ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

Enhancement of Biogenic Sulfide Production in a Packed-Bed Bioreactor Via Critical Inoculum Design and Carrier Material Selection

Matthew J.L. McMahon, Andrew J. Daugulis

Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada K7L 3N6; telephone: 613-533-2784; fax: 613-533-6637; e-mail: daugulis@chee.queensu.ca

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ABSTRACT: Sulfate reducing bacteria (SRB) are commonly used in environmental bioprocesses for the treatment of acid mine drainage and sulfate wastewaters. Biogenic H_2S is also a potential source of H_2 fuel with the recent development of H_2S splitting technologies. In this study, a sulfate reducing packed bed bioreactor (PBR) capable of rapidly achieving high volumetric productivities was developed using a novel method of rational inoculum design and the selection of improved biomass carrier materials. An inoculum with initial composition of ~95% *Desulfovibrio desulfuricans* (ATCC 7757) and 5% SRB consortium was designed based on the pure strain's superior immobilization potential and the SRB consortium's superior kinetics. Diatomaceous earth (DE) pellets, porous glass beads, polyurethane foam and bone char were evaluated as potential biomass carrier materials. The DE pellets immobilized the most biomass and were employed in two packed bed bioreactor fermentations. Using the designed inoculum and DE pellets, a packed bed bioreactor achieved a volumetric productivity of 493 mol $H_2S\,m^{-3}\,day^{-1}$ (based on a 308 mL working volume) with a dissolved sulfide concentration of 9.9 mM. This occurred after 8.3 days of operation and represents a tenfold reduction in the start-up period compared to other sulfate reducing PBRs described in the literature.

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KEYWORDS: bioreactor; consortium; *Desulfovibrio desulfuricans*; immobilized; sulfate reducing bacteria

Introduction

Sulfate reducing bacteria (SRB) play a key role in the anaerobic degradation and eventual mineralization of organic matter. They utilize a wide variety of small molecular weight substrates to reduce sulfate to sulfide (HS^-, H_2S) with concomitant production of bicarbonate alkalinity. Lactate, butyrate, ethanol and methanol are common SRB substrates that are produced during the anaerobic degradation of organic matter. The catabolic metabolism of SRB is described by Equation (1) for the oxidation of a generic carbohydrate substrate (Widdel, 1988):

$$2CH_2O + SO_4^{2-} \xrightarrow{SRB} 2HCO_3^- + H_2S \qquad (1)$$

The metabolic properties of SRB are currently exploited in a variety of environmentally focused bioprocesses including the treatment of sulfate/sulfite waste streams (Colleran et al., 1995; Lens et al., 2003), treatment of acid mine drainage (Neculita et al., 2007) and the bioremediation of recalcitrant xenobiotics (Boopathy et al., 1998). With the emergence of H_2S splitting technologies (Ni et al., 2006; Ohashi et al., 1998), H_2S also has the potential to serve as a precursor molecule for H_2 fuel. This represents a novel approach to biological H_2 production with potential advantages compared to the low H_2 yields of dark fermentation (Angenent et al., 2004) and the endothermic process of steam reforming biogas CH_4 to H_2.

Due to the intrinsically slow growth rates of anaerobic bacteria, immobilized cell bioreactors have become the de facto standard for high-rate SRB bioprocesses regardless of the intended application. The most recent studies have employed packed bed bioreactors (PBR) due their operational robustness and decreased maintenance requirements (Alvarez et al., 2006, 2007; Baskaran and Nemati, 2006; Kolmert and Johnson, 2001).

A common measure of SRB bioreactor performance is the volumetric rate of sulfate reduction (mol $SO_4^{2-}\,m^{-3}\,day^{-1}$) or sulfide production (mol $H_2S\,m^{-3}\,day^{-1}$), the latter being a more conservative measure despite their theoretical equivalence. Volumetric rates as high as 677 mol $SO_4^{2-}\,m^{-3}\,day^{-1}$ (65 g $SO_4^{2-}\,L^{-1}\,day^{-1}$) have been achieved in PBRs only after gradual increases in the loading rates with start-up periods in excess of 100 days (Stucki et al., 1993). In order to increase the

Correspondence to: A.J. Daugulis

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viability of sulfate reducing bioprocesses, a drastic reduction in the start-up period is required.

Two key design criteria affect PBR volumetric productivity: the choice of carrier material and the inoculum composition. The ability to readily immobilize large volumes of biomass is crucial to rapidly 'seeding' the PBR and reducing start-up times. Numerous carrier materials are currently used for SRB immobilization which suggests that a single, high performance immobilization material has yet to be identified. The second criterion, inoculum composition, plays a significant role in bioreactor performance due to the constituent species' intrinsic kinetics and immobilization potential (i.e., predisposition for attached growth and biofilm formation). Despite the seemingly obvious implications of inoculum composition, recent studies have continued to use undefined consortia (Alvarez et al., 2006, 2007; Baskaran and Nemati, 2006; Selvaraj et al., 1997) with little regard for the inoculum's characteristics so long as it exhibits sulfate reducing activity.

