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Microbial Removal of Sulfur Dioxide from a Gas Stream with Net Oxidation to Sulfate

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ABSTRACT

A study has been conducted of the feasibility of utilizing the sulfate reducing bacterium *Desulfovibrio desulfuricans* and the chemoautotroph *Thiobacillus denitrificans* as a basis of a microbial process for the removal of sulfur dioxide from a gas with net oxidation to sulfate. In reactors-in-series, SO_2 was reduced to H_2S in the first stage by *D. desulfuricans*. The H_2S was then stripped with nitrogen and sent to a second stage where it was oxidized to sulfate by *T. denitrificans*. A sulfur balance demonstrated complete reduction of SO_2 to H_2S in the first stage and complete oxidation of H_2S to sulfate in the second stage.

Index Entries: Sulfur dioxide; NO_x; *Thiobacillus denitrificans;* sulfate reducing bacteria; flue gas desulfurization.

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. On 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

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damage to ecosystems, acid rain causes major damage to materials such as metals, limestone, marble, and mortar. Sulfur dioxide and related pollutants in the atmosphere have also been linked to various catagories of human diseases (*3–5*).

A major concern is the very large amounts of SO_2 emitted, especially in metropolitan areas where power generation plants are concentrated. For example, a typical 1000 MW boiler burning 3.5% sulfur coal will emit more than 600 tons of SO_2/d (*6*). Various estimates of total emissions have been made. The general consensus is that the US emissions from the electric utility industry in 1980 were over 18 million tons (*7*). Similar data for other parts of the world are not readily available. However, emissions are heavy in all industrialized countries, particularly in areas such as the Ruhr Valley in West Germany and the Tokyo area in Japan.

There are several engineering solutions to this problem, although none alone satisfies all of the desired technical and economic requirements. There are two basic approaches to addressing the problem of SO_2 emissions: desulfurize the feedstock prior to or during combustion; or scrub the resultant SO_2 from the boiler flue gases. Although feedstock desulfurization and allied technologies (e.g., coal liquefaction) are of considerable interest, it is the flue gas processing alternative that will be addressed here.

The most commercially important flue gas desulfurization technology at present is the use of solid, throwaway adsorbents such as limestones and dolomites that have affinity for acid gases like SO_2. This type of process results in the production of large amounts of calcium sulfate ($CaSO_4$), which can represent a significant disposal problem. In addition, little or no removal of oxides of nitrogen (NO_x) is achieved.

Several of the more promising technologies under development combine SO_2 and NO_x removal. These include two regenerable dry scrubbing processes, the copper oxide process and the NOXSO process. In regenerable dry scrubbing processes, as the name implies, flue gases are contacted with a dry sorbent resulting in the chemisorption of SO_2. The sulfated sorbent is subsequently regenerated using a reducing gas such as hydrogen, carbon monoxide, or methane. Regeneration of the copper oxide sorbent produces a concentrated stream of sulfur dioxide. In the copper oxide process, NO_x is catalytically reduced to elemental nitrogen with ammonia. In the NOXSO process, the sorbent consists of sodium aluminate ($NaAlO_2$) on gamma alumina. The sorbent also adsorbs or chemisorbs NO_x from flue gas. The NO_x chemisorption product is unstable above 400°C. During regeneration, heating the sorbent in air to 600°C produces a concentrated NO_x stream. Subsequent treatment of the sorbent with a reducing gas produces a mixture of SO_2, H_2S, and elemental sulfur (*6,8,9*).

Regenerable dry scrubbing processes, such as the NOXSO or copper oxide processes, offer considerable advantages over the use of throwaway

adsorbents. Primary among these are reduced costs for chemical makeup and the simultaneous removal of SO_2 and NO_x. However, both the copper oxide and NOXSO processes produce a concentrated stream of SO_2 or SO_2 and H_2S, respectively, that must be disposed of or recovered in a separate process. New technology is needed to process these concentrated sulfur gas streams from regenerable dry scrubbing processes to produce a usable byproduct that can be easily recovered, stored, and transported.

