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Some studies of cadmium adsorption using *Aspergillus niger*, *Penicillium austurianum*, employing an airlift fermenter

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Abstract

Aspergillus niger and *Penicillium austurianum* were produced on a rotary shaker washed, dried, and sieved. The 1 g of each biomass were introduced into tiny columns and Erlenmeyer flasks of 50 ml volume, to study the effect of agitation, cadmium in solution concentration and a wide range of pH. The samples were analyzed using atomic absorption spectroscopy (Shimadsu AA67) at 228.8 nm. The adsorption data of *A. niger* were fitted to Langmuir and Freundlich models and R^2 higher than 0.97 and 0.98 obtained. In a different set of experiments the effects of cadmium concentration in solution, temperature, aeration velocity and mixed culture of 1:1 and 3:1 ratio employing a glass made airlift fermenter were investigated. The results show that the operation temperature of 22 ± 1 °C and pH 4 and 5 are suitable for biosorption, using active fungi. It was also found that 2 vvm aeration is reasonably sound to produce biomass and biosorption using *A. niger* in the airlift reactor.

Keywords: Heavy metal; Cadmium removal; Fungi

1. Introduction

The influence of heavy metals on the global pollution through activities such as mining operation, industrialization and urbanization have enhanced the potential of heavy metals in the ecosphere [1]. A large number of industries including, electronics, plating, battery, pigment and ammunition manufacture, release heavy metals like cadmium, lead and zinc, etc. in waste streams. The effects of physico-chemical nature of the environment have key role on determining interactions with and the toxicity of the metals [2]. Holan et al. [3] point out the harmfulness of the toxicity levels. In Japan people living around Minimata bay suffered neurological illness by eating sea fish and shellfish contaminated with methyl-mercury. Further, the inhabitants around Jinstsu river suffered painful Itai–Itai after eating rice grown in contaminated soil irrigated by river polluted with cadmium, lead and zinc. Stringent environmental rules and EEC [4] guide-line permits German industrial waste water to contain only 0.5 mg Cd/l [5]. Filamentous fungi are of increasing importance in biotechnological processes to produce a wide variety of products such as primary and secondary metabolites [6,7] and leaching of metal ores [8,9]. Many papers in recent years have appeared in the literature to address the significance of biosorption phenomenon [10–14]. Biosorption of various metals especially heavy and other environmentally harmful species by micro-organisms like fungi appears to be promising for clean-up of waters [15,16], especially since conventional physical and chemical techniques of removing soluble metal waste (such as ion-exchange, precipitation, electrochemical treatment, and evaporative recovery) are generally very expensive when the contaminant concentration are in the range of 10–100 ppm. Processes to clean-up waste waters which depend on inactive or immobilized biomass as exchange site, mixed cultures [17,18], and fungi [19,20] are generally used.

In the present study *Aspergillus niger*, *Penicillium austurianum* in inactive, active and mixture form were used to examine their adsorption capacity. Cadmium, an important environmental pollutant [21] was used as the test metal in the form of cadmium sulfate ($3CdSO_4{\cdot}8H_2O$) which is to be absorbed by micro-organisms [22]. *Penicillium austurianum* and *A. niger* after 48 h of cultivation were removed from the medium, dried, crushed, sieved and 1 g of each was introduced into a series of test tubes using a wide range of cadmium concentration and pH of 4 and 5, respectively. The experimental data of inactive *A. niger* were fitted to the Langmuir and Freundlich models and R^2 values higher than 0.98 and 0.97 were obtained. Therefore, it was thought desirable to investigate the effects of the initial cadmium concentration, medium composition, temperature, ratio of fungi mixture, and shaker agitation on the adsorption capacity of fungi. The effects of initial cadmium concentration, medium

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Nomenclature

a	effective interfacial area (mm^{-1})
b	Langmuir isotherm constant (l/mg)
C	concentration of metal in solution (mg/l)
C_e	metal concentration in eluent (mg^{-1})
C_{eq}	equilibrium concentration of adsorbate (mg/l)
C_i	initial concentration of metal (mg/l)
d	bubble diameter (mm)
H_C	height of continuous phase (mm)
H_D	height of dispersed phase (mm)
K	constant, relative indicator of adsorption capacity
K_r	constant of adsorption rate (per mg/metal per min)
n	constant (temperature dependent)
$1/n$	constant, intensity of the reaction
q_e	specific metal adsorbed (mg adsorbed/mg adsorbent)
Q_m	maximum metal adsorbed (mg adsorbed/mg adsorbent)
t	time (s)
V_g	superficial dispersed phase velocity (mm/s)

Greek letters

ε_g	gas hold-up
θ	surface area (binding sites)

Subscripts

B	bubble
C	continuos phase
D	dispersed phase
e	equilibrium
g	referring to gas phase
i	initial

composition, aeration velocity and ratio of fungi mixture on the adsorption capacity of active fungi adsorbing cadmium were verified.

2. Materials and methods

2.1. Organisms

The strains used in the present study were *A. niger* and *P. austurianum* species, and a mixture of both. The strain was maintained at 30 °C on 2% malt extract agar (MEA). Precultures were routinely cultured every 2 months, prior to experimental use on the same medium; spore suspension of 3–4 weeks old was used as inoculum.

2.2. Culture conditions and medium

All cultures including precultures were prepared in Erlenmeyer flasks of 500 ml volume with 200 ml effective volume. Production medium composed of beet root molasses, corn steep liquor (CSL) and trace elements. The glucose content of molasses and dry solid (DS) of CLS was 20 and 9.6 g/l, respectively. The initial pH of the media producing *A. niger* and *P. austurianum* were 5 and 4, respectively. The inoculum for all the experimental culture was 7% (v/v) and cultivated at 30 °C. An Adolf–Kuhner orbital shaker was used to cultivate biomass for 48 h which is used in inactive form. A reaction time of 24 h was required to produce precultures to be used in the airlift reactor. The medium was introduced to Erlenmeyer flasks under sterile conditions, and were stoppered with cotton bungs and covered with aluminum seals. The density of the spore suspension was adjusted to give an absorbance of 0.78 nm corresponding to about 10^7 spores per ml.

2.3. Feed composition

In order to grow fungi on an economical carbon source, beet root molasses were used (20 g/l). Corn steep liquor was used as a nitrogen source, 120 ml/l (≈8% dry weight) ≈9.6 g/l. Cadmium sulfate ($3CdSO_4$ $8H_2O$) of desirable concentration was prepared. The pH of the initial feed was adjusted to 4 and 5 for *P. austurianum* and *A. niger* by NaOH addition.

Chemicals: all the chemicals used were of reagent grade.

