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Removal of Carbonyl Sulfide and Hydrogen Sulfide from Synthesis Gas by *Chlorobium thiosulfatophilum*

RAHUL BASU,[1] K. THOMAS KLASSON,[2] EDGAR C. CLAUSEN,*,[1] AND JAMES L. GADDY[1]

[1]*University of Arkansas, Department of Chemical Engineering, Fayetteville, AR 72701; and* [2]*Oak Ridge National Laboratory, Oak Ridge, TN*

ABSTRACT

The anaerobic, photosynthetic bacterium *Chlorobium thiosulfatophilum* utilizes CO_2 as its carbon source and operates at the mesophilic temperature of 30°C. It requires incandescent light for growth and compounds such as H_2S, S°, $S_2O_3^{2-}$, or H_2 as a source of electrons. Of these compounds, H_2S as sulfide is the preferred electron donor, with other compounds utilized only when sulfide has been depleted from the medium. The organism is also capable of indirectly utilizing carbonyl sulfide (COS), since COS reacts with water to form CO_2 and H_2S. This work presents kinetic information on the rate of growth of *C. thiosulfatophilum*, as well as the rates of uptake of both H_2S and COS. The growth is dependent on light intensity according to a Monod type relationship.

Index Entries: Carbonyl sulfide; hydrogen sulfide; *Chlorobium thiosulfatophilum;* desulfurization; pollutants.

Notation: B, Constant, h^{-1} lux; I_o, Light intensity, lux; K_t, Constant lux; μ, Specific growth rate, h^{-1}; μ_m, Maximum specific growth rate, h^{-1} (Constant in Eq. (5)); μ'_m, Maximum specific growth rate, h^{-1} (Constant in Eq. (6)); X, Dry weight cell concentration, mg l^{-1}; X_o, Initial dry weight cell concentration, mg l^{-1}.

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

INTRODUCTION

Synthesis gas, a product of coal gasification, typically contains 1–2 vol% sulfur, with 95–99% 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 H_2S and COS are typically present in only small quantities, they pose serious problems for both process equipment and the environment. They are corrosive to both iron and steel (*4*), and COS is a precursor to the formation of sulfur oxide derivatives, which are highly regulated environmental pollutants. H_2S and COS also act as poisons to the catalysts used in the downstream processing of synthesis gas to produce liquid and gaseous products.

Present desulfurization technology employs absorption processes using ammonia, alkanolamines, or alkaline salt solutions (*3*). The chromia-alumina catalyst is used for the removal of COS from synthesis gases containing large amounts of carbon monoxide (*3*). Biological processes may serve as an environmentally sound alternative to the traditional desulfurization processes. If the biological process recovers sulfur as valuable elemental sulfur, it may become an economical choice as well.

Thiobacillus denitrificans (*5*) and *Chlorobium thiosulfatophilum* (*6–10*) in laboratory studies biologically remove hydrogen sulfide from gas streams. *Thiobacillus denitrificans* grows both anaerobically and aerobically on sulfide as the energy source producing intracellular sulfur granules or sulfate under sulfide-limiting conditions. *Chlorobium thiosulfatophilum,* a photosynthetic bacterium, uses sulfide as the electron donor and produces extracellular sulfur granules or sulfate. There has been no evidence to show that COS can be directly used as an energy source or electron donor by sulfur bacteria, but recent studies have shown that COS might possibly be co-metabolized by some CO-utilizing bacteria (*11*).

This paper presents results focused on direct and indirect conversion of gaseous H_2S and COS to elemental sulfur, cell yields after growth on sulfide, and light limitation by *C. thiosulfatophilum.*

MATERIALS AND METHODS

Microorganism and Medium

Chlorobium thiosulfatophilum (ATCC 17092) was obtained from the American Type Culture Collection (Rockville, MD). It was grown on a basal medium containing (per 100 mL): yeast extract (Difco), 0.5 g; Pfennig's minerals solution (*12*), 5 mL; Pfennig's trace metals solution (*13*), 0.1 mL; and sodium bicarbonate, 0.4 g. The first four ingredients were combined with deionized water and boiled under a N_2/CO_2 (80/20 %v/v) atmosphere for 2 min. The solution was allowed to cool to room temperature prior to the addition of sodium bicarbonate. Seventy-five milliliters of this medium

was then added to 150 mL serum bottles (Wheaton, Millville, NJ) under anaerobic conditions using a Hungate technique (*14*). Butyl rubber stoppers and aluminum crimp-seals (Wheaton) were used to seal the bottles before steam sterilization at 2 atm (15 psig) for 20 min.

