ELSEVIER

Journal of Hazardous Materials 49 (1996) 165–179

JOURNAL OF HAZARDOUS MATERIALS

Heavy metal removal by caustic-treated yeast immobilized in alginate

Yongming Lu, Ebtisam Wilkins *

Department of Chemical and Nuclear Engineering, University of New Mexico, Albuquerque, NM 87131-1341,U.S.A.

Received 20 September 1995; accepted 25 November 1995

Abstract

Saccharomyces cerevisiae yeast biomass was heated in 0.75 M NaOH at 70–90 °C for 10–15 min to increase its biosorption for heavy metals, and then immobilized in alginate gel. Biosorption for Cu^{2+}, Cd^{2+} and Zn^{2+} on alginate gel, native yeast, native yeast immobilized in alginate gel, and caustic-treated yeast immobilized in alginate gel, were all compared. Immobilized yeasts (native yeast and caustic-treated yeast) could be reactivated and reused in a manner similar to ion-exchange resins. Immobilized caustic-treated yeast has high heavy metal biosorption capacity and high metal removal efficiency over a rather wide pH region. The biosorption isotherm of immobilized caustic-treated yeast was studied and empirical equations were obtained. The initial pH of polluted water affected the metal removal efficiency in extreme pH regions, and the biosorption capacity almost remained constant over a wide pH range. The equilibrium biosorption appeared to be temperature independent in the range from 7 °C to 45 °C at low initial metal concentration.

Keywords: Alginate; Biosorption; Caustic-treated; Heavy metal; Immobilization; Removal; Yeast

1. Introduction

The discharge of heavy metals into the environment and municipal sewers by the mining, metallurgical, electroplating, electronic, metal-finishing, nuclear and other industries, constitutes one of the major causes of land and water pollution. Conventional physico-chemical treatment methods, which include precipitation-filtration, ion ex-

* Corresponding author. Tel: (1-505) 277-5906 or 2928; fax: (1-505) 277-5433; e-mail: wilkins@bootes.unm.edu

PII S0304-3894(96)01754-2

change, reverse osmosis, oxidation-reduction, electrochemical recovery, membrane separation and other techniques are often ineffective or uneconomical when the heavy metal concentrations in the polluted environment are in the range of 10–100 mg l^{-1} and the permissible concentrations are less than 1 mg l^{-1} [1]. The search for new and innovative technologies has focused attention on the metal-removal capacities of various biological materials, such as bacteria, yeasts, filamentous fungi, algae, and plant cells [2–5].

Although viable (living) microorganisms have shown promise for wastewater treatment applications, their use has been limited, particularly to treating AMD (acid mine drainage) waters. These systems often require the addition of nutrients and hence increase BOD (biochemical oxygen demand) or COD (chemical oxygen demand) in the effluent, and the maintenance of a healthy microbial population may be difficult due to metal toxicity, unsuitable water pH, and other negative environmental factors. In addition, culture acclimation results in reduced metal uptake [6]. Biosorption is the accumulation of metals without active uptake. Biosorption can be considered as a collective term for a number of passive accumulation processes which in any particular case may include ion exchange, coordination, complexation, chelation, adsorption and microprecipitation. It may occur even when the cell is metabolically inactive, such as when it has been killed by chemical or physical means [7,8]. The advantages of using non-viable cells are numerous. Killed cells may be stored or used for extended periods at room temperature without decay occurring.

For use in industrial or technical operations, natural microbial biomass has several disadvantages in that it may cause problems in the operation of reactors by blocking flow lines and clogging filters, while separation of biomass and effluent can be difficult and expensive [9]. Immobilization of biomass in a polymeric matrix can yield beads or granules with optimum size, mechanical strength, rigidity and porosity characteristics [10]. Several immobilization techniques have been reported [11–14]. Polysaccharide-based biomaterials, such as alginate, also have binding sites for divalent cations because of the presence of amino, carboxyl, phosphate and sulfate functional groups within them. Calcium alginate, one of the popular entrapment agents in immobilization technology, has been one of the most extensively investigated biopolymers for binding heavy metals from diluted aqueous solutions [15,16]. Alginate is a linear polyuronate obtained from marine algae and contains variable amounts of D-manuronic acid and L-guluronic acid, which can be crosslinked by using calcium ion as a crosslinking agent because it can be selectively bound between sequences of polyguluronosyl residues [17]. Alginate is used as an entrapment material to immobilize yeast in this study.

Microorganisms can accumulate metabolic and non-metabolic metals by precipitating or binding the metals onto cell walls or cell membranes owing to the presence of carboxyl, hydroxyl, phosphoryl and other negatively charged sites in anionic walls. As might be expected, intracellular materials, which will be released after physical or chemical treatments, can also bind metal ions, due to the presence of reactive groups, to metal ions in them. Some methods of killing cells may actually improve the biosorption properties of the biomass [7]. Treatment of biomass with NaOH and other alkaline reagents has been demonstrated to increase the capacity of certain biomass to adsorb metals [18,19]. Caustic treatment of biomass has the advantages that it destroys autolytic enzymes that cause putrification of biomass, and, inter alia, removes lipids and proteins

that mask reactive sites [20]. Caustic treatment of biomass can result in solubilization and subsequent loss of biomass that have metal binding capacity, but most base-soluble biomass can be reconstituted by adding acid to attain a neutral pH [19].

Biomass can be produced specially for biosorbent production through fermentation. However, the cost of such custom fermentation can be too high to produce an economically competitive waste treatment product. Most companies seeking to commercialize biosorbents for waste treatment have sought suitable biomass that is a by-product of pharmaceutical and enzyme manufacturing. However, the by-product microorganism is almost always a proprietary strain of the pharmaceutical or enzyme manufacturer. This factor can discourage the biotechnology firm from supplying the biomass. On the other hand, the ready availability of waste *Saccharomyces cerevisiae* yeast biomass from classical food fermentation industries (e.g. breweries) makes the use of this product a viable proposition.