We have recently described a microbial/Claus process for the conversion of SO_2 to H_2S to elemental sulfur as a means of byproduct recovery or disposal from regenerable dry scrubbing processes (*10*). Specifically, the sulfate reducing bacterium *Desulfovibrio desulfuricans* in anaerobic and nonaseptic cultures has been used to remove SO_2 from a gas with reduction to H_2S. Nonaseptic operation resulted in the development of a significant population of mixed heterotrophic bacteria in the culture in addition to the *Desulfovibrio*. Under SO_2-limiting conditions no sulfite accumulated in the culture medium and SO_2 was completely reduced to H_2S, which was stripped from the reactor with nitrogen. The culture medium contained mineral salts, glucose, and trace vitamins. In pure culture, sulfate reducing bacteria do not normally utilize carbohydrates as carbon or energy sources but are restricted to compounds such as ethanol, acetate, pyruvate, and lactate. These are recognized as endproducts of anaerobic carbohydrate metabolism by fermentive bacteria. However, vigorous growth of *D. desulfuricans* was observed in mixed cultures on glucose. Working cultures containing greater than 5×10^8 cells/mL were determined by microscopic counts to be 50% *Desulfovibrio*. Apparently, the mixed heterotrophs in the culture utilized glucose and produced pyruvate, lactate, or other endproducts. These fermentive endproducts then served as carbon and energy sources for *D. desulfuricans*. Pure cultures of sulfate reducing bacteria also require redox-poising agents to maintain strict anaerobic conditions; mere exclusion of oxygen is not sufficient. However, in these experiments, no redox-poising agents were used. Apparently, the mixed heterotrophs in the culture scavenged all of the available oxidants and kept the redox potential sufficiently low to favor the growth of the *Desulfovibrio*.

A process has been proposed whereby a concentrated SO_2 stream from a dry regenerable scrubbing process (such as the copper oxide process) is split with 2/3 of the SO_2 reduced to H_2S by contact with a culture of sulfate reducing bacteria (*10*). The resulting H_2S is then combined with the remaining SO_2 and used as the feed to a Claus reactor to produce elemental sulfur (*11,12*). However, a microbial process can potentially impact on the overall problem of flue gas desulfurization and NO_x removal in other ways as well. The most attractive option is the simultaneous removal of SO_2 and NO_x from flue gas by contact with one or more microbial cultures. In this regard, we propose to expand the work described above with

cultures of sulfate reducing bacteria to include the autotroph *Thiobacillus denitrificans* either in coculture or in cultures-in-series to effect the simultaneous removal of SO_2 and NO_x from a gas stream.

T. denitrificans is a strict autotroph and facultative anaerobe first described in detail by Baalsrud and Baalsrud (*13*). Under anaerobic conditions, nitrate may be used as a terminal electron acceptor with reduction to elemental nitrogen. Thiosulfate, elemental sulfur, and sulfide may be used as energy sources with oxidation to sulfate; however, sulfide is an inhibitory substrate. Sublette and Sylvester (*14–17*) have demonstrated that *T. denitrificans* may be readily cultured aerobically or anaerobically in batch or continuous reactors on H_2S (g) under sulfide-limiting conditions. Complete removal of H_2S from feed gases was observed with complete oxidation of H_2S to sulfate that accumulated in the culture media. Stable reactor operation was demonstrated for up to 7 d in batch cultures and 26 d in continuous cultures at reactor loadings as high as 4–5 mmoles H_2S oxidized/h-g biomass. Maximum H_2S loading of the biomass was estimated at 5.4–7.7 mmoles H_2S/h-g biomass under anaerobic conditions and 15.1–20.9 mmoles H_2S/h-g biomass under aerobic conditions. Indicators of reactor upset were determined and recovery from upset demonstrated. Sublette and Sylvester also reported that heterotrophic contamination of *T. denitrificans* cultures had no effect on H_2S oxidation.

Conceptually, sulfate reducing bacteria and *T. denitrificans* can be used in coculture or in reactors-in-series to bring about the net oxidation of SO_2 to sulfate, as shown in Eqs. (1) and (2)

