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Hexavalent Chromium Removal by a *Paecilomyces sp* Fungal

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1. Introduction

The strong impact of hexavalent chromium on the environment and on the human health demand suitable technologies to neutralize the hazard of chromium. The traditional technologies used for the remediation of environment contaminated with Cr (VI) are based on physical and chemical approaches, which require large amounts of chemical substances and energy. Such methodologies have proved complete expensive on a large-scale application at contaminated sites, and also they have generated hazardous by-products (Cervantes *et. al*., 2001). Bioremediation, a strategy that uses living microorganisms, is essentially proposed to clean up the environment from organic pollutants. However, since there is an evidence that several microorganisms possess the capability to reduce Cr (VI) to relatively toxic Cr (III), bioremediation gives immense opportunities for the development of technologies for the detoxification of soil contaminated with Cr (VI) as an alternative to existing physical-chemical remediation technologies (Cervantes *et al*., 2001).

Chromium is an essential micro-nutrient in the diet of animals and humans, as it is indispensable for the normal sugar, lipid and protein metabolism of mammals. Its deficiency in the diet causes alteration in lipid and glucose metabolism in animals and humans. Chromium is included in the complex named glucose tolerance factor (GFC) (Armienta-Hernández and Rodríguez-Castillo, 1995). On the other hand, no positive effects of chromium are known in plants and microorganisms. However, elevated levels of chromium are always toxic, although the toxicity level is related to the chromium oxidation state. Cr (VI) not only is highly toxic to all forms of living organisms. It is mutagenic for bacteria, mutagenic and carcinogenic for humans and animals, but also, it is involved in causing birth defects and the decrease of reproductive health (Marsh and McInerney, 2001). This metal may cause death in animals and humans, if ingested in large doses. The LD_{50} for oral toxicity in rats is from 50 to 100 mg/kg for Cr (VI) and 1900-3000 mg/kg for Cr (III). Cr (VI) toxicity is related to its easy diffusion across the cell membrane in prokaryotic and eukaryotic organisms and subsequent Cr (VI) reduction in cells, which gives free radicals that, may directly cause DNA alterations as well as toxic effects. Cr (III) has been estimated to be from 10 to 100 times less toxic than Cr (VI), because cellular membranes appear to be quite impermeable to most Cr (III) complexes. Nevertheless, intracellular Cr (III), which is the terminal product of the Cr (VI)-reduction, forms amino acid nucleotide complexes *in vivo*, whose mutagenic potentiality is not fully known (Gutiérrez Corona, *et al*., 2010).

It is well known that prokaryotes are more resistant to Cr (VI) than eukaryotes. Toxic chromium effects on bacteria, algae and plants have been reviewed by Wong and Trevors (1988). On the contrary, scant information is available about the impact of the chromium on the structure and diversity of soil microbial communities. In many studies, it has been difficult to assess the toxicity of chromium to soil microorganisms, because the environments examined were often polluted at the same time with organic pollutants and/or different heavy metals. In a soil chronically polluted with chromium (about 5000 mg/kg of soil) by leather tannery activity, the oxygenic phototrophic microorganisms and heterotrophic bacterial communities were both affected by chromium. Nitrogen-fixing cyanobacteria were not detected in contaminated soil with Cr using the MPN test, and data obtained from enriched cultures for nitrogen-fixing cyanobacteria showed that this, belonging to the Nostoc group was present, but they had a low number of heterocyst. The size of the cultivable heterotrophic bacterial community was not affected by chromium pollution, but there was a relationship between the percentage of chromate-tolerant bacteria and the level of chromium in the soil (Anjana *et al*., 2007). The ability of some microorganisms for interact with different Cr forms makes them attractive in the context of environmental biotechnology. In this sense, the use of microbial biomass for the removal of Cr from industrial wastewater and polluted water has already been recognized. The properties of some microorganisms for both: tolerate and reduce Cr (VI) enable their application in biotechnological process focusing on detoxification of Cr (VI). Cr resistance has been described in bacteria and fungi isolated from Cr-polluted environments. Yeast strains isolated include *Candida* and *Rhodosporidium* genera, but in these, the general mechanism of chromate resistance is related to limited ion uptake, rather than to chemical reduction of the toxic species (Baldi, *e. al*., 1990; Pepi and Baldi, 1992). However, other yeasts such as *Candida utilis* (Muter, *et al*., 2001) and *Candida maltose* (Ramírez-Ramírez, *et al*., 2004), showed partial ability to reduce Cr (VI) and also the capability to accumulate Cr in the biomass. Recent reports have also examined Cr (III) and Cr (VI) uptake and accumulation by different filamentous fungi (Acevedo-Aguilar, *et al*., 2008; Fukuda, *et al*., 2008; Srivastava and Thakur, 2007; Morales-Barrera and Cristiani-Urbina, 2008). The present study report the isolation and identification of a *Paecilomyces* sp fungal strain that exhibits high resistance level, resistance, biosorption and reduction potential to Cr (VI).

2. Materials and methods

2.1 Microorganism and chromate resistance test

A chromate-resistant filamentous fungus was isolated from polluted air with industrial vapors, in Petri dishes containing modified Lee's minimal medium (LMM, Lee, *et al*., 1975) [with 0.25% KH_2PO_4, 0.20% $MgSO_4$, 0.50% $(NH_4)_2SO_4$, 0.50% NaCl, 0.25% glucose] supplemented with 500 mg/L $K_2Cr_2O_7$; the pH of the medium was adjusted and maintained at 5.3 with 100 mMol/L citrate-phosphate buffer. The cultures were incubated at 28°C for 7 days. The strain was identified based on their morphological structures such color, diameter of the mycelia, and microscopic observation of formation of spores (Kirk, *et al*., 2001). Chromate-resistant tests of the isolated strain, filamentous fungus *Paecilomyces* sp, were performed on liquid LMM containing the appropriate nutritional requirements and different concentrations of Cr (VI) (as potassium dichromate), and determining the dry weight. The isolation was carried out near of Chemical Science Faculty, located in the city of San Luis Potosí, Mexico.

2.2 Preparation of biosorbent

The biomass was obtained by growth the fungus in thioglycollate broth (8 g/L) at 28°C with constant shaking (100 rpm). After of 4 days of incubation, the fungal biomass was obtained by filtration on Whatman filter paper No. 1. Later, the fungal biomass was centrifuged (3000 rpm, 5 min), washed 3 times with trideionized water, dried (80°C, 12 h) in bacteriological stove, ground in mortar and stored in amber glass bottles at room environment until use.

2.3 Preparation of stock solution

An aqueous stock solution (1000 mg/L) of Cr (VI) ions was prepared using $K_2Cr_2O_7$ salt. pH of the solution was adjusted using 0.1 N HCl or NaOH. Fresh dilutions were used for each study.

