Asian Journal of Chemistry Vol. 20, No. 8 (2008), 6075-6080

Adsorption Capability of Lead, Nickel and Zinc by Exopolysaccharide and Dried Cell of *Ensifer meliloti*

AMIR LAKZIAN*, ALI R. BERENJI†, ESMAEL KARIMI and SOLMAZ RAZAVI
Department of Soil Science, Faculty of Agriculture
Ferdowsi University of Mashhad, Mashhad 91779, Iran
Fax: (98)(511)8787430; Tel: (98)(511)8795612-7 (Ext. 294)
E-mail: alakzian@yahoo.com; lakzian@ferdowsi.um.ac.ir

In this study, the adsorption capability of lead, nickel and zinc by exopolysaccharide (EPS) and dried cell of *Ensifer meliloti* (formerly *Sinorhizobium meliloti*) which nodulates alfalfa has been investigated. The results showed that dried cells had less adsorbing tendency compared to EPS of *Ensifer meliloti* MS-125. 0.02 g of EPS adsorbed 89, 85 and 66 % of lead, nickel and zinc, respectively, presented in 50 mg L^{-1} solutions. Maximum adsorption of lead, nickel and zinc were 110, 54 and 94 mg g^{-1} EPS, respectively, estimated by using Langmuir model.

Key Words: *Ensifer meliloti*, *Sinorhizobium meliloti*, Exopolysaccharide, Biosorption, Heavy metals, Langmuir model.

INTRODUCTION

Accumulation of heavy metals in ecosystems due to metal mining activities, electrical equipment production, application of artificial colours and pesticides, tannery industries, chemical fertilizers and municipal sewage disposal has concerned scientists and officials[1]. In the past decades, concern about releasing these pollutants to aquatic ecosystems has increased. In order to decrease the concentration of heavy metals, many treatments have been used to remove metal ions from sewage sludge before releasing to the environment. The common treatments are chemical precipitation, ion-exchange, reverse osmoses, electrical dialysis and photo-remediation[2]. The recent technologies for removing toxic metals from aquatic media lead to study on biosorbents. Biosorbents are the biological materials capable of removing heavy metals from aquatic environments[3]. Bacteria, fungi and yeast can take up these toxic metals and can be considered as biosorbent[4-7]. A natural, abundant and cheap microbial biomass can sometimes uptake heavy metals

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†Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad-91779, Iran; E-mail: berenji@wali.um.ac.ir

selectively[8]. Recently, the application of microorganisms and their extracellular polymeric substances or exopolysaccharides (EPS) as biosorbents has been studied. For example, during growth, *Rhizobium etli* M4 can bound *ca.* 60 % of its weight as manganese ions[3].

The purpose of this study is to investigate adsorption capacity of lead, nickel and zinc by dried cells and EPS of *Ensifer meliloti*.

EXPERIMENTAL

Bacterial strain: In order to evaluate the production of EPS of *Ensifer meliloti* (formerly *Sinorhizobium meliloti*), 15 isolates were selected from Tehran Soil and Water Research Center and cultivated at pH = 6 in yeast extract mannitol agar (YEMA) culture medium. Then, *Ensifer meliloti* isolates were evaluated on the basis of EPS production. The results showed that all isolates had different ability in producing EPS. The maximum amount of EPS was obtained by MS-125 isolate. Therefore, MS-125 isolate was selected for this study.

Isolation of exopolysaccharide (EPS): The cellular biomass and EPS were produced in 1 L of yeast extract mannitol broth (YEMB) culture medium (at 30 °C, 150 rpm shaker for 48 h after logarithmic phase). Cellular biomasses were separated by using a 17000 rpm centrifuge at 4 °C, then dried at 40 °C and weighted. In order to separate the soluble EPS, cold ethanol (in ratios of 3:1) were added to cell free cultures. Then, saturated sodium chloride (in ratios of 1:2) were added to samples which were kept for 20 min at -20 °C. The polysaccharides were precipitated in the sample, separated, dried at 40 °C and weighted[3].

Biosorption studies: Binding capability of EPS and dried cell for lead, nickel and zinc were examined by adding 0.02 g of EPS and dried cell into a 10 mL solution of each metal and of a combination of three metals at 30 °C. A pH of 6.0 was selected for the biosorption studies because the maximum biosorption of heavy metals on biomass has been observed[9-11] at around pH = 6.0. Residual concentration in each sample was determined after 20, 50, 90, 150, 210 and 1080 min using a Shimadzu AA-670 atomic absorption spectrophotometer.

