BIOSORPTION OF LEAD (II) ON MODIFIED BARKS EXPLAINED BY THE HARD AND SOFT ACIDS AND BASES (HSAB) THEORY

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Chemical modification of Douglas fir bark and its subsequent utilization in adsorption of Pb(II) from aqueous solutions was investigated. The polysaccharidic moiety of barks was functionalized by periodate oxidation and derivatized after reductive amination in the presence of aminated oligo-carrageenans. Pb(II) adsorption isotherms of derivatized barks were then determined and compared to the capabilities of crude barks using the Langmuir adsorption model in terms of affinity (*b*) and maximum binding capacity (q_{max}). Compared to crude barks, the derivatization of barks by oligo-carrageenans resulted in significant enhancements of q_{max} and *b* by up to x8 and x4, respectively. The results obtained from crude barks on chemically grafted carboxylic and sulfated barks are discussed and interpreted through the Hard and Soft Acids and Bases (HSAB) theory.

Keywords: Douglas fir barks; Chemical modification; biosorption; Langmuir isotherm; Lead

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INTRODUCTION

The ability of barks to bind heavy metal ions from polluted waters and the impact of various operating factors on their properties have been largely described (Harman *et al.* 2008). Our previous work (Martin-Dupont *et al.* 2002) has demonstrated heavy metal binding capabilities of Douglas fir barks and the possibility to enhance their lead(II) binding capacities. Metal sequestration during biosorption follows complex mechanisms that mainly involve ionic interactions. Recently we demonstrated that the lead binding capacities of crude barks could be successfully increased through the covalent grafting of aminated oligogalacturonans (Astier *et al.* 2010).

Several chemical groups of biomass can attract and sequester metal ions: acetamido, amino, amido, sulfhydryl, sulfate, and carboxyl (Gardea-Torresdey *et al.* 1990). The role of these ion exchanger groups has been already studied (Martin-Dupont *et al.* 2004). This is the case of barks functionalized with a stilbene derivative whose sulfonate group enhances the sorbent selectivity for lead(II). Furthermore, the role of sulfate in lead(II) biosorption has been pinpointed in recent studies on carrageenans extracted from red algae (Yipmantin *et al.* 2011; Veroy *et al.* 1980).

The study aim was first to convert crude bark into a specific lead(II) biosorbent through the covalent grafting of aminated oligo-carrageenans. In a second part, the bio-

sorption capabilities of chemically modified barks were compared to crude and oligo-galacturonan-modified barks and interpreted through the HSAB theory (Pearson 1987).

EXPERIMENTAL

Materials

Douglas fir (*Pseudotsuga menziesii*) bark samples were obtained from a local sawmill in the Limousin region (France). Barks were dried in a ventilated oven (40 °C), ground (particle size <200 µm), and stored in desiccators at room temperature.

Chemicals

All solvents and reagents were purchased from Aldrich, Acros, or VWR. Milli-Q deionized water (Millipore system) was used throughout all experiments. Chloroform was distilled over CaH_2. Other solvents were used without further purification.

Bark Functionalization

Bark functionalization was realized according previous work (Astier *et al.* 2010).

Oligo-carrageenan synthesis and derivatization with spacer

Oligo-carrageenans were obtained by acid hydrolysis of commercial iota-carrageenan extracted from red algae. 1 g of iota-carrageenan was added to 100 mL of distilled water, and pH was adjusted to 4 with HCl (0.1 M). The mixture was stirred in an oven at 80 °C for 20 h. Oligomers of high degree of polymerization were removed by precipitation with 3 volumes of ethanol. Monomers were removed by size exclusion chromatography (Biogel P2, eluent: water). The purified fraction was concentrated, lyophilized, and analyzed by mass spectrometry.

