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Effect of flow rate on heavy metal accumulation by rotating biological contactor (RBC) biofilms

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Immobilized biofilms are effective in heavy metal removal. The current studies investigated the use of rotating biological contactor (RBC) biofilms in treatment of a wastewater containing cadmium, copper and zinc, each at a concentration of 100 mg L^{-1}. In particular, the influence of hydraulic retention time (HRT) on metal accumulation was studied. Longer HRTs (>12 h) were associated with greater metal removal than short HRTs, particularly with regard to cadmium and zinc. The system was also shown to operate successfully over an extended period of time, at an HRT of 24 h, with removal efficiencies of approximately 34%, 85% and 57% for Cd^{2+}, Cu^{2+} and Zn^{2+} respectively after 5–8 weeks contact. *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 244–250.

Keywords: biofilms; rotating biological contactor (RBC); heavy metals; flow rates; biosorption; hydraulic retention time (HRT)

Introduction

Heavy metals are a common cause of pollution. They include several elements essential for growth, reproduction and/or survival of living things, some with no known biological function and many with economic, industrial and/or military uses [13]. Unlike toxic organic compounds, metals are non-degradable and tend to accumulate in the environment [2]. Their discharge into the environment by a number of industries, including mining, nuclear and electronic industries, constitutes one of the major causes of land and water pollution [6,12] and results in high concentrations of the metals relative to normal background levels [22]. Important heavy metal pollutants include cadmium, tin, lead, copper, iron, mercury, nickel, zinc and chromium [1,22].

Conventional methods of treatment, such as chemical precipitation and ion exchange, are becoming increasingly expensive, especially where large volumes of effluent with relatively low metal concentrations are involved [25,26]. Furthermore, several of these methods have also been reported to be industrially impractical due to difficulties encountered in treating the solid waste generated [14].

In recent years there has been an increasing interest in the use of microorganisms, in particular in immobilized systems, to treat heavy metal-polluted wastes [17,19]. They can accumulate trace levels of heavy metal ions, many toxic, from aqueous solutions and play a major role in the modification, activation and detoxification of heavy metals. Although metals cannot be broken down into other products, they may, as a result of biological action, undergo changes in valence and/or conversion into organometallic compounds [16]. Both these processes are considered as detoxification mechanisms since volatilization and removal of the metal may result.

Immobilized systems owe their success, about one order of magnitude greater than suspended systems, to the much higher surface areas and biological mass concentration achievable [20]. They capitalize on the ability of mixed cultures of microorganisms to adhere to inert supports and form biofilms, the physical attachment preventing biomass washout, thereby providing higher loading rates than suspended systems [7,10]. Biofilms employed in wastewater treatment systems appear to be resistant to inhibitory and toxic materials, for example heavy metals [8]. The tolerance of biofilms to high metal concentrations may be due to their ability to precipitate insoluble metal salts outside the cells as sulfides, oxides or hydroxides [3]. The high affinity for metallic cations of the exopolysaccharide components of the glycocalyx has been exploited in certain wastewater treatment plants [4]. The anionic nature of the polymers may inhibit the entrance of cationic molecules into the biofilm by acting as a molecular sieve and an ionic exchange matrix [4].

Immobilized-cell bioreactor technology provides a cost-effective means for eradication of pollutants at their point of origin [21]. This technology is applied in the rotating biological contactor (RBC). This system relies on the development of an active biofilm on rotating surfaces [24]. Metals are removed from solution by biosorption to the biofilm which may be periodically replaced upon saturation. Alternatively, the metals may be desorbed either into a smaller volume and appropriately disposed of, or recovered for reuse, in the case of valuable metals [23].

A long-term laboratory-scale study was set up to investigate the applicability of RBCs for treating heavy metal-polluted wastewaters containing high concentration of Cd^{2+}, Cu^{2+} and Zn^{2+}. An initial study investigated the effect of disc rotational speed on heavy metal accumulation by RBC biofilms (in press). The current study aimed to determine the influence of flow rates and the associated hydraulic

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retention times (HRTs) on metal accumulation by the biofilms.

