Biofilm-Associated Metal Bioremediation

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Abstract

One of the biggest challenges to the developing societies is metal pollution, especially in the regions of mining and plating that is affecting people worldwide. The use of conventional strategies in removing the waste is expensive and generates a large amount of toxic wastes, thereby affecting the environment adversely. This has resulted in the drift from the normal strategies to the use of eco-friendly strategies for the removal of metallic wastes being present within the soil. This technique of remediation uses the microbial organisms or microbial biomass that helps in detoxifying the soil from the toxic effects of inorganic metallic salts and heavy metals. Microbial biomass mainly comprises of extracellular polymeric substances (EPS) which increases the efficiency of metal sequestering for the purpose of field bioremediation. The EPS is released by the microbial cells for the purpose of self-defense which mainly occurs during various environmental stresses such as starvation, temperature, pH, and other physiological or rheological stress conditions. It contains a large amount of anionic charge hence it causes large sequestering of metallic ions. Thus this chapter will focus on the biofilm-associated bioremediation of heavy metals, and the mechanism which is helping the process to occur.

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8.1   Introduction

In order to maintain the standards of the environmental regulatory bodies, significant strategies are to be undertaken to reduce the organic and inorganic pollutants that contaminate ecosystems (Trapani et al. 2010). Heavy metals are natural high density elements that are required in various biological processes in trace amounts. Recalcitrant and toxic compounds containing heavy metals are a result of numerous urban and enhanced industrial activities. Accounting to its ease in solubility, products from pharmaceuticals, pesticides, plastics, rubbers, tanneries, and organic chemical industries lead to the accumulation of metals like Pb^{2+}, Cr^{2+}/ Cr^{3+}, Cd^{2+}, Ni^{2+}/ Ni^{4+}, Zn^{2+}, Cu^{2+}, Hg^{2+}, etc. that further triggers bio-magnifications (Lakherwal 2014). Metals tend to be toxic on accumulation, as they are nondegradable and can only be altered to different valance states and transformed by sorption, methylation etc. So this nonbiodegradability and the rising accumulation of free forms of heavy metals can lead to irreversible and indigenous health issues that affect bones, kidney, heart, and even brain. They can pose mutagenic, cytotoxic, or even carcinogenic effects (Ray and Ray 2009). The common physicochemical practices to reduce the load of these heavy metal ions include coagulation, reverse osmosis, chemical precipitation, nanofiltration, ultrafiltration, etc. But these procedures are not very favorable due to high reagent demand, costing, operational effectiveness, high energy demand, etc. Thus, for sustainable environmental detoxification, to reduce heavy metal ions to a safer level of concentration, there is a need to undertake environment friendly methods and strategies (Prasad and Prasad 2012). Here, microbial communities of bacteria, microalgae, fungi, and yeast play a vital role in degradation, neutralization, and mineralization as processes of remediation in order to remove organic and inorganic terrestrial and aquatic pollution of heavy metal ions. Efficient utilization of microbial metabolism in place of physicochemical procedures is a safe and efficient approach (Pandey and Jain 2002). Sorption of metal ions is an electrostatic or covalent interaction of the metal with peripherally available functional group on the sorbent. When such sorbents are of biological origin of eukaryotic or prokaryotic microbial biomass, such phenomenon is called biosorption (Comte et al. 2008; Lau et al. 2005). An environment-friendly approach is thus biofilm-mediated bioremediation. In order to thrive in stringent environmental conditions like high temperature, pH, salinity, and heavy metal-contaminated site, the microbial community produces extracellular polymeric substances (EPS) (de Carvalho 2018). EPS are biosynthetic polymers made up of mainly polysaccharides, proteins, and uronic acid that are produced in light of self-defense against environmental stress. The EPS composition hinders the metal ion penetration

into the cellular surface and thereby helps in sequestration of the metals. Biofilms are formed by the association of single or various species of bacteria, algae, or fungi, and its subsequent structure and composition of EPS produced can alter on the basis of environmental conditions (Yin et al. 2019).

As biofilms are capable to absorb, sequester, and immobilize various environmental pollutants (namely heavy metal ions), hence biofilm-mediated bioremediation is an effective approach. Thus, the attempt of this chapter is to focus on survival strategy of the microorganisms in the impacted (heavy metal contaminated) environment, the protective and beneficial approach of biofilms for the producing community, the biosynthesis of the polymer, i.e., the biofilm's life cycle and thereby metal ion to the biofilm interaction. Finally, in order to understand the mechanisms involved in metal ion uptake by the EPS, the interaction mechanism is also studied. Lastly, the scope and miscellaneous uses of biofilm and possible adverse environmental impacts have also been discussed.

8.2   Heavy Metals and Their Toxicity

As an important part of the global ecobiological system and vital component of metabolic pathways, heavy metals are sufficient even in trace amount. Such are the metals with atomic numbers more than 20 and of metallic density more than 5 g/cm^3 with distinct chemical characteristics. Majority are present in all ecosystems in varying concentrations along with trace quantities of essential metals like Mn, Ni, Zn, Mg, Cu, etc. They tend to have prolonged half-lives and resist biodegradation, thus are potent toxic to the ecosystem (Hussain et al. 2013). Environmental accumulation of these metals as a result of overloaded byproducts and toxic sludges from industries over long period of time is a serious matter of concern now.