2.4. Biomass

The mycelia biomass of *A. niger* and *P. austurianum* after 48 h of cultivation on malt extract at 30 °C and 150 rpm on rotating shaker including the inactive and preculture cultivation were used throughout the present investigations. The biomass of *A. niger* and *P. austurianum* were washed with distilled water of desired pH after constant pH was attained from the elute, separated onto filter paper, and placed in petri dishes in an electric oven at 42 °C for 48 h to dry. The dried biomass was then crushed in a blender and sieved so as to obtain particles of 14–16 BSS mesh (1.2–1.4 mm) size. The dried and sieved mycelial preparations were used to verify the adsorption capacity of the fungi in inactive form.

2.5. Metal solutions

Stock solutions of 58, 112, 255, 508 and 810 ppm of cadmium solutions were prepared. The working metal solutions were prepared in glass distilled water. The pH of the metal solution was adjusted to desirable values of 4 and 5 with 0.1 M NaOH and 0.1 M hydrochloric acid for *P. austurianum* and *A. niger*, respectively. The samples were analyzed using an atomic absorption spectrometer (Shimadsu AA670) at 228.8 nm throughout the experimental work. A calibration chart was also constructed and used to obtain cadmium concentration.

2.6. Metal sorption experiments

A batch equilibration method was employed to study the adsorption of cadmium by inactive *P. austurianum* and *A. niger* biomass. The period of contact between the biomasses and the metal solutions was varied to examine the rate of adsorption. A set of 50 ml Erlenmeyer flasks containing 5 ml of 112 ppm metal solution was used and 1 g of sieved biomass was contacted with the metal solution by incubating the flask in rotary shaker at 150 rpm for 1 h. In order to determine the adsorption rate the flasks were removed from shaker at intervals of 5 min. In separate studies, 12 test tubes containing 1 g of sieved biomass and 5 ml metal solution of 112 ppm strength was mixed for 40 min and the test tubes checked at 5 min intervals. The content of the flasks and test tubes were filtered and the filtrate analyzed for residual metal concentration. In order to check the effect of pH on the adsorption capacity samples of the *P. austurianum* and *A. niger* were conditioned at different pH ranging between 1 and 7 with distilled water of desired pH until the pH of washed water was constant. The biomass was dried prior to experimentation. In order to check the effect of physico-chemical properties of the medium on adsorption capacity of *A. niger* and *P. austurianum* for cadmium, the fungi were cultivated on 2% malt extract broth and beet root molasses (20 g/l of glucose) adding to it 120 ml/l corn steep liquor of 8% dry solid. The orbital shaker was agitated at 150 rpm and maintained at 30 °C for 48 h. The metal solutions of varying concentrations of 58, 112, 255, 508 and 810 ppm were used to examine the effect of initial metal ion in solution. The pH of the metal solutions was adjusted to 4 and 5 for *P. austurianum* and *A. niger*, respectively. Experimentally optimized pH range of 3.5–4 and 4.5–5 were found for *P. austurianum* and *A. niger*, respectively. For simplicity solutions at pH 4 and 5 were used to precondition the biomasses in order to avoid any changes in the initial pH of the metal ion solutions during biosorption [23]. All the above mentioned experiments were carried out in triplicate and averaged to ensure the accuracy of results.

The mechanism of metal uptake by microbial cells is not yet well understood. The real attachment of the metal ions on the cells surface may include physical adsorption (biosorption), ion-exchange, or chemisorption. To describe uptake of heavy metals ion by active and inactive micro-organisms adsorption isotherms of Freundlich and Langmuir models were used.

$$q_e = K_f C_{eq}^{1/n} \quad \text{(Freundlich isotherm)} \tag{1}$$

$$q_e = \frac{bQ_m C_{eq}}{1 + bC_{eq}} \quad \text{(Langmuir isotherm)} \tag{2}$$

It may be assumed during the adsorption process, desorption is negligible and that the process is irreversible, [24]. Therefore, the kinetic analysis suggested by Langmuir [25] for gas adsorption may be applied for this case. Vincent at al. [26] wrote an equation for the residual metal concentration unabsorbed as a function of time, $C(t)$:

$$\frac{dC(t)}{dt} = -K_r C(t)[1 - \theta(t)] \tag{3}$$

where K_r is the rate constant and $\theta(t)$ is the fraction occupied binding sites at time t, as

$$\theta(t) = \frac{C_i - C(t)}{C_i - C_{eq}} \tag{4}$$

where, C_i and C_{eq} are the initial and equilibrium concentration of metal. Incorporating Eq. (4) into Eq. (3) and integrating, Eq. (5) is obtained:

$$I_n \frac{[C]}{[C - C_{eq}]} = I_n \frac{[C_i]}{[C_i - C_{eq}]} + \frac{[C_{eq}]}{[C_i - C_{eq}]} K_1 T \tag{5}$$

Therefore, a plot of $I_n[C/(C - C_{eq})]$ against 't' should yield a straight line, from the slope of which 'K_r' may be calculated, provided K_r is independent of 'θ'. The applicability of Eq. (5) to the data of metal uptake obtained as a function of time can be tested by determining rate constants (K_r) graphically and by calculations.

