CHAPTER FIVE

REUSABILITY OF ALGAL BIOMASS IN A FIXEB BED REACTOR

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The immobilized biomass of *Sargassum baccularia* has been shown to have high metal uptake properties and thus can be considered as a potential biosorbent. Chapter 3 detailed the kinetic and equilibrium data necessary for mathematical modelling to predict the breakthrough curve for the biomass in a fixed bed column. For practical application, a detailed investigation on the reusability of the biomass is important.

Previous work by Tan (1999) on the reusability of *Sargassum* biomass has focussed on batch application using EDTA and HCl as desorbing agents. Although freely suspended biomass may have better contact with the adsorbates during the biosorption process, the biomass suspension is normally not the practical form for direct use in the process. The biomass is best immobilized to enhance its stability, mechanical strength, the ease of handling and reusability in terms of separation of the biosorbent from the treated influent. However, in a batch configuration biomass deterioration may occur due to agitation. Accordingly, the continuous configuration offer greater metal binding capacity and higher efficiency and are more readily adapted to automation than batch

with immobilized biomass are therefore best designed frequently designed for fixed bed reactor (Chang *et al.*, 1998).

Although the effectiveness of *Sargassum baccularia* as a biosorbent for heavy metal removal is well established, commercial acceptance of a treatment process based on biosorption will largely depend on its ability to compete with existing technologies on a cost basis. Since the reusability of *Sargassum baccularia* in a batch configuration has been studied and results has indicated the potential to be reused in multiple cycles of adsorption-desorption processes, the scope of the present work encompasses the reusability of the biomass in a fixed bed column. The dynamics of biosorption in a fixed bed column was conducted to determine column breakthrough time, column saturation time, and adsorption and desorption efficiency. The mathematical model elucidated in Chapter 4 was also applied to predict breakthrough curves for the immobilized biomass in multiple cycles of treatment. EDTA was used as the desorbing agent to strip bound copper ions because cell damage was minimal in comparison to HCl (Chu *et al.*, 1997; Tan, 1999). The present work will also concentrate in optimizing the concentration of EDTA to remove copper ions based on elution time from the column and subsequent adsorption-desorption efficiency.

5.1 Fixed-Bed Biosorption Dynamics

The characteristics as well as effectiveness of the fixed-bed biosorption were evaluated with the breakthrough time (t_b), adsorption efficiency (a_e), desorption efficiency (d_e), total adsorption efficiency (Q_T) and length of unused bed (LUB). All these parameters can be determined from the adsorption and desorption curves of the immobilized biomass of *Sargassum baccularia.*

For all these parameters, t_b can be defined as the time span during which the effluent concentration of copper was under 10% of copper concentration in the feed. The Q_T value is the ratio of total amount of copper adsorbed at the time of saturation versus total amount of copper flow in for the same time interval. The LUB value, frequently used for scale up estimation, represents the fraction of the bed which is not utilized at the breakthrough time.

According to Ruthven's definition;

$$LUB = L(1 - \frac{t_b}{t^*}) \qquad (5.1)$$

where

LUB = Length of Unused Bed (cm)

L = Length of column (cm)

t_b = Breakthrough time (h)

t* = Time when effluent is 50% of feed concentration (min)

For more efficient mass transfer or less mass transfer resistance, the value of t_b is closer to t*, resulting in a smaller value for LUB. The value of LUB depends very much on

mass transfer rate, flow rate and the shape of the equilibrium curve (Chang *et al.* 1998) but is assumed to be independent of total bed length (Ruthven, 1984). Hence, a large scale unit can also be designed according to the LUB value obtained from small scale laboratory tests for the same superficial velocity and particle size (Chang *et al.* 1998) as well as to determine how multiple cycles of treatment may affect the mass transfer rate simply by analyzing the values of t_b, t^*, a_e, d_e, Q_T and LUB.

