Indonesia. Open Microbiol J 3:58–66. https://doi.org/10.2174/1874285800903010058

Appia AC, Guiliani N, Ratouchniak J, Bonnefoy V (1999) Characterization of an operon encoding two c-type cytochromes, an aa3-type cytochrome oxidase, and rusti-cyanin in *Thiobacillus ferrooxidans* ATCC 33020. Appl Environ Microbiol 65(11):4781–4787. https://doi.org/10.1128/AEM.65.11.4781-4787.1999

Baker BJ, Banfield JF (2003) Microbial communities in acid mine drainage. FEMS Microbiol Ecol 44(2):139–152. https://doi.org/10.1016/S0168-6496(03)00028-X

Baker AC, Dopson M (2007) Life in acid: pH homeostasis in acidophiles. Trends Microbiol 15 (4):165–171. https://doi.org/10.1016/J.TIM.2007.02.005

Bhattacharyya A, Stavitski E, Dvorak J, Martinez CE (2013) Redox interactions between Fe and cysteine: spectroscopic studies and multiplet calculations. Geochim Cosmochim Acta 122:89–100. https://doi.org/10.1016/j.gca.2013.08.012

Brandl H, Bosshard R, Wegmann M (2001) Computer-munching microbes: metal leaching from electronic scrap by bacteria and fungi. Hydrometallurgy 59(2):319–326. https://doi.org/10.1016/S0304-386X(00)00188-2

Brandl H, Lehmann S, Faramarzi MA, Martinelli D (2008) Biomobilization of silver, gold, and platinum from solid waste materials by HCN-forming microorganisms. Hydrometallurgy 94 (1):14–17. https://doi.org/10.1016/j.hydromet.2008.05.016

Bryner LC, Anderson R (1957) Microorganisms in leaching sulfide minerals. Ind Eng Chem 49 (10):1721–1724. https://doi.org/10.1533/9781845694616.341

Cao B, Ahmed B, Kennedy DW, Wang Z, Shi L, Marshall MJ, Fredrickson JK, Isern NG, Majors PD, Beyenal H (2011) Contribution of extracellular polymeric substances from Shewanella sp. HRCR-1 biofilms to U(VI) immobilization. Environ Sci Technol 45(13):5483–5490. https://doi.org/10.1021/es200095j

Cao K, Li DX, Yang YM (2012) The research of leaching copper from printed circuit boards in microbial metabolic under the action of electric field. North Environ 25:49–53. https://doi.org/10.1081/ESE-200034763

Chen H, Zhao J, Dai GL (2011) Silkworm exuviae – a new nonconventional and low-cost adsorbent for removal of methylene blue from aqueous solutions. J Hazard Mater 186(2):1320–1327. https://doi.org/10.1016/j.jhazmat.2010.12.006

Choi MS, Cho KS, Kim DS, Kim DJ (2004) Microbial recovery of copper from printed circuit boards of waste computer by *Acidithiobacillus ferrooxidans*. J Environ Sci Health A 39:2973–2982. https://doi.org/10.1081/ESE-200034763

Colmer AR, Hinkle ME (1947) The role of microorganisms in acid mine drainage: a preliminary report. Science 106(2751):253–256. https://doi.org/10.1126/science.106.2751.253

Cui JR, Zhang LF (2008) Metallurgical recovery of metals from electronic waste: a review. J Hazard Mater 158(2):228–256. https://doi.org/10.1016/j.jhazmat.2008.02.001

Druschel GK, Baker BJ, Gihring TM, Banfield JF (2004) Acid mine drainage biogeochemistry at Iron Mountain, California. Geochem Trans 5(2):13–32. https://doi.org/10.1063/1.1769131

Elbehti A, Brasseur G, Lemesle-Meunier D (2000) First evidence for existence of an uphill electron transfer through the bc1 and NADH-Q oxidoreductase complexes of the acidophilic obligate chemolithotrophic ferrous ion-oxidizing bacterium *Thiobacillus ferrooxidans*. J Bacteriol 182 (12):3602–3606. https://doi.org/10.1128/JB.182.12.3602-3606.2000

Gan M, Zhou SG, Li MY, Zhu JY, Liu XX, Chai LY (2015) Bioleaching of multiple heavy metals from contaminated sediment by mesophile consortium. Environ Sci Pollut Res 22 (8):5807–5816. https://doi.org/10.1007/s11356-014-3759-x

Garcia-Moyano A, Gonzalez-Toril E, Aguilera A, Amils R (2007) Prokaryotic community composition and ecology of floating macroscopic filaments from an extreme acidic environment, Rio Tinto (SW, Spain). Syst Appl Microbiol 30(8):601–614. https://doi.org/10.1016/j.syapm.2007.08.002

Gehrke T, Telegdi J, Thierry D, Sand W (1998) Importance of extracellular polymeric substances from *Thiobacillus ferrooxidans* for bioleaching. Appl Environ Microbiol 64(7):2743–2747. https://doi.org/10.1128/AEM.64.7.2743-2747.1998

Gonzalez-Toril E, Llobet-Brossa E, Casamayor EO, Amann R, Amils R (2003) Microbial ecology of an extreme acidic environment, the Tinto River. Appl Environ Microbiol 69(8):4853–4865. https://doi.org/10.4028/www.scientific.net/AMR.71-73.13

Grengg C, Mittermayr F, Baldermann A, Bottcher ME, Leis A, Koraimann G, Grunert P, Dietzel M (2015) Microbiologically induced concrete corrosion: a case study from a combined sewer network. Cem Concr Res 77:16–25. https://doi.org/10.1016/j.cemconres.2015.06.011

Gu X, Wong JWC (2004) Identification of inhibitory substances affecting bioleaching of heavy metals from anaerobically digested sewage sludge. Environ Sci Technol 38(10):2934–2939. https://doi.org/10.1021/es0347134

Gu WH, Bai JF, Dong B, Zhuang XN, Zhao J, Zhang CL, Wang JW, Shih K (2017) Enhanced bioleaching efficiency of copper from waste printed circuit board driven by nitrogen-doped carbon nanotubes modified electrode. Chem Eng J 324:122–129. https://doi.org/10.1016/j.cej.2017.05.024

Gurung A, Chakraborty R (2009) The role of *Acidithiobacillus ferrooxidans* in alleviating the inhibitory effect of thiosulfate on the growth of acidophilic Acidiphilium species isolated from acid mine drainage samples from Gorubathan, India. Can J Microbiol 55(9):1040–1048. https://doi.org/10.1139/W09-062

Haanela A, Johnson DB (2014) Microorganisms in subterranean acidic waters within Europe's deepest metal mine. Res Microbiol 165(9):705–712. https://doi.org/10.1016/j.resmic.2014.07.007

Havlik T, Orac D, Petranikova M, Miskufova A, Kukurugya F, Takacova Z (2010) Leaching of copper and tin from used printed circuit boards after thermal treatment. J Hazard Mater 183 (1):866–873. https://doi.org/10.1016/j.jhazmat.2010.07.107

Hong Y, Valix M (2014) Bioleaching of electronic waste using acidophilic sulfur oxidising bacteria. J Clean Prod 65:465–472. https://doi.org/10.1016/j.jclepro.2013.08.043

Hose LD, Palmer AN, Palmer MV, Northup DE, Boston PJ, DuChene HR (2000) Microbiology and geochemistry in a hydrogen-sulphide-rich karst environment. Chem Geol 169(3):399–423. https://doi.org/10.1016/S0009-2541(00)00217-5

Huo YT, Chang ZD, Li WJ, Liu SX, Dong B (2015) Reuse and valorization of vanadium and tungsten from waste V_2O_5–WO_3/TiO_2 SCR catalyst. Waste Biomass Valoriz 6:159–165. https://doi.org/10.1007/s12649-014-9335-2

Ilyas S, Anwar MA, Niazi SB, Afzal Ghauri M (2007) Bioleaching of metals from electronic scrap by moderately thermophilic acidophilic bacteria. Hydrometallurgy 88(1):180–188. https://doi.org/10.1016/j.hydromet.2007.04.007

Ilyas S, Lee JC, Chi RA (2013) Bioleaching of metals from electronic scrap and its potential for commercial exploitation. Hydrometallurgy 78:138–143. https://doi.org/10.1016/j.hydromet.2012.11.010

Isıldar A, Vossenberg JVD, Rene ER, Hullebusch EDV, Lens PNL (2016) Two-step bioleaching of copper and gold from discarded printed circuit boards (PCB). Waste Manag 57:149–157. https://doi.org/10.1016/j.wasman.2015.11.033

Jowkar MJ, Bahaloo-Horeh N, Mousavi SM, Pourhossein F (2018) Bioleaching of indium from discarded liquid crystal displays. J Clean Prod 180:417–429. https://doi.org/10.1016/j.jclepro.2018.01.136

Kimura S, Bryan Christopher G, Hallberg Kevin B, Johnson DB (2011) Biodiversity and geochemistry of an extremely acidic, low-temperature subterranean environment sustained by chemolithotrophy. Environ Microbiol 13(8):2092–2104. https://doi.org/10.1111/j.1462-2920.2011.02434.x

Li Y, Li H (2014) Type IV pili of Acidithiobacillus ferrooxidans can transfer electrons from extracellular electron donors. J Basic Microbiol 54:226–231. https://doi.org/10.1002/jobm.201200300

Liang GB, Mo YW, Zhou QF (2010) Novel strategies of bioleaching metals from printed circuit boards (PCBs) in mixed cultivation of two acidophiles. Enzym Microb Technol 47(7):322–326. https://doi.org/10.1016/j.enzmictec.2010.08.002

Liang CL, Xia JL, Nie ZY, Yang Y, Ma CY (2012) Effect of sodium chloride on sulfur speciation of chalcopyrite bioleached by the extreme thermophile *Acidianusmanzaensis*. Bioresour Technol 110:462–467. https://doi.org/10.1016/j.biortech.2012.01.084

Liu H, Yin H, Dai Y, Dai Z, Liu Y, Li Q, Jiang H, Liu X (2011) The co-culture of *Acidithiobacillus ferrooxidans* and *Acidiphilium acidophilum* enhances the growth, iron oxidation, and CO_2 fixation. Arch Microbiol 193(12):857–866. https://doi.org/10.1007/s00203-011-0723-8

Macalady JL, Jones DS, Lyon EH (2007) Extremely acidic, pendulous cave wall biofilms from the Frasassi cave system, Italy. Environ Microbiol 9(6):1402–1414. https://doi.org/10.1111/j.1462-2920.2007.01256.x

Marhual N, Pradhan N, Kar R, Sukla L, Mishra B (2008) Differential bioleaching of copper by mesophilic and moderately thermophilic acidophilic consortium enriched from same copper mine water sample. Bioresour Technol 99(17):8331–8336. https://doi.org/10.1016/j.biortech.2008.03.003

Marra A, Cesaro A, Rene ER, Belgiorno V, Lens PNL (2018) Bioleaching of metals from WEEE shredding dust. J Environ Manag 210:180–190. https://doi.org/10.1016/j.jenvman.2017.12.066

Miller JD, McDonough PJ, Portillo HQ (1981) Electrochemistry in silver-catalyzed ferric sulfate leaching of chalcopyrite. Keaton, M.C. (Ed.), Process and Fundamental Consideration of Selected Hydrometallurgical Systems. New York: 327–338

Mishra D, Kim DJ, Ralph D, Ahn JG, Rhee YH (2008a) Bioleaching of metals from spent lithium ion secondary batteries using *Acidithiobacillus ferrooxidans*. Waste Manag 28(2):333–338. https://doi.org/10.1016/j.wasman.2007.01.010

Mishra D, Kim DJ, Ralph DE, Ahn JG, Rhee YH (2008b) Bioleaching of spent hydroprocessing catalyst using acidophilic bacteria and its kinetics aspect. J Hazard Mater 152:1082–1091. https://doi.org/10.1016/j.jhazmat.2007.07.083

Natarajan KA (1992) Effect of applied potentials on the activity and growth of *Thiobacillus ferrooxidans*. Biotechnol Bioeng 39(9):907–913. https://doi.org/10.1002/bit.260390905

Okabe S, Odagiri M, Ito T, Satoh H (2007) Succession of sulfur-oxidizing bacteria in the microbial community on corroding concrete in sewer systems. Appl Environ Microbiol 73(3):971–980. https://doi.org/10.1128/AEM.02054-06

Okibe N, Johnson DB (2004) Biooxidation of pyrite by defined mixed cultures of moderately thermophilic acidophiles in pH-controlled bioreactors: significance of microbial interactions. Biotechnol Bioeng 87(5):574–583. https://doi.org/10.1002/bit.20138

Panda S, Akcil A, Mishra S, Erust C (2017) Synergistic effect of biogenic Fe3+ coupled to S° oxidation on simultaneous bioleaching of cu co, Zn and as from hazardous pyrite ash waste. J Hazard Mater 325:59–70. https://doi.org/10.1016/j.jhazmat.2016.11.050

Pathak A, Morrison L, Healy MG (2017) Catalytic potential of selected metal ions for bioleaching, and potential techno-economic and environmental issues: a critical review. Bioresour Technol 229:211–221. https://doi.org/10.1016/j.biortech.2017.01.001

Peng A-A, Liu H-C, Nie Z-Y, Xia J-L (2012) Effect of surfactant Tween-80 on sulfur oxidation and expression of sulfur metabolism relevant genes of *Acidithiobacillus ferrooxidans*. Trans Nonferrous Metals Soc China 22(12):3147–3155. https://doi.org/10.1016/S1003-6326(12)61767-1

Pradhan N, Nathsarma K, Rao KS, Sukla L, Mishra B (2008) Heap bioleaching of chalcopyrite: a review. Miner Eng 21(5):355–365. https://doi.org/10.1016/j.mineng.2007.10.018

Priya A, Hait S (2018) Feasibility of bioleaching of selected metals from electronic waste by *Acidiphilium acidophilum*. Waste Biomass Valoriz 9:871–877. https://doi.org/10.1007/s12649-017-9833-0

Quatrini R, Johnson DB (2018) Microbiomes in extremely acidic environments: functionalities and interactions that allow survival and growth of prokaryotes at low pH. Curr Opin Microbiol 43:139–147. https://doi.org/10.1016/j.mib.2018.01.011

Rawlings DE (2005) Characteristics and adaptability of iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. Microb Cell Factories 4 (1):13–21. https://doi.org/10.1186/1475-2859-4-13

Roberts DJ, Nica D, Zuo G, Davis JL (2002) Quantifying microbially induced deterioration of concrete: initial studies. Int Biodeterior Biodegradation 49(4):227–234. https://doi.org/10.1016/S0964-8305(02)00049-5

Sun YW, Shao Y, Peng J, Liu LG, Teng BT (2013) A basic site-switched structure conversion of neutral red for selective DNA recognition. Sensors Actuators B Chem 188:242–248. https://doi.org/10.1016/j.snb.2013.07.034

Urbieta MS, Gonzalez TE, Bazan AA, Giaveno MA, Donati E (2015) Comparison of the microbial communities of hot springs waters and the microbial biofilms in the acidic geothermal area of Copahue (Neuquen, Argentina). Extremophiles 19(2):437–450. https://doi.org/10.1007/s00792-015-0729-2

Van der Zee FP, Bisschops IAE, Lettinga G, Field JA (2003) Activated carbon as an electron acceptor and redox mediator during the anaerobic biotransformation of azo dyes. Environ Sci Technol 37(2):402–408. https://doi.org/10.1021/es025885o

Vera M, Schippers A, Sand W (2013) Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation – Part A. Appl Microbiol Biotechnol 97(17):7529–7541. https://doi.org/10.1007/s00253-013-4954-2

Vincke E, Boon N, Verstraete W (2001) Analysis of the microbial communities on corroded concrete sewer pipes – a case study. Appl Microbiol Biotechnol 57(5):776–785. https://doi.org/10.1007/s002530100826

Vlasceanu L, Sarbu SM, Engel AS, Kinkle BK (2000) Acidic cave-wall biofilms located in the Frasassi Gorge, Italy. Geomicrobiol J 17(2):125–139. https://doi.org/10.1080/01490450050023809

Wang J, Bai J, Xu J, Liang B (2009) Bioleaching of metals from printed wire boards by Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans and their mixture. J Hazard Mater 172(2–3):1100–1105. https://doi.org/10.1016/j.jhazmat.2009.07.102

Wang SH, Zheng Y, Yan WF, Chen LX, Mahadevan GD, Zhao F (2016) Enhanced bioleaching efficiency of metals from E-wastes driven by biochar. J Hazard Mater 320:393–400. https://doi.org/10.1016/j.jhazmat.2016.08.054

Wang SH, Xie YL, Yan WF, Wu XE, Wang C-T, Zhao F (2018) Leaching of vanadium from waste V_2O_5-WO_3/TiO_2 catalyst catalyzed by functional microorganisms. Sci Total Environ 639:497–503. https://doi.org/10.1016/j.scitotenv.2018.05.168

Watanabe K, Manefield M, Lee M, Kouzuma A (2009) Electron shuttles in biotechnology. Curr Opin Biotechnol 20(6):633–641. https://doi.org/10.1016/j.copbio.2009.09.006

Xiang Y, Wu PX, Zhu NW, Zhang T, Liu W, Wu JH, Li P (2010) Bioleaching of copper from waste printed circuit boards by bacterial consortium enriched from acid mine drainage. J Hazard Mater 184(1):812–818. https://doi.org/10.1016/j.jhazmat.2010.08.113

Yang T, Xu Z, Wen JK, Yang LM (2009) Factors influencing bioleaching copper from waste printed circuit boards by *Acidithiobacillus ferrooxidans*. Hydrometallurgy 97(1):29–32. https://doi.org/10.1016/j.hydromet.2008.12.011

Zeng WM, Qiu GZ, Zhou HB, Liu XD, Chen M, Chao WL, Zhang CG, Peng J (2010) Characterization of extracellular polymeric substances extracted during the bioleaching of chalcopyrite concentrate. Hydrometallurgy 100(3):177–180. https://doi.org/10.1016/j.hydromet.2009.11.002

Zhang SL, Forssberg E (1997) Mechanical separation-oriented characterization of electronic scrap. Resour Conserv Recycl 21:247–269. https://doi.org/10.1016/S0921-3449(97)00039-6

Zhang CF, Katayama A (2012) Humin as an electron mediator for microbial reductive dehalogenation. Environ Sci Technol 46(12):6575–6583. https://doi.org/10.1021/es3002025

Zhou S, Gan M, Zhu JY, Li Q, Jie SQ, Yang BJ, Liu XD (2015) Catalytic effect of light illumination on bioleaching of chalcopyrite. Bioresour Technol 182:345–352. https://doi.org/10.1016/j.biortech.2015.02.010

Zhu NW, Xiang Y, Zhang T, Wu PX, Dang Z, Li P, Wu JH (2011) Bioleaching of metal concentrates of waste printed circuit boards by mixed culture of acidophilic bacteria. J Hazard Mater 192(2):614–619. https://doi.org/10.1016/j.jhazmat.2011.05.062

Ziegler S, Ackermann S, Majzlan J, Gescher J (2009) Matrix composition and community structure analysis of a novel bacterial pyrite leaching community. Environ Microbiol 11(9):2329–2338. https://doi.org/10.1111/j.1462-2920.2009.01959.x

Chapter 15
Potential of Free Floating Macrophytes for Bioremediation of Heavy Metals- A Conceptual Review

Punita Parikh and Krupa Unadkat

Abstract Ecosystem balance and ecological restoration occurred naturally that helped to maintain sustainability in ecosystem. The biodiversity plays a significant role in biogeochemical cycling and balancing the ecosystem. Anthropogenic activities for development and indiscriminate use of technology lead to exploitation of natural resources and imbalance of ecosystem. Pollutants containing various organic and inorganic components including heavy metals into aquatic ecosystem lead to their bioaccumulation and bioaugmentation. This could be reverted by conservation of natural resources including biodiversity and utilizing their potential in bioremediation. The present paper is a conceptual review of researches carried out to assess the efficiency of free floating macrophytes in bioremediation of heavy metals. A periodic assessment of systematic literature can bring about the possibility of effective utilization of aquatic plants in water treatment and pollution reduction thereby ecosystem balance.

Keywords Bioremediation · Free floating macrophytes · Heavy metals

1 Introduction

1.1 Aquatic Angiosperm Diversity in Wetlands

In ancient time aquatic and wetland ecosystem were considered as unproductive, worthless and sometimes detrimental, subsequently it illuminated with specific ecological features, values and functions (Mishra and Tripathi 2009). The backbone of aquatic ecosystem is its diversity of flora and fauna. Aquatic flora increases productivity as well as asseverate the balance of an aquatic ecosystem. Though global acceptance of aquatic biodiversity as sustainable life supports system, it hardly acquires attention for scientific studies. Hence, potential of aquatic plants remains quiescent since prehistoric times.

P. Parikh (✉) · K. Unadkat
Department of Botany, The MS University of Baroda, Vadodara, Gujarat, India

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_15

1.1.1 Global Distribution of Aquatic Macrophytes

Aquatic macrophytes have a world-wide distribution, almost all the biogeographic regions of the world comprise of greater diversity of these plants. The habitats of aquatic macrophyte exhibit the most diversified, productive and conglomerate portions of water bodies. To a common man, aquatic macrophytes are those which are of substantial size to see with a naked eye. This diversified autotroph includes Green algae (division Chlorophyta), Yellow-green algae (division Xanthophyta) and Blue green algae (Cyanobacteria). Non vascular plants of Bryophytes (Hornworts and Musci) and Vascular plants belonging to Pteridophytes as well as Angiosperms.

Among various groups of plants mentioned above, vascular macrophytes were recorded as early as the mid1800s by Darwin (1859). Further, Aquatic Macrophytes of Angiosperm group., comprises of 250,000–400,000 angiosperm species, of which only 2443 species are Aquatic. Ecologically, they are classified as emergent macrophytes (plants with roots and most part of stem submersed in soil and leaves extending into the air e.g. *Typha* sps.), submerged macrophytes (plants growing completely submerged under the water and roots in close association with substrate e.g. *Hydrilla* sps.), Rooted floating leaved (plants rooted with the substratum at the bottom with long petiole and large leaf lamina floats on the facet of water e.g. *Nymphaea* sps.) and free-floating macrophytes (plants mostly small sized that usually float on the water surface e.g. *Lemna* and *Wolffia* spp). Representative genera of Aquatic macrophytes found globally are *Sagittaria, Alisma, Butomus, Brasenia, Cabomba, Callitriche, Ceratophyllum, Scirpus, Carex, Myriophyllum, Elodea, Vallisneria, Juncus, Lemna, Utricularia, Nelumbo, Nymphaea, Nuphar, Spartina, Eichhornia, Potamogeton, Ranunculus, Sparganium,* and *Typha.*

Chambers et al. (2008) had studied Global diversity of aquatic macrophytes in freshwater at a global scale in which they recorded highest species diversity (984 sps.) of vascular aquatic macrophytes in Neotropics of which 188 species belonged to the family Podostemaceae which contributed largely towards the diversity of the macrophytes in this region. The study also envisaged that the diversity was intermediate in the Orient (664 sps), Nearctic (644 sps) and Afrotropic (614 sps), lower in Palearctic 497 sps and Australasia (439 sps) which still subsides in the region of Pacific and Oceanic islands (108 sps.). Very few vascular macrophytes were confined to the Antarctica bioregion and that to only in the sub-Antarctic freshwaters.

The largest and solely found aquatic family of angiosperms in terms of number of species and genera was Podostemceae. In the Tropics, the plants of this family are generally found in fast flowing waters. Moreover, in terms of species richness, Cyperaceae and Poaceae were distributed all over the world except Antarctica. In contrast to fresh waters, where Aquatic macrophytes show worldwide distribution, there are only few marine submerged Angiosperms that are found growing in Coastal areas in shallow waters throughout the world. Among these *Zostera* and *Phyllospadix*belong to the family Zosteraceae are commonly found Aquatic macrophytes.

1.1.2 Aquatic Angiosperm Diversity in Wetlands of India

India is endowed with variety of geographical land, wide range of climatic condition, fertile soil, tranquil spread of meadows and dense forest. Aquatic ecosystem and terrestrial ecosystem both have their exclusive significance. The unique ecological features that provide services to humanity as well as numerous products are wetland ecosystem (Prasad et al. 2002). The land transition between aquatic and aquatic ecosystem is wetland ecosystem which is considered as third amongst most productive ecosystem. In India wetland ecosystem comprises of most of the natural water bodies in major (rivers, coastal lagoons) and artificial wetlands (ponds, lakes, irrigated fields) (Ramsar 2013). Such areas support a broad extent of water resources including cold, warm, brackish and marine water results in vast natural habitats with rich in biodiversity as compare to other countries. The wetland ecosystem provides key services such as nutrient removal, toxic retention, flood control, biodiversity maintenance and carbon sequestration (Turner et al. 2000). Wetlands of India are ranging from warm and arid zone of Gujarat and Rajasthan to the tropical monsoon-based regions of Central India, from warm and arid zone of Gujarat and Rajasthan to the tropical monsoon-based regions of Central India, from cold arid zones of Laddakh to wet Imphal. In India estimated wetlands are 4.1 million hectares (0.44%) which includes both natural and artificial wetlands.

Aquatic angiosperm plant comprises very flexible and complex morphology which results in numerous structural modifications than terrestrial plants. These modifications of aquatic plants result in phenotypic variations which might have adaptive significance. So, these plants pose challenge for taxonomist in their identification. Indian flora fluctuates from one geographical region to another so, flora is essential tool to study plants and their utilities at various geographical areas.

However, literature on plant biodiversity in wetlands of Gujarat especially on Baroda district of Gujarat is scarce. Though, few studies had been carried out earlier on this aspect by Phatak and Satakopan (1956–1957) who had made extensive studies on Harni pond with particular reference to the phenology of the Angiosperms and Cryptogams. Thereafter in 1961, Chavan and Sabnis reported 32 species of Cyperaceae from Baroda district and enlisted them as Amphibious hydrophytes. As mentioned by the same authors (1960) that they found a lacuna on the data on the ecology of the vegetation in and around running water, carried out floristic composition and seasonal changes in the vegetation along the bank of River Vishwamitri at Baroda District. They had recorded 151 plants belonging to 45 families. More systematic study on Hydrophytes of Savli taluka of Baroda district had undertaken by Chavan and Padate 1962. There were 68 species reported that belonged to 53 genera and 34 families.

Dave and Krishnayya during 2000–02 assessed vegetation cover in and around Harni pond. They noticed that changes have occurred in the overall floristic composition growing in and around Harni pond at Baroda due to the impact of anthropogenic pressure over the past 45 years. Unadkat (2017) carried out Biodiversity study on wetlands of Vadodara district of Gujarat state. She documented a total of

94 aquatic plant species belonging to emergent, submerged, rooted with floating leaves and free-floating hydrophytes in the study. When the data was compared with earlier reports for the same wetlands, it showed that population of aquatic plants in terms of diversity was declining in Vadodara region to the point of extinction and it was recommended that these aquatic plants should be conserved to uphold the rich biodiversity for Vadodara region for the welfare of humanity.

Saini and Kumar (2017) had stated that seasonal and monthly variation were responsible for appearance and diversity of aquatic community of rainy water wetlands of Yamuna Nagar, Haryana. They have recorded of 59 species belonging to 37 families. Maximum number of species was recorded for family Poaceae and the minimum for family Adiantaceae.

1.1.3 List of Commonly Occurring Aquatic Angiosperms

Aquatic plants are adapted to survive in freshwater or marine water environments. Aquatic Macrophytes are various groups of aquatic plants which have substantial size to be visible by naked eye. They either grow in or surrounding the water. Thus, they are excellent indicator of frequently changing environmental condition of aquatic body. Hence, composition of macrophytes community can reflect great sensitivity and biological integrity of aquatic ecosystems. The appreciation of the positive ecological values of Macrophytes has led to a better perception of their diversity and role in natural ecosystem for their scientific management.

Aquatic macrophytes comprises of macroalgae of the divisions Chlorophyta (green algae), Xanthophyta (yellow-green algae) and Rhodophyta (red algae) and the “blue-green algae” (cyanobacteria); Bryophyta (mosses and liverworts); Pteridophyte (ferns); and Spermatophyta (seed-bearing plants). Aquatic plants are phylogenetically well dispersed across the angiosperms. Aquatic angiosperm is the largest group and usually found in floating, emergent, submerged and wetland condition. The colloquial examples of aquatic angiosperms include water lily, water hyacinth, lotus, floating heart and so forth. Sea grasses such as *Thalassia* and *Zostera* are only angiosperms capable of growing completely submerged in seawater. The largest aquatic angiosperm is amazon water lily and smallest is the minute duckweed (Table 15.1).

1.1.4 Free Floating Macrophytes

The Macrophytes generally remain in contact with air and water but not within the contact of soil are known as free floating Macrophytes. They have ability to freely float on the superficies of water due to the presence of aerenchymatous tissue. Well developed stem, Co_2 and O_2 intake partly from air and water, presence of root hairs lying at right angle to the axis of the main root and occurrence of heterophylly are some common features of ecological interest of these plants. *Wolffia, Lemna,*

Table 15.1 List of Aquatic Angiosperms of common occurrence. (Unadkat 2017)

Sr. No.	Botanical name	Common name	Family	Morpho-ecological group
1	*Spirodela polyrrhiza* (L.) Schleid.	Duckweed	Araceae	Floating
2	*Aponogeton natans* (L.) Engl. & K. Krause	Cape pond weed	Aponogetanaceae	Floating
3	*Lemna trisulca* L.	Star duckweed	Araceae	Floating
4	*Ipomoea aquatica* Forssk.	Water spinach	Convolvulaceae	Floating
5	*Ranunculus aquatilis*	Water-crowfoot	Rananculaceae	Floating
6	*Nymphoides indica* (L.) Kuntze	Water snowflake	Menyanthaceae	Floating
7	*Hygroryza aristata* (Retz.) Nees ex Wight & Arn.	–	Poaceae	Floating
8	*Echhornia crissepes*	Water hyacinth	Pontederiaceae	Floating
9	*Pistia stratiotes*	Water lettuce	Araceae	Floating
10	*Nelumbo nucifera* Gaertn.	Indian lotus	Nelumbonaceae	Floating anchored
11	*Nymphea nouchali* Burm.f.	Star lotus	Nymphaeaceae	Floating anchored
11	*Ceratophyllum submersum* L.	Hornwort	Ceratophyllaceae	Submerged
12	*Utricularia vulgaris* L.	Bladderworts	Lentibulariaceae	Submerged
13	*Zannichellia palustris* L.	Horned pondweed	Potamogetonaceae	Submerged
14	*Hydrilla verticillata* (L. f.) Royle	Waterweed	Hydrocharitaceae	Submerged
15	*Vallisneria spiralis* L.	Tap grass	Hydrocharitaceae	Submerged
16	*Limnophyton obtusifolium* (L.) Miq.	Blunt arrowhead	Alismataceae	Submerged
17	*Gomphrena celosioides* Mart.	Gomphrena weed	Amaranthaceae	Submerged
18	*Pomatogeton crispus* L.	Curly leaf pond weed	Potamogetonaceae	Submerged
19	*Potamogeton perfoliatus* L.	Perfoliate pondweed	Potamogetonaceae	Submerged
20	*Limnophila heterophylla*	Marsh weeds	Plantagenaceae	Submerged
21	*Sagittaria sagittifolia*	Arrowhead	Alismataceae	Wetland
22	*Chloris barbata* Sw.	Windmill grass	Poaceae	Wetland
23	*Dactyloctenium aegyptium* (L.) Willd	Egyptian grass	Poaceae	Wetland
24	*Colocasia esculenta* (L.) Schott	Elephant's ear	Araceae	Wetland
25	*Amaranthus spinosus* L.	Spiny amaranth	Amaranthaceae	Wetland
26	*Sphaeranthus indicus L.*	Gork hmundi	Asteraceae	Wetland
27	*Peristrophe paniculata* (Forssk.) Brummitt	Panicled Foldwing	Acantharean	Wetland
28	*Limnophila gratioloides* R. Br.	–	Scrophulariaceae	Wetland

(continued)

Table 15.1 (continued)

Sr. No.	Botanical name	Common name	Family	Morpho-ecological group
29	*Bergia ammannioides*	Jerry water fire	Elatinaceae	Wetland
30	*Cressa cretica* L.	European water clover	Convolvulaceae	Wetland
31	*Phyla nodiflora* (L.) Greene	Turkey tangle fogfruit	Verbenaceae	Wetland
32	*Elaeocarpus variabilis* Zmarzty	Chorphone	Elaeocarpaceae	Wetland
33	*Aeschynomene indica* L.	Curly indigo	Fabaceae	Wetland
34	*Malvastrum coromandelianum (L.) Garcke*	False mallow	Malvaceae	Wetland
35	*Melgmium indica*	–	Malvaceae	Wetland
36	*Melgmium indica*	–	Malvaceae	Wetland
37	*Paspalidium geminatum* (Forssk.) Stapf	Egyptian panicgrass	Poaceae	Wetland
38	*Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap	Camelthorn	Fabaceae	Wetland
39	*Eliocharis dulsis*	Water chestnut	Cyperaceae	Wetland
40	*Scripus articulatus* L.	Apurau	Cyperaceae	Wetland
41	*Ischaemum rugosum* Salisb.	Wrinkle duck-beak	Poaceae	Wetland
42	*Cyperus difformis* L.	Flat sedge	Cyperaceae	Wetland
44	*Ammannia multiflora* Roxb.	Red stem	Lythraceae	Wetland
45	*Alternanthera philoxeroides*	Alligator weed	Amaranthaceae	Wetland
46	*Xanthium spinosum* L.	Prickly burweed	Asteraceae	Wetland
47	*Cyperus* sp.	–	Cyperaceae	Wetland
48	*Verbascum chinense* (L.) Santapau	Velvet Plant	Scrophulariaceae	Wetland
49	*Hyptis suaveolens* (L.) Poit.	Pig nut	Lamiaceae	Wetland
50	*Abutilon indicum* (L.) sweet	Indian mallow	Malvaceae	Wetland
51	*Croton abaitensis* Baill.	–	Euphorbiaceae	Wetland
52	*Barleria prionitis* L.	Porcupine flower	Acanthaceae	Wetland
53	*Woodfordia fruticosa* (L.) Kurz	Dhaura	Lythraceae	Wetland
54	*Achyranthes aspera* L.	Prickly chaff flower	Amaranthaceae	Wetland
55	*Lindenbergia muraria* (Roxburgh ex D. Don) Brühl	–	Orobanchaceae	Wetland
56	*Polygonum glabrum* willd	Dense flower knotweed	Polygonaceae	Wetland
57	*Eleocharis atropurpurea* (Retz.) J. Presl & C. Presl	Purple spike rush	Cyperaceae	Wetland
58	*Paspalum disticum* L.	Ginger grass	Poaceae	Wetland
59	*Urena lobata* L.	Cesar weed	Malvaceae	Wetland

(continued)

Table 15.1 (continued)

Sr. No.	Botanical name	Common name	Family	Morpho-ecological group
60	*Echinops echinatus* Roxb.	Indian globe thistle	Asteraceae	Wetland
61	*Blumea laciniata* (Wall. Ex Roxb.) DC.	Cut leaf Blumea	Asteraceae	Wetland
62	*Blumea lacera* (Burm.f.) DC.	Karonda	Asteraceae	Wetland
63	*Blumea* sp.	–	Asteraceae	Wetland
64	*Spilanthes acmella* L.	Toothache plant	Asteraceae	Wetland
65	*Cocculus hirsutus* L.	Broom creeper	Menispermaceae	Wetland
66	*Lindernia oppositifolia* (L.) Mukerjee	Yellowseed false pimpernel	Linderniaceae	Wetland
67	*Vernonia cinerea* (L.) less.	Little ironweed	Asteraceae	Wetland
68	*Aura lanata* (L.) Juss.	Gorakh-boonti	Amaranthaceae	Wetland
69	*Fimbristylis bisumbellata* (Forssk.) Bubani	Grasslike fimbry	Cyperaceae	Wetland
70	*Crotalaria herpetoclada* Rossberg	Rattlepods	Fabaceae	Wetland
71	*Phyllanthus virgatus* G. Forst.	Narrow piss weed	Phyllanthocin	Wetland
72	*Euphorbia prostate*	Prostrate sandmat	Euphorbiaceae	Wetland
73	*Rungia pectinata* (L.) Nees	Comb Rangia	Acanthaceae	Wetland
74	*Tridax procumbens* L.	Jayanthi	Asteraceae	Wetland
75	*Sida alba* L.	Broomweed	Malvaceae	Wetland
76	*Triumfetta rhomboidea* Jacq.	Burr bush	Malvaceae	Wetland
77	*Cyperus haspan L.*	Papyrus sedges	Cyperaceae	Wetland
78	*Solanum tampicense* Dunal	Yellow-fruit nightshade	Solanaceae	Wetland
79	*Ludvigia perennis* L.	Water-primrose	Onagraceae	Wetland
80	*Vernonia cinerea* (L.) less.	–	Asteraceae	Wetland
81	*Phyllanthus amarus* Schumach. & Thonn.	Carry-me seed	Phyllanthaceae	Wetland
82	*Alternanthera ficoidea (L.) R.Br. Ex DC.*	Sanguinarea	Amaranthaceae	Wetland
83	*Paspalum distichum* L.	Biscuit grass	Poaceae	Wetland
84	*Ludvigia octavalvis* L.	Willow prime rose	Onagraceae	Wetland
85	*Hygrophila polysperma* (Roxb.) T. Anderson	Gokulakanta	Acanthaceae	Wetland
86	*Ammania baccifera* L.	Monarch red stem	Lythraceae	Wetland
87	*Alternanthera pungens kunth.*	Khaki weed	Amaranthaceae	Wetland

(continued)

Table 15.1 (continued)

Sr. No.	Botanical name	Common name	Family	Morpho-ecological group
88	*Commelina communis* L.	Asiatic dayflower	Commelinaceae	Wetland
89	*Equisetum ramosissimum* subsp. Debile	Snake grass	Equisetaceae	Wetland
90	*Caesulia axillaris* L.	Pink node flower	Asteraceae	Wetland
91	*Typha angustifolia L.*	Narrowleaf cattail	Poaceae	Wetland

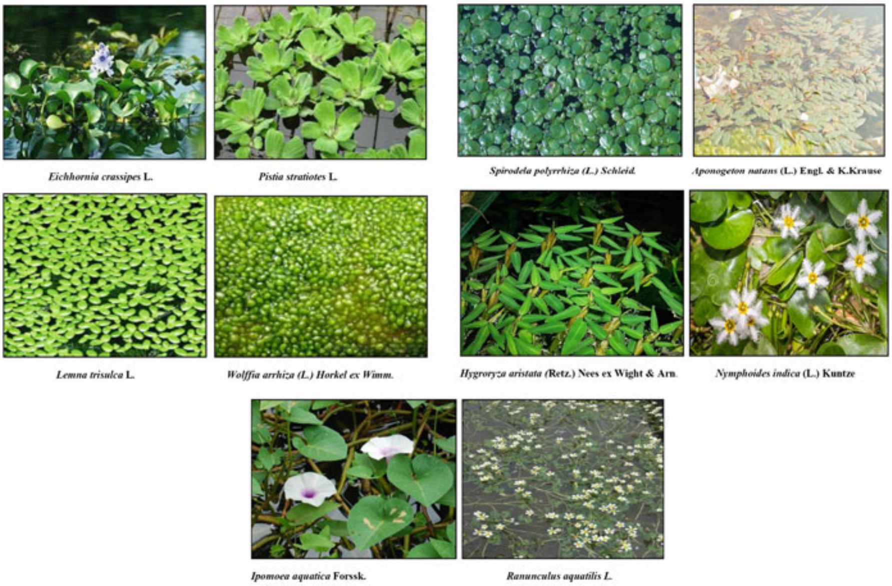

Eichhornia crassipes L.
Pistia stratiotes L.
Spirodela polyrrhiza (L.) Schleid.
Aponogeton natans (L.) Engl. & K.Krause
Lemna trisulca L.
Wolffia arrhiza (L.) Horkel ex Wimm.
Hygroryza aristata (Retz.) Nees ex Wight & Arn.
Nymphoides indica (L.) Kuntze
Ipomoea aquatica Forssk.
Ranunculus aquatilis L.

Fig. 15.1 Free-floating macrophytes of Indian wetlands

Spirodella, Eichhornia and *Pistia*, *Ranunculus aquatilis,* belongs to this category. Figure 15.1 represents plants of free-floating groups found in wetlands of India.

1.1.5 Significance of Aquatic Biodiversity in Ecosystems

Diverse group of aquatic habitats ranging from fresh water (lakes, ponds, reservoirs, rivers, streams, groundwater, and wetlands) to marine (ocean, estuaries, salt marshes, coral reefs, mangroves and algal colonies) ecosystems contemplated as the eco-sensitive and socio-economically important systems. The overall health of our environment is well maintained and supported largely by enormous economic and inventive value of a particular Aquatic ecosystem. Aquatic habitats nurture wide variety of organisms providing them with nutrition, materials, breeding ground etc. which are essential for sustaining life in the ecosystem. (King and Brown 2010). These critical elements of dynamic processes of Earth are the fundamentals for human economies and health. Humans rely on food, medicines and other materials available in aquatic habitats since long. Rivers, lakes, and estuaries are hubs for wildlife and are important for navigation, industries and recreation (Article, National Geographic, Aquatic ecosystem).

