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BIOLOGICAL PRODUCTION OF ETHANOL FROM COAL

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SUMMARY

Two batch and one continuous reactor study involving Clostridium ljungdahlii were carried out. First, the effects of H_2 partial pressure on growth, CO and H_2 uptake and product formation by C. ljungdahlii were investigated in batch culture. Over the concentration range studied, it was observed that CO was preferentially utilized in favor of H_2. It was also seen that increasing H_2 partial pressures increased the ratio of ethanol to acetate.

Previous kinetic studies with Clostridium ljungdahlii at CO partial pressures of 1.6 atm and below have shown zero order equations for both the specific growth rate, μ, and the specific uptake rate, q, in terms of dissolved CO tension. When an experimental study was carried out over an increased CO partial pressure range (0-4.27 atm), zero order reactions were again obtained, indicating that the Monod saturation constants, K_p and K_p', were quite small and that substrate inhibition was not apparent over this CO partial pressure range.

Finally, a two-stage CSTR system was successfully operated with C. ljungdahlii in which growth occurred in the first stage and ethanol production occurred in the second stage. Acetate production was completely eliminated in the second stage reactor by the addition of benzyl viologen, coupled with

nutrient limitation and pH shift. However, it was seen that ethanol production in this arrangement was not as efficient as product formation in the first reactor, so that additional research is required to find the optimum reducing agent concentration and other operating conditions in the second stage of the CSTR series system.

INTRODUCTION

Clostridium ljungdahlii, a strain isolated from animal waste in the University of Arkansas laboratories, converts CO, CO_2, and H_2 in synthesis gas to ethanol and acetate by the reactions:

$$6\ CO + 3\ H_2O \rightarrow C_2H_5OH + 4\ CO_2 \quad (1)$$

$$2\ CO_2 + 6\ H_2 \rightarrow C_2H_5OH + 3\ H_2O \quad (2)$$

$$4\ CO + 2\ H_2O \rightarrow CH_3COOH + 2\ CO_2 \quad (3)$$

$$2\ CO_2 + 4\ H_2 \rightarrow CH_3COOH + 2\ H_2O \quad (4)$$

Although three components of synthesis gas are viable substrates for ethanol and acetate production by C. ljungdahlii, little is known about the preference of the bacterium for one substrate over another. Preliminary results at H_2 partial pressures below 0.26 atm showed that CO was not preferentially utilized in favor of H_2 (PETC report No. 8-89, September 1989). However, it is unknown whether there is a preferred substrate at H_2 partial pressures above 0.26 atm.

Since acetate is produced in conjunction with growth and energy production by the bacterium, its production is favored over ethanol production, which requires energy for formation. In fact, batch studies without culture manipulation showed a 20:1 ratio of acetate to ethanol. Various techniques have been employed in the University of Arkansas laboratories to significantly

improve this ratio. The addition of low concentrations (50 ppm) of reducing agent to the liquid medium in batch culture resulted in the production of equimolar amounts of ethanol and acetate. Molar product ratios in the CSTR have been as high as 3 moles ethanol per mole of acetate by utilizing pH shift and alternate medium constitutents.

Although there has been significant success in improving the product ratios in favor of ethanol production, the improved ratios have always been coupled with culture distress and even the loss of culture viability soon after the favorable product ratios were attained. Thus, a method needs to be developed for the C. ljungdahlii system that results in significant ethanol production while, at the same time, maintaining a viable culture for an extended (or even indefinite) period of time.

Another important consideration is the effect of substrate on product (ethanol:acetate) ratio. Su *et al.*[1] showed that the H_2 concentration in the medium plays an important role in regulating the ratio of ethanol to acetate by C. thermocellum. The ratio of ethanol to acetate on a molar basis increased from 0.7 to 1.7 when adding H_2 to C. thermocellum digesting cellulose at 1 atm in comparison to experiments without H_2 present. When the partial pressure of H_2 was increased to 2.5 atm, the ratio of ethanol to acetate increased to 2.0. Similarly, the ratio of ethanol to acetate increased for C. ljungdahlii when employing a H_2 partial pressure of 0.26 atm in comparison to lower H_2 partial pressures. Again, however, the effects of H_2 partial pressure above 0.26 atm is unknown.

A mass transfer/kinetic study on CO alone was carried out previously over a restricted CO partial pressure range of 0 - 1.6 atm. This pressure restriction was necessary because of the type of reactors employed in the

batch studies. Zero order equations in terms of dissolved CO tension were obtained for both the specific uptake rate and the specific growth rate:

$$q = q_{max} = 43.37 \text{ mmol CO/g·cell·hr}$$

$$\mu = \mu_{max} = 0.04 \text{ hr}^{-1}$$

In order to obtain a more accurate picture of the effects of CO on growth and uptake, the CO partial pressure range needs to be extended. Perhaps CO will be found to be an inhibitor at increased CO partial pressures or the Monod saturation constants, K_p and K_p', may be found to be more significant at higher partial pressures

The purpose of this report is to present new experimental findings in batch and continuous culture concerning the above topics. First, the effects of H_2 partial pressure on product formation is presented. Secondly, the results of a kinetic study using CO as the substrate over an extended pressure range are presented and discussed. Finally, the results of a two-stage continuous stirred tank reactor study in which acetate production is completely eliminated from the second stage are shown.

THE EFFECTS OF H_2 PARTIAL PRESSURE ON THE PERFORMANCE OF C. LJUNGDAHLII.

Batch experiments were carried out to measure the effects of increased H_2 partial pressure on growth, substrate uptake and product formation by C. ljungdahlii. The batch experiments were carried out in serum bottles inoculated with C. ljungdahlii into basal medium supplemented with 0.01 percent yeast extract. The experiments were carried out at an initial pH of 5.0 using cysteine hydrochloride as the reducing agent. The gas phase consisted of CO and CO_2 at 1 atm into which various partial pressures of H_2 were added. The partial pressures of H_2 utilized were 0.15, 0.2, 0.25, 0.29, and 0.40 atm, which correspond to 6, 8, 9.6, 11.4, and 15.4 mmole H_2. The quantity of CO was held constant in the experiments at 44 mol percent.

The effects of increased H_2 partial pressure on the growth of C. ljungdahlii on CO, CO_2, H_2 is shown in Figure 1. As is noted in the figure, the H_2 partial pressure had essentially no effect on the rate of growth of C. ljungdahlii nor the maximum attained cell concentration at H_2 partial pressures up to 0.40 atm. However, the lag phases were different for the various partial pressures, although lag phase did not increase with H_2 partial pressure. It should be noted that the maximum cell concentration and the rate of growth after the lag phases were essentially identical. It is also noteworthy to mention the slight declines in cell concentration seen after the maximum was reached during the experiment. This result is probably due to the low level of yeast extract used in the experiment which limits growth but helps promote ethanol formation.

The effect of increased H_2 partial pressure on CO uptake by the bacterium is shown in Figure 2. As was seen in Figure 1, all bottles showed the same rate of CO uptake after the lag phase, and reached complete CO utilization except at the highest H_2 partial pressure. The time required for the onset of CO utilization was about 145 hrs for a partial pressure of 0.15 atm, 80 hrs for partial pressures of 0.2 and 0.25 atm, 215 hrs for a partial pressure of 0.29 atm and 260 hrs for a partial pressure of 0.4 atm.

H_2 uptake in Figure 3 paralleled CO uptake in Figure 2. As is seen by the slopes of the curves in Figure 3, the rate of H_2 uptake increased with increasing H_2 partial pressure. The exception to this rule again occurs at a 0.15 atm partial pressure. This result agrees well with results presented previously for varying CO partial pressure. All bottles but one again yielded complete H_2 conversion. However, the onset of H_2 uptake occurred between 25 and 50 hrs after the onset of CO conversion. This result is contrary to the

results obtained at lower H_2 partial pressures (PETC report 8-89, September 1989), but agrees with results obtained in continuous culture where H_2 utilization is often seen to stop when adequate utilization (90 percent conversion) of CO is not obtained. Thus, CO is the preferred substrate at higher H_2 partial pressures and in continuous culture.

The effects of increased H_2 partial pressure on ethanol and acetate formation by the bacterium are shown in Figures 4 and 5. As noted in comparing Figures 4 and 5, ethanol and acetate formation occurred at essentially the same time as the onset of growth and substrate uptake. The maximum ethanol concentrations increased with increasing partial pressure, except at a H_2 partial pressure of 0.15 atm (see Figure 4). The ethanol concentration decreased with time after reaching a maximum. If the results obtained at H_2 partial pressure of 0.15 atm are excluded, it appears that increasing H_2 partial pressure resulted in increased ethanol information. However, this increase in ethanol production could be attributed to additional substrate conversion from H_2 rather than a shift in reaction pathway from ethanol to acetate. A look at acetate formation should be useful in answering this question.

As is noted in Figure 5, a single maximum acetate concentration of about 11 mmol was obtained. The acetate concentration decreased after reaching a maximum at the highest partial pressure. Thus, by increasing the ethanol concentration in Figure 4 while the acetate concentration was constant (Figure 5), the ratio of ethanol to acetate increases with increasing H_2 partial pressure.

