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Biosorption of Cu (II) onto chemically modified waste mycelium of *Aspergillus awamori*: Equilibrium, kinetics and modeling studies

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ABSTRACT

The biosorption potential of chemically modified waste mycelium of industrial xylanase-producing strain *Aspergillus awamori* for Cu (II) removal from aqueous solutions was evaluated. The influence of pH, contact time and initial Cu (II) concentration on the removal efficiency was evaluated. Maximum biosorption capacity was reached by sodium hydroxide treated waste fungal mycelium at pH 5.0. The Langmuir adsorption equation matched very well the adsorption equilibrium data in the studied conditions. The process kinetic followed the pseudo-firs order model.

Key words: *Aspergillus awamori*, biomass modification, biosorption, Cu (II) removal, waste fungal mycelium

Introduction

For their toxicity and possibilities for bioaccumulation by food chain, environmental pollution with heavy metals became probably the major ecological problem of the modern society. One of such heavy metal is Cu (II), thought it is essential to higher life, and is toxic at higher concentrations (ECOTOX, 2005). The excessive intake of Cu (II) by man leads to serious health problems such as severe mucosal irritation, capillary, hepatic, renal and central nervous damages (Kalavathy et al., 2005).

Traditional physical and chemical methods for removing dissolved heavy metal ions from waste waters have significant disadvantages, because of incomplete metal removal, expensive equipment and potential risk of the generation of hazardous by-products (Volesky, 1990; Veglio & Beolchini, 1997; Tsezos M, 2001). The biosorption of metallic ions by non-living microbial biomass offers an alternative to the bioremediation of industrial effluents as well as the recovery of metals contained in other media. Many microorganisms such as sulfate-reducing bacteria (White & Gadd, 2000), actinomycetes (Kujan et al., 2005), fungi (Kapoor & Viranghavan, 1997; Tzekova et al., 2000; Bhainsa & D'Souza, 2008), yeasts (Huang et al., 1990; Wang & Chen, 2006), algae (Kratochvil & Volesky, 1998), wood rotting *Basidiomycetes* (Gabriel et al., 2001), agricultural wastes (Basci et al., 2004; Aksu & Isoglu, 2005), crab and shell biomass (Dahiya et al., 2008) were studied as prospective Cu (II) biosorbents. Searching for low cost and inexpensive microbial biosorbents for Cu (II) removal focused attention on waste fungal mycelium. Unfortunately Cu (II) biosorption studies were not carried out with waste fungal biomasses, but usually with especially cultivated fungal strains which then were thermally killed. Filamentous fungi belonging to genera *Aspergillus*, *Penicillium* and *Rhizopus* are intensively used in fermentation industries for

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RESEARCH ARTICLE

producing enzymes, antibiotics and other bioproducts and large amounts of waste fungal mycelium are produced annually. Only Fourest & Roux (1992) and Gulati et al. (2002) studied biosorption of Cu, Ni, Zn, Cd and Pb by waste fungal mycelium of *Aspergillus terreus* and *Rhizopus arrhizus*, obtained as by-products from industrial fermentation processes.

The aim of present study was to evaluate for the first time the biosorption potential of waste mycelium of industrial xylanase producing strain *Aspergillus awamori* for Cu (II) removal from aqueous solutions and optimization of process parameters to reach effective heavy metal removal.

Materials and Methods

Preparation of biosorbent

Waste mycelium of the industrial strain *A. awamori* was harvested by filtration at the end of the fermentation process for production of a complex enzyme preparation with a leading xylanase activity (Ilieva et al., 2002). The waste mycelium was killed by autoclaving at 121°C for 20 min, washed thoroughly with deionized water and dried in an oven at 80°C for 10 h. Then it was powdered to particles of uniform size of about 100 μm. This powdered biomass was used in further biosorption experiments as control sample.

Biosorbent treatment procedures

Formaldehyde treatment was carried out as described by Kapoor & Viranghavan (1997): 1 g of powdered biosorbent was treated with 20 ml of formaldehyde and 40 ml of formic acid. The mixture was agitated on a rotary shaker for 5 h at 150 rpm.

Ethanol treatment was carried out as described by Drake et al. (1996): 1g of powdered biosorbent was suspended in 65 ml of ethanol and 0.6 ml of concentrated hydrochloric acid. The mixture was shaken for 5 h at 150 rpm.

