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The Biological Production of Ethanol from Synthesis Gas

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ABSTRACT

There has been considerable interest recently in the production of liquid fuels from coal through direct or indirect liquefaction. A culture has been isolated from animal waste that is capable of producing ethanol and acetate from carbon monoxide and from hydrogen and carbon dioxide, the major components of synthesis gas. This paper presents results of batch and continuous laboratory studies with this culture. Special efforts are directed toward maximizing the ratio of ethanol to acetate.

Index Entries: Synthesis gas; carbon monoxide; ethanol; acetate; clostridia.

NOMENCLATURE

D	dilution rate	(h^{-1})
H	Henry's law constant	(atm • L/mmol)
K_La	volumetric mass transfer coefficient	(h^{-1})
N	amount of component	(mmol)
P	partial pressure	(atm)
t	time	(h)
V	volume	(L)
X	cell concentration	(mg/L)

Subindices or Superindicies

ACH	acetic acid
CO	carbon monoxide

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ETOH	ethanol
G	gas
H_2	hydrogen
L	liquid

INTRODUCTION

Synthesis gas, composed mainly of carbon monoxide, hydrogen, and carbon dioxide, can be used as a major intermediate in the production of chemicals and fuels from a wide variety of raw materials. The gasification of solid fuels such as coal and biomass, the catalytic reforming of natural gas, or the partial oxidation of heavy oils produce synthesis gas suitable for further processing (*1*). Many traditional catalytic processes are being developed for the conversion of synthesis gas components to more desirable chemicals (*2*), but interest has recently arisen in the biological transformation of these compounds, (*3*) in view of certain advantages offered by microbial processes over chemical conversions, such as, efficiency and specificity.

Autotrophic growth on a gaseous mixture of hydrogen and carbon dioxide is well known for several bacteria, such as, acetogens and methanogens (*4,5*). The ability of homoacetogens to grow on carbon monoxide as an energy source was first reported by Lynd and Zeikus (*6*). The distinctive feature of the autotrophic pathway of these microorganisms seems to involve the reduction of a molecule of carbon dioxide to a methyl group and then its combination with a molecule of carbon monoxide and CoA to form acetyl-CoA (*4*). This combination of reactions has been designated as the acetyl-CoA pathway (*7*). In the case of clostridial fermentations, it has been proposed that acetyl-CoA is the central intermediate, (*8*) as well as the only source of substrate level phosphorylation in acetogenic clostridia during unicarbonotrophic growth (*9*).

Organisms have been identified in the literature that are capable of converting gaseous mixtures of CO, H_2, and CO_2 to organic acids (such as acetic and butyric acids) and to methane (*10*). Although many anaerobic, facultatively anaerobic, and even some strictly aerobic microorganisms form various amounts of ethanol from glucose (*11*), no organism was known to form ethanol autotrophically from synthesis gas components. Last year, the isolation of a strict anaerobic mesophilic bacterium from animal waste was reported to be capable of converting CO, H_2, and CO_2 to a mixture of acetate and ethanol (*12*). Preliminary identification studies have indicated that the bacterium has a strong possibility of being a new clostridium species (*13*). It is likely that in the same manner as with other clostridia growing on sugars, ethanol and acetate are formed from acetyl-CoA by this organism, with the product distribution highly dependent on the regulation of electron flow (*14*).

The purpose of this paper is to present preliminary results obtained with the newly isolated bacterium, both in batch and continuous reactor

Table 1
Liquid Medium Composition Employed

	per 100 mL
Pfenning's mineral solution	5.0 mL
B-vitamins	0.5 mL
Yeast extract	0.01 g or 0.1 g
Pfenning's trace metal solution	0.1 mL
$Na_2S \cdot 9H_2O$, 2.5% solution	2.0 mL
Cysteine-HCl, 3.5% solution	2.0 mL
Resazurin, 0.1% solution	0.1 mL

studies. Approximate cell yields and proposed stoichiometries for the formation of acetate and ethanol from synthesis gas components are presented. The effect of the yeast extract concentration in the fermentation broth on the specific rates of growth and substrate uptake, as well as on the product ratios, is discussed.

EXPERIMENTAL PROCEDURES

Microorganism

The bacterium employed was isolated from chicken waste by S. Barik and E. Johnson in the University of Arkansas laboratories (*12*). The bacterium grows well on synthesis gas components and fructose, producing ethanol and acetic acid. No significant growth has been observed on glucose or formic acid. No other products besides carbon dioxide, ethanol, and acetic acid have been identified. Growth on a mixture of 80% CO and 20% CO_2 is as fast as on synthesis gas. However, on 100% CO, a longer lag phase is observed. Very slow growth occurs on a mixture of only H_2 and CO_2 by bacteria acclimated to synthesis gas. When growing on fructose or CO alone, CO_2 is produced along with small amounts of H_2. In all cases, very low levels of yeast extract are required for growth and substrate uptake. The level of yeast extract, in turn, seems to affect the length of the lag phase, the total cell concentration achieved, and the product ratios.

Batch Equipment and Procedures

Media preparation was carried out in an atmosphere of 80% N_2 and 20% CO_2, as described by Hungate (*15*) and Lungdahl and Wiegel (*16*). The medium composition is given in Table 1. The initial pH was adjusted to 5.0 with HCl. (The pH during a batch experiment dropped continuously to levels around 3.6–3.8). After pH adjustment, the medium (200 mL/bottle) was then transferred to bottles, 1216 ± 2 mL in total volume. The bottles were sealed with butyl rubber stoppers and aluminum seals and autoclaved at 121°C for 20 min.

Reducing solutions (cysteine-HCl and sodium sulfide) were added to the bottles prior to inoculation with a seed culture grown in similar conditions as described above. The bottles were then filled with the desired gas composition and pressure. Argon (200 mL) was always injected to act as an inert component to allow accurate determination of total pressure changes in the bottles. The bottles were then placed in a shaker incubator (100 rpm) at 37°C during experimentation. Sampling of the gas composition, optical density, pH, acetate, and ethanol concentrations was carried out at appropriate intervals. Typically, 0.4 mL gas samples were withdrawn using gas-tight syringes, whereas 2.5 mL of liquid were anaerobically sampled employing disposable sterile syringes.

Continuous Equipment and Procedures

Continuous reactor experiments were carried out in a New Brunswick Bioflo C.30 chemostat modified for use under anaerobic conditions with continuous flow of liquid and gas. The reactor vessel was 750 mL nominal volume with a liquid culture volume of approximately 350 mL at the operating conditions employed. A detailed description of the system and the anaerobic techniques for startup and continuous operation have been given previously (*17,18*).