DETERMINATION OF MASS TRANSFER AND KINETIC PARAMETERS

Figures 6 and 7 show results from a batch synthesis gas fermentation using C. ljungdahlii. Initial CO partial pressures ranging from 1.44 atm to 4.27 atm were utilized in smaller batch reaction vessels in order to determine the effects of increased CO pressure on cell growth and substrate uptake. Basal medium containing 0.01% yeast extract was used in all experimental runs.

Figure 6 shows cell concentration profiles for the various CO partial pressures. As is noted, cell growth followed the same patterns for all CO partial pressures. As expected, the lag phase increased with CO partial pressure. The maximum cell concentration increased with increasing CO partial pressure. Figure 7 shows CO utilization with time as a function of initial CO partial pressure. As is noted, because of the lag phases the time for complete CO utilization increased with CO partial pressure. The rate of CO utilization, as obtained from the slopes of the plots, was essentially constant with initial CO partial pressure.

MASS TRANSFER CONSIDERATIONS

The conversion of CO, CO_2 and H_2 in synthesis gas to ethanol and acetate by C. ljungdahlii is a multi-phase process. First, the gas phase substrate must be transferred from the bulk gas phase to the liquid phase. Dissolved gas is then taken up by the solid organisms and converted to a liquid phase product. A small amount of the dissolved substrate is utilized for the growth of the bacteria, with the majority converted to ethanol or acetate.

The overall conversion scheme may be visualized using the following expression:

$$CO_{(g)} \xrightarrow{r} CO_{(l)} \xrightarrow{r} \begin{matrix}\text{Ethanol}\\ \text{Acetate}\\ \text{Cells}\end{matrix} \qquad (5)$$

In Equation (5), the gaseous substrate, in this case carbon monoxide ($CO_{(g)}$), is transported to the liquid phase ($CO_{(l)}$) through mass transport (t) after which it undergoes biocatalytic reaction (r) to ethanol, acetate and cells. In batch culture, the rate of transport (t) may be described by the following equation:

$$t = \frac{K_L a}{H} \left[P^G_{CO} - P^L_{CO}\right] \qquad (6)$$

where $K_L a$ is the overall mass transfer coefficient times the interfacial surface area (a necessary factor due to the virtual impossibility of of separately measuring the gas/liquid interfacial area), H is the Henry's law constant for carbon monoxide in the liquid culture, and $\left[P^G_{CO} - P^L_{CO}\right]$ is the driving force, a function of the carbon monoxide partial pressure in the gas (g) and liquid (l) phases.

Equation (6) may be incorporated into a carbon monoxide mass balance for the gas phase which states that the rate of change in the number of moles of CO in the gas phase equals the rate of transport from the gas phase to the liquid phase, expressed as:

$$\frac{dN^G_{CO}}{dt} = - \frac{K_L a}{H} \left[P^G_{CO} - P^L_{CO}\right] V_L \qquad (7)$$

In Equation (7) the negative sign indicates the disappearance of CO from the gas phase. The liquid volume (V_L) is included in the equation for units agreement, since the mass transfer coefficient, $K_L a/H$, is expressed in mmol CO/atm•L•hr.

As the fermentation proceeds in batch culture, the cell population grows to a point at which it consumes CO as quickly as it enters the liquid phase.

At this point the overall reaction becomes mass transfer limited and the CO partial pressure in the liquid phase is reduced to zero. Under these limiting conditions, Equation (7) reduces to:

$$\frac{dN_{CO}^{G}}{dt} = - \frac{K_L a}{H} (P_{CO}^{G}) V_L \qquad (8)$$

Upon rearrangement, Equation (8) allows the graphical determination of $\frac{K_L a}{H}$ as the slope of a plot of P_{CO}^{G} as a function of $- \frac{1}{V_L} \frac{dN_{CO}^{G}}{dt}$ (shown in Figure 8). As is noted, for the mass transfer limited region of Figures 8, $K_L a/H$ equals 23.88 mol CO/atm•L•hr. Once $K_L a/H$ is determined, it can be used to calculate values for P_{CO}^{L} for the time during which the fermentation is not mass transfer limited. It should be noted that the value of $K_L a/H$ differs significantly from the result found earlier in different reaction vessels. This is expected since the mass transfer characteristics of the two reactors are different.

REACTION KINETICS

The second segment of Equation (5), representing the rate of biocatalytic reaction of carbon monoxide to products, may be expressed as:

$$r = q_{CO} X \qquad (9)$$

where q_{CO} is the specific CO uptake rate and X is the cell concentration in the liquid medium. When incorporated into a CO balance for the liquid phase, the following relation results:

$$\frac{K_L a}{H} (P_{CO}^{G} - P_{CO}^{L}) V_L - q_{CO} X V_L = \frac{dN_{CO}^{L}}{dt} = \frac{dP_{CO}^{L}}{dt} \frac{V_L}{H} \qquad (10)$$

Equation (10) states that the rate of change of CO in the liquid phase

(expressed as $\frac{dN_{CO}^{L}}{dt}$ or in terms of the CO partial pressure in the liquid, $\frac{dP_{CO}^{L}}{dt}\ \frac{V_L}{H}$) is equal to the rate of transport into the liquid phase minus the rate of disappearance from the liquid phase resulting from biocatalytic conversion.

From this relation, the specific uptake rate of CO, q_{CO}, may be determined for use in a descriptive kinetic model (previously presented in PETC Report No. 5-89). Additionally, the specific growth rate (μ) may be analogously defined as the time rate of change of cells per unit of cells present in the culture:

$$\mu = \frac{1}{X}\ \frac{dX}{dt} \tag{11}$$

When placed in kinetic models and accounting for substrate inhibition, the following equations are obtained as a function of the CO partial pressure in the liquid:

$$\mu = \frac{\mu_{max}\ P_{CO}^{L}}{K_p + P_{CO}^{L} + (P_{CO}^{L})^2/W} \tag{12}$$

and

$$q_{CO} = \frac{q_{max} P_{CO}^{L}}{K_p' + P_{CO}^{L} + (P_{CO}^{L})^2/W'} \tag{13}$$

where μ_{max} and q_{max} are the maximum specific growth and carbon monoxide uptake rates, respectively, K_p and K_p' are the liquid carbon monoxide partial pressures at which μ and q_{CO} are at one-half their maximum values, and W and W' are substrate inhibition parameters the magnitude of which is inversely proportional to the level of substrate inhibition.

Upon inversion and rearrangement of Equations (12) and (13) relations conducive to graphical representation are obtained:

$$\frac{P_{CO}^{L}}{\mu} = \frac{(P_{CO}^{L})^2}{\mu_{max} W} + \frac{P_{CO}^{L}}{\mu_{max}} + \frac{K_p}{\mu_{max}} \qquad (14)$$

and

$$\frac{P_{CO}^{L}}{q_{CO}} = \frac{(P_{CO}^{L})}{q_{max} W'} + \frac{P_{CO}^{L}}{q_{max}} + \frac{K_p'}{q_{max}} \qquad (15)$$

Figures 9 and 10 show how the calculated specific rates and the calculated partial pressures in the liquid phase fit Equations (14) and (15). Also shown are the results from the previous kinetic studies, shown as the short straight lines in the plots. For initial CO gas phase partial pressures at the current experimental levels (4.27 atm and below) no substrate inhibition was detectable and graphically, the intercepts were essentially zero giving a zero order reaction with respect to the partial pressure of CO in the liquid phase. The resulting values for μ_{max} and q_{max} were 0.079 hr^{-1} and 65.43 $\frac{\text{mmol CO}}{\text{gcell}\cdot\text{hr}}$, respectively. Thus, $q = q_{max} = 65.43$ mmol CO/g·cell hr and $\mu = \mu_{max} = 0.079\ hr^{-1}$. The resulting zero order Monod model provides a a simple model for reactor design.

The maximum specific uptake rate, q_{max}, may be expressed either in terms of the grams of CO or the grams of carbon per gram of cells per hour as

$$q_{max} = 65.43 \text{ mmol CO/g}\cdot\text{cell}\cdot\text{hr}$$
$$= 1.83 \text{ g CO/g}\cdot\text{cell}\cdot\text{hr}$$
$$= 0.79 \text{ g C/g}\cdot\text{cell}\cdot\text{hr}$$

This compares to the growth of S. cerevisiae, an ethanol producing yeast, upon glucose with a specific carbon uptake rate of $q_{max} = 0.27$ g C/g·cells·hr^2, and

the bacterial conversion of glucose to ethanol by Zymomonas mobilis at a rate of q_{max} = 0.67 g c/g•cells•hr[3].

It should be noted that the results of the present study represent a 51 percent increase in the maximum specific uptake rate, q_{max}, and a 98 percent increase in the maximum specific growth rate, μ_{max}. With these new results, the bacterium is now performing in a similar fashion as with other co-utilizing bacteria.

CONTINUOUS CSTR STUDIES

Although there has been significant success in improving the product ratios in favor of ethanol production, the improved ratios have always been coupled with culture distress and even the loss of culture viability soon after the favorable product ratios were attained. Thus, a method needs to be developed for the C. ljungdahlii system that results in significant ethanol production while, at the same time, maintaining a viable culture for an extended (or even indefinite) period of time.

