African Journal of Biotechnology Vol. 8 (17), pp. 4163-4172, 1 September, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Biosorption characteristics of *Aspergillus fumigatus* in removal of cadmium from an aqueous solution

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Accepted 23 July, 2009

Nineteen fungal species were isolated from soil contaminated with industrial wastes of which *Aspergillus* species were the most dominant. The growth of the isolates was notice by Cd concentration in growth medium, thus about 20% of the isolates can grow up to 50 mg Cd/100 ml medium and only *Aspergillus fumigatus* and *Penicillium chrysogenum* can grow at 100 mg Cd with growth decrease of 88.2 and 99.4%, respectively. The results revealed that the living biomass of the isolates were more efficient to biosorb Cd than their dried powdered biomass by 15 - 44%. The formulation of yeast peptone glucose (YPG) medium fortified the isolates by ingredients favored the best growth yields that have the highest Cd biosorption, compared to yeast malt extract (YM) and sabourad (Sb) media. The dried *A. fumigatus* biomass was the most efficient than other tested fungi. The influence of different treatments of dried *A. fumigatus* biomass on its Cd biosorption activity, indicated that 0.5 N NaOH and autoclaving was the most efficient treatment (3 fold increase as compared to untreated). The biosorption of Cd by treated *A. fumigatus* biomass was considerably influenced by the pH value of the biosorption medium, contact time, biomass levels and Cd concentration. Thus, 98% of Cd was absorbed in biosorption medium containing 10 mg Cd and 100 mg dried treated biomass/100ml bidistilled water at pH 5 after 90 min of contact, nitric acid (0.05 N) was the best Cd eluent (99.8%) as compared to the other eluents. The desorbed *A. fumigatus* biomass was successfully reused for 5 consecutive times for Cd biosorption with decrease reached to 28% at the 5th reuse.

Key words: Biosorption, cadmium, *Aspergillus fumigatus*, industrial wastes, biomass.

INTRODUCTION

Mobilization of heavy metals in the environment due to industrial activities is a serious concern due to the toxicity of these metals in humans and other life forms. Removal of toxic heavy metals from industrial waste waters is essential from the stand point of environmental pollution control (Guangyu and Thiruvenkatachari, 2003; Say et al., 2003; Svecova et al., 2006). Among the toxic heavy metals, mercury, lead and cadmium called the big three are in the limelight due to their major impact on the environment (Volesky, 1994). Cadmium is introduced into the bodies of water from smelting, metal plating, cadmium-nickel batteries, phosphate fertilizer, mining, pigments, stabilizers, allay industries and sewage sludge (Tilaki and Ali, 2003). Discharges containing cadmium, in particular, are strictly controlled due to the highly toxic nature of this element and its tendency to accumulate in the tissues of living organisms (Dianati-Tilaki et al., 2004). The harmful effects of cadmium include a number of acute and chronic disorders, such as *itai-itai* disease, renal damage, emphysema, hypertension, and testicular atrophy (Leyva-Ramos et al., 1997).

Conventional techniques for the removal of heavy metals from wastewater, such as chemical precipitation, ion exchange, activated carbon adsorption and separation processes have limitations and become inefficient and expensive especially when the heavy metal concentration is less than 100 ppm (Yan and Viraraghavan, 2001). The ability of microorganisms to accumulate metal ions from aqueous solutions has been widely reported (Tangaromsuk et al, 2002; Lopez-Errasquin and Vazquez, 2003; AL-Garni, 2005; Svecova et al., 2006; EL-Sherif et al., 2008; Zamil et al., 2009).

Living and dead cells of fungi are able to remove heavy metals ions from aqueous solutions (Kapoor and

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Viraraghavan, 1995) and the use of dead cells seems to be more advantages than using living cells (Gadd, 1992). In multicomponents systems (biosorbents), biosorption of heavy metal ions depends not only on the specific surface properties of the biomass and on the physicochemical parameters of the solution, say, temperature, pH, initial metal-ion concentration and biomass concentration, but also on features of those components, as well as, the cultural conditions of the organisms (Macaskie and Dean, 1984; Wilde and Benemann, 1993; Sag and Kutsal, 1995a, b). The efficiency of biosorption can be increased by different physical and chemical pretreatments of the microbial biomass (Puranik and Pakniker, 1999; Kapoor et al., 1999).

Biotechnological exploitation of biosorption technology for removal of heavy metal(s) depends on the efficiency of the regeneration of biosorbent after metal desorption. Therefore non-destructive recovery by mild and cheap desorbing agents is desirable for regeneration of biomass for use in multiple cycles (Gupta et al., 2000). The objective of the present work was to elucidate the capacity of fungi isolated from industrially polluted soil to tolerate and adsorb cadmium, as well as, the effect of some cultural conditions of the fungi to biosorb the metal ions. The influence of some physicochemical parameters on biosorption of cadmium by the most potent fungus biomass and pretreatment of the biomass, as well as desorption and reuse of the biomass were studied.

