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Biological Conversion of Synthesis Gas

Final Report
August 31, 1990 - September 3, 1993

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Work Performed Under Contract No.: DE-FG21-90MC27225

**For
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Office of Fossil Energy
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September 1993

ACKNOWLEDGMENT

Financial support for this work has been provided by the Morgantown Energy Technology Center, U. S. Department of Energy under grant number DE-FG21-90MC27225.

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1.0 INTRODUCTION

Synthesis gas, a mixture of primarily CO, H₂ and CO₂, is a major building block in the production of fuels and chemicals. The gas is derived from non-gaseous raw materials such as coal, shale oil, tar sands, heavy residue and biomass. The composition of synthesis gas is dependent upon the raw material used and the gasification process. Coal-derived gas is rich in CO and H₂, with lower concentrations of CO₂ and CH₄ and traces of H₂S and COS (1).

Synthesis gas may be used in the production of both gaseous and liquid fuels. The conventional method of producing gaseous fuel first employs the catalytic shift reaction. In this reaction, CO and H₂O are converted into H₂ and CO₂. Following the removal of excess CO₂ and sulfur contaminants such as H₂S and COS, the H₂ and CO₂ are catalytically methanated to CH₄ and H₂O. After drying, the product gas contains 95-98% CH₄.

Most methanation processes require temperatures of 300-700°C and operating pressures of 3-20 atm (2). The catalysts used for the methanation step include nickel and potassium-based catalysts, as well as mixtures of iron/chromium oxides and zinc/copper oxides (3,4). Poisons for these catalysts include chlorine and sulfur (5,6). In addition, chemical catalytic processes are known to produce by-products such as methanol, formaldehyde and acetic acid (7).

A wide variety of both liquid and gaseous fuels may be produced from synthesis gas using Fischer-Tropsch synthesis including light hydrocarbons (methane, ethane), fuel range hydrocarbon distillates and heavy waxes. The stoichiometry for product formation may be ideally represented by the equation:

However, based on actual yields in a Synthol entrained flow reactor, the overall stoichiometry is better characterized by the empirical relation (8):

As is noted in Equations (1.1) and (1.2), about 2 moles of H_2 are required for every mole of CO .

The H_2/CO ratio in synthesis gas is dependent upon both the grade of coal used in the feed and the type of gasifier employed. Modern gasifiers produce synthesis gas with a H_2/CO ratio as low as 0.6. Therefore, an increase in the H_2 content of synthesis gas is usually necessary prior to Fischer-Tropsch synthesis, which requires coupling water gas shift and gasification.

The utilization of coal to produce liquid fuels by Fischer-Tropsch synthesis is a multi-step process. After gasification at 700-2000°C (9), the raw gas is quenched and scrubbed to condense tars and oils and to remove dust and water soluble materials from the gas stream such as phenols, chloride, ammonia, cyanide, thiocyanate, and perhaps some H_2S (10). A catalytic water-gas shift conversion at about 225°C is then used to adjust the H_2/CO ratio:

A high H_2/CO ratio is not only necessary to satisfy the stoichiometry of the Fischer-Tropsch synthesis, but is beneficial in controlling carbon deposition and reducing associated catalyst deactivation (11).

Following shift conversion, the gas contains primarily H_2 , CO , CO_2 , H_2O , sulfur gases and possibly methane, although small amounts of higher aliphatic hydrocarbons and light oils are sometimes present. The sulfur is present as H_2S and COS , with smaller amounts of organic sulfides or mercaptans. These "acid gases", including CO_2 , are removed using a variety of processes, including Claus, absorption, or liquid phase oxidation processes. The sulfur

concentration must be reduced to less than 0.1 ppm to protect sensitive downstream catalysts (10). Following purification, the gas is then available for conversion to C₁-C₃₅ hydrocarbons in a Fischer-Tropsch synthesis reactor at 225-365°C.

There are several limitations with water gas shift, purification and Fischer-Tropsch synthesis. Each of these operations are conducted in separate steps, at different conditions requiring intermediate adjustment of temperature or pressure, which results in a very complex and expensive process. Also, these processes are carried out at elevated temperatures and pressures, and thus suffer from low thermal efficiencies. The catalysts used in the Fischer-Tropsch synthesis are particularly sensitive to the presence of sulfur gases or carbon deposition and deteriorate rapidly. Carbon deposition, as was mentioned previously, is enhanced by low H₂/CO ratios in the synthesis gas. Finally, more specific catalysts are required to avoid production of a broad array of chemicals and fuels and to improve raw material yields from Fischer-Tropsch synthesis.

1.1 Biological Synthesis Gas Conversion

Biological processes may be utilized as an alternative to the traditional methods of producing fuels from raw synthesis gas. Biological processes operate at near ambient temperatures, and yields and energy efficiencies are high. Many microorganisms (biocatalysts) are not sensitive to the presence of low concentrations of toxic gas compounds found in synthesis gas (12,13). In addition, biological reactions are very specific, and often a single product is produced as a result of biosynthesis.

A single reaction vessel can often be used to carry out several biological reactions in series or parallel. Therefore, one reactor might be

used for water gas shift, gas purification (sulfur and CO₂ removal) and product formation (Fischer-Tropsch synthesis or methanation) by utilizing a co-culture or mixed culture. As is shown in Figure 1.1, the process for biological production of hydrocarbons from synthesis gas could be very simple. Following gasification, the gas temperature would be reduced to 95-135°F, with the opportunity for heat recovery and improved energy efficiency. The cool gases would then be bubbled through a biological reactor, where a culture of microorganisms would be maintained to produce H₂ and remove sulfur gases and CO₂. Cultures may be developed that utilize CO, CO₂ and H₂ as substrates to produce hydrocarbons, such that adjustment of the H₂/CO ratio in the synthesis gas is not essential. Also, the removal of CO₂ may not be necessary if a liquid product is produced in the reactor. High conversions in the reactor will be necessary to avoid recycle. Therefore, significant potential exists for developing a simple, more economical biological process for hydrocarbon production from coal.

The disadvantages of biological processes include slower reactions and the need for sterile conditions to prevent contamination. When utilizing synthesis gas, sterility is insured by the presence of CO, which is toxic to most microorganisms. Biological reactions involving gaseous substrates may be operated under mass transfer limiting conditions, where mass transfer controls the rate of transport of gaseous reactants and, consequently, reactor size. Reactor rates have been found to be proportional to pressure, and the use of special bioreactors at moderate pressures (150 psia) have resulted in residence times for near complete conversion of synthesis gas of only a few minutes (14,15). Therefore, fast reaction rates and small reactors can be achieved by the appropriate bioreactor design.

Coal → Methane Conversion Process

CONVENTIONAL

BIO-CONVERSION

Figure 1.1. Comparison of Conventional and biological routes for methane production from coal.

1.2 Brief Statement of Purpose

The purpose of the proposed research is to develop a technically and economically feasible process for biologically producing H_2 from synthesis gas while, at the same time, removing harmful sulfur gas compounds. This research builds upon previous research studies carried out in the University of Arkansas laboratories where the technical and economic feasibility of biologically producing CH_4 from synthesis gas components has been demonstrated. Six major tasks are being studied:

1. Culture development, where the best cultures are selected and conditions optimized for simultaneous hydrogen production and sulfur gas removal;
2. Mass transfer and kinetic studies in which equations necessary for process design are developed;
3. Bioreactor design studies, where the cultures chosen in Task 1 are utilized in continuous reaction vessels to demonstrate process feasibility and define operating conditions;
4. Evaluation of biological synthesis gas conversion under limiting conditions in preparation for industrial demonstration studies;
5. Process scale-up where laboratory data are scaled to larger-size units in preparation for process demonstration in a pilot-scale unit; and
6. Economic evaluation, where process simulations are used to project process economics and identify high cost areas during sensitivity analyses.

The bioconversion studies are built upon previous experimental work performed under DOE contract. A strong foundation in gaseous substrate fermentation with a major emphasis on continuous bioreactor design and operation has been achieved. Although the technical feasibility of biological

methane production from synthesis gas has been demonstrated, several important pieces of information are needed prior to recommending a demonstration unit for this biological conversion. These process development studies can be grouped into three categories:

1. Culture development under industrial conditions;
 2. Investigation of alternate reactor systems to minimize capital costs;
- and
3. Process scale-up measurements.

Culture development studies under industrial conditions involve operating the cultures under less than optimum conditions in order to define operating limits for the cultures. The effects of trace quantities of oxygen in the feed on the strict anaerobes will be quantified. In addition, synthesis gas contaminants other than sulfur gases will be added to the gas stream to measure the effects of these materials on cell growth, substrate consumption and product formation. The effects of various CO/H₂ ratios on product yield and fermentation rates will be measured. Finally, efforts will be directed toward developing a cheap minimal media for synthesis gas fermentation.

An economic evaluation of various reactor alternatives has shown that the use of traditional mass transfer equipment yields high capital costs due to relatively poor gas-liquid mass transfer. A reaction system with mass transfer capabilities that minimize capital costs is thus sought. Various reaction systems are potential candidates and will be tested, including modified trickle-bed reactors and pulse and airlift fermenters. Also, various compounds will be added to the fermentation media in an effort to increase the mass transfer coefficient by changing physical properties.

Scale-up measurements involve several experiments. First, physical properties of the fermentation media will be evaluated under a variety of fermentation conditions. Two physical properties, medium viscosity and surface tension, are thought to change significantly with increasing cell density during the fermentation. Methods for handling these changes during process scale-up will be addressed. SEcondly, the limiting conditions of gas-liquid fermentation need to be defined. The maximum operable cell density and the performance of the cultures under extreme agitation rates, cell densities, operating pressure and high product concentrations will be evaluated. Finally, the scale-up correlations used in the economic analysis will be tested in larger bench-scale continuous reactors prior to recommending orocess demonstration in a PDU.

The production of hydrogen from CO and water can be carried out biologically by the facultative photosynthetic bacterium *Rhodospirillum rubrum* as an alternative to the water gas shift reaction. H₂ production is essentially in adjusting the H₂/CO ratio for further utilization of the syngas. Experiments in the University of Arkansas laboratories have shown that this bacterium is fast growing, exhibits fast CO uptake and is table in the presence of high CO and sulfur gas compositions. *R. rubrum* requires a tungsten light source for growth; however, the actual chemical reaction can be carried out in the dark. Therefore, only a small bioreactor for bacterial growth is required, thereby reducing capital and radiant energy costs.

Sulfur gas removal may be carried out by the anaerobic bacterium *Chlorobium thiosulfatophilum* (16,17). *C. thiosulfatophilum*, a phototropic sulfur bacterium, has the advantage of producing sulfur which could be easily

separated as a solid by-product. It has also been shown to utilize COS in conjunction with the chemical reaction

on *C. thiosulfatophilum* is superior to other sulfur gas-utilizing species such as *Thiobacillus denitrificans*, which produces sulfate as the product in H_2S conversion (18,19).

2.0 BIOLOGICAL H_2 PRODUCTION AND SULFUR GAS REMOVAL

2.1 Biological Water Gas Shift

Since very little has been published concerning the biological conversion of CO and water to H_2 and CO_2 according to the reaction of Equation (1.3), the initial screening process was based mostly upon previous work conducted in the University of Arkansas laboratories.

In the past, two photosynthetic purple bacteria, *Rhodospirillum rubrum* and *Rhodopseudomonas gelatinosa* have been found to be capable of carrying out the water gas shift reaction. *R. rubrum* carries out the reaction both in the light and in the absence of light, but requires light for growth. *R. gelatinosa*, on the other hand, carries out the reaction in the dark and utilizes CO as a carbon source for growth. Since both of these organisms were not originally identified as H_2 producers in the literature, other bacteria with similar physiologies may also have H_2 producing capabilities. Thus, bacteria with the following characteristics were sought as possible water gas shift biocatalysts:

1. be a purple, non-sulfur photosynthetic bacterium in the *Rhodospirillaceae* family;
2. be capable of anaerobic growth in the light;
3. have an optimal temperature of 25-40°C; and
4. show documented growth on simple substrates such as CO_2 or acetate.

A list of bacteria satisfying the above criteria is shown in Table 2.1. Based upon this list, three organisms (one from each genus) were selected for further study in the laboratory. The choice of organisms was based upon the ability of the organisms to use simple acids as substrates and optimal growth conditions. Together with *R. rubrum* and *R. gelatinosa* (see Table 2.1), a representative collection of bacteria from the Rhodospirillaceae family was thus obtained. Each of the bacterial cultures was obtained from the American Type Culture Collection (Rockville, MD).

2.2 Sulfur Gas Utilization

A number of microbial processes for the oxidation of H_2S to SO_4^{2-} or elemental sulfur have been reported in the literature. The sulfur bacteria comprise the phototropic and the chemolithotrophic bacteria, and the growth conditions may be anaerobic to aerobic. Based upon literature information, the list presented in Table 2.2 was compiled with the following considerations in mind:

1. the bacterium must be capable of anaerobic growth and sulfide reduction;
2. the bacterium must show elemental sulfur accumulation outside the cell;
3. the bacterium must have an optimal temperature of 25-40°C; and
4. the bacterium must have shown documented growth on simple substrates such as CO_2 or acetate.

Of the five choices shown in Table 2.2, only 2 were selected for further study at present: *Chlorobium thiosulfatophilum* and *C. phaeobacteroides*. *C. thiosulfatophilum* was obtained from ATCC (Rockville, MD) and *C. phaeobacteroides* was kindly supplied by Dr. T. Bergstein, a visiting scientist

Table 2.1

Bacteria Capable of Carrying Out the Biological
Water Gas Shift Reaction

ATCC #	Name	Optimum Temperature (°C)
<u>Rhodospirillum</u>		
25903	<u>rubrum</u>	30-35
25093	<u>tenuis</u> *	30
14031	<u>molischianum</u>	30
27871	<u>photometricum</u>	---
15798	<u>fulvum</u>	30
<u>Rhodopseudomonas</u>		
17001	<u>palustris</u>	30-37
19567	<u>viridis</u>	25-30
25092	<u>acidophilia</u>	25-30
17011	<u>gelatinosa</u> **	30
11166	<u>capsulata</u>	25-30
17023	<u>sphaeroides</u>	25-30
<u>Rhodomicrobium</u>		
17100	<u>vannielii</u>	30

* - Also named - Rhodocyclus tenuis

** - Also named - Rhodocyclus gelatinosus

at Washington University, St. Louis, MO. *Ectothiorhodospira abdelmalekii* was found not to be suitable for sulfur gas conversion due to its alkaline growth conditions at pH 8.5 and high salinity (14-16%) requirements. *Thiobacillus denitrificans* has also been initially rejected, not being phototrophic and mainly producing sulfate as the end product. *Chloroflexus aurantiacus* has not been selected for immediate study, but future plans may include studies of this organism. *C. aurantiacus* is capable of reducing S^{2-} to elemental sulfur and does not produce substantial amounts of sulfate. However, only 50% of the initial sulfur present in the medium was accounted for as elemental sulfur in one study.

Table 2.2

Microorganisms Capable of Reducing H_2S to Elemental Sulfur

ATCC #	Name
35917	<u><i>Ectothiorhodospira abdelmalekii</i></u>
	<u><i>Chlorobium</i></u>
17092	<u><i>thiosulfatophilum</i></u>
N/A	<u><i>phaeobacteroides</i></u>
29362	<u><i>Chloroflexus aurantiacus</i></u>
23642	<u><i>Thiobacillus denitroficans</i></u>

In addition to the reduction of H_2S , the removal of carbonyl sulfide (COS), also present in the synthesis gas, is also desired. COS spontaneously undergoes a reaction with water to produce H_2S and CO_2 as described by the equation:

Thus, the removal of COS may be seen as a removal of H₂S produced according to Equation (1.4). It has also been proposed that CO-utilizing microorganisms may utilize COS due to their molecular similarities. Three cultures, *Peptostreptococcus productus* (ATCC 35244), *Rhodospirillum rubrum* (ATCC 25903) and *Eubacterium limosum* (ATCC 8486) are presently being considered as possible COS-utilizing organisms. Both *P. productus* and *E. limosum* are anaerobic organisms capable of consuming CO and producing acetate. Acetate may also be used as the required carbon source for organisms carrying out the water gas shift reaction. *R. rubrum* is the obvious first choice since it is one of the most promising microorganisms for the water gas shift reaction studies. Other cultures may include selected H₂S-reducing cultures, since H₂S is a product in the reaction between COS and water (Equation 1.4).

3.0 MATERIALS AND METHODS

3.1 Organisms and Culture

Rhodospirillum rubrum, ATCC 25903, was obtained from the American Type Culture Collection, Rockville, MD. The medium used for stock cultures, transfers and experiments contained per 100 mL: Pfennig mineral solution (20), 5 mL; Pfennig trace metal solution (20) with the addition of 10 mg/l Na₂SeO₃ (21), 0.1 mL; B-vitamin solution (21), 0.5 mL; yeast extract (Difco), 0.1 g; NaCH₃COO·3H₂O, 0.58 g; NH₄Cl, 0.27 g; and NaHCO₃, 0.4 g. Stock cultures were kept anaerobically under tungsten light at 30°C and transferred every 2-3 weeks. Seed cultures used for inoculation of the bioreactors were prepared by transferring 5 mL of stock culture to 75 mL of fresh medium reduced with 1.5 mL of Na₂S (2.5%). Typically the seed culture was grown for 48 hours before an aliquot of 5 mL was used for inoculation of the reactor.

The medium for the studies with *C. thiosulfatophilum* contained per 100 mL: Pfennig mineral solution (20), 5 mL; Pfennig trace metals solution (20), 0.1 mL; B-vitamins solution (21), 0.5 mL; yeast extract (Difco), 0.05 g; and NaHCO_3 , 0.4 g. Before sterilization, 75 mL of medium were added to each bottle under a N_2/CO_2 (80%/20%) atmosphere. The bottles were autoclaved at 15 psig for 20 minutes. Once sterile, the bottles were flushed with He/CO_2 (60%/40%) to remove any traces of O_2 and inoculated with 5 mL of seed culture. Gaseous H_2S was then added to the bottles in the desired amounts. The seed culture was grown for 72 h before being used for inoculation into the bottles.

3.2 Experimental Screening Procedures

All experiments have been carried out using a basal medium containing per 100 mL: Pfennig mineral solution, 5 mL; Pfennig trace metal solution, 0.1 mL; B-vitamin solution, 0.5 mL; yeast extract (Difco), 0.05 or 0.1 g; and NaHCO_3 , 0.25 or 0.4 g (see Table 3.1 for solution compositions). All ingredients (except for NaHCO_3) were mixed with distilled water, heated and boiled for 2 min under anaerobic conditions. After cooling and the addition of NaHCO_3 , the medium was dispensed into serum bottles (100 or 150 mL nominal volume). The bottles were capped with butyl rubber stoppers and aluminum crimp seals. Anaerobic conditions were maintained throughout the preparation by working under an atmosphere of N_2/CO_2 (75%/25%) or H_2/CO_2 (75%/25%). Half-filled bottles were steam-sterilized at 15 psig for 20 min.

If strict anaerobicity was necessary, 2 mL of reducing agent solution (2.5% $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ or cysteine-HCl $\cdot\text{H}_2\text{O}$) were added per 100 mL of medium before inoculation. In addition to above medium, this basal medium was supplemented with specific carbon substrates or growth factors as the various cultures required. A 5% inoculum was typically employed in these batch screening experiments and the pH was maintained close to 7.0.

Table 3.1

Composition of Solutions

<u>Pfennig Mineral Solution</u>	<u>g/L</u>
KH ₂ PO ₄	10.0
MgCl ₂ ·6H ₂ O	6.6
NaCl	8.0
NH ₄ Cl	8.0
CaCl ₂ ·2H ₂ O	1.0
 <u>Pfennig Trace Metal Solution</u>	 <u>g/L</u>
ZnSO ₄ ·7H ₂ O	0.1
MnCl ₂ ·4H ₂ O	0.03
H ₃ BO ₃	0.3
CoCl ₂ ·6H ₂ O	0.2
CuCl ₂ ·H ₂ O	0.01
NiCl ₂ ·6H ₂ O	0.02
Na ₂ MoO ₄ ·2H ₂ O	0.03
FeCl ₂ ·4H ₂ O	1.5
Na ₂ SeO ₃	0.01
 <u>B-Vitamin Solution</u>	 <u>mg/L</u>
Biotin	20
Folic acid	20
Pyridoxal HCl	10
Lipoic acid	60
Riboflavin	50
Thiamine HCl	50
Ca-D-pantothenate	50
Cyanocobalamin	50
P-aminobenzoic acid	50
Nicotinic acid	50

In monitoring the progress of the experiments, gas phase analyses were performed using gas chromatography, liquid phase sulfide concentrations were monitored with a sulfide probe, cell density was measured by optical density on a spectrophotometer and pH was measured by employing a Corning pH meter. Liquid and gas samples, 1.5 mL and 0.4 mL, respectively, were withdrawn from the reactors during the fermentation and analyzed for cell concentration and gas phase composition. The dry cell weight concentration for Rhodospirillaceae were obtained by measuring light scatter at 540 nm in a Spectronic 21 (Milton Roy Co., Rochester, NY) and converting it to cell density using a calibration curve ($DCW(mg/l) = 395.4 \cdot ABS_{540}$).

The presence of elemental sulfur crystals makes measurement of cell concentration by optical density difficult. Two methods have thus been used for measuring the cell concentration of *C. thiosulfatophilum*: direct o.d. measurement at 580 nm and o.d. measurement of the pigment chlorophyll at 670 nm. For the measurement of pigment, 1 mL of solution was first centrifuged at 15,000 rpm for 4 min. The supernatant water was then discarded. Methanol (1 mL) was then added to dissolve the chlorophyll present in the cells, followed by centrifugation for an additional 2 min. Methanol (1.5 mL) was then added to the new supernatant and the absorbance measured at 670 nm.

