

Role of Bacteria in Bioremediation of Heavy Metals

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A total of nineteen metal-resistant and non-resistant bacteria from activated sludge treating both metal-contaminated industrial effluents and municipal wastewater were isolated and identified. Among these isolates, *Pseudomonas pseudoalcaligenes* and *Micrococcus luteus* were selected for further investigations due to their high copper and lead biosorption capacities. Cells of *M. luteus* could be used for at least five alternate biosorption and desorption cycles without loss of copper removal capacity. Immobilization of *M. luteus* in 2% calcium alginate and 10% polyacrylamide gel bead increased copper uptake by 61%. *M. luteus* and *P. pseudoalcaligenes* may have potential applications in removing and recovering copper and lead respectively from industrial effluents. In this paper we review the role of bacteria in Bioremediation of Heavy metals.

Keywords: Bacteria, Bioremediation, heavy metals.

Environmental problems associated with heavy metals are very difficult to solve in contrast to organic matters because incineration or biodegradation can transform the latter. As a fact, most of heavy metals have toxic effects on living organisms when exceeding a certain concentration. Furthermore, some heavy metals are being subject to bioaccumulation and may pose a risk to human health when transferred to the food chain (USEPA, 1987). The scientific literature reveals that heavy metals (such as Lead, Mercury, Arsenic, Cadmium etc.) has been recognized as a potent human toxins with reports of many diseases, such as brain damages and mental disabilities associated with ingestion dating to the last century. In the last two decades environmental interests has induced much

research concentration and focused on the effects of toxic metals on the ground water because they ultimately reach and accumulate in plants and animals tissues (Environ, 1988). According to the water standards used by the World Health Organization, levels of heavy metals such as lead ions in wastewater must be controlled and reduced to set value (USEPA, 1986). It is recognized that mine wastes have been generated for several centuries, and mining activity has accelerated significantly during the 20th century.

Chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis and solvent extraction are the most commonly used techniques for the removing of heavy metals ions from dilute solutions. Furthermore, these techniques of purification are often inappropriate or expensive, particularly when unwanted heavy metals are present in very low concentration or in large solution volume. For instance, some of the proposed methods for removal of heavy metals, like adsorption on zeolites, clay minerals, and

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synthetic organic ion exchangers, have its shortcomings. Zeolites tend to decompose in the presence of high pH; clays cannot sustain selectivity at high sodium concentration (Amphlett, 1964). Furthermore, these traditional techniques present significant disadvantages, such as energy requirements, and are very expensive when the contaminant contents are in the range of 10-100 mg/l (Wilde and Benemann, 1993). Thus, for many years chemical engineers have been attracted to new techniques of water treatment requiring less energy and less pollutant. One of these techniques is the use of micro-algae and microorganisms as to degrade or detoxify hazardous elements. Avery and Tobin (1992) reported the use of *Saccharomyces cerevisiae* in order to adsorb divalent metal cations in dilute solutions into the cell walls, both alive or denatured. They reported particularly high selectivity for Sr²⁺ atoms. Khoshmanesh and co-workers (1995, 1997) used green microalgae in the removal of heavy metals from polluted waters. Maeda and Skaguchi (1990) have indicated the importance of algae and their role in the bioremediation of toxic metals. Aladhab and co-workers (1997) used the same technique as part of a process that was proposed to remove traces of radioactive elements found in water. The algae applied in the process were collected from fresh water coming from a hot spring located in Al-Ain in the Emirate of Abu Dhabi.

Toxic metal wastes from defense-related activities, industry, and municipal sources have routinely entered the environment through disposal in landfill sites or by accidental release in accidents. These practices have resulted in surface contamination problems, transport to groundwater, and/or bioaccumulation of radionuclides and toxic metals. Metals such as Cs, Sr, Cd, and, to a lesser extent, Co are prevalent in soils near industrial centers at concentrations up to 50 u.g of Cs/g, 350 u.g of Cd/g, and 500 ug of Sr/g (31). As cocontaminants, toxic metals are often inhibitory to other bioremediative processes, e.g., hydro-carbon degradation .

Microbial communities are of primary importance in bioremediation of metal-contaminated soils and represent a substantial proportion of the *in situ* biomass and metabolic diversity. The structure and diversity of soil

microbial communities have been shown to change in soil in the presence of toxic metals. Microorganisms can alter metal chemistry and mobility through reduction, accumulation, mobilization, and immobilization. Since metal ion species are generally more readily soluble in acidic environments, acidogenic microbial metabolic activities may contribute to the introduction of metals into groundwater from contaminated soils.

