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COS Degradation by Selected CO-Utilizing Bacteria

Scientific Note

K. D. SMITH, K. T. KLASSON, M. D. ACKERSON, E. C. CLAUSEN,* AND J. L. GADDY

University of Arkansas, Department of Chemical Engineering, Fayetteville, AR 72701

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INTRODUCTION

Synthesis gas, a major product of coal gasification, typically contains 1–2 sulfur by volume, with 95–99% or more of the sulfur present as H_2S (*1,2*). Typical levels of carbonyl sulfide (COS) in coal-derived synthesis gas range from 0.03–0.07% by volume (*3*), although COS concentrations have been observed in the 5–10% range (*1,2*).

Although COS is present in only small quantities, it poses serious problems to equipment and the environment. COS is corrosive to both iron and steel (*4*), and is a precursor to the formation of sulfur oxide derivatives, which are highly regulated environmental pollutants. COS also poses significant problems to downstream catalysts as a catalyst poison. Examples of catalysts for downstream processing of synthesis gas to methane include nickel and potassium-based catalysts, as well as mixtures of iron/chromiumn oxides and zinc/copper oxides (*5,6*). Poisons for these catalysts include chlorine and sulfur (*7,8*).

COS is currently removed from synthesis-gas streams by amine-based adsorption at atmospheric pressure, hot carbonate adsorption systems at moderate pressures, or physical-solvent adsorption systems at higher

*Author to whom all correspondence and reprint requests should be addressed.

pressures (*9*). A less capital- and energy-intensive alternative to adsorption might be the removal of sulfur gases using biological processes. H_2S may be removed from gas streams by employing *Thiobacillus denitrificans* (*10*) or *Chlorobium thiosulfatophilum* (*11*). Recent observations in the University of Arkansas laboratories showed that, in consequence of its similar structure, COS might be degraded by CO-utilizing bacteria.

The purpose of this paper is to present the initial results of tests of various CO-utilizing bacteria for their possible ability to degrade COS. The optimum conditions for each culture were employed as noted in the literature or determined in the laboratory. The respective rates at which COS was degraded by each culture were compared to the rate of chemical reaction of COS with water:

$$COS + H_2O \rightarrow H_2S + CO_2 \quad (1)$$

Gas-phase composition was held constant in comparing the different cultures.

CO CONVERSION BY BACTERIAL CULTURES

Four CO-utilizing bacteria were chosen to be tested for their ability to degrade COS:

1. *Peptostreptococcus productus*
2. *Eubacterium limosum*
3. *Clostridium ljungdahlii*
4. *Rhodospirillum rubrum*

Of the above, *C. ljungdahlii, P. productus,* and *E. limosum* convert CO to acetate by the equation

$$4\,CO + 4\,H_2O \rightarrow 2\,HCO_3^- + CH_3COO^- + 3\,H^+ \quad (2)$$

P. productus was isolated from anaerobic sewage-digester sludge and has been reported to have a doubling time of 1.5 h at 37°C and pH 7 (*12*). *E. limosum* exhibited a doubling time of 7 h under the same conditions (*13*). *P. productus* has been shown to be able to tolerate H_2S and COS concentrations as high as 20% by volume (*14*).

C. ljungdahlii was recently isolated from chicken waste at the University of Arkansas (*15*) and, in addition to Reaction 2, carries out the following reaction (*16*):

$$6\,CO + 7\,H_2O \rightarrow C_2H_5OH + 4\,HCO_3^- + 4\,H^+ \quad (3)$$

The CO conversion to ethanol is favored at low pH and under nutrient limitation.

R. rubrum is a photosynthetic bacterium that is capable of converting CO and H_2O to HCO_3^-, H^+, and H_2 by the following reaction (*17*):

$$CO + 2H_2O \rightarrow HCO_3^- + H_2 + H^+ \quad (4)$$

R. rubrum does not utilize CO for growth, but is able to utilize a wide variety of carbon sources, including acetate, malate, and yeast extract. Tungsten light is required for growth, but is not required for CO utilization (*18*). *R. rubrum* was shown to be inhibited by COS concentrations above 6% (*14*).

