The European Journal of Mineral Processing and Environmental Protection
Vol.3, No.1, 1303-0868, 2003, pp. 36-41

ejmp&ep

Removal of chromium(VI) ions from synthetic solutions by the fungus *Penicillium canescens*

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Received 4 June 2002; accepted 3 January 2003

ABSTRACT

Penicillium canescens has demonstrated the ability to bind high amount of chromium(VI) from aqueous solutions. Cr(VI) adsorption capacity increases with the time during the first 4h and then levels off toward the equilibrium adsorption capacity. Biosorption of Cr(VI) ions reached equilibrium in 4h. Cr(VI) ions binding *Penicillium canescens* was clearly pH dependent. Cr(VI) loading capacity increased with increasing pH under acidic conditions, presumably as a function of Cr(VI) speciation and due to the H^+ competition at same binding sites. The adsorption of Cr(VI) ions reached a plateau value at around pH 6.0. The maximum adsorption capacity of Cr(VI) ions onto the fungal biomass was 34.8mg/g. Elution of Cr(VI) ions was performed using 0.5M HCl. The fungus *Penicillium canescens* could be used for six cycles for biosorption.

Keywords: Cr(VI); Fungal biomass; Heavy metal-binding; Biosorption; *Penicillium canescens*

1. INTRODUCTION

Chromium is hazardous because it affects human physiology, accumulates in the food chain and causes several diseases. The stricter environmental regulations related to the discharge of heavy metals make it necassary to develop processes for their removal from wastewater (Sittig, 1979). Heavy metals are commonly removed by chemical precipitation, ion-exchange, adsorption, solvent extraction and reverse osmosis processes (Grau and Bisang, 1995; Singh and Tiwari 1997; Ouki and Neufeld, 1997; Denizli *et al.*, 1999).

These methods have several disadvantages such as unpredictable metal ions removal, high material cost, and the generation of toxic sludges which are often difficult to dewater and also require extreme caution in their disposal. Due to these disadvantages, there is a need for novel treatment methods for the removal of heavy metal ions from wastewater (Kapoor *et al.*, 1999). Application of biosorption method to the treatment of wastewaters containing heavy metal ions has been given significant attention recently by the research community (Loren, 1979; Gadd, 1996; Mittar *et al.*, 1992; Park *et al.*, 1999; Say *et al.*, 2001). These methods become inefficient and expensive especially when the concentration of the heavy metal ion is low, the order of 1 to 100mg/l.

In the concept of biosorption, several chemical processes may be involved, such as adsorption, ion exchange, and covalent bonding with the biosorptive sites of the microorganisms including carboxyl, hydroxyl, sulphydryl, amino and phosphate groups (Frurest and Volesky, 1997). Fungal cell walls and their components have major role in the biosorption (Tsezos *et al.*, 1997; Hafez *et al.*, 1997; Gardea-Torresdey *et al.*, 1998). Fungal biomass can also take up considerable quantities of heavy metals from aqueous solution by adsorption or a related process, even in the absence of physiological activity (Gadd and White, 1989).

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Many fungal species such as *Rhizopus arrzhius, Penicilium spinulum, Phanerochaete chrysosporium* and *Aspergillus niger* have been extensively studied for heavy metal biosorption and the process mechanism seems to be dependent upon species (Zhou and Kiff, 1991; Saglam *et al.*, 1999; Mowll and Gadd, 1983; Krantz-Rülcker *et al.*, 1996).

The objective of the present research was to study the effect of pH, time and heavy metal concentration on biosorption of chromium(VI) ions using *Penicillium canescens* biomass. Elution-reuse of biomass was also evaluated.

2. MATERIALS AND METHODS

2.1. Cell line and medium

The fungus used was *Penicillium canescens* (2195) which was isolated from soil. The microorganism was maintained by subculturing on potato-dextrose-agar plates. Fungal biomass was cultivated in liquid medium using the shake flask method. Spores and mycelium from the potato-dextrose-agar spread-plate cultures were transferred to 250ml Erlenmeyer flasks containing 100ml of potato-dextrose broth. Once inoculated flasks were incubated on an orbital shaker at 120rpm for 7 days at 22±2°C. After incubation, the biomass was harvested from the medium by filtration and washed several times with distilled water, it was then dried at 90°C in an oven for 24h. The dried sample was then ground using a blender and sieved to pass through a 100mesh (150µm) sieve to obtain uniform particle size.

