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Role of microorganisms in bio-removal of heavy metals

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Abstract

Background and objective: Increasing metal contamination in the environment, foods, and drinking water due to industrial activities is one of the most concerns all over the world. Among all chemical, physical, and biological techniques used for reducing these contamination, bio-removal approach has attracted interest as a green ecofriendly, inexpensive, and simple method for decontamination of metals from soil, foodstuffs, and water. As a matter of interest, biological decontamination of heavy metals in food is discussed in the current review paper.

Results and conclusion: After an introduction about importance and concerns about heavy metal pollutions, main reported microorganisms for bio-removal of Lead, Cadmium, Mercury, and Arsenic are addressed. Then, the main influencing variables on biosorption efficiency including pH, bacterial concentration, temperature, and contact time have been reviewed. Finally, maximum permitted levels of the pollutants in foods and feeds, and their effect on human health are reported. Choosing a suitable microorganism under appropriate condition is important in heavy metals bio-removal. In addition, matrix of the media (food, drinking or waste water, and soil) is another important issue. Other than providing optimum external variables for the microorganisms, having lipids, carbohydrate, and other necessary nutrients in the matrix would increase the biosorption effectiveness.

Keywords: Bacteria, beneficial microorganisms, bio-decontamination, fungi, heavy metals, process variables

1. Introduction

Water contamination by heavy metals and their hazardous impact on human health is one of drastic concerns in recent decades [1]. Lead, Cadmium, Arsenic, and Mercury are among the most dangerous heavy metals [2]. Mercury accumulation in drinking water has been reported in some countries such as Pakistan and Iran [3,4]. Effects of Mercury on the body depends on various factors including exposure time, route of exposure (e.g., water, food, atmosphere), and its chemical form (inorganic or organic) [5]. Mercury is soluble in lipids (especially in form of Hg^{2+}), and is able to enter the human cells, where it shows its negative effects. It can also cause neurological disorders in human [6]. Lead and Cadmium are the most hazardous contaminants in our environment [7,8]. Cadmium exposure would cause hypertension, lung and renal disorder, hepatic injuries, and teratogenic effects [8]. Arsenic presence in foods cause cell poisoning, some organs' failure, and may develop cancer. Inorganic Arsenic is more toxic than organic form [9]. According to the World Health Organization, allowance dose of Arsenic in

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drinking water is 10 µg/L. Water contamination by Arsenic is reported in some countries such as Bangladesh (10-50 µg/L) [10], and Pakistan (10 µg/L) [11].

Some physical and chemical [12,13], and also biological methods using microorganisms [2,14] have been used for heavy metal removal. Biosorption has some advantages, so that it is inexpensive, selective, effective, and efficient [15]. It involves use of bacteria, yeasts, and molds. *Saccharomyces cerevisiae* is a valuable biosorbent due to its usage in fermented foodstuffs and beverages [16]. Several studies carried out about heavy metal biosorption by this valuable yeast [17-19]. Potency of *S. cerevisiae* for heavy metals' removal is determined by type of heavy metal, the yeast biomass, pH, temperature, contact time, and culture media [20,21]. Mechanism of action in bio-removal process is a controversial issue. For *S. cerevisiae*, it is done by surface charges, so that *S. cerevisiae* poses phosphodiester bridges in the cell wall that makes negative surface charges on the cells [22].

In general, absorption of heavy metals by micro-organisms are done via two pathways: bio-accumulation and biosorption. Microbial cell wall contains proteins, polysaccharides, and lipids with various functional groups (e.g., hydroxyl, carbo-xylate, phosphate, and amine), that able to bind heavy metals [2,20]. It has been reported that addition of phosphate to the photosynthetic microorganism's cell wall will increase the cells' ability to absorb heavy metals. It is due to esteri-fication of hydroxyl groups by phosphoric acid in the cell wall [2]. On the other hand, bioremediation occurs by stimulation of microbial growth that uses some contaminants such as solvents and oils in the environment as food and energy source. These microorganisms will convert the contaminants to water and carbon dioxide [15, 16]. In this review paper, the biosorbent microorganisms able to red-uce contamination of Lead, Cadmium, Mercury, and Arsenic are reported. In addition, effective vari-ables in biosorption process are discussed.

