RESEARCH PAPER | Biology | Volume : 4 | Issue : 3 | Mar 2014 | ISSN - 2249-555X

Studies on Biosorption Process for Removing Heavy Metals from Aqueous Effluent by A Bacterial Extracellular Polymeric Substance

KEYWORDS: Heavy Metals, Biosorption, EPS

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ABSTRACT *Bioremediation of heavy metal pollution remains a major challenge in environmental Biotechnology. Some industrial processes results in the release of heavy metals into aquatic systems. This has led to increasing concern about the effect of toxic heavy metals as environmental pollutants. One of the approaches considered for application involves biosorption either to biomass or to isolated biopolymers, as a more economical, effective and safe alternative to conventional processes. Many bacterial polysaccharides have been shown to bind heavy metals with varying degrees of specificity and affinity. The adsorption of heavy metals by extracellular polymeric substances (EPS) is non-metabolism, energy independent and can be caused by interaction between metal cations and negative charge of acidic functional groups of EPS.*

In this work, a experiment was carried out to investigate the ability of adsorption of three different metals – Cd , Zn and Cu – by an EPS produced by a strain of Pseudomonas sp. EPS concentration and adsorption time were varied, in order to determine the ideal conditions to remove these metal cations from aqueous solutions. Results showed that the EPS is efficient to adsorb cadmium, zinc, and copper from the system. The extracellular polymeric substance produced by Pseudomonas sp was shown to be a good adsorbent, capable to remove cadmium, zinc, and copper from an aqueous system, with removal efficiencies of 66.4, 69.8, and 72.2% of each metal, respectively.

Introduction

Earth's surface comprises of 70% water is the most valuable natural resource existing on our planet. Without this invaluable compound, the life on the Earth would not exist. Although this fact is widely recognized, pollution of water resources is a common problem being faced today. Heavy metal pollution occurs directly by effluent outfalls from industries, refineries and waste treatment plants and indirectly by the contaminants that enter the water supply from soil/ ground water systems and from the atmosphere via rain water. (Vijayaraghavan et al., 2008). Among toxic substances reaching hazardous levels are heavy metals. (Vieira djet al., 2000). Heavy metals are the group of contaminants of concern, which comes under the inorganic division. Exposure to heavy metals has been linked with developmental retardation, various cancers, kidney damage, and even death in some instances of exposure to very high concentrations (Lokeshwari et al., 2007).

The anthropogenic sources of heavy metals include wastes from the electroplating and metal finishing industries, metallurgical industries, tannery operations, chemical manufacturing, mine drainage, battery manufacturing, leather tanning industries, fertilizer industries, pigment manufacturing industries, leachates from landfills and contaminated ground water from hazardous waste sites (Faisal et al., 2004). Industrial processes utilizing metal as catalysts have generated large amounts of aqueous effluents that contain high levels of heavy metals. These heavy metals include cadmium, chromium, cobalt, copper, manganese, mercury, nickel, silver, and zinc, arsenic, lead. 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 that have adapted to the toxic concentrations of heavy metals\ and become "metal resistant".

At present, metal-polluted industrial effluents are mostly treated by chemical methods, such as chemical precipitation, electrochemical treatment, and ion exchange. These methods provide only partially effective treatment and are costly to implement and use, especially when the metal concentration is low. The alternative use of microbe-based biosorbents for the removal and recovery of toxic metals from industrial effluents can be an economical and effective method for metal removal.

Bioremediation technologies in general should be relatively inexpensive and simple because of the low added value associated with their commercial application (Gutnick and Bach, 2000). Heavy metal accumulation processes by biological cells are grouped together under the general term of "biosorption". The mechanisms of biosorption may involve intracellular uptake and storage via active cationic transport systems, surface binding or some undefined mechanisms. It was reported that microorganisms become adapted to these environments by acquisition of specific resistance systems (Yilmaz, 2003). The need for economical, effective and safe methods for removal of heavy metals from wastewater has directed attention to EPS produced from algae, bacteria, fungi and yeast. EPS plays a crucial role in the metal biosorption process. The adsorption of heavy metals by EPS is non-metabolism, energy independent and can because by interaction between metal cationic and negative charge of acidic functional groups of EPS. In addition, this substance is important in the formation and maintenance of bacterial biofilms. Toxic metals bind to biofilm exopolymers, facilitating metal transport and ameliorating metal toxicity. Thus, binding of heavy metals by EPS is thought to be an important mechanism in the natural detoxification of heavy metal-contaminated sites and in heavy metal bioremediation (Salehizadeh and Shojaosadati, 2003; Kachlany et al., 2001).