When human body gets exposed to elevated amounts of these metals, they bind to various biomolecules and exert toxicity via different mechanisms like disrupting enzymatic functions, hinder in process of DNA formation and production of reactive oxygen species (ROS) (Yu 2001; Gauthier et al. 2014). Metals like lead, cadmium, and mercury are able to interact with cell membrane ligands, disrupt channels, and confer metabolic disturbances and cytotoxicity (Tavares and Carvalho 1992). Methylation or harmful thiol formation by chromium and arsenic, binding of cadmium to certain protein to render it dysfunctional, saturation and lipid peroxidation due to iron, lead causing free radical imbalance are few of the toxicological manifestations of heavy metal ions (Jaishankar et al. 2014).

Various plants and animal diseases have been reported due to metal toxicity. A prominent and burning example is the Minamata Bay (Japan) tragedy, where due to the Minamata disease, several people lost lives on consumption of shellfish that were heavily mercury contaminated. Zinc plays a vital role in plant growth and its defense mechanism. Upon accumulation, it reacts with oxygen and acids to produce potential toxic compounds harming the plants (Fosmire 1990). According to WHO recommended guidelines, arsenic concentration above 10μg/L might cause bronchitis, liver cirrhosis, or even cancer. Dietary intake of cobalt above 6μg may cause
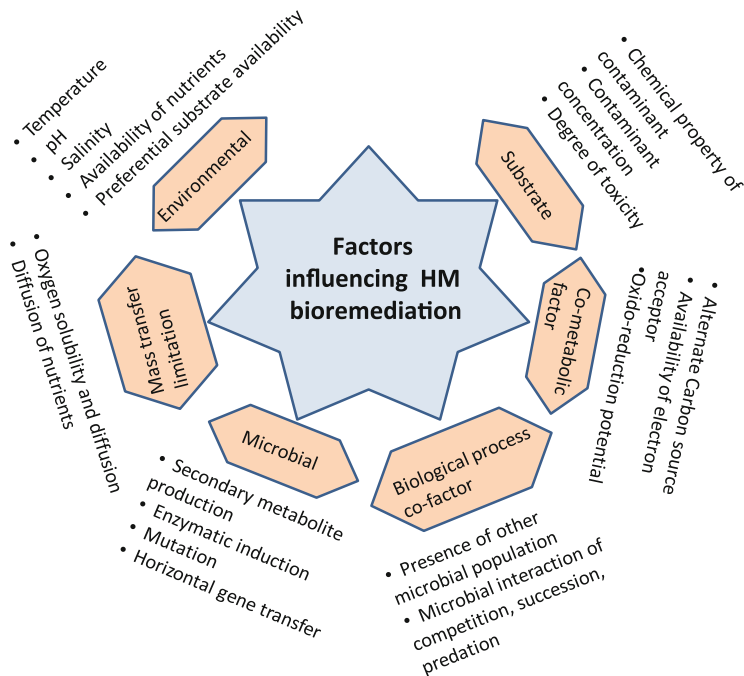
Fig. 8.1 Factors influencing bioremediation of heavy metals (HM)

chronic asthma or pneumonia. Renal and hepatic systems, and even the immune system are affected on overexposure to lead or copper ions (Flora et al. 2012).

Taking into consideration the cost, effectiveness, and end products produced after the physical treatments like flocculation, ion exchange, electrodialysis, etc. to reduce these metal ion concentrations to safer limits in the environment, it urges for imperative need to find environment-friendly and economic strategies (Alluri et al. 2007). Many microbes can thrive in metal contaminated sites and potentially accumulate soluble or particulate metal ions. Microbial biomasses of fungi, yeasts, and bacteria act as bioabsorbents of these heavy metal ions (Das et al. 2008). To survive in stringent environment, these eukaryotic and prokaryotic biomasses produce biosynthetic polymers that act as a shield and help in sequestering the metal ions via bio-immobilization or biotransformation (Fig. 8.1).

8.3   Biofilm: Composition and Structure

8.3.1   Composition

Biofilm is a cluster of microorganisms of same or different species that organize to attach themselves on living or nonliving surfaces while shielding themselves with a protective EPS layer from antibiotic agents, predators, or environmental stress.

