

BIOREACTOR DESIGN FOR SYNTHESIS GAS FERMENTATIONS

K. T. Klasson, M. D. Ackerson, E. C. Clausen, and J. L. Gaddy
Department of Chemical Engineering
University of Arkansas
Fayetteville, AR 72701

ABSTRACT

Fermentation of slightly soluble gaseous substrates, such as CO and H₂, requires the transport of the substrate from the gas phase, through the liquid phase, and into the solid phase for conversion. These reactions are generally mass transport limited, and bioreactor designs must achieve high mass transfer coefficients, as well as high cell concentrations, to minimize reactor volume. Immobilized cell systems are ideal for these fermentations, and operation at high pressure facilitates gas solubility and faster mass transfer. This paper compares the performance of a continuous stirred tank reactor, a bubble column reactor, and a trickle-bed reactor for the conversion of CO, CO₂, and H₂ in coal synthesis gas into methane using a tri-culture of *Rhodospirillum rubrum*, *Methanobacterium formicicum*, and *Methanosarcina barkeri*. *R. rubrum* is a photosynthetic bacterium, and special provisions for supplying light for growth of this organism are necessary. Mass transfer coefficients are compared and intrinsic kinetics presented. Gas retention times of a few minutes have been achieved for complete conversion of the gaseous substrate.

INTRODUCTION

Synthesis gas, a mixture of primarily CO, H₂ and CO₂, is a major building block in the production of fuels and chemicals. The gas may be produced from several sources, including coal, oil shale, tar sands, heavy residues, biomass or natural gas. Most synthesis gas is produced today by catalytic reforming of natural gas, although the partial oxidation of heavy liquids is also practiced (Graboski, 1984). Only a small percentage of the synthesis gas currently produced is by gasification of solid fuel. However, because of the large reserves of coal in the United States (300 year supply at the current consumption rate (Specks and Klussman, 1982)), synthesis gas production from coal will become an important technology in the future.

Coal gasification, which is a combination of pyrolysis and combustion reactions, (Simbeck *et al.* 1982), produces a gas consisting of more than 50 percent H₂ and CO, the balance being a mixture of CO₂, CH₄, H₂S, COS and nitrogen compounds. The actual composition depends upon process conditions and the coal that is employed. The raw gas has a low to medium Btu content, with a heating value of 160-450 Btu/SCF, depending on whether air or oxygen is used during gasification (Coffin, 1984). Following quenching and purification, the synthesis gas contains 25-35 percent H₂, 40-65 percent CO, 1-20 percent CO₂, 0-7 percent CH₄ and other compounds in small quantities.

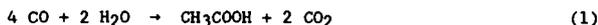
Catalytic processes may be used to convert syngas into a variety of fuels and chemicals, such as, methane, methanol, formaldehyde, acetic acid, etc. (Courty and Chaumette, 1978). Microorganisms may also be used to convert synthesis gas components into fuels and chemicals. Biological processes, although generally slower than chemical reactions, have several advantages over catalytic processes, such as higher specificity, higher yields, lower energy costs and generally greater resistance to poisoning. Furthermore, the irreversible character of biological reactions allows complete conversion and avoids thermodynamic equilibrium relationships.

The purpose of this paper is to present data for the development of optimal bioreactor concepts for syngas fermentations. Laboratory data for continuous culture experiments for the conversion of synthesis gas components into methane and ethanol are presented. Various bioreactor schemes for synthesis gas fermentations have been investigated and mathematical models that define intrinsic kinetics and mass transfer relationships are developed. Methods to predict reactor performance and gas retention times for the CSTR and immobilized cell reactor are presented.