MATERIALS AND METHODS

Organisms

Peptostreptococcus productus, Strain U-1, was supplied by M. P. Bryant, University of Illinois, Department of Dairy Science. *Eubacterium limosum,* Strain 8486, and *Rhodospirillum rubrum,* Strain 25903, were obtained from the American Type Culture Collection, Rockville, MD. *Clostridium ljungdahlii,* Strain PETC, was isolated from chicken waste in the University of Arkansas laboratories.

Medium

The medium for the bacteria per 100 mL, was as follows: yeast extract (Difco), 0.10 g; Pfennig's mineral solution (*19*), 5 mL; Pfennig's trace metal solution (*19*) with the addition of 10 mg/L Na_2SeO_3 (*15*), 0.1 mL; B-vitamins (*20*); resazurin (0.1%), 0.1 mL; and $NaHCO_3$, 0.25 g. The medium pH was 7.0 for *P. productus, E. limosum,* and *R. rubrum,* and 5.0 for *C. ljungdahlii.* The anaerobic techniques for the preparation and use of the medum were developed by Hungate (*21*) and modified by Bryant (*22*) and Miller and Wolin (*23*). Cysteine hydrochloride (2.5% solution) was used as the reducing agent for the basal medium (2 mL of solution in 100 mL of medium) in each of the bacterial studies and was added prior to inoculation of the sterile medium.

Fermentation Equipment

The batch fermentations were carried out in glass serum bottles (Wheaton Glass Co., Vineland, NJ), 150-mL nominal size. Gas-impermeable butyl rubber septum-type stoppers and aluminum crimp seals (Bellco Glass Co., Millville, NJ) were used to seal the bottles. When sealed, the bottles were gas-tight, yielding anaerobic conditions under the experimental conditions employed. The bottles were sterilized by autoclaving at 15 psig for 20 min.

Agitation during the experiments was provided by a New Brunswick Scientific (New Brunswick, NJ) shaker incubator at 100–150 rpm. The bottles were placed horizontally in the incubator and periodically removed for about 3 min during sampling. Tungsten light was supplied to the *R. rubrum* cultures during the fermentation, whereas the fermentations

involving the other bacteria were carried out in the dark. The experiments with *R. rubrum* were conducted at 30°C; all the other cultures were incubated at 37°C.

Seed cultures were prepared prior to each experiment in 150-mL serum bottles containing the medium and then inoculated with 5 mL of stock culture. The gas phase in the seed cultures was a mixture of 80% CO and 20% CO_2 at 1 atm. The seed cultures were placed in the shaker incubator at 100–150 rpm for 24 h in order to acclimate the culture to experimental conditions, reduce the lag-phase periods, and help to achieve repetition in successive experiments.

Once the bottles containing 75 mL of medium were reduced, they were inoculated with 5 mL of seed culture, and synthesis gas (76 mL of a mixture of 24% H_2, 65% CO, and 11% CO_2 at 1 atm) was introduced. The bottles were flushed with the gas mixture through sterilized cotton filters and needle-tubing connectors for 3 min. A needle was placed in the rubber septum to provide an outflow vent during the gassing procedure. Methane (20 mL) was added to each bottle as a tracer gas. The tracer gas was not consumed by the bacteria and enabled the calculation by chromatographic analysis of molar quantities in the gas phase of the bottles.

Once the addition of the gas phase was complete, the bottles were sampled for initial cell concentration and placed in the shaker incubator. The growth was then monitored and, at staggered times, one of the bottles was regassed according to the procedure described above; however, this time COS (97.5% purity) was also injected into the bottles to create an atmosphere similar to a synthesis-gas mixture containing 5% COS in the gas phase. The bottle was then returned to the incubator. By staggering the times of regassing and COS addition, different "initial" cell concentrations were obtained. A liquid sample was withdrawn to measure the cell concentration.

