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Variations in the Bioaccumulation Rates of Heavy Metal by Immobilized and Free Gram-negative and Gram-positive Bacterial Cells Isolated From an aerated lagoon

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ABSTRACT

The uptake of heavy metals by all immobilized microbial cell strains (bacterial beads: bacterial cells in alginate) is better than by free cells. The average removal percentage for all six metals by Gram-positive bacterial beads showed that *Bacillus megaterium* (81%), *Bacillus flexus* (73%), *Bacillus amyloliquefaciens* (70%) and *Bacillus cereus* (66%). While Immobilized Gram-negative cells of *Pseudomonas veronii* and *Rahnella aquatilis* were good for the uptake of metals such as copper, nickel and cobalt. *Rahnella aquatilis* and *Pseudomonas veronii* accumulated Cu, Ni and Co by respectively (91.5%, 77 %), (90.1%, 85 %) and (75%, 74%), while Pb was only accumulated by *Pseudomonas veronii* (67 %). On the other hand, *Sphingobacterium daejeonense* and *Roseomona saquatica* were relatively weak in accumulating heavy metals. On the other hand, the uptake of heavy metals by all free microbial cell strains possessed no significant difference by varying metals type and bacterial strains, except Co and Pb were poorly accumulated. Immobilization in alginate beads provides a protective environment for these agents for sufficient time of heavy metals removal. Immobilized systems currently offer various advantages over free systems. *Rahnella aquatilis* was reported for the first time in this research as a potential organism for the bioaccumulation of some toxic heavy metals from domestic wastewaters.
Keywords: *Bacillus megaterium,* Gram–ve, Gram-positive, Heavy metals, Immobilization, *Pseudomonas veronii, Rahnellaaquatilis*.

INTRODUCTION

Heavy metals in different environmental compartments can be hazardous to ecosystems. The metals Cd, Pb and Zn among other heavy metals are main contaminants of ecosystems in Egypt (Chen *et al*., 2010). The ability of microbial strains to grow in the presence of heavy metals would be helpful in the wastewater treatment where microorganisms are represented to the inhibitory effect of heavy metals, which is a common phenomenon that occurs in the biological treatment of wastewater and sewage (Tsai *et al.,* 2013). Some heavy metals are toxic to bacteria grown in the surrounding polluted water. Immobilization technique may provide a protective shelter for such cells to help their growth survival and toxic metals accumulation. Immobilized Cells are usually entrapped in matrices such as alginate, polyacrylamide and agarose. In an immobilized system growth and production phases can be decoupled and controlled by chemical and physical stress conditions. This allows cells to be retained for extended periods, with alternate renewal growth and secondary metabolite production cycles (Covarrubias *et al.*, 2012).

The present study aims at applying the immobilization technique in order to offer a biological and chemical stability technique that may have a potential in wastewater treatment systems, and help in the bioremediation potential of immobilized microbial cells.

MATERIALS AND METHODS

Study site

The domestic wastewater treatment system of Ismailia was selected to carry out this study.

Heavy metal analysis

The heavy metals (Ni, Cu, Mn, Pb, Mg, and Co) were determined according to APHA standard methods, (2005) using Atomic Absorption Spectrometry.

Gram-negative and Gram-positive Bacterial Cells Isolated From an aerated lagoon

Bacillus megaterium, Bacillus amyloliquefaciens, Bacillus cereus, Bacillus flexus Pseudomonas veronii, *Rahnellaaquatilis*, *Roseomonasaquatica* and *Sphingobacteriumm daejeonense*were used and determined by previous study (Rashad, 2014).

