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Sugar-beet pulp pectin gels as biosorbent for heavy metals: Preparation and determination of biosorption and desorption characteristics

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ABSTRACT

The present work reports the feasibility of using sugar-beet pectin gels for the removal of heavy metals from aqueous solutions. Sugar-beet pectin hydro- and xerogels were tested in the batch biosorption and desorption of cadmium, lead and copper. Pectins were successfully extracted and demethylated from the sugar-beet pulp, an agricultural residue, and gelled in the presence of $CaCl_2$. The stability of the hydro- and xerogel pectin beads made them suitable for biosorption of heavy metals in different conditions. Biosorption data were fitted to the pseudo-second order kinetic model and the Langmuir isotherm model, obtaining the corresponding parameters. Treated and untreated beads were characterized using FTIR and SEM to determine possible binding mechanisms. The main mechanisms involved were ion exchange with calcium of gel structure and chelation or complexation with carboxyl groups. After biosorption, calcium in the gels was substituted by metal cations reorganizing the structure of the gel matrix in a way that was visible using scanning electron microscopy. HNO_3 0.1 M was the best eluant for the reutilization of the gels and recovered all the adsorbed metal unlike HCl and H_2SO_4. Sugar-beet pectins could be used as an efficient biosorbent for the treatment and recovery of Cu, Pb and Cd from wastewater.

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1. Introduction

Heavy metals are highly recalcitrant elements with a high potential to pollute water resources that can be accumulated and concentrated in living tissues along the food chain. Copper, lead and especially cadmium are a sanitary and ecological threat, even at very low concentrations. The recovery of these metals from effluents not only ensures that they do not reach the environment but also help to preserve natural resources. Furthermore, in the case of copper, the steadily increasing market price is another incentive for a profitable recovery.

Traditional methods for recovering heavy metals from effluents are generally expensive or inadequate to treat highly diluted effluents. These methods include: chemical precipitation and filtration, redox reactions, electrochemical treatments, reverse osmosis, ion exchange, adsorption and evaporation. Biosorption is a cost effective alternative that can be appropriate in the purification of effluents with low metal concentrations and can also be used to remove other pollutants such as dyes or organic compounds. It is a property of certain types of organic matter or biomass (biosorbents) to passively bind metals on chemically active sites or functional groups [1]. The type of biomass used determines the metal uptake and the selectivity of the recovery process. The use of dead biomass makes the process nutrient-independent, faster and increases the metal uptake [2].

Different models can describe the biosorption kinetics, such as the pseudo-first order model proposed by Lagergren and the pseudo-second order model proposed by Ho and McKay [3]. The maximum metal uptake and the affinity of the biomass for a certain metal can be obtained from the sorption isotherms. Although there are different isotherm models, the Langmuir model is, by far, the most used in simple systems.

Recently attention has been addressed towards byproducts or wastes from large scale industrial operations and agricultural waste materials based on their availability, high efficiency, easy handling and low cost [4]. Sugar-beet (*Beta vulgaris* L.) pectins can be obtained from sugar-beet pulp, a residue of the sugar processing industry. Compared to other pectins obtained from other sources, like citrus, apple and sunflower pectins, sugar-beet pectins have the advantage that the raw material is already dried and does not depend on stationality. Sugar-beet pulp is sold as animal feed at very low prices and is readily available for revalorization. Just in Spain more than 200,000 tons are generated per year (Grupo Ebro Puleva). Sugar-beet pulp has a high pectin content (15–30%), but these pectins have poor gelling properties compared to citrus and apple pectins due to their high degree of methylation and low molecular weight. Moreover, sugar-beet pectins have not been extensively used in traditional applications in the food industry,

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mainly because of their high neutral sugar and low galacturonic acid content [5]. Therefore, the development of alternative applications for these pectins, such as biomass immobilization and the biosorption of heavy and precious metals, is highly desirable.

Pectins are polysaccharides of the middle lamella and primary cell wall in which they are crosslinked with cellulose and hemicellulose fibers. The structure of pectin is complex and can vary depending on the source and the extraction method. It is a polysaccharide composed of galacturonic acid units with α(1,4) bonds, which constitute the "smooth regions". In the "hairy regions", the rhamnose units in the carbon skeleton are branched with secondary chains, mainly arabinans, normally lost during extraction. Other residues are: methanol, acetic acid, phenolic acid, and amides. Ferulic acid is a characteristic group of sugar-beet pectins. Therefore, the main functional groups of pectin are: hydroxyl, carboxyl, amide and methoxyl. These functional groups have been traditionally associated to heavy metal binding, especially carboxyl groups with a great biosorption and heavy metal removal potential [1]. Additionally, pectins in granulated form such as gel beads can be used for continuous applications such as fixed-bed columns. This makes sugar-beet pectins an interesting alternative to similar polysaccharides such as alginate that are already widely used and accepted.

Most of the pectins present in the sugar-beet pulp are high methoxyl and have more than 50% of methoxylated residues. They gel at low pH values and in the presence of a high concentration of soluble solids. The resulting gels dissolve quickly in water and have a soft consistency and therefore have no application in the biosorption of metals or in the immobilization of biomass. Low-methoxyl pectins have less than 50% of methoxylated residues and can be obtained from high-methoxyl pectins by demethylation. These pectins are sensitive to gelation with divalent cations such as calcium according to the "egg-box model" proposed by Rees but methoxyl groups are an impediment for the formation of the calcium bridges [6]. Their gels are stable in aqueous solutions and can be used in similar applications like those of alginate including biomass immobilization and heavy and precious metal biosorption, among others [7].

Demethylation occurs at low temperatures and in an alkaline media through a base-hydrolysis of the esther groups (saponification) [5,8]. At neutral or alkaline pH values pectin degradation takes place by the β-elimination of the glycosidic bond that is adjacent to the esterified units of the galacturonic acid. The degradation increases with temperature and is parallel to the demethylation process. Different types of pectin demethylation methods can be used: acid, alkali, ammonia and enzyme treatments [8]. Harel et al. [7] proposed a sugar-beet pectin demethylation method using ammonia that yielded gels with enough mechanical strength and insolubility, suitable for biosorption applications.

After biosorption, the elution of metals could be interesting for the reutilization of exhausted biomass and the recovery of the adsorbed metals. Desorption can be carried out by proton exchange using acids, by exchange with other ions (for example $CaCl_2$) or by chelating agents (EDTA). An efficient eluant is one that desorbs the metal completely without deteriorating the biomass in case it will be reused.

The aim of this work was to determine the effectiveness of sugar-beet pectin gels for the biosorption and desorption of Cd^{2+}, Pb^{2+} and Cu^{2+} from aqueous solutions. There are few studies in the literature related to metal binding with sugar-beet pectins, and only one with calcium gels for one metal, cadmium [7,9,10]. Studies concerning to desorption and reuse of metal-loaded biosorbents are also very rare and none address the use of pectins or their gels. In the present work, sugar-beet pectins were extracted and demethylated to obtain hydro- and xerogels which were used for the biosorption and desorption of three heavy metals: cadmium, lead and copper. The stability of the gel beads in aqueous solutions was tested and the optimum conditions for the biosorption were determined. The biosorption data were fitted to the pseudo-second order kinetic model, and the Langmuir isotherm model, and the corresponding parameters were obtained. This equilibrium and kinetic information is useful to design a full-scale batch process and to predict biosorbent performance and effectiveness. In order to confirm the demethylation and determine possible metal binding mechanisms after metal biosorption, the pectins and their gels were characterized using infrared and microscopy analysis. Finally, the best desorbent of the metal-loaded xerogels was chosen from three types of inorganic acids (HCl, H_2SO_4 and HNO_3 0.1 M).

2. Materials and methods

2.1. Biosorbents

Azucarera Ebro Agrícola provided the sugar-beet pulp from the Toro plant in Zamora, Spain. The pulp was collected directly from the final drying line to ensure freshness. Sugar-beet pectin was extracted based on the protocol proposed by Harel et al. [7]. The sugar-beet pulp was washed repeatedly with tap water and filtered with cheesecloth to remove the molasses. After that, a 5% pulp suspension in 0.3 M H_2SO_4 was heated during 4 h at 80 °C in a water bath. The solids were filtered and the remaining liquid was treated with a solution of 95% ethanol until a pectin precipitate was formed. This ethanol solution becomes impregnated with alcohol soluble residues and cannot be reused. The pectin was filtered and washed repeatedly with ethanol solution: twice with 70% ethanol, and once with 85% and 95% ethanol successively, with enough volume to cover the pectin precipitate in each step. The remaining solid was dried in a stove at 35 °C and ground in an agate mill. The remaining ethanol solution from the last wash (95%) can be mixed and reused with the ethanol of the initial precipitation step after extraction.

