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Microbial Reduction of SO_2 as a Means of Byproduct Recovery from Regenerable, Dry Scrubbing Processes for Flue Gas Desulfurization

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ABSTRACT

A project is under way at the University of Tulsa to investigate the reduction of SO_2 to H_2S by sulfate reducing bacteria (SRB) in co-culture with mixed fermentative heterotrophs. We have previously demonstrated that SO_2 is completely reduced to H_2S (contact times of 1-2 s) in cultures in which no redox poising agents were required and glucose served as the ultimate source of carbon energy. We have proposed that such a microbial process could be coupled with a Claus reactor to recover elemental sulfur as a byproduct of regenerable, dry scrubbing processes for flue gas desulfurization.

The development of this process concept has continued with a study of the use of molasses as a source of carbon and reduced nitrogen, identification of important non-SRB heterotrophs in process cultures, and the identification of the end products of carbohydrate fermentation that serve as carbon and energy sources for the SRB and identification of the end products of SRB metabolism.

Index Entries: *Desulfovibrio desulfuricans;* sulfur dioxide; flue gas desulfurization; sulfate-reducing bacteria; hydrogen sulfide.

INTRODUCTION

With the continual increase in the utilization of high sulfur fossil fuels (particularly coal and sour petroleum crudes), the release of airborne sulfur dioxide (SO_2) into the environment has become a critical problem.

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Upon release into the atmosphere, SO_2 may react photochemically or catalytically with other atmospheric contaminants to form sulfur trioxide (SO_3), sulfuric acid (H_2SO_4), and various salts of sulfuric acid that form the chief constituents of acid precipitation, also known as "acid rain" (*1,2*). The effects of acid rain on ecosystems have been reported to include: increased leaching of nutrients from plant foliage and soil, interference with decomposition processes, and disruption of the nitrogen cycle. In addition to damage to ecosystems, acid rain causes major damage to materials, such as metals, limestone, marble, and mortar. Sulfur dioxide and related pollutants have also been linked to various categories of human diseases (*3,4*).

There are several engineering solutions to the problem of SO_2 emissions from the combustion of sulfur-bearing fuels although none alone satisfy all of the desired technical and economic requirements. The most commercially important flue gas desulflurization technology at present is the use of solid, throwaway adsorbents, such as limestones and dolomites, that have an affinity for an acid gas like SO_2. This type of process results in the production of large amounts of calcium sulfate ($CaSO_4$), which represents a significant disposal problem. In addition, little or no removal of oxides of nitrogen (NO_x) is achieved.

Several more promising technologies for flue gas desulfurization are under development that combine SO_2 and NO_x removal. These include two dry, regenerable scrubbing processes, the copper oxide process (*5*), and the NOXSO process (*6*). Dry, regenerable scrubbing processes offer considerable advantage over the use of throwaway adsorbents. Primary amount these are reduced costs for chemical makeup and the simultaneous removal of SO_2 and NO_x. However, regeneration of the sorbent in both the copper oxide and NOXSO processes produces a concentrated stream of SO_2 or SO_2 and H_2S (hydrogen sulfide), respectively, that must be disposed of or recovered in a separate process. New technology is needed to process these concentrated sulfur gas streams from dry, regenerable scrubbing processes to produce a usable byproduct that can be easily recovered, stored, and transported.

It has been 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 (*7*). Beneficial cross-feeding resulted in vigorous growth of *D. desulfuricans,* which used SO_2 as a terminal electron acceptor with complete reduction of SO_2 to H_2S. Sulfate reducing bacteria (SRB) cannot use simple sugars (such as glucose) as carbon and energy sources (*8*). Apparently, the fermentative heterotrophs that developed in these cultures as a result of nonaseptic operation utilized glucose and produced fermentative end products that served as carbon and energy sources for *D. desulfuricans.* Sulfate-reducing bacteria are also strict anaerobes; exclusion of oxygen is not sufficient to ensure growth of pure cultures. Redox poising agents are generally required to maintain a redox potential in the medium

of -150 to -200 mV. However, in the working culture described above, no redox-poising agents were required. One possible explanation is that mixed non-SRB heterotrophs in the culture scavenged oxidants and, thus, kept the redox potential sufficiently negative to favor the growth of *D. desulfuricans* (*7*).

