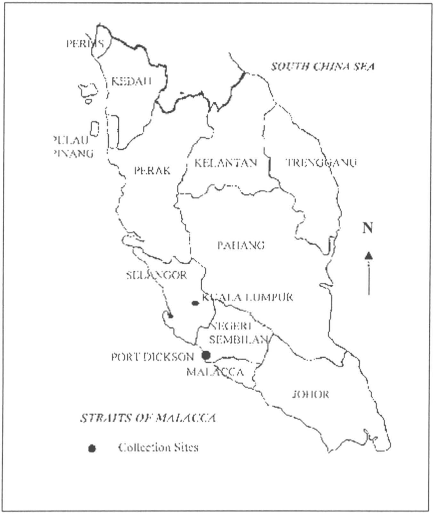
APPENDIX I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APPENDIX II

PARAMETER LIMITS OF EFFLUENTS OF STANDARDS A & B

Parameter	Units	Std A	Std B
Temperature	^0C	40	40
pH	-	6.0 - 9.0	5.5 - 9.0
BOD_5 at 20^0C	mg/L	20	50
COD	mg/L	50	100
Suspended Solids	mg/L	50	100
Mercury	mg/L	0.005	0.05
Cadmium	mg/L	0.01	0.02
Chromium, Hexavalent	mg/L	0.05	0.05
Arsenic	mg/L	0.05	0.10
Cyanide	mg/L	0.05	0.10
Lead	mg/L	0.10	0.50
Chromium, Trivalent	mg/L	0.20	1.00
Copper	mg/L	0.20	1.00
Manganese	mg/L	0.20	1.00
Nickel	mg/L	0.20	1.00
Tin	mg/L	0.20	1.00
Zinc	mg/L	1.00	1.00
Boron	mg/L	1.00	4.00
Iron	mg/L	1.00	5.00
Phenol	mg/L	0.001	1.00
Free Chloride	mg/L	1.00	2.00
Sulphide	mg/L	0.50	0.50
Oil and Grease	mg/L	Not Detectable	10.00

Source: Environmental Quality (Sewerage and Industrial Effluents) Regulations 1978
Regulation 8(1), 8(2), 8(3)

PARAMETER LIMITS IF EFFLUENTS OTHER THAN OF STANDARDS A & B

Parameter	Units	Standard
Temperature	^0C	45
pH	-	5.0 - 9.0
BOD_5 at 20^0C	mg/L	400
COD	mg/L	1000
Suspended Solids	mg/L	400
Mercury	mg/L	0.10
Cadmium	mg/L	1.00
Chromium, Hexavalent	mg/L	2.00
Arsenic	mg/L	2.00
Cyanide	mg/L	2.00
Lead	mg/L	2.00
Chromium, Trivalent	mg/L	10.00
Copper	mg/L	10.00
Manganese	mg/L	10.00
Nickel	mg/L	10.00
Tin	mg/L	10.00
Zinc	mg/L	10.00
Boron	mg/L	-
Iron	mg/L	50.00
Phenol	mg/L	5.00
Free Chloride	mg/L	-
Sulphide	mg/L	2.00
Oil and Grease	mg/L	100.00

Source: Environmental Quality (Sewerage and Industrial Effluents) Regulations 1978
Regulation 11(5) (b)

APPENDIX III

Fixed-bed biosorption and elution of copper on polyvinyl alcohol-immobilized *Sargassum* seaweed biomass

K. F. Tan, K. H. Chu, B. Sen Gupta, M. A. Hashim*

*Author for correspondence.
Fax: +60-3-7568940 Email: h1mohali@umcsd.um.edu.my

K. F. Tan, M. A. Hashim
Institute of Postgraduate Studies and Research
University of Malaya
50603 Kuala Lumpur, Malaysia

K. H. Chu
School of Engineering and Science
Swinburne University of Technology
P.O. Box 218, Hawthorn
Victoria 3122, Australia

B. Sen Gupta
School of Chemical Engineering
Queen's University of Belfast
5AG Belfast, Northern Ireland, U.K.

Running head: Copper biosorption by immobilized *Sargassum* seaweed biomass

Key words: Biosorption; immobilized biomass; seaweed; polyvinyl alcohol; fixed-bed

Abstract

Copper biosorption by inactivated biomass of the brown marine alga *Sargassum baccularia* immobilized in polyvinyl alcohol (PVA) beads was investigated. PVA-immobilized biomass beads were packed in a laboratory-scale fixed-bed column and subjected to three consecutive cycles of copper loading and elution. Bound copper was eluted with solutions containing a range of EDTA concentrations. Up to 100% of bound copper was consistently recovered from immobilized biomass using 4 mM EDTA solution in repeated loading/elution cycles, implying passive binding of copper to the cell wall of the seaweed biomass. The PVA-immobilized biomass beads were shown to be robust and stable with little decrease in the copper uptake capacity under dynamic flow conditions. The excellent reusability of the new biosorbent could lead to the development of a viable metal bioremediation technology.