The acid-extracted sugar-beet pectin had a high methylation degree and was not suitable for calcium gelation, therefore the pectin had to be demethylated. The demethylation method was adapted from the methods proposed by Harel et al. [7] and Le Cerf et al. [11]. A solution of 2% pectin in deionized water was stirred for at least 2 h and, as described for the extraction process, precipitated with 95% ethanol and filtered with cheesecloth. The pectin was cooled to 4 °C and a solution of ammonia 1 M at the same temperature was added until a 2% pectin solution was obtained. This solution was stirred until homogenized and kept 12 h without stirring at 4 °C. As described earlier, it was precipitated with a 70% ethanol solution, filtered and washed again twice with the ethanol solution. After filtering a third time it was left 6 h stirring in the 70% ethanol solution. For the last washes it was filtered and resuspended in a 85% and 95% ethanol solution successively. The remaining solid was dried in a stove at 35 °C and ground with an agate mortar.

The demethylation procedure was effective in increasing the calcium sensitivity of the pectins and the preparation of calcium pectate beads. Solutions of different concentrations of pectin (1.5%, 2%, 3.5% and 5%) were dropped into different cooled 1 M $CaCl_2$ solutions (0.2, 0.6 and 1 M) to determine the influence of pectin and calcium concentrations on the gelation process. Visual and tactile ("finger test", according to [8]) evaluation determined that the optimum gelation conditions were 1.5% pectin dropped on a 4 °C 1 M $CaCl_2$ solution. The viscous solution was pressed through a syringe (internal diameter of 0.5 mm) to ensure good reproducibility. The beads were kept at 4 °C at least 24 h in the same solution. Excess $CaCl_2$ was rinsed with distilled water. The diameter of the hydrogel beads formed was 3 ± 0.2 mm. The beads were air dried at room temperature (23 ± 1 °C) to obtain the xerogel beads, that measured approximately 1.4 ± 0.2 mm of diameter.

In order to determine the stability at different pH and stirring conditions, hydrogel (0.3 g) and xerogel (0.01 g) beads without biomass were placed in contact during 48 h with a battery of solutions (50 ml) at different initial pH values: 1, 2, 3, 4, 6 and 8, adjusted with diluted H_2SO_4, HCl or NaOH, with and without orbital stirring. Initial and final weights of the gels before and after treatment were recorded. Final pH values and calcium concentration in solution were also measured. Calcium concentration was measured by AAS analysis using a PerkinElmer 1100B Atomic Flame Absorption Spectrometer.

2.2. Biosorption experiments

Biosorption experiments were carried out at room temperature ($23 \pm 1\,^{\circ}C$) with monometallic solutions prepared with chemical reagents of analytical grade: $CdSO_48/3H_2O$, $Pb(NO_3)_2$ and $CuSO_45H_2O$ from stock solutions of 1000 mg/l of Cd^{2+}, Pb^{2+}, and Cu^{2+}. In the case of lead, nitrate instead of sulfate was used to avoid metal precipitation, though the effect of this anion is negligible [12]. The initial pH value of the metal solutions was adjusted with diluted 0.1 M H_2SO_4 for Cd and Cu, HNO_3 for Pb, and 0.1 M NaOH as needed. For the optimum pH studies, 75 ml of metal solution (100 mg/l) were placed in contact with the pectin gel beads (1 g/l) in an orbital shaker at 150 rpm at the following initial pH: 3, 4 and 6 for cadmium, and 3, 4 and 5 for lead and copper. Special care was taken to select values that were below each metal's hydroxide precipitation pH for the metal concentrations used in this study, to ensure that the metal uptake was only due to biosorption and not chemical precipitation. Due to their different levels of hydration, the amount of hydro- and xerogels used in each experiment was calculated with respect to their pectin content. 1 g/l pectin corresponds to 3 g of hydrogel beads or 0.1 g of xerogel beads. This allowed discarding the water content of the gels and estimating the metal uptakes as a function of the actual amount of pectin. Therefore, biosorption data per gram of biosorbent refer to the amount of pectin contained in the gels and not to the gel weight.

The initial pH values with the highest metal uptakes per gram of pectin were chosen to continue with the experimentation. The metal recovery of different initial pectin concentrations (0.5, 1 and 2 g/l) was compared. The gel beads were put in contact with the metal solutions and liquid samples were taken at different times (0, 15, 60, 120, 240, 480 and 1400 min) for pH and AAS analysis. The initial pH values with the highest metal uptakes per gram of pectin beads were chosen. All the experiments were performed in duplicate.

In all cases, the metal uptake was calculated from the following equation:

$$q_t = \frac{C_0 - C_t}{B} \quad (1)$$

where q_t is metal uptake at time t (mmol/g of pectin); C_0 the initial metal concentration (mmol/l); C_t the metal concentration at time t (mmol/l); B is the biomass concentration (g/l).

Based on previous studies, the proton consumption during the kinetic experiments was calculated from the change in pH with respect to the initial pH values using the expression [12]:

$$H^+\text{consumed/g of biomass} = \frac{10^{-\text{initial pH}} - 10^{-\text{final pH}}}{\text{g of biomass}} \quad (2)$$

2.3. Biosorption kinetics study

Experimental data of the optimum pH studies were fitted to the pseudo-second order kinetic model. The following equation describes the pseudo-second order kinetics in its linearized form [3,13]:

$$\frac{t}{q_t} = \frac{1}{k_2 q_2^2} + \frac{1}{q_2} t \quad (3)$$

where q_2 is the maximum metal uptake for the pseudo-second order kinetics (mmol/g of pectin) and k_2, the pseudo-second order rate constant (g/mmol min).

The values of q_2 and k_2 can be deduced from the linear representation of t/q_t vs. t.

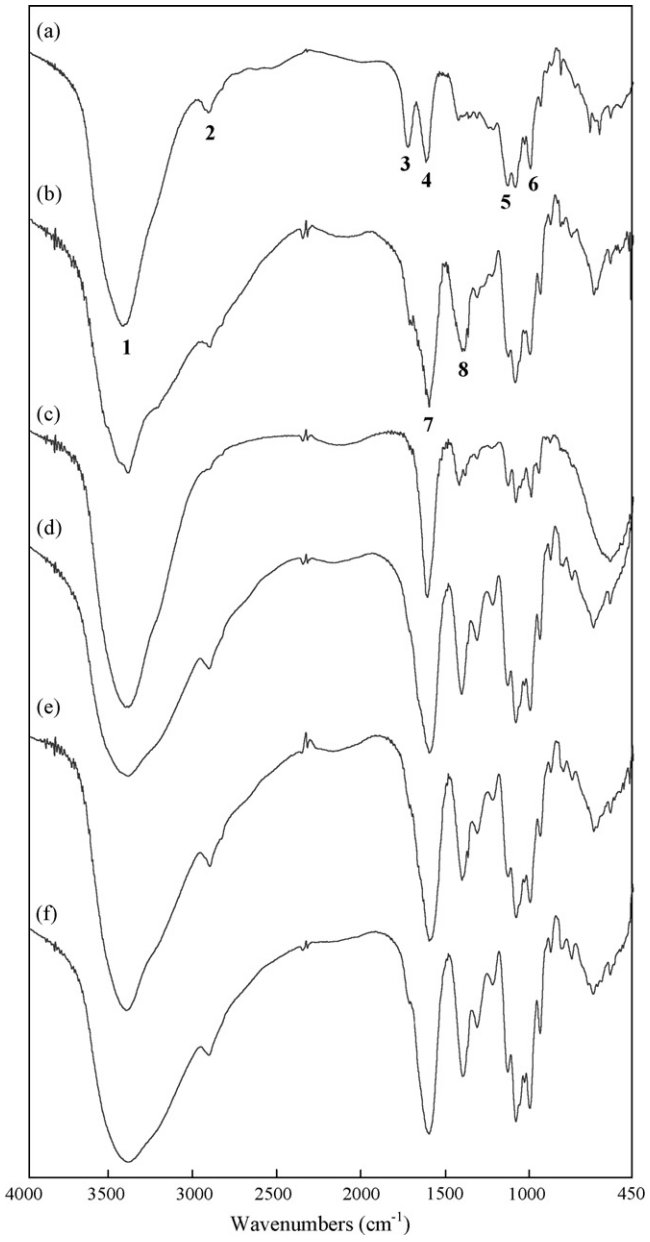

Fig. 1. FTIR spectra of native sugar-beet pectin (a), demethylated pectin (b) and calcium xerogels without metal (c) and with Cd (d), Pb (e), and Cu (f). The most relevant stretching bands have been highlighted: (1) O–H; (2) C–H; (3 and 4) C=O asymmetric and symmetric; (5) C–O–C of $-C_5O$; (6) C–O of C–O–C and C–OH.

