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Biosorption of Nickel Using Filamentous Fungi

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ABSTRACT

Nickel (Ni) uptake capability from aqueous solutions was studied in a filamentous fungi strains group of *Rhizopus* sp., *Penicillium* sp. *Aspergillus* sp., *Trichoderma* sp., *Byschoclamyss* sp., and *Mucor* sp. The metal uptake of a *Rhizopus* sp. strain, which has the highest uptake capacity, was corroborated by electron microscopy; no Ni deposits were observed on the cell wall, but rather a homogeneous accumulation was seen on the cell surface. The influence on the capacity of metal uptake by environmental parameters such as pH, temperature, time, and the interference of other ions in the solution, was also studied. Nickel accumulation by the selected strains is fast, occurring in less than 30 min, and does not require a microorganism's active metabolism to take place. Sorption isotherms were established for the selected fungi, in order to determine the maximum metal uptake capacity. The sorption isotherms were fixed to the mathematical models of Freundlich and Langmuir, obtaining better performance on the Langmuir model.

INTRODUCTION

High quantities of toxic metals are frequently associated with effluents originating from numerous industrial operations. Because of their toxicity, they must be removed by means of different physical, chemical, or biological technologies *(1,2,3,5)*. One of the best biological options is biosorption. Biosorption refers to the passive metal uptake by different forms of biomass, which may be dead or alive. Filamentous fungi may better suit this purpose than other microbial groups, because of their high tolerance toward metals, wall binding capacity, and intracellular metal uptake capabilities. Unlike intracellular metal uptake, binding of metals to cell surface components is a very fast process. Efficiency of biosorption

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reactions is modified by environmental conditions such as temperature, pH, ionic strength of the media, and so on *(6,8)*.

The purpose of this research was to evaluate nickel (Ni) biosorption capacity by different biomass of strains of filamentous fungi, and the role of some environmental conditions in the process efficiency. Metal uptake capabilities and other biosorbent characteristics were calculated by sorption isotherms.

MATERIALS AND METHODS

Microorganisms

For a previous work *(9)*, the biosorbents used in this research were selected from a group of Colombian native strains of filamentous fungi belonging to the genus *Penicillium* sp., *Trichoderma* sp., *Rhizopus* sp., *Mucor* sp., *Byssochlamys* sp., *Paecelomyces* sp., and *Aspergillus* sp. These strains were obtained from Andes University Culture Collection (Bogotá DC, Colombia). That work resulted in the selection of four strains with potential for Ni biosorption: *Penicillium* sp BARE1, *Mucor* sp 0620, *Rhizopus* sp 0145, and *Rhizopus* sp 0101.

Culture Media and Reagents

Maintenance and biomass growth media were Malta Extract Agar and Malta Extract Broth (Oxoid, Basingstoke, UK). Metal source was a stock obtained from a Ni tritisol standard from Merck. In washing biomass and biosorption reactions, 1 *M* Tris-HCl buffer, pH 7.0 was used. Changes in pH were performed by adding HCl or NaOH. For electron microscopy sample fixing, phosphate buffer (pH 7.4, plus glutaraldehyde 2.5%) was used. Ethanol solutions in increasing concentrations (30, 50, 90, 95, 100% v/v) were used to remove residual water from these samples.

Biosorption Assays

Biomass Production

Each strain was inoculated in a solid medium and the slants were cultivated for 5 d (25 +/−2°C). From these cultures, a spore solution (10^6 spores/mL) was obtained and used to inoculate flacks with 400 mL of Malta Extract Broth. These media were cultivated for 5 d in a rotary shaker (150 rpm) at 25 +/−2°C.

Biomass Preparation

The harvested biomass was washed 3× in buffer (1 *M* Tris-HCl, pH 7.0). Residual water was removed by vacuum filtration using 0.7-μm filters, and the biomass was stored at 4°C.

Biosorption Reaction

Metal solutions were poured in 125-mL flasks with the required wet wt of biomass. The initial concentration of metal was adjusted to give different concentrations between 10 and 300 ppm. Final volume reaction was completed with 1 *M* Tris-HCl buffer, pH 7.0. In all experiments except the kinetics studies, exposure time was 6 h. The reactions were carried out in a rotary shaker (150 rpm) at room temperature (20+/−2°C).

