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Sulfur Gas Tolerance and Toxicity of CO-Utilizing and Methanogenic Bacteria

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ABSTRACT

Anaerobic bacteria have been shown to be capable of converting CO, H_2, and CO_2 in synthesis gas to valuable products, such as acetate, methane, and ethanol. However, synthesis gas also contains small quantities of sulfur gases such as H_2S and COS, that may inhibit the performance of these organisms. This paper compares the performance of several CO-utilizing and methanogenic bacteria in converting CO, CO_2, and H_2 to products in the presence of various concentrations of H_2S and COS. The sulfur gas toxicity levels, growth, substrate uptake, and product formation for each organism are compared.

Index Entries: Sulfur gas toxicity; methanogens; CO-utilizing bacteria.

INTRODUCTION

In gasifying coal to produce synthesis gas, the sulfur present in coal is converted to H_2S, COS, CS_2, and mercaptans. As much as 99% of the sulfur in synthesis gas exists as H_2S, with the balance as organic sulfur. Most of the organic sulfur is present as COS, with the other forms at minor concentration levels (*1*). Total organic sulfur concentrations in synthesis gas typically range from 0.03–0.07 vol% (*2*). Total sulfur concentrations in synthesis gas typically range from 1–2 vol% (*3*).

The presence of sulfur compounds in synthesis gas causes serious potential problems in the application and use of the gas. If the gas is used

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directly in a combustion process, the sulfur is converted to SO_x and becomes an environmental problem in exhaust gases. The sulfur gases are also toxic to many catalysts in subsequent liquefaction or methanation steps. As an example, Fleming and Primack (*4*) noted that the sulfur concentration must be reduced to levels less than 0.1 ppm in order to protect sensitive downstream catalysts during Fischer-Tropsch synthesis. Thus, the removal of gaseous sulfur compounds is usually essential in the utilization of synthesis gas from high-sulfur and some low-sulfur coals.

In the past five years, considerable interest has evolved in utilizing the components of synthesis gas to produce fermentation products, particularly alcohols, organic acids, CO_2/H_2, and methane. It is known that many bacteria are quite rugged in their ability to grow and metabolize substrates in extreme environments (*5,6*). However, the effects of sulfur gases on the performance of CO-, CO_2- and H_2- utilizing organisms have not been adequately studied.

The purpose of the study reported in this paper was to investigate the effects of H_2S and COS on growth, substrate uptake, and product formation by bacteria used in synthesis gas fermentations. Specifically, the performance of the strict anaerobes, *Peptostreptococcus productus, Rhodospirillum rubum, Methanobacterium formicicum,* and *Methanosarcina barkeri,* was monitored in the presence of sulfur gas concentrations between 0 and 39.5 vol% (the maximum sulfur gas concentration at a pressure of 10 atm with complete conversion of other synthesis gas components).

BIOLOGICAL SYNTHESIS GAS CONVERSION

As mentioned previously, the components of synthesis gas may be used to produce a variety of fermentation products, including organic acids, alcohols, and methane. A discussion of the reactions and bacteria used in the bioconversions has been presented earlier (*7,8*). The following paragraphs address only the bacterial systems utilized in the present study as representative organisms for converting synthesis gas components to fermentation products.

Conversion of CO to Acetate by *P. productus*

Of the many bacteria capable of converting CO to acetate, the strict anaerobe *P. productus* was chosen for sulfur tolerance studies because of its fast rate of growth and high tolerance to CO. *P. productus* forms acetate from CO by the equation (*9*)

$$4\,CO + 4\,H_2O \rightarrow 2\,HCO_3^- + CH_3COO^- + 3\,H^+ \quad (1)$$

Although *P. productus* is also capable of utilizing CO_2 and H_2 for acetate production, CO is the preferred substrate (*10*).