In order to study the influence of initial concentrations of lead, nickel and zinc on binding capability of EPS, 30, 50, 100, 150 and 200 mg L^{-1} of three elements were prepared and 0.02 g polysaccharides were added to plastic vessels containing 10 mL of mentioned solutions and then incubated at 30 °C. In maximum adsorption time (20 min), residual concentrations were determined by atomic absorption spectrophotometer.

Data treatment: Isothermal adsorption of lead, nickel and zinc were studied by using Langmuir model. The Langmuir equation is valid for monolayer sorption onto a surface with a finite number of identical sites and is given by eqn. 1:

$$q_{eq} = \frac{Q^0 bC_{eq}}{1 + bC_{eq}} \quad (1)$$

where Q^0 is the maximum amount of the metal ion per unit weight of cell to form a complete monolayer on the surface bound at high C_{eq} (mg g^{-1}) and b is a constant related to the affinity of the binding sites. Q^0 represents a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in the comparison of adsorption performance, particularly in cases where the sorbent did not reach its full saturation in experiments. Q^0 and b can be determined from the linear plot of C_{eq}/q_{eq} *vs.* C_{eq}.

RESULTS AND DISCUSSION

Adsorption capacity of EPS and dried cells: The adsorption capacity of EPS of *Ensifer meliloti* MS-125 for lead, nickel and zinc showed that EPS had a higher capacity in comparison with the dried cells. The maximum adsorption of the three metals has been observed in 20 min after adding EPS or dried bacterial cells to the metal solutions. After 20 min, the adsorption pattern of lead, nickel and zinc did not change, *i.e.*, to the end of the experiment (1080 min). 0.02 g EPS of *Ensifer meliloti* MS-125 adsorbed 89, 85 and 66 % of lead, nickel and zinc, respectively, presented in 50 mg L^{-1} solutions of each metal, but the dried bacterial cells adsorbed 73, 41 and 44 %, respectively. It has been reported that EPS of *Enterobacter cloaceae* has chelated with 66 % of cadmium ion available in a 100 mg Cd L^{-1} solution[12]. It seems that quality and quantity of EPS, metal concentration and environmental factors affect the adsorption patterns. It is important to mention that the maximum and minimum adsorption occurred for lead and zinc, respectively. EPS and also functional groups involved in adsorption of bacterial cells had higher intention to adsorb lead in comparison with zinc. The adsorption capability of manganese, lead, copper, nickel and cobalt by *Rhizobium etli* M4 and their EPS has been investigated by Foster *et al.*[3]. It has been reported that lead was adsorbed more than other elements by bacterial cells and EPS of *Rhizobium etli* M4. It has been also shown that the adsorption capability of EPS is greater than that of the bacterial cells of *Rhizobium etli* M4.

Competitive biosorption: Simultaneous presence of the three elements (Pb, Ni and Zn) had a considerable effect on adsorption pattern of EPS and dried cells of *Ensifer meliloti* MS-125. The results have shown that even in presence of all three elements, lead had the maximum adsorption by EPS and dried bacterial cells and zinc had the lowest adsorption level. In this settings, 98, 92 and 46 % of lead, nickel and zinc, respectively, were adsorbed by EPS while 88, 44 and 27 % of the metal ions were adsorbed by the dried bacterial cells.

It has been found that the adsorption of lead and nickel would increase in presence of all the three elements. In addition, zinc adsorption pattern was completely different from lead and nickel ones. Thirty and 39 % of zinc has been adsorbed in presence of lead and nickel, respectively, by EPS and dried cells. Foster *et al.*[3] have also reported that simultaneous presence of metals in a solution would change the adsorption pattern of heavy metals on EPS and of *Rhizobium etli* cells due to the interactions of the elements and their competition for occupying binding sites. Special functional groups such as structural polysaccharides of fungi, amide and phosphate of nucleotide acids and carboxyl and sulphate in algae polysaccharides intensively adsorb heavy metals. Tobin *et al.*[13] have explained the relation between the adsorption of metal ions and phosphate, carboxylate and other functional groups of *Rhizopus arrhizus* biomass.

Effects of initial concentration of metal ions on the biosorption capacity: Fig. 1 depicts the effect of initial concentration of the metals on their adsorption by EPS of *Ensifer meliloti* MS-125. The results reveal that the amount of adsorption increased by higher metal concentrations. The adsorption trend of the three elements is almost similar up to 50 mg L^{-1}. In higher concentrations, however, lead is adsorbed more than nickel and zinc, while the adsorption trend of nickel and zinc are similar. This fact also indicates the higher tendency of functional groups to uptake lead. Adsorption of metals on a microbial absorbent is controlled by initial metal concentrations in samples[14]. As shown in Fig. 1, lead, nickel and zinc adsorption patterns are affected by their initial concentrations, but it is obvious that the increasing trend would continue until the active adsorbing sites become saturated. This increasing trend may depend on some properties of the metal sorbates (*e.g.* ionic size, reduction potential of the metal) and the properties of the biosorbent[15] (*e.g.* structure, functional groups and surface area).