Mostly composed of water, the EPS is made of mostly homo or hetero polysaccharides, proteins, nucleic acids, humic acid, lipids, as well as low molecular weight non-polymeric substances. FTIR spectroscopic analysis shows predominantly polysaccharides and proteins as the components of biofilm (Mosharaf et al. 2018). This microbial mucoid polysaccharide helps in cell aggregation, maturation of microcolonies, attachment to surface, and water-holding capacity and protects the cell cluster from stringent environment (Czaczyk and Myszka 2007). They are best differentiated not on the basis of origin but on the basis of separation, as soluble or bound EPS (Pal and Paul 2008). Ionic bridges that interlink the polysaccharide chains are due to uronic acid and other divalent metallic cations, which also entrusts the acidic character of EPS (Majumdar et al. 2013; Dogan et al. 2015) .The exopolysaccharide composition differs with diverse microbial aggregates, phase of growth, and its extraction method, like production of capsular EPS during the log phase of growth, whereas slime is formed during stationary phase (Wingender et al. 1999). EPS with polysaccharide backbone of repetitive units of same or different monosaccharides are differentiated on the basis of monomer units and linkages. The heteropolysaccharides are composed of units of N-acetylglucosamine or N-acetylgalactoseamine, D-glucose, L-rhamnose with intermittent presence of glycerol, phosphate, or acetyl groups, whereas homopolysaccharides are classified as Fructan, polygalactan, α-D-glucan, and β-D-glucan (Sharon 1966). Variety of EPS are produced upon alteration of the polysaccharide backbone by change in polymeric length due to variations in side chains and functional groups (Whitfield 1988). Those EPS with abundant anionic functional groups are reported to be better heavy metal remediator and a suitable biosorber. Moreover, as a nonliving adsorbent, EPS is considered more useful than living microorganisms to avoid pathogenecity concerns (Gavrilescu 2004). Some of the anionic bacterial EPS reported are xanthan (*Xanthomonas campestris*), galactopol (*Pseudomonas oleovorans*), hyaluronan (*Pseudomonas aeruginosa*), gellan (*Sphingomonas paucimobilis*), alginate (*Azotobacter vinelandii*) (Freitas et al. 2009, 2011). PsI polysaccharide produced by *Pseudomonas aeruginosa* is reported to maintain biofilm architecture and is responsible for intracellular and cell surface adhesion (Ma et al. 2012).

eDNA or extracellular DNA is a constituent of EPS that are formed from lysed cells within the biofilm. Recent studies show that these eDNA are important constituents of the EPS as they maintain the structural integrity of the biofilm, enhance cell–cell communication, and act as nanofilaments for electron transfer (Martins et al. 2010). These eDNA ranges in length of 1000–10,000 bp (Romero et al. 2018).

8.3.2 EPS Synthesis

The EPS is synthesized intra- or extracellularly depending upon the type of polysaccharide it produces. Precisely considering bacterial EPS biosynthesis, it is mostly seen homopolysaccharides are produced extracellularly when responsible precursors are transferred from substrate to the growing end of polysaccharide chain by suitable
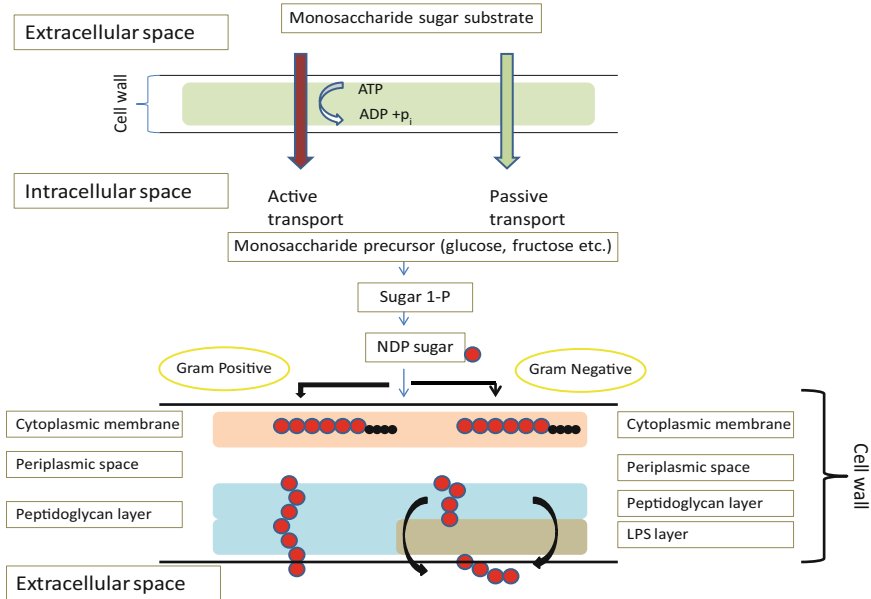
Fig. 8.2 Biosynthesis of EPS in Gram-positive and Gram-negative bacteria. Initiating with substrate diffusion, conversion within the cytoplasm, and thereby transport via polysaccharide assembly for the growing chain in the cytoplasmic membrane, elongation of the assembled polysaccharide in the peptidoglycan, maturation and transportation by ABC-dependent or Wzx–Wzy pathway to the extracellular environment

enzymes. The polysaccharide then matures and assembles itself with varying branches (Boels et al. 2001).

Comparatively a complex sequence occurs during the intracellular synthesis and transport of the polysaccharides. Regulatory molecules of interconnected metabolic pathways and various enzymes are involved during such synthesis process. It involves active or passive passage of substrate sugar into the cell which then gets catabolized by phosphorylation or periplasmic oxidation (Freitas et al. 2011). Intracellular synthesis involves the assembly of the high molecular weight, hydrophilic polymer in the cytoplasm and its transport via the cell membrane. The Gram-negative bacteria is studied to biosynthesize via the following pathways (Fig. 8.2).