The objective of this study was to develop a sulfate reducing PBR that was capable of achieving high volumetric productivities within a significantly decreased start-up period. The aspect of carrier material selection was addressed by evaluating four candidate materials. Polyurethane foam and Poraver™ porous glass beads were selected as benchmark materials that had been used extensively in previous studies. Bone char and Celite™ R-635 diatomaceous earth pellets were selected as novel materials for SRB immobilization. The effect of inoculum composition was investigated by comparing the kinetics and immobilization potential of a pure culture (*Desulfovibrio desulfuricans*) and an SRB consortium. The pure culture and consortium were evaluated independently and as a mixed inoculum over a range of initial fractional compositions.

Materials and Methods

Growth Medium and Sulfate Reducing Bacteria

Postgate C (PC) medium (Postgate, 1984) was used for all batch and continuous PBR fermentations. It contained per liter of distilled water: 0.5 g KH_2PO_4, 1 g NH_4Cl, 4.5 g Na_2SO_4, 0.06 g $MgSO_4{\cdot}7H_2O$, 0.06 g $CaCl_2{\cdot}H_2O$, 10 g sodium lactate (60% w/w syrup), 1 g yeast extract, 0.1 g sodium thioglycollate, 0.004 g $FeSO_4{\cdot}7H_2O$ and 5.0 g sodium citrate dihydrate. Initial pH was adjusted to 6.5 using 3 M NaOH and 1 M HCl but was otherwise not adjusted during the experiments.

D. desulfuricans subsp. *desulfuricans* (ATCC 7757) was obtained from the American Type Culture Collection (Manassas, VA). The SRB consortium was enriched from a municipal landfill leachate sample obtained at a depth of 15 m and was graciously provided by Dr. G. Voordouw of the University of Calgary, Alberta. The consortium was initially enriched using a defined lactate-sulfate medium and then on PC medium until successive transfers exhibited constant yields of biomass (X, cell dry weight (CDW)) to sulfate reduced ($Y_{X/S} = 0.11$ g CDW g^{-1} SO_4^{2-}). Each transfer (2%, v/v) occurred after the culture had reached stationary phase with a dissolved sulfide concentration of 15–20 mM. Enrichments and stock cultures were performed in 100 mL liquid volume serum bottles under anaerobic conditions. All work was performed aseptically using 121°C steam sterilization and 0.20 μm filtration methods as required.

Analytics

Suspended biomass concentrations were measured at 600 nm using a Biochrom Ultrospec 3000 UV/Vis spectrophotometer and correlated to CDW. Dissolved sulfide (H_2S, HS^-) was measured at 480 nm according to the CuS precipitation reaction described by Cord-Ruwisch (1985). Dissolved sulfate was measured at 420 nm according to the $BaSO_4$ precipitation reaction described by Kolmert et al. (2000).

Lactate was measured at 210 nm using a Waters 2487 UV–Vis HPLC equipped with an Atlantis dC_{18} 5 μm, 40 mm × 150 mm column. The mobile phase was 20 mM $NaH_2PO_4{\cdot}2H_2O$ (pH 2.7) at 1.2 mL min^{-1}.

Bacterial immobilization was confirmed by scanning electron microscopy (SEM). After an 11 days PBR fermentation, the bioreactor was drained and R-635 pellets were taken from different points along the bioreactor and pre-treated for SEM imaging. The pellets were immersed in aqueous solutions of 25%, 50%, 75%, and 100% (v/v) histological grade alcohol for 20 min each. The dehydrated samples were coated with SEM grade silver paint, affixed to aluminum stubs, dried and micrographed with a LEO 1530 field emission SEM at an accelerator voltage of 3.5 kV and a working distance of 3–6 mm.

Carrier Materials

Polyurethane foam plugs (6.35 mm D × 12.17 mm H; \$US 5.18 L^{-1}) were used as purchased from Fisher Scientific. Bone char (5 × 8 mesh; \$US 1.99 L^{-1}) was purchased from Ebonex (Detroit, MI) soaked in acid for 1 h (2 L 1 M HCl kg^{-1} bone char), rinsed in distilled water and dried at 80°C to remove carbonate alkalinity. Porous glass beads (1–2 mm D; \$US 0.36 L^{-1}) were donated by Poraver North America (Barrie, Ontario). Celite™ R-635 diatomaceous earth pellets (6.35 mm D × 12.7 mm H, \$US 13.30 L^{-1}) were donated by World Minerals (Santa Barbara, CA). Glass beads and R-635 pellets were rinsed in distilled water and dried at 80°C before use.