List of symbols

b	Langmuir constant (l/mg)
C	concentration of copper in solution in column outlet (mg/l)
C_e	equilibrium concentration of copper in solution (mg/l)
C_i	initial concentration of copper in solution (mg/l)
i	cycle number
k	Freundlich constant ([mg copper/g biomass][l/mg]$^{1/n}$)
n	Freundlich constant
q	copper uptake (mg copper/g biomass)
q_m	Langmuir maximum uptake capacity (mg copper/g biomass)
R^2	correlation coefficient
V	volume of solution (l)
W	mass of biomass (g)

Abbreviations

EDTA	ethylenediaminetetraacetic acid
ICP-AES	inductively coupled plasma atomic emission spectrophotometer
ID	internal diameter of column
MW	molecular weight
PVA	polyvinyl alcohol

1

Introduction

Improper treatment of waste streams containing heavy metal ions from mining operations, metal plating and electronic device manufacturing operations leads to the discharge of these ions to the environment, causing serious pollution problems. Since heavy metals are extremely toxic even in trace quantities to plant and animal life, highly efficient treatment methods are needed to remove metal contaminants from waste streams in order to meet increasingly stringent environmental quality standards. Conventional treatment methods such as chemical precipitation and reverse osmosis become inefficient or expensive when the metals are present in trace quantities. The process of sorption appears to be one of the few alternatives available for such situations. Many investigators have studied biosorption of metal contaminants from water using various microbial and plant biomass [1]. Among these materials, marine macro-algae, commonly known as seaweeds, have attracted much attention. Seaweeds are a source of easily available biomass since they proliferate abundantly in the oceans. Inactivated biomass of seaweeds has been shown to possess impressive binding capacities for a range of toxic metal ions [2-18].

Full-scale adsorption processes for wastewater treatment are commonly carried out in continuous flow systems such as fixed-beds. Design and scale-up procedures as well as process equipment for this type of operation are readily available. It is therefore desirable to develop biosorption processes for metal removal based on the fixed-bed configuration. Although seaweed biomass in its native form has been used in fixed-bed studies [19-21], the biomass in general has a tendency to disintegrate and swell, causing operational problems such as clogging and pressure drop fluctuations.

To be of practical use, seaweed biomass can be immobilized on porous solids or chemically cross-linked to increase its stability and mechanical strength. In this context we recently immobilized biomass of the brown seaweed, *Sargassum baccularia*, using polyvinyl alcohol (PVA) as the immobilization matrix [22]. The reusability of the PVA-immobilized biomass beads was investigated by conducting multiple cycles of copper loading and elution in batch systems with hydrochloric acid and EDTA solutions as the eluants.

In this work the biosorption property of PVA-immobilized *Sargassum* biomass was further evaluated using a laboratory-scale fixed-bed column. Experimental results and analyses that quantified the amounts of copper loaded onto and eluted from the immobilized biomass in three consecutive cycles of fixed-bed operations were presented. The ability of solutions containing a range of EDTA concentrations to regenerate copper-laden biomass for reuse was examined.

2

Materials and methods

2.1

Seaweed biomass

Samples of the brown seaweed *Sargassum baccularia* were collected from a beach located on the west coast of Peninsular Malaysia. The biomass was washed thoroughly to remove adhering particles and dried to a constant weight. It was then ground and sieved to a size range of 106-250 μm.

2.2

Biomass immobilization

The immobilization protocol of Chen and Lin [23] was adopted in this work to immobilize *Sargassum* biomass using polyvinyl alcohol (PVA, MW = 72,000) obtained from Fluka, Switzerland. Biomass particles were mixed with an aqueous PVA solution (15% w/v) to produce a mixture with a solid-liquid ratio of approximately 150 g/l. The resulting mixture was transferred to a syringe and pressured to drop into a gently stirred saturated boric acid solution to form spherical beads of approximately 0.35 cm in diameter. The beads were then transferred to a sodium phosphate solution for hardening, followed by washing with deionized water to remove any residual chemicals.

2.3

Batch biosorption

Batch experiments were conducted to evaluate the biosorption equilibrium isotherms of copper. PVA-immobilized biomass beads containing 0.1 g dry weight biomass were suspended in flasks containing copper solutions with concentrations of between 20-150 mg/l prepared by dissolving $Cu(NO_3)_2 \bullet 3H_2O$ (Fluka, Switzerland) in deionized water. Two separate sets of copper solutions with initial pH of 3.0 and 6.0 were prepared. The solutions were incubated for 24 h in a rotary shaker at 200 rpm and 25 °C. Upon equilibrium, samples were taken from each flask and filtered through 0.45 μm membranes. The final concentration of the unbound copper in the supernatants was determined with an inductively coupled plasma atomic emission spectrophotometer (ICP-AES, Baird ICP 2000, USA). Copper loading on the biomass, expressed as mg copper/g dry biomass, was calculated from a mass balance:

$$q = \frac{V}{W}(C_i - C_e) \qquad (1)$$

The Langmuir (Eq. (2)) and Freundlich (Eq. (3)) isotherm models are by far the most common expressions used to describe equilibrium data on q vs. C_e:

$$q = \frac{q_m b C_e}{1 + bC_e} \qquad (2)$$

$$q = kC_e^{1/n} \qquad (3)$$

A non-linear regression procedure was used to estimate the isotherm parameters, q_m and b in the Langmuir model and k and n in the Freundlich model, from the experimental equilibrium data at pH 3 and pH 6.