(a) ABC transporter-dependent pathway: polymerization occurs at the inner cell membrane, in the cytoplasm side (Cuthbertson et al. 2009) (i) the Wzx–Wzy-dependent pathway, wherein the polymer monomeric units are accumulated at the inner face of the cytoplasmic membrane and polymerized at the periplasm.
(b) Wzx–Wzy-dependent pathway: The monomer units are assembled in the inner face of the cell membrane, whereas polymerization takes place in the cytoplasm.

(c) Synthase-dependent pathway: Here the stand is secreted across the cell wall membrane, where the homopolymers are translocated and polymerization takes place with a single precursor of sugar and single synthase protein (Table 8.1).

8.3.3 Biofilm Structure and Its Formation

Unlike the planktonic growth, the development of biofilm has distinct and significant mechanism. The biofilm formation and growth are ubiquitous to bacterial cells and occur sometimes under diverse stringency. The biofilm formation, its attachment, and maturation mostly requires a moist environment. Attachment surface may be biotic or abiotic, and their interaction interface can be of any form of solid-liquid, liquid-liquid, or solid-air. Different stages of microbial life cycle, is reported to produce various forms of biofilm.

The overall process of formation involves four distinct steps initiating with the attachment of the planktonic forms of microbes to a moist surface by Van der Waals or electrostatic forces of attraction. Following it is the procedure of microcolony formation, maturation, and development of biofilm and finally detachment from the surfaces (Jamal et al. 2018).

- *Initial surface attachment*: Intricate study on biofilm formation reports the initial attachment of the microbes on a moist surface. Their arrival instigates a formation of conditioning layer with the organic matters within a short span of time (Qi and Christopher 2019). The microbial population attaches itself to the surface with its pili, fimbriae, by van der Waals force or via electrostatic forces of attraction. The adhesion to the surface brings about cohesive forces among the cells to expand the film formation.
- *Colony formation*: The intercellular signaling calls for rapid cell multiplication and thus microcolony formation. The spatial arrangement of the clustered microcolonies determine the architecture of the biofilm. The side chains of the formed EPS by these colonies further strengthens the biofilm's anchoring to the surface. This confirms the further maturation at that surface (Randrianjatovo-Gbalou et al. 2017).
- *Architectural maturation*: Intracellular signaling, replication, and nutrient transfer increase the microbial population to the desired density (Vasudevan 2014). Maturation-specific genes are expressed during this stage of development that is facilitated by the phenomenon of Quorum Sensing. This three-dimensional biofilm development can produce as thick as few inches of film by some bacteria.
- *Microbial detachment*: Biofilm detachment may be caused by physical forces of shear strain like natural abrasion or hydrodynamic forces which erodes off biofilm layers (Webb 2009). This passive dispersion of sloughing might occur due to aged biofilm, deprivation of nutrients, natural erosion, etc. But regardless of the detachment process, the conditioning layer tends to remain attached to the substratum, chopping off the outer layer with saccharolytic enzymes produced by the microorganisms (Fig. 8.3).

