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BIOREMEDIATION OF HEAVY METALS IN A SYNTHETIC WASTEWATER USING A ROTATING BIOLOGICAL CONTACTOR

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Abstract—Immobilised microorganisms provide a potential system for the treatment of metal-contaminated waters. This study investigates the efficiency of a rotating biological contactor (RBC) in the treatment of waters contaminated with cadmium, copper and zinc in multiple sorption–desorption cycles. Each sorption cycle extended over a period of 12 weeks at an HRT of 24 h to determine the efficiency of the system over a protracted period of time. The removal pattern observed in the initial cycle, namely Cu ⩾ Zn > Cd, was repeated in both subsequent cycles. After completion of each cycle metals were successfully desorbed by means of an acid wash. The sorption ability of the biofilm was not adversely affected by the desorption process as evidenced by the similar metal removal rates obtained in each of the three sorption cycles. These results suggest that RBCs can be used successfully in the treatment of high-strength metal-contaminated wastewaters. © 2001 Elsevier Science Ltd. All rights reserved

Key words—rotating biological contactor, biofilms, heavy metals, sorption, desorption, cadmium, copper, zinc

INTRODUCTION

The contamination of the environment by heavy metals is of growing concern because of the numerous health risks to animals and humans following exposure. Common sources of metal polluted wastes include electroplating plants, metal finishing operations, as well as many mining, nuclear and electronics industries. All of these contribute to anomalously high concentrations of metals in the environment relative to the normal background levels (Neytzell-De Wildes, 1991).

The effectiveness of commonly employed methods of treating heavy metal-polluted wastes, including, amongst others, precipitation and ion exchange, remains limited (Kratochril *et al*., 1997), particularly for dilute metal wastes (Corder and Reeves, 1994; Kratochril *et al*., 1997). In the last decade an alternative treatment method, viz. biosorption, has been developed (Volesky, 1990; Matheikal *et al*., 1991; Williams *et al*., 1998). Biosorption involves the accumulation of heavy metals by biological material either by metabolically mediated methods or by purely physico-chemical means (Fourest and Roux, 1992). Unlike physical and chemical treatments, biosorption generally does not entail high operational costs and many potential sources of suitable biological material are cheaply and readily available (Hobson and Poole, 1988; Wood, 1992). Furthermore, a common problem associated with conventional treatment, namely the difficulties encountered in treating the solid waste subsequently generated (Environmental Management Division, 1991), does not exist since biological treatment concentrates the wastes into smaller volumes which are subsequently easier to dispose of appropriately (Bhide *et al*., 1996).

The rotating biological contactor (RBC) represents a viable means for the secondary treatment of both municipal and industrial wastewaters (Banerjee, 1997) by exploiting the advantages of both fixed film and suspended growth systems. Although RBCs entail high initial capital costs, their low costs of operation and maintenance as well as their simple process control, increase their economic viability (Borchardt, 1971; Wu and Smith, 1982; Wheatley, 1984). Metals are removed by biosorption onto the microbial biofilm and the metal-loaded biomass may either be periodically removed for controlled disposal or suitably treated to recover sorbed metals such that

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the biofilm may be re-used in multiple cycles (Hutchins *et al.*, 1986).

This study aimed to determine the suitability of RBC biofilms in treating a synthetic wastewater containing cadmium, copper and zinc in multiple sorption–desorption cycles. Previous investigations determined the appropriate disc rotation speed (Costley and Wallis, 1999) and hydraulic retention time (Costley and Wallis, 2000) associated with both maximum biofilm growth and metal removal. This study investigates the rejuvenative capacity of the biofilm, in terms of its reusability, as well as the ability to recover sorbed metals.

MATERIALS AND METHODS

Rotating biological contactor

A laboratory-scale RBC (Fig. 1) was built and operated as described previously (Costley and Wallis, 2000). Polystyrene wedges were attached to the discs to facilitate biofilm sampling. A 40% immersion level was maintained throughout the experiment with outflow pipes ensuring that this level was not exceeded, maintaining a working volume of approximately 10 l. The rotational speed of the discs was maintained at 10 rev min^{-1} since previous experiments had shown that this speed did not disrupt the biofilm and provided enough turbulence to keep the heavy metals in suspension and thus in contact with the immobilized biomass (Costley and Wallis, 1999).

Synthetic effluent

Synthetic effluent was prepared with the following composition: 10% (v/v) nutrient broth (Biolab, Diagnostics Pty Ltd., Midrand, South Africa) (COD $< 2.5\,mg\,l^{-1}$) spiked with the required aliquots of each of the three metal stock solutions to obtain final metal ion concentrations of $100\,mg\,l^{-1}$. Metal salts (Analar grade, BDH Ltd., Dorset, UK) used to make stock solutions ($10{,}000\,mg\,l^{-1}$) were as follows: $CdCl_2{\cdot}2.5H_2O$, 19.5313 g; $CuCl_2{\cdot}2H_2O$, 26.8097 g; and $ZnSO_4{\cdot}7H_2O$, 43.9754 g. The insolubility of $ZnCl_2$ at high concentrations caused $ZnSO_4{\cdot}7H_2O$ to be used.