Control experiments were also conducted to assess the ability of PVA to remove copper from aqueous solution. Biomass-free PVA beads were subjected to the same batch biosorption experiments described above. The results indicated that copper uptake by biomass-free PVA beads was negligible.

2.4

Fixed-bed biosorption

Immobilized biomass beads were packed in a glass column with an ID of 1.6 cm to a bed height of about 13 cm. The column was maintained at 25 °C which was equipped with an external water jacket for temperature control. A copper solution of 20 mg/l adjusted to pH 6 was pumped through the column at a flow rate of 1 ml/min using a liquid chromatography pump (Perkin Elmer, USA). Samples were collected at the column outlet at fixed time intervals with a fraction collector and analyzed for copper by the ICP-AES. A breakthrough curve was obtained by plotting copper concentration in the column outlet against time. When the beads were fully saturated with copper, the copper solution flow was switched to deionized water to flush out unbound copper from the column. Eluant containing EDTA was then pumped through the column at a flow rate of 1 ml/min to elute the bound copper from the beads. EDTA solutions were prepared by dissolving the disodium salt of ethylenediaminetetraacetic acid obtained from Fluka, Switzerland in deionized water. Samples were collected at the column outlet at fixed time intervals and analyzed for copper by the ICP-AES. An elution curve was obtained by plotting copper concentration in the column outlet against time. At the end of the elution step, deionized water was pumped into the column to displace any remaining EDTA solution which might interfere with the next copper loading. The biomass beads, now free of copper and eluant, were ready for reuse. The above experimental procedures constituted one cycle of copper loading and elution. EDTA solutions of 2, 4 and 6 mM were used as the eluant. Three consecutive cycles of copper loading and stripping were conducted with each of the EDTA solutions, yielding a total of nine fixed-bed experiments.

In each cycle the amount of copper loaded onto immobilized biomass upon complete saturation of the fixed-bed and the amount of copper eluted from metal-laden biomass were estimated by evaluating the areas associated with the respective breakthrough and elution curves. These values were used to calculate the copper reloading and elution efficiencies of multiple cycles in a fixed-bed column as defined in Eqs. (4) and (5):

$$\text{Reloading efficiency} = \frac{\text{Amount of metal loaded in Cycle } (1+i)}{\text{Amount of metal loaded in Cycle } 1} \times 100\% \qquad (4)$$

$$\text{Elution efficiency} = \frac{\text{Amount of metal eluted in Cycle } i}{\text{Amount of metal loaded in Cycle } i} \times 100\% \qquad (5)$$

Equation (4) can be used to assess the reusability of an adsorbent by comparing metal uptake in subsequent cycles to metal uptake by fresh adsorbent in the first cycle while Eq. (5) assesses the metal recovery efficiency of an eluant within a loading/elution cycle.

3

Results and discussion

3.1

Batch biosorption

In order to describe the equilibrium of copper biosorption on immobilized biomass beads accurately, it is important to allow sufficient contact time for batch systems to reach equilibrium due to the possible presence of mass transfer limitations within porous beads. Dynamic studies showed that copper uptake reached 90% of the final equilibrium position in about 10 h (data not shown). A contact time of 24 h employed for all the batch biosorption studies reported here was thus more than sufficient to allow the biosorption step to reach equilibrium. The equilibrium data measured at pH 3 and 6 are shown as symbols in Fig. 1. Copper uptake at pH 6 was relatively higher than that at pH 3 over the solution concentration range examined in this study. The binding of metal ions to inactivated seaweed biomass is a passive process and occurs by ion exchange processes involving a finite number of binding sites on the cell wall of the biomass.

Lower copper uptake at acidic pH values may be attributed to competition between Cu^{2+} and protons for the same binding sites on the immobilized biomass.

The equilibrium data shown in Fig. 1 were fitted to the Langmuir and Freundlich isotherm equations as defined in Eqs. (2) and (3) by a non-linear regression analysis. Regressed values of the isotherm parameters and correlation coefficient R^2 are listed in Table 1. The best fit isotherms predicted by these estimates were plotted in lines in Fig. 1. According to the Langmuir model, the maximum uptake capacity of the immobilized biomass for copper at pH 6 is about 40.3 mg/g dry biomass which is bigger than that at pH 3. The R^2 values, a measure of the degree of fitness, indicate that the Freundlich model exhibited a better fit to the equilibrium data than the Langmuir model especially at pH 6.

