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BIOSORPTION OF LEAD(II) IONS BY DEAD BACTERIAL BIOMASS ISOLATED FROM MINE WATER

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Palabras clave: *Biosorción, Plomo, Biomasa bacteriana*

ABSTRACT

Lead and its different chemical forms have been widely used in industry over the past years and the result is the high pollution detected in water and soil environments. Lead (II) resistant microorganisms were isolated from liquid samples collected from Mina Asientos, Cochabamba-Bolivia. Eleven strains were isolated in solid media with 200 ppm Pb(II) and 4 strains were selected for metal removal in liquid samples. One strain presented the highest Pb(II) removal values for 250 ppm Pb(II). The selected strain designated as MA-4 was subjected to phylogenetic studies and showed 99.9 % sequence similarity with *Pseudomonas monteilii*. The biosorption follows Langmuir and Freundlich isotherm models, with q_{max} and K_f values of 166.67 and 11.09, respectively. These results implied a strong binding capacity to dead biomass and its potential application in biosorption process.

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RESUMEN

El plomo y sus diferentes formas químicas se han utilizado ampliamente en la industria durante los últimos años y el resultado es la alta contaminación detectada en el agua y el suelo. Se aislaron microorganismos resistentes al plomo (II) de muestras líquidas recolectadas en Mina Asientos, Cochabamba-Bolivia. Se aislaron once cepas en medio sólido con 200 ppm de Pb (II) y se seleccionaron 4 cepas para remoción de metales en muestras líquidas, una cepa

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presentó los valores de remoción de Pb (II) más altos para 250 ppm de Pb (II). La cepa seleccionada designada como MA-4 se sometió a estudios filogenéticos y mostró una similitud de secuencia del 99,9% con *Pseudomonas monteilii*. La biosorción sigue los modelos de isotermas de Langmuir y Freundlich, con valores de q_{max} y K_f de 166,67 y 11,09, respectivamente. Estos resultados implicaron una fuerte capacidad de unión a la biomasa muerta y su potencial aplicación en el proceso de biosorción.

INTRODUCTION

Heavy metals are naturally occurring elements in nature and environmental contamination and human exposure results in most of the cases from anthropogenic activities. This major environmental problem is the result of increased use of metals and chemicals in process industries generating large quantities of effluents that contain high level of toxic heavy metals that persists in the environment due to their non-degradable nature. They have harmful effects on human health and its presence on aqueous water streams have become an important problem to be solved. Several factors have to be considered for heavy metals toxicity such as dose, route of exposure and chemical species, but also we have to take in consideration the age, gender, genetics and nutritional status of expose individuals. Different conventional methods have been studied and applied to remove or reduce heavy metal contamination from aqueous effluents [1-3].

Heavy metal bioremediation from human contamination activities is an effective alternative to physicochemical methods. Conventional methods for removing metals from aqueous solutions include different chemical based treatments. The biggest disadvantages of these methods are an incomplete removal, a low selectivity and a high energy and money consumption. Biological approach has great potential being feasible economically. Biosorptive processes are more applicable due low cost and environment friendly manner. Microbial populations in metal polluted environments adapt to toxic concentrations of heavy metals developing great resistance to this type of contamination. Biological processes can include adsorption, accumulation and enrichment mechanisms of metal removal, e.g. biosorption and bioflocculation by whole cells or microbial metabolites [4-6].

Different materials have been investigated as biosorbents for the removal of heavy metals. The tested materials can be classified into the following categories: bacteria (e.g. *Pseudomonas stutzeri*), fungi (e.g. *Penicillum sp*.), yeast (e.g. *Saccharomyces cerevisae*), algae (e.g. *Spirogyra sp*.) and other polysaccharides materials (e.g. bacterial exopolysaccharides). Mechanisms of cell sorption are independent of cell metabolism: they are based upon physicochemical interactions between metal and functional groups of the cell wall. Metal biosorption by inactivate biomaterials was involved in the complexation between metal ions and various functional groups, such us amino, carboxyl, phosphate, hydroxyl, etc. present on the cell surface. A wide variety of living and dead biomass offers an economical alternative for sorption technologies [7-10].

