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METAL RECOVERY FROM *SACCHAROMYCES CEREVISIAE* BIOSORPTION COLUMNS.

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SUMMARY

The bioaccumulation of metal chlorides (Cu, Zn, Co, Cd, Ni and Cr) to immobilised *S. cerevisiae* was studied in packed-bed continuous flow columns. The metals were eluted from the columns using 0.1 M HCl, with a desorption of $\geq$ 90 % being attained. Reusability of the biomass was demonstrated. Mixed metal solutions were applied and selective binding and recovery was achieved between copper and cobalt.

INTRODUCTION

Toxic metals are increasingly being diverted into the environment from industrial waste water. The waste water from electroplating, tanning and mining industries often contain excessive amounts of metals such as cadmium, cobalt, copper and chromium. The impact of these metals on the environment and their accretion through the food chain has promoted research in developing alternative, efficient and low cost waste water purification systems. Microorganisms are potent biosorbent materials and may provide the technology required for metal removal and concentration (Nourbakhsh *et al*, 1994). Bioremediation has both the potential application for environmental regulation and economic recovery of the metals. Biosorbents may be utilised where more expensive physico-chemical methods are not feasible and are of particular value with large volumes of waste water containing relatively low metal concentrations (Volesky, 1987). While the bioremediation of metals is acknowledged and

well documented, it's full industrial potential has yet to be realised (Lakshmanan, 1986; Broda 1992). An effective metals removal plant would require a biomass which is readily available, economical, has a high uptake capacity, allows for selective recovery and is reusable.

Metal uptake by a biosorbent comprises two phases; a metabolism independent surface binding and an energy dependant intracellular influx (Norris and Kelly, 1977; Gadd 1986). Adsorption to the yeast cell wall is rapid, with the adsorptive capacity being determined by the structural organisation of the cell wall protein-carbohydrate complex (Davidova and Kasparova, 1992: Brady *et al.*,1994). Potential binding sites include phosphate, hydroxyl, carboxyl and other functional groups (Tobin *et al.*, 1990). These multiple, non-equivalent sorption sites have varying affinities for different ions, for which the cations compete (Davidova and Kasparova, 1992). Physical sorption to cell wall structures should be reversible and allow for metal recovery (Kuyunak and Volesky, 1988). The objectives of this study was to investigate the binding of metal chlorides (Cu, Co, Zn, Cd, Ni and Cr) to immobilized *Saccharomyces cerevisiae* in packed-bed continuous-flow columns and to determine recovery and concentration potential and the reusability of the biomass. Two continuous flow columns in series were used to demonstrate selective binding. The metals were eluted from the columns using dilute acid.

MATERIALS and METHOD

Commercial preparations of *S. cerevisiae* were obtained from Anchor yeast. $CuCl_2.2H_20$, $CoCl_2.6H_2O$, $CdCl_2.H_2O$ and were obtained from Merck, $ZnCl_2$ from BDH, $CrCl_2.6H_2O$ from Reidel-de Haen and $NiCl_2.6H_2O$ from Saarchem. N,N,N',N'-tetramethyl-ethylenediamine (TEMED), polyacrylamide and N,N' methylene-bis-acrylamide were purchased from Sigma. Ammonium persulphate, NaCl, NaOH and HCl were supplied by Saarchem. Ultra-pure deionized water, purified by a Milli-Q water system, was used in all experiments.

Immobilisation: Yeast immobilisation as adapted by Brady and Duncan (1994) was as follows: 3.75 g acrylamide monomer and 0.2 g N,N'-methylene-bis-acrylamide were dissolved in 12 ml deionized water. *S. cerevisiae* (5 g) was washed with 10 ml water and then suspended in 10 ml 0.9 % NaCl. The acrylamide solution and the cell suspension were mixed together with 1 ml TEMED and 2.5 ml ammonium persulphate. The reaction mixture was cooled and allowed to polymerize, after which it was passed through a 30 - mesh sieve. The resultant biomass was washed with water.

Columns: LKB columns were packed with 20 ml of the immobilised yeast slurry and conditioned by passing 20 ml 0.1 M HCl, 20 ml 0.05 M NaOH and 40 ml water through the column. Single metal applications of 200 μmoles/l and a volume of 500 ml were passed through the columns at a flow rate of 1 ml per minute. The eluent was collected in 10 ml fractions. The metals were then eluted from the columns with 50 ml 0.1 M HCl, collected in 5 ml fractions. The columns were reconditioned by eluting with 20 ml 0.05 M NaOH and 20 ml water. The metals were then reapplied. Mixed metal solutions were passed through two columns set in series and these columns were desorbed separately.

Analysis: A GBC 909 atomic absorption spectrophotometer was used for metal analysis.

RESULTS and DISCUSSION

The metal ions were effectively removed from solution by the yeast biomass (table 1). The biomass removed the metals until reaching a saturation threshold, following which uptake declined rapidly. A typical bioaccumulation profile is shown in figure 1. The metals were desorbed from the biomass using 0.1 M HCl. Initial recovery of copper, cobalt and cadmium was 100 %. The high recovery by mild acid elution suggests accumulation by passive binding to the cell walls of *S. cerevisiae*. The recovered metals were concentrated in small volumes. For example, copper, zinc and cobalt were collected into 10 ml aliquots, representing a 50 fold reduction from the initial volume. Chromium was not eluted by 0.1 M HCl. The optimum pH for chromium adsorption is below 2 (Nourbakhsh *et al*, 1994), implying the need for an alternative desorption protocol. Increasing the concentration of HCl (1 M) gave a 34 % chromium recovery. The inability to recover chromium by mild acid treatment could lead to selective desorption between chromium and the other metals examined. The columns were reconditioned and the metals reapplied to assess the reusability

Table 1 : Metal uptake on 20 ml *S. cerevisiae* columns and metal recovery*.

