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Development of magnetic biosorbents for metal uptake

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Two types of magnetic biosorbent were prepared by novel protocols from epichlorhydrin-cross-linked *Saccharomyces cerevisiae* cell walls and their biosorption characteristics were compared to those of non-magnetic cell walls. The magnetic biosorbents I and II were capable of binding Cu^{2+} maximally to 225 and 50 μmol/g, Cd^{2+} to 90 and 25 μmol/g and Ag^{+} to 80 and 45 μmol/g respectively. These values compare with 400, 125 and 75 μmol/g, respectively, for non-magnetic cell walls.

Introduction

Concerns over the significant and long-term environmental hazards posed by heavy metals have focused attention towards their disposal and prompted research into developing novel processes for removal and/or recovery of such elements (Blackwell *et al.*, 1995; Leusch *et al.*, 1995; McHale and McHale, 1994). The use of microbial cells as biosorbents for metals offers a potential alternative to existing methods for decontamination and/or recovery of toxic/precious metals from waste streams (Blackwell *et al.*, 1995; de Rome and Gadd, 1991; Strandberg *et al.*, 1981). However, microbial based-biosorbents must be technologically and economically competitive with existing processes if they are to be used on an industrial scale (Brierley *et al.*, 1986; Holan *et al.*, 1993).

Yeasts possess an acknowledged potential for accumulating a range of metal cations (Blackwell *et al.*, 1995) and large amounts of this metal can remain associated with the yeast cell wall (Strandberg *et al.*, 1981). Protein and carbohydrate fractions of yeast cell walls have previously been shown to be involved in binding Cu^{2+}, Cd^{2+} and Co^{2+} ions (Brady and Duncan, 1994). Isolated components of yeast cell walls (mannans, glucans and chitin) were also observed to accumulate greater quantities of metal than intact cell walls (Brady *et al.*, 1994).

The use of microbial-based biosorbents for industrial applications has been hindered by problems associated with both growth requirements if living cells are involved and physical characteristics of the material (McHale and McHale, 1994). Clearly, toxic or inhibitory components will preclude use of viable cell systems. Low mechanical strength of the biomass can cause difficulties associated with separation of the biomass from effluents which, in turn, contribute to limitations in process design. A further problem is associated with fragmentation of the biomass causing flow restrictions in continuous-flow contact vessels. Cross-linking or immobilisation technologies may overcome many of these problems (McHale and McHale, 1994) by reinforcing the biomass for process applications and may also enhance the sorption performance (Leusch *et al.*, 1995; Ting and Teo, 1994).

Recently there has been considerable interest in the potential of magnetic immobilisation systems for biotechnology applications ranging from protein recovery to enzyme and DNA purification (O'Brian *et al.*, 1996) and improved fermentation performances with magnetically immobilised biocatalysts (Brady *et al.*, 1996; Ivanova *et al.*, 1996). Processes for metal recovery from waste streams using both metabolising and non-metabolising cultures in magnetic biosorbent systems have been described (Elwood *et al.*, 1992; Lloyd and Macaskie, 1996; Sly *et al.*, 1993; Thomas and Macaskie, 1996; Wong and Fung, 1997). In metabolising cell systems metal precipitation by reaction with metabolically liberated phosphate or sulphide ions results in magnetically responsive biosorbent. This approach has been shown to be applicable to a wide range of metals and removal levels of up to 200 μmol Hg^{2+}/g cells have been achieved in large scale tests

(Ellwood *et al.*, 1992) although the application of this system is limited to non-toxic effluents. Alternative strategies have involved adsorption of cells to various oxide surfaces with relatively low cell loadings of the order of 10% w/w (Shabtai and Fleminger, 1994; Sly *et al.*, 1993; Wong and Fung, 1997). Thus, while good metal binding to the cells has been achieved, overall biosorbent uptake levels were decreased and the durability of the biosorbent in large scale applications remains largely untested.

Non-magnetic particulates can be magnetised through a process known as seeding (Dauer and Dunlop, 1991). This facilitates rapid and simple removal of particulates, in this context the biosorbent, from solution using a magnetic separator or a permanent magnet. For example in high gradient magnetic separation (Kolm *et al.*, 1975), the magnetic traction force has been employed to capture magnetic particles upon a magnetised fibre filtration matrix (Dauer and Dunlop, 1991). The advantages of using magnetic supports include both facile, selective recovery and recycling of magnetically immobilised adsorbents (Halling and Dunnill, 1980; Wong and Fung, 1997).

