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Microbial Reduction of Sulfur Dioxide with Pretreated Sewage Sludge and Elemental Hydrogen as Electron Donors

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ABSTRACT

It has been demonstrated that heat- and alkali-pretreated sewage sludge may serve as an electron donor and carbon source for SO_2 reduction by *Desulfovibrio desulfuricans*. A continuous *D. desulfuricans* culture was operated for 6 mo with complete reduction of SO_2 to H_2S. The culture required only minor amounts of mineral nutrients in addition to pretreated sewage sludge. It has also been shown that the sulfate-reducing bacterium *Desulfotomaculum orientis* can be grown on H_2 as an energy source, CO_2 as a carbon source, and SO_2 as a terminal electron acceptor. Complete reduction of SO_2 to H_2S was observed.

Index Entries: Sulfur dioxide; *Desulfovibrio desulfuricans; Desulfotomacululm orientis;* flue-gas desulfurization.

INTRODUCTION

We have previously demonstrated that the sulfate-reducing bacterium, *Desulfovibrio desulfuricans* may be grown in mixed culture with fermentative heterotrophs in a medium in which glucose served as the only carbon source. Beneficial crossfeeding resulted in vigorous growth of *D. desulfuricans,* which used SO_2 (1.0% SO_2, 5% CO_2, balance N_2) as a terminal electron acceptor with complete reduction of SO_2 to H_2S with only 1–2 s of contact time. Sulfate-reducing bacteria (SRB) cannot use simple sugars

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(such as glucose) as carbon and energy sources. However, the fermentative heterotrophs that developed in these cultures as a result of septic operation utilized glucose and produced fermentative end products (ethanol and lactic acid), which served as carbon and energy sources for *D. desulfuricans*. Sulfate-reducing bacteria are also strict anaerobes; mere exclusion of oxygen is not sufficient to support growth of pure cultures. Redox-poising agents are generally required to maintain a redox potential in the medium of −150 to −250 mV. However, in *D. desulfuricans* working cultures, no redox-poising agents were required (*1,2*).

The authors have proposed that the concentrated SO_2 stream obtained from certain regenerable processes for flue-gas desulfurization, such as the copper oxide process, may be split with two-thirds of the SO_2 reduced to H_2S by contact with a culture of sulfate-reducing bacteria (*1,3*). The resulting H_2S may then be combined with the remaining SO_2 and used as feed to a Claus reactor to produce elemental sulfur (*4,5*).

An economic evaluation of this microbial SO_2-reduction process concept was reported comparing microbial SO_2-reduction to conventional catalytic SO_2 hydrogenation with H_2 generation from methane (*6*). This analysis showed that the microbial reduction process is not competitive with conventional SO_2-reduction techniques when glucose (DE95 corn hydroysate) is utilized as the feedstock electron donor. However, the microbial process could be competitive with lower cost feedstocks.

Here an investigation is reported of two cheaper feedstocks or carbon and energy sources (electron donors) for SO_2-reducing cultures that have the potential for significantly lowering the costs of raw materials. These feedstocks are municipal sewage sludge and $CO_2(g)/H_2(g)$. Heat- and alkali-pretreated sewage sludge has been used as a carbon and energy source for a continuous SO_2-reducing culture of *Desulfovibrio desulfuricans*. Also SO_2-reducing batch cultures of *Desulfotomaculum orientis* have been grown with CO_2 as a carbon source and H_2 as an energy or electron donor.

MATERIALS AND METHODS

Sewage Sludge as an Electron Donor for SO_2 Reduction

D. desulfuricans (ATCC 13541) was obtained from the American Type Culture Collection (Rockville, MD). Stock cultures were maintained as previously described (*1*).

A continuous SO_2-reducing culture of *D. desulfuricans* with a feed of raw sewage sludge and/or yeast extract was developed as follows. First a batch culture of *D. desulfuricans* was developed septically in a complex medium as previously described and operated in a fed-batch mode with daily additions of 1.0% glucose (*1*). Sulfate was the terminal electron acceptor. This culture received a 300 mL/min N_2 purge. When the H_2S con-

Table 1
Effect of Heat/Alkali Treatment
on Suspensions of Sewage Sludge[a]

	Before treatment	After treatment
MLSS (mg/L)	5800	4370
Soluble COD (mg/L)[b]	70	4400
Soluble protein (mg/L)[b]	24	550

