Microbial bioremediation of heavy metals

Ana Volarić[1], Zorica Svirčev[1], Dragana Tamindžija[2] and Dragan Radnović[1]

[1]University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, Novi Sad, Serbia
[2] University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, Novi Sad, Serbia

Abstract

Heavy metal pollution is one of the most serious environmental problems, due to metal ions persistence, bioavailability, and toxicity. There are many conventional physical and chemical techniques traditionally used for environmental clean-up. Due to several drawbacks regarding these methods, the use of living organisms, or bioremediation, is becoming more prevalent. Biotechnological application of microorganisms is already successfully implemented and is in constant development, with many microbial strains successfully removing heavy metals. This paper provides an overview of the main heavy metal characteristics and describes the interactions with microorganisms. Key heavy metal resistance mechanisms in microorganisms are described, as well as the main principles and types of heavy metal bioremediation methods, with details on successful pilot scale bioreactor studies. Special attention should be given to indigenous bacteria isolated from the polluted environments since such species are already adapted to contamination and possess resistance mechanisms. Utilization of bacterial biofilms or consortia could be advantageous due to higher resistance and a combination of several metabolic pathways, and thus, the possibility to remove several heavy metals simultaneously. Novel technologies covered in this review, such as nanotechnology, genetic engineering, and metagenomics, are being introduced to the field of bioremediation in order to improve the process. To conclude, bioremediation is a potentially powerful solution for cleaning the environment.

Keywords: biotransformation; pollution; biotechnology; heavy metal resistance.

Available on-line at the Journal web address: http://www.ache.org.rs/HI/

REVIEW PAPER

UDC: 504.5+549.25:351.777.6

Hem. Ind. **75 (2)** 103-115 (2021)

1. INTRODUCTION

As a result of industrialization, the environment is being polluted with various types of hazardous wastes. Microorganisms have a crucial role in the pollutants fate in the environment, as they play a part in pollutant transport, distribution, properties, transformation, and mineralization. They have been successfully used for remediation of various types of environmental pollutants, including diverse organic compounds as well as heavy metals [1,2]. Organic compounds can be completely degraded by the microbial metabolism, resulting in the removal from the environment. Contrary, heavy metals persist in the environment due to their non-degradable nature [3]. As such, heavy metals cannot be completely degraded or eliminated, but only transformed by a microbial activity to a less toxic, bioavailable, or mobile form. Microorganisms can also adsorb and accumulate metals inside the cells, which can then be more easily removed from the contaminated environment. Thus, microorganisms are a valuable asset in combat against widespread pollution, with significant practical applicability.

In total, there are 53 heavy metals. Out of these, 22 are of some biological importance. Due to low solubility, tin (Sn), cerium (Ce), gallium (Ga), zirconium (Zr), and thorium (Th) do not exhibit biological influence. Trace elements with low toxicity and important roles in physiological, biochemical, and metabolic processes in living organisms (*e.g.* co-factors for some enzymes, micronutrients, regulators of osmotic pressure, etc.) are iron (Fe), molybdenum (Mo), and manganese (Mn). Heavy metals zinc (Zn), nickel (Ni), copper (Cu), vanadium (V), cobalt (Co), wolfram (W), and chromium

Corresponding author: Dragana Tamindžija; University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, Novi Sad, Serbia
E-mail: dragana.cucak@dh.uns.ac.com
Paper received: 15 September 2020
Paper accepted: 21 March 2021
https://doi.org/10.2298/HEMIND200915010V

(Cr) are considered toxic with high to moderate importance as trace elements. Arsenic (As), silver (Ag), antimony (Sb), cadmium (Cd), mercury (Hg), lead (Pb), and uranium (U) have limited beneficial functions and are considered toxic [4]. The most common metals found in the contaminated areas are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), and mercury (Hg) [5]. Most heavy metals are naturally present in the environment originating from pedogenetic processes of weathering of parent materials and are usually found in trace amounts in insoluble forms, not available for uptake by living organisms. Various anthropogenic activities are causing the disturbance and acceleration of slowly occurring natural geochemical cycles of metals, leading to accumulation of one or more heavy metals at concentrations higher than defined background values [6]. With the rapid expansion of many industries (*e.g*. mining, surface finishing, energy and fuel production, metallurgy, electroplating, *etc.*), wastes containing heavy metals are discharged into the environment [7]. Some heavy metals essential for plant growth (*e.g*. Cu, Mn, Co, Fe, Mo, Ni, and Zn) are introduced to soil in the form of fertilizers. Fertilizers can contain trace amounts of other heavy metals, such as Cd and Pb [6]. Several common pesticides used extensively in the past were based on the compounds containing Cu, Hg, Mn, Pb, or Zn. Certain animal wastes such as solids or slurries used in agriculture as fertilizers can contain elevated levels of Cu and Zn, which act as growth promoters in the pig and poultry industry. Long-term irrigation with wastewater containing low heavy metal concentrations can lead to accumulation of metals in soil.

