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RESEARCH ARTICLE

BIOSORPTION OF LEAD (II) FROM AQUEOUS SOLUTIONS BY THREE FUNGAL BIOMASSES

¹Ismael Acosta-Rodríguez, ¹Adriana S. Rodríguez-Pérez, ²Víctor M. Martínez-Juárez,
^{*1}Juan F. Cárdenas-González, ¹José F. Navarro-Castillo, and ¹María Eugenia Torre-Bouscoulet

¹Universidad Autónoma de San Luis Potosí, Facultad de Ciencias Químicas, Centro de Investigación y de Estudios de Posgrado, Laboratorio de Micología Experimental. Av. Dr. Manuel Nava No. 6, Zona Universitaria, 78320 San Luis Potosí, S.L.P. México

²Área Académica de Medicina Veterinaria y Zootecnia, Instituto de Ciencias Agropecuarias. Universidad Autónoma del Estado de Hidalgo, Zona Universitaria, Rancho Universitario Km 1. C.P. 43600, Tulancingo de Bravo Hidalgo, México

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ABSTRACT

The bio sorption of lead (II) on three fungal biomasses: *Mucor rouxii*-1, *Mucor rouxii*-2, and *Aspergillus flavus* were studied in this work. It was found that the biomasses of the three fungi were very efficient removing the metal in solution, using Dithizone, reaching the next percentage of removals: 96.1%, 91.5%, and 57%, for *M. rouxii*-1, *M. rouxii*-2, and *A. niger*, respectively. The highest adsorption was obtained at pH 4.0-5.0, at 28°C after 24 hours of incubation, with 1 g/100 mL of fungal biomass. It is concluded that the studied biomasses, can be used for the treatment of wastewater, specifically in metal removal heavy as lead (II).

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INTRODUCTION

Heavy metals are potentially toxic elements, whose presence in the environment has increased significantly in recent decades, mainly by the action of man. Metal contamination is an important to living things environmental threat, since various metals that are essential micronutrients such as copper and zinc, are toxic in high concentrations, while others such as cadmium, lead, mercury and arsenic are toxic to minimal doses (Tejada-Tovar *et al.*, 2015). Lead (Pb) and its derivatives are widely distributed in the environment: air, drinking water, rivers, lakes and oceans, soil, plants, animals, etc. This metal is used in the manufacture of pigments, coatings, containers, ointments, batteries electrical (Dao and Beardall, 2016) and currently it has numerous applications in industry Metallurgical: arms ammunition, metal bearings, cable cover, sheet lead, solders, pigments, and ceramic glaze.

*Corresponding author: Juan F. Cárdenas-González

Universidad Autónoma de San Luis Potosí, Facultad de Ciencias Químicas, Centro de Investigación y de Estudios de Posgrado, Laboratorio de Micología Experimental. Av. Dr. Manuel Nava No. 6, Zona Universitaria, 78320 San Luis Potosí, S.L.P. México

He Occupational exposure limit (TLV-TWA) has been of 50- μg lead/ m^3 (ATSDR, 2014). In addition, the phenomenon known as "pica" which it occurs in children who suck toys or objects paints or wrappers based lead salts is another source of pollution to consider (Sanz-Gallén and Marqués, 1995). Lead and Mercury were reported to easily cross the placenta and enter the fetal circulation of the blood (Iyengar and Rapp, 2001). Exposure to low levels of Pb may affect calcium transfer in human placental syncytiotrophoblasts (Lafond *et al.*, 2004), and has the indirect effect of brain development strain (Vejrup *et al.*, 2014), and more recently, it was detected in umbilical cord serum samples of mother-newborn pairs in Shengsi islands face the Yangtze River estuary and Hangzhou Bay in China (Mengling *et al.*, 2016). However, the use of lead as anti-knock additive is what most contributed to their accumulation in the environment (Lara-Viveros *et al.*, 2015), because the metal from the gas pump means 76% of its emissions (Moline *et al.*, 1999). Notable evidence of damage induced by lead exposure forced to in 1980 in the United States, use should be restricted tetraethyl lead in gasoline, as a control measure to reduce pollution environmental, so now the population It exposed and intoxicated with metal concentrates