Table 8.1 Microbial EPS: homopolysaccharide and heteropolysaccharide

| EPS	Class	Monomers	Linkages	Example of EPS-producing microorganisms	Reference
Levan	Homopolysaccharide	Fructose	β-2,6	*Halomonas smyrnensis*, *Streptococcus salivarius*, *Streptococcus mutans*	Sima et al. (2011) and Czaczyk and Myszka (2007)
Curdlan	Homopolysaccharide	Glucose	β-1,3 & β-1,2	*Bradyrhizobium japonicum*, *Streptococcus pneumoniae*	McIntosh et al. (2005) and Czaczyk and Myszka (2007)
Pullulan	Homopolysaccharide	Glucose	α-1,4	*Aureobasidium pullulans*	Özcan et al. (2014), Cheng et al. (2011) and Elshafie et al. (2017)
Reuteran	Homopolysaccharide	Glucose	α-1,4	*Lactobacillus reuteri 121*	Kralj et al. (2002)
Cellulose	Homopolysaccharide	Glucose	β-1,4	*Gluconacetobacter hansenii*	Sun et al. (2010) and Shah and Brown (2005)
Alternan	Homopolysaccharide	Glucose	α-1,3 & α-1,6	*Streptococcus salivarius*	Simpson et al. (1995)
Xanthan	Heteropolysaccharide	Glucose as backbone and glucuronic acid as side chain	(1→4)-β-D-glucan	*Xanthomonas campestris*	Schatschneider et al. (2013)
Alginate	Heteropolysaccharide	β-D-Mannurosyl & α-L-guluronosyl	1–4 bonds	*Pseudomonas aeruginosa*, *Azotobacter vinelandii*	Czaczyk and Myszka (2007)
LAB EPS	Heteropolysaccharide	Fructose, rhamnose, glucose, and galactose	1–6; 1–4; 1–2; 1–3 bonds	*Lactobacillus* sp.	Nishimura (2014)
Sphingans (gellan, rhamsan, welan)	Heteropolysaccharide	Glucose, rhamnose and glucoronic acid	1–3; 1–4 bonds	*Sphingomonas elodea*, *Sphingomonas* sp. *ATCC53159*	Pollock (1993) and Coleman et al. (2008)
Schizophyllan	Homopolysaccharide	Glucose	β-1,3 with β-1,6 branching	*Schizophyllum commune ATCC38548*	Joshi et al. (2016)
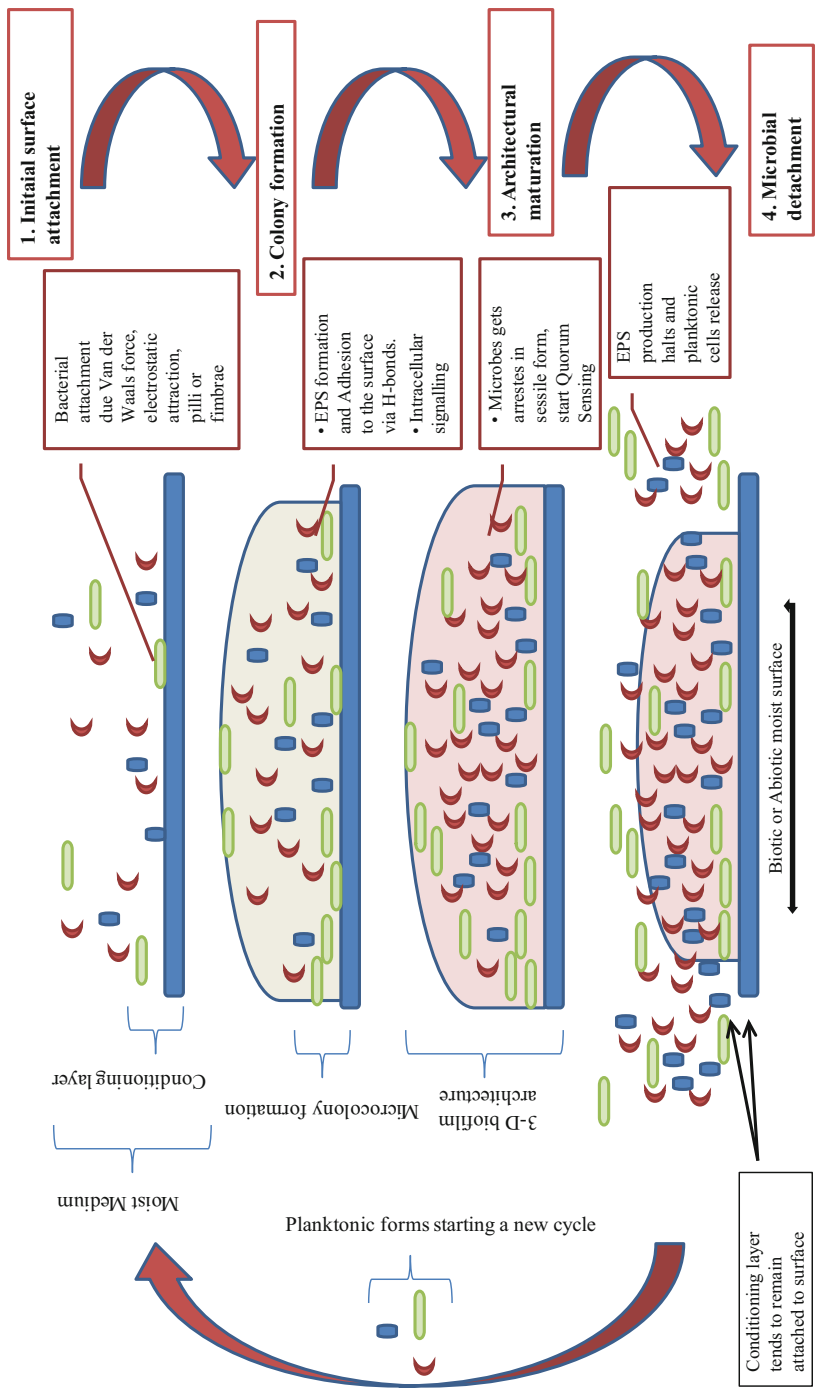
Fig. 8.3 Stepwise events of microbial biofilm formation

8.4   Biofilm-Producing Microbiota

Biofilm producing microbial biomass have been widely reported for heavy metal bioremediation. The microbial population predominantly includes bacteria along with fungi, microalgae, and yeast. Mostly the metal sequestration takes place by adsorption or bioaccumulation. These metal-resistant strains are efficient producers of biosynthetic polymers (EPS), and their immobilized forms are better-off with metal interaction and its reaction kinetics as reports suggest that production of EPS enhances due to attachment to a substrate (Vandevivere and Kirchman 1993).