The composition of the synthetic effluent, in terms of COD concentration and metal ion concentrations, was based on the results from a preliminary study (results not shown) conducted on a number of metal-contaminated industrial effluents from the Pietermaritzburg area. In general, the effluents tested were found to be nutrient limiting and contained a number of metals including cadmium, copper, iron, lead, mercury and zinc at concentrations ranging from < 5 to $> 83\,mg\,l^{-1}$.

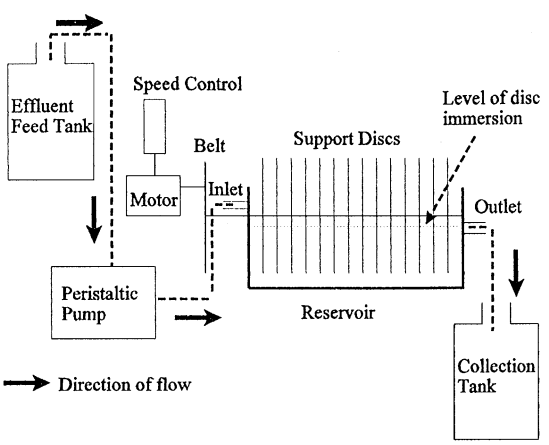

Fig. 1. Diagram of the rotating biological contractor and the associated process control equipment.

Biofilm development

The initial inoculum consisted of activated sludge obtained from the Hammarsdale sewage works, Kwazulu-Natal, South Africa. Enrichments were conducted in half-strength nutrient broth spiked with aliquots of each of the three metal ion stock solutions. A series of enrichments were conducted using increasing metal ion concentrations of 1, 10 and $100\,mg\,l^{-1}$ to obtain an acclimatised population.

The RBC was filled with half-strength nutrient broth inoculated with a second stage enrichment culture to obtain a 25% (v/v) inoculum and biofilm development monitored using scanning electron microscopy (SEM). This concentration of nutrient broth was used to provide both carbon and energy source and to minimise complexation between the metals and the organic constituents in the medium. A series of experiments conducted prior to set up of the RBC confirmed that the nutrient broth did not interfere with the free heavy metal ion concentration (results not shown).

The RBC was operated in fed-batch mode. Each week 2 l of the bulk liquid was withdrawn and replaced with fresh half-strength nutrient broth. After a period of 4 weeks, during which temperatures $< 19°C$ were recorded, biofilm growth remained limited. A heating element was therefore placed in the bioreactor to maintain the medium temperature at 26°C in an attempt to stimulate biofilm growth. No attempt to control temperature had been made prior to this 4 week period as we were attempting to mimic environmental conditions as close as possible and further more it was our desire to design a bioreactor which required minimal control and hence low running costs. The biofilm was allowed to develop for a further 3 weeks in fed-batch mode.

Multiple sorption–desorption cycles

Long term sorption cycles followed by desorption cycles were conducted to assess the loading and desorption efficiency of the biofilms, as well as the rejuvenation capacity of the biofilms. The experimental design was as follows: Sorption cycle one (84 d), Desorption cycle one (48 h); Sorption cycle two (84 d), Desorption cycle two (18 h); Sorption cycle three (84 d), Desorption cycle three (12 h).

Sorption cycle one (SC1). Synthetic effluent was prepared and added to the RBC following development of a mature biofilm (approximately 7 weeks). A calibrated Watson Marlow peristaltic pump was used to regulate the flow rate of fresh effluent into the RBC in a direction parallel to the rotating shaft and perpendicular to the discs. No attempt was made to control the temperature of the effluent. Treated effluent was collected in a receiving tank. Previous experiments (results not shown) determined HRTs < 24 h to be insufficient for effective metal removal, consequently the bioreactor was run for a period of 84 days, equivalent to 84 cycles, at a flow rate of 6.9 ml min^{-1} giving a retention time of 24 h.

Samples (1.5 ml) of both the influent and effluent were taken daily for analysis of actual composition by atomic absorption using a Varian Spectr AA-200 Series Atomic Absorption Spectrophotometer equipped with a Varian SPS-S auto-sampler (Varian Australia Pty Ltd., Mulgrave, Victoria). Because of the analytical accuracy of the method used and also because of the complete mixing ensured by the disc rotation speed employed, only single determinations

were made on each sample. All samples were centrifuged at 12,800 g for 10 min and then diluted with distilled water to within the detection range of the experiment. Minimum and maximum air temperatures in the laboratory and the temperature of the effluent were recorded for each HRT. Daily pH values were also recorded.