A calibration curve for cell concentration in mg/L as a function of optical density at 580 nm is shown in Figure 3.1 and a calibration for cell concentration based upon chlorophyll content in mg/L as a function of optical density at 670 nm is shown in Figure 3.2. As is noted, both calibrations yielded straight lines. These calibration curves were constructed at a late stage in the fermentation when all of the elemental sulfur had been converted

Figure 3.1 Cell Concentration Calibration Curve as a Function of Optical Density at 580 nm for *C. thiosulfatophilum*.

Figure 3.2 Cell Concentration Calibration Curve (Based upon Chlorophyll Content) as a Function of Optical Density at 670 nm for *C. thiosulfatophilum*.

to sulfate. Thus, no interference of sulfur on the direct cell concentration measurements at 580 nm was observed.

Gas phase CO, CO₂ and H₂ analyses were performed on a gas chromatograph (Perkin-Elmer Sigma 300, Norwalk, CT) using an 1.8 m stainless steel column packed with Carbosphere, 60/80 mesh. The oven temperature was held constant at 135°C while the injector and thermal conductivity detector temperatures were 175°C. Helium was used as the carrier gas at a flow rate of 40 mL/min.

The gas phase H₂S and COs concentrations were measured by gas chromatography using a Hewlett-Packard (Kennett Square, PA) HP 5890 Series II gas chromatograph and HP 7673 integrator equipped with a thermal conductivity detector. The column was a 1/8" x 6' teflon column packed with Chromosorb 107, 80/100 mesh (Altech Assoc., Inc., Deerfield, IL). The oven temperature was 80°C, and the detector and injector temperatures were kept at 175°C. the carrier gas was helium at a flow rate of 30 mL/min. Liquid phase sulfur concentrations (H₂S(l), HS⁻, S²⁻) were measured using a Corning Silver/Sulfide Electrode (Corning Glass Works, Medfield, MA) and an Orion Specific Ion meter (Orion Research, Inc., Cambridge, MA).

3.3 Batch Bioreactors and Operating Conditions

Batch fermentations were carried out in 150 mL glass serum bottles (Wheaton Glass Co., Millville, NJ). The bottles were sealed using butyl rubber septum stoppers and aluminum crimp seals (Bellco Glass Co., Vineland, NJ). Before sterilization, 75 mL of medium were added to each bottle under a He/CO₂ (75%/25%) atmosphere. The bottles were autoclaved at 15 psig for 20 minutes.

Once sterile, the bottles were reduced with 1.5 mL of Na₂S (2.5%) and inoculated with 5 mL of seed culture. After inoculation the desired gas

phases were introduced by flushing with CO/CO₂ (80%/20%) for 3 minutes using an appropriate check valve to achieve the desired pressure. Argon (20 mL) was then added to each bottle as an inert tracer gas with a gas-tight syringe. This procedure allowed calculation of the amounts of each gas component through the fermentation.

The experiments were carried out in a modified incubator shaker (Model G25, New Brunswick Scientific Co., Edison, NJ) at 150 rpm and 30°C. Tungsten light, necessary for growth, was supplied in excess in the shaker.

The experiments involving light intensity were also carried out in a modified incubator shaker (Model G25, New Brunswick Scientific Co., Edison, NJ) at 150 rpm and 30°C. Tungsten light, necessary for growth, was supplied in the shaker. 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 Company, Rochester, NY) in the lid to control light intensity. Light intensities in the boxes were measured with a LX-101 Digital Lux Meter (Cole-Parmer, Chicago, IL).

3.4 Continuous Bioreactor and Operating Conditions

The continuous stirred-tank reactor used was a New Brunswick Scientific (Edison, NJ) BioFlo II fermenter equipped with temperature, pH and agitation control. The liquid working volume was 1250 mL and the overhead gas volume was 350 mL. Illumination necessary for growth was supplied by two tungsten lights (40W) directed towards the glass fermentation jar. Experiments were carried out at 30°C and pH 7. The feed gas used was a mixture of H₂, Ar, CO and CO₂ (20/15/55/10%) and was continuously fed to the reactor. Liquid feed was also supplied on a continuous basis. A schematic of the equipment set-up is shown in Figure 3.3.

Figure 3.3. Schematic Set-up of the CSTR Reactor System using *R. rubrum*
 Legend: 1- Feed reservoir; 2- Feed pump; 3- Fermenter;
 4- Gas supply tank; 5- Rotameter; 6- Effluent reservoir;
 7- Exit gas.

Typically experiments were started with constant agitation rate and gas flow rate. during an experimental run, the liquid flow rate was reduced from a high to low setting. This procedure induced an increase in cell concentration and CO conversion with time as the culture strived to reach a steady state for growth limited by medium composition, liquid flow rate and illumination. The conversion of CO was monitored and experiments were considered complete when the gas conversion leveled off. The liquid flow rate was then increased and the cell concentration allowed to decrease to a low level before starting a new experiment.

An effort has been made to develop a mixed culture of *R. rubrum* and *C. thiosulfatophilum*. 75 ml of basal medium with the addition of ammonium chloride and sodium acetate (medium used for *R. rubrum*) was added to 150 ml serum bottles. 1.5 ml of 2.5% sodium sulfide was then added to reduce any traces of oxygen. The bottles were purged with CO/CO₂ (80%/20%) for five minutes and 5 ml of H₂S were added. Pure cultures of *R. rubrum* and *C. thiosulfatophilum* (5 ml each) were added to the bottles.

It was observed that for all experiments the performance of the mixed culture was not as good as with pure cultures in terms of H₂ production, CO uptake, and H₂S consumption. The rates were much slower than when using the pure cultures. While for pure cultures, complete CO uptake and H₂S consumption required between 24 and 48 hours, for the mixed culture it required about 7 days.

In the future, it is proposed to utilize pure cultures in CSTRs in series for both H₂ production and H₂S removal. In this arrangement, the first CSTR containing *C. thiosulfatophilum* will consume H₂S (or at least reduce the level

of H₂S to tolerable levels). The second CSTR in series containing *R. rubrum* will produce H₂ from CO. While the gas stream passes from the first reactor to the second, the liquid streams in the CSTRs will be separate and parallel.

3.5 Analytical Techniques

Liquid and gas samples, 1.5 mL and 0.4 mL, respectively, were withdrawn from the reactors during the fermentation and analyzed for cell concentration and gas phase composition. The dry cell weight concentration for *R. rubrum* was obtained by measuring light scatter at 540 nm in a Spectronic 21 (Milton Roy Co., Rochester, NY) and converting it to cell density using a calibration curve ($DCW(mg/l) = 395.4 \cdot ABS_{540}$). Cell concentration measurements for *C. thiosulfatophilum* are discussed later.

Gas analyses of CO, CO₂ and H₂ were performed on a gas chromatograph (Perkin-Elmer Sigma 300, Norwalk, CT) using an 1.8 m stainless steel column packed with Carbosphere, 60/80 mesh. The oven temperature was held constant at 135°C while the injector and thermal conductivity detector temperatures were 175°C. Helium was used as the carrier gas at a flow rate of 40 mL/min.

The gas phase H₂S concentrations were measured by gas chromatography using a Hewlett-Packard (Kennett Square, PA) HP 5890 Series II gas chromatograph and an HP 7673 integrator equipped with a thermal conductivity detector. The column was a 1/8" x 6' teflon column packed with Chromosorb 107, 80/100 mesh (Altech Assoc., Inc., Deerfield, IL). The oven temperature was 80°C, and the detector and injector temperatures were kept at 175°C. The carrier gas was helium at a flow rate of 30 mL/min. Liquid phase sulfur concentrations (H₂S(l), HS⁻, S²⁻) were measured using a Corning Silver/Sulfide Electrode (Corning Glass Works, Medfield, MA) and an Orion Specific Ion meter (Orion Research, Inc., Cambridge, MA).

4.0 CULTURE DEVELOPMENT

4.1 Water Gas Shift Studies

The initial water gas shift work concentrated on growing lyophilized cells in simple basal medium with the addition of acetate and biocarbonate under a H_2/CO_2 atmosphere (75%/25%) by successive transfer. After good growth had repeatedly been obtained in this medium, small amounts of CO were added to the gas phase at a partial pressure of approximately 0.16 atm. If hydrogen was produced in these initial studies, a transfer was made to identical medium and CO was again added after growth was observed.

The results of these initial experiments with the four Rhodospirillaceae are shown in Table 4.1. As is noted, all of the cultures except *R. gelatinosa* were identified as H_2 -producers under the conditions employed. All of the organisms showed good growth on acetate, with H_2 production the most prominent so far in cultures reduced with cysteine-HCl. So far the most promising microorganisms are *R. palustris*, together with previously studied *R. rubrum*. *R. rubrum* has previously been shown to grow and completely convert all CO present in the gas phase to hydrogen at initial CO partial pressures up to 2 atm (22).

Continued studies, however, where gas phase compositions and stoichiometric relationships were closely followed, showed that even though substantial amounts of H_2 were produced, CO was not consumed. These experiments were conducted in triplicate.

Based upon documented research conducted with *R. rubrum* (23), it is likely that the observed H_2 production was based on a nitrogen system which liberates H_2 from organic substrates (such as acetate) under ammonia or N_2 limitations. This theory is in agreement with the results of the present

Table 4.1

Initial Results of Water Gas Shift Reaction Studies

<u>Culture</u>	<u>Reducing Agent</u>	<u>Growth</u>	<u>H₂ Production</u>	<u>Microscope Observation</u>
<u>R. tenuis</u>	Na ₂ S	++	ns	Thin rod-shaped, form large dense mats and clusters
	Cysteine-HCl	++	+	
	None	++	ns	
<u>R. palustris</u>	Na ₂ S	+++	ns	Thin crooked rod shaped, form small star-like clusters
	Cysteine-HCl	+++	+++	
	None	+++	+++	
<u>R. gelatinosa</u>	Na ₂ S	++	ns	Thin rod shaped, non-descriptive
	Cysteine-HCl	++	--	
	None	++	ns	
<u>R. vanniellii</u>	Na ₂ S	+	ns	Lemon shaped, flagellated, form chains and aggregates
	Cysteine-HCl	++	++	
	None	++	ns	

ns - not studied

- - negative test (no H₂ production)

+ - positive results; ++ more positive; +++ highly positive results

study, where H₂ production was initiated after substantial growth had been observed. It is likely that cell growth was accompanied by a limitation of ammonia and with the subsequent onset of H₂ production.

More recent experiments were directed toward utilizing a modified medium (see Table 4.2). In this medium, the yeast extract content was reduced and NH₄Cl was added to inhibit the formation of H₂ from acetate. The results from these experiments showed that no H₂ production occurred in the modified medium (see Table 4.3). Good growth was observed for *R. palustris* and slight growth was observed for *R. vaniellii* and *R. tenue*. The weak growth observed with the two latter strains is possibly related to the reduction of yeast extract in the new medium. Growth may be improved by acclimating the cultures to H₂ production/CO consumption. In addition, a small effort has been focused on the apparent production of H₂ from organic substrates such as acetate. Acetate may be seen as a liquid intermediate which may be produced from CO by cultures such as *Peptostreptococcus productus*, (24), according to Equation (4.1):

The produced acetate could then be converted to H₂ by several members of the Rhodospirillaceae family.

4.1.1 H₂ Production from Acetate

Several members of the Rhodospirillaceae family have been investigated for possible production of H₂ from acetate. The acetate may be seen as a liquid intermediate which may be produced by e.g. *P. productus*. The production of H₂ from a variety of organic substrates has previously been shown in the literature (25-27). The supply of nitrogen and chemical configuration were shown to strongly affect H₂ production in these cases.

Table 4.2
Modified Media Compositions

	Original Medium per 100 mL	Modified Medium per 100 mL
Pfennig Minerals Solution	5 mL	5 mL
Pfennig Trace Metal Solution	0.1 mL	0.1 mL
B-vitamins Solution	0.5 mL	0.5 mL
Yeast Extract	0.1 g	0.005 g
NaHCO ₃	0.25 g	0.25 g
NH ₄ Cl	--	0.27 g

For solutions composition and medium preparation, see reference (2)

Table 4.3
Results of Water Gas Shift Reaction Studies
Using a Modified Medium

<u>Culture</u>	<u>Growth</u>	<u>H₂ Production</u>
<i>R. tenuis</i>	++	--
<i>R. palustris</i>	+++	--
<i>R. vaniellii</i>	+	--

- negative test (no H₂ production)
- + positive results, ++ more positive; +++ highly positive results

Since it is generally considered that the enzyme nitrogenase is responsible for the release of H₂ in photosynthetic bacteria (28-30), experiments were conducted with various nitrogen sources according to the following test pattern:

Cultures: *Rhodospirillum rubrum* (ATCC 25903)
Rhodopseudomonas palustris (ATCC 17001)
Rhodocyclus gelatinosus
 (*Rhodopseudomonas gelatinosa*)(ATCC 17001)

Nitrogen Source: Glutamic acid, 0.106% (w/v)
 NH₄Cl, 0.038%
 Urea, 0.021% (base nitrogen content the same)

Carbon Source: None other than CO₂
 Sodium acetate 0.576%

Each of the above organisms were evaluated for H₂ production using a certain nitrogen source with or without acetate addition. For additional medium constituents and preparation see Table 4.4 and reference (31).

Table 4.4

Medium Composition, for H₂ Production
 from Acetate per 100 mL

Minerals Solution	5 mL
Pfennig Trace Metals Solution	0.1 mL
B-vitamins Solution	0.5 mL
Yeast Extract	0.02g
NaHCO ₃	0.25g
Nitrogen Source	as indicated in text
Sodium Acetate	as indicated in text

The minerals solution contained 10.0 g/L KH₂PO₄, 6.6 g/L MgCl₂·6H₂O, 8.0 g/L NaCl, and 1.0 g/L CaCl₂ 2H₂O. For other solutions see reference (10)

4.1.1.1 Bioreactor and Operating Conditions

Batch fermentations were carried out in 150 mL glass serum bottles (Wheaton Glass Co., Millville, NJ). The bottles were sealed using butyl

rubber septum stoppers and aluminum crimp seals (Bellco Glass Co., Vineland, NJ). Before sterilization, 75 mL of medium were added to each bottle under a He/CO₂ (75%/25%) atmosphere. The bottles were autoclaved at 15 psig for 20 minutes.

Once sterile, the bottles were inoculated with 5 mL of seed culture. The seed cultures had been previously growing in conditions similar to the experiment before they were used. Argon (20 mL) was then added to each bottle as an inert tracer gas with a gas-tight syringe. This procedure allowed calculation of the amounts of H₂ through the fermentation.

The experiments were carried out in a modified incubator shaker (Model G25, New Brunswick Scientific Co., Edison, NJ) at 150 rpm and 30°C. Tungsten light, necessary for growth, was supplied in excess in the shaker.

4.1.1.2 Analytical Techniques

Liquid and gas samples, 2.5 mL and 0.4 mL, respectively, were withdrawn from the reactors during the fermentation and analyzed for cell concentration and gas phase composition. Cell growth was obtained by measuring light scatter at 540 nm in a Spectronic 21 (Milton Roy Co., Rochester, NY).

Gas analyses were performed on a gas chromatograph (Perkin-Elmer Sigma 300, Norwalk, CT) using an 1.8 m stainless steel column packed with Carbosphere, 60/80 mesh. The oven temperature was held constant at 135°C while the injector and thermal conductivity detector temperatures were 175°C. Helium was used as the carrier gas at a flow rate of 40 mL/min. The acetate concentration was analyzed with a Varian (Walnut Creek, CA) model GC-3400 gas chromatograph and a model DS-604 data station. The GC was equipped with a 3 mm x 1.8 m, 100/200 mesh, Poropak QS (Alltech, Deerfield, IL) column and a flame ionization detector. The oven temperature was maintained at 190°C, the

detector and injector temperatures were both 240°C, and the carrier gas was helium at a flow rate of 40 mL/min.

4.1.1.3 Results

The results of the acetate fermentations conducted with *R. rubrum* are displayed in Figures 4.1-4.6. As noted in the figures, the growth (OD₅₄₀) increased sharply and then leveled off towards the end of the fermentation. The highest optical density reached was 1.2 in the medium supplemented with NH₄Cl and sodium acetate (or acetic acid) (see Figure 4.4). The only H₂ production with this culture was observed in the medium supplemented with glutamic and acetic acids (Figure 4.2). The amount produced was 0.4 mmol after 200 hrs, but decreased to 0.2 mmol after 500 hrs. Apparently the produced H₂ was being consumed during the latter part of the fermentation.

In Figures 4.7-4.12 the results for the fermentations using *R. palustris* are displayed. In general, the medium supplemented with acetate supported the best growth. In addition, the culture in the medium with only glutamic acid grew quite well. The highest optical densities ranged from 1.2-1.4. The production of H₂ was observed in several cases (see Figures 4.7, 4.8, 4.10, 4.12) with the most promising results for the experiment with glutamic and acetic acids (Figure 4.8). In this experiment the culture produced 1.4 mmol of H₂ in 200 hrs and showed no signs of H₂ disappearance. As is noted in Figure 4.8, growth (OD), H₂ production and acetate consumption almost ceased after 200 hrs of fermentation.

The experiments conducted with *R. gelatinosus* showed similar patterns for growth and H₂ production as with the other two purple bacteria. The results are shown in Figures 4.13-4.18. As with *R. palustris*, the best growth with *R.*

Figure 4.1. *R. rubrum* grown in the presence of glutamic acid.

Figure 4.2. *R. rubrum* grown in the presence of glutamic and acetic acids.

Figure 4.3. *R. rubrum* grown in the presence of NH_4Cl .

Figure 4.4. *R. rubrum* grown in the presence of NH_4Cl and acetic acid.

Figure 4.5. *R. rubrum* grown in the presence of urea.

Figure 4.6 *R. rubrum* grown in the presence of urea and acetic acid.

Figure 4.7 *R. palustris* grown in the presence of glutamic acid.

Figure 4.8 *R. palustris* grown in the presence of glutamic and acetic acids.

Figure 4.9 *R. palustris* grown in the presence of NH_4Cl .

Figure 4.10 *R. palustris* grown in the presence of NH_4Cl and acetic acid.

Figure 4.11 *R. palustris* grown in the presence of urea.

Figure 4.12 *R. palustris* grown in the presence of urea and acetic acid.

Figure 4.13 *R. gelatinosus* grown in the presence of glutamic acid.

Figure 4.14 *R. gelatinosus* grown in the presence of glutamic and acetic acids.

Figure 4.15 *R. gelatinosus* grown in the presence of NH_4Cl .

Figure 4.16 *R. gelatinosus* grown in the presence of NH_4Cl and acetic acid.

Figure 4.17 *R. gelatinosus* grown in the presence of urea.

Figure 4.18 *R. gelatinosus* grown in the presence of urea and acetic acids.

gelatinosus was obtained in medium supplemented with glutamic and/or acetic acids. The typical growth obtained was an OD of 1.3-1.7. The highest H₂ production, 0.9 mmol, occurred in the medium with glutamic and acetic acids after 150 hrs (see Figure 4.14). This production level remained constant throughout the rest of the fermentation.

To summarize, all of the cultures produced H₂ most efficiently in the medium with glutamic and acetic acids. The largest H₂ production, 1.4 mmol, was observed using *R. palustris*. H₂ production rates for this case are presented later in this report.

4.2 H₂S Removal Studies

The two most likely bacterial candidates capable of converting H₂S to elemental sulfur have been investigated for use in a co-culture with *R. rubrum*. These two bacteria, *Chlorobium thiosulfatophilum* and *Chlorobium phaeobacteroides*, are both members of the green sulfur photosynthetic bacteria family. Both bacteria were subjected to parametric studies covering growth and H₂S uptake in order to select the best bacterium for further study.

The conditions studied and overall results of these studies are shown in Table 4.5. As is noted in the table, both bacteria are very similar in behavior for the conditions studied. Both grew well in basal medium with or without yeast extract present. Hydrogen consumption was noted for both bacteria during the latter part of the fermentation as the H₂S (added as Na₂S) was depleted from the medium. Only *C. thiosulfatophilum* has been tested so far for the utilization of acetate (a substrate for *R. rubrum*) or acetate plus NH₄Cl. *C. thiosulfatophilum* was not inhibited by acetate and, in fact, grew on acetate as a carbon source. The bacterium also grew on basal medium supplemented with NH₄Cl and acetate.

Table 4.5

Initial Screening of Growth Conditions of H₂S Reducers

<u>Condition Studied</u>	<u>C. thiosulfatophilum</u>	<u>C. phaeobacteroides</u>
Is yeast extract needed for growth?	No	No
Does it grow in basal medium	Yes	Yes
Is acetate used?	Yes	--
Does acetate inhibit growth?	No	--
Does it use H ₂ as reducing agent?	Yes	Yes
Does it grow in basal medium supplemented with NH ₄ Cl and acetate?	Yes	--

A dash indicates that the condition has not yet been studied.

Later studies with the two bacteria have shown that *C. phaeobacteroides* often fails to grow on basal medium, while *C. thiosulfatophilum* always grows in basal medium. Also, *C. phaeobacteroides* will thus be chosen as the bacterium for H₂S conversion to elemental sulfur.

4.3 COS Degradation

4.3.1 COS Degradation by Selected CO-Utilizing Bacteria

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 H₂S (32,33). Typical levels of carbonyl sulfide (COS) in coal-derived synthesis gas range from 0.03-0.07% by volume (34), although COS concentrations have been observed in the 5-10% range (32,33).

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 (35), 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/chromium oxides and zinc/copper oxides (36,37). Poisons for these catalysts include chlorine and sulfur (38,39).