Conversion of CO and Water to CO_2 and H_2 by *R. rubrum*

R. rubrum is a photosynthetic bacterium capable of carrying out the biological water gas shift reaction (*11*)

$$CO + H_2O \rightarrow CO_2 + H_2 \quad (2)$$

The bacterium *Rhodoseudomonas gelatinosa* also carries out the reaction. Although *R. rubrum* does not utilize CO for growth, the bacterium is capable of growing on a number of other carbon sources, including yeast extract and acetate produced by *P. productus*. Tungsten light is required for growth, but is not required for CO utilization. *R. rubrum* was chosen for the sulfur toxicity studies because of its superior growth rate in comparison to *R. gelatinosa*.

Conversion of H_2 and CO_2 by Methanogens

Most methanogens are capable of producing methane from CO_2 and H_2 by the equation (*13*)

$$4\,H_2 + CO_2 \rightarrow CH_4 + 2\,H_2O \quad (3)$$

Two representative methanogens were chosen for the sulfur toxicity studies, *M. formicicum* and *M. barkeri*, owing to observed differences in the rate of substrate and CO tolerance by the two bacteria. *M. formicicum* has been shown to uptake H_2 and CO_2 to produce CH_4 much faster than *M. barkeri*. However, nearly complete inhibition of H_2 uptake by *M. formicicum* was observed at a CO partial pressure of 0.76 atm, whereas *M. barkeri* showed only minimally decreased rates of uptake at the same CO partial pressure (*14*).

MATERIALS AND METHODS

Microorganisms

Peptostreptococcus productus, strain U-1, and *Methanosarcina barkeri* were kindly supplied by M. P. Bryant, Department of Dairy Science, University of Illinois, Urbana, IL. *Rhodospirillum rubrum*, Strain 25903, and *Methanobacterium formicicum*, Strain 33274, were obtained from the American Type Culture Collection, Peoria, IL.

Media

The medium for the bacteria, shown in Table 1, was a basal medium containing vitamins, minerals, salts, and yeast extract. Sodium bicarbonate was added to the medium as a buffer for pH control. Yeast extract and sodium acetate served as carbon sources for the growth of *R. rubrum*. The

Table 1
Composition of Basal Media

Ingredient	per 100 mL
Yeast Extract	0.1 g
Phennig's Mineral Solution	5 mL
Phennig's Trace Metal Solution	0.1 mL
B-vitamins	0.5 mL
$NaHCO_3$	0.35 g
NH_4Cl	0.27 g[a]
$Na\ CH_3\ COO \cdot 3\ H_2O$	0.6 g[a]

[a] *R. rubrum* only.

anaerobic techniques for the preparation and use of the media were developed by Hungate (*15*) and modified by Bryant (*16*) and Balch and Wolfe (*17*). Sodium sulfide was used as the reducing agent for the basal medium in each of the bacterial studies.

Fermentation Equipment

The batch fermentations were carried out in glass serum bottles (Wheaton Glass Co., Millville, NJ), 150 mL nominal size. Gas impermeable butyl rubber septum-type stoppers and aluminum crimp seals (Bellco Glass Co., Vineland, NJ) were used to seal the bottles. When sealed, the bottles were completely gas tight, yielding extremely anaerobic conditions under 3–4 atm of pressure. The bottles were sterilized by autoclaving at 15 psig for 20 min.

Agitation during the experiments was provided by a New Brunswick (New Brunswick, NJ) shaker incubator set at 100–150 rpm. The bottles were placed horizontally in the incubator at 30 or 37 °C and only 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.

Seed cultures were prepared in 150 mL serum bottles containing the medium prior to each experiment and then inoculated with 5 mL of stock culture. For *M. formicicum,* the gas phase in the bottles consisted of H_2/CO_2 (75%/25%) at 1 atm. The gas phase used for the *P. productus* and *R. rubrum* 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. A seed culture was not prepared for *M. barkeri* owing to its extremely slow growth rate. The inocula for the *M. barkeri* experiments were taken directly from stock cultures maintained in the nonshaker incubator.

