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BINARY BIOSORPTION OF Cu(II)-Pb(II) MIXTURES ONTO PINE NUTS SHELL IN BATCH AND PACKED BED SYSTEMS

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Abstract

The study of binary biosorption of Pb(II) and Cu(II) was performed by pine nuts shell. Results were compared with the single biosorption of Pb(II) and the single biosorption of the Cu(II). Experiments were performed in batch stirred and in a continuous system. The presence of lead caused a slightly decrease of the copper biosorption yield and can be related to the copper equilibrium uptake. This antagonistic action can be related to competition phenomena between metal ions in solution for active sites presented on the pine nut shell. The affinity of Pb(II) for pine nuts shell was higher than that of Cu(II). The extended Langmuir model and the extended Sips model were used to fit experimental data from binary experiments carried out in batch stirred system. Both models correctly fitted biosorption equilibrium data in range of concentrations studied. Differences obtained by both models were small, however, extended Sips model reproduced lightly better experimental results tan the extended Langmuir one. In the binary system, the maximum biosorption capacities obtained were 2.22 and 7.14 mg/g for Cu(II) and Pb(II) respectively. Finally, breakthrough curves in single systems for Cu(II) and Pb(II) were reported and they were compared with breakthrough curves for binary metal solutions. The competition between ions in the column were evaluated according to some obtained parameters, as biosorption capacity, amount of metal loading on pine nuts shell surface, total biosorption yield, breakthrough and exhausted times etc.

1. Introduction

Stone Pine (*Pinus pinea* L.) produces pine nuts that are edible seeds, which are highly appreciated all over the Mediterranean area. Spain is the main pine nut producing country with a 75% of the whole world production (Cárcel et al., 2012).

The nut comprises of a creamy white kernel of ovoid shape covered by a thin brown film inside a hard woody shell, which is arranged inside the pinecone. Once it has been extracted from the cone, the main operation of the processing is the cracking of the shell to get the fruit, a white and clean grain. In order to perform this operation the industrial cracking process is carried out using roller crackers, which compresses nuts between two cylinders. After the cracking and separation of the shell and the grain, the pine nut shell remains as an industrial waste. For this reason, the study of a use of this waste is important.

For almost 50 years in developed countries, intensive agriculture and industrial activity have shown up the release of important quantities of pollutants (especially, of heavy metals). During the operation and processing of these activities considerable volumes of residues (liquid and solid) are generated. Thus, high concentrations of heavy metals may be accumulated in soils and water (Lebeau et al., 2002; Sowinski et al., 2016). The liquid residues (or wastewaters) contain heavy metals, arsenic and other inorganic species in concentrations that exceed the

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local legal threshold values (Hansen et al., 2010). Thus, governments have established environmental restrictions with regard to the quality of wastewater, forcing industries to remove of metals from their effluents before discharging. Copper and lead are two of the toxic heavy metals which are common pollutants of the environment. Copper is an essential element for life, but at high concentrations copper is toxic to living organisms including soil microorganisms (Andreazza et al., 2010; Bansal et al., 2016). However lead has no known essential biological function, and all lead compounds are potentially harmful or toxic; all measured effects of lead are adverse, including those related to survival, growth, learning, reproduction, development, behavior, and metabolism (Ahmad et al., 2009; Chellvarajoo et al., 2017).

Different treatment alternatives exist such as chemical precipitation, electrodialysis, ion exchange, and adsorption. All of these technologies facilitate the removal of the inorganic compounds from the wastewater with either high or low efficiency but all are associated with important costs such as reagent addition, power consumption, limited life time of equipment, and general operational costs (Hansen et al., 2010). Biosorption as an alternative and effective technology has been widely studied over recent years, because of its wide range of target pollutants, high sorption capacity, excellent performance, ecofriendly nature and low operating cost (Farooq et al., 2010; Sud et al., 2008). Compared with traditional technologies, biosorption has advantages such as technically feasible and use of cheap material as biosorbents and lately, the study of biosorption has turned into the search for low-cost adsorbents (Veglio and Beolchini, 1997; McKay et al., 1999; Gupta et al., 2009). These are typically residues from industry or agriculture, which have been used in the treatment of industrial wastewater (Chong and Volesky, 1996; Yu and Kaewsarn, 1999; Doyurum and Celik, 2006; Fiol et al., 2006; Martínez et al., 2006; Bulut and Tez, 2007; Calero et al., 2010; López-Mesas et al., 2011; Xu and Chen, 2012).

While much research has been carried out on the uptake of single species of metal ions by low cost biosorbent, little attention seems to have been given to the study of metal ion mixtures (Andreazza et al., 2010). Nevertheless, as it is very improbable to have just one metal species in solution, recent research studies have been extended to systems concerning two or more metals (Mehta and Gaur, 2005; Romera et al., 2006; Khani, 2013; Vijayaraghavan and Joshi, 2014). These studies have focused mainly on the effect of a metal on the sorption capacity of another metal by the biomass and the affinity of the biomass for both metals. In this sense, the study of multimetallic systems is more complex than of monometallic systems, as in these systems are competition phenomena between metals by the biomass active sites (Volesky, 2003; Pehlivan and Altun, 2008; Pehlivan et al., 2009). On the other hand, although recent studies on biosorption have shown that pine nuts shell

can be used as potential biosorbent for the removal heavy metals (Sag et al., 1996; Li et al., 2001; Calero et al., 2011). These studies have evaluated the biosorption capacity of the pine nuts shell in single metal batch systems. However, there is little knowledge about binary biosorption of metals ions, and less about these systems in continuous flow.

In the present work, kinetic and equilibrium biosorption parameters were obtained from the application of mathematical models. Besides, the interaction between the metals ions in competitive studies for their biosorption on the pine nuts shell was placed on continuous removal of both metals from single and binary solutions assessed from experiments in a packed bed column. The physicochemical characteristics of biosorbent were evaluated.