The system was maintained at 37°C and pH 4.0 during the entire experiment. The agitation rate was set at 400 rpm. The gas flowing into the reactor consisted of a synthetic mixture (Air Products and Chemicals, Inc., Allentown, PA) of 18.5% H_2, 15.4% Ar, 56.1% CO, and 10% CO_2 at a constant flow rate of 3.5 mL/min. The liquid feed composition was the same as in Table 1, with only cysteine-HCl as the reducing agent (no addition of sodium sulfide).

Analytical Procedures

Cell concentrations (in mg/L) were determined by comparing optical density readings with a standard calibration curve at 580 nm in a Bausch and Lomb Spectronic 21 spectrophotometer. Gas compositions were obtained by gas chromatography with a 6 ft Carbosphere 60/80 mesh column in a two-step temperature program (30°C and 125°C). Liquid analyses were performed by gas chromatography on previously acidified samples in a 2 ft column packed with Porapak QS at 140°C. n-Butanol was used as the internal standard during liquid phase analysis after verifying that *n*-butanol was not present as a product.

RESULTS AND DISCUSSION

Bath Fermentation Profiles

Batch fermentation experiments with the newly isolated microorganism in glass bottles under externally controlled conditions did not show

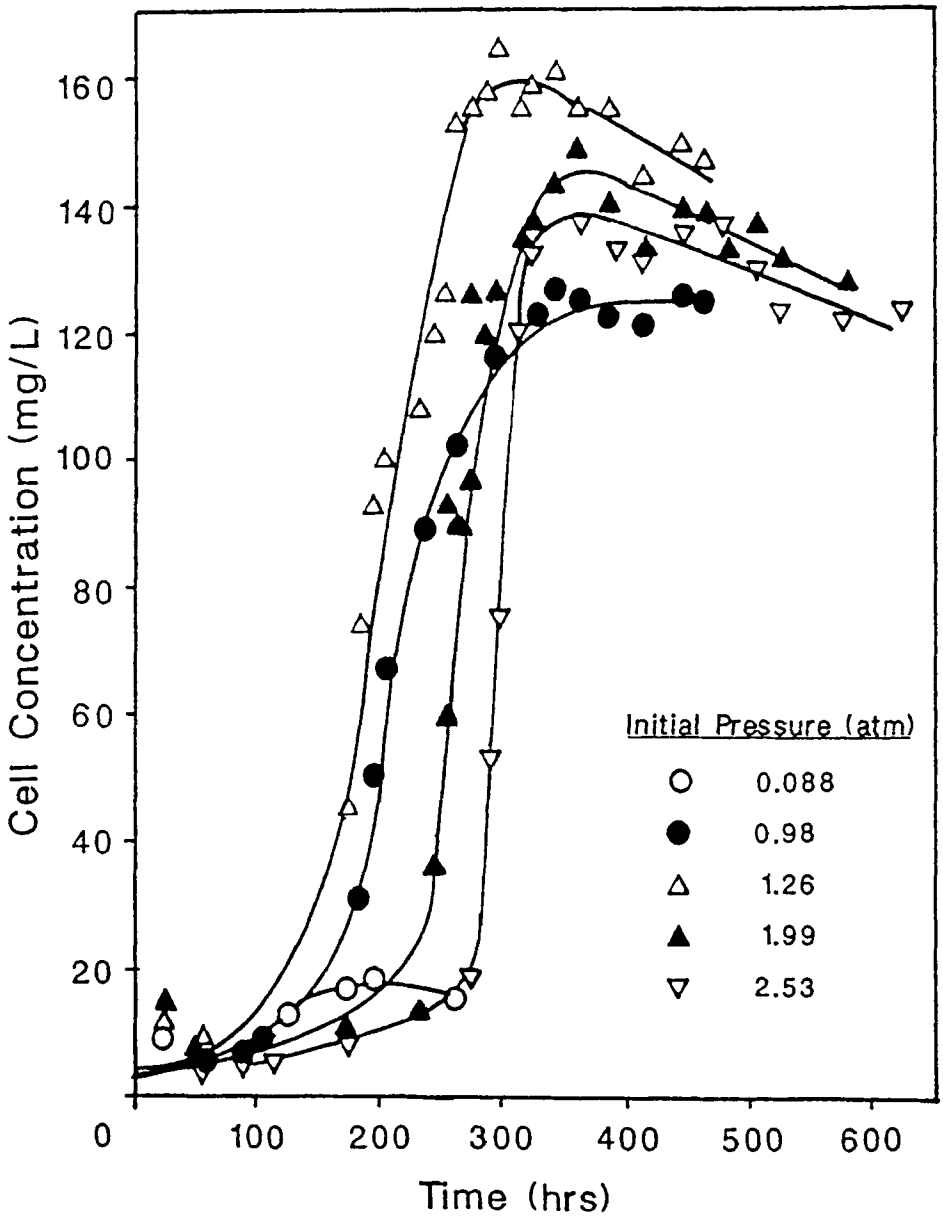

Fig. 1. Cell concentration as a function of time with 0.01% yeast extract in batch culture.

a high degree of reproducibility. This behavior is typically found with most clostridia (*19*) and although the assessment of environmental conditions in an ever-changing substrate and product concentration system is difficult, batch fermentations provide important information in evaluating overall performance. The results presented here summarize the most often encountered patterns during the studies so far conducted.

Figure 1 presents the cell concentration profiles obtained for various initial synthesis gas pressures using 0.01% yeast extract. As noted, the cell