primarily in industries or work activities employing lead compounds, in their workers or to nearby towns same (Calderón-Salinas and Maldonado-Vega, 2008; Arbabet *et al.*, 2015). For other hand, there are reports that mention some microorganism possess skills acquisition metals heavy, thanks to contain in their cell wall various chelating groups such as components carboxyls, phosphates, amides, thiols, hydroxyls, chitin, gluco-proteins, which play a role important in the biosorption of heavy metals (Arbabi *et al.*, 2015; Enamorado *et al.*, 2011): *Bacillus* sp isolated from soil (Tunali *et al.*, 2006), lead-resistant microorganisms, isolated from industrial simple (Chatterjee *et al.*, 2012), *Bacillus subtilis* CCEBI 1032, *Pseudomonas aeruginosa* CCEBI 1044, *Kluyveromyces marxianus* CCEBI 201 biomass (Tur-Naranjo *et al.*, 2013), the yeast *Saccharomyces cerevisiae* (PauroRoque *et al.*, 2009) and *Candida albicans* (Baysal *et al.*, 2009), the fungi *Aspergillus niger* O-5 (Enamorado *et al.*, 2011), *Aspergillus versicolor* (Mufedah and Nazareth, 2012), some mucorales (Cárdenas González *et al.*, 2012), *Penicillium purpurogenum* (Say *et al.*, 2003), indigenous fungal strains (Faryal *et al.*, 2007), *Aspergillus niger* and *Penicillium* sp (Ahmad *et al.*, 2006), and the basidiomycete *Phanerochaete chrysosporium* (Morales-Fonseca *et al.*, 2010). The algae *Lobophoravariegata* (Lamouroux) (Jha *et al.*, 2009), and *Enteromorpha* (Hammud *et al.*, 2016), and activated carbon fibers (Leyva-Ramos *et al.*, 2011). The objective of this work was to study the removal of Lead (II) in solution by the fungal biomass of *Mucor rouxii-1*, *Mucor rouxii-2*, and *Aspergillus flavus*.

MATERIAL AND METHODS

Microorganism, culture for biomass obtaining, and lead resistance test

A chromate-resistant filamentous fungi were isolated from polluted air from fuel station, near of Chemical Science Faculty, located in the city of San Luis Potosí, México, in Petri dishes containing modified Lee's Minimal Medium [LMM, Lee *et al.*, 1975] [with 0.25% KH_2PO_4 , 0.20% MgSO_4 , 0.50% $(\text{NH}_4)_2\text{SO}_4$, 0.50% NaCl, 0.25% glucose] supplemented with 500 mg/L $\text{Pb}(\text{NO}_3)_2$; the pH of the medium was adjusted and maintained at 5.3 with 100 mMol/L citrate-phosphate buffer. The fungi were grown at 28°C in an agitated and aerated liquid media containing thioglycolate broth, 8 g/L. After 7 days of incubation, the cells were centrifuged 3000 rpm for 10 min, washed twice with trideionized water and then dried at 80°C for 4 h in an oven. Finally, the fungal biomass was milled and stored in an amber bottle in the refrigerator until their use. Lead-resistant tests of the isolated strains, filamentous fungi *Mucor rouxii-1*, *Mucor rouxii-2*, and *Aspergillus niger*, were perform on liquid LMM containing the appropriate nutritional requirements and different concentrations of Lead (II) (as lead nitrate), and the dry weight was determined. Tree dependent experiments were carried out and the mean value was shown.

Lead (II) solutions

For analysis, were prepared a series of solutions of Lead (II) $\text{Pb}(\text{NO}_3)_2$ of 100 mg/L, pH was adjusted with nitric acid and/NaOH, and the quantity of biomass added to each flask was of 1 g/100 mL for the lead's solution. It taken samples at different times, the biomass is removed for centrifugation

(3000 rpm/5min) and the supernatant is analyzed to define the ion metal concentration.

Determination of Lead(II)

The concentration of lead ions in solution was determined by the colorimetric method of Dithizone, with which a complex is formed dithizonate of lead cherry red color, which read at an absorbance of 510 nm with a minimum detectable concentration of lead solution of 1.0 $\mu\text{g}/10$ mL of dithizone (Greenberg *et al.*, 1992).