Biofilm samples attached to polystyrene sheeting covering the relevant discs were removed weekly and fixed in 3% (v/v) glutaraldehyde, washed twice in 0.05 M cacodylate buffer for 10 min and dehydrated in an alcohol series (10 min each in 30%, 50%, 70%, 80%, 90% and 3×10 min in 100%). The samples were then critical point dried in a Hitachi HCP-2 Critical Point Drier (CPD) and viewed in a Hitachi S-570 SEM after gold–palladium sputter coating.

Desorption cycle one (DC1). After completion of SC1 (84 d), the RBC was drained, washed with distilled water to remove any traces of synthetic effluent and then treated as outlined in Table 1, DC1. After washing with distilled water the bioreactor was filled with a 0.1 M hydrochloric acid (HCl) solution (10 l) and run in batch mode for a period of 3 h. The bioreactor was then drained and exposed to 0.1 M HCl solution (10 l) for 3 h in batch mode, after which a third aliquot of 0.1 M HCl (10 l) was added and the RBC run in batch mode for 18 h. The bioreactor was subsequently drained and exposed to a 0.5 M HCl solution for a period of 12 h and then rinsed with distilled water for 12 h in the operating mode. Samples (in triplicate) were taken after each water or acid wash for AAS analysis. The results from each wash were pooled to obtain the total amount of metal ions desorbed during DC1.

Desorption performance was expressed as a percentage of initial biosorbent loadings and was determined as follows (Atkinson *et al.*, 1998):

$$\text{desorption efficiency} = \frac{\text{quantity desorbed}}{\text{quantity adsorbed}^*} \times 100\%$$

quantity adsorbed* refers to the difference in metal concentrations between the influent and the resulting effluent based on the AAS results obtained during the relevant sorption cycle.

Samples of the biofilm were taken during the desorption procedure and prepared for SEM analysis.

Sorption cycle two (SC2). After completion of DC1, the biomass was exposed to fresh effluent at a hydraulic retention time of 24 h and the experiment repeated as described for SC1 (84 d). From SC1 it was clear that metal removal fluctuated very little after 7 days of commencement of the sorption cycle. Subsequently, samples for AAS analysis were taken on days 1–7 and thereafter on a weekly basis for a period of 12 weeks. Biofilm samples were removed weekly and prepared for SEM analysis.

Table 1. Details of extractants and procedures used in the desorption cycles

Desorption cycle (DC)	Conditions[a]
DC1	0.1 M HCl for 3 h 0.1 M HCl for 3 h 0.1 M HCl for 18 h 0.5 M HCl for 12 h DW[b] for 12 h
DC2	0.5 M HCl for 3 h 0.5 M HCl for 12 h DW for 3 h
DC3	0.5 M HCl for 3 h 0.5 M HCl for 3 h 0.5 M HCl for 3 h DW for 3 h

[a] All washes (acid and distilled water) were carried out in batch-mode using 10 l volumes of extractant.
[b] Distilled water (DW).

Desorption cycle two (DC2). After 12 weeks the RBC was drained, washed and then treated as per Table 1, DC2. Samples (1.5 ml) were collected after each relevant acid or water wash and analysed for metal ion concentrations. Mass balances, calculated on the basis of metal ion concentrations in the desorbent solution and volume of desorbent used, were used to estimate the total quantities of metal desorbed.

Sorption cycle three (SC3). The RBC was run for a third time with fresh effluent at a HRT of 24 h for another period of 84 days (84 cycles). Samples for AAS analysis were taken as per SC2.

Prior to initiation of desorption cycle three, the RBC was stopped and biomass samples attached to the polystyrene liner (equivalent to 25% of the total area colonised) removed from both sides of discs 3, 6, 9 and 12. The biomass was removed from the polystyrene which was shown to shrink on drying resulting in erroneous readings, dried to constant weight at 60°C, the weight recorded and used to estimate the total biomass, in grams, per disc. The biomass was then digested in 10 ml 4 M HCl/HNO_3 on a sand digester and the digests diluted to 250 ml with distilled water prior to AAS analysis. The results obtained were used to estimate the amount of metal associated with the biomass. Results were expressed as milligrams metal per gram biofilm.

A similar analysis was conducted on sludge collected over each of the last four cycles (cycles 81–84) of SC3. The final effluent produced during each cycle was filtered through two, pre-dried Ashless Whatmann filter papers (Number 40). The filters were placed in a furnace at 500°C for 3 h and then digested as for the biomass and analysed for the presence of the three metals under study.