It has been recently shown in the University of Arkansas laboratories that some co-utilizing bacteria are capable of degrading COS (40). The results of these initial tests with various co-utilizing bacteria comparing their ability to degrade COS in the presence of CO are shown in this report. 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:

The gas phase composition was held constant in comparing the different cultures.

4.3.1.1 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

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 (24). *E. limosum* exhibited a doubling time of 7 h under the same conditions (42). *P. productus* has been shown to be able to tolerate H₂S and COS concentrations as high as 20% by volume (42).

C. ljungdahlii was isolated from chicken waste at the University of Arkansas (43) and, in addition to Reaction 2, carries out the following reaction (44):

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

R. rubrum, as mentioned previously, is a photosynthetic bacterium that is capable of converting CO and H₂O to HCO₃⁻, H⁺, and H₂ by the following reaction (45):

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 (46). *R. rubrum* was shown to be inhibited by COS concentrations above 6% (42).

4.3.1.2 Materials and Methods

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.

The medium for the bacteria was the same as the original medium of Table 4.2 with the addition of 0.1 mL of 0.1% resazurin. The medium pH was 7.0 for *P. productus*, *E. limosum*, and *R. rubrum*, and 5.0 for *C. ljungdahlii*. The experimental techniques employed in these batch comparison studies were as described previously (31,40).

4.3.1.3 Results and Discussion

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 Figures 4.19-4.22. The disappearance of CO in the medium alone is also shown for comparison purposes. As is noted in the figures, the

Figure 4.19 CO uptake by *P. productus* in the presence of COS.

Figure 4.20 CO uptake by *E. limosum* in the presence of COS.

Figure 4.21 CO uptake by *C. ljungdahliae* in the presence of COS.

Figure 4.22 CO uptake by *R. rubrum* in the presence of COS.

rate of CO consumption increased with inoculum size. Furthermore, all organisms yielded essentially complete conversion for all inoculum sizes in 25 h or less. CO was not degraded in the medium alone.

The uptake of COS in the presence of CO by the bacteria is shown in Figures 4.23-4.26. *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 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 Figures 4.19-4.26 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 COS were very low. It is possible that 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 (Figure 4.27). At least-squares fit forced through the origin yields a slope of 0.055 mol COS/mol CO. This ratio of initial rates may be compared to the initial amounts of gases present, which were approximately 0.06 mol COs/mol CO in all experiments. This would indicate similar kinetic utilization modes for both CO and COS,

Figure 4.23 COS degradation by *P. productus* in comparison to the medium alone.

Figure 4.24 COS degradation by *E. limosum* in comparison to the medium alone.

Figure 4.25 COS degradation by *C. ljungdahliae* in comparison to the medium alone.

Figure 4.26 COS degradation by *R. rubrum* in comparison to the medium alone.

Figure 4.27 Initial COS-utilization rate as a function of initial CO-utilization rate for *P. productus*, *E. limosum* and *R. rubrum*.

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.

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 H₂O. *Clostridium ljungdahlii*, on the other hand, was not found to have an ability to degrade COS. H₂S was detected as a major gas-phase product, but stoichiometric product yields have not yet been determined.

COS degradation by *C. ljungdahlii* (see Figure 4.25) 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.

It was observed during the COS degradation studies that H₂S 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.

4.3.2 Indirect COS Utilization by Chlorobia

COS undergoes a chemical reaction with water to produce H₂S and CO₂ by the equation:

Thompson *et al.* 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. They showed that the rate constant varied with temperature by the equation:

$$k = 1.06 \times 10^{12} e^{-22710/RT} \quad (4.5)$$

where k is the first order rate constant, sec⁻¹;

R is the ideal gas constant, 1.987 cal/gmole°C; and

T is the absolute temperature, °K.

At 37°C, the rate constant is thus calculated as 0.37 h⁻¹.

C. thiosulfatophilum can utilize COS indirectly, by utilizing the H₂S produced by the chemical reaction in Equation (4.2). If this is indeed a satisfactory process, it will eliminate the need for an additional organism (such as *Rhodospirillum rubrum* or *Peptostreptococcus productus*) for COS degradation.

5.0 MASS TRANSFER/KINETIC CONCEPTS WITH RHODOSPIRILLACEAE

5.1 Specific Carbon Monoxide Uptake Rate for *R. rubrum*

In converting gaseous substrates by microorganisms present in a liquid phase, the gas must be transported from the bulk gas phase to the cell wall. It is widely recognized (47) that the main resistance to this transport, for

sparingly soluble gases such as CO and O₂, is the liquid film at the gas/liquid interface. All other transport phenomena are of lesser magnitude. The rate of transport then becomes proportional to the concentration driving force across the film. If the liquid phase concentration of CO is expressed in terms of partial pressure, Equation (5.1) may be written.

$$\text{Rate of transport} = \frac{K_L a}{H} (p_G - p_L^*) V_L \quad (5.1)$$

In Equation (5.1), p_L^* is the partial pressure of CO in the gas phase which in equilibrium with the bulk liquid phase concentration of CO. For the sealed bioreactors used in this study, the rate of transport changes with time due to cell growth and consumption of CO by the cells. If the CO consumption rate in the liquid is very low, the driving force, $p_G - p_L^*$, is also small and the CO uptake rate is considered to be kinetically limited. On the other hand, if the reaction rate in the liquid is high and the CO is consumed as it enters the liquid, p_L^* approaches zero and the rate of uptake depends only upon the mass transfer rate. According to Equation (5.1), the rate of transport is then proportional to the partial pressure of CO in the gas phase times the liquid volume. In the unique case of gas consumption by a growing microbial culture, both of the rate limiting regions are present during the fermentation, given that the initial cell concentration is sufficiently low.

Since the solubility of CO in water (and medium) is low, it may be safely assumed that the amount of CO present in the liquid phase is negligible compared to the CO in the gas phase, n_{CO} . Thus, Equation (5.1) may be rewritten as:

$$\frac{1}{V_L} \left(\frac{dn_{CO}}{dt} \right) = \frac{K_L a}{H} (p_G - p_L^*) \quad (5.2)$$

The disappearance of CO in the liquid phase due to microbial uptake may be expressed as proportional to the cell density in the liquid. The proportionality constant, q , is generally called the specific uptake rate and is defined in Equation (5.3).

$$\text{moles of CO consumed} = q \times V_L \quad (5.3)$$

As for most microbial systems, the rate of substrate uptake by the cells, q , is dependent upon the availability of the substrate and thus the concentration of the dissolved gas becomes the primary variable. If the general assumption is made that substrate inhibition is present, Equation (5.4) may be used to describe the CO uptake per dry cell weight.

$$q = \frac{q_m p_L^*}{K_p + p_L + (p_L^*)^2 / W} \quad (5.4)$$

Equation (5.4) is the well known Monod model (48) rewritten for substrate uptake and modified by Andrews (49) to include substrate inhibition.

The determination of the parameters in Equation (5.4) is not straightforward since the partial pressure of CO in the liquid phase is difficult to measure. However, by using the data obtained late in the batch fermentation, the mass transfer coefficient, $K_L a$, may be determined using Equation (5.2) and the assumption that p_L^* is zero. This value of $K_L a$ may then be used to calculate p_L^* for the part of the batch fermentation which is not under mass transfer limiting conditions. A simple stepwise procedure may be formulated for the determination of the parameters $K_L a$, q_m , K_p and W as follows.

1. Determine q from the experimental data and Equation (5.3) for the entire fermentation.

2. Determine $K_L a$ from the experimental data during the latter part of the fermentation using Equation (5.2) and the assumption that p_L^* is equal to zero.
3. Calculate p_L^* in the early part of the fermentation using the value of $K_L a$ found in Step 2 and Equation (5.2).
4. Rearrange Equation (5.4) to form Equation (5.5) so that the parameters are easily determined by plotting p_L^*/q as a function of p_L^* using a second order equation to the fit data by the method of least squares.

$$\frac{p_L^*}{q} = \frac{K_p}{q_m} + \frac{p_L^*}{q_m} + \frac{(p_L^*)^2}{q_m W} \quad (5.5)$$

This technique has previously been demonstrated in detail by Vega et al. (50) for the fermentation of CO to acetate by *P. productus*.

Two sets of experiments (Experiments 1 and 2) were conducted with different initial CO partial pressures. By conducting two experiments, the reproducibility of the experimental and analytical techniques was verified and a model covering a rather wide range of CO partial pressures was developed. In addition to the experiments with CO, a third experiment was conducted with various gas phase compositions (see Table 5.1) in a medium containing no acetate and 0.06% yeast extract. No H₂ production was observed in the bottles without CO, but cell growth remained essentially constant for all gas phase compositions. This result helps to verify that CO is utilized in H₂ production but is not utilized for cell growth.

The increase in cell concentration (Experiments 1 and 2) with time within each experiment was identical for all CO pressures studied (see Figure 5.1). However, the cell concentration profiles varied between experiments due to a

Figure 5.1. Cell Concentration Profiles in Experiments 1 and 2.

Table 5.1

Experiment 3 - effect of gas phase composition on cell growth
and hydrogen production (inoculum size = 16 mg/l.)

Gas Phase Composition (mol percent)	Maximum Cell Concentration, 86 hr (mg/l)	Hydrogen Production
CO ₂ /He (25%/75%)	209	-
CO ₂ /N ₂ (25%/75%)	206	-
CO ₂ /CO (25%/75%)	208	++
CO ₂ /CO (50%/50%)	214	+
CO ₂ /He/N ₂ (33%/34%/33%)	202	-

longer lag phase in one of the experiments. The differences in lag phase between the experiments may be caused by different inoculum histories. The CO was completely consumed by *R. rubrum* in both experiments (see Figures 5.2 and 5.3), with fermentation times for complete conversion ranging from 40 to 120 hours. The solid curves in Figures 5.2 and 5.3 correspond to the smooth curves used when estimating derivatives which were needed for the calculations of the CO uptake rate in Equation (5.2). As the CO was consumed, H₂ was produced in nearly stoichiometric amounts. The H₂ production with time is shown in Figures 5.4 and 5.5. As is seen, the reactor with the highest initial CO pressure produced the most H₂. The dashed curves in Figures 5.3 and 5.4 have only been added to emphasize trends in the raw data. A H₂ yield on CO of 0.87 mol/mol was found by examining all of the collected data (see

Figure 5.2. CO Partial Pressure Profiles for *R. rubrum* in Experiment 1.

Figure 5.3. CO Partial Pressure Profiles for *R. rubrum* in Experiment 2.

Figure 5.4. H_2 Production for *R. rubrum* in Experiment 1.

Figure 5.5. H_2 Production for *R. rubrum* in Experiment 2.

Figure 5.6). This corresponds to 87% of the theoretical yield from Equation (1.3). Two possible explanations for this low yield may be that *R. rubrum* may use some of the electrons for other than H₂ production or that some of the CO may have been used as a carbon source by the cells.

Figure 5.7 was generated in order to illustrate the determination of the mass transfer coefficient. In Figure 5.7, the estimation K_La is performed only with data from Experiment 1. The slope of the straight line is equal to K_La/H and if a value of 1120 l atm/mol is used for the Henry's law constant (51), K_La may be determined as 9.7 hr⁻¹ for this experiment. For the second experiment a K_La of 14.4 was obtained in a similar fashion. With the values of K_La, the partial pressure of CO in the liquid may be found as described in Step 3 above and the plot suggested in Step 4 may be generated (see Figure 5.8). In Figure 5.8, the initial lag phase before exponential growth has been eliminated for all cases. As noted, all of the data may be represented by a single quadratic curve. From the coefficients in the equation of the curve, the parameters in Equations (5.4) and (5.5) may be easily calculated. The final expression correlating the specific uptake rate of CO by *R. rubrum* is described in Equation (5.6).

$$q = \frac{0.055 p_L^*}{0.45 + p_L^* + \left(p_L^*\right)^2/0.106}, \quad (p_L^* < 1.1 \text{ atm}) \quad (5.6)$$

In examining Equation (5.6), it is seen that the maximum specific uptake rate of CO, q_m, is 0.055 mol/g,hr and the Monod constant, K_p, is 0.45 atm. In addition, the substrate inhibition constant, W, has a value of 0.106 atm, indicating that CO significantly inhibits CO uptake. In fact, the highest specific uptake rate found from Equation (5.6) is 0.011 atm, occurring at an equilibrium partial pressure of 0.218 atm.

Figure 5.6. Estimation of the H₂ yield on CO for *R. rubrum* in Experiments 1 and 2.

Figure 5.7. Determination of the Mass Transfer Coefficient for *R. rubrum* in Experiment 1.

Figure 5.8. Determination of the Kinetic Parameters for CO Uptake by *R. rubrum* according to the Proposed Model (Experiments 1 and 2).

In comparing the rate of H₂ production on CO by *R. rubrum* with production by other organisms, it is seen that *R. rubrum* has a specific H₂ production rate (equal to 87% of the CO uptake rate) of 0.004-00.009 mol/g, hr over an equilibrium partial pressure range of 0.1-1.0 atm. In a review article, Vignais et al. (52) reported maximal H₂ production rates by a variety of photosynthetic bacteria on various substrates. The highest reported H₂ production rate was 0.010 mol/g,hr for *Rhodopseudomonas capsulata*, strain B10, grown on lactate in nitrogen-limited culture. Bott et al. (53) reported a H₂ production rate of 2.4×10^{-3} - 3.6×10^{-3} mol/g, hr for *Methanosarcina barkeri* grown on 2.5-10% CO at pH 7 and 37°C in the presence of propyl iodide and 2-bromoethanesulfonate.

5.2 The Effect of Light Intensity on Growth for *R. rubrum*

Studies of bacterial growth are often conducted in batch fermentation vessels or chemostats with one limiting nutrient, usually carbon. Light is generally not considered a nutrient but may be the growth limiting factor in the photosynthetic growth of suspended cells. The role of light intensity and its influence on growth and product formation has mainly been studied with algae (54-56) and various mathematical models have been proposed (57) and tested. The most often used correlation is a Monod type equation using the mean light intensity as the limiting "nutrient."

All purple bacteria are potentially capable of growing anaerobically in the light with CO₂ as the carbon source and inorganic compounds as electron donors. However, the purple bacteria can also utilize acetate (or other carbon sources) under similar conditions for photoheterotrophic growth. The acetate is largely converted into a reserve material, poly-β-hydroxybutyric

acid, in stepwise reactions which may be summarized as:

The reduction power needed (H) may be supplied from the TCA cycle, with the anaerobic oxidation of acetate according to the equation:

The necessary ATP is furnished through the photochemical reaction:

The overall reaction obtained by combining Equations (5.7-5.9) yields:

As is seen in Equation (5.10), most of the carbon present in acetate is converted to poly- β -hydroxybutyrate and very little is lost as CO_2 . However, since poly- β -hydroxybutyrate itself is not cell material, further carbon will be lost as CO_2 in the sequential steps.

Since the generation of energy and thus growth is dependent upon light it should be possible to treat the light intensity as a "substrate" and utilize a Monod type expression to predict the specific growth rate (μ) with light intensity (54,57,58) This relationship is shown in Equation (5.11):

$$\mu = \frac{\mu_m I}{K_I + I} \quad (5.11)$$

However, the light intensity (I) is very different from a substrate since it varies throughout the bulk of the liquid. For dense cultures it is therefore necessary to use either an average specific growth rate (57) or, if the circulation of liquid throughout the bottle is high, an average light intensity (54,56). The resulting equations when using an average specific growth rate and an average light intensity are shown below in Equations (5.12) and (5.13).

$$\mu_{avg} = \frac{1}{L} \int_0^L \mu(I_z) dz \quad (5.12)$$

$$I_{avg} = \frac{1}{L} \int_0^L I_z dz \quad (5.13)$$

I_z may be described by the well-known Beer-Lambert law

$$I_z = I_0 e^{-\epsilon z X} \quad (5.14)$$

where I_0 is the incoming light intensity, z and X are the light path distance and the cell concentration, respectively, and ϵ is the extinction coefficient.

If, on the other hand, the cell concentration is low and the agitation vigorous in a small vessel, it may be assumed that the effect of light intensity on the specific growth rate may be expressed in terms of incoming light intensity (see Equation (5.15)). This is true if the mixing time is faster than the growth rate. In other words, the cells will be in a region (volume) close to the incoming light, often enough not to be affected by the reduced light throughout the culture. In the case of dense cultures, this region becomes very small and the incoming light is quickly reduced, so that it is likely the cells will enter this smaller region often.

$$\mu = \frac{\mu_m I_0}{K_I + I_0} \quad (5.15)$$

In Equation (5.15), μ may be estimated as $1/X)(dX/dt)$ during the initial part of a batch experiment when the cell concentration is low. Equation (5.15) may be rearranged to yield a linear form as:

$$\frac{1}{\mu} = \frac{1}{\mu_m} \left(\frac{1}{I_0} \right) + \frac{K_I}{\mu_m} \quad (5.16)$$

Equation (5.16) may be used to estimate the constants μ_m and K_I using a straight line regression fit.

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 and these cell concentration profiles are shown in Figure 5.9 for one of the experiments. As is noted in Figure 5.9, growth increased with increasing light intensity. The initial specific growth rate for each reactor was estimated with increasing light intensity. 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. A typical growth curve is shown in Figure 5.10 and, as is seen in the insert in Figure 3.10, the slope (μ) of the curve decreases with time as the culture gets more dense and the incoming light is significantly reduced. The initial specific growth rates determined by the procedure demonstrated in Figure 5.10 for the various light intensities are shown in Table 5.2.

Using the values in Table 5.2 and the relationship presented in Equation (5.16), the values for the parameters μ_m and K_I were found to be 0.052 h^{-1} and 140 lux, respectively. This indicates a maximum specific growth rate of 0.052 h^{-1} for the conditions employed and a 50% reduction of the maximum growth rate at a light intensity of 140 lux. In Figure 5.11, the initial specific growth rate is plotted as a function of light intensity. The curve in Figure 5.11 is obtained from the empirical model

Figure 5.9. Cell concentration profiles at various light intensities for *R. rubrum*.

Figure 5.10. Cell concentration profile and determination of initial specific growth rate for *R. rubrum* in an experiment using a light intensity of 507 lux.

Figure 5.11. The effect of light intensity on the initial specific growth rate for *R. rubrum*

$$\mu = \frac{0.052 I_0}{140 + I_0} \quad (5.17)$$

Table 5.2

The Effect of Light Intensity (I_0) on Initial Specific Growth Rate (μ_{init}) for *R. rubrum*

Experiment I		Experiment II	
I_0 (lux)	$\mu_{init}(h^{-1})$	I_0 (lux)	$\mu_{init}(h^{-1})$
198	0.0328	114	0.0236
346	0.0388	202	0.0282
521	0.0439	329	0.0334
661	0.0469	430	0.0355
839	0.0464	507	0.0375

As is noted, a good correlation is obtained between the raw data and the predicted values obtained from the correlation. The specific growth rate asymptotically approaches the maximum specific growth rate of $0.052 h^{-1}$ as predicted by the model and, at a light intensity of 2660 lux, the specific growth rate is 95% of the maximum. The effect of light intensity on growth and the chlorophyll content of *R. rubrum* was also studied by Holt and Marr (59). They found a similar shaped curve as presented in Figure 5.11 when plotting the specific growth rate as a function of light intensity. In continuous stirred tank reactor experiments using a malate-glutamate medium (the preferred medium for *R. rubrum*), specific growth rates of 0.13 - $0.14 h^{-1}$ for light intensities above 4300 lux were obtained. A very sharp decrease in the specific growth rate below 1000 lux was also observed. Later, Cohen-

Bazire and Sistrom (60) used the data from Holt and Marr together with additional data from experiments with *Rhodospseudomonas spheroides* to describe the specific growth rate as a function of light intensity. According to their preliminary results, the specific growth rate could be described as $\mu_m - B/I$, where I is the light intensity and B is an empirical constant. Direct comparisons of the present results with data in the literature are difficult due to differences in medium, reactor shape, light intensity measurements and light source. Qualitatively, however, the effect of light intensity on the specific growth rate appears to follow a Monod type shaped curve for all of the systems studied. This clearly indicates the importance of light intensity in photosynthetic microbial systems and the resemblance between light intensity and a limiting substrate.

In addition to the light limiting culture experiments, experiments were also conducted with excess light in which acetate and ammonia concentrations were limited. Cell growth is plotted as a function of acetate and ammonia consumption in Figures 5.12 and 5.13, respectively. A straight line fit through the origin was used in both cases to find the observed cell yield on acetate and ammonia. The cell yields were calculated to 0.42 g/g and 13 g/g for acetate (as acetic acid) and ammonia (as NH_3), respectively. Based upon an empirical chemical formula for bacterial cells ($\text{CH}_{1.833}\text{N}_{0.225}\text{O}_{0.385}$) (61), approximately 55% of the carbon in acetate was incorporated into cell mass, with the balance going to CO_2 .

5.3 The Effect of Temperature on the Kinetics of *R. rubrum*

The models of Equations (5.6) and (5.17) were obtained at the published optimum temperature of 30°C . However, from observing the performance of other microorganisms as a function of temperature it was often found that the

Figure 5.12. Determination of the cell yield on acetate for *R. rubrum*

Figure 5.13. Determination of the cell yield on ammonia for *R. rubrum*

no growth was observed at 37°C. The experimental growth region was chosen for analysis in order to eliminate differences in inoculum and culture acclimation effects often present in the lag phase. In the exponential phase, the plot of $\ln X$ as a function of time should yield a straight line. As is noted in Figure 5.14, three experiments (corresponding to three initial partial pressures) were run at each temperature. In analyzing the data, it is seen that the highest cell concentrations ($\ln X$) occurred at the lower temperatures of 25 and 30°C. Slightly depressed growth occurred at 32 and 34°C. If a single straight line is drawn through the truly exponential region of the data, the slope of the line is found to be 0.035 h^{-1} , which is equal to the specific growth rate, μ . Thus, any temperature between 25 and 34°C is suitable for the growth of *R. rubrum*.