2.4. Biosorption isotherm studies

The isotherms were obtained using the chosen initial pH (6 for cadmium, 4 for lead and 5 for copper). A 0.5 g/l pectin concentration was used according to preliminary studies shown in this work. 50 ml of metal solution were mixed with the hydro- and xerogels

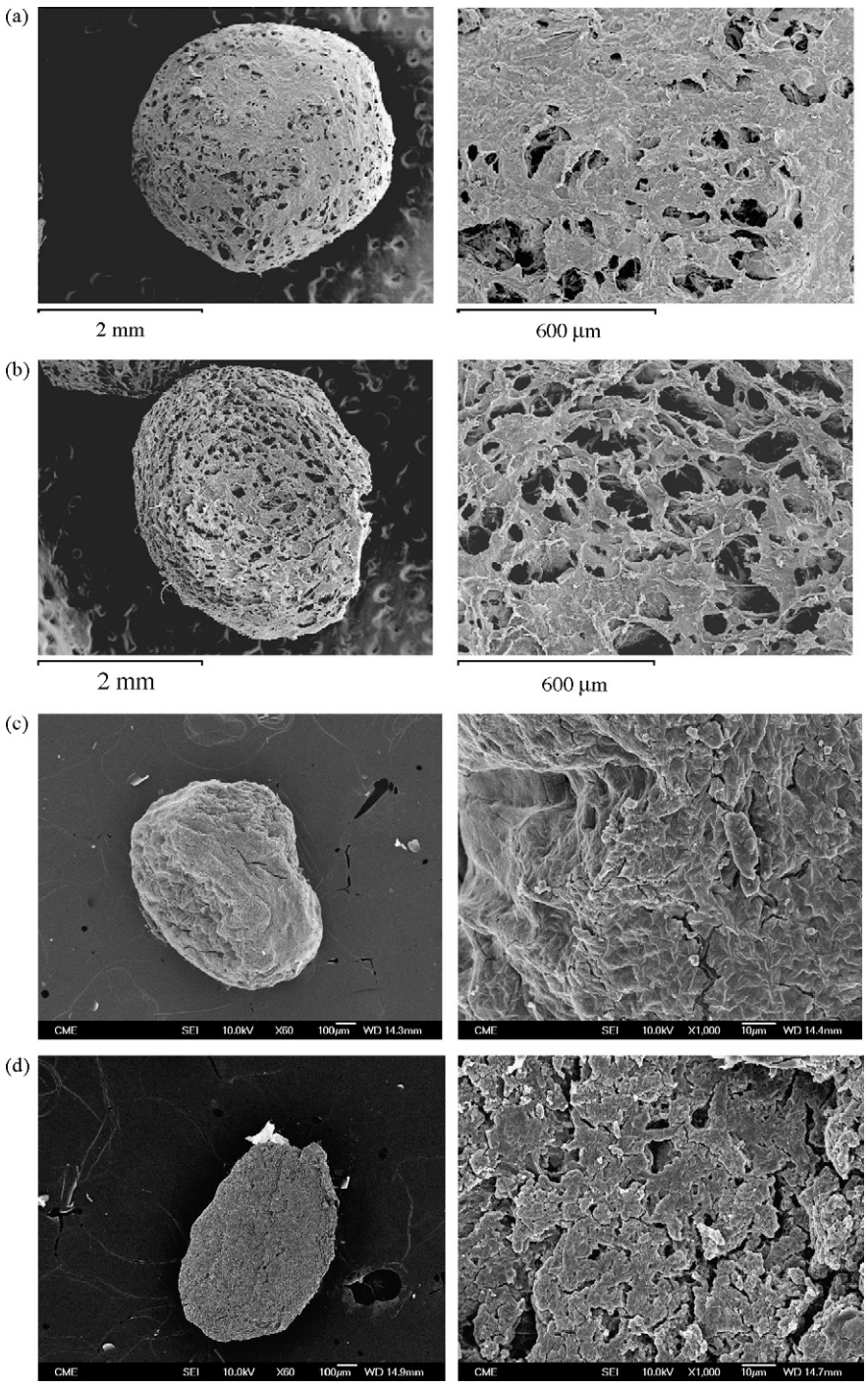

Fig. 2. SEM micrographs of the surface (a) and the inner part (b) of lyophilized sugar-beet pectin hydrogel beads. FESEM micrographs of the surface (c) and the inner part (d) of sugar-beet pectin xerogel beads.

at different metal concentrations: 10, 25, 50, 100 and 150 mg/l. The experiments were stopped after 120 min to ensure that the equilibrium concentrations were reached.

The results were then fitted to the Langmuir isotherm model, which can be expressed in its linearized form as [15]:

$$\frac{C_e}{q_e} = \frac{C_e}{q_{max}} + \frac{1}{bq_{max}} \quad (4)$$

where q_e is the metal uptake at equilibrium time (mmol/g of pectin); q_{max} the maximum metal uptake of the Langmuir isotherm (mmol metal/g of pectin); C_e heavy metal concentration at the equilibrium (mmol metal/l); b the equilibrium constant (l/mmol metal).

The sorption parameters can be calculated from the isotherm using the linear expression of the Langmuir model (C_e/q_e vs. C_e). The equilibrium constant indicates the affinity between the biomass and a given metal, the greater its value the greater the affinity.

2.5. Metal desorption

Desorption studies were carried out with 0.1 M HCl, H_2SO_4 and HNO_3 in a similar way to the biosorption studies. After the adsorption test, metal-loaded xerogels were filtered, weighed and placed in contact with the elution acid solutions at a concentration of 1 g

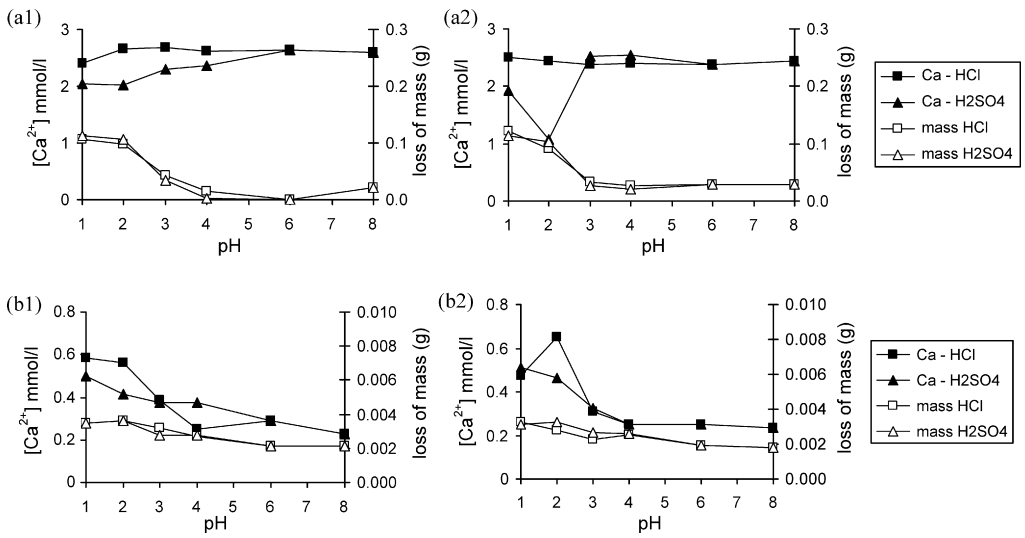

Fig. 3. Calcium release and mass loss of hydrogel (a) and xerogel (b) pectin beads at different initial pH values and in the presence of HCl and H_2SO_4 with (1) and without (2) stirring.

of gel/l. Metal concentration was determined in samples removed at 2, 5, 10, 30 and 60 min until it remained constant.

2.6. Biosorbent characterization

FTIR (Fourier transform infrared spectroscopy) analyses with KBr discs containing 2% finely ground sample were performed in a MIDAC Prospect-IR spectrophotometer. The spectral data were processed with Nicolet OMNIC E.S.P software. Infrared spectra were recorded in the region of 500–4000 cm^{-1} at a resolution of 4 cm^{-1}. In order to observe the morphology, lyophilized and xerogel beads were coated with a thin layer of gold, or graphite in the case of samples containing lead, and examined under a scanning electron microscope, SEM-EDX (JEOL JSM-6400). A double coating with graphite and then gold was used to observe the samples with a field emission scanning electron microscope, FESEM (JEOL JSM-6335-F).

