1 of 2

BIOLOGICAL CONVERSION OF SYNTHESIS GAS

Topical Report: Limiting Conditions/Scale-up

by

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September 1993

Work performed under Grant No. DE-FG21-90MC27225

for

U. S. Department of Energy
Morgantown Energy Technology Center
Morgantown, WV

by

University of Arkansas
Fayetteville, AR 72701

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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.

TABLE OF CONTENTS

	<u>Page</u>
ACKNOWLEDGEMENT.....	i
LIST OF TABLES.....	ii
LIST OF FIGURES.....	iii
1.0 INTRODUCTION.....	1
1.1 Biological Synthesis Gas Conversion.....	3
1.2 Brief Statement of Purpose.....	6
1.2.1 Purpose.....	9
2.0 MATERIALS AND METHODS.....	9
2.1 Organisms and Culture.....	9
2.2 Batch Bioreactors and Operating Conditions.....	10
2.3 Continuous Bioreactor and Operating Conditions.....	10
2.4 Analytical Techniques	14
3.0 EFFECTS OF CELL CONCENTRATION ON PHYSICAL PROPERTIES	14
4.0 EFFECTS OF O ₂ AND H ₂ S ON THE PERFORMANCE OF R. RUBRUM	22
4.1 CO Uptake in the Absence of H ₂ S and O ₂	27
4.2 H ₂ S Effects.....	31
4.3 O ₂ Effects.....	41
5.0 SCALE-UP STUDIES.....	55
5.1 Baseline Studies in the 1.25 L CSTR	55
5.1.1 Agitation Rate Study	61
5.1.2 Temperature Study	61
5.1.3 Liquid Flow Rate Study	65
5.1.4 Light Intensity Study	65
5.1.5 Gas Flow Rate Study	75
5.2 Scale-up to 2.5, 5.0 and 14.0 L CSTRs	75
6.0 CONCLUSIONS	96
7.0 LITERATURE CITED	97

LIST OF TABLES

	<u>Page</u>
3.1 Relating Density to Temperature for <i>R. rubrum</i> and <i>C. thiosulfatophilum</i>	20
3.2 Relating Viscosity to Temperature for <i>R. rubrum</i> and <i>C. thiosulfatophilum</i>	23
4.1 Values of n and $P_{H_2S, \max}$ for the Modified Andrews Equation at Different H_2S Concentrations	43
4.2 Lag Phase for CO Consumption at Different $P_{CO, i}$ and $P_{O_2, i}$	56
4.3 Values of n and $P_{O_2, \max}$ for the Modified Andrews Equation at Different O_2 Concentrations	58
5.1 Performance of the CSTR with the Sulfur Recovery Using <i>C.</i> <i>thiosulfatophilum</i>	81
5.2 Performance of the CSTR with the Sulfur Settler and Cell Recycle Using <i>C. thiosulfatophilum</i>	94
5.3 The Effect of Agitation Rate on Different Size Reactors.....	95