We have proposed that the concentrated SO_2 stream obtained from the copper oxide process may be split, with two-thirds of the SO_2 reduced to H_2S by contact with culture of sulfate-reducing bacteria (*7*). 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 (*9,10*). Alternatively, a stream of H_2S and SO_2, such as obtained by regeneration of the sorbent in the NOXSO process, may be conditioned to produce the specific H_2S/SO_2 ratio (2/1) required by a Claus reactor.

The development of this process is continuing. Here we report on a study of the use of molasses as a source of carbon and reduced nitrogen, identification of important non-SRB heterotrophs in process cultures, and identification of the end products of carbohydrate fermentation that serve as carbon and energy sources for the SRB and the identification of the end products of SRB metabolism.

MATERIALS AND METHODS

Organism and Culture

D. desulfuricans (ATCC 13541) was obtained from the American Type Culture Collection (Rockville, MD). Stock cultures were maintained as previously described (*7*).

Working cultures of *D. desulfuricans* were developed as previously described and operated in a fed-batch mode with daily additions of either glucose (10 g/L) or molasses (30 g/L) (*7*). The molasses used in this study was plantation brand blackstrap molasses (Allied Old English, Inc., Port Reading, NJ). According to the distributor, this molasses contained 55 wt% carbohydrates and 10.5 wt% ash. In each case, as previously described, cultures were developed nonaseptically in a complex medium, followed by change over to a minimal medium with sulfate as the terminal electron acceptor and finally, change over to the same minimal medium with SO_2 (1% SO_2, 5% CO_2, and the balance N_2) as the terminal acceptor. Typically, a feed rate of 0.78 mmol/h SO_2 to a 1–2 L culture was used (*7*).

A number of experiments were conducted in which, after 2–3 d of operation on SO_2, molasses or glucose addition was terminated and the the behavior of the reactor monitored. The purposes of these experiments were to relate the utilization of molasses sugars to the time course of SO_2 reduction and relate the concentrations of end products of carbohydrate fermentation by non-SRB fermentative heterotrophs and the end products of *D. desulfuricans* metabolism to the time course of SO_2 reduction, and estimate the total SO_2 reduced per unit weight of sugar utilized.

Analytical

Analytical methods for quantitating H_2S in the reactor outlet gas and glucose, biomass protein, total sulfide, sulfite, and ammonia nitrogen in the reactor medium have been described in previous publications (*7,11*).

Total Kjeldahl nitrogen was determined using a Digesdahl Digestion Apparatus obtained from the Hach Chemical Co. (Loveland, CO). Total reducing sugars were determined by the Nelson's Test (*12,13*).

Samples from molasses-fed cultures contained sucrose, a nonreducing sugar, as well as the reducing sugars, fructose and glucose. Therefore, total sugars were determined by treatment of samples with invertase (to hydrolyze sucrose), followed by the Nelson's test. The pH of appropriately diluted samples from molasses-fed cultures, as well as glucose and sucrose standards, was adjusted to 4.6 with citric acid–sodium citrate buffer. Approximately 1.6 mg/mL invertase (Sigma Chemical Co., St. Louis, MO) was then added and the samples heated to 50°C for 1 h with periodic mixing. The Nelson's test was then applied to obtain the total sugars.

The sucrose concentration in molasses-fed cultures was calculated by subtracting the reducing sugar concentration (determined before invertase treatment) from the total sugar concentration (determined after invertase treatment). The fructose concentration in molasses-fed cultures was calculated by subtracting the glucose concentration from the reducing sugar concentration.

The oxidation-reduction potential of culture media was monitored with a Chemcadet millivolt meter with a Wastewater ORP electrode, both obtained from Cole-Parmer, Chicago, IL.