Yeast cells killed by extreme chemical and physical condition may also show very different metal accumulating properties compared to the original yeast. The aim of this study was to investigate metal biosorption properties of alginate gel, native yeast, immobilized native yeast, and immobilized yeast which had been heated in NaOH solution and then entrapped in alginate gel. The adsorption isotherm and the effect of pH and temperature on the biosorption were studied in detail for immobilized caustic-treated yeast.

2. Experimental

2.1. Preparation of biosorbent beads

Yeast biomass, *Saccharomyces cerevisiae* (bakers yeast, type II), and alginic acid (sodium salt with high viscosity, from *macrocystis pyrifera* (kelp)) were obtained from Sigma Chem.

Native yeast (6 g) was mixed with 20 ml 0.75 M NaOH, and the resulting solution was heated to 70–90 °C for 10–15 min. After the temperature of the solution returned to ambient, 4 M HCl was used to adjust the pH to 6 to reconstitute the base-soluble biomass [19]. The product was then mixed with 30 ml 3.33% (w/w) sodium alginate solution. The immobilized caustic-treated yeast (ICY) was prepared by dispensing drops of the mixture, which was cut by using a needle, through a 1 ml syringe (2 mm ID) into $CaCl_2$ (1.5% w/v) solution. The immobilized yeast (IY) was prepared with the same method and the same recipe as ICY (12% w/w yeast and 2% w/w alginate), but without the alkali treatment and base-soluble biomass reconstitution process. The alginate gel bead was also prepared by dispensing the sodium alginate (4% w/w) drops through a 1 ml syringe into $CaCl_2$ (1.5% w/v) solution.

All 3 types of biosorbent beads were cured overnight in the $CaCl_2$ solution, rinsed with deionized water (DW), and soaked in 0.5 M HCl solution for more than 24 h for consistency with the biosorbent reactivation process (the immobilized native yeast (IY) was denatured and is no longer viable in this acid treatment process). The beads were then soaked in deionized water for more than 24 h, and the water was changed. This

soaking process was repeated several times until the pH of the water (after 24 h) reached 3.5. All 3 types of biosorbent beads prepared in this study had diameters of 2.3 ± 0.2 mm.

2.2. Batch experiments

2.2.1. Equilibration Time

Biosorption is generally considered to be a rapid physical and chemical phenomenon. The overall rate of binding (diffusion plus reaction) depends primarily on diffusivity. Preliminary experiments were performed to determine equilibrium time in a 400 ml beaker for native yeast, alginate gel bead, immobilized yeast, and immobilized caustic-treated yeast. Biosorbent beads (alginate gel, IY, or ICY) or native yeast, 0.4 g dry weight, was added to 200 ml well-mixed metal solution of either Cu^{2+}, Cd^{2+} or Zn^{2+} with initial pH 5.0 at ambient temperature (23 ± 1 °C). Samples were periodically withdrawn and assayed for metal content. The equilibrium times found were used for other experiments.

2.2.2. Calculation

Metal-laden gel beads, collected after equilibrium was reached in batch experiments, were dried at about 110 °C for 2 h and weighed. The amount of metal adsorbed was determined by the difference between the initial metal-ion concentration and the final one after equilibrium was reached. Metal concentrations were quantified using a Perkin-Elmer atomic absorption spectrophotometer Model 460. For comparison, the digested biomass was also used to determine the quantity of metal adsorbed. The procedure was as follows: 5 ml concentrated HNO_3 (70%) and 10 ml deionized water were added to the dried beads in a 125 ml Erlenmeyer flask, and the mixture was heated at boiling point for about 15 min. The mixture was centrifuged, and the solid was rinsed with deionized water and centrifuged again. The solution was diluted to a fixed volume using deionized water for metal content assay.

2.2.3. Comparison of Biosorbents

The metal biosorption properties of native yeast, alginate gel bead, immobilized yeast, and immobilized caustic-treated yeast were studied by adding 0.2 g (dry weight) of beads or native yeast to 100 ml of metal solution of either Cu^{2+}, Cd^{2+} or Zn^{2+} with initial pH 5.0, and the initial concentration was in the range from 16–18 mg l^{-1} at ambient temperature (23 ± 1 °C). The beads were then dried and weighed after equilibrium was reached. For native yeast, samples were separated in a high-speed centrifuge before the metal-ion content assay.

2.2.4. Metal Desorption and Biosorbent Reactivation

Immobilized yeasts (IY or ICY), 0.2 g dry weight, were added to 100 ml of metal solution of either Cu^{2+}, Cd^{2+} or Zn^{2+} with an initial pH of 5.0; and the initial concentration was in the range 16–18 mg l^{-1} at ambient temperature (23 ± 1 °C). Following initial biosorption of metals, the metal-laden beads were soaked in 25 ml 0.5 M HCl for more than 24 h for desorption of metals, rinsed with deionized water, soaked

in deionized water for more than 24 h, and the water was changed. The soaking process was repeated several times until the pH of the water (after 24 h) reached 3.5, before the next biosorption experiment. The initial metal concentration and that after equilibrium were assayed for, each time, and the biosorbent beads were dried and weighed after the sixth reuse cycle.