Fermentation Conditions

The batch fermentations were carried out in glass serum bottles. When sealed, the bottles were completely gas tight, yielding anaerobic conditions once the oxygen had been removed from the bottle. Experiments were started by flushing sterile medium containing bottles with a He/CO_2 mixture (80/20 %v/v), after which 20 mL of methane were added with a glass syringe to each bottle as a tracer gas. The bottles were then inoculated with 5 mL of pregrown seed culture, and, finally, H_2S or COS was added to the bottles in different amounts with a syringe. The bottles were placed in a modified shaker incubator (Model G25, New Brunswick Scientific Co., Edison, NJ) equipped with tungsten light (λ = visible range) and controlled at a temperature of 30°C and an agitation rate of 150 rpm. For the light limited growth study, each bottle was placed horizontally into a dark box, contained in the shaker incubator, with a light reduction filter (Kodak Wratten Gelatin Filter, Eastman Kodak Co., Rochester, NY) in the lid to control light intensity.

Analytical Techniques

Liquid and gas samples, 2.5 and 0.4 mL, respectively, were withdrawn from the reactors during cultivation and analyzed for liquid and gas phase concentrations. The dry cell weight concentration was obtained from chlorophyl data by centrifuging 1 mL of liquid sample for 5 min at 15,000 rpm, discarding the top phase and resuspending the cells in 1 mL of methanol to extract the chlorophyl. After an additional centrifugation cycle, the green colored methanol extract was combined with 1.5 mL of fresh methanol. The absorbance was measured at 670 nm in a Spectronic 21 spectrophotometer (Milton Roy Co., Rochester, NY) and converted to dry weight cell concentration using a calibration curve.

Gas analyses were performed on a gas chromatograph (Hewlett-Packard 5890 Series II gas chromatograph and HP 3396A integrator, Avondale, CA) using a 3 mm × 1.8 m PTFE column packed with Chromosorb 107, 80/100 mesh (Alltech, Deerfield, IL). The oven temperature was maintained at 80°C, whereas the injector and thermal conductivity detector temperatures were 175°C. Sulfide in the liquid phase was measured in sulfide antioxidant buffer (SAOB) (Orion Res. Inc., Cambridge, MA) using a Corning Silver/Sulfide electrode (Corning Glass Works, Medfield, MA) and a Corning double junction reference electrode in conjunction with an Orion specific ion meter, model 407A (Orion Res. Inc.). Light intensities were measured with a LX-101 digital lux meter (Cole-Parmer, Chicago, IL).

THEORY

The green sulfur bacteria *Chlorobium thiosulfatophilum* (*6–10*) and *Chlorobium phaeobacteroides* (*15*) have been shown to oxidize H_2S to sulfate in a reaction sequence best described by

$$H_2S = S° + 2H^+ + 2e^- \quad (1)$$

$$2S° + 3H_2O = S_2O_3^{2-} + 6H^+ + 4e^- \quad (2)$$

$$S_2O_3^{2-} + 5H_2O = 2SO_4^{2-} + 10H^+ + 8e^- \quad (3)$$

The electron source gained through the reactions is used, in large part, for growth on CO_2. The indirect bacterial uptake of COS is likely to follow a similar pattern with the first reaction being that of the reaction of COS with water (*16*):

$$COS + H_2O = H_2S + CO_2 \quad (4)$$

The formed H_2S is then utilized by the bacteria according to Eqs. (1)–(3).