The objective of the present study was to evaluate the biosorption of lead (II) metal ions on dead biomass from microorganisms isolated from mine wastewater.

EXPERIMENTAL

Chemicals

Chemicals were purchased either from Merck or Sigma. All other chemicals used in the present study were of analytical grade.

Isolation and identification of lead-resistant bacteria from mine effluents

Lead-resistant microorganisms were isolated from water samples collected from Mina Asientos, Cochabamba-Bolivia. Isolated microorganisms were grown in 3% (w/v) TSB (Tryptic Soy Broth) medium supplemented with 0.5 %(w/v) peptone, 0.5 % (w/v) yeast extract and Pb(II) (Lead nitrate) at 200 ppm.

In order to obtain samples for species determination, deoxyribonucleic acid (DNA) extraction, 16S rDNA gene amplification and sequencing of the PCR products were carried out at Secugen (Spain). The analysis of the DNA sequences was completed with the BLAST server of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) using the BLAST algorithm and the BLASTN program for the comparison of a nucleotide query sequence against a nucleotide sequence database. 16S rDNA sequence analysis was performed with MEGA X software package by using the neighbor-joining method with bootstrap values based on 1000 replications.

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For the phylogenetic trees, only sequences from the strains whose names have been validly published were taken into account.

Characterization of the isolate was done by Gram' stain reaction [11]. Biochemical characteristics were screened by the corresponding API system (bioMerieux) [12]. Substrate fermentation was evaluated by API 50CH (bioMerieux).

Dead biomass preparation

The bacterial strains selected were studied in liquid medium containing: 3% (w/v) TSB, 3 % (w/v) urea, 3% (w/v) glucose and 0.015 ($mg \cdot L^{-1}$) $CaCl_2$. The microorganisms were grown under different values of pH (6, 6.5, 7), temperature (15, 25 y 35 °C) and orbital agitation speed (100, 150 and 200 rpm) for 72 hours. Samples were taken every 12 hours. Biomass was harvested at 3000 rpm for 10 min, after drying at 105 °C for 12 hours. Biomass was measured by dry weight.

Microorganisms were grown under optimal conditions previously determined. Bacterial biomass was harvested by centrifugation for 10 min at 3000 rpm, rinsed with sterile water and dried for 12 hours at 105ºC, afterwards dead biomass was immobilized in alginate beds. The immobilization via entrapment was carried out as follows: Na-alginate (3.0 g) was dissolved in distilled water (40 mL) and bacterial dead biomass was added in it. The volume of the mixture was increased to 100 mL and stirred to obtain a uniform mixture. The mixture was introduced into a 0.5 M $CaCl_2$ solution through a syringe under magnetic stirring at room temperature. The obtained beads were stirred in this solution for 60 min, stored at 4°C, and washed with distilled water several times before use.

Lead biosorption

Test solution containing Pb(II) ions was prepared from analytical grade chemical lead nitrate, $Pb(NO_3)_2$ (99% Sigma Aldrich). The concentration of metal ions, 250 ppm, was prepared from a stock solution of 1000 $mg \cdot L^{-1}$. Before mixing with the biomass, the pH of each test solution was adjusted to the required value. Percentage of metal absorbed was measured at 5, 10, 15 and 30 minutes.

Batch biosorption test

Biosorption experiments were carried out with different concentrations of lead aqueous solutions (200, 300, 400, 500, 800 and 1000 $mg \cdot L^{-1}$) at a pH value of 4. Each solution was mixed with 1.0 $g \cdot L^{-1}$ biomass. Five mililiters samples were collected and the biomass recovery was by centrifugation. A sample of the supernatant was taken for measuring lead remaining in solution by flame atomic adsorption spectrophotometry (Perkin Elmer AAnalyst 200). All measurements were performed in duplicate.