Separation

The biosorbent material was removed by vacuum filtration using 0.7-μm filters. The metal concentration within the biomass was calculated from the difference between metal concentration before and after metal uptake. Metal concentration was determined by adsorption spectroscopy, in a Perkin-Elmer 5100 PC, (Norwalk, CT), using an acethylene-air flame. For electron microscopy, biomass samples were taken and stored in dH_2O at 4°C *(12,15)*.

Kinetic Biosorption Assays

These experiments were carried out using biomass of the microorganisms *Rhizopus* sp 0101, *Rhizopus* sp 0145, *Mucor* sp 0620, and *Penicillium* sp BARE1. For each microorganism, flasks were disposed with 2 g of biomass (wet wt), and the final volume was adjusted with Tris buffer, pH 7.0. Initial metal concentration was 10 ppm. Reaction was finished at different exposure times: 0.5, 1, 2, 4, 6, 8, 16, and 24 h. Each assay included an unique negative control that was sampled at 24 h.

Sorption Isotherms and Metal Uptake Capacity

Sorption isotherms were carried out using *Rhizopus* sp 0101 biomass. Harvested and washed biomass was dried at 40°C, for 12 h, to get a constant weight. Biomass particle size was adjusted to 0.85–1.16 mm in diameter. Initial metal concentrations were 10, 20, 50, 100, 200, 300, and 400 ppm. A 1 g/L biomass concentration was used. Isotherms were performed at pH 3.0, 5.0, 7.0. In pH, temperature, and sodium chloride experiments, a negative control without biomass was included.

Electron Microscopy and X-ray Elemental Analysis (SEM-EDX)

Biomass samples from isotherm assays were taken for electron microscopy and X-ray elemental analysis. Samples were dried at 40°C, until constant weight, and then covered with gold for later scanning *(10,11)*. Analyses were carried out in a Cambridge Instruments Stereoscan 240 electron microscope with an X-ray analytic system, EDAX.

pH Influence

pH influence in Ni uptake was assayed with pH values between 1.0 and 11.0. pH-s of the reaction media (1 *M* Tris-HCl) were adjusted using

HCl or NaOH. In each experiment, 2 g of biomass (wet wt) was placed in 50 mL of reaction media.

Temperature Influence

The temperature values evaluated were 4, 20, 40, and 60°C. In each assay, 2 g (wet wt) of biomass were used in 50 mL of solution.

Sodium Chloride Concentration Influence

Influence of this ion concentration in Ni uptake were performed in assays which contained 1.5 g (wet wt) and 10 ppm of Ni. Reaction media were supplemented with NaCl in the following concentrations: 100, 1000, 5000, and 10,000 ppm.

RESULTS AND DISCUSSION

Kinetics Biosorption Assays

Figure 1 shows the metal removal for each exposure time, determined as the percentage of metal removed. For both *Rhizopus* sp 0101 and 0145, Ni uptake rates were the highest between 1 and 2 h of exposure. *Rhizopus* sp 0101 reached its maximum metal uptake rate after 30 min, and *Rhizopus* sp 0145 reached equilibrium after 2 h. *Penicillium* sp Barel and *Mucor* sp 0620 kinetics were different: Both had lower metal uptake rates than the two strains of *Rhizopus*. *Penicillium* sp Barel was at equilibrium after 6 h, and *Mucor* sp 0620 reached its maximum metal uptake after 24 h.

Rhizopus sp 0101 and *Rhizopus* sp 0145 kinetics were typical of the biosorption process, and were established as passive reactions nondependent on metabolism. High metal uptake rates reached in free nutrient media are characteristic of passive biosorption *(15)*. Metabolism-dependent intracellular uptake, in which metal ions are transported into cells across the cell wall, may be slower than passives ones. This could explain the observed *Mucor* 0620 and *Penicillium* sp Barel kinetics *(8,12,14,15)*.

