Chapter 1

Bioremediation of Polluted Waters Using Microorganisms

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Abstract

Water pollution is an issue of great concern worldwide, and it can be broadly divided into three main categories, that is, contamination by organic compounds, inorganic compounds (e.g., heavy metals), and microorganisms. In recent years, the number of research studies concerning the use of efficient processes to clean up and minimize the pollution of water bodies has been increasing. In this context, the use of bioremediation processes for the removal of toxic metals from aqueous solutions is gaining considerable attention. Bioremediation can be defined as the ability of certain biomolecules or types of biomass to bind and concentrate selected ions or other molecules present in aqueous solutions. Bioremediation using microorganisms shows great potential for future development due to its environmental compatibility and possible cost-effectiveness. A wide range of microorganisms, including bacteria, fungi, yeasts, and algae, can act as biologically active methylators, which are able to at least modify toxic species. Many microbial detoxification processes involve the efflux or exclusion of metal ions from the cell, which in some cases can result in high local concentrations of metals at the cell surface, where they can react with biogenic ligands and precipitate. Although microorganisms cannot destroy metals, they can alter their chemical properties via a surprising array of mechanisms. The main purpose of this chapter is to provide an update on the recent literature concerning the strategies available for the remediation of metal-contaminated water bodies using microorganisms and to critically discuss their main advantages and weaknesses. The focus is on the heavy metals associated with environmental contamination, for instance, lead (Pb), cadmium (Cd), and chromium (Cr), which are potentially hazardous to ecosystems. The types of microorganisms that are used in bioremediation processes due to their natural capacity to biosorb toxic heavy metal ions are discussed in detail. This chapter summarizes existing knowledge on various aspects of the fundamentals and applications of bioremediation and critically reviews the obstacles to its commercial success and future perspectives.

Keywords: Metals, microorganisms, bioremediation, polluted water

1. Introduction

Environmental contamination by heavy metals from anthropogenic and industrial activities has caused considerable irreparable damage to aquatic ecosystems. Sources include the mining and smelting of ores, effluent from storage batteries and automobile exhaust, and the manufacturing and inadequate use of fertilizers, pesticides, and many others. The metals and metalloids that contaminate waters and are most commonly found in the environment include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, silver, gold, and nickel. These metals are the subject of concern due to their high toxicity. Apart from being hazardous to human health, they also have an adverse effect on the fauna and flora, and they are not biodegradable in nature. Thus, there is a need to seek new approaches in developing treatments to minimize or even eliminate metals present in the environment.

Several different physicochemical and biological processes are commonly employed to remove heavy metals from industrial wastewaters before their discharge into the environment [1]. Conventional physicochemical methods such as electrochemical treatment, ion exchange, precipitation, osmosis, evaporation, and sorption are not cost-effective, and some of them are not environmentally friendly [2, 3]. On the other hand, bioremediation processes show promising results for the removal of metals, even when present in very low concentrations where physicochemical removal methods fail to operate. Furthermore, this is an eco-compatible and economically feasible option. The bioremediation strategy is based on the high metal binding capacity of biological agents, which can remove heavy metals from contaminated sites with high efficiency. In this regard, microorganisms can be considered as a biological tool for metal removal because they can be used to concentrate, remove, and recover heavy metals from contaminated aquatic environments [4]. Several studies have been conducted using microorganisms for the uptake of heavy metals in polluted waters as an alternative strategy to conventional treatments [5–7]. Bioremediation by microorganisms is very useful due to the action of microorganisms on pollutants even when they are present in very dilute solutions, and they can also adapt to extreme conditions. Although the mechanisms associated with metal biosorption by microorganisms are still not well understood, studies show that they play an important role in the uptake of metals and that this action involves accumulation or resistance.

In this chapter, previously published research data on the potential of the microorganisms that have been used for the bioremediation of metals is discussed. In-depth descriptive information on the bioremediation process uses various microorganisms, including algae and bacteria, and the mechanisms of action, bioremediation efficiency, and current applications are provided together with suggestions to overcome the limitations associated with their large-scale and more efficient application. Future prospects for the potential use of genetic engineering techniques to develop prominent recombinant novel microorganism variants that are more efficient and improvements in the operation conditions of bioremediation technologies will also be discussed and explored.

2. Status of heavy metal pollution

The term "heavy metal" generally refers to metallic elements with an atomic weight higher than that of Fe (55.8 g mol^{-1}) or density greater than 5.0 g cm^{-3}, and these metals are naturally present in the environment. However, some metals with an atomic weight lower than that of Fe, for example, Cr, and others which are considering metalloids, such as As and Se, are also commonly referred to as heavy metals [8]. Heavy metals can play a role as micronutrients, such as Cu, Fe, Mn, Mo, Zn, and Ni, but they can also be toxic to humans, e.g., Hg, Pb, Cd, Cu, Ni, and Co [9], depending on the exposure levels.