The purpose of this work was to test new methods for the preparation of non-viable magnetic biosorbents by chemical crosslinking techniques at high cell loadings (up to ca. 80% w/w). Non-viable biomass was selected in order to obviate potential toxicity problems. Using novel protocols, fine magnetic seed particles of iron (II, III) oxide (magnetite) and silanised magnetite were attached to the cell wall envelopes, making them magnetic and amenable to separation. The treated envelopes were further processed by cross-linking using epichlorhydrin and the uptake of copper, cadmium and silver from aqueous solution compared with that of non-magnetite cross-linked cell wall envelopes.

Materials and methods

Materials

Saccharomyces cerevisiae was obtained from Prague Breweries and used as a 15% (w/v) slurry. Iron (II, III) oxide (magnetite) and silanised magnetite were obtained from the Department of Biochemistry and Biotechnology, Institute of Landscape Ecology, Ceské Budéjovice, The Czech Republic. $CuSO_4 \cdot 5H_2O$, $Cd(CH_3COO)_2 \cdot 2H_2O$, Ag_2SO_4, ether and acetic acid were from Lachema Brno, The Czech Republic. Epichlorhydrin (1-chlor-2,3-epoxypropan) was from Merck-Schuchardt, München, SRN. Acetone, ethanol and sodium hydroxide were from Penta Chrudim, The Czech Republic.

Preparation of *S. cerevisiae* cell wall envelopes (primary biomass)

S. cerevisiae biomass suspension was suspended in 1 M NaOH (1:2; v/v) and boiled for 3–4 hours to facilitate protein removal. The suspension was centrifuged for 20 min at 1,200 *g* followed by resuspension in deionised distilled water and recentrifugation. This process was repeated twice. The biomass was then suspended in 1 M NaOH again and the procedure repeated (boiling time 2 hours). The biomass was resuspended in deionised distilled water, and finally rinsed successively in ethanol, acetone and ether. It was then dried for 24 h at room temperature. The resulting biomass was a soft, light-brown powder.

Preparation of non-magnetic biosorbent

S. cerevisiae cell wall envelopes were immobilized using a method modified from Dostalek *et al.* (1994). The primary biomass (10 g) was suspended in 20 ml distilled water. After 10 min, 2 ml of a 40% aqueous solution of NaOH was added and mixed. After 20 min, 1.5 ml epichlorhydrin was added and again mixed well. This suspension was left in a closed vessel for 60 min and then heated to 50°C for 60 min. The suspension was neutralised in 0.35 M acetic acid and washed several times with deionised distilled water. The biosorbent particles were mixed with water in a high speed homogenizer. These soft particles were dried in the same way as the primary biomass. The resulting biosorbent particles were separated into size ranges by screening. The principal particle size was from 25 to 60 mesh (250–707 μm) and this fraction was used for uptake experiments.

Preparation of magnetic biosorbents

(a) Biosorbent with non-covalently binding magnetite ("magnetic biosorbent I"): The primary biomass (10 g) was mixed with 3 g magnetite and the remaining protocol was identical to the preparation of non-magnetic biosorbent.

(b) Biosorbent with covalently binding magnetite ("magnetic biosorbent II"): This biosorbent was prepared using the following protocol derived from Safarik *et al.* (1995). Magnetite (10 g) was suspended in 1.6 M HNO_3 and boiled in a closed vessel at 100°C for 60 min. After thorough washing with distilled water, 40 ml 10% (w/v) aqueous solution of 3-amino-propyltriethoxysilane (adjusted to pH 4.0 with 2 M HCl) was added to the sedimented magnetite. The suspension was stirred on a water bath at 80°C for 4 h, after which the silanized magnetite was thoroughly washed with deionised

distilled water. The primary biomass (10 g) was mixed with the silanised magnetite (3 g dry weight). The remaining protocol was identical to the preparation of non-magnetic biosorbent.

Uptake experiments

For each adsorption isotherm, a series of 250 ml Erlenmeyer flasks was prepared with 100 ml of metal-bearing solution (Cu^{2+}, Cd^{2+} or Ag^{+}) in the concentration range 10–100 mg/l. 0.1 g of biosorbent was added to each flask and contacted overnight (16 hours) on a rotary shaker at room temperature. Samples were removed from each flask and the metal concentration remaining in solution was determined using atomic absorption spectrometry (AAS). Control experiments using particles of magnetite and silanised magnetite were included in each test.

