Chapter 2
Microbes: "A Tribute" to Clean Environment

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Abstract Due to industrial development, the amount and variety of hazardous substances added to the environment has increased drastically. Bioremediation is the process of using microorganisms or other life forms to consume and breakdown environmental pollutants in comparatively safe products. Because bacteria have a fast rate of population growth and are constantly evolving, they can adapt to live off materials and chemicals that are normally poisonous to other species. Some bacteria can remove chlorine from carcinogenic materials, digest pesticides, and have the ability to decolorize various xenobiotic dyes through microbial metabolism. Other microbes used for biological decolorization are red yeasts like *Rhodotorula rubra, Cyathus bulleri, Cunninghamella elegans,* and *Phanerochaete chrysosporium, Actinobacteria, Cyanobacteria, Flavobacteria, Deinococcus-thermus, Thermotogae, Firmicutes*, *Staphylococcus*, and *Proteobacteria*. Construction of strains with broad spectrum of catabolic potential with heavy metal-resistant traits makes them ideal for bioremediation of polluted environments in both aquatic and terrestrial ecosystems. The transfer of genetic traits from one organism to another paves way in creating Genetically Engineered Microorganisms (GEMs) for combating pollution in extreme environments making it a boon to mankind by cleaning up the mess that has created in nature.

Keywords Pollution • Nano-bioremediation • Clean environment • Designer microbes • Microbial cleaners

1 Introduction

Over the last 150 years, the number of organic chemicals released into the environment has increased dramatically (Schwarzenbach et al. 2010) leaving an unprecedented chemical footprint on earth. Many groundwater contaminations result from

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point sources, originating from accidents or contaminations at industrial sites. These contaminations typically form plumes with high concentrations of pollutants (μg/L to mg/L range). Alternatively, chemicals may enter groundwater through widespread application in agriculture or release from sewage treatment into rivers. Here, pesticides, pharmaceuticals, or consumer care products are introduced as nonpoint sources and typically occur in much smaller concentrations (micropollutants in ng/L to μg/L range) (Richardson and Ternes 2011).

Biodegradation is one of the most favored and sustainable means of removing organic pollutants from contaminated aquifers but the major steering factors are still surprisingly poorly understood. Microorganisms play a fundamental role in the environment. Their role is the result of complex biogeochemical processes by consortia of microorganisms and the function of individual species is not clear in many cases (OECD 2015).

2   Rationale of Using Microbes for Clean Environment

Microbes are ubiquitous in nature and are being exposed to the continuous release of more and more recalcitrant xenobiotic compounds into the environment. These microbes inhabiting polluted environments are armed with various resistance and catabolic potentials. The catalytic potential of microbes in nature is enormous, and this is advantageous to mankind for a cleaner and healthier environment through bioremediation.

The contamination cleanup strategy called bioremediation using naturally occurring or genetically modified microbes to clean up dirt and pollution is gaining importance, as scientists devise new ways to use bugs against mercury, oil spills, radioactive waste, and more.

In general, potential microbes with broad spectrum of activities from their native habitat have been screened, characterized, genetically modified, and released back to their native habitat for better performance. By such studies, the core problem of pollution is tactfully attacked and benefits of decontamination add healthy atmosphere to mankind.

The overall rationale for using any microbes is similar for all types of products. Living microbes are capable of enzymatically degrading substances associated with soil and/or bad odor. Thus, products containing spores (dormant microbes) have to allow for a germination step first to the vegetative state to become physiologically active. Microbial action is aimed at controlling odor and to support the cleaning action of detergents (Kumar and Gopal 2015).

Some microorganisms produce a broad range of extracellular enzymes including proteases, cellulases, amylases, and ureases which can degrade organic high molecular weight substances in soil. As opposed to cleaners with added enzymes, microbes can further metabolize some of these degradations products. Substances creating odor problems such as NH_3 can be metabolized, or the formation of H_2S may be avoided by transforming SO_3 into S_2. The purified degrading enzymes, nitrilase,

azoreductases, and organophosphate hydrolases could be effectively used in industry for the treatment of effluents. The systems developed are eco-friendly and economical and hence could effectively be integrated with physicochemical methods for pollution control.

The microbes used in the cleaning products are also claimed to outcompete unwanted microorganisms in colonizing surfaces by using up the nutrients provided in the soil and from polluted surfaces. Other microbes can directly inhibit the growth of unwanted microbes, for example, by lowering the pH. Producers claim a long-term effect because microorganisms will stay on the treated surface (as spores) and hinder recolonization by unwanted microbes.