LIST OF FIGURES

	<u>Page</u>
1.1 Comparison of Conventional and Biological Routes for Methane Production from Coal.....	5
2.1 Schematic Set-up of the CSTR Reactor System Using <i>R. rubrum</i>	12
3.1 The Effect of Cell Concentration Density for <i>R. rubrum</i> and <i>C. thiosulfatophilum</i>	16
3.2 The Effect of Temperature on Density of Water, <i>R. rubrum</i> and <i>C. thiosulfatophilum</i>	18
3.3 Test of Logarithmic Relationship Between Density and Inverse Absolute Temperature.....	19
3.4 The Effect of Cell Concentration on Viscosity for <i>R. rubrum</i> and <i>C. thiosulfatophilum</i>	21
3.5 Test of Logarithmic Relationship Between Viscosity and Inverse Absolute Temperature.....	24
3.6 The Effect of Cell Concentration on Surface Tension for <i>R. rubrum</i> and <i>C. thiosulfatophilum</i>	25
3.7 The Effect of Temperature on Surface Tension for <i>R. rubrum</i> and <i>C. thiosulfatophilum</i>	26
4.1 Determination $K_L a/H$ for the Batch Bottle Experiments.....	28
4.2 Specific CO-Uptake Rate at Different $P_{CO,i}$ (No H_2S or O_2 Present).....	29
4.3 Illustration of Modified Monod Expression for CO Uptake (No H_2S or O_2 Present).....	30
4.4 Effects of H_2S on Cell Density ($P_{CO,i} = 0.8$ atm).....	32
4.5 Effects of H_2S on Cell Density ($P_{CO,i} = 1.0$ atm).....	33
4.6 Effects of H_2S on CO-uptake by <i>R. rubrum</i> ($P_{CO,i} = 0.8$ atm).....	34
4.7 Effects of H_2S on CO-uptake by <i>R. rubrum</i> ($P_{CO,i} = 1.0$ atm).....	35
4.8 Effects of H_2S on H_2 Production ($P_{CO,i} = 0.8$ atm).....	36
4.9 Effects of H_2S on H_2 Production ($P_{CO,i} = 1.0$ atm).....	37
4.10 Estimation of $Y_{H_2/CO}$ in the Presence of H_2S	38
4.11 Moles of H_2S as a Function of Time ($P_{CO,i} = 0.8$ atm)	39
4.12 Moles of H_2S as a Function of Time ($P_{CO,i} = 1.0$ atm)	40
4.13 Modeling Scheme	42
4.14 Experimental q Compared to Calculated q from the Modified Andrews Equation	44
4.15 Experimental q Compared to Calculated q from the Modified Andrews Equation	45
4.16 Effects of O_2 on Cell Density ($P_{CO,i} = 0.8$ atm)	46
4.17 Effects of O_2 on Cell Density ($P_{CO,i} = 1.15$ atm)	47
4.18 Effects of O_2 on CO uptake by <i>R. rubrum</i> ($P_{CO,i} = 0.8$ atm).....	48
4.19 Effects of O_2 on CO uptake by <i>R. rubrum</i> ($P_{CO,i} = 1.15$ atm).....	49
4.20 Effects of O_2 on H_2 Production ($P_{CO,i} = 0.8$ atm)	50
4.21 Effects of O_2 on H_2 Production ($P_{CO,i} = 1.15$ atm).....	51
4.22 Estimation of $Y_{H_2/CO}$ in the Presence of Oxygen	52
4.23 Moles of O_2 as a Function of Time ($P_{CO,i} = 0.8$ atm).....	53
4.24 Moles of O_2 as a Function of time ($P_{CO,i} = 1.15$ atm).....	54
4.25 Lag Periods for CO Consumption by <i>R. rubrum</i> in the Presence of O_2	57
4.26 Experimental q Compared to Calculated q from the Modified Andrews Equation.....	59

4.27	Experimental q Compared to Calculated q from the Modified Andrews Equation.....	60
5.1	The Effects of Agitation Rate on Cell Concentration Using <i>R. rubrum</i> in the 1.25 L CSTR.....	62
5.2	The Effects of Agitation Rate on CO Conversion Using <i>R. rubrum</i> in the 1.25 L CSTR.....	63
5.3	The Effects of Agitation Rate on H ₂ Production Using <i>R. rubrum</i> in the 1.25 L CSTR.....	64
5.4	The Effects of Temperature on Cell Conversion Using <i>R. rubrum</i> in the 1.25 L CSTR.....	66
5.5	The Effects of Temperature on CO Conversion Using <i>R. rubrum</i> in the 1.25 L CSTR.....	67
5.6	The Effects of Temperature on H ₂ Production Using <i>R. rubrum</i> in the 1.25 L CSTR.....	68
5.7	The Effects of Liquid Flow Rate on Cell Concentration Using <i>R. rubrum</i> in the 1.25 L CSTR	69
5.8	The Effects of Liquid Flow Rate on CO Conversion Using <i>R. rubrum</i> in the 1.25 L CSTR.....	70
5.9	The Effects of Liquid Flow Rate on H ₂ Production Using <i>R. rubrum</i> in the 1.25 L CSTR.....	71
5.10	The Effects of Light Intensity on Cell Concentration Using <i>R. rubrum</i> in the 1.25 L CSTR.....	72
5.11	The Effects of Light Intensity on CO Conversion Using <i>R. rubrum</i> in the 1.25 L CSTR	73
5.12	The Effects of Light Intensity on H ₂ Production Using <i>R. rubrum</i> in the 1.25 L CSTR.....	74
5.13	The Effects of Gas Flow Rate on Cell Concentration Using <i>R. rubrum</i> in the 1.25 L CSTR.....	76
5.14	The Effects of Gas Flow Rate on CO Conversion Using <i>R. rubrum</i> in the 1.25 L CSTR.....	77
5.15	Schematic Diagram of the Experimental Set-up for the 14.0 L Fermenter.....	80
5.16	The Effect of Gas Retention Time on Cell Concentration, CO Conversion and H ₂ Production Using <i>R. rubrum</i> in the 2.5 L CSTR.....	83
5.17	The Effect of Liquid Dilution Rate on Cell Concentration, CO Conversion and H ₂ Production Using <i>R. rubrum</i> in the 2.5 L CSTR.....	84
5.18	The Effect of Agitation Rate on Cell Concentration, CO Conversion and H ₂ Production Using <i>R. rubrum</i> in the 2.5 L CSTR.....	85
5.19	Long Term Behavior of the 2.5 L CSTR Using <i>R. rubrum</i>	87
5.20	The Effects of Gas Retention Time on Cell Concentration, CO Conversion and H ₂ Production Using <i>R. rubrum</i> in the 5.0 L CSTR.....	88
5.21	The Effect of Agitation Rate on Cell Concentration, CO Conversion and H ₂ Production Using <i>R. rubrum</i> in the 5.0 L CSTR.....	90
5.22	The Effect of Gas Retention Time on Cell Concentration, CO Conversion and H ₂ Production Using <i>R. rubrum</i> in the 14.0 L CSTR.....	91
5.23	Long Term Behavior of the 14.0 L CSTR Using <i>R. rubrum</i>	93

