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Binding of divalent metal cations by sugar-beet pulp

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The binding of divalent metal cations (Ca^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) by sugar-beet pulp was studied. In the presence of 0.1 M $NaNO_3$ the level of metal cation uptake was found to reach its maximum value very rapidly with the speed increasing both with the sugar-beet pulp concentration and with increasing initial pH of the suspension. Using a pH-metric method and by comparing binding isotherms drawn by measuring the free cation concentration after equilibration, a clear scale of decreasing selectivity was found as follows: $Cu^{2+} \sim Pb^{2+} \gg Cd^{2+} \sim Zn^{2+} > Ni^{2+} > Ca^{2+}$. Binding followed the Langmuir-type isotherm except for Ca^{2+}. Adsorption may contribute to the binding phenomenon in addition to ion exchange, which may include electrostatic interactions and even chelation in the case of the more strongly bound cations. Sugar-beet pulp, which is cheap and highly selective, therefore seems to be a promising substrate to entrap heavy metals in aqueous solutions. Copyright © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The standards imposed on industries that discharge heavy metals in their waste effluents are continually tightened due to increasing industrial production and updated knowledge regarding the toxicities of heavy metals that enter the human food chain after accumulating in plants and animals (Förstner & Wittmann, 1983). Conventional methods to reach imposed values of heavy metal concentrations in industrial effluents have shown their limits and are often extremely expensive. The biosorption phenomenon (Volesky, 1990) has provided an alternative treatment of industrial effluents from that of the traditional physico-chemical methods. It involves the use of natural substrates that are provided, for example, by agriculture (Kumar & Dara, 1982; Marshall *et al.*, 1993), forestry waste products (Vazquez *et al.*, 1994), micro-organisms (Brady & Duncan, 1994; Brady *et al.*, 1994), humic substances (Kerndorff & Schnitzer, 1980), lichens (Richardson, 1995), mycorrhizal fungi (Galli *et al.*, 1994) or mariculture (Xue *et al.*, 1988; Holan *et al.*, 1993; Holan & Volesky, 1994). Biosorption is assumed to be metabolism-independent due to the use of non-living biomass. Metal ion uptake may therefore involve complexation, coordination, chelation, ion exchange, adsorption and inorganic microprecipitation according to the type of substrate (Volesky, 1990). In contrast, metal-ion binding to living cells occurs either through surface adsorption or through intracellular accumulation. Significant practical limitations to living biomass-employing methods to treate wastewaters arise from the inhibition of the biomass growth when the metal cation concentrations are too high and/or when significant amounts of metal cations are sorbed. However, methods for water treatment that employ non-living biomass are not complicated by such considerations.

Sugar-beet pulp, the by-product of the sugar industry, is rich in pectic and cellulosic substances, >40% and ~20% of the dry matter respectively. Sugar-beet pulp is very cheap and its production reaches 14×10^6 tonnes of dry matter each year in the European Community. Up to now, its main use has been incorporation in animal feeds. However, several

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*Author to whom correspondence should be addressed. Tel: (33) 2 40 67 50 60; fax: (33) 2 40 67 50 66; e-mail: thibault@nantes.inra.fr potential ways to enhance its value have already been investigated (Broughton *et al.*, 1995), such as production of dietary fibers (Bertin *et al.*, 1988; Michel *et al.*, 1988), pectins (Ralet *et al.*, 1991) or arabinans (Broughton *et al.*, 1995) and, more recently, the use of galacturonic acid for the production of ferulic acid (Micard *et al.*, 1994) and detergents (Petit *et al.*, 1993).

Another application may be the use of sugar-beet pulp as a natural sorbent to entrap heavy metals from aqueous solutions. Sugar-beet pulp exhibits high cation exchange capacity due to the presence of carboxyl functions on galacturonic acids, which are the main component of pectins. Sugar-beet pectins exhibit high affinity towards metal cations with a clear scale of selectivity as follows: $Cu^{2+} \sim Pb^{2+} \gg Zn^{2+} > Cd^{2+} \sim Ni^{2+} \geq Ca^{2+}$ (Dronnet *et al.*, 1996). The cell wall of *Nitella flexilis*, which possess a high amount of pectins, displays high affinity to metal cations and a significant fraction of copper ions can strongly bind to two carboxylate functions in equatorial positions (inner-sphere complex) whilst zinc and calcium ions can only form outer-sphere complexes, thus explaining the higher affinity of pectins towards copper ions (Van Cutsem & Gillet, 1983).

To date, relatively few investigations of metal cation binding by sugar-beet pulp have been performed, however, in one study, beet pulp was studied for decontamination of radioactive wastewater (Langenhorst *et al.*, 1961). The effluent was treated by percolation through a column and the decontamination of solutions loaded with ^{140}Ba and ^{140}La chloride with levels as high as 99% was found to depend on the experimental conditions (flow rate, effluent volume). More recently, the sorption by low-cost adsorbents of hexavalent chromium was examined (Sharma & Forster, 1994). By comparing adsorption isotherms, sugar-beet pulp was found to bind higher quantities of Cr(VI) than sugar-cane bagasse and maize cob, but lower quantities than sawdust. The results were confirmed by regression analysis of parameters calculated from the Langmuir approach, which gave a maximum binding capacity of $1.47 \times 10^{-4}\,mol\,g^{-1}$ at pH 2. Data obtained after treatment of experimental results by the Freundlich approach also confirmed the respective degrees of uptake. These types of application appear to be promising and could make biosorption a new alternative method for adding value to sugar-beet pulp.

In the current work, the binding of Ca^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} by sugar-beet pulp was studied. Initially, a scale of selectivity was set up by a rapid pH-measurement method. The quantification of the metal fraction bound to the substrate was determined by potentiometry or by means of a dye spectrophotometric method and allowed the influence of the initial pH of the medium and the effects of sugar-beet pulp and of metal cation concentrations on the binding level to be studied.