2.2.5. Study of ICY

The effect of temperature on the biosorption was studied by keeping batch reactors at 7 ± 1 °C, 23 ± 1 °C or 45 ± 1 °C. All solutions added to the reactor were prewarmed or precooled. The effect of initial pH on the biosorption was studied by adding HCl (1–4 M) or NaOH (1–4 M) to adjust the initial pH of the solution. No buffer was included. In order to prevent assay error due to the hydrolysis of metals at high pH, the initial metal concentration was assayed before pH adjustment (the concentration change owing to the volume increase after pH adjustment was negligible). The biosorption isotherm was studied by using different concentrations of the metals ranging from 2–1000 mg l^{-1} with fixed biosorbent density (2 g l^{-1}), or by using different biosorbent density (2–6 g l^{-1}) and fixed initial metal concentration (14–18 mg l^{-1}). The initial pH was 5.0, and the experiments were completed at ambient temperature (23 ± 1 °C).

3. Results and discussion

Preliminary experiments were carried out to determine the equilibrium time for biosorption. It has been found that most of the biosorption occurred within the first 5–10 min and a minimal decrease of metal concentration is observed after 60 min for the native yeast. This is because almost no diffusion resistance exists in the native yeast system. For alginate gel beads and immobilized yeast systems (IY and ICY), more than 90% of the metal biosorption was completed within 3 h and equilibrium was reached after 24 h. Hence, 36 h was used as equilibrium time for alginate gel beads and immobilized yeasts (IY and ICY); 2 h was used as equilibrium time for native yeast. Fig. 1 shows the experimental data for immobilized caustic-treated yeast.

The metal biosorption properties of alginate gel, native yeast biomass, immobilized native yeast, and immobilized caustic-treated yeast were compared. The average results are listed in Table 1. Alginate gel accumulated more metal ions compared to the other 2 types of biosorbent. Yeast immobilized in the alginate gel reduced the quantity of heavy metal binding to the biomass, as compared to the biosorption by the native yeast, by about 10–25%. The decrease of the amount of metal adsorbed occurred probably because of the cross-linking of potential metal-binding sites with alginate gel, and masking of active sites due to the higher density of the biomass. However, this negative effect is negligible compared to the advantage of immobilization in industrial operations.

Yeast treated with hot NaOH obviously increased the amount of metal adsorption by about 6–26% compared to biosorption by the native yeast, even though both negative factors were also present due to immobilization. On the other hand, the increase of the biosorption capacity due to the presence of alginate with the highest metal biosorption capacity in immobilized caustic-treated yeast is insignificant because it is only 14% of

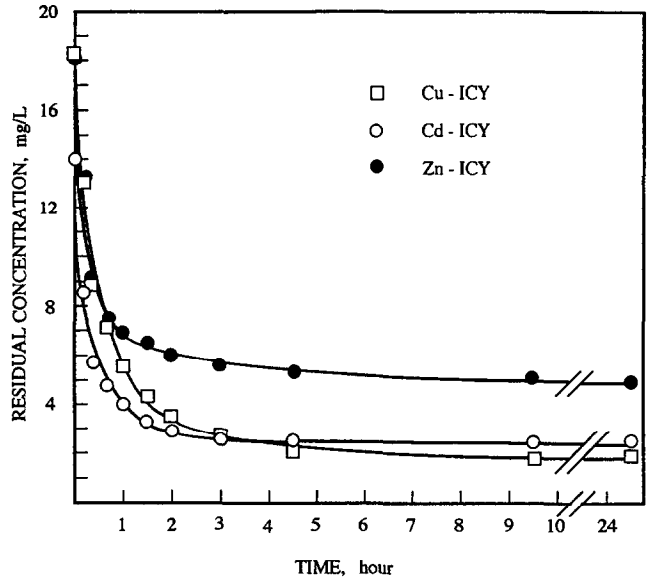


Fig. 1. Rate of metal biosorption of Cu^{2+}, Cd^{2+} and Zn^{2+} ions on immobilized caustic-treated yeast (ICY) with initial pH 5.0 at 23 ± 1 °C.

the total weight. The large proportion of yeast biomass in the gel beads also makes it economically a promising biosorbent to recover heavy metal from dilute solutions. Brady et al. also found a significant increase in the amount of copper ion adsorbed to yeast biomass with hot NaOH treatment [18]. However, the granular biosorbent prepared by filtering the hot alkali treated yeast solution has a copper-binding capacity 27% lower than that of native yeast biomass [18].

Hot alkali treatment dissolves a considerable amount of biomass which would not be retained during normal filtration, such as manno-protein [21], from the cell, and these base soluble materials also have metal-binding capacity. Hence, the advantage found in this study of reconstituting base soluble biomass by adding acid is significant. Because the immobilized biosorbent has the advantage of being macroscopic in size and, therefore, can be retained by a simple mesh without the requirement of expensive separation units, caustic-treated yeast immobilized in alginate was studied in detail.

Table 1
Metal biosorption quantity (mg g^{-1} dry mass) of different types of biosorbents with initial concentration 17 ± 1 mg l^{-1} and pH 5.0 at 23 ± 1 °C. (IY-immobilized native yeast, ICY-immobilized caustic-treated yeast)

Metal ions	Alginate gel	Native yeast	IY	ICY
Cu^{2+}	9.01	7.02	6.32	8.46
Cd^{2+}	7.50	5.85	4.44	6.20
Zn^{2+}	7.08	4.54	3.73	5.72