Triethyl phosphate treatment was carried out as described by Fu & Viraraghavan (2002): 1 g of powdered biosorbent was heated in a mixture of 40 ml triethyl phosphite and 30 mL nitromethane under reflux conditions for 6 h.

Pyridine and acetic anhydride treatment was carried out as described by Tzekova et al. (2006): 1 g of powdered biosorbent was suspended in 50 mL mixture of pyridine and acetic anhydride (12:88 ratio) and heated for 2 h at 60ºC.

Sodium hydroxide treatment was carried out by suspending 1 g of powdered biosorbent in 20 ml 0.5 M sodium hydroxide and boiled for 15 min.

Dimethyl sulfoxide (DMSO) treatment was carried out by suspending 1 g of powdered biosorbent in 20 ml 50% (v/v) DMSO solution and boiled for 15 min.

On the end of each procedure the treated biosorbent was separated by filtration through filter paper Whatmann No.1, washed twice with deionized water and dried at 80ºC for 8 h.

Preparation of Cu (II) solution and analysis of Cu (II) concentration

A stock solution (1000 mg/L) of Cu (II) was prepared by dissolving the adequate amount of $CuSO_4.5H_2O$ (Merck, Darmstadt, Germany) in deionized water. For metal biosorption experiments Cu (II) solutions of different concentrations (25, 50, 75 and 100 mg/L) were prepared by adequate dilution of the stock solution in deionized water.

The Cu (II) concentration in the solution before and after biosorption was measured by direct titration method with EDTA and Fast Sulphon Black F as an indicator (Jeffery et al., 1989).

Biosorption studies

In order to evaluate the effect of pH, initial Cu (II) concentration, and contact time series of biosorbtion experiments were carried out in a batch system. The pH of metal solution was adjusted to values between 2.0 and 6.0 using 0.1M HCl or 0.1M NaOH. Biosorption of Cu (II) was carried out in 250 ml Erlenmeyer flasks by adding 0.1 g biosorbent to 100 mL metal solution of desired pH value at 20°C, on a rotary shaker 150 rpm for 180 min. On the end of the biosorption process biosorbent was separated from the solution by filtration and residual Cu (II) concentration was measured as listed above.

All experiments were carried out in triplicate. For all graphical representation, the mean values of three independent experiments were considered and standard deviations within triplicate were too small to be plotted as error bars (<1%).

Determination of Cu (II) uptake and removal efficiency

The Cu (II) uptake at equilibrium was calculated by the simple difference method (Volesky, 1990):

$$q = \frac{(C_i - C_f)V}{W} \qquad (1)$$

where **q** is the Cu (II) uptake (mg/g); **V** is the volume of the solution in the flask (L); $\mathbf{C_i}$ and $\mathbf{C_f}$ - initial and final concentration of Cu (II) in the solution (mg/L); **W** is the mass of biosorbent (g).

DOI: 10.69085/jbb20122163

RESEARCH ARTICLE

The removal efficiency is calculated as:

$$R = \frac{(c_i - c_f)}{c_i}.100, \quad \% \qquad (2)$$

where: $\mathbf{c_i}$ and $\mathbf{c_f}$ denote respectively initial concentration of Cu (II) and final residual concentration of Cu (II) at the moment **t**, in mg/L.

Biosorption equilibrium modeling

The biosorption of Cu (II) onto chemically modified waste mycelium of *A. awamori* was characterized using Langmuir and Freundlich isotherms (Li et al., 2009).

The Langmuir model corresponds to the following equation:

$$q_e = q_m \frac{bC_e}{1 + bC_e} \qquad (3)$$

where $\mathbf{C_e}$ - metal concentration in solution at equilibrium (mg/L); $\mathbf{q_m}$ – maximum amount of metal adsorbed per unit of dry biomass (mg/g); **b** - the binding stability constant (l/mg). The Freundlich equation is of the form:

$$q_e = KC^{1/n} \qquad (4)$$

where **K** and **1/n** are Freundlich adsorbent constant and exponent characterizing the system. For fitting the experimental date, the Langmuir model was linearized as follows:

$$\frac{1}{q_e} = \frac{1}{q_m b}.\frac{1}{C_e} + \frac{1}{q_m} \qquad (5)$$

The Freundlich isotherme was also linearized as follow:

$$\lg q_e = \lg K + \frac{1}{n}\lg C_e \qquad (6)$$

Kinetic studies

In order to analyse kinetic of Cu (II) biosorption onto chemically modified waste mycelium of *A. awamori*, pseudo-first order (Ho & McKay, 1999), pseudo-second order (Chiou & Li, 2002; Sag & Aktay, 2002) and Morris-Weber kinetic models (Weber & Morris, 1963) were applied.