Habitat diversity and their resources shows absolute correlation with the biodiversity of a particular ecosystem which is true for Aquatic ecosystem as well. They perform multiple functions and are the most precious and are essential for the integrity of biotic communities. The earth's biological diversity is substantially supported by Aquatic ecosystems and they share a huge proportion of biological productivity. The quality of human life is also corelated with the resources of Aquatic ecosystems. For instance, to process our waste products, we depend on inhabitants (like fungi and bacteria) of surface water that continuously degrades hazardous toxins and nutrients that we flush into nearby water resources or into our sewage systems.

Aquatic plants in fresh waters are the most essential components of food web dynamics. Though phytoplankton and epiphytic algae are present in almost all fresh water systems, much of the food is derived from the aquatic plants after they are dead. Detritus that are formed after the death of these plants is consumed by detritivores like insects, invertebrates and larger crustaceans which are in turn being eaten up by bluegill sunfish which is the dominant littoral forage fish. The top predators depend on this forage fish for food. Thus, to sustain Aquatic food chain, Aquatic plants play a key role.

Freshwater ecosystems like ponds and lakes, streams and river and wetlands have many uses to humans including his way of living. For e.g. to harness powers to generate electricity, dams are built across these resources. They serve as important resources in economic activity of the any country/state where farmers transport surface and groundwater resources to their fields. This generate huge income in terms of fiscal value every year. The building of dams and barrages helps controlling flood and movement of goods respectively. Moreover, sustaining crops via irrigation practices, fresh water ecosystems provide food through fisheries. Apart from this, Freshwater ecosystems naturally share resources between habitats. The ecosystems in rivers and streams from higher elevation carry nutrients and other salts from other fresh water bodies like streams and wetlands located at lower elevation which subsequently get deposited to the ocean. The ecosystem of freshwater type help enabling many migrating species like salmon to bring nutrients from the marine ecosystem to upstream freshwater systems. Seasonally, these fresh waterways can also exchange nutrients. Having high density than warm water, cold water sinks to the bottom where temperature is comparatively steady. With the commencement of winter season, the temperature of air drops and the water closer to the surface fall below the temperature at bottom. As a consequence of this, heated water from the bottom rise and cold water from the surface sinks. Along with this, the nutrients are churned from the bottom and are brought to the surface.

Similarly, wetland ecosystem provides number of invaluable services to mankind. In regions where there is high intensity rainfall occurs over a short period, wetlands stop spreading out and slowing down floodwater, also reduces severity of downstream flood, simultaneously can limit the extent of erosion. Because of their effectiveness in absorbing water during wet period and release during dry period, wetlands act as sponges. Thus, regulates flow of streams. Wetlands can slowdown velocity of runoff and thus are able to trap and retain sediments. The presence of wetland vegetation also assimilates nitrates and phosphates through action of anaerobic bacteria. Apart from all these, wetlands contribute large share towards maintenance and supporting floral and faunal biodiversity.

Unfortunately, fresh and marine biodiversity are at a greater risk due to certain factors like species overexploitation, industrial, urban and Agricultural land pollution, Habitat modification/alterations due to construction of dams, deforestation, converting marshy habitats and other water bodies for different purposes (Basem 2020) Both, artificial and natural alteration in the environment bring about increased susceptibility of valuable aquatic resources. These changes ultimately result in the extinction of the species / populations and decreased in population size of Aquatic biota. Therefore, in order to insulate and preserve our valuable Aquatic resources we should implement and follow conservation strategies which would not only help maintaining the balance of nature but also make available the resources for forthcoming generations.

These conservation approaches involves restoration of damaged Aquatic habitats and species population, development of Aquatic bioreserves to conserve existing biodiversity in the habitats, building up large as well as small scale biosphere reserves targeted for conservation of Aquatic biodiversity within an area, watershed management practices, tree plantation in catchment area, avoiding establishment of industrial area and thermal power plants near Aquatic bodies, identifying threatened or endangered aquatic species and increasing public awareness towards biodiversity (Kumar and Asija 2009).

1.2 Heavy Metal Pollution in Aquatic Environment

1.2.1 Occurrence of Heavy Metals in the Environment

Crustal rock and the minerals it found in those rocks are the ultimate and primary headspring of heavy metals in the environment including Aquatic bodies of both the types; Fresh and Marine waters (Brathwaite and Rabone 1985) Various types of host rocks contain Zinc(Zn), Copper (Cu), Lead (Pb), Mercury (Hg), Cadmium (Cd), Nickel (Ni), Silver (Ag), Chromium(Cr), Iron (Fe), Selenium(Se), Titanium (Ti) and Arsenic (As) in varying amount. Natural weathering of these host rocks and liberation of metals from their indigenous spheres significantly contributes towards heavy metal pollution in the environment. (Masindi and Khathutshelo 2018) Among the listed heavy metals; Zn is mainly located in sedimentary rocks that includes limestone, dolostone, salt and shale. Zinc carbonate ($ZnCO_3$), known as calamine or smithsonite is commonly found as Zn ore (Peter and Tharsika 2017). The host rocks of Cu are Igneous, Sedimentary, Metamorphic types. In amalgamation with sulphur oxides and oxygen, it is found as Sulphides and sulphur oxides respectively. Its combination with cobalt, Ni, Pb, Ag and Fe forms complex Cu minerals. Pb is found mainly in sedimentary rocks (including limestone, dolostone, salt and shale), igneous and metamorphic rocks. $ZnCO_3$ known as calamine or smithsonite is a common Zn ore. A Pb sulphide mineral is known as Galena (Peter and Tharsika 2017).

Hg is found in pure form in found in volcanic rocks, but is usually occur in the mineral called cinnabar which is chemically known as Hg sulphide. Cd is principally found in rocks of sedimentary type, the amount being much higher than that in in igneous or metamorphic rocks (except nonferrous metallic ores of Zn, Pb and Cu (ICAD). It occurs naturally in Earth's curst in combination with Zn. In air, it gets released through volcanoes and forest fires. Rocks of Igneous and Metamorphic type contain Ag. Native elemental mineral form is rare. In this state it is often associated with quartz, gold, Cu, sulphides, arsenide of other metals and other Ag minerals.

Cr which is Naturally occurring is typically found associated with ultramafic igneous and metamorphic rocks like peridotites and serpentinites, and their derived soils (Chrysochoou et al. 2016). Earth's most important Fe ore deposits are found in sedimentary rocks. They are formed from chemical reactions which combines Fe and oxygen in marine and fresh waters. Hematite (Fe_2O_3) and magnetite (Fe_3O_4) are the two most important minerals found in the deposits as Fe oxides. The Heavy metal Se is mainly concentrated in sedimentary rocks like shales, sandstones, limestones, and phosphorite rocks. Ti metal is usually present in igneous rocks and in the sediments derived from them and is not found as the free element. It is found in the minerals rutile (TiO_2), ilmenite ($FeTiO_3$), and sphene, and is present in titanites and in many Fe ores.

Barium (Ba) is a trace element occurring in igneous and sedimentary rocks in association with potassium- bearing minerals in the Earth's crust and it becomes available to the aquatic environment through chemical weathering of rocks and minerals (Liguori et al. 2016). Ultramafic rocks possess two principle minerals that contain Ni. These are olivine (High amount of Ni) and orthopyroxene (small amount of Ni). These minerals are the prime source of the Ni (Lightfoot 2017).

The heavy metal As is largely present in Sedimentary Rocks of Limestones, Sandstones, Shales and clays Phosphorites and Sedimentary ores of iron and manganese. The highest amount of As is associated with sulphide minerals and metal oxides, specifically iron oxides (Pal 2015).

Apart from this natural sources of Heavy metals, forest fires, volcanic eruption, biogenic sources, sea salt spray and wind-borne particles cause natural emission of heavy metals that may occur under different and indubitable environmental conditions. Anthropic activities such as industries, wastewater generated from different sectors, agriculture, mining etc. has paramount share in polluting different compartments with heavy metals and from there they take entry in the sediment as well (Huang et al. 2020).

In Aquatic ecosystems, sediment act as supply for the source as well as sink of heavy metals. After entering into the Aquatic systems, most of the heavy metals get quickly deposited into the sediment, get concentrated in much higher amount than in the waters of Aquatic bodies and in the sediment their concentration reaches at the peak level in the water body of Aquatic systems (Shyleshchandran et al. 2018; Liu et al. 2018). Because of the deposition of Heavy metals into the sediments, the characteristics of the sediment is most likely get changed both in terms of its physicochemical or hydrological conditions. As a result, heavy metals present in the sediment may either get removed from the surface on which they are adsorbed or resuspend and this in turn cause secondary pollution in Aquatic bodies.

In coastal ecosystem, metals exist in two states., 1. Dissolved in the water gauge or 2. Deposited in the sediment beds. These states of metals depend on certain physico-chemical characters like pH, conductivity, salinity and organic matter and also on the nature of chemical species. Metals such as Pb, As, Cd, Cr, Hg, Ni are the key indicators of anthropogenic pressure in coastal environment (Idris 2008; Cuculic et al. 2009). They get discharged into the Aquatic environment through storm water and effluent discharges of Industrial waste. Thus, are of the potential risk to the Aquatic Environment.

1.2.2 Sources of Heavy Metals in Aquatic Bodies

There is a significant contamination of fresh water resources as well as artificial eutrophication of lakes and reservoirs and an accelerating accumulation of toxic metals in human food chain. This has assumed serious proportion in the twentieth century and water, one of the most precious and vital components of the biosphere, is being constantly polluted.

The various sources of different heavy metal contamination in the environment are Agriculture pesticides, smelting, mining, metallurgical industrial waste, pharmaceutical fertilizers, Industrial smoke, Waste disposal, Corrosion and so forth.

Erosion of soil is also a potential source of heavy metals in water. During run-off caused by the erosion heavy metals that have bound to soil sediments are picked up and many a times particularly during rain fall wastes carrying heavy metals are washed into poor drainage systems and also into nearby aquatic bodies and thus distributed into Aquatic systems (Taiwo et al. 2011). In addition to this, fine colloidal particles called as Aerosols also carry various pollutants like heavy metals and smoke cloud. Emissions from chimneys of different industries as well as solid particles present in the smoke get deposited on land or water bodies (Akpor et al. 2014).

The industrial sources of Heavy metals are listed in Table 15.2. Anthropogenic emissions of heavy metals have a major share in contributing heavy metals in the

Table 15.2 List of some heavy metals and their industrial sources. (Trivedi and Goel 1984)

Heavy metals	Industrial source
Aluminum (Al)	Coal-fired power plants or incinerators, paper industries
As (As)	Textile, electrical, wood and wood products, paper manufacturing units, paint industries
Barium (Ba)	Paper industries, textile and garment industries
Cd (Cd)	Electroplating factory, Cd-Ni batteries preparation, control rods, shields within nuclear reactors, television phosphors, paint industries
Cr (Cr)	Mines, electroplating, leather tanning, industrial coolants, paint industries
Cobalt (Co)	Paper industries, textile and garment industries
Copper (Cu)	Paint industries, pesticides and fertilizer, textile and garment industries
Fluoride (F)	Food industries, fluoride-containing pesticides industries, fluorinated pharmaceuticals companies, Teflon pans
Cesium (Cs)	Nuclear power plants, nuclear powered satellites or submarines
Iron (Fe)	Paper industries
Pb (Pb)	Paint industries, pesticide industries, battery manufacturing, crystal glass preparation, textile and garment industries
Manganese (Mn)	Paper industries
Hg (Hg)	Pharmaceuticals and electronics, thermal power plants, medical waste, paint industries
Ni (Ni)	Stainless steel manufacturing industries, electroplating factory discharge, automobile batteries, paint industries
Se (Se)	Energy generation, metal and oil refining, mining
Ag (Ag)	Photographic and imaging industry
Ti (Ti)	Continental steel and tube company
Vanadium(V)	Mining, electroplating industry, alloy
Zinc (Zn)	Smelting and electroplating industries, paint industries, textile and garment industries

environment that ends up in wastewater. Various industrial products including metals like Cd, Pb, Mn, Cu, Zn, Cr, Hg, As, Fe and Ni are discharged in waste. Some of the human sources of heavy metals in wastewater. Wastewater (effluents) from metal finishing, electroplating, Textiles, metal mining and smelting activities and nuclear power are the commonest anthropogenic fount of heavy metals that contaminate Aquatic bodies. Also, the wet sludge that is generated in the cleaning processes and in the treatment of wastewater produced in substantial quantities contain toxic metals in high amount (Cushnie 1985).

After functioning and operation of nuclear power plants, the nuclear effluent contains metals like Cu and Zn which is directly get discharged into surface water as well as ground water that pollute the entire Aquatic ecosystems (Begum et al. 2011).

1.2.3 Responses of Aquatic Macrophytes to Heavy Metals

Some metals like Cu and Zn are required because of their role as micronutrients needed for growth and upkeep by aquatic plants. But excessive amounts of these metals can become toxic to plants. In response to heavy metal exposure, aquatic plants, like terrestrial plants develop basic strategies to grow in metal contaminated environment and they either grow as metal excluders or metal indicators or accumulators (Baker and Walker 1990). Wide range of aquatic plant species, such as water hyacinths *Eichhornia* sp., (*E. crassipes, E. azurea, E. diversifolia, E. paniculata*), *Salvinia* sp., (*S. molesta, S auriculata, S. minima*), water lettuce (*Pistia stratiotes*), giant duckweed, duckweed (*Lemna minor), Azolla* sp., submerged species such as *Potamogeton* sp. *Myriophyllum* sp. emergent species like *Typha* sp. *Scirpus* sp. *Limnocharis flava, Spartina* sp.*, Cyperus* sp. *and Phragmites* sp. capabilities to reduce the concentration of heavy metals from polluted water bodies (Soda et al. 2012; Rodriquez and Brisson 2015) because of this potential aquatic plant species have gained attention to use in phytoremediation process. Accumulation capacities of aquatic plants may vary from floating plants to emergent plants.

Aquatic macrophytes respond to heavy metals towards biochemical parameters after they accumulate metal ions from the environment which are very much specific -specific. These responses depend on inherent characteristic of plants (Nayek et al. 2010). Loss in chlorophyll pigment (due to chloroplast destruction and replacement of Mg^{+2} of chloroplast by metal ion), Reduced sugar (corresponded with the inhibition of photosynthesis or photosynthetic inhibition or respiration stimulation for more energy requirement in metal stress condition. (John et al. 2008). In many Aquatic macrophytes increase in total protein because of higher synthesis of metal binding content due to the higher synthesis of metal binding proteins also known as phytochelatins, high proline accumulation (due to increased biosynthesis of proline, hyperactivity of antioxidant activities of catalase; CAT and peroxidase; POD (due to over-production of reactive oxygen species (ROS) related to excess metal accumulation in leafy parts of aquatic macrophyte (Nayek et al. 2010). However, ascorbate peroxidase (APX) may reduced or remained unchanged which may be due to variation in the enzyme response that may vary with the plant species which produce free radicles (Mazhoudi et al. 1997).

Aquatic plants cannot show avoidance towards unwanted changes in the surrounding environment. When concentration of heavy metals exceeds optimal level, they trigger a sweeping physiological and biochemical amendment and also exert adverse impacts on the plants both directly and indirectly. Heavy metals are known to modify metabolic pathways of plants subjected to metal ions. This may prove even detrimental to these plants. Heavy metals induce oxidative stress by producing reactive oxygen species (ROS), Reduced or photosynthesis inhibition, respiration and disintegration of cell organelles and finally the death of plants (Zhang et al. 2017; Sooksawat et al. 2013; Sneller et al. 2000).

Apart from this, restarted growth (specially roots), chlorosis and necrosis of leaf like symptoms observed in plants exposed to heavy metals represent some phytotoxic responses followed by abscission as well as senescence that ultimately results into less nutrient uptake and which in turn interfere with the acquired biomass. For instance, Cd and Cu cause many abnormal structures related to morphological (such as breaking of roots, necrosis and colony disintegration, root break-up) and physiological (such as photosynthesis, pigment synthesis and enzyme activity) parameters of aquatic plants (Khellaf and Zerdaoui 2010; Xing et al. 2010). However, these responses depend on various factors like metal concentration, time of exposure, presence of more than one metal ion in the solution etc. For e.g., *Eichhornia crassipes* at 6 mg/l As concentration caused death on exposure to the metal at 8 days period but the plants are able to survive in Zn metal exposure at the same concentration i.e. 6 mg/l (Hasan et al. 2007). An Emergent Aquatic plant *Typha capensis* in the presence of As, Hg, Cd and Pb (multimetal solution) showed inhibition in the absorption (Wiafe et al. 2019). Nirogen metabolism which plays a crucial role in regulating growth and development of plant right from metabolism to distribution of resources among various plant parts is severely affected once heavy metal enter accumulated by plant body.

Like terrestrial plants, Aquatic Plants respond to detrimental effects of heavy metals through several mechanism. At first, they sense this heavy metal stress stimuli, alert transduction and transmission of signals into the cell, activating congruous measures to escape the negative effects heavy metal stress by harmonising molecular, physiological and biochemical status of the cell.

1.2.4 Adaptive Strategies of Aquatic Macrophytes

Plants possess a sophisticated and interrelated network of defence strategies due to which they can either avoid or tolerate intoxication to heavy metals. Physical fence are the first line of developing shield against metals in plants. These include presence of thick cuticle and trichomes (which are biologically active tissues), cell walls and also symbiosis with mycorrhiza (in terrestrial plants.) (Hall 2002; Wong et al. 2004; Harada et al. 2010). Structures like trichomes can store heavy metal ions; thus, helps in detoxification process and they also secrete secondary metabolites which protect the plants against hazardous effects of metals.

On the other hand, if heavy metal ions enter into cells and tissues of plants; they commence several cellular defence mechanisms which weaken and nullify harmful effects of metal ions. The primary way to endure or neutralize toxicity of metal ions, the plants synthesize various biomolecules such as low-molecular weight proteins like phytochelatins (PCs) and metallothionein (MT) which function as metal chelators. These chelators are the best designated sulphur-containing metal-binding ligands. They are known to contribute to metal homeostasis, the purpose being again detoxification. This they accomplished by buffering cytosolic metal concentrations (Verkleij and Sachat 1990). Heavy metals are known to bind to the thiol (–SH) that have low molecular weight showing a very high affinity for metals specially those which are toxic. These low molecular weight thiols are categorized as Biological thiols. Glutathione (GSH) and cysteine are known biological thiols. GSH works as a substrate for phytochelatin synthesis and which help detoxifying heavy metals like Cd and Ni (Freeman et al. 2004). Once PCs production in stimulated in response to heavy metal stress they form complexes with toxic metal ions in the cytosol and consequently get subsequently transported them into the vacuole (Salt and Rauser 1995). Another key element in this defence mechanism is the production of metallothioneins (MTs) metal complex at the intra- and intercellular level. This is followed by heavy metal removal from sensitive sites or legand metal sequestration into vacuoles. Thus, first by chelation of metal ions and then by sequestration in vacuoles, plants remain protected from deleterious effect of metals.

Though heavy metal accumulation in aquatic plants causes several morphological, biochemical, and ultrastructural abnormalities, chloroplast destruction, changes in nitrogen metabolism inactivation of enzymes and alteration in normal physiological and metabolic pathways; the plants develop a very potential mechanism to combat with toxicity induced by heavy metals. In response to heavy metal stress, plants are furnished with a repository mechanism to impede heavy metal toxicity.

Also, in response to heavy metal stress, determining plants' biochemical responses play a key role in revealing mechanisms that have role in adaptation, tolerance and expression of sensitivity towards the stress. Generation of Reactive Oxygen Species (ROS) is the primary response of plants including Aquatic inhabitants due to the auto oxidation of heavy metal which is redox-active. This ROS cause damage to the biomolecules. But as an adaptive strategy, plants develop a set of defence mechanisms that deal with oxidative stress. This mechanism is complex mechanism also known as ROS scavenging mechanisms. They occur at cellular as well as molecular level. These mechanisms not only slow down the process of oxidation of biomolecules and reduce damage to cell oxidation yet increase plant resistance towards heavy metals (Sharma and Dubey 2005). One of the prime constitutions of plant defence system includes antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) and low-molecular weight quenchers (cysteine, ascorbic acid, thiols, proline (Sharma and Dubey 2005). α-tocopherol, carotenoids, phenolic and nitrogen compounds (Singh et al. 2010). These enzymes have protective roles against oxidative damage (Nayek et al. 2010).

Heavy metal stress triggers the activity of these antioxidative enzymes which decrease the level of H_2O_2 that helps minimising the damage to cell membranes. Also, MDA which is produced by lipid peroxidation under the condition of distress also get enhanced. This increased MDA content play a key role that Pbs to adaptation and thus plants are able to overcome the stress finally Pbs to the survival.

In addition to this, Carboxylic acids and amino acids, such as citric, malic, and histidine (His), are potential ligands produced by the plants and thus play a vital role in detoxification and tolerance towards metal ions (Rauser 1999; Hall 2002) Heat shock proteins (HSPs) that normally show enhanced expression in antiphon to the growth of number of organisms at temperatures above the ideal temperature required for their growth; now known to express under heavy metal stress (Verling 1991; Lewis et al. 1999). HSPs not only function as molecular chaperones in normal protein folding and assembly, but may also function that provides shield and reconstruction of damaged proteins under the condition heavy metal stress. Apart from this, Metal-binding proteins and peptides in plants are known to enhance metal tolerance specifically Cd, Hg, and Pb.

A heterocyclic amino acids Proline forms fundamental part of proteins. It has osmoprotection and metal chelator properties which enables the plant to tolerate heavy metal stress. The antioxidative properties of Proline helps protecting the enzymes from denaturation due to the stress and also stabilize protein, regulates cytosolic acidity (Gajewska and Sklodowska 2007) repair the damage of chlorophyll (Carpena et al. 2003) and provide resources of nitrogen and energy (Chandrashekhar and Sandhyaran 1996). Under heavy metal stress, plants normally accumulate Proline which is an indication of tolerance towards metal ion stress.

However, the situation becomes worst when plant cells are confronted with metal ions to such an extent that may Pb to cell damage and Pb to death of the plant. To confront with the stress, plants are known to develop molecular responses that make them enable to tolerate the stress. For this, they possess metal transporter that are involved in metal absorption and homeostasis; thus possess significant role in tolerance. These include heavy metal (or CPx-type) ATPases, cation diffusion facilitator (CDF), family proteins (Williams et al., 2000) and the Zinc-iron permease (ZIP) family (Guerinot 2000).

1.3 Utilization of Free Floating Macrophytes in Bioremediation of Heavy Metals

1.3.1 Mechanism of Heavy Metal Bioaccumulation in Aquatic Plants

Since Aquatic bodies are continuously being exposed to contaminants such as pesticides, Herbicides, Fungicides, Heavy metals and other Hydrocarbon compounds due to Anthropogenic inputs, plants inhabitants of such ecosystem develop a variety of mechanism to deal with the pollutants. The mechanism by which they uptake, translocate and accumulate micronutrients in their cell; the same mechanism is involved with Heavy metals from Aquatic environment. For this reason, Phytoremediation technology has been widely used for the removal of contaminants including heavy metals from water and soil. This mechanism follows phytoextraction, Phyto stabilisation, rhizofilltration, and phytovolatilization (Wani et al. 2017).

Phytoextraction

This method which is also known as phytoaccumulation is mainly employed to remove pollutants from soil, sediment, wastewater and sludge. It involves uptake of pollutants through roots and then translocate them in their above ground plant parts such as stem and leaves that can be harvested and finally burnt to gain energy and recovery as well as recycle of absorbed metals from the ash (Erakhrumen 2017).

Phytostabilization

Phytostabilization is the most suited method for soil, sludge and sediment. It involves the basic concept that specific chemicals produced by the plants; in place of their degradation immobilize the contaminants. This is an effective way to prevent relocation of contaminants to groundwater or their entry into food chain (Cunningham et al. 1996). In this method, plants act as an impediment of percolation of water within the soil. Sorption, precipitation, reduction in metal valence etc. are the important properties of this method which is mainly used to treat metals like Cu, Zn, Cr, As and Cd (Kunito et al. 2001). By this method one can prevent movement of contaminants, it is best suited for preserving and restoring our soil and valuable Ground water resources. It is the safest option to clean the environment as it does not interfere with the natural obstruct environment. The disadvantage of this method is that movement of metals like contaminants is restricted but they continue to stay in the soil (Zeng et al. 2018).

Rhizofiltration

The roots play major role in this method and it makes usage of plants to ad/absorb the pollutants. Thus, it restricts movement of heavy metals like pollutant in groundwater. Aquatic plants with long fibrous root systems can be used in rhizofiltration since pH change in the rhizosphere as well as exudate from roots Pbs to precipitation of heavy metals onto the root surface. This can be productively used for various industrial effluents, radioactive contaminants and metals. Change or alteration of pH in the rhizospheric zone and exudates from root helps the heavy metal precipitation on the root surface the (Raskin and Ensley 2000).

Phytovolatilization

In this method, plants after absorbing a contaminant converts into a volatile compound and then transpire them into surrounding atmosphere through stomata and other transpiring surfaces. This method is more suited for plants like *Brassica napus* and *Brassica juncea* are appropriate species for the phytovolatilization of Se. Hg and Se are the Heavy metals that can be readily remediated by phytovolatilization process (Karami and Shamsuddin 2010).

Plants have also evolved highly specific mechanisms to translocate and store micronutrients. These same mechanisms are also involved in the uptake, translocation, and storage of toxic elements, whose chemical properties simulate those of essential elements. Plants possess and use a variety of mechanisms to deal with the contaminations especially heavy metals, hydrocarbon compounds and man –made chemicals such as herbicides, fungicides, pesticides. Plants sequester them in their cell walls. Plants chelate these contaminations in the soil in inactive forms or complex those in their tissues and can store them in vacuoles, away from the sensitive cell cytoplasm where most metabolic processes occur. Organics may be degraded in the root zone depending on their properties of plants or taken up, followed by degradation, sequestration, or volatilization. TEC which is the most common pollutant of Groundwater (Newman and Reynolds, 2004), atrazine, a herbicide (Burken and Schnoor 1997), TNT type of explosive (Hughes et al. 1997), MTBE, a fuel additive (Davis et al. 2003) and PCBs (polychlorinated biphenyls) can be Successfully emended using phytoremediation methods. Phytoremediation is an emerging technology that uses plants to remove contaminants from soil and water.

1.3.2 Heavy Metal Bioaccumulation Capacity of Free Floating Macrophytes

Aquatic macrophytes fit perfectly for phytoremediation of Heavy metals from Aquatic ecosystem because of their rapid growth, high productivity in terms of biomass, robustness and resistance towards toxic components in the environment. The use of such macrophytes is a good alternative for decontaminant the water bodies loaded with heavy metals. Aquatic plants can accumulate substantial amount of heavy metals through active and passive absorption but the capacity of accumulation by different plant organ such as root, stem and leaves varied a lot (Harguinteguy et al. 2014; Cai et al. 2018). Also, the studies on heavy metal accumulation by Aquatic macrophytes have reported that the accumulation capacity is maximum in submerged plants followed by free floating plants and is minimum in Emergent plants but this capability is highly influenced by Aquatic environment as well as plant species (Harguinteguy et al. 2014).

Eichhornia crassipes, Pistia stratiotes, Salvinia auriculata and *Lemna minor* are commonly occurring free floating aquatic plant species in wetlands of India. The experimental data on accumulation efficiency of different heavy metals has proved that these plants have great potential to clean up Aquatic Environment.

Eichhornia crassipes

This species is known to be highly tolerant to various pollutant including Heavy metals and possess high magnitude of absorption of metals particularly Cd, Pb, Hg, Cu, Ni and Zn. The accumulation of Cd-(97.5%), Hg-(99.9%), Pb-(83.4%) and Ni-(95.1%) was reported when the initial concentration of Cd: 0.24, Hg: 4.971, Pb: 1.199, Ni: 3.34 mg/l was present in industrial wastewater (Fazal et al. 2015). The magnitude of absorption for water hyacinth was estimated at 0.24 kg/ha for Cd, 5.42 kg/ha for Pb, 21.62 kg/ha for Cu, 26.17 kg/ha for Zn, and 13.46 kg/ha for Ni in Erh-Chung constructed wetlands of Taiwan (Liao and Chang 2004).

The plants exposed to Wastewater from steel effluents containing Al, Pb, AS, Cd, Cu had accumulated (removed) Al-(73%), Pb-(73%,) As-(74%), Cd-(82.8%), Cu-(78.6%) (Aurangzeb et al. 2014). Further, Cr accumulation had increased from 63% to 80% on 3rd and 9th day respectively while Zn accumulation had increased from 67% to 96% and 100% on 9th, 12th and 15th day respectively on exposure of the plant to the stock solution of the metal ions (Swarnalatha and Radhakrishnan 2015). This study had shown that water hyacinth is a felicitous candidate for phytoremediation of waste water polluted with many heavy metals.

Salvinia auriculata
When three species of Salvinia, viz. *S. natans, S. molesta* and *S. auriculata* was analyzed for checking their accumulation capacity for heavy metals, S. molesta showed maximum accumulation of Hg, Ni and Pb in the concentration of 18,575 ppm, 18,875 ppm and 18,275 ppm respectively. The results of the study inferred that *S. natans, S. molesta and S. auriculata* have the inherent capacity for the accumulation of appreciable quantities of heavy metals (Kumari et al. 2016). Wolff et al. (2012) have reported a multiple level approach in which Salvinia auriculata responses to Cd pollution in aquatic ecosystems.

The results indicated that S. auriculata can be explored as a bioindicator and can be further tested for biomonitoring programs where an aquatic ecosystem contaminated by Cd. Additionally, Polechonska et al. (2019) was investigated 10 trace elements (As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn) in *Salvinia natans*. The results indicated that the plant may be considered good accumulator for Cu, Fe, Ni and Zn and hyperaccumulator for Mn.

Pistia stratiotes
When the harmful impacts of eight potentially toxic trace elements (Ag, Cd, Cr, Cu, Hg, Ni, Pb and Zn) were examined for Pistia stratiotes, it was found to present differential accumulation and tolerance levels for different metals at similar treatment conditions. It is a good accumulator of Cu although other elements can also be captured by the plant (Odjegba and Fasidi 2004). Radu et al. (2018) evaluate the possibility to remove heavy metals Cd, Zn, Cr and Ni from wastewater using two aquatic plants, water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*).

These plants possessed outstanding abilities to metabolize and bioaccumulate heavy metals from various polluted aquatic environments. The results obtained by Farnese et al. (2014) indicated that water lettuce (*Pistia stratiotes)* has a high potential of being a bioindicator in As-contaminated aquatic environments. There was an escalation of Cu metal ion reported in the fronds and simultaneously it triggers transient oxidative defense reactions. Thus, the reports affirm the fact that the plant *L. minor* proved to be an ideal for its use in clean up technology of wastewater and can readily be used for industrial wastewater after its pretreatment. The sensitivity characteristics of the plant can be used for Biomonitoring of Industrial wastes.

Lemna minor
According to Obermeier et al., 2015 detoxification potential of *Lemna minor* for organic pollutants was high and increased significantly with incubation. Cu was accumulated in the fronds at high levels, and transient oxidative defence reactions were triggered. This work confirmed the significance of *L. minor* for the removal of Cu from water and the conjugation of the selective herbicide pethoxamide. It would be ideal to use it for attributions such as polishing the water after pre-treatment or even using its sensitivity characteristics for biomonitoring. Another comparison is made between *P. stratiotes, L. minor* and *Spirodela intermedia* for 15 days

laboratory experiments, where they obtained high absorption values in all the plants. Although the *L. minor* species did not survive at the end of the experiment due to vegetal cover in the water layer that prevents the increase of oxygen dissolved in the recipient, Pbing to the death of individuals (Miretzky et al. 2004).

1.3.3 Bioaccumulation Factor and Metal Accumulation Index

One of the important concepts in environmental risk assessment is Bioaccumulation Factor and Bio-Concentration Factor as they offer quantitative information regarding capability of an organism to take up a pollutant from the water.

Bioaccumulation factor is defined as the capacity of a particular plant species to concentrate/accumulate metal ion into its tissue from the metal contaminated environment (Ladislas et al. 2012). While calculating BAF dry weight of metal ion accumulating plant species is taken into account. On the basis of substratum i.e. from soil to Terrestrial plants; from sediments to Rooted Hydrophytes and from Aquatic water bodies to Aquatic plants, BAF is calculated using the following formula (Wilson and Pyatt 2007).

$$\text{Bioaccumulation factors (BAF)} = \frac{[\mathrm{X}]_{\text{organism}}}{[\mathrm{X}]_{\text{water}}}$$

Where $[\mathrm{X}]_{\text{organism}}$ is the concentration of the element X in the examined organism. $[\mathrm{X}]_{\text{water}}$ is the concentration of X in the water.

BAF is dependent on the ambient concentration of heavy metal in the surrounding environment (DeForest et al. 2007). Bioaccumulation factor (BAF) is often treated as spatially constant because toxic elements like heavy metals vary in wide range amongst plants as well as in the environment. It is used to measure concentration of metals in aquatic organisms / plants and is expressed in the unit L/kg.

Certain important characteristics make aqueous macrophytes most suitable candidates for the accumulation of metals. These may include their biomass, leaves and epiphytes surviving on these macrophytes, that provide large surface area for precipitation of certain pollutants like metals their absorption and finally sequestration. The accumulation efficiency of hydrophytes depends on characteristics of particular plant species and metal ion concentration in the water (Kadukin et al. 1982; Sood et al. 2012). For e.g. Some free-floating plants like *Eichhornia crassipes* showed high level of Cu accumulation while in *Ceratophyllum demersum* high concentration of Iron was detected. Most Aquatic plants have tremendous capacity to accumulate various metal ions in their plant parts from water. Research had proved that the Accumulation capacity in some Aquatic plants is*: Lemna gibba* > *Potamogeton pectinatus* > *Ceratophyllum demersum* > *Eichhornia crassipes* > *Najas armata* > *Phragmites australis.* This proves that these plant species are suitable candidates that can be explored in biomonitoring survey tool for heavy metal pollution marker and in biological treatment of polluted water contaminated with heavy metals like Fe, Cu, Cd, Zn, Ni, Pb etc. (Ali and Zyada 2015).

By calculating BAF, one can identify metal hyperaccumulator plant species. It provides scope for decontamination of Aquatic bodies from metal pollution thereby reducing pollution load. The plant species especially those which have BAF values greater than one, are appropriate for phytoextraction. The BAF values for hyperaccumulators is >1 mg/kg^{-1} whereas for accumulator and excluder plant species, it is <1 mg/kg^{-1}.

For any aquatic ecosystem, the amount of metals in water / sediments are referred as sensitive indicators (Jain et al. 2005). This is because the amount/concentration is directly related to their toxic impacts on that particular ecosystem. Therefore various measures such as Contamination factor (Cf), (Edet and Ofong 2002); Contamination index (Cd) (Nayek et al. 2010), modified degree contamination index (mCd) Devanesan et al. (2017) and Metal accumulation index (MAI) (Yasser et al. 2018) are used either singly or in combination with assessment of heavy metal enrichment and contamination in water and sediments. In particular, MAI is used to evaluate comprehensive pursuance of heavy metal accumulation in plants (Fig. 15.2).

The metal accumulation index (MAI) was determined by evaluating the overall performance of heavy metal accumulation in plants.

$$\mathrm{MAI} = \sum_{j=1} Ij$$

$$\mathrm{Ij} = \mathrm{X}/\Sigma\mathrm{X}.$$

Where, N is the total number of metals analyzed
Ij is the sub-index for variable j. Ij can be further defined,
x is its Σx is the mean concentration of an element and standard deviation

The capacity of hydrophytes for accumulating considerable amounts of metals is depends on characteristics of specific species of plants and metal ion content of water (Kadukin et al. 1982; Sood 2012). Among various hydrophytes studied for Idku Lake along the north and south sectors (El-Amier et al. 2018), *E. crassipes* (A plant of free-floating type) revealed paramount accumulation of all the heavy metals under investigation.

Some free-floating plants like *Eichhornia crassipes* showed high level of copper while in *Ceratophyllum demersum* high concentration of Iron was detected. Bioaccumulation factor values showed that the trend of accumulation of most metals was *Lemna gibba* > *Potamogeton pectinatus* > *Ceratophyllum demersum* > *Eichhornia crassipes* > *Najas armata* > *Phragmites australis* which make them suitable candidate to be used in biomonitoring survey as good tool for heavy metal pollution marker, in biological treatment of polluted water.(Fe, Cu, Cd, Zn, Ni, Pb) (Ali and Zyada 2015).

MAI was determined by evaluating the overall performance of heavy metal accumulation in plants.

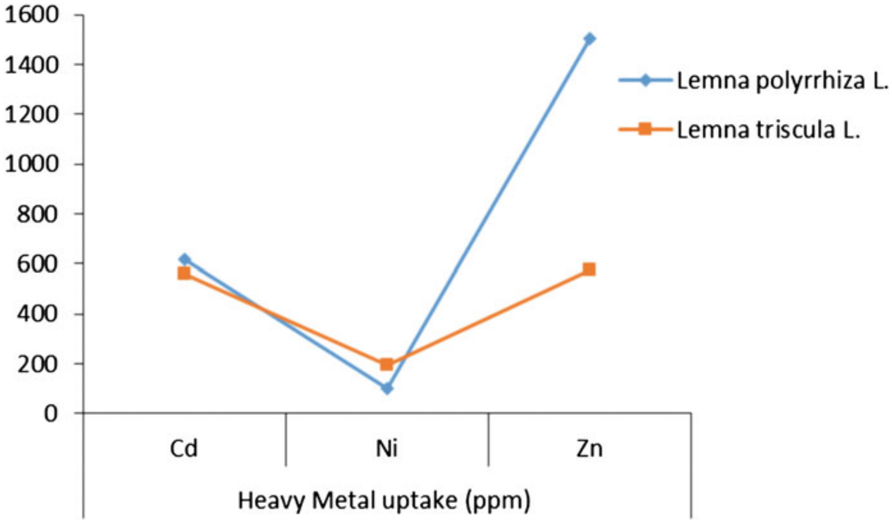


Fig. 15.2 Bioaccumulation of Cd, Ni and Zn metals after 9 days of exposure by 2 Lemna species. (Source: Unadkat 2017)

Aquatic macrophytes are powerful tools that can be explored to reduce the concentration of heavy metals from water bodies and have drawn a lot of attention worldwide. They possess tremendous capacity to accumulate/concentrate various metal ions in their plant parts and for this reason they are used in phytoremediation of heavy metal ions from both, industrial as well as municipal wastewater.

2 Conclusion

Anthropogenic emissions of heavy metals have causes widespread pollution of the natural ecosystems. Various industrial activities like metal finishing, electroplating, Textiles, metal mining and smelting activities and nuclear power discharge inorganic pollutant as well as organic pollutants to the natural ecosystem. These pollutants are resistant to degradation and represent an ongoing toxicological threat to both wildlife and human beings. Aquatic macrophytes have greater metal hyperaccumulation mechanisms, which is of particular importance in the context of the potential use of this plant in bioremediation process. Bioremediation has grown into a green, attractive and promising alternative to other conventional techniques in treating large volumes of wastewater with low concentration of pollutants as it can be more cost effective too.

References

Akpor OB, Ohiobor GO, Olaolu TD (2014) Heavy metal pollutants in wastewater effluents: sources, effects and remediation. Adv Biosci Bioeng 2(4):37–43

AliNafea EM, Zyada MA (2015) Biomonitoring of heavy metals pollution in Lake Burullus, Northern Delta, Egypt. Afr J Environ Sci Technol 9(1):1–7

Aurangzeb N, Nisa S, Bibi Y, Javed F, Hussain F (2014) Phytoremediation potential of aquatic herbs from steel foundry effluent. Braz J Chem Eng 31(4):881–886. https://doi.org/10.1590/0104-6632.20140314s00002734

Baker AJ, Walker PL (1990) Ecophysiology of metal uptake by tolerant plants: heavy metal tolerance in plants. In: Shaw AJ (ed) Evolutionary aspects. CRC Press, Boca Raton

Baseem SM (2020) Water pollution and aquatic biodiversity. Biodiversity Int J 4(1):10–16

Begum N, Sayeswara HA, Naik KL (2011) Seasonal variations of phytoplankton diversity in Bethur pond near Davangere, Karnataka. Environ Ecol 29(3):1355–1359

Brathwaite RL, Rabone SDC (1985) Heavy metal sulphide deposits and geochemical surveys for heavy metals in New Zealand. J R Soc NZ 15(4):363–370

Burken JG, Schnoor JL (1997) Uptake and metabolism of atrazine by poplar trees. Environ Sci Technol 31:1399–1406

Cai SW, Shen ZW, Ni ZH, Li YF, Liu B, Dai Y, Zhao J (2018) Metal distribution in water, sediment, submerged plant, and fish from an Urban River in Zunyi, Southwest of China. Fresenius Environ Bull 27:1627–1633

Carpena RO, Vazquez S, Esteban E, Fernandez-Pascual M, Rosario de Felipe M, Zornoza P (2003) Cadmium–stress in white lupin: effects on nodule structure and functioning. Plant Physiol Biochem 161:911–919

Chambers P, Lacoul P, Murphy KJ, Thomaz SM (2008) Global diversity of aquatic macrophytes in freshwater. Hydrobiologia 595:9–26

Chandrashekhar KR, Sandhyaran S (1996) Salinity induced chemical changes in *Crotalaria striata* DC. Indian J Plant Physiol 1:44–48

Chavan AR, Padate SN (1962) The hydrophytes of Savali Taluka. J M. S. Univ Baroda XI(3):63–78

Chavan AR, Sabnis SD (1961) A study of the hydrophytes of Baroda and environs. J Indian Bot Soc XL(1):121–130

Chrysochoou M, Theologou E, Bompoti N (2016) Occurrence, origin and transformation processes of geogenic chromium in soils and sediments. Curr Pollution Rep 2:224–235. https://doi.org/10.1007/s40726-016-0044-2

Cuculic V, Kwokal CNZ, Mlakar M (2009) Natural and anthropogenic sources of Hg, Cd, Pb, Cu and Zn in seawater and sediment of Mljet National Park, Croatia. Estuar Coast Shelf Sci 81 (3):311–320

Cunningham SD, Huang JW, Chen J, Berti WR (1996) Abstracts of papers of the American Chemical Society. American Chemical Society, vol. 212, p 87

Cushnie GC (1985) Electroplating wastewater pollution control technology. Noyes Publication, Park Ridge, pp 375–377

Darwin CR (1859) On the origin of species by means of natural selection. J. Murray, London

Davis LC, Erickson LE, Narayanan N, Zhang Q (2003) Modelling and design of phytoremediation. In: Phytoremediation: transformation and control of contaminants. Wiley, New York, pp 663–694

Devanesan S, Alsalhi MS, Vishnubalaji R, Alfuraydi AA, Nehad MAM, Murugan K, Shaban RMM, Nicoletti M, Benelli G (2017) Rapid biological synthesis of silver nanoparticles using plant seed extracts and their cytotoxicity on colorectal cancer cell lines. J Clust Sci 28:595–605

DeForest DK, Brix KV, Adams WJ (2007) Assessing metal bioaccumulation in aquatic environments: the inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration. Aquat Toxicol 84:236–246

Edet AE, Offiong OE (2002) Evaluation of water quality pollution indices for heavy metal contamination monitoring. A study case from Akpabuyo-Odukpani area, Lower Cross River Basin (southeastern Nigeria). Geo J 57:295–304

El-Amier YA, El-Alfy MA, Nofa MM (2018) Macrophytes potential for removal of heavy metals from aquatic ecosystem, Egypt: using metal accumulation index (MAI). Plant Arch 18 (2):2131–2144

Erakhrumen AA (2017) Phytoremediation: an environmentally sound technology for pollution prevention, control and remediation in developing countries. Educ Res Rev 2:151–156

Farnese FS, Oliveira JA, Lima FS, Leao GA, Gusman GS, Silva LC (2014) Evaluation of the potential of *Pistia stratiotes* L. (water lettuce) for bioindication and phytoremediation of aquatic environments contaminated with arsenic. Braz J Biol 74(3–1):37–49

Fazal A, Mustafa HSB, Hasan E, Anwar M, Tahir MHN and Sadaqat HA (2015) Inter relationship and path coefficient analysis among yield and yield related traits in sesame (Sesamum indicum L.). Nature Sci 13:27–32.

