

**REMOVAL AND RECOVERY OF HEAVY METALS FROM
SYNTHETIC SOLUTIONS AND ELECTROPLATING EFFLUENTS
USING YEAST AND THE WATER FERN *Azolla filiculoides***

THESIS

**Submitted in Fulfilment of the Requirements
for the Degree of**

DOCTOR OF PHILOSOPHY

**in the Department of Biochemistry and Microbiology
Faculty of Science
Rhodes University**

by

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NOVEMBER 1997

ABSTRACT

The aims of the project were twofold. The initial objective of the study, based on previous results, was to develop an economically viable methodology for immobilizing yeast cells for the treatment of heavy metal-laden waste water. The non-viable yeast cross-linked by 13% (w/v) formaldehyde/1N HNO₃ exhibited satisfactory mechanical strength and rigidity in a continuous-flow column operation. No apparent disruption of the biomass after repeated use was observed. The cost of immobilizing 1kg dry yeast pellets was estimated at less than US\$1. Zn uptake capacity of FA-cross-linked pellets, on batch trials, remained similar to that of raw yeast, reflecting that the immobilizing procedure did not hinder its metal removing capacity. In column studies, cation metals were effectively removed by the yeast pellets from aqueous solution at natural pHs, and then recovered completely by washing the pellets *in situ* with 0.1M HCl. The recovered metals were concentrated in such small volumes that recycling or precipitation of them was facilitated. The metal uptake capacity of the regenerated biomass remained constant in comparison with cycle 1, indicating that reuse of the yeast would be possible.

In the case of Cr⁶⁺, a gradual breakthrough curve of Cr in the column profile was noted, with a simultaneous reduction of Cr⁶⁺ to Cr³⁺. However, Cr⁶⁺ in the effluent can be markedly minimised either by accumulation onto the biomass or reduction to its trivalent form. Desorption of bound Cr⁶⁺ with either alkali or salt could not accomplish the regeneration of the biomass. A combination of reduction and desorption with FA/HNO₃ appeared promising in regeneration of the saturated biomass at 4°C.

The metal sorption capacities of the yeast pellets, on a batch or a fixed-bed system are relatively lower than that of documented sorbents. Apparently more of the yeast pellets would be required for treating a certain volume of waste effluent, than with other sorbents. Therefore *Azolla filiculoides* was examined as a suitable sorbent for this purpose. This constitutes the second part of the project.

Azolla filiculoides, a naturally-abundant water fern, was screened for its metal sorption and

recovering capacities, mechanical stability, flow-permeability and reusability. The azolla biomass appeared to have fulfilled the required mechanical criteria during the repeated sorption-desorption column operations. It is water-insoluble and appears flexible under pressure when rinsed with water. These characters are of crucial importance in a continuous-flow system since a column can be operated at high flow rates without apparent compact of the biomass and pressure loss. Therefore, immobilization of the biomass can be avoided.

The sorption isotherm data, obtained from batch removal of Cr^{6+} , showed that the sorption process was effective, endothermic and highly pH dependent. Considerable amounts of Cr^{6+} were accumulated at the optimum pHs of 2-2.5. Column sorption of Cr^{6+} at a low flow rate and pH of 2.5 showed optimum performance with a total Cr uptake of 50.4mg/g at 60% saturation of the biomass. Removal of Cr^{6+} from an electroplating effluent using an azolla column was deemed reasonably satisfactory, although the uptake declined slightly. Desorption of bound Cr^{6+} with various desorbents was incomplete, which resulted in a low regeneration efficiency of about 50%. However, removal and recovery of Cr^{3+} using the azolla column was than that of Cr^{6+} . Desorption of Cr^{3+} from the spent biomass column was accomplished with the recovery of 80% using 0.5N H_2SO_4 . The regeneration efficiencies for Cr^{3+} removal were up to 90% and demonstrated that the biomass is reusable.

Cation metal uptake capacities of azolla, obtained either from batch or column experiments, are reasonably high in comparison with other sorbents. The uptake of Ni or Zn ions from solution is pH dependent showing the optimum pH of around 6 to 6.5, under the current experimental conditions. The sorption kinetics for cation metals was rapid with about 80% of the bound Ni ions being taken up in the first 10 min. The character of rapid binding is extremely important in a column sorption process, especially on a large scale since it favours an optimum uptake of metals at high flow rates. The Ni or Zn uptakes in column sorption were not markedly affected when the flow rates were increased from 80ml/h up to 800ml/h for the 5g biomass used.

The cation heavy metals removed from waste effluents were recovered in a concentrated solution of small volume. The desorption of bound Ni and Zn ions from the saturated biomass

was accomplished with either 0.2N HCl or H₂SO₄ that resulted in recoveries of more than 95%. The metals recovered, in the case of Ni and Zn, are identical to that of plating agents eg. nickel sulphate or chloride, so that recycling of the metals is possible. An effluent-free, closed loop of Ni or Zn treatment system was proposed, whereby the Ni or Zn ions can be recycled to the plating bath whilst the purified water is fed back to the rinse tanks.

Ca and Mg ions, commonly present in the electroplating effluents, appeared to affect sorption of heavy metals by azolla when metal concentrations were relatively low, presumably through its competitive binding for the shared sites on surfaces of azolla.

The data obtained from column sorption of Ni and Zn follows the BDST model well, enabling the application of the model to predicting design parameters for scale-up of the biosorption column system.

It is interesting that the values of metal uptake, expressed in molar quantities, obtained on respective single-metal solutions and the multiple metal system, are similar, implying that the mechanisms involved in the sorption of all metal cations are similar and that the binding sites on surfaces of azolla are probably shared by all cation metals. The surface of the biomass provides sites for metal binding estimated in the range of 0.45-0.57mmol/g, based on the current experiments. The biomass has a surface area of 429 m²/g and water retention of 14.3 ml/g. The functional groups on the surface of azolla were partially identified using chemical modification and metal binding comparison. Among the functional groups examined, carboxyl groups, provided by amino acids and polysaccharides, appeared to play an important role in metal cation binding. The infrared spectra of the samples support this conclusion.

TABLE OF CONTENTS

CONTENTS	PAGE
Title page	
Abstract	i
Table of Contents	iv
List of Figures	x
List of Tables	xv
Acknowledgements	xviii
Abbreviations	xix
1. General Introduction	1
1.1 Water and metal pollution	1
1.2 Electroplating Effluents	2
1.3 Conventional Treatment of Metal-laden Waste Effluent in Metal Finishing Industries	4
1.3.1 Chemical Precipitation	4
1.3.1.1 Hydroxide Precipitation	4
1.3.1.2 Sulfide Precipitation	6
1.3.2 Cementation	6
1.3.3 Reverse Osmosis	7
1.3.4 Evaporation	7
1.3.5 Electrolysis	8
1.3.6 Solvent Extraction	8
1.3.7 Ion Exchange	9
1.4 Bioremediation of Metal-laden Effluents	9
1.5 Biosorption by Microorganisms: Metal recovery	11
1.6 Microbial Sorbents	12
1.7 Plant Biomass	14
1.8 Research Aims	20
2 Sorption of Metals by Formaldehyde Cross-linked Yeast Biomass	22
2.1 Introduction	22

2.2	Existing Techniques for Immobilizing Microorganism	22
2.2.1	Entrapment in Polymers	22
2.2.2	Attachment to Inert Support or Self-formed Pellets	25
2.2.3	Cross-linked with Formaldehyde	27
2.3	Methods and Materials	28
2.3.1	Materials	28
2.3.2	Immobilization	28
2.3.3	Determination of Structural Properties	29
2.3.4	Metal Determination	30
2.3.5	Adsorption Isotherms	30
2.3.6	Column Operation	31
2.3.7	Desorption of Cr^{6+} with Various Desorbents	32
2.4	Results and Discussion	32
2.4.1	Stability of the Cross-linked Biomass	32
2.4.2	Morphological Structure of Formaldehyde Cross-linked Yeast	33
2.4.3	Comparison of sorption isotherms of FA-pellets and raw yeast	35
2.4.4	Column Sorption of Cu, Zn and Cd from Aqueous Solution	36
2.4.5	Desorption and Recovery of Cation Metals	39
2.4.6	Column Sorption of Cr^{6+} from Electroplating Effluent	40
2.4.7	Desorption of Cr^{6+}	43
2.4.7.1	Desorption with NaCl or NaOH	43
2.4.7.2	Reduction and Desorption of Cr^{6+}	44
2.5	Conclusions	46
3	Batch Removal of Cr^{6+} from Aqueous Solution using <i>Azolla filiculoides</i>	47
3.1	Introduction	47
3.2	Materials and Methods	48
3.2.1	Biomass	48
3.2.2	Heavy Metals	48
3.2.3	Adsorption Isotherms	48
3.2.4	Kinetic Studies	49
3.3	Results and Discussion	49
3.3.1	Adsorption Isotherms	49
3.3.2	Effect of pH	51
3.3.3	Kinetics	53
3.3.4	Reduction of Cr^{6+} to Cr^{3+}	55
3.3.5	Sorption of Cr^{3+}	56
3.3.6	Mechanical Stability	58

3.3.7	Comparison with Other Sorbents	58
3.4	Conclusions	59
4	Column Removal of Chromium(VI) from Aqueous Solutions and Electroplating Effluent	61
4.1	Introduction	61
4.2	Methods and Materials	61
4.2.1	Materials	61
4.2.2	Assay Methods	62
4.2.3	Preparation of Columns	62
4.2.4	Regeneration of Exhausted Column Biomass after Cr^{6+} Sorption	63
4.2.4.1	Desorption of Cr^{6+} with 0.2M NaOH and 1M NaCl / 0.1M NaOH	63
4.2.4.2	Reduction and Desorption of Cr^{6+} with $\text{Na}_2\text{S}_2\text{O}_5$ / H_2SO_4	63
4.2.5	Regeneration of Exhausted Column Biomass after Cr^{3+} Sorption	63
4.2.5.1	Desorption of Cr^{3+} with H_2SO_4 and HCl	63
4.2.5.2	Reconditioning of Azolla Biomass	64
4.2.6	Regeneration efficiency	64
4.3	Results and Discussion	64
4.3.1	Sorption of Cr^{6+}	64
4.3.1.1	Effect of pH	65
4.3.1.2	Effect of Flow Rate	67
4.3.1.3	Effect of Mass of Azolla	68
4.3.1.4	Treatment of Plating Effluent	68
4.3.2	Reduction of Cr^{6+} to Cr^{3+}	70
4.3.3	Gradual Breakthrough of Cr^{6+} in Column Operation	71
4.3.4	Desorption of Cr^{6+}	72
4.3.4.1	Desorption of Cr^{6+} with 0.5M NaOH or 1M NaCl/0.1M NaOH	72
4.3.4.2	Reduction and Desorption of Cr^{6+}	73
4.3.5	Sorption and Desorption of Cr^{3+}	74
4.3.5.1	Sorption of Cr^{3+}	74
4.3.5.2	Desorption of Cr^{3+}	76
4.4	Conclusions	77
5	Removal and Recovery of Ni from Aqueous Solutions and Electroplating Effluent using <i>Azolla filiculoides</i>	79
5.1	Introduction	79
5.2	Methods and Materials	80
5.2.1	Materials	80
5.2.2	Assay Methods	80

5.2.3	Adsorption Isotherms	80
5.2.4	Kinetic study	81
5.2.5	Preparation of Columns	81
5.2.6	Regeneration of Column Biomass	82
5.3	Results and Discussion	82
5.3.1	Adsorption Isotherms	82
5.3.2	Kinetics	84
5.3.3	Column Removal of Ni	85
5.3.4	Effect of Flow Rates	86
5.3.5	Effect of Influent pHs and Repeated Sorption of Ni	87
5.3.6	Effect of Influent Ni Concentration	89
5.3.7	Effect of Varying Mass of Azolla	90
5.3.8	BDST Equation	91
5.3.9	Treatment of Plating Effluent	93
5.3.10	Desorption and Recovery of Ni	96
5.3.11	Recycling of Ni and Waste Water	98
5.3.12	Comparison with Other Sorbents	100
5.4	Conclusions	102
6	Removal and Recovery of Zn by Azolla Biomass	103
6.1	Introduction	103
6.2	Methods and Materials	103
6.2.1	Materials	103
6.2.2	Assay Methods	104
6.2.3	Adsorption Isotherms	104
6.2.4	Kinetic Study	104
6.2.5	Preparation of Columns	104
6.3	Results and Discussion	105
6.3.1	Adsorption Isotherms	105
6.3.2	Kinetics	107
6.3.3	Column Removal of Zn	109
6.3.3.1	Effect of Flow Rates	109
6.3.3.2	Effect of Influent pHs	111
6.3.3.3	Effect of Influent Zn Concentration	112
6.3.3.4	Reusability of the Biomass for Zn Removal	112
6.3.4	BDST Model Analysis	113
6.3.5	Treatment of Plating Rinse Effluent	117
6.3.6	Desorption and Recovery of Zn	119
6.3.7	Recycling of Zn and Waste Water	121

6.4	Conclusions	121
7	Column Competitive Sorption of Multi-metals by Azolla	123
7.1	Introduction	123
7.2	Methods and Materials	124
7.2.1	Materials	124
7.2.2	Assay for Metals	124
7.2.3	Preparation of Columns	124
7.3	Results and Discussion	124
7.3.1	Column Removal of Cd	124
7.3.2	Competitive Sorption of Multi-metals	126
7.3.2.1	Sorption of Ni, Zn and Cd at pH 6.6	126
7.3.2.2	Sorption of Cr ³⁺ , Ni, Zn and Cd	128
7.3.2.3	Effect of Ca on Column Sorption of Ni, Zn and Cd	130
7.3.2.4	Effect of Mg on Column Sorption of Ni, Zn and Cd	132
7.4	Conclusions	134
8	Partial Characterization of <i>Azolla filiculoides</i>	136
8.1	Introduction	136
8.2	Biochemical Composition	136
8.2.1	Methods and Materials	136
8.2.1.1	Protein (Nitrogen) Determination	136
8.2.1.2	Lipid Determination	137
8.2.1.3	Ash Determination	138
8.2.1.4	Total Carbohydrates	138
8.2.2	Results and Discussion	138
8.3	Physical Characterization	139
8.3.1	Determination of Specific Surface Area	139
8.3.1.1	Methods and Materials	139
8.3.1.2	Results and Discussion	139
8.3.2	Determination of Water Retention	141
8.3.2.1	Methods and Materials	141
8.3.2.2	Results and Discussion	141
8.4	Chemical Modification of Azolla Biomass	142
8.4.1	Introduction	142
8.4.2	Methods and Materials	142
8.4.2.1	Amino Group Modification	144
8.4.2.2	Amino and Hydroxyl Group Modification	144
8.4.2.3	Carboxyl Group Modification	144

8.4.3	Results and Discussion	145
8.4.3.1	Ni and Cr ⁶⁺ Uptake of Amino & Hydroxyl Modified Azolla	145
8.4.3.2	Comparison of Ni and Cr ⁶⁺ Uptake of Carboxyl-Modified Azolla with That of Native	147
8.5	Infrared Analysis of Azolla Biomass	150
8.5.1	Introduction	150
8.5.2	Methods and Materials	150
8.5.3	Results and Discussion	151
8.6	Conclusions	156
9	General Discussion	157
9.1	Cross-linked Yeast Biomass	157
9.2	<i>Azolla filiculoides</i>	158
9.3	General comments	161
	Appendices	162
	References	163

LIST OF FIGURES

	PAGE
Figure 1.1 Appearances of (A) fresh and (B)dried <i>Azolla filiculoides</i>	19
Figure 2.1 Scanning electron micrographs of the surface of yeast cells cross-linked by FA. (A), fresh biomass; (B), after exposure to Zn solution; (C), after exposure to Cr^{6+} solution.	35
Figure 2.2 Comparison of sorption isotherms for FA-immobilized yeast-Zn and native yeast-Zn systems at 18°C. Incubation time, 5 h; biomass, 4g/l.	36
Figure 2.3 Breakthrough curves for repeated removal of Cu by 10g yeast from solution. Flow rate, 50ml/h; infl. Cu, 127mg/l; infl. pH, 5.3.	37
Figure 2.4 Breakthrough curves for repeated removal of Zn by 10g yeast from solution. Flow rate, 50ml/h; infl. Zn, 130.7mg/l; infl. pH, 6.1.	38
Figure 2.5 Breakthrough curve for removal of Cd by 10g yeast from solution. Flow rate, 50ml/h; infl. Cd, 112.4mg/l; infl. pH, 6.7.	38
Figure 2.6 Recovery of cation metals from saturated yeast biomass with 0.1M HCl. Flow rate, 50ml/h.	40
Figure 2.7 Breakthrough curves for column removal of Cr^{6+} by 10g yeast biomass from EPE1 at varying infl. pHs. (A), at pHs of 4 and 7.5; (B), at pHs of 2.5 and 3; flow rate, 50ml/h; infl. Cr^{6+} , 30mg/l.	42
Figure 2.8 Breakthrough curves for scale-up column removal of Cr^{6+} by 150g yeast biomass from EPE1 at infl. pHs 2.5 and 3. Flow rate, 50ml/h; infl. Cr^{6+} , 30mg/l.	43
Figure 2.9 Desorption of Cr^{6+} with varying desorbents. Flow rate, 50ml/h.	44
Figure 2.10 Recovery of Cr^{6+} from saturated yeast biomass with 0.1% FA/1N HNO_3 . A, at 4° C; B, at 18°C; C, at 18°C, scale-up 1; D, at 18°C, scale-up 2.	45
Figure 3.1 Adsorption isotherms of azolla- Cr^{6+} system at 18°C after 5 h incubation. Biomass, 4g/l.	50
Figure 3.2 Effect of pH and initial concentrations on TCr removal. Biomass, 4g/l.	52
Figure 3.3 Effect of contact time on the residual Cr^{6+} and TCr concentrations at pH 2.	54

Figure 3.4	Reduction of Cr^{3+} by azolla at different pHs and initial Cr^{3+} concentrations after 5 h incubation at 18°C. Biomass, 4g/l.	56
Figure 3.5	Adsorption isotherms of azolla- Cr^{3+} system. Biomass, 4g/l.	57
Figure 4.1	Breakthrough curves for column removal of TCr and Cr^{6+} from solutions at infl. pHs 2 and 3. Flow rate, 80ml/h; infl. Cr^{6+} , 100g/l; biomass, 5g.	66
Figure 4.2	Breakthrough curves for column removal of TCr and Cr^{6+} from solutions at flow rates 80ml/h and 160ml/h. Infl. pH, 2.5; infl. Cr^{6+} , 100g/l; biomass, 5g.	67
Figure 4.3	Breakthrough curves for column removal of TCr and Cr^{6+} at infl. pH 2.5 and flow rate 160ml/h. Infl. Cr^{6+} , 100mg/l; biomass, 10g.	68
Figure 4.4	Breakthrough curves for column removal of TCr and Cr^{6+} from EPE2 at infl. pH 2.5 and flow rate 160ml/h. EPE2 Cr^{6+} , 18mg/l; biomass, 10g.	69
Figure 4.5	Breakthrough curves for column removal of TCr, Cr^{6+} and Ni from EPE3 at infl. pH 3 and flow rate 80ml/h. EPE3 Cr^{6+} , 20.5mg/l, Ni, 2mg/l; biomass, 5g.	70
Figure 4.6	Desorption and recovery of chromium from Cr^{6+} -laden biomass column with various desorbents. A1 , $\text{Na}_2\text{S}_2\text{O}_5$, Cyc.1; A2 , $\text{Na}_2\text{S}_2\text{O}_5$, Cyc.2; B , NaCl/ NaOH; C , NaOH.	73
Figure 4.7	Breakthrough curves for repeated column removal of Cr^{3+} for 5 cycles. Infl. Cr^{3+} , 100mg/l; biomass, 5g.	75
Figure 4.8	Desorption and recovery of Cr^{3+} from Cr^{3+} -laden biomass column with various desorbents. A1 , 0.5N H_2SO_4 ; A2 , 0.2N H_2SO_4 ; B1 , 0.5N HCl; B2 , 0.2N HCl.	77
Figure 5.1	Adsorption isotherms for azolla-Ni system at 18°C. Incubation time, 5 h; biomass, 4g/l.	83
Figure 5.2	Effect of contact time on the sorption of Ni by azolla at pH 6.5. Biomass, 4g/l; (A), sampled for 24 h; (B), sampled for 2 h.	84
Figure 5.3	Breakthrough curves for column removal of Ni by azolla with varying flow rates. Infl. Ni, 100mg/l; infl. pH, 7.0; biomass, 5g.	87
Figure 5.4	Breakthrough curves for column removal of Ni by azolla for 4 cycles. Flow rate, 480ml/h; infl. pH, 7.0; biomass, 5g.	88
Figure 5.5	Scanning electron micrograph of used biomass after sorption of Ni.	89

Figure 5.6	Breakthrough curves for removal of Ni with varying mass of azolla. Infl. Ni, 100mg/l; infl. pH, 7.0; flow rate, 480ml/h.	91
Figure 5.7	BDST curves at various breakthrough levels with varying flow rates. Infl. pH, 7.0.	92
Figure 5.8	Comparison of breakthrough curves for removal of Ni from EPE4 and aqueous solution. Infl. pH, 7.0; flow rates, 480ml/h; biomass, 5g.	95
Figure 5.9	Breakthrough curves for removal of Ni from EPE5. Infl. pH, 7.1; infl. Ni, 12mg/l; Ca, 22mg/l; Mg, 38.5mg/l; flow rate, 480ml/h; biomass, 5g.	95
Figure 5.10	Ni recovery with H ₂ SO ₄ and HCl of varying concentrations. A1 , 0.2N HCl, cyc.1; A2 , 0.2N H ₂ SO ₄ , cyc.2, A3 , 0.2N HCl, cyc.3; A4 , 0.2N H ₂ SO ₄ , cyc.4; B , 0.1N HCl; C , 0.1N H ₂ SO ₄ .	97
Figure 5.11	Recoveries of Ni and Ca with 0.2N HCl, after treatment of EPE4.	97
Figure 5.12	Schematic installation for electroplating rinse effluent treatment. A , plating bath; B , catching bath; C , rinse tank 1; D , rinse tank 2; E , biomass column; F , saturated column in regeneration; G , activated carbon filter for removing impurities; H , acids container.	99
Figure 5.13	Breakthrough curve for removal of Ni by 5g activated carbon. Infl.pH, 7.0; infl. Ni, 100mg/l; flow rate, 480ml/h.	101
Figure 6.1	Adsorption isotherms for azolla-Zn system at 18°C. Incubation time, 5 h; biomass, 4g/l.	106
Figure 6.2	Effect of contact time on the sorption of Zn by azolla at pH 6.0. Biomass, 4g/l.	108
Figure 6.3	Breakthrough curves for column removal of Zn by azolla with varying flow rates. Infl. Zn, 100mg/l; infl. pH, 6.2; biomass, 5g.	110
Figure 6.4	Breakthrough curves for column removal of Zn by azolla at influent pH 6.2 and 4. Biomass, 5g.	111
Figure 6.5	Breakthrough curves for column removal of Zn by azolla for 6 cycles. Flow rate, 480ml/h; infl. Zn, 100mg/l; infl. pH, 6.2; biomass, 5g.	112
Figure 6.6	Typical breakthrough curves for removal of Zn with varying bed heights. Infl. Zn, 100mg/l; inf. pH, 6.2; flow rate, 480ml/h.	115
Figure 6.7	BDST curves at 50% (A) and 30% breakthrough (B) with varying flow rates and influent pHs.	116

Figure 6.8	Breakthrough curves for removal of Zn from EPE6. Infl. pH, 6.7; infl. Zn, 30mg/l; Ca, 24mg/l; flow rates, 480ml/h; biomass, 5g.	118
Figure 6.9	Zn recovery with H ₂ SO ₄ and HCl of varying concentrations. A, 0.2N H ₂ SO ₄ ; B, 0.1N H ₂ SO ₄ ; C, 0.2N HCl; D, 0.1N HCl.	120
Figure 6.10	Recoveries of Zn, Ca and Mg with 0.2N HCl, after treatment of EPE6.	121
Figure 7.1	Breakthrough curve for column removal of Cd by 5g azolla at influent pH 6.6. Infl. Cd, 100mg/l; flow rate, 480ml/h.	125
Figure 7.2	Breakthrough curves for column removal of Ni, Zn and Cd by 5g azolla at influent pH 6.6. Infl. Ni, 58mg/l; Zn, 74mg/l; Cd, 122mg/l; flow rate, 480ml/h; C _t , metal conc. at a given time; C ₀ , infl. metal conc..	127
Figure 7.3	Breakthrough curves for column removal of Cr ³⁺ , Ni, Zn and Cd by 5g azolla at influent pH 4.5. Infl. Cr ³⁺ , 50mg/l; Ni, 56.2mg/l; Zn, 75.8mg/l; Cd, 114.2mg/l; flow rate, 160ml/h; C _t , metal conc. at a given time; C ₀ , infl. metal conc..	130
Figure 7.4	Breakthrough curves for column sorption of Ca, Ni, Zn and Cd by 5g azolla at influent pH 6.5. Infl. Ca, 43.5mg/l; Ni, 57.7mg/l; Zn, 77.0mg/l; Cd, 127.0mg/l; flow rate, 480ml/h; C _t , metal conc. at a given time; C ₀ , infl. metal conc..	132
Figure 7.5	Breakthrough curves for column sorption of Mg, Ni, Zn and Cd by 5g azolla at influent pH 6.4. Infl. Mg, 33.3mg/l; Ni, 58.5mg/l; Zn, 75.0mg/l; Cd, 140.0mg/l; flow rate, 480ml/h; C _t , metal conc. at a given time; C ₀ , infl. metal conc..	134
Figure 8.1	Chemical modification of amino (A & B) and carboxyl groups (C & D).	143
Figure 8.2	Effect of chemical modification of amino groups on Ni removal capacities of azolla biomass. The biomass was tested at incubation Ni concentrations of 100 and 800mg/l respectively.	146
Figure 8.3	Effect of chemical modification of amino groups on Cr ⁶⁺ removal capacities of azolla biomass. The biomass was tested at incubation Cr ⁶⁺ concentrations of 100 and 800mg/l respectively.	147
Figure 8.4	Effect of chemical modification of carboxyl groups on Ni removal capacities of azolla biomass. Ni concentrations of 100 and 800mg/l were used in the sorption tests respectively.	149
Figure 8.5	Effect of chemical modification of carboxyl groups on Cr ⁶⁺ removal capacities of azolla biomass. Cr ⁶⁺ concentrations of 100 and 800mg/l were used in the sorption tests respectively.	149

- Figure 8.6** Infrared spectra of protonated or Cd loaded *S. fluitans*. str.: stretching; chel. str.: chelate stretching; def.: deformation (Taken from Fourest & Volesky, 1996). 153
- Figure 8.7** Infrared spectra of native and carboxyl-modified azolla biomasses. **A**, native; **B**, modified by glycine ethyl ester - carbodiimide; **C**, modified by methyl iodide. 154
- Figure 8.8** Infrared spectra of native and metal-loaded azolla biomasses. **A**, native; **B**, loaded with Zn; **C**, loaded with Ni. 155

LIST OF TABLES

	PAGE
Table 1.1 Characteristics of plating waste effluent discharge from local electroplating shops.	3
Table 1.2 Metal residues in hard water at different pHs in hard water.	5
Table 1.3 Cd uptake capacity of various sorbents.	15
Table 1.4 Cr(III) uptake capacity of various sorbents.	15
Table 1.5 Cr(VI) uptake capacity of various sorbents.	16
Table 1.6 Ni uptake capacity of various sorbents.	16
Table 1.7 Cu uptake capacity of various sorbents.	17
Table 1.8 Zn uptake capacity of various sorbents.	17
Table 2.1 Levels of metals present in electroplating effluent (EPE1), compared to stipulated criteria (mg/l).	28
Table 2.2 Granule stabilities of the yeast pellets cross-linked with differing FA/0.1M HCl ratios.	33
Table 2.3 Column removal of cation metals from aqueous solutions with 10g biomass at a flow rate of 50ml/h.	37
Table 2.4 Cation metal recovery with 120ml of 0.1M HCl.	39
Table 2.5 Column removal of Cr ⁶⁺ from EPE1.	41
Table 2.6 Chromium recovery with various desorbents.	44
Table 3.1 Langmuir constants of Cr ⁶⁺ -azolla binding.	50
Table 3.2 Final pH at different Cr ⁶⁺ concentrations after 5 h incubation.	51
Table 3.3 Cr ³⁺ produced after 5 h incubation at pH 1.5 and varying initial Cr ⁶⁺ concentration.	53
Table 3.4 Langmuir constants of Cr ³⁺ -azolla binding at 18°C.	58
Table 3.5 Summary of sorptive potential of various sorbents.	59

Table 4.1	Characteristics of EPE2 and EPE3.	62
Table 4.2	Column removal of Cr^{6+} with varying pH, flow rate and mass of azolla.	65
Table 4.3	Cr(VI) recovery with various desorbents.	72
Table 4.4	Column removal of Cr^{3+} by 5g azolla at influent Cr^{3+} of 100mg/l with varying pHs and flow rates.	75
Table 4.5	Cr(III) recovery with various desorbents in volume of 150ml.	76
Table 5.1	Characteristics of EPE4 and EPE5.	80
Table 5.2	Langmuir constants of Ni-azolla binding at 18°C.	83
Table 5.3	Column removal of Ni with varying flow rates, influent pHs and Ni concentrations.	85
Table 5.4	Column removal of Ni at infl. pH 7 and infl. Ni 100mg/l with varying mass of azolla and flow rate.	90
Table 5.5	Calculated constants of the BDST equation.	93
Table 5.6	Column removal of Ni from EPE4 and EPE5 with 5g azolla at flow rate of 480ml/h.	94
Table 5.7	Ni recovery with various desorbents.	96
Table 5.8	Ni uptake capacities of various sorbents on batch scale.	101
Table 6.1	Characteristics of EPE6.	104
Table 6.2	Langmuir constants of Zn-azolla binding at 18°C.	106
Table 6.3	Zn uptake capacities of various sorbents on batch scale.	107
Table 6.4	Column removal of Zn with varying flow rates, influent pHs and Zn concentrations.	109
Table 6.5	Constants determined for the BDST equation.	115
Table 6.6	Column removal of Zn from EPE6 with 5g azolla.	118
Table 6.7	Zn recovery with various desorbents.	120
Table 7.1	Column removal of Cd by various biosorbents.	125

Table 7.2	Column metal uptake capacities at 60% saturation from single and mixed metal systems.	128
Table 7.3	Uptake of Cr ³⁺ , Ni, Cd and Zn during column sorption on a multi-metal system at various stages.	129
Table 7.4	Uptake of Ca, Ni, Cd and Zn during column sorption on multi-metal system. at various stages.	131
Table 7.5	Comparison of metal uptakes on mixed metal systems with and without Ca at saturation.	131
Table 7.6	Uptake of Mg, Ni, Cd and Zn during column sorption on multi-metal system at various stages.	133
Table 8.1	Biochemical composition of azolla, in comparison with other water plants.	138
Table 8.2	Specific surface area of azolla biomass based on MB adsorption method.	140
Table 8.3	Specific surface area of azolla, in comparison with other biosorbents based on MB adsorption method.	140
Table 8.4	Swelling characteristics of azolla, in comparison with a marine algae.	141

ACKNOWLEDGEMENTS

The author wishes to thank the following people for their contribution to this thesis:

- Professor John R. Duncan for his invaluable support, guidance and encouragement throughout this project.
- The staff of the Department of Biochemistry and Microbiology at Rhodes University, for the years of support and assistance they provided. In particular, Mrs. Joan Miles for her secretary help.
- My wife for her support, understanding and patience, and assistance throughout my career.
- Mr. Robin Cross and Mrs. Shirley Pinchuck, Electron microscopy Unit at Rhodes University, for assistance with the Electron microscopy; Professor J.H. Neethling and Miss Lorinda Wu, Department of Physics at University of Port Elizabeth, for assistance of the Infrared Spectrometer.
- My colleagues, Brendan Wihelmi, Robert P. van Hille for their help with collecting samples.
- I am grateful to Professor Don Hendry for proof reading this thesis.
- The Water Research Commission of South Africa for its personal financial support, without which this research would not have been possible.

ABBREVIATIONS

BDST	- Bed-depth-service-time
ca.	- Approximately
Ca	- Ca^{2+}
Cd	- Cd^{2+}
Co	- Co^{2+}
C_t	- Metal concentration at a given time
Cu	- Cu^{2+}
DI	- Distention index
EM	- Electron microscopy
EPE	- Electroplating effluent
FA	- Formaldehyde
GA	- Glutaraldehyde
GEE-Car	- Glycine ethyl ester-carbodiimide
h	- Hour
HAA	- Acetylacetone
Hg	- Hg^{2+}
HTTA	- Thenoyltrifluoroacetone
Infl.	- Influent
IR	- Infrared ray
K	- K^+
MI	- Methyl iodide
MB	- Methylene blue
Mg	- Mg^{2+}
NaDDC	- Sodium diethyldithiocarbamate
Na	- Na^+
Ni	- Ni^{2+}
Pb	- Pb^{2+}
PEI	- Polyethylene imine
PVA-A	- Polyvinylalcohol-Na-alginate

PVA-P	- Polyvinylalcohol-Na-orthophosphate
PVF	- Polyvinyl formal
RO	- Reverse osmosis
SA	- Succinic anhydride
SAA	- S-acetylmercaptosuccinic anhydride
SEM	- Scanning electron microscopy
SI	- Sodium iodide
SSP	- Slightly soluble sulphide precipitation
SSSP	- Soluble sulphide precipitation
TCr	- Total chromium concentration
wt	- Weight
Zn	- Zn^{2+}

1 GENERAL INTRODUCTION

1.1 WATER AND METAL POLLUTION

Water is the most important of natural resources on the earth, but is unfortunately a limited resource. Per capita water supplies worldwide are already a third lower than they were 25 years ago due mainly to a population increase of 1.8 billion people. During the next 30 years, the world population is projected to increase to at least eight billion or more, resulting in an increase in the demand for water by more than 650 percent (Giacasso, 1996).

Water availability and quality are of paramount importance for socioeconomic growth in South Africa. South Africa is approaching a crisis point where available water resources will no longer be able to meet the demand (Odendaal, 1989). Rapid urbanisation and industrialization will substantially increase pressures on the quality of available water, thereby threatening the usability of these supplies. Today industry uses more than 40% of total water withdrawals in developed countries; the comparable figure in developing countries is less than 10% (Giacasso, 1996). Thus industrial development will also lead to more water quality problems. With such a high demand for the limited quantity of potable water it is necessary to prevent or at least limit its contamination with pollutants.

One commonly encountered group of pollutants is toxic metals. Heavy metals exert toxicity to humans, aquatic life and the environment in a number of ways. Their ability to bind a variety of organic ligands can cause denaturation of proteins, including enzymes, disruption of cell membranes and decomposition of essential metabolites, and many can act as antimetabolites towards essential nutrients (Gadd, 1986; Passow & Rothstein, 1960).

Some of the heavy metals are essential for growth of both prokaryotic and eukaryotic organisms, but also have a comprehensive toxic effect on cells if present in higher concentrations. Others do not fulfill any physiological functions but cause metal toxicity within the cell at much low concentrations (Gadd & Griffiths, 1978). Heavy metal

contamination of the lowest strata of the food chain will have a cumulative effect through the food chain. Fish and microorganisms are more sensitive to the effect of metal interaction than humans, and hence the suggested criteria for river and dam water are more strict than for drinking water.

Because of the slow natural process of metal formation, metals are considered to be a non-renewable resource and should be managed with care (Brady & Duncan, 1992). If metals could be reclaimed from waste effluents or losses during metal processing could be reduced, the opportunity is presented to produce cheaper goods with higher profits. The metal pollutants can originate from a variety of sources such as nuclear power and fuel generating plants, metal finishing and electroplating processes, mining, refining and smelting industries as well as tannery and textile industries (Guan *et al*, 1993).

1.2 ELECTROPLATING EFFLUENTS

The variety of processes and methods of operation in the metal finishing industry give rise to a wide range of effluent composition. The plating baths contain high concentrations of potential polluting metals, whilst the rinse water, although being relatively lower in concentration of heavy metals, can contribute a significant load because of their large volumes. In the UK it was estimated that the metal finishing industry used 4% of the estimated total industrial water usage of which 80% was discharged as effluent (Wild, 1987). The electroplating processes likely to be carried out are degreasing, pickling, dipping (acid or alkali), stripping, etching, brightening and polishing. Among them the predominant sources of metal contaminants are found from (Pollution Group, 1987):

1. alkali cleaning to remove oil and grease;
2. acid descaling and pickling of basis metals to ensure satisfactory adhesion of the deposit in the plating stage;
3. metal plating solution from cyanide complexes of Zn, Cd, Cu, Ag⁺, Au⁺ and Au³⁺.
4. metal plating solutions based on the acid salts of Ni, Cu and Zn;

5. chromium compound-based coating on Al, Cd, Zn, Ni and Zn;
6. tin plating based on acid or alkaline complexes;
7. sulphuric acid anodizing of aluminum;
8. accidental overflows of solutions or possible leaks as well as the periodic discharge of exhausted process solutions.

Table 1.1 presents the typical plating effluent characteristics of 16 electroplating factories surveyed in Hong Kong (Chiu *et al*, 1987).

Table 1.1 Characteristics of plating waste effluent discharge from local electroplating shops

Plating operation	Factory		mg/l						
	area (m ²)	pH	Cr	CN	Cu	Ni	Zn	Al	Ag
Cr ⁶⁺ , Ni & Zn	60	4.0	33	6	4	168	250	—	—
Cu, Ni & Cr ⁶⁺	70	5.2	20	4	2	30	—	—	—
Al anodizing	100	2.7	—	—	—	—	—	230	—
Cr ⁶⁺ , Ni & Cr ⁶⁺	120	2.5	31	4	2	13	—	—	—
Ni & Zn	150	8.2	1	—	—	95	10	—	—
Cu, Ni, Cr ⁶⁺ & Ag ⁺	150	1.7	9	1	—	8	—	—	2
Cu, Ni & Cr ⁶⁺	200	4.5	25	6	3	230	—	—	—
Cu, Ni, Cr ⁶⁺ & brass	200	7.0	40	1	11	365	15	—	—
Ni, Cr ⁶⁺ & Au ³⁺	300	5.6	38	—	—	3	—	—	—
Cu, Ni, Cr ⁶⁺ & brass	400	7.7	5	5	30	25	10	—	—
Range	20-1000	1.7 - 8.2	1-40	1-6	1-30	3-365	4-250	10-230	2-3

1.3 CONVENTIONAL TREATMENT OF METAL-LADEN WASTE EFFLUENTS IN METAL FINISHING INDUSTRIES

Metal finishing operations use large quantities of water for cleaning and rinsing processes to produce corrosion-resistant, wear-resistant or decorative finishes and deposits by the use of a wide range of metal species. As only 30-40% of all metals used in the electroplating process can be effectively plated onto the articles (Grebenyuk *et al*, 1996), its waste effluents are responsible for the supply of toxic and corrosive heavy metals to the environment. With the increasing worldwide awareness of the effects of pollution on the environment and stringent legislative measures, the industry is generally responding in an attempt to create a better image. A number of treatment techniques have been developed and are utilized for water purification and metal recovery (Dean *et al*, 1972). Treatment methods include chemical precipitation, cementation, electrolysis, solvent extraction, reverse osmosis, and ion exchange.

1.3.1 CHEMICAL PRECIPITATION

1.3.1.1 HYDROXIDE PRECIPITATION

The most generally applied treatment method, particularly where complex chemical compounds are not involved and economic recovery is not a consideration, is the lime treatment plant, because of its relatively low cost as the precipitant. Removing such metals as Cu, Zn, Fe^{3+} , Mn, Ni and Co requires almost complete precipitation as the hydroxides with no special modification. However, to effectively reduce Cu to a concentration of 1mg/l from an original concentration of 50mg/l, a large excess of lime is required (up to 15 times as the stoichiometric excess) (Spearot & Peck, 1984). A disadvantage with the use of lime results from its low solubility, leading to the requirement for a carefully designed handling and dosing system and the generation of large volumes of sludge (Wild, 1987) and the costs of hazardous landfill are increasing daily. For Cd, Pb and Hg, precipitation may be incomplete, however, and a modified flowsheet employing soda ash (for Pb) or sodium sulfite (for Cd or

Hg) may be required. Where Cr^{6+} is present, reducing the metal with sulfur dioxide, ferrous sulfate, or metallic iron before lime treatment is necessary. In the case of alkaline effluents (Zn, Cu or Au cyanide), chlorination may be needed to break down complex organic metallic compounds before chemical treatment (Dean *et al*, 1972). Where strong acidic wastes exist, part of the neutralization with limestone may be somewhat less expensive than lime.

However, limestone must be evaluated carefully for each acid waste since it may not be as effective as theoretically indicated owing to particle coating, the need for fine grinding, and pH limitation of calcium carbonate (Dean *et al*, 1972).

Metal salts from simple inorganic compounds will tend to become insoluble in the neutral pH range, but not all metals will precipitate on neutralization and not all metals will precipitate at the same pH point and to the same extent (Pollution group, 1987). The initial problem is the selection of the optimum pH for precipitation. Some of metals are amphoteric, and therefore are soluble at alkaline pH values; examples of such metals are aluminium and zinc. Other metals require a relatively high pH to reach minimum solubility; this category would include Ni and Cu. Table 1.2 lists the metal content in solutions at various pH values. Chelating agents, organic acid salts, and the various wetting agents sometimes encountered in a mixed rinse-water stream may make the quantitative precipitation of the various metals impossible (Pollution group, 1987).

Table 1.2 Metal residues in tab water at different pHs (Pollution group, 1987)

pH	mg/l					
	Fe^{3+}	Ni	Cr^{3+}	Zn	Cd	Cu
6.5	0.8	19.2	17.8	18.5	19.2	11
7.0	0.4	18.9	13.7	17.8	18.4	5.8
8.0	0	10.8	7.1	9.1	15.2	2.4
8.5	0	2.3	5.0	1.6	4.8	1.7
9.0	0	0.6	3.4	1.5	0.9	1.2
10.0	0	0	0.3	8.4	0	0.4

1.3.1.2 SULFIDE PRECIPITATION

Sulphide precipitation has been demonstrated to be an effective alternative to hydroxide precipitation for removing various heavy metals from industrial effluents (Pollution group, 1987). The high reactivity of sulphide with metal ions and the insolubility of the metal sulphides over a broad pH range are attractive features compared with the hydroxide precipitation process. Sulphide precipitation can also achieve low metal solubilities in the presence of certain complexing and chelating agents.

In the soluble sulphide precipitation (SSP) process, the soluble reagents Na_2S or NaHS are added to the waste water so that operational problems with this process in controlling reagent demand to prevent overdoses and the associated odour problem have been overcome by the use of a sulphide ion selective electrode to control the addition of the soluble sulphide. The slightly soluble sulphide precipitation (SSSP) process uses a freshly prepared ferrous sulphide solution (prepared by reacting FeSO_4 and NaHS) as the source of sulphide ions needed to precipitate the metals from waste water. As sulphide ions are consumed, additional FeS will dissociate to maintain the equilibrium concentration of sulphide ions. Because most metals are less soluble than ferrous sulphide, they will precipitate as metal sulphides. An advantage of the SSSP is the absence of any detectable H_2S odour. Secondly, the SSSP will reduce hexavalent chromium to the trivalent state under the same conditions required for metal precipitation thus eliminating the need to segregate and pretreat chrome waste streams. Disadvantages of SSSP are that the process requires considerably greater than stoichiometric reagent consumption and produces a significantly greater volume of sludge than either the hydroxide or SSP processes.

The potential hazard of sulphides coming in contact with acids with the evolution of toxic H_2S fumes emphasises the need to control a low residue in the discharge.

1.3.2 CEMENTATION

Cementation is the displacement of a metal from solution or the reduction of any ionic species by contact with a metal in the higher electromotive series, particularly where metal recovery is desirable. It can be used for precipitating silver from photographic precessing solutions, the precipitation of Cu from printed circuit etching wastes, the reduction of hexavalent chrome in plating effluents, and chrome-inhibited cooling water. Using Zn dust as a precipitant for gold and silver from cyanide solutions is a widely used cementation process that has interesting potential for recovering precious metals with less valuable species (Pollution group, 1987).

1.3.3 REVERSE OSMOSIS (RO)

RO is a technique which involves the use of a high-pressure system to force pure water through a membrane, leaving behind a concentrated chemical solution (Wild, 1987). Plating salts in the rinse effluents can be concentrated up to 20 times by RO and returned to the plating bath. The final concentration at which the plating chemicals are recycled is limited by the osmotic pressure of the solution, hence in practice RO has to be used in conjunction with evaporation (Pollution group, 1987). Because RO membranes reject most organic brighteners along with the plating salts, RO cannot be used for copper pyrophosphate, chromium and cyanide rinse water and dual Ni application. Another difficulty with RO is the maintaining membrane performance. The pH must be kept within prescribed limits to ensure a reasonable life and additional steps are necessary to prevent the accumulation of biomatter as fine particles from fouling the membranes (Binnie & Partners, 1987). The capital, operating and maintenance costs of RO system are comparatively high.