A two-stage CSTR system has been developed for this purpose. The first reactor (volume of 350 mL) was used primarily for the growth of C. ljungdahlii. The liquid feed to this reactor consisted of 0.02 percent yeast extract in basal medium at pH 4.5, with a flow rate of 360 mL/day. In addition, synthesis gas was fed to the reactor to supply carbon and energy sources for growth. Because this reactor was designed primarily for growth, significant acetate production was also expected. The second reactor (volume of 1.44 L) was used primarily for ethanol production by C. ljungdahlii cells formed in the first reactor. No additional liquid was fed to the second reactor so that nutrients were limiting. In addition, the pH was adjusted to pH 4.0 and the reducing agent benzyl viologen was added at a concentration of

10-20 ppm. Synthesis gas was bubbled into the second CSTR for conversion to ethanol. It was hoped that only ethanol would be produced from the cells in the second CSTR. However, because the conditions in the second reactor were not conducive for growth, the efficiency of ethanol production in the second CSTR was in doubt. An experiment was thus run with the above system to determine the overall performance of this two-stage system and to determine the suitability of the second stage in efficiently producing ethanol by utilizing the pH shift, nutrient limitation and benzyl viologen addition. The results are summarized below.

OPERATING CONDITIONS

The reactor volumes and liquid flow rates were held constant at the values shown above throughout the experimental study. In addition, the feed to the first reactor always consisted of 0.02 percent yeast extract in basal medium. The pH varied a bit in each reactor, but was essentially 4.5 in Reactor A (the first reactor in series) and 4.0 in Reactor B.

The gas flow rate to Reactor A varied from 2.34 mL/min during start-up to a high of 3.40 mL/min on day 53. However, during most of the experimental study the gas flow rate was held constant at 2.76 mL/min. Similarly, the gas flow rate to Reactor B varied from a low of 1.79 mL/min during start-up to 2.20 mL/min. Adjustments in these gas flow rate were made periodically in an attempt to improve reactor performance but, as noted, were not significant adjustments.

Benzyl viologen at a concentration of 20 ppm was added to Reactor B on day 13. The higher concentrations utilized in previously batch studies (30-50 ppm) were found to be excessive in continuous culture, resulting in culture death. The concentrations of benzyl viologen was lowered to 10 ppm on day 45. Initially, no benzyl viologen was added to Reactor B.

RESULTS AND DISCUSSION

Cell concentration profiles for the two reactors are shown in Figures 11 and 12. As is noted in Figure 11, after a brief start-up period, the cell concentration varied about a cell concentration of approximately 300 mg/L from 270 to 317 mg/L until day 65. A temperature decrease in the constant temperature room is then thought to be responsible for the significant drop in cell concentration seen after day 60. The cell concentration in Reactor B was also seen to gradually increase during start-up until the addition of benzyl viologen on day 13. A cell concentration of 340 mg/L was seen at this point. The reducing agent benzyl viologen, while yielding reduced conditions necessary for ethanol production, also resulted in the death of cells particularly at high concentrations (a high concentration may be as little as 20 ppm, as is noted in Figure 12). The cell concentration decreased to only 250 mg/L throughout the addition of 20 ppm benzyl viologen. When 10 ppm benzyl viologen was used instead of 20 ppm, the cell concentration in Reactor B also decreased, but only to a 280 mg/L concentration. Again, the results after day 60 are due to the temperature upset.

The conversions of CO and H_2 in the synthesis gas feed to Reactors A and B are shown in Figure 13 and 14. As is noted in Figure 13, the conversions of both CO and H_2 remained constant at 90-95 percent throughout the whole experimental study (excluding start-up and the temperature upset). Both the CO and H_2 conversions decreased as a result of benzyl viologen addition in Reactor B (see Figure 14), regardless of whether 20 ppm or 10 ppm benzyl viologen was used. This phenomenon has been seen previously in the University of Arkansas laboratories, and may signal that the efficiency of the cells in utilizing substrate or forming product is reduced in the presence of benzyl viologen or other reducing agents.

Product formation in the reactors are shown in Figures 15-17. As expected, acetate formation was much greater in Reactor A than ethanol formation due to the growth-enhancing conditions employed. Concentrations of approximately 3.5 g/L acetate and 0.5 g/L ethanol were formed except during start-up and after the temperature upset. This translates into a molar ratio of 0.19 moles ethanol per mole acetate, or just over 5 moles acetate produced per mole of ethanol.

Figure 16 shows the overall product concentration profile from the two-stage system. As is noted, the concentration of ethanol stayed approximately constant at about 1 g/L, with peak concentrations of about 1.3 g/L. These concentration levels have been exceeded in the past. The concentration of acetate, however, fell with the addition of benzyl viologen, especially when using 20 ppm of the reducing agent. A better idea of what happened upon the addition of benzyl viologen can be seen in Figure 17, where the concentrations of ethanol and acetate produced in Reactor A are subtracted from the overall product concentrations. It can be clearly seen that only ethanol was produced in the second reactor. As a matter of fact, the negative acetate concentrations indicate that acetate was actually being consumed in the second reactor. The only negative aspect of this result is the hope that the ethanol concentrations would have been higher. This probably can be accomplished by varying the benzyl viologen concentration.

The efficiency of cells producing acetate (and small amounts of ethanol) along with growth can be compared to reduced cells producing ethanol only by looking at the steady-state specific product yields from substrate. A rough estimate of these quantities can be obtained by dividing the amount of product produced by the substrate utilized and the cell concentration. The combined

product in Reactor A was found to be approximately 4 g/L (3.5 g/L acetate and 0.5 g/L ethanol) from Figure 15. The ethanol production in Reactor B was about 0.5 g/L (Figure 17). The average cell concentration in Reactor A was about 300 mg/L (Figure 11) and the average in Reactor B, although tough to estimate, was about 280 mg/L (Figure 12). The substrate utilization in Reactor A was approximately 2.76 mL/min at a 95 percent conversion (see Figure 13), and the substrate utilization in Reactor B was approximately 1.88 mL/min at a 50 percent conversion (see Figure 14). Although these are only "ball park" estimates, calculations show a specific productivity estimate of

$$\frac{4 \text{ mg/L}}{(2.76 \text{ mL/min})(0.95)(300 \text{ mg/L})} = 0.051$$

in Reactor A and a specific productivity estimate of

$$\frac{0.5 \text{ mg/L}}{(1.88 \text{ mL/min})(0.5)(280 \text{ mg/L}} = 0.0019$$

in Reactor B thus, the cells were more efficient at more efficient at producing product in Reactor A.

FUTURE WORK

Based upon the above results and rough productivity estimates, several things are worth noting:

1. Research needs to be conducted on producing the highest possible cell concentration in Reactor A so that the ethanol production and concentration in Reactor B can be high. The acetate produced in Reactor A can be removed in a side stream. Thus, the addition of other nutrient sources (with or without synthesis gas) should be studied.

2. Ethanol formation is apparently not as efficient per cell in Reactor B as acetate formation in Reactor A. This could mean that the viability of the

cells is affected by the presence of benzyl viologen. Lower concentrations of benzyl viologen should be investigated, with and without the pH shift, as well as other reducing agents, to help increase the viability of the cells in Reactor B.

3. Other reactor schemes may be used, but only after the present mode of operation is more clearly understood.

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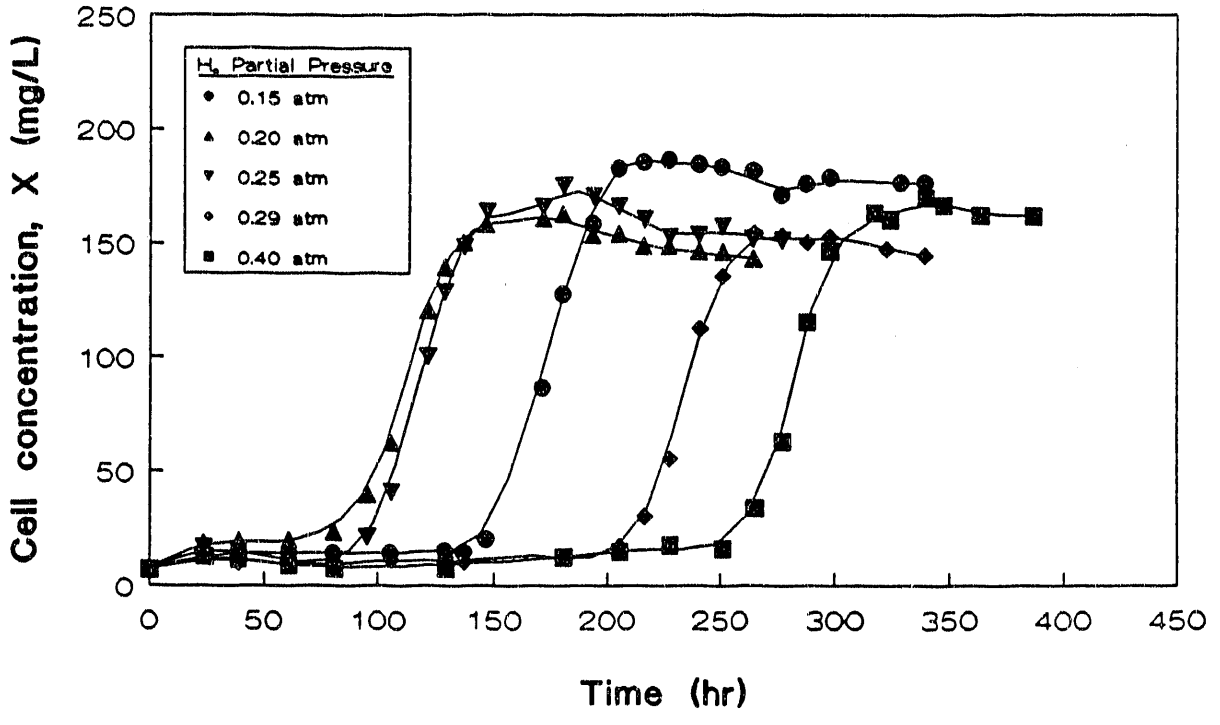

Figure 1. Effect of H_2 partial pressure on cell growth by *C. ljungdahlii* grown on CO and H_2.

