BIOREMOTION OF TOXIC METALS FROM WATER BY SPORES OF *Bacillus* SG1

BIOREMOCIÓN DE METALES TÓXICOS DE AGUAS POR ESPORAS DE *Bacillus* SG1

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ABSTRACT

Selective biosorption of Pb and As ions by spores were investigated. Microorganism used was Bacillus spp. SG1 isolated from marine sediment. The optimum pH value for Pb and As biosorption was found to be in the range of 4 to 7. Spores showed high affinity for binding to these metals in single aqueous solutions. High absorption was observed for Pb and As with initial concentration of 4 mg/L and under these conditions the sorption isotherms follow the classical Langmuir pattern with a regression value of 0.89 and 0.88 for Pb and As respectively. For practical purposes, a simple biofilter built with sand and spores coated with manganese, resulting in an effective 99% and 98% removal of Pb and as from water within 1 hour time period. This application is affordable and seems to be useful for low income communities of rural areas for treating contaminated water.

Key words.- Biosorption, Adsorption, Bacillus SG1, Toxic metals, Contaminated water, Redox reactions, Biofilter.

RESUMEN

Se investigó el proceso de biosorción selectiva de Pb y As por esporas bacterianas. El microorganismo utilizado fue Bacillus spp. SG1 aislado del ambiente marino. Los valores de pH óptimo para la adsorción de estos metales tóxicos se encontraron situarse entre 4 y 7. Las esporas mostraron una alta afinidad por estos metales en soluciones acuosas. Una adsorción muy alta de Pb y As se observó a concentraciones iniciales de 4 mg/L y bajo estas condiciones las isotermas de sorción siguen el patrón clásico de Langmuir con un valor de regresión de 0.89 y 0.88 para Pb y As respectivamente. En términos de una simple aplicabilidad práctica se habilitaron botellas de plástico conteniendo una mezcla de arena y esporas activadas con manganeso, resultando en una remoción efectiva del 99% y 98% de Pb y As del agua en el periodo de 1 hora. Esta simple aplicación puede ayudar a resolver a descontaminar el agua de bebida de origen subterráneo utilizado en comunidades rurales.

Palabras clave.- Biosorción, Adsorción, Bacillus SG1, Metales tóxicos, Agua contaminada, Reacciones redox, Biofiltros.

INTRODUCTION

Virtually all metals, including the essential metal micronutrients, are toxic to aquatic organisms as well as humans if exposure levels are sufficiently high. Metals are introduced into aquatic systems as a result of weathering of soils and rocks, from volcanic eruptions and from a variety of human activities involving the mining, processing, or use of metals and/or substances that contain metal

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contaminants [15]. Heavy metals can not be decomposed in situ by biological means. Many metals are essential for biological functions, whereas it is not known whether some others such as Pb, Cr, As, Cd, Al, Ag, Sn, Au, Sr have essential biological functions [4, 5 y 7].

It is therefore necessary to remove these toxic metals from water due to effects of disturbing environmental quality and being harmful to human health. Biological removal of heavy metals contaminants from aquatic effluents offers great potential when metals are present in trace amounts. Many microbial species such as bacteria, fungi, yeast and algae are know to be capable of adsorbing heavy metals on their surface and/or accumulating within their structure [1, 8, 9, 10 y 18].

It is possible that microorganisms can be used in the removal of toxic metal ions from the water and even in the recovery of them by using these adsorption properties of the microorganisms. Physical adsorption or ion exchange at the living or non-living cell surface is very rapid and occur in a short time after microorganisms come into contact with heavy metal ions. Accumulation occurs in living cells and is slow, related to metabolic activity [14]. Although there are a number of studies on removal of heavy metals, the knowledge of the applications of biosorbents for clean water provision at urban and rural areas has not been yet fulfilled.

It is well known that bacteria from many genera in fresh and marine waters and sediments catalyze the conversion of manganese between oxidation states, mainly between + II and + IV [12 y 15]. On the other hand, many organisms produce specialized macromolecules which catalyze the redox reactions (direct catalysis), and in some cases it has been shown that genes and enzymes are involved [2 y 17].

Marine *Bacillus* sp. strain SG-1, which was isolated from a near-shore sediment enrichment culture, is one bacterium that has been extensively studied in terms of Mn(II) and Co(II) oxidation [3 y 16]. In this bacterium, it is the spores that catalyze Mn(II) and Co(II) oxidation under environmentally relevant pH, temperature, and metal concentration conditions. The spores also bind a variety of other heavy metals, such as Cd and Zn. Mn(II)-oxidizing activity is located on the spore coats and apparently is catalyzed by a protein. Recent evidence suggests that a spore surface protein related to the multicopper oxidase family of proteins is involved in Mn(II) oxidation [13 y 17]. Consistent with this idea, low amounts of Cu(II) stimulate Mn(II) oxidation.

Therefore, in this study we were interested in the removal of heavy metals from water taking advantage of the principles of microbial transformations such as redox conversions. Our aim was to study the adsorption feasibility of toxic metals on the spores of *Bacillus* and to provide a simple method to treat contaminated water.

MATERIALS AND METHODS

Preparation, purification, and pretreatment of SG-1 spores.