2. Material and methods

2.1. Pine nuts shell

The pine nuts shell used in the present study is supplied by Carsan Biocombustibles S.L. factory from Granada (Spain). Particles of pine nuts shell were milled with an analytical mill (IKA MF-10) and <1 mm fraction was chosen for the biosorption tests without any pre-treatment.

2.2. Metal solutions

Stock solutions of 1000 mg·L^{-1} for Pb(II) and Cu(II) were prepared, by dissolving desired amount of Pb(NO$_3$)$_2$ and Cu(NO$_3$)$_2$·3H$_2$O in 500 mL of distilled water. After, rest of solutions of different concentrations was prepared by appropriate dilution of both above stock solutions.

2.3. Experimental procedure

The biosorption tests were performed by batch and continuous techniques in monometallic and bimetallic systems.

2.3.1. Bach experiments

The batch adsorption experiments were conducted in a thermostatic shaker at 25 ºC with 100 mL of the aqueous solutions of Cu(II) and Pb(II) ions and 1 g of the pine nuts shell. The pH is initially adjusted to 5 and it is kept constant in this value with 0.1 M HCl and 0.1 M NaOH solutions. After 120 min the final Pb(II) and Cu(II) concentrations were analyzed using Atomic Absorption Spectrometry (Perkin-Elmer Model AAnalyst 200). The adsorption experiments were conducted to analyze the effect of lead in biosorption of copper, so, experiments were performed changing initial copper concentration from 10 to 300 mg·L^{-1} and for three initial lead concentrations (20, 50, and 100 mg·L^{-1}).

2.3.2. Column experiments

The column adsorption experiments were performed in a fixed-bed column at constant temperature (25ºC). The column was a jacket glass

tube with an internal diameter of 1.5 cm and a length of 23 cm packed. Glass spheres of 5 mm diameter were placed at the bottom of the column prior the active biomass bed to ensure homogenous distribution of the feeding solution. A determined weight of pine nuts shell (corresponding to a determined bed height) was packed into the column. Glass beads are not mixed with adsorbent. The density and porosity of pine nuts shell bed are approximately 0.74 g·cm⁻³ and 40.0%, respectively.

The experiments were conducted by pumping the metal solution from a 10 L storage tank in up flow mode through the fixed-bed column with a peristaltic pump (Dinko model D21 V) at different flow rates. The pH value in the storage tank was adjusted to 5.0 (this value was considered the optimum for the biosorption process of copper in previous studies (Martín-Lara et al., 2012; Calero et al., 2013a) using a 0.1 M HCl and 0.1 M NaOH solutions. Samples of the column effluent were collected at predefined regular time intervals and were analyzed by atomic absorption spectrometry (Perkin-Elmer Model AAnalyst 200) for determining the final Pb(II) and Cu(II) concentrations. Operation of the column was stopped after a determined operation time (around 260 min).

3. Mathematical description

One of the difficulties in studying the adsorption of metal ions from wastewaters is the presence of a multitude of metals. When in the aquatic system there are more of one metal, may occur a competition between metals. So that, the evaluation, interpretation and representation of results is more complex than in single systems, and usually, can be occur several types of responses (Sag and Kutsal, 1996; Puranik and Paknikar, 1999; Li et al., 2001; Apiratikul and Pavasant, 2006; Srivastava et al., 2006; Ofomaja et al., 2010; Shouaib et al., 2011; Al-Quahtani, 2012):
- the effect to bimetallic-system is higher than effect to each metal present in the mixture (synergistic behavior).
- the effect to bimetallic-system is lower than effect to each metal present in the mixture (antagonistic behaviour).
- the effect to bimetallic-system is not higher neither lower than effect to each metal present in the mixture (without interaction).

3.1. Biosorption equilibrium of binary system Cu(II) - Pb(II) in batch systems

Equilibrium models for multimetallic systems attempt to provide the relation between the retained amount of one component and the concentration of other components, whether they remain in solution as if they have been retained.

Most of isotherms proposed to describe this type of isotherms are based on three considerations (Apiratikul and Pavasant, 2006; Aksu, 2002; Hammaini et al., 2002):

1. sorption is competitive: models are developed under concept to the original model of Langmuir, where a binding site can be occupied by a single sorbate.

2. sorption is not competitive: models are developed under assumption that sorbates can be retain simultaneously in the same binding site.

3. sorption is partially competitive: models are developed under assumption that a sorbate can be binded a free same binding site or one occupied by other sorbate.

Isotherms based on competitive sorption are the most commonly used to describe bimetallic systems and can be classified in two groups according to relation with isotherms for a monometallic system (Bellot et al., 1993; Sag et al., 1998; Aksu et al., 2006):
a. Competitive isotherms related only to parameters of single isotherms: competitive Langmuir model (often called extended Langmuir model), competitive Freundlich model (often called extended Freunlich model) etc.
b. Competitive isotherms related to parameters of single isotherms and correction factors: competitive modified Langmuir model, competitive modified Freundlich model etc.

The two models applied in this work are described below. These models have been selected to be the most commonly used to describe multimetallic systems and to be those who best represented results obtained in this work.