In this direction, a new terminology named "microbial cleaners" is coming into play. Microbial cleaners are the specially designed groups of bacteria and fungi that are capable of cleaning the polluted sites (Spök 2009).

In commercial contexts, microbial cleaners are mainly applied for odor control in cases where conventional cleaners are considered less efficient. The rationale is that microbes causing problems in hospitals are outcompeted by the microbes used in the cleaner which would render disinfection unnecessary. Besides hard surface cleaning, these products are also used for cleaning carpets and upholstery. Specialty products are used for cleaning drains, pipes, and grease traps in order to remove deposits, and also in industrial production in the washing of machine parts, as well as for oil spills on masonry or concrete.

Products based on Effective Microorganisms (EM®) represent a special type in terms of product design, producer, production process, and marketing. An inoculum including a combination of bacteria and fungi is manufactured by licensed companies mainly based in Japan and marketed worldwide by specialized EM vendors and health food shops.

The same and similar combinations of microbes are used for various outdoor and indoor purposes including soil enhancement, composting, as a feed additive and for cleaning. EM cleaners are not only applied in all the areas described above but recommended for a much broader range of indoor cleaning applications including tiling, stove, refrigerator, pots and pans, bio-waste container, living spaces, wooden floors, closets, wardrobes, shoe cabinets, leather clothes, glass doors, washing machines, dishwashers, doormats, cars, and even as laundry detergent (Spök 2009).

Microbial cleaners are covered under Environment and Health Legislation by EU Directive 2000/54/EC which regulates the minimum requirements for the protection of workers from risks related to biological agents.

Employers (e.g., manufacturers and blenders of microbial products, professional cleaning service companies, other companies employing cleaning personnel) are required to conduct a risk assessment, including the classification of the microorganisms used into one of four risk groups based on the pathogenic potential. Potential allergenic or toxigenic effects and exposures also have to be considered (Directive 2000).

Only microbes which belong to risk group 1 are not considered to pose any hazards to human health. The use of microbes classified in risk group 2 or higher requires notification to the national competent authorities and preventive measures

by the employer. The type of risk mitigation measures largely depends on the particular risk group and exposure scenario.

Manufacturers claim that microbes classified into risk group 2 or higher are neither used nor considered for application in cleaners.

The microorganisms that are considered as biocides include two *Bacillus* spp. including *B. subtilis* which is frequently used in microbial cleaners. These microbes are listed as biocides in the Annex to Regulation 1451/2007.

Microbial cleaners are environmentally sound. Most microbial cleaner products contain much lower levels of acids and surfactants. Microbial products used in commercial and industrial contexts for cleaning drains, pipes, and grease traps are less alkaline and indicate a potential for reducing the amount of organic solvents used. This is also true for solvent-free microbial degreasing of parts in industrial manufacturing. According to manufacturers, the preventive character of microbial action is also potentially beneficial for the environment as microbes are being active as long as there is sufficient nutrients and water on the surface. When lacking nutrients or water, certain microbes can survive as spores which can germinate and become physiologically active again if nutrients and water becomes available to them. If used on a regular basis, for instance, in grease traps and drain pipes the formation of sediments and odor is reduced which renders the need to use environmentally harmful cleaning products unnecessary.

The most frequently used microbes are members of the genus group*Bacillus, Bifidobacterium, Lactobacillus, Rhodopseudomonas*, and *Saccharomyces*. Some producers are specialized on combinations of different *Bacillus* sp. spores instead of using vegetative cells; as spores allow for a longer shelf life up to 1 year (Spök 2009).

3   Bioremediation Through Genetic Engineering of Microbes

Bioremediation involves using genetically altered living organisms that give them taste for toxins to eat the contaminants. Bioremediation explores gene diversity and metabolic versatility of microorganisms (Fulekar 2009). Such microbes are deployed to purge sites of contaminants such as polychlorinated biphenyls (PCBs), oil, radioactive waste, gasoline, and mercury.

Microbes play a very important role in the mineralization of pollutants either by natural selection or through recombinant DNA technology making bioremediation process an extension of normal microbial metabolism. The recombinant DNA technology explores PCR, anti-sense RNA technique, site-directed mutagenesis, electroporation, and particle bombardment techniques.