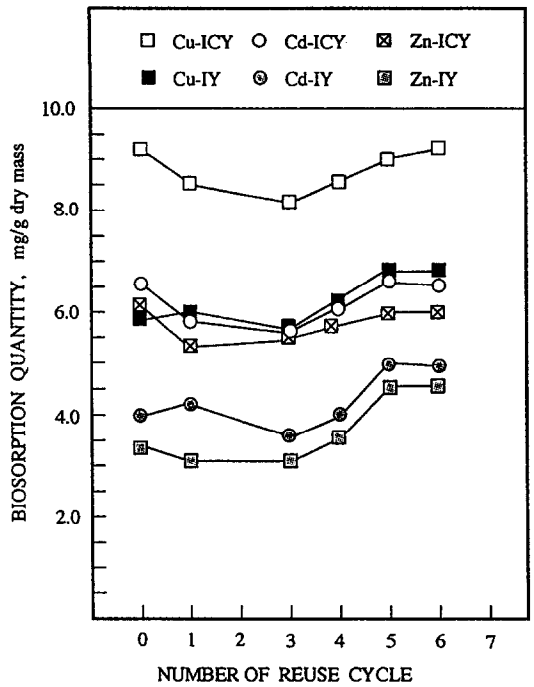


Fig. 2. Biosorption of metal ions on immobilized native yeast (IY) and immobilized caustic-treated yeast (ICY) reactivated and reused in the manner of ion-exchange resins with initial concentration in the range of 16–18 mg l^{-1} and pH 5.0 at 23 ± 1 °C.

For immobilized biomass, it is possible to reactivate the biosorbent and recover the loaded metals after desorption in a manner similar to ion-exchange resins. However, the biosorption mechanisms, and hence the desorption mechanisms, for biosorbent are much more complicated than those for ion-exchange resins or conventional physical and chemical adsorption materials. Several factors, such as type of biosorbent, type and concentration of desorbent, and type of metal ions, can affect the desorption efficiency. HCl was used as a desorbent by Mattuschka et al. to recover Cr^{3+}, Pb^{2+} and Ag^{+} ions loaded on *Streptomyces noursei* biomass [22]. It was found that more than 90% of metal ions (Cu^{2+}, Cd^{2+} or Zn^{2+}) were desorbed when 0.5 M of HCl was used, and no significant metal recovery increase was observed when the concentration of HCl was higher than 0.5 M, which is the usual concentration used in ion-exchange operations.

The results for Cu^{2+}, Cd^{2+} and Zn^{2+} biosorbed by immobilized yeasts (IY and ICY) are shown in Fig. 2 for 6 reuse cycles. No decrease in the amount of metal adsorbed and no biomass loss were found in the experimental period. The immobilized yeasts (IY and ICY) tended to shrink as the metal accumulated, resulting in a smaller and more dense particle. The fluctuations of biosorption capacity were caused by several factors, including small differences in initial metal concentrations for each cycle and hence differences in concentrations after equilibrium, which affects the amount of metal adsorbed significantly, especially in low concentration (biosorption isotherm), and desorption operation and assay error.

The effect of metal concentration after equilibrium on the metal biosorption of immobilized caustic-treated yeast can be described by the Langmuir and Freundlich adsorption isotherms (Fig. 3 and Fig. 4). The empty cycles stand for the experimental data obtained by changing the initial metal concentration (2–1000 mg l^{-1}) with fixed biosorbent density (2 g l^{-1}), and the shaded cycles stand for the experimental data obtained by changing the biosorbent density (2–6 g l^{-1}) and fixing the initial metal concentration (14–18 mg l^{-1}). Fig. 3c and Fig. 4c show that, for immobilized caustic-treated yeast, the Freundlich isotherm fits the data better than the Langmuir isotherm for Zn^{2+}. Generally, Langmuir and Freundlich adsorption isotherms could describe both physical and chemical adsorption phenomena. However, it is impossible that biosorption capacity increases with equilibrium concentration exponentially at high concentrations because biosorption saturation is physically reasonable. As shown in Fig. 4, 2 different sets of model parameters for different concentration regions are used for the Freundlich isotherm in order to get a better fit. The concentration at which model parameters change is about $1^{*}10^{-4}$ M (4, 10 and 6 mg l^{-1} for Cu^{2+}, Cd^{2+} and Zn^{2+}, respectively).

As shown in the saturation biosorption data from the Langmuir isotherm equations, the copper ion was preferentially adsorbed compared to other metal ions. The series of decreasing sorption is $Cu^{2+} > Cd^{2+} > Zn^{2+}$ for the separation experiment ($0.0286 < 0.0413 < 0.114$).

Fig. 5a demonstrates that the metal biosorption depends on the biomass density. With higher biomass densities, the amount of metal accumulated per dry mass decreases. With increasing biomass density, nevertheless, more metal can be removed from solution (Fig. 5b). The relations between the amount of metal accumulated per gram of dry mass and the metal concentrations after equilibrium are also shown in Fig. 3 and Fig. 4. The results demonstrate the validity of both isotherms in another way. Because the initial concentration of Cd^{2+} (14 mg l^{-1}) was lower than that of Zn^{2+} and Cu^{2+} (both were 18 mg l^{-1}), the amount of Zn^{2+} accumulated per gram of dry mass is higher than that of Cd^{2+} corresponding to the cell density owing to the higher concentration of Zn^{2+} after equilibrium. But the biosorption preference corresponding to the equilibrium concentration is also in the order of $Cu^{2+} > Cd^{2+} > Zn^{2+}$ (Fig. 3 and Fig. 4).