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.

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 H₂S conversion (18,19).

1.2.1 Purpose

The purpose of this report is to present results from process scale-up and limiting conditions studies aimed at eventually developing a commercialized process for H₂ conversion and H₂S removal. Studies demonstrating the effects of cell concentration on physical properties are presented. In addition, long term studies with *R. rubrum* under limiting operating conditions are presented, along with *R. rubrum* CSTR studies in 1.25, 2.0, 5.0 and 14.0 L reactors.

2.0 MATERIALS AND METHODS

2.1 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.

2.2 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/CO_2 (75%/25%) atmosphere. The bottles were autoclaved at 15 psig for 20 minutes.

Once sterile, the bottles were reduced with 1.5 mL of Na_2S (2.5%) and inoculated with 5 mL of seed culture. After inoculation the desired gas phases were introduced by flushing with CO/CO_2 (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).

2.3 Continuous Bioreactor and Operating Conditions

The continuous stirred-tank reactor used was a New Brunswick Scientific (Edison, NM) 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 2.1.

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

Figure 2.1. 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.

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.

2.4 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).

3.0 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 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% CO₂, 20.04% H₂,
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) (22), 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 (23). 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 3.1. 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

Figure 3.1. The Effect of Cell Concentration on Density for *R. rubrum* and *C. thiosulfatophilum*.

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.

As is shown in Figure 3.2, 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 3.3), using the equation:

$$\ln \rho = \ln \alpha + \frac{\beta}{T} \quad (3.1)$$

$$\text{or} \quad \rho = \alpha e^{\beta/T} \quad (3.2)$$

Constants for Equations (3.1) and (3.2) for water and the bacterial systems are shown in Table 3.1.

The effects of cell concentration on viscosity at 25°C is shown in Figure 3.4. 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:

$$\ln \mu = \ln \gamma + \frac{\delta}{T} \quad (3.3)$$

Figure 3.2. The Effect of Temperature on Density of Water, *R. rubrum* and *C. thiosulfatophilum*.

Figure 3.3. Test of Logarithmic Relationship Between Density and Inverse Absolute Temperature.

Table 3.1

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

$$\text{or} \quad \mu = \gamma e^{\delta/T} \quad (3.4)$$

A plot of this data is shown in Figure 3.5, and constants for these equations are shown in Table 3.2. As is noted in Figure 3.5, a single straight line can accurately describe the viscosity relation of Equation (3.3) for the systems studied.

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 3.6 and 3.7. 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 3.6). The surface tension decreased with temperature for all systems (see Figure 3.7), with water having the highest surface tension, followed by two bacterial systems.

4.0 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,

Table 3.2

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

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

<u>System</u>	γ	δ
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

Figure 3.5. Test of Logarithmic Relationship Between Viscosity and Inverse Absolute Temperature.