2. INTERACTION OF HEAVY METALS WITH MICROORGANISMS

Microbial populations are often capable of surviving environmental stresses caused by accumulation of heavy metals, through the selection of the fittest and formation of heavy metal tolerant and/or resistant microbial populations [8]. Toxicity of metals, among other factors, depends on their chemical speciation, bioavailability, absorbed dose, route of exposure, and characteristics of the exposed individual [9,10]. Metal speciation and bioavailability are very important from the aspect of biological interactions. The speciation of any metal in nature depends on the combined effects of pH, redox potential, and ionic strength. Bioavailability of metals is a complex phenomenon, influenced by many physical, chemical, and biological factors such as pH, eH, ion exchange capacity, organic matter content, clay content, presence of ligands, metal concentration, and speciation [16,17].

Some heavy metals are essential for the normal function of a microbial cell, such as iron and copper contained in cytochrome c oxidase [13], and manganese, which is important for oxygen production during photosynthesis [14]. However, metals can be very toxic to cells, as a result of their affinity to bind to many cellular components, which can lead to cell membrane disruption, DNA damage, inhibition of transcription and translation, inhibition of enzymatic activities, protein denaturation, and inhibition of cell division. Environmental pollution caused by the heavy metal release can change microbial soil communities, with an inhibitory effect on microorganisms in most cases [15]. Some studies found that the culturable number of various microbial groups in the contaminated soil samples is decreased, compared to a number in uncontaminated samples [16]. Contrary, other studies did not find a statistically significant correlation between the metal concentration in soil (Cr) and cultivable bacteria count nor with the percentage of chromate tolerant cultivable bacteria [17,18]. The functional diversity and composition of the microbial community can be also assessed by using the Biolog-Microplate method [19], restriction fragment length polymorphism (RFLP) based on 16S rRNA sequence of microbes [20,21] as well as the PCR-DGGE analysis [22]. In a polluted environment, the response of microbial communities is determined by the metal type, nature of the substrate (pH, for example, which affects metal bioavailability), and microorganism species. In some cases strains both from polluted and clean environments have a similarly high level of heavy metal resistance indicating the presence of constitutive or intrinsic resistance mechanisms [23]. Microbial resistance mechanisms can be encoded by chromosomal or mostly, plasmid genes [24,25]. Microorganism species that possess plasmid-encoded resistance mechanisms are more important for eventual applications in bioremediation because those genes can easily become available for other microbial species through the horizontal gene transfer. There are five main heavy metal resistance mechanisms (Fig. 1): (I) extracellular (permeability) barrier, (ii) oxidation/reduction of heavy metal ions, (iii) intracellular sequestration, (iv) extracellular sequestration, and (v) efflux (active transport) of metal ions [26].

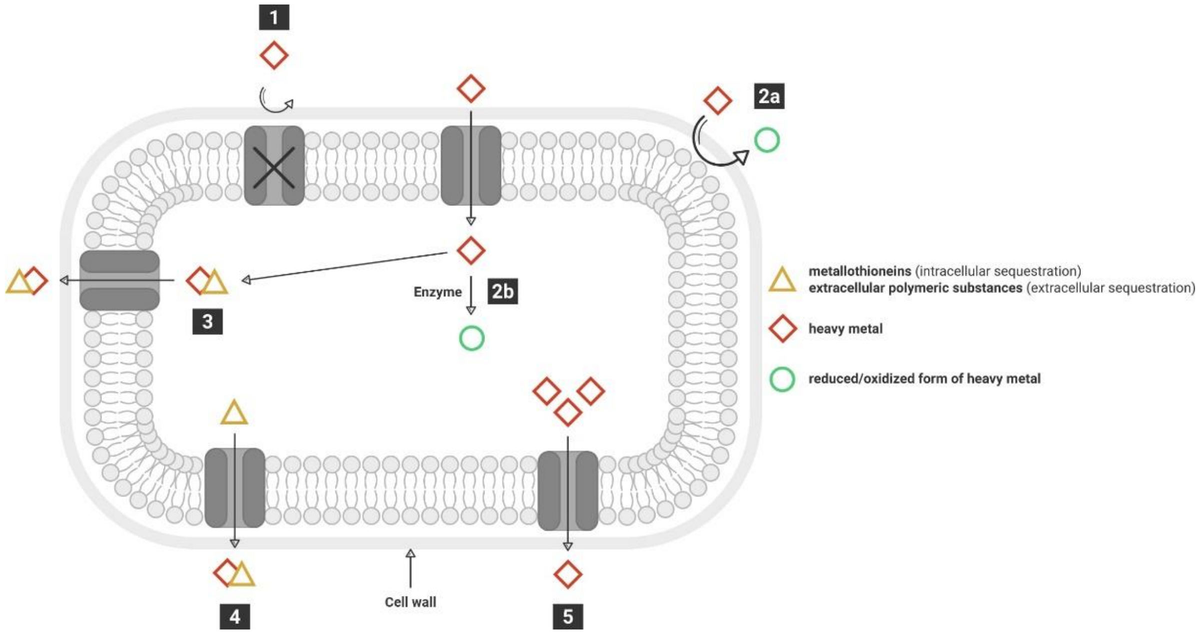

Figure 1. Five main heavy metal resistance mechanisms: (1) extracellular barrier – mutation of gene involved in transport; (2a) extracellular enzymatic oxidation/reduction; (2b) intracellular enzymatic oxidation/reduction; (3) intracellular sequestration; (4) extracellular sequestration; (5) efflux of metal ions.