Over the past decade, the consumption of metals and chemicals in the process industries has increased dramatically. Industrial uses of metals such as metal plating, tanneries, industrial processes utilizing metal as catalysts, have generated large amount of aqueous effluents that contain high levels of heavy metals. These heavy metals include cadmium, chromium, cobalt, copper, iron, manganese, mercury, molybdenum, nickel, silver, and zinc. Metal-polluted industrial effluents discharged into sewage treatment plants could lead to high metal concentrations in the activated sludge. Microbial populations in metal polluted environments contain microorganisms which have adapted to toxic concentrations of heavy metals and become "metal-resistant" (Kasan and Baecker, 1989).

At present, metal-polluted industrial effluents are mostly treated by the chemical methods, such as chemical precipitation, electrochemical treatment and ion exchange. These methods provide only partially effective treatment and are costly to implement, especially when the metal concentration is low. The alternative use of microbe-based biosorbents. Thus, Biosorption, (which is removal using sorption onto living/dead microorganisms) for the removal and recovery of toxic metals from industrial effluents can be economical and effective methods for metal removal. The metal removal ability of microorganisms including bacteria (Chua *et al.*, 2000), microalgae and fungi (Lo *et al.*, 1999) has been studied extensively. The heavy metal removal capacity is higher than those conventional methods and the uptake of heavy metals can be selective (Unz and Shuttleworth, 1996). Microbial cells can also be supplied inexpensively as waste in industrial fermentation processes as well as biological wastewater treatment plants (Volesky and May-Phillips, 1995).

Salient Features of the Biosorbent

Highly porous; specific surface area of 256 sq. m per g.

1. Exhibits high uptakes for the tested toxic trace metals (for eg., 570% and 690% of the best uptake values reported in the literature at the corresponding equilibrium solution metal concentrations, for cobalt and nickel, respectively).
2. Can be used over a wide temperature (30 to 45 C) and pH (2 to 8) ranges with little change in uptake.
3. Sorbed metal can be recovered through desorption.
4. Can be re-used; also has a good shelf-life.
5. Good handling characteristics; can be used in any reactor configuration (i.e. mixed tank, fixed bed and others).
6. Inexpensive and relatively simple to prepare.

Studies were also carried out to characterize the metal biosorption behavior of the metal resistant and non-resistant bacteria in an activated sludge process treating both municipal and metal-contaminated industrial wastewater. Heavy metals studied include Copper, nickel, zinc, lead and chromium. Two bacterial strains, *Pseudomonas pseudo-alcaligenes* and *Micrococcus luteus* that are effective in removing metals, were selected. The effects of initial metal concentration, pH, competing cations, desorption and immobilization on the metal biosorption capacities of the two selected strains have been studied extensively.

METHODS

Procedure

Procedures for the isolation and identification

Fresh activated sludge was collected from the return sludge channel at a local sewage treatment works. The activated sludge was serially diluted with distilled and deionized (DDI) water. Aliquots (0.1 ml) were spread on nutrient agar and cultivated in Shell Lab model 2020 incubator at 30°C for three days. The isolated colonies were identified by MIDI Sherlock Microbial Identification System and API 20NE as well as 20E systems. For copper, nickel, zinc, lead and chromium resistant bacteria, aliquots (0.1 ml) of serially diluted sludge were spread over nutrient

agar containing 100 mg/l of the metals and incubated at 30°C for three days. The isolated colonies were identified. Each of these nineteen isolates were screened for biosorption of chromium, copper, lead, nickel and zinc with initial metal concentration of 100 mg/l and cell concentration of 1-2 g dry cell/l at pH 5.

Biosorption isotherms

To determine the metal biosorption isotherms, batch biosorption experiments were conducted in polypropylene bottles using *Pseudomonas pseudoalcaligenes* and *Micrococcus luteus*. These two strains (1-2 g dry cell/l) were suspended in solutions containing different metal concentrations. After 24 h of incubation at pH 5 and 25°C, samples were taken from the solutions, centrifuged (8000 rpm), decanted and the supernatant analyzed for metal by flame or graphite furnace atomic absorption spectroscopy.

To determine the effect of pH on copper and lead biosorption, the metal solutions and the bacterial suspensions were adjusted separately to the desirable pH (2.0-6.0) by 0.1 M NaOH and 0.1M HNO₃. They were then mixed and incubated at initial metal concentration 100 mg/l at 25°C on an orbital shaker for 24 h.

