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BIOSORPTION OF Pb(II) BY *Thiobacillus ferrooxidans*

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Key words: metal recovery, heavy metals

ABSTRACT

Mainly due to the commercial value and the environmental impact that the heavy metals cause, a number of studies has been conducted to determine the possibility of removing and recovering them from diluted solutions. The traditional methods for removing metals have several disadvantages when they are present in concentrations lower than 100 mg/L. In this low concentration range, *biosorption*, which uses biological materials as adsorbents, has been considered an alternative method. In this work, variables such as pH and biomass chemical treatment have been studied for their effect on the capacity of lead biosorption by *Thiobacillus ferrooxidans*. Studies to determine the time for lead adsorption were carried out as well. Results indicated that a capacity as high as 443 mg of Pb(II)/g of dry biomass can be obtained at a temperature of 25 °C and pH 5 when cells under chemical treatment were used, and that the biosorption process occurred quickly within 30 minutes.

Palabras clave: recuperación de metales, metales pesados

RESUMEN

Debido principalmente al valor comercial y al impacto que los metales pesados causan, se han realizado muchos estudios sobre la posibilidad de removerlos y recuperarlos de soluciones diluidas. Los métodos tradicionales para la remoción tienen varias desventajas cuando los metales están presentes en concentraciones menores de 100 mg/L. La *biosorción*, que implica el uso de materiales biológicos como adsorbentes, ha sido considerada como un método alternativo. En este trabajo, se estudió el efecto de variables como pH y tratamiento químico de la biomasa en la capacidad de biosorción de Pb(II) por *Thiobacillus ferrooxidans,* así como el tiempo de biosorción. Los resultados indican que pueden ser obtenidos hasta 443 mg de Pb(II)/g de biomasa seca cuando el sistema se trabaja a pH 5, 25 ºC y las células se tratan con NaOH 0.075 M. También se encontró que el proceso ocurre en los primeros 30 minutos.

INTRODUCTION

Current technologies for the removal and recovery of of metals and other toxic substances of industrial interest usually produce wastes with high concentrations of them. These wastes are an important source of environmental pollution. Pollution of water, air and soil by heavy metals is one of the most severe environmental problem, and a very

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difficult one to solve. The most common sources of water pollution with heavy metals are petroleum processes, energy generating plants, electrodeposition of metals and metallurgic processes.

So far, there are a number of technologies for the removal and recovery of heavy metals from diluted solutions. The traditional methods for removal include precipitation, oxidation/reduction, ionic exchange, filtration, electrochemical processes, membrane separations, and evaporation. These methods imply several disadvantages including high cost, incomplete removal, low selectivity, high energy consumption, and the generation of toxic slurries that are difficult to eliminate when used with concentrations of metals that are below 100 mg/L (Cushnie 1985, Paknikar *et al.* 1993). On the other hand, the current environmental legislation has established maximum permissible limits for the discharge of metal-containing waste streams. In the case of lead, the maximum permissible limit is 1ppm.

Alternative methods of metal removal and recovery for concentration range 0-100 ppm based in biological materials have been considered (Modak and Natarajan 1995, Volesky and Holan 1995). Certain types of microbial biomass can retain relatively high quantities of metals by means of passive processes known as biosorption, which depends on the affinity between the metallic species or its ionic forms and the binding sites on the molecular structure of the cellular wall (Raras 1995).

The biosorption process is relatively fast and the fact that it is a surface phenomenon facilitates the removal of the metal and the subsequent use of the material as biosorbent. An important aspect of the operation is that it can be carried out when the cell is metabolically inactive or even dead. One advantage of using microorganisms in an inactive way is that its propagation and use can be separate processes (Volesky 1990).

There is a current need to evaluate alternative biosorbents that could produce basic information for the development of new processes or select systems that improve the processes of removal of heavy metals in solution. The potential of *Thiobacillus ferrooxidans* as a biosorbent for Cu(II) and Ni(II) has been reported (Duarte *et al.* 1997, Ruiz-Manriquez *et al.* 1998). In this work, the potential for Pb(II) is showed. The effects of lead toxicity in humans include hypertension and brain damage, beside effects in livestock and aquatic fauna (Foster and Wase 1997).