The oligo-carrageenan mixture (100 mg) was dissolved in 1 mL of water, then was added to 3 mL of a methanolic solution containing 10 eq. of *tert*-butyl 3-aminopropylcarbamate (211 mg) and 16 eq. of $NaBH_3CN$ (121 mg). The mixture was stirred at 80 °C for 1 h and evaporated to dryness. The solid was taken up with 10 mL of water. Excess of amine was removed by 8 washes with chloroform (8 x 20 mL). The reducing agent was removed by size exclusion chromatography (Biogel P2, eluent: water). Deprotection was carried out by dilute TFA at room temperature during 20 min.

Bark Grafting

Periodic oxidation and reductive amination of bark

The procedure was adapted from our previous work (Astier *et al.* 2010). The first chemical step consisted of aldehyde group generation. To this end, the cellulosic and hemicellulosic moieties of Douglas fir bark were oxidized by *per*iodic acid. Douglas fir bark (6 g) was suspended in a 0.157 M $NaIO_4$ aqueous solution. In order to avoid radical-induced depolymerization, the reaction was conducted in the dark in the presence of propanol-1 as radical scavenger with a 9:1 (v/v) $NaIO_4$/propanol-l ratio. The reaction mixture was stirred at room temperature for 7 days, and then reaction was stopped by destruction of excess periodate with ethylene glycol. The oxidized product was rinsed

with milli-Q water and air-dried at 40 °C. In a second step, 200 mg of dialdehyde bark (DAB) were derivatized by reductive amination with aminated oligo-carrageenan mixture solution (0.026 M solution, assuming that DP = 4). $NaBH_3CN$ at a concentration of 0.064 M was used as reducing agent and pH was adjusted to 6.5; the resulting reaction mixture was stirred at room temperature for 5 days. Derivatized barks were rinsed with milli-Q water, air-dried at 40 °C, and stored in desiccators.

Determination of substitution degrees

Acidity was determined using the Boehm method (Boehm 1966). 50 mg of crude or modified barks were suspended in 10 mL of 0.1 M $NaHCO_3$ solution and stirred during 72 hours under nitrogen. Suspensions were filtered on sintered glass (porosity 3); excess alkali was back-titrated by 0.1 N HCl. The substitution degree (DS) of bark (Table 2) was then estimated by using the following formula:

$$DS = \frac{\dfrac{n_{H^+}(\textit{aminated bark}) - n_{H^+}(\textit{NaBH}_3\textit{CN bark})}{DP}}{n_{CHO}(\textit{dialdehyde bark})} \times 100 \quad (1)$$

in which the quantity n_{H+}**(***aminated bark*) is the acid content of aminated barks (eq.g^{-1}), n_{H+}(*$NaBH_3CN$ bark*) is that of the barks treated with the reducing agent without amine (eq.g^{-1}), n_{CHO} (*dialdehyde bark*) is the content of aldehyde functions of barks after *per*iodic oxidation (mol.g^{-1}), and DP is the average number of glucidic residues of aminated oligo-carrageenan.

Characterization

Mass spectrometry

Electrospray ionization (ESI-MS) data acquisition was performed on an ESI ion trap (IT) instrument (LCQ Advantage, Thermo Electron, USA). Samples were dissolved in 1:1 MeOH-H_2O at a concentration of 100 µg.mL^{-1}. Their introduction was performed at a flow rate of 2.5 µL.min^{-1} in negative or in positive ionisation mode. MALDI Mass measurements were performed on an Auto flex III MALDI-TOF/TOF spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a Smart beam laser (355 nm, 200 Hz) in negative ionization mode, with a reflector detection. Oligosaccharide samples were deposited onto a polished steel MALDI target plate and mixed in a 1:1 ratio (v/v) with N,N-dimethylaniline (DMA)/2,5-dihydroxybenzoic acid (DHB) matrix solution (DHB 100 mg.mL^{-1} in H_2O/ACN/DMA (49.9:49.9:0.2)). Acquisition parameters (laser power, pulsed ion extraction, etc.) were optimized for each sample.

