Fossil

Biological Upgrading of Coal-Derived Synthesis Gas

Final Report

**S. Barik
E.R. Johnson
C.W. Ko
E.C. Clausen
J.L. Gaddy**

October 1986

Work Performed Under Contract No.: DE-AC21-84MC21011

**For
U.S. Department of Energy
Office of Fossil Energy
Morgantown Energy Technology Center
Morgantown, West Virginia**

**By
University of Arkansas
Fayetteville, Arkansas**

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ABSTRACT

The technical feasibility of the biological conversion of coal synthesis gas to methane has been demonstrated in the University of Arkansas laboratories. Cultures of microorganisms have been developed which achieve total conversion in the water gas shift and methanation reactions in either mixed or pure cultures. These cultures carry out these conversions at ordinary temperatures and pressures, without sulfur toxicity.

Several microorganisms have been identified as having commercial potential for producing methane. These include a mixed culture of unidentified bacteria; P. productus which produces acetate, a methane precursor, and Methanothrix sp., which produces methane from acetate. These cultures have been used in mixed reactors and immobilized cell reactors to achieve total CO and H₂ conversion in a retention time of less than two hours, quite good for a biological reactor. Preliminary economic projections indicate that a biological methanation plant with a size of 5×10^{10} Btu/day can be economically attractive.

ACKNOWLEDGMENT

Financial support for this work was provided by the Morgantown Energy Technology Center, Department of Energy on contract No. DE-AC21-84MC21011.

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1.0 INTRODUCTION

The United States has massive reserves of coal, enough to last about 500 years at the current rate of consumption. However, the sulfur content of much of this coal is so high that environmental regulations prevent its burning, or the energy content is so low that the transportation and combustion of the coal is economically prohibitive. These problems must be resolved prior to the utilization of this abundantly available natural resource.

Coal may be converted to a more convenient energy source and a valuable chemical feedstock by gasifying the coal and removing undesirable components. In a gasification process, the coal is gasified by adding steam and oxygen at 0-70 atm and at temperatures as high as 1500°C. A gas consisting of more than 50 percent hydrogen and carbon monoxide is produced. A typical range of literature values for synthesis gas compositions from many gasification processes is shown in Table 1.1. In addition to CO and H₂, small amounts of CO₂, CH₄, H₂S and COS are also produced.

TABLE 1.1

Typical Composition of Gasifier Gas*

<u>Component</u>	<u>Percent of Total</u>
Hydrogen	25-36
Carbon Monoxide	43-65
Carbon Dioxide	1-20
Methane	0-7
Sulfur as H ₂ S or COS	0-1

*average if data taken from Westfield Lurgi gasifier, Shell Kopper gasifier, and Cool Water Gasification Project, Energy Progress, 1982.

To upgrade the synthesis gas, the gas typically undergoes acid gas removal to remove the sulfur-bearing compound and carbon dioxide and catalytic water-gas shift reaction where carbon monoxide and H₂O are converted to carbon dioxide and hydrogen. Following purification to remove undesired carbon dioxide and hydrogen sulfide, the hydrogen and carbon monoxide are catalytically methanated to methane and water. The water is then removed to give a gas that is 95-98 percent methane with a higher heating value of 980⁺ Btu per SCF.

1.0.1 Catalytic Coal Gas Methanation

Three basic steps are used in catalytically upgrading and purifying the raw coal gas resulting from the gasification of coal to pipeline quality methane:

1. Water-gas shift conversion;
2. Acid gas removal and sulfur recovery; and,
3. Methanation and drying.

The water-gas shift conversion reaction is used to convert carbon monoxide and water to carbon dioxide and hydrogen by the reaction:

Hydrogen and carbon dioxide are reacted in the methanation step by two main reactions:

Sulfur removal is required in most processes to protect the nickel-based catalysts from sulfur poisoning. After drying and acid gas removal, the resulting pipeline-quality gas is 95-98 percent methane.

The temperatures required in most methanation processes are 300-450°C at pressures of 40-250 psi.¹ The Exxon process employs gasification and methanation in a single step at 700°C.^{2,3} Most catalysts used are nickel-based; however, the Exxon process utilizes a potassium-based catalyst.^{2,3} Poisons for these catalysts include chlorine and sulfur.^{4,5} One source also points out that free carbon, nitrogen and oxygen are potential nickel-based catalyst poisons.⁶

Capital costs for shift conversion, acid gas removal and sulfur recovery, and methanation and drying typically constitute 20-30 percent^{7,8} of the total capital costs in most coal gasification processes. Typical projected plant sizes cited in the literature produce about 125 million ft³/day requiring \$2.3 billion in total capital costs. Total costs for gasification range from \$5-6/million Btu.⁹ Methanation costs are usually not separated from other gasification costs and are, therefore, difficult to assess. One isolated source lists the total costs for converting carbon monoxide and hydrogen to methane at \$6-10/million Btu.¹⁰ A more reasonable estimate of the total cost of methanation is probably 25 percent of the gasification costs, or about \$1.50/million Btu.

Because high temperatures and pressures are required in the catalytic gasification process, losses in thermal efficiency and high energy costs occur. Thus, a process employing lower temperatures and pressures for even a portion of the overall process scheme would be advantageous. Also, a process that is not particularly sensitive to the presence of sulfur and other toxic materials is desirable.

As an alternative to catalytic conversion, synthesis gases may be upgraded to a higher methane concentration by contacting the gases with anaerobic bacteria.¹¹ These biological processes occur at low operating temperatures and pressures and are capable of converting CO, CO₂ and H₂ to methane. Because of the lower operating temperatures, efficiencies are expected to be quite high. The process should not be particularly sensitive to sulfur and other materials found to inhibit catalytic gasifica-

tion. If sulfur sensitivity is found, resistance to sulfide toxicity may be developed or the offensive material can probably be removed biologically.

1.1 Biological Coal Gas Conversion to Methane

Anaerobic digestion has been used for many years to treat domestic and industrial wastes. Recently, anaerobic digestion has been receiving attention as a method of producing methane gas for energy usage. An anaerobic digestion culture contains a wide variety of organisms capable of converting many substrates to methane. As an example, it is now well established that microorganisms derived from an anaerobic sewage sludge digester can carry out the water-gas shift reaction anaerobically, i.e. the conversion of CO and H₂O into CO₂ and H₂.¹² Furthermore, two other components of coal gas, hydrogen and CO₂, are well known primary substrates for methane bacteria^{13,14} and can be readily converted to methane. Thus, an inexpensive method for upgrading synthesis gas would be to bubble the gas through an anaerobic digestion culture acclimated to methane production from coal gas. Since coal gasification units must be large to be economical, very large biological reactors must be employed for upgrading the coal gas. These large biological reactors mean high capital investments, so that reaction kinetics and rate constants must be maximized to reduce reactor costs.

Inside the single stage reactor, carbon monoxide would be converted to methane precursors, and these products will be converted to methane, all using a single culture. Alternatively, a series of reactors may be employed, one for precursor production and one for methane production. Finally, a co-culture of organisms may be used to improve upon mixed culture performance. In all of these systems, the pressure and temperature will remain low in comparison to catalytic methanation processes, typically from 1-10 atm and 25-75°C. The pressure should be a strong factor in determining the performance of this reaction system, and special attention should be given to this parameter.

1.1.1 Microbiology of Biological Methanation

The primary reactions in the biological conversion of coal gas are the formation of methane precursors and the biomethanation reactions. The organisms Rhodopseudomonas gelatinosa and Rhodospirillum rubrum utilize CO and H₂O to produce CO₂ and H₂ by the reaction:

The organisms Peptostreptococcus productus, Acetobacterium woodii, and Eubacterium limosum produce acetate by the reaction:

Methanosarcina sp. utilizes the CO₂ and H₂ to produce CH₄ by the reaction:

Methanothrix soehngenii utilizes acetate to produce CH₄ by the reaction

It may also be possible to convert CO directly into methane. Methanobacterium thermoautotrophicum has been reported to produce methane according to Equation 15:

The growth of this organism was reported to be very slow and was inhibited by high concentrations of CO. It has also been reported that other methanogenic bacteria can convert CO and H₂ to CH₄ according to the equation:

All of the above organisms can be isolated from natural sources. Sewage sludge from anaerobic treatment plants and animal rumen are the sources from which most anaerobic bacteria have been isolated. Sewage sludge usually contains a consortium of various methanogenic bacteria. In anaerobic digestion processes, 80 percent of the methane is produced from acetate by Equations (1.4) and (1.5). Some of the above organisms are maintained in pure culture collections. Therefore, coal gas conversions might be carried out with pure cultures or with mixed cultures, isolated from natural sources, and acclimated to the toxic environment of carbon monoxide.

All of these organisms function at optimal temperatures of 30–70°C and ordinary pressures, although higher pressures have been used successfully.²⁰ Processes to produce methane from coal gas using the above microorganisms would be very simple. The synthesis gas is introduced into a reactor containing a culture of the organisms where the CO, CO₂, and H₂ are converted into CH₄, followed by separation of the excess CO₂, to produce pipeline quality gas. The culture might consist of a mixture of microorganisms to carry out all the above reactions, a pure culture of a single organism to carry out reaction (1.6), or a co-culture of two bacteria to carry out the series of reactions (1.1) and (1.3) or (1.4) and (1.5).

Since biological reactions are much slower than chemical reactions, retention time and reactor volume are important considerations in developing an efficient and economical process. Cultures that result in reaction kinetics that give high yields and complete conversion are also important to conserve raw materials and avoid recycle.

The biological conversion of coal gas is a heterogeneous biocatalytic reaction, involving gas, liquid, and solid (microorganism) phases. The mass transfer of the slightly soluble CO and H₂ from the gas phase through the liquid and into the solid phase, as well as the kinetics of the biological reactions, are important to the understanding and design of these systems. The conversion in the reactor is a function of the microorganism cellular concentration, the gas concentration in the liquid phase, and the liquid phase mass transfer coefficient. Therefore, reactors that develop high cell densities and high mass transfer rates will be most effective. Under certain conditions, these reactions are expected to be mass transfer limited. Therefore, operation at high pressure, where gas solubilities are higher, is expected to enhance the overall reaction rates.

1.2 Purpose

The objectives of this two-year study were seven-fold:

- a. to study the conversion of coal synthesis gas to a higher value fuel in a single-stage mixed culture obtained from sewage sludge;
- b. to screen various organisms capable of producing methane precursors from CO in coal gas;
- c. to optimize growth conditions for the production of methane precursors from CO;
- d. to carry out the conversion of coal gas components to methane in a stagewise system in order to improve upon single-stage conversion;
- e. to carry out culture enhancement studies and co-culture studies as a potential improvement over series operation;
- f. to determine the toxicity effects of sulfur gases on the performance of mixed and pure culture organism; and,
- g. to assess the preliminary economic feasibility of biologically converting coal gas to methane in pure and mixed culture.

Each of these tasks have been discussed in detail in topical reports submitted to the Morgantown Energy Technology Center. The final report will be used to compile this data and determine the technical and economic feasibility of biological coal gas conversion. Recommendations for future work will be presented and discussed.

2.0 SINGLE-STAGE CONVERSION

The simplest biological system for the conversion of coal synthesis gas to methane consists of a reactor containing a single mixed culture for methanation. The coal gas would simply be bubbled through the culture maintained at 37°C and at ambient pressures or above. The inoculum for the culture originates from domestic sewage sludge, which contains a wide range of organisms capable of converting many substrates. The culture is slowly acclimated to CO by gradual conditioning to the feed. The CO converting and methane producing bacteria eventually become the dominant organisms in the mixed culture.

Reactor designs for mixed culture methanation may consist of stirred tank reactors or immobilized cell (fixed film) reactors. The immobilized cell reactor has the advantages of no agitation and higher conversions, if a suitable population of organisms can be attached to the packing. Both of these reaction systems will be used in determining the feasibility of CO and H₂ conversion to methane in a single-stage mixed culture.

2.1 Equipment and Procedures

2.1.1 Reaction Equipment

Since the major components of coal synthesis gas, carbon monoxide and hydrogen, are only slightly soluble in water, a reactor must be chosen so as to promote mass transfer between the gas and liquid. Of the reactor choices available, the traditional stirred tank reactor seems to be a simple, but obvious, choice for these studies. A constant volume of liquid can be used to hold the mixed culture of organisms and any necessary nutrients required by the organisms. Gas may be introduced to this liquid culture continuously and the conversion of the CO and H₂ monitored. Thus, a stirred tank reactor was chosen for this study. Later studies will utilize other reactors, including immobilized cell reactors.

Single-state mixed flow reactors, constructed of steel and aluminum reinforced plexiglass, are used in studies conducted at low agitation rates. The reactor, shown in Figure 2.1, is equipped to handle continuous gas flow at pressures as high as 100 psi. Agitation of the liquid culture is provided by air-powered magnetic stirrers. Synthesis gas, a mixture of CO, CO₂, H₂, CH₄ and H₂S, enters the reactor through a gas sparger and is distributed through the mixed culture. The product gas, containing a higher concentration of methane, exits through the top of the reactor. Nutrients are periodically added to the reactor in a liquid through an inlet in the reactor side wall. Spent liquid media is also removed through this port. The reactor is operated in a room with constant temperature controlled at 37°C.

For studies requiring agitation rates exceeding 200 rpm, a New Brunswick Model C30 chemostat is employed. Again, continuous introduction of coal gas is utilized, while varying the agitation rate from 0-1000 rpm. The chemostat is equipped with temperature and agitation control, while allowing for pH monitoring and control. Since a glass vessel is employed, all experiments utilizing this vessel have to be carried out at atmospheric pressure. For high agitation rates and increased pressures, a 1 liter high pressure Parr reactor has been used.

Figure 2.1. Single-stage reactor.

A significant effort has been made to insure safe operation of the reaction system and to prevent worker exposure to toxic gases. The reaction system is housed in a constant temperature explosion-proof environment maintained at 37°C. A gas monitoring system is used to monitor the concentration of H₂S, CO and H₂ in the room. If an unsafe condition occurs in the room, a warning siren is activated, the room is exhausted, and feed gas is vented to the atmosphere. In addition, laboratory personnel working in the area wear sensitivity badges to warn of toxic gas exposure, and are instructed of procedures to be used upon entering the reaction area.

A schematic of the system used to transfer gas from high pressure cylinders to the reactors is shown in Figure 2.2. The gas is first passed through an air-actuated shut-off valve, which stops the gas flow during times of toxic gas build-up. Air is shut off to the valve in case of emergency by solenoid control from the gas monitoring system. During normal operation, the gas is passed through the air-actuated valve to flow metering valves.

The desired flow rate is set on the metering valves and monitored using gas rotameters. The gas is passed through a sampling loop and to the reactors. The product gas from the reactors is passed through a pressure gauge, another sampling loop, and finally, through a pressure regulating valve. The gas flow rate is then monitored through a wet test meter and vented to the atmosphere.

As noted, three separate feeding systems are available for the separate operation of three reaction systems. In addition, the system can be operated with various mixtures of the coal gas components. For example, pure CO can be used in starting the experiments, gradually introducing the gas mixture. Mixing tees are used to gradually change from one gas mixture to another.

2.1.2 Culture Acclimation

Sewage sludge from a primary anaerobic digester was used as the inoculum for the mixed culture study. The temperature was maintained at 37°C and atmospheric pressure. The agitation rate was held at 200 rpm while maintaining strict anaerobic conditions. Initial inoculation attempts involved bubbling a mixture of carbon monoxide, hydrogen and nitrogen slowly through 250 ml of sludge in order to acclimate the culture to coal gas. Since CO is toxic to most organisms, this brought about a rise in pH due to cell death.

Following this unsuccessful start-up attempt, an equal quantity of pH 7.3 buffer solution was added to the sludge. With this change, the introduction of the gas did not cause a pH change. Culture acclimation was a very slow procedure, requiring approximately 3 months of gradual gas introduction into the buffered sludge culture. During this time, the reactor was operated in both batch and continuous modes. After the acclimation period, synthetic coal gas containing the compositions shown in Table 2.1 was used. These compositions were used as a typical compositions from coal gasification processes. H₂S will be added in later studies.

Figure 2.2. Synthesis gas reaction system.

TABLE 2.1

Laboratory Synthetic Coal Gas Composition

	Mole Percent	
	Gas I	Gas II
Carbon Monoxide	45	65
Hydrogen	30	22
Carbon Dioxide	15	11
Methane	10	2

2.1.3 Operational Procedures

The day to day operational procedures in attaining steady state rate data were much the same as those used during start-up. After choosing a gas retention time, coal gas was bubbled through the culture for at least 5 retention times. The gas composition at the reactor inlet and outlet was monitored on a daily basis. Periodically, the pH of the liquid culture was checked and nutrients were added as needed. The composition of the nutrient solution is shown in Table 2.2. The gas phase during nutrient addition consisted of a 80:20% N₂:CO₂ mixture. Pfenning's mineral solution is a basal salts mixture consisting of nitrogen as ammonia, phosphate and chloride. Pfenning's trace metal solution contains iron, zinc, copper, cobalt, and

TABLE 2.2

Nutrient Solution for Single Stage Mixed Culture Operation

	Per 100 ml Aqueous Solution
Pfenning's mineral solution	5.0 ml
B-vitamins	0.5 ml
Yeast extract	0.1 g
Pfenning's trace solution	0.1 ml
NaHCO ₃	0.35 g

nickel salts, as well as sodium molybdate. When fresh nutrient solution was added, the extracted effluent was analyzed for pH, ammonia, adenosine triphosphate (ATP), cell density, and volatile fatty acids.

Steady-state was achieved after obtaining consistent gas compositions for at least two consecutive retention times. Again, this procedure required 5-10 retention times between changes in operating conditions.

The variables studied in the experimental study were gas flow rate and agitation rate while holding the other variables constant. The gas flow rate was adjusted to give retention times (based upon liquid volume) from 1.9 hours to 16.7 hours and the agitation rate ranged from 200 to 600 rpm. The liquid volume of the culture was 1 liter in the low agitation rate studies and 300 ml in the higher agitation rate studies. The pH was maintained at 7.3 and the temperature was held at 37°C.

2.1.4 Analytical Procedures

Several analytical procedures were used on a routine basis to monitor the progress of the biomethanation experiments. On a daily basis, inlet and outlet gas flow rates and compositions were measured. On a periodic basis, usually bi-weekly, the liquid culture was analyzed for bacterial growth, pH, ammonia, ATP and volatile fatty acids. These experimental methods will be described briefly in Appendix A.

2.2 Results and Discussion

Three experimental studies were carried out using single stage biomethanation during the contract period:

1. A study of the effect of agitation rate on conversion;
2. A study of the effect of gas flow rate (retention time) on culture performance; and,
3. A study of the effect of pressure on the performance of the mixed culture.

The results of these studies are presented and discussed in the following sections.

2.2.1 Agitation Rate Studies

The major components of coal synthesis gas, hydrogen and carbon monoxide, are only slightly soluble in water. The Henry's Law constants for CO and H₂ at 30°C are 6.20×10^4 atm/mole fraction and 7.29×10^4 atm/mole fraction, respectively.²¹ It is thus expected that there will be a mass transfer limitation in transferring CO and H₂ from the bulk gas phase to the liquid phase prior to reaction. A high rate of agitation could be necessary to achieve adequate conversions in the reaction system.

Experiments were carried out at variable agitation rates ranging from 200 to 600 rpm. At 200 rpm, the agitation is gentle to moderate and at 600 rpm the agitation is brisk. The gas composition was as described in Table 2.1 with the gas retention time initially held constant at 7.6-8.3 hr. (gas flow rate of 42-46 ml/hr through the 350 ml liquid culture). As mentioned previously, the reaction temperature was held constant at 37°C and the reaction pressure was 1 atm.

Table 2.3 shows a summary of the data taken at agitation rates ranging from 200 to 600 rpm after 3 months of acclimation. Carbon balances between inlet and outlet streams are quite good when the differing flows are considered. As noted, the conversion of CO at 500 and 600 was about 99 percent, and the conversion of H₂ was 90 percent at 500 rpm and 100 percent at 600 rpm. The conversion of CO at 400 rpm was 70 percent and 38 percent at 200 rpm.

TABLE 2.3

Single Stage Conversion Experiments at Variable Agitation Rates
and (3 months acclimation time)

Average Gas Retention Time (hrs)	Agitation Rate (rpm)	Gas Concentration (mole percent)							
		CO		CH ₄		CO ₂		H ₂	
		In	Out	In	Out	In	Out	In	Out
8.3	200	46.5	28.6	9.5	17.5	15.2	26.5	28.7	27.0
8.3	400	48.2	13.4	9.5	18.5	15.2	44.3	27.1	23.9
8.3	500	45.2	1.5	9.8	32.6	15.4	62.4	29.8	3.6
7.6	600	45.3	0.2	9.5	34.9	14.6	64.9	30.6	0

As is shown in the table, the CO conversion increased almost linearly with agitation rate up to an agitation rate of 500 rpm. Hydrogen conversion, on the other hand, is somewhat slower and also reached complete conversion at an agitation rate of 500 rpm. As the agitation rate is increased, the concentration of substrate (CO and H₂ in the liquid phase) decreases. Therefore, the differences in conversion are primarily the result of differences in concentration (Henry's law constant, initial concentration, etc.) as well as differences in microorganisms. Thus, high agitation rates are needed to provide good mass transfer between the insoluble substrate and the organisms.

In order to monitor culture stability and change, the experiments of Table 2.3 were repeated using the same culture after about 8 months of continuous gas flow through the culture. The gas retention time in these studies was 1.94 hrs (180 ml/hr through the 350 ml liquid culture), while varying the agitation rate from 200 to 600 rpm. Again, the gas composition, temperature, and pressure were held constant as before.

The results of these experiments are shown in Tables 2.4 and 2.5. As is noted in Table 2.4, the compositions of the gas streams were nearly the same as in the prior results even when using a gas flow rate over 4 times the rate in the previous experiments. The culture has continued to improve its ability to grow in the presence of CO and convert CO and H₂. Visual observation shows a greater number of cells in the later culture causing an enhancement of the rate.

TABLE 2.4

Single-Stage Gas Compositions Using the Stirred Tank Reactor
(8 months Acclimation Time)

Agitation (rpm)		CO,%	H ₂ ,%	CO ₂ ,%	CH ₄ ,%
200	In	43.38	29.86	15.79	11.09
	Out	44.82	18.01	21.70	14.80
300	In	43.24	29.56	15.01	10.65
	Out	25.74	11.15	39.35	22.93
400	In	45.20	27.01	15.25	11.42
	Out	6.71	3.84	55.40	33.25
500	In	43.88	29.63	14.91	10.81
	Out	4.48	2.81	57.99	33.76
600	In	44.53	28.33	14.88	11.19
	Out	1.66	4.81	60.67	31.85

Coal gas conversion data, as shown in Table 2.5, indicate a 90 percent conversion of both CO and H₂ at an agitation rate of 500 rpm. Again, the flow rate of coal gas is essentially doubled over the previous study, indicating marked improvement in the culture with further acclimation time. Thus, a 2 hour retention time at 500 rpm and atmospheric pressure are adequate conditions for nearly complete coal gas conversion.

TABLE 2.5

Single Stage Conversion of CO and H₂ Using the Stirred-Tank Reactor
(8 months Acclimation Time)

Agitation (rpm)	Gas retention time (h)	O.D. 580 nm	% Conversion	
			CO	H ₂
200	1.94	2.25	0	36.69
300	1.94	2.35	40.48	62.28
400	1.94	2.30	85.16	85.78
500	1.94	2.55	89.80	90.52
600	1.94	2.15	96.28	83.03

The experiments as shown in Table 2.5 were repeated one additional time at the same gas retention time after 1 year of acclimation. These results are shown in Table 2.6. As noted, the CO conversions stayed essentially constant at a given agitation rate. The H₂ conversions, on the other hand, increased considerably to over 90 percent at 500 rpm, and nearly 100 percent at 600 rpm. As expected, the culture has continued to improve with acclimation time, although not as significantly as in the past.

TABLE 2.6

Conversion of CO and H₂ in a Stirred-Tank Reactor At Various Rates of Agitation.
(1 year Acclimation Time)

Agitation (rpm)	Conversion*	
	% CO	H ₂
200	8.24	49.14
300	31.65	56.06
400	83.23	82.27
500	89.10	93.0
600	94.25	97.10

*Coal gas mixture contained 65% CO, 22% H₂, 11% CO₂ and 2% CH₄. Gas flow rate was approximately 3 ml.min⁻¹ and gas retention time was approximately 1.94 hr.

2.2.2 Variable Gas Flow Rate Studies

A second study was undertaken to measure the effect of gas flow rate (retention time) on CO and H₂ conversion. A constant agitation rate of 500 rpm was used. These experiments took place at approximately the same time of the earlier agitation rate experiments. Again, improvement over these results is expected with additional culture acclimation and improvement.

Tables 2.7 and 2.8 show the results of experiments carried out at 500 rpm and various retention times. Essentially no difference is seen in the conversion at retention times greater than 8 hours. At a 6.7 hour retention time the CO conversion was 88 percent and the H₂ conversion was 90 percent.

As the retention time was lowered from 3.7 hours to 1.9 hours, the CO conversion decreased to 23 percent and the H₂ conversion fell below 10 percent. Apparently, cell growth is maximized near a 4 hour retention time.