$$SO_2 \rightleftharpoons H_2S \quad \text{Sulfate reducing bacteria} \tag{1}$$

$$H_2S \rightleftharpoons SO_4^{-2} \quad \textit{T. denitrificans} \tag{2}$$

Various options would exist for the recovery of this sulfate and its disposal or utilization as a byproduct. As with processes that use throwaway adsorbents, the disposal of this sulfate could have a negative impact on process economics. However, this potential disposal problem could be offset by the advantage of some degree of NO_x removal by *T. denitrificans*. As noted above, *T. denitrificans* utilizes nitrate as an oxidant in the absence of oxygen with reduction to elemental nitrogen. Nitric oxide (NO) has been shown to be an intermediate in the reduction of nitrate by this organism (*18–20*). Ishaque and Aleem (*19*) and Baldensperger and Garcia (*20*) have demonstrated that whole cells of *T. denitrificans* will catalyze the reduction of NO to elemental nitrogen with a concomitant oxidation of thiosulfate (electron donor). However, their experiments utilized "resting cells;" that is, the cells were not actively growing and reproducing. It is unknown as to whether NO will support the growth of *T. denitrificans* as a terminal electron acceptor. Nitrogen dioxide (NO_2) is not an intermediate in nitrate reduction by *T. denitrificans*. However, NO_2 would react with water in a microbial culture to produce nitric acid and make nitrate available to the organisms. Therefore, it is anticipated that NO_2 could be utilized in the

support growth to *T. denitrificans* under anaerobic conditions with reduction to elemental nitrogen.

The high reaction rates and mild reaction conditions offered by a microbial process can conceivably yield technical and economic advantages over conventional processes for SO_2/NO_x removal. An experimental program and economic analysis has been undertaken that will allow the conceptualization of the most advantageous means by which a microbial process can impact on the overall problem of flue gas desulfurization and NO_x removal. We report here on a study of the net oxidation of SO_2 to sulfate by *Desulfovibrio desulfuricans* and *T. denitrificans* in series reactors and in coculture. Other work dealing with the optimization of SO_2 reduction by *D. desulfuricans* and an investigation of NO reduction by *T. denitrificans* are ongoing and will be reported at a later date.

MATERIALS AND METHODS

Organisms and Culture

Stock Cultures

Desulfovibrio desulfuricans (ATCC 13541) was obtained from the American Type Culture Collection, Rockville, MD. Stock cultures were maintained anaerobically and aseptically on a complex glucose medium, as previously described (*10*). In this medium, sulfate was the terminal electron acceptor and ammonium ion the source of reduced nitrogen. The carbon and energy source for the *D. desulfuricans* was derived from the yeast extract, peptone and beef extract components.

Thiobacillus denitrificans (ATCC 23642) was also obtained from the ATCC. Stock cultures were grown anaerobically and aseptically in a thiosulfate maintenance medium, as previously described (*14*). In this medium, thiosulfate was the electron donor, nitrate the terminal electron acceptor, bicarbonate the source of carbon, and ammonium ion the source of reduced nitrogen.

Operation of Reactors-in-Series

Working cultures of *D. desulfuricans* for SO_2 reduction were developed, as described elsewhere (*10*). These cultures consisted of *D. desulfuricans* and mixed heterotrophs in coculture in a glucose minimal medium. The mixed non-SRB heterotrophs in these cultures developed simply from nonaseptic operation of the reactor and consisted primarily of short, gram-negative rods. These heterotrophs have not been further defined as yet. As noted previously, these cultures contained greater than 5×10^8 cells/mL, in which approximately 50% were crescent shaped cells indicative of *Desulfovibrio*. Sulfur dioxide fed to the cultures served as the terminal electron acceptor for the *D. desulfuricans*. The cultures were anaerobic

in that there was no oxygen feed to the reactor and the culture was continually stripped with nitrogen (270 mL/min). The SO_2 feed gas contained 0.99% SO_2, 4% CO_2, and balance nitrogen. The purpose of the CO_2 was to ensure a continuous source of carbon for *T. denitrificans* in the reactor downstream. Cultures were generally maintained in a Marubishi MD 300 bench scale fermenter (2 1 culture) at 30°C and pH 7.0, as described previously (*10*). The off-gas from the *D. desulfuricans* reactor was fed to the *T. denitrificans* reactor.