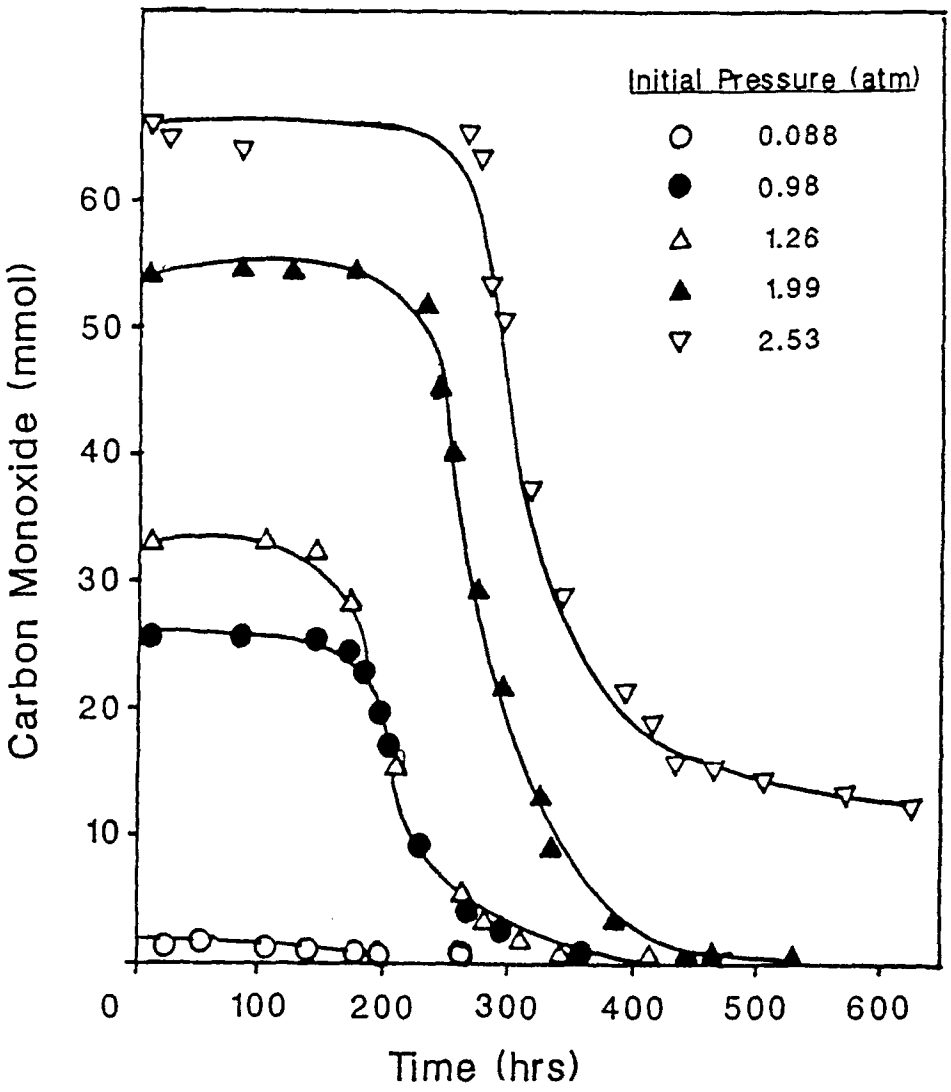

Fig. 2. Carbon monoxide disappearance with time with 0.01% yeast extract in batch culture.

concentration increased rather sharply after a lag phase period that was generally longer with increasing initial pressure. The maximum cell concentration was not proportional to the amount of substrate available. Instead, a maximum cell concentration was achieved when the initial pressure was around 1.30–1.40 atm. A period of decrease in the medium optical density was observed at the end of the batch process. At higher yeast extract concentrations, a slight increase in the total cell concentrations achieved was generally observed, as well as a decrease in the lag phase period.

The disappearance of carbon monoxide and hydrogen during the batch fermentation for various initial pressures is shown in Figs. 2 and 3, respectively. In the experiment, the bottle with the highest initial gas pressure

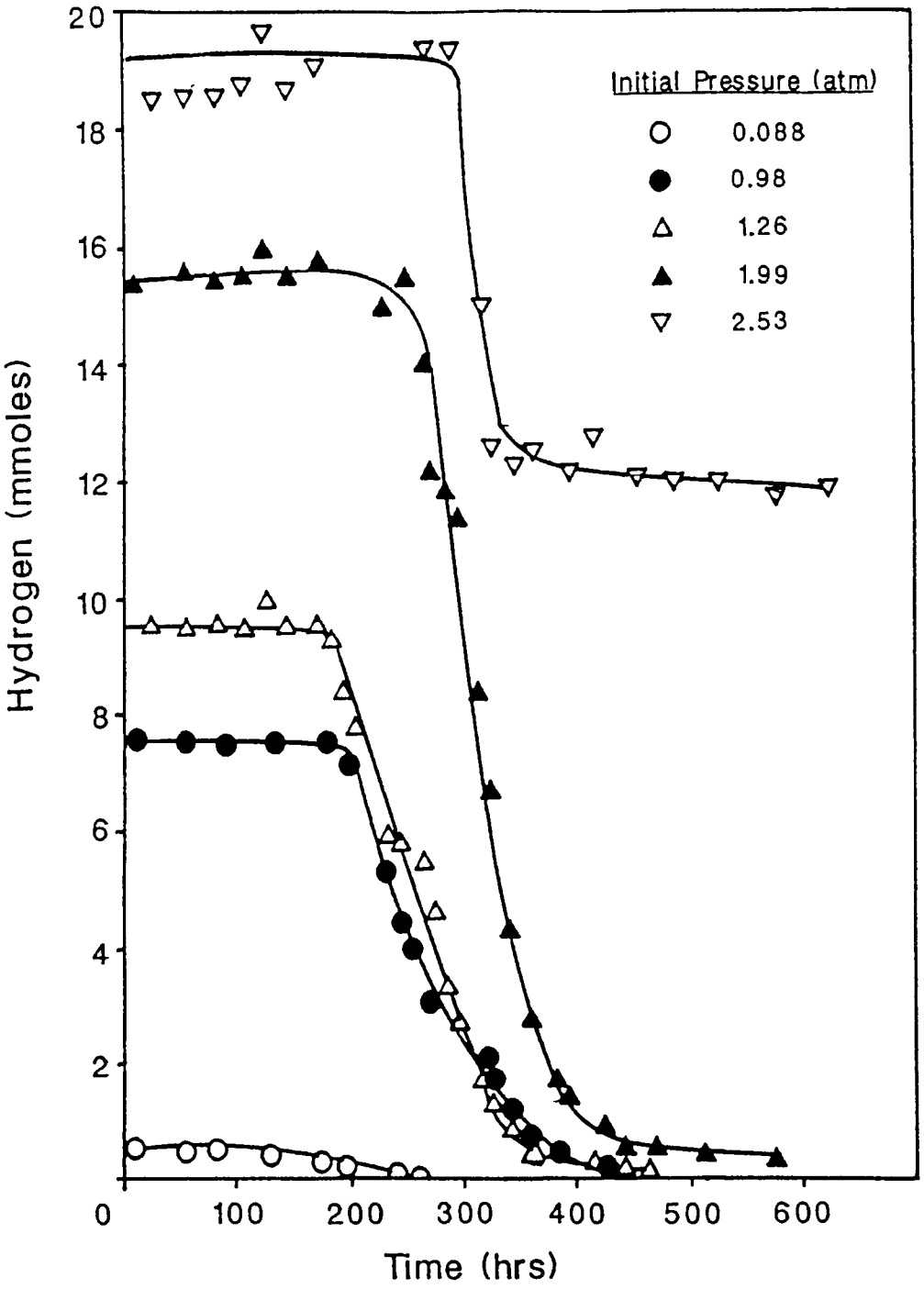

Fig. 3. Hydrogen disappearance with time with 0.01% yeast extract in batch culture.