The values of Q_T, a_e and d_e can be obtained by integrating the curve under the adsorption and desorption curves in Figure 5.1 and Figure 5.2 respectively. These symbols can be represented as:

Q_T = Total adsorption efficiency (%)

$$\frac{\text{Amount of copper adsorbed (mg)} \times 100\%}{\text{Amount of copper in feed (mg)}} \quad (5.2)$$

a_e = Adsorption efficiency (%)

$$\frac{\text{Amount of copper adsorbed in higher cycles (mg)} \times 100\%}{\text{Amount of copper adsorbed in first cycle (mg)}} \quad (5.3)$$

d_e = Desorption efficiency (%)

$$\frac{\text{Amount of copper desorbed in one cycle (mg)} \times 100\%}{\text{Amount of copper adsorbed in one cycle (mg)}} \quad (5.4)$$

Integration involves solving the general problem of

$$I = \int_a^b f(x)dx \quad (5.5)$$

The problem can be visualized as determining the area under the curve in Figures 5.1 and 5.2 which is defined by a function. A simple numerical approach consists of approximating the area as a series of trapezoids. Thus the sum of the trapezoids represents an estimate of the area,

$$I \cong (x_1 - x_0)\frac{f(x_0)+f(x_1)}{2} + (x_2 - x_1)\frac{f(x_1)+f(x_2)}{2} + \cdots + (x_n - x_{n-1})\frac{f(x_{n-1})-f(x_n)}{2} \qquad (5.6)$$

Two other widely used formulas to evaluate equally spaced points are Simpson's 1/3 rule and Simpson's 3/8 rule (Chapra, 1998),

$$I \cong (x_2 - x_0)\frac{f(x_0)+4f(x_1)+f(x_2)}{6} \qquad (5.7)$$

$$I \cong (x_3 - x_0)\frac{f(x_0)+3f(x_1)+3f(x_2)+f(x_3)}{8} \qquad (5.8)$$

To evaluate the amount of copper adsorbed or desorbed from the respective curves in Figures 5.1 and 5.2, Equations 5.6 and 5.7 were used.

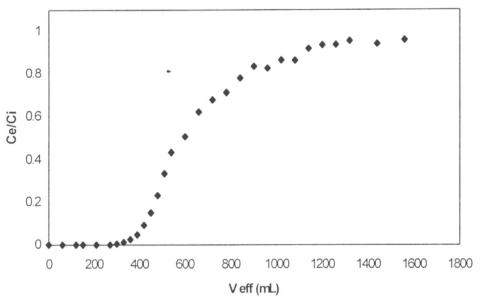

Figure 5.1: Typical breakthrough curve for PVA immobilized biomass of *S. baccularia* in a fixed bed column. Area under the curve multiply with Ce/Ci represents copper that is unadsorbed.

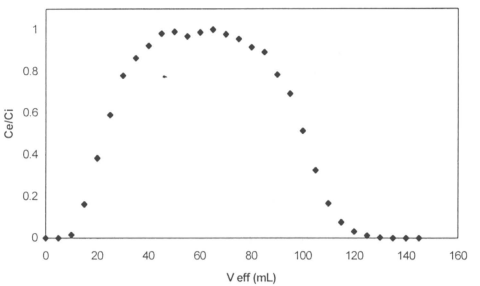

Figure 5.2: Typical elution curve for PVA immobilized biomass of *S. baccularia* in a fixed bed column. Area under the curve multiply with Ce/Ci represents copper that is eluted.

5.2 Materials and Methods

5.2.1 Materials

Algal biomass of *Sargassum baccularia* that were harvested, stored and processed as described in Sections 3.3.1 to 3.3.2 were employed for the multiple adsorption-desorption experiments. Analytical grade reagents were used in the experiments (Fluka, Switzerland). The pH of the solutions was measured and adjusted by using a calibrated pH meter (Metrohm, Switzerland).

A chromatographic column with an I.D. of 1.5 cm and column length of 20 cm together with a variable plunger (Pharmacia) was used for this study. Copper and EDTA solutions were pumped through the column by using a LC-6A liquid chromatographic pump (Shidmazu, Japan) with a programmable flow rate. The effluent samples were collected at the column outlet through a programmable fraction collector (Model FC 204, Gilson).

Copper obtained from the fraction collector were analyzed by Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES) using a Baird ICP 2000 (Baird, Switzerland) instrument. Copper standard solutions were prepared by using ICP reagent grade obtained from Fluka (Switzerland).