EXPERIMENTAL

Beet pulp

Raw sugar-beet pulp (kindly supplied by Générale Sucrière, France) was ground with a hammer mill (linear velocity of $100\,m\,s^{-1}$) until it passed a 2 mm screen and then it was sieved. The fraction in the range between 250 and 500 μm, corresponding to ~31% of the initial pulp weight, was kept for further experiments. In order to inactivate endogenous enzymes and to discard residual mono- and oligomers, the retained fraction was immersed in boiling 70% ethanol for 20 min and then collected on a sintered glass filter (porosity 2); the residue was resuspended in 70% ethanol and extracted at room temperature until the filtrate was sugar-free as proved by the phenol sulfuric acid test (Dubois *et al.*, 1956). The resulting pulp was dried by solvent exchange (95% ethanol and acetone) and finally air-dried (yield = 28% from the initial ground pulp). This material is referred to as SBP in the text.

The acidic form (Bertin *et al.*, 1988) was obtained by stirring SBP ($10\,g\,l^{-1}$) at 4°C in an aqueous solution of 0.01 M HCl overnight. SBP was then washed with ultrapure water until the disappearance of chloride was observed, as indicated by the silver nitrate test, and then dried by solvent exchange and by air-drying (material recovered = 95%).

Chemical characterization

Moisture content was determined by drying at 120°C for 2 h. All compositions are given on a moisture-free basis. The ash content was determined by incineration at 550°C overnight followed by 1 h at 900°C.

SBP was hydrolyzed by 72% sulfuric acid (1 h, 20°C), diluted to 1 M and heated (3 h, 100°C). Galacturonic acid (GalA) was then quantified colorimetrically by the automated m-hydroxydiphenyl method (Thibault, 1979). Neutral sugars were identified and quantified by GLC after reduction and acetylation (Englyst & Cummings, 1984). Methanol and acetic acid were determined by HPLC after saponification (Voragen *et al.*, 1986). The degrees of methylation (DM) and acetylation (DAc) were calculated as the molar ratios of methanol and acetic acid to GalA, respectively.

Swelling capacity (SC) was measured by the bed volume technique (Kuniak & Marchessault, 1972). Dry SBP (100 mg) was weighed in a glass cylinder and left overnight at 25°C in an excess of 0.1 M $NaNO_3$. Results were expressed as millilitres of swollen sample per gram of initial dry matter. Measurements were carried out in triplicate.

The water-retention capacity (WRC) was determined by centrifugation (MacConnell *et al.*, 1974). The samples were soaked in 0.1 M $NaNO_3$ for 16 h at 4°C and centrifuged for 1 h at 18 000 *g*. The supernatants were carefully removed and the residues left for 1 h on sintered glass (porosity 2). The pellets were weighed, dried for 2 h at 120°C and weighed again. Results were expressed as grams of water per gram of dry residue. Measurements were made in triplicate.

Specific surface area was determined in duplicate by the BET method (Brunauer, 1945) using a Gemini III 2375 Surface Area Analyser (Micromeritics® Instrument Corporation, USA) with nitrogen as the analysis gas.

The cation exchange capacity (CEC) is the total number of ionic sites. It was determined with SBP in the acidic form (100 mg in 50 ml of 0.1 M $NaNO_3$) by pH titration with 0.1 M NaOH and was expressed as mequiv. g^{-1}.

Chemicals

Metal cations (collectively referred to as Me^{2+} in the text) were used as their chloride or nitrate salts: $Cu(NO_3)_2{\cdot}2.5H_2O$, $Cu(Cl)_2{\cdot}2H_2O$, $Cd(NO_3)_2{\cdot}4H_2O$ and $Ni(NO_3)_2{\cdot}6H_2O$ were supplied by Aldrich, $Pb(NO_3)_2$ and $Ca(NO_3)_2{\cdot}4H_2O$ by Merck and $Zn(NO_3)_2{\cdot}7H_2O$ by Fluka. Tetramethylmurexide (TMMX) was purchased from Sigma and used without further purification.

pH-measurement method

pH measurements were performed at 25.0±0.1°C. The pH meter LPH430T (Radiometer Analytical SA, France) was equipped with a combined pH electrode (type U402-S7/120, Ingold) and a temperature probe (XT 130, Radiometer). Calibration was performed before each experiment. SBP (2 g l^{-1}) in the acidic form was stirred for 1 h in 0.1 M $NaNO_3$ to allow hydration and the pH adjusted to 5.1 by adding 0.1 M NaOH. pH measurements were performed either by a continuous mode or by a batch method. The continuous mode was performed as follows: addition of a constant quantity of a 0.01 M solution of Me^{2+}, 15 min of stirring, 15 min of rest, pH measurement on the supernatant, new addition of Me^{2+}, stirring (15 min), rest (15 min), pH measurement and so on. The batch method was carried out by adding various quantities of a 0.01 M solution of Me^{2+} to SBP suspensions (2 g l^{-1}), stirring for 24 h and then measuring the pH of the supernatants after 15 min of rest. The experimental results are presented by plotting the pH variation corrected for dilution (ΔpH) against the ratio $[Me^{2+}]_t/[C_p]$ ranging from 0 to 1.6, where $[Me^{2+}]_t$ is the total metal cation concentration in equiv. l^{-1} and $[C_p]$ is the concentration of SBP in equiv l^{-1} as determined from the following equation:

$$[C_p]\,(\text{equiv.}\,l^{-1}) = [\text{SBP}]\,(g\,l^{-1}) \times \text{CEC}\,(\text{equiv.}\,g^{-1})$$

Metal cation binding

Experiments were carried out in batch assays. Suspensions of SBP in the acidic form at given concentrations were stirred to complete hydration in 0.1 M $NaNO_3$ at 25.0±0.1°C for 1 h and then the pH was adjusted to a stable initial value by adding 0.1 M NaOH. After 1 h of stirring, varying amounts of a 0.01 M solution of Me^{2+} were added to the suspensions and the mixtures were stirred for 2 h.