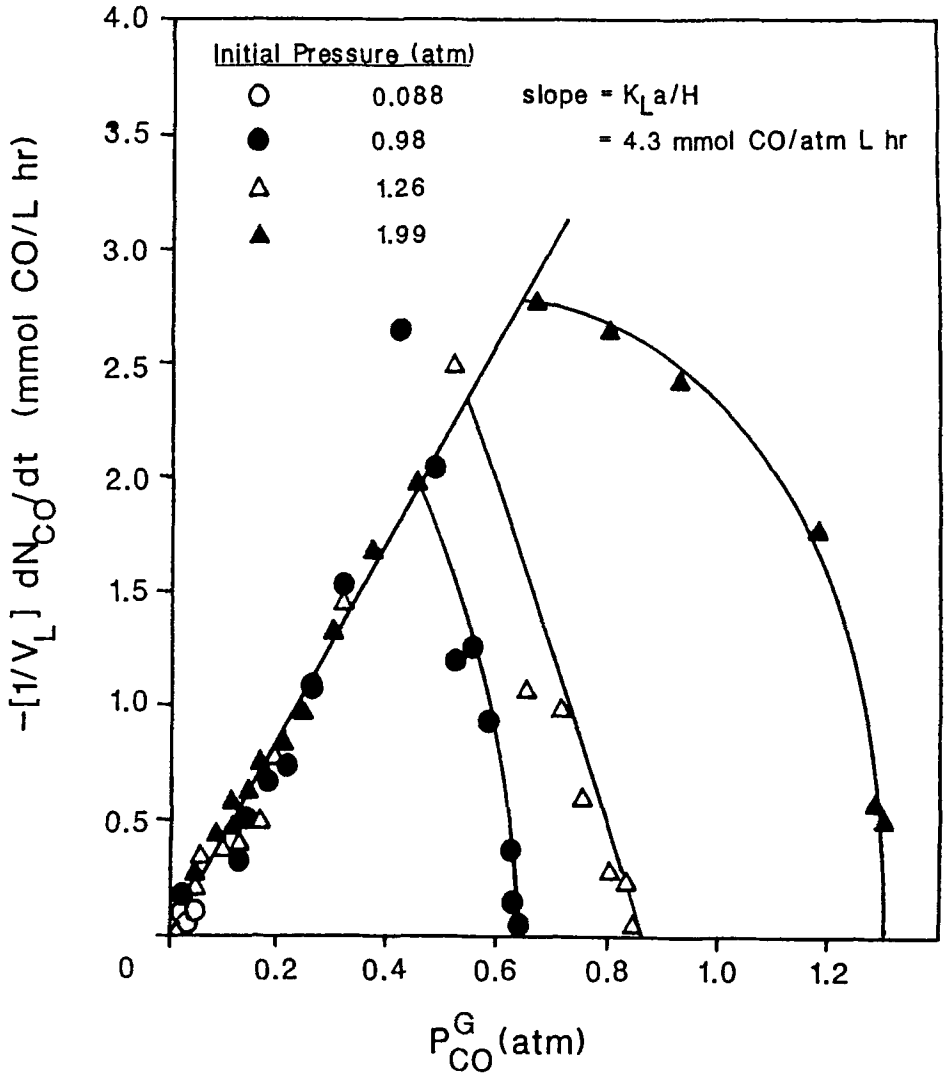

Fig. 4. Determination of the volumetric mass transfer coefficient for carbon monoxide in batch culture.

did not achieve complete conversion of the gaseous substrates. The utilization of slightly soluble gaseous substrates (such as CO and H_2) in shaken bottles becomes mass transfer limited at a certain level of cell concentration as has been described previously (*17,20*). Under these conditions, the volumetric rate of carbon monoxide uptake is proportional to the partial pressure of carbon monoxide in the gas phase, as is shown in Fig. 4 for the four bottles that fully utilized the substrates. The ratio of the mass transfer coefficient to the Henry's law constant for carbon monoxide, K_La/H, is found to be 4.3 mmol CO/atm L h from the slope of the best fit line through the data. A similar plot can be obtained for hydrogen.

The dissolved carbon monoxide and hydrogen concentrations are, therefore, kept at a level very close to zero as soon as the fermentation

becomes mass transfer limited. The batch fermentation can be considered to be substrate limited throughout most of the process. Although hydrogen is consumed simultaneously along with carbon monoxide during the batch process, it is easily observed that hydrogen utilization does not occur until the dissolved carbon monoxide concentration falls to a very low level. Any decrease in the microbial uptake rate of carbon monoxide, which leads to increased levels of dissolved carbon monoxide (*see*, for example, the bottle at highest pressure in Figs. 2 and 3), immediately results in a sudden stop of hydrogen uptake. This behavior can be understood in terms of a preferred substrate (CO) or in the well known inhibitory effect of carbon monoxide on hydrogenase activity (*21,22*).

The production of acetate and ethanol during a batch fermentation followed typical patterns of primary metabolites. Figure 5 shows the acetate produced as a function of time for various initial pressures. Parallel profiles were obtained for ethanol produced although at lower levels. The amount of acetate obtained increased with the amount of substrate consumed, and, for most of the fermentation, the cell concentration increase was proportional to the acetate produced (data not shown). Both acetate and ethanol concentrations reached a maximum, after which their concentration in the fermentation broth decreased. The length of this period of product decrease and its magnitude were not very repetitive in experiments under similar conditions. The fate of the acetate and ethanol consumed has not been ascertained.

The production of ethanol and acetate occurred simultaneously and a rather constant ratio of products was maintained along the batch fermentation. Figure 6 presents the ethanol produced as a function of the acetate produced during batch fermentations with various initial yeast extract concentrations. A molar ratio of ethanol acetate of about 1:9 was obtained for the three lower levels of yeast extract employed, whereas a ratio of 1:22 occurred for yeast extract levels of 0.1 and 0.2% after short initial period with a higher ethanol-to-acetate ratio. These ratios were found to be rather consistent in all of the batch experiments performed to date, and do not seem to be affected by the initial pressure employed.