The pseudo-first order equation of Lagergren was expressed as follows:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \qquad (7)$$

where $\boldsymbol{q_e}$ and $\boldsymbol{q_t}$ are the adsorption capacity at equilibrium and time ***t***, respectively (mg/g), $\mathbf{k_1}$ is the rate constant of pseudo first-order adsorption (l/min).

The pseudo-second order equation was expressed as follows:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \qquad (8)$$

where $\mathbf{k_2}$ is the rate constant of pseudo-second order adsorption (g/mg.min).

The Morris-Weber equation was expressed as follows:

$$q_e = K_d.t^{1/2} \qquad (9)$$

where; $\mathbf{q_e}$ is the concentration of sorbed ion (mmol/g) at time **t**, and $\mathbf{K_d}$ is the rate constant for intra-particle transport (mg/g.min$^{1/2}$).

Results and Discussion

Biosorption is defined as the property of different biomaterials to accumulate metal ions by adsorption on the cell surface. The metal uptake capacity of biosorbents, in particular fungal mycelium, is a function of surface chemistry of the microbial cells. The major constituents of fungal cell wall are carbohydrates such as chitin and chitosan, polyuronide and polyphosphates, lipids and proteins (Siegel et al., 1990). These compounds served as donors of hydroxyl, carboxyl, amino and phosphate groups, which act as metal binding active sites of the biosorbents. To elucidate the role of above mentioned groups in Cu (II) removal process and to enhance the removal efficiency, biosorbent was modified by chemical pretreatment with formaldehyde, triethyl phosphate, pyridine and acetic anhydride, sodium hydroxide and DMSO. The results obtained are shown in Figure 1. As seen formaldehyde, ethanol, triethyl phosphate, pyridine and acetic anhydride treatments decreased Cu (II) uptake by 16.17%, 41.50%, 18.20% and 38.22%, respectively. Sodium hydroxide and DMSO treatments increased Cu (II) uptake by 48.20% and 20.05%, respectively.

The formaldehyde treatment of biosorbent caused methylation of amino groups on the cell walls and in this way prevented their participation in the metal binding process. Ethanol treatment of biosorbent caused esterification of carboxyl groups and demonstrated the strongest inhibitory effect on the biosorbtion in comparison with other treatment procedures. Treating the biosorbent with a mixture of triethyl phosphate and nitromethane caused esterification of phosphates and also demonstrated negative effect on the

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RESEARCH ARTICLE

process' effectiveness, bit it was less pronounced in comparison with esterification of carboxyl groups. Esterification of hydroxyl groups by pyridine and acetic anhydride treatment caused reduction of metal removal capacity of biosorbent, which was comparable with those caused by esterification of carboxyl groups.

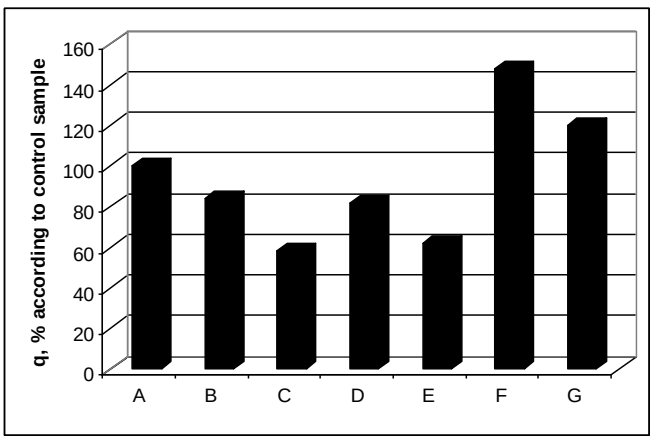

Figure 1. *Cu (II) bisorption by chemically modified biosorbent of A. awamori. V = 100 ml; W = 1 g/L; t = 180 min; c_0 = 50 mg/L; t = 20^{0}C;* ***A*** *- control heat inactivated sample,* ***B*** *- formaldehyde treatment,* ***C*** *- ethanol treatment,* ***D*** *- triethyl phosphate treatment,* ***E*** *- pyridine and acetic anhydride treatment ,* ***F*** *- sodium hydroxide treatment,* ***G*** *- DMSO treatment.*

Based on the obtained results it could be assumed that carboxyl groups played a key role in Cu (II) removal from aqueous solutions, followed by hydroxyl groups, phosphate and amino groups. Carboxyl and hydroxyl groups are negatively charged and probably the electrostatic attraction was the major mechanism involved in Cu (II) biosorption at the studied conditions.