[a]One hundred grams per liter wet-packed sludge suspended in mineral salts medium, pH adjusted to 12.0 with 10*N* NaOH, autoclaved at 121°C for 30 min, and then pH adjusted to 7.0 with 6*N* H_3PO_4.
[b]Concentration in supernatant after centrifugation at 5000 x *g* and 4°C for 15 min.

centration exceeded 1000 parts per million on a volume basis (ppmv), the biomass was harvested by centrifugation and resuspended in a medium consisting of (in m*M* unless otherwise indicated): Na_2HPO_4 (8.5); KH_2PO_4 (13.2); $MgCl_2$ (7.4); NH_4Cl (3.7); $FeCl_3$ (0.25); Balch vitamin solution (2.0 mL/L); trace element solution (15.0 mL/L); Na_2SO_4 (42.3), and yeast extract (11.2 g/L) at pH 7.0. The Balch vitamin solution and the trace element solution have been described previously (*7,8*). This culture was initially operated in a fed-batch mode with daily additions of yeast extract (11.2 g/L) and subsequently in a continuous mode (*see* details of continuous operation *below*) with this same medium as feed at times supplemented with 100 g/L of wet-packed sewage sludge (without pretreatment). Sewage sludge was obtained from the recycle from the secondary settler of a municipal activated sludge treatment system in Tulsa, OK. The sludge was harvested from fresh samples by centrifugation at 5000×g and 25°C, and washed once with the medium described above (minus yeast extract) before use. These initial experiments demonstrated that raw sewage sludge would not support sulfate reduction, although yeast extract did. Evidently, *D. desulfuricans* and the mixed heterotrophs could not utilize the predominantly insoluble carbon and energy sources of the raw sewage sludge. These observations led to pretreatment of the sewage sludge to facilitate solubilization of the sludge biosolids.

A culture medium composed of pretreated sewage sludge was prepared as follows: 100 g of wet-packed sludge was suspended in 1 L of the above-described medium (without yeast extract or sulfate). The pH was then adjusted to 12.0 with 10*N* NaOH, and the suspension autoclaved at 121°C for 30 min. After cooling, the pH was readjusted to 7.0 with 6*N* H_3PO_4. Table 1 shows the MLSS, soluble chemical oxygen demand (COD), and protein concentrations before and after treatment of the sludge suspensions. As seen in Table 1, heat/alkali pretreatment solubilized a significant fraction of the sludge biosolids.

A continuous SO_2-reducing culture with a feed of heat/alkali pretreated sewage sludge was developed as follows. Sulfate-reducing biomass from the *D. desulfuricans* culture growing on yeast extract described above was harvested by centrifugation at 5000×g and 25°C. The biomass was then resuspended in 1.5 L of a filtered (Whatman No. 1 filter paper) preparation of pretreated sewage sludge medium described above in a B. Braun Biostat M fermenter. The feed for the fermenter consisted of unfiltered pretreated sewage sludge medium. The feed reservoir (1 L Erlenmeyer) was chilled with ice in an insulated container to slow subsequent microbial activity, which might reduce the concentration of fermentable substrates in the feed. Feed was pumped to the fermenter by a B. Braun FE 211 diaphragm pump initially at a rate of 12.0 mL/h resulting in a dilution rate of 0.19 d^{-1}. On day 59, the volumetric feed rate was reduced to 8.0 mL/h (0.13 d^{-1}) and remained at this level for the duration of the first experiment. Effluent from the fermenter was continuously removed by peristaltic pump by means of a 1/4-in stainless-steel tube at the culture surface that withdrew mixed liquor from the reactor as the volume increased with feed delivery.

The culture was maintained at pH 7.0 and 30°C. The agitation rate was 200 rpm. The culture received two gas feeds: 308 mL/min N_2 (to strip H_2S) and 9.8 mL/min of 1.0% SO_2, 5% CO_2, balance N_2. This corresponded to a molar SO_2 feed rate of 0.236 mmol/h.

The feed and effluent were continuously monitored for MLSS, soluble COD, and total and soluble protein. Sulfite was determined periodically in the culture medium, and H_2S concentration was routinely determined in the outlet gas. Other analyses performed periodically were volatile fatty acids, carbohydrates, lipids, and enumeration of *D. desulfuricans*.