Figure 3.6. The Effect of Cell Concentration on Surface Tension for *R. rubrum* and *C. thiosulfatophilum*.

Figure 3.7. The Effect of Temperature on Surface Tension for *R. rubrum* and *C. thiosulfatophilum*

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 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.

4.1 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 4.1-4.3 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 4.1 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)

$$q = \frac{q_m P_{CO}^L}{K_m + P_{CO}^L + \left(P_{CO}^L\right)^2/W} \quad (4.1)$$

was rearranged to yield

$$\frac{P_{CO}^L}{q} = \frac{K_m}{q_m} + \frac{1}{q_m} \left(P_{CO}^L\right) + \frac{1}{W q_m} \left(P_{CO}^L\right)^2 \quad (4.2)$$

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

Figure 4.1. Determination of $K_L a/H$ for the Batch Bottle Experiments.

Figure 4.2. Specific CO-uptake Rate at Different P_{CO_2} (no H_2S or O_2 present).

Figure 4.3. Illustration of Modified Monod Expression for CO Uptake (no H_2S or O_2 present).

A plot of the experimental data, rearranged to fit Equation (4.2), is shown in Figure 4.3. The modified Monod equation for CO-uptake by *R. rubrum* without the presence of CO is thus:

$$q = \frac{0.018 P_{CO}^L}{0.2 + P_{CO}^L + \left(P_{CO}^L\right)^2 / 0.82}, \text{ mol/gcell}\cdot\text{hr} \quad (4.3)$$

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

4.2 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 4.4-4.9 show representative cell, CO and H₂ concentration profiles for representative initial CO partial pressures. As is noted in Figures 4.4 and 4.5, 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 4.10, 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 4.11 and 4.12 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 4.4-4.9 were modeled using the procedure outlined previously. An equation of the form

Figure 4.4. Effects of H_2S on cell density [$P_{\text{CO}_2} = 0.8 \text{ atm}$].

Figure 4.5. Effects of H_2S on cell density [$P_{\text{CO}_2} = 1.0 \text{ atm}$].

Figure 4.6. Effects of H_2S on CO-uptake by *R. rubrum* [$P_{CO_2} = 0.8$ atm].

Figure 4.7. Effects of H_2S on CO-uptake by *R. rubrum* [$P_{CO_2} = 1.0 \text{ atm}$].

Figure 4.8. Effects of H_2S on H_2 Production [$P_{\text{co}_2} = 0.8 \text{ atm}$].

Figure 4.9. Effects of H_2S on H_2 Production [$P_{\text{CO}_2} = 1.0 \text{ atm}$].

Figure 4.10. Estimation of $Y_{\text{H}_2/\text{CO}}$ in the presence of H_2S .

Figure 4.11. Moles of H_2S as a Function of Time [$P_{CO_2} = 0.8$ atm]

Figure 4.12. Moles of H_2S as a Function of Time [$P_{CO_2} = 1.33$ atm]

$$q = \frac{q_m P_{CO}^L}{K_S + P_{CO}^L + (P_{CO}^L)^2/W} \left(1 - \frac{P_{H_2S}}{P_{H_2S, \max}} \right)^n \quad (4.4)$$

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 (4.3) and the constants n and $P_{H_2S, \max}$ were to be determined. Figure 4.13 illustrates the procedure for determining n and $P_{H_2S, \max}$. Table 4.1 shows the results of the modeling, and Figures 4.14 and 4.15 show comparisons of experimental data with the results of the model.

4.3 O₂ Effects

Experiments with *R. rubrum* were similarly carried out in the presence of O₂ partial pressures ranging from 0-0.05 atm. Figures 4.16-4.21 show representative cell, CO and H₂ concentration profiles for representative initial CO partial pressures. As is noted in Figures 4.16 and 4.17, 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 4.18-4.21. 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 4.22). This value is consistent with the value of 0.84 mol/mol obtained in the H₂S study. Contrary to H₂S, Figures 4.23 and 4.24 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.

Figure 4.13. Modeling Scheme.