A wide variety of microorganisms have been shown to produce various polysaccharides and other biopolymers which exhibit metal-binding properties. For electrostatic interactions, the binding of cations to bacterial biopolymers generally occurs through interaction with negatively charged functional groups such as: uronic acids, phosphoryl groups associated with membrane components, or carboxylic groups of aminoacids. In addition to electrostatic interactions, there may also be cationic-binding by positively charged polymers, or coordination with hydroxyl groups (Gutnick and Bach, 2000).Isolated biopolymers for heavy metal remediation have not been applied on a large scale, although synthetic polymers have been used for various precipitation treatments (Gutnick and Bach, 2000). This research was carried out to show the potential of biosorption of Zn, Cd and Cu by an EPS produced from Pseudomonas sp., using factorial experimental design.

The MIDC area of Akola city is located near National Highway -6, that include number of industries. The effluent of that industries having various hazardous heavy metal that are directly or indirectly affect the health of animal as well as human being. The Morna river of Akola city is fully dumped by industrial waste that consists of significant metal contaminant. Wastewaters from these industries include metal ions having permanent toxic effect. So there is need to develop feasible and useful treatment methods to purify industrial wastewaters. Removal and recovery of such heavy metals are very important with respect to environmental and economical considerations.

The designed physical and chemical methods for removal of metal ions from effluents are commercially impractical, because of high operating cost and difficult techniques.. The removal of heavy metals from our environment especially wastewaters is now shifting from the use of conventional adsorbents to the use of bio-sorbents. Biosorption consists of a group of applications, which involve the detoxification of hazardous substances instead of transferring them from one medium to another by means of microbes and plants This study was carried out to characterize the metal biosorption behavior of bacteria in an activated sludge process treating metal-contaminated industrial wastewater The ultimate goals of this study were to develop novel and economical processes for removing and recovering of heavy metal.

Material and Methods

❖ **Collection of sample:**

Effluent samples were collected from the different industries of Akola city. Samples was collected in sterile plastic container and taken immediately to the laboratory and maintained at 2-8 °C for further studies.

❖ **Isolation and screening of bacteria:**

The screening of metal tolerant Pseudomonas sp. was carried out by broth method or plate diffusion method. Isolated organism was identified on the basis of their morphological, biochemical and cultural characteristics by adopting standard methods.

❖ **Biomass cultivation:**

Isolated metal tolerant Pseudomonas sp was cultivated in the form of biomass on shaker for incubation with suitable medium. The high yield of biomass production was evaluated in the presence of different sugar sources (like glucose, sucrose, maltose etc).The carbohydrate source with appropriate concentration providing optimum biomass production was carried ahead for further study.

Isolation of extra cellular polymeric substances:

Isolated of metal tolerant Gram negative bacterial strain was subjected for isolation of extracellular polymeric substance (EPS) by acetone precipitation technique. The isolated EPS was stored in phosphate buffer to bioremediation the present metal in sample.

❖ **EPS Yield**

Percentage yield of EPS was calculated by formula:

Percentage yield of EPS = dry wt. of EPS / dry wt. of cell + EPS wt.

The percentage yield of EPS will help to recognize a correlation between amounts of EPS essential to remove heavy metal present in effluent.

❖ **Biosorption experiment:**

Different concentration of EPS were combined with heavy metal containing sample and incubated on shaker with a constant speed of 300 rpm and left to equilibrate samples were collected at predefined time intervals and the amount of metal in the supernatant was determine

The percentage of biosorption was evaluated by following formula:

Percentage of biosorption = (Initial – Final metal concentration) x 100 / initial metal concentration

❖ **Experimental design**

The biosortpion of Cd^{2+}, Zn^{2+}, and Cu^{2+} from aqueous solutions was evaluated by a three level factorial experiments (Table 1).