In the second of two experiments of this type, after steady state was achieved, the SO_2 feed rate was increased stepwise until the specific activity of *D. desulfuricans* was exceeded as indicated by accumulation of sulfite in the culture medium. Combined with an enumeration of *D. desulfuricans*, this allowed an estimation of the maximum specific activity of the organism for SO_2 reduction under those growth conditions (mmol SO_2-reduced/h/10^{11} cells).

Hydrogen as an Electron Donor for SO_2-Reducing Cultures

D. orientis (ATCC 19365) was obtained from the American Type Culture Collection (Rockville, MD). Stocks were grown in 100-mL septum bottles in a mineral salts medium consisting of (in m*M* unless otherwise indicated): KH_2PO_4 (2.2); NH_4Cl (9.3); NaCl (17.1); $MgSO_4$ (18.3); Na_2SO_4 (7.0); $CaCl_2 \cdot 2H_2O$ (0.68); $NaHCO_3$ (11.9); $Fe(NH_4)_2(SO_4)_2$ (0.18); trace element solution (1.0 mL/L); and Wolfe's vitamin solution (1.0 mL/L) at pH 7.3. The trace element solution and Wolfe's vitamin solution are described elsewhere (*9*). Bottles contained 15 mL of medium and were gassed with

$H_2/CO_2/N_2$ (5:5:90, v/v/v). Stocks were incubated at 30°C. When H_2 depletion was indicated by gas chromatography, the bottles were regassed.

SO_2-reducing cultures (1.5 L) of *D. orientis* were prepared by growing the organism septically in a B. Braun Biostat M at pH 7.3 and 30°C in the mineral salts medium described above supplemented with 5.9 mL of 60% sodium lactate (Sigma Chemical Co., St. Louis, MO). Cultures were inoculated with fresh stocks (15 mL) with demonstrated H_2-utilizing capability. Lactate was replenished as it was utilized (5.9 mL every 2 d) until the biomass total protein concentration was 0.2–0.3 g/L. At this time, the biomass was harvested by centrifugation at $5000 \times g$ and 25°C, and resuspended in 1.5 L of a sulfate-free mineral salts medium consisting of (in m*M* unless otherwise indicated): KH_2PO_4 (2.2); NH_4Cl (9.3); NaCl (17.1); $MgCl_2$ (10.1); $CaCl_2 \cdot 2H_2O$ (0.68); $NaHCO_3$ (11.9); $FeCl_3$ (0.12); trace element solution (1.0 mL/L); and Wolfe's vitamin solution (1.0 mL/L). After resuspension of biomass, the fermenter received gas feeds of 140 mL/min 5% CO_2, balance N_2; 70 mL/min H_2; and 7.5–9.6 mL/min of 1.0% SO_2, 5% CO_2, balance N_2. The molar feed rates of SO_2 were 0.19–0.24 mmol/h. Cultures were maintained under these conditions at pH 7.3 and 30°C for 15–30 d during which time the outlet gas was monitored for H_2S, and the culture medium analyzed to demonstrate growth of *D. orientis* under these conditions.

Analytical

Table 2 summarizes gas chromatographic conditions for analysis of H_2 and H_2S in reactor outlet gases and volatile fatty acids in medium samples. Mixed liquid suspended solids (MLSS) were determined by filtering known volumes of culture medium samples through tared Whatman GF/C glass-fiber filters as described elsewhere (*10*). CODs were determined using Hach Chemical Co. (Loveland, CO) premeasured reagent vials. Biomass protein was determined by the Bradford method with bovine serum albumin (Sigma Chemical Co.) as a standard (*11*). Bradford reagent was obtained from Bio-Rad (Richmond, CA). Samples containing whole microbial cells were sonicated prior to analysis. The sonication procedure has been described previously (*8*). Total carbohydrates were determined by the Orcinol method with glucose as a standard (*12*). Total lipids in the *D. desulfuricans* culture feed and effluent were determined gravimetrically via two-stage extraction with diethyl ether followed by evaporation of the solvent. Ammonium ion was determined by the Nessler's method as described elsewhere (*10*). *D. desulfuricans* was enumerated by the most probable number technique (*13*) using BTI-SRB medium from Bioindustrial Technologies, Inc. (Grafton, NY).