Once the bottles containing 75 mL of medium were reduced with 1.5 mL of 2.5% sodium sulfide solution, they were inoculated with 5 mL of

seed culture, and the desired gas phase (76 mL) was then introduced. The bottles were flushed with the gas mixture for 2–3 min through sterilized cotton filters and needle-tubing connectors. A needle was placed in the rubber septum to provide an outflow vent during the gassing procedure. In experiments with *P. productus,* 20 mL of methane was added to each bottle as a tracer gas. The tracer gas was not consumed by the bacteria and enabled the calculation of molar quantities in the gas phase of the bottles from chromatographic analysis. In experiments with *R. rubrum, M. barkeri,* and *M. formicicum,* argon (20 mL) was used as the tracer gas. Finally, variable quantities of H_2S (99.5% purity) or COS (97.5% purity) were then injected into the bottles as required.

Once the addition of the gas phase was complete, the bottles were sampled for initial gas composition and placed in the shaker incubator. A liquid sample was withdrawn from the seed culture to measure the cell concentration. The initial cell concentration of the bottles was calculated by diluting the value obtained from the seed culture measurement.

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

Analytical Techniques

Cell concentrations for *P. productus* and *M. formicicum* were determined by measuring liquid culture turbidity at 580 nm on a Bausch and Lomb (Milton Roy Co., Rochester, NY) spectrophotometer and converting to cell density using a calibration curve. Turbidity measurements for *R. rubrum* were performed at 540 nm. Cell concentration measurements were not made for *M. barkeri* since the cells tended to clump together during the fermentation, thus making cell density measurements difficult.

A Corning (Corning, NY) pH meter, Model 140, was used for measuring the pH of liquid samples withdrawn from the bottles. The pH was controlled in a range of 6.7–7.0 by adding small amounts (1 mL or less) of 10% sodium bicarbonate solution when necessary. The sodium bicarbonate solution was prepared anaerobically and was sterilized by autoclaving for 20 min.

Two methods were required for the determination of the gas phase composition, depending upon the components present in the gas phase. Both methods were developed for a Perkin-Elmer (Norwalk, CT) Sigma 300 gas chromatograph. A 1.8 m column packed with Porapak Q, 100/120 mesh was used for the determination of CO, CH_4, and CO_2. In this procedure, the oven temperature was maintained at 30–40°C, the detector and injector temperatures were both 175°C, and the carrier gas used was helium at a flow rate of 40 mL/min. A 1.8 m column packed with Carbosphere, 60/80 mesh, allowed the determination CO, H_2 CH_4, CO_2 and Ar compositions. The detector and injector temperatures were the same in this procedure, as outlined above. Temperature programming was necessary to allow adequate separation of the peaks in a reasonable period of time.

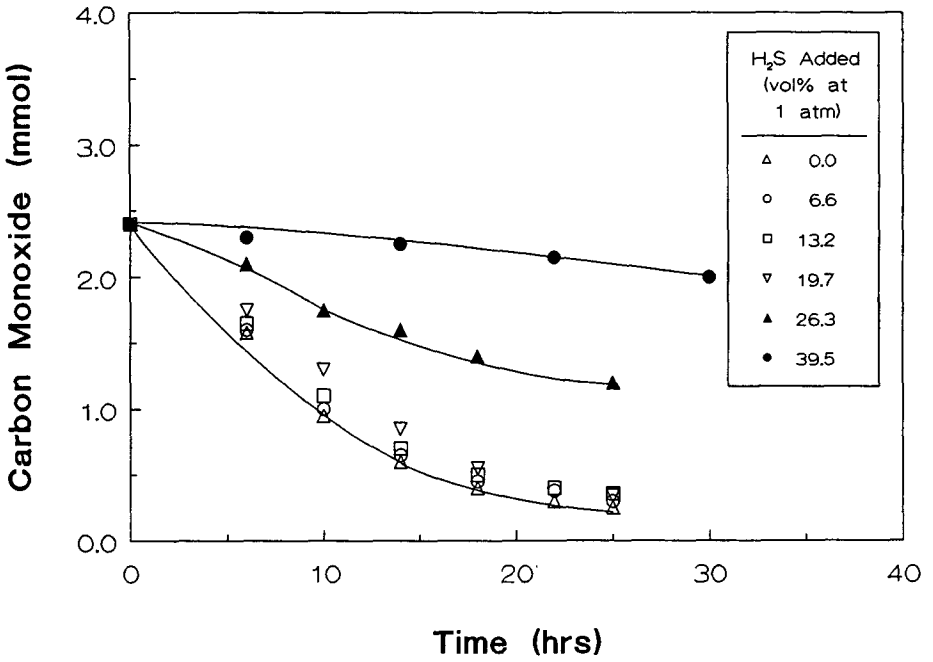

Fig. 1. CO disappearance as a function of time for *P. productus* in the presence of various concentrations of H_2S.