Table 4.1

Values of n and $P_{H_2S,max}$ for the Modified Andrews Equation
at Different H_2S Concentrations

$P_{H_2S,i}$ (atm)	n	$P_{H_2S,max}$ (atm)	
0.0068	1	0.025	$P_{CO,i} < 1.0 \text{ atm}$
		0.016	$P_{CO,i} > 1.0 \text{ atm}$
0.0086	1	0.025	$P_{CO,i} < 1.0 \text{ atm}$
0.012	1	0.025	$P_{CO,i} < 1.0 \text{ atm}$
		0.020	$P_{CO,i} > 1.0 \text{ atm}$
0.018	1	0.030	$P_{CO,i} > 1.0 \text{ atm}$

Figure 4.14. Experimental q Compared to Calculated q From the Modified Andrews Equation.

Figure 4.15. Experimental q Compared to Calculated q From the Modified Andrews Equation.

Figure 4.16. Effects of O_2 on Cell Density [$P_{CO_2} = 0.8$ atm].

Figure 4.17. Effects of O_2 on Cell Density [$P_{\text{CO}_2} = 1.15 \text{ atm}$].

Figure 4.18. Effects of O_2 on CO Uptake by *R. rubrum* [$P_{CO_2} = 0.8 \text{ atm}$].

Figure 4.19. Effects of O_2 on CO Uptake by *R. rubrum* [$P_{CO_2} = 1.15 \text{ atm}$].

Figure 4.20. Effects of O_2 on H_2 Production [$P_{\text{CO}_2} = 0.8 \text{ atm}$].

Figure 4.21. Effects of O_2 on H_2 Production [$P_{CO_2} = 1.15 \text{ atm}$].

Figure 4.22. Estimation of $Y_{(\text{H}_2/\text{CO})}$ in the presence of Oxygen.

Figure 4.23. Moles of O_2 as a Function of Time [$P_{CO_2} = 0.8$ atm].

Figure 4.24. Moles of O₂ as a Function of Time [P_{CO₂} = 1.0 atm].

Table 4.2 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.

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

$$t_{\text{lag}} = a P_{\text{O}_2, i} + b \left(P_{\text{O}_2, i} \right)^2 \quad (4.5)$$

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

$$t_{\text{lag}} = 5699.8 P_{\text{O}_2, i} - 49095.7 \left(P_{\text{O}_2, i} \right)^2 \quad (4.6)$$

The experimental data of Figures 4.16-4.21 were also modeled using the procedure described previously. An equation of the form

$$q = \frac{q_m P_{\text{CO}}^L}{K_S + P_{\text{CO}}^L + \left(P_{\text{CO}}^L \right)^2 / W} \left(1 - \frac{P_{\text{O}_2}}{P_{\text{O}_2, \text{max}}} \right)^n \quad (4.7)$$

was used in modeling the data with q_m , K_S and W again determined previously. Table 4.3 shows the results of the modeling and Figures 4.26 and 4.27 show comparisons of the experimental data with the results of the model

5.0 SCALE-UP STUDIES

5.1 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

Table 4.2

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

LIST OF TABLES

	<u>Page</u>
3.1 Relating Density to Temperature for <i>R. rubrum</i> and <i>C. thiosulfatophilum</i>	20
3.2 Relating Viscosity to Temperature for <i>R. rubrum</i> and <i>C. thiosulfatophilum</i>	23
4.1 Values of n and $P_{H_2S, \max}$ for the Modified Andrews Equation at Different H_2S Concentrations	43
4.2 Lag Phase for CO Consumption at Different $P_{CO, i}$ and $P_{O_2, i}$	56
4.3 Values of n and $P_{O_2, \max}$ for the Modified Andrews Equation at Different O_2 Concentrations	58
5.1 Performance of the CSTR with the Sulfur Recovery Using <i>C.</i> <i>thiosulfatophilum</i>	81
5.2 Performance of the CSTR with the Sulfur Settler and Cell Recycle Using <i>C. thiosulfatophilum</i>	94
5.3 The Effect of Agitation Rate on Different Size Reactors.....	95

Figure 4.25. Lag periods for CO Consumption by *R. rubrum* in the Presence of O_2 .

Table 4.3

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 4.26. Experimental q Compared to Calculated q From the Modified Andrews Equation.

Figure 4.27. Experimental q Compared to Calculated q From the Modified Andrews Equation.

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.