SYNTHESIS GAS FERMENTATIONS

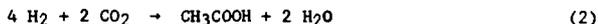
Methane Production

Methane may be produced by methanogenic bacteria from either acetate or H₂ and CO₂, both of which may be produced from syngas components. Acetate may be produced by several anaerobic bacteria, including Peptostreptococcus productus, (Barik *et al.* 1987; Lorowitz and Bryant, 1984), Acetobacterium woodii (Kerby *et al.* 1983) Clostridium thermoaceticum (Wood *et al.* 1982; and Kerby and Zeikus, 1982) and Eubacterium woodii (Genthner and Bryant, 1982), which produce acetate by the reaction:



Among these bacterial species, P. productus utilizes CO very rapidly with a doubling time of less than two hours, and can grow with as much as 90 percent CO in the gas phase (Barik *et al.* 1987).

Many anaerobic bacteria, including P. productus, are known to produce acetate from H₂ and CO₂, (Mayer *et al.* 1977; Sleat *et al.* 1985; and Balch *et al.* 1977), which produces a homoacetic fermentation by anaerobically oxidizing hydrogen and reducing CO₂ according to the equation:



Two species of purple non-sulfur bacteria, Rhodospseudomonas gelatinosa (Uffen, 1976; and Dashkevich and Uffen, 1979) and Rhodospirillum rubrum (Breed *et al.* 1977) are known to perform the water gas shift reaction to produce H₂ as follows:



R. gelatinosa grows under strict anaerobic conditions on the dark with CO as the only carbon and energy source, although growth is stimulated by the addition of trypticase. R. rubrum requires tungsten light and the presence of a carbon source other than CO (sugars, acetate, yeast extract etc.) for growth. In comparing these two species, R. rubrum grows faster and reaches higher cell concentrations that uptake CO more rapidly. R. rubrum has also been found to tolerate small amounts of oxygen and sulfur compounds often present in synthesis gas.

Almost all methanogenic bacteria, including Methanospirillum hungatii, Methanobacterium formicicum, Methanobrevibacter smithii, Methanosarcina barkeri, utilize CO₂ and H₂ to produce CH₄ according to (Thauer *et al.* 1977; Balch *et al.* 1979; and Zehnder *et al.* 1981:



Methane may also be produced from acetate by Methanosarcinaceae sp., such as Methanosarcina barkeri, as well as Methanothrix soehngenii (Jones et al. 1987). While Methanosarcina barkeri will utilize acetate only in the absence of other preferred substrates (such as H₂ and CO₂), Methanothrix sp. does not utilize H₂ and CO₂ and growth and methane formation is observed exclusively in the presence of acetate (Huser et al. 1982). Both microorganisms show comparable specific growth rates at low acetate concentrations (< 3mM). However, from the Monod saturation constants (K_s = 0.7 mmol/l for Methanothrix and 5 mmol/l for M. barkeri), it is expected that at low acetate concentrations Methanothrix will give faster rates and predominate.

From the above, it can be seen that the production of methane from syngas is a two-step process; formation of the methane precursors (acetate or hydrogen) and the biomethanation of the precursor. These reactions may be carried out in separate stages or as a CO culture in the same reactor. Compatibility of the cultures with substrates and products is essential for an efficient process.

Methane Production from Acetate. In order to produce methane from synthesis gas through acetate, CO (and possibly CO₂ and H₂) is first converted to acetate using the bacterium P. productus. The acetate is then reacted to methane using either Methanothrix sp. or M. barkeri.

In order to develop a successful co-culture, both the acetogenic and methanogenic bacteria must have resistance to CO toxicity. Studies of the CO uptake rate with time for P. productus using various initial CO partial pressures showed an increase in the rate of reaction with increasing partial pressure up to a partial pressure of 1.6 atm. At a partial pressure of 2.5 atm, however, the culture failed to utilize the gas after a short initial period of uptake. At 2.5 atm, the dissolved CO concentration reached toxic levels due to insufficient cell mass to keep the reaction mass transfer-limited. Studies have shown that both growth and CO uptake by P. productus are inhibited at dissolved CO tensions above 0.16-0.8 atm (Vega et al. 1989). Higher gas phase CO partial pressures may be employed as long as a sufficient number of cells are present to keep the dissolved CO tension low.