2.4 Biosorption studies

The biosorption capacity of fungal biomass was determined by contacting various concentrations (100 - 1000 mg/L) of 100 mL Cr (VI) solution in 250 ml Erlenmeyer glass flasks, with 1 g of biomass. The mixture was shaken in a rotary shaker at 120 rpm followed by filtration using Whatman filter paper No. 1. The filtrate containing the residual concentration of Cr (VI) was determined spectrophotometrically at 540 nm after complexation with 1, 5 Diphenylcarbazide (Eaton *et. al.*,1995), Cr (III) with Chromazurol S (Pantaler and Pulyaeva,1985) and Cr total by total by Electrothermal Atomic Absorption Spectroscopy (Eaton *et. al.*,1995). For the determination of rate of metal biosorption by biomass from 100 mL (at 200, 400, 600, 800 and 1000, mg/L), the supernatant was analyzed for residual Cr (VI) after the contact period of 1-12 hours. The effect of pH and temperature on Cr (VI) sorption by fungal biomass, was determined at pH values of 1, 2, 3, and 4, 28°C, 40°C, 50°C and 60°C, respectively. The effect of different doses of biomass ranging from 1 to 5 g/L, with 100 mg/L of Cr (VI) concentrations was determined.

2.5 Culture conditions in liquid media

Cultures in 100 mL of sterile LMM [amended with 50 mg/L Cr (VI)] inoculated with 5×10^5 spores/mL were incubated at 28°C for 48 h. Then, cells were aseptically separated by centrifugation at 2,000 rpm (4°C) for 10 min, and washed twice with sterile trideionized water to eliminate culture medium components and cell debris. The cell pellet was resuspended in 3 mL of sterile trideionized water by shaking in a vortex mixer for 30s, and was then transferred to 100 mL of fresh LMM amended with 50 mg/L Cr (VI). At various times during the course of incubation, 1 mL aliquots were removed and centrifuged at 5,000 rpm for 10 min to sediment the cells; the supernatant fluid was used to determine the concentration of hexavalent, trivalent or total Cr.

2.6 Determination of hexavalent, trivalent, and total Cr

Hexavalent Cr and trivalent Cr were quantified by a spectrophotometric method employing diphenylcarbazide and chromazurol S, respectively (Eaton *et al.*, 1995; Pantaler and Pulyaeva, 1985), total Cr was determined by electrothermal atomic absorption spectroscopy (Eaton *et al.*, 1995).

The values shown in the results section are the mean from three experiments carried out by triplicate.

2.7 Bioremediation assay

Two 250 ml Erlenmeyer glass flasks, with 5 g of fungal biomass, were add with 20 g of contaminated earth with 50 mg Cr (VI)/g earth of tannery (Celaya , Guanajuato, México), and the volume was complete to 100 mL with trideionized water. The mixture was shaken in a rotary shaker at 120 rpm followed by filtration using Whatman filter paper N°1. The filtrate containing the residual concentration of Cr (VI) was determined with 1, 5 diphenylcarbazide (Eaton *et al*., 1995).

3. Results and discussion

3.1 Isolation and identification of a fungal strain capable of removing Cr (VI)

The fungal strain isolated was able to growth on LMM supplemented with 2000 mg/L of Cr (VI) (Figure 1). This indicates that this fungus developed the Cr (VI) resistance and probably the Cr (VI) is being reduced in the polluted air. A variety of microorganisms with the Cr (VI) resistance and Cr (VI) reducing ability have been isolated from effluents of tanneries (Seng and Wang, 1994; Dark, *et al*., 2004; Fukuda, *et.al*., 2008). Colonies of the isolated fungal strain grew rapidly and mature within 3 days. *Paecilomyces* sp are thermopile and can grow well at temperatures as high as 50° and 60°C. The colonies are flat, powdery or velvety in texture. The initial color is white, and becomes yellow, yellow-green, pink, or violet. The reverse is dirty white or buff. A sweet aromatic color may be associated with older cultures. Septate hyaline hyphae, conidiophores, phialides, conidia, and chlamidospores are observed. Conidiophores (3-4 µm wide and 400-600 µm long) are often branched and carry the phialides at their tips. The phialides are swollen at their bases and taper towards their apices. They are usually grouped in pair or brush-like clusters. Conidia are unicellular, hyaline to darkly colored, smooth or rough, oval to fusoid, and form long chains. Chlamidospores are occasionally present. With different concentrations of Cr (VI) include changes in morphologies, showing slower growth and least conidiation (Figure 2) (Kirk, *et al*., 2001).

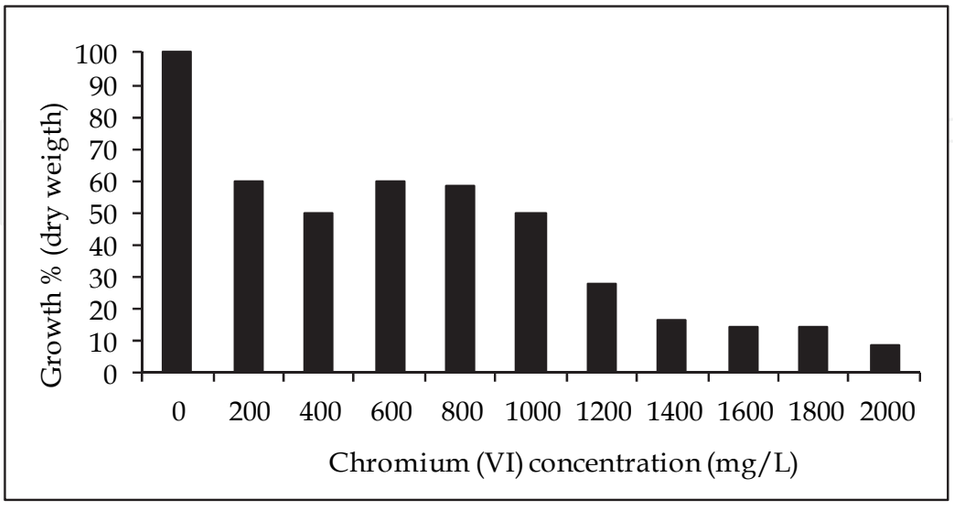


Fig. 1. Growth in dry weight of *Paecilomyces* sp with different concentrations of Cr (VI). 1x10^5 spores/mL, 28°C, 7 days of incubation, 100 rpm.

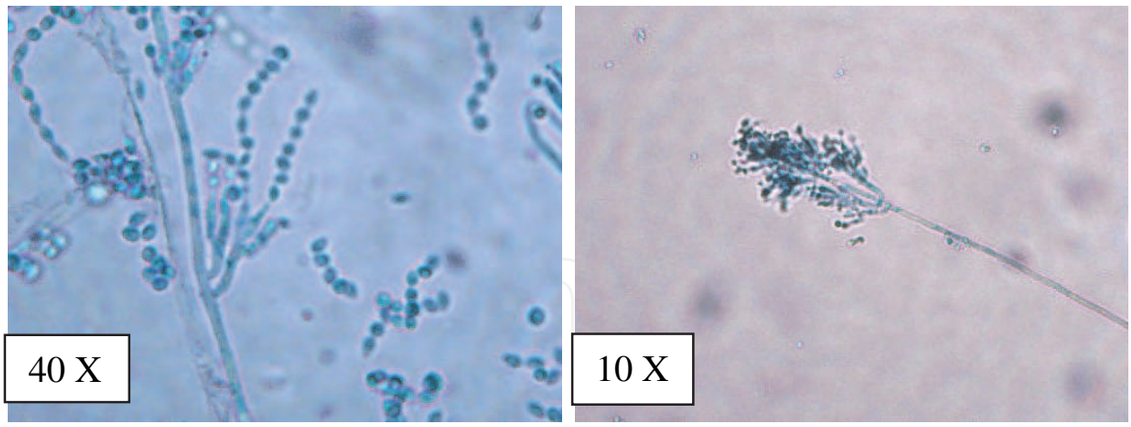


Fig. 2. Microscopic morphology of the fungus *Paecilomyces* sp. In absence and presence of 500 mg/L of Cr (VI), respectively.