Freeman JL, Persans MW, Nieman K, Albrecht C, Peer W, Pickering IJ, Salt DE (2004) Increased glutathione biosynthesis plays a role in nickel tolerance in Thlaspi nickel hyperaccumulators. Plant Cell 16:2176–2191

Gajewska E, Skłodowska M (2007) Differential biochemical responses of wheat shoots and roots to nickel stress: antioxidative reactions and proline accumulation. Plant Growth Regul 54:179–188

Guerinot ML (2000) The ZIP family of metal transporters. Biochem Biophys Acta Biomem 1465:190–198

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53 (366):1–11

Harada E, Kim JA, Meyer AJ, Hell R, Clemens S, Choi YE (2010) Expression profiling of tobacco leaf trichomes identifies genes for biotic and abiotic stresses. Plant Cell Physiol 51 (10):1627–1637

Harguinteguy CA, Cirelli AF, Pignata ML (2014) Heavy metal accumulation in leaves of aquatic plant *Stuckenia filiformis* and its relationship with sediment and water in the Suquía river (Argentina). Microchem J 114:111–118

Hasan SH, Talat M, Rai S (2007) Sorption of cadmium and zinc from aqueous solutions by water hyacinth (*Eichchornia crassipes*). Bioresour Technol 98:918–928

Huang Z, Liu C, Zhao X, Dong J, Zheng B (2020) Risk assessment of heavy metals in the surface sediment at the drinking water source of the Xiangjiang River in South China. Environ Sci Eur 32:1–9

Hughes HB, Shanks J, Vanderford M, Lauitzen J, Bhadra R (1997) Transformation of TNT by aquatic plants and plant tissue cultures. Environ Sci Technol 31:266–271

Idris AM (2008) Combining multivariate analysis and geochemical approaches for assessing heavy metal level in sediments from Sudanese harbours along the Red Sea coast. Microchem J 90 (2):159–163

Jain AK, West TO, Yang X, Post WM (2005) Assessing the impact of changes in climate and CO2 on potential carbon sequestration in agricultural soils. Geophys Res Lett 32:1–4. https://doi.org/10.1029/2005GL023922. issn: 0094-8276

John R, Ahmad P, Gadgil K, Sharma S (2008) Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. Plant Soil Environ 54(6):262–270

Karami A, Shamsuddin ZH (2010) Phytoremediation of heavy metals with several efficiency enhancer methods. Afr J Biotechnol 9:3689–3698

Kadukin AI, Krasintseva VV, Romanova GI, Tarasenko LV (1982) Concentration of Iron, Manganese, Zinc, Copper and Chromium in Some Aquatic Plants. Hydrobiol J 18:64–67

Khellaf N, Zerdaoui M (2010) Growth response of the duckweed *Lemna gibba* L. to copper and nickel phytoaccumulation. Ecotoxicology 19:1363–1368

King J, Brown C (2010) Integrated basin flow assessments: concepts and method development in Africa and South-east Asia. Freshw Biol 55:127–146

Kumar U, Asija MJ (2009) Biodiversity: principle and conservation. Vedams eBooks, New Delhi

Kumari S, Kumar B, Sheel R (2016) Bioremediation of heavy metals by serious aquatic weed, *Salvinia*. Int J Curr Microbiol Appl Sci 5(9):355–368

Kunito T, Saeki K, Oyaizu K, Mutsumoto S (2001) Characterization of copper resistant bacteria communities in copper contaminated soils. Eur J Soil Biol 37:95–102

Ladislas L, El-Mufleh A, Gérente C, Chazarenc F, Andrès Y, Bechet B (2012) Potential of aquatic macrophytes as bioindicators of heavy metal pollution in urban stormwater runoff. Water, Air, and Soil Pollut 223(2):877–888. https://doi.org/10.1007/s11270-011-0909-3

Lewis S, Handy RD, Cordi B, Billinghurst Z, Depledge MH (1999) Stress proteins (HSPs): methods of detection and their use as an environmental biomarker. Ecotoxicology 8:351–368

Liao S, Chang N (2004) Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan. J Aquatic Plant Manag 42:60–68

Lightfoot PC (2017) Petrology and geochemistry of the Sudbury igneous complex Nickel Sulfide Ores and Impact Melts: Origin of the Sudbury Igneous Complex presents a current state of understanding on the geology and ore deposits of the Sudbury Igneous Complex. Elsevier, pp 190–295

Liguori Bianca TP, Almeida Marcelo GDE, Rezende CEDE (2016) Barium and its importance as an Indicator of (Paleo) productivity. An Acad Bras Ciênc 88(4):2093–2103. (Annals of the Brazilian Academy of Sciences). https://doi.org/10.1590/0001-3765201620140592

Liu M, Zhong J, Zheng X, Yu J, Liu D, Fan C (2018) Fraction distribution and leaching behaviour of heavy metals in dredged sediment disposal sites around Meiliang Bay, Lake Taihu (China). Environ Sci Pollut Res 25:9737–9744

Masindi V, Khathutshelo LM (2018) Chapter: Heavy metals. In: Environmental contamination by heavy metals. Intech Open Publisher

Mazhoudi S, Chaoui A, Ghorbal MH, Ferjani E (1997) Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum*, Mill). Plant Sci 127:129–137

Miretzky P, Saralegui A, Cirelli AF (2004) Aquatic macrophytes potential for the simultaneous removal of heavy metals (Buenos Aires, Argentina). Chemosphere 57(8):997–1005

Mishra VK, Tripathi VD (2009) Accumulation of chromium and zones from aqueous solution using water hyacinth. J Hazard Mater 164(2–3):1059–1063

Nayek S, Gupta S, Saha R (2010) Effects of metal stress on biochemical response of some aquatic macrophytes growing along an industrial waste discharge channel. J Plant Interact 5(2):91–99

Newman LA, Reynolds CM (2004) Phytodegradation of organic compounds. Curr Option Biotechnol 15:225–230

Obermeier M, Schroder CA, Helmreich A, Schroder BA (2015) The enzymatic and antioxidative stress response of Lemna minor to copper and a chloroacetamide herbicide. Environ Sci Pollut Res 22(23):18495–18507

Odjegba VJ, Fasidi IO (2004) Accumulation of trace elements by *Pistia stratiotes*: implications for phytoremediation. Ecotoxicology 13(7):637–646

Pal P (2015) Introduction to the arsenic contamination problem. In: Groundwater arsenic remediation: treatment technology and scale

Peter R, Tharsika T (2017) Articles Earth sciences museum resources rocks and Minerals British Geological Survey, NERC

Phatak VG, Satakopan S (1957) Plant types of the ponds of the plains around Baroda. I- pond vegetation at harni (Baroda). J M.S. Univ Baroda 6:11–40

Polechonska L, Klink A, Dambiec M (2019) Trace element accumulation in *Salvinia natans* from areas of various land use types. Environ Sci Pollut Res 26:30242–30251

Prasad SN, Ramachandra TV, Ahalya N, Sengupta TA, Kumar AK, Tiwari VS, Vijayan L (2002) Conservation of wetlands of India – a review. Trop Ecol 43(1):173–186

Radu VM, Ionesscu P, Diacu E, Ivanov AA (2018) Removal of heavy metals from aquatic environments using water hyacinth and water lettuce. Rev Chim (Bucharest) 68(12):2765–2767

Ramsar S (2013) The list of wetlands of international importance. The Secretariat of the Convention on Wetlands, Gland, Switzerland

Raskin I, Ensley BD (2000) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, pp 53–70

Rauser RW (1999) Structure and function of metal chelators produced by plants. Cell Biochem Biophys 31:19–48

Rodriguez M, Brisson J (2015) Pollutant removal efficiency of native versus exotic common reed (*Phragmites australis*) in North American treatment wetlands. Ecol Eng 74:364–370

Saini S, Kumar N (2017) Assessment and identification of aquatic diversity of wetlands of Yamuna Nagar district, Haryana, India. Int J Life Sci SciRes 3(4):1253–1261

Salt DE, Rauser WE (1995) Mg ATP-dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiol 107:1293–1301

Sharma P, Dubey RS (2005) Lead toxicity in plants. Braz J Plant Physiol 17:35–52

Shyleshchandran MN, Mohan M, Ramasamy EV (2018) Risk assessment of heavy metals in Vembanad Lake sediments (south-west coast of India), based on acid-volatile sulfde (AVS)-simultaneously extracted metal (SEM) approach. Environ Sci Pollut Res 25:7333–7345

Singh R, Tripathi RD, Dwivedi S, Kumar A, Trivedi PK, Chakrabarty D (2010) Lead bioaccumulation potential of an aquatic macrophyte *Najas indica* are related to antioxidant system. Bioresour Technol 101:3025–3032

Sneller EC, Van Heerwaarden LM, Schat H (2000) Toxicity, metal uptake, and accumulation of phytochelatins in silene vulgaris exposed to mixtures of cadmium and arsenate. Environ Toxicol Chem 19(12):2982–2986

Soda S, Hamada T, Yamaoka Y, Ike M, Nakazato H, Saeki Y, Sakurai Y (2012) Constructed wetlands for advanced treatment of wastewater with a complex matrix from a metal-processing plant: bioconcentration and translocation factors of various metals in *Acorus gramineus* and *Cyperus alternifolius*. Ecol Eng 39:63–70

Sood A, Uniyal PL, Prasanna R, Ahluwalia AS (2012) Phytoremediation Potential of Aquatic Macrophyte, Azolla. AMBIO 41:122–137

Sooksawat N, Meetam M, Kruatrachue M, Pokethitiyook P, Nathalang K (2013) Phytoremediation potential of charophytes: bioaccumulation and toxicity studies of cadmium, lead and zinc. J Environ Sci 25(3):596–604

Swarnalatha K, Radhakrishnan B (2015) Studies on removal of Zn and Cr from aqueous solutions using water hyacinth. Pollution 1(2):193–202. https://doi.org/10.7508/PJ.2015.02.007

Taiwo AM, Adeogun AO, Olatunde KA, Adegbite K (2011) Analysis of groundwater quality of hand-dug wells in peri-urban areas of Obantoko, Abeokuta, Nigeria for selected physiochemical parameters. Pac J Sci Technol 12:527–534

Trivedi RK, Goel PK (1984) Chemical and biological methods for water pollution status. Environmental Publication, Karad

Turner RK, van der Bergh JCJM, Soderqvist T, Barendregt A, van der Straaten J, Maltby E, van Ierland EC (2000) Ecological-economic analysis of wetlands: scientific integration for management and policy. Ecol Econ 35(1):7–23

Unadkat KV (2017) Phytoremediation of heavy metal ions by selected aquatic weeds. PhD thesis. Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda

Verkleij JA, Schat C (1990) Mechanisms of metal tolerance in higher plants. In: Shaw AJ (ed), Heavy metal tolerance in plants: Evolutionary aspects, CRC Press, INC. Florida, Boca Raton, pp 179–193

Verling E (1991) The roles of heat shock proteins in plants. Plant Mol Biol 42:579–620

Wani RA, Ganai BA, Shah MA, Uqab B (2017) Heavy metal uptake potential of aquatic plants through phytoremediation technique – a review. J Bioremediat Biodegrad 8:404–409

Wiafe S, Buamah R, Essandoh H, Lawrence D (2019) Assessment of *Typha capensis* for the remediation of soil contaminated with As, Hg, Cd and Pb. Environ Monit Assess 191:346. https://doi.org/10.1007/s10661-019-7484-2

Williams LE, Pittman JK, Hall JL (2000) Emerging mechanisms for heavy metal transport in plants. Biochim Biophys Acta Biomembr 1465(1–2):104–126

Wilson B, Pyatt FB (2007) Heavy metal bioaccumulation by the important food plant, Olea europaea L., in an ancient metalliferous polluted area of Cyprus. B Environ Contam Tox 78:390–394

Wolff G, Pereira GC, Castro EM, Louzada J, Coelho FF (2012) The use of Salvinia auriculata as a bioindicator in aquatic ecosystems: biomass and structure dependent on the cadmium concentration. Braz J Biol 72(1):71–77

Wong HL, Sakamoto T, Kawasaki T, Umemura K, Shimamoto K (2004) Down-regulation of metallothionein, a reactive oxygen scavenger, by the small GTPase OsRac1 in rice. Plant Physiol 135(3):1447–1456

Xing W, Huang W, Liu G (2010) Effect of excess iron and copper on physiology of aquatic plant Spirodela polyrrhiza (L.) stilled. Environ Toxicol 25:103–112

Yasser B, Hassan E, Yasser A (2018) Screening of phytase producing fungi isolated from syrian soil. J Bio Innov 7(3):462–474

Zeng P, Guo Z, Cao X, Xiao X, Liu Y, Shi L (2018) Phytostabilization potential of ornamental plants grown in soil contaminated with cadmium. Int J Phytoremediation 20:311–320

Zhang T, Lu Q, Su C, Yang Y, Hu D, Xu Q (2017) Mercury induced oxidative stress, DNA damage, and activation of antioxidative system and Hsp70 induction in duckweed (Lemna minor). Ecotoxicol Environ Saf 143:46–56

Chapter 16
Bioremediation of Heavy Metals Using *Salvina Molesta* – A Freshwater Aquatic Weed

T. G. Nithya, P. Snega Priya, and M. Kamaraj

Abstract Heavy metal toxicity and the threat of their bioaccumulation in the food chain is one of the major environmental problems faced by our modern society. This challenge can be overcome by a promising environmental-friendly and cost-effective method using plants as a bioremediation system ie., phytoremediation process. Aquatic ferns in particular exhibit exorbitant potential to remove various contaminants including heavy metals, organic compounds, radionuclides from the environment. Among various aquatic macrophytes, *Salvinia* a free-floating aquatic fern holds a distinct position because of high productivity and tolerance to a wide range of temperatures. *Salvinia* is a genus of floating ferns belonging to the family *Salviniaceae* and it is a potentially growing freshwater weed causing disturbances to the aquatic habitats and is under the urge of eradication in developed countries. At present, giant *Salvinia* is one of the most widespread, environmentally, economically, and socially destructive invasive weed worldwide. Though these aquatic weeds are a disturbing factor to the environment they possess significant bioremediation properties. In the present study bioremediation of various heavy metal contaminants using *S molesta* will be discussed with relevant data structure and patterns of study and also the significance of weeds as a potential bioremediation agent will be elucidated.

Keywords *Salvinia molesta* · Phytoremediation · Aquatic ferns · Eco friendly · Heavy metals

---

T. G. Nithya (✉)
Department of Biotechnology, SRM Institute of Science and Technology, Kattankulathur, Tamilnadu, India

P. Snega Priya
Department of Medical Microbiology, SRM Medical College Hospital and Research Center, Kattankulathur, Tamilnadu, India

M. Kamaraj
Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_16

1 Introduction

Water pollution is a worldwide problem that leads to the cause of death and disease. According to researchers, the improper disposal of waste causes contamination of groundwater. The strongest water pollutants are insecticides, pollutants from livestock operations, volatile organic compounds, food processing waste, and chemical waste. Heavy metals are the most dangerous type of chemicals since they impose serious health hazards in all living organisms. The most common heavy metal contaminants are Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Lead (Pb), Nickel (Ni), and Zinc (Zn) (Lasat 2002). The heavy metal pollution in the environment is presently a global challenge. Heavy metal toxicity and the threat of their bioaccumulation in the food chain is one of the major environmental problems faced by our modern society. The noteworthy reason for heavy metal pollution is a discharge of contaminated sewage and effluents from industries (Prasannakumari et al. 2014).

In recent years the studies related to pollutant uptake and degradation are being a subject of interest. Many scientist are working hard towards decreasing the level of heavy metal pollution. Some of the most used methods of removal of contaminants are chemical precipitation, adsorption, ionic exchange, and electrochemical filtration. Most of these methods are costly and inefficient at low concentrations. These drawbacks in these methods demand the development of new low-cost water treatment methods. Therefore for the removal of heavy metal contaminants from the environment, the most preferred method is phytoremediation. Phytoremediation is defined as the capability of plants to degrade and reduce the pollutant from the environment. These plants can accumulate the toxic contaminants within themselves and also capable to destroy them (Dipu 2013).

Phytoremediation uses specific plants that can accumulate various heavy metals like lead, chromium, arsenic, etc., and its radioisotopes, which helps in cleaning soil and water contaminated with heavy metals. The recent work on the capability of certain chelating agent that helps in the removal of heavy metal by the plant is highly promising for commercial use in the future (Ghosh and Singh 2015). Aquatic ferns have the capability of degradation of contaminants.

Among various species, *Salvinia*, a free-floating aquatic fern holds a distinct position because of high productivity and tolerance to a wide range of temperatures (Olguin et al. 2003). *Salvina Molesta* is a free-floating weed that is native to South-Eastern Brazil. It is a highly accelerated vegetative reproduction and has earned the title of various serious weed in most regions outside its natural habitat. It can chock up the rivers and canals and has both direct and indirect effects on the aquatic ecosystem (Sreerama Kumar et al. 2005). It grows in the slow-moving water (or) still water like ponds, lakes, rivers, and prefers nutrient-rich water such as eutrophic water and polluted water. It grows best at pH less than 7.5 (Arzani et al. 2007) and the optimum temperature range is 25–36 °C (Lesniewicz et al. 2006). *Salvina molesta* can be used for the treatment of blackwater effluent in an eco-friendly sewage system (Moore and Ramamoorthy 1984; Prichard et al. 1984).

2 Phytoremediation

2.1 Definition

The phytoremediation term was coined in 1991. It is a combination of a Greek word consist of ‘Phyto’ meaning plants and the Latin word ‘Remedium’ meaning to remove the evil. The primary facts about phytoremediation were derived from a diversity of researches that includes oil spill management and accumulation of heavy metals by plants (EPA 2000). There are many definitions given by scientists about phytoremediation. Taking all into account, phytoremediation can be defined as a debouching technology where the plants were used to remove the hazardous contaminant from the polluted environment. Thus this process will be useful in the development of the quality of the surroundings (Tangahu et al. 2011). US Environmental protection agency 2000, states that phytoremediation is a cost-effective and new-fangled technology that can be used as an effective alternative over other technologies.

2.2 Methods of Phytoremediation

The mechanism of the plant in the removal of contaminants such as heavy metals is diverse. In the remediation of soil and water plants use six main mechanisms to make the environment clean. (Ali et al. 2013; Dixit et al. 2015).

The six main mechanisms are as follows: (Fig. 16.1)

- Phytoextraction
- Phytofiltration
- Phytostabilization
- Phytodegradation
- Phytovolatilization
- Rhizodegradation

2.2.1 Phytoextraction

The phytoextraction is the process by which the plant absorbs the contaminants from soil or water through the roots and transfers to shoot and get stored for gaining energy and nutrients (Erakhrumen and Agbontalor 2007; Erdei et al. 2005).

2.2.2 Phytofilteration

The Phytofilteration is defined as the process by which the plant is capable of sequestering or filtering the contaminants from water. (Sumiahadi and Acar 2018).

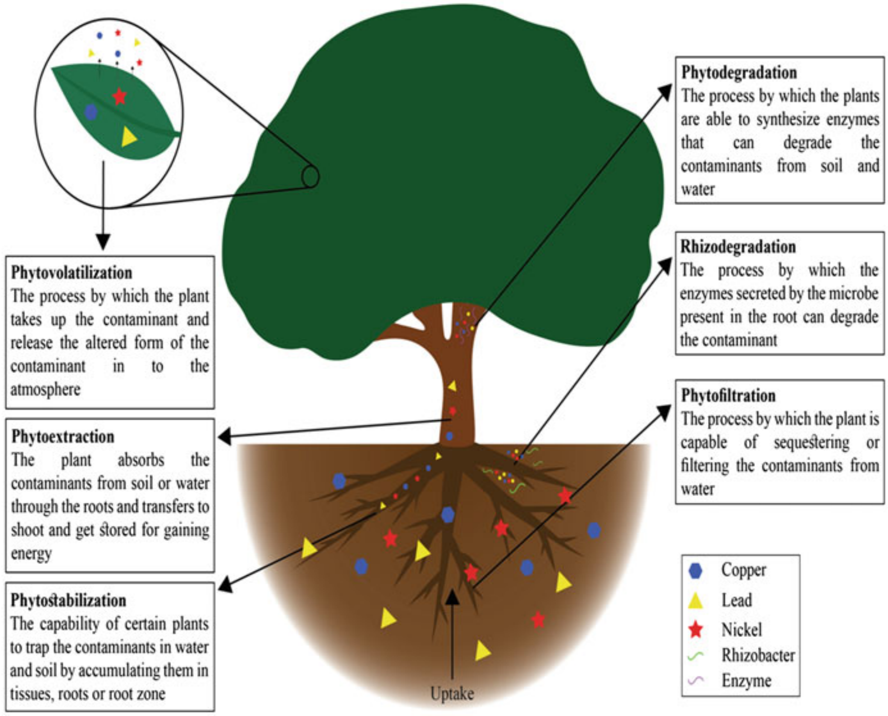


Fig. 16.1 Methods of phytoremediation

The rhizofiltration is defined as the process in which the contaminants can be precipitated in the root of the plant which can be helpful in the process of cleaning the wastewater that includes industrial effluents and heavy metals (Erakhrumen and Agbontalor 2007). Once the plant accumulates the contaminant it can be easily harvested and disposed of to the other medium, hence it’s an easier and efficient method.

2.2.3 Phytostabilization

The phytostabilization is defined as the capability of certain plants to trap the contaminants in water and soil by accumulating them in tissues, roots, or root zone. This resists the movement of the contaminants and further contamination. The removal of contamination from soil and sediments would be efficiently done using this technique. The natural environment is not involved and it is safer (Erdei et al. 2005).

2.2.4 Phytodegradation

Phytodegradation is defined as the process by which the plants can synthesize enzymes that can degrade the contaminants from soil and water (Sumiahadi and Acar 2018). This method has a higher ability to degrade the contaminants faster and effectively.

2.2.5 Phytovolatalization

Phytovolatalization is defined as the process by which the plant takes up the contaminant and releases the altered form of the contaminant into the atmosphere. Thus it reduces the level of contamination. The process is carried out when the plant uptakes the water along with contaminants. The leaves can absorb the contaminant and convert into a gaseous state further released in the air at low concentration (Erakhrumen and Agbontalor 2007; Erdei et al. 2005).

2.2.6 Rhizodegradation

Many microorganism are present in the roots of plants. They have a symbiotic relationship with particular plants. The rhizodegradation is defined as the process by which the enzymes secreted by these microbes help in the degradation of heavy metals and the treatment of wastewater. (Sumiahadi and Acar 2018). Plant growth-promoting rhizobacteria (PGPR) is one of such microbes which helps in the removal of heavy metal in bio augmented plants (Afzal et al. 2014).

2.3 Selection of Plants for Remediation

Many kinds of research have described the ability of plants as an accumulator of heavy metals from soil and water which are contaminated. Since the efficacy of remediation depends on certain properties and functions of the bioremediating plant, it is important to select the appropriate one for the treatment (Sumiahadi and Acar 2018).

According to Lasat (2000), The consideration for selection are the following:

- The biomass of the plant, the remediation rate of heavy metal is proportional to the biomass of the plant.
- Protection of Ecosystem, Native species must be selected which can be helpful in bio conservation.
- The deep-rooted plants would be appropriate for the contamination of soil and water which helps the removal of contaminants that are invasive.

- The shallow-rooted plants are preferred for surface contamination of soil and water.

3 Heavy Metal Toxicity

3.1 *Definition*

The heavy metals are defined as the elements or components which rectify the properties of metal and possess an atomic number greater than 20. Metals are naturally occurring elements found in soil. However, when the normal level exceeds, the metal can be toxic to animals, plants, and microbes (Lasat 2000). The more common and principal heavy metals which account to the contamination of the environment include arsenic (As), Strontium (Sr), Caesium (Cs), Uranium (U) (Raskin et al. 1997), Mercury (Hg), Chromium (Cr), lead (Pb), Manganese (Mn), Nickel (Ni), Cobalt (Co), Cadmium (Cd) Lead (Pb) and zinc (Zn) (Raskin et al. 1997; Lasat 2000). Among these certain metals is an inevitable micronutrient for the development of plants. Zn, Cu, Mn, and Ni are very important for the growth, and Cd, Pb, and Hg are important for the functioning of the plant (Gaur and Adholeya 2004).

3.2 *Sources of Heavy Metals in the Environment*

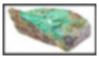
There are two main sources of heavy metals which include; (Ali et al. 2013) (Table 16.1)

- Natural source.
- Man-made source (Anthropogenic sources).

3.3 *Health Hazards in Heavy Metal Toxicity*

The consumption of higher amounts of heavy metal leads to severe health effects in human health (Table 16.2).

Table 16.1 Sources of heavy metals from natural and man-made sources

Heavy metals	Natural source	Man-made source
Arsenic 	Rocks and soils	Pesticides and wood preservative
Copper 	Volcanic dust	Pesticides and fertilizers
Cadmium 	By-product of zinc	Paints, phosphate fertilizers
Chromium 	Rocks, soil, volcanic dust	Tanneries, steel industries, fly ash
Mercury 	Earth's crust, emissions from volcanoes.	Medical waste, coal combustion
Nickel 	Earth core	Industrial effluents, steel alloys, automobiles batteries
Lead 	Soil	Battery manufacturing, insecticides, and herbicides

4 Bioremediation of Heavy Metal Toxicity by *Salvina molesta*

4.1 Aquatic Plants in Phytoremediation

The aquatic environment is a cost-efficient and wealthy cleaning technique for the removal of contamination of the large area. The plants of the aquatic ecosystem are found to be accumulators of contaminants and heavy metals naturally (Pratas et al. 2014). This method is found to be effective and provides a higher benefit (Guittonny-Philippe et al. 2015). The selection of aquatic plant is very important for the effective removal of contaminants (Fritioff and Greger 2003). In recent times the phytoremediation using aquatic plants have become an enormous technique to reduce the contaminants. An interesting factor is that the roots of aquatic plants are extensive in absorbing contaminants like heavy metals and can accumulate them. So the aquatic plants serve as the best choice of phytoremediation (Mays and Edwards

Table 16.2 health hazards in heavy metal toxicity

Metals/ metalloids	Disease/disorders	Reference
Arsenic (As)	Skin disorder, gangrene, kidney, and bladder cancer.	Buhari Muhammad et al. (2016)
Copper	Alopecia, anemia, arthritis, hypothyroidism, heart attack, etc.,	Jagdish Kumar et al. (2018)
Cadmium (Cd), zinc (Zn)	Acute-respiratory, gastrointestinal, kidney damage.	Mohammad et al. (2008)
Mercury (Hg)	Affects immune system, kidney malfunction, nervous disorder	Mukesh and Anil (2005)
Nickel	Headache, nausea, respiratory disorder, sometimes death.	Rendall et al. (1994)
Lead (Pb)- present in the soil	Brain damage	Hussain and Kecili (2020)
Cyclodines	Liver toxicity, affects central nervous system, headache, nausea, fatigue, eye irritation, and skin rash.	Hussain and Kecili (2020)

2001). There are many types of aquatic plants used in phytoremediation which include free-floating plants, water hyacinth, *Salvinia* (aquatic fern), water lettuce, duckweed, submerged aquatic plants, and emerged aquatic plants. In this chapter, the *Salvinia* species will be discussed elaborately.

4.2 Salvinia *Species*

Salvinia is a water fern, a free-floating macrophyte extensively scattered in aquatic ecosystems. It has the capability of reproducing quickly and has the ability to settle widespread colonies in lesser time. *Salvinia* can make its population double within 3 to 5 days under favorable conditions (Henry-Silva and Camargo 2006). It has a high growth rate with wide distribution and is easy to handle. Various noxious entities have supported the application of *Salvinia* to be used in phytoremediation (Gardner and Al-Hamdani 1997).

Salvinia species, especially *S. molesta* and *S. natans,* are possibly used in phytoremediation as it has extensive capacity for removal of heavy metals due to the rapid growth rate and sufferance to toxic pollutants (Dhir 2009; Dhir et al. 2011). It can effectively be used for the treatment of different kinds of wastewater treatment (Abd-Elnaby and Egorov 2012).*Salvinia* roots have a higher rate of metal accumulation.

Accumulation of toxic metal in S. *natans* and S. *minima* reduces with increasing concentration of phosphate, perhaps heavy-metal uptake increase with the addition of sulfur (Hoffmann et al. 2004). The presence of suitable environmental conditions along with certain nutrients and chelators will determine the hyper-accumulation of heavy metals *Salvinia* (Olguin et al. 2003). Different species of the *Salvinia* (water

Table 16.3 Removal rate of metals by *Salvinia* species

Metals/metalloids	Conditions (Initial concentrations)	Results	Reference
Cd, Ni, Pb, Zn		Removal rate:	Iha and Bianchini Jr (2015)
	Cd-(0.03 mgL^{-1})	Cd-(0.0045 mgm^{-2})	
	Ni-(0.40 mgL^{-1})	Ni-(0.0595 mgm^{-2})	
	Pb-(1.00 mgL^{-1})	Pb-(0.1423 mgm^{-2})	
	Zn-(1.00 mgL^{-1})	Zn-(0.4046 mgm^{-2})	
Zn, Cu, Ni, and Cr	15 mgL^{-1} initial concentration. 10 g biomass of five plants.	Removal rate:	Dhir et al. (2011)
		Zn-(84.8%)	
		Cu-(73.8%)	
		Ni-(56.8%)	
		Cr-(41.4%)	
Cu, Cr, Pb, Cd	0, 20, 40, 80, 160 M concentrations of $NiCl_2$	Accumulation of Ni is 16.3 mgg^{-1}	Fuentes et al. (2014)
Ni	Initial concentration	After treatment	Ranjitha et al. (2016)
	Cu-(1.092 ± 0.026)	Cu-(2.035 ± 0.014)	
	Cr-(2.201 ± 0.0024)	Cr-(1.052 ± 0.022)	
	Pb-(2.974 ± 0.018)	Pb-(1.924 ± 0.012)	
	Cd-(0.251 ± 0.017)	Cd-(0.018 ± 0.018)	
Pb, Ni, Cu, Zn, Mn, Fe, Cr, Cd	Effluents: Coal mine	Removal rate	Lakra et al. (2017)
		Pb-(96.96%)	
		Ni-(97.01%)	
		Cu-(96.77%)	
		Zn-(96.38%)	
		Mn-(96.22%)	
		Fe-(94.12%)	
		Cr-(92.85%)	
		Cd-(80.99%)	

fern) are an excellent accumulator of Iron, Cadmium, Nickel, Manganese, Zinc, and Lead as reported by several studies, and their details are reported in Table 16.3.

4.3 Salvina molesta

Salvina molesta is known as giant *Salvinia*, a free-floating fern in the family *Salvinia* ceae. The name *Salvinia* originates from the Latin word meaning troublesome. This species is distributed along with countries like Africa, the Indian subcontinent, South

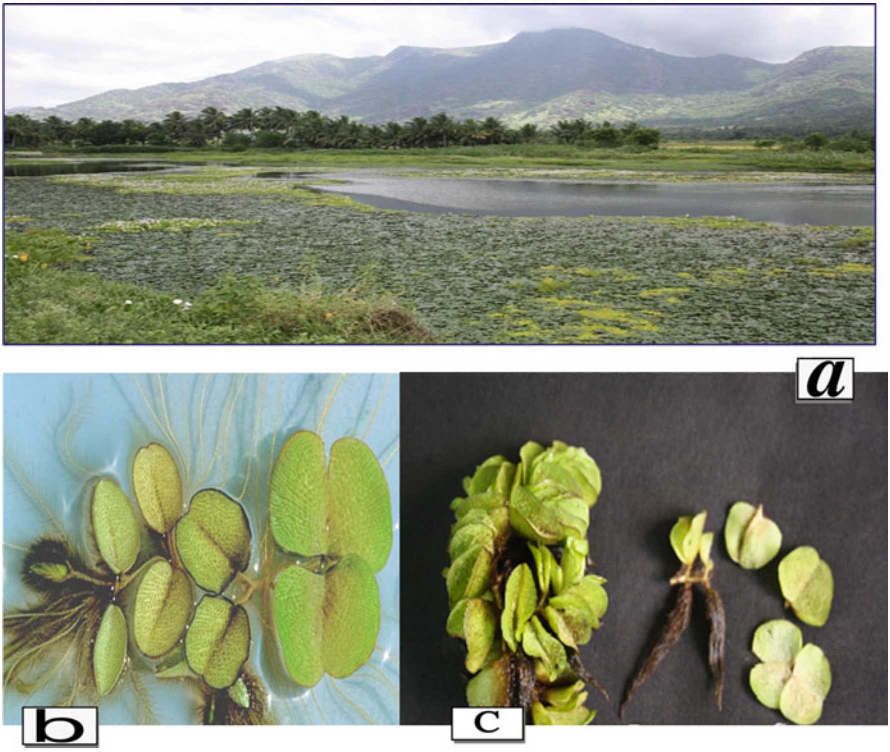


Fig. 16.2 Collection site and images of *Salvinia molesta*. (**a**) *Salvinia molesta* distribution in the aquatic ecosystem. (**b**) Ventral view of *Salvinia molesta* leaf. (**c**) *S.molesta* whole plant with stem and hairy root

East Asia, Australia, southern USA, and New Zealand. In India, the *Salvinia molesta* is widely distributed in the Kanyakumari district Fig. 16.2 (Thomson and Room 1986). *Salvinia* is included in the monogeneric family. *Salvina molesta* is a free-living *pteridophyte*. Each plant is composed of two floating green leaves and a set of brown leaves which is submerged and filiform in shape. It is coated with hairs with a velvety appearance at the surface of the leaves that absorb water and nutrients and act as a root. It was identified initially as the *S. auriculata* but later in 1972, it has been reclassified into male sporocarps or fruiting bodies (Nelson 2015). The ability of *S. molesta* in the removal of Cd, As, Pb, and Cu, etc., has been reported (Dipu 2013).

Ranjitha et al. (2016) has also confirmed that *S. molesta* is capable of reducing heavy metals released from industrial effluents. This plant accumulates all the heavy metals in the roots and during this process, they do not report any deviation from the regular growth process. This process of phytoremediation was termed as rhyzofiltration. This plant is also found to have 50–60% of lipids which can be extracted and used for biodiesel production. (George and Gabriel 2017).

4.3.1 Taxonomy of *Salvina molesta*

Domain: *Eukaryota.*
Kingdom: *Plantae*
Subkingdom: *Tracheobionta*
Phylum: *Pteridophyta*
Class: *Liliopsida*
Order: *Hydropteridales*
Family: *Salvinia*ceae
Genera: *Salvinia*
Species: *Salvina molesta*

Common names: Giant *Salvinia*, Karibaweed, water moss, water fern, Australianazolla, water spangles, African pyle.

Habitat: The habitat of *S. molesta* can be either still or slow flowing water such as lakes and dams.

4.4 Subspecies of Salvina molesta

There are 12 main subspecies in *Salvinia molesta.* They are *Salvinia natan, Salvinia oblongifolia, Salvinia cucullata,Salvinia biloba Radii, Salvinia herzogii, Salvinia auriculata, Salvinia minima Baker, Salvinia radula, Salvinia sprucei, S. martynii. Spruce, S. hastata Desv.*

4.5 Phytoremediation Process of Salvina molesta *Against Heavy Metals*

Salvina molesta can perform phytoremediation of heavy metals in the aquatic ecosystem due to its higher growth rate and capable of collecting nutrients from the source. *S. molesta* can accumulate toxic metals such as lead, cadmium, and copper (Mitchel 1972; Hariyadi et al. 2013). The species of *Salvinia* performs an efficient rhizofiltration method to precipitate toxic metals from the contaminated water. They are hyperaccumulators of heavy and toxic metals naturally. Therefore they could be used in the phytoremediation process of a polluted water body.

Many kinds of research have studied the efficiency of *Salvina molesta* in phytoremediation of wastewater. *S. molesta* removes phosphorous and heavy metals like Cd, Cu, Ni, Zn, Fe, and Pb from the wastewater. This treated water can be used for irrigation, gardening, cleaning, and washing and the plant can be helpful to recover the accumulated metals for commercial use (George and Gabriel 2017) (Fig. 16.3).

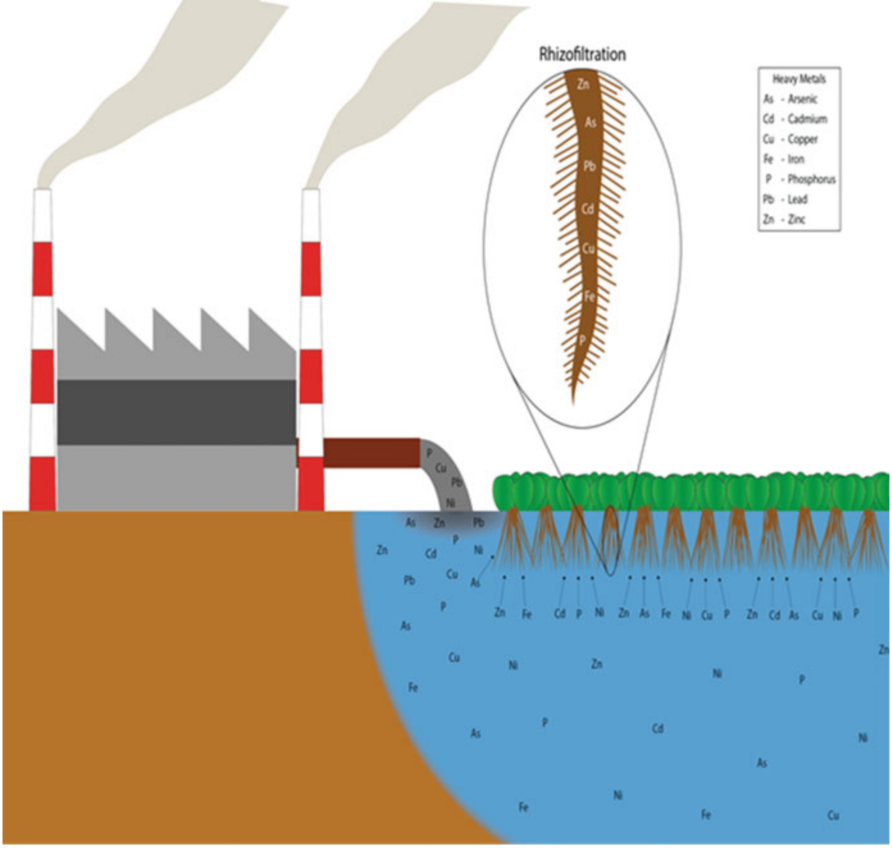


Fig. 16.3 The Rhizofiltration of heavy metals by *Salvinia molesta*

One classic example is phosphorous regulation by phytoremediation. Phosphorus is used as a macronutrient by plants. Phosphorous major constituent in ADP and ATP which is very important for energy storage and in the process of photosynthesis and respiration. It is also needed in building their biochemical structural component like nucleic acids during their growth (Tisdale et al. 1993). Hence during their growth period, these plants need a high amount of phosphorus. Therefore, the required amount of phosphate or orthophosphate is taken up by the wastewater. Therefore, the phytoremediation system helps to regulate the phosphate concentration in the water body.