The procedure described above was followed for each of the batch experiments. The same batch of medium was used for every bottle in an experiment to eliminate any variable caused by irregularities in the medium.

Analytical Techniques

Cell concentrations were determined by measuring liquid-culture turbidity at 540 or 580 nm on a Bausch and Lomb (Milton Roy Co., Rochester, NY) spectrophotometer and converting to cell density using a calibration curve. Precautions were taken to ensure anaerobicity during sampling. A Corning (Corning, NY) pH meter, Model 140, was used for measuring the pH of liquid samples withdrawn from the bottles.

Gas-phase analyses of CO, CH_4, CO_2, H_2S, and COS were performed with a thermal conductivity detector in a Varian (Sunnyvale, CA) 3400 gas chromatograph. The column was a 3.2-mm×1.8-m Teflon™ column packed with Chromosorb 107, 80/100 mesh (Alltech, Deerfield, IL). The column temperature was 80°C and the detector and injector temperatures were 175°C. The carrier gas was helium at a flow rate of 30 mL/min.

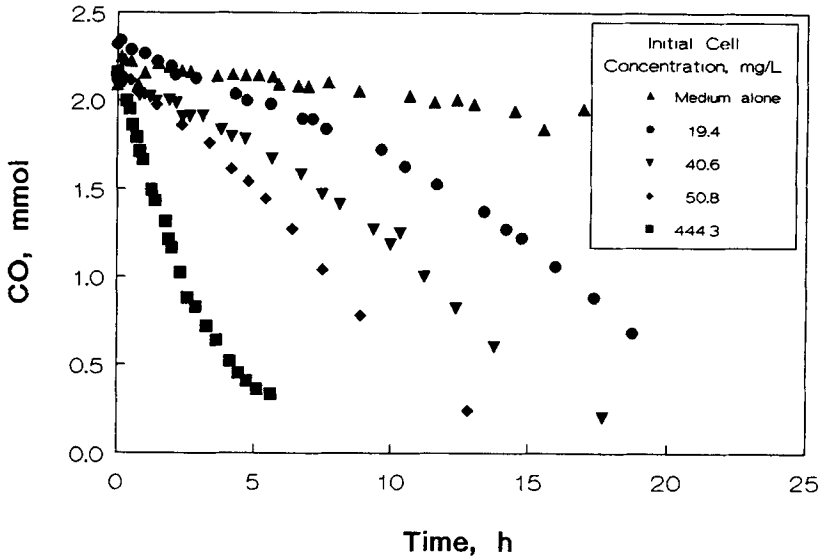

Fig. 1. CO uptake by *P. productus* in the presence of COS.

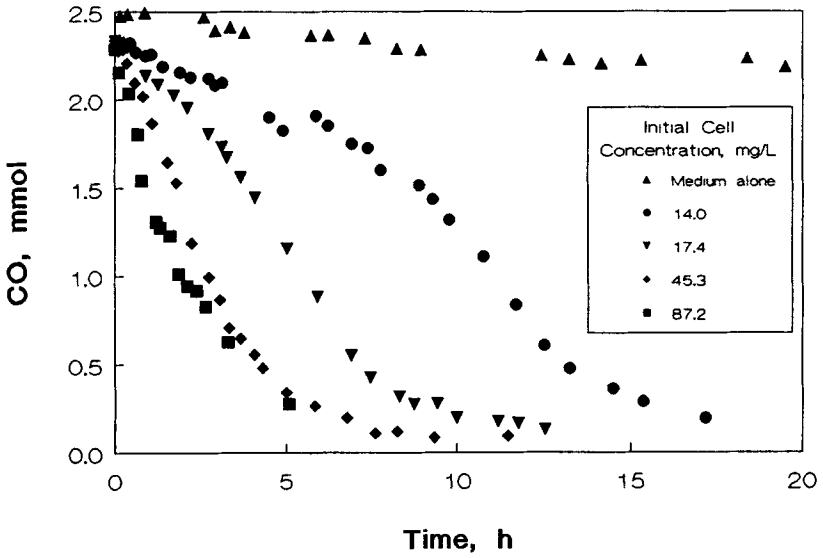

Fig. 2. CO uptake by *E. limosum* in the presence of COS.