Kinetic and Immobilization Studies

Batch fermentations for kinetic characterization of *D. desulfuricans* and the SRB consortium were conducted in

New Brunswick Scientific BioFlo I bioreactors (2 L liquid volume) stirred at 90 RPM by two Rushton turbines. All fermentations were conducted at $34 \pm 1^{\circ}C$ under anaerobic (N_2) conditions with an initial cell concentration of ~ 0.050 g CDW L^{-1}. The inoculum consisted of various ratios (0–100%) of growth phase *D. desulfuricans* and the SRB consortium. Batch kinetic data was used to calculate biomass to sulfate yields ($Y_{X/S}$), specific growth rates (μ) and specific rates of sulfate reduction (Q_s).

Comparisons of carrier materials and the immobilization potential of various inoculum compositions were performed by circulating 2 L stationary phase SRB cultures through glass column PBRs for 20 h at 200 mL h^{-1} at ambient conditions (Fig. 1). The amount of immobilized biomass was estimated by performing a mass balance on the liquid culture cell density at $t = 0$ and $t = 20$ h.

The upflow PBR dimensions were 38 cm × 4.5 cm with a total volume of 615 mL. Butyl rubber sampling ports were located at the inlet, midpoint and outlet of the bioreactor. Prior to use, the PBRs were aseptically packed with carrier materials and purged with N_2. Liquid feed rates were controlled with an IMED Gemini-1 digital peristaltic pump. PBR temperatures were controlled by circulating thermostated water through Tygon tubing coils. The entire apparatus was covered in removable foam insulation.

Packed Bed Bioreactors Studies

Two continuous fermentations, aimed at achieving high volumetric productivities, were similarly inoculated by circulating 2 L SRB cultures through PBRs for 48 h at 200 mL h^{-1}. After inoculation, the PBRs were fed with PC medium at an initial feed rate of 20 mL min^{-1} and maintained at $38 \pm 1^{\circ}C$. When the outlet dissolved sulfide had reached the inhibitory concentration of ~15 mM (Okabe et al., 1992; Reis, 1992; Reis et al., 1991) the feed rate was increased step-wise to approximately twice the previous rate. This was repeated until feed rates of up to 640 mL h^{-1} were attained, equivalent to a hydraulic rate time (HRT) of 37.5 min or a dilution rate (D) of 1.6 h^{-1} based on packed void volume.

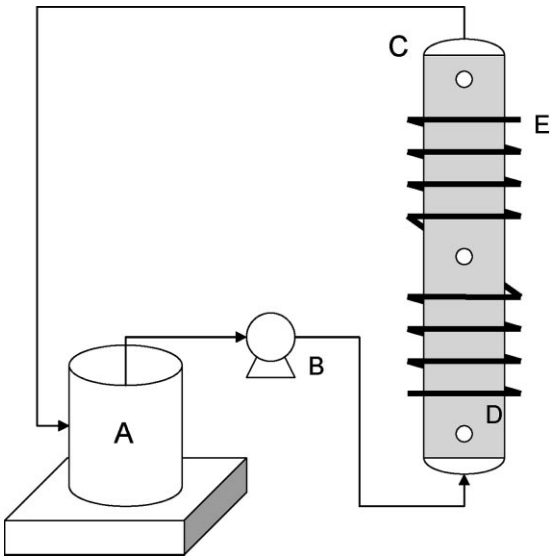

Figure 1. Schematic diagram of the experimental setup for carrier material and immobilization potential studies. During continuous PBR fermentations, the BioFlo bioreactor (A) was eventually replaced with a carboy of PC medium and the effluent was sent to a separate holding vessel. Legend: (A) BioFlo stirred tank bioreactor; (B) digital peristaltic pump; (C) packed bed bioreactor; (D) sampling port; (E) Tygon tubing heating coils (insulation not shown).

Results and Discussion

Carrier Material Comparison

Stationary phase *D. desulfuricans* cultures were circulated through PBRs containing four different carrier materials to compare the materials' respective ability to immobilize SRB biomass (Fig. 2).

Poraver™ porous glass beads were selected as a benchmark material because of their frequent use in the literature for SRB immobilization (Alvarez et al., 2006; Kolmert et al., 1997; Kolmert and Johnson, 2001; Nagpal et al., 2000). Of the four materials evaluated, Poraver™ immobilized the least amount of *D. desulfuricans* (0.083 g CDW). The 1–2 mm beads were easily crushed, which diminishes their applicability at larger scales. Although it was the cheapest of the materials evaluated, Poraver™'s poor immobilization capacity and low durability precluded its use in subsequent continuous PBR fermentations. It was encouraging, however, that three other materials had been identified that exhibited improved immobilization capacity compared to this benchmark material.