Materials and methods

Enrichment for metal-acclimatized microorganisms

A three-step enrichment procedure was conducted on an initial inoculum (25%, v/v), consisting of activated sludge obtained from the Hammarsdale sewage works, KwaZulu-Natal, South Africa. The same ratio of inoculum to fresh growth medium was used at each step to obtain a metal-acclimatized microbial population. The Hammarsdale sewage treatment plant serves a highly industrialized region and receives a wide diversity of metal ion-containing effluents including those of the electroplating, textile and several other industries, as well as animal wastes. Sludge from this facility would thus provide an excellent source of microorganisms resilient to a wide range of chemical toxins, including each of the heavy metals under study and hence no other inoculum sources were sought. Media for all enrichments consisted of 10% (v/v) nutrient broth (Biolab Diagnostics Pty Ltd, Midrand, South Africa) spiked with appropriate aliquots from concentrated metal salt solutions to obtain final concentrations of 1, 10 or 100 mg L^{-1} for the first, second and third enrichments respectively.

Metal salt solutions

Metal (10 g L^{-1}) salt solutions used were: $CdCl_2 \cdot 5H_2O$ (19.5315 g), $CuCl_2 \cdot 2H_2O$ (26.8097 g) and $ZnSO_4 \cdot 7H_2O$ (43.9754 g). The sulphate salt of zinc was used because of the insolubility of $ZnCl_2$ at the high concentrations required. All chemicals were Analar grade (BDH Ltd, Dorset, UK.

Synthetic effluent

A synthetic effluent (pH 5.5–6.5) was formulated using 10% (v/v) nutrient broth supplemented with appropriate aliquots of each heavy metal stock solution to obtain final concentrations of 100 mg L^{-1}.

Rotating biological contactor

A 14-disc, single-stage rotating biological contactor was constructed (dimensions as per Table 1). Discs (12.5 cm radius) were made of plastic, to which segments of polystyrene were attached to facilitate biofilm sampling. The discs were mounted on an axle such that approximately 40% of the total disc surface area was submerged. Outflow pipes ensured that this level was not exceeded. The tank, in shape, closely approximated the dimensions of the submerged portion of the discs to prevent short circuiting and to force a thin film of fluid to pass over the disc surfaces [5]. The volume of the tank was 10 L.

Table 1 Dimensions of the rotating biological contactor

Length of trough	41.0 cm
Width of trough	29.0 cm
Depth of trough	14.5 cm
Diameter of discs	25.0 cm
Thickness of discs	0.3 cm
Distance between discs	1.9 cm
Distance between outer edge of discs and wall of trough	2.0 cm

The rotational speed of the discs was maintained at 10 rpm unless otherwise stated. This speed was selected so as not to shear the biomass from the discs and to provide enough turbulence to ensure the heavy metals were kept in contact with the immobilized biomass.

Biofilm development

Synthetic effluent was inoculated with second-stage enrichment cultures to obtain a 25% (v/v) inoculum and final volume of 10 L. This was then fed into the reactor which was operated in fed-batch mode for 4 weeks at ambient laboratory temperatures (19–26°C). Poor biofilm development prompted insertion of a heating element which maintained medium temperature at 26°C. The biofilm was allowed to develop for a further 3 weeks after which the heating element was removed and heavy metal accumulation experiments were initiated.

Examination of biofilms by scanning electron microscopy (SEM)

Samples of the biofilm attached to polystyrene were taken on a weekly basis during biofilm development and during the heavy metal accumulation experiments and prepared for scanning electron microscopy (SEM). Previous experiments (results not shown) determined that disc position had no effect on biofilm development and hence only one disc (disc 7) was sampled in order to preserve the overall integrity of the biofilms in the RBC. The samples were fixed in 3% (v/v) glutaraldehyde, washed twice with 0.05 M cacodylate buffer for 10 min per wash and then dehydrated through a series of ascending concentrations of ethanol in distilled water (30%, 50%, 70%, 80%, 90% and three changes in 100%; 10 min each). The samples were critical point dried in a Hitachi HCP-2 Critical Point Drier (CPD), mounted on metal stubs and sputter coated with gold-palladium prior to examination in a Hitachi S-570 SEM (Hitachi Ltd, Tokyo, Japan).

