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Original Research

Fungal Biomass for Cu(II) Uptake from Aqueous Systems

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Abstract

At present, heavy metals pollution is becoming a more and more serious problem for the environment and public health. Removal of heavy metals and metalloids from aqueous solutions is generally achieved through several processes such as chemical precipitation/neutralization, solvent extraction, ion exchange, membrane separation, reverse osmosis, or adsorption. These processes are efficient in removing the bulk of metals from solution at high or moderate concentrations. For lower metal concentrations, and in case of pollution with nanoparticles, these processes are expensive, and they are replaced by alternative methods (such as biosorption) that use different natural materials of biological origin. The objective of the present study is to investigate the use of fungal biomass as the biosorbent for the removal of Cu(II) from an aqueous solution that contains copper sulfide nanoparticles. The capacity to retain Cu(II) from aqueous suspension used is determined and compared for the following fungal strains: *Aspergillus oryzae*, *Aspergillus niger*, *Fusarium oxysporum*, and *Polyporus squamosus*. The results obtained show that the specific copper uptake varies between 1.66 mg/g and 7.52 mg/g. The maximum specific copper uptake value (7.52 mg/g) was obtained for the fungus strain *Fusarium oxisporum* MUCL 791. Analysis of the IR spectra revealed that the mechanism of the copper uptake by fungal biomass involves interactions between copper ions and hydroxyl, amino, carboxyl, and carbonyl groups from fungal biomass surface depending on the types of fungal strain. Desorption studies showed that copper recovery efficiency varied between 82 and 86% when acidic solutions were used as eluents.

Keywords: copper removal, fungal strain, bioleaching, biosorption

Introduction

Heavy metal pollution represents a major problem, with a major impact on the environment and life itself [1-3]. Many wastes and wastewaters contain heavy metals. They result from numerous industries such as: energy and fuel production, electroplating, metal surface treating, surface finishing industry, fertilizer and pesticide industry, electrolysis, leather industry, photography, electro-osmosis, aerospace, atomic energy installation, mining, and smelting. Wastewaters of these industries contain metals classified as: toxic (Cr, Hg, Zn, Pb, Sn, Ni, Cd, As, Cu, Co), precious (Ru, Pd, Au, Pt, Ag), and radionuclides (Am, Ra, U, Th) [4, 5]. Wastes with heavy metal content

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are discarded directly or indirectly in the environment, causing important environmental problems and negative effects on organisms, they and are becoming less and less exhaustible.

The main characteristics of heavy metals toxicity are the following: they present toxicity over a long period of time, there are some heavy metals that can be transformed into more toxic forms in the environments (such as Hg), bioaccumulation and the bioconcentration of heavy metals hase negative effects on live organisms, heavy metals can be transformed from one species into another by oxidation state changing, and they are not degradable using conventional methods such as biotreatment. The toxicity of heavy metals can occur even at low concentrations (1-10 mg/L) (for example Hg and Cd are toxic at concentrations lower than 0.001-0.1 mg/L).

The small size and large surface areas of nanoparticles increase their potential to cross cell membranes. Because they have similar dimensions with cellular components (e.g., ribosome), they may allosterically interact with protein and nucleic acids, determining the destruction of vital processes (i.e. gene translation and transcription, enzyme function). Their large surface area increases the chemical reactivity of the nanoparticles, consequently they could act as carriers for other contaminants. All these properties and characteristics of nanomaterials determine risks for environment and health.

Copper has many applications due to its important physical and mechanical properties, such as good corrosion resistance, and electrical and thermal conductivity. Consequently, large volumes of wastewaters and wastes that contain copper result from industry. The potential sources of copper in industrial effluents include electrical equipment, pulp and paper mills, the fertilizer industry, metal cleaning and plating baths, wood pulp production, primer paints, and fungicides [6]. In these wastewaters copper can be found in dissolved state or in the form of nanoparticles of its compounds [7].

Although copper is an essential nutrient to humans and other life forms, it is known to be one of the heavy metals that is most toxic to living organisms. Therefore, copper must be removed from wastewaters before being discharged to the environment.