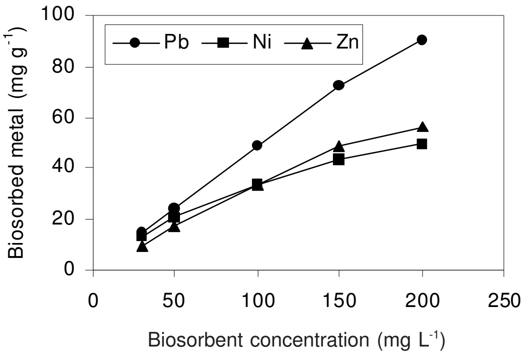

Fig. 1. Effect of initial concentrations of nickel, lead and zinc on their adsorption by EPS of *Ensifer meliloti* MS-125 after a 20 min incubation (pH = 6)

Langmuir adsorption isotherms: The equlibria of biosorption of heavy metals were modelled using adsorption-type isotherms. According to the literature[16-19], the Freundlich and Langmuir models were used to describe the biosorption equilibrium. Fig. 2 shows the Langmuir plots for adsorption of Pb, Ni and Zn by EPS of *Ensifer meliloti* MS-125. The Langmuir constants (Q^0 and b) along with the correlation coefficients (R^2), were calculated from the plots (Fig. 2) and are presented in Table-1. The regression correlation coefficients for all metal ion-EPS systems are very high.

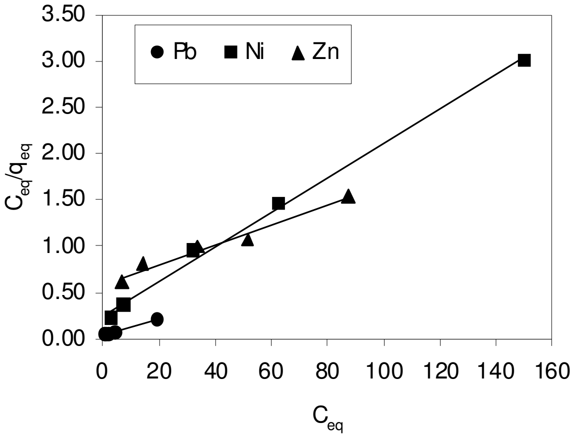

Fig. 2. Langmuir isotherms for adsorption of nickel, lead and zinc by EPS of *Ensifer meliloti* MS-125 (pH = 6)

TABLE-1
LANGMUIR ISOTHERM MODEL CONSTANTS FOR ADSORPTION OF LEAD, NICKEL AND ZINC BY EPS OF *Ensifer meliloti* MS-125

Heavy metal	Q^0 (mg g^{-1})	b (L mg^{-1})	R^2
Pb	110	0.269	0.9803
Ni	54	0.075	0.9956
Zn	98	0.018	0.9602

After processing Langmuir model, maximum adsorption of lead, nickel and zinc was estimated to be 110, 54 and 94 mg g^{-1} EPS, respectively. As shown in Fig. 2, EPS of *Ensifer meliloti* MS-125 had a high affinity and capacity to adsorb lead in comparison with nickel and zinc. Therefore, consequent results reveal that the order of the adsorption of lead, nickel and zinc by EPS of *Ensifer meliloti* MS-125 is followed by Pb > Zn > Ni.

Conclusion

The aim of this work is to find the biosorption characteristics of *Ensifer meliloti* MS-125 for the removal of lead, nickel and zinc ions. Experiments were performed as a function of initial metal ion concentration and time at pH = 6. The results showed that EPS was a good adsorbing medium for the metal ions and had a high adsorption capacity for the treatment of wastewater containing lead, nickel and zinc ions.

The Langmuir adsorption model was used for the mathematical description of the biosorption of lead, nickel and zinc to EPS and the isotherm constants were evaluated to compare the biosorptive capacities of EPS for the three metal ions.

ACKNOWLEDGEMENTS

This project has been supported by Department of Soil Science, Ferdowsi University of Mashhad. The authors thank the members of Soil and Water Research Institute in Tehran for their contribution.

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(*Received*: 8 November 2007; *Accepted*: 1 July 2008) AJC-6650