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A mathematical process model for cadmium precipitation by sulfate-reducing bacterial biofilms

Christopher White[1], John S. Dennis[2] & Geoffrey M. Gadd[1]
[1]*Division of Environmental and Applied Biology, Biological Sciences Institute, School of Life Sciences, University of Dundee, Dundee DD1 4HN, UK (*author for correspondence: e-mail: g.m.gadd@dundee.ac.uk)*
[2] *Department of Chemical Engineering, University of Cambridge, New Museums Site, Pembroke Street, Cambridge, CB2 3RA, UK*

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This paper is dedicated to the memory of Dr Chris White who died suddenly, shortly after completion of this article.

Key words: biofilms, cadmium, metal accumulation, process model, sulfate-reducing bacteria

Abstract

Sulfate-reducing bacterial (SRB) biofilms were grown in a flowcell in which the biofilm was grown on a fixed area of support which was supplied with recirculating medium of defined composition, volume and circulation rate. Utilization rates for substrates, production rates for products and material mass-balances for substrates and Cd were determined and a mathematical model constructed based on theoretical considerations and experimental data. The rate of sulfate reduction was zero-order with respect to sulfate concentration and unaffected by the presence of 250 μM Cd. However, Cd reacted with the sulfide produced by the SRB to produce solid CdS, removing sulfide from solution. A significant fraction of colloidal CdS was formed which flocculated relatively slowly, limiting the overall rate of Cd bioprecipitation. Experiments using chemically-synthesised colloidal CdS indicated that the biofilm did not influence colloidal Cd flocculation but stimulated sedimentation of the CdS precipitate once flocculated. A mathematical model of bioprecipitation was developed in which the CdS formation rate was determined by two steps: sulfide production by the biofilm and colloidal CdS flocculation. This model accurately predicted the behaviour of further experimental runs which indicated the adequacy of the overall process description. The model also indicated that the rate of sulfate reduction and the rate of flocculation were the key variables in optimising the biofilm system for metal removal.

Introduction

Sulfate-reducing bacteria (SRB) have been used to treat toxic metal pollution in a number of bioreactor configurations including stirred-tank (White & Gadd 1997, 1998) or fixed-bed reactors (Hammack & Edenborn 1992) with some operating on a commercial scale (Barnes et al. 1991, 1994). However, a potential stumbling block confronting further development is the comparatively long residence time and large working volume of such bioreactors. One potential route to reducing both the residence time and working volume is the development of optimised biofilm reactors (White & Gadd 1998, 2000; Smith & Gadd 2000). However, this requires a greater understanding of the processes occurring in biofilm growth and interactions with toxic metals than is presently available. SRB biofilms may interact with toxic metals in solution in a number of ways. Metal sulfide precipitation occurs in both biofilm and free-living SRB cultures (White & Gadd 1996a, b, 1997, 1998, 2000; White et al. 1998) and other significant interactions with biofilms include metal hydroxide precipitation due to raised pH (Hartley et al. 1996) and biosorption by cell surfaces (Gadd 1992a, b, 2000, 2001; Gadd & White 1993). Extracellular polymeric substances (EPS) and other biomolecules produced by free-living or biofilm SRB may bind both soluble toxic metals (Beech & Cheung 1995; Videla 1994; Bridge et al. 1999) and particulates (Flemming 1995; Vie-

ira & Melo 1995). In a previous study, SRB biofilms grown with 200 μM Cd in a stirred-tank bioreactor accumulated CdS and their protein and polysaccharide content simultaneously increased indicating that sulfide precipitation and entrapment by extracellular polymers occurred (White & Gadd 1998). However, the stirred-tank bioreactor system only permitted study of a limited range of variables. The present study was aimed at quantitative determination of the interactions between sulfate-reducing bacterial biofilms, metabolisable substrates and a toxic metal (Cd) in solution. It made use of a purpose-designed flowcell constructed to contain a defined area of biofilm with a predetermined volume of medium and flow velocity. A mathematical process model was developed in parallel with experimental work which enhanced the quantitative conclusions of the study and enabled prediction of the key variables for further optimisation.

Materials and methods

Organisms and culture maintenance

The mixed SRB biofilm culture was isolated in our laboratory (White & Gadd 1998; White et al. 1998) and maintained in batch culture in screw-top tubes of SL10 medium (Widdel & Pfennig 1982). Biofilm growth was maintained by sequentially subculturing growth attached to 5x20 mm glass slips (White & Gadd 1998). To provide larger amounts of inoculum culture, a sterile 60 $\times$ 10 mm polystyrene coupon ("Plastikard", Slaters Ltd, Matlock, UK) was placed in a continuous culture system comprising a 10 ml syringe body closed with a rubber stopper. Medium was fed into this via the Luer fitting and exited via a syringe needle piercing the stopper. It was inoculated by adding 2 glass slips from batch culture and incubating without medium flow for 48 h after which continuous flow was started at 2 ml h^{-1} (D = 0.2 h^{-1}). This flow rate maintained biofilm growth but washed out planktonic growth. SL10 medium, pH 6.5 and 20 °C, was supplied from a 1 l reservoir which was continuously deaerated with sterile N_2 (White & Gadd 1998).

Biocell culture

Experimental biofilms were cultured in a purpose-designed flow cell (Figure 1). This comprised four main components; a top-plate, base plate, spacer and the support on which the biofilm itself was grown. The top and base plates both consisted of 10 mm thick polycarbonate sheets 125 mm wide and either 200 or 600 mm long. The spacer plate comprised a 3 mm thick polycarbonate sheet, otherwise of the same dimensions as the top and base plates. This plate had a slot 30 mm wide and either 100 or 500 mm long respectively at its centre which acted as a liquid channel in the assembled flowcell. The top-plate was pierced by 6 mm stainless steel inlet and outlet pipes for medium supply and egress. The biofilm support was held between the base plate and spacer and comprised a 300 μm thick "Plastikard" sheet. The assembly was sealed by butyl rubber O-rings and held together by stainless steel bolts. The flowcell was inoculated by aseptically removing two coupons from 21 day continuous syringe-body cultures and placing each in a pre-sterilized 30 ml plastic universal bottle containing 25 ml of SL 10 medium. Approximately 0.5 ml of 0.5 mm diameter sterile glass beads (Merck UK, Lutterworth) were added and the head-space was filled with sterile N_2 and sealed. The bottle was then vortexed at moderate speed (approximately 200 rpm) for 60 sec to suspend the biofilm. Two 25 ml aliquots of suspended biofilm were aseptically added to the sterile, anaerobic, reduced SL10 medium in a reservoir bottle which comprised a 1 l "Duran" screw-cap glass bottle (Merck UK, Lutterworth) equipped with inlets and outlets for sparging N_2 and recirculating medium and a sterile 25 mm magnetic stirrer follower. The inoculum suspension was incubated for 12 h at 20 °C with stirring (500 rpm) and sparging with sterile N_2 but without recirculation through the biocell. After this, the suspension was recirculated through the biocell for 24 h to allow colonisation. The inoculated reservoir was then replaced with one containing fresh medium which was recirculated to allow batch biofilm growth for 48 h after which it was replaced with a stirred, N_2-sparged continuous-flow reservoir of 200 ml working volume containing SL10 medium which was supplied continuously at a dilution rate of 0.2 (40 ml) h^{-1}. This dilution rate washed out suspended SRB culture minimising suspended growth. Continuous culture was maintained for 14 days to pre-grow mature biofilm. For experimental purposes, 14 day-old biofilm was transferred to 200 mm biocells. The 600 mm flowcell was disassembled and the portion of "Plastikard" membrane carrying biofilm was cut into 140 mm lengths. These were placed in individual 200 mm flowcells which were assembled and filled with anaerobic medium by passing 50 ml of sterile, anaerobic, reduced SL10 medium through the cell. 1 l of sterile, anaerobic, pre-reduced SL10 medium was