RESULTS AND DISCUSSION

CO Uptake

The experiments were designed so that CO and COS served as cosubstrates for the organisms. The utilization of CO in the presence of COS by the four bacteria is shown in Figs. 1–4. The disappearance of CO in the medium alone is also shown for comparison purposes. As is noted in the figures, the rate of CO consumption increased with inoculum size. Fur-

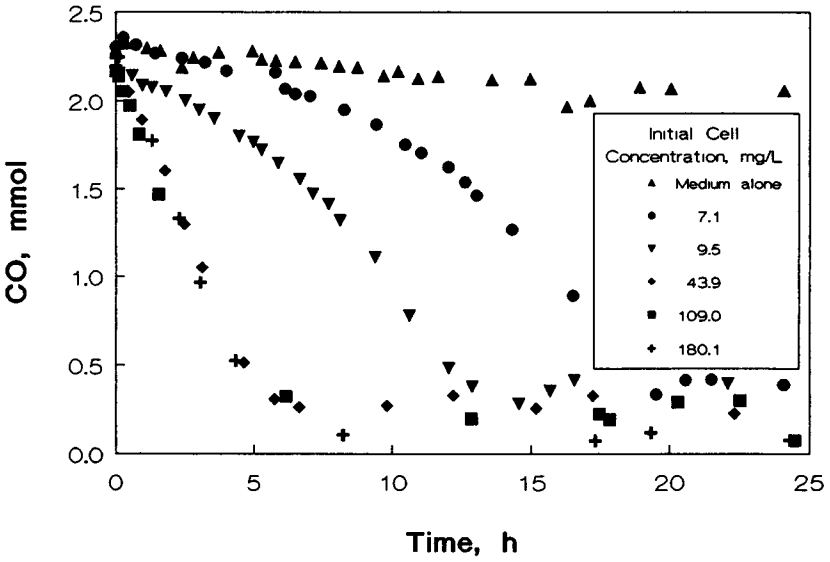

Fig. 3. CO uptake by *C. ljungdahlii* in the presence of COS.

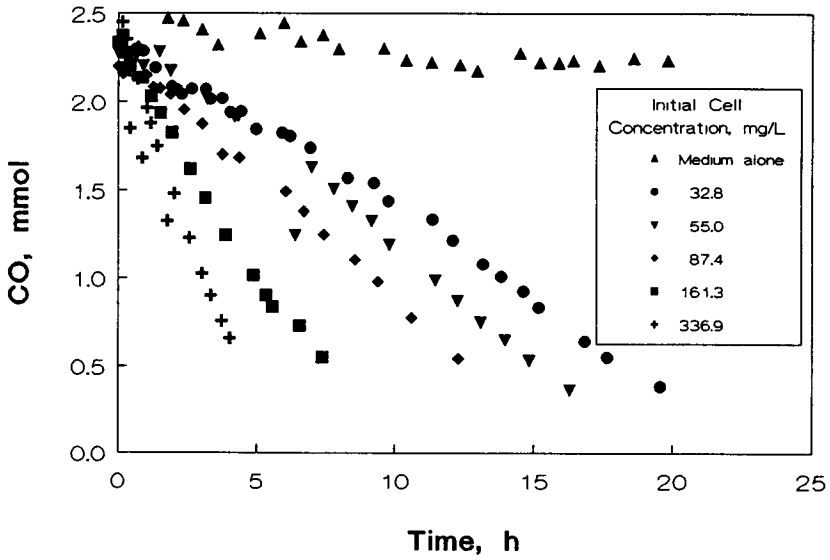

Fig. 4. CO uptake by *R. rubrum* in the presence of COS.

thermore, all organisms yielded essentially complete conversion for all inoculum sizes in 25 h or less. CO was not degraded in the medium alone.