Chemical precipitation, ion exchange, electrochemical treatment, membrane technologies, reverse osmosis, and sorption are methods that can be applied to remove copper from wastewaters. All these removal methods have advantages and disadvantages. The main disadvantages of these methods are: they produce large amounts of sludge that need to be disposed, they can be expensive, and they can only lead to incomplete removal of copper [8].

Sorption is one of the most commonly used methods for copper or other heavy metals removal from wastewaters. Depending on the materials used in sorption processes, there are: adsorption on inorganic materials such as ferric oxide and silica [9], titania [10], alumina [11], selenium nanoparticles [12], activated carbon [13], activated carbon from *Eucalyptus camaldulensis Dehn.* bark [14], and other materials such as kaolin [15], medicinal stones [16], clays [17], zeolites [18], sand [19], thermal power plant ash [20], biosorption [21-28], and chemiosorption [29].

Biosorption is a new and less expensive method for removal of heavy metals such as copper(II) from wastewaters, even in dilute solutions [30]. Among biosorbents, fungal biomass has been recognized for the removal of heavy metals and radionuclides from polluted environments [31]. This is due to the fact that fungal cell walls mainly consist of polysaccharides, proteins, and lipids, with many functional groups that can be used in metals bonding. They also often exhibit marked tolerance toward metals and other adverse conditions such as low pH.

Several fungal biosorbents such as *Trametes versicolor* [32], *Aspergillus niger* [31, 33], *Pleurotus ostreatus* (a macro-fungus) [34], *Mucor indicus* [35], *Penicillium* sp. [36], *Pleurotus pulmonarius* [37], *Schizophyllum commune* [38], *Rhizopus arrhizus* [38], *Aspergillus flavus* [39], *Aspergillus versicolor* [40], *Pycnoporus sanguineus* (a white-rot fungus) [41], *Fusarium* sp. [42], *Cladosporium cladosporioides* [43], *Gliomastix murorum* [43], and *Bjerkandera* [43] were used in copper removal from wastewater.

The main objective of our study was to compare the removal capacity of five different fungal strains in copper removal from wastewaters. These selected fungal strains are *Aspergillus oryzae*, *Aspergillus niger*, *Fusarium oxysporum*, and *Polyporus squamosus*, and copper was used in the form of copper sulfide nanoparticles.

Experimental Procedures

The fungal strains were propagated on 39 g potato dextrose agar (PDA)/l and 0.1 g yeast extract/L for 5-7 days at 30ºC. Copper(II) uptake studies were carried out in liquid minimal medium containing 30 g/L dextrose, 10 g/L peptone, 0.4 g/L KH_2PO_4, 0.2 g/L KH_2PO_4, and 0.2 g/L $MgSO_4 \cdot 7H_2O$. pH of the growth medium was 6.24. After inoculation, flasks were incubated at 150 rpm for 8 days at 30ºC using a Thermoshake Incubating Orbital Shaker from Gerhardt GmbH & Co., Germany.

In this study copper was used in the form of copper sulfide nanoparticles. These nanoparticles were previously obtained and characterized [44-46]. CuS nanoparticles used have an average diameter of about 20-30 nm [46].

All the chemicals used were of analytical grade from Merck, Germany.

The copper content in the final solution was determined after the filtration of biomass with the use of atomic absorption spectroscopy (AAS) (AAS Multi-Element with Continuum Source ContraAA 700 from Analytik Jena AG Company, Germany).

IR Spectrum of dried biomass (at 70ºC for 8 hours) that grew in the absence and presence of copper was recorded in the 400-4000 cm^{-1} area with an FT-IR 620 Spectrometer (Jasco, Japan).

Table 1. Quantity (g) of biomass obtained without and with copper in growth liquid medium.