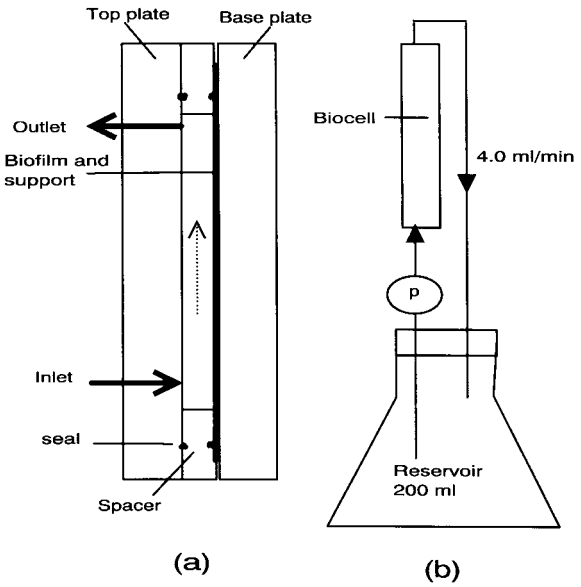

Figure 1. Diagram showing (a) the construction of the flowcell and (b) the medium recirculation through the flowcell. Arrows indicate the direction of liquid flow, P = pump.

then recirculated through the cell for 24 h. Previous trials had indicated that this transfer procedure did not adversely affect biofilm activity and allowed the experimental material to be confined to one surface of defined area (the "Plastikard" sheet) and allowed a greater standardisation of biofilm material than was achieved by growing cultures separately in 200 mm flowcells.

Recirculated batch experiments

The cell was flushed to remove bulk-phase sulfide by passing through 50 ml of SL10 medium which had been deaerated but from which Na_2S was omitted. This ensured that all of the sulfide present in the system originated from sulfate reduction during the experiment. The recirculating medium was then replaced by 200 ml of deaerated SL10 medium, pH 6.0 at 20 °C, without Na_2S, contained in a 250 ml conical flask. Control runs contained no additional metal while experimental runs contained 250 μM $CdCl_2$. Recirculation was then started at 4.0 ml min^{-1}. The medium flask was sparged with N_2 (3-5 ml min^{-1}) to maintain anaerobic conditions. 1.0 ml samples of liquor were removed at intervals and analysed for Cd, sulfate and organic acids. Further 0.5 ml samples were removed by syringe and immediately analysed for dissolved sulfide. Samples of the head-space gases were removed by syringe and also analysed immediately. At the end of the experiment, the flowcell was disassembled and the loose precipitated material gently washed off with 50 ml of distilled water, separated from the wash water by centrifugation (1200 g, 5 min) and stored at −20 °C. Biofilm samples of known area (1 cm^2) were also harvested at the end of each run.

Colloidal CdS formation

To assay the formation of colloidal CdS, 1.0 ml samples were removed at suitable intervals and fractionated as follows. The precipitated solid CdS was separated by centrifugation (1200 g, 2 min) and the supernatant decanted. 100 μl of 5.0 M NaCl was added to the supernatant to raise the ionic strength and enhance flocculation of colloidal CdS, incubated for 5 min and then separated by further centrifugation at higher speed (7000 g, 2 min). The supernatant was decanted and contained any remaining dissolved Cd salts. In order to determine the role of the biofilm in filtering or flocculating colloidal Cd, a mixed colloidal and precipitated CdS dispersion was prepared by adding 500 μmol l^{-1} $CdCl_2$ to a 10 mM Na_2S solution in 200 ml volumes of rapidly stirred SL10 medium in the biocell circuit. The sodium sulfate in this medium was replaced by an equimolar concentration of NaCl so that sulfate reduction was not supported but a high ionic strength was maintained. The artificial colloid suspension was then recirculated through biocells containing biofilms or "Plastikard" with no biofilm, sampled at intervals and analysed to determine the fate of the Cd.

Analytical methods

Cd was assayed by atomic absorption spectrophotometry (AAS) following digestion of solid fractions in 6 M HNO_3 at 90 °C for 1 h (White & Gadd 1995, 1998). Protein was extracted by vortexing the coupon in 1.0 or 2.0 ml of 0.5 M NaOH with the addition of approximately 0.5 cm^3 of 0.5 mm diameter glass beads to resuspend the biofilm followed by incubating the biofilm suspension for 30 min to extract protein which was assayed by the Bradford method (White & Gadd 1998). Carbohydrate was assayed using the anthrone method (Herbert et al. 1971) after vortexing in distilled water with glass beads to suspend the biofilm material. Sulfide was assayed polarographically (White & Gadd 1996b). Head space gases were analysed by gas chromatography (GC) using a Varian 90-P GC with a Porapak Q 80/100 1320 mm column at 25 °C.

Results

Substrate utilisation, sulfate reduction and sulfide production

Lactate utilisation was continuous and all of the lactate supplied (4.0 mmol) was used during the course of the experiment. There was no difference between the rate of lactate utilisation in the presence or absence of Cd. Acetate was not utilised by the SRB biofilm cultures to any significant extent and the build-up of acetate in the liquor approximately matched the amount of lactate used (Figure 2a). Sulfate utilisation apparently proceeded at a constant rate over the experimental period and was also unaffected by the presence of Cd (Figure 2a). As a result, the stoichiometry of sulfate and lactate utilisation did not differ significantly between cultures with and without Cd, being (mol lactate:mol sulfate) 1.89 ± 0.29:1 and 1.73 ± 0.20:1 respectively (determined by linear regression, which was significant at probabilities of 0.0004 and 0.00006 respectively). This was only marginally lower than the theoretical value of 2:1 for lactate oxidation to acetate (Postgate 1984) and was further evidence of the minor contribution of acetate-utilising organisms to sulfate reduction by the biofilm. Methanogenesis by this mixed culture was also negligible as methane was not detected in the head-space gases. While the presence of Cd in the medium did not affect the rate of sulfate reduction, it resulted in a lower concentration of dissolved sulfide in the circulating medium. The initial sulfide concentration following flushing with sulfide-free medium was close to zero in both the presence and absence of Cd (Figure 2b). In the absence of Cd, the sulfide concentration rose rapidly reaching a final concentration of 5.0 ± 0.7 mM. With Cd present, the final sulfide concentration was similar (4.6 ± 0.5 mM) but was delayed due to an initial period of 45–60 min when it remained close to zero. The subsequent rates of sulfide production appeared to be comparable in the presence and absence of Cd (Figure 2a, b). The difference in sulfide concentration between the Cd-containing and control cultures at the end of this lag period was approximately the same as the initial Cd concentration which is consistent with the "missing" sulfide having reacted stoichiometrically with the Cd to form CdS.

pH and Eh

The pH of the medium increased progressively over the course of the experiment (Figure 3) as a result of sulfate reduction (Postgate 1984; White & Gadd 1996b). However, the pH remained too low for significant precipitation of $Cd(OH)_2$ to occur (Figure 3) (Svehla 1996). The medium E_h also became more negative as sulfate reduction progressed and sulfate was converted to sulfide (Figure 3).