Ca^{2+}, Ni^{2+} and Zn^{2+} activity was determined by means of a dual-wavelength spectrophotometric method, using TMMX as an activity probe for Me^{2+} (Kwak & Joshi, 1981; Dronnet *et al.*, 1996). After equilibration (see next subsection), absorbances of TMMX complexes were measured, with a S-1000 spectrophotometer (Secomam, France), on supernatants which had been filtered off using 0.45 μm filtration units Analypore® (OSI, France). Cd^{2+}, Cu^{2+} and Pb^{2+} activity was directly determined on the reaction mixture by potentiometry using ion-selective electrodes (XS 300, XS 290 and XS 310, respectively, Radiometer) and a saturated calomel electrode (XR 100, Radiometer) equipped with a protection extension (XL 100, Radiometer) filled with 1 M KNO_3 and connected to a potentiometer (LPH430T, Radiometer). Calibration was carried out before each experiment with standard cadmium, copper and lead nitrate solutions in the presence of 0.1 M $NaNO_3$.

Activities were calculated from known concentrations in calibration solutions using the Debye–Hückel theory formula (Dronnet *et al.*, 1996). The same procedure was employed to determine free ion concentrations, $[Me^{2+}]_f$, from measured activities in assays. Potentiometric measurements were also performed with filtered supernatants as analyzed in the dual-wavelength spectrophotometric method. No significant difference of $[Me^{2+}]_f$ was found between filtered and non-filtered supernatants, showing that filtration did not disturb the free cation fraction measurement.

The experimental data obtained by activity measurements are presented as binding isotherms $[Me^{2+}]_b/[C_p]$ vs. $[Me^{2+}]_t/[C_p]$, where $[Me^{2+}]_b$ is the bound metal cation concentration in equiv. l^{-1}. Total binding of the added metal cation to the single carboxyl functions would occur according to a 'stoichiometric isotherm' symbolized by a straight line on the graphs. The 'stoichiometric isotherm' reaches a plateau when all the carboxyl functions carried by pectins are saturated by metal cations, according to the ratio of one divalent cation per two carboxyl functions.

Kinetic assays

Cu^{2+} and Ni^{2+} binding by SBP was studied in the range 5 min to 20 h at 25.0±0.1°C. $[Me^{2+}]_f$ was determined after filtration of the supernatants. Experiments were performed with SBP with an initial pH of 7.2 at either 1.82 or 7.27 g l^{-1} (corresponding to 1 and 4 mequiv. l^{-1}, respectively), and with $[Me^{2+}]_t/[C_p]$ values of 0.1 or 1.

Theoretical interpretation according to the Langmuir-type isotherm

The data were fitted to a linearized form of the Langmuir isotherm (Langmuir, 1918):

$$\frac{[\mathrm{Me}^{2+}]_f}{[\mathrm{Me}^{2+}]_L} = \frac{1}{(K_L.\mathrm{Me_bmax})} + \frac{[\mathrm{Me}^{2+}]_f}{\mathrm{Me_bmax}} \qquad (1)$$

where $[\mathrm{Me}^{2+}]_L$ is the amount of metal cation bound at equilibrium per gram of SBP. K_L (l. equiv.$^{-1}$) is the Langmuir constant related through the Arrhenius equation to the energy of adsorption and thus represents the adsorption equilibrium constant, and $\mathrm{Me_bmax}$ (equiv. g^{-1}) is the maximum binding capacity.

RESULTS AND DISCUSSION

Biomass

The main components of SBP are compiled in Table 1. Arabinose, glucose (mostly from cellulose) and GalA were the main components. The high amount of arabinose and the high degree of acetylation (52) are typical features of pectins from sugar-beet pulp. This composition is in agreement with previous published results (Micard *et al.*, 1994; Renard *et al.*, 1994).

The CEC value (0.55 mequiv. g^{-1}) was close to previously reported values (Bertin *et al.*, 1988; Ralet *et al.*, 1991; Renard *et al.*, 1994) (Table 2). This experimental value is in very good agreement with the value that can be calculated (0.545 mequiv. g^{-1}) from the GalA content and the DM, showing that the methylester-free galacturonosyl units of pectic chains can fully account for the ionic binding of SBP. A specific surface area of 3.05 m^2 g^{-1} was found by the BET method.

Hydration properties were measured for SBP in conditions known to give minimal hydration values (Renard *et al.*, 1994). Hydration capacities were in the same range as values already found (Bertin *et al.*, 1988; Michel *et al.*, 1988; Ralet *et al.*, 1991; Renard *et al.*, 1994). SC measurements were also performed with varying $[\mathrm{Me}^{2+}]_t$ up to 0.1 M in the presence of 0.1 M $\mathrm{NaNO_3}$: addition of metal cations was found to lower SC values to 11 ml g^{-1}, suggesting that the presence of divalent cations resulted in some ionic cross-linking. No significant difference of SC values was observed either between the metal cations or with varying concentrations of metal cation. Lower SC values (6.2 ml g^{-1}) were found in distilled water for *Ascophyllum nodosum* (Holan & Volesky, 1994).