Immobilization of bacterial cells in calcium alginate gel

From bacterial suspension in sodium alginate, beads of the same size were formed by direct instilling (using a syringe and a needle 0.9 mm in diameter) to a 0.2 M solution of calcium chloride (II). The immobilizate was left in the solution of calcium chloride (II) at a temperature of 4°C for 20 min for hardening, and then rinsed with sterile distilled water (Subhashini *et al.*, 2011). Analysis of heavy metals concentration of (Ni, Cu, Mn, Pb, Mg, and Co) in the bio-sorption medium was determined by Atomic absorption spectrophotometer. Specific metal uptake (q) by biomass (mg g^{-1}) was calculated as described by (Zhang *et al*., 1998):

$$q = V \times (Ci - Cf) / 1000\, W$$

Where V is the volume of the solution in the contact batch flask, Ci is the initial concentration of lead in the solution (mg ml^{-1}), Cf is the final concentration of lead in the solution and W is the mass of adsorbent (g).

Similarly biosorption efficiency R (%) can be calculated as, R = Ci-Cf/Ci x100%.

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Optimization for heavy metal removal

Temperature, pH, biomass, and heavy metal concentrations are the factors which affects the biosorption process. Particularly, pH, biomass concentration (Gong *et al*., 2005) and heavy metal concentration (Kiran *et al*., 2005) on biosorption experiments. Maximum removal of metals by bacterial strains occurs at 27°C (Gulay *et al.*,2003). *Bacillus* sp., and *Pseudomonas* sp. have the ability to adsorb maximum Cu, Cd and Pb at pH 7 (Rani *et al.*, 2010) which, similar to the results of Wang and Chen, 2006 and Blackwell *et al.* 1995. This pH range is widely accepted as being optimal for metal uptake of almost all types of biomass.

The maximum metal ion concentration was 30 µg/l for all bacterial strains according to growth measurement against different concentration of heavy metals (Puranik *et al.*, 1999).

Liquid cultures were pre-incubated in 100 ml metal-deficient nutrient broth until they reached mid-log phase and 1 ml bacterial sample was transferred in to 50 ml nutrient broth supplemented with heavy metal ions in 250 ml Erlenmeyer shake flasks. Cultures were incubated at 27^{o}C on an environmental rotary shaker incubator. Samples of each culture were collected after 96 h that maximum uptake of metals was noted at 96 hrs. After which there was no uptake of metal ions (Subhashini *et al.*, 2011). The samples were centrifuged for 10 min. at 6000 rpm and the supernatant was used for residual metal analysis by using atomic absorption spectrophotometer (AAS). The amount of the metal ion removed by the bacteria was determined by the difference between the initial and residual concentrations and the values were expressed as percentages of the controls at the time of the cell harvest. All the experiments were performed in duplicates and the average values were determined.

The effect of immobilized bead number on metal removal was studied (Subhashini *et al*., 2011). The maximum removal of metal obtained with 300 beads. Thus, the removal efficiency increases with increase in bead number. This is because the number of viable cells in the beads is high with increase in beads.

RESULTS

Heavy metals

Comparatively, highest removal of the heavy metals were manganese 99 %, copper 77 % and iron 70 %. On the other hand, poor removal efficiencies occurred for magnesium 12 % and nickel 30% (Fig. 1). Level of heavy metals (µg/ l) in the sludge of the aerated lagoon system in (Fig. 1) indicates that the results do not comply with the national regularity standards.

In this study the uptake of heavy metals by all immobilized microbial cell strains (bacterial beads: bacterial cells in alginate) is better than by free cells excluding few bacterial strains that removed by free cells was little higher than bacterial beads as observed by Mn, Co and Pb (Fig. 2).

The removal ratio of Mn by free cells as well as bacterial beads was relatively higher and compact for most bacterial strains. Co and Pb showed a lower percentage of removal for both bacterial beads and free cells. Cu, Ni and Mg, were significantly removed by bacterial beads higher than free cells (Fig. 2).

