

Oleaginous seeds, press-cake and seed husks for the biosorption of metals

J. Boucher, C. Chabloz, O. Lex and I. W. Marison

ABSTRACT

The press-cake (PC) of oleaginous species (*Brassica napus*, *Moringa oleifera*, *Glycine max*) has been investigated as a biosorbent for metals removal from contaminated water.

Sorption isotherms for copper (Cu) have been established for different pH values and fitted with Langmuir isotherms. The maximum sorption capacity was observed at pH 5 ($q_{\max} = 11.5$ mg/g). The press-cake has been fractionated into water-soluble components, husks and the remaining solid residue. From these fractions the husks are responsible for the largest part of the sorption activity ($q_{\max} = 36.6$ mg/g). The sorption mechanism was shown to be complex, involving proton exchange, chemisorption and calcium exchange. FTIR experiments showed that hydroxyl groups are involved in the metal binding.

The findings show that in the removal of toxic metals, the use of oleaginous seed by-products represent a low cost, domestic and environmentally friendly technology, from which the metals, in concentrated form, may be readily recovered.

Key words | biosorbent, husks, metals, press-cake, rapeseed, water treatment

J. Boucher

C. Chabloz

O. Lex

Ecole Polytechnique Fédérale de Lausanne (EPFL),
Laboratory of Chemical and Biochemical
Engineering,
CH-1015 Lausanne,
Switzerland

I. W. Marison (corresponding author)

School of Biotechnology,
Dublin City University, Glasnevin,
Dublin 9, Ireland
Tel.: +353 1 700 83 93
Fax: +353 1 700 54 12
E-mail: ian.marison@dcu.ie

ABBREVIATIONS

C^*	Concentration of metal in the aqueous phase at equilibrium [mg/L]
K	Affinity constant for the Langmuir model [L/g]
m	mass [mg]
q_{\max}	Maximum adsorption capacity for the Langmuir model [mg/g]
q^*	Concentration of metal sorbed on the biosorbent at equilibrium [mg/g]
Y	Sorption efficiency [%]
V	Volume [L]
EDS	Electron dispersive spectroscopy
FTIR	Fourier-transform infrared spectroscopy
HOP	Hydrophobic organic pollutant
PC	Press-cake
SEM	Scanning electron microscopy
WS	White sediment
XPS	X-ray photoelectron spectroscopy

doi: 10.2166/aqua.2008.044

INTRODUCTION

Waste streams containing low-to-medium levels of heavy metals are often encountered in metal plating facilities, mining operations, landfill leachates, fertilizers and electronic device manufacturers. To remove metals from metal laden wastewater, a number of methods have been proposed, including chemical precipitation (Matlock *et al.* 2002), ion exchange (Erdem *et al.* 2004), membrane separation (Atamanenko *et al.* 2003), solvent extraction (Silva *et al.* 2005), electrodeposition (Kongsricharoern & Polprasert 1996) and adsorption (Park & Kim 2005). The need to find affordable and simple technologies for the treatment of metal laden waste water at small scale and/or in developing countries has been pointed out in recent years and attention directed to *biosorption*, based on the metal binding capacities of many biological materials, of which many reviews have been published over the last ten years (Veglio & Beolchini 1997; Volesky 2001).

Press-cake (PC) is a secondary by-product of the oil mill industry, resulting from the pressing of oleaginous seeds. It consists of a solid residue made of the residual crushed seed constituents and a non-negligible amount of oil trapped in the solid matrix. PC of oleaginous seeds represents an enormous amount of agricultural material which is presently poorly valorised. Indeed, for rapeseed, PC represents about 60% of the original weight of the seeds (Bell 1995). PC contains between 36 to 44% crude proteins. However, many oilseeds also contain high levels of anti-nutritive factors such as glucosinolates, eruric acid, phytate or tannins, as well as hull residues (Davin 1983), which may suppress the value of the meal if sold as a protein supplement to animal feed (Bell & Keith 1991; Liu *et al.* 1994; Li *et al.* 2002; Zukalova & Vasak 2002). As a consequence, considerable research is aimed at finding new ways to valorise PC, such as soil amendment (Anikwe 2000), composting for agro-productions (Zervakis *et al.* 1996; Manios 2004) and production of biofuels or biogas (Jimenez & Gonzalez 1991; Kostov *et al.* 1995; Staubmann *et al.* 1997). Having a high energy content, PC may be incinerated to derive thermal energy (Salunkhe *et al.* 1992; Natarajan *et al.* 1998; Manios 2004).

In a previous work (Boucher *et al.* 2007), the retention of hydrophobic organic pollutants (HOP) in PC was described. In the case of the HOP tested, retention was the consequence of absorption in the residual oil trapped within the PC matrix. PC is a very heterogeneous material composed of various fractions as shown in Table 1.

Table 1 | Composition of PC divided in the cotyledon + hypocotyls (corresponding to the flesh of the seeds - WS) and husks. Data are from Niewiadomski (Niewiadomski 1990) in % of moisture free, oil free matter; * data for calcium was measured in the PC (cotyledon + hypocotyls + husk)

	Rapeseed cotyledon + hypocotyl [% (w/w)]	Rapeseed husks [% (w/w)]
Lignin	0.7	25.5
Cellulose	4.7	13.7
Hemicellulose	9.4	19.0
Pectin	8	12
Starch	2.1	0.4
Sucrose	7.7	1.9
Proteins	50.7	18.1
Calcium	0.66*	

Like all vegetal biomass, press-cake is composed of cellulose, lignin and hemi-cellulose; thus PC harbours many potential metal binding functional groups, which makes PC a promising biosorbent for removing metals from contaminated waters. Indeed, lignin (Dizhbite *et al.* 1999; Garcia-Valls & Hatton 2003), cellulose (Ogiwarai & Kubota 1969; Shukla & Sakhardande 1991), hemicellulose and pectins (Nawirska 2005), phytic acid (Martin & Evans 1986, 1987), as well as many proteins (Mejare & Bulow 2001), are all compounds present in rapeseed PC which have been described as having metal binding properties. Incidentally, many fruit/seed by-products have also been described as having biosorption properties (Okieimen & Onyenkpa 1989; Johnson *et al.* 2002; Dupont *et al.* 2003; Saaed & Iqbal 2003; Teixeira Tarley & Zezzi Arruda 2004; Nawirska 2005). However the sorption capacity of PC from various plant species for metal ions has not been previously reported and it is hoped that the present work will contribute in filling this gap.