5.2.2 Methods

Multiple adsorption-desorption studies were conducted in a continuous configuration as depicted in Plate 5.1. The experiments were conducted at a constant room temperature of 25^0C. The chromatographic column was packed with the biomass beads to a bed

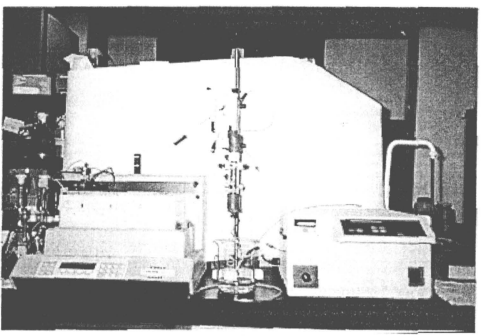

Plate 5.1: Laboratory set up for fixed bed column

depth of 13 cm and a probe was inserted into the column to agitate the packed bed so as to ensure that no air bubbles were trapped. The variable plunger was then slowly inserted into the column. Before the experiments were conducted, the chromatographic column and the liquid pump were purged off trapped air using a special syringe. This was to ensure that was no internal circulation of fluid in the column because this may upset the experiments.

For adsorption studies, feed solution containing copper at 20 mg/L was prepared and pumped through the fixed bed at a constant flow rate of 1.0 mL/min. This was to allow equilibrium to be attained and diffusion of the solute into the micro-porous structure of the PVA-biomass biosorbent. The effluent samples for ICP analysis were collected periodically in special test tubes using the Gilson programmable fraction collector.

When the fixed bed was saturated due to the continuous loading of copper solutions, the column was rinsed by passing approximately 500 mL of distilled water through the bed at a flow rate of 5 mL/min. The desorption studies were conducted by eluting the column with EDTA at concentrations of 2, 4 and 6 mM and at a constant flow rate similar to the above. The effluent samples from the desorbing solution were similarly collected using the Gilson programmable fraction collector and suitability diluted prior to the ICP analysis. After completion of the desorption experiments, the column was again rinsed with distilled water. The biomass beads inside the packed bed were taken out and replaced by fresh biomass before another set of experiments.

5.3 Results and Discussion

5.3.1 Multiple Adsorption/Desorption (A/D) Studies

As mentioned above, for multiple adsorption and desorption (A/D) studies, bed depth of 13 cm with a flow rate of 1 mL/min was used. Regeneration was accomplished by eluting the saturated column with EDTA at three different concentrations for a total of three cycles. Treated biomass was completely replaced by fresh biomass beads that were packed into the column for subsequent treatments. The breakthrough curves are depicted in Figures 5.3 to 5.5. All treatments with EDTA resulted in a reduction of the breakthrough time with subsequent treatments. Tables 5.1 to 5.3 summarize the effect of EDTA on the dynamic properties of the immobilized biomass in a fixed bed reactor.

Table 5.1: The effect of A/D cycles on Cu adsorption using EDTA at 2 mM

Cycle	t_b (hr)	Q_T (%)	LUB
1.1	3.99	35.1	5.78
1.2	3.35	31.9	5.91
1.3	3.03	25.9	5.74

With 2mM EDTA as the desorbing agent, breakthrough time was reduced from approximately 4 hours in Cycle 1.1 to 3 hours in Cycle 1.3, a drop of 24%. The LUB values in Table 5.1 remained essentially unchanged at 5 cm, indicating that EDTA at 2 mM did not alter mass transfer characteristics although total adsorption efficiency was reduced by 9.2% from 35.1% in Cycle 1.1 to 25.9% in Cycle 1.3 which is considered negligible.

Desorption with EDTA at 4 mM as well as 6 mM also resulted in the reduction of breakthrough time with subsequent treatments (Tables 5.2 and 5.3). However, breakthrough time using EDTA at 6 mM resulted in a drop of almost 30.3% in comparison to 24% by EDTA 2 mM and 19% by EDTA at 4 mM. From Table 5.2, Q_T values remained approximately 30% with LUB values of 4 cm, indicating that the mass the transfer properties remained unchanged with EDTA 4 mM. However, at 6 mM, the values of LUB and Q_T were affected (Table 5.5). It is possible that at higher concentrations, EDTA may affect the mass transfer properties of the immobilized biomass of *Sargassum baccularia*.