The sulfur green bacterium *C. thiosulfatophilum* requires both reduced sulfur and incandescent light for autotropic growth. Since the growth is light dependent, it is logical to treat light intensity as a "substrate." Light intensity has been used as a growth-limiting factor to predict growth rates for photosynthetic bacteria using a Monod type expression (*7,17*) or other empirical growth expression (*18*):

$$\mu = \mu_m \cdot I_o / (K_I + I_o) \quad (5)$$

$$\mu = \mu_m' - B/I_o \quad (6)$$

RESULTS AND DISCUSSION

Chlorobium thiosulfatophilum utilizes CO_2 as a carbon source, but also requires tungsten light and electron donors such as H_2S, S°, $S_2O_3^{2-}$, or H_2 for growth. In the presence of H_2S, the sulfide is converted to elemental sulfur during growth.

To illustrate this interdependence of light, carbon source and electron donor a plot of the natural log of the ratio of the cell concentration compared to the initial cell concentration as a function of time is shown in Fig. 1 for various initial H_2S levels. This plot should yield straight lines for each initial H_2S level, the slopes of these lines corresponding to the specific growth rate, μ, for each H_2S concentration. However, as is noted in Fig. 1, essentially a single straight line is obtained for all of the H_2S levels. This result indicates that something other than H_2S is limiting the growth rate. Since the solubilities of both H_2S and CO_2 are high in water (at neutral pH), and the cell concentration was fairly low, it is likely that the light supply limited cell growth in these experiments.

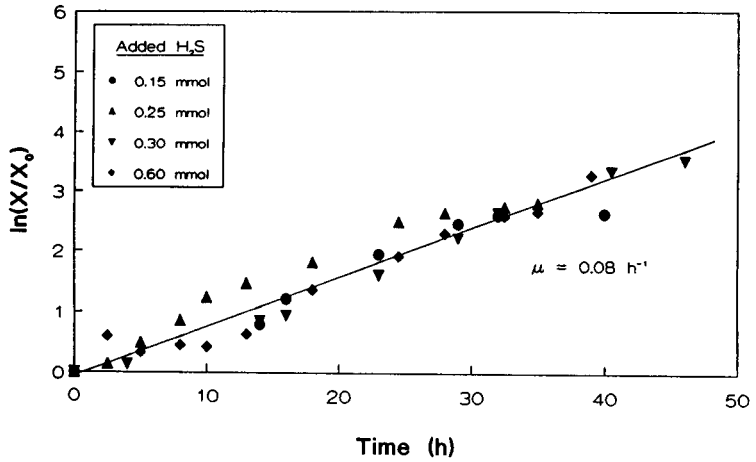

Fig. 1. Determination of the specific growth rate of *C. thiosulfatophilum* at various initial H_2S levels.

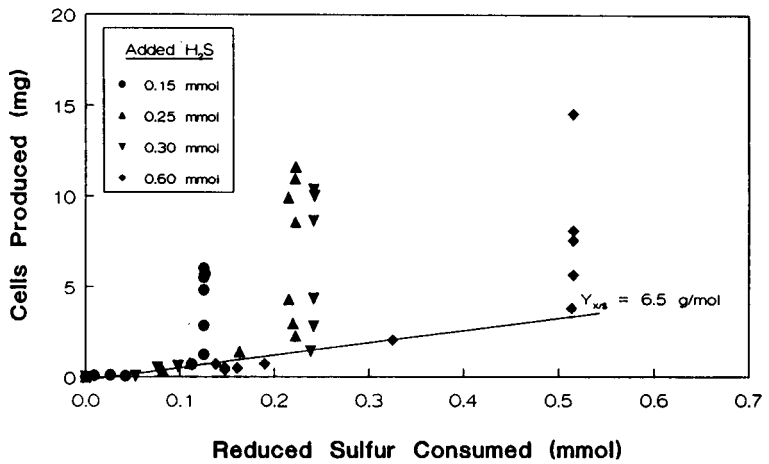

Fig. 2. Cell production by *C. thiosulfatophilum* as a function of sulfide consumption.