COS Degradation

The uptake of COS in the presence of CO by the bacteria is shown in Figs. 5–8. *P. productus, E. limosum,* and *R. rubrum* showed an ability to degrade COS faster than the reaction of COS with water in the medium. *C. ljungdahlii,* on the other hand, was not shown to utilize or degrade COS

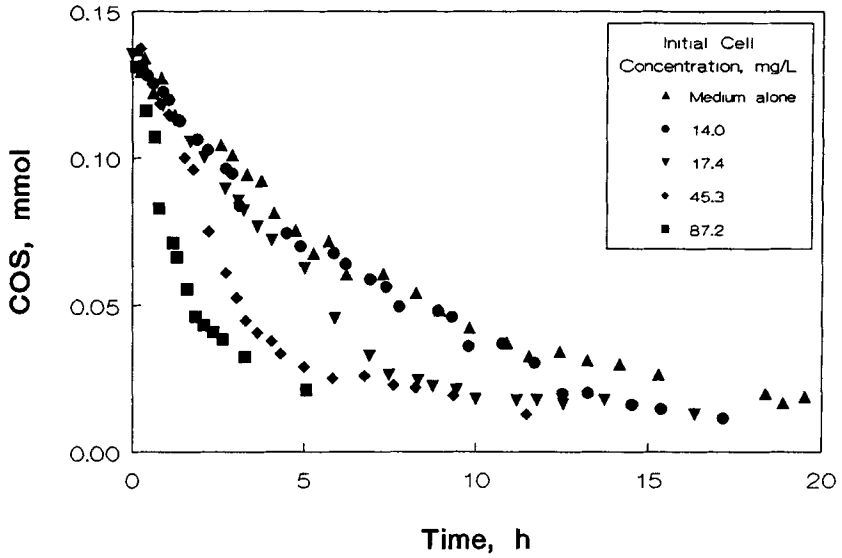

Fig. 5. COS degradation by *P. productus* in comparison to the medium alone.

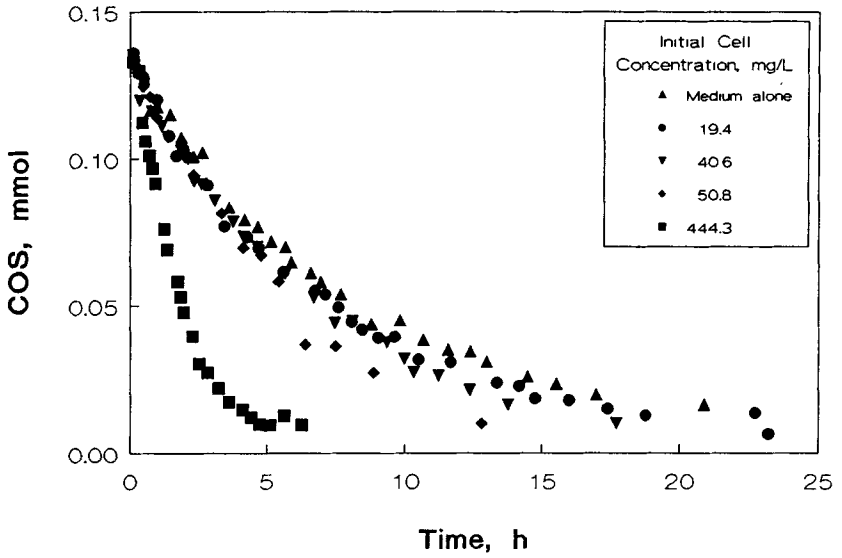

Fig. 6. COS degradation by *E. limosum* in comparison to the medium alone.

any faster than medium alone. It should be pointed out that the COS profiles with medium alone were run at identical pH levels and temperatures as the CO-COS degradation experiments. Thus, for experiments with *P. productus* and *E. limosum*, a medium at pH 7 and 37°C was used, and for experiments with *C. ljungdahlii* a medium at pH 5 and 37°C was used. A medium at pH 7 and 30°C was used for experiments with *R. rubrum*.

