EQUILIBRIUM AND KINETICS STUDIES OF COPPER (II) BIOSORPTION BY *RHIZOPUS MICROSPORUS*

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Abstract

In this work a biosorption route is developed using dead and viable biomass from Rhizopus microsporus to recover copper (II) from aqueous solutions. Batch sorption experiments were conducted at 25◦C, biosorbent concentration within the range of 2.0 to 2.5 mg/L and copper (II) concentrations within the range of 25 to 350 mg/L. Two methodologies regarding the pH were compared, either allowing this parameter to vary freely or controlling its value throughout the batch. The latter methodology led to an increase in the biosorption capacity from 7.61 to 20.38 mg/g at the optimum pH of 5. Both Langmuir and Freundlich adsorption models fitted well the equilibrium data. Biosorption kinetics showed very good fitting to pseudo first and pseudo second-order models, with equilibrium being approached within 4 hours. Biomass proved to maintain its sorption efficiency for at least three regeneration cycles using HCl (0.1M) as eluent.

Key words: Biosorption, copper, dead fungal biomass, desorption, kinetics

1. Introduction

The industrial and urban development, associated with the progressive increase in the consumption of natural resources, has been the cause of many hazards to the environment and human health due to the emission of pollutants to the air, soil and water(PRAKASHAM et al., 1999; MALIK, 2004). Consequently, one of today's prominent issues is to make industrialization compatible with the preservation of nature and mankind. The development of processes for waste treatment is part of this effort, since it is not always possible to eliminate or reduce the formation of pollutants in industrial activities. Ideally, the treated waste should be harmless to the environment and suitable for reuse in the industrial process or recovery as a valuable product.

Heavy metal pollution is an important environmental problem (WANG and CHEN, 2009), because of its widespread use, its high toxicity, no biodegradability and tendency to accumulate throughout the food chain (ÇABUK et al., 2007). Mining tails dams are worrying sources of heavy metal pollution. The ponds within these dams interact with the surrounding environment as they contain dilute solutions of heavy metals, in many cases above safe limits. Since heavy metals are also a valuable resource, their recovery and recycle assumes even greater significance (MALIK, 2004). This is particularly true for copper. This heavy metal finds various applications in contemporary society, particularly in the construction industry and in the manufacture of electronic components. Similarly to other heavy metals, in dissolved form it can cause several physiological problems or even death (REZAEI et al., 2011). In Brazil, large amounts of copper processing waste with a content of about 0.07% of the metal are deposited in dams that house large amounts of water. Promoting copper leaching from the mine tailings to the liquid phase followed by copper recovery is considered a promising way of making the dam environmentally safer while recovering the metal. This paper is concerned with the copper recovery from the liquid phase.

Several methods exist for recovering metal from aqueous media such as ion exchange, chemical precipitation, electrochemical techniques, evaporation and reverse osmosis

(PERPETUO et al., 2011). However, in some instances these processes may not be applied due to high cost, low recovery efficiency from diluted systems, sludge generation, or environmental impacts related to reactants or energy consumption. In this context, biosorption appears as an alternative technology to detoxify and recover heavy metals from dilute aqueous solutions. The main advantages of biosorption include low operation costs, low chemical and/or biological sludge volumes and high efficiency in detoxifying effluents (MONTAZER-RAHMATI et al., 2011).

Biosorption has been conducted with any of a wide range of organisms (NAJA et al., 2010), such as bacteria (LOUKIDOU et al., 2004), fungi (ÇABUK et al., 2007), algae (BORBA et al., 2006), yeast (HAN et al., 2006) and plants (SARIN and PANT, 2006). When selecting a biomass source for a particular application, availability and biosafety are important criteria. Therefore, it is suitable to use microorganisms that are native to the area where the process is to be applied, as long as its biomass has adequate sorption behavior. Some of the most important features are a favorable sorption isotherm, fast metal uptake kinetics and biomass stability throughout sorption-desorption cycles.

Biosorption is the result of a passive physical-chemical interaction between ions in solution and biomass. It is particularly the cell wall structure of the organism which is responsible for this phenomenon (MONTAZER-RAHMATI et al., 2011). Many authors report that the biosorption mechanism may be ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation, precipitation and most probably a combination of these (VEGLIO and BEOLCHINI, 1997; SCHIEWER and VOLESKY, 1996). The biomass sorption behavior may be evaluated in terms of thermodynamic and kinetic aspects. The former establishes the possible metal uptake in the biomass which is attained from a solution, being usually expressed by sorption isotherms. The latter determines the sorption rate as a function of the departure from thermodynamic equilibrium. Several parameters influence isotherm and kinetic behavior, such as pH, temperature, condition of the biomass, biomass type, ionic strength and competition between metal ions (NAJA et al., 2010; VIJAYARAGHAVAN and YUN, 2008).

Biosorption isotherms may be experimentally determined from batch experiments where biomass and a metal-containing solution are brought into contact for a sufficient time for equilibrium to be attained. Vijayaraghavan and Yun (VIJAYARAGHAVAN and YUN, 2008) mention two different methodologies in biosorption experiments commonly used by authors regarding the pH, one in which the parameter is controlled and the other one in which the pH is allowed to vary freely. Nevertheless, very few publications address and compare biosorption potential with both methodologies (FOUREST and ROUX, 1992; HOLAN et al., 1993; FOUREST et al., 1994). Determination of biosorption kinetics relies on the same type of experiments, but metal uptake is measured along time.

In the present study the biosorption potential of copper (II) ions by the biomass of *Rhizopus microsporus*, a naturally occurring fungus in copper mining areas, is evaluated. The analysis is performed by means of batch experiments for determining biosorption isotherms using two different methodologies, with and without pH control. The effects of pH and autoclaving

pretreatment upon biosorption capacity and biosorption kinetics are assessed. The reusability of the biosorbent throughout successive sorption-desorption cycles are investigated.

