Chapter 26
Exploration of Heavy Metal Removal by Bacteria – Current State and Future Prospects

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INTRODUCTION

Water is undeniably the most valuable natural resource existing on our planet without which the existence of life is impossible. Although strict environmental regulations with regard to contaminants discharged from industrial operations are being introduced, still the condition is very pathetic particularly for potable water. Water is polluted mainly by direct and indirect sources. Heavily polluted water as effluents from various industries, refineries and waste water plants constitutes the direct sources whereas indirect sources include contaminants that enter the water supply from soils/ground water systems and from the atmosphere via rain water. These contaminants can be organic as well as inorganic. Some organic water pollutants include industrial solvents, volatile organic compounds, insecticides, pesticides and food processing wastes, etc. Inorganic water pollutants include metals, fertilizers and acidity caused by industrial discharges, etc.

Large number of industries uses metals including metallurgical, mining, electronic, metal finishing and electroplating. The presence of metal ions in final industrial effluents is detrimental to both lower and higher organisms. The metals have a tendency to accumulate to toxic levels and cause ecological damage [1]. Mercury, lead, cadmium and chromium (VI) are regarded as important toxic metals. Some radionuclides such as uranium exhibit high toxicity and radioactivity even at small concentration.The maximum contaminant level (MCL) standards for heavy metals established by United States Environment Protection Agency [2] are summarized in Table 1.

Table 1: Maximum contaminant level (MCL) of metals according to United States Environment Protection Agency

Sr. No	Heavy metal	Toxicities	MCL (mg/L)
1	Arsenic	Skin manifestations, visceral cancers, vascular disease	0.050
2	Cadmium	Kidney damage, renal disorder, human carcinogen	0.01
3	Chromium	Headache, diarrhea, nausea, vomiting, carcinogenic	0.05
4	Copper	Liver damage, Wilson disease, insomnia	0.25

5	Nickel	Dermatitis, nausea, chronic asthma, coughing, human carcinogen	0.20
6	Zinc	Depression, lethargy, neurological signs and increased thirst	0.80
7	Lead	Damage the fetal brain, diseases of the kidneys, circulatory system, and nervous system	0.006
8	Mercury	Rheumatoid arthritis, and diseases of the kidneys, circulatory system, and nervous system	0.00003

Strategies for Heavy Metal Removal

Extensive research in the field of heavy metal removal has brought to the forefront two important methods: biotic and abiotic. Abiotic method includes adsorption, precipitation, ion exchange, membrane filtration and electro dialysis technologies. Biotic method includes biosorption and bioaccumulation as the principle methods. A diagrammatic representation of various methods for heavy metal removal is described in figure 1.

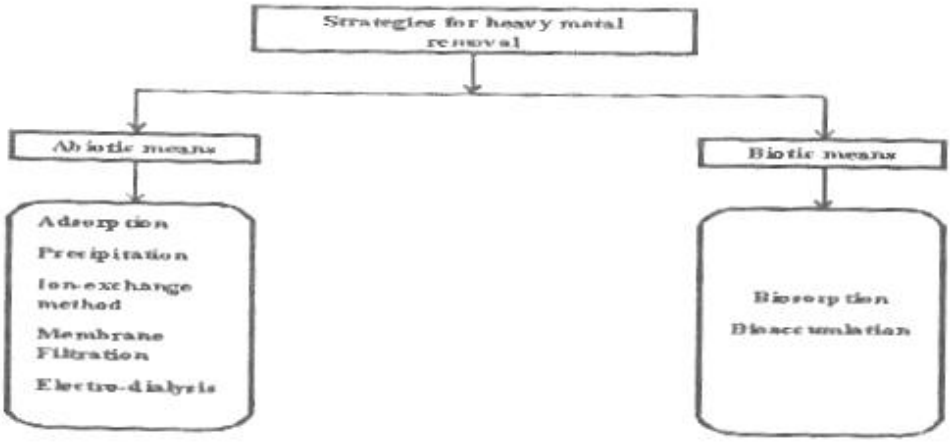

Fig. 1: Various methodologies for heavy metal removal

Abiotic methodologies

Various abiotic methods for removal of heavy metals, principles involved and related description are given below:

Adsorption

Adsorption is a separation process in which certain components of the fluid phase are transferred to the surface of the solid adsorbents. When a solid surface is exposed to a fluid phase, the molecules from the bulk of the fluid phase have tendency to accumulate or concentrate at the surface of a solid. The phenomenon of the enrichment of chemical substances at the surface of a solid is called 'adsorption'. All adsorption performance processes depends on solid-liquid equilibria and on mass transfer rates. Most adsorbents are highly porous materials, and

adsorption takes place primarily on the walls of the pores or at the specific sites inside the particle. Separation occurs because differences in molecular weight, shape, or polarity cause some molecules to be held more strongly on the surface than others or because the pores are too small to admit the larger molecules. The overall rate of adsorption is controlled by the rate of diffusion of solute within the capillary pores of the adsorbent and varies with square root of the contact time with the adsorbent. The adsorption operation can be batch, semi-batch and continuous. However adsorption method of heavy metal removal is nonspecific and cannot be employed for selective removal of metal ions.

Precipitation

The heavy metal contents of wastewaters can be effectively removed to acceptable levels by precipitating the metal in an insoluble form.Heavy metals are typically precipitated from waste water as hydroxides, sulfides or sometime sulfates and carbonates. Precipitation by hydroxide formation is the most common heavy metal precipitation method. The precipitation typically follows the reaction:

$$M^{+n} + nOH^{-} \longleftrightarrow M(OH)_n$$

Many heavy metals are amphoteric. Therefore their solubility reaches a minimum at a specific pH (different for each metal). Particular should be taken to destroy the complexing agent if it is present in the water. For precipitation of heavy metals as sulphides, addition of sulphide ions is required. However, this method of heavy metal removal has its limitations like evolution of H_2S if the pH is not carefully maintained in the alkaline region.

Ion-exchange

Ion-exchange method of heavy metal removal is also one of the important strategies for heavy metal removal from polluted water. Ion exchange is a water treatment method where one or more undesirable contaminants are removed from water by exchange with another non-objectionable or less objectionable substance. Both the contaminant and the exchanged substance must be dissolved and have the same type (+,-) of electrical charge. Typically, ion exchangers are ion exchange resins, zeolites, montmorillonite, clayand soil humus. They can be cationic exchanger, anionic exchanger or both. Depending on their chemical structure, they can be selective as well as non-selective. Ion exchange is a reversible process and the ions exchanger can be regenerated or loaded as required. These exchanges take place without any physical alteration to the ion exchange material. The process has some disadvantages in that there are substances occurring in some water (such as organic matter or

Fe^{3+} ions) which can foul the resin and decrease the overall efficiency of the process.

Membrane filtration

Membrane filtration has received considerable attention for the treatment of inorganics, since it is capable of removing not only suspended solid and organic compounds, but also inorganic contaminants such as heavy metals.

Membranes provide physical barriers that permit the passage of materials only up to a certain size, shape or character. Depending on the size of the particle that can be retained, various types of membrane filtration such as ultrafiltration, nanofiltration and reverse osmosis can be employed for heavy metal removal.

Ultrafiltration is a pressure-driven process that removes metals and other pollutants from water. Reverse osmosis depends on ionic diffusion to effect the separation. Nano filtration functions similarly to RO, but is generally targeted to remove only divalent and larger ions.

Electro-dialysis

Electrodialysis uses electrical current as the main driving force in matter separation. This limits the possible solutes targeted for recovery separation to charged particles and is rarely used for heavy metal pollution removal.