Extracellular barrier, including cell membrane, cell wall, and extracellular polymers, is responsible for metal resistance by preventing the entrance of metal ions inside the cell [27]. Living or dead bacterial cells can adsorb metal ions by binding them to different moieties of the cell wall or capsule (carboxyl, amino, phosphate, and hydroxyl groups) [28]. Changes in the plasma membrane permeability, such as mutations of genes involved in transport, could prevent the entry of metal ions in the cell [29]. **Efflux or active transport of metal ions** outside of the cell is a widely present heavy metal resistance mechanism, which prevents accumulation of toxic ions inside the cell [29]. Following the metal uptake, some microorganisms sequestrate metal ions **intracellularly** using cysteine-rich peptides, called metallothioneins [26,30]. After the sequestration, the complexed metal can be transported out of the cell or stored in intracellular granules. **Extracellular sequestration** of metals can be carried out by binding to extracellular polymeric substances (EPS) or some other electronegative components of the cell membrane [31,32], metal precipitation [27], and with the help of biosurfactants and siderophores [31]. **Oxidation and reduction** lead to the formation of less toxic metal forms. In some metals higher oxidation state is more toxic (*e.g*. Hg, Cr, Se), while in others a higher state is less toxic (*e.g*. As). Many species, such as *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens,* and *Escherichia coli*, are capable of reducing highly toxic hexavalent chromium to its biologically inert, less toxic trivalent form [8,17,33-38]. Besides chromium, it is reported that bacteria can reduce mercury [39], iron [40], arsenic [41], uranium [42], manganese [43], molybdenum [44], and other metals, and oxidize As [45,46].

3. REMOVAL OF HEAVY METALS FROM THE ENVIRONMENT

3. 1. Conventional remediation techniques

Physico-chemical remediation techniques such as excavation and disposal to a landfill, the containment of contaminated areas, incineration, chemical oxidation or reduction, extraction of pollutants with organic solvents, electroreclamation, and vitrification are already in use [47]. The excavation and disposal technique simply moves contaminated soil to another place and may create risks in excavation and transport of the contaminated soil [48]. Besides this, it is extremely hard to find new landfills for contamination disposal. The technique based on containing the contamination is a temporary solution, with the contamination remaining on site and requiring constant monitoring. A better approach would be to eliminate contaminants, or in the case of heavy metals, convert them to less toxic forms. However, those techniques can be very expensive, complicated, and they lack public acceptance. The most commonly used techniques, in the case of heavy metal removal from the soil, are immobilization of metals by increasing the pH value, and the opposite process termed soil washing with acids, in order to increase metal solubility, followed by flocculation to remove metal ions [31]. In the case of sediment remediation, it is usually necessary to remove the sediment and treat it elsewhere, during which process the metal mobility and toxicity could be increased. Metal removal from aquatic systems can be achieved by using a wide range of techniques, including flocculation, precipitation, complexation, ion exchange, reverse osmosis, and electrochemical recovery. The downside of all techniques discussed is expensiveness, alongside with possible ineffectiveness, and negative ecological side effects.

3. 2. Bioremediation

Bioremediation is a process of biotransformation of the environment altered by contaminants, to its original state by using organisms to degrade pollutants [49]. The main focus is on the use of microorganisms and the variety of their metabolic processes to clean up the environment. The advantages of bioremediation are low cost and the use of natural processes for the pollution removal [50,51]. However, these processes can be time-consuming and affected by various environmental factors, such as climate and geological conditions [52]. Other drawbacks regarding applications of bioremediation processes are formation of possibly more toxic byproducts, limited effectiveness, and the specificity of biological processes [53]. As it was said earlier, heavy metals cannot be degraded, but only transformed from one oxidation form to another. When their oxidation state is altered, heavy metals can become (i) more water-soluble and removed by leaching, (ii) less toxic, (iii) less water-soluble, leading to their precipitation, or (iv) volatilized and removed from the contaminated area [49]. Distribution of heavy metals in the environment is regulated, to a large degree, by microbial activities [54]. Microorganisms used for bioremediation can be indigenous to the contaminated site, or isolated from elsewhere and introduced to the site [53]. The process of adding microorganisms to the site, in order to increase the removal of hazardous substances, is known as bioaugmentation [55]. For bioremediation to be successful, it is necessary to strictly control environmental conditions on the contaminated site (*i.e*. by adding the nutrients, controlling the temperature or the pH), due to the microbial sensitivity to even small fluctuations of some factors. There are two types of bioremediation (Fig. 2), based on the method of removal and transportation of toxic waste [53]:

1. ***In situ* bioremediation.** In this type of bioremediation, removal of the contaminated substrate is not needed. The process involves supplying oxygen and nutrients to the system to stimulate naturally occurring microbial population. *In situ* bioremediation can be intrinsic, based on the stimulation of indigenous microbial communities, and engineered, which involves the introduction of microorganisms to the site.