The positive effect of sodium hydroxide treatment may be due to removal of lipids, proteins and other impurities on the cell surface, which made active binding sites inaccessible for Cu (II) biosorption. According to Huang et al. (1990) the increased Cu (II) biosorption capacity of sodium hydroxide treated biosorbents may be explained by the removal of proteins, which make non-adsorbable protein – metal complexes with Cu (II) ions. Of course synergism of both processes can not be excluded. Other authors (Huang et al., 1990; Lu & Wilkins, 1996; Göksungur et al. 2005) also reached enhancement of metal removal capacity of alkali treated biosorbents, which is in accordance with our results.

Extraction of lipids with DMSO also increased Cu (II) removal potential of fungal biosorbent, but the positive effect was weaker in comparison with effect caused by sodium hydroxide treatment. All further examinations were carried out with biosorbent treated with sodium hydroxide.

pH is probably the major factor influencing metal biosorbtion process (Volesky, 1990; Esposito et al., 2003). On the one hand the dissociation of active binding sites on the biosorption surface, respectively electrostatic attraction between metal ions and binding sites, depends on the pH. On the other hand processes like precipitation of heavy metals and complexation also strongly depends on pH. The effect of initial pH on the Cu (II) removal by sodium hydroxide treated waste mycelium of *A. awamori* was evaluated ranging from pH 2.0 to pH 4.0. The results obtained are shown in Figure 2.

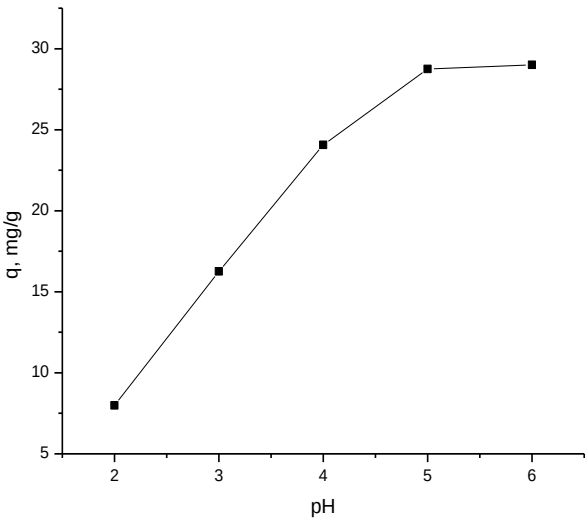

Figure 2. *Effect of pH on Cu (II) biosorption onto sodium hydroxide treated biosorbent of A. awamori. V = 100 mL; W=1g/L; t = 180 min; c_0 = 50 mg/L; t = 20^{0}C.*

As seen maximum Cu (II) uptake 28.75 mg/g with removal efficiency 57.50% was reached at pH 5.0. At pH values higher than 6.0 Cu (II) ions precipitated because of the high concentrations of hydroxyl anions in the biosorption solution. The fungal biomass consists mainly of chitin, chitosan, proteins and other compounds, which served as donors of carboxyl and amino groups (Siegel et al., 1990; Gochev et al., 2010), which act as binding sites for Cu (II) ions. The increasing of pH caused deprotonation of these groups and thus formed negatively charged sites for electrostatic attraction of positively charged metal ions.

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RESEARCH ARTICLE

For optimization of process parameters and enhancement of Cu (II) removal the effect of contact time at different initial metal concentrations were studied. The results obtained are shown in Figure 3.