Table 1. Main components of sugar-beet pulp in the acidic form

Component	Dry matter (mg g^{-1})
Rhamnose	22
Fucose	1
Arabinose	229
Xylose	15
Mannose	13
Galactose	56
Glucose	206
Galacturonic acid	200
Methanol (DM)	19 (52)
Acetic acid (DAc)	35 (52)
Ash	11

DM (degree of methylation in %) and DAc (degree of acetylation in %) are the number of methyl-esterified galacturonosyl residues and acetylated galacturonosyl residues for 100 galacturonosyl residues.

Table 2. Main physico-chemical parameters of sugar-beet pulp in the acidic form

Parameter	Value
CEC (mequiv. g^{-1})	0.55
Bulk density (g l^{-1})	335
Swelling capacity (ml g^{-1})	17.9
Water-retention capacity (g g^{-1})	17.2
Specific surface area (m^2 g^{-1})	3.05

Leaching of material was observed during binding assays and was due to a slight aqueous extraction of polysaccharides, which is amplified by stirring. The GalA loss was always below 5% of the initial amount of GalA in the SBP. This loss decreased as the binding increased before reaching a plateau at a value depending on the metal cation. Moreover, the GalA loss was found to be lower for Cu^{2+} and Pb^{2+} than for the other cations, suggesting that the ionic cross-linking is higher with the two first metal cations. The loss of GalA was not considered in the calculation of the bound cation fraction values by SBP due to its negligible value. Such a loss of polysaccharides (alginates) was also observed for marine algae during binding of metals (De Carvalho *et al.*, 1994).

Kinetics of binding

Preliminary studies were performed by pH measurements of the SBP in the acidic form, either by a batch method (Fig. 1a) or by a continuous method (Fig. 1b). Experiments were carried out with SBP suspended in 0.1 M $\mathrm{NaNO_3}$ in order to minimize the Donnan exclusion phenomenon.

The pH-measurement method was based on the fact that the exchange of the protons at the ionic sites with Me^{2+} leads to an increase of the proton concentration in solution, which is more marked for the more strongly bound metal cations. It can therefore be assumed that the more the pH decreases, the higher the affinity of pectins for a given Me^{2+}.

pH variations were found to be similar and to follow the same features whatever the experimental conditions.

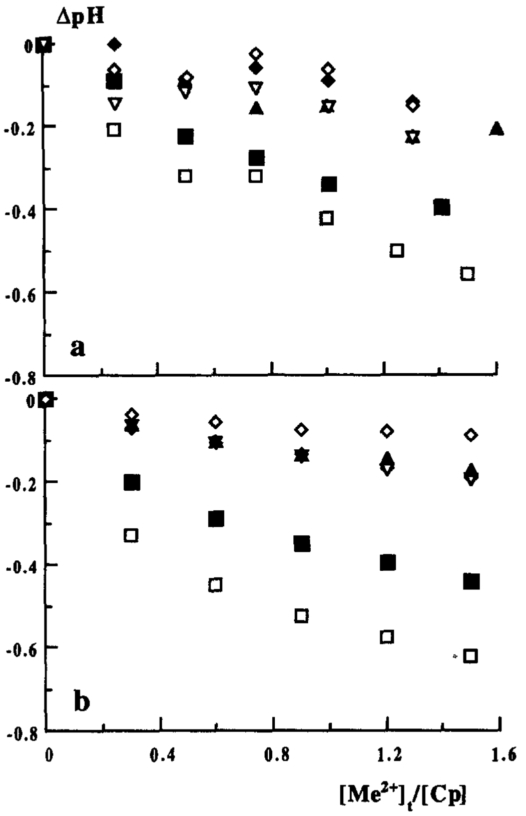

Fig. 1. Effect of metal cation binding on the pH of SBP suspensions at an initial pH~5.1 and at 3.64 g l^{-1} in 0.1 M $NaNO_3$ at 25°C: (□) Cu^{2+}, (■) Pb^{2+}, (▽) Cd^{2+}, (◆) Zn^{2+}, (▲) Ni^{2+}, (◇) Ca^{2+}; (a) by the batch method, (b) by the continuous method.

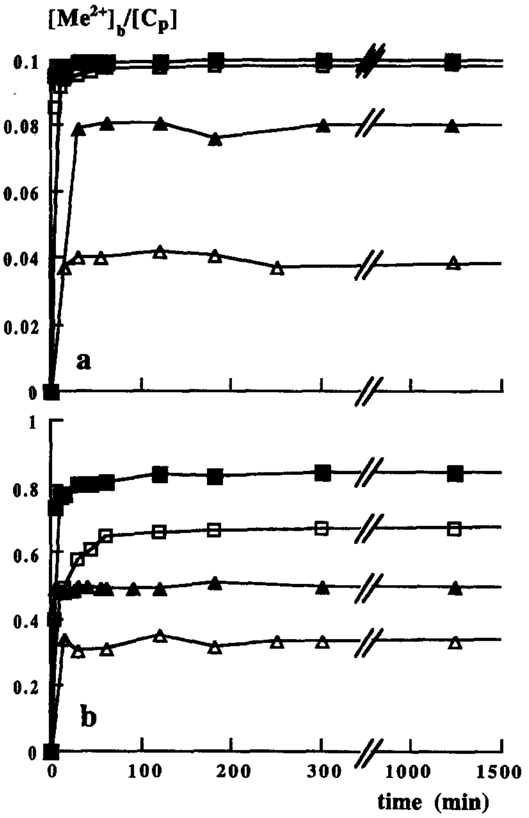

Fig. 2. Time course of the binding by SBP in 0.1 M $NaNO_3$ at an initial pH~7.2 of (□) Cu^{2+} and (△) Ni^{2+}. Empty symbols: [SBP] = 1.82 g l^{-1}; full symbols: [SBP] = 7.27 g l^{-1}. $[Me^{2+}]_t/[C_p] = 0.1$ (a) and 1 (b).