2.7. Experimental procedure for simultaneous production of biomass and biosorption in fermenter

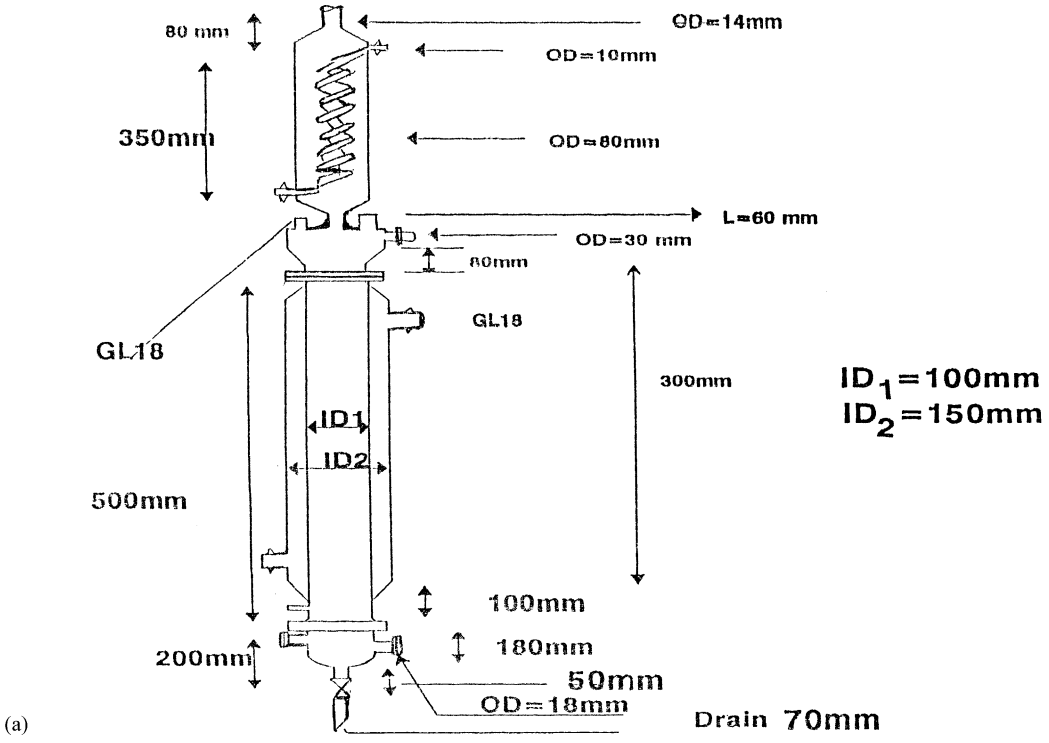
The cadmium sulfate solution was carefully prepared for each set of experimental work. The pH of each medium was measured using a digital pH meter (Corning type NE-1604). Initially, samples were collected and checked precisely to ensure no contamination was present in the solution. The predetermined quantity of metal solution was added gently to the bioreactor. The live steam was introduced through one of the nozzles of the air chamber for 45 min and the dilution due to the steam condensation was almost constant about at 0.5 l, to make-up the total working volume to 5 l for all the experiments performed. The steam inlet was shut off and a little air was allowed into the bioreactor through the bottom chamber to prevent the metal solution leakage to the bottom of air chamber, which also enhances the cooling rate. Chilled water was circulated in the jacket of the airlift bioreactor and condenser to decrease the cooling time, and preventing any losses in the fermenter volume during cooling and fermentation. The fermentation temperature was controlled at appropriate temperature, e.g. 30 °C. Inoculum addition to the airlift bioreactor was performed under sterile conditions. The pH of the medium was adjusted to about 4 and 5 for *P. austurianum* and *A. niger*, respectively. The fermenter was operated in semibatch manner. The flow of air was monitored using a precaliberated rotameter. The bubble breakdown in the disengagement section (due to area expansion) was incomplete and many bubbles circulated through the downcomer (due to recirculation) and some escaped with undesirable gases produced during processing through the condenser. Siegel et al. [27] found that the gas recirculation rate is dependent on the fluid residence time, liquid level in

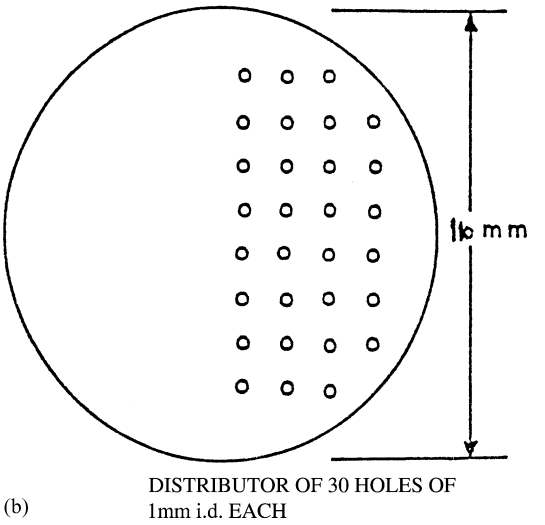
the gas–liquid separator. Verlaan et al. [28] have reported that the recycled gas constitutes less than 10% of the fresh gas in the riser. In order to study the hydrodynamic characteristics of the split airlift bioreactor. The clear liquid height (H_C) and the height of dispersion (H_D) were measured to estimate the dispersed phase hold-up (ε_G) as defined in Eq. (6).

$$\varepsilon_G = \frac{H_D - H_C}{H_D} \tag{6}$$

2.8. Airlift fermenter details

The schematic diagram of the glass made airlift fermenter of 100 mm inside diameter and 800 mm height is depicted in Fig. 1(a). The diameter of the holes on the sparger was 1 mm and the holes were drilled on a square pitch of 6 mm center to center distance. The holes were drilled on nearly half of the stainless steel plate of 1 mm thickness (sparger)





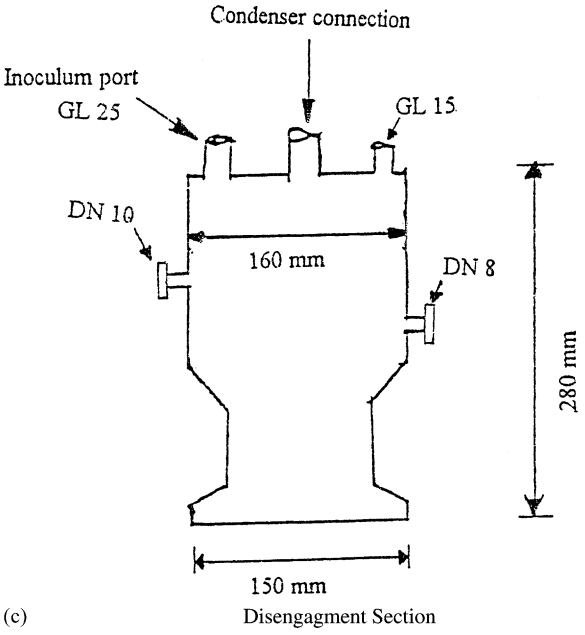


Fig. 1. (a) Schematic diagram of airlift fermenter. (b) Distributor of 30 holes of 1 mm i.d. each; (c) disengagement section.

which formed the riser side of the main body, and the number of holes were 28. The air was introduced through a chamber of 100 mm length and internal diameter. The chamber was flanged and connected to the main body of the fermenter placing the sparger in between the flanges. The jacketed glass made airlift fermenter was divided in two parts (riser and downcomer) by a 5 and 100 mm thickness and width slab, respectively. The PTFE made slab was 600 mm in height. The main body of the bioreactor was flanged to the disengagement section and condenser was connected at the top of disengagement. The condenser surface area used was 0.3 m^2 to arrest any out going vapor to maintain the bioreactor volume for the entire experiments constant. Details pertaining to the distributor and disengagement sections are depicted in Fig. 1(b) and (c), respectively. Aeration was controlled by a flowmeter. A filtration arrangement using 0.2 μm mesh size for the inlet and outlet air was used. Circulating water mixture through the bioreactor jacket and maintaining about 30 °C for each batch controlled the process temperature.

3. Results

3.1. Effect of pH

pH is a key parameter in most biological processes and controls the growth and/or the adsorption capacity of substances. Fig. 2 shows that under acidic conditions the adsorption of cadmium by both of the species was quite low. There was an increase in cadmium adsorption with an increase in the pH from 2 to 5 and beyond which no real changes in cadmium adsorption was observed. The observations of present studies supports the idea that the initial pH of the metal solution plays a vital role in the metal adsorption in general and in particular in cadmium biosorption using *A. niger* and *P. austurianum*. The results obtained indicate that the optimum pH range for adsorption of cadmium in solution by *P. austurianum* and *A. niger* was between 3.5–4 and 4.5–5, respectively.