S. molesta presents enzymatic antioxidant defense mechanisms to reduce toxicity and is more effective in the floating leaves. To prevent oxidative damage produced by toxic metals *S. molesta* has many defense mechanisms such as antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), and peroxidases) that eliminate Reactive Oxygen Species in subcellular compartments. These enzymes play an important role in aquatic ferns for the tolerance of heavy metals (Farnese et al. 2014; Andrade et al. 2016; da Silva et al. 2018).

Pavithra and Kousar (2016) studied the potentiality of *S. molesta* in the treatment of wastewater released from textiles and proved that *S. molesta* has the capability of reducing the harmful heavy toxic metals. Ng and Chan (2017) have researched the Palm oil mill effluent (POME) wastewater treatment using *S. molesta* plant. Their observation suggested that the water quality of POME has increased after treatment with *S. molesta*. Similarly (Ng et al. 2017) have cultured the *S. molesta* in raceway pond for about 16 days. The wastewater samples were collected after cultivation and quality analysis was done for phosphate, nitrate, ammonia, nitrogen, and chemical oxygen demand. The observation has shown that *S.molesta* has accounted for 95% phosphate removal lowering its value to 0.17 mg/l, nitrate lowered to 0.50 mgL^{-1}, and ammonia to 2.62 mgL^{-1}. The efficiency of COD removal was found to be 39%. This study agrees with the report achieved by Xie and Yu (2003) who have defined that the plant in the aquatic system can absorb and accumulate phosphorus and they can utilize it for their growth.

Abeywardhana et al. (2017) has demonstrated the removal of heavy metal from the water discharged from industries using *S. molesta*. In accordance with their experiment the nitrogen removal efficiency was found to be 73.34%, chromium 81.66%, Copper 69.81%, iron 65.2%, nickel 66.39%, lead 74.85% and phosphate was found to be 72.63%. From this result, it is evident that *S.molesta* can be used in an efficient phytoremediation process.

George and Gabriel (2017) has inferred the proficiency of *S.molesta* in the reduction of heavy metal level in the wastewater of municipality from industrial sites around India. A further study by Tangahu and Putri (2017) has inspected the efficiency of *S. molesta* in reducing COD and BOD. This study BOD reducing the efficiency of 21% and COD reducing efficiency to 42%. Besides, Hanafiah et al. (2018) have suggested the ability of *S. molesta* in the removal of ammonical nitrogen and solid effluents through phytoremediation. Moreover, da Silva et al. (2018) experimented on the role of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX) in *S. molesta* tolerance to Arsenite and performance of this plant in the remediation of contaminated water. The results of the research show that *S. molesta* has a greater tendency to reduce arsenite toxicity from wastewater.

Sreekumar and John (2018) validated the effectiveness of *S. molesta* plants in the reduction of heavy metals (Zn and Cd) from wastewater. Research by Ugya et al. (2019) demonstrated the efficacy of macrophytes in phytoremediation of wastewater produced from the dye industry. They authenticated that *S. molesta* has the highest reduction efficacy for phenol and ammoniacal nitrogen compared to other macrophytes. This outlines the roles of *S. molesta* in the degradation of organic, inorganic, metals, and heavy metals contaminants in the phytoremediation of wastewater regardless of its source.

5 Conclusion

Phytoremediation is an accustomed and eco-friendly design for the removal of toxic substances present in nature. Traversing into the understanding of the working of it would certainly increase our realization, thus making us go for the suitable process of phytoremediation. The appropriate plant species for the removal of heavy metals in sewage water can also be allocated according to the waste material and toxic elements.

Phytoremediation manifests favorable results in cleaning up the environment of various toxic elements, though it requires more research attempts. The water treated with phytoremediation can be used for various purposes like irrigation; gardening and cleaning. Thus phytoremediation accounts for the conservation of water. According to previous studies, it was highly notable that *S.molesta* is capable of removing heavy metal contaminants from wastewater. This plant could also be helpful as a bio-indicator for the determination of heavy metal pollution in wastewater. Furthermore, researches have to be carried out and will be useful in more understanding of *S. molesta* in phytoremediation. The use of *S.molesta* will be a promising approach in the removal of toxic elements and aids in bio-conservation.

References

Abd-Elnaby A, Egorov M (2012) Efficiency of different particle sizes of dried *Salvinia* natan in the removing of cu and oil pollutants from water. J Water Chem Technol 34:143–146. https://doi.org/10.3103/s1063455x12030046

Abeywardhana MLDD, Bandara NJGJ, Rupasinghe SKLS (2017) Proceedings of the 22′. Session III – waste management and pollution control. Proceedings of the 22nd international forestry and environment symposium of the Department of Forestry and Environmental Science. University of Sri Jayewardenepura, Sri Lanka, pp 233. https://doi.org/10.31357/fesympo.v22i0.3457

Afzal M, Khan QM, Sessitsch A (2014) Endophytic bacteria: prospects and applications for the phytoremediation of organic pollutants. Chemosphere 117:232–242

Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals – concepts and applications. Chemosphere 91:869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075

Andrade HM, Oliveira JA, Farnese FS, Ribeiro C, Silva AA, Campos FV, Neto JL (2016) Arsenic toxicity: cell signalling and the attenuating effect of nitric oxide in *Eichhornia crassipes*. Biol Plant 60:173–180. https://doi.org/10.1007/s10535-015-0572-4

Arzani A, Zeinali H, Razmjo K (2007) Iron and magnesium concentrations of mint accessions (Mentha spp.). Plant Physiol Biochem 45:323–329. https://doi.org/10.1016/j.plaphy.2007.03.023

Buhari Muhammad L, Sulaiman Babura R, Vyas NL, Sulaiman B, Harishu Umar Y (2016) Role of biotechnology in phytoremediation. J Bioremed Biodegr. https://doi.org/10.4172/2155-6199.1000330

da Silva AA, de Oliveira JA, de Campos FV, Ribeiro C, dos Santos Farnese F, Costa AC (2018) Phytoremediation potential of *Salvina Molesta* for arsenite contaminated water: role of antioxidant enzymes. Theor Exp Plant Physiol. https://doi.org/10.1007/s40626-018-0121-6

Dhir B (2009) *Salvinia*: an aquatic fern with potential use in phytoremediation. Environ Int J Sci Technol 4:23–27

Dhir B, Sharmila P, Saradhi PP, Sharma S, Kumar R, Mehta D (2011) Heavy metal induced physiological alterations in *Salvinia natans*. Ecotoxicol Environ Saf 74:1678–1684. https://doi.org/10.1016/j.ecoenv.2011.05.009

Dipu S (2013) Phytoremediation of heavy metals from industrial effluent using constructed wetland technology. Appl Ecol Environ Sci 1:92–97. https://doi.org/10.12691/aees-1-5-4

Dixit R, Wasiulah MD, Pandiyan K, Singh UB, Sahu A, Shukla R, Singh BP, Rai JP, Sharma PK, Lade H, Paul D (2015) Bioremediation of heavy metals from soil and aquatic environment. An overview of principles and criteria of fundamental processes. Sustainability 7:2189–2212

EPA (U.S. Environmental Protection Agency) (2000) Introduction to phytoremediation. State Environmental Protection Agency, Ohio, p 104

Erakhrumen, Agbontalor A (2007) Review phytoremediation: an environmentally sound technology for pollution prevention, control and remediation in developing countries. Edu Res Rev 2 (7):151–156

Erdei L, Mezosi G, Mecs I, Vass I, Foglein F, Bulik L (2005) Phytoremediation as a program for decontamination of heavy-metal polluted environment. Acta Biologica Szegediensis 49:75–76

Farnese FS, Oliveira JA, Lima F, Lea GA, Gusman GS, Silva LC (2014) Evaluation of the potential of *Pistia stratiotes* L. (water lettuce) for bioindication and phytoremediation of aquatic environments contaminated with arsenic. Braz J Biol 74:108–112. https://doi.org/10.1590/1519-6984.01113

Fritioff A, Greger M (2003) Aquatic and terrestrial plant species with potential to remove heavy metals from stormwater. Int J Phytoremediation 5:211–224

Fuentes II, Espadas-Gil F, Talavera-May C, Fuentes G, Santamaría JM (2014) Capacity of the aquatic fern (*Salvinia minima* Baker) to accumulate high concentrations of nickel in its tissues and its effect on plant physiological processes. Aquat Toxicol 155:142–150. https://doi.org/10.1016/j.aquatox.2014.06.016

Gardner JL, Al-Hamdani SH (1997) Interactive effects of aluminum and humic substances on *Salvinia*. J Aquat Plant Manag 35:30–34

Gaur A, Adholeya A (2004) Prosfects of arbusclar mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr Sci 4:528–534

George GT, Gabriel JJ (2017) Phytoremediation of heavy metals from municipal wastewater by *Salvina molesta* Mitchell. Haya Saudi J Life Sci 3:108–115. https://doi.org/10.21276/haya

Ghosh M, Singh SP (2015) A review on phytoremediation of heavy metals and utilization of it's by products. Asian J Ener Environ 6:214–231

Guittonny-Philippe A, Petit ME, Masotti V, Monnier Y, Malleret L, Coulomb B, Laffont-Schwob I (2015) Selection of wild macrophytes for use in constructed wetlands for phytoremediation of contaminant mixtures. J Environ Manag 147:108–123

Hanafiah MM, Megat Mohamad NHS, Aziz NIHA (2018) *Salvina molesta* and *Pistia stratiotes* as phytoremediation agents in sewage wastewater treatment. Sains Malaysiana 47(8):1625–1634. https://doi.org/10.17576/jsm-2018-4708-01

Hariyadi B, Yanuwiadi B, Polii S (2013) Phytoremediation of arsenic from geothermal power plant waste water using *Monochoria Vaginalis*, *Salvina molesta* and *Colocasa esculenta*. Int J Biosci (IJB) 6:104–111. https://doi.org/10.12692/ijb/3.5.104-111

Henry-Silva GG, Camargo AFM (2006) Efficiency of aquatic macrophytes to treat Nile tilapia pond effluents. Sci Agric 63:433–438. https://doi.org/10.1590/s0103-90162006000500003

Hoffmann T, Kutter C, Santamaria J (2004) Capacity of *Salvinia minima* Baker to tolerate and accumulate as and Pb. Eng Life Sci 4:61–65. https://doi.org/10.1002/elsc.200400008

Hussain CM, Kecili R (2020) Future of environmental analysis. Mod Environ Anal Tech Poll:381–398. https://doi.org/10.1016/B978-0-12-816934-6.00014-X

Iha DS, Bianchini I Jr (2015) Phytoremediation of Cd, Ni, Pb and Zn by *Salvinia minima*. Int J Phytoremediation 17:929. https://doi.org/10.1080/15226514.2014.1003793

Jagdish Kumar A, Inchulkar SR, Bhagat S (2018) An overview of copper toxicity relevance to public health. Eur J Pharm Med Res 11:232–237

Lakra KC, Lal B, Banerjee T (2017) Decontamination of coal mine effluent generated at the Rajrappa coal mine using phytoremediation technology. Int J Phytoremediation 19:530–536. https://doi.org/10.1080/15226514.2016.1267698

Lasat MM (2000) Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. J Hazard Subst Res:21–25

Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31:109–120

Lesniewicz A, Jaworska K, Zyrnicki W (2006) Macro- and micro-nutrients and their bioavailability in polish herbal medicaments. Food Chem 99:670–679. https://doi.org/10.1016/j.foodchem.2005.08.042

Mays P, Edwards G (2001) Comparison of heavy metal accumulation in a natural wetland and constructed wetlands receiving acid mine drainage. Ecol Eng 16:487–500

Mitchel DS (1972) The kariba weed *Salvina molesta*. Fern Gazette 10:251–252

Mohammad IL, He Z-l, Peter Stoffela J, Yang X-e (2008) Phytoremediation of heavy metals polluted soils and water; progresses and perspectives. J Zhejang Univ Sci B 9(3):210–220. https://doi.org/10.1631/jzus.b0710633

Moore JW, Ramamoorthy S (1984) Heavy Metals in natural waters. Applied monitoring and impact assessment. Springer, New York, p 16

Mukesh D, Anil K (2005) Treatment of waste from metal processing and electrochemical industries. Biotreat Indus Efful:145–155. https://doi.org/10.1016/B978-075067838-4/50014-2

Nelson LS (2015) Giant and common *Salvinia*. Best management practices handbook. US Army Engineer Research and Development Center

Ng YS, Chan DJC (2017) Wastewater phytoremediation by *Salvina molesta*. J Water Pro Eng 15:107–156

Ng YS, Samsudin NIS, Chan DJC (2017) Phytoremediation capabilities of Spirodela polyrhiza and *Salvina molesta* in fish farm wastewater: a preliminary study. IOP Conf Ser Mater Sci Eng. https://doi.org/10.1088/1757899X/206/1/012084

Olguin EJ, Rodriguez D, Sanchez G, Hernandez E, Ramirez ME (2003) Productivity, protein content and nutrient removal from anaerobic effluents of coffee wastewater in *Salvinia minima* ponds, under subtropical conditions. Acta Biotechnol 23: 259–270. https://doi.org/10.1002/abio.200390033

Pavithra M, Kousar H (2016) Potential of *Salvina molesta* for removal of sodium in textile wastewater. J Bioremed Biodegrad 7. https://doi.org/10.4172/2155-6199.1000364

Prasannakumari AA, Gangadevi T, Jayaraman PR (2014) Absorption potential for heavy metals by selected ferns associated with Neyyar River (Kerala), South India. Int J Environ Sci 5:270–276

Pratas J, Paulo C, Favas PJ, Venkatachalam P (2014) Potential of aquatic plants for phytofiltration of uranium-contaminated waters in laboratory conditions. Ecol Eng 69:170–176

Prichard E, Mackay GM, Points J (1984) Inorganic Analytes, sample preparation in trace analysis: a structured approach to obtaining reliable results. Royal Society of Chemistry, Cambridge, pp 1–39

Ranjitha J, Raj A, Kashyap R, Vijayalakshmi S, Donatus M (2016) Removal of heavy metals from industrial effluent using *Salvina molesta*. Int J Chem Tech Research 5:608–613

Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–228

Rendall REG, Philips JI, Renton KA (1994) Death following exposure to nickel from a metal arc process. Ann Occup Hyg 38:921–930

Sreekumar A, John J (2018) Removal of pollutants from pestilent water using selected. Hydrophytes 11(7):1618–1621

Sreerama Kumar P, Ramani S, Amani, Singh SP (2005) Natural suppression of the aquatic weed, *Salvina Molesta* D. S. Mitchell, by two previously unreported fungal pathogens. J Aquat Plant Manage 43:105–107

Sumiahadi A, Acar R (2018) A review of Phytoremediation technology: heavy metals uptake by plants. IOP Conf Series Earth Environ Sci:142. https://doi.org/10.1088/1755-1315/142/1/012023

Tangahu BV, Putri AP (2017) The degradation of bod and cod of batik industry wastewater using *Egeria Densa* and *Salvina molesta*. Jurnal Sains & Teknologi Lingkungan 2: 82–91.

Tangahu BV, Abdullah SRS, 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 2011:939161. https://doi.org/10.1155/2011/939161

Thomson PA, Room PM (1986) Taxanomy and control of *Salvinia molesta*. Nature 6063:581–584

Tisdale SL, Nelson W, Beaton JD, Havlin J (1993) Elements required in plant nutrition, soil fertility and fertilizers. MacMillan Publ. Co., New York, pp 48–49

Ugya AY, Hua X, Ma J (2019) Phytoremediation as a tool for the remediation of wastewater resulting from dyeing activities. Appl Ecol Environ Res 2:3723–3735. https://doi.org/10.15666/aeer/1702

Xie Y, Yu D (2003) The significance of lateral roots in phosphorous (P) acquisition of water (*Eichhornia crassipes*). Aquat Bot 75:311–321. https://doi.org/10.1016/s0304-3770(03)00003-2

Chapter 17
Mycoremediation: Fungal-Based Technology for Biosorption of Heavy Metals – A Review

Abate Ayele, Setegn Haile, Digafe Alemu, Tamiru Tesfaye, and M. Kamaraj

Abstract Environmental pollution due to the development of industries in recent times is a great threat to living beings. Different industries released heavy metals into the water body. These heavy metals can be toxic, carcinogenic, and cause threats to human beings and the aquatic environment. So, there is a serious challenge to remove heavy metals from wastewater. Biosorption of heavy metals using fungus or mycoremediation is a widely used technology due to low cost, high biomass, and eco-friendly nature. When compared to fungal biomass with other biosorbents, fungal biomasses are more accessible as a waste product from industry and give economic advantages. Most fungi applied to remove heavy metals are non-pathogenic and can be simply used without any safety issues. Fungal biosorption largely depends on various factors such as pH, metal ion, and biomass concentration and to a limited extent on temperature. This review article focused on collected and disseminated facts on different fungal adsorbents which are important for the removal of heavy metals and offer information about biosorption mechanisms as well as a functional group of fungus involved for the removal of different heavy metals.

Keywords Biosorption · Fungi · Heavy metals · Mycoremediation · Functional groups

---

A. Ayele · S. Haile · D. Alemu · T. Tesfaye
Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

M. Kamaraj (✉)
Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

Bioprocess and Biotechnology Center of Excellence, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_17

1 Introduction

The environment comprises complex variables that include air, water, and land. Their helpful association forms a basis for the presence of the human being along with other living things, namely, plants, animals, and microbes. But, knowledge and scientific advances in the form of industrial societies have contributed to severe environmental contamination of air, soil, and water, which are considered to be an indispensable part of human life. Increasing humane population, development, and rapid mechanizations are recognized as important challenges to the groundwater resources management in developing countries (Selvi et al. 2019).

Heavy metal pollution in the environment causing serious health menaces to the ecosystem due to its various levels of accumulation and toxicity (Tangahu et al. 2011). Although there are different heavy metal remediation methods, biological sorption has become a promising alternative to the existing expensive methods and has more advantages mainly in terms of cost, environmental benignity, easy and safe operation, green chemistry concept, possible recovery, and better removal at low concentration (Sharma et al. 2018). The most well-known biological sources for the utilization of heavy metal sorption methods are plants, fungi, bacteria, and algae (Kaur et al. 2019). Among these, the fungal biomass has been perceived for the overwhelming of heavy metals sorption on polluted environments due to their nature of cell wall composition which comprises of 80–90% of polysaccharides, proteins, and lipids with numerous important functional groups that can be utilized in metal binding (Dhankhar and Hooda 2011). They likewise regularly show stamped resilience toward metals and other antagonistic conditions, for example, low pH (Simonescu and Ferdeş 2012).

Fungal biomass has received a lot of consideration as a biosorbent due to a high level of cell wall material, which increases the variety of functional groups, engaged with heavy metal binding, and in this way builds the metal sequestration capacity of organisms (Dhankhar and Hooda 2011). However, nowadays, this technology is not extensively applied to all over the world and needs to be given much attention and extent. As a result, the current review mainly focused on various aspects of the removal of heavy metals using different fungus, factor influencing, and biosorption mechanisms as well as functional groups involved in biosorption.

2 Mycoremediation

Mycoremediation is one part of bioremediation that uses fungi to restore, degrade, and repair contaminated environments (Mani and Kumar 2013; Asiriuwa et al. 2013; Hamba and Tamiru 2016). Mycoremediation is using fungi in bioremediation, and the long threads (hyphae) interact with soil particles, roots, and rocks forming a filamentous body which are known to tolerate heavy metals and can adjust themselves to grow under various extreme conditions of pH, temperature, nutrient accessibility, and high metal concentrations (Aly et al. 2018). This new technology held the ability for eliminating contaminants of heavy metals from the polluted environment by channeling them to the fruit bodies for removal (Okhuoya 2011; Deshmukh et al. 2016). The key to mycoremediation is determining the right fungi species to target a specific pollutant through either chelation, adsorption, accumulation, or transformation (Thenmozhi et al. 2013; Hamba and Tamiru 2016). Fungi are the dominating living cell in soil, and they have been broadly used for the bioremediation of metal contamination in such environments (Hoshino and Morimoto 2008; Harms et al. 2011).

Fungi have an advantage over bacteria for the bioremediation of polluted environments due to the abovementioned reasons such as the unique nature of its biomass, hyphal network, and longer lifecycle. Moreover, fungi that resist metal compete with native bacteria in hostile conditions (Sun et al. 2012). Broader metabolic competence of fungi offers their various applications in the elimination of different pollutants. Polysaccharides and proteins containing carboxyl, hydroxyl, sulfate, phosphate, and amino groups for binding metal ions are the main components of the cell walls of fungi (Maheswari and Murugesan 2009). These functional groups are responsible for providing the ligand atoms to make complexes with metal ions for attracting and retaining metals in the biomass. The selection of metal-tolerant fungi from a polluted environment screens them for their metal removal potential and further bioaugmentation of promising soil fungi native to the metal-contaminated soil may be a successful strategy for the appropriate site-specific bioremediation. Several researchers have also suggested that studies on the wild-type fungal strains are required that can tolerate high metal concentration, can remove metal from the surrounding matrix, and thus can lead to an efficient bioremediation process (Gentry et al. 2004; Mukherjee et al. 2010).

3 Factors Influencing Biosorption of Heavy Metals Using Fungi

The biosorption process depends on different factors that influence biosorption mechanisms. pH, initial concentration of heavy metal, the dosage of biomass, temperature, and contact time are the main factors for the elimination of metal ions (Veglio and Beolchini 1997).

3.1 The Influence of pH

pH is one of the major factors that affect heavy metal uptake from the environment. Most of the heavy metal removal and uptake by fungus species was favorable at acidic pH shown below in Table 17.1. Due to lower pH levels, heavy metals tend to

Table 17.1 Some fungal species used for removal of heavy metals

S/ No	Fungal species	Heavy metals	Dosage (g/L)	Initial concentration (mg/L)	Time (Min)	pH	Temp (°C)	Removal efficiency (%)	Adsorption capacity (mg/g)	References
1.	*Aspergillus niger*	Cd (II)	1.33	75	–	6.5	30	97	–	Munawar et al. (2018)
2.	*Penicillium canescens*	As(III)	–	100	240	5	20	–	26.4	Say et al. (2003)
		Hg(II)	–	100	240	5	20	–	54.8	
		Cd(II)	–	100	240	5	20	–	102.7	
		Pb(II)	–	100	240	5	20	–	213.2	
3.	*Penicillium chrysogenum*	Cd (II)	2	1	240	5	60	91	180	Alothman et al. (2019)
		Cu (II)	2	1	120	6	60	53	190	
		Pb(II)	2	1	240	5	60	56	180	
4.	*Aspergillus ustus*	Cd (II)	2	1	60	5	60	84	185	
		Cu (II)	2	1	240	6	60	52	185	
		Pb(II)	2	1	240	7	30	42	190	
5.	*Aspergillus niger*	Cd (II)	5.22	89.93	–	6.01	–	98	2.2	Amini and Younesi (2009)
		Ni (II)	5.22	89.93	–	6.01	–	80	1.6	
		Pb(II)	5.22	89.93	–	6.01	–	99	4.7	
		Cr (VI)	1	3000	1440	3	27	72.2	–	Santhi and Guru (2014)
6.	*Polyporus squamosus*	Hg (II)	–	47.39	254.9	5.3	20	35.37	3.54	Uzun and Şahan (2017)
7.	*Aspergillus fumigatus*	Pb (II)	10	100	7200	5	–	92	–	Sharma et al. (2016)
8.	*Aspergillus tamarii NRC 3*	Cu (II)	5/50 ml	120	60	5	30	96.75	–	Saad et al. (2019)
9.	*Rhodotorula mucilaginosa*	Cu (II)	–	200	60	5	30	90	26.2	Salvadori et al. (2014)

10.	*Trichoderma* sp. *BSCR02*	Cr (VI)	1.6	200	120	5	35	96.38	–	Smily and Sumithra (2017)
11.	*Aspergillus niger MSR4*	Cr (VI)	2	62.5	37.5	2	27	63.82	–	Samuel et al. (2015)
12.	*Circinella* sp.	Ni (II)	5	0.5257	60	6	40	–	–	Alpat et al. (2010)
13.	*Trichoderma asperellum*	Cd (III)	–	100	–	9	–	76.17	10.75	Mohsenzadeh and Shahrokhi (2014)
14.	*Trichoderma harzianum*	Cd (III)	–	100	–	9	–	69	–	
15.	*Trichoderma tomentosum*	Cd (III)	–	100	–	9	–	66.19	5.23	
16.	*Saccharomyces cerevisiae*	Cd (II)	0.1	50	60	4–5	20–25	67	–	Thippeswamy et al. (2014)
		Pb (II)	0.1	50	60	4–5	20–25	61	–	
		Ni (II)	0.1	50	60	4–5	20–25	64	–	
		Cr (VI)	0.1	50	60	4–5	20–25	63	–	
		Cu (II)	0.1	50	60	4–5	20–25	57	–	
		Zn (II)	0.1	50	60	4–5	20–25	53	–	
		Cd (II)	0.2	20	30	8.5	25	84.80	–	Hamza et al. (2010)
		Zn (II)	0.2	20	30	6	25	66.14	–	
		Cu (II)	0.2	20	30	6	25	45.65	–	
17.	*Penicillium* sp.	Pb (II)	2	145	7200	5	25	95.24	–	Kurç et al. (2016)
18.	*Aspergillus niger*	Cr(VI)	2	300	–		40	–	97.1	Ren et al. (2018)
19.	*Penicillium janthinellum*	Pb(II)	0.2	123	65	4.5	30	76	–	Aytar et al. (2014)
		Ni (II)	1.6	33	89	4.5	30	47	–	

form free ionic species, more protons available to saturate metal-binding sites. This means that the adsorbent surface is further positively charged at higher hydrogen ion concentrations. The change in pH improves the biosorption rate as reducing pH provides high numbers of the free binding sites and increases metal uptakes (Dwivedi 2012). Thorium removal by *Aspergillus fumigatus* was reported at pH 4 with maximum metal ion removal capacity (Bhainsa and D'Souza 2009).

3.2 The Influence of Initial Concentration of Heavy Metal

The preliminary amount of heavy metal plays a significant role that determines molecular mass transfer resistances between the molecule and sorbent (Khataee et al. 2013). The influence of the initial amount on metal ion biosorption is analyzed by using the initial concentration of chromium [Cr (III)/Cr (VI)] ranging from 0 to 500 mgL^{-1}. Reduction of Cr (IV) to Cr (III) was observed over 200 mgL^{-1} initial concentration which proves that initial concentration also matters with fungal biomass and contact time (Kim et al. 2011). *Trichoderma versicolor* fungal biomass analyzed for biosorption of chromium (VI) was observed at the preliminary amount of 15–30 mgL^{-1}, and chromium uptake was observed at the range of 2.34 mgg^{-1} in the study conducted by Aksu et al. (2007).

3.3 The Influence of Temperature

Temperature is one of the known factors to influence the solidity of the cell wall and its arrangement and can also cause ionization of chemical moieties. These factors may concurrently influence the binding sites on isolated fungal species causing a reduction in heavy metal elimination (Congeevaram et al. 2007). Temperature affects the adsorption of heavy metals by fungi (Kabbout and Taha 2014). As mentioned in Table 17.1, most of the heavy metal biosorption using different fungal species was optimum at the temperature between 20 and 30 °C.

3.4 The Influence of Contact Time

Contact time is the length of time the fungi stay in the contaminant. It also plays an effective role in fungal biosorption indicating the efficiency of biosorbent to remove pollutants from a contaminated environment. The efficiency of metal adsorption of the dead mass of *Aspergillus niger* was evaluated, and quick removal of metal ions on the interchange in the functional groups presents on the fungal cell surface at the initial reaction period and further reduction into metal ion adsorption due to intracellular aggregation of the metal particles. Adsorption rate at an initial 5 min for metal ion (zinc, cobalt, and cadmium) was quick until it reaches equilibrium condition (Yousefi et al. 2015). For example, Godlewska-Żyłkiewicz et al.'s (2019) experiment showed that the effectiveness of biosorption of Pd (II) and Pt (II) on *Aspergillus* sp. within 15 min reached 97% and 91%, respectively.

3.5 The Influence of Biomass Dosage

Fungal biomasses are applicable to eliminate heavy metals from the contaminated terrestrial and aquatic ecosystem. According to Godlewska-Żyłkiewicz et al.'s (2019) study, the efficiency of biosorption of ions increases as the mass of biomass increases to a value of 0.013 g (0.1 g of wet mass). This is probably the influence of a higher number of binding sites on the surface of the biosorbent. The efficiency of biosorption was constant in the range of 0.013–0.066 g of biomass dosage demonstrating the formation of balance among the ions bound to the biosorbent and those residual in the solution. According to Saad et al.'s (2019) study, the amount of copper (II) uptake by *Aspergillus tamarii* NRC 3 biomass was improved as the preliminary amount of biomass increased, and the uptake of Cu^{+2} was recoded (92.40%) in a liquid containing 5 g of wet weight biomass (Table 17.1).

4 Biosorption Mechanisms of Heavy Metals Using Fungi

Microorganisms that are continually exposed to heavy metal stress develop adapting mechanisms to the metal contaminants. Mechanisms involved in biosorption are often classified based on varied criteria: (1) based on cell metabolism, they're classified as metabolism-dependent and non-metabolism-dependent, while (2) based on the location where the metal elimination from liquids happens, biosorption can be classified as extracellular accumulation/precipitation, cell surface sorption/precipitation, and intracellular accumulation (Bellion et al. 2006; Vankar and Bajpai 2008; Dhankhar and Hooda 2011; Thakur et al. 2015; Shakya et al. 2015; Siddiquee et al. 2015; Bahobil et al. 2017). Different mechanisms involved in biosorption phenomena are summarized in Fig. 17.1.

Generally, two mechanisms are proposed for overwhelming heavy metal resistance in fungi. The first mechanism is an extracellular (chelation and cell membrane binding) sequestration, and the second is intracellular physical sequestration of metal by binding to proteins or other ligands to prevent it from harming the metal-sensitive cellular targets. Along these lines, extracellular mechanisms are mainly implied within the avoidance of metal entry, whereas intracellular sequestration systems intend to downsize the metal stress within the cytosol (Baldrian 2003; Anahid et al. 2011). In the extracellular mechanism, different organic molecules that do not belong to the matrix of the cell membrane are excreted by the fungal cell to chelate metal ions. Binding to the cell membrane of fungus is named biosorption (Fawzy

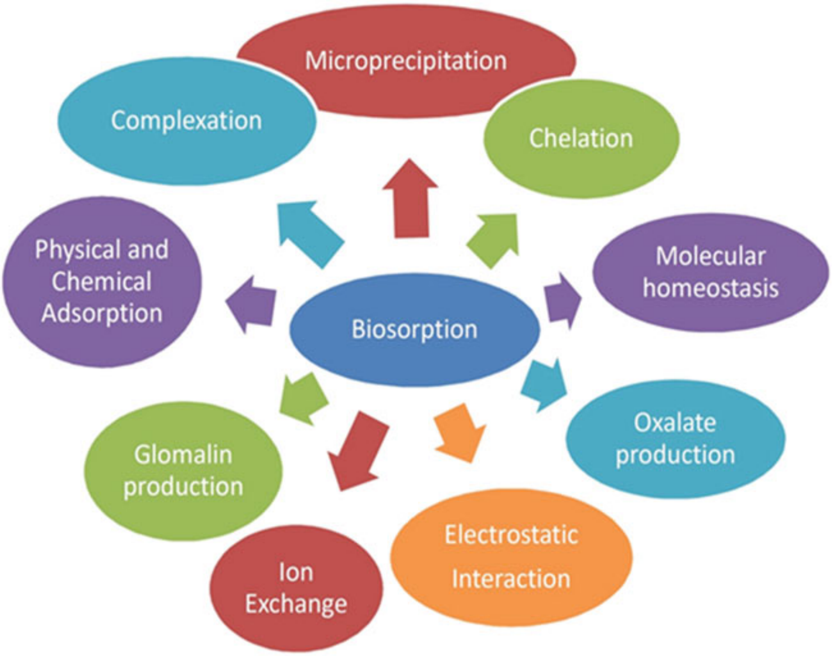


Fig. 17.1 Different mechanisms involved in biosorption phenomena. (Asgher 2012; Kumar et al. 2019)

et al. 2017). The sequestering or binding of metal using fungal biomass is a great extent due to the cell wall containing different polysaccharides, proteins and lipids with varying functional moieties such as hydroxyl, amine, carboxyl, and phosphates (Ahemad and Kibret 2013).

4.1 *Extracellular (Chelation and Cell-Wall Binding) Sequestration*

The fungal cell wall surfaces thus have important protective properties and so may act as a barrier, controlling the uptake of solutes into the cell including potentially toxic metal species (Gadd 1993). The rigid cell wall of the fungi consists of chitin, inorganic ions, lipids, nitrogen-containing polysaccharides, polyphosphates, and proteins. There are two ways in which these cell wall components help to sorb heavy metals. (i) The first barrier can excrete substances such as organic acids and proteins capable of immobilizing heavy metals. (ii) The second barrier responsible for (unspecific) bonding heavy metals to the cell wall (Shakya et al. 2015; Ayangbenro and Babalola 2017).

The fungal cell wall is composed of chitin (a long straight linear homopolymer of beta-1,4-linked N-acetylglucosamine), glucan, mannan, proteins, and other different polymers that have carboxyl, phosphoryl, hydroxyl, amino, amine, and imidazole functional groups at the surface of fungus (Ramrakhiani et al. 2011). The fungal cell wall has a multilaminate, microfibrillar structure. Ultrastructural studies have revealed two phases: (1) an outer layer consisting of glucans, mannans, or galactans and (2) an inner microfibrillar layer, the crystalline properties of which are conferred by the parallel arrangement of chitin chains, sometimes of cellulose (a polymer of D-glucopyranose) chains or in certain yeasts (Sağ 2001).

The main functional groups in fungal biomass that can participate in sorption are electronegative groups such as hydroxyl or sulfhydryl groups, anionic groups such as carboxyl or phosphate groups, and nitrogen-containing groups such as amino groups. Carboxyl and phosphate groups are considered to be significant binding sites for many toxic heavy metals (Tobin et al. 1994; Viraraghavan and Srinivasan 2011). The metal absorbed using the cell wall has been comprehensively based on two mechanisms: absorbed directly using functional groups like phosphate, carboxyl, amine, and phosphate diester compounds. The second uptake mechanism results from physicochemical inorganic interactions directed by adsorption phenomena (Kapoor and Viraraghavan 1995; Pakshirajan et al. 2013).

Another group of heavy metal-binding compounds produced by fungi is melanins, which are fungal pigments that improve survival under different environmental stresses. Fungal melanins are found inside and/or outside the cell walls where electron-dense deposits and granules are observed. Granules might be released into the external medium and be named "extracellular melanin" even though this is generally of identical composition to cell wall-associated melanin. Fungal phenolic polymers and melanins contain phenolic units, peptides, sugars, aliphatic hydrocarbons, and unsaturated fatty acids and along these lines possess many potential metal-binding sites (White et al. 1995). Fungal melanins can effectively adsorb overwhelming heavy metals, including zinc and lead, in the bioremediation of soil. Comparative with sand materials, dehydrated biomass of melanized fungi brought about 50–200 times more metal absorbs in terms of surface area (Cordero et al. 2017). Chitin and chitosan present in fungal cell walls can also sequester metal ions. Zinc was adsorbed more actively on the outer layer of *Rhizopus arrhizus*, especially on its chitin/chitosan components (Viraraghavan and Srinivasan 2011).

4.1.1 Ion Exchange

Ion exchange is the substitution of an ion in a solid phase in contact with a solution by another ion (Bahafid et al. 2017). The functional groups present on the cell surface cooperate through the metal ion known as an ion-exchange mechanism, for the uptake of metal ions from the environment and decontaminated sites (Meena and Busi 2018).

4.1.2 Precipitation

Precipitation is defined as the creation of insoluble inorganic metal precipitates. This might be more simply understood when metals are attached to extracellular polymeric elements excreted by eukaryotic microorganisms, especially yeast and fungi. The precipitates might be made and persist in contact with or inside the microbial cells or may be independent of the compact phase of the microbial cell (Bahafid et al. 2017). Precipitation of metals outside the cell occurs on the surface of the cell and in the solution. Precipitation is considered to be a metabolically dependent process when the fungal cell secretes such substances that favor precipitation; else, it is independent because no intracellular secretions are involved, and metal surface chemical interactions induce precipitation (Shakya et al. 2015).

4.2 Intracellular Sequestration of Heavy Metals

Due to their nature of cell wall and fast growth rate, fungi reduce the environmental pollutants into less toxic forms. This process is through metabolic activity within living organisms (Bahobil et al. 2017) through chemical reactions such as oxidation, reduction, methylation, and dealkylation; they can transform toxins through volatilization and reduce the metal toxicity (Siddiquee et al. 2015). They also transfer excess metals to fungi mycelium and plant symbiosis by cytoplasmic vesicles and vacuoles.

4.2.1 Glomalin Production

Glomalin appears to be effective in sequestering different heavy metals, particularly Cu, Pb, and Cd found in high concentrations in polluted dust (Gonzalez-Chavez et al. 2004). Arbuscular mycorrhizal fungi (AMF) produce glomalin, initially believed to be a secreted hydrophobin and later identified as a spore- and cell wall-localized 60-kilodalton heat shock protein, which looks to play a role in heavy metal immobilization (Ferrol et al. 2016). Regardless of the possibility of the secretion of organic acids such as chelators, glomalin, a glycoprotein exuded by arbuscular mycorrhizal fungi, has been proposed to play a role in metal immobilization, as illustrated in Fig. 17.2 (González-Guerrero et al. 2009).

4.2.2 Molecular Homeostasis

Uptake of essential metals through the mycorrhizal pathway starts through the cell membrane of the extraradical mycelium. Genome sequencing and transcriptomic analyses of *Rhizopus irregularis* brought in the identification of various genes

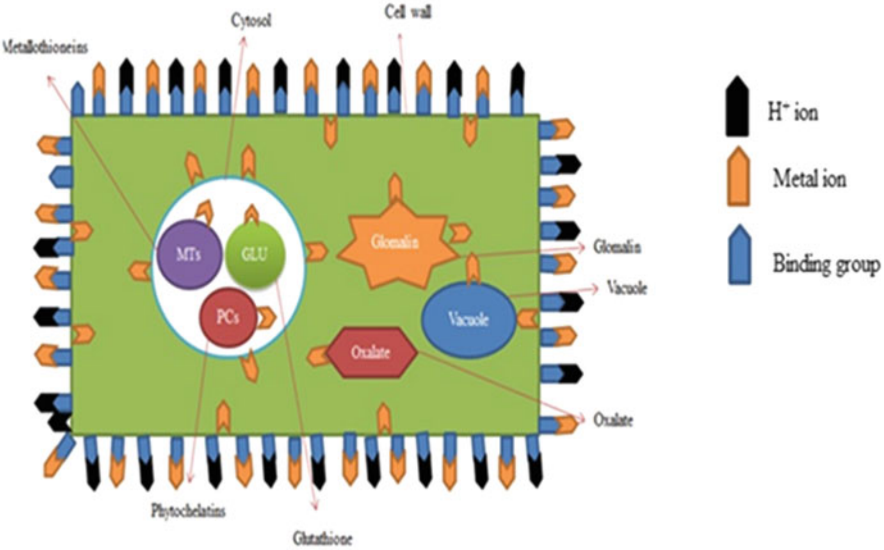


Fig. 17.2 Mechanism of heavy metal adsorption by fungi. (Meier et al. 2012)

encoding putative transport proteins mediating the uptake of Zn, Fe, Cu, and Mn from the soil solution (Ferrol et al. 2016). The fungus *Botrytis cinerea* was challenged to heavy metal (copper, nickel, zinc, cadmium) stress and secreted large panel of proteins, oxidoreductases and cell-wall degrading enzymes which are modified in response to metals (Cherrad et al. 2012).

4.2.3 Intracellular Chelation

Heavy metal chelation through high-affinity ligands by cytosol is potentially a very useful mechanism of heavy metal detoxification and resilience. Potential ligands include amino acids and organic acids, and two classes of peptides, phytochelatins and metallothioneins. Glutathione (GSH) and GSH oligomers, such as phytochelatins, are also likely to be associated with heavy metal obstruction in arbuscular mycorrhizal fungi, as illustrated in Fig. 17.2 (Baldrian 2003; Ferrol et al. 2016). Intracellular chelation of metals in the cytosol by a range of ligands (glutathione, metallothioneins) and increased efflux from the cytosol out of the cell or into sequestering compartments are also key mechanisms conferring resistance. Mechanisms reduce the uptake of metals into the cytosol by extracellular chelation through extruded ligands and binding onto cell wall components (Bellion et al. 2006).

4.2.4 Oxalate Production

Metal immobilization through insoluble metal oxalate formation is a process of marked ecological significance being engaged with fungal survival, mineral formation, pathogenesis, biodeterioration, soil weathering, and metal detoxification (Kaewdoung et al. 2016). As illustrated in Fig. 17.2, the production of oxalic acid by fungi provides a means of immobilizing soluble metal ions, or complexes, as insoluble oxalates decreasing bioavailability and conferring tolerance. Jarosz-Wilkolazka and Gadd (2003) studied oxalate-producing white-rot species, *Bjerkandera fumosa*, *Trametes versicolor*, and *Phlebia radiata*, and a brown-rot species, *Fomitopsis Pinicola*, were used for solubilization of metals (Jarosz-Wilkolazka and Gadd 2003). Filamentous fungi, compared with bacteria, secrete a large number of organic acids, for example oxalic, tartaric, and citric acids. The secreted oxalic acid can react with Pb (II) to form primarily lead oxalate and insoluble Pb minerals. Then, the enhanced biosorption via forming a new border of the cell wall prevents the transportation of Pb (II) into hypha (Tian et al. 2018).