MATERIALS AND METHODS

Microorganisms and maintenance

19 fungal species were isolated from soil contaminated with Industrial wastes at Yanbooh city, Saudi Arabia, purified and identified (Frey et al., 1979; Moubasher, 1993; Watanabe, 2002; CBS, 2006) using Czapek's-dox and potato dextrose agar (PDA) media. The fungi were maintained on solid PDA at 2°C, for use as stock culture, on PDA slants at 4°C.

Fungal growth screen

Cadmium tolerance of the 19 fungal isolates was determined by measuring the mycelial biomass. Each fungus was grown in 250 ml Erlenmeyer flasks containing 100 ml of YPG liquid medium, which consist of (g/l): 3 yeast extract, 10 peptone and 20 glucose. The medium was amended with different concentrations of cadmium (10, 25, 50 and 100mg Cd/100 ml). The cultures were inoculated with 7 mm mycelial discs removed from the margins of 7 day old colonies and incubated at 28°C in an orbital shaker at 150 rpm for 5 days. The mycelia were harvested by filtration through a nitrocellulose filter with a pore size of 0.45 µm (Millipore) and were dried overnight at 65°C to determine the dry weight. 3 replications of all assays were performed.

Cadmium biosorption by living and dried biomass

100 mg of the dried grinded fungal biomass and an equivalent weight of living fungal biomass, after calculation of the moisture content of the biomass, were dispensed in 250 ml Erlenmeyer flasks containing 10 mg cadmium/100 ml of deionized distilled water shaked at 120 rpm for 60 min, thereafter the biomass (living or dead) was removed by centrifugation and the residual cadmium concentration was measured in the filtrate.

Preparation of the powdered dried cells (biomass)

The developed fungal growth was separated from the culture medium by centrifugation at 6000 rpm for 15 min (J-2/C plus centrifuge). Harvested growth was washed twice with deionized distilled water and dried in an oven at 80°C for 48 h. The dried sample was then ground, using a blender and sieved to pass through a 100 mesh sieve to obtain uniform particle size.

Metal solution

A stock cadmium ion solution (1000 mg/l) was prepared by dissolving cadmium nitrate (Fisher Scientific Ltd) in deionized distilled water, shaking for 15 min at 100 rpm and then left to stand for 24 h to obtain complete dissolution. Stock solution was diluted with deionized distilled water to obtain the necessary concentrations (Budavari et al., 1989). Solutions were adjusted to desired pH values using 0.1 N NaOH and 0.1 N HNO_3. The cadmium concentration was determined with atomic absorption spectrophotometer (Unicam 929AA).

Metal absorption studies

A batch equilibrium method was used to determine sorption of cadmium by the tested fungi(us). All biosorption experiments were conducted in 250 ml Erlenmeyer flasks containing 100 ml of the tested cadmium solution. Powdered biomass (100 mg, unless otherwise stated) were exposed to metal solution for 60 min (otherwise stated) at 25 ± 2°C on a rotary shaker 120 rpm. The biomass was separated by centrifugation at 10000 rpm for 10 min and residual cadmium concentration was measured in the supernatant. Metal adsorbed by the biomass (mg metal/g biomass) was calculated (Volesky and May-Phillips, 1995) as:

$$Q = V (Ci – Cf) / 1000 \times M$$

Where: Q = cadmium uptake (mg Cd / mg biomass), V = suspension cadmium volume (ml), Ci = initial Cd concentration (mg/l), Cf = finial Cd concentration (mg/l), M = quantity of dry biomass (mg).

Cadmium removal under different nutritional conditions

As the compostion of the medium may have a direct effect on metal uptake, cadmium uptake was analyzed under different nutritional conditions. Mycelia of the tested fungi produced after 5 days of growing under shaked conditions(150 rpm) at 28 ± 2°C on examined liquid media(g/l), 3 yeast extract, 10 pepton, 20 glucose (YPG), 10 peptone, 40 glucose(Sabouraud, Sb) and 3 yeast extract, 10 malt extract(YM), were harvested and dried. Thereafter, the dry weights of the fungal growth and the absorption of cadmium by dried grinded biomass were determined and the assays were determined in triplicates.

Biomass treatment

The most efficient *A. fumigatus* of cadmium absorption was allowed

to grow on YPG medium for 5 days, therafter, the fungal growth was separated and dried in an oven. For each 500 mg dry weight the following treatments were carried out for 15 min, autoclaving, 0.5 N NaOH and boiling , 0.5 N NaOH and autoclaving, 0.5 N KOH and autoclaving, 0.5 M NaCl, detergent, 10% formaldehyde, 10% acetic acid and 0.1 N HCl. The biomasses were separated by centrifugation and then washed with generous amounts of deionized water till the pH of the wash solution was in the near neutral range (7.0 - 7.2). After washing the biomass was dried at 65°C for 12 h, weight, powdered and sieved to pass through a 100 mesh sieve. The powdered biomass residue obtained was referred to as " pretreated biomass ". Both untreated and pretreated biomasses were used in metal uptake.

Effect of pH and contact time

The pretreated biomass with 0.5 N NaOH and autoclaving, the best treatment for Cd absorption, was suspended in cadmium solution (10 mg/100 ml) with different pH values(3,4,5,6), for different time intervals (0 - 480 min), on a rotary shaker at 120 rpm. Thereby the absorption of cadmium was assayed.