The aims of the present work were to (1) investigate metal sorption by oleaginous seeds and PC and identify which fraction is responsible for biosorption and (2) describe the mechanisms responsible for the sorption of the active fraction.

MATERIALS AND METHODS

Materials

Fresh rapeseed PC resulting from cold pressing of rapeseed (*Brassica napus*) seeds was obtained from Eco-Energie SA (Etoy, Switzerland). Soybean PC resulting from hot pressing (100°C) of soybean (*Glycine max*) seeds was obtained from Florin AG (Muttens, Switzerland). Moringa PC resulting from cold pressing of dehusked Moringa (*Moringa oleifera*) seeds was obtained from Optima Environnement SA (Nyon, Switzerland). Rapeseed seeds (*Brassica napus*) were provided by the Station Fédérale de Recherches Agronomiques de Changins (Switzerland).

When required PC or seeds were ground to the desired granulometry using a blender (IKA Model A11 Basic, Switzerland). Unless otherwise stated all experiments were undertaken using the 200–630 µm fraction.

Copper solutions (250 mg/L) were prepared fresh before each experiments from CuSO₄ (Fluka Chemie N°61230, Buchs, Switzerland). The pH was adjusted to the desired value with 0.1 M HCl or 0.1 M NaOH.

PC fractionation and washing

In order to remove all soluble material from PC, it was first washed four times with deionised water, until the washing solution was free from any colouration, protein, phytic acid and glucosinolate. A quantity of dry PC (350 mg) was introduced in a 50 ml tube containing 35 ml deionised water and agitated for 10 min using a magnetic stirrer. After 30 min centrifugation at 2,500 g, 100 ml of the supernatant was discarded and 100 ml of fresh water added to enable a second washing of the PC pellet. The resulting washed PC was used for all sorption experiments.

The husks were separated from the rest of the PC by centrifugation since they sediment to the bottom of the centrifugation tube, beneath the finer particles of PC hereafter named “white sediment” (WS). Four successive centrifugations were required in order to completely separate the husks from the WS. The washed husks were oven dried at 80°C for 24 h, stored in a dessicator and used for biosorption studies.

Determination of the sorption activity of PC, “white sediment” and husks

Sorption studies were performed at room temperature in 250 ml polypropylene flasks, with a wide opening in order to allow the introduction of a pH electrode, a burette and the possibility to take samples. The target biomass fraction and remaining washing water were adjusted to the desired pH with a measured volume of HCl 0.1 M or NaOH 0.1 M. A precise volume of metal solution (of a given concentration and pH) was then added to the flask and the pH adjusted to the target value under stirring. Phases were separated by centrifugation and a 10 ml sample removed for further analysis. The concentration of metal sorbed on the biosorbent (q [mg/g]) is calculated at equilibrium (q^*) from a mass balance (Equation (1)):

$$q^* = \frac{V_{\text{aq,tot}}}{m} (C_{M,\text{aq}}^0 - C_{M,\text{aq}}^*) \quad (1)$$

where $V_{\text{aq,tot}}$ is the total volume of liquid in the system, m is the mass of the sorbent, $C_{M,\text{aq}}^0$ [mg/L] is the initial aqueous concentration of metals and $C_{M,\text{aq}}^*$ [mg/L] the aqueous equilibrium concentration. For a given condition, the sorption efficiency (Y) was calculated from:

$$Y = 100 \left(1 - \frac{C_{\text{aq}}^*}{C_{\text{aq}}^0} \right) \quad (2)$$

In order to obtain the isotherm this procedure was repeated, by addition of further CuSO₄ solution, and Langmuir coefficients calculated by fitting the Langmuir model (Equation (2)) using MATLAB® software. The effect of contact time on sorption was studied sampling the solution over a period of 2 hours.

The influence of changing ionic strength during experiments, resulting from the addition of acid and base to maintain a constant pH, was checked by performing experiments at different ionic strengths (ionic strength was adjusted to the desired value with NaCl). As previously described in the literature (Lee *et al.* 1998), salt concentration was not observed to have an effect on biosorption until 1 g/L NaCl, a value higher than that reached during our experiments.

Desorption experiments

Desorption experiments were performed by contacting the Cu loaded sorbent with a desorption solution (phase ratio: 10 g/L), HCl 0.1 M or deionised water. After mixing (2 hours) and phase separation, the metal concentration was determined in the supernatant and the percentage of metal recovered was calculated. All measurements were performed in triplicate.

Determination of functional groups responsible for biosorption

The functional groups present in husks were characterized by Fourier-transform infrared (FTIR) spectroscopy. Samples were prepared by embedding whole seeds in methylmethacrylate resin (Technovit®, Zürich, CH), and preparing thin sections using a microtome (20 μm thickness). Samples were characterised using a spectrum spotlight FTIR image system (Perkin Elmer, Shelton, USA) in transmission mode

and *Spectrum v5.0.1* software was used for recording spectra and performing mappings. FTIR measurements were performed on crude husks and husks contacted with copper in order to investigate changes in the spectra. Electron microscopy (SEM), coupled to an electron dispersive spectroscope (EDS) was used for microanalysis of the same sample after gold deposition.

Samples for X-ray Photoelectron Spectroscopy Analysis (XPS) were prepared by attaching a very thin layer of freshly prepared husks to an adhesive tape placed on a sample holder. The XPS studies were carried out with a Perkin-Elmer PHI 5500 ESCA System, as described elsewhere (Figueira *et al.* 1999).

Analytical techniques

Metal concentrations were determined using an atomic absorption spectrometer (Shimadzu AA-6650, Reinach, Switzerland). Prior to measurements, samples were filtered through single use 0.2 μm syringe filters (ethylene oxide). Analysis were performed in triplicate.