2. Materials and methods

2.1. Preparation of inoculum and biomass

Rhizopus microsporus was collected and isolated from the copper tailing area of a mine in the amazon region. The fungus was maintained on potato dextrose agar (PDA). *Rhizopus microsporus* was cultivated for 15 days in PDA medium in 500mL Erlenmeyer's flasks for the production of a spore suspension. A saline solution (0.9% w/v) was used to collect spores, resulting in a concentration of 10^6 spores/mL determined in a neubauer chamber. The suspension was then mixed with glycerol solution (20% w/v) and stored inside 2 mL cryotubes in an ultrafreezer at -800°C. These spores were used as inocula for the production of biomass.

The fungus was cultivated in a liquid medium composed of malt extract (10 g/L), glucose (20 g/L) and yeast extract (10 g/L). Erlenmeyer flasks (500 mL) containing 100mL of culture medium were inoculated with 1 mL of spore suspension and incubated in a rotary shaker (150 rpm) at 28^0C. Fungal mycelium was harvested after 20 hours incubation, separated from the culture medium, washed several times with Milli-Q water and homogenized using a commercial blender. For the preparation of dead biomass an appropriate amount of viable biomass was autoclaved at 121 °C for 20 minutes.

2.2. Copper solution treatment and measurements

All copper (II) solutions were prepared by dissolving analytical grade $CuCl_2 \cdot 2H_2O$ in Milli-Q water. Copper (II) solutions were diluted to the range of 1 to 5 mg/L using an acid solution of HNO_3 (3.25% v/v). Copper concentration was determined by flame atomic absorption spectrophotometry (AAS) (Shimadzu model GFA-7000A).

2.3. Biosorption procedure

Experiments were conducted with dead biomass, otherwise specified. Batch experiments were conducted by introducing wet biomass (in the range of 2.0 - 2.5 g/L of dry mass) in plastic flasks (50 mL) generally containing a volume of 30 mL copper (II) solutions of known initial pH and copper concentration. The flasks were agitated at 150 rpm in an incubator shaker at 25°C for at least 12 hours to reach sorption equilibrium. The fungal biomass was separated from the metal solution by vacuum filtration through membranes of 0.45 □m (Millipore). The resulting metal solution was analyzed. The biosorption capacity (Q_{Eq}) was calculated using the following mass balance equation (1):

$$Q_{Eq} = \frac{V(C_0 - C_{Eq})}{m_s} \qquad (1)$$

where C_0 is the initial metal concentration (mg/L), C_{Eq} the equilibrium metal concentration (mg/ L), V the solution volume (L) and m_s is the dry fungal mass (g).

2.4. pH control procedure

Two different methodologies were used regarding the pH. In the methodology referred as "pH free", the metal solution pH was adjusted before addition of biomass and not controlled thereafter. In the other methodology, referred as "pH control", manual adjustments occurred every hour until the pH remained constant. The flasks remained under constant temperature and agitation for another 12 hours to assure that the equilibrium has been reached. For both methodologies the pH was adjusted using 0.1 M HCl and 0.1 M NaOH solutions.

2.4.1. Effect of autoclaving pretreatment

To evaluate the effect of autoclaving pretreatment the biosorption capacities of viable and dead biomasses, were compared. Biosorption experiments were conducted at initial copper concentration of 100 mg/L at initial pH 5, which was not controlled during the experiment.

2.4.2. Effect of pH

Biosorption experiments were conducted in the pH range of 1 to 5 with copper (II) solutions with initial concentration of 100 mg/L, with both "pH free" and "pH control" methodologies. Simulations of chemical equilibrium using the software CHEAQS Pro showed that possible precipitation of copper hydroxides takes place for pH values above 5.7. Therefore, most experiments were conducted at a pH of 5 or lower.

2.4.3. Biosorption isotherms

Biosorption isotherms were obtained using the "pH free" and "pH control" methodologies at pH values of 1 and 5. The copper (II) solutions initial concentrations were within the range of 25 to 350 mg/L and 25 to 200, respectively.

2.4.4. Sorption-desorption cycles

Consecutive sorption and desorption batch experiments were performed in order to investigate the biosorbent regeneration ability. Sorption cycles were composed of a 12 h sorption process followed by a 4h desorption one. Initial Copper (II) concentrations were 50 and 100 mg/L. Following each sorption process, copper (II) loaded biomass was separated by vacuum filtration through 0.45 □m membranes and suspended into 30 mL of HCl 0.1 M eluent solution to remove the metal, then thoroughly washed with Milli-Q water and placed into metal solution for the next biosorption cycle, which was conducted 3 times. The copper (II) concentration in the solution after each sorption and desorption processes was determined. The pH was allowed to vary freely throughout the experiment.

2.4.5. Kinetics studies

Batch kinetic experiments were performed with 40 mL of copper (II) solutions with a initial concentration of 150 mg/L at pH 5, which was not controlled during the experiment. Samples of 0.5 mL of supernatant were collected at 5, 10, 15, 30, 60, 120, 240 and 1440 minutes.

