Journal of Industrial Microbiology & Biotechnology (1997) 18, 414–421

An internal sedimentation bioreactor for laboratory-scale removal of toxic metals from soil leachates using biogenic sulphide precipitation

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An internal-sedimentation bioreactor was employed to provide biomass feedback and process intensification in a laboratory-scale sulphide-bioprecipitation system for toxic metals (Cd, Cr, Cu, Mn, Ni, Zn) present in acid leachates from metal-contaminated soil. Biomass feedback was improved by addition of a cationic polymer flocculant and the activity of the sulphate-reducing bacterial culture was increased by the addition of cornsteep in addition to the ethanol used as carbon/energy substrate. A mass-balance was carried out for carbon and sulphur in the system. Sulphate reduction in the reactor was able to remove acidity at moderate sulphate concentrations up to 50 mM although it was insufficient at the highest levels tested. When presented with a simulated toxic metal-containing leachate, the reactor was able to precipitate metals efficiently under all of the conditions of sulphate concentration and pH tested, producing an effluent with metal concentrations suitable for environmental discharge.

Keywords: sulphate-reducing bacteria; bioremediation; biotechnology; toxic metals; bioreactors; metal sulphides; metal leachate

Introduction

There is an increasing interest in the application of microbial biotechnology to the remediation of land and waters contaminated with toxic metals. The technologies which have been applied to this include biosorption by living or inactive biomass [5,9,12] and precipitation by microbial metabolic products using a number of systems of varying technical complexity, from natural or artificial wetlands [7,8,11,20] to engineered bioreactors [3,9,17,19]. The present study was undertaken as part of the development of a multi-stage biological process for the treatment of toxic-metal-contaminated land. The integrated process comprised two components: biological leaching of metals from soil using sulphur-oxidising bacteria under aerobic conditions and the subsequent removal of sulphate, acidity and toxic metals from the leachate by the anaerobic action of sulphate-reducing bacteria. The present study focussed on the integration of sulphate reduction into the biological process. Alkalisation and precipitation of toxic metals resulted from bacterial reduction of sulphate to sulphide, low dissociation constants of H_2S in aqueous solution and low solubility of toxic metal sulphides [7,8,11,17]. Ethanol as a carbon/energy source with the addition of a complex nitrogen source (cornsteep) was found previously to be an efficient nutrient regime for sulphate reduction and the substrate/sulphate stoichiometry and hydraulic retention time of the reactor were the significant variables controlling process efficiency [17,18].

Simple continuous-flow stirred tank reactors (CSTR) were not considered adequate for operation using soil leachates as the maximum sulphate concentration treatable using such a reactor was approximately 20 mM and almost complete sulphate reduction was required for removal of acidity to minimise alkali addition [17]. The reactor used for treating leachate, therefore, required biomass feedback as a means of increasing the activity over that obtainable from a simple CSTR and had to be well-mixed in order to avoid the production of pH gradients by progressive sulphate reduction during plug-flow. Previous laboratory-scale applications of bacterial sulphate reduction for metal removal utilised stirred-tank reactors, with or without biomass feedback [12,13,16], and showed that information derived from this type of reactor can be applied to other scaled-up systems including stirred-tank and sludge-blanket reactors [2]. The present study was carried out with the aim of developing a protocol utilising an internal sedimentation bioreactor for the sulphate-reducing component of the integrated metal treatment process on a laboratory scale.

An internal sedimentation bioreactor is a feedback bioreactor in which high biomass concentrations are achieved by dividing the reactor into two zones. The lower zone is a stirred-tank reactor and the stirring is limited to this zone by a set of baffles (Figure 1a). The upper zone is therefore unstirred and a large fraction (dependent on flow-rates and reactor geometry) of the solids entering this zone sediments under gravity and returns to the lower zone. The compact, one-piece design of this reactor makes it particularly suitable for small-scale operation. Nutrient and other feeds are supplied to the lower zone and there are two outflows: one in the upper zone consisting of partially clarified liquor and one in the lower active zone of concentrated biomass. The flows through the reactor are shown in Figure 1b. The biomass concentration in the active zone is determined by the rate of growth, dilution rate and the proportional outflow via the two routes so that:

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Received 29 October 1996; accepted 31 March 1997