Figure 5.15 shows the calculation of the yield of H_2 from CO , $Y_{p/s}$, for the four temperatures. Previous results have shown a product yield of 0.87 mol/mol at 30°C, and the theoretical yield is 1.0 mol/mol from Equation (5.6). The slope of the line in Figure 3.15 is 0.96, which is 96% of theoretical. Also, there is seen to be no effect of temperature on the product yield. No explanation is offered for the increase in the yield in the temperature study compared with previous results at 30°C.

A plot of P_{CO}^G/q as a function of P_{CO}^G is shown in Figure 5.16 for the four temperatures. As is noted, a single curve is essentially obtained for temperatures of 30, 32 and 34°C, indicating that the specific uptake rate is the same at these temperatures. (An exception to this observation is one set of data at 34°C which gives lower uptake rates and thus higher values of P_{CO}^G/q . Also, the rather steep curvature for one set of data at 30°C is due to the lag phase). At 25°C, it is seen that the specific uptake rate is lower

reported optimum was, in fact, not really an "optimum" temperature, but instead a "preferred" temperature of the two or three temperatures tested. Thus, the effects of temperature on growth and uptake by *R. rubrum* need to be studied to establish true temperature optima in terms of maximizing the specific growth and uptake rates. Furthermore, since biological water gas shift may not be carried out under strictly isothermal conditions in industrial reactors, the effect of temperature on the performance of the bacterium needs to be well understood.

The purpose of this study is to present results on the effect of operating temperature on the performance of *R. rubrum*. The operating temperature was varied between 25 and 37°C in an effort to quantify the effects of temperature on the specific growth rate, the yield of H₂ from CO and specific uptake kinetics.

Five temperatures were considered in the experimental study: 25, 30, 32, 34 and 37°C. As was mentioned previously, the reported "optimum" temperature was 30°C. Thus, temperatures both above and below the optimum were considered. The lowest temperature chosen was 25°C since it is well known that reaction rate generally increases with temperature, so that the specific rates of both growth and uptake would be expected to be quite low at temperatures below 25°C. The maximum temperature studied was 37°C since no growth was obtained at this temperature. Small temperature increments between 30 and 37°C were used since it was expected that these small increases in temperature might significantly affect bacterial growth and CO uptake.

The effects of temperature on cell concentration in the exponential growth phase is shown in Figure 5.14, where the natural logarithm of the cell concentration is plotted as a function of time for four temperatures. Again,

Figure 5.14. Growth of *R. rubrum* in the Experimental Growth Phase as a Function of Temperature.

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Figure 5.15. Calculation of the Yield of H₂ from CO by *R. rubrum* as a Function of Temperature.

Figure 5.16. Comparison of Specific Uptake Kinetics for *R. rubrum* as a Function of Temperature.

for all three data sets, indicating that 25°C would be an inappropriate temperature for catalyzing the reaction of Equation (5.6).

Thus, the growth of *R. rubrum* may be satisfactorily carried out at 25 and 30°C, while uptake and thus the conversion of CO best occurs at temperatures of either 30, 32 or 34°C. The reported optimum of 30°C is well suited for both growth and CO uptake. More importantly, small upward variations in temperature in industrial processes will not negatively affect CO uptake or H₂ production.

5.4 The Kinetics of *R. rubrum* in the Continuous Stirred Tank Reactor

The purpose of this study was to determine the CO conversion kinetics by *R. rubrum* in a continuous stirred tank reactor and to illustrate the potential for using *R. rubrum* as a model system for the mass transfer determinations. The experiments were conducted in a fashion which allowed for separation of mass transfer and kinetic aspects of the fermentation.

An overall non-steady state CO balance over the CSTR may be written as:

$$\left(\frac{P_{Ar}^I G^I}{R T} \right) \left[\left(\frac{P_{CO}^I}{I} \right) - \left(\frac{P_{CO}^G}{G} \right) \right] - \left(\frac{V_G}{R T} \right) \frac{dP_{CO}^G}{dt} = r_{CO} V_L \quad (5.18)$$

where r_{CO} is the volumetric CO mass transfer/uptake rate as defined in Equation (5.19).

$$r_{CO} = K_L a \left(C_L^* - C_L \right) = \left(\frac{K_L a}{H} \right) \left(P_{CO}^G - P_{CO}^* \right) = Xq \left\{ P_{CO}^* \right\} \quad (5.19)$$

The function $q \left\{ P_{CO}^* \right\}$ in the above equation describes the microbial kinetics and may be as simple as a Monod-type expression or may include additional terms.

Based on gas analysis, r_{CO} may be calculated from Equation (5.18) for the entire fermentation. As the cell concentration increases with time, the system moves from a kinetic limited condition to a mass transfer limited state. At this point the CO conversion (and r_{CO}) becomes constant even though the cell concentration still increases. The mass transfer coefficient can now be calculated using Equation (5.19) with the assumption that $P_{CO}^* \ll P_{CO}^G$. It is important to realize that the growth of *R. rubrum* is not limited by the CO supply but by liquid constituents, liquid flow rate and illumination. The same equation may then be used to estimate the dissolved CO $\left[P_{CO}^*\right]$ for the earlier part of the fermentation. Finally, with the calculated values of r_{CO} , P_{CO}^* and the measured values of X , Equation (5.19) may again be used to evaluate the kinetic function, $q \left\{P_{CO}^*\right\}$.

Experiments were conducted using five different agitation rates (300-700 rpm in 100 rpm increments). Gas composition and cell concentrations were measured. In Figure 5.17, representative CO conversion and cell concentration profiles for three of the five experiments are displayed. As is noted in the figure, an increase in the agitation rate resulted in a higher value for the maximum conversion at a given agitation rate. A maximum CO conversion of 38% was obtained using an agitation rate of 300 rpm compared to a 58% CO conversion at 700 rpm. The maximum CO conversion, indicated by the plateau obtained for the CO conversion profiles (see Figure 5.17), corresponds to CO mass transfer limiting conditions in the fermenter. It is clear that mass transfer limiting conditions are in effect during this time since the CO conversion leveled off even though cell concentration continued to increase. Thus, the cell concentration in the fermenter was not limited by CO transport

Figure 5.17. Cell Concentration and CO Conversion Profiles in the CSTR for Agitation Rates of 300, 500, and 700 RPM (*R. rubrum*).

but by the factors described above. By the same token, the maximum CO conversion was not limited by cell concentration but by agitation rate, gas flow rate and gas inlet composition.

Based on the gas phase analysis, the volumetric CO transfer rate (r_{CO}) was calculated according to Equation (5.18) for the mass-transfer limited region of the fermentation (time > 45-60 h). The overall mass transfer coefficients were then calculated using Equation (5.19) and the assumption that $P_{CO}^* \ll P_{CO}^G$. The estimation K_La values ranged from 15 to 35 h^{-1} for the agitation rate range of 300-700 rpm (see Table 5.3). For agitation rates between 400-600 rpm, only small changes in the K_La values were observed. The most likely explanation for this result is that the position of the impellers relative to the liquid level and the effect of vortex mixing is of great importance. Visual inspection showed a clear change in flow patterns between 300 and 400 rpm, and 600 and 700 rpm.

By using the calculated values of r_{CO} (from Equation (5.18) and the estimated values of K_La (see Table 5.3), the dissolved CO concentration $\left[P_{CO}^*\right]$ was calculated for the initial (and assumingly kinetic limited) part of the fermentation using Equation (5.19). A representative result is shown in Figure 5.18 for an agitation rate of 500 rpm. As is noted, P_{CO}^* was approximately zero after 45 hours, corresponding to mass transfer limited operation. In addition to calculating P_{CO}^* , the values for the specific CO uptake rate (q) equal to r_{CO}/X , were also calculated (see Figure 5.19). Starting of P_{CO}^* , the data indicated that the specific CO uptake rate increased sharply with increasing values of P_{CO}^* . Above a dissolved CP pressure of 0.05 atm, the specific uptake rate decreased almost linearly with increasing CO partial pressure. It is evident from the result that CO has an inhibitory

Figure 5.18. Measured CO Partial Pressure and Calculated Dissolved CO Tension in the CSTR for an Agitation Rate of 500 RPM (*R. rubrum*).

effect on the CO uptake (see Figure 5.19). Thus, an attempt was made to fit the data to two inhibition models based on the Monod equation, one commonly used for substrate inhibition, another mostly used to account for product inhibition.

Table 5.3

Mass Transfer Coefficients Calculated at Final Conversion Values
for Various Agitation Rates
(*R. rubrum*)

Agitation Rate (rpm)	P_{CO}^* (atm)	r_{CO} (mol/h L)	$K_L a$ (h ⁻¹)
300	0.2804	$3.731 \cdot 10^{-3}$	14.9
400	0.2307	$4.431 \cdot 10^{-3}$	21.5
500	0.2134	$4.337 \cdot 10^{-3}$	22.8
600	0.2142	$4.549 \cdot 10^{-3}$	23.8
700	0.1679	$5.322 \cdot 10^{-3}$	35.5

$$q = \frac{q_m \cdot P_{CO}^*}{K_p + P_{CO}^* + \left[P_{CO}^* \right]^2 / W} \quad (5.20)$$

$$q = \frac{q_m \cdot P_{CO}^*}{K_p + P_{CO}^*} \left[1 - \frac{P_{CO}^*}{P_m} \right] \quad (5.21)$$

Equation (5.20) corresponds to Andrews modification of the Monod relationship to include substrate inhibition (49) and Equation (5.21) is the Monod expression, modified with a linear inhibition term. Equation (5.20) has recently been used to describe CO uptake rate by *R. rubrum* in batch culture.

Figure 5.19. Measured Specific Uptake Rate as a Function of Calculated Dissolved CO Tension for Agitation Rates of 300–700 RPM (*R. rubrum*).

Non-linear regression analysis was used to obtain the parameters in Equations (5.20) and (5.21). The result of this analysis indicated that Equation (5.20) was unsuitable for the data since the regression predicted negative values for at least one parameter. This was considered inconsistent with the development of Equation (5.20). The solid curve in Figure 5.19 is the best fit determined according to Equation (5.21) and corresponds to the expression:

$$q = \frac{0.0146 \cdot P_{CO}^*}{0.0053 + P_{CO}^*} \left[1 - \frac{P_{CO}^*}{0.68} \right] \quad (5.22)$$

This equation is very sensitive to the independent variable P_{CO}^* . For low values of P_{CO}^* , the part of the equation corresponding to the Monod model is dominating, while for higher values of P_{CO}^* , the linear inhibition term is more prevalent. It may be predicted from the equation that CO uptake completely inhibited by CO partial pressures of 0.68 atm and above.

The dashed curve in the same figure was generated based on previous results obtained in batch culture using similar experimental conditions. As is noted in Figure 5.19, the correlation previously found for batch data does not fit the data obtained in the CSTR experiments. The CO uptake rate in batch culture was less inhibited by dissolved CO and q approached zero as P_{CO}^* approached infinity. However, both models predict a maximum specific CO uptake rate of approximately 0.011-0.012 mol/g,h. The difference in batch versus continuous data may be explained by CO acclimation. Batch experiments were typically started with CO partial pressures up to 1.6 atm. A long lag phase was always present at these pressures, but the cultures grew eventually. All experiments conducted in the CSTR were started at a maximum CO partial

pressure of 0.5 atm. If higher initial CO partial pressures had been used in the CSTR studies, perhaps the results in the CSTR and in batch culture would have been similar.

The hydrogen yield was constant throughout the CSTR experiments at 0.88 mol/mol (see Figure 5.20). This value is 88% of the theoretical value obtained from the stoichiometry of Equation (1.3). In batch culture, the yield was estimated to 0.87 mol/mol.

5.5 Rate of H₂ Production by *R. palustris*

R. palustris, grown on a basal medium supplemented with glutamic and acetic acids, has shown promising results in releasing H₂ during growth. This section will concentrate on determining the rate of the production in the experiment.

The rate of production of many growth-related products in microbial systems may be described by the equation:

$$\frac{d(PV_L)}{dt} = \nu \cdot XV_L \quad (5.23)$$

where ν is the specific production rate, X is the cell concentration V_L is the liquid volume, P is the product concentration and t is time. The specific production rate, ν , may be a function of substrate and product concentrations, but for simplicity it will be assumed to be constant. If the derivative, $d(PV_L)/dt$, the cell concentration, X , and the liquid volume V_L , are known a value of ν may be found in Equation (5.23). For the case of hydrogen production from acetate by *R. palustris*, Equation (5.23) may be rewritten as:

$$\frac{dH_2}{dt} = \nu \cdot X \cdot V_L \quad (5.24)$$

Figure 5.20. Determination of H₂ Yield for *R. rubrum* in the CSTR.

Batch cell concentration, H₂ production and liquid volume profiles are plotted for *R. palustris* in Figure 5.21. Also shown in the figure are smoothed curves of this data indicating experimental trends. Utilizing these smooth curves, the rates of hydrogen consumption dH_2/dt , were calculated. Figure 5.22 was constructed by plotting dH/dt as a function of XV_L . As is seen in the figure, a straight line may be used to predict the data for the initial part of the fermentation. The slope of the line, 0.002 mol/g,hr, is equal to the specific H₂ production rate, ν , by the cells. This value is considerably lower than the values of 0.004-0.009 mol/g,hr obtained for *R. rubrum* carrying out the water gas shift reaction. This result indicates that the water gas shift reaction using *R. rubrum* is the preferred choice over H₂ production from acetic acid in the presence of glutamic acid using *R. palustris*.

6.0 MASS TRANSFER AND KINETIC CONCEPTS WITH CHLOROBIVM THIOSULFATOPHILLUM

The anaerobic, photosynthetic bacterium *Chlorobium thiosulfatophilum* has been chosen from among several bacteria for its ability to convert H₂S to elemental sulfur. The bacterium utilizes CO₂ as its carbon source and operates at the mesophilic temperature of 37°C. The bacterium requires tungsten light for growth and compounds such as H₂S, elemental sulfur or H₂ as a source of reducing power. Of these latter three compounds, H₂S as sulfide is the preferred source of reducing power, with H₂ and elemental sulfur utilized only when sulfide has been depleted from the medium. The organism is also capable of indirectly utilizing COS, since COS reacts with water to form CO₂ and H₂S by the equation (62):

Thus, the H₂S formed by Equation (1.4) is utilized by *C. thiosulfatophilum* following the reaction of COS and water.

Figure 5.21. Cell concentration, H_2 production and liquid volume profiles for *R. palustris* grown on glutamic and acetic acids.

Figure 5.22. Determination of the average initial specific H_2 uptake rate for *R. palustris* grown on glutamic and acetic acids.

6.1 Growth of *C. thiosulfatophilum* in the Presence of H₂S

As was mentioned previously, *C. thiosulfatophilum* utilizes CO₂ as a carbon source, but also requires tungsten light and a source of reducing power such as H₂S, H₂ or elemental sulfur for growth. In the presence of H₂S, the sulfide is converted to elemental sulfur during growth.

To illustrate this interdependence of light, carbon source and reducing power 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 Figure 6.1 for various initial H₂S levels. This plot should yield straight lines for each initial H₂S level, the slopes of these lines corresponding to the specific growth rate, μ , for each H₂S concentration. However, as is noted in Figure 6.1, essentially a single straight line is obtained for all of the H₂S levels. This result indicates that something other than H₂S is limiting the reaction. In this case, it is obvious that H₂S and CO₂ are present in excess and that the light intensity is limiting cell growth. It is thus expected that a Monod relationship for specific growth rate as a function of light intensity will be obtained.

The cell production by *C. thiosulfatophilum* as a function of the total sulfide concentration (H₂S (g), H₂S (l) and related species) is plotted in Figure 6.2 in order to determine the yield of cells from sulfide. As is noted, a single straight line is obtained for all of the initial H₂S levels, at least until H₂S is depleted from the system. When all of the H₂S is depleted from the medium, the culture continues to grow, utilizing elemental sulfur as the source of reducing power. This onset of elemental sulfur utilization is represented in Figure 6.2 by the vertical cell production data for each initial H₂S level.

Figure 6.1. Determination of the Specific Growth Rate of *C. thiosulfatophilum* at Various Initial H_2S Levels.

Figure 6.2. Cell Production by *C. thiosulfatophilum* as a Function of Sulfide Consumption.

As is noted in Figure 6.2, a cell yield on sulfide of 9.2 mg cells/mmol sulfide is obtained. This yield is greater than typical yields of cells from glucose by the yeast *Saccharomyces cerevisiae*, where a yield of 5.4-9.0 mgcells/mmol glucose is obtained.

A determination of Monod kinetics for H₂S uptake by *C. thiosulfatophilum* was not possible due to difficulties in following the various sulfur species and the problem associated with sulfur disappearance into the rubber septa sealing the batch reactors.

6.2 Loss of H₂S into Rubber Septa

Previous studies with H₂S uptake by various species of chlorobia have generated data which are difficult to quantify and model since H₂S is known to diffuse through the rubber septa sealing the batch reactors. Although H₂S disappearance into butyl rubber is relatively small in comparison to H₂S uptake by the bacteria, it would be helpful if the average amount of H₂S leaving the batch system through the stopper could be quantified.

If a first order rate of disappearance of H₂S (both gas and liquid species) is assumed, the following equation may be written:

$$- \frac{d(\text{sum H}_2\text{S})}{dt} = k P_{\text{H}_2\text{S}}^G \quad (6.1)$$

In Equation (6.1), $-\frac{d(\text{sum H}_2\text{S})}{dt}$ represents the rate of disappearance of all sulfur species into the butyl rubber stoppers, $P_{\text{H}_2\text{S}}^G$ represents the gas phase partial pressure of H₂S and k represents the first order rate constant.

Equation (6.1) may be integrated to yield:

$$- \Delta (\text{sum } \Delta \text{H}_2\text{S}) = k \int_0^t P_{\text{H}_2\text{S}}^G dt \quad (6.2)$$

Thus a plot of $-\Delta (\text{sum } \Delta \text{H}_2\text{S})$ as a function of $\int_0^t P_{\text{H}_2\text{S}}^G$ should yield a

straight line with a slope k if the disappearance can indeed be represented by a first order relationship.

Figure 6.3 shows such a plot for two initial H_2S levels in water (no culture), housed in serum stoppered batch reactors. As is noted, a reasonable straight line can be fitted through the data. The slope of this line, k , is $0.0470 \text{ mmol/atm}\cdot\text{h}$. This relationship can now be used to correct experimental data for H_2S disappearance through the butyl rubber stoppers.

6.3 Indirect COS Uptake by *C. Thiosulfatophilum*

As was mentioned previously, COS undergoes a chemical reaction with water to produce H_2S and CO_2 by the equation:

Thompson *et al.* (62) 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 $15-40^\circ\text{C}$. They showed that the rate constant varied with temperature by the equation:

$$k = 1.06 \times 10^{12} e^{-22710/RT} \quad (6.3)$$

where k is the first order rate constant, sec^{-1} ;

R is the ideal gas constant, $1.987 \text{ cal/gmole}^\circ\text{C}$; and

T is the absolute temperature, $^\circ\text{K}$.

At 37°C , the rate constant is thus calculated as 0.37 h^{-1} .

C. thiosulfatophilum can utilize COS indirectly, by utilizing the H_2S produced by the chemical reaction in Equation (1.4). If this is a satisfactory process, it will eliminate the need for an additional organism (such as *Rhodospirillum rubrum* or *Peptostreptococcus productus*) for COS degradation.

Figure 6.3. Determination of the First Order Rate Constant for H_2S Disappearance into Butyl Rubber Septa.

Figures 6.4-6.9 show cell growth, gas phase COS uptake and gas phase production and uptake profiles for indirect COS utilization by *C. thiosulfatophilum*. Two experiments were performed: one with 0.2, 0.9 and 0.6 mmol of COS initially; and one with 0.3, 0.5 and 0.8 mmol of COS initially.

In examining the cell concentration profiles of Figures 6.4 and 6.5, it is seen that typical cell concentration profiles are obtained even though COS is utilized by an indirect route. It also appears that the lag phase is slightly larger for the highest initial COS levels.

Figures 6.6 and 6.7 present gas phase COS concentration profiles for the two experiments. Also shown in each figure are the results of an experiment using the highest COS level (0.6 mmol and 0.8 mmol, respectively) and no culture. As is noted in the two figures, the rate of disappearance of COS from the batch reactors, as determined from the slopes of the COS concentration profiles, is identical with and without culture regardless of the initial COS level. This result indicates that the rate limiting step in COS uptake is the reaction of COS with water by Equation (1.4). Thus, the actual uptake of COS does not depend upon the initial concentration of COS, at least until the initial COS level reaches very high inhibitory levels.

Figures 6.8 and 6.9 show gas phase H₂S production (by the chemical reaction) and uptake profiles for the two experiments for *C. thiosulfatophilum*. As in Figures 6.6 and 6.7, profiles without culture are also shown at the highest initial COS levels. As is noted in Figures 6.8 and 6.9, the profiles without culture showed the gradual accumulation of H₂S with time. The production of H₂S by Equation (1.4) is indeed a relatively slow reaction. As is noted in the experimental runs with culture, the H₂S produced

Figure 6.4. Cell Concentration Profiles for the Indirect Uptake of COS by *C. thiosulfatophilum* (Experiment 1).

Figure 6.5. Cell Concentration Profiles for the Indirect Uptake of COS by *C. thiosulfatophilum* (Experiment 2).

Figure 6.6. Gas Phase COS Uptake Profile for *C. thiosulfatophilum* (Experiment 1).