Determination of nitrogen content

The nitrogen content of primary biomass, non-magnetite biosorbent and magnetic biosorbents I and II was determined using a Kjetec AUTO 1030 Analyser.

Results and discussion

The main components (and typical weight percentages) in the *S. cerevisiae* cell wall are mannan (31%), glucan (28.8%), protein (13%) and lipid (8.5%) (Korn and Northcote, 1960; Northcote and Horne, 1952). In the present study the primary biomass was derived from waste brewing yeast by treatment to remove the nucleic acid, cell organelles and the majority of cell wall protein. The nitrogen contents of the primary biomass, non-magnetic biosorbent, magnetic biosorbent I and magnetic biosorbent II (as a % of dry weight) were 0.24, 0.23, 0.18 and 0.18 respectively. These values correspond to protein concentrations in the range 1.5–1.0 percent dry weight (Markham, 1942), confirming that the majority of protein has been removed. The polysaccharides from the yeast cell wall (mannans, glucans and chitin) remain as the principal components of this biomass, and it is the negatively charged reactive sites (hydroxyl and carboxyl and other groups) of these polysaccharides that are involved in the binding of metal ions (Strandberg *et al.*, 1981; Tobin *et al.*, 1990; Volesky and Holan, 1995).

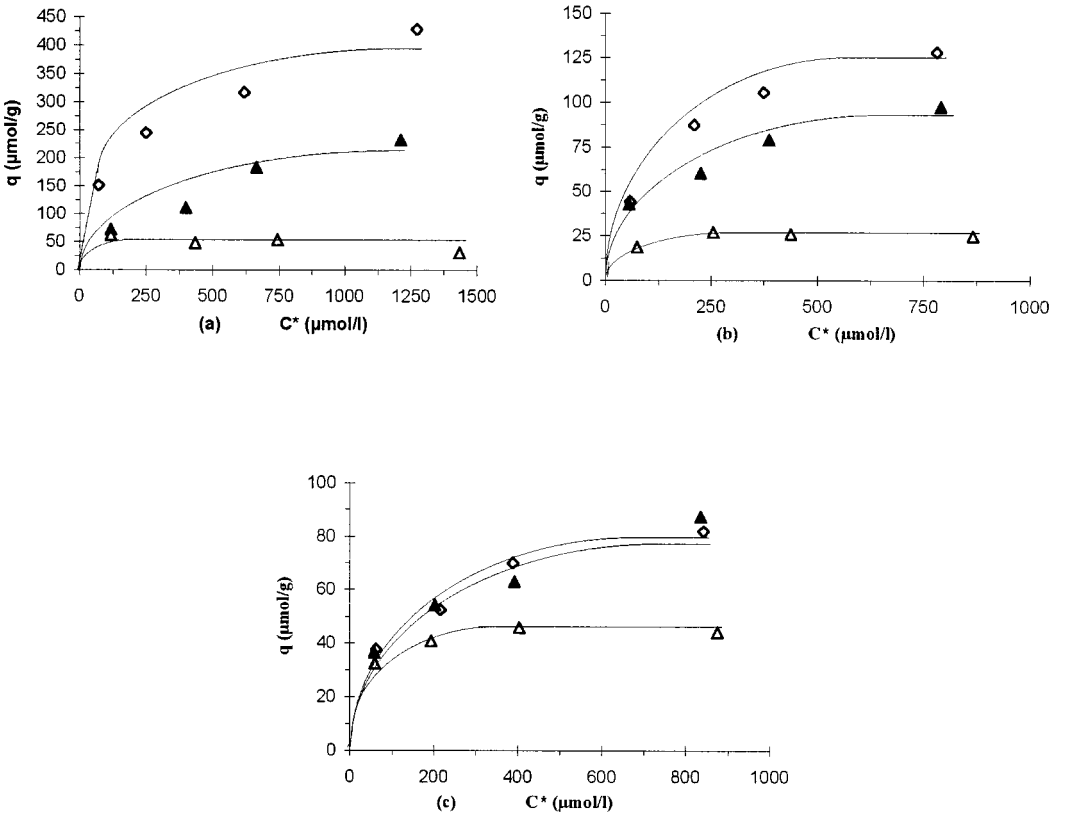

Figure 1 Equilibrium sorption isotherms for (a) copper, (b) cadmium and (c) silver. (◇) Non-magnetite biosorbent, (▲) magnetite biosorbent I, (△) magnetite biosorbent II.