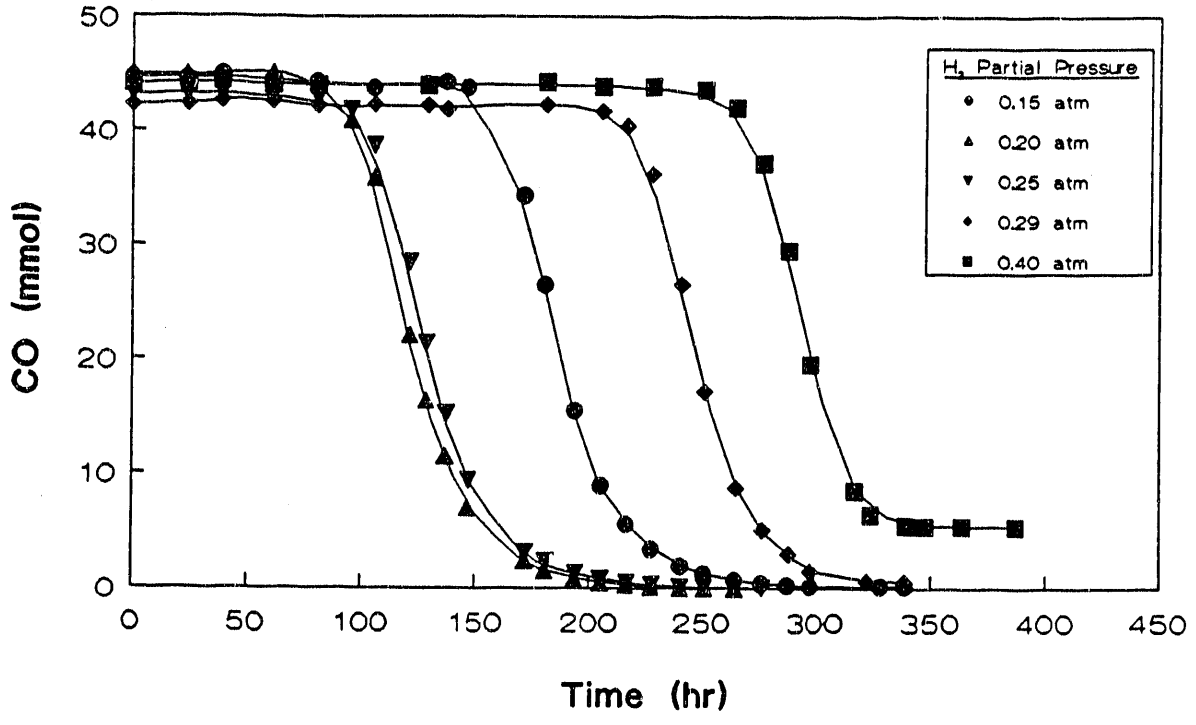

Figure 2. Effect of H_2 partial pressure on CO uptake by *C. ljungdahlii* grown on CO and H_2.

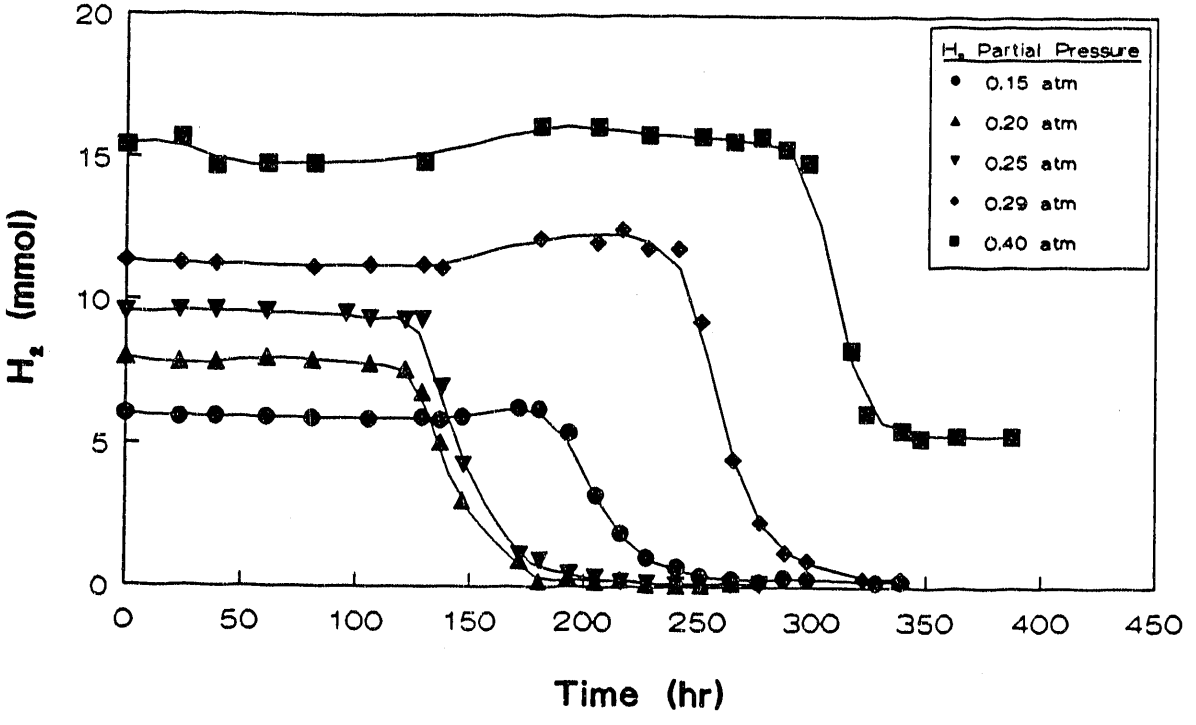

Figure 3. Effect of H_2 partial pressure on H_2 uptake by *C. ljungdahlii* grown on CO and H_2.

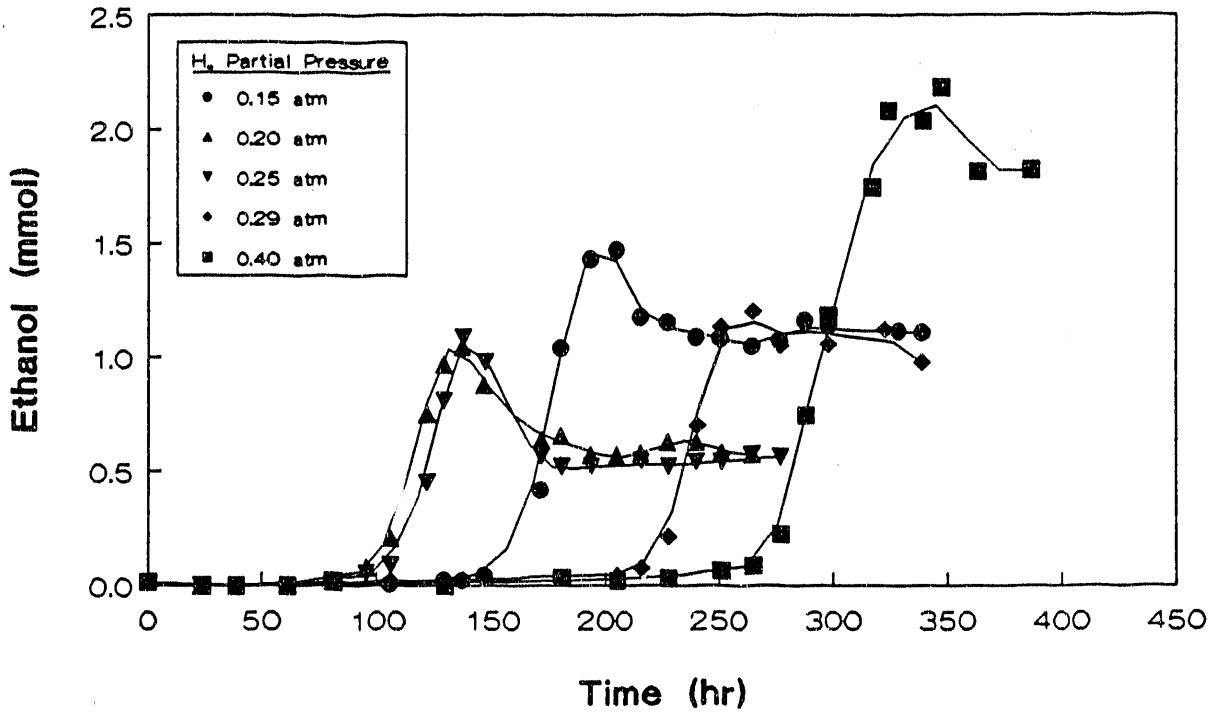

Figure 4. Effect of H_2 partial pressure on ethanol production by *C. ljungdahlii* grown on CO and H_2.