Effect of pretreated biomass level

Pretreated biomass (20 - 110mg) was exposed to 100 ml of cadmium solution(10 mg/100 ml) at the optimum pH 5 for 90 min and pH 6 (less optimum) for 180 min on a rotary shaker at 120 rpm. Thereby, the residual cadmium in the supernatant after centrifugation at 10000 rpm for 10 min was measured.

Elution of cadmium ions biosorbed by pretreated biomass and regeneration of pretreated biomass

The adsorbed cadmium by the pretreated biomass at pH 5 for 90 min was eluted by the hanging in 100 ml/250 ml Erlenmeyer flasks containing distilled water, 0.05 N nitric acid, 0.1 M $CaCl_2$, 0.1 M NaCl, 0.1 M sodium carbonate and 0.1 N HCl. The flasks were shaked (120 rpm) for 2 h at 25 ± 2°C, thereafter the pretreated biomass was isolated by centrifugation and the eluted cadmium in the supernatant was measured. 0.05 N nitric acid solution was used to elute metal ions from pretreated biomass in subsequent experiment to study the reuse of biomass in biosorption. Use of 0.05 N HNO_3 solution as an elutant deposits H^+ ions on the biomass surface. Excessive amounts of H^+ can reduce the metal biosorption capacity of biomass. Therefore, reuse of the fungal biomass in biosorption after elution of biosorbed metal ions will require H^+ ions to be removed from the biomass. Washing the biomass with deionized water can remove H^+ ions. 2 methods were used to regenerate the biomass after eluting the biosorbed cadmium ions. The first involved washing the biomass with deionized water till the pH of the wash solution was in the range of 5.0 - 5.4. In the second regeneration method, the above biomass (washed by deionized water) shaken in 0.01 M NaOH for 30 min at 120 rpm. The regenerated biomass was air dried and its ability to biosorb cadmium ions was examined. The biosorption-elution of biosorbed metal ion-regeneration of biomass cycle was repeated 5 times.

RESULTS AND DISCUSSION

Fungal isolates and their tolerance to cadmium

19 fungal species (*Aspergillus flavus, A. fumigatus*, *A. niger, A. sydowii, A. terreus, A. ustus, A. versicolor, Cephaliophora irregularis, Cladosporium cladosporioides, Fusarium heterosporum, F. oxysporum, Paecilomyces variotii, Papulaspora irregularis, Penicillium citrinum, P. chrysogenum, Phoma humicola, Setosphaeria rostrata, Trichoderma longibrachiatum, T. viride*) were isolated from industrially polluted soil with heavy metals, which included, 20 copper, 15 zinc, 2.8 lead and 1.86 cadmium (mg/kg dry soil). The species of the genus *Aspergillus* were the most dominant (37%), while *Fusarium*, *Penicillium* and *Trichoderma* species were less frequent (10.5%). However, the other genera were represented by one species. These indicated that *Aspergillus* species are more tolerant to the types and quantities of the industrial wastes of Yanbooh industrial city, Saudi Arabia. All the isolates (Table 1) can grow up to 25 mg Cd/100 ml medium with growth yields decreases ranged between (62.66-99.68%). At 50 mg Cd, about 20% of the species failed to grow. At this Cd level, *A. fumigatus* was the most resistant, it showed 77.7% growth decreases, while the others showed decreases between 82.2 - 99.4%. While, 100 mg Cd/100 ml medium proved to be lethal for all isolates except *A. fumigatus* (growth decrease 88%) and *P. chrysogenum* (99% growth decrease). These results indicate that the response of the moulds to cadmium concentrations is not dependent on the genus of the fungus but to its species and strains, as there are morphological and physiological differences between fungal, genera, species and strains, and therefore, their different response to the concentrations of the heavy metal ions. Thus, as *A. fumigatus* was the most tolerant to Cd concentrations up to 100 mg Cd/100 ml medium, *A. flavus, A. niger* and *A. sydowii* showed growth decrease of more than 94% at only 25 mg Cd, while *A. niger* and *A. flavus* failed to grow at 50 mg Cd. The same pattern was also indicated within the species of *Fusarium*, *Penicillium* and *Trichoderma*. This indicated that the more tolerant species have the mechanisms and physiological adaptation to resist the higher Cd concentrations and so avoid its toxic effect. In accordance with these findings, it was reported that the genera, *Aspergillus, Penicillium* and *Trichoderma* are of high capacity to biosorb cadmium and other heavy metals (Volesky, 1990; Zafar et al., 2007; Lopez-Errasquin and Vasques, 2003). It was also reported that microorganisms isolated from contaminated environments with heavy metals they have adapted to such environments (Lopez-Errasquin and Vasques, 2003; Cernansky et al., 2007).

Cadmium absorption by living and dead biomass

To determine the tolerance of the fungus to high levels of metal ions and its capacity to biosorb that metal by its biomass either living or dead (dried), the following experiment conducted by hanging 100 mg dry biomass/100 ml Cd solution (10 mg) and equivalent living biomass to that

Table 1. Effect of different Cd concentrations in the growth of tested fungi.

