RESEARCH ARTICLE

Biosorption of Heavy Metals from Aqueous Solution using Bacterial EPS

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Manuscript details:

Received: 05 November, 2014
Revised : 25 November, 2014
Revised received: 12 December, 2014
Accepted: 16 December, 2014
Published: 30 December, 2014

Editor: Dr. Arvind Chavhan

Citation this article as:
Gawali Ashruta A, Nanoty VD, Bhalekar UK (2014) Biosorption of Heavy Metals from Aqueous Solution using Bacterial EPS, *Int. J. of Life Sciences*, 2(4): 373-377.

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 processes such as precipitation, coagulation, ion exchange, electrochemical and membrane processes. Many bacterial polysaccharides have been revealed to join heavy metals with changeable degrees of specificity and resemblance The adsorption of heavy metals by extracellular polymeric substances (EPS) is energy independent, non-metabolism, and can be caused by contact between negative charge and metal cations of acidic functional groups of EPS. Isolated biopolymers have not been practical on a huge range for heavy metal remediation.

In this work, a experiment was carried out to investigate the ability of adsorption of three different metals – Zn^{2+}, Pb^{2+}, Cr^{6+}, Ni^{2+}, Cu^{2+}, Cd^{2+} and Co^{2+} by an EPS produced by consortium gram-negative bacteria. EPS concentration and adsorption time were wide-ranging, in order to determine the ultimate environment to remove these metal cations from aqueous solutions. Outcome showed that the EPS is proficient to adsorb cadmium, zinc, and copper from the system. The extracellular polymeric substance produced by consortium gram-negative bacteria were shown to be a good adsorbent, capable to remove zinc, lead, chromium, nickel, copper, cadmium and cobalt from an aqueous system, with removal efficiencies of 77.15, 78.18, 74.48, 66.63, 71.00, 72.71 and 76.12% of each metal, respectively.

Keywords: Heavy metals, Bioremediation, EPS,

INTRODUCTION

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 & from the atmosphere via rain water. Among toxic substance reaching hazardous levels are heavy metals (Vieira *and Volesky,* 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.

The anthropogenic sources of heavy metals consist of wastes from the electroplating and metal finishing industries, metallurgical industries, manufacturing, leather tanning industries, fertilizer industries,tannery operations, chemical manufacturing, mine drainage, battery pigment manufacturing industries, leachates from landfills and contaminated ground water from hazardous waste sites (Faisal *and Hasnain,* 2004). Industrial processes utilizing metal as catalysts have generated bulky amounts of aqueous effluents that include 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 show the way to high metal concentrations in the activated sludge. Microbial populations in metal-polluted environments contain microorganisms that have adapted to the noxious concentrations of heavy metals and become "metal resistant".

At present, metal-polluted industrial effluents are frequently treated by chemical methods, such as chemical precipitation, electrochemical treatment, and ion exchange. These methods supply only to some extent effective treatment and are costly to put into operation 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 becaused 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^{2+}, Pb^{2+}, Cr^{6+}, Ni^{2+}, Cu^{2+}, Cd^{2+} and Co^{2+} by a EPS produced from consortium gram-negative bacteria *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 those industries having various hazardous heavy metals 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 wastes that consist of significant metal contaminant. Wastewaters from these industries contain metal ions having everlasting toxic effect. So there is necessitating developing realistic and useful treatment methods to get rid of impurities industrial wastewaters. Removal and recovery of such heavy metals are very important with respect to environmental and cheap to run considerations.

The considered physical and chemical methods for removal of metal ions from effluents are commercially not practical, because of high in commission cost and tricky techniques. The elimination of heavy metals from our surroundings especially wastewaters is now variable from the use of conventional adsorbents to the use of bio-sorbents. Biosorption consists of a group of applications, which involve the detoxification of dangerous substances as an alternative of transferring them from one medium to another by means of microbes and plants This study was conceded out to exemplify the metal biosorption performance of bacteria in an activated sludge process treating metal-contaminated industrial wastewater The eventual goals of this study were to build up narrative and efficient processes for removing and recovering of heavy metal.

MATERIAL AND METHODS

Preparation of stock solution (Artificial effluent)

Aqueous solutions of metals were prepared in the laboratory. Stock solutions (1000 mg/L) were prepared from the following salts; Cd(II) from $Cd(NO_3)_2$. $4H_2O$, Cr(VI) from K_2CrO_7, Cu(II) from $Cu(NO_3)_2$ $5H_2O$, Ni(II) from $Ni(NO_3)_2$. $6H_2O$, Pb(II) from $Pb(NO_3)_2$ and Co(II) from $Co(NO_3)_2$ in double distilled H_2O. Working solutions were prepared by diluting the stock solutions to the desired concentrations in ddH_2O for metal ion sorption study and nitric acid, hydrochloric acid and sodium hydroxide for pH adjustment. Fresh dilutions and metal solutions were used in each sorption study. Concentrations of Cd, Cr, Cu, Ni, Pb, Co, Zn in the medium were determined by atomic absorption spectrophotometer (AAS; PerkinElmer 1100B).