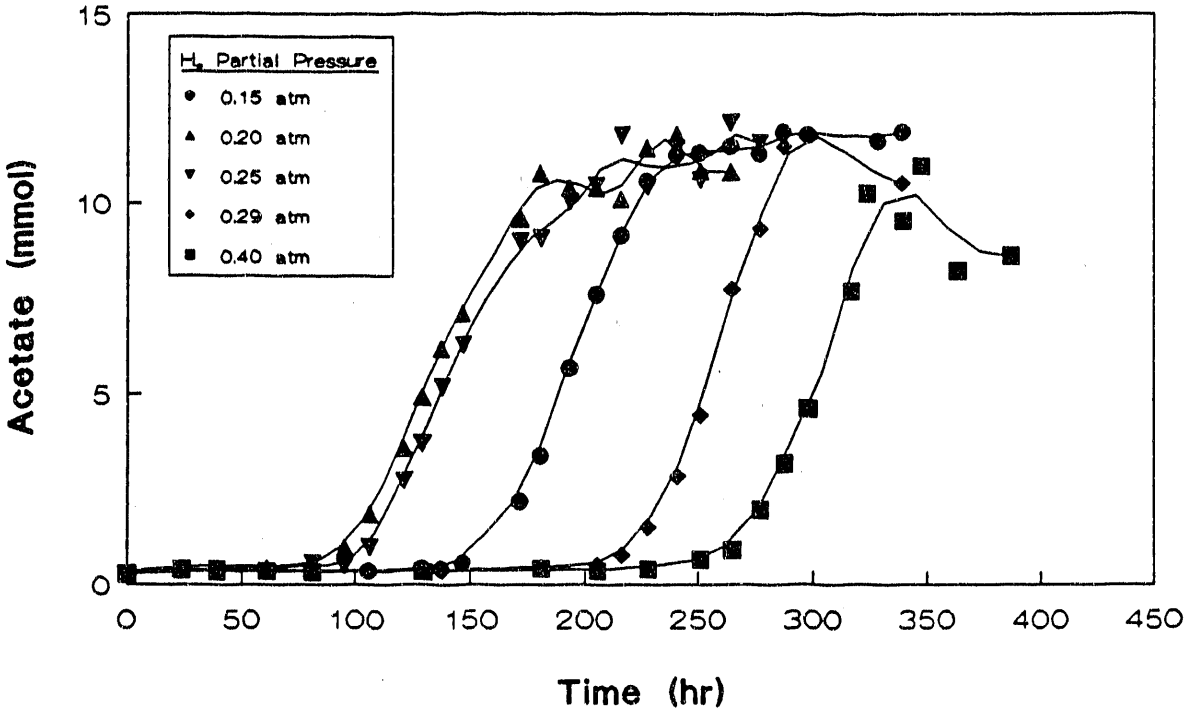

Figure 5. Effect of partial pressure on acetate production by *C. ljungdahlii* grown on CO and H_2.

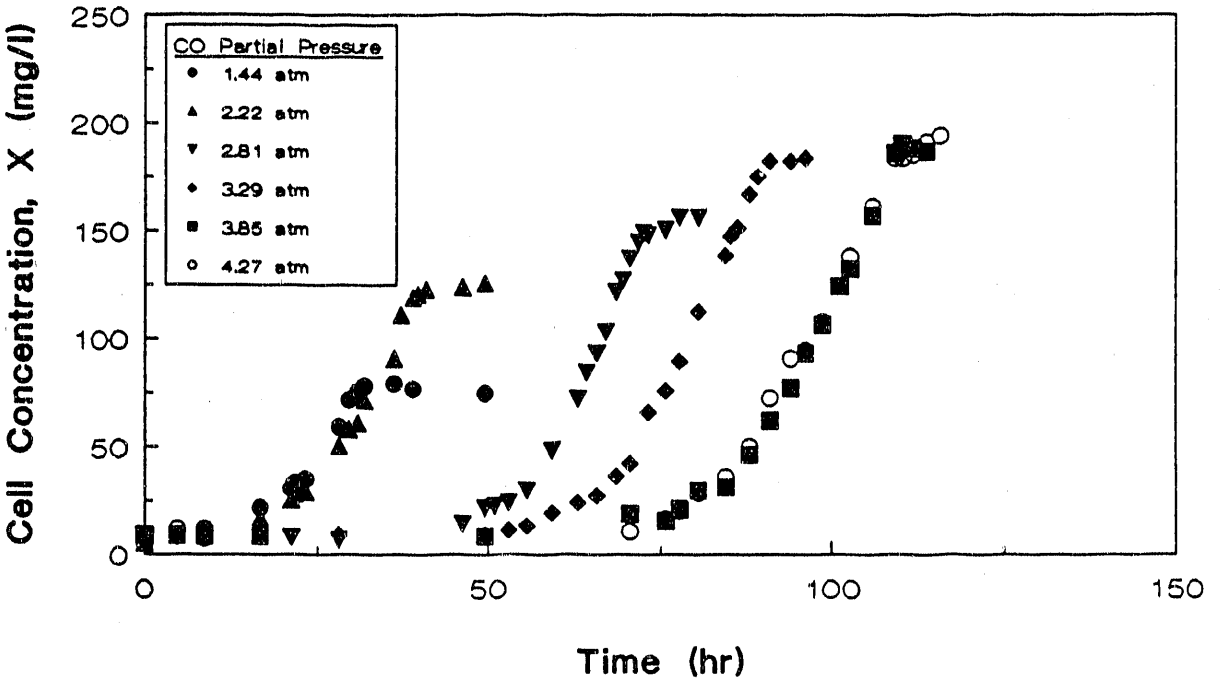

Figure 6. Effect of CO partial pressure on cell growth by *C. ljungdahlii* grown on CO.

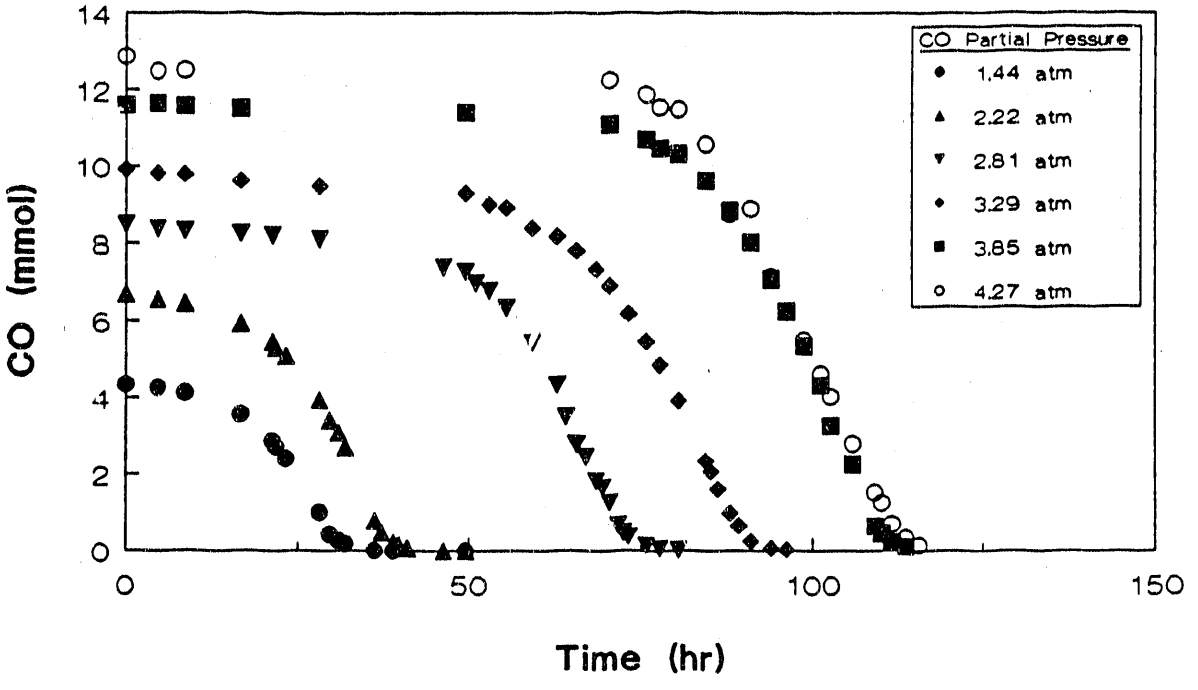

Figure 7. Effect of CO partial pressure on CO uptake by *C. ljungdahlii* grown on CO.