In analyzing the results presented in Figs. 1-8, it was noted from the slopes of the curves that the rate of COS degradation for *P. productus* and *E. limosum* tended to decrease when the concentrations of both CO and

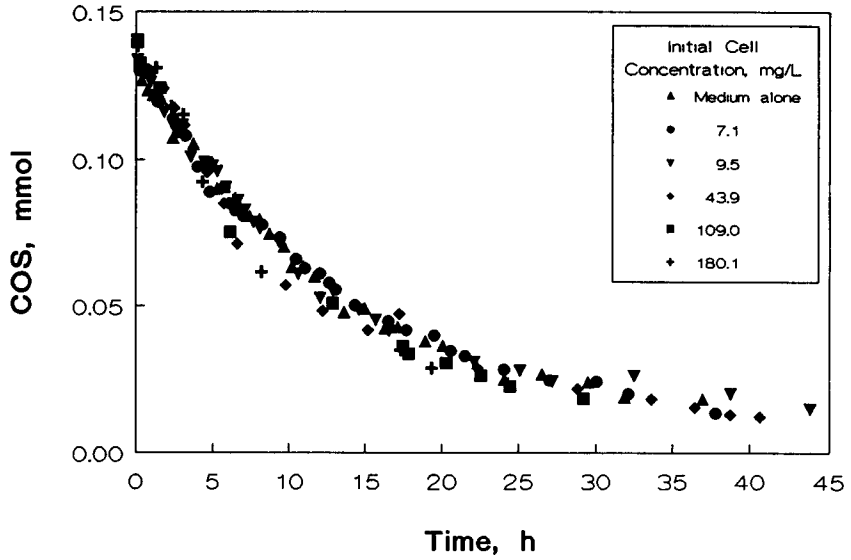

Fig. 7. COS degradation by *C. ljungdahlii* in comparison to the medium alone.

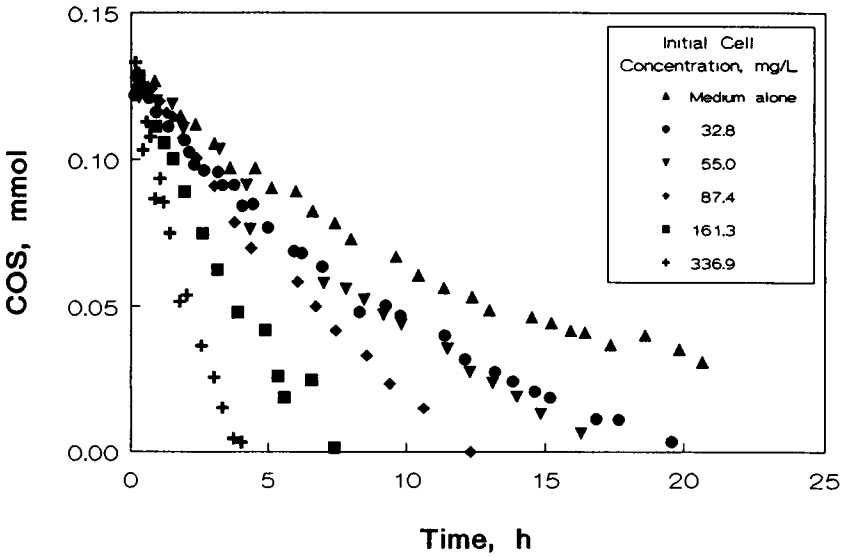

Fig. 8. COS degradation by *R. rubrum* in comparison to the medium alone.