Mould	Cadmium concentrations(mg/100 ml medium)				
	0	10	25	50	100
	D.wt (mg/100 ml medium)	% of growth decrease	% of growth decrease	% of growth decrease	% of growth decrease
A. flavus	743	82.77	86.41	100	100
A. fumigatus	798	25.56	62.66	77.69	87.97
A. niger	618	99.19	99.68	100	100
A. sydowii	298	85.57	94.30	96.64	100
A. ustus	427	43.33	75.64	82.20	100
A. versicolor	860	28.02	71.16	97.79	100
A. terreus	658	23.86	79.18	98.18	100
Cephaliophora irregularis	512	17.19	71.09	99.41	100
Cladosporium cladosporioides	286	48.60	73.43	98.95	100
Fusarium heterosporum	332	47.29	88.55	100	100
F. oxysporum	610	85.90	89.34	96.56	100
Paecilomyces variotii	445	32.81	87.64	97.53	100
Papulaspora irregularis	406	74.63	76.35	99.26	100
Penicillium citrinum	494	38.46	71.26	98.58	100
P. chrysogenum	733	30.97	77.90	97.54	99.05
Phoma humicola	525	63.81	82.29	99.24	100
Setosphaeria rostrata	582	45.36	71.65	83.85	100
Trichoderma longibrachiatum	731	67.85	85.50	100	100
T. viride	696	43.68	73.13	97.41	100

dry, (after calculating the moisture content), in 250 ml Erlenmeyer flasks, shaked at 25 ± 2°C for 60 min. The data (Table 2, Figure 1) indicated that the living biomass of the tested fungi were more efficient to biosorb Cd than their dried biomasses by percentages ranged between about 15-44%. The results also revealed that no relationship between the efficiency of living biomass and its corresponding dried biomass to absorb Cd ions. The biosorption by the 2 forms (living and dried) of biomass was dependent on the fungal species and may on its strains. These may due to the differences between the mechanism of biosorption in both living and dead microbial biomass. However, dead biomass is more advantages than living one (Volesky, 1990; Gupta et al., 2000; Zhou and Kiff, 1991; Bayramoglu et al., 2003). *A. fumigatus* of the 19 fungal species proved to be the most efficient from the points of its tolerance to high Cd levels in its growth medium and its efficiency to biosorb Cd by its living and dried biomass. Species of aspergilli especially *A.fumigatus*, *A. flavus* and *A. niger* were successfully used for the biosorption of heavy metals ions (Soylak et al, 2006, Akar and Tunail, 2006, Dacera and Babel, 2008).

Cadmium removal under different nutritional conditions

The results (Table 3) indicated that yeast peptone glucose medium (YPG) fortified the tested fungi by nutrient, qualitatively and quantitively in favour of the growth and the formation of metal ions absorbing surfaces (groups) as compared to yeast malt (YM) medium, while Sabouraud (Sb) was not so. These results indicated that yeast extract, as the common compound in YPG, YM media, fortified the moulds by macro and micronutrients that enhance the formation of metal binding groups. The results revealed that *A .fumigatus* dry biomass was the most efficient to biosorb Cd, even when grown on any of the tested media, as compared to the other tested fungal species, while *T. viride* came in the second order (less than 15% as *A. fumigatus*). All these results indicated that biosorption of Cd by the test fungi was dependent in the fungal species and its strain, as well as, in the cultural conditions. It was reported (Volesky, 1990) that of the factors that play important roles in metal ions uptake cultural conditions (components of the fermentation medium), culture age, cultivation conditions of temperature, pH and aeration.

Effect of treatment of *A. fumigatus* biomass

The pretreatment of dried fungal biomass by different compounds (Figure 2) resulted in loss in the weight of dried biomass ranged between 13-50%. Acid (hydrochloric or acetic acid) pretreatment resulted in drastic de-

Table 2. Cadmium uptake by living and dried biomass of the tested fungi.

Mould	Cadmium uptake (%)		Efficiency (%)
	Living biomass	Dead biomass	
A. flavus	13.5	9.8	27.41
A. fumigatus	39.1	27.8	28.90
A. niger	16.7	10.3	38.32
A. sydowii	18.7	11.5	38.50
A. ustus	17.4	10.4	40.23
A. versicolor	18.7	13.5	27.81
A. terreus	16.8	11.2	33.33
Cephaliophora irregularis	21.9	17.9	18.26
Cladosporium cladosporioides	13.0	7.3	43.85
Fusarium heterosporum	11.8	9.1	22.88
F. oxysporum	17.1	12.8	25.15
Paecilomyces variotii	18.7	15.9	14.97
Papulaspora irregularis	22.1	18.7	15.38
Penicillium citrinum	19.9	13.7	31.16
P. chrysogenum	23.9	16.3	31.80
Phoma humicola	16.9	9.7	42.60
Setosphaeria rostrata	14.9	10.2	31.54
Trichoderma longibrachiatum	21.5	16.7	22.33
T. viride	23.4	18.7	20.09

Efficiency = [(Adsorbed Cd by living biomass – Adsorbed Cd by dead biomass) /Adsorbed Cd by living biomass] x 100.