3. Results and discussion

3.1. Characterization of the sugar-beet pectins and their gels

The success of the H_2SO_4 pectin extraction procedure was confirmed by FTIR analyses (Fig. 1) that were interpreted according to Kamnev et al. [16]. These authors defined the following characteristic regions: the ν(O–H) stretching bands of the hydroxyl groups from 3100 to 3600 cm^{-1}; the stretching bands of the C–H (from 2800 to 3000 cm^{-1}); the fingerprint region, that is characteristic of each type of pectin, under 2000 cm^{-1}, that includes the bands of the sugar ring (C5O–) between 950 and 1200 cm^{-1}; and the region of the carboxyl groups (–COOH) between 1200 and 1800 cm^{-1}. In the latter region, Synytsya et al. [17] identified the glycosidic bond at 1146 cm^{-1}. Sun and Hughes [18] have identified the sugar ring band at similar ranges (from 990 to 1100 cm^{-1}) and the stretching of the C–O bond of the C–OC and C–OH from 1016 to 1050 cm^{-1}. These same authors mention that the band of aromatic alcohols of lignin precursors appears at 1516 cm^{-1}, which, according to Synytsya et al. [19], corresponds to the ferulic acid that is characteristic of the sugar-beet pectin. However, it is not observed in our study probably because of the interference of other bands.

The FTIR spectra of the acid-extracted and ammonia-demethylated pectin (Fig. 1b) show a variation in the characteristic –COOH bands: the non-ionized (methylated or protonated) –COOH stretching peaks at 1744 and 1637 cm^{-1} in the extracted pectin shift after the demethylation process to a characteristic symmetrical and asymmetrical COO– stretching at 1612 and 1406 cm^{-1}. This shift in the peaks confirmed a decrease of the number of methylated –COOH groups. The resulting pectins also gelled by charge compensation with acids and were base-soluble, that is also characteristic of low-methoxyl pectins.

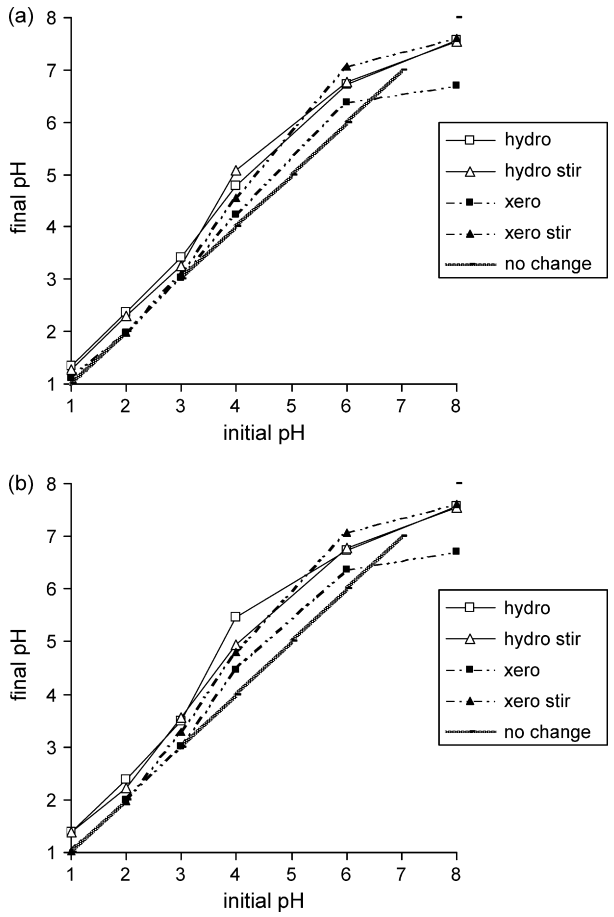

Fig. 4. Final pH values of hydrogel (*hydro*) and xerogel (*xero*) pectin beads in solutions at different initial pH values and in the presence of HCl (a) and H_2SO_4 (b) with (*stir*) and without stirring.

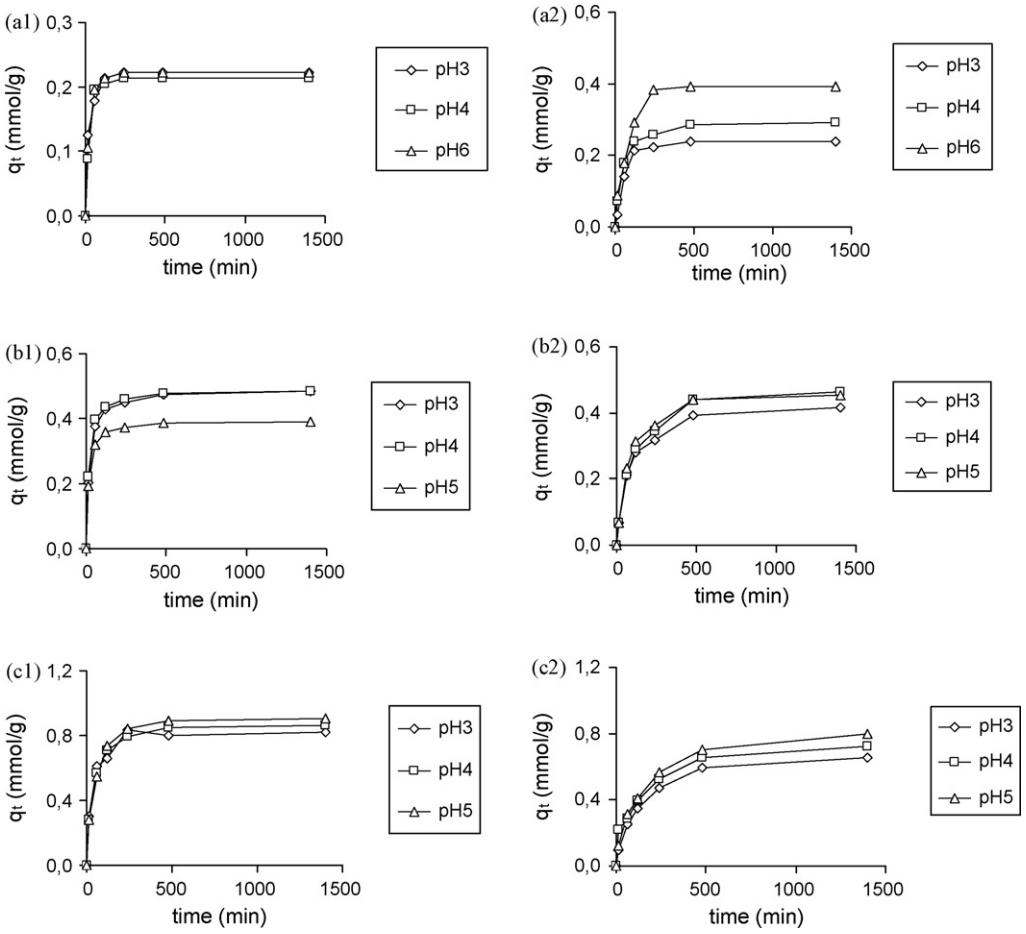

Fig. 5. Biosorption uptake of Cd (a), Pb (b) and Cu (c) with sugar-beet pectin hydrogels (1) and xerogels (2) at different initial pH values.

Hydrogel beads were lyophilized for SEM observation (Fig. 2a and b) and presented a high surface and inner porosity. However, according to Óuwerx et al. [20], lyophilization creates artifacts by collapsing the walls of pores and it is improbable that the gel beads have this sponge-like structure in the hydrated state. Xerogel beads presented a more compact structure with cracks (Fig. 2c and d).

Gelation modified the FTIR peaks of the demethylated pectin that correspond to the –COOH residues (Fig. 1a and c), indicating that these were the main groups involved in the process. Chen et al. [21], using the same technique with alginate, observed the same changes in the FTIR spectra due to calcium binding.

Calcium, as a divalent metal cation, has a precipitation effect on the polysaccharides that contain carboxyl groups such as pectin or alginate and can be removed from solution and retained in the gel structure, according to the "egg-box model" [22]. According to Dronnet et al. [10], the interaction of non-gelled pectins in solution with calcium is of electrostatic nature and the bonding of each cation implies a stoichiometric union with two carboxyl groups. Another option is the formation of a chelating structure of calcium with only one carboxyl group, like the one proposed by Nakamoto [23].

A study of the chemical and mechanical stability of the hydro- and xerogels was made. Fig. 3 shows the variation of the bead mass and the amount of calcium released by the hydrogels (a) and xerogels (b) with different initial pH values adjusted with H_2SO_4 or HCl, and under different stirring conditions. Both hydro- and xerogel beads became more stable at pH values higher than 4 since the gel mass and the amount of calcium released became constant independently of the type of acid or stirring conditions. The amount of released calcium decreased at pH values equal or greater than 4 indicating a greater stability of the beads in these conditions. Therefore, the stability of the hydro- and xerogel pectin beads made them suitable for experiments of heavy metal biosorption in different conditions preferably at pH values equal or greater than 4. The xerogels were more stable than the hydrogels: they released less calcium because of their compact structure and there was no important loss of bead mass regardless of the type of acid and initial pH values. Drying the pectin beads, a simple procedure, not only improved their stability in solution, but also the dehydration facilitated the handling and conservation of the beads.