TABLE 2.7

Single Stage Gas Compositions at 500 rpm
(Atmospheric Pressure)

Average Gas Retention Time (hrs)	Gas Concentration (mole percent)							
	CO		CH ₄		CO ₂		H ₂	
	In	Out	In	Out	In	Out	In	Out
16.7	44.2	2.7	11.0	38.4	14.8	57.0	30.0	1.9
11.1	45.6	2.5	11.2	39.6	15.3	56.6	28.0	1.3
8.3	45.2	1.5	9.8	32.6	15.4	62.4	29.8	3.6
6.7	44.1	5.5	11.7	36.6	14.7	55.0	29.5	2.9
5.6	42.6	5.1	10.2	37.5	14.4	53.2	32.8	4.2
4.8	46.5	7.4	13.1	35.2	15.6	53.6	24.8	3.8
4.2	45.1	6.8	11.1	37.1	15.0	50.9	28.8	5.2
3.7	42.0	22.8	10.2	13.6	13.6	32.4	34.1	31.2
2.8	41.9	27.6	12.8	21.3	14.0	24.3	31.3	26.9
1.9	43.2	33.2	11.0	13.3	14.8	28.0	31.0	25.5

TABLE 2.8

Single Stage Conversion Experiments at 500 rpm
(Atmospheric Pressure)

Retention Time, hours	CO Conversion, %	H ₂ Conversion, %
16.7	93.9	93.7
11.1	94.5	95.4
8.3	96.7	87.9
6.7	87.5	90.2
5.6	88.0	87.2
4.8	84.1	84.7
4.2	84.9	81.9
3.7	45.7	8.5
2.8	34.1	14.1
1.9	23.2	17.7

It is interesting to compare the results of the mixed culture conversions with time. At a constant conversion of 1.9 hrs and an agitation rate of 500 rpm, Table 2.8 yields a CO conversion of 23.2 percent and a H₂ conversion of 17.7 percent. When using the culture that was acclimated to coal gas for an additional 8 month period, both the CO and H₂ conversions reached 90 percent. Thus, a minimum retention time of 1.9 hours can be utilized with the mixed culture.

This surprising result of increased conversion ability has been addressed by Andrews et al. when measuring the effects of cells on oxygen transfer.^{22,23} They note that there is a chemical enhancement due to the uptake of oxygen by cells, direct uptake by cells of oxygen from the gas phase and a hydrodynamic effect that is equally important with dead cells or inert particles. Thus, a more highly acclimated culture yields higher cell numbers that can increase CO and H₂ uptake in this mass transfer limited system.

2.2.3 Effect of Pressure

Table 2.9 gives the final product composition for the biological conversion in a mixed culture. As noted, almost total conversion of both CO and H₂ results. A product gas consisting of about 40 percent methane and 60 percent CO₂ results, with only small quantities of CO and H₂. To produce pipeline quality methane, the biological reactor must be followed by a CO₂ separator.

TABLE 2.9

Product Gas from Biomethanation

Gas Concentration (mole percent)

	<u>Inlet</u>	<u>Exit</u>
CO	45	2.5
H ₂	30	1.3
CO ₂	15	56.6
CH ₄	10	39.6

The mixed bacterial culture was subjected to increased pressures in batch studies at 0-100 psig. A coal gas mixture containing 65 percent CO, 22 percent H₂, 11 percent CO₂ and two percent CH₄ was used as the feed gas. CaCO₃ (1.5g/300 ml) was added for pH control during the experiments. Agitation was provided at 400 ± 20 rpm.

The data in Table 2.10 indicate that cultures have adapted well to high pressures. The time required for 85-95 percent CO and H₂ conversions has shortened due to culture adaptation. For example, the conversion of CO and H₂ at 50 psig were both greater than 95 percent within 48 hours compared to 8 days reported one month previously. More than 88 percent of the CO and 90 percent of the H₂ were converted within 52 hours at 60 psig.

TABLE 2.10

Batchwise Coal Gas Conversion by the CO-adapted Mixed Bacterial Cultures Using a High Pressure Reactor at Various Pressures*

Pressure (psig)	Time required for max. conv. (hours)	Maximum Conversion, %		Maximum CH ₄ formed (mole %)
		CO	H ₂	
20	39.33	84.33	88.5	25.85
30	53.0	96.68	89.56	42.47
40	36.08	85.59	91.80	23.18
50	48.75	95.30	96.18	26.88
60	52.0	88.94	93.02	23.06
100	52.0	9.80	20.80	4.39

* Agitation was provided at 400 ± 20 rpm.

The production of the methane, however, was adversely affected by increased pressure. The level of CH₄ reached 42 percent at 30 psig, but at higher pressures, acetate accumulation was imminent and methane formation was lower. CaCO₃ was very effective in maintaining the pH between 6.8-7.3 during the experiments.

These results indicate that the CO-converting bacterial species can be subjected to high pressures and still function well. Although the methane bacteria are known to withstand higher pressures, their failure to metabolize acetate to CH₄ is unexplained. Further adaptation of these bacteria or pure cultures of methane producing bacteria will highlight the cause for this aberrating behavior of the methanogens.

2.2.4 Mass Transfer Limitations

Assuming that the reaction rate is totally mass transfer limited and that there is no gas phase resistance, the rate of conversion, Q, is given by:

$$\frac{dC_A}{dt} = K_{1a}C^* = \frac{\text{moles of gas in} - \text{moles of gas out}}{\text{volume of liquid}} = Q_{CO} \quad (2.1)$$

where

C_A = concentration of reactant (either CO or H₂);
 t = reaction time;
 K_{1a} = mass transfer coefficient;
 a = interfacial area;
 $C^* = \frac{P_g}{H}$ = interfacial composition;
 P_g = interfacial pressure; and
 H = Henry's Law constant

In this equation, the concentration in the bulk liquid phase is assumed to be zero.

For CO, $P_g \approx 0.45$ atm, $H = 6.73 \times 10^4 \frac{\text{atm}}{\text{mole fraction}}$

and C^* is computed to be $3.711 \times 10^{-4} \frac{\text{gmole CO}}{1}$.

Rearrangement of Equation (2.1) gives:

$$K_{1a} (\text{hr}^{-1}) = \frac{Q_{CO}}{3.711 \times 10^{-4}} \frac{\frac{\text{moles}}{1 \cdot \text{hr}}}{\frac{\text{mole}}{1}} \quad (2.2)$$

For H₂, $P_g = 0.3$ atm, $H = 7.44 \times 10^4$ atm/mole fraction and C^* is calculated

to be $= 2.238 \times 10^{-4} \frac{\text{gmole H}_2}{1}$

From Equation (2.1):

$$K_{1a} (\text{hr}^{-1}) = \frac{Q_{H_2}}{2.238 \times 10^{-4}} \frac{\frac{\text{moles}}{1 \cdot \text{hr}}}{\frac{\text{moles}}{1}} \quad (2.3)$$

In correlating the mass transfer coefficients and the flow rate, Eckenfelder ²⁴ has suggested a general form:

$$K_{1a}V = \rho F^\alpha \quad (2.4)$$

where ρ and α = empirical constants

F = the gas flow rate

V = the reactor volume

Figure 2.3 shows a plot of $\ln K_{1a}$ as a function of gas flow rate from the data in Table 2.7 above a 4 hour retention. As noted, a single straight line is obtained for both H₂ and CO. The data do not fit for retention times for less than 4 hours.

Figure 2.3. Correlation between mass transfer coefficient and gas flow rate.

The following general equation is obtained at 500 rpm:

$$K_1a = 6.66F^{0.0066} \quad (2.5)$$

where F = gas flow rate, cm^3/hr

K_1a = mass transfer coefficient, hr^{-1}

In analyzing the results of Figure 2.3, it is noted that a constant intercept of 1.9 is found yielding a single equation for both H_2 and CO as is shown in Equation (2.5). It is interesting to examine this result further.

Boussinesq indicates that the mass transfer coefficient, K_1 , can be related to the diffusivity of the gas inside the liquid, D , the ascending terminal velocity of the bubbles, V_B , and the bubble diameter, d_B , by the equation:

$$K_1 = 1.13 (DV_B/d_B)^{1/2} \quad (2.6)$$

Since CO and H_2 are fed as a gas mixture into the reactor, the ascending velocity, V_B , and the bubble diameter, d_B , should be the same for both bases. Since a single straight line is found in Figure 2.3, the diffusivities, D , of both gases should be nearly the same. Indeed this is found to be the case.

3.0 SURVEY OF SHIFT CONVERSION REACTIONS AND OPTIMIZATION

One of the most interesting results of the mixed culture study is the improved culture performance with increased acclimation time. After an 8 month period of operation, the retention time for complete conversion decreased from 8 hours to 4 hours. After an additional 4 months, the time for complete conversion decreased to 2 hours. Thus, the gas flow rate can be increased four fold at the same conversion with increased acclimation time.

The major reason for this dramatic improvement was the enrichment of the culture. Organisms important for CO and H₂ conversion were becoming more dominant in the mixed culture, and thus the population of the more dominant CO and H₂ utilizing organisms increased. Therefore, the mixed culture became a more pure culture of organisms capable of converting CO to H₂.

Because acclimation times are long (especially when dealing with a toxic gas such as CO) and great improvement can come from increased acclimation time, pure cultures of CO converting bacteria were screened for their ability to convert CO and H₂ to methane intermediates. CO converters were chosen since the natural sources such as sewage sludge and animal rumen are deficient in these bacteria, thus requiring long acclimation times. Intermediate conversion of CO and H₂O to both acetate and CO₂ and H₂ were considered since pure culture methane bacteria are able to use both products as substrates. The reactions of converting CO to methane intermediates were shown previously in Equations 1.1 and 1.4.

Once these pure culture organisms are chosen, optimization studies will be undertaken to yield the best growth and production conditions. These organisms will then be combined with pure culture methanation bacteria in building a co-culture or stagewise system. Improvements in rates over mixed culture performance is expected since these organisms will be the best organisms for CO and H₂ conversion to methane.

3.1 Materials and Methods

3.1.1 Experimental

Six species of bacteria were screened for their ability to convert CO in synthesis gas to methane intermediates:

- o Rhodopseudomonas gelatinosa
- o Rhodospirillum rubrum, 2 species
- o Peptostreptococcus productus
- o Acetobacterium woodii
- o Eubacterium limosum

As mentioned previously, three of these species produce H₂ and CO₂ and 3 produce acetate.

3.1.1.1 Sources of Organisms

Peptostreptococcus productus, strain U-1 and Acetobacterium woodii were kindly supplied by Professor M. P. Bryant, University of Illinois,

Urbana, IL 61801. Rhodopseudomonas gelatinosa and Rhodospirillum rubrum (Strains I and II) were obtained from the American Type Culture Collection, Rockville, MD 20852.

3.1.1.2 Media and Conditions of Cultivation

The media and conditions of cultivation for the above organisms are shown in Appendix A.

3.1.2 Analytical Procedures

Several analytical procedures were used on a routine basis to monitor the progress of the culture screening experiments. On a daily basis, gas compositions were measured. On a periodic basis the liquid culture was analyzed for bacterial growth, pH, ammonia, ATP and volatile fatty acids. These experimental methods are described briefly in Appendix A.

3.2 Screening

As noted earlier, Rhodopseudomonas gelatinosa and the two species of Rhodospirillum rubrum convert CO to CO₂ and H₂ by the reaction:

Peptostreptococcus productus and Acetobacterium woodii convert CO to acetate and CO₂ by the reaction:

Both of these reactions yield methane by the same overall stoichiometry:

so that the rates of conversion are the only important parameters. It should be noted in the traditional biomethanation reaction that approximately 80 percent of the methane formed comes through acetate.

A comparison of the 5 anaerobic bacteria used in the production of methane intermediates is shown in Table 3.1. The results of these studies were accumulated over a period of many months. Since the bacteria were originally grown on sugars, gradual acclimation to CO was required. The first column in Table 3.1 shows the name of the bacteria. As noted, two species of R. rubrum were used. Column 2 shows the maximum optical density obtained with a given composition of CO in the gas phase. The optical density is used to give a comparison of cell growth at a given time during the fermentation, and thus can be used to predict the ability to convert CO.

Column 3 shows the conditions of growth, including the recommended optimum temperature and any special growth conditions. All of the bacteria were strict anaerobes, which means that all oxygen (including the oxygen in water) needs to be removed to allow growth and production.

TABLE 3.1

Survey of Shift Conversion Organisms

Organism	Maximum Optical Density ¹	Conditions of growth	Doubling time	Time Required to utilize CO (days)	Max % CO Tolerance	Major Products
<u>P. productus</u>	0.83(50) ²	37°C, dark	4-6 hours	4(50) ²	90	Acetate + CO ₂
<u>R. gelatinosa</u>	0.16(20)	20°C, dark	30 hours	12(20)	30	H ₂ + CO ₂
<u>R. rubrum</u> (I)	0.46(50)	30°C, tungsten light	35 hours	11(50)	60	H ₂ + CO ₂
<u>R. rubrum</u> (II)	1.0(50)	30°C, tungsten light	24 hours	10(50)	60	H ₂ + CO ₂
<u>A. woodii</u>	0.50(25)	37°C, dark	28 hours	7(25)	Tested up to 40	Acetate + CO ₂

1. Initial O.D. subtracted.

2. Growth with % CO and % CO utilization

P. productus, Peptostreptococcus productus

R. gelatinosa, Rhodopseudomonas gelatinosa

R. rubrum, Rhodospirillum rubrum

A. woodii, Acetobacterium woodii

Column 4 shows the doubling time or time required to double the number of cells during the fermentation. The doubling time for these bacteria is a function of acclimation time and agitation rate. Like the mixed culture bacteria, the transfer of CO from the bulk gas phase into the liquid controls the rate of reaction. In these experiments no agitation was provided, so that the doubling times reported are less than optimum. However, the experimental conditions were identical so that the doubling times can be directly compared.

Column 5 presents the time to completely utilize either 25 or 50 percent CO. Again, the reaction time is a function of agitation rate and acclimation time so that these times can be much shorter. The rates were measured under identical conditions.

The maximum CO tolerance is shown in Column 6. CO tolerance increases with acclimation so that many successive transfers of the cultures over several months were required to reach these levels. The CO tolerance can be important in using coal gases richer in CO and reducing acclimation time.

The major products percent in the fermentation are shown in Column 7. Minor by-products include propionate and butyrate.

In comparing optical densities, it is seen that P. productus and R. rubrum (II) produce nearly twice as many cells on 50 percent CO, so that their cell densities are lower.

In comparing growth conditions, it is seen that P. productus and A. woodii require temperatures of 37°C for optimum production, while the other organisms have an optimum temperature of 30°C. Both species of R. rubrum are photosynthetic bacteria and require tungsten light for growth. The other bacteria can grow in the dark. The requirement for tungsten light is a major disadvantage since fermentations in the light are difficult to carry out.

The doubling time for P. productus is much lower than for the other organisms, requiring only 4-6 hours. The doubling times for the two R. rubrum species are 24 and 35 hours. Doubling times for the other organisms are quite long since these bacteria are utilizing only 20-25 percent CO.

The maximum CO tolerance of P. productus is 90 percent, while reaching 60 percent for both species of R. rubrum. The maximum tolerance of CO for R. gelatinosa and A. woodii are 30 and 40 percent, respectively.

In deciding which organisms to study further, the important results of Table 3.1 must be compared. The most important result shown in Table 3.1 is the time to utilize 50 percent CO. These results indicate that P. productus should be used to produce acetate and either species of R. rubrum should be used to produce H₂ and CO₂.

The second major factor is the CO tolerance P. productus is again the most impressive organism producing acetate, and either species of R.

rubrum should be utilized to produce CO₂ and H₂. The growth conditions of R. rubrum make them unsatisfactory for large-scale fermentation, but their results are so far superior to R. gelatinosa that they must be considered if CO₂ and H₂ are to be produced.

Thus, the overall conclusions are to use P. productus for acetate production at 37° C or R. rubrum (II) (due to its higher optical density) for CO₂ and H₂ production at 30°C. These organisms will be studied further in an optimization study.

3.3 Optimization Results

3.3.1 Peptostreptococcus Productus Optimization Studies

The optimization study for P. productus including nutrient requirements, pH, temperature and CO tolerance for this bacterium have been determined and are summarized in Table 3.2. This process required several additional months beyond the screening studies. CO tolerance is especially influenced by increased acclimation time. The media as listed in Table A.4 has been changed only in the amount of 2.5 percent sodium sulfide required. The recommended media suggests 2.0 ml of 2.5 percent sodium sulfide, whereas the optimum media of Table 3.2 suggests only 1.0 ml. The CO tolerance of P. productus is up to 90 percent CO. P. productus grows and produces acetate in the presence of other coal gas constituents such as CH₄, CO₂, and H₂. The optimum pH is 7.4 at a temperature of 37°C. The major products of CO metabolism are acetate and CO₂ with trace amounts of propionate and butyrate also produced. As noted earlier, the bacteria is strictly anaerobic.

Experiments have shown that P. productus is also able to utilize H₂ and CO₂ in producing acetate by the equation:

This reaction occurs only after the CO is depleted, so that it is a secondary reaction. It is interesting to note that if acetate were the desired intermediate product, the yield of acetate from coal gas could be increased by employing Equation (3.2) in a second series vessel. This reaction would take place after CO is utilized in the first vessel, producing acetate and CO₂. Acetate tolerance by the organisms would have to be measured and a higher level developed, if necessary.

TABLE 3.2

Optimum Growth Requirements of Peptostreptococcus productus

1. Media:	per 100 ml
Pfenning's mineral	5.0 ml
Pfenning's trace metal	0.1 ml
B-vitamins	0.5 ml
Na ₂ SeO ₃ (0.1%)	0.1 ml
Resazurin (0.1%)	0.1 ml
Yeast extract	0.2 g
Sodium biocarbonate	0.35 g
Sodium sulfide (2.5%)	1.0 ml
2. CO tolerance:	up to 90%
3. pH:	7.4
4. Temperature:	37°C
5. Major product from CO:	Acetate + CO ₂
6. Use H ₂ and CO ₂ :	Yes
7. Strictly anaerobic:	Yes

3.3.2 Rhodospirillum Rubrum (II) Optimization Studies

The optimum growth requirements for Rhodospirillum rubrum have been completed and the results are summarized in Table 3.3. As noted, the media as presented in Table A.3 is the same as the optimum media with two exceptions. The optimum media requires 0.002 g EDTA per 100 ml instead of 1.0 ml of 0.425 percent EDTA. Also, 0.09 g of Pfenning's mineral and 0.1 m of Pfenning's trace metal solution is required for optimum performance for every 100 ml of media. This replaces 1.0 ml of Pfenning's mineral solution in the original recommended media.

By successive transferring, the CO tolerance of R. rubrum (II) has been increased to 90 percent. R. rubrum also utilized CO in the presence of other coal gas constituents such as CH₄, CO₂ and H₂. The optimum pH range is from 6.6 to 7.0 at a temperature of 30°C. The major products from CO metabolism are CO₂ and H₂ with negligible utilization of the resulting CO₂ and H₂. As noted previously, R. rubrum is a strict anaerobe.

The other growth requirement of the bacteria is a tungsten light source. As mentioned in the topical report for Task B, this can represent a major hurdle to using R. rubrum in large-scale fermentation studies since a lighted fermentation is difficult to carry out. This requirement should be kept in mind during future studies and may cause R. rubrum to be eliminated during future work.

TABLE 3.3

Optimum Growth Requirements of Rhodospirillum rubrum (II)

1. Media:	Per 100 ml
Malic acid	0.25 g
Yeast extract	0.10 g
Ammonium sulfate	0.125 g
MgSO ₄ , 7 H ₂ O	0.02 g
CaCl ₂ , 2 H ₂ O	0.007 g
EDTA	0.002 g
KH ₂ PO ₄	0.06 g
K ₂ HPO ₄	0.09 g
Pfenning's mineral	0.1 ml
Pfenning's trace metal	0.05 ml
B-vitamins	0.75 ml
Neutralize with dilute NaOH	
Gas phase N ₂ :CO ₂	80%-20%
2. CO tolerance:	up to 90%
3. pH:	6.6-7.0
4. Temperature:	30°C
5. Major product from CO:	H ₂ + CO ₂
6. Use of H ₂ and CO ₂ :	none-negligible
7. Strictly anaerobic:	Yes
8. Other growth requirement:	Tungsten light source

4.0 STAGewise UPGRADING

As an alternative to single stage conversion, pure culture organisms may be used to convert coal gas to methane using a co-culture of organisms or in a stagewise manner. Stagewise conversion refers to using an organism such as P. productus or R. rubrum to convert CO to methane precursors in one vessel, followed by conversion to methane in a second vessel. By separating the organisms, each reaction may be operated at the optimum conditions of pH, pressure, temperature and feed rate without interference from non-contributing organisms. In addition, the rates of reaction for pure cultures are expected to be much higher than for mixed cultures or pure cultures. The disadvantage, of course, is the need for an additional reaction vessel, which must be offset by an improvement in reaction rate.

The purpose of this study was to determine the feasibility of stagewise upgrading of gasification coal gas using bacterial cultures and various reactor systems in comparison to single stage mixed culture conversion. Several experiments were designed to study the bacterial conversion of coal gas to methane specifically via the acetate pathway. Two different reactor systems were employed in the study, and the conversion of coal gas or CO and H₂ was studied using either mixed or pure cultures of bacteria. Bacterial growth, conversion of CO, H₂ and CO₂ and product formation were monitored during these studies and specific conclusions were made during these experiments.

4.1 Materials and Methods

The materials and methods used in the stagewise upgrading studies were essentially identical to the methods described previously in Sections 2.1 and 3.1. The media used for the CO enrichment, the stirred-tank reactor and high pressure reactor studies, the immobilization studies, and the acetate enrichment studies was the same as for P. productus, listed in Table A.4. In establishing the CO utilizing enrichment, sewage sludge was used as the primary inoculum. A mixture of 50 percent CO and 50 percent 80:20-N₂:CO₂ was used for the establishment and maintenance of CO-degrading and CH₄-forming enrichments. The enrichments were also grown on coal gas when required.

For the stirred-tank and high pressure reactor studies, media exchanges were made under strict anaerobic conditions inside an anaerobic chamber. Small amounts of medium (up to 50 ml) or sodium bicarbonate, if needed, were injected using a sterile syringe fitted with a one-way valve. Media exchange during the batch immobilized column reactor studies were performed in an anaerobic chamber or using special devices fitted with quick connections.

For the establishment and maintenance of acetate enrichments, acetate (40-80 mM) was used as the sole carbon source. The optimum conditions for the growth of Methanosarcina sp. are listed in Table 4.1. For the growth of Methanosarcina sp., the gas phase was first flushed with 80:20 H₂-CO₂ and then adjusted to 40 psig.

TABLE 4.1

Optimum Growth Requirements for *Methanosarcina* sp. (Strain MS)

<u>Components</u>		<u>g/liter</u>
Minerals I	K_2HPO_4	6.0
Minerals II	KH_2PO_4	6.0
	$(NH_4)_2SO_4$	6.0
	NaCl	12.0
	$MgSO_4$	2.6
	$MgCl_2$	4.0
Trace Metals*III	$CoCl_2$	0.10
	Na_2MoO_4	0.24
	$NiCl_2$	0.24

*Nitrilotriacetate (1.5g) may be added to III; pH to 6.5 with KOH and then add to other minerals.

		<u>mg/liter</u>
B-vitamins IV	Biotin	2.0
	Folic Acid	2.0
	Pyridoxine-HCl	10.0
	Thiamine-HCl	5.0
	Riboflavin	5.0
	Nicotinic Acid	5.0
	Ca-pantothenate	5.0
	Vitamin B-12	0.10
	P Amino Benzoic Acid	5.0
	Thioctin Acid	5.0

Use per 100 ml

5.0 ml of I
5.0 ml of II
0.1 ml of III
0.5 ml of IV

NH_4Cl 0.27 g
Sod. acetate 0.15 g
 $NaHCO_3$ 0.40 g
 $FeSO_4$ 0.001 g
Resazurin (0.1%) 0.1 ml

Mix and adjust pH to 6.9 with dilute HCl, boil under $N_2:CO_2$ (80:20) and dispense to bottles as required. Autoclave; pressurize to 40 psi with $H_2:CO_2$).

Temperature optimum: 37°C
Strictly anaerobic conditions.

4.1.1 Immobilization Techniques

A variety of bio-support materials including ceramic rings, glass beads, and Rasching rings were chosen for this study. The organic compounds used for coating the packing materials included agar, starch, sodium alginate, calcium alginate, pectin and carrageenan gel. In preparing the packings, the bio-support materials were first washed and dried in an oven. Recommended concentrations of the coating materials, as shown in the literature, were prepared and the bio-support materials were immersed in the melted coating materials. After a few minutes, the excess coating material was drained off and the bio-support materials were allowed to dry at room temperature. After attaining complete dryness, the materials were treated with 3 percent glutaraldehyde.

For the batch studies using serum stoppered bottles, the bio-support materials were introduced to the bottles aseptically and anaerobically. For the studies involving packed column reactors, the materials were packed in a pre-sterilized column, and then contacted with sterile N_2 - CO_2 to attain anaerobiosis. Media and inoculum were added after these procedures were completed.

When wood chips were used as the packing material, the column reactor was packed with wood chips and the entire assembly was sterilized with 3 percent glutaraldehyde. Prior to packing, the wood chips were treated with 20 percent ethanol and boiled for 4-6 hours to remove soluble phenolics and other organics. Alcohol from the wood chips was removed by washing with distilled water. The wood chips were air dried at room temperature before packing into the reactor. The column assembly was made anaerobic by passing N_2 - CO_2 through the column for several hours before bacterial inoculation.

Coal gas was supplied as the feed gas. Liquid samples and gas samples were routinely analyzed to determine bacterial conversions. Bacterial attachment to the packing material was monitored regularly.