3.2 Studies with fungal biomass

3.2.1 Effect of pH and incubation time

Figure 3 shows the adsorption of Cr (VI) by 1.0 g/100 mL of fungal biomass as a function of time at pH of 1.0, 2.0, 3.0 and 4.0, for initial Cr (VI) concentration of 100 mg/L. The metal removal was found to be 100% at 9 hours and 79.2% at 10 hours, with pH 1.0 and 2.0, respectively. Aqueous phase pH governs the speciation of metals and also the dissociation of active functional sites on the sorbent. Hence, metal sorption is critically linked with pH.

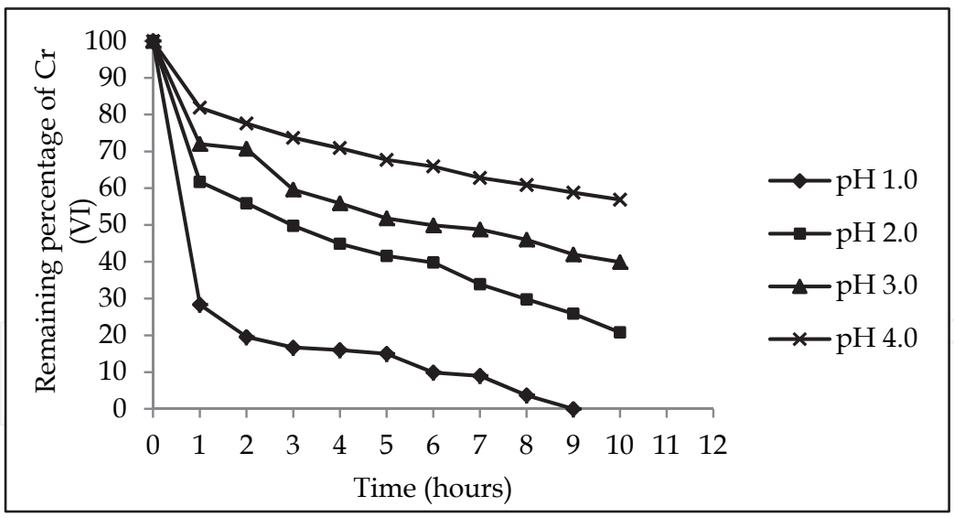


Fig. 3. Effect of pH and incubation time on the removal of 100 mg/L Cr (VI). 28°C. 1 g of fungal biomass. 100 rpm.

Not only different metals show different pH optima for their sorption but may also vary from one kind of biomass to the other (Tewari *et al*., 1995; Ucun *et al*., 2002). It can be observed from the figure that the uptake of Cr (VI) decreases with increase in pH. In general, the Cr (VI) adsorption by different biosorbents have shown similar trend and the optimum pH 1.0 has been reported (Nourbakhsh *et.al*., 1994). The literature has reported an optimal pH for the

removal of Cr (VI) by the fungi *Rhizopus arrhizus* and *Saccharomyces cerevisiae* in a range of 1.5-2.5, at 4 h (Nourbakhsh *et al*., 1994), although most show a pH optimum of removal in the range of 2.0 to 3.0 (Tewari *et al*., 2005; with *Mucor hiemalis*; Sag and Aktay, 2002, for *Rhizopus arrhizus*, both at 24 h, Bai and Abraham, 2001; with *Rhizopus nigricans*, at 8 h). The highest sorption capacity of mandarin shell for Chromium (VI) was at pH 1.0 and the decrease in sorption capacity with increase in pH may be attributed to the changes in metal speciation and the dissociation of functional groups on the sorbent. Ucun *et al*., (2002) have reported that the pH dependence of metal uptake could be largely related to the various functional groups on the adsorbent surface along with metal solution chemistry.

3.2.2 Effect of temperature

Temperature dependence of the adsorption process is associated with several thermodynamic parameters. Figure 4 shows an increasing trend of Cr (VI) removal with the rise in temperature from 28 to 60°C. Results that is consistent with those of Park *et al*., (2004), who observed that at 45°C and 24 h, adsorption occurs for the same metal with *Aspergillus niger*, and Leyva-Ramos *et al*., (2005) for the removal of cadmium (II) with corn cob (40 °C and 5 days), but differ from 35°C and 24 h reported by Sag and Aktay (2002) for *Rhizopus arrhizus*, and with those reported for mandarin flax husk (Zubair, *et al*., 2008). The increase in Cr (VI) uptake may be due to creation of some new sorption sites on the sorbent surface or the increased rate of intraparticle diffusion of sorbate ions into the pores of adsorbent at higher temperature, as diffusion is an endothermic process (Das, *et al*., 2000).

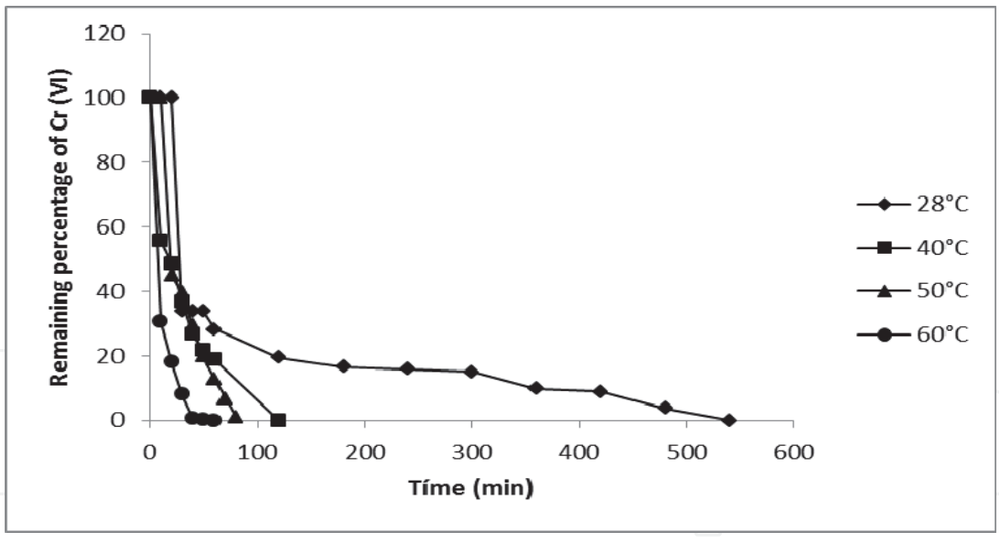
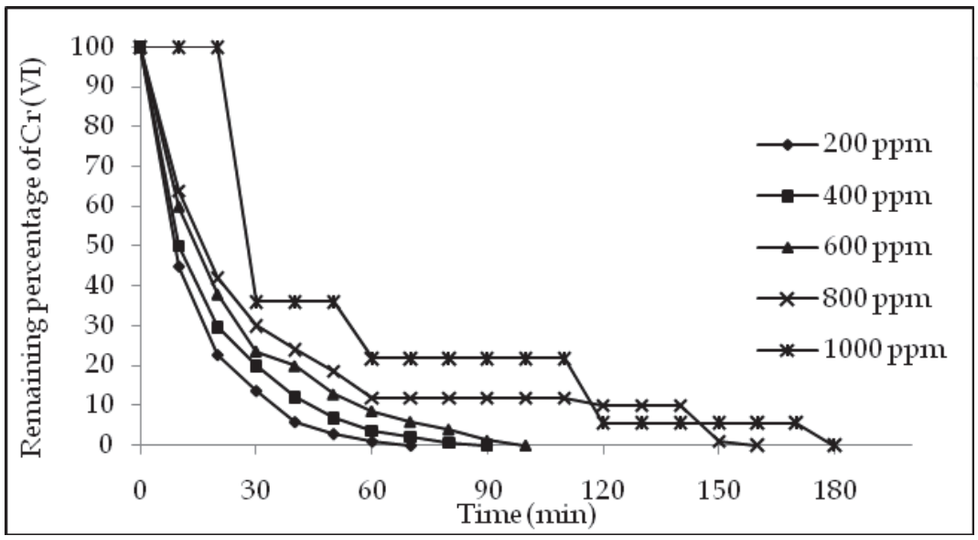