Table 1. High and low levels of factor

FACTOR	LEVEL		
	-1	0	1
Time of adsorption (h)	9	15	24
Concentration of EPS (g/L)	0.01	0.50	1
Metal	Cd	Zn	Cu

The experimental design is known as an III^f experiment, where III denotes the number of levels and f denotes the number of factors: concentration of EPS – C_{EPS}, time of adsorption – t, and metal – M1, M2, M3 (cadmium, zinc and copper, respectively). Forty experiments were carried out, plus 2 blanks for each metal

Results and Discussion

Results for Cd^{2+}, Zn^{2+}, and Cu^{2+} uptake are shown in Table 2. The removal efficiency (η) was defined as

$$\eta = CM - CMF / CM$$

Where C_M and C_{MF} are, respectively, the initial and final concentrations of metal species

The metal uptake by EPS (q) was defined as:

$$q = C_{M} - C_{MF} / C_{EPS}$$

Where C_M and C_{MF} are, respectively, the initial and final concentrations of metal species (mg/L), and C_{EPS} is the concentration of EPS (g/L).

Table 2. Experimental factorial design results for Cd^{2+}, Zn^{2+}, and Cu^{2+} uptake

FACTOR		Cd^{2+}		Zn^{2+}		Cu^{2+}	
t	C_{EPS}	q (mg/g)	Removal Efficiency (%)	q (mg/g)	Removal Efficiency (%)	q (mg/g)	Removal efficiency (%)
-1	-1	3235	64.7	3127.8	62.5	3424.6	68.5
-1	+1	132.9	66.4	139.6	69.8	144.6	70.1
-1	0	249.1	64.7	245.2	60.4	268.1	69.7
+1	-1	3049.2	61.0	3122.4	62.4	3433.3	68.6
+1	+1	128.9	64.5	128.3	64.1	139.5	69.2
+1	0	244.8	63.6	250.8	65.2	262.3	68.2
0	-1	3368.3	67.3	3121.7	62.4	3425.3	68.5
0	+1	127.6	63.8	123.6	61.8	137.4	68.7
0	0	245.2	63.7	245.6	63.8	259.6	67.5

Loaëc et al. (1997) studied the metal uptake capacity of an exopolysaccharide produced by Alteromonas macleodii subsp. fijiensis. For an exopolysaccharide concentration of 1.0

Volume : 4 | Issue : 3 | Mar 2014 | ISSN - 2249-555X

g/L and initial concentrations of lead , cadmium, and zinc of 50 mg/L, they obtained 100 % of removal efficiencies. Nevertheless, as the biosorbent concentration is higher than the maximum used in this work (0.25 g/L), the metals uptakes were smaller: 316mg/g of lead, 125 mg/g of cadmium, and 75 mg/g of zinc.

Salehizadeh and Shojaosadati (2003) obtained metal uptakes around 700 mg/g of lead, and 400 mg/g of zinc and copper, with 0.25 g/L of a polysaccharide produced by Bacillus firmus, and removal efficiencies of 98.3 %, 74.9 % and 61.8 % of lead, cupper, and zinc, respectively. As mentioned before, an increase in EPS values leads to an increase in the removal efficiency.

In this work removal efficiencies of 67.3 %, 69.8 % and 70.1% of cadmium, zinc, and copper, respectively.

Conclusions

The extracellular polymeric substance produced by Pseudomonas sp was shown to be a good adsorbent, capable to remove cadmium, zinc, and copper from an aqueous system, with removal efficiencies of 67.3, 69.8, and 70.1% of each metal, respective. Times of adsorption (8 h, 16 h, and 24 h) did not influence the responses, indicating that in this interval of time the equilibrium of adsorption was already reached. In the other hand, concentration of EPS is an important factor to be evaluated, as its increase leads to an increase in removal efficiency and to a decrease of metal uptake

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