2.2. Biosorption of chromium ions

Biosorption of Cr(VI) from synthetic wastewaters containing single metal ions was investigated in batch experiments. The effect of pH on the biosorption capacity of the fungal biomass with Cr(VI) ions was investigated in the pH range 2.0-7.0 at 20°C. A 100mg sample of the biomass was washed twice with 0.01M HCl using a centrifuge to remove any soluble materials or metal ions that may be present on the biomass. Each Cr(VI) solution (100mg/l) was prepared in 150mM NaCl solution (50ml) and dry fungal biomass was transferred to this medium and agitated magnetically at 100rpm. All water used in the experiments was purified using a Barnstead (Dubuque, IA) ROpure® LP reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion exchange packed-bed system. The resulting purified water (deionized water) has a specific resistance of 18MW. After swelling of the fungal biomass (about 20min) the pH of the solution was adjusted with 0.1M NaOH or 0.1M HCl. During the biosorption experiment the pH of the medium was controlled with a pH probe. The effect of the initial Cr(VI) concentration on the biosorption was studied at pH 5.0 as described above except that the concentration of Cr(VI) solution in adsorption medium was varied between 10 and 750mg/l. After biosorption, the biomass was separated by centrifugation from the medium. Analysis for Cr(VI) content in the supernatants and controls were performed by graphite furnace atomic absorption spectrophotometer (AAS 5EA, Carl Zeiss Technology, Zeiss Analytical Systems, Germany). The instrument response was periodically checked with known metal solution standards. Each experiment was performed in triplicate for quality control and statistical purposes. For each set of data present, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were calculated for each set of samples in order to determine the margin of error. The amount of adsorbed Cr(VI) ions per gram biomass was obtained by using the following expression.

$$Q = [(C_o - C) V] / m \quad (1)$$

Where Q is the amount of Cr(VI) ions adsorbed on the biomass (mg/g). C_o and C are the concentrations of the Cr(VI) ions in the solution (mg/l) initially and after biosorption. V is the volume of the medium (l) and m is the amount of the fungal biomass (g).

In order to determine the reusability of the dry fungal biomass, repeated adsorption-elution cycles were performed six times by using the same fungal biomass. Elution of Cr(VI) ions was performed using 0.5M HCl solution. Fungal biomass carrying 34.8mg Cr(VI)/g were placed in this desorption medium and stirred at 250rpm for 2 hours at room temperature. After eluting, the biosorbed Cr(VI) ions biomass was regenerated by washing with deionized water. The final Cr(VI) ion concentration in the aqueous phase was determined by using an AAS. The desorption ratio was calculated from the amount of Cr(VI) ions initially loaded on the biosorbents and the final Cr(VI) ions concentration in the desorption medium.

3. RESULTS AND DISCUSSION

3.1. Biosorption time

As seen here, chromium adsorption capacity increases with the time during the first 4 h and then levels off toward the equilibrium adsorption capacity (Figure 1). Adsorption of Cr(VI) was rather fast especially when the Cr(VI) concentration was high. This is due to the high complexation rate between the Cr(VI) and the metal complexing groups on the surface of biomass. The fungal cell walls have a negative charge due to the arrangement of the carboxyl and phosphate groups of the cell walls. The phosphate-containing teichoic acid in the cell wall of the fungi is primarily responsible for metal binding (Tobin *et al.*, 1994). Mass transfer limitations were also overcome by high driving force, which was the concentration difference of Cr(VI) between the liquid and the biomass phases, in the case of high Cr(VI) concentration.

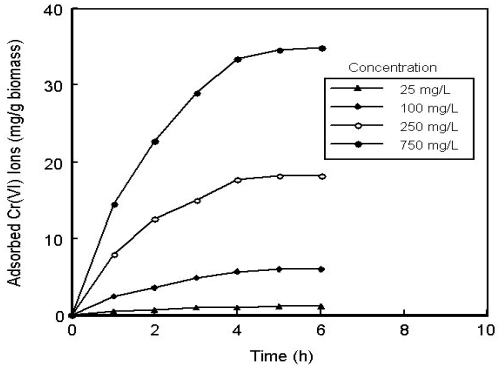

Figure 1. Biosorption times of Cr(VI) ions on biosorbents from aqueous solutions: pH: 5.0, T: 20°C