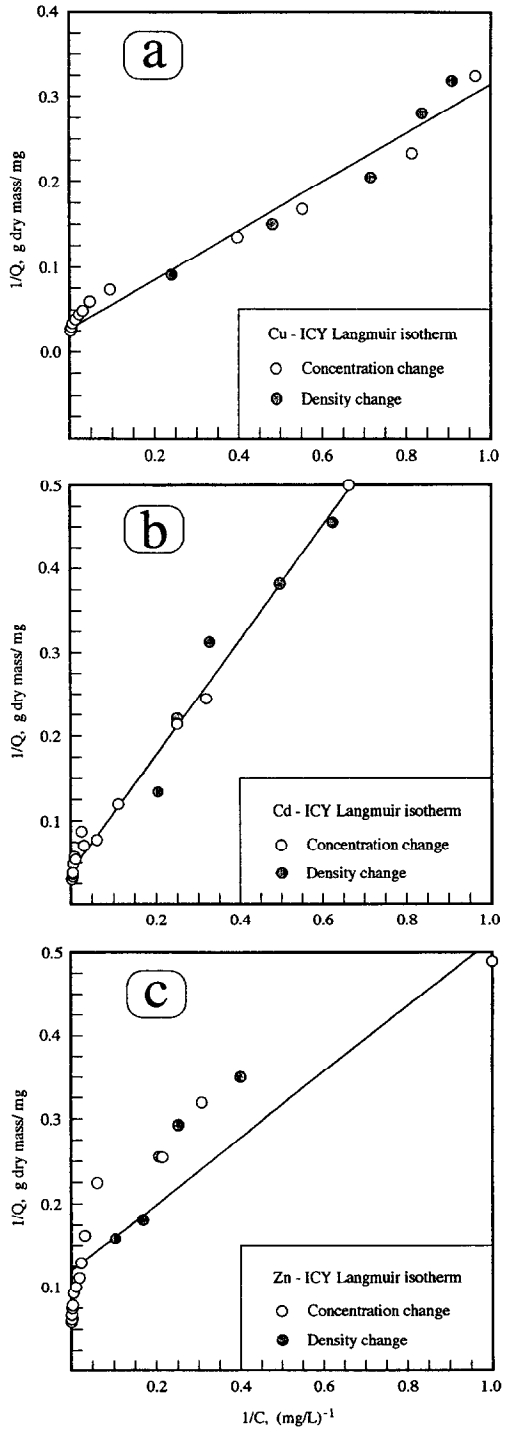
Metal removal was significantly affected by the initial pH of the metal solution due to cation competition effects with the hydronium ion (H^{+}). Copper, cadmium and zinc biosorption by immobilized caustic-treated yeast did not occur below pH 3, but increased rapidly above pH 3, and levelled off at about pH 4 (Fig. 6). In the experiments, it was found that the metal ions hydrolyzed at pH above 6.5, 9.0 and 8.0

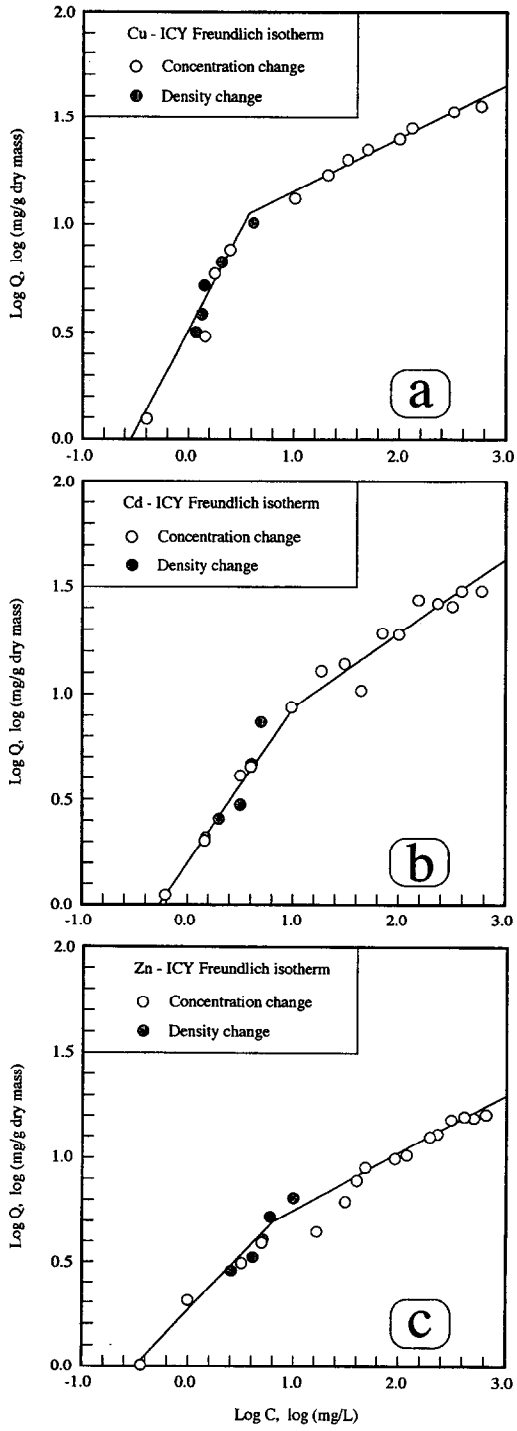
Fig. 3. Biosorption of Cu^{2+}, Cd^{2+}, Zn^{2+} ions on immobilized caustic-treated yeast (ICY) and Langmuir adsorption isotherm equations

$1/Q = 0.285/C + 0.0286$ for Cu^{2+}, $1/Q = 0.681/C + 0.0413$ for Cd^{2+}

$1/Q = 0.401/C + 0.114$ for Zn^{2+}

Q is the amount of metal adsorbed per gram of dry mass (mg g^{-1} dry mass) and C is the metal concentration after equilibrium (mg l^{-1}).


a
1/Q, g dry mass/ mg
0.4
0.3
0.2
0.1
0.0
0.2
0.4
0.6
0.8
1.0
Cu - ICY Langmuir isotherm
Concentration change
Density change
b
1/Q, g dry mass/ mg
0.5
0.4
0.3
0.2
0.1
0.2
0.4
0.6
0.8
1.0
Cd - ICY Langmuir isotherm
Concentration change
Density change
c
1/Q, g dry mass/ mg
0.5
0.4
0.3
0.2
0.1
0.2
0.4
0.6
0.8
1.0
Zn - ICY Langmuir isotherm
Concentration change
Density change
1/C, (mg/L)-1