IR and NMR spectroscopies

Crude, dialdehyde, and modified barks were characterized by infrared (IR) spectroscopy with a Perkin-Elmer Spectrum 1000 FT-IR spectrometer in the 600 to 4000 cm^{-1} range.

^1H Nuclear magnetic resonance (NMR) spectroscopy was performed with a Bruker DPX-400 spectrometer. Chemical shifts are reported as δ in parts per million.

Biosorption Experiments

Lead(II) biosorption

Biosorption experiments were carried out in batch conditions by adding 10 mg of barks to 10 mL of a single metal aqueous solution ($Pb(NO_3)_2$) at concentrations ranging from 0 to 2000 ppm (19.31 meq.L^{-1}). The initial pH of each metal solution was adjusted to 5 by dropwise addition of 0.1 N HNO_3. Suspensions were shaken at room temperature during 2 h to ensure equilibrium. Adsorbent was finally separated from the solution by vacuum filtration through a sintered glass filter (porosity 3). The maximum lead adsorption capacity q_{max} and the Langmuir constant *b* were then graphically obtained from the Langmuir adsorption isotherms methodology as previously described (Harman *et al.* 2008).

Metal ion analytical determinations

Concentrations of residual PbII in filtrate were determined by atomic absorption spectrometry at 283.6 nm with a Perkin Elmer AAnalyst 400 spectrophotometer, in the concentration range 0 to 10 ppm (0 to 96.5 µeq.L^{-1}). The amount of adsorbent-bound lead was calculated from the difference between initial and final concentrations of the metal ion in solution.

RESULTS

Chemical Modification of Barks

The general procedure used for bark modification is described in Fig.1.

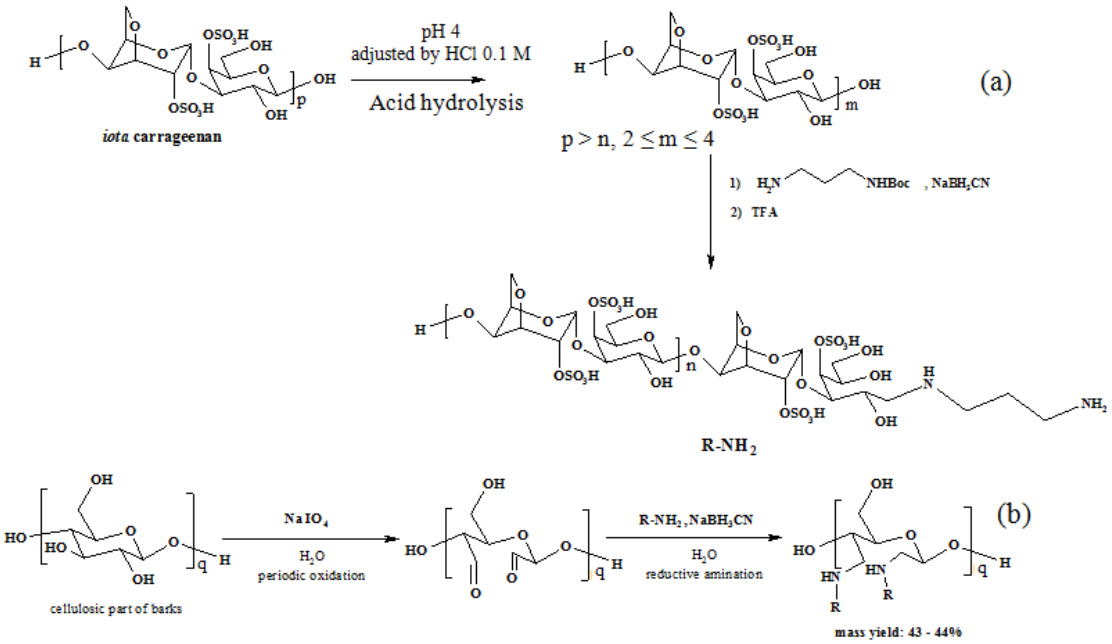


Fig. 1. (a) Hydrolysis and derivatization steps of carrageenan, (b) Functionalization and derivatization steps of the polysaccharidic moieties of Douglas fir bark