RESULTS AND DISCUSSION

Fungal strains tolerance to lead (II)

The cells of the isolated strains: *Mucor rouxii-1*, *Mucor rouxii-2*, and *Aspergillus niger*, grew on LMM supplemented with 2 g/L of lead (II), about 29%, 31.8%, and 12.4%, of growth relative to controls (210, 201, and 193 mg of dry weight without metal) was obtained (Figure 1) and, therefore probably are resistant to the metal. Different microorganisms that are lead resistant have been isolated from different contaminated sites: Tunali *et al.*, (2006), reported a bacterial strain of *Bacillus* sp., isolated from soil with 1 g/L of Lead; Lead-resistant microorganisms, isolated from industrial simple, with different Lead concentrations (Chatterjee *et al.*, 2012), *P. chrysosporium*, tolerant to 10.000 mg/L of lead acetate (Morales-Fonseca *et al.*, 2010), *A. niger*, *Penicillium austrianum*, *S. cerevisiae*, *Mucor arcinoides*, and *Trichoderma reesei*, which grew with 100 mg/L of lead (Faryal *et al.*, 2007), *A. niger* RH 17 and *A. niger* RH 18, which showed maximum resistance up to 6000 and 7000 mg/L, respectively (Awofolu *et al.*, 2006), the cyanobacterium *Gloeocapsa* sp., grow at 20 mg/L (Raungsomboon *et al.*, 2008), and the aquatic macrophyte *Salvinia auriculata*, with 1-10 mg/L (Espinosa-Quñones *et al.*, 2009). This results indicate a change in the microflora, by an increase in the contamination by heavy metals in the places studies (Tejada *et al.*, 2015).

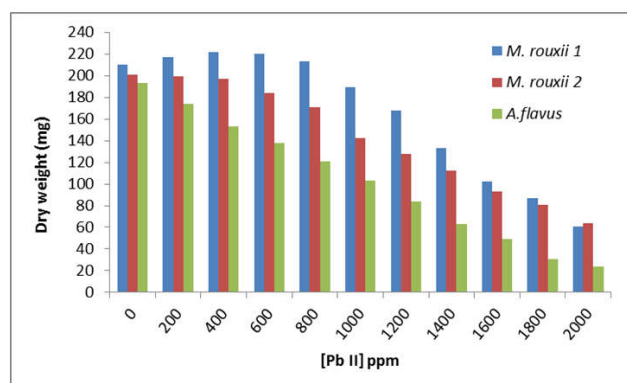


Figure 1. Growth in dry weight of *M. rouxii-1*, *M. rouxii-2*, and *A. flavus* with different concentrations of Pb (II), 1×10^5 spores/mL, 28°C, seven days of incubation, 100 rpm.

The effect of incubation time and pH

Figure 2 and 3 shows the effect of contact time and pH on biosorption of Pb(II) ions (100 mg/L) to the dried fungal biomass, it was found that the highest removal occurred at 24 h of incubation and pH 4.0-5.0 (96.1%, 91.5%, and 57%,

for *M. rouxii*-1, *M. rouxii*-2, and *A. niger*, respectively), and these results resemble those reported by *A. niger* (Awofolu *et al.*, 2006), corncob of *Zea mays* (Jiménez *et al.*, 2015), the yeast *S. cerevisiae* (Pauro Roque., 2009), and *Aspergillusniger*O-5 (Enamorado *et al.*, 2011), and they are different for indigenous fungal strains (Faryalet *et al.*, 2007) and for macrofungus *Lactariusscrobiculatus* (Anayurt *et al.*, 2009). The pH has been identified as one of the most important parameter that is effective on metal sorption. It is directly related with competition ability of hydrogen ions with metal ions to active sites on the biosorbentsurface (Leyva-Ramos *et al.*, 2011).

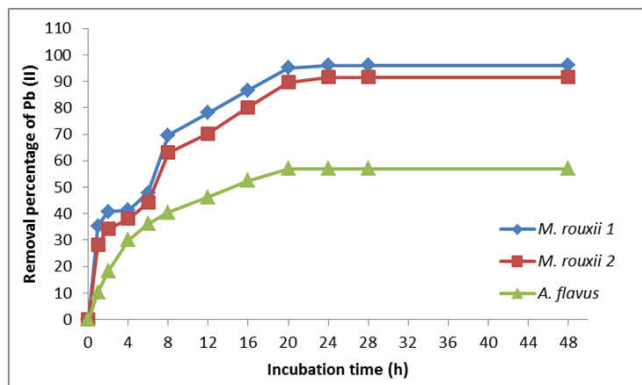


Figure 2. The effect of incubation time on the biosorption of lead (II).100mg/LPb(II), 28°C, 100 rpm, pH 4.0, g of fungal biomass of *M. rouxii*-1, *M. rouxii*-2, and *A. flavus*.