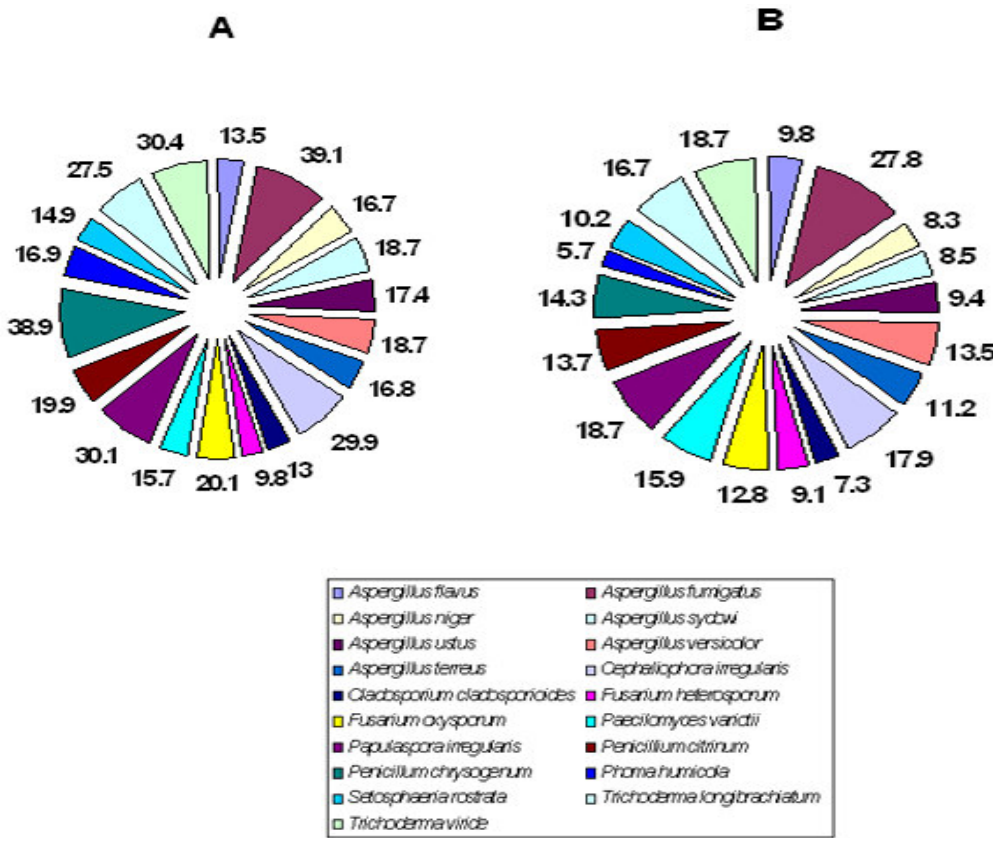

Figure 1. Cadmium uptake (%) by living (A) and dead (B) fungal biomass.

Table 3. Effect of YPG, YM and Sb media on dry weight yields (mg/100ml) and their efficiency on Cd uptake (%).

Mould	Growth Media						Av. D.wt. (mg)	Av. Cd uptake
	YPG		YM		Sb			
	D.wt. (mg)	Cd uptake	D.wt. (mg)	Cd uptake	D.wt. (mg)	Cd uptake		
Aspergillus flavus	8.57	13.5	5.63	9.9	6.34	8.5	6.8	10.6
A. fumigatus	8.64	27.8	7.59	24.6	4.67	21.9	7.0	24.8
A. niger	7.54	16.7	6.75	13.7	5.34	11.5	6.5	14.0
A. sydowi	2.46	18.7	3.73	15.1	3.05	13.2	3.1	15.7
A. ustus	1.92	17.4	1.64	14.1	1.53	12.8	1.7	14.8
A. versicolor	7.07	18.7	4.25	13.4	3.86	12.2	5.1	14.8
A. terreus	6.72	16.8	6.00	13.7	4.29	13.2	5.7	14.6
Cephaliophora irregularis	4.25	21.9	3.82	17.9	2.61	15.8	3.6	18.5
Cladosporium cladosporioides	2.39	13.0	2.07	11.0	1.67	9.8	2.0	11.3
Fusarium heterosporum	2.54	11.8	2.62	9.1	2.30	7.7	2.5	9.5
F. oxysporum	6.72	17.1	4.36	12.4	3.82	10.8	5.0	13.4
Paecilomyces variotii	5.41	18.7	3.58	14.6	2.60	13.0	3.9	15.4
Papulaspora irregularis	4.76	22.1	4.19	18.4	2.23	17.6	3.7	19.4
Penicillium citrinum	5.57	19.9	3.09	13.5	2.95	12.5	3.9	15.3
P. chrysogenum	6.37	23.9	4.08	16.9	2.73	14.9	4.4	18.6
Phoma humicola	5.98	16.9	4.67	12.7	4.45	11.6	5.0	13.7
Setosphaeria rostrata	4.64	14.9	3.99	12.8	4.36	11.5	4.3	13.1
Trichoderma longibrachiatum	7.67	21.5	6.28	18.9	5.51	17.0	6.5	19.1
T. viride	7.56	23.4	4.35	21.0	5.42	19.1	5.8	21.2