Total protein was determined using the Bradford method with BSA as standard. Phytic acid was determined by mixing 200 μl sample with 400 μl of 3% HCl followed by shaking for 5 min at 80°C (Xu *et al.* 1992). After centrifugation at 20,000 rpm for 10 min, 250 μl of the supernatant was mixed with 250 μl HCl 3%, 4 ml Wade reagent (1 mmol FeCl_3 , 10 mmol sulfosalicylic acid) and 3.5 ml water. This mixture was immediately measured against the diluted reagent solution spectrophotometrically at 510 nm. The concentration of phytic acid was calculated from a calibration curve obtained using a standard solution of phytic acid dodecasodium salt in water diluted with 3% HCl.

RESULTS AND DISCUSSION

Metal biosorption to the press-cake of oleaginous plants

Preliminary experiments to screen PC originating from the three oleaginous species rapeseed, soybean and moringa, for the ability to adsorb copper, showed that all three could retain between 65–81% of the applied copper (Figure 1). Due to the good retention and ready availability of fresh PC, rapeseed PC was used for all further experiments.

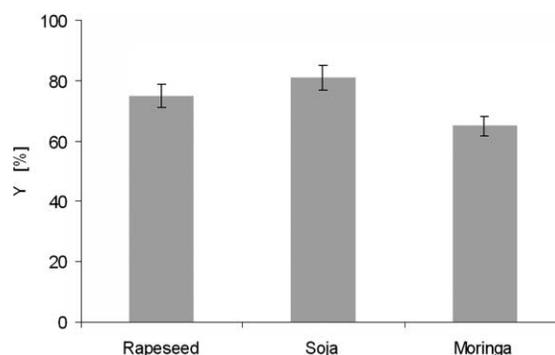


Figure 1 | Comparison of the sorption efficiency (Y) for copper of PC from three plant species. Biosorbent (350 mg) was contacted with 35 ml copper sulphate solution (100 mg/L; pH 5) with shaking at 120 rpm for one hour.

In subsequent experiments, the sorption of five metallic species: copper, nickel, zinc, lead and cadmium was demonstrated for rapeseed PC (Figure 2) with sorption efficiencies between 62–77% under the experimental condition tested (the efficiency as defined here, depends on the initial concentration and the adsorbent dose), thereby demonstrating the potential for using PC from oleaginous plants as biosorbent for a range of metallic species.

In order to understand the sorption mechanism further, the PC was fractionated and equilibrium constants determined for each fraction. All further experiments were performed with rapeseed PC, and copper as model metal species.

Fractionation of rapeseed press-cake

PC is a very heterogeneous material consisting of seed debris, which is mainly formed from crushed seed storage tissues (cotyledons and/or endosperm), and seed protective

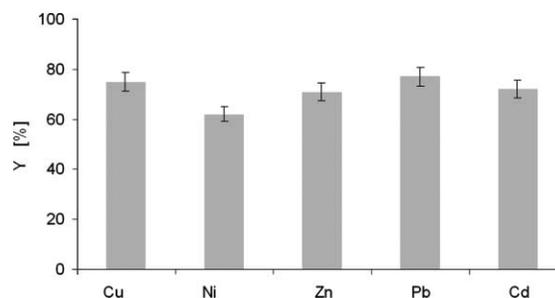


Figure 2 | Comparison of the sorption efficiency (Y) of rapeseed PC for a range of metallic species. Rapeseed PC (350 mg) was contacted with 35 ml metal solution (100 mg/L; pH 5) and shaken at 120 rpm for one hour.

coat, often called testa or husk. The separation and characterization of the diverse fractions constituting PC is a pre-requisite in order to study the contribution of each of these fractions in the uptake of metals.

When contacted with an aqueous phase, PC separates into a solid fraction and a solubilised fraction (Figure 3). Indeed PC is made of many components which are highly soluble such as phytic acid, glucosinolates and many proteins (Al-Asheh *et al.* 1998; El-Batal & Abdel Kareem 2001; Ghodsvali *et al.* 2005), which confers to the water contacted with PC a yellowish appearance. The protein and phytic acid content was determined after each washing. Most of the soluble material is recovered during the first washing step, and after 4 washings no detectable amount of the compounds tested could be detected in the soluble fraction.

Following removal of the soluble components, the solid fraction could be readily separated by centrifugation to yield husks (black flakes) and a white sediment (hereafter named WS) consisting of all non-soluble components of crushed seeds, i.e. the flesh of the seeds, made of the cotyledons and hypocotyl. WS is composed of (i) lipids, essentially residual oil (17% [w/w]) in the form of triacylglycerols and lipids associated with cell membranes; (ii) non-soluble proteins, particularly oleosins which constitute up to 20% total seed proteins (Murphy & Cummins 1989), and (iii) fibres, defined as the skeletal remains of plant cell walls (De Vried *et al.* 1999), constituted of polysaccharides (cellulose, hemicellulose and pectin) and lignin.

A proximate composition of PC (cotyledon + hypocotyl and husks) based on analysis and literature values is given in Table 1. The husks represent 25% (w/w) of the PC and have a mean diameter of $46 \pm 17 \mu\text{m}$. This data shows that PC is a complex material containing mainly organic

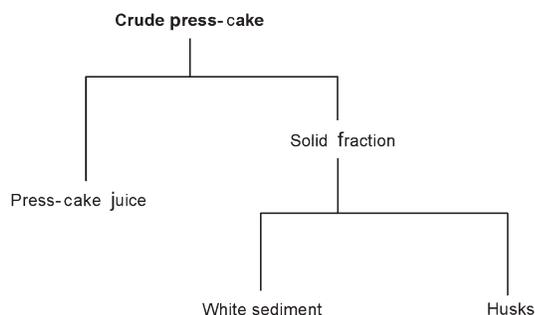


Figure 3 | Fractionation of PC by washings and centrifugation.

residues made of several polar functional groups, such as carboxyl and amines in proteins and, hydroxyl groups in cellulose and lignin (Dizhbite *et al.* 1999; Taty-Costodes *et al.* 2003). Carboxyl and carbonyl groups are especially present in hemi-cellulose and pectins, together with hydroxyl groups (Zhang & Kamdem 2000). In biomaterials these groups can be involved in chemical bonding and are responsible for the cation exchange capacity (Volesky 2004). Since hydroxyl and carboxyl groups are often considered to be responsible for biosorption in seed materials and are very abundant in vegetal fibres, they are expected to be particularly abundant in the husk fraction.