Table 2: Advantages and disadvantages of abiotic method for heavy metal removal

Sr. No	Method	Advantages	Disadvantages
1	Adsorption	Wide scope of application	Nonselective, saturable
2	Precipitation	Simple, cheap	For high concentrations, difficult separations, generates sludge
3	Ion exchange	Effective, Possibility of Metal recovery	Sensitive to particles, expensive
4	Membrane filtration	Efficient	Limited lifetime of membrane
5	Electrodialysis	Possibility of Metal recovery	For high concentrations, expensive

Biotic methodologies

Biosorption

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent normally water) containing dissolved species to be sorbed. Due to high affinity of sorbent for the sorbate specie, the latter is attracted and removed by different mechanisms. The process continues till equilibrium is established between amount of solid-bound sorbate species and its portion remaining in the solution. Biosorption is advantageous with respect to other conventional method. Major advantages include high efficiency, low cost, minimization of chemical and biological sludge, possibility of metal recovery and regeneration of bio-sorbent [3]. However its major disadvantage in saturation of biosorbent and the consequent decrease in the efficiency of process. The mechanism of biosorption is a complicated process. The mechanism of metal biosorption is influenced by status of biomass (living or dead), type of biomaterial, properties of metal solution chemistry, pH etc. The metal biosorption process by living cell is a two-step process. In the first step, metal ions are adsorbed to the surface of cell by interactions between metals and functional group displayed on surface of cell. All the metal ions before gaining access to the cell membrane and cell cytoplasm come across the cell wall. The cell wall consists of variety of polysaccharide and proteins and hence offers a number of active sites capable of binding metal ions. Metal uptake by non-living cell is mainly in the passive mode and occurs in two stages: passive uptake which takes place immediately and active uptake which takes place slowly.

Bacteria as biosorbent: The ability of some microorganism to accumulate metallic elements was witnessed in early 1980's. Numerous research reports have been published from toxicological points of view, but these were concerned with the accumulation due to the active metabolism of living cells, the effects of metal on the metabolic activities of the microbial cell and the consequences of accumulation on the food chain [4].However, further research has revealed that inactive/deadmicrobial biomass can passively bind metal ions via various physicochemical mechanisms. With this new finding, research on biosorption became active with numerous biosorbents of different origins being proposed for the removal of metals/dyes. Researchers have understood and explained that biosorption depends not only on the type or chemical composition of the biomass, but also on the external physicochemical factors and solution chemistry. Many investigators have been able to explain the mechanisms responsible for biosorption, which may be one or combination of ion exchange, complexation, coordination,

adsorption, electrostatic interaction, chelation and micro precipitation[5]. A number of bacterial species have shown promising results for heavy metal biosorption. A comprehensive list of various metals biosorbed by bacteria is given as table 3.

Table 3: Bacteria reported for heavy metal removal by biosorption

Sr. No	Bacteria	Metal	Reference
1	Aeromonascaviae	Chromium (VI)	[6,7]
2	Bacillus coagulans		[8]
3	Bacillus licheniformis		[9].
4	Bacillus megaterium		[8]
5	Bacillus thuringiensis		[10]
6	Chryseomonasluteola		[11]
7	Pseudomonas sp.		[12]
8	Staphylococcus xylosus		[12]
9	Bacillus subtilis IAM 1026	Copper	[13]
10	Enterobacter sp. J1		[14]
11	Micrococcus luteus IAM 1056		[13]
12	Pseudomonas aeruginosa PU21		[15]
13	Pseudomonas cepacia		[16]
14	Pseudomonas putida		[17]
15	Pseudomonas stutzeri IAM 12097		[13]
16	Sphaerotilusnatans		[18]
17	Streptomyces coelicolor		[19]
18	Thiobacillusferrooxidans		[20]
19	Thiobacillusferrooxidans		[21]
20	Aeromonascaviae	Cadmium	[6,7]
21	Bacillus circulans		[22]
22	Pseudomonas putida		[17]
23	Staphylococcus xylosus		[12]
24	Pseudomonas aeruginosa PU21		[15]
25	Staphylococcus xylosus		[12]
26	Streptomyces rimosus		[23]
27	Bacillus sp. (ATS-1)	Lead	[24]
28	Corynebacteriumglutamicum		[25]
29	Enterobacter sp. J1		[14]
30	Pseudomonas aeruginosa PU21		[15]
31	Pseudomonas putida		[26]
32	Streptomyces rimosus		[23]
33	Streptoverticilliumcinnamoneum		[27]
34	Lactobacilliusbulgaricus		[28]
35	Bacillus thuringiensis	Nickel	[29]
36	Streptomyces rimosus		[23]