2. Heavy metals' bi-removal

Lead (Pb) exists in the Earth's crust with its four different isotopes. In nature, it is rarely found in metal form and almost is in combination with other elements. Lead and its compounds are used in a variety of industries like battery, plastics, printing, radiator, welding, paint, and cosmetics. Lead may enter our body through consumption of contaminated water and food [23]. About 94% of lead accumulates in the bones and the teeth in adults. This element can cause harmful effects in the blood, gastrointestinal tract, and cardiovascular, renal, and nervous systems [24]. The International Agency for Research on Cancer (IARC) classifies lead in Group 2A (probably carcinogenic to humans) [25]. Several microorganisms able to refine heavy metals by various mechanisms including aggregation, bio-adsorption, and biotransformation [17,26-28].

To isolate resistant *Pseudomonas* sp. in absorption of lead from oil effluents, 24 strains of *Pseudomonas* under different growth conditions were studied. Erythromycin (15 µg) and chloramphenicol (30 µg) were used as antibiotics. The bacteria were grown at 40 °C, pH = 6, and shaking rate of 100 rpm. The author found that Mso1 strain was able to remove 40% of lead during 24 h [29,30].

Corynebacteria are mostly found in plants and animals. In humans, they can be found in skin, respiratory, gastrointestinal, and urogenital tract. All species of *Corynebacteria* are pathogenic. Although, the most well-known species associated with human diseases is *Corynebacterium diphtheria*. It is an aerobic gram-positive bacillus, and its cell wall consists of galactose, arabinose, and diamino pimelic acid. Corynebacterium is reported as one of the potent bacteria for heavy metals' bio-removal. Potency of *C. glutamicum* from lysine fermentation process for lead biosorption from synthetic wastewater was studied. The biomass bound to lead (2.74 mmol/g) at pH = 5. This study revealed that *C. glutamicum* is a helpful bacterium for lead bio-removal [31].

Flavobacteriua are gram-negative and rod-shaped bacteria. *Flavobacterium heparinum* and *F. sasangense* are the most common strains. In Korea, *F.*

sasangense was added to polluted wastewater to remove heavy metals at 30 °C and pH = 7.5-8, through which about 50% of heavy metals were removed by the bacterium in the matrix [32].

Nocardia are acid fast bacteria with fungal appearance due to having filament. They are hardly gram-positive by hot staining. There are 85 species of Nocardia, pathogenic and non-pathogenic [34]. In study of El-Gendy et al., 50% of heavy metals were removed by *Nocardiopsis* sp. MORSY1948, and *Nocardia* sp. MORSY2014 at pH = 7 and 40 °C (3% biosorbent dosage) [33].

Azotobacter species are gram-negative bacteria found in soil and water. They are motile and aerobe, form thick-walled cysts that make capsule sludge, and live in soil that release ammonium ions in the soil. *Azotobacter chroococcum* was discovered in 1901 by a Dutch microbiologist. It is a potent bacterium used for bioremediation of heavy metals in agricultural soil [34-36].

Aspergillus species are found commonly around the world. Some species are pathogenic and many others have food, beverage, and industrial applications. *Aspergillus flavus*, *A. parasiticus*, and *A. fumigatus* are pathogenic fungi, while *A. niger* and *A. oriza* are beneficial species. Study of Ren et al. showed that lead, copper, cadmium, and zinc were removed by *A. niger* in soil up to 46%, 90%, 50%, and 20%, respectively [37].

Lactic acid bacteria absorb heavy metals on surface of their cell wall or accumulate them inside their cells [38]. Singh et al. showed that *Lactobacillus acidophilus* was able to bind and remove heavy metals from water after 4 h [39]. Elsanhoty and Al-Turki reported that *L. Acidophilus* has a high ability to decrease heavy metals concentration in water [40]. In another study, 10^{12} CFU/ml *L. acidophilus* was used to reduce concentration of mercury in milk, through which 72% bio-removal was achieved after 4 days [26].