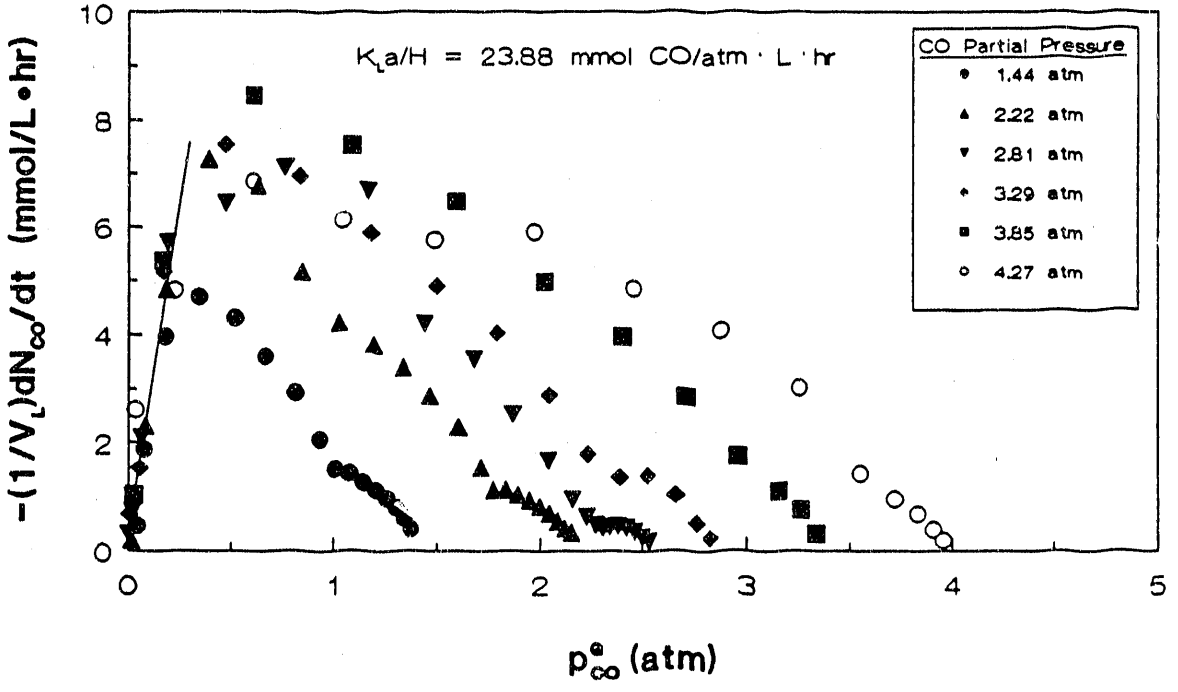

Figure 8. Volumetric mass transfer coefficient for the fermentation of carbon monoxide by *C. ljungdahlii*.

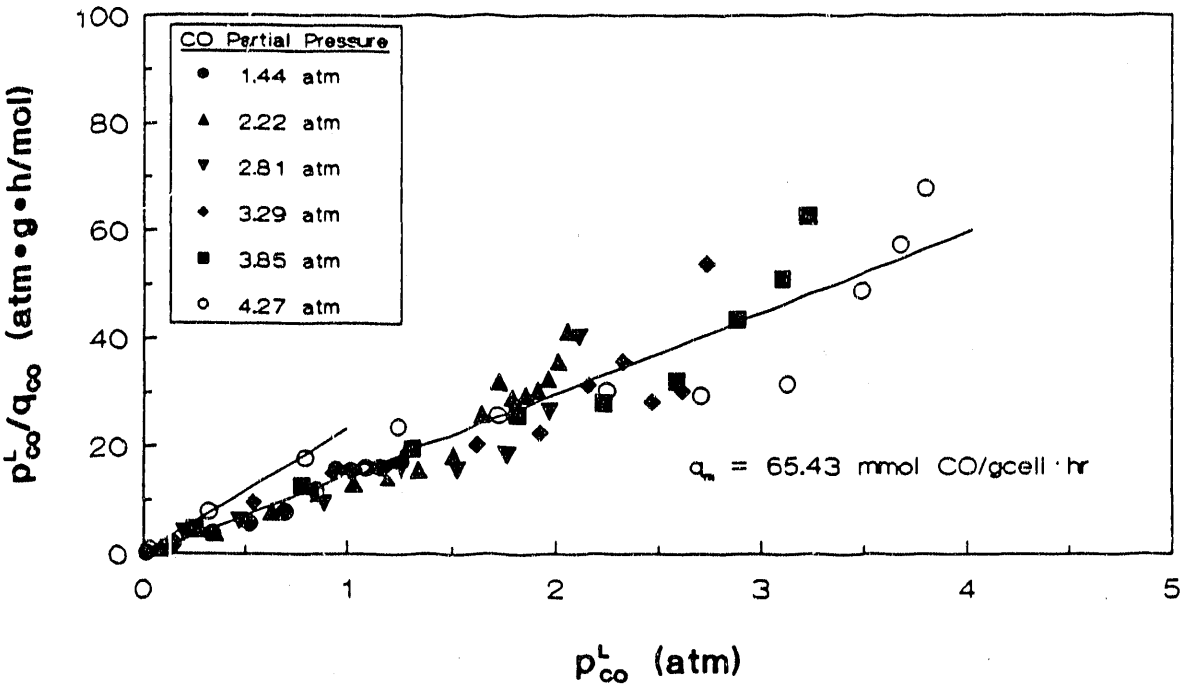

Figure 9. Monod model for the rate of CO uptake by *C. ljungdahlii* in batch fermentation.

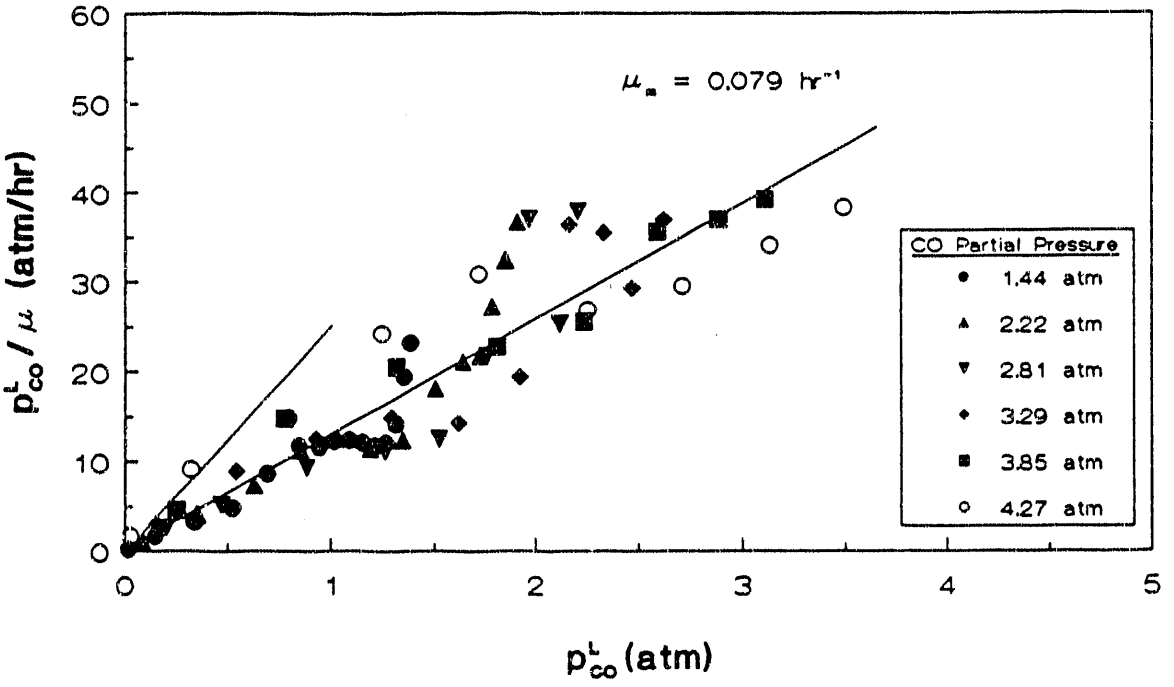

Figure 10. Monod model for the rate of cell growth by *C. ljungdahlii* in batch fermentation.