MATERIALS AND METHODS

Biosorbent

Metabolically inactive cells of *Thiobacillus ferrooxidans* were used as biosorbent. These cells were produced using a NBS-75 fermentator. The inoculum was produced from a collection strain (ATCC19859) in the Biotechnology Laboratory of the University of Sonora in a NBS-BIOFLO1 fermentator. In both cases a modified 9K growth medium was used (Barron and Lueking 1990). The batches were developed at a pH of 1.9, 30 °C and 300 rpm. The cells were harvested, cleaned following the procedure reported by Shuler and Tsuchiya (1975), and dried according the procedure published by Goodhue *et al.* (1986).

Chemical treatment

For the experiments where the effect of chemical treatment was analyzed, the cells were treated with 0.075 M NaOH for a period of 10 minutes using 10 mL of NaOH solution. The cells were then centrifuged, using a refrigerated Biofuge 17R from Baxter, for a period of 10 minutes at 11400 g. The resulting cells were resuspended in deionized water and centrifuged again. This operation was repeated until a neutral pH was obtained. Finally, the remaining cells were suspended in a sodium perchlorate solution.

Solutions

For the preparation of lead solutions, lead nitrate ($Pb(NO_3)_2$) 99.5 % reactive grade from SIGMA was used. This solutions were usually prepared at concentrations of 25, 50, 75, 100, and 150 mg of lead per liter of solution. As a background electrolyte, sodium perchlorate ($NaClO_4 \cdot H_2O$) 86.1 % reactive grade, from SIGMA, was used in a concentration of 0.02 M. These solutions were made using deionized water and the pH was adjusted using either 0.1 N NaOH or 0.01 N HCl.

Lead biosorption

An experimental system that consisted of an agitated tank reactor was used. This reactor was operated in a batch mode. The reactor was equipped with a pH electrode and it sat on a thermomagnetic plate which was used to provide an agitation of 300 rpm. The reactor (50 mL beaker) was filled with 25 mL of solution with a different initial lead concentration each time (25, 50, 75, 100, and 150 mg of lead per liter of solution). A predetermined concentration of *T. ferrooxidans* biomass (0.2 mg of dry cells / mL) was used. Each experiment was run for two hours, which was enough time to achieve a steady state. All the experiments were carried out at a temperature of 25 °C. The pH was controlled throughout the experiment adding either 0.1 N NaOH or 0.01 N HCl solutions. As a dependent variable, the biosorption capacity (mg of Pb(II) /g of dry biosorbent) was used. The independent variable used was the concentration of metal in solution (mg of Pb(II) / L of solution).

Lead quantification

The samples were collected and filtered using Millipore filters of 0.22 micrometers. The filtrate was collected for lead analysis and, periodically, the membranes were also analyzed to detect the presence of lead. The lead analysis was conducted using a Perkin Elmer 3110 atomic absorption spectrophotometer. This instrument was initially calibrated with known concentration and then samples of unknown concentration were identified.

Biosorption evaluation

The evaluation of the biosorption was carried out considering the equilibrium relations (adsorption isotherms) obtained under different operating conditions. The equilibrium relations were considered favorable when a significant amount of biosorption occurred. These relations were mathematically modeled to obtain a maximum capacity and desorption constant values.

RESULTS

Effect of pH

The effect of the pH on the capacity for lead biosorption by *T. ferrooxidans* biomass was studied. In the case of biomass without chemical treatment the results are shown in **figure 1**. The experiments were carried at pH values of 3, 4, and 5. At pH values of 4 and 5 a favourable isotherm was obtained, with a maximum capacity of biosorption of 215.5 and 216.7 mg Pb(II) / g of dry biosorbent, respectively. For pH 3, a linear adsorption was observed (r^2 = 0.96). In the case of chemical treated biomass a maximum capacity of 174 and 443 mg of Pb(II) /g of dry biosorbent was obtained at a pH 4 and 5, respectively. At a pH 3 a direct linear adsorption was observed. **Figure 2** shows a comparison between the isotherms for the three cases.