Polyurethane foam was also selected as a benchmark carrier material due to its frequent use for SRB immobilization

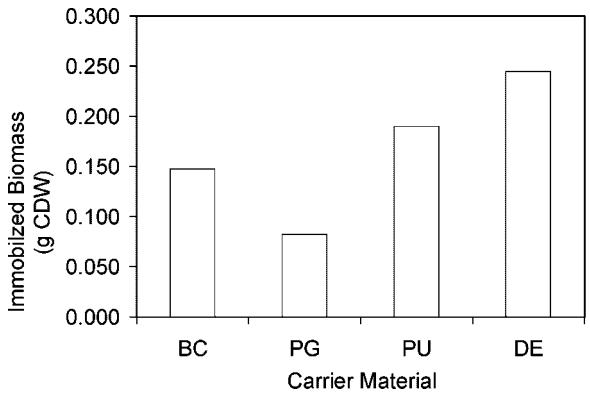

Figure 2. Biomass immobilized (g CDW) on different carrier materials after circulating 2 L stationary phase *Desulfovibrio desulfuricans* cultures through 615 mL PBRs for 20 h. BC, bone char; PG, Poraver™ porous glass beads; PU, polyurethane foam; DE, R-635 diatomaceous earth pellets.

(Cadavid et al., 1999; Cattony et al., 2005; Silva et al., 2006; Stucki et al., 1993). Polyurethane foam immobilized the second largest amount of biomass (0.190 g CDW) compared to the other three materials in this study. Unfortunately, its sponge-like structure had poor gas permeability. This was an undesirable property for future experiments involving the use of an N_2 strip gas to remove dissolved sulfide and enhance conversion (McMahon and Daugulis, 2008). Due to the potential for gas entrainment, polyurethane foam was used only for short-term experiments to evaluate the immobilization potential of *D. desulfuricans* and the SRB consortium.

Bone char had been selected as a novel candidate material due to its previous success in immobilizing anaerobic bacteria over a range of bioprocesses including butanol production (Qureshi et al., 2005) and treatment of petrochemical wastewater (Patel and Madamwar, 2002).

Compared to the other three materials, bone char immobilized a moderate amount of *D. desulfuricans* (0.147 g CDW) but was very brittle and crumbled under normal handling conditions. In addition, bone char contained 7–9% (w/w) $CaCO_3$ that if not removed by acid pretreatment, increased the medium pH and severely limited SRB activity in serum bottle cultures (data not shown). Due to these drawbacks and moderate immobilization capability, bone char was not considered for use in subsequent PBR fermentations.

Like bone char, Celite^{TM} R-635 was selected as a novel candidate material due to its previous use in a variety applications including bioscrubbers (Sorial et al., 1998; Wright, 2005) and wastewater treatment (Bertin et al., 2004; Durham et al., 1994; Kim et al., 2002; Peres et al., 1999). In this study the R-635 pellets immobilized the most biomass (0.245 g CDW) and exhibited other properties desirable in a carrier material. SEM micrographs of R-635 pellets revealed a highly microporous structure well suited for bacterial immobilization with an average pore size (manufacturer reported) of 20 μm. The pellets themselves were durable, inert and designed for use at larger scales. R-635 pellets were thus selected for use in all subsequent continuous PBR fermentations.

Microbial Systems Characterization

Batch fermentations of *D. desulfuricans* and the SRB consortium were conducted to compare their microbial kinetics (Table I). With respect to reaction stoichiometry both microbial systems had similar biomass yield coefficients ($Y_{X/S}$) of ~0.11 g CDW g^{-1} SO_4^{2-}. Sulfate reduction was coupled to lactate oxidation at a ratio of ~0.47 mol SO_4^{2-} mol^{-1} lactate in both microbial systems and was indicative of incomplete oxidation with stoichiometric production of acetate according to Equation (2). Previous work with both microbial systems confirmed reduction of sulfate to sulfide at a 1:1 molar ratio (McMahon and Daugulis, 2008). The acetate by-product represents a residual COD which requires mineralization before effluent discharge. The use of a two-stage sulfate reduction process has been previously described in the literature for mineralization of the residual acetate (Deswaef et al., 1996)

$$2CH_3CHOHCOO^- + SO_4^{2-} \xrightarrow{SRB} 2CH_3COO^- + 2HCO_3^- + H_2S \quad (2)$$

Although the microbial systems shared a similar reaction stoichiometry, the SRB consortium's specific rate of sulfate reduction (Q_s, g SO_4^{2-} g^{-1} CDW h^{-1}), was twice as fast as that of *D. desulfuricans* (Table I). The SRB consortium's kinetic advantage was anticipated as the enrichment method of serial transfers, by design, selected for species with superior μ and Q_s values under the imposed conditions.