Working cultures of *T. denitrificans* were developed anaerobically and non-aseptically on thiosulfate as described by Sublette and Sylvester (*14, 17*). Thiosulfate is inhibitory to H_2S oxidation by *T. denitrificans* (*14*). Therefore, prior to receiving the off-gas from the *D. desulfuricans* reactor, *T. denitrificans* cells were harvested by centrifugation at 4900×g at 25°C for 10 min. Cells were then resuspended in 1.4 L of fresh thiosulfate medium (without thiosulfate) and transferred to a B. Braun Biostat M fermenter. Gas feed from the *D. desulfuricans* reactor was then initiated. The culture was maintained at 30°C and pH 7.0, as described previously (*14*). The off-gas of the *T. denitrificans* reactor was transferred to a Precision Scientific Wet Test Meter that measured cumulative gas flow. The *T. denitrificans* reactor was operated under sulfide-limiting conditions. In other words, at all times the H_2S feed rate was less than the maximum specific activity of *T. denitrificans* for H_2S oxidation.

Typically, the *D. desulfuricans* and *T. denitrificans* reactors were maintained in series for 72–96 h with periodic sampling of the reactor media and outlet gases.

D. desulfuricans *and* T. denitrificans *in Coculture*

In this experiment, SO_2 was fed to a reactor containing *D. desulfuricans*, *T. denitrificans*, and mixed heterotrophs where SO_2 was reduced to H_2S by the *D. desulfuricans*. The H_2S thus produced was immediately oxidized by *T. denitrificans* to sulfate that accumulated in the culture.

The coculture was developed as follows. First, a working culture of *D. desulfuricans* and mixed heterotrophs was developed, as described above, with the exception that the culture medium was a lactate minimal medium given in Table 1 that was supplemented with nutrients for *T. denitrificans*. Lactate was substituted for glucose because of reports in the literature that *T. denitrificans* is inhibited by low concentrations of glucose (*21*). (Tolerance of glucose concentrations in excess of 10 g/L was subsequently demonstrated in our laboratories.) Prior to the introduction of SO_2, the biomass was harvested by centrifugation as described above and resuspended in the same medium without sulfate. When the temperature of the culture medium stabilized at 30°C, SO_2 was introduced. The same SO_2 gas mixture as described above was used. The culture was also purged with 270 mL/min of nitrogen to strip out H_2S. Three mL of 80% sodium lactate was added to the culture every 4 h. The culture was operated in

Table 1
Modified Minimal Medium for the Growth
of *D. desulfuricans* and *T. denitrificans* in Coculture

Component	Per liter
Sodium lactate (80% by wt)	1.5 mL
Na_2SO_4	1.5 g
Balch vitamin solution[a]	2.0 mL
Na_2HPO_4	1.2 g
KH_2PO_4	1.8 g
$MgSO_4 \cdot 7H_2O$	0.4 g
NH_4Cl	0.5 g
$CaCl_2$	0.03 g
$MnSO_4$	0.02 g
$FeCl_3$	0.02 g
$NaHCO_3$	1.0 g
KNO_3	5.0 g
Heavy metal solution[a]	15.0 mL
Mineral water	50.0 mL

[a]The Balch vitamin solution (*10*) and the heavy metal solution (*14*) have been described elsewhere.

this manner for 24 h. At the end of this time, a slurry of *T. denitrificans* cells (1.0–1.5 g cell mass by dry wt) previously grown on thiosulfate were introduced into the reactor. The reactor was then operated for another 24 h while the off-gas of the reactor was monitored for H_2S.

Analytical

Gas Analysis

All gas analyses were conducted by gas chromatography, as described previously (*10,14*). A thermal conductivity detector was used that had a detection limit for H_2S of 50 parts per million by volume (ppmv) with a sample size of 0.5 mL. For quick, approximate determination of H_2S concentration in the outlet gas from either reactor, Gastec Analyzer tubes (Gastec Corp., Yokohama, Japan) were used. The ranges of these tubes for H_2S detection were in four classes: 5–60 ppmv, 60–120 ppmv, 100–1600 ppmv, and 0.1–0.2%.

Analysis of Culture Media

Biomass protein was determined by sonication followed by colorimetric analysis by the micro-Folin method, as previously described (*14,22,23*).

Total sulfide was determined by ion specific electrode (*14*). Elemental sulfur was determined by its reaction with cyanide to produce thiocyanate, which was in turn quantitated as $Fe(SCN)_6^{-3}$ (*24*). Sulfite was determined spectrophotometrically by reacting sulfite with formaldehyde in

sulfuric acid (*25*). Sulfate was determined turbidometrically by precipitation as barium sulfate (*26*).