For each biosorbent, sorption isotherms in the form of metal uptake (q) as a function of final solution concentration (C*) are shown in Fig. 1 for Cu^{2+}, Cd^{2+} and Ag^{+}. In all cases metal uptake is given as μmol per gram of primary biomass as the biosorbents contain ca. 23% of magnetite or silanised magnetite. The Langmuir sorption model (Langmuir, 1918) was used to calculate the maximum adsorption capacities (q_{max}) at saturation for each of the biosorbents which are cited in the following section.

The particles of magnetite and the silanised magnetite did not accumulate metal (results not shown). The non-magnetite biosorbent had the highest sorption capacity for Cu^{2+} and Cd^{2+}, with uptake levels of ca. 400 and 125 μmol/g and Langmuir q_{max} values of 609.0 and 163.7 μmol/g for Cu^{2+} and Cd^{2+} respectively (see Fig. 1(a) and (b)). Magnetite biosorbent II proved to be a poor biosorbent of these metals, under the present experimental conditions, with uptake levels of ca. 50 and 25 μmol/g and q_{max} values of 55.1 and 30.3 μmol/g for Cu^{2+} and Cd^{2+} respectively. However, magnetite biosorbent I had uptake levels of ca. 225 and 90 μmol/g and q_{max} values of 371.4 and 118.3 μmol/g (ca. 60 and 70% of the values for the non-magnetite biosorbent) for Cu^{2+} and Cd^{2+} respectively (see Fig. 1(a) and (b)).

The non-magnetite biosorbent and magnetite biosorbent I exhibit similar equilibrium sorption isotherms for Ag^{+} uptake (see Fig. 1(c)), with uptake levels of ca. 75 and 80 μmol/g and q_{max} values of 98.3 and 100.1 μmol/g respectively. Ag^{+} was accumulated to the least extent by magnetite biosorbent II with a maximum uptake level of ca. 45 μmol/g and a q_{max} value of 51.0 μmol/g.

The reduction in uptake for magnetite biosorbents I and II, when compared to that of the non-magnetite biosorbent, is likely due to the magnetite particles blocking a certain percentage of binding groups. This may also affect the affinity of each binding site for metal if sites comprise more than one cell wall group acting together (Tobin *et al.*, 1990).

The difference in uptake values for magnetite biosorbents I and II can be attributed to differences in preparation. For biosorbent I magnetite was simply entrapped within the biomass matrix during cross-linking with epichlorhydrin. The bound magnetite particles would subsequently be expected to block a proportion of binding sites on the adsorbent surface. The silanised magnetite particles used in biosorbent II however are covalently bound to the cell wall envelopes leading to stronger, more stable bonds with the yeast polysaccharides and greater mechanical strength of the biosorbent. These particles may directly block potential metal binding sites and also interfere with metal-biomass interactions at other sites due to stereochemical hindrance by the silane chain.

It is interesting that the sorption capacity for silver was approximately identical for the non-magnetite biosorbent and magnetite biosorbent I (Fig. 1(c)). Ag^{+} uptake by magnetite biosorbent II is also reduced to a lesser degree than that for either Cu^{2+} or Cd^{2+} which suggests that a different mechanism of biosorption may be involved. Ag^{+} binding possibly involves the conversion of Ag^{+} to Ag^{o}, the elemental form of silver, which is then entrapped within the biosorbent matrix rather than bound by the polysaccharide. In this case, blocking of binding sites by magnetite or stereochemical hindrance by the silane chain would not be expected to interfere with Ag^{+} uptake to the same extent as it would for Cu^{2+} or Cd^{2+}.

In summary, the methods described here show potential for development of crosslinked magnetic biosorbent. The crosslinking protocol results in biosorbent that has undiminished metal binding capacity as compared to native biomass (Leusch *et al.*, 1995; Ting and Teo, 1994). Similarly, the magnetising methods used for magnetic biosorbent I cause no decrease in silver accumulation potential while for Cu^{2+} and Cd^{2+} binding capacity remains at 60% and 70% respectively. Overall, the present uptake levels are comparable with those of metabolism-mediated biosorption systems (Ellwood *et al.*, 1992; Thomas and Macaskie, 1996) and non-immobilised biosorbents which typically exhibit binding levels of 100–400 μmol/g cells (Blackwell *et al.*, 1995; Tobin *et al.*, 1990; Volesky and Holan, 1995). Furthermore, this biosorbent has the advantages of increased rigidity and non-susceptibility to effluent toxins. The present cell loadings (ca. 80% w/w) compare favourably with levels achieved by simple adsorption techniques of ca. 10% (Shabtai and Fleminger, 1994; Wong and Fung, 1997). Investigation of the mechanical strength and magnetic separation characteristics of this biosorbent is underway.