Routine monitoring of H_2S in the outlet gas was done by gas chromatography as described above. However, more accurate chemical methods were also employed for sulfur balances in which H_2S in the outlet gas was

Table 2
Chromatographic Conditions for Analysis of H_2 and H_2S in Fermenter Outlet Gases and Volatile Fatty Acids in Medium Samples

Analyte	H_2	H_2S	Volatile fatty acids
Instrument	HP 5890	HP 5890	HP 5840
Column	20 ft x 1/8 in id stainless steel; 100/200 Hay Sep D	10 ft x 1/8 in id Teflon; 80/100 Porapak QS	2 m x 1.8 mm id glass 80/120 Carbopack B-DA/ 4% Carbowax 20 M
Carrier gas	He, 30 mL/min	He, 30 mL/min	N_2, 24 mL/min
Temperatures:			
Column oven	40°C (2 min) then 24°C/min 120°C max	90°C	175°C
Injection oven	100°C	120°C	200°C
Detector oven	140°C	120°C	200°C
Detector	Thermal conductivity	Thermal conductivity	Flame ionization
Standard	Pure H_2	Primary standard 1.001% H_2S (Matheson Gas Co.)	Gravimetrically prepared; all samples and standards contained 0.03*M* oxalic acid

precipitated as ZnS and analyzed spectrophotometrically. Reactor outlet gas was bubbled into 400 mL of 0.1% zinc acetate for 2 h. Two reagents were required for colorimetric analysis of the precipitated sulfide, DMPD reagent, and ferric reagent. The DMPD reagent was prepared by dissolving 1.0 g of *N,N*-dimethyl-*p*-phenylenediamine sulfate (Sigma Chemical Co.), 1.0 g Zn $(CH_3COO)_2 \cdot 2H_2O$, and 50 mL of concentrated H_2SO_4 in distilled water and diluted to 1 L. Ferric reagent was prepared by dissolving 5.0 g $FeCl_3 \cdot 6H_2O$ in 20 mL of distilled water. Suspensions of ZnS were analyzed by mixing 5.0 mL of the ZnS suspension (or a suitable dilution) with 4.9 mL DMPD reagent followed by immediate addition of 0.1 mL of ferric reagent. The absorbance at 660 nm was then read after at least 10 min of incubation at room temperature.

Sulfide stocks were prepared by washing $Na_2S \cdot 9H_2O$ with distilled water, drying at room temperature, and dissolving approx 8 g in anoxic 0.01*N* NaOH to a total vol of 1 L. Stocks were standardized by titration with 0.01*M* lead perchlorate using a sulfide ion-specific electrode to detect the end point as previously described (*8*).

RESULTS AND DISCUSSION

Sewage Sludge as an Electron Donor for SO_2 Reduction

During start-up of the continuous *D. desulfuricans* SO_2-reducing culture with pretreated sewage sludge feed, the H_2S concentration in the reactor outlet gas was about 6000 ppmv after 24 h. This H_2S production was much too high to account for in terms of SO_2 reduction alone. Over the next 48 h, the H_2S concentration declined steadily until the concentration was 200–250 ppmv, where it remained for the duration of the experiment. This extra H_2S production, over and above that produced by SO_2 reduction, has been attributed to the metabolism by nonSRB heterotrophs of sulfur-containing substrates (probably S-containing amino acids) produced during heat/alkali treatment of sewage solids. These soluble substrates were present at very high concentrations during start-up.

Figures 1–3 document the feed and effluent MLSS, soluble COD, and total and soluble protein during the entire course of the experiment. Time zero corresponds to the initiation of SO_2 feed. As seen in Figs. 1–3, the culture was subject to variations in the feed composition with regard to soluble COD and total protein. Since the method of preparation of the feed was not changed during the course of the experiment, these variations have been attributed to variations in the sludge as obtained from the municipal sewage treatment system. Despite these variations, the culture was very stable with respect to SO_2 reduction. No upsets (as indicated by