Extended Langmuir isotherm

Assuming that solid surface is uniform and all sorbate ions present in solution compete by the same binding superficial sites, the extended Langmuir isotherm can be represented by Eq. (1) (Srivastava et al., 2006):

$$q_{ei} = \frac{b_i\, q_{max}\, C_{ei}}{1 + \sum_{j=1}^{N} b_j\, C_{ej}} \qquad (1)$$

where the subscript "i" refers to the species to which it applies the model and subscript "j" refers to each of the "N" species present in the system, q_{ei} is the metal uptake at equilibrium (mg metal "i"/g of the biosorbent), C_{ei}, the equilibrium concentration of the metal "i" in the solution (mg/L), q_{max} is the maximum capacity (mg/g) and b_i is the Langmuir affinity constant for metal "i"(L/mg). The values of q_{max} and b_i can be obtained by fitting of equation with experimental data for a multimetallic system. For a binary system Eq. (1) is broken down into Eqs. (2, 3):

$$q_{e1} = \frac{b_1\, q_{max}\, C_{e1}}{1 + b_1\, C_{e1} + b_2\, C_{e2}} \qquad (2)$$

$$q_{e2} = \frac{b_2\, q_{max}\, C_{e2}}{1 + b_1\, C_{e1} + b_2\, C_{e2}} \qquad (3)$$

Those equations would be resolved together to obtain model parameters. In this model the q_{max} value is exclusively for metals and due to assumption discussed above about surface uniformity and competition by the same binding site of sorbates. When maximum capacities change for different multimetallic systems, one possible explanation is that the active sites are not homogeneous, i.e. some places are specific to certain heavy metals. However, this fact is not consistent with Langmuir model considerations.

Extended Sips isotherm

One of model most used to study the equilibrium of multicomponent systems is the combination between Langmuir and Freundlich models, developed by Sips (1948) (Sips, 1948). This model shows a better reproduction of experimental data using additional parameters. The equation can be given by Eq. (4) (Alimohamadi et al., 2005):

$$q_{ei} = \frac{q_{mi}\, b_i\, C_{ei}^{\frac{1}{n_i}}}{1 + \sum_{j=1}^{N} b_j\, C_{ej}^{\frac{1}{n_j}}} \qquad (4)$$

where $q_{m,i}$ (mg/g), b_i ($(L/mg)^{1/ni}$) and n_i are the model constants.

That for a binary system can be decomposed into Eqs. (5, 6):

$$q_{e1} = \frac{q_{m1}\, b_1\, C_{e1}^{\frac{1}{n_1}}}{1 + b_1\, C_{e1}^{\frac{1}{n_1}} + b_2\, C_{e2}^{\frac{1}{n_2}}} \qquad (5)$$

$$q_{e2} = \frac{q_{m2}\, b_2\, C_{e2}^{\frac{1}{n_2}}}{1 + b_1\, C_{e1}^{\frac{1}{n_1}} + b_2\, C_{e2}^{\frac{1}{n_2}}} \qquad (6)$$

where q_{m1}, b_1, n_1 and q_{m2}, b_2 and n_2, are model constants for the first and second component respectively.

Both models have been adjusted using Excel for Windows by minimizing of percentage of Marquardt standard deviation (MPSD) that responds to Eq. (7):

$$MPSD = 100 \sqrt{\frac{1}{d-p} \sum_{j=1}^{N} \left(\sum_{i=1}^{d} \left(\frac{q_{e,ji,exp} - q_{e,ji,cal}}{q_{e,ji,exp}} \right)^2 \right)} \qquad (7)$$

where d is the number of experimental data points, p is the number of parameters in the isotherm equation, N is the total number of metal ions, $q_{e,exp}$ is the experimental metal uptake at equilibrium and $q_{e,cal}$ the calculated metal uptake at equilibrium.

Besides this parameter, it is also estimated the sum of squared residuals (SSR), represented by Eq. (8):

$$SSR = \sum_{j=1}^{N} \left(\sum_{i=1}^{d} \left(q_{e,ji,exp} - q_{e,ji,cal} \right)^2 \right) \qquad (8)$$

3.2. Biosorption of mixture Cu(II) - Pb(II) in a packed bed column

After batch studies of biosorption in binary system Cu(II) – Pb(II), binary sorption experiments were carried out in a packed bed column with different fractions of Cu(II) and Pb(II) to analyze the effect of presence of both metals in solution. From data obtained in previous tests (Calero et al., 2011, 2013a), it has performed two types of experiments:

I. With experimental conditions which optimize percentage of copper removal: Q = 2 mL·min^{-1}, $C_{i\,total}$ = 40 mg/L (Cu/Pb 20/20 mg·L^{-1}) and biosorbent dose = 15 g.

II. With experimental conditions which optimize biosorption capacity of copper: Q = 6 mL·min^{-1}, $C_{i\,total}$ = 100 mg/L (Cu/Pb 50/50 mg·L^{-1}) and biosorbent dose = 5 g.

The breakthrough curves of experiments type I and type II were represented and the most significant parameters of breakthrough curves were obtained from these according to equations 9 to 14.

- Volume of the effluent, V_{ef} (mL) (Eq. 9):

$$V_{ef} = Q\, t_{total} \qquad (9)$$

where t_{total} is the total flow time (min) and Q is the volumetric flow rate (mL·min^{-1}).

- Total mass of metal biosorbed (the area under the breakthrough curve) for a defined feed concentration (Eq. 9):

$$q_{total} = \frac{Q}{1000} \int_{t=0}^{t=t_{total}} C_R\, dt \qquad (10)$$

where q_{total} is the total mass of metal biosorbed (mg) and C_R is the concentration of metal removal (mg·L^{-1}).

- Total amount of metal ions sent to the column (Eq. 11):

$$m_{total} = \frac{C_i\, Q\, t_{total}}{1000} \qquad (11)$$

- Total metal removal (% R):

$$\%\ Removal = \frac{q_{total}}{m_{total}} 100 \qquad (12)$$

- The amount of metal biosorbed at equilibrium or biosorption capacity, q_e (mg of metal sorbed/g of sorbent), and the equilibrium metal concentration, C_e (mg·L^{-1}):

$$q_e = \frac{q_{total}}{w} \qquad (13)$$

$$C_e = \frac{m_{total} - q_{total}}{V_{ef}} 1000 \qquad (14)$$

where w is the mass of the biosorbent (g).