Effect of Inoculum Composition on Kinetics

Additional 2 L batch fermentations were conducted to evaluate the effect of inoculum composition on specific growth rates. A nonlinear trend between specific growth rate and the % consortium in the initial inoculum was observed (Fig. 3).

The importance of these results was not fully appreciated until considered in conjunction with the microbial systems' immobilization potential (Fig. 4). The immediate implication was that the inclusion of even small fractions of consortium in the initial inoculum dramatically increased the specific growth rate relative to that of the pure species. The nonlinear relationship was attributed to the exponential nature of microbial growth whereby the kinetically superior SRB consortium rapidly became the dominant population in suspended growth batch fermentations. The SRB consortium's superior kinetics rates may have been partially due to an increased resistance to sulfide inhibition. This characteristic may have been selected for during the enrichment process by allowing the cultures to consistently reach inhibitory dissolved sulfide concentrations (15–20 mM) between transfers.

Table I. Batch growth kinetic properties for *Desulfovibrio desulfuricans* and an SRB consortium.

	$Y_{X/S}$ (g CDW g^{-1} SO_4^{2-})	μ (h^{-1})	Q_s (g SO_4^{2-} g^{-1} CDW h^{-1})
Desulfovibrio desulfuricans	0.10–0.12	0.07–0.09	0.58–0.90
SRB consortium	0.11	0.12–0.13	1.1–1.2

Fermentations were at 34°C and performed in duplicate.

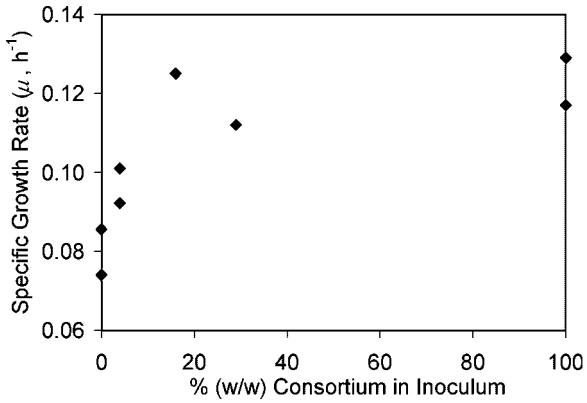

Figure 3. Specific growth rates (μ, h^{-1}) of 2 L SRB batch fermentations (34°C) as a function of initial inoculum composition. Zero percent consortium corresponds to 100% *Desulfovibrio desulfuricans*.

As the microbial kinetic data was obtained from suspended growth cultures, some discretion is required in applying it to attached growth systems as kinetic behavior may diverge significantly from suspended cell data (Okabe, 1992). For instance, recent studies have indicated that sulfide inhibition in SRB is a function of the biomass morphology (biofilm, granular or suspended) with some SRB biofilm systems operating continuously at sulfide concentrations as high as 36 mM (Celis-Garcia et al., 2007; Kaksonen et al., 2004a). Due to the comparative difficulty in obtaining biofilm kinetic parameters, the use of suspended cell data remains the most convenient method of making initial comparisons between cell lines.

Effect of Inoculum Composition on Immobilization Potential

Based solely on kinetics, the previous experiments suggested that the SRB consortium would be the preferred microbial system in suspended growth bioreactors. In attached growth PBRs however, immobilization potential has an equally important role in the overall bioreactor performance. Thus, immobilization potential was evaluated for the SRB consortium and *D. desulfuricans* separately and as various mixed inoculums (Fig. 4). Using both polyurethane foam and R-635 pellets as carrier materials, a nonlinear inverse relationship between immobilized biomass and the % consortium in the initial inoculum was observed. *D. desulfuricans* had a considerably better immobilization potential compared to the SRB consortium. In a trend similar to that of Figure 3, the addition of even small fractions of SRB consortium to the inoculum was apparently sufficient for the consortium to become the dominant population as demonstrated by the sharp decrease in the immobilization potential.