5 Functional Groups on the Surface of Fungi for Heavy Metal Removal

Features responsible for the functional group's biosorption of metal ions are determined by Fourier-transform infrared spectroscopy (FTIR). The mechanism of biosorption was investigated by measuring the zeta potential and by performing FTIR, and most of the wavenumber is ranged from 400–4000 cm^{-1}, and FTIR spectra were measured on KBr pellet (Aytar et al. 2014). The bonding mechanism between heavy metals and fungal biomass can be determined by interpreting the FTIR absorption spectrum (Simonescu and Ferdeş 2012). For instance, from Table 17.2 below, the fungal species consist of a common functional group to remove heavy metals, especially ⁻OH, C⁻H, and some of the species consist of unique functional groups, namely, thiol. Uptake of metal by a fungus has been enhanced by electrostatic attraction to anion functional groups on the cell surface, resulting in the passive attraction of various metal cations (Tsekova et al. 2006). The biosorption capacity of the fungal biomass of *Pleurotus mutilus* for biosorption of Fe^{+3} and Mn^{+2} was evaluated in Table 17.2. Different functional groups, alcohol, hydroxyl, amino, and carboxyl on biosorbent which are responsible for biosorption of positively charged metal ion (Fe^{+3} and Mn^{+2}) with negatively charged functional groups, were reported by Madani et al. (2015). Functional groups on the biomass surfaces can be modified by various pretreatment methods such as acid, base, and thermal treatment (Fig. 17.3) for the effective adsorption of heavy metals. The surface modification impacts the functional groups on the surface of biomass to enhance the adsorption capacity of biomass (Yin et al. 2018; Kumar et al. 2019).

Table 17.2 The fungal functional group involved in heavy metal removal

S/ No	Fungus species	Heavy metals	Wave number (cm^{-1})	Functional group involved	References
1.	*Beauveria bassiana*	Zn(II), Cu(II), Cd(II), Cr(VI), Ni(II)	3407.0	C–OOH and –OH	Gola et al. (2016)
			2924.7	N–H	
			2854.7	C–H	
			1742.7	C–O	
			1643.6	(C–H bending, CH_3	
			1408.3	C–H	
			1041.0	C–O–C	
2.	*Acremonium pinkertoniae*	Cu(II)	3420	–OH	Zapotoczny et al. (2007)
			2920, 2850	C–H	
			1650	C=O (amide I)	
			1557, 1539	N-H (amide II)	
			1317	C–N (amide III)	
			1155, 1112	C–O–C, C–C	
			1076, 1044	C–O, C–C	
			820	C–H	
3.	*Galerina vittiformis*	Cr(VI), Pb(II), Cd(II)	1658 ± 5, 1253 ± 5	Oxalic acid	Damodaran et al. (2013)
			2550 ± 5	Thiol group	
		Cu(II), Zn(II)	2550 ± 5	Thiol group	
4.	*Trametes versicolor*	Cd(II)	3421	–OH	Manna et al. (2018)
			2928	–CH	
			1644,	–C=0	
			1064	–C=C–	
			1378	$-CH_3$	
5.	*Penicillium janthinellum*	Cu(II)	3420.39	–OH	Cai et al. (2016)
			2927.32	CH_2	
			1733.42	C=O	
			1259.49	PO_3	
			882.09	CO_3^{-2}	
		Pb(II)	3420.62	–OH	
			2927.87	CH_2	
			1733.46	C=O	
			1318.22	CH_2	
			1258.81	PO_3	
			882.21	CO_3^{-2}	
		Cd(II)	3420.62	–OH	
			3420.62	CH_2	

(continued)

Table 17.2 (continued)

S/ No	Fungus species	Heavy metals	Wave number (cm^{-1})	Functional group involved	References
			3420.62	C = O	
			1254.41	CO$_3^{-2}$	
	Pleurotus mutilus	Fe(III), Mn(II)	3425	O-H N-H	Madani et al. (2015)
			2924	O−H C−H	
			1645	C=O N−H C=C	
			1087	C−O	
			<800	P=O	

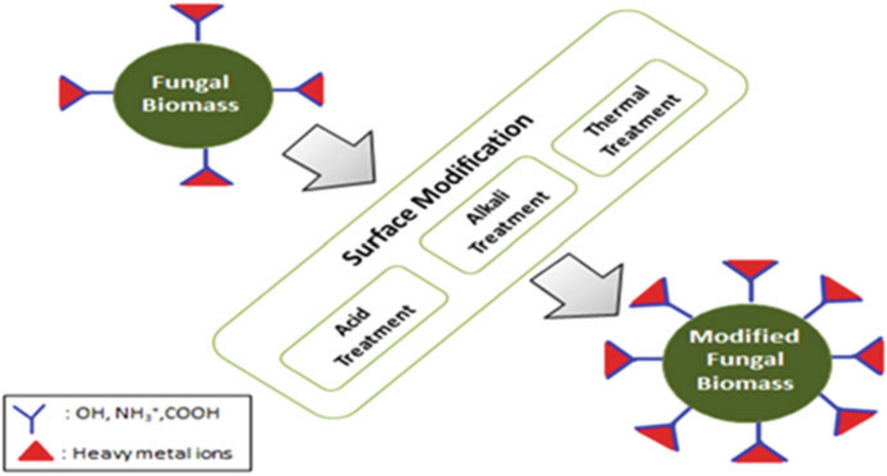


Fig. 17.3 Surface modification of biomass for efficient of heavy metal removal. (Yin et al. 2018; Kumar et al. 2019)

6 Conclusion

In the present review, the application of fungal biomass for the removal of heavy metal ions has been reviewed extensively. The process of heavy metal removal has many attractive features including the removal of metal ion over a relatively broad range of temperature and pH. Biosorption performance of different fungal biosorbents was studied by different researchers, and they provide decent arguments for the implementation of biosorption technologies for heavy metal removal from solutions and to understand the mechanism responsible for biosorption. The efficiency of heavy metal removal by fungus is influenced by several parameters including pH, initial concentration, contact time, temperature, and biomass dosage. Biomass has been found as a natural source containing different functional sites, with the ability for adsorption of metal ions. There is still a challenge for specific adsorption due to the heterogeneity of the cell fungal surface. More studies should be carried out for a better understanding of heavy metal removal by biosorption processes.

Conflict of Interest The authors declare that there is no conflict of interest regarding the publication of this manuscript.

References

Ahemad M, Kibret M (2013) Recent trends in microbial biosorption of heavy metals: a review. Biochem Mol Biol 1:19–26. https://doi.org/10.12966/bmb.06.02.2013

Aksu Z, Kılıç NK, Ertuğrul S, Dönmez G (2007) Inhibitory effects of chromium (VI) and Remazol Black B on chromium (VI) and dyestuff removals by Trametes versicolor. Enzyme Microb Technol 40:1167–1174

Alothman ZA, Bahkali AH, Khiyami MA, Alfadul SM, Wabaidur SM, Alam M, Alfarhan BZ (2019) Low-cost biosorbents from fungi for heavy metals removal from wastewater. Sep Sci Technol:1–10

Alpat S, Alpat SK, Çadirci BH, Özbayrak Ö, Yasa İ (2010) Effects of biosorption parameter: kinetics, isotherm and thermodynamics for Ni (II) biosorption from aqueous solution by Circinella sp. Electron J Biotechnol 13:4–5

Aly MM, Alzahrani N, Amashah RH, Jastaniah SD (2018) Bioremediation of hazardous heavy metals from solutions or soil using living or dead microbial biomass. J Pharm Biol Sci 13:75–80

Amini M, Younesi H (2009) Biosorption of Cd(II), Ni(II) and Pb(II) from aqueous solution by dried biomass of *Aspergillus niger*: application of response surface methodology to the optimization of process parameters. Clean Soil Air Water 37:776–786

Anahid S, Yaghmaei S, Ghobadinejad Z (2011) Heavy metal tolerance of fungi. Sci Iran 18:502–508

Asgher M (2012) Biosorption of reactive dyes: a review. Water Air Soil Pollut 223:2417–2435

Asiriuwa OD, Ikhuoria JU, Ilor EG (2013) Myco-remediation potential of heavy metals from contaminated soil. Bull Environ Pharmacol Life Sci 2:16–22

Ayangbenro AS, Babalola OO (2017) A new strategy for heavy metal polluted environments: a review of microbial biosorbents. Int J Env Res Pub He 14:1504

Aytar PNAR, Gedikli SERAP, Buruk YELIZ, Cabuk A, Burnak N (2014) Lead and nickel biosorption with a fungal biomass isolated from metal mine drainage: box–Behnken experimental design. Int J Environ Sci Technol 11:1631–1640

Bahafid W, Joutey NT, Asri M, Sayel H, Tirry N, Ghachtouli NE (2017) Yeast biomass: an alternative for bioremediation of heavy metals. In: Morata A, Loira I (eds) Yeast – industrial applications. IntechOpen, Rijeka. https://doi.org/10.5772/intechopen.70559

Bahobil A, Bayoumi RA, Atta HM, El-Sehrawey MM (2017) Fungal biosorption for cadmium and mercury heavy metal ions isolated from some polluted localities in KSA. Int J Curr Microbiol Appl Sci 6:2138–2154

Baldrian P (2003) Interactions of heavy metals with white-rot fungi. Enzyme Microb Technol 32:78–91

Bellion M, Courbot M, Jacob C, Blaudez D, Chalot M (2006) Extracellular and cellular mechanisms sustaining metal tolerance in ectomycorrhizal fungi. FEMS Microbiol Lett 254:173–181

Bhainsa KC, D'Souza SF (2009) Thorium biosorption by *Aspergillus fumigatus*, a filamentous fungal biomass. J Hazard Mater 165:670–676

Cai CX, Xu J, Deng NF, Dong XW, Tang H, Liang Y et al (2016) A novel approach to the utilization of the fungal conidia biomass to remove heavy metals from the aqueous solution through immobilization. Sci Rep 6:36546. https://doi.org/10.1038/srep36546

Cherrad S, Girard V, Dieryckx C, Gonçalves IR, Dupuy JW, Bonneu M, Poussereau N (2012) Proteomic analysis of proteins secreted by *Botrytis cinerea* in response to heavy metal toxicity. Metallomics 4:835. https://doi.org/10.1039/C2MT20041D

Congeevaram S, Dhanarani S, Park J, Dexilin M, Thamaraiselvi K (2007) Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. J Hazard Mater 146:270–277

Cordero RJ, Vij R, Casadevall A (2017) Microbial melanins for radioprotection and bioremediation. Microb Biotechnol 10:1186

Damodaran D, Balakrishnan RM, Shetty VK (2013) The uptake mechanism of cd(II), Cr(VI), cu (II), Pb(II), and Zn(II) by mycelia and fruiting bodies of *Galeria vittiformis*. Biomed Res Int:1–11

Deshmukh R, Khardenavis AA, Purohit HJ (2016) Diverse metabolic capacities of fungi for bioremediation. Indian J Microbiol 56:247–264

Dhankhar R, Hooda A (2011) Fungal biosorption – an alternative to meet the challenges of heavy metal pollution in aqueous solutions. Environ Technol 32:467–491

Dwivedi S (2012) Bioremediation of heavy metal by algae: current and future perspective. J Adv Lab Res Biol 3:195–199

Fawzy EM, Abdel Motaal FF, Zayat SAE (2017) Biosorption of heavy metals onto different eco-friendly substrates. J Toxicol Environ Health Sci 9(5):35–44

Ferrol N, Tamayo E, Vargas P (2016) The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. J Exp Bot 67:6253–6265

Gadd G (1993) Interactions of fungi with toxic metals. New Phytol 124:25–60

Gentry T, Rensing C, Pepper IAN (2004) New approaches for bioaugmentation as a remediation technology. Crit Rev Environ Sci Technol 34:447–494

Godlewska-Żyłkiewicz B, Sawicka S, Karpińska J (2019) Removal of platinum and palladium from wastewater by means of biosorption on Fungi *Aspergillus* sp. and yeast *Saccharomyces* sp. Water 11:1522

Gola D, Dey P, Bhattacharya A, Mishra A, Malik A, Namburath M, Ahammad SZ (2016) Multiple heavy metal removal using an entomopathogenic fungi *Beauveria bassiana*. Bioresour Technol 218:388–396

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

González-Guerrero M, Benabdellah K, Ferrol N, Azcón-Aguilar C (2009) Mechanisms underlying heavy metal tolerance in arbuscular mycorrhizas. In: Azcón-Aguilar C, Barea J, Gianinazzi S, Gianinazzi-Pearson V (eds) Mycorrhizas - functional processes and ecological impact. Springer, Berlin/Heidelberg. https://doi.org/10.1007/978-3-540-87978-7_8

Hamba Y, Tamiru M (2016) Mycoremediation of heavy metals and hydrocarbons contaminated environment. Asian J Nat Appl Sci 5:2

Hamza SM, Ahmed HF, Ehab AM, Mohammad FM (2010) Optimization of cadmium, zinc and copper biosorption in an aqueous solution by *Saccharomyces cerevisiae*. J Am Sci 6:597–604

Harms H, Schlosser D, Wick LY (2011) Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nat Rev Microbiol 9:177–192. https://doi.org/10.1038/nrmicro2519

Hoshino YT, Morimoto S (2008) Comparison of 18S rDNA primers for estimating fungal diversity in agricultural soils using polymerase chain reaction-denaturing gradient gel electrophoresis. Soil Sci Plant Nutr 54:701–710. https://doi.org/10.1111/j.1747-0765.2008.00289.x

Jarosz-Wilkolazka A, Gadd GM (2003) Oxalate production by wood-rotting fungi growing in toxic metal-amended medium. Chemosphere 52:541–547

Kabbout R, Taha S (2014) Biodecolorization of textile dye effluent by biosorption on fungal biomass materials. Phys Procedia 55:37–444. https://doi.org/10.1016/j.phpro.2014.07.063

Kaewdoung B, Sutjaritvorakul T, Gadd GM, Whalley AJS, Sihanonth P (2016) Heavy metal tolerance and biotransformation of toxic metal compounds by new isolates of wood-rotting fungi from Thailand. Geomicrobiol J 33:283–288

Kapoor A, Viraraghavan T (1995) Fungal biosorption — an alternative treatment option for heavy metal bearing wastewaters: a review. Bioresour Technol 53:195–206

Kaur H, Rajor A, Kaleka AS (2019) Role of phycoremediation to remove heavy metals from sewage water: review article. J Environ Sci Technol 12:1–9

Khataee AR, Vafaei F, Jannatkhah M (2013) Biosorption of three textile dyes from contaminated water by filamentous green algal Spirogyra sp.: kinetic, isotherm and thermodynamic studies. Int Biodeterior Biodegrad 83:33–40

Kim EJ, Park S, Hong HJ, Choi YE, Yang JW (2011) Biosorption of chromium (Cr (III)/Cr (VI)) on the residual microalga *Nannochloris oculata* after lipid extraction for biodiesel production. Bioresour Technol 102:11155–11160

Kumar A, Kumar V, Singh J (2019) Role of fungi in the removal of heavy metals and dyes from wastewater by biosorption processes. In: Yadav A, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi. Fungal Biology. Springer, Cham, pp 397–418

Kurç MA, Güven K, Korcan E, Güven A, Malkoc S (2016) Lead biosorption by a moderately halophile *Penicillium* sp. isolated from çamalti saltern in Turkey. Life Sci Biotechnol 5:13–22

Madani A, Chergui A, Selatnia A (2015) Biosorption of Fe and Mn ions from aqueous solution by a *Pleurotus mutilus* fungal biomass. J Chem Pharm Res 7:19–26

Maheswari S, Murugesan AG (2009) Remediation of arsenic in soil by Aspergillus nidulans isolated from an arsenic-contaminated site. Environ Technol 30:921–926

Mani D, Kumar C (2013) Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. Int J Environ Sci Technol 11:843–872

Manna A, Sundaram E, Amutha C, Vasantha VS (2018) Efficient removal of cadmium using edible fungus and its quantitative fluorimetric estimation using (Z)-2-(4H-1, 2, 4-Triazol-4-yl) iminomethylphenol. ACS Omega 3:6243–6250

Meier P, Borie F, Bolan N, Cornejo P (2012) Phytoremediation of metal-polluted soils by Arbuscular Mycorrhizal Fungi. Crit Rev Environ Sci Technol 42:741–775

Meena H, Busi S (2018) Biosorption of dye and heavy metal pollutants by fungal biomass: a sustainable approach. In: Prasad R (eds) Mycoremediation and environmental sustainability. Fungal Biology. Springer, Cham, pp 253–271

Mohsenzadeh F, Shahrokhi F (2014) Biological removing of cadmium from contaminated media by fungal biomass of *Trichoderma* species. J Environ Health Sci Eng 12:102

Mukherjee A, Das D, Mondal SK, Biswas R, Das TK, Boujedaini N, Khuda-Bukhsh AR (2010) Tolerance of arsenate-induced stress in Aspergillus niger, a possible candidate for bioremediation. Ecotoxicol Environ Saf 73:172–182

Munawar R, Mughal EU, Sadiq A, Mukhtar H, Zafar, MN, Mumtaz, MW, ... Yousaf Z (2018) Biotechnology: a powerful tool for the removal of cadmium from aquatic systems. Sains Malays 47:27-34.

Okhuoya JA (2011) Mushrooms: what they are and what they do. Inaugural Lecture Ser 114

Pakshirajan K, Izquierdo M, Lens PNL (2013) Arsenic (III) removal at low concentrations by biosorption using *Phanerochaete chrysosporium* pellets. Sep Sci Technol 48:1111–1122

Ramrakhiani L, Majumder R, Khowala S (2011) Removal of hexavalent chromium by heat-inactivated fungal biomass of *Termitomyces clypeatus*: surface characterization and mechanism of biosorption. Chem Eng J 171:1060–1068

Ren B, Zhang Q, Zhang X, Zhao L, Li H (2018) Biosorption of Cr(vi) from aqueous solution using dormant spores of *Aspergillus niger*. RSC Adv 8:38157–38165

Saad AM, Saad MM, Ibrahim NA, El-Hadedy D, Ibrahim EI, Hassan HM (2019) Evaluation of Aspergillus tamarii NRC 3 biomass as a biosorbent for removal and recovery of heavy metals from contaminated aqueous solutions. Bull Natl Res Cent 43:1–9

Sağ Y (2001) Biosorption of heavy metals by fungal biomass and modeling of fungal biosorption: a REVIEW. Sep Purif Methods 30:1–48

Salvadori MR, Ando RA, Oller do Nascimento CA, Corrêa B (2014) Intracellular biosynthesis and removal of copper nanoparticles by dead biomass of yeast isolated from the wastewater of a mine in the Brazilian Amazonia. PLoS One 9(1):e87968. https://doi.org/10.1371/journal.pone.0087968

Samuel MS, Evy AAM, Chidambaram R (2015) Isotherm modelling, kinetic study and optimization of batch parameters using response surface methodology for effective removal of Cr (VI) using fungal biomass. PLoS One 10(3):e0116884. https://doi.org/10.1371/journal.pone.0116884

Santhi R, Guru V (2014) Biosorption of hexavalent chromium using Aspergillus niger dead biomass and its optimization studies. Int J Current Microbiol Appl Sci 3:669

Say R, Yilmaz N, Denizli A (2003) Removal of heavy metal ions using the fungus *Penicillium canescens*. Adsorpt Sci Technol 21:643–650

Selvi A, Rajasekar A, Theerthagiri J, Ananthaselvam A, Sathishkumar K, Madhavan J, Rahman PKSM (2019) Integrated remediation processes toward heavy metal removal/recovery from various environments – a review. Front Environ Sci 7:66. https://doi.org/10.3389/fenvs.2019.00066

Shakya M, Sharma P, Meryem SS, Mahmood Q, Kumar A (2015) Heavy metal removal from industrial wastewater using fungi: uptake mechanism and biochemical aspects. J Environ Eng 142:C6015001. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000983

Sharma R, Sharma AK, Kumar R (2016) Removal of Pb by fungus *Aspergillus fumigatus*. Int J Basic Appl Biol 3:55–59

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. 3Biotech 8:216

Siddiquee S, Rovina K, Azad SA (2015) Heavy metal contaminants removal from wastewater using the potential filamentous fungi biomass: a review. J Microb Biochem Technol 7:384–395

Simonescu CM, Ferdes M (2012) Fungal biomass for Cu (II) uptake from aqueous systems. Pol J Environ Stud 2(1):1831–1839

Smily JRMB, Sumithra PA (2017) Optimization of chromium biosorption by fungal adsorbent, Trichoderma sp. BSCR02 and its desorption studies. HAYATI J Biosci 24:65–71

Sun J, Zou X, Ning Z, Sun M, Peng J, Xiao T (2012) Culturable microbial groups and thallium-tolerant fungi in soils with high thallium contamination. Sci Total Environ 441:258–264

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:1–31

Thenmozhi R, Arumugam K, Nagasathya A, Thajuddin N, Paneerselvam A (2013) Studies on Mycoremedation of used engine oil contaminated soil samples. Adv Appl Sci Res 4:110–118

Thakur Y, Kumar M, Singh S (2015) Microbial biosorption as a green technology for bioremediation of heavy metals. Res J Pharm Biol Chem Sci 6:1717–1724

Thippeswamy B, Shivakumar CK, Krishnappa M (2014) Study on heavy metals biosorption ability of *Saccharomyces cerevisiae*. Int J Biol Res 2:106–115

Tian D, Jiang Z, Jiang L, Su M, Feng Z, Zhang L, Wang S, Zhen L, Hu S (2018) A new insight into lead (II) tolerance of environmental fungi based on a study of *Aspergillus niger* and *Penicillium oxalicum*. Environ Microbiol 21:471–479

Tobin JM, White C, Gadd GM (1994) Metal accumulation by fungi: applications in environmental biotechnology. J Ind Microbiol 13:126–130

Tsekova K, Christova D, Ianis M (2006) Heavy metal biosorption sites in Penicillium cyclopium. J Appl Sci Environ Manage 10:117–121

Uzun Y, Şahan T (2017) Optimization with response surface methodology of biosorption conditions of Hg (II) ions from aqueous media by *Polyporus Squamosus* fungi as a new biosorbent. Arch Environ Prot 43:37–43

Vankar PS, Bajpai D (2008) Phyto-remediation of chrome-VI of tannery effluent by *Trichoderma* species. Desalination 222:255–262

Veglio F, Beolchini F (1997) Removal of metals by biosorption: a review. Hydrometallurgy 44:301–316

Viraraghavan T, Srinivasan A (2011) Fungal biosorption and biosorbents. In: Microbial biosorption of metals. Springer, Dordrecht, pp 143–158

White C, Wilkinson SC, Gadd GM (1995) The role of microorganisms in biosorption of toxic metals and radionuclides. Int Biodeterior Biodegrad:17–40

Yin K, Wang Q, Lv M, Chen L (2018) Microorganism remediation strategies towards heavy metals. Chem Eng J 360:1553–1563

Yousefi J, Shahram S, Zadeh N (2015) Effect of contact time and temperature on biosorption of heavy metals from aqueous solution. Int J Rev Life Sci 5:1406–1411

Zapotoczny S, Jurkiewicz A, Tylko G, Anielska T, Turnau K (2007) Accumulation of copper by *Acremonium pinkertoniae*, a fungus isolated from industrial wastes. Microbiol Res 162:219–228

Part III
Impact and Risk Assessments

Chapter 18
Geospatial Modelling of Air Pollution and its Impact on Health of Urban Residents Using Spatial Models: A Review

M. Prashanthi Devi, Janani Selvaraj, and Harathi Dayalan

Abstract Air quality is a very important factor in projecting or representing the status of environment and health of any region particularly urban areas. Air pollution studies analysing the quality of air deliver strategic information to the decision-making process and play a significant role in the implementation of the policies that influence the air quality of a region. Majority of the air pollution models that simulate the distribution of pollutants consider various physical and environmental characteristics that include wind direction, speed, temperature, etc., which help in determination of the air pollution trajectory. The integration of these models in GIS gives a geophysical dimension to the air quality information by relating the actual pollution concentrations to the health of plant and human life in that location. Over the recent years, several efforts have been made to map traffic-related emission and determine pollution patterns in urban areas using GIS. The use of GIS as a tool to illustrate the spatial patterns of emission and to visualize the impact of congestion on human health has long been attempted. To simulate the impact of air quality in terms of transportation and land use policy changes, several integrated models can be performed. GIS is a dynamic tool when combined with statistical analysis to map traffic-related air pollution and to generate predictive models of pollution surfaces. These models are useful to develop decisions based on monitored pollution data and exogenous information. With this background, a review on GIS-based methods to evaluate the impact of air pollution on human health has been presented. The complexity of using GIS for integrated air quality mapping and its impact on human health lies in many domains. Understanding the relationships between health, environment, geology, hydrology, air pollution studies, agronomy and their dependencies in a spatial phenomenon is the major crux. Spatial explorative models that can determine the relationships between the environment and high pollution

---

M. Prashanthi Devi (✉) · J. Selvaraj
Department of Environmental Science and Management, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

H. Dayalan
Department of Zoology, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu, India

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_18

concentrations across various demographic layers can help in identifying hotspots that demand special investigation or monitoring. Data visualization which otherwise means illustrating complex information through a map provides a dynamic insight to help the authorities plan future strategies.

Keywords Geospatial modelling · Air quality monitoring · Air quality epidemiological studies · Spatial modelling approaches

1 Introduction

Air quality is an important determinant of health and several studies have documented the wide range of adverse effects of ambient air pollution on human health in various parts of the world. In specific, outdoor air pollution emissions originate from both natural and anthropogenic sources. Though in most natural sources forest fires and dust storms contribute to local air pollution especially in prone arid regions, the contribution from human activities far exceeds those of natural sources.

Poor urban planning, rapid urban sprawl and overdependence on private vehicle transport is a major factor responsible for accelerated pollution emissions. Adverse health consequences due to air pollution can occur as a result of both short- or long-term exposure. The pollutants with the strongest impacts on human health are particulate matter (PM), ozone (O_3), nitrogen dioxide (NO_2) and sulphur dioxide (SO_2). Exposure to these airsborne pollutants may cause both acute and chronic effects on human health resulting in damage of different systems and organs. The health effects range from minor upper respiratory irritation to chronic respiratory and heart disease, lung cancer, acute respiratory infection and asthmatic attacks (Raaschou-Nielsen et al. 2013).

The International Agency for Research on Cancer (IARC) working group have unanimously classified outdoor air pollution, specifically particulate matter, as carcinogenic to humans (IARC Group 1), based on sufficient evidence of carcinogenicity in humans and experimental animals and strong mechanistic evidence (Loomis et al 2014). The findings state that, an increased risk of lung cancer was observed in various cohort and case-controlled studies, including millions of people and many thousands of lung cancer cases from Europe, North America and Asia.

2 Air Quality Monitoring

Policy makers have been installing monitoring stations or systems across many cities (Kanaroglou et al. 2005; Jerrett et al. 2005) for legislatory purposes. Pollution sensors have also been placed by researchers to assess the personal exposure to air pollution in places of their interest (Semanjski et al. 2016a, b; Gillis et al. 2016), such as major roads with heavy traffic and industrial and residential sites. The recorded measurements at these stations or locations can only quantify the air pollution level in that particular region or nearby small areas. In order to estimate the concentrations of the air pollutants at unmonitored areas using the available measurements, a variety of methods, including spatial averaging, nearest neighbour, inverse distance weighting (IDW), kriging, land-use regression (LUR) modelling, dispersion modelling, neural networks, etc., have been proposed by researchers (Xingzhe et al. 2017).

Several study designs have opted for the use of a single methodology to estimate the concentrations of air pollutants (Ritz et al. 2000; Ritz et al. 2007; Miller et al. 2007; Brauer et al. 2008; Henderson et al. 2007; Beckerman et al. 2012), while some have attempted a combination of several methods for pollution estimation at different geographical scales (Brunekreef et al. 2009; Beelen et al. 2007).

With the rapid development of pollution-detecting sensors and global positioning system (GPS) technology over the recent decades, mobile sensing systems with devices integrated with these systems have been extensively applied in urban areas. Hence this leads to an increase in the intensity of pollutant measurements, but does not discount the sparse measurements since the coverage of the mobile sensing systems mainly explore specific areas or provide roads wise information (Xingzhe et al. 2017).

The regular measurement of precise ground level $PM_{2.5}$ concentrations is of great significance to epidemiological studies whereby it provides valuable information for an effective management and forecasting of air quality (Al-Saadi et al. 2005). Air quality monitoring networks have been established in many industrial countries to measure pollutant concentration at different locations on a daily or hourly basis. The temporal resolution and accuracy of air quality data vary depending on the methods of measurement (Cyrys et al. 2001). Point measurements collected at monitoring units are not necessary representative of regional concentrations. The regional variability of air pollutants is difficult to assess from point measurements alone due to various other impending factors (van Donkelaar et al. 2010).

Ground-based measurements are generally considered to be accurate and they provide measurements for relatively small areas around the point stations. Often, the limited spatial coverage and irregular distribution of ground-based monitoring stations largely restrict the study on space–time dynamics of air pollution and its impact on human health and the environment (Tian and Chen 2010). Application of satellite data to observe the surface air quality has advanced considerably (Martin 2008; Hoff and Christopher 2009). In fact, global aerosol observations from satellite remote sensing has proven to substantially improve estimates of population that are exposed to $PM_{2.5}$.

Regression models were developed by researchers to find whether any correlation exists between MODIS-derived AOD products with ground-measured $PM_{2.5}$ (Diao et al. 2019). North American Land Data Assimilation System (NLDAS) data was also integrated to account for the meteorological variables while estimating the ground-level $PM_{2.5}$ from AOD. It was observed that the relationships varied for different AOD products and also for different regions in the USA.

Several statistical tools including hybrid multi-criteria decision-making (MCDM) theories have been used in spatial analysis to identify optimum locations for new stations to generate more accurate air quality scenarios in Tehran city in Iran (Kazemi-Beydokhti et al. 2019).

3 Air Quality Epidemiological Studies

The adverse effects of ambient air pollution on health are large, ranging from hypertensive disorders and neurodegeneration to respiratory and cardiovascular diseases. The impacts of air pollution vary depending on the period of exposure, and a significant number of epidemiological studies have been conducted for investigating both the short- and long-term effects of exposure (Ostro et al. 1996; Sahsuvaroglu et al. 2009) .

Since the 1990s, multi-city time series studies have provided strong evidences associating short-term exposure of air pollution to adverse health events (in terms of mortality and morbidity). For example, the National Morbidity, Mortality, and Air Pollution Study (NMMAPS) in the USA and the Air Pollution and Health: A European Approach (APHEA) study in Europe have found a 0.21% and a 0.6% increase in total mortality per 10 units' elevation in particulate concentrations, respectively (Samoli et al. 2003; Peng et al. 2005).

The influence of elevated ozone associated with smoke (EOAS) on paediatric asthma was observed in the different regions of the USA (Pratt et al. 2019) in which the researchers have implemented Monte Carlo approach to assess the quantitative burden. The study also was used to estimate the median number of excess paediatric asthma emergency departments (ED) in the continental USA between 2005 and 2014. From the study observations, significant increase in paediatric asthma ED visits that are associated with enhanced ozone exposure from smokes were made. Also, it has been observed that the effect of ozone due to wildlife fires can have more impact on cardiopulmonary health.

The impact of $PM_{2.5}$ exposures on paediatric asthma health by analysing a cohort study of 11 years of data had observed a higher impact of $PM_{2.5}$ during the initial developmental years of a child's life. It was also reported that the impacts varied by confounders including age, race/ethnicity and insurance, which contributed to asthma health disparities among children along with the level of exposure to $PM_{2.5}$ (Oyana et al. 2019).

Mudiyanselage and Tripathi (2019) employed various methodologies to examine the associations between asthma/COPD morbidity and their determinants. By analysing individual effects and combined effects, they explored the potential risk factors per meteorological factor, the direct and indirect factors of air pollution, the socio-economic factors and the contributing characteristics of the physical environment. They found that meteorological factor such as relative humidity, physical factors such as proximity to roads, and road density, social factor such as firewood use and geographical factor such as elevation played a vital role in predicting morbidity from asthma and COPD.

In areas where the levels of pollution were relatively low, Chang et al. (2019) examined associations between asthma morbidity and local ambient air pollution. A time series analysis of asthma hospital admissions and fine particulate matter pollution ($PM_{2.5}$) in central Mississippi during 2003–2011 were conducted. It showed an estimated 7.2 times increase in daily all-age asthma emergency room admissions per 10 $\mu g/m^3$ increase in the 3-day average of $PM_{2.5}$ levels.

In India, particulate matter, that is, PM_{10}, is regularly monitored at Centre Pollution Control Board operated sites across the country. These data were utilized to estimate relative risk (RR) of all-cause mortality cases in Chennai and Delhi (Health Effects Institute Research Report 2011). The contributory factors being many owed the large uncertainty in estimating premature mortality due to ambient $PM_{2.5}$ exposures.

Firstly, there are very few $PM_{2.5}$ monitoring sites in some of the major cities and many of them do not have consistent quality-controlled data for a long enough period. Moreover, these sites are located in urban areas that leaves most of India unmonitored. Further highly variable aerosol fine mode fraction across space and time (Dey and Di Girolamo 2010) suggests the difficulty in inferring $PM_{2.5}$ from particulate matter measurements especially PM_{10}. Secondly, very limited cohort study exists to specifically quantify cause-specific RR from ambient $PM_{2.5}$ exposure in India. Extrapolation of relative risk (RR) in a study conducted recently in India for household air pollution exposure to that of ambient $PM_{2.5}$ exposure (Balakrishnan et al. 2013; Smith et al. 2014) does not provide the right solution either due to the insufficient data of ambient $PM_{2.5}$ exposures through India.

Thirdly, recent estimates of premature mortalities using integrated exposure risk function (IER) considered uniform baseline mortality across India and raw estimate of satellite based $PM_{2.5}$ in the study on global burden of diseases (GBD 2013; Murray et al. 2015). In order to address the issues aforementioned, researchers have made use of satellite based $PM_{2.5}$ estimates to calculate the relative risk of the premature mortality caused due to chronic obstructive pulmonary disease (COPD), ishchemic heart disease (IHD) and lung cancer throughout India (Sourangsu and Sagnik 2016).

The broad-area coverage of satellites may allow atmospheric remote sensing thereby offering a unique opportunity to monitor air quality at all levels including continental, national and regional scales. Satellite estimates could be an alternative indicator of air quality in pollution-based epidemiological studies (Zhiyong and Ranga Rao 2009).

4 Spatial Modelling Approaches in Air Quality Epidemiological Studies

The major challenges involving the health effects of air pollution from industrial, traffic and other conventional and non-conventional heating sources are being the focus of considerable public and scientific attention. The modelling approaches used for assessing the impact of exposure on human health largely depends on the kind of data sources and how the data collection process is happening over space and/or time. Data used in studies to evaluate the exposure–risk relationship generally include disease events, measurements on pollutants or other risk factors and the reference population at risk, along with other covariates describing socio-demographic features.

Air pollution concentrations are measured at routine intervals at a number of monitoring sites across a region of interest producing point reference data or are simulated by numerical models over a preset grid of locations. On the other hand, health data are mostly collected at an aggregated level and are usually associated with irregularly shaped administrative regions. Epidemiological investigations where the associations between disease occurrence and environmental risk factors over aggregated groups are studied rather than at individual levels are termed as ecological studies (Cameletti et al. 2019).

In statistical epidemiology, health data are usually collected either as count data or at individual level (case event data). In the latter case, two major types of studies can be distinguished. Those based on analysing the spatial pattern of case event locations using point processes (Grell et al. 2015; Diggle 1990, 2003; Pinto Junior et al. 2015), and the other is cohort studies which are useful in quantifying the health effects of long-term exposure (months or years) to pollution (Molitor et al. 2006; Hoek et al. 2013).

There are several challenges involved in studies related to the case event data. Firstly, the data are not easily available involving high costs to obtain due to the large amount of data required and the anticipated expenses using the GIS and global positioning system (GPS)-based devices for survey and data collection (Nuckols et al. 2004). Secondly, the location of the case home address could not be possibly related to the disease aetiology. For instance, the disease could have been contracted at the work place because of occupational exposure or could be related to the amount of time spent by individuals in several indoor and outdoor microenvironments characterised by different pollutant concentrations.

In this regard some authors have proposed models for personal exposure based on longitudinal panel studies, time-activity diary data or GPS-enabled individual exposure monitor (McBride et al. 2007; Zidek et al. 2007 and Blangiardo et al. 2011). As a result of these drawbacks related to individual data, either time series or areal unit study designs have been suggested to quantify the short- and long-term health impacts of air pollution. These studies utilise population level summaries rather than individual level data and cannot be used to quantify individual level cause and effect.

While looking into the modelling framework related to areal level data, the first statistical challenge regards the spatial misalignment between health and exposure data. Here the consequence that pollutant concentration has to be scaled up at the area level, while being measured at finite number of point-referenced monitoring stations which is a procedure known as change of support (Gelfand et al. 2010). A simple solution would be to compute the average of the point measurements collected for each area, possibly using distance or population-based weights (Elliott et al. 2007; Madsen et al. 2008; Young et al. 2009). However, this approach may not be feasible when the monitoring stations network is sparse, that is, in areas without monitoring stations, or when there is a strong spatial heterogeneity of the considered pollutants.

Therefore, estimation of the area-level concentrations through weighted averages over grid level concentrations is possible by using deterministic atmospheric dispersion models (Bell 2006; Rushworth et al. 2014; Lee and Sarran 2015). Another alternative approach would be model-based perspective, where a spatial statistical model can be built which combines pollutant measurements from stations with the output of numerical dispersion models in a data fusion approach (Fuentes et al. 2006; Peng and Bell 2010; Sahu et al. 2010; Berrocal et al. 2010; Pannullo et al. 2015; Moraga et al. 2017). Under this modelling framework, other than accounting for spatial correlation and measurement error, additional covariates like meteorological variables can also be included.

The authors of this paper conducted a study where the satellite aerosol concentrations are linked to lung cancer incidences using spatial regression models. The lung cancer incident data was aggregated and sorted for each taluk and the age standardized incidence rates were calculated. The remotely sensed grid data of the $PM_{2.5}$ concentrations was acquired from the Socioeconomic Data and Applications Centre (SEDAC) of NASA. The Global Annual $PM_{2.5}$ grid data sets from MODIS, MISR and SeaWiFS Aerosol Optical Depth (AOD) represent a series of three-year running mean grids (1998–2012) of fine particulate matter (solid particulate and liquid droplets) derived from a combination of the above satellite retrievals.

Together, the grids provide a continuous surface of $PM_{2.5}$ concentrations per cubic metre, that is, particulate matter 2.5 micrometres or smaller, for health and environmental research. A global decadal (2001–2010) mean $PM_{2.5}$ concentration grid was also produced. The raster grids have a cell resolution of 6 arc-minutes (0.1 degree or approximately 10 km at the equator) and cover the global land surface. The decadal $PM_{2.5}$ concentration grids were used for the present study. Using zonal statistics, the mean $PM_{2.5}$ value for each taluk was computed and utilized for further analysis. In addition, the age standardized tobacco and bidi smoking prevalence rates were incorporated to the present assessment and the data was acquired from the study conducted by Fu et al. (2014) (Tables 18.1, 18.2, 18.3 and Fig. 18.1).

The statistically significant positive association between standardized lung cancer incidence rates and aerosol particle pollution can be taken as indicator of a potential air pollution effect. Additionally, the MODIS aerosol data lends itself to population-based exposure assessment and the ecological approach. Seamless remote sensing images represent aggregate exposure better than averaging ground measurements

Table 18.1 Model estimation parameters of the OLS model

Ordinary Least squares regression				
Variable	Coefficients	Std.Error	t-Statistic	p
CONSTANT	−1.18031	0.6509689	−1.813159	0.07982
Log_FR	−0.2720628	0.1253441	−2.170527	0.03801
aspr bidi	−0.1204884	1.080237	−0.1115389	0.91193
aspr cig	6.917924	1.864985	3.709373	0.00084
PM	0.07577523	0.02887251	2.624476	0.01352

Table 18.2 Model estimation parameters of the spatial lag model

Spatial lag regression				
Variable	Coefficients	Std.Error	t-Statistic	p
rho	0.2045582	0.1851351	1.104913	0.2692
CONSTANT	−1.00853	0.6022152	−1.674699	0.09399
Log_ER	−0.2687533	0.1137567	−2.362529	0.01815
aspr_bidi	−0.203SS96	0.9902273	−0.2059019	0.836S7
aspr cig	5.562523	2.018134	2.75627	**0.00585**
PM	0.06775406	0.02667802	2.539696	**0.01109**

Table 18.3 Model estimation parameters of the spatial error model

Spatial error regression				
Variable	Coefficients	Std. Error	t-Statistic	P
CONSTANT	−1.116116	0.6368439	−1.752573	0.07968
Log_ER	−0.2610312	0.1149888	−2.270057	0.0232
aspr_bidi	−0.05601217	1.069062	−0.0523938	0.95821
aspr_cig	6.768067	1.903514	3.555565	**0.00038**
PM	0.07200134	0.02754872	2.6136	**0.00896**
Lambda	0.1170615	0.211161	0.5543709	0.57932

over a limited number of monitoring sites. This reduces the error in spatially variant data rendering more accuracy to the geostatistical interpolation caused by uneven spatial distribution of monitors. Also remotely sensed data of high resolution offers tremendous spatial coverage suitable for regional air quality monitoring, pollution event warning and environmental health studies.

