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TOPICAL REPORT

HIGH PRESSURE SYNTHESIS GAS CONVERSION

Task 3: High Pressure Profiles

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212

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TABLE OF CONTENTS

	<u>Page</u>
1.0 INTRODUCTION.....	1
1.1 Purpose.....	3
2.0 BIOCONVERSION OF SYNTHESIS GAS.....	4
2.1 Gas-Liquid Mass Transfer Concepts.....	4
2.2 Bioreactors for Synthesis Gas Fermentations.....	6
2.2.1 Stirred-Tank Reactor.....	6
2.2.2 Immobilized Cell Column (ICR).....	1
2.3 Effects of Pressure.....	10
3.0 EXPERIMENTAL PROCEDURES.....	15
4.0 RESULT AND DISCUSSION.....	18
4.1 Initial Start-up Data.....	18
4.2 Operational Difficulties.....	23
4.3 <i>C. ljungdahlii</i> Studies after Correction of Operational Difficulties.....	24
4.4 <i>R. rubrum</i> Studies.....	28
4.5 Improved Start-up with <i>C. ljungdahlii</i>	34
4.6 Stabilized Operation.....	43
4.7 Gas Flow Rate Profiles.....	47
5.0 CONCLUSIONS.....	52
6.0 REFERENCES.....	53

LIST OF TABLES

	<u>Page</u>
3.1 Media Used in High Pressure CSTR Study with <i>C. ljungdahlii</i>	17
3.2 Medium Composition for <i>R. rubrum</i>	19
4.1 Operating Conditions for <i>R. rubrum</i> in the Trickle Bed Reactor.....	33
4.2 Operating Conditions Used in Figures 4.14-4.16 for the High Pressure CSTR with <i>C. ljungdahlii</i>	42
4.3 Summary of <i>C. ljungdahlii</i> Performance at Increased Pressure.....	48

LIST OF FIGURES

	<u>Page</u>
2.1 Model Verification and Evaluation of the Volumetric Mass Transfer Coefficient in a CSTR.....	8
2.2 Syngas Conversion as a Function of Pseudo Retention Time and Mass Transfer Coefficient in a CSTR.....	9
2.3 Syngas Conversion as a Function of Pseudo Retention Time and Mass Transfer Coefficient in an ICR.....	12
2.4 CO Consumption as a Function of Time at Increased CO Partial Pressure.....	14
2.5 Productivity as a Function of Total Pressure and Pseudo Retention Time in a CSTR.....	16
4.1 Cell Concentration Measurements During Start-up of the High Pressure CSTR with <i>C. ljungdahlii</i>	20
4.2 Products Concentration Measurements During Start-up of the High Pressure CSTR with <i>C. ljungdahlii</i>	21
4.3 CO and H ₂ Conversions During Start-up of the High Pressure CSTR with <i>C. ljungdahlii</i>	22
4.4 Cell Concentration Measurements in the High Pressure CSTR with <i>C. ljungdahlii</i> (P = 150 psig).....	25
4.5 Product Concentration Measurements in the High Pressure CSTR with <i>C. ljungdahlii</i> (P = 150 psig).....	26
4.6 CO and H ₂ Conversions in the High Pressure CSTR with <i>C. ljungdahlii</i> (P = 150 psig).....	27
4.7 Cell Concentration Measurements in the High Pressure Trickle Bed Reactor with <i>R. rubrum</i>	29
4.8 Acetate Concentration Measurements in the High Pressure Trickle Bed Reactor with <i>R. rubrum</i>	30
4.9 CO Conversions in the High Pressure Trickle Bed Reactor with <i>R. rubrum</i>	31
4.10 Hydrogen Concentration Measurements in the High Pressure Trickle Bed Reactor with <i>R. rubrum</i>	32
4.11 Steady State Cell Concentration Profiles in the High Pressure Trickle Bed Reactor with <i>R. rubrum</i> at 100 psig.....	35
4.12 Steady State CO Conversions Profiles in the High Pressure Trickle Bed Reactor with <i>R. rubrum</i> at 100 psig.....	36
4.13 H ₂ Yields from CO and H ₂ O by <i>R. rubrum</i> in the High Pressure Trickle Bed Reactor at 100 psig.....	37
4.14 Cell Concentration Measurements in the High Pressure CSTR with <i>C. ljungdahlii</i> During Gradual Pressure Acclimation.....	39
4.15 CO and H ₂ Conversions in the High Pressure CSTR with <i>C. ljungdahlii</i> During Gradual Pressure Acclimation.....	40
4.16 Product Concentration Measurements in the High Pressure CSTR with <i>C. ljungdahlii</i> During Gradual Pressure Acclimation.....	41
4.17 CO and H ₂ Conversions by <i>C. ljungdahlii</i> at 40 psig (G = 6 SCCM, L = 0.2 mL/min).....	44
4.18 Cell Concentration Profile for <i>C. ljungdahlii</i> at 40 psig (G = 6 SCCM, L = 0.2 mL/min).....	45
4.19 Product Concentrations for <i>C. ljungdahlii</i> at 40 psig (G = 6 SCCM, L = 0.2 mL/min).....	46

	<u>Page</u>
4.20 CO and H ₂ Conversions by <i>C. ljungdahlii</i> at 40 psig for Various Gas Flow Rates (L = 0.2 mL/min).....	49
4.21 Cell Concentration Profile for <i>C. ljungdahlii</i> at 40 psig for Various Gas Flow Rates (L = 0.2 mL/min).....	50
4.22 Product Concentrations for <i>C. ljungdahlii</i> at 40 psig for Various Gas Flow rates (L = 0.2 mL/min).....	51

1.0 INTRODUCTION

It has recently been demonstrated that the components of coal synthesis gas can be converted biologically into liquid fuel (ethanol). Biocatalytic processes have several advantages over catalytic processes, such as higher specificity, higher yields, and lower energy costs. These microorganisms have been shown to be resistant to poisoning by sulfur gases. Also, the irreversible nature of biological reactions allows complete conversion without thermodynamic equilibrium limitations.