Table 5.2: The effect of A/D cycles on Cu adsorption using EDTA at 4 mM

Cycle	t_b (hr)	Q_T (%)	LUB
2.1	7.05	31.5	3.89
2.2	6.25	31.2	4.04
2.3	5.74	29.8	4.76

Table 5.3: The effect of A/D cycles on Cu adsorption using EDTA at 6 mM

Cycle	t_b (hr)	Q_T (%)	LUB
3.1	8.95	56.9	4.98
3.2	8.52	48.9	3.31
3.3	6.24	34.6	2.90

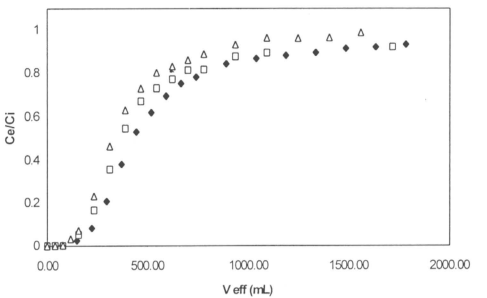

Figure 5.3: Adsorption profiles for PVA immobilized biomass of *S. baccularia* with EDTA at 2 mM. (♦, Cycle 1.1; □, Cycle 1.2; △, Cycle 1.3)

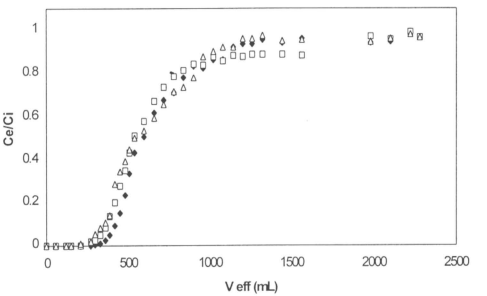

Figure 5.4: Adsorption profiles for PVA immobilized biomass of *S. baccularia* with EDTA at 4 mM. (♦, Cycle 2.1; □, Cycle 2.2; △, Cycle 2.3)

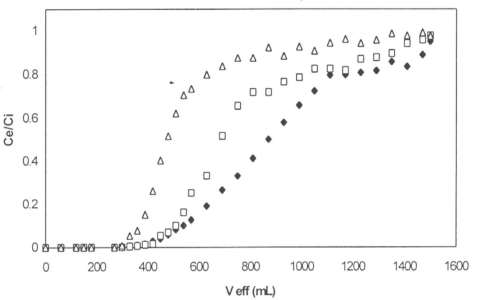

Figure 5.5: Adsorption profiles for PVA immobilized biomass of *S. baccularia* with EDTA at 6 mM. (♦, Cycle 3.1; □, Cycle 3.2; △, Cycle 3.3)

5.3.2 Adsorption and Desorption Efficiencies

The adsorption and desorption efficiency for the PVA immobilized biomass of *Sargassum baccularia* in a fixed bed column was investigated using EDTA as the main desorbent. The optimum concentration of EDTA was tested in the range of 2,4 and 6 mM by eluting the column with EDTA once it is saturated with copper ions. Figures 5.6 to 5.8 depict the desorption curves using EDTA at three different strengths.

According to Chu *et al.* (1997) and Tan (1999), for a desorbent to be considered efficient, it has to fulfill two significant requirements:

1) Complete desorption in each cycle
2) Metal uptake capacity of the adsorbent remains unchanged in successive cycles

Adsorption efficiency a_e as defined in Equation 5.3 allows one to assess the reusability of an adsorbent by comparing metal uptake in subsequent cycles to metal uptake by the virgin adsorbent in the first cycle. Equation 5.4 that describes desorption efficiency d_e can be used as a parameter to determine whether a desorbent is able to meet the first requirement.

Figures 5.9 to 5.11 shows the experimental results obtained from three consecutive cycles of copper adsorption-desorption using EDTA at 2,4 and 6 mM. The open bars depict the amount of copper adsorbed while the solid bar the amount of copper desorbed.