Fig. 4. Effect of temperature on the removal of 100 mg/L Cr (VI). 1 g of fungal biomass. 100 rpm

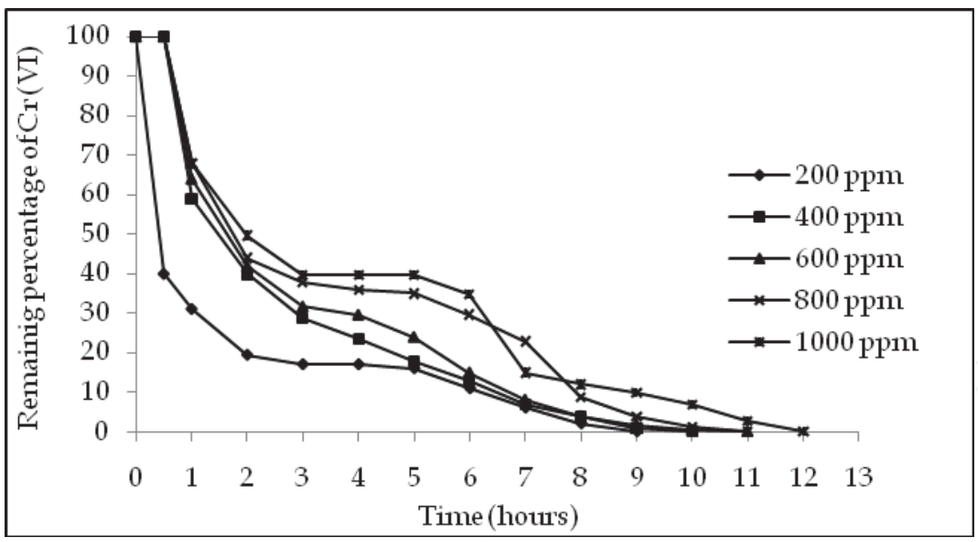
3.2.3 Effect of Cr (VI) concentration

The time taken to remove 200 mg/L chromium solution was 70 min. But as the chromium concentration increased, the percentage of chromium biosorption progressively decreased from 100% in 100 mg/L to 80% in 1000 mg/L solution, to 60°C (Figure 5a), and to 28°C, 200 and 1000 mg/L of the metal was remove in 9 and 12 hours, respectively (Figure 5b). This appears to be due to the increase in the number of ions competing for the available binding

sites in the biomass and also due to the lack of binding sites for the complexation of Cr ions at higher concentration levels. At lower concentrations, all metal ions present in the solution would interact with the binding sites and thus facilitated 100% adsorption. At higher concentrations, more Cr ions are left unabsorbed in solution due to the saturation of binding sites (Ahalya *et al*. 2005).



(a)



(b)

Fig. 5. Effect of Cr (VI) concentration on the removal of the metal. 1 g of fungal biomass. 100 rpm. a. - 60°C. b. - 28°C.

3.2.4 Effect of biomass concentration

We studied the removal of 1000 mg/L of Cr (VI) with various concentrations of fungal biomass at 60°C, finding that to higher concentration of biomass, is better the biosorption of Cr (VI), because the metal is removed at 70 minutes using 5.0 g of biomass (Figure 6). If we

increasing the amount of biomass, also increases the removal of Cr (VI) in solution, since there are more metal biosorption sites, because the amount of added biosorbent determines the number of binding sites available for metal biosorption (Cervantes *et al*., 2001). Similar results have been reported for biomass *Mucor hiemalis* and *Rhizopus nigricans*, although the latter with 10 g of biomass (Tewari *et al*., 2005, Bai and Abraham, 2001), but are different from those reported by Zubair *et al*., (2008), for mandarin flax husk biomass, who report an optimal concentration of biomass of 100 mg/L.

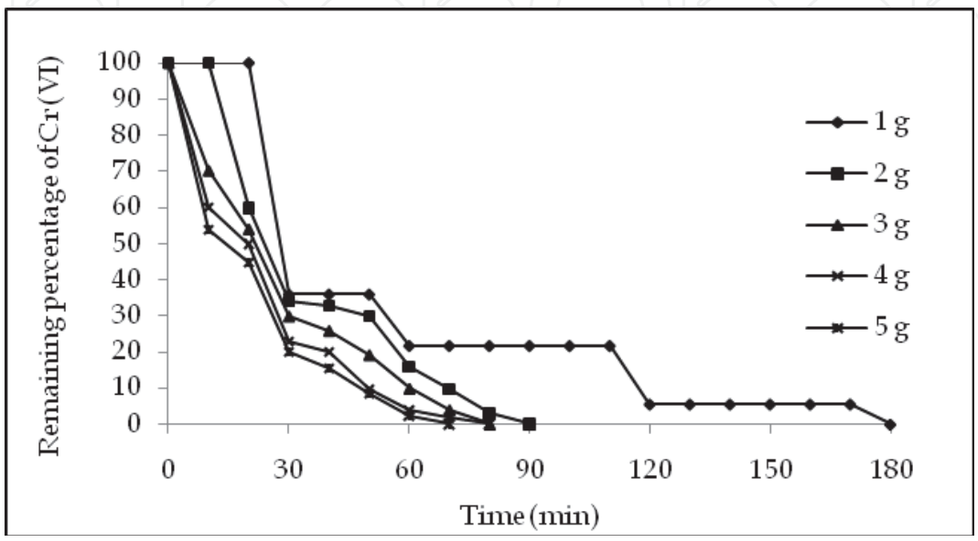


Fig. 6. Effect of biomass concentration on the removal of 1.0 g/L of Cr (VI). 100 rpm. 60°C.

Finally, Table 1 shows the adsorption efficiency of Cr (VI) by different biomass of microorganisms which shows that the biomass of *Paecilomyces* sp reported in this study is the most efficient in the removal of metal.