Amino oligo-carrageenan synthesis

Oligo-carrageenans were obtained by acid hydrolysis (HCl) of commercial iota-carrageenan (Viebke *et al.* 1995). This class of polysaccharides exhibits a backbone of alternating D-galactopyranose whose C-4 hydroxy groups are substituted by sulfate ester groups (G4S) and 3,6-anhydro-D-galactose with the C-2 hydroxy groups substituted by sulfate ester groups (DA2S). After elimination of oligo-carrageenans with high degree of polymerization (DP) by ethanol precipitation and removal of monomers by size exclusion chromatography on Biogel P2, a mixture of oligo-carrageenans (65% mass yield) was obtained. The average DP was determined by MALDI mass spectrometry. (Fig. 2).

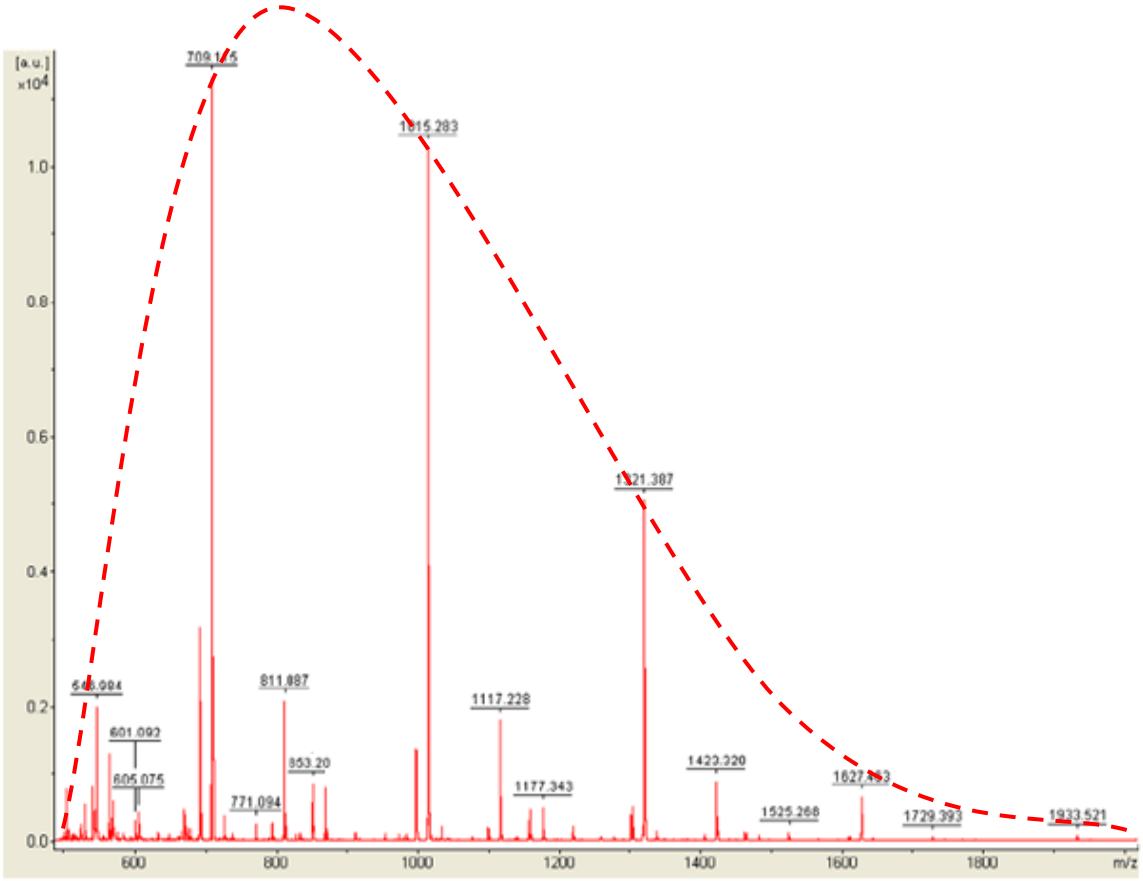
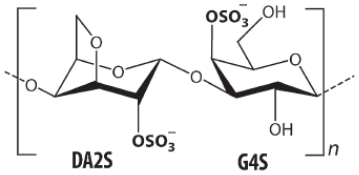