Comparison of four different hydraulic retention times (HRTs)

After development of a substantial biofilm, as determined by SEM, the reactor was drained, rinsed with deionised water, and re-filled with fresh synthetic effluent. An influent feed tank was attached to a previously calibrated Watson Marlow (Watson Marlow Ltd, Falmouth, Cornwall, UK) peristaltic pump 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. Treated effluent was collected in a receiving tank. Four HRTs (3, 6, 12 and 24 h) were implemented, each for a total of 24 h and hence eight sets of samples were collected when the HRT = 3 h; four sets when the HRT = 6 h; two sets when the HRT = 12 h; and one set when the HRT = 24 h. The respective flow rates were approximately 55.5, 27.8, 13.9 and 6.9 ml min^{-1}.

Samples (1.5 ml) of the influent and the effluent were taken at the end of each cycle at each respective HRT and the amount of each metal adsorbed was determined by atomic absorption spectrometry (AAS) with a Varian Spectr AA-200 Series Atomic Absorption Spectrophotometer equipped with a Varian SPS-S auto-sampler (Varian Australia Pty Ltd, Mulgrave, Victoria).

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 (Varian Australia Pty Ltd, Millgrave, Victoria).

Biofilm sorption capacity

The results of preliminary experiments indicated that an HRT of 24 h was most effective and so this was employed for the remainder of the investigation. The reactor was run for a period of 84 days, equivalent to 84 cycles, at a flow rate of 6.9 ml min^{-1}. Samples (1.5 ml) of both the influent and effluent were removed daily for AAS analysis.

Results

Biofilm structure

Biofilm development prior to addition of the heating element was limited, changing little over a 4-week period. Although some colonization had occurred it was very sparse, the cells occurring in small clumps (Figure 1a). Closer examination of the cell clusters revealed both rod-shaped and coccoid bacteria attached to the polystyrene sheeting by means of extracellular strands (Figure 1b). The prevailing growth medium temperatures (generally $<18°C$) were apparently too low to support vigorous biofilm development.

An increase in medium temperature stimulated biofilm development. Although colonization was still patchy, investigation of the cell clusters indicated the presence of numerous different morphotypes, including yeast-like organisms, and bacterial rods and cocci (Figure 1c). The presence of filamentous organisms in numerous clusters was also noted (Figure 1d) and their abundance increased with time. Closer examination of these clusters showed a mixture of rods and cocci intermingled with the filamentous organisms (Figure 1e).

After 7 weeks the discs had developed a moderately thick biofilm which appeared relatively even (Figure 1f). The polystyrene surfaces were not easily discernible because the surfaces were completely covered with amorphous biological material. After initial development of the confluent biofilm all subsequent cell growth occurred on top of the extracellular products of earlier cells to form a multilayered biofilm. Biofilm development appeared to proceed unhindered after the metal-containing effluent was passed through the system, the biofilm remained intact and visible