The major disadvantage of biological processes is slow reaction rates. Retention times of several hours are common for most fermentations, compared with retention times of seconds for catalytic conversions. Such long reaction times would be prohibitive when converting large volumes of synthesis gas. However, equivalent retention times of minutes have been achieved for synthesis gas fermentations, and reaction times of seconds are believed to be possible with the concepts that will be used in this study. With retention times of seconds, the rapid commercialization of the biological production of liquid fuels from synthesis gas would be inevitable.

Recently, an anaerobic mesophilic bacterium was isolated that is capable of converting CO, H₂, and CO₂ into ethanol (Klasson *et al.* 1989; Barik *et al.* 1987). Identification and characterization studies have shown this organism to be a new clostridial strain; named *Clostridium ljungdahlii* (Vega *et al.* 1989a). Ethanol is produced from CO/H₂O or CO₂/H₂ as follows:

Acetic acid is also produced according to:

Work with this culture has demonstrated that the production of ethanol is favored under non-growth conditions. Therefore, the maintenance of conditions not conducive to growth, such as non-optimal temperature or pH and the limitation of nutrients and carbon sources, have resulted in enhancing the ethanol production by sixty times. When operating a continuous reactor where non-growth conditions are maintained, acetate production has been completely eliminated.

Synthesis gas fermentations require the transport of the gaseous substrate from the gas phase, through the liquid phase, and into the solid microorganism for reaction. This heterogeneous system is generally mass transfer limited due to the very low solubilities of H_2 and CO . As will be shown later, the transport rate (and the reaction rate), $\frac{dN_S^G}{dt}$, under mass transfer limited conditions is given by:

$$\frac{dN_S^G}{V_L dt} = \frac{K_L a}{H} P_S^G \quad (1.5)$$

where N_S^G - mols of gas transported from gas phase

V_L - liquid volume of reactor

t - time

$K_L a$ - mass transfer coefficient

H - Henry's law constant

P_S^G - partial pressure of substrate in gas phase

As noted in Equation (1.5), the reaction rate is directly proportional to the partial pressure of the gaseous substrates. Hence, the reaction rate and

reactor size are proportional to total pressure in the fermenter. There will, of course, be an upper limit of pressure that the microorganisms can withstand and the cell concentration must match the increase in pressure. As shown later, pressures of 10 atm have been achieved successfully in our laboratory in Parr reactors, without continuous liquid flow. These experiments have confirmed that the reaction rate is proportional to pressure as shown by Equation (5), at least up to 10 atm, the limit of present experimental equipment. Most organisms will withstand much higher pressures than 10 atm, and reaction times of a few seconds would be possible at a pressure of 100 atm, for example. The pressure limits of these organisms are not known, but are probably much higher than present experimental equipment can achieve. Higher pressure fermenters cannot be purchased, and must be fabricated and assembled.

The purpose of this research project is to build and test a high pressure fermentation system for the production of ethanol from synthesis gas. The fermenters, pumps, controls, and analytical system were procured or fabricated and assembled in our laboratory. This system was then used to determine the effects of high pressure on growth and ethanol production by *C. ljungdahlii*. The limits of cell concentration and mass transport relationships were found in CSTR and immobilized cell reactors (ICR). The minimum retention times and reactor volumes were found for ethanol production in these reactors.

1.1 Purpose

The purpose of this report is to present operating data *C. ljungdahlii* at increased pressure as a function of the major operating variables. The start-up of the reactor system is first discussed, followed by steady state operation at a chosen pressure of 40 psig.

2.0 BIOCONVERSION OF SYNTHESIS GAS

The production of liquid fuel from synthesis gas will require processing large quantities of gas. Therefore, to obtain reasonable reactor sizes and favorable costs, the gas retention times must be short for high conversion levels. The choice of a suitable bioreactor for synthesis gas fermentations will be a matter of matching reaction kinetics with the capabilities of the various reactors. It has been found that for these slightly soluble gases, the rate of mass transfer usually controls the reactor size (Vegaet *al.* 1989b, 1989c). Mass transfer capabilities of the reactor must be balanced with the cell density achieved. The proper reactors for these systems will likely be ones that achieve high mass transfer rates and high cell densities.

2.1 Gas-Liquid Mass Transfer Concepts

The transfer of gas phase substrates in fermentation systems involves three heterogeneous phases: the bulk gas phase, the culture medium (liquid) and microbial cells (solid) suspended in the medium. The reactants, present in the gas phase, must be transported across the gas-liquid interface and diffuse through the culture medium to the cell surface to be consumed by the microbes. In general, a combination of the following resistances can be expected (Bailey and Ollis, 1986):

1. Diffusion through the bulk gas to the gas-liquid interface.
2. Movement across the gas-liquid interface.
3. Diffusion of the solute through the relatively unmixed liquid region (film) adjacent to the bubble and into the well-mixed bulk liquid.
4. Transport of the solute through the bulk liquid to the stagnant film surrounding the microbial species.