Bacillus sp. strain SG1, isolated by Nealson and Ford [11] was grown at room temperature and maintained in K medium in 1-liter flasks placed on a rotary shaker at 150 rpm. K medium contains (per liter of 75% seawater) 2 g of peptone and 0.5 g of yeast extract. After autoclaving, 20 ml of 1 M filter-sterilized N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid (HEPES) buffer (pH 7.4 to 7.8) and 0.1 ml of 1 M filter-sterilized $MnCl_2$ were added per liter. For agar plates, 15 g of agar per liter was added before autoclaving.

Usually more than 95% of the bacteria produced endospores within 5 days. Fully sporulated cultures consisted of spore clumps and few individual spores, as observed by phase-contrast microscopy. Spores were harvested by centrifugation at 16,000 × g and 4°C for 10 min and were purified to remove precipitated Mn oxides and adsorbed trace metals from the spore surface as follows. Spore pellets were washed with water, homogenized and recollected by centrifugation. Spores were then homogenized and treated with 0.01 M ascorbic acid with shaking for 10 min, washed three times with 1 M NaNO3-0.01 M EDTA, and finally washed five times with Milli-Q water. After homogenization, spores were stored in Milli-Q water at 4°C; spores in the suspensions usually formed aggregates. The number of spores in a suspension was determined by direct counting with a counting chamber after dispersion of clumps. A total of 44 small squares were counted. The average number of spores in each small square was 24, and the standard deviation was 5.8.

Control of manganese oxidation

The bacterial spores were grown, harvested by centrifugation and cleaned with deionized water. A solution of $MnCl_2$ (10mM) with 75% seawater and a HEPES buffer at pH 7.5 were inoculated with a thick suspension of spores. The suspension showed a change in spore color from light to dark.

Experiments with spores alone

Adsorption properties of Mn-oxidized spores for biosorption of different metal ions in single-component aqueous solution were measured with standard batch equilibrium experiments. The concentrations of the heavy metal ions ranged from of 100 to 400 mg/L that was obtained from stock solutions of 1 g/L Pb^{2+} ($Pb(NO_3)_2$) and 1g/L As ($H_2AsO_4^-$). In the initial experiment the effect of solution pH on biosorption of metal ions was studied. For this purpose aqueous solutions containing 4 mg/L of investigated metal ions, adjusted to the predetermined pH values ranging from 2 to 8, were used.

Adsorption was then started by adding 1.0 g of Mn-oxidized spores into 100 mL metal solution in 500 mL Erlenmeyer flasks. The flasks were sealed with Parafilm and agitated on a rotary shaker at 200 rpm and 25 °C during 8 h. All adsorption tests were overall run in duplicate and relative deviation obtained was less than 5 %. Controls without the adsorbent were also run in parallel. Furthermore, the adsorption isotherm experiments were performed under the same conditions but only at pH 7, which was determined as the optimal one. The solution samples were taken at predetermined time intervals during 10 h for evaluation of sorption kinetics. The metal ion concentrations adsorbed onto spores were calculated from the difference between metal concentration before and after the biosorption process

Experiments with spores and sand

Preparation of biofilter and reaction set-up.- Dried spores (40 g) were mixed with sand (200 g) of uniform size (±2 mm diameter). The mixture was placed in a 2 L plastic soda bottle with holes on top and the bottom. The top hole was connected with PVC tube to water supply and the bottom hole was connected to the sampling device with valves. Water containing 100 μM $MnCl_2$ is placed on the bottle and the bottom the sand. The water was drained out of the reactor, analyzed for Mn and the reactor was ready to use. A reservoir able to hold 10L of water supply was attached to the inflow pipe to supervise the operation.

Measuring of sorption capacity of biofilter for Pb and As.- Water contaminated with 4 mg/l Pb^{2+} ($Pb(NO_3)_2$) and 4 mg/L As ($H_2AsO_4^-$) and buffered to maintain a neutral pH was placed in the reservoir (single solution for metal) and let it standing for 1 hour then flow trough the reactor. Samples of 100 ml of filtered water were collected at 15 minutes intervals. The analysis of Pb and As ions were carried out by atomic absorption spectrophotometer at 0.001 ppm sensitivity level.

RESULT AND DISCUSSION

Effect of pH and concentration on metal biosorption.

Spores of Bacillus spp. SG1 was the strain utilized for biosorption of Pb and as dissolved in water. Sorption of heavy metals from aqueous solutions depends on properties of adsorbent and molecules of adsorbate transfer from the solution to the solid phase. It has been also reported that biosorption capacities for heavy metals are strongly pH sensitive and that adsorption increases as solution pH increases. Initial investigation of biosorption capability of Mn-oxidized spores for Pb and As (4 mg/L) each in single solution at different values of pH (2 to 8) is presented in Fig. 1.

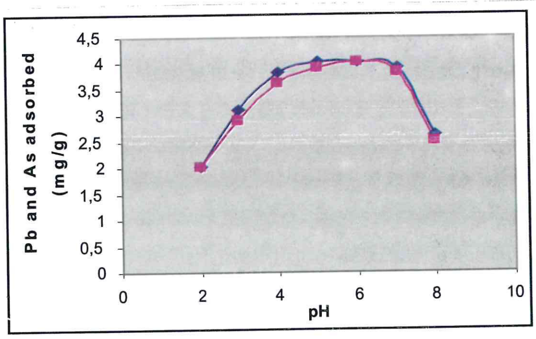

Fig. 1 *Effect of pH on biosorption of Pb and As onto Mn-oxidized spores.*

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Recepción de originales: Agosto 2007
Aceptación de originales: Octubre 2007