COS were very low. It is possible that the rate of COS degradation is strongly linked to the rate of CO utilization in these organisms, meaning that when the rate of CO utilization decreases, the rate of COS degradation could exhibit a corresponding decrease. If the difference in initial rates of COS degradation with cells and in medium alone is plotted as a function of the difference in CO uptake rates by the cells and in medium alone for *P. productus, E. limosum,* and *R. rubrum,* a nearly linear trend is observed (Fig. 9). A least-squares fit forced through the origin yields a slope of 0.055

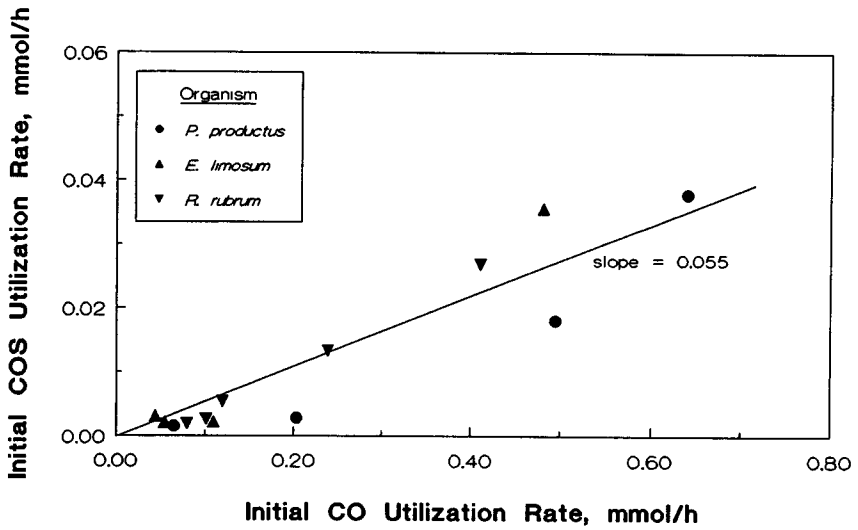

Fig. 9. Initial COS-utilization rate as a function of initial CO-utilization rate for *P. productus, E. limosum,* and *R. rubrum.*

mol COS/mol CO. This ratio of initial rates may be compared to the initial amounts of gases present, which were approx 0.06 mol COS/mol CO in all experiments. This would indicate similar kinetic utilization modes for both CO and COS, with COS possibly being "used" in place of CO. However, since the initial ratios (mol COS/mol COS) were kept constant in all experiments and isotope labeling was not employed, this conclusion is speculative.

COS degradation by *C. ljungdahlii* (*see* Fig. 7) may have been limited by the low operating pH (pH 5). In comparing the rates of COS degradation by the medium alone, it seen that the rate (slope of curve) at pH 5 is much lower than the rate at pH 7. Perhaps *C. ljungdahlii* would have shown the ability to degrade COS if an initial pH of 7.0 were utilized.

In any case, it has been demonstrated that COS can be degraded by some CO-utilizing organisms faster than in medium alone. Particularly effective was *R. rubrum,* which completely degraded the added COS in <20 h for cell concentrations above 55 mg/L. Possible mechanisms for degradation include, but are not limited to, dual substrate utilization of both COS and CO. Studies to elucidate the reaction mechanism and microbial kinetics are in progress.

Product Formation

It was observed during the COS degradation studies that H_2S was a major product formed in the gas phase for each of the three bacteria capable of degrading COS (data not shown). No other gas-phase products were formed, and liquid-phase products were not detected or measured. Product yields in verifying process stoichiometry are under study.

CONCLUSIONS

The results of this study support the hypothesis that several organisms that are capable of utilizing CO are also capable of degrading CO-like compounds, such as COS. Three organisms, *Peptostreptococcus productus, Rhodospirillum rubrum,* and *Eubacterium limosum,* were shown to metabolize COS at rates much higher than that of the reaction of COS with H_2O. *Clostridium ljungdahlii,* on the other hand, was not found to have an ability to degrade COS. H_2S was detected as a major gas-phase product, but stoichiometric product yields have not yet been determined.

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