One of the biggest concerns associated with the treatment of heavy metals by *in situ* microbial reduction is the possibility of changes caused by environmental conditions that may influence metal speciation [56]. A reduced form of the metal in an ecosystem can be reoxidized by either physicochemical or biological processes, becoming toxic again. Therefore, it is important to monitor metal speciation to ensure that the chemical form does not change over time.

2. ***Ex situ* bioremediation.** This type of bioremediation requires excavation of the contaminated soil and sediment or pumping of the groundwater. Based on the state of the contaminant, *ex situ* bioremediation can be classified as:
   - *solid phase system* - includes organic (leaves, animal manures, and agricultural wastes) and problematic wastes (domestic and industrial wastes, sewage sludge, and municipal solid wastes). This kind of treatment includes landfarming, composting, and biopiles.

     Landfarming is a simple process that includes excavation of the contaminated soil, spreading it over a prepared bed, and tilling until the pollutants are degraded. It is based on the stimulation of indigenous microbial communities and their aerobic activities in the superficial layer of the soil.

     Composting involves mixing of the contaminated soil with organic wastes (such as manure and agricultural waste), which provide nutrients for microorganisms.

     Biopiles presents a hybrid method of landfarming and composting.
   - *slurry phase system* – the contaminated substrate is mixed with water in a large tank (bioreactor) at constant mixing in order to keep the microorganisms in contact with the pollutants. This kind of bioremediation can be performed by processes in bioreactors, or by bioventing, biosparging, and bioaugmentation.

Bioreactors. In bioreactors, bioremediation of soil, sediment, water, or sludge is conducted through an engineered containment system, which enables greater biodegradation, due to easily manageable conditions.

Bioventing. This technique is based on pumping of air and nutrients through wells to the contaminated soil into the unsaturated zone, in order to stimulate microbial communities.

Biosparging. Biosparging involves air injection below a water table to increase oxygen and nutrient levels in the saturated zone and groundwater, thus increasing the activity of microorganisms.

Bioaugmentation. This method is based on addition of microorganisms to the contaminated matrix, either indigenous or exogenous. Most soils after prolonged exposure to pollutants already possess a microbial community resistant to that pollution, in which case addition of exogenous microorganisms is not needed.