Contamination by heavy metals causes many deleterious effects, which affect not only fauna and flora but also human health [10, 11]. Heavy metal ions have a strong electrostatic attraction and high binding affinities with the same sites that essential metal ions normally bind to in various cellular structures, causing destabilization of the structures and biomolecules (cell wall enzymes, DNA and RNA), thus inducing replication defects and consequent mutagenesis, hereditary genetic disorders, and cancer [12]. Heavy metals are notable contaminants because they are toxic, nonbiodegradable in the environment, and easily accumulated in living organisms [13]. The contamination of waters with heavy metals occurs through natural and anthropogenic activities, mainly related to industrialization. Table 1 shows the natural and anthropogenic sources of some of the most widespread study heavy metals as environmental pollutants, together with a brief list of their adverse health effects and their applications [14]. Although studies on bioremediation generally consider the total amount of metal present in the environment, the toxicity of these metals is dependent on their chemical form. The wide range of chemical forms in which heavy metals can be present in the environment includes cationic/anionic species and complexes (hydroxylated or complexed to Cl), and their oxidation state varies depending on the medium pH and composition.

Heavy metals contaminated in soil can accumulate and persist for long periods of time and may be harmful to vital processes involved in microbial nutrient cycling [15]. The toxicity and mobility of heavy metals are strongly dependent on their chemical form and specific binding properties. Changes in the environmental conditions in soils, such as acidification and variations in the redox potential, can cause the mobilization of heavy metals from the solid phase to the liquid phase, thereby allowing the potential contamination to the plants grown in these soils [16]. In water bodies, a heavy metal in relatively high concentrations affects the biota due to its toxicity, or it can be bioaccumulated, which increases its effect further along

Element	Contamination sources		Uses	Adverse health effects
	Natural	Anthropogenic		
Cd	Zn and Pb minerals, phosphate rocks	Mining waste, electroplating, battery plants	Automobile exhaust	Respiratory, cardiovascular, renal effects
Cr	Chromite mineral	Electroplating, metal alloys, industrial sewage, anticorrosive products	Pesticides, detergents	Mental disturbance, cancer, ulcer, hypokerotosis
Cu	Sulfides, oxides carbonates	Electroplating, metal alloys, domestic and industrial waste, mining waste, pesticides	Most uses are based on electrical conductor properties	Anemia and other toxicity effects induced indirectly through interaction with other nutrients
Pb	Galena mineral	Battery plants, pipelines, coal, gasoline, pigments	Batteries, alloys	Neurotoxic
Ni	Soils	Metal alloys, battery plants, industrial waste, production of vegetable oils	Batteries, electronics, catalysts	Skin allergies, lung fibrosis, diseases of the cardiovascular system
Zn	Minerals (sulfides, oxides, silicates)	Metal alloys, pigments, electroplating, industrial waste, pipelines	Fertilizers, plastics, pigments	Abdominal pain, nausea, vomiting and diarrhea, gastric irritation, headache, irritability, lethargy, anemia

Table 1. Contamination sources, uses, and adverse health effects of some heavy metals

the food chain. The progressive increase in the concentration of a contaminant such as a metal, as it advances in the food chain, is known as biomagnification. This occurs due to the need for a large number of organisms from lower trophic levels to feed a member of a higher trophic level and thus contaminants that cannot be metabolized but are fat soluble can accumulate in the fatty tissues of living organisms.

Various studies have been conducted to minimize or eliminate the heavy metals present in the environment. Conventional processes include precipitation, reverse osmosis, adsorption onto activated carbon or alumina, and redox processes [17]. However, these technologies are considered to be inefficient because of expensive cost [12]. In bioremediation by microorganisms typically employing one type of organism or a consortium of microorganisms, high toxic chemicals are converted into less toxic chemicals by biological means [18]. The technology makes use of the metabolic potential of microorganisms to clean up contaminated environments [19] and has been proposed as an attractive alternative owing to its lower cost and higher efficiency [20] compared with other physicochemical methodologies [12]. Microorganisms can decompose or transform hazardous substances into less toxic metabolites or degrade them to nontoxic end products. Microorganisms can also survive in contaminated habitats because they are metabolically able to exploit contaminants as potential energy sources [11].

In bioremediation, microorganisms with biological activity, including algae, bacteria, fungi, and yeast, can be used in their naturally occurring forms.

The number of publications on the use of microorganisms for the removal of heavy metals in contaminated environments has been increasing steadily over the past 10 years. Figure 1 shows the main types of microorganisms used in these processes, based on a search for papers reporting microorganisms and bioremediation studies, indexed in the ISI Web of Science for the period of 2004 to 2014. It can be observed in Figure 1 that the microorganisms that have been most commonly used are bacteria and fungi, although yeast and algae are also frequently applied.

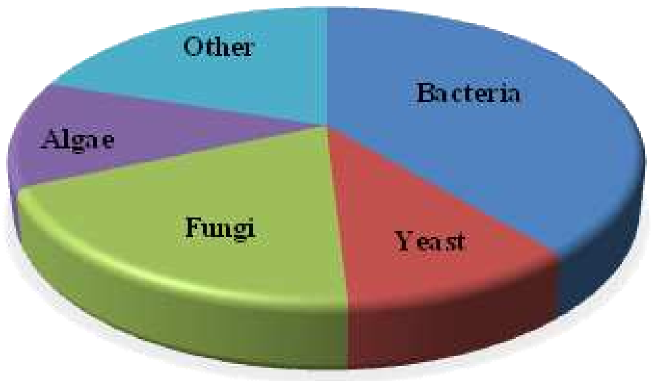

Figure 1. Types of microorganisms used in bioremediation processes.