The major advantage of developing genetically engineered microbes is that the modified organism has a higher degradative capacity and can even degrade the recalcitrant molecules. It is an effective, safe, and economical technique for bioremediation (Singh et al. 2014).

In a recent study, researchers developed a modified *E. coli* bacterium that allowed it to not only survive in mercury but also to remove it from waste sites. The genes produce proteins called metallothionein and polyphosphate kinase that allow the bacterial cells to develop a resistance to mercury and to accumulate large amounts of the heavy metal within the organism. Mercury is a toxic heavy metal and can be converted into methylmercury, a more toxic form; and no natural organism can bio-remediate it. These transgenic bacteria sequester mercury contamination before the natural bacteria converts it into toxic methylmercury. These transgenic microbes are used in the form of filters and are added (bio-augmentation) at the contaminated and polluted site to remove the toxic metal (Kumar and Gopal 2015).

Thus, bio-augmentation is a type of bioremediation that involves adding organisms directly to the open environment. The microbes that are not able to adapt the environment die quickly and simply provide more nutrients for the indigenous bacteria to feed on.

There are some microbes that develop special proteins to protect themselves from potentially toxic nanoparticles in their own environment. These microbial proteins can be used to improve water quality on a large scale. In another study, bacteria were isolated from an abandoned mine excrete proteins that cause metal nanoparticles to aggregate. The bacteria bind and immobilize the metals in the form of nanoparticles which are potentially toxic to the bacteria.

Similarly, S-reducing bacteria can cause the precipitation of zinc metal and form nanoparticles. These nanoparticles are able to move freely because they are so small (2–6 nm diam.) and can redissolve if conditions change (Dixit et al. 2015).

Microbes such as bacteria, fungi, and algae play an important role in the biore-mediation of xenobiotic compounds like dyes and plastic including pesticides and insecticides like morpholine, methyl parathion, organophosphorus compounds, and benzimidazoles (Tang et al. 2007).

In a study, different pure isolates of *Pseudomonas* sp. were characterized for complete and partial mineralization of morpholine, methyl parathion, and other organophosphorus pesticides and fungicides that causes oil hydration by both aromatic and aliphatic hydrocarbon degradation. There are some other bacteria such as *Serratia* sp. and *Bacillus* sp. that have been characterized for their ability to degrade benzimidazole compounds and effectively decolorize distillery and textile mill effluents, respectively. The other species, *Pseudomonas A3, Pseudomonas putida, P. aeruginosa,* and *Serratia marinorubra*, have been used for complete mineralization of broad-spectrum fungicides in soil.

These microbes including *Trichoderma viridae* are also capable of degradation of commercial textile mill azo reactive dyes like Black B, Turq Blue GN, Yellow HEM, Red HEFB, Navy HER, number of mono, bi, poly azo dyes, and triphenylmethane dyes like Methyl red, Acid black, Acid brown, Acid green, Sudan black, and Crystal violet to name a few.

For the detoxification of heavy metal and biosorption, *Bacillius* sp. was found to be effective in reducing hexavalent chromium to its nontoxic trivalent form. The cultures of *Azotobacter* sp. and *Leuconostoc* sp. are cloned for their extracellular

polysaccharide (EPS) production for biosorption of many heavy metals like cadmium, zinc, arsenate, and chromium from polluted samples.

Likewise, aliphatic and aromatic hydrocarbons including crude oil and nitro-aromatic compounds are also successfully degraded by a wide range of *Pseudomonas* sp. and *Raulstonia* sp. All these specialized bacteria are encoded with a catabolic plasmid which encoded the genes for hydrocarbon degradation.

Microbes are also capable of decolorization and deodorization of highly polluted river water. There are various potential bacteria and fungi that have been isolated, characterized, and effectively used for bioremediation. The treated water can be used for agriculture, industry, and aquarium and for other household purpose (Kumar and Gopal 2015). Thus, till date bioremediation is the most reliable and eco-friendly technique for the treatment of hazardous waste effluents.

4   Microbes Causing Bioremediation

These include a diverse array of microbes isolated from various environmental habitats. Some of them are identified as active members of consortium including *Acinethobacter, Actinobacter, Acaligenes, Arthrobacter, Bacillins, Berijerinckia, Flavobacterium, Methylosinus, Mycrobacterium, Mycococcus, Nitrosomonas, Nocardia, Penicillium, Phanerochaete, Pseudomonas, Rhizoctomia, Serratio, Trametes*, and *Xanthofacter*. Each of these individual microbes is not capable of complete biodegradation but these works synergistically for the complete mineralization under both aerobic and anaerobic conditions (Singh et al. 2014).