5. Transport through the second unmixed liquid film associated with the microbes.
6. Diffusive transport across the liquid/solid boundary and into the microbial floc, mycelia, or particle, if appropriate. When the microbes take the form of individual cells, this resistance disappears.
7. Transport across the cell envelope to the intracellular reaction site.

As with the conventional chemical engineering analysis of absorption processes, mass transfer through the bulk gas phase is assumed to be instantaneous. Also, when individual cells are suspended in a medium, the liquid film resistance around the cells is usually neglected with respect to other resistances, because of the minute size and the enormous total surface of the cells (Finn, 1954). Thus, for the transfer of sparingly soluble gases, such as CO, the primary resistance to transport may be assumed to be in the liquid film at the gas-liquid interface. The rate of transport from the gas phase must be equal to the rate of consumption in the liquid phase, given by a Monod relationship:

$$\frac{d N_S^G}{V_L dt} = \frac{X q_m P_S^L}{K_p' + P_S^L + (P_S^L)^2/W'} = \frac{K_L a}{H} (P_S^G - P_S^L) \quad (2.1)$$

where X is cell concentration and q_m , K_p' , and W' are Monod constants. The right side of this equation represents the gas transport rate into the liquid phase.

Equation (2.1) shows that a bioreactor for these gaseous systems must operate in either of two regimes. In one case, sufficient cells are present to react more solute, but the mass-transfer rate cannot keep pace. Therefore, the liquid phase concentration P_S^L , goes to zero and the reactor is mass

transport limited. The cell concentration and rate of consumption are limited by the ability of that particular reactor to transfer substrate. In the other case, sufficient substrate can be supplied, but the cell concentration does not allow consumption at an equal rate. Then the liquid phase concentration is not zero (with possible inhibitory effects) and the rate is limited by the cell concentration in that particular bioreactor. Obviously, the best bioreactor is one that will achieve high cell concentrations and high mass transfer rates.

2.2 Bioreactors for Synthesis Gas Fermentations

Since large volumes of syngas must be processed, continuous reactors are dictated. Stirred-tank reactors achieve high mass transfer rates, but require substantial energy input for agitation. Immobilized cell reactors achieve high cell concentrations, without agitation, and are promising for these applications.

2.2.1 Stirred-Tank Reactor

The traditional CSTR assumes complete mixing and uniform concentrations through the bulk liquid phase. For syngas fermentations, the gas must be sparged into the liquid phase, be consumed, with any excess and product gases leaving the top of the liquid and eventually the reactor. High gas flow rates are required and nearly complete conversion of substrate is necessary. Conversely, only small liquid flow rates, essential to supply nutrients and remove liquid products, are necessary. Consequently, high cell concentrations should be possible. In most cases, the reactor volume will be controlled by the necessary gas retention time to achieve the desired conversion of substrate. Relatively high agitation rates will be required to promote transfer of the slightly soluble gas substrate.

Mass transfer coefficients, necessary for prediction of CSTR performance and scale-up, may be obtained from an analysis of the operation under mass-transfer limited conditions. A material balance around the CSTR with perfect mixing gives the relationship defining concentrations:

$$\frac{1}{Y_0} = \frac{1}{Y_i} + \frac{V_L}{Y_i} \frac{K_L a}{H} \frac{P_I^G}{n_I} \quad (2.2)$$

Equation (2.2) is expressed in terms of an inert component, whose quantity and partial pressure do not change through the system. Therefore, to simplify the model, concentrations are in the ratio of substrate to inert (Y), with Y_0 at the outlet and Y_i at the inlet. P_I^G is the partial pressure of inert in the gas stream and n_I is the molar flow rate of inert.

The agreement of this model with experimental data is shown in Figure 2.1. Good agreement is achieved with a linear relationship. The slope of this line gives the mass transfer coefficient, $K_L a/H = 30$. A model including Equation (2.2), as well as material balances for the gases flowing into the reactor and equilibrium relationships for the gas phase CO_2 with the bicarbonate and the pH level in the liquid, has been developed (Vega et al. 1989c). Solutions of the model for various volumetric mass transfer coefficients and various total operating pressures are shown in Figure 2.2. As observed, increases in the mass transfer coefficient or in total operating pressure lead to higher reactor productivities. Due to the perfect mixing in a CSTR, complete conversion is only possible when the gas flow rate is very low.

Figure 2.2 shows that with a mass transfer coefficient of 100, a pseudo retention time of one hour would result in a conversion of 80 percent. This retention time is the liquid volume divided by the gas flow rate and is the limit that defines reaction volume. Alternatively, a retention time of 2 hours is necessary for conversion of 90 percent of the entering gas.

Figure 2.1. Model Verification and Evaluation of the Volumetric Mass Transfer Coefficient in a CSTR.

Figure 2.2. Syngas Conversion as a Function of Pseudo Retention Time and Mass Transfer Coefficient in a CSTR.