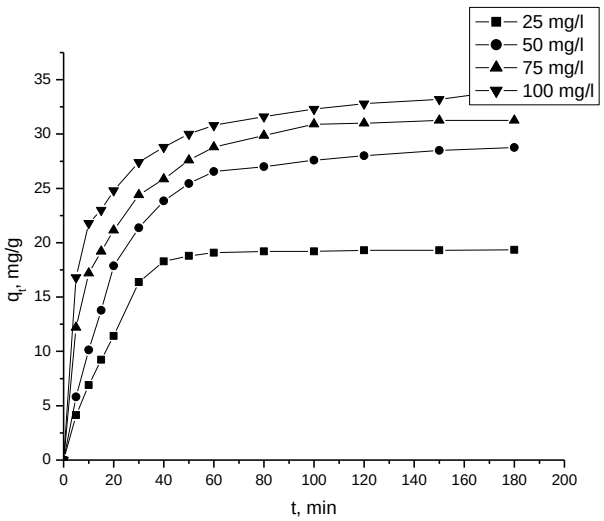

Figure 3. *Effect of contact time on Cu (II) biosorption onto sodium hydroxide treated biosorbent of A. awamori. V = 100 mL; W = 1g/L; t = 180 min; t = 20^{0}C.*

As seen at all of the studied initial Cu (II) concentrations biosorption process can be divided into two phases. The first rapid phase passed during the first 60 min, followed by second slower phase until the equilibrium was reached at 180 min. The rapid phase is due to the availability of enough free active metal binding sites on the biosorbent surface. Later during the second phase the number of free binding sites decreased and as a result of competition among Cu (II) the biosorption rate and effectiveness decreased.

In order to investigate the mechanism of sorption, the rate constants of biosorption process were determined by using Legergren first order and a pseudo-second order kinetic models. The values of the first and second order rate constants are listed in Table 1.

The results obtained indicated that the adsorption of Cu (II) onto sodium hydroxide modified waste mycelium of *A. awamori* followed the pseudo-first order kinetic model.

The kinetics of Cu (II) biosorption onto fungal mycelium was also studied by Morris-Weber kinetic model. According to this model, a plot of q_t versus $t^{1/2}$ could predict the sorption mechanism of Cu (II) ions. If the intraparticle diffusion is the sole rate determining step, the plot of q_t vs. $t^{1/2}$ should be linear. If the data exhibit multi-linear plots, two or more steps influenced the absorption processes.

Table 1. *First order (k_1) and second order (k_2) rate constants for Cu (II) biosorption by sodium hydroxide treated biosorbent of A. awamori.*

c_0, mg/l	k_1, min^{-1}	R^2	k_2, g/mg.min	R^2
25	$7{,}74.10^{-2}$	0,9952	$5{,}31.10^{-4}$	0,9747
50	$4{,}27.10^{-2}$	0,9989	$8{,}75.10^{-4}$	0,9978
75	$3{,}49.10^{-2}$	0,9976	$3{,}46.10^{-3}$	0,9987
100	$2{,}83.10^{-2}$	0,9920	$4{,}86.10^{-3}$	0,9921

As seen (Figure 4) the data points were related by two or three straight lines, indicating three different kinetic mechanisms. The initial curved region corresponds to the external surface uptake, the second stage relates the gradual uptake reflecting intraparticle diffusion as the rate liming step and final plateau region indicates equilibrium uptake.

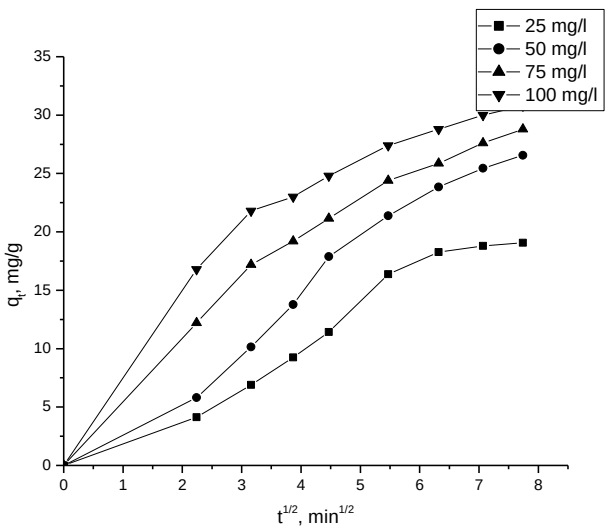

Figure 4. *Weber–Morris plots for biosorption of Cu (II) onto sodium hydroxide treated biosorbent of A. awamori. V = 100 mL; W = 1g/L; t = 180 min; t = 20^{0}C.*

The application of the biosorption technique in commercial scale requires proper quantification of the sorption equilibrium for process simulation. For this purpose the Langmuir and Freundlich isotherm models are usually used (Abdelwahab, 2007; Li et al., 2009). The linear plots of Langmuir and Freundlich isotherms are shown in Figure 5 and Figure 6, respectively.