Fig. 2. Negative MALDI-TOF mass spectra of oligo-carrageenans mixture obtained by acid hydrolysis

The most intense ions at m/z 709.115 and 811.087 correspond to $[(DA2S\text{-}G4S)_2\text{-}3SO_3+H_2O\text{-}H]^-$ and $[(DA2S\text{-}G4S)_2\text{-}2SO_3+H_2O+Na\text{-}2H]^-$ (Table 1). The reducing end of oligo-carrageenans was then converted into amine prior to reaction with dialdehyde barks. This methodology has been used in the determination of the configuration of 3,6-anhydrogalactose in galactans (Navarro and Stortz 2003). Attachment of amino group was performed through reductive amination of oligosaccharides anomeric carbon in presence of N-Boc-1,3-diaminopropan, followed by removal of the protective N-Boc group.

Table 1. Main Ions Identified for the Oligo-carrageenans *m*/*z*

m/z			SO_3	H	Na^+
	DA2S	G4S			
709.115	2	2	-3	3	-
811.087	2	2	-2	2	1
853.200	3	2	-4	4	-
1015.283	3	3	-5	5	-
1117.228	3	3	-4	4	1
1321.387	4	4	-7	7	-
1423.320	4	4	-6	6	1
1525.268	4	4	-5	5	2
1627.463	4	4	-4	4	3
1729.393	4	4	-3	3	4
1933.521	5	5	-6	6	3

ESI mass spectrometry of native, N-Boc protected, and unprotected oligo-carrageenans showed the efficiency of chemical reactions. Structural modification of pure $[(DA2S\text{-}G4S)]_2$ (data not shown) oligo-carrageenan was also observed by positive ESI mass spectrometry. N-Boc protected and unprotected aminopropyl-$[(DA2S\text{-}G4S)]_2$ oligo-carrageenan spectra showed the expected signals at m/z = 1117.1 ($[M\text{-}6SO_3]^+$) and m/z = 1073.3 ($[(M\text{-}5SO_3]^+$), respectively. Furthermore, modifications of oligosaccharides were confirmed with ^{1}H NMR spectra. The fixation of N-Boc aminopropyl chain was established by ^{1}H NMR analysis by the appearance of a signal at 1.47 ppm with an integrated intensity of 9 protons (Fig. 3).