Fungus strain	Initial copper concentration (mg/L)				
	0	25	50	75	100
Aspergillus oryzae ATCC 11489	13.18	9.96	10.57	11.53	14.25
Aspergillus oryzae ATCC 20423	27.99	25.53	21.87	18.71	14.20
Aspergillus niger ATCC 15475	20.96	17.28	17.02	16.57	15.40
Fusarium oxisporum MUCL 791	25.04	20.10	15.24	12.75	11.45
Polyporus squamosus	23.07	26.21	23.46	20.28	17.83

Copper Uptake

The comparative bioleaching and bioaccumulation of copper ions by five fungal strains was investigated as a function of initial copper concentration and biomass growth. The results are expressed as units of dried cell mass (X_m: g/L), bioaccumulated metal ion concentration at the end of growth (C_{acc}: mg/L), specific copper uptake as a function of copper ions per unit of dry weight (q_m: mg/g), and the percentage uptake efficiency. This parameter (percentage uptake efficiency) is defined as the ratio of bioaccumulated copper ion concentration at the end of the growth period to the initial copper concentration.

Optimal pH for Copper Uptake by Fungal Biomass

Fungal strains were inoculated into 100 mL solutions containing 25 mg Cu(II)/L with an initial pH of 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, and 7.5 in order to determine the optimal pH for heavy metal removal. The medium pH was varied using HCl 1M and NH_4OH (25%, w/w) solutions, and it was determined using type C830, a multiparameter analyzer. The mixtures were shaken at 150 rpm until reaching equilibrium.

Optimal Temperature for Copper Uptake by Fungal Biomass

Fungal strains were inoculated into 100 mL solutions containing 25 mg Cu(II)/L with an initial temperature of 20, 25, 30, 35, and 40ºC in order to determine the optimal temperature for heavy metal removal. The mixtures were shaken at 150 rpm until reaching equilibrium.

Desorption Studies

At the end of the biosorption process, 100 mL HNO_3 0.05M solution was added to the fungal biomass loaded with copper ions in order to eliminate copper ions retained by the fungal biomass. The samples were stirred in a Thermoshake Incubating Orbital Shaker at 30ºC for 24 hours. The mixture was filtered to separate the biomass and the filtrate was used to determine the concentration of copper ions desorbed.

Results and Discussion

Copper Uptake

Aspergillus oryzae, *Aspergillus niger*, and *Fusarium oxisporum* are filamentous fungi which are ubiquitous in natural environments and widely used in industrial processes. *Polyporus squamosus* is a basidiomycete bracket fungus edible when it is young. This fungus is known to grow rapidly in liquid culture medium.

Comparative studies for the determination of copper uptake by five fungus strains were performed in batch systems using suspensions with the initial copper ion concentrations varying between 25 and 100 mg/L. Copper was used in the form of copper sulfide (CuS) nanoparticles. An inverse correlation between copper concentration and biomass growth was observed (Table 1). From this table it can be seen that by increasing the copper ion concentration, the final biomass is reduced substantially, excepting the case of fungus strain *Polyporus squamosus*. The quantity of biomass obtained for this fungal strain increases with the concentration of copper ions, reaching a level of 23.46 g. For higher copper concentrations in suspensions it was observed that the quantity of *Polyporus squamosus* biomass grown is lower than in the absence of copper in the liquid culture medium.

Copper uptake yields and specific copper uptake (q_m) are compared in Table 2. Specific copper uptake as a function of copper ions per unit of dry weight (q_m: mg/g) was calculated using the following equation:

$$q_m = \frac{(C_i - C_e) \cdot V}{m} \quad (1)$$

where:

q_m – copper uptake per unit of dry biomass (mg/g)
C_i – initial copper concentration (mg/L)
C_e – equilibrium copper concentration (mg/L)
V – suspension volume (L)
m – quantity of dry biomass (g)

Uptake efficiency was calculated from the amount of copper ions absorbed on the fungus strain and the amount of copper ions available in the synthetic solution used, as per the following equation:

Table 2. Removal of copper by fungal strains, incubated for 8 days at 30ºC with shaking at 150 rpm.