2.5. Adsorption equilibrium models

Langmuir and Freundlich adsorption models were used to represent the experimental data of the biosorption isotherms. In the former model a monolayer is assumed to deposit on a homogeneous surface without interaction among sorbed molecules (SEADER et al., 2011). In the latter model stronger binding sites are occupied first and the binding strength decreases with increasing degree of site occupation (SEADER et al., 2011). The Langmuir (2) and Freundlich isotherm equations (3) are respectively:

$$\frac{1}{Q_{Eq}} = \frac{1}{q_{max} K_L C_{Eq}} + \frac{1}{q_{max}} \quad (2)$$

$$\ln Q_{Eq} = \ln K_f + \frac{1}{n} \ln C_{Eq} \quad (3)$$

where Q_{Eq} is the biosorption capacity at equilibrium (mg/g), constant q_{max} represents the monolayer saturation at equilibrium or maximum amount of copper adsorbed per biomass weight (mg/g), C_{Eq} (mg/L) is the copper (II) concentration at equilibrium, K_L is the Langmuir adsorption constant (L/mg), K_F ($mg^{(n-1)/n}$ $L^{1/n}$ /g) and n are the Freundlich adsorption constants.

2.6. Kinetics of copper (II) biosorption

Kinetics of copper (II) biosorption was adjusted both to the linear first-order Lagergren model, in which the rate of occupation of adsorption sites is proportional to the number of unoccupied sites (MICHALAK et al., 2013), as well as to a pseudo second-order model, in which the rate of occupation of adsorption sites is proportional to the square of the number of unoccupied sites (HO, 2006) , as shown in equations (4) and (5), respectively.

$$ln(Q_{Eq} - Q_t) = ln(Q_{Eq}) - K_1 t \quad (4)$$

$$\frac{t}{Q_t} = \frac{1}{K_2 Q_{Eq}^2} + \frac{1}{Q_{Eq}} t \quad (5)$$

where K_1 is the Lagergren rate constant of sorption (1/min), K_2 is the pseudo second-order rate constant of sorption (g/mg min) and Q_t is the biosorption extent (mg/g) at time t.

2.7. Statistical Analysis

Initially a set of biosorption experiments were carried out to determine the variance components of the biosorption capacity variable. A nested (hierarchical) design analysis was used as shown in figure 1.

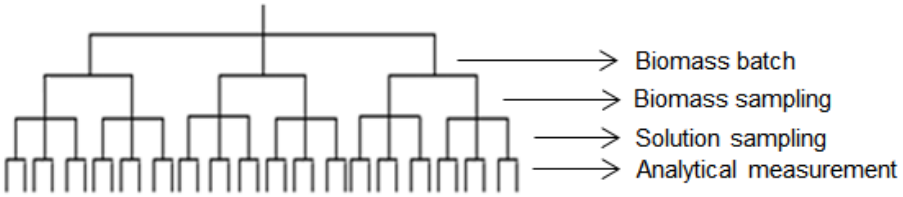

Figure 1. Nested design of the variance components.

It was found that the main source or experimental error is due to the biomass batch factor. Therefore to reduce the error every experiment was performed using one single biomass batch.

All the biosorption experiments were carried out in duplicate with respect to the biomass sampling and in duplicate in relation to the analytical reading. Some experiments (effect of pH and autoclave pretreatment) were carried in triplicate with respect to the solution treatment. Data are presented as the mean values of these independent trials with standard deviation error bars. The significance of these factors on the copper concentration was assessed with the method of analysis of variance (ANOVA) with a level of significance (α) of 5%.

3. Results and discussion

3.1. Effect of autoclaving pretreatment

The biosorption capacity for viable and dead biomass was 6.68 ± 1.10 and 6.10 ± 1.55 mg/g, respectively. The ANOVA gives a P-Value of 15.43%, higher than the significance level of 5%. Therefore pretreating the biomass with temperatures above 121 ^{o}C does not affect the interaction between copper (II) and *Rhizopus microsporus*. Many authors investigated the effect of pretreating the biosorbent on biosorption of microorganisms. The biosorption capacity of dead cells may be greater, equivalent to or less than that of living cells (KAPOOR and VIRARAGHAVAN, 1995). It was found that the autoclaving pretreatment slightly inhibit the biosorption of copper and nickel by *Pseudomonas aeruginosa* (SAR et al., 1999) while it enhanced the biosorption of copper by *Hypocrea lixii* (SALVADORI et al., 2013).

3.2. Effect of pH

It was found that the pH values decreased during biosorption for solutions in the initial pH range of 2 to 5. At equilibrium the pH values converged to 1.78 and 3.76, respectively. The phenomenon of pH reduction was also reported by other authors (HAN et al., 2006; FOUREST and ROUX, 1992; BENAÏSSA and ELOUCHDI, 2011). It may be explained by the displacement of hydrogen cations in the fungal cell wall matrix by the copper ions in the

solution. This is consistent with the hypothesis that ion exchange is one of the main mechanisms in the biosorption of copper (II) by *Rhizopus microsporus*.

The solution pH is perhaps the most important parameter for biosorption (HAN et al., 2006). The effect of pH on the biosorption capacity, using the "pH free" methodology, is shown in figure 2. The pH values are reported as the equilibrium values. It was found that the biosorption capacity increases with the pH. The maximum biosorption capacity was observed at equilibrium pH values ranging from 3.65 to 3.76, corresponding to the initial pH of 4 and 5 respectively. Tukey's test (α = 5%) showed that there were no significant differences on the biosorption capacity within this pH range. Nevertheless the small difference in sorption can be explained by the convergence of pH values in the equilibrium in these trials.

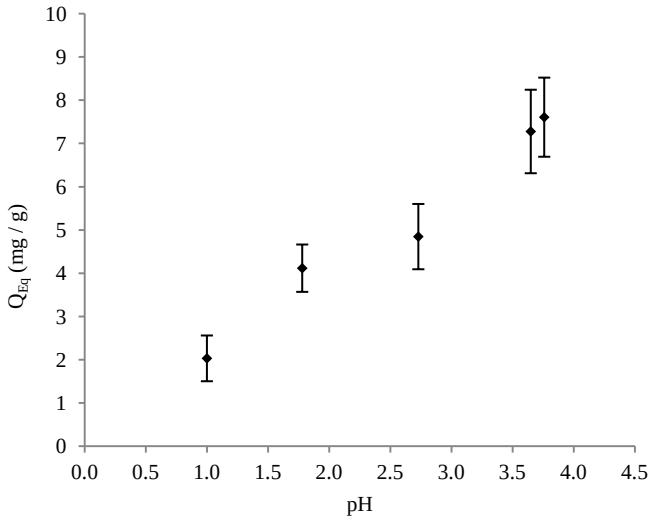

Figure 2. Effect of pH in biosorption capacity, "pH free" methodology. The equilibrium pH values are reported.