Figure 2 gives some indication of which metals are used in bioremediation processes employing microorganisms, and chromium, copper, cadmium, and lead together account for 70% of applications, although nickel and zinc are also used. Other metals that are used to a lesser extent include arsenic and mercury.

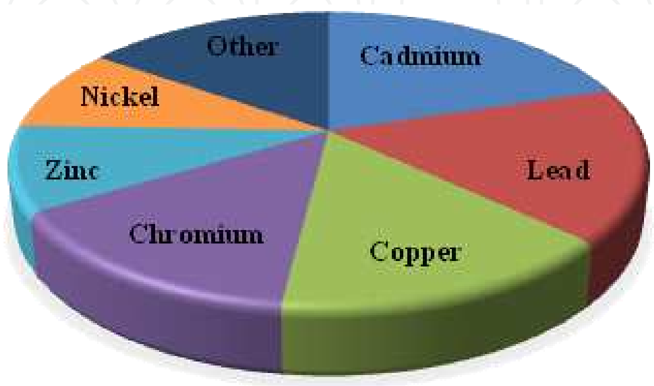

Figure 2. Metals used in bioremediation process employing microorganisms.

3. Types of organisms used in bioremediation

Typically, bioremediation is based on the cometabolism action of one organism or a consortium of microorganisms [18]. In this process, the transformation of contaminants presents a little efficiency or no benefit to the cell, and therefore this process is described as nonbeneficial biotransformation [21, 22]. Several studies have shown that many organisms (prokaryotes and eukaryotes) have a natural capacity to biosorb toxic heavy metal ions [23]. Examples of microorganisms studied and strategically used in bioremediation treatments for heavy metals include the following: (1) bacteria: *Arthrobacter* spp. [24], *Pseudomonas veronii* [25], *Burkholderia* spp. [26], *Kocuria flava* [27], *Bacillus cereus* [28], and *Sporosarcina ginsengisoli* [29]; (2) fungi: *Penicillium canescens* [30], *Aspergillus versicolor* [31], and *Aspergillus fumigatus* [32]; (3) algae: *Cladophora fascicularis* [33], *Spirogyra* spp. and *Cladophora* spp. [34], and *Spirogyra* spp. and *Spirullina* spp. [35]; and (4) yeast: *Saccharomyces cerevisiae* [36] and *Candida utilis* [37].

Prokaryotes (bacteria and archaeans) are distinguished from eukaryotes (protists, plants, fungi, and animals). The cellular structure of eukaryotes is characterized by the presence of a nucleus and other membrane-enclosed organelles. Also, the ribosomes in prokaryotes are smaller (70S) than in eukaryotes (80S) [38]. The way in which microorganisms interact with heavy metal ions is partially dependent on whether they are eukaryotes or prokaryotes, wherein eukaryotes are more sensitive to metal toxicity than prokaryotes [12]. The possible modes of interaction are (a) active extrusion of metal, (b) intracellular chelation (in eukaryotes) by various metal-binding peptides, and (c) transformation into other chemical species with reduced toxicity. For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products [39]. Bacteria and higher organisms have developed mechanisms associated with resistance to toxic metals and rendering them innocuous [20]. Several microbes, including aerobes, anaerobes, and fungi, are involved in the enzymatic degradation process. Most of bioremediation systems are run under aerobic conditions, but anaerobic conditions make it possible microbial organisms to degrade otherwise recalcitrant molecules [39].

Because several different types of pollutants can be present at a contaminated site, various types of microorganisms are required for effective remediation. Some types of microorganism are able to degrade petroleum hydrocarbons and use them as a source of carbon and energy. However, the choice of the organisms employed is variable, depending on the chemical nature of the polluting agents, and needs to be selected carefully as they only survive in the presence of a limited range of chemical contaminants. The efficiency of the degradation process is related to the potential of the particular microorganism to introduce molecular oxygen into the hydrocarbon and to generate the intermediates that subsequently enter the general energy-yielding metabolic pathway of the cell. Some bacteria search the contaminant and move toward it because they flexibly exhibit the potential as a chemotactic response [40].

Numerous microorganisms can utilize oil as a source of food, and many of them produce potent surface-active compounds that can emulsify oil in water and facilitate its removal [21]. Bacteria that can degrade petroleum products include species of *Pseudomonas*, *Aeromonas*, *Moraxella*, *Beijerinckia*, *Flavobacteria*, *Chrobacteria*, *Nocardia*, *Corynebacteria*, *Modococci*, *Strepto-*

myces, *Bacilli*, *Arthrobacter*, *Aeromonas*, and cyanobacteria [40] and some yeasts [21]. For example, *Pseudomonas putida* MHF 7109 can be isolated from cow dung microbial consortia for the biodegradation of selected petroleum hydrocarbon compounds, such as benzene, toluene, and o-xylene (BTX) [23].