5 Conclusion

In this paper, several spatial tools to quantify the effects of air quality on health have been presented. The major challenge involved in the study of spatial exposure assessment is the collection of the precise air quality information and the representative health data as they need to be scaled to a single spatial unit in order to apply

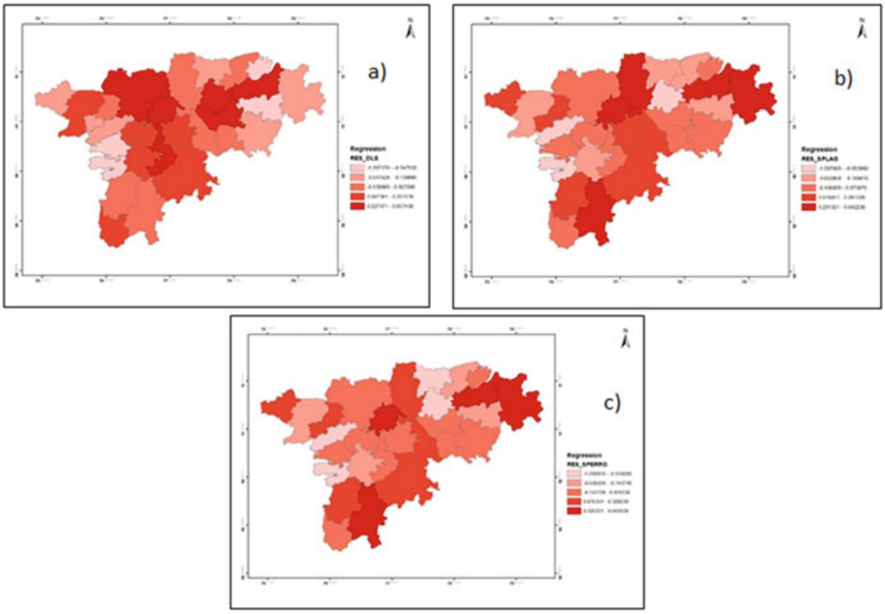


Fig. 18.1 Map depicting the residuals of the (**a**) OLS model (b) spatial lag model (**c**) spatial error model

higher spatial statistical methods. Point or case event data preserve exact spatial information that concerns the disease occurrences, though most times the use of residential address is poorly related to individual exposure. An example of point process methods in spatial epidemiology is when case-control matched data help to investigate the health status of people living around potential environmental pollution sources, such as an incinerator. Despite there exists challenges like ecological fallacy associated with areal level study designs, they are far more diffused in spatial epidemiology than studies based on case event data, due to their immediate availability. In fact, count data are more closely matched with the background population at risk, and exposure measurements are more reliably expressed as average exposure at small area level than as individual level exposure measurements.

References

Al-Saadi J, Szykman J, Pierce RB, Kittaka C, Neil D, Chu DA et al (2005) Improving national air quality forecasts with satellite aerosol observations. Bull Am Meteorol Soc 86(9):1249––1261

Balakrishnan K, Ghosh S, Ganguli B, Sambandam S, Bruce N, Barnes DF, Smith KR (2013) State and national household concentrations of PM2.5 from solid cook fuel use: results from measurements and modelling in India for estimation of the global burden of disease. Environ Heal Glob Access Sci 12(1):77

Brauer M, Lencar C, Tamburic L, Koehoorn M, Demers P, Karr C (2008) A cohort study of traffic-related air pollution impacts on birth outcomes. Environ Health Perspect 116:680–686

Beckerman BS, Jerrett M, Finkelstein M, Kanaroglou P, Brook JR, Arain MA, Sears MR, Stieb D, Balmes J, Chapman K (2012) The association between chronic exposure to traffic-related air pollution and ischemic heart disease. J Toxicol Environ Health A 75(7):402–411. https://doi.org/10.1080/15287394.2012.670899

Beelen R, Hoek G, Fischer P, van den Brandt PA, Brunekreef B (2007) Estimated long-term outdoor air pollution concentrations in a cohort study. Atmos Environ41:1343–1358

Bell ML (2006) The use of ambient air quality modeling to estimate individual and population exposure for human health research: a case study of ozone in the Northern Georgia region of the United States. Environ Int 32(5):586–593

Berrocal VJ, Gelfand AE, Holland DM (2010) A Spatio-temporal downscaler for output from numerical models. JABES 15:176–197. https://doi.org/10.1007/s13253-009-0004-z

Blangiardo M, Hansell A, Richardson S (2011) A Bayesian model of time activity data to investigate health effect of air pollution in time series studies. Atmos Environ 45:379–386

Brunekreef, Bert, Beelen, Rob, Hoek, Gerard, Schouten, Leo, Bausch-Goldbohm, Sandra, Fischer, Paul, Armstrong, Benedict, Hughes, Edward, Jerrett, Michael, Brandt, Piet (2009) Effects of long-term exposure to traffic-related air pollution on respiratory and cardiovascular mortality in the Netherlands, The NLCS-AIR study. Research report (Health Effects Institute). 139:5–71. discussion 73

Cameletti et al (2019) Bayesian modelling for spatially misaligned health and air pollution data through the INLA-SPDE approach. Spat Stat 31.(2019:100353

Chang HH, Pan A, Lary DJ et al (2019) Time-series analysis of satellite-derived fine particulate matter pollution and asthma morbidity in Jackson. MS Environ Monit Assess 191:280. https://doi.org/10.1007/s10661-019-7421-4

Cyrys J, Dietrich G, Kreyling W, Tuch T, Heinrich J (2001) PM2.5 measurements in ambient aerosol: comparison between Harvard Impactor (HI) and the Tapered Element Oscillating Microbalance (TEOM) system. Sci Total Environ 278(1–3):191–197

Dead M How do we know and what does it mean? Methods used in the comparative risk assessment of household air pollution. Annual review of public health. 35(1):185–206

Dey S, Di Girolamo L (2010) A climatology of aerosol optical and microphysical properties over the Indian subcontinent from 9 years (2000–2008) of Multiangle Imaging Spectroradiometer (MISR) data. ||nt J Geophys Res 115(2010)

Diggle P (1990) A point process modelling approach to raised incidence of a rare phenomenon in the vicinity of a prespecified point. J Roy Statist Soc Ser A 153(3):349–362

Diggle P (2003) Statistical analysis of spatial point patterns. In: Mathematics in biology. Arnold

Elliott P, Shaddick G, Wakefield JC, Hoogh CD, Briggs DJ (2007) Long-term associations of outdoor air pollution with mortality in Great Britain. Thorax 62(12):1088–1094

Fu SH, Jha P, Gupta PC, Kumar R, Dikshit R et al (2014) Geospatial analysis on the distributions of tobacco smoking and alcohol drinking in India. PLoS One 9(7)

Fuentes M, Song H-R, Ghosh SK, Holland DM, Davis JM (2006) Spatial association between speciated fine particles and mortality. Biometrics 62(3):855–863

GBD Risk Factors Collaborators* , Forouzanfar MH, Alexander L, Anderson HR, Bachman VF, Biryukov S, Brauer M, Burnett R, Casey D, Coates MM, Cohen A, Delwiche K (2013) Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013, Lancet 2015, 386 (10010): 2287–2323. https://doi.org/10.1016/S0140-6736(15)00128-2

Gelfand A, Diggle P, Fuentes M, Guttorp P (eds) (2010) Handbook of spatial statistics. Chapman & Hall

Gillis D, Semanjski I, Lauwers D (2016) How to monitor sustainable mobility in cities? Literature review in the frame of creating a set of sustainable mobility indicators. Sustainability 8:29

Grell K, Diggle P, Frederiksen K, Schüz J, Cardis E, Andersen P (2015) A three-dimensional point process model for the spatial distribution of disease occurrence in relation to an exposure source. Stat Med 34:3170–3180

Henderson SB, Beckerman B, Jerrett M, Brauer M (2007) Application of land use regression to estimate long-term concentrations of traffic-related nitrogen oxides and fine particulate matter. Environ Sci Technol 41:2422–2428

Hoek G, Krishnan R, Beelen R, Peters A, Ostro B, Brunekreef B, Kaufman J (2013) Long-term air pollution exposure and cardio-respiratory mortality: a review. Environ Health 12(1):12–43

Hoff R, Christopher SA (2009) Remote sensing of particulate matter air pollution from space: have we reached the promised land? J Air Waste Manage Assoc 59:642–675. https://doi.org/10.3155/1047-3289.59.6.642

Jerrett M, Buzzelli M, Burnett RT, DeLuca PF (2005) Particulate air pollution, social confounders, and mortality in small areas of an industrial city. Soc Sci Med 60:2845–2863

Kanaroglou PS, Jerrett M, Morrison J, Beckerman B, Arain MA, Gilbert NL, Brook JR (2005) Establishing an air pollution monitoring network for intra-urban population exposure assessment: a location-allocation approach. Atmos Environ 39:2399–2409

Kirk R. Smith, Nigel Bruce, Kalpana Balakrishnan, Heather Adair-Rohani, John Balmes, Zoë Chafe, Mukesh Dherani, H. Dean Hosgood, Sumi Mehta, Daniel Pope, Eva Rehfuess, (2014) Millions Dead: How Do We Know and What Does It Mean? Methods Used in the Comparative Risk Assessment of Household Air Pollution, Annual Review of Public Health 35:1, 185–206

Kazemi-Beydokhti M, Abbaspour R, Kheradmandi M, Bozorgi-Amiri A (2019) Determination of the physical domain for air quality monitoring stations using the ANP-OWA method in GIS. Environ Monit Assess 191. https://doi.org/10.1007/s10661-019-7422-3

Lee D, Sarran C (2015) Controlling for unmeasured confounding and spatial misalignment in long-term air pollution and health studies. Environmetrics 26(7):477–487

Loomis D, Huang W, Chen G (2014) The International Agency for Research on Cancer (IARC) evaluation of the carcinogenicity of outdoor air pollution: focus on China. Chin J Cancer 33 (4):189–196. https://doi.org/10.5732/cjc.014.10028

Madsen L, Ruppert D, Altman NS (2008) Regression with spatially misaligned data. Environmetrics 19(5):453–467

Martin RV (2008) Satellite remote sensing of surface air quality. Atmos Environ 42(34):7823–7843

McBride SJ, Williams RW, Creason J (2007) Bayesian hierarchical modeling of personal exposure to particulate matter. Atmos Environ 41(29):6143–6155

Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson GL et al (2007) Long-term exposure to air pollution and incidence of cardiovascular events in women. N Engl J Med 356:447–458

Minghui Diao, Tracey Holloway, Seohyun Choi, Susan M. O’Neill, Mohammad Z. Al-Hamdan, Aaron Van Donkelaar, Randall V. Martin, Xiaomeng Jin, Arlene M. Fiore, Daven K. Henze, Forrest Lacey, Patrick L. Kinney, Frank Freedman, Narasimhan K. Larkin, Yufei Zou, James T. Kelly & Ambarish Vaidyanathan (2019) Methods, availability, and applications of PM2.5 exposure estimates derived from ground measurements, satellite, and atmospheric models, Journal of the Air &Waste Management Association, 69:12, 1391–1414. https://doi.org/10.1080/10962247.2019.1668498

Molitor J, Molitor N-T, Jerrett M, McConnell R, Gauderman J, Berhane K, Thomas D (2006) Bayesian modeling of air pollution health effects with missing exposure data. Am J Epidemiol 164(1):69–76

Moraga P, Cramb SM, Mengersen KL, Pagano M (2017) A geostatistical model for combined analysis of point-level and area-level data using INLA and SPDE. Spat Statist 21:27–41

Mudiyanselage R, Tripathi N (2019) Geostatistical predictive modelling for asthma and chronic obstructive pulmonary disease using socioeconomic and environmental determinants. Environ Monit Assess 191. https://doi.org/10.1007/s10661-019-7417-0

Murray C et al (2015) Global, regional and national comparative risk assessment of behavioural, environmental, occupational and metabolic risks or clusters of risks in 188 countries 1990–2013: a systematic analysis for the GBD background. Lancet 6736:1–27

Nuckols JR, Ward MH, Jarup L (2004) Using geographic information systems for exposure assessment in environmental epidemiology studies. Environ Health Perspect 112(9):1007–1015

Ostro B, Sanchez J, Aranda C, Eskeland G (1996) Air pollution and mortality: results from a study of Santiago. Chile J Expo Anal Environ Epidemiol 6:97–114

Oyana T, Podila P, Relyea G (2019) Effects of childhood exposure to PM2.5 in a Memphis pediatric asthma cohort. Environ Monit Assess 191. https://doi.org/10.1007/s10661-019-7419-y

Pannullo F, Lee D, Waclawski E, Leyland AH (2015) Improving spatial nitrogen dioxide prediction using diffusion tubes: a case study in West Central Scotland. Atmos Environ 118:227–235

Peng RD, Bell ML (2010) Spatial misalignment in time series studies of air pollution and health data. Biostatistics 11(4):720–740

Peng RD, Dominici F, Pastor-Barriuso R, Zeger SL, Samet JM (2005) Seasonal analyses of air pollution and mortality in 100 US cities. Am J Epidemiol 161(6):585–594

Pinto Junior JA, Gamerman D, Paez MS, Fonseca Alves RH (2015) Point pattern analysis with spatially varying covariate effects, applied to the study of cerebrovascular deaths. Stat Med 34 (7):1214–1226

Pratt J, Gan R, Ford B, Brey S, Pierce J, Fischer E, Magzamen S (2019) A national burden assessment of estimated pediatric asthma emergency department visits that may be attributed to elevated ozone levels associated with the presence of smoke. Environ Monit Assess 192:261. https://doi.org/10.1007/s10661-019-7420-5

Raaschou-Nielsen O et al (2013) Air pollution and lung cancer incidence in 17 European cohorts: prospective analyses from the European study of cohorts for air pollution effects (ESCAPE). Lancet Oncol 14:813–822

Ritz B, Yu F, Chapa G, Fruin S (2000) Effect of air pollution on preterm birth among children born in southern California between 1989 and 1993. Epidemiology 11(5):502–511

Ritz B, Wilhelm M, Hoggatt KJ, Ghosh JKC (2007) Ambient air pollution and preterm birth in the environment and pregnancy outcomes study at the University of California. Los Angeles Am J Epidemiol 166(9):1045–1052

Rushworth A, Lee D, Mitchell R (2014) A spatio-temporal model for estimating the long-term effects of air pollution on respiratory hospital admissions in Greater London. Spat Temp Epidemiol 10:29–38

Sahsuvaroglu T, Jerrett M, Sears MR, McConnell R, Finkelstein N, Arain A, Newbold B, Burnett R (2009) Spatial analysis of air pollution and childhood asthma in Hamilton, Canada: comparing exposure methods in sensitive subgroups. Environ Health 8:14

Sahu S, Gelfand A, Holland D (2010) Fusing point and areal level space-time data with application to wet deposition. J R Statist Soc Ser 59(1):77–103

Samoli E, Touloumi G, Zanobetti A, Le Tertre A, Schindler C, Atkinson R, Vonk J et al (2003) Investigating the dose-response relation between air pollution and total mortality in the aphea-2 multicity project. Occup Environ Med 60(12):977–982

Semanjski I, Bellens R, Gautama S, Witlox F (2016a) Integrating big data into a sustainable mobility policy 2.0 planning support system. Sustainability 8:1142

Semanjski I, Lopez Aguirre AJ, De Mol J, Gautama S (2016b) Policy 2.0 platform for Mobile sensing and incentivized targeted shifts in mobility behavior. Sensors 16:1035

Sourangsu C, Sagnik D (2016) Cause-specific premature death from ambient PM2.5 exposure in India: estimate adjusted for baseline mortality. Environ Int 91:283–290

Tian J, Chen D (2010) A semi-empirical model for predicting hourly ground level fine particulate matter (PM2.5) concentration in southern Ontario from satellite remote sensing and ground-based meteorological measurements. Remote Sensing Environ 114:221–229

Van Donkelaar A, Martin RV, Brauer M, Kahn R, Levy R, Verduzco C et al (2010) Global estimates of ambient fine particulate matter concentrations from satellite-based aerosol optical depth: development and application. Environ Health Perspect 118:847–855. https://doi.org/10.1289/ehp.0901623

Xingzhe X, Semanjski I, Gautama S, Tsiligianni E, Deligiannis N, Rajan RT, Pasveer F, Philips W (2017) A review of urban air pollution monitoring and exposure assessment methods ISPRS. Int J Geo Inf 6:389. https://doi.org/10.3390/ijgi6120389

Young LJ, Gotway CA, Yang J, Kearney G, Du Clos C (2009) Linking health and environmental data in geographical analysis: it's so much more than centroids. Spat Temp Epidemiol 1 (1):73–84

Zhiyong H, Ranga Rao K (2009) Particulate air pollution and chronic ischemic heart disease in the eastern United States: a county level ecological study using satellite aerosol data. Environ Health 8:26

Zidek JV, Shaddick G, Meloche J, Chatfield C, White R (2007) A framework for predicting personal exposures to environmental hazards. Environ Ecol Stat 14(4):411–431

Chapter 19
Health Impacts of Contaminated Water in India: Coping Strategies for Sustainable Development

J. V. Arun and A. Premkumar

Abstract Humans driven by insatiable consumeristic demands cause divergence in our ecological balance. Rapid industrialization and urbanization create fissures in environmental quality and human health. Degradation of the environment because of incompetent management of water resources is the major urban environmental problems confronting developing economies like India. Groundwater in India is mainly contaminated with geogonic pollutants, Fluoride and Arsenic. Among the states, river stretches in Maharashtra and Assam are the extremely polluted ones. The study primarily intends to evaluate the impact of water pollution on human health. Drinking water contamination accounts for a substantial increase in the rates of Fluorosis, Acute Diarrhoeal Diseases, Typhoid, and Cholera which engulf the Indian public health system. The assessment carried out reveals that diarrhoea is the major waterborne disease in India. The main reasons for the cause of diarrhoeal cases were estimated using regression models. Further, the study suggests ways and means control of water contamination.

Keywords Water · Pollution · Health · Disease · Diarrhoea

1 Introduction

Rapid growth in population, urbanization, and industrialization has created differences in environmental quality and human health. Immediate consequences of environmental degradation take the form of damage to human health and most importantly, damage costs in developing nations are higher than those in developed

---

J. V. Arun (✉)
Assistant Professor, Department of Economics, Government Arts College for Men (Autonomous), Chennai, Tamil Nadu, India

A. Premkumar
Research Scholar, Department of Economics, Sacred Heart College (Autonomous), Tirupattur, Tamil Nadu, India

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_19

nations (David and Warford 1993). Incompetent management of water resources is the major urban environmental problems looming developing economies. Earth's surface is covered by 70% of water but only 1% of its water is available as fresh drinking water. Being a finite resource, water should be preserved and utilized efficiently. But studies show that there is an increasing trend in water pollution (Schwarzenbach et al. 2010; Ambast et al. 2006). Every day, sewage and industrial waste to the extent of 2 million tons are discharged into the world's water (UN WWAP 2003). In developing countries, 70% of industrial wastes are disposed into the water where they contaminate existing water supplies (UNESCO 2009). Further, surface water is tainted with heavy metals, persistent organic pollutants, nutrients, and nitrates that harm health. Climate change also affects the availability and quality of water required for basic human needs, thus undermining the basic rights to safe drinking water (World Water Development Report 2020). The linkage between water quality and public health is well established (Koonan and Khan 2010; Tyagi 2009). The disposal of untreated or partly treated industrial and municipal sewage into water bodies or on land results in water pollution (Bhullar 2013; Chaplin 2011). This has a very serious impact on drinking water quality. A broader understanding of the health and water sector implies that water-borne diseases are mostly linked to insufficient sanitation (Coleman 2011). Therefore the issue of water contamination can only be addressed if sanitation, health, and environment are considered as its intrinsic components.

According to a Lancet report on pollution and health (2017), worldwide an estimated 1.8 million people died because of polluted water in the year 2015 and out of which 0.64 million from India alone. Composite Water Management Index (2018), claims that 70% of water supply in India is contaminated resulting in approximately 0.2 million deaths every year and the nation has been ranked way below at 120th place among 122 countries in the water quality index. These figures highlight the fact that the magnitude of the water pollution problem is significantly high in India. In particular, the incidence of water contamination is higher in urban areas (India Infrastructure Report 2011). The major source of water pollution in India is ascribed to the discharge of untreated waste from commercial institutions, dumping of industrial waste matter, and run-off from agricultural fields. Some of the other reasons being leaky water pipe joints in areas where the water pipe and sewage line pass close together. In some cases, water gets polluted at the source due to the inflow of sewage into the source (Chakraborty et al. 2004). Surface and groundwater pollution pose a severe threat to mankind due to its unswerving impact on human health. Moreover, Sustainable Development Goal (SDG) target 6.1 calls for all-inclusive access to safe and affordable drinking water (WHO 2019). India, as a signatory to the SDGs needs to work out its priorities to achieve the target by 2030. In consideration of the facts and figures, the study aims to assess the impact of water pollution on human health in India.

2 Water Pollution

India accounts for approximately 4% of the water resources of the world (Kaur et al. 2012) and the estimated utilizable water is about 1123 km^3 (CWC 2010). Out of which, water from the surface is estimated at 690 km^{3,} and groundwater has been assessed to be at 433 km^3. But agricultural runoff, sewage, and industrial waste discharge have resulted in deterioration of water quality. In general, groundwater is less susceptible to contamination than surface water bodies (Chakraborty and Mukhopadhyay 2014). Groundwater depletion and pollution are due to the geogenic process which is a naturally happening phenomenon. Declining groundwater quality can also be attributed to over-exploitation as it results in saline water intrusion in the coastal areas (Chinchmalatpure et al. 2019). Fluoride and Arsenic are the two naturally occurring toxic chemicals that contaminate groundwater in India. A high concentration of fluoride causes fluorosis resulting in weak bones and teeth whereas arsenic is a carcinogen. In India, about 40 million people are residing in arsenic contamination zone whereas the presence of fluoride beyond the permissible limit of 1.5 mg/liter in groundwater has been reported in several parts of the nation (CGWB 2018). Karnataka, Rajasthan, and Tamil Nadu are the three states with the highest level of fluoride contamination in their drinking water sources whereas Assam has the most number of districts with the excess presence of arsenic in groundwater followed by Punjab and Uttar Pradesh (NHP 2019).

The major part of surface water is rivers, wetlands, and creeks. India is endowed with a network of rivers but they are used as carriers of wastewater. Organic and bacterial contaminations are the critical pollution factors of rivers which lead to water-borne diseases. These demands an in-depth study on state-wise polluted river stretches (Fig. 19.1) which were mapped with the support of Geographic Information System (GIS). The states are classified into 5 categories viz., states with (i) <10 (ii) 11–20 (iii) 21–30 (iv) 31–40 and (v) >40 polluted rivers. It reveals that Maharashtra and Assam have more than 40 polluted rivers followed by Kerala and Madhya Pradesh which has been slotted under [21–30] category. Domestic sewage, garbage dumping, idol immersion during the festival are the major causes of water contamination in Maharashtra (Gangotri and Khobragade 2017). In the case of Assam, the generation of waste from industries and municipal bodies has polluted the river water quality (Girija et al. 2007). High pH values are related to acidic behavior in various locations of Kerala due to the discharge of industrial waste into the river (Muruga Lal Jeyan and Akhila 2017). Uncontrollable use of insecticides, pesticides in cement factories, lime kilns process results in water pollution in Madhya Pradesh (Mishra 2017). The states having many polluted rivers in the range [11–20] are Goa, Gujarat, Karnataka, Odisha, Uttar Pradesh, and West Bengal. It is heartening to note that 22 States/UTs have less than 10 polluted river stretches. Dumping of solid and hazardous waste in wetlands has severely polluted them leading to severe health impacts (NEP 2006). Overall, rivers and other surface water sources are facing serious threat due to letting loose of untreated effluents.

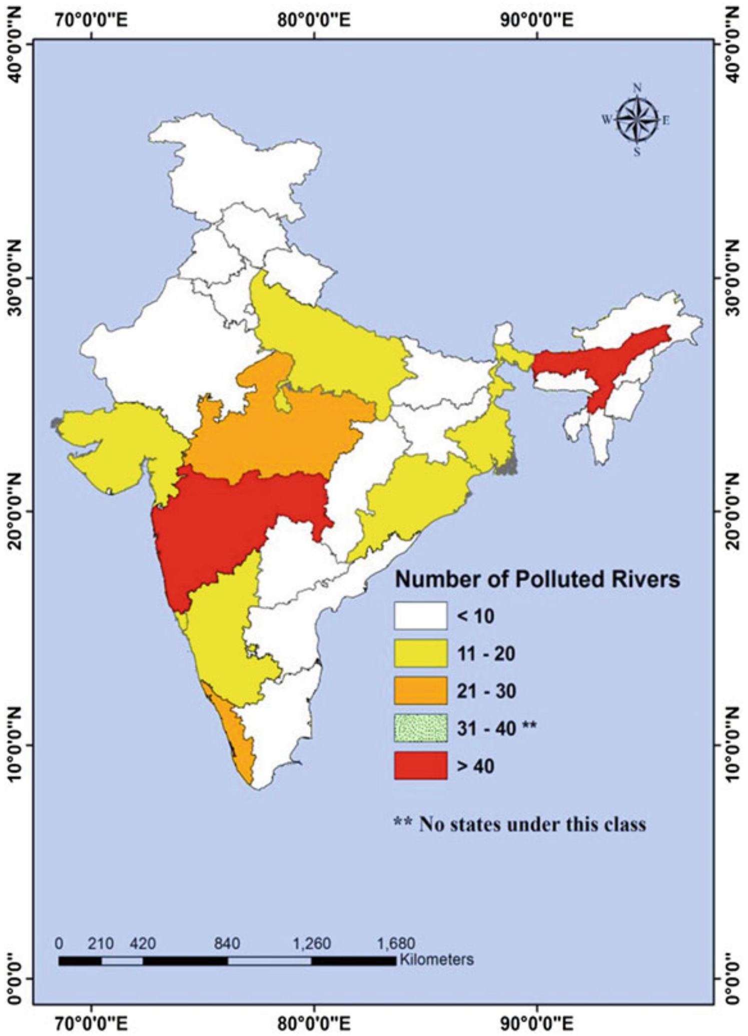


Fig. 19.1 State wise Polluted River Stretches
Source: Developed by the authors
Note: Based on Lok Sabha Unstarred Question No-165

Therefore, increasing surface water pollution warrants the abatement of pollution and conservation of rivers.

3 Health Impacts of Water Pollution

It is a well-known fact that polluted water leads to water-borne diseases and its burden on human health is heavy and sometimes fatal. Furthermore, health costs incurred due to water pollution are quite substantial. The presence of geogenic pollutant arsenic in groundwater causes cancer, endocrine disruptor, and skin problems. Drinking water sources contaminated with fluoride ion creates disorders such as dental and skeletal fluorosis. In India alone, 0.87 million dental and 0.26 million skeletal suspected cases were identified. Out of these, Karnataka itself accounts for 62.67% of dental cases followed by Rajasthan and Tamil Nadu with 27.07% and 2.27% respectively. The same scenario prevails for skeletal cases; Karnataka has 73.7%, Rajasthan 14.10%, and Tamil Nadu 5.16% (NHP 2019). Besides, pesticides and other hazardous chemical contaminants in water result in reproductive and developmental problems (WHO 2003). Globally, 1.8 million people consume fecally contaminated water for drinking purposes (WHO/UNICEF 2014). Approximately 3.1% of all deaths and 3.7% of DALYs (Disability Adjusted Life Years) were caused due to unsafe water, sanitation, and hygiene (WHO 2013). Therefore, Fecal Coli contamination is an important issue to be addressed.

Each year, contaminated water causes 4 billion diarrhoeal cases and as a result, 2.2 million deaths occur (WHO 2000). In India, half a million children die every year because of diarrhoea (UNICEF 2000). Cholera, Dysentery, Hepatitis, Typhoid and Acute Diarrhoeal Disease (ADD) is the most common water-borne diseases that affect a large population in the tropical regions. Out of which, the prevalence of Acute Diarrhoeal Disease, Typhoid and Cholera are predominant in India. To completely understand the enormity of the situation, those three major health diseases were discussed in detail for the period 2009–2018. The Table 19.1 reveals that the country was able to control cholera to a greater extent which is not so with typhoid as it shows an increasing trend. Only in recent years (2017–18), the growth rate of diarrhoeal cases has started to decline but it remains as the leading waterborne disease to combat. The reasons behind more number of diarrhoeal cases may be

Table 19.1 Number of Diarrhoea, Typhoid & Cholera Cases

Years	Diarrhoea	Typhoid	Cholera
2009	11,984,490	1,039,311	3482
2010	10,742,327	1,084,885	5155
2011	10,231,049	1,062,446	2341
2012	11,701,755	1,477,699	1583
2013	11,413,610	1,650,145	1130
2014	11,673,018	1,736,687	844
2015	12,913,606	1,937,413	913
2016	13,923,275	2,222,695	841
2017	13,416,748	2,264,453	508
2018	13,194,775	2,308,537	651

Source: Authors tabulation of National Health Profile Statistics, Government of India

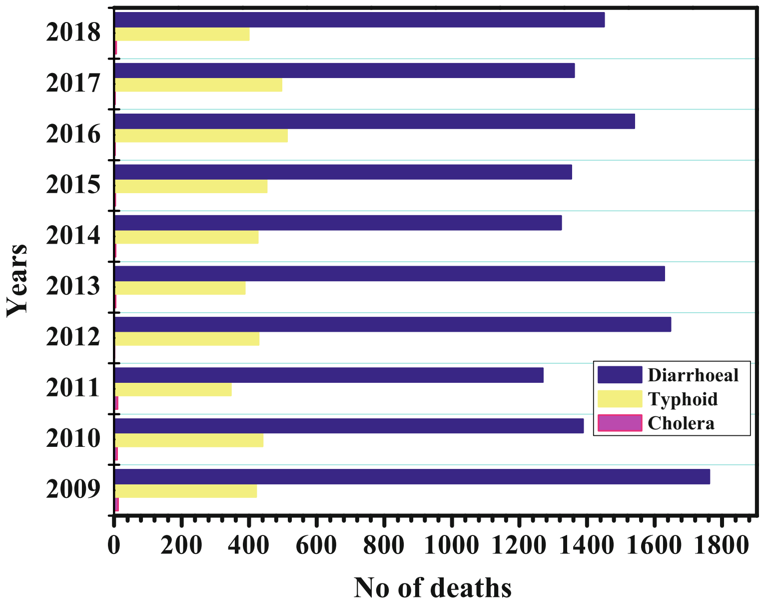


Fig. 19.2 Number of deaths due to Diarrhoea, Typhoid & Cholera
Source: Developed by the Authors
Note: Based on National Health Profile Statistics, Government of India

partly due to excess runoff during monsoon season and water contamination caused by pathogens such as *E-coli*. In 2018, West Bengal (17.06%) followed by Uttar Pradesh (9.91%) is the states with the most number of diarrhoeal cases and in the case of Typhoid, West Bengal (10.89%) trailed Uttar Pradesh (33.11%). As far as Cholera is concerned, it is higher in the states of Uttar Pradesh, Delhi, and West Bengal with a percentage of 23.50%, 20.58%, and 19.35% respectively (NHP 2019). Put together, Uttar Pradesh is the worst affected state with a large number of cases whereas water borne diseases are the common morbidity among the flood-prone population in West Bengal (Joshi Pooran et al. 2011) (Fig. 19.2).

With the most number of deaths, diarrhoea is the major waterborne disease in India followed by Typhoid and Cholera. It may be noted that there is a reduction in the death rate of these diseases probably due to an increase in health awareness among the people. Sometimes, cholera can cause life-threatening forms of diarrhoea. Maharashtra and Haryana are the only two states that encountered cholera deaths in 2018. Among the states in India, the maximum number of diarrhoea and typhoid deaths has been registered in Uttar Pradesh and West Bengal. In particular, diarrhoea kills more children than malaria, measles, and AIDS combined (Liu et al. 2012). About 88% of diarrhoeal deaths are due to unsafe water, inadequate sanitation, and insufficient hygiene (UNICEF 2006). The paper attempts to evaluate the variable's influence on diarrhoea that will be useful for policy formulations.

4 Model Specification

Studies have shown unsafe water sources, inadequate sanitation, and rainfall have interdependent effects on diarrhoea (Fink et al. 2011; Bhavnani et al. 2014). As diarrhoea is the most common waterborne disease in India, it is all the more important to estimate the risk of diarrhoea from those variables. In this study, a multiple regression was carried out to probe whether water from unprotected well/ spring, annual rainfall, latrine usage method, and types of household drainage system could significantly predict diarrhoeal cases in India. The study was carried out for the year, 2018. The data used in the analysis were sourced from the India Meteorological Department, National Sample Survey 76th round on Drinking Water, Sanitation, Hygiene, and Housing Condition in India and National Health Profile. Table 19.2 provides the definitions of the variables used in the model.

Table 19.3 provides the values of R^2, adjusted R^2, F- ratios, and Coefficients. R^2 tells the proportion of variation in outcome variable that can be explained by the predictor variables and in this case, $R^2 = 0.60$ (60%) of the variability. Adjusted R^2

Table 19.2 Description of variables

Variables	Definitions
Diarrhoea[a]	Diarrhoea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual).
Unprotected well/ spring[a]	A well not secluded from runoff water or not protected from bird droppings and animals was classified as unprotected well. A spring which was not protected or exposed to outside pollution was called unprotected spring.
Annual rainfall[c]	Rainfall is expressed as the depth to which it can cover a horizontal projection of the earth's surface if there is no loss by evaporation, runoff or infiltration. It is expressed in terms of mm or cm.
Latrine usage[a] regularly	If household members generally used latrine, it was considered that the member had used latrine regularly.
Occasionally	If a member did not use latrine regularly but used it from time to time, it was considered that the member had used latrine occasionally.
Never	If a member never used latrine or rarely used it, it was considered that the member had never used latrine.
Household drainage system[a] underground	Septic tank is an excreta collection device consisting of a water-tight settling tank, which is normally located underground.
Covered/open Pucca	Pucca structure was defined as a structure whose walls and roofs were made of pucca materials.
Open Katcha	A structure which had walls and a roof made of non-pucca materials were considered as a katcha structure.
No drainage	No formal system of carrying off household wastewater and liquid waste.

Source: [a]WHO, [b]NSS 76th round GoI, [c]India Meteorological Department

Table 19.3 Coefficients

Variables	Coef.	Std. Err.	P > t
UNWS	0.2590903	0.1150423	0.033[a]
AR	0.1769154	0.101522	0.093[b]
LUREG	11.04218	6.51888	0.102
LUOCC	11.43825	6.525314	0.091[b]
LUNEV	11.13357	6.567273	0.102
HDSUN	−8.950086	6.449945	0.177
HDSCOP	−8.93124	6.449612	0.178
HDSOK	−8.996964	6.454805	0.175
HDSND	−8.90876	6.45112	0.179
_cons	−208.6369	414.2019	0.619
Source	**SS**	**Df**	**MS**
Model	275.446008	9	30.605112
Residual	179.106303	26	6.88870395
Total	454.552311	35	12.9872089
Number of obs	**36**		
F(9, 26)	**4.44**		
Prob>F	**0.0013**		
R- squared	**0.6060**		
Adj R- squared	**0.4696**		

Source: Computed data

[a]Significant at 5%

[b]Significant at 10%

enhances data accuracy by adjusting the statistic based on the number of independent variables in the model (Kothari and Gaurav 2019). The F- ratios reveals that the overall regression model is a good fit for the table. The Coefficients explain the extent to which the individual predictor variables contribute to the regression model.

The functional form of the model can be expressed as:

$$\mathrm{Dia}_{I\,1} + {}_1\mathrm{UNWS} + {}_2\mathrm{AR} + {}_3\mathrm{LUREG} + {}_4\mathrm{LUOCC} + {}_5\mathrm{LUNEV} + {}_6\mathrm{HDSUN} + {}_7\mathrm{HDSCOP} + {}_8\,\mathrm{HDSOK} + {}_9\,\mathrm{HDSND} + {}_i$$

Where, $\mathrm{Dia}_\mathbf{i}$ = number of diarrhoeal cases; UNWS = unprotected well/spring; AR = annual rainfall; LUREG = regular usage of latrine; LUOCC = occasional usage of latrine; LUNEV = never used latrine; HDSUN = underground drainage system; HDSCOP = covered/open pucca drainage system; HDSOK = open katcha drainage system; HDSND = no drainage system; α = constant; β = vector of coefficients and, ε_i = error term.

The results indicate water taken from unprotected well/spring statistically significantly predicted the dependent variable (number of diarrhoeal cases) at 5% level of significance whereas annual rainfall and occasional usage of latrine positively affect the diarrhoeal cases at 10% level of significance. Our finding that unimproved water source (i.e., unprotected well/spring) increases diarrhoeal cases are broadly inconsistent with other studies (Pruss et al. 2002; Hashizume et al. 2008; WHO/UNICEF

2011). However, we found that there exists a complex interaction between sanitation practices (i.e., latrine usage) and diarrhoea. For instance, occasional usage of the latrine was a risk factor but 'never used latrine' is insignificant. The reasons for this inconsistency maybe because of the fact bacterial pathogens exploit inconsistent household sanitation practices than without one. Additionally, our results show that the level of rainfall increases the risk of diarrhoea which is inconsistent with the studies of Drayna et al. (2010) and Singh et al. (2001). With regards to the correlation between contaminated water and diarrhoea; improved water facilities, better sanitation practices and patterns of rainfall are the major defining contexts to be considered. The study encompasses major health hazards caused by different kinds of water pollutants (biological and chemical) and in line with this; the following section reviews policy formulations and coping mechanisms for sustainability.

5 Policy Instruments

The institutional framework is required to mitigate the health risks caused by untreated drinking water. On the human rights to safe drinking water; UN Committee on Economic, Social and Cultural Rights (2002) argued on the legal bases that it is necessary to decrease the risk of a water-related disease. UN reaffirmed the recognition in its subsequent resolutions – The Human Right to Water and Sanitation, 2010/11. Besides the UN, other global agencies through their decisions and scheme of action engaged in combating the ill effects of water contamination. These include the Dublin Statement on Water and Sustainable Development (1992), World Health Assembly Resolution- Drinking Water, Sanitation, and Health (2011) and 6th World Water Forum-Ministerial Declaration (2012).

The Government of India (GoI) has enacted a good number of laws to control water pollution. Notably, the Water (Prevention and Control of Pollution) Act, 1974 and Water (Prevention and Control of Pollution) Cess Act, 1977 has provided the legal framework for the prevention and control of water pollution resulting from the disposal of untreated sewage into water bodies. But the Water Cess Act aimed at generating more revenue rather than limiting the usage of water by industries (Chakraborty and Mukhopadhyay 2014). Environment Protection Act, 1986, lay down standards for the discharge of pollutants from the industries so that the quality of water is not affected. The Policy Statement for Abatement of Pollution, 1992, highlighted the need for a sewage treatment system in water polluted areas. However, studies on the reduction in water pollution level suggest that charges of pollution tax should be higher than the rate of water cess to achieve prescribed water quality standards (Murty and Kumar 2011). Water Quality Assessment Authority set up by the Ministry of Environment and Forests has issued the Uniform Protocol on Water Quality Monitoring Order, 2005, and the Uniform Drinking Water Monitoring Protocol, 2013. Ministry of Environment and Forests (MoEF) launched the National Lake Conservation Plan in 2008 to restore the lake water quality in the country. Similarly, to conserve water bodies, the Ministry of Water Resources (MoWR) launched the ‘Repair, Renovation, and Restoration of water bodies’ scheme in 2009 with domestic support and external assistance. To cut short the pollution levels in the Ganga river basin, World Bank has approved a loan of $400 million to National Mission for Clean Ganga (NMCG) for the period 2020–2025 and this is in addition to the already received $600 million for the project until 2021. Government initiatives like the Clean Ganga project, 2009 will help the government to overcome surface water pollution issues. In 2013, GoI launched Atal Bhujal Yojana (ABHY) which focuses on interventions to improve groundwater quality. Consequently, the project with the World Bank started in 2018, has been planned until 2023. Studies have shown that there is a significant correlation between non-availability of piped water, absence of flushed toilets, and morbidity due to diarrhoea. National Urban Health Mission, 2013, provided a framework to expand water and sanitation facilities to effectively address mortality and morbidity associated with diarrhoea. But the implementation of these instruments has been poor as they are non-binding in nature (Cullet and Bhullar 2015).

6 Coping Strategies

Even after enacting many instruments, the country is still not able to combat water pollution levels. In particular, wastewater released into drains causes water pollution which acts as breeding grounds for mosquitoes and other pathogens. These bacterial microbes result in the heightened occurrence of diarrhoea whereas appropriate hygiene behaviors can lessen the frequency of diarrhoeal diseases. Community ownership, participation, education, and empowerment can induce collective behavioral change which is required to achieve better water quality management. Moreover, the integration of community water supply and sanitation programs is vital to achieving safe water supplies and environmental sanitation. But there is a lack of coordination between the departments concerned. Therefore, inter-sectoral cooperation should be strengthened amid all authorities and stakeholders. In a nation like India, the cost of treating sewage waters is on the higher side which is beyond the fiscal means of towns and municipal bodies. Economically viable and socially acceptable resource recovery technology like biological wastewater treatment can be adopted to negate financial constraints.