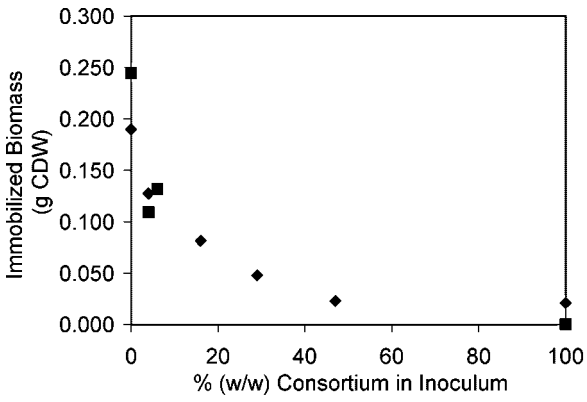

Figure 4. Immobilized biomass (g CDW) in a PBR as a function of initial inoculum composition. 0% consortium corresponds to 100% *Desulfovibrio desulfuricans*. Carrier materials were polyurethane foam (◆) and R-635 pellets (■).

D. desulfuricans had been selected, in part, for its rapid kinetics and attached growth characteristics (Beyenal and Lewandowski, 2004; Chen et al., 1994; Konishi et al., 1996; Lee et al., 2006; Lopes et al., 2006; Okabe et al., 1994). In serum bottles cultures, *D. desulfuricans* formed thick, viscous biofilms ~48 h after reaching stationary phase. The SRB consortium, in contrast, produced only a small amount of rapidly settling flocs less than 1 mm in diameter with no indication of biofilm formation. Despite the absence of visible biofilm it was not believed that the consortium was entirely incapable of attached growth. SRB are notorious for producing biofilms in natural environments and engineered systems such as pipeline networks (Chen et al., 1994). This would suggest that the landfill leachate sample from which the SRB consortium was derived might have originally contained an SRB population more predisposed to attached growth. The enrichment process, in retrospect, was not well suited to isolate for attached growth species. Biofilm forming SRB would have been at a competitive disadvantage by directing energy and carbon resources towards extracellular polysaccharide (i.e., biofilm) formation at the expense of cellular replication. Transferring samples by syringe would also have selected for suspended growth rather than attached growth species.

An initial inoculum composition of approximately 95% SRB *D. desulfuricans* and the balance SRB consortium was chosen for use in all subsequent PBR fermentations. The immobilization of large masses of SRB during the PBR start-up period was considered imperative to warrant a small loss of initial kinetic performance. It was anticipated that the extracellular polysaccharide material produced by *D. desulfuricans* would serve as a biofilm foundation layer onto which the rapidly growing SRB consortium could colonize. Continuous flow conditions would then naturally select for the optimal balance between the slower growing *D. desulfuricans* and the faster growing consortium. This was qualitatively confirmed by inoculating a serum bottle culture with a sample from a PBR that had been operating for 11 days. The culture exhibited sulfate reducing activity, but did not form the characteristic biofilms of *D. desulfuricans*, suggesting that it was no longer the

dominant species. Future work has been proposed that will use molecular methods to track population shifts and provide quantitative confirmation of this hypothesis in a manner similar to that described by Kaksonen et al. (2004b).

Continuous PBR Fermentations

After the identification of a superior carrier material and inoculum composition, two continuous PBR fermentations were conducted using R-635 pellets and a ~95:5 *D. desulfuricans*:SRB consortium inoculum. The objective of both fermentations was to achieve a high volumetric productivity with a minimal start-up period. The second fermentation also evaluated the PBR's response and recovery from an induced process shutdown as a test of the system's robustness.

Throughout both fermentations, the effluent dissolved sulfide concentration was relatively constant within the range of 10–15 mM (Fig. 5). When feed rates were increased, there was a transient decrease in dissolved sulfide concentration due to dilution effects. As the biomass inventory within the PBR adjusted to changes in feed rates, the dissolved sulfide concentration returned to its original steady-state value. The increase in dissolved sulfide concentration along the second half of the PBR was negligible and indicated that the majority of the biological activity (i.e., sulfate conversion) was occurring within the first half of the PBR. Such behavior is typical of inhibited PBRs where the accumulation of a toxic product significantly diminishes kinetic rates along the length of the bioreactor (Chen et al., 1994; Daugulis and Swaine, 1987). As anticipated, the region with the highest biological activity (i.e., bioreactor inlet) contained the most immobilized biomass on the R-635 pellets (Fig. 6A–C).

Given that negligible conversion was occurring within the second half of the bioreactor, volumetric productivities were calculated based on 50% of the total PBR volume (308 mL) and dissolved sulfide concentration at the middle sampling port. Volumetric productivity (mol $H_2S\,m^{-3}\,day^{-1}$) time course plots for both fermentations (Figs. 7 and 8) were similar in nature and suggested good reproducibility of