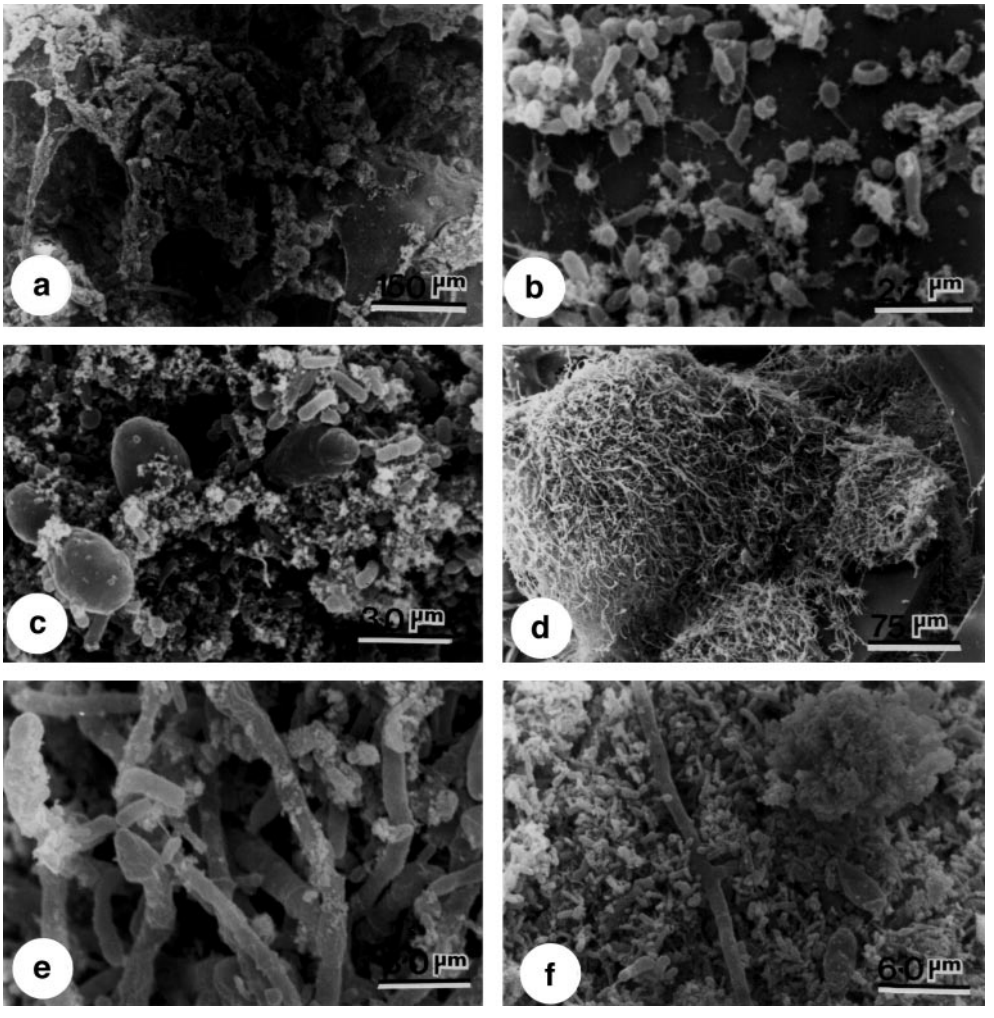

Figure 1 Scanning electron micrographs showing development of the biofilm with time. (a) and (b) Week 4; (c) and (d) week 5 (one week after heating element had been installed); and (e) and (f) week 7.

increases in biofilm thickness were noted (data not shown). No significant detachment occurred during the 84-day experimental period.

Comparison of four different hydraulic retention times (HRTs)

Shorter retention times (<12 h) were clearly ineffective for mixed metal removal (Figure 2). Although some copper removal occurred, levels were generally below 30%, whilst for cadmium and zinc negative removal rates were recorded.

Longer retention times were associated with slightly increased levels of heavy metal removal. At an HRT of 12 h, approximately 2.67% Cd^{2+}, 18.03% Cu^{2+} and 5.11% Zn^{2+} was removed. Although still low, the amounts of all three metals removed, particularly cadmium and zinc, were markedly increased compared with the negative results recorded at the shorter HRTs.

At an HRT of 24 h, removal of all three metals was substantially improved with average values of 11.9%, 39.98% and 11.95% being recorded for Cd^{2+}, Cu^{2+} and Zn^{2+}, respectively. For both Cd^{2+} and Zn^{2+} the removal efficiency doubled, while Cd^{2+} removal showed a five-fold increase.

pH increased with an increase in contact time between the biofilm and the effluent (Table 2). The pH values were notably higher when an HRT of 24 h was used compared to the pH values recorded when an HRT of 3 h was implemented.