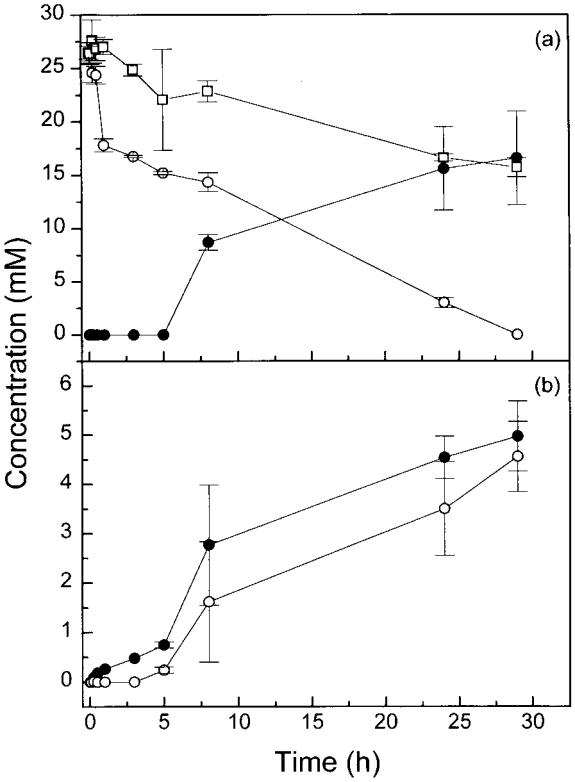

Figure 2. Changes in concentration of (a) (○) lactate, (●) acetate and (□) sulfate and (b) net sulfide production in flowcell circuits with (○) 250 μM Cd or (●) no Cd (control) during batch incubation in the flowcell. 200 ml of medium was recirculated at 4.0 ml min^{-1}. All points are the mean of 4 replicates and the bars indicate the standard error of the mean (SEM).

Cd removal

Cd was removed from the liquid phase (comprised of dissolved and colloidal Cd compounds) in two phases, a rapid phase lasting approximately 40 min in which approximately 98% was removed and a subsequent slower phase, lasting a further 120 min in which the remainder was removed (Figure 4a). It became clear that this was not a simple process. A visible yellow coloration, typical of CdS (Svehla 1996), appeared in the liquor within 1–2 min after starting recirculation of the Cd-amended medium. It was found that some yellow coloration persisted after centrifugation (2 min, 1200 g) of samples taken during Cd precipitation. Further

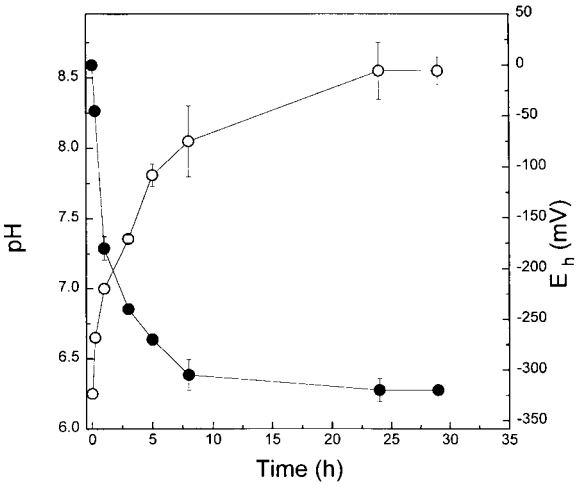

Figure 3. Changes in (○) pH and (●) E_h during the course of batch incubation of the flowcell cultures. 200 ml of medium was recirculated at 4.0 ml min^{-1} . There was no added sulfide in this medium. Each point is the mean of 4 experimental determinations and the bars indicate the SEM.

Cd was precipitated from these samples following the thawing of frozen stored supernatants. This suggested that some of the CdS was present in a colloidal form. Dark-field microscopy supported this by showing the presence of light-scattering particles. Experimental samples of recirculating Cd-containing medium were fractionated into soluble, colloidal and precipitated Cd using the centrifugation procedure described. Soluble Cd disappeared over a period of approximately 30–40 min incubation in the biofilm cell. Most or all of the CdS was initially formed as colloidal particles but over a period of approximately 60–70 min this colloidal material was almost completely flocculated (Figure 4b). At the end of the experiment (29 h incubation) negligible amounts of Cd (0.3 μmol total) remained in solution or as colloid and most of the Cd was precipitated. The precipitated CdS was in two parts; a smaller fraction (4.4 μmol) was within the biofilm itself and a larger fraction (42.6 μmol) was present as precipitated material in the biocell or in circulation. Protein and carbohydrate were also present in the precipitated material, which suggested that it was at least in part derived from sloughed, biofilm-associated CdS. The total Cd recovered in all of the fractions was 47.3 μmol or 94.6% of the 50 μmol initially present in 200 ml of medium. To investigate the role of the biofilm in colloid flocculation and precipitate removal, a mixed dispersion of colloidal and precipitated CdS was prepared by adding 500 μmol l^{-1} of Cd to an excess (10 mM) of sodium sulfide (Svehla 1996).

Table 1. Cd distribution in biocell following recirculation of Cd-containing medium

Fraction	Cd (μmol)	
	+ Biofilm	Control (no biofilm)
Dissolved $CdCl_2$	0.05	0.04
Suspended colloidal CdS	6.62	11.40
Suspended CdS precipitate	22.60	47.80
Settled CdS precipitate	55.83	49.82
Coupon/biofilm Cd content	10.19	0.32
Total	95.27	108.54

The total Cd added was 100 μmol (200 ml at 500 μM concentration) in a recirculating volume of 200 ml and the total contact time was 29 h. The control runs contained a "Plastikard" support but no biofilm. All values are the mean of 4 determinations and the errors were less than 5% throughout.