37	Arthrobacternicotianae IAM 12342	Thorium	[30]
38	Bacillus licheniformis IAM 111054		[30]
39	Bacillus megaterium IAM 1166		[30]
40	Bacillus subtilis IAM 1026		[30]
41	Corynebacteriumequi IAM 1038		[30]
42	Corynebacteriumglutamicum IAM 12435		[30]
43	Micrococcus luteus IAM 1056		[30]
44	Nocardiaerythropolis IAM 1399		[30]
45	Zoogloearamigera IAM 12136		[30]
46	Pseudomonas sp. (strain MTCC 3087)	Thorium, Uranium	[31]
47	Citrobacter freudii	Uranium	[32]
48	Arthrobacternicotianae IAM 12342		[30]
49	Bacillus licheniformis IAM 111054		[30]
50	Bacillus megaterium IAM 1166		[30]
51	Bacillus subtilis IAM 1026		[30]
52	Corynebacteriumequi IAM 1038		[30]
53	Corynebacteriumglutamicum IAM 12435		[30]
54	Micrococcus luteus IAM 1056	Uranium	[30]
55	Zoogloearamigera IAM 12136		[30]

Mechanism of bacterial biosorption: The bacterial cell wall is the first component that comes into contact with metal ions/dyes, where the solutes can be deposited on the surface or within the cell wall structure [33,34]. Since themode of solute uptake by dead/inactive cells is extracellular, the chemical functional groups of the cell wall play vital roles in biosorption. Due to the nature of the cellular components, several functional groups are present on the bacterial cell wall, including carboxyl, phosphonate, amine and hydroxyl groups [34, 35]. As they are negatively charged and abundantly available, carboxyl groups actively participate in the binding ofmetal cations. Several dye molecules, which exist as dye cations in solutions, are also attracted towards carboxyl and other negatively charged groups. Golab and Breitenbach [36] indicated that the carboxyl groups of the cell wall peptidoglycan of *Streptomyces pilosus* were responsible for the binding of copper. Also, amine groups are very effective at removing metal ions, as it not only chelates cationic metal ions, but also adsorbs anionic metal species or dyes via electrostatic interaction or hydrogen bonding. Kang and co-workers [37] observed that amine groups protonated at pH 3 and attracted negatively charged chromate ions via electrostatic interaction. Vijayaraghavan and Yun confirmed that the amine groups of *C. glutamicum* were responsible for the binding of reactive dye anions via electrostatic attraction [38]. In general, increasing the pH increases the overall negative charge on the

surface of cells until all the relevant functional groups are deprotonated, which favors the electrochemical attraction and adsorption of cations. Anions would be expected to interact more strongly with cells with increasing concentration of positive charges, due to the protonation of functional groups at lower pH values. The solution chemistry affects not only the bacterial surface chemistry, but the metal/dye speciation as well. Metal ions in solution undergo hydrolysis as the pH increases. The extent of which differs at different pH values and with each metal, but the usual sequence of hydrolysis is the formation of hydroxylated monomeric species, followed by the formation of polymeric species, and then the formation of crystalline oxide precipitates after aging [39]. For example, in the case of nickel solution, López et al. indicated that within the pH range from 1 to 7, nickel existed in solution as Ni2+ ions (90%); whereas at pH 9, Ni^{2+} (68%), $Ni_4OH_4^{4+}$ (10%) and $Ni(OH)^+$ (8.6%) co-existed [40]. The different chemical species of a metal occurring with pH changes will have variable charges and adsorbability at solid–liquid interfaces. In many instances, biosorption experiments conducted at high alkaline pH values have been reported to complicate evaluation of the biosorbent potential as a result of metal precipitation [41].