Fungus strain	Initial copper concentration	X_m	C_{acc}	q_m	Uptake efficiency
	(mg/L)	(g/L)	(mg/L)	(mg/g)	(%)
Aspergillus oryzae ATCC 11489	25	9.87	17.91	1.81	71.62
	50	8.74	39.68	4.54	79.36
	75	9.43	57.61	6.11	76.81
	100	15.38	71.65	4.66	71.65
Aspergillus oryzae ATCC 20423	25	9.24	21.15	2.29	84.62
	50	13.33	44.1	3.31	88.21
	75	11.83	62.5	5.29	83.33
	100	13.45	73.6	5.47	73.6
Aspergillus niger ATCC 15475	25	8.88	20.86	2.35	83.46
	50	11.13	37.54	3.37	75.01
	75	11.35	51.79	4.56	69.05
	100	15.35	74.75	4.87	74.75
Fusarium oxisporum MUCL 791	25	9.35	21.09	2.26	84.37
	50	11.23	43.77	3.90	87.53
	75	10.40	60.17	5.78	80.23
	100	9.96	72.37	7.52	72.37
Polyporus squamosus	25	12.35	20.53	1.66	82.12
	50	11.80	42.31	3.59	84.62
	75	11.18	59.6	5.33	79.47
	100	10.98	79.66	7.25	79.26

$$\text{Uptake efficiency}(\%) = \frac{\text{mg Cu(II) removed}}{\text{mg Cu(II) available}} \cdot 100 = \frac{\text{mg Cu(II) available} - \text{mg Cu(II) residual}}{\text{mg Cu(II) available}} \cdot 100 \quad (2)$$

From Table 2 it can be seen that the presence of low concentrations of copper in solution did not result in significant specific uptake. The increasing copper ion concentration resulted in increased uptake for copper, but decreased uptake efficiency. Although the presence of increased concentrations of copper ions inhibited fungal growth, the net copper uptake increased when the initial copper ion concentration in solution was 50 mg/L. This trend was observed for the following fungus strains: *Aspergillus oryzae* ATCC 11489, *Aspergillus oryzae* ATCC 20423, *Fusarium oxisporum* MUCL 791, and for *Polyporus squamosus*. For the fungus strain *Aspergillus niger* ATCC 15475 was observed that the copper uptake decreased with increasing copper ion concentrations in solution, but for the copper concentration equal to 100 mg/L it was observed that the uptake efficiency was higher than determined for the solution with 75 mg Cu(II)/L.

The results obtained are consistent with literature data that mention a specific uptake of copper variation between 1.46 mg/g by *Kluyveromyces marxianus* [47] and 90 mg/g by *Thiobacillus ferrooxidans* [48]. In our experiments we observed that the specific copper uptake varies between 1.66 mg/g and 7.52 mg/g. The maximum specific copper uptake value was obtained in the case of fungus strain *Fusarium oxisporum* MUCL 791 in the presence of copper ion solution with 100 mg/L concentration.

Effect of Time on Copper Uptake by Fungal Biomass

Copper removal efficiency as a function of time of fungal biomass from solution containing nanoparticles of CuS, is demonstrated in Fig. 1.

From Fig. 1 it can be seen that the copper biosorption by fungi is a relatively lent process and almost reached the maximal level after 5 days. The maximum level of copper retained was registered in the case of *Aspergillus oryzae* ATCC 20423. A value of 88.21% for copper removal efficiency was obtained for this fungal strain. These results are similar to literature data that mention copper removal efficiency values of: 60% for *Aspergillus niger* [49], 51.77% for *Pleurotus ostreatus* [34], up to 90% for *Kluyveromyces marxianus* and *Candida* sp. [43], and 70% for *Aspergillus niger* pretreated [50].

Optimal pH for Copper Uptake by Fungal Biomass

pH value influences the chemistry of heavy metals solution, and it represents one of the most important factors in metal ions adsorption. Consequently, the effects of varying pH between 3.0 and 7.5 on removal efficiency of Cu(II) by the five fungus strains were examined (Fig. 2).

The results presented in Fig. 2 showed that the biosorption of Cu(II) ions is dependent on the pH of the solution. A gradual increase in the removal efficiency of Cu(II) was observed for each fungal strain used. The cell surface copper binding sites and the availability of copper in the solution are influenced by pH. Little biosorption of Cu(II) ions was observed at low pH due to the competition between hydrogen ions and copper ions on the biosorption active sites [35]. The increase of pH resulted in an increased negative charge on the surface of the cell, which favored electrochemical attraction and adsorption of copper. The biosorption of Cu(II) couldn't be studied above pH 7.5 since the copper hydroxide starts to precipitate in the liquid.