Desorption cycle three (DC3). After 12 weeks operation the RBC was drained, washed and then treated as outlined in Table 1, DC3. Samples for AAS analysis were collected as described for DC2. Biofilm samples (equivalent to 25% of the total area colonised), attached to the polystyrene liner, were removed from discs 3, 6, 9 and 12 on completion of the desorption cycle. The biofilm was then removed from the polystrene, and dried to constant weight at 60°C and the weight was recorded. An approximate weight of the total biomass per disc was then extrapolated from these recorded values.

RESULTS AND DISCUSSION

Multiple sorption–desorption cycles

Sorption cycle one (SC1). The long-term metal biosorption capacity of the biomass employed is important if the system is to become a viable means of treating metal-polluted wastewaters, and compete successfully with traditional physico-chemical methods. Initial studies aimed to investigate the ability of the biofilm to remove metals continuously over an extended period of time (12 weeks). The biofilm clearly showed the metal-binding capacity: copper > zinc > cadmium (Fig. 2). During the first 3 weeks of the experiment removal of cadmium and zinc was relatively low, on average, removal efficiencies of $\approx 15\%$ and $\approx 25\%$ for Cd^{2+} and Zn^{2+}, respectively were recorded. A sizeable increase

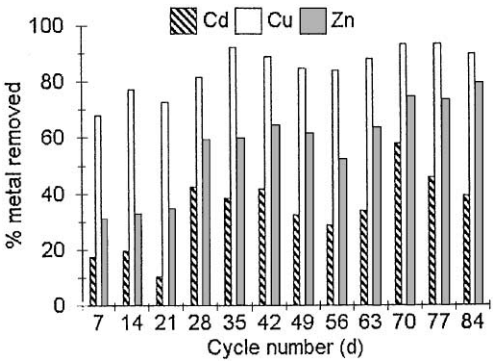

Fig. 2. Percentage metals removed from a synthetic effluent containing Cd^{2+}, Cu^{2+} and Zn^{2+} by the biofilm over 84 days (sorption cycle one).

in the removal of both these metals was noted after 4 weeks contact, at which stage removal efficiencies of $\approx 42\%$ and $\approx 59\%$ were recorded for Cd^{2+} and Zn^{2+}, respectively. The removal of copper remained relatively high throughout the experiment. During the first week of contact an average removal efficiency of $\approx 58\%$ was recorded. Thereafter weekly removal efficiency averaged $\approx 84\%$. Similar high values for copper accumulation have been quoted for organisms which produce sheaths or extracellular materials, which have been shown to protect bacteria from the toxic effects of metals (Bitton and Freihofer, 1978) and also to play a role in metal accumulation (Brown and Lester, 1979). SEM electron micrographs (not shown) revealed the presence of such material which may account for the high copper accumulation evidenced.

Removal of both copper and zinc from the synthetic wastewater proceeded at a relatively constant rate over the last 3 weeks of the experiment, suggesting that the biofilm may have reached an equilibrium as far as uptake of these metals was concerned. However, cadmium removal efficiencies decreased over the last 3 weeks, from $\approx 57\%$ removal recorded during week 10 to $\approx 39\%$ removal recorded during week 12 (Fig. 2), suggesting that the loading capacity of the biofilm with respect to cadmium was declining, suggesting possible saturation of the biofilm. The fact that cadmium has no known metabolic function (Ting *et al.*, 1991) and is known to be toxic at very low concentrations (Trevors *et al.*, 1986) may also have contributed to the low sorption capacity evidenced. Cells are able to withstand the presence of such metals by means of various resistance mechanisms, which may either prevent initial uptake of the ion or provide a means of expelling any absorbed ion from the cell, subsequently resulting in low sorption capacities of the ion in question.

The blue colouration of the biofilm increased in intensity as the amount of accumulated copper increased. This first became noticeable after 7 days contact between biofilm and the effluent, providing visual confirmation of the analytical results and showing the uniformity of metal ion permeation. The colour change may have been due to the formation of one or more copper oxides or hydroxide minerals on/in the biofilm (McLean *et al.*, 1990).

Desorption cycle one (DC1). The ability and ease of recovery of sorbed metals from the biomass is vital if the process is to be viable. Metal ion recovery/desorption was investigated using HCl as the desorbent. The ability of dilute acids to elute metal ions is important in detoxification processes, because it can be used to remove the metal ions from a loaded biomass (Cotoras *et al.*, 1992). Matheikal *et al.* (1991) postulated that the high concentrations of protons made available by the HCl may dislodge bound metals from active sites, by making the bond between the metal and the biosorbent labile. Numerous studies have shown the metal desorption properties of HCl; Bux *et al.* (1995) for example, showed HCl to be an effective desorbent of both Cd^{2+} and Zn^{2+}. It is used in industry since it is relatively cheap. In its dilute form (≈ 0.1 M), it is not only effective for removal of heavy metals but also causes little damage to the biomass, consequently permitting multiple adsorption–desorption cycles to be conducted with the same biofilm material. This substantially improves the economics of biomass technical applications (Tsezos, 1983). However at higher concentrations (>1 M), or with prolonged exposure, there may be a damage to the biomass (Gadd, 1992).