4.1   Use of Microbes in Cleaning Products

Chemical-based cleaning products are commonly used throughout the world in both industrial and domestic use. Some of the common examples of the synthetic cleaning solutions are sodium hypochlorite, sodium hydroxide, and ammonium hydroxide. Since they are highly reactive, they are not environmental friendly and can affect human health. Some studies have reported that mixing of these products liberates toxic chlorine and ammonia gas that cause acute poisoning and illness (Nazaroff and Weschler 2004).

To overcome the environmental health hazard, microbial-based cleaning products are recently developed and used in many developed countries like the UK, the USA, etc. These products contain various strains of microorganisms as active ingredients in place of synthetic chemicals. These products would be in great demand in the near future and the global market may reach up to USD 9.32 billion dollars by the year 2017 PR Web (2011).

Both the vegetative cells and the spores are used in the cleaning products and treatment applications. The most common microbial species used are *Bacillus* sp.

like *B. subtilis, B. licheniformis*, and *B. amyloliquefaciens. B. polymyxa* strains have also been used as production organisms for topical antibiotics (Adisesh et al. 2011; Gelmetti 2008).

The other bacterial genera used in cleaning agents are *Achromobacter*, *Actinobacter*, *Alcaligenes*, *Arthrobacter*, *Rhodopseudomonas*, *Rhodobacter*, and *Lactobacillus* sp. (Wassenaar and Klein 2008). Some of these bacteria degrade various xenobiotic compounds while others degrade textile azo dyes (Xingzu et al. 2008).

Some of the cleaning products also use fungal species like *Saccharomyces* and *Candida* species. They have the potential to be effectively used in the biodegradation of variety of hazardous chemicals (Xiuyan et al. 2011; Harms et al. 2011).

Besides, microbial metabolites such as enzymes like amylases, proteases, and lipases are also used in detergent products. This is done to improve their activity at lower water temperature and more alkaline pH levels (Kirk et al. 2002). For example, *B. subtilis* strains have been engineered to express some of these modified genes and a number of recombinant lipase enzymes have been produced using engineered-*Bacillus* and *Aspergillus* species (Hasan et al. 2010).

5 Mechanism of Microbial Mineralization: Biodegradation

During biodegradation, the breakdown and transformation of insoluble organic toxic constituents into the less toxic soluble inorganic compounds takes place with the help of microbial enzymes. Bioremediation is a step-wise process where the intermediate compounds are converted into carbon-di-oxide, water, and other inorganic compounds. The biodegradation takes place under both aerobic and anaerobic conditions. Under aerobic conditions, oxygen acts as a terminal electron acceptor whereas under anaerobic metabolism, nitrate, sulfate, and bicarbonate acts as a terminal electron acceptor.

There are several factors that affect the rate of biodegradation such as soil moisture, oxygen availability, soil pH, availability of nutrients, contaminant concentration, and the presence of suitable microbes. The optimum conditions of these factors enhance the rate of biodegradation by microbes. Oxygen plays a critical role in the bio-degradative process. Research studies have shown that aerobic indigenous microorganisms play a key role in degradation of petroleum oils (Cai et al. 2013).

5.1 Xenobiotic Compounds: Aerobic Biodegradation Pathway

In the bacterial respiration, oxygen is the most common electron acceptor. In aerobic biodegradation of aromatic compounds, oxygen plays an important dual role: firstly, it acts as an electron acceptor for the aromatic pollutants, and secondly it activates the substrate with the help of oxygenation reactions. Some polluted

environments are often noxious such as aquifers, aquatic sediments, and submerged soils and require alternative electron acceptors such as nitrate, Fe(III), and sulfate (Cao et al. 2009).

Some of the common xenobiotic compounds include petroleum hydrocarbons, chlorinated aliphatics, benzene, toluene, phenol, naphthalene, fluorine, pyrene, chloroanilines, pentachlorophenol, and dichlorobenzenes. All these compounds are rapidly and potentially degraded by the aerobic degradation process and finally release carbon-di-oxide, water, residues along with some biomass. Many bacterial consortia capable of growing on these chemicals degrade toxic compounds to nontoxic compounds (Shimao 2001).