1.3.4 EVAPORATION

Evaporative distillation is applicable to a number of plating process operations and may be operated at atmospheric pressure or as a vacuum recovery system. A typical chrome loop

evaporative recovery system has been described by Kolesar (1972). Fumes from the plating bath are evaporated, in conjunction with the overflow from the rinse tank which has passed through a cation exchanger for Cr^{3+} , Fe^{3+} , Ni and other metallic contaminants removal. Because of the thermal energy costs of operation, evaporators are not usually considered for recovery of plating chemicals from diluted waste waters. Reverse osmosis or ion exchange can be used to preconcentrate the rinse water prior to evaporation.

1.3.5 ELECTROLYSIS

In the electrolytic method of recovery and removal, the passage of an electrical current between two electrodes which are suspended in the solution, results in the deposition of metals on the cathode. Although in principle the technique of electrolytic removal of metals from solution is simple, the selection and control of operating conditions is extremely important (Linkson, 1987). The optimum operation requires a highly concentrated metal solution and the high levels of metal removal needed to meet environmental standards can not be achieved in practice. Therefore it was suggested that it should be used in combination with other treatments such as ion exchange (Basta & Short, 1983).

1.3.6 SOLVENT EXTRACTION (SE)

The liquid ion exchange, used in chemical and metallurgical industries, involves extracting a particular metal from solution by contacting the solution with an organic reagent. This reagent reacts preferentially with the heavy metal of interest and converts it to a form which is soluble in appropriate organic solvents. Four chelating reagents were found to offer the best potential for the extraction of heavy metals like Cu, Ni, Cr, Cd and Zn, namely: acetylaceton (HAA); thenoyltrifluoroacetone (HTTA); sodium diethyldithiocarbamate (NaDDC); 8-hydroxyquinoline (8HQ). The HTTA and NaDDC show non-selective separating capacity for all the metals whilst the HAA exhibits partially selective extraction only for Cu. A severe economic limitation to this recovery method was the inability of any of the chelating agents to be reused once they had been stripped with 2.4M HCl because of loss of extraction

capabilities (Clevenger & Novak, 1983). At current prices, the costs of the chelating agent needed to recover a metal is higher than the value of the metal. These costs do not include the routine chemicals and operating costs.

1.3.7 ION EXCHANGE

With the rapid rise in the price of precious metals during the past few years, recovery of even minute amounts of these elements has become of greater interest to metal finishers. Although precious metals are recovered easily from concentrated solutions by reduction and precipitation or electrolysis, solutions containing a few parts per million are difficult to handle by these methods. However the use of ion-exchange resins makes it possible to concentrate dilute solutions so that they can be subjected to common refining procedures to recover the metals.

Application of a strongly basic resin to the recovery of gold from a cyanide solution was initiated in 1953 where a capacity of up to 0.297g of gold per gram of resin was attained (Waitz, 1982). Because the eluting system results in a very dilute recovered solution and the eluting agent (acetone) is hazardous, incineration has become the most popular method of recovering gold from ion-exchange resins. Work on recovery of silver and platinum has also been reported (Waitz, 1982). The use of resins solely for purifying waste waters is, in most cases, inappropriate because of the high price of the materials although in some instances the cost may be partially offset by the metal recovered (Volesky, 1987).

1.4 BIOREMEDIATION OF METAL-LADEN EFFLUENT

Conventional technologies for removal of metals from industrial waste solutions in some cases may be incomplete or considerably expensive, especially when the metals are dissolved in large volumes of solution at relatively low concentrations ($< 50\text{mg/l}$) (Dean *et al*, 1972; Shumate *et al*, 1978; Spearot & Peck, 1984; Sharma & Forster, 1996 b). Biotechnological approaches to the abatement of toxic metal pollution can potentially offer an alternative or

adjunct to the existing metal treatment technologies (Gadd & White, 1993). The use of biological treatment systems to remove or reduce the concentrations of heavy metals from metal-contaminated effluents or sites is one of the applications of bioremediation. Such systems have numerous applications, including clean up of ground water, soil, lagoons, mining water and industrial processing effluents. There are tremendous market opportunities for bioremediation including process-waste treatment, industrial lagoons, municipal-landfill leachate and general hazardous spills. Revenue estimates derived from these applications add an additional US\$2-4 billion to the bioremediation market worldwide (Caplan, 1993). It has been estimated that bioremediation will have a long, steep growth curve ahead before levelling out in 10-15 years. This will be an interesting period from a business perspective and will probably follow a similar pattern seen in the microcomputer industry during the 1980s (Caplan, 1993).

Microorganisms are known to play an important role in the solubilization, accumulation, transport and deposition of metals in the environment (Hutchins *et al*, 1986). Hydrogen sulfide is produced by sulfate-reducing bacteria such as *Desulfovibrio* spp., *Desulfotomaculum* spp., and *Desulfomonas pigra* (Gadd & White, 1993). The solubility of most metal sulfides is extremely low and they are readily precipitated as metal sulfides. Addition of sewage to effluents enhances the activity of aerobic, heterotrophic bacteria; this results in oxygen depletion and subsequently promotes growth of the sulfate reducer (Hutchins *et al*, 1986). The process has since expanded to a commercial pilot-scale using an 1800m³ concrete reactor built by Parques BV (Balk, The Netherlands), and it is capable of treating 7000m³/day and has been in operation since 1992 (Gadd & White, 1993).

Other microorganisms excrete hydrogen peroxide to precipitate metal cations as oxides on the cell surface, while the production of oxalic acid by fungi leads to the precipitation of insoluble metal oxalate crystals on the cell surface and in the medium (Hughes & Poole, 1989). A large and varied group of bacteria are able to cause the deposition of Fe(OH)₃ and MnO₂ from mine effluents by oxidation and deposition on their extracellular polymers. The iron-oxidizing or-depositing bacteria include the genera *Gallionella*, which produces a

twisted stalk made up of filaments of iron(III) hydroxides, and *Siderocapsa* which deposits iron in mucoid capsules (Hughes & Poole, 1989).

The applications of biological treatment vary from large-scale processes such as the removal of metals from mine wetlands and sewage sludge to much smaller operations involving the recovery of precious metals from process or waste effluents from electroplating and jewellery industries. The recovery processes using surface-binding of biomass has numerous advantages over other methods (Hughes & Poole, 1989). Sources of the biomass can be identified as either the byproducts of biotechnological industries or naturally abundant biomaterials, with which significant savings in costs can be achieved.

1.5 BIOSORPTION BY MICROORGANISMS: METAL RECOVERY

It is generally agreed that the ultimate remediation is attained only when the metal becomes concentrated to the point that it can either be returned to the process or resold. This aspect of the operation deals with the recovery of the metals, which ideally should go hand in hand with the removal aspect, making the overall process an ultimately effective procedure for controlling the utilization of metals by industries in their technological processes (Volesky & Holan, 1995). Traditional methods of metal recovery from waste streams, such as ion exchange and electrolysis, have not proved cost effective especially in the low concentration ranges (Volesky, 1987; Duncan *et al*, 1995). Biotechnology based processes can however play a role in this field, through bioprecipitation and biosorption.

The term “biosorption” is now frequently used to encompass uptake by biomass via an energy-independent, physico-chemical based interactions between metal ions and the surfaces of biomass (Gadd, 1990). The utilization of surface binding of biomass rather than internalization into microbial cells is of importance in recovery of precious metals, since removal of the metals out of the cells is not possible. There are also numerous advantages of using non-viable microorganisms in metal-laden industrial effluents. Inactive cells are not subject to metal toxicity, nor do they need nutrients supply and culture maintenance.

Moreover, the dead or denatured cells can be cheaply obtained in large quantities as byproducts of the biotechnology industries. The chemical composition of microbial cell walls or envelopes largely determines the sorptive property of non-viable microorganisms (Volesky, 1987). The cell walls and envelopes of microbial biomass are mainly composed of polysaccharides and proteins, and offer abundant ligands for metal interaction, eg. carboxylate, hydroxyl, sulphhydryl, phosphate and amino groups. Surface biosorption, a process based on ion-exchange, coordination, complexation, chelation, adsorption and microprecipitation, occurs relatively rapidly and can be reversible (Ting *et al*, 1991; Volesky, 1987).

1.6 MICROBIAL SORBENTS

In evaluating a commercially viable biosorbent, the following criteria can be applied (Hughes & Poole, 1989):

1. The organism must be immobilized or granulated readily;
2. It must have a metal removal efficiency over 99%;
3. It must have high surface binding capacity;
4. It must be effective over a wide pH range;
5. The metal must be removed readily (by treatment with acid or an appropriate ligand), and the organism must be unaffected by at least 100 recycles;
6. The process must be cheaper than ion exchange or solvent extraction methods.

The sources of microbial sorbents so far documented can be classified into 3 groups: algae, fungi and bacteria.

Fungal mycelial byproduct, such as *Rhizopus arrhizus*, *Mucor miehei* and *Penicillium chrysogenum*, from fermentation industries has a considerable affinity for metals (eg Zn, Cd, Ni, Pb, Cr⁶⁺ and Ag⁺) and can be used in treatment of metal-laden effluents (Fourest *et al*, 1994). The maximum metal uptake of 0.97mmol/g for Pb was observed at pH 7. pH

neutralization during metal sorption enhanced Zn sorption by these fungi (0.57, 0.52 and 0.33mmol/g, respectively), compared with the metal uptake values (0.24, 0.08 and 0.05mmol/g, respectively) at natural pHs (5.8, 3.9 and 4.0, respectively).

The derived cell-wall product from *Bacillus subtilis* exhibits high metal uptake capacities for most metals on bench scale (Beveridge, 1986). *Pseudomonas mendocina* AS302 was selected out of 80 different strains by using a metal enrichment-screening test showing a high sorptive capacity for several metals without specificity (Dies *et al*, 1995). It was found to accumulate as much as 108mg/g of uranium, 75mg/g of gold and 24mg/g of nickel in batch tests.

Another inexpensive source of biosorbents, which is available in copious quantities, comes from the oceans as seaweeds, representing many types of marine macroalgae (Volesky & Holan, 1995). Brown algae in particular are suited for binding metal ions, probably due to their high polysaccharides content (Holan & Volesky, 1994). *Ascophyllum nodosum* exhibits as high sorptive capacity for lead (210mg/g), demonstrating better performance than that of the ion-exchange resin IRA-400 (Volesky & Holan, 1995). The biosorption has two phases: a fast (<4 seconds) reaction and a much slower metal intake (2 hours). The first phase is attributed to surface sorption, mainly based on ion exchange with the participation of the carboxyl groups of uronic acids. The second phase represents the internalization of metal ions to cell structure (Volesky & Holan, 1995). Cu ions was found to be adsorbed not only by ion-exchange but also by additional covalent bonding with the carboxyl groups (Crist *et al*, 1990).

Various species of yeast, eg. *Saccharomyces cerevisiae*, also have the ability to sequester a wide range of heavy metals. The brewer's yeast strain of *S. cerevisiae* possessed the most pronounced ability to remove uranyl ions from solution in both its living and dead form. Metal uptake values of the later were comparable to the values observed for dead *R. arrhizus* and other *Rhizopus* species (Volesky & May-Phillips, 1995). The non-viable brewer's yeast was found to have accumulated 0.4mmol uranium/g cells at an equilibrium concentration of 0.7mM at the optimum pH 4-5 on batch scale, whereas the binding of Cd, Zn and Cu appeared to be relatively weak, as indicated by the initial slopes of the corresponding

isotherms (Volesky & May-Phillips, 1995). Dead Baker's yeast (Lallemand) exhibited a high uptake of Zn (0.47 mmol/g) at an equilibrium concentration of 2.6mM and a low uptake of Cu (< 0.1 mmol/g) (Volesky & May-Phillips, 1995). Accumulation of Co, Cu and Cd from aqueous solutions by live cells *S. cerevisiae*, during a biphasic process, was reported (Norris & Kelly, 1977; Brady & Duncan, 1994 b). The first, which was rapid and metabolism-independent, was metal binding to the cell surface; this was followed by metabolism-dependent, progressive uptake of relatively large amounts of metals. A granular biomass derived from waste *S. cerevisiae* of fermentation industries, by heating and alkali and treatment, was developed by Brady *et al* (1994). The granular biomass was effectively used in fixed-bed reactors to recover heavy metals from artificial solutions and industrial wastewater.

The cell wall of *S. cerevisiae* contains abundant glucan, mannan and protein, and trace of chitin. Each of the major cell wall components contributes specific binding ligands such as carboxylate, hydroxyl, phosphate and amino groups (Stoll, 1996). Of particular interest are the negatively charged carboxyl groups considered to be the dominant binding sites on surface of cell wall for heavy metals sorption (Ashkenazy *et al*, 1996).

Assessment of sorption performance for existing biosorbents are crucial in selecting the industrially-suitable ones for application. Experimentation and preliminary testing of a solid-liquid sorption system are usually based on two types of investigations: (1) equilibrium batch sorption tests and (2) dynamic continuous-flow operations (Volesky & Holan, 1995). Two widely accepted equilibrium sorption isotherm models for single solute systems used in the literature are the Langmuir and Freundlich equations, which will be discussed in detail in next chapters.

1.7 PLANT SORBENTS

A variety of biomass derived from plants reportedly have the potential to absorb and adsorb heavy metals from aqueous mediums. Among them, the bark of *Pinus sulvestris*, sphagnum moss peat, leaf mould, water hyacinth eg. *Potamogeton lucens* exhibited the most pronounced

metal uptake capacities for removing both cation and anion heavy metals (Table 1.3-1.8). The main advantages of using plant-derived biomass is twofold: (1) They are abundantly available in nature thus the cost can be even lower than those from other biological sources; (2) They are generally water-insoluble and their granules possess the desired mechanical strength and rigidity therefore avoiding tedious procedure of immobilization for a continuous-flow process.

In Table 1.3 - 1.8, only the data from sorption of Cd, Cr³⁺, Cr⁶⁺, Ni, Zn and Cu, which are commonly encountered in electroplating effluents, are included. Although the experiment conditions on which the data were obtained were varied, a fact can be reflected that some potential plants, especially aquatic species, can take up as many heavy metals as microorganisms do.

Table 1.3 Cd uptake capacity of various sorbents

Sorbent	X _m (mg/g)	pH	Max.conc. (mg/l)	Dose (g/l)	Reference
Activated carbon	11.1	6.3-7.2	100	5	Larsen & Schierup, 1981
Straw	7.7	6.3	100	5	Larsen & Schierup, 1981
Peat (eutrophic & oligotrophic)	20.2-22.5	5.6	1124	50	Gosset <i>et al</i> , 1986
Rice hulls	21.4	-	40	-	Roy <i>et al</i> , 1992
<i>Potamogeton lucens</i>	61.4	5.0-6.6	-	2	Schneider & Rubio, 1995

X_m maximum metal uptake capacity.

Max. conc. maximum metal concentrations used in the experiments.

Dose sorbent concentration.

- not specified

Table 1.4 Cr³⁺ uptake capacity of various sorbents

Sorbent	X _m (mg/g)	pH	Max.conc. (mg/l)	Dose (g/l)	Reference
Iminodiacetic acid-cellulose	48.9	5	80	1.5	Chan <i>et al</i> , 1992

Table 1.5 Cr⁶⁺ uptake capacity of various sorbents

Sorbent	X _m (mg/g)	pH	Max.conc. (mg/l)	Dose (g/l)	Reference
Activated carbon (Filtrisorb-500)	145.0	2.7	1000	4	Sharma & Forster, 1994
Activated carbon (Filtrisorb-500)	57.7	-	250	-	Huang & Wu, 1977
Coconut shell- based activated carbon	20	2.5	80	-	Alaerts <i>et al</i> , 1989
Sphagnum moss peat	119.0	1.5	1000	4	Sharma & Forster, 1993
Leaf mould	43.0	2	1000	4	Sharma & Forster, 1994
Saw dust	10.1	4.0	>800	60	Bryant <i>et al</i> , 1992
Compost	101	4.2	500	-	Sharma & Forster, 1994
Rice hulls	164.3	-	410	-	Roy <i>et al</i> , 1992

Table 1.6 Ni uptake capacity of various sorbents

Sorbent	X _m (mg/g)	pH	Max.conc. (mg/l)	Dose (g/l)	Reference
Activated carbon	8.1	6.5-7.3	100	5	Larsen & Schierup, 1981
Straw	6.4	6.5	100	5	Larsen & Schierup, 1981
Peat (eutrophic & oligotrophic)	11.2	4.5	587	50	Gosset <i>et al</i> , 1986
Iminodiacetic acid-cellulose	20.8	7	80	1.5	Chan <i>et al</i> , 1992
<i>Potamogeton lucens</i>	22.9	5.0-6.6	-	2	Schneider & Rubio, 1995
<i>Salvinia herzogii</i>	14.4	5.0-6.6	-	2	Schneider & Rubio, 1995
<i>Eichhornia crassipes</i>	11.6	5.0-6.6	-	2	Schneider & Rubio, 1995

Table 1.7 Cu uptake capacity of various sorbents

Sorbent	X _m (mg/g)	pH	Max.conc. (mg/l)	Dose (g/l)	Reference
Activated carbon	13.8	5.4-5.9	100	5	Larsen & Schierup, 1981
Straw	6.4	5.4-5.0	100	5	Larsen & Schierup, 1981
Peat (eutrophic & oligotrophic)	12.1	4	635	50	Gosset <i>et al</i> , 1986
Saw dust	21	5.5	>600	60	Bryant <i>et al</i> , 1992
Iminodiacetic acid-cellulose	20.5	4	80	1.5	Chan <i>et al</i> , 1992
Water hyacinth roots (<i>Eichhornia crassipes</i>)	20.9	5.5	200	5	Low <i>et al</i> , 1994
<i>Potamogeton lucens</i>	40.8	5.0-6.6	-	2	Schneider & Rubio, 1995
<i>Salvinia herzogii</i>	19.7	5.0-6.6	-	2	Schneider & Rubio, 1995
<i>Eichhornia crassipes</i>	23.1	5.0-6.6	-	2	Schneider & Rubio, 1995

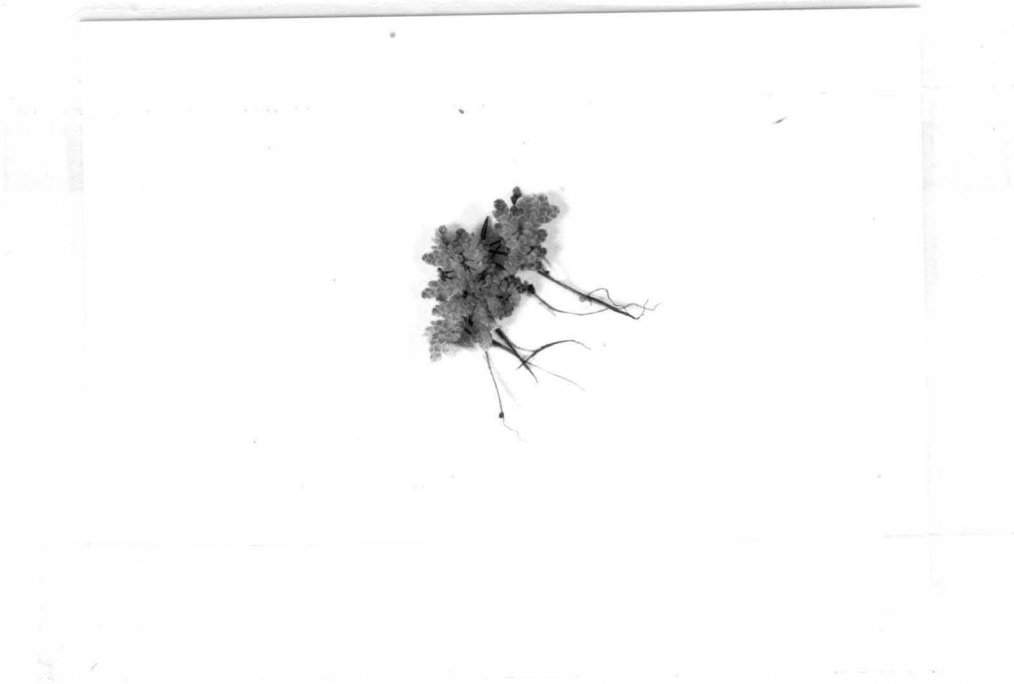
Table 1.8 Zn uptake capacity of various sorbents

Sorbent	X _m (mg/g)	pH	Max.conc. (mg/l)	Dose (g/l)	Reference
Activated carbon	6.2	6.3-6.8	100	5	Larsen & Schierup, 1981
Straw	5.3	6.3	100	5	Larsen & Schierup, 1981
Peat (eutrophic & oligotrophic)	11.1	6.7	654	50	Gosset <i>et al</i> , 1986
Modified bark	43.5	6.1	1045	10	Garballah & Kilbertus, 1994
<i>Potamogeton lucens</i>	32.4	5.0-6.6	-	2	Schneider & Rubio, 1995
<i>Salvinia herzogii</i>	18.1	5.0-6.6	-	2	Schneider & Rubio, 1995
<i>Eichhornia crassipes</i>	19.2	5.0-6.6	-	2	Schneider & Rubio, 1995

Azolla is a genus of small aquatic ferns containing a symbiotic blue-green algae. This fern-algae association has been shown to be capable of sustaining growth on nitrogen free media. *Azolla* is of interest not only as a botanical curiosity, but through its importance in nature, both as a weed and as a fertilizer. In areas where water resources are limited, the growth of aquatic plants has caused some concern. Of particular interest are free-floating plants such as *Azolla*, *Salvinia*, and *Eichhornia* which are able to colonize areas of open water. These plants can form dense mats over water surfaces on which terrestrial plants are able to grow to form a type of sudd vegetation. This is undesirable since the decreasing area of open water imposes limitations on water usage and exerts negative effects on the oxygen content of the water and harms the aquatic ecology (Ashton & Walmsley, 1976). Unlike *Salvinia* and *Eichhornia*, *Azolla* can fulfill its nitrogen requirement through the assimilation of atmospheric nitrogen by its algae symbiot. Potentially *azolla* can easily colonize water bodies which are deficient in nitrogen and unsuitable for growth of other aquatic plants.

An *azolla* plant consists of a short, branched, floating stem, bearing roots which hang down into the water (Fig 1.1). The stem and branches are covered with small, alternate, overlapping leaves. Each leaf is bilobed, the upper lobe contains the green pigment chlorophyll, whilst the lower lobe lacks chlorophyll and is colourless. The algae, *Anabaena azollae*, is closely associated with the apical meristem of the fern and grows in union with the fern. As leaf primordia are formed by the meristem, filaments of the algae become entrapped within the developing cavities in the dorsal lobes of each leaf. The algae is restricted to this micro-environment during its growth and dies off when the leaf senesces (Ashiton, 1982).

A



B

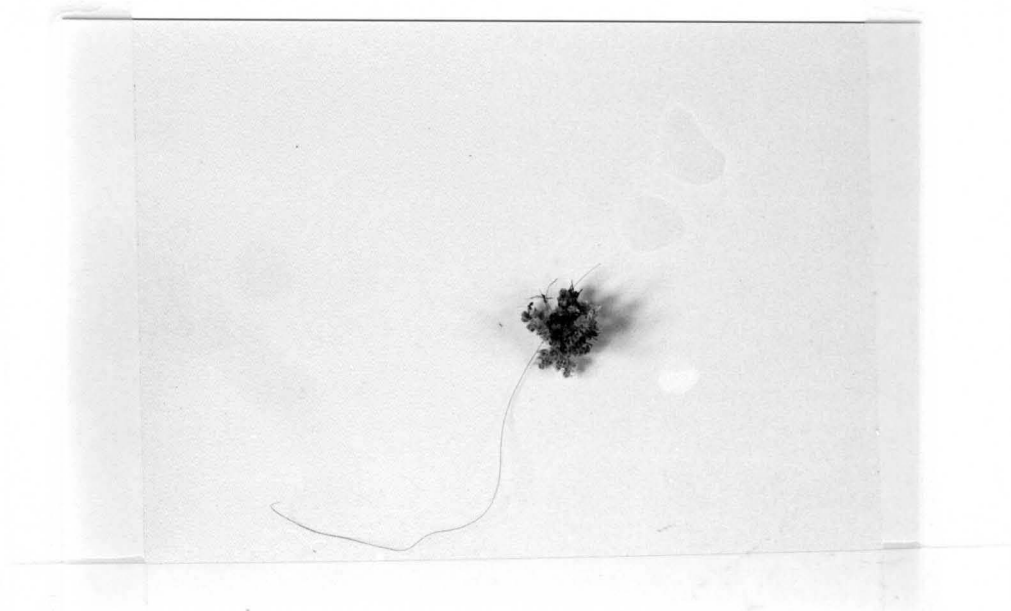


Figure 1.1 Appearances (10 x magnification) of fresh (A) and dried (B) *Azolla filiculoides*.

It has been known that the azolla absorb a wide range of nitrates, phosphates and other nutrients (Priel, 1995). Reports on utilizing azolla species in removing toxic metals have recently demonstrated that heavy metals can also be accumulated from either polluted water or soil by absorption into the plants. *Azolla pinnata* was used to reclaim mercury-contaminated soil by Mishra *et al* (1987). The plant was inoculated on surfaces of water in which the contaminated soil had been added. Up to 54.5% of the mercury in the soil was effectively either taken up or converted into a volatile form by direct or indirect action of the azolla. Use of the aquatic plant in absorbing metals from waste water has also been documented (Jain *et al*, 1989). After incubation with 1-8ppm Fe and Cu for 14 days, 9.7mg Fe and 7.7mg Cu were taken up by one gram of biomass, showing the concentration factors of 68.9 and 58.4 respectively.

The application of the aquatic plants for remediation of metal-laden waste water, by means of absorption, is limited (Priel, 1995), due to the slow process of removing metals in the ponding system and the difficulties in regeneration of large amounts of exhausted biomass. Attempts have been made to use the dried form of azolla in filter containers and it was deemed a more efficient process in removing metals from waste water than a ponding system (Priel, 1995).

1.8 RESEARCH AIMS

A detailed knowledge from previous studies of the mechanistic and biochemical aspects of heavy metal removal by non-viable yeast cells was the basis for an attempt to explore the possible application of yeast biomass in a fixed-bed column system for treatment of metal-laden effluents. The investigation was focused on developing an economically-viable immobilizing methodology for preparing pellets of potential microorganisms, yeast in this case, for column operation. The column performances of the yeast pellets in terms of metal uptake, stability and reusability were examined.

The second aim of this research was to explore the potential application of the water fern, *Azolla filiculoides*, for removal and recovery of heavy metals from electroplating effluents. The project was stimulated by evidence that *Azolla filiculoides* was able to absorb metals from polluted aqueous environments. Due to the meagre information concerning the sorption performances of the water fern, the following aspects were investigated:

1. Maximum metal uptake capacities, sorptive kinetics and pH dependence of the sorption isotherms, in batch experiments.
2. Column performances of the azolla biomass, with respect to metal uptake, effects of pHs, flow rates and mass of sorbent, desorption and recovery, regeneration and reusability of the biomass, and finally attempts at recycling of the metals and water.
3. Partial characterization of the azolla biomass.

It was hoped that detailed information of metal removal and recovery by *Azolla filiculoides*, obtained in this project will facilitate the implementation of a novel alternative bioremediation process.

2. SORPTION OF METALS BY FORMALDEHYDE CROSS-LINKED YEAST BIOMASS

2.1 INTRODUCTION

The yeast, *Saccharomyces cerevisiae*, has previously been shown to accumulate heavy metals from water (Norris & Kelly, 1977; Brady & Duncan, 1994 b; Brady *et al*, 1994). As a waste product of numerous fermentation industries it may be inexpensively obtained in large quantities and, providing a reliable source for large-scale application in bioremediation processes.

Microbial biomass consists mostly of small cells or particles of low density, poor mechanical strength and little rigidity. Use of the native biomass in conventional processing operations for treatment of metal-bearing waste water is not satisfactory because of solid/liquid separation problems. It is generally agreed that immobilization of the biomass can bestow ideal size, mechanical strength, rigidity and porous characteristics to the microbial materials (Brierley & Brierley, 1993).

2.2 EXISTING TECHNIQUES FOR IMMOBILIZING MICROORGANISMS

The methods for immobilizing microbial cells can be classified into three categories: entrapping, cross-linking and carrier-attaching methods. Of these, the entrapment within polymers has been most extensively investigated.

2.2.1 ENTRAPMENT IN POLYMERS

In alginate: Immobilization of *S. cerevisiae* with alginate for metal removal was investigated by de Rome & Gadd (1991) using 1.5% w/v of alginic acid and baker's yeast. The alginate-yeast suspension was pumped through a capillary tube (internal diameter 1mm)

and dropped into a solution of 50mM CaCl₂ in which the alginate gelled as beads. The beads were deemed as stable and water-insoluble. The entrapped yeast showed good metal uptake capacity in batch operation with 166µmol/g for Cs, 41µmol/g for Sr and 345µmol/g for U. Desorption of bound metals from the immobilized yeast with mineral acids was efficient, with 80% recoveries for U, but only 5-10% for Sr and Cs. In a continuous-flow system the alginate-entrapped yeast only showed decreased efficiency in uptake down to 30-70% of those recorded for batch tests. It was noted that prolonged washing with mineral acids destroyed the integrity of the beads leading to loss of yeast cells. A detrimental effect of high pH on the alginate system has also been reported (Klein & Vorlop, 1985). The alginate itself has been known as a good adsorptive matrix for metal accumulation (Sag *et al*, 1995). It was observed that a great amount of Hg and Cd was accumulated by cell-free alginate matrix, which exhibited percentage metal removal values equivalent to those of the free biomass and exceeded those of alginate entrapped *Chlorella homosphaera* (Tobin *et al*, 1993). Results from a gold sorption study indicated that 40% Au recovery was associated with the presence of the alginic matrix (da Costa & Leite, 1991).

In silica gel: The use of silica-entrapped algal cells in column operation for sequestering trace amounts of Pb, Cu, Zn and Cd was evaluated by Mahan & Holcombe (1992). The results from column adsorption showed that Pb and Cu were preferentially extracted from aqueous solutions, while Cd and Zn were weakly bound to the resin and exhibited better metal uptake only in the solutions that contained lower amount of light metals such as Ca, Mg, Na and K ions. The competitive effects of Ca, Mg, Na and K ions on removal of heavy metals (Zn and Cd) by the silica-algae biomass were marked and it could be concluded that silica-immobilized biomass has little selectivity for metal removal from waste waters.

In polyvinylformal (PVF): Immobilized within a PVF matrix, inactive mycelia of *R. arrhizus* showed preferential capacity of metal uptake to that of native microbial cells (Tzezos & Deutschmann, 1990). The PVF solution was prepared by adding 1g PVF powder to 20ml of dichloromethane followed by mixing with 1g of native biomass. Following solvent evaporation the biosorbent consisting of biomass embedded in a pliable PVF matrix was

obtained. A cells loading of 50% w/w resulted in a hard product that could be cut or ground to produce biosorbent particles with ideal sizes. The biosorbent with 50-80% w/w cell loading exhibited percentage removal of Cd approaching that of the free cells, whilst a 30% lower metal uptake by PVF immobilized *R. arrhizus* was shown in a uranium removal experiment (Tzezos & Deutschmann, 1990). The immobilization of biomass with PVF did not markedly block or hinder binding of metals to the microbial cells, nor did it contribute to the uptake of Cd ion (Tobin *et al*, 1993). The PVF appears to act as an inert and non-inhibitory supporting matrix with which high metal uptake capacity and satisfactory mechanical strength can be maintained in a continuous-flow process for treatment of metal-bearing effluents.

In polyvinylalcohol-Na-orthophosphate (PVA-P) and polyvinylalcohol-Na-alginate (PVA-A). The immobilization of *S. cerevisiae* with PVA-P and PVA-alginate were prepared by Stoll & Duncan (1997). Those two PVA-based beads fulfilled the necessary physical requirements, viz. that of mechanical strength, rigidity, and porosity. The Cu-accumulating property of PVA-A immobilized yeast in a column operation exceeded that of the PVA-P type showing a value of Cu uptake of 5mg/g from 1 litre solution, while the PVA-P only removed Cu at 1mg/g.

In polysulfone: A commercialized biosorbent, designated BIO-FIX was fabricated and utilized to remove metal contaminants from acidic mine waste waters (Jeffers & Corwin, 1993). The BIO-FIX beads were prepared by dissolving high density polysulfone pellets in dimethylformamide, blending dried *Sphagnum peat moss* into the solution and injecting the slurry into water. The beads prepared from a solution containing 100g/l polysulfone and 250-300g of biomass per litre exhibited a suitable combination of sorptive kinetics and physical stability. Batch and continuous tests demonstrated that BIO-FIX readily sorbed As, Cd, Pb and other toxic metals from acid mine drainage waters. Selectivity for toxic heavy metals over Ca and Mg was demonstrated. The beads exhibited excellent metal sorption and handling characteristics in stirred tanks, column reactors, and a low-maintenance passive system. Cyclic tests indicated that the beads continued to extract metal ions after repeated

loading-elution cycles.

In polyethyleneimine-glutaraldehyde (PEI-GA): A *Bacillus* species, which is a byproduct from an enzyme producer, was treated with hot NaOH, and immobilized using PEI and GA (Brierley & Brierley, 1993). The best Cu-loading (154mg/g) onto immobilized *Bacillus* in batch test was obtained with a PEI:GA:biomass ratio of 2:1:40. The loading was over double that obtained with the same PEI:GA:biomass ratio using biomass that was not treated with caustic prior to immobilization. Lower Cu-loading (88mg/g) was found with increased biomass fraction at a ratio of 2:1:150, indicating an enhanced effect by PEI-GA immobilization on Cu binding.

By applying the same procedure, *S.cerevisiae* was immobilized with a PEI:GA:biomass ratio of 1.2:1:40 and utilized in column operations for removal of Cu (Stoll & Duncan, 1997). Cu was effectively removed from solution with a Cu-loading of more than 10mg/g. The enhanced performance was observed in a reaccumulation cycle.

In polyacrylamide: Polyacrylamide is a widely used immobilization matrix for microbial cells for laboratory biosorption studies. By immobilizing *S. cerevisiae* in polyacrylamide gel and packing into columns, Cu, Co and Cd were removed from aqueous solutions yielding effluents with no detectable heavy metals (Duncan *et al*, 1995). In the use of Cu, a removal efficiency of 65% was determined with binding of 20mg Cu per g dry mass of yeast. The metals were eluted from the column using 0.1M HCl, with a desorption of > 90% being attained (Wihelmi & Duncan, 1995).

2.2.2 ATTACHMENT TO INERT SUPPORT OR SELF-FORMED PELLETS

Self-formed fungal pellets: An advantage with some fungal systems is that many can be grown in the form of pellets which have analogous properties to immobilized particles (White & Gadd, 1990). *R. arrhizus*, *P. italicum*, *P. chrysogenum* and *A. niger* were maintained on malt extract agar at 25°C and the mycelial pellets were harvested after 96 h culture by

filtration through a 63 μm nylon mesh. Excess water was removed by draining, followed by pressing with three changes of absorption paper. Pressed biomass of 10g was contained within an air-lift reactor and a thorium solution was supplied upwards at 200ml/h with an internal secondary circulation being supplied by an air-lift. Air-lift columns containing *R. arrhizus* and *A. niger* biomass were the most effective biosorbents with loading capacities of 0.5 and 0.6mmol/g respectively (116 and 138mg/g). The efficiency of Th sorption by *A. niger* was markedly reduced in the presence of other inorganic solutes while Th uptake by *R. arrhizus* was relatively unaffected (White & Gadd, 1990). However, the pellets eventually showed some disintegration resulting in an increased resistance to liquid flow, and similarities in density between the biomass and the liquid medium made continuous operation difficult (White & Gadd, 1990).

Biofilm on inert support: A technically simpler approach is the utilization of biofilms having metal sorption capacity (Cotoras *et al*, 1993). A PTFE column containing 150g of 3mm glass beads was packed with culture medium and inoculated with *Bacillus* sp. F1. The bioreactor was operated batchwise with medium recirculation. After incubation, the culture was drained from the column and the attached biomass was rinsed twice with distilled water. During the biosorption phase, a 0.2mM CuSO_4 solution was pumped through the bioreactor at a flow rate of 9ml/min. Cu was appreciably accumulated by the biofilm until it reached saturation. Desorption with H_2SO_4 solution at pH 1 appeared successful. However, the biofilm became saturated earlier in the 2nd cycle, indicating deteriorated biosorption capacity (Cotoras *et al*, 1995). The metal loading and desorption caused alterations of the bacterial surface, but the biomass remained attached on the inert support and it could be reused in a new sorption cycle.

This bacterial strain was also able to colonize different inert surfaces, namely PVC, polyethylene and polyethylene-tere-phthalate, graphite, volcanic minerals and porous ceramic. These results are encouraging and show that biofilm-based processes could be used for future industrial applications.

2.2.3 CROSS-LINKING WITH FORMALDEHYDE

Dried brown marine algae, *Ascophyllum nodosum*, demonstrated a high uptake of Cd and Pb (as much as 30% of the biomass dry weight) from aqueous solutions (Volesky & Holan, 1995). Application of the raw material in batch scale resulted marked leaching of cellular polysaccharides into the solution (de Carvalho *et al*, 1994). Therefore the raw material was cross-linked by formaldehyde-HCl (FA) before being applied to batch and column experiments to eliminate the leaching of soluble components like alginate from the cell wall (Schiewer *et al*, 1995). The procedure was preceded by adding 50g raw biomass in a mixed solution consisting of 1 part of 37% of formaldehyde and 2 parts of 3.7% of HCl (Schiewer *et al*, 1995). No appreciable loss of initial weight was observed during batch tests with the formaldehyde-HCl cross-linked algae.

Multiple criteria establish the basis for successful immobilization, i.e. maximum amount of biomass and minimum amount of immobilizing agent, metal uptake capacities in comparison with that of native forms, high porosity with good particle strength favourable for continuous processing and regenerability and durability in repeated use (Gadd & White, 1993). In addition to the technical requirements economic consideration are paramount in the exploitation of biomass in metal removal/recovery systems. It should be borne in mind that biosorbent immobilization may constitute an additional and significant economic cost which preclude its use in large scale application under certain conditions (White & Gadd, 1990). In some developing countries, reagents like polysulfone, polyacrylamide, polyethyleneimine and glutaraldehyde, etc., have to be imported from overseas therefore gel entrapment with such chemicals on industrial scale could be prohibitively expensive. Moreover, limits to rates of diffusion into particles may be another problem in gel entrapment (Duncan *et al*, 1995). In this regard the development of a simpler, low-cost microbial immobilization technology for continuous treatment of metal-laden waste water was therefore undertaken in present study.

2.3 METHODS AND MATERIALS

2.3.1 MATERIALS

The yeast, *S. cerevisiae*, was chosen as starting material because it has been shown to be able to accumulate a wide range of heavy metals and can be obtained cheaply as a byproduct of fermentation industries. An electroplating effluent, designated as EPE1, was collected from a factory (PE Plating, South Africa) operating a chromium-plating line (Table 2.1).

Formaldehyde, HCl, $\text{CuCl}_2 \cdot 7\text{H}_2\text{O}$ and ZnSO_4 were obtained from Saarchem, SA and CdSO_4 from Riedel-deHaen, Germany. All the chemicals were analytical grade.

Table 2.1 Levels of metals present in EPE1, compared to stipulated national drinking water and aquatic ecosystem criteria (mg/l) (Kempster *et al*, 1980)

	pH	Cu	Zn	Cd	Cr^{6+}	Ni
Drinking water	Neutral	1.0	5.0	0.01	0.05	0.05
Dam/ River	Neutral	0.005	1.0	0.003	0.05	0.05
EPE1	7.1-7.3	< 0.8	0.8-1.2	< 0.05	29.0-31.0	4.0-5.0

2.3.2 IMMOBILIZATION

Formaldehyde was chosen as the cross-linking agent for yeast immobilization because of its low-price in the local market and the excellent fixative ability for biomaterials. The protocol is a modification of the method outlined by Schiewer *et al* (1995). Formaldehyde is a powerful reducing agent especially in the presence of alkali (Parker & Vaughan, 1958). It has been widely used for disinfecting (germicide and fungicide), coagulating rubber latex, hardening gelatin plates and rendering casein, albumin and gelatin insoluble (Merck Index, 1968). Formaldehyde tends to form polymerization products with itself while in solution and can develop some bifunctional polymers. The polymers can function as a cross-linking agent.

Cross-linking could also occur from the formation of ethers from the natural biosaccharides present in the biomass via aldehydes, formaldehyde, dialdehyde and acrolein (Volesky & Holan, 1995). Since the aldehydes are more readily oxidized when exposed to air, the presence of a mineral acid (eg 1% HCl) can stabilize the aldehydes in solution by a reversible reaction.

The cells were extensively washed with distilled water to remove any remaining alcohol. After being oven-dried at 60°C for 8 h, the yeast was crumbled to form granules of 3-5mm in diameter. The immobilizing solution consisted of 37% (w/v) FA mixed with 0.1M HCl at varying ratio. Various volumes of FA were mixed with 0.1M HCl to obtain the required FA concentrations ranging from 1 to 15% (w/v) in 0.1M HCl. The immobilizing solution of 130ml was added to 100g pellets (right above the biomass) and incubated them for 5-10 minutes at room temperature. The pellets were again dried at room temperature after the reaction was completed. The consumption of immobilizing solution was approximately 78ml per 100g dried yeast.

2.3.3 DETERMINATION OF STRUCTURAL PROPERTIES

Scanning electron microscopy (JEOL JSM 180 SEM) of gold coated biosorbent samples provided information regarding the porosity of the pellets and the surface area available for metal binding.

Subjective stability tests were performed on the cross-linked biomass. Mechanical strength of the pellets was estimated by resistance to degradation and structural damage as a result of high hydrodynamic pressure in column operations. Water solubility of the pellets was evaluated by incubating them in water for 5 days. Poor stability was reflected by disintegration of the pellets after they were used in column operation, whilst stable preparation retained their integrity.

2.3.4 METAL DETERMINATION

Concentrations of total Cr, Cu, Zn and Cd were determined by atomic absorption spectrophotometry (GBC Scientific Equipment, Australia). The Cr^{6+} was measured colorimetrically using 1,5-diphenyl carbazide (Saarchem, SA) method (Anon, 1985). In an acid solution, Cr^{6+} reacts with 1,5-diphenyl carbazide to give a red-violet colouration, which can be determined by measuring absorbance at $\lambda = 540\text{nm}$ using a spectrophotometer (UV 160A, Shimadzu, Japan). The difference between the TCr and Cr^{6+} two values was taken as Cr^{3+} concentration.

2.3.5 ADSORPTION ISOTHERMS

Adsorption isotherms have commonly been used to describe experimental results for the uptake of metal ions by sorbent. The langmuir equation is the simplest model used for adsorption phenomena of one component, which relies on a postulated chemical or physical (or both) between solute and vacant sites on the adsorbent surface (Sag & Kutsal, 1996 b). In this study, batch sorption of Zn by FA-immobilized yeast pellets and native yeast were carried out at pH 6.0 and 18°C on a rotary shaker for five hours using 250ml conical flasks. A neutral pH of 6.0 was chosen because an optimum metal uptake could be attained without precipitation of the metal. A biomass of 4g/l and Zn solution of 100ml were used in all cases for both FA-pellets and raw yeast. The pH values of the solutions were set at 6.0 prior to the experiments, checked and adjusted every hour during the incubation by addition of either H_2SO_4 or NaOH. The isotherm studies were performed by varying the initial Zn concentrations (C_0) from 50mg/l to 600mg/l. After shaking the flasks for five hours, the reaction mixtures were filtered through Whatman no.1 filter paper and the filtrate was analysed for Zn concentration (C_e). The following Langmuir sorption model was employed for the estimation of maximum metal uptake (X_m) when these could not be reached in the experiment (Hayward & Trapnell, 1964; Holan *et al*, 1993):

$$C_e/q_e = 1/(X_m b) + C_e/X_m$$

Where X_m is indication of maximum sorption capacity and b is the Langmiur constant, the ratio of the sorption/desorption rates, related to energy of adsorption through the Arrhenius equation (Holan *et al*, 1993). q_e is the metal uptake (mg Cr/g of biomass) at equilibrium, and C_e is the equilibrium concentration of chromium (mg/l).

2.3.6 COLUMN OPERATION

Glass columns of 16mm internal diameter were used in the experiment. Yeast pellets of 10g (dry wt) immobilised by FA were rehydrated in distilled water for 1 hour and then packed into the column of 1.6 x 20cm (bed volume: 40.2ml). Feed solutions were pumped upwards through the column at a flow rate of 50ml/h. As precipitation of respective metals could occur at various pHs, the influent pHs were kept at the natural values of their solutions. The elute was collected in 10ml fractions. The working temperature was kept at approximately 18°C.