3.3 Studies with fungal alive

3.3.1 Effect of pH

Figure 7 shows the effect of varying pH (4.0, 5.3, and 7.0, maintained with 100 mMol/L citrate-phosphate buffer.) on the rate of Cr (VI) removal. The rate of chromium uptake and the extent of that capture were enhanced as the pH falls from 7.0 to 4.0. The maximum uptake was observed at pH 4.0 (96% at 7 days), 96%, Liu *et. al*., (2007) and Bai and Abraham, (2001) reported maximum removal at 100 mg/L Cr (VI) solution using *Mucor racemosus* and *Rhizopus nigricans* with pH optimum of 0.5-1.0, and 2.0 respectively, Sandana Mala *et.al*., (2006) at pH 5.0 for Cr (VI) with *Aspergillus niger* MTCC 2594, Rodríguez *et. al*., (2008) at pH 3.0-5.0 for Pb^{+2}, Cd^{+2} and Cr^{+3} with the yeast *Saccharomyces cerevisiae*, Park *et. al*., (2004) at pH 1-5 for Cr (VI) with brown seaweed *Ecklonia*, Higuera *et. al*., (2005) at pH 5.0 for Cr (VI) with the brown algae *Sargassum* sp, and Fukuda *et. al*., (2008) at pH 3.0 for Cr (VI) with *Penicillium* sp. In contrast to our observations, Prasenjit and Sumathi (2005), reported maximum uptake of Cr (VI) at pH 7.0 with *Aspergillus foetidus*, Puranik and Paknikar (1985) reported an enhanced uptake of lead, cadmium, and zinc, with a shift in pH from 2.0 to 7.0 using a *Citrobacter* strain, and a decrease at higher pH values. Al-Asheh and Duvnjak (1995) also demonstrated a positive effect of increasing pH in the range 4.0-7.0 on Cr (III) uptake using *Aspergillus carbonarius*. At low pH, the negligible removal of chromium may be due to the

competition between hydrogen (H+), and metal ions Srivasta and Thakur (2007). At higher pH (7.0), the increased metal removal may be due to the ionization of functional groups and the increase in the negative charge density on the cell surface. At alkaline pH values (8.0 or higher), a reduction in the solubility of metals may contribute to lower uptake rates.

Biosorbent	Capacity of adsorption (mg/g)	References
Aspergillus foetidus	2	Prasenjit and Sumathi (2005)
Aspergillus niger	117.33	Khambhaty et al. (2009)
Aspergillus sydowi	1.76	Kumar et al. (2008)
Rhizopus nigricans	47	Bai and Abraham (2001)
Rhizopus oligosporus	126	Ariff et al. (1999)
Rhizopus arrhizus	11	Bai and Abraham (1998)
Rhizopus arrhizus	78	Aksu and Balibek (2007)
Rhizopus sp.	4.33	Zafar et al. (2007)
Mucor hiemalis	53.5	Tewari et al. (2005)
Paecilomyces sp	1000	(Present study)
Bacillus coagulans	39.9	Srinath et al. (2002)
Bacillus megaterium	30.7	Srinath et al. (2002)
Zoogloea ramigera	2	Nourbakhsh et al. (1994)
Streptomyces noursei	1.2	Mattuschka and Straube (1993)
Chlorella vulgaris	3.5	Nourbakhsh et al. (1994)
Cladophora crispate	3	Nourbakhsh et al. (1994)
Dunaliella sp.	58.3	Donmez and Aksu (2002)
Pachymeniopis sp.	225	Lee et al. (2000)

Table 1. Capacity of biosorption of different microbial biomass for removal Cr (VI) in aqueous solution.

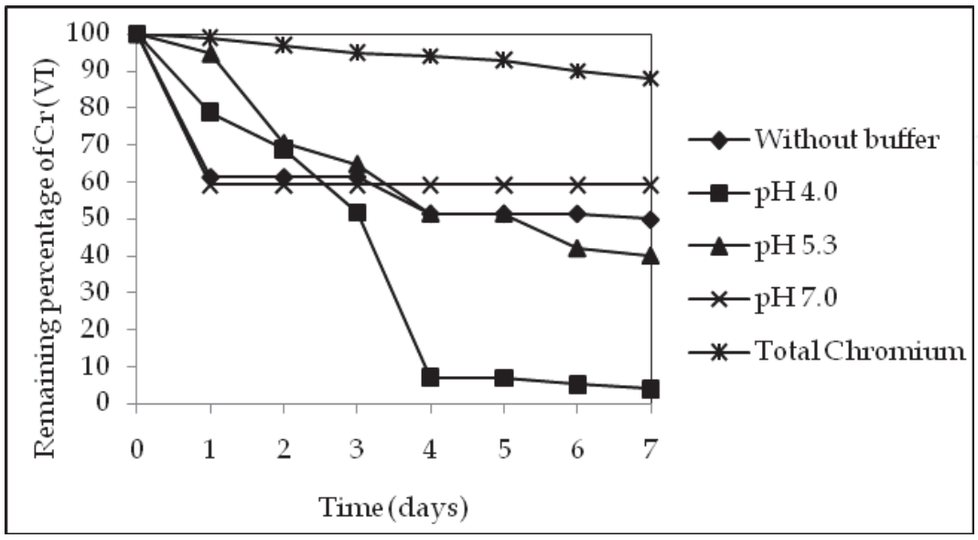


Fig. 7. The effect of pH on Chromium (VI) removal by *Paecilomyces* sp. 50 mg/L Cr (VI), 100 rpm, 28ºC.

3.3.2 Effect of cell concentration

The influence biomass in the removal capacity of Cr (VI) was depicted in Figure 8. From the analyzed (38, 76, and 114 mg of dry weight) the removal capacity was in the order of 99.17%, 97.95%, and 97.25%, respectively. In contrast to our observations, the most of the reports in the literature observe at higher biomass dose resulted in an increase in the percentage removal [1, 3, 7, 8, 19, and 22]. To higher biomass concentration, there are more binding sites for complex of Cr (VI) (e.g. $HCrO^{-4}$ and Cr_2O7^{-2} ions) (Seng and Wang, 1994; Cervantes *et. al*., 2001). However it did not show in our observations.

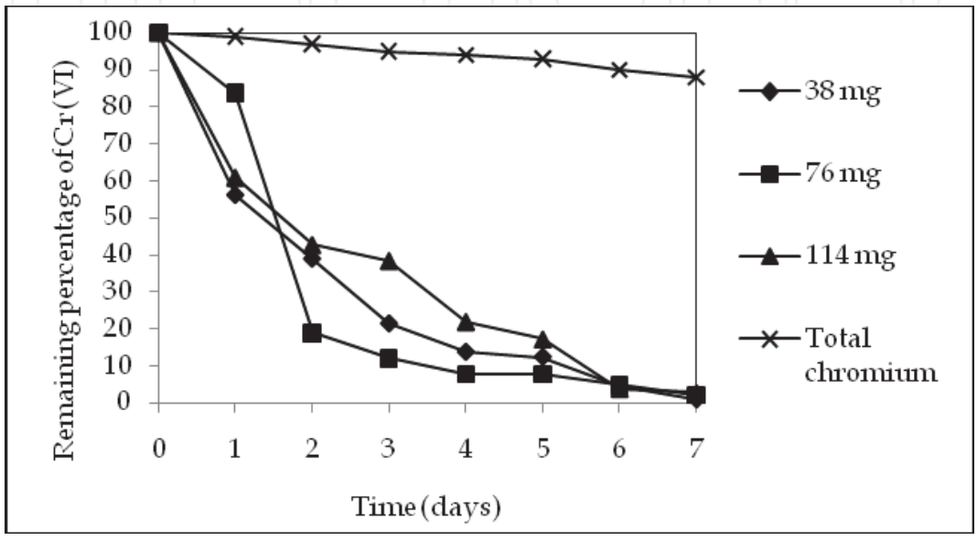


Fig. 8. The effect of cell concentration on the removal of 50 mg/L Cr (VI), 100 rpm, 28°C, pH 1.0.

