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Autotrophic Growth of *Desulfotomaculum Orientis* with Reduction of Sulfur Dioxide

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ABSTRACT

It has been demonstrated that the sulfate-reducing bacterium *Desulfotomaculum orientis* can be grown in batch cultures on elemental hydrogen (H_2) as an energy source, carbon dioxide (CO_2) as a carbon source, and sulfur dioxide (SO_2) as a terminal electron acceptor. At sufficiently high H_2 partial pressures, complete reduction of SO_2 to H_2S was observed with 1–2 s of gas-liquid contact time. The maximum specific activity for SO_2 reduction was 6.5 mmol SO_2/h-g of total biomass protein. The stoichiometry of SO_2 reduction with H_2 as the electron donor has been determined under these conditions. At low H_2 partial pressures, SO_2 was both oxidized to sulfate and reduced to hydrogen sulfide (H_2S).

Index Entries: Sulfur dioxide; *Desulfotomaculum orientis*; flue-gas desulfurization.

INTRODUCTION

We have previously demonstrated that the sulfate-reducing bacterium *Desulfovibrio desulfuricans* can be grown in mixed culture with fermentative heterotrophs in a medium in which glucose served as the only carbon source and SO_2 as the terminal electron acceptor with complete reduction of SO_2 to H_2S (*1*).

It has been 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

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hydrogen sulfide (H_2S) by contact with a culture of sulfate-reducing bacteria (*1,2*). The resulting H_2S could then be combined with the remaining SO_2 and used as feed to a Claus reactor to produce elemental sulfur (*3,4*).

An economic evaluation of this microbial SO_2-reduction process concept was reported comparing microbial SO_2 reduction with glucose as the electron donor to conventional catalytic SO_2 hydrogenation with H_2 generation from methane. This analysis showed that the microbial reduction process is not competitive with conventional SO_2-reduction methods when glucose (DE95 corn hydroysate) is utilized as the feedstock electron donor. However, it was shown that a microbial process could be competitive with lower cost feedstocks (*5*).

Carbon dioxide and H_2 have been investigated as feedstocks for batch SO_2-reducing cultures of *Desulfotomaculum orientis*. Growth of *D. orientis* has been demonstrated on H_2 as an energy source, CO_2 as a carbon source, and SO_2 as the terminal electron acceptor. The maximum specific activity of *D. orientis* for SO_2 reduction and the stoichiometry of SO_2 reduction with H_2 as the electron donor have been determined. The effect of H_2 starvation of SO_2 reduction has also been investigated.

MATERIALS AND METHODS

Organism and Stock Culture

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); $CaCl_2/2H_2O$ (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 (*6*). 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. The bottles were regassed when the H_2 depletion were indicated by gas chromatography.

SO_2 Reduction by *D. orientis* at High Partial Pressure of H_2

Sulfur dioxide-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 total biomass protein concentration was 0.2–0.3 g/L. At this time the biomass was harvested by centrifugation at 5000*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); $CaCl_2/2H_2O$ (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).

In initial experiments, 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. All gases entered the reactor culture through a common glass-fritted sparger. The molar feed rates of SO_2 were 0.19–0.24 mmol/h. The volume percent of H_2 overall in the gas feed was about 32%. The reactor operating pressure was approximately atmospheric. Therefore, in these initial experiments the inlet H_2 partial pressure was about 0.32 atm. 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.

Specific Activity of *D. orientis* for SO_2 Reduction

In the experiments described above the reactors were operated under SO_2-limiting conditions. In other words, SO_2 was fed to the reactor at a molar flowrate that did not exceed the biomass capacity for SO_2 reduction. A study was conducted to determine the maximum specific activity of the *D. orientis* working cultures for SO_2 reduction. The SO_2 feed rate (1.0% SO_2, 5% CO_2, balance N_2) was increased stepwise until sulfite (the aqueous form of SO_2) began to accumulate in the culture medium. The feed rate at which sulfite began to accumulate together with the biomass protein concentration in the culture allowed the maximum specific activity to be estimated. The other feed gases consisted of 140 mL/min 5% CO_2, balance N_2 and 74 mL/min H_2.

Stoichiometry of Microbial SO_2 Reduction with H_2

The stoichiometry of SO_2 reduction by *D. orientis* with respect to H_2 was determined as follows. An SO_2-reducing culture of *D. orientis* was produced as described above. Once growth on $H_2/CO_2/SO_2$ was established, the gas feed composition was changed to 29.0 mL/min 10% H_2, 5% CO_2, balance N_2; 73 mL/min 5% CO_2, balance N_2; and 9.4 mL/min 1.0% SO_2, 5% CO_2, balance N_2. The overall volume percent H_2 in the total feed gas was therefore reduced more than tenfold to 2.6%. This was done so that changes in the H_2 concentration in the feed gas resulting from contact with the culture could be more accurately measured. The H_2 consumed was determined by gas chromatography analysis of the outlet gas stream (*see below*).