Biosorption isotherms

The biosorption equilibrium isotherm was obtained by the Freundlich model (Eq. 1) and the Langmuir model (Eq. 2), respectively [13].

$$q = K_f C_e^{1/n} \qquad (1)$$

Where K_f and n are the distribution coefficient and a correction factor, respectively. By plotting the linear form of Eq. (1), $\ln q = (1/n)(\ln C_e + \ln K_f)$, the slope is the value of 1/n and the intercept is equal to $\ln K_f$. For Langmuir:

$$q_{eq} = \frac{q_{max} b C_e}{1 + b C_e} \qquad (2)$$

The linear form of Langmuir is

$$\frac{C_{eq}}{q_{eq}} = \frac{1}{q_{max} b} + \frac{C_{eq}}{q_{max}} \qquad (3)$$

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Where q_{max} is the Langmuir constant ($mg \cdot g^{-1}$) reflecting the maximum adsorption capacity of the metal ion per unit weight of biomass to form a complete monolayer on the surface bound at high C_{eq}. The value of Langmuir constant b ($L \cdot mg^{-1}$) represents a ratio of adsorption rate constant to desorption rate constant, which also gives an indication of the affinity of the metal for binding sites on the biosorbent. q_{max} and b can be determined from the linear form of Langmuir equation (3) by plotting C_{eq}/q_{eq} vs. C_{eq}.

Metal analyses

The effect of initial concentrations on metal adsorption was calculated using the following equation:

$$Metal\ adsorbed\ (\%) = \frac{C_e}{C_i} \times 100 \qquad (4)$$

The specific metal adsorption *q* was calculated using the following equation (5):

$$q_e(mg/g) = \left[\frac{C_i - C_e}{M}\right] \times V \qquad (5)$$

Where q_e is the specific metal biosorption (mg metal/g biomass), *V* is the volume of the metal solution (L), C_i and C_e are the initial and equilibrium concentration of metal (mg metal$\cdot L^{-1}$), respectively, and M is the dry weight of the biomass (g).

After the biosorption, the pellet obtained by centrifugation was resuspended with 25 ml of distilled water, and then concentrated nitric acid was added. The solution was heated at 200 °C for two hours or until the organic matter was dissolved. Finally, the solution was filtered and conserved to measure lead in the atomic adsorption spectrophotometer.

RESULTS AND DISCUSSION

Identification and characterization of lead-resistant bacteria from mine effluents

Eleven morphologically distinct metal tolerant bacterial strains were isolated in solid medium from water samples collected in Mina Asientos, Cochabamba-Bolivia. Four of those strains presented high biosorption values. One strain, designated as MA-4, presented better growth at solid medium supplemented with up to 200 ppm Pb(II) and the highest Pb(II) biosorption value in liquid samples up to 250 ppm Pb(II). The isolate coded as MA-4 presented white circular colonies with wavy margins. Strain MA-4 showed Gram negative bacilli shaped colonies. Biochemical test reveal positive production of triptophane desaminose, D-xylose, D-galactose, D-mannose and D-fucose. Growth occurs with D-galactose and D-fucose and its negative for the rest of substrates tested by API 50CH.

The isolate was identified with 16S rDNA sequencing analysis. A comparison of the DNA sequence with sequences in the National Center for Biotechnology Information (NCBI) database with BLAST software showed 99.99 % sequence identity of strain MA-4 with the published 16S rDNA sequences of *Pseudomonas monteilii* (Fig. 1). The strain was then designated as *Pseudomonas monteilii* MA-4. The *Pseudomonas* genus has the highest number of recognized species and different members have been reported for its application in different bioremediation processes. High-quality draft genome sequence of type strain *Pseudomonas monteilii* DSM 14164T was recently reported [14]. Different strains of *P. monteilii* have been reported for its contaminant removal ability from liquid samples [15, 16].

Biosorption of lead by dead biomass

Eleven strains were isolated in solid media and based on its grown on solid media supplemented with Pb(II), 4 were selected for biosorption tests with liquid samples: strain MA-1, strain MA-3, strain MA-4 and strain MA-5. Strain MA-4 showed the highest result in metal absorbed, after 30 minutes; therefore this strain was selected for further studies and designated as *P. monteilii* MA-4 based on phylogenetic analysis. Optimal bacterial growth was observed at pH value of 7, orbital agitation speed of 100 rpm and temperature value of 35 ºC for *P. monteilii* MA-4. Maximum adsorption capacity ($mg \cdot g^{-1}$) for lead by *P. monteilii* MA-4 dead biomass was 250 $mg \cdot g^{-1}$ at 30 min when immobilized in alginate beads (Figure 2).