Figure 1 illustrates a gradual stepwise procedure where CO partial pressures as high as 10 atm are successfully employed. The pressure was gradually increased in this study only after the cell concentration increased in order to keep the process mass transfer-limited. The effects of CO on methanogens may be illustrated using the bacterium M. barkeri. The consumption of H₂ with time for M. barkeri at various initial CO partial pressures is shown in Figure 2. As noted, the time for consumption essentially doubled when increasing the CO partial pressure from 0 to 0.59 atm. This result is expected due to the well-known inhibitory effect of CO on hydrogenases. Similar results were obtained for Methanothrix sp. It is thus essential for hydrogen-utilizing organisms such as methanogenic bacteria that a low dissolved CO concentration be maintained.

A second factor in evaluating methane production from synthesis gas through acetate is the potential inhibitory effects of acetate on both P. productus by product inhibition and methanogens by substrate inhibition. It has been found that acetate concentrations of 20-25 g/L may be successfully employed with P. productus without appreciable product inhibition. Figure 3 shows the effect of acetate concentration on methane production by M. barkeri in batch culture. As noted, the methane production increased with increasing acetate concentration up to 6 g/L acetate, with some inhibition noted at the 6 g/L level. At acetate concentrations above 6 g/L, however, methane production was severely inhibited. Similar inhibitory effects were seen with Methanotherix sp. where methane production was slowed at 9 g/L acetate and stopped at 12 g/L.

Figure 4 shows the methane productivity in an immobilized cell reactor (ICR) employing Methanotherix sp. at various feed acetate concentrations. The column was operated over a period of nearly 250 days by gradually increasing the inlet acetate concentration and flow rate as cell growth allowed. As noted in the figure, a maximum inlet acetate concentration of 10 g/L was successfully employed at a methane productivity of 5 VVD. Further increases in the acetate concentration were not possible, even with the simultaneous addition of high concentrations of yeast extract. Thus, comparatively low productivities, even in an ICR, result for methane production from acetate.

Co-culturing of P. productus and methanogens utilizing acetate was not found feasible due to the slow rate of growth and low acetate tolerance of methanogens. P. productus totally dominated the co-cultures, producing acetate much faster and in higher concentrations than the methanogens were capable of utilizing. The methanogens were thus inhibited by acetate and the co-culture could not be sustained. The production of methane through acetate will, therefore, require separate reaction vessels.

Methane Production from H₂ and CO₂. An alternative route involves the conversion of CO and H₂ by R. rubrum, followed by conversion of all the H₂ and CO₂ to methane using either M. formicicum or M. barkeri. It has been found that H₂ production by R. rubrum is essentially unaffected by CO partial pressures up to 2.0 atm. Therefore, as with P. productus, the limiting factor in CO utilization by R. rubrum is the ability to maintain a high cell concentration and, consequently, a low dissolved CO tension in the liquid phase. M. formicicum has been shown to be able to uptake H₂ and CO₂ to produce methane much faster than M. barkeri. However, in order to form a successful co-culture, M. formicicum must be able to tolerate low levels of dissolved CO. The utilization of H₂ by M. formicicum in the presence of various CO partial pressures is shown in Figure 5. As noted, nearly complete inhibition of H₂ uptake was found at a CO partial pressure of only 0.76 atm. The inhibition of CO on M. barkeri might enable a rapid rate of uptake of H₂.

R. rubrum is a photosynthetic bacteria requiring tungsten light for growth, but not for CO uptake. Figure 6 shows the growth and consumption of CO with time at various light intensities for R. rubrum. As shown, the cell growth rate increased with light intensity up to 1490 lux, however, no further enhancement was found at higher intensities. CO consumption was essentially

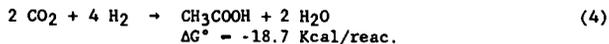
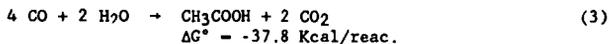
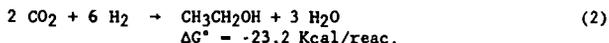
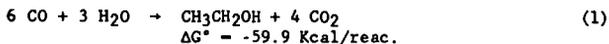
unaffected by the presence of light. Methanogens have been found to be unaffected by the presence of light.