Nitrate was determined by the cadmium reduction method using gentistic acid in place of *N*-(1-napthyl)-ethylenediamine in the color development step (*26*). Ammonium ion was determined by the Nessler method without distillation (*26*). Reagents for both the nitrate and ammonium ion determinations were obtained from Hach Chemical Co.

Viable counts of *T. denitrificans* were determined by plate counts on thiosulfate agar incubated anaerobically, as described previously (*14*). Counts of *D. desulfuricans* were made microscopically, identifying crescent shaped cells using a Corpuscle Counting Chamber (Hausser Scientific). This method was verified by comparison to a three-tube most probable number method that is a modification of American Petroleum Institute Standard Practice, Bulletin 38 (*27*). Total heterotrophs were quantitated by plate counts on nutrient agar (Difco labs) incubated anaerobically at 30°C.

RESULTS AND DISCUSSION

Net Oxidation of SO_2 to Sulfate by Reactors-in-Series

In a typical experiment (Exp. #062) SO_2 was fed to the *D. desulfuricans* reactor at a molar flow rate of 0.78 mmoles/h, resulting in steady concentration of H_2S of 800 ppmv in the reactor off-gas. In Exp. #062, 69.0 mmoles of SO_2 were fed to the reactor, whereas 63.7 mmoles of H_2S were detected in the off-gas during the course of the experiment. In the three performed experiments of this type, the ratio of H_2S produced to SO_2 consumed averaged 0.95. As indicated in the previous section, the detection limit for the gas chromatograph used to analyze the off-gas for H_2S was about 50 ppmv. Therefore, the H_2S analyses used in these calculations may have underestimated the actual H_2S concentration by about 6%. The true H_2S/SO_2 ratio is, therefore, very likely 1.0. In other words, all SO_2 was converted to H_2S. Other details of the performance of a *D. desulfuricans* reactor with an SO_2 feed have been reported elsewhere (*10*).

The off-gas of the *D. desulfuricans* reactor was fed to the *T. denitrificans* reactor. There was no accumulation of sulfite in the liquid phase of the *T. denitrificans* reactor, indicating that there was no SO_2 in the feed gas to the *T. denitrificans* reactor. Under sulfide-limiting conditions, the sulfide concentration in the culture medium was less than 1 μM. No elemental sulfur was detected. However, sulfate accumulated in the medium as H_2S was removed from the feed gas (Fig. 1). The H_2S concentration in the outlet gas averaged less than 2 ppmv, as estimated by low range Gastec Analyzer tubes. Removal of H_2S from the feed gas was accompanied by growth as evidenced by the accumulation of biomass protein (Fig. 2) and the con-