3.3.3 Effect of initial Cr (VI) concentration

As seen in Figure 9, when the initial Cr (VI) ions concentration increased from 50 mg/L to 200 mg/L, the percentage removal of metal ions decreased. This was due to the increase in the number of ions competing for the available functions groups on the surface of biomass. Our observations are like to the most of the reports in the literature (Bai and Abraham, 2001; Seng and Wang, 1994; Beszedits, 1988; Park *et. al.,* 2004; Sahin and A. Öztürk, 2005; Liu, *et. al*., 2007; Rodríguez, *et. al*., 2008; Park *et. al*., 2004; Higuera Cobos *et. al*., 2005).

3.3.4 Effect of carbon source

Figures 10a and 10b, shows that the decrease of Cr (VI) level in culture medium of *Paecilomyces* sp occurred exclusively in the presence of a carbon source, either fermentable (glucose, sucrose, fructose, citrate) or oxidable (glycerol). In the presence of glucose, other inexpensive commercial carbon sources like unrefined sugar and brown sugar or glycerol, the decrease in Cr (VI) levels occurred at a similar rate, at 7 days of incubation are of 99.17%, 100%, 94.28%, 81.5, and 99%, respectively, and the other carbon sugar were fewer effectives. On the other hand, incubation of the biomass in the absence of a carbon source did not produce any noticeable change in the initial Cr (VI) concentration in the growth medium. These observations indicated that in culture of the fungus a carbon source is required to provide the reducing power needed to decrease Cr (VI) in the growth medium. Our

observations are like to the report of Acevedo-Aguilar, *et. al*., (2008) and Prasenjit and Sumathi (2005), with glucose like carbon source, and are different to the observations of Srivasta and Thakur (2007) with *Aspergillus* sp and *Acinetobater* sp, who observed how the main carbon source the sodium acetate.

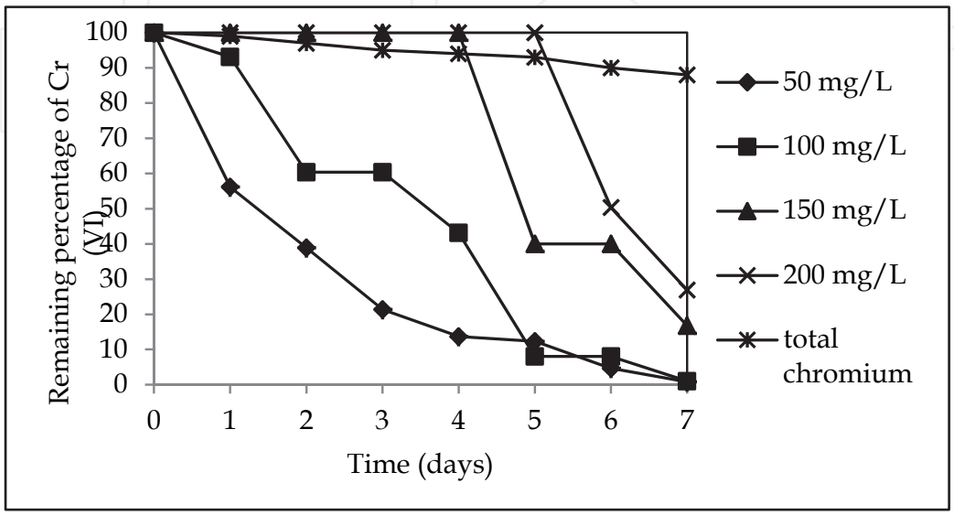


Fig. 9. The effect of the concentration of Cr (VI) in solution on the removal, 100 rpm. 28°C, pH 4.0.

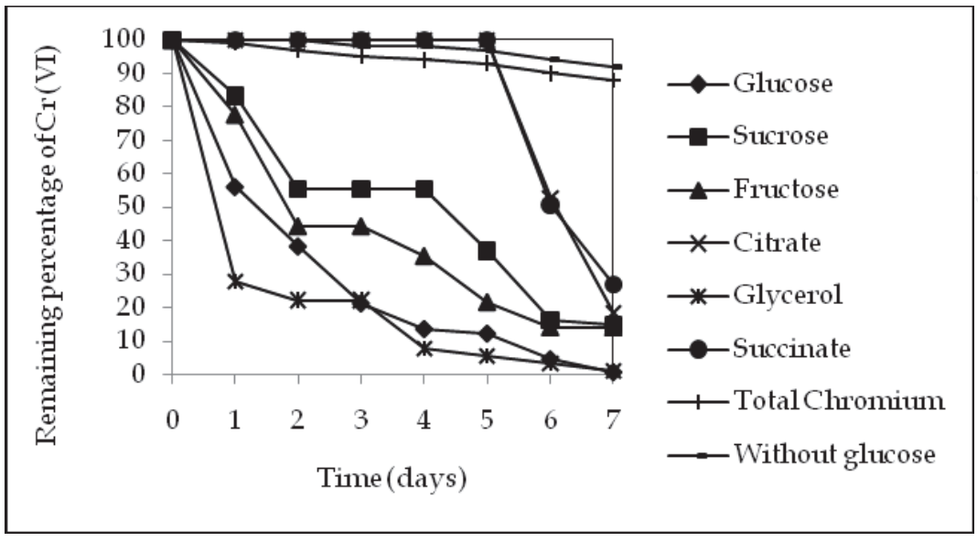


Fig. 10. (a) Influence of carbon source on the capability of *Paecilomyces* sp to decrease Cr (VI) levels in the growth medium. 100 rpm, 28ºC, pH 4.0