References

Blackwell, KJ, Singleton, I and Tobin, JM (1995). *J Appl Microbiol Biotechnol* **43**: 579–584.

Brady, D and Duncan, JR (1994). *Enzyme Microb Technol* **16**: 633–638.

Brady, D, Nigam, P, Marchant, R, McHale, L and McHale, AP (1996). *Biotech Lett* **18**: 1213–16.

Brady, D, Stoll, AD, Starke, L and Duncan, JR (1994). *Biotechnol Bioeng* **44**: 297–302.
Brierley, JA, Goyak, GM and Brierley, CL (1986). Considerations for Commercial Use of Natural Products for Metals Recovery. In: *Immobilisation of Ions by Bio-Sorption*, H Eccles and S Hunt, eds, pp 105–117. Chichester, England: Ellis Horwood.
Dauer, RR and Dunlop, EH (1991). *Biotech Bioeng* **37**: 1021–1028.
Dostalek, P, Pilarek, D and Tobin, J (1994). IUMS Congresses 1994, 7th Intl Congress Bacteriol Appl Microbiol Division, July 3–8 (1994), Prague, Czech Republic.
Ellwood, DC, Hill, MJ and Watson, JHP (1992) Pollution Control using Microorganisms and Magnetic Separation. In: *Microbial Control of Pollution*, JC Fry, GM Gadd, RA Herbert, CW Jones and IA Watson-Craik eds, 48th Symposium of the SGM, pp 89–112. Cambridge University Press.
Halling, PJ and Dunnill, P (1980). *Enzyme Microb Technol.* **2**: 2–10.
Holan, ZR, Volesky, B and Prasetyo, I (1993). *Biotechnol Bioeng* **41**: 819–825.
Ivanova, V, Hristov, J, Dobreva, E, Al-Hassan, Z and Penchev, I (1996). *Appl Biochem Biotechnol* 59: 187–98.
Kolm, H, Obertuffer, J and Kelland, D (1975). *Sci Am*: 46–54.
Korn, ED and Northcote, DH (1960). *Biochem J* **75**: 12–17.
Lloyd, JR and Macaskie, LE (1996) *Appl Environ Micro* **62**: 578–582.
Langmuir, I (1918). *J Am Chem Soc* **40**: 1361–1403.
Leusch, A, Holan, ZR and Volesky, B (1995). *J Chem Tech Biotechnol* **62**: 279–288.
Markham, R (1942). *Biochem J* **36**: 790.
McHale, AP and McHale, S (1994). *Biotech Adv* **12**: 647–652.
Northcote, DH and Horne, RW (1952). *Biochem J* **5**: 232–238.
O'Brian, SM, Thomas, ORT and Dunnill, P (1996). *J Bacteriol* **50**: 13–25.
Rome, L de and Gadd, GM (1991). *J Ind Microbiol* 7: 97–104.
Safarik, I, Safarikova, M and Vrchotova, N (1995). *Collect Czech Chem Commun* **60**: 34–42.
Shabtai, Y and Fleminger, G (1994). *Appl Environ Microbiol* **60**: 3079–3088.
Sly, LI, Arunpairojana, V and Dixon, DR (1993). *Water* (June) pp. 38–40.
Strandberg, GW, Shumate, SE and Parrott, JR (1981). *Appl Environ Microbiol* **41**: 237–245.
Thomas, RAP and Macaskie, LE (1996). *Environ Sci Technol* **30**: 2371–2375.
Ting, YP and Teo, WK (1994). *Bioresource Technol* **50**: 113–117.
Tobin, JM, Cooper, DG and Neufeld, RJ (1990). *Enzyme Microb Technol* **12**: 591–595.
Volesky, B and Holan, HR (1995). *Biotechnol Prog* **11**: 235–250.
Wong, PK and Fung, KY (1997). *Enzyme Microb Technol* **20**: 116–121.

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