- *Exhausted time*: When the volume of the fluid begins to flow through the column, the mass-transfer zone varies from 0% of the inlet concentration (corresponding to the solute-free sorbent) to 100% of the inlet concentration (corresponding to the total saturation). From a practical point of view, the exhausted time, t_{ex}, is established when the concentration in the effluent is higher than 90-95% of the inlet concentration;

- *Service or breakthrough time*: The breakthrough time, t_b, is established when the metal concentration in the effluent reaches a determined value, generally related to the permitted disposal limit for each metal.

The experimental results for seconds experiments (Figs. 5b, 6b and 7b) have been fitted to three models show through non-linear regression, using the Marquardt algorithm.

Thomas model

The Thomas model is a mathematical description which given of the performance of a cation exchange column for the case in which the rate of the exchange is determined by a second order law (Thomas, 1944).

$$\frac{C}{C_i} = \frac{1}{1 + \exp\left(\frac{k_{Th}}{Q}(q_0 m - C_i V_{ef})\right)} \qquad (15)$$

where C is the solute concentration in the liquid phase in mg/L, C_i is the initial metal concentration in the solution, mg/L, k_{Th} is the Thomas rate constant in mL/min·mg and q_0 is the maximum concentration of the solute in the solid phase in mg/g.

Yoon-Nelson model

This model introduces a theoretical model that addressed the breakthrough of a contaminant and shows a reproduction of experimental data using additional parameters (Eq. 16) (Yoon and Nelson, 1984).

$$\frac{C_i}{C} = \frac{1}{1 + e^{k_{YN}(\tau - t)}} \qquad (16)$$

where k_{YN} is the Yoon and Nelson's proportionality constant in min-1 and τ is the required time to retain 50% of the initial adsorbate in min.

Dose-Response model

This is a modified model of Thomas or Bohart-Adams model. This model describes more adequately the column kinetics for metal removal and minimizes the error resulting from use of the Thomas model, especially at lower or higher time periods of the breakthrough curve (Yan and Viraraghavan, 2001).

$$\frac{C}{C_i} = 1 - \frac{1}{1 + \left(\frac{C_i V_{ef}}{q_0 m}\right)^a} \qquad (17)$$

where a is the constant of the Dose-Response model.

4. Results and discussion

4.1. Characterization of pine nuts shell

Table 1 shows the physicochemical characteristics for the pine nuts shell. Characterization of biosorbent is vital to understanding the metal binding mechanism onto biomass. Elemental analysis results shown that pine nuts shell was composed of 46.30% C, 6.92% H, 0.45% N and 46.28% O. on the other hand, the moisture content of pine nuts shell was of 6.36%. Respect to physical properties, pine nut shell shown values inside of range of other agroindustrial residues: the BET surface area was 0.579 m²·g⁻¹, the pore volume was 0.973 cm³·g⁻¹ and the pore diameter 54.4 Å.

About the potentiometric titrations, pine nuts shell showed a high total concentration of acid-basic sites, assuming an acid character with a pH_{pzc} value of 6.11. Finally, in the FTIR spectra numerous peaks were appreciated indicating the complex nature of the material. The analysis of peaks showed the presence of carboxyl and hydroxyl groups characteristic of these types of waste. These physic-chemical properties (high surface area, high total concentration of acid-basic sites, the presence of some characteristic groups…) make that pine nuts shell could be a good biosorbent.

The most of obtained values for physic-chemical properties are in the same range that other similar biosorbents. For example, Calero et al., 2013b studied the olive tree pruning and they obtained values of 0.631 m²/g for BET surface area, 1.500 cm³/g for total volume pore and an elemental composition of 51.27, 6.75, 0.70 and 41.23 % for Carbon, Hydrogen, Nitrogen and Oxygen respectively. Besides they obtained the same main groups in the biosorbent as responsible of biosorption properties. Ronda et al., (2014) studied the olive stone and the pine bark and they obtained values of 0.418 and 0.560 m²/g for BET surface area of olive stone and pine bark respectively, pH_{pzc} values of 5.17 and 4.84 for olive stone and bine bark respectively and an elemental composition of 52.34, 7.11, 0.03 and 40.47 % for olive stone and 48.15, 5.51, 0.38 and 45.91 % for pine bark for Carbon, Hydrogen, Nitrogen and Oxygen respectively.

All these biosorbents showed good properties as biosorbent of metals. The higher affinity of biomass for the lead ions that for the copper one was also obtained by Hawari and Mulligan (2007).

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Table 1. Physico-chemical characterization of pine nuts shell

| *Physical characterization*	
BET surface area	0.579 m^2/g
Pore volume	0.973 cm^3/g
Pore diameter	54.4 Å
Particle size	< 1mm
Potentiometric titrations	
Total concentration of acid-basic sites	0.428 mmol/g
Concentration of carboxylic group	0.240 mmol/g
pH$_{PZC}$	6.11
Elemental analysis	
Carbon	46.30 %
Hydrogen	6.92 %
Nitrogen	0.45 %
Sulphur	0.05%
Oxygen	46.28 %
FTIR analysis	
Wavenumbers, cm^{-1}	**Group**
3330	O-H
2922	C-H$_n$ aliphatic
1727	C=O
1506	C=C
1250	C-O carboxylic or ester
1030	C-O alcoholic
897 and 667	C-H$_n$ aliphatic or aromatic

This behavior was obtained by the most authors that revealed that in biosorption studies of multimetallic systems, the functional groups presented more affinity by some ions that by others (Han et al., 2006).

4.2. Batch biosorption form single and binary Pb(II)-Cu(II) solution onto pine nuts shell

The optimal value of pH was determined in previous studies of both metals separately, and the value obtained was 5 (Calero et al., 2010; Martín-Lara et al., 2012). Besides, at this pH, it has not occurred precipitation processes for any of two metals. First it have performed experiment with mixtures Cu(II)/Pb(II) (5/5, 20/20, 50/50 and 100/100 mg·L^{-1}), keeping constant a value of pH 5 and the behaviour of the mixture Pb(II)-Cu(II) with respect to the single-metal biosorption was analyzed.