Dilute HCl was clearly effective as a metal-desorbing agent in the present investigation (Fig. 3). Aldor *et al.* (1995) highlighted the need to consider the process concentration ratio (CR), which is the "*ratio of the metal concentration in the eluate to the metal concentration at which the biomass was initially loaded*", when assessing the efficiency of a particular desorbent. Consequently, desorption performance was expressed as a percentage of initial

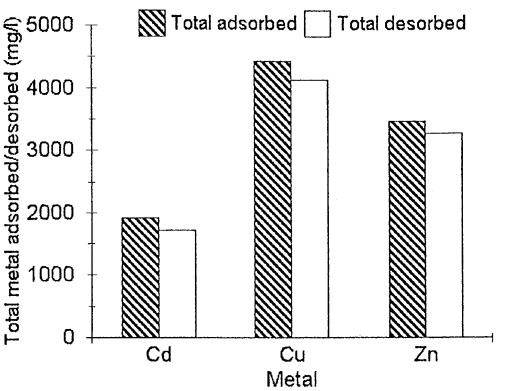

Fig. 3. Concentration of total metals adsorbed by the biomass (sorption cycle one) and subsequently desorbed by 0.1 M HCl (desorption cycle one).

biosorbent loadings, i.e. as a percentage of the total metal adsorbed as determined by AAS. Desorption efficiencies of all the three metals were high: 88.8% of the total Cd^{2+}, 93.3% of the total Cu^{2+} and 94.0% of the total Zn^{2+} adsorbed, was desorbed on contact with the dilute acid. Similar recovery rates were reported by Chang *et al.* (1997) who managed to recover more than 80% Cd^{2+} and 98% Cu^{2+} after elution of the biomass with 0.1 M HCl.

Metal that was not extracted by acid (i.e. unrecoverable metal) might be trapped intracellularly, or alternatively may be associated with any biofilm which detached and was subsequently washed out during the sorption period (Wong *et al.*, 1993; Wehrheim and Wettern, 1994). The high recovery rates of all the three metals suggests accumulation by passive binding to the cell walls and associated extracellular material of the microorganisms comprising the biofilm, and that the ions on the cell surfaces were coupled with ligands, which were easily substituted with H^+ ions (Matheikal *et al.*, 1991; Bux *et al.*, 1995). Electron dispersive X-ray (EDX) analysis (SEM and TEM) confirmed that all the three metal ions were bound to the cell walls and extracellular polysaccharide material, with little or no metal within the cells (results not shown).

When recovering adsorbed metal species through desorption, it is essential to employ the highest solid to liquid ratio possible, so as to produce the highest possible concentration of metal in the desorbent (Tsezos, 1983). The volume of desorbent (50 l) introduced into the bioreactor corresponded to approximately 6% of the total effluent treated (840 l), thereby producing a highly concentrated solution at substantially reduced volumes, which are easier to handle.

After each acid wash the biofilm clearly became more decolourised. After the initial acid wash the biofilm changed from a deep blue to a light green shade. On completion of the desorption process the biofilm was brown and very slimy with no signs of the blue colouration (evidence of copper absorption to the biofilm) visible. The colour change of the biofilm from blue to brown suggested large-scale desorption of surface-bound copper.

There was also a noticeable decrease in biofilm thickness after completion of the desorption procedure suggesting detachment of part of the biofilm. Prior to desorption, biofilms were between 1.5 and 2.5 cm thick; after desorption all that remained was a thin, though entire, coating on the support discs. EM investigations of the ultrastructure of the biofilm prior and subsequent to metal desorption illustrated a noticeable change in the microbial population of the biofilm. Prior to desorption, a well defined population of bacteria, yeast and filamentous organisms were present (Fig. 4(A)); however after desorption close examination of the biofilm revealed the predominance of filamentous organisms, with few bacteria or yeast cells present (Fig. 4(B)).