3.2. Metal adsorption

Fig. 3 shows that the adsorption of cadmium solution of 112 ppm using *P. austurianum* and *A. niger* at pH values of 4 and 5 and operating temperature of 30 ± 1 °C. Using the orbital shaker rotating at 150 rpm the systems reached equilibrium in 15 and 8 min, respectively. It was observed that using both the fungi, the rate of adsorption was very rapid with 80 and 83% cadmium uptake by *P. austurianum* and *A. niger* occurring within the first 4–5 min of the experiment. The rate constant K_r of cadmium adsorption by *P. austurianum* and *A. niger* was calculated using Eq. (5) and also determined graphically by plotting $I_n[C/(C - C_e)]$ versus 't' (plot not shown). The values of rate constants for *P. austurianum* and *A. niger* adsorbing cadmium, determined graphically were 1.08 and 0.65 1/mg cadmium per min, respectively. The corresponding calculated values of K_1 were 0.7 ± 0.15 and 1.3 ± 0.535 per mg/metal per min, respectively.

3.3. Effect of cadmium concentration

Figs. 4 and 5 show that increasing initial cadmium concentration in solution decreases the adsorption period and reduces equilibrium time. Employing inactive mass of *A. niger* treated with 810 ppm initial concentration of cadmium in solution takes 4 min to equilibrate and about 42% of the cadmium is adsorbed. Using 58 ppm cadmium in solution takes 15 min to equilibrate and 44% of the cadmium is adsorbed. When *P. austurianum* is treated with 58 and 810 ppm cadmium in solution takes 15 and nearly 4 min to equilibrate and 69 and 42% reduction in cadmium is achieved, respectively. This may be explained by the possibility that increasing the cadmium strength in solution, the free sites of fungi are occupied by metal ions much faster than when the solution is dilute.

3.4. Effect of adsorption period

Fig. 6 shows that by increasing processing period approximately more than 8 and 15 min has no effect on the

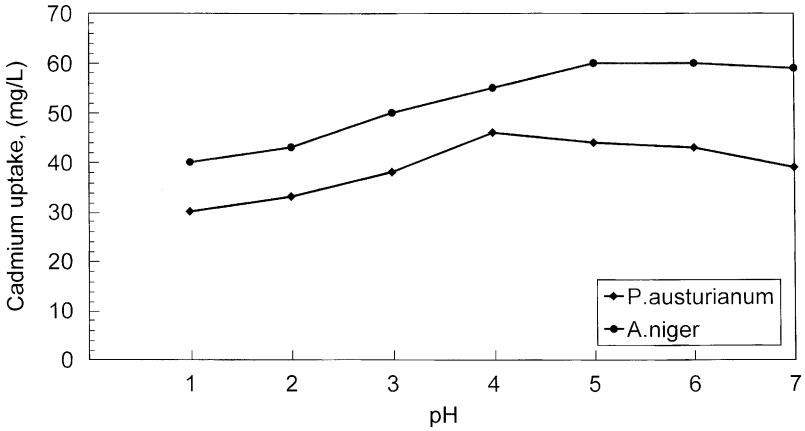


Fig. 2. Effect of pH on adsorption of cadmium by inactive *P. austurianum* and *A. niger*.

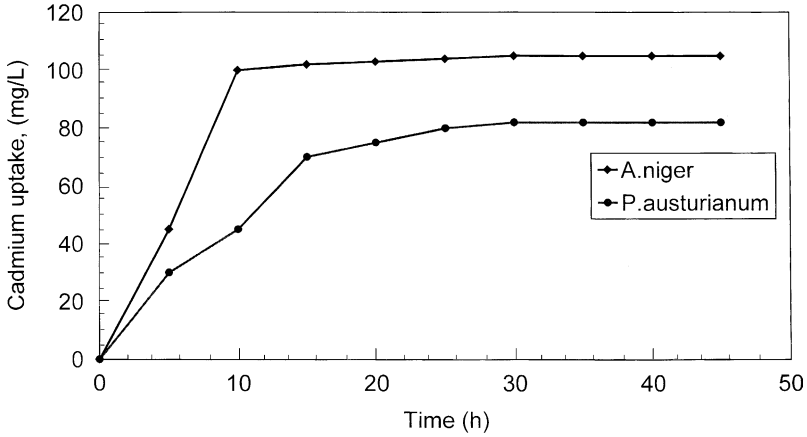


Fig. 3. Effect of shaker rotation on adsorption of cadmium by inactive *P. austurianum* and *A. niger*.

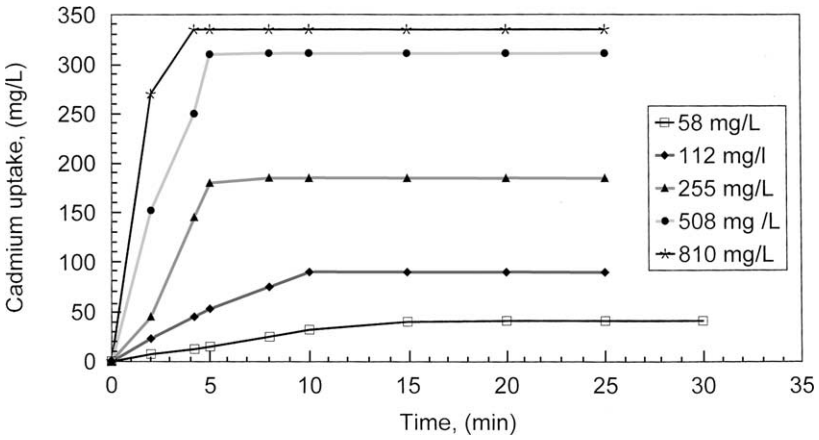


Fig. 4. Effect of cadmium concentration on adsorption time using inactive *A. niger*.

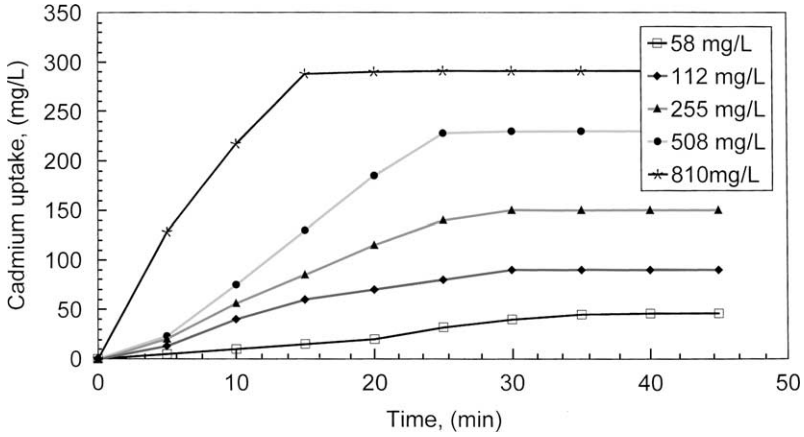


Fig. 5. Effect of cadmium concentration on adsorption period using inactive *P. austurianum*.

