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REUSABILITY OF IMMOBILISED *SACCHAROMYCES CEREVISIAE* WITH SUCCESSIVE COPPER ADSORPTION-DESORPTION CYCLES.

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SUMMARY

Immobilised *Saccharomyces cerevisiae* in batch reactors effectively removed copper from solution with a binding equilibrium of 70 % being attained within 20 minutes of contact. Maximum uptake was between pH 3 to 5 (V_{max} = 24.1 μmol/g) and was substantially reduced at pH 2. Bound copper was readily recovered by addition of 1.0 M HCl ($\leq$2 % v/v). In adsorption-desorption studies metal removal and recovery was high, and uptake was increased with repeated use. Electron microscopy confirmed that no morphological changes occur to the cells during repeated adsorption-desorption.

INTRODUCTION

Increasing amounts of heavy metals are being released into limited water supplies during industrial processing such as mining and electroplating, promoting research in the development of alternative and cost-effective waste-water purification technology. Bioremediation is an approach which has the potential application to decontaminate and to recovery metals from these waste-waters (Volesky and Holan, 1995). Metal removal mechanisms are varied, including adsorption, absorption and precipitation, any one or a combination of which may be operational at any particular time. Adsorption is generally a rapid cell surface binding and the efficiency is dependent on the structural organization of the cell wall (Davidova, 1992), biosorbent and metal concentration, and metal solution chemistry.

Saccharomyces cerevisiae has been utilized as the biosorbent due to its ability to accumulate heavy metals, retain its integrity and withstand harsh environments (Brady and Duncan, 1994). As a by-product of fermentation processes it is readily availability as a cost effective biosorbent. The biosorbent has the potential for high metal uptake capacity, metal selectivity and metal recovery. The yeast cells were immobilised to improve the ability to recover and regenerate the biosorbent, making it mechanical suitable for batch and packed-bed reactors. The ability to desorb and recover bound metal from the sorbent would benefit the economics of the process (Tsezos, 1984; de Rome and Gadd, 1991). The eluting solution should be

non-toxic, achieve maximum recovery using minimal quantities at the lowest possible concentration. Rate of desorption is dependent on the hydration of metal ions, the cell wall microstructure and the binding strength (Crist *et al*, 1994). The biosorbent should not be damaged during the adsorption, desorption or regeneration processes to allow reuse (McLean *et al*, 1994).

The objective of this study was to investigate copper accumulation by an immobilised *S. cerevisiae* biosorbent in batch and column reactors. The ability of potential eluting solutions for recovery of bound metal was tested and the reusability of the biosorbent assessed. Electron microscope studies allowed observation of any morphological changes to the cell during repeated adsorption-desorption cycles.

MATERIALS and METHOD

Commercial preparations of *S. cerevisiae* were obtained from Anchor Yeast Inc (production strain, 90 % cell viability). $CuCl_2.2H_20$, $CaCl_2.2H_2O$, Ca_2CO_3 and $CaHCO_3$ were purchased from Merck. KOH, NaOH, KCl, H_2SO_4, HNO_3 and HCl were supplied by Saarchem. Ultra-pure deionized water was purified by a Milli-Q water system.

Immobilisation: Yeast immobilisation in polyacrylamide gel was as described previously (Wilhelmi and Duncan, 1995).

Adsorption profiles: Immobilised biomass (1g) was weighed into conical flasks. The metal solution (10 ml of 200 μmol/l) was then added. For determination of binding rates the contact times were 0.5, 1, 2, 3, 4, 5, 10, 20, 30 and 60 minutes with shaking at ambient temperature. Post incubation 5 ml of the reaction mixture was filtered (HA 0.45 micron nylon filters - Micron Separations Inc.) under vacuum and the filtrate analysed for metal. Equilibrium studies of copper removal were conducted over the range of 50 to 2000 μmol/l. Incubation was as above with shaking for 30 minutes. Post-contact the mixtures were centrifuged at 500 g x 5 min, the supernatant was removed and analysed for metal. The determinations were repeated 5 times per concentration. For kinetic characterization, the results were analysed using Michaelis-Menten binding isotherms. Km and V_{max} values were determined using Lineweaver-Burk and Hanes-Wolf transformation plots.

pH profiles: The pH of the copper solution was adjusted using NaOH or HCl prior to incubation with the biosorbent. The pH range evaluated was from pH 2 to 6. Batch reactor incubation was as described above for equilibrium studies. The initial copper concentration was 200 μmol/l.