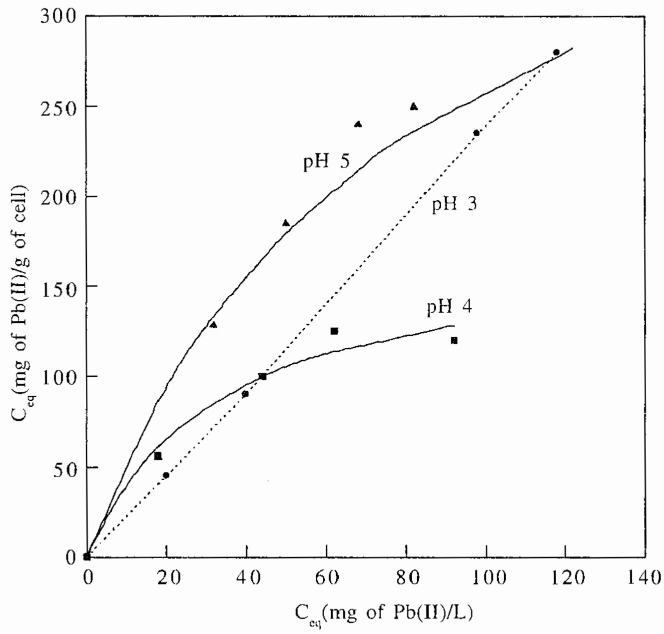

Fig. 2. Effect of pH on Pb(II) biosorption by chemically treated *T. ferrooxidans*. Biomass was treated with 0.075 M NaOH , 0.2 mg of cells/ml, 25 °C. Data for pH 4 and 5 were adjusted using Langmuir´s model (continuous lines)

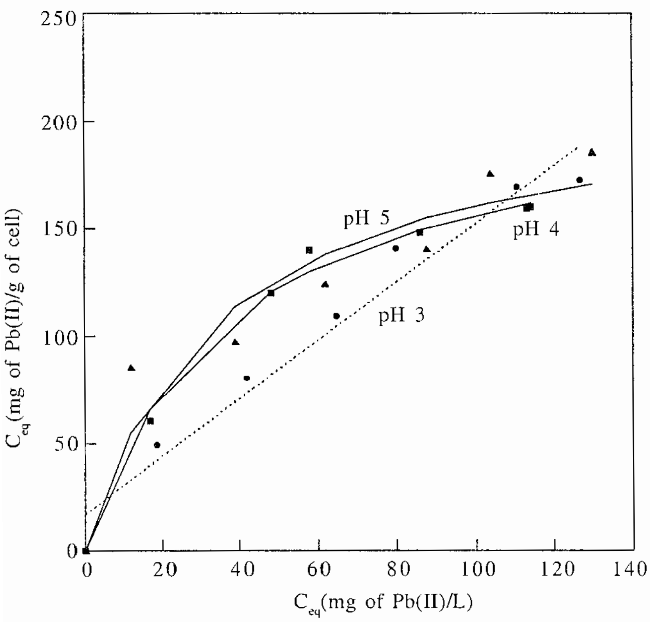

Fig. 1. Effect of pH on Pb(II) biosorption by *T. ferrooxidans*. Untreated biomass was used at 25 °C. 0.2 mg of cells/ml. Data for pH 4 and 5 were adjusted using Langmuir´s model (continuous lines)

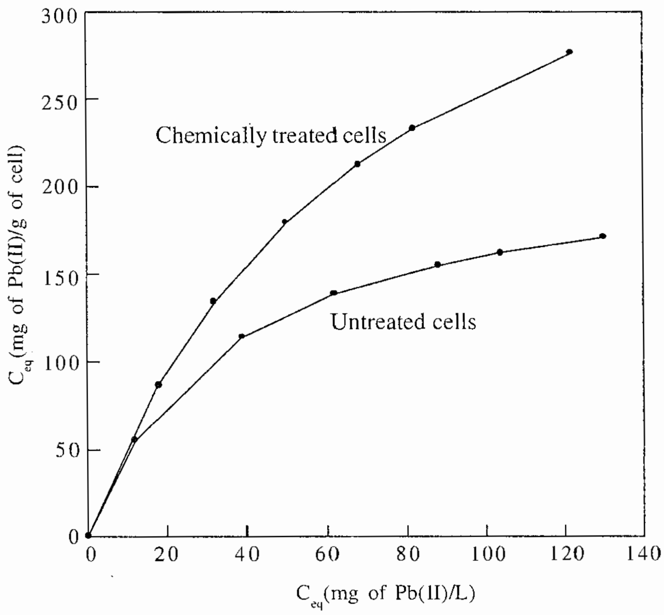

Fig. 3. Effect of treatment with 0.075 M NaOH on Pb(II) biosorption by *T. ferrooxidans*. Experiments were run at pH 5 and 25 °C. Data were adjusted using Langmuir´s model

TABLA I. VALUES OF EXPERIMENTAL EQUILIBRIUM CONSTANTS

pH	Chemical treatment	Desorption constant (1) K_d (mg/L)	Maximum capacity (1) q_{max} = (mg/g)	Slope (2)
3	NO	-	-	1.53
3	YES	-	-	2.37
4	NO	37.9	215.5	-
4	YES	33.0	174.1	-
5	NO	35.1	216.7	-
5	YES	73.1	443.0	-

(1) Constants obtained using Langmuir´s model
(2) Slopes obtained by regression

Effect of chemical pretreatment

The effect of chemical treatment in the capacity for lead biosorption by *T. ferrooxidans* biomass was evaluated with respect to untreated biomass. The results can be observed in **figure 3**. As can be seen, the difference in the capacity for Pb(II) biosorption is significant (227 mg Pb(II) for pH 5 in favour of chemical treated biomass).