Sorption Isotherms and Metal Uptake Capacity

Isotherm results were adjusted to the Langmuir and Freundlich models. Figure 2 shows the typical isotherm pattern obtained with all tested materials. Table 1 shows these parameters, which were evaluated according to the least-fitting method, using the experimental Cf and q values *(12,15)*. As seen in Table 1, correlations obtained with Langmuir model were better than Freundlich ones, except for pH 6.0. The q_{10} (uptake capacity at Cf = 10 ppm) at pH 7.0, calculated from Langmuir and Freundlich models were 21 mg/g and 6.8 mg/g, respectively; experimental q_{10} was 16 mg/g. The q_{200} (uptake capacity at Cf = 200 ppm) at pH 7.0, calculated from Langmuir and Freundlich models, were 30 mg/g and 15.6 mg/g, respectively, experimental q_{200} was 41 mg/g.

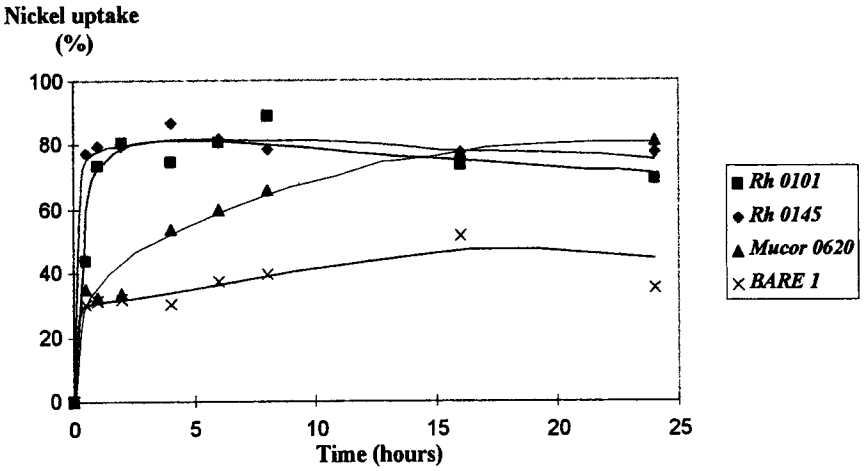

Fig. 1. Kinetics of nickel biosorption. Initial metal concentration was 10 ppm. Metal uptake was expressed as removal percentage for each exposition period. The evaluated strains were *Rhizopus 0101, Rhizopus 0145, Mucor 0620, Penicilluim BARE1.*

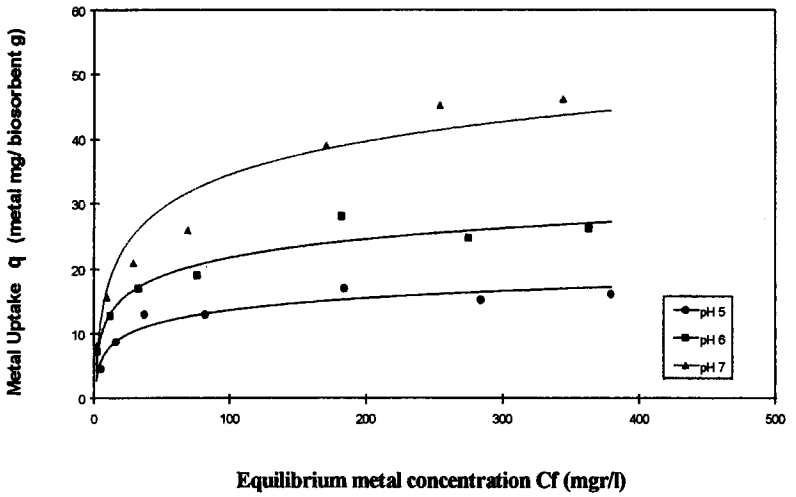

Fig. 2. Nickel Biosorpion Isotherms. Assays were performed with the strain *Rhizopus 0101.* Biomass was treated as was indicated in the text. Biomass final concentration was 1g/L dried weight.

The experimental q_{max} obtained at pH 7.0 was 45 mg/g, was greater than that reported by Fourest et al. *(17)*, who found a $q\text{-}_{max}$ of 22.2 mg/g for a strain of *Rhizopus arrhizus.* This difference may be explained by specific characteristics of the evaluated strains, although other important parameters, such as particle size, were not reported *(17).*

Electron Microscopy and X-ray Elemental Analysis (SEM-EDX)

Electron microscopy is a powerful tool for biosorbent characterization, because it permits determination of metal accumulation by the bio-

Table 1
Nickel Biosorption Isotherm: Langmuir and Freunlich Model Parameters

	Langmuir model			Freundlich model		
pH	q_{max}	K_d	R^2	n	K	R^2
5.0	16.6	0.071	0.994	3.04	6.98	0.952
6.0	22.3	0.18	0.941	3.82	6.16	0.959
7.0	31.6	0.20	0.92	3.65	3.65	0.855

Assays were performed with the strain *Rhizopus* 0101. Biomass was treated as indicated in the text. Biomass final concentration was 1g/L dried wt.