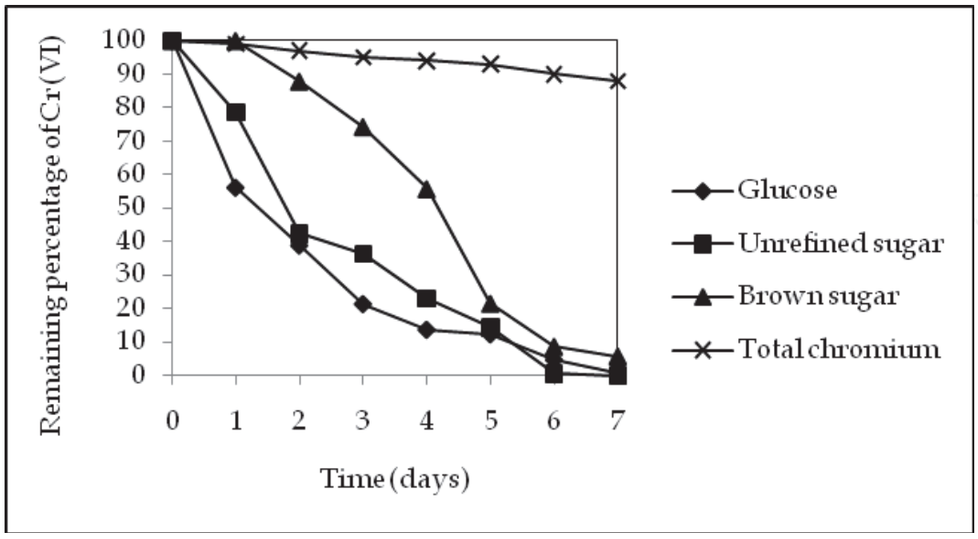


Fig. 10. (b) Influence of commercial carbon sources and salt on the capability of *Paecilomyces* sp to decrease Cr (VI) levels in the growth medium. 100 rpm, 28ºC, pH 4.0

3.3.5 Time course of Cr (VI) decrease and Cr (III) production

The ability of the isolated strain to lower the initial Cr (VI) of 50 mg/L, and Cr (III) production in culture medium was analyzed. Figure 11A show that *Paecilomyces* sp exhibited a remarkable efficiency to diminish Cr (VI) level with the concomitant production of Cr (III) in the growth medium (indicated by the formation of a blue-green color and a white precipitate, and its determination by Cromazurol S, (Figure No. 11 B) (Pantaler and Pulyaeva, 1985). Thus, after 7 days of incubation, the fungus strain caused a drop in Cr (VI) from its initial concentration of 50 mg/L to almost undetectable levels. As expected, total Cr concentration remained constant over time, in medium without inoculum. These observations indicate that *Paecilomyces* sp strain is able to reduce Cr (VI) to Cr (III) in growth medium amended with chromate. There are two mechanisms by which chromate could be reduced to a lower toxic oxidation state by an enzymatic reaction. Currently, we do not know whether the fungal strain used in this study express and Cr (VI) reducing enzyme(s). Further studies are necessary to extend our understanding of the effects of coexisting ions on the Cr (VI) reducing activity of the strain reported in this study. Cr (VI) reducing capability has been described in some reports in the literature (Smith et. al., 2002; Sahin and A. Öztürk, 2005; Muter *et. al*., 2001; Ramírez-Ramírez *et. al*., 2004; Acevedo-Aguilar, *et. al*., 2008; Fukuda et. al., 2008). Biosorption is the second mechanism by which the chromate concentration could be reduced, and 1 g of fungal biomass of *Paecilomyces* sp is able to remove 1000 mg/L of Cr (VI) at 60°C, at 3 hours of incubation (Figure 4), because the fungal cell wall can be regarded as a mosaic of different groups that could form coordination complexes with metals, and our observations are like to the most of the reports in the literature (Bai and Abraham, 2001; Seng and Wang, 1994; Ramírez-Ramírez *et. al*., 2004; Acevedo-Aguilar, *et. al*., 2008; Fukuda *et. al*., 2008; Prasenjit and Sumathi, 2005).

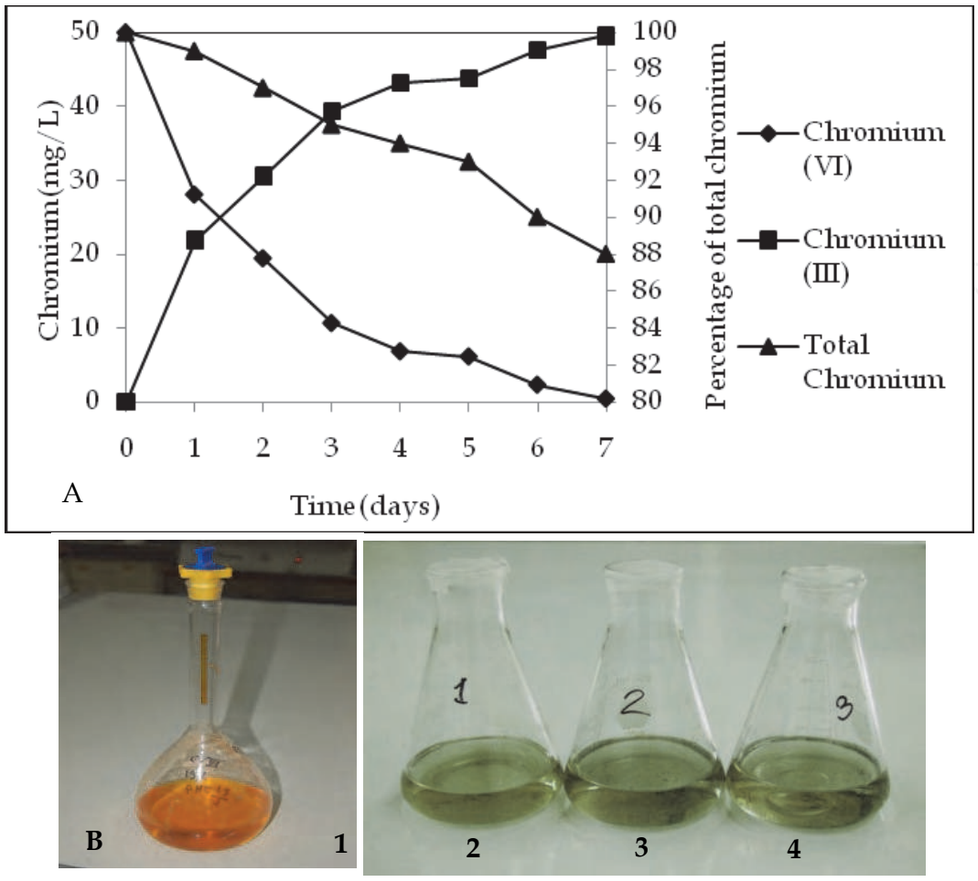


Fig. 11. Time-course of Cr (VI) decrease and Cr (III) production in the spent medium of culture initiated in Lee´s minimal medium, amended with 50 mg/L Cr (VI), 100 rpm, 28ºC, pH 4.0 (**A**). B. - Appearance of the solutions. Total Cr coupled with the biomass, after different incubation times in the presence of Cr (VI). 1. - Standard solutions of Cr (VI) (1.0 g/L, pH= 1.0). 2.-25 mg/L 3.-50 mg/L 4.-100 mg/L

3.3.6 Removal of Cr (VI) in industrial wastes with fungal biomass

We adapted a water-phase bioremediation assay to explore possible usefulness of strain of *Paecilomyces* sp, for eliminating Cr (VI) from industrial wastes, the mycelium biomass was incubated with non sterilized contaminated soil containing 50 mg Cr (VI)/g, suspended in LMM, pH 4.0. It was observed that after eight days of incubation with the *Paecilomyces* sp biomass, the Cr (VI) concentration of soil sample decrease fully (Figure 12), and the decrease level occurred without change significant in total Cr content, during the experiments. In the experiment carried out in the absence of the fungal strain, the Cr (VI) concentration of the soil samples decreased by about of 18% (date not shown); this might be caused by indigenous microflora and (or) reducing components present in the soil. The chromium removal abilities of *Paecilomyces* sp are equal or better than those of other reported strains, for example *Candida maltose* RR1 (Ramírez-Ramírez *et. al*., 2004). In particular, this strain was superior to the other strains because it has the capacity for efficient chromium reduction under acidic conditions. Most other Cr (VI) reduction studies were carried out at neutral pH (Fukuda *et. al*., 2008; Greenberg *et. al*., 1992). *Aspergillus niger* also has the ability to reduce

and adsorb Cr (VI) (Fukuda *et. al*., 2008). When the initial concentration of Cr (VI) was 500 ppm, *A. niger* mycelium removed 8.9 mg of chromium/g dry weight of mycelium in 7 days. In the present study, *Paecilomyces* sp, remove 50 mg/g, (pH, 4.0 and 8 days).