A first group of cations (Ca^{2+}, Ni^{2+}, Cd^{2+} and Zn^{2+}) could be characterized by a low and quasi-linear decrease of pH. The second group (Cu^{2+} and Pb^{2+}) showed much more pronounced pH variations, which tended to a plateau in the final stages. This plateau was more marked by the continuous method, which also gave more reproducible results. No significant difference of the ΔpH magnitudes was found between the two experimental approaches, suggesting that the cation binding reached equilibrium rapidly. Indeed, no significant pH variation was observed after 30 min.

Kinetic assays, performed with Cu^{2+} and Ni^{2+}, confirmed that the maximum of the binding level occurred rapidly (< 1.5 h) irrespective of the SBP quantity and of the metal cation, with more than 72% of the final binding level reached within 10 min (Fig. 2). This level seemed to be reached more rapidly but at lower values for Ni^{2+} than for Cu^{2+}. This shows that the lower the binding level, the easier/faster the uptake, indicating that the binding process could be more complex for the more strongly bound cations. Thus, activity of metal cations was measured after 2 h of stirring.

Experimental data of the binding of Cu^{2+} and Ni^{2+} were plotted in terms of the following pseudo first-order rate expression:

$$\ln \frac{[Me^{2+}]_t}{[Me^{2+}]_f} = kt \qquad (2)$$

where k is the initial rate constant of binding (min^{-1}). Linear plots of $\ln([Me^{2+}]_t/[Me^{2+}]_f)$ vs. time were obtained for both cations (plots for Cu^{2+} only shown in Fig. 3). This demonstrates the applicability of equation (2) to different $[Cu^{2+}]_t$ and different SBP concentrations. The k values obtained in the case of Cu^{2+} ions are indicated in Table 3; the k values of Ni^{2+} ions are not shown since the high binding rate of Ni^{2+} ions precluded reliable results.

At a given $[Cu^{2+}]_t$, the k values presented no significant difference whatever the concentration of SBP, as expected from pseudo first-order kinetics, while they decreased with increasing $[Cu^{2+}]_t$ for a given SBP concentration. Similar results have already been reported for the sorption of Pb^{2+} by coconut-shell carbon, granular-activated carbon (Arulanantham *et al.*, 1989) and biogas resident slurry (Namasivayam & Yamuna, 1995). However, the initial rate of cobalt binding by *Ascophyllum nodosum* has been reported to increase with increasing initial concentration of cobalt (Kuyucak & Volesky, 1989).

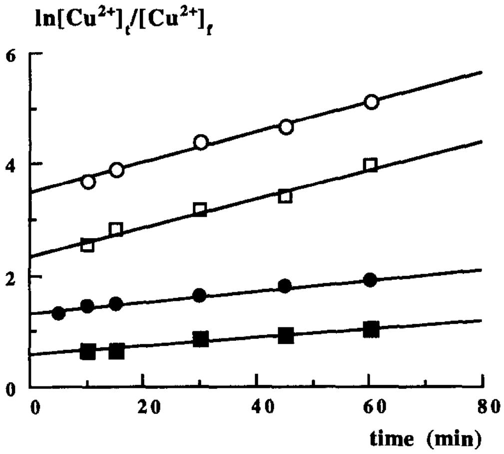

Fig. 3. Pseudo first-order kinetic plots of the binding of Cu^{2+} by SBP in 0.1 M NaNO$_3$ at an initial pH~7.2: (□) [SBP]=1.82 g l^{-1}, (○) 7.27 g l^{-1}. [Me^{2+}]$_t$/[C_p]=0.1 (empty symbols) and 1 (full symbols).

Table 3. Copper binding rate constants

[SBP](g l^{-1})	[Cu^{2+}]$_t$/[C_p]	Rate constant k (min^{-1})×100
1.82	0.1	2.6
	1	0.8
7.27	0.1	2.8
	1	1.0

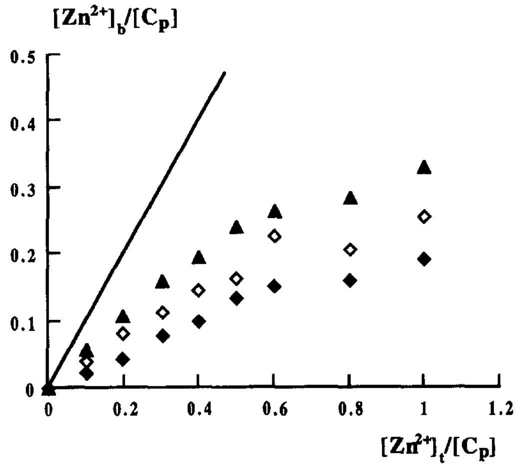

Fig. 4. Influence of initial pH on the binding isotherms of Zn^{2+} by SBP at 3.64 g l^{-1} in 0.1 M NaNO$_3$ and at an initial pH (◆) 3.5, (◇) 4.6 and (▲) 7.2; (———) stoichiometric isotherm.

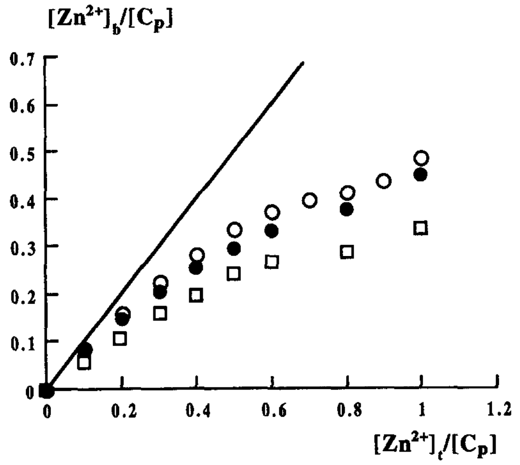

Fig. 5. Influence of [SBP] on the binding isotherms of Zn^{2+} by SBP at an initial pH~7.2 in 0.1 M NaNO$_3$, with [SBP] at (□) 3.64, (●) 7.27 and (○) 14.55 g l^{-1}; (———) stoichiometric isotherm.