SO_2 Reduction by *D. orientis* at Low Partial Pressure of H_2

Following the investigation of H_2/SO_2 stoichiometry described above the feed gas was changed again to 11.6 mL/min 10% H_2, 5% CO_2, balance N_2; 64.7 mL/min N_2; and 9.3 mL/min 1.0% SO_2, 5% CO_2, balance N_2. At the same time 44.3 mL/min of the outlet gas was diverted to a caustic scrubber to remove H_2S then recycled back to the reactor. The purpose of this experiment was to reduce the H_2 throughput in the culture. These actions had the effect of both reducing the molar H_2 feed rate and the volume percent H_2 in the total feed gas to about 1.3%. Therefore the inlet H_2 partial pressure was about 0.013 atm. The reactor was operated in this manner for 21 d.

As detailed below this experiment produced unexpected results with respect to the observed H_2/SO_2 ratio. These results seemed to be related to the low H_2 partial pressure in the culture. These effects were further investigated with a new *D. orientis* culture produced as described above, first on lactate/sulfate and subsequently on $H_2/CO_2/SO_2$. Initially the gas feed to this culture consisted of 30.5 mL/min 10% H_2, 5% CO_2, balance N_2; 80.5 mL/min N_2; and 9.3–11.8 mL/min 1.0% SO_2, 5% CO_2, balance N_2 (0.025 atm H_2). After 10 d the H_2 feed rate was reduced to 15.9 mL/min 10% H_2, 5% CO_2, balance N_2 (0.015 atm H_2). This feed condition was maintained for another 21 d. On the 32nd d of operation, the H_2 feed was stopped completely. A feed of 15.9 mL/min 10% H_2, 5% CO_2, balance N_2 was reinitiated on the 38th d for 24 h (0.015 atm H_2).

At the conclusion of this experiment, the culture was harvested by centrifugation and resuspended in fresh sulfate-free medium. The culture than again received a gas feed of 15.9 mL/min 10% H_2, 5% CO_2, balance N_2; 80.5 mL/min N_2; and 11.8 mL/min 1.0% SO_2, 5% CO_2, balance N_2 (0.015 atm H_2). On the 21st d of operation the feed gases were changed to 32.4 mL/min H_2; 91.0 mL/min 5% CO_2, balance N_2; and 9.3 mL/min 1.0% SO_2, 5% CO_2, balance N_2 (0.24 atm H_2) for the duration of the experiment.

Analytical

Table 1 summarizes gas chromatographic conditions for analysis of H_2 and H_2S in the reactor outlet gas. Ammonium ion was determined by the Nesslers method (*7*). Sulfite was determined spectrophotometrically by reacting sulfite with formaldehyde in sulfuric acid (*8*). Biomass protein was determined by sonication followed by colorimetric analysis by the Bradford method with bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a standard (*9*). Bradford reagent was obtained from Bio-Rad (Richmond, CA). Sulfate was determined turbidometrically following precipitation with $BaCl_2$ (*7*).

While routine monitoring of H_2S in the reactor outlet gas was done by gas chromatography, more accurate chemical methods were employed

Table 1
Chromatographic Conditions for Analysis of H_2 and H_2S in Fermenter Outlet Gases

Analyte	H_2	H_2S
Instrument	HP 5890	HP 5890
Column	20′ × 1/8 ″ ID Stainless steel; 100/200 Hay sep D	10′ × 1/8 ″ ID Teflon; 80/100 Porapak QS
Carrier gas	He, 30 mL/min	He, 30 mL/min
Temperatures		
Column oven	40°C (2 min) then 24°C/min 120°C max	90°C
Injection oven	100°C	120°C
Detector oven	140°C	120°C
Detector	Thermal conductivity	Thermal conductivity
Standard	Pure H_2	Primary standard 1.001% H_2S (Matheson Gas Co.)

for sulfur balances. Reactor outlet gas was bubbled into 400 mL of 0.1 wt% zinc acetate for 2 h where H_2S was precipitated as ZnS. Precipitated sulfide was then analyzed colorimetrically by the methylene blue method as described previously (*6*).