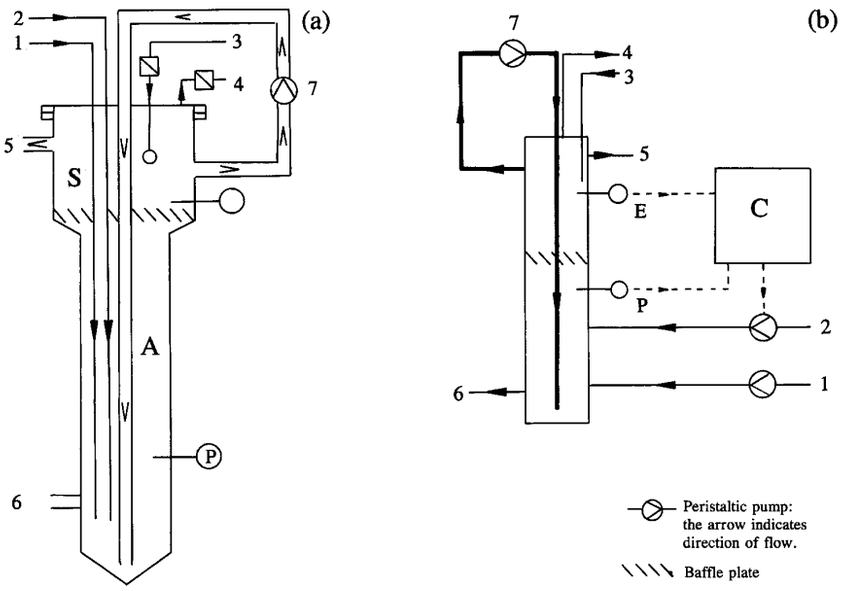

Figure 1 Diagram of the internal sedimentation bioreactor used for biprecipitation showing: (a) detail of the vessel; and (b) diagram of the controls and feeds for the bioreactor. The material flows are labelled respectively: A, active zone containing concentrated biomass and solids (this zone was stirred by recirculating the liquor); S, sedimentation zone containing clarified liquor; (1), medium feed; (2), alkali feed; (3), N_2 sparger; (4), gas outlet; (5), weir (dilute liquor outlet); (6), concentrated culture outlet; and (7), recirculation loop. The data-acquisition/control components are labelled: (P), pH probe; (E), redox (E_h) probe and (C) computerised data acquisition/control system (see text for details). Main feeds are shown as continuous fine lines, recirculation as continuous thick lines and data channels as discontinuous lines: arrows indicate the direction of flow.

$$dx/dt = \mu x - cDx - (1 - c)Dhx \quad (1)$$

where x = biomass concentration; μ = specific growth rate; c = fractional flow through concentrated outflow ($0<c<1$); D = dilution rate; h = dilution factor ($0<h<1$). At steady state $dx/dt = 0$ so that:

$$\mu = cD + (1 - c)Dh = AD \quad (2)$$

where $A = c(1 - h) + h$.

Since h is effectively a constant at moderate flow rates, A is determined by c, which can be readily manipulated by controlling the proportional flow through the concentrated outflow stream and allowing the remainder to exit via the partially clarified, dilute stream [14]. The aim of this work was to evaluate the use of sulphate-reducing bacteria cultured in this bioreactor for bioprecipitation of metals from an acidic sulphate-rich soil leachate which was produced by the bioleaching component of the integrated process. As operation of the reactor depends on sedimentation of solids, the ability of a number of commercial flocculating agents to enhance this was investigated using batch systems and in the reactor itself. In addition to characterising the effects of significant variables including organic nitrogen [18], on the operation of the reactor, the effects of chromate and temperature were also studied using appropriate batch and chemostat cultures.

Materials and methods

Organisms and culture

The organisms used were a sulphate-reducing mixed bacterial culture derived from several environmental sources and selected for suitable properties including rapid rates of sulphate reduction and metal tolerance [17]. The organisms were maintained in continuous chemostat culture on SRB1 medium comprising (g L^{-1}): ethanol, 2.4; Na_2SO_4, 1.8; KH_2PO_4, 0.25, NH_4Cl, 1.0; yeast extract (Oxoid Ltd, Basingstoke, Hants, UK), 1.0; $CaCl_2{\cdot}H_2O$, 0.06; $MgCl_2{\cdot}6H_2O$, 0.06; $FeCl_3$, 0.004. A solution (1.0 ml L^{-1}) comprising 50 mM each of Cd, Co, Cr, Cu, Mn, Ni, and Zn chlorides was also added. The inflow pH was 6.0. The dilution rate was 0.05 h^{-1} and the temperature and pH of the broth were 20°C and 7.4 respectively. No control was required for pH maintenance. Inoculum for the internal sedimentation reactor was provided by aseptically transferring 100 ml of active culture from this chemostat to the reactor under anaerobic conditions.