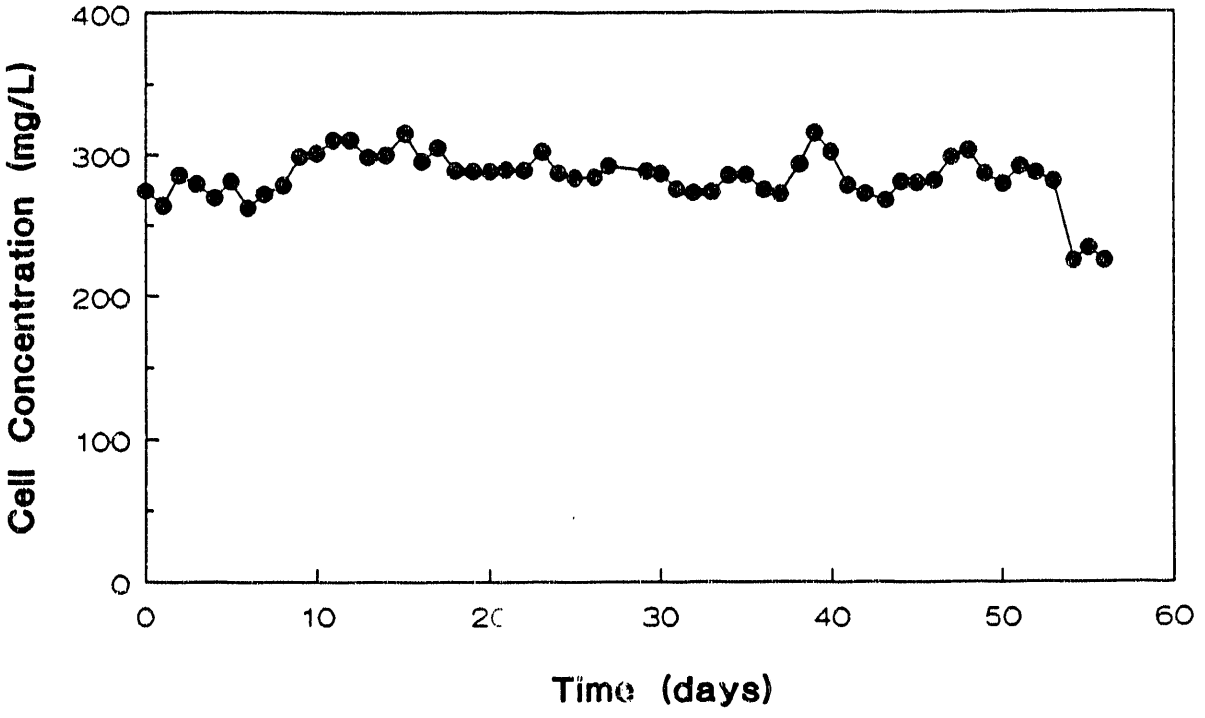

Figure 11. Cell Concentration Profile in Reactor A in a Two-Stage CSTR System Using *C. ljungdahlii*.

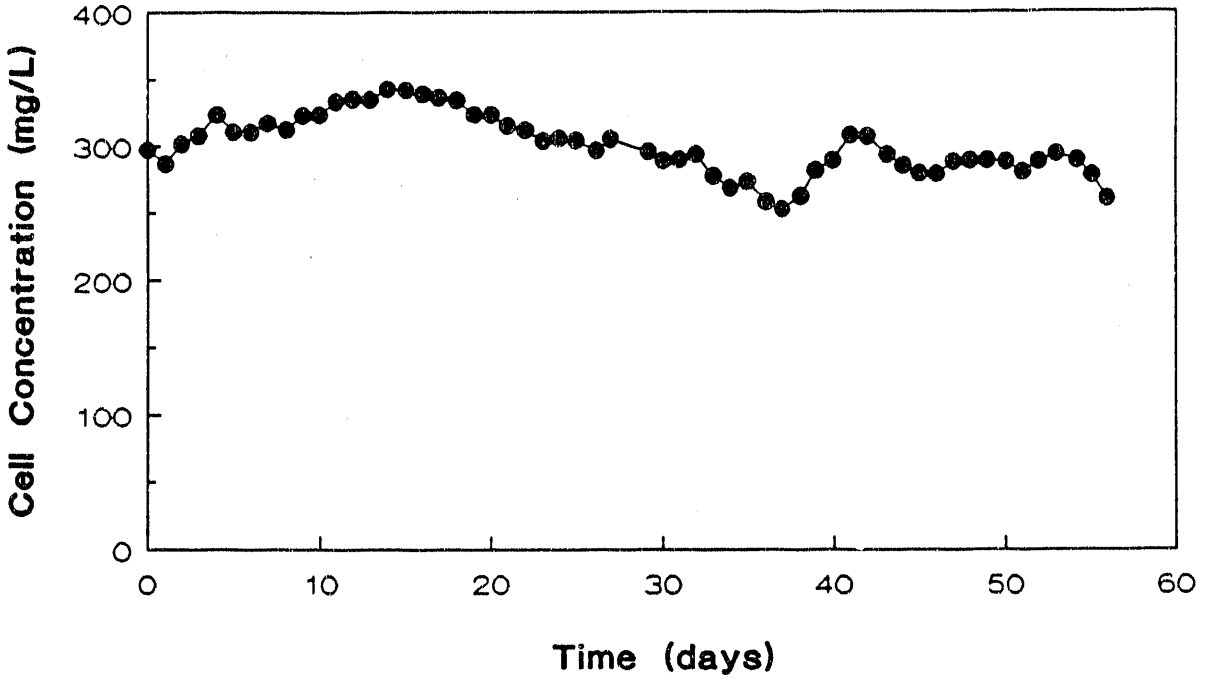

Figure 12. Cell concentration profile in Reactor B in a two-stage CSTR system using *C. ljungdahlii*.

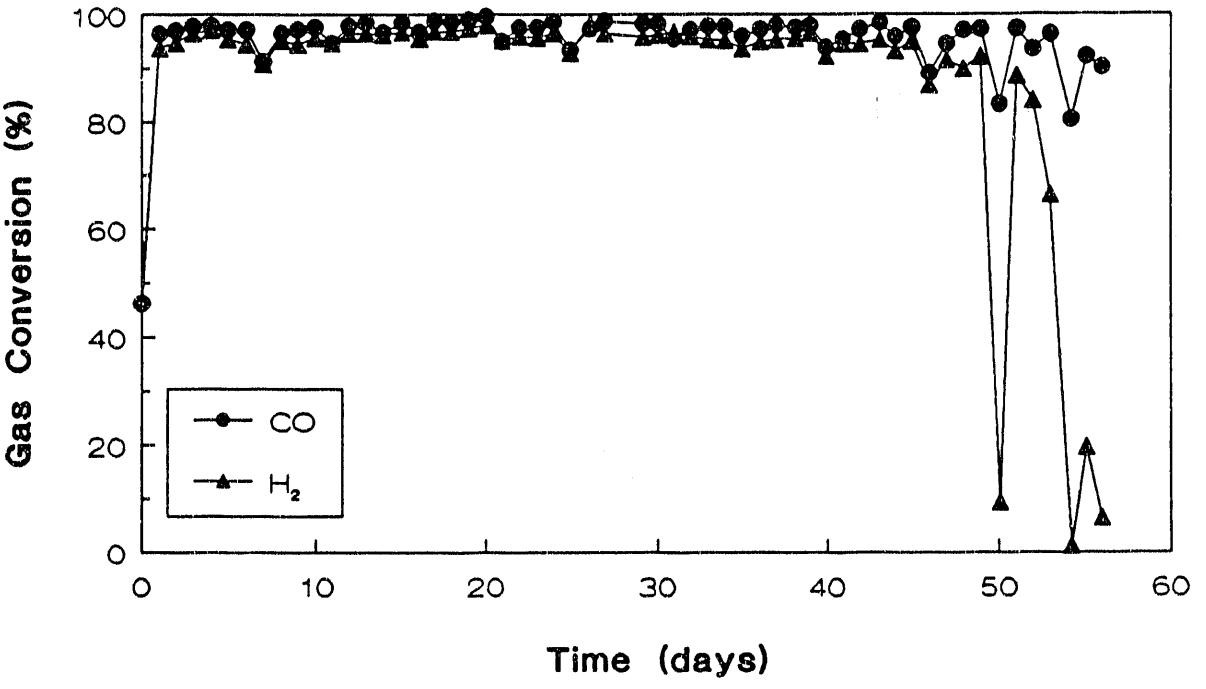

Figure 13. CO and H_2 conversion in Reactor A in a two-stage CSTR system using *C. ljungdahlii*.

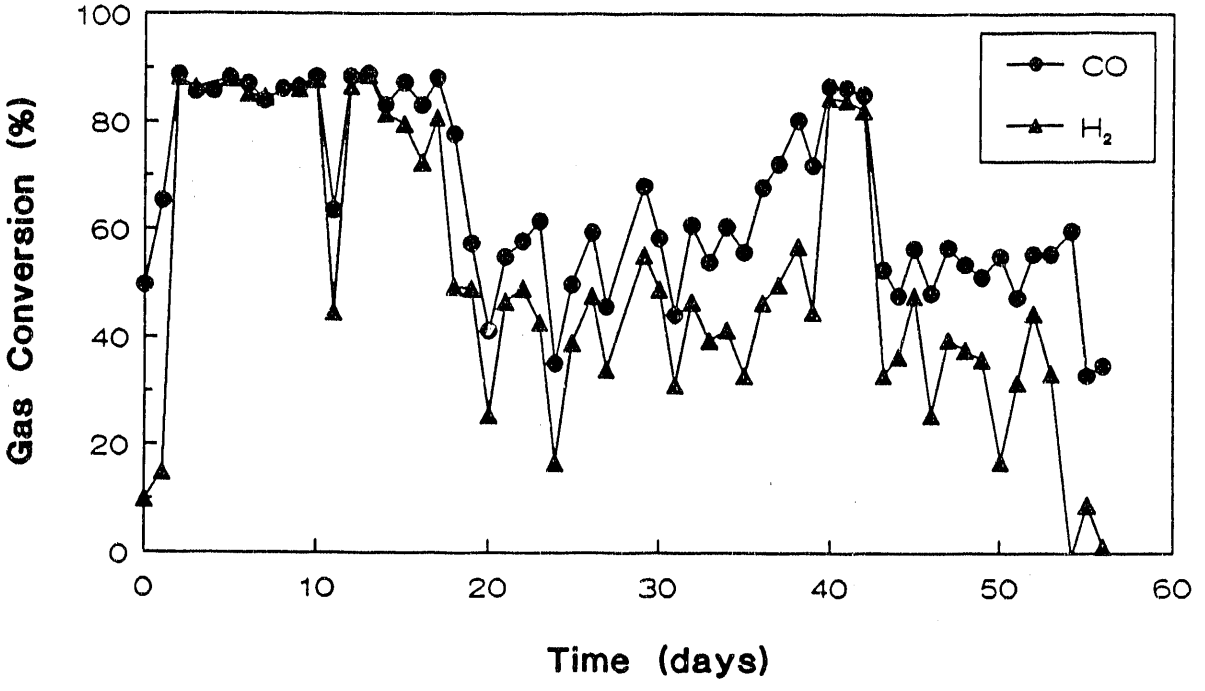

Figure 14. CO and H_2 conversion in Reactor B in a two-stage CSTR system using *C. ljungdahlii*.