A similar optimum initial pH in the range of 4 to 6 on biosorption capacity was reported in a number of other studies using the "pH free" methodology, as in the biosorption of copper (II) by *Phanerochaete chrysosporium* (IQBAL and EDYVEAN, 2004; SAY et al., 2001), *Hypocrea lixii* (SALVADORI et al., 2013) and by *Streptomyces coelicolor* (OZTÜRK et al., 2004).

The pH can affect the metal sorption in two ways. The first one is through speciation, altering the availability of the metal ion at higher pH values (TUBBING et al., 1994). Our calculations show that for aqueous solutions of copper speciation is negligible in the pH range studied. For instance, at a pH value of 6, about 96% of the copper is in the form of the copper (II) ion, whereas a pH of 5 and lower, more than 99% of the copper is present as the free ion. The second one is associated with the possible protonation of functional groups present in the cell wall at low pH values, giving an overall positive charge to the biomass and, therefore, reducing its biosorption capacity for metallic ions (VIJAYARAGHAVAN and YUN, 2008; SAY et al., 2001). The results obtained for pH 1 and 2 are clearly in agreement with the latter mechanism as it indicates that low pH values inhibit metal biosorption.

The effect of pH was also investigated using the “pH control” methodology. Figure 3 shows that biosorption increases with pH. In this methodology the pH range is extended, so a relatively large biosorption capacity (20.38 mg/g) is obtained at pH 5. Other authors also described an optimal pH around 5.0 using the “pH control” methodology, such as in the biosorption of copper, zinc and cadmium by *Ulva lactuca* (ARECO et al., 2012) and copper by *Phanerachaete chysosporium* (SAY et al., 2001). The results reinforce the statement that the pH is an important factor on metal biosorption.

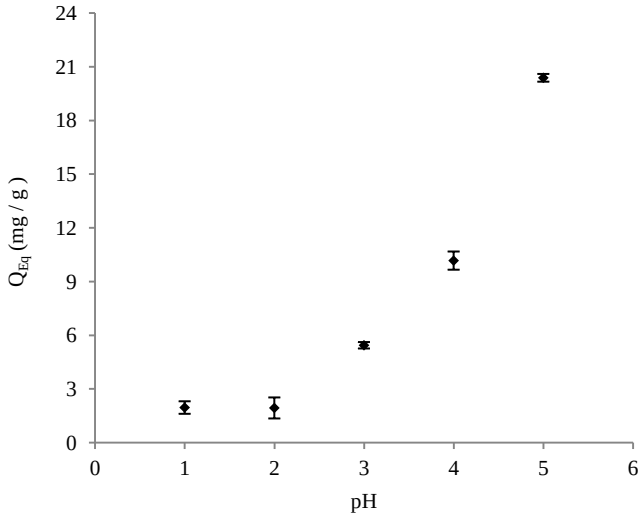

Figure 3. Effect of pH in biosorption capacity for the “pH control” methodology.

Comparing both methodologies a great discrepancy in biosorption capacities for initial pH values above 4 is observed. For instance, the biosorption capacity for pH 5 was 2.7 times larger with “pH control” than without it, as the value of the “pH free” system converged to pH value of 3.7 as shown in figure 2. The only disadvantage of pH control for practical applications is the cost related to reactant addition.

3.3. Biosorption isotherms

Biosorption isotherms conducted at initial Copper (II) concentrations of 25 to 350 mg/L for pH 1 and pH 5 are shown in figure 4. For the isotherm following the “free pH” methodology, the initial pH was 5 and the equilibrium pH values were within the range of 3.15 to 4.00. The pH decrease was larger for the more concentrated solution as a result of a higher exchange between copper (II) and hydrogen ions.

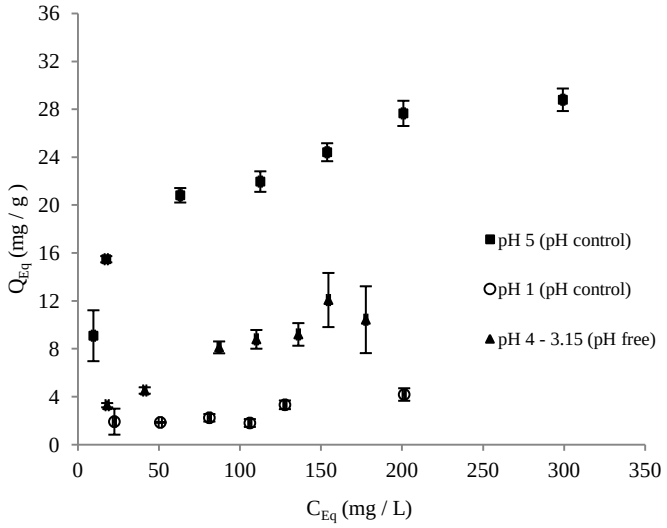

Figure 4. Biosorption isotherms. For the free pH experiment, the initial pH was 5 and the final pH varied from 3,15 to 4,00 with the solution copper concentration.