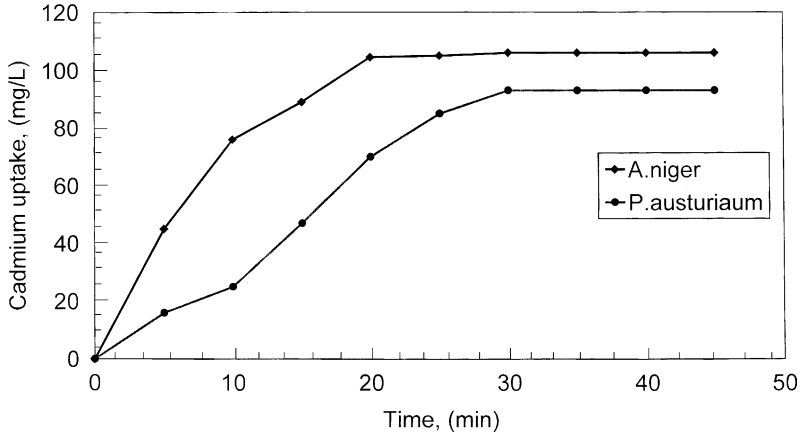


Fig. 6. Effect on time on adsorption of cadmium using inactive *A. niger* and *P. austurianum*.

adsorption extent of the cadmium removal by *A. niger* and *P. austurianum* using cadmium concentration of 112 ppm strength. The results of the present work is on the parallel line with the findings of Huang [15]. This shows that the cell wall texture of *A. niger* may differs from that of *P. austurianum* which consequently affect the rate and extent of cadmium sorption.

3.5. Effect of medium composition

The effect of medium composition on the production of biomass of fungi and consequently on the isotherm behavior of cadmium adsorption is depicted in Figs. 7–10. Using 2% malt extract in the medium to produce *A. niger* and *P. austurianum* under the same appropriate experimental conditions reduces 4–10% cadmium more than the medium consisting of molasses and CSL. It may be explained that adsorption behavior depends on the combined effect of composition of cellular wall texture, and cultivating medium. The results of the present study are similar to that of Gadd [29,30]. The experimental data were fitted to the Freundlich equation and Langmuir model. It is observed that both the models well fit the data with $r^2 = 0.98$ (Langmuir), 0.99 (Freundlich) using *A. niger* and malt extract. Further data of *P. austurianum* and malt extract with $r^2 = 0.98$ (Langmuir), and 0.99 (Freundlich) were obtained.

3.6. Inoculum

A solution of *A. niger* containing 6.2×10^7/ml spore was used with respect to all the experiments performed using *A. niger*. The spore counting was undertaken using The Neobar lamel [31] method. The Inoculum was about 7%. The same procedure was followed to count the spore of *P. austurianum* which amounted to 3.6×10^8/ml, composing 7% of the medium. The same amount of spore was used as inoculum for all the experiments using *P. austurianum*.

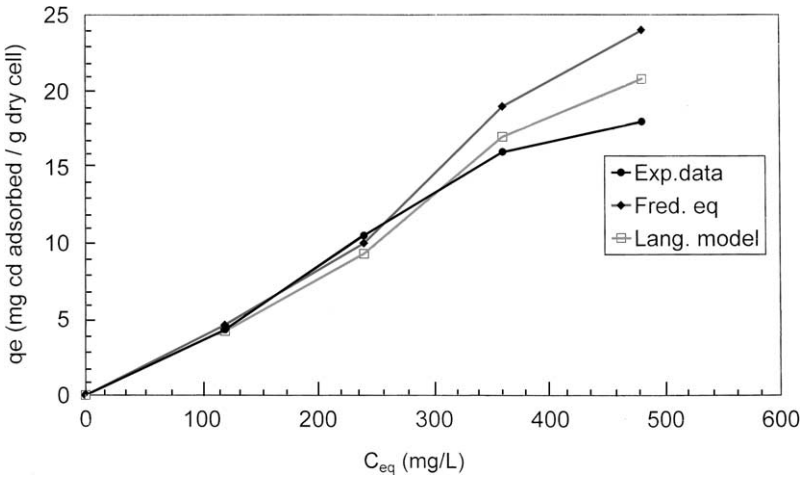


Fig. 7. Isothermal behavior of inactive *A. niger* adsorbing cadmium (using malt extract).

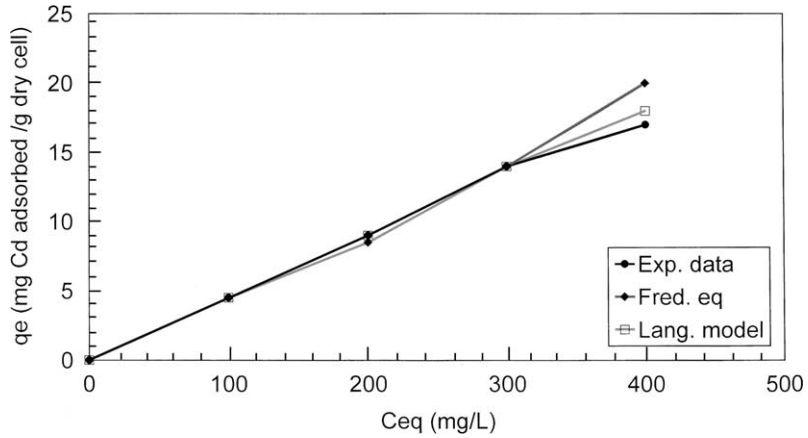


Fig. 8. Isothermal behavior of inactive *P. austurianum* adsorbing cadmium (using malt extract).

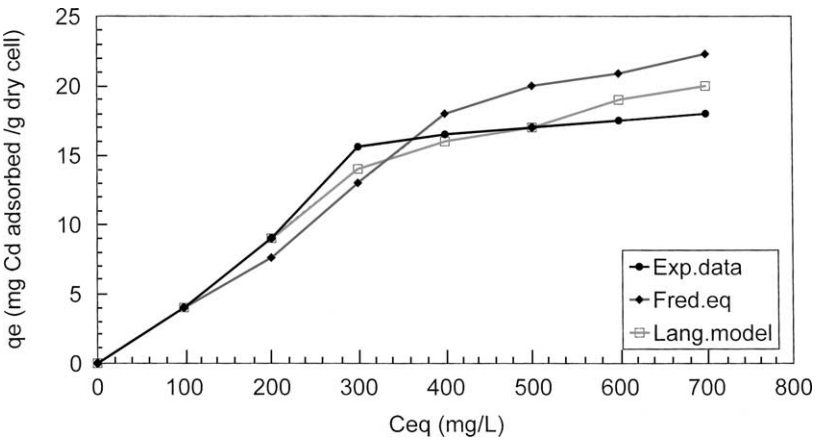


Fig. 9. Isothermal behavior of inactive *A. niger* adsorbing cadmium (using molasses).