RESULTS AND DISCUSSION

SO_2 Reduction by *D. orientis* at High Partial Pressure of H_2

With an inlet H_2 partial pressure of 0.32 atm, *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 flowrate of 0.185 mmol/h, the H_2S concentration in the outlet gas (total volumetric flowrate of 218 mL/min) averaged 340 parts per million by volume (ppmv). Table 2 shows the result of sulfur balances performed at various times during the course of several batch experiments under these conditions. Complete conversion of SO_2 to H_2S was indicated. Sulfite concentrations in the bulk aqueous phase were relatively constant and averaged less than 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. 1.

Table 2
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
B4	0.200	0.194	0.97
		0.209	1.05
		0.192	0.96
		0.189	0.95
		0.188	0.94
B5	0.206	0.202	0.98
		0.211	1.02
	0.191	0.184	0.96
		0.187	0.98
		0.185	0.97
		0.177	0.93
		0.184	0.96

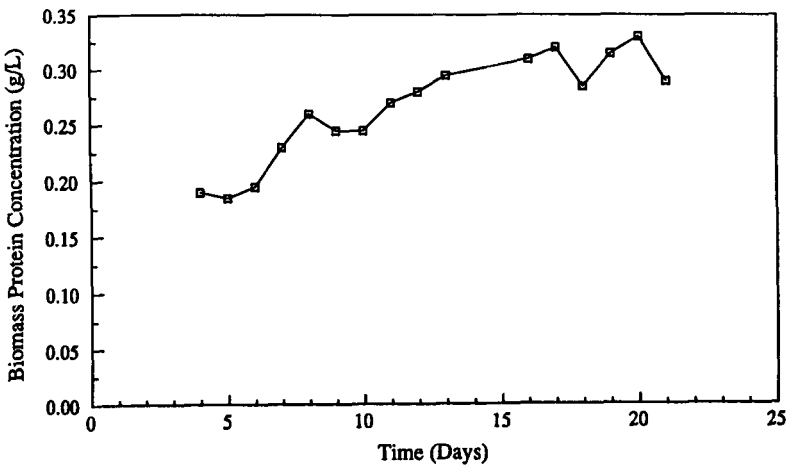

Fig. 1. Biomass protein concentration in a batch culture of *D. orientis* receiving a $H_2/CO_2/SO_2$ feed. H_2 partial pressure was 0.32 atm.

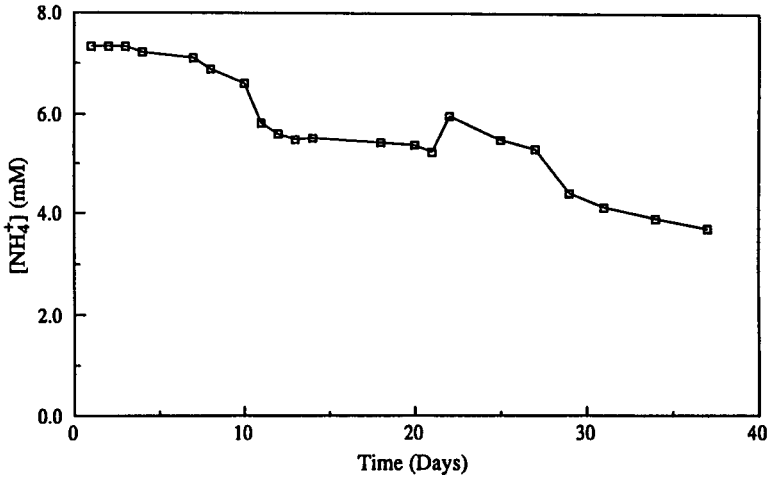

Fig. 2. Ammonium ion concentration in a batch culture of *D. orientis* receiving a $H_2/CO_2/SO_2$ feed. H_2 partial pressure was 0.32 atm.

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. 2).

In order to firmly establish 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 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. 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 (0.32 atm H_2). The 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 because of the decrease in total gas flow. 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. 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 (<5 mg/L) 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.

Table 3
Stoichiometry of H_2 Consumption
in an SO_2-Reducing Culture of *D. orientis*

Experiment	SO_2 Feed rate, mmol/h	H_2S Production rate, mmol/h	H_2S/SO_2
E1	0.230	0.770	3.35
E2	0.224	0.719	3.21

Specific Activity of *D. orientis* Cultures for SO_2 Reduction

When the SO_2 feed rate was increased stepwise to a *D. orientis* working culture (1.5 L) containing a total of 148 mg of biomass protein, sulfite accumulation was observed when the SO_2 feed rate (1.0% SO_2) was 39 mL/min (0.96 mmol SO_2/h). Therefore, the specific activity was 6.5 mmol SO_2/h-g of total biomass protein. The upset could not be reversed by increasing the H_2 feed rate. Only a reduction in the SO_2 feed rate could result in a decrease in the sulfite concentration.