This suspension was circulated through biocell circuits containing either blank "Plastikard" or "Plastikard" supporting inactive biofilms. Flocculation of colloidal Cd was not affected by the presence of the biofilm, as seen in the comparison of Figures 5a and 5b. To extend this result, Figure 5c shows the rate of removal of colloidal material when a growing biofilm is present and is generating sulfide in order to precipitate the cadmium which had a starting concentration equal to that in Figures 5a and 5b. However, the sedimentation of flocculated CdS precipitates from suspension was enhanced by the presence of a biofilm (Figure 5a, b). The final mass-balance showed clearly that the biofilm trapped significant amounts of Cd and that the presence of the biofilm also led to a greater accumulation of both biofilm-bound and unattached sedimented CdS in the biocell (Table 1). Much of the precipitated CdS was sedimented in the flow cell rather than other sections of the system as it was unstirred and its larger cross-sectional area compared to the tubing carrying recirculating material resulted in relaxed flow conditions and enhanced the sedimentation of suspended solids. Finally, Figures 6a and b show the rate of generation of sulfide by biofilms of thicknesses 200 and 550 μm, respectively. It is noteworthy that the presence of cadmium, at the concentrations used (250–500 μM), did not appear to inhibit growth.

Theoretical analysis of sulfate utilization and sulfide production

It was assumed that the biofilm could be approximated as a uniform slab of material and the standard expressions governing diffusion and reaction in such a geometry were utilised, as described in Appendix

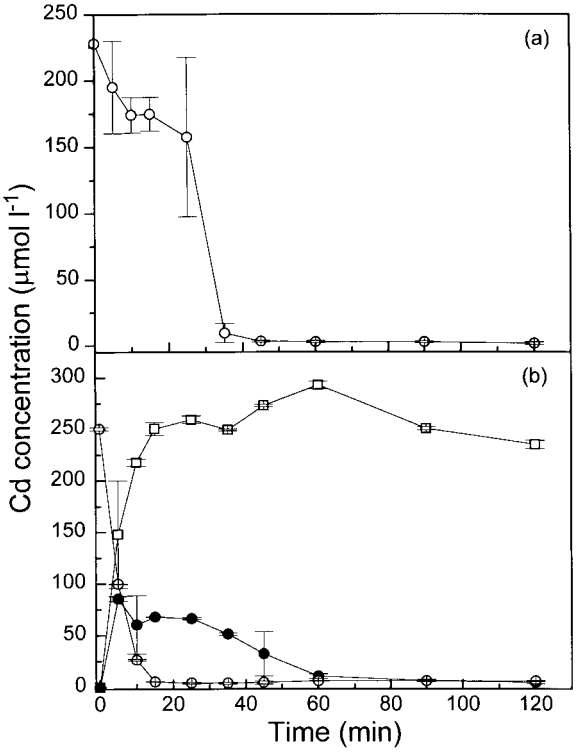

Figure 4. (a) Cd removal by an SRB biofilm, initial Cd concentration 250 μM. (b) Speciation of Cd during removal from solution by SRB biofilms, showing (○) soluble Cd^{2+} (●) colloidal CdS and (□) suspended CdS precipitate to show their interconversion over time. 200 ml of medium was recirculated at 4.0 ml min^{-1} . There was no added sulfide in this medium. Each point is the mean of 3 determinations and the bars indicate the SEM.

1. Figure 6a shows how the sulfate concentration decreased with time in a batch biofilm experiment in the absence of Cd. The experiment was conducted on a 14 day old biofilm which, from experimental determinations involving sectioning and staining, was shown to have an active thickness of about 200 μm (data not shown). By inspection of Figure 6a, the following conclusions were drawn:

(a) Over the range of sulfate concentrations between 5 mM and 0.5 mM the observed sulfate utilisation, and hence production of sulfide, was zero order, as seen by the linear decrease with time.

(b) In the experimental system shown in Figure 6a, the total volume of liquid was 200 cm^3, and the area of biofilm was 30 cm^2. Accordingly, the rate of sulfide production *per unit area of biofilm*, assuming well-stirred conditions, R, is 0.91 μmol cm^{-2} h^{-1}. Let it be assumed that there is complete penetration of the biofilm. For zero-order kinetics with complete penetration of the biofilm, calcu-

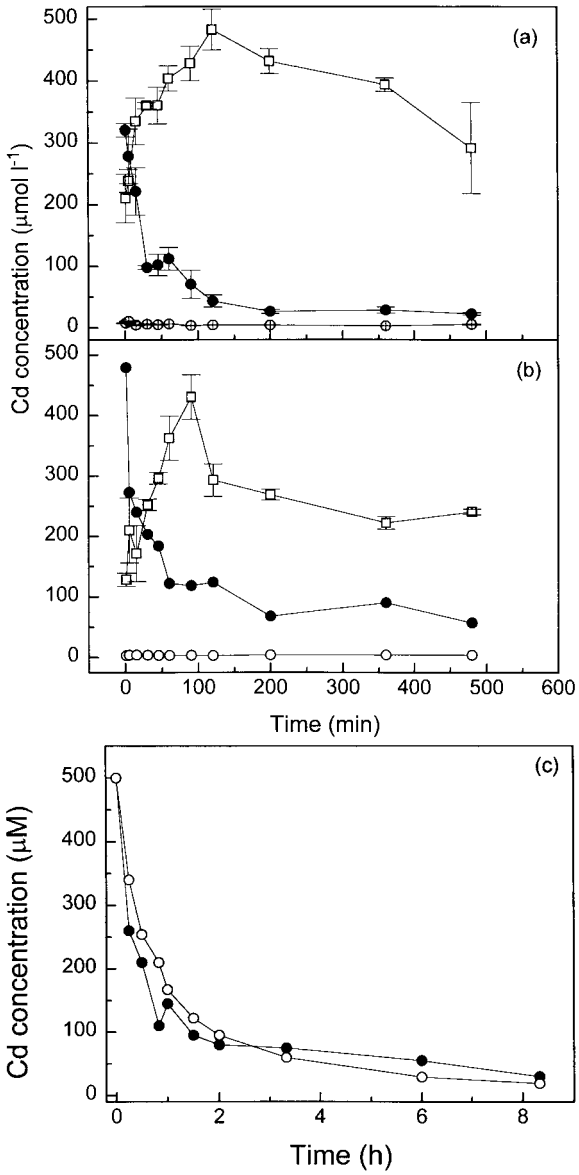

Figure 5. Speciation of Cd following mixing 500 μM $CdCl_2$ and 10 mM Na_2S at pH 6.5 in a flowcell circuit with recirculation at 4 ml min^{-1} (a) with the flowcell containing a "Plastikard" sheet but no biofilm (control) and (b) with a "Plastikard" sheet with biofilm growth. The Na_2S was added to the medium in the reservoir flask at assembly and Cd was added to the stirred medium at time = 0 min. (○), soluble Cd^{2+} (●), colloidal CdS and (□) precipitated CdS. All points are the mean of 4 determinations and the bars indicate SEM. (c) A comparison of (○) model and (●) experimental time-course data for metal removal by an SRB biofilm. Experimental points are the mean of 4 determinations and errors are less than ±3% throughout.