Biosorption kinetics

The biosorption kinetics was studied using the experimental set up with a lead concentration of 100 mg of Pb(II)/L and a chemically treated biomass concentration of 0.2 mg of cells/mL of solution. The temperature was set at 25 ºC, the pH of the solution was 5, and a volume of 250 mL of solution was used. The resulting data was analyzed using the Skidmore model (Skidmore *et al.*1990) and they are shown in **figure 4**, where an estimated k value of 0.0087 L/mg-min was obtained.

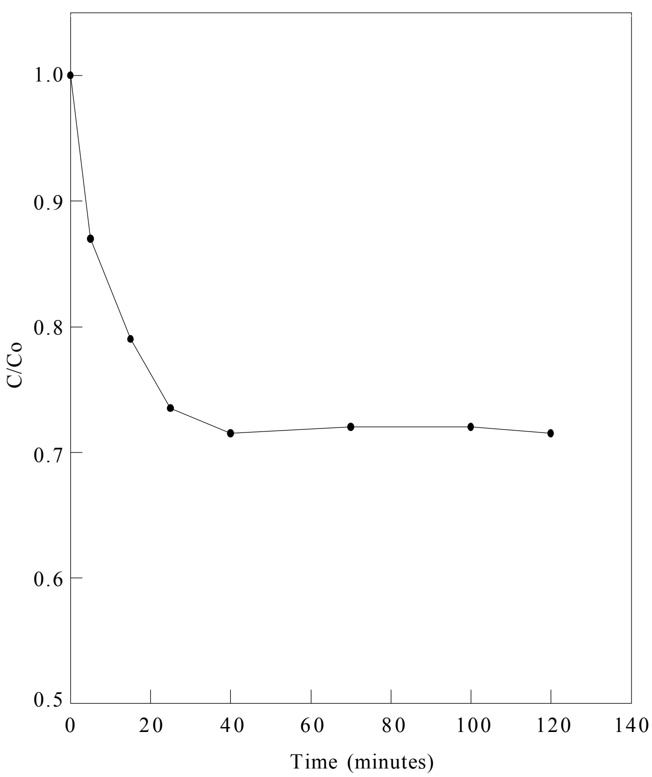

Fig. 4. Biosorption kinetic of Pb(II) by *T. ferrooxidans*. Experiments were run at pH 5, 25 °C. 0.2 mg of biomass/ml, 100 mg Pb(II)/L

Equilibrium constants

Finally, **table I** shows the values of the different equilibrium constants obtained under the different experimental conditions described above. To obtain these constants, curve fit and regression features in SigmaPlot v. 2.01 was used.

DISCUSSION

The work reported by Volesky and Holand (1995), identifies organisms that have potential as adsorbents and establishes, also, references of adsorbent capacities for these kind of processes. From the results obtained in this work and its comparison with the above reference (189 and 601 mg of Pb(II)/g of dry biosorbent, for the case of *Bacillus subtilis*), it can be said that *Thiobacillus ferrooxidans* is an organism that has a good capacity for Pb(II) biosorption when is treated with NaOH.

The pH of the solution has a significant effect on the capacity for metal biosorption, mainly due to the protonization that occurs at low values of pH and to the effect it has on the chemistry of the solution. However, in this work and in agreement with what has been reported by Fourest and Roux (1992) lead biosorption was slight inhibited by protons and maximal uptake ocurred at pH 5

Also, in agreement with what has been reported by Brierley and Brierley (1993), an appropriate physical or chemical pretreatment of the biomass has positive effects on its capacity for biosorption of metals. This effect could be explained by the increase in the availability of binding sites or by the removal of polysaccharides that block the access to the binding sites. It could also result from a change of permeability in the cellular wall, in the case of bioaccumulation. For the system studied, the increment in the capacity was significant when the biomass was chemically treated.

Also it can be said that the biosorption process occurs rapidly (30 min), which suggest that it is a surface phenomenon.

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