8.4.1   Bacteria in Bioremediation of Heavy Metals

The lab-scale efficiency of microbial biomass varies with the onsite physicochemical and other environmental factors (Ayangbenro and Babalola 2017). Almost all bacterial cells, due to varying size, cellular composition like teichoic acid, N-acetylglucosamine, or N-acetyl muramic acid, their robust adaptivity, and metabolic secretion are able to produce biofilms and thrive in harsh environments. This helps the bacterial biomass to accomplish efficient removal of the nonbiodegradable heavy metals (Hassan et al. 2010; Özer and Özer 2003). Numerous species have been reported as important biosorbents like *Listeria monocytogenes* (Colagiorgi et al. 2017), *Pseudomonas fluorescens* (Lopez et al. 2000)*, Pseudomonas putida, Sphingomonas* sp. (Douterelo et al. 2018), *Micrococcus luteus* (Puyen et al. 2012), *Staphylococcus xylosus* (Aryal et al. 2010), and metabolically superior consortium of *Acinetobacter* sp. and *Arthrobacter* sp. (De et al. 2008), *Acinetobacter* sp. IrC1 and *Cupriavidus* sp. IrC4 (Irawaiti et al. 2018). The teichoic acid on the bacterial cell wall is the potential site for bacterial chemisorptions (Mosa et al. 2016). Upon bacterial exposure to contaminants, enzymatic induction calls for remediation by metal chelation or electrostatic interaction. Reports suggests that zeolite-immobilized *Desulfovibrio desulfuricans* used in a batch reactor show metal removal efficiency of 90.1% 98.2%, and 99.8%, of Ni, Cu, and Cr^{6+}, respectively (Kim et al. 2015). Also efficient Pb reduction by *Bacillus megaterium* (2.13–0.03 mg/L), Cr reduction (1.38–0.08 mg/L) by *Aspergillus niger*, and Cd reduction (0.4–0.03 mg/L) by *Bacillus subtilis* has been reported by Abioye et al. (2018).

8.4.2   Fungi in Bioremediation of Heavy Metals: Mycoremediation

Fungi are the decomposers that are involved in vital nutrient cycling in the nature and adapt to harsh conditions similar to that of bacteria. But the bacterial and fungal glycan and other biosynthetic enzymes differ in sequence homology (Sheppard and Howell 2016). Yet these organisms are capable of biofilm formation by clustering and attaching to a surface or entrapping itself inside the EPS to shield from environmental stringency. Reports suggest that in the course of biofilm formation, the fungal ability of surface attachment is due to the presence of hydrophobin

protein. This protein signals the adhesion of fungal spore on hydrophobic surfaces (Pérez-Mendoza et al. 2011). Various members those are able to form biofilm are species of *Neocosmopora* and *Acremonium* (Douterelo et al. 2018), *Candida albicans* (Rajendran et al. 2016), and those with prominent metal removal efficacy are *Coprinopsis atramentaria* reported to bioaccumulate 94.7% of 800 mg/L of Pb^{2+} (Luna et al. 2016), *Candida sphaerica* has removal efficiency of Pb, Zn, Fe of 79%, 90%, and 95%, respectively. Reduction of Cr (VI) to Cr (III) by yeast strains of *Rhodotorula mucilage, S. cerevisiae, Pichia guilliermondii*, and *Yarrowia lipolytica* has also been reported (Chatterjee et al. 2012). Fungi are studied to biosorp and accumulate heavy metals by means of ion-exchange or complexation mainly in their fruit bodies (Ogbo and Okhuoya 2011). Fructification, age of mycelia, and metal exposure time-span determine the degree of removal efficacy (Floudas et al. 2012).

8.4.3   Algae in Bioremediation of Heavy Metals: Phycoremediation

Algae are the organisms that produce a large quantity of biomass, able to survive in toxic environments with low nutrient availability. The most prominent members with effective heavy metal bioremediative ability are those of microalgae. Their various cell surface chemical moieties are the metal binding sites like with amide, carboxyl, hydroxyl groups (Abbas et al. 2014). Algae like bacteria and fungi, bioremediate heavy metals by adsorption, cellular integration of the toxicants or by degradation (Chabukdhara et al. 2017). Among the various algal groups, heavy metal phycoremediation by biosorption is best reported by Phaeophyta (brown algae) (Brinza et al. 2007; Oyedepo 2011). Table 8.1 enlists the heavy metal remediating bacteria, fungi, and algae (Table 8.2).