Metal-saturated yeast biomass was regenerated (desorbed) by washing the columns downwards with 120ml 0.1M HCl at a flow rate of 50ml/h. The metal uptake capacities of the biomass were defined as the amount of metals taken up by one gram of biomass at 60% saturation of the breakthrough curves. After acid washing, the column biomass was reconditioned with 0.05M NaOH (incubated for 5 min) to reverse the acidity of the biomass which was followed by two bed volumes of distilled water. The term of regeneration efficiency outlined by Martin and Ng (1987) was employed to evaluate reusability of the biomass. The regeneration efficiency was defined as the ratio of metal uptake of reaccumulation to that of the first cycle.

Scale-up of column removal of Cr^{6+} with 150g FA cross-linked yeast from EPE1 was performed using a perspex column of 3.6 x 49cm (bed volume: 499ml), at a flow rate of 1 l/h and pHs of 2.5 and 3 respectively.

2.3.7 DESORPTION OF Cr^{6+} WITH VARIOUS DESORBENTS

The first elutions were carried out downwards with 1M NaCl/0.05M NaOH or 0.1M NaOH respectively at a flow rate of 50ml/h. The second elution involved reduction of Cr^{6+} to Cr^{3+} by FA followed by desorption of the Cr^{3+} with 1N HNO_3 . Washes of the Cr^{6+} with 20ml portions of a mixture of 0.1% FA and 1N HNO_3 (mix immediately before use) were performed. The desorbing solution was left in the column for 6 h before being released. The batch washes were repeatedly carried out 4-5 times until the metal concentrations of the elute were below 100mg/l. Further washes of the used biomass with the acids could no longer effectively decrease the metal content in the elute.

2.4 RESULTS AND DISCUSSION

2.4.1 STABILITY OF THE CROSS-LINKED BIOMASS

The 13-15% FA/0.1M HCl cross-linked biomass yielded the most stable and water-insoluble pellets. The cell loading (weight of dried yeast before immobilization versus that after immobilization) was found to be 97.8%. Reuse of the pellets in column operation did not appear to affect the physical properties of the pellets while the granules were still rigid and retained integrity after 5 days incubation. Table 2.2 shows the diverse stabilities of the cross-linked biomass at varying ratios of FA to 0.1M HCl.

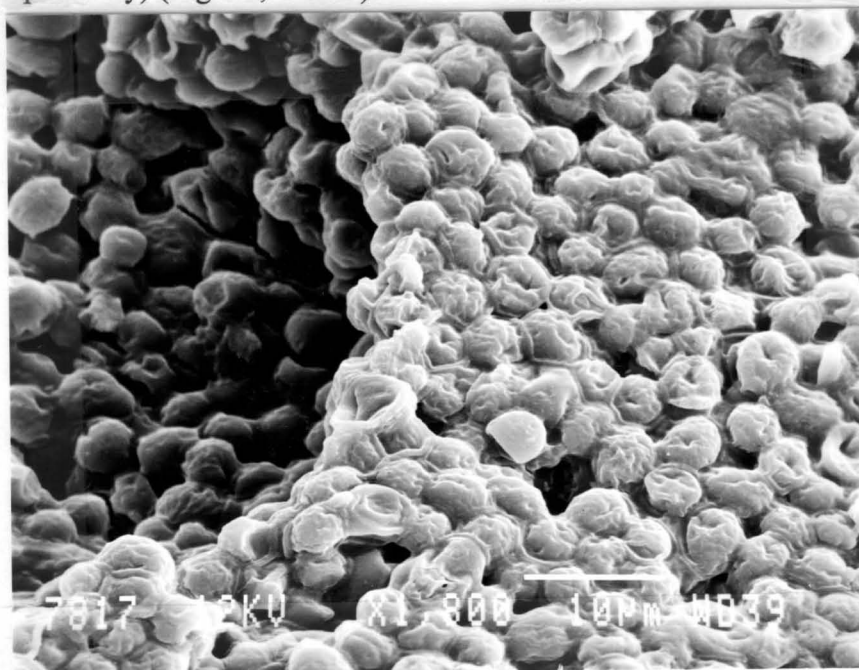
Table 2.2 Granule stabilities of the yeast biomass cross-linked with differing FA/0.1M HCl ratios

FA/0.1M HCl (%)	Rinse time (min)	Solubility in cross-linking solution	Solubility in water	Granule stability (subjective test)	Cell loading
1-8	10	Soluble	-	Not applicable	-
10.5	10	Slightly soluble	Insoluble	Hard	93.7
13	10	Insoluble	Insoluble	Hard	97.8
13	5	Insoluble	Insoluble	Hard	-
15	10	Insoluble	Insoluble	Hard	97.5
15	5	Insoluble	Insoluble	Hard	-

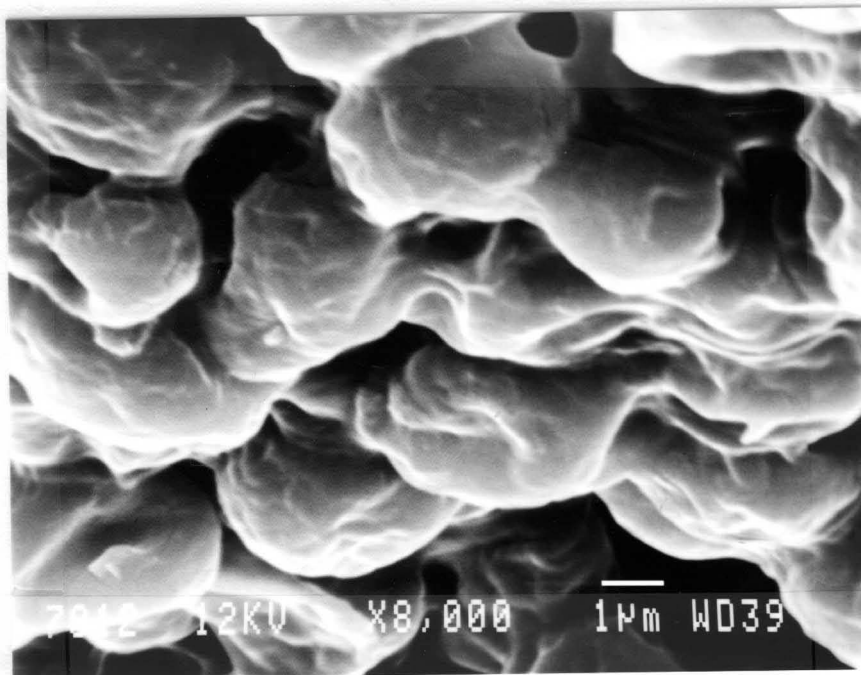
2.4.2 MORPHOLOGICAL STRUCTURE OF THE FA-CROSS LINKED YEAST

The formaldehyde cross-linked yeast biomass was visualized using SEM (Fig 2.1, A-D). The yeast cells were fixed to each other to form a close structured, yet porous aggregate, under the immobilizing conditions. At low magnification (1800 times) the formaldehyde cross-linked yeast biomass appeared as solid compact pellets (Fig 2.1, A). Under high magnification (8000 times) pores in the exterior of the pellets become apparent (Fig 2.1, B). No apparent morphological alterations were observed after uptake of Zn and Cr⁶⁺ from solutions (7.2mg/g and 5.1mg/g respectively) (Fig 2.1, C & D).

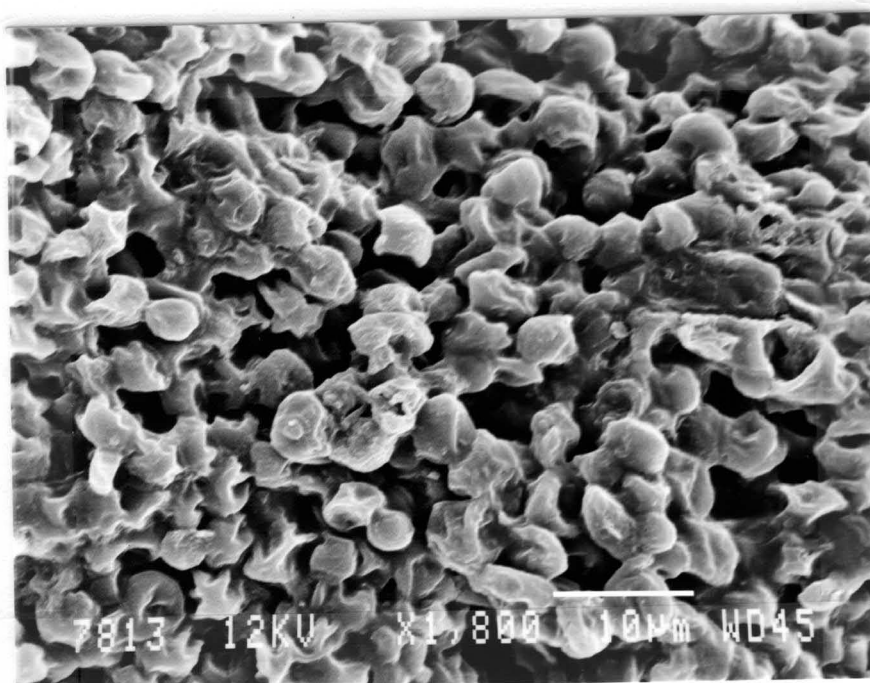
A.



B.



C.



D.

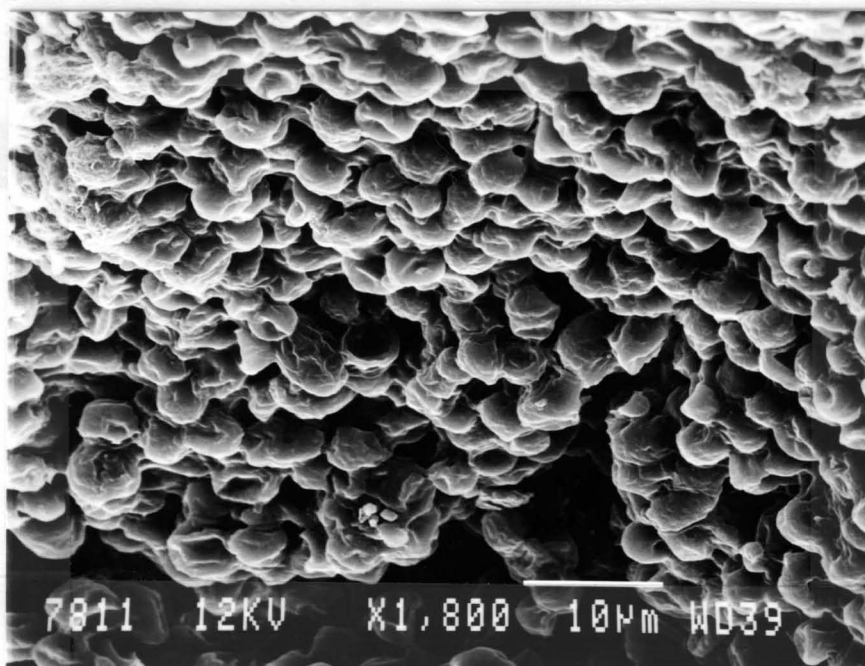


Figure 2.1 Scanning electron micrographs of the surfaces of yeast cells cross-linked by FA. (A), fresh yeast pellets, at 1800 x magnification; (B), fresh yeast pellets, at 8000 x magnification; (C), after exposure to Zn solution, at 1800 x magnification; (D), after exposure to Cr^{6+} solution, at 1800 x magnification.

2.4.3 COMPARISON OF SORPTION ISOTHERMS OF FA-IMMOBILIZED YEAST PELLETS WITH THAT OF RAW YEAST

Isotherm tests of FA-immobilized pellets and native yeast were carried out to show the effect of immobilization on metal sorption. The isotherm data showed good compliance with the Langmuir equation (Fig 2.2). The correlation coefficients, r^2 , for FA-pellets and native yeast, were 0.97 and 0.96 respectively indicating a strong positive relationship between the equilibrium Zn concentration, C_e , and the metal uptake, q_e . Maximum Zn sorption capacities of FA-pellets and raw yeast, based on the experimental data, were computerized to be 17.9 and 18.6mg/g respectively. The b values for FA-pellets and raw yeast were 0.0094 and 0.0124 respectively. No apparent decrease in metal uptake capacity of the FA-immobilized pellets was observed. The slight difference in their capacities could be due to the fact that the raw

yeast, which dissolved completely in the incubation solutions, presented larger surface area while FA-pellets remained in granule.

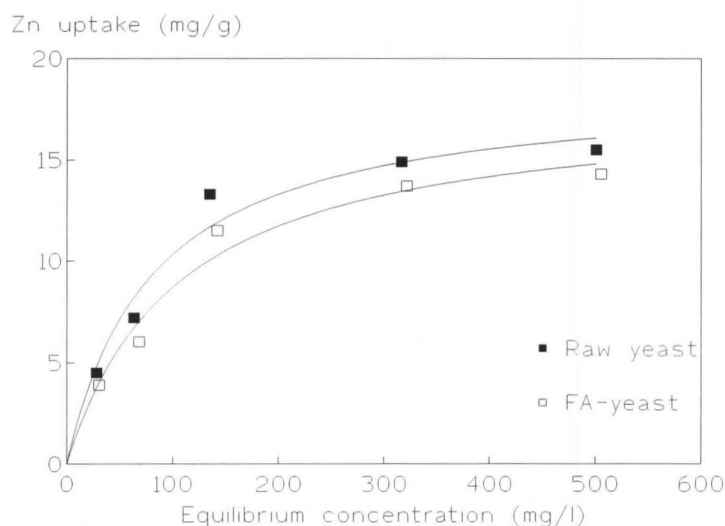


Figure 2.2 Comparison of sorption isotherms for Zn for FA-immobilized yeast and raw yeast systems. Temperature, 18°C; incubation time, 5 h; biomass, 4g/l.

2.4.4 COLUMN SORPTION OF Cu, Zn AND Cd FROM AQUEOUS SOLUTIONS

The metal cations were effectively removed from aqueous solutions by the biomass column at a flow rate of 50ml/h and the natural pHs of their solutions. The influent metal concentrations used in this experiment are around 100mg/l, which were commonly adopted by a number of researchers (Larsen & Schierup, 1981). The metal uptake of the biomass at 60% saturation for Cu, Zn and Cd were found to be 8.0mg/g, 7.1mg/g and 14.0mg/g respectively (Fig 2.3-2.5 and Table 2.3). Values of the metal uptake capacities can also be obtained on molar base (Table 2.3), giving 0.13mmol/g for Cu, 0.11mmol/g for Zn and 0.13mmol/g for Cd. The values, ranging from 0.11-0.13mmol/g might be adopted to estimate the concentration of binding sites on the surfaces of the biomass for these divalent cation metals, under the experiment conditions, regardless of respective atomic masses. The effects of pH changes in

column operations on the onset of the breakthrough for respective metals were significant indicating the ion-exchange nature of the biosorptive process. The decline of pH could provide an easy control measurement for termination of a column operation.

Table 2.3 Column removal of cation metals from aqueous solutions with 10g biomass at a flow rate of 50ml/h

Metal	Infl. metal conc. (mg/l) ^a	Infl. pH	Metal uptake (mg/g) ^b	Metal uptake (mmol/g) ^b
Cu	127.0	5.3	8.0	0.13
Cu ^c	127.0	5.3	8.1	0.13
Zn	130.7	6.1	7.1	0.11
Zn ^c	130.7	6.1	7.2	0.11
Cd	117.0	6.7	14.0	0.13

^a the influent metal concentrations were made at approximately 2mM for Cu and Zn, and 1mM for Cd.

^b at 60% saturation of the biomass.

^c cyc.2

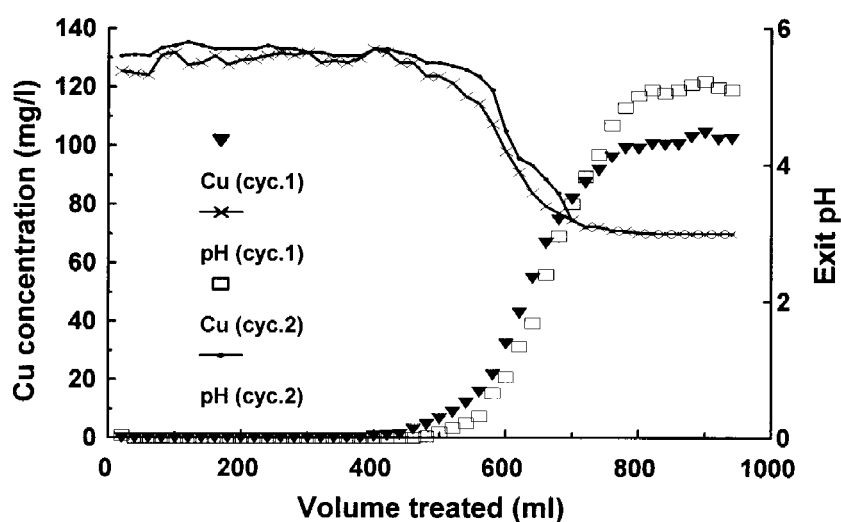


Figure 2.3 Breakthrough curves for repetitive removal of Cu from solution by 10g yeast. Flow rate, 50ml/h; infl. Cu, 127mg/l; infl. pH, 5.3; bed volume, 40.2ml.

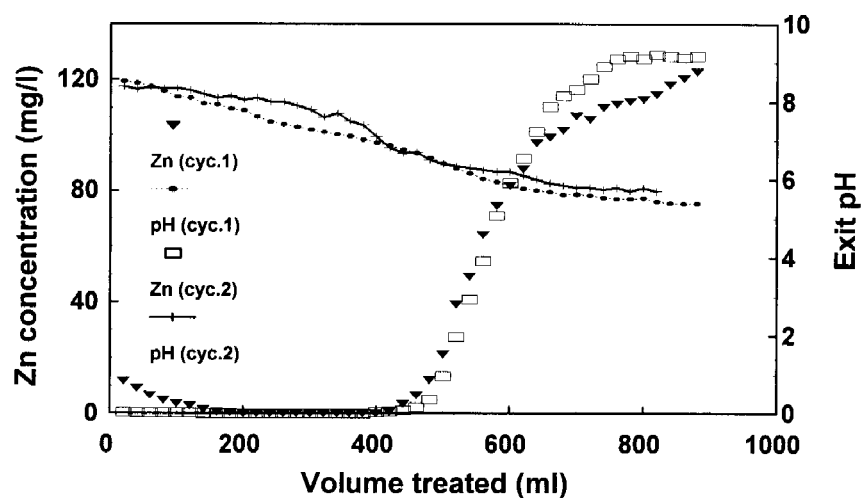


Figure 2.4 Breakthrough curves for repeated removal of Zn by 10g yeast from solution. Flow rate, 50ml/h; infl. Zn, 130.7mg/l; infl. pH, 6.1; bed volume, 40.2ml.

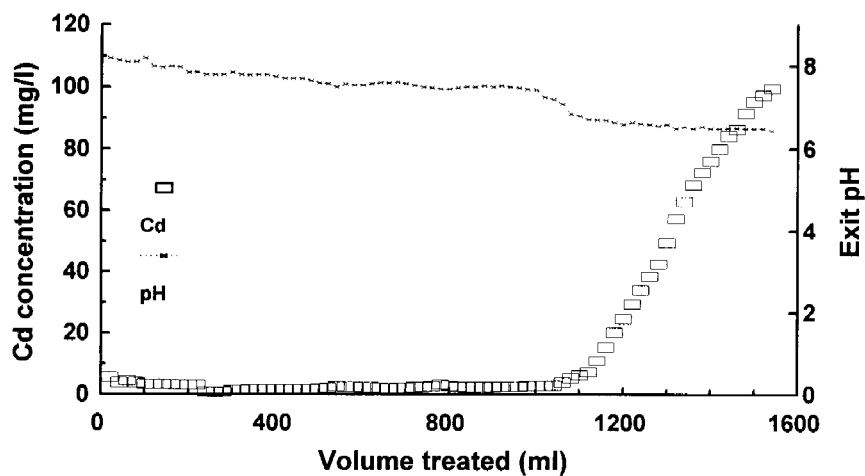


Figure 2.5 Breakthrough curve for removal of Cd by 10g yeast from solution. Flow rate, 50ml/h; infl. Cd, 112.4mg/l; infl. pH, 6.7; bed volume, 40.2ml.

2.4.5 DESORPTION AND RECOVERY OF CATION METALS

Recovery of 93-97% of the cation metals was accomplished using 0.1M HCl in volume of 120ml (Fig 2.6 and Table 2.4). The bound metals were desorbed and concentrated to levels as high as 1500mg/l for Cd and 1000mg/l for Cu and Zn. Recycling of them would thus be possible or, alternatively, precipitation of the concentrated metals can more easily be carried out. Reaccumulation of Zn and Cu by the regenerated biomass remained constant with the metal uptake of 7.2mg/g for Zn and 8.1mg/g for Cu demonstrating the potential for reuse of FA cross-linked yeast biomass in treatment of cation metal-laden wastewater (Figs 2.3 & 2.4 and Table 2.3). Reconditioning the column biomass with 0.05M NaOH also appeared to effectively reverse its acidity and maintained the metal uptake capacities.

Table 2.4 Cation metal recovery with 120ml of 0.1M HCl

Metal	Metal uptake (mg)	Metal desorbed (mg)	Recovery %	Regeneration efficiency %
Cu	87.1	81.0	93.0	100
Zn	76.7	74.8	97.5	100
Cd	144.9	138.5	95.6	-

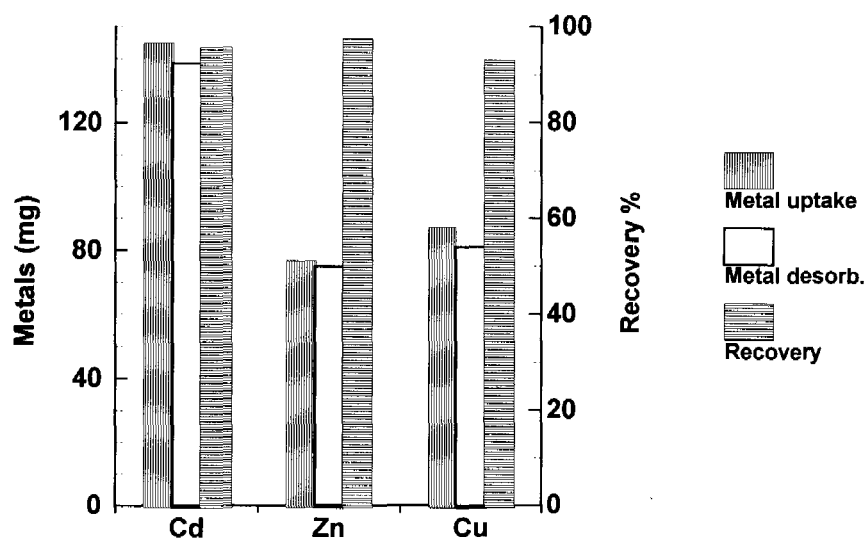


Figure 2.6 Recovery of cation metals from saturated yeast biomass with 0.1M HCl. Flow rate, 50ml/h.

2.4.6 COLUMN SORPTION OF Cr^{6+} FROM ELECTROPLATING EFFLUENT

The yeast pellets were applied to treatment of EPE1 which contains 30mg/l Cr^{6+} , at flow rate of 50ml/h and varying influent pHs. Removal of TCr (total Cr) by yeast biomass appeared to be highly pH-dependent (Fig 2.7 and Table 2.5). Enhanced TCr uptake was accomplished with decreasing influent pHs. Unlike cation metal accumulation, gradual breakthroughs of the TCr and Cr^{6+} at all pHs occurred in the column elution profiles (Fig 2.7). At an optimum influent pH of 2.5, the TCr uptake capacity, calculated from the breakthrough curves at 60% saturation, was 6.3mg/g while less metal was accumulated at increasing influent pHs (Table 2.5). However further accumulation of Cr^{6+} on the semi-saturated biomass can be expected before it reaches full saturation.

In the scale-up of the column operations substantial amount of TCr and Cr^{6+} had been accumulated and removed from EPE1 at 20% saturation at pHs of 2.5 and 3.0 respectively (Fig 2.8 & Table 2.5). It was seen that reduction of Cr^{6+} to Cr^{3+} occurred during the sorption

process, resulting in leaching of Cr^{3+} in the elute (Fig 2.8). The TCr concentration in the treated EPE1 was found to be 4mg/l which still exceeded the stipulated criteria for effluent discharge. Thus EPE1 was re-treated using the same procedure, but it did not eliminate the remaining chromium ions, since most of the chromium ions leached from the column in treatment 1 was Cr^{3+} , which obviously can not be effectively accumulated at such a low pH. The reduction of Cr^{6+} to its trivalent form, along with sorption, was found to exist in the removal of Cr^{6+} by sorbents such as activated carbon, sphagnum moss peat and leaf mould (Sharma & Forster, 1993, 1995, 1996). The phenomenon was once again revealed in the present study that there was appreciable amount of Cr^{6+} being converted by the yeast biomass at pH 2.5, probably due to some unknown reducing group on surface of the biomass or the remaining FA from cross-linking procedure. Little Ni from EPE1 had been removed during the column operation at low pH (Fig 2.8).

Table 2.5 Column removal of Cr^{6+} from EPE1

Infl. conc. (mg/l)	Infl. pH	Flow rate (ml/h)	Biomass (g)	Cr^{6+} removal (mg/g)	TCr uptake (mg/g) ^a	Volume treated (l) ^a
30	2.5	50	10	ND	6.3	3.1
30 ^b	2.5	50	10	ND	5.1	2.4
30	3.0	50	10	ND	5.0	2.3
30	4.0	50	10	ND	3.7	1.7
30	7.5	50	10	ND	0.3	0
30	2.5	1000	150	2.94 ^c	2.6 ^c	>15
30	3.0	1000	150	1.85 ^d	1.7 ^d	>10

^a at 60% saturation of the biomass.

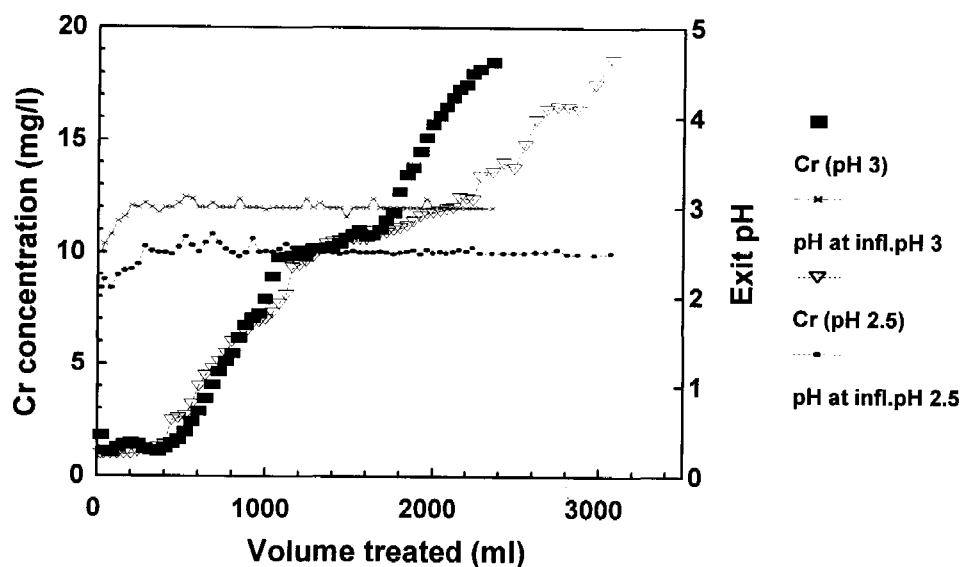
^b cyc. 2.

^c at 20% saturation of the biomass.

^d at 25% saturation of the biomass.

ND: not determined.

A.



B.

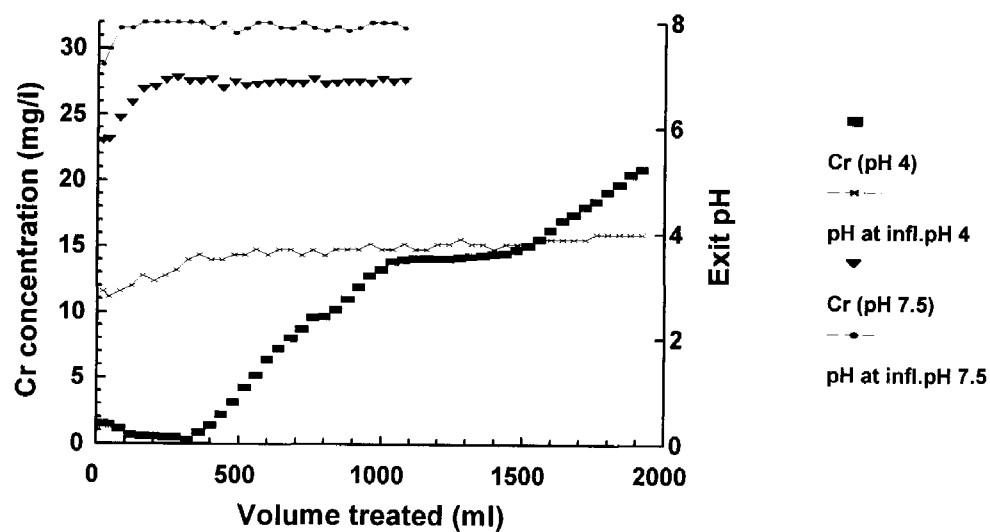


Figure 2.7 Breakthrough curves for column removal of Cr^{6+} by 10g yeast biomass from EPE1 at varying infl. pHs. (A), at pHs of 4 and 7.5; (B), at pHs of 2.5 and 3; flow rate, 50ml/h; infl. Cr^{6+} , 30mg/l; bed volume, 40.2ml.

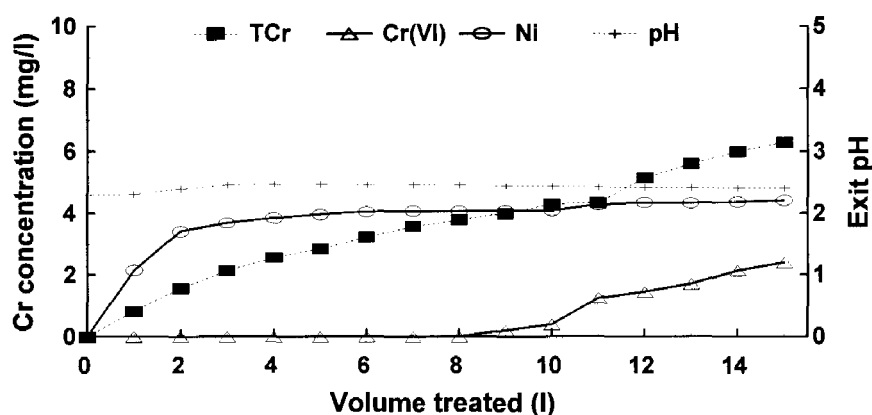


Figure 2.8 Breakthrough curves for scale-up 1 column removal of Cr^{6+} by yeast biomass from EPE1 at infl. pH 2.5. Biomass, 150g; flow rate, 1 l/h; infl. Cr^{6+} , 30mg/l; infl. Ni, 4.5mg/l.

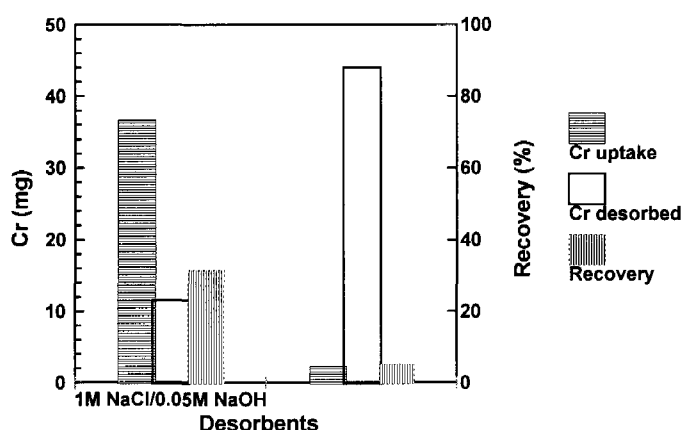
2.4.7 DESORPTION OF Cr^{6+}

2.4.7.1 DESORPTION WITH NaCl OR NaOH

After washing with 0.1M NaOH, very little Cr^{6+} (2.3mg) had been recovered with a recovery of only 5.2% (Fig 2.9 and Table 2.6). Furthermore the liquor leached from the column was brown coloured during the alkaline desorption and extraction of yeast constituents by alkaline was thought possible. Elution through the column with 1M NaCl/0.05M NaOH improved the desorption efficiency (11.6mg) but resulted only in a recovery of 31.5% (Fig 2.9).

Table 2.6 Chromium recovery with various desorbents

Desorbent	Cr ⁶⁺ uptake (mg)	TCr desorbed (mg)	Volume used (ml)	Recovery (%)	Regeneration efficiency (%)
1% FA/1N HNO ₃ ^a	49.7	35.9	120	72.2	83.7
1% FA/1N HNO ₃	41.1	23.1	120	56.1	-
1% FA/1N HNO ₃ ^b	393.8	203.4	1000	51.7	-
1% FA/1N HNO ₃ ^c	247.5	95.4	1000	38.6	-
1M NaCl/0.05M NaOH	36.7	11.6	120	31.5	-
0.1M NaOH	44.0	2.3	120	5.2	-

^a at 4 °C.^b scale-up 1^c scale-up 2**Figure 2.9** Desorption of Cr⁶⁺ with 1M NaCl/0.05M NaOH and 0.1M NaOH. Flow rate, 50ml/h.

2.4.7.2 REDUCTION AND DESORPTION OF Cr⁶⁺

The methodology was based on the assumption that Cr⁶⁺ which was bound to yeast biomass during sorption could be reduced by either certain reducing groups on the surface of the biomass or by introducing the reductant, eg. FA, and subsequently desorbed by acids. The

reason for using FA as a reductant in desorption was twofold: firstly it is a cheap and strong reductant; secondly it can further enhance the mechanical stability of the pellets for successive operations. The results of desorption with FA/1N HNO₃ is presented in Fig. 2.10 and Table 2.6. Among the regenerating agents the combination of FA and HNO₃ at 4°C exhibited the best desorbing capacity with a recovery of 72.2%, and a regeneration efficiency of 83.7%. Applying the same combination of FA and HNO₃ at 18°C, on small and large scale, resulted less Cr recovery (38-56%) than that at low temperature. The sorption of TCr onto biomass, eg sphagnum peat moss, is generally considered to be an endothermic, chemisorptive procedure (Sharma & Forster, 1993), the desorption of Cr therefore should be enhanced by lowering the reaction temperature. It would be worthwhile to further investigate the methodology for achieving better desorption of Cr ions, which in turn would facilitate the subsequent reuse of the biomass in treatment of Cr-laden waste effluents.

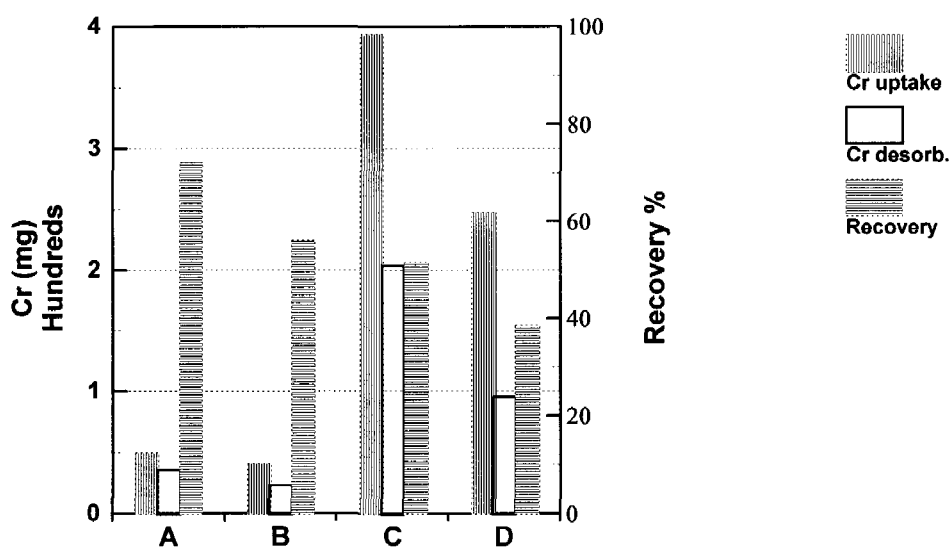


Figure 2.10 Recovery of Cr⁶⁺ from saturated yeast biomass with 0.1% FA/1N HNO₃. **A**, at 4° C; **B**, at 18°C; **C**, at 18°C, scale-up 1; **D**, at 18°C, scale-up 2.

2.5 CONCLUSIONS

This study presents a novel method in immobilizing *S. cerevisiae* as a biosorbent for treatment of metal-laden waste effluent, in which the immobilizing cost can be reduced to an affordable extent on industrial scale. The cost of immobilizing 1 kg dry yeast pellets was estimated at less than US\$1. The non-viable yeast cross-linked by 13% FA/1N HNO₃ exhibits satisfactory mechanical stability in continuous-flow operation. No apparent disruption of the biomass after reuse was observed. The Zn uptake capacity of FA-cross-linked pellets, on batch trials, remained similar to that of native yeast, reflecting that the immobilizing procedure did not hinder its metal removing capacity. In column studies, Cu, Zn and Cd were removed from aqueous solutions by the yeast pellets at the natural pH of their solutions. Recovery of more than 93% of the bound metals were achieved by washing the column biomass with 0.1M HCl. The recovered metals were concentrated in small enough volumes that recycling them can be carried out. The metal uptake capacity of the regenerated biomass remained constant in comparison with cycle 1 thereby reuse of the yeast would be possible.

In the case of Cr⁶⁺, a gradual breakthrough curve of Cr in column profile was noted, with which reduction of Cr⁶⁺ to Cr³⁺ occurring. However, Cr⁶⁺ in EPE1 can be significantly minimised either by accumulation onto the biomass or reduction to its trivalent form so that detoxification of Cr⁶⁺-laden wastewater can be accomplished. Desorption of bound Cr⁶⁺ with either alkaline or salt was not accomplished. A combination of reduction and desorption with FA/HNO₃ however, appeared promising in regeneration of the saturated biomass at 4°C, whereas applying the same procedure at room temperature did not show satisfactory results, reflecting the thermochemical effect on binding of the metal to yeast biomass.

3. BATCH REMOVAL OF Cr^{6+} FROM AQUEOUS SOLUTION BY *Azolla filiculoides*

3.1 INTRODUCTION

Chromium is one of the most toxic heavy metals present in industrial waste water. Two forms of chromium, hexavalent and trivalent, are often identified in electroplating and tanning factories, and the former is generally thought as the more toxic and mutagenic (Coleman & Paran, 1991). Cr^{6+} is typically present as an anion, either chromate (CrO_4^{2-}) or dichromate ($\text{Cr}_2\text{O}_7^{2-}$), in aqueous solution. In practice, the anion species are normally reduced to trivalent chromic ions and then precipitated as chromic hydroxides using $\text{Ca}(\text{OH})_2$. However, it is a process which only gives incomplete removal of chromium, has a high chemical requirement and produces a voluminous, toxic sludge which may pose disposal problems. Although ion-exchange and activated carbon adsorption are other available options, relatively high capital investment and maintenance costs are required during operation (Sharma & Forster, 1994). Therefore there is a need for the development of lowcost, easily available materials which can adsorb chromium economically. In this regard, the search for new and innovative treatment technology has been focused on various biosorbents such as sphagnum moss peat, compost, leaf mould, coconut shell-based activated carbon, coconut husk and palm pressed fibre. Their metal-binding capacities and possibilities and limitations for application are documented elsewhere (Alaerts *et al*, 1989; Tan & Lee, 1993; Sharma & Forster, 1993, 1994).

One of the naturally-abundant, potential sorptive biomass is *Azolla filiculoides* (see Section 1.7). It is a lowcost and water-insoluble sorbent, and could be used for remediation of metal-laden waste effluents, provided it is capable of removing heavy metals effectively. In addition, the development of an azolla-based biosorbent for waste water treatment, especially in developing countries, may benefit both environmental problems, in removing heavy metals from water using this environmental weed. The study described in this chapter investigates the potential of removing Cr^{6+} from aqueous solution and electroplating effluent using dry azolla biomass on batch scale as a part of series of studies on bioremediation of Cr^{6+} -laden

electroplating effluents.

3.2 MATERIALS AND METHODS

3.2.1 BIOMASS

Fresh *Azolla filiculoides* was collected from local dams near Grahamstown, South Africa. The plant was washed with distilled water, dried in oven for 6 hours at 60°C and milled to a gritty consistency. Particles between 2-3mm in length was selected as the experimental biomass. The stock biomass was kept in dark at 18°C .

3.2.2 HEAVY METALS

Analytical grade reagents were used in all cases. Stock hexavalent and trivalent chromium solutions (1000mg/l) were prepared in distilled water as $\text{K}_2\text{Cr}_2\text{O}_7$ and CrCl_3 (Merck, Germany), respectively. The concentration of total chromium (TCr) was determined by atomic absorption spectrophotometry (GBC Scientific Equipment, Australia). The concentration of hexavalent chromium was measured colorimetrically using 1,5-diphenyl carbazide (Saarchem, SA) method (Anon, 1985) as described in Section 2.3.4. The difference between the two values was taken as trivalent chromium concentration.

3.2.3 ADSORPTION ISOTHERMS

Sorption isotherms were carried out at 18°C on a rotary shaker for five hours using 250ml conical flasks. The Cr^{6+} and Cr^{3+} solutions (100ml) were adjusted to various initial pH values before addition of the biomass(4g/l). The pH values of the solutions were checked and adjusted every hour during the incubation by addition of either H_2SO_4 or NaOH . The isotherm studies were performed by varying the initial Cr^{6+} or Cr^{3+} concentrations (C_0) from 20mg/l to 1000mg/l and the pH from 1.5 to 6 for Cr^{6+} or 3 to 5 for Cr^{3+} . The pH values of the incubation solutions were chosen because it has been reported that Cr^{6+} can be preferably adsorbed at

low pHs and Cr^{3+} removed at higher pHs (Sharma & Forster, 1993, 1994). After shaking the flasks for five hours, the reaction mixtures were filtered through Whatman no.1 filter paper and the filtrate was analysed for Cr^{6+} , TCr and pH. The same isotherm tests were repeated at 25°C and 32°C respectively to determine the effect of temperature on the sorption of Cr^{6+} to azolla biomass. The Langmuir sorption model, described in Chapter 2.3.5, was used for estimation of the maximum metal uptake (X_m).

3.2.4 KINETIC STUDIES

Azolla biomass of 1.2g was thoroughly mixed with 300ml of Cr^{6+} solutions of different concentrations ranging from 100mg/l to 800mg/l. The suspensions were shaken for 48 hours at 18°C using 500ml conical flasks. Samples of 2ml were collected at varying times and analysed as previously described.

3.3 RESULTS AND DISCUSSION

3.3.1 ADSORPTION ISOTHERMS

Sorption equilibrium was established when most of the chromium ions were adsorbed onto azolla after five hours agitation. The isotherm data showed good compliance with the Langmuir equation (Table 3.1). The correlation coefficients, r^2 , for the 1.5 to 4 pH range, were found to be more than 0.92, indicating a strong positive relationship between the equilibrium Cr^{6+} concentration (C_e) and the metal uptake (q_e). The isotherm data have been used to calculate the maximum sorption capacity of azolla by substituting the required equilibrium concentrations in the Langmuir equation. The metal uptake increased with increasing equilibrium metal concentrations and with lowering pH values, except for pH 1.5 at which it showed lower metal uptake than those at pHs 2, 3 and 4. The X_m for Cr^{6+} at pH 2 and 18°C, was 70.6mg/g, whilst the highest values of sorption capacity (120mg/g) was obtained at 32°C.

3. BATCH REMOVAL OF Cr^{6+} BY *Azolla*

The effect of temperature on metal uptake was significant, indicating that the sorption of Cr^{6+} on azolla is endothermic (Table 3.1). The value b is related to the energy of sorption and reflects the “strength or affinity” of the sorbent for the solute at relatively low concentrations (Hollan *et al*, 1993). It did not show any set trend with either increase or decrease in pHs of the incubating solutions, in the sorption isotherms (Fig 3.1).