As intensive agriculture impacts the quality of water, optimum utilization of fertilizers and insecticides should be encouraged to maintain the standard of water quality. Encouraging bio-degradable pesticides and adopting organic farming will result in the reduction of groundwater pollution from agricultural chemicals. Particular attention should be directed towards the management of river basins for the maintenance of the ecosystem. Environmental awareness in the industrial sector has increased but the enforcement and regulation of policy instruments is the issue to be dealt with. Therefore, the emphasis is to be placed on facilitating common effluent treatment plants to ensure the clustering of polluting industries. Taxes on polluting agents provide incentives to industries for adopting the least cost pollution abatement technologies. But an increase in Research and Development (R&D) expenditure is important to construct a cleaner production process. Also, collaboration with private entities will strengthen plans to develop eco-friendly technologies. To develop a harmonized control mechanism, integrated water resource management is necessary that includes water conservation, water harvesting, and waste management. Above all, it has to be realized that the existing policy framework has to be amended or adopted to be in line with the latest standards and principles to control the water-related disease.

7 Conclusion

All these information are explaining the fact that water pollution poses a stern warning to human health. Harmful consequences of contaminated water to human health are already being felt and could become worse in the days to come if it goes unchecked. India's water consumption per head is on the rise along with increased demand for water-intensive products. Therefore, it becomes much more important to conserve the water resources and not to pollute them with non-degradable wastes. India has made substantial progress in combating the health impact of water pollution. Despite the various policy decisions and actions taken by the pollution governing body, India is still vulnerable to water pollution. Enactment of laws and creating institutional structures are not sufficient enough to address the impending environmental problem. The government should strengthen the institutions by providing more autonomy to deal with the tribulations of water pollution. Participation of all the stakeholders in the management of water resources should be encouraged and eco-engineering can also be taken up for rejuvenating the waterways of the country. Above all, reducing water pollution to safe limits will reduce economic losses arise due to illness.

References

Ambast SK, Tyagi NK, Raul SK (2006) Management of declining groundwater in the trans indo-Gangetic plain (India): some options. Agric Water Manag 82(3):279–296

Bhavnani D, Goldstick Jason E, Cevellos W, Trueba G, Eisenberg Joseph NS (2014) Impact of rainfall on Diarrhoeal diseases risk associated with unimproved water and sanitation. Am J Trop Med Hyg 90(4):701–705

Bhullar L (2013) Ensuring safe municipal wastewater disposal in India: is there a legal basis? J Environ Law 25(2):235–260

Chakraborty D, Mukhopadhyay K (2014) Water pollution and abatement policy in India: a study from an economic perspective, Global issues in water policy 10. Springer, Dordrecht

Chakraborty D, Sengupta MK, Rahman MM, Ahmad S, Chowdhury UK, Mukherjee SC (2004) Groundwater arsenic contamination and its health effects in Ganga-Meghna-Brahmaputra plain. J Environ Monit 6:74–83

Chaplin SE (2011) Indian cities, sanitation and the state: the politics of the failure to provide. Environ Urban 23(1):57–70

Chinchmalatpure AR, Bisweswar G, Shrvan K, David CD, Vibhute Sagar D (2019) Groundwater pollution through different contaminants: Indian scenarion. In: Dagar JC et al (eds) Research developments in saline agriculture. Springer, Singapore, pp 423–459

Coleman RM (2011) The human right of sanitation for all: a study of India. Pac McGeorge Glob Bus Dev. L.J. 24:267–302

Cullet P, Bhullar L (2015) Sanitation Law in India- An Overview In: Cullet, Philippe and Bhullar, Lovleen (eds.), Sanitation Law and Policy in India- An Introduction to Basic Instruments Oxford: Oxford University Press, pp 6–16

David P, Warford JJ (1993) World without end: economic environment and sustainable development. Oxford University Press, World Bank, Oxford

Drayna P, McLellan SL, Simpson P, Li SH, Gorelick MH (2010) Association between rainfall and pediatric emergency department visits for acute gastrointestinal illness. Environ Health Perspect 118:1439–1443

Fink G, Gunther I, Hill K (2011) The effect of water and sanitation on child health: evidence from the demographic and health surveys 1986–2007. Int J Epidemiol 40:1196–1204

Gangotri N, Khobragade K (2017) Study of water quality of Sion Lake, Mumbai, Maharashtra. Sch J Eng Technol 5(8):413–415

Girija TR, Chandan M, Chandramouli V (2007) Water quality assessment of an untreated effluent impacted urban stream: the Bharalu tributary of the Brahmaputra River, India. Environ Monit Assess 130:221–236

GOI (2006) National environment policy 2006. Ministry of Environment and Forest, Government of India

GOI (2018) Central ground water board. Ministry of Water Resources RD & GR, Government of India, Faridabad

GOI (2019) National health profile. Central Bureau of Health Intelligence, Ministry of Health and Family Welfare, Government of India, New Delhi

Hashizume M, Wagatsuma Y, Faruque AS, Hayashi T, Hunter PR, Armstrong B, Sack DA (2008) Factors determining vulnerability to diarrhoea during and after severe floods in Bangladesh. J Water Health 6:323–332

IDFC (2011) India infrastructure report 2011, water, policy and performance for sustainable development. Oxford University Press

Joshi Pooran C, Sonia K, Aribam Bijaya S, Prashant K, Aoust Olivia S, Singh Mongjam M, Michael M, Guha SD (2011) Recurrent floods and prevalence of diarrhoea among under five children: observations from Bahraich district, Uttar Pradesh, India. Glob Health Action 4

Kaur R, Wani SP, Singh AK, Lal K (2012) Wastewater production, treatment and use in India, Country report India, India

Koonan S, Khan AH (2010) Water, health and water quality regulation. In: Cullet P, Gowlland-Gulatieri A, Madhav R, Ramanathan U (eds) Water law for the twenty-first century: national and international aspects of water law reform in India. Routledge, Abingdon, pp 287–310

Kothari CR, Gaurav G (2019) Research methodology: methods and techniques, 4th edn. New Age International Publishers, New Delhi

Li L, Johnson Hope L, Simon C, Jamie P, Susana S, Lawn Joy E, Igor R, Harry C, Cibulskis R, Mengying Li MSPH, Colin M, Robert B (2012) Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 379 (9832):2151–2161

Ministry of Water Resources (2010) Central water commission annual report 2010–11. Ministry of Water Resources, Government of India

Mishra A (2017) Impact of pollutants on water quality of Satna River Madhya Pradesh India. Eur J Biotechnol Biosci 5(2):66–69

Murty MN, Kumar S (2011) Water pollution in India: an economic appraisal. In: India infrastructure report 2011: water policy and performance for sustainable development. IDFC/Oxford University Press, New Delhi, pp 285–298

Muruga Lal Jeyan JV, Akhila R (2017) Consistent evaluation of Largest River of Kerala to restrict water limitations at ten locations. Indian J Sci Res 17(1):132–136

National Sample Survey (NSS) (2018) Report no. 584: drinking water, sanitation, hygiene and housing condition in India, NSS 76th round (July–December 2018)

Philippe C, Bhullar L (2015) Sanitation law in India- an overview in: cullet, Philippe and Bhullar, Lovleen (eds.). Oxford University Press, Sanitation Law and Policy in India- An Introduction to Basic Instruments Oxford, pp 6–16

Pruss A, Kay D, Fewtrell L, Bartram J (2002) Estimating the burden of disease from water, sanitation, and hygiene at a global level. Environ Health Perspect 110:537–542

Schwarzenbach Rene P, Thomas E, Hofstetter Thomas B, von GU, Bernhard W (2010) Global WaterPollution and human health. The Annual Review of Environment and Resources 35:109–136

Singh RB, Hales S, de Wet N, Raj R, Hearnden M, Weinstein P (2001) The influence of climate variation and change on diarrhoeal disease in the Pacific Islands. Environ Health Perspect 109:155–159

Tyagi PC (2009) Water pollution and contamination. In: Iyer R (ed) Water and the laws in India. Sage, New Delhi, pp 329–355

UN WWAP (2003) United Nations world water assessment programme. The world water development report 1: water for people, water for life. UNESCO, Paris

UNESCO (2009) Water in a changing world. The United Nations world water development report 3. United Nations Educational, Scientific and Cultural Organization, Paris

UNESCO (2020) The United Nations world water development report 2020. United Nations Educational, Scientific and Cultural Organization, France

UNICEF (2006) Annual report 2006, United Nations international children’s fund. United Nation, New York

WHO (2003) Guidelines for drinking water quality, 3rd edn. World Health Organization, Geneva

WHO (2013) The world health report 2013, research for universal health coverage. World Health Organization, Geneva

WHO and UNICEF (2000) Global water supply and sanitation assessment 2000 report. World Health Organization and United Nations Children’s Fund Joint Monitoring Programme for Water Supply and Sanitation

WHO and UNICEF (2011) WHO/UNICEF joint monitoring Programme (JMP) for water supply and sanitation, Types of Drinking-Water Sources and Sanitation

WHO and UNICEF (2014) Progress on drinking water and sanitation. World Health Organization, Geneva

Chapter 20

The Potential of Biotechnology on Soil Quality by Minimizing Agrochemical Impact to Ensure Sustainable Agriculture-A Review

Solomon Abera

Abstract Ensuring sustainable agriculture by producing quality and adequate plant products for the long-term with minimum influences on the environment without compromising with the soil quality is a critical scientific goal. Once soil health is confirmed it can maintain environmental values, sustain biological functions, and encourage animal and plant health. Over the years, farmers and private investors have been using agrochemicals intensively, which influences ecosystem and plant productivity. The usual approach to improving crop productivity is by conventional methods and use of synthetic agrochemicals, which are a slow process and pollute the environment respectively. The rapid development of biotechnological tools like genotyping, genetic engineering, and transcriptional profiling speed up the classical approach and are ecofriendly. Genome-wide transcriptional profiling approaches have been used successfully to investigate the regulation of abiotic influences on plants: like gene regulation responses to plant nutrient uptake in roots of different species and so on. These investigations have implications for adaptation in plants for their respective purposes. This current chapter is focused on a recent biotechnological approach for the identification of microbes applicable in biofertilizers, identification of genes from microbial strains involved in biofertilizers application, the role of plant molecular breeding as well as genetic engineering to minimize agrochemical impact on sustainable agriculture.

Keywords Biotechnology · Rhizosphere microbial · Environmental safety · Soil quality · Sustainable agriculture

---

S. Abera (✉)
Department of Biotechnology, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia
e-mail: solomon.abera@aastu.edu.et

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6_20

1 Introduction

Soil is a complex ecosystem and contains diverse microflora that influences its properties. For agriculture, the soil is the basis and universal medium for food production. Besides its universal role as a medium, soil serves in facilitating gaseous exchange, the flow of energy, detoxification of pollutants, and mediating water and nutrient interchange (Larson and Pierce 1994). Soil quality determines its ability to maintain productivity, minimize the impact on the environment and human health. The terms soil quality and soil health are frequently used interchangeably to describe the capability of the soil to support plant growth (Harris and Bezdicek 1994). Soil quality has been defined as "constant capability of the soil to do as a vigorous living system, in the ecosystem, to continue biological productivity, encourage the quality of water and air, maintain animal, plant and human wellbeing" (Avidano et al. 2005). From a wider perspective, soil can do and used as its potential (Doran and Safley 1997). As the criteria of MDS (minimum data set), soil to be quality various physical, chemical, and biological parameters should be fulfilled (Fig. 20.1).

Starting from cultivation, various activities of human beings are related to the depletion of soil quality in several ways. Extensive chemical inputs as agrochemicals (synthetic fertilizers and pesticides) have contributed as the major cause of declining soil health. The soil health parameters (biological, chemical, and physical) are influenced via agrochemicals. Based on the FAO report, the world population will be increased multifold soon, and we need more food without adversely affecting our environment. To ensure additional food supply, farming is the central resort via using soil as the lone medium. So, the care of soil quality is very important to fulfill

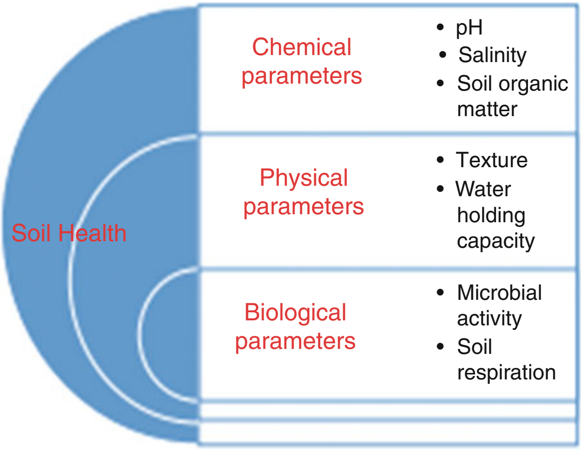


Fig. 20.1 Parameters of soil health. A complete assessment of soil health is based on three classes of soil parameters i.e., physical, chemical, and biological. It may be seen that various parameters are influencing each other and thus soil microbial activity is affected by the physical and chemical health of the soil. SOM soil organic matter. (Reproduced with permission from Prashar and Shah, 2016, Copyright © 2016, Springer International Publishing Switzerland)

the global food demands. However, the main method for this was conducted by the intensive application of agrochemicals to improve nutrient availability and control crops from different types of pests. This has created different environmental, economic as well as social problems. Among these problems, the soil is an environmentally most sensitive part. Extensive and unjustified agrochemicals application has also caused vast soil pollution.

Agrochemicals do affect the functional and structural variability of microbe's populations. The effects may be range from short reversible to long irreversible changes. Generally, agrochemical's inputs seem to give fast benefit for crop-yield improvement so far their constant usage results in a radical impact on soil parameters. Therefore, looking at other ecofriendly biotechnological approaches is the only choice to ensure sustainable agricultural rehearses via keeping soil quality. This chapter by addressing the present status of agrochemicals' impact on soil health focused on recent biotechnological approach for identification of biofertilizer microbial spp., identification of genes from microbial strains involved in biofertilizers application, the role of plant molecular breeding, genomics, and genetic engineering to minimize agrochemical impact on sustainable agriculture.

2 Effect of Synthetic Fertilizers on Soil Parameters

For standard yield and growth plant requires 16 vital elements in the right proportions. Constant crop cultivation leads to a reduction of these nutrients from the soil whereas their availability is a prerequisite to sustaining crops. The common method to add the nutrients in cultivated soils is via the use of artificial fertilizers. Parallel with increasing food productions, the demand for chemical fertilizer has been continuously growing with time. Global fertilizer demand of the three main fertilizer nutrients phosphorus, nitrogen, and potassium reached 186.67 million tons in 2016, increasing by 1.4% over 2015 consumption levels while the need for nitrogen, phosphorus, and potassium is predicted to grow yearly on an average by 1.5, 2.2, and 2.4% respectively from 2015 to 2020 (FAO 2017).

However, the influences of synthetic fertilizers on the soil environment are very serious. Permanent use of synthetic NPK fertilizers has a noticeable effect on physical features of soil like texture; chemical characteristics like salinity, pH; biological parameters like N mineralization, and soil respiration in different crops like corn and wheat (Wu et al. 2012). NPK fertilizers have also a negative impact on dehydrogenase enzymatic activities. For instance, in NPK applied soils compared with organic-manure added soils, the activity of this enzyme in the soil is negatively distressed via triggering copper contamination (Xie et al. 2009). Considerably lower enzymatic activities have been detected in soil treated with synthetic fertilizer than organic fertilizer treated for spring cereals cultivation (Balezentiene and Klimas 2009). Therefore, it indicated that the application of synthetic NPK fertilizers commonly leads to the declining of most common soil enzyme activities and also creates unwanted changes in N concentrations and organic carbon in the soil (SOC).

Several studies were reported about a significant increase in microbial communities, SOC, and enzymatic functions in soils, fertilized by organic manure when compared with those treated by synthetic inorganic fertilizers to support crops like mustard and wheat (Yang et al. 2011).

Furthermore, the diversity of gram-negative bacteria that contains several plant-friendly clusters like *Pseudomonas* is adversely influenced due to the extensive use of inorganic synthetic fertilizers on wheat and rice (Wu et al. 2012). The poisonous impact of heavy metals like cypermethrin, copper, cadmium, and on soil microbial communities has been evaluated while the microorganisms' sensitivity to these contaminants was documented to be greater in soils fertilized with synthetic chemical fertilizers (Xie et al. 2009). Thus, chemical fertilizers surely interrupt the soil microbes concerning the functional and structural variability of soil species, which causes the reduction of the nutrient content of cultivated soils.

3 A Consequence of Extreme Use of Pesticides on Soil

Pesticides are bioactive substances encompassing varied types of chemicals including insecticides, herbicides, fungicides, rodenticides, growth regulators of plant, nematicides, defoliants, and desiccant-agents to avoid the premature drop of fruits, fruit thinning agent, and also chemicals used postharvest to avoid crop-loss during transportation or storage. The majority of presently applied pesticides are artificial chemicals. These artificial pesticides can be classified by different considerations like target pest, active ingredients composition, soil persistency, entry-mode into target pest, method of preparation, and toxicity level of active molecule (Zacharia 2011). However, classification due to the chemical composition of the active ingredient provides a summary of the nature and behavior of particular pesticides. Rationally, a pesticide would not disturb the non-target organisms, would be biodegradable, cheap, besides having a low half-life. However, most pesticides have chronic and acute toxicity levels that considered them as biocides (Zacharia 2011). Several pesticides were identified as a highrisk to environmental habitats in the soil (Sattler et al. 2006). So, the influence of pesticides on essential non-targeted soil microorganisms and biodiversity is a major concern (Fig. 20.2).

When the importunity of pesticides in the soil is compared with plants or animals, it persists for extended times in soil (Edwards 1975). The half-life of pesticide measures the time that the applied pesticide degrades in soil or water (EPA 2012). A pesticide with a longer half-life is the more probable to leach in the soil. Organochlorines pesticides contain more than four chlorine atoms in each molecule make their break down procedure too slow and are considered to be the common persistent pesticides in the surroundings. For instance, among organochlorine pesticides like p,p-dichlorodiphenyltrichloroethane(DDT) and toxaphene considered as persistent pesticides also persist in the food chain systems and consequently increase the risk of human health and environments (EPA 2000). Due to the high dosage, the toxicity effect of pesticides may be considered very high. For instance, insecticides

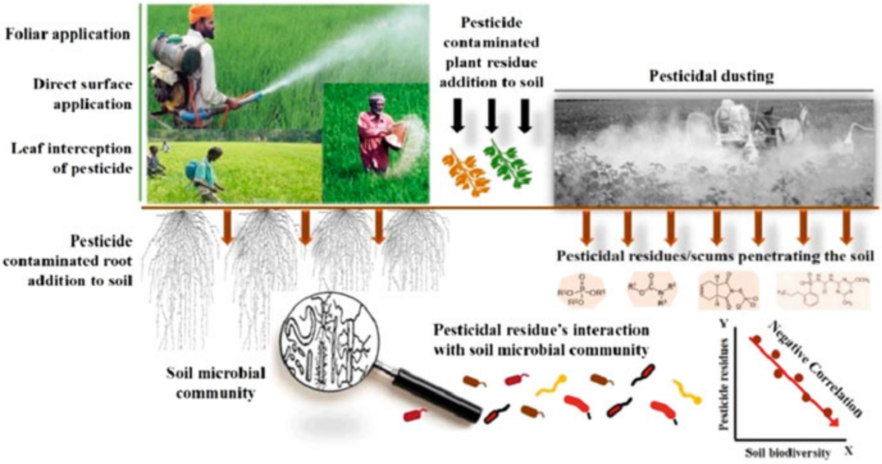


Fig. 20.2 A schematic illustration depicting the response and effects of pesticides on soil microbial communities and biodiversity. (Reproduced from Meena et al. 2020)

such as unden and lindene when used at 125 and 156 gha^{-1} concentration inhibited vegetable crop production and microbial activities (Glover-Amengor and Tetteh 2008). Likewise, nitrification has been inhibited at 20 kg ha^{-1}of hexazinone^{1}butat 5 and 10 kgha^{-1} concentration of this pesticide enhanced decay of cellulose as well as the rate of ammonification in soddy podzolic soil (Bliev et al. 1985). Additionally, when hexazinone used at the concentration of 5 kgha^{-1}it's residual in the soil reached to zero stage within 450 days but due to 10 kgha^{-1} application zero level residual detected after 750 days. Approximately, 2 million tons of pesticides are used yearly while by the year 2020, the world pesticide demand has been expected to rise to 3.5 million tons (Sharma et al. 2019). Therefore, it can be decided when pesticides are applied in high concentration, they influence soil microbial functions and population, soil parameters, nutrient balance, and availability in soil.

For the last 20 years, numerous research groups were aggressively involved in studying the variations in soil parameters and also shift in the micro-biome structure of cultivated soils because of continued pesticide application. The response of soil micro-biome to numerous types of synthetic pesticides varies and depends on different factors like the nature of pesticides, kinds of microorganisms within the soil, and soil properties. For instance, for the same concentration of pesticide, the number of resistant communities will increase and at the same time, the sizes of sensitive microorganisms decrease because of low competition (Chen et al. 2001). In other cases, a consistent decrease in microbial diversity was reported after the subsequent application of pesticides (Lupwayi et al. 2009). When soil becomes controlled by only a few functional microorganisms because of the influence of used chemical pesticide this may affect the overall and several biological activities of soil. For instance, some herbicide like butachlor temporarily varied the rhizospheric microbial population (Kalyanasundaram and Kavitha 2012). Therefore, it can be decided that the population size and structure of microorganisms in the soil can be determined by the number of applications, dosage, type of pesticide, soil quality (nutrient content and physical parameters), and type of microorganisms. However, it was confirmed all soil parameters (physical, chemicals, and biological) influenced via the application of any type of pesticide. Generally, agricultural industrialization needs agricultural management and a critical focus of agrochemical's impact on soil parameters. With the help of biotechnological tools, some countries like China proposed a strategy like zero growth of pesticide and chemical fertilizer use (Shuqin and Fang 2018). In this chapter within the following topics, the potential of biotechnology is addressed to minimize the impact of agrochemicals on soil qualities.

4 Biotechnological Approach to Resolve Agrochemicals' Effect on Soil Quality

4.1 Biofertilizers

Biofertilizer is a preparation of latent or live cells of efficient microbial strains like nitrogen-fixing, phosphate mobilizing, phosphate solubilizing, plant growth-promoting etc., which can be applied on soil, seed, or composting areas to increase the number of these microorganisms and speed up the different microbial process to improve the level of the accessibility of nutrients as a form that can be simply taken by the plant (NIIR BOARD 2004). Microorganisms used for biofertilizer contribute a very important role in refining soil fertility by solubilizing insoluble components and fixing atmospheric nitrogen. They also support root development via augmenting hormonal-synthesis routes and plant tolerance to abiotic and biotic stress. They are ecofriendly and cost-effective. Microorganisms like fungi, bacteria, and blue-green algae are used as biofertilizers. To increase their shelf-life, carrier materials like mulch and lignite powder are applied for packing. Therefore, biofertilizers have paramount significance in sustainable agriculture in reducing problems related to the use of synthetic fertilizers. Contrary to synthetic fertilizers, biofertilizers'usage in soil was proven to enhance several soil properties and functions. The use of biofertilizers for agriculture has a long history which passes to a different generation of farmers like a conventional approach. However, to get an appropriate result from biofertilizer we should apply biotechnological techniques like identification of appropriate microbial species, identification of genes from microbial strains, which are involved in converting the unavailable form of nutrients into available forms, and so on. There are different research works which confirmed the role of a biotechnological approach to increase biofertilizers application for sustainable agriculture.

4.1.1 Biotechnology and Biofertilizer

In addition to the phenotypic studies by physiological and biochemical characterization, the DNA based molecular characterization (one of the biotechnological tool) study provides more appropriate insight for the phylogenetic classification of organisms into numerous taxonomic levels. However, the traditional methods of classification (morphological and biochemical) grouped all isolates in a few categories like either Gram-negative or Gram-positive in the case of bacteria. The sequences of rRNA, especially 16S rRNA gene have much prominence with reference to the evolutionary studies of bacteria. In addition to having the extremely conserved region, 16S rRNA gene also includes a mutable part which is a useful tool to categorize different genera. The conserved part of this gene is important for designing the primers for PCR of several taxa at various levels of taxonomy starting from one strain to complete phyla (Vetrovsky and Baldrian 2013). From 16S rRNA gene-based molecular characterization study, *Pseudomonas* and *Acinetobacter* species were identified to dominate bacterial genera among the population of phosphate solubilizing bacteria in rhizospheric soil of different plants (Munir 2018). In that study, an up-scaling in root number was found upon inoculation with identified phosphate solubilizing bacteria in the existence as well as in the nonexistence of pesticides.

In another study, from the samples collected from root, leaves, and stems of 10 rice cultivars, 12 species were identified as plant growth-promoting bacteria (PGPB) using nif (nitrogen-fixing) gene-specific primer(Sang et al. 2014). These diazotrophic bacteria were characterized using molecular markers and effectively applied to stimulate plant development and inducing fungal resistance in several plants within a short period of time. In another study 9 g – positive bacterial strains were characterized by 16S rRNA gene sequencing and phylogenic analysis among legumes'rhizospheric soil led to the identification of plant growth-promoting bacteria (PGPR) (Hayat et al. 2013). In this study, the application of the identified strain RH-5 as biofertilizer was reported to upsurge crops grains and biomass (beans; 50%, wheat; 40–45%) above control. In similar research by 16S rRNA gene sequencing and phylogenic analysis zinc-tolerant bacteria with PGP(plant growth-promoting) properties; *Acinetobacterhaemolyticus, Enterobacter cloacae, Serratiamarcescens and Citrobacterfreundii* have been identified. When these isolates are applied as a biofertilizer showed Phosphorus (P)solubilization activity and phytoremediation for reclamation of soil at high $ZnSO_4$ concentrations (Neha et al. 2013). In another observation isolate CNE215 from nodules and CRE1 from roots identified by molecular diversity of polymerase chain reaction (PCR) amplified 16S rRNA evaluated under field conditions and showed up to 22.5% increasing in grain yield of chickpea when they applied as biofertilizer over uninoculated control (Sain et al. 2013).

Endophytic bacteria *Pseudomonas and Rahnella* isolates proposed as potential microbial biofertilizers for sustainable banana production via the application of DNA based molecular characterization (Ngamau et al. 2012). Using the aid of nucleic acid and in silico similarity search analysis, four endophytic bacteria isolates identified and showed remarkable phosphate solubilizing activity (Seung et al. 2013). These PGPB isolates demonstrated their positive effects on development and growth in rice crops. Hence, the potential of biotechnology has an unlimited impact on characterization, and identification, and improvement of phosphorous solubilizing microorganism (PSM) and nitrogen-fixing bacteria for efficient nutrient uptake as well as promoting plants growth when they applied as biofertilizer in cultivated soils by confirming sustainable agriculture within a short period and decrease of chemical fertilizers pollution on the soil.

4.1.2 Plant Molecular Breeding for Soil Quality Improvement

Quantitative traits are genetically influenced by many genes and each gene has a relatively small influence on the phenotype but is mainly controlled by environmental factors (Buckler et al. 2009). A chromosomal region related to a marker that affects a quantitative trait was defined as a quantitative trait locus (QTL) (Geldermann 1975). In the period 1980–1990 molecular maps were constructed for various plant species. The complication of molecular map construction has developed from the RFLP maps of the 1980s to PCR-based markers of the 1990s to more integrated maps, due to the result of using diverse types of molecular markers including genic markers, over the past decade. Linkage maps have been used in gene mapping for major genes and QTL. In addition to mapping, a marker-trait association for NUE through quantitative real-time polymerase chain reaction (RT-PCR) technology has been using (Sinha et al. 2018).

There is a crucial demand for the release of nutritious, high-yielding, resource use-efficient, and different stress-tolerant crop varieties, especially in cereal crops, which are the main source of human, to go with the incredible human population growth. The yields of cereal grain had seen notable progress in the green revolution. This enhancement in production was mainly realized by high-input-responsive varieties demanding more synthetic fertilizers and pesticides. This kind of approach adversely affected our sustainable agricultural system in the past three to four decades. So, now it is a time to find a way that yields can be improved with limited applied inputs, especially the inputs adversely affecting our sustainable agriculture. In experimental managed plots, Nitrogen (N) fertilizer recovery in a 1 year averaged 57% for wheat, 65% for maize, and 46% for rice (Xu et al. 2015). However, in farming practices did not reflect the efficiencies got in the experimental plot. For example, N retrieval effectiveness on average ranges from 30 to 55% under irrigated conditions and from 20 to 30% for farmer practice fields under rainfed environments (Liu et al. 2016).

Due to these limitations of the crop in the uptake of the given fertilizer inputs, breeding crop cultivars for the improvement of nutrient use efficiency (NuUE) becomes a prerequisite to minimize the quantity of nutrient application goes to ecosystems, reduce fertilizer application, decreasing input costs, and improving cereal yield with assuring sustainable agriculture via maintaining soil quality.

Therefore, the identification, characterization, and development of crop varieties with higher yield under low input conditions become a high breeding priority. The traits involved in nutrient absorption, transport, and mobilization identified with the recent plant molecular breeding tools like molecular linkage genetic maps, quantitative trait locus (QTL) mapping technologies, and high-throughput single nucleotide polymorphism (SNP) markers for its easiness and high precision (Feng et al. 2018). In addition now to advance plant breeding for NuUE, researchers have been accessing massive online organized plant breeding and genomic resources including quality genome sequences (Du et al. 2017) thick SNP maps (Ebana et al. 2012) free databases of genomic information, and wide germplasm collections (Agrama et al. 2010). Several research works have been done with the help of plant molecular breeding tools via the use of these available resources for the improvement of NPK nutrients use efficiency. Currently in order to exploit the NuUE QTLs *Pup1* is the best model for exploiting the NuUE QTLs, which is evaluated in several genetic backgrounds under field environments (Vinod and Heuer 2012).

4.1.3 Nitrogen Use Efficiency Improvement

As Nitrogen is the first an essential element for many aspects to improve plant productivity higher amounts of nitrogen fertilizer has been using continuously, which led to decreased nitrogen use efficiency (NUE) and increased environmental pollution (Bouwman et al. 2002). A high rate of NH_3 losses up to 56% of the applied N by volatilization was reported under several agroecosystems (Zaman et al. 2013). Leaching of NO_3 by rain or irrigation water and its change into nonaccessible form by microorganisms is another factor to reduce its uptake by crop plants. Nitrous oxide (N_2O) greenhouse gas formed due to extreme use of nitrogen fertilizer depletes the ozone layer (Malyan et al. 2016). (Fig. 20.3) outlines the cycle of nitrogen in the environment, that is, how N enters to soil and losses into the environment.

Exploring the available varieties by integrating classical plant breeding techniques with advanced biotechnological tools to increase NUE of a plant is a valuable method. Several research efforts have been done to detect best N use-efficient genotypes and superior grain yield (GY) parameters (Singh et al. 2015). In rice crop (Obara et al. 2004), QTL regions linked with panicle weight and panicle number on chromosome number 2 that includes a regulator gene (*GS1*) for glutamine synthetase activity have been identified. Via applying this QTL rice plants have been selected for their best panicle number, great in tillering capability, and overall panicle weight in small N rates. Genomic regions, which are identified by different researchers as shown in Table 20.1, associated with the high product at low nitrogen amounts will be valuable in marker-assisted breeding for the release of improved NUE crop variety within a short period.

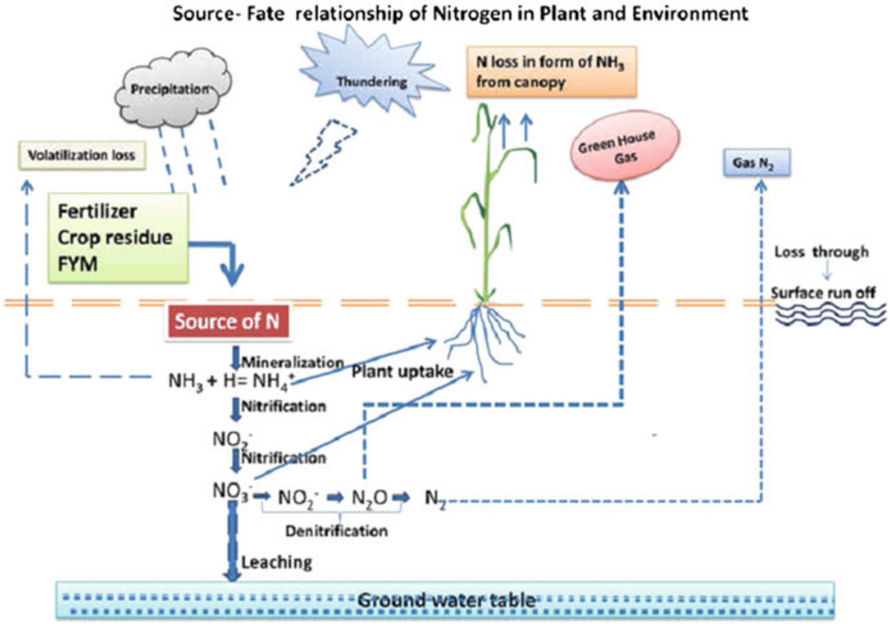


Fig. 20.3 The cycle of N in the environment. (Reproduced from Ranjan and Yadav, 2019, Copyright © Informa UK limited)

4.1.4 Phosphorus Use Efficiency Improvement

One-third of the world's cultivable lands have deficient of phosphorus (P) to fulfill the requisite for best plant development and growth (MacDonald et al. 2007). To overcome this P deficiency, artificial fertilizers have been applying in higher amounts that influence the soil parameters. So, identifying and developing for low phosphorus-tolerant (LPT) varieties are the main parameter for high phosphorus use efficiency improvement in crop production. Due to their grain yield, 6 LPT genotypes of rice were identified as IET5854, Rasi, IET14554, IET15328, IET17467, and PRH122 with the aid of molecular characterization (Krishnamurthy et al. 2010). From rice mapping population and several groups, two cultivars (DJ123 and Mudgo) were developed with careful selection procedures in the laboratory stage to minimize the requirement for extensive field evaluations. This used to detect best traits contributed intolerance of low P (Yugandhar et al. 2017; Mahender et al. 2017; Chithrameenal et al. 2017).Via mutation induction, one of the molecular breeding approaches, for the development of resistance variety for both abiotic and biotic stress conditions (Panigrahy et al. 2014) by using ethane methanesulfonate (EMS) 4 LPT varieties were identified. Various QTLs and genes controlling agronomic traits associated to LPT had been identified and categorized by researchers as shown in Table 20.1. For instance, QTLs for P insufficiency tolerance have been identified from the rice population by crossing P-efficient Kasalath (*indica* landrace) and

Table 20.1 QTLs identified to control nitrogen and phosphorus use-efficiency

Sl. No	Traits	Population	Cross	References
1	Plant height	DHs	IR64/Azucena	Fang and Wu (2001)
2	Grain number, low tiller number, and strong culm	*Ipa*	OsSPL14	Jiao et al. (2010)
3	N uptake (NUP), grain yield, biomass yield, N use efficiency (NUE)	CSSLs	9311/Nipponbare	Zhou et al. (2017)
4	Twelve physiological and agronomic traits	RILs	IR64/Azucena	Nguyen et al. (2016)
5	Glutamine synthetase, panicle number per plant, panicle weight	NILs	Koshihikari/ Kasalath	Obara et al. (2001)
6	Root dry weight, shoot dry weight, biomass	RILs	Zhenshan97/ Minghui 63	Tong et al. (2006)
7	Grain protein content	RIL	Morex × Barke	Pasam et al. (2012), Han et al. (2016)
8	Total grain number, total leaf nitrogen, total shoot nitrogen, nitrogen uptake, specific leaf nitrogen	RILs	IR69093-4-3-2/ IR72	Laza et al. (2006)
9	Yield	RIL	Morex × Barke	Han et al. (2016)
10	Relative root dry weight, spikelet number per panicle, spikelet fertility, 1000-grain weight	ILs	Shuhui 527 86 × Minghui	Cho et al. (2007)
11	Grain protein content		Lewis × Karl	Mickelson et al. (2003)
12	Total plant nitrogen, nitrogen-use efficiency	DHs	IR64/Azucena	Senthilvel et al. (2008)
	Phosphorus			
13	Grain yield per plant, biomass, harvest index, etc.	RILs	*IR64/INRC10192*	Srividya et al. (2010)
14	Grain yield per plant, grain number per panicle	RILs	*Zhenshan 97/HR5*	Tong et al. (2011)
15	Nitrogen deficiency tolerance and nitrogen-use efficiency	RILs	*Zhenshan 97* and *Minghui 63*	Wei et al. (2012)
16	Total plant nitrogen, nitrogen dry matter production efficiency, nitrogen grain production efficiency, total grain number	RIL	*Dasanbyeo/ TR22183*	Piao et al. (2009)

P-inefficient Nipponbare (*japonica* cultivar) (Wissuwa et al. 1998). The *Pup1* gene, important for phosphorus uptake, was also identified and characterized by DNA based molecular techniques (Chin et al. 2011).

4.1.5 Potassium (K) Use Efficiency Improvement

The accessibility of potassium inside the soil plays a major role in crop grain quality and yield (Wang and Wu 2015). Thus, the characterization of potassium use efficiency (KUE) in a crop is vital and requests to develop genotypes for low K conditions. Numerous research works were conducted via integrating conventional and plant molecular breeding for the characterization of KUE in crops. In double haploid (DH) population containing 123 lines taken fromAzucena (*japonica* variety) andIR64 (*indica* variety) of rice at a hydroponic investigation (Wu et al. 1998) found three QTLs related with shoot and root dry weight in limited K conditions. These QTLs are used to improving K uptake and K use efficiency in the plant. Numerous QTLs controlling agronomic traits related KUE had been identified and characterized by different researchers.

Hence, improvement of NuUE (nutrient use efficiency) is a crucial component for agronomic and environmental perspective current trends in plant molecular breeding as one of the biotechnological tools able to accelerate the realizing of the polygenic character of complex traits. Therefore, integrating advanced plan molecular breeding with the conventional breeding to increase the efficient use of balanced PNK nutrient elements via maintaining soil fertility as well as the soil quality is one of the critical approaches in sustainable agriculture.

4.2 Omics Approach for Eco-Friendly Agriculture

Since various crop traits are quantitative (controlled by multiple genes interacting with smaller effect) the QTL mapping progressed for identification of many genes to assist conventional breeding via marker-assisted selection and breeding by design (Peleman and Van der Voort 2003) have shown good outcomes. However, QTL-analysis methods have limitations of accuracy to handle the plant's inherent complexities such as genotype-environment interactions, polygenic control, and epistasis. So, supporting QTL-analysis with the omics approach is critical for the enhancement of plant genetic analysis and efficient breeding to full fill the world food demand without environmental effluence. There are different omics technologies to realize for advanced crop improvement in the genomic era such as genomics, transcriptomics, proteomics, and metabolomics.

4.2.1 Genomics

Genomics nowarose to the sequencing and mapping of genome with the detail annotation information found in the genetic sequence. It may be categorized into structural and functional genomics for efficient functional analysis. Structural genomics applicable for initial phase of genome analysis that tells the wholegenome sequence of each organism and the functional genomics make thedetections ofgenes' functions (Leister 2005). Proteomics,Transcriptomics and metabolomics are the main tools of genomics. All these considered as post-genomic technologies and used to characterizing sets of molecules' data generated by genomes. Since genomics includes everything starting from gene sequencing to gene function annotation all other omics under the umbrella of genomics. Due tocomplex nature of omics data the analysis is relay on information technology aidvia bioinformatics tools.

Genomics played great role for the enhancement of crop production at it is intricate in sequencing of different crop species via advanced sequence technology. Since genomics referred the studying of genetic information and geneorganization in the genome it applied to identify the biological function of genes via collecting as well as analyzing genomic data. Among other organisms the genomes of plants'are described very wellby their gene content, genome size, level of polyploidy and repetivesequences (Campos-de Quiroz 2002).

When genome sequence data is accessible among genomic techniques genome annotation used to find the potential putative genes and allocating functions for them. Commonly this is performed by in silico, with the support of computer based programs either in ab initio (based on gene signal like start and stop codon) orhomology based approach.So, this helps classifying genes based on their allocatedfunction and use them for crop enhancement in both biotic and a biotic stress environment. Among the abiotic stress conditions plant NuU Eimprovement is one of the main targets, which is benefited from genomic technology. For this achievement in plant several genomes were sequenced with high quality starting from rice the smallest genome (389 Mb) (IRGSP 2005) till wheat the largest genome size (14,500 Mb) (Guan et al. 2019) and available for public use.

4.2.2 Transcriptomics

Transcriptomics is the investigation of gene expression within a particular environment through a varied range of cellular responses. The identification and characterization of putative genes involved in any agronomic important trait instead of the genome can be investigated via mRNAs analysis. It detects the expression range of specific RNAs among thousands of RNAs and produces a snapshot picture of genes translated within a given event by the available several technology such as serial analysis of gene expression (SAGE) (Velculescu et al. 1995) RNAseqand DNA microarray (Schena et al. 1995). Detail study of the molecular basis of Nutrient use efficiency (NuUE) would allow us to offer important evidence for crop production improvement by biotechnological approaches. The current advances in transcriptomics techniques shift our method of putative genes identification, which contributes a vital role in the NuU Efrom marker-based into direct gene identification by their expression level (Liu et al. 2016; Rose et al. 2017; Zhou et al. 2017). Recently 688 now NuUE-responsive genes for 12 nutrient type's identified and available at NuUE web resource are shown in Fig. 20.4 (Kumar et al. 2018).