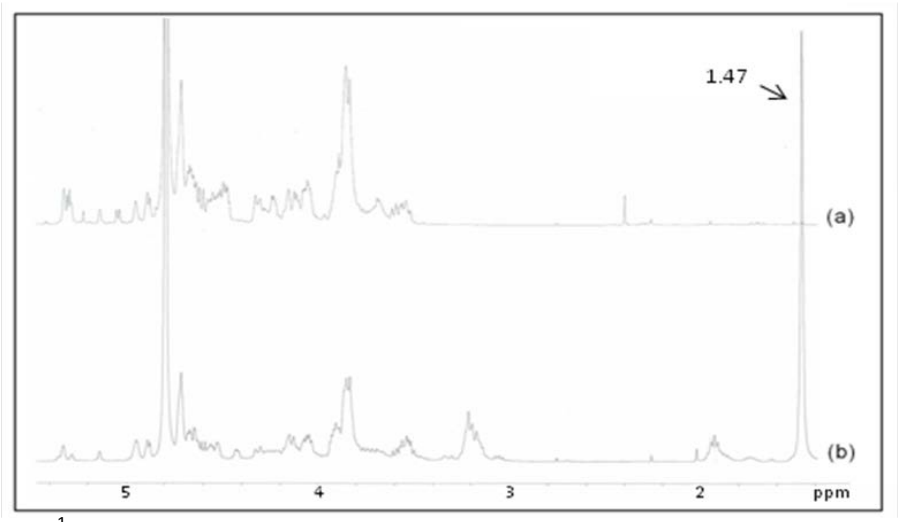


Fig 3. ^{1}H NMR spectra of (a) oligo-carrageenans and (b) oligo-carrageenans modified by *tert*-butyl 3-aminopropylcarbamate

Deprotection of Boc-protected amino group by diluted TFA was then confirmed by the disappearance of the signal at 1.47 ppm and no significant change in the osidic region of the spectrum.

Bark derivatization and characterization

Functionalization of bark involved the formation of dialdehyde functions in the polysaccharidic moieties by periodate ($NaIO_4$) oxidation. The methodology is expected to produce high aldehyde functional group content while preventing extensive degradation of bark. Typical aldehyde content of 8.00 mmol.g^{-1}could be estimated through the Canizzaro reaction (Pommerening *et al.* 1992; Klein-Koerkamp *et al.* 2009). Grafting of each aminated oligo-carrageenan was performed by reductive amination according to the procedure described in the experimental section. With value of 4.75 (Table 2), the degree of substitution is small but consistent with our previous data (Astier *et al.* 2010).

Table 2. Substitution Degrees and Mass Yield of Grafted Barks.

Bark	Carrageenans	Galacturonans *
Acidity of attached oligosaccharides (mmol.g^{-1})	2.877	3.017
DS (%)	4.75	7.68
Mass yield	82	80

* Astier *et al.* 2010

FT-IR spectra of dialdehyde and functionalized barks are presented in Fig. 4.

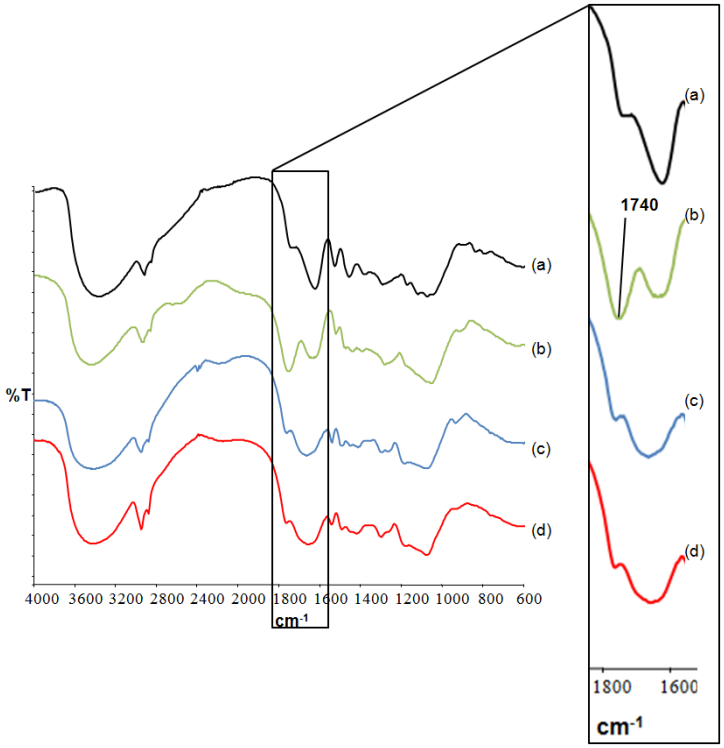


Fig. 4. FT-IR Spectra of crude barks (a), dialdehyde barks (b), barks grafted with oligo-galacturonic acid (c - Astier *et al.*, 2010), and oligo-carrageenans (d).