Bacterial beads of *Rahnella aquatilis* was relatively best strain that accumulate Cu (91.5%), Ni (90.1%) and Mn (85.8%) as *Pseudomonas veronii* top strain that uptake Pb (67.5%), Co (67.5%) and Mg (97.7%). The average removal for all six metals by bacterial beads Showed that *Pseudomonas veronii* (81%) and *Rahnella aquatilis* (73%) were best strains that reasonably uptake all 6 metals. *Sphingobacterium daejeonense* (43%) and *Roseomona saquatica* (38%) were relatively weak accumulating metals (Fig3). On the other hand, the uptake of heavy metals by all free microbial cell strains possessed no significant difference by varying metals type and bacterial strains, except Co and Pb were poorly accumulated. In addition, *Roseomonas aquatic* and *Sphingobacterium daejeonense* were relatively weak accumulating metals (Fig. 4).

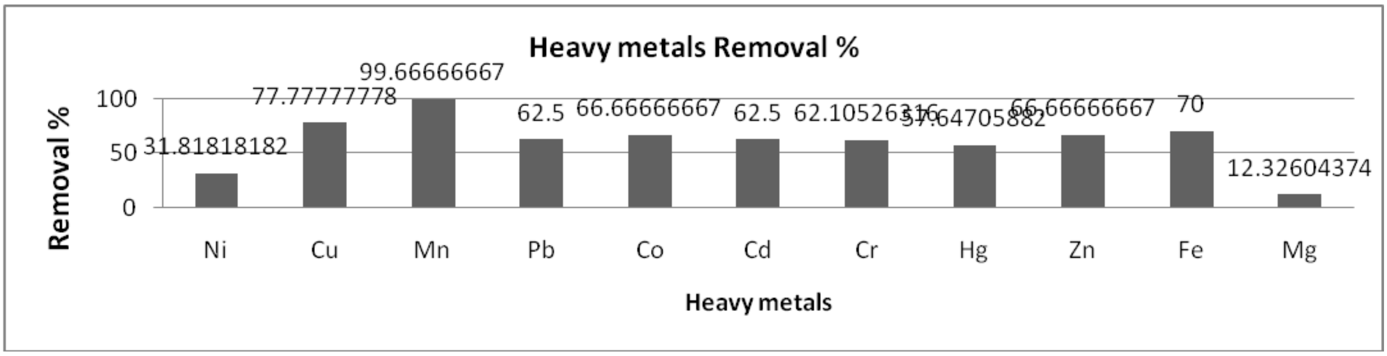

Figure (1): Removal measurement of heavy metals in the aerated lagoon system in Ismailia, Metals removal ratio by free and immobilized cells of bacterial strains.