Cell production by *C. thiosulfatophilum* as a function of the total sulfide (H_2S (g), H_2S (l), HS^- and S^{2-}) consumption is plotted in Fig. 2 in order to determine the yield of cells from sulfide. As is noted, a single straight line is obtained for all of the initial H_2S levels, at least until H_2S is depleted from the system. When all of the H_2S is depleted from the medium, the culture continues to grow, utilizing elemental sulfur as the electron donor. This onset of elemental sulfur utilization is represented in Fig. 2 by the vertical cell production data for each initial H_2S level.

As is also noted in Fig. 2, a cell yield on sulfide of 6.5 g cells/mol sulfide is obtained. This yield compares well with the values found by Sublette and Sylvester (*5*), who reported yields of 5.3 and 13.4 g/mol for

aerobic and anaerobic growth of *T. denitrificans*, respectively. The estimated theoretical yield for complete conversion of sulfide to elemental sulfur and biomass was 15 g/mol, based on reaction stoichiometry (*10*).

Light Limiting Growth

If nutrients (minerals, metals, and vitamins) are supplied in excess, H_2S, CO_2, and light are factors that may limit the growth rate for *C. thiosulfatophilum*. To determine the growth kinetics under light-limiting conditions, two batch experiments were conducted with a set of five reactors per experiment. Using natural grey light reduction filters, various incoming light intensities were achieved. The cell concentration (X) was obtained as a function of time. Growth increased with increasing light intensity up to a light intensity of about 1000 lux. The initial specific growth rate for each reactor was estimated as the slope of the line from a plot of ln(X) as a function of time (Fig. 3).

Using the specific growth rates from Fig. 3 and the relationship presented in Eqs. (5) and (6), the values for the parameters μ_m, K_I, μ'_m, and B were found to be 0.152 h^{-1}, 351 lux, 0.0986 h^{-1} and 7.185 h^{-1} lux, respectively. In Fig. 4, the initial specific growth rate found from the experimental data is plotted as a function of light intensity. The curves in Fig. 4 were obtained from the empirical models. As is noted, the correlation described in Eq. (6) fit the experimental data rather well (r^2 = 0.93; all data points considered).

Indirect COS Uptake

COS undergoes a chemical reaction with water to produce H_2S and CO_2 as described in Eq. (4). Thompson et al. (*16*) studied the reaction and showed that the kinetics of the reaction could be described by a first order irreversible rate expression over a temperature range of 15–40°C, and that the rate constant followed an Arrhenius correlation.

Chlorobium thiosulfatophium can utilize COS indirectly, by utilizing the H_2S produced by the chemical reaction described by Eq. (4). If this is a satisfactory process, it will eliminate the need for an additional organism for complete removal of COS and H_2S in synthesis gas.

To study indirect COS utilization, two experiments were conducted with various initial amounts of COS (no H_2S was added in these experiments). Typical cell concentration profiles were obtained even though COS was utilized by an indirect route. It appeared, however, that the lag phase was slightly longer for the highest initial COS levels (data not shown). The amounts of COS and H_2S in the gas phase during the fermentation have been plotted as functions of time in Figs. 5–8. It should be noted that the legends in these figures refer to the amounts of initially added gas; the measured amount of H_2S or COS in the gas-phase at time zero is always lower since the gases dissolve rapidly in the liquid phase.