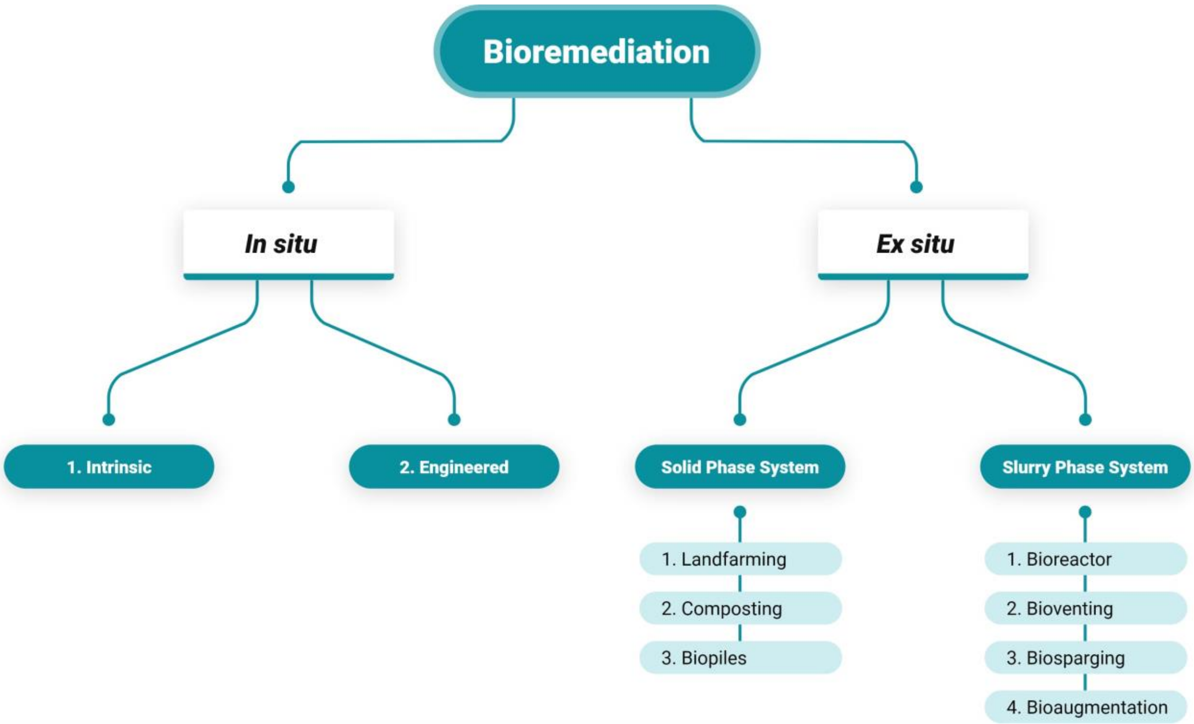

Figure 2. Overview of bioremediation methods.

3. 2. 1. Biotechnological applications of heavy metal resistant microorganisms

The ability to resist and detoxify heavy metals has been proven under laboratory conditions for many bacterial species, which can potentially be successfully used in bioremediation of heavy metal contamination. For successful design of a bioreactor system (Fig. 3) for heavy metal bioremediation, it is important to pay attention to the following factors: existence of a microbial population capable of detoxifying the contaminant, availability of the contaminant to the microbial cells, and the environmental factors, such as the type of soil, pH, temperature, and the presence of oxygen and nutrients [57]. Regarding the choice of the microbial population capable of heavy metal detoxification, use of the indigenous microorganisms would be the best solution, since they are already adapted to higher concentrations of heavy metals and other conditions in the contaminated substrate. In order to isolate such microorganisms, the soil, wastewater, or other samples that should be decontaminated from heavy metals, could be treated in a bioreactor. After the incubation, microorganisms could be isolated and used for creation of heavy metal resistant culture collections. To increase the number and variability of resistant microorganisms, some strains isolated from the other sources could be added into the culture collection. The best sources of heavy metal resistant microorganisms are mines, containing naturally high concentrations of various metals, and thus having highly resistant microbial communities. The use of a consortium consisting of resistant microorganisms could be more effective in heavy metal bioremediation than the use of a single species or strain [58,59]. The main advantages of the consortium over single strains are a diversity of metabolic processes and resistance mechanisms, and the possibility to remove various heavy metals simultaneously.

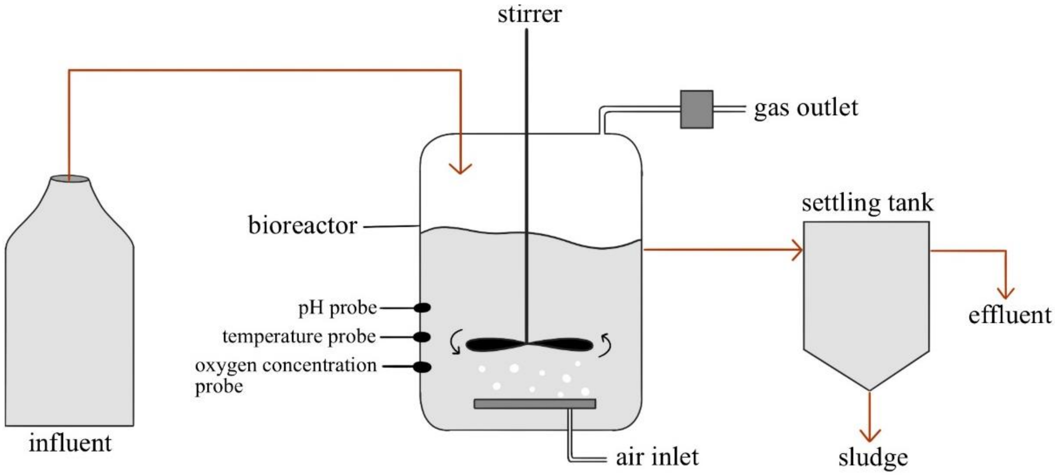

Figure 3. Scheme of a general bioreactor system for wastewater treatment

One of the successful studies was conducted on a continuous hexavalent chromium biological removal from wastewater by anaerobic-aerobic activated sludge process [60]. The bioreactor used in this study had two zones – aerobic, with the air pump, and anaerobic, with a stirrer, which are linked together by two holes on the bottom of the anaerobic tank. The influent containing hexavalent chromium is primarily added into the anaerobic tank, followed by passing through to the aerobic tank. In this experiment, a rapid increase in the chromium concentration in the effluent was observed during the first few days after the addition of higher Cr(VI) concentrations into the influent. After a short period of adaptation of the bacterial community to the new conditions, the concentration of chromium in the effluent significantly decreased, with the final values around 0 mg dm^{-3}. This system has been proven effective since the chromium removal efficiency was above 97% at influent Cr(VI) levels of 60 mg day^{-1}. From this experiment it can be concluded that for successful remediation by a continuous system, it is important to avoid sudden increases in the Cr(VI) concentrations in the influent, ensuring the effluent water quality.