2.2.2 Immobilized Cell Column (ICR)

Column fermenters, with immobilized or suspended cells, offer the advantages of high cell densities and plug flow operation. These systems do not require mechanical agitation, with mixing provided by counter flow of gas and liquid. Energy for mixing is supplied by gas pressure drop and such systems are potentially more economical than the CSTR. Packed columns also offer the advantages of high surface to volume ratios and high mass transfer rates with reduced back-mixing.

Whole cell immobilization techniques can be classified into two major groups, entrapment and carrier binding (Vega *et al.* 1988). Entrapment includes both enclosure of a catalyst behind a membrane or within a gel structure. Carrier binding includes all methods where there is a direct binding of cells to water-soluble carriers by physical adsorption or by ionic and/or covalent bonds. Potential mass transfer limitations are always present with entrapment systems, either across the gel matrix or gel occlusion, or across the system membrane. On the other hand, the carrier-binding methods allow direct contact between the fermentation broth and the biocatalyst, with potentially enhanced mass transfer rates.

Microorganisms can be immobilized to insoluble biosupport materials by two methods: crosslinking and adsorption. Crosslinking, or covalent bonding, involves the use of a chemical agent, like glutaraldehyde or cyanuric chloride, to link the cells to the support. The chemical reaction is between the hydroxyl or lipid groups in the cell wall and a durable coating, like gelatin or agar, applied to the packing. Adsorption is the physical (occasionally ionic) attachment of the cell to the support. This method has been found to be effective for some small bacteria that can adhere to crevices in a support like wood chips.

In these reactors, the microorganisms are in direct contact with the substrate, minimizing diffusional resistance. These packed columns operate close to plug flow and, thereby, offer kinetic advantages for these reactions. Cells attached to the support grow and multiply into a film, which may be several layers of cells in thickness. In fact, cell overgrowth can result in completely filling the interstitial spaces, such that channeling may be a problem. Therefore, high cell densities and low retention times are possible.

By combining a material balance along the column with the rate expression for gas transport into the liquid phase, the following expression for the ratio of partial pressures of gaseous reactant entering and leaving the reactor is obtained:

$$\ln \frac{P_S^0}{P_S^1} = \frac{K_L a}{H} \frac{\epsilon_L h R T S}{G} \quad (2.3)$$

where ϵ_L is the fraction of liquid in the column, h is the height of the column, S is the cross-sectional area of the column, R is the ideal gas constant, T is the absolute temperature, and G is the gas flow rate. A plot of $\ln Y_i/Y_0$ vs $ShRT/G$ yields a straight line with slope $K_L a \epsilon_L / H$. The numerical solution (Runge-Kutta) of the differential equations that describe the system was solved for other operating conditions and is shown in Figure 2.3. Experimental data are given for $K_L a \epsilon_L / H$ of 13.5.

The immobilized cell column achieves higher rates of specific CO conversion than the stirred-tank reactor without the need for more expensive mechanical agitation. More importantly, at the same mass transfer coefficients as in the CSTR, conversions are substantially higher. For example, at $K_L a / H = 100$, the conversion at a one hour retention time is 95

Figure 2.3. Syngas Conversion as a Function of Pseudo Retention Time and Mass Transfer Coefficient in an ICR.

percent, compared to 80 percent for the CSTR. Alternatively 90 percent conversion could be achieved in a retention time only 30 minutes in the ICR. The major disadvantage of the ICR is the lack of flexibility in operating conditions since the contacting capabilities are mainly fixed with the design of the column dimensions and packing.

2.3 Effects of Pressure

Under mass transfer limited conditions, the liquid phase composition, P_S^L , in Equation (2.1) becomes zero and the reaction rate reduces to:

$$\frac{d N_S^G}{V_L dt} = \frac{K_L a}{H} P_S^G \quad (1.5)$$

For maximum productivity, bioreactors for synthesis gas fermentations should achieve high cell concentrations and always operate under mass transfer limitations. Consequently, the reactor volume will be proportional to an increase in total pressure, as shown by Equation (1.5).

Experiments have been conducted at elevated pressures with synthesis gas. If the pressure of the culture is suddenly increased to as little as three atm, the culture fails. This failure is due to inhibitory effects of higher CO concentrations in the liquid phase, rather than pressure limitations of the cells (Vega *et al.* 1989b). Dissolved CO tensions of 0.8 atm have been found to be inhibitory.

Figure 2.4 shows the results of experiments where the pressure was increased gradually in a stepwise fashion. As noted, this culture performed well at pressures as high as 10 atm. The CO consumption was even more rapid at 10 atm than at lower pressures. Therefore, acclimation of the cultures and higher cell concentrations are necessary for operation at elevated pressure. The pressure limit of existing laboratory fermentation equipment is 10 atm.

Figure 2.4. CO Consumption as a Function of Time at Increased CO Partial Pressures.

The foregoing data show that Equation (1.5) holds and that reaction rate is proportional to pressure, at least up to 10 atm. The effect of elevated pressure on the retention time is given in Figure 2.5. For a retention time of 30 min for the ICR at one atm, the reaction rate, Q , is ten times higher at 10 atm. Consequently, the equivalent retention time can be reduced to 3 minutes for this bioreactor. If the pressure could be increased to 100 atm, the equivalent retention time would be reduced to 18 seconds.