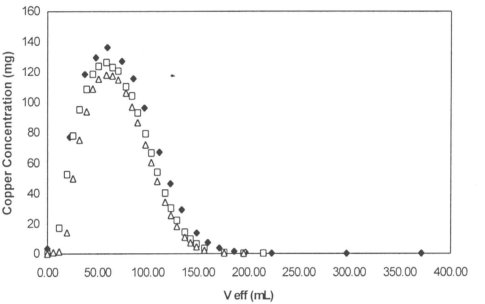

Figure 5.6: Desorption profiles for PVA immobilized biomass of *S. baccularia* with EDTA at 2 mM. (♦, Cycle 1.1; □, Cycle 1.2; △, Cycle 1.3)

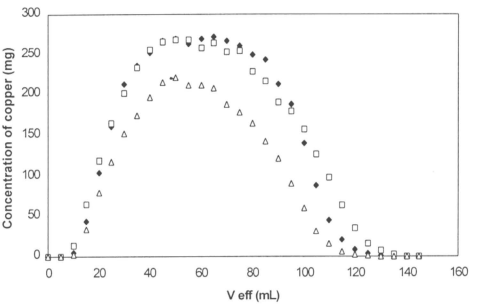

Figure 5.7: Desorption profiles for PVA immobilized biomass of *S. baccularia* with EDTA at 4 mM. (♦, Cycle 2.1; □, Cycle 2.2; △, Cycle 2.3)

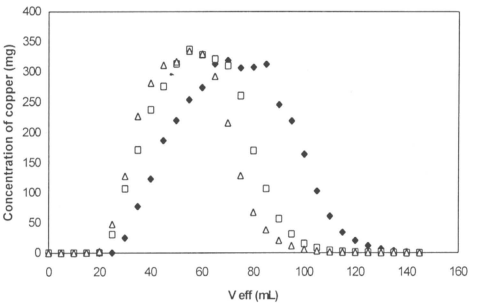

Figure 5.8: **Desorption profiles for PVA immobilized biomass of *S. baccularia* with EDTA at 6 mM. (♦, Cycle 3.1; □, Cycle 3.2; △, Cycle 3.3)**

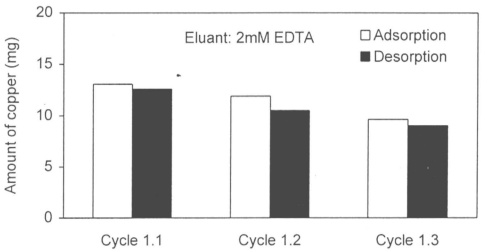

Figure 5.9: **Three consecutive cycles of copper adsorption-desorption using EDTA at 2 mM as desorbing agent (□, amount adsorbed; ■, amount desorbed)**

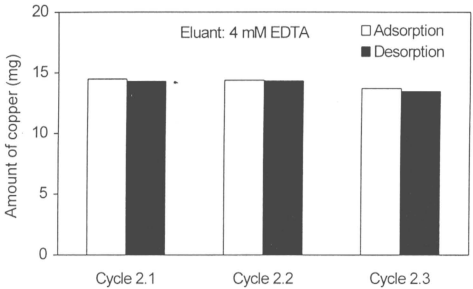

Figure 5.10: **Three consecutive cycles of copper adsorption-desorption using EDTA at 4 mM as desorbing agent (□, amount adsorbed; ■, amount desorbed)**

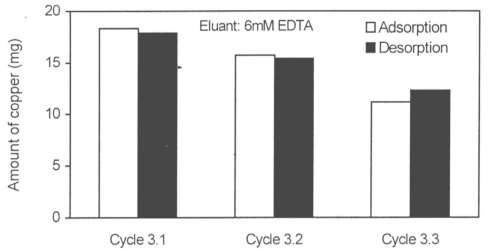

Figure 5.11: **Three consecutive cycles of copper adsorption-desorption using EDTA at 6 mM as desorbing agent (□, amount adsorbed; ■, amount desorbed)**

Biosorption of copper ions by immobilized biomass of *S. baccularia* is actually adsorption by complexation with negatively charged groups of cell wall where the biomass can be regarded as a simple biosorbent. An almost complete elution by EDTA (Table 5.4) suggests that the bioaccumulation of copper ions is by a passive surface adsorption and not metabolism mediated intracellular uptake. This phenomenon enables the surface bound copper to be extractable by EDTA treatment, thus restoring the binding sites and making the biosorbent available for further biosorption processes.