Influence of pH on binding

Zn^{2+} binding increased with initial pH values (Fig. 4) as already reported in the literature for different substrates and other cations (Van Cutsem & Gillet, 1983; Darnall *et al.*, 1986; Holan *et al.*, 1993; Namasivayam & Yamuna, 1995). Indeed, binding isotherms of Zn^{2+} increased as the initial pH of the suspension varied from 3.5 to 7.2.

As the pH is lowered, the overall surface charge on SBP will become increasingly less negative, which reduces the attraction of positively charged metal cations. Increasing the initial pH therefore increases the interactions of divalent metal cations with SBP. As the maximal binding level can be reached when all of the carboxyl functions are neutralized, the binding assays were performed at pH ~7.2.

This pH dependence of the binding showed that ion exchange is involved in the binding mechanism of metal cations by sugar-beet pulp.

Influence of the sugar-beet pulp concentration on binding

Binding isotherms obtained for Zn^{2+} tended towards the 'stoichiometric isotherm' as the concentration of SBP increased (Fig. 5). Nevertheless, this increase diminished with SBP concentration. The same feature was found when SBP concentration increased from 3.64 to 14.55 g l^{-1} (2 and 8 mequiv. l^{-1}), with an intensity depending on the metal cation (Fig. 6). Indeed, the difference of binding between the two groups of metal cations became less marked with higher pulp concentration since binding levels increased more drastically for Cd^{2+}, Zn^{2+}, Ni^{2+} and Ca^{2+} than for the two more strongly bound cations.

An increase of the binding with the substrate concentration was also obtained when uptake of metal ions was carried out with polymers in solution such as polygalacturonate (Jellinek & Sangal, 1972) or pectins (Dronnet *et al.*, 1996) in the presence of supporting monovalent salts. This can be explained by the competitive interactions between Me^{2+} and Na$^+$ for galacturonate becoming more favourable for the divalent cation when the ratio [Me^{2+}]/[Na$^+$] increases and because the divalent cation exhibits higher affinity towards SBP than the monovalent cation.

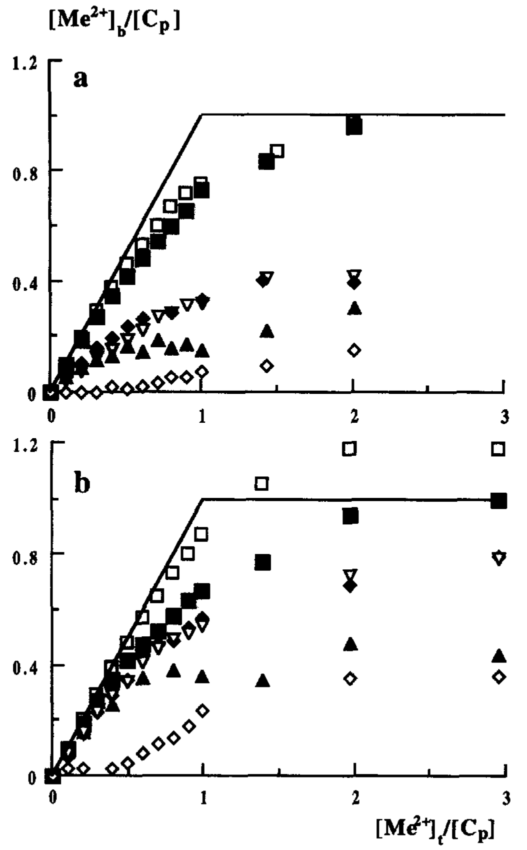

Fig. 6. Influence of the metal cation on the binding isotherms of SBP in 0.1 M $NaNO_3$ at 25°C at an initial pH~7.2: (□) Cu^{2+}, (■) Pb^{2+}, (▽) Cd^{2+}, (◆) Zn^{2+}, (▲) Ni^{2+}, (◇) Ca^{2+}; (———) stoichiometric isotherm. (a) 3.64 g l^{-1} and (b) 14.55 g l^{-1}.

As shown in Fig. 6b, binding isotherms may exceed the 'stoichiometric isotherm' in the case of the more strongly bound cations. Nevertheless, the exact stoichiometry, by referring to carboxyl functions, was not reached with the concentrations of SBP used here. The concentration of SBP needed for a total saturation of carboxyl functions by Cu^{2+} was determined by extrapolation of the curved plot $[Cu^{2+}]_b$ vs. [SBP] for a ratio $[Cu^{2+}]_t/[C_p] = 1$, i.e. where two carboxylates bind one copper ion, to the maximum binding capacity (0.55 mequiv. g^{-1}) (not shown). The complete binding of Cu^{2+} by carboxylates would occur for [SBP]~50 g l^{-1}.

Scale of selectivity

Binding isotherms of the metal cations for SBP concentrations of 3.64 and 14.55 g l^{-1}, with an initial pH of ~7.2, are presented in Fig. 6, respectively. Such binding isotherms facilitate comparison of the binding of different metal cations to one another and the binding of metal cations by soluble polymers such as pectins (Garnier *et al.*, 1994; Dronnet *et al.*, in press). Experiments made with Cu^{2+} in the chloride and nitrate forms exhibited no significant difference. The sorption of metal cations was therefore assumed to be independent of the type of inorganic anionic counterparts in the salts.