Bioreactor construction and operation

The reactor was constructed of glass using *Quickfit* fittings (Merck, Lutterworth, Leics, UK). The total volume was 1200 ml with a working volume (active zone) of 400 ml. The active zone was 6 cm in diameter with the upper zone expanded to 10 cm in diameter. Two separate ports were fitted in the upper zone and one in the lower zone to accommodate pH and redox probes. The outlets in both active (concentrated stream) and sedimentation (dilute stream) zones were integral to the construction. The baffles comprised two vertical acrylic plates resting on the shoulder of the expanded portion by means of lugs. Vertical convective flows were prevented by baffles attached to these at 45° to the horizontal. The active zone was stirred by recirculating partially-clarified liquor withdrawn from the settling zone (Figure 1a). The reactor base was tapered to form a 90° cone at the lower end to aid mixing by this recirculation

current. Secondary circulation (300 ml min^{-1}) was maintained by means of a Watson-Marlow (Falmouth, Cornwall, UK) 505S peristaltic pump. Feeds were provided into the active zone and samples were withdrawn directly from this zone. Nitrogen was sparged into the upper zone to maintain an O_2-free headspace while avoiding convective mixing. Feeds were not sparged and all material entering the reactor was at equilibrium with the atmosphere. Samples were removed via a dip-tube inserted into the upper part of the sedimentation zone or via the lower outlet for the active zone. The pH and E_h of the culture were monitored by means of glass and platinum electrodes respectively (Russell pH Ltd, Auchtermuchty, Fife, UK). Since pH was controlled, the pH probe was inserted into the lower port to monitor the active zone. The redox probe was inserted via one of the upper probe ports, allowing ease of removal as this probe required frequent cleaning. The pH signal was fed through a high-impedance buffer amplifier and both signals were input via an Analog Devices HPL-TC conditioning panel to an Analog Devices RTI-820 A/D board (Calex Instrumentation Ltd, Leighton Buzzard, Herts, UK) inserted into a Viglen PC. The monitoring/control software was Labtech Notebook (Adept Scientific Software, Letchworth, Herts, UK). Experimental cultures were maintained at 20°C on SRB1 medium, with the pH lowered to 4.0 by addition of 5 M HCl in order to simulate leachate conditions. Although sulphate-reduction was an important mechanism resulting in alkalization, additional control of reactor pH (to levels required in individual experiments, usually around pH 6–7) was achieved by addition of 5 M NaOH, using Labtech Notebook to control the alkali pumps. The hydraulic residence time of the active zone was 10 h and the solids residence time was approximately 500 h, and these conditions were maintained except where varied for experimental purposes.

Comparison of flocculants

Sulphide sludge was obtained from the effluent of a reactor operating without flocculant addition. It was separated by settling, resuspended in simulated leachate at approximately 20 mg ml^{-1} and homogenised using a glass homogeniser. Samples of various commercial flocculants were obtained from Allied Colloids Ltd (Bradford, Yorkshire, UK). These included cationic (LT22, LT22S and LT24 with 20%, 20% and 7% cationic content respectively), anionic (LT25, LT26 and LT27 with 20%, 40% and 30% anionic content respectively) and non-ionic (LT20) flocculants. Each flocculant was prepared as a 1% (w/v) dispersion by mixing 0.1 g with 1.0 ml of methanol and then adding 9.0 ml of distilled deionised water. To compare the effects of the various flocculating agents, 10.0 ml of sludge suspension was prepared by mixing 1.0 ml of the stock suspension with 9.0 ml of simulated leachate. 0.1 ml of 1% flocculant stock was added, mixed well and decanted into a 10-ml measuring cylinder. The volume of settled sludge was read at intervals. A control sludge suspension was prepared without flocculant. To assess the effect of flocculant concentration, 10.0 ml volumes of sludge suspension were mixed as above with a suitable volume of 1% flocculant or of a diluted solution and decanted into 10-ml measuring cylinders. The volume of settled sludge was monitored as above. All flocculation trials were replicated four-fold.

Effect of flocculants on reactor operation

The conditions were as those described for maintenance of the internal sedimentation reactor culture except that, where required, a cationic flocculant, Magnafloc T-24 (Allied Colloids) and/or cornsteep (Merck 'Fermtech' supplied by BDH, Lutterworth, Leics, UK) was added. Two millilitres of a 500 mg L^{-1} solution of Magnafloc T-24 were added daily to the active zone of the reactor and cornsteep was added in the medium, at a concentration of 1/10 of the ethanol concentration (0.45 g L^{-1}). Samples were taken at 24-h intervals for analysis.