Table 5.4: Adsorption and desorption efficiency. Initial copper concentration of 20 mg/L at pH 6, column length 13 cm and flow-rate at 1 mL/min

	Adsorption efficiency (%)	Desorption 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

Table 5.4 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.

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. In Figure 5.14, the elution curves for three different EDTA concentration is compared.

EDTA at 6 mM exhibited the shortest elution time of 120 minutes. The difference between EDTA at 4 mM and 2 mM in comparison to 6 mM are approximately 5 and 251 minutes. This suggest that EDTA at 6 mM did not significantly improve elution time when compared to EDTA at 4 mM. Nevertheless, exposure of the immobilized biomass of *S. baccularia* to EDTA at three different concentrations resulted in a decrease in copper uptake with subsequent cycles.

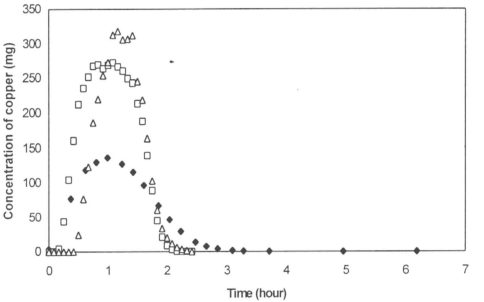

Figure 5.12: **Elution curves for three different EDTA strengths. (♦, [EDTA] = 2 mM; □, [EDTA] = 4 mM; △, [EDTA] = 6 mM)**

5.4 SEM and EDAX Analysis

5.4.1 SEM

Scanning Electron Microscope (SEM) was used to study the texture, topography and surface features of the immobilized biomass of *S. baccularia*. The magnification of SEM covers the range of 0.1 μm to 1 μm. Due to SEM's high spatial resolution and high depth of field, it is an ideal tool to examine and interpret microstructure of the immobilized biomass.

5.4.2 SEM Specimen Preparation

For use in SEM, the immobilized biomass was cleaned, degreased and coated with insulating materials. The immobilized biomass was cleaned to remove grease and hydrocarbon. Ultrasonic cleaning in acetone, trichloromethane, toluene and alcohol were also utilized.

Specimen coating was carried out to insulate or semi-insulate the samples. This is necessary because exposure to electron beam may result in charge build-up and this will result in diverse variation in samples. In this present work, the immobilized biomass was sputter coated with gold-palladium alloy which forms a thinner and more continuous film. It is also less granular and will reduce electron interference. Other coating materials are also available. They are:

1) gold, which is expensive and highly grainy, requires thick film for continuity;
2) silver, which has high grain size that result in high conductivity and
3) aluminium, which is cheaper but tends to oxidize.

Using SEM analysis, the surface structure of the PVA immobilized bead of *S. baccularia* was analyzed. Plates 5.2 and 5.3 show the microstructure of the virgin PVA matrix with macro and micropores evident. These indicate that biomass immobilization with PVA as a support matrix is a highly porous structure, enabling the diffusion of copper ions into the inner parts of the PVA matrix and subsequent attachment of copper ions onto the functional groups present on the surface of the biomass. The porous structure of the matrix also increases the surface area available to sequester copper ions. The filamentous structure in Plates 5.4 and 5.5 shows the biomass of *S. baccularia* entrapped onto the PVA matrix.

5.4.3 EDAX

EDAX (Energy Dispersive Analysis of X-ray) analysis was performed using the Phillips SL 30 coupled with a LEICA S440 SEM to analyze the chemical composition of the sample surface. X-ray microanalysis can be considered as a quantitative and non-destructive technique that allows *in situ* detection of elements as low as 10^{-19} g.

Analysis with SEM and EDAX was carried out simultaneously for the immobilized biomass. Samples from the fixed bed column were taken from the upper section of the bed.