The application of biotechnology to the treatment of heavy metals is a relatively new subject. A better understanding of the processes through which microorganisms capture heavy metals, particularly the metabolism and detoxification pathways, has been accumulated. It can help the solution with maximum efficiency in dealing with environmental problems associated with heavy metal contamination [41]. The changes arising from the biotechnological approach include bioleaching, bioextraction, biosorption, bioencapsulation, and bioremediation [42]. In this regard, genetic engineering is a fundamental approach to modulate the metabolic pathways of these microorganisms and to inhibit the toxic the action of the metals by the modulated activity. The modified microorganisms can change the inorganic form into the organic form by some reactions, for instance, by transforming the metals through oxidation–reduction reductions, thus increasing the solubility.

Besides the increase of the solubility by microorganisms modifying microorganisms to increase their resistance through factors involving the solubility of heavy metals, their interaction with other factors (e.g., complexation reactions, changes in pH, sorption, precipitation, bioaccumulation, and encapsulation) can result in increased solubility or render the heavy metals inert in the environment [18]. Genetic engineering can be applied to modify the microorganisms and achieve interesting features such as accelerated growth, tolerance to extreme environmental conditions and pH variations, and low cost cultivation. Recent studies have demonstrated the ability of certain fungi (e.g., *Aspergillus* and *Penicillium*) and some yeasts (e.g., *S. cerevisiae*) to remove heavy metals from certain environments. The species *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces boulardii*, *Enterococcus faecium*, and *Staphylococcus aureus* have also been used for the removal of heavy metals from water bodies [43]. The process of metal accumulation on the cell surface is dependent on the metabolic activity of the microorganism as well as the characteristic of cell surface, and it is known as bioaccumulation [44]. It has been noted that metal ions interact with the proteins necessary for the proper functioning of the cell structure, affecting its metabolic functions. Genetic engineering, which allows the improvement of the metabolic structure of microorganisms, enables the high accumulation of metals or reduces the toxicity of metals, thereby promoting the decontamination of water bodies.

Many papers on bioremediation with wild or genetic modified microorganisms have been published over the years. Figure 3 shows the data obtained from a search of the web covering a period of 20 years (1995–2014), which deal with the development of methodologies for the decontamination of environments containing various heavy metals.

With the recent advances in genetic engineering, it is now relatively easy to construct genetically engineered microorganisms (GEMs) through reshuffling the genes, promoters, etc., and this can enhance their performance *in situ*. Several GEMs have been successfully constructed and experimentally tested for efficient bioremediation under laboratory conditions [45]. Recombinant DNA techniques can be used to enhance the ability of an organism to metabolize a xenobiotic through the detection of genes associated with degradation, transforming them