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Langmuir and Freundlich models are commonly used to describe the biosorption process; both equations were applied for *P. monteilli* MA-4. Langmuir model makes several assumptions, such monolayer adsorption and constant adsorption energy, while Freundlich equation deals with heterogeneous surface adsorption. The Langmuir model and Freundlich equation parameters were derived from the fitting experimental points q_{max} and K_f values obtained in this study were 166.67 and 11.09, respectively (Table 1). The correlation coefficients obtained for lead ion biosorption were for Langmuir model 0.0127 and for Freundlich model 0.44.

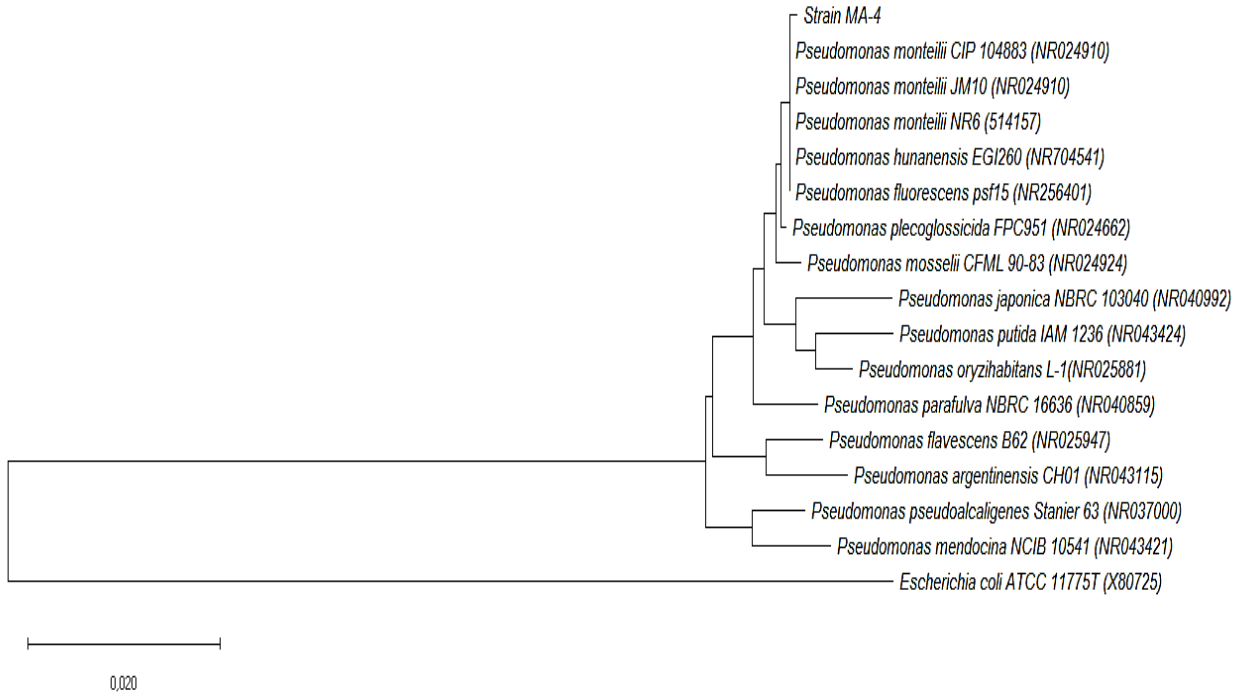

Figure 1. *Phylogenetic tree of members of the genus Pseudomonas, based on 16S rRNA gene sequences. The tree was constructed using the neighbor-joining method with bootstrap values based on 1000 replications. Escherichia coli ATCC 11775T was used as the out group. Accession numbers are given in parentheses.*