Stoichiometries and Yields

The stoichiometry for the formation of acetate from carbon monoxide, as well as hydrogen and carbon dioxide, has been well-established for many acetogenic bacteria (*4*). These reactions are

$$4\,CO + 2\,H_2O \rightarrow CH_3COOH + 2\,CO_2$$
$$\Delta G^\circ = -37.8\ \text{Kcal/g mole}\ CH_3COOH \quad (1)$$

$$2\,CO_2 + 4\,H_2 \rightarrow CH_3COOH + 2\,H_2O$$
$$\Delta G^\circ = -18.6\ \text{Kcal/g mole}\ CH_3COOH \quad (2)$$

The lack of an autotrophic ethanol producing microorganism has not allowed the determination of the stoichiometries of ethanol formation.

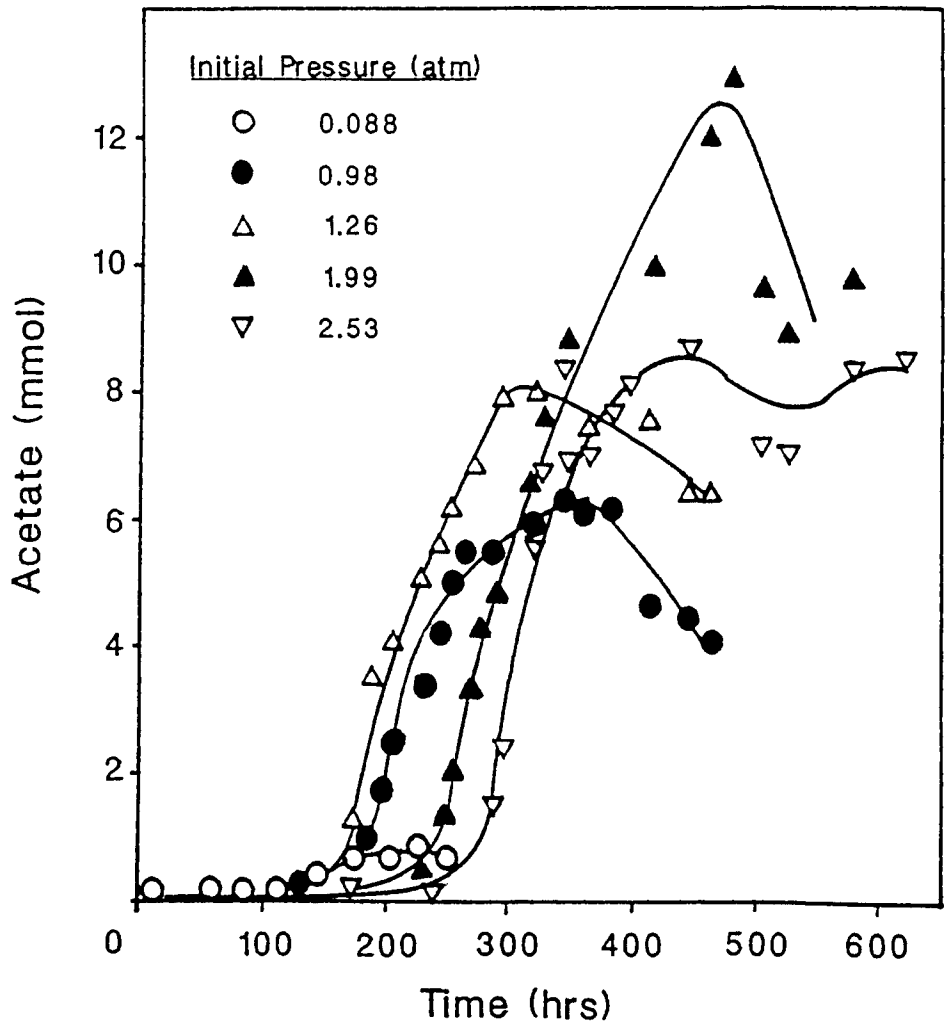

Fig. 5. Acetate produced as a function of time with 0.01% yeast extract in batch culture.

From a similarity standpoint with respect to Eqs. (1) and (2), the following reactions may be considered

$$6\,CO + 3\,H_2O \rightarrow C_2H_5OH + 4\,CO_2$$
$$\Delta G^\circ = -59.9\ \text{Kcal/g mole}\ C_2H_5OH \quad (3)$$
$$2\,CO_2 + 6\,H_2 \rightarrow C_2H_5OH + 3\,H_2O$$
$$\Delta G^\circ = -23.2\ \text{Kcal/g mole}\ C_2H_5OH \quad (4)$$

Although it is not possible from the experimental data presented earlier to isolate ethanol production from acetate formation in order to assess the ethanol yields on the synthesis gas components, an indirect indication of the validity of Eqs. (1-4) can be inferred. Combining Eqs. (1-4) yields

$$6\,\Delta N_{ETOH} + 4\,\Delta N_{ACH} = -(\Delta N_{CO} + \Delta N_{H2}) \quad (5)$$