Figure 6.7. Gas Phase COS Uptake Profile for *C. thiosulfatophilum* (Experiment 2).

Figure 6.8. Gas Phase H_2S Production (from COS) and Uptake Profile by *C. thiosulfatophilum* (Experiment 1).

Figure 6.9. Gas Phase H_2S Production (from COS) and Uptake Profile by *C. thiosulfatophilum* (Experiment 2).

by the chemical reaction was not immediately consumed by the culture. Consumption did not occur due to the low initial cell density. It should be remembered that H_2S is required for growth. There was no evidence that COS could be directly utilized by *C. thiosulfatophilum*. There is also some evidence to suggest that COS may even be inhibitory to the growth of the bacterium and its ability to utilize COS.

Figure 6.10 shows a plot of the increased total sulfur (COS (g), H_2S (g), total sulfide species) profiles for the data without culture in Figures 6.6-6.9. It is assumed in this plot that all COS in the liquid phase had been converted to H_2S . As is noted in the figure, one experiment showed essentially constant total sulfur, while the other experiment showed that the total sulfur content decreased slightly with time.

Figure 6.11 shows the same profile as in Figure 6.10 now corrected for H_2S disappearance into the stopper by the results of Figure 6.3. As is noted in Figure 6.11, now one set of data shows increasing total sulfur with time, while the other data set shows essentially constant total sulfur with time. Obviously, sulfur is not always lost to the stoppers, thus complicating the correction for H_2S disappearance.

Figure 6.12 utilizes the same data to determine the actual experimental stoichiometry of Equation (1.4). According to Equation (1.4), a slope of 1.0 should result when H_2S production (corrected for disappearance into the stoppers) is plotted against COS consumption. As is noted in Figure 6.12, a slope of 0.94 mol/mol is attained, thereby verifying the stoichiometry of Equation (1.4).

The kinetics of the chemical reaction in Equation (1.4) may also be determined from the data of Figures 6.6-6.9. The rate of disappearance of COS from the gas and liquid phases may be written as:

Figure 6.10. Measured Total Sulfur Profile without Culture for COS
Conversion to H_2S .

Figure 6.11. Measured Total Sulfur Profile without Culture for COS Conversion to H_2S (Corrected for H_2S Disappearance into Rubber Stopper).

Figure 6.12. Determination of Stoichiometry for Equation 1.4.

$$-\frac{d}{dt} \left(n_{\text{COS}}^G + n_{\text{COS}}^L \right) = k_t \cdot c_{\text{COS}}^L \cdot v_L = k_t \cdot \frac{P_{\text{COS}}}{H} \cdot v_L \quad (6.4)$$

Assuming that $k_t \ll K_L a$, $P_{\text{COS}}^L = P_{\text{COS}}^G$ at equilibrium

Therefore,

$$-\frac{d}{dt} \left(\frac{P_{\text{COS}}^G \cdot v_G}{RT} + \frac{P_{\text{COS}}^G \cdot v_L}{H} \right) = k_t \cdot \frac{P_{\text{COS}}^G}{H} \cdot v_L \quad (6.5)$$

Thus, a plot of $-\left[\frac{1}{v_L} \right] \frac{d}{dt} \left[P_{\text{COS}}^G \left[\left(\frac{H}{RT} \right) v_G + v_L \right] \right]$ as a function of P_{COS}^G

should yield a straight line of slope k_t . In this plot,

$$\frac{H}{RT} = \frac{55.10}{0.08206 \cdot (273.15 + 30)} = 2.215 \text{ (dimensionless)}$$

Such a plot is shown in Figure 6.13. As is noted, a rate constant of 0.243 h^{-1} is attained, which compares well with the values of 0.144 h^{-1} at 30°C or 0.339 h^{-1} at 37°C obtained by Thompson *et al.* (62). Smith (63) obtained a value of 0.25 h^{-1} under the exact same experimental conditions as the experiment in Figure 6.13.

6.4 Growth Kinetics for *C. thiosulfatophilum* Using Light as the

Limiting Substrate

In determining growth kinetics for *C. thiosulfatophilum*, 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 and these cell concentration profiles are shown in Figure 6.14 for one of the experiments. As is noted in Figure 6.14, growth increased with increasing

Figure 6.13. Determination of the First Order Reaction Rate Constant for the Reaction: $\text{COS} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{S} + \text{CO}_2$.

Figure 6.14. Cell Concentration Profiles for *C. Thiosulfatophilum* as a Function of Incoming Light Intensity.

light intensity up to a light intensity of about 1000 lux. The initial specific growth rate for each reactor was estimated with increasing light intensity. 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, shown in Figure 6.15.

Using the values from Figure 6.15 and the relationship presented in Equation (5.16), the values for the parameters μ_m and K_I were found to be 0.152 h^{-1} and 351 lux, respectively. This indicates a maximum specific growth rate of 0.152 h^{-1} for the conditions employed and a 50% reduction of the maximum growth rate at a light intensity of 351 lux. In Figure 6.16, the initial specific growth rate is plotted as a function of light intensity. The curve in Figure 6.16 is obtained from the empirical model

$$\mu = \frac{0.152 I_0}{351 + I_0} \quad (6.6)$$

As is noted, a fairly good correlation was obtained between the raw data and the predicted values obtained from the correlation. The specific growth rate asymptotically approached the maximum specific growth rate of 0.152 h^{-1} as predicted by the model.

In comparing the Monod equations for *C. thiosulfatophilum* and *R. rubrum*, it is seen that the maximum specific growth rate, μ_m , for *C. thiosulfatophilum* is 3 times the value for *R. rubrum* (0.152 h^{-1} versus 0.052 h^{-1}). In addition, the value for K_I for *C. thiosulfatophilum* was 2.5 times the value for *R. rubrum* (351 lux vs 140 lux). This latter result means that a 50% reduction in the maximum specific growth rate occurs at a much higher light intensity for *C. thiosulfatophilum* than *R. rubrum*.

Figure 6.15. Determination of Initial Specific Growth Rates for *C. Thiosulfatophilum* at Various Light Intensities.

Figure 6.16. Comparison of Monod Model with Experimental Data for the Light Intensity Study with *C. Thiosulfatophilum*.

A comparison of specific growth rates for the two bacteria as a function of light intensity is shown in Table 6.1. As expected, *C. thiosulfatophilum* consistently showed higher specific growth rates than *R. rubrum* at a given light intensity. This may occur because *C. thiosulfatophilum* utilizes both chemical energy (H_2S , S) and light energy to supply energy for growth, while *R. rubrum* utilizes light energy only as a source of energy for cell growth.

Table 6.1

Calculated Specific Growth Rates for *R. rubrum* and
C. thiosulfatophilum from Monod Equations

Light Intensity (lux)	Specific Growth Rate, μ (h^{-1})	
	<u><i>R. rubrum</i></u>	<u><i>C. thiosulfatophilum</i></u>
50	0.014	0.019
100	0.022	0.034
200	0.031	0.055
300	0.035	0.070
500	0.041	0.089
700	0.043	0.101
1000	0.046	0.113
1500	0.048	0.123

7.0 H_2S CONVERSION BY CHLOROBIIUM THIOSULFATOPHILUM

The anaerobic, photosynthetic bacterium *Chlorobium thiosulfatophilum* is able to convert H_2S and COS in synthesis gas to elemental sulfur. The bacterium grows on CO_2 as its carbon source at $30^\circ C$. In the absence of sulfide, the formed elemental sulfur is converted to sulfate. Thus, bioreactor designs must incorporate sulfur removal as an integral part of the bioprocess.

7.1 Conversion Without Sulfur Removal

In this initial study, *C. thiosulfatophilum* was used to convert H₂S to elemental sulfur in a continuous stirred tank reactor (CSTR) with continuous gas and liquid feed. Sulfur removal was not part of this initial system design, but will be an added feature in future work. The gas used in this study contained 2.52 percent H₂S, 10.00 percent CO₂, 14.99 percent CH₄ (as a tracer) and 72.49 percent He. The liquid flow rate to the 1380 mL reactor volume ranged from 10.8-23.6 mL/min and was a variable in the study. The initial gas flow rate was 11.6 standard mL/min, although it was also changed twice during the study. The temperature was maintained at 31°C and the agitation rate was held at 200 rpm in the Bioflo reactor.

Cell density was monitored by the chlorophyll method and gas composition was monitored by gas-solid chromatography. Light at 2200 lux was supplied using two 40W tungsten light bulbs on the outside of the glass reactor vessel. The light intensity was measured at the front of the reactor vessel, but was not uniform around the reactor.

Figures 7.1 and 7.2 show cell concentration and H₂S conversion measurements in the CSTR with *C. thiosulfatophilum*. Several changes were made during the course of the experiment as is noted on the two figures. Complete H₂S conversion was noted at a gas flow rate of 11 standard mL/min (2.09 hr gas retention time). The gas conversion fell at increased gas flow rates, mainly due, however, to temperature control problems.

Two problems were noted in operating the system. First, the use of external light in the temperature controlled room caused a temperature increase inside the reactor, thus lowering the H₂S conversion and cell concentration. Secondly, a yellowish color was observed in the reactor after

Figure 7.1. Cell Concentration Measurements for H_2S Conversion by *C. thiosulfatophilum* in the CSTR.

Figure 7.2. H₂S Conversion Measurements for H₂S Removal by *C. thiosulfatophilum* in the CSTR.

several hours of operation due to the accumulation of sulfur in the reactor. Both of these problems will be addressed in future studies.

A chiller will be installed in the cooling water line to help control the cooling water temperature. Light intensity will be controlled by installing a variable autotransformer with the lights.

Sulfur will be removed from the system by employing a separatory funnel and a recycle loop between the reactor and the separator. The cell culture with suspended sulfur will be pumped into the separatory funnel. Another pump will recirculate the cell culture back to the reactor. An overflow line on the funnel will take care of the flow imbalances between the pumps. Settled sulfur will be removed from the bottom of the funnel as needed.

7.2 H₂S Conversion in the CSTR With Sulfur Recovery by Gravity Separation

A CSTR system has been designed and operated for the conversion of H₂S (and later COS) to elemental sulfur. The fermenter is a 1.25 L New Brunswick Scientific Bioflo chemostat equipped with agitation and temperature control. The fermenter has been modified to operate under strict anaerobic conditions with both continuous gas and liquid flow. The fermenter is maintained at 29-31°C by housing the system in a constant temperature environment. Light is supplied to the culture using 2 60W bulbs mounted approximately 8 in from the reactor. Liquid effluent from the reactor contains elemental sulfur, which may be separated from the medium by gravity, if desired. A 475 mL separatory flask with a side arm is used for the rapid separation of solid sulfur from the balance of the effluent.

The system was first operated without sulfur recovery at a constant gas flow rate of 20 standard mL/min (62.5 min gas retention time), while varying the dilution rate (inverse liquid retention time), D , from 0.4 to 2.0 days⁻¹.

The gas contained 2.5 percent H_2S , 10 percent CO_2 and 15 percent CH_4 in helium. The use of two 60 W bulbs for light intensity had been shown in preliminary studies to give a high yield of elemental sulfur while minimizing SO_4^{2-} formation. Figure 7.3 shows the results of the liquid dilution rate study with cell concentration, H_2S conversion and liquid sulfur species (S^{2-} , SO_4^{2-} and S^0) concentrations plotted as a function of dilution rate, D . As is noted in the figure, the H_2S conversion was essentially 100 percent for all of the dilution rates studied. The exception to this result is the dip in conversion found while operating at dilution rates of $0.7 - 1.0 \text{ days}^{-1}$. Cell concentration showed typical Monod behavior with dilution rate increasing from 350 mg/L at a 0.4 days^{-1} dilution rate to 450 mg/L at a 1.3 days^{-1} dilution rate before falling gradually as the dilution rate was further increased. The dissolved S^{2-} concentration, corresponding to the accumulated H_2S dissolved in the liquid phase, remained near zero for all dilution rates. Almost all of the sulfur product was present as elemental sulfur (400 - 1000 mg/L), while the sulfate concentration remained at 0 - 100 mg/L. In fact, the SO_4^{2-} concentration was 0 at dilution rates below 1.20 days^{-1} .

A second study was performed in the CSTR without sulfur recovery in which the gas retention time was varied at a constant liquid dilution rate of 1.25 days^{-1} . Figure 7.4 shows the results of this study, where cell concentration, H_2S conversion and sulfur species concentrations are plotted as a function of gas retention time. As is noted in the figure, the H_2S conversion increased almost linearly with gas retention time, ranging from just under 60 percent at a 20 min gas retention time to 100 percent at a 75 min gas retention time. As expected, the cell concentration was not affected by gas retention time, maintaining a constant concentration of 350 mg/L. Similarly, the liquid phase

Figure 7.3. Performance of CSTR without Sulfur Recovery using *C. thiosulfatophilum* at Various Liquid Dilution Rates (Gas Retention Time: 62.5 min).

Figure 7.4. Performance of CSTR without Sulfur Recovery using *C. thiosulfatophilum* at Various Gas Retention Times (Liquid Dilution Rate: 1.25 days⁻¹).

S^{2-} concentration stayed very low at 0-50 mg/L, the SO_4^{2-} concentration remained low at 0-60 mg/L and the elemental sulfur concentration ranged from 600-800 mg/L.

Finally, a third study was performed in the CSTR, but this time with the sulfur recovery device. As is shown in Figure 7.5, cell concentration, H_2S conversion and sulfur species concentrations are plotted as a function of gas retention time for a liquid dilution rate of 1.25 days^{-1} . In this study, the H_2S conversion ranged from 60-100 percent, reaching 100 percent conversion at a gas retention time of 45 min. Thus, conversions were higher at a given retention time with sulfur recovery. The cell concentration was constant at 350 mg/L, a similar level as in the previous study. The S^{2-} and SO_4^{2-} concentrations were again quite low and the sulfur concentration in the effluent (not the total sulfur produced) ranged from 200-350 mg/L. Thus, sulfur recovery significantly improves H_2S conversion and thereby sulfur production.

Future studies with the reactor and sulfur separator will include cell recycle to increase the concentration of cells inside the reactor. A hollow fiber membrane system compatible with the sulfur species is being sought. It is also believed that increased pressure will improve the performance of the *C. thiosulfatophilum* system.

Additional experiments were carried out in the stirred tank reactor with the sulfur settler at varying light intensities and gas flow rates to observe maximum H_2S removal. A schematic of the equipment set-up is shown in Figure 7.6. Results from the study are summarized in Table 7.1. As is noted, the gas retention time for complete H_2S removal was minimized at 20 min when using two 200 W light sources. Also, it is observed that the S^{2-} concentration

Figure 7.5. Performance of CSTR with Sulfur Recovery using *C. thiosulfatophilum* at Various Gas Retention Times (Liquid Dilution Rate: 1.25 days⁻¹).

Figure 7.6. Schematic Diagram of the Experimental Set-up for H_2S Removal and Sulfur Production

Table 7.1

Performance of the CSTR with Sulfur Recovery Using *C. thiosulfatophilum* at Different Light Intensities
(Liquid Dilution Rate: 1.25 days⁻¹)

Light	Gas Retention Time, min	Cell Conc. mg/L	% H ₂ S Conversion	S ²⁻ mg/L	SO ₄ ²⁻ mg/L	S° mg/L
2X60W	43	339	99.2	0	1	292
2X75W	43	409	99.9	1	182	327
2X75W	36	433	99.8	2	240	327
2X75W	31	460	97.8	0	183	402
2X100W	31	463	99.5	2	214	449
2X100W	27	441	95.5	2	135	516
2X150W	27	391	99.5	2	117	496
2X150W	24	408	98.5	2	89	572
2X150W	22	412	96.5	2	62	501
2X200W	22	422	99.9	2	100	539
2X200W	20	453	99.9	2	115	440

remained essentially at zero. It was also possible to maintain the SO_4^{2-} concentration at levels below 100 mg/L. The sulfur concentration in the liquid at this gas retention time remained between 500-600 mg/L. Higher light intensities are expected to permit higher H_2S removal rates. However, because of physical limitations in providing sufficient reactor cooling, experiments were not carried out at higher light intensities.

After determining the optimum liquid dilution rate, gas flow rate, and light intensity to maximize H_2S removal in the system, an experiment was performed for an extended time period at these optimum conditions (1.25 days-1 liquid dilution rate, 19.6 min gas retention time, and two 200W light sources) to establish the stability of the process. The results of this experiment over a period of 393 hrs are shown in Figure 7.7. It can be seen from this figure that the reactor performance was quite stable during this period. However, the settler was not efficient in removing all of the elemental sulfur particles present in the reactor effluent. The effluent contained 425-550 mg/L elemental sulfur. The H_2S removal rate during this operation was 57 micromol/min/L which is about 1.5 times the maximum value reported by Kim and Chang.

7.3 H_2S Conversion with Cell Recycle and Sulfur Recovery

The continuous stirred tank reactor system was modified to include both sulfur recovery and cell recycle using ceramic tubular membranes (Membralox ceramic membranes, U.S. Filter, Warrendale, PA; membrane area: 55 cm^2). Ceramic membranes were chosen due to their resistance to H_2S and other sulfur species. The sulfur recovery filter (5.0 micron pore size) was first installed in front of the cell recycle filter (0.2 micron pore size) due to the larger size of the sulfur particles in comparison to cells. But however,

Figure 7.7. Long Term Behavior of the CSTR with Sulfur Recovery using *C. thiosulfatophilum* (Liquid Dilution Rate: 1.25 days⁻¹; Gas Retention Time: 19.6 min)

the sulfur recovery filter was found to block after 15-30 minutes of operation. It is possible to regenerate the filter by backflushing with water. The frequent backflushing disturbs the reactor system and makes the process impractical.

The continuous stirred tank reactor system was also operated along with a sulfur settler and a polysulfone hollow fiber membrane module (0.45 micron microfiltration cartridge, 465 cm² membrane area, fiber lumen i.d. 0.75 mm, A/G Technology, Needham, MA) for cell recycle. Results for four different cases (denoted as Case I to Case IV) of operation are presented in Table 7.2. In case I, the reactor was operated with the settler only (recycle rate = 70 mL/min) and no cell recycle membrane was installed. In case II, the settler and the hollow fiber module were in the same loop (recycle rate = 450 mL/min) and the liquid discharge from the reactor was taken completely as the membrane permeate. This causes gradual accumulation of cells in the reactor and the reactor performance in terms of H₂S conversion drops. In case III, 50 percent of the reactor discharge was taken as bleed and 50 percent as membrane permeate. This caused improvement in reactor performance. However, keeping the settler and the filter in the same loop with a high recycle rate causes a higher concentration of suspended S⁰ in the reactor. In case IV, the settling of sulfur was improved by separating the cell recycle (recycle rate = 450 mL/min) and the sulfur settling (recycle rate = 70 mL/min) loops. Fifty percent of the reactor discharge was as bleed. This change resulted in a significant improvement in H₂S conversion with a lower gas retention time.

8.0 EFFECTS OF CELL CONCENTRATION ON PHYSICAL PROPERTIES

As a first step in scaling up the bioprocesses for sulfur gas removal and shift conversion, the effects of cell concentration on density, viscosity and

Table 7.2

Performance of the CSTR with the Sulfur Settler and Cell
Recycle Using *C. thiosulfatophilum*

Time h,	Light Intensity	D, day ⁻¹	Gas RT Min	X, mg/L	%H ₂ S conv.	SO ₄ ²⁻ mg/L	S° mg/L	S ²⁻ mg/L
Case I								
96	2X60W	1.12	36	605	99.6	47	655	1
Case II								
24	2X60W	1.12	36	802	98.7	58	1275	1
48	2X60W	1.12	36	1595	99.9	107	1762	1
72	2X60W	1.12	31	1782	99.7	108	1488	1
96	2X60W	1.12	27	2125	92.6	42	2733	1
120	2X60W	1.12	27	2554	94.0	25	3255	1
144	2X60W	1.30	27	2799	79.1	4	2294	1
168	2X60W	1.30	27	3244	70.0	4		1
192	2X75W	1.30	27	3300	88.8	8	1795	1
Case III								
24	2X75W	1.30	27	3993	80.4	1	2016	1
48	2X75W	1.30	27	3190	77.0	4		1
72	2X75W	1.30	27	2854	79.0	0.5	3046	1
96	2X75W	1.30	27	2324	79.3	4	2381	1
168	2X75W	1.30	27	1911	94.0	0.5	2317	1
192	2X75W	1.30	27	1887	96.1	0.5	1684	1
Case IV								
24	2X75W	1.30	27	2036	99.9	6	757	1
48	2X75W	1.30	24	2149	99.8	76	591	1
144	2X75W	1.30	20	1778	98.1	5	876	2
168	2X75W	1.30	20	1754	98.1	7	852	7

Case I : Reactor with settler (recycle rate = 70 mL/min)

Case II : Reactor with cell recycle and settler in the same loop (recycle rate = 450 mL/min).

Case III: Reactor with cell recycle and settler in the same loop (recycle rate = 450 mL/min) and 50% bleed.

Case IV : Reactor with cell recycle (recycle rate = 450 mL/min) and settler (recycle rate = 70 mL/min) in different loop and 50% bleed.

surface tension were determined. Logarithmic functions for density as a function of inverse absolute temperature were found for water, *R. rubrum* cells in medium and *C. thiosulfatophilum* cells in medium, although a different relationship was found for each of the three systems. A single logarithmic function was found for viscosity as a function of inverse absolute temperature for the three systems. No simple relationship was found for surface tension as a function of temperature.

Little is known about the effects of bacterial cells and medium on physical properties. In fact, various literature references show a variety of effects on physical properties, including both decreases and increases with cell concentration. In order to accurately scale-up bioprocesses, information must be gathered on the effects of bacterial type, cell and medium concentration, and temperature on physical properties. Studies were thus carried out using both *Rhodospirillum rubrum* and *Chlorobium thiosulfatophilum* at various cell concentrations in measuring the effects of temperature on density, viscosity and surface tension.