Effect of cations on lead biosorption

The effects of copper and nickel on lead biosorption of *P. pseudoalcaligenes* were also studied by adding 0.2 to 2.0 mmol/l copper or nickel metal ions into the lead solution.

Biosorption and desorption cycles

Cells of *M. luteus* with final concentration at 1.5 g dry cell/l were suspended in 25 ml solutions containing 0.787 mmol Cu²⁺A. The pH of the resulting mixtures was adjusted to 6.0 by HNO₃ and NaOH. The mixture was shaken at 250 rpm and 25°C for six hours. The copper sorbed biomass was centrifuged, rinsed with DDI water and resuspended in 10 cm³ 0.05 M H₂SO₄ for 45 minutes in order to recover the copper ions from cells. The change of metal concentration during desorption was measured by taking samples from the solution. The regenerated biomass was again suspended in copper solutions for the next biosorption run. These biosorption and desorption steps were repeated five times. The copper concentrations in the supernatants were determined after biosorption and 234 desorption.

Immobilization studies

Cells of *M. luteus* were harvested during the exponential phase of growth and resuspended in solution containing 4% sodium alginate, 18.2% acrylamide and 1.8% N,N'-methylene-bis-acrylamide. The resulting mixture was dropped into solution containing 4% calcium chloride, 0.1% N,N,N',N'-tetramethylethylene-diamine and 0.1% ammonium persulfate. The drops gelled and formed particles with diameter $2.3 \text{ mm} \pm 0.3 \text{ mm}$ upon contact with the calcium chloride solution. The immobilized cell beads were left in the solution at 4°C with constant and gentle stirring for at least two hours to complete gel formation. The beads were then transferred into fresh nutrient (Oxid Nutrient Broth) for 12 hours for further cell growth. Finally, the beads were taken out, washed with DDI water and stored at 4°C before use. The immobilized cell beads were prepared under aseptic conditions.

A total of nineteen metal-resistant and non-resistant bacteria from activated sludge treating both metal-contaminated industrial effluents and municipal wastewater were isolated and identified till date. These includes both Gram-positive (e.g. *Micrococcus*) and Gram-negative (e.g. *Pseudomonas*) bacteria. The biosorption capacity of these strains for five different heavy metals (copper, nickel, zinc, lead and chromium) was determined at pH 5 and initial metal concentration 100 mg/L. *Pseudomonas pseudoalcaligenes* and *Micrococcus kiteus* were found to be capable of removing significant amounts of copper and lead. Hence, they were selected for further investigations. Langmuir adsorption isotherms adequately represented the distribution of copper and lead for both species. The study of pH effect on metal removal for both species indicated that the metal biosorption increased with increasing pH from 2 to 6. The effect of competing cations on biosorption of *P. pseudoalcaligenes* was studied. Sulphuric acid (0.05 M) was the most efficient desorption medium among the eleven reagents tested. Over 90% of copper sorbed on the cells of *M. Metis* could be recovered by washing with 0.05 M sulphuric acid within five minutes. The biosorbent was used for at least five biosorption and desorption cycles without loss of copper removal capacity. Immobilization of *M. luteus* in 2%

calcium alginate and 10% polyacrylamide gel beads increased copper uptake by 61 %.

DISCUSSION

Isolation and identification

Seven species of metal-resistant bacteria and twelve dominant species of metal non-resistant bacteria were isolated from activated sludge. Isolation of microbial strains from activated sludge or other waste streams for biosorption of heavy metals has been studied extensively. All the heterotrophic non-resistant isolates from the activated sludge showed wide varieties of species including Gram-negative and positive. Most of them were rods and a few bacteria were filamentous and coccus. *Pseudomonas*, *Bacillus* and *Aeromonas* species are commonly found in activated sludge. As for the metal-resistant bacteria, both Gram-negative (e.g. *Pseudomonas cepacia*) and Gram-positive (e.g. *Bacillus pumilus*) were identified which were widely isolated by other researchers (Kasan and Baecker, 1989). The *Pseudomonas* spp. are well documented for their resistance to chemical antimicrobial agents. One yeast strain was also identified (*Candida albicans*).