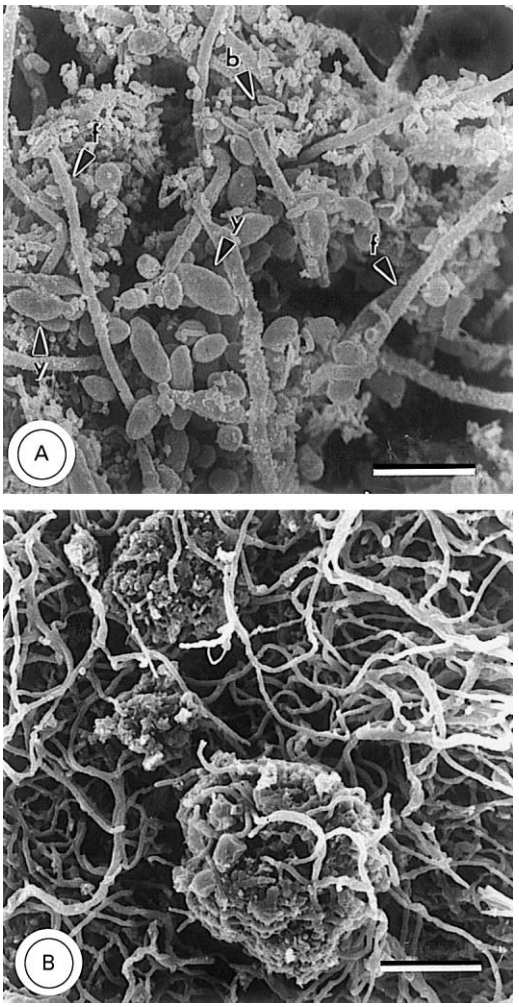

Fig. 4. (A) Biofilm prior to desorption cycle one (DC1). Note the presence of numerous bacteria (b), yeast (y) and filamentous organisms (f). Bar represents 6 μm. (B) Biofilm after DC1. Note the distinct lack of bacterial cells. Bar represents 11 μm.

Sorption cycle two (SC2). For a biological metal removal system to have potential application at an industrial scale, reusability of the biomass is vital (Gadd, 1992; McHale and McHale, 1994). Upon completion of DC1 the regenerative properties of the biomass were investigated. The metal-decontaminated biofilm was re-exposed to fresh effluent for a second 84 day period to monitor the effect of the acid treatment on its metal sorption capacity.

The metal uptake values obtained upon re-exposure of the regenerated biomass to the effluent revealed that the acid treatment had not affected the metal binding capacity of the biomass (Fig. 5), uptake of all the three metals occurring unimpeded after washing with HCl. Within 1 week of re-exposure to the fresh effluent the biofilm had developed the blue colouration, indicative of the presence of copper. Bacteria were quick to recolonise

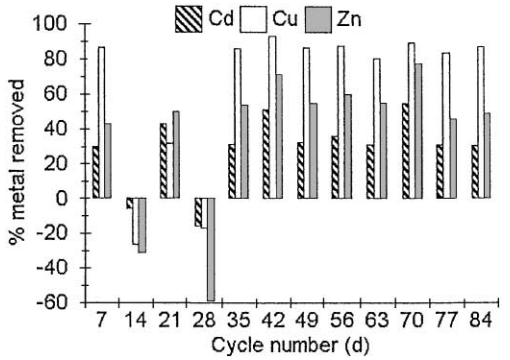

Fig. 5. Percentage metals removed from a synthetic effluent containing Cd^{2+}, Cu^{2+} and Zn^{2+} by the regenerated biofilm over 84 days (sorption cycle two).

the disc surfaces on re-exposure of the acid-washed biofilm to the synthetic effluent. Electron microscopy revealed the presence of numerous bacteria as well as yeast in association with filamentous organisms which were no longer found to be predominant (Fig. 6), suggesting that the acid treatment had not hampered the biological activity of the biofilm in any significant way. This was confirmed by the continued growth of the biofilm, evident in the increase in biofilm thickness (data not shown) over the remaining 11 weeks.

After 12 weeks contact, total average removals from the synthetic effluent of approximately 33.7%, 73.7% and 50.8% for Cd^{2+}, Cu^{2+} and Zn^{2+}, respectively were recorded. These values compared favourably with those recorded during the initial run (34.0% for Cd^{2+}, 84.6% for Cu^{2+} and 57.3% for Zn^{2+}).

During the 12 week contact period there were two occasions (day 14 and 28) when the RBC stopped and discs became stationary. Both interruptions were less than 24 h long and were either due to power failures or due to problems with the motor. It must be noted that the supply of synthetic wastewater to the RBC was not interrupted. On both occasions the exposed biofilm (representing $\approx 60\%$ of the biomass) became dehydrated and increased metal concentrations in the outflow liquid were recorded resulting in apparent negative metal removal (see Fig. 5, days 14 and 28), possibly due to desorption of previously sorbed metal ions. The biofilm was able to recover quickly from such perturbations; within 24 h of reactivation of the RBC removal rates for all the three metals returned within the range recorded prior to the disruption and the hydrated state of the biofilm was re-established. Clearly, the biofilm was able to withstand periodic perturbations without exhibiting any long-term deleterious effects on removal rates. None of these breakdown periods extended beyond 24 h.