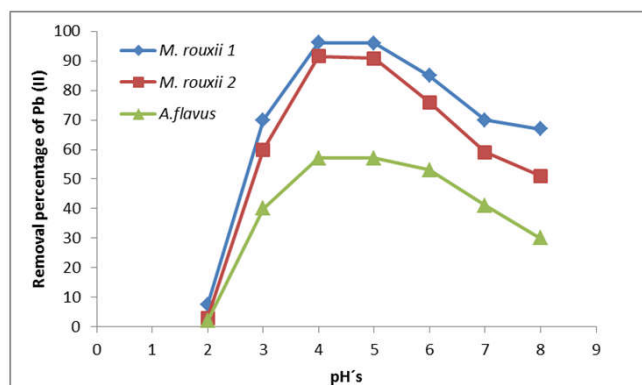


Figure 3. The effect of pH on the biosorption of Pb (II).100 mg/L Pb (II), 28°C, 100 rpm, 1g of fungal biomass of *M. rouxii*-1, *M. rouxii*-2, and *A. flavus*.

Effect of temperature

Figure 4 shows the effect of varying temperatures (28°C, 37°C, and 42°C), the maximal adsorption capacity was found at 28°C, (96.1%, 91.5%, and 57%, respectively), and the adsorption capacity of dried biomasses were 96.1%, 91.5%, and 57%, at 28°C, for *M. rouxii*-1, *M. rouxii*-2, and *A. niger*, respectively, they are similar to the reported for indigenous fungal strains (Faryal *et al.*, 2007) and for macrofungus *Lactariusscrobiculatus* (Anayurt *et al.*, 2009), and are different for *Candida albicans* biomass (Baysal *et al.*, 2009) and urea-modified *Triticumaestivum* (cotton) biomass (Farooq *et al.*, 2010). The temperature of the adsorption medium could be important for energy-dependent mechanisms in metal biosorption by microorganisms. Energy independent

mechanisms are less likely to be affected by temperature since the process responsible for biosorption is largely physicochemical in nature.

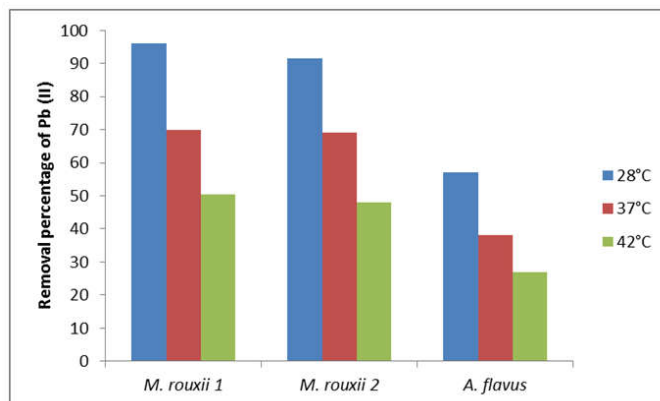


Figure 4. The effect of temperature on the biosorption of Pb (II).100 mg/L Pb (II), pH 4.0, 100 rpm, 1g of fungal biomass of *M. rouxii*-1, *M. rouxii*-2, and *A. flavus*

Effect of initial lead (II) concentration

Biosorption capacities of the fungal biomasses for the lead(II) ions, were studied as a function of the initial Pb(II) ions concentration between 200 and 1000mg/L in the biosorption medium (Figure 5), and the percentage of adsorption decreased, when ions concentration increased. A similar type of trend was reported for the removal of Pb(II) from aqueous solution by sorption on indigenous fungal strains (Awofolu *et al.*, 2006), the cyanobacterium *Gloeocapsa* sp., (Raungsomboon *et al.*, 2008), *Trametesversicolor* ATCC 13488 (Solis Pacheco *et al.*, 2015), are different for corncob of *Zea Mays* (Jiménez *et al.*, 2015), and squid *Ommastrephesbartrami* melanin (Chen *et al.*, 2009). These results may be explained to be due to the increase in the number of ions competing for the available binding sites and because of the lack of active sites on the biomass at higher concentrations (Wang *et al.*, 2001).