Table 3.1 Langmuir constants of Cr^{6+} -azolla binding

Incubation pH	Temperature (C°)	X_m (mg/g)	b ($\times 10^{-3}$)	Correlation (r^2)
1.5	18	19.7	6.58	0.93
2	18	70.6	3.05	0.99
2	25	72.0	2.65	0.98
2	32	120.2	0.97	0.96
3	18	61.6	3.06	0.99
4	18	20.5	6.67	0.94
6	18	14.0	2.72	0.81

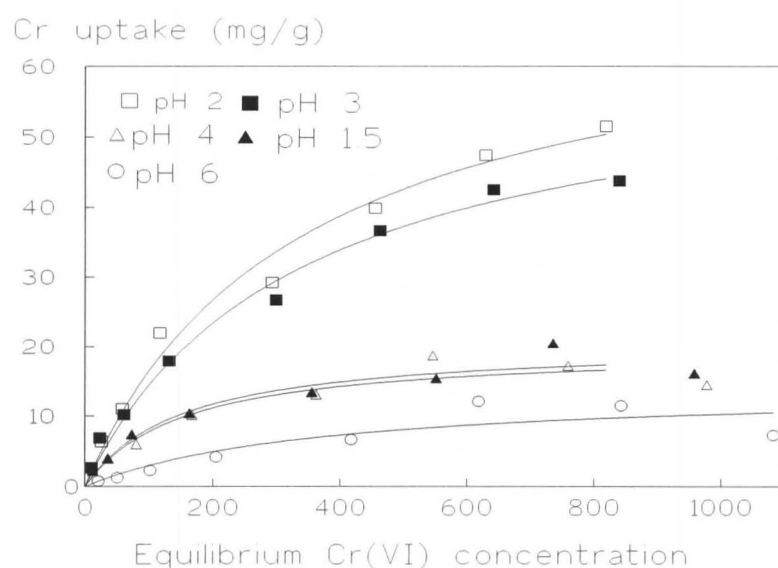


Figure 3.1 Adsorption isotherms of azolla- Cr^{6+} system at 18°C and varying pHs after 5 h agitation. Biomass, 4g/l.

3.3.2 EFFECT OF pH

It was noted in a preliminary trial that the pH in both blank and metal solutions was raised from an initial pH of 4.0 by azolla biomass during incubation (Table 3.2). To ensure that the sorption procedure was carried out at the required pH, adjustment of pH each hour was performed. Similar phenomena were observed with other sorbents such as leaf mould, activated carbon and sphagnum moss peat (Sharma & Forster, 1993, 1994 & 1996 a). The reason for the increase in pH could be due to the presence of certain unknown groups (presumably oxo groups) on the surface of the biomass, which buffered the solution. However, less increase was observed at very low initial pH (pH 1.5) due to the fact that the changes in hydroxyl and proton concentrations were insufficient to cause any increase in pH when the solution was highly acidic (Sharma & Forster, 1994).

Table 3.2 Final pH at different Cr^{6+} concentrations after 5 hours incubation

	Cr^{6+} (mg/l)								
	0	20	50	100	200	400	600	800	1000
pH	7.2	7.5	7.5	7.3	6.9	6.6	6.4	6.3	6.2

The optimum removal of Cr^{6+} occurred at pH 2-3 for all initial concentrations, while at each pH value, the removal efficiency decreased when initial Cr^{6+} concentrations were increased (Fig3.2). Data concerning optimum pHs of other sorbents was reported in the similar range: activated carbon, pH 2.5; sphagnum moss peat, pH 1.5; compost, pH 1.5; leaf mould, pH 2.0 and coconut husk fibre, pH 2.05 (Sharma & Forster, 1994).

Removal of Cr^{6+} ions was apparently pH dependent. This is due to the nature of the chemical interaction of metal ions with the surface of azolla and is probably related to the overall surface charge of the biomass. As the pH is increased, however, the overall surface charges will become negative, which will expel the approach of an anion metal (Sag & Kutsal, 1996

b). One exception is the sorption of Cr^{6+} at pH 1.5 which showed a lowered X_m , in comparison with those at pHs of 2 and 3. It is also noted that large quantities of Cr^{6+} had been reduced to its trivalent form at pH 1.5, which accounted for 36 - 77% of the initial Cr^{6+} concentrations used in the isotherm study (Table 3.3). The simultaneous reduction of Cr^{6+} at pH of 1.5 can be one of the factors interfering the sorption, possibly by decreasing the amount of Cr^{6+} ions available for binding. However, a detailed understanding to the phenomenon is difficult when information concerning ligands on surface of the biomass and mechanism by which the sorption occurs is meagre.

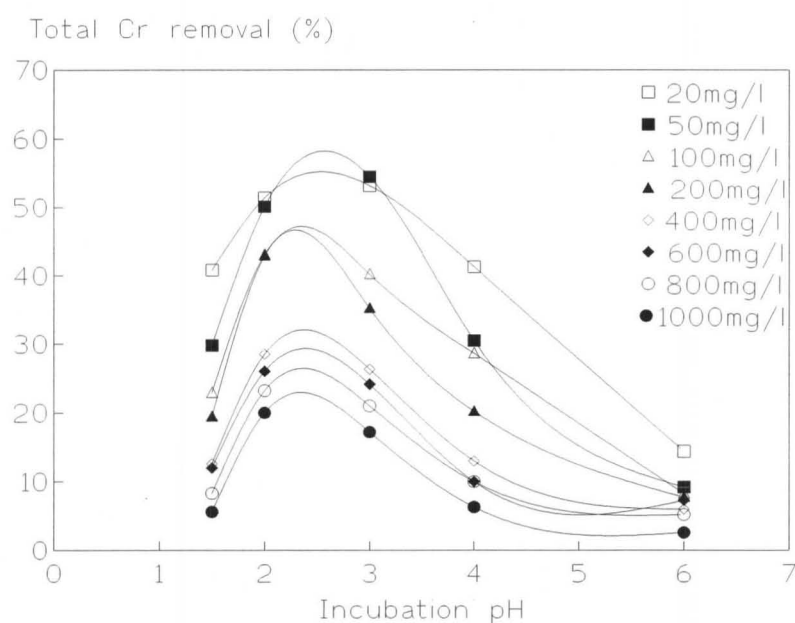


Figure 3.2 Effect of pHs and initial Cr^{6+} concentrations on the total Cr removal at 18°C. Biomass, 4g/l

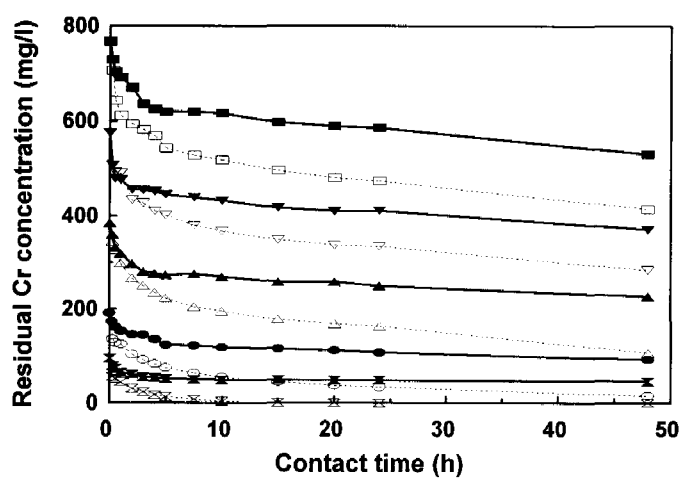
Table 3.3 Cr^{3+} produced after 5 h incubation at pH 1.5 and varying initial Cr^{6+} concentration

Ini. Cr^{6+} conc. (mg/l)	20.7	51.8	103.5	207	414	621	828	1035
Cr^{3+} conc. (mg/l)	12.3	36.4	79.8	125.6	199.8	262.3	322.9	381.4
Cr^{3+} / ini. Cr^{6+} (%)	59.2	70.2	77.1	60.7	48.3	42.2	39.0	36.9

3.3.3 KINETIC STUDIES

It can be seen that most of chromium ions were rapidly bound onto azolla in the first 5 h and this was followed by a gradual sorption throughout the rest of the period (Fig 3.3). The slopes of the plot are biphasic and suggest that more than one type of mechanism is involved in the binding procedure. A similar phenomenon was found in a study using yeast as a biosorbent (Stoll, 1996). The first phase, which was achieved within 10-20 min, mainly represents metal binding on surface of the sorbent. This stage of “immediate solute uptake” is of more practical importance, in for example, a continuous-flow process system. The second phase, is suggested to be a chemisorption-dominated procedure, occurring mostly in internal pores of the biomass, in the case where non-viable biomass was used (Sag & Kutsal, 1996). A simultaneous reduction of Cr^{6+} to Cr^{3+} occurred during the stage reconfirming the chemical nature of the sorption (Fig 3.3) (Sharma & Forster, 1994).

A



B

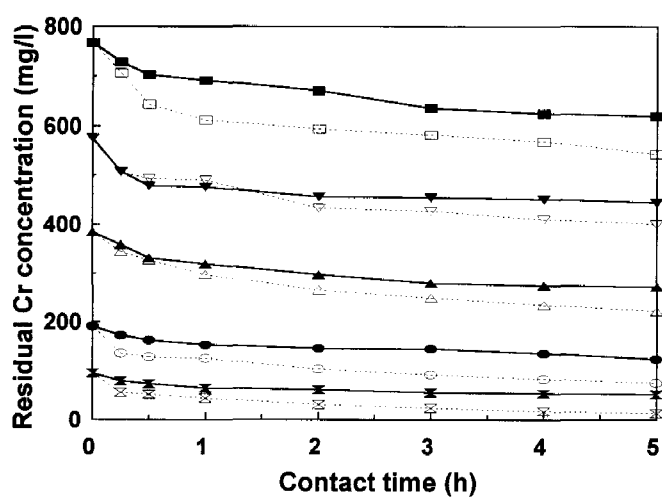
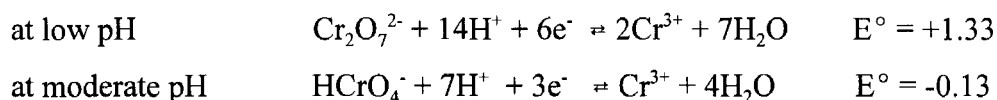


Figure 3.3 Effect of contact time on the residual Cr^{6+} and TCr concentrations at pH 2. Filled symbols, TCr; empty symbols, Cr^{6+} ; (A), sampled for 48 h; (B), sampled for 5 h.

3.3.4 REDUCTION OF Cr^{6+} TO Cr^{3+}

Reduction of Cr^{6+} to Cr^{3+} is easier to conduct in acidic media than in alkaline media, as indicated by standard potential data (Moeller *et al*, 1989):



It was seen that a significant amount of Cr^{6+} had been reduced to trivalent form at low pH (especially at pH 1.5) during isotherm tests, which is possibly attributed to the effect of highly concentrated protons (Fig. 3.4). However, appreciable reduction can also be noted even at a moderate pH of 6 (Fig 3.4), suggesting the presence of reducing groups on surface of the biomass (since reduction of Cr^{6+} to Cr^{3+} is not likely to occur at neutral pHs as indicated by the standard potential data). In kinetic experiments, it appeared that more Cr^{6+} ions had been reduced at pH 2.0, when the incubation time was increased than that at the onset of the sorption, implying that the reduction was seemingly a time-dependent process (Fig 3.3). This phenomenon was confirmed by a number of researchers during their Cr^{6+} sorption studies on miscellaneous sorbents eg. leaf mould, sphagnum peat moss and activated carbon (Sharma & Forster, 1993, 1994 & 1996 a). The presence of reducing groups for Cr^{6+} on surfaces of sorbents is well documented but their identification is as yet unclear. Reduction of Cr^{6+} to its trivalent form can also contribute to the detoxification of Cr^{6+} -laden waste waters since Cr^{3+} was less toxic than the hexavalent form (Coleman & Paran, 1991).

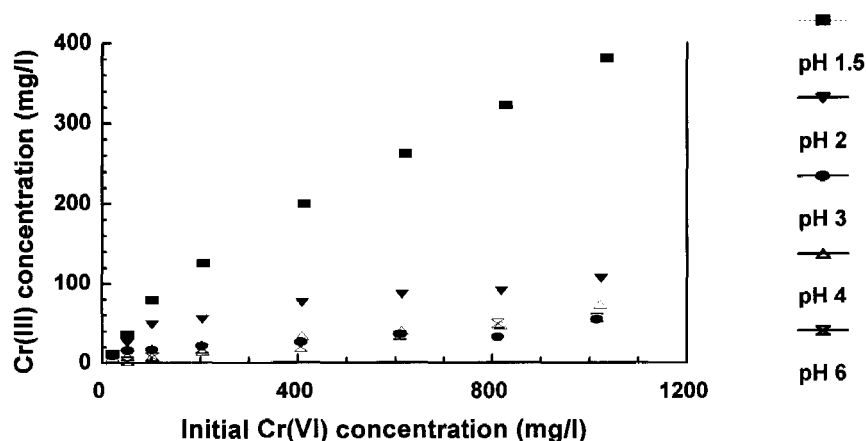


Figure 3.4 Reduction of Cr^{3+} by azolla at different pHs and initial Cr^{3+} concentrations after 5 h incubation at 18°C. Biomass, 4g/l.

3.3.5 SORPTION OF Cr^{3+}

Cr^{3+} , although less toxic than hexavalent chromium, has been classified as one of the moderate toxicity contaminants to aquatic life and the stipulated safety criteria is less than 0.1mg/l (Kempster *et al*, 1980). Furthermore Cr^{3+} , when it is disposed to environment, may be oxidised by a number of microorganisms, plants and inorganic compounds at neutral pHs in nature (Cited by Brady, 1992). Therefore, removal of Cr^{3+} ions from any waste waters before discharge is obviously necessary. In determining the maximum Cr^{3+} sorption capacity of azolla biomass, isotherm tests were conducted, with varying initial Cr^{3+} concentrations (20-800mg/l) at pHs 3, 4 and 5 (Fig 3.5). A pH of 5 was found to be the highest value at which isotherms can be performed since precipitation occurred at pH higher than 5.5 even at low Cr^{3+} concentration (Losi *et al*, 1994). The data from the isotherms followed Langmuir model well resulting in a X_m of 27.8mg/g for Cr^{3+} with azolla at pH 5 (Table 3.4). The b values did not show any trend when the incubation pHs varied. The Cr^{3+} sorption capacities of azolla

3. BATCH REMOVAL OF Cr^{6+} BY *Azolla*

varied depending on the incubation pHs. The sorption of Cr^{3+} by azolla is seemingly affected by proton concentrations in an adverse way to that described in Section 3.4.2 for sorption of Cr^{6+} . Chemical interaction of the cation metal with the ligands on the surface of azolla may once again partially explain this situation. It is likely that protons alter the overall surface charge of the biomass and thereby affect the interaction with the ligands (Sag & Kutsal, 1996). The sorption of Cr^{3+} , in general, was thought to be similar to that for other cation metals such as Ni, Zn and Cu (Sharma & Forster, 1994).

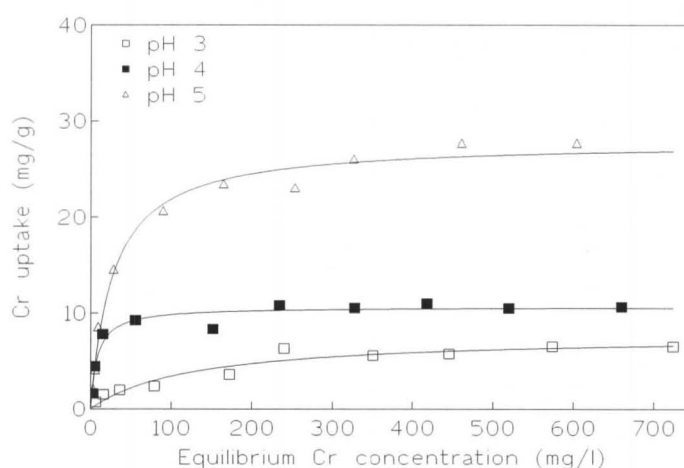


Figure 3.5 Adsorption isotherms of azolla- Cr^{3+} system at 18°C and varying pHs. Biomass, 4g/l.

Table 3.4 Langmuir constants of Cr^{3+} -azolla binding at 18°C

Incubation pH	X_m (mg/g)	b ($\times 10^{-3}$)	Correlation (r^2)
3	7.7	7.98	0.93
4	10.7	124.39	0.94
5	27.8	36.77	0.99

3.3.6 MECHANICAL STABILITY

The azolla biomass was water-insoluble at low pH conditions during the batch agitation for as long as 48 h. In all cases no disruption in its integrity after incubation was observed by visual test. The biomass was therefore deemed to be suitable for a fixed-bed column process.

3.3.7 COMPARISON WITH OTHER SORBENTS

Table 3.5 presents a summary of various sorbents used for sorption of Cr^{6+} . Comparison has been made in terms of the maximum sorption capacities X_m and the optimum pH for sorption. It is clear from these data that some materials such as activated carbon, sphagnum moss peat, compost and azolla exhibit much greater sorption capacities over other sorbents. However, a potential of any material as a sorbent will have to be evaluated from an economic perspective. In most cases, activated carbon will have to be purchased commercially and the cost varies from place to place. On the other hand azolla can either be collected from dams, rivers and wetlands in large quantity or cultivated easily where azolla has not been introduced. Taking this into consideration, azolla biomass could be one of the lowest-cost candidates for removal of Cr^{6+} in treatment of heavy metal laden waste water.

Table 3.5 Summary of sorption potential of various sorbents for Cr(VI)

Sorbent	X_m (mg/g)	Optimum pH	Tem. (°C)	Max.conc. (mg/l) ^a	Reference
Activated carbon (Filtrisorb-400)	145.0	2.7	25	1000	Sharma & Forster, 1996 a
Sphagnum moss peat	119.0	1.5	25	1000	Sharma & Forster, 1993
Compost	101.0	4.2	25	500	Sharma & Forster, 1994
<i>Azolla</i>	120.2	2.0	32	1000	Present study
<i>Azolla</i>	70.6	2.0	18	1000	Present study
Activated carbon (Filtrisorb-400)	57.7	-	-	250	Huang & Wu, 1977
Leaf mould	43.0	2.0	25	1000	Sharma & Forster, 1994
Coconut husk fibres	29.0	2.1	-	80	Tan <i>et al</i> , 1993
Coconut shell-based activated carbon	20.0	2.5	-	50	Alaerts <i>et al</i> , 1989
Palm pressed fibres	14.0	2.0	-	80	Tan <i>et al</i> , 1993
Saw dust	10.1	4.0	27	800	Bryant <i>et al</i> , 1992

^a maximum Cr^{6+} concentration used in the experiments.

3.4 CONCLUSIONS

On the basis of the data obtained in present study, the following conclusions can be drawn. *Azolla filiculoides* is able to accumulate a considerable amount of Cr^{6+} from aqueous solution. It showed a maximum sorption capacity of 70.6mg/g at 18°C and 120.2mg/g when the temperature was increased to 32°C, which suggests that the Cr^{6+} sorption process was endothermic. Binding of Cr^{6+} is highly pH dependant, the optimum pH being 2.0. Less Cr^{6+}

3. BATCH REMOVAL OF Cr^{6+} BY Azolla

was accumulated by azolla at a mild pH of 6.0. At pH of 1.5, a large fraction of Cr^{6+} was reduced to its trivalent form, a species which is poorly adsorbed by azolla at this pH. Reduction of Cr^{6+} can to certain extent also be detected at pH 6.0 probably due to the presence of some reducing groups on surfaces of the biomass. Cr^{3+} can be reasonably accumulated by azolla at a mild pH of 5.0 with a sorption capacity of 27.8mg/g. Better removal could be expected at pH values higher than 5 but Cr^{3+} precipitates out of solution under those conditions. Dried azolla biomass showed good physical properties in the batch tests and appeared to be suitable for a fixed-bed column process in removing Cr^{6+} from chromium contaminated effluents. This will be discussed in next chapter.

4 COLUMN REMOVAL OF HEXAVALENT CHROMIUM FROM AQUEOUS SOLUTIONS AND ELECTROPLATING EFFLUENTS

4.1 INTRODUCTION

Batch studies on azolla- Cr^{6+} and Cr^{3+} binding has shown that azolla is a potentially strong sorbent for removing Cr^{6+} and Cr^{3+} from aqueous solution at low pH (Zhao & Duncan, 1997, b). However, batch processes are usually limited to the treatment of small quantities of waste water and the regeneration procedure of the exhausted biomass is quite difficult. Therefore it is necessary to consider other modes of contacting the solid sorbents and the waste water in applying the system to large-scale treatment. The other option is continuous flowthrough system in which three types of contacting systems are usually encountered: (1) fixed-bed; (2) pulsed-bed; (3) fluidised-bed processes. Among them, fixed-bed system are most widely used and the effluent is continuously in contact with fresh sorbent and therefore the driving force to reach equilibrium is high (McKay, 1981). In this regard, an investigation on the potential of a continuous-flow column system for treatment of Cr^{6+} -contaminated effluents was undertaken. The aim of this section is to examine the effects of pHs, flow rates and mass of azolla on column capacities of removing Cr^{6+} as well as Cr^{3+} from either aqueous solution or electroplating effluents. The possibilities of desorption with alternative desorbents and the reusabilities of the azolla biomass for treatment of Cr^{6+} -laden wastewater were also investigated.

4.2 METHODS AND MATERIALS

4.2.1 MATERIALS

Azolla biomass was collected and prepared as described in Chapter 3.3.1. All the chemicals were analytical grade. Potassium dichromate (Merck, Germany) and chromium chloride (Reidel-de Haen, Germany) were used as the source of hexavalent and trivalent chromium solutions. $\text{Na}_2\text{S}_2\text{O}_5$, NaCl, NaOH, HCl, H_2SO_4 and HNO_3 were obtained from Saarchem, SA.

The electroplating effluents, designated as EPE2 and EPE3, were collected from a factory (PE Plating, South Africa) operating a chromium-plating line (Table 4.1).

Table 4.1 Characteristics of EPE2 and EPE3

		Cr(VI)	Ni	Zn	Cd	Cu	Fe	Pb
	pH	mg/l						
EPE2	6.1	18	0.5	0	0	0	0	0
EPE3	5.9	20.5	2	0	0	0	0	0

4.2.2 ASSAY METHODS

Hexavalent and trivalent chromium were determined as described in section 3.3.2.

4.2.3 PREPARATION OF COLUMNS

Glass columns of 25mm internal diameter were used in the operation. Uniformly milled *azolla* biomass of 5g in its dry form was inserted from top of the columns and rinsed with distilled water. The dimensions of packed columns for 5g wet biomass was 2.5 x 12 cm. Feed solutions were pumped upwards through the column using a peristaltic pump (Watson Marlow, 504S). Flow rates were maintained as required. The eluate was collected in 20ml fractions. The working temperature was kept at approximately 18°C. The saturation point for the column operation was defined as the volume when the eluate concentrations of Cr^{6+} reached 60% of the influent concentrations. The TCr uptake was defined as the mg of Cr^{6+} and Cr^{3+} adsorbed by one gram of dry biomass. The Cr^{6+} removal was the mg of Cr^{6+} both taken up and reduced by one gram of dry biomass.

4.2.4 REGENERATION OF EXHAUSTED COLUMN BIOMASS AFTER Cr^{6+} SORPTION

The saturated or semi-saturated *azolla* biomass were regenerated by washing the columns with various desorbents. The regeneration efficiency was described in Chapter 2.3.6.

4.2.4.1 DESORPTION OF Cr^{6+} WITH 0.2M NaOH AND 1M NaCl/0.1M NaOH

The elution was carried out down-flow with 0.2M NaOH or 1M NaCl respectively at a flow rate of 80ml/h (16ml/h.g). Fractions of 20ml were collected.

4.2.4.2 REDUCTION AND DESORPTION OF Cr^{6+} WITH $\text{Na}_2\text{S}_2\text{O}_5$ /HCl OR H_2SO_4

This step involves in reduction of Cr^{6+} to Cr^{3+} by $\text{Na}_2\text{S}_2\text{O}_5$, which was followed by desorption of the cation chromium with acids. The Cr^{6+} -saturated biomass was washed with 35ml mixture of $\text{Na}_2\text{S}_2\text{O}_5$ and acids which was enough to rinse the biomass. Solutions of 0.5M $\text{Na}_2\text{S}_2\text{O}_5$ and 0.5M HCl or 0.5M H_2SO_4 were used as reductant and desorbent respectively, and mixed immediately before loading. $\text{Na}_2\text{S}_2\text{O}_5$ was chosen as reductant in the experiment because it is a strong and cheap reducing agent in South Africa market. The desorbing solution was left in the column for about 6 hours before being released so as to give enough time for Cr^{6+} to react with the reductant $\text{Na}_2\text{S}_2\text{O}_5$. The batch washes were repeatedly carried out 3-4 times until the concentrations of the elute were below 100mg/l. Finally the column biomass was rinsed with 2 bed volumes of distilled water.

4.2.5 REGENERATION OF EXHAUSTED COLUMN BIOMASS AFTER Cr^{3+} SORPTION

4.2.5.1 DESORPTION OF Cr^{3+} WITH H_2SO_4 AND HCl

Washes of the Cr^{3+} -loaded biomass with 35ml acids were performed by a down-flow through

the columns. The contact time for biomass with acids was 6 h as it was seen that very little chromium ion had been washed out in less than 5 h of incubation.

4.2.5.2 RECONDITIONING OF *Azolla* BIOMASS

After acid washes, the column biomass was reconditioned with one bed volume of 0.1M NaOH (incubating for 20 min) to increase the pH of the biomass. This was followed by a 2 bed volume wash with distilled water.

4.2.6 REGENERATION EFFICIENCY

The TCr uptake capacity and regeneration efficiency of biomass were described in Chapter 2.3.6.

4.3 RESULTS AND DISCUSSION

4.3.1 SORPTION OF Cr^{6+}

The performances of column biomass for sorption of Cr^{6+} were estimated by running several columns at different influent pHs, flow rates and with varying mass of *azolla* (Table 4.2). The TCr uptake and Cr^{6+} removal, and the treated volume at 60% exhaustion determined from column operations were compared.

Table 4.2 Column removal of Cr^{6+} with varying pH, flow rate and mass of azolla

Infl. conc. (mg/l)	Infl. pH	Flow rate (ml/h)	Biomass (g)	Cr^{6+} removal (mg/g) ^a	TCr uptake (mg/g) ^a	Cr^{3+} produced (mg/g)	α^b	Volume treated (litre) ^a
100	3.0	80	5	18.3	17.1	1.2	0.07	1.4
100	2.0	80	5	48.5	35.7	12.8	0.36	2.8
100	2.0	160	5	28.6	23.4	5.2	0.22	1.7
100 ^c	2.0	160	5	14.2	11.8	2.3	0.20	0.9
100	2.5	80	5	50.4	41.5	8.8	0.21	3.0
100	2.5	160	5	24.0	21.6	2.4	0.11	1.8
100	2.5	160	10	36.9	34.1	2.9	0.08	5.2
18 ^d	2.5	160	10	26.3	21.3	5.0	0.23	18.0
20.5 ^e	3.0	80	5	ND	ND	1.9	0.09	ND

^a at 60% saturation of the column biomass^b ratio of reduction to adsorption^c cycle 2^d EPE2^e EPE3

ND: not determined

4.3.1.1 EFFECT OF pH

The effects of varying influent pHs on sorption of Cr^{6+} by azolla biomass are shown in Figs. 4.1, 4.2 and Table 4.2. Although gradual breakthrough of TCr and Cr^{6+} at all pHs and flow rates occurred in the column elution profiles, the metal uptake, calculated from the breakthrough curves at 60% saturation of the column biomass, were 41.5mg/g, 35.7mg/g and 17.1mg/g, at pH 2.5, 2.0 and 3.0 respectively. However, further accumulation of Cr^{6+} by the semi-saturated biomass can also be expected before it reaches full saturation (Figs 4.1-4.3). The results from column sorption are in agreement with the previous batch isotherm studies on azolla- Cr^{6+} system, which showed that the optimum pH for azolla- Cr^{6+} binding are 2-2.5 (Zhao & Duncan, 1997 b). The treated volumes of Cr^{6+} -solution before 60% saturation were 3.0, 2.8 and 1.4 litres at influent pHs of 2.5, 2.0 and 3.0 respectively showing the similar

trend to that for TCr uptake (Figs 4.1, 4.2 and Table 4.2).

The pH of the treated solution was raised up by the biomass during a column process, when using a Cr^{6+} solution of pH 3. The exit pH was found at 4-5 throughout the operation. Uptake of TCr at a influent pH of 3 was lower than that at influent pHs of 2 and 2.5 (Table 4.2). In previous batch experiments, low TCr sorption capacities such as 20.5mg/g (at pH 4) and 14mg/g (at pH 6) were obtained, which reflected that sorption of Cr^{6+} by *azolla* biomass at mild or neutral pHs is limited (Zhao & Duncan, 1997 b). Some sorbents which were capable of buffering the Cr^{6+} - medium during sorption have also been reported (Sharma & Forster, 1994). Raising of pH values by the biomass did not occur in other column operations (pHs 2.0 and 2.5) probably due to the fact that the changes in hydroxyl and proton concentrations induced by *azolla* were insufficient to cause any increase in pH when the solution was highly acidic. This phenomenon is in agreement with that shown on batch tests in Chapter 3.4.2.

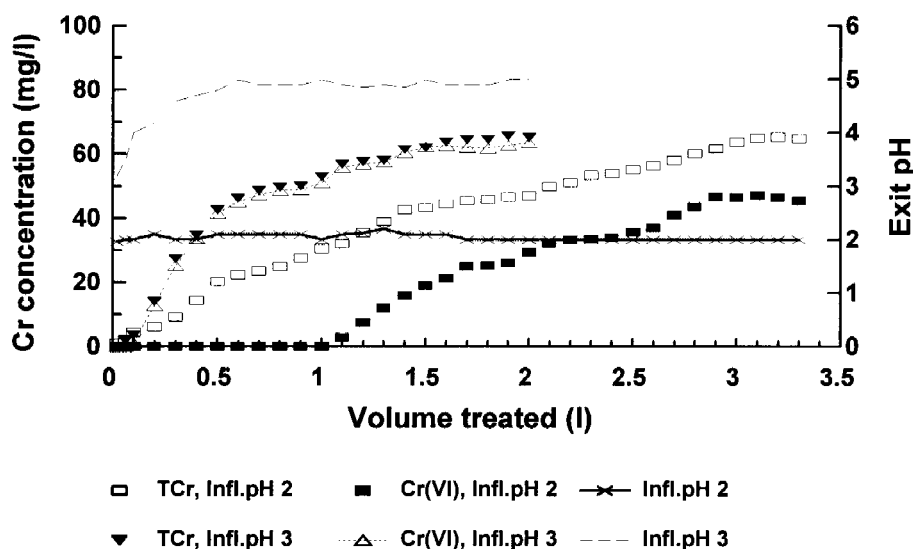


Figure 4.1 Breakthrough curves for column removal of TCr and Cr^{6+} from aqueous solution at infl. pHs 2 and 3. Flow rate, 80ml/h; infl. Cr^{6+} , 100g/l; biomass, 5g.

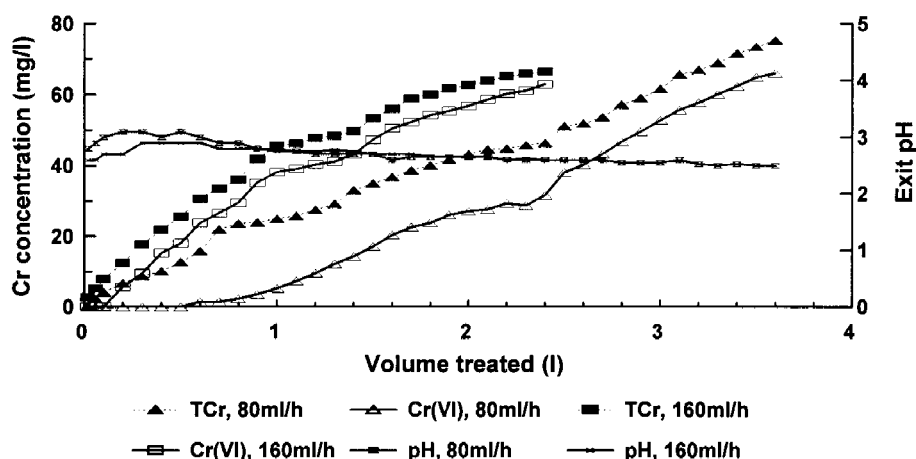


Figure 4.2 Breakthrough curves for column removal of TCr and Cr^{6+} from aqueous solution at flow rates 80ml/h and 160ml/h. Infl. pH, 2.5; infl. Cr^{6+} , 100g/l; biomass, 5g.

4.3.1.2 EFFECT OF FLOW RATE

Sorption of Cr^{6+} at higher flow rate (160ml/h) resulted in earlier breakthrough of Cr^{6+} in the elution profile (Fig 4.2). The metal uptake at breakthrough, at pH 2.5 and 160ml/h, was 21.6mg/g showing the decrease in sorption capacity by about 50% in comparison with a flow rate of 80ml/h. A lower volume of treated solution before breakthrough at 160ml/h can also be seen in Fig 4.2 and Table 4.2 in comparison with that at 80ml/h. Negative effects of increasing flow rates on Cr^{6+} sorption was also observed elsewhere when using sphagnum moss peat (Sharma & Forster, 1995). Contact time for Cr^{6+} with the sorbent appeared to affect binding of Cr^{6+} to a certain extent. For a flow rate of 80ml/h the contact time was estimated as 0.44 h at which a better TCr uptake was attained than that at a contact time of 0.22 h (at a flow rate of 160ml/h).

4.3.1.3 EFFECT OF MASS OF *Azolla*

Doubling the amount of biomass in a sorption column, as expected, improved the sorption by 73% in terms of the volume treated before 60% saturation (Fig 4.3). The TCr uptake and Cr^{6+} removal, when a biomass of 10g was used, were 34.1 and 36.9mg/g respectively which differ from those values with 5g biomass (Table 4.2). It should also be borne in mind that the contact time had actually been doubled when amount of biomass was increased from 5 to 10g at the constant flow rate, which resulted in better uptake.

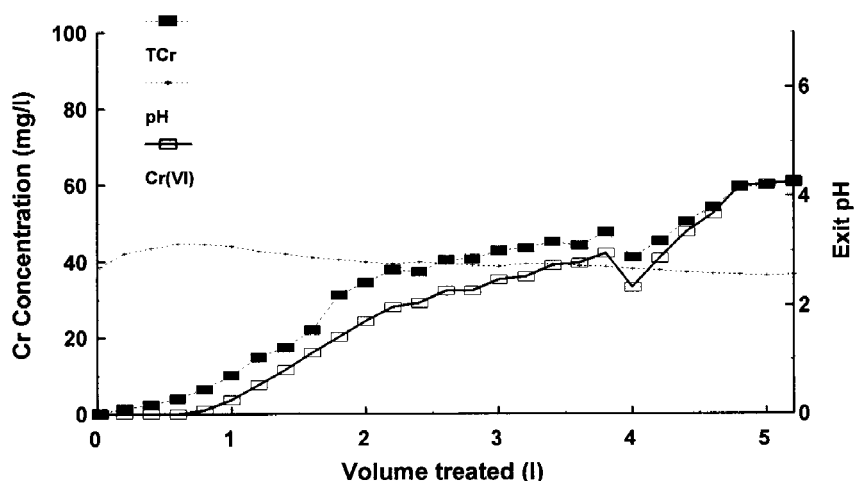


Figure 4.3 Breakthrough curves for column removal of TCr and Cr^{6+} at infl. pH 2.5 and flow rate 160ml/h. Infl. Cr^{6+} , 100mg/l; biomass, 10g.

4.3.1.4 TREATMENT OF PLATING EFFLUENT

The removal of Cr^{6+} from EPE2 (Cr^{6+} concentration: 18mg/l) by 10g biomass at pH 2.5 is presented in Fig 4.4. Complete removal of Cr^{6+} in the first 6 litres (by either sorption on azolla or reduction to trivalent form) can be seen and the volume of plating effluent treated before breakthrough was found to be 18 litres. The TCr uptake, calculated from the breakthrough curve, was 21.3mg/g for TCr and 26.3mg/g for Cr^{6+} respectively. The TCr

uptake from EPE2 is 37.5% lower than that resulted from using a synthetic solution containing 100mg/l Cr^{6+} . The varied influent Cr^{6+} concentrations used in those two operation, 18mg/l for EPE2 and 100mg/l for the other, can be attributed to the difference in their TCr uptake. In addition, other anionic ions or organic compounds present in EPE2 could also affect the sorption process resulting in a declined TCr uptake.

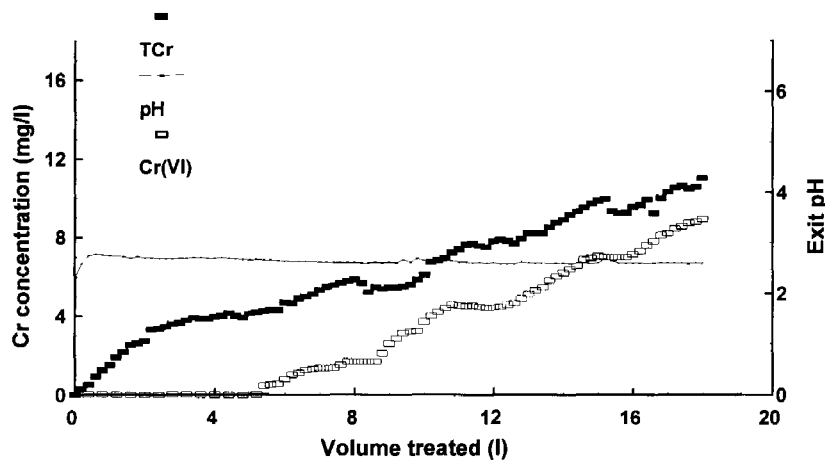


Figure 4.4 Breakthrough curves for column removal of TCr and Cr^{6+} from EPE2 at infl. pH 2.5 and flow rate 160ml/h. EPE2 Cr^{6+} , 18mg/l; biomass, 10g.

The removal of TCr, Cr^{6+} and Ni by 5g biomass at a influent pH 3 and a flow rate of 160ml/h from EPE3 is shown in Fig. 4.5. A TCr uptake of 20.7mg/g was achieved at 20% saturation with 5.3 litre effluent having been treated. Unlike that in Fig. 4.1, the exit pH of treated EPE3 had not been raised by the biomass, for some unknown reasons.

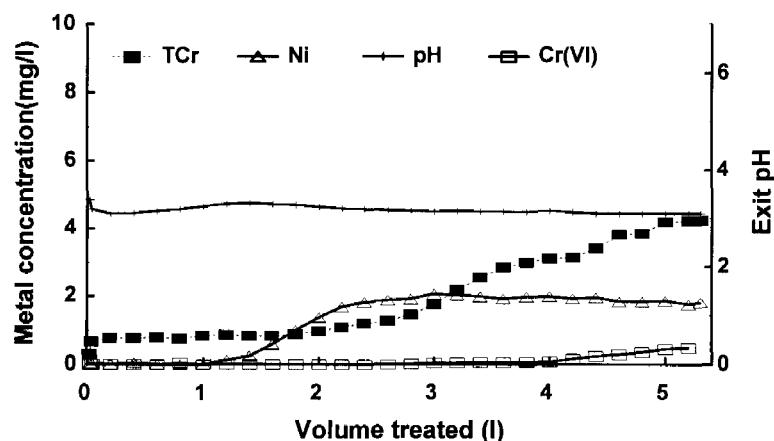


Figure 4.5 Breakthrough curves for column removal of TCr, Cr^{6+} and Ni from EPE3 at infl. pH 3 and flow rate 80ml/h. EPE3 Cr^{6+} , 20.5mg/l, Ni, 2mg/l; biomass, 5g.

4.3.2 REDUCTION OF Cr^{6+} TO Cr^{3+}

Reduction, along with sorption processes, were postulated to exist in the removal of Cr^{6+} by sorbents such as activated carbon, sphagnum moss peat and leaf mould (Sharma & Forster, 1994-1996; Huang & Wu, 1977; Tan *et al*, 1993). The present study once again revealed this phenomenon showing that the reduction of Cr^{6+} to its trivalent form by azolla did occur in column operation at pH 2.0 and 2.5, resulting in a leaching of variable amounts of Cr^{3+} . The reduction to sorption ratio (α), was introduced to evaluate the reduction process and is shown in Table 4.2. It appeared that less Cr^{6+} ions had been reduced when the flow rates were increased from 80 to 160ml/h, with the α values decreasing from 0.36 to 0.22 for pH 2.0 and from 0.21 to 0.11 for pH 2.5 (Table 4.2). This may suggest that the reduction reaction probably occurred at internal surfaces of the biomass, and that the sorption of Cr^{6+} takes place in both internal and outer surfaces. When the flow rate is increased (shortening of contact time), less Cr^{6+} diffuses into the internal pores to get reduced there, while the sorption process in internal pores will also be limited to a certain extent, resulting in earlier breakthrough of Cr^{6+} uptake.

4.3.3 GRADUAL BREAKTHROUGH OF Cr^{6+} IN COLUMN OPERATIONS

Gradual breakthrough of Cr^{6+} was once again observed in azolla column operations at all influent pHs and flow rates. The phenomenon of gradual breakthrough, from an application viewpoint, is of importance because a column run is always terminated at some chosen values of metal concentration. Therefore total available metal removal capacity of the sorbent can not be fully utilised in single fixed-bed runs. A number of researchers have also observed the similar gradual breakthrough of Cr^{6+} in their studies on column sorption of Cr^{6+} with activated carbon, leaf mould, sphagnum moss peat, coconut husk and palm pressed fibres, where physical and chemical sorption are deemed to be the predominant mechanisms for removal of Cr^{6+} (Huang & Wu, 1977; Tan *et al*, 1993; Sharma & Forster, 1995, 1996). In the present studies, the following facts have been noted:

1. The sorption process of azolla with Cr^{6+} is endothermic (temperature dependent).
2. The sorption of Cr^{6+} by azolla is relatively slow in comparison with other cation metals (Chapter 5 & 6), indicating a nature of chemisorption (Hayward & Trapnell, 1964);
3. Desorption of Cr^{6+} from the exhausted biomass was difficult, suggesting strong bonds were formed between the sorbent and ligand;
4. An appreciable amount of Cr^{6+} has been converted to Cr^{3+} during column operation, showing that chemical reactions occurs;

These observations support the conclusion that chemisorption plays a dominant role in the sorption of Cr^{6+} with azolla. Since the chemisorption is generally slower than the physical one, a minimum reaction time could be required for metal binding. When the contact time for biomass and ligand was not long enough or even shortened, gradual breakthrough of Cr^{6+} would occur irrespective of whether the binding sites on azolla are saturated or not.

Nevertheless, in the case of porous sorbents, eg azolla, penetration of adsorbate to the interior of the sorbents may also be an extremely slow process (Hayward & Trapnell, 1964).

Unfortunately the gradual breakthrough could not always be improved by lowering the flow rate because the reduction process would be enhanced at low flow rates inducing more

leaching of Cr^{3+} (Fig 4.2).

4.3.4 DESORPTION OF Cr^{6+}

4.3.4.1 DESORPTION OF Cr^{6+} WITH 0.5M NaOH OR 1M NaCl/0.1M NaOH

Alkaline brine and caustic soda were reportedly the effective regeneration agents for desorbing chromate ions from exhausted anion exchange resins (Cited by Sengupta & Clifford, 1986). Desorption of chromium ions from an exhausted column consisting of sphagnum moss peat with 1M NaOH was attempted by Sharma & Forster (1995). However, the desorption efficiency attained was considerably lower than that for exchange resins, and the regeneration efficiency was less than 40%. In the present experiment, desorption of the bound chromium ions with 0.5M NaOH or 1M NaCl/0.1M NaOH using volume of 120ml appeared unsuccessful showing the recoveries of only 6.5 and 12.3% respectively (Table 4.3). This may exclude the possibility that anion exchange regulates the binding. Furthermore the liquor leached from the biomass was strongly brown coloured during the alkaline desorption and it was thought that extraction of *azolla* constituents by alkali occurred.

Table 4.3 Chromium(VI) recovery with various desorbents

Desorbents	Cr^{6+} uptake (mg)	Cr desorbed (mg)	Volume used (ml)	Recovery (%)	Regeneration efficiency (%)
0.5M NaOH	147.5	9.6	120	6.5	-
1M NaCl/0.1M NaOH	99.2	12.2	120	12.3	-
0.5M $\text{Na}_2\text{S}_2\text{O}_5$ /0.5M HCl, cyc.1	154.4	77.9	120	50.5	50.5
0.5M $\text{Na}_2\text{S}_2\text{O}_5$ /0.5M HCl, cyc.2	84.2	39.86	120	47.3	-

4.3.4.2 REDUCTION AND DESORPTION OF Cr^{6+}

The methodology was based on the assumption that Cr^{6+} which was bound onto *azolla* biomass during sorption could be reduced by either some unknown reducing groups on the surface of the biomass or by introducing the reductant, $\text{Na}_2\text{S}_2\text{O}_5$, and thereby be desorbed by acids, since the binding of Cr^{3+} on sorbents at acidic pHs is low (Zhao & Duncan, 1997, b & c). The results of desorption with $\text{Na}_2\text{S}_2\text{O}_5$ plus H_2SO_4 were presented in Fig. 4.6 and Table 4.3. A recovery of about 50% was achieved with 3 batch washes of total volume of 120ml (contact time: 6 h). The regeneration efficiency of only 50.5% indicates that the TCr uptake capacity of the used biomass has been diminished by half, due to either incomplete regeneration (about half of the adsorbed Cr ions remained in the biomass) or a deteriorated capacity for removal of chromium ions by the regeneration process. Based on the fact that the most bound Cr^{3+} ions, shown in Table 4.5, was recovered by 0.5N H_2SO_4 , a combination of reductant and desorbent can be potentially feasible for recovery of Cr^{6+} . One of the barriers to an optimum desorption will assumably be the incomplete reduction of Cr^{6+} to Cr^{3+} , which leads to a unsatisfactory recovery. Further investigation on using more effective reductants and suitable reducing methodologies, will be needed, which would in turn facilitate the application of the sorption technology in chromium-laden waste water treatment.