8.5   Metal–Microbe Interaction and EPS-Mediated Strategies for Remediation

The physical methods involved to clean up the environment from metal accumulation involve high-cost techniques and production of toxic byproducts. This calls for cost-effective and environment-friendly biological methods. Exploiting the microbial metabolism is a gentle approach to combat heavy metal toxicity. But not all the time these metals pose to be toxic to the microbes. The concentration of metal and microbes, mode of interaction with the metals as active or passive uptake and subsequently develop metal tolerance or resistance. (Alluri et al. 2007). This microbial metal tolerance can be specific or nonspecific. Mercury methylation is an example of specific tolerance that produces volatile toxic methyl mercury, whereas nonspecific tolerance may be constitutive or inducible. Production of cysteine-rich metallothionine peptides by *Pseudomonas putida* or *E. coli* is an example of nonspecific inducible resistance (Rajendran et al. 2003). On the other hand,

Table 8.2 List of the heavy metal remediating bacteria, fungi, and algae

Metal	Bacteria	Fungi	Algae
Fe	*Rhodobacter capsulatus*	*Aspergillus flavus* (Bano et al. 2018) *Pleurotus ostreatus* (Arbanah et al. 2012)	*Chlorella vulgaris* (Kwarciak-Kozlowska et al. 2014)
Pb	*Methylobacterium organophylum* (Bharagava and Mishra 2018) *Pseudomonas putida* (Chen et al. 2005)	*Agaricus bisporus* (Frutos et al. 2016) *Pleurotus florida* (Prasad et al. 2013)	*Nostoc* sp. (Kumaran et al. 2011) *Corallina mediterranea* (Ibrahim 2011)
Zn	*Pseudomonas putida* (Pardo et al. 2003) *Bacillus firmus* (Salehizadeh and Shojaosadati 2003)	*Schizophyllum commune* (Javaid and Bajwa 2008) *Pleurotus ostreatus* (Arbanah et al. 2012)	*Sargassum* sp., *Ulva* sp., *Padina* sp., *Gracilaria* sp. (Sheng et al. 2004) *Scenedesmus quadricauda* (Bayramoglu and Arica 2009)
Cr (VI)	*Bacillus laterosporous* (Zouboulis et al. 2004) *Bacillus cereus* (Nayak et al. 2018) *Bacillus circulans* MN1 (Chaturvedi 2011)	*Rhizopus arrhizus* (Shoaib et al. 2013) *Pleurotus ostreatus* (Arbanah et al. 2012)	*Isochrysis galbana* (Kadimpati et al. 2013) *Sargassum polycystum* (Senthilkumar et al. 2010)
Cd	*Bacillus laterosporous* (Zouboulis et al. 2004) *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Bacillus cereus* (Kafilzadeh et al. 2013)	*Alternaria alternata* (Bahobil et al. 2017) *Aspergillus flavus* (Cardenas-Gonzalez et al. 2017)	*Ceramium virgatum* (Sari and Tuzen 2008) *Caulerpa fastigiata* (Sarada et al. 2014)
Cu	*Desulfovibrio desulfuricans* (Kim et al. 2015) *Micrococcus luteus* (Puyen et al. 2012)	*Agaricus bisporus* (Frutos et al. 2016) *Termitomyces clypeatus* (Ramrakhiani et al. 2011)	*Chlorella kessleri* (Horvathova et al. 2009) *Caulerpa lentillifera* (Apiratikul and Pavasant 2008)
Ni	*Pantoea agglomerans* and *Enterobacter asburiae* (Bhagat et al. 2016) *Desulfovibrio desulfuricans* KCTC5768 (Congeevaram et al. 2007)	*Aspergillus versicolor* (Taştan et al. 2010) *Schizophyllum commune* (Javaid and Bajwa 2008)	*Chlorella vulgaris* (Klimmek et al. 2001) *Spirogyra hyaline* (Kumar and Oommen 2012)

exopolysaccharide production is a nonspecific tolerance. Adsorption, bioleaching, bioaccumulation, and redox transformations are the modes of interaction among the microorganisms to uptake metal ions.

8.5.1   EPS-Mediated Metal Biosorption: Mechanism, Advantages, and Disadvantages

Among various biotransformation procedures, bioadsorption has been seen to be more feasible due to its inherent advantages and applicability. Unlike bioaccumulation or redox transformations, biosorption is a metabolism-independent procedure which can be achieved by both live and dead biomass via different physicochemical procedures (Vijayaraghavan and Yun 2008). While it is a challenge for the live cells beyond lethal concentration of metals or in restricted nutrient availability, to survive metabolically actively, dead cells on the other hand are independent of such constrain to sequester metal by adsorption and its storage (Aksu 2005). Moreover, since the dead biomass only accumulate the metals within, without involving it in metabolic processes, so they can be profitably desorbed and the metal ions can be utilized, acting as reversible ion exchanger. Even though these microbial biomass (live or dead) are efficient and economical solution for metal removal, but its efficiency of application on industrial scale is still a constraint which needs to be worked upon further (Wang and Chen 2009).

8.5.2   Strategies of Heavy-Metal and EPS Interaction and Its Remediation

The exopolysaccharides are made up of repetitive sugar units that are responsible for metal chelation by interacting with their functional groups via ion exchange, physical sorption, or precipitation mechanisms. The charged metal ions interact with anionic EPS for the presence of ionizable carboxylate, phosphate, and amine groups on the EPS backbone (Liu and Fang 2002). The hydroxyl, carboxyl, and amino functional groups aid in metal binding and stabilize the metal–polymer interaction by forming co-ordination bonds (Cozzi et al. 1969).