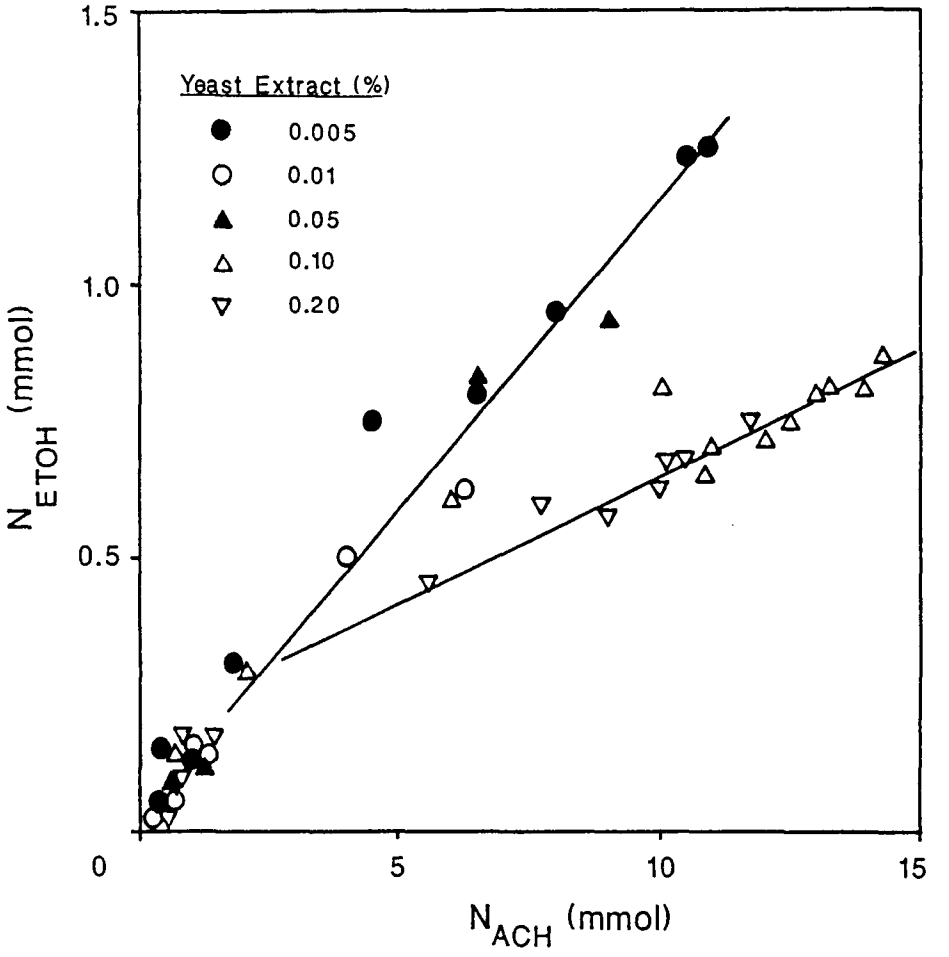

Fig. 6. Product distribution for various initial yeast extract concentrations in batch culture.

The stoichiometrical relationship of Eq. (5) has been tested in Fig. 7 for data at various initial pressures. As observed, all of the data fall along a straight line of slope very close to one, in agreement with the stoichiometries proposed in Eqs. (1–4). It should be mentioned, however, that some experiments, especially those at high initial pressures, resulted in slopes significantly lower than one (0.68–0.8). In all of these cases in question, carbon material balances could not be closed. No explanation is available yet for the lack of stoichiometric agreement in some of the higher initial pressure experiments.

Cell yields on carbon monoxide and hydrogen have not been accurately determined yet. Preliminary results suggest that about 3.5% of the carbon monoxide is converted to cell material (0.035 g cell/g CO) during most of the batch fermentations. This result agrees well with other acetogenic autotrophic bacteria (*17*). The occurrence of growth on some components of yeast extract is possible, but has not been positively demonstrated.

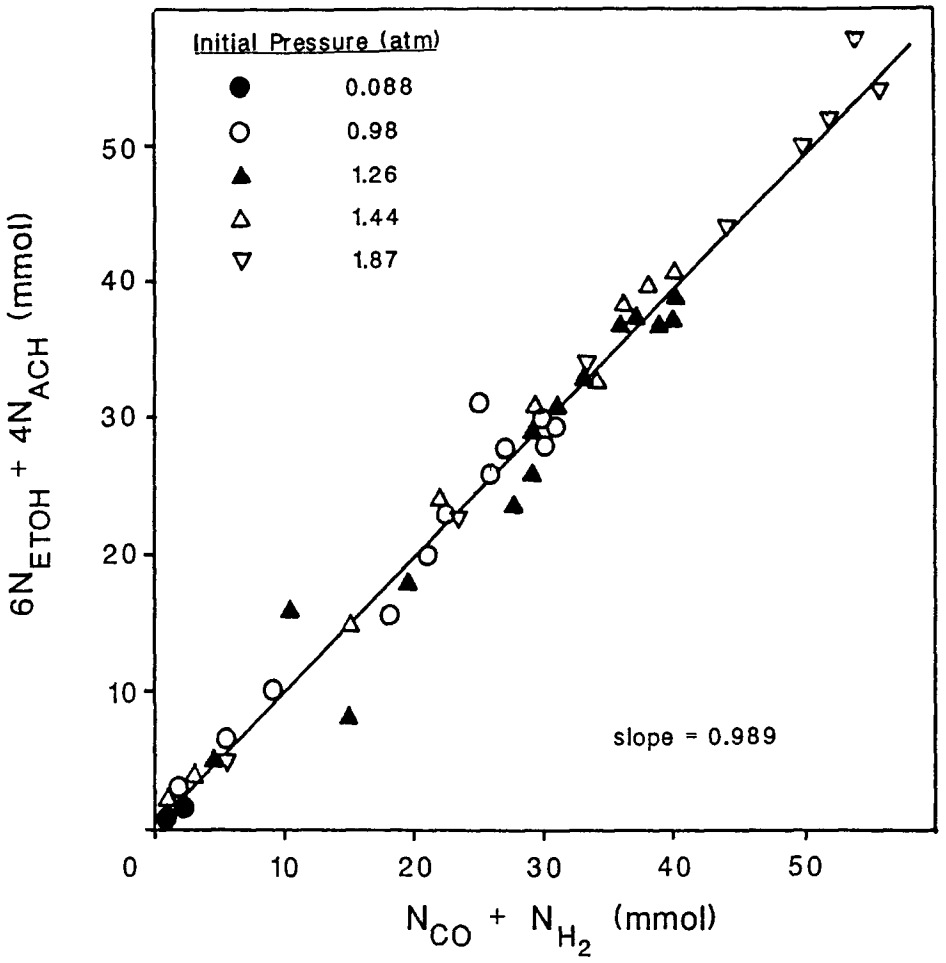

Fig. 7. Testing of the proposed stoichiometries for ethanol and acetate production from CO and H_2 in Eq. (5).

Continuous Fermentation in a CSTR

A continuous experimental run was carried out with the newly isolated microorganism for a period of 36 d at constant agitation rate (400 rpm) and gas flowrate (3.5 mL/min). The results obtained for cell concentration, substrate conversion levels, product concentrations in the effluent, and product molar ratios of ethanol to acetate are given as functions of the operating time in Figs. 8, 9, 10, and 11, respectively. In these figures, four regions can be identified as a result of different externally applied conditions.