Aldehyde functionalization is clearly demonstrated through the changes of absorption bands at 1735 cm^{-1} (C=O stretching of aldehydes). The characteristic NH_2 absorption bands from 3300 to 3500 cm^{-1} did not show up because of the overlap with the large OH band from 3200 to 3600 cm^{-1} as well as those of the sulfur-oxygen absorption bands because of the too low substitution degree.

Lead(II) Binding Properties of Crude and Oligo-carrageenan-grafted Barks

Adsorption of lead onto crude and grafted Douglas fir barks was studied through the Langmuir isotherm methodology. The Langmuir parameters, q_{max} (maximum lead adsorption capacity) and b (Langmuir constant, *i.e.* affinity), were graphically deduced from the data presented in Fig. 5.

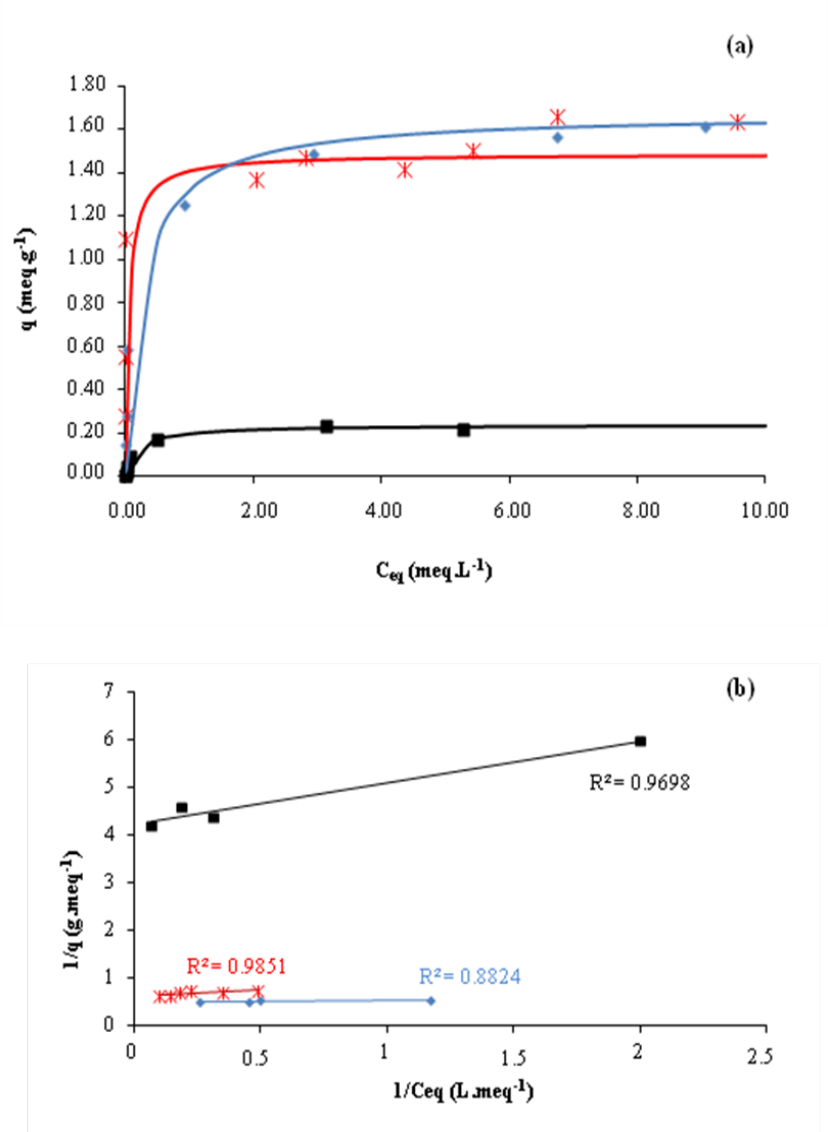


Fig. 5. Lead(II) adsorption on barks: (a) isotherms, (b) Langmuir linearization; ■ Crude barks; barks grafted with ◆ oligo-galacturonic acids (Astier *et al.*, 2010), ✳ oligo-carrageenans