4.2 Results and Discussion

The coal-derived synthesis gas can be upgraded to obtain a higher Btu gas using microbial cultures. A combination of bacterial growth manipulation and the use of appropriate reactor designs will be highly beneficial for the future industrialization of this process. Bacterial screening studies as reported in previous topical reports indicated that both mixed and pure bacterial cultures possessed metabolic potential to convert CO and H_2 or synthesis gas to methanogenic precursors, which were then converted to high Btu methane. The present study was undertaken to determine specific reactor systems which will enhance bacterial capabilities in the conversion of coal gas. The results obtained from several experiments including cell immobilization and reactor pressure studies demonstrate the possible potential of microbial enhancement of coal gas conversion to high Btu fuel gas.

4.2.1 Coal Gas Conversion by Bacteria Using a Mixed Flow Fermentor

A mixed culture established from sewage sludge inoculum and maintained on 50 percent CO metabolized coal gas at a fast rate in preliminary

batch experiments. Microscopic observations revealed that this culture had a predominantly coccus-type organism presumed to be CO-utilizing bacteria, and several methane producing bacteria, tentatively identified as Methanothrix - and Methanosarcina - like organisms. The presumed CO-degrading bacteria had a morphotype identical to P. productus, a pure culture known to metabolize CO. This mixed culture was used to determine the conversion of coal gas using a one-liter Bio-flo fermentor.

The New Brunswick one-liter (Model C-30) fermentor was modified to grow and utilize anaerobic bacteria, with both batch and continuous gas flow. Sample ports for gas, bicarbonate feed septa and Swagelok quick connects were installed for easy handling. All of the connections were made airtight using a teflon seal. Butyl rubber or glass tubing was used as required. The liquid outlet port was closed using a melted glass tube connected with a butyl rubber tube. The sample port was also clamp closed. The reactor was sterilized by autoclaving, followed by flushing to remove air with O₂-free N₂ and CO₂. Media transfer and bacterial inoculations were made in an anaerobic chamber (Coy Laboratory Products, Inc., MI).

The basal salts media used in the reactor was essentially the same as listed for P. productus in Table A.4. The anaerobic media was dispensed in 50 ml or 75 ml amounts in serum stoppered bottles and autoclaved. Media was reduced with sodium sulfide (2.5 percent) prior to use. Cultures were grown in serum stoppered bottles prior to inoculation into the reactor. The gas supply was connected to the reactor by quick connections fitted to a flow meter. Liquid samples (~ 10 ml) were removed through the sample outlet, and the outlet was then closed immediately. Sterile sodium bicarbonate (7 percent) was added using a syringe with a one-way valve. The pH of the culture was maintained between 6.8 and 7.4.

Agitation and gas flow rates were changed as required. bacterial cell density, pH, volatile fatty acids, (VFA) profile and gas measurements were performed on a daily basis or as required. Fresh basal media was exchanged at 5 to 7 day intervals, or as needed.

4.2.1.1 Coal Gas Conversion by the Mixed Culture

The mixed bacteria were grown in a mixed flow reactor as a batch culture. Coal gas containing 45 percent CO, 30 percent H₂, 15 percent CO₂ and 10 percent CH₄ was fed to the reactor up to a maximum level. The medium was first inoculated with a log phase culture inside an anaerobic chamber. Bacterial growth and the conversion of CO and H₂ were monitored at regular intervals. Agitation was provided at 300 rpm. The results, reported in Table 4.2, show the bacterial metabolism of CO and H₂. Within 36 hours, the bacterial conversions of CO and H₂ were 98 percent and 88 percent, respectively. These results also showed that the bacterial conversion of CO started immediately (within 30 minutes); but H₂ conversion did not occur until 4 hours after inoculation. The CO and H₂ conversions were also accompanied by the formation of methane, which reached a level of 37 percent. During this period, the bacterial growth increased from 0.75 to 1.6. Acetate accumulated up to 0.5 g/liter.

TABLE 4.2

Bacterial Conversion of Coal Gas in a Mixed Flow
Reactor Used as a Batch Culture*

	Gas Composition, Mole Percent			
	CO	H ₂	CH ₄	CO ₂
After feeding	45.50	26.89	11.0	15.5
Output after 36 hours	0.90	3.4	36.8	57.8
Percent conversion after 36 hours	98.1	87.4		

* Agitation was provided at 300 rpm
Coal gas contained 45 percent CO, 30 percent H₂, 15 percent CO₂
and 10 percent CH₄

In later studies, this bacterial enrichment also converted CO and H₂ at similar rates when a coal gas containing a mixture of 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄ was used as the feed gas. The results of these two experiments show that the mixed culture has tremendous potential to metabolize CO and H₂ and can be used for the conversion of coal gas to methane.

4.2.1.2 Coal Gas Conversion by the Mixed Culture Using Continuous Gas Flow

The CO-degrading mixed culture was grown continuously in a mixed flow fermentor, and the conversion of CO and H₂ was studied using various gas retention times and different rates of agitation. All the results reported here were taken during the early phase of the growth of the bacterial cultures. The media was inoculated with a log phase culture using an anaerobic chamber. The experiments were performed with a very well-adapted mixed culture which was maintained on 50 percent CO for at least one year.

In the first experiment, a coal gas mixture containing 45 percent CO, 30 percent H₂, 15 percent CO₂ and 10 percent CH₄ was fed continuously at a flow rate of 3 ml/min (gas retention time was approximately 1.94 hr). Bacterial growth, gas compositions (inlet/outlet), ATP and VFA profiles were monitored on a daily basis. These experiments were designed to

determine the effect of agitation and/or high cell density in the reactor on CO and H₂ conversion. As noted in Tables 4.3 and 4.4 the initiation of the bacterial conversion of CO was immediate which was enhanced with the increase in bacterial growth. Within 3 days of inoculation, more than 84 percent of the CO was converted to acetate and/or CH₄. In contrast, H₂ was not utilized until after 48 hours and reached a maximum within the next 2 days.

The correlation between bacterial growth and conversion of H₂ and CO is shown in Figure 4.1. Although the initial conversion of CO and H₂ was directly dependent on bacterial growth, there seems to be little effect on the conversion when the bacterial growth reached an optical density of 2.0 or more under the conditions provided. As can be seen from this figure, the conversions of H₂ and CO reached a maximum of 85 percent, after which the conversion was perhaps limited by mass transfer.

The effect of agitation on bacterial conversion of CO and H₂ was also studied using the CO-adapted culture in a continuous stirred-tank reactor. A basal salt media was inoculated with the bacterial culture and coal gas was fed continuously at a flow rate of 3 ml/min. The initial agitation was maintained at 400 rpm. The bacterial cultures were allowed to grow to an optical density of about 2.0 and then the agitation was changed. After at least 4-5 gas retention times in the reactor, the gas composition was analyzed until a constant conversion of CO and H₂ was reached. As can be seen from Tables 4.5 and 4.6, the CO and H₂ conversions were directly correlated with agitation. There was no conversion of CO at an agitation rate of 200 rpm but reached a level of about 97 percent at 600 rpm. At 200 rpm, the H₂ conversion was about 40 percent but reached to 91 percent at an agitation of 500 rpm. The data in Table 4.6 clearly suggests that the conversions of CO and H₂ are dependent entirely on mass-transfer rather than bacterial cell density in a reactor.

In another study, the effect of gas flow rate (retention time) on bacterial conversion of CO and H₂ was determined. A constant agitation rate of 500 rpm was used. These experiments were performed with the mixed culture that was adapted to CO only for 8-10 months.

Tables 4.7 and 4.8 show the results of experiments carried out at 500 rpm and various retention times. Essentially no difference is seen in the conversion at retention times greater than 8 hours. At a 6.7 hour retention time, the CO conversion was 88 percent and the H₂ conversion was 90 percent.

As the retention time was lowered from 3.7 hours to 1.9 hours, the CO conversion decreased to 23 percent and the H₂ conversion fell below 10 percent. Apparently, cell growth is maximized near a 4 hour retention time.

It is interesting to compare the results of the mixed culture conversions with time. At a constant conversion of 1.9 hours and an agitation rate of 500 rpm, Table 4.8 yields a CO conversion of 23.2 percent and a H₂ conversion of 17.7 percent. When using the culture that was acclimated to coal gas for an additional 8 month period, both the CO and H₂ conversions reached 90 percent. Thus a minimum retention time of 1.9 hours can be utilized with the mixed culture.

TABLE 4.3

Single-Stage Conversion of CO and H₂ Using a Continuous
Stirred-Tank Reactor: Gas Composition with Growth*

Growth** 580 nm	Gas Compositions, mole %				
		CO,	H ₂	CO	CH ₄
0.65	In	45.09	27.07	15.24	11.17
	Out	20.64	28.73	38.72	10.55
0.75	In	45.09	27.07	15.24	11.17
	Out	13.45	27.56	46.51	11.21
1.15	In	45.36	26.22	15.50	11.66
	Out	10.53	24.69	50.05	13.59
1.275	In	45.36	26.22	15.50	11.66
	Out	9.85	23.75	50.91	13.50
1.60	In	45.15	28.44	15.09	10.37
	Out	6.42	21.70	54.31	16.90
2.30	In	45.20	27.01	15.25	11.42
	Out	6.71	3.84	55.40	33.45
3.24	In	44.74	27.99	14.64	10.75
	Out	6.02	4.90	55.89	30.59

*Gas retention time was maintained at approximately 1.94 hr and
agitation was provided at 400 rpm.

**The initial O.D. was 0.31

TABLE 4.4

Single-Stage Conversion of CO and H₂ Using a Continuous
Stirred-Tank Reactor: Conversion with Growth*

Time (Days)	Growth** 580, nm	% Conversion	
		CO	H ₂
1	0.65	54.23	0
1	0.75	70.28	0
2	1.15	76.80	5.84
2	1.275	78.28	9.43
3	1.60	84.12	28.52
4	2.30	85.16	85.79
5	3.24	86.55	82.50

* The gas retention time was maintained at approximately 1.94 hr
and the agitation was kept constant at 400 rpm.

** The initial O.D. was 0.31

Figure 4.1. Conversion of CO and H₂ as a function of bacterial growth in a continuous stirred-tank reactor.

TABLE 4.5

Single Stage Conversion of CO and H₂ Using a Continuous Stirred-Tank Reactor: Gas Composition with Agitation Rate*

Agitation (rpm)		Gas Compositions, mole %			
		CO,	H ₂	CO ₂	CH ₄
200	In	43.38	29.86	15.79	11.09
	Out	44.82	18.01	21.70	14.80
300	In	43.24	29.56	15.01	10.65
	Out	25.74	11.15	39.35	22.93
400	In	45.20	27.01	15.25	11.42
	Out	6.71	3.84	55.40	33.25
500	In	43.88	29.63	14.91	10.81
	Out	4.48	2.81	57.99	33.76
600	In	44.53	28.33	14.88	11.29
	Out	1.66	4.81	60.67	31.84

* The gas retention time was maintained at approximately 1.94 hr. The bacterial cells were allowed to grow first before the readings were recorded.

TABLE 4.6

Single Stage Conversion of CO and H₂ Using a Continuous Stirred-Tank Reactor: Conversion with Agitation Rate

Agitation (rpm)	Gas retention time (h)	O.D. 580 nm	% Conversion	
			CO	H ₂
200	1.94	2.25	0	39.69
300	1.94	2.35	40.48	62.28
400	1.94	2.30	85.16	85.78
500	1.94	2.55	89.80	90.52
600	1.94	2.15	96.28	83.03

TABLE 4.7

Single Stage Compositions of Coal Gas Obtained From a
CO-adapted Mixed Culture at 500 rpm and
Atmospheric Pressure

Average Gas Retention Time (hrs)	CO		Gas Concentration (mole percent)				H ₂	
	In	Out	In	Out	In	Out	In	Out
16.7	44.2	2.7	11.0	38.4	14.8	57.0	30.0	1.9
11.1	45.6	2.5	11.2	39.6	15.3	56.6	28.0	1.3
8.3	45.2	1.5	9.8	32.6	15.4	62.4	29.8	3.6
6.7	44.1	5.5	11.7	36.6	14.7	55.0	29.5	2.9
5.6	42.6	5.1	10.2	37.5	14.4	53.2	32.8	4.2
4.8	46.5	7.4	13.1	35.2	15.6	53.6	24.8	3.8
4.2	45.1	6.8	11.1	37.1	15.0	50.9	28.8	5.2
3.7	42.0	22.8	10.2	13.6	13.6	32.4	34.1	31.2
2.8	41.9	27.6	12.8	21.3	14.0	24.3	31.3	26.9
1.9	43.2	33.2	11.0	13.3	14.8	28.0	31.0	25.5

TABLE 4.8

Single-Stage Conversion of Coal Gas by a CO-adapted
Mixed Culture at 500 rpm and Atmospheric Pressure

Retention Time, hours	CO Conversion, %	H ₂ Conversion, %
16.7	93.9	93.7
11.1	94.5	95.4
8.3	96.7	87.9
6.7	87.5	90.2
5.6	88.0	87.2
4.8	84.1	84.7
4.2	84.9	81.9
3.7	45.7	8.5
2.8	32.1	14.1
1.9	23.2	17.7

This surprising result of increased conversion ability has been addressed by Andrews et al., when measuring the effects of cells on oxygen transfer.^{25,26} They note that there is a chemical enhancement due to the uptake of oxygen by cells, direct uptake by cells of oxygen from the gas phase and a hydrodynamic effect that is equally important with dead cells or inert particles. Thus, a more highly acclimated culture yields higher cell numbers that can increase CO and H₂ uptake in this mass transfer limited system.

4.2.1.3 Coal Gas Conversion by *P. productus* in a Batch Culture

P. productus was grown in a stirred-tank reactor as a batch culture. Coal gas containing 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄ was feed to the reactor up to a maximum level. Bacterial growth, and the conversion of CO and H₂ were monitored on a regular basis. Agitation was provided at 250 rpm. The results, reported in Table 4.9, show that *P. productus* rapidly utilized CO. Within 24 hours, more than 75 percent of the CO was converted to acetate and CO₂. Within 48 hours, the conversion was more than 92 percent. The bacterial growth also increased from an optical density to 2.10 to 2.9 during 24 hours. Hydrogen conversion did not occur until after 24 hours of incubation, but then H₂ started to disappear slowly. At the end of 48 hours, only 7.43 percent of the H₂ was metabolized by *P. productus*. Acetate (1 mol/4 mol of CO consumed) and CO₂ were the only products produced during bacterial metabolism.

TABLE 4.9

Single Stage Conversion of Coal Gas to Acetate
by *P. productus* When Grown as a Batch Culture
in a Stirred-Tank Reactor

	Gas Composition, mole percent			
	CO	H ₂	CH ₄	CO ₂
After Feed	66.91	18.58	2.34	11.72
Output After 2 days	5.81	17.20	2.76	40.34
Percent Conversion After 2 days	92.26 (2)*	7.43 (2)		

Coal gas contained 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄

* Days

Agitation was provided at 250 rpm.

4.2.1.4 Coal Gas Conversion by *P. productus* Using Continuous Gas Flow

The bacterial conversion of coal gas was studied using a one-liter Bio-flo fermenter under continuous gas flow. The effect of gas retention

time and different rates of agitation on the conversion of CO and H₂ were studied using P. productus in a stirred-tank reactor. During these experiments, all of the data was collected during the log phase or early lag phase growth of P. productus. Media exchange was made every 5-7 days or as required. Synthetic coal gas containing 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄ was used as the feed gas. Gas compositions were determined until a steady-state had been reached. Sodium bicarbonate (7 percent) was added to the culture when required and the pH was maintained between 6.8 and 7.4.

In the first experiment, a constant gas retention time of 1.46 hours was selected and the agitation rate was varied from 200-600 rpm. The results, reported in Table 4.10 shows that the CO conversion was dependent on the rates of agitation. Complete conversion of CO occurred when an agitation rate of 600 rpm was provided. The bacteria could convert more than 88 percent of the CO even at 400 rpm. There was no conversion of H₂ during this experiment. It is known that P. productus does not use H₂ unless all the CO has been depleted and so this result was not unexpected. Acetate was the only product formed during the bacterial metabolism of CO. Bacterial growth followed a normal pattern and P. productus dominated more than 99 percent of the bacterial population when small amounts of contamination occurred after few days of the reactor start-up.

Similar experiments with P. productus at a 1.56 hour gas retention time showed 92 and 88 percent conversions of CO at 850 and 600 rpm, respectively. However, the cultures were not very well acclimated at this time, suggesting that bacterial adaptation to specific substrates and conditions are necessary before steady-state results could be obtained.

In the second experiment, an agitation rate of 500 rpm was selected and the gas retention times were varied between 0.246 to 1.46 hours. Gas measurements were made until a steady-state had been reached. The results of this experiment, presented in Table 4.11, show the conversion of CO at various gas retention times but at a fixed agitation rate of 500 rpm. The bacterial conversion of CO was more than 92 percent at a 1.46 hour gas retention time. The conversion increased with a decrease in gas flow rate. More than 56 percent of the CO was converted at a gas flow rate of 12.5 ml/min. The bacteria could tolerate a gas flow rate of 22 ml/min (0.24 hour gas retention time). It should be noted here that the doubling time of P. productus, when grown under a 50 percent CO atmosphere, is about 2-3 hours. Such high gas flow rates as provided during this experiment did not cease bacterial growth or CO metabolism by P. productus, indicating its extreme tolerance to toxic carbon monoxide. As these experiments were run for less than an 18 hour period, H₂ conversion by P. productus was not noticed.

TABLE 4.10

Conversion of Coal Gas to Acetate by P. productus,
When Grown in a Stirred-Tank Reactor at Various
Rates of Agitation*

Agitation (rpm)	Gas Retention Time (hours)	Synthesis Gas Conversions, mole %	
		CO	H ₂
200	1.46	25.7	0
300	1.46	59.0	0
400	1.46	88.6	0
500	1.46	93.2	0
600	1.46	100.0	0

* Coal gas contained 65% CO, 22% H₂, 11% CO₂ and 2% CH₄

In the third experiment, two gas retention times were selected and the conversion of CO was studied by changing the rates of agitation. The data in Table 4.12 confirms that CO conversion was completely dependent on the rates of agitation. At a 0.9 hour gas retention time, the CO conversion was almost negligible at 200 rpm but increased with increasing agitation. More than 95 per cent of the CO was converted at 600 rpm but complete conversion was achieved at 700 rpm. The CO conversion with a gas retention time of 1.46 hour was higher than of a 0.9 hour gas retention time during all the agitation rates. A conversion of more than 92 percent occurred at 500 rpm, and complete conversion occurred at 600 rpm. The conversion of H₂ was almost negligible during these experiments.

When the CO conversions were calculated on a per gm of dry cell weight basis, the maximum rate of conversion (29.58 mmoles of CO/gm dry cell weight/hour) occurred at 500 rpm and 0.63 hour gas retention time. At 600 rpm and a 1.45 hour gas retention time the corresponding value was 22.75 mmoles of CO/gm dry cell/hour.

Thus, from these experiments, it can be seen that P. productus has a tremendous potential to convert toxic CO even at very high gas flow rates (up to 22 ml/min). Reactor agitation rates were found to be an important factor in the bacterial metabolism of CO, mainly due to mass-transfer effects.

TABLE 4.11

Conversion of Coal Gas* to Acetate by P. productus When
Grown in a Continuous Stirred-Tank Reactor at Various
Gas Retention Times at Constant Agitation (500 rpm).

Gas Retention Time (Hours)	Coal Gas Conversion, Percent	
	CO	H ₂
1.46	92.08	0
0.90	75.48	0
0.629	69.62	0
0.433	56.48	0
0.246	21.39	0

* Coal gas contained 65% CO, 22% H₂, 11% CO₂ and 2% CH₄
Agitation was provided at 500 ± 10 rpm

TABLE 4.12

Conversion of Coal Gas to Acetate by P. productus, When Grown in a Continuous Stirred-Tank Reactor at Various Agitation Rates and Two Gas Retention Times.

Agitation (rpm)	Gas Retention Time (hours)	CO Conversion, Percent
200	1.46	14.53
	0.90	2.13
300	1.46	52.84
	0.90	39.10
400	1.46	86.88
	0.90	46.13
500	1.46	92.08
	0.90	75.48
600	1.46	100.00
	0.90	95.30
700	1.46	100.00
	0.90	100.00

* Coal gas contained 65% CO, 22% H₂, 11% CO₂ and 2% CH₄.
The conversion of H₂ was negligible at all the agitation periods.

4.2.2 High Pressure Reactor Studies

The effect of pressure on coal gas conversion using the CO-adapted mixed culture was studied using a Parr pressure reactor (Parr Instrument Company, Moline, IL). The mixed bacterial cultures were grown in a reactor containing 300 ml of basal salts medium. A coal gas mixture containing 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄ was used as the feed gas. The pressure of the reactor was adjusted by installing a one-way pressure check-valve on the gas outlet line. Agitation was provided at 400 rpm. CaCO₃ (1.5 g/300 ml) was used to control pH. As reported in Table 4.13 the conversions of CO and H₂ were 91.8 and 89.1 percent at atmospheric pressure. At 25 psi, the conversion of CO was much higher (93 percent), but decreased with increased pressure, converting only 9.8 percent of the CO at 100 psi. H₂ conversion at atmospheric pressure was 89.1 percent, but also decreased with increased pressure and dropped to 20.8 percent at 100 psi. The methanogens were most adversely affected by the increased pressure and methane production dropped considerably. The lower methane production was perhaps attributed to the drop in pH occurring during operation, caused by the build-up of organic acids.

TABLE 4.13

Single Stage Conversion of Coal Gas* by a Mixed Culture in
a High Pressure Reactor Operated at Different Pressures

Pressure	Gas Composition, Mole Per Cent			
	CO	H ₂	CH ₄	CO ₂
Atmospheric	5.37 (91.8)**	2.41 (89.1)	23.26	44.54
25 PSI	4.80 (92.7)	4.04 (81.7)	17.15	60.39
50 PSI	8.99 (86.2)	6.93 (68.5)	21.19	53.55
100 PSI	58.67 (9.8)	17.43 (20.8)	4.39	17.48

* Coal gas contained 65% CO, 22% H₂, 11% CO₂ and 2% CH₄.

** Percent conversion

The reactor was under batch conditions and was run at 400 rpm.

Table 4.14 shows the initial maximum rates of CO conversion at various pressures. The rates of CO conversion increased from 3.366 mmoles/hr/liter at 20 psig to 8.610 mmoles/hr/liter at 50 psig, but declined to 5.957 when the culture was subjected to a pressure of 60 psig. The conversion of H₂ did not occur during the 8-12 hour period and so the initial rate of H₂ conversion could not be determined.

When the culture was adapted to high pressure (40-60 psi) by growing for several months, the conversion of CO and H₂ were very rapid at 40 and 60 psig. At 40 psig more than 91 percent of the CO was converted within 24 hours. The corresponding CO conversion at 60 psig were 21.25 and 8.70 mmoles/hr/liter. These values were higher than the values obtained previously indicating the benefits of increased acclimation times. H₂ conversion was similarly higher, with more than 90 percent conversions occurring at both 50 and 60 psig.

The increase in the CO conversion rates at 40 and 60 psig occurred only when the cultures were grown at these pressures for a very long time. This indicates that longer culture adaptation at higher pressures may be required in order to obtain high gas conversion rates.

From these high pressure studies, it can be concluded that the bacterial cultures can be adapted to higher pressures when needed. Coal gas conversion under these conditions will be beneficial because at higher reactor pressures more quantities of CO could be metabolized by the microorganisms per unit time.

TABLE 4.14

Batchwise Coal Gas Conversion by a CO-adapted Mixed Bacterial Culture Using a High Pressure Reactor at Various Pressures

Pressure, psig	Maximum Rate of CO Conversion (mmole/hr/liter)
20	3.366
30	5.390
50	8.610
60	5.957

Agitation was provided at 400 ± 20 rpm

The results obtained during continuous experiments under higher reactor pressures were not encouraging. The bacterial cultures did not perform as well as when they were grown under batch system. The main reasons for lower conversion rates were probably due to:

1. The addition of larger gas bubbles which were not dispersed as evenly as needed to overcome mass transfer problems.
2. The major constituents of the media such as yeast extract might have been consumed too quickly, and unless replaced, bacterial growth would cease; and,
3. The maintenance of pH was another problem under the operating conditions at higher reactor pressures.

These problems have now been corrected and it is expected that the cultures will function well using the new operating systems.

4.2.3 Acetate Conversion by Methanosarcina sp. and an Acetate-Utilizing Enrichment

A Methanosarcina sp. (Strain MS) and an acetate-utilizing enrichment were used to study the conversion of acetate in batch culture experiments. Methanosarcina sp. (Strain MS) is a strict anaerobic bacteria which grows on H_2 - CO_2 , methanol, and methyl amines, producing methane. This bacterium also uses small amounts of acetate as its carbon and energy source to produce CH_4 , but bacterial adaptation to acetate is required. The optimum conditions for the growth of Methanosarcina sp. are listed in Table 4.1. Anaerobic media (40 ml) were dispersed in serum-stoppered bottles (125 ml capacity) and were sterilized by autoclaving at $120^\circ C$. The media was reduced with 0.5 ml of sodium sulfide (2.5%) and was inoculated with 3 ml

of a log phase culture of Methanosarcina sp. Acetate (40 mM) was added to some bottles. The gas phase for these bottles was maintained at 80:20 N₂ and CO₂ (1 atm). When H₂-CO₂ was used as the energy source, the gas phase was first flushed with 80:20 H₂-CO₂ and then adjusted to 40 psig. The bottles were incubated in a 37°C temperature controlled incubator shaker. Agitation was provided at 100 rpm. The results of this experiment are reported in Table 4.15. When Methanosarcina sp. was grown with H₂-CO₂, the bacterium produced CH₄ very rapidly. Almost all of the H₂ was utilized and a concentration of more than 54 percent methane accumulated in the gas phase.