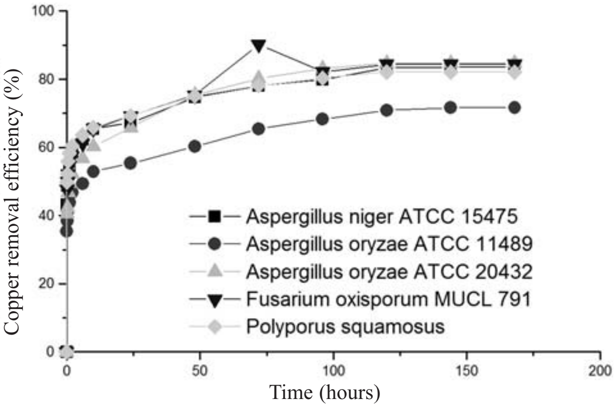

Fig. 1. Copper removal efficiency as a function of time.

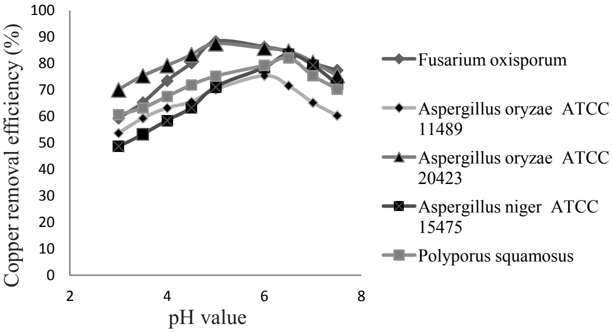

Fig. 2. Copper removal efficiency as a function of pH value.

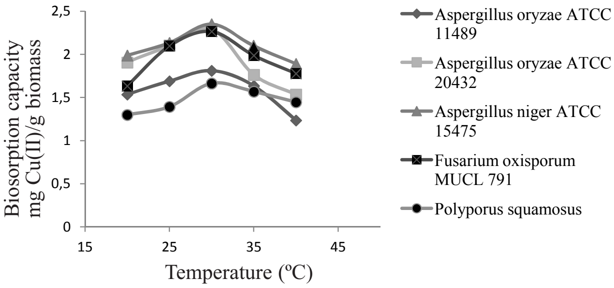

Fig. 3. Fungal biosorption capacity as a function of temperature value.

Fusarium oxisporum has the ability to absorb the maximum copper level at pH 5, *Aspergillus oryzae* ATCC 11489 at pH equal with 6, *Aspergillus oryzae* ATCC 20423 at a pH value of about 5.5, and *Aspergillus niger* ATCC 15475 and *Polyporus squamosus* at pH equal with 6.5. Consequently, the highest biosorption of copper occurs at different pH values depending on the fungal strain. The optimal pH range for copper uptake is between 5 and 6.5, which is widely accepted as being optimal for almost all types of fungal biomass.

The results obtained are in concordance with literature data that mentioned the following values of pH for maximum Cu(II) adsorption: 6.5 for *Aspergillus niger* [33], 5 for *Trametes versicolor* [49], 5.5 for *Penicillium hirsutum* [51], and 6 for preatreated *Aspergillus niger* biomass [50].

Optimal Temperature for Copper Uptake by Fungal Biomass

The effect of temperature was evaluated in the biosorption process of Cu(II) by fungal biomass in experiments carried out at temperatures of 20, 25, 30, 35, and 40ºC using suspensions containing 25 mg Cu(II)/L. The suspension volume used was 100 mL, the time to reach equilibrium was 6 days, and the shaking was at 150 rpm. The results obtained are presented in Fig. 3.

From Fig. 3 it can be seen that copper biosorption depends on temperature. The experimental results indicate an increase of biosorption capacity with increasing temperature from 20 to 30ºC. The highest increase of biosorption capacity was observed for *Fusarium oxisporum* MUCL 791, being 0.6314 mg Cu(II)/g biomass, and the lowest increase of biosorption capacity (0.2757 mg Cu(II)/g biomass) was registered for *Aspergillus oryzae* ATCC 11489. This illustrates that copper biosorption is an endothermic process in the respective temperature range. The favorable effect of higher temperature can be attributed to: higher affinity of Cu(II) for binding centers, the growing of binding centers from biomass surface as a result of reorientation of fungal biomass cell wall components, ionization of chemical groups from cell wall, adsorbent diffusion with high speed in boundary layer, and in internal pores of biomass particles due to the decrease of liquid viscosity with the increase of temperature. However, it was observed that an increase of temperature from 30 to 40ºC determines a decrease of biosorption capacity, probably due to damages to binding centers of biomass.