Metal desorption: Batch reactor incubations were as described above. Post-contact the reaction mixtures were treated with potential eluting solutions. The acids HNO_3, HCl and H_2SO_4 (0.01-0.1 M) sequentially decreased the pH and desorption profiles were generated. The volumes and concentration of acid used were recorded. Ca_2CO_3, $CaHCO_3$, $CaCl_2.2H_2O$, KCl, KOH and NaOH solutions (1 M) were added to the reaction mixtures in aliquots of 0.1 ml, 0.5 ml and 1.0 ml.

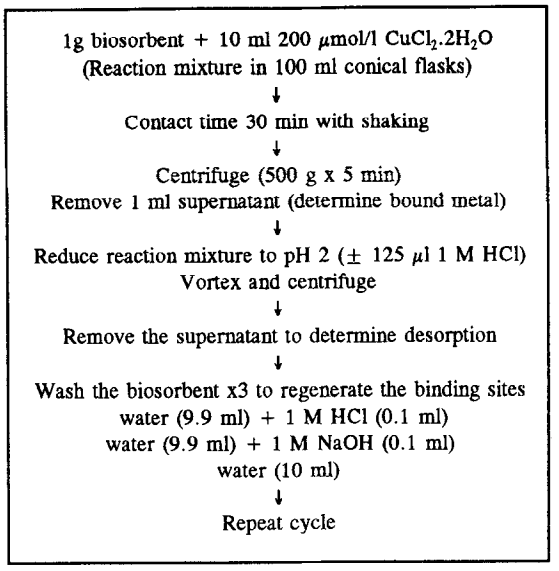

Figure 1: A flow-diagram of the batch reactor adsorption-desorption protocol.

Reusability of the biosorbent: Refer to Figure 1 for the batch reactor reusability protocol. Biosorption columns (LKB) were prepared as described previously (Wilhelmi and Duncan, 1995). Eight adsorption-desorption cycles were investigated for both batch and biosorption column reactors.

Transmission electron microscopy: Control, copper exposed and copper plus acid treated *S. cerevisiae* cells were prepared for transmission electron microscopy using standard techniques (Cross, 1989). The sections were examined under a JEOL 100 CX transmission electron microscope.

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

RESULTS

Metal uptake progressively increased up to 20 minutes, reaching an adsorption equilibrium of 65 to 70 % metal removal (Figure 2). The rate of metal removal and relatively low copper concentration suggest a metabolism independent biosorption to the cell wall. Higher concentrations and contact times would lead to internalization, which would be inappropriate for a removal and recovery process. Controls using polyacrylamide gel with no *S. cerevisiae* accumulated an average of 1.5 % of the copper in solution and the nylon filters did not retain any metal. Equilibrium studies of copper removal were investigated over varying concentration ranges. Biosorption followed Michaelis-Menten kinetics, with the Km for copper binding being 413 μmol/l and the V_{max} 24.1 μmol/g (Figure 3). These constants may be of value if comparing other metals or biomass if all other parameters remain consistent. They may also aid as a guideline for designing of a bioremediation reactor. Since low Km values are an indication of high affinity a system such as a biosorption column which has low contact times may be applicable in such cases.

The bioaccumulation of divalent cations is pH dependent. By adjusting the pH of the metal solution prior to biosorbent contact, a profile of optimum pH for copper removal was generated. Copper removal from solution by *S. cerevisiae* was maximized in the pH range 3 to 5 (60-75 %) and was substantially reduced at pH 2 (5 %). The lower pH leads to H^+ ion competition for binding sites, effectively neutralising the sites and inhibiting metal binding.

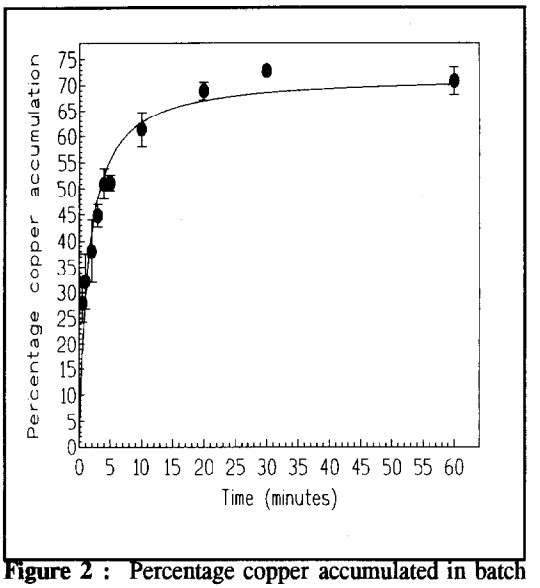

Figure 2 : Percentage copper accumulated in batch reactors by immobilised *S. cerevisiae* as a function of time (n=5, ± SD). The initial copper concentration was 200 μmol/l.