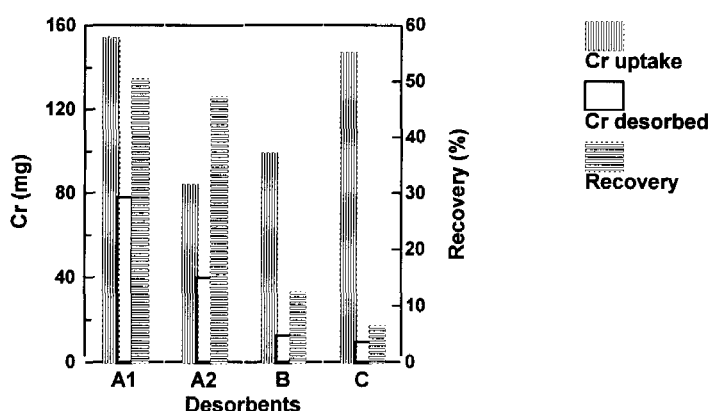


Figure 4.6 Desorption and recovery of chromium from Cr^{6+} -laden biomass column with various desorbents. **A1**, $\text{Na}_2\text{S}_2\text{O}_5$, cyc.1; **A2**, $\text{Na}_2\text{S}_2\text{O}_5$, cyc.2; **B**, NaCl/NaOH ; **C**, NaOH .

4.3.5 SORPTION AND DESORPTION OF Cr^{3+}

4.3.5.1 SORPTION OF Cr^{3+}

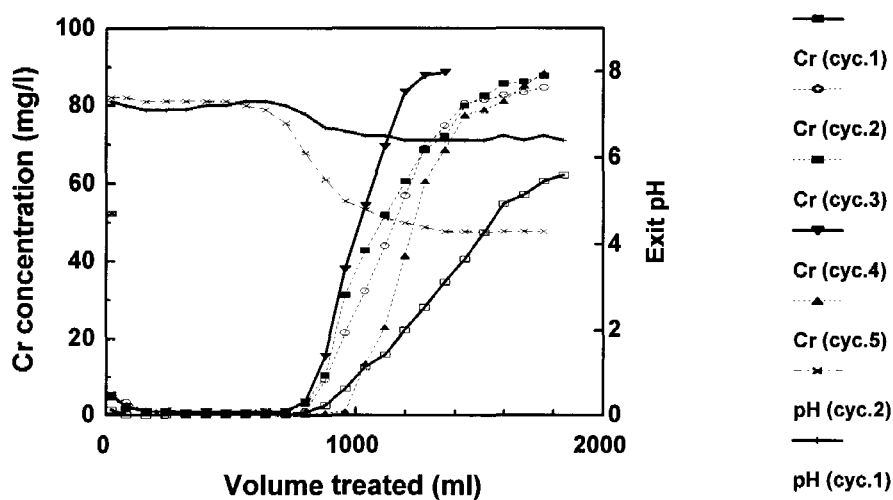
In conventional treatment of Cr^{6+} -laden plating effluent, reduction of Cr^{6+} to Cr^{3+} by reductants such as SO_2 , $\text{Na}_2\text{S}_2\text{O}_5$, Na_2SO_3 and NaHSO_3 , etc., is generally carried out prior to its subsequent precipitation step (Pollution group, 1987). However not all metal will be precipitated on neutralization and the removal of Cr^{3+} depends on the adjusted pH, type of precipitants used and duration of settling time. It was reportedly shown that the Cr^{3+} concentration remained at 13.7-15mg/l after precipitation at pH 7.0 (Pollution group, 1987).

In this regard, the column performances of *azolla* for removing Cr^{3+} ions was therefore examined. The regeneration efficiency, metal uptake and the volume treated at 60% saturation, at pHs 5.0 and 3.5 and flow rates 80ml/h and 160ml/h respectively, are summarised in Table 4.4 and Fig 4.7. The Cr^{3+} uptake and the regeneration efficiency for cycles 2-5, although slightly lower than that for cycle 1, are reasonably consistent irrespective of the number of cycles. This may suggest that reuse of the biosorbent in repeated column removal of Cr^{3+} from aqueous medium may be viable. Increasing flow rates from 80 to 160ml/h did not markedly decrease the Cr^{3+} uptake which suggested that the flow rate can be increased to this level without lowering the column efficiency (Table 4.4). The pH profile of cycle 2 was lower than that of cycle 1, after regeneration of the used biomass, which may explain the difference in metal uptake between cycle 1 and 2.

An early breakthrough of Cr^{3+} and a low Cr^{3+} uptake of 9.2mg/g in a column operation at an influent pH of 3.5 was noted, confirming the negative effect of low pH on Cr^{3+} sorption (Table 4.4).

Table 4.4 Column removal of Cr^{3+} by 5g azolla at influent Cr^{3+} of 100mg/l at varying pHs and flow rates

Cycle	Infl. pH	Flow rate (ml/h)	Cr^{3+} uptake (mg/g) ^a	Volume treated (ml) ^a	Regeneration efficiency (%)
	3.5	80	9.2	0.58	-
1	5.0	80	25.5	1.76	-
2	5.0	80	21.3	1.22	83.4
3	5.0	80	22.3	1.20	87.5
4	5.0	80	18.9	1.06	73.9
5	5.0	80	23.2	1.28	91.0
	5.0	160	19.2	1.24	-

^a at 60% saturation of the column biomass**Figure 4.7** Breakthrough curves for repeated column removal of Cr^{3+} for 5 cycles. Infl. Cr^{3+} conc., 100mg/l; biomass, 5g.

4.3.5.2 DESORPTION OF Cr^{3+}

Desorption of Cr^{3+} with either H_2SO_4 or HCl at varying concentrations was carried out and the results are presented in Fig 4.8 and Table 4.5. A recovery of 80% Cr^{3+} was accomplished with a number of washing using 0.5N H_2SO_4 in a total volume of 150ml (4 portions), whilst desorption with either 0.2N H_2SO_4 or 0.5N HCl only resulted in 50-60% recovery respectively probably due to the fact that chromium sulphate has higher solubility in water than its chloride (0.34 and 0.22mol chromium in 100ml H_2O respectively, CRC Handbook of Chemistry and Physics, 1980-1981). Similar result for desorption of Cr^{3+} from immobilised *S. cerevisiae* column were reported by Wilhelmi and Duncan (1995) in which only 34% recovery was obtained using 1M HCl . The complexity of desorption of Cr^{3+} further supports the assumption that chemisorption is probably responsible for binding of chromium to the biomass. However, further investigation is needed in illustrating the mechanisms regulating the binding of chromium to the biosorbent.

Table 4.5 Cr^{3+} recovery with various desorbents in volume of 150ml

Desorbents	Cr^{3+} uptake (mg)	Cr^{3+} desorbed (mg)	Recovery (%)
0.2N H_2SO_4	126.5	64.4	50.0
0.5N H_2SO_4	117.8	96.7	82.1
0.2N HCl	113.2	38.6	35.0
0.5N HCl	134.8	82.0	60.9

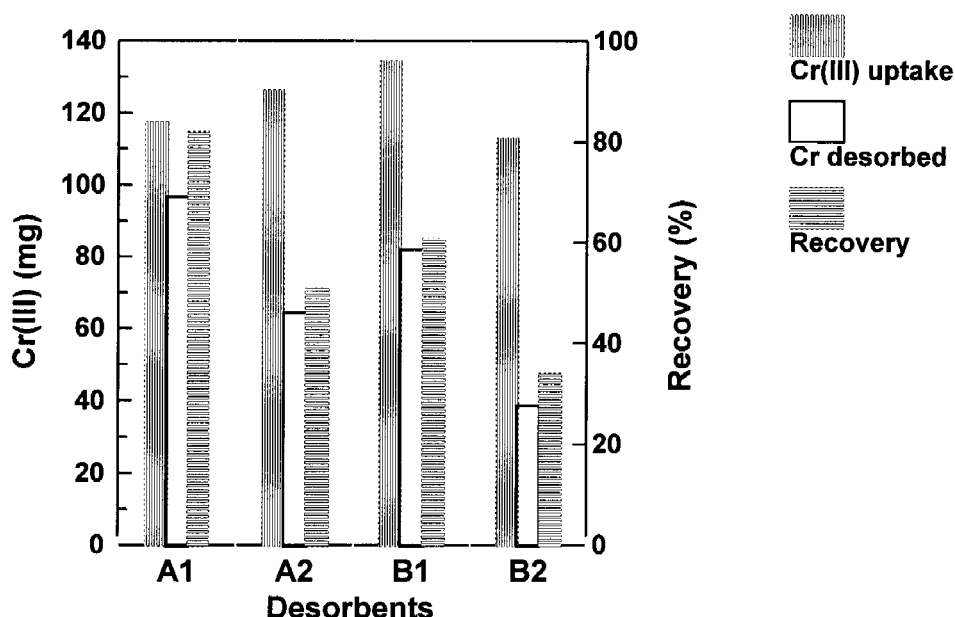


Figure 4.8 Desorption and recovery of chromium from Cr^{3+} -laden biomass column with various desorbents. **A1**, 0.5N H_2SO_4 ; **A2**, 0.2N H_2SO_4 ; **B1**, 0.5N HCl ; **B2**, 0.2N HCl .

4.4 CONCLUSIONS

The dried form of azolla biomass, repeatedly used in present study, showing good insolubility in water and stability at low pHs and can be applied to a continuous flow system for treatment of chromium-laden wastewater without immobilization. It is able to remove a significant amount of Cr^{6+} from either aqueous solution and electroplating effluent at pH 2.0-2.5 in a fixed-bed column process. Desorption of bound Cr^{6+} with 0.5M NaOH or 1M NaCl /0.1 M NaOH proved unsuccessful, whilst a combination of 0.5M $\text{Na}_2\text{S}_2\text{O}_5$ /0.5M HCl , at a contact time of 6 h, appeared promising yet incomplete with a regeneration efficiency of 50%. Further work is needed to improve the reduction and desorption process in order that a optimum regeneration efficiency can be attained. As an alternative to the direct sorption of Cr^{6+} from

water, the trivalent chromium ions can be removed effectively by *azolla* biomass at pH 5. More efficient removal at pHs higher than 5 can be expected. Desorption of Cr^{3+} from the saturated biomass column was accomplished with the recovery of 80% using 0.5N H_2SO_4 which shows a greater desorbing ability to that of HCl. The metal uptake capacity of *azolla* biomass remained similar to the first run after completion of five cycles of sorption-desorption. Regeneration efficiency for Cr^{3+} removal of up to 90% also confirmed the reusability of *azolla* biomass in the column operation. Therefore, it may be concluded that the *azolla* biomass has promise for use in bioremediation of Cr^{6+} -contaminated wastewater.

5. REMOVAL AND RECOVERY OF NICKEL FROM AQUEOUS SOLUTION AND ELECTROPLATING RINSE EFFLUENT USING *Azolla filiculoides*

5.1 INTRODUCTION

Nickel, well verified in mammals for its role in carcinogenicity (Reith & Brogger, 1983), is being widely used in electroplating industries to enhance the value of product by providing aesthetic appearance, hardness and corrosive resistance (Pollution group, 1987). As only 30 to 40 per cent of all metals used in electroplating process are effectively utilised (plated onto the articles), its waste effluents are responsible for some of the supply of heavy metal pollutants to the environment (Greenyuk *et al*, 1996). Chemical precipitation with lime or caustic soda is one of the most conventional treatments, where recovery of metals or water is not a consideration. However, to effectively decrease metals to acceptable levels requires a large excess of chemicals, which generates volumetric sludge and increases cost (Spearot & Peck, 1984). Other available treatments such as ion-exchange, electrolysis and reverse osmosis require high capital investment and running costs.

It has been demonstrated that certain plants, being cheaply available in nature, have the potential to effectively remove heavy metals from dilute wastewater by a sorption process thus offer an alternative to the existing technologies (Scott, 1992). Studies focusing specifically on Ni removal are rare, as previous investigations mostly covered the sorption of Ni in a range of other metals and the information is mostly relevant to the description of metal accumulation by living plants or to the toxic effects of metals on their metabolism (Holan & Volesky, 1994). Although the previous results revealed the promising potential of *Azolla filiculoides* in accumulating hexavalent and trivalent chromium in batch and continuous flow systems, its capability of removing divalent cation metals eg. Ni remained unknown (Zhao & Duncan, 1997, b & c). Therefore this section will examine the sorption and desorption of Ni using the azolla biomass and explored the possibilities of recycling the plating rinse waters as well as the nickel.

5.2 METHODS AND MATERIALS

5.2.1 MATERIALS

The azolla biomass was collected and prepared as described in Chapter 3.3.1. Analytical grade reagents were used in all cases. Stock nickel solution (1000mg/l) was prepared in distilled water as NiCl_2 (Merck, Germany). All working solutions were prepared by diluting the stock solution with distilled water. NaOH, HCl and H_2SO_4 were obtained from Saarchem, South Africa. Activated carbon was provided by Saarchem, South Africa. The electroplating effluents, designed as EPE4 and EPE5 (Table 5.1), were collected from a factory (PE Plating, South Africa) operating an acid-Ni-plating line. The pH values in Table 5.1 are those for a particular sample of effluent. The effluent pH varied between 6.7 - 7.3.

Table 5.1 Characteristics of EPE4 and EPE5

	pH	Ni	Ca	Mg	Cr(VI)	Zn	Fe
		mg/l					
EPE4	7.0	20	21	ND	0	0	0
EPE5	7.1	12	22	38.5	0	0	0

ND: not determined

5.2.2 ASSAY METHODS

The Ni, Ca and Mg concentrations were determined by atomic absorption spectrophotometry (GBC Scientific Equipment, Australia).

5.2.3 ADSORPTION ISOTHERMS

Batch sorption tests were carried out at 18°C on a rotary shaker using 250ml conical flasks. Nickel solutions (100ml) were adjusted to the required initial pH values before addition of the

biomass (4g/l). The pH values of the solution were checked and adjusted every hour during the incubation by addition of either H₂SO₄ or NaOH. The isotherm studies were performed by varying the initial Ni concentrations from 20mg/l to 1000mg/l and the pHs from 3.0 to 6.5. The highest pH adopted in the isotherm study was 6.5, since precipitation of nickel ions reportedly occurred at pHs higher than 6.7 (Holan & Volesky, 1994). After the flasks were agitated for five hours, the reaction mixtures were filtered through Whatman no.1 filter paper and the filtrate was analysed for residual Ni concentrations. The Langmuir sorption model was employed for the estimation of maximum metal uptake (X_m) where they could not be reached in the experiment (Gosset *et al*, 1986; Holan *et al*, 1993).

$$C_e/q_e = 1/(X_m b) + C_e/X_m$$

Where X_m and b are Langmuir constants, indicative of maximum adsorption capacity and a measure of adsorption energy respectively. q_e is the metal uptake (mg Ni/g of biomass), C_e is the equilibrium concentration of nickel (mg/l).

5.2.4 KINETIC STUDIES

Azolla biomass of 1.2g was thoroughly mixed with 300ml of nickel solutions of varying concentrations ranging from 20mg/l to 800mg/l. The suspensions were shaken for 24 hours at 18°C using 500ml conical flasks. Aliquots of 2ml were collected at required times and analysed as previously described.

5.2.5 PREPARATION OF COLUMNS

Glass columns of 25mm internal diameter were used in the operation. Uniformly milled *azolla* biomass of 5g in its dry form was inserted from the top and rinsed with distilled water. Feed solutions were pumped upwards through the column using a peristaltic pump (Watson Marlow, 504S). Flow rates were maintained as required. The elute was collected in 20ml fractions. The working temperature was kept at approximately 18°C. The breakthrough point

for the column operation was defined as the volume when the exit concentrations of Ni reached 60% of the influent concentrations. The term, column efficiency, was introduced to compare the Ni uptake of each column operation at 60% saturation, against that at a flow rate of 80ml/h, pH 7.0.

5.2.6 REGENERATION OF COLUMN BIOMASS

The saturated *azolla* biomass was regenerated *in situ* by washing the column with acids. The elution was carried out downwards with 0.1-0.2N either H₂SO₄ or HCl respectively at a flow rate of 80ml/h (16ml/h.g). 20ml fractions were collected. After acid washing, the columns were reconditioned with 35ml 0.1M NaOH (incubated for 20 min) to reverse acidity of the biomass which was followed by 2 x 35ml distilled water. The reconditioning of the regenerated biomass was thought necessary because pH values of the acid-washed biomass were extremely low (pH 1-2) with the result that metal uptake capacity was markedly decreased. The terms, metal uptake capacity and regeneration efficiency (ratio of the regenerated metal uptake capacity to the original one), were described in Chapter 2.3.6, and used here to evaluate the efficiency and the reusability of the biomass for column removal of Ni.

5.3 RESULTS AND DISCUSSION

5.3.1 ADSORPTION ISOTHERMS

The nickel isotherms followed the Langmuir model well with the correlation coefficients higher than 0.97 (Fig 5.1 and Table 5.2). The removal of Ni from aqueous solution was more efficient with increasing incubating pHs (Fig 5.1). The best pH for the sorption of Ni on *azolla* biomass was found to be 6.5, under the present experimental conditions. Although higher pHs may give as good a removal of Ni as at pH 6.5, metal precipitation may occur during incubation when a metal concentration as high as 1000mg/l of Ni was used. Hence pH of 6.5 was the highest used on batch tests in this study. The maximum Ni uptake by *azolla* at

pH 6.5 was found to be 43.4mg/g, whilst lower amounts of Ni had been removed at pH 3 (22.4mg/g), and pH 5 (32.4mg/g) (Table 5.2). The b value, a indication of the “strength or affinity” of the sorbent for metals at relatively low metal concentrations, was found to increase as the incubation pHs rises, which implies that removal of Ni at high pH could be more complete than that at low pH.

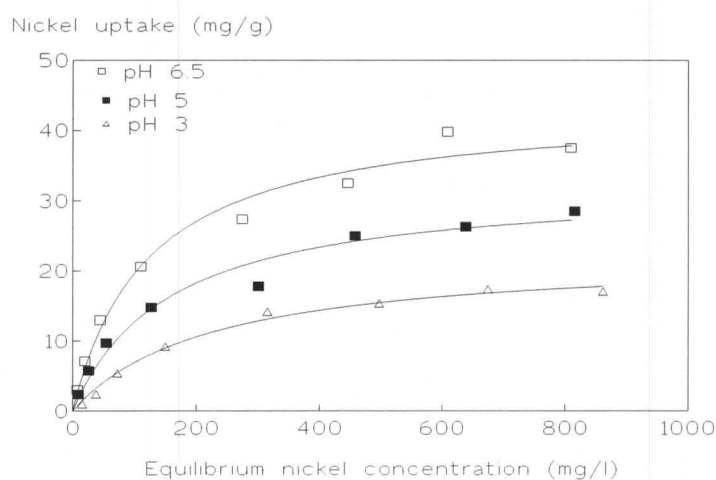


Figure 5.1 Adsorption isotherms for azolla-Ni system. Temperature, 18°C; incubation time, 5 h; biomass, 4g/l.

Table 5.2 Langmuir constants of Ni-Azolla binding at 18°C

pH	X_m (mg/g)	b ($\times 10^{-3}$)	Correlation (r^2)
3.0	22.4	4.50	0.99
5.0	32.4	6.45	0.97
6.5	43.4	8.27	0.98

5.3.2 KINETICS

Rapid removal of Ni by *azolla* was observed with about 80% of the bound metal adsorbed in the first 10 minutes, followed by a more gradual sorption course (Fig 5.2). The character of rapid sorption is of importance in a continuous-flow treatment system because it enables an optimum metal uptake at high flow rates.

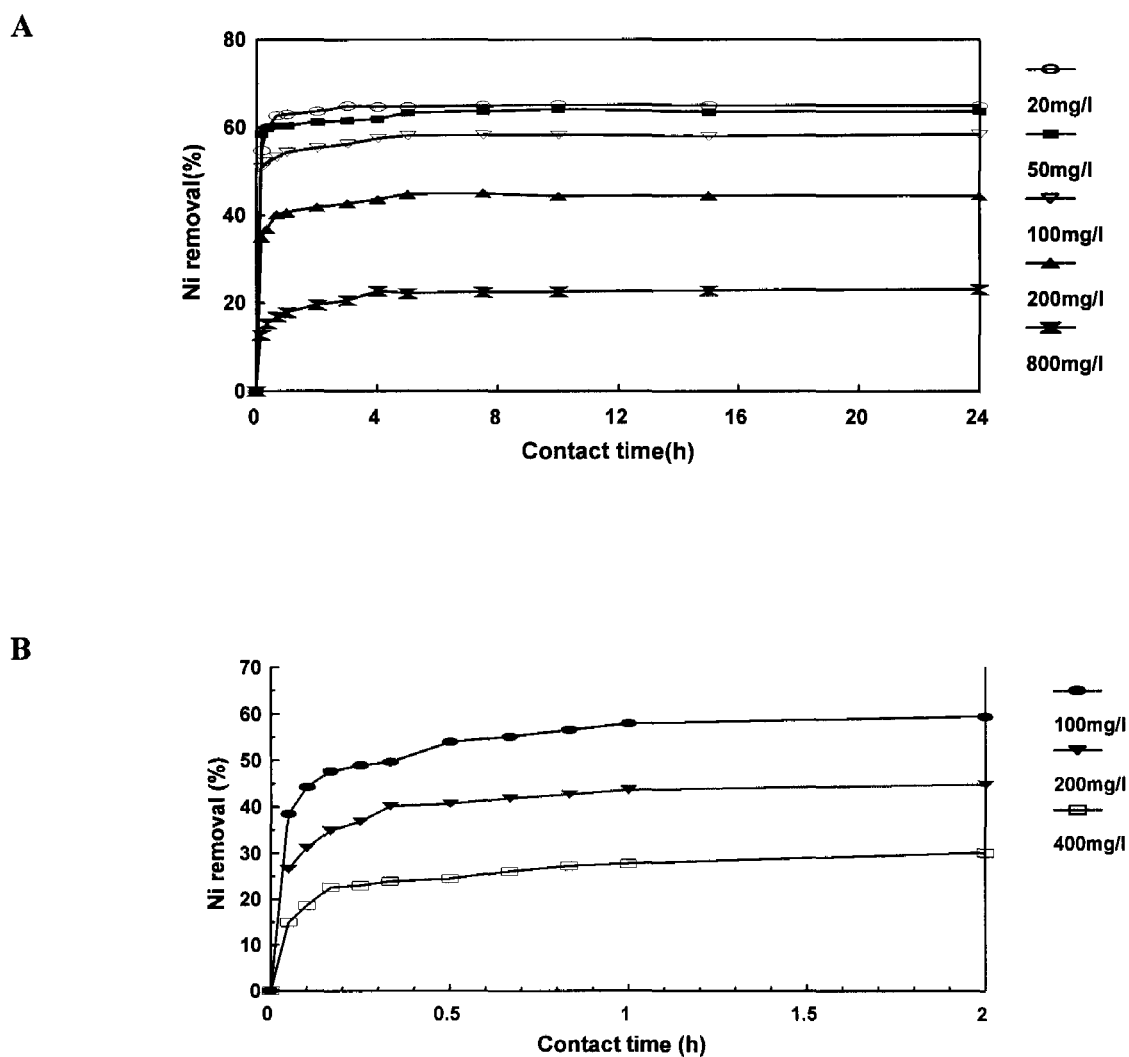


Figure 5.2 Effect of contact time on the sorption of Ni by *azolla* biomass at pH 6.5. Biomass, 4g/l. (A), sampled for 24 h; (B), sampled for 2 h.

5.3.3 COLUMN REMOVAL OF Ni

Column performance for sorption of Ni was evaluated by comparing the metal uptake and the volume treated at 60% saturation of the biomass with varying flow rates, mass of *azolla*, influent pHs and Ni concentrations in the column operations (Figs 5.3, 5.4 & 5.6 and Table 5.3). It was observed that precipitation of Ni did not occur at pH 7.0 in the preparation of nickel chloride solution, since the natural pH of the dissolved NiCl_2 solution was found to be 7.3. The pH values of the electroplating rinse effluent were 6.7 - 7.3. Therefore, the influent pH value in column operations was defined at the neutral condition.

Table 5.3 Column removal of Ni by 5g *azolla* biomass with varying flow rates, influent pHs and Ni concentrations

Cycle	Infl. Ni (mg/l)	Infl. pH	Flow rate (ml/h)	Ni uptake (mg/g) ^a	Column efficiency (%) ^{a, b}	Volume treated (l) ^a
	100	7.0	80	27.9	100	1.52
	100	7.0	160	20.9	75.0	1.16
	100	7.0	240	24.6	88.1	1.32
	100	7.0	320	22.1	79.0	1.22
1	100	7.0	480	26.1	93.6	1.50
	100	7.0	800	21.6	77.4	1.20
2	100	7.0	480	21.4	76.7	1.16
3	100	7.0	480	23.8	85.3	1.30
4	100	7.0	480	25.2	90.2	1.36
	100	5.0	480	14.7	52.4	1.00
	10	7.0	480	15.4	55.2	8.40
	20	7.0	480	19.2	68.8	5.40

^a at 60% saturation of the column biomass

^b the Ni uptake at 80ml/h was defined as 100%

5.3.4 EFFECT OF FLOW RATES

The column performance, in terms of Ni uptake and the treated volume at 60% saturation of the biomass, was not markedly affected by increasing flow rates from 80 up to 800ml/h in the operations at an influent pH of 7. However, the highest Ni uptake capacity (27.9mg/g) was found to be at a low flow rate of 80ml/h (Fig 5.3 and Table 5.3). The value of Ni uptake on column operation is lower than that from batch tests (Table 5.2), due possibly to the fact that different Ni concentrations were used in column and batch experiments. The incubation Ni concentrations on isotherm tests, at which Ni uptake of 43.4mg/g was obtained, were as high as 1000mg/l, whereas a influent Ni concentration of 100mg/l was used in column operations. The column efficiencies at those flow rates, although varied did not show any significant decreasing trend with increasing flow rates upto 800ml/h. A flow rate of 480ml/h was chosen as one of the working parameters in the successive column studies because it is a much high flow rate and at which one of the highest Ni uptake was attained. The rapid removal of Ni from solution can be predicted with the data from kinetic experiments in which high sorption rates were obtained that 87% of the bound metal had been removed in 10 minutes (Fig 5.2). The contact time within the bed for Ni with *azolla* at a flow rate of 480ml/h was estimated as 4.4 minutes. This information is particularly useful in designing a scale-up of the sorption technology for treatment of Ni-laden effluents.

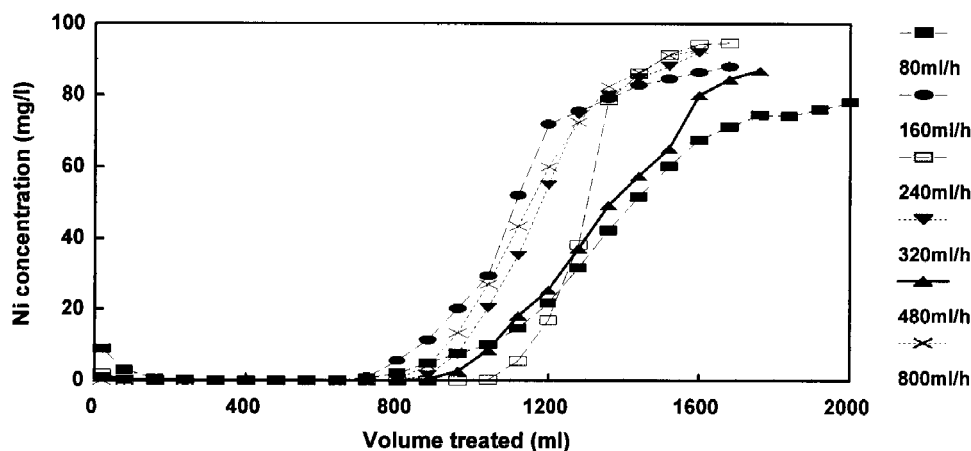


Figure 5.3 Breakthrough curves for column removal of Ni by 5g azolla with varying flow rates. Infl. Ni, 100mg/l; infl. pH, 7.0.

5.3.5 EFFECT OF INFLUENT pHs AND REPEATED SORPTION OF Ni

Accumulation of metals in fixed bed columns is largely dependent on the pH of the influent solution (Stoll & Duncan. 1997). It was seen in the present study that lowering the pH value of the influent to 5 resulted in an earlier breakthrough in the sorption profile with a nickel uptake of 14.7mg/g, showing a column efficiency of only 52.4% (Table 5.3). On the other hand, adjusting the influent pH to 7 seen in the cycle 2-4 (Fig 5.4), maintained its sorption capacity in the repeated column operations. Reconditioning of the biomass with 0.1M NaOH, was shown to effectively reverse the acidity of the biomass resulting from desorption as shown by the pH profile for cycle 2 and consequently maintained its sorption capacity (Table 5.3), presumably through increasing porosity and alkalinity of the biomass. The enhanced porosity of the biomass by alkali reconditioning may be indicated by an enlarged wet volume of the biomass in comparison with that of biomass without alkali treatment (about 10-20% increase in volume was observed in all cases). The modification of the biomass with mild alkaline could provide increased surface area available for metal sorption, thereby improving the sorption capacity. On the other hand, residual alkali may otherwise remain behind in the

biomass pores after washing, thereby influencing the biomass-metal binding patterns possibly through microprecipitation on its surface as appears to be the case from Fig 5.5. The effect of caustic precipitation, within the biomass should not be overestimated since, as noted by Brady *et al*, (1994), some forms of caustic treated biomass have decreased capacities for accumulating metals.

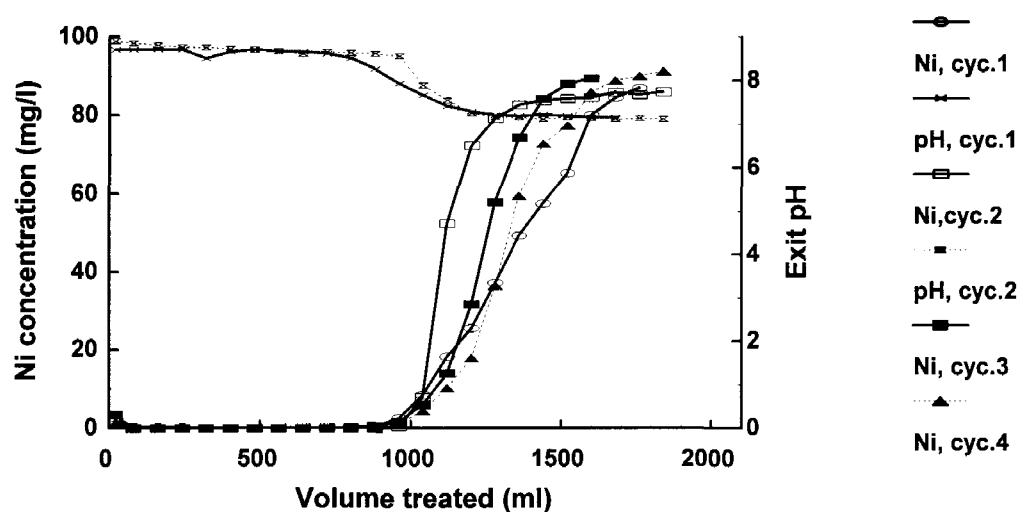


Figure 5.4 Breakthrough curves for column removal of Ni by 5g *azolla* for 4 cycles. Flow rate, 480ml/h; infl. Ni, 100mg/l; infl. pH, 7.0.

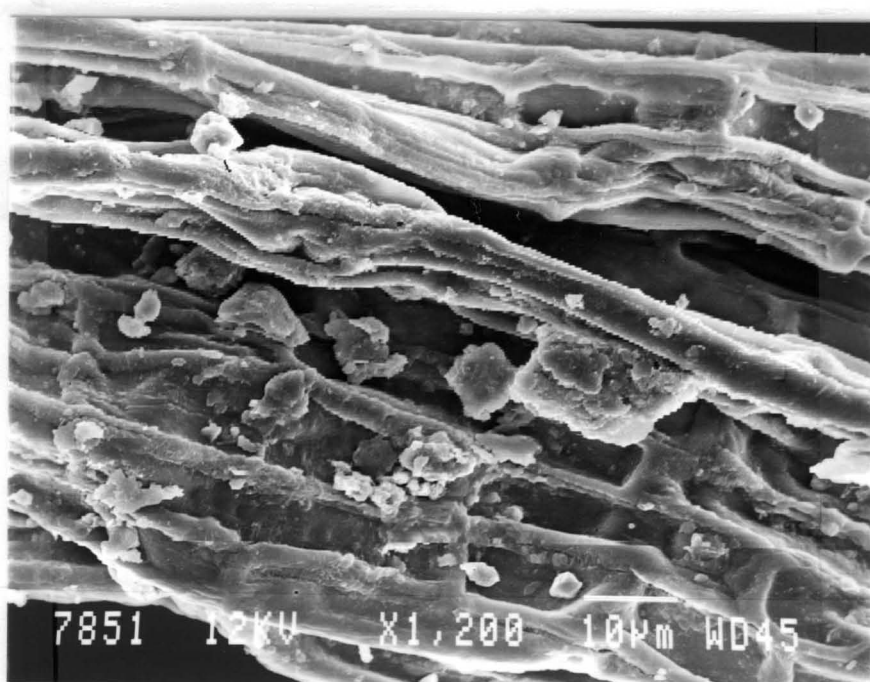


Figure 5.5 Scanning electron micrograph of used azolla after sorption of Ni.

5.3.6 EFFECT OF INFLUENT Ni CONCENTRATION

In considering the fact that the most encountered Ni concentrations in electroplating rinse effluents ranges from 10 to 20mg/l (Pollution Group, 1987), the corresponding influent Ni concentrations were chosen in evaluating the metal uptake capacity of azolla biomass. It can be seen that changes in the feeding Ni concentration affected column performances of the azolla biomass (Table 5.3). At influent Ni concentrations of 10 and 20mg/l, Ni uptake of 15.4 and 19.2mg/g was obtained respectively, indicating a decreasing trend in the values of Ni uptake with lowering Ni concentration in the feeding solution. This trend was also reflected by the result from isotherm study in Chapter 5.3.1. On the other hand, though not proportionally, much more volume of Ni-laden solution had been treated before 60% saturation of the biomass, 8.4 and 5.4 litres at the influent Ni concentrations of 10 and 20mg/l respectively.

5.3.7 EFFECT OF VARYING MASS OF *Azolla*

Varying the amount of biomass in column operations at a flow rate of 480ml/h resulted in a proportional increased volume of treated Ni-laden solution of 0.82, 1.48 and 2.36 litres for 2.5, 5 and 7.5g biomass respectively (Table 5.4 and Fig. 5.6). The calculated metal uptake at 60% saturation remained unchanged in the three column operations giving the values of 25.7, 26.2 and 28.7mg/g for 2.5, 5 and 7.5g biomass respectively (Table 5.4). The resulting trend was confirmed by other column operations at flow rate of 800ml/h (Table 5.4). This characteristic in column performance is of particular importance in designing a scale-up column system for metal treatment, because the volume of treated wastewater can be estimated through the positive relationship between the amount of biomass used and the volume of wastewater treated, at a specified metal concentration.

Table 5.4 Column removal of Ni at influent pH 7.0 and influent Ni concentration of 100mg/l with varying mass of *azolla* and flow rate

Flow rate (ml/h)	Biomass (g)	Ni uptake (mg/g) ^a	Column efficiency (%) ^b	Volume treated (l) ^a
480	2.5	25.7	91.8	0.82
480	5.0	26.2	93.6	1.50
480	7.5	28.7	102.5	2.36
800	2.5	25.8	92.4	0.76
800	5.0	21.6	77.4	1.20
800	7.5	26.4	94.4	2.10

^a at 60% saturation of the column biomass

^b the metal uptake at 80ml/h shown in Table 5.3 was defined as 100%

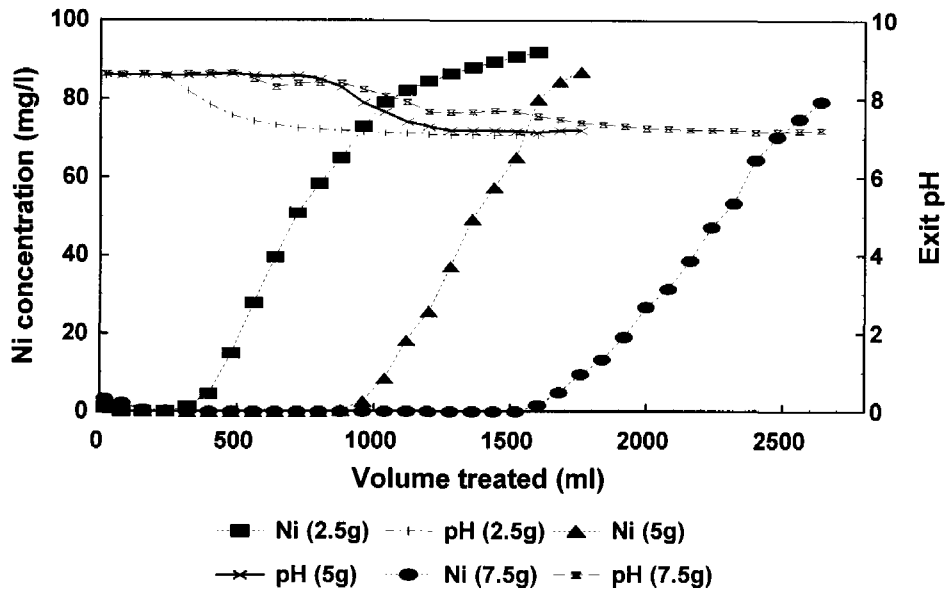


Figure 5.6 Breakthrough curves for removal of Ni with varying mass of azolla. Infl. Ni, 100mg/l; infl. pH, 7.0; flow rate, 480ml/h.

5.3.8 BDST EQUATION

The most common model used to correlate service time (t) with other design parameters in column systems is the Bed-Depth-Service-Time (BDST) model (Sharma & Forster, 1996). The modified form of the Bohart and Adams model states that the service time of a column is given by

$$t = (N_0 / C_0 U) H - (1 / k C_0) \ln[(C_0 / C_t) - 1]$$

where t is service time (h) to breakthrough; N_0 is sorption capacity (mg solute/l sorbent); C_0 is initial solute concentration (mg/l); U is linear flow rate (m/h); H is depth of sorbent bed (m); k is rate constant of sorption (l/mg.h) and C_t is outlet concentration (mg/l).

By plotting t against H from experimental data, N_0 can be evaluated from the slope of the

graph and k from the intercept at $t = 0$. At 50% breakthrough ($C_0 / C_t = 2$), the logarithmic term reduces to zero and the expression can be written as $t_{50} = (N_0 / C_0 U)H$. Thus, a t_{50} / H curve is a straight line passing through the origin, provided the data follow the model (Fig. 5.7). The values of N_0 , determined from the equation at different flow rates, together with other constants were summarised in Table 5.5. The estimated values of adsorption capacity at full saturation of the biomass, N_0 , is consistent with the observed values from column operations (Table 5.3). Values at 30% breakthrough were also constructed to show the consistency of the equation. The use of BDST model in this way provides a realistic description of the accumulation of Ni by azolla biomass and the empirical data can be adopted in predicting the bed depth or service time for a specified set of influent characteristics for the scale-up of the biosorption column (Muraleedharan *et al*, 1994).

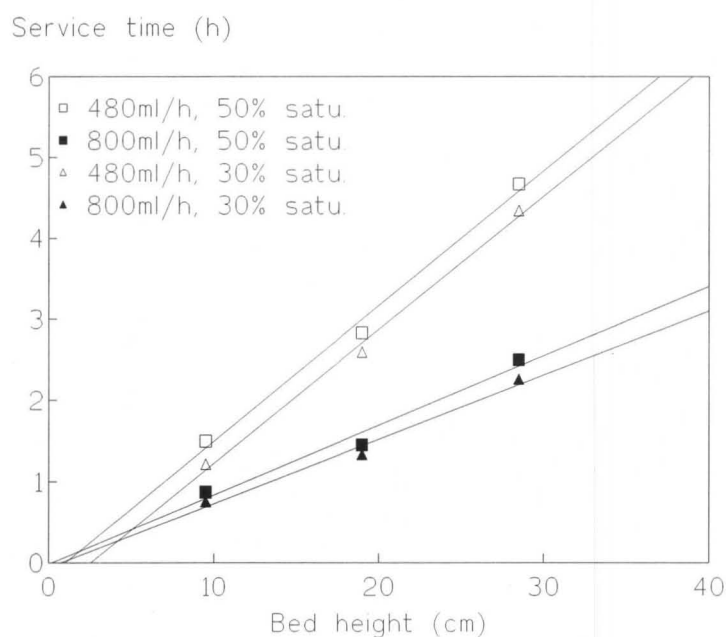


Figure 5.7 BDST curves at various breakthrough levels with varying flow rates. Infl. pH, 7.0.

Table 5.5 Calculated constants of the BDST equation

Flow rate (ml/h)	Slope	Breakthrough (%)	Intercept (y)	Intercept (x)	N_0 (mg/g)	r^2
480	16.684	50	-0.170	1.02	30.4	0.992
800	8.553	50	-0.017	0.20	25.9	0.972
480	16.447	30	-0.417	2.50	-	0.995
800	7.895	30	-0.058	0.70	-	0.982

5.3.9 TREATMENT OF PLATING RINSE EFFLUENT

The removal of Ni from plating rinse effluent (EPE4) by 5g biomass at pH 7 is presented in Fig 5.8 and Table 5.6. Complete removal of Ni from EPE4 in the first 3 litres was observed with a metal uptake of 14.6mg/g at 60% saturation. It was seen that together with Ni the Ca ion present in rinse water was also adsorbed in the column, which probably accounted for the decreased Ni uptake (24.1%) of the biomass, in comparison with the column efficiency for Ca-free Ni solution (Table 5.6). The presence of Ca appeared to affect the accumulation of Ni somehow, but the *azolla* biomass still showed the priority for Ni as seen in the elution profile, in which further accumulation of Ni proceeded whilst sorption of Ca reached saturation (Fig 5.4). Ca was only bound to the biomass in limited quantity (3.9mg/g) at full saturation thereby suggesting that the biosorbent is partially selective for Ni under present experimental conditions.

Another sample of rinse effluent, namely EPE5, was characterized by a lower concentration of Ni (10mg/l) and high contents of Ca and Mg (Table 5.6). At 60% saturation, a Ni uptake capacity of 12.9mg/g was attained with 5.8 litres of EPE5 being treated (Fig 5.9). The Ni uptake capacity was found lower than that of for Ca-free Ni solution by 16.2% (Table 5.6). The Ca accumulated, 12.1mg/g, from EPE5 was higher than that from EPE4, due probably to the low Ni concentration present in EPE5 (Fig 5.9). It was postulated that Ca ions, probably

share some of the binding sites on surface of *azolla* with Ni and imposed competitive effects on sorption of Ni. The sorption of Ca appeared to be enhanced when Ni concentration was lower. The effect of Mg of high concentration present in EPE5 on Ni sorption was relatively minor, showing a Mg uptake of 4.1mg/g. The Mg ions were accumulated simultaneously with Ni and Ca at beginning of the treatment, thereafter displaced from the biomass while sorption of Ni and Ca continues (Fig 5.9). It should be also noted that the interfering effects from other unknown components present in the effluent on the sorption capacity of *azolla* can not be excluded under conditions of the experiment.