There was an increase of the final pH of the solutions at initial pH between 4 and 6 after contact with the hydro- and xerogels (Fig. 4). This change in pH increased with stirring, and was similar independently of the type of acid and gel. There was an increase of the proton consumption until the initial pH was equal to the p*K*a of the gels, which is between 3.6 and 4.1 for pectins [24]. After this value, the proton consumption continued but decreased until proton release occurred at the initial pH value of 8. This buffering effect was due to the association and dissociation of the pectin functional groups, mainly carboxyl groups. The tendency towards deprotonation at initial pH values above the p*K*a of the biomass favors biosorption since there is an increase of negatively charged binding sites that are available and attract positively charged metal ions in solution.

3.2. Kinetic studies

When the biomass was in contact with the metal solution, the metal concentration decreased and the metal uptake increased

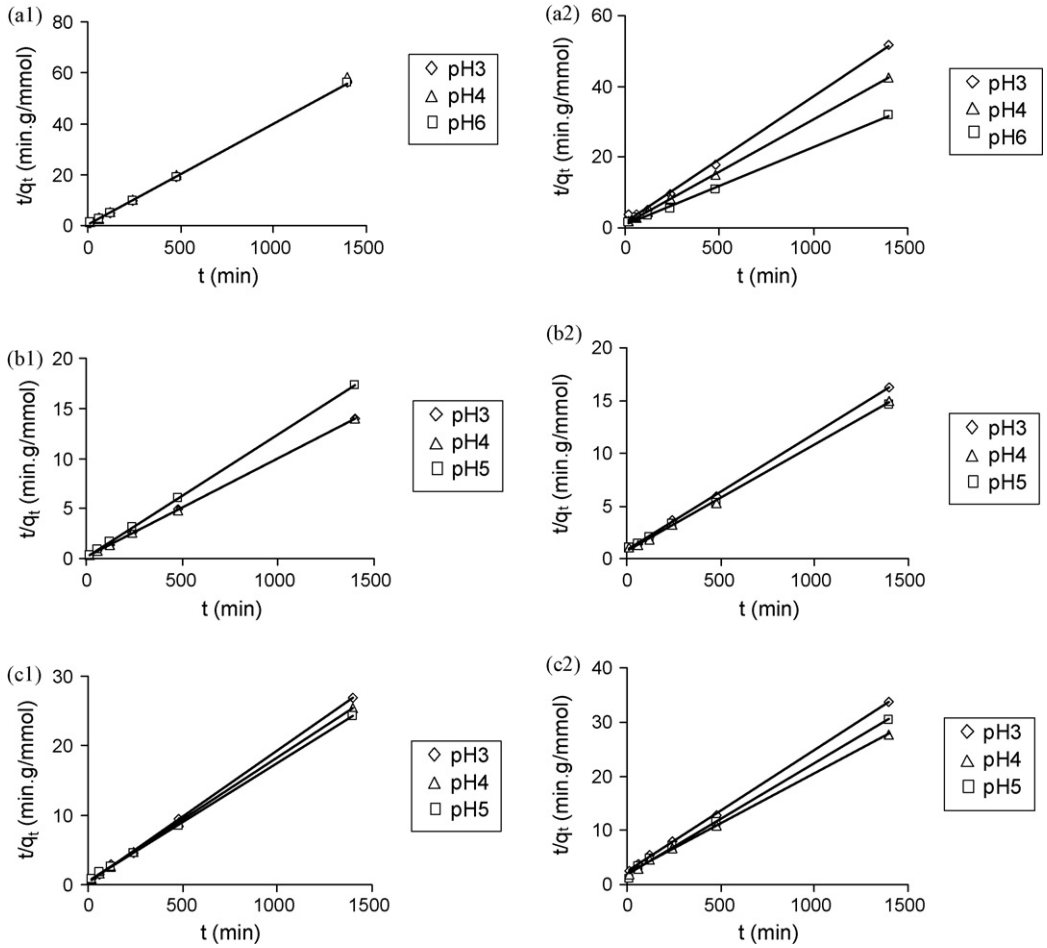

Fig. 6. Pseudo-second order kinetic fit for biosorption of Cd (a), Pb (b) and Cu (c) with hydrogel (1) and xerogel (2) pectin beads.

until the gels became saturated and an equilibrium concentration was reached in 8 h for the biosorption with the xerogels and in about 2 h for cadmium, and 8 h for lead and copper for the hydrogels (Fig. 5).

In order to define the sorption efficiency of Cd, Pb and Cu ions onto pectin gels, the experimental data of biosorption were fitted to pseudo-second order kinetic model. Fig. 6 depicts the excellent fitting of metal uptake to this model. Table 1 shows the calculated values of kinetic parameters for cadmium, lead and copper biosorption with pectin hydro- and xerogel beads at different initial pH values. High values of regression coefficients ($R^2 > 0.99$) indicated the applicability of this kinetic model to the cadmium, lead and copper biosorption on pectin gels.

Table 1
Kinetic parameters of pseudo-second order model for the biosorption of Cd, Pb and Cu with sugar-beet pectin gels.

Metal	Gels	pH	q_2 (mmol/g)	k_2 (g/mmol min)	R^2
	Hydro	3, 4 and 6	0.224	1.45×10^{-3}	0.999
	Xero	3	0.250	3.6×10^{-4}	0.997
Cd		4	0.302	7.04×10^{-4}	0.999
		6	0.407	1.50×10^{-3}	0.998
	Alginate	6	0.235	0.0237	0.989
	Hydro	3 and 4	0.488	7.83×10^{-3}	0.999
		5	0.395	4.40×10^{-3}	0.999
Pb	Xero	3	0.438	1.15×10^{-3}	0.999
		4 and 5	0.479	1.58×10^{-3}	0.999
	Alginate	5	0.503	7.6×10^{-3}	0.986
	Hydro	3	0.832	0.0290	0.999
		4	0.886	0.0237	0.999
		5	0.928	0.0257	0.999
Cu	Xero	3	0.705	3.22×10^{-3}	0.999
		4	0.768	5.10×10^{-3}	0.999
		5	0.855	5.72×10^{-3}	0.997
	Alginate	5	0.675	0.0145	0.996

Optimum pH values for the biosorption presented the highest kinetic uptakes. Generally, higher pH values favor biosorption, since the biomass is deprotonated when the pH is above the p*K*a, as discussed earlier. At lower pH values, the protonated functional groups could induce an electrostatic repulsion of the metal cations. Therefore, if more than one pH value presented the same metal uptake, as in the case of cadmium with the hydrogels, the highest was chosen in order to favor the pectin's negative charges and the attraction of the metal ions. Accordingly, the pH values used to continue with the experimentation with both the hydro- and the xerogels were 6 for cadmium, 4 for lead and 5 for copper. Nevertheless, the differences between the kinetic parameters of the hydro- and xerogels are small. From the point of view of an industrial application of the gels for effluent treatment, the use of xerogels present more advantages for their easier handling and conservation.

Table 1 also compares the kinetic parameters of the pectin hydro- and xerogels with alginate xerogels from previous studies in similar experimental conditions [14]. According to this table, pectin xerogels show greater cadmium kinetic uptakes. Both hydro- and xerogels presented greater uptakes for copper than alginate xerogels. With respect to the kinetic rates, the process was faster with the hydrogels, since the structure was less compact and more hydrated, except for cadmium. In the case of copper, pectin hydrogels presented faster kinetic rates than alginate xerogels.

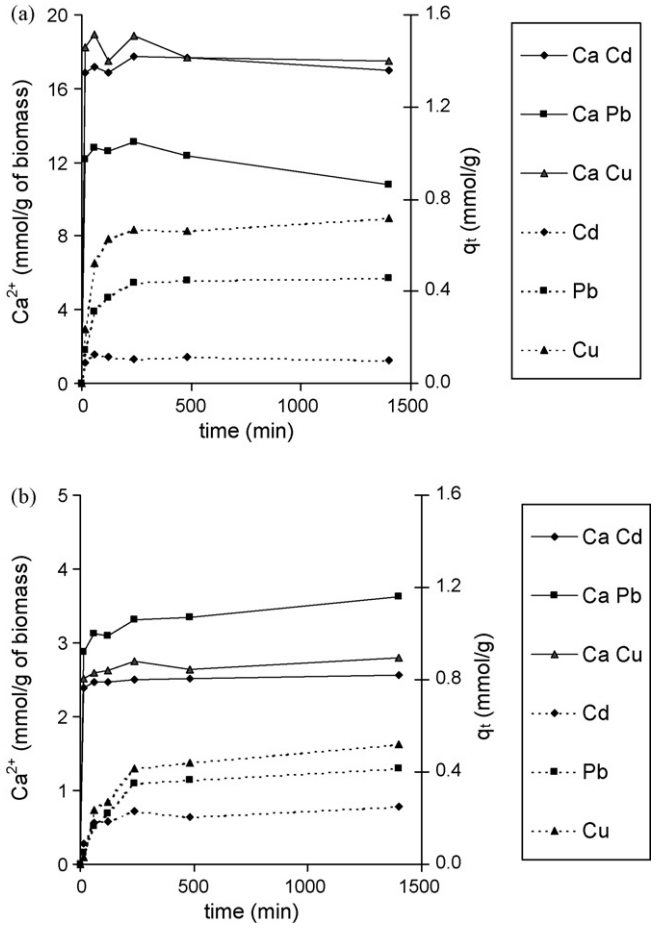

Fig. 7. Relationship between calcium release and the biosorption of Cd (initial pH 6), Pb (initial pH 4) and Cu (initial pH 5) with pectin hydrogels (a) and xerogels (b) (1 g/l).