Figure 1 shows the total percentage removal and the percentage for each metal at different concentrations of copper and lead. Results show that increasing of initial concentrations of metals in solution, decreases the percentage removal of each metal and the total. It is also observed that the lead has more affinity for pine nuts shell than copper, because the percentage removal of lead is always higher in all bimetallic systems, although at lowest concentrations (5/5) the percentage removal of both metals was similar.

Besides, the percentage removal of copper decreases more strongly than of lead when the total initial concentration of metals increases. To show more clearly these results, Fig. 2 shows the biosorption capacity of Pb(II) and Cu(II) versus total initial concentration of both metals. The biosorption capacity increased with increasing metal ion concentration and the increase was a lot higher for lead.
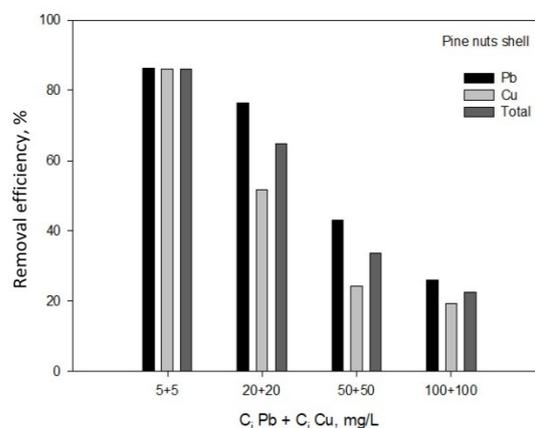
Fig. 1. Effect of presence of lead in copper biosorption by pine nuts shell at different concentrations of both metals

The binding strength of a metal ion to the biomass is dependent upon other different factors such

as hydration effects, hydrolysis effects, ionic rate, the electronegativeness or covalent index of a metal ion.
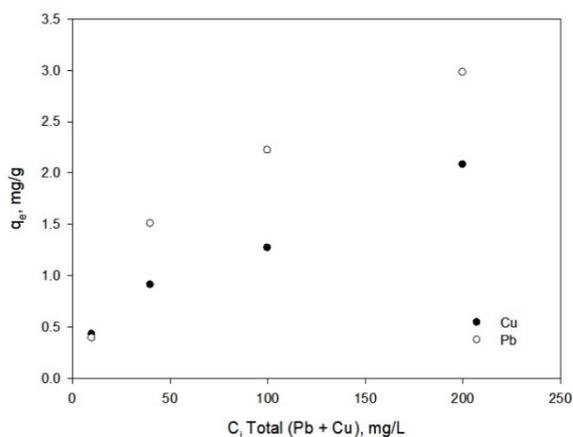
Fig. 2. Biosorption capacity of lead and copper by pine nuts shell versus initial concentration of both metals

In general, the larger the effective hydrated radius, the larger the hydration energy of an ions; the hydrolysis constants shown a linear relationship with the adsoption capacity; ions with larger ionic rate or covalent index usually have a higher affinity for biosorbents, etc. So, it is produced more competitiveness between ions with similar characteristics (Igbal and Edyvean, 2004; Aksu et al., 2006; Han et al., 2006; Chen et al., 2007; Fagundes-Klen et al., 2007; Hawari et al., 2007; Vilar et al., 2008; Remenarova et al., 2012).

Table 2 illustrates values of ionic radius, electronegativeness and covalent index of both metals present in binary system used (Donbebe et al., 2006). Ionic properties of lead are higher than copper ones. So, the ionic radius for lead was 1.20 and for copper 0.69; the covalent index for lead was 11.1 and for copper 5.56. Values for lead were approximately twice more than copper ones, and it may justify above results.

Table 2. Comparison of some physical-chemical properties (electronegativeness, ionic radius, covalent index and molecular weight) of copper and lead ions

Properties	*Lead*	*Copper*
Electronegativeness, X_m	2.33	1.90
Ionic radius, r	1.20	0.69
Covalent index, $C_I = X_m^2(r + 0.85)$	11.1	5.56
Molecular weight, g/mol	207.20	63.55

Effects of the presence of lead ions on the biosorption of copper ions were investigated in terms of equilibrium isotherm. Data of single metals (single lead and copper) were used to predict the binary biosorption data using the single metal biosorption parameters reported in a previous work (Calero et al., 2011). In Fig. 3 it is observed that although the influence of both metals was not very important, the equilibrium uptake of Cu(II) increased slowly with presence of Pb(II). In the single system, the maximum uptake obtained was 4.20 mg/g for Cu(II), while the uptake obtained in the binary metal solution were 1.82, 1.95 and 2.17 mg/g for at the lead concentrations of 20, 50 and 100 mg/L respectively. This inhibitory effect of Pb(II) ions on the equilibrium Cu(II) uptake seems simple to explain. The presence of the other component develops a competition for the active sites on the surface and some sites are occupied by the second component. As a consequence, the first component has a smaller number of accessible active sites and its uptake is decreased.
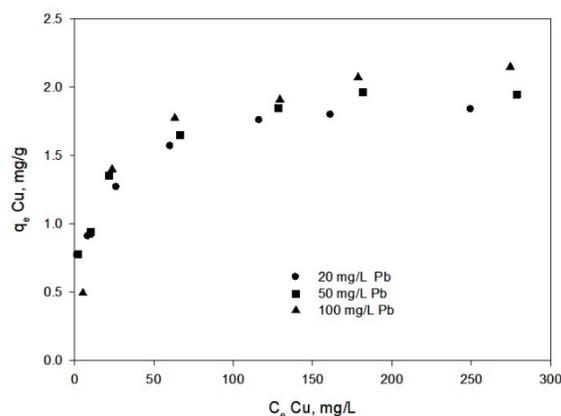
Fig. 3. Values of copper biosorption capacity versus copper equilibrium concentration in solution for each of initial lead concentrations tested using pine nuts shell

These results have been fitted two models: extended Langmuir isotherm and extended Sips isotherm. The values of constant and parameter of fit for each model are summarized in Table 3. Extended Sip model is better obeyed by the binary system than the Langmuir one (lower values of SSR), although differences between two models are not significant. To analyze better results, in Table 4 are shown values of biosorption capacity of Cu(II) and Pb(II) obtained in single metal system in previous works (Calero et al., 2011) and values obtained with extended Sips model for binary system.