Table 5.6 Column removal of Ni from EPE4 and EPE5
with 5g *azolla* at a flow rate of 480ml/h

Infl. Ni (mg/l)	Infl. Ca (mg/l)	Infl. Mg (mg/l)	Infl. pH	Ni uptake (mg/g) ^a	Column efficiency (%) ^b	Volume treated (litre) ^a
10	0	0	7.0	15.4	55.2	8.4
20	0	0	7.0	19.2	68.8	5.4
20 ^c	21	ND	7.0	14.6	52.2	4.2
12 ^d	22	38.5	7.1	12.9	46.2	5.8

^a at 60% saturation of the column biomass

^b the Ni uptake at 80ml/h shown in Table 5.3 was defined as 100%

^c EPE4

^d EPE5

ND: not determined

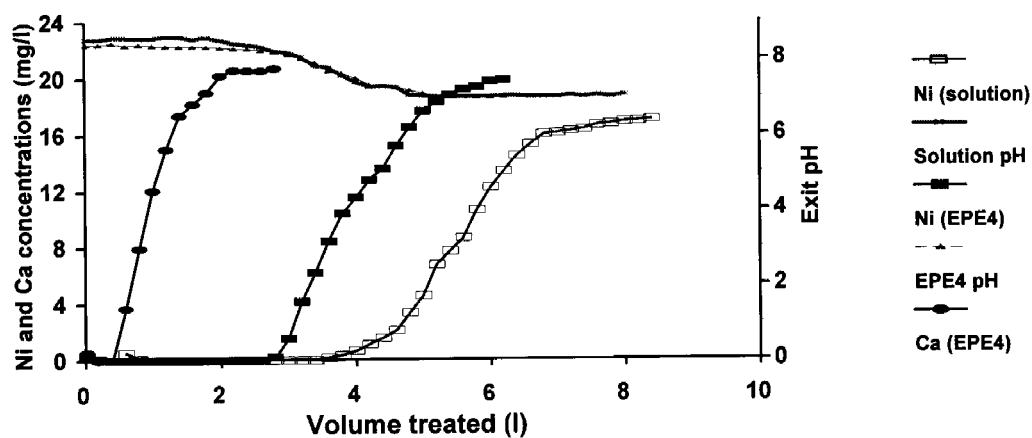


Figure 5.8 Breakthrough curves for removal of Ni from EPE4 and aqueous solution. Infl. pH, 7.0; infl. Ni, 20mg/l; Ca, 21mg/l; flow rates, 480ml/h; biomass, 5g.

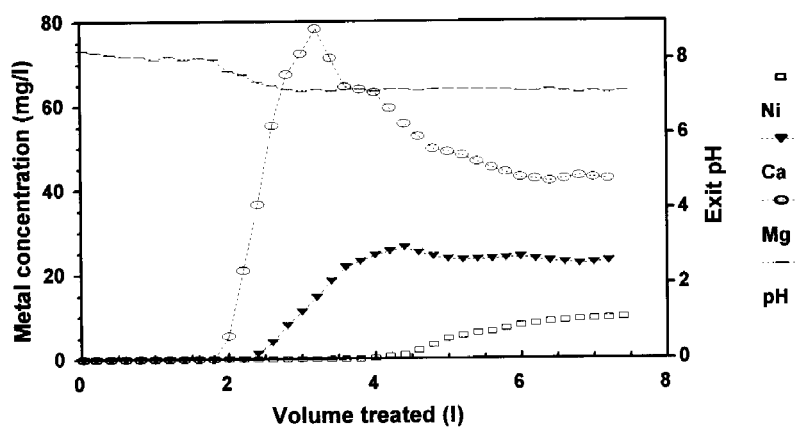


Figure 5.9 Breakthrough curves for removal of Ni from EPE5. Infl. pH, 7.1; infl. Ni, 12mg/l; Ca, 22mg/l; Mg, 38.5mg/l; flow rates, 480ml/h; biomass, 5g.

5.3.10 DESORPTION AND RECOVERY OF Ni

Desorption of Ni with different concentrations of HCl or H₂SO₄ was carried out and the results are presented in Table 5.7. Recoveries of up to 95% of Ni was accomplished using 0.2N HCl or H₂SO₄ in volume of 120ml, whilst desorption with 0.1N HCl or H₂SO₄ only achieved 60-70% recovery in the same volume of desorbing solution. A greater volume of desorbing solution were required in completion of the desorption with 0.1N acids. Both of the acids at a concentration of 0.2N showed good Ni-desorbing capacity in regeneration of the exhausted biomass. The regeneration efficiencies show that even after completion of four alternating cycles, the metal-removal capacity was not markedly affected and that the azolla biomass is reusable (Fig 5.10 and Table 5.3).

Recovery of Ni from the saturated biomass with 0.2N HCl, after EPE4 treatment, was nearly complete (Fig 5.11). However, together with Ni, Ca was also desorbed by the acid, which obviously introduced an impurity into the recovered Ni solution.

Table 5.7 Ni recoveries with various desorbents in a volume of 120ml

Cycle	Desorbent	Ni uptake (mg)	Ni desorbed (mg)	Recovery (%)	Regeneration efficiency (%)
	0.1N HCl	122.5	76.0	62.0	-
	0.1N H ₂ SO ₄	115.8	77.6	67.0	-
1	0.2N HCl	152.3	143.6	94.3	-
2	0.2N H ₂ SO ₄	119.6	113.6	95.0	81.9
3	0.2N HCl	124.8	118.1	94.6	91.1
4	0.2N H ₂ SO ₄	134.1	127.9	95.3	90.1
EPE4	0.2N HCl	81.2	79.8	98.2	-

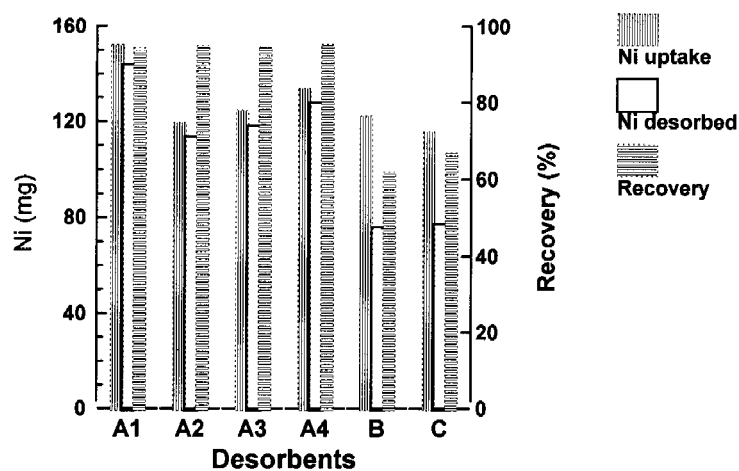


Figure 5.10 Ni recovery with H_2SO_4 and HCl of varying concentrations. A1, 0.2N HCl, cyc.1; A2, 0.2N H_2SO_4 , cyc.2; A3, 0.2N HCl, cyc.3; A4, 0.2N H_2SO_4 , cyc.4; B, 0.1N HCl; C, 0.1N H_2SO_4 .

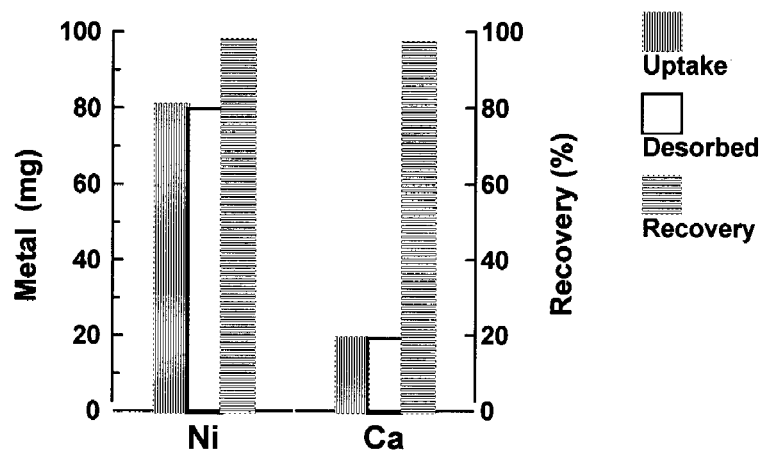


Figure 5.11 Recoveries of Ni and Ca with 0.2N HCl, after treatment of EPE4.

5.3.11 RECYCLING OF Ni AND WASTE WATER

The accumulated Ni ions were recovered in the same chemical form as the original plating agents used in the electroplating factory (nickel sulphate or chloride) and concentrated at a level (over 1000mg/l) that is high enough that feeding it back to the Ni-plating bath would be possible. The possible impurities in the recovered Ni solution will be mainly the organics which appear to be leached from *azolla* biomass during desorption, but this can be eliminated by pumping the solution through a activated carbon column, a commonly adopted procedure in preparing plating agents in electroplating shops. Therefore precipitation of the concentrated Ni solution and dumping of the heavy metal-laden sludge could be avoided.

In a common electroplating line, articles are transported from the plating bath to the catching bath and then to the rinse tanks (Fig 5.12). The amount of electrolytes in catching bath, which are carried over from the plating bath, are so high that reuse of the rinse water in catching bath directly to plating bath is possible. However, metals and waste water in rinse tanks are the major concerns in treatment of plating effluent. A simplified Ni and water recycling technology is proposed and illustrated in Fig 5.12. Columns filled with *azolla* biomass could be situated next to the rinse tank 1. The effluent from rinse tank 1 (C) can be continuously circulated through the working column, designated as E, with saturated column (F) being regenerated with acids. Concentrated Ni solution can be fed back to the plating bath (A), after being filtered by a carbon column (G). The purified wastewater from the working column can also be recycled to rinse tanks (C & D) thus reducing water consumption in electroplating shops. The on-site technology for Ni-treatment thus enables the extraction of pure Ni ions from rinse water and recycling of the metal back to plating bath (Fig 5.12), which is not only environmentally beneficial but also economically profitable. The development of effluent-free, closed cycles of water supply for electroplating production has been considered as an ultimate aim when screening a new water purification methods (Greenyuk *et al*, 1996). To the existing water purification methods, concentration and extraction of heavy metals by *azolla*-based sorption technology could be implemented as an efficiently and economically viable process.

This diagram proposed here is only a primary frame for treatment of metal-bearing waste water using the biosorbent. Further modification and improvement of the proposed technology are thus necessary in industrial practice. In some cases, multi-stage processes through combination of the sorption means with other technologies eg chemical precipitation could otherwise be more applicable.

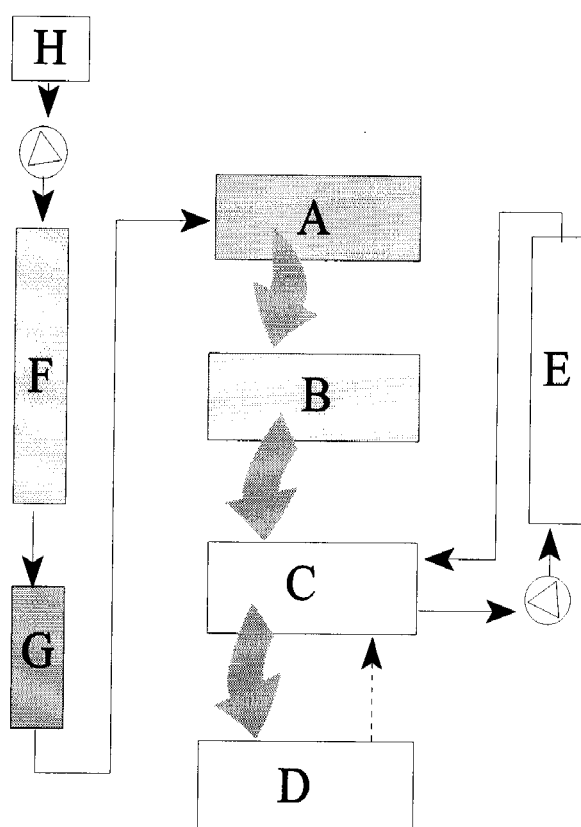


Figure 5.12 Schematic installation for electroplating rinse effluent treatment. **A:** plating bath; **B:** catching bath; **C:** rinse tank 1; **D:** rinse tank 2; **E:** biomass column; **F:** saturated column for regeneration; **G:** activated carbon filter for removing impurities; **H:** acids container.

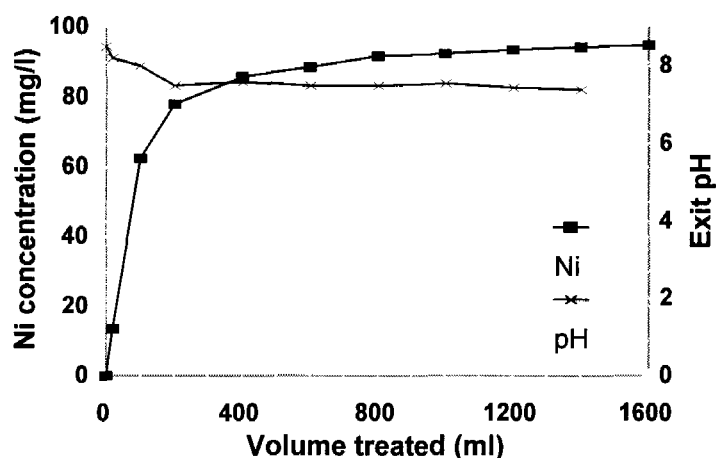
5.3.12 COMPARISON WITH OTHER SORBENTS

In order to assess the viability of a treatment process based on sorption, the metal uptake capacities of the test sorbents must be compared with those of other sorbents under similar conditions. Table 5.8 shows a summary of various sorbents that have been used for batch removal of Ni. The comparison has been made in terms of maximum uptake capacities, X_m . Among the sorbents, azolla appears to have the superior potential for Ni removal on batch scale.

Activated carbon is usually considered to be the sorbent against which others are assessed (Sharma & Forster, 1996). Column studies on removal of Ni by activated carbon has not been reported. In order to compare column performance of azolla with that of activated carbon, a column operation for removal of Ni with 5g activated carbon was carried out under identical conditions. A lower Ni uptake of 3.3mg/g (for activated carbon) at full saturation was exhibited (Fig 5.13), which is in consistent with that of carbon in batch test (Tabla 5.8). The unsatisfactory performance of activated carbon can be partially explained by the fact that the surface of activated carbon is essentially nonpolar although a slight polarity may rise from surface oxidation (Ruthven, 1984).

Table 5.8 Ni uptake capacities of various sorbents on batch scale

Sorbent	Xm (mg/g)	pH	Max.conc. (mg/l)	Dose (g/l)	Reference
Activated carbon	8.1	6.5-7.3	100	5	Larsen & Schierup, 1981
Straw	6.4	6.5	100	5	Larsen & Schierup, 1981
Peat	11.2	4.5	587	50	Gosset <i>et al.</i> , 1986
Iminodiacetic acid-cellulose	20.8	7	80	1.5	Chan <i>et al.</i> , 1992
<i>Potamogeton lucens</i>	22.9	5.0-6.6	-	2	Schneider & Rubio, 1995
<i>Salvinia herzogii</i>	14.4	5.0-6.6	-	2	Schneider & Rubio, 1995
<i>Eichhornia crassipes</i>	11.6	5.0-6.6	-	2	Schneider & Rubio, 1995
<i>Azolla filiculoides</i>	43.4	6.5	1000	4	Present study

**Figure 5.13** Breakthrough curves for removal of Ni with 5g activated carbon. Infl. pH, 7.0; infl. Ni, 100mg/l; flow rate, 480ml/h.

5.4 CONCLUSIONS

The data from batch experiment on the *azolla*-Ni system provided fundamental information in terms of optimum pH and maximum metal uptake, for continuous-flow treatment of metal-laden electroplating effluents.

A significant amount of Ni (14.6 - 27.9mg/g) can be removed from either aqueous solution and electroplating effluent at neutral pH in a column process. The metal uptake was found to be 27.9 mg/g at 60% saturation of the biomass and a flow rate of 80ml/h, whilst a better metal uptake capacity of about 30mg/g was observed at 80% saturation. No marked reduction in the column performances was observed when increasing flow rates up to 800ml/h. Ca ions present in the electroplating effluents appeared to be a competitive interference with binding of Ni on the biomass, especially when Ni concentrations were low. The effect of Mg ions on Ni sorption appeared to be relatively minor. The data obtained from column operations follows the BDST model well thereby enabling the application of the model to predicting design parameters for scale-up of the biosorption column system.

Complete desorption of bound Ni with 0.2N H₂SO₄ and HCl in volume of 120ml was accomplished with a recovery of 95%, whilst the acids at 0.1N only resulted in 60-70% recovery. Data on regeneration efficiency for four cycles indicates that the metal uptake capacity of the biomass had not been markedly affected by repeated operations and regeneration process. The reusability of *azolla* biomass for metal-treatment purpose is suggested to be viable.

An effluent-free, closed loop of Ni treatment system was proposed whereby the Ni ions can be recycled to the plating bath with the purified water being fed back to the rinse tanks. The data from the present studies showed that *azolla* biomass has promising potential in remediation of Ni-laden effluents.

6. REMOVAL AND RECOVERY OF Zn By *Azolla filiculoides*

6.1 INTRODUCTION

In the previous Chapters (3 & 5), the water fern exhibited excellent sorption capacities for anion and cation metals at optimum pHs, and was used to effectively recover Ni ions from waste water. However, performances of the biosorbent for removing other metal cations of interest remains unclear and can differ greatly from each other. Adoption of the parameters obtained from sorption of Ni for treatment of other cation metals may not be applicable. It was therefore deemed necessary to re-confirm the general applicability of the biosorbent by examining another cation heavy metal, Zn, with regard to sorption capacity and use in recovery.

In addition, acid-Zn-plating lines are one of the main electroplating operations around world and the rinse effluent from these plants is one of the sources of the high amount of Zn encountered in many electroplating discharges. The plating agents used in the Zn-plating-lines are zinc chloride or sulphate therefore recovering and recycling of Zn metal by the similar technology applied in previous chapter could be possible. Treatment of Zn-containing effluent in this chapter focused on sorption and desorption of Zn using azolla biomass in batch and continuous-flow processes.

6.2 METHODS AND MATERIALS

6.2.1 MATERIALS

The azolla biomass was collected, prepared as described in Section 3.3.1. Analytical grade reagents were used in all cases. Stock zinc solution (1000mg/l) was prepared in distilled water with ZnSO₄ (Merck, Germany). All working solutions were prepared by diluting the stock solution with distilled water. NaOH, HCl and H₂SO₄ were obtained from Saarchem,

SA. The electroplating effluent, designated as EPE6 (Table 6.1), was collected from a factory (Silverton Radiator, South Africa) operating an acid-Zn-plating line.

Table 6.1 Characteristics of EPE6

	pH	Zn	Ca	Mg	NH ₄	Ni	Fe
		mg/l					
EPE6	6.7	30	24	64.6	60	0	0

6.2.2 ASSAY METHODS

Zn, Ca and Mg concentrations were determined by atomic absorption spectrophotometry (GBC Scientific Equipment, Australia). Ammonium content was detected using a spectroquant test kit obtained from Merck, Germany (Merck Manual, 1995).

6.2.3 ADSORPTION ISOTHERMS

Batch sorption tests were carried out as described in Section 5.2.3

6.2.4 KINETIC STUDIES

Kinetic experiments were conducted as described in Section 5.2.4.

6.2.5 PREPARATION OF COLUMNS

Columns and *azolla* biomass were prepared, operated and regenerated as described in Section 5.2.5 & 5.2.6. Three columns of the same diameter (25mm) were used in the experiments for BDST modelling. Biomass of 2.5, 5 and 7.5g were packed into each of them respectively. The bed heights of the columns were adjusted, corresponding to the amounts of biomass

contained, to be 0.095, 0.19 and 0.285m respectively. The wet biomass was highly flexible that adjustment of the bed height was easily performed.

6.3 RESULTS AND DISCUSSION

6.3.1 ADSORPTION ISOTHERMS

Equilibrium sorption isotherms of Zn by azolla at pH 3, 4 and 6 are shown in Fig 6.1 and Table 6.2. The isotherms follow the Langmuir adsorption pattern, as indicated by the correlation coefficients (higher than 0.98). The Zn uptake increases when the initial incubating concentrations are raised, as long as binding sites are not saturated. The maximum Zn uptake by azolla at the highest pH of 6, under the experiment conditions, was calculated to be 45.2mg/g (Table 6.2). The constant b which as previously indicated can serve as an indicator of the isotherm rise in the region of lower residual Zn concentrations, which reflects the “strength or affinity” of the sorbent for the solute (Hollan *et al*, 1993). In other word, a higher value of b indicates that the sorption of metal would be more complete than those of lower b values. Sorption of Zn at pH of 6 not only resulted in a high Zn uptake, but also a high b value of the isotherm, thereby showing an increased capacity of azolla for adsorbing metal from dilute solutions. Sorption of Zn at pHs higher than 6 would possibly result in better removal of Zn but the contribution of metal precipitation to the overall removal could be apparent, thus rendering the interpretation of data difficult.

Table 6.3 Shows a summary of various sorbents used for batch removal of Zn from aqueous solution. Among them, azolla biomass appears to have superior potential for sequestering Zn ions from solutions. ZnSO_4 , instead of ZnCl_2 , was used for preparing the working solutions in the present experiment, because of its higher solubility at pH 6 than that of ZnCl_2 (CRC Handbook, 1980-1981). In a preliminary trial, precipitation of Zn out of the solution was observed during preparation of a 2mM ZnCl_2 solution at a pH 5.5. Therefore, the documented value of Zn uptake capacity of 43.5mg/g by bark of *Pinus sylvestris* (Table 6.3) which was obtained using ZnCl_2 at pH of 6.1, probably needs to be re-confirmed.

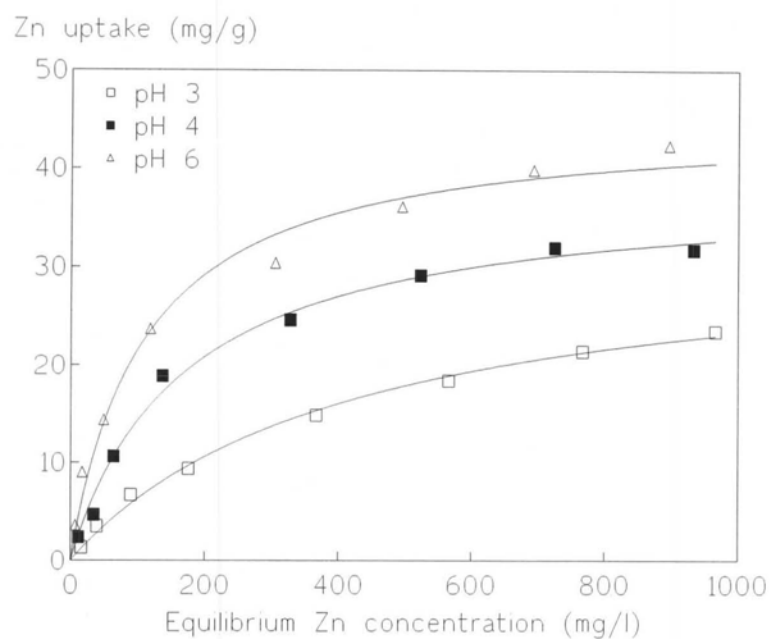


Figure 6.1 Adsorption isotherms for Zn-azolla system at 18°C. Incubation time, 5 h; biomass, 4g/l.

Table 6.2 Langmuir constants of Zn-azolla binding at 18°C

Incubation pH	X_m (mg/g)	b ($\times 10^{-3}$)	Correlation (r^2)
3.0	33.2	2.34	0.99
4.0	38.4	5.96	0.99
6.0	45.2	9.16	0.99

Table 6.3 Zn uptake capacities of various sorbents

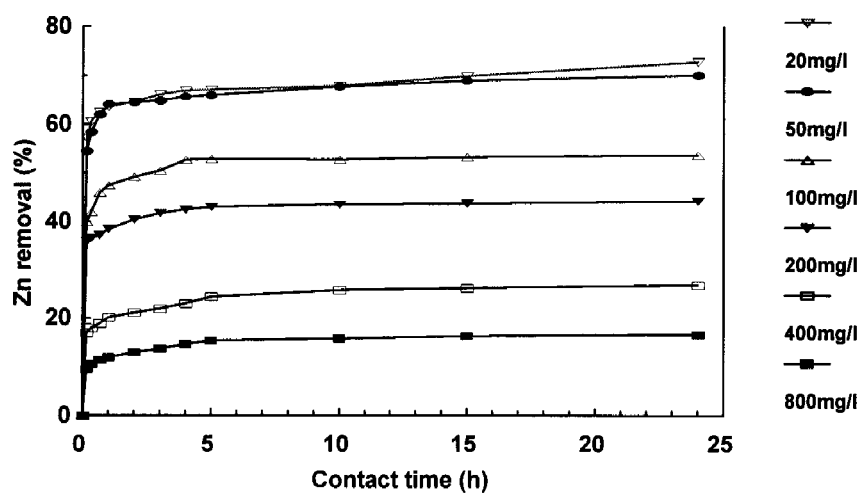
Sorbent	X _m (mg/g)	pH	Max.conc. (mg/l)	Dose (g/l)	Reference
Activated carbon	6.2	6.8	100	5	Larsen & Schierup, 1981
Straw	5.3	6.3	100	5	Larsen & Schierup, 1981
Peat	11.2	4.7	587	50	Gosset <i>et al</i> , 1986
Bark of <i>Pinus sylvestris</i>	43.5 ^a	6.1	1045	10	Gaballah & Kilbertus, 1994
<i>Potamogeton lucens</i>	32.4	5.0-6.6	-	2	Schneider & Rubio, 1995
<i>Salvinia herzogii</i>	18.1	5.0-6.6	-	2	Schneider & Rubio, 1995
<i>Eichhornia crassipes</i>	19.2	5.0-6.6	-	2	Schneider & Rubio, 1995
<i>Azolla filiculoides</i>	45.2	6.0	1000	4	Present study

^a Zn solution was made with ZnCl₂.
Dose biomass concentration.
- not specified.

6.3.2 KINETICS

The equilibrium sorption of Zn was rapidly reached at varying initial incubating concentrations at pH 6 (Fig 6.2). The percentage removal of the solute in the first 3 minute incubation was 70, 69.1 and 55 for the incubating concentrations of 100, 200 and 400mg/l respectively, showing the similar kinetics of metal binding to that of Ni in Chapter 5 (Fig 5.3).

A.



B.

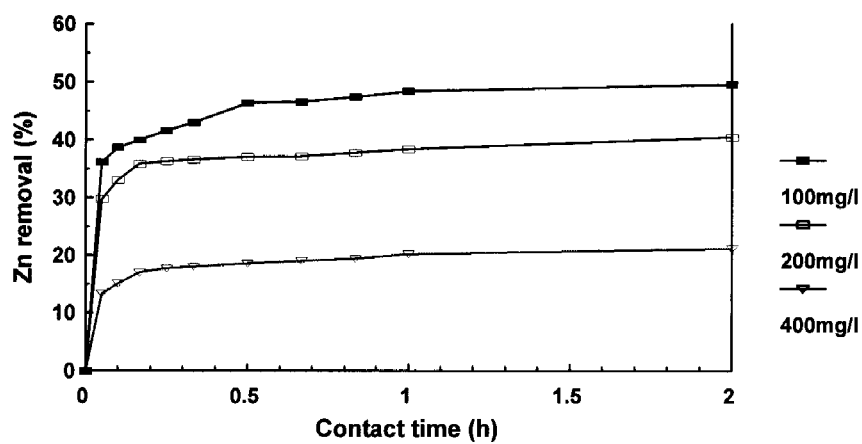


Figure 6.2 Effect of contact time on the sorption of Zn by azolla biomass at pH 6.0 and 18°C. Biomass, 4g/l. (A), sampled for 24 h; (B), sampled for 2 h.

6.3.3 COLUMN REMOVAL OF Zn

Column performances for removal of Zn were evaluated by comparing the Zn uptake and the volume treated at 60% saturation of the biomass with varying flow rates, mass of azolla, influent pHs and Zn concentrations. Those data are summarised in Table 6.4 and presented in Figs 6.3 - 6.6.

Table 6.4 Column removal of Zn with varying flow rates, influent pHs and Zn concentrations

Cycle	Infl. Zn (mg/l)	Infl.pH	Flow rate (ml/h)	Zn uptake (mg/g) ^a	Column efficiency (%) ^b	Volume treated (l) ^a
	100	6.2 ^c	160	28.2	100.0	1.46
1	100	6.2	480	30.4	107.8	1.60
	100	6.2	800	25.8	91.5	1.40
2	100	6.2	480	29.1	103.2	1.50
4	100	6.2	480	26.3	93.3	1.40
6	100	6.2	480	28.2	100.0	1.50
	100	4.0	160	23.3	94.3	1.20
	100	4.0	480	26.6	82.6	1.30
	50	6.2	480	28.5	101.1	2.90

^a at 60% saturation of the column biomass.

^b the metal uptake at 160ml/h was defined as 100%.

^c the influent pH was 6.2 as this was the natural pH of ZnSO₄ solution.

6.3.3.1 EFFECT OF FLOW RATES

Column operations on removal of Zn at three different flow rates were presented in Fig 6.3. At flow rates of 160 and 480ml/h, the column biomass showed similar performance to that of Ni, resulting in Zn uptake of 28.2 and 30.4mg/g, and treated volume of 1.46 and 1.6 litres, respectively. A slightly earlier breakthrough curve can be seen, when the flow rate was

increased to 800ml/h, resulting a 8.5% lower Zn uptake at the breakthrough point (Table 6.4). It appears that a flow rate of 800ml/h would probably be the upper limit below which the optimum uptake can be attained, under current experimental conditions. Flow rate is a critical characteristic in evaluating sorbents for continuous-treatment of metal-laden effluents on industrial scale. Some of the microorganisms which showed high metal sorption capacities in batch tests have failed to be applied to continuous-flow processes to purify waste water, because of a marked pressure loss (increasing resistance to flow) during operation and/or lower metal uptake compared to that on batch tests capacities when flow rates were increased (de Rome & Gadd, 1991; Muraleedharan *et al*, 1994).

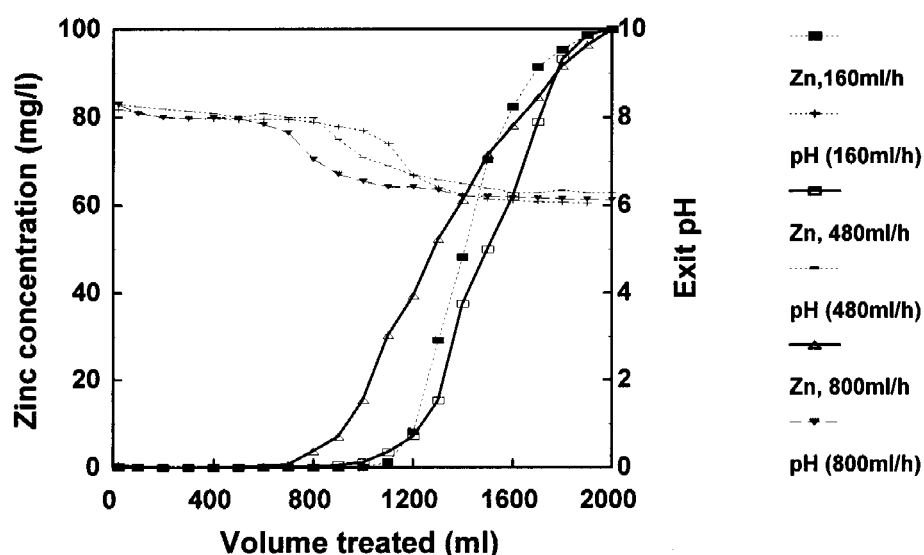


Figure 6.3 Breakthrough curves for column removal of Zn by 5g *azolla* at varying flow rates. Infl. Zn, 100mg/l; infl. pH, 6.2.

6.3.3.2 EFFECT OF INFLUENT pHs

The effect of varying influent pHs on column performances of removing Zn was shown in Fig 6.4 and Table 6.4. Sorption of heavy metals on biosorbents is generally regarded as a pH-dependent procedure (Stoll & Duncan, 1997). This was seen in the case of Ni sorption with azolla in Chapter 5, in which a column efficiency of only 52.4% was attained when the influent pH was lowered by 2 units (from 7 to 5). It is interesting that, as shown in Fig 6.4, lowering the influent pH to 4 (also 2 units less) did not result in a significant decrease in Zn uptake compared to that at pH 6.2. The column efficiency at an influent pH of 4 and flow rate of 480ml/h was 94.3%. The performances of an azolla column at a low pH can be predicted with the data from the isotherm studies in Section 6.3.1, in which a maximum Zn uptake capacity of 38.4mg/g was exhibited at a incubating pH of 4 which accounted for 85% of the Zn uptake capacity at pH 6 (Table 6.2).

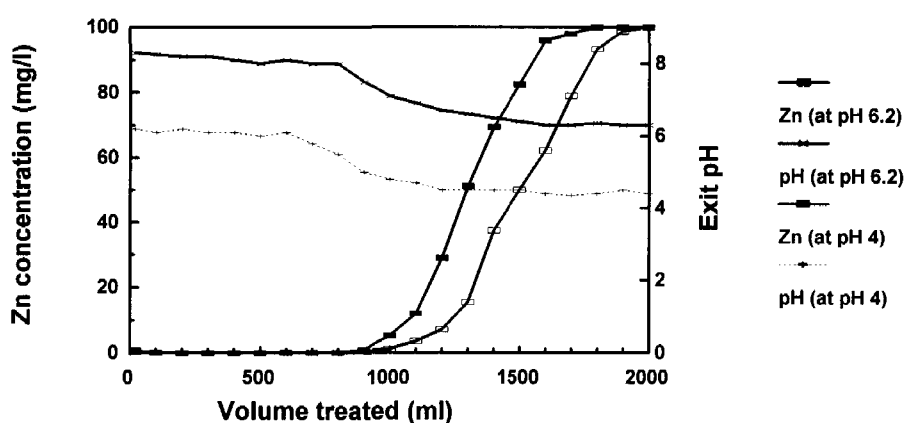


Figure 6.4 Breakthrough curves for column removal of Zn by 5g azolla at influent pH 6.2 and 4.0. Infl. Zn, 100mg/l; flow rate, 480ml/h.

6.3.3.3 EFFECT OF INFLUENT Zn CONCENTRATION

As the most common Zn levels in electroplating effluents range from 15 to 50mg/l (Pollution group, 1987), the column performances of azolla for removing Zn should be tested at corresponding metal levels, with those of electroplating effluents. Unlike Ni sorption, the Zn uptake capacity of the biomass at a lowered influent Zn concentration of 50mg/l remained constant (Table 6.4). Further experiments are needed to elucidate the phenomenon. At the breakthrough, the Zn uptake was 28.5mg/g with a column efficiency of 101%, and the treated volume was proportionally increased to 2.9 litres.

6.3.3.4 REUSABILITY OF THE BIOMASS FOR Zn REMOVAL

Table 6.4 and Fig 6.5 present the data for repeated sorption of Zn by regenerated azolla biomass for 6 cycles. All the column experiments were operated under the identical conditions. Similar Zn uptake values and volumes of treated solution were attained by each of the operations, which indicated the reusability of the biomass for sorption of Zn from aqueous medium.

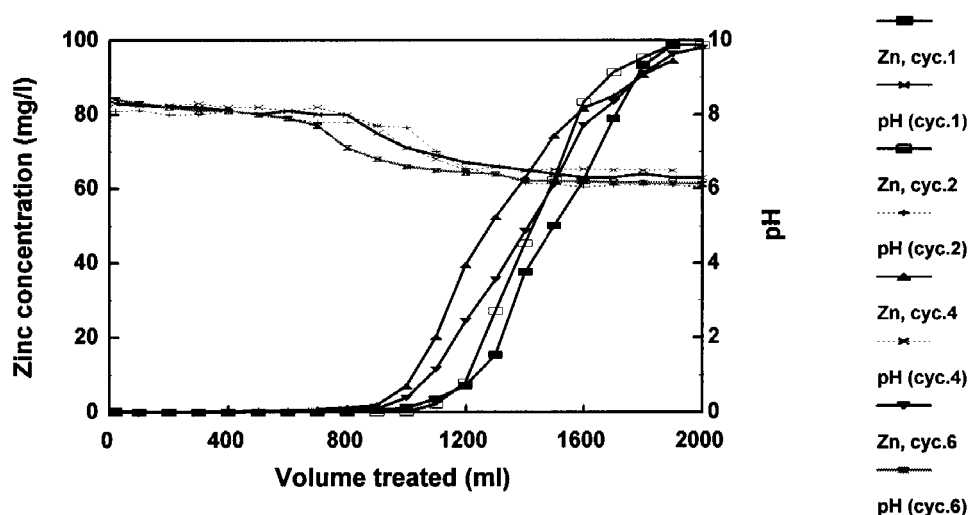


Figure 6.5 Breakthrough curves for column removal of Zn by 5g azolla for 6 cycles. Flow rate, 480ml/h; infl. Zn, 100mg/l; infl. pH, 6.2.

6.3.4 BDST MODEL ANALYSIS

The sorption capacity of a given sorbent for metals is usually determined by carrying out an sorption isotherm. However, isotherms can not give accurate scale-up data in fixed or fluidised bed processes, since sorption in a flow column is not at equilibrium. The optimum operating capacity and contact time must be determined *in vivo* to decide upon the best column dimensions and the number of units needed for continuous treatment (McKay & Bino, 1990).

Models for design of fixed bed adsorbers have been developed and these included the mathematical analysis and prediction of the shape of the breakthrough curve, termed MTZ model, and the height equivalent of a theoretical plate, HETP model (Cited by McKay & Bino, 1990). The most commonly used data analysis method has been thought as the Bed-Depth-Service-Time (BDST) model (Bohart & Adams, 1920). It offers, for suitable sorption systems, the simplest approach and most rapid prediction of optimum adsorber design (McKay & Bino, 1990). The methodology for constructing the BDST model in this study was described in Section 5.3.8.

The values of N_0 , determined from the equation at different flow rates, influent pHs and concentrations, together with other constants were summarised in Table 6.5. Fig 6.6 shows typical column sorption profiles with bed height of 0.095, 0.19 and 0.285m, in which 2.5, 5 and 7.5g of *azolla* biomass were contained, respectively. Varying the bed height and amount of biomass in column operations at a flow rate of 480ml/h resulted in a proportional increased volume of treated Zn solution (Fig 6.7). The estimated values of adsorption capacity at full saturation of the biomass, N_0 , is consistent with the observed values from earlier column operations (Table 6.4 and 6.5). The use of the BDST model in this way provides a realistic description of the adsorption of Zn by *azolla* biomass and the empirical data can be adopted in predicting the bed depth or service time for a specified set of influent characteristics for the scale-up of the sorption column (Muraleedharan *et al*, 1994).

By modifying the equation the values of constants obtained from the plot can be extrapolated for alternative influent concentrations and flow rates, A simplified form of the Bohart-Adams model is:

$$t = aH + b,$$

where the slope, $a = N_0 / C_0 U$ and the intercept at Y axis, $b = (1 / kC_0) \ln[(C_0 / C_i) - 1]$.

When a new influent Zn concentration, other than the one used in the development of constants, is applied to the column system, the equation can be modified by utilizing the new slope and intercept, $a' = (C_0 / C_0') a$ and $b' = b(C_0 / C_0') + \ln[(C_0' / C_i') - 1] / \ln[(C_0 / C_i) - 1]$, respectively.

Where C_0 and C_0' are the old and new influent concentrations; C_i and C_i' the old and new outlet concentrations.

The value of service time t determined experimentally at the influent Zn concentrations of 50 and 150mg/l at 50% breakthrough, were 8.96 and 2.82 h respectively, which are close to the values predicted with the BDST model (9.51 and 3.18 h) showing that this model applied well for azolla-Zn adsorption process under the specified conditions (Table 6.5). However, predicting service time and bed height at varying flow rates and influent pHs with limited data, appears less practical. More empirical information is needed for establishing modelling parameters with regard to pH and flow rate changes in column operations. It should be borne in mind that the data used in constructing the BDST model herein, were obtained using synthetic solutions rather than electroplating effluents. The electroplating effluents usually contain a variety of metals, alkali-earth elements, organic or inorganic compounds, etc.. These impurities existing in the effluents could interfere with the sorption of heavy metals and consequently result in an inaccurate prediction for the treatment of industrial effluents. Applying the BDST model *in situ* to treatment of electroplating effluents therefore needs to be handled with care.

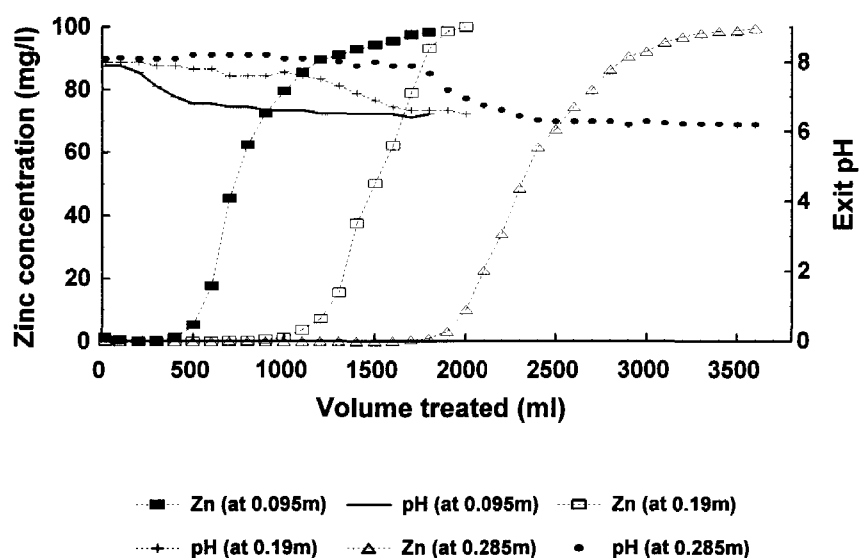
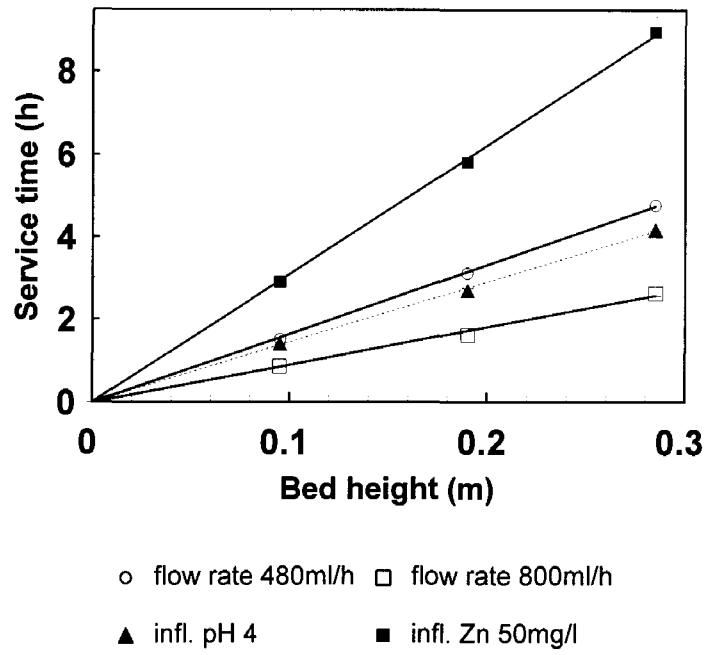


Figure 6.6 Typical breakthrough curves for removal of Zn with varying bed heights. Infl. Zn, 100mg/l; infl. pH, 6.2; flow rate, 480ml/h.

Table 6.5 Constants determined for the BDST equation

Flow rate (ml/h)	Infl. pH	Infl. Zn (mg/l)	Slope	Break-through (%)	Intercept (y)	Intercept (x)	N_0 (mg/g)	r^2
480	6.2	100	17.2	50	-0.13	0.007	31.3	0.99
480	6.2	100	15.4	30	-0.10	0.007	-	0.99
800	6.2	100	9.3	50	-0.06	0.006	28.3	0.99
800	6.2	100	9.2	30	-0.25	0.027	-	0.99
480	4.0	100	14.5	50	0.009	-0.0007	26.5	0.99
480	4.0	100	13.4	30	-0.07	0.005	-	0.99
480	6.2	50	31.8	50	-0.14	0.0004	28.9	0.99
480	6.2	50	31.7	30	-0.48	0.015	-	0.99
480	6.2	150	10.0	50	0.04	0.02	27.2	0.99

A



B

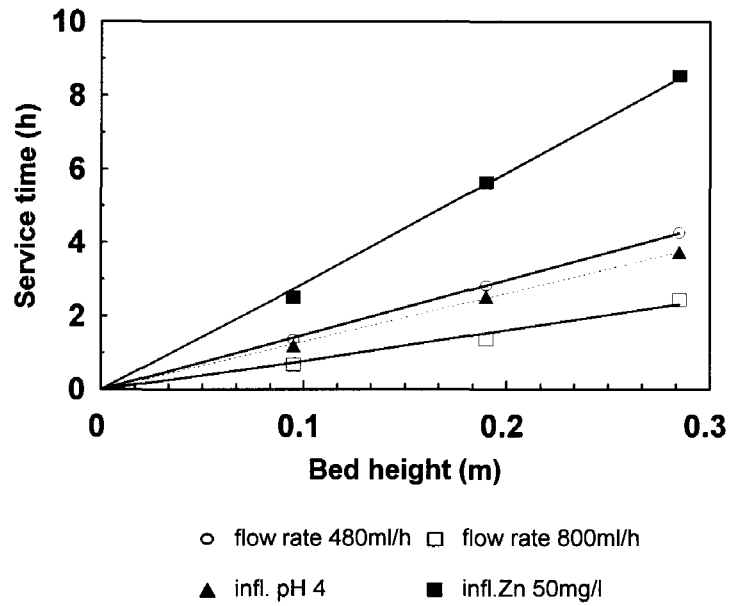


Figure 6.7 BDST curves at 50% (A) and 30% breakthrough (B) with varying flow rates and influent pHs.