Models providing 'first principle' description of the mechanisms underlying metal biosorption by seaweed biomass including ion exchange, electrostatic interactions and surface complexation phenomena have recently been developed [24-27]. Despite the sound theoretical basis of these models, their practical applicability is limited because extensive work is required for model calibration. By contrast, simple phenomenological models such as the Langmuir and Freundlich models have been used in numerous studies to quantify and predict metal binding to seaweed biomass [8,11,12,17]. Due to their mathematical simplicity, these phenomenological models can be easily incorporated in a larger system model such as a continuous flow fixed-bed process model. It is however important to note that the assumptions associated with the conceptual developments of the Langmuir and Freundlich models are rarely satisfied in metal-biomass systems.

3.2

Fixed-bed biosorption

A copper solution of 20 mg/l adjusted to pH 6 was pumped through a fixed-bed column at a flow rate of 1 ml/min to load copper onto PVA-immobilized biomass under dynamic flow conditions. The performance of a fixed-bed biosorption column is best depicted in terms of the so-called breakthrough curve which is simply a plot of copper concentration in the column outlet against time, as shown in Fig. 2. All the breakthrough curves obtained in this work exhibited the typical 'S' shape curve of a fixed-bed column; an initial period of zero or minimal copper at the column outlet followed by a gradual increase in copper concentration that would ultimately reach the initial copper concentration of the feed solution. At this point, the immobilized biomass is considered fully saturated with copper. The amount of copper loaded onto the immobilized biomass upon column saturation can be estimated from the area above the breakthrough curve. In practice, the feed solution flow is terminated when metal concentration in the column outlet reaches discharge limits.

Upon saturation of the biosorption column, deionized water was used to remove unbound copper from the column. The copper-loaded biomass was then eluted by eluants containing EDTA. EDTA, a strong metal chelating agent, has previously been shown to be effective in stripping bound copper from PVA-immobilized biomass in batch systems [22]. The amount of copper eluted can be estimated from the area bound by the elution curve.

To assess the reusability of PVA-immobilized biomass as well as the elution efficiency of EDTA solutions under dynamic flow conditions, three sets of fixed-bed biosorption experiments each comprising three consecutive cycles of copper loading and elution were conducted. For each set of the experiments the same copper loading conditions were maintained but the elution step was varied by using solutions containing 2, 4 and 6 mM EDTA. Typical elution profiles are shown in Fig. 3. Copper concentration in the column outlet increased rapidly after a short period of time to a maximum of about 330 mg/l in the case of elution by 6 mM EDTA solution, followed by a rapid decline in copper concentration that eventually reached zero. Figure 3 shows that the elution process using 4 mM and 6 mM EDTA as the eluant was efficient and capable of producing solutions containing high concentrations of copper. In the case of elution with 2 mM EDTA, a much broader elution curve was observed.

The amount of copper loaded upon saturation of the column and the amount of copper eluted in each cycle estimated from the respective breakthrough and elution curves are shown in Fig. 4. The results in Fig. 4 were used to calculate the reloading and elution efficiencies according to Eqs. (4) and (5) (Table 2).

Table 2 shows that the elution efficiencies for the three sets of experiments ranged from 88% to 96% (2mM EDTA), 98%-100% (4 mM EDTA) and 98%-110% (6 mM EDTA). An elution efficiency greater than 100% (Cycle 3.3) indicates the stripping of bound copper that was not eluted in the preceding cycles. It is clear that almost complete elution of bound copper was readily achieved with 4 mM and 6 mM EDTA solutions.

A slight reduction in the reloading efficiency of the immobilized biomass was observed with 4mM EDTA solution as the eluant (95% reloading efficiency in Cycle

2.3). On the other hand, when 6 mM EDTA solution was used as the eluant, copper uptake by the immobilized biomass in Cycle 3.3 was reduced to 61% of the original copper uptake observed in Cycle 3.1. The relatively large reduction in copper uptake in Cycles 3.2 and 3.3 may be attributed to the adverse effect of EDTA on the biosorption property of the immobilized biomass. The presence of excess EDTA in the biosorption column could have altered the configuration of copper binding sites on the biomass, resulting in a reduced number of binding sites available for copper uptake in Cycles 3.2 and 3.3. For the given biosorption column it is therefore recommended that EDTA solution with a strength of 4mM be used as the eluant in order to ensure complete elution of bound copper from the immobilized biomass and negligible reduction in the copper uptake capacity.

The relatively low reloading efficiency observed in Cycles 1.3 was not due to the adverse effect of EDTA because a low strength EDTA solution of 2 mM was not expected to cause serious damage to copper binding sites on the biomass. The decrease in copper uptake capacity in this case may be attributed to the low desorption efficiency observed in Cycle 1.2. Copper taken up in Cycle 1.2 that was not eluted would reduce the number of binding sites available for copper uptake in Cycle 1.3.

The effluent of the fixed-bed column containing relatively high concentrations of EDTA-copper complexes requires further treatment. In principle it can be treated in electrochemical systems [28,29] to recover the metal in solid form and EDTA for reuse in the elution step of the biosorption column.