RESULTS AND DISCUSSION

The effects of H_2S and COS on substrate uptake by the four anaerobic bacteria are shown in Figs. 1–8. As noted, the sulfur gas concentrations employed ranged from 0–39.5 vol%, corrected to 1 atm total pressure. As stated earlier, each of the bottles used in the experiments contained Na_2S in the liquid phase as a reducing agent. Assuming complete dissociation, the equivalent H_2S concentration in the gas phase for the Na_2S addition is 6.6% at 1 atm. Thus, the bacteria are actually capable of metabolizing their respective substrates at slightly higher sulfur gas concentrations than shown in the figures. It should be noted that, although the results presented in this paper are reproducible, their intended use at this time is for qualitative comparisons of sulfur toxicity in anaerobic bacteria.

The effects of H_2S and COS on CO conversion to acetate by *P. productus* are shown in Figs. 1 and 2. As with all the bacteria in this study, neither H_2S nor COS was utilized by the bacteria for growth or as a substrate. As noted, H_2S and COS affected CO uptake almost identically. Only small differences in uptake were noted at sulfur gas concentrations below 20%. Uptake was severely inhibited at a concentration of 26.3% and essentially stopped with 39.5% sulfur gas.

The effect of sulfur gas on CO conversion to CO_2 and H_2 by *R. rubrum* are shown in Figs. 3 and 4. H_2S only marginally affected CO uptake, even

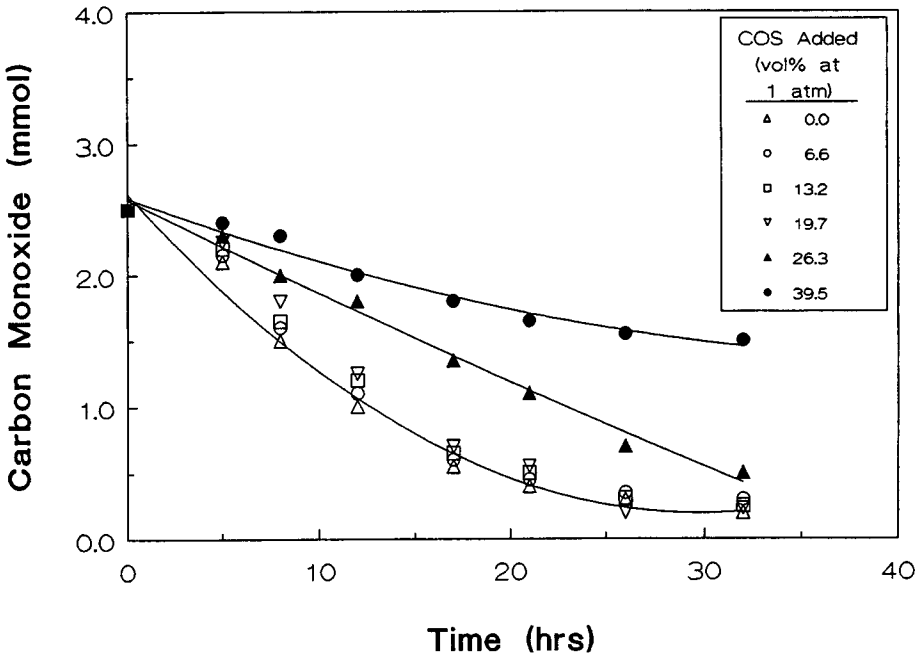

Fig. 2. CO disappearance as a function of time for *P. productus* in the presence of various concentrations of COS.