Cu - ICY Freundlich isotherm
Concentration change
Density change
a
Cd - ICY Freundlich isotherm
Concentration change
Density change
b
Zn - ICY Freundlich isotherm
Concentration change
Density change
c
Log Q, log (mg/g dry mass)
Log C, log (mg/L)
2.0
1.5
1.0
0.5
0.0
-1.0
0.0
1.0
2.0
3.0

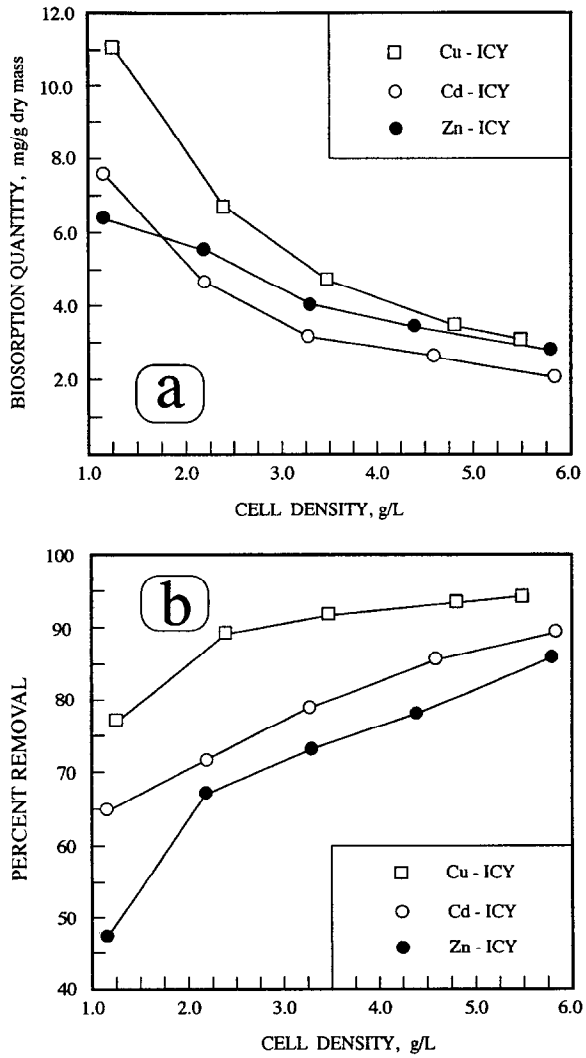


Fig. 5. The effect of biosorbent density on the biosorption characteristic of immobilized caustic-treated yeast (ICY) with initial metal concentration of 18, 14, 18 mg L^{-1} for Cu^{2+}, Cd^{2+} and Zn^{2+}, respectively, and pH 5.0 at 23 ± 1 °C.

Fig. 4. Biosorption of Cu^{2+}, Cd^{2+}, Zn^{2+} ions on immobilized caustic-treated yeast (ICY) and Freundlich adsorption isotherm equations

$\log Q = 0.943 \log C + 0.500$ for Cu^{2+} $(C < 4 mg l^{-1})$, $\log Q = 0.250 \log C + 0.900$ for Cu^{2+} $(C > 4 mg l^{-1})$

$\log Q = 0.743 \log C + 0.193$ for Cd^{2+} $(C < 10 mg l^{-1})$,

$\log Q = 0.343 \log C + 0.593$ for Cd^{2+} $(C > 10 mg l^{-1})$

$\log Q = 0.536 \log C + 0.268$ for Zn^{2+} $(C < 6 mg l^{-1})$, $\log Q = 0.275 \log C + 0.475$ for Zn^{2+} $(C > 6 mg l^{-1})$

Q is the amount of metal adsorbed per gram of dry mass (mg g^{-1} dry mass) and C is the metal concentration after equilibrium (mg l^{-1}).

for Cu^{2+}, Cd^{2+} and Zn^{2+}, respectively. However, the deposit disappeared after the addition of immobilized caustic-treated yeast due to its acidic characteristic during acid treatment (pH was about 3.5). Partially due to the acidic characteristic of immobilized caustic-treated yeast, the pH after equilibrium changed little (from 3 to 4) for the initial pH range from 3 to 9. A similar phenomenon was found in all other experiments, such as in the biosorbent reuse and the biosorption isotherm. Fig. 6 also shows that there are second increases of metal removal at pH about 6.5, 7 and 8 for Cu^{2+}, Zn^{2+} and Cd^{2+} respectively. This may suggest the presence of chemical precipitation due to hydrolysis of metal ions. The phenomenon that the biosorption quantity is almost maintained constant in the initial pH region from 4 to 6.5, from 4 to 7 and from 4 to 8 for Cu^{2+}, Zn^{2+} and Cd^{2+} respectively suggests that the biosorption isotherm equations obtained from the experiment with initial pH 5.0 can be used for those pH regions.