4

Conclusions

Non-living biomass of *Sargassum baccularia* immobilized in polyvinyl alcohol beads has been demonstrated as an effective biosorbent for the removal of copper from aqueous solution. The PVA matrix showed negligible uptake of copper. The batch studies showed that copper uptake by PVA-immobilized biomass increased with increasing solution pH. Experimental equilibrium isotherms could be well fitted by the Langmuir and Freundlich models. The fixed-bed studies demonstrated the robustness of the immobilized biomass beads in repeated biosorption/elution cycles. It was established that eluant containing 4mM EDTA could be used to regenerate the immobilized biomass beads resulting in up to 100% copper recovery and that no significant reduction in the copper uptake capacity was observed. The present study showed that elution conditions should be optimized under dynamic flow conditions in order to ensure complete metal elution and negligible decrease in the metal uptake capacity of immobilized biomass packed in fixed-bed columns.

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Table 1. Equilibrium isotherm parameters for copper biosorption on immobilized *Sargassum* biomass at 25 °C.

	pH = 3	pH = 6
Langmuir model		
q_m (mg/g)	31.15	40.31
b (l/mg)	0.28	1.14
R^2	0.9796	0.9480
Freundlich model		
k ([mg/g][l/mg]$^{1/n}$)	13.39	21.10
n	5.19	5.75
R^2	0.9650	0.9926

Table 2. Reloading and elution efficiencies according to Eqs. (4) and (5).

	Reloading efficiency (%)	Elution efficiency (%)
EDTA = 2 mM		
Cycle 1.1	-	96
Cycle 1.2	91	88
Cycle 1.3	74	94
EDTA = 4 mM		
Cycle 2.1	-	99
Cycle 2.2	99	100
Cycle 2.3	95	98
EDTA = 6 mM		
Cycle 3.1	-	98
Cycle 3.2	86	98
Cycle 3.3	61	110

Figure captions

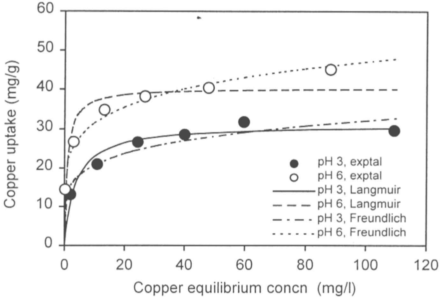
Fig. 1. Copper biosorption equilibrium data on PVA-immobilized *Sargassum* biomass and equilibrium isotherms predicted by the Langmuir and Freundlich models at pH 3 and pH 6.

Fig. 2. A typical breakthrough curve of fixed-bed column packed with PVA-immobilized *Sargassum* biomass. C_i = 20 mg/l, flow rate = 1 ml/min, amount of biomass = 14.4 g, pH = 6 and temperature = 25 °C.

Fig. 3. Elution of copper with eluants containing 2, 4 and 6 mM EDTA. Eluant flow rate = 1 ml/min.

Fig. 4. Amounts of copper loaded and eluted in three cycles of fixed-bed operation. C_i = 20 mg/l, feed/eluant flow rate = 1 ml/min and eluant: (a) = 2 mM EDTA, (b) = 4 mM EDTA, (c) = 6 mM EDTA.

Fig. 1 Tan et al.



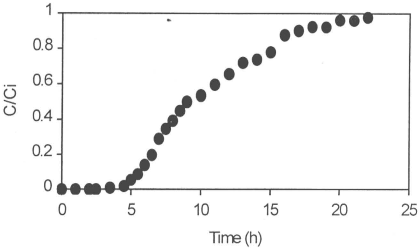

C/Ci
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
Time (h)

Fig. 3 Tan et al.

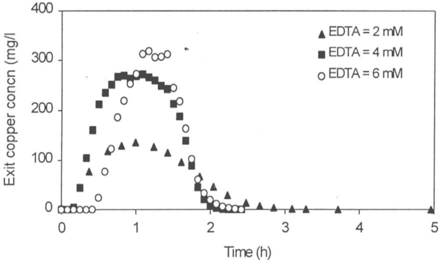
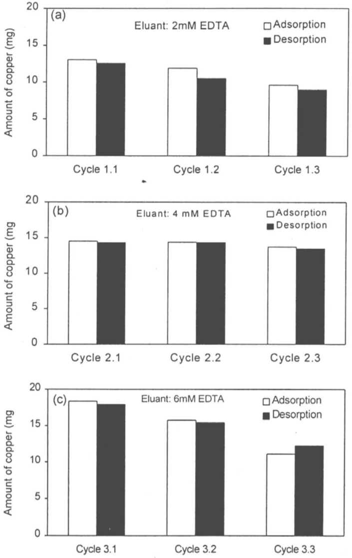


Fig. 4 Tan et al.