Techniques of enhancing biosorption capabilities: Modification of the binding sites on a biomass seems to enhance the biosorption capacities by multiple folds. Carboxyl, amine, phosphonate, sulfonate and hydroxyl groups have become well established as being responsible for metal binding. As the density of these groups is low, most biosorbents show low sorption capacities. Various procedures are available for the enhancement of these functional groups on the biomass. In general, futile/less important functional groups can be converted into active binding groups via several chemical treatment methods. Jeon and Höll used chloroacetic acid to introduce carboxyl in the place of hydroxyl groups [42]. Carboxylated biomass was treated with ethylenediamine followed by carbodiimide to form aminated biomass. Li and researchers employed citric acid to modify an alkali-saponified biomass, which increased the total acidic sites, but a decrease of basic sites. In particular, they reported that biomass modified using 0.6 mol/L citric acid at 80 °C for 2 h exhibited cadmium uptake capacity twice than that of the raw biomass [43].

Many studies have focused on enhancing the active binding sites to improve the biosorption; however, less attention has been paid to the inhibition sites. For instance, amine groups are responsible for the

binding of dye anions via electrostatic interaction; whereas the presence of negatively charged groups, such as carboxyl, may repel dye anions.

Another efficient way for the introduction of functional groups onto the biomass surface is the grafting of long polymer chains onto the biomass surface via direct grafting or polymerization of a monomer. However, very little research has focused specifically on this aspect. Deng and Ting [44-47] worked extensively with polyethylenimine, composed of a large number of primary and secondary amine groups, which when cross-linked with biomass exhibited good biosorption abilities towards chromium (VI), copper, lead, nickel and arsenic.

Genetic interventions are also employed for the improvement of biosorbent action as they have the potential improve or redesign microorganisms. Genetic modification is a potential solution to enhance the selectivity as well as the accumulating properties of the cells [48]. Higher organisms respond to the presence of metals, with the production of cysteine-rich peptides, such as glutathione (GSH) [49], phytochelatins (PCs) and metallothioneins (MTs) [50] which can bind and sequester metal ions in biologically inactive forms [51]. The overexpression of MTs in bacterial cells will result in an enhanced metal accumulation and; thus, offers a promising strategy for the development of microbial-based biosorbents for the remediation of metal contamination [48]. In addition to the high selectivity and accumulation capacity, Pazirandeh and coworkers demonstrated that the uptake by recombinant *E. coli* (expressing the *Neurosporacrassa* metallothionein gene within the periplasmic space) was rapid. Greater than 75% Cd uptake occurred in the first 20 min, with maximum uptake achieved in less than 1 h [48]. However, the expression of such cysteine-rich proteins is not devoid of problems, due to the predicted interference with redox pathways in the cytosol. More importantly, the intracellular expression of MTs may prevent the recycling of the biosorbents, as the accumulated metals cannot be easily released [52]. Chen and Georgiou suggested a solution to bypass this transport problem by expressing MTs on the cell surface [53]. Sousa and coworkers demonstrated the possibility of inserting MTs into the permissive site 153 of the LamB sequence [54].The expression of the hybrid proteins on the cell surface dramatically increased the whole-cell accumulation of cadmium. Also, the expression of proteins on the surface offers an inexpensive alternative for the preparation of affinity adsorbents [55].

Attempts to create *recombinant bacteria* with improved metal binding capacity have so far been restricted to mostly Escherichia coli.