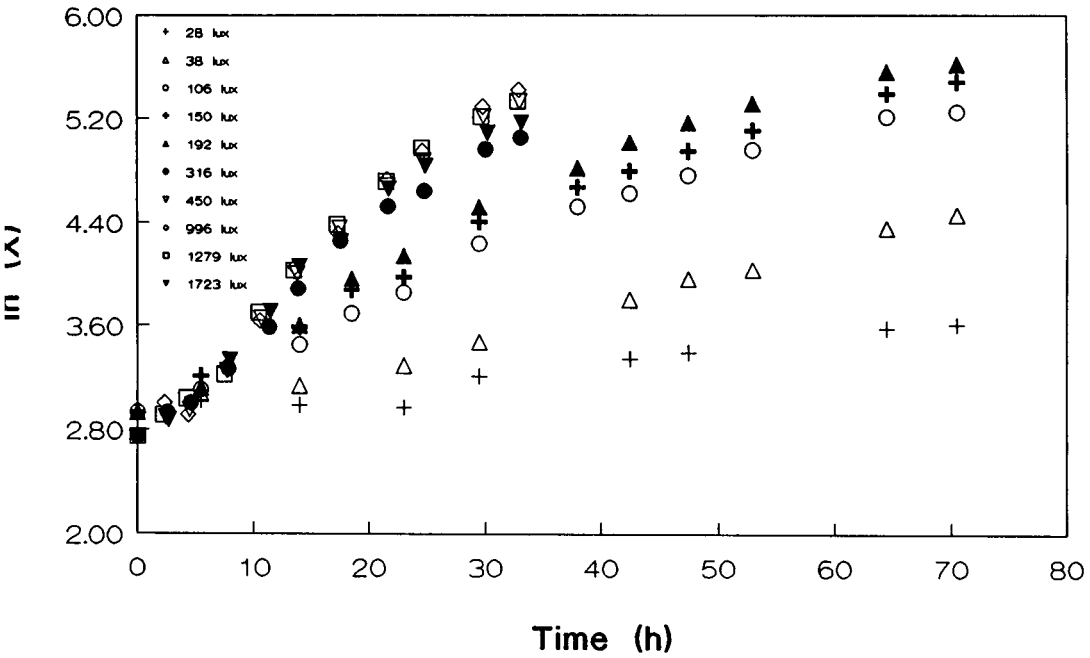

Fig. 3. Determination of initial specific growth rates for *C. thiosulfatophilum* at various light intensities.

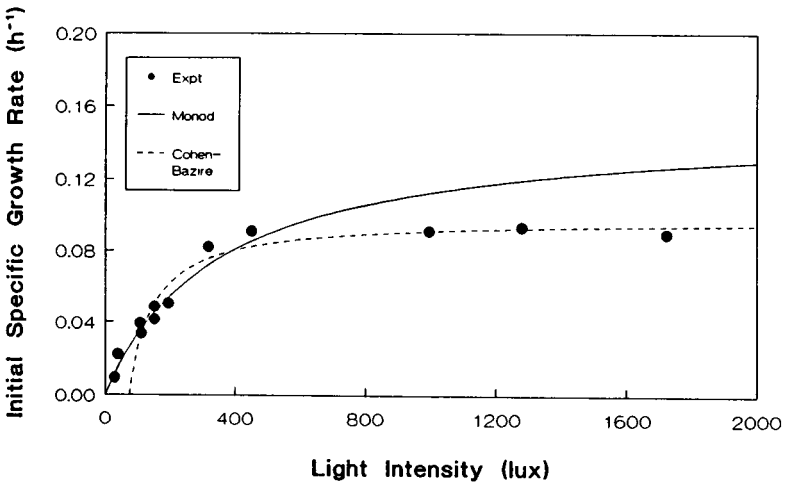

Fig. 4. Comparison of models with experimental data for the light intensity study with *C. thiosulfatophilum*.

As is noted in Figs. 5 and 6, the rate of disappearance of COS from the batch reactors, with and without culture, is identical for similar initial COS levels. This result indicates that the rate limiting step in indirect COS uptake is the reaction of COS with water as described by Eq. (4).

Gas phase H_2S accumulation profiles for the two experiments are shown in Figs. 7 and 8. Profiles without cultures are also shown at the

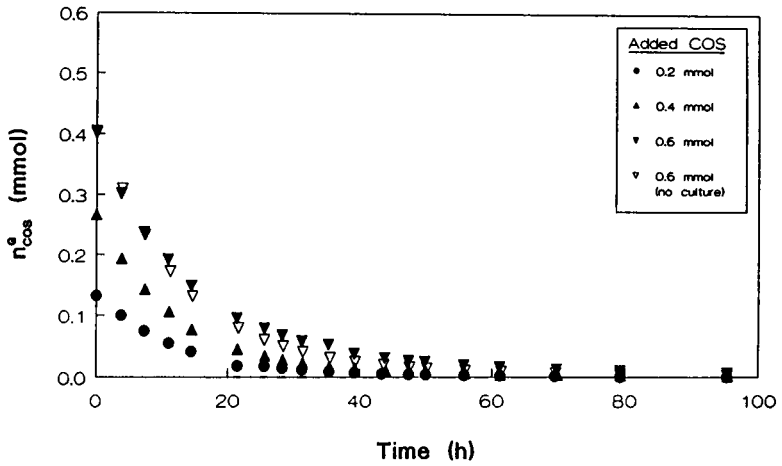

Fig. 5. Gas phase COS uptake profiles for *C. thiosulfatophilum* at various levels of COS (Experiment 1).