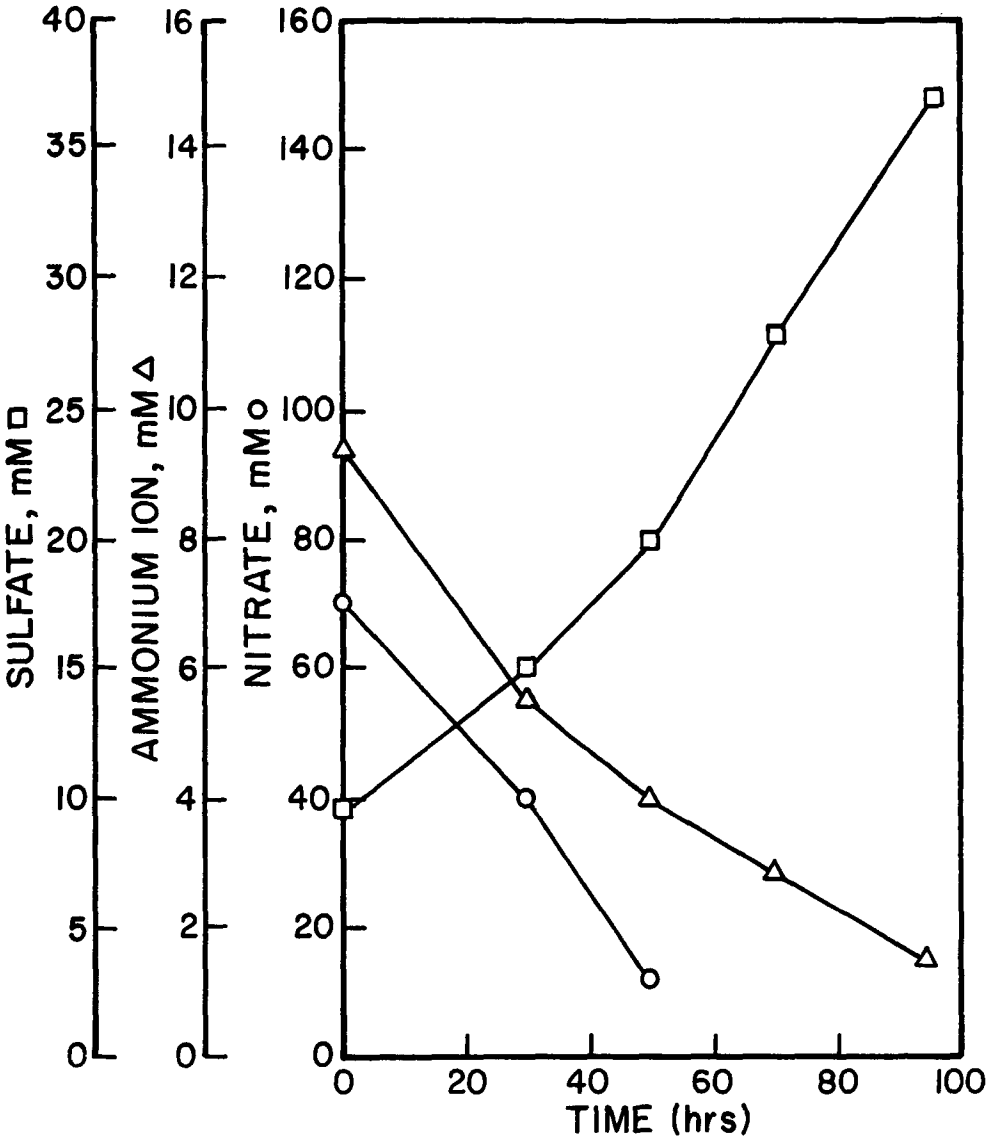

Fig. 1. Concentrations of sulfate (SO_4^{-2}), ammonium ion (NH_4^+) and nitrate (NO_3^-) in a *T. denitrificans* reactor receiving an H_2S feed from a *D. desulfuricans* reactor.

sumption of nitrate (Fig. 1) and ammonium ion (Fig. 1). The *T. denitrificans* count and total heterotroph count were also seen to increase as H_2S was removed from the reactor feed gas (Fig. 2). (Thiosulfate minimal medium contains no source of organic carbon; heterotrophs grow only at the expense of waste products and products of cell lysis of *T. denitrificans* (*17*).)

Sublette and Sylvester (*14*) have shown that the anaerobic oxidation of H_2S by *T. denitrificans* is an acid producing process and requires alkali addition to maintain pH at 7.0. In all of the experiments conducted in this