A period of 4 d was maintained at the beginning of the experiment using batch liquid culture and 0.01% yeast extract (Region I). During this period, the cell concentration increased rapidly, achieving high conversions in a short period of time. Acetate and ethanol accumulated in the reactor to

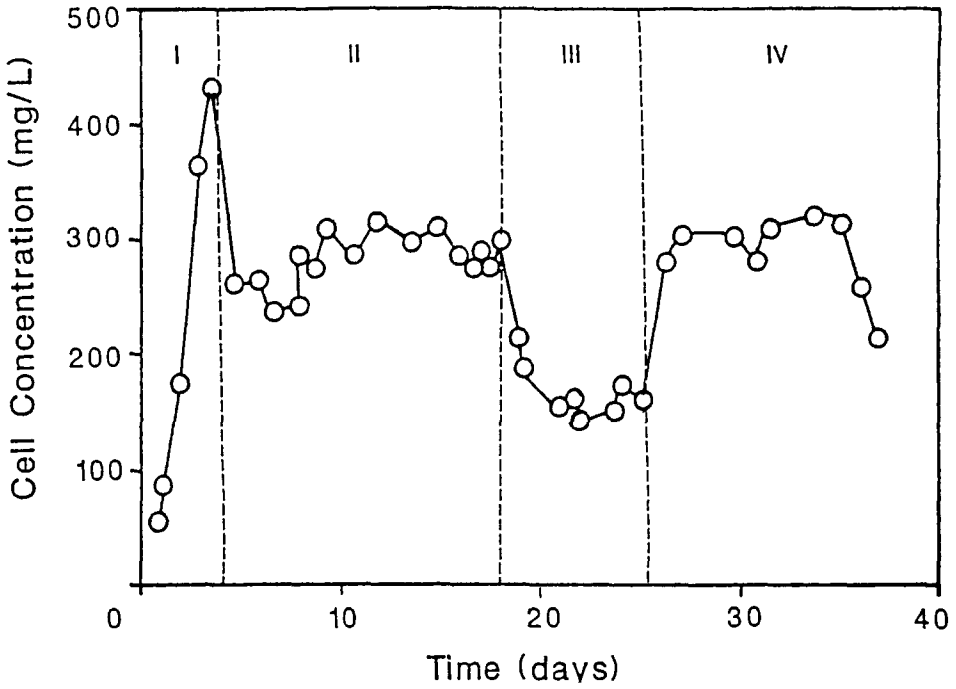

Fig. 8. Cell concentrtiton as a function of operating time in the CSTR.

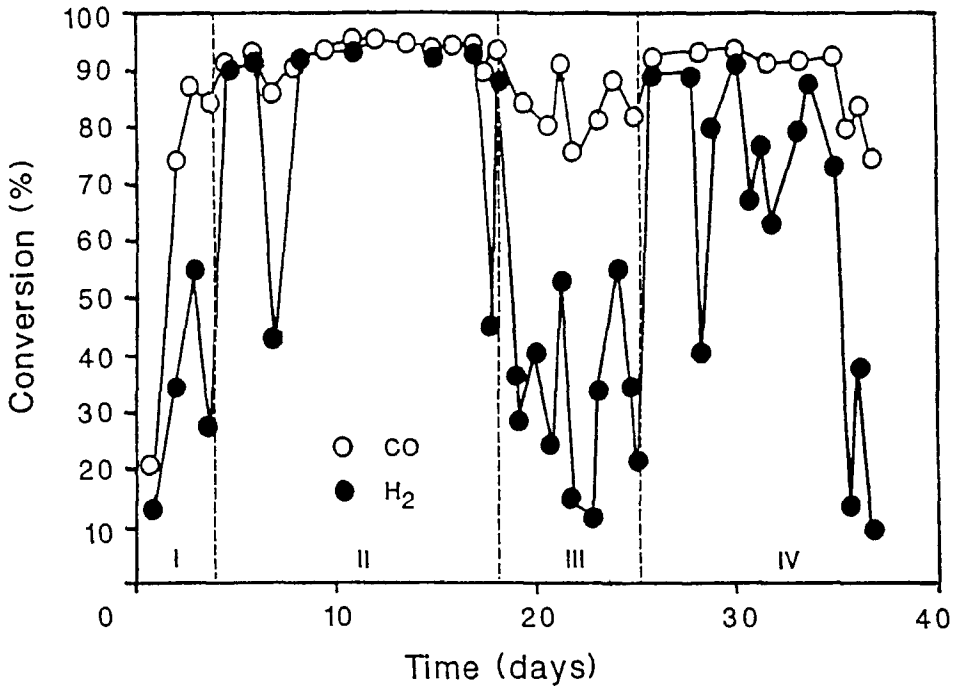

Fig. 9. Carbon monoxide and hydrogen conversion as a function of operating time in the CSTR.

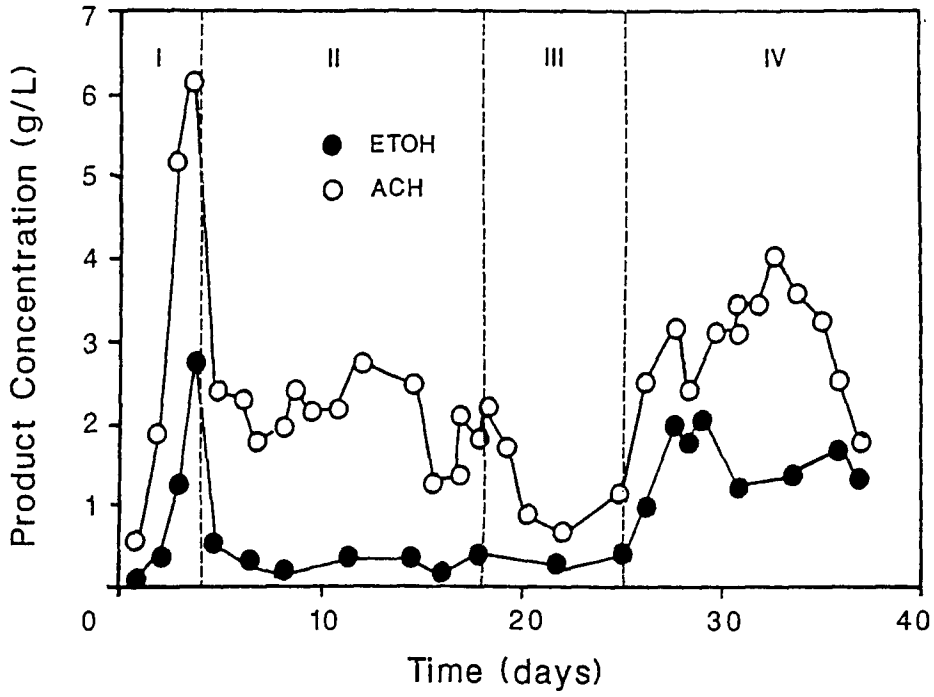

Fig. 10. Ethanol and acetate concentrations in the reactor as a function of operating time in the CSTR.