Effect of chromate on reactor operation

Simple continuous stirred-tank reactors utilising SRB1 medium with 20 mM sulphate and 40 mM ethanol were fed the same medium containing 100 μM and subsequently 200 μM potassium chromate. Samples were removed at intervals and analysed for sulphate, sulphide, chromate and protein. E_h and pH were also monitored during the experiment. These variables were used to observe any change in the state of the reactor.

Effect of temperature

These experiments were carried out in batch culture. The medium (SRB1) was made up in 11 batches and dispensed as 20-ml aliquots in Universal bottles which were sealed using butyl-rubber serum caps. Oxygen-free N_2 was sparged into the headspaces using hypodermic needles inserted through the caps and the bottles were sterilised by autoclaving them (121°C, 15 min). Inoculum for experimental batch cultures was prepared by adding 2.0 ml of the chemostat culture to a bottle and incubating it for 48 h at 20°C. Two millilitres of inoculum culture were withdrawn and added to the experimental culture in 20 ml of SRB1 medium, pH 6.0, containing 20 mmol L^{-1} sodium sulphate, 40 mmol L^{-1} ethanol and 0.3 g L^{-1} cornsteep in screw-top tubes. The cultures were then mixed thoroughly and initial samples were then taken from one set. The remaining cultures were incubated at temperatures of 4, 12, 20, 30 and 37°C for 72 h and then sampled and assayed for sulphate, sulphide and protein. All treatments were replicated four-fold.

Analytical methods

Protein concentration was used as an estimate of biomass present in cultures. One millilitre of culture was homogenised with a glass homogeniser, pipetted into a test-tube, 200 μl of 4 M NaOH added and mixed and extracted for 10 min, shaking the culture to resuspend cells after 5 and 10 min incubation. The solids were then separated by centrifugation (9000 × *g*, 5 min) and 100-μl aliquots were assayed using the Bradford method [4] with 5 ml of reagent, standardising with 1 mg ml^{-1} bovine serum albumin (Sigma, Poole, Dorset, UK). Sulphide was assayed by DC polarography using a Metrohm 663 VA stand (Metrohm UK Ltd, Buckingham, Bucks, UK) and Eco-Chimie μ-Autolab controller and software (Windsor Scientific Ltd, Slough, Berks, UK) [17]. Sulphate was assayed by ion

chromatography (IC) using a Metrohm 690 ion chromatograph equipped with an electrical conductivity detector and a Metrohm 'Supersep' IC anion column with borite-mannite eluent [17]. Metals were analysed by atomic absorption spectrophotometry [17].

Results and discussion

Effect of temperature

Sulphide production in anaerobic batch culture was maximal at 30°C (Figure 2) which agrees with previous findings for mesophilic sulphate-reducing bacteria [15]. However, the maximum was not well-defined and the sulphide concentration increased by 50% between 5 and 20°C although it doubled between 20 and 30°C. The protein concentration increased over the whole temperature range so that the sulphide produced per mg of protein decreased, especially between 30 and 37°C (Figure 2). This suggests that the dominance of sulphate-reducing bacteria in the mixed culture was best maintained at the lower end of the temperature range employed. As growth-dependent variations in culture conditions are negligible in batch cultures during early growth, estimated batch variables can be representative of bioreactor operations [1]. These data were therefore taken as indicating an optimum operating temperature of 20°C for sulphide production.

Comparison of flocculating agents

Figure 3 shows the time-courses of sludge settling in the presence of various flocculants. The best performances were given by the cationic flocculants LT24 and LT22S, and the non-ionic flocculant LT20. It was apparent from inspection that the flocculants which performed poorly produced a diffuse floc with little overall reduction in volume and would therefore not aid settling in the reactor.