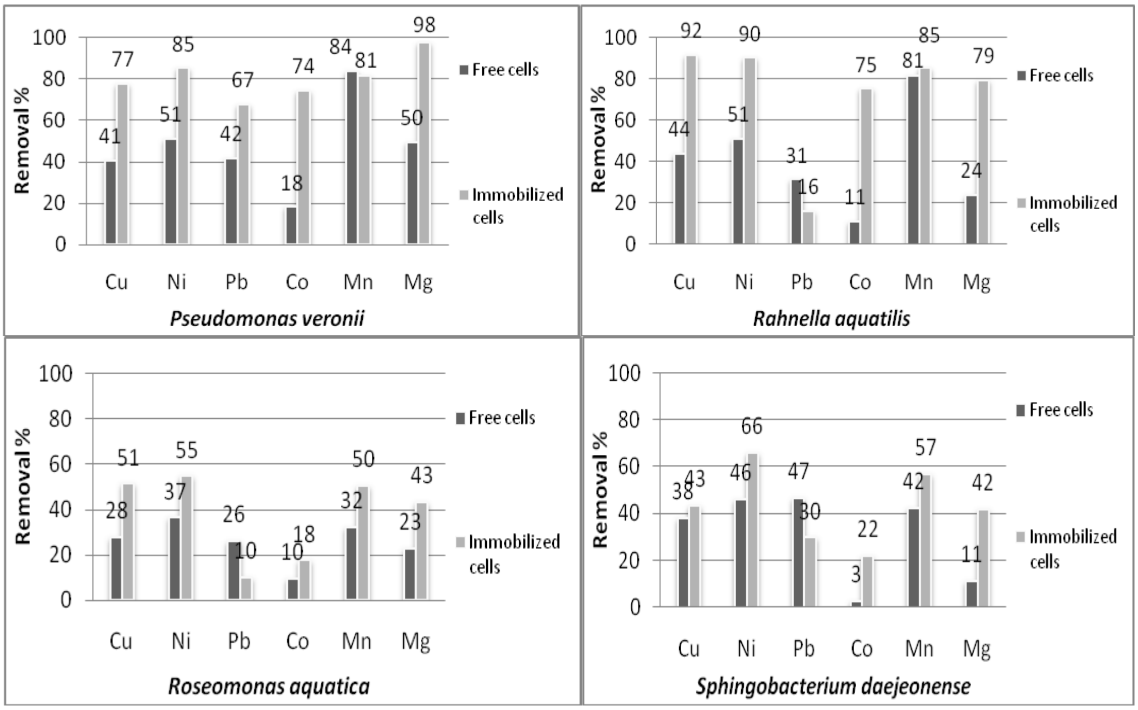

Figure (2): Various metal removal percentage by free and immobilized cells of different Bacterial species.

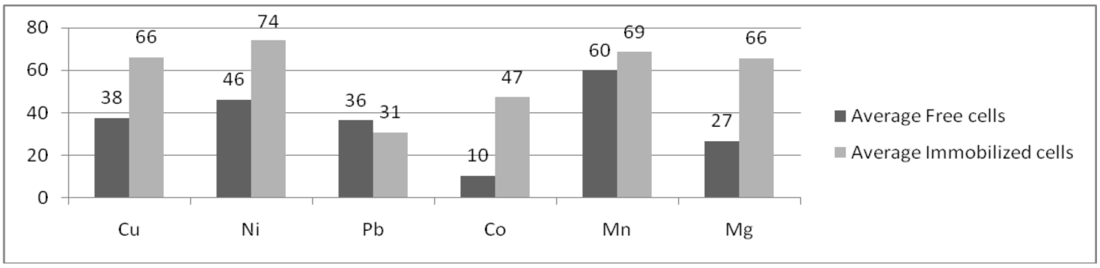

Figure (3): Average removal of metals by free and immobilized cells of all four bacterial strains.

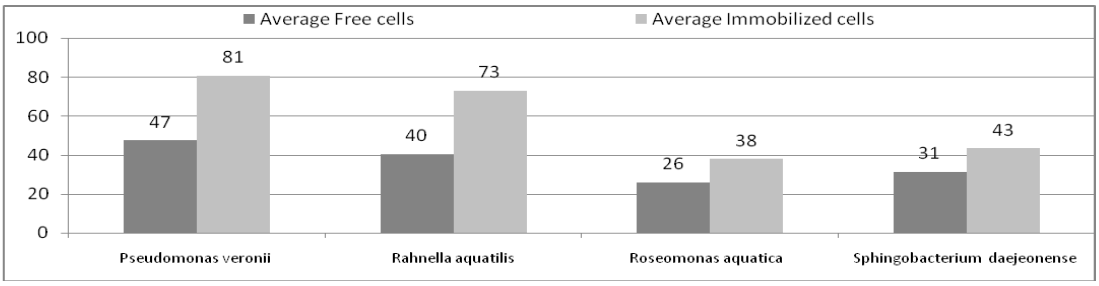

Figure (4): Average removal for all six metals by free and immobilized cells of bacterial strains.

DISCUSSION

Whole cell immobilization is convenient, has a short preparation time and is relatively economical. Covarrubias *et al*. (2012) demonstrated that wastewater microbial populations are responsible for reducing populations of biological agents added for wastewater treatment. Immobilization in alginate beads provided a protective environment for these agents for sufficient time to allow removal of heavy metals. Zaki *et al.* (2008) showed that immobilized cells are protected from the toxicity and were less sensitive to the majority of the phenolic compounds tested in compare to the free cells. However, cells stored in Ca-alginate showed the highest sensitivity in compare to other tested entrapment gels. Ca-alginate was chosen as the most suitable candidate material in the present context. Because, it could be stored at least 8 weeks at 4 ºC. Thus, indicating that the presence of alginate did not impair cell metabolism and, indeed, assisted its maintenance (Zaki, *et al.* 2008).