5.1.1 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 6.1-6.3. 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 5.1). The CO conversion increased sharply with agitation rate, reaching a maximum conversion of about 75 percent at 600 rpm (see Figure 5.2). The H_2 production rate also reached a maximum of about 100 $\mu\text{mol}/\text{min}$ at 600-700 rpm (see Figure 5.3). An agitation rate of 600 rpm was chosen for further study.

5.1.2 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 .

Figure 5.1. The Effects of Agitation Rate on Cell Concentration using *R. rubrum* in the CSTR

Figure 5.2. The Effects of Agitation Rate on CO Conversion using *R. rubrum* in the CSTR

Figure 5.3. The Effects of Agitation Rate on H₂ Production using *R. rubrum* in the CSTR

The effects of temperature on culture performance are shown on Figures 5.4-5.6. Cell concentration was a maximum at 25°C (see Figure 5.4). However, the CO conversion reached a maximum of 80 percent over a temperature range of 30-34°C (see Figure 5.5) and the H₂ production reached a maximum at 32°C (see Figure 5.6). Thus a temperature of 32°C was chosen for further study.

5.1.3 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 5.7-5.9. 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 5.7). Liquid flow rate showed little effect on CO conversion (Figure 6.8) or H₂ production (Figure 5.9). A liquid flow rate of 850 mL/day was chosen for further study.

5.1.4 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 5.10-5.12. As is noted in Figure 5.10, 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

Figure 5.4. The Effects of Temperature on Cell Concentration using *R. rubrum* in the CSTR

Figure 5.5. The Effects of Temperature on CO Conversion using *R. rubrum* in the CSTR

Figure 5.6. The Effects of Temperature on H₂ Production using *R. rubrum* in the CSTR

Figure 5.7. The Effects of Liquid Flow Rate on Cell Concentration using *R. rubrum* in the CSTR

Figure 5.8. The Effects of Liquid Flow Rate on CO Conversion using *R. rubrum* in the CSTR

Figure 5.9. The Effects of Liquid Flow Rate on H₂ Production using *R. rubrum* in the CSTR

Figure 5.10. The Effects of Light Intensity on Cell Concentration using *R. rubrum* in the CSTR

Figure 5.11. The Effects of Light Intensity on CO Conversion using *R. rubrum* in the CSTR

Figure 5.12. The Effects of Light Intensity on H₂ Production using *R. rubrum* in the CSTR

offered, light intensity had some effect on CO conversion (Figure 5.11) and H₂ production (Figure 5.12). For economical reasons, a low light intensity range of 1050-1150 lux was chosen for further study.

5.1.5 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 5.13 and 5.14. As is noted in Figure 5.13, gas flow rate had no effect on cell concentration. Gas flow rate significantly affected CO conversion (see Figure 5.14) decreasing nearly linearly (with scatter) with gas flow rate. The conversion ranged from 80 percent to 30 percent throughout the course of the study. H₂ 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.

5.2 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.

Figure 5.13. The Effects of Gas Flow Rate on Cell Concentration using *R. rubrum* in the CSTR

Figure 5.14. The Effects of Gas Flow Rate on CO Conversion using *R. rubrum* in the CSTR

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. 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 5.15.

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 5.1 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

Figure 5.15. Schematic Diagram of the Experimental Set-up for the 14.0 L Fermenter

Table 5.1

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 ⁰ 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

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 5.16 shows results 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 5.17 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 5.18 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

Figure 5.16. The Effect of Gas Retention Time on Cell Concentration, CO Conversion and H₂ Production using *R. rubrum* in the 2.5 L CSTR

Figure 5.17. 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 5.18. The Effect of Agitation Rate on Cell Concentration, CO Conversion and H₂ Production using *R. rubrum* in the 2.5 L CSTR

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 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 5.19 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 5.20 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

Figure 5.19. 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 5.20. The Effect of Gas Retention Time on Cell Concentration, CO Conversion and H₂ Production using *R. rubrum* in the 5.0 L CSTR

2 of 2

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

Figure 5.21 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 5.22 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

Figure 5.21. The Effect of Agitation Rate on Cell Concentration, CO Conversion and H₂ Production using *R. rubrum* in the 5.0 L CSTR

Figure 5.22. The Effect of Gas Retention Time on Cell Concentration, CO Conversion and H₂ Production using *R. rubrum* in the 14.0 L CSTR

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 5.23 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 5.3 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.

Figure 5.23. 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 5.2

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 5.3

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

6.0 CONCLUSIONS

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.

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