Figure 4 shows that the biosorption capacity increased with the copper (II) concentration in solution. All isotherms, independently of the initial pH and whether it was controlled or not, showed similar behavior. The derivative of the biosorption capacity function with respect the copper concentration variable, given as $\frac{dQ_{Eq}}{dC_{Eq}}$, presented a large increase in lower concentrations of metal followed by a discrete increase while the solution concentration was raised further. The isotherm at constant pH 5 increased sharply from 9.1 to 20.8 mg/g followed by a minor increase to 28.8 mg/g. This phenomenon can be explained by the saturation of the active sites present in biomass due to higher metal presence in the solution (ÖZER et al., 2009).

Both Langmuir and Freundlich adsorption models fitted well the experimentally determined isotherms, as shown graphically in figure 5 and 6 and from the determination coefficient (R^2) of the linear regressions shown in table 1. Langmuir model presented good adjustments with pH 5 and pH 1 isotherms, with R^2 values of 0.966 and 0.839, respectively. For the “pH free” isotherm both models presented similar R^2 values of 0.944 and 0.965 respectively.

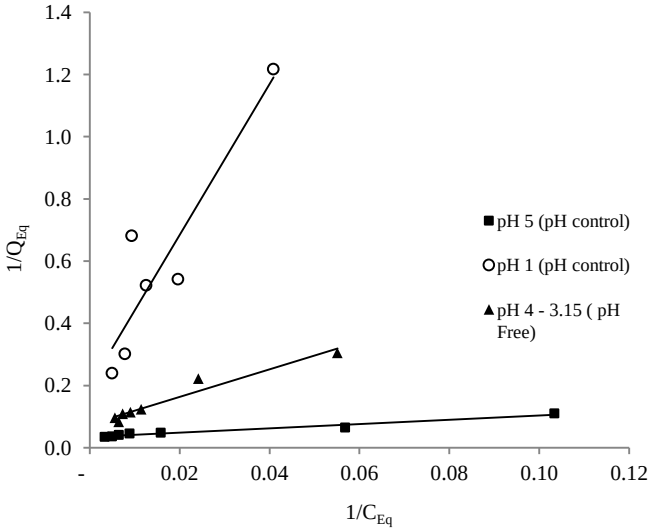

Figure 5. Langmuir linear plot.

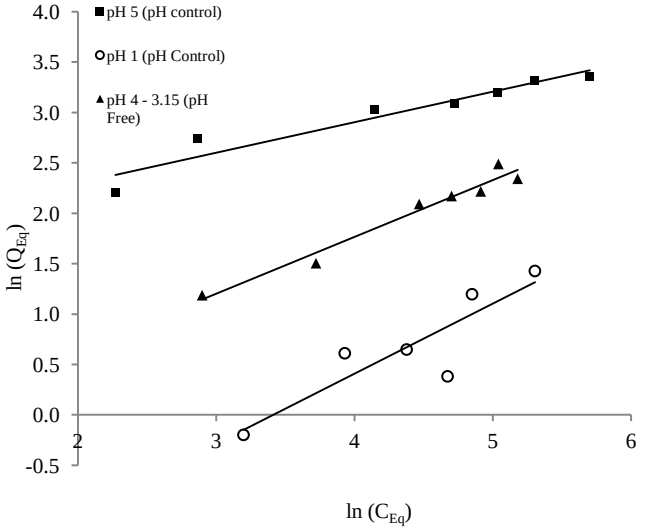

Figure 6. Freundlich linear plot.

Table 1. Parameters and determination coefficient for Langmuir and Freundlich adsorption models

	Langmuir			Freundlich		
	K_L (L/mg)	q_{max} (mg/g)	R^2	K_f	n	R^2
pH 5 (pH Control)	0.031	32.47	0.966	5.45	3.31	0.923
pH 1 (pH Control)	0.008	5.03	0.839	0.24	2.06	0.786
Initial pH 5 (pH free)	0.017	13.41	0.944	0.61	1.77	0.965

As biosorption isotherms show the distribution of solute between the liquid and solid phases in equilibrium (HAN et al., 2006), for a metal removal process a curve with a steep initial slope is desired (KRATOCHVIL and VOLESKY, 1998). Such a slope is represented as the constant K_L in Langmuir's model. A high K_L value indicates a low residual metal concentration in liquid phase in equilibrium with a high metal concentration in the biomass.

Similarly, a low inclination of the isotherm is desirable for metal desorption from the biosorbent (VOLESKY, 2001). Another important model parameter is the q_{max}, as it corresponds to the highest theoretical possible sorbate uptake (KRATOCHVIL and VOLESKY, 1998). A high value for this parameter is desirable for sorption and a low value for desorption. Therefore, in order to develop a process involving sorption-desorption cycles, it is desirable to obtain a high difference between these parameters in sorption and desorption isotherms. Table 1 shows that the pH 5 isotherm (pH controlled) presented the highest values for K_L and q_{max} of 0.031 L/g and 32.5 mg/L, respectively. In opposition, the pH 1 isotherm yielded values of 0.008 L/g and 5.0, respectively. This considerable difference could be usefully explored for a practical application of biosorption using an acid solution as eluent.

Although these adsorption models are able to fit quite well the experimental equilibrium data of biosorption, they do not necessarily reflect the actual phenomena taking place on the ionic or molecular level (VOLESKY, 2007). Nevertheless the parameters obtained from these models (table 1) may provide information on metal-uptake capacities between different biosorbents or operational conditions. Values of Langmuir's and Freundilich's constants obtained for the biosorption of copper (II) by different microorganism are listed in table 2. By comparing with the parameters in table 1, one sees that *Rhizopus microsporus* is among the four best microorganisms for biosorption as far as the sorption isotherm behavior is concerned.

Table 2. A comparison of Langmuir and Freundlich parameters of different biomasses for Copper (II).