Stoichiometry of Microbial SO_2 Reduction with H_2

The purely chemical reduction of SO_2 with H_2 would be described by Eq. (1).

$$3\,H_2 + SO_2 \rightarrow H_2S + 2H_2O \quad (1)$$

In a *D. orientis* culture operating on a feed of $H_2/CO_2/SO_2$, a H_2/SO_2 ratio of slightly more than 3.0 would be expected since some reducing equivalents produced by H_2 oxidation would be required to reduce CO_2 for the production of biomass. Experimentally, the H_2/SO_2 ratio was found to be 3.28 (Table 3).

SO_2 Reduction by *D. orientis* at Low Partial Pressures of H_2

Following the investigation of H_2/SO_2 stoichiometry the H_2 feed rate was reduced and a portion of the outlet gas was recycled as described in the Materials and Methods. During this time H_2/SO_2 ratios of 0.9–1.5 were observed. In order to determine whether these observations were related to the low H_2 partial pressure (0.013 atm) in this experiment, a new culture *D. orientis* culture was produced as described in the previous section which was again operated with a low partial pressure of H_2 (0.025 and then 0.015 atm) in the gas feed. The results of this experiment are shown in Table 4. As seen in Table 4 the H_2/SO_2 ratios were again seen to be lower than

Table 4
Stoichiometry of the Oxidation/Reduction of SO_2 in a *D. orientis* Culture with a $H_2/CO_2/SO_2$ Feed at Low H_2 Partial Pressure (Exp. E1)[a]

Day	H_2 consumed, mmol/h	SO_2 in, mmol/h	SO_4^{-2}, mmol/h	H_2S mmol/h	H_2/SO_2	H_2/H_2S
4	0.424	0.228			1.86	
5	0.527	0.228			2.31	
11	0.406	0.290			1.40	
12	0.382	0.290			1.32	
18	0.344	0.290	0.183		1.19	
19	0.230	0.290	0.150		0.79	
21		0.290	0.174	0.136		
25		0.290	0.132	0.129		
26	0.310	0.290	0.167	0.148	1.07	2.09
32	0	0.290	0.195	0.060		
33	0	0.290	0.235	0.051		
37	0	0.290	0.202	0.052		
39	0.355	0.290	0.151	0.109	1.22	3.25

[a]The H_2 partial pressure was 0.025 atm for the first 10 d and 0.015 atm thereafter until H_2 feed was terminated on d 32. The H_2 partial pressure was again 0.015 atm on d 39.

expected based on earlier experiments. However, the H_2/SO_2 ratios were higher at the higher H_2 partial pressure (0.025 atm) in the feed gas. On the 13th d of operation, sulfate was found in the culture medium (24 m*M*). This sulfate had accumulated over the previous 12 d of operation on a $H_2/CO_2/SO_2$ feed and continued to accumulate for the duration of the experiment.

Analysis of the outlet gas for H_2S showed less than stoichiometric production of H_2S based on the SO_2 feed rate (Table 4). However, during this time, the $(SO_4^{-2} + H_2S)/SO_2$ ratio averaged 1.02. Therefore, SO_2 was both reduced to H_2S and oxidized to sulfate. This explains the depressed H_2/SO_2 ratios observed under these and similar reaction conditions. Less than stoichiometric utilization of H_2 was observed because only a fraction of the SO_2 was reduced to H_2S. The remainder was oxidized to sulfate.

On day 32 of this experiment, the H_2 feed was eliminated. The reactor continued to receive a gas feed of 11.8 mL/min 1% SO_2, 5% CO_2, balance N_2 and 80.5 mL/min N_2. During this time, H_2S continued to be produced but at a slower rate (Table 4). The rate of sulfate accumulation increased and the $(SO_4^{-2} + H_2S)/SO_2$ ratio averaged 0.92. A feed of 15.9 mL/min 10% H_2, 5% CO_2, balance N_2 was reinitiated on the 38th d. As seen in Table 4, the rate of sulfate accumulation decreased and H_2S production increased in response to the renewed availability of H_2. The response of this reactor to the elimination of H_2 feed was markedly different from the