Low molecular weight carboxylic acids in culture media were identified and quantitated by gas chromatography with a FID detector. A glass column (2 m × 2 mm ID) was used with a stationary phase of 80/120 Carbopack B-DA/4% Carbowax 20M (Supelco, Bellefonte, PA). The column temperature was 175°C, and the injector and detector temperatures were 200°C. The carrier gas was nitrogen delivered at a flow rate of 24 mL/min. All samples contained 0.3*M* oxalic acid. Metabolites were identified and quantitated by comparison of chromatograms of medium samples with standard solutions of known compounds.

Ethanol and lactic acid were determined using diagnostic kits from Sigma Chemical Co. (St. Louis, MO). Ethanol analysis was based on oxidation by alcohol dehydrogenase. Lactic acid analysis was based on the oxidation of lactate by lactate dehydrogenase.

Identification of Non-SRB Heterotrophs

Non-SRB heterotrophs from both glucose and molasses-fed cultures were identified as follows. Using a sterile loop, samples from test cultures were streaked for isolation on Bacto Nutrient agar plates (Difco Labs, Detroit, MI) and incubated at 30°C, both aerobically and anaerobically. Anaerobic plates were incubated in sealed Gas Pak anaerobic jars with

Table 1
Typical Composition of Cane Molasses[a]

Component	Content, %
Sucrose	33.4
Glucose + Fructose	21.9
Organic nonsugars	19.6
Ash	9.8
Water	16.0

[a] According to (*16*).

catalytic removal of oxygen (Becton Dickinson and Co., Cockeysville, MD). After 24 h incubation, isolated colonies were restreaked and incubated as before. This process was repeated until a pure culture was indicated by colony morphology and Gram stain. Isolated strains were then identified by whole cell fatty acid analysis (*14,15*) by Microbial ID, Inc. (Newark, DE). These identifications were confirmed, where possible, by use of Enterotube II and/or Oxi-Ferm diagnostic media (Roche Diagnostic Systems, Nutley, NJ).

RESULTS AND DISCUSSION

Growth of *D. desulfuricans* and Mixed Heterotrophs on Molasses with Reduction of SO_2 to H_2S

In the US, the cost of starch hydrolysate, sucrose, and cane molasses are comparable when compared on a $/ton of carbohydrate basis (*6*). Starch hydrolysate and sucrose in bulk represent relatively pure sources of easily fermentable sugars. On the other hand, the composition of cane or beet molasses depends on several factors, including location of cultivation, soil type, climate, and processing. A typical cane molasses composition is shown in Table 1. As seen in Table 1, molasses also contains a high concentration of organic nonsugars. These factors combine to make molasses an undesirable feedstock for fermentations in this country. However, in Europe, the cost of starch hydrolysate and sucrose are much higher than molasses, resulting in much greater use of molasses as a feedstock (*16*). For this reason, we have investigated the reduction of SO_2 to H_2S by cultures of *D. desulfuricans* and mixed heterotrophs in which molasses was used as the ultimate source of carbon and energy.

It was observed that working cultures of *D. desulfuricans* could be prepared with molasses in a manner identical to that used with glucose. Vigorous growth was observed in either complex or minimal media (*7*), with molasses as the source of carbohydrate. Cultures (1.5 L) were maintained for up to 2 wk batch-wise, with the daily addition of 30 g/L molasses at an