Use of biofilms in bioremediation of heavy metals is also a very potent and promising method due to providing high biomass concentrations, while the bacteria can stay in the system for an unlimited time, thus providing a chance to adapt to various unfavorable conditions [61,62]. For the treatment of chromium solutions in one of the studies, *Arthrobacter viscosus* was chosen, due to its high production of exopolysaccharides and the ability of biofilm formation [61]. For the two tested concentrations of chromium of 10 and 100 mg dm^{-3} the removal efficiency was 100% during the first 26 days and 6 days, respectively. After that, the removal efficiency started to decrease. At the end of the experiment (226 days for 10 mg dm^{-3} and 104 days for 100 mg dm^{-3}), removal efficiencies were 36% and 38%, respectively. The authors of this study have concluded that with the increase in the chromium concentration, the removal ability decreases [61]. A possible explanation for this result is in the toxicity which hexavalent chromium has on living cells at high concentrations. Therefore, the influent concentration has to be optimized for each individual system. In order to improve the removal efficiency and activity period, biofilms are usually supported on some materials, such as granular activated carbon (GAC), zeolite, kaolin, etc. It was shown that even more successful could be combining these carriers, such as GAC and zeolite [63]. In this study, the chromium removal achieved by using an *Arthrobacter viscosus* biofilm supported on GAC was 19 %, similarly to the use of the biofilm supported on zeolite (18 %). However, better performances were reached when GAC and zeolite were used together (42 % of the Cr(VI) was removed). The main amount of the metallic ion in solution is adsorbed on GAC covered with biofilm. This allows more even distribution of the remaining ions on the surface of zeolite covered with biofilm. With zeolite not being as good adsorber as GAC, the combination of these two materials leads to the better heavy metal removal percentage [63].

In another study, it was shown that the bacterial strain *Acidithiobacillus ferooxidans* has the ability to completely remove hexavalent chromium at concentrations in the range 1 - 5 mg dm^{-3}, while the removal efficiency decreased to only 43% at 10 mg dm^{-3} Cr(VI) [64].

For the removal of arsenic in the form of arsenate (As^{5+}), a laboratory glass column was used as a fixed bed anaerobic reactor with commercially available sand as a biomass carriers [65]. The reactor was inoculated with sulfate-reducing bacteria, later proven to be the species of the genera *Desulfovibrio* and *Desulfomicrobium*. At first, under high sulfide concentrations arsenic was not removed, while after addition of 100 and 200 mg dm^{-3} Fe^{2+}, the arsenic removal increased to 63% and 80%, respectively. Further increase in the removal to ~96% was achieved by a decrease in the dissolved sulfide concentration and maintenance of the Fe^{2+} concentration at 200 mg dm^{-3} [65].

A completely automated pilot plant for the removal of mercury from industrial wastewater was designed and consisted of a pre-treatment and nutrient amendment units followed by a bioreactor and activated carbon filter, and supplied with measuring/control devices [66]. The plant was inoculated with 7 mercury-resistant *Pseudomonas* strains. Over the whole testing period of 8 months, 98% of the inflow mercury was removed [66].

Removal of mercury was also reported in a hybrid bioreactor for combined cadmium/mercury bioremediation [67]. This hybrid bioreactor comprised two systems: (i) anaerobic-anoxic-aerobic, and (ii) photoautotrophic, organized in 8 units: (1) depositional tank planted with macrophytes, (2) anaerobic tank, (3) overflow pool (used for reduction of suspended particles/materials), (4) settling tank (for further reduction of suspended materials), (5) anoxic bed, (6) aerobic bed, (7) clarification tank (also used for reduction of suspended materials), and (8) photoautotrophic system. The hybrid bioreactor supported both heterotrophic and autotrophic organisms and the presence of various bacterial species, *Cladophora* sp., diatoms, and cyanobacteria was observed. The average removal efficiencies achieved in this bioreactor were 79% for Cd and 62% for Hg. Advantages of this system are in mineralization of the sludge, its treatment by the photoautotrophic system, and conversion into organic fertilizer thus avoiding possible toxic sludge pollution.

A *Thiobacillus ferooxidans* strain was used for bio-dissolution of nickel-cadmium batteries in two percolator systems [68]. The first system was a sulfuric acid bioreactor with *T. ferooxidans* immobilized on elemental sulfur. The acidic medium produced in the first system was pumped to the second system containing Ni-Cd batteries. Extraction percentages at the end of the experiment were 100% for cadmium and 96.5% for nickel. This system has a great potential for use as a first step in recycling of nickel-cadmium batteries, thus preventing contamination arising from discarded batteries.

A laboratory scale anaerobic baffled bioreactor was successfully used for the treatment of acidic and zinc-containing wastewater [69]. The bioreactor was divided into four compartments and filled with sulfate-reducing bacteria. Throughout the process, the zinc removal efficiency was always higher than 99% achieved by Zn precipitation in the first compartment. Thus, a possibility for Zn recovery was provided without losing high amounts of sulfate-reducing bacteria, which may be reseeded from other compartments without a significant influence on the overall system [69].