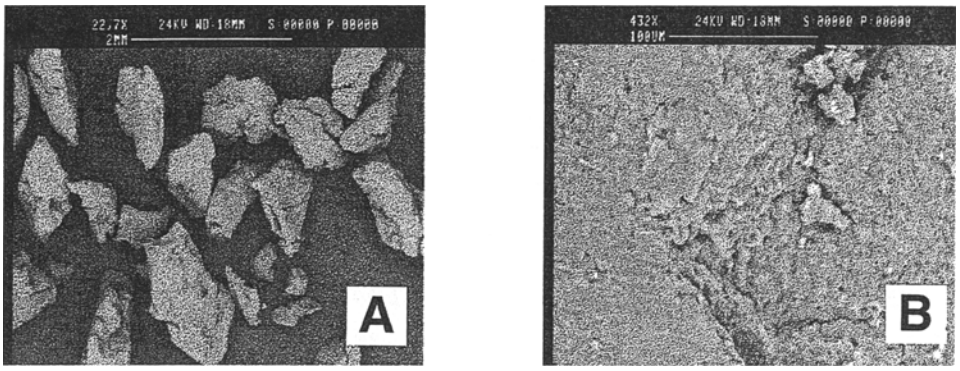

Fig. 3. *Rhizopus sp 0101* Biosorbent Morphology. Electronic Microphotographs of *Rhizopus sp 0101* Biosorbent. **(A)** 22.7 X. **(B)** 432 X. Analysis were carried out in a Cambridge Instruments Stereoscan 240 electron microscope.

mass. EDX scan is useful for determination of metal distribution in the biosorbent *(12,13)*. Figure 3 shows the biosorbent morphology, which is an amorphous granule. The surface of the granule is irregular, with large area for metal–granule interaction. The nature of this kind of biosorbent facilitates its use in either fixed-bed or fluidized-bed reactors.

Samples from *Rhizopus* sp 0101 isotherm experiments were scanned. Nickel was found in all of them. It seems that metal deposition is homogeneous, since no precipitates were detected (Fig. 4).

Influence of pH

The pH is one of the most important factors in metal biosorption success **(4)**. pH influence was evaluated for *Rhizopus* sp 0101, *Rhizopus* sp 0145, *Mucor* sp 0620, and *Penicillium* sp Barel. In all evaluated microorganisms, Ni biosorption is inhibited by increasing hydrogen ion concentration. With pH 5.0, reaction efficiency decreased; at lower pH, it was completely inhibited. On the other hand, neutral and alkaline pH values contributed to reaction efficiency. Decreased Ni uptake obtained at pH 3.0