An experiment was performed in a continuous stirred-tank reactor to study the simultaneous conversion of CO₂ and H₂ directly to CH₄ employing a co-culture of *R. rubrum* and *M. formicicum*. Since the organisms have different optimum temperatures, the lower temperature, 30°C, was chosen for study. Figure 7 shows the CH₄ and CO production with time since start-up in the CSTR. Following a significant period of methanogen acclimation, almost complete conversion of both CO and H₂ occurred after 300 hours of operation. The methane production rate shown in Figure 7 reached a steady-state level after 350 hours of operation of about 1.6 mmole CH₄/hr, which represents a methane yield from CO, and H₂ and CO₂ of about 96 percent of theoretical. The system was operated with a retention time of one hour and stable operation was monitored for several weeks.

Ethanol Production

While many anaerobic, facultatively anaerobic and even some strictly aerobic microorganisms form various amounts of ethanol from glucose (Wiegel, 1980), no organisms were known to form ethanol autotrophically from synthesis gas components. In 1987, a strict anaerobic mesophilic bacterium was isolated that was capable of converting CO, H₂ and CO₂ to a mixture of acetate and ethanol (Barik *et al.* 1987). Identification and characterization studies have shown that the bacterium is a new clostridial species, named *Clostridium ljungdahlii*, Strain PETC, in honor of Dr. Lars G. Ljungdahl for his work on clostridia and acetogens (Clausen and Gaddy, 1988). *C. ljungdahlii* is a gram-positive, motile, rod-shaped anaerobic bacterium which sporulates infrequently. In addition to synthesis gas components, it is capable of growing on xylose, arabinose and fructose. As with other class I clostridia, it is expected that ethanol and acetate are formed through acetyl-CoA as the central intermediate (Rogers, 1986).

The overall stoichiometry for the formation of ethanol and acetate from CO and H₂/CO₂ has been established as (Vega *et al.* 1989):



Under usual laboratory conditions, *C. ljungdahlii* produces acetate as the major product, with only small quantities of ethanol present in the product stream. Figures 8 and 9 show the acetate and ethanol production profiles as a function of yeast extract concentration. The data show an ethanol/acetate

ratio of only 0.5. It is also noted that yeast extract has an influence on the product ratio, which leads to the hypothesis that high ethanol production is non-growth related. An examination of the acetyl-CoA pathway shows that production of acetate is balanced in ATP, while ethanol production results in a net consumption of ATP which would not support growth of the bacteria. Therefore, studies to minimize acetate production have concentrated upon factors which would not support growth of the bacteria. Therefore, studies to minimize acetate production have concentrated upon factors which regulate the growth of the organism.

Control of Growth Rate Parameters. *C. ljungdahlii* grows well and produces ethanol and acetate within a pH range of 4-6 with typical anaerobic media. Figure 10 shows the product distribution for *Clostridium* sp. with various initial yeast extract concentrations in batch culture. As noted, a molar ethanol to acetate ratio of 1:0 was obtained for yeast extract concentrations between 0.005 and 0.05 percent. The normal ratio of 1:22 results under more favorable growth conditions when employing 0.1 and 0.2 percent yeast extract. Studies with a defined medium of only vitamins, minerals and salts showed similar results in increasing the product ratio to about .1.

Recent research has shown that the presence of reducing agents in the liquid media of *Clostridium* fermentations has brought about an increase in solvent formation (Rao and Muetharsan, 1987; 1988). Reducing agents apparently cause altered electron flow, which direct carbon flow and acid to alcohol production. Reducing equivalents are directed to the formation of NADH which, in turn, resulted in increased alcohol production. Batch experiments were carried out with *C. ljungdahlii* by adding small quantities of reducing agents (30, 50 and 100 ppm) to assess the feasibility of increasing the ethanol to acetate ratio. The experiment carried out with 100 ppm of reducing agents resulted in very limited growth in all cases. On the other hand, 50 ppm and 30 ppm concentrations were successful in improving the ethanol to acetate ratio in some cases, as is shown in Table 1. The experiment with benzyl-viologen at a concentration of 30 ppm produced 3.7 mmol of ethanol with a ratio of 1.1, the highest ratio observed in batch experiments. It is interesting to mention that those reducing agents that improved the product ratio always resulted in slower growth rates of the bacteria, as could be expected from decreased ATP formation.