APPENDIX IV

BIOSORPTION OF COPPER IONS BY *SARGASSUM* BIOMASS

Tan Kong Fei[1], Chu Kim Hoong[2], Bhaskar Sen Gupta[3], Mohd Ali Hashim[1*]

*Author for correspondence,
Fax: +603-79675319

[1]Department of Chemical Engineering
University of Malaya
50603 Kuala Lumpur, Malaysia

[2]School of Engineering Science and Technology
Swinburne University of Technology
P.O. Box 218, Hawthorn
Victoria 3122, Australia

[3]Bhaskar Sen Gupta
School of Chemical Engineering
Queen's University of Belfast
5AG, Belfast, Northern Ireland, U.K.

Keywords: biosorption, elution, ion exchange, immobilised, PVA

Abstract

Inactivated biomass of *Sargassum baccularia*, immobilised into polyvinyl alcohol (PVA) was studied for its capability to remove and recover copper in a continuous system. Elution of bound copper ions were achieved using EDTA at 2 mM and 4 mM. An almost complete removal of copper ions by EDTA indicates that copper was passively adsorbed by the biosorbent. Optimal elution time was attained after 140 mL of EDTA at 4 mM was pumped into the column at 1 mL/min. However, exposure of the biosorbent to EDTA diminished its performance slightly in subsequent copper uptake. A two parameter mathematical model was employed to predict the breakthrough curve of *S. baccularia* in the fixed bed column. Predicted results were in good agreement with experimental data.

INTRODUCTION

Copper is widely used as an industrial metal especially in the manufacturing of various alloys. It is also used in metal plating and in the production of copper wire. However, copper present in industrial effluents that are discharged prior to treatment may promote a variety of undesirable effects on humans. Accidental ingestion of food or beverages contaminated by copper may result in gastrointestinal disturbances such as diarrhoea and weight loss (Salmon and Wright, 1971) while absorption of excess copper may result in "Wilson's disease" (Volesky, 1990). Although existing technologies are available to address heavy metal pollution, the cost effectiveness of these technologies remains limited (Kratochvil *et al.*, 1997; Aksu *et al.*, 1998; Sar *et al.*, 1999).

Metal remediation by biosorption appears to be one of the few alternatives available for such situations. Of particular interest are micro-organisms, especially marine algae as they proliferate abundantly in the oceans, matures rapidly, is relatively cheap to process and has high metal accumulation capability (Zhou *et al.*, 1998; Figueira *et al.*, 1999; Yang and Volesky, 1999). The feasibility of using inert marine algae as biosorbents also offers several advantages like low operating cost, minimisation of disposable sludge volume, high detoxifying efficiency for very dilute effluents and no nutrient requirement (Kratochvil and Volesky, 1998).

Among the huge diversity of marine algal biomass, brown seaweeds of the genus *Sargassum* species have been studied for their excellent capacity to bind and selectively sequester metals (Hashim *et al.*, 2000; Kratochvil and Volesky, 1998; Kratochvil *et al.*, 1998; Lee and Volesky, 1997; Chu *et al.*, 1997). Accumulation of metals can occur by means of metabolism mediated metal uptake or physico-chemical biosorption. However, on-going investigations into the biosorption properties of *Sargassum* species suggest ion exchange to be the main mechanism responsible for metal uptake (Schiewer, 1999; Fourest and Volesky, 1996).

For a successful application in the remediation of heavy metal wastewater, most biosorbents are immobilised to enhance their mechanical strength and chemical stability. The most convenient configuration is that of a packed column, which offers a number of advantages such as high yield operations, easy scale-up from laboratory scale procedures (Aksu *et al.*, 1998), purification of high volumes of wastewater and easily adapted to automation (Volesky, 1990). In this present work, the biosorptive behaviour of *Sargassum baccularia* immobilised with polyvinyl alcohol (PVA) was studied in a packed bed laboratory scale column. A series of multiple adsorption and elution of copper was conducted to assess the stability of copper biosorption by the immobilised algal biomass. Structural integrity and surface morphology of the immobilised biomass was examined using SEM after successive elution with EDTA. Comparison between the immobilised biosorbent exposed to EDTA at two various concentration was also examined to assess visual damage on the biosorbent apart from a quantitative analysis by performing a mass balance on the amount of copper adsorbed and eluted. The effect of ion exchange was examined with an EDAX instrument.

MATERIALS AND METHODS

Biosorbent

Samples of the brown seaweed *Sargassum baccularia* were collected from a beach located on the west coast of Peninsular Malaysia. The biomass was washed with distilled deionized waster thoroughly to remove adhering particles and dried to a constant weight at 60^0C. It was then ground and sieved to a size range of 106-250 μm.

Biomass immobilisation

The immobilisation protocol of Chen and Lin (1994) was adopted in this work to immobilise *Sargassum* biomass using polyvinyl alcohol (PVA) with a molecular weight of 72,000 obtained from Fluka, Switzerland. Biomass particles were mixed with an aqueous PVA solution (15% w/v) to produce a mixture with a solid-liquid ratio of approximately 150 g/L. The resulting mixture was transferred to a syringe and pressured to drop into a gently stirred saturated boric acid solution to form spherical beads of approximately 0.35 cm in diameter. The beads were then cured in 1.0 M of sodium phosphate solution, followed by washing with deionized water to remove any residual chemicals.