Alkanes consisting of long carbon chain and straight structures are more prone to aerobic biodegradation. Aerobic degradation pathway of alkane degradation is basically the oxidation of the terminal methyl group into a carboxylic acid through an alcohol intermediate, and finally completes mineralization through β-oxidation pathway (Zhang and Bennett 2005).

The aerobic degradation process of aromatic compound involves their oxidation by molecular oxygen, and then it enters into Krebs cycle and β-oxidation (Wilson and Bouwer 1997). During aerobic respiration, microorganisms use oxygen to hydroxylate the benzene ring, resulting in the subsequent fission of the ring. The enzymes involved in these processes are mono- and di-oxygenase that incorporate one or two atoms of oxygen, respectively, into the ring.

5.2 Anaerobic Biodegradation Pathway

The anaerobic biodegradation pathway is followed by the microorganisms when the pollutants are highly recalcitrant (increase in halogenation) and cannot be mineralized by aerobic pathways. Under anaerobic conditions, the biodegradation of xenobiotic compound produces carbon-di-oxide, methane, water, residues, and biomass (Jayasekara et al. 2005). Some examples of recalcitrant molecules include polychlorinated biphenyls (PCBs), chlorinated dioxins, and some pesticides like DDT.

Anaerobic bacteria perform reductive dehalogenation either by a gratuitous reaction or by a new type of anaerobic respiration. This process reduces the degree of chlorination and makes the product more accessible for mineralization by aerobic bacteria (Van Agteren et al. 1998).

During anaerobic degradation, reductive dehalogenation is the first step of degradation of PCBs (Poly chlorinated biphenyl); dehalogenation is done under anaerobic conditions where organic substrates act as electron donors. PCBs accept electrons to allow the anaerobic bacteria to transfer electrons to these compounds. Anaerobic bacteria are capable of degrading xenobiotics that are present in various anaerobic habitats.

The major groups of anaerobic bacteria responsible for degrading xenobiotic compounds include *Acidovorax, Bordetella, Pseudomonas, Sphingomonas, Variovorax, Veillonella alkalescens, Desulfovibrio* spp., *Desulfuromonas michi-*

ganensis, Desulfitobacterium halogenans, D. oleovorans, G. metallireducens, and *D. acetonicum.* Anaerobic sulfate-reducing and methanogenic condition can be applied to isolate pure culture of anaerobic bacteria (Zhang and Bennett 2005). Anaerobes can also utilize substituted and complex aromatic compounds in the way that do not perturb the benzene nucleus itself. The list of selected xenobiotic compounds and their degrading bacterial species are presented in Table 2.1.

Table 2.1 List of xenobiotic compounds and their degrading bacterial species (Agrawal and Shahi 2015)

Target compounds	Bacteria degrading the compounds
PAH (polycyclic aromatic hydrocarbons) compounds	
Naphthalene	*Streptomyces* spp. isolates AB1, AH4, and AM2, strain QWE-35
	Pseudomonas sp. CZ2 and CZ5
	Pseudomonas stutzeri strain B1SMN1
	Achromobacter sp.
	Enterobacter sp.
	Geobacillus sp. SH-1
	Rhodococcus
	Pseudomonas putida S2
	Bacillus fusiformis (BFN)
	Paenibacillus
	Novosphingobium naphthalenivorans sp.
	Polaromonas naphthalenivorans sp. nov.Strain CJ2
	Bacillus naphthovorans strain MN-003
	Staphylococcus sp. strain MN-005 and *Micrococcus* sp.
	Neptunomonas naphthovorans
Phenanthrene	*Pseudomonas* sp. Ph6
	Massilia sp. Strain Pn2
	Sphingobium sp. Strain PNB
	Sphingomonadaceae PHPY *and Rhodobacteraceae* SK
	Mycobacterium sp.
	Brevibacillus sp. PDM-3
	Vibrio parahaemolyticus
Anthracene	*Microbacterium* sp. *strain SL10*
	Martelella sp. *AD3*
	Ochrobactrum sp. VA1
	Rhodococcus opacus 412
	Ps. aeruginosa and Ps. citronellolis
PCP (pentachlorophenol)	*Kocuria* sp. CL2
	Comamonas testosteroni CCM 7530
	Sphingobium sp. UG30
	Bacillus cereus (DQ002384), *Serratia marcescens strain*
	Sphingomonas chlorophenolica

Table 2.1 (continued)