The results (Table 3) show that adsorption behavior of Pb^{2+} ion on both crude and grafted barks can be satisfactorily described by Langmuir isotherms (Fig. 4(b)). Quantitatively, the grafting of oligo-carrageenans onto barks could increase the value of q_{max} by up to 8 times. Furthermore, and compared to crude barks, grafting of oligo-carrageenan onto barks increased their affinity for lead by a factor 4.

Table 3. Langmuir Parameters of Crude and Modified Barks

Bark	Crude	Oligo-carrageenans	Oligo-galacturonans *
q_{max} (meq.g^{-1})	0.24	1.49	1.78
b (L.meq^{-1})	4.79	18.14	3.71
R^2	0.9698	0.9851	0.8824

* Astier *et al*., 2010.

DISCUSSION

The results show an increase of adsorption capacities of the modified barks. This property could be justified by the chemical modification of barks that introduces on their surface a larger number of ion exchange sites. Our chemical modification route of barks, based on periodic oxidation, makes it possible to modulate the nature of the adsorption sites. The choice of the ion exchanger group is guided by the nature of the particulate ion to be removed. Although many authors consider both sulfate and carboxylate as hard bases, these functional groups differ in size, electronegativity, etc. (Table 4).

Table 4. Atomic Parameters of Oxygen, Sulfur, and Carbon

atom	S	C	O
electronegativity	2.58	3.40	3.44
atomic radius (nm)	0.088	0.065	0.047

These parameters establish the hardness sequence $R\text{-}COO^- > R\text{-}SO_3^-$. According to the Pearson's theory of hard and soft acids and bases, carboxyl and sulfate groups are hard and intermediate-to-hard bases, and lead is an intermediate-to-soft metal. In the HSAB theory, hard metals adsorption involve mainly electrostatic interactions with small oxygen-containing ligands such as carboxyl groups, while soft metals adsorption involve mainly interactions with sulfur-containing ligands such as sulfate (Forstner and Wittman 1981; Niu et al. 2010).

In the case of grafted oligo-galacturonans, if the values of adsorption capacities actually increased, the affinity values remain stable, which is in perfect agreement with the HSAB theory. The grafting of a family of ion exchange groups, in the case of oligo-galacturonans, carboxylic acids, enhances the capacity of ion exchange by increasing the number of ion exchange sites. The grafted sites are identical, and they will present the same affinity for the same metal, regardless of their number. In contrast, grafting of oligo-carrageenans introduces sulfate on the surface of barks, and multiplying affinity values by 4 for lead. Such a result increases the interest of our approach and is explained

by the HSAB theory. In fact, lead is a metal ion of moderate strength, and that is why it mainly interacts with ion exchange groups of medium strength such as sulfate groups. Carboxylate groups, which are harder, present a lower affinity for lead.

CONCLUSION

1. Ours work demonstrates the ability of modified barks to have a selective affinity against Pb(II). This affinity owes itself to the nature of ion-exchange groups naturally present or grafted onto barks. In the last case, adsorption capacities and affinity can be increased by factors 8 and 4, respectively.
2. Moreover, our results are consistent with the Hard and Soft Acid and Base theory proposed by Pearson to explain the phenomenon of heavy metal sorption onto by-products from a natural origin, *e.g.* barks.
3. Lastly, our strategy offers the experimenter the opportunity to direct the selectivity of modified bark to adsorb heavy metal by diversifying the grafted chemical groups. Then, selective ion exchangers with enhanced capacities of adsorption could be easily obtained after specific chemical modifications of agricultural or forestry by-products.

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