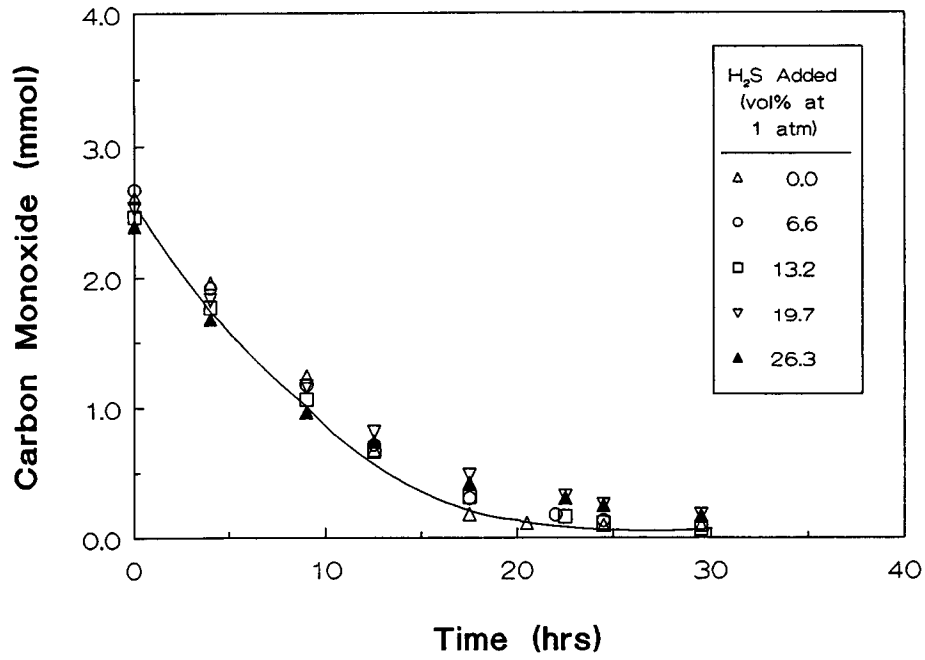

Fig. 3. Effect of various concentrations of H_2S on CO uptake by *R. rubrum*.