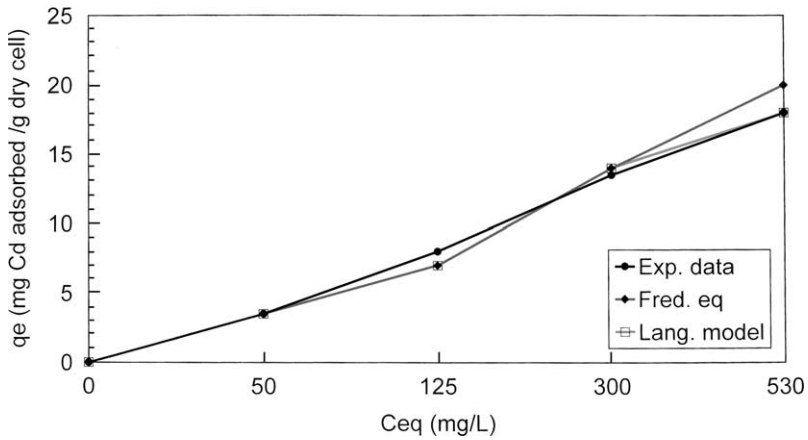


Fig. 10. Isothermal behavior of inactive *P. austurianum* adsorbing cadmium (using molasses).

3.7. Effect of aeration

The effect of aeration on the growth and biosorption of *A. niger* and *P. austurianum* was performed in the range of 1–4 volume of air per volume of medium per minutes (vvm). The pH of the medium using *A. niger* and *P. austurianum* was 5 and 4, respectively. Otherwise the rest of the experimental conditions including operating temperature throughout the investigation using the fungi were identical. The dry weight of fungi obtained was measured after each aeration

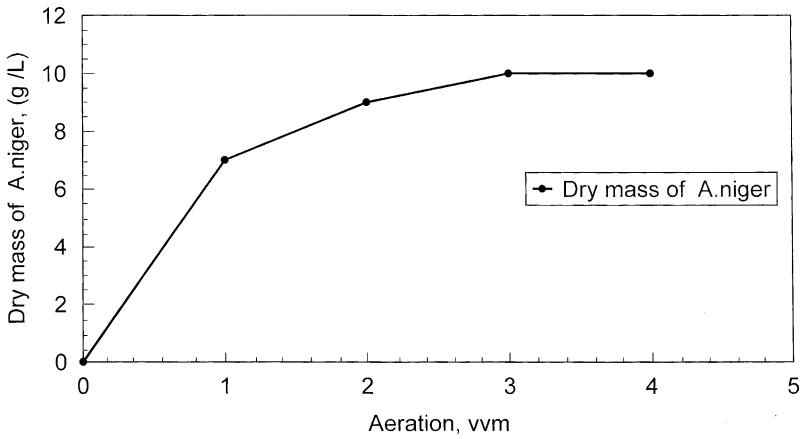


Fig. 11. Effect of aeration on dry mass of *A. niger*.

velocity for *A. niger* and *P. austurianum*, respectively. The effect of aeration velocity on the amount of dry mass of *A. niger* produced is depicted in Fig. 11. It can be observed by increasing the aeration velocity the dry weight of biomass of *A. niger* increases. Increasing aeration velocity more than 2 vvm decreases the biomass dry weight. It may be due to combined effect of evaporation of high food value medium and at the same time very rapid growth of *A. niger* at initial stages of exponential growth phase resulting in starvation at later stages. The entire experimental work concerning aeration was performed in semibatch mode, the air being sparged continuously. For spherical gas bubbles, the effective liquid interfacial area a is given by the following equation:

$$a = \frac{6\varepsilon_g}{d_B} \tag{7}$$

where a is used in calculation of $k_L a$. However, it depends on the procedure used in obtaining over all mass transfer coefficients. It was observed the initial hold-up values for each V_g was higher than the final hold-up under the same condition (results not shown). The increase of biomass production in the course of operation result in decreasing the hold-up values. The same result is observed in other multiphase reactors like slurry reactors [32–35].

3.8. Effect of initial cadmium concentration

Effect of different cadmium concentration 12, 26, 44 and 78 ppm on biosorption of *A. niger* and *P. austurianum* are depicted in Figs. 12 and 13, respectively. The cultivation temperature was maintained at about 30 ± 1 °C. The batch operation was carried out for more than 50 h and sample was collected at every 3 h interval for cadmium estimation.

3.9. Effect of optimized pH

By advancing the process, pH of the medium using *A. niger* decreased to nearly 2.5, and *P. austurianum* to 3.5, respectively. It may be because of production of citric and oxalic acids during the course of operation. As the pH of the medium decreased below 4 and 5 the uptake percentage

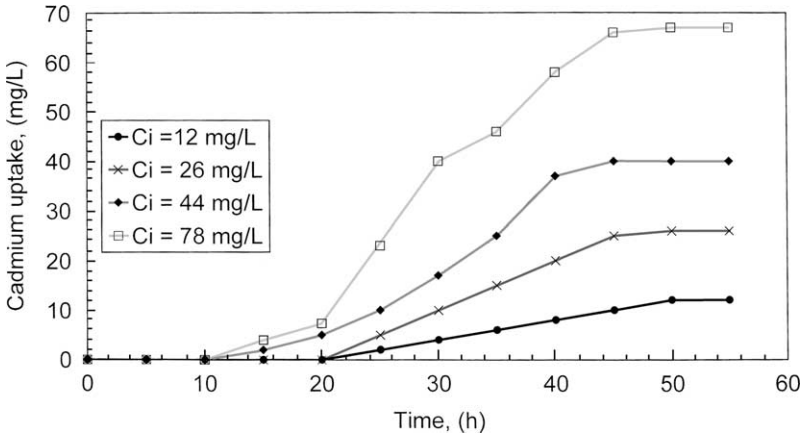


Fig. 12. Effect of initial concentration of cadmium on biosorption of *A. niger*.