Effect of flocculant concentration

The cationic flocculant LT24 was used in this experiment as it gave the greatest sedimentation rate. The maximum effect was retained down to concentrations of 0.001% (w/v)

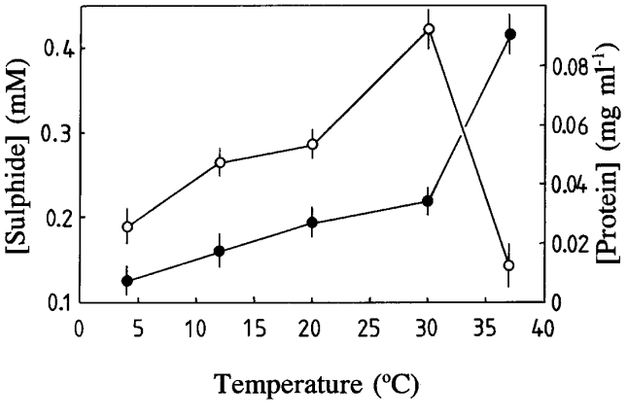

Figure 2 Effect of temperature on the production of (○) sulphide and (●) biomass during 72-h incubation by a mixed sulphate-reducing bacterial population in anaerobic batch culture (20 ml SRB1 medium containing 20 mM sodium sulphate, 40 mM methanol and 0.3 g L^{-1} cornsteep, pH 6.0). Each point is the mean of four separate determinations and bars indicate the standard error of the mean (s.e.m.).

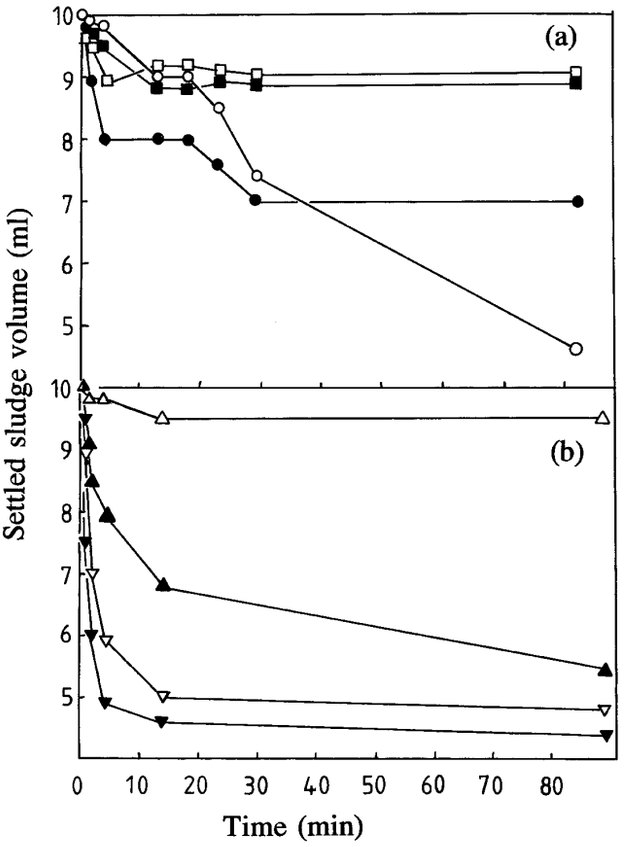

Figure 3 Time-course of homogenised sulphide sludge settling. The settled sludge volumes were measured after incubation in the presence of 0.1 mg ml^{-1} of flocculant for the time intervals indicated. The flocculants were: (a) no-flocculant (control) (○); anionic polymeric flocculants LT25 (●); LT26 (□); LT27 (■); and (b) cationic flocculants LT22 (△); LT22S (▽); or LT24 (▼); or non-ionic polymeric flocculants (LT20) (▲). The settled volumes were measured in measuring cylinders. The error throughout was less than 0.7 ml: error bars have been omitted for the sake of clarity.

and flocculation was still effective at the lowest concentrations tested (Figure 4). The volumes of all of the flocculated samples were in the region of 2.5–2.8 ml after 15 min incubation, while the volume of sludge in a control (without flocculant) was 4.6 ml. These results indicate that varying the flocculant concentration mainly affected the rate of aggregation and settling.

Effects of cornsteep and flocculant on bioreactor operation

The addition of flocculant to the reactor resulted in greater biomass concentration and ethanol utilisation, with all ethanol being utilised, but little increase in sulphate utilisation (Table 1). The ratio of the protein concentrations in the two zones (active : sedimentation) increased from approximately 9 : 1 in the absence of flocculant to 12.5 : 1 in the presence of flocculant and to 13.7 : 1 where both flocculant and cornsteep were added, showing that the presence of flocculant, but not cornsteep, aided biomass retention. With flocculant only, sulphate reduction was apparently limited by the availability of ethanol as all was utilised. When cornsteep was added in addition to the flocculant, there was