Biosorption activity of the solid fractions of rapeseed press-cake

The sorption of copper by PC biomass was very rapid regardless of the metal concentration (Figure 4), with most of the metal uptake occurring during the first few minutes and sorption equilibrium achieved within a period of 30 min.

However, as the copper concentration is raised, the sorption efficiency (Y) progressively decreases from 95% for $C_0 = 10 \text{ mg/L}$ to 57% for $C_0 = 250 \text{ mg/L}$ (C_0 is the initial concentration of copper in the solution), even though the sorption equilibrium was always attained within 30 min. In order to confirm whether sorption equilibrium decline was due to saturation of sorption sites, sorption isotherms were established.

Since metal sorption was accompanied by a decrease in pH (data not shown), pH was continuously adjusted during

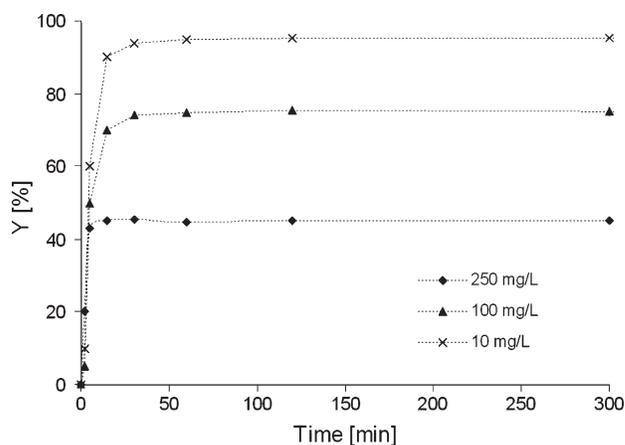


Figure 4 | Sorption efficiency (Y) of copper to rapeseed PC from solutions of different initial concentration (C_0 of 10, 100 and 250 mg L^{-1}) by 10 g/L PC as function of contact time.

experiments in order to establish valid sorption isotherms at constant pH values. Isotherms for pH 3, 4 and 5 are shown in Figure 5, while higher pH values could not be tested due to poor solubility of copper under these conditions. Data were fitted with a Langmuir isotherm and show that the maximal sorption capacity (Table 2) is at pH 5 ($q_{\max} = 13.4 \text{ mg}_{\text{Cu}}/\text{g}$). From the results, it can be concluded that Cu uptake by the PC biomass is via chemically equilibrated and saturable mechanism.

Comparison of sorption of copper to various PC fractions and ground seeds

In order to determine the contributions of each PC fraction on the biosorption of copper, sorption isotherms were established for PC, husks, WS and ground seeds, at pH 5, and fitted with a Langmuir model (Figure 6).

The Langmuir parameters are given in Table 2. The high specific uptake capacity of the husks for copper ($36.6 \text{ mg}_{\text{Cu}}/\text{g}$) as compared to that of the crude PC ($13.4 \text{ mg}_{\text{Cu}}/\text{g}$) and WS ($8.6 \text{ mg}_{\text{Cu}}/\text{g}$) shows that husks are the most active fraction responsible for PC biosorption properties. Sorption of Cu to ground seeds was observed to be comparable to that observed for PC. These results are in agreement with literature data, reporting husks from diverse species, such as wheat, rice, black gram, melon or moringa, as possible metal biosorbents (Okieimen & Onyenkpa 1989; Dupont *et al.* 2003; Saaed & Iqbal 2003; Teixeira Tarley & Zezzi Arruda 2004; Kumari

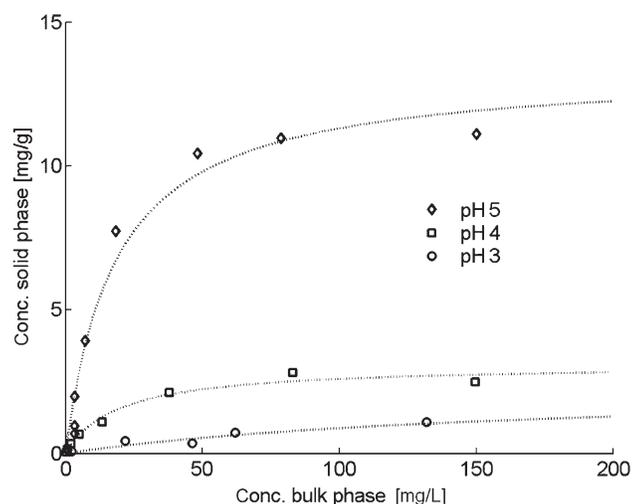


Figure 5 | Isotherms for the sorption of copper on washed PC, at pH 3, 4 and 5, fitted with the Langmuir model (Langmuir coefficients are given in Table 2).

Table 2 | Langmuir parameters for the different sorption isotherms; q_{\max} [mg/g] and K [L/g]. The Langmuir Equation is defined as $q = q_{\max}KC/(1 + KC)$

		q_{\max}	K
PC	pH 5	13.4	0.05
	pH 4	3.2	0.05
	pH 3	1	0.05
Husks	pH 5	36.6	0.2
	pH 4	13.7	0.06
	pH 3	1	0.05
WS	pH 5	8.6	0.1
Seeds	pH 5	10.7	0.06

et al. 2006). The q_{\max} is even close to that reported for algae such as Sargassum algal biosorbent ($q_{\max} = 59 \text{ mg}_{\text{Cu}}/\text{g}$), which are considered the best metal-sorbing type of biomass (Vieira & Volesky 2000).

Due to the high sorption efficiency and high homogeneity subsequent work focused on the characterization of copper biosorption to rapeseed husks and the determination of the sorption mechanism.