Cadmium (Cd) cannot be found pure in nature. Cadmium is found in zinc ores and widely used in batteries. The chemical form of cadmium affects its absorption and distribution in different organs of the body [41]. Nutritional deficiencies such as iron deficiency increase cadmium absorption through food. Taking zinc as a dietary supplementation reduces the absorption of this element from the gastrointestinal tract. Inhaling the high concentrations of cadmium cause lung damage, pneumonia and pulmonary edema and prolonged exposure to small amounts of this element in the air would cause lung cancer, as well as accumulation of cadmium in the kidneys and eventually kidney disease [42]. Cadmium poisoning affects our body's metabolism and causes skeletal changes, bone pain, osteomalacia, or osteoporosis. Nutritional deficiencies in calcium, protein and vitamin D increase the sensitivity of bones to the effects of cadmium [43]. Cadmium would enter our body through the consumption of contaminated water and food. Many plants absorb cadmium from the soil and also high concentrations of cadmium can be stored in the liver and kidneys of animals and therefore in the food chain [44]. The International Agency for Research on Cancer classifies cadmium in Group 1 (Carcinogenic to humans) [25].

Mercury (Hg) is a heavy metal that is liquid at room temperature. This element is released in nature from erosion of the earth's crust, volcanic activity, and washing of the soil and rocks. Moreover, human activities such as mining, smelting metals, industrial and agricultural effluents, burning coal, and fossil fuels cause mercury entrance into the environment [43]. Different mercury salts have different applications in bacterial and fungal disinfection process, and making laxatives and diuretics. Mercury in its elemental form is fat-soluble and can easily penetrate into biological membranes, especially the blood-brain barrier, which results in inactivation of preplasmic and cytoplasmic enzymes within the cells [45]. Air is the main source of Hg contamination in food, drinking water, and environment. Fortunately, amount of mercury in most of foods are below the allowance limit, but fish and marine foods are the main source of Hg contamination especially in form of methyl-mercury [46]. Mercury is a potent toxic element that would concentrate in food chain and will make adverse effects in human after long-term exposure. It can cause many health problems like kidney, heart, and lung failure, depression, autoimmune disorder, fatigue, and sleep deprivation [47]. Sulfation of heavy metals is preferable for their detoxification because this form of metals is biologically inaccessible and precipitate [48]. In this regard, some microorganisms such as cyanobacteria and photosynthetic microalgae can convert it insoluble form [46]. Bio-removal of Hg by *L. acidophilus* [50-52], *B. cereus* [51], *S. cerevisiae* [18,21] *Citrobacter* ssp. [53], *Chlorella vulgaris* [54], and *Rhizopus arrhizus* [55] were reported.

Various viable organisms such as fungi and algae are able to decrease concentration of arsenic in aqueous solutions. Different bacteria use different mechanisms to survive in arsenic rich situations. These microorganisms can convert toxic form of As to its lower toxic insoluble form. It is done by the activity of oxidative organisms on mineral containing sulfides. Arsenic is able to disrupt the cell membrane. However, the cells take a defensive mechanism such as forming a defensive cell membrane and producing substances to destruct the target pollutant [56]. Bacillus, Streptomyces, and Pseudomonas are among arsenic absorbing bacteria. These biosorbents decrease amount of As by binding, oxidizing, volatilizing, immobilizing, and transforming [57].

Some of microbial metabolites, redox potential, and methylation can be used against As. Anaerobic biosorbents act as electron receptor/donor to deliver CO_2 to the cell. Microorganisms can convert arsenate to arsenic and/or use arsenate as electron receptor [58]. Moreover, chemical or physical properties of the microbial metabolites is changed, so that they could immobilize As. Microorganisms able to freeze and therefore stabilize their metabolites that immobilize the hydroxide deposits. They must stabilize the metabolites to inactivate arsenic [59]. In bio-stimulation, stimulation of microorganisms through adding growth substrates and nutrients (e.g., nitrogen, carbon) with electron acceptor/donor is occurred. Carbon as an energy source may be used to stimulate the micro-organism's growth. Although, the process is affected by other factors such as pH, electric conductivity, contact time, temperature etc. [60]. Maximum permitted levels of Pb, Cd, Hg, and As in food and feed are presented in Table 1 [61].