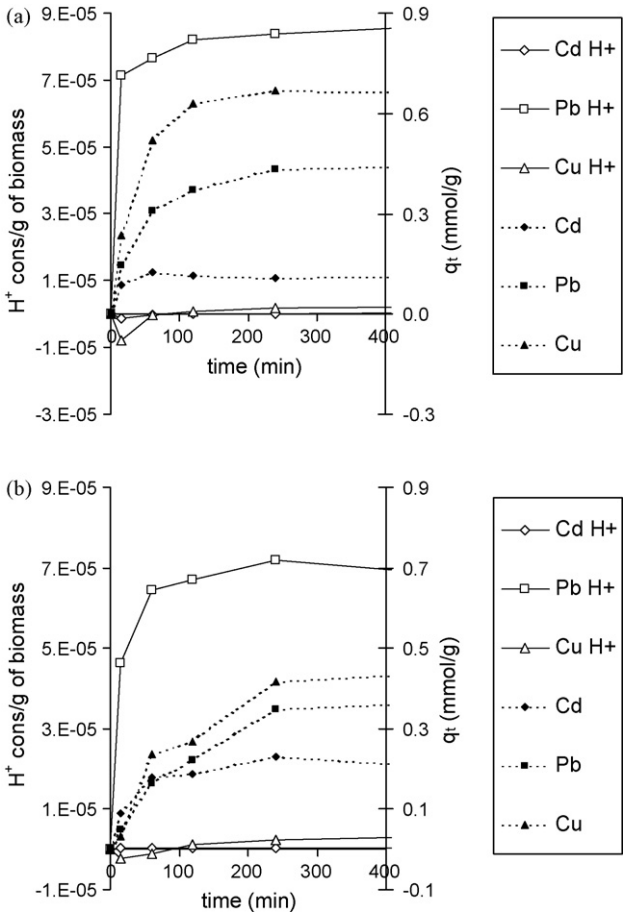

Fig. 8. Relationship between proton consumption and metal uptake during the biosorption of Cd (initial pH 6), Pb (initial pH 4) and Cu (initial pH 5) with pectin hydrogels (a) and xerogels (b).

The kinetic uptakes and rates of the biosorption with pectin gels followed this order: Cu > Pb > Cd, similar to that obtained by Dronnet et al. [10] for the selectivity of the sugar-beet pulp and non-gelled pectins for these metals. Copper presented the highest kinetic parameters, most probably due to its smaller ionic size with respect to cadmium and lead.

3.3. Calcium release and proton consumption during biosorption

Fig. 7 shows the amount of calcium released with respect to the amount of metal removed during cadmium, lead and copper biosorption with sugar-beet pectin gels at the optimum initial pH values (6 for cadmium, 4 for lead and 5 for copper). This amount was greater than that released in blank assays with gels in deionized water as described in the chemical and mechanical stability assays (Section 3.1). The results shown in Fig. 7 suggest a possible ion exchange between calcium from the gels and metals in solution. Table 2 shows the final mmol of calcium released per mmol of adsorbed metal, and the correlation coefficients of the amount of calcium released per gram of pectin gel and the corresponding metal uptakes.

According to this table, calcium release was related to the amount of cadmium, lead and copper adsorbed per gram of biomass, confirming that calcium was exchanged with metals in solution. Chen et al. [21] also observed this exchange between alginate calcium and adsorbed copper and lead using FTIR and XPS. The exchange between cadmium, lead and copper with calcium has also been documented by Dronnet et al. [10] during the biosorption with sugar-beet pulp and non-gelled sugar-beet and citrus pectins using potentiometric and spectrophotometric methods.

Fig. 8 shows proton consumption with respect to metal uptake onto pectin gels. During lead adsorption there was a slight decrease of the proton concentration, which suggests a possible competition between Pb^{2+} cations and protons for pectin binding sites. During the biosorption of cadmium and copper, the proton concentration

Table 2
Final amounts of calcium released and protons consumed per mmol of adsorbed metal onto sugar-beet pectin gels.

Gel	Metal	Ca^{2+} released/Me^{2+} adsorbed (mmol)	R^2 Me–Ca	H^+ cons/Me^{2+} adsorbed (mmol)	R^2 Me–H
Hydro	Cd	173	0.963	3×10^{-6}	0.0369
	Pb	24	0.755	2×10^{-4}	0.873
	Cu	24	0.780	3×10^{-6}	0.576
Xero	Cd	10	0.834	2×10^{-6}	0.932
	Pb	9	0.741	2×10^{-4}	0.806
	Cu	5	0.667	6×10^{-6}	0.862

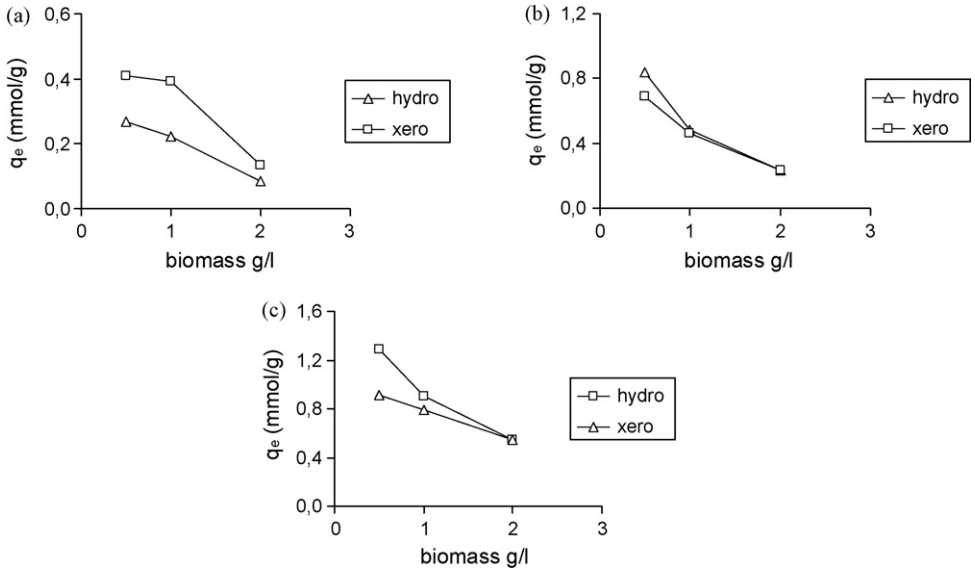

Fig. 9. Relationship between biomass concentration and metal uptake during the biosorption of Cd (a), Pb (b), and Cu (c) with sugar-beet pectin gels.

in solution is practically constant and only small variations at initial times were observed when the gels were placed in contact with the solution. This is an indication that protons did not compete with these metals for pectin binding sites.

Based on these results, the biosorption of cadmium, lead and copper is mainly due to ion exchange and complexing between divalent cations (Cd^{2+}, Pb^{2+}, and Cu^{2+}) in solution and calcium (Ca^{2+}) chelated or linked to carboxylic groups in the polymeric structure of pectin gel.

3.4. Influence of pectin concentration

A series of experiments were carried out to determine the influence of the biomass concentration. In this case, the optimum pectin dosage corresponded to the least amount of biomass tested with the highest metal uptake: 0.5 g/l for both the hydro- and the xerogel (Fig. 9). This behaviour is typical for biosorption: when the biomass concentration increases, the Cd, Pb and Cd removal efficiency (metal uptake per gram of biomass) decreases (Table 3). This phenomenon has been widely recorded and some of the proposed hypotheses state that it is due to a decrease in the availability of the biomass due to biomass particles electrostatic interactions, interference of metal binding sites or mixing problems [25].

Table 3
Influence of pectin concentration on the uptake capacity of cadmium, lead and copper.