	Uptake	µmoles/g**	Recovery	%
METAL	Run 1	Run 2	Run 1	Run 2
Copper	27.9	27.0	100	91
Cobalt	49.7	50.0	100	99
Cadmium	31.6	37.3	100	100
Nickel	33.7	38.7	100	91
Zinc	40.7	49.9	89	61
Chromium	28.6	++	34***	++

* Typical results are reported.
** Bioaccumulation results expressed as µmoles metal per gram wet weight *S. cerevisiae*.
*** Chromium recovered with 1.0 M HCl.

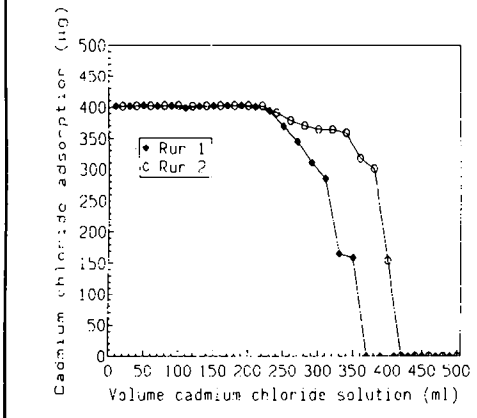

Figure 1 : Bioaccumulation of cadmium chloride on a 20 ml immobilised *S. cerevisiae* column. The cadmium chloride was desorbed by 0.1 M HCl, the column was reconditioned and cadmium chloride reapplied to determine reusability.

of the biomass. Bioaccumulation remained constant or was increased with the second application of metal. Cadmium, nickel and zinc exhibited substantial increases in adsorption to the biomass (table 1). This beneficial consequence of the adsorption-desorption process demonstrates the potential for continuous utilisation of the biomass. Reconditioning the columns with NaOH also appeared to benefit the uptake capacity of the biomass. Cadmium was initially accumulated at a rate of 31.6 µmoles/g and 37.3 µmoles/g on the second application (figure 1). All adsorbed cadmium was recovered by 0.1 M HCl elution, a 33 fold reduction in volume observed for both applications. Cobalt was accumulated the most successfully of the metals, with an initial 99.9 % and then a 100 % removal from the applied 500 ml solutions. Zinc recovery was reduced after the second application, possibly a result of internalisation of the metal.

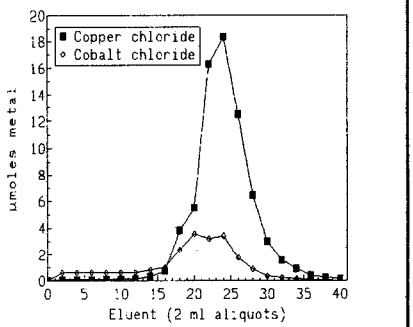

Figure 2a : Desorption of copper chloride and cobalt chloride from a 20 ml immobilised *S. cerevisiae,* column 1 of two columns in series. Hydrochloric acid (0.1 M) was used to desorb the metals. Results are expressed as µmoles metal recovered per 2 ml eluent. The predominant metal eluted from this first column was copper.

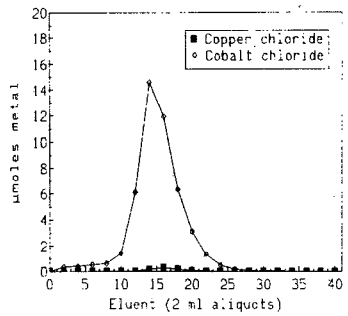

Figure 2b : Desorption of copper chloride and cobalt chloride from a 20 ml immobilised *S. cerevisiae,* column 2 of the two column series. Hydrochloric acid (0.1 M) was used to desorb the metals. Two ml fractions were collected. The predominant metal recovered from this column was cobalt.

Mixed metal samples of two metal species were passed through two columns set up in series to determine the extent of selective uptake of the cations due to different affinities for sorption binding sites on the biomass. The columns were washed separately with 0.1 M HCl to determine metal accumulated per column. Copper displaced all metals from the first column, with the exception of chromium. Copper displaced zinc with a binding ratio of 6:1. Cadmium was also displaced by copper, however substantial mixing occurred and selective recovery of metals was not achieved. Copper bound preferentially to cadmium at a ratio of 2:1. Selective binding was most clearly demonstrated between the metals copper and cobalt. Copper bound preferentially to the first column (binding ratio of 4:1) and cobalt was displaced onto the second column. The metal recovered and concentrated from the first column was > 75 % copper (figure 2a). The second of the two column series bound predominantly cobalt and a 99 % pure cobalt solution was obtained (figure 2b). While the success of selective binding and recovery of metals from mixed metal solutions is dependant on the metals and their initial concentration in solution, the process did illustrate the potential for selective binding to biosorbents and the possible recovery of metals from these solutions.

CONCLUSION

The immobilised *S. cerevisiae* packed-bed biosorption columns effectively removed metals from aqueous solutions. The bioaccumulated metals were recovered by 0.1 M HCl elution. The desorption protocol utilised a minimum quantity of acid to yield concentrated, low volume metal eluents. The successful elution of metals from the biosorption columns implied passive binding to sorption sites on the cell wall. The biomass was reusable. The adsorption-desorption process did not adversely affect the uptake capacity of the biosorbent and the uptake of specific metals was enhanced with continuous use. Selective binding to *S. cerevisiae* was demonstrated. The potential to reuse biosorbents and the ability to selectively recover metals from aqueous solutions could lead to the development of a viable, cost effective metal bioremediation technology.

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