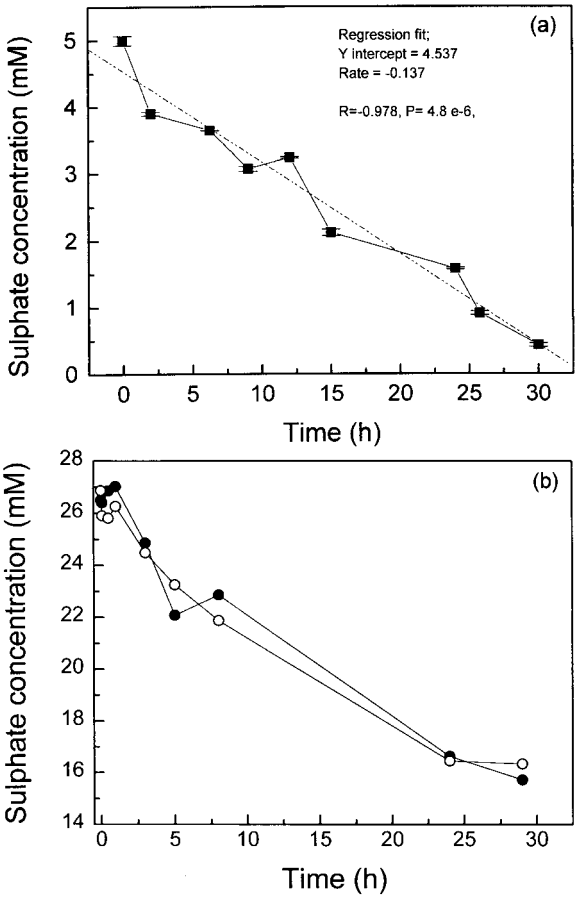

Figure 6. (a) Sulfate utilisation by SRB biofilms in the absence of Cd at an initial sulfate concentration of 5 mM. (b) Sulfate utilisation by SRB biofilms with (○) no Cd (control) and (●) 250 μM Cd. Each point is the mean of 4 determinations and the bars indicate the SEM. Regression was calculated using MS Origin software.

lations presented in Appendix 1 show that R = L v_m, where L is the active biofilm thickness and v_m is the specific rate of sulfide production per unit volume of biofilm. With L = 200 μm, the value of v_m is calculated as R/L = 45.7 mol m^{-3} h^{-1}. Again referring to Appendix 1, for complete penetration, the value of the parameter $\gamma = (2\ S_b\ D/L^2\ v_m)^{0.5}$ must be greater than unity. Taking the bulk sulfate concentration, S_b = 5 mM and the diffusivity of sulfide in biofilm as about 80% of that in water, D = 2.39×10^{-6} m^2 h^{-1}, the value of γ is about 3.6 which is greater than unity. This calculation was repeated assuming that there is incomplete penetration for which γ would have to be less than unity. The upshot is that this latter assumption gives γ which is greater than one and is therefore an absurd result. Accordingly, it can be concluded that the biofilm was completely penetrated and that v_m = 45.7 mol m^{-3} h^{-1}. This is in very close agreement with the value of 44.3 mol m^{-3} h^{-1} estimated in Appendix 1 from work performed by Nielson (1987).

Figure 6b shows the results from a similar experiment, except that, in this case, the biofilm was 550 μm thick, the experiment was performed in both the presence and absence of cadmium and the initial concentration of sulfate was higher. It can be seen that the presence of cadmium has no effect on the rate of sulfide production by the biofilm, at the concentrations used and over a time period of 29 hours. It was found that straight lines could be used to correlate these data (r^2 = 96.3 and 97.4 for the results in the absence and in the presence of Cd, respectively), despite the possibility of some curvature. Assuming that the plots are linear, and the kinetics are therefore zero order, it was found that v_{max} for the experiments was 52.6 mol m^{-3} h^{-1} and that there was complete penetration of the biofilm. The value of v_{max} is in good agreement with that found from Figure 6a.

The simple form of the kinetic expressions for the production of sulfide make further analysis of the system and its scale-up a tractable proposition. Of course, at large biofilm thicknesses, or with higher cell concentrations within the film, than those used here could well result in diffusion limitations. However, our experiments do show the order of magnitudes of the key parameters which would be needed in design.

Capture of cadmium by the biofilm

In the biofilm experiments, described in the previous section, the rate of production of sulfide was sufficient to react with all of the cadmium ions originally present in times of 0.75 h or less. However, results shown in Figures 4 and 5 show that the rate of removal of colloidal cadmium sulphide from solution occurs over a time period larger than this. For example, in Figure 5c, which shows the concentration of colloidal cadmium sulfide, at 0.75h, the colloidal concentration still amounts to 40% of the cadmium present. The results shown in Figure 5 show that the biofilm appears to have very little impact on the rate of removal of colloidal material from suspension, as noted earlier. However, we attempted to model the system to attempt to understand the circumstances in which filtration of colloidal material by the film might be important and which might, therefore improve the rate of removal of suspended cadmium sulfide.

In order to model the system, it was assumed that the rate of cadmium removal consisted of:

(a) precipitation of colloidal metal sulfide by the instantaneous reaction of soluble cadmium ions with soluble sulfide in the bulk solution, followed by either
(b) flocculation of the colloidal particles to form precipitated CdS, or
(c) capture of colloidal CdS by the biofilm acting as a filter.

It was assumed that both (b) and (c) can proceed simultaneously, albeit at different rates, depending on the physical conditions of the experiment. With these assumptions, the following equations were derived to describe the system.

Removal of soluble Cd ions

Let R be the rate of sulfide production per unit nominal area of biofilm, A be the nominal area of biofilm and C be the concentration of soluble cadmium ions in the system. If it is assumed that the whole system comprising the biofilm cell and its reservoir approximates to a batch stirred tank, then:

$$V\frac{dC}{dt} = -R\ A \tag{1}$$

where V is the total volume of the biocell plus the reservoir. Hence:

$$C = C_0 - (R\ A/V)t \tag{2}$$

where C_0 is the starting concentration of cadmium ions. Equation (1) holds until C has fallen to zero at time $t_1 = C_0/(R\ A/V)$.

Formation and removal of suspended colloidal CdS

As noted above, it is assumed that the colloidal material can either be captured by the biofilm acting as a filter or flocculates in free solution to form precipitated CdS which drops out of suspension. The flocculation of colloids is a complex process. In the simple treatment given here, it is assumed that the flocculation rate is driven by the particle-particle collision frequency which is, in turn, dependent on the mean rate of fluid shear, G, in the system. Such shear-driven or orthokinetic flocculation is described by the following equation for equal-sized spheres:

$$\frac{dn}{dt} = -(2/3)\alpha d^3\ Gn^2 \tag{3}$$

where α is the fraction of collisions which lead to a permanent aggregate and d is the diameter of the colliding particles with a number concentration n. Clearly, a flocculating system contains particles of different sizes with the particle size distribution changing as the flocculation proceeds. In the crude analysis presented here, it was assumed that the colloidal CdS can be characterised by one mean particle size, d, and that the result of a fruitful collision between two particles of size d leads to a new particle which is not a colloid and is therefore precipitated from the system. Accordingly, if the molar concentration of colloidal material is N, $N = n\ \rho\ \pi\ d^3/6$, where ρ is the molar density of a particle of diameter d. Thus, equation (3) becomes:

$$\frac{dN}{dt} = -(4\alpha/\rho\pi)G\ N^2 = k_c\ N^2. \tag{4}$$

In addition to flocculation, it was assumed that colloidal CdS can be captured by the biofilm acting as a filter, with filtration coefficient, λ. A theoretical approach for the estimation of λ is given in Appendix 2 which shows that:

$$\lambda = [1.5\ b\ D\ (9\xi)^{-1/3}]\ [6\ (1-\varepsilon)L/d_p]^{2/3}/\Gamma(4/3) \tag{5}$$

where b is the channel width, D is the Stokes-Einstein diffusion coefficient for particles of colloidal CdS, $\xi = D\ a/2\ U_1$, where 2a is the free height of the channel (width between plates less the height occupied by the biofilm) and U_1 is the maximum stream velocity (equal to 1.5 times the average flow velocity) and Γ is the gamma function. To account for the total area of biofilm actually exposed, the biofilm has been supposed to be of nominal height L' and to consist of spherical elements of diameter d_p and with a voidage of ε in the film between spherical elements. It should be noted that L' might be greater than the *active* height L.