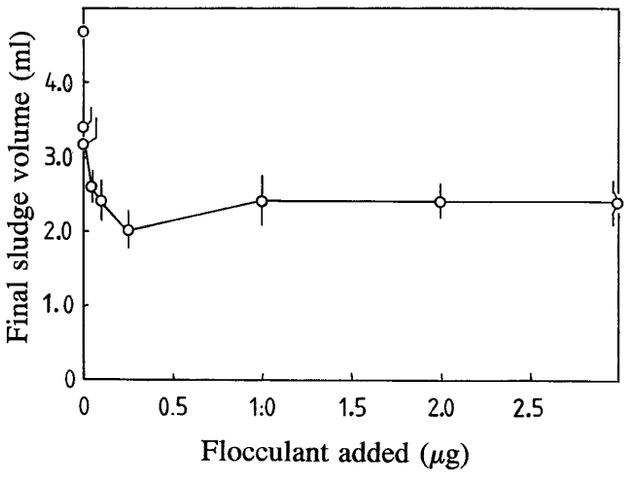

Figure 4 Effect of flocculant (LT24) concentration on settled sludge volume. The conditions were as described for Figure 3 and the settled volume was measured after 15 min incubation. Each point is the mean of four separate determinations, bars indicate s.e.m.

Table 1 The effects of cornsteep and flocculant on operation of the internal sedimentation bioreactor

Component	Component concentrations occurring under different treatments		
	Control	Flocculant	Flocculant plus cornsteep
Sulphate (mM)	43.7 ± 2.70	36.31 ± 3.14	5.78 ± 2.70
Sulphide (mM)	0.51 ± 0.12	0.49 ± 0.07	1.22 ± 0.05
Ethanol (mM)	6.39 ± 2.05	0.00 ± 0.00	6.78 ± 2.77
Protein (mg ml^{-1})			
active	0.18 ± 0.03	0.23 ± 0.01	0.20 ± 0.03
sedimentation	0.21 ± 0.01	0.018 ± 0.00	0.012 ± 0.00

The control culture was grown at 20°C on SRB1 medium containing 100 mM of both sulphate and ethanol and no additional organic nitrogen sources. Where indicated, cornsteep (0.45 g L^{-1}) and/or 'Magnafloc' LT24 flocculant (1.25 mg L^{-1} day^{-1}) were added to this basal medium. Samples were taken after 10 days equilibration and sampling was repeated over 4 successive days of operation. All values shown are the mean ± standard error (four replicates). Protein determinations were carried out on samples from both the active and sedimentation zones of the reactor, which contained the concentrated and dilute culture respectively.

no effect on biomass concentration but sulphate reduction increased, and was accompanied by a decrease in the ethanol utilisation (Table 1). This suggested that cornsteep alleviated ethanol-limitation of the culture. Previous batch and chemostat studies indicated that addition of cornsteep increased both biomass and sulphate reduction by a similar mixed culture, apparently as a result of reduced ethanol utilisation for biosynthesis [18]. However, it appeared that the long solids retention time (approximately 500 h) of the internal sedimentation bioreactor masked any effect of growth rate on biomass concentration although the flocculating agent improved retention of biomass by the reactor. Consequently, when cornsteep was added in addition to flocculant, there was a significant increase in sulphate reduction but no further increase in biomass concentration.

Fate of chromate and dissolved oxygen

There was a decrease in biomass concentration but no detectable loss of sulphate-reducing activity as a result of the addition of chromate to bioreactors at either 100 or 200 μmol L^{-1} (Table 2). This suggested that the effect of chromate on sulphate-reducing components of the culture was minimal although it may have shown some toxicity towards other strains present. However, the reactor readily removed Cr(VI) from the medium and none was detected using atomic absorption spectrophotometry in the outflow or reactor vessel (Table 2). This is consistent with Cr(VI) being reduced to Cr(III) by biological and/or chemical mechanisms followed by precipitation as sulphide in the reactor (see below). Although the leachate was not sparged with N_2, dissolved O_2 entering the active zone appeared to be adequately removed by the facultative components of the culture and/or reduced compounds as the working E_h was continuously below −300 mV.