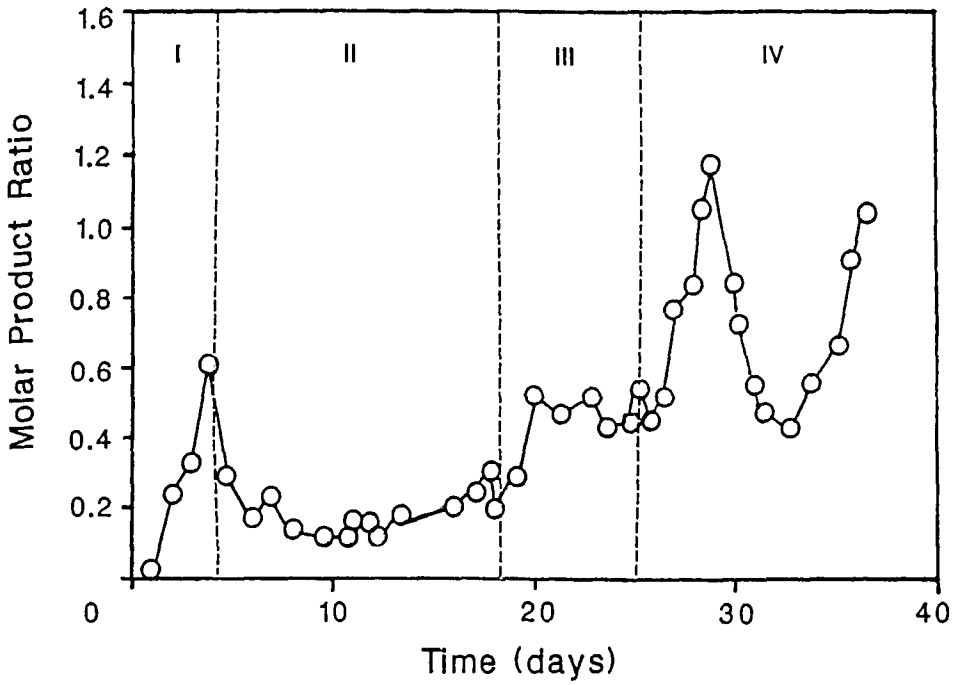

Fig. 11. Ethanol/acetate molar ratio as a function of operating time in the CSTR.

about 6 g/L and 2.8 g/L, respectively, with the molar ratio continuously increasing up to about 1:1.6. At the end of Region I, the conversion levels of the substrates dropped, probably because of a lack of nutrients or product inhibition.

Continuous liquid feed containing 0.1% yeast extract was then started at a dilution rate of 0.069 h^{-1} (Region II). The cell concentration achieved at steady state was 300 mg/L. The conversion levels of carbon monoxide and hydrogen stabilized at about 92%. From previous experience with the same equipment (*17*), it was known that these conversions corresponded to gas–liquid mass transfer limited operation at the conditions of agitation and gas flowrate employed. The system was, therefore, under substrate limitation, with both of the dissolved gas concentrations very close to zero. Approximately 0.2 g/L ethanol and 2.3 g/L acetate were obtained, yielding a very unfavorable molar ratio of about 1:5.

After 11 d of operation, the yeast extract concentration in the feed was lowered to 0.01%, holding all other variables constant (Region III). The cell concentration in the reactor decreased to around 150 mg/L along with marked reductions in the conversion levels. As was observed in batch culture, the presence of significant dissolved carbon monoxide in the fermentation broth had a large effect on the hydrogen uptake (hydrogen conversions mimic carbon monoxide conversions at much lower values). The reactor, in this region, was not substrate limited but possibly nutrient limited. The specific rate of carbon monoxide uptake under these conditions was around 75 mmoles CO/gcell · h. The ethanol concentration in the effluent was not affected by the lower yeast extract concentration (although the ethanol production/U of cell concentration doubled), whereas the acetate concentrations dropped to about 1 g/L. The molar ratio thus improved to about 1:2.

In Region IV, the dilution rate was decreased to $D = 0.031\ h^{-1}$ while maintaining the same yeast extract concentration in the feed (0.01%). The decrease in the liquid flow rate imposed a further decrease in the supply of yeast extract to the reactor. Nevertheless, the cell concentration increased to around 200 mg/L, forcing an even lower yeast extract concentration available per cell. The conversion levels improved somewhat due to the higher cell concentrations although the mass transfer limited operation of Region II was not achieved. The product concentrations increased as a result of the lower dilution rate but the most spectacular change was observed in the product molar ratio which oscillated between 1:2 and 1:0.8.

In general, the continuous fermentation results indicate the importance of yeast extract and dilution rate on the product distribution and the stability of the system. Studies involving stability of other clostridial cultures under various operating conditions such as substrate concentration and dilution rate (*23*), nitrogen and phosphate limitations (*24,25*), and so on, have often shown oscillatory behavior and contradictory results with regard to improvements in the solvents/acids ratios. In our experiments, yeast extract seems to provide stability, increase the specific rates of car-

bon monoxide and hydrogen uptake, and favor the formation of acetate. Low levels of yeast extract and low dilution rates promote ethanol formation, although these conditions lead to an oscillatory pattern in the product distribution. In order to adequately assess the influence of yeast extract components, efforts have been initiated to grow the microorganism in defined media in a manner similar to Drake et al. (*26,27*).

CONCLUSIONS

The autotrophic production of mixtures of ethanol and acetic acid from synthesis gas components (CO, H_2, and CO_2) have been demonstrated in batch and continuous cultures with a recently isolated microorganism. An indirect method has been used to assess the stoichiometries for the formation of ethanol and acetic acid from carbon monoxide and from hydrogen and carbon dioxide with experimental data gathered along batch fermentations at various initial pressures. A rather constant ratio of ethanol vs acetate during batch fermentations was obtained. This ratio seems to be affected by the initial yeast extract concentration but not by the initial gas pressure in the bottles.

The continuous operation of a CSTR with independent gas and liquid feed streams has demonstrated the importance of yeast extract concentration on the stability and product distributions under externally controlled conditions. Specific rates of carbon monoxide uptake as high as 75 mmol CO/g cell h has been achieved under nutrient limited operation. The optimum molar product ratio of ethanol to acetate was obtained at the lowest yeast extract concentration and the lowest liquid dilution rate employed in this study. Equimolar amounts of both products in the fermentation broth have been achieved although in an oscillatory manner. The effect of other environmental variables such as pH, eH, electron flow modificators, and of substrate and product inhibitors is currently under study. The ultimate goal of the work is the achievement of a continuous homoethanolic fermentation of synthesis gas components.

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