			Langmuir		Freundlich		
Biosorbent	Initial pH	pH control	K_L (L/g)	q_{max} (mg/g)	K_f ($mg^{(n-1)/n}\ L^{1/n}/g$)	n	Reference
R.microsporus (Autoclaved)	5	No	0.02	13.41	0.61	1.77	Present work
R. microsporus (Autoclaved)	5	Yes	0.03	32.47	5.45	3.31	Present work
R. microsporus (Autoclaved)	1	Yes	0.01	5.03	0.25	2.06	Present work
C.cladosporioides (Inactivated)	6	N/A	0.10	9.43	1.67	2.78	(LI et al., 2009)
G.murorum (Inactivated)	6	N/A	0.09	10.90	1.72	2.56	(LI et al., 2009)
Bjerkandera sp. (Inactivated)	6	N/A	0.08	13.20	1.74	2.27	(LI et al., 2009)
A. niger (NaOH pretreated and Dried)	6	No	0.19	6.35	1.47	2.89	(KAPOOR et al., 1999)
A. niger (NaOH pretreated and Dried)	5	No	0.30	4.69	1.47	0.79	(KAPOOR et al., 1999)
H. lixii (Live)	5	No	0.01	7.20	0.44	2.27	(SALVADORI et al., 2013)
H.lixii (Dried)	5	No	0.03	8.00	0.59	2.56	(SALVADORI et al., 2013)
H. lixii (Autoclaved)	5	No	0.04	19.00	1.37	1.96	(SALVADORI et al., 2013)
A. niger ($Ca(NO_3)_2$ pretreated)	4	No	N/A	N/A	0.87*	0.49*	(MULLEN et al., 1992)
M. rouxxi ($Ca(NO_3)_2$ pretreated)	4	No	N/A	N/A	0.74*	0.55*	(MULLEN et al., 1992)
G. lucidum (No pretreatment)	5	Yes	0.90	28.45	9.97	2.03	(RAO et al., 1993)
G. lucidum (NaOH pretreated and	5	Yes	0.21	65.50	9.65	1.77	(RAO et al., 1993)

Dried)							
A. Niger (NaOH pretreated and Dried)	5	Yes	0.02	10.11	0.23	1.23	(RAO et al., 1993)
Treated sludge (NaOH pretreated and Dried)	5	Yes	2.76	16.31	9.44	4.90	(RAO et al., 1993)
P. crysosporium (Dried)	6	Yes	N/A	26.55	N/A	N/A	(SAY et al., 2001)
Beer yeast (HCl pretreated and dried)	5	No	0.14	1.45	0.02*	2.11*	(HAN et al., 2006)
Enteromorpha prolifera (Dried)	2	No	0.04	42.37	6.39	2.85	(ÖZER et al., 2009)
Enteromorpha prolifera (Dried)	3	No	0.03	52.08	6.55	2.32	(ÖZER et al., 2009)
Enteromorpha prolifera (Dried)	4	No	0.03	57.14	8.97	2.98	(ÖZER et al., 2009)
Enteromorpha prolifera (Dried)	5	No	0.03	52.63	7.11	2.85	(ÖZER et al., 2009)

* Unit used (mmol)

3.4. Sorption and desorption cycles

The regeneration of biomass is required to enable practical application of a biosorption process, as it influences both economic and environmental performances of the process (AKAR et al., 2007). In order to test the reusability of the biosorbent, the biosorption capacity was determined by repeating sorption-desorption cycles for three consecutive times. The results obtained for each cycle for two levels of initial copper (II) concentration (50 and 100 mg/L) are shown in figure 7. No significant changes in the biosorption capacity were found for level 2 experiments for three consecutive cycles, while level 1 experiments exhibited small variations in the response between cycles. Such difference may be attributed to experimental error, as the initial metal concentration should not influence de regeneration of the biomass. Similar results in which the biosorption capacity was maintained using HCl as eluent (concentration range of 0.01 to 0.1 M) were reported for five sorption-desorption cycles for the biosorption of cadmium by *Ascophyllum nodosum* (HOLAN et al., 1993) and copper by *Trametes versicolor* (BAYRAMOGLU et al., 2003) and for four cycles for the biosorption of lead by *Aspergillus paraciticus* (AKAR et al., 2007).

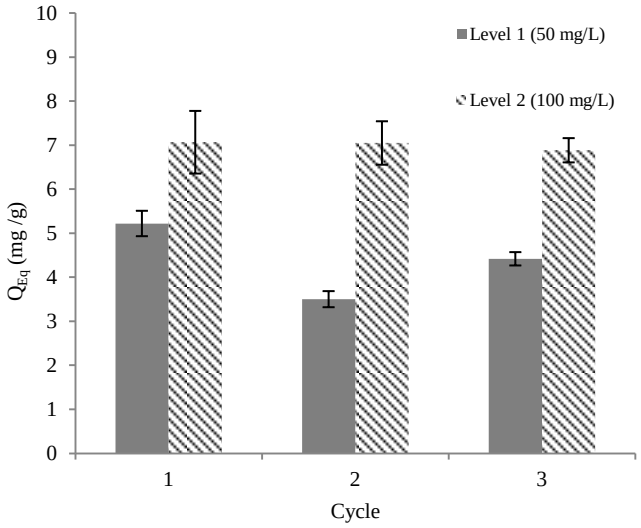

Figure 7. Biosorption capacity at sorption stage for 3 successive cycles.

The copper remaining in the biosorbent after each desorption step, given as the capacity of biosorption, is shown in figure 8. One concludes that the biosorption capacity did not change across three sorption-desorption cycles.