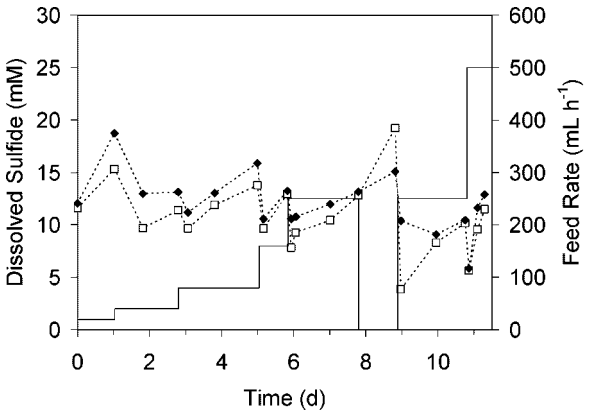

Figure 5. Dissolved sulfide concentrations (dashed lines) measured at the middle (□) and outlet (◆) of the PBR for continuous fermentation #2. Concentration profiles were typical of both PBR fermentations. Feed rate (solid line) was increased from 20 to 500 mL h^{-1} with a shutdown period at 7.8 days.

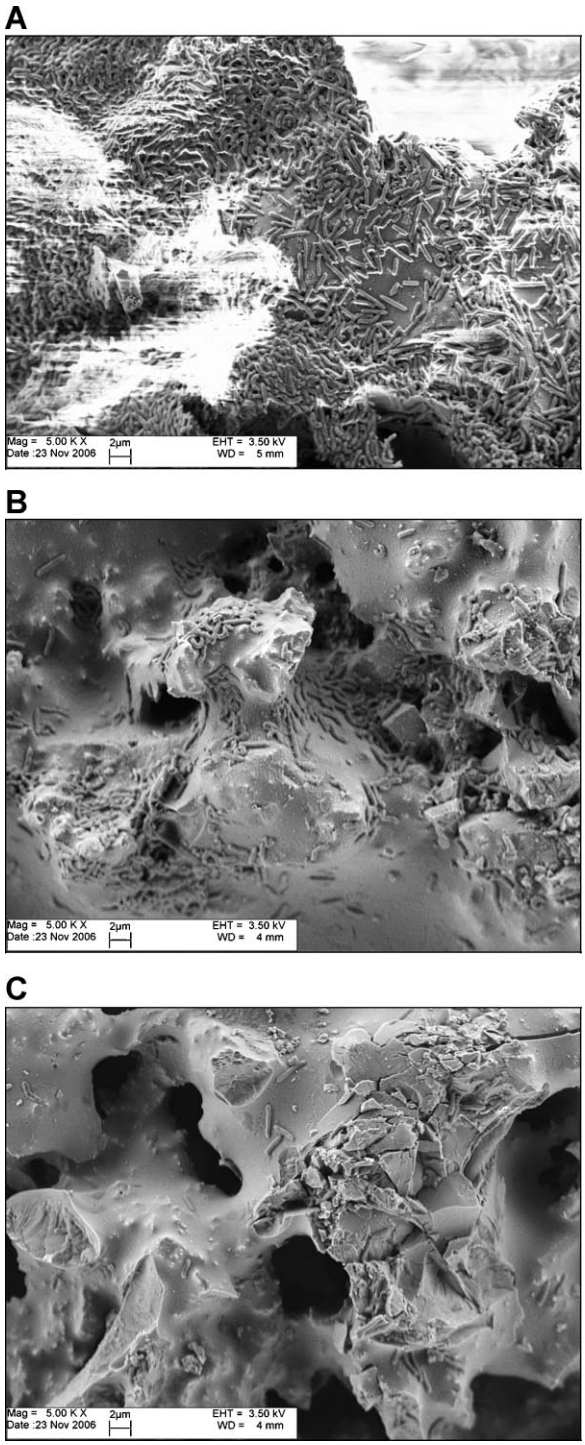

Figure 6. Scanning electron micrographs (5,000×) of the surface of R-635 pellets retrieved from the inlet (A), midpoint (B) and outlet (C) of a PBR after an 11 days fermentation. Scale bar shows 2 µm.

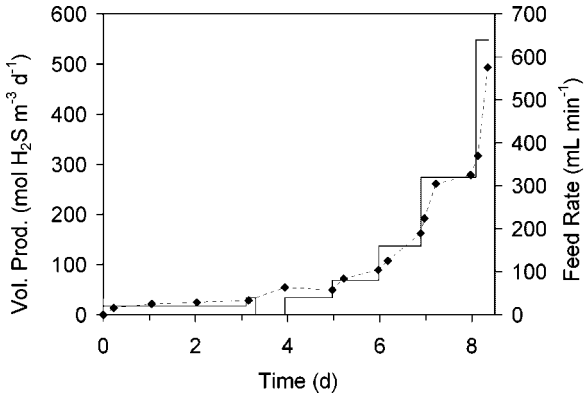

Figure 7. Volumetric sulfide productivity-time course plot (◆) for PBR fermentation #1. Feed rate (solid line) was increased from 20 to 640 mL h^{-1}. Productivity values were based on a 308 mL bioreactor working volume.