Sulphate reduction and pH control

The reactor was operated using inflow sulphate concentrations of 10 mM (pH 3), 50 mM (pH 2) and 191 mM (pH 2). Sulphate was supplied as sodium sulphate and the pH adjusted separately by adding concentrated HCl (Analar). Both the sulphate concentrations and pH values used were derived from experimental results of soil-leaching tests (data not shown). Ethanol utilisation at 10 and 50 mM sulphate was almost complete (only traces were detectable). Sulphate (5–6 mM) remained unreduced at both of these inflow concentrations and sulphide concentrations in solution were similar. Since the amount of sulphate reduced at 50 mM was thus approximately nine-fold greater than at 10 mM, it appeared that removal of sulphide as precipitated sulphides and H_2S gas eliminated the excess sulphide produced at the higher sulphate concentration. At an inflow sulphate concentration of 191 mM, the reduction of sulphate and oxidation of ethanol both appeared to be limited by the biological activity of the system and utilisation was incomplete with final concentrations of 93.2 mM sulphate and 365.8 mM ethanol remaining (Table 3). Since sulphate reduction removed acidity from the system the additional alkali requirement for pH control was minimal at 10 mM sulphate and accounted for a very small proportion of the acidity (4.46 mEq compared to 100 mEq of acid present at pH 2) at 50 mM. However, a considerably greater amount of alkali was required at 191 mM sulphate,

Table 2 Stirred-tank reactor operation in the presence and absence of chromate

CrO_{4in}^{2-}	CrO_{4out}^{2-}	SO_{4out}^{2-}	S_{out}^{2-}	pH	E_h (mV)	Protein (μg ml^{-1})
0	0	0.30	1.37	7.9	−491	66
0.10	0	0.32	1.32	8.0	−581	65
0.20	0	0.33	1.34	7.2	−530	38

The mixed sulphate-reducing culture was grown at 20°C in a chemostat on SRB1 medium containing 20 mM sulphate, 40 mM ethanol and 0.2 g L^{-1} cornsteep, at a dilution rate of 0.05 h^{-1} for 10 days prior to sampling on 4 successive days from duplicate cultures. All concentrations except that of protein are quoted in mM.

Table 3 Substrate and product concentrations and parameters for different streams of the internal-sedimentation bioreactor

Variable	Measured values in reactor inflow and outflow streams			
	Inflow	Out_{dilute}	$Out_{concentrated}$	Off gases
pH	2.0	6.3	6.3	
E_h (mV)	200	–415	<–415	
Flow rate (ml h^{-1})	28.8	28.8	0.63	720
D (h^{-1})	0.050	0.050	0.001	
Sulphate (mM)	191.5	93.2	93.2	
Soluble sulphide (mM)	0	1.31	1.31	
H_2S gas (mmol L^{-1})	0	0	0	0.29
Ethanol (mM)	500.0	365.8	365.8	ND
Acetate (mM)	0	33.5	33.5	ND
CO_2 (mM)	0	ND	ND	0.44

The reactor was equilibrated at 20°C for 10 days and samples were removed daily over 3 subsequent days of operation. Values shown are the mean of three measurements or of logged readings over the same period: the error on measurements throughout was less than 10%. Inflow values were measured using samples removed from the medium feed to the reactor. The liquor outflow samples for the dilute (clarified liquor) and concentrated (concentrated solids and active culture) streams were taken from the outlets of the sedimentation and active zone respectively (Figure 1). Off gas samples were removed by syringe from a butyl rubber section of the gas outlet.

although the initial pH was also 2, so that pH control was mainly achieved by addition of alkali. Therefore, at 10 and 50 mM sulphate, an effective operating pH could be maintained in the bioreactor by sulphate reduction with significant alkali addition only required under more extreme conditions.

Carbon and sulphate utilisation

A more complete analysis of the carbon and sulphur metabolites was carried out at 191 mM sulphate where both sulphate and ethanol were present in excess, as were other nutrients (Table 3). As the protein concentration under these conditions was only slightly greater than at 10 mM sulphate (0.23 and 0.19 mg ml^{-1}, respectively), the biomass concentration of the active zone of the reactors was probably the major limiting factor to sulphate reduction. A proportion of the ethanol supplied was unused due to the large excess supplied: ethanol was therefore a major carbon component of the exit liquor. The only soluble carbon product detected in the liquor was acetate. Dissolved bicarbonate was not detectable although CO_2 was present in the off-gases where it represented a significant carbon flux (Table 3). An approximate mass balance for carbon is also given in Table 4 where approximately 70–80% of the carbon input is accounted for. It is probable that a significant amount of carbon was present in the solids as biomass and carbonates, which were not assayed. Soluble sulphide was present in the effluents but no other dissolved reduced sulphur compounds were detectable by polarography. Sulphide was also present as H_2S in the gas phase but these two sulphide flows only accounted for approximately 10% of the sulphate reduced (Tables 3, 4). This suggests that the main sink of sulphide produced was solid sulphides. Although available methods for determination of sulphide in the solid phase did not yield reliable results when applied to the reactor solids (data not shown), approximate values calculated from the solids dry weight suggested a value for sulphide present as solid in the range 30–50 mmol L^{-1} of working (active zone) reactor volume. On this basis, the percentage of sulphide accounted for would be similar to that of carbon (Table 4).