Table 1. Peak Levels for Ethanol Production and the Molar Ratio
(ETOH/ACH at 30 and 50 ppm Reducing Agent Concentrations)

Reducing agent	(50 ppm)		(30 ppm)	
	EtOH(mmol)	ETOH/ACH	ETOH(mmol)	ETOH/ACH
Control	0.60	0.12	1.40	0.24
Sodium thioglycolate	1.30	0.20	1.30	0.25
Ascorbic acid	1.50	0.24	1.50	0.25
Menthyl Viologen	1.90	0.20	2.50	0.40
Benzyl Viologen	1.25	0.21	3.70	1.10

Recently, the connection between sporulation and increased solventogenesis has been identified (Jones *et al.* 1982; Long *et al.* 1984; Gattschal and Morris, 1981). Under certain conditions, which are strain-dependent, a shift of the bacteria into a sporulation phase is accompanied by morphological changes (elongation of the cells) and the production of solvents rather than acids. A batch experiment with *C. ljungdahlii* was conducted on the premise that by forcing the culture to grow at a reduced rate, sporulation could be induced with an accompanying improvement in ethanol production. Synthesis gas was used as the primary carbon substrate. However, the complex nutrient yeast extract was replaced by various sugars and starches which, in previous studies, promoted sporulation of *Clostridium thermosaccharolyticum* (Pheil and Ordal, 1967). Table 2 summarizes the results obtained for each of the nutrients studies, along with the maximum values obtained for cell concentration, ethanol concentration and molar product ratios. As noted, the highest product ratios were obtained for cellobiose and rhamnose, with product ratios over 3 times the ratio obtained in the presence of yeast extract. Ethanol and cell concentrations were highest in the presence of cellobiose and galactose, where the ethanol concentrations were over 4 times the value obtained in the presence of yeast extract and the cell concentrations were 20 percent greater. Thus, cellobiose as a nutrient produces not only higher ratios of ethanol to acetate, but also higher concentrations of ethanol and cells.

Table 2. Summary of Results with Nutrient Sources Bringing About Sporulation

Nutrient	Maximum		ETOH/ACH molar ratio
	Cell Conc (mg/L)	ETOH (mmol)	
Yeast Extract	140	0.13	0.13
Cellobiose	170	0.56	0.45
Rhamnose	135	0.31	0.44
Galactose	168	0.53	0.36
Starch	130	0.27	0.36

Continuous Stirred-Tank Reactor Performance. An obvious method to produce high ethanol ratios is to operate two continuous reactors in series, with the first used to promote cell growth, while the second reactor is used for increased ethanol production. A pH shift between the reactors from 4.5 to 4.0, as well as a dilution rate shift, are used to cause the onset of ethanol production while, at the same time, causing growth to cease. Media constituents to promote growth can be added to the first reactor, and constituents to promote ethanol production at the expense of acetate can be added to the second reactor.

Figure 11 shows the molar product ratios for both stirred-tank reactors. yeast extract (0.02 percent) was added to the liquid medium of Reactor A (first in the series initially and cellobiose later). Ethanol concentrations in Reactor B increased to nearly 3 g/L and seemed to be stimulated somewhat by the use of cellobiose as the nutrient for cell growth. Substrate CO and H₂ conversions were essentially 100 percent in Reactor A, and fluctuated somewhat in Reactor B. The product ratio increased with time in both reactors, reaching a value of about 1.0 in Reactor A and a value of about 1.5 in Reactor B. The addition of cellobiose seemed to improve the product ratio over yeast extract. By subtracting the product concentrations produced in Reactor A, an ethanol ratio of 4 moles is obtained in Reactor B.