Isolation and screening of bacteria:

The screening of metal tolerant gram-negative bacteria 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 gram-negative bacteria were 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 bioremediate 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 regular speed of 300 rpm and gone 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 Zn^{2+}, Pb^{2+}, Cr^{6+}, Ni^{2+}, Cu^{2+}, Cd^{2+} and Co^{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)	2	8	24
Concentration of EPS (g/100 ml)	0.5	1	2

RESULTS AND DISCUSSION

Table 2: Experimental factorial design results for Zn^{2+}, Pb^{2+}, Cr^{6+}, Ni^{2+}, Cu^{2+}, Cd^{2+} and Co^{2+} uptake

Factor		Zn^{2+}		Pb^{2+}		Cr^{6+}		Ni^{2+}		Cu^{2+}		Cd^{2+}		Co^{2+}	
Time	C_{EPS}	Q	E (%)	q	E (%)	q	E (%)	q	E (%)	q	E (%)	q	E (%)	q	E (%)
-1	-1	3.5	70	3.6	72.43	3.5	69	3.2	63.32	3.2	63.13	3.2	64.42	3.6	72.03
-1	0	3.8	76.22	3.9	78.13	3.7	73.13	3.2	64.28	3.5	70.09	3.5	70.54	3.8	75.19
-1	1	3.9	77.15	4.0	79.03	3.7	74.48	3.3	66.63	3.6	71	3.6	72.71	3.8	76.12
0	-1	3.8	75.54	3.9	78.18	3.6	71.04	3.3	65.11	3.5	70.84	3.5	70	3.8	75.18
0	0	3.7	73.43	3.8	76.02	3.5	70.11	3.2	64.13	3.5	69.31	3.4	68.64	3.7	74.01
0	1	3.7	73.18	3.5	70.25	3.5	70.98	3.1	62.03	3.5	69.98	3.5	69.8	3.7	74.61
1	-1	3.8	75	3.9	77.39	3.6	72.22	3.3	65.03	3.4	68.31	3.4	68.39	3.7	74.23
1	0	3.5	70.09	3.8	75.05	3.5	70.01	3.0	60.03	3.2	63.81	3.3	65	3.7	73.33
1	1	3.6	71.49	3.9	77.22	3.5	70.18	3.2	63.89	3.2	64.72	3.3	65.65	3.7	74.04

Results for Zn^{2+}, Pb^{2+}, Cr^{6+}, Ni^{2+}, Cu^{2+}, Cd^{2+} and Co^{2+} uptake are shown in Table 2. The removal efficiency (η) was defined as.

$$\eta = C_M - C_{MF}/C_M \times 100$$

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).

All over it was consummate that individual bacterial EPS removed all the seven heavy metal ions significantly and exhibited preference order of $Pb^{2+} > Zn^{2+} > Co^{2+} > Cr^{6+} > Cd^{2+} > Cu^{2+} > Ni^{2+}$.

Other studies that involved Cu^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Cr^{6+}, Ni^{2+} and Pb^{2+} in mixture solutions also reported similar biosorption profiles. Yu *et al.,* (2007) reported the biosorption profiles results of their studies to be $Pb^{2+} > Zn^{2+} > Co^{2+} > Cr^{6+} > Cd^{2+} > Cu^{2+} > Ni^{2+}$ for *Pseudomonas sps*. All of these observations are consistent with our present study.

This biosorption profile, however, is not applicable to all organisms and conditions. Contrasting our study Da Costa and Duta (2001) reported a reverse profile by Bacillus biomass: $Cd^{2+} > Cu^{2+} > Pb^{2+}$. While in consistent with our present study are other researchers' biosorption profile report, which was $Pb^{2+} > Cd^{2+} > Cu^{2+}$ wherein Cd^{2+} was biosorbed to a greater extent than Cu^{2+} (Kim *et al.*, 2007). This can be explained by differences in selectivity of biosorbents for metals in solutions, which has been more extensively studied by Premuzic *et al.,* (1991).

CONCLUSIONS

The extracellular polymeric substance produced by consortium gram-negative bacteria were shown to be a good adsorbent, capable to remove zinc, lead, chromium, nickel, copper, cadmium and cobalt from an aqueous system, with removal efficiencies of 77.15, 78.18, 74.48, 66.63, 71.00, 72.71 and 76.12% of each metal, respective. Times of adsorption (2 h, 8h, 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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