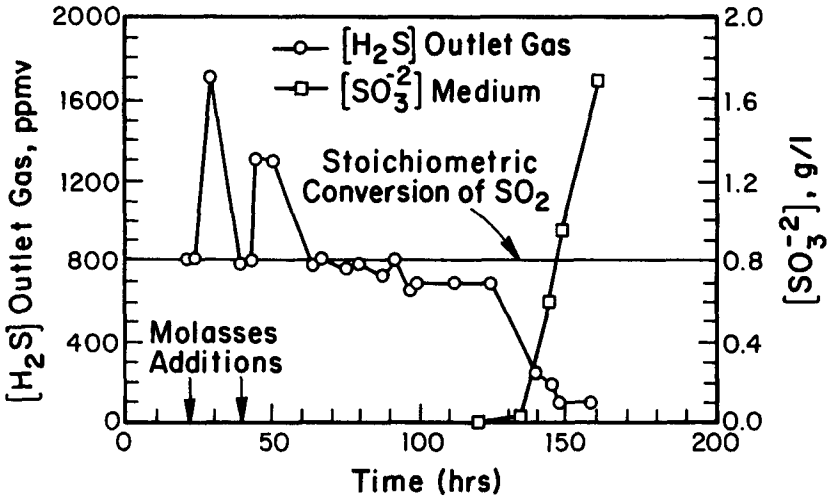

Fig. 1. Concentration of H_2S in the outlet gas (ppmv) and sulfite concentration (g/L) in the medium of a *D. desulfuricans* reactor following molasses feed. (Time zero corresponds to the initiation of SO_2 feed.)

SO_2 feed rate of 0.78 mmol/h. Complete reduction of SO_2 to H_2S was indicated. Interestingly, however, greater than stoichiometric conversion of SO_2 to H_2S was observed. As shown in Fig. 1, the production of H_2S surged after each addition of molasses and did not return to normal (stoichiometric) for 20–24 h. This has been attributed to the presence of sulfate-S in the molasses, which provided a source of terminal electron acceptor in addition to the SO_2 feed. Although difficult to analyze turbidometrically because of dark color, aqueous solutions of the molasses feedstock were found to contain significant amounts of material precipitated by $BaCl_2$. This excess production of H_2S, combined with the variability of the composition of molasses, could cause process control problems when a steady stream of H_2S is required (feed to a Claus reactor, for example).

A number of experiments were conducted in which, after 2–3 days of operation on SO_2, molasses addition was terminated and the behavior of the reactor monitored. Figure 2 shows the concentration of total sugars, sucrose, fructose, and glucose following the termination of molasses addition. Figure 1 shows the corresponding output of H_2S from the reactor and the concentration of sulfite in the culture medium. As shown in Fig. 2, the total sugars are essentially depleted 10 h after the last molasses addition. However, the reactor continued to reduce SO_2 to H_2S, with no sulfite accumulation for an additional 93 h after the terminal addition of molasses. The probable explanation is that the sugars were metabolized by the mixed non-SRB heterotrophs much faster than the *D. desulfuricans* could utilize the end products of the fermentation of those sugars. Therefore, the *D. desulfuricans* continued to have these fermentative end products available as carbon and energy sources long after the sugars disappeared

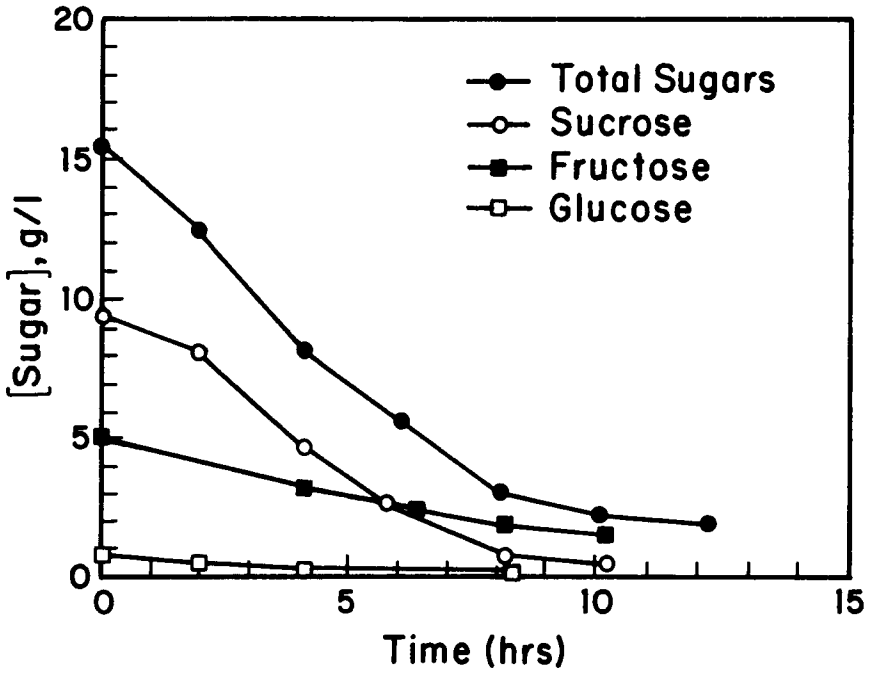

Fig. 2. Sugar concentrations in a *D. desulfuricans* reactor following molasses feed. (Time zero corresponds to the last molasses addition.)

from the medium. This has been confirmed in glucose-fed cultures, as discussed below.

