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

SC Costley and FM Wallis

School of Applied Environmental Sciences (Microbiology and Plant Pathology), University of Natal, Pietermaritzburg, South Africa

Immobile 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 (\(\geq 12\) 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.

Immobile 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].

Immobile-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 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 (\(\geq 12\) 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)
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 Hammersdale 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 Hammersdale 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\(_2\)·5H\(_2\)O (19.5315 g), CuCl\(_2\)·2H\(_2\)O (26.8097 g) and ZnSO\(_4\)·7H\(_2\)O (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 poly styrene 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of trough</td>
<td>41.0 cm</td>
</tr>
<tr>
<td>Width of trough</td>
<td>29.0 cm</td>
</tr>
<tr>
<td>Depth of trough</td>
<td>14.5 cm</td>
</tr>
<tr>
<td>Diameter of discs</td>
<td>25.0 cm</td>
</tr>
<tr>
<td>Thickness of discs</td>
<td>0.3 cm</td>
</tr>
<tr>
<td>Distance between discs</td>
<td>1.9 cm</td>
</tr>
<tr>
<td>Distance between outer edge of discs and wall of trough</td>
<td>2.0 cm</td>
</tr>
</tbody>
</table>

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).