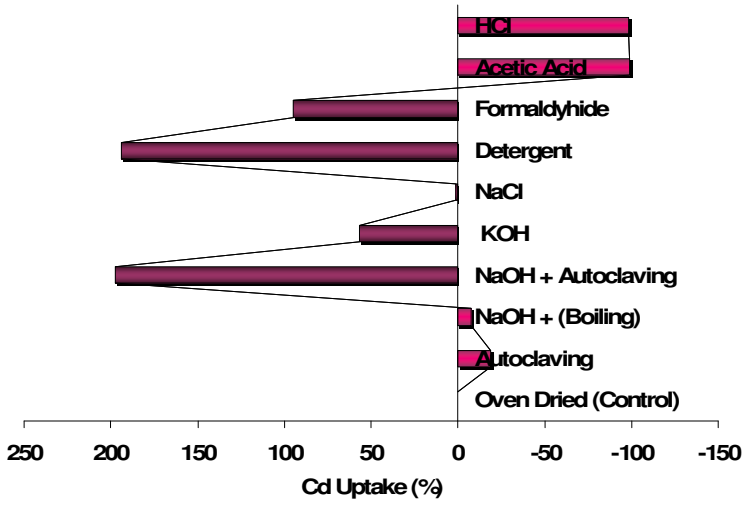

Figure 2. Cadmium uptake (%), as compared to control (untreated), by different treated *A. fumigatus* biomasses.

crease of Cd biosorption(99, 98.4%, respectively) as compared to oven dried biomass (control), this indicated that the acids destroyed the absorbing groups and their positive ions(H^+) may covalently bonded to the absorbing surfaces. However, formaldehyde pretreated biomass showed about 95% increase in Cd biosorption activity, this is due to the change in oxidation reduction potential of the negatively charged biosorbing surfaces. While detergent treated biomass showed about 2.9 fold increase for Cd absorption, this finding may attributed to the highly surface tension of the detergent that may expose biosorbing groups that increase available absorbing charges for metal ions absorption. On the other hand, alkali (NaOH, KOH) and autoclave pretreated *A. fumiga-*

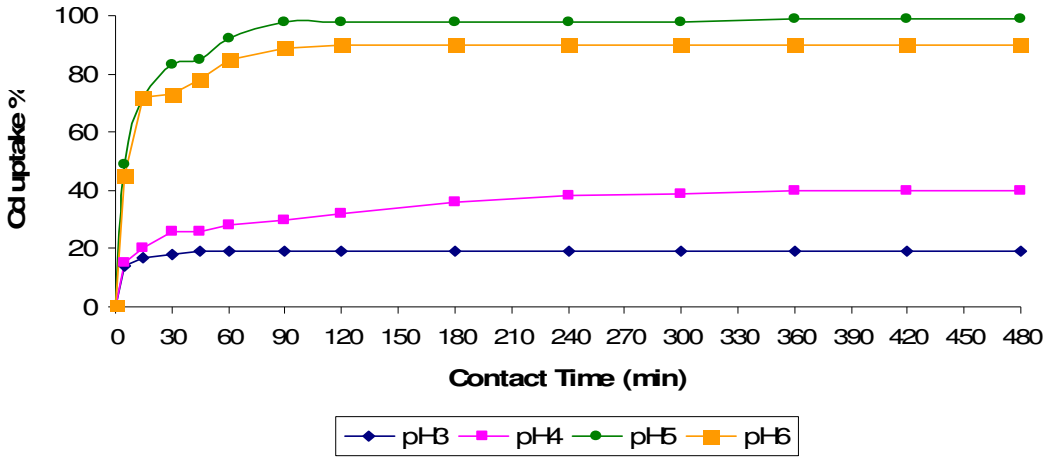

Figure 3. Effect of pH at different contact periods on cadmium uptake (%) by pretreated (0.5 N NaOH and autoclaving) *A. fumigatus* biomass.

tus biomass resulted in significantly increased Cd absorption (197.6, 56.83%, respectively). In accordance with these findings, it was reported that the dried biomass act as ion exchange resins and its biosorbing capacity depends on the available charges on the cell surface that bind to the biosorbent metal ions. So, the capacity of the biomass can be increased by physical and chemical treatments, which led to removal, hiding or exposing chemical groups that binding or exchange with the adsorbed metal ions. Heating and boiling are of the physical factors, while detergents, organic solvents, alkali and acids of the chemical factors (Greene and Darnell, 1990; Volesky, 1990; Puranik and Pakniker, 1999; Kapoor and Viraraghavan, 1998; Kapoor et al., 1999; Gupta et al., 2000).