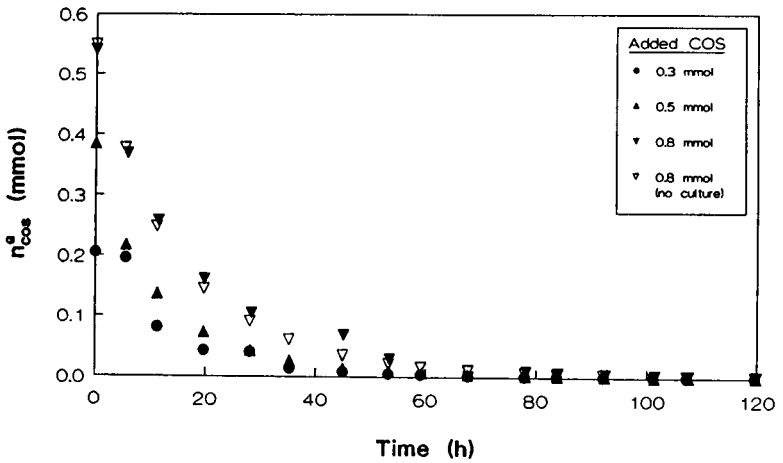

Fig. 6. Gas phase COS uptake profiles for *C. thiosulfatophilum* at various levels of COS (Experiment 2).

highest initial COS levels. As is noted in Figs. 7 and 8, the profiles in runs without culture showed the gradual accumulation of H_2S with time. The production of H_2S by Eq. (4) is indeed a relatively slow reaction. As is noted in the experimental runs with culture, the H_2S produced by the chemical reaction was not immediately consumed by the culture. The cells were able to utilize H_2S faster than it was being formed through the chemical reaction only after sufficient growth was attained. Eventually the gas phase composition showed no H_2S indicating that all the formed H_2S was consumed by the growing cells. It should be remembered that H_2S is required for growth. There was no evidence that COS could be directly utilized by *C. thiosulfatophilum*.

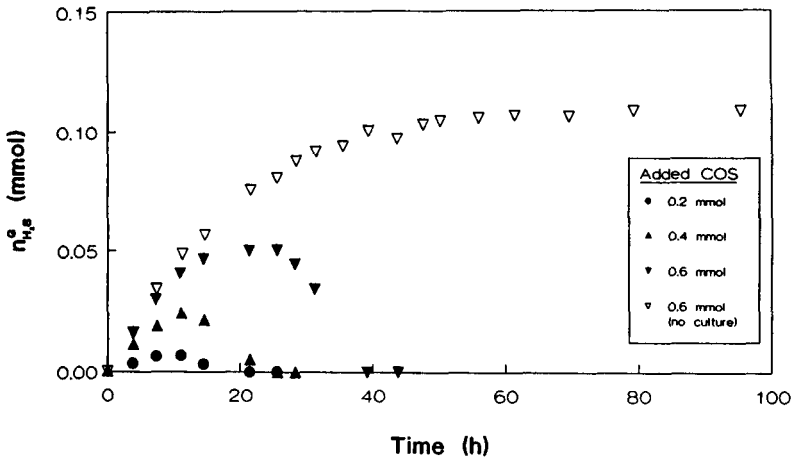

Fig. 7. Gas phase H_2S accumulation for *C. thiosulfatophilum* at various levels of COS (Experiment 1).