From the foregoing arguments, the equation describing the formation and removal of colloidal material is:

$$\frac{dN}{dt} = R\ A/V - k_c\ N^2 - (\lambda/V)N \tag{6}$$

with N = 0 at t = 0. Clearly, beyond a time t_1, the term R A/V must be removed as then no further formation of colloidal material can occur. Although the analytical integration of Equation (6) is straightforward, the resulting expressions are rather cumbersome, and so it was integrated numerically.

Comparison of theory with experimental observations

Figures 5a and b show the progress of colloid removal for the case in which there is no colloid generation and in the absence and presence of biofilm, respectively. For this case, the governing equation is Equation (6) with R A/V = 0 for all t and with N = N_0 at t = 0 as the starting condition. This was fitted to the results in Figure 5a with N_0 = 500 μM, V = 0.21 l and with the filtration coefficient, λ, calculated from eq. (5) by using rough estimates of the parameters contained in that expression, estimated as follows:

CdS particle diameter	0.01 μm
Brownian diffusivity, D	1.62×10^{-7} m^2 h^{-1} (estimated from the Stokes-Einstein equation)
Flow width, b	3 cm
Active biofilm height, L	550 μm (with L$'$ taken equal to L)
Free flow height, 2a	2 mm (allowing for biofilm plus substrate)
Mean flow velocity	0.111 cm s^{-1}
Max. flow velocity, U_1	0.167 cm s^{-1}
ξ	1.35×10^{-11} m^2
Biofilm element dia., d_p	100 μm
Biofilm voidage, ε	0.4
Γ(4/3) (from tables)	0.894

Hence:

λ/V	0.124 h^{-1}.

Of course, the values of ε and d_p are hypothetical at this stage and the size of a Cd particle has been used as a fitting parameter.

Figure 5c shows the theoretical curve fitted to the experimental results of colloid removal against time for a growing biofilm, using k_c – related to the effectiveness of collisions between colloids, α, through Equation (4), above – as a fitting parameter. Although not shown in Figure 5c, it was found that there was no significant difference between the experimental results for the cases with and without the biofilm. Figure 5c shows reasonable agreement between theory and experiment using a value of k_c of 3.5×10^{-3} (μM h)$^{-1}$. Other results also showed that the theoretical curves do not alter very much when the value of λ/V varies between the estimated value of 0.124 h^{-1} and zero. This reinforces the fact that particle capture by filtration appears to be very much a secondary process when compared with flocculation in free solution in this experimental system.

Discussion

From the theoretical analysis and experimental results the rate at which sulfate was reduced at the levels of sulfate considered in this study (0–35 mM) was zero order with regard to sulfate concentration and, for fresh biofilms, did not appear to be hindered by the build-up of insoluble CdS over short time periods (the duration of experimental runs was 30 h). Calculations also indicated that the biofilm was completely penetrated by substrates throughout these experiments. For times in excess of this (as would occur in operational reactors) it is not possible to say at present how insoluble metal sulfide accumulation would affect sulfide production.

The capture of cadmium from solution involved formation of colloidal CdS in suspension, a phenomenon which has not been previously reported in SRB systems. This was followed by flocculation of the colloid in free solution to form precipitated material and/or capture of the colloid by direct filtration through the biofilm. In the system under investigation, free solution flocculation appeared to dominate as a mechanism for CdS removal. Most or all of the CdS occurred initially as colloidal material which flocculated to form a solid CdS precipitate. The formation of colloidal CdS was rapid and mainly controlled by sulfide production resulting from SRB metabolism while flocculation proceeded more slowly and was the effective rate-determining step in the overall process of bioprecipitation. Inclusion of this slow step in the mathematical process model resulted in a good fit to experimental data while ignoring the flocculation step resulted in a model which predicted more rapid bioprecipitation than actually occurred. This indicated that the two-step model was a complete description of the processes occurring in bioprecipitation. Unexpectedly, the presence of the biofilm did not have any observable effect on the rate at which the colloidal CdS was flocculated. This implies that flocculation occurred primarily in the circulating bulk phase and that filtration by the biofilm was only a minor process with no soluble polymers produced capable of acting as flocculants. However, once flocculated, precipitated CdS settled out of the circulating liquor faster in the presence of a biofilm which suggests that precipitated solid CdS was entrapped by the biofilm EPS at this stage rather than as colloidal material. This is consistent with previous studies where biofilms cultured on coupons suspended in a stirred-tank system accumulated CdS via a similar deposition mechanism (White

& Gadd 1998) as was kaolinite (Vieira & Melo 1995). Formation of colloidal metal sulfide requires the presence of excess sulfide (Svehla 1996) and significant amounts of colloidal CdS were formed in the flow-cell during bioprecipitation by an SRB biofilm despite the very low bulk phase sulfide concentrations (Figure 4). This implies that the reaction between Cd^{2+} and H_2S took place in a hydrodynamic boundary layer at the surface of the biofilm where diffusion from the biofilm resulted in a higher concentration of H_2S than in the bulk phase. However, the experimental system used did not permit the fine-scale investigation of the biofilm/liquid boundary needed to confirm this.

The bioreactor model was developed using a process outline from the experimental data presented here and parameters obtained from both our experimental data and that in the literature. The model predictions were in good agreement with experimental data so that it would appear that colloidal sulfide formation, colloid flocculation and precipitate entrapment were the significant processes occurring in the system and were described adequately in the model. The biocell allowed measurement of metal, sulfate, sulfide and organic substrate (lactate) utilisation by a defined area of biofilm under defined conditions which was essential to develop mass-balances for these materials and to provide parameters for the mathematical model. We conclude that the key parameters that determined the overall rate of bioprecipitation were the rate of sulfide production and the rate of colloid flocculation both of which would be candidates for optimisation in a working bioreactor.