8.5.3   Types of EPS and Its Remediation Strategies

8.5.3.1 Dead Biomass EPS

EPS of a dead biomass and favorable environmental condition gives maximum metal uptake capacity as a biosorbent. An acidic pH is mostly favorable for metal uptake as an overall positive charge is created due to lowered pH that protonates the functional group which interacts well with the negatively charged EPS (Sultan et al. 2012). However, the adsorption capacity of immobilized EPS is head over dead biomass EPS. Freundlich–Langmuir adsorption model in a research report suggested that in acidic pH of 2, copper ions were maximally chelated by dead biomass EPS with a concentration of 26 mg/g of EPS where the metal load was 91.66 ppm (Ozdemir et al. 2005).

8.5.3.2 Homogeneous EPS

Many single species microbiota are reported to thrive in multi-metal contaminated sites, bind to or sequestrate metal specifically or nonspecifically, by either tolerance or resistance. Cells along with their produced polysaccharide together are seen to be more efficient in adsorption of heavy metals. Various microbes and specially bacteria have been widely reported for such bioremediating activity like *Methylobacterium organophilum* (copper and lead removal) (Kim et al. 1996), *Rhizobium radiobacter* (biosorption of zinc and lead) (Wang et al. 2013), microalgae *Halomonas* sp. (calcium, iron, magnesium, aluminum) (Gutierrez et al. 2012), cyanobacteria *Anabaena spiroids* (manganese) (Freire-Nordi et al. 2005), etc.

8.5.3.3 Immobilized EPS

Studies on specificity and reaction kinetics have shown the excellency of the immobilization techniques. Attachment of microbial cells on a surface enhances not only EPS production but also metal binding ability without elevating the growth rate (Vandevivere and Kirchman 1993). The EPS secreted by *Paenibacillus polymyxa* immobilized on agar beads (Hassiba et al. 2014) and *Chryseomonas luteola* immobilized on alginate beads (Ozdemir et al. 2005) are able to efficiently adsorb various metals like cadmium, cobalt, nickel cobalt, etc.

The test microorganism and the metal concentration are more of a concern than other factors of colony size, procedure of metal–microbe interaction, etc. (Cruz-Vega et al. 2008). Hence for isolating a powerful bioremediating microbe, such criteria must be essentially considered.

8.6   Challenges with Biofilm and Future Prospects

The aggregation of microbial population called biofilms, able to attach to various surfaces, are explicitly studied for environmental pollution remediation. An economical as well as sensitive application of the ubiquitous exopolysaccharide (EPS) produced by the microbial biomass is an efficient approach to metal ion bioremediation. Along with the various environmental factors, the EPS matrix composed of various polysaccharides, nucleic acids, humic acid, and proteins helps in formation and stabilization of the biofilm, defining its 3D structure. These biofilm-forming microbial population adapt to thrive in harsh conditions; hence, several dynamics governs the EPS efficacy of metal removal. As discussed before, the charge composition of EPS, types, quantity, and charge distribution of the metal pollutants, environmental factors of temperature, pH, and salinity are all, in combination, determining factors of the onsite sorption efficacy of the biopolymer. Moreover, the lab-based efficacy must also be replicated at the actual site of pollution which needs to be monitored and checked for proper desorption of the metals from the polymer for sustainable remediation. Desired modification of the polymer for immobilization and improved selectivity for metals can be made of EPS hybrids with ceramic, porous silica, etc. (Kariminiaae-Hamedaani et al. 2003; Shi et al. 2011). Selection of anionic EPS producers and mixed culture EPS gives better

results in bioremediation, but scaling up the technology, out from laboratory as field trials, needs more research for successful application (Kiliç et al. 2015).

Microbial biofilms are also utilized in several promising ways as in production of microbial fuel cell (MFC) that oxidizes organic matter to produce electricity, as bioemulsifier and biocapsule, as biofilm traps and microbial canaries (Moreno-García et al. 2018). As an emerging scope, Biofilm-Integrated Nanofiber Display (BIND) is a nano-biotechnological approach to develop programmable biomaterials like adhesive biofilm that are able to bind various surfaces (Nguyen et al. 2014).

Uncontrolled growth of biofilms on suitable surfaces are nowadays a potent matter of concern in the health sector. The growth of biofilm on medical instruments, surface of catheters, and medical implants may cause nosocomial infections, on teeth (plaque), it is also implicated in the otitis media (an acute ear infection). Even avid growth of these biofilms are seen over household items like cutting board, toilet sink, wash basin, etc., which need to be checked and sanitized. Biofilm formation is a major concern in the food industry as they cause havoc product spoilage with growth of pathogenic bacteria, mechanical blockage, and post processing contamination. Hence, to combat with such biofilm problems, effective and systematic antibiofilm strategies are to be undertaken for large-scale application.

The great versatility of the microflora helps in with proficient method of metal ion sequestration and restoration of contaminated ecobiological systems. Thus, in order to effectively use EPS for bioremediation, it must be studied properly from its formation, maturation, EPS–metal binding, and therefore sustainably remove heavy metals from the environment.

Conflict of Interest A mutual declaration by all the authors states that there is no conflict of interest.

Moreover, this chapter does not contain any study conducted upon human or animals, by any authors.

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