Target compounds	Bacteria degrading the compounds
Chloroaniline	*Acinetobacter baylyi*
	Delftia tsuruhatensis H1
	Acinetobacter baumannii CA2
	Pseudomonas putida CA16
	Klebsiella sp. CA17
Phthalate	*Achromobacter denitrificans*
	Arthrobacter sp. C21
	Agrobacterium sp. JDC-49
	Ochrobactrum sp. JDC-41
	Enterobacter sp. T5
	Rhodococcus sp. L4
Pesticides	
Endosulfan compounds	*Paenibacillus* sp. *ISTP10*
	Achromobacter xylosoxidans strain C8B
	Stenotrophomonas maltophilia and Rhodococcus erythropolis
	Klebsiella oxytoca KE-8
	Klebsiella pneumonia
2,4-D (2,4-dichlorophenoxyacetic acid)	*Maribacter* sp.
	Delftia sp.
	Pseudomonas putida
	Comamonas koreensis
DDT (Dichlorodiphenyltrichloroethane)	*Pseudoxanthobacter liyangensis* sp. nov
	Novosphingobium arabidopsis sp. nov.
	Alcaligenes sp.
	Serratia marcescens DT-1P
Halogenated organic compounds	
Vinyl chloride	*Micrococcus species*
	Sphingopyxis sp.
	Pseudomonas aeruginosa
Herbicides	
Atrazine	*Raoultella planticola*
	Bacillus subtilis
	Rhodococcus sp.
	Arthrobacter sp.
	Nocardioides sp.
Propanil	*Xanthomonas* sp., *Acinetobacter calcoaceticus*
	Rhodococcus sp., and *Pseudomonas* sp.
Petroleum products	*Acinetobacter* sp.
	Pseudomonas, Achromobacter, Bacillus, and *Micromonospora*
	Dietzia strain
	Flavobacterium sp. *Acinetobacterium calcoaceticum*, and *Pseudomonas aeruginosa*

Table 2.1 (continued)

Target compounds	Bacteria degrading the compounds
Azo dyes	*Morganella* sp.
	Sphingomonas sp.
	Proteus hauseri
	Staphylococcus arlettae

The sulfate reducing bacteria play a major role in degrading crude oil (Barton and Hamilton 2007). These groups of bacteria are obligate anaerobes that utilize sulfate as final electron acceptor during anaerobic respiration and generate hydrogen sulfide from the reduction of sulfate (Sahrani et al. 2008).

The process of conversion of biodegradable materials to gases like carbon dioxide, methane, and nitrogen compounds is called mineralization. Mineralization process is completed, when all the biodegradable biomass is consumed and all the carbon is converted into carbon dioxide (Kyrikou and Briassoulis 2007).

Rhodococcus RHA1 and *Arthrobacter keyseri* 12B bacteria play a major role in the degradation of 3,4-dihydroxybenzoate (Hara et al. 2007). For the degradation of chlorinated compounds, bacteria take several paths simultaneously for the removal of five chlorine atoms leading to the formation of phenol and finally mineralization to methane and carbon-di-oxide. Biogas usually methane is generated from anaerobic digestion (Holm-Nielsen et al. 2009).

6 Mechanisms of Bioremediation

There are various mechanisms of bioremediation like biosorption, metal–microbe interactions, bioaccumulation, bio-mineralization, biotransformation, and bioleaching. Microorganisms remove the heavy metals from soil by using chemicals for their growth and development. They are capable of dissolving metals and reducing or oxidizing transition metals. The different methods used by microbes to restore environment are oxidation, binding, immobilization, volatilization, and transformation of heavy metals. The microbes protect themselves from the toxic chemical pollutants through developing self-defense mechanisms including formation of outer cell-membrane protective material including hydrophobic or solvent efflux pumps. For instance, plasmid-encoded and energy-dependent metal efflux systems involving ATPases and chemiosmotic ion/proton pumps are reported for As, Cr, and Cd resistance in many bacteria (Roane and Pepper 2000).

6.1 Bioremediation Through Adsorption

Microorganisms can remove the heavy metals by the mechanism of bio-adsorption. The microbes have unique binding sites at their cellular structure without the use of energy. Amongst the various reactive compounds associated with bacterial cell

walls, the extracellular polymeric substances have significant effects on acid-base properties and metal adsorption (Guiné et al. 2006). Studies on the metal-binding behavior of extracellular polymeric substances (EPS) revealed a great ability to complex heavy metals through various mechanisms, which include proton exchange and micro-precipitation of metals (Comte et al. 2008; Fang et al. 2010).