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Appendix 1. Derivation of kinetic expressions

Rate equations

Various workers (Atkinson & Davies 1974; Bailey & Ollis 1986) have considered the problem of diffusion and reaction in a pellet of biocatalyst when Michaelis Menten kinetics obtains. Here, the rate of reaction, v, is given by:

$$v = v_m S/(K_m + S) \tag{1.1}$$

with v_m being the maximum rate of reaction when the substrate concentration, S, is much larger than K_m. It is possible to define parameters ϕ and β, such that, for a slab-type geometry:

$$\phi = L[v_m/(K_m D)]^{0.5}$$

$$\beta = S_b/K_m$$

where S_b is the bulk concentration of substrate (assuming there to be no external mass transfer resistance in the liquid film at this stage) and D is the effective diffusivity of the substrate in the biofilm. Clearly, ϕ is a form of Thiele modulus but related only to the first order part of the Michaelis Menten equation. It is possible (Atkinson & Davies, 1974) to define another parameter, ψ, such that:

$$\psi = [\phi\beta/(1+\beta)][\beta - \ln(1+\beta)]^{-0.5}/\sqrt{2},$$

whence the effectiveness factor for slab geometry, η, is given by:

$$\eta = 1 - \left[\frac{\tanh\phi}{\phi}\{(\psi/\tanh\psi) - 1\}\right] \quad \text{for } \psi \leq 1 \tag{1.2}$$

or

$$\eta = (1/\psi) - \left[\frac{\tanh\phi}{\phi}\{(1/\tanh\psi) - 1\}\right] \quad \text{for } \psi \geq 1 \tag{1.3}$$

To proceed, it is necessary to estimate typical values of these parameters. Previous experiments reported on films of *Desulfovibrio* in sulfate limitation (Nielsen 1987) suggest that $K_m \approx 1.4\ \mu M$, $v_m = X.V_{max}$, where X is the biomass density and V_{max} is the maximum rate based on unit mass of biomass, with $V_{max} \approx 0.75$ mmol/g cells/h and $X \approx 59$ kg/m^3, and D

$\approx 2.39 \times 10^{-6}$ m^2/h. In Nielsen's (1987) work, v_m is therefore 44.3 mol/m^3 biofilm/h. Also, $\phi = 11.5$ for L = 100 μm and $\beta = 714$, for $S_b = 1$ mM. Accordingly, $\psi = 0.306$ and the effectiveness factor, $\eta = 0.997$.

The practical interpretation of this result is that the observed rate of reaction for a biofilm, R, is given by:

$$R = \eta v_m S_b/(K_m + S_b) = \eta v_m \beta/(1 + \beta) \approx \eta v_m$$

given that β is so large. That is, it is reasonable to model the sulfate reduction as a zero order process.

For zero order kinetics, it is very easy to show that, in the absence of external film mass transfer, the relevant equation is:

$$\frac{d^2W}{dx^2} = 2/\gamma^2 \tag{1.4}$$

where $\gamma^2 = (2S_bD)/L^2v_m$, $W = S/S_b$ and $x = z/L$, where z is the distance into the film from the interface between and liquid and the biomass. The solution depends on whether or not there is complete penetration of the film by the substrate. Thus:

Complete penetration of the biofilm

Here, the boundary conditions are $W = 1$ at $x = 0$ and $dW/dx = 0$ at $x = 1$. The resulting solution is, therefore:

$$W = 1 - 2x/\gamma^2 + x^2/\gamma^2. \tag{1.5}$$

This solution holds until the point at which $W = 0$ at $x = 1$. That is, when $\gamma \geq 1$. The rate of reaction is given by $-(D\ dS/dz)_{z=0} = -(D\ S_b/L)\ dW/dx)_{z=0} = Lv_m$. The effectiveness factor is clearly unity for this condition.

Incomplete penetration of the biofilm

Here, the concentration falls to zero at some distance $L_f < L$. Let $x_f = L_f/L$, so that $dW/dx = 0$ at $x = x_f$. The resulting solution to Equation (1.4) is:

$$W = 1 - 2x/x_f + x^2/x_f^2 \tag{1.6}$$

with $x_f = \gamma$. The rate of reaction is given by $-(D\ dS/dz)_{z=0} = -(D\ S_b/L)\ dW/dx)_{z=0} = (2v_mDS_b)^{0.5}$ and so the effectiveness factor, $\eta = (2v_mDS_b)^{0.5}/v_mL = \gamma$.

In the case under consideration, $\gamma = 3.3$. This means that there is a uniform rate of substrate utilisation within the pellet down to $z = L$ and that the effectiveness factor is unity. The rate of reaction per unit area of biofilm is therefore Lv_m.

Appendix 2. Estimation of the filtration constant (λ)

In this treatment, it is assumed that colloidal CdS has a particle size below 0.1 μm so that the primary filtration mechanism is by Brownian diffusion of the particles to the surface of the biofilm. Once a particle reaches the biofilm surface, it is assumed to be captured. That is, the "sticking probability" is unity. It is also assumed that, over the short period of the batch experiments, sloughing of biofilm elements is insignificant. This latter aspect cannot be neglected for continuous experiments over long periods of time and for subsequent design of practical reactors.

Consider a longitudinal section of the biocell, parallel to the direction of flow, and let the gap between the two plates, after subtraction of the nominal biofilm height, be 2a. Let y be a coordinate normal to the biofilm, such that y = 0 marks the outer edge of the biofilm and y = 2a, the opposite plate (which is not covered with biofilm). Let z be the coordinate in the direction of flow. At z = 0, the flow enters from the reservoir with colloid concentration N_0 and flow rate v. Finally, let u be the velocity at distance y from the biofilm and U_1 be the maximum velocity at y = a, assuming the flow to be laminar.

From simple laminar flow theory:

$$u = U_1(2ay - y^2)/a^2. \tag{2.1}$$

A simple mass balance on a small element of liquid yields:

$$u\frac{\partial N}{\partial z} = -D\frac{\partial^2 N}{\partial y^2}, \tag{2.2}$$

where D is the Stokes–Einstein diffusivity for colloidal particles of diameter d. As Equations (2.1) and (2.2) currently stand, an analytical solution is impossible. However, if one assumes that the contact time is relatively short (about 2.5 minutes compared with a filtration time constant of the order of several hours), all of the concentration change occurs near the surface of the biofilm where y is small. Now, near the wall, since $(y/a)^2 \to 0$, $u \to 2U_1y/a$. Accordingly, Equation (2.2) simplifies to:

$$y\frac{\partial N}{\partial z} = -\xi\frac{\partial^2 N}{\partial y^2}, \tag{2.3}$$

where $\xi = D\ a/2\ U_1$. For these short contact times, the boundary conditions become:

$$N = N_0 \text{ for } z = 0 \text{ and } y > 0$$

$$N = N_0 \text{ for } y = \infty \text{ and z finite}$$

$$N = 0 \text{ for } y = 0 \text{ and } z > 0.$$

The final condition reflects the assumption that the film acts as a total sink for the CdS and that adsorbed CdS does not exert any adverse effect on this assumption. By re-writing the above equation using the reduced variables $\Theta = (N_0 - N)/N_0$ and $\sigma = y/[(9\xi z)^{1/3}]$, it becomes an ordinary differential equation such that:

$$\frac{d^2\Theta}{d\sigma^2} + 3\sigma^2\frac{d\Theta}{d\sigma} = 0 \qquad (2.4)$$

which is readily integrated and solved using the boundary conditions. Further manipulation yields the average flux, F, of the particles to the biofilm as:

$$F = [1.5DN_0(9\xi Z)^{-1/3}]/\Gamma(4/3) \qquad (2.5)$$

where Γ is the gamma function and Z is the nominal total length of the biofilm in the z direction. Clearly, then, the filtration coefficient λ, is given by:

$$\lambda N_0 = FZb \qquad (2.6)$$

where b is the nominal width of the film, so:

$$\lambda = [1.5bD(9\xi)^{-1/3}]Z^{2/3}/\Gamma(4/3). \qquad (2.7)$$

However, the biofilm has an undulating surface and so to take Z as the nominal length of the biocell (10 cm) is likely to lead to a significant underestimate of the filtration capacity. To make a better estimate, the following assumptions were made; the biofilm consists of roughly spherical elements of biomass of diameter, d_p; the voidage in the biofilm (between the spherical elements) is ε, and the distance from the membrane to the outer edge of the biofilm remains as L.