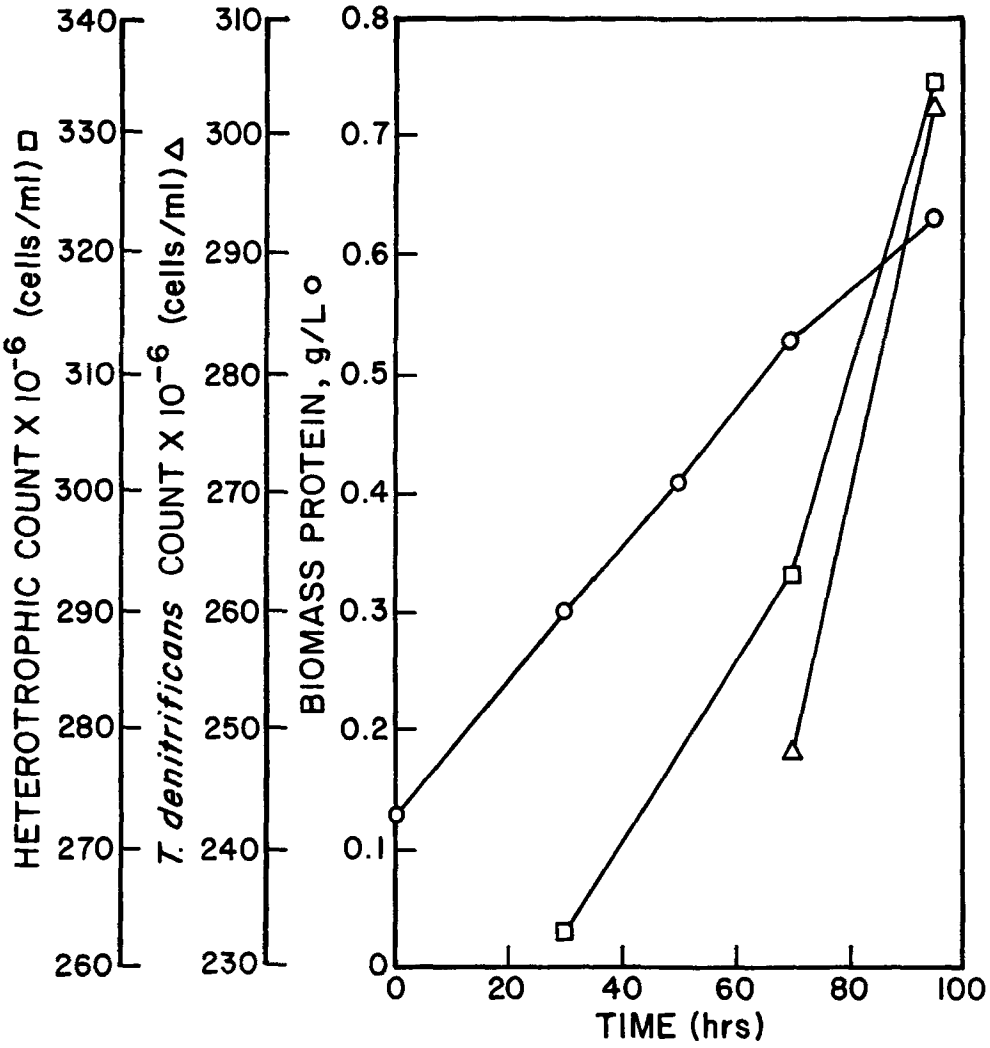

Fig. 2. Biomass protein concentration and *T. denitrificans* and total heterotroph counts in a *T. denitrificans* reactor receiving an H_2S feed from a *D. desulfuricans* reactor.

work, however, acid addition was required to maintain a pH of 7.0 in the *T. denitrificans* reactor. For example, in Exp. #062, a total of 66.1 meq of acid were required for the oxidation of 63.7 mmoles of H_2S. This led to speculation that there was an alkaline component in the off-gas from the *D. desulfuricans* reactor that was not only supplying the alkalinity needed to neutralize the acid produced by the oxidation of H_2S in the *T. denitrificans* reactor, but also requiring further acid addition to maintain the proper pH in the culture medium. An attempt was made to identify this alkaline component in the off-gas through gas chromatography, infrared spectroscopy, and ultraviolet spectroscopy. None of these analyses resulted in the identification of any species other than H_2S, N_2, CO_2, and water in the feed gas to the *T. denitrificans* reactor.

Table 2
Summary of Observations:
Hydrogen Sulfide Oxidation by *T. denitrificans* Batch Cultures

Exp. no.	H_2S oxidized, mmoles	SO_4^{-2} produced, mmoles	NO_3^- consumed, mmoles	NH_4^+ consumed, mmoles	Biomass protein produced, g
062	63.7	64.2	151.7	11.2	0.41
090	86.2	87.9	168.1	11.2	0.55
091	60.4	61.4	101.4	10.8	0.45

Table 3
Stoichiometry of Hydrogen Sulfide Oxidation
by *T. denitrificans* in Batch Reactors

	mole/mole			g Biomass protein/ mol H_2S
Exp. no.	SO_4^{-2}/H_2S	NO_3^-/H_2S	NH_4^+/H_2S	
062	1.01	2.38	0.17	6.4
090	1.02	1.95	0.13	6.4
091	1.02	1.68	0.18	7.5
Average	1.02	1.99	0.16	6.8