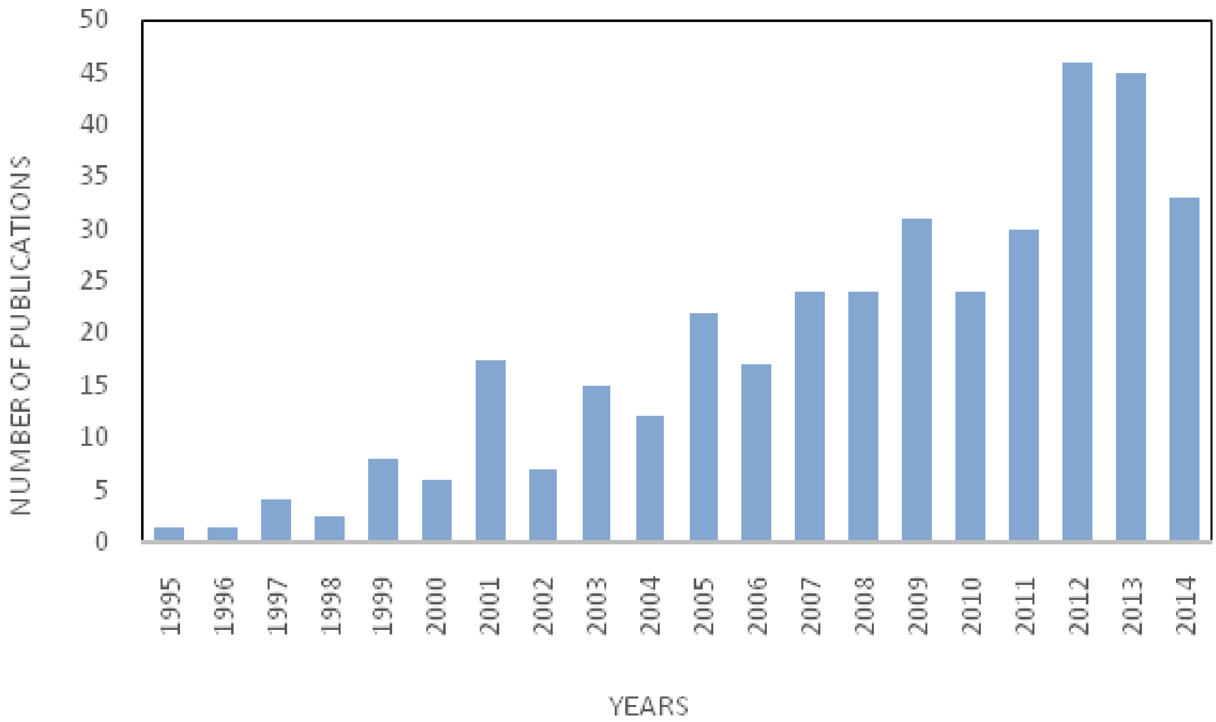

Figure 3. Scientific publications on bioremediation using microorganisms.

into appropriate bioremediation agents. Recombinant DNA technology explores the use of different approaches including PCR, antisense RNA technique, and site-directed mutagenesis.

Engineered strains of *Deinococcus geothermalis* have been developed for the bioremediation of environments containing mixed radioactive waste at high temperatures. Recombinant strain of *Acenitobacter baumanii* was found to enhance degradation rates at sites contaminated with crude oil [45]. In the presence of metals, some higher organisms produce cysteine-rich peptides, such as glutathione (GSH), phytochelatins (PCs), and metallothioneins (MTs), which can bind and sequester metal ions in biologically inactive forms. The overexpression of MTs in recombinant bacterial cells resulted in enhanced metal accumulation, thus offering a promising strategy for the development of microbial-based biosorbents [12].

Recent studies show that certain GEMs have increased ability to metabolize specific chemicals such as hydrocarbons and pesticides [12, 23].

Genetic engineering techniques and studies on the metabolic potential of microorganisms have allowed the design of genetically modified microorganisms capable of degrading specific contaminants. This approach offers an opportunity to create an artificial combination of genes that do not exist together in nature. The most commonly used techniques include engineering with single genes or operons, pathway construction, and alternation of the sequences of existing genes [22]. Genetic and biochemical techniques, such as PCR, *in situ* hybridization, and use of antibodies, can also contribute greatly to our knowledge regarding the potential activity of the microorganisms present at polluted sites. DNA tests can indicate the presence of particular microbes potentially involved in biodegradation, and the use of enzyme-specific antibodies can reveal the induction of catabolic enzymes. Changes in the composition of bacterial populations may be observed during treatment, and differences can be noted in comparison with nonpolluted sites. DNA probes targeting specific genetic sequences, i.e., the genes responsible for the degradation ability of the microorganism, can be used to characterize a contaminated site throughout the bioremediation program, to determine the overall community structure and catabolic activity [46].

The first two genetically modified bacterial strains were *Pseudomonas aeruginosa* (NRRL B-5472) and *P. putida* (NRRL B-5473), and these contained genes for naphthalene, salicylate, and camphor degradation. *Pseudomonas fluorescens* HK44, which can degrade naphthalene, represents the first example of a microorganism genetically engineered for bioremediation purposes [22]. The associated research demonstrated that the genes responsible for the naphthalene degradation pathway were arranged under a common promoter, which resulted in the simultaneous degradation of naphthalene [22]. Other authors have shown that some bacteria, such as *Geobacter metallireducens*, can remove uranium from drainage waters in mining operations and from contaminated groundwater [21].

4. Mechanisms associated with bioremediation by microorganisms

Figure 4 shows the major groups of microorganisms commonly used for the bioremediation of metals, which include bacteria, microalgae, fungi, and yeast.

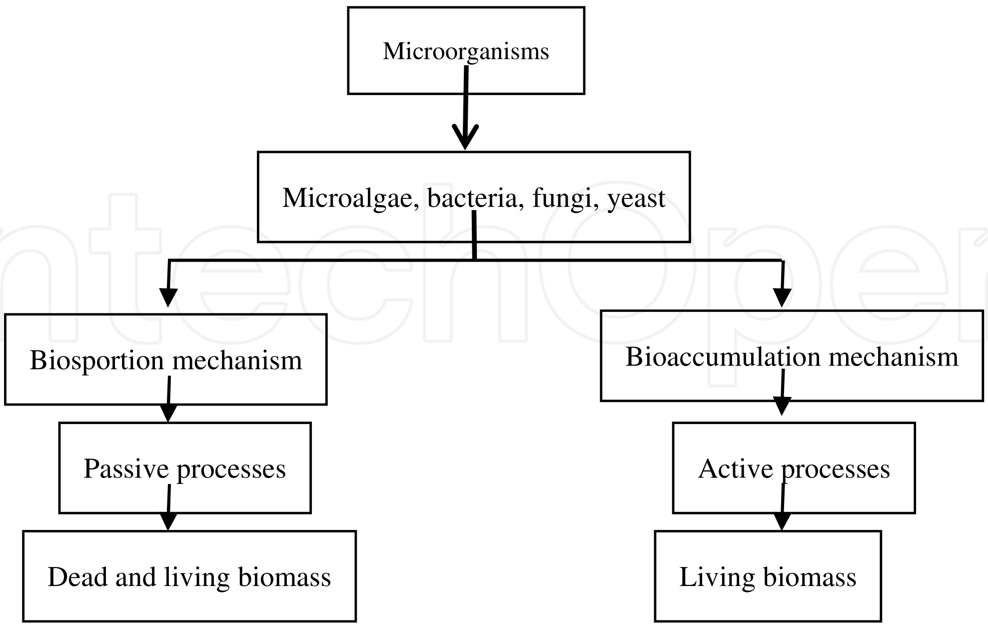

Figure 4. Microorganisms employed in the bioremediation and processes/mechanisms involved in the case of dead and living biomass.