The specific productivity steadily improved to levels of 250-300 mmole ethanol/gcell·day throughout the experiment, which is a 30-fold improvement over specific productivities in a single CSTR.

BIOREACTOR DESIGN

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 (Vega *et al.* 1989a, 1989b). 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. These concepts will be expanded in this and the following section.

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.

It can be shown that the substrate rate per unit of reactor volume, $\frac{dN_S^G}{V_L dE}$, is given in terms of the gas phase partial pressures as:

$$\frac{d N_S^G}{V_L dt} = \frac{K_L a}{H} (P_S^G - P_S^L) \quad (1)$$

where N_S^G - moles substrate transferred from the gas phase, V_L is the volume of the liquid phase, t is time, K_L is the overall mass transfer coefficient, a is the gas-liquid interfacial area per unit volume, H is Henry's law constant, P_S^G is the partial pressure of the substrate in the bulk gas phase, and P_S^L is the partial pressure (dissolved tension) of the substrate in the liquid phase ($P_S^L = HC_L$). 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:

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

where X is cell concentration and q_m , K'_p , and W' are Monod constants.

Equation (2) 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 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 concentrations in that particular bioreactor. Obviously, the best bioreactor is one that will achieve high cell concentrations and high mass transfer rates.

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. Trickle-bed columns, where the gas is the continuous phase and the liquid flows over packed internals, is a unique means of increasing the mass transfer for these systems.

Stirred-Tank Reactor. The traditional CSTR assumes complete mixing and uniform concentrations throughout 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 near 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:

$$\frac{1}{Y_0} = \frac{1}{Y_1} + \frac{V_L}{Y_1} \frac{K_L a}{H} \frac{P_I^G}{n_I} \quad (3)$$

Equation (3) is expressed in terms of an inert component, whose quantity and partial pressure does 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_1 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 for $P_{\text{productus}}$ is shown in Figure 12. 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 (3), 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.* 1989b). Solutions of the model for various volumetric mass transfer coefficients and various total operating pressures are shown in Figures 13 and 14, respectively. Experimental data at 1 atm and a mass transfer coefficient of 30 are also included in the figures. As observed, increases in the mass transfer coefficient or in total operating pressure leads 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 13 shows that with a mass transfer coefficient of 100, a pseudo retention time of one hour would result in a conversion of 80 percent. From Figure 14, the retention time could be reduced to 6 minutes at 10 atm for the same amount of CO converted. The use of the model allows the extrapolation of performance of the CSTR system and will permit preliminary economic evaluation of an industrial scale process when coupled with suitable equations for scale-up of properties such as the mass transfer coefficient.

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 being 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:

$$\ln \frac{P_S^0}{P_S^1} = \frac{K_L a}{H} \frac{\epsilon_L h R T S}{G} \quad (4)$$

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 $\ln Y_1/Y_0$ vs $ShRT/G$ yields a straight line with slope $K_L a \epsilon_L / H$. The numerical solution (Runge-Kutts) of the differential equations that describe the system were solved for other operating conditions and are shown in Figure 15. 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 percent, compared to 80 percent for the CSTR. Alternatively, 80 percent conversion could be achieved in a retention time only 3 min 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.

CONCLUSIONS

The fermentation of coal synthesis gas has been demonstrated to methane and ethanol. Two pathways for the indirect production of methane from synthesis gas have been evaluated. Production through acetate as an intermediate is limited by acetate inhibition of methanogens. Production through H_2 with a co-culture of R. rubrum with methanogens gives faster rates without inhibition.

Ethanol can be produced from synthesis gas with a new species of Clostridium isolated from animal waste. The ratio of ethanol to acetate in the product stream is affected by many variables including pH, nutrient composition and the introduction of reducing agents to alter electron flow. High ethanol ratios are favored by non-growth conditions. Product ratios of 4:1 (ethanol to acetate) are achieved in a two-stage continuous culture with pH and dilution rate shift.