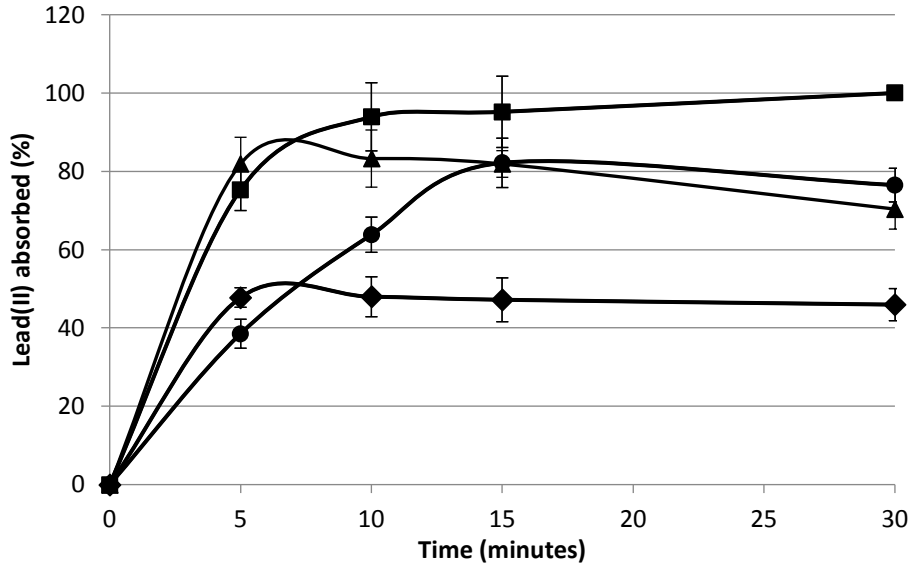

Figure 2. *Lead biosorption in liquid media tests. (■) strain MA-4,(▲) strain MA-3, (●) strain MA-1, (♦) strain MA-5.*

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Table 1. *Langmuir and Freundlich isotherm values for the biosorption of lead (II) ion onto P. monteilii MA-4.*

Freundlich model			**Langmuir model**		
K_f (L·g^{-1})	**1/n**	**R^2**	**q_{max} (mg·g^{-1})**	**b (L·mg^{-1})**	**R^2**
11.09	0.44	0.984	166.67	0.0127	0.985

The coefficient b in Langmuir equation is a measure of the stability of the complex formed between metal ions and biomass under specific experimental conditions. The small b value obtained in this research implied strong binding of metal ions to dead biomass. Maximum metal uptake capacity of biomass q_{max} was found as 166.67 mg·g^{-1} for biosorption of Pb(II). These lead adsorption capacities can be compared with those reported for different bacteria like *Corynobacterium glutamicum* 567.7mg·g^{-1}, *Phanerochaete chrysosporium* 134 mg·g^{-1} and *Pseudomonas aeruginosa ASU 6a* 123 mg·g^{-1}. *P. monteilli* MA-4 can be considered a bioremediation agent for lead(II) removal and therefore have great potential application in bioremediation processes [17].

CONCLUSION

The recovery and remediation of Pb(II) is nowadays a research topic that demands attention. There are several traditional physicochemical treatments developed but microbial remediation is a promising strategy. Different microorganisms so far have been reported for its ability of Pb(II) removal from water and soil environments. Lead biosorption by members of the *Pseudomonas* members are well documented with quite rapid biosorption times using intact cells, thermolyzed, chemically treated and dead cells. This study indicates that dead biomass of *Pseudomonas monteilii* MA-4 can be used effectively as a biosorbent for the removal of Pb(II) from aqueous solutions. Maximum removal up to 250 ppm of Pb(II) was observed within first 30 minutes when immobilized in alginate beads. The adsorption equilibrium data fitted well with Langmuir and Freundlich models for metal ions in the concentration range studied. The results demonstrate that dead biomass of *P. monteilii* MA-4 is a potential support to be used as a promising biosorbent for the removal of Pb(II) ions from aqueous solutions.

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