The present study reports the potential of immobilized cells of *Bacillus megaterium, Pseudomonas veronii* and *Rahnella aquatilis* for the uptake of toxic metals such as copper and nickel and cobalt. *Bacillus flexus*, *Bacillus amyloliquefaciens* and *Bacillus cereus* were relatively moderate accumulating all six metals. *Sphingobacterium daejeonense* and *Roseomonas aquatica* were relatively weak accumulating metals. *Rahnella aquatilis* was reported for the first time in this research as a potential organism for the bioaccumulation of the aforementioned heavy metals from domestic wastewaters. Wong and So (1993) isolated *Pseudomonas putida,* from electroplating effluent, showing that it accumulated Cu, up to 6.5% dry weight, from a Cu containing solution. Other investigators have demonstrated the capabilities of several bacteria in removing uranium, Cd, Pb, and other toxic metals from polluted effluents.

Generally, immobilization has a short preparation time and is relatively economical. Covarrubias, et al (2012) demonstrates that wastewater microbial populations are responsible for reducing populations of biological agents added for wastewater treatment. Immobilization in alginate beads provides a protective environment for these agents for sufficient time to allow removal of heavy metals (Zaki, et al. 2008).

Iqbal and Edyvean, (2007) developed immobilization system that have a promising potential for practical applications to remove heavy metals from industrial effluents. High metal loading capacity, good mechanical strength, ease of handling, high porosity, regeneration ability and low cost availability of the immobilization matrix are the key features of this biosorbent. Subhashini, et al (2011) showed that immobilized beads are potential in removal of Cu (II) from contaminated water, which is environmentally friendly. Tsai et al (2013) demonstrates the benefits of using a cell-immobilized system to treat high concentrations of a toxic xenobiotic, i.e. benzene, and those of using a cell-suspended system to treat a relatively non-toxic xenobiotic, i.e. toluene, by Pseudomonas sp. Abdel Hameed and Ebrahim (2007) claimed that immobilization results in better bio-catalyst stability and increased productivity over free systems.

CONCLUSION

The average removal for all six metals by bacterial beads Showed that *Bacillus megaterium, Pseudomonas veronii* and *Rahnell aaquatilis* were best strains that reasonably uptake all 6 metals.Also, *Bacillus flexus, Bacillus amyloliquefaciens* and *Bacillus cereus* were relatively moderate accumulating all 6 metals. *Sphingobacterium daejeonense* and *Roseomonas aquatica* were relatively weak accumulating metals. On the other hand, the uptake of heavy metals by all free microbial cell strains possessed no significant difference by varying metals type and bacterial strains, except Co and Pbwere poorly accumulated.

Immobilization in alginate beads provides a protective environment for these agents for sufficient time of heavy metals removal. Immobilized systems currently offer various advantages over free systems. Bacteria exposed to high levels of heavy metals in their environment have adapted to this stress by developing various resistance mechanism.

These mechanisms could be utilized for detoxification and removal of heavy metals from polluted environment. According to these results, the present study evaluates that the identified bacteria were used to remediate heavy metal contaminated wastewater excluding *Roseomonas aquatic* and *Sphingobacterium daejeonense*.

FURTHER INVESTIGATIONS

Eventually, one can also predict through these results, whether the uptake is metabolism-dependent or an adsorption phenomenon. Hence the use of other techniques can clarify all predictions made from the metal biosorption profiles of the microbes.

The biosorption process is critically linked to a number of factors that affect the biosorption capacity (pH, Biomass concentration, Initial metal ion concentration and Temperature). Hence, it would be necessary to determine the kinetic properties of biosorption, which are significant for determining the time required to reach equilibrium and to evaluate the maximum adsorption capacity.

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