Characterisation of copper biosorption to rapeseed husks

Influence of pH

Isotherms for the sorption of copper to rapeseed husks were established at different pH values (Figure 7). The Langmuir

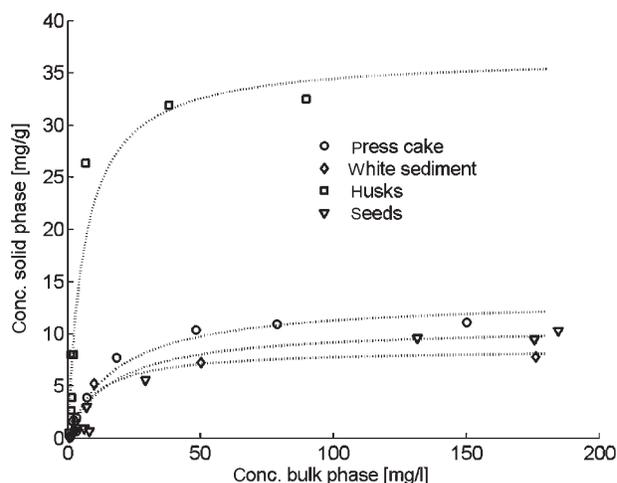


Figure 6 | Isotherms for the sorption of copper on washed PC, "white sediment", husks and ground seeds at pH 5, fitted with the Langmuir model (Langmuir coefficients are given in Table 2).

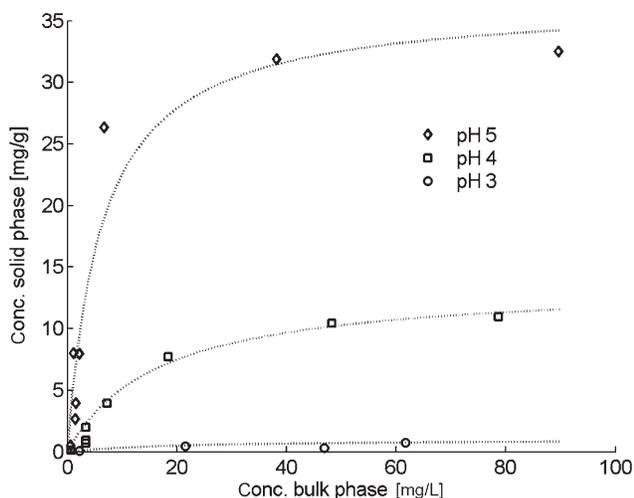


Figure 7 | Isotherm for the sorption of husks at pH 3, 4 and 5, fitted with the Langmuir model (Langmuir coefficients are given in Table 2).

adsorption parameters for the adsorption capacity (q_{\max}) and the adsorption affinity (K) were calculated from this data and are presented in Table 2. As previously shown for sorption to PC (Figure 5), it appears that the efficiency of copper ion sorption to husks is controlled by the pH of the reaction mixture, with poor sorption at pH 2. Since the availability of negatively charged groups at the biosorbent surface is necessary for the sorption of metals to occur, under acidic conditions few sites are expected to be available, due to competition between Cu^{2+} ions and $\text{H}^+/\text{H}_3\text{O}^+$ ions, as well as protonation of functional groups at pHs below their specific pKas. Sorption was observed to increase as the pH of the solution increased with an optimum for Cu removal at pH 5. Similar results for Cd, Cr, Cu, Fe, Ni, Pb and Zn sorption to diverse biosorbents has been reported to occur within the same pH optimum range (Saeed & Iqbal 2003). For pH values above 5, the weak solubility of copper, resulting in the precipitation of copper hydroxides, hindered sorption experiments.

Desorption studies

As a consequence of the pH dependency of biosorption, lowering the pH was expected to help regeneration and recycling of the spent biosorbent, in a similar way to that reported elsewhere (Agarwal *et al.* 2006). Desorption experiments using acid (0.1 M HCl) or deionised water as eluent yielded Cu recoveries of $0.7 \pm 0.1\%$ and

$102.6 \pm 5.3\%$ respectively. The desorption at acidic pH is consistent with the low copper binding observed at pH 3 due to protonation of functional groups below their pKa, resulting in displacement of the bound copper into solution. Moreover the absence of any significant desorption when water was used as desorption solution shows that the bonding of copper to the husk is stable and physical bonding can thus not be postulated as a major sorption mechanism. Indeed, if the adsorption is by physical bonding then the loosely bound solute can be easily desorbed with distilled water in most cases. However, if the mode of sorption is by chemical bonding or ion exchange, or a combination of both, then desorption is affected by stronger desorbents, such as acid or alkali solutions (Agarwal *et al.* 2006).

Proton and ion exchange

The biosorption of copper was accompanied by a decrease in pH. Thus for the determination of sorption isotherms, NaOH was constantly added in order to keep the pH at a constant value. This proton release during biosorption strongly suggests an ion exchange mechanism. The volume of NaOH needed to control the pH was recorded during the experiment, thereby allowing calculation of the amount of H^+ that are transferred to the solution during biosorption. As shown in Figure 8, the proton liberation is proportional to the amount of sorbed copper. If the biosorption was strictly a proton exchange mechanism, the slope of the curve would be expected to have a value of 2 as observed by Crist (Crist *et al.* 1990) for algal biosorbents. However, in

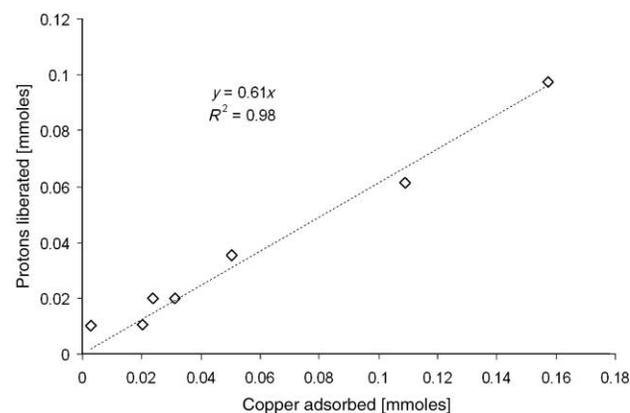


Figure 8 | Liberation of protons during biosorption on rapeseed husks as a function of the amount of sorbed copper (pH 5).

our case, a value of 0.62 was measured at pH 5, which suggests that proton exchange is not the sole mechanism involved. Chemisorption, which does not yield proton liberation may also be involved, particularly at higher pH values, when the pH is above the pKa of functional groups.