With these short reaction times, the reactor volume to convert 90 percent of the synthesis gas from a 50×10^6 CF per day Texaco gasifier would be only about 20 ft. in diameter by 20 ft. high. This nominal reactor size makes the economics of this technology very favorable. However, present laboratory fermentation equipment does not permit conducting these higher pressure experiments.

3.0 EXPERIMENTAL PROCEDURES

The high pressure CSTR was started up using the bacterium *C. ljungdahlii*. Since start-up in continuous vessels with this bacterium has been slow in the past, it was expected that the start-up at increased pressure could also be time consuming.

As is shown in Table 3.1, three media were employed in the start-up studies. Medium I, a mixture of vitamins, minerals and metals found particularly effective in growing *C. ljungdahlii* and producing ethanol in favor of acetate, was used from time 0 to 235 hr. Medium II, with slightly modified compositions in comparison to Medium I, was used to reduce the formation of precipitates in the system from time 236 hr to 869 hr. Finally, Medium III, which was a basal medium without yeast extract supplemented with $(\text{NH}_4)_2 \text{HPO}_4$, was used as a simple medium to promote growth during time 870 hr forward. The culture was grown and transferred to the CSTR using procedures developed previously.

Figure 2.5. Productivity as a Function of Total Pressure and Pseudo Retention Time in a CSTR.

Table 3.1

Media Used in High Pressure CSTR Study
with *C. ljungdahlii*

<u>Component</u>	<u>Concentration (g/L)</u>		
	I	II	III
(NH ₄) ₂ HPO ₄		2.0	2.0
MgCl ₂ •6H ₂ O		0.5	0.3
CaCl ₂ •2H ₂ O		0.2	0.05
KCl		0.15	0.15
NaCl		0.2	0.2
PFN-Trace Metals		10 mL	5 mL
JRP-vitamins		1 mL	1 mL
B-vitamins		0.25 mL	0.25 mL
KH ₂ PO ₄		--	--
NH ₄ Cl		--	--
H ₃ PO ₄		1.0	0.875 mL
		(adjust to pH 4.5)	(adjust to pH 4.5)
Cysteine HCl (2.5%)		10 mL	10 mL

The CSTR was initially started with a liquid medium flow rate of 1700 mL/day, but was very quickly changed to a flow rate of 800 mL/day when good growth was not immediately observed in the reactor. The synthesis gas flow rate was 3.09 standard cm³/min during the study.

In addition, the photosynthetic bacterium *Rhodospirillum rubrum* was used for the demonstration of a rapid start-up of a high pressure gas fermentation system since the gas phase substrate (CO) is not used by this bacterium for growth. Direct pressuring is thus possible. For *R. rubrum* growth is dependent upon light intensity and the liquid medium composition. *R. rubrum* was used in the high pressure in Table 3.2. Sodium acetate and yeast extract served as the carbon and energy sources for the growth of *R. rubrum*.

4.0 RESULTS AND DISCUSSION

4.1 Initial Start-up Data

Cell concentration measurements, product concentration measurements and CO and H₂ conversions are shown as a function of time during start-up in Figures 4.1-4.3. Also shown in the figures are the operating pressures which were changed several times during the start-up period. As is noted in Figure 4.1, the cell concentrations were relatively low, ranging from 100-500 mg/L. Typical cell concentrations in the CSTR at atmospheric pressure are typically 400 mg/L. There was no correlation evident between cell concentration and pressure, indicating that a stable population had not yet been reached. Nevertheless, it was demonstrated that *C. ljungdahlii* could grow at increased pressure, at least to 120 psig.

Product concentrations, shown in Figure 4.2, showed essentially equal and low concentrations of ethanol and acetate. This result has been found to be

Table 3.2

Medium Composition for *R. rubrum*

<u>Component</u>	<u>Quantity /L</u>
Ammonium Chloride	2.70 g
B-Vitamins	5.00 mL
PFN-Minerals	50.00 mL
PFN-Trace Metals	1.00 mL
Sodium Bicarbonate	4.00 g
Sodium Acetate	5.76 g
Yeast Extract	0.50 g

Figure 4.1. Cell Concentration Measurements During Startup of the High Pressure CSTR using *C. ljungdahlii*.

Figure 4.2. Products Concentration Measurements During Start-Up of the High Pressure CSTR using *C. ljungdahlii*.

Figure 4.3. CO and H₂ Conversions During Start-Up of the High Pressure CSTR using *C. ljungdahlii*.

typical of the *C. ljungdahlii* fermentation during start-up. CO and H₂ conversions, shown in Figure 4.3 were also typically quite low during start-up (0-40%), but are expected to improve dramatically once a stable population has been established.

4.2 Operational Difficulties

Numerous operational difficulties were encountered with the high pressure system during initial start-up which have been corrected. First, it was discovered that the high pressure pump could not deliver low enough medium flow rates to effectively start the reactor. Also, the pump developed leaks around the seal due to precipitation problems in the medium, upon the introduction of NaOH for pH control. The medium was modified as was shown in Table 3.1, a timer was added to the system and the pump was replaced with a spare HPLC pump which can deliver liquid flow rates as low as 0.1 mL/min. In addition, a three-way valve was installed in-line to permit easier repair of the pump, if necessary.

A second problem involved the CSTR vessel itself. It is believed that the Parr reaction vessel may not have allowed good mass transfer of gas to the liquid because of its vertical design. Information was sought from Parr relative to internal mixing in the reactor and the possibility of obtaining substitute reaction vessels.