6.2   Bioremediation Through Physio-Bio-Chemical Mechanism

Biosorption is a process of involving higher affinity of a biosorbent towards any sorbate usually metal ions, and this process is continued till equilibrium is established between the two phases or components (Das et al. 2008). There are some microbes like *Saccharomyces cerevisiae* that acts as a biosorbent for the removal of Zn(II) and Cd(II) through the ion exchange mechanism (Talos et al. 2009). *Cunninghamella elegans* emerged as a promising sorbent against heavy metals released by textile wastewater (Tigini et al. 2010). Heavy metal degradation is an energy expenditure process.

Some fungi such as *Klebsiella oxytoca*, *Allescheriella* sp., *Stachybotrys* sp., *Phlebia* sp. *Pleurotus pulmonarius*, and *Botryosphaeria rhodina* have metal-binding potential (D'Annibale et al. 2007). Pb(II) contaminated soils can be biodegraded by fungal species like *Aspergillus parasitica* and *Cephalosporium aphidicola* with biosorption process (Akar et al. 2007). In a study, mercury-resistant fungi (*Hymenoscyphus ericae, Neocosmospora vasinfecta*, and *Verticillum terrestre*) were able to bio-transform a Hg(II) state to a nontoxic state (Kelly et al. 2006).

Many of the contaminants are hydrophobic and are absorbed by the microbes through the secretion of some biosurfactant and direct cell-contaminant association. Biosurfactants form stronger ionic bonds with metals and form complexes before being desorbed from soil matrix to water phase due to low interfacial tension (Thavasi 2011).

Microbes mobilize the heavy metals from the contaminated sites by leaching, chelation, methylation, and redox transformation of toxic metals. Heavy metals can never be destroyed completely, but the microbial process transforms their oxidation state or organic complex, so that they become water-soluble, less toxic, and precipitated (Garbisu and Alkorta 2001). Microorganisms use heavy metals and trace elements as terminal electron acceptors and reduce them through the detoxification mechanism. Microorganisms remove heavy metals through the mechanisms which they employ to derive energy from metals redox reactions to deal with toxic metal through enzymatic and non-enzymatic processes.

Microorganisms adopt different defense systems like exclusion, compartmentalization, complex formation, and synthesis of binding protein and peptides to reduce the stress developed by toxic metals (Gómez Jiménez-T et al. 2011). Heavy metal accumulation by microorganisms can be studied by the expression of metal-bind-

ing protein and peptides (phytochelatins and metallothionein) (Cobbett and Goldsbrough 2002).

Synechococcus sp. (cynobacterial strains) has been reported with the expression of the smtA gene and production of metal-binding protein. *Ralstonia eutropha* has been genetically modified to express mouse metallothionein on the cell surface and decrease the toxic effect of the Cd(II) in the contaminated sites. Expression of different proteins and peptides by the *Escherichia coli* regulates the range of accumulation of cadmium (Mejare and Bulow 2001).

6.3   Molecular Mechanisms in Bioremediation

There are various mechanisms involved in the removal of heavy metals by microorganisms. A genetically engineered bacterium *Deinococcus geothermalis* reduces mercury at high temperatures due to the expression of mer operon from *E. coli* coded for Hg^{2+} reduction (Brim et al. 2003). Another mercury-resistant bacteria *Cupriavidus metallidurans* strain MSR33 was genetically modified by introducing a pTP6 plasmid that provided genes (merB and merG) regulating mercury biodegradation along with the synthesis of organomercurial lyase protein (MerB) and mercuric reductase (MerA) (Rojas et al. 2011)). Modification of *Pseudomonas* strain with the pMR68 plasmid with novel genes (*mer*) made that strain resistant to mercury (Sone et al. 2013). Two different mechanisms for mercury degradation by bacteria (*Klebsiella pneumonia* M426) are mercury volatilization by reduction of Hg(II) to Hg(0) and mercury precipitation as insoluble Hg due to volatile thiol (H_2S) (Essa et al. 2002). Genetic engineering of *Deinococcus radiodurans* which naturally reduces Cr(IV) to Cr(III) has been done for complete toluene (fuel hydrocarbon) degradation by cloned genes of *tod* and *xyl* operons of *Pseudomonas putida* (Brim et al. 2006). Microbial metabolites like metal bound coenzymes and siderophores are mainly involved in the degradation pathway (Penny et al. 2010).