Bioremediation can be separated into two categories, biosorption and bioaccumulation. Biosorption is a passive adsorption mechanism that is fast and reversible [6, 49]. The metals are retained by means of physicochemical interaction (e.g., ion exchange, adsorption, complexation, precipitation, and crystallization) between the metal and the functional groups present on the cell surface [6, 47–50]. Several factors can affect the biosorption of metals, such as pH, ionic strength, biomass concentration, temperature, particle size, and presence of other ions in the solution [48]. Both living and dead biomass can occur for biosorption because it is independent of cell metabolism. On the other hand, bioaccumulation includes both intra- and extracellular processes where passive uptake plays only a limited and not very well-defined role [6]. Therefore, living biomass can only occur for bioaccumulation.

Table 2 shows a comparison of the main parameters associated with biosorption and bioaccumulation processes. In general, the biosorption process needs inexpensive cost because the biomass can be obtained from industrial waste, and it can be regenerated and reused in many cycles. Bioaccumulation, on the other hand, needs expensive cost because the process occurs in the presence of living cells in which reuse is limited. Also, important factors to be considered include selectivity of metals and the potential for regeneration. The selectivity in biosorption is generally low because the bind only occurs by physicochemical interaction. It can be increased through modification of the biomass. Nevertheless, processes involving bioaccumulation generally perform better than those involving biosorption.

The structure of the cell wall of a microorganism contains various macromolecules, such as polysaccharides and proteins, with a high number of charged functional groups, including carboxyl, imidazole, sulfydryl, thioether, phenol, carbonyl, amide, ester sulfate, amino, and hydroxyl groups [51–53]. The positively charged metal present in the solution gravitates toward these functional groups and adsorption occurs. The form in which microorganisms are cultivated can influence the cell wall composition, and this can be exploited to improve the adsorption capacity of the microorganisms [6]. Bacteria can remove heavy metals from wastewater via functional groups, such as ketones, aldehydes, and carboxyl groups present in their cell walls and thereby produce less chemical sludge [54]. Both gram-positive and gram-negative bacteria are used for the uptake of metals. Green, red, and brown algae are also used as biosorbents. Some functional presents in bacteria such as uronic acid of carboxyl groups and sulfate groups, xylans, galactans, and alginic acid are capable of performing ion exchange. The advantage of using algae as biosorbents is that they generally do not produce toxic substances, unlike other microorganisms such as bacteria or fungi [55].

Fungi and yeasts also used for the adsorption. The most advantage of fungi is highly variable, ranging in size from mushrooms to microscopic molds. They are easy to grow and produce a substantial biomass. The cell walls of fungi are rich in polysaccharides and glycoproteins, which contain, for instance, amine, imidazole, phosphate, sulfate, sulfhydryl, and hydroxyl groups [56, 57]. However, the cell walls of yeasts contain a microfibrillar structure composed of more than 90% polysaccharides. The main groups present in these walls are amine, hydroxide, carboxyl, sulfate, and phosphate groups [58].

Characteristics	Biosorption	Bioaccumulation
Cost	Usually low. Biomass can be obtained from industrial waste. Cost associated mostly with transportation and production of biosorbent.	Usually high. The process occurs in the presence of living cells that have to be supported.
pH	The solution pH strongly affects the sorption capacity of heavy metals. However, the process can occur within a wide pH range.	Significant change in pH can strongly affect living cells.
Selectivity	Poor. However, this can be increased by modification/biomass transformation.	Better than in the case of biosorption.
Rate of removal	Most mechanisms occur at a fast rate.	Slower rate than in the case of biosorption because intercelluar accumulation takes a long time.
Regeneration and reuse	Biosorbents can be regenerated and reused in many cycles.	Reuse is limited due to intercellular accumulation.
Recovery of metals	With an adequate eluent the recovery of heavy metals is possible.	Even if possible, biomass cannot be used for other purposes.
Energy demand	Usually low.	Energy is required for cell growth.

Table 2. Comparison of biosorption and bioaccumulation processes [51].

Most heavy metals cannot be biodegraded and they tend to accumulate in the microorganism [59]. Several factors influence metal accumulation, such as the degree of exposure, metal concentration, temperature, and salinity, and therefore it is difficult to obtain detailed information on how the accumulation occurs in the bioremediation [60]. The process of accumulation is complex and varied according the pathway of metabolism is regulated by the metal concentration [61]. Mechanisms of metal ion uptake based on surface binding and metals ions entering the cell membrane have been proposed [62–65].