Finally, it was observed that the adjustable check valve system, used to control system pressure, allows the pressure to fluctuate too much about the desired pressure. This problem is being studied with a solution to be resolved soon.

4.3 *C. ljungdahlii* Studies After Correction of Operational Difficulties

The numerous technical problems that were encountered in starting the reactor with *C. ljungdahlii* in the CSTR have been corrected. *C. ljungdahlii* was next utilized in the CSTR at 150 psig for an extended time period.

Cell concentration measurements, product concentration measurements and CO and H₂ conversions at 150 psig are shown as a function of time in Figures 4.4-4.6. The reactor volume in these studies was 600 mL and the volume in the watch glass was 210 mL. Thus, the total system volume was 510 mL. With a total pressure of 150 psig, the gas flow rate was 2 mL/min, the liquid medium rate was 0.2 mL/min and the agitation rate was varied from 200-500 rpm. As is noted in Figure 4.4, the cell concentration remained constant but at a relatively low level indicating that CO was probably inhibiting growth. Typical cell concentrations in the CSTR at atmospheric pressure were approximately 400 mg/L. The information presented in Figure 4.4 indicates that a stepwise process of gradually increasing pressure and cell concentration, while keeping the dissolved CO concentration low, is necessary. Data with this technique will be presented later.

Product concentrations, shown in Figure 4.5, showed that ethanol was the dominant product probably since CO was not zero in the liquid phase. The maximum ethanol concentration was 4.7 g/L, with the corresponding acetate concentration reaching 0.4 g/L. These concentrations are unfortunately lower than expected at 150 psig. CO and H₂ conversions, shown in Figure 4.6, were quite variable. Cell concentrations, product concentrations and conversions are expected to improve dramatically once a stable population has been established. The key seems to be to establish a high, stable cell

Figure 4.4. Cell Concentration Measurements in the High Pressure CSTR with *C. ljungdahlii* ($P = 150$ psig).

Figure 4.5. Product Concentration Measurements in the High Pressure CSTR with *C. ljungdahlii* ($P = 150$ psig).

Figure 4.6. CO and H₂ Conversions in the High Pressure CSTR with *C. ljungdahlii* ($P = 150$ psig).

concentration which will permit high CO and H₂ conversions and high product concentrations. As mentioned previously, this technique is presently being used.

4.4 *R. rubrum* Studies

As was mentioned previously, the *R. rubrum* system was presented to demonstrate rapid start-up with a system not requiring gradual pressure acclimation. This is possible with the *R. rubrum* system since cell concentration does not depend upon the CO concentration at all, but is instead dependent upon the carbon source in the liquid medium and the light supply. Furthermore, it has been established for this organism that CO conversion is independent of light intensity.

Preliminary results with *R. rubrum* in the high pressure trickle bed reactor are shown in Figures 4.7-4.10. Since the conditions were changed often in these initial studies, the conditions employed in the trickle bed are shown in Table 4.1.

Cell concentration measurements in Figure 4.7 show that, as expected, light intensity alone set the cell concentration in the reactor. After the initial start-up (Section A and part of Section B), a cell concentration of about 450 mg/L was obtained when using 40 W bulbs and the steady state cell concentration had not yet been reached with the 60 W bulbs. Acetate, the carbon source for the growth of *R. rubrum*, was not completely depleted during the studies and thus did not limit the cell concentration (see Figure 4.8).

The CO conversions, shown in Figure 4.9 show a 40 percent conversion with the 40 W bulbs at 80 psig, little to no CO conversion with the 25 W bulbs at 50 psig and a 35 percent conversion with 50 psig and 50 w bulbs. The H₂

Figure 4.7. Cell Concentration Measurements in the High Pressure Trickle Bed Reactor with *R. rubrum*.

Figure 4.8. Acetate Concentration Measurements in the High Pressure Trickle Bed Reactor with *R. rubrum*.

Figure 4.9. CO Conversions in the High Pressure Trickle Bed Reactor with *R. rubrum*.

Figure 4.10. Hydrogen Concentration Measurements in the High Pressure Trickle Bed Reactor with *R. rubrum*.

Table 4.1

Operating Conditions for *R. rubrum* in the
Trickle Bed Reactor

<u>Operating Conditions</u>	<u>Range in Figures 4-7</u>				<u>E</u>
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	
Pressure, psig:	0	10-80	50	50	50
Gas Flow Rate, mL/min:	2	2	1-2	1	1
Liquid Flow Rate, mL/min:	0	0.55	0.17	0.03-0.2	0.2
Recycle Rate, mL/min:	500	500	500	500	500
Light Intensity, W (bulbs)	40	40	25	40	60

concentrations, shown in Figure 4.10, were stoichiometric, but are a bit difficult to follow.

Additional steady state results with *R. rubrum* in the high pressure trickle bed reactor at a pressure of 100 psig are shown in Figures 4.11-4.13. As is noted, the gas flow rate was varied from 3-6 mL/min (STP), while the liquid flow rate was held constant at 0.2 mL/min, the light intensity was 60 W, and the recycle rate was 500 mL/min.

Cell concentration measurements in Figure 4.11 show that, as expected, light intensity alone set the cell concentration in the reactor. A cell concentration of about 300 mg/L was obtained when using the 60 W bulbs. Acetate, the carbon source for the growth of *R. rubrum*, was not completely depleted during the studies and thus did not limit the cell concentration.