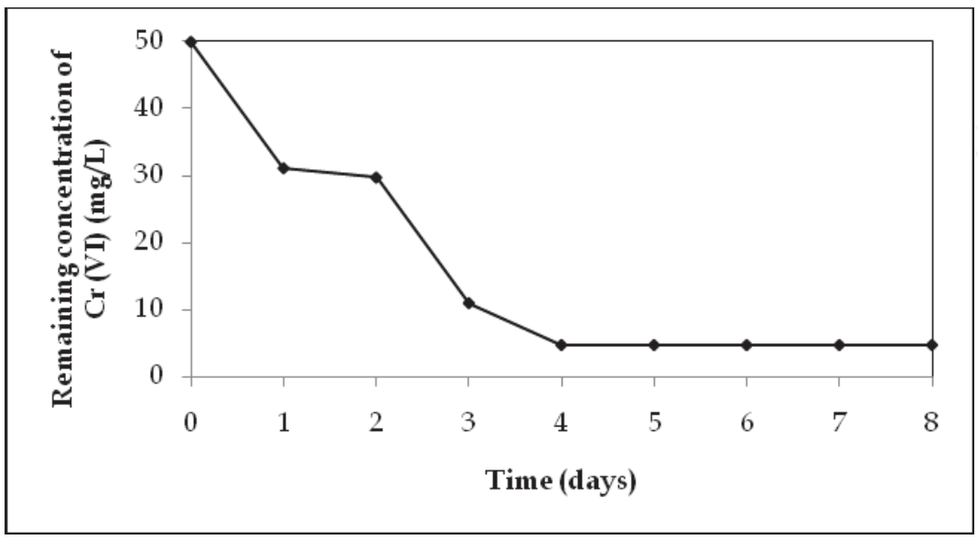


Fig. 12. Removal of Chromium (VI) in industrial wastes incubated with the fungal biomass. 100 rpm, 28ºC, pH 4.0, 50 g of contaminated soil (50 mg Cr (VI)/g soil).

Reports on applications of microorganisms for studies of bioremediation of soils contaminated with chromates are rare. One such study involved the use of unidentified bacteria native to the contaminated site, which are used in bioreactors to treat soil contaminated with Cr (VI). It was found that the maximum reduction of Cr (VI) occurred with the use of 15 mg of bacterial biomass per g of soil (wet weight), 50 mg per g of soil molasses as carbon source, the bioreactor operated under these conditions, completely reduced 5.6 mg/Cr (VI) per g of soil at 20 days (Jeyasingh and Philip, 2004). In another study using unidentified native bacteria-reducing Cr (VI) of a contaminated site, combined with *Ganoderma lucidum*, the latter used to remove by biosorption Cr (III) formed. The results showed that the reduction of 50 mg/L of Cr (VI) by bacteria was about 80%, with 10 g / L of peptone as a source of electrons and a hydraulic retention time of 8 h. The Cr (III) produced was removed using a column with the fungus *G. lucidum* as absorber. Under these conditions, the specific capacity of adsorption of Cr (III) of *G. Lucidum* in the column was 576 mg/g (Krishna and Philip, 2005). In other studies, has been tested the addition of carbon sources in contaminated soil analyzed in column, in one of these studies was found that the addition of tryptone soy to floor to add to with 1000 mg/L of Cr (VI) increase reduction ion, due to the action of microorganisms presents in the soil, although such action is not observed in soil with higher concentrations (10.000 mg/L) of Cr (VI) (Tokunaga *et al*., 2003). Another study showed that the addition of nitrate and molasses accelerates the reduction of Cr (VI) to Cr (III) by a native microbial community in microcosms studied, in batch or in columns of unsaturated flow, under conditions similar to those of the contaminated zone. In the case of batch microcosms, the presence of such nutrients caused reduction of 87% (67 mg/L of initial concentration) of Cr (VI) present at the start of the experiment, the same nutrients, added to a column of unsaturated flow of 15 cm, added with 65 mg/L of Cr (VI) caused the reduction and immobilization of the10% of metal, in a period of 45 days (Oliver *et al*., 2003).

4. Conclusion

A fungal strain resistant to Cr (VI) and capable of removing the oxyanion from the medium was isolated from the environment near Chemical Science Faculty, located in the city of San Luis Potosí, Mexico. The strain was identified as *Paecilomyces* sp, by macro and microscopic characteristics. It was concluded that application of this biomass on the removal of Cr (VI) in aqueous solutions can be used since 1 g of fungal biomass remove 100 and 1000 mg/100 mL of this metal after one and three hours of incubation, and remove 297 mg Cr (VI) of waste soil contaminated, and this strain showed the capacity at complete concentrations reduction of 50 mg/L Cr (VI) in the growth medium after 7 days of incubation, at 28°C, pH 4.0, 100 rpm and a inoculum of 38 mg of dry weight. These results suggest the potential applicability of *Paecilomyces* sp for the remediation of Cr (VI) from polluted soils in the Fields.

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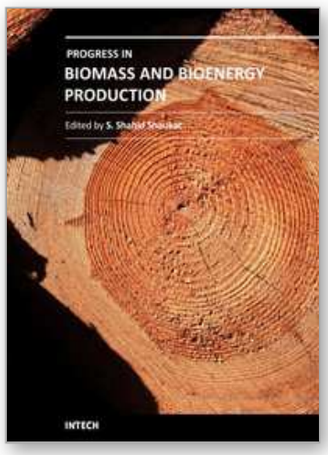

Progress in Biomass and Bioenergy Production
Edited by Dr. Shahid Shaukat

ISBN 978-953-307-491-7
Hard cover, 444 pages
Publisher InTech
Published online 27, July, 2011
Published in print edition July, 2011

Alternative energy sources have become a hot topic in recent years. The supply of fossil fuel, which provides about 95 percent of total energy demand today, will eventually run out in a few decades. By contrast, biomass and biofuel have the potential to become one of the major global primary energy source along with other alternate energy sources in the years to come. A wide variety of biomass conversion options with different performance characteristics exists. The goal of this book is to provide the readers with current state of art about biomass and bioenergy production and some other environmental technologies such as Wastewater treatment, Biosorption and Bio-economics. Organized around providing recent methodology, current state of modelling and techniques of parameter estimation in gasification process are presented at length. As such, this volume can be used by undergraduate and graduate students as a reference book and by the researchers and environmental engineers for reviewing the current state of knowledge on biomass and bioenergy production, biosorption and wastewater treatment.

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