Effect of pH at different contact periods on biosorption

The effect of pH 3, 4, 5, 6 at different contact periods (up to 480 min) on biosorption of Cd by pretreated *A. fumigatus* biomass with 0.5 N NaOH and autoclaving (Figure 3) indicated that the more acidic pH 3 was not favorable for Cd ions biosorption, not exceeded 20. However, at less acidic pH 4, biosorption activity was doubled (40%). On the other hand, pH 5 proved to be the most favorable for metal ions biosorption (99%) and as the pH raised to 6 non-significant decrease of Cd adsorption was recorded. It was reported that pH value has important role in metal ions biosorption, where the active biosorbing groups have the ability to accept or loss of protons that depend mainly in the pH value (Pinghe et al., 1999; Yalcinkaya et al., 2002). Also, it affects metal ions solubility and ionization of the biosorbing groups in the biomass surfaces (Fourest and Volesky, 1996). It was also reported that high acidity makes the biosorbent surfaces accept protons (H^+) and therefore reduce their ability to adsorb positive Cd ions and oppositely as acidity decrease to its optimum value, which differ from biomass to another and type of adsorbed metal ions, the adsorbing surfaces saturated with negative charges, resulted increased efficiency to bind and adsorb metal ions of positive charges (Fourest and Roux, 1992; Sadowski, 2001; Bayramoglu *et al*., 2003). In accordance with our finding that the highest Cd biosorption by pretreated *A. fumigatus* biomass lie between pH 5 and 6, it was reported (Kacar et al., 2000; Say et al., 2003; Svecova et al., 2006; Mashitah et al., 2008), that pH between 5 and 6 was optimum for Cd biosorption by different fungi.

As for the contact time of biosorbent at the tested pHs with 10 mg Cd/100 ml, the data revealed that the required time to attain equilibrium between the adsorbing surfaces and Cd ions was 45 min at pH 3, 6 h at pH 4, 90 min at pH 5 and 3 h at pH 6. These findings indicated that the equilibrium time depend in many factors of which pH value of the biosorbent liquid. The fluctuation of equilibrium period was recorded by many workers and depend in many factors of which the type and nature of the biomass, metal ions, and physicochemical properties of the biosorption process (Saglam et al., 1999; Kacar et al., 2000; Abu Al-Rub, et al., 2004; Herrero et al., 2005; Bayramoglu et al., 2006; EL-Sherif et al., 2008; Parameswari et al., 2009).

Effect of pretreated biomass level

The results (Figure 4) indicated that 100 mg of pretreated *A. fumigatus* biomass was optimum for the highest biosorption of Cd ions (10mg/100 ml), at both optimum pHs (5,6). Lower biomass levels were concomitant with parallel decrease in biosorption activity and the same figure was recorded as the biomass increased to 110 mg. These indicate that the number of adsorbing negative charges on the pretreated biomass level was parallel with

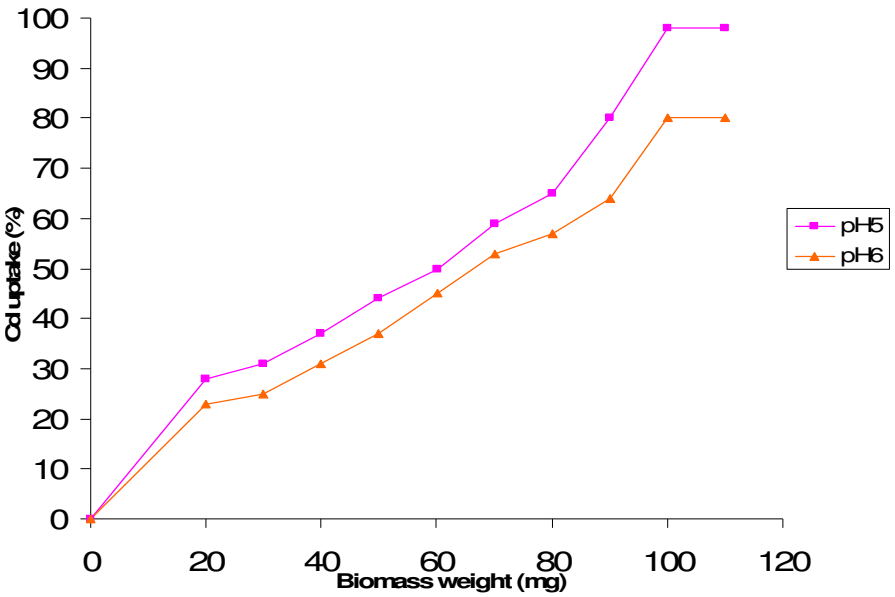

Figure 4. Effect of pretreated *A. fumigatus* biomass weights on cadmium uptake (%) at pH 5 and pH 6.

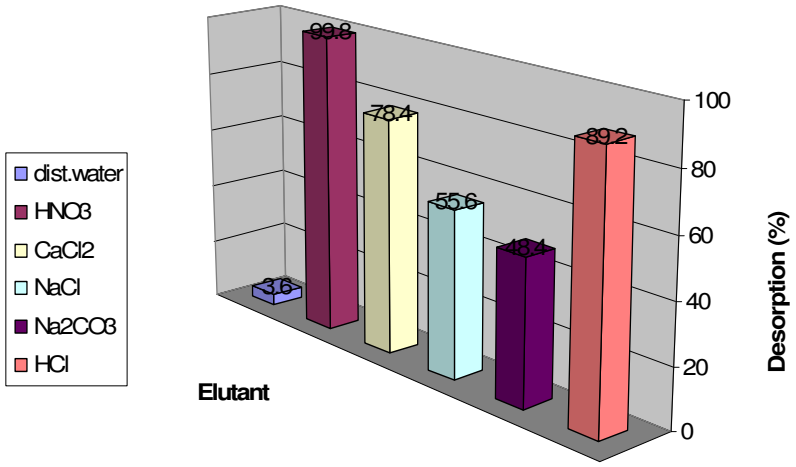

Figure 5. Effect of different elutants on cadmium desorption.