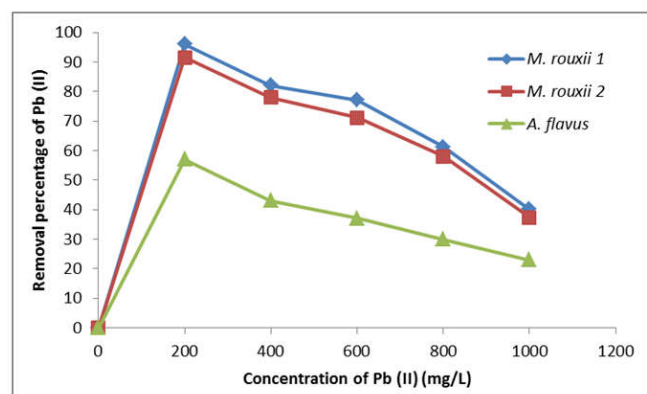


Figure 5: The effect of initial Pb (II) concentration on the biosorption of Pb (II). pH 4.0, 100 rpm, 1g of fungal biomass of *M. rouxii*-1, *M. rouxii*-2, and *A. flavus*

Effect of initial biomass concentration

The influence of biomass on the removal capacity of Pb (II) was depicted in Figure 6. If we increase the amount of biomass also increases the removal of the metal in solution (100% of

removal, with 5 g of fungal biomass, at 8, 12, and 16 hours, respectively), for the fungal biomasses analyzed, with more biosorption sites of the same, because the amount of added biosorbent determines the number of binding sites available for metal biosorption (Rezaee *et al.*, 2005). Similar results have been reported for macrofungus *L. scrobiculatus* (Anayurt *et al.*, 2009), urea-modified *Triticumaestivum* biomass (Farooq *et al.*, 2010), and loess clay based copolymer (Heet *et al.*, 2012).

M. rouxii-1, *M. rouxii*-2, and *A. flavus*, respectively, and with HCl 0.5M about of 87%, 86%, and 85%, for the three fungal biomasses analyzed. Similar results have been reported for corncob of *Zea Mays* (Jiménez *et al.*, 2015), squid *Ommastrephesbartrami* melanin (Farooq *et al.*, 2010), and loess clay based copolymer (He *et al.*, 2012). This indicates that those fungal biomasses a reasonable natural source to absorb heavy metal contaminants.

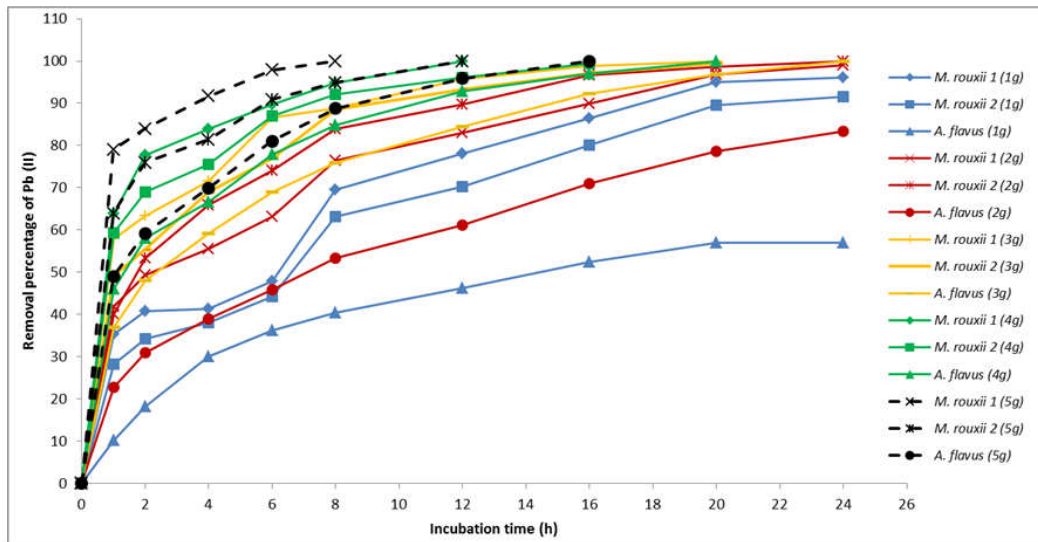


Figure 6. The effect of initial biomass concentration on the biosorption of Pb (II). pH 4.0, 100 rpm, 1-5 g of fungal biomass of *M. rouxii*-1, *M. rouxii*-2, and *A. flavus*