It is clearly illustrated that pine nuts shell has higher affinity by lead approximately three times more, thus in single system, as in binary system. The biosorption capacities values of copper and lead in single system were 4.293 and 11.261 mg/g respectively, and in binary systems 2.224 and 7.142 mg/g respectively. These values are related to size and molecular weight of ions, so, taking into account the molecular weight and comparing values in mmol/g both values are very similar: 0.0675 and 0.0543 mmol/g for copper and lead ions in single system and 0.0350 and 0.0345 mmol/g for copper and lead ions in binary system. However, the regulations of heavy metals levels in wastewater are usually presented in mg/g and for it results are shown commonly with this unit. The equilibrium data for binary biosorption of copper and lead ions may be analyzed in form of

biosorption capacity of one metal ion in the presence of the other metal ion, Q_m and to the biosorption capacity for the same metal ion when it is present alone in the solution, Q_s, (Ofomaja et al., 2010). So that, there are three possible cases:

$$\frac{Q_m}{Q_s} > 1$$

The biosorption is promoted by the presence of the other metal ion.

$$\frac{Q_m}{Q_s} = 1$$

There is no observable net interaction.

$$\frac{Q_m}{Q_s} < 1$$

The biosorption is suppressed by the presence of the other metal ion.

In this case, the value obtained of Q_m/Q_s for copper ions is approximately half a unit (0.518), suggesting that lead produces interaction onto copper when both metal ions are present in solution. The value obtained for lead ions (0.634) is also lower than unity but higher that for copper one, suggesting that presence of copper in solution reduces less the biosorption of lead.

Plots of 3-D surfaces are one of most common methods used to represent equilibrium data of multimetallic systems. For binary mixtures, it is represented in X and Y axes the equilibrium concentrations of both metals and in Z axis the biosorption capacity of the metal analyzed. In Figure 4 the two (equilibrium) metal concentrations were plotted against the copper uptake and the biosorption surface was obtained by extended Sips model. In this figure are represented in X and Y axes the equilibrium concentrations of copper and lead respectively, and biosorption capacity of lead in Z axe. It is observed that the model reproduce acceptably experimental results, according with fitting parameters obtained in previous analysis.

These results are in agreement with those found in literature about the study of multimetallic systems biosorption using different biosorbents, particularly to binary systems Cu(II) – Pb(II). Most of authors agree that multicomponent systems study is complex due to interaction and competition processes between different metals present in solution, besides of the complex procedure to obtain experimental isotherms. On the other hand, some of proposed models are, sometimes, too simple to describe the complexity of multimetallic systems, and other, too complex to be used. However, there are numerous models that can be used for biosorption systems with several species in solution, which is most common on industrial effluents (Sag and Kutsal, 1996; Aksu, 2002; Hammaini et al., 2002; Apiratikul and Pavasant, 2006; Srivastava et al., 2006; Remenarova et al., 2012).

4.3. Biosorption of mixture Cu(II) – Pb(II) in a packed bed column

The breakthrough curves of experiments type I for mono and bi-component solutions (Figure 5a, 6a and 7a) were practically as a horizontal line it indicated that pine nuts shell removed practically the total of metals presented in solution. The breakthrough curves of experiments type II for bi-component solutions (Fig. 7b) show different shapes than those observed for mono-component test (Figs. 5b and 6b), this can be attributed to the direct influence of metal cations that compete for a limited number of binding sites on biomass. Most significant parameters of breakthrough curves were obtained. Table 5 shows results for copper and lead respectively, both single like in binary systems.

Furthermore, Table 5 shows parameters of total breakthrough curves in binary system Cu/Pb. From experiments type I (Figs. 5a, 6a and 7a), values of percentage removal nearly to 100% were obtained for copper and lead ions. It also observed that biosorption capacity of pine nuts shell for copper was generally less than that of lead ions, thus for single metals as when they are together in solution, although differences are not significant.
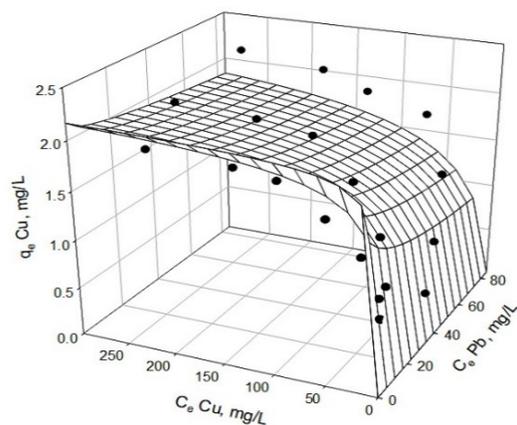