Desorption cycle two (DC2). DC1 required 48 h (two cycles) to complete, i.e. the metal adsorption process was interrupted/shutdown for 48 h; clearly unacceptable if an economically viable, continuous system, with potential application on an industrial scale is desired. Hence, in an attempt to decrease the shutdown period, the concentration of the desorption agent, HCl, was increased from 0.1 to 0.5 M. Figure 7 compares the percentage metals desorbed in DC1 and DC2. Desorption efficiency of both Cu^{2+} and Zn^{2+} appeared to have increased slightly when the higher concentration of the desorption agent was used. However closer inspection showed that the desorption efficiencies were not substantially different. For instance, DC1 (0.1 M HCl) resulted in 93.3% recovery of bound copper, whereas DC2 (0.5 M HCl) resulted in 93.7% recovery. Similarly with Zn^{2+}, DC1 resulted in 94.0% recovery of bound zinc, whereas DC2 recovered 96.4% of this metal. The recovery of Cd^{2+} appeared to have diminished in DC2, but again the difference in recovery rates was small (89.8% vs. 83.2%). It must be noted that although recovery percentages did not differ to any great extent, on employment of the stronger desorbing agent, recovery times were 50% shorter with the stronger acid.

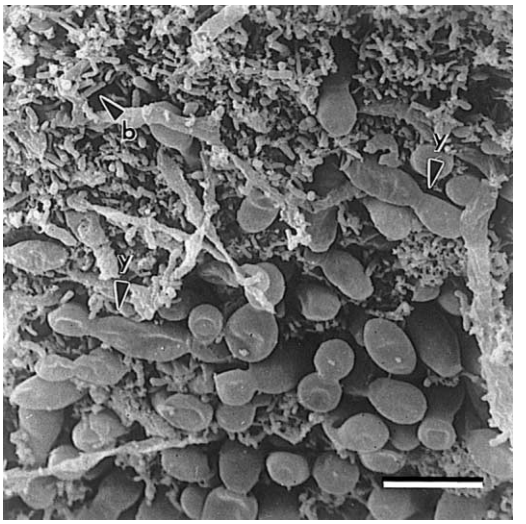

Fig. 6. Biofilm 7 days after initiation of sorption cycle two. Note the presence of bacteria (b) and yeast (y). Bar represents 6 μm.

The recovery of Cd^{2+} for both DC1 and DC2 was greater than 83% and that for both Cu^{2+} and Zn^{2+} was greater than 93%. The biofilm apparently exhibited a greater loading capacity for copper and zinc than for cadmium, providing a possible explanation for the higher removal rates of the former two elements from the biofilm. Conversely, a greater proportion of the total Cd^{2+} removed from the effluent during sorption may have been accumulated intracellularly, as opposed to simple surface binding, and may not have been recovered by the conventional non-destructive desorption procedures employed.

Sorption cycle three (SC3). Average metal ion removal efficiencies of 30.4% for Cd^{2+}, 81.8% for Cu^{2+} and 49.6% for Zn^{2+} were recorded after 12 weeks. Comparison with results recorded after the same period during SC1 and SC2 (Table 2), showed little substantial differences suggesting that the use of 0.5 M HCl had not adversely affected the sorption capacity of the biofilm. Where removal efficiency had been reduced the reduction was small (<6%). Any changes in percentage metal removal may be due to structural changes in the microbial cells of the biofilm known to be induced by HCl (Wong *et al*., 1993). Structural changes in the biofilm would, in turn, affect surface binding of the metal ions by altering the sorption sites available.

Analysis of the biofilm at the end of SC3 showed it to contain, on average, $3.4 \pm 2\%$ of the dry weight as cadmium, $14.4 \pm 6\%$ as copper and $6.4 \pm 2\%$ as zinc. Samples of the sludge revealed comparable results: $\approx 2.6\%$ cadmium, $\approx 11.2\%$ copper and $\approx 6.2\%$ zinc. It must be noted that very little sludge was collected, on average less than 40 mg sludge was collected per cycle suggesting that the contribution of the sludge to the total metal removed was minimal.

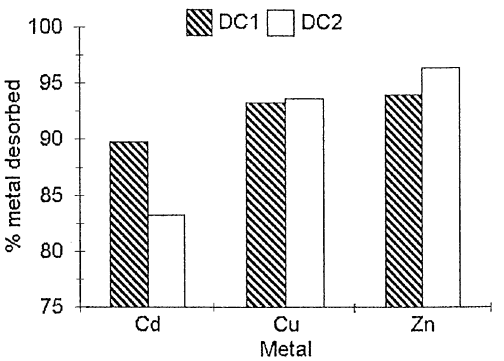

Fig. 7. Comparison of the percentage Cd^{2+}, Cu^{2+} and Zn^{2+} desorbed after desorption cycles one—DC1 (0.1 M HCl) and two—DC2 (0.5 M HCl).