The approximate amount of SO_2 that can be reduced per unit weight of sugar in molasses cultures was estimated from the amount of SO_2 reduced from the time of the last molasses addition until sulfite began to accumulate in the medium. This was found to be approximately 0.22 g SO_2/g sugar.

The Kjeldahl nitrogen content of the molasses was found to be 6470 ppm. It was observed that the organic nitrogen in the molasses furnished sufficient reduced nitrogen to these cultures so that NH_4^+ could be eliminated from the medium formulation. In those experiments in which culture were allowed to proceed to an upset condition, following a terminal addition of molasses, nitrogen was shown not to be a limiting factor.

Material Balances for SO_2 Reduction in Glucose-Fed *D. desulfuricans* Reactors

In order to evaluate the microbial reduction of SO_2 to H_2S from both a technical and economic point of view, it is necessary to understand the flow of carbon from glucose (or other carbohydrates) to the fermentative non-SRB heterotrophs in the process culture, to the sulfate-reducing bacterium (*D. desulfuricans*), and finally to the end products of the process.

Working cultures of *D. desulfuricans* were prepared on glucose, as described previously (*7,11*). During growth on SO_2, the culture medium was sampled periodically and analyzed by gas chromatography. As shown in Figs. 3 and 4, the following metabolites of glucose were identified: ethanol lactic acid, acetate, proprionate, butyrate, and isobutyrate. Ethanol and lactic acid are common end products of the anaerobic fermentation of glucose. As will be shown subsequently, all of the non-SRB heterotrophs identified in these process cultures are known to produce lactic acid and/or ethanol from glucose. Lactic acid and ethanol are also recognized as carbon and energy sources for *D. desulfuricans* (*8*). Acetate, propionate, isobutyrate, and butyrate have also been observed as end products of anaerobic glucose fermentation. In addition, acetate is the end product of the oxidation of lactic acid and ethanol by *D. desulfuricans*. (Carbon dioxide is also formed from lactate.)

As seen in Figs. 3 and 4, a marked increase in ethanol, lactate, acetate, and butyrate concentrations followed fed batch glucose additions. In some cases, glucose addition was terminated and the behavior of the reactor monitored until an upset condition was produced. As seen in Fig. 5, the lactic acid concentration fell essentially to zero within 24 h of the last glucose addition. The ethanol concentration declined more gradually from 2–3 g/l to zero in about 96–120 h. When ethanol was depleted, sulfite began to accumulate in the reactor medium, and the H_2S concentration in the reactor outlet gas declined, indicating less than stoichiometric conversion of SO_2 to H_2S. Acetate is seen in Fig. 6 to have accumulated, while ethanol was depleted. Material balances for two such experiments are given in Table 2; however, CO_2 production was not determined.

It appears that the fermentation of glucose by non-SRB heterotrophs primarily produced ethanol and lactic acid which, in turn, were used by *D. desulfuricans* as carbon and energy sources. As noted above, following the termination of glucose addition, reactor failure, as indicated by sulfite accumulation, corresponded directly with ethanol depletion. Acetate appears to be the primary end product of ethanol and lactic acid metabolism by *D. desulfuricans*. The exact role of propionate, butyrate, and isobutyrate is as yet unclear.