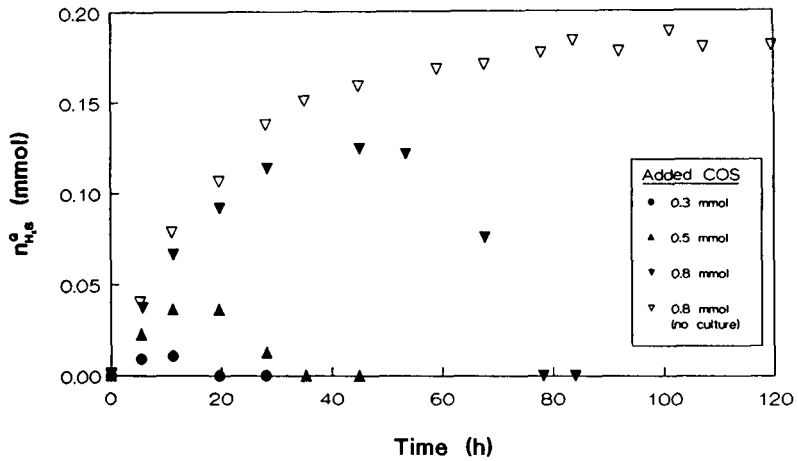

Fig. 8. Gas phase H_2S accumulation for *C. thiosulfatophilum* at various levels of COS (Experiment 2).

Even though mass transfer measurements were not done in these experiments, past experience in these reactors indicate that the mass transfer rate of COS exceeds the rate of reaction. The first-order reaction rate constant for the reaction of Eq. (4) was determined as 0.24 h^{-1} from the uninoculated reactors. This value compares well with the values of Smith (*19*), who found the rate constant affected by pH and temperature, and with values reported by Thomson et al. (*16*). Cell production by *C. thiosulfatophilum* as a function of the total sulfide and carbonyl sulfide consumed are shown in Fig. 9. The cell yield from these experiments was found to be 9.7 g/mol.

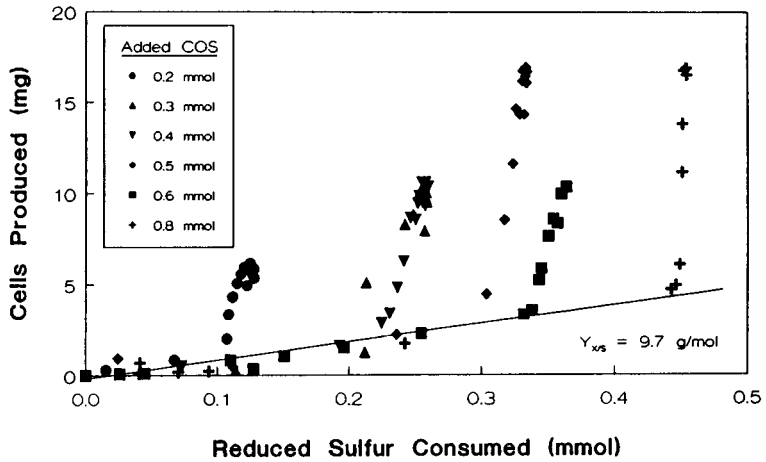

Fig. 9. Cell production by *C. thiosulfatophilum* as a function of total sulfide and carbonyl sulfide consumed.

CONCLUSIONS

COS and H_2S were successfully removed from the gas phase by the bacterium *C. thiosulfatophilum* for levels up to at least 0.17 atm (maximum gas phase COS at t = 0 in Fig. 6). The cell yield on sulfide (when the end product was extracellular elemental sulfur) was calculated as 6.5 g cells/mol H_2S. Similar experiments with COS showed a cell yield of 9.7 g/mol. The growth of *C. thiosulfatophilum* may be modeled in terms of incoming light intensity using a Monod equation:

$$\mu = 0.152 \cdot I_o/(351 + I_o) \quad (8)$$

or by a Cohen-Bazire relationship:

$$\mu = 0.0985 - 7.185/I_o \quad (9)$$

Equation (9) fits the experimental data very well ($r^2 = 0.93$) compared to the Monod model ($r^2 = 0.9$) except at very low light intensities. The rate limiting step in the uptake of COS was the reaction of COS with water to form H_2S and CO_2. The first-order rate constant was determined to be 0.24 h^{-1}.

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