For a given SBP concentration, the level of the binding isotherm depends on the metal cation. The same scale of selectivity as obtained by pH measurements was found by titration of the free cation fraction after equilibrium:

$$Cu^{2+} \geq Pb^{2+} \gg Cd^{2+} \sim Zn^{2+} > Ni^{2+} > Ca^{2+}$$

For all cations, the binding isotherms reached a plateau at increasing binding levels with increasing values of $[Me^{2+}]_t/[C_p]$, except for Ca^{2+}. Moreover, the more strongly bound cations, Cu^{2+} and Pb^{2+}, reached a higher plateau, close to the stoichiometry, for a higher $[Me^{2+}]_t$ than Cd^{2+}, Zn^{2+} and Ni^{2+}. The saturation of the ionic sites started at lower initial metal ion concentrations and at a lower binding extent for the more weakly bound ions. Ca^{2+} binding started at a value of $[Ca^{2+}]_t/[C_p]$~0.3 and followed a concave-shaped course up to ~1 where the plateau was directly reached, indicating that the binding of Ca^{2+} is difficult at low concentration but becomes easier as $[Ca^{2+}]_t$ is raised. The same feature was noted for the system Ca^{2+}/sugar-beet pectins/0.1 M $NaNO_3$, for which apparent association constants were found to be more than 10 times lower than for other systems of metal cations/pectins (Dronnet *et al.*, 1996).

Similar selectivity scales have already been found with various substrates. The microalgae *Chlorella regularis* (Darnall *et al.*, 1986) and *Chlorella fusca* (Wehrheim & Wettern, 1994) exhibit the order of affinity for divalent cations $Cu^{2+} > Zn^{2+} > Ba^{2+}$ and $Cu^{2+} \geq Pb^{2+} \gg Cd^{2+}$, respectively, while $Cu^{2+} \gg Zn^{2+} \geq Cd^{2+}$ was found in the case of *Ascophyllum nodosum* (De Carvalho *et al.*, 1994). Affinity of rice milling by-products (hulls and bran) for cations decreased in the order $Cu^{2+} \gg Zn^{2+} > Co^{2+} > Ni^{2+}$ and this was ascribed to the presence of phytic acid and proteins (Marshall *et al.*, 1993).

Thus, pH measurements and binding isotherms show the same selectivity scale with the pulp and with solutions of sugar-beet pectins (Dronnet *et al.*, 1996), suggesting that no difference of selectivity occurs between the extracted form of the polymer and its cross-linked form inside the plant cell wall. However, binding isotherms of SBP were higher and reached higher plateaus than those of sugar-beet pectins. Furthermore, the binding level of metal cations may be higher than the level of the stoichiometric isotherm.

Langmuir-type isotherms

While the experimental data obtained to establish binding isotherms can be plotted in terms of Scatchard

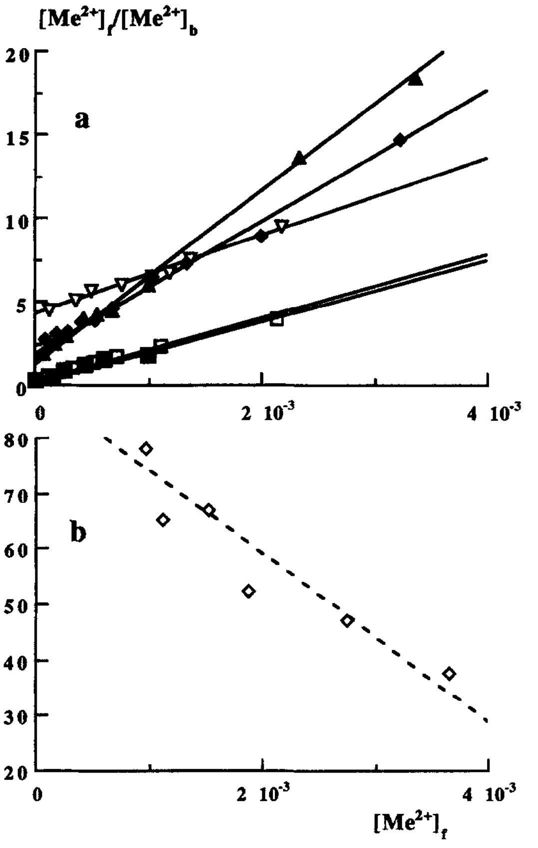

Fig. 7. Linearized Langmuir plots for sugar-beet pulp at 3.64 g l^{-1} in 0.1 M $NaNO_3$ at 25°C at an initial pH~7.2. (a) (□) Cu^{2+}, (■) Pb^{2+}, (▽) Cd^{2+}, (◆) Zn^{2+}, (▲) Ni^{2+}; (b) (◇) Ca^{2+}.

and Hill plots in the case of soluble polymers (Dronnet *et al.*, 1996), data obtained from metal cation binding by SBP were plotted in terms of the linearized Langmuir isotherm according to equation (1). The Langmuir isotherm basically approximates to monomolecular adsorption of gases and it is widely applied to experimental data in the case of adsorption from solution by a surface-active solid where random adsorption occurs onto independent sites (Langmuir, 1918).

Linear plots of $[Me^{2+}]_f/[Me^{2+}]_L$ vs. $[Me^{2+}]_f$ were obtained showing that the Langmuir adsorption model is well suited to describe the phenomenon of divalent metal cation binding by SBP (Fig. 7a), except for Ca^{2+} (Fig. 7b). The Me$_b$max and K_L constants determined from Langmuir plots are presented in Table 4. The values of Me$_b$max/CEC are also presented in Table 4; they correspond to the degree of saturation of the ionic sites.