Fixed bed operation

Immobilised biomass beads were packed in a glass column with an ID of 1.6 cm to a bed height of about 13 cm. A copper solution of 20 mg/L was pumped through the column at a flow rate of 1 mL/min using a liquid chromatography pump (Perkin

Elmer, USA). Samples were collected at the column outlet at fixed time intervals with a fraction collector and analysed for copper by the ICP-AES. When the beads were fully saturated with copper, copper solution flow was switched to deionized water to remove unbound copper ions from the column. Eluant containing EDTA at 2 M and 4 mM was then pumped through the column at a flow rate of 1 mL/min to elute bound copper ions from the beads. EDTA solutions were prepared by dissolving the disodium salt of ethylenediaminetetraacetic acid obtained from Fluka, Switzerland in deionized water. Samples were collected at the column outlet at fixed time intervals and analysed for copper by the ICP-AES. At the completion of the elution step, residual EDTA solution inside the column was displaced by pumping deionized water into the column. A total of three consecutive cycles was conducted for each EDTA concentration, yielding six fixed bed data.

SEM and EDAX

Samples of the immobilised *Sargassum baccularia* beads were taken from the top of the column at the completion of each multiple cycles of sorption and elution experiments. The immobilised beads were then freeze dried prior to instrumental analysis. The structure of the immobilised bead, both before and after exposure to loading and elution solutions was examined under a scanning electron microscope (SEM). The samples were sputter coated with gold-palladium alloy for 3 minutes, then viewed, at 10kV and 25 kV of accelerating voltage by a LEICA S 440 scanning electron microscope.

Energy dispersive analysis of X-rays (EDAX) were obtained in order to determine possible ionic exchange between copper ions and other divalent ions present on the immobilised bead by analysing the chemical composition on the sample surface.

EDAX can be considered as quantitative and non destructive technique that allows in situ detection of elements. EDAX spectra of the samples were obtained using a Phillips SL 30 EDAX instrument.

Fixed bed modelling

To model the breakthrough patterns of the immobilised beads in the fixed bed column, a two-parameter model described by Belter *et al.*, (1988) and Brady *et al.*, (1999) was used. The model relates change in effluent concentration to column residence time through two parameters:

1) A characteristic time, t_0
2) A standard deviation, σ

Equation (1) describes the model:

$$\frac{C_e}{C_i} = \frac{1}{2}(1 + erf[\frac{t - t_0}{\sigma t_0 \sqrt{2}}]) \qquad (1)$$

where

C_e = effluent concentration

C_i = influent concentration

erf[x] = error function of x

A breakthrough curve can be obtained by calculating t_0 and a representative value of σ. The characteristic time, t_0 is equivalent to the time when the effluent concentration is half the influent concentration. Thus, the standard deviation, σ is computed by fitting Equa. (1) to the experimental time concentration data.

RESULTS AND DISCUSSION

Fixed bed column studies

Biosorption of copper ions by the immobilised algal biomass is very much influence by its solution pH. From batch studies (data not shown), copper uptake by *S. baccularia* increased when pH of the biosorption medium was increased from 3 to 6. When solution pH was adjusted to pH 7, precipitation was observed. Temperature was kept constant at 25^{0}C. Fixed bed column studies were performed under these optimum conditions.

The performance of the immobilised algal biomass in the fixed bed column is best summarised by the resulting breakthrough curves shown in Figs. 1 and 3. Both figures shows copper biosorption curves for cycles 1 to 3. The curves exhibited a characteristic S shape, which is typical for adsorption process in fixed bed operations. For Fig. 1, breakthrough occurred after approximately 250 mL of copper solution was pumped into the column whereas for Fig. 3, breakthrough began when 300 mL of copper solution had passed into the column. Breakthrough was essentially completed when 2.0 L of solution had passed into the column for both figures. From Fig. 1 and Fig. 3, there was observable difference in the breakthrough curves for cycles 2 and 3. Each shifted towards the left, resulting in earlier breakthrough and saturation. This indicates that the performance of the biosorption column may have been affected when the immobilised algal biomass was exposed to EDTA.

A typical elution curve when 2 mM EDTA was employed as the desorbing agent is depicted in Fig. 2. Copper concentration from the column outlet in Cycle 3 increased rapidly in a brief interval to maximum value of 140 mg/L. For cycles 1 and 2, the respective copper concentration were 119 mg/L and 125 mg/L. Elution for all three cycles was completed when approximately 250 mL of desorbing solution had passed into the column. When 4 mM EDTA was used to strip bound copper ions, elution was essentially completed when 140 mL desorbing solution had passed into the column. This is shown in Fig. 4. Duration was shortened by as much as 90 minutes, corresponding to an improvement of 78.6 % when compared to 2 mM EDTA. A copper mass balance was also performed for the multiple cycles of adsorption and elution studies. Results are expressed as adsorption and elution efficiency (Hashim *et al.*, 2000; Chu *et al.*, 1997), with data presented in Table 1.