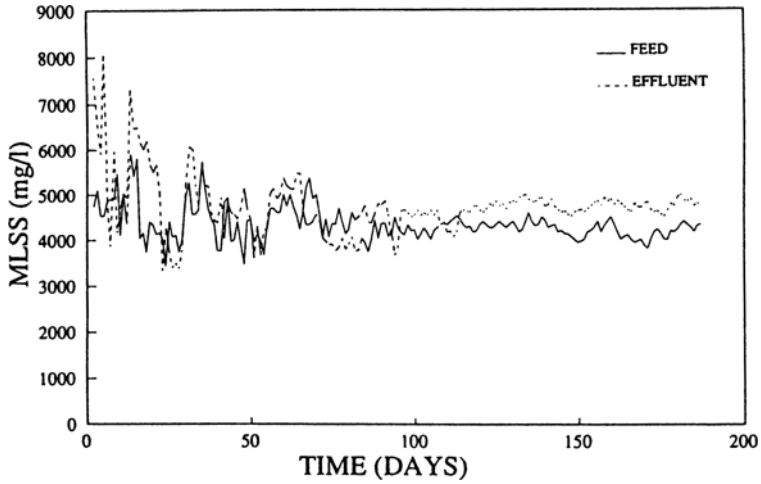

Fig. 1. MLSS concentrations in the feed and effluent of a continuous SO_2-reducing culture operating with a feed of heat/alkali-pretreated sewage sludge.

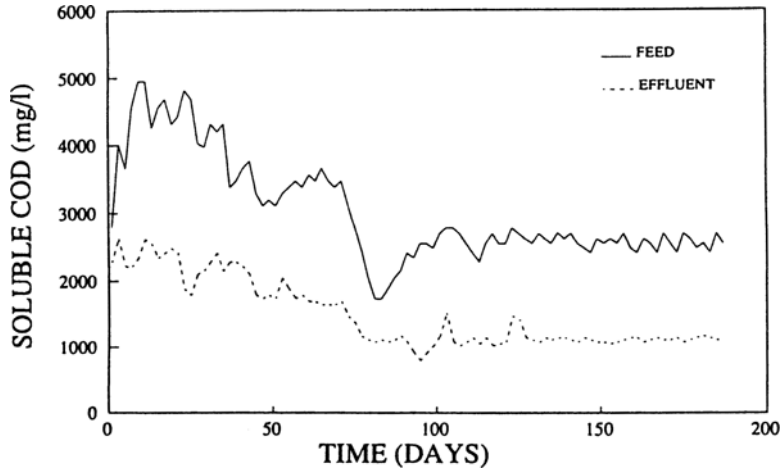

Fig. 2. Soluble COD concentrations in the feed and effluent of a continuous SO_2-reducing culture operating with a feed of heat/alkali-pretreated sewage sludge.

accumulation of sulfite in the culture medium) were observed, and as shown in Table 3, complete reduction of SO_2 to H_2S was observed. Since about 100 d, the reactor has operated with about a 10% increase in MLSS from feed to effluent. Note, however, the roughly 50% decrease in total protein. During the last 60 d, there has been a 60% decrease in the feed-soluble COD compared to the effluent. As seen in Fig. 3, there has been a similar decrease in the soluble protein comparing the feed to the effluent.

Feed and effluent filtrates were analyzed for total carbohydrates, carboxylic acids, and lipids at about 130 d. The total feed and effluent were

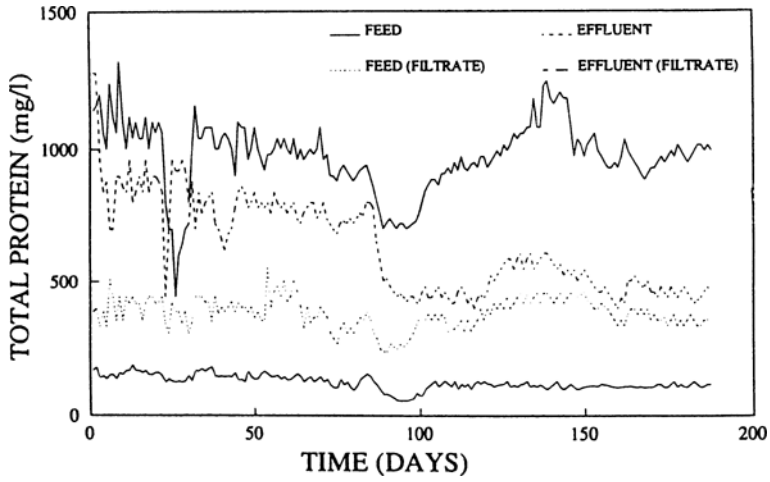

Fig. 3. Protein concentrations in the feed and effluent of a continuous SO_2-reducing culture operating with a feed of heat/alkali-pretreated sewage sludge.