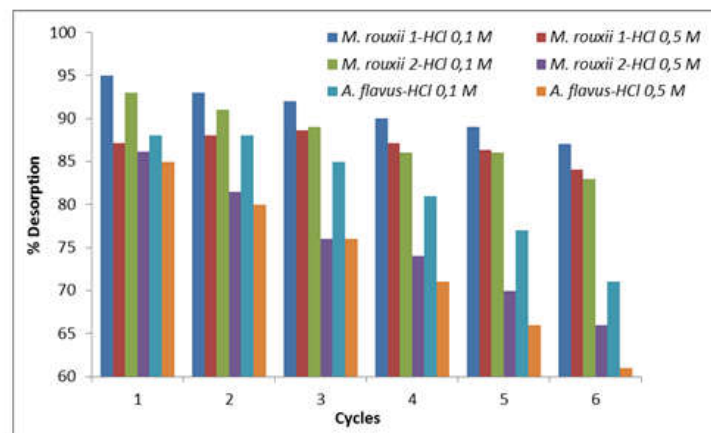


Figure 7. Effect of different concentration of HCl on desorption of Pb (II). 100 mg/L of Pb (II), 1g of fungal biomass, 100 rpm. 72 h. 28°C

Desorption

The recovery of heavy metals from metal-laden biomass has been approached by utilizing various desorption agents, including HCl, H₂SO₄, Na₂CO₃, EDTA, and mercaptoethanol (Farooq *et al.*, 2010; Chang *et al.*, 1994; Nakajima *et al.*, 1993). Among these approaches, decreasing the pH value using HCl and EDTA appears to have had the best desorption efficiency.

To determine the optimal HCl concentrations for metal desorption, the amount of metals released from the fungal biomasses at different concentrations of HCl was observed in Figure 7. Pb(II) can be almost completely recovered by washing twice with 0.1M HCl, about 95%, 93%, and 88%, for

Application on natural water

The fungal biomasses were successfully used for the removal of lead from water samples of "Tanque Tenorio", located southeast of the city, in the municipality of Soledad de Graciano Sánchez, San Luis Potosí, S.L.P., México, and is a lagoon of wastewater collection, which receives a high volume of wastewater discharges, of which 60% are from urban areas and 40% of industrial origin (note that the industrial area of San Luis Potosi has more than 253 companies, among which mining-metallurgical, textile and chemical (SEDECO 2004), and is a risk to health in surrounding communities (Sarabia Melendez *et al.*, 2011). The mean results of water quality before and after biosorption of studied water samples are shown in Table 1. It may be seen that after biosorption of

Pb(II), this was reduced between 84.75% and 88.63%, for *M. rouxii-2* and *M. rouxii-1*, and 49% for *A. flavus*, showing the efficiency of the biosorbents for the removal of Pb (II) ions from Lagoon water samples, and the results are similar for lead removal with Pb-resistant microorganisms, isolated from industrial simple (Chatterjee *et al.*, 2012), *A. niger* RH 17 and *A. niger* RH 18 (Faryalet *et al.*, 2007), *Phanerochaete chrysosporium* (Morales-Fonseca *et al.*, 2010), urea-modified *Triticum aestivum* biomass (Wang *et al.*, 2001), *Gossypium hirsutum* (cotton) waste biomass (Riaz *et al.*, 2009), and for Shell of Yuca and Name Tejada *et al.*, 2016).

Table 1. Removal of Pb (II) of natural water contaminated with 263 mg/L of Pb (II), 1 g of fungal biomass, 100 rpm, 28°C, different pH (adjusted), 48 h of incubation

pH	Percentage of Removal (%)		
	<i>M. rouxii-1</i>	<i>Mucor rouxii-2</i>	<i>Aspergillus flavus</i>
2.0	29.2	27.7	22.0
4.0	88.17	85.47	49.0
6.0	88.63	84.75	48.0

Conclusion

In this study, Pb (II) uptake by different fungal biomasses was investigated. The performance of the biosorbents was examined as a function of the operating conditions, in particular incubation time, pH and initial metal ion concentration and fungal biomass. The experimental evidence shows a strong effect of the experimental conditions. Maximum biosorption capacity values showed that some biosorbents used are very effective in recovery or removal of lead ion from aquatic systems. When the ease of production and economical parameters are concerned, it was observed that the fungal biomasses analyzed, are a very promising biomaterial for removal or recovery of the metal ion studied.

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