the results. In both cases, volumetric productivities up to 493 mol H_2S m^{-3} day^{-1} were achieved within 11.3 days after start-up at 20 mL h^{-1} (Table II). These results are a significant decrease in the start-up period associated with high volumetric productivities. Stucki et al. (1993) and Selvaraj et al. (1997) both achieved comparable productivities after start-up periods greater than 100 days using more elaborate PBR systems that included recycle loops, sulfide stripping columns and online pH control. Differences in operational parameters such as HRT, substrate and pH may account for some differences in overall performance (Kaksonen et al., 2004a). More recently however, Baskaran and Nemati (2006) used a single-pass, lactate fed PBR similar to that used in this study but achieved a volumetric productivity of only 57 mol SO_4^{2-} m^{-3} $days^{-1}$ after 119 days of operation. This suggests that the use of serially enriched, poorly immobilizing SRB consortia is a key reason for the extended start-up periods associated with the previous studies.

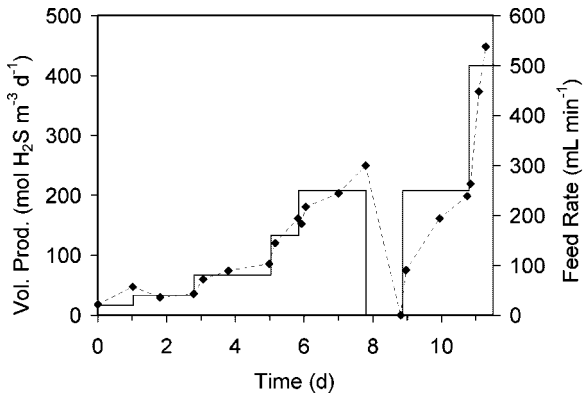

Figure 8. Volumetric sulfide productivity-time course plot (◆) for PBR fermentation #2. Feed rate (solid line) was increased from 20 to 500 mL h^{-1}. Productivity values were based on a 308 mL bioreactor working volume.

Table II. Summary of the highest volumetric sulfide productivities in two separate PBR fermentations.

PBR fermentation	Feed rate (mL h^{-1})	Dilution rate (h^{-1})	Dissolved sulfide (mM)	Vol. prod. (mol H_2S m^{-3} day^{-1})	Start-up period (days)
#1	640	1.60	9.9	493	8.3
#2	500	1.25	11.5	448	11.3

Postgate medium contained 32 mM sulfate and 54 mM lactate. Productivity values were based on a 308 mL bioreactor working volume.

During the second fermentation, the PBR's ability to recover from a 24 h shutdown period was evaluated by decreasing the feed rate from 250 to 0 mL h^{-1} and operating at ambient temperature (Figs. 5 and 8). The shutdown period resulted in total substrate depletion with dissolved sulfide concentrations of 30–15 mM along the length of PBR. After the feed rate was re-started at 250 mL h^{-1}, some biomass sloughing was observed but performance was quickly restored to its previous level within 2 days and then doubled to 448 mol H_2S m^{-3} day^{-1} (at 500 mL h^{-1}). The bioreactor's rapid recovery after a period of substrate depletion with highly inhibitory sulfide concentrations was an indication of the operational advantages that can be obtained by using R-635 pellets in conjunction with the designed inoculum composition: namely, excellent immobilization capacity and the ability to sustain microbial growth under adverse conditions.

Conclusions

Results from this study have demonstrated the largely unrecognized importance of inoculum design for improving the performance of sulfate reducing PBRs. The standard method of suspended growth serial transfer enriched for a kinetically favorable SRB consortium that reduced sulfate ($Q_s = 1.2$ g SO_4^{2-} g^{-1} CDW h^{-1}) at twice the specific rate of the benchmark species *D. desulfuricans*. Unfortunately, the consortium exhibited negligible immobilization potential compared to the biofilm forming *D. desulfuricans*. By combining these two microbial systems, a mixed inoculum was designed that exploited the advantages of the individual cell lines. Celite™ R-635 diatomaceous earth pellets were shown, for the first time, to be a suitable carrier material for SRB and outperformed other known SRB carrier materials such as Poraver™ porous glass beads and polyurethane foam.

Using an inoculum with an initial composition of ~95% *D. desulfuricans* and 5% consortium, a Celite™ R-635 filled PBR was able to achieve a volumetric sulfide production rate of 493 mol H_2S m^{-3} day^{-1} (HRT = 38 min; $D = 1.6$ h^{-1}) after only 8.3 days of operation. This represents an order of magnitude decrease in start-up time compared to other PBR

studies that achieved similar volumetric rates after approximately 100 days.

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