Fig. 4. Experimental values of copper biosorption capacity and biosorption surface obtained by extended Sips model for pine nuts shell
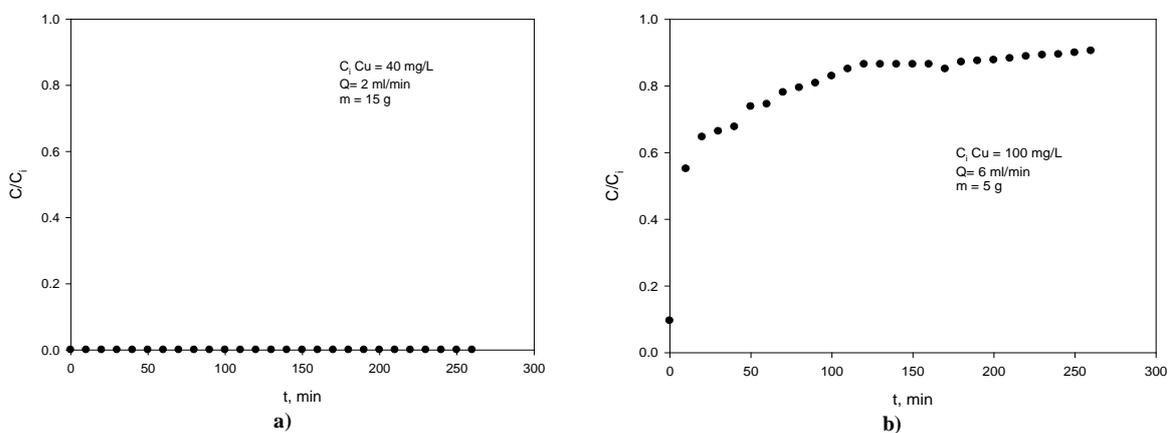
From experiments type II (Figs. 5b, 6b and 7b), different behaviours of biosorbent were obtained for copper and lead when they are together in solution. So, for single systems, biosorption capacity for lead (20.25 mg·g^{-1}) is higher than copper one (7.14 mg·g^{-1}), indicating the higher affinity of lead for pine nuts shell. On the other hand, the breakthrough time was practically the initial time and the bed exhaustion time was 240 min for copper while for lead, the breakthrough time was 35 min and the exhaustion of column were not attained. Comparing results when two metals are together (Table 5), the total biosorption capacity (11.62 mg·g^{-1}) is higher than for copper, but lower than for lead.

**Table 3.** Constant values and fitting parameters of copper and lead for Langmuir and extended Sips models

Extended Langmuir model					
q_{max} mg·g^{-1}	b_{Cu}	b_{Pb} L·mg^{-1}	MPSD	SSR	
3.067±0.210	0.512±0.092	1.551±0.149	56.88	49.39	
Extended Sips model					
q_{mCu}	q_{mPb} mg·g^{-1}	n_{Cu}	n_{Pb}	b_{Cu}	b_{Pb} (L·mg^{-1})$^{1/n}$	MPSD	SSR
2.224±0.406	7.142±0.758	1.312±0.174	1.930±0.151	0.485±0.048	0.703±0.095	34.94	21.24

**Table 4.** Biosorption capacities values of copper and lead in single and binary systems

Single isotherm q_e, mg/g		*Isotherm in binary system q_e,mg/g*		Q_m/Q_s	
Cu	**Pb**	**Cu**	**Pb**	**Cu**	**Pb**
4.293±0.182	11.261±0.469	2.224±0.406	7.142±0.758	0.518	0.634

Fig. 5. Breakthrough curve for copper: a) Q = 2 mL·min^{-1}, C$_i$ = 40 mg·L^{-1}; biosorbent dose = 15 g;
b) Q = 6 mL·min^{-1}, C$_i$ = 100 mg·L^{-1}; biosorbent dose = 5 g
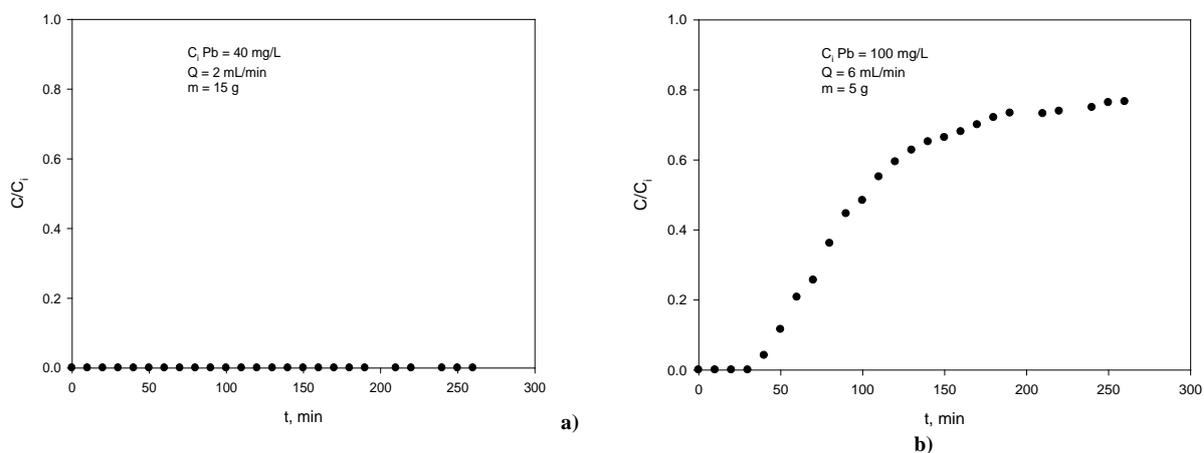
Fig. 6. Breakthrough curve for lead: a) Q = 2 mL·min^{-1}, C$_i$ = 40 mg·L^{-1};
biosorbent dose = 15 g; b) Q = 6 mL·min^{-1}, C$_i$ = 100 mg·L^{-1}; biosorbent dose = 5 g
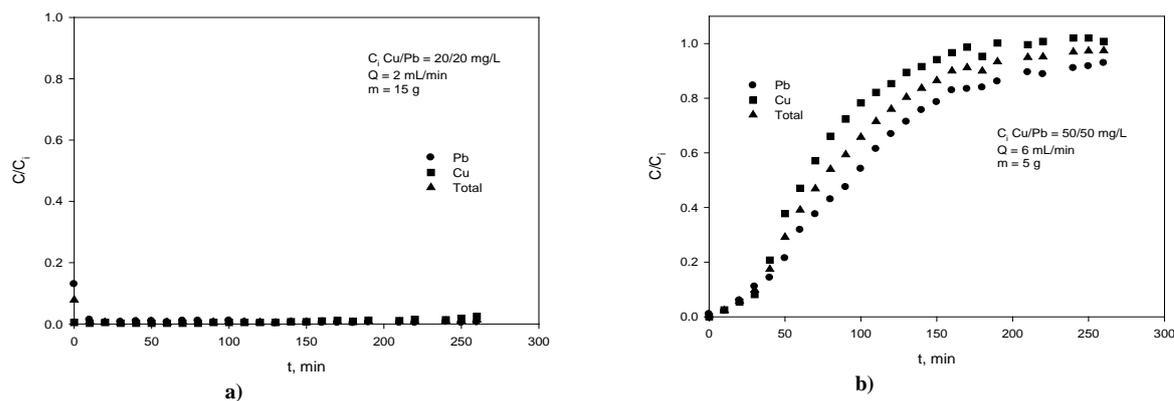
Fig. 7. Breakthrough curve for copper and lead in binary system: a) Q = 2 mL·min^{-1},
C$_i$ total = 40 mg·L^{-1} (Cu/Pb = 20/20 mg·L^{-1}): biosorbent dose = 15 g; b) Q = 6 mL·min^{-1},
C$_i$ total = 100 mg·L^{-1} (Cu/Pb = 50/50 mg·L^{-1}); biosorbent dose = 5 g