Table 3
Sulfur Balances in *D. desulfuricans* Continuous SO_2-Reducing Reactor Operated on Feed of Heat/Alkali Pretreated Sewage Sludge

Day	SO_2 feed rate, mmol/h	H_2S production rate, mmol/h	H_2S/SO_2
21	0.205	0.204	1.00
22	0.205	0.209	1.02
47	0.222	0.224	1.01
65	0.222	0.219	0.99
68	0.236	0.229	0.97
85	0.236	0.232	0.98

also analyzed for lipids. Results are shown in Table 4. As noted above, in SO_2-reducing cultures of *D. desulfuricans* in which glucose served as the ultimate carbon and energy source, acetic acid and other volatile fatty acids were produced as end products of glucose metabolism by *D. desulfuricans* and the mixed heterotrophs in the culture. In the cultures described here, the exact carbon sources for *D. desulfuricans* were not known. It was also not known whether these carbon sources came directly from the pretreated sewage sludge, were produced by fermentation of the solubilized sludge components by the mixed heterotrophs in the culture, or both. As seen in Table 4, very little net production of carboxylic acids was observed when solubilized sewage sludge served as the carbon and energy source. The data with regard to lipid analysis suggest underutilization of the lipid fraction despite significant solubilization.

Table 4
Total Carbohydrates, Carboxylic Acids, and Total and Filtrate Lipids in Feed and Effluent of Continuous SO_2-Reducing Culture

	Feed		Effluent	
Analysis	Filtrate	Total	Filtrate	Total
Carbohydrates	0.98 g/L		0.53 g/L	
Carboxylic acids:				
Acetic	73 mg/L		59 mg/L	
Propionic	2 mg/L		ND[a]	
Isobutyric	ND[a]		8 mg/L	
Butyric	ND[a]		5 mg/L	
Isovaleric	ND[a]		22 mg/L	
Lipids				
Dried at 60°C	0.24 g/L		0.18 g/L	
Dried at 40°C	0.27 g/L		0.22 g/L	
Dried at 60°C		0.38 g/L		0.27 g/L

[a]Not detected.

A second continuous SO_2-reducing culture operating on a feed of pretreated sewage sludge was initiated with virtually identical results. This culture is currently being used to study biomass recycle and improvements in volumetric productivity that will be reported at a later date. However, one experiment conducted with this culture should be reported here, that is, the determination of the maximum specific activity of *D. desulfuricans* for SO_2 reduction under these culture conditions. An MPN count (in triplicate) of sulfate-reducing bacteria in the process culture resulted in a count of 4.5×10^7 cells/mL. At an SO_2 molar feed rate of 0.50 mmol/h, this culture (1.5 L) began to accumulate sulfite in the culture medium. Therefore, the maximum specific activity for SO_2 reduction was 0.74 mmol SO_2/h—10^{11} cells. This compares to 1.7 mmol SO_2/h—10^{11} cells obtained for SO_2 reduction by this organism in mixed culture with glucose as the carbon and energy source (*1*).

H_2 as an Electron Donor for SO_2 Reduction

D. orientis was successfully grown in mineral salts medium with a gas feed of CO_2, H_2, and SO_2. Hydrogen was the energy source, carbon dioxide the carbon source, and SO_2 the terminal electron acceptor. With a molar SO_2 flow rate of 0.185 mmol/h, the H_2S concentration in the outlet gas (total volumetric flow rate of 218 mL/min) averaged 340 ppmv. Table 5 shows the result of sulfur balances performed at various times during the course of three batch experiments. Complete conversion of SO_2 to H_2S is

Table 5
Sulfur Balances in *D. orientis* SO_2-Reducing Batch Cultures

Experiment	SO_2 feed rate, mmol/h	H_2S production rate, mmol/h	H_2S/SO_2
B1	0.165	0.173	1.05
B2	0.237	0.236	1.00
		0.241	1.02
		0.236	1.00
B3	0.185	0.189	1.02
		0.182	0.98
		0.184	0.99