The pH at which the second metal removal quantity increase occurs is in the order of $Cu^{2+} < Zn^{2+} < Cd^{2+}$. This order is the same as that of the solubility products of metal hydroxides, $Cu(OH)_2$ ($K_{sp} = 10^{-19.8}$) < $Zn(OH)_2$($K_{sp} = 10^{-16.8}$) < $Cd(OH)_2$($K_{sp} = 10^{-13.9}$) [23]. This supports the suggestion that there is the mechanism of chemical precipitation at the high initial pH region, although the equilibrium pH is acidic. On the other hand, the second increase could not be infinite because of the limit of the amount of metal ion added. Also, at very high pH (say 10–11), the alginate dissolved and the

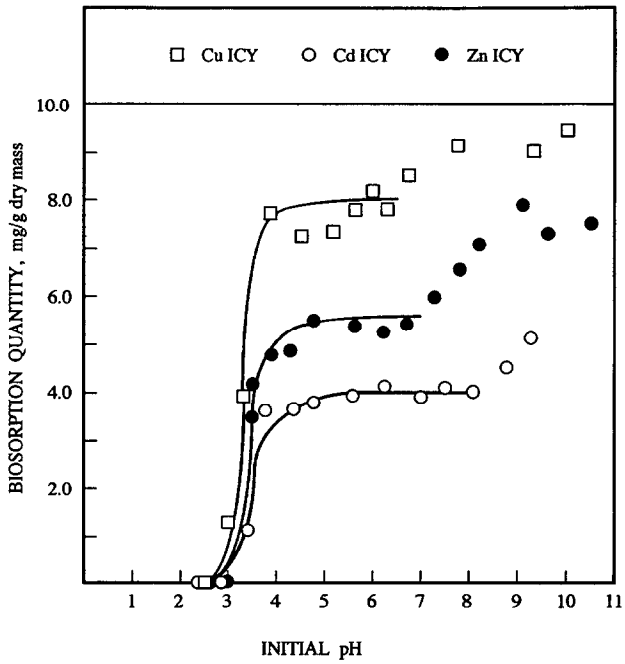


Fig. 6. The effect of initial pH of metal solution on the biosorption of immobilized caustic-treated yeast (ICY) with initial metal concentration of 20, 13, 18 mg l^{-1} for Cu^{2+}, Cd^{2+} and Zn^{2+}, respectively, and pH 5.0 at 23 ± 1 °C.

Table 2
Effect of temperature on the metal biosorption quantity (mg g^{-1} dry mass) of immobilized caustic-treated yeast (ICY) with initial concentration of 18, 14 and 18 mg l^{-1} for Cu^{2+}, Cd^{2+} and Zn^{2+} respectively and pH 5.0 at 23 ± 1°C

	Temperature (°C)			Average equilibrium conc.	Calculated values	
Species	7 ± 1°C	23 ± 1°C	45 ± 1°C	(mg l^{-1})	Langmuir	Freundlich
Cu ICY	6.45	6.69	7.52	2.5	7.01	7.50
Cd ICY	4.44	4.58	4.25	4.3	5.01	4.61
Zn ICY	5.24	5.53	5.41	5.9	5.50	4.80

yeast was unimmobilized [24]. The fact that immobilized caustic-treated yeast works well in the low pH region suggests that it is a promising biosorbent for AMD waters and other waters with natural pH. Because the concentration of Cd^{2+} after equilibrium was lower than that of Zn^{2+} due to the lower initial concentration of Cd^{2+} (13 mg l^{-1}) compared to that of Zn^{2+} (18 mg l^{-1}), the amount of Zn^{2+} accumulated per gram of dry mass at the levelled pH region was higher than that of Cd^{2+}.

In the biosorption isotherm experiment, it was found that the higher the concentration after equilibrium, the more the metal ions adsorbed, and the lower the pH after equilibrium, although the pH difference was not significant because of the logarithmic relation between molar concentration and pH. This phenomenon demonstrates that the ion-exchange mechanism is present in the biosorption process.

The effect of temperature on the metal biosorption property of ICY is shown in Table 2. Because the concentration of Zn^{2+} after equilibrium was higher than that of Cd^{2+} due to the difference of initial concentration, the amount of Zn^{2+} accumulated per gram of dry mass was higher than that for Cd^{2+} corresponding to the same temperature. It is seen from Table 2 that the difference of biosorption data due to the change of temperature is negligible, hence the biosorption is temperature independent. A similar result was found by Kuhn et al. for the accumulation of cadmium by immobilized *Zoogloea ramigera* 115 at lower initial concentration (< 50 mg l^{-1}) [14]. The biosorption rate increased with temperature if the initial concentration of Cd^{2+} was higher than 50 mg l^{-1}. That is probably because of the increase of chemical adsorption and diffusion rates with temperature. No effect of temperature on the equilibrium biosorption and no relationship between the Cd^{2+} adsorption rate and temperature were found [14]. The data calculated using the isotherm equations obtained from the experiment are also listed in Table 2, and show the validity of both isotherms.

4. Conclusions

Immobilized yeasts (IY and ICY) could be reactivated and reused in a manner similar to ion-exchange resins. No metal biosorption quantity decrease and biomass loss were found after 6 reuse cycles. Yeast biomass immobilized in alginate reduced the quantity of heavy metal binding to the biomass, as compared to biosorption by native yeast, by about 10–25%. However, it is negligible compared to the advantage of immobilization

in industrial operations. Caustic-treated yeast immobilized in alginate enhanced the quantity of metal biosorption. Base soluble biomass which also had metal binding capacity was reconstituted by adding acid to adjust the pH to neutral. Because of its high heavy metal biosorption capacity and high metal removal efficiency over a wider acidic pH region, this caustic-treated yeast immobilized in alginate is promising for use in heavy metal removal from aqueous solutions.

The effects of metal concentration after equilibrium was reached on the biosorption property of immobilized caustic-treated yeast could be described by the Langmuir and Freundlich adsorption isotherms. For the Freundlich isotherm, it was found to be better to use 2 different sets of model parameters for different concentration regions, rather than to use 1 set of parameters only.

Heavy metal biosorption on the immobilized caustic-treated yeast was temperature independent at low initial metal concentration. On the other hand, the initial pH of the heavy metal solution affected the metal-removal efficiency significantly owing to cation competition with the H^+ ion in drastic pH regions. Cu^{2+}, Cd^{2+} and Zn^{2+} biosorption by immobilized caustic-treated yeast did not occur below pH 3, but increased rapidly above pH 3, levelled off at pH 4, and almost maintained constants in the initial pH region from 4 to 6.5, from 4 to 7 and from 4 to 8 for Cu^{2+}, Zn^{2+} and Cd^{2+} respectively. This phenomenon suggests that the biosorption isotherm equations obtained from the experiment with the initial pH 5.0 can be used for those levelled pH regions. In the high pH region, the chemical precipitation of metal ions was found.