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RESEARCH ARTICLE

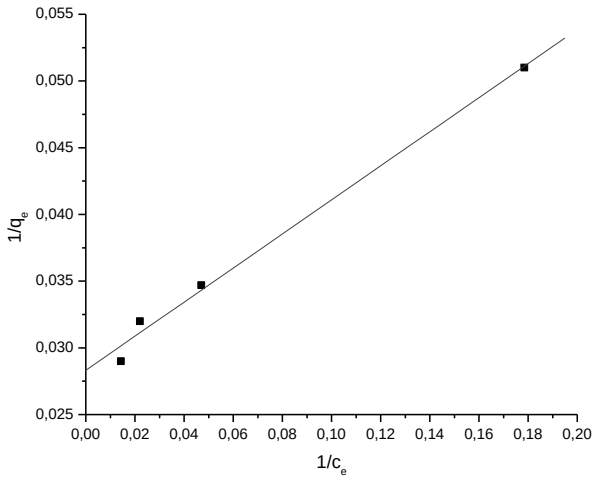

Figure 5. *Linear plot of Langmuir isotherm for biosorption of Cu (II) onto sodium hydroxide treated biosorbent of A. awamori.*

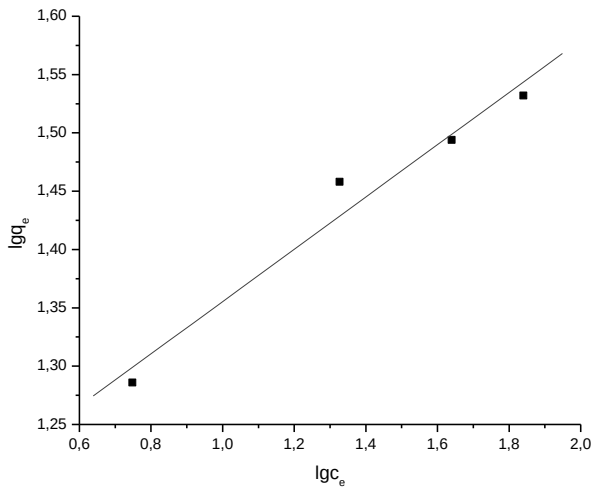

Figure 6. *Linear plot of Freundlich isotherm for biosorption of Cu (II) onto sodium hydroxide treated biosorbent of A. awamori.*

The parameters of Langmuir and Freundlich adsorption isotherms, evaluated from the linear plots and the correlation coefficients are listed in Table 2.

The values of correlation coefficients for both models indicated that the studied process fitted better to the Langmuir equation, which means that the biosorption of Cu (II) onto chemically modified waste mycelium of *A. awamori* was a monolayer onto a surface containing finite number of identical sites.

Table 2. *First order (k_1) and second order (k_2) rate constants for Cu (II) biosorption by sodium hydroxide treated biosorbent of A. awamori.*

Model	Constants	R
Langmuir	q_{max} = 35.97 mg/g b = 0.136 l/mg	0.9966
Freundlich	K_F = 13.31 1/n = 0.231 n = 4.32	0.9852

The Cu (II) biosorption capacity of sodium hydroxide modified waste mycelium of *A. awamori* was relatively higher in comparison with other fungal biosorbents such as *A. niger*, *A. terresus* and *A. flavus* (Akar & Tunali, 2006; Mukhopadhyay et al., 2007).

Conclusion

Biosorption capacity of *A. awamori* waste fungal mycelium for Cu (II) removal from aqueous solutions was enhanced by chemical treatment with 0.5 M sodium hydroxide. Carboxyl and hydroxyl groups played a key role as Cu (II) binding sites. The maximum metal uptake was reached at pH 5.0 and the kinetic of Cu (II) biosorption onto chemically modified waste mycelium of *A. awamori* followed the pseudo-first order model. The experimental data at studied conditions fitted well to the Langmuir isotherm model and maximum Cu (II) uptake 35.97 mg/g was calculated.

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RESEARCH ARTICLE

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