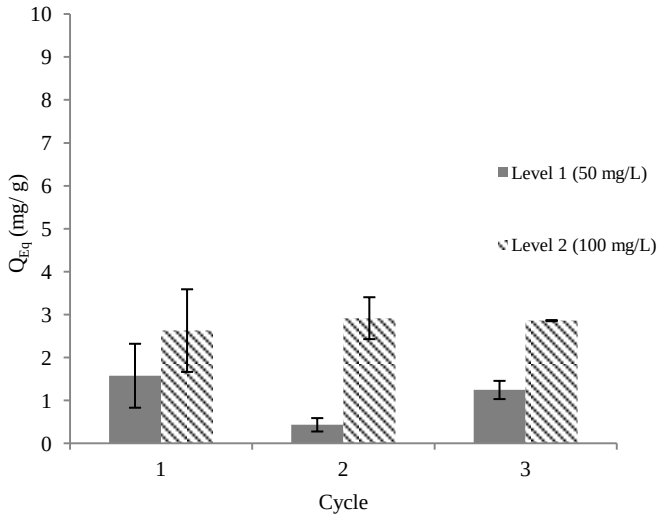

Figure 8. Biosorption capacity at desorption stage for 3 successive cycles.

Figure 9 shows simultaneously the results of the 3 cycles with the isotherms of pH 1 and 5 according to the Langmuir model obtained above. It is observed that such results correspond to equilibrium as given by the isotherms, differences may be attributed to different batches of biomass; this suggests that the interaction of copper with *Rhizopus microsporus* is a reversible process. Other authors also reported the reversibility of the biosorption process (GHAED et al., 2013; AKSU et al., 1992; MICHALAK et al., 2013).

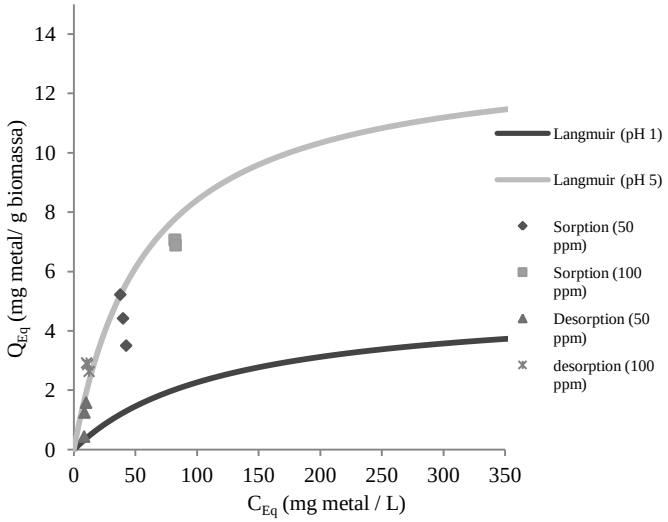

Figure 9. Biosorption capacity for the three regeneration cycles compared to the Langmuir model isotherms.

One of the main points in this process is that the biomass weight loss after the three cycles was 0.1 ± 3.2% (the error is probably due to measurement inaccuracy of dry mass) suggesting that the *Rhizopus microsporus* biomass is resistant against the eluent‘s acidic attack. Vijayaraghavan et al. reported a considerable weight loss of approximately 42 % in the desorption of the dye Reactive black 5 by Corynebacterium glutamicum using NaOH (0.01M) as eluent.

3.5. Kinetics studies

Kinetic parameters are of great importance for the implementation of practical decontamination processes of wastewater contaminated with heavy metals (AZIZIAN, 2004). In this study the biosorption extent (Q_t) of copper (II) was determined as a function of contact time in batchwise operation. The results are shown in figure 10. An increase in biosorption extent over time is observed, showing that the biosorption process is very fast at the beginning. After 4 hours the biosorption extent reached 97% of the biosorption capacity value (equilibrium). The literature reports a wide range of time intervals needed for equilibrium for the biosorption of copper (II). *Aspergillus niger* approached equilibrium within 6 hours (KAPOOR et al., 1999), *Cladosporium cladosporioides*, *Gliomastix murorum* and *Bjerkandera sp* within 2 hours (LI et al., 2009) and immobilized *Phanerochaete chrysosporium* within 1 hour (IQBAL and EDYVEAN, 2004). There are many parameters that influence the kinetics such as temperature, the structural properties both of the support and the biosorbent, agitation speed, the properties of the ion under study and the initial concentration of ionic species (BAYRAMOGLU et al., 2003). Therefore a quantitative comparison of biosorption kinetics must consider the many variables involved.

The biosorption kinetic data was tested against pseudo-first order and pseudo-second order kinetic models, as shown in Figures 11 and 12. The determination coefficients for the regressions are presented in table 3. It is observed that the data fit very well to both models. Values of R^2 were 0.992 for the pseudo-first order and 0.999 for the pseudo-second order

model. Other authors also reported good fitting with both models as in the biosorption of copper by *Cladosporium cladosporiodes* (LI et al., 2009) and by *Hypocrea lixii* (SALVADORI et al., 2013).

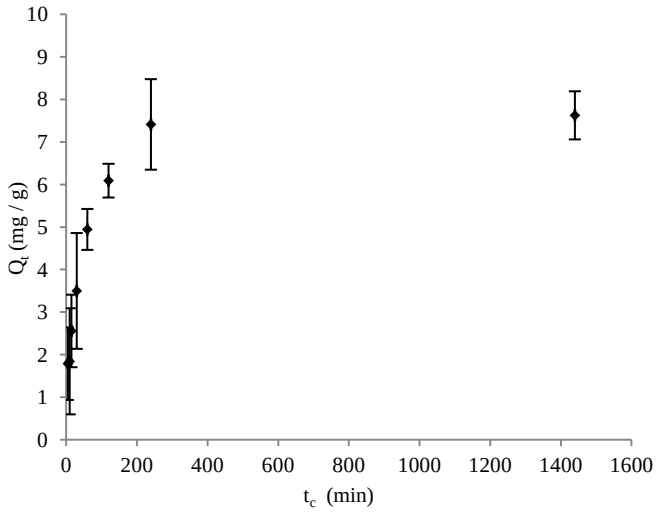

Figure 10. Biosortpion extent as a function of time. Initial copper (II) concentration of 150 mg/L, initial pH 5, agitation speed of 150 rpm and temperature of 25°C.