When Methanosarcina sp. was grown with acetate as the energy source, the production of methane was very low and acetate was not utilized as rapidly as H₂-CO₂. Therefore, adaptation experiments on acetate using Methanosarcina sp. were necessary where, in addition to 40 mM acetate, the gas phase had 10 psig of H₂CO₂ (80:20). After full growth, the culture was transferred to identical growth conditions after which they were grown only with acetate to study acetate metabolism. However, this process did not considerably enhance the acetate degradation by Methanosarcina sp.

An acetate-cleaving enrichment containing mostly Methanothrix sp., cleaved acetate to CH₄ (Table 4.16). This enrichment was originally obtained from a sewage sludge inoculum, and has been maintained on 80 mM acetate during the last year. The medium used for this enrichment was identical to the medium used for growing P. productus and contained 0.1 percent yeast extract as a growth supplement.

Media (40 ml) was inoculated with 5 ml culture and was incubated at 37°C. Acetate was added as required. Bacterial growth and gas analysis were performed periodically. The results, reported in Table 5.15 show that the enrichment is capable of forming methane from acetate, with the optimum concentrations of acetate approximately 160 mM. Bacterial growth and methane formation by this enrichment were not affected up to a 160 mM acetate concentration. Although concentrations of 320 and 400 mM inhibited bacterial growth and methanogenesis, it is highly possible to adapt the enrichment to such higher concentrations of acetate.

In conclusion, this enrichment has tremendous potential to metabolize acetate formed during coal gas metabolism by P. productus and their co-culture could serve a potential means of biological conversion of coal gas to CH₄ in a single stage reactor.

TABLE 4.15

Production of Methane by Methanosarcina sp. (Strain MS)
Using H₂-CO₂ or Acetate as Methanogenic Precursors

Days	Gas compositions, mole%		
	H ₂	CO ₂	CH ₄
1. <u>With H₂-CO₂ (80:20)</u>			
0	64.69	34.92	0
4	1.68	43.20	54.29
2. <u>With acetate (40 mM)</u>			
0	--	14.97	0
9	--	14.90	3.0

All values are means of triplicate samples.

TABLE 4.16

Production of Methane by an Acetate-Utilizing Enrichment*
Growing on Various Concentrations of Acetate

Acetate (mM)	Max. O.D. (580 nm)	Methane, mole % formed in		
		7 days	15 days	28 days
40	0.388 (7)**	28.28	30.52	31.70
80	0.525 (10)	31.25	40.81	41.58
160	0.655 (15)	26.24	54.01	53.99
320	0.458 (28)	17.44	27.83	53.10
400	0.325 (28)	8.95	24.87	30.57

* The acetate enrichment had a predominant population of Methanothrix soehngenii - like organism

** Days took to reach maximum growth (optical density).

All values are means of triplicate samples

4.2.4 Immobilization of Bacterial Cells to Various Bio-support Materials

Several bio-support materials, both uncoated and coated with organic compounds (gelatin and agar in various concentrations) were incubated with the mixed bacterial culture in order to determine cell immobilization and selection of proper bio-support materials. Forty-five ml of basal salts medium (+ 0.1% yeast extract) were dispensed in serum stoppered bottles along with bio-support materials and were inoculated with 5 ml of mixed culture. The gas phase (2 atm) was adjusted to 50 percent CO, the balance being N₂ and CO₂. The bottles were incubated at 37°C and at periodic intervals the gas phase was analyzed for CO disappearance.

The bio-support materials were closely checked for cell attachment. The results of this experiment are reported in Table 4.17. As expected, the bacterial cultures grew well and utilized CO within 10 days irrespective of the presence of bio-support materials and treatment with 3% glutaraldehyde. But methane formation was inhibited in the presence of the glutaraldehyde-treated bio-support materials. Perhaps glutaraldehyde inhibited the metabolism of methane bacteria, resulting in acetate accumulation.

H₂ was also utilized within 10 days in almost all the samples, except in samples containing glass beads and treated with 3 percent glutaraldehyde. A close examination of the bio-support materials revealed no indication of cell attachment. In many cases, the cells were flocculated in the bio-support materials but gentle vibrations released them as free cells. Many other organic compounds such as carrangenan gel, polyacrylamide absorbents, sodium and calcium-alginates were also tested in cell immobilization experiments, but none of the above bio-support or organic coating materials showed promise for immobilization.

4.2.5 Coal Gas Conversion in an Immobilized Cell Reactor (ICR) Using Mixed Bacterial Cultures

The feasibility of bacterial immobilization was determined using a mixed culture known to metabolize coal gas to methane. A column reactor (4' x 9" O.D) was packed with gelatin-agar (20:1.5%) coated porous ceramic bio-support materials as described earlier (Section 4.1.1) that were previously treated with 3 percent glutaraldehyde. After the column reactor was made anaerobic, the reactor was inoculated with cultures from the Bio-flo reactor. Coal gas containing 45 percent CO, 30 percent H₂, 15 percent CO₂ and 10 percent CH₄ was bubbled through the reactor. The gas compositions at the entrance and exit, as well as the gas flow rates, were determined on a daily basis. Liquid samples were measured for cell density, pH and VFA profiles. The cells were allowed to grow at the expense of CO and covalently bound to the packing materials before coal gas was fed on a continuous basis. The bacterial growth and the conversion of CO and H₂ are summarized in Table 4.18. The conversion of CO and H₂ in the ICR were poor; conversions greater than 50 percent were not obtained either for CO or H₂. The shift in flow rates did not effectively influence the rate of conversion, probably due to negligible mass transfer of CO into solution. Also, there was no evidence of bacterial attachment to the packing materials.

Table 4.17 Conversion of CO by Mixed Bacterial Cultures Inoculated on Various Support Materials for Cell Immobilization*.

Bio-support materials	Gas Composition, percent**							
	5d				10d			
	H ₂	CO	CO ₂	CH ₄	H ₂	CO	CO ₂	CH ₄
Glass beads, untreated	2.83	9.5	39.28	7.18	3.6	0	43.04	16.19
Glass rings, untreated	6.55	3.25	38.58	6.57	3.8	0	37.56	17.33
Ceramic, untreated	3.54	5.27	41.66	7.12	0	0	43.24	15.65
Rushing rings, untreated	5.41	8.06	41.27	7.94	0	0	46.96	15.02
Glass rings, treated ***	5.19	21.92	29.78	6.03	0	0	47.01	10.11
Glass beads, treated	9.96	23.54	31.16	2.03	7.30	6.5	45.66	0.62
Ceramic, treated	10.96	14.27	33.66	0.44	0	0	48.58	1.68

*The medium (45 ml) contained in 120 ml serum bottles was inoculated with 5 ml of mixed cultures.

**0 day values are mean of 3 bottles: H₂, 2.46%; CO 51.99%; CO₂, 7.75%; CH₄, 1.53%

***Biosupport materials were coated with 25% galatin, or 20% gelatin and 1.5% agar and treated with 3% glutaraldehyde.

All values are mean of duplicate or triplicate samples

Several other attempts to achieve cell-immobilization using different packing and other organic coating materials have been unsuccessful. From these attempts it was felt that the major factor of the poor performance of the immobilized cell reactor was due to the failure in achieving high density cell attachment to the packing materials. In an effort to increase bacterial attachment, the reactor was subjected to a pressure of 20-30 psi and run batchwise. Although this process was continued for several weeks, no improvements in cell immobilization were seen. At all times, the bacteria grew as free cells and lived as clumps.

TABLE 4.18

Bacterial Conversion of Coal Gas in an ICR as a
Function of Flow Rate

Flow Rate ml.min ⁻¹	Gas Retention (h)	Max O.D. (580 nm)	CO Conv. %	H ₂ Conv. %
22.0	1.02	1.30	15.0	30.0
15.0	1.5	2.25	25.0	42.0
10.0	2.25	1.90	45.0	49.0
8.0	2.81	1.50	29.0	36.0

4.2.6 Coal Gas Conversion in an Immobilized Cell Reactor (ICR) Using P. productus

In an effort to immobilize P. productus, a reactor (total volume ~ 250 ml) was packed with ash wood chips and the entire assembly was sterilized with 3 percent glutaraldehyde. The wood chips were first boiled with 20 percent ethanol for 4-6 hours to remove soluble phenolics and other organics. Wood chips were chosen to trap cells into the tiny interstitial spaces inside the chips since cross-linking attempts with the mixed culture were not effective. The reactor was made anaerobic by passing oxygen-free N₂-CO₂ and then was inoculated with a log phase culture of P. productus previously grown on coal gas using a Bio-flo reactor. The culture was recycled through the wood chip filled reactor for several hours, and the optical density of the recycled liquid was taken every 15-30 minutes. As shown in Figure 4.2, the optical density of the culture was reduced from 1.45 to 0.92 within 100 minutes. This large reduction in optical density of the culture indicated that a large number of bacterial cells have been attached to the highly porous wood chip materials. The reactor was then continuously fed with a basal salts media used to grow P. productus (Table A.4). A coal gas containing 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄ was then bubbled through the reactor using various gas flow rates.

Bacterial adaptation in the ICR was a slow process requiring a minimum 3 weeks of gradual liquid media and gas introduction into the reactor.

Figure 4.2. Initial adsorption of *P. productus* on the support material (wood chips).

Steady-state was achieved after obtaining a consistent gas composition for at least two consecutive retention times. This procedure required 5-10 retention times between changes in operating conditions. At steady-state, the gas flow rates and compositions were monitored at the entrance and exit of the reactor. The cell density, pH and ammonia nitrogen were also measured. An illustration of the constancy of the CO conversion at three different gas residence times is in Figure 4.3. The stable, steady-state operation of the immobilized-cell bioreactor was achieved for an extended period of time (nearly three months).

Since the major goal of this study was to find an effective method of cell immobilization and to determine the rate of coal gas conversion, the result of this experiment is, therefore, shown based on the rate of coal gas conversion rather than the rate of acetate production. As mentioned earlier, P. productus produces acetate according to Equation (2.2). If desired, under ideal conditions (no contamination of the system) the rate of acetate production can be determined from the rate of CO conversion, since, according to Equation (1.4), for every 4 moles of CO converted, one mole of acetate is produced.

The experimental profile of coal gas conversion at various retention times is shown in Tables 4.19 and 4.20. Essentially no CO was converted at retention times lower than 0.2 hour. As noted, at a 6.25 hour gas retention time, the CO conversion was 76.6 percent. As the retention time decreased, the coal gas conversion also decreased, suggesting that the reaction was mass-transfer controlled. At gas retention times lower than 0.5 hours, the CO conversion fell below 10 percent.

TABLE 4.19

Conversion of Coal Gas to Acetate by P. Productus in an Immobilized Cell Reactor at Various Gas Retention Times

Gas retention time (hrs)	Maximum CO Conversion percent
0.5	9.9
0.833	22.2
1.25	36.2
2.5	51.5
5.0	66.6
6.25	76.6

Figure 4.3. Conversion of CO by P. productus immobilized in porous wood chips as a function of gas retention time.

TABLE 4.20

Conversion of CO and H₂ to Acetate by P. productus in an Immobilized Cell Reactor at Various Gas Retention Times

Gas retention time (hrs)	CO*		CH ₄		CO ₂		H ₂	
	In	Out	In	Out	In	Out	In	Out
0.5	68.4	61.6	3.1	4.4	11.4	19.4	15.5	13.4
0.833	70.3	54.7	0.87	4.9	11.4	23.3	15.5	12.3
1.25	70.2	44.8	2.5	4.1	10.7	21.4	15.7	9.9
2.5	70.8	34.3	3.0	5.8	11.7	28.7	14.4	7.7
5.0	71.3	23.8	2.6	12.2	10.8	45.3	14.3	4.6
6.25	70.5	16.5	2.8	17.1	10.9	48.2	15.1	7.1

* Gas composition was presented in mole %.

As discussed earlier, P. productus uses most of the CO before H₂ utilization becomes significant. In this process, however, due to the continuous feeding of the coal gas to the reactor, abundant CO was always present in the reactor and, therefore, the H₂ conversion was expected to be insignificant in comparison to CO utilization becomes significant. In this process, however, due to the continuous feeding of the coal gas to the reactor, abundant CO was always present in the reactor and, therefore, the H₂ conversion was expected to be insignificant in comparison to CO utilization. In contrast, a significant amount of H₂ was converted probably due to the reactor contamination by H₂ utilizing bacteria. In addition to high H₂ conversion, methane was also produced in an unexpectedly high percentage, up to 17 percent. The production of methane in the reactor system is most likely related to the presence of methanogenic bacteria as contaminants.

Figure 4.4 presents the CO conversion profile of the ICR as a function of gas retention time based on the liquid volume. The maximum CO productivity (5.59 VVD) was obtained at a retention time of 1.25 hours. The productivity increased from 3.79 VVD to 5.59 VVD with respect to gas retention time until a value of 2.3 VVD was reached at a retention time of 6.25 hours.

As presented in Appendix B, productivity is directly related to the reciprocal of the volume. The volume of the liquid culture inside the reactor is equal to the void volume and is, therefore, less than the volume of the packed-bed reactor. Thus, the CO productivities based on the total volume of the reactor (shown in Figure 4.5) were lower than the productivities (VVD) based on liquid volume, but followed almost the same

Figure 4.4. Rate of utilization of CO (based on liquid volume) as a function of gas retention time using P. productus in an immobilized cell reactor.

Figure 4.5. Rate of utilization of CO (based on reactor volume) as a function of gas retention time using P. productus in an immobilized cell reactor.

type of pattern as the productivities based on the liquid volume. Beginning with 1.45 VVD at a gas retention time of 0.5 hours, the productivities increased until they reached a maximum of 2.31 VVD, which occurred at a gas retention time of 1.25 hours.

Since the efficiency of the ICR is directly related to the number of immobilized cells, the results using this reactor system can be further improved if a larger number of cells were immobilized. The re-inoculation of the reactor with a thick culture of P. productus provides more cells in the reactor and, therefore, enhances the immobilized cell population. The survival and the activity of the immobilized cells are also dependent upon the availability of nutrient. At large media flow rates some of the immobilized bacteria are removed from the reactor, and at small media flow rates the cell activity decreases. Thus, introducing the media at its optimum flow rate could result in higher immobilized cell population. Furthermore, if the reactor becomes contaminated, P. productus might fail to compete with these organisms, thus decreasing its metabolic activity. The use of the ICR has various advantages over a CSTR in the conversion of coal gas, such as its high performance and the elimination of high cost of agitation. An efficient ICR is, therefore, a desirable reactor in the use of biological conversion of coal gas.

The above described system operated for several months without any major problems. However, the control of pH was an obstacle during these experiments. The pH had to be maintained between 6.8 to 7.4 with the addition of appropriate amounts of sodium bicarbonate.

Visual observations of the free cells in the liquid culture (taken from the ICR) under the microscope revealed that large numbers of unknown microbes were present. At the same time an effort was made to check the purity of the attached cells onto the wood chips. A small amount of the wood chips from inside the reactor was placed in a bottle, and after all the free cells were removed by washing the wood chips with media, the bottle containing wood chips and media was shaken vigorously for a few minutes. The liquid sample taken from this bottle was observed under the microscope. The result suggested that a large number of P. productus (about 80 percent) were present in the sample. The lower percentage of the contamination among the immobilized cells could explain the fact that a major population of P. productus was still alive and active.

Thus the initial attempt to immobilize bacterial cells converting coal gas have been a success. From the observations made, it is presumed that glutaraldehyde did not serve well as the cross-linking agent; other chemicals such as cyanuric chloride were not used. Adsorption appears to be a good immobilization technique, although higher cell densities are necessary to increase the conversion of CO.

4.2.7 Conversion of Acetate by a Mixed Culture Using an ICR

The conversion of acetate was studied using an immobilized cell reactor. A column was packed with ceramic saddles and connected to a plastic gas collecting bag for this purpose. The column was filled with an anaerobic sewage digester sludge (~ 270 ml) (total volume ~ 450 ml) into which 2 g/l sodium acetate was added. This column was run batchwise for

several weeks. At every 3-5 days, about 150 ml fresh basal salts medium amended with 2 g/l acetate was pumped to the reactor and left as a batch reactor. This procedure was followed to facilitate the establishment of acetate-degrading bacteria in a higher population. Gas measurements (total volume, and gas compositions) and liquid sample analyses were performed on a daily basis. In the beginning, it was also required to add some rumen fluid (5 percent) and/or 0.1 percent yeast extract to the reactor which were used as a growth supplement for the bacterial species.

After a few months, an acetate solution (2-5 g/liter) was pumped at 3-12 hour retention times. Gas analyses showed that CH_4 and CO_2 were the major products formed during acetate metabolism. The concentration of methane varied from 40-70 percent when 2 g/l acetate and various retention times were provided. The methane concentration increased up to 80 percent when using 5 g/l acetate at a 12 hour retention time. Acetate metabolism was complete (100 percent) between 6 and 12 hour retention times, but fell below 50 percent when a 3 hour retention time and 5 g/l acetate was provided. The reactor was very stable over a 6 month period. The performance of the reactor also increased with the adaptation of bacterial cultures.

Two bacterial species, Methanosarcina sp. (Strain MS) and an acetate enrichment containing mainly Methanothrix sp. have been shown to convert acetate to CH_4 using batch culture experiments. The bacteria could be used in an ICR system. The success of the mixed cell immobilization in the acetate conversion indicates that an ICR could be used as an alternative to a CSTR in converting acetate produced by P. productus during coal gas conversion. The use of pure cultures could also enhance the efficiencies of the bacterial conversion of acetate.

4.2.8 Metabolic Products

P. productus produced only acetate during CO metabolism according to Equation (1.4). Acetate produced by P. productus was at a rate of 1 mol acetate produced/4 mol of CO consumed, and in some experiments, acetate accumulated up to 15 g/liter in the culture media. Small amounts of butyrate and propionate were also detected during the bacterial metabolism of CO . In the experiments with mixed bacterial cultures including pressure studies, methane was the main product formed during CO metabolism. In these experiments, the accumulation of acetate was below 2 g/liter. Methanogenic bacteria metabolized acetate to methane and CO_2 . In an acetate fed immobilized cell reactor, the methane concentration reached a maximum of 80 percent.

5.0 CULTURE ENCHANCEMENT

The purpose of this study was to determine and improve the growth conditions of pure bacterial cultures converting coal gas to acetate and/or methane. Several experiments were designed to study the bacterial conversion of coal gas by single or mixed culture systems using a single-stage reactor. Bacterial growth, conversion of CO and product formation were monitored during these studies and specific conclusions have been made.

Two pure cultures of bacteria, Peptostreptococcus productus and Rhodospirillum rubrum were chosen for this study. A methane bacteria, Methanosarcina sp. (Strain MS) which metabolizes H_2 -CO₂, methanol and methyl amines to methane, was used in some experiments. Upon adaptation, this bacterium also uses small amounts of acetate to produce methane. Another acetate enrichment containing a Methanothrix soehngenii - like organism, which cleaves acetate to methane, was grown with P. productus to determine if a co-culture of these organisms can be obtained. P. productus or the known mixed cultures were grown in a one liter Bio-flo reactor at various rates of agitation and gas retention times. Batch experiments were also performed to determine maximum substrate utilization by the pure or the mixed co-culture systems.

This chapter describes the results of the experiments conducted to determine the feasibility of culture upgrading using the single-stage reactor system. P. productus was grown extensively in a stirred-tank reactor at various agitation rates and gas-retention times. Bacterial growth, CO utilization and product formation were used as the major criteria in determining the ability of P. productus or the co-cultures in the biomethanation of coal gas.

5.1 Materials and Methods

The materials and methods utilized in culture enhancement have been previously described in Sections 2.1 and 3.1.

5.2 Results and Discussions

Preliminary bacterial screening studies indicated that P. productus and R. rubrum possessed metabolic potential to convert CO or coal gas to methanogenic precursors. Both P. productus and R. rubrum were tested for their ability to grow on higher concentrations of CO, various compositions of synthetic coal gas and their tolerance to sulfur toxicants. P. productus has been grown in a CSTR reactor at various rates of agitation and gas flow rates. Methane producing bacteria such as Methanosarcina sp. and Methanothrix soehngenii containing enrichments were co-cultured with P. productus to determine the feasibility of using a single-stage reactor system during the biconversion of coal gas to methane. The following results were obtained from a series of experiments conducted to determine the future use of an appropriate co-culture in a single-stage reactor system.

5.2.1 Co-culture Experiments

In an attempt to obtain a methanogenic co-culture with CO-utilizing P. productus, Methanosarcina sp. and the acetate enrichment were inoculated to the tubes containing this bacterium. CO (50%, 2 atm) was introduced following the same method as described above and the balance gas was adjusted with 80:20 N₂-CO₂. All of the tubes were incubated at 37°C. The results are reported in Table 5.1. P. productus metabolized CO well. Within 6 days, almost 100 percent of the CO was converted in the samples with and without Methanosarcina sp. The metabolism of CO in the presence of the acetate enrichment was slightly slower; however, more than 72 percent of the initial CO was utilized within this period.

Microscopic observations showed that Methanosarcina sp. did not grow well in the presence of CO, but could produce CH₄ from acetate during bacterial metabolism. In contrast, the acetate enrichment containing Methanothrix sp. grew well in the presence of 50 percent CO and produced methane from acetate. At the end of 7 days, the gas phase contained CH₄ when P. productus and the acetate culture were grown together. Both Methanosarcina sp. and the acetate enrichment did not metabolize CO when incubated in the absence of P. productus.

TABLE 5.1

Metabolism of CO to Methane by P. productus Grown with 50 Percent CO in Co-culture With Methanosarcina sp. and an Acetate-Utilizing Enrichment

Culture	Maximum OD (580 nm)	% CO Recovered 0d	6d	Maximum CH ₄ mole %, (days)
<u>P. productus</u>	0.85 (6)*	44.54	1.05	0
<u>P. productus</u> + <u>Methanosarcina</u> sp.	0.66(6)	49.57	0	16.76(6)*
<u>P. productus</u> + Acetate Enrichment	0.69(7)	56.20	16.15	12.31(7)

* days

These results indicate that methanogenic co-cultures can be obtained with P. productus. Recent experiments using a CSTR indicate that Methanosarcina sp. is very suitable in batch culture, but that Methanothrix sp. grows better and cleaves acetate during continuous gas flow. Moreover, Methanothrix showed greater tolerance to CO than Methanosarcina sp. as indicated from the growth experiments.

No attempt has been made to obtain a co-culture with R. rubrum. As R. rubrum grows only in the presence of light and makes only H₂-CO₂ from

CO, a methanogen known to utilize H_2 -CO₂ such as Methanospirillum hungatii could be a suitable bacteria to be co-cultured with this bacterium.

5.2.2 Bacterial Metabolism of Coal Gas

Both P. productus and R. rubrum were grown with the two coal gas mixtures in studying the bacterial conversion of CO in batch culture experiments. As mentioned previously, coal gas Mix I contained 45 percent CO, 30 percent H₂, 15 percent H₂ and 10 percent CH₄; and coal gas Mix II had 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄. Basal salts media (45 ml) was dispensed in 125 ml serum stoppered bottles, followed by inoculation with 5 ml of a log phase culture of P. productus and 3 ml of a culture of R. rubrum. Coal gas (2 atm) was introduced using a special gas manifold. P. productus was incubated flat at 37°C, whereas the R. rubrum cultures were grown at 30°C under tungsten light. Bacterial growth, gas analysis and VFA profiles were monitored at periodic intervals.

Both cultures grew very well in the presence of coal gas mixtures in spite of the changes in the gas compositions. In the presence of coal gas Mix II, a maximum cell density of 0.455 was obtained for P. productus in 6 days, whereas the maximum cell density for R. rubrum was 2.935 in 7 days. This higher cell density, however, had no influence on the rate of CO metabolism.

The CO conversion for both the bacteria grown in the presence coal gas Mix II are presented in Figure 5.1. As can be seen from this figure, the initial rates of CO conversion by both bacteria were similar during the first 8 days, after which the rate was slower for R. rubrum. This decrease in rate could be due to the presence of higher amounts of H₂ (above 40 percent), which must be inhibiting CO metabolism. However, within 6 days, 83.2 and 77.1 percent of the CO were metabolized by P. productus and R. rubrum, respectively. Hydrogen was not metabolized by P. productus until the CO level dropped below 12 percent, but about 50 percent of the H₂ was metabolized within the next 10 days. R. rubrum does not utilize H₂, so that H₂ accumulated to a level of 43 percent in the gas phase. P. productus produced only acetate (1 mol of acetate/4mol CO metabolized) but R. rubrum metabolized CO to CO₂ and H₂. No other volatile acids were detected in the samples during the 21 days of incubation.

The bacterial growth, conversion of CO and product formation by both of the bacteria were identical to these results when gas Mix I was used in some experiments.

5.2.3 Biological Conversion of Coal Gas in a Continuous Stirred-Tank Reactor

The bacterial conversion of coal gas was studied using a New Brunswick one liter (Model C-30) fermentor. The reactor was modified to use strictly anaerobic bacteria and could be used for batch experiments or for continuous gas flow systems. Sample ports for gas sampling, bicarbonate feed septum and Swagelok quick connects were installed for easy handling. All connections were made airtight using Teflon seals. Butyl rubber or glass tubing was used as required. The liquid outlet port was

Figure 5.1. Metabolism of coal gas (Gas Mix II) by two bacterial cultures.

sealed with melted glass tubing connected with a clamped butyl rubber tube. The reactor was sterilized by autoclaving and then the air was flushed from the system with O₂-free N₂ and CO₂. Media transfer and bacterial inoculation were made inside an anaerobic chamber (Coy Laboratory Products, Inc. MI). The anaerobic media was dispensed in 50 ml or 75 ml amounts in serum stoppered bottles and then autoclaved. The media was reduced with sodium sulfide (2-5 percent) before use. Cultures were grown in serum stoppered bottles and all additions, including inoculation, were performed inside the anaerobic chamber. The gas supply was made through quick connection fitted to a flow meter. Liquid samples (~ 10 ml) were taken using the sample outlet port and closed immediately. Sterile sodium bicarbonate (7 percent) was added using a syringe with a one-way valve. The pH of the culture was kept within a range of 6.8 - 7.4.