Suppose there are n biomass elements per unit volume of biofilm. Consider a nominal length l of biofilm in the z direction and unit width (normal to the y-z plane). Then, considering the volume of biomass gives:

$$l.1(1 - \varepsilon)L = n\pi d_p^3/6,$$

whence

$$n = 6l(1 - \varepsilon)L/\pi d_p^3$$

and so the actual area of biofilm associated with the length l is $n\pi d_p^2 = 6l(1 - \varepsilon)L/d_p$. Now, the *nominal* area was $l \times 1$ and so the value of Z in Equation (2.7) ought to be $[6l(1 - \varepsilon)L/d_p]/l = 6(1 - \varepsilon)L/d_p$. Accordingly, the value of the filtration coefficient is given by:

$$\lambda = [1.5bD(9\xi)^{-1/3}][6(1 - \varepsilon)L/d_p]^{2/3}/\Gamma(4/3). \qquad (2.8)$$

References

Atkinson B & Davies IJ (1974) The overall rate of substrate uptake (reaction) by microbial films. Part I – A biological rate equation. Trans. Inst. Chem. Eng. 52: 248

Bailey JE, Ollis DF (1986) Biochemical Engineering Fundamentals. McGraw Hill, Chicago

Barnes LJ, Janssen FJ, Sherren J, Versteegh JH, Koch RO & Scheeren PJH (1991) A new process for the microbial removal of sulphate and heavy metals from contaminated waters extracted by a geohydrological control system. Trans. Inst. Chem. Eng. 69: 184–186

Barnes LJ, Scheeren PJM & Buisman CJN (1994) Microbial removal of heavy metals and sulphate from contaminated groundwaters. In Means JL & Hinchee RE (eds) Emerging technology for bioremediation of metals (pp 38–49). Lewis Publishers, Boca Raton

Beech IB & Cheung CWS (1995) Interactions of exopolymers produced by sulphate-reducing bacteria with metal ions. Int. Biodet. Biodeg. 35: 59–72

Bridge TAM, White C & Gadd GM (1999) Extracellular metal-binding activity of the sulphate-reducing bacterium *Desulfococcus multivorans*. Microbiol. 145: 2987–2995

Flemming H-K (1995) Sorption sites in biofilms. Wat. Sci. Technol. 32: 27–33

Gadd GM (1992a) Heavy metal pollutants: Environmental and biotechnological aspects. In Lederberg J (ed) Encyclopedia of microbiology (pp 351–360). Academic Press, San Diego

Gadd GM (1992b) Microbial control of heavy metal pollution. In Fry JC, Gadd GM, Herbert RA, Jones CW & Watson-Craik IA (eds) Microbial control of pollution (pp 59–88) Cambridge University Press, Cambridge

Gadd GM (2000) Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. Curr. Opinion Biotechnol. 11: 271–279

Gadd GM (2001) Accumulation and transformation of metals by microorganisms. In Rehm H-J, Reed G, Puhler A & Stadler P (eds) Biotechnology, a multi-volume comprehensive treatise, Volume 10: Special processes (pp 225–264). Wiley-VCH Verlag GmbH, Germany

Gadd GM & White C (1993) Microbial treatment of metal pollution – a working biotechnology? Trends Biotechnol. 11: 353–359

Hammack RW & Edenborn HM (1992) The removal of nickel from mine waters using bacterial sulphate-reduction. Appl. Microbiol. Biotechnol. 37: 674–678

Hartley AM, House WA, Leadbeater BSC & Callow ME (1996) The use of microelectrodes to study the precipitation of calcite upon algal biofilms. J. Coll. Interface Sci. 183: 498–505

Herbert D, Phipps PJ & Strange RE (1971) Chemical analysis of microbial cells. Methods Microbiol. 5: 210–344

Nielsen P (1987) Biofilm dynamics and kinetics during high-rate sulphate reduction under anaerobic conditions. Appl. Environ. Microbiol. 53: 27–33

Postgate JR (1984) The sulphate-reducing bacteria. Cambridge University Press, Cambridge

Smith WL & Gadd GM (2000) Reduction and precipitation of chromate by mixed culture sulphate-reducing bacterial biofilms. J. Appl. Microbiol. 88: 983–991

Svehla G (1996) Vogel's qualitative inorganic analysis. Longman, Singapore

Videla HA (1994) Biocorrosion of nonferrous metal surfaces. In Geesey GG, Lewandowski Z & Flemming HK (eds) Biofouling and biocorrosion in industrial water systems (pp 231–243). Lewis Publishers, Boca Raton

Vieira MJ & Melo LF (1995) Effect of clay particles on the behaviour of biofilms formed by *Pseudomonas fluorescens*. Wat. Sci. Technol. 32: 45–52

White C & Gadd GM (1995) Determination of metals and metal fluxes in algae and fungi. Sci. Tot. Environ. 176: 107–115

White C & Gadd GM (1996a) A comparison of carbon/energy and complex nitrogen sources for bacterial sulphate-reduction: potential applications to bioprecipitation of toxic metals as sulphides. J. Indust. Microbiol. Biotechnol. 17: 116–123

White C & Gadd GM (1996b) Mixed sulphate-reducing bacterial cultures for bioprecipitation of toxic metals: factorial and response-surface analysis of the effects of dilution rate, sulphate and substrate concentration. Microbiol. 142: 2197–2205

White C & Gadd GM (1997) An internal sedimentation bioreactor for laboratory-scale removal of toxic metals from soil leachates using biogenic sulphide precipitation. J. Indust. Microbiol. Biotechnol. 18: 414–421

White C & Gadd GM (1998) Accumulation and effects of cadmium on sulphate-reducing bacterial biofilms. Microbiol. 144: 1407–1415

White C & Gadd GM (2000) Copper accumulation by sulphate-reducing bacterial biofilms and effects on growth. FEMS Microbiol. Lett. 183: 313–318

White C, Sharman AK & Gadd GM (1998) An integrated microbial process for the bioremediation of soil contaminated with toxic metals. Nature Biotechnol. 16: 572–575

Widdel F & Pfennig N (1982) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids: 2. Incomplete oxidation of propionate by *Desulfobulbus propionicus* gen-nov, sp-nov. Arch. Microbiol. 131: 360–365