Thus optimal temperature for the Cu(II) biosorption process by all fungal strains used is 30ºC. The results obtained are consistent with the results mentioned in literature data [52].

Mechanisms Involved in Copper Uptake by Fungal Biomass

The mechanism involved in the adsorption process has an important role in the case of pollutant removal from environments. Adsorption is divided into two: one is due to the forces of physical nature named van der Waals forces, and one where adsorbed molecules are held to the surface by valence force of the same type as those occurring between bound atoms in molecules, named chemisorption.

In order to determine the characteristic functional groups responsible for biosorption of copper ions, FT-IR spectroscopy was applied. The bonding mechanism between copper and fungal biomass can be determined by interpreting the infrared absorption spectrum.

In the case of *Aspergillus oryzae* ATCC 20423, the fungal strain FTIR spectra are presented in Fig. 4.

From this figure it can be seen that all five FTIR spectra present distinct peaks at 3,393, 2,926, 1,638, 1,404, and 1,073 cm^{-1}. The broad and strong band at 3,393 cm^{-1} can be attributed to overlapping of –OH and –NH stretching. The band at 2926 cm^{-1} is attributed to the C-H stretching vibrations. The strong peak at 1638 cm^{-1} can be due to a C=O stretching in carboxyl or amide groups. The peak at 1404 cm^{-1} is attributed to N-H bending in the amine group. The band observed at 1073 cm^{-1} was assigned to the CO stretching of alcohols and carboxylic acids. Thus, *Aspergillus oryzae* ATCC 20423 biomass contains hydroxyl, carboxyl, and amine groups on its surface.

From Fig. 4 it can be seen that the stretching vibration of the OH group shifted from 3,393 cm^{-1} to 3,418 cm^{-1} (4b), to 3,398 cm^{-1} (4d), to 3,406 cm^{-1} (4e). These results revealed that chemical interactions between the copper ions and the hydroxyl groups occurred on the biomass surface. The carboxyl peak observed for unloaded biomass at 1638 cm^{-1} is shifted to 1,634 cm^{-1} or 1,629 cm^{-1}. This decrease in the wave number of the peak characteristic for the C=O group from carboxylic acid revealed that interactions with the carbonyl functional group are present between biomass and copper ions. These results indicate that the free carboxyl groups changed into carboxylate, which occurred during the reaction of the metal ions and carboxyl groups of the biosorbent.

No changes in frequency were observed in the C-H and -NH_2 groups of biomass after copper biosorption. In addition, all FTIR spectrum of *Aspergillus oryzae* ATCC 20423 loaded with copper ions contain bands at 533, 529, and 525 cm^{-1}, which can be attributed to Cu-O stretching modes.

Fig. 5 presents superposition of FT-IR Spectra of *Poyporus squamosus* grown without copper, and in the presence of medium culture with 25, 75, and 100 mg Cu(II)/L.

From the analysis of IR spectra presented in Fig. 5 it can be seen that the mechanism of copper uptake by *Polyporus squamosus* involves interactions between copper ions and hydroxyl, amino, and carbonyl groups from fungal biomass surface since shifting of peaks characteristic to these groups were registered.

Similar FT-IR results were obtained for all fungal strains used. These results are very close to those reported for the biosorption Pb(II), Cd(II), and Cu(II) onto *Botrytis cinerea* fungal biomass [53] and Pb(II) and Cd(II) from aqueous solution by macrofungus (*Lactarius scrobiculatus*) biomass [54].