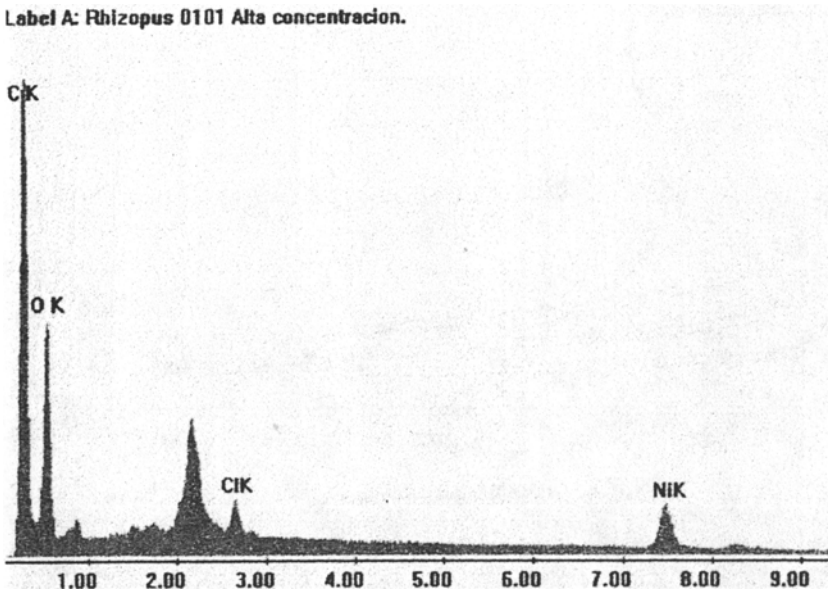

Fig. 4. *Rhizopus sp 0101* Biosorbent EDX-Scan. Analysis were carried out in a Cambridge Instruments Stereoscan 240 electron microscope with an X-ray analytic system EDAX. C, O, Cl, and Ni peaks are shown.

or lower is the result of H^+ ions binding to the reactive groups in the biomass. pH values greater than 8.0 lead to ionization of amine, imidazole, and phosphate groups, increasing biomass reactivity *(4,8,17)*. pH of metal solutions were adjusted with Tris. In addition, negative controls for each pH value showed that, in the evaluated metal concentration (10 ppm), there were not interferences caused by metal precipitation. These results agree with the solubility Ni diagram, in which Ni (in concentrations greater than 8 ppm) precipitates as hydroxide at pH values higher than 9.0 *(16)*.

Influence of Temperature

For all microorganisms evaluated, the results indicate (data not shown) that the temperature does not affect Ni uptake, as reported in the literature. For instance, Brady and Duncan *(4)*, working in Cu^{+2} bioaccumulation by *S. cerevisiae*, observed that the temperature does not influence metal uptake in the range of 5 to 40°C.

Influence of Sodium Chloride on Nickel Uptake

The results obtained with these experiments are depicted in Fig. 5. High sodium chloride concentrations affected the biosoption process by decreasing metal uptake up to 20%. This effect is caused by the high ionic strength of the solution, and because sodium ions are competing with Ni for negative reactive sites in the biosorbent. This effect is very important,

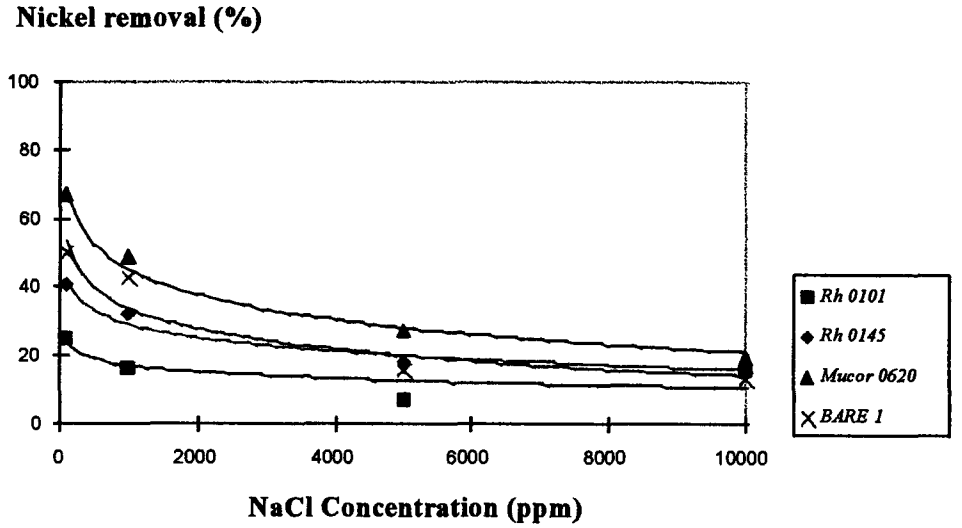

Fig. 5. Sodium Chloride Effect in Biosorption Initial metal concentration was 10 ppm. Metal uptake was expressed as removal percentage for each NaCl concentration. The evaluated strains were *Rhizopus 0101, Rhizopus 0145, Mucor 0620, Penicilluim BARE1.*

because industrial effluents are very complex solutions with different metallic ions. These results agree with the report of Corder and Reeves *(18)*, in which a biomass of cyanobacteria decreases its Ni uptake capacity when it was used as Ni biosorbent in media containing sodium ions *(1,5,6)*.

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