Gel	Metal	Dosage (g/l)	q_e (mmol/g)	q_e (mg/g)
Hydro	Cd	0.5	0.267	30.0
		1	0.222	25.0
		2	0.085	9.5
	Pb	0.5	0.840	174.0
		1	0.483	100.0
		2	0.236	49.0
	Cu	0.5	1.29	82.0
		1	0.905	57.5
		2	0.543	34.5
Xero	Cd	0.5	0.409	46.0
		1	0.391	44.0
		2	0.133	15.0
	Pb	0.5	0.685	142.0
		1	0.463	96.0
		2	0.236	49.0
	Cu	0.5	0.913	58.0
		1	0.795	50.5
		2	0.547	34.8

3.5. Biosorption isotherms

The isotherms of the biosorption of cadmium (initial pH of 6), lead (initial pH of 4) and copper (initial pH of 5) with sugar-beet pectin gels are presented in Fig. 10. Table 4 shows their fit to the Langmuir model and the corresponding parameters. Hydrogel metal uptakes followed the same order of the kinetic uptakes: Cu ≥ Pb > Cd. On the other hand, the order of the xerogel uptakes was: Cd > Cu ≥ Pb. This difference could be due to differences in the preparation process, hydration and particle size. In general, hydrogels presented greater uptakes than xerogels, except for cadmium biosorption. Table 4 also compares the Langmuir parameters for the pectin gels with those of alginate xerogels obtained from experiments with similar experimental conditions [14]. The pectin gels showed greater cadmium and lead uptakes than alginate.

A greater equilibrium constant b indicates a stronger bond between metal and biomass [26]. The sugar-beet pectin gels presented higher affinities for lead and copper than alginate, but similar affinities for cadmium. The order of the constants was Pb ≫ Cu ≫ Cd, that agrees with that proposed by Kartel et al. [9] and with the rule of Irving Williams on the stability of the complexes formed by metal cations and oxygenated donor groups in the gels [10]. These were the hydroxyl and carboxyl groups of the galacturonic acids of the pectin gel matrix. According to different authors, the order of selectivity is determined by the size and disposition of the functional groups in the binding sites of the biomass [2,22]. Additionally, the pectin gels presented higher affinities for lead and copper than the alginate xerogels (Table 4).

3.6. Desorption studies

Fig. 11 shows the elution curves of metal-loaded gels with three different mineral acids: 0.1 M HCl, H_2SO_4 and HNO_3. 0.1 M HNO_3 was the most efficient eluant for cadmium (30 min), lead (60 min) and copper (120 min), since it recovered 100% of metal adsorbed on pectin gel beads. In this figure, metal-loaded gels were those obtained from the kinetic studies (Section 3.2) at initial pH values for the solutions of 6 for cadmium, 4 for lead and 5 for copper.

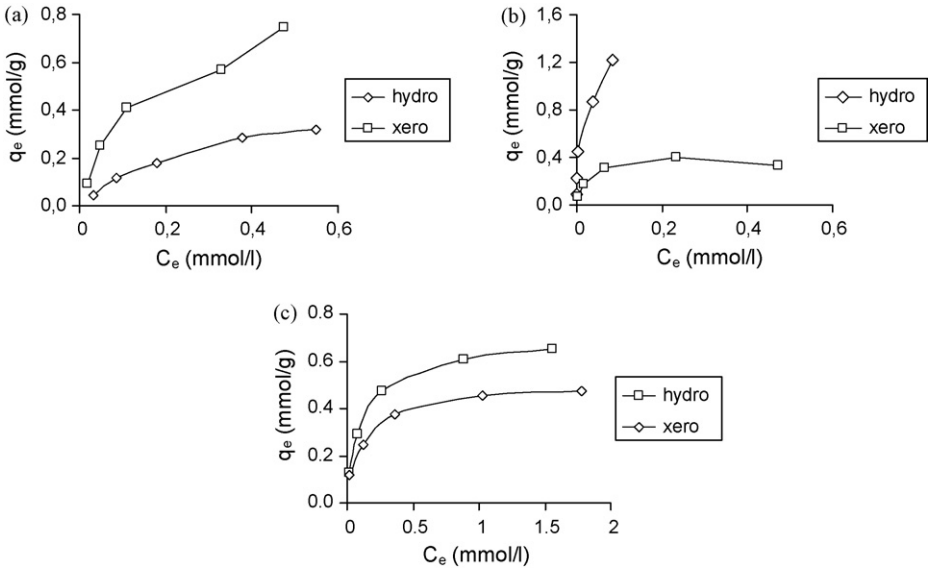

Fig. 10. Sorption isotherms for Cd (a), Pb (b), and Cu (c) of sugar-beet pectin hydro- and xerogels (at 0.5 g of pectin/l and initial solution pH of 6 for Cd, 4 for Pb and 5 for Cu).

Table 4
Langmuir parameters of the biosorption of Cd, Pb and Cu with sugar-beet pectin gels.

Metal	Gel	q_{max} (mmol/g)	q_{max} (mg/g)	b (l/mmol)	R^2
Cd	Hydro	0.2746	30.9	1.61	0.991
	Xero	0.5063	56.9	1.75	0.965
	Alginate	0.2752	30.9	1.42	0.975
Pb	Hydro	0.6268	129.9	143.7	0.998
	Xero	0.4022	83.3	64.1	0.975
	Alginate	0.4022	83.3	64.1	0.975
Cu	Hydro	0.6870	43.7	10.0	0.998
	Xero	0.4921	31.3	8.00	0.999
	Alginate	1.20	76.3	2.64	0.991

According to the Langmuir parameters presented in Table 4, cadmium presented the lowest affinities for the gels, contributing in part to the shorter recovery times. Sekhar et al. [27] also found that this acid, at the same concentration, was a better eluant of lead than HCl from immobilized plant biomass (IPBFIX). H_2SO_4 could not be used as a desorbent for lead due to the low solubility of lead sulfate. On the other hand, it is interesting to note that HCl only recovered 20% of the adsorbed cadmium.

Nevertheless, our copper recovery results are different from those obtained in other studies. For example, Lau et al. [28] found that the best eluant for this metal from brown algae was H_2SO_4 0.1 M. Gupta et al. [29], using the green alga *Spirogyra*, obtained the best recovery with HCl 0.1 M. For Chen and Yang [30], the best desorbent of cadmium, lead and copper from the brown alga *Sargassum* was 0.2 mM HCl. Therefore, the variables that affect the selection

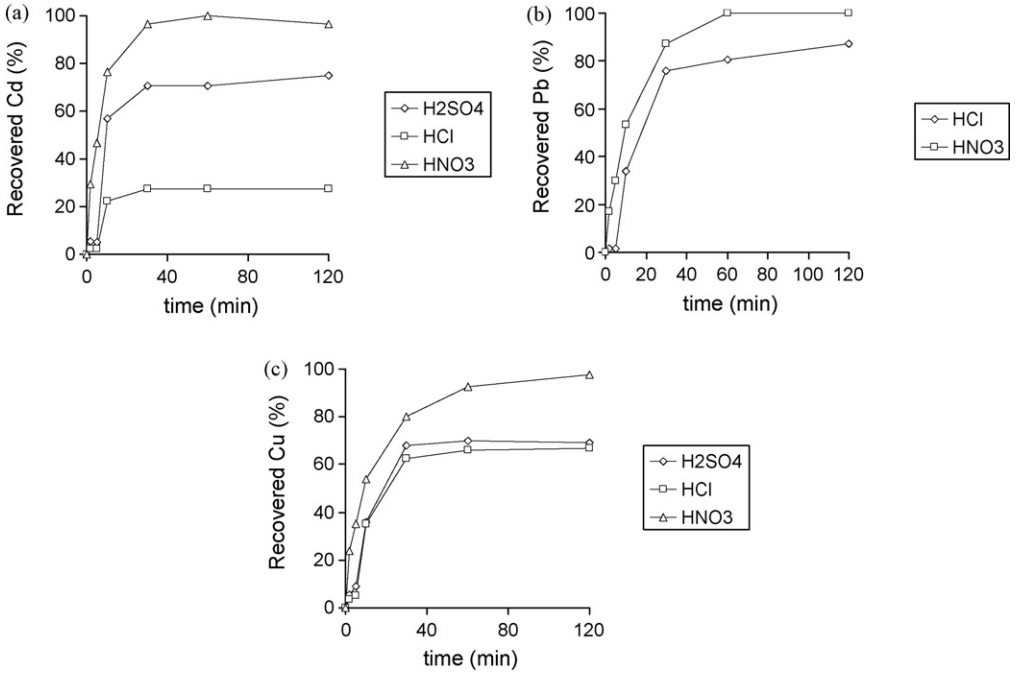

Fig. 11. Elution of Cd (a), Pb (b) and Cu (c) from sugar-beet pectin xerogels using different acids: 0.1 M HCl, H_2SO_4 and HNO_3.