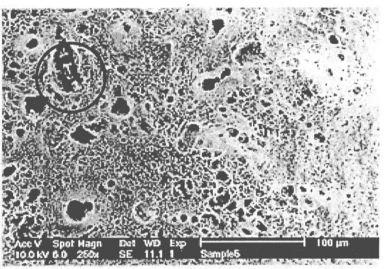

Plate 5.2: SEM photo for PVA immobilized biomass of *S. baccularia*. Circle indicates *macropore* of the PVA matrix. (Magnification: 250x)

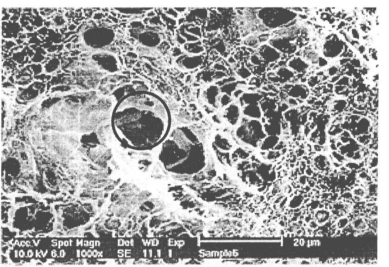

Plate 5.3: SEM photo for PVA immobilized biomass of *S. baccularia*. Circle indicates *micropore* of the PVA matrix. (Magnification: 1000x)

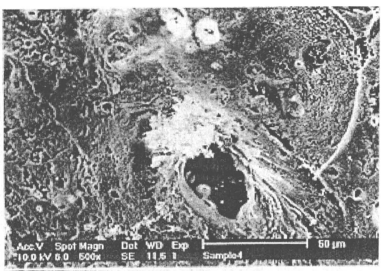

Plate 5.4: **SEM photo showing the filamentous structure of *S. baccularia* biomass entrapped unto the surface PVA matrix. (Magnification: 500x)**

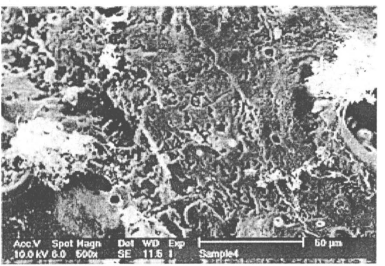

Plate 5.5: **SEM photo showing the unequal distribution of the biomass unto the surface of the PVA matrix. (Magnification: 500x)**

Plate 5.6 shows the chemical constituents that forms the background spectrum for the surface of a virgin PVA immobilized biomass of *S. baccularia*. The letters K, L and M refers to the shells inside an atom where the orbiting electrons are arranged. Electrons in K shell will need the highest energy to be removed from the respective atom as it is closest to the nucleus of the atom, followed by L and M. The letter C denotes carbon, O for oxygen, Mg for magnesium, Al for aluminium, Si stands for silicon, Os means osmium and Au refers to aurum- or gold. Osmium and aurum were used for SEM analysis, hence the presence of the spectrum in Plate 5.6. Since the biomass is biological in nature and polyvinyl alcohol (PVA) was used as the immobilization matrix, this explains the high spectrum peaks for carbon and oxygen. The peak at 1.79 KeV refers to silica which is used to encrusts the algal cell wall (Remacle, 1990).

In Plates 5.7 and 5.8, corresponding to peaks at 1.29 KeV and 3.66 KeV are magnesium and calcium. Plate 5.7 is the spectrum for the virgin PVA immobilized biomass while Plate 5.8 shows the spectrum for the immobilized biomass treated with EDTA and distilled water. Both metallic ions play an important role in the normal biological function of a typical alga. Magnesium is required for enolase activity and in phosphate transfer enzymes (Huber *et al.*, 1990) while calcium is an important constituent of cell membranes and cell walls (Suhasini *et al.*, 1999; Huber *et al.*, 1990).

In Plate 5.9, corresponding to peaks at 0.98 and 8.11 KeV are copper. Plate 5.9 is the spectra for the immobilized biomass treated with copper. The EDAX spectrum for the PVA immobilized biomass of *S. baccularia* before copper uptake exhibits a distinctive magnesium and calcium peak whereas after copper uptake, the copper signal

is predominant with near or complete absence of both magnesium and calcium signals. This indicate a strong possibility that both magnesium and calcium ions may have been exchanged by copper ions from the biosorbent. The release of calcium ions with the uptake of copper ions have also been reported with other biosorbents such as *Ganoderma lucidum* (Muraleedharan *et al.*, 1994) and the uptake of cobalt ions in the alga, *Ascophyllum nodosum* (Kuyucak and Volesky, 1989).

Ion exchange was also reported by Suhasini *et al.*, (1999) when calcium ions were exchanged by cobalt ions using *Rhizopus sp.* as their biosorbent. The amount of calcium was estimated by elution to be around 4 mmoles/g of the biosorbent while the amount of cobalt sorbed was near 4.39 mmoles/g of the biosorbent. This suggests a near stoichiometric exchange of calcium ions by cobalt ions. However, in this present work, no quantitative experiments were conducted to determine the amount of copper ions exchanged by magnesium and calcium ions.