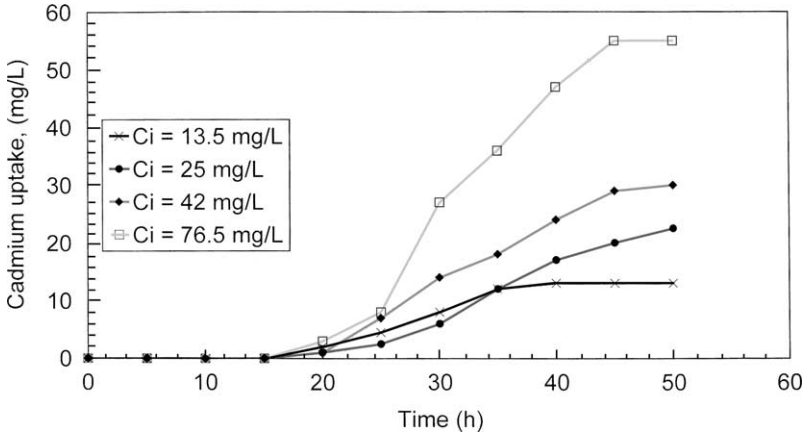


Fig. 13. Effect of Initial concentration of cadmium on biosorption of *P. austurianum*.

of cadmium also decreased. Therefore, further investigation was carried out to obtain the range of optimized pH values. And it was found to be in the range of 4 and 5 using *P. austurianum* and *A. niger*, respectively. The effect of controlled pH of 4 and 5 using *P. austurianum* and *A. niger* on biosorption of cadmium is depicted in Figs. 14 and 15. Reduction in pH decreases solubility of metals in the medium and in addition availability of electrical charge for biosorption may be reduced. It is exhibited in Fig. 15 by maintaining the process pH about 4 for *P. austurianum* the biosorption efficiency is increased, from 70 to 78%. Fig. 14 shows that at pH 5 using *A. niger* the uptake is increased from 83 to 97.8%. The results of the present investigation is on the parallel line as reported by Gadd [29].

3.10. Effect of process temperature

Fig. 16 shows that by increasing the process temperature more than $22 \pm 0.5\,^{\circ}C$, the percentage of cadmium removal is decreased. The biosorption of cadmium is achieved in two steps; initially some cadmium concentration is reduced due to diffusion of metal to the surface of active biomass in airlift fermenter. Further, adsorption is progressed because of metabolites activities, which is a slow process. At $22 \pm 0.5\,^{\circ}C$ cadmium uptake is about 97%. By increasing the process temperature further to $37\,^{\circ}C$, the biosorbed cadmium percentage decreases to 78 under the same conditions. The phenomena may be explained, as the surface diffusion is an exothermic type of reaction. Increasing the medium temperature decreases the cadmium percentage removal although the reaction rate increases according to Arrhenius equation. It is also possible that the temperature of the medium increases, denaturation also increases.

3.11. Effect of mixed cultures

Mixed cultures of 1:1 and 3:1 ratio of *A. niger* and *P. austurianum* were used to examine the biosorption of cadmium in the aforesaid airlift bioreactor. Using *P. austurianum* and *A. niger* 1:1 at their optimized pH values of 4 and 5 for the fungi, respectively. Macroscopic observation confirms that growth of *A. niger* is more rapid than the *P. austurianum* almost in both the pH. It can be seen that at pH 5 and 4 about 84 and 79% Cd removal is achieved. Though *A. niger* in

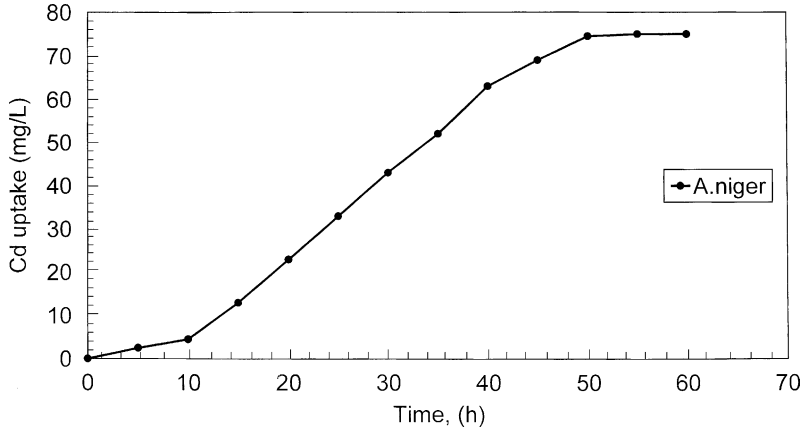


Fig. 14. Effect of optimized pH on biosorption of cadmium using *A. niger* (Ci = 78 mg/l, pH 5).

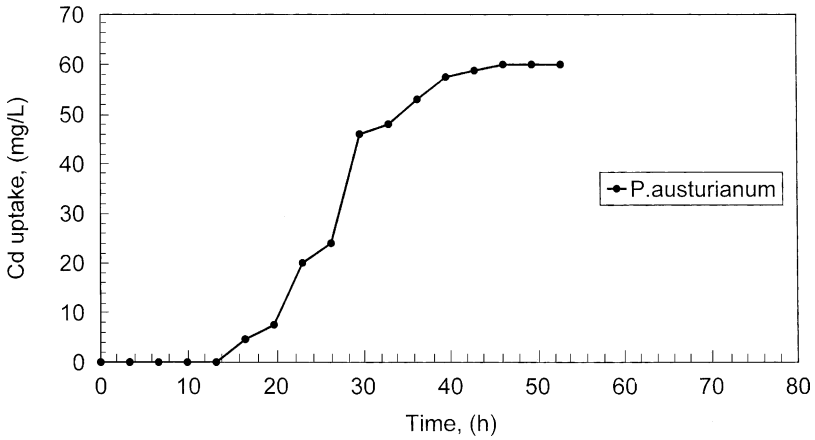


Fig. 15. Effect of optimized pH on biosorption of cadmium using *P. austurianum* (Ci = 76.5 mg/l, pH 4).

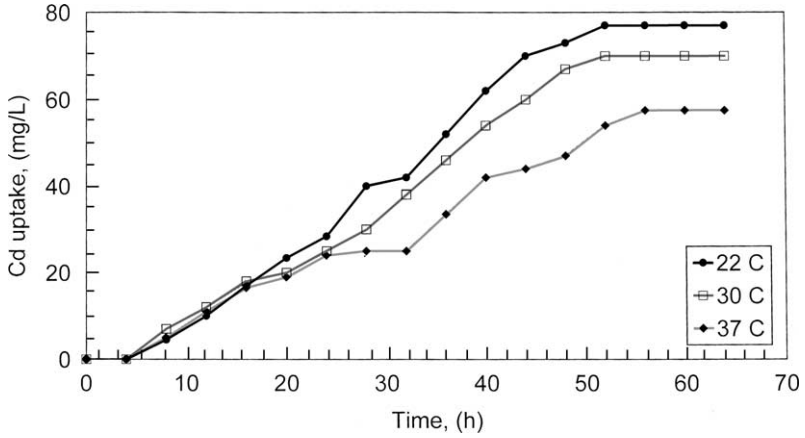


Fig. 16. Effect of temperature on biosorption on cadmium using *A. niger* (Ci = 78 mg/l, pH 5).