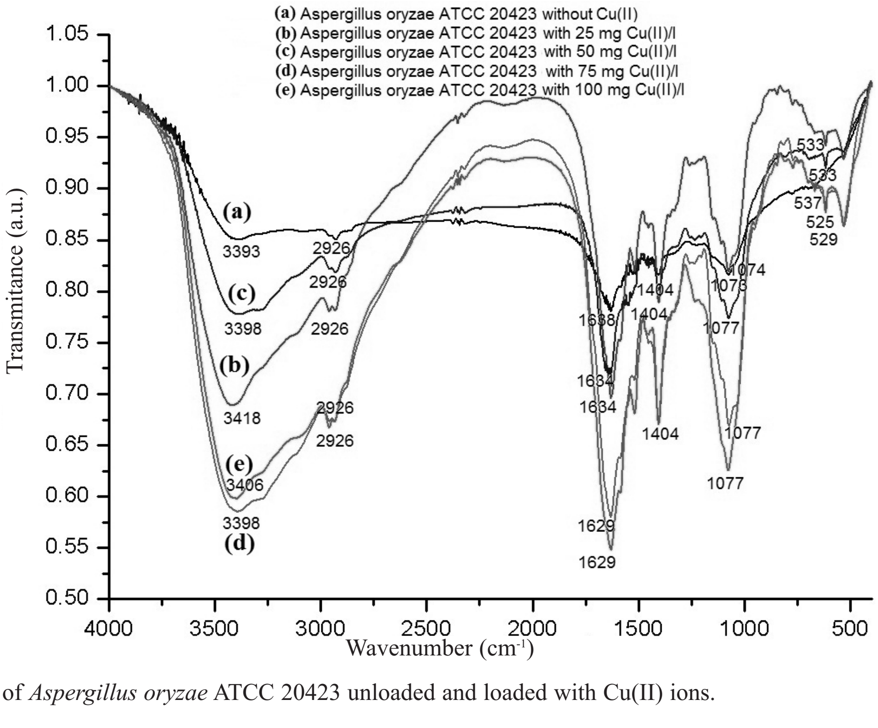

Fig. 4. FT-IR spectra of *Aspergillus oryzae* ATCC 20423 unloaded and loaded with Cu(II) ions.

Desorption Studies

In order to be used in an industrial scale biosorption process, the biosorbents have to be easily regenerated and used in a high number of sorption-desorption cycles without biomass denaturation and with the same removal efficiency. This is possible only by using suitable eluents. Therefore, for a biosorption process to be completed it has to be followed by a desorption process with an important role in pollutant recovery. Desorption is important when biomass obtaining and regeneration are expensive, and therefore brings about an increase in the cost of the process. A successful desorption process requires selecting a suitable eluent. This depends on the type and the mechanism involved in the biosorption process.

As a result, at the end of the copper biosorption process biomass loaded with copper was used in desorption processes. The following copper recovery efficiency: 81.23% for *Aspergillus oryzae* ATCC 20423, 83.45% for *Aspergillus oryzae* ATCC 11489, 82.23% for *Fusarium oxisporum* MUCL 791, 85.67% for *Aspergillus niger* ATCC 15475, and 84.89% for *Polyporus squamosus,* was obtained by adding the fungal biomass loaded with copper to the HNO_3 0.05M solution. The copper recovery efficiency varied between 82% and 86%. The highest recovery efficiency was registered for the *Aspergillus niger* fungal strain.

From these experiments, a final conclusion can be drawn: the desorption and recovery of copper ions can be performed using acidic solutions. Thus, the fungal biomass can be used in other copper ion removal cycles from wastewaters.

Conclusions

From the comparative study on copper removal capacity of fungal strains *Aspergillus oryzae* ATCC 20423, *Aspergillus oryzae* ATCC 11489, *Fusarium oxisporum* MUCL 791, *Aspergillus niger* ATCC 15475, and *Polyporus squamosus,* it can be concluded that the removal capacity of these fungal strains depends on copper concentration, time, pH value, and temperature. The copper removal capacity varies between 69% and 88%. The highest value of copper removal capacity was obtained for *Aspergillus oryzae* ATCC 20423. The copper biosorption by fungal biomass is a relatively lent process and reached almost its maximal level after 5 days. In the case of the fungal strains selected, the optimal temperature for Cu(II) biosorption process was 30ºC, and the optimal pH value depends on the fungal strain used. Copper recovery efficiency varied between 82 and 86% when acidic solutions were used as eluents. The highest recovery efficiency was registered for *Aspergillus niger* fungal strain.