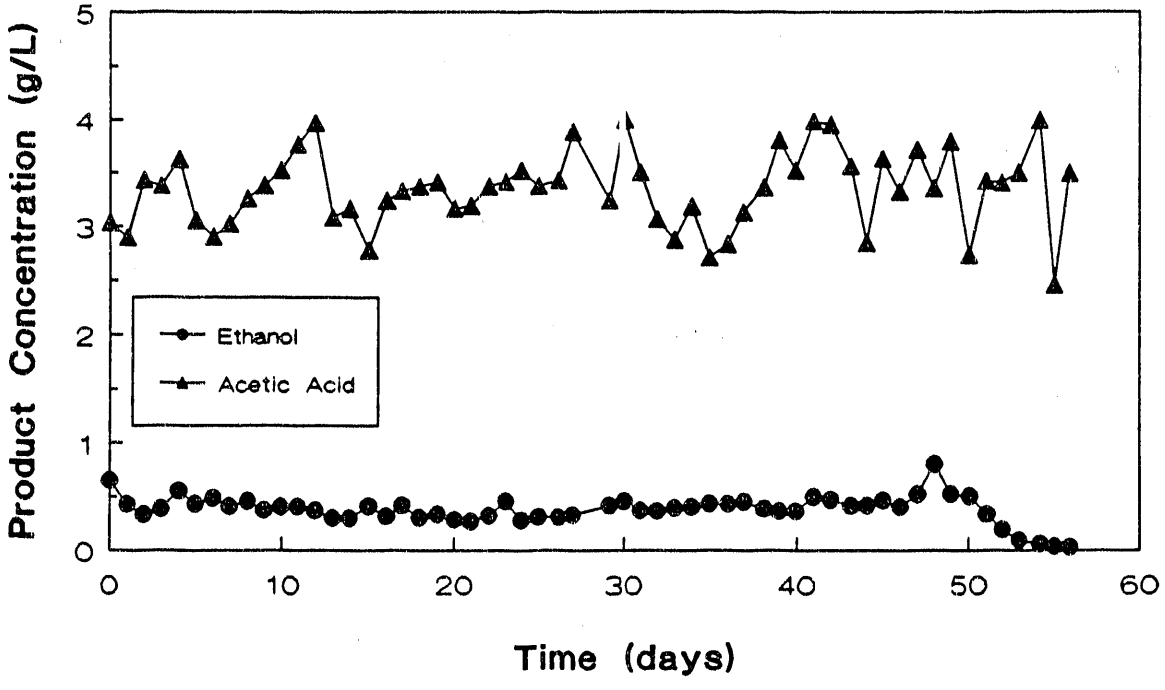

Figure 15. Production of ethanol and acetate by *C. ljungdahlii* in Reactor A of a two-stage CSTR system.

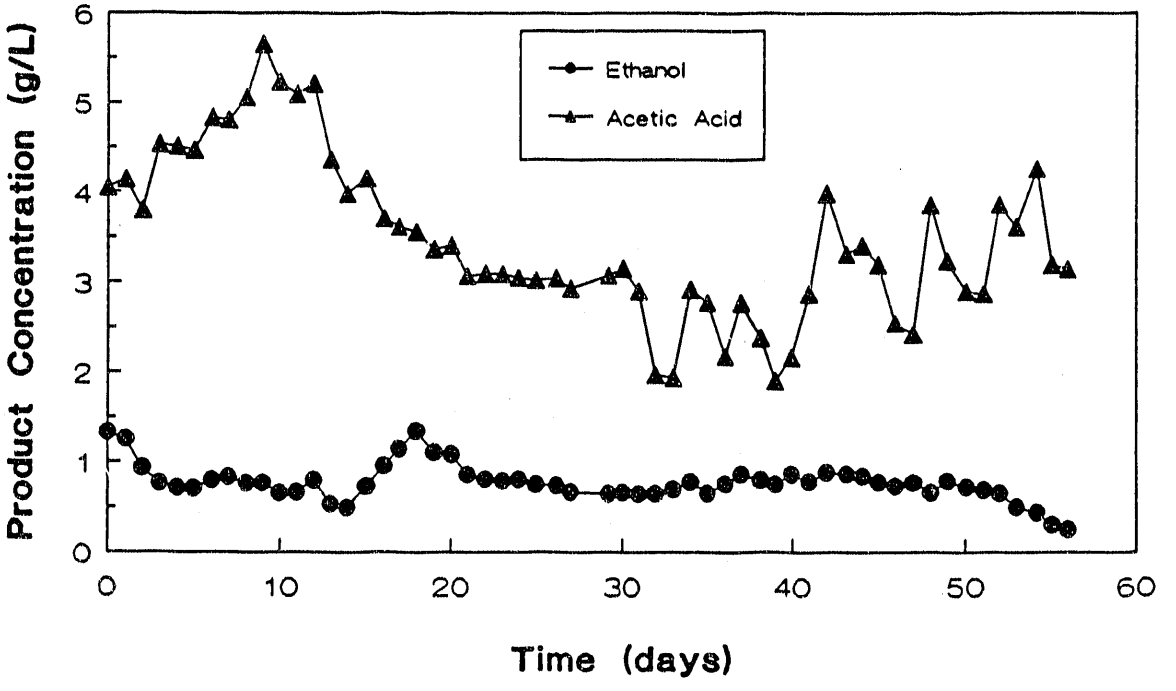

Figure 16. Product concentration profiles for the overall two-stage reactor system using *C. ljungdahlii*.

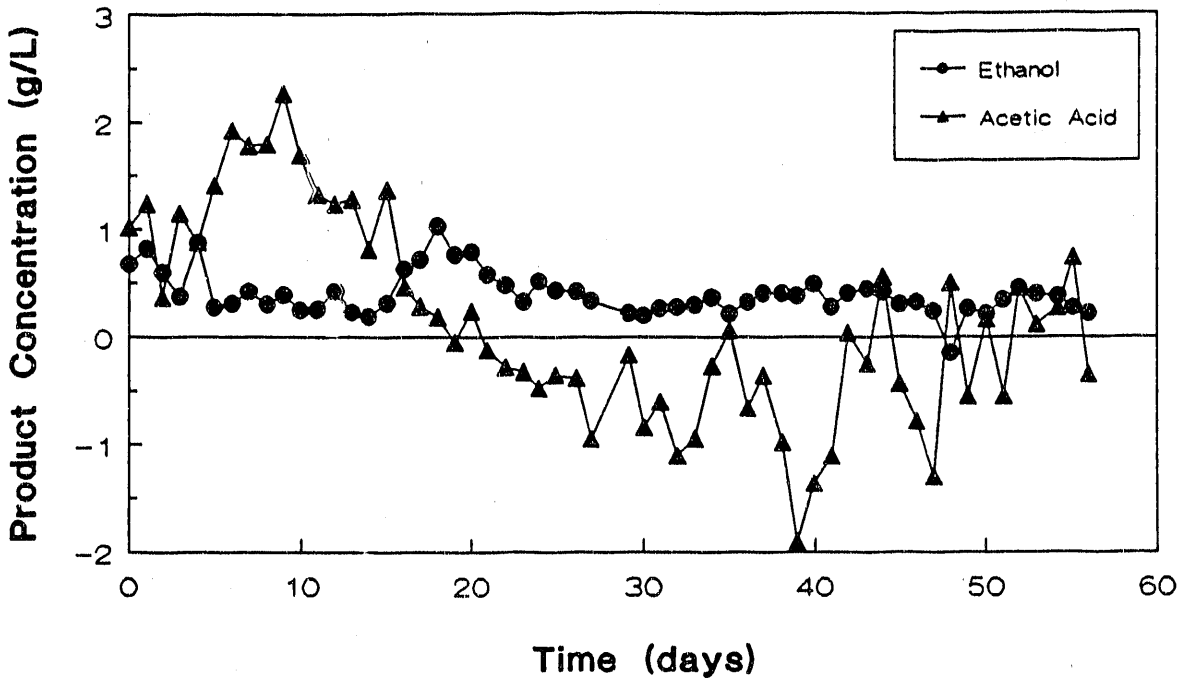

Figure 17. Production of ethanol and acetate by *C. ljungdahlii* in Reactor B of a two-stage CSTR system.

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