Agitation and gas flow rates were changed as required. Bacterial cell density, pH, Volatile Fatty Acid (VFA) profile and gas composition measurements were performed on a daily basis or as required. Bacterial cultures were checked daily under a phase contrast microscope and contaminated cultures were discarded immediately. Fresh basal media was exchanged at 5 to 7 day intervals or as needed.

5.2.3.1 Bacterial Conversion of Coal Gas Using a Batch Culture

The bacterial metabolism of coal gas was determined in a one liter Bio-flo fermenter as a batch culture. P. productus was grown alone or in co-culture with Methanosarcina sp. and an acetate enrichment known to cleave acetate to CH₄. Coal gas Mix II was used for determining the rates of CO conversion by P. productus and methane formation during the mixed culture experiments. The results of these experiments are summarized below.

5.2.3.1.1 Coal Gas Conversion by P. productus

P. productus was grown in a stirred-tank reactor as a batch culture and coal gas containing 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄ was fed to the reactor up to a maximum level. Bacterial growth and the conversion of CO and H₂ were monitored on a regular basis. Agitation was provided at 250 rpm. The results are reported in Table 5.2. As reported in this table, P. productus rapidly utilized CO. Within 24 hours more than 75 percent of the CO was converted to acetate and CO₂. Within 48 hours, the CO conversion was more than 92 percent. The bacterial growth also increased from an optical density of 2.10 to 2.9 within 24 hours. The conversion of H₂ did not occur until 24 hours of

TABLE 5.2

Single Stage Conversion of Coal Gas to Methane by P. productus
Co-culture With an Acetate Enrichment When Grown as a Batch
Culture in a Stirred-Tank Reactor

	Gas Composition, Mole %			
	CO	H ₂	CH ₄	CO ₂
<u>Only P. productus</u>				
After feed	66.91	18.58	2.34	11.72
Output	5.81	17.20	2.76	40.34
% Conversion	92.26(2)*	7.43(2)		
<u>P. productus + Acetate Enrichment</u>				
After feed	70.87	15.72	1.97	10.74
Output	8.31	10.70	27.34	53.15
% Conversion	88.28(1)	32.0(1)		

* days

incubation but then H₂ disappeared very slowly. At the end of 48 hours, only 7.43 percent of the H₂ was metabolized by P. productus. Acetate (1 mol/4mol CO consumed) and CO₂ were the only products produced during bacterial metabolism.

These results show that P. productus has tremendous potential to metabolize CO and can be used for the production of an important chemicals such as acetate from toxic carbon monoxide.

5.2.3.1.2 Conversion of Coal Gas by P. productus and Methanosarcina sp.

In an attempt to co-culture P. productus with Methanosarcina sp., 250 ml of a full-grown culture of P. productus in the stirred-tank reactor was removed and replaced by 200 ml of reduced media and 50 ml of full-grown culture of Methanosarcina sp. Coal gas was fed to the reactor as above and the culture was grown as a batch culture. Agitation was provided at 300 rpm. The results are presented in Figure 5.2. The bacterial conversion of CO was improved and P. productus metabolized about 82 percent of the CO within 24 hours. The conversion of H₂ began within 2 hours, and in 24 hours more than 85 percent of the H₂ was converted. It should be mentioned here that this case Methanosarcina, and not P. productus, was mainly responsible for H₂ conversion. Methanosarcina sp. also produced methane from acetate stoichiometrically and the level reached a gas phase concentration of 25.6 percent within a 24 hour period.

Figure 5.2. Conversion of coal gas by a CO culture, *P. productus* and *Methanosarcina* sp. when grown in a stirred-tank reactor as a batch culture.

5.2.3.1.3 Coal Gas Conversion by *P. productus* and an Acetate Enrichment

An acetate enrichment mostly containing *Methanothrix soehngenii* - like organisms which cleaved acetate to methane was grown along with *P. productus* to determine whether a co-culture of these organisms can be obtained. This enrichment was established in the University of Arkansas biotechnology laboratory from an anaerobic digester sludge inoculum and has been maintained on 80 mM acetate for over one year. Similar to the previous methods, 250 ml of the full-grown culture of *P. productus* was removed and was replaced by 200 ml of reduced media and 50 ml of a full-grown culture of the acetate enrichment. Coal gas was fed to the reactor as described above and the culture was grown as a batch culture with an agitation rate of 300 rpm.

As with previous experiments, *P. productus* metabolized CO very rapidly and within 24 hours about 88 percent of the CO was metabolized. Perhaps some methanogens present in the mixed culture slowly utilized H₂, such that 32 percent of the H₂ was metabolized within this period. Methane reached a concentration of 27 percent in the gas phase, suggesting rapid metabolism of acetate.

From the above discussions, it is concluded that *P. productus* is one of the best organisms that can convert CO and H₂O to acetate. A co-culture of this organism with a H₂-CO₂ utilizing methanogen or acetate-cleaving bacterial cultures could lead to a single-stage reactor system where the coal gas can be easily transformed to methane. As *P. productus* does not metabolize H₂-CO₂ in the presence of excess CO, and cannot metabolize acetate, a co-culture of appropriate organisms is an advantageous method during coal gas conversion to methane. The results presented also indicate that *P. productus* can utilize CO much faster if H₂ and acetate can be removed from the media as they are produced. *P. productus* has not been tested for its metabolic tolerance to acetate or H₂ which might be limiting its capabilities in metabolizing carbon monoxide.

5.2.3.2 Bacterial Conversion of Coal Gas Using the Continuous Stirred-Tank Fermenter and Continuous Gas Flow

The bacterial conversion of coal gas was studied using a one liter Bio-flo fermenter under continuous gas flow. *P. productus* was grown alone or in the presence of *Methanosarcina* sp. or an acetate-degrading enrichment to determine the potential of the bacterial cultures to convert coal gas to methane. Both agitation and gas flow rates were changed as required. Coal gas Mix I and Mix II were used for these studies. As the rates of CO conversion and other observations related to bacterial growth were similar with both gas mixtures, only the results obtained with Mix II are presented below.

TABLE 5.3

Conversion of Coal Gas to Acetate by P. productus, When
Grown in a Continuous Stirred-Tank Reactor at
Various Rates of Agitation

Agitation (rpm)	Gas Retention Time (hours)	Coal Gas Conversion, mole %	
		CO	H ₂
200	1.46	25.7	0
300	1.46	59.0	0
400	1.46	88.6	0
500	1.46	93.2	0
600	1.46	100.0	0

* Coal gas contained 65% CO, 22% H₂, 11% CO₂ and 2% CH₄

5.2.3.2.1 Coal Gas Conversion by P. productus

P. productus was grown in a stirred-tank fermenter and the conversions of CO and H₂ were studied using various gas retention times and at different rates of agitation. All of the continuous gas flow experiments were performed during the log phase or early log phase of P. productus. Media exchange was made every 5-7 days or as required. Synthetic coal gas containing 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄ was used as the feed gas. Gas compositions were determined until a steady-state had been reached. Sodium bicarbonate was added when required to maintain the pH between 6.8 to 7.4.

In the first experiment, a gas retention time of 1.46 hour was used and the agitation rate was changed from 200-600 rpm. The results reported in Table 5.3 show that the CO conversion was dependent on the rates of agitation. Complete conversion of CO occurred when an agitation rate of 600 rpm was provided. The bacteria could convert more than 88 percent of the CO even at 400 rpm. There was no conversion of H₂ during this experiment. P. productus obtains hydrogen for acetate formation from H₂O and does not use H₂ unless all of the CO has been depleted. Acetate was the only product formed during the bacterial metabolism of CO. Bacterial growth followed a normal pattern and P. productus dominated more than 99 percent of the bacterial population when small amounts of contamination occurred after a few days of the reactor start-up.

Similar experiments with P. productus at a 1.56 hour gas retention time showed 92 and 88 percent conversions of CO at 850 and 600 rpm, respectively. However, the cultures were not very much acclimated at this time, suggesting that bacterial adaptation to specific substrates and conditions are necessary before steady state results could be obtained.

In the second experiment, an agitation of 500 rpm was selected and the gas retention times were changed from 0.246 to 1.46 hours. Gas measurements were made until a steady state had been reached. The results of this experiment, presented in Table 5.4, shows the conversion of CO at various gas retention times but at a fixed agitation rate of 500 rpm. The bacterial conversion of CO was more than 92 percent at a 1.46 hour gas retention time. The conversion increased with a decrease in gas flow rate. More than 56 percent of the CO was converted at a gas flow rate of 12.5 ml/min. The bacteria could tolerate a gas flow rate of 22 ml/min (0.24 hr gas retention time). It should be noted here that the doubling time of P. productus, when grown under a 50 percent CO atmosphere, is about 2-3 hours. Such high gas flow rates as provided during this experiment did not cease bacterial growth or CO metabolism by P. productus indicating the extreme tolerance of P. productus to toxic carbon monoxide. As these experiments were run for less than an 18 hour period, H₂ conversion by P. productus was not noticed.

TABLE 5.4

Conversion of Coal Gas* to Acetate by P. productus, When Grown in a Continuous Stirred-Tank Reactor at Various Gas Retention Times and Constant Agitation (500 rpm)

Gas Retention Time (Hours)	Coal Gas Conversion, mole %	
	CO	H ₂
1.46	92.08	0
0.90	75.48	0
0.629	69.62	0
0.433	56.48	0
0.246	21.39	0

* Coal gas contained 65% CO, 22% H₂, 11% CO₂ and 2% CH₄
Agitation was provided at 500 ± 10 rpm

In the third experiment, two gas retention times were selected and the conversion of CO was studied by changing the agitation. The data in Table 5.5 shows that CO conversion was completely dependent on the rates of agitation. More than 95 percent of the CO was converted at 600 rpm but complete conversion was achieved at 700 rpm. The CO conversion with a gas retention time of 1.46 hours was higher than with a 0.9 hour gas retention time during all the agitation rates. A conversion of more than 92 percent occurred at 500 rpm, and complete conversion was found at 600 rpm. The conversion of H₂ was almost negligible during these experiments.

When the CO conversion rates were calculated on a gm dry cell weight basis, the maximum conversion (23.58 m moles of CO/gm dry cell weight/hour) occurred at 500 rpm and a 0.63 hour gas retention time. At 600 rpm and a 1.45 hour gas retention time, the corresponding value was 22.75 m moles of CO/gm dry cell/hour.

Thus, from these experiments, it can be seen that P. productus has a tremendous potential to convert toxic CO even at very high gas flow rates (up to 22 ml/min). Reactor agitation rates were found to be an important factor in the bacterial metabolism of CO, mainly due to mass-transfer problems.

TABLE 5.5

Conversion of Coal Gas to Acetate by P. productus,
When Grown in a Continuous Stirred-Tank Reactor at
Various Agitation Rates and Two Gas Retention Times

Agitation (rpm)	Gas Retention Time (hours)	CO Conversion, mole %
200	1.46	14.53
	0.90	2.13
300	1.46	52.84
	0.90	39.10
400	1.46	86.88
	0.90	46.13
500	1.46	92.08
	0.90	75.48
600	1.46	100.00
	0.90	95.30
700	1.46	100.00
	0.90	100.00

* Coal gas contained 65% CO, 22% H₂, 11% CO₂ and 2% CH₄
The conversion of H₂ was negligible at all the agitation periods.

5.2.3.2.2 Coal Gas Conversion by P. productus and Methanosarcina sp.

The continuous conversion of coal gas by a P. productus and Methanosarcina sp. co-culture was studied using a stirred-tank reactor. Bacterial cultures were grown in a mineral medium supplemented with 0.1 percent yeast extract. After full growth of only P. productus in the reactor, 250 ml culture was removed and replaced by 200 ml of fresh media and 50 ml of log phase Methanosarcina culture. A coal gas containing 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄ was used as

the feed gas. Two gas retention times (1.56 and 0.73 hr) were chosen for these experiments and the agitation was varied from 200 to 850 rpm as indicated in Table 5.6. A minimum of 3-4 gas retention times were required to reach optimum gas conversions and gas measurements were taken until a steady-state had been reached.

The conversions of both CO and H₂ are reported in the table. At a 1.56 hour gas retention time, the CO conversion was negligible at a minimum agitation rate of 250 rpm, but increased with the increasing rates of agitation. A maximum conversion of 92 percent was achieved at 850 rpm and 1.56 hour gas retention time. CO conversion using a 0.73 hour gas retention time was always lower than the CO conversion at a 1.56 hour retention time. The maximum CO conversion with the 0.73 hour retention time was only 87 percent. The conversion of H₂ at the 1.56 hour retention time was higher than the conversion at 0.73 hour and reached a level of 94 percent at 800 rpm. The maximum methane formation at a 1.56 and 0.73 hour gas retention times was 18 and 5 percent, respectively. Coal gas was supplied up to 11 ml/min, but had no adverse effects on P. prinductus which converted more than 40 percent of the CO at 700 rpm at this flow rate.

The lower conversion of H₂ and the formation of low levels of CH₄ at the 0.73 hour gas retention time could be due to the loss of activity of Methanosarcina sp. Apparently, Methanosarcina sp. could not tolerate the high concentrations of CO and started to disappear from the reactor when gas flow rates greater than 6 ml/min were provided. The utilization of H₂ and acetate were, therefore, inhibited leading to low CH₄ formation.

TABLE 5.6

Single State Conversion of Coal Gas to Methane by
P. productus in Co-culture with Methanosarcina sp.
 Grown in a Continuous Stirred-tank Reactor at 2
 Gas Retention Times and at Variable Agitation Rates*

Agitation (rpm)	Gas Retention Time (hours)	% Conversion**		Max CH ₄ ** Formed, mole %
		CO	H ₂	
200	1.56	1.0	40.0	4.62
	0.73	n.d.	n.d.	n.d.
250	1.56	13.7	49.0	6.50
	0.73	12.4	31.4	2.88
300	1.56	32.3	59.2	10.03
	0.73	23.3	44.8	4.90
400	1.56	69.8	83.2	14.25
	0.73	54.7	39.8	6.42
500	1.56	87.1	84.3	16.91
	0.73	60.2	17.5	3.98
600	1.56	88.0	77.5	15.13
	0.73	80.6	29.0	4.41
700	1.56	88.0	77.5	17.77
	0.73	82.0	23.0	4.64
800	1.56	90.2	93.5	17.26
	0.73	86.9	32.0	4.73
850	1.56	91.7	87.6	15.85
	0.73	87.3	30.9	5.10

* Feed gas composition was 65% CO, 22% H₂, 11% CO₂ and 2% CH₄

** A minimum of 3-4 gas retention times were required to reach steady-state conversion n.d. Not determined.

5.2.3.2.3 Coal Gas Conversion by P. productus and an Acetate Enrichment

The conversion of coal gas by a mixture of P. productus and an acetate cleaving enrichment was studied using continuous gas flow and at various rates of agitation. P. productus was grown in a stirred-tank fermenter with an acetate-utilizing enrichment in a similar manner as described for the growth of P. productus and Methanosarcina sp. in Section 4.4.2.2. A coal gas containing 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄ was used as the feed gas. Bacterial growth, gas compositions

and VFA profiles were monitored at regular intervals. All the results reported here are obtained during the log or early log phase growth of the bacteria, and gas analyses were performed until steady-state has been reached.

In the first experiment, a gas retention time of 1.45 hour was selected and agitation was varied from 250-700 rpm. The results of this experiment are reported in Table 5.7. P. productus converted CO at a faster rate and the bacterial conversion of CO increased with the increase in agitation and reached 93 percent at 700 rpm. CO conversion at lower agitation rates were presumably mass-transfer limited. Interestingly, H₂ conversion occurred and almost 80 percent of the H₂ was converted at high agitation rates. The conversion of H₂ probably was by the methanogenic bacteria present in the acetate enrichment and not by P. productus or Methanothrix sp. The methane reached up to 20 percent, but at some agitation rates, lower values of methane were seen. Methanothrix usually converts acetate to methane, but rather slowly, which could explain the incomplete acetate conversion with changing agitation rates. This bacteria, however, unlike Methanosarcina sp., has more potential in the conversion of acetate to methane in the presence of higher concentrations of CO. Microscopic observations also revealed that P. productus

TABLE 5.7

Coal Gas Conversion to Methane by P. productus in Co-culture
With an Acetate-Cleaving Mixed Culture Grown in a
Continuous Stirred-Tank Fermentor*

Agitation Rate rpm	Percent Conversion		Maximum CH ₄ formation (mole %)
	CO	H ₂	
250	21.36	21.82	3.55
300	38.74	54.60	7.85
400	72.94	72.19	20.37
500	79.40	82.47	9.07
600	91.10	85.10	12.83
700	93.0	78.69	14.86

* Gas retention time was 1.45 hr.

constituted more than 90 percent of the bacterial population in the reactor indicating its adaptability with other bacterial strains.

In another experiment, P. productus and the acetate-cleaving enrichment were grown in a stirred-tank reactor at a fixed agitation rate of 500

rpm and variable gas flow rates (Table 5.8). P. productus metabolized CO at similar rates as was observed in previous experiments, with a conversion of more than 89 percent at a 2.7 hour gas retention time. The rates of CO conversion decreased with higher gas flow rates. The conversion of H₂, perhaps by methanogenic bacteria present in the enrichment, was more than 72 percent during each agitation. Methanothrix sp. also converted acetate to CH₄.

Thus, from the above discussions, it can be concluded that P. productus can be co-cultured with other groups of bacteria. Methanothrix sp. seems to be more tolerant to CO than Methanosarcina sp., which perhaps needs more adaptation to the conditions in which P. productus is being grown. An ideal tri-culture system containing P. productus, and pure cultures of Methanosarcina sp. and Methanothrix sp. would be highly desirable in the conversion of coal gas to CH₄ in a single-stage reactor, where CO, H₂ and a portion of the CO₂ will be metabolized to methane via the acetate pathway. The outlet gas containing 60-75 percent CH₄ and

TABLE 5.8

Coal Gas Conversion to Methane by P. productus in Co-culture With An Acetate-Cleaving Mixed Culture Grown in a Continuous Stirred-Tank in a Continuous Stirred-Tank Fermenter at Various Gas Retention Times and at 500 rpm

Gas Retention Times (hours)	% Conversion		Maximum CH ₄ Formation (mole %)
	CO	H ₂	
2.7	89.0	82.10	24.01
1.45	79.4	82.47	15.49
0.98	74.8	83.8	16.96
0.68	71.8	72.3	18.68

25-40 percent CO₂ can be purified to obtain methane for fuel purposes. However, in the absence of a pure Methanothrix culture, the use of a co-culture containing P. productus and an acetate-enrichment seems logical in the biomethanation of coal gas.

5.2.4 Determination of Bacterial Dry Cell Weight

P. productus and an acetate enrichment containing Methanothrix sp. were grown in a stirred-tank reactor. Synthetic coal gas containing 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄ was used as the feed gas. A gas retention time of 1.45 hr and an agitation rate of 500 rpm were used for this experiment.

The co-culture containing P. productus could convert more than 85 percent of the CO and H₂ at 500 rpm and a gas retention time of 1.45 hour. Methanothrix sp. utilized acetate and produced up to 24 percent CH₄ at the above specified conditions. Bacterial growth was determined at various time intervals and an undiluted culture (5-15 ml) was filtered using a previously weighed Millipore filter (0.45 µm pore size) unit. The filter paper containing bacterial cells was first washed with distilled water (~ 10 ml) and then dried in an oven at 110°C until a constant weight had been obtained. The results are reported in Figure 5.3. An equation was obtained from this graph by the least square method:

$$W = 0.51 (O.D.) - 0.066 \quad (5.1)$$

where

W = cell dry weight, g/L and

OD = optical density at 580 nm

This equation will be used in future experiments for this co-culture, in order to calculate the CO conversion rates per unit of biomass.

5.2.5 Metabolic Products

P. productus produced only acetate during CO metabolism according to Equation (1.3). Acetate production by P. productus was at a rate of 1 mol acetate produced/4 mol CO consumed and an accumulation of up to 15 g/liter of acetate was detected during some experiments. Small amounts of butyrate and propionate also accumulated during the bacterial metabolism of CO. R. rubrum metabolized CO to equimolar amounts of CO₂ and H₂ according to Equation (1.1). No other volatile fatty acids were detected during the experiments. In the co-culture experiments, methane was the main product of CO metabolism. In the presence of Methanosarcina sp., H₂ and CO₂ were metabolized to methane. This bacterium also converted a portion of the acetate to the methane. When P. productus was co-cultured with Methanothrix sp., the bacteria converted acetate to methane. Some methanogens present in this mixed culture also metabolized H₂ and CO₂ to CH₄. In co-culture experiments, methane accumulated to a level of up to 24 percent. In continuous gas flow rate experiments, methane production had not reached steady-state, as the conditions were changed as soon as CO metabolism had reached a steady-state condition. This probably led to incomplete conversion of acetate formed during CO metabolism by P. productus. However, from these preliminary results, a suitable condition can be worked out for a co-culture system which can metabolize coal gas to methane.

6.0 TOXICITY STUDIES

Depending upon the sulfur content of coal, the coal synthesis gas following gasification may contain sulfur gases, typically present as H_2S or COS. Typical levels of these sulfur gases are less than 1 percent, with maximum levels of about 2 percent possible. The effects of these sulfur gases on the growth and performance of mixed and pure cultures should be known. In catalytic processes, sulfur is a known poison for nickel, potassium, iron/chromium oxide, and zinc/copper oxide catalyst.^{4,5}

The purpose of this report is to present the results from batch fermentation results using mixed and pure cultures in the presence of varying concentrations of H_2S and COS. The mixed bacterial culture used in converting coal gas to methane in a single stage is used, in addition to the CO-utilizers Peptostreptococcus productus and Rhodospirillum rubrum. These two pure cultures were studied since screening experiments have shown that these two organisms are the most promising in producing methane precursors.²⁷

In the toxicity studies, the effects of the sulfur gases on both growth and metabolism is studied, using sulfur gas concentrations of 0-10 percent by volume. Concentrations of sulfur gases in excess of the maximum COS and H_2S concentrations are studied, in order to determine the concentrations at which total inhibition of growth and metabolism is found.

The results of these experiments will be very valuable in determining if pre-treatment of the gas stream is needed prior to biological conversion. If little or no toxicity is found at normal levels of sulfur gases, removal of the gases will only be necessary to meet pollution standards. If toxicity is found, suitable biological techniques for sulfur gas removal may be utilized.

Two pure cultures of bacteria, Peptostreptococcus productus and Rhodospirillum rubrum were chosen for this study. These two bacterial cultures have been shown to have significant potential in converting coal gas and/or CO to methanogenic precursors. Three sulfur gases, H_2S , COS, and SO_2 were used for this study. The gases in various concentrations (0 - 10%) were mixed with CO and the bacterial growth and metabolism of CO were monitored. The effect of sulfur gases on the product formation during CO metabolism was also evaluated.

6.1 Materials and Methods

The materials and methods used in these toxicity studies are essentially described in Sections 2.1 and 3.1. In addition, the following modifications to these experiments were made.

Sulfur gases were added using a sterile syringe fitted with one-way valves. The cultures were then vortexed for a few seconds before incubation. These operations were carried out in a hood and all the tubes were checked for gas leakage. The final pressure for the tubes or the bottles was adjusted to 2 atm.

Control tubes with only CO or coal gas were run simultaneously during each experiment. The cultures of P. productus and mixed cultures were

incubated (flat) in the dark at 37°C. In some experiments, P. productus tubes were agitated at 200 rpm using a temperature controlled New Brunswick Incubator Shaker (Model G-25). All incubations of R. rubrum were performed under tungsten light at 30°C.

6.2 Results and Discussion

Two pure bacterial cultures, P. productus and R. rubrum and a mixed culture isolated from sewage sludge were grown with 50 percent CO, in the presence of 0-10 percent concentrations of sulfur gases. Bacterial growth, CO metabolism and product formation were chosen as the main criteria for culture evaluation in determining sulfur gas tolerance.

6.2.1 H₂S

The effect of H₂S on bacterial growth CO metabolism and product formation was studied. R. rubrum and a mixed culture were included for this purpose. Several batch culture experiments were performed to determine the toxic effects of H₂S on the growth and metabolism of CO by these bacterial species. Serum stoppered Balch tubes containing 9 ml of basal salts media (+0.1% yeast extract) in triplicate were inoculated with 1 ml of a log phase culture of P. productus and mixed cultures or 0.5 ml log phase culture of R. rubrum, previously not exposed to H₂S. The gas phase (2 atm) contained 50 percent CO, 0-10 percent H₂S and the balance was adjusted with 80 percent N₂ and 20 percent CO₂. The tubes inoculated with P. productus and the mixed culture were incubated at 37°C, while R. rubrum was grown at 30°C under tungsten light. Bacterial growth and gas measurements were performed as required. Aliquots of liquid samples were frozen for VFA (volatile fatty acids) analyses at periodic intervals.

6.2.1.1 Bacterial Growth

The growth of P. productus in the presence of 0-10 percent H₂S and 50 percent CO are shown in Figure 6.1 and Table 6.1. As can be seen from Figure 6.1 there was a slight inhibition in the growth of P. productus with 1 and 2 percent H₂S, but no growth occurred in the presence of 5 and 10 percent H₂S. Although no initial lag was observed in the presence of H₂S, bacterial growth after a 6 hour period became slower in the presence of 2 percent or more H₂S, the culture took 8 days to reach a maximum optical density of 0.38 in comparison to an optical density of 0.46 in 4 days at 1 percent H₂S, the culture took 8 days to reach a maximum optical density of 0.38 in comparison to an optical density of 0.46 in 4 days at 1 percent H₂S (Table 6.1).