Comparison between copper uptake and copper eluted in Table 1 indicates that elution efficiency ranged from 88 % to 96 % with 2 mM EDTA and 98% to 100% when 4 mM EDTA was used. It is obvious that an almost complete elution was readily attainable with 4 mM EDTA. The effective elution of copper ions from immobilised *S. baccularia* suggests that copper ions were physicochemically sequestered on its surface. However, subsequent cycles of copper uptake showed a slight reduction after exposure to both EDTA concentrations. According to Chu *et al.*, (1997), the apparent reduction in copper uptake when EDTA was used may be attributed to the dissolution of biomass components containing copper binding sites.

SEM and EDAX analysis

Figure 5 shows a typical SEM micrograph of a virgin immobilised algal biomass at x1000 magnification. The presence of macropore and micropore on the surface of the immobilised algal biomass indicates a very porous structure, which is very important for mass transfer. An SEM micrograph of the biosorbent after copper uptake is presented in Fig. 6, which revealed the presence of a precipitate on the biomass surface. Figures 7 and 8 shows the surface structure of the immobilised algal biomass after three continuous cycles of adsorption and elution with EDTA at 2 mM and 4 mM respectively. Visual analysis with SEM indicates that the external morphology of the biosorbent remained relatively unchanged.

Figure 9 presents a typical EDAX spectra for immobilised *S. baccularia* before copper uptake. Corresponding to peaks at 1.3 KeV and 3.7 KeV are magnesium and calcium ions. Both ions play a very important role in the normal biological function of typical algae. According to Huber *et al.*, (1990), magnesium is required for enolase activity and in phosphate transfer enzymes while calcium is an important constituent of cell membranes and cell walls (Suhasini *et al.*, 1999). In Fig. 10, EDAX spectra for immobilised *S. baccularia* after copper uptake exhibited predominant copper peaks at 0.98 KeV and 8.1 KeV. The distinctive magnesium and calcium peaks in Fig. 9 are absent in Fig. 10. This suggests a strong possibility that ion exchange had occurred and both magnesium and calcium ions had been replaced by copper.

Fixed bed modelling

For fixed bed modelling, Brady *et al.*, (1999) and Brady and Tobin (1995) represented t_0 and σ with constants described in Equations (2) and (3):

$$t_0 = \frac{k_1}{Q} \tag{2}$$

$$\sigma^2 = \frac{k_2 Q}{L} \tag{3}$$

where

Q = column flow-rate (mL/min)

L = column length (m)

k_1 & k_2 = constants

According to Equa. (2) and Equa. (3), a linear relationship exists whereby the constants k_1 and k_2 can be obtained. Constant k_1 was obtained from a series of breakthrough curves operaed at constant feed concentration and bed depth while varying the flow-rate. Constant k_2 was obtained by minimising the objective function, F which represents the sum of squares of the relative deviations between the model and experimental data for different values of sigma, σ:

$$\text{Minimise F} = \sum_{n=1}^{i} (X_e - X_m)^2 \qquad (4)$$

Once the minimised value of σ is obtained, constant k_2 can be evaluated with Equa. (3).

Figures 11 (a) and (b) are two parameter plots for immobilised *S. baccularia* in a fixed bed column. Bed depth of the column was maintained at 13 cm with 20 mg/L of copper solution as feed concentration. Flow-rates were in the range of 1 mL/min to 2 mL/min. Correlation coefficient, R^2, is a measure of the degree of fitness.

Theoretical breakthrough curves generated by the model are compared with corresponding experimental curves under operating conditions both within and outside of calibration. Figure 12 show breakthrough curves at different bed depths while Fig. 13 depicts breakthrough curves at different feed concentrations. In both experiments, the performance of the model within and beyond calibration range is undisputed. Good agreement between theoretical and experimental data indicates that the two parameter model can be employed for scale up and design of fixed bed reactor within its limitations.

CONCLUSION

Removal and recovery of copper in a fixed bed column was readily achieved by immobilised *S. baccularia*. Visual analysis with SEM indicates that polyvinyl alcohol as the immobilisation matrix is chemically stable. No structural and morphological changes on the surface of the immobilised biosorbent were observed after three multiple cycles of adsorption and desorption studies. However, mass balance performed on copper uptake and copper eluted from the column indicates that exposure to EDTA, a strong chelating agent, may have damage the functional groups responsible for copper binding. Analysis with EDAX suggest that calcium and magnesium may have been exchanged with copper during biosorption. The mechanism of copper biosorption by immobilised *S. baccularia* is probably a combination of passive adsorption and ion exchange. Column breakthrough curves predicted by the two parameter model is in good agreement with experimental data. Clearly, the two parameter has potential to be used as a simplified model for scale up and design of fixed bed reactors.