Table 4 Carbon and sulphur mass balances

	Carbon or sulphur flux through the reactor (mmol L^{-1} h^{-1})				
	Liquor			Off gases	Total outflow
	Inflow	Out_{dilute}	$Out_{concentrated}$		
Carbon					
ethanol	14.40	10.50	0.23	0	10.70
acetate	0	0.96	0.02	0	0.98
CO_2	0	ND	ND	0.32	0.32
Total carbon	28.80	23.10	0.50	0.32	23.92
Sulphur					
sulphate	5.50	2.70	0.06	0	2.80
sulphide (sol.)	0	0.07	0	0	0.07
H_2S (gas)	0	0	0	0.21	0.21
(sulphide solids)*	0	0.30	0.06	0	0.36
Total sulphur	5.50				3.40

The mass balances were calculated using data from Table 3. All carbon and sulphur inputs were added in the nutrient stream and the total outflow shown for each component is the sum of the fluxes in the individual outflow streams. *Due to poor analytical reproducibility for sulphides in the solid phase, the mass-balance was constructed assuming 1/3 of the solid dry weight to be sulphide. ND = not determined.

Table 5 Internal sedimentation bioreactor effluent metal concentrations compared with environmental target levels

Metal	Concentration (μM)		
	Effluent	Target maximum*	
		Groundwater	Drinking water
Cd	0	0.05	0.05
Co	4.0	3.38	0.98
Cr	3.3	3.85	0.98
Cu	0	3.17	0.79
Mn	278.0	NA	0.93
Ni	16.5	2.56	0.86
Zn	1.0	12.30	11.56

Internal sedimentation bioreactor cultures were equilibrated for 10 days using SRB1 medium containing 100 mM sulphate, 200 mM ethanol and 1.8 g L^{-1} cornsteep and 200 μM of each metal except for Cd (40 μM) and Mn (500 μM). Samples were removed over 3 subsequent days for analysis: mean values are shown, the relative standard error being less than 5% throughout. The target concentrations (*) are based on EC standards [10]. NA = not available (a standard was not given for Mn concentrations in groundwater because this element was not regarded as a potential hazard [10]).

Metal removal

Cd, Cr, Cu, Mn, Ni, Pb and Zn were all supplied at concentrations around those obtained in soil-bioleaching experiments (data not shown). With the exception of Mn and, to a lesser extent, Ni these were removed with >95% efficiency (Table 5). Bioprecipitation of these two elements was less efficient than that of the other target metals at lower concentrations in chemostat cultures [17] (and was also lower in another study [20]), which suggests that this is an effect of their chemistry rather than factors such as the high Mn concentration. The solubility products of both Mn and Ni sulphides are some orders of magnitude higher than those of the other metals present but appear to be still sufficiently low to allow efficient removal as sulphides [6]. The relatively poor performance in this case was probably the result of interaction with other components in the system. However, it is clear that the reactor was capable of removing metals from the stimulated leachate with effluent liquor concentrations of most metals (except nickel) being acceptable for environmental discharge [10] (Table 5).

Conclusions

An internal sedimentation bioreactor had several features which rendered it suitable for sulphide bioprecipitation of toxic metals from soil leachates. The internal sedimentation mechanisms, operating with the addition of flocculating agents, allowed the development of high concentrations of biomass. This in turn permitted almost complete reduction of sulphate concentrations up to 50 mM although reducing activity appeared to be less adequate at higher concentrations. Efficient sulphate reduction at <50 mM also maintained the pH of the reactor close to a suitable operating value, minimising input of alkali and accumulation of sodium which could otherwise limit the recycling of process water. However, the results indicated that alkali addition would be necessary to maintain a working pH at higher sulphate concentrations. The main purpose of the sulphate-reducing component within the integrated process was to immobilise the toxic metals removed from the soil by the leaching stage. It was clear that the reactor was able to perform this function efficiently for simulated acid leachate.

Acknowledgements

GM Gadd gratefully acknowledges financial support from the BBSRC Biochemical Engineering Link Programme (BCE03292). This work was carried out as part of the LINK project 'Development of an integrated bioremediation route for contaminated land and liquid effluents' in collaboration with British Nuclear Fuels plc, Preston, Lancs, UK and Viridian Bioprocessing Ltd, Whitstable, Kent, UK.

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