Initial results with R. rubrum in the presence of 0-10 percent H₂S and 50 percent CO are not shown here. The growth of R. rubrum was slower even in the presence of 1 percent H₂S and no growth occurred above a concentration of 1 percent H₂S. Therefore, in experiment 2, R. rubrum was grown in the presence of 0.25, 0.5, 0.75 and 1 percent H₂S and 50 percent CO.

Figure 6.1. Growth of *P. productus* at various levels of H_2S .

TABLE 6.1

Metabolism of CO by P. productus in the presence of H₂S*

% H ₂ S	Max. Growth(d)**	Max. CO Conversion % (d)
0	0.45(4)	96.7(7)
1	0.46(4)	86.5(7)
2	0.38(8)	83.0(9)
5	0.26(9)	27.4(9)
10	0.11(4)	8.2(9)

* values are means of duplicate tubes

** minus O.D. at 0 time

The results are reported in Figure 6.2. Bacterial growth was slower with increasing concentrations of H₂S. The cell densities were at least 26, 42, 50 and 67 percent lower with 0.25, 0.5, 0.75 and 1 percent H₂S concentrations than the control (no H₂S) samples. The doubling times of the bacteria were 19.2, 24.0, 28.8, 32.4 and 57.6 hours, respectively, when grown with 0, 0.25, 0.5, 0.75 and 1 percent H₂S.

In another experiment (expt. #3), R. rubrum was grown in serum stoppered Balch tubes containing 9 ml basal media and 50 percent CO (2 atm). After 72 h of growth the gas phase in the tubes was flushed with sterile N₂:CO₂ (80:20) and was exchanged with 50 percent CO (2 atm) and 0-2 percent H₂S. As can be seen from Figure 6.3, the presence of H₂S adversely affected bacterial growth even under these conditions. High cell density (starting O.D. ~ 1.5) did not affect the H₂S toxicity, and bacterial growth ceased completely. Conventional growth occurred when the culture was grown with only 50 percent and no H₂S. This observation was in contrast to the bacterial metabolism of CO by pre-grown cultures of R. rubrum as will be discussed later (see Section 6.2.1.2).

The mixed culture grew well in the presence of H₂S (Table 6.2). No inhibition of bacterial growth was noticed up to a 2 percent concentration of H₂S but growth decreased slightly in the presence of 5 and 10 percent H₂S.

Figure 6.2. Growth of *R. rubrum* in the presence of different concentrations of H₂S.

Figure 6.3. Growth of *R. rubrum* when H_2S (0-2%) and CO (50%, 2 atm) were added to 72-hour pregrwon (+ CO) cultures.

TABLE 6.2

Growth of Mixed Cultures Grown with 50 percent CO
and Various Concentrations of H₂S

Days	Optical Density* at 580 nm With 50% CO and H ₂ S(%)				
	0	1	2	5	10
0	0.366	0.366	0.366	0.366	0.366
1	0.60	0.60	0.576	0.534	0.492
2	0.588	0.57	0.57	0.54	0.498
5	0.588	0.48	0.468	0.45	0.42
7	0.558	0.486	0.48	0.432	0.45

* Values are means of duplicate samples.

A decrease of only 10-17 percent in cell density was noticed in the presence of 5 and 10 percent H₂S.

From the foregoing discussions, it can be concluded that the presence of lower concentrations of H₂S does not affect bacterial growth. All three cultures tested in this study could tolerate at least up to 1 percent H₂S and so are suitable to be used in the biomethanation of coal gas.

6.2.1.2 Metabolism of CO

The presence of H₂S had a similar effect on the bacterial metabolism of CO by all three CO-utilizing cultures used in this study. As with bacterial growth, the presence of 1 percent H₂S had very little effect on the conversion of CO by *P. productus*, but its presence at higher concentrations inhibited CO metabolism by this bacterium (Figure 6.4). More than 80 percent of the CO was utilized by this bacterium within 5 days in the presence of up to 2 percent H₂S. The corresponding values at 5 and 10 percent H₂S were 36 and 3 percent, respectively, during this period. At a 10 percent H₂S level, more than 75 percent of the CO was not utilized even after 21 days of incubation.

The presence of H₂S had an inhibitory effect on CO metabolism by *R. rubrum*. This bacterium could not utilize CO in the presence of more than 1 percent H₂S but below 1 percent, CO metabolism by this bacterium proceeded at similar rates as in the control tubes. More than 95 percent of the CO was metabolized in the presence of 0.25 - 1 percent H₂S at the end of 17 days of incubation (Table 6.3). In another experiment, when 1 and 2 percent H₂S was added to 72 hour pre-grown cultures, CO metabolism proceeded without any inhibition, despite no bacterial growth (see discussions

Figure 6.4. Effect of H_2S concentrations on the metabolism of CO by P. productus.

above). In this case, more than 95 percent of the CO was metabolized within 10 days, even in the presence of 2 percent H₂S (Figure 6.5). These results with *R. rubrum* indicate that bacterial growth, but not the metabolism of CO, is mostly affected by the presence of H₂S. Under some conditions, H₂S toxicity could be avoided by first growing bacterial cells to a higher cell density prior to their exposure to this toxic gas.

The effect of H₂S on the metabolism of CO by the mixed bacterial culture is shown in Figure 6.6. As can be seen from this figure, the rate of CO metabolism was decreased with an increase in the level of H₂S. Up to 2 percent H₂S had very little effect on CO conversion, but at 5 and 10 percent H₂S levels, the metabolism of CO started after 5 days of incubation and proceeded very slowly. At the end of 23 days, 30.6 and 36.9 percent of the CO had not been metabolized by the mixed cultures in the presence of 5 and 10 percent H₂S, respectively.

TABLE 6.3
Metabolism of CO by *R. rubrum* Grown With
Various Concentrations of H₂S

Conc. of H ₂ S, %	Maximum O.D. (600 nm)	Gas Compositions*, mole %			
		0 day		17 days	
		CO	CO	CO ₂	H ₂
0	2.55	50.93	2.49	26.58	27.35
0.25	1.89	54.80	4.29	26.59	31.46
0.50	1.45	53.13	2.12	27.75	29.70
0.75	1.28	52.57	3.75	27.97	27.16
1.0	0.86	54.22	5.39	27.11	27.82

* Gas phase (2 atm) was adjusted using Leur-lok syringes equipped with one-way valves.

Thus, the effect of lower concentrations of H₂S on bacterial metabolism of CO is very negligible. The experiments with pre-grown *R. rubrum* cultures also suggest that the metabolism of CO in the presence of H₂S may not be as adversely affected as the bacterial growth. H₂S toxicity can be avoided through culture manipulation.

6.2.2 COS

The toxicity of COS on the bacterial growth and CO metabolism was also studied using two pure bacterial cultures, *P. productus* and *R. rubrum* and the mixed culture known to utilize carbon monoxide. Several experiments were conducted to determine the degree of COS toxicity during bac-

Figure 6.5. Metabolism of CO by *R. rubrum* when H₂S (0-2%) and CO (50%, 2 atm) were added to 72-hour pregrown (+CO) cultures

Figure 6.6. Bacterial conversion of CO in the presence of H₂S using mixed culture.

terial metabolism of CO. The general plan of the experimental procedures was the same as described for H₂S under Section 6.2.1. Basal salts media (9 ml) in triplicate were inoculated with log phase culture of the bacterial and were incubated as described under Section 6.2.1. CO (50 percent, 2 atm) and COS (0-5 percent) were added to the cultures using a gas manifold system or special syringes fitted with one-way valves. Gas analyses and liquid sampling were performed as described previously.

6.2.2.1 Bacterial growth

The growth of P. productus on 50 percent CO and 0-5 percent COS is reported in Figure 6.7. Complete inhibition of growth occurred in the presence of 5 percent COS, but at 1 and 2 percent COS the bacterial growth was reduced by 20 and 24 percent, respectively, than with the cultures grown with only 50 percent CO. Interestingly, there appeared to be no distinct lag phase during bacterial growth at 1 and 2 percent COS, suggesting that COS is not adversely toxic to the organism at these concentrations.

The effect of COS on the bacterial growth of R. rubrum was studied using batch culture experiments. Bacterial growth was strongly affected by the presence of higher concentrations of COS. The growth of R. rubrum ceased completely at 5 percent COS. With 1 and 2 percent COS, the bacterial growth was retarded by 45 and 60 percent, respectively, in comparison to the control samples (Figure 6.8). In contrast to the growth of P. productus, initial growth of R. rubrum exhibited a slight lag with 2 percent COS. The lower cell concentrations of the bacterium in the presence of 1 and 2 percent COS perhaps suggests that COS is toxic to the growing cells and interferes with the growth mechanism of the bacterium. However, no attempt was made to add COS to pre-grown cultures of R. rubrum to determine whether COS toxicity can be reduced.

In contrast to the P. productus and R. rubrum cultures, the mixed culture grew well in the presence of up to 5 percent COS (Table 6.4). There was no difference in the cell densities of the bacterial culture grown in the presence of 50 percent CO and 0-5 percent COS. The bacterial cell density was only 20 percent lower when the cultures were grown with 50 percent CO and 1 percent COS.

From the above discussions, it can be concluded that COS is more toxic than H₂S and bacterial growth is affected by the presence of even 1 percent COS. The mixed culture is found to be more tolerant to COS than H₂S. However, the presence of lower concentrations of COS will probably have little effect on the bacterial growth as examined during this study.

6.2.2.2 Metabolism of CO

The presence of higher concentrations of COS affected CO conversion by P. productus. The conversion of CO ceased completely in the samples with 5 percent COS additions (Figure 6.9). However, there was no effect on CO conversion at a concentration of 1 percent COS, and the rates of CO metabolism were identical to the samples containing no COS. Complete conversion of CO by this bacterium occurred within 12 days in the samples with 0 and 1 percent COS. At a 2 percent COS concentration, CO conversion

Figure 6.7. Bacterial growth of *P. productus* in the presence of 50% CO and at different levels of COS.

Figure 6.8. Bacterial growth of *R. rubrum* in the presence of 50% CO and at different levels of COS.

Figure 6.9. Bacterial growth of CO by *P. productus* when grown in the presence of COS.

was slower but more than 95 percent of the CO disappeared after 21 days of incubation.

TABLE 6.4

Growth of Mixed Cultures Grown With 50 Percent CO and Different Concentrations of COS

Days	Optical Density* at 580 nm, With 50 Percent CO and COS (%)				
	0	1	2	5	10
0	0.366	0.366	0.366	0.366	0.366
1	0.60	0.60	0.60	0.588	0.48
2	0.588	0.57	0.588	0.57	0.492
5	0.588	0.54	0.492	0.474	0.45
7	0.558	0.492	0.48	0.48	0.42

* Values are means of duplicate samples

The addition of COS also affected CO metabolism by *R. rubrum* (Figure 6.10). In contrast to the bacterial growth in the presence of 1 percent COS, *R. rubrum* exhibited no inhibition in transforming CO to CO₂ and H₂ grown at this concentration of COS. Within 12 days, complete conversion of CO occurred in the samples with 0 and 1 percent COS. With a 2 percent COS concentration, the conversion was decreased by about 10 percent in comparison to the control tubes. The metabolism of CO was strongly inhibited by the presence of 5 percent COS and even after 21 days of incubation, less than 20 percent of the CO was metabolized by this bacterium.

The presence of COS had adverse effects on CO metabolism by the mixed cultures (Figure 6.11). CO disappeared slowly in the presence of 1 and 2 percent COS. Complete disappearance of CO was noticed within 10, 14 and 21 days in the samples containing 0, 1 and 2 percent COS, respectively. At 5 and 10 percent COS levels, CO metabolism was inhibited considerably for the first 5-7 days but then the cultures started to metabolize CO. At the end of 23 days, 5.7 and 29.6 percent of the CO were not metabolized by the bacteria grown in the presence of 5 and 10 percent of the CO were not metabolized by the bacteria grown in the presence of 5 and 10 percent COS.

In summary, the toxic effects of COS on the bacterial metabolism of CO were more pronounced than at similar concentrations of H₂S. However, as with H₂S, lower concentration of COS showed no inhibitory effects on the bacterial metabolism of CO. A concentration of about 5 percent and above was found to be inhibitory to the catabolism of CO. Surprisingly, the mixed culture was found to be more tolerant to COS than the other two cultures used in this study.

Figure 6.10. Bacterial conversion of CO by *R. rubrum* when grown in the presence of COS.

Figure 6.11. Bacterial conversion of CO in the presence of COS using a mixed culture.

6.2.3 SO₂

Two pure bacterial cultures, P. productus and R. rubrum were tested for their tolerance and ability to metabolize CO in the presence of 0-10 percent SO₂. The general plan for this study was essentially the same as described for H₂S under Section 6.2.1. Basal salts media (9 ml) in triplicates were inoculated with a log phase culture of P. productus and R. rubrum and were incubated as described previously. Carbon monoxide (50 percent, 2 atm) and SO₂ (0-10 percent) were added to the cultures using gas manifold systems or special syringes fitted with one-way valves. Bacterial growth, CO metabolism and product formation were determined at periodic intervals.

6.2.3.1 Bacterial Growth

The effect of SO₂ on bacterial growth was studied using P. productus in a batch culture experiment. The results of this experiment are reported in Figure 6.12. The initial growth of P. productus in the presence of 1 percent SO₂ was slightly slower, although no distinct lag phase was seen. Bacterial growth became linear after 24 hours of incubation. At a 2 percent SO₂ concentration, bacterial lag was extended to 7 days, and bacterial cell density was more than 60 percent lower than the control tubes even after 18 days of incubation. Complete inhibition of growth occurred when 5 and 10 percent SO₂ were added to the gas phase. The inhibiting effect on the bacterial growth of R. rubrum in the presence of SO₂ was very adverse (Figure 6.13). As reported in this figure, a lag of more than 12 hours occurred during bacterial growth in the presence of 1 percent SO₂. At this level of SO₂, the cell density was at least 30 percent lower than the cultures grown only on 50 percent CO at the end of 10 days. R. rubrum could not grow at all when concentrations of 2-10 percent SO₂ were added to the gas phase.

The data presented in Figures 6.12 and 6.13 suggest that SO₂, even at lower concentrations, exhibit toxicity to the bacterial cultures. Bacterial growth was totally affected in the presence of more than 1 percent SO₂. Sulfur dioxide was thus found to be the most toxic gas among the gases (H₂S, COS and SO₂) used in this study. Bacterial growth of R. rubrum was also found to be affected more than the growth of P. productus in the presence of SO₂.

6.2.3.2 Metabolism of CO

The presence of 1 percent SO₂ in the gas phase adversely affected the CO utilization by P. productus (Figure 6.12). At the end of 7 days, the conversion of CO was at least 26 percent lower than the control samples. CO metabolism by this bacterium in the presence of 2 percent SO₂ occurred only after 10 days; but only 36 percent of the CO was metabolized at the end of 18 days of incubation. At SO₂ concentrations of 5 percent or more, CO metabolism was completely inhibited.

Interestingly, the CO metabolism by R. rubrum in the presence of 1 percent SO₂ was not inhibited at all, despite lower cell densities (Figure 6.13). The presence of 2-10 percent concentrations of SO₂ in the gas phase completely inhibited the metabolism of CO. The bacterium did not

Figure 6.12. Growth and metabolism of CO by *P. productus* in the presence of 1% SO₂.

Figure 6.13. Growth and metabolism of CO by *R. rubrum* in the presence of 1% SO₂.

utilize CO in the presence of 2-10 percent SO₂ even after 21 days of incubation.

Thus, SO₂ is toxic to the bacterial cultures and the metabolism of CO was affected at concentrations of 2-10 percent SO₂. The toxicity of SO₂ on pre-grown cells was not determined; but bacterial tolerance data to 1 percent SO₂ is very encouraging. Since the total sulfur gas (H₂S, COS and SO₂) concentrations in coal gas are unlikely to exceed 2 percent, no problems are anticipated in feeding coal gas containing sulfur to the bacterial cultures.

6.2.4 Metabolic Products

P. productus produced only acetate during CO metabolism according to Equation (1.4) and the presence of sulfur gases (H₂S, COS and SO₂) had no influence on this mechanism. Similarly, R. rubrum metabolized CO to H₂ and CO₂ according to the Equation (1.1). R. rubrum metabolized CO to equal molar amounts of CO₂ and H₂ (Table 6.3), and the presence of the above sulfur gases did not influence this transformation. No other volatile fatty acids or alcohols were produced by this bacterium in the presence of toxic sulfur compounds.

The presence of H₂S and COS affected the mixed cultures in metabolizing CO to CH₄. Without the presence of H₂S or COS, the mixed cultures metabolized CO to CH₄ through the acetate pathway. But, both H₂S and COS had probable adverse effects on the methanogens, since methane formation from acetate did not occur or occurred very slowly. H₂S had a tremendous effect on CH₄ formation which was completely inhibited in the presence of 5 and 10 percent H₂S. Only as much as 3 percent CH₄ accumulated in these cultures in comparison to 15-18 percent CH₄ formed in the presence of 0-2 percent H₂S.

Similarly, methanogenesis was very much retarded in the presence of more than 2 percent COS. Although CO metabolism in the presence of 2 percent COS was complete within 23 days, only 8 percent CH₄ was formed in comparison to 18 percent CH₄ when no COS was present. The corresponding CH₄ values for 5 and 10 percent COS at 23 days were 9 and 2 percent, respectively. Acetate and small amounts of propionate and butyrate accumulated in the medium where methanogenesis was retarded by the presence of toxic sulfur gases.

7.0 PRELIMINARY ECONOMIC EVALUATIONS

Based upon the performance of mixed and pure culture reactions for the biological conversion of coal synthesis gas to methane in laboratory scale studies, an economical analysis of a 5.0×10^{10} Btu/day biological methanation process has been conducted. The analysis includes the fixed capital investment for methanation in 1986 dollars and the operating costs excluding the value of the coal gas raw material. The operating costs are calculated on the basis of dollars per million Btu, excluding the coal gas cost.

For a coal gasification plant using a traditional catalytic methanation process, the total costs for gasification range from \$5-6/million Btu.⁷ One isolated source lists the total costs for catalytically upgrading carbon monoxide to methane at \$6-10/million Btu.¹⁰ A more reasonable estimate of the total cost of methanation is probably about \$1.50/million Btu.³ The economic feasibility of biological methanation processes will thus be judged based upon this value.

7.1 Description of Study Cases

7.1.1 Basic Reactions and Cases Studied

As listed in Table 7.1, four reactions were utilized in this study:

- the water gas shift reaction carried out by R. rubrum or R. gelatinosa;
- acetate production from CO using P. productus;
- methane production from CO₂ and H₂ by a variety of methanogens; and,
- methane production from acetate by a Methanothrix enrichment.

These reactions provided a basis for various design cases for economic analysis.

TABLE 7.1

Basic Reactions in Biological Methanation

Microorganism	Reaction
<u>R. rubrum</u> , <u>R. gelatinosa</u>	$\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2$
<u>P. productus</u> (P.P.)	$4\text{CO} + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + 2\text{CO}_2$
Methanogens (M.S.)	$\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$
Methanothrix	$\text{CH}_3\text{COOH} \rightarrow \text{CO}_2 + \text{CH}_4$

Case 1 involved using a pure culture of P. productus to produce acetate from CO, followed by a second reactor using Methanothrix to convert acetate to methane. Case 2 involved using a co-culture of P. productus and methanogens such as Methanosarcina to produce methane and acetate from CO, CO₂ and H₂, followed by a second reactor utilizing Methanothrix to produce methane from acetate. Case 3 involved using a mixed culture involving all four reactions and one reactor. Because the CO transfer from the gas phase to the liquid phase is expected to be the limiting step of the overall reaction, the first reactor is the major cost factor in Cases 1 and 2. The second reactors in Cases 1 and 2 are expected to be small and no energy input requirement for the reaction is expected due to the faster liquid phase reactor. Therefore, in this analysis, the second reactor is neglected for economic purposes.

7.1.2 Methane Production in the Ideal Cases

Because there are several reactions involved in each case, the methane production for a given CO conversion may be different from case to case. The feed coal gas composition may also play an important role. By assuming that acetate can be completely converted to methane and CO₂, Case 1 yields 0.25 mole of methane produced for every mole of CO converted, independent of the composition of coal gas, since hydrogen is not utilized in the reactions. However, for Cases 2 and 3, 21.75/65 moles of methane are produced per mole of CO converted, based upon a coal gas composition of 65 percent CO and 22 percent H₂. This value changes to 0.5 moles of methane produced per mole of CO converted if the coal gas composition has a H₂:CO ratio of unity. These values are obtained by sample calculation shown in Appendix C.

7.1.3 Economical Cases Studied

In this economical study, all three cases are evaluated in a CSTR, with scale-up based on the same retention time. However, only the data for the two highest agitation rates are included because they can support CO conversions of 90 percent. For the immobilized cell reactor, all the retention times used in the experimental study will be evaluated in the economic evaluation. Because the CO transfer from the gas phase into the liquid phase is the rate limiting step, the benefits of operating at higher mass transfer rates are also studied by increasing the operating pressure to give a higher mass transfer driving force. In the economic evaluation studies using 75 psia and 150 psia, gas pressures are also given for every case, assuming that the CO conversions are the same as at atmosphere pressure.

7.1.4 Approach to Economic Comparisons

Prior to comparing reactors for converting coal gas to methane, key variables for performance and scale-up must be estimated. For the stirred-tank reactor, the power consumption of the agitators in the presence of gas sparging must be estimated. The performance of larger stirred-tank and immobilized cell reactors must then be projected from laboratory data. Section 3-6 discusses power consumption, performance and scale-up in industrial-scale fermentation reactors.

Cost estimations for the various reaction schemes using both stirred-tank and immobilized cell reactors are discussed in Section 7.6. Reactor pressures of 1 atm, 75 psi and 150 psi are considered. Capital costs for reaction vessels are compared, as well as operating costs for utilities, labor, fixed charges, depreciation, and materials costs.

7.2 Estimation of the Stirred-Tank Reactor Power Consumption

Because the stirred-tank reactor power input has not been measured, the power consumption had to be estimated using known correlations. There are correlations between the impeller Reynolds number and the power number published for some commonly used impeller types and geometries. However, none of the known relationships are shown for the magnetically driven impeller used in the stirred-tank reactor experiments. The power consumption for the stirred-tank reactor was estimated by knowing the dimensions and configuration of the stirred-tank reactor, and applying correction factors to known correlations. The mass transfer rate of known systems can be calculated by the estimated power input and can be compared with the mass transfer rate determined by the overall CO mass balance. A more reasonable power consumption for the stirred-tank reactor can then be decided by comparison.

7.2.1 Power Consumption Estimation by Assuming the Configuration of the Stirred-Tank Reactor Impeller

7.2.1.1 The Known Correlation of Impeller Reynolds Number and Power Number

The power number, N_p , as a function of the impeller Reynolds number N_{Re} , for the commonly used flat-blade turbine, paddle, and marine propeller are illustrated in Figure 7.1. The dimensionless Reynolds number, N_{Re} , is defined as:

$$N_{Re} = \frac{n D_i^2 \rho}{\mu}, \quad (7.1)$$

where n = agitation rate,
 D_i = impeller diameter,
 ρ = fluid density, and
 μ = fluid viscosity

The dimensionless power number, N_p , is defined as:

$$N_p = \frac{P_{gc}}{N^3 D_i^5 \rho} \quad (7.2)$$

where P = power input,
 gc = conversion factor, $32.2 \frac{\text{lbmft}}{\text{lbsec}^2}$

and n, D_i , and ρ are defined as above
 Correlations in laminar, transition and turbulent regions are shown for flat blade, paddle, and marine impellers.

Figure 7.1. N_p vs. N_{Re} ; the power characteristics are shown by the power number, N_p , and the modified Reynolds number, N_{Re} , of single impellers on a shaft. The lower part of the figure shows the geometrical ratios of representative types of impellers: turbine, paddle, and marine propeller. The right-hand side of the lower part indicates another impeller configuration on the same shaft.

7.2.1.2 Geometry of the Stirred-Tank Reactor and Physical Properties of Solution

The stirred-tank reactor included an inside diameter of 7.6 cm, with four 0.8 cm wide baffle plates. The culture volume was 325 ml, equivalent to 7.16 cm of liquid height. The impeller diameter was 5.3 cm, with a width of 0.9 cm. The distance between the bottom of the impeller and the fermenter bottom, H , was 1.5 cm. If the impeller is considered to be a flat-blade turbine, the length of one arm of blade, L_i , is estimated as 2.1 cm. The culture solution was assumed to have a density of 1 g/ml and a viscosity of 2 centipoise (approximating the physical properties of water).

7.2.1.3 Correction for a Different Reactor Configuration in the Stirred-Tank Reactor

By specifying the agitation speed and assuming the stirred-tank reactor has the standard configuration shown in Figure 3.1, the power number and the power input for the ungasged system, P^* , can be determined. The difference between the standard configuration and the stirred-tank reactor system configuration can be found by multiplying a correction factor, f_c , as suggested by Aiba, et al.²⁸ defined by the equation,

$$f_c = \left[\frac{G(1) G(2)}{G_S(1) G_S(2)} \right]^{1/n} \quad (7.3)$$

where $G(i)$ is the i th geometry ratio of the Bio-flo configuration,
 $G_S(i)$ is the i th geometry ratio of the standard configuration shown in Figure 3.1; and,
 n is the total number of different geometrical ratios

It has been found that f_c is 0.6778 for a flat-blade turbine, 0.5459 for paddle agitator and 0.5167 for marine propeller assumption.