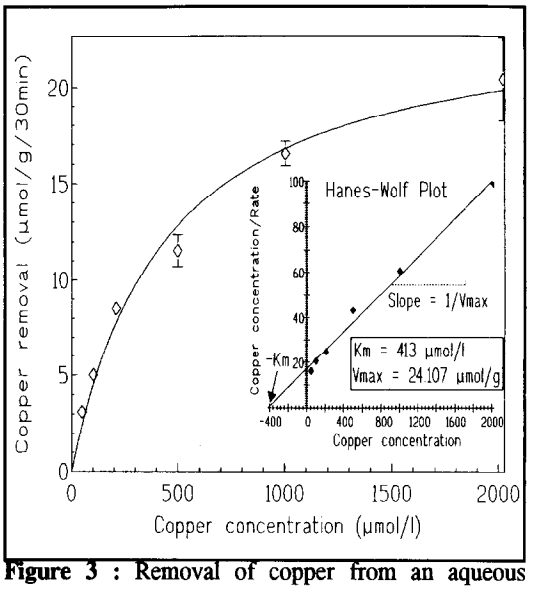

Figure 3 : Removal of copper from an aqueous solution by immobilised *S. cerevisiae* in batch reactors (n=5, ± SD). Km and V_{max} were calculated as 413 μmol/l and 24.1 μmol/g respectively.

Post accumulation of the copper to the biosorbent, a range of potential eluting solutions were investigated for their metal recovery potential. By determining the copper bound to the biosorbent, the desorption efficiency of the eluting solution was assessed. The desorption protocol was effective for solutions capable of rapid desorption. Most success was achieved using the mineral acids. By sequential lowering of the pH a copper recovery of ≥ 80 % was achieved at pH 2. HCl, H_2SO_4 and HNO_3 effectively desorbed bound copper by addition of nominal volumes of 1.0 M solutions to the remaining 9.0 ml reaction mixture (Table 1). Recovery of bound metal from the biosorbent is important if the process is continuous and if the biomass is to be reused. $CaHCO_3$, $CaCl_2.2H_2O$, Ca_2CO_3 and KCl were relatively ineffective at recovering copper and desorbed 32.1 %, 24.9 %, 14.3 % and 9.3 % respectively (Table 1). NaOH and KOH increased copper adsorption. NaOH was used for biosorbent regeneration post acid desorption. It neutralised the biosorbent pH and had the beneficial side-effect of enhancing metal removal efficiency.

Adsorption-desorption was investigated over 8 cycles to assess the reusability of the immobilised *S. cerevisiae*. Copper removal from solution in batch reactors increased from an initial 65 % to between 70 and 85 % efficiency in the latter cycles (Figure 4). Desorption was achieved by reducing the pH using HCl. Recovery of metal was high with an average recovery of ≥ 85 % over the repeated cycles. No apparent damage was done to the biosorbent and no adverse effect on uptake or recovery of the metal was observed. The immobilised biomass was easily recovered and regenerated in this investigation. Adsorption-desorption studies using free cell suspensions were unsuccessful due to the significant loss

Table 1: Percentage desorption of copper from immobilised *S. cerevisiae*.

Eluting Solution 1 M	Volume ml	Desorption %	SD
H_2SO_4	0.20	85.0	± 4.3
HCl	0.13	80.4	± 5.5
HNO_3	0.25	89.5	± 4.3
$CaHCO_3$	1.0	32.1	± 6.1
Ca_2CO_3	1.0	14.3	± 2.6
KCl	1.0	9.3	± 1.8
$CaCl_2.2H_2O$	1.0	24.9	± 4.2
Water control	0.5	1.2	± 0.8