R. rubrum and *C. thiosulfatophilum* were grown in continuous culture to various cell concentrations by varying the light intensity to the reactor. A New Brunswick Bioflo II-C fermenter was used as the CSTR in each bacterial study. The media for *R. rubrum* and *C. thiosulfatophilum* were as described previously. The operating conditions for the *R. rubrum* system were as follows:

Liquid Flow Rate:	7.08, 3.54 mL/hr
Gas Flow Rate:	3.95 Standard mL/min
Gas Composition:	9.95% CO ₂ , 20.04% H ₂ , 15.27% Ar (tracer), 54.75% CO
Reactor Temperature:	30°C

The operating conditions for the *C. thiosulfatophilum* system were as follows:

Liquid Flow Rate: 16.2 mL/hr
Gas Flow Rate: 8.4 mL/min
Gas Composition: 2.52% H₂S, 10.00% CO₂,
14.99% CH₄ (tracer), 72.49% He
Reactor Temperature: 31°C

Density (specific gravity) was measured using the ASTM standard test for specific gravity of creosote fractions and residues (D-369-84) (64), which is identical in substance to the Standard Method of Determination of the Specific Gravity of Distillation Fractions. Viscosity and surface tension were measured using the technique presented by Findlay (65). An Ostwald viscometer was used for viscosity measurement and a 250 mm borosilicate glass capillary tube was used to measure surface tension.

The effects of cell concentration for both *R. rubrum* and *C. thiosulfatophilum* on density at 25°C is shown in Figure 8.1. The available cell concentration range for *R. rubrum* was 400-1800 mg/L, while the available cell concentration range for *C. thiosulfatophilum* was only 0-700 mg/L. *R. rubrum* obviously is able to grow to higher cell densities in the CSTR under similar conditions. This phenomenon does not necessarily mean that the global rate of conversion is greater for *R. rubrum*. As is noted in the figure, the density for the *R. rubrum* system was less than the density for *C. thiosulfatophilum*. The density for *R. rubrum* ranged from about 1.000 - 1.001 g/mL. The density for the *C. thiosulfatophilum* system ranged from 1.002-1.003 g/mL, increasing gently with cell concentration.

Figure 8.1. The Effect of Cell Concentration on Density for *R. rubrum* and *C. thiosulfatophilum*.

As is shown in Figure 8.2, the density of water, *R. rubrum* and *C. thiosulfatophilum* each decreased with increasing temperature. The density of *R. rubrum* was the highest (at both cell concentrations shown), followed by *C. thiosulfatophilum*, followed by water. The density of each of these systems may be fit by a logarithmic function with inverse absolute temperature (see Figure 8.3), using the equation:

$$\ln \rho = \ln \alpha + \frac{\beta}{T} \quad (8.1)$$

or
$$\rho = \alpha e^{\beta/T} \quad (8.2)$$

Constants for Equations (8.1) and (8.2) for water and the bacterial systems are shown in Table 8.1.

The effects of cell concentration on viscosity at 25°C is shown in Figure 8.4. As was similarly noted for density, the viscosity of the *R. rubrum* system was generally greater than for the *C. thiosulfatophilum* system, probably since *R. rubrum* grows to higher cell concentrations. In general, the viscosity for the two systems ranged from 0.895-0.925 cp over the cell concentration range. Viscosity data at various temperatures were again fit to an equation of the form:

$$\ln \mu = \ln \gamma + \frac{\delta}{T} \quad (8.3)$$

or
$$\mu = \gamma e^{\delta/T} \quad (8.4)$$

A plot of this data is shown in Figure 8.5, and constants for these equations are shown in Table 8.2. As is noted in Figure 8.5, a single straight line can accurately describe the viscosity relation of Equation (8.3) for the systems studied.

Figure 8.2. The Effect of Temperature on Density of Water, *R. rubrum* and *C. thiosulfatophilum*.

Figure 8.3. Test of Logarithmic Relationship Between Density and Inverse Absolute Temperature.

Table 8.1

Relating Density to Temperature for R. rubrum
and C. thiosulfatophilum

$$\ln \rho = \ln \alpha + \beta/T$$

<u>System</u>	<u>α</u>	<u>β</u>
Water (literature)	0.9113	26.786
<u>R. rubrum</u> (950 mg/L)	0.9153	27.309
<u>R. rubrum</u> (1750 mg/L)	0.9582	13.395
<u>C. thiosulfatophilum</u> (525 mg/L)	0.9059	29.720

temperature range: 22-37°C

Figure 8.4. The Effect of Cell Concentration on Viscosity for *R. rubrum* and *C. thiosulfatophilum*.

Figure 8.5. Test of Logarithmic Relationship Between Viscosity and Inverse Absolute Temperature.

Table 8.2

Relating Viscosity to Temperature for R. rubrum
and C. thiosulfatophilum

$$\ln \mu = \ln \gamma + \frac{\delta}{T}$$

<u>System</u>	<u>γ</u>	<u>δ</u>
Water (literature)	1.19 E-03	1971.73
Water (measured)	3.53 E-03	1647.15
<u>R. rubrum</u> (950 mg/L)	1.52 E-03	1907.56
<u>R. rubrum</u> (1750 mg/L)	2.82 E-03	1720.94
<u>C. thiosulfatophilum</u> '525 mg/L)	1.36 E-03	1936.38

The effects of cell concentration at 25°C and temperature on surface tension for the *R. rubrum* and *C. thiosulfatophilum* systems are shown in Figures 8.6 and 8.7. The surface tension for *R. rubrum* ranged from 68-71 dyne/cm over the cell concentration range studied, and the surface tension ranged from 70-72 dyne/cm over the cell concentration range studied (see Figure 8.6). The surface tension decreased with temperature for all systems (see Figure 8.7), with water having the highest surface tension, followed by two bacterial systems.

9.0 EFFECTS OF O₂ AND H₂S ON THE PERFORMANCE OF R. RUBRUM

Batch experiments were carried out in serum stoppered bottles to quantitatively determine the effects of oxygen and H₂S on CO-uptake by *Rhodospirillum rubrum* in performing the water gas shift reaction. Small quantities of oxygen may be present in syngas especially during process start-up and during maintenance. Low concentrations of H₂S (1-2%) have been observed in most syngas streams. The experimental study showed that *R. rubrum* can tolerate oxygen and, in fact, consumes it prior to CO-utilization. H₂S is not consumed and slows CO-uptake at increased (0.013 atm) partial pressure. Models were developed for both systems.

Syngas is known to contain approximately 1 percent H₂S, along with CO, CO₂, H₂ and CH₄. Similarly, the syngas may become contaminated with oxygen, particularly during reactor start-up and during maintenance. Previous studies with the water-gas shift bacterium *Rhodospirillum rubrum* have shown that the bacterium is tolerant of small quantities of oxygen, but the effects of oxygen on CO-consumption are unknown. Similarly, *R. rubrum* is known to be tolerant of H₂S, with high concentrations of H₂S negatively affecting CO-uptake. Batch experiments were thus carried out to determine the effects of H₂S and O₂ on

Figure 8.6. The Effect of Cell Concentration on Surface Tension for *R. rubrum* and *C. thiosulfatophilum*.

Figure 8.7. The Effect of Temperature on Surface Tension for *R. rubrum* and *C. thiosulfatophilum*

CO-uptake by *R. rubrum*. The results of these experiments were quantified by using Monod equations modified by adding terms for CO, H₂S and O₂ inhibition. The techniques used in determining kinetic expressions previously shown for other gas-phase substrate bacterial systems including *R. rubrum* were utilized.

9.1 CO Uptake in the Absence of H₂S and O₂

Experiments were first performed without H₂S and O₂ in the gas phase to verify the Monod equation for CO-uptake by *R. rubrum*. Figures 9.1-9.3 show the treatment of the experimental data in obtaining the kinetic equation. CO partial pressures ranging from 0.68-1.33 atm were utilized. Figure 9.1 notes that a mass transfer coefficient, K_La/H, of 8.4 mmol/L·hr atm was obtained for the batch serum bottle reactors.

The modified Monod model with CO inhibition (Andrew's equation)

$$q = \frac{q_m P_{CO}^L}{K_m + P_{CO}^L + \left(P_{CO}^L\right)^2 / W} \quad (9.1)$$

was rearranged to yield

$$\frac{P_{CO}^L}{q} = \frac{K_m}{q_m} + \frac{1}{q_m} \left(P_{CO}^L\right) + \frac{1}{W q_m} \left(P_{CO}^L\right)^2 \quad (9.2)$$

which is a second-order equation in P_{CO}^L .

A plot of the experimental data, rearranged to fit Equation (9.2), is shown in Figure 9.3. The modified Monod equation for CO-uptake by *R. rubrum* without the presence of CO is thus:

$$q = \frac{0.018 P_{CO}^L}{0.2 + P_{CO}^L + \left(P_{CO}^L\right)^2 / 0.82}, \text{ mol/gcell}\cdot\text{hr} \quad (9.3)$$

Figure 9.1. Determination of $K_L a/H$ for the Batch Bottle Experiments.

Figure 9.2. Specific CO-uptake Rate at Different P_{CO_2} (no H_2S or O_2 present).

Figure 9.3. Illustration of Modified Monod Expression for CO Uptake (no H_2S or O_2 present).

This equation agrees quite well with the previously determined kinetic expression.

9.2 H₂S Effects

Experiments were next carried out in the presence of H₂S. Initial H₂S partial pressures ranging from 0 - 0.013 atm were studied. Figures 9.4-9.9 show representative cell, CO and H₂ concentration profiles for representative initial CO partial pressures. As is noted in Figures 9.4 and 9.5, the cell concentration was unaffected by H₂S except at the highest initial H₂S partial pressure. CO-uptake and H₂ production were inhibited by H₂S except at the lowest initial H₂S partial pressure.

As is noted in Figure 9.10, the yield of H₂ from CO, $Y_{H_2/CO}$, was constant with initial H₂S partial pressure. The yield was 0.84 mol/mol, which is less than the theoretical yield of 1.0, but typical of the yields obtained without H₂S present. Figures 9.11 and 9.12 show that H₂S was not consumed by the bacterium, with the concentration remaining at the initial H₂S partial pressure throughout the course of the experiment.

The experimental data of Figures 9.4-9.9 were modeled using the procedure outlined previously. An equation of the form

$$q = \frac{q_m P_{CO}^L}{K_S + P_{CO}^L + \left(P_{CO}^L\right)^2/W} \left[1 - \frac{P_{H_2S}}{P_{H_2S, \max}}\right]^n \quad (9.4)$$

was used in modeling the effects of H₂S partial pressure on CO-uptake. The constants q_m , K_S and W were shown previously in Equation (9.3) and the constants n and $P_{H_2S, \max}$ were to be determined. Figure 9.13 illustrates the procedure for determining n and $P_{H_2S, \max}$. Table 9.1 shows the results of the

Figure 9.4. Effects of H_2S on cell density [$P_{\text{CO}_2} = 0.8 \text{ atm}$].

Figure 9.5. Effects of H_2S on cell density [$P_{\text{CO}_2} = 1.0 \text{ atm}$].

Figure 9.6. Effects of H_2S on CO -uptake by *R. rubrum* [$P_{\text{CO}_2} = 0.8$ atm].

Figure 9.7. Effects of H_2S on CO-uptake by *R. rubrum* [$P_{\text{co},i} = 1.0 \text{ atm}$].

Figure 9.8. Effects of H_2S on H_2 Production [$P_{CO_2} = 0.8 \text{ atm}$].

Figure 9.9. Effects of H_2S on H_2 Production [$P_{\text{CO}_2} = 1.0 \text{ atm}$].

Figure 9.10. Estimation of $Y_{(H_2/CO)}$ in the presence of H_2S .

Figure 9.11. Moles of H_2S as a Function of Time [$P_{CO_2} = 0.8$ atm]

Figure 9.12. Moles of H_2S as a Function of Time [$P_{\text{CO}_2} = 1.33$ atm]

Figure 9.13. Modeling Scheme.

Table 9.1

Values of n and $P_{\text{H}_2\text{S},\text{max}}$ for the Modified Andrews Equation
at Different H_2S Concentrations

$P_{\text{H}_2\text{S},i}$ (atm)	n	$P_{\text{H}_2\text{S},\text{max}}$ (atm)	
0.0068	1	0.025	$P_{\text{CO},i} < 1.0 \text{ atm}$
		0.016	$P_{\text{CO},i} > 1.0 \text{ atm}$
0.0086	1	0.025	$P_{\text{CO},i} < 1.0 \text{ atm}$
0.012	1	0.025	$P_{\text{CO},i} < 1.0 \text{ atm}$
		0.020	$P_{\text{CO},i} > 1.0 \text{ atm}$
0.018	1	0.030	$P_{\text{CO},i} > 1.0 \text{ atm}$

modeling, and Figures 9.14 and 9.15 show comparisons of experimental data with the results of the model.

9.3 O₂ Effects

Experiments with *R. rubrum* were similarly carried out in the presence of O₂ partial pressures ranging from 0-0.05 atm. Figures 9.16-9.21 show representative cell, CO and H₂ concentration profiles for representative initial CO partial pressures. As is noted in Figures 9.16 and 9.17, the maximum cell concentrations at each initial CO partial pressure were essentially unaffected by the presence of oxygen. The presence of oxygen, however, strongly affected the length of the lag phases as noted in Figures 9.18-9.21. Oxygen affected the rates of CO uptake and H₂ production far less than H₂S, as noted by the constant slopes in the figures after the lag phases.

The yield of H₂ from CO, $Y_{H_2/CO}$, was constant at 0.82 mol/mol, regardless of initial O₂ concentration (see Figure 9.22). This value is consistent with the value of 0.84 mol/mol obtained in the H₂S study. Contrary to H₂S, Figures 9.23 and 9.24 show that O₂ was indeed consumed by *R. rubrum* in the experimental study. The O₂ consumption time matched the length of the lag phase for CO consumption, indicating that CO conversion and O₂ consumption cannot occur concurrently.

Table 9.2 presents information on the lag phases for the experiments. Shown in the tables are the initial CO partial pressure, the initial O₂ partial pressure, the length of the lag phase, and the cell concentrations at the beginning and end of the lag phases. The length of the lag phase was proportional to the initial oxygen partial pressure. Also, growth occurred during oxygen consumption.

Figure 9.14. Experimental q Compared to Calculated q From the Modified Andrews Equation.

Figure 9.15. Experimental q Compared to Calculated q From the Modified Andrews Equation.

Figure 9.16. Effects of O_2 on Cell Density [$P_{CO_2} = 0.8$ atm].

Figure 9.17. Effects of O_2 on Cell Density [$P_{CO_2} = 1.15 \text{ atm}$].

Figure 9.18. Effects of O_2 on CO Uptake by *R. rubrum* [$P_{CO_2} = 0.8 \text{ atm}$].

Figure 9.19. Effects of O_2 on CO Uptake by *R. rubrum* [$P_{CO_2} = 1.15 \text{ atm}$].

Figure 9.20. Effects of O_2 on H_2 Production [$P_{\text{CO}_2} = 0.8 \text{ atm}$].

Figure 9.21. Effects of O_2 on H_2 Production [$P_{CO_2} = 1.15 \text{ atm}$].

Figure 9.22. Estimation of $Y_{(\text{H}_2/\text{CO})}$ in the presence of Oxygen.

Figure 9.23. Moles of O_2 as a Function of Time [$P_{O_2} = 0.8$ atm].

Figure 9.24. Moles of O_2 as a Function of Time [$P_{\text{CO}_2} = 1.0$ atm].

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Table 9.2

Lag Phase for CO Consumption at Different $P_{CO,i}$ and $P_{O_2,i}$

$P_{CO,i}$ (atm)	$P_{O_2,i}$ (atm)	$t_{LAG}(h)$	$X_{LAG,i}(mg/L)$	$X_{LAG,i}(mg/L)$
0.8	0.0087	46	71.62	473
0.8	0.027	113	71.62	658
0.8	0.047	146	71.62	726
1.0	0.0087	50	72.60	495
1.0	0.027	117	72.60	744
1.15	0.0087	48	66.50	415
1.15	0.028	128	66.50	944
1.15	0.048	169	66.50	1063

The length of the lag phase was modeled as a function of the initial O₂ partial pressures by the equation:

$$t_{\text{lag}} = a P_{\text{O}_2, i} + b \left(P_{\text{O}_2, i} \right)^2 \quad (9.5)$$

where t_{lag} is in h and $P_{\text{O}_2, i}$ is in atm. The resulting equation is:

$$t_{\text{lag}} = 5699.8 P_{\text{O}_2, i} - 49095.7 \left(P_{\text{O}_2, i} \right)^2 \quad (9.6)$$

The experimental data of Figures 9.16-9.21 were also modeled using the procedure described previously. An equation of the form

$$q = \frac{q_m P_{\text{CO}}^L}{k_S + P_{\text{CO}}^L + \left(P_{\text{CO}}^L \right)^2 / W} \left[1 - \frac{P_{\text{O}_2}}{P_{\text{O}_2, \text{max}}} \right]^n \quad (9.7)$$

was used in modeling the data with q_m , K_S and W again determined previously. Table 9.3 shows the results of the modeling and Figures 9.26 and 9.27 show comparisons of the experimental data with the results of the model

10.0 SCALE-UP STUDIES

10.1 Studies in the 1.25 L CSTR

The effects of important operating parameters on culture performance by *R. rubrum* in the CSTR (no cell recycle) were assessed. A 1.25 L Bioflo IIC reactor was used in the initial studies. The reactor was equipped with continuous gas and liquid flow; temperature, pH and agitation rate control; and was lighted with two 60 W lamps placed approximately 15 cm from the reactor. Light intensity was controlled using a powerstat. The reactor volume was 1.6 L and the liquid culture volume was 1.25 L.

Figure 9.25. Lag periods for CO Consumption by *R. rubrum* in the Presence of O_2 .

Table 9.3

Values of n and $P_{O_2, \max}$ for the Modified Andrews Equation
at Different O_2 Concentrations

$P_{O_2, i}$ (atm)	n	$P_{O_2, i}$ (atm)		
0.0087	2	0.017	$P_{CO, i} < 1.0 \text{ atm}$	
		0.013	$P_{CO, i} > 1.0 \text{ atm}$	
0.028	2	0.044	$P_{CO, i} < 1.0 \text{ atm}$	
		0.035	$P_{CO, i} > 1.0 \text{ atm}$	
0.048	2	0.067	$P_{CO, i} < 1.0 \text{ atm}$	
		0.060	$P_{CO, i} > 1.0 \text{ atm}$

Figure 9.26. Experimental q Compared to Calculated q From the Modified Andrews Equation.

Figure 9.27. Experimental q Compared to Calculated q From the Modified Andrews Equation.

10.1.1 Agitation Rate Study

The effects of agitation rate on culture performance was studied first. Based upon earlier successful experimental studies, the gas flow rate was set at 7 SCCM (2.98 hr gas retention time), the liquid flow rate was 450 mL/day (0.015 hr^{-1} dilution rate), the temperature was 30°C and the light intensity to the reactor was 1150 lux per bulb (2300 lux total). The agitation rate was varied from 200-800 rpm.

The effects of agitation rate on culture performance are shown in Figures 10.1-10.3. As is noted, cell concentration increased nearly two-fold over the course of the agitation rate study, reaching a maximum of about 1200 mg/L at an agitation rate of 700 rpm (see Figure 10.1). The CO conversion increased sharply with agitation rate, reaching a maximum conversion of about 75 percent at 600 rpm (see Figure 10.2). The H_2 production rate also reached a maximum of about $100 \mu\text{mol}/\text{min}$ at 600-700 rpm (see Figure 10.3). An agitation rate of 600 rpm was chosen for further study.

10.1.2 Temperature Study

The effects of temperature on culture performance was studied at a gas flow rate of 5.2 SCCM (4.0 hr gas retention time), a liquid flow rate of 440 mL/day (0.015 hr^{-1} dilution rate), a temperature of 30°C , an agitation rate of 600 rpm and a light intensity of 1050 lux per bulb (2100 lux total). The temperature was varied from 25 to 36°C .

The effects of temperature on culture performance are shown on Figures 10.4-10.6. Cell concentration was a maximum at 25°C (see Figure 10.4). However, the CO conversion reached a maximum of 80 percent over a temperature range of $30\text{-}34^\circ\text{C}$ (see Figure 10.5) and the H_2 production reached a maximum at 32°C (see Figure 10.6). Thus a temperature of 32°C was chosen for further study.

Figure 10.1. The Effects of Agitation Rate on Cell Concentration using *R. rubrum* in the CSTR

Figure 10.2. The Effects of Agitation Rate on CO Conversion using *R. rubrum* in the CSTR

Figure 10.3. The Effects of Agitation Rate on H₂ Production using *R. rubrum* in the CSTR

Figure 10.4. The Effects of Temperature on Cell Concentration using *R. rubrum* in the CSTR

Figure 10.5. The Effects of Temperature on CO Conversion using *R. rubrum* in the CSTR

Figure 10.6. The Effects of Temperature on H₂ Production using *R. rubrum* in the CSTR

10.1.3 Liquid Flow Rate Study

The effects of liquid flow rate (liquid dilution rate) on culture performance was studied at a gas flow rate of 7.8 SCCM (2.67 hr gas retention time), a temperature of 32°C, an agitation rate of 600 rpm and a light intensity of 1050 lux per bulb. The liquid flow rate was varied from 500-2200 mL/day (0.017-0.073 hr⁻¹ dilution rate).