Moreover, as shown in Table 1 and SEM/EDS analysis (Figure 9), husk shows a high level of associated calcium. In a manner similar to that reported for Cocoa shells in acidic conditions (Meunier *et al.* 2003), it was observed that calcium was liberated in the aqueous solution when copper sorbed to the rapeseed husks. Although the stoichiometry of the process could not be established, our results (data not shown) clearly indicate that calcium exchange was

responsible for copper biosorption, at least to the same extent as proton exchange. The application of other analytical techniques, such as titration combined with on-line measurement of pH, calcium and copper using ion selective electrodes (Gondar *et al.* 2006) would be very valuable for the quantitative determination of the contribution of calcium and proton exchange in the total biosorption uptake.

Investigation of functional groups - FTIR analysis

The binding behaviour suggests that to some extent, carboxyl groups may be responsible for the binding of copper, since the ionization constants of carboxyl groups range between 3 and 4 (Gardea-Torresdey *et al.* 1996). At lower pH values, the carboxyl groups are protonated, thereby reducing the probability of binding to any positively charged ions, whereas at higher pH, the carboxylate (COO^-) ligands attract the positively charged ions and binding occurs (Horsfall & Spiff 2004). However husks are complex biomatrices, having a high amount of fibre (Table 1), which suggests that a high number of hydroxyl groups are also present.

FTIR experiments were undertaken in order to gain a better insight of the functional groups involved in copper biosorption to rapeseed husks, since information can be obtained about the nature of chemical groups on the surface of the biosorbent, as well as on the nature of the binding between the biosorbent and the metallic species.

A broad band between $3,000$ and $3,750\text{ cm}^{-1}$ was observed, corresponding to the stretching of $-\text{OH}$ groups on the rapeseed husks due to both constitutive $-\text{OH}$ (cellulose and lignin for example) and adsorbed water to the husk surface. Peaks around $2,900\text{ cm}^{-1}$ indicate the presence of C-H links. A peak at $1,743\text{ cm}^{-1}$ represents the stretching of carbonyl groups (Teixeira Tarley & Zezzi Arruda 2004). Peaks in the $1,300$ – $1,700\text{ cm}^{-1}$ region indicate the presence of proteins (carboxyls, amide) and phenolic compounds, in particular the band at $1,739\text{ cm}^{-1}$ is assigned to carboxyl stretching vibration associated with carboxylic acids (Zhang & Kamdem 2000). The wide peak in the $1,000$ – $1,200\text{ cm}^{-1}$ domain is typical of cellulosic compounds (Zhang & Kamdem 2000).

When comparing the FTIR spectrum of the crude husk (reference) with husks which had been in contact with

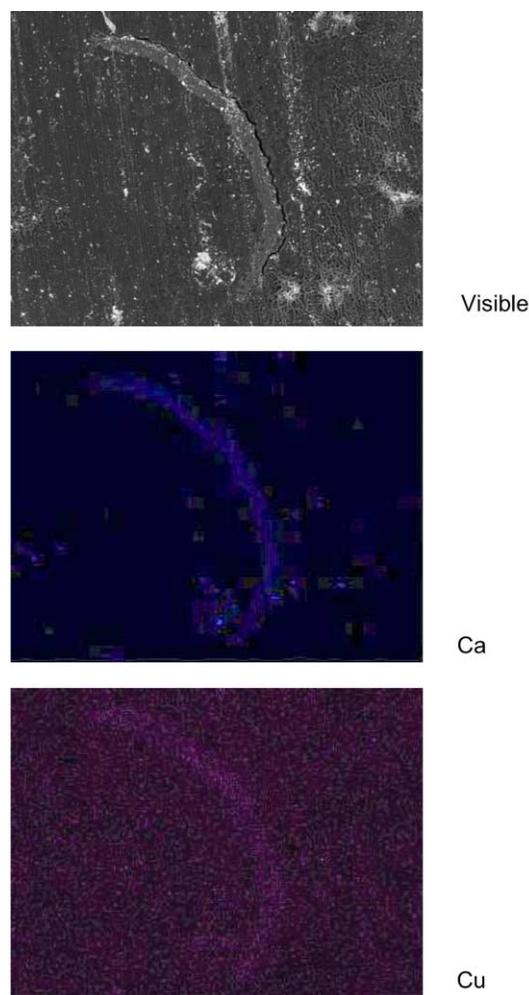


Figure 9 | Microanalysis of rapeseed husks performed with a SEM coupled with an electron dispersive spectroscopy (EDS). The sample is approx. $400\ \mu\text{m}$ long.

copper, the occurrence of a new peak around $3,500\text{ cm}^{-1}$ indicates a beginning of structuration of the -OH band, which may be attributed to bonding of copper to some -OH groups: O-Cu, either in hydroxyl or carboxyl groups.

Moreover, X-ray photoelectron spectroscopy (XPS) was also undertaken on Cu treated husks and indicated that the Cu is present in an oxidized form on the sample. Indeed, the position and broadness of the band measured for copper on the husk (933.4 eV) corresponds to an oxidized form of copper (between 933.5 and 934 eV) and not to the metallic form (932.5 to 932.9). This result is consistent with the previous results showing that Cu is associated with -O in the biomatrix.

CONCLUSIONS

Of the various fractions of rapeseed seeds tested for copper biosorption, husk appears to be the most efficient fraction, with sorption efficiencies comparable to the best biosorbents described in the literature (Volesky & Holan 1995).

Our results reflect the complexity of rapeseed husks as a biosorbent for copper with several sorption mechanisms being involved together with several functional groups. There is clear evidence that proton exchange takes place together with calcium exchange. FTIR and XPS data suggest that Cu on the biosorbent is in an oxidised form and probably linked with hydroxyl or carboxyl groups.

Other metals were also successfully demonstrated to sorb to rapeseed husks however, the sorption mechanisms require investigation for each specific case.

Rapeseed husks not only have interesting biosorption efficiencies, they also present additional advantages. The protein content of this material is low (Table 1), which is advantageous over the protein-rich algal and fungal biomass commonly described as metal biosorbents by other authors (Volesky & Holan 1995). Indeed, proteinaceous materials are likely to be unstable and putrefy under moist conditions, which renders transport and use of the biosorbent impractical, especially for continuous processes.

Due to their structure, husks need no pre-processing and could be used directly in a packed column for continuous sorption. Moreover, desorption of metals from metal-bearing PC could be successively achieved by

lowering the pH, thereby allowing re-use of the sorbent for several sorption cycles.