It could be related to batch experiments, i.e., the presence of copper inhibits the lead removal. Finally, in this case, the breakthrough time was 50 min and the bed exhaustion time was 170 min. Table 6 reported values of the models parameters from fitting models for experiments type II (Figs. 5b, 6b and 7b) through non-linear regression. Although, it was noted that results obtained of biosorption capacity with the models (q$_0$) were, in some cases, different to experimental results, in general, models fitted adequately the experimental data, with high values of r^2 and low values of SSR. It is observed that Dose-Response is the best model that represented the system, with the highest values of r^2 (>0.975 in all cases) and the lowest values of SSR (<0.074 in all cases). The differences between the other two models (Thomas and Yoon-Nelson models) are not significant.

5. Conclusions

Results showed that pine nut shell was an effective biosorbent for the removal of copper and lead in single and binary systems. In batch mode and binary system, the total percentage removal was higher that copper removal, but lower that lead removal in all mixtures tested.

For example, at initial concentration 20+20 mg·L^{-1} (Pb+Cu), the approximately total percentage removal was 65%, and approximately percentages removal for copper and lead were 52 and 77% respectively. So, in continuous mode, the effect observed was similar, the biosorption capacity for lead, copper and two metals together are 20.25 mg·g^{-1}, 7.14 mg·g^{-1}, and 11.62 mg·g^{-1}, respectively.

That was related to the competence between metal cations for the same active sites of the biomass. The experimental results were analyzed using the extended Langmuir and Sips models. Sips model fitted better the results, although differences between two models are not significant. Dose-Response is the best model that represented the system in packed bed column.

Table 5. Characteristic parameters of copper, lead and total breakthrough curves

	V$_{eff}$, mL	q$_{total}$, mg	m$_{total}$, mg	R, %	q$_e$, mg/g	C$_e$, mg/L	t$_b$, min	t$_{ex}$, min
Copper breakthrough curve								
Figure 5a	520	17.84	18.72	95.30	1.19	1.70	--	--
Figure 5b	1560	35.69	179.77	19.85	7.14	92.36	--	240
Figure 7a	520	10.53	10.61	99.24	0.70	0.158	-	-
Figure 7b	1560	20.76	75.50	27.50	4.15	35.09	50	140
Lead breakthrough curve								
Figure 6a	520	28.65	28.65	100.0	1.91	0.00	-	-
Figure 6b	1560	101.26	197.64	51.23	20.25	61.78	35	-
Figure 7a	520	14.97	15.18	98.62	0.998	0.410	-	-
Figure 7b	1560	63.96	92.04	69.49	12.79	17.00	90	-
Total breakthrough curve								
	520	25.59	25.79	99.22	1.71	0.385	-	-
	1560	58.12	167.54	34.69	11.62	70.14	50	170

Table 6. Thomas, Yoon-Nelson and Dose-Response model parameters at different inlet Pb(II) concentrations

Model	Parameters	Figure 5b	Figure 6b	Figure 7b		
				Cu	Pb	Total
Thomas	k_{Th}, mL/mg·min	0.139	0.182	0.869	0.506	0.318
	q_0, mg/g	0.403	14.876	4.063	5.838	9.759
	r^2	0.889	0.881	0.988	0.979	0.985
	q_{total}, mg	2.02	74.38	20.31	29.19	48.80
	$\sum[(C_i/C)_{exp}-(C_i/C)_{cal}]^2$	0.234	0.244	0.038	0.052	0.043
Yoon and Nelson	k_{YN}, min^{-1}	0.014	0.018	0.043	0.025	0.032
	τ_{cal}, min	3.4	123.9	67.7	97.3	81.3
	τ_{exp}, min^{-1}	8.0	110.0	65.0	95.0	75.0
	r^2	0.889	0.881	0.988	0.979	0.985
	$\sum[(C_i/C)_{exp}-(C_i/C)_{cal}]^2$	0.234	0.244	0.038	0.052	0.043
Dose-Response	a	0.630	1.935	2.975	2.307	2.524
	q_0, mg/g	1.042	13.312	3.766	5.282	8.862
	q_{total}, mg	5.21	66.56	18.83	26.41	44.31
	r^2	0.977	0.975	0.996	0.997	0.998
	$\sum[(C_i/C)_{exp}-(C_i/C)_{cal}]^2$	0.016	0.052	0.013	0.074	0.006

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