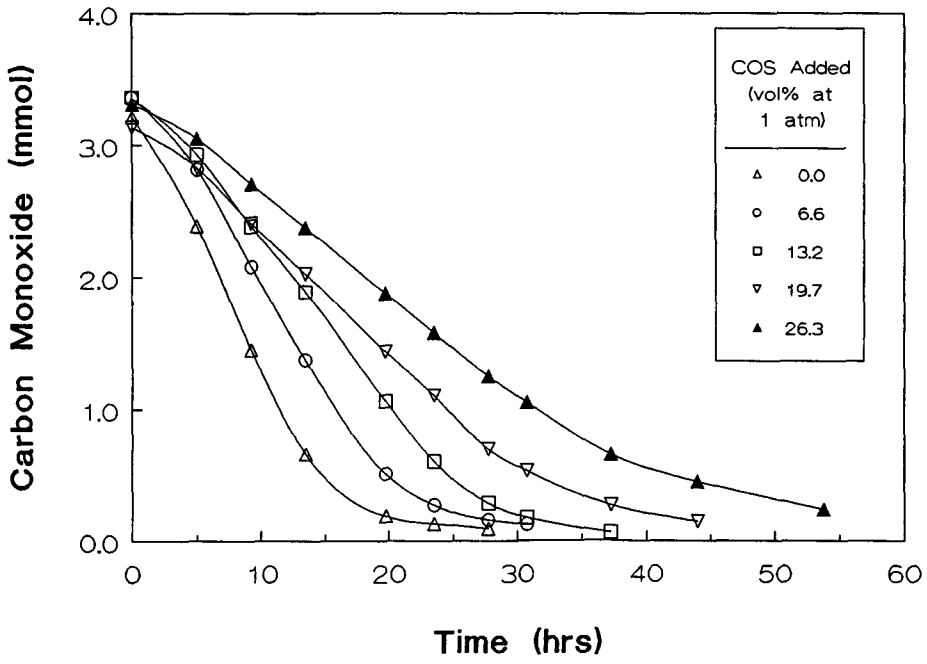

Fig. 4. Effect of various concentrations of COS on CO uptake by *R. rubrum.*

at concentrations of 26.3%. The yield of H_2 from CO, $Y_{P/S}$, was constant regardless of H_2S concentration at 0.99 mol/mol (99% of theoretical). COS, on the other hand, inhibited CO uptake by *R. rubrum,* even at a concentration of 6.6%, as noted in the increased times for complete conversion with increasing COS concentration. The yield of H_2 on CO, $Y_{P/S}$, remained constant at 0.96 mol/mol for all COS concentrations employed.

Figures 5 and 6 show the results of H_2 and CO_2 conversion to CH_4 by *M. barkeri,* according to Eq. (3), in the presence of H_2S and COS. As noted in the figures, H_2 uptake by *M. barkeri* was essentially unaffected by the presence of either of the sulfur gases up to a concentration of 26.3%. Yields of CH_4 from H_2, $Y_{P/S}$, were also unaffected by the presence of sulfur gases, remaining constant at 0.23 mol/mol (96% of theoretical) in the presence of H_2S and 0.21 mol/mol (84% of theoretical) in the presence of COS.

As noted in Figs 7 and 8, H_2 conversion to CH_4 by *M. formicicum* was significantly inhibited by the presence of H_2S and COS. H_2 was consumed in the presence of 26.3% H_2S or 13.2% COS. Significant inhibition was observed even at 6.6% sulfur gas concentrations. However, as with the other bacteria, the product yields were not affected by the presence of sulfur gas. $Y_{P/S}$ remained constant at 0.31 (higher than theoretical) regardless of H_2S concentration and 0.25 (theoretical) regardless of COS concentration.

A summary of the maximum H_2S and COS concentrations not significantly affecting substrate uptake by the four bacteria is shown in Table 2.

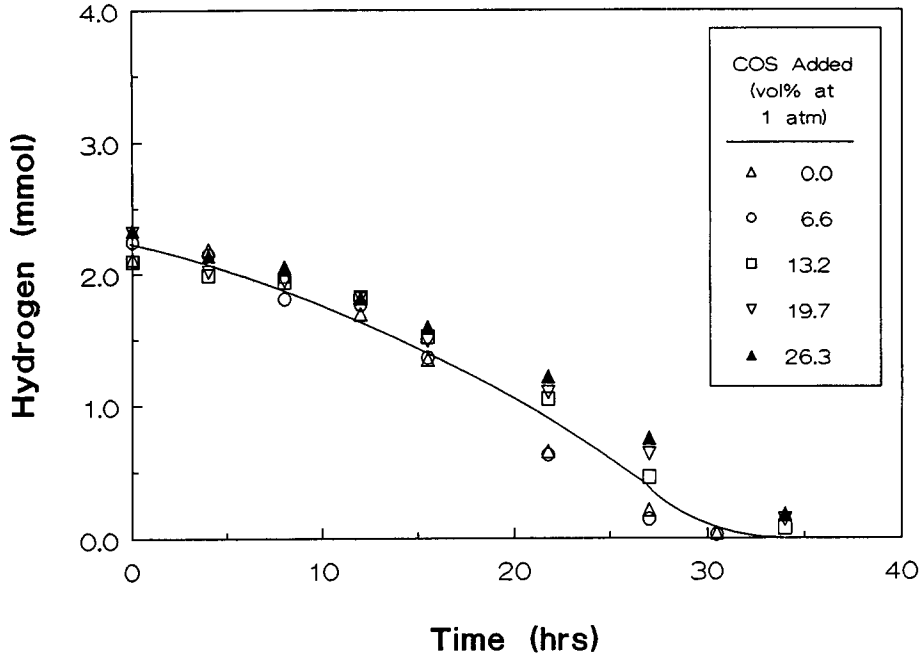

Fig. 5. H_2 consumption as a function of time for various concentrations of H_2S (*M. barkeri*).