This is because *E. coli* greatly facilitates genetic engineering experiments and it is found to have more surface area per unit of cell mass, which potentially should give higher rates of metal removal from solution [56]. Nevertheless, a Gram-positive surface display system also possesses its own merits compared to Gram-negative bacteria [57,58]: (a) translocation through only one membrane is required, and (b) Gram-positive bacteria have been shown to be more rigid and; therefore, less sensitive to shear forces [59] due to the thick cell wall surrounding the cells, which potentially make them more suitable for field applications, such as bioadsorption. Samuelson and coworkers generated recombinant *Staphylococcus xylosus* and *Staphylococcus carnosus* strains, with surface-exposed chimeric proteins containing polyhistidyl peptides. Both strains of staphylococci gained improved nickel-binding capacities due to the introduction of the H1or H2 peptide into their surface proteins[58]. Owing to their high selectivity, genetically engineered biosorbents may prove very competitive for the separation of toxins and other pollutants from dilute contaminated solutions.

Desorption: Biosorption is a process of treating pollutant-bearing solutions to make it contaminant free. However, it is also necessary to be able to regenerate the biosorbent. This is possible only with the aid of appropriate elutants, which usually results in a concentrated pollutant solution. Therefore, the overall achievement of a biosorption process is to concentrate the solute, i.e., sorption followed by desorption. Desorption is of utmost importance when the biomass preparation/generation is costly, as it is possible to decrease the process cost and also the dependency of the process on a continuous supply of biosorbent. A successful desorption process requires the proper selection of elutants, which strongly depends on the type of biosorbent and the mechanism of biosorption. Also, the elutant must be nondamaging to the biomass, less costly, environmental friendly and effective. Several investigators have conducted exhaustive screening experiments to identify appropriate elutants for this process. Of these, the Kuyucak and Volesky [60] examined several chemical agents to desorb Co^{2+} from cobalt-laden *Ascophyllum nodosum*, and identified $CaCl_2$ in the presence of HCl as a suitable elutant.

The performance of an elutant also strongly depends on the type of mechanism responsible for the biosorption. For instance, electrostatic attraction was found to be the main mechanism responsible for the biosorption of negatively charged dye anions to a positively charged cell surface [61]. Therefore, it would be logical to make the cell surface

negative using alkaline solutions to repel the negatively charged reactive dyes [62].

The ultimate purpose of a biosorption process is to concentrate solute. Very high concentrations, in the order of 10 times higher than that of the initial solute, can commonly be expected by the end of elution process. The recovery of a solute from these high concentrated solutions can be accomplished using another process, such as precipitation or electrowinning. Binupriya and coworkers desorbed Reactive blue MR from dye-loaded *Trametes versicolor* using ethanol and; thereby suggested that Reactive blue MR-rich ethanol medium can be distilled to remove dyes and the recovered dye can be used as low-grade dyes in colored glass, plastic and ceramic industries [63].

Bioaccumulation

Bioaccumulation can be defined as the uptake of toxicants by living cells. The toxicant can transport into the cell, accumulate intracellularly, across the cell membrane and through the cell metabolic cycle [64]. However the overall cost of bioaccumulation is high because the cell maintenance is cost prone. Also the effects of extreme pH conditions, time consumption, little possibility of regeneration and reuse and the requirement of external metabolic energy makes the process of bioaccumulation uncommon.

FUTURE PROSPECTS

Bacterial biomass represents an efficient and potential class of biosorbents for the removal of both dyes and metal ions. Unfortunately, the difficulties in reusing the microbial biomass, as well as the poor selectivity, hinder their applications under real conditions. Although some attempts have been made at the commercialization of biosorption for wastewater treatment, the progress is very modest considering that there has been more than a decade of fundamental research. The important features required for the successful application of biosorption technology to real situations include, but are not limited to:

- Screening and selection of the most promising biomass, with sufficiently high biosorption capacity and selectivity.
- Optimizing the conditions for maximum biosorption, including optimization of pH, temperature, ionic strength and co-ion effects, etc
- Improving the selectivity and uptake via chemical and/or genetic modification methods.

- Examining the mechanical strength of biomass and if insufficient for reuse, improving rigidity by proper immobilization or other chemical methods.
- Testing the performance of biosorbents under different modes of operation.
- Analyzing the behavior of biosorbent for use with real industrial effluents and, simultaneously analyzing the impact of water quality on the biosorption uptake of the specific pollutant of interest.

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