7.2.1.4 The Correction for Power Input for Gassed Systems

The ratio of the power requirements for a gassed system to the requirements of an ungasged system was found to be a function of the aeration number, N_a , and the type of impeller by Ohyama, et al.²⁹ The aeration number is defined as the apparent velocity of gas through the cross-sectional area of the vessel divided by the tip velocity of impeller. The dimensionless aeration number, N_a , is defined as,

$$N_a = \frac{F}{n D_i^3} \quad (7.4)$$

where F = aeration rate, and
 n and D_i^3 are as defined above

Figure 7.2 shows a plot of P_g/P as a function of the aeration number for various impeller types. As noted, the power for the gassed system is always less than the power requirements for an ungasged system, varying from 20 percent to 100 percent of the ungasged power requirements. In this study, the large retention time for coal gas conversion forces the

- A: Flat-blade turbine ($n_p=8$)
 B: Vaned disk ($n_p=8$)
 C: Vaned disk ($n_p=6$)
 D: Vaned disk ($n_p=16$)
 E: Vaned disk ($n_p=4$)
 F: Paddle
 $D_i/D_1=3$
 $W_b/D_1=0.1$
 $D_i/H_1=3$

Figure 7.2. Power requirements for agitation in a gassed system. The ordinate and abscissa are the degree of power decrease, P_g/P , and the aeration number, N_a . Parameters are the types of impellers, whose representative geometrical ratios in agitated vessels are also shown in the figure.

aeration number to become very small (e.g. $N_a = 2.45 \times 10^{-4}$ at the 200 rpm and a 0.73 hour retention time). At such small aeration numbers, the power required in a gassed system essentially equals the power in an ungassed system.

7.2.1.5 The Result of Power Consumption Estimated by N_{Re} vs N_p

The power consumption per unit volume of culture vs the agitation rate for the Bio-flo reactor assuming flat-blade turbine behavior, paddle agitator behavior and marine propeller behavior are listed in Table 7.2. The power per unit volume, P_g/V , ranges from 0.13 hp/m³ at 200 rpm to 13.6 hp/m³ at 810 rpm for the flat-blade turbine assumption. Similar calculations show a range of 0.04-2.5 hp/m³ for the paddle agitator, and a range of 0.02-0.9 hp/m³ for the marine propeller assumption.

7.2.2 Estimate of the CO Liquid Phase Mass Transfer Rate From Power Input Estimates

The mass transfer of CO from the gas phase to the cells is usually limited by the mass transfer of CO from gas liquid interface to the bulk liquid³⁰. Therefore, the CO transfer is controlled by the mass transfer resistance of the liquid phase, which is made up of the interfacial area per unit volume of liquid, a , and the liquid phase mass transfer coefficient k_L . Therefore, the overall CO transfer rate is $k_L a (C_f - C_L)$, where C_f is the CO concentration at the interface and C_L is CO concentration in the bulk liquid phase.

7.2.2.1 Correlation of Interfacial Area

It has been found by Calderbank³¹ that for low impeller Reynolds numbers, the interfacial area per unit volume of solution, a , is

$$a = a_0 = 1.44 \frac{(P/V)^{0.4} \rho_c^{0.2}}{\sigma^{0.6}} \frac{(V_s)^{1/2}}{V_t} \quad (7.5)$$

where P/V is the power input per unit volume of fluid (erg/sec);
 ρ_c is the density of fluid in C.G.S. units;
 σ is the surface tension in C.G.S. units;
 V_s is the superficial feed gas velocity; and,
 V_t is the terminal velocity of bubbles freely rising.

For high impeller Reynolds numbers $Re^{0.7} \frac{(ND)^{0.3}}{V_s} > 2000$

the following relationship holds:

$$\log \frac{2.3a}{a_0} = 1.95 \times 10^{-5} Re^{0.7} \frac{(ND)^{0.3}}{V_s} \quad (7.6)$$

In this equation, a_0 can be determined by Equation (7.5).

The surface tension of air and water at 37°C is about 70.44 dynes/cm. It has been found that the terminal velocity of freely rising bubbles is nearly constant over the bubble size range used in the fermentation

Table 7.2 Stirred-Tank Reactor Power Consumption Estimation from N_{Re} vs N_p

N	RPM	200	300	400	500	600	700	800
$N_{Re} = \frac{ND_i^2 \rho}{\mu}$		468.1	702.3	936.4	1170.4	1404.5	1638.6	1872.6
Assume Impeller is Flat-Blade Turbine								
$N_p^* = \frac{\rho N^3 D_i^5}{g_c}$		3.0	3.3	3.7	4.05	4.3	4.7	4.9
HP		6.231x10 ⁻⁵	2.313x10 ⁻⁵	6.151x10 ⁻⁴	1.315x10 ⁻³	2.412x10 ⁻³	4.187x10 ⁻³	6.515x10 ⁻³
$P_g = P = P^* f_c = 0.6778 P^*$	HP	4.223x10 ⁻⁵	1.568x10 ⁻⁴	4.169x10 ⁻⁴	8.913x10 ⁻⁴	1.635x10 ⁻³	2.838x10 ⁻³	4.496x10 ⁻³
P_g/V	HP/m ³	0.130	0.483	1.283	2.742	5.031	8.732	13.588
Assume Impeller is Paddle								
$N_p^* = \frac{\rho N^3 D_i^5}{g_c}$		1.1	1.1	1.1	1.1	1.1	1.1	1.1
HP		2.285x10 ⁻⁵	7.714x10 ⁻⁵	1.829x10 ⁻⁴	3.570x10 ⁻⁴	6.172x10 ⁻⁴	9.800x10 ⁻⁴	1.462x10 ⁻³
$P_g = P = P^* f_c = 0.549 P^*$	HP	1.247x10 ⁻⁵	4.211x10 ⁻⁵	9.985x10 ⁻⁵	1.949x10 ⁻⁴	3.369x10 ⁻⁴	5.350x10 ⁻⁴	7.981x10 ⁻⁴
P_g/V	HP/m ³							
Assume Impeller is Marine Propeller								
$N_p^* = \frac{\rho N^3 D_i^5}{g_c}$		0.54	0.5	0.48	0.46	0.44	0.42	0.41
HP		1.122x10 ⁻⁵	3.506x10 ⁻⁵	8.691x10 ⁻⁵	1.494x10 ⁻⁴	2.467x10 ⁻⁴	3.741x10 ⁻⁴	5.452x10 ⁻⁴
$P_g = P = P^* f_c = 0.5767 P^*$	HP	5.797x10 ⁻⁶	1.812x10 ⁻⁵	4.981x10 ⁻⁵	7.719x10 ⁻⁵	1.275x10 ⁻⁴	1.933x10 ⁻⁴	2.817x10 ⁻⁴
P_g/V	HP/m ³	0.018	0.856	0.138	0.238	0.392	0.595	0.867

industry.³¹ A value of the terminal velocity of 0.87 ft/sec has been reported by Harberman, et al.³² In this study, the largest impeller Reynolds number is only 1872 at 800 rpm. Thus, there is no need to correct a_0 using Equation (7.6).

Table 7.3 lists values of A for all of the stirred-tank studies with the assumption of impeller types as described in Figure 7.1. The interfacial areas range from 0.01-0.06 cm²/cm³, depending upon agitation rate, type of agitator and type of fermentation.

7.2.2.2 Estimation of CO Mass Transfer Coefficients

It also has been found by Calderbank and Moo-Young³³ that agitation intensity, bubble size and bubble free rising velocity have no effect on the liquid phase mass transfer coefficient, k_L . For bubble sizes having less than a 2.5 mm diameter, k_L can be calculated by

$$k_L = 0.31 (S_c)^{-2/3} \frac{\mu_{CG}}{\rho_C}^{1/3} \quad (7.7)$$

where S_c is the Schmidt number = $\mu/\rho_C D$
 D is the solute diffusivity

The CO diffusivity in water has been estimated by the method of Wilke and Chang as 1.006×10^{-6} cm²/sec at 37°C.³⁴ With the assumption that the fluid density is 1 g/cm³ and the viscosity is 2 centipoise, the CO liquid phase mass transfer coefficient, k_L , is estimated as 0.00528 cm/sec.

7.2.3 Estimation of the CO Liquid Phase Mass Transfer Rate from the Overall Mass Balance

If P_i is the partial pressure of CO in the entering gas stream and P_o is the partial pressure of CO in the existing gas stream, then the CO transfer rate is described as

$$\frac{dC}{dt} = \frac{F}{V} \frac{P_i - P_o}{RT} = \frac{V_s}{h} \frac{P_i - P_o}{RT} \quad (7.8)$$

Where F is the gas volume flow rate, V is the volume of solution, V_s is the gas superficial velocity, and h is the height of solution. From the transfer equation, the CO transfer rate can be described as:

$$\frac{dC}{dt} = (k_a)G \rightarrow L (C_f^0 - C_L) = \frac{V_s}{h} \frac{P_i - P_o}{RT} \quad (7.9)$$

where C_L is the CO concentration in the bulk liquid and usually can be assumed as zero. C_f is the CO equilibrium concentration at the bubble surface and can be estimated by Henry's law as,

$$C_f = \frac{\bar{P}}{H} \quad (7.10)$$

Where $\bar{P}$ is the average partial pressure of CO in the bubble and H is the Henry's law constant. Because the CO concentration inside the bubble is going to drop dramatically during bubble ascension, a log mean partial pressure of CO can be used to estimate C_f .

Table 7.3 Interfacial Area Per Unit Volume of Solution as cm^3/cm^3
for the Stirred-Tank Reactor Studies

RPM	Turbine Assumption	Paddle Assumption	Marine Propeller Assumption
Case 1. P.P. 1.45 hr. retention time $V_S = 4.506 \times 10^{-5}$ ft/sec			
400	0.0319	0.0180	0.0131
500	0.0432	0.0235	0.0163
600	0.0551	0.0293	0.0199
700	0.0716	0.0353	0.0235
Case 2A. P.P. + M.S. 1.5 hr. retention time $V_S = 4.240 \times 10^{-5}$ ft/sec			
400	0.0310	0.0175	0.0127
500	0.0420	0.0228	0.0158
600	0.0535	0.0284	0.0193
700	0.0667	0.0342	0.0228
800	0.0796	0.0401	0.0265
Case 2B. P.P. + M.S. 0.73 hr retention time $V_S = 8.946 \times 10^{-5}$ ft/sec			
400	0.0450	0.0254	0.0184
500	0.0609	0.0332	0.0229
600	0.0777	0.0413	0.0280
700	0.1009	0.0497	0.0331
800	0.1156	0.0583	0.0418
Case 3. Mixed Culture 1.94 hr. retention time $V_S = 3.363 \times 10^{-5}$ ft/sec			
300	0.0186	0.0110	0.0079
400	0.0276	0.0156	0.0113
500	0.0374	0.0203	0.0140
600	0.0476	0.0253	0.0172

$$C_f = \frac{P_i - P_o}{H \ln \frac{P_i}{P_o}} \quad (7.11)$$

Therefore,

$$(k_L a)_{G+L} = \frac{\ln(P_i/P_o) V_s H}{h RT} \quad (7.12)$$

For CO, H is found to be 6.73×10^{-4} atm/mole fraction, or 0.0374 atm/mole/L using water as the solvent. Therefore, at 37°C,

$$(k_L a)_{G+L} = 1.470 \times 10^{-3} \frac{\ln \frac{P_i}{P_o} V_s}{h} . \quad \text{The } k_L a \text{ estimated by this method}$$

and estimated by P/V for the propeller configuration assumption are listed in Table 7.4. The marine propellers assumption gives the smallest P/V and $k_L a$ for the three types of impellers shown in Figure 7.1.

7.2.4 Determination of Stirred-Tank Reactor Power Input

As was shown in Table 7.4, the $k_L a$ estimated by an overall CO balance is about 144 to 196 times less than the value predicted by assuming the CSTR impeller has a marine impeller configuration. This indicates that the agitation efficiency of the stirred-tank reactor is much worse than the marine impeller when considering the agitation rates. However, in reality, it couldn't be 144 to 196 times less in $k_L a$, because this results in 748832 to 537824 times less than the calculation in Equation (7.5). The correction factor for the different reactor configurations used in the power consumption estimation, Equation (7.3), could be overestimated, because nearly all of the geometry ratios of the stirred-tank reactor are smaller than the standard configuration, so that treating the effect of every ratio the same may not be proper. Furthermore, the CSTR impeller is set at the bottom of the reactor and no correction for this orientation has been given.

Neglecting the bulk liquid CO concentration may result in an underestimation of $k_L a$ by an overall mass balance. However, even if C_L is one-half of C_f , the $k_L a$ only doubles. When C_L is greater than one-half of C_f , it is difficult to say that the liquid phase mass transfer is the limiting step. Even if C_L is 0.9 C_f , $k_L a$ may increase 10 times, but in that condition, liquid phase mass transfer no longer affects the reaction. Thus, the agitation rate loses its influence on the reaction rate which is not true in this case. Therefore, the major difference must come from overestimating the power input. When the CSTR power input is estimated to be 1/10 of the value predicted by a marine propeller configuration, this gives a reduction in $k_L a$ estimated by P/V of 2.5 percent.

Table 7.4 Comparison of $K_L a$ Estimated from the Overall Mass Balance and from the P/V Calculation

RPM	CO Conversion %	P_i/P_0	$(k_L a)$ Eq. C (sec ⁻¹)	$k_L a$ (marine propeller) (sec ⁻¹)	$(k_L a)$ (marine propeller) ($k_L a$) G+L
Case 1. P.P. retention time 1.45 hr. $V_S = 1.37 \times 10^{-3}$ cm/sec					
400	72.94	3.695	3.682×10^{-7}	6.916×10^{-5}	187.8
500	79.40	4.854	4.450×10^{-7}	8.606×10^{-5}	193.4
600	91.10	7.303	5.601×10^{-7}	1.051×10^{-4}	187.6
700	93.0	14.286	7.057×10^{-7}	1.241×10^{-4}	175.9
Case 2A. P.P. + M.S. retention time 1.56 hr. $V_S = 1.276 \times 10^{-3}$ cm/sec average 185.6					
400	69.8	3.311	3.137×10^{-7}	6.706×10^{-5}	213.8
500	87.1	7.752	5.365×10^{-7}	8.342×10^{-5}	155.5
600	88.0	8.333	5.554×10^{-7}	1.019×10^{-4}	183.5
700	88.0	8.333	5.554×10^{-7}	1.204×10^{-4}	216.8
800	90.2	10.204	6.085×10^{-7}	1.399×10^{-4}	229.9
Case 2B. P.P. + M.S. retention time 0.73 hr. $V_S = 2.726 \times 10^{-3}$ cm/sec average 196.4					
400	54.7	2.208	4.433×10^{-7}	9.715×10^{-5}	219.2
500	60.2	2.513	5.157×10^{-7}	1.209×10^{-4}	234.4
600	80.6	5.155	9.178×10^{-7}	1.478×10^{-4}	161.0
700	82.0	5.556	9.597×10^{-7}	1.748×10^{-4}	182.1
800	86.9	7.634	1.179×10^{-6}	2.207×10^{-4}	187.2
Case 3. Mixed Culture retention time 1.9 hr. $V_S = 1.026 \times 10^{-3}$ cm/sec average 176.8					
300	40.48	1.680	1.093×10^{-7}	4.171×10^{-5}	381.6
400	85.16	6.739	4.019×10^{-7}	5.966×10^{-5}	148.4
500	89.80	9.804	4.809×10^{-7}	7.392×10^{-5}	153.7
600	96.20	26.882	6.933×10^{-7}	9.082×10^{-5}	131.0
					average 144.4

7.3 Performance of the Stirred-Tank Reactor

7.3.1 The Performance of the Stirred-Tank Reactor at Atmospheric Pressure

The performance of stirred-tank reactor operated at atmospheric pressure is tabulated in Table 7.5. As noted, the CO conversion, methane produced, methane in the outlet stream of the reaction and power per unit volume are given for each of the cases presented in Chapter 2. Only the performance for the two highest agitation rates are considered, in order to yield CO conversions near 90 percent. Because there is 2 percent CH₄ in the coal gas, the methane in the outlet stream should be the sum of methane produced and methane in the feed.

7.3.2 Estimation of the Performance of the Stirred-Tank Reactor at Higher Pressures

It has been shown in the laboratory that the biological conversion of methane can occur at pressures as high as 100 psi. According to the mass transfer equation, Equation (7.9), the increase in pressure will yield the same ratio of mass transfer rate without the need of increased power input because the increased pressure produces the same ratio of increase of driving force through Henry's law. Therefore, the performance of the stirred-tank reactor at 75 psi and 150 psi pressure can be estimated at the same CO conversion and power input per unit volume. The methane production and methane concentration in the outlet stream can be increased by the ratio of pressure to atmosphere pressure. However, this conclusion is true only if the liquid phase mass transfer is still the limiting step.

7.4 Performance and Mass Transfer in the Immobilized Cell Reactor

7.4.1 Performance of the Immobilized Cell Reactor

The only case studied in the laboratory using the immobilized cell reactor is Case 1, the conversion of CO to methane via P. productus at atmospheric pressure. The volume of the column reactor employed in the study was 450 ml, with a 5 cm inside diameter. The solution volume is 250 ml, due to the presence of packing inside the reactor. The performance of the immobilized cell reactor at 1 atm pressure is listed in Table 7.6. Retention time in the table is based upon solution volume as opposed to empty column volume.

As noted, the CO conversion in this study is much smaller than in the stirred-tank reactor study, due to lower mass transfer rate without agitation. However, in this type of reactor, the mass transfer rate could be increased over the scale-up ratio when the same gas retention time and same geometric ratio are used in the scale-up. Therefore, the effect of scale-up on the mass transfer rate must be studied to give a proper CO conversion.

7.4.2 Mass Transfer in an Immobilized Cell Reactor

There are no mass transfer rate correlations for packed bed immobilized cell reactors. However, for an unpacked column with bubble aeration, there is a correlation proposed by Aiba²⁸ as:

Table 7.5 Performance of the Stirred-Tank Reactor at One Atmosphere Pressure

Case 1. P.P. retention time = 1.45 hr = 1.468×10^{-4} gmole feed gas/min = 9.542×10^{-5} gmole CO/min
 2.936×10^{-6} gmole CH₄/min

RPM	CO conversion	CH ₄ produced	CH ₄ in outlet	P/V (Hp/m ³)
600	91.1%	2.173×10^{-5} gmole/min	2.467×10^{-5} gmole/min	0.0392
700	93.0%	2.219×10^{-5} gmole/min	2.513×10^{-5} gmole/min	0.0595

Case 2A. P.P. + M.S. retention time = 1.56 hr = 1.365×10^{-4} gmole feed gas/min = 8.873×10^{-5} gmole CO/min
 2.73×10^{-6} gmole CH₄/min

700	88.0%	2.613×10^{-5} gmole/min	2.886×10^{-5} gmole/min	0.0595
800	90.2%	2.678×10^{-5} gmole/min	2.951×10^{-5} gmole/min	0.0867

Case 2B P.P. + M.S. retention time = 0.73 hr = 2.917×10^{-4} gmole/feed gas/min = 1.896×10^{-4} gmole CO/min
 5.834×10^{-6} gmole/CH₄/min

700	82.0%	5.202×10^{-5} gmole/min	5.785×10^{-5} gmole/min	0.0595
800	86.9%	5.513×10^{-5} gmole/min	6.096×10^{-6} gmole/min	0.0867

Case 3. mixed culture retention time = 1.94 hr = 1.098×10^{-4} gmole feed gas/min = 7.137×10^{-5} gmole CO/min
 2.196×10^{-6} gmole CH₄/min

500	89.80%	2.144×10^{-5} gmole/min	2.364×10^{-5} gmole/min	0.0238 Hp/m ³
600	96.28%	2.299×10^{-5} gmole/min	2.519×10^{-5} gmole/min	0.0392 Hp/m ³

Table 7.6 The Performance of the Immobilized Cell Reactor at 1 atm Pressure

Retention Time hr	Feed Gas Flow Rate gmole/min	CO Feed Flow Rate gmol/min	CH ₄ Feed Flow Rate gmole/min	CO Conversion %	CH ₄ Produced gmole/min	CH ₄ Flow Rate in Product stream gmole/min
0.5	3.276×10^{-4}	2.129×10^{-4}	4.258×10^{-6}	9.9	5.269×10^{-6}	9.527×10^{-6}
0.8333	1.966×10^{-4}	1.278×10^{-4}	3.932×10^{-6}	22.2	7.093×10^{-6}	1.103×10^{-5}
1.25	1.310×10^{-4}	8.515×10^{-5}	2.620×10^{-6}	36.2	7.706×10^{-6}	1.033×10^{-5}
2.5	6.552×10^{-5}	4.259×10^{-5}	1.310×10^{-6}	51.5	5.483×10^{-6}	6.793×10^{-6}
5.0	3.276×10^{-5}	2.129×10^{-5}	6.552×10^{-7}	66.6	3.545×10^{-6}	4.200×10^{-6}
6.25	2.621×10^{-5}	1.704×10^{-5}	5.242×10^{-7}	76.6	3.263×10^{-6}	3.787×10^{-6}

$$k_L a \propto \frac{Fh}{d_B^{3/2} V_B^{1/2} V} \quad (7.13)$$

Where F = gas flow rate;
 h = liquid depth;
 d_B = bubble diameter; and
 V_B = ascending terminal velocity of bubbles

It is reasonable to use this correlation for the immobilized cell reactor with a safety factor to correct for the presence of packing on mass transfer. Because the bubble diameter and ascending terminal velocity of the bubbles are usually constant, $k_L a$ can be considered as a function of Fh/V . Scale-up based on a constant retention time means a constant F/V . However, the liquid height h , is increased by the scale-up ratio (V_2/V_1) to the one-third power. Therefore $k_L a$ increases with the scale-up ratio as $(k_L a)_2 = (V_2/V_1)^{1/3} (k_L a)_1$. This conclusion has been confirmed by the studies of Moo-Young and Blanch.³⁵ The $k_L a$ for a tower fermenter is correlated as

$$k_L a = \alpha V_S^B \quad (7.14)$$

Where V_S is the superficial gas velocity expressed in terms of meters per second. The values of B are in the region of unity (0.89 ~ 1.0), whereas the values of α depend upon the nature of additives. If scale-up is based on the same geometrical ratio and the same gas retention time, V_S again is increased with the one-third power of the scale-up ratio, $(V_2/V_1)^{1/3}$. Thus, $k_L a$ is increased with the scale-up ratio of $(V_2/V_1)^{0.89/3} \sim 1/3$, and the CO conversion is also increased. However, the increase of CO conversion could result a lower average driving force, $C_f - C_L = C_f$, since a log average is used to determine the equilibrium CO concentration in the bubble as described in Equation (7.11).

7.4.3 Pseudo-Immobilized Cell Reactor

By assuming that the CO conversion is proportional to $(k_L a) \cdot (C_f - C_L)$ or $k_L a \cdot C_f$, the value of $k_L a C_f$ for a specified CO conversion can be determined by an overall CO balance on the immobilized cell reactor as described by Equation (7.12). After adjustment of the C_f value for the specified CO conversion by Equation (7.11), the $k_L a$ required to reach the specified CO conversion can be determined, and thus the scale-up ratio. The pseudo reactor size to reach a CO conversion of 99 percent is listed in Table 7.7. In this table, the reactor size of the unpacked column is determined first followed by packed column Size A conservative correction factor of 3 times the unpacked column size is assumed for estimating the size of the packed column. For a 99 percent CO conversion, $\ln P_i/P_0$ and C_{f2} are 4.605 and 3.736 mole/L, respectively.

The performance of a pseudo-immobilized cell reactor is listed in Table 5.3. The conversion in the system is 99 percent based upon the information of Table 7.8. The cost estimation will be based on this performance instead of that of the 450 ml laboratory immobilized cell reactor.