Table 1- Maximum permitted levels (MRL) of common heavy metals in foods

Pb (mg/kg)	Hg (mg/kg)	Cd (mg/kg)	As (mg/kg)
0.01-1	0.001-0.1	0.003-2	0.01-0.5

3. The most potent microorganisms in bio-removal of heavy metals

Most of the scientific reports are about Lactobacillus and yeast. Different microorganisms are used to remove heavy metals from water and food, among them bacteria are a large useful group [62]. Knowing the appropriate microorganisms for bio-removal purposes is of great importance. For cadmium removal, are *L. acidophilus*, *Bifidobacterium lactis*, *B. longum*, and *L. fermentum* have been introduced. These bacteria are also able to remove lead [63]. *L. acidophilus* was reported to remove cadmium from milk with high efficiency (75%) [26]. In general, *Lactobacillus* sp. have a high absorption capacity for heavy metals due to high proportion of peptidoglycan and teichoic acid in their cell walls [64]. It has been shown that lactic acid bacteria have high tendency to absorb heavy metals such as lead and cadmium in water and food [65]. Since these bacteria have a negative surface charge, they are appropriate to bind cations [66]. The candidate microorganisms in heavy metals' bio-removal are included to *L. acidophilus* sp. [26,59,66,67], *L. plantarum* [65,68], *B. coagulans* [66], *L. bulgaricus* [69], *L. bulgaricus* [64], *B. subtilis* [70], *L. fermentum* [71], *L. casei* [72], *B. lactis* [73], *L. buchneri*, *B. longum*, and *L. rhamnosus* [66,74]. *S. cerevisiae* is an ideal microbial model for identifying the mechanisms of biosorption, especially at cellular level [75]. Biosorption of lead and cadmium [17,19,20], Hg [21,26], Cr [75,76], Se [77], Ni and Co [78], and Cu [79] by *S. cerevisiae* has been studied.

4.Effective factors in microbial biosorption

4.1.pH

pH is one of the main factors in heavy metal removal. The biosorption process depends on pH. It is reported that by increasing pH in a controlled range, cadmium removal is increased [2,80].

In high acidic and alkali medium, metal complexes may be destroyed. The optimal pH for metals absorption is different, but in the most studies the highest removal was observed at pH 5-7 by bacteria [80-82]. Bacterial cell wall may be damaged at low or high pH, and they would not be able to bind heavy metals [80]. Among all reported *Lactobacilli* sp., *L. fermentum* is the most sensitive bacterium to pH [82]. Optimum pH for the absorption of different elements is different. For example, the optimum pH for Cu removal by *S. cerevisiae* is 5, whereas for Uranium is 4-5 [2,83]. However, the maximum bioremediation of heavy metals by *S. cerevisiae* is occurred at pH > 5 [2,84].

4.2. Biomass concentration

Bio-removal, as a surface process, occurs after binding of metal cations to the anionic groups on the bacterial surface and the yeast cells' wall [2,16]. By increasing the biomass concentration, a faster bio-removal is occurred [63]. As mentioned above, *Lactobacillus* strains have been reported to have a high affiliation to heavy metals [86,87].

4.3.Temperature

For an efficient biosorption, temperature should be in the optimum range. High temperature kills the bacteria and low temperature inactivates them [63]. The highest bio-removal capacity has been observed in the range of 15-40 °C for bacteria [85,86]. As a general rule, the optimum bio-removal temperature is determined by bacteria spp., medium, and type of heavy metal [2]. The highest biosorption capacity of *S. cerevisiae* for Pb, Ni, and Cr removal was observed in the range of 15-40 °C [48]. Pb and Ni was reported to be eliminated at 25 to 40 °C [16].

4.4.Contact time

A short contact time would decrease the bio-removal efficiency [63]. Different bacteria show different bio-removal ability and the bacteria should be compatible with the medium and also the metal ions [66]. It is clear that by increasing the contact time, more metal ions bind to the micro-organism, by which the bio-removal efficiency would increase [26].

5. Conclusion

Bioremediation is an effective, green, ecofriendly, inexpensive, and easy technique for decontamination of heavy metals from soil, food, and water. In order to increase the bio-removal efficiency, some technical aspects should be considered. Selection of appropriate microorganism is of great importance in heavy metals' bio-removal. The selected strain should be highly tolerant with fast and stable growth. In addition, matrix of the media (food, drinking or waste water, and soil) should be optimized in term of lipid, carbohydrate, and the other nutrients required for growth of the microorganisms. The processing factors including pH, contact time, temperature, biomass amount, and metals' concentration are of external parameters with significant effect in the yield of bio-removal. Stability of the binding between the microorganism and the pollutant should be monitored due to the possible risk of release in the gastrointestinal tract. However, further research is needed for better understanding of the mechanisms as well as improving the equilibrium and the kinetic models for heavy metals' bio-removal by micro-organisms. Importantly, innovation in bio-techniques is needed to reduce the overall operating cost and enhance the final efficiency.

6. Conflict of interest

The authors declare that there is no conflict of interest.

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