These results confirm the previous assumption regarding the pH-dependence of metal cation binding and are illustrated by the binding of Zn^{2+} for which Me$_b$max increased, together with K_L, with increasing initial pH. Me$_b$max values increased with the concentration of SBP for each metal cation, confirming results already shown by binding isotherms. Parameters of the Langmuir-type isotherm equation allowed the quantitative scale of selectivity $Cu^{2+} > Pb^{2+} > Zn^{2+} > Cd^{2+} > Ni^{2+}$ to be drawn. This selectivity order, which corresponds to the one determined by comparison of binding isotherms, is also shown with polycarboxylate-containing substrates following the Langmuir-type isotherm (Kuyucak & Volesky, 1989; Marshall *et al.*, 1993; Sampedro *et al.*, 1995). Values of Me$_b$max found with SBP were found to be lower than values obtained for the measurements on other substrates where different experimental conditions were used, such as 0.74 mequiv. of Cd^{2+} per gram of a non-living brown alga (Winter *et al.*, 1994) and 1.21, 0.75 and 0.23 mequiv. of Cu^{2+}, Zn^{2+} and Ni^{2+}, respectively, per gram of rice bran (Marshall *et al.*, 1993).

Whatever the SBP concentration, much higher values of the binding equilibrium constant K_L were found for Cu^{2+} and Pb^{2+} than for Cd^{2+}, Ni^{2+} and Zn^{2+}. Furthermore, values of K_L increased with increasing SBP concentration for the latter cations, whereas they decreased for Cu^{2+} and Pb^{2+}. Such a trend reinforced

Table 4. Langmuir isotherm parameters

Cation	[SBP](g l^{-1})	Initial pH	K_L(l equiv.$^{-1}$)	Me$_b$max (mequiv. g^{-1})	Me$_b$max/CEC
Cu^{2+}	3.64	7.2	10970	0.54	0.99
	14.55	7.2	8200	0.62	1.12
Pb^{2+}	3.64	7.2	6610	0.46	0.83
	14.55	7.2	6570	0.58	1.05
Cd^{2+}	3.64	7.2	620	0.38	0.77
	14.55	7.2	750	0.47	0.86
Zn^{2+}	3.64	3.5	1120	0.16	1.65
	3.64	4.6	1160	0.19	0.53
	3.64	7.2	1700	0.27	0.48
	7.27	7.2	2170	0.47	0.85
	14.55	7.2	2360	0.49	0.92
Ni^{2+}	3.64	7.2	300	0.15	0.27
	14.55	7.2	410	0.36	0.65
Ca^{2+}	—	—	—	—	—

the assumption that the mechanisms governing the binding to SBP are more complex for Cu^{2+} and Pb^{2+} than for the more weakly bound cations. This feature differs from the evolution of Me_bmax, which increased together with the concentration of SBP, irrespective of the metal cation, as already observed. It is also not in agreement with the tendency of the apparent association constant $K_{H^{-1}}$ calculated from the Hill approach in the case of interactions of Me^{2+} with sugar-beet pectins (Dronnet *et al.*, 1996). Indeed, whatever the metal cation, $K_{H^{-1}}$ values decreased with increasing pectin concentration in the absence of supporting salt while they increased in the presence of 0.1 M $NaNO_3$ (Dronnet *et al.*, 1996).

The binding level of Cu^{2+} and Pb^{2+} is higher than the stoichiometry for the highest SBP concentration, suggesting that not only electrostatic interactions were responsible for the binding. As shown in Table 4 in the case of Zn^{2+}, the value of the ratio Me_bmax/CEC found for $pH = 3.5$ illustrated the fact that metal cations compete with protons for binding when all of the ionic sites are not neutralized. The lower the dissociation coefficient, the higher the competition between metal cations and protons, and the lower the binding equilibrium constant K_L.

Phenomena other than electrostatic interactions could occur in the cation binding inside the cell wall. Indeed, adsorption and/or chelation involving hydroxyl functions, close to carboxylates, may increase the binding level of cations such as Cu^{2+} and Pb^{2+} in addition to the electrostatic interactions taking place in the binding of Ca^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+}. Complexation of metals with neutral mono- and polysaccharides in solution, undoubtedly involving hydroxyl functions, has already been reported (Angyal, 1989).

CONCLUSION

Binding of metal cations to sugar-beet pulp occurs according to a clear scale of selectivity, which decreases as follows: $Cu^{2+} \geq Pb^{2+} \gg Cd^{2+} \sim Zn^{2+} > Ni^{2+} > Ca^{2+}$. This scale agrees with previous selectivity scales established for solutions of sugar-beet pectins or for substrates carrying carboxyl functions (Angyal, 1989; Dronnet *et al.*, 1996). Binding was influenced by pH and the amount of sugar-beet pulp: the higher the initial pH of the suspension, and the higher the concentration of SBP, the higher the binding levels. Maximum uptake of metal cations was reached very quickly (< 1.5 h) and binding of metal cations by sugar-beet pulp was most likely to be first order. The sorption of divalent metal cations by SBP seemed to involve adsorption phenomenon in addition to ion exchange and electrostatic interactions may be completed by chelation for the more strongly bound cations, as in the case of the binding of divalent cations by the soluble form of sugar-beet pectins (Dronnet *et al.*, 1996).

SBP is a very cheap industrial by-product, readily obtainable in large quantities and exhibiting excellent binding capacities with limited leaching of pectins during the binding process. All of these factors make sugar-beet pulp a potential substrate for decontamination of metal-charged wastewaters. Furthermore, its binding capacities could be easily improved. For instance, saponification of the methyl-esterified galacturonosyl units or oxidation of hydroxyl functions may significantly increase the cationic exchange capacity. On the other hand, simple chemical cross-linking reactions may increase the rigidity of the matrix and may provide a new material with higher binding capacity and lower hydration properties.

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