A novel perspective approach in bioremediation is the next generation industrial biotechnology (NGIB) based on extremophilic bacteria [70]. The NGIB should overcome disadvantages of the current industrial biotechnology, such as heavy consumption of fresh water and energy, microbial contaminations, difficulty to develop fully automated processes, difficult and expensive product separation and purification, etc. The strains used in NGIB should be resistant to other microbial contaminations and grow rapidly under harsh conditions (*e.g*. very low or high temperatures, pH, osmotic pressure; growth on unusual substrates, or in the absence of water). Many bacterial groups, such as acidophiles, alkaliphiles, thermophiles, psychrophiles, xerophiles, methanotrophs, and halophiles possess one or more of the required characteristics and their biotechnological utilization is yet to be investigated.

Extremophilic microorganisms belonging to Archaea have been poorly studied in respect to bioremediation [71]. It is known that the strain *Sulfolobus acidocaldarius*, belonging to the domain Archaea, is capable of oxidizing arsenite to arsenate [72]. Another example of a heavy metal resistant Archaea is *Sulfolobus solfataricus*, having a mercury reductase [73]. It is necessary to further investigate this group of microorganisms, as it is possible that they would show greater efficacy in bioremediation under harsh conditions than the Eubacteria.

3. 2. 2. Novel technologies in bioremediation

Genetic engineering. Bioremediation with the use of genetically engineered bacteria is an emerging and promising technology based on addition of genes for metal homeostasis, survival in abiotic and biotic stresses, biodegradative enzymes, metal chelators and transport genes, metal uptake regulator genes, and risk-mitigating genes in the bacterial genome [74]. The advantage of such bacterial species is in the tolerance and resistance to heavy metal pollution. However, these species can be more vulnerable to impacts of various environmental stresses due to the addition of foreign genes. Besides, the competition for nutrients and other resources between genetically engineered and indigenous species can become a serious problem. Indigenous species can be superior because they are already adapted to the environmental conditions in the contaminated areas, and resistant strains are selected due to the exposure to heavy metal induced stress. On the other side, genetically engineered bacterial species can prevail because the addition of specific genes provides the advantage over the indigenous strains. The best solution is to use naturally occurring microorganisms for constructing the recombinant bacteria, because once genetically engineered, this species will have the same genetic base as the naturally occurring species, but will also be improved by the addition of the resistance genes. For example, cloning and expression of chromate efflux gene *chrA* from chromate resistant environmental isolate *B. pseudomycoides* in *B. subtilis* resulted in its improved survival [75]. A modified gene expression for metallothionein in *Ralstonia eutropha* CH34 led to improved bioremediation of cadmium under laboratory conditions [76]. Modifications in metalloregulatory protein ArsR in an *Escherichia coli* strain were shown to be responsible for successful bioremediation of arsenic in contaminated drinking water and groundwater [77]. A modified Hg^{2+} transporter in *E. coli* JM109 induced the increased resistance to mercury under laboratory conditions [78]. Modifications of genes encoding MerR, CadC, ZntR, P*mer*, P*cadA*, and P*zntA* proteins in *Pseudomonas fluorescens* OS8, *Escherichia coli* MC1061, *Bacillus subtilis* BR161, and *Staphylococcus aureus* RN4220 were shown to be responsible for cadmium, lead, zinc, and mercury biosensing in water-suspensions and extracts of soil [79].

Nanotechnology. Remediation of heavy metals can be achieved by the use of nanotechnology. A wide range of nanoparticles (iron, gold, silver, etc.) is known to enhance microbial activities with respect to bioremediation [80]. However, the use of toxic chemicals in the physical and chemical synthesis of nanomaterials is limiting applications of such materials in bioremediation. Nanoparticles produced by bacterial enzymatic activities are far more superior, exhibiting higher catalytic reactivity, better specific surface area, and improved contact between the enzyme and metal [81]. Nanoparticles are created by enzymatic processes when the bacteria uptake the metal ions from the environment and turn them into element metals. Biosynthesis of nanoparticles can be intracellular, following the uptake of metal ions, or extracellular, which involves trapping the metal ions on the cell surface, followed by enzymatic transformation. It was reported that *Rhodococcus* sp. can synthesize Au nanoparticles intracellularly [82], *Bacillus cereus* can synthesize Ag nanoparticles intracellularly [83], while magnetite is synthesized by *Shewanella oneidensis* extracellularly [84]. There are many other examples of nanoparticles synthesized by microbial activities [81]. Such nanoparticles can be used for the removal of heavy metals. For example, iron nanoparticles, among the mostly used nanoparticles in bioremediation, can reduce highly toxic hexavalent chromium into its innocuous trivalent form [85–87]. Bimetal oxide magnetic nanomaterials ($MnFe_2O_4$ and $CoFe_2O_4$) have shown higher adsorption capacity and removal of As^{3+} and As^{5+} than Fe_3O_4, possibly due to the increased content of surface hydroxyl groups in bimetal oxide nanoparticles [88]. Also, it was reported that the removal of Pb^{2+} from wastewater can be achieved rapidly *via* adsorption onto γ-Fe_2O_3 nanoparticles [89].