an increase of metal ions as a result to increasing of biosorbent surfaces till attaining equilibrium, therefore any increase in biomass level is not accompanied by increasing adsorption efficiency for the lower number of the adsorbed metal ions, as compared to the available charges on the biomass surface (Sampedro et al., 1995; Tawfik et al., 2005; Mecedo et al., 2003; El-Morsy, 2004; AL-Garni, 2005; Mashitah et al., 2008).

Elution of biosorbed Cd from pretreated biomass and reuse of the biosorbent

The biosorbed Cd ions were eluted from pretreated biomass using various elutants. Figure 5 compares the removal efficiency of various chemicals in eluting biosorbed Cd ions. Deionized water was able to elute biosorbed ions only to a limited extending (3.6%), indicating the strong affinity that biomass possesses towards the metal ions. 0.05 N nitric acid solution was able to effectively elute the biosorbed metal ions (99.8%) and to a lesser extend HCl (89.2%). Ca^{2+} and Na^{+} ions in solutions ($CaCl_2$, NaCl, Na_2CO_3) at a concentration of 0.1 M were able to elute Cd ions (78.4, 55.6 and 48.4%, respectively). Possibly due to ion-exchange reactions and Ca^{2+} as a divalent cation as Cd was more efficient more the monovalent Na^{+}. The biomass was used for 5 cycles of biosorption-elution of biosorbed Cd ions (using 0.05 N

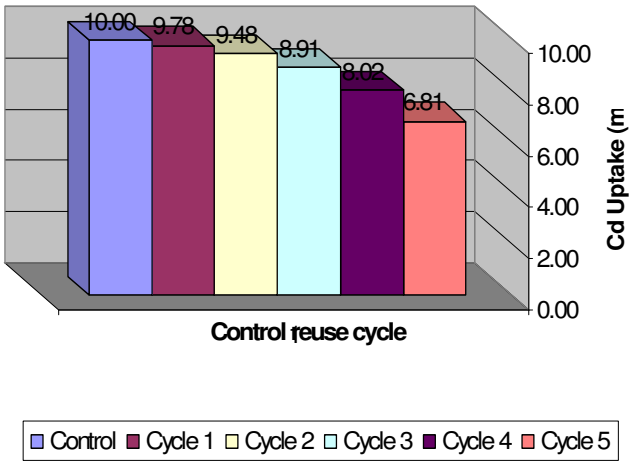

Figure 6. The 5th reuse of desorbed *A.fumigatus* pretreated biomass for Cd absorption.

nitric acid), regeneration of biomass to study the changes in metal biosorption with subsequent usage. Figure 6 shows that biomass lost a portion of its Cd biosorption capacity after cycle 1, but was able to retain the residual Cd biosorption for another 4 cycles. The results show that the desorption (elution) efficiency were decreased by about 15% at the 5th cycle, while absorption capacity of Cd ions was decreased by about 29% under the same conditions. These findings clearly show that the pretreated dry *A. fumigatus* biomass can be used repeatedly without significantly losing the adsorption capacity. In accordance with our results several solutions were used for elution of adsorbed metal ions, as diluted mineral acids, carbonates, bicarbonate, chlorides, sulphate, deionized water etc, (Kuyucak and Volesky, 1988; Gupta et al., 2000; Say et al., 2003) and 0.05 N nitric acid used successfully in elution of adsorbed Cd on *A. niger* biomass. The decrease in adsorption capacity as a result to repeated elution by nitric acid and readsorption may due to the antagonistic effect of elutants on binding surface on the biomass (Hashim et al., 2000). Several workers indicated the reuse of desorbed biomass for readsorption of metal ions for several times (Kapoor et al., 1999; Hashim et al., 2000; Say et al., 2003; Rangsayatorn et al., 2004).

Conclusion

A. fumigatus, of 19 fungal species isolated from soil contaminated with industrial wastes, was the most tolerant to Cd level up to 100 mg/ 100 ml medium. The living fungal biomass was more efficient to biosorb Cd than its dried biomass. The biosorption of Cd by *A. fumigatus* was considerably influenced by the composition of the growth medium. The biosorption of Cd by dried biomass of *A. fumigatus* was considerably influenced by its treatment, pH value of the biosorption medium, contact time, biomass level and Cd concentration under these conditions 98% of Cd was absorbed. The biosorbed Cd can be eluted and the desorbed *A. fumigatus* pretreated dried biomass can be successively reused for Cd biosorption for 5 consecutive times.

ACKNOWLEDGEMENT

The authors are thankful to the Ministry of Higher Education, King Abdulaziz University, Deanship of Scientific Research, Jeddah, Saudi Arabia for the financial support to carry out this research work.

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