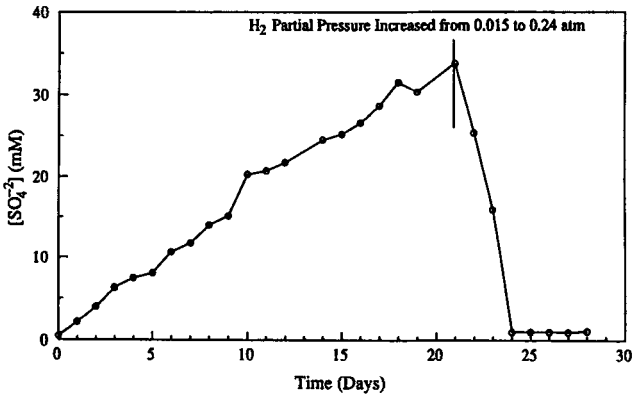

Fig. 3. Sulfate concentrations in a batch *D. orientis* culture receiving a $H_2/CO_2/SO_2$ feed, first at low H_2 partial pressure (0.015 atm) and subsequently at a higher (0.24 atm) partial pressure.

reactor described above operating at a much higher inlet H_2 partial pressure. The key difference between the two experiments is the apparent condition of starvation for an energy source (H_2) in this experiment and the resulting change in SO_2 metabolism in the organism.

At the conclusion of this experiment, biomass was harvested by centrifugation and resuspended in fresh autotrophic medium. The culture then again received a gas feed of $H_2/CO_2/SO_2$ where the H_2 partial pressure was again 0.015 atm. As seen in Fig. 3, sulfate was again seen to accumulate although at somewhat lower rates (Table 5) than in the previous experiment. The average $(H_2S + SO_4^{-2})/SO_2$ ratio observed was 0.98; therefore, all SO_2 was again either oxidized to sulfate or reduced to H_2S.

The H_2/H_2S ratio averaged 3.2. Referring to Eq. (1), it appears that the fraction of the SO_2 feed reduced to H_2S was reduced at the expense of H_2 oxidation only. The electron acceptor for SO_2 oxidation to sulfate is unknown. If the electron acceptor is SO_2, 3 mol of SO_2 would be oxidized for every 1 mol of SO_2 reduced according to the Eq. (2).

$$4H_2O + 4SO_2 \rightarrow 3SO_4^{-2} + H_2S + 6H^+ \quad (2)$$

However, production of H_2S from this reaction would result in a H_2/H_2S ratio of less than 3.0.

On the 21st d of this experiment, the H_2 partial pressure in the gas feed was increased to 0.24 atm. As shown in Fig. 3, the sulfate concentration in the culture medium immediately began to decline. After three days under this feed condition, the sulfate concentration was less than 1 m*M*. Therefore, with increased availability of H_2 as an energy source, SO_2 oxidation ceased and both the influent SO_2 and accumulated sulfate were used as electron acceptors with reduction to H_2S.

Table 5
Stoichiometry of the Oxidation/Reduction of SO_2 in a *D. orientis* Culture with a $H_2/CO_2/SO_2$ Feed at Low H_2 Partial Pressure (Exp. E2)[a]

Day	H_2 consumed, mmol/h	SO_2 in, mmol/h	SO_4^{-2}, mmol/h	H_2S mmol/h	H_2/SO_2	H_2/H_2S
1		0.228	0.141			
2		0.290	0.104			
3		0.290	0.116			
5		0.290	0.045			
6	0.608	0.290	0.156	0.187	3.25	2.10
7	0.791	0.290	0.070	0.238	3.32	2.73
8	0.538	0.290	0.130	0.179	3.01	1.86
9		0.290	0.069	0.193		
11		0.290	0.030			
12		0.290	0.071	0.216		
14		0.290	0.075	0.189		
15		0.290	0.032	0.206		
16		0.290	0.075	0.196		
17		0.290	0.127			
18		0.290	0.146			
22		0.185	−0.557			
23		0.228	−0.545	0.522		
24		0.228	−0.673			
25		0.228	0			
26		0.228	0			
27		0.290	0			
28		0.290	0	0.287		
33		0.290	0	0.306		
35		0.290	0	0.284		

[a]The H_2 partial pressure was 0.015 atm for the first 21 d of operation and 0.24 atm thereafter until the experiment was terminated.

CONCLUSIONS

Sulfur dioxide was reduced to H_2S by cultures of *Desulfotomaculum orientis*, in which H_2 was the energy source and CO_2 was the carbon source. Complete reduction of SO_2 to H_2S was observed at inlet H_2 partial pressure of 0.24–0.32 atm. However, at tenfold lower H_2 partial pressures both H_2S and sulfate were obtained as products.

These results indicate that a new mode of sulfur metabolism in *D. orientis*, in which SO_2 is both reduced to H_2S and oxidized to sulfate can be induced by H_2 starvation. In fact, data suggest that perhaps H_2 can be eliminated as an energy source with SO_2 acting as both electron donor and electron acceptor.

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