3.2. Effect of pH on the biosorption capacity

The medium pH affects the solubility of metals and the ionisation state of the functional groups (carboxylate and phosphategroups) of the fungal cell wall. It is reported that chitin as cell wall component of fungi is also responsible for metal sorption. In all filamentous fungi except Oomycetes, chitin (poly-N-acetylglucos-amine) was found as a major constituent of microfibrils (Tsezos and Volseky, 1982). Chitosan (deactylated poly-N-acetyl glucosamine) is thought to be responsible for biosorption due to the nitrogen site of the chitosan amine group (Guibal *et al.*, 1995). The carboxyl and phosphate groups carry negative charges that allow the fungal cell wall components to be potential scavengers of metal ions. But it should be noted that chromium(VI) exists in aqueous solution as oxo-anion (CrO_4^{2-} or $Cr_2O_7^{2-}$) with an overall charge of -2 (Gardea-Torresdey *et al.*, 1998). If there is an electrostatic interaction between the metal ion and the fungal biomass, a negatively charged ion will not bind to a negatively charged ligand. This could be the case if the carboxylate groups are the binding sites on the fungal biomass. Therefore, the binding sites on the fungal biomass responsible for this heavy metal ion uptake may only bind positively charged ions. Hence, in addition of phosphate-containing teichoic acid in the cell wall of the fungi, positively charged amino groups on the fungal biomass

could be responsible for the binding of Cr(VI) ions. The maximum biosorption of Cr(VI) on the biomass was observed at around pH 6.0. The amount of adsorbed Cr(VI) ions on the dry fungal biomass at pH 6.0 was found to be 6.1mg/g for (Figure 2). There was an increase in the adsorption amount of Cr(VI) ions per unit weight of fungal biomass with increasing pH from 2.0 to 6.0, but it seemed to reach a constant value at pH greater than 6.0. At acidic pH (pH 2.0), protonation of the cell wall components adversely effected the biosorption capacity of the fungal biomass, but its effect became minor with increasing pH in the medium. With an increase in pH, the negative charge density on the cell surface increases due to the deprotonation of the metal binding sites and thus increases biosorption. The pH-dependent trend also suggests that Cr(VI) ions that can be bound by the fungal biomass may also be recovered by reducing the pH. Several researchers have investigated the effect of pH on biosorption of heavy metals by using different kinds of microbial biomass. For example, Gardea-Torresdey *et al.* studied the adsorption of chromium ions on the biomass of Medicago sativa and they observed that the optimum pH for the biosorption was 6.0 (Gardea-Torresdey *et al.*, 1998).

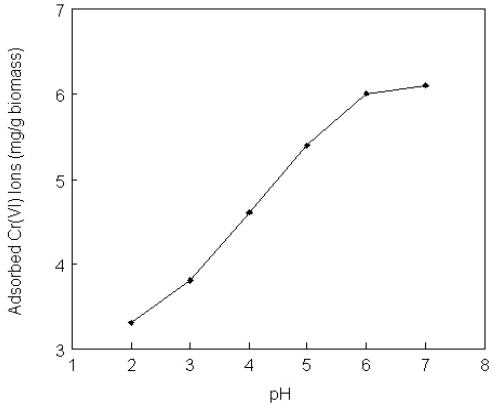

Figure 2. Effect of pH on biosorption of Cr(VI) ions on biosorbents from aqueous solutions: Initial concentration of Cr(VI): 100mg/l, T: 20°C

3.3. Effects of equilibrium concentration of Cr(VI) ions

The biosorption capacity of Cr(VI) onto the fungal biomass *Penicillium canescens* is shown in Figure 3. The biosorption capacity of the biomass increased first with increasing the equilibrium concentration of Cr(VI) and reached a saturation value. This saturation value is around 500mg/l. The maximum adsorption capacity of the dry fungal biomass in the studied range was 34.8mg/g.