Biosorption of heavy metals

Among the 19 species tested, species *Micrococcus luteus* have the highest copper (biosorption capacity, mg metal/g dry biomass). The copper biosorption capacity of all the isolates is compared. For lead, the species *Bacillus subtilis*, *Pseudomonas pseudoalcaligenes* and *Micrococcus luteus* were about 0.3 mmol/g. Wong and Kwok (1992) and Wnorowski (1991) reported that heavy metal biosorption capacity of metal-resistant strains was higher than that of non-resistant strains. metal-resistant strains did not necessarily remove metals better than non-resistant strains. This suggested that metal removal (binding) could not be mainly attributed to their metal resistance mechanism. Among all the isolates, species (*Pseudomonas pseudoalcaligenes* and *Micrococcus luteus*) were the most effective bacteria in removing copper and lead from the aqueous solutions.

Effect of pH

The pH effect on copper removal by species *Micrococcus luteus* and lead biosorption

by species *Pseudomonas pseudoal-caligenes* showed great similarity. As the metal removal capacities of both species increased with increasing solution pH. At lower pH, biosorption of hydrogen ions was pre-ferred over that of metal ions, but at higher pH, more metal ions would be taken up. The pH biosorption studies were not conducted at pH values above 6, because insoluble metal hydroxide precipitated from the solution at higher pH values, making true biosorption studies impossible. The pH profile suggested that metal binding to cell walls and external surfaces was very likely the dominant removal process.

Effects of competing cations

The adsorption isotherms of lead ion in the presence of copper and nickel ions are studied. The presence of copper decreased the amount of lead adsorbed. This interference in biosorption of lead by copper ions indicated the possibility of overlap in the sorption sites. In the case of nickel inhibition), an insignificant decrease of lead uptake was observed. Lead may be preferentially adsorbed by the biomass or they were adsorbed at different binding sites.

Desorption studies

Eleven different desorption media were chosen and tested in the course of the present work. They include distilled deionized water (DD1), competing counter-ions, proton exchangers and complexing agents. Agents with the potential for competitive ion exchange reaction are sodium and calcium salts. Mineral acids (sulphuric acid) and organic acids (oxalic and cit-ric acids) were considered to be proton exchanging agents. Complexing agents included the chelating agents like EDTA, NTA, polyphosphate, as well as compounds containing chlo-ride, carbonate and bicarbonate anions. The effectiveness of using complexing agents as desorbing media is determined by the stability constants between the complexing ligands and the cationic metal ions. If they were greater than the constants for the sites on the bacte-rial surface and the metal ions, more metals can be chelated and retained by the desorption agent, and vice versa. For the competing counter-ions and proton exchangers, the strength of desorbing power was decided by the competitive abilities between the target metal ions and other cations presented for the active sites on the biomass surface.

Biosorption and desorption cycles

Repeated biosorption and desorption operations were performed to examine the reusability and metal recovery efficiency of the biomass. Due to the consideration of the post-treatment of recovery of metals from concentrated desorbing media, 0.05 M sulphuric acid was used for recovery of copper ions as their sulphate form can be recovered directly in the process of electrolysis. Five consecutive regeneration cycles were conducted with *M. luteus* using 0.05 M sul-phuric acid as desorbing medium. The copper biosorption capacity of the bio-mass had no significant difference from cycle 1 to cycle 5. For all runs, more than 90% of sorbed copper could be recovered and concentrated in a small volume of acid. The results indicated that *Micrococcus* sp. possess high reuse potential for removal and recovery of copper ions from wastewater.

Immobilization studies

The high surface to volume ratio of the microbial cells allow a better contact between the biosorbent surface and soluble ions and molecules (Butter *et al.*, 1996). However, because of the difficulty of separating cells from the solution phase, cell immobilization procedures may be required for the development of large-scale recovery processes. The most common strategies used to attain this goal include the colonization of cells on a solid substrate i.e. biofilm, or their immobilization in a polymeric or porous matrix (Rowe and Scott, 1996).

Cells of *M luteus* were immobilized in gel beads to remove copper ions from waste-water. Polyacrylamide-entrapped cells have good mechanical properties and are inert to microbial degradation (Nakajima *et al*, 1982). Calcium alginate is also a well known biopolymer. It is biocompatible, chemically resistant, inexpensive and easy to regenerate. Besides, it has a loose structure for overcoming diffusion limitations and provides a good model system for adsorption. Hence, the combined calcium alginate-polyacrylamide-based immobilization matrix offers a promising potential for whole cell immobilization in order to detoxify the metals-bearing solutions. Figure 10 depicts that immobilization of cells increased copper removal by 61 % compared with the control which contained no cells.

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