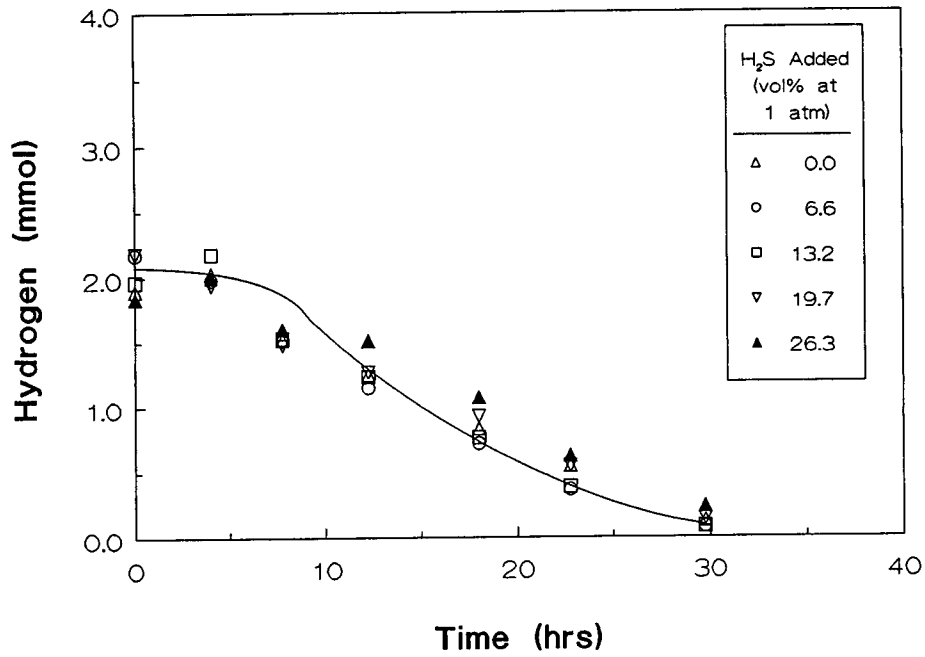

Fig. 6. H_2 consumption as a function of time for various concentrations of COS (*M. barkeri*).

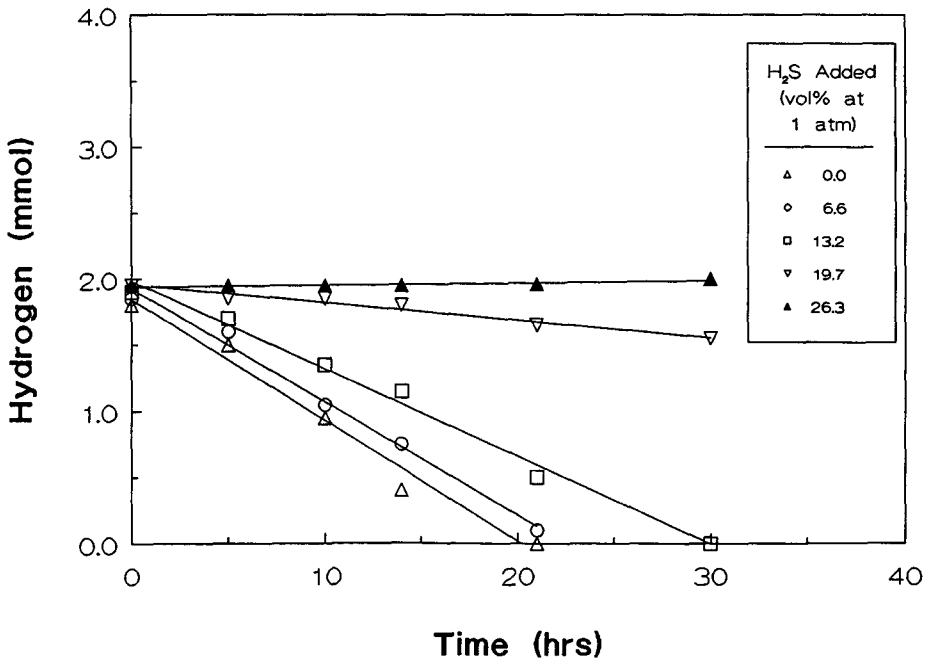

Fig. 7. H_2 consumption as a function of time for various H_2S concentrations (*M. formicicum*).