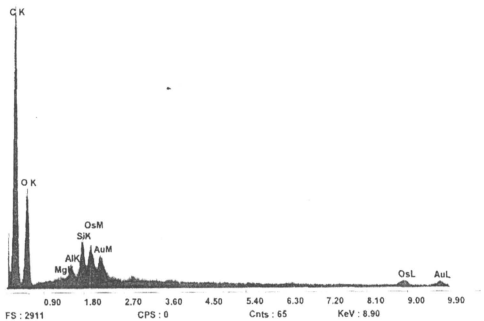

Plate 5.6: EDAX analysis for virgin PVA immobilized biomass of *S. baccularia*.

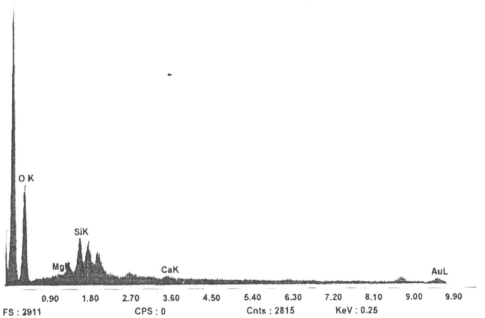

Plate 5.7: EDAX analysis for virgin PVA immobilized biomass of *S. baccularia* with magnesium and calcium peaks.

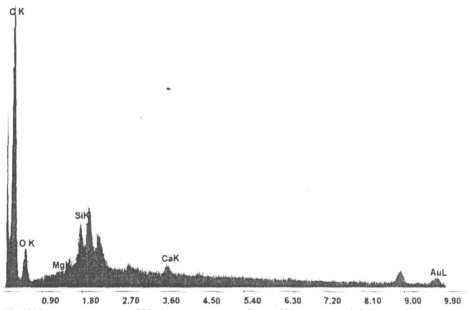

Plate 5.8: EDAX analysis for PVA immobilized biomass of *S. baccularia* treated with EDTA with magnesium and calcium peaks.

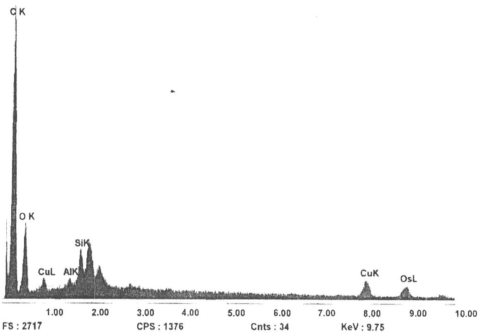

Plate 5.9: EDAX analysis for PVA immobilized biomass of *S. baccularia* loaded with copper solutions showing copper peaks but absence of magnesium and calcium peaks.

Conclusion

The Length of Unused Bed (LUB) method revealed that mass transfer properties was sensitive to EDTA at 6 mM. After 3 cycles of continuous A/D studies, LUB values varied from approximately 5 cm in Cycle 3.1 to 2.9 cm in Cycle 3.3. However, LUB values remained essentially unchanged at 5 and 4 cm for EDTA at 2 and 4 mM respectively.

By varying the concentration of EDTA at 2,4 and 6 mM, the optimum concentration was found to be 4 mM. All three different concentrations successfully removed 90% bound copper ions from the biomass surface for three consecutive cycles of adsorption-desorption studies. An almost complete elution of bound copper ions by EDTA suggests that the mechanism of copper uptake by *S. baccularia* is not by metabolism mediated intracellular uptake, rather by passive surface adsorption. However, EDTA is a strong chelating agent that is capable of damaging and modifying cell walls, hence the drop in adsorption efficiency for the biosorbents in subsequent cycles of adsorption-desorption studies.

Analysis with SEM showed the porous structure of the PVA immobilized biomass of *S. baccularia*. EDAX results suggest that the absence of calcium and magnesium peaks at 1.29 and 3.6 KeV after the biosorbents were loaded with copper maybe due to ionic exchange between copper with calcium and magnesium on the biomass surface.