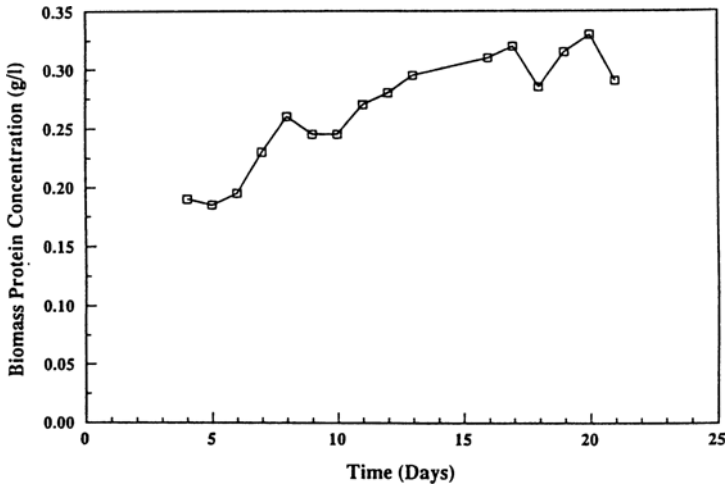

Fig. 4. Biomass protein concentration in a batch SO_2-reducing culture (B3) of *D. orientis* operating with a feed of CO_2, H_2, and SO_2.

indicated. Sulfite concentrations in the bulk aqueous phase were relatively constant and averaged <5 mg/L.

As SO_2 was removed from the feed gas and reduced to H_2S, the biomass protein in these reactors was seen to increase as shown in Fig. 4. The data are somewhat erratic because of a tendency for the biomass to adhere to the walls of the vessel; however, a clear upward trend is evident, indicating growth of the organism on $H_2/CO_2/SO_2$. Ammonium ion, a source of reduced nitrogen for the organism, was seen to decrease as SO_2 was removed from the feed gas (Fig. 5).

In order to establish firmly that SO_2 reduction in these cultures was occurring at the expense of the H_2 oxidation, experiments were conducted in batch SO_2-reducing cultures in which the H_2 feed was turned off and the results observed. If reducing equivalents required for SO_2 reduction

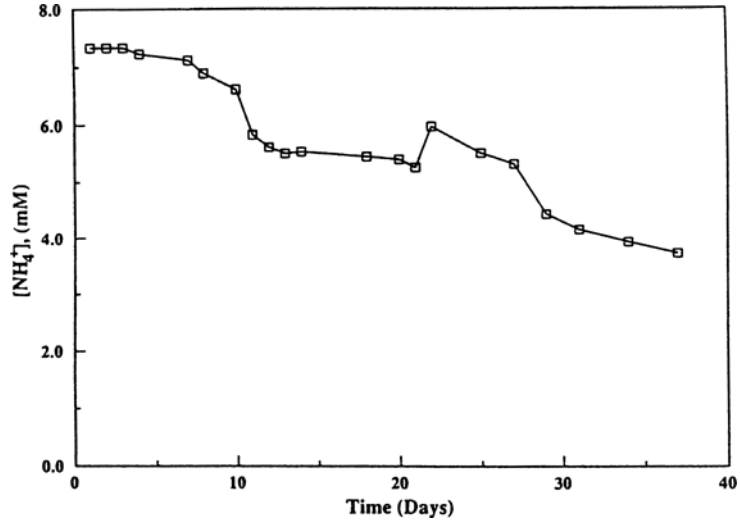

Fig. 5. Ammonium ion concentration in a batch SO_2-reducing culture (B3) of *D. orientis* operating with a feed of CO_2, H_2, and SO_2.

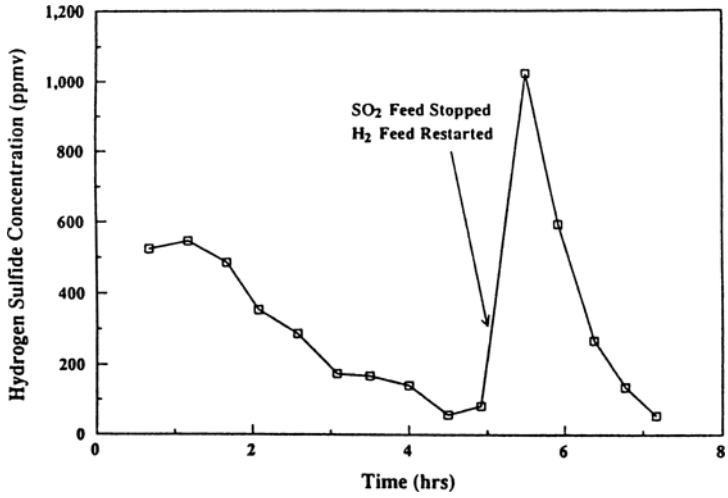

Fig. 6. Outlet H_2S concentration from a batch SO_2-reducing culture of *D. orientis* following cessation of H_2 feed.