The CO conversions, shown in Figure 4.12, varied significantly even though long periods of time were allowed to reach steady-state. Similarly, the H₂ production yields varied from 0-1.0 (stoichiometric). It is felt that the use of higher gas flow rates might result in more stable operation of *R. rubrum* in the reactor.

In general, the operation of the high pressure trickle bed reactor was very good within the limitations of the system. Thus, the *R. rubrum* system (without light limitation) illustrates the potential of the high pressure gas fermentation system.

4.5 Improved Start-up with *C. ljungdahlii*

Previous studies with *C. ljungdahlii* in the high pressure CSTR showed that the system could operate successfully at pressures as high as 150 psig. The cell concentration in these studies, however, was only 100 mg/L, a

Figure 4.11. Steady State Cell Concentration Profiles in the High Pressure Trickle Bed Reactor with *R. rubrum* at 100

Figure 4.12. Steady State CO Conversions in the High Pressure Trickle Bed Reactor with *R. rubrum* at 100 psig.

Figure 4.13. H_2 Yields from CO and H_2O by *R. rubrum* in the Hig Pressure Trickle Bed Reactor at 100 psig.

concentration that is only 25 percent of the typical concentration in the CSTR at atmospheric pressure. Furthermore, the CO and H₂ conversions were quite variable and generally around the 50 percent level. Higher cell concentrations are imperative to maintain mass transfer limiting conditions inside the CSTR at elevated pressure. A very gradual experimental program was thus initiated in an effort to maintain higher cell concentrations and gas conversions at elevated pressure.

Cell concentration measurements, CO and H₂ conversions, and product concentration measurements are shown as a function of time for various operating conditions in Figures 4.14-4.16. A legend for the operating conditions shown in the figures is presented in Table 4.2. The reactor volume in these studies was 600 mL and the volume in the watch glass was 210 mL. Thus, the total system volume was 810 mL. The gas flow rate ranged from 2-6 mL/min, the liquid medium rate was 0.2-0.29 mL/min and the agitation rate was 500 rpm. As is noted in Figure 4.14, the cell concentration remained relatively constant at 400-600 mg/L in Regions A and B before falling to near zero in Phase C. This represents an unsuccessful start-up of the culture. During this start-up to 35 psig, the CO and H₂ conversions were generally less than 50 percent (see Figure 4.15). A more successful start-up is shown in Regions C-G, where the pressure was very gradually increased from 5-32 psig over a time period of 1200 hr. The cell concentration ranged from 200-600 mg/L, but still unfortunately decreased with pressure, although not to as great an extent as was shown previously (see Figure 4.14). The CO conversions were maintained, however, at 70-80 percent, while the H₂ conversions ranged from 50-80 percent (see Figure 4.15). This procedure is thought to be much better than the more rapid start-up of earlier studies.

Figure 4.14. Cell Concentration Measurements in the High Pressure CSTR with *C. ljungdahlii* During Gradual Pressure Acclimation.

Figure 4.15. CO and H₂ Conversions in the High Pressure CSTR with *C. ljungdahlii* During Gradual Pressure Acclimation.

Figure 4.16. Product Concentration Measurements in the High Pressure CSTR with *C. ljungdahlii* During Gradual Pressure Acclimation.

Table 4.2

Operating Conditions Used in Figures 4.14-4.16 for the High
PressureCSTR with *C. ljungdahlii*

<u>Region</u>	<u>Conditions</u>
A	Gas flow rate (G) = 6 mL/min, total pressure (P) = 5 psig, liquid flow rate (L) = 0.2 mL/min
B	G reduced from 6-2 mL/min over time, P = 12.5 psig, L = 0.2 mL/min
C	G reduced from 5-3 mL/min over time, P = 35 psig, L = 0.2 mL/min
D	G increased from 3-5.2 mL/min over time, P = 5 psig, L = 0.2 mL/min
E	G = 4 mL/min, P = 5 psig, L = 0.2 mL/min
F	G = 4 mL/min, P = 16 psig, L = 0.24 mL/min 60 mL inoculum added at end of Region F
G	G = 5 mL/min, P = 16 psig, L = 0.24 mL/min 180 mL inoculum grown on 2 g/L trypticase, peptone and 1/2 modified basal medium added during the latter stages of Region G
H	G = 6 mL/min, P = 32 psig, L = 0.24 mL/min

The product concentrations in these gradual start-up studies was 6-12 g/L acetate and 0-1 g/L ethanol (see Figure 4.16). This poor ratio is of little concern, however, since it is well known that the culture will eventually shift to ethanol. This more gradual procedure will be utilized in subsequent studies at elevated pressure.

4.6 Stabilized Operation

Operating data obtained for *C. ljungdahlii* in the high pressure CSTR at 40 psig are shown in Figures 4.17-4.19. The gas flow rate to the system was 6 mL/min (at standard conditions) and the liquid flow rate was 0.2 mL/min. The agitation rate in these studies was 400-450 rpm and the temperature was maintained at 37°C and the reactor volume was 600 mL.