5. Considerations regarding metal uptake capacity of microorganisms

The pathway via which the metal binds to a specific site of the biomass is of great importance in relation to the efficiency of a bioremediation process. For example, the ingestion of sediments by microorganisms is considered a principal route of exposure to metals, although free metal ions in sediment pore waters are generally considered to be the most bioavailable form of metals. Thus, metal accumulation is affected by the feeding behavior of microorganisms [61]. After the ingestion of heavy metals, a process of metal excretion and/or detoxify begins to avoid potential toxic effects. However, microorganisms will not suffer the toxic effects of the presence of metals when they are stored in detoxified forms [61]. Moreover, the metal–biomass interaction is dependent on the type of metal that can bind to oxygen-containing or S- and N-containing ligands. Although this may be a simple overview of the mechanisms involved, it can act as a starting point for proposing new approaches related to the efficiency of metal uptake by microorganisms [50].

Otherwise, microorganisms can synthesize metal binding proteins, such as MTs or PCs, and the proteins are strongly related to the capacity of metal adsorption, accumulation, and resistance [50]. In particular, metalloproteins are a large group of these proteins, which play an important role mainly in regulating the amount of metals within the cells.

Metal binding proteins present outside of cell membrane attract metal ions exist in solution and assist the transport to cytosol, where metallochaperones (specialized protein chelators) transfer metals to the appropriate receptor protein. The binding sites of the metal binding proteins have been improved to other protein, such as heterologous metalloproteins by using genetic technique. Some researchers developed heterologous metalloproteins with higher affinity and metal-binding capacity and/or specificity and selectivity, which was expressed in bacteria to improve their capacity to adsorb metals [50]. The technique changing the proteins on the cell surface, into heterogeneous one by using recombinant DNA has emerged as a novel approach to enhance the capacity of adsorption. Both bacteria and yeasts have been investigated for this purpose. A wide diversity of metal-binding proteins, such as glutathione (GSH), GSH-related phytochelatins (PCs), cysteine-rich metallothioneins (MTs), and synthetic phytochelatins (ECn), have been used to enhance the bioaccumulation of heavy metals [66]. For example, the recombinant bacterial strain cloned mercury operon, which coded the regulatory gene (MerR) and other genes involved in the transport, was constructed. The strain showed high resistant to mercury by the detoxification of mercury ions within the cell [66].

The expression of metal-binding proteins or peptides in microorganisms to enhance heavy metal accumulation and/or tolerance has great potential. Several different peptides and proteins have been explored [20, 50]. Different resistance mechanisms can be activated, for example, the production of peptides of the family of metal binding proteins, such as MTs or phytochelatins (PCs); the regulation of the intracellular concentration of metals, with the expression of transporters of proteins of ligand–metal complexes from the cytoplasm to the inside of vacuoles; and the efflux of metal ions by ion channels present in the cell wall. The genes to show the tolerance toward toxicity of metals are often encoded on the transposons or plasmids, which facilitate their dispersion from cell to cell [12]. The tolerance is caused by either the activity bacterial metal resistance result from either the active efflux pumping of the toxic metal out of the cell or enzymatic detoxification (generally via redox chemistry) where a toxic ion is converted into a less toxic or less available metal ion.

Several metal-binding peptides have been studied with the aim of increasing Cd resistance or accumulation by *E. coli* cells. Naturally occurring Cd-binding proteins and peptides, such as MTs and PCs, are very rich in cysteine residues. In addition, histidines are known to have high affinity for transition metal ions such as Zn^{2+}, Co^{2+}, Ni^{2+} and Cu^{2+}. Therefore, various peptides comprising different sequences of cysteines or histidines was used to bind Cd [20], and consequently Cd tolerance and accumulation could be enhanced in *E. coli* cells. It would be of interest to evaluate Cd-binding peptides and proteins engineered into more environmentally robust bacteria, such as *Pseudomonas*, for their potential use in bioremediation [20].

Hexavalent chromium is mobile, highly toxic, and considered as a priority environmental pollutant. Chromate reductases found in chromium-resistant bacteria have the potential for use in bioremediation process because they are known to catalyze the reduction of Cr(VI) to Cr(III) [67]. The enzymatic reduction of Cr(VI) to Cr(III) involves the transfer of electrons from electron donors, like NAD(P)H, to Cr(VI) with the simultaneous generation of reactive oxygen species (ROS) [67]. Microorganisms that have the ability to reduce Cr(VI) are referred as chromium-reducing bacteria (CRB). Gram-positive CRB shows to have significant tolerance to the toxicity of Cr(VI) even at high concentrations, whereas gram-negative bacteria are much more sensitive to Cr(VI) [67]. Some genes responsible for resistance to Cr(VI) have been determined in bacteria. For example, the chrR gene located on the chromosome of *P. aeruginosa* confers resistance to chromate. *Ochrobactrum tritici* contains several genes associated with chromate resistance, namely, chrB, chrA, chrC, chrF, and ruvB. The presence of enzymes that play a role in reducing Cr(VI) have been reported for different microorganisms. The enzymes such as quinone reductases, nitroreductases, and NADPH-dependent enzymes vary in their ability to transform chromate and involve different pathways. Several bacteria reduce Cr(VI) through membrane-bound reductases, such as flavin reductase, cytochromes, and hydrogenases. These enzymes can form part of the electron transport system and use chromate as the terminal electron acceptor [67]. Table 3 shows ability of typical microorganisms (algae, bacteria, fungi, and yeasts) to remove heavy metals from certain environments [68–80]. As can be seen, a wide range of microorganisms have been considered for the development of efficient technology for the removal of heavy metal ions from polluted effluents.