Metagenomics. It is of great importance to monitor the structure of microbial communities during the process of bioremediation. In the past, it presented a huge problem, due to the fact that only a small fraction of species (less than 1%) present in the soil is culturable under laboratory conditions [90]. The scientific developments have brought metagenomics, a method based on the analysis of the complete DNA extracted from the soil sample [91]. It is assumed that the isolated DNA, termed the soil metagenome, represents the collective DNA of all indigenous microorganisms from the sample [92,93]. The general procedure for metagenomic research includes several steps: (i) enrichment of samples, (ii) extraction of genomic DNA, (iii) construction of DNA library, (iv) screening of the target genes and (v) expression of the product of the target gene [94].

With the use of metagenomics, microbial diversity as well as the potential for heavy metal removal can be determined [95,96]. This approach allows scientists to investigate the overall metabolic capacity of a microbial community, including specific groups of genes encoding proteins responsible for heavy metal transformations. In addition, metagenomics provides the possibility to compare polluted with unpolluted sites in terms of functional diversity and community structures. For example, it was observed that functional diversity was higher in the community from an unpolluted soil sample, in comparison to the one in a soil polluted with Cd [97]. This finding implicates that pollution leads to changes in the functional diversity (usually decreasing it) and community structures over time.

4. CONCLUSION

Conventional remediation techniques have many drawbacks, including the secondary contamination of the environment with even more toxic pollutants, making them less suitable for further use. Therefore, turning to green remediation techniques is of great importance. Very potent tools in bioremediation of heavy metals are various microorganism species, capable of detoxifying a wide range of metal contaminants, due to versatile metabolic activities. There is already a vast number of laboratory scale studies of heavy metal removal, but currently the field studies are fewer. However, some of the developed bioreactor systems were proven to be very efficient in the removal of metal contamination. Beside bacterial species already used in such systems, it is important to investigate Archaea, as a very potent group of various extremophilic microorganisms. Several novel technologies, such as genetic engineering and nanotechnology are being developed and utilized in order to improve bioremediation processes. Overall, bioremediation represents a very powerful tool for the clean-up of the environment in the future.

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SAŽETAK

Bioremedijacija teških metala pomoću mikroorganizama

Ana Volarić[1], Zorica Svirčev[1], Dragana Tamindžija[2] i Dragan Radnović[1]

[1]*Univerzitet u Novom Sadu, Prirodno-matematički fakultet, Departman za biologiju i ekologiju, Trg Dositeja Obradovića 2, Novi Sad, Srbija*

[2]*Univerzitet u Novom Sadu, Prirodno-matematički fakultet, Departman za hemiju, biohemiju I zaštitu životne sredine, Trg Dositeja Obradovića 3, Novi Sad, Srbija*

(Pregledni rad)

Zagađenje teškim metalima je jedan od najozbiljnijih problema u životnoj sredini zbog perzistencije, biodostupnosti i toksičnosti. Brojne konvencionalne fizičke i hemijske metode se tradicionalno koriste za čišćenje životne sredine. S obzirom na to da ove metode imaju nekoliko značajnih mana, upotreba živih organizama, odnosno bioremedijacija, postaje sve zastupljenija. Biotehnološka primena mikroorganizama se već uspešno sprovodi i konstantno unapređuje, a mnogi bakterijski sojevi uspešno uklanjaju teške metale. Ovaj rad pruža pregled osnovnih karakteristika teških metala i opisuje njihovu interakciju sa mikroorganizmima. Opisani su ključni mehanizmi rezistencije mikroorganizama na teške metale, kao i osnovni principi i tipovi metoda bioremedijacije, sa posebnim osvrtom na pilot studije sa bioreaktorima. Posebnu pažnju bi trebalo obratiti na autohtone bakterije izolovane iz sredina prirodno zagađenih teškim metalima, jer su takve vrste već adaptirane na zagađenje i imaju razvijene mehanizme rezistencije. Upotreba biofilma ili konzorcijuma može biti efikasnija, zbog veće rezistencije i kombinacije nekoliko metaboličkih puteva, te samim tim mogućnosti za uklanjanje više teških metala istovremeno. Nove tehnologije opisane u ovom radu, poput nanotehnologije, genetičkog inženjerstva i metagenomike se sve više uvode u polje bioremedijacije u cilju poboljšanja procesa. Prema tome, bioremedijacija predstavlja moćno rešenje za čišćenje životne sredine.

Ključne reči: biotransformacija; zagađenje; biotehnologija; rezistencija na teške metale