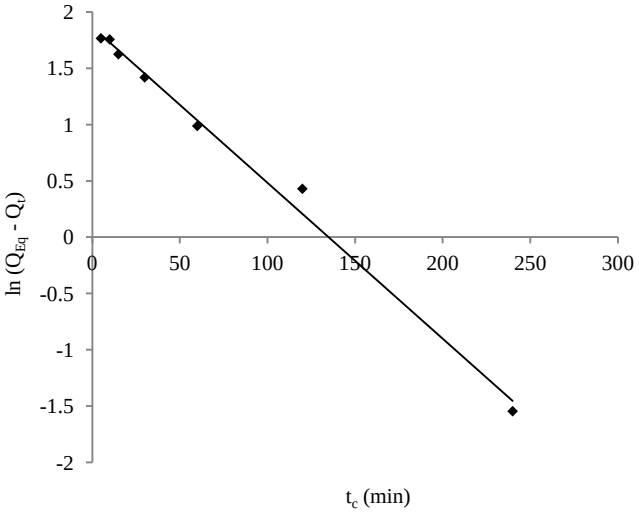

Figure 11. Linear plot of the pseudo-first order kinetic model.

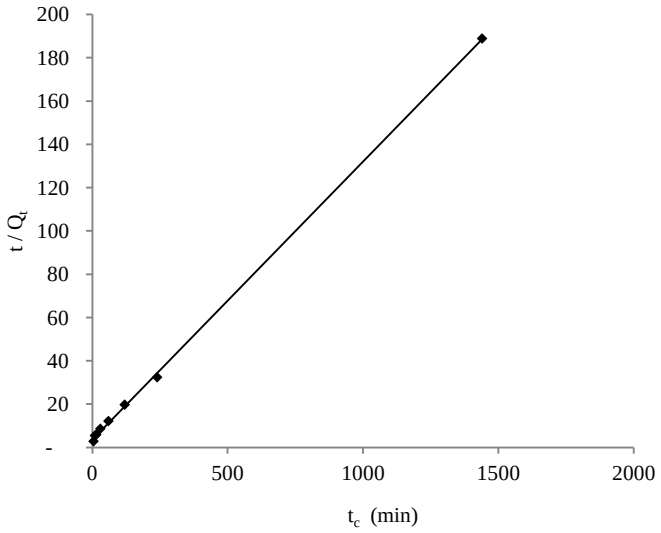

Figure 12. Linear plot of the pseudo-second order kinetic model.

Table 3. Parameters and determination coefficients for pseudo-first and pseudo-second order adsorption models

Pseudo-first order		Pseudo-second order	
K_1 (1/min)	R^2	K_2 (g / (mg min))	R^2
1.38.10-2	0.992	4.53E-03	0.999

4. Conclusion

The biomass of *Rhizopus microsporus* was found to be suitable for the biosorption of copper (II). The control of pH throughout the biosorption process was important to assure a high copper uptake. Under free pH the pH dropped to about 3, reducing substantially the metal uptake. Good results for the desorption process were found at pH 1. Both Langmuir and Freundlich adsorption models agreed well with the biosorption isotherms. The time required to approach equilibrium was approximately 4 hours. Both pseudo-first and pseudo-second order models presented very good fitting with kinetic data. The decrease of pH during biosorption process suggests that ion exchange is one of the main mechanisms involved in the interaction of copper with the fungus cell wall. It was found that an increase in pH greatly enhances the biosorption capacity, as its values varied from 1.95 mg/g to 20.38 mg/g for final pH 1 and 5, respectively. Biosorption at pH values higher than 5 were not carried out as metal precipitation may occur. The difference in sorption behavior at pH values of 1 and 5 was explored in a process involving the regeneration of the biosorbent in a concentrated acid eluent. The biomass was submitted to three sorption-desorption cycles. It was found that HCl (0.1M) is a proper eluent, as the sorption efficiency and the physical stability of the biomass were not altered. No significant weight loss was observed in the process; hence it could be a

suitable system for industrial application. The sorption-desorption cycles study also demonstrated that the biosorption of copper by *Rhizopus microsporus* is a reversible process. In order to try to enhance biosorption, autoclaving treatment was tested. However no significant effect was found in the biosorption capacity. It may be concluded that pretreated *Rhizopus microsporus* can be used as an effective source of biomass for copper (II) removal and recovery from aqueous solutions. Since this microorganism occurs naturally in the environments of copper contaminated areas, a simple yet biosafe process may be applied.

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Nomenclature

C_0 : Initial metal concentration (mg/L)

C_{Eq} : Equilibrium metal concentration (mg/ L)

K_1 : Lagergren rate constant of sorption (1/min)

K_2 : Pseudo second-order rate constant of sorption (g / (mg. min))

K_f : Freundlich adsorption constant ($mg^{(n-1)/n}\ L^{1/n}$ /g).

K_L : Langmuir adsorption constant (L/mg)

m_s : Dry fungal mass (g)

n : Freundlich adsorption constant

Q_{Eq} : Biosorption capacity at equilibrium (mg/g)

q_{max} : Monolayer saturation at equilibrium (mg / g)

Q_t : Biosorption extent (mg/g)

R^2: Determination coefficient

t: Time (min)

V : Solution volume (ml)

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