6.3.5 TREATMENT OF PLATING RINSE EFFLUENT

The electroplating rinse effluent (EPE6), sampled from an acid Zn-plating line, was characterised by high amount of Mg, Ca and ammonium (Table 6.1). Complete removal of Zn in the first 1.5 litres was observed and the Zn uptake at 60% and 84% saturation were found to be 15.2 and 17.4mg/g respectively (Fig 6.8 and Table 6.6). An earlier and gradual breakthrough curve for removal of Zn was exhibited in Fig 6.8 showing the possible effect of interfering ions present. The value of Zn uptake from EPE6, which contained high amounts of Ca, Mg and NH_4 , was however considered to be fairly good. The *azolla* biomass showed priority in uptake of Zn, rather than the hardness ions and ammonium, thus it was thought to be partially selective in waste effluent treatment.

Ca ions were bound with the biomass at beginning of the column operation, followed by a slight displacement from the *azolla* when Zn sorption proceeded (Fig 6.8). The amount of Ca accumulated at 60% saturation was 5.1mg/g, a similar value to that observed for Ni sorption (Fig 5.8 and Table 6.6). Ca was reportedly a major interfering ion found in treatment of electroplating rinse effluent where a cation-exchanger KU-2-8 was used (Grebenyuk *et al*, 1996). It led to a decrease in the sorbent capacity progressing with each regeneration cycles therefore it was suggested that softened water be used for the rinsing operation.

Mg ions were also primarily accumulated by the biomass for the first litre, but thereafter the majority of the bound Mg ions was displaced when Zn and Ca sorption proceeded (Fig 6.8). At 60% saturation, the bound Mg ions were found to be 8.7mg/g. A limited amount of ammonium (3.8mg/g) was also adsorbed by the column biomass before the breakthrough point which was followed by a minor displacement from the biomass (Fig 6.8). All three compounds seemingly experienced a similar adsorption pattern: primarily being taken up and thereafter displaced. However, the amount of these compounds displaced does not account for that accumulated, resulting in a varied uptake of the respective compounds. It appeared that the impurities present in EPE6 were accumulated by *azolla* biomass at the expense of Zn removal. The hardness ions, particularly at high concentrations, seemingly imposed negative

effects on removal of Zn, presumed via competitive sorption to binding sites on azolla biomass surfaces.

Table 6.6 Column removal of metals from EPE6 (pH 6.7) with 5g azolla at 60% saturation

Metal	Infl.conc. (mg/l)	Uptake (mg/g)
Zn	30.0	15.2
Ca	24.0	5.1
Mg	64.6	8.7
NH ₄	60.0	3.8

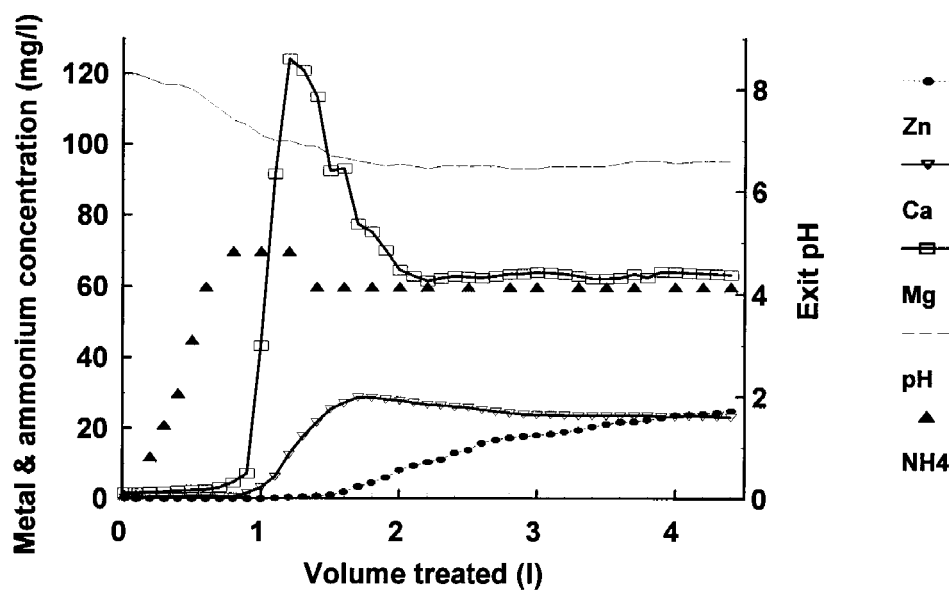


Figure 6.8 Breakthrough curves for removal of Zn from EPE6. Infl. pH, 6.7; infl. Zn, 30mg/l; Ca, 24mg/l; Mg, 64.6mg/l; NH₄, 60mg/l; flow rate, 480ml/h; biomass, 5g.

6.3.6 DESORPTION AND RECOVERY OF Zn

Desorption of Zn were carried out by batch washes in a total volume of 120ml at various concentrations of HCl or H₂SO₄. Recoveries of Zn by H₂SO₄ were complete (96.8 and 98.7% respectively), whereas the desorption by 0.1 or 0.2N HCl appeared slightly less efficient, giving the recoveries of 85.7 and 88% respectively (Fig 6.9). This could be explained by the fact that the solubilities of zinc sulphate is higher than that of zinc chloride (CRC Handbook, 1980-1981). Zn uptake capacities of the regenerated biomass for six cycles remained constant thus demonstrating the reusability of the biomass in the treatment of Zn effluent (Table 6.7). Recovery of Zn from the saturated biomass with 0.2N HCl, after EPE6 treatment, was complete (Fig 6.10). Similar to Ni recovery with HCl in Chapter 5.3, all metals adsorbed on the biomass were washed out by the acid.

The impurities eg. Ca and Mg ions, present in the recovered solution, may make recycling of metals complicated. This problem could consequently be avoided by using the recycled rinse water, in which the amount of impurities should have been greatly eliminated.

Alternatively, the biosorption system can also be jointly operated with other technical process eg. electrolysis under certain circumstances, to recover the pure form of the metals. Chemical precipitation of heavy metals from the concentrated metal medium would be more easily carried out where recovery of the metals is not of concern.

Table 6.7 Zinc recovery with various desorbents

	Desorbent	Ni uptake (mg)	Ni desorbed (mg)	Recovery (%)	Regeneration efficiency ^a (%)
Cyc.1	0.1N HCl	155.0	132.8	85.7	-
Cyc.2	0.2N H ₂ SO ₄	148.5	141.2	95.1	95.7
Cyc.4	0.2N H ₂ SO ₄	138.5	136.7	98.7	86.5
Cyc.6	0.2N H ₂ SO ₄	146.4	140.5	96.0	92.8
EPE6	0.2N HCl	86.9	85.4	98.3	-

^a cyc.1 is defined as 100%.

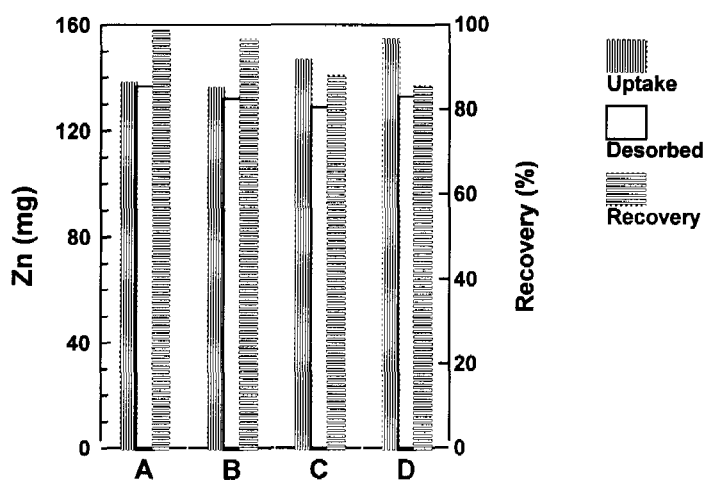


Figure 6.9 Zn recovery with H₂SO₄ and HCl of varying concentrations. A, 0.2N H₂SO₄; B, 0.1N H₂SO₄; C, 0.2N HCl; D, 0.1N HCl.

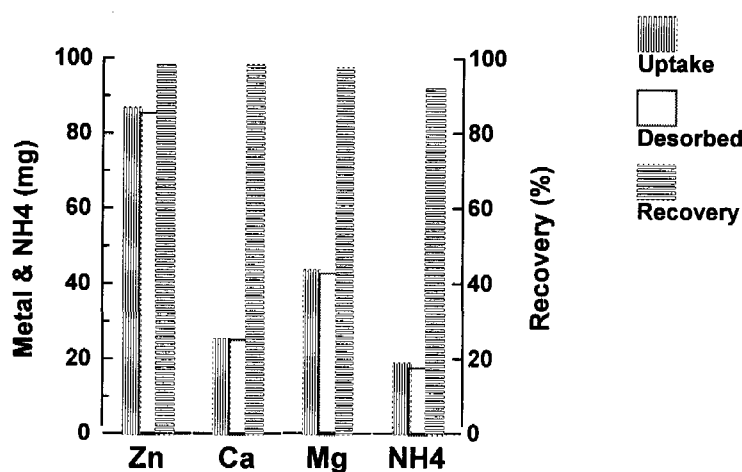


Figure 6.10 Recovery of Zn, Ca, Mg and NH_4 with 0.2N HCl, after treatment of EPE6.

6.3.7 RECYCLING OF Zn AND WASTE WATER

Data obtained in this chapter have clearly shown that the performances of *azolla* biomass in treating Zn effluent, either on batch or column scale, are similar to that in treatment of Ni effluent with a few exceptions. It is suggested that the technology for recycling metal and water, proposed in Section 5.3. for Ni recycle, could be applied to Zn effluent treatment with the modifications suggested in this section.

6.4 CONCLUSIONS

Azolla biomass, tested for removal of Zn on batch and column scale, once again exhibited its effectiveness in removal of metal from both solution or electroplating effluent. The biomass appeared to have good mechanical stability and flow-permeability throughout all column

operations. Similar to Ni sorption seen in Chapter 5, high Zn uptake capacities of *azolla* biomass, from batch and column trials at neutral pHs and high flow rates, were obtained. Unlike what was found in the Ni experiments, lowering the influent pH to 4 did not affect Zn uptake greatly in column operations. Column performances of *azolla* biomass for sorption of Zn followed the BDST model well. The service time for a given influent concentration can be predicted by the model established using the experimental data. Recovery of Zn was coupled with the procedure for regeneration of spent biomass. Complete desorption of bound Zn with 0.2N sulphuric or hydrochloric acids enabled a successful regeneration of the exhausted biosorbent. The biosorbent was repeatedly used for six cycles without marked decrease in its Zn uptake capacity. Ca and Mg found in Zn electroplating effluents at high concentrations, can be competitively accumulated at the expense of Zn sorption, imposing a somewhat negative effect on removal of Zn from electroplating rinse effluents.

7. COLUMN COMPETITIVE SORPTION OF MULTIPLY METALS BY *Azolla filiculoides*

7.1 INTRODUCTION

Data obtained from previous sections clearly demonstrated the excellence of azolla biomass in removal and recovery of heavy metals from electroplating rinse effluents (Zhao & Duncan, 1997 a-e). However, most of the experiments have been based on one-metal aqueous systems. Even when the effluents were examined and the influence of other ions in the effluent, eg. Ca, Mg and NH_4 , was determined, the results did not reveal whether there were any mutual interferences of the heavy metals of interest, nor indicate the sorption preference of the biomass for heavy metals which may exist in many complex effluents. There are various ways of expressing the effect of additional sorbate species on the sorption process (de Carvalho *et al*, 1995). Batch sorption in a multi-metal solution will provide fundamental data for this purpose, while column operation on a mixed-metal medium can reveal more practical information for treatment of metal-laden effluents.

In electroplating shops, rinse effluents discharged from each plating lines are frequently pooled together prior to drainage, to facilitate settling of the metals. Treatment of a combined electroplating effluent containing mixed metals using azolla columns would be a novel technology for the treatment of this effluent.

Experiments in this section present a quantitative approach, namely metal displacement in column operations, to studying the sorption capacities of azolla biomass from solutions consisting of 3 or 4 metals of interest.

7.2 METHODS AND MATERIALS

7.2.1 MATERIALS

The azolla biomass was collected, prepared as described in Section 3.3.1. Analytical grade reagents were used in all cases. Stock metal solutions (1000mg/l) was prepared in distilled water with NiCl₂, ZnSO₄ (Saarchem, SA), CrCl₃, CdSO₄ (Riedel-De Haen, Germany), CaCl₂ and MgCl₂ (Merck, Germany). All working solutions were prepared by diluting the stock solution with distilled water. NaOH, HCl and H₂SO₄ were obtained from Saarchem, SA.

7.2.2 ASSAY FOR METALS

Ni, Zn, Cd, TCr, Ca and Mg concentrations were determined by atomic absorption spectrophotometry (GBC Scientific Equipment, Australia).

7.2.3 PREPARATION OF COLUMNS

Columns and azolla biomass were prepared and operated as described in Section 5.2.5.

7.3 RESULTS AND DISCUSSION

7.3.1 COLUMN REMOVAL OF Cd

Column sorption performance of azolla was initially tested with cadmium solution under identical conditions to that for Ni and Zn sorption, since Cd is one of the competing heavy metals found in many electroplating effluents. Although similar behaviours of the biomass in Cd removal can be expected to that found in the previous Ni and Zn sorption experiments, the current operation provided data for column removal of cadmium, against which other experiments could be assessed. Fig 7.1 and Table 7.1 present the data for column sorption of Cd. The Cd uptake was 54.4mg/g and the treated volume of Cd-laden solution was 2.8 litres.

A dried brown algae, *Ascophyllum nodosum* cross-linked by formaldehyde, has been applied to column tests for Cd removal (Volesky & Prasetyo, 1994). An average Cd uptake capacity of 30mg/g was found under specified conditions. Barley straw, *Hordeum vulgare* L., adsorbed 7.5mg/g of Cd from aqueous solution under similar experimental conditions to the present one (Larsen *et al*, 1981). Comparison of azolla biomass with other sorbents can not be directly performed since the experimental conditions and their physical characteristics varied (Table 7.1). However, azolla biomass appeared to have good potential for removing Cd, among the biosorbents documented (Table 7.1).

Table 7.1 Column removal of Cd by various biosorbents

Sorbent	Flow rate (ml/h.g)	Infl. pH	Infl. Cd (mg/l)	Saturation (%)	Cd uptake (mg/g)	Reference
Barley straw (<i>Hordeum vulgare</i> L.)	444-200	6.8	100	100	7.5	Larsen & Schierup, 1981
Brown algae (<i>A. nodosum</i>)	-	5.5	10	50	30.0	Volesky & Prasetyo, 1994
<i>Azolla filiculoides</i>	96	6.6	100	60	54.4	Present study

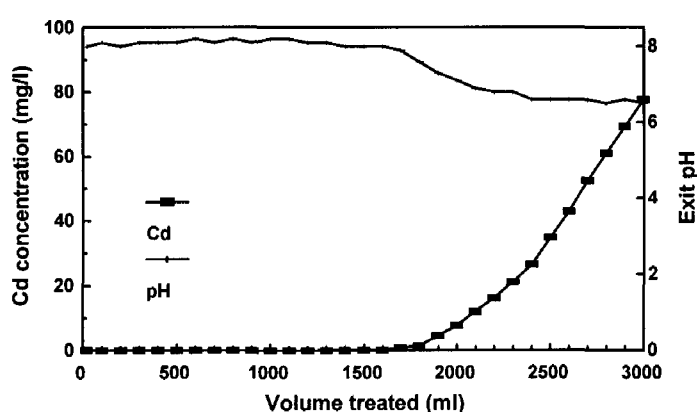


Figure 7.1 Breakthrough curve for column removal of Cd by 5g azolla at influent pH 6.6. Infl. Cd, 100mg/l; flow rate, 480ml/h.

7.3.2 COMPETITIVE SORPTION OF MULTIPLE METALS

7.3.2.1 SORPTION OF Ni, Zn AND Cd AT pH 6.6

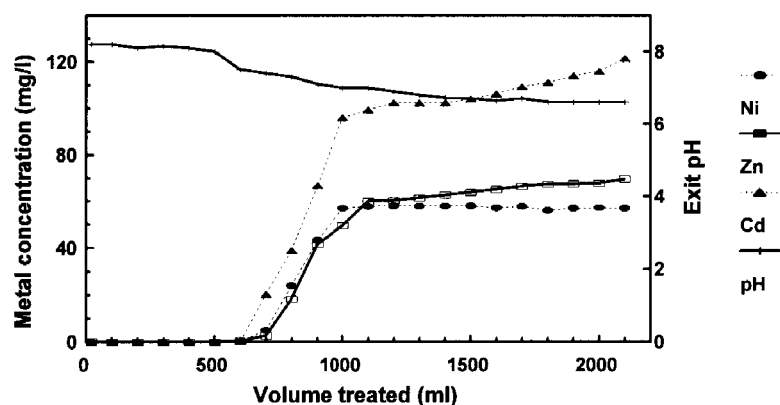
Initially a combined solution of Ni, Zn and Cd was used because the natural pHs at which they are dissolved are similar (7.0, 6.2 and 6.6, respectively). The feed solution was made 1mmol/l for each metal and the resultant pH was 6.6. The start of breakthrough for all 3 metals occurred simultaneously after a treated volume of 600ml, after while the solution pH started to decline (Fig 7.2). The elution profiles for all metals are quite similar, displaying a rapid breakthrough which was followed by a gradual saturation. Although none of the three metals was displaced by others during the sorption, the metal uptake capacities for these three, expressed in molar quantities at 60% saturation, are 0.17, 0.21 and 0.20mmol/g, indicating a slight affinity preference for the cation metals, $Zn = Cd > Ni$, under these conditions.

Table 7.2 presents data of metal uptakes for each metals, adopted from previous experiments on single-metal systems, which supports the above conclusion. The total metal uptake in the multi-metal system was 18.8% higher than that in the single-metal one, probably due to the fact that a solution containing high total concentration of mixed metal (254mg/l) was used in this experiment.

The metal uptake capacities in molar quantities in respective single systems were constant (0.45-0.48mmol/g for Ni, Zn and Cd), under identical conditions except that pHs range from 6.2-7.0 (Chapters 5 & 6). These values might be used as an indication of the available binding sites on the surface of the biomass for divalent cation heavy metals examined, under the specified conditions (Sharma & Forster, 1994). While affinity of the biomass for respective cation metals varies slightly depending on their species and the influent metal concentrations thereof, similar mechanisms appeared to predominate in the sorption of all cation metals, since the metal uptakes in molar quantities from each single-metal solutions are relatively close to that for total metal uptake from a multi-metals solution (Table 7.2). It also implies

that all cation metals may share the binding sites on surfaces of azolla and competitively interfere with each other, when present at different concentrations in a multi-metal system.

A



B

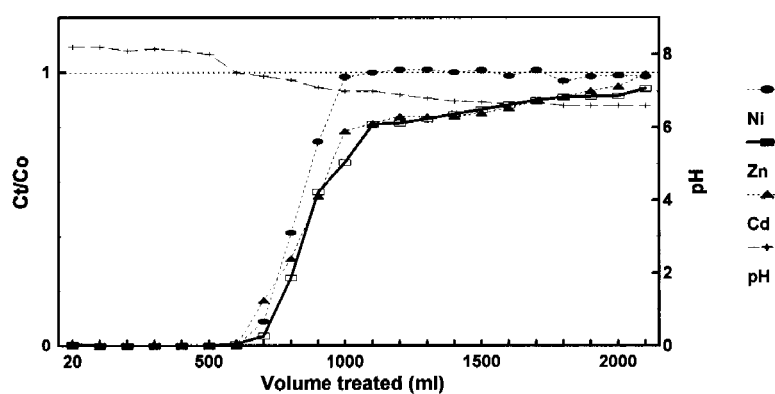


Figure 7.2 Breakthrough curves for column removal of Ni, Zn and Cd from a multi-metal system by 5g azolla at influent pH 6.6. Infl. Ni, 58mg/l; infl. Zn, 74mg/l; infl. Cd, 122mg/l; flow rate, 480ml/h; C_t , metal conc. at a given time; C_0 , infl. metal conc.. (A), metal conc. is plotted on y-axis; (B), ratio of C_t to C_0 is plotted on y-axis.

Table 7.2 Column metal uptake capacities at 60% saturation from single and mixed metal systems

Metal	Single metal			Mixed metals			
uptake	Ni	Zn	Cd	Ni	Zn	Cd	Total
mg/g	26.1	30.4	54.4	10.1	13.5	21.9	45.5
mmol/g	0.45	0.47	0.48	0.17	0.21	0.20	0.57

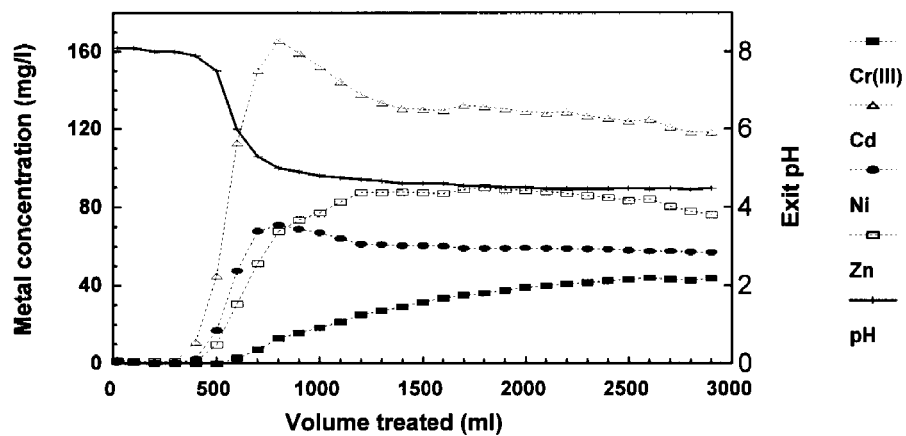
7.3.2.2 SORPTION OF Cr, Ni, Zn AND Cd

An attempt at column operations using a solution consisting of Cr^{3+} , Ni, Zn and Cd at their natural pH was unsuccessful, since Cr^{3+} precipitated out of the solution at the pH (5.5) of the metal solution. Lowering the solution pH to 4.5 was found to stabilize Cr^{3+} ions in the solution. Flow rate of 160ml/h, instead of 480ml/h shown in Fig 7.2, was used in this column operation because uptake of Cr^{3+} by azolla is a rather slow process compared to that of other cation metals. Fig 7.3 shows the competitive sorption on the four-metal system (1mmol/l for each of the metals) at a influent pH of 4.5. All metals present were adsorbed by the biomass from the medium for a treated volume of first 500ml, thereafter the bound Ni and Cd ions were displaced from the column biomass to a certain extent (Fig 7.3). Unlike Ni and Cd, the bound Zn ions was displaced from biomass to a lesser extent and at a later stage, thereby reflected that binding of Zn with the sorbent appears to be slightly stronger and more stable than that of Ni and Cd (Table 7.3). Decline of solution pH occurring simultaneously with the displacement could not be the direct factor causing the displacement, since no displacement of Ni or Zn had been observed during the column sorption on single-metal mediums at influent pH 5 or 4 (Fig 6.4). It appeared that the further sorption of Cr^{3+} was attained at the expense of other metals. Higher affinity of Cr^{3+} for the biosorbent could be the possible reason for displacement of other heavy metals at the experimental pH. The total metal uptake, expressed in molar quantities, at the treated volumes of 700, 1500 and 2900ml are similar (0.516, 0.522 and 0.495mmol/g respectively), implying that the increased uptake of Cr^{3+} had compensated for the amount of Ni, Zn and Cd displaced (Table 7.3).

Table 7.3 Uptake of Cr^{3+} , Ni, Cd and Zn during column sorption on a multi-metal system at various stages

Volume treated (ml)	mg/g					mmol/g				
	Cr^{3+}	Ni	Zn	Cd	Total	Cr^{3+}	Ni	Zn	Cd	Total
700	7.7	6.3	10.2	11.8	36.0	0.15	0.11	0.16	0.11	0.516
1500	12.1	5.0	9.3	7.0	33.4	0.23	0.09	0.14	0.06	0.522
2900	14.9	4.3	6.7	3.6	29.5	0.29	0.07	0.10	0.03	0.495

A



B

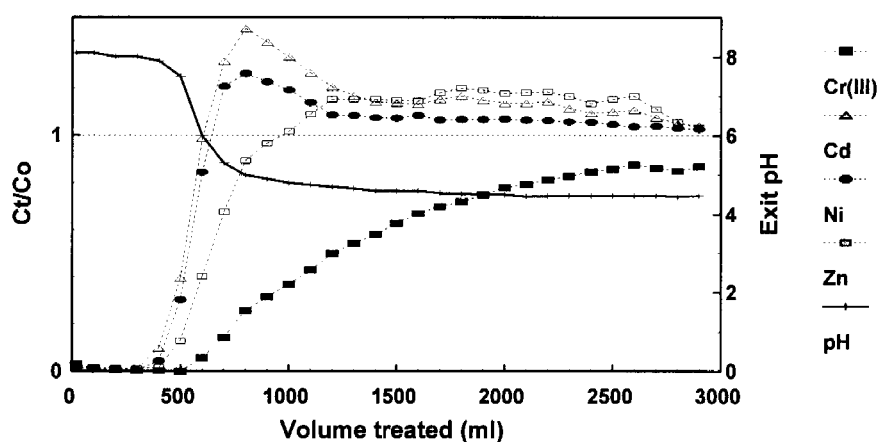


Figure 7.3 Breakthrough curves for column removal of Cr^{3+} , Ni, Zn and Cd by 5g azolla at influent pH 4.5. Infl. Cr^{3+} , 50mg/l; Ni, 56.2mg/l; Zn, 75.8mg/l; Cd, 114.2mg/l; flow rate, 160ml/h; C_t , metal conc. at a given time; C_0 , infl. metal conc.. (A), metal conc. is plotted on y-axis; (B), ratio of C_t to C_0 is plotted on y-axis.

7.3.2.3 EFFECT OF Ca ON COLUMN SORPTION OF Ni, Zn AND Cd

Since Ca is always present in plating effluents column sorption of Ca, Ni, Zn and Cd was conducted to examine the effect of Ca on sorption of the others, under identical experimental conditions to that presented in Fig 7.2 except for the presence of Ca ions. The Ca ions, after being primarily adsorbed to the column, were subsequently displaced from biomass when sorption of other metals proceeded (Fig 7.4). From this data it could be calculated that the amount of Ca ions displaced only accounted for 50-60% of that adsorbed at beginning of the profile. The Ca uptake at end of sorption was 0.053mmol/g accounting for 10% of the total metal uptake (Table 7.4). The uptake of Ni, Zn and Cd at saturation was 0.53mmol/g, 18% lower than that obtained on a medium without Ca ions (Table 7.5). An inhibitory effect of Ca on sorption of heavy metals by azolla therefore occurred, even though it was at relatively low ratio of Ca to heavy metals. The negative effect of Ca ions on sorption of heavy metals from electroplating effluents by a cation exchange resin (KU-2-8), or by azolla biomass has

previously been reported and discussed (Greenyuk *et al*, 1996).

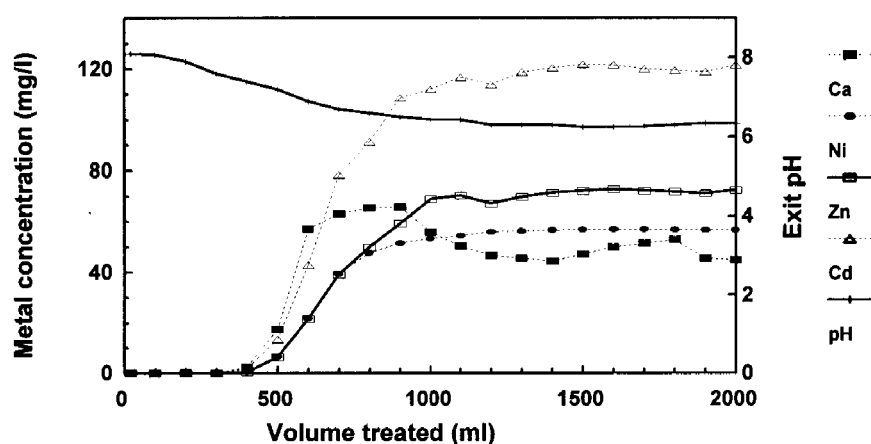
Table 7.4 Uptake of Ca, Ni, Cd and Zn during column sorption on multi-metal system at various stages

Volume treated (ml)	Metal uptake (mmol/g)					Total Ni, Zn & Cd
	Ca	Ni	Zn	Cd		
700	0.103	0.134	0.167	0.156	0.560	0.458
1500	0.066	0.144	0.194	0.176	0.580	0.514
2900	0.053	0.145	0.201	0.182	0.581	0.528

Table 7.5 Comparison of metal uptakes on mixed metal systems with and without Ca at saturation

System	Metal uptake mmol/g			Ni, Zn & Cd
	Ni	Zn	Cd	
Ni, Zn & Cd	0.175	0.245	0.226	0.646
Ca, Ni, Zn & Cd	0.145	0.201	0.182	0.528

A



B

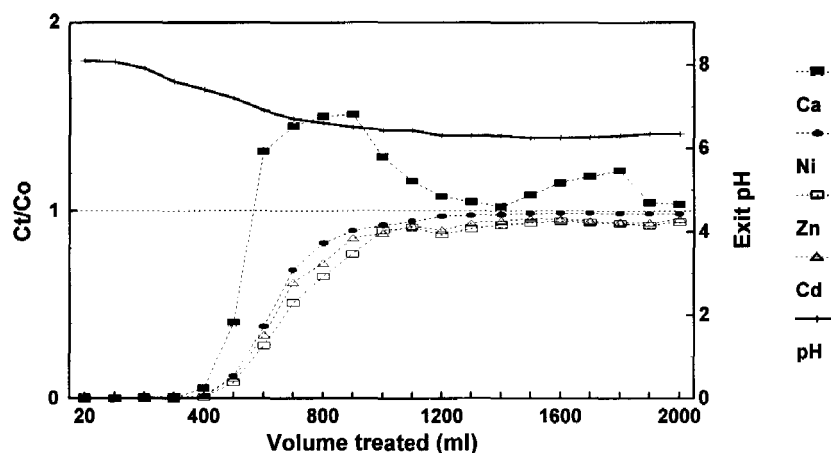


Figure 7.4 Breakthrough curves for column sorption of Ca, Ni, Zn and Cd by 5g azolla at influent pH 6.5. Infl. Ca, 43.5mg/l; Ni, 57.7mg/l; Zn, 77.0mg/l; Cd, 127.0mg/l; flow rate, 480ml/h; C_t , metal conc. at a given time; C_0 , infl. metal conc.. (A), metal conc. is plotted on y-axis; (B), ratio of C_t to C_0 is plotted on y-axis.

7.3.2.4 EFFECT OF Mg ON COLUMN SORPTION OF Ni, Zn AND Cd

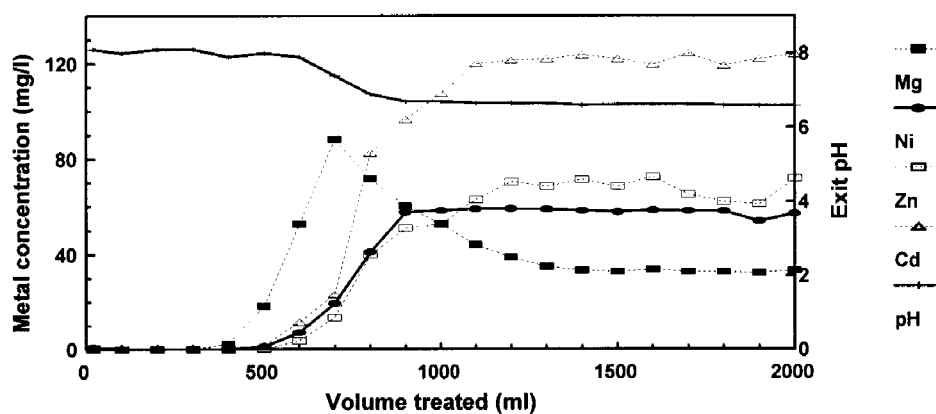
Another commonly encountered hardness ion in plating effluent is Mg. Its effect on sorption of Ni and Zn has been discussed in previous sections (Chapter 5 & 6), which shows that it could compete with heavy metals for binding sites on surface of azolla when Mg concentration is relatively high. A competitive sorption test was conducted in order to determine the effect of Mg on sorption of the others, at a low ratio of Mg concentration to that of heavy metals. As shown in Fig 7.4, the displacement of bound Mg ions from biomass was nearly complete in that the Mg uptake at end of the experiment was only 0.0004mmol/g (Table 7.6). The heavy metal uptake at saturation was not much altered (0.631mmol/g), compared to the value obtained on Mg-free solution (0.646mmol/g). It reflects that the column sorption capacity for heavy metals was not affected by the presence of Mg ions, when the Mg concentration was relatively low. On the other hand, the Mg uptake of 0.358mmol/g seen in the previous section (Table 6.6), which led to a decreased removal of Zn, suggests that the interfering effect on sorption of heavy metal occurs when the Mg ions concentration is increased (65mg/l in that case).

7. COMPETITIVE SORPTION OF MULTI-METALS BY *Azolla*

Table 7.6 Uptake of Mg, Ni, Cd and Zn during column sorption on multi-metal system at various stages

Volume treated (ml)	Metal uptake mmol/g					
	Mg	Ni	Zn	Cd	Total	Ni, Zn & Cd
700	0.086	0.150	0.177	0.193	0.606	0.520
1500	0.0009	0.156	0.213	0.232	0.602	0.601
2900	0.0004	0.158	0.225	0.249	0.631	0.631

A



B

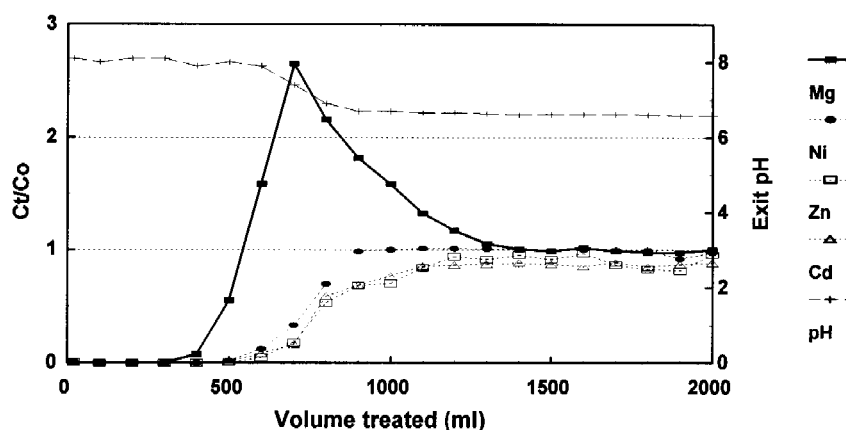


Figure 7.5 Breakthrough curves for column sorption of Mg, Ni, Zn and Cd by 5g azolla at influent pH 6.4. Infl. Mg, 33.3mg/l; Ni, 58.5mg/l; Zn, 75.0mg/l; Cd, 140.0mg/l; flow rate, 480ml/h; C_t , metal conc. at a given time; C_0 , infl. metal conc.. (A), metal conc. is plotted on y-axis; (B), ratio of C_t to C_0 is plotted on y-axis.

7.4 CONCLUSION

Removal of cation heavy metals from the multi-metal aqueous systems by *Azolla filiculoides* in a fixed-bed column was effective. A total metal uptake of 0.57mmol/g for Ni, Zn and Cd at 60% saturation, at an influent metal concentration of 3mmol/l, was found. After being primarily adsorbed to the column, Cd, Ni and Zn were subsequently displaced to a certain extent from biomass, probably by a further sorption of Cr^{3+} which appeared to have a stronger binding with the biomass than the other metals. The Ca ions, though present at a relatively low concentration in a multi-metal system, appeared to interfere with the sorption of other metals to a certain extent, presumably via a competitive binding to the available sites on surface of azolla. The negative effect of Mg ions on sorption of heavy metals was shown minor, when its concentration was relatively low. Since the concentrations of Ca and Mg ions present in plating effluents varies from factory to factory, attention should therefore be paid to the presence and concentrations of these ions when applying the sorption technology *in situ* to treatment of plating effluents.

7. COMPETITIVE SORPTION OF MULTI-METALS BY Azolla

Similar to Ni and Zn (0.45 and 0.47 respectively), the Cd uptake by azolla from a single-metal system, was 0.48mmol/g, implying that the available binding sites for divalent cation heavy metals on surfaces of azolla could be similar and in the range of 0.45-0.48mmol/g. Similar mechanisms appear to dominate the sorption of all cation metals, and the binding sites on surface of the biomass are possibly shared by all the cation metals examined in this study. Based on these data, it can be postulated that multiple cation heavy metals in an effluent could be effectively removed by the azolla-sorption technology in a similar way to the treatment of single-metal containing effluent, developed in the previous sections.

8 PARTIAL CHARACTERIZATION OF *Azolla filiculoides*

8.1 INTRODUCTION

The overall performance of azolla biomass in removing and recovering heavy metals has been demonstrated in previous chapters. However, the knowledge about the sorbent, in terms of physical properties, chemical structure and composition, and nature of the metal binding process, remain meagre. These features of the sorbent are important if it is to be used as a competitive product in water purification and metal reclamation. Understanding of the fundamental properties of the biosorbent can also be helpful in modelling and predicting its sorption characteristics, as well as in attempts to improve its complexing properties by biological, chemical and engineering processes (Fourest & Volesky, 1996). In this regard, partial characterization of azolla biomass, qualitatively or quantitatively, was carried out in the present chapter.

8.2 BIOCHEMICAL COMPOSITION

Biochemical composition of azolla in terms of concentrations of protein, lipids, carbohydrates and ash, was determined. Comparison among the similar types of water plant, *Potamogeton lucens*, *Salvinia herzogii* and *Eichhomia crassipes*, is also presented.

8.2.1 METHODS AND MATERIALS

8.2.1.1 PROTEIN (Nitrogen) DETERMINATION

The Kjeldahl method (AOAC Official Methods of Analysis, 1984) was used for determination of total nitrogen. The nitrogen of proteins and amino acids is converted quantitatively by sulphuric acid to ammonium sulphate at presence of Hg as catalyst. The ammonia is then distilled when NaOH is added, and subsequently absorbed by acids.

To 1g azolla sample in a Kjeldahl flask, was added 1.2g HgSO_4 , 15g K_2SO_4 and 25ml H_2SO_4 . The sample was digested for 2 hours at 250°C . When the digested mixture had cooled down to ca. 25°C , 50ml distilled water and 25ml 8% $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ were added to precipitate Hg. One volume of 50ml 45% NaOH was added to the digested sample to ensure that the solution was strongly alkaline. The Kjeldahl flask was immediately connected to a distillation apparatus and distilled for 15min, with the steam bubbling through the digest. The distillate was collected in 80ml 0.02N HCl and titrated with 0.015N NaOH to neutral pH. The volume of NaOH consumed during titration was substituted into the equation below to determine the total nitrogen expressed as % N. Blank flasks were distilled and titrated for correction of the determination. The determination was repeated three times and the average value was reported as the final value.

$$\% \text{ N} = \frac{[(\text{ml std acid} \times \text{normality acid}) - (\text{ml std NaOH} \times \text{normality NaOH})] \times 1.4007}{\text{g sample}}$$

Protein content of azolla was estimated using a multiplication factor of 6.25 as the ratio of protein : nitrogen is 16 : 1.

8.2.1.2 LIPID DETERMINATION

Biomass (5g of 20-mesh or finer) was rinsed with 15ml 70% alcohol in a 250ml centrifuge bottle for few minutes to moisten all particles. The sample was incubated in a water bath at $75\text{--}80^\circ\text{C}$ for 15 min. Alcohol (27ml) was added immediately with vigorously shaking for 2 min. When the sample had cooled down, 45ml ether was added to the mixture followed by vigorous shaking for 5 min. The reaction mixture was then centrifuged at ca. 2000 rpm for 10 min and decanted into a 250ml beaker. The precipitate was re-extracted with three 20ml portions of ether. The combined alcohol-ether extracts were evaporated just to dryness on a steam bath and any remaining moisture driven off in an oven at 100°C for 5 min. The dry extract was redissolved in ca. 15ml CHCl_3 and filtrated through an asbestos mat and a layer of sand into a previously weighed heat-resistant dish. Three more washes were repeated with

10ml CHCl₃ each. The combined filtrate was finally evaporated on steam bath and dried in an oven at 100°C for 90 min. The dry filtrate was weighed as lipids (AOAC Official Methods of Analysis, 1984).

8.2.1.3 ASH DETERMINATION

A sample of 2g in a porcelain crucible was placed in a temperature controlled furnace preheated to 600°C for 2 h. When the reaction was completed, the crucible was transferred directly to desiccator, cooled, and weighed immediately (AOAC Official Methods of Analysis, 1984).

8.2.1.4 TOTAL CARBOHYDRATES

The total carbohydrate was estimated from the difference between the total weight of dry solids and the weight of protein, lipids and ash (Scheider & Rudo, 1995).

8.2.2 RESULTS AND DISCUSSION

Values of protein, lipids, carbohydrates (estimated) and ash for azolla are presented in Table 8.1. Comparatively high value of lipids and low content of protein, among the species members, was found.

Table 8.1 Biochemical composition of azolla, in comparison with other water plants

	<i>Azolla filiculoides</i> (%)	<i>P. lucens</i> (%)	<i>S. herzogii</i> (%)	<i>E. crassipes</i> (%)
Protein	10.1	21.7	11.5	10.0
Lipids	3.6	0.9	1.1	0.7
Ash	14.4	11.4	10.2	20.3
Carbohydrates	71.9	66.0	77.2	69.0

8.3 PHYSICAL CHARACTERIZATION

Physical characterization was carried out in terms of specific surface area and water retention of the azolla biomass.

8.3.1 DETERMINATION OF SPECIFIC SURFACE AREAS

In many scientific and industrial applications of dispersed materials, the specific surface area of the material is an important characteristic, especially for sorption application (van den Hull & Lyklema, 1968). In this study, the term “specific surface area” refers to the surface area for binding of cations or any positively charged molecules.

8.3.1.1 METHODS AND MATERIALS

The specific surface area was measured using the methylene blue (MB) adsorption method (van den Hull & Lyklema, 1968; Scheider & Rubio, 1995), which has been commonly used for evaluation of sorbents. A volume of 100ml of various concentrations of methylene blue solution (Merck, Germany) was shaken for 1 hour with 0.2g azolla. The final concentrations of the dye in the supernatant were determined spectrophotometrically at 660nm. Preliminary experiments indicated that this period was sufficient to attain equilibrium. The specific surface area was calculated from the maximum adsorption capacity assuming a cross sectional area of 108Å for the methylene blue molecule (van den Hull & Lyklema, 1968).

8.3.1.2 RESULTS AND DISCUSSIONS

Table 8.1 presents the specific surface areas determined by dye adsorption method. At the initial MB concentration of 600mg/l, the maximum MB loading was found and the specific surface area was estimated as 429.4m²/g under present experimental conditions. This high surface area is probably one of the most important factors which contributes to its metal uptake capacities. *Azolla filiculoides*, among its related species, showed the highest surface

area (Table 8.3). Information on surface area of other biosorbents is scarce. It should also be pointed out that this method of calculating surface area may not be entirely accurate but gives comparative values.

Table 8.2 Specific surface area of azolla biomass based on MB adsorption method

Initial MB conc.(mg/l)	Final MB conc. (mg/l)	MB loading (mg/g)	Specific surface area (m ² /g)
50	1.3	24.4	44.5
100	1.3	49.4	90.4
200	4.1	98.0	179.1
400	97.0	151.5	276.9
600	130.1	235.0	429.4
800	409.0	195.5	357.3
1000	614.4	192.8	352.4

Table 8.3 Specific surface area of azolla, in comparison with other biosorbents based on MB adsorption method (Scheider & Rudo, 1995)

	<i>Azolla filiculoides</i>	<i>P. lucens</i>	<i>S. herzogii</i>	<i>E. crassipes</i>
Specific surface area (m ² /g)	429.4	415.0	270.0	250.0

8.3.2 DETERMINATION OF WATER RETENTION

8.3.2.1 METHODS AND MATERIALS

Swelling characteristics (distention index, swelling ratio and volume of absorbed solvent) which are important in process design were obtained from the weights and volumes of dry and swollen biomass (Holan *et al*, 1993). Dry azolla (5g) was swollen in cylinder with distilled water for 12h, the excess water was decanted and the cylinder degassed under low pressure. The volume was then measured.