Acknowledgements

The authors would like to thank Prof. Bruce M. Thomson, Prof. Ric Morris and Mr. Mark Schmidt of the Department of Civil Engineering for advice on atomic absorption spectrophotometry procedures.

References

[1] S.E. Shumate, II., G.W. Strandberg and J.R. Parrott Jr., Biotechnol. Bioeng. Symp., 8 (1978) 13.
[2] D. Brady and J.R. Duncan, Bioaccumulation of metal cations by *Saccharomyces cerevisiae*, in A.E. Torma, M.L. Apel and C.L. Brierley (Eds.), Biohydrometallurgical Technologies, Vol. 2, Proc. of An International Biohydrometallurgy Symposium, WY, USA, 1993, The Minerals, Metals and Materials Society, 1993, pp. 711–724.
[3] C.L. Brierley, Geomicrobiol. J., 8 (1991) 201.
[4] C.L. Brierley, in H.L. Ehrlich and C.L. Brierley (Eds.), Metal immobilization using bacteria, Microbial Mineral Recovery, McGraw-Hill, NY, USA, 1990, pp. 303–323.
[5] A.A. Pradhan and A.D. Levine, Water. Sci. Technol., 26 (9–11) (1992) 2145.
[6] S. Ghosh and S. Bupp, Water Sci. Technol., 26 (1–2) (1992) 227.
[7] N. Kuyucak and B. Volesky, Biotechnol. Lett., 10 (2) (1988) 137.
[8] M. Tsezos, Can. Metall. Q., 24 (2) (1985) 141.
[9] M. Tsezos, Adsorption by microbial biomass as a process for removal of ions from process or waste solutions, in H. Eccles and S. Hunt (Eds.), Immobilization of Ions by Biosorption, Ellis Horwood, Chichester, 1986, pp. 201–218.

[10] M. Tsezos, Engineering aspects of metal binding by biomass, in H.L. Ehrlich and C.L. Brierley (Eds.), Microbial Mineral Recovery, McGraw-Hill, NY, 1990, pp. 325–339.
[11] T.H. Jeffers and R.R. Corwin, Waste water remediation using immobilized biological extractants, in A.E. Torma, M.L. Apel and C.L. Brierley (Eds.), Biohydrometallurgical Technologies, Vol. 2, Proceeding of An International Biohydrometallurgy Symposium, WY, USA, 1993, The Minerals, Metals and Materials Society, 1993, pp. 1–13.
[12] A.M. Khalid, A.M. Shemsi, K. Akhtar and M.A. Anwar, Uranium biosorption by *Trichoderma harzianum* in polyester foam beads, in A.E. Torma, M.L. Apel and C.L. Brierley (Eds.), Biohydrometallurgical Technologies, Vol. 2, Proceeding of An International Biohydrometallurgy Symposium, WY, USA, 1993, The Minerals, Metals and Materials Society, 1993, pp. 309–317.
[13] L. De-Rome and G.M. Gadd, J. Ind. Microbiol., 7 (1991) 97.
[14] S.P. Kuhn and R.M. Pfister, J. Ind. Microbiol., 6 (1990) 123.
[15] L.K. Jang, W. Brand, M. Resong, W. Mainieri and G.G. Geesey, Environ. Prog., 9 (1990) 269.
[16] L.K. Jang, G.G. Geesey, S.L. Lopez, S.L. Eastman and P.L. Wichlacz, Water Res., 24 (1990) 889.
[17] I.W. Sutherland, Alginates, in D. Byrom (Ed.), Biomaterials: Novel Materials From Biological Sources, Stockton Press, NY, 1991, pp. 308–331.
[18] D. Brady, A. Stoll and J.R. Duncan, Environ. Technol., 15 (1994) 429.
[19] C.L. Brierley and J.A. Brierley, Immobilization of biomass for industrial application of biosorption, in A.E. Torma, M.L. Apel and C.L. Brierley (Eds.), Biohydrometallurgical Technologies, Vol. 2, Proceeding of An International Biohydrometallurgy Symposium, WY, USA, 1993, The Minerals, Metals and Materials Society, 1993, pp. 35–44.
[20] T.R. Muraleedharan and C. Venkobachar, Biotechnol. Bioeng., 35 (1990) 320.
[21] B.J. Catley, Isolation and analysis of cell walls, in I. Campbell and J.H. Duffus (Eds.), Yeast: A Practical Approach, IRL Press. Ltd., Oxford, 1988, pp. 163–183.
[22] B. Mattuschka, K. Junghans and G. Straube, Biosorption of metals by waste biomass, in A.E. Torma, M.L. Apel and C.L. Brierley (Eds.), Biohydrometallurgical Technologies, Vol. 2, Proceeding of An International Biohydrometallurgy Symposium, WY, USA, 1993, The Minerals, Metals and Materials Society, 1993, pp. 125–132.
[23] K. Schwitzgebel and D.M. Manis, Removal of chromate, cyanide, and heavy metals from wastewater, in D.L. Wise and D.J. Trantolo (Eds.), Process Engineering for Pollution Control and Waste Minimization, Marcel Dekker, NY, 1994, pp. 535–556.
[24] J. Klein and K-D Vorlop, Immobilization techniques - cells, in M. Moo-Young (Ed.), Comprehensive Biotechnology, Vol. 2, Pergamon Press, Oxford, 1985, pp. 203–224.