The medium temperature did not increase or decrease by more than 1°C during investigations into the effect of HRT on metal accumulation (results not shown). Laboratory minimum and maximum temperatures also did not vary greatly (<2°C).

Sorption capacity of the biofilm

Figure 3 illustrates daily percentage removal of cadmium (Figure 3a), copper (Figure 3b) and zinc (Figure 3c) at an HRT of 24 h over the 12-week experimental period. For both Cd^{2+} and Zn^{2+} an increase of 100–300% in average metal removal efficiency was noted after 4 days contact, at which time removal of these metal ions had increased to 21.7% and 31.1% respectively. After 4 days, Cu^{2+} removal showed an increase of approximately 40%; from 49.6% on day 4 to 68.1% on day 5. These increases corresponded to an increase in pH (Table 2). Up to day 21 metal removal remained relatively stable for Cu^{2+} and Zn^{2+}, but decreased for Cd^{2+}.

A second significant increase in Cd^{2+} and Zn^{2+} removal occurred 4 weeks after initial contact with the biomass. Cadmium removal increased from approximately 15.6% (average daily removal prior to day 28) to >30% (average daily removal after day 28) and zinc removal from approximately 32.8% to >60% over the same time span. Copper removal increased from approximately 72.6% to >84%. The pH increased from 5.81 to 6.19 (Table 3).

Removal of all three metals continued over the following 8 weeks and after 12 weeks the average removal efficiencies recorded for Cd^{2+}, Cu^{2+} and Zn^{2+} were 34.0%, 84.6% and 57.3% respectively. Removal of both copper and zinc was relatively stable over the last 3 weeks of the experiment, whereas that of cadmium was persistently erratic. Over the last 8 weeks of the experimental run, the pH did not fluctuate more than 0.5 unit.

Medium temperatures remained between 21–23°C and laboratory temperatures did not vary by more than 4°C (results not shown).

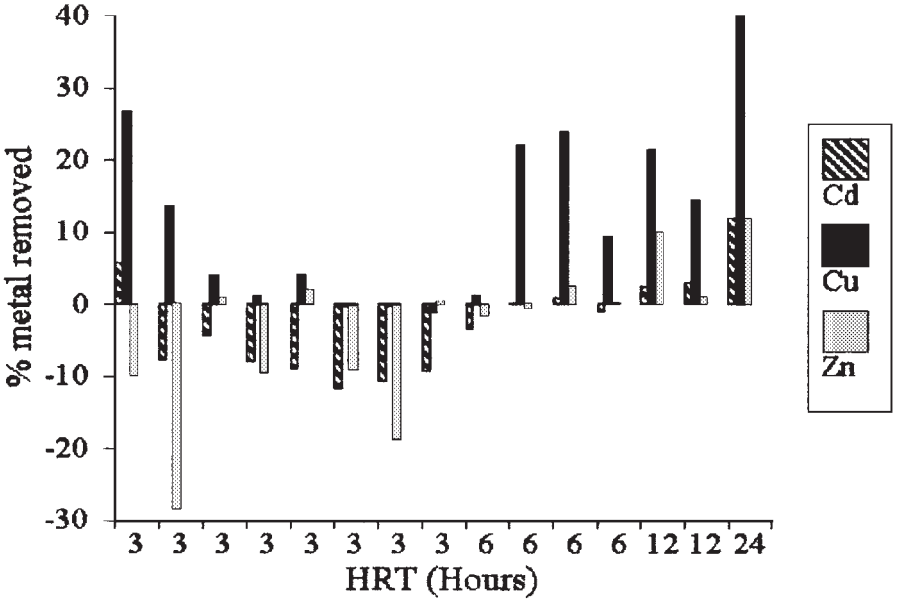

Figure 2 Percentage of metals removed at four different hydraulic retention times (HRTs). Each HRT was implemented for a total of 24 h. Hence eight cycles were run when the HRT = 3 h; four when the HRT = 6 h; two when the HRT = 12 h; and one when the HRT = 24 h. All cycles were completed using the same biofilm.