Similar values for cadmium were recorded by Aiking *et al*. (1984) who reported *Klebsiella aerogenes* to contain 2–4% of their dry weight as cadmium and by Campbell and Martin (1990) who found fungal biomass to contain 2–3% of their dry weight as cadmium. Higher values have been reported, for example a *Bacillus* sp. used in the AMT-Bioclaim™ system (Hutchins *et al*., 1986), was found to contain 21.4% of the dry weight as cadmium. Much higher values for copper removal have been quoted. Dunn and Bull (1983) reported values of $30 \pm 0.6\%$ of the biomass dry weight due to accumulated copper. Similar values were quoted for copper accumulation by two *Zoogloea* spp. (Friedman and Dugan, 1968) and for *Sphaerotilus natans* (Sidorowicz *et al*., 1979). A range of values have been recorded for zinc. Hutchins *et al*. (1986) reported a *Bacillus* sp. to contain 13.7% zinc per dry gram biomass whilst Volesky and Holan (1995) quoted values of between 1.4% and 4% for *Saccharomyces cerevisiae*.

Desorption cycle three (DC3). Metal desorption efficiencies similar to those associated with the previous two desorption cycles were recorded, even though the total desorption period was reduced to 12 h. Approximately 87.9% of the total Cd^{2+}, 91.1% of the total Cu^{2+} and 96.4% of the total Zn^{2+} sorbed by the biomass were recovered. Here also the most effective step was the initial acid wash. In all the three cases approximately 50% desorption occurred within the first 3 h of acid contact (Table 3). Almost 100% desorption of copper had occurred by the end of the second acid wash since the amount desorbed after the third acid wash was relatively small when compared to the previous two washes. Water caused little desorption of any of the metals and hence served purely as a rinse step prior to re-exposure of the biofilm to fresh effluent.

The acid washes caused a change in biofilm appearance similar to that observed during both previous desorption cycles. This was most apparent

Table 2. Comparison of average percentage metal removal recorded for each sorption cycle after 12 weeks contact time with a synthetic effluent containing Cd^{2+}, Cu^{2+} and Zn^{2+}

Sorption cycle (SC)	$\%Cd^{2+}$ removed	$\%Cu^{2+}$ removed	$\%Zn^{2+}$ removed
SC1	34.0	84.6	57.3
SC2	33.7	73.7	50.8
SC3	30.1	81.8	49.7

Table 3. Metal desorbed after each 3 h acid and water wash (desorption cycle three)

Desorption step	Amount of Cd^{2+} desorbed (mg)	Amount of Cu^{2+} desorbed (mg)	Amount of Zn^{2+} desorbed (mg)
HCl wash 1	1053.6	2178.8	2202.8
HCl wash 2	495.2	2203.6	902.8
HCl wash 3	363.5	86.2	344.1
Distilled water	2.1	15.4	4.4

during the initial HCl wash, during which more than 50% of the copper was desorbed, resulting in a loss of the characteristic blue colour of the biofilm. The change in biofilm appearance was accompanied by a noticeable reduction in biomass, from an average of 15.7 g dry weight per disc prior to acid exposure to 4.7 g per disc after completion of the desorption cycle. This was due primarily to the detachment of part of the biofilm on exposure to the acid solution as well as the desorption of metals from the biofilm. Electron microscopy revealed a noticeable reduction in the number of bacterial cells in the biofilm following the desorption process, as had occurred in both previous desorption cycles (results not shown).

CONCLUSIONS

A metal ion affinity series was identified during the initial sorption run, namely Cu > Cd > Zn and this was confirmed in the subsequent two runs, both in terms of metal preference and quantity of metal removed. Metals were successfully recovered with an inexpensive and simple reagent; dilute HCl (0.1 M). The metals were released rapidly from the biofilm, suggesting that accumulation was extracellular, and the sorption capacity of the biofilm did not appear to be adversely affected, as indicated by the subsequent high metal removal percentages recorded on its re-exposure to fresh effluent. A higher concentration of HCl (0.5 M) resulted in a faster desorption process, which would be economically favourable, and did not appear to damage the biofilm and its sorption capacity in any way. Short-term disruptions to the system did not appear to affect its long-term performance, the system recovering within one cycle (24 h) following a perturbation.

The high metal sorption capacities recorded, particularly for copper, indicate that the biofilm of a RBC could be used as a tool to remove and recover metals from wastewaters. Its rejuvenation capacity, expressed in its re-use in multiple sorption–desorption cycles without loss of sorption capacity, as well as the high recovery rates of previously sorbed metals, clearly improves the potential of the system for use in industrial-scale metal clean-up operations. Further studies on the feasibility of using this bioreactor for large-scale operation are needed.

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