Identification of Non-SRB Heterotrophs in *D. desulfuricans* Reactors

As noted previously, the non-SRB heterotrophs responsible for carbohydrate fermentation and the production of carbon and energy sources suitable for the sulfate-reducing bacteria arise in working cultures simply from nonaseptic operation of the reactor. In order to avoid variability, it will eventually be necessary to use a standard inoculum for these cultures. Therefore, we have begun to identify the non-SRB heterotrophs that develop in these reactorss. Those bacteria identified thus far are

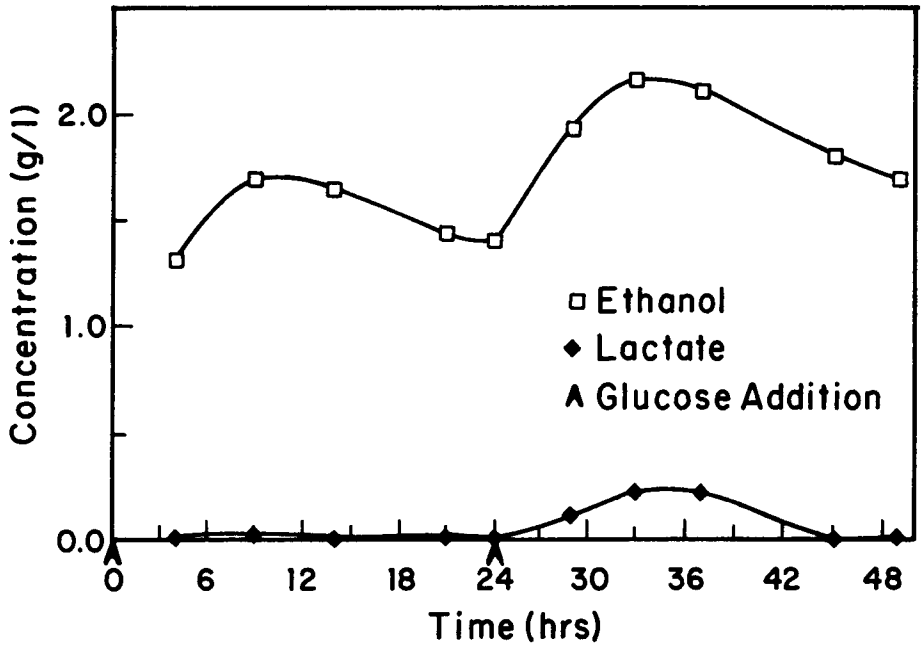

Fig. 3. Ethanol and lactic acid concentrations in a glucose-fed *D. desulfuricans* reactor operating on a SO_2 feed.

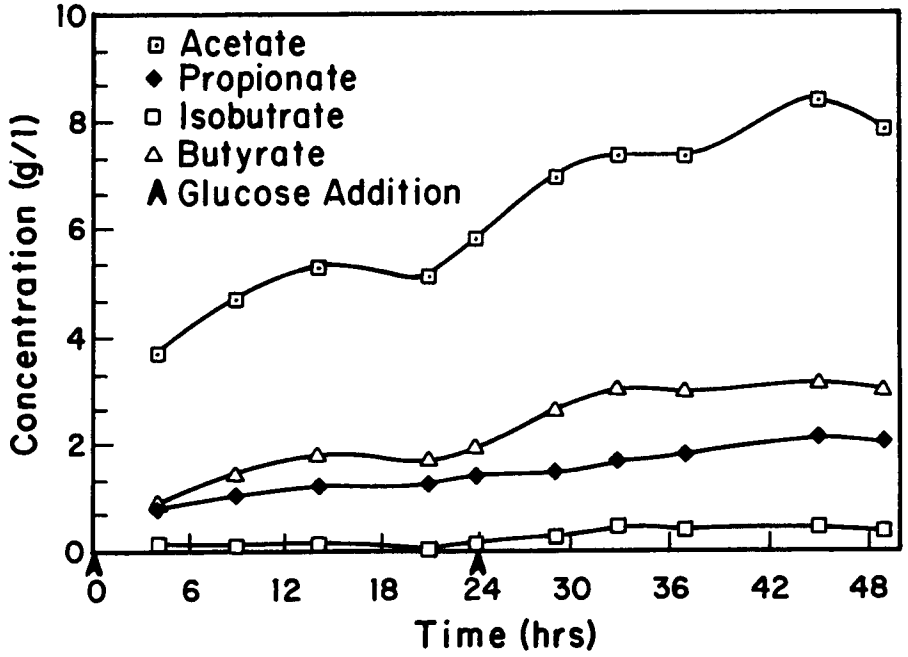

Fig. 4. Acetate, propionate, butyrate, and isobutyrate concentrations in a glucose-fed *D. desulfuricans* reactor operating on a SO_2 feed.