One of the main advantages of using *Polyporus squamosus* fungal strain in heavy metal removal from wastewaters is that their size, texture and other characteristics are conducive to its development as adsorbent without the need to immobilize or develop a sophisticated reactor configuration as needed in the case of microorganisms.

From the analysis of the IR spectra, it can be seen that the mechanism of copper uptake by fungal biomass involves interactions between copper ions and hydroxyl, amino, carboxyl, and carbonyl groups from fungal biomass surface depending on the types of fungal strain.

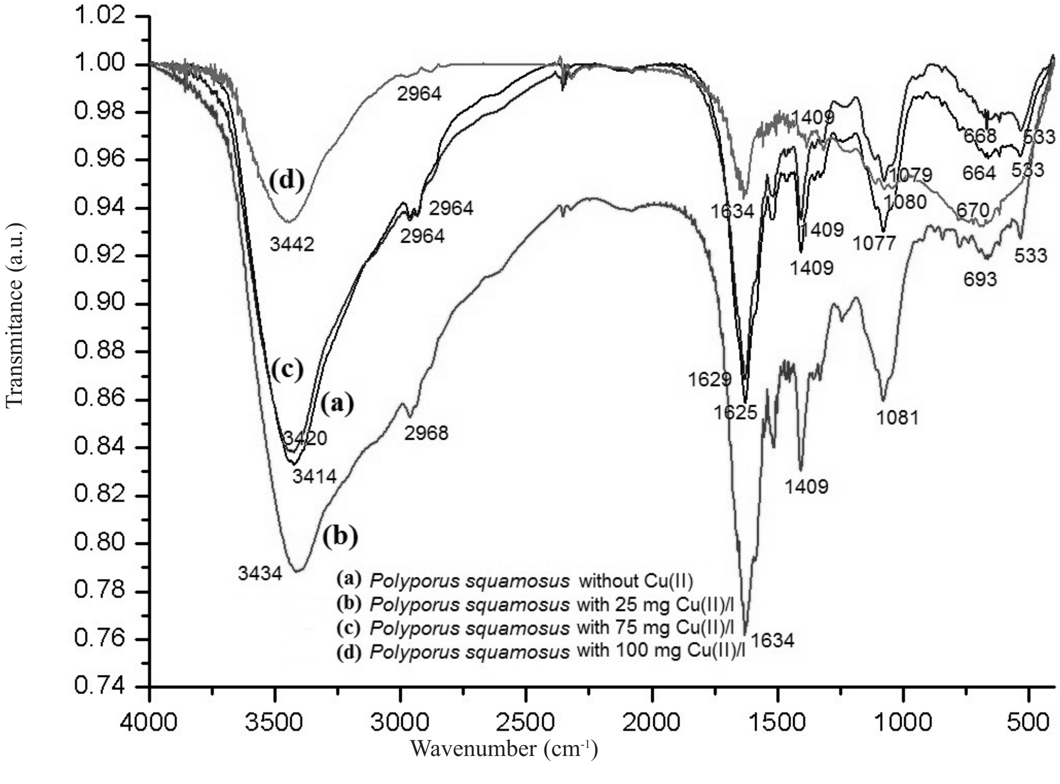

Fig. 5. FT-IR spectra of *Polyporus squamosus* unloaded and loaded with Cu(II) ions.

As a result, the fungal biomass can be used successfully in the removal of heavy metals from wastewaters, even from diluted solutions and in the form of nanoparticles, as they generally have high growth and reproduction speed, and can use a large number of carbon sources (even from waste). They can be relatively easily selected and obtained with high yield and good composition. In addition, biomass production does not depend on climate and seasonal variations. Another advantage of using fungal biomass to reduce heavy metal content from wastewaters is its ability to adapt to adverse environmental conditions such as low pH value. This is a special advantage because it is well known that a real industrial wastewater contains more than just one metal, but also organic compounds, and other inorganic compounds such as acids, bases, and salts.

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