7   Designer Microbes: Biotechnological Intervention in Bioremediation

Genetic engineering offers an advantage of constructing microbial strains which can withstand adverse stressful situations and can be used as bioremediators under various complex environmental conditions. A recent development in this field is "microbial biosensors" that measures the degree of contamination in contaminated sites quickly and accurately.

A list of selected genetically engineered bacteria for removal of heavy metals is presented in Table 2.2.

Table 2.2 Selected genetically engineered bacteria for remediation of heavy metals (Divya and Kumar 2011)

GEB	Heavy metal	Removal efficiency (%)
E. coli strain	As	100
E. coli JM109	Hg	96
Methylococcus capsulatus	Cr^{6+}	100
P. fluorescens 4F39	Ni	80

GEB genetically engineered bacteria, *As* arsenic, *Hg* mercury, *Cr* chromium, *Ni* nickel

Till date various biosensors have been designed to evaluate heavy metal concentrations like mercury (Hg), cadmium (Cd), nickel (Ni), copper (Cu), and arsenic (As) (Verma and Singh 2005; Bruschi and Goulhen 2006). Besides, genetic engineering of endophytes and rhizospheric bacteria for plant-associated degradation of pollutants in soil is also considered to be one of the most promising new technologies for remediation of metal contaminated sites (Divya and Kumar 2011). Bacteria like *Escherichia coli* and *Moreaxella* sp. expressing phytochelatin 20 on the cell surface have been shown to accumulate 25 times more Cd or Hg than the wild-type strains (Bae et al. 2003). However, one major obstacle for utilizing these GEMs in hostile field conditions is sustaining the recombinant bacteria population in soil, with various environmental conditions and competition from native bacterial populations (Wu et al. 2006).

Further, the molecular approaches have been applied to only limited bacterial strains like *Escherichia coli*, *Pseudomonas putida*, *Bacillus subtilis*, etc. This means other microorganisms need to be explored for their application in heavy metal bioremediation through molecular intervention.

8   Nano-bioremediation: Recent Approach

The nanoparticles that enhance microbial activity to remove toxic pollutants are called "nano-bioremediation." Another concept of "bio-nanotechnology" is defined as the bio-fabrication of nano-objects or bifunctional macromolecules that are used as tools to construct or manipulate nano-objects.

The major advantage of using nano-based technologies is that they reduce both the costs of cleaning up contaminated sites at a large scale and the process time. Wide physiological diversity, small size, genetic manipulability, and controlled cultivability make microbial cells ideal producers of nanostructures. They may range from natural products, such as polymers and magnetosomes, to engineered proteins or protein constructs, such as virus-like proteins (VLP) and tailored metal particles (Sarikaya et al. 2003).

Deinococcus radiodurans, a radioactive-resistant organism, has the ability to withstand radiation well beyond the naturally occurring levels, thus it is used in radioactive waste cleanup initiatives in the USA (Brim et al. 2000).

Nanoscale modified biopolymers have now replaced the metal-chelating polymers that require toxic solvents for their synthesis and ultrafiltration for their separation.

Here, the polymers can be recovered by changing the environmental surrounding like pH, temperature, etc. These are manufactured by genetic and protein engineering of microorganisms, and their size can be controlled at the molecular level (Vishwanathan 2009). This innovative technique would be a promising tool to address the escalating problem of heavy metal as well as organic contaminants in the environment (Dixit et al. 2015).

9   Conclusions

There is a lot of research going on for combating the pollution throughout the globe. Microbes represent an effective tool for bioremediation and for clean-up environment. They can degrade almost all types of pollutants, heavy metals, industrial effluents, xenobiotics, and other recalcitrant molecules of complex nature. There are numerous biotechnological tools like genetic engineering, nano-bioremediation, bio-nanotechnology, and nanoscale modified biopolymers that can be used to further improve the efficacy of microbial cells in cleaning up the waste from the environment. Construction of strains with broad spectrum of catabolic potential with heavy metal-resistant traits makes them ideal for bioremediation of polluted environments for both aquatic and terrestrial ecosystems. The transfer of genetic traits from one organism to another paves way in creating Genetically Engineered Microorganisms (GEMs) for combating pollution in extreme environments making it a boon to mankind by cleaning up the mess that has created in nature.

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