As is noted in Figure 4.17, the conversions of both CO and H₂ were quite steady over nearly 600 hr of operation, with a CO conversion of about 70 percent and a H₂ conversion of about 60 percent. The cell concentration over the same period, shown in Figure 4.18, was also fairly steady, with an average value of about 180 mg/L. The lack of a constant cell concentration at a given pressure has been the major operating problem in previous studies. In the past, the cell concentration has gradually and steadily fallen throughout the course of an experiment at increased pressure. This problem has been corrected as evidenced by the steady cell concentration at 40 psig for nearly 600 hr. Figure 4.19 shows the product ethanol and acetate concentrations throughout the study. Although these concentrations are quite constant, the average acetate concentration is about 8.5 g/L, while the average ethanol concentration is only 0.04 g/L. Product ratio continues to be a problem in this increased pressure system.

Figure 4.17. CO and H₂ Conversions by *C. ljungdahlii* at 40 psig
(G = 6 sccm, L = 0.2 mL/min)

Figure 4.18. Cell Concentration Profile for *C. ljungdahlii* at 40 psig
(G = 6 sccm, L = 0.2 mL/min)

Figure 4.19. Product Concentrations for *C. ljungdahlii* at 40 psig
(G = 6 sccm, L = 0.2 mL/min)

Table 4.3 presents a summary of steady state data for *C. ljungdahlii* at pressures ranging from 5 to 80 psig. In these studies the liquid flow rate was maintained at 0.2 mL/min and the gas flow rate was varied from 6 to 18 standard mL/min (sccm). In general, the CO and H₂ conversions decreased with gas flow rate, but were not affected in any pattern by pressure. For example, at a gas flow rate of 6 sccm, the CO conversion was 76 percent at 80 psig, 72 percent at 60 psig and 70 percent at 40 psig. The cell concentrations generally decreased with pressure, ranging from 438 mg/L at 5 psig to 136 mg/L at 80 psig. The ethanol concentrations were unfortunately low at each condition, showing a maximum of 0.25 g/L at 20 psig with a gas flow rate of 12 sccm. The acetate concentration ranged from 2-10 g/L, with no clear pattern established for acetate concentration with pressure or gas flow rate.

4.7 Gas Flow Rate Profiles

The gas flow rate or gas retention time is known to be the most important parameter affecting gas conversion (and possibly product ratio) in the CSTR. Liquid flow rate or dilution rate is known to set the cell concentration in the CSTR, at least at atmospheric pressure.

An operating pressure of 40 psig was chosen as a representative pressure at which to study the effects of gas flow rate on increased pressure operation with *C. ljungdahlii*. The liquid flow rate was set at 0.2 mL/min (0.02 hr⁻¹ liquid dilution rate). The effects of gas flow rate on gas conversion, cell concentration and products concentration at 40 psig are shown in Figures 4.20-4.22. As is noted in Figure 4.20, the conversions of both CO and H₂ were shown to decrease gently with increasing gas flow rate. The CO and H₂ conversions were both about 60 percent at a gas flow rate of 6 SCCM (100 min

Table 4.3

Summary of *C. ljungdahlii* Performance at Increased Pressure

Pressure psig	G sccm	L mL/min	X mg/L	% Conv. CO	% Conv. H ₂	EtOH g/L	HAc g/L
5	6.00	0.2	437.9	58.95	41.37	0.00	2.09
20	12.00	0.2	111.8	44.01	13.32	0.25	6.04
40	6.00	0.2	179.1	69.82	59.26	0.04	8.56
40	12.00	0.2	200.1	34.38	9.16	0.00	9.64
40	15.00	0.2	240.0	25.50	10.79	0.07	9.41
40	18.00	0.2	298.7	29.25	2.84	0.00	7.70
60	6.00	0.2	150.1	72.34	64.48	0.02	10.10
80	6.00	0.2	136.3	75.71	52.72	0.00	8.81

Figure 4.20. CO and H₂ Conversions by *C. ljungdahlii* at 40 psig for Various Gas Flow Rates ($\bar{L} = 0.2$ mL/min)

Figure 4.21. Cell Concentration Profile for *C. ljungdahlii* at 40 psig for Various Gas Flow Rates ($L = 0.2$ mL/min)

Figure 4.22. Product Concentrations for *C. ljungdahlii* at 40 psig for Various Gas Flow Rates (L = 0.2 mL/min)

gas retention time). At a gas flow rate of 18 SCCM (33.3 min gas retention time). At a gas flow rate of SCCM (33.3 min as retention time), the CO conversion was 25 percent and the H₂ conversion was about 5 percent.

Cell concentration was shown to increase very slowly with gas flow rate (see Figure 4.21), probably due to the measured availability of carbon as the flow rate is increased. The cell concentration increased from about 150 mg/L to 240 mg/L as the flow rate was increased from 6 to 18 sccm. Finally, the ethanol and acetate concentrations were 0 and 9 g/L (see Figure 4.22), indicating that the medium utilized was not suitable for high pressure operation. Higher total product concentrations and higher ethanol concentrations were expected. A long term medium study is thought to be necessary to obtain higher product ratios.

5.0 CONCLUSIONS

Cell concentration, gas conversion and product concentration profiles were presented for *C. ljungdahlii* as a function of gas flow rate, the variable which affects bacterium performance the most. This pressure was chosen as a representative pressure over the 0-150 psig operating pressure range for the bacterium. Increased pressure negatively affected ethanol productivity probably due to the fact that medium composition was designed for atmospheric pressure operation. Medium development at increased pressure is necessary for high pressure development of the system.

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