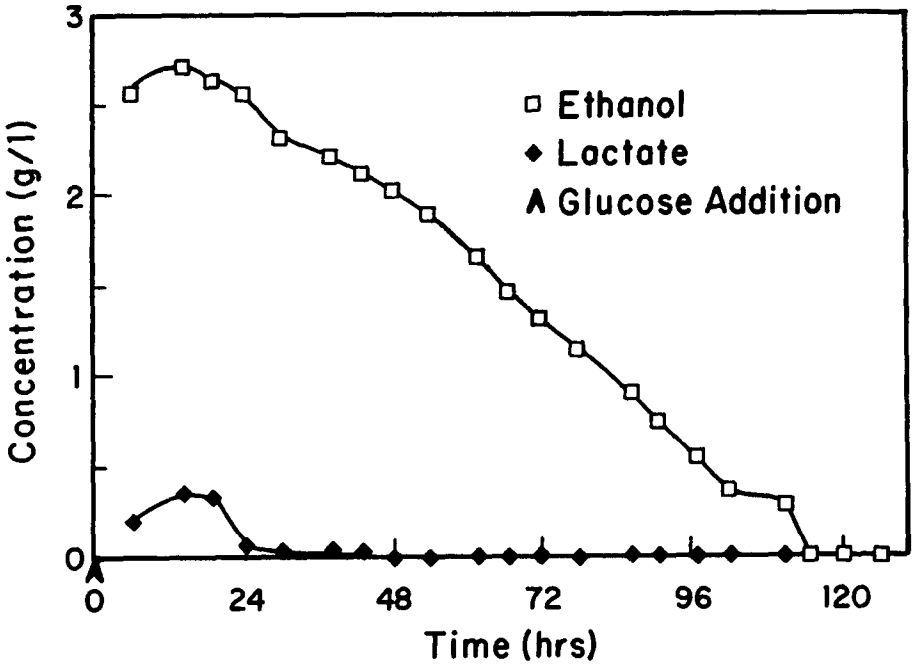

Fig. 5. Ethanol and lactic acid concentrations in a glucose-fed *D. desulfuricans* reactor with a SO_2 feed, following the terminal addition of glucose.