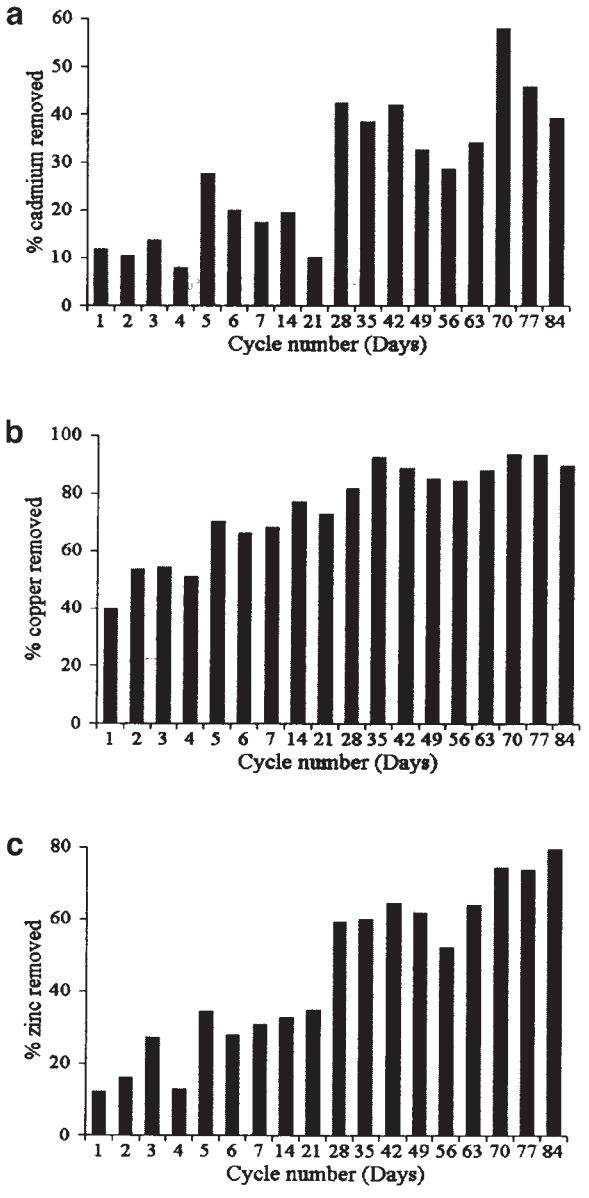

Figure 3 Percentage of metals removed over 84 days from a synthetic effluent containing Cd^{2+}, Cu^{2+} and Zn^{2+} (all at an initial concentration of 100 mg L^{-1}) by the biofilm. (a) Cadmium; (b) copper; and (c) zinc.

Discussion

Recent reports have highlighted the fact that although biosorption is a rapid process, insufficient residence times markedly decrease metal removal levels [31]. The contact time between metal and biomass is very important in determining metal removal efficiencies [30]. Too short an HRT will result in low removal rates, whereas too long an HRT will not be economically feasible. In order for a biological system to compete successfully with conventional physicochemical methods of treatment, the shortest possible hydraulic retention time associated with the most efficient removal rates is required. Consequently, four different HRTs, 3 h, 6 h, 12 h and 24 h, were tested, over a period of 24 h, in an attempt to optimise metal removal in a 10-L capacity RBC operating at 10 rpm. No attempt was made to control the pH or temperature as one of the primary aims of the entire investigation was to develop a system which could work with minimal control.

Table 2 pH values recorded during investigations into the effect of HRT on heavy metal accumulation

Cycle[a]	pH at HRT (h)			
	3	6	12	24
1	4.13	4.14	4.42	5.04
2	4.28	4.29	4.47	
3	4.35	4.36		
4	4.40	4.41		
5	4.49			
6	4.52			
7	4.59			
8	4.63			

[a]One cycle is the completion of the respective HRT; ie cycle 1, HRT = 3 h is completion of a 3-h cycle and cycle 2, HRT = 3 h is the completion of the second 3-h cycle.