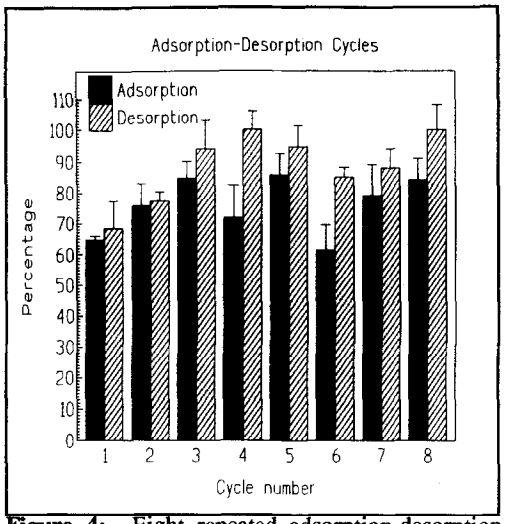

Figure 4: Eight repeated adsorption-desorption cycles in batch reactors determined the reusability of the immobilised *S. cerevisiae* biosorbent. The initial copper concentration was 200 μmol/l and was desorbed using HCl (n=5, ±SD).

of cells between each cycle. Accumulation profiles of a biosorption column demonstrated the reusability of the biosorbent over 8 cycles. Initially the column removed all the copper from solution. At saturation there was a rapid decline in metal accumulation, following which the metal was recovered and concentrated by elution with 0.1 M HCl. The column was regenerated and reused. Copper accumulation by the biosorption column was initially 31.2 μmol/g *S. cerevisiae*. This declined slightly over the following 2 cycles, possibly due to a nominal loss of cells from mechanical shearing. Thereafter the column stabilised and the accumulation capacity increased substantially in the latter cycles with up to 47.8 μmol/g being accumulated per cycle. The improved uptake was possibly due to an increase in the number of metal binding sites made accessible to the metal ions during the adsorption-desorption process. Copper recoveries of ≥ 85 % were achieved and the copper was concentrated up to 40 fold.

Cells exposed to copper and dilute acid treatment were observed under transmission electron microscopy to determine the extent of any structural damage to the cell integrity, morphological changes and to assess the type of bioaccumulation. Untreated cells were used as controls (Figure 5A) because of the harsh conditions of electron microscopy which also involves the exposure to metals. From the randomly selected micrographs no damage on copper exposure could be observed. The cells exposed to copper were characterised by dark, electron dense cell walls (Figure 5B) in comparison to the controls. Cells exposed to copper and adsorption-desorption cycles were more spherical in appearance and did not have electron

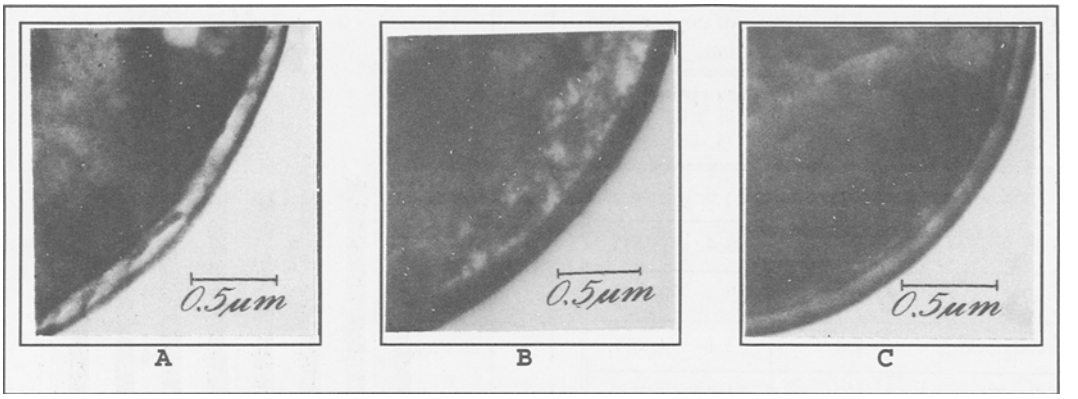

Figure 5 : Transmission electron micrographs of cross-sectional *S. cerevisiae* cells. **A** Control cell. **B.** Cell exposed to copper with an electron dense cell wall. **C.** Cell exposed to copper and HCl desorption.

dense cell walls (Figure 5C). The micrographs suggest a metabolism independent surface binding, which would allow rapid adsorption and recovery of bound metal and thus repeated use of the biosorbent.

CONCLUSION

S. cerevisiae effectively removes copper from aqueous solutions. The process is reversible and bound metal was most successfully recovered using HCl, H_2SO_4 and HNO_3. Removal and recovery efficiencies of the immobilised biosorbent improved with up to 8 repeated adsorption-desorption cycles in batch and column contactors. Electron micrographs supported the mechanism of surface binding and no damage to the cells was detected. The biosorbent showed the potential for reuse in continuous bioremediation systems.

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