came from H_2 oxidation, the cessation of H_2 feed would produce a reduction in the outlet H_2S concentration and an accumulation of sulfite in the culture medium. As shown in Figs. 6 and 7, this was indeed the case. Before the H_2 feed was turned off, the total gas feed rate in these experiments was 220 mL/min consisting of 9.6 mL/min of 1.0% SO_2, 5% CO_2, balance N_2; 71 mL/min H_2; and 140 mL/min of 5% CO_2, balance N_2. The

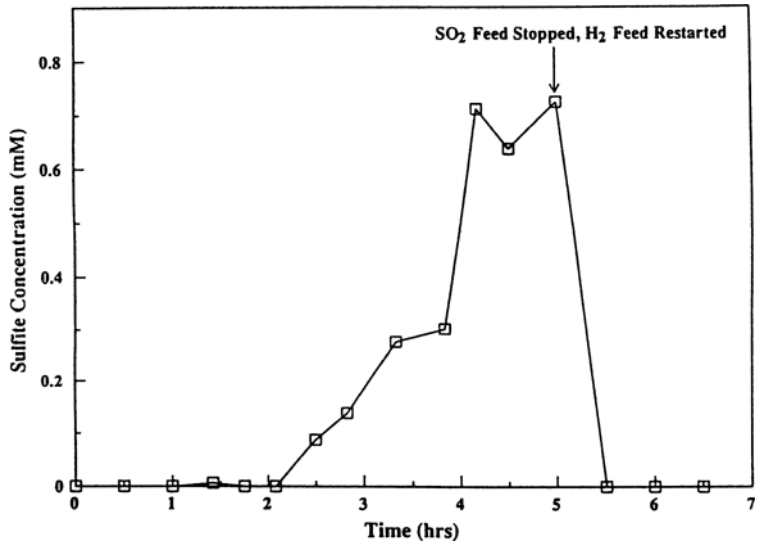

Fig. 7. Sulfite concentration in a batch SO_2-reducing culture of *D. orientis* following cessation of H_2 feed.

H_2S concentration in the outlet gas was about 340 ppmv. When the H_2 feed was turned off, the H_2S concentration in the outlet gas increased to 500 ppmv owing to the decrease in total gas flow. As seen in Fig. 6, the H_2S concentration then decreased dramatically over the next 4 h. At the same time, sulfite was observed to accumulate in the culture medium (Fig. 7). Five hours after the H_2 feed was turned off, the SO_2 feed was turned off, and the H_2 feed restarted. Consequently, the sulfite concentration returned to very low levels within 30 min, and there was a corresponding transient surge in H_2S production. After another 3.5 h the SO_2 feed was restarted at the original feed rate. The H_2S concentration in the outlet gas then returned to about 340 ppmv, representing stoichiometric reduction of SO_2 to H_2S.

One last observation is worthy of some discussion. In roughly half of the *D. orientis* SO_2-reducing cultures studied, significant levels of volatile fatty acids accumulated. For example, acetic acid accumulated to concentrations of 1.0% by weight in some cultures. Lesser amounts of butyric acid (0.2%), propionic acid (0.004%), isobutyric acid (0.01%), isovaleric acid (0.001%), and valeric acid (0.0005%) were observed. In those cultures where these carboxylic acids were found, it could be shown that their accumulation was directly linked to SO_2 reduction. When SO_2 feed was stopped, accumulation of volatile fatty acids ceased. When SO_2 feed was restarted, acids again accumulated. Whether this behavior can be correlated with the appearance of other bacteria in the culture or is a function of culturing conditions is not yet known, and will be reported on at a later date.

CONCLUSIONS

Sulfur dioxide was readily reduced to H_2S by contact with a mixed culture of *D. desulfuricans* in which a heat- and alkali-pretreated municipal sewage sludge served as a carbon and energy source for the culture. Sulfur dioxide was also reduced to H_2S by mixed cultures of *D. orientis*, in which H_2 was the energy source and CO_2 was the carbon source. These results suport the technical feasibility of the use of these feedstocks as electron donors for SO_2 reduction. These feedstocks are also potentially more economically than carbohydrate feedstocks used previously as electron donors in these cultures. Future work will concentrate on maximizing the volumetric productivity of both of these reactor systems.

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