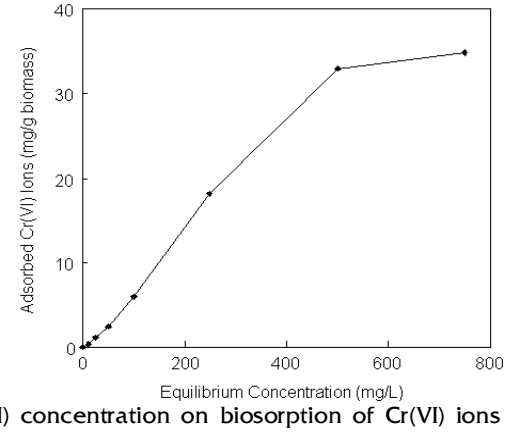

Figure 3. Effect of initial Cr(VI) concentration on biosorption of Cr(VI) ions on biosorbents from aqueous solutions: pH: 5.0, T: 20°C

The adsorption capacities of the fungal biomass obtained with chromium are comparable with the values reported in the previous studies. The biosorption capacity of the *Medicago sativa* (African Alfalfa shoots) was 7.24mg chromium(III) per gram dry biomass (Gardea-Torresdey *et al.*, 1998). The adsorption capacity of *Chlorella vulgaris* was 23.6mg for Cr(VI) per g dry biomass (Aksu and Kutsal, 1990). Sag and Kutsal have used *Rhizopus arrhizus* microorganisms for Cr(VI) adsorption (Sag and Kutsal, 1996). The equilibrium adsorption capacity was obtained in the range of 21-90mg/g dry weight of microorganisms. Aggarwal et al studied adsorption of chromium onto granulated activated carbon and they obtained maximum adsorption capacity as 70mg/g (Aggarwal *et al.*, 1999). Nourbaksh et al investigated biosorption of chromium(VI) ions on the different filamentous funguses including *C. vulgaris, C. crispata, R. arrhizus, S. cerevisiae and Z. ramigera* and maximum adsorption capacity was found to be 4.5mg/g (Nourbakhsh *et al.*, 1994). The comparison of the biosorption capacities of dried fungal biomass used in this work with those reported in the literature shows that these microorganisms are suitable for this purpose.

3.4. Reuse of biosorbents

To be useful in metal ion recyling prosesses, metal ions adsorbed should be easily desorbed under suitable conditions and adsorbents should be used many times in order to decrease material cost. Elution experiments were performed using 0.5M HCl solution as the elution agent. The dry fungal biomass loaded Cr(VI) ions was placed within the elution medium and the amount of Cr(VI) ions eluted in 2 hours was measured. It must be pointed out that in biosorption (i.e., binding of Cr(VI) ions onto fungal biomass) is completely reversible. More than 90% of the adsorbed Cr(VI) ions was desorbed in all cases. This means that HCl breaks down the interaction forces between Cr(VI) ions and binding sites onto the surface of the fungal biomass. Table 1 shows the adsorption-elution values of Cr(VI) ions by dry fungal biomass after several cycles of consecutive adsorption and desorption. This table clearly shows that the dry fungal biomass can be used repeatedly without loosing significantly their adsorption capacities for Cr(VI) ions.

Table 1
Cr(VI) ions adsorption capacity of dry fungal biomass after repeated adsorption-elution cycle. Initial concentration of Cr(VI) ions: 100mg/l, pH: 5.0, T: 20°C

Cycle No	Adsorption mg/g	Desorption %
1	34.8	92.0
2	34.2	94.2
3	33.4	93.7
4	33.0	95.3
5	32.6	94.8
6	32.0	94.6

4. CONCLUSIONS

Heavy metal ions are known to be toxic and especially copper, cadmium, arsenic, mercury, chromium, lead, nickel, selenium, silver and zinc are released into the environment in quantities that pose a risk to living systems. Removal of heavy metal ions from aquatic systems is carried out by classical method of adsorption technique. In this work, *Penicillium canescens* has been successfully used as the biosorbent for removal of heavy metal ions from aqueous solutions. The mechanism and the kinetics of heavy metal species biosorption on the fungal biomass depends on the experimental conditions particularly time, medium pH and heavy metal ions concentration. We have shown that the fungal biomass *Penicillium canescens* have high adsorption capacity for Cr(VI) ions. This adsorption capacity was as high as 34.8mg/g at 750mg/l initial concentration of Cr(VI) ions. The biosorbents can be regenerated and reused by acid treatment. The fungal biomas were reused in six times with negligible decrease in biosorption capacities. The adsorption results of this study indicate the possibilities that exist in

the clean up of the environment with the use of natural resources. In addition, the substantially lower cost of the fungal biomass also indicates a great potential for the removal of heavy metal ions from aqueous systems.

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