Table 3 pH values recorded during the 84-day cycle employing an HRT of 24 h

Cycle number (days)	pH
1	5.04
7	6.37
14	5.71
21	5.81
28	6.19
35	6.50
42	6.67
49	6.91
56	6.89
63	6.81
70	6.93
77	6.81
84	6.80

The experimental results indicated that highest metal removal levels occurred when a 24-h HRT was used. A possible explanation for the negative removal capacities recorded for both cadmium and zinc at retention times <12 h may be desorption and resolubilisation of some of the metal ions sorbed during the adaptation period. This may be due to increased competition for available adsorption sites from the other metal ion species present [11]. The presence of either copper or zinc or a mixture of these metals affects the binding of cadmium to biomass [16]. Since both copper and zinc were present, it could be that these metal ions out-competed cadmium ions for available adsorption sites, resulting in low removal of the latter. Furthermore, a low external pH reduces both surface binding and intracellular influx [29] due to the presence of hydrogen ions which compete successfully with other cations for binding sites and hence occupy many potential metal binding sites, resulting in poor metal biosorption results [15]. Uptake, by a biomass, of both cadmium [9] and zinc [23] can be affected adversely by a low pH. The pH during the current investigation (HRTs <12 h) remained below 4.6

and hence may have contributed to the initial negative removal rates obtained.

Longer retention times (>12 h) resulted in more effective metal removal than did high flow rates with their associated short HRTs. The increase in pH (from <4.5 to >5.5) with this regime may have been conducive to the higher removal rates of all three metals since optimal removal of cadmium [25], copper [23] and zinc [18] occurs at pH values >5.

The biofilm clearly showed the metal-binding preference: copper > zinc > cadmium. Copper removal was approximately twice that for zinc and almost three times that of cadmium. The relatively high removal levels recorded for copper and zinc could be explained on the basis that both these metals have well known metabolic functions, and hence were absorbed with greater efficiency than was cadmium. The latter, with no known metabolic functions [27], could adversely affect a series of cellular functions [28], thereby showing a more pronounced toxic action. Consequently cells may exhibit resistance mechanisms to enable them to withstand high concentrations of such metals, and hence exhibit low sorption capacities. The resistance mechanism employed may either prevent initial uptake of an ion, or alternatively may provide a means of expelling the ion from the cell should it be absorbed intracellularly.

Removal of both copper and zinc was relatively stable 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 levels were still fluctuating over the last 3 weeks, suggesting either: weak binding of cadmium to the biofilm; the maximum sorption capacity of the biofilm for cadmium had not yet been reached; sorption sites were saturated due to binding of the competing ions Cu^{2+} or Zn^{2+}; or alternatively, repeated sorption/desorption of Cd^{2+} ions was occurring.

Neither laboratory nor effluent temperatures appeared to influence metal accumulation by the biofilm. However low effluent temperatures did adversely affect establishment of the biofilm, particularly in its early stages, and this explained the slow colonization of the polystyrene observed prior to heating of the effluent.

The long-term biosorption capacity of the biomass employed is important if the system is to become a viable means of treatment and compete successfully with physicochemical methods. The potential of an RBC for treating heavy metal-polluted waters was confirmed by the continued removal of cadmium, copper and zinc over the 12-week experimental period. To be commercially viable, the immobilized biomass should also possess the capacity for re-use in multiple adsorption-desorption cycles. Investigations in this regard are currently underway in our laboratory and initial results have illustrated that firstly, more than 88% of the total Cd^{2+} accumulated, 93% of the total Cu^{2+} accumulated and 94% of the total Zn^{2+} accumulated can be recovered by means of inexpensive eluting agents (including 0.1 M HCl) and secondly, the intact biofilms from which the sorbed metal ions have been desorbed can be re-used in subsequent sorption cycles without any apparent reduction in the sorption capacity of the biofilm. Preliminary SEM-linked electron dispersive X-ray analysis (EDX) investigations show that the metals are predominantly associated with the surface of the cells and with an extracellular polysaccharide material present while TEM investigations have indicated the presence of some metal within the cells (paper submitted for publication).

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