Bioreactors that achieve high mass transfer rates and high cell concentrations are desirable for synthesis gas fermentations. Methods to determine mass transfer coefficients for CSTR and ICR reactors have been developed. High pressure has been found to increase the reaction rate proportionately. Models for these bioreactors show high conversion of gaseous substrate can be achieved in a retention time of a few minutes.

LIST OF REFERENCES

- Bailey, J. E. and D. F. Ollis, Biochemical Engineering Fundamentals, McGraw-Hill, New York (1986).
- Balch, W. E., S. Schoberth, R. S. Tanner, and R. S. Wolfe, "Acetobacterium, New Genus of Hydrogen-Oxidizing, Carbon-Dioxide-Reducing Anaerobic Bacteria," Int'l. J. Syst. Bacteriology, 27, 355-361 (1977).
- Barik, S., E. R. Johnson, E. C. Clausen, and J. L. Gaddy, "Conversion of Coal Synthesis Gas to Methane," Energy Progress, 7, 157 (1987).
- Barik, S., J. L. Vega, E. R. Johnson, E. C. Clausen, and J. L. Gaddy, "Methanation of Synthesis Gas Using Biological Processes," CRC Series, (1987).
- Breed, R. S., E. G. D. Murray, and N. R. Smith, Bergey's Manual of Determinative Bacteriology, The Williams and Wilkins Company, Baltimore, MD (8th ed.) (1987).
- Clausen, E. C. and J. L. Gaddy, "Advanced Studies of the Biological Conversion of Synthesis Gas to Methane," Topical Report 1: Reactor Optimization, Performed on METC Contract DE-AC21-86MC23281, U. S. Department of Energy, March 1988.
- Coffin, J. M., "Industrial Coal Gasification: Applications and Economy," Energy Progress, 4, 131-137 (1984).
- Courty, Ph. and P. Chaumette, "Syngas: A Promising Feedstock in the Near Future," Energy Progress, 7, 23-30 (1987).
- Dashekovicz, M. P. and R. L. Uffen, "Identification of a Carbon Monoxide-Metabolizing Bacterium as a Strain of Rhodopseudomonas gelatinosa," International Journal of Systematic Bacteriology, 29, 145-148 (1979).
- Finn, R. K., "Agitation-Aeration in the Laboratory and in Industry," Bacteriol. Rev. 18, 154-274 (1954).
- Genthner, B. R. S. and M. P. Bryant, "Growth of Eubacterium limosum with Carbon Monoxide as the Energy Source," Appl. Environ. Microbiol. 43, 70-74 (1982).
- Gottschal, J. C. and J. G. Morris, Biotechnology Letters, 3, 525-530 (1981).
- Graboski, M. S., "The Production of Synthesis Gas from Methane, Coal, and Biomass. In Catalytic Conversion of Synthesis Gas and Alcohols to Chemicals, R. G. Herman (ed.) Plenum Press, New York, pp. 37-50 (1984).
- Huser, B. A., K. Wuhmann, and A. J. B. Zehnder, "Methanotherix soehgenii gen. nov. sp. nov., a New Acetotrophic Non-hydrogen-oxidizing Methane Bacterium," Arch. Microbiol. 132, 1-9 (1982).