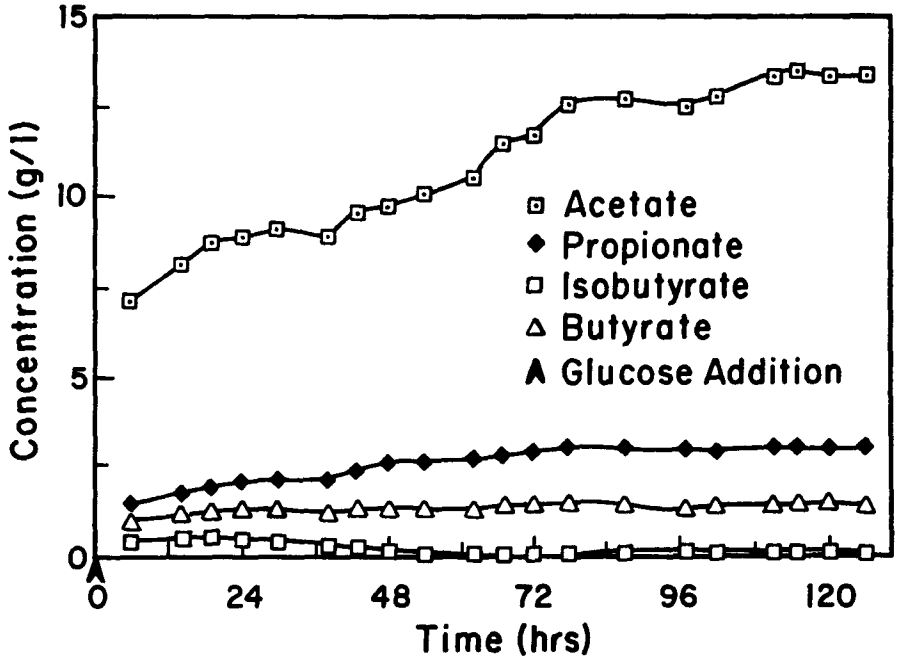

Fig. 6. Acetate, propionate, butyrate, and isobutyrate concentrations in a glucose-fed *D. desulfuricans* reactor with a SO_2 feed, following the terminal addition of glucose.

Table 2
Material Balances for SO_2 Reduction
of Glucose-Fed *D. desulfuricans* Reactors

	Exp. A	Exp. B
Glucose utilized	45.0 g	30.0 g
SO_2 reduced	7.9 g	5.6 g
Acetate produced	15.7 g	11.1 g
Propionate produced	3.6 g	3.3 g
Butyrate produced	1.7 g	0.5 g
Isobutyrate produced	0.12 g	0.08 g
SO_2/Glucose, g/g	0.18	0.19
SO_2/Glucose, mol/mol	0.49	0.52
Acetate/Glucose, g/g	0.35	0.37
Acetate/Glucose, mol/mol	1.04	1.06

listed in Table 3. Also given in Table 3 are the end products of anaerobic fermentation of glucose by each of these organisms, as given by Bergey's Manual (*17*). Based on the analysis given above, those organisms that produce ethanol, and to a lesser extent lactate, predominate in these cultures.

CONCLUSION

In summary, it has been observed that molasses will support the reduction of SO_2 to H_2S by *D. desulfuricans* in coculture with mixed heterotrophs. However, the sulfur contained in the molasses results in H_2S production in excess of that expected, based on the SO_2 feed. H_2S production was seen to surge after each molasses addition. This excess H_2S production could cause process control problems when a steady stream of H_2S is required (feed to a Claus reactor, for example). The organic nitrogen content of the molasses was sufficient as a nitrogen source for the culture. The "yield" of SO_2, reduced per gram of sugar during growth on molasses, was estimated to be 0.22 g SO_2/g sugar vs 0.19 g SO_2/g sugar in glucose-fed cultures.

Material balances in glucose-fed cultures indicate the fermentation of glucose primarily to ethanol by non-SRB heterotrophs in the culture and subsequent oxidation of ethanol to acetate by *D. desulfuricans*. To a lesser extent, propionate, butyrate, and isobutyrate accumulated in SO_2-reducing cultures. The incorporation of strains of sulfate-reducing bacteria, capable of utilizing acetate, and other low molecular weight carboxylic acids would improve the "yield" of SO_2 reduced per gram of sugar. Finally, 10 different non-SRB heterotrophs have been identified from *D. desulfuricans* working cultures. This is the first step in formulating a standard inoculum for these cultures.

Table 3
Non-SRB Heterotrophs Found in a *D. desulfuricans* Reactor Operating with a SO_2 Feed and Their Respective End Products of Glucose Fermentation

Organism	Endproducts[a]
Enterococcus faecium	lactate, ethanol, and acetate
Escherichia coli	lactate, acetate, and formate
Citrobacter freundi	lactate, acetate, and formate
Citrobacter diversus	lactate, acetate, and formate
Klebsiella pneumoniae	2,3-butanediol, lactate, acetate ethanol, and formate
Klebsiella pneumoniae ozaenae	2,3-butanediol, lactate, acetate, ethanol, and formate
Enterobacter agglomerana	2,3-butanediol, lactate, acetate, ethanol, and formate
Clostridium bifermentaus	isobutyrate, isovalerate, isocaprate, butyrate, ethanol, propanol, and isobutanol
Salmonella arizonae	lactate, acetate, and formate
Enterobacter cloacae	2,3-butanediol, lactate, acetate, ethanol, and formate

[a]According to (*17*).

ACKNOWLEDGMENTS

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