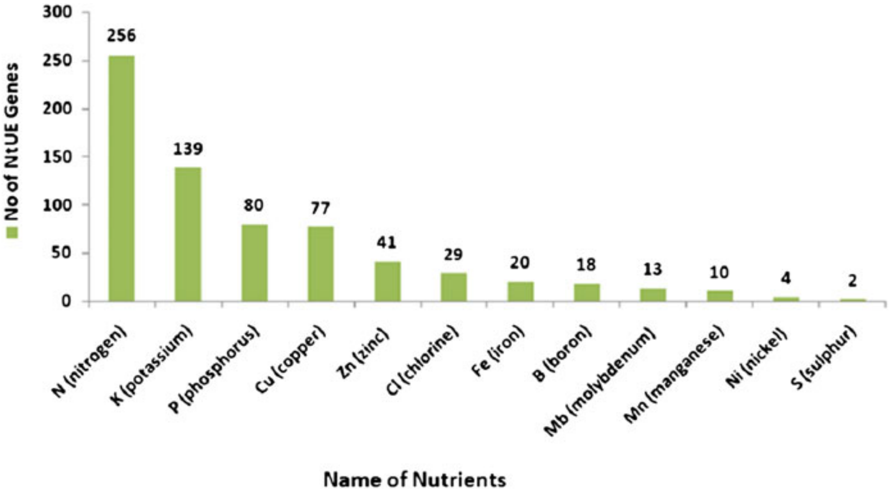


Fig. 20.4 Distribution of macronutrient- and micronutrient-responsive genes corresponding to their nutrient type in the nutrient use efficiency (NtUE) web resource. (Copyright: © 2018 Kumar et al. Reproduced under the terms of the Creative Commons Attribution License)

Generally, the plant omics approach is used to realize the molecular and genetic base of all biological practices in plants that are important for both abiotic and biotic conditions including plant nutrient deficiency environment. This allows effective manipulation of plants in the release of new improved cultivars with better quality in a short period (Vassilev et al. 2005). Genomics also allows investigating several genes within the same time (Struik et al. 2007) and minimizes the gap among genotype and phenotype (Campos-de Quiroz 2002). For designed breeding approach omics particularly transcriptomics played a great role never before in order to select the best genotypes from the breeding population. With genomic approaches, marker-assisted selection slowly changed into genomics-assisted breeding (Varshney et al. 2005). Genomics study has also effectively identified numerous metabolic pathways as well as provided genes or direct markers for agronomically important traits. These identified genes beside marker application are used as bases for genetic engineering. The ability to understand genomics is useable for the developments of new plant investigative tools, which minimize chemicals application in disease control as well as soil fertility improvement.

4.3 Plant Genetic Manipulation for Reduction of Agrochemical Usage

4.3.1 Transgenic Crop for Herbicide and Insecticide Minimization

Transgenic crop or genetically modified crop (GMC) has been widely used since it first commercialized in the mid-1990s at several countries for four major crops such as cotton, canola, soybean, and maize. Since the main traits of commercialized transgenic crops are genetically modified for insect resistance (GM IR) tolerance to insecticides and Genetically modified for herbicide resistance (GM HR) tolerant to herbicides. GM crop technology played a notable positive impact on the environment so far via minimizing pesticides active ingredients applied as shown in Table 20.2. Worldwide, in 2014 GM technology has contributed to an important role to minimize the negative impact related with pesticides (herbicide & insecticide) in the GM devoted areas by reducing 581.4 million kg of active ingredient compared with areas planted with conventional crop (Brookes and Barfoot 2016). In this study when the environmental impact related to these pesticides on a common transgenic plan, as measured by the Environmental Impact Quotient (EIQ) indicator, improved by 18.5%.

The environmental improvements from the GM IR traits have commonly gained from reduced use of insecticides. However, the gains of environmental improvements from GM HT traits came from the combination of changes in the profile of herbicides used, which favor more environmentally products and via facilitated changes in farming systems. The obtainability of a minimum cost, glyphosate herbicide (broad-spectrum), and change of farming system from traditional to reduced/no-till (RT/NT) cultivation systems in GM crop growing countries have decreased the amount of greenhouse gas (GHG) releases from tractor fuel. Tillage minimization due to glyphosate resistance crops (GRCs) in addition to the reduction

Table 20.2 GM herbicide-tolerant crops: Summary of active ingredient usage and associated EIQ changes 1996–2015 source. (Reproduced with permission from Brookes and Barfoot 2016, Copyright © 2016 Brookes and Barfoot. Published with license by Taylor & Francis)

Country	Change in active ingredient use (million Kg)	% Change in the amount of active ingredient used	%Change in EIQindicator
GM HT canola			
US	−2.9	−33.9	−47.0
Canada	−18.3	−18.6	−31.5
Australia	−0.5	−2.8	−2.3
Aggregate impact: All countries	−21.7	−17.2	−29.3
GM HT sugar beet			
USand Canada	+2.0	+32.5	+0.1

Note: Negative sign = reduction inusage or EIQ improvement. Positive sing = increase usage or worse EIQ value of (GHG) emissions from tractor it has two positive environmental impacts. First, intensive tillage for weed management is the most perpetual environmental damage via assisting loss of topsoil due to water and wind, which is challenging to recover within centuries. This can be remediated byRT/NT farming practice. Second, carbon and moisture loss from soil can also be triggered by tillage. Thus, tillage reduction is certified by glyphosate resistance crops has had important positive environmental impacts via decreasing soil losses and enhancing carbon and soil moisture-holding. Tillage reduction in glyphosate resistance crops from 1996 to 2012 resulted in retaining 204 billion t of CO_2 being released into the atmosphere (Barfoot and Brookes 2014).

4.3.2 RNA Interference (RNAi) Based Technology for Reduction of Insecticide Application

RNA interference as one of a biological practice inhibits gene regulation via sRNA (small RNA) molecules at the level of transcriptional/ post-transcriptional, either by chromatin modifications inhibition or by the reduction of lifespan or translation possible of the marked mRNA. The initiator of gene silencing is double-stranded RNA (dsRNA) produced from a foreign source like transgene and virus or an endogenous genomic source. The process RNAi based gene silencing is used in agriculture to control plant diseases, which need intensive pesticide in conventional plant protection. To control plant damage by diseases RNAi as gene silencer applied by host-induced gene silencing (HIGS) or via artificially synthesized dsRNA/ NonTransformativeRNAi. The fast advancement of RNAi based technology for disease control makes it easily available in the commercial market. For this achievement, several research works have been done as one of the biotechnological tools via clearly verifying the mechanism of RNAi based technology starting from laboratory to field test conditions.

Simple RNA interference pathways, which are common in several eukaryotic cells depend on a diverse number of sRNAs to regulate mRNA degradation/ translation within a sequence-specific order (Castel and Martienssen 2013; Borges and Martienssen 2015). First, sRNAs are produced from internally synthesized or externally supplied dsRNAs, which are set 20–30 nucleotide (nt) long via ribonuclease III enzymes named DICER in several organisms or DICER-like (DCL) in plants and fungi. Second, the sRNA added into particular members of a protein family called AGO (ARGONAUTE) within the cytoplasm. Third, parallel with the untwisting of the RNA duplex and passenger RNAstrand release, RNA-induced silencing complex (RISC) assembled by the involvement of additional proteins. Forth, in the cytoplasm, the reserved guide RNA strand indicates RISC complex into a targeted complementary RNA. Due to the biological situation, target detection goes to triggering DNA methylation to TGS (transcriptional gene silencing) or RNA destabilization and/or translational inhibition to PTGS (post-transcriptional gene silencing). RNAi pathways and components were studied critically in Drosophila melanogaster model insect and RNA interference pathway genes were also identified for all insects within highly variable environmental RNAi (Cooper et al. 2019).

Insect Control Through HIGS (Host-Induced Gene Silencing)

Different transgenic crops express dsRNAs with homology to genes encoding pathogenicity of, viruses, fungi, bacteria oomycetes, insects nematodes, and (Cai et al. 2018; Rosa et al. 2018). The double-stranded RNAs are synthesized via the host plant and finally perform in the target organism as a host-induced gene silencing (HIGS; Nowara et al. 2010) (Fig. 20.5). Efforts have been done to clarify how HIGS is triggered in insects by transgene regulation in the plant. Findings reveal that HIGS in insects is triggered more successfully via unprocessed and long dsRNA than by plant-processed sRNA (Zhang et al. 2015).

Among the successful HIGS in the year 2016 transgenic targeting western corn rootworm (WCR) has been accepted by the CFIA (Canadian Food Inspection Agency). For this product, SmartStax PRO was given as a commercial name and approved in 2017 via the EPA (US Environmental Protection Agency). The Resistancesystem in this product is mediated by a dsRNAdesigned for specific of

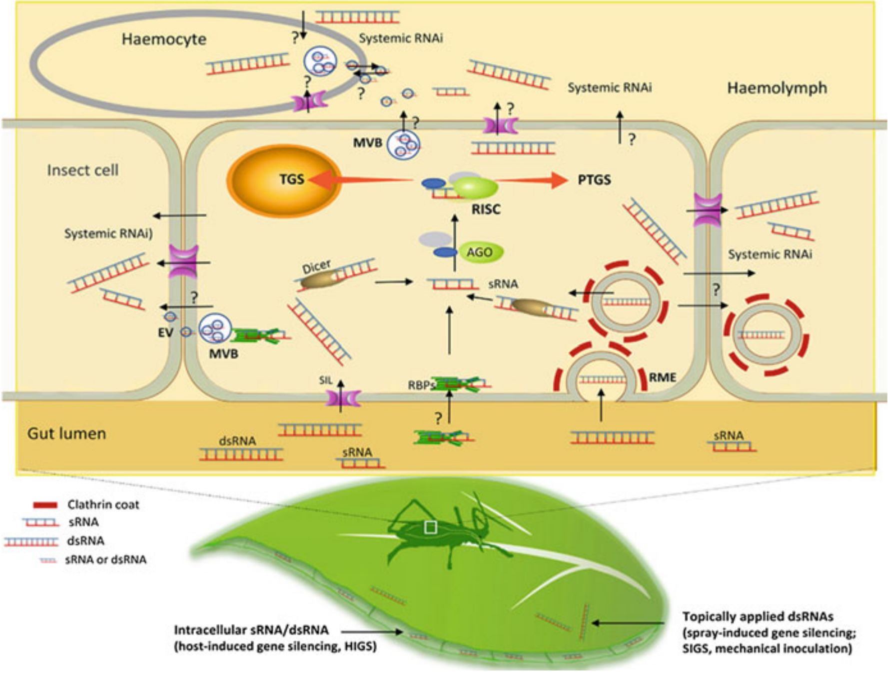


Fig. 20.5 Schematic representation of RNAi-mediated plant protection from insect predation. (Reproduced from Liu et al. 2019, Copyright ©Elsevier, under the provisions of Creative Commons CC-BY license)

targets SUCROSE-NON-FERMENTING7 gene of WCR (DvSnf7) with the arrangement of two insecticidal proteins encoded via the CRYSTAL (Cry) genes Cry34Ab1 and Cry3Bb1, which were taken from (BT) *Bacillus thuringiensis* (Head et al. 2017). Snf7 protein used in the ESCRT (Endosomal Sorting Complex Required for Transport) complex that plays a vital role within the cell as a housekeeping gene via regulating the transport, internalization, lysosomal degradation, and sorting of transmembrane proteins.

Diseases and pests worldwide 20–40%estimated case of crop yield worldwide, among these insects causing a major percentage of this loss through plant disease transmission of plant and direct damage (Douglas 2018). For the last 50 years, control of crop disease has relied severely on the application of broad-spectrum chemicals. However, misuse of these chemicals has directed to the resistance of more than 500 insect species as well as caused environmental pollution (De Andrade and Hunter 2016). RNAi-based approaches could offer an environmentally friendlier technique to control disease-causing pests and reduce the use of synthetic pesticides. Furthermore, since RNA interference can be extremely specific (Cooper et al. 2019), farmers can able to control invasions of crop pests without influencing useful insects and other important soil microflora. In addition, relevant use of dsRNA may not cause an environmental hazard asdsRNAs are rapidly degraded in water and soil with a half-life under 72 h or 30 h, respectively (Fischer et al. 2017).

Insect Control by NonTransformativeRNAi

Since the real application of HIGS strategies has some questions due to its GMO-nature in various countries, high cost, and a long time to develop. For example, the commercial accessibility cultivar “HoneySweet,” which was released to *Plum pox virus* (PPV) resistant took 20 years (Scorza et al. 2013). There is also a shortage of transformation protocols for major cultivated plants that causes a delay in the development of RNAiGM plants (Mitter et al. 2017). So, to overcome this problem another strategy via the use of in vitro produced sRNAs to trigger RNA interference in plant pests was developed (Gu and Knipple 2013). RNA sprays that targeting gene silence without genetic transformation called SIGS (spray-induced gene silencing) or nontransformaticRNAi showed good results on fungi (McLoughlin et al. 2018) viruses (Niehl et al. 2018). Since nontransformaticRNAi-based technology silence genes without the insertion of a foreign gene in the plant genome will not remain considered as GM products (Fig. 20.6). So, beyond to time-saving, it increased public receipt of RNAi based tech for disease protection Table 20.2 (Cagliari et al. 2018). NontransformaticRNAi-based technology silence genes also easy and cost effect (Table 20.3).

The research conducted in the previous decades concerning the practice of nontransformaticRNA interference in plant protection; it is supposed that this technique will rapidly spread to growers as dsRNA based products (Cagliari et al. 2018; Mat Jalaluddin et al. 2018). WhileRNAi is not presently practical in every delivery method (San Miguel and Scott 2016) this technology has a great possibility,

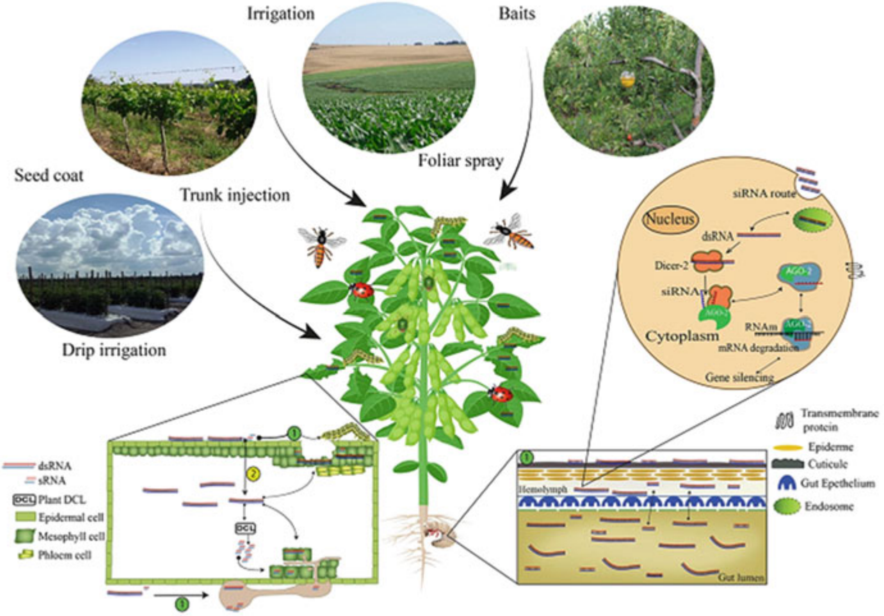


Fig. 20.6 Non-transformative delivery strategy routes for RNAi-based gene silencing induction. The first step to achieving successful RNAi-based gene silencing results via non-transformative approaches is the selection of the RNAs (dsRNA or siRNA) delivery strategy: Foliar spray, trunk injection, irrigation, drip irrigation, seed coat, baits, and powder or granules for soil applications. Once the RNAs are delivered, the insects and pathogens need to internalize the RNAs molecules, and this process can occur (1) directly or (2) indirectly. Direct uptake occurs when the organisms get in contact with the RNAs molecules during application or feed on tissues containing the RNA molecules on the surface. However, when the RNA molecules are absorbed, translocated in the plant vascular system, and taken up by the organism, the process is classified as indirect uptake. Inside the organism system, the cell uptake of dsRNA can be mediated by transmembrane channel proteins such as sid- or endocytosis. The RNAi-based gene silencing depends on the release at cellular levels of dsRNA or siRNA molecules. When dsRNAs are unloaded in the cytoplasm, these molecules are processed into siRNA fragments by an enzyme called Dicer 2 (DCR-2). The siRNA fragments are then incorporated into the RISC complex (RNA-induced Silencing Complex), which contains the Argonaute 2 (AGO-2) protein, and, in a sequence-specific manner, bind to a complementary messenger RNA (mRNA), cleave it, prevent protein formation, and thus affect target organism survival. (Reproduced from Cagliari et al. 2019, Copyright © 2019 Cagliari, Dias, Galdeano, dos Santos, Smagghe and Zotti, under the provisions of the Creative Commons Attribution License (CC BY))

particularly for diseases and insect control via avoiding pesticide resistance as well as environmental pollution problems.

Table 20.3 Different features affecting the development of RNAi-based products: Transformative vs. Non-transformative methods. (Reproduced from Cagliari et al. 2019, Copyright © 2019 Cagliari, Dias, Galdeano, dos Santos, Smagghe and Zotti, under the provisions of the Creative Commons Attribution License (CC BY))

	Strategy	
Future	Transformative	Non-transformative
Development time	High	Low
Development costs	High	Low
Feasibility according to culture	Unviable for some plant species	Viable for all
Delivery of sRNA	Continuous	Transient
Feasibility according to the pest	Most pest can be targeted due to the continuous dsRNA supply feature	Not all pest can be targeted due to recalcitrant features
Development of resistance	High	Low
Regulator process	Extensive	Simple
Acceptance by consumer	Low	High

5 Conclusion and Future Perspectives

Industrialized agriculture has increased the application of agrochemicals in an unprecedented manner. The augmented use of these synthetic chemicals has influenced the soil parameters as far as the major soil species, nutrient content, functions of enzymes within the soil, functional and structural variability of micro-organism populations are concerned. These observations project on agricultural management and the strategy of a critical emphasis on the influence of industrialized agriculture on soil quality as indispensable needs of the hour. The time-consuming practice of the conventional methods and the negative implications related to high quantity use of synthetic agrochemicals are serious concerns. In another way, the rapid development of biotechnological tools like genotyping, genetic engineering, and genomics offers considerable advantages in opposite to classical approaches to reduce the agrochemical impact on soil quality, ultimately to ensure sustainable agriculture. Among one of the biotechnological tools, genotyping microbial strains can improve phosphorous solubilizing microorganism (PSM) and nitrogen-fixing bacteria for efficient nutrient uptake as well as promoting plant growth. Plant molecular breeding also improves NuUE, which is a crucial component for agronomic and environmental perspective, interlaced with an accelerated polygenic nature understanding of complex traits. Genomics studies can identify various metabolic pathways and genes or direct markers for agronomically important traits. These identified genes may also remain used for genetic engineering of plant species. As part of biotechnological tools, RNAi based technology could be applied for the protection of different diseases as an eco-friendly approach. Beyond the experimental level, the real application of biotechnological techniques as the alternative approach of agrochemicals must be the future focus of biotechnology to ensure sustainable agriculture.

References

Agrama HA, Yan W, Jia M, Fjellstrom R, McClung AM (2010) Genetic structure associated with diversity and geographic distribution in the USDA rice world collection. Nat Sci 2:247–291

Avidano L, Gamalero E, Cossa GP, Carraro E (2005) Characterization of soil health in an Italian polluted site by using microorganisms as bioindicators. Appl Soil Ecol 30:21–33

Balezentiene L, Klimas E (2009) Effect of organic and mineral fertilizers and land management on soil enzyme activities. Agron Res 7(Special issue I):191–197

Barfoot P, Brookes G (2014) Key global environmental impacts of genetically modified (GM) crop use 1996–2012. GM Crops 5:149–160

Bliev UK, Martynov AN, Zarkov AV, Maximova LI (1985) Effect of verpa preparation on the fertility of soddypodzolic soil. Agrochimica 2:97–100

Borges F, Martienssen RA (2015) The expanding world of small RNAs in plants. Nat Rev Mol Cell Biol 16:727–741

Bouwman AF, Boumans LJM, Batjes NH (2002) Emissions of N2O and NO from fertilised fields: summary of available measurement data. Glob Biogeochem Cycles 16:6–13

Brookes G, Barfoot P (2016) Environmental impacts of genetically modified (GM) crop use 1996–2014: impacts on pesticide use and carbon emissions. GM Crops Food 7(2):84–116. https://doi.org/10.1080/21645698.2016.1192754

Buckler ES et al (2009) The genetic architecture of maize flowering time. Science 325:714–718

Cagliari D, Santos EAD, Dias N, Smagghe G, Zotti M (2018) Modulating gene expression – abridging the RNAi and CRISPR-Cas9 technologies. In: Singh A, Khan MW (eds) Nontransformative strategies for RNAi in crop protection. IntechOpen, London, pp 1–18. https://doi.org/10.5772/32009. Cappelle K, De Oliveira CFR, Van Eynde B, Christiaens O, Smagghe

Cai Q, He B, Kogel KH, Jin H (2018) Cross-kingdom RNA trafficking and environmental RNAi-nature's blueprint for modern crop protection strategies. Curr Opin Microbiol 46:58–64

Cagliari D, Dias NP, Galdeano DM, dos Santos EÁ, Smagghe G and Zotti MJ (2019) Management of Pest Insects and Plant Diseases by NonTransformative RNAi. Front Plant Sci 10:1319. https://doi.org/10.3389/fpls.2019.01319

Campos-de Quiroz H (2002) Plant genomics: an overview. Biol Res 35:385–399

Castel SE, Martienssen RA (2013) RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. Nat Rev Genet 14(2):100–112

Chen SK, Edwards CA, Subler S (2001) Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. Soil Biol Biochem 33:1971–1980

Chin JH, Gamuyao R, Dalid C, Bustamam M, Prasetiyono J, Moeljopawiro S, Wissuwa M, Heuer S (2011) Developing rice with high yield under phosphorus deficiency: *Pup1* sequence to application. Plant Physiol 156:1202–1216

Chithrameenal K, Vellaikumar S, Ramalingam J (2017) Identification of rice (*Oryza sativa* L.) genotypes with high phosphorus use efficiency (PUE) under field and hydroponic conditions. Indian Res J Genet Biotechnol 9:23–37

Cho YI, Jiang WZ, Chin JH, Piao Z, Cho YG, McCouch S, Koh HJ (2007) Identification of QTLs associated with physiological nitrogen use efficiency in rice. Mol Cell 23:72–79

Cooper AM, Silver K, Zhang J, Park Y, Zhu KY (2019) Molecular mechanisms influencing efficiency of RNA interference in insects. Pest Manag Sci 75:18–28

De Andrade EC, Hunter WB (2016) RNA interference – natural gene-based technology for highly specific pest control (HiSPeC). RNA Interference. https://doi.org/10.5772/61612

Doran JW, Safley M (1997) Defining and assessing soil health and sustainable productivity. In: Pankhurst C, Doube BM, Gupta VVSR (eds) Biological indicators of soil health. CAB International, Wallingford, pp 1–28

Douglas AE (2018) Strategies for enhanced crop resistance to insect pests. Annu Rev Plant Biol 69:637–660

Du H, Yu Y, Ma Y, Gao Q, Cao Y, Chen Z, Ma B, Qi M, Li Y, Zhao X et al (2017) Sequencing and denovo assembly of a near complete *indica*rice genome. Nat Commun 8:15324

Ebana K, Yonemaru J, Fukuoka S, Iwata H, Kanamori K, Namiki N, Nagasaki H, Yano M (2012) Genetic structure revealed by a whole-genome single nucleotide polymorphism survey of 5 diverse accessions of cultivated Asian rice (*Oryza sativa* L.). Breed Sci 60:390–397

Edwards CA (1975) Factors that affect the persistence of pesticides in plants and soils. Pure Appl Chem 42:39–56

EPA U.S. (2000) Persistent bioaccumulative and toxic (PBT) chemical program. http://www.epa.gov/pbt/pubs/pestaction.htm#Introduction

EPA U.S. (2012) Types of pesticides. http://www.epa.gov/pesticides/about/types.htm

Fang P, Wu P (2001) QTL x N-level interaction for plant height in rice (Oriza sativa L.). Plant Soil 236:237–242

FAO (2017) World fertilize trends and outlook to 2020

Feng B, Chen K, Cui Y, Wu Z, Zheng T, Zhu Y, Ali J, Wang B, Xu J, Zhang W et al (2018) Genetic dissection and simultaneous improvement of drought and low nitrogen tolerances by designed QTL pyramiding in rice. Front Plant Sci 9:306

Fischer JR, Zapata F, Dubelman S, Mueller GM, Uffman JP, Jiang C, Jensen PD, Levine SL (2017) Aquatic fate of a double-stranded RNA in a sediment–water system following an over-water application. Environ Toxicol Chem 36(3):727–734

Geldermann H (1975) Investigations on inheritance of quantitative characters in animals by gene markers. I. Methods. Theor Appl Genet 46:319–330

Glover-Amengor M, Tetteh FM (2008) Effect of pesticide application rate on yield of vegetables and soil microbial communities. West Afr J App Ecol 12:1

Gu L, Knipple DC (2013) Recent advances in RNA interference research in insects: implications for future insect pest management strategies. Crop Prot 45:36–40. https://doi.org/10.1016/j.cropro.2012.10.004

Guan J, Garcia DY, Zhou et al (2019) The battle to sequence the bread wheat genome: a tale of the three kingdoms. Genomics Proteomics Bioinformatics. https://doi.org/10.1016/j.gpb.2019.09.005

Han M, Wong J, Su T, Beatty PH, Good AG (2016) Identification of nitrogen use efficiency genes in barley: searching for QTLs controlling complex physiological traits. Front Plant Sci 7:1–7

Harris RF, Bezdicek DF (1994) Descriptive aspects of soil quality health. In: Doran JW, Coleman DC, Bezdicek DF, Stewart BA (eds) Defining soil quality for a sustainable environment, SSSA special publication, vol 35. SSSA, Wisconsin, pp 23–36

Hayat R, Rabia K, Muhammad E, Iftikhar A, Akira Y, Safdar A (2013) Molecular characterization of soil bacteria for improving crop yield in Pakistan. Pak J Bot 45(3):1045–1055

Head GP, Carroll MW, Evans SP, Rule DM, Willse AR, Clark TL, Storer NP, Flannagan RD, Samuel LW, Meinke LJ (2017) Evaluation of SmartStax and SmartStaxPRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. Pest Manag Sci 73:1883–1899

IRGSP (International Rice Genome Sequencing Project) (2005) The map-based sequence of the rice genome. Nature 436:793–800

Jiao Y, Wang Y, Xue D, Wang J, Yan M, Liu G, Dong G, Zeng D, Lu Z, Zhu X, Qian Q, Li J (2010) Regulation of OsSPL14 by OsmiR156 defines ideal plant architecture in rice. Nat Genet 42:541–544

Kalyanasundaram D, Kavitha S (2012) Effect of butachlor on the microbial population of direct sown rice. World Acad Sci Eng Technol 69:853–855

Krishnamurthy P, Sreedevi B, Ram T, Padmavathi G, Kumar RM, Rao PR, Rani NS, Latha PC, Singh SP (2010) Evaluation of rice genotypes for phosphorus use efficiency under soil mineral stress conditions. Oryza 47:29–33

Kumar A, Pandeya A, Malik G et al (2018) A web resource for nutrient use efficiency-related genes, quantitative trait loci and microRNAs in important cereals and model plants. F1000Research 7:673. https://doi.org/10.12688/f1000research.14561.1

Larson WE, Pierce FJ (1994) The dynamics of soil quality as a measure of sustainable management. In: Defining soil quality for a sustainable environment, Special publication. Soil Science Society America, pp 37–51

Laza MR, Kondo M, Ideta O, Barleen E, Imbe T (2006) Identification of quantitative trait loci for d13C and productivity in irrigated lowland rice. Crop Sci 46:763–773

Leister D (2005) Plant functional genomics. Food Products Press, New York

Liu S, Jaouannet M, Dempsey DMA, Imani J, Coustau C, Kogel KH (2019) RNA-based technologies for insect control in plant production. Biotechnol Adv 39:107463

Liu Z, Zhu C, Jiang Y, Tian Y, Yu J, An H, Tang W, Sun J, Tang J, Chen G et al (2016) Association mapping and genetic dissection of nitrogen use efficiency-related traits in rice (*Oryza sativa* L.). Funct Integr Genomics 16:323–333

Lupwayi NZ, Harker KN, Dosdall LM, Turkington TK, Blackshaw RE, O'Donovan JT, Carcamo HA, Otani JK, Clayton GW (2009) Changes in functional structure of soil bacterial communities due to fungicide and insecticide applications in canola. Agric Ecosyst Environ 130:109–114

MacDonald GK, Bennett EM, Potter PA, Ramankutty N (2007) Agronomic phosphorus imbalances across the world's croplands. Proc Natl Acad Sci USA 108:3086–3091

Mahender A, Anandan A, Pradhan SK, Singh ON (2017) Traits-related QTLs and genes and their potential applications in rice improvement under low phosphorus condition. Arch Agron Soil Sci 64:449–464

Malyan SKA, Bhatia A, Kumar DK, Gupta R, Singh SS, Kumar R, Tomer O, Kumar, Jain N (2016) Methane production, oxidation and mitigation: a mechanistic understanding and comprehensive evaluation of influencing factors. Sci Total Environ 572:874–896. https://doi.org/10.1016/j.scitotenv.2016.07.182

Mat Jalaluddin NS, Othman RY, Harikrishna JA (2018) Global trends in research and commercialization of exogenous and endogenous RNAi technologies for crops. Crit Rev Biotechnol 39:67–78. https://doi.org/10.1080/07388551.2018.1496064

McLoughlin AG, Wytinck N, Walker PL, Girard IJ, Rashid KY, De Kievit T et al (2018) Identification and application of exogenous dsRNA confers plant protection against Sclerotiniasclerotiorum and Botrytis cinerea. Sci Rep 8:1–14. https://doi.org/10.1038/s41598-018-25434-4

Meena RS, Kumar S et al (2020) Impact of agrochemicals on soil microbiota and management: a review. Land 9:34. https://doi.org/10.3390/land9020034

Mickelson S, See D, Meyer FD, Garner JP, Foster CR, Blake TK, Fischer AM (2003) Mapping of QTL associated with nitrogen storage and remobilization in barley (Hordeumvulgare L.) leaves. J Exp Bot 54:801–812

Mitter N, Worrall EA, Robinson KE, Xu ZP, Carroll BJ (2017) Induction of virus resistance by exogenous application of double-stranded RNA. Curr OpinVirol 26:49–55. https://doi.org/10.1016/j.coviro.2017.07.009

Munir I (2018) Phosphate solubilizingbacteria: their isolation, characterization and impacton plant growth. Dissertation. University of the Punjab

Neha M, Garima G, Prabhat NJ (2013) Assessment of mineral phosphate-solubilizing propertiesand molecular characterization of zinc-tolerant bacteria. J Basic Microbiol 52:549–558

Ngamau C, Nyambura N, Viviene NM, Akio T, Catherine WM (2012) Solation and identification of endophytic bacteria ofbananas (*Musa* spp.) in Kenya and their potential asbiofertilizers for sustainable banana production. Afr J Microbiol Res 6(34):6414–6422

Nguyen HTT, Dang DT, Pham CV, Bertin P (2016) QTL mapping for nitrogen use efficiency and related physiological and agronomical traits during the vegetative phase in rice under hydroponics. Euphytica 212:473–500

Niehl A, Soininen M, Poranen MM, Heinlein M (2018) Synthetic biology approach for plant protection using dsRNA. Plant Biotechnol J 16:1679–1687. https://doi.org/10.1111/pbi.12904

NIIR BOARD (2004) Bacterial biofertilizers for sustainable crop production. www.arpnjournals.com/jabs/research_papers/rp.../jabs_0512_396

Nowara D, Gay A, Lacomme C, Shaw J, Ridout C, Douchkov D, Hensel D, Kumlehn J, Schweizer P (2010) HIGS: host-induced gene silencing in the obligate biotrophic fungal pathogen Blumeria graminis. Plant Cell 22:3130–3141

Obara M, Kajiura M, Fukuta Y, Yano M, Hayashi M, Yamaya T, Sato T (2001) Mapping of QTLs associated with cytosolic glutamine synthetase and NADH- glutamate synthase in rice (Oryza sativa L.). J Exp Bot 52:1209–1217

Obara M, Sato T, Sasaki S, Kashiba K, Nagano A, Nakamura I, Ebitani T, Yano M, Yamaya T (2004) Identification and characterization of a QTL on chromosome 2 for cytosolic glutamine synthetase content and panicle number in rice. Theor Appl Gene 110:1–11

Paleman JD, Van der Voort JR (2003) Breeding by design. Trends Plants Sci 8:330–334

Panigrahy M, NageswaraRao D, Yugandhar P, SravanRaju N, Krishnamurthy P, Voleti SR, Ashok Reddy G, Mohapatra T, Robin A, Singh AK et al (2014) Hydroponic experiment for identification of tolerance traits developed by rice Nagina 22 mutants to low-phosphorus in field condition. Arch Agron Soil Sci 60:565–576

Pasam RK, Sharma R, Malosetti M, Eeuwijk FAV, Haseneyer G, Kilian B, Graner A (2012) Genome-wide association studies for agronomical traits in a worldwide spring barley collection. BMC Plant Biol 12:1–22

Piao Z, Li M, Li P, Zhang C, Wang H, Luo Z, Lee J, Yang R (2009) Bayesian dissection for genetic architecture of traits associated with nitrogen utilization efficiency in rice. Afr J Biotechnol 8:6834–6839

Prashar P, Shah S (2016) Impact of fertilizers and pesticides on soil microflora in agriculture. In: Lichtfouse E (eds) Sustainable agriculture reviews. Sustainable Agriculture Reviews, vol 19. Springer, Cham. https://doi.org/10.1007/978-3-319-26777-7_8

Ranjan R, Yadav R (2019) Targeting nitrogen use efficiency for sustained production of cereal crops. J Plant Nutri 42(9):1086–1113

Rosa C, Kuo YW, Wuriyanghan H, Falk BW (2018) RNA interference mechanisms and applications in plant pathology. Annu Rev Phytopathol 56:581–610

Rose TJ, Kretzschmar T, Waters DLE, Balindong JL, Wissuwa M (2017) Prospects for genetic improvement in internal nitrogen use efficiency in rice. Agronomy 7:70–85

Sain R, Singh S, Dudeja GR, Kumar V (2013) Isolation characterization, and evaluation of bacterial root and nodule endophytes from chickpea cultivated in northern India. J Basic Microbiol 55:74–81. https://doi.org/10.1002/jobm.201300173

San Miguel K, Scott JG (2016) The next generation of insecticides: DsRNA is stable as a foliar-applied insecticide. Pest Manag Sci 72:801–809. https://doi.org/10.1002/ps.4056

Sang HJ, Mayank A, Gururani B, Se-Chul C (2014) Isolation and characterization of plant growth promoting endophyticdiazotrophic bacteria from Korean rice cultivars. Microbiol Res 169:83–98

Sattler C, Kachele H, Verch G (2006) Assessing the intensity of pesticide use in agriculture. Agric Ecosyst Environ 119:299–304

Schena MD, Shalon RW, Davis P, Brown O (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270:467–470

Scorza R, Callahan A, Dardick C, Ravelonandro M, PolakJ MT et al (2013) Genetic engineering of plum pox virus resistance: “HoneySweet” plum-from concept to product. Plant Cell Tissue Organ Cult 115:1–12. https://doi.org/10.1007/s11240-013-0339-6

Senthilvel S, Vinod KK, Malarvizhi P, Maheswaran M (2008) QTL and QTL × environment effects on agronomic and nitrogen acquisition traits in rice. J Integr Plant Biol 50:1108–1117

Seung HJ, Narayan CP, Jian XD, Young SK, Bong-Sik Y, Seung HY (2013) Biocontrol activity of bacillusamyloliquefaciens CNU114001 against fungal plant diseases. Mycobiology 41 (4):234–242

Sharma A et al (2019) Worldwide pesticide usage and its impacts on ecosystem. SN Appl Sci 1:1446. https://doi.org/10.1007/s42452-019-1485-1

Shuqin JIN, Fang ZHOU (2018) Zero growth of chemical fertilizer and pesticide use: china’s objectives, progress and challenges. J Resour Ecol 9(1):50–58

Singh H, Verma A, Ansari MA, Shukla A (2015) Physiological response of rice (*Oryza sativa* L.) genotypes to elevated nitrogen applied under field conditions. Plant Signal Behav 9:29–45

Sinha SK, Sevanthi VAM, Chaudhary S, Tyagi P, Venkadesan S, Rani M, Mandal PK (2018) Transcriptome analysis of two rice varieties contrasting for nitrogen use efficiency under chronic n starvation reveals differences in chloroplast and starch metabolism-related genes. Genes (Basel) 11:20–45

Srividya A, Vemireddy LR, Hariprasad AS, Jayaprada M, Sakile S, Puram VRR, Anuradha G, Siddiq EA (2010) Identification and mapping of landrace derived QTL associated with yield and its components in rice under different nitrogen levels and environments. Int J Plant Breed Genet 4:210–227

Struik PC, Cassman KG, Koornneef M (2007) A dialogue on interdisciplinary collaboration to bridge the gap between plant genomics and crop sciences. In: Spiertz JHJ, Struik PC, van Laar HH (eds) Scale and complexity in plant systems research: gene-plant-crop relations. Springer, Dordrecht, pp 317–326

Tong HH, Mei HW, Yu XQ, Xu XY, Li MS, Zhang SQ, Luo LJ (2006) Identification of related QTLs at late developmental stage in rice (Oryza sativa L.) under two nitrogen levels. Acta Genet Sin 33:458–467

Tong HH, Chen L, Li WP, Mei H, Xing Y, Yu X, Xu X, Zhang S, Luo L (2011) Identification and characterization of quantitative trait loci for grain yield and its components under different nitrogen fertilization levels in rice (*Oryza sativa* L.). Mol Breed 28:495–509

Varshney RK, Graner A, Sorrels ME (2005) Genomics-assisted breeding for crop improvement. Trends Plants Sci 10:621–630

Vassilev D, Leunissen J, Atanassov A et al (2005) Applications of bioinformatics in plant breeding. Biotechnol Biotechnol Equip 19:139–152

Velculescu VE, Zhang L, Vogelstein B, Kinzler KW (1995) Serial analysis of gene expression. Science 270:484–487

Vetrovsky T, Baldrian P (2013) The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. PLoS One 8(2):e57923. https://doi.org/10.1371/journal.pone.0057923

Vinod KK, Heuer S (2012) Approaches towards nitrogen- and phosphorus-efficient rice. AoB Plants 28:1–18

Wang Y, Wu WH (2015) Genetic approaches for improvement of the crop potassium acquisition and utilization efficiency. Curr Opin Plant Biol 25:46–52

Wei D, Cui K, Ye G, Pan J, Xiang J, Huang J, Nie L (2012) QTL mapping for nitrogen-use efficiency and nitrogen-deficiency tolerance traits in rice. Plant Soil 359:281–295

Wissuwa M, Yano M, Ae N (1998) Mapping of QTLs for phosphorus-deficiency tolerance in rice (*Oryza sativa* L.). Theor Appl Genet 97:777–783

Wu P, Ni JJ, Luo AC (1998) QTLs underlying Rice tolerance to low-potassium stress in Rice seedlings. Crop Sci 38:1458–1462

Wu F, Gai Y, Jiao Z, Liu Y, Ma X, An L, Wang W, Feng H (2012) The community structure of microbial in arable soil under different long-term fertilization regimes in the Loess Plateau of China. Afr J Microbiol Res 6:6152–6164

Xie W, Zhou J, Wangb H, Chen X, Lu Z, Yu J, Chen X (2009) Short-term effects of copper, cadmium and cypermethrin on dehydrogenase activity and microbial functional diversity in soils after long-term mineral or organic fertilization. Agric Ecosyst Environ 129:450–456

Xu X, Liu X, He P (2015) Yield gap indigenous nutrient supply and nutrient use efficiency for maize in China. PLoS One 10:e0140767

Yang YH, Chen DM, Jin Y, Wang HB, Duan YQ, Guo XK, He HB, Lin WX (2011) Effect of different fertilizers on functional diversity of microbial flora in rhizospheric soil under tobacco monoculture. Acta Agron Sin 37:105–111

Yugandhar P, Veronica N, Panigrahy M, NageswaraRao D, Subrahmanyam D, Voleti SR, Mangrauthia SK, Sharma RP, Sarla N (2017) Comparing hydroponics, sand, and soil medium to evaluate contrasting Rice Nagina 22 mutants for tolerance to phosphorus deficiency. Crop Sci 57:1–9

Zacharia JT (2011) Identity, physical and chemical properties of pesticides. In: Stoytcheva M (ed) Pesticides in the modern world – trends in pesticides analysis. Intech Publisher, Rijeka, pp 1–18. https://doi.org/10.5772/17513

Zaman M, Saggar S, Stafford AD (2013) Mitigation of ammonia losses from urea applied to a pastoral system: the effect of nBTPT and timing and amount of irrigation. N Z Grassland Assoc 75:121–126

Zhang J, Khan SA, Hasse C, Ruf S, Heckel DG, Bock R (2015) Pest control. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. Science 347 (6225):991–994

Zhou Y, Tao Y, Tang D, Wang J, Zhong J, Wang Y, Yuan Q, Yu X, Zhang Y (2017) Identification of QTL associated with nitrogen uptake and nitrogen use efficiency using high throughput genotyped CSSLs in rice (*Oryza sativa* L.). Front Plant Sci 8:1166

Index

J. Aravind et al. (eds.), *Strategies and Tools for Pollutant Mitigation*,
https://doi.org/10.1007/978-3-030-63575-6