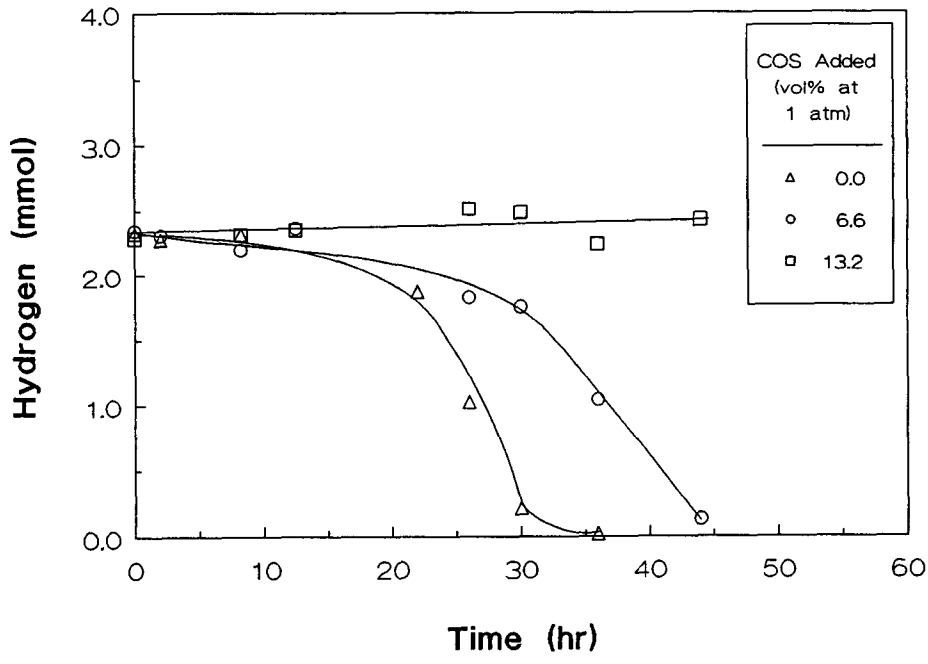

Fig. 8. H_2 consumption as a function of time for various COS concentrations (*M. formicicum*).

Table 2
Summary of Substrate Uptake by the Bacteria in the Presence of H_2S and COS

Organism	Maximum sulfur concentration not affecting substrate uptake, vol %	
	H_2S	COS
P. productus	19.7	19.7
R. rubrum	26.3	<6.6
M. barkeri	26.3	26.3
M. formicicum	<6.6	<6.6

As noted, *P. productus* and *M. barkeri* are not affected by either sulfur gas up to a 20% concentration. *R. rubrum* is adversely affected by COS at low concentrations and *M. formicicum* is inhibited by both H_2S and COS at low concentrations.

Cell Growth

Although the effects of sulfur gases on cell growth are not shown in order to conserve space, it should be noted that the effects of H_2S and COS on growth were similar to the results obtained for substrate uptake. Sulfur gases only marginally affected the growth of *P. productus* and *M. barkeri.* However, both H_2S and COS inhibited the growth of *R. rubrum* and *M. formicicum* even at low sulfur gas concentrations.

CONCLUSIONS

Batch toxicity studies have shown that both CO-utilizing bacteria (*P. productus* and *R. rubrum*) are capable of converting CO to products in the presence of significant amounts of H_2S or COS. *M. barkeri,* a methanogen capable of converting either acetate of H_2/CO_2 to methane, was found to also be tolerant of H_2S or COS at concentrations up to 39.5%. *M. formicicum,* on the other hand, was found to be significantly affected by sulfur gases in the 5–10% concentration range. Thus, it is recommended that mixed cultures employing CO-utilizing and methanoogenic bacteria for synthesis gas conversion to methane use both *M. barkeri* and *M. formicicum* for methane formation.

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