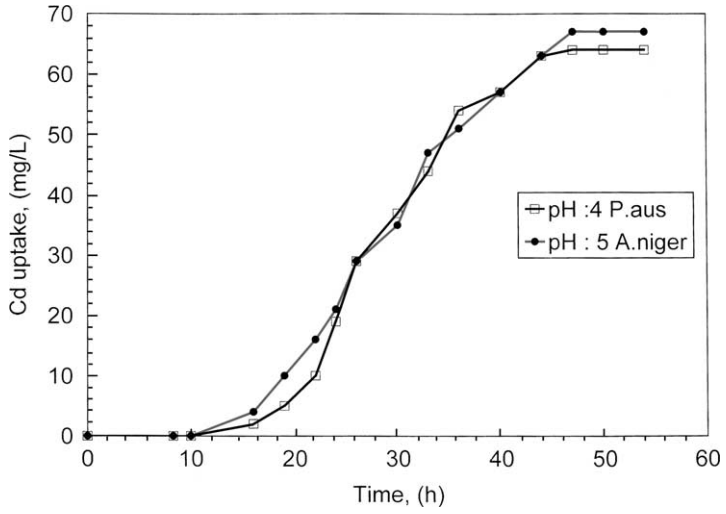


Fig. 17. Effect of mixed culture 1:1, *A. niger* and *P. austurianum* on cadmium biosorption (Ci = 78 mg/l, pH 4 and 5).

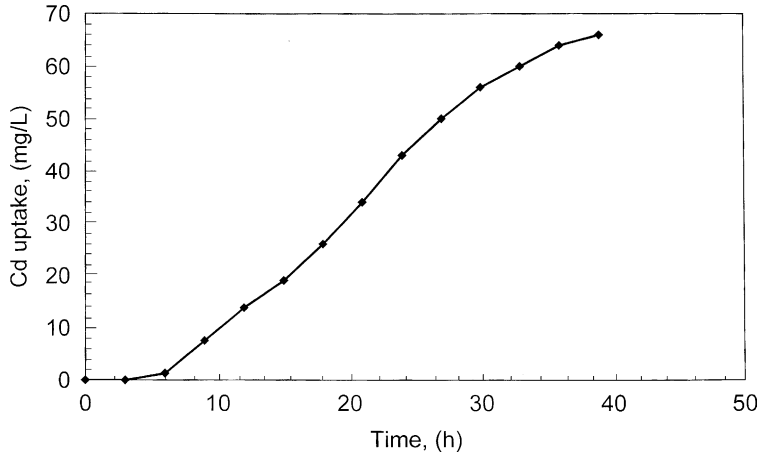


Fig. 18. Effect of mixed culture 3:1, *A. niger* and *P. austurianum* on cadmium biosorption (Ci = 78 mg/l, pH 5).

both the circumstance has predominant growth however at pH 5 shows reasonably higher cadmium removal. The result is depicted in Fig. 17. It can be seen that cadmium biosorption by *A. niger* declines by 4% under the same conditions at pH 4. The result of the present study is on the similar line with the findings of Huang et al. [15,36]. They showed that the reduction in metal-up take pattern with growth was because of drop in pH. The variation in the sorption capacity of fungi with pH was explained by Huang et al. [37] on the basis of proton-competitive adsorption reactions. Fig. 18 shows that using mixed cultures of 3:1 *A. niger* to *P. austurianum*, the biomass of *A. niger* in the medium increases and as a result the cadmium uptake increases to 89%, which shows 5% enhancement with respect to 1:1 ratio. It seems that combined effect of pH variation and production of different organic material by both of the fungi affect the cadmium biosorption uptake.

4. Discussion

The pH values of most of the industrial waste waters containing heavy metals are acidic [38]. The pH optima obtained in the present studies were in the range of 3.5–4 and 4.5–5 for active and inactive biomasses of *P. austurianum* and *A. niger*, respectively. The adsorption of cadmium by *P. austurianum* and *A. niger* are influenced by the cadmium solution pH indicating that the process was governed by an ion-exchange mechanism. The low level of metal adsorption at low pH can be attributed to the increased concentration of hydrogen (H^+) and hydronium (H_3O^+) ions competing for metal binding sites on the biomass [39]. The increase in the level of adsorption by biomasses with increasing pH could be due to combined effects of (i) less ionic competition (ii) and decrease in solubility of cadmium at higher pH values. Ionic size induces adsorption of hydrolyzed species and the biomass removes a greater quantity of the metal. By increasing the pH of the medium the concentration of the H_3O^+ ions decreases, therefore, sufficient monomeric sites of chitin/chitosan remains free and are occupied by ions of Cd^{2+}. The result of the present study is on the similar line with the findings of Fourest and Roux [40], Huang [15], and Volesky et al. [41].

Data exhibited in Fig. 3 shows that adsorption of cadmium by *P. austurianum* and *A. niger* is reasonably fast. A number of steps are involved in the adsorption mechanism which deal with the transfer of cadmium from solution to the biosorbent surface [42]. The first step, bulk transport of cadmium ions in the solution phase, is usually rapid because of mixing and direction of flow [39]. The second step, film transport, involves diffusion of the metal through a hydrodynamic boundary layer around the biosorbent surface. The third step, actual adsorption of metal ions by the active sites of the biomass is considered to be rapid, equivalent to an equilibrium reaction [43]. Cadmium biosorption by *A. niger* and *P. austurianum* a good mixing of solute and biosorbents in the system suppresses the kinetic limitation due to first step and enhances the kinetics appear to be more effectively influenced by the transfer of metal from solution to active sites on the biosorbent surface. It has been before reported that the biosorption needs a few min to reach 90% of the total metal adsorption. Fourest and Roux [40] reported that in 20 min more than 90% zinc and nickel was taken up by *R. arrhizus* at pH 5–6 and 6.5–7, respectively. In the present study in some cases more than 90% uptake of cadmium by *A. niger* and was achieved in 4 min. These findings are supported by the plots of Eq. (5). In addition, as the plots obtained are linear ($r^2 = 0.98$)] the rate constant for metal adsorption (K_r) is more or less independent of the surface coverage [26]. These data can be employed for scaling up the process and the space time for *A. niger* and *P. austurianum* could be adjusted to 4 and 15 min, respectively.

Equilibrium sorption isotherm examinations exhibited that metal adsorption by *A. niger* and *P. austurianum* is a chemically equilibrated and securable mechanism [44].

Thus, sorption increased with the initial metal concentration as long as binding sites are free. The linear transformation of the adsorption data using Freundlich and Langmuir models allowed computation of the metal adsorption capacities ($r^2 \geq 0.98$ for respective models). Equal conformity of these data to the Freundlich and Langmuir adsorption models indicated that the biosorption of cadmium in the present study could be characterized as a monolayer, single site type phenomenon with no interaction between sorbed metals and the heterogeneous surface [25,42].

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