The effects of liquid flow rate on culture performance are shown in Figures 10.7-10.9. As expected, cell concentration was significantly affected by liquid flow rate, reaching a maximum of 1200 mg/L at a liquid flow rate of 850 mL/day (0.028 hr⁻¹ dilution rate) (see Figure 10.7). Liquid flow rate showed little effect on CO conversion (Figure 10.8) or H₂ production (Figure 10.9). A liquid flow rate of 850 mL/day was chosen for further study.

10.1.4 Light Intensity Study

The effects of light intensity on culture performance was studied at a gas flow rate of 9.1 SCCM (2.29 hr gas retention time), a liquid flow rate of 850 mL/day, a temperature of 32°C and an agitation rate of 600 rpm. The total light intensity, supplied by two 60 W bulbs, ranged from 500-5000 lux.

The effects of light intensity on culture performance are shown in Figures 10.10-10.12. As is noted in Figure 10.10, light intensity had little to no effect on cell concentration, with the cell density controlled by the liquid medium concentration and flow rate. Although there is no explanation offered, light intensity had some effect on CO conversion (Figure 10.11) and H₂ production (Figure 10.12). For economical reasons, a low light intensity range of 1050-1150 lux was chosen for further study.

Figure 10.7. The Effects of Liquid Flow Rate on Cell Concentration using *R. rubrum* in the CSTR

Figure 10.8. The Effects of Liquid Flow Rate on CO Conversion using *R. rubrum* in the CSTR

Figure 10.9. The Effects of Liquid Flow Rate on H₂ Production using *R. rubrum* in the CSTR

Figure 10.10. The Effects of Light Intensity on Cell Concentration using *R. rubrum* in the CSTR

Figure 10.11. The Effects of Light Intensity on CO Conversion using *R. rubrum* in the CSTR

Figure 10.12. The Effects of Light Intensity on H₂ Production using *R. rubrum* in the CSTR

10.1.5 Gas Flow Rate Study

The effects of gas flow rate (gas retention time) on culture performance was studied at a liquid flow rate of 1000 mL/day (0.033 hr^{-1} liquid dilution rate), a temperature of 32°C , an agitation rate of 600 rpm and a light intensity per bulb of 1150 lux (2300 lux, total). The gas flow rate was varied from 7-20 SCCM (1.04-2.98 hr gas retention time).

The effects of gas flow rate on culture performance are shown in Figures 10.13 and 10.14. As is noted in Figure 10.13, gas flow rate had no effect on cell concentration. Gas flow rate significantly affected CO conversion (see Figure 10.14) decreasing nearly linearly (with scatter) with gas flow rate. The conversion ranged from 80 percent to 30 percent throughout the course of the study. H_2 production increased and then levelled off with increasing flow rate. A gas flow rate of 11 SCCM (1.89 hr gas retention time) may be used to achieve a 70 percent CO conversion.

10.2 Scale-Up to 2.5, 5.0 and 14.0 L CSTRs

A scale-up of the process for hydrogen production using *R. rubrum* is necessary to evaluate the economic feasibility against the conventional process. Most scale increases in industrial practice are conducted by keeping selected parameters constant with the change in scale. The most common parameters used are: (1) equal power/volume ratio; (2) equal K_{La} ; (3) equal shear; (4) equal mixing; and (5) combination of K_{La} , shear and mixing. Most methods assume geometric similarity, though in practice minor adjustments are made to keep all relevant parameters within desired ranges.

The objective of this scale-up study in the stirred tank fermenters was to verify the conditions for bacterial cell growth and the desired level of CO conversion to produce hydrogen developed in a 1.25 L bench-scale fermenter.

Figure 10.13. The Effects of Gas Flow Rate on Cell Concentration using *R. rubrum* in the CSTR

Figure 10.14. The Effects of Gas Flow Rate on CO Conversion using *R. rubrum* in the CSTR

The method of equal power/volume ratio has been used for this fermentation system as a primary scale-up parameter. The mass transfer coefficients were estimated for each reactor under the condition of ~70 percent CO conversion.

The continuous stirred tank reactors (CSTRs) used for the experiments were two New Brunswick Scientific (Edison, NJ) BioFlo IIc fermenters (2.5 L and 5.0 L) equipped with temperature, pH and agitation control and a Virtis model 43-100 fermenter (Virtis Co., Gardiner, NY). The agitation system in each of the reactors consisted of two disk type turbine impellers. The liquid working volumes in the reactors were 2.5, 5.0 and 14.0 L, respectively, and the overhead gas volumes were 0.8, 1.6 and 6.0 L, respectively. Illumination necessary for growth was supplied by two tungsten lights (60 W lamps for BioFlo reactors and 100 W lamps for the Virtis fermenter). The incident light intensities on the outer wall of the reactors were measured with a LX-101 digital lux meter (Cole-Parmer, Chicago, IL), and were 2500, 5000 and 9000 lux for the three reactors, respectively. Experiments were carried out at pH 7 and at a temperature of 32°C. The feed gas was a mixture of H₂, Ar, CO, CO₂ (20/15/55/10% v/v) and was continuously fed to the reactors. Liquid medium was also supplied on a continuous basis.

Typically experiments were started with constant agitation rate, gas flow rate, light illumination and liquid flow rate. The cell concentration, hydrogen production and percentage of carbon monoxide conversion were monitored and several data were collected to establish each steady state condition.

Experiments were performed to establish the scale-up procedure at optimum operating conditions for cell growth, percent carbon monoxide conversion and hydrogen production obtained from the 1.25 L reactor. Light illumination was

increased linearly with the liquid working volume of the reactors. The agitation rate was determined by the equal power/volume rule. Once each reactor was operated under the optimum condition determined previously, experiments were performed to investigate carbon monoxide conversion and hydrogen production at different gas flow rates under the operating condition. A time duration study was also performed to show the stability of the 2.5 L and 14.0 L reactors at the optimum operating condition.

A concentrated medium (seven times concentrated) along with sterile deionized water was used for the 14.0 L Virtis fermenter. The filtered water was used to maintain the liquid dilution rate. A sterile filter with a porosity of 0.2 μm (Gelman, Ann Arbor, Michigan) was used for the filtration of deionized water. Prior to filtration the water was purged with helium for 4 hours in order to remove any dissolved gas. A schematic of the equipment set-up for this reactor is shown in Figure 10.15.

The equal power/volume rule was applied for the scale-up of the geometrically similar continuous stirred tank reactors (CSTR) for hydrogen production using *R. rubrum*. Table 10.1 presents the geometric dimensions of the reactors used for this scale-up study. In the first stage of the scale-up a 2.5 L CSTR was used. The operating conditions were chosen based on the performance of a 1.25 L CSTR. The liquid dilution rate was chosen by keeping the liquid retention time constant. The light requirement was in the range of 500 - 1100 lux for the 1.25 L reactor, therefore 2500 lux light energy was sufficient for the 2.5 L reactor. Since the liquid working volume was doubled it was expected that the gas flow rate may also be doubled for the same level of CO conversion. However, the gas flow rate was increased by 25 percent for the same level of CO conversion in the 2.5 L CSTR. Figure 10.16 shows results

Figure 10.15. Schematic Diagram of the Experimental Set-up for the 14.0 L Fermenter

Table 10.1

Performance of the CSTR with Sulfur Recovery using *C. thiosulfatophilum* at Different Light Intensities
(Liquid Dilution Rate: 1.25 days⁻¹)

Light	Gas Retention Time, min	Cell Conc. mg/L	% H ₂ S Conversion	S ²⁻ mg/L	SO ₄ ²⁻ mg/L	S° mg/L
2X60W	43	339	99.2	0	1	292
2X75W	43	409	99.9	1	182	327
2X75W	36	433	99.8	2	240	327
2X75W	31	460	97.8	0	183	402
2X100W	31	463	99.5	2	214	449
2X100W	27	441	95.5	2	135	516
2X150W	27	391	99.5	2	117	496
2X150W	24	408	98.5	2	89	572
2X150W	22	412	96.5	2	62	501
2X200W	22	422	99.9	2	100	539
2X200W	20	453	99.9	2	115	440

Figure 10.16. The Effect of Gas Retention Time on Cell Concentration, CO Conversion and H₂ Production using *R. rubrum* in the 2.5 L CSTR

from the gas flow rate variation studies in this reactor. In these studies, the liquid dilution rate, D , was maintained at 0.67 days, the agitation rate was 540 rpm, the temperature was 32°C and the light intensity was 5000 lux (2 x 2500 lux). At a gas flow rate of 30 sccm (83.33 min gas RT), a 70 percent CO conversion was obtained. As the gas flow rate was increased to 89 sccm (28.1 min gas RT), the CO conversion dropped to 24 percent. Hydrogen production increased linearly at low gas flow rate. At higher gas flow rates, the hydrogen production rate remained constant. Also, fluctuations in hydrogen production were observed at a 74 sccm gas flow rate (33.78 min gas RT).

Figure 10.17 presents the data for the liquid dilution rate variation study with the 2.5 L reactor. The gas retention time was 83 min, with the other conditions the same as in the previous study. A 40 percent improvement in CO conversion was observed as the liquid dilution rate was tripled. However, the cell concentration also gradually decreased. The liquid dilution rate was varied in order to show the effect of liquid dilution rate on CO conversion. The hydrogen production rate was stable as the liquid dilution rate was varied. The CO conversion was 50 percent at a liquid dilution rate of 0.21 day⁻¹. As the liquid dilution rate was increased to 0.67 day⁻¹, the CO conversion improved to 70 percent.

Figure 10.18 presents the agitation rate variation study with the 2.5 L reactor. The liquid dilution rate was again 0.67 days⁻¹, the gas retention time was 83 min, the temperature was 32°C and the light intensity was 2 x 2500 lux. The hydrogen production rate was low at low agitation rates. As the agitation rate was increased to 540 rpm or above the CO conversion reached 70 percent and the hydrogen production rate was also improved. A high agitation

Figure 10.17. The Effect of Liquid Dilution Rate on Cell Concentration, CO Conversion and H_2 Production using *R. rubrum* in the 2.5 L CSTR

Figure 10.18. The Effect of Agitation Rate on Cell Concentration, CO Conversion and H₂ Production using *R. rubrum* in the 2.5 L CSTR

rates the CO conversion and hydrogen production rate were also improved. At high agitation rates the CO conversion and hydrogen production rate remained constant. The cell concentration was about 1200 mg/L. The optimum agitation rate was comparable with the value obtained by the rule of constant power per unit volume. The optimum agitation rate for the 2.5 L fermenter was 540 rpm. As it was also the calculated agitation rate based on constant power required per unit volume.

Figure 10.19 shows the stability of the 2.5 L fermenter for 664 hours of operation, running at optimum operating conditions (83 min gas retention time, $D = 0.067 \text{ days}^{-1}$, 72°C , 540 rpm and $2 \times 2500 \text{ lux}$ light intensity). The CO conversion was practically constant at 70 percent. There were small fluctuations in the hydrogen production rate and cell concentration. The average cell concentration was 1250 mg/L. The average hydrogen production rate was $425 \mu\text{mol} \cdot \text{min}^{-1}$.

The optimum operating conditions of the 1.25 L reactor were also utilized for the scale-up to a 5.0 L CSTR. The effects of gas flow rate and agitation rate were studied in this reactor. Figure 10.20 shows the gas flow rate variation between 25 and 140 sccm (200 and 35.7 min gas RT). The dilution rate was 67 days^{-1} , the agitation rate was 950 rpm, the temperature was 32°C and the light intensity was $2 \times 5000 \text{ lux}$. As the gas flow rate was increased, the CO conversion was linearly decreased and the hydrogen production rate was increased. The cell concentration was constant, except in the beginning when there was a mechanical problem with the nutrient pump. There was a small drop in cell concentration during that period. The average cell concentration was 1280 mg/L. The optimum gas flow rate for a 70 percent CO conversion was 60 sccm (83.33 min gas RT) for the 5.0 L CSTR. A comparison of the 2.5 L and 5.0

Figure 10.19. Long Term Behavior of the 2.5 L CSTR using *R. rubrum*
(Liquid Dilution Rate: 0.67 days^{-1} ; Gas Retention
Time: 83 min; Agitation Rate: 540 rpm)

Figure 10.20. The Effect of Gas Retention Time on Cell Concentration, CO Conversion and H₂ Production using *R. rubrum* in the 5.0 L CSTR

L fermenters shows that the gas rate can be doubled as the working volume is doubled for the same level of CO conversion.

Figure 10.21 represents the agitation rate varied from 300 to 800 rpm, while the other parameters were fixed. The gas retention time was 83 min, while maintaining the other parameters at the values of the previous study. Based upon the equal power per unit volume rule, the calculated agitation rate for the 5.0 L reactor is 490 rpm. The calculated agitation rate was compatible with the experimental data. The CO conversion and hydrogen production were improved as the agitation rate was increased. At high agitation rates the CO conversion and hydrogen production rate were constant. The cell concentration essentially constant during this set of experiments. The hydrogen production rate was $1000 \mu\text{mol}\cdot\text{min}^{-1}$ with a gas flow rate of 60 sccm, an agitation rate of 490 rpm and a 70 percent CO conversion.

Figure 10.22 shows the gas flow rate variation studies in a 14 L fermenter. In this study, $D = 0.68 \text{ days}^{-1}$, the temperature was 37°C , the light intensity was $2 \times 5000 \text{ lux}$, and the agitation rate was 400 rpm. As the gas flow rate increased the CO conversion gradually decreased. The cell concentration was quite stable at 1200 mg/L. The hydrogen production rate was linearly increased as the gas flow rate was increased. The CO conversion dropped to 46 percent at a gas flow rate of 402 sccm (34.83 min gas RT). A 70 percent CO conversion was obtained with a 150 sccm gas flow rate (93.33 min gas RT). The hydrogen production rate was increased to $5100 \mu\text{mol}\cdot\text{min}^{-1}$ as the gas flow rate was increased to 402 sccm (34.83 min gas RT). The agitation rate was also calculated based on equal power per unit volume rule. A constant agitation rate of 400 rpm was used for the 14 L CSTR.

Figure 10.21. The Effect of Agitation Rate on Cell Concentration, CO Conversion and H₂ Production using *R. rubrum* in the 5.0 L CSTR

Figure 10.22. The Effect of Gas Retention Time on Cell Concentration, CO Conversion and H₂ Production using *R. rubrum* in the 14.0 L CSTR

Figure 10.23 shows the stability of the 14.0 L fermenter for 440 hours of operation, running at optimum operating condition ($D = 0.68 \text{ days}^{-1}$, GRT = 93 min, $T = 32^{\circ}\text{C}$, agitation rate = 400 rpm). The nutrient used was a seven times concentrated medium, and deionized, filtered water was used to maintain the liquid dilution rate. After 140 hours of operation the reactor was toxicated and therefore the CO conversion, cell concentration and hydrogen production rate gradually dropped. The reactor was restored by replacing the regular medium and the operation was continued for another 150 hours. A five times concentrated medium was replaced with deionized and filtered water for 150 hours of operation. The CO conversion was maintained at about 70 percent with a gas flow rate of 150 sccm, a concentrated medium flow rate of 1400 mL day^{-1} and a water flow rate of 8160 mL day^{-1} . The total liquid dilution rate was 0.68 day^{-1} . The hydrogen production rate was $2500 \mu\text{mol} \cdot \text{min}^{-1}$ during the experiment.

The mass transfer coefficients were found to be similar for the four reactors. They were 106.0, 121.24, 122.82 and 116.43 h^{-1} for the 1.25, 2.5, 5.0 and 14.0 L reactors, respectively. The mass transfer coefficient obtained from the 14.0 L reactor was averaged at the constant gas retention time of 93.3 min. Table 10.3 represents the percent CO conversion and product H_2/CO obtained with different agitation rates. At low agitation rates the reactors were less productive. The maximum productivity was obtained at the desired condition with high mass transfer coefficient.

11.0 ECONOMIC EVALUATIONS

The ultimate test for a biological process is an economic evaluation based upon the technical information gathered. An evaluation of biological water gas shift using *R. rubrum* has been performed to process 50 million

Figure 10.23. Long Term Behavior of the 14.0 L CSTR using *R. rubrum*
 (Liquid Dilution Rate: 0.68 days⁻¹; Gas Retention
 Time: 93 min; Agitation Rate: 400 rpm)

Table 10.2

Performance of the CSTR with the Sulfur Settler and Cell
Recycle Using *C. thiosulfatophilum*

Time h,	Light Intensity	D, day ⁻¹	Gas RT Min	X, mg/L	%H ₂ S conv.	SO ₄ ²⁻ mg/L	S° mg/L	S ²⁻ mg/L
Case I								
96	2X60W	1.12	36	605	99.6	47	655	1
Case II								
24	2X60W	1.12	36	802	98.7	58	1275	1
48	2X60W	1.12	36	1595	99.9	107	1762	1
72	2X60W	1.12	31	1782	99.7	108	1488	1
96	2X60W	1.12	27	2125	92.6	42	2733	1
120	2X60W	1.12	27	2554	94.0	25	3255	1
144	2X60W	1.30	27	2799	79.1	4	2294	1
168	2X60W	1.30	27	3244	70.0	4		1
192	2X75W	1.30	27	3300	88.8	8	1795	1
Case III								
24	2X75W	1.30	27	3993	80.4	1	2016	1
48	2X75W	1.30	27	3190	77.0	4		1
72	2X75W	1.30	27	2854	79.0	0.5	3046	1
96	2X75W	1.30	27	2324	79.3	4	2381	1
168	2X75W	1.30	27	1911	94.0	0.5	2317	1
192	2X75W	1.30	27	1887	96.1	0.5	1684	1
Case IV								
24	2X75W	1.30	27	2036	99.9	6	757	1
48	2X75W	1.30	24	2149	99.8	76	591	1
144	2X75W	1.30	20	1778	98.1	5	876	2
168	2X75W	1.30	20	1754	98.1	7	852	7

Case I : Reactor with settler (recycle rate = 70 mL/min)

Case II : Reactor with cell recycle and settler in the same loop (recycle rate = 450 mL/min).

Case III: Reactor with cell recycle and settler in the same loop (recycle rate = 450 mL/min) and 50% bleed.

Case IV : Reactor with cell recycle (recycle rate = 450 mL/min) and settler (recycle rate = 70 mL/min) in different loop and 50% bleed.

Table 10.3

The Effect of Agitation Rate in Different Size Reactors

1.25 L Reactor				
Agitation Rate, rpm	Gas Flow Rate sccm	Gas Retention Time (min)	% CO Conversion	H ₂ /CO
200	7.035	178	20.56	0.70
300	7.035	178	26.55	0.90
400	7.035	178	39.96	1.20
500	7.035	178	60.00	2.375
600	7.035	178	81.35	5.37
700	7.035	178	72.55	3.53
800	7.035	178	65.75	2.60
2.5 L Reactor				
300	29.00	86	17.65	0.79
400	29.65	84	53.20	2.09
500	29.57	85	60.94	2.45
540	29.03	86	71.10	3.70
600	29.00	86	71.26	3.78
700	29.24	85	72.23	3.74
800	32.05	78	65.91	2.99
5.0 L Reactor				
300	61.95	81	29.43	1.01
400	61.71	81	54.37	2.05
490	59.13	85	70.24	3.68
500	60.79	82	67.58	3.10
600	60.33	83	69.21	3.44
700	60.69	82	67.79	3.30
800	60.89	82	68.53	3.25
14.0 L Reactor				
400	150.75	93	72.02	3.15

standard cubic feet of synthesis gas per day (50 MMSCFD). The gas is assumed to consist of 65 percent (by volume) CO, 20 percent H₂, 4 percent CH₄ and 11 percent CO₂, a typical concentration for coal-derived synthesis gas. The CO conversion is assumed to be 95 percent, the growth yield is 0.4 g cell/g glucose and the operating liquid phase tension is 0.2 atm. A summary of these design assumptions is shown in Table 11.1. Several cases were considered:

- a) CSTRs without light
- b) CSTRs with light
- c) countercurrent ICRs, no light
- d) countercurrent ICRs with light
- e) improved strain in CSTR
- f) improved strain in ICR.

11.1 Evaluations with *R. rubrum*

Table 11.2 presents the capital costs of the major equipment items for H₂ production from synthesis gas using CSTRs as the reactors of choice. Light requirements are not considered. As is noted, the total capital cost is \$6.221 million and the fixed capital investment is estimated as \$18.663 million or \$0.107/MSCF synthesis gas. Table 11.3 shows the projected economics for H₂ production from syngas, with each operating cost shown in dollars per MSCF synthesis gas. The total H₂ cost is estimated at \$0.672/MSCF synthesis gas. Tables 11.4 and 11.5 show similar results when considering the light requirements for *R. rubrum*. The fixed capital investment increases to \$29.6 million and the H₂ cost increases to \$1.30/MSCF synthesis gas.