Table 2 summarizes the stoichiometry of H_2S oxidation, as determined in the three experiments of this type completed. In a typical experiment (Exp. #062) the oxidation of 63.7 mmoles of H_2S was accompanied by the production of 66.4 mmoles of sulfate and 0.41 g of biomass protein and the consumption of 151.7 mmoles of nitrate and 11.2 mmoles of NH_4^+. Table 3 expresses the stoichiometric data as ratios with respect to H_2S oxidized. The average SO_4^{-2}/H_2S ratio is seen to be slightly greater than 1.0. This could be because of a small underestimation of H_2S concentration in the feed gas by gas chromatography, as described above. The other stoichiometric ratios relative to H_2S may also be underestimated for the same reason. The stoichiometries with respect to ammonium ion, nitrate, and biomass protein differ somewhat from that reported by Sublette and Sylvester (*14*) for batch anaerobic conditions. Greater NH_4^+ and NO_3^- utilization and biomass production per mole of H_2S oxidized were observed in the experiments reported here. This was probably a result of the nonaseptic operation of the *T. denitrificans* reactors. The experiments reported by Sublette and Sylvester were conducted under aseptic conditions with no heterotrophic contamination. In the *T. denitrificans* cultures used in these experiments, the average heterotrophic contamination accounted for approximately 50% of the total biomass in the reactors (Fig. 2). This

could easily account for the higher yields of biomass and the greater consumption of ammonium ion and nitrate seen in these experiments.

Net Oxidation of SO_2 by a Coculture of *D. desulfuricans* and *T. denitrificans*

A limited number of experiments of this type have been conducted. Therefore, the following results should be considered preliminary.

In Exp. #061, a *D. desulfuricans* culture was developed in a modified lactate minimal medium (Table 1), as described above. An SO_2 feed of 0.78 mmoles/h was initiated following a changeover to the same medium without sulfate. This resulted in a steady production of H_2S and an average concentration in the outlet gas of 800 ppmv. Within 1 min of the addition of *T. denitrificans* cells to the reactor, the H_2S concentration in the off-gas dropped from 800 ppmv to less than 2 ppmv. No sulfite, sulfide, or elemental sulfur were detected in the culture medium. This condition was maintained for 24 h, at which time the experiment was terminated.

CONCLUSIONS

The net oxidation of SO_2 to sulfate has been demonstrated utilizing *D. desulfuricans* and *T. denitrificans* cultures in series. Sulfur dioxide was reduced to H_2S in the *D. desulfuricans* stage; H_2S, in turn, was oxidized to sulfate in the *T. denitrificans* stage. Sulfate accumulated in the medium of the *T. denitrificans* reactor.

The next step in the development of a microbial SO_2/NO_x process is to incorporate NO_x in the feed to the system. In a two-stage system for combined SO_2/NO_x removal, the first stage must be the sulfate reducing bacteria reactor. (If the *T. denitrificans* reactor was the first stage, sulfite would accumulate to toxic levels.) Therefore, the microbial population in this stage must be tolerant of NO_x. The steady state liquid phase concentration of NO in the first stage would be quite low considering the low solubility of NO in water and its relatively low concentration in flue gas. It is unknown what effect this low concentration of NO would have on *D. desulfuricans* or the mixed heterotrophs that would compose the first stage culture. This is currently under investigation in our laboratories. The solubility of NO_2 in water is much higher than that of NO. However, it is anticipated that the nitrate resulting from reaction of NO_2 with water in the first stage may be scavenged by heterotrophs in the culture and utilized as a terminal electron acceptor. In experiments in our laboratories, nitrate added to nonaseptic *Desulfovibrio* cultures has quickly disappeared.

In a series reactor scheme, the second stage containing the *T. denitrificans* culture would be operated on a sulfide-limiting basis. Otherwise, sulfide would accumulate to toxic levels in the liquid phase (*14*). There-

fore, the electron acceptor (NO, NO_2, or NO_3^-) must be in stoichiometric excess. In most cases, this would require supplementing the culture medium with nitrate. With NO and NO_2 from the gas competing with the intermediates of nitrate reduction for access to the appropriate enzymes in the biomass, complete removal of NO_x would not be anticipated. Because of the low solubility of NO, more vigorous gas–liquid contacting would likely be required in this second stage, compared to the first stage.

A single-stage process may be feasible in which flue gases are contacted with a mixed culture of *Desulfovibrio*, *T. denitrificans*, and mixed heterotrophs. However, as noted above, the results obtained thus far regarding SO_2 oxidation to sulfate in cocultures are encouraging but must be considered preliminary at this stage.

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