Distention index (DI) was calculated from the ratio: $DI = V_s/W_d$. Where V_s is the volume of the biomass after swelling and W_d is the weight of the dry biomass.

The swelling ratio is $Q = W_s/W_d$, where W_s is the weight of swollen biomass. The weight of absorbed solvent (WAS) was calculated as the ratio $WAS = (W_s - W_d)/W_d$.

8.3.2.2 RESULTS AND DISCUSSIONS

The value of water retention obtained showed a high capacity of azolla to absorb water, approximately five times the mass value of the dry plant (Table 8.4). The value of DI, indication of expansion during swelling, was found to be quite high (14.3ml/g) compared to *A.nodosum*. This character is probably attributed to the large surface area and abundant content of carbohydrates of azolla. Information on swelling property of the biomass is another crucial parameter in designing a biomass-based reactors for wastewater treatment.

Table 8.4 Swelling characteristics of azolla, in comparison with a marine algae

Biomass	Dry weight (g)	Swollen weight (g)	Swollen volume(ml)	DI (ml/g)	Q	WAS (g/g)
<i>Azolla</i>	5.0	32.6	71.5	14.3	6.5	5.5
<i>A.nodosum</i> ^a	1.399	6.75	6.2	4.4	4.8	3.9

^a taken from Holan *et al*, 1993

8.4 CHEMICAL MODIFICATIONS OF *Azolla* BIOMASS

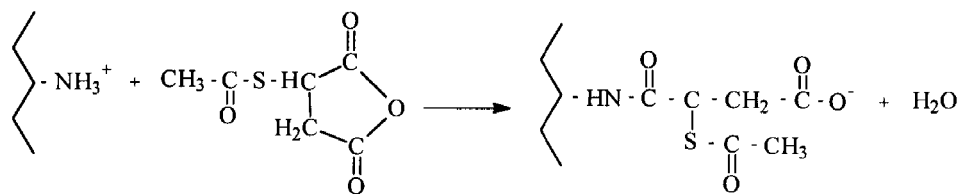
8.4.1 INTRODUCTION

Chemical modification of cells prior to metal binding reactions has been used by numerous researchers to determine which of the chemical groups located on surfaces of biomass are involved in the binding (Beveridge & Murray, 1980; Tobin *et al*, 1990; Brady & Duncan, 1994). The application of these methods to *azolla* biomass could reveal not only which types of ligands are available for metal binding, but also whether inexpensive chemical modifications could be used to enhance metal removal. As drastic chemical modification could cause overall alterations to the plant cell structure, all the modification procedures adopted were carried out at mild conditions. The modified *azolla* biomass was subsequently tested for Ni and Cr⁶⁺ uptake capacities in batch experiments at varying metal concentrations as described previously (Sections 3.2.3 & 5.2.3). Ni and Cr⁶⁺ were chosen as the cation and anion representatives of metals found in plating effluents. The only difference was that the pHs of the mixture were not adjusted during the incubation so that the final pH of the solution mirrored the reaction pH to produce the modified biomass. In some cases chemical modification procedure affects metal binding of the biomass by means of acidifying or alkalifying surface of the biomass, resulting in pH changes of the incubation solution. This can only be reflected when the pH of the incubation solution is not adjusted. The metal uptake capacities of modified *azolla* biomass and native one were compared.

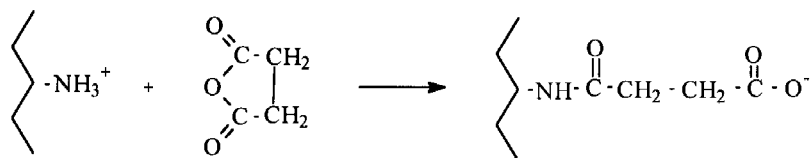
8.4.2 METHODS AND MATERIALS

The modification reactions are illustrated in Fig 8.1.

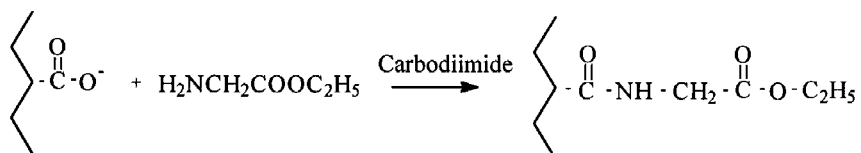
(A) S-Acetylmercaptosuccinic anhydride addition



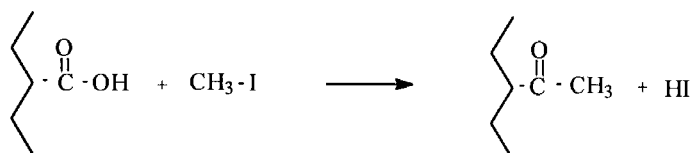
(B) Succinic anhydride addition



(C) Glycine ethyl ester addition



(D) Methyl addition

**Figure 8.1** Chemical modification of amino(A & B) and carboxyl groups (C & D).

8.4.2.1 AMINO GROUP MODIFICATION

(A) **S-Acetylmercaptosuccinic anhydride addition to amino groups** (Beveridge & Murray, 1980). Amino groups were chemically modified by the addition of S-acetylmercaptosuccinic anhydride (Sigma) so that both a carboxyl and a sulphhydryl group were introduced to the biomass, while the amino group was neutralized (Fig 8.1). The anhydride was dissolved in ethanol to yield a 20mM solution. A 20 ml aliquot of this solution was added to a suspension of biomass (2g in 180ml water) to produce a final anhydride concentration of 2mM. The reaction was carried out under nitrogen at a constant pH of 6.8 with stirring for 6 h at 22° C. The modified biomass was washed three times with distilled water and then dried at 37° C.

(B) **Succination of the amino groups** (Doyle *et al*, 1980) Biomass of 2g (in 250ml 1M NaCO₃, pH 8.0) was succinylated by adding 3.6g solid succinic anhydride (Sigma) for 10 min. The modified biomass was washed with distilled water, and resuspended in 1M hydroxylamine (pH 8.0) to remove o-acetyl groups. The acetylated biomass was finally washed with water and dried at 37° C.

8.4.2.2 AMINO AND HYDROXYL GROUP MODIFICATION

Biomass of 2g was treated with 400ml 0.05M sodium iodoacetate (Sigma), maintained at pH 8.0 for 6 h at 22° C, then washed with water and dried at 37° C. This reagent typically attaches to amino groups at low or neutral pHs but may also bind to phenolic or hydroxyl groups at higher pH (Beveridge & Murray, 1980).

8.4.2.3 CARBOXYL GROUP MODIFICATION

(A) **Esterification of carboxyl groups** (Beveridge & Murray, 1980). Carboxyl groups were blocked by glycine ethyl ester (Sigma) by means of the carbodiimide reaction, in which the negative charges were neutralized. Biomass (2g) was incubated with a total volume of 200ml

of 0.5M glycine ethyl ester and 0.2M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrfochloride (Sigma). The reaction mixture was maintained at 22° C and continuously stirred for 6 h at pH 4.75, and subsequently washed and dried at 37° C.

(B) Methylation of carboxyl groups (Tobin *et al*, 1990). The methyl iodide (May & Baker, England) treatment results principally in esterification of biomass carboxyl groups, thereby neutralizing them. Methyl iodide is a strong alkylating agent and may also result in the displacement of H-atoms on -NH₂ and -OH groups. The expected effect is to inhibit carboxyl groups from participating in the uptake process. In this method 2g azolla were stirred with 200ml methyl iodide for 4 h at 22° C, and subsequently washed and dried at 37° C.

8.4.3 RESULTS AND DISCUSSIONS

The effects of chemical modifications of the surface groups on Ni and Cr⁶⁺ are presented in Figs 8.2 - 8.5.

8.4.3.1 Ni AND Cr⁶⁺ UPTAKE OF AMINO AND HYDROXYL GROUP MODIFIED *Azolla*

The variations in metal uptake capacities of the modified biomass, for both cationic and anionic metals, compared to that of the native azolla, are minor and do not show any increased or decreased trend (Figs 8.2 & 8.3). Beveridge & Murray (1980) modified the cell wall of *Bacillus subtilis* using the same methodology and found that it did not decrease the metal uptake values. They came to the conclusion that the amino groups were not considered to be potent chelators for most metals which appears to be similar for azolla. In the case of SA modification the initial and final incubation pHs were lower than those in other modifications implying the acidifying effect on metal binding by SA modification.

The modification of hydroxyl groups of azolla, by SI in this experiment, did not make any

difference in metal uptake capacities of the native and the modified azolla (Figs 8.2 & 8.3). *Azolla filiculoides* contains a large amount of carbohydrates (71.9% of its composition, Table 8.1), which represents a high content of hydroxyl groups. The hydroxyl groups will get negatively charged at pHs above their isoelectric points, thereby they could contribute to the binding of cation metals under certain conditions. The contradictory result obtained here could probably due to inappropriate blockage of the hydroxyl groups on surface of azolla at the neutral pH of 8.0, since it was suggested that the hydroxyl groups may be bound by SI at higher pHs (Beveridge & Murray, 1980). Other suitable blocking methodology is needed to elucidate the function of hydroxyl groups in binding of metals.

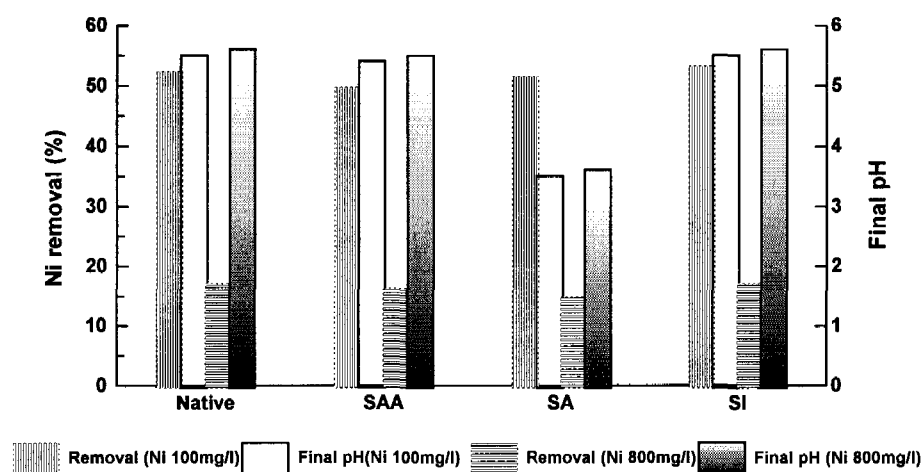


Figure 8.2 Effect of chemical modification of amino groups on Ni removal capacities of azolla biomass. The biomass was tested at incubation Ni concentrations of 100 and 800mg/l respectively. **Native**, unmodified azolla; **SAA**, azolla modified by S-acetylmercaptosuccinic anhydride; **SA**, azolla modified by succinic anhydride; **SI**, azolla modified by sodium iodoacetate.

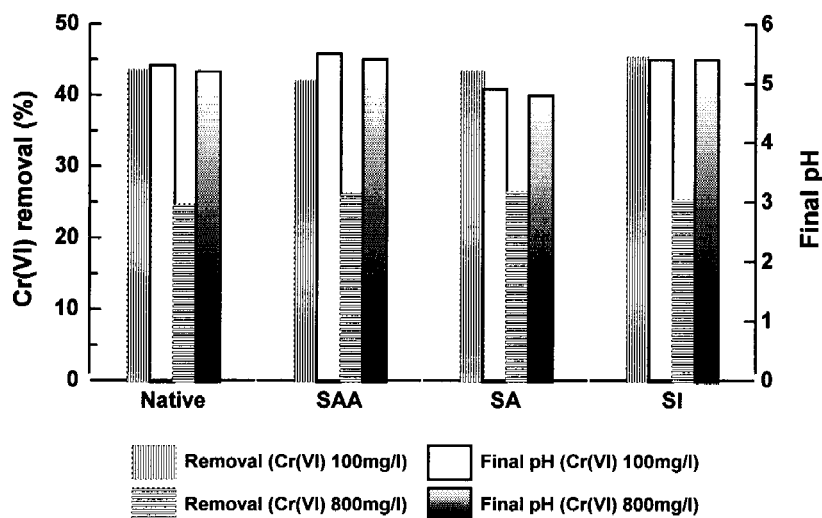


Figure 8.3 Effect of chemical modification of amino groups on Cr^{6+} removal capacities of azolla biomass. The biomass was tested at incubation Cr^{6+} concentrations of 100 and 800mg/l respectively. **Native**, unmodified azolla; **SAA**, azolla modified by S-acetylmercaptosuccinic anhydride; **SA**, azolla modified by succinic anhydride; **SI**, azolla modified by sodium iodoacetate.

8.4.3.2 COMPARISON OF Ni AND Cr^{6+} UPTAKE OF CARBOXYL-GROUP-MODIFIED Azolla

Two well established means of esterification of carboxyl groups were adopted in this study. The use of carbodiimides is of the mostly recommended because of its high degree of specificity and mild pH requirement (Gardea-Torresdey *et al*, 1990). Moreover, no exogenous group is introduced into the wall of the biomass therefore avoiding a complicated interpretation of the data. The final pHs of the incubation solutions of modified azolla were similar to that of native one so that the effect of pH on the metal uptake was minor.

Chemical modification of the carboxyl groups of the azolla surface had a profound effect on metal uptake. As Figs 8.4 & 8.5 show, the blocking of carboxyl groups diminishes Ni but increases Cr^{6+} uptake to a large degree. Up to 80% reduction in Ni and 50% increase in Cr^{6+} uptake were observed.

Carboxyl groups were identified as providing the major sites on the surface of bacteria, seaweed and alga for metal deposition (Beveridge & Murray, 1980; Tobin *et al*, 1990; Gardea-Torresdey *et al*, 1990; Fourest & Volesky, 1996). Crist *et al* (1981) presented evidence that metallic cation binding to *Vaucheria* sp. occurred at least in part by an ion-exchange mechanism and found hydrogen ions were displaced as metal cations were adsorbed by the alga. These results also indicated that cation metals adsorbed to algal surfaces by electrostatic attraction to negative sites such as carboxylate anions (Gardea-Torresdey *et al*, 1990). Data obtained here on Ni sorption agrees with the statement.

Results from the present work suggest that ionized carboxyl groups on the azolla surface may play a minor role in Cr^{6+} binding. The increase in Cr^{6+} uptake may be explained by the decrease in overall negative charge on azolla surface after modification of carboxyl groups. The reduction in anionic charge would favour the anionic chromium complex interaction with the ligands on azolla surface.

Though the structural constituents of the wall of azolla is not yet clear, the contents of protein, lipids and total carbohydrates of the water fern, would provide useful information for further elucidation of the binding mechanism (Table 8.1). Amino acids and polysaccharides in the cell wall could provide functional groups eg. carboxyl, amino and sulphate groups for metal binding.

The binding of metals with biomass, or biosorption, arises from not only the binding to carboxyl groups but also the coordination of the metal ions to a number of functional groups on surface of biomass (Gardea-Torresdey *et al*, 1990). These coordinating groups (provided by proteins, lipids and carbohydrates) include amino, thioether, sulfhydryl, carboxyl, imidazole, phosphate, phenolic, hydroxyl, and amide moieties etc. The contribution of these functional groups to metal binding can be seen in the current work, since remarkable metals had still been taken up by azolla when the carboxyl groups of the biomass was chemically blocked (Figs 8.4 & 8.5). Further investigation on binding of metals by these potential ligands is obviously necessary.

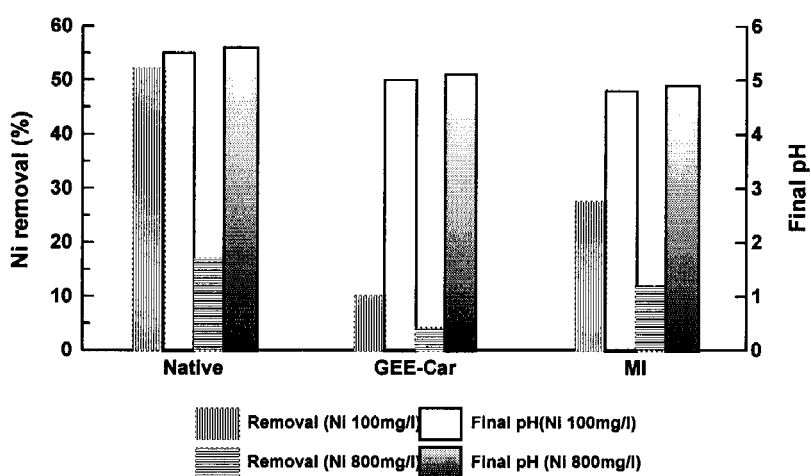


Figure 8.4 Effect of chemical modification of carboxyl groups on Ni removal capacities of azolla biomass. Ni concentrations of 100 and 800mg/l were used in the sorption tests respectively. **Native**, unmodified azolla; **GEE-Car**, azolla modified by glycine ethyl ester and carbodiimide; **MI**, azolla modified by methyl iodide.

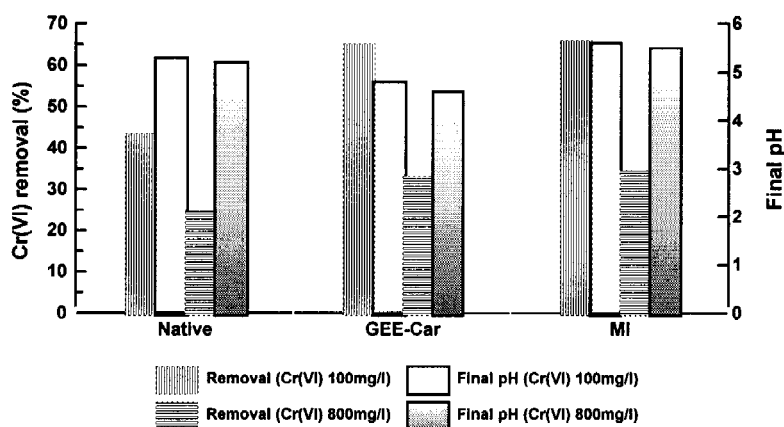


Figure 8.5 Effect of chemical modification of carboxyl groups on Cr⁶⁺ removal capacities of azolla biomass. Cr⁶⁺ concentrations of 100 and 800mg/l were used in the sorption tests respectively. **Native**, unmodified azolla; **GEE-Car**, azolla modified by glycine ethyl ester and carbodiimide; **MI**, azolla modified by methyl iodide.

8.5 INFRARED ANALYSIS OF *Azolla* BIOMASS

8.5.1 INTRODUCTION

Infrared (IR) analysis has been used in the past to investigate the mechanism of gold and cobalt biosorption by the biomass of *S. Natans*, *A. Nodosum* and *S. Fluitans* (Kuyucak & Volesky, 1989, a & b; Fourest & Volesky, 1996; Stoll, 1996). The examination of IR spectra of compounds or materials can aid a chemical investigation in many ways. Functional groups on a sample of interest could be identified, or partially interpreted by measuring the absorption bands arising from infrared fundamental vibrations (stretching and bending) in a given environment (Furniss *et al*, 1978). Structural modifications closer to the absorption centre will affect the energy associated with the absorption, and lead to a shift of the absorption band to higher or lower frequencies. These frequency shifts, however, have been found to lie within defined limits and the numerical value often provides valuable information on the structural environment of the associated group (Furniss *et al*, 1978). In this regard, the examination of IR spectra of *azolla* biomass was undertaken in this section so as to provide a clue to the identity of the functional groups that are responsible for metal sorption. In practice, an IR spectrum of a pure sample (a compound) will have fairly sharp and well-resolved absorption bands, whilst the spectra of a crude preparation or a material that contain many different kinds of molecules will display broad and poorly resolved absorption bands because of many absorption bands that are present (Williams & Fleming, 1966).

8.5.2 METHODS AND MATERIALS

Infrared spectra of various preparations of *azolla* biomass were recorded on a Magna-IR spectrometer 550 (Nicolet Instrument Corporation, USA). Approximately 1mg of sample and 100mg KBr are ground together finely, dried to remove moisture, and pressed under elevated pressure for 45min into a small clear disc.

It was reported that the raw *S. fluitans* biomass, used as a control on IR analysis, had been

found to be loaded with alkali and alkaline earth ions eg. Na, K, Mg and Ca therefore no obvious variation in the IR spectrum could be detected between the metal-free and the metal-loaded samples (Fourest & Volesky, 1996). It was further suggested that the native biomass under IR examination be washed to remove the salts and protonated with 0.1M HCl. This approach allowed the identification of two absorbance peak shifts, characteristics of coordination compounds between carboxyl groups and heavy metals (Fourest & Volesky, 1996). In the present work therefore, the native control biomass was prepared by washing with 0.1M HCl and then rinsed extensively with deionized water. This was designated as control for IR analysis. Another batch of unmodified azolla biomass, before being subjected to IR analysis, was exposed to Ni, Zn and Cr⁶⁺ solutions (800mg/l) for 5 h on batch scale as described in Sections 3.2.3 & 5.2.3, so as to get the biomass saturated with metals. The chemically modified azolla was also subjected to IR examination.

8.5.3 RESULTS AND DISCUSSION

Infrared spectra of native, carboxyl-modified, and metal-loaded azolla biomass are presented in Figs 8.7. and 8.8. The spectra from the azolla samples have a high similarity with those of algae, *Sargassum fluitans*, which was examined by Fourest & Volesky in 1996 (Fig 8.6). The similarity in IR spectra may indicate the similar composition for both types of biomass and allow a comparison between the two biosorbents. The spectrum of the native azolla displays a absorbance peak at 1736.02 cm⁻¹ caused by C = O stretching probably in a carboxyl group (Figs 8.7 & 8.8). The metal-loaded azolla biomass exhibit spectra with a shift of the carbonyl stretching band from 1736 to 1619 cm⁻¹. The shift was considered to be typical of the complexation of the carbonyl group by dative coordination (Nakamoto, 1986), with the metals in this case. The spectra of carboxyl-modified azolla display the similar shift of the carbonyl stretching band from 1736 cm⁻¹ to 1619 cm⁻¹, but the differences in between are not as large as the metal-loaded ones (Fig 8.8). The shifts of carbonyl stretching for azolla are generally not as significant as that for algae, but can be discerned by comparing the absorbance of the samples at these two regions with that of native one.

The most apparent alterations in the spectra of the metal-loaded or modified samples, compared to the native one, are the two peaks that emerged at 480 and 540 cm^{-1} respectively. Absorbance bands arising in regions of 400 to 600 cm^{-1} are probably induced by inorganic salts and derived compounds (Williams & Fleming, 1966), which could possibly be metals and blocking reagents in this case.

Data based from IR analysis on azolla biomass agrees with the results obtained on algae (Fourest & Volesky, 1996) and yeast (Ashkenazy *et al*, 1997), that carboxyl groups on surface of biosorbents could be one of the main ligands for the bulk of metal sorption. Since carboxylic acids or carboxylates are weak acids or salts of weak acid, they will only get negatively charged at neutral or higher pHs and function as a ligand, in a manner of weak cation exchanger, for binding of cation metals (Fourest & Volesky, 1996). Unlike that in the case of strong acids, the carboxyl groups are likely to be protonated at the lowered ambient pHs (much lower pHs are needed to protonate a strong acid), leading to releasing the bound cations. This character of carboxyl groups can be seen throughout the present study (Chapters 4-7), where the Ni, Zn, Cd as well as Cr^{3+} were bound to azolla at a neutral or higher pH, and desorbed by lowering the pHs. The importance of the carboxyl ligands on surface of azolla in sorption technology may therefore be that it makes the desorption of bound metals with acids possible and eases the metal recovery procedure.

However, in order to fully interpret the spectra obtained and to define each functional groups on surface of azolla, further diagnostic investigation on binding of metals with azolla using combined chemi-physical and biological techniques are apparently needed.

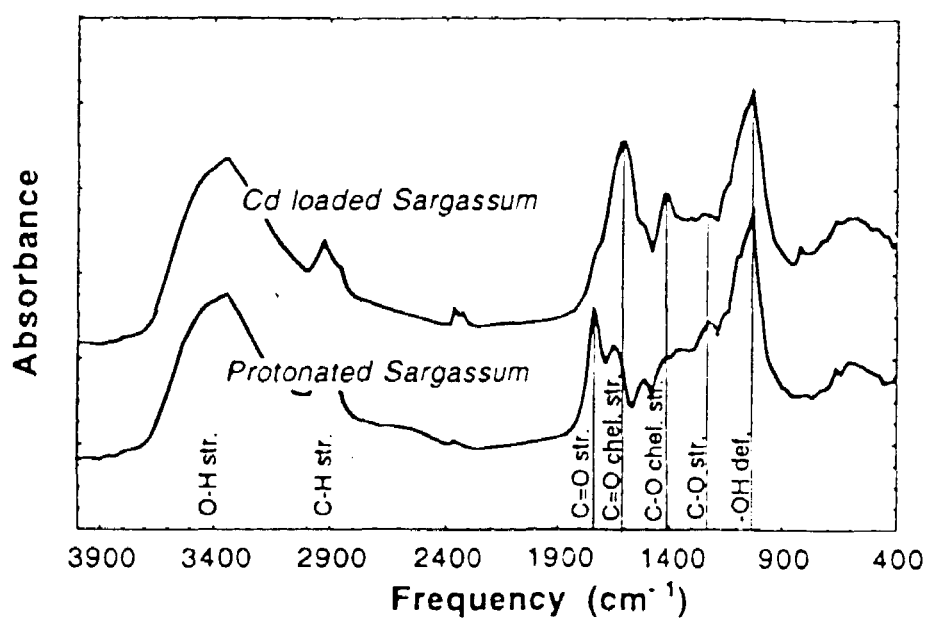


Figure 8.6 IR spectra of protonated or Cd loaded *S. fluitans*. str.: stretching; chel. str.: chelate stretching; def.: deformation (Taken from Fourest & Volesky, 1996).

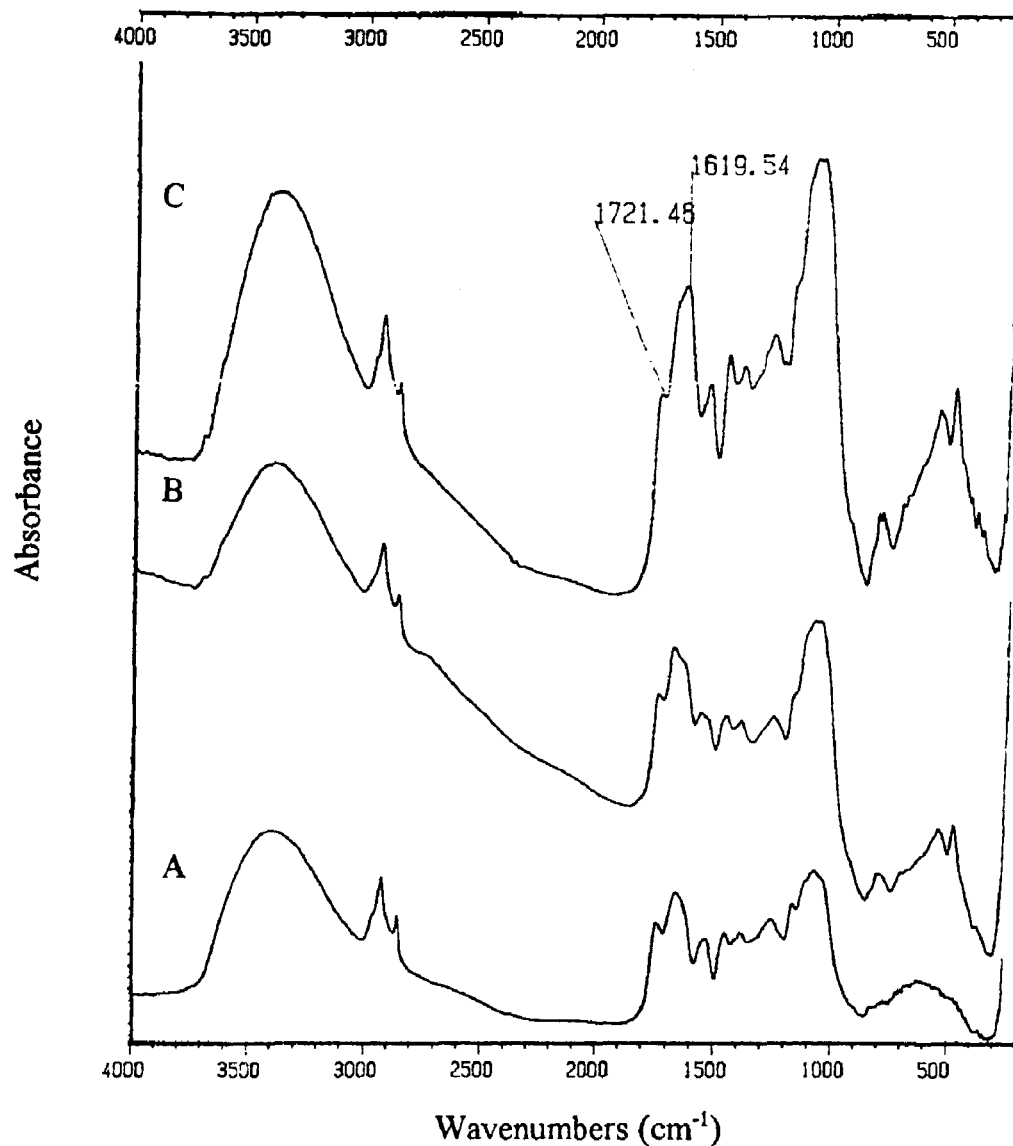


Figure 8.7 IR spectra of native and carboxyl-modified *azolla* biomass. **A**, native; **B**, modified by glycine ethyl ester - carbodiimide; **C**, modified by methyl iodide.

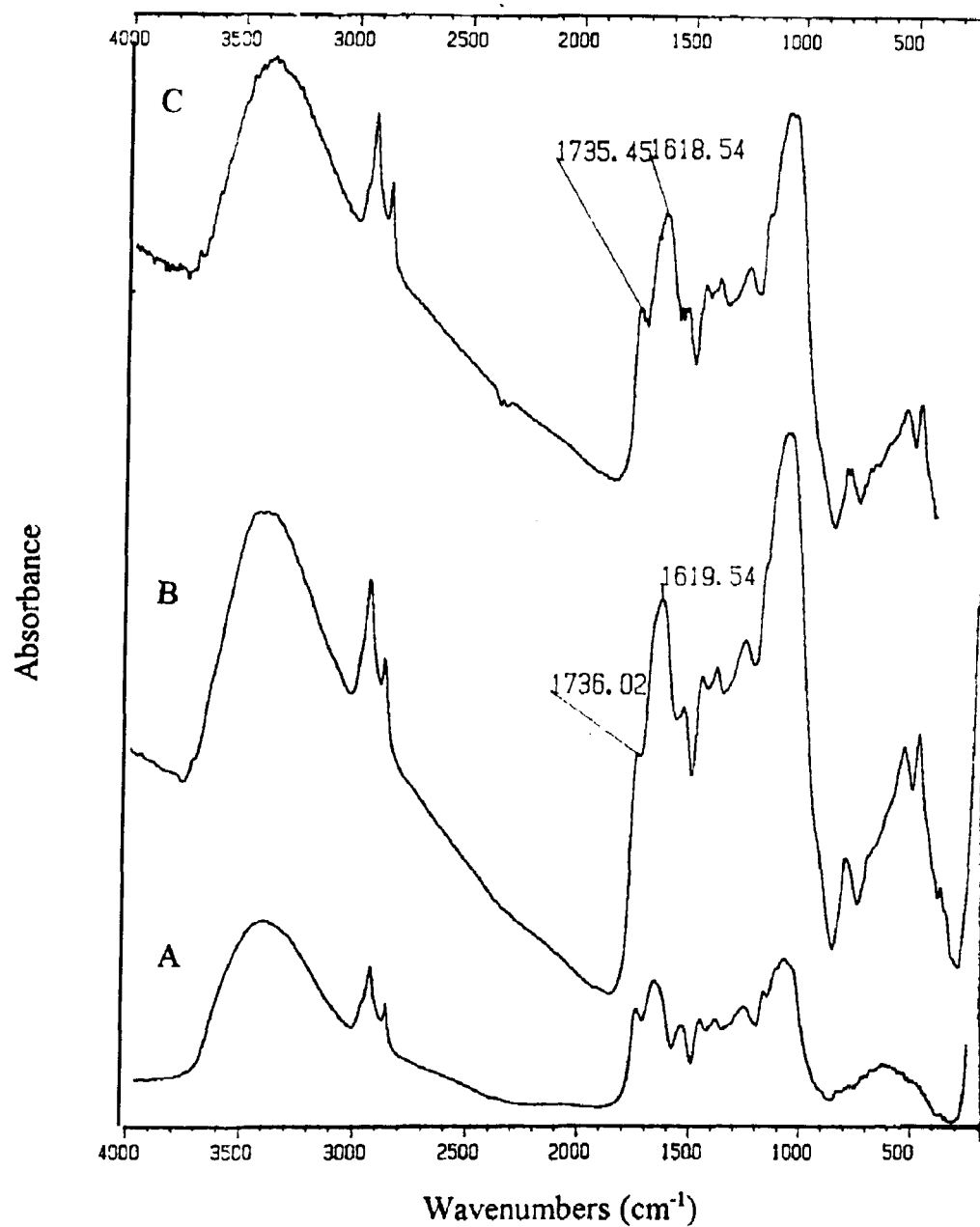


Figure 8.8 IR spectra of native and metal-loaded azolla biomass. A, native; B, loaded with Zn; C, loaded with Ni.

8.6 CONCLUSIONS

Results presented in this chapter provide a comprehensive understanding of the nature of metal binding of azolla biomass.

The biomass has a large surface area and high water retention capability in comparison with its species members. The biochemical compositions of the biomass was determined in terms of protein, lipids, total carbohydrates and ash. The functional groups on surface of azolla were partially identified using chemical modification and subsequently metal binding comparison tests. Among the functional groups examined, carboxyl groups, provided by amino acids and polysaccharides, appeared to play an important role in metal cation binding. The infrared spectra of the samples support the conclusion resulting from the modification experiments but more information is needed for detailed interpretation of the mechanisms for metal binding.

9. GENERAL CONCLUSIONS

9.1 CROSS-LINKED YEAST BIOMASS

Immobilization techniques are the critical procedure in application of some potential microorganisms to remediation of the heavy metal-laden waste effluents on industrial scale. A suitable immobilizing method will not only maintain or improve the efficiencies of the microbes for removing metals, but will also meet the economic requirements of the industries. A novel method for immobilizing yeast biomass has been designed and tested for both the efficiency and the cost. The non-viable *Saccharomyces cerevisiae*, cross-linked by 13% formaldehyde/1N HNO₃, shows satisfactory mechanical stability and rigidity in repeated sorption-desorption operations, in a fixed-bed column system. The metal uptake capacity of FA-cross-linked pellets, on batch trials, remained similar to that of raw yeast, reflecting that the immobilizing procedure did not hinder its metal removing ability. Cu, Zn and Cd ions were effectively removed by the cross-linked yeast pellets from solutions and then completely recovered with acid.

Moreover, Cr⁶⁺ in electroplating effluent was significantly minimised either by accumulation onto the biomass or reduction to its trivalent form so that detoxification of Cr⁶⁺-laden wastewater can be accomplished. Desorption of bound Cr⁶⁺ with either alkaline or salt could not be accomplished. A combination of reduction and desorption with FA/HNO₃ appeared promising in regeneration of the saturated biomass at 4°C, whereas applying the same procedure at room temperature did not show satisfactory results, reflecting the thermochemical effect on binding of this metal to yeast biomass.

The cost of immobilizing 1kg dry yeast pellets is estimated at less than US\$ 1, a cost at which an application of the immobilizing techniques, on a large scale, will be possible. The metal sorption capacities of the yeast pellets, in a fixed-bed system, are relatively low, in comparison with that of *Azolla filiculoides*. Comparison of the column sorption capacities between the immobilized yeast pellets and the native cells could not be directly performed,

due to the fragility of native yeast cells. It is clear that a larger amount of the yeast biomass would be required for treating a certain volume of industrial effluent, than that required when using azolla.

9.2 *Azolla filiculoides*

Azolla filiculoides, a naturally-abundant water fern, has been screened, for its metal sorption capacities, stability and reusability. The physical appearances of azolla had an extensive surface area accommodating possible binding sites for metals. The dry form of azolla biomass exhibits numerous characteristics which facilitate its application for treatment of metal-laden waste effluents. The water-insolubility and flexibility shown by the biomass, when contained in a column, are of crucial importance in a continuous-flow system since a column can be operated at high flow rates without apparent compacting of the biomass and pressure loss. The biomass appeared to have fulfilled the required mechanical criteria during the repeated sorption-desorption column operations.

The isotherm data, obtained from batch removal of Cr^{6+} , showed that the sorption process was effective, endothermic and highly pH dependent. Considerable amounts of Cr^{6+} were accumulated at the optimum pHs of 2-2.5. Column sorption of Cr^{6+} at a low flow rate (80ml/h) and pH of 2.5 showed an optimum performance with a total Cr uptake of 50.4mg/g at 60% saturation of the biomass. Removal of Cr^{6+} using an azolla column from an electroplating effluent was deemed reasonably satisfactory, although the uptake was slightly decreased than that from synthetic solutions. In most cases, column performances for removing heavy metals from industrial effluents, can not be expected to attain the same levels as that resulting from using a synthetic metal solution. The possible presence of various interfering ions and organic compounds in the effluents will consequently affect the sorption process. Desorption of bound Cr^{6+} with various desorbents was incomplete, which resulted in a low regeneration efficiency of about 50%. Further work is needed to improve the desorption process in order that a better regeneration efficiency can be attained. Removal and recovery of Cr^{3+} using the azolla column was, however, promising. Desorption of Cr^{3+} from the spent

biomass column was accomplished with the recovery of 80% using 0.5N H₂SO₄. The metal uptake capacity of azolla biomass remained similar to the first run after completion of five cycles of sorption-desorption. Regeneration efficiency for Cr³⁺ removal of up to 90% also demonstrated the reusability of azolla biomass in the column operation for removing Cr³⁺.

Cation metal uptake capacities of azolla, obtained either from batch (i.e. 43.4mg Ni /g; 45.2mg Zn /g) or column (i.e. 27.9mg Ni /g; 30.4mg Zn /g at 60% saturation) experiments, are reasonably high in comparison with other sorbents or biosorbents. The uptake of Ni or Zn ions from solution is pH dependent showing an optimum pH of 6 to 6.5, under the experimental conditions. The sorption of Ni and Zn ions in batch trials was rapid with about 80% of the bound Ni ions being taken up in the first 10 min. The character of rapid binding by azolla is extremely important in a column sorption process, especially on a large scale since it enables the optimum uptake of metals at high flow rates. The Ni or Zn uptakes in column sorptions were not markedly affected when the flow rates were increased from 80ml/h up to 800ml/h for the 5g biomass used.

The cation heavy metals removed from waste effluents, have been recovered in a concentrated, low volume solution. The desorption of bound Ni and Zn ions from the saturated biomass was accomplished with either 0.2N HCl or H₂SO₄ and resulted in recoveries of more than 95%. The form of the metals recovered, in the case of Ni and Zn, are identical to that of plating agents eg. nickel sulphate or chloride, so that recycling of the metals is possible. Alternatively, the biosorption system can also be jointly operated with other technical processes eg. electrolysis under certain circumstances, to recover the pure form of metals. Chemical precipitation of the recovered heavy metals can be greatly facilitated from the concentrated metal medium where recovery of the metals is not concerned. An effluent-free, closed loop of Ni or Zn treatment system was proposed whereby the Ni or Zn ions can be recycled to the plating bath whilst the purified water being fed back to the rinse tanks.

Ca and Mg ions, commonly present in the electroplating effluents, appeared to affect sorption

of heavy metals by azolla when metal concentrations were relatively low, presumably through competitive binding for the shared sites on surface of azolla. When concentrations of heavy metals are relatively high, the effect of Mg ions on sorption of heavy metals appeared minor.

The data obtained from column sorptions of Ni and Zn follows the BDST model well enabling the application of the model to predicting design parameters for scale-up of the biosorption column system. The service time for a given influent concentration can be predicted by the model established using the experimentally available data.

It is interesting that the values of metal uptake, expressed in molar quantities, obtained during column operations on respective single-metal solutions and the multiple metal system, are similar, implying that the mechanisms involved in the sorption of all metal cations are similar and the binding sites on surfaces of azolla are probably shared by all cation metals. The binding sites on surfaces of the biomass for metal cations are estimated as in range of 0.45-0.57mmol/g, under the specified conditions. A slight sorption preference of the Azolla for metal cations can be defined as: $\text{Cr}^{3+} > \text{Zn} = \text{Cd} > \text{Ni}$.

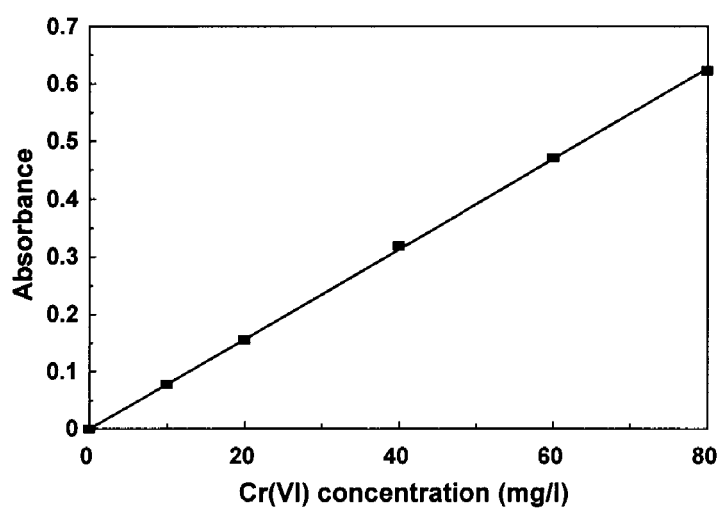
The biomass has a large surface area and high water retention capability, in comparison with members of similar species. The biochemical compositions of the biomass was determined in terms of protein, lipids, total carbohydrates and ash. The functional groups on surface of azolla were partially identified using chemical modification and metal binding comparison. Among the functional groups examined, carboxyl groups, provided by amino acids and polysaccharides, appeared to play an important role in metal cation binding. The infrared spectra of the samples support the conclusion resulting from the modification experiments but more diagnostic information is needed in order to fully elucidate the mechanisms for metal binding.

The economic factor in the application of azolla to treatment of waste effluents on large scale is encouraging. The water fern is abundantly distributed around the world, especially in South America, South Asia and South Africa. In some areas where the species has not been

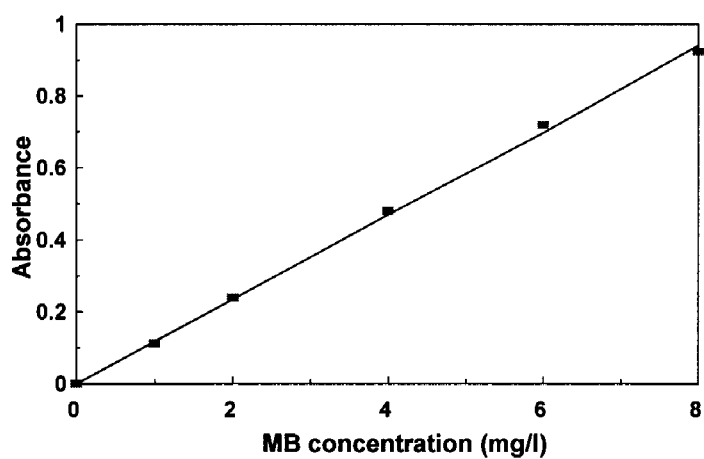
introduced, it can also be easily cultivated in dams, rivers and wet-lands, regardless of their ambient conditions. The main cost of the biosorbent would be the labour expenses for collection and preparation. It could therefore be one of the lowest-cost biosorbents available for this purpose.

9.3 GENERAL COMMENTS

In present work, the remediation of metal-laden effluents by selected biosorbents have been investigated with regard to metal sorption and recovery, regeneration of the biosorbent, and recycle of the metals as well as the water. The performances of azolla biomass during the whole process are satisfactory. The biomass fulfilled the basic requirements of a sorbent for treatment of metal-laden effluents, with the attractive advantage in economic terms. Therefore, biosorption using the azolla biomass can be a viable alternative to the existing technologies for small to medium sized metal finishing industries in future.



Appendix 1 Cr(VI) standard curve.



Appendix 2 Methylene blue standard curve.

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