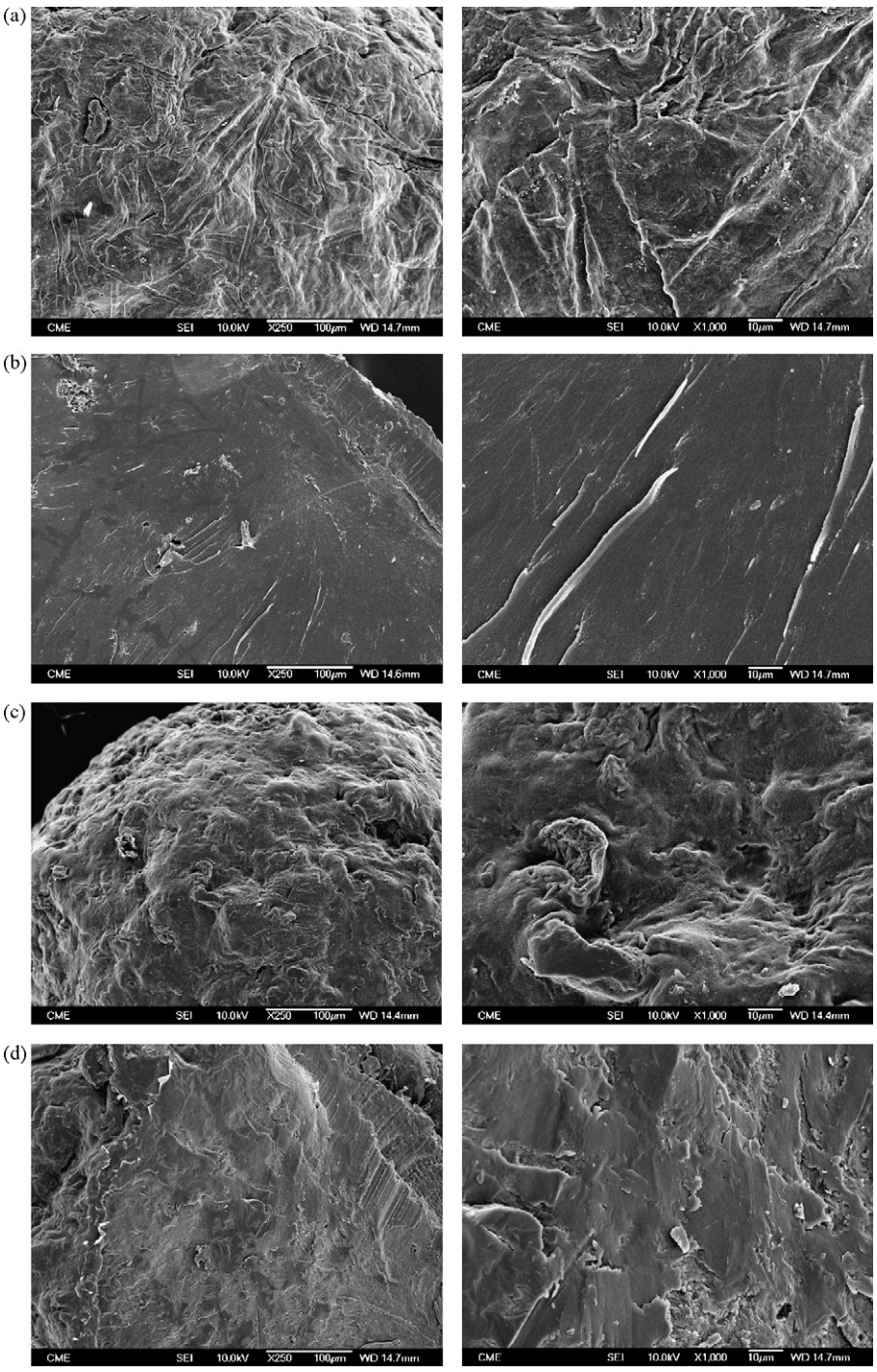

Fig. 12. FESEM micrographs of a sugar-beet pectin xerogel beads after cadmium biosorption (a and b) and after rehydration only with water (c and d). Surface (a and c) and inner part (b and d) of the beads.

of an adequate desorbent for metal recovery are: type of biomass, metal used and concentration of eluant.

3.7. Characterization of the sugar-beet pectin gels after heavy metal uptake

According to the SEM-EDX analysis, cadmium, lead and copper had a uniform distribution on the surface and interior of the sugar-beet pectin xerogels after biosorption (results not shown). The beads with adsorbed metal did not show the cracks on the surface and interior that had the gels before biosorption (Figs. 2 and 12). This could indicate a different complexation mechanism of calcium and the recovered metal, in which ion is substituted by cadmium, lead and copper. It appears that after biosorption the structure is more smooth, compact and uniform. Part of this effect could be due to rehydration, in fact, gels rehydrated only with water still presented a less uniform structure than those with metal (Fig. 12). On the other hand, Óuwerx et al. [20], prepared alginate beads using different divalent cations and found that calcium alginate gels were less stable than those prepared with cadmium and copper. The adsorbed metals could form bridges with the pectin chains similar to those of calcium according to the Rees “egg-box” model and

would constitute a visual confirmation of what has been observed by other authors after the binding of cadmium, lead and copper with polysaccharides of the cell wall such as alginate and pectin using different spectroscopic techniques [10,21,31].

FTIR provided more information about the possible binding mechanisms of cadmium, lead and copper with pectin gels (Fig. 1). The spectra of the gels after biosorption were interpreted according to Kamnev et al. [16], and compared with those of the gels without metal. After cadmium recovery there was a displacement of the bands that corresponded to the asymmetric (from 1628 to 1617 cm^{-1}) and symmetric (from 1439 to 1422 cm^{-1}) stretching of carboxyl groups (Fig. 1d).

Fig. 1e shows the FTIR spectra of the sugar-beet pectin gels treated with lead. As for cadmium biosorption, there was a displacement of the asymmetric (from 1628 to 1617 cm^{-1}) and symmetric (from 1439 to 1421 cm^{-1}) carboxyl stretching bands. In this case, there was also a modification of the hydroxyl stretching band from 3415 to 3425 cm^{-1} that was not observed after cadmium binding. This additional displacement suggests that lead forms stronger bonds with the biomass, moreover, lead has the highest affinity constant values and cadmium the lowest (Table 4). Raize et al. [32] also reached this conclusion with brown algae, using EDS, FTIR and XPS. These authors proposed chelation, besides ion exchange as an additional mechanism for the biosorption of lead. According to Rendleman [22], this cation is highly reactive, contributing to the formation of complexes with the pectin matrix. According to Figueira et al. [33] and Fuks et al. [34], another indication of chelating reactions would be the decrease between the distance of the two carboxyl bands, that was observed after the sorption of the three metals, but this reaction was more pronounced for lead. After copper binding, the asymmetric (from 1628 to 1617 cm^{-1}) and symmetric (from 1439 to 1419 cm^{-1}) stretching bands of the carboxyl groups were also modified (Fig. 1f). The hydroxyl FTIR band presented less displacement than with lead (from 3415 to 3409 cm^{-1}).

These FTIR results confirmed the participation of the carboxyl groups in the cadmium, lead and copper biosorption with sugar-beet pectins as confirmed by Dronnet et al. [10] with this technique and by Harel et al. [7] using NMR. Additionally, Khotimchenko et al. [35] observed that de-esterification of citrus pectins, that increases the number of available carboxyl groups, increased their lead uptake. In the case of sugar-beet pectin gels, the binding process occurs by ion exchange between calcium and protons and by chelation or complexation with one or two carboxyl groups as explained earlier. Finally, according to Kartel et al. [9], the participation of the different biosorption mechanisms depends greatly on the experimental conditions and the processing and pretreatment of the pectins, and therefore this would limit the numerical comparison of biosorption parameters among different studies.

4. Conclusions

Sugar-beet pectin hydro- and xerogels revalue an agricultural residue. Both are efficient biosorbents for cadmium, lead and copper and can be easily desorbed for their reutilization. They were successfully obtained from pectin extracted and demethylated from the sugar-beet pulp.

The kinetic metal uptakes and rates of biosorption with pectin gels followed this order: Cu > Pb > Cd and the order of the Langmuir constant was: Pb ≫ Cu > Cd. Lead formed the most stable bonds with binding sites of pectin due to its higher affinity for both hydro- and xerogels. Compare to alginate, sugar-beet pectin gels presented higher metal affinities and can be an interesting alternative to the former biosorbent.

The biosorption mechanism is mainly based on ion exchange and complexing between divalent cations in solution (Cd^{2+}, Pb^{2+}, and Cu^{2+}) and calcium (Ca^{2+}) chelated or linked to carboxylic groups in the polymeric structure of pectin gel. During biosorption, the adsorbed metals replaced calcium in the gels reorganizing their structure. These metals were completely recovered from the xerogels using 0.1 M HNO_3.

Sugar-beet pectin gels are an adequate granulated biomass with the necessary chemical and mechanical resistance for heavy metal biosorption and desorption. Xerogels, with better conservation and handling than hydrogels, can be specially indicated for further applications in continuous processes for metal decontamination of industrial effluents.

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