- Jones, D. T. et al. "Solvent Production and Morphological Changes in Clostridium acetobutylicum Induced by Viologen Dyes," Applied and Environmental Microbiology, 53, No. 6, 1232-1235 (June 1987).
- Jones, W. J. D. P. Nagle, Jr., and W. B. Whitman, "Methanogens and the Diversity of Archaeobacteria," Microbiol. Rev. 51, 135-177 (1978).
- Kerby, R., W. Niemczura, and J. G. Ziekus, "Single Carbon Catabolism in Acetobacterium woodii and Butyribacterium methylotrophicum by Fermentation and ¹³C Nuclear Magnetic Resonance Measurement," J. Bacteriol. 155, 1208-1218 (1983).
- Kerby, R. and J. G. Ziekus, "Growth of Clostridium thermoaceticum on H₂/CO₂ or CP as Energy Source," Curr. Microbiol. 132, 1-9 (1982).
- Long, S et al. "Isolation of Solvent Production, Clostridial Stage and Endospore Formation in Clostridium acetobutylicum." Applied Microbiology and Biotechnology, 20, 256-261 (1984).
- Lorowitz, W. H. and M. P. Bryant, "Peptostreptococcus productus Strain that Grows Rapidly with CO as the Energy Source," Appl. Environ. Microbiol. 47, 961-964 (1984).
- Mayer, F., R. Lurz, and S. Schoberth, "Electron Microscopic Investigation of the Hydrogen-Oxidizing Acetate-Forming Anaerobic Bacterium Acetobacterium woodii," Arch. Microbiol. 112, 207-214 (1977).
- Pheil, C. G. and Z. G. Ordal, "Sporulation of the Thermophilic Anaerobes," Applied Microbiology, 51, No. 4, 893-898 (1967).
- Rao, G. and R. Murtharasan, "Altered Electron Flow in a Reducing Environment in Clostridium acetobutylicum," Biotechnology Letters, 10, No. 2, 129-132 (1988).
- Rao, G. and R. Mutharasan, "Altered Electron Flow in Continuous Cultures of Clostridium acetobutylicum Induced by Viologen Dyes," Applied and Environmental Microbiology, 53, No. 6, 1232-1235 (June 1987).
- Rogers, P., "Genetics and Biochemistry of Clostridium Relevant to Development of Fermentation Processes." Advances in Applied Microbiology, 31, 1-60 (1986).
- Simbeck, D. R., R. L. Dickenson, A. J. Moll, "Coal Gasification, An Overview," Energy Progress, 2, 42-46 (1982).
- Sleat, R., A. Mah, and R. Robinson, "Acetoanaerobium noterae: New Genus, New Species, An Anaerobic Bacterium that Forms Acetate from Hydrogen and Carbon Dioxide," Intl. J. Syst. Bacteriol. 35, 10-15 (1985).
- Specks, R. and A. Klussmann, "German Hard Coal Conversion Projects," Energy Progress, 2, 60-65 (1982).
- Thauer, R. K., K. Jungnermann, and K. Decker, "Energy Conservation in Chemotrophic Anaerobic Bacteria," Bacteriol. Rev. 41, 100-180 (1977).

Uffen, R. L., "Anaerobic Growth of a Rhodospseudomonas Species in the Dark with Carbon Monoxide as Sole Carbon and Energy Substrate," Proc. Natl. Acad. Sci. U.S.A. **73**, 3298-3302 (1976).

Vega, J. L., S. Prieto, B. B. Elmore, E. C. Clausen, and J. L. Gaddy, "The Biological Production of Ethanol from Synthesis Gas," Appl. Biochem and Biotech. **20**, 781 (1989).

Vega, J. L., E. C. Clausen, and J. L. Gaddy, "Biofilm Reactors for Ethanol Production," Enzyme Microb. Technol. **10**, 403 (1988).

Vega, J. L., E. C. Clausen, and J. L. Gaddy, "Study of Gaseous Substrate Fermentations: Carbon Monoxide to Acetate. 1. Batch Culture," Biotechnol. Bioeng. (accepted for publication) (1989a).

Vega, J. L., G. M. Antorrena, E. C. Clausen, and J. L. Gaddy, "Study of Gaseous Substrate Fermentations: Carbon Monoxide to Acetate. 2. Continuous Culture," Biotechnol. Bioeng. (accepted for publication) (1989b).

Wiegel, J., "Formation of Ethanol by Bacteria. A Pledge for the Use of Extreme Thermophilic Anaerobic Bacteria in Industrial Ethanol Fermentation Processes." Experientia, **36**, 1434-1446 (1980).

Wood, H. G., H. L. Drake, and S. Hu, "Studies with Clostridium thermoaceticum and the Resolution of the Pathway Used by Acetogenic Bacteria that Grow on Carbon Monoxide or Carbon Dioxide and Hydrogen." Proc. Biochem. Sym. 29-56 (1982).