Microorganism	Type	Metal	Reference
Algae	*Ascophyllum nodosum*	Pb, Ni	68
		Pb, Cu, Cd, Zn	69
	Chlorella pyrendoidosa	U	70
	Cladophora fascicularis	Pb	33
	Fucus vesiculosus	Cr	71
		Pb	68
		Cd	72
	Hydrodictylon, *Oedogonium*, and *Rhizoclonium* spp	V, As	73
	Spirogyra spp. and *Cladophora* spp.	Pb, Cu	34
	Spirogyra spp. and *Spirullina* spp.	Cr, Cu, Fe, Mn, Zn	35
Bacteria	*Bacillus cereus*	Cr	28
	Burkholderia species	Cd, Pb	26
	Kocuria flava	Cu	27
	Pseudomonas veronii	Cd, Zn, Cu	25
	Sporosarcina ginsengisoli	As	29
	Stenotrophomonas spp.	Au	74

Microorganism	Type	Metal	Reference
Fungi	*Agaricus bisporus*	Cd, Zn	75
	Aspergillus fumigatus	Pb	32
	Aspergillus versicolor	Cr, Ni, Cu	31
	Aspergillus, *Mucor*, *Penicillium* and *Rhizopus*	Cd, Cu, Fe	77
	Aspergillus niger, Aspergillus foetidus, and *Penicillium simplicissimum*	Ni, Co, Mo, V, Mn, Fe, W, Zn	78
	Ganoderma lucidum, Penicillium spp.	Ar	76
	Penicillium canescens	Cr	30
Yeast	*Candida tropicalis*	Cd, Cr, Cu, Ni, Zn	79
	Candida utilis	Cd	37
	Pichia guilliermondii	Cu	79
	Saccharomyces cerevisiae	Cr, Ni, Cu, Zn	36
	Streptomyces longwoodensis	Pb	80

Table 3. Sorption potential of certain microorganisms to remove heavy metals.

6. Conclusions

Natural and anthropogenic activities generate large quantities of aqueous effluents containing toxic metals. Many studies have been conducted in recent decades aimed at lowering metal concentrations derived from natural resources. In addition, considerable effort has been made to develop efficient and cost-effective technologies and apply them to industrial wastewater treatment. The potential for microorganisms to remove metals from solutions through passive and active mechanisms has been shown to be an interesting approach to metal uptake in polluted waters, and the efficiency of such processes is dependent on the experimental conditions, the target pollutant and various other factors.

The application of this type of bioremediation process in large scale remains, however, a challenge, and a preventive approach to metal pollution problems is therefore encouraged. Further investigations aimed at the identification of the mechanisms involved the characterization of biosorbents, and advances in genetic engineering are required.

Many microorganisms can break down metals naturally, but this is not a sufficient solution on a global scale. Therefore, as a means to resolve this problem, engineered microorganisms can be developed with the help of genetic engineering. A better understanding of the way in which both eukaryotes and prokaryotes metabolize heavy metals and the detoxification pathways will help future researchers to deal with this type of environmental problem with maximum efficiency. The choice of the most promising type of biomass must be made, taking into account its cost and availability, and this is necessary on an industrial scale. The microorganisms should be easy to obtain and to cultivate. For example, industrial-scale application would not be of interest if the microorganism is difficult to cultivate, a rare species or a species in danger of extinction.

Although some progress has been made in the recognition of the importance of microorganisms for the decontamination of polluted waters, some important points still need to be addressed. However, a new challenge has emerged for science. Thus, further studies need to focus on the development of new clean environmentally acceptable technologies with commercial feasibility.

Nomenclature

BTX	Benzene, toluene, and *o*-xylene
CAPES	Coordination of Improvement of Higher Education Personnel
CNPq	National Council for Scientific and Technological Development
DNA	Deoxyribonucleic acid
ECn	Synthetic phytochelatins
FAPEG	Research Foundation of the State of Goiás
FAPEMIG	Research Foundation of the State of Minas Gerais
GEM	Genetically engineered microorganisms
GMMs	Genetically modified microorganisms
GSH	Glutathione
MTs	Metallothioneins
NRRL B-5472	*Pseudomonas aeruginosa*
NRRL B-5473	*Pseudomonas putida*
PCR	Polymerase chain reaction
PCs	Phytochelatins
RNA	Ribonucleic acid

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