Table 11.1

Design Assumptions

Design Basis: 50 MM SCF/day synthesis gas

Syngas Composition:

CO: 65%
H₂: 20%
CH₄: 4%
CO₂: 11%

CO conversion: 95 percent

Culture: *R. rubrum*

Growth Yield: 0.4 g cell/g Glucose

Specific gas uptake rate: 0.055 gmole/g cell hr

Operating Pressure: 17 atm

Operating P_{CO}^L : 0.1 atm

Table 11.2

Capital Equipment Requirements

Case A: *R. rubrum*, CSTRs, no light

Ractors, 6 CSTRs, 1200 m³, 17 atm
 $K_{La} = 177 \text{ hr}^{-1}$, 1500 hp for agitation
Cost: \$6,045,000 (1993, delivered)

Medium pumps, 6, 11 hp, reciprocating
Cost: \$176,000 (1993, delivered)

Fixed Capital Investment

3 X \$6,221,000 = \$18,663,000
= \$0.107/MSCF synthesis gas

Table 11.3

Economic Evaluation

Case A: *R. rubrum*, CSTRs, no light

	\$/MSCF Synthesis Gas
Capital	0.107
Chemicals and Energy	
Electricity	0.193
Chemicals and Steam	<u>0.287</u>
Total	0.480
Labor and Supervision	0.010
Maintenance (4% FCI)	0.043
Taxes and Insurance (3% FCI)	0.032
Depreciation	0.107
Total H ₂ Cost	\$ 0.672/MSCF = Synthesis Gas

Table 11.4

Capital Equipment Requirements

Case B: *R. rubrum*, CSTRs, Light Required

Reactors, 6 CSTRs, 1200 m ³ , 17 atm
K _{La} - 177 hr ⁻¹ , 1500 hp for agitation
Cost = \$6,045,000 (1993, delivered)
Medium Pumps, 6, 11 hp, reciprocating
Cost = \$176,000 (1993, delivered)
Light Source, 18277 KW, Change every half year for 10 years
Cost \$3,655,000
Fixed Capital Investment
3 x 9,876,000 = \$29,628,000
= \$0.169/MSCF Synthesis Gas

Table 11.5

Economic Evaluation

Case B: *R. rubrum*, CSTRs, Light Required

Capital	\$/MSCF Synthesis Gas
	0.169
Chemicals and Energy	
Electricity - Agitation and Pumps	0.193
Electricity - Light	0.526
Chemicals and Steam	<u>0.287</u>
	1.006
Labor and Supervision	0.010
Maintenance (4% FCI)	0.068
Taxes and Insurance (3% FCI)	0.051
Depreciation	0.169
Total H ₂ Cost	\$1.304/MSCF Synthesis Gas

Table 11.6

Capital Equipment Requirements

Case C: *R. rubrum*, Countercurrent ICRs, no light

Reactors, 5, 3.5 m diameter, 20 m High, 10 Columns, 17 atm 1 inch porcelain Berl Saddles
Cost = \$2,106,000
Medium Pumps, 5, 30 hp, reciprocating
Cost = \$263,000
Fixed Capital Investment
3 x 2 \$369,000 = \$7,107,000
= \$0.041/MSCF = Synthesis Gas

Tables 11.6-11.9 show results using an immobilized cell reactor with and without light. Without light the cost of H₂ is \$0.459/MSCF syngas (see Table 11.7, and with light the cost of H₂ is \$1.00/MSCF syngas. It is apparent from these analyses that higher rates of CO utilization would improve the economics and that the light requirements for *R. rubrum* cannot be economically supplied by electricity.

11.2 Evaluations with ERIH₂

Engineering Resources, Inc. (ERI) has identified an organism for water gas shift which has much higher rates of CO utilization (and thus H₂ production) and does not require light for conversion. The economics for this bacterium ERIH₂, in CSTRs and ICRs are shown in Tables 11.10-11.13. In the CSTR, ERIH₂ requires a H₂ production cost of \$0.269/MSCF synthesis gas or \$0.436/MSCF hydrogen. In the ICR, ERIH₂ requires a H₂ production cost of \$0.119/MSCF syngas or \$0.193/MSCF H₂.

Hydrogen removal from CO₂ was next considered in the economics. There are a number of processes to remove CO₂ from gas streams. The separation is based on one of the following four principles (66):

- (1) CO₂ is weakly acidic, forming H₂CO₃ when dissolved in water. This permits the use of liquid alkaline solutions, either regenerable or nonregenerable, for absorption of CO₂.
- (2) CO₂ is soluble in water and in many organic liquids. Processes based on solubility as opposed to chemical affinity are also used.
- (3) The molecular size and structure of CO₂ permit it to be selectively absorbed on solid adsorbants, particularly molecular sieves.
- (4) The acidic nature or size and structures of CO₂ in some mixtures permit it to be separated by the use of permeable membranes.

Table 11.7

Economic Evaluation

Case C: *R. rubrum*, Countercurrent ICRs, no light

Capital	\$/MSCf Synthesis Gas 0.041
Chemicals and Energy	
Electricity - pumps	0.003
Chemicals and Steam	<u>0.287</u>
Total	0.290
Labor and Supervision	0.010
Maintenance (4% FCI)	0.016
Taxes and Insurance (3% FCI)	0.012
Depreciation	0.041
<hr/>	
\$0.459/MSCF = Synthesis Gas	

Table 11.8

Capital Equipment Requirements

Case D: *R. rubrum*, Countercurrent ICRs, with light

Reactors, 5, 3.5 m diameter, 20 m height, 17 atm 1 inch porcelain Berl Saddles
Cost = \$2,106,000
Medium pumps, 5, 30 hp, reciprocating
Cost = \$263,000
Light sources, 18207 KW, Change every half year for 10 years
Cost = \$3,665,000
Fixed Capital Investment
3 X 6,034,000 = \$18,102,000
= \$0.103/MSCF Synthesis Gas

Table 11.9

Economic Evaluation

Case D: *R. rubrum*, Countercurrent ICRs, with light

	\$/MSCF Synthesis Gas
Capital	\$0.103
Chemicals and Energy	
Electricity - Pumps	0.003
Electricity - Light	0.526
Chemicals and Steam	<u>0.287</u>
	0.816
Labor and Supervision	0.01
Maintenance (4% FCI)	0.041
Taxes and Insurance (3% FCI)	0.031
Depreciation	\$0.103
	\$1.001/MSCF Synthesis Gas

Table 11.10

Capital Equipment Requirements

Case E: ERIH_2 , CSTRs

Reactors, 3 CSTRs, 1200m^3 , 17 atm
$K_{\text{La}} = 205\text{ hr}^{-1}$, 500 hp for agitation
Cost: \$3,031,000 (1992, delivered)
Medium pumps, 3, 11 hp, reciprocating
Cost: \$88,000 (1992, delivered)
Fixed Capital Investment
= $3 \times \$3,119,000 = \$9,357,000$
= \$0.053/MSCF synthesis gas

Table 11.11
Economic Evaluation
Case E: ERIH₂, CSTRs

	\$/MSCF synthesis gas
Capital	0.053
Chemicals and Energy	
Electricity	0.097
Chemicals and Steam	<u>0.072</u>
Total	0.169
Labor and Supervision	0.010
Maintenance (4% FCI)	0.021
Taxes and Insurance (3% FCI)	0.016
Depreciation	0.053
Total H ₂ Cost	\$0.269/MSCF syngas or \$0.436/MSCF H ₂

Table 11.12
Capital Equipment Requirements
Case F: ERIH₂

ICR Columns, 2, 3.2 m dia x 20 m high, 17 atm,
packed with 1 in Berl saddles
Cost: \$1,136,000 (1992, delivered)

Medium pumps, 2, 40 hp, reciprocating pumps
Cost: \$98,000 (1992, delivered)

Fixed Capital Investment
= 3 x \$1,234,000 = \$3,702,000
= \$0.021/MSCF synthesis gas

Table 11.13

Economic Evaluation

Case F: ERIH₂, ICRs

	\$/MCSF synthesis gas
Capital	0.021
Chemicals and Energy	
Electricity	0.002
Chemicals and Steam	<u>0.072</u>
Total	0.074
Labor and Supervision	0.010
Maintenance (4% FCI)	0.008
Taxes and Insurance (3% FCI)	0.006
Depreciation	0.021
Total H ₂ cost	\$0.119/MSCF syngas or \$0.193/MSCF H ₂

The amount of CO₂ removed by solid adsorbants is small compared to that removed by liquid absorbant systems. Permeable membrane separation is of minor commercial importance (66). For the solvent system, the CO₂ content of the liquid phase increases in direct proportion to the partial pressure of CO₂ in the vapor phase. For the chemical reaction system, the equilibrium pressure increases only slightly with loading of the liquid over the lower range of solution loadings, but curves upward sharply as the loading approaches the stoichiometric limit set by the chemical reaction. It is obvious that at high available partial pressures of CO₂, as in this application, the solvent system is capable of attaining a higher loading of the scrubbing solution and, therefore, will require lower solution circulation rates for the same degree of CO₂ removal.

Another important advantage of the solvent system is a regeneration. The ratio of stripping vapor/solution required to strip the solution to a very low residual CO₂ content will be substantially less for the solvent system than for the chemical system. Thus a stripped solvent system will generally be capable of scrubbing the gas to be treated to a lower level of residual CO₂. Compared to a chemical system, the total energy required for the solvent system per mole of CO₂ removed is generally lower and is composed of power for circulating the solution and, in many cases, for refrigeration since the solubility of CO₂ is greatly increased by operating at less than ambient temperature.

Table 11.14 lists the typical operating conditions of various CO₂ removal processes (66). As discussed above, solvent systems involving sulfinol, selexol, rectisol, purisol, and fluror appear to be most suitable. The feed gas in this application will contain about 45 percent CO₂ and a total

Table 11.14

Typical Operating Conditions of Various CO₂ Removal Processes

Process	Solution Composition	Net Loading (scf/gal)	CO ₂ in Feed Gas by Volume	Operating Conditions		Utilities	
				Pressure (lb/in ² gauge)	Temperature (°F)	Heat	Power
Catacarb	Varies	3-5.5	2-27	100-1200	100-260	Low	Medium
Benfield	K ₂ CO ₃ + promoter	3-5.5	2-27	100-2000	100-260	Low	Medium
Vetrocoke	Varies	Not known	2-27	100-1200	120-212	Low	Medium
Conventional MEA	15-20%	2.5-3.5	0.5-10.0	25-950	80-130	High	High
High-loaded MEA	30-40%	7	15-25	400-900	130-160	Medium	Medium
Conventional DEA	20-25%	2-3	0.5-0.8	175-500	80-130	High	High
High-loaded DEA	25-27%	5-5.4	2.0-20	175-950	80-130	Medium	Medium
SNPA:DEA	25-30%	5.5-6.2	2.0-30	200-2000	80-130	Medium	Medium
DGA	65-70%	5-7	5.0-20	150-950	80-130	Medium	Medium
Sulfinol	Varies	8-16	2.0-30	300-1000	100-160	Medium	Medium
Selexol	DMPEG	11-15	2.0-45	500-1400	100-160	Low	Low-Medium
Rectisol	Methanol	5-20	2.0-35	300-1200	-5 to -100	Low	Medium
Purisol	NMP	5-10	5.0-35	450-1200	40-105	Low	Medium
Fluor	Propylene carbonate		5.0-53				

*/Depends on solubility and recompression of flash gas

Note: NMP: n-methyl 2-pyrrolidone

DMPEG: dimethyl ether of polyethylene glycol

Sulfinol solvent: sulfolane, di-isopropanol amine

pressure of 400 psi, or 180 psi CO₂ partial pressure. The majority of these solvents can also simultaneously remove H₂S, and some solvents such as sulfinol and selexol even remove COS. The processes using these solvents have the advantages of a low solvent circulation rate, low plant costs, low utilities costs, high effectiveness for the removal of COS, CS₂ and HS, low solvent degradation and low corrosion rates (66). The major disadvantage of these processes is the requirement of a royalty. Thus, there is not much published information available to estimate CO₂ removal costs.

In this study, the estimation of CO₂ removal costs is based on the only published material and energy balance information for the sulfinol process (67). Figure 11.2 shows the system flow chart of the sulfinol process and Table 11.15 lists the material and energy balance information. The calculation will be based on Gas "E", since it has exactly the same CO₂ partial pressure as in this application (18 percent in 1000 psi, or 180 psi). The feed gas flow rate in Table 11.15 is 100 MMSCFD, or 18 MMSCFD of CO₂ load. In this application, the feed gas will be 1.6175 x 50 MMSCFD with 45 percent CO₂, or a 36.4 MMSCFD CO₂ load. Thus, the amount treated is roughly double the published data. The lower total pressure and higher CO₂ concentration in the feed should not affect the process operation much since it is CO₂ that is absorbed. Based on the above discussion, the major operation cost will be for the solution circulation pump energy and steam in the regenerator boiler.

Table 11.16 shows the equipment and their costs for CO₂ removal. As is noted, an absorber, regenerators, reboilers and condensers are required, along with associated pumps. The total fixed capital investment is \$11,847,000 or \$0.068/MSCF of synthesis gas. Table 11.17 shows an economic evaluation for CO₂ removal alone. The total CO₂ removal cost is estimated as \$0.369/MSCF of synthesis gas or \$0.598/MSCF of hydrogen.

Figure 11.1 Typical Equilibrium Lines for Chemical and Physical Solvents
(Eickmeyer, *et al*)

Figure 11.2 System Flow for Sulfinol Process (Goar)

Table 11.15

Typical Circulation and Reboiler System Rates
for Sulfinol Process

Item	Rates for Sulfinol Process				
	Gas "A"	Gas "B"	Gas "C"	Gas "D"	Gas "E"
Contactor pressure, psia	1,000	1,000	1,000	1,000	1,000
Feed-gas temp., °F	110	110	110	110	110
Feed-gas flow (dry), MMSCFD	100	100	100	100	100
Feed-gas composition, mole %					
H ₂ S	0.65	20.1	20.10	51.5	0.10
CO ₂	8.73	2.0	2.00	3.5	18.00
N ₂	2.37	1.4	1.40	8.6	0.70
C ₁	87.90	71.5	63.01	25.8	80.94
C ₂	0.35	2.0	8.43	5.8	0.17
C ₃		1.7	3.71	3.2	0.05
C ₄		1.1	0.82	1.6	0.04
C ₅		0.2	0.53		
Total	100.00	100.00	100.00	100.00	100.00
COS, grains/100 scf	3.0	7.3	7.3	8.4	
RSH, grains/100 scf	2.1	1.5	1.5	3.10	
H ₂ S/CO ₂ ratio	0.0744	10.05	10.05	14.71	0.0056
Sweet gas H ₂ S content, grains/100 scf	<0.25	<0.25	<0.25	<0.25	<0.25
Sweet gas CO ₂ content, mole %	<1.0	<1.0	<1.0	<1.0	<2.0
Sweet gas total sulfur, grains/100scf	<1.0	<1.0	<1.0	<1.0	<1.0
Solution circulation rate, gpm at 100°F	1,483	1,748	1,790	2,366	2,167
Solution net pickup, scf acid-gas/gal	4.39	8.78	8.57	16.14	5.24
Reboiler steam rate, lb/hr	69,740	111,440	125,960	156,010	99,600
Reboiler steam rate, lb/gal solution	0.78	1.06	1.17	1.10	0.77

*Includes 0.02 mole % aromatics. The calculated acid gas produced from Gas "C" could contain 1.0 mole % hydrocarbons, which would contain 0.20 mole % of C₅⁺ fraction, based on the H₂S portion only. This acid gas could be processed in a sulfur plant without any further treatment. The Sulfinol system for Gas "E" was designed to leave 2.0 mole % CO₂ in the sweet gas, while reducing the H₂S content to 0.25 grains/100 scf.

Table 11.16
Capital Costs for CO₂ Removal

Absorber	
9.8 min liquid retention time	
Cost: \$568,000	
Regenerator	
9.8 min liquid retention time	
Cost: \$568,000	
Pumps	
7-300 hp reciprocating pumps	
Cost: \$613,000	
Reboiler	
Total area: 55,000 ft ² (5 units of 11,000 ft ² each)	
Cost: \$1,543,000	
Condenser	
Cost: \$657,000	
Fixed Capital Investment	
- 3 x \$3,949,000 = \$11,847,000 = \$0.068/MSCF synthesis gas	

Table 11.17
Economic Evaluation for CO₂ Removal

Capital	\$/MSCF synthesis gas
Chemicals and Energy	0.068
Electricity	0.041
Steam	0.191
Chemicals	<u>0.054</u>
Total	0.286
Labor and Supervision	0.010
Maintenance (4% FCI)	0.003
Taxes and Insurance (3% FCI)	<u>0.002</u>
Total CO ₂ Removal Cost	\$ 0.369/MSCF syngas
	or \$ 0.598/MSCF H ₂

The revised economics for H₂ production using ERIH₂ including CO₂ removal are shown in Tables 11.18 and 11.19. The total H₂ cost ranges from \$0.772/MSCF of H₂ for the ICR case to \$1.231/MSCF of H₂ for the CSTR case. Thus, the economic projections indicate that H₂ can be produced biologically for 19.3¢/MSCF without H₂ removal and for 77.2¢/MSCF if H₂ separation by the sulfinol process is employed.

12.0 CONCLUSIONS

Based upon the results of this culture screening study, *R. rubrum* is recommended for biocatalysis of the water gas shift reaction and *C. thiosulfatophilum* is recommended for H₂S conversion to elemental sulfur. Both bacteria require tungsten light for growth and can be co-cultured together if H₂S conversion is not complete (required concentration of at least 1 ppm), thereby presenting H₂ uptake by *C. thiosulfatophilum*.

COS degradation may be accomplished by utilizing various CO-utilizing bacteria or by indirectly converting COS to elemental sulfur after the COS first undergoes reaction to H₂S in water. The second alternative is probably preferred due to the low expected concentration of COS relative to H₂S.

Mass transfer and kinetic studies were carried out for the *Rhodospirillum rubrum* and *Chlorobium thiosulfatophilum* bacterial systems. *R. rubrum* is a photosynthetic anaerobic bacterium which catalyzes the biological water gas shift reaction:

C. thiosulfatophilum is also a photosynthetic anaerobic bacteria, and converts H₂S and COS to elemental sulfur.

Table 11.18

Economic Evaluation Including CO₂ RemovalCase G: ERIH₂, CSTRs Utilized

	\$/MSCF synthesis gas
Capital	0.021
Chemicals and Energy	0.455
Labor and Supervision	0.020
Maintenance (4% FCI)	0.024
Taxes and Insurance (3% FCI)	0.018
Depreciation	0.121
Total H ₂ Cost	\$ 0.759/MSCF syngas or \$ 1.231/MSCF H ₂

Table 11.19

Economic Evaluation Including CO₂ RemovalCase H: ERIH₂ ICRs Utilized

	\$/MCSF synthesis gas
Capital	0.089
Chemicals and Energy	0.360
Labor and Supervision	0.020
Maintenance (4% FCI)	0.004
Taxes and Insurance (3% FCI)	0.003
Depreciation	0.021
Total H ₂ Cost	\$ 0.476/MSCF syngas or \$ 0.772/MSCF H ₂

Batch studies with *R. rubrum* have demonstrated that CO utilization can be modeled by the equation:

$$q = \frac{0.055 P_L^*}{0.45 + P_L^* + \left(P_L^*\right)^2/0.106} \quad (5.6)$$

A similar equation was found for CO utilization in continuous (CSTR) culture:

$$q = \frac{0.0146 P_{CO}^*}{0.0053 + P_{CO}^*} \left(1 - \frac{P_{CO}^*}{0.68} \right) \quad (5.22)$$

The growth of *R. rubrum* in terms of incoming light intensity is given by the equation:

$$\mu = \frac{0.052 I_o}{140 + I_o} \quad (5.17)$$

The growth of *R. rubrum* may be satisfactorily carried out at 25° and 30°C, while CO uptake and thus the conversion of CO best occurs at temperatures of either 30°, 32° or 34°C.

The rate of conversion of COs and H₂O to CO₂ and H₂S may be modeled by a first order rate expression. The rate constant at 30°C was found to be 0.243 h⁻¹. The growth of *C. thiosulfatophilum* may be modeled in terms of incoming light intensity using a Monod equation:

$$\mu = \frac{0.152 I_o}{351 + I_o} \quad (6.6)$$

Comparisons of the growth of *R. rubrum* and *C. thiosulfatophilum* shows that the specific growth rate of *C. thiosulfatophilum* is much higher at a given light intensity.

The CSTR with sulfur recovery and cell recycle using *C. thiosulfatophilum* was shown to be very effective in removing H₂S from syngas streams. A gas retention time of only 20 min was required to remove essentially 100 percent of the H₂S from a synthetic syngas stream containing 2.5 percent H₂S. Just as important essentially 100 percent of the sulfur product was produced as elemental sulfur instead of sulfate. The *C. thiosulfatophilum* system will be very useful along with *R. rubrum* in removing H₂S and converting CO and water to H₂.

The effects of cell density from *R. rubrum* and *C. thiosulfatophilum* on density, viscosity and surface tension were determined. In addition, the effects of small concentrations of oxygen and H₂S were quantitatively determined for *R. rubrum*. Finally, the scale-up of the *R. rubrum* process in reactors ranging from 1.25 L to 14.0 L was successfully performed. The power/unit volume scale-up parameter is appropriate for this biological system.

Based upon the results of the economic analyses, it is seen that the light requirements for *R. rubrum* cannot be economically satisfied with electricity. Furthermore, higher specific CO uptakes rates are desirable. The bacterium ERIH₂, isolated at Engineering Resources, Inc., does not require light and has very high specific uptakes rates. The economic evaluation with ERIH₂ indicates that H₂ recovery and for 77¢ MSCF if H₂ separation by the sulfinol process is employed.

13.0 NOMENCLATURE

C	concentration	mol/L
G	volumetric gas flow rate	L/h
H	Henry's law constant for CO	L atm/mol
I	Light intensity	lux
K_I, K_p	Monod saturation constant	lux
K_{La}	overall mass-transfer coefficient	h^{-1}
n	number of moles	mol
P	partial pressure	atm
q	specific CO uptake rate by cells	mol/g h
r	volumetric CO mass-transfer/uptake rate	mol/h L
R	ideal gas law constant	L atm/mol °K
t	time	h
T	absolute temperature	°K
V	volume	L
W	Substrate inhibition constant	atm
X	cell concentration	g/L
z	distance	
ϵ	extinction coefficient	
μ	specific growth rate	h^{-1}

Superscripts and Subscripts

*	equilibrium value
Ar	argon, inert tracer gas
CO	carbon monoxide
G	gas phase

13.0 NOMENCLATURE (continued)

I,o inlet conditions

L liquid phase

m maximum

z any position

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