The successful application of the PC of oleaginous seeds as a biosorbent suggests an inexpensive, environmentally friendly method for the removal of copper and other metals from aqueous media. Combustion of the resulting press-cake would yield considerable energy while enabling the metals to be recovered in concentrated form.

REFERENCES

- Agarwal, G. S., Bhuptawat, H. K. & Chaudhari, S. 2006 Biosorption of aqueous chromium(VI) by *Tamarindus indica* seeds. *Bioresour. Technol.* **97**(7), 949–956.
- Al-Asheh, S., Lamarche, G. & Duvnjak, Z. 1998 Investigation of copper sorption using plant materials. *Water Qual. Res. J. Canada* **33**(1), 167–183.
- Anikwe, M. A. N. 2000 Amelioration of a heavy clay loam soil with rice husk dust and its effect on soil physical properties and maize yield. *Bioresour. Technol.* **74**(2), 169–173.
- Atamanenko, I. D., Kryvoruchko, A. P., Yurlova, L. Y. & Kornilovich, B. Y. 2003 Concentration of heavy metal by pressure-driven membrane methods. *Desalination* **158**(1–3), 151–156.
- Bell, J. M. 1995 Meal and by-product utilization in animal nutrition. In: Kimber, D. & McGregor, D. I. (eds) *Brassica Oilseeds: Production and Utilization*. Cab International, Oxon, UK, p. 301.
- Bell, J. M. & Keith, M. O. 1991 A survey of variation in the chemical composition of commercial canola meal produced in western canadian crushing plants. *Can. J. Anim. Sci.* **71**, 469–480.
- Boucher, J., Steiner, L. & Marison, I. W. 2007 Biosorption of atrazine in the press-cake of oil seeds. *Water Res.* **41**(15), 3209–3216.
- Crist, R. H., Martin, J. R., Guptill, P. W. & Eslinger, J. M. 1990 Interaction of metals and protons with algae. 2. Ion exchange in adsorption and metal displacement by protons. *Environ. Sci. Technol.* **24**, 337–342.
- Davin, A. 1983 Fractionation of oleaginous seed meals by screening and characterization of the product. *Qual. Plant. Plant Foods Hum. Nutr.* **33**, 153–160.
- De Vried, J. W., Prosky, L., Li, B. & Cho, S. 1999 A historical perspective on defining dietary fibers. *Cereal Foods World.* **44**, 367–369.
- Dizhbite, T., Zakis, G., Kizima, A., Lazareva, E., Rossinskaya, G., Jurkjane, V., Telysheva, G. & Viesturs, U. 1999 Lignin – a useful bioresource for the production of sorption-active materials. *Bioresour. Technol.* **67**(3), 221–228.
- Dupont, L., Bouanda, J., Dumonceau, J. & Aplincourt, M. 2003 Metal ions binding onto a lignocellulosic substrate extracted from wheat bran: a NICA-Donnan approach. *J. Colloid Interface Sci.* **263**(1), 35–41.

- El-Batal, A. I. & Abdel Karem, H. 2001 Phytase production and phytic acid reduction in rapeseed meal by *Aspergillus niger* during solid state fermentation. *Food Res. Int.* **34**(8), 715–720.
- Erdem, E., Karapinar, N. & Donat, R. 2004 The removal of heavy metal cations by natural zeolites. *J. Colloid Interface Sci.* **280**(2), 309–314.
- Figueira, M. M., Volesky, B. & Mathieu, H. J. 1999 Instrumental analysis study of iron species biosorption by Sargassum biomass. *Environ. Sci. Technol.* **33**, 1840–1846.
- Garcia-Valls, R. & Hatton, T. A. 2003 Metal ion complexation with lignin derivatives. *Chem. Eng. J.* **94**(2), 99–105.
- Gardea-Torresdey, J. L., Tiemann, K. J., Gonzalez, J. H., Henning, J. A. & Townsend, M. S. 1996 Ability of silica-immobilized *Medicago sativa* (alfalfa) to remove copper ions from solution. *J. Hazard. Mater.* **48**(1–3), 181–190.
- Ghodsvali, A., Khodaparast, M. H. H., Vosoughi, M. & Diosady, L. L. 2005 Preparation of canola protein materials using membrane technology and evaluation of meals functional properties. *Food Res. Int.* **38**(2), 223–231.
- Gondar, D., Iglesias, A., Lopez, R., Fiol, S., Antelo, J. M. & Arce, F. 2006 Copper binding by peat fulvic and humic acids extracted from two horizons of an ombrotrophic peat bog. *Chemosphere* **63**(1), 82–88.
- Horsfall, M., Jr. & Spiff, A. I. 2004 Studies on the effect of pH on the sorption of Pb²⁺ and Cd²⁺ ions from aqueous solutions by *Caladium bicolor* (Wild Cocoyam) biomass. *Electron. J. Biotechnol.* **7**(3), 313–323.
- Jimenez, L. & Gonzalez, F. 1991 Study of the physical and chemical properties of lignocellulosic residues with a view to the production of fuels. *Fuel* **70**(8), 947–950.
- Johnson, P. D., Watson, M. A., Brown, J. & Jefcoat, I. A. 2002 Peanut hull pellets as a single use sorbent for the capture of Cu(II) from wastewater. *Waste Manage.* **22**(5), 471–480.
- Kongsricharoen, N. & Polprasert, C. 1996 Chromium removal by a bipolar electro-chemical precipitation process. *Water Sci. Technol.* **34**(9), 109–116.
- Kostov, O., Tzvetkov, Y., Kaloianova, N. & Van Cleemput, O. 1995 Cucumber cultivation on some wastes during their aerobic composting. *Bioresour. Technol.* **53**(3), 237–242.
- Kumari, P., Sharma, P., Srivastava, S. & Srivastava, M. M. 2006 Biosorption studies on shelled *Moringa oleifera* Lamarck seed powder: removal and recovery of arsenic from aqueous system. *Int. J. Mineral Process.* **78**(3), 131–139.
- Lee, S. H., Jung, C. H., Chung, H., Lee, M. Y. & Yang, J.-W. 1998 Removal of heavy metals from aqueous solution by apple residues. *Process. Biochem.* **33**(2), 205–211.
- Li, D., Pengbin, X., Liming, G., Shijun, F. & Canghai, H. 2002 Determination of apparent ileal amino acid digestibility in rapeseed meal and cake processed at different temperatures using the direct and difference method with growing pigs. *Arch. Anim. Nutr.* **56**, 339–349.
- Liu, Y.-G., Zhou, M.-Q. & Liu, M.-L. 1994 A survey of nutrients and toxic factors in commercial rapeseed meal in China and evaluation of detoxification by water extraction. *Anim. Feed Sci. Technol.* **45**(3–4), 257–270.
- Manios, T. 2004 The composting potential of different organic solid wastes: experience from the island of Crete. *Environ. Int.* **29**(8), 1079–1089.
- Martin, C. J. & Evans, W. J. 1986 Phytic acid–metal ion interactions. II. The effect of pH on ca(II) binding. *J. Inorganic Biochem.* **27**(1), 17–30.
- Martin, C. J. & Evans, W. J. 1987 Phytic acid: divalent cation interactions. V. titrimetric, calorimetric, and binding studies with cobalt(ii) and nickel(ii) and their comparison with ot. *J. Inorganic Biochem.* **30**(2), 101–119.
- Matlock, M. M., Howerton, B. S. & Atwood, D. A. 2002 Chemical precipitation of heavy metals from acid mine drainage. *Water Res.* **36**(19), 4757–4764.
- Mejare, M. & Bulow, L. 2001 Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. *Trends Biotechnol.* **19**(2), 67–73.
- Meunier, N., Laroulandie, J., Blais, J. F. & Tyagi, R. D. 2003 Cocoa shells for heavy metal removal from acidic solutions. *Bioresour. Technol.* **90**(3), 255–263.
- Murphy, D. J. & Cummins, I. 1989 Seed oil-bodies: isolation, composition and role of oil-body apolipoproteins. *Phytochemistry* **28**(8), 2063–2069.
- Natarajan, E., Nordin, A. & Rao, A. N. 1998 Overview of combustion and gasification of rice husk in fluidized bed reactors. *Biomass Bioenerg.* **14**(5–6), 533–546.
- Nawirska, A. 2005 Binding of heavy metals to pomace fibers. *Food Chem.* **90**(3), 395–400.
- Niewiadomski, H. 1990 *Rapeseed: Chemistry and Technology*. Elsevier Science Publishers, Amsterdam.
- Ogiwarai, Y. & Kubota, H. 1969 Combination of cellulosic materials and metallic ions. *J. Polymer Sci. Part A-1* **7**, 2087–2095.
- Okieimen, F. E. & Onyenkpa, V. U. 1989 Removal of heavy metal ions from aqueous solutions with melon (*Citrullus vulgaris*) seed husks. *Biol. Wastes* **29**(1), 11–16.
- Park, S.-J. & Kim, Y.-M. 2005 Adsorption behaviors of heavy metal ions onto electrochemically oxidized activated carbon fibers. *Mater. Sci. Eng. A* **391**(1–2), 121–123.
- Saaed, A. & Iqbal, M. 2003 Bioremoval of cadmium from aqueous solution by black gram husk (*Cicer arietinum*). *Water Res.* **37**, 3472–3480.
- Salunkhe, D. K., Chavan, J. K., Adsule, R. N. & Kadam, S. 1992 *World Oilseeds: Chemistry, Technology, and Utilization*. Von Nostrand Reinhold, New York.
- Shukla, S. R. & Sakhardande, V. D. 1991 Removal of metal ions using dyed cellulosic materials. *Dyes and Pigments* **17**(2), 101–112.
- Silva, J. E., Paiva, A. P., Soares, D., Labrincha, A. & Castro, F. 2005 Solvent extraction applied to the recovery of heavy metals from galvanic sludge. *J. Hazard. Mater.* **120**(1–3), 113–118.
- Staubmann, R., Foidl, G., Foidl, N., BGubitz, G. M., Lafferty, R. M., Arbizu, V. M. V. & Steiner, W. 1997 Biogas production from *Jatropha curcas* press-cake. *Appl. Biochem. Biotechnol.* **63**(5), 457–467.
- Taty-Costodes, V. C., Fauduet, H., Porte, C. & Delacroix, A. 2003 Removal of Cd(II) and Pb(II) ions, from aqueous solutions, by adsorption onto sawdust of *Pinus sylvestris*. *J. Hazard. Mater.* **105**(1–3), 121–142.

- Teixeira Tarley, C. R. & Zezzi Arruda, M. A. 2004 Biosorption of heavy metals using rice milling by-products. Characterisation and application for removal of metals from aqueous effluents. *Chemosphere* **54**(7), 987–995.
- Veglio, F. & Beolchini, F. 1997 Removal of metals by biosorption: a review. *Hydrometallurgy* **44**, 301–316.
- Vieira, R. H. S. F. & Volesky, B. 2000 Biosorption: a solution to pollution? *Int. Microbiol.* **3**, 17–24.
- Volesky, B. 2001 Detoxification of metal-bearing effluents: biosorption for the next century. *Hydrometallurgy* **59**, 203–216.
- Volesky, B. 2004 *Sorption and Biosorption*. BV Sorbex Inc, Montreal-St-Lambert, Quebec, Canada, pp. 316.
- Volesky, B. & Holan, Z. R. 1995 Biosorption of heavy metals. *Biotechnol. Prog.* **11**, 235–250.
- Xu, P., Price, J., Wise, A. & Aggett, P. J. 1992 Interaction of inositol phosphates with calcium, zinc, and histidine. *J. Inorganic Biochem.* **47**, 119–130.
- Zervakis, G., Yiatras, P. & Balis, C. 1996 Edible mushrooms from olive oil mill wastes. *Int. Biodeterior. Biodegrad.* **38**(3–4), 237–243.
- Zhang, J. & Kamdem, P. 2000 FTIR characterization of copper ethanalamine – wood interaction for wood preservation. *Holzforschung* **54**, 119–122.
- Zukalova, H. & Vasak, J. 2002 The role and effects of glucosinolates of Brassica species – a review. *Rostlinna Vyroba* **48**(4), 175–780.

First received 3 February 2007; accepted in revised form 29 October 2007