Table 7.7 Pseudo Immobilized Cell Reactor Size Estimation

450 ml Immobilized Cell Reactor							Scaled-up Immobilized Cell Reactor						
V_2 Retention Time, hr	V_2 CO Conversion	P_i/P_0	C_{F1} mole/L	V_{s1} cm/sec	$(k_L a)_1$ sec ⁻¹	$C_{F1}(k_L a)_1$ mole/sec L	$C_{F2}(k_1 a)_2$ mole/sec L	$(k_L a)_2$ Sec ⁻¹	$\frac{(k_L a)_2}{(k_L a)_1}$	V_2/V_1	V_2 Uncorrected m ³	V_2 Corrected m ³	
0.5	9.9	1.110	16.487	7.074×10^{-3}	4.718×10^{-8}	7.779×10^{-7}	7.779×10^{-6}	2.082×10^{-6}	44.13	85941	38.67	110.01	
0.8333	22.2	1.285	15.386	4.244×10^{-3}	6.801×10^{-8}	1.046×10^{-6}	4.655×10^{-6}	1.249×10^{-6}	18.36	6189	2.795	8.355	
1.25	36.2	1.567	14.007	2.829×10^{-3}	8.121×10^{-8}	1.130×10^{-6}	3.112×10^{-6}	8.330×10^{-7}	10.26	1080	0.486	1.458	
2.5	51.5	2.062	12.368	1.415×10^{-3}	6.544×10^{-8}	8.094×10^{-7}	1.556×10^{-6}	4.165×10^{-7}	6.36	257.3	1.161	0.348	
5.0	66.6	2.944	10.555	7.074×10^{-4}	4.958×10^{-8}	5.233×10^{-7}	7.779×10^{-7}	2.082×10^{-7}	4.20	74.1	0.033	0.099	
6.25	76.6	4.274	9.165	5.659×10^{-4}	5.253×10^{-8}	4.814×10^{-7}	6.222×10^{-7}	1.665×10^{-7}	3.17	31.9	0.014	0.042	

Table 7.8 Performance of Pseudo Immobilized Cell Reactor at Atmospheric Pressure

Retention Time hr	Reactor Size m ³	Solution Volume m ³	Feed Gas Flow Rate gmole/min	CO Feed Flow Rate gmole/min	CH ₄ Feed Flow Rate gmole/min	CH ₄ Produced gmole/min	CH ₄ Flow Rate in Product Stream gmole/min
0.5	110.01	61.12	80.09	52.06	1.602	12.855	14.49
0.8333	8.355	4.542	3.650	2.373	0.073	0.5873	0.6603
1.25	1.458	0.31	0.4246	0.2760	0.00849	0.06831	0.0768
2.5	0.348	0.1933	0.05066	0.03293	1.013x10 ⁻³	8.150x10 ⁻³	9.163x10 ⁻³
5.0	0.099	0.055	7.207x10 ⁻³	4.685x10 ⁻³	1.441x10 ⁻⁴	1.160x10 ⁻³	1.304x10 ⁻³
6.25	0.042	0.0233	2.442x10 ⁻³	1.587x10 ⁻³	4.884x10 ⁻⁵	3.928x10 ⁻⁴	4.416x10 ⁻⁴

7.5 Scale-Up

7.5.1 Scale-up Basis

The scale-up basis for economic analysis is a biological coal gas conversion plant producing 5×10^{10} Btu/day. Methane is assumed to be the only heating source in the product. Because the heat of combustion of methane is 212,798 kcal/g-mole or 382,036 Btu/lb mole, the required production rate of methane is 90.65 lbmole/min or 41200 g-mole/min to meet the design requirement of 5.0×10^{10} Btu/day.

7.5.2 Scale-up Principle

The scale-up is based on the same geometrical ratio and same performance (same retention time and CO conversion of the stirred-tank reactor and immobilized cell reactor). It should be noted that in the fermentation industry, the scale-up is usually based on some superficial gas velocity instead of the same retention time.³⁶ This scale-up basis results from excess oxygen in these fermentations and very small retention times to maintain a high oxygen transfer driving force in bench-scale studies. However, the total conversion of CO is the major purpose of this study. Therefore, the scale-up is based upon using the same retention time as in the laboratory studies.

7.5.3 Scale-up Criteria for the CSTR

The same values for P/V , $k_L a$, and the mean liquid velocity at a particular point in the vessel can all be used as the criteria of scale-up in a CSTR.³⁶ However, the most frequently used parameter is the power input per unit volume of solution, $(P/V)^{30}$, because the physical properties are the major factors for the gas-liquid interfacial area. A constant P/V results in the same $k_L a$ for the scale-up based on the same superficial gas velocity. However, by carefully examining the gas-liquid interface area correlation, Equation (7.5), the scale-up based on constant retention time and constant P/V leads to an increase in $k_L a$. As mentioned by Calderbank and Moo-Young³³, k_L and V_t are constant for all possible criteria of scale-up, but the superficial gas velocity, V_s , will be increased by the scale ratio to the $1/3$ power, $(V_2/V_1)^{1/3}$. This means that the scale-up based on the same P/V will increase $k_L a$ with the scale-up ratio by $(V_2/V_1)^{1/6}$ times. From the view of mass transfer, the same $k_L a$ is a more proper scale-up criteria. Using this criteria $(P/V)_1^{0.4}(V_s)_1^{0.5}$ is equal to $(P/V)_2^{0.4}(V_s)_2^{1/2}$ and $(V_s)_2$ equals $(V_2/V_1)^{1/3}V_{s1}$. This results in the reduction of the power input per unit volume by $(P/V)_2 = \frac{(P/V)_1}{(V_2/V_1)^{2.5/6}}$.

This result can be realized from the standpoint of a much longer bubble-liquid contact time since the bubble ascent velocities are the same. However, using the conservative approach, a constant P/V is used in these scale-up studies.

7.5.4 Scale-up Results of the CSTR

The results of the scale-up of the stirred-tank reactor at atmospheric pressure are listed in Table 7.9. Using the previous assumptions for operation at 75 and 150 psi, these scale-up results are listed

Table 7.9 Scale-Up Results of the CSTR at 1 ATM

Agitation Rate			Scale-up Ratio	Total Solution	Total Power	Total Feed Rate	
RPM	P/V	$\frac{\text{HP}}{\text{M}^3}$	$\frac{\text{O}_2}{(\text{gmole/min})}$	TV_2/V_1	Vol. $\text{TV}_2(\text{m}^3)$	$\text{TP}_2(\text{HP})$	$\text{TF}_2 (\text{gmole/min})$
Case 1. P.P. R.T. = 1.45 hr $F_1 = 1.468 \times 10^{-4}$ gmole/min							
600	0.0392	2.467×10^{-5}	1.670×10^9	5.428×10^5	2.128×10^4	2.452×10^5	
700	0.0595	2.513×10^{-5}	1.639×10^9	5.327×10^5	3.170×10^4	2.406×10^5	
Case 2A. P.P. + M.S. R.T. = 1.56 hr $F_1 = 1.365 \times 10^{-4}$ gmole/min							
700	0.0595	2.886×10^{-5}	1.428×10^9	4.641×10^5	2.761×10^4	1.949×10^5	
800	0.0867	2.951×10^{-5}	1.396×10^9	4.537×10^5	3.934×10^4	1.906×10^5	
Case 2B. P.P. + M.S. R.T. = 0.73 hr $F_1 = 2.917 \times 10^{-4}$ gmole/min							
700	0.0595	5.785×10^{-5}	7.122×10^8	2.315×10^5	1.377×10^4	2.077×10^5	
800	0.0867	6.096×10^{-5}	6.759×10^8	2.197×10^5	1.905×10^4	1.972×10^5	
Case 3C. Mixed Culture R.T. = 1.94 hr $F_1 = 1.098 \times 10^{-4}$ gmole/min							
500	0.0238	2.364×10^{-5}	1.743×10^9	5.665×10^5	1.348×10^4	1.914×10^5	
600	0.0392	2.519×10^{-5}	1.636×10^9	5.317×10^5	2.084×10^4	1.796×10^5	

in Table 7.10 and 7.11, respectively. In all three tables, 4 cases are presented using P. productus, P. productus plus a methane bacteria at two retention times, and the mixed culture. Total power requirements and total feed rates are presented for each case.

7.5.5 Scale-up Results of the Immobilized Cell Reactor

As described in Table 7.8, the immobilized cell reactor has been scaled-up to the mass transfer optimized size for a specified retention time. For a specified retention time, a reactor volume larger than the optimized size cannot increase the CO conversion and excess volume is wasted. However, the retention time can be reduced in this situation to fully utilize its volume and thus reduce the reactor size. Unfortunately, the lowest retention time in this study is only 0.5 hour. There is no information available leading to any retention times lower than 0.15 hour. In this study, only 0.5 and 0.8333 hour retention times with the mass transfer optimized size and a fixed reactor volume of 100 M³ are studied because too many reactors are not economical. The scale-up of a 1000 M³ reactor with the same geometry ratio results in a column height of 30 M which is acceptable. The results of scale-up at 1 atm, 75 psia, and 150 psia are listed in Tables 7.12, 7.13, and 7.14.

7.6 Cost Estimation

7.6.1 Basis of Cost Estimation

Because methanation costs are usually not separated from other gasification costs, operating cost estimations in this study are shown as the cost per million Btu required during methanation (no gas purification). The material costs are limited to the media costs only. The costs for the coal gas feed are excluded.

In the cost estimation, the following general rules are used. The plant is assumed to be operating 365 days per year. The capital costs are reflected as operating costs by assuming a five year return. The capital cost estimation is based on the Chilton method,³⁷ with the applicable factors listed in Table 7.15. As noted in the table, the fixed capital costs are estimated at 3.08 times the delivered equipment costs. Equipment purchased costs are based on the updated cost factors published in 1985³⁸. The inflation rate for 1985 and 1986 are assumed to be 3 percent each year. The delivery charge is assumed to be 5 percent of the purchased cost. Stainless steel is used as the material of construction for the reactors from pressures of atmosphere to 150 psia. Because the largest available agitator is 300 HP, 300 HP per reactor is used as the upper limit for the CSTR. In the operating cost estimation, labor and supervision, maintenance, taxes and insurance, and depreciation are assumed to be 5 percent, 5 percent, 2 percent and 8 percent of the total capital costs, respectively.

Table 7.10 Scale-Up Results of the CSTR at 75 psi

Agitation Rate		Scale-up Ratio		Total Solution	Total Power	Total Feed Rate
RPM	P/V $\frac{\text{HP}}{\text{M}^3}$	CH_4 (gmole/min)	TV_2/V_1	Vol. $\text{TV}_2(\text{m}^3)$	$\text{TP}_2(\text{HP})$	TF_2 (gmole/min)
Case 1 P.P. R.T. = 1.45 hr $F_1 = 7.492 \times 10^{-4}$ gmole/min						
600	0.0392	1.259×10^{-4}	3.344×10^8	1.087×10^5	4.261×10^3	2.505×10^5
700	0.0595	1.282×10^{-4}	3.214×10^8	1.045×10^5	6.218×10^3	2.408×10^5
Case 2A. P.P. + M.S. R.T. = 1.56 hr $F_1 = 6.964 \times 10^{-4}$ gmole/min						
700	0.0595	1.472×10^{-4}	2.799×10^8	9.097×10^4	5.413×10^3	1.949×10^5
800	0.0867	1.505×10^{-4}	2.738×10^8	8.899×10^4	7.715×10^3	1.907×10^5
Case 2B. P.P. + M.S. R.T. = 0.73 hr $F_1 = 1.488 \times 10^{-3}$ gmole/min						
700	0.0595	2.952×10^{-4}	1.396×10^8	4.537×10^4	2.700×10^3	2.077×10^5
800	0.0867	3.110×10^{-4}	1.325×10^8	4.306×10^4	3.733×10^3	1.972×10^5
Case 3. Mixed Culture R. T. = 1.94 hr $F_1 = 5.60 \times 10^{-4}$ gmole/min						
500	0.0238	1.206×10^{-4}	3.416×10^8	1.110×10^5	2.642×10^3	1.913×10^5
600	0.0392	1.285×10^{-4}	3.206×10^8	1.042×10^5	4.085×10^3	1.795×10^5

Table 7.11 Scale-Up Results of the CSTR at 150 PSI

Agitation Rate		Scale-up Ratio		Total Solution	Total Power	Total Feed Rate
RPM	P/V $\frac{HP}{M^3}$	Q_4 (gmole/min)	TV_2/V_1	Vol. $TV_2(m^3)$	$TP_2(HP)$	TF_2 (gmole/min)
Case 1. P.P. R.T. = 1.45 hr $F_1 = 1.468 \times 10^{-3}$ gmole/min						
600	0.0392	2.518×10^{-4}	1.538×10^8	5.317×10^4	2.084×10^3	2.451×10^5
700	0.0595	2.564×10^{-4}	1.507×10^8	5.223×10^4	3.108×10^3	2.407×10^5
Case 2A. P.P. + M.S. R.T. = 1.56 hr $F_1 = 1.393 \times 10^{-3}$ gmole/min						
700	0.0595	2.954×10^{-4}	1.399×10^8	4.457×10^4	2.652×10^3	1.949×10^5
800	0.0867	3.012×10^{-4}	1.368×10^8	4.446×10^4	3.855×10^3	1.906×10^5
Case 2B. P.P. + M.S. R.T. = 0.73 hr $F_1 = 2.976 \times 10^{-3}$ gmole/min						
700	0.0595	5.901×10^{-4}	6.982×10^7	2.269×10^4	1.350×10^3	2.078×10^5
800	0.0867	6.218×10^{-4}	6.626×10^7	2.154×10^4	1.868×10^3	1.972×10^5
Case 3C. Mixed Culture R.T. = 1.34 hr $F_1 = 1.120 \times 10^{-3}$ gmole/min						
500	0.0238	2.411×10^{-4}	1.709×10^8	5.554×10^4	1.322×10^3	1.914×10^5
600	0.0392	2.569×10^{-4}	1.604×10^8	5.213×10^4	2.043×10^3	1.796×10^5

Table 7.12 Scale-Up Results of the Immobilized Cell reactor at 1 atm.

Retention Time hr.	Optimized Reactor Size			1000m ³ Volume Reactor			
	Reactor Size m ³	CH ₄ In Outlet gmole/min	Scale-Up Ratio TV _{R2} /V ₁	No. of Reactors	CH ₄ In Outlet gmole/min	Scale-Up Ratio TV ₂ /V ₁	No. of Reactors
0.5	110.01	14.49	2843.3	2844	131.67	312.9	313
0.8333	8.355	0.660	62424.2	62425	79.003	521.5	522

Table 7.13 Scale-Up Results of the Immobilized Cell reactor at 75 psi

Retention Time hr.	Optimized Reactor Size			1000m ³ Volume Reactor			
	Reactor Size m ³	CH ₄ In Outlet gmole/min	Scale-Up Ratio TV _{R2} /V ₁	No. of Reactor	CH ₄ In Outlet gmole/min	Scale-Up Ratio TV ₂ /V ₁	No. of Reactor
0.5	110.01	74.577	552.4	553	671.77	61.33	62
0.8333	8.355	3.367	12236.4	12237	403.07	102.22	103

Table 7.14 Scale-Up Results of the Immobilized Cell reactor at 150 psi

Retention Time hr.	Optimized Reactor Size			1000m ³ Volume Reactor			
	Reactor Size m ³	CH ₄ In Outlet gmole/min	Scale-Up Ratio TV _{R2} /V ₁	No. of Reactor	CH ₄ In Outlet gmole/min	Scale-Up Ratio TV ₂ /V ₁	No. of Reactor
0.5	110.01	149.2	276.1	277	1343.6	30.6	31
0.8333	8.355	6.735	6117.1	6118	806.2	51.1	52

TABLE 7.15
Factors Used in the Chilton Method

Item	Factor	of Item	Factor of Item #1
1. Delivered equipment	1.0	#1	1.0
2. Installed equipment	1.43	#1	1.43
3. Process piping - third	0.30	#2	0.429
4. Instrumentation - some	0.05	#2	0.0715
5. Buildings and site development - outdoor	0.1	#2	0.143
6. Auxiliaries - major addition	0.05	#2	0.0715
7. Outside lines - short	0.02	#2	0.0286
8. Total physical plant costs: Sum of item 2 through 7			2.17
9. Engineering and construction - simple	0.2	#8	0.434
10. Contingencies	0.2	#8	0.434
11. Size factor-large commercial unit	0.02	#8	0.434
12. Total fixed plant cost: Sum of Item 8 through Item 11			3.08

The effects of media recycle and the need for media supplementation are not available at this time. Furthermore, the composition of the media can also be different from the laboratory studies, substituting media from a cheaper source. Therefore, in these estimations, the media costs are estimated by the media used in the laboratory study with a three-month retention time. The Chemical cost of 1M^3 medium is listed in Table 7.16.³⁹ As noted, a cost of $\$23.77/\text{M}^3$ results. However, the majority of water can be reused. The chemical cost of medium can thus be reduced to $\$16.77/\text{M}^3$. The sterilization temperature for the medium is 120°C . Based on the enthalpy of saturated liquid water at 120°C and a $\$4.00/10^6$ Btu heating value, the sterilization cost is around $\$1.60/\text{M}^3$. Therefore, the total cost to prepare 1M^3 medium is $\$18.37$. However, as mentioned above, this value will probably be reduced considerably with increased laboratory study.

7.6.2 Cost Estimation for the CSTR

The cost of the CSTR operated at 1 atm, 175 psi, and 150 psi for three different cases are listed in Tables 7.17-7.28. Total capital costs are presented at two agitation rates for each case studied, including reactors and agitators. Operating costs include utilities, labor and supervision, maintenance, taxes and insurance, depreciation and material costs. Total operating costs are included with and without operating costs for media exchange. As noted previously, only minor media costs are expected by substituting a cheap media and by utilizing media recycle.

Table 7.16 Cost of 1 m³ of Laboratory Media

Chemical	Amount	Unit Cost, \$	Cost, \$
Distilled H ₂ O	950.0 liter	0.008/liter	7.60
Yeast extract *	1000 g	103.25/10Kg	10.325
Sodium sulfide	500 g	0.12/lb	0.132
Sodium bicarbonate	3500 g	17.05/100lb	1.313
			19.370
Pfenning mineral			
KH ₂ PO ₄	500g	121.30/20kg	0.6065
Mg Cl ₂ ·6H ₂ O	330g	0.14 $\frac{1}{2}$ /lb	0.105
NaCl	400g	0.29/lb	0.255
NH ₄ Cl	400g	18.00/100lb	0.158
CaCl ₂ ·2H ₂ O	50g	15.30/ton	0.008
			1.133
Pfenning trace mineral			
ZnSO ₄ ·7H ₂ O	0.1g	26.5/100lb	0.000058
MnCl ₂ ·4H ₂ O	0.03g	0.61/lb	0.000040
H ₃ BO ₃	0.3g	569.0/ton	0.000188
CoCl ₂ ·6H ₂ O	0.2g	415/lb	0.001826
CuCl ₂ ·H ₂ O	0.01g	153.0/ton	0.000002
NiCl ₂ ·6H ₂ O	0.02g	1.05/lb	0.000046
Na ₂ MoO ₄ ·2H ₂ O *	0.03g	4.87/lb	0.000324
FeCl ₂ ·4H ₂ O	1.5g	176.00/ton	0.000290
			0.006
B-vitamins			
Biotin	100mg	5.50/g	0.550
Folic acid *	100mg	517.50/kg	0.05175
Pyridoxal hydro-chloride	50mg	29.00/kg	0.00145
Lipoic acid	300mg	103.95/50g	0.62370
Riboflavin	250mg	43.00/kg	0.01075
Thiamine hydro-chloride	250mg	27.00/kg	0.00675
Calcium-D-Pantothenate	250mg	10.5/kg	0.002625
Cyanocobalamin	250mg	8.00/gram	2.00
P-Aminobenzoic acid	250mg	9.60/kg	0.0024
Nicotinic acid	250mg	7.50/kg	0.001875
Na ₂ SeO ₃ *	50mg	67.25/500g	0.006725
			3.258
* SIGMA 1986			\$23.77/1m ³ medium

Table 7.17 Cost Estimation for the CSTR

Case 1: Methane Production through Peptostreptococcus productus at 14.7 psi

Capital Costs

Agitator size: 300 HP, $\$1.734 \times 10^4$

Agitation Rate RPM	TVR (m ³)	TP (HP)	Nb. of Reactors	Tank Vol. (m ³)	Delivered Cost One Reactor, \$	Total Equipment Cost $\times 10^6$	Capital Cost $\times 10^6$
600	6.785×10^5	2.364×10^4	79	8589	1.468×10^6	115.972	357.194
700	6.659×10^5	3.522×10^4	118	5643	1.137×10^6	134.166	413.231

Operating Costs

Agitation Rate RPM	Utilities	Labor and Supervision		Maintenance		Taxes and Insurance	
	TP(HP)	$\$/\text{mmBtu}$	$\$/\text{mmBtu}$	$\$/\text{mmBtu}$	$\$/\text{mmBtu}$	$\$/\text{mmBtu}$	$\$/\text{mmBtu}$
600	2.364×10^4	9.264	0.508	17.860	0.979	7.144	0.391
700	3.522×10^4	13.804	0.756	20.662	1.132	8.265	0.453

Agitation Rate RPM	Depreciation	Operating Costs Without Material Costs		Material Costs		Total Operating Costs	
	$\$/\text{mmBtu}$	$\$/\text{mmBtu}$	$\$/\text{mmBtu}$	TV ₂ (m ³)	$\$/\text{mmBtu}$	$\$/\text{mmBtu}$	$\$/\text{mmBtu}$
600	28.576	1.566	80.7	5.428×10^5	39.668	2.174	6.596
700	33.058	1.811	96.451	5.327×10^5	38.928	2.133	7.418

Note: TVR = Total Reactor Volume
 TP = Total HP required
 TV₂ = Total Solution Volume

Table 7.18 Cost Estimation for the CSTR:

Case 1: Methane Production through Peptostreptococcus productus at 75 psi

Capital Costs

Agitator size: 300 HP, $\$1.734 \times 10^4$

Agitation Rate RPM	TVR (m^3)	TP (HP)	No. of Reactors	Tank Vol. (m^3)	Delivered Cost One Reactor, \$	Total Equipment Cost $\times 10^6$	Capital Cost $\times 10^6$
600	1.359×10^5	4.734×10^3	16	8494	1.458×10^6	23.328	71.850
700	1.306×10^5	6.909×10^3	23	5678	1.141×10^6	26.243	80.828

Operating Costs

Agitation Rate RPM	Utilities			Labor and Supervision		Maintenance		Taxes and Insurance	
	TP(HP)	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6$	$\$/\text{mmBtu}$
600	4.734×10^3	1.855	0.102	3.593	0.197	3.593	0.197	1.417	0.079
700	6.909×10^3	2.708	0.148	4.041	0.221	4.041	0.221	1.617	0.089

Agitation Rate RPM	Depreciation		Operating Costs Without Material Costs		Material Costs			Total Operating Costs	
	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^{-6}/\text{yr}$	$\$/\text{mmBtu}$	TV ₂ (m^3)	$\times 10^{-6}/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$
600	5.748	0.315	16.226	0.890	1.08×10^5	7.944	0.435	24.17	1.324
700	6.466	0.354	18.873	1.033	1.045×10^5	7.637	0.418	26.51	1.453

Note: TVR = Total Reactor Volume
 TP = Total HP required
 TV₂ = Total Solution Volume

Table 7.19 Cost Estimation for the CSTR:

Case 1: Methane Production through Peptostreptococcus productus at 150 psi

Capital Costs

Agitator size: 300 HP, $\$1.734 \times 10^4$

Agitation Rate RPM	TVR (m^3)	TP (HP)	No. of Reactors	Tank Vol. (m^3)	Delivered Cost One Reactor, \$	Total Equipment Cost $\times 10^6$	Capital Cost $\times 10^6$
600	6.646×10^4	2.316×10^3	8	8308	1.439×10^6	11.512	35.457
700	6.529×10^4	3.453×10^3	12	5441	1.112×10^6	13.344	41.100

Operating Costs

Agitation Rate RPM	Utilities			Labor and Supervision		Maintenance		Taxes and Insurance	
	TP(HP)	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6$	$\$/\text{mmBtu}$
600	2.316×10^3	0.908	0.050	1.773	0.097	1.773	0.097	0.709	0.039
700	3.453×10^3	1.353	0.074	2.055	0.113	2.055	0.113	0.822	0.045

Agitation Rate RPM	Depreciation		Operating Costs Without Material Costs		TV ₂ (m^3)	Material Costs		Total Operating Costs	
	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$		$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^{-6}/\text{yr}$	$\$/\text{mmBtu}$
600	2.837	0.155	8.0	0.438	5.317×10^4	3.886	0.213	11.886	0.651
700	3.288	0.180	9.573	0.525	5.223×10^4	3.817	0.209	13.39	0.734

Note: TVR = Total Reactor Volume
 TP = Total HP required
 TV₂ = Total Solution Volume

Table 7.20 Cost Estimation for the CSTR:

Case 2A: Methane Production from the Co-culture at 14.7 psi, 1.56 hr retention time

Capital Costs

Agitator size: 300 HP, $\$1.734 \times 10^4$

Agitation Rate RPM	TVR (m ³)	TP (HP)	No. of Reactors	Tank Vol. (m ³)	Delivered Cost One Reactor, \$	Total Equipment Cost $\times 10^6$	Capital Cost $\times 10^6$
700	5.801×10^5	3.068×10^4	103	5632	1.136×10^6	117.008	360.385
800	5.67×10^4	4.371×10^4	146	3884	9.066×10^6	132.364	407.681

Operating Costs

Agitation Rate RPM	Utilities	Labor and Supervision	Maintenance	Taxes and Insurance
TP(HP)	$\times 10^6/\text{yr}$ $/\text{mmBtu}$	$\times 10^6$ $/\text{mmBtu}$	$\times 10^6/\text{yr}$ $/\text{mmBtu}$	$\times 10^6$ $/\text{mmBtu}$
700	3.068×10^4 11.859 0.659	18.019 0.987	18.019 0.987	7.208 0.395
800	4.371×10^4 17.133 0.939	20.384 1.117	20.384 1.117	8.154 0.447

Agitation Rate RPM	Depreciation	Operating Costs Without Material Costs	Material Costs	Total Operating Costs
$\times 10^6/\text{yr}$ $/\text{mmBtu}$	$\times 10^6/\text{yr}$ $/\text{mmBtu}$	TV ₂ (m ³) $\times 10^6/\text{yr}$ $/\text{mmBtu}$	$\times 10^6/\text{yr}$ $/\text{mmBtu}$	$\times 10^6/\text{yr}$ $/\text{mmBtu}$
700	28.831 1.580	83.936 4.608	4.641×10^5 33.916 1.858	117.052 6.452
800	32.614 1.787	98.669 5.407	4.537×10^5 33.156 1.817	131.825 7.223

Note: TVR = Total Reactor Volume
 TP = Total HP required
 TV₂ = Total Solution Volume

Table 7.21 Cost Estimation for the CSTR:

Case 2A: Methane Production for the Co-culture at 75 psi, 1.56 hr retention time

Capital Costs

Agitator size: 300 HP, $\$1.734 \times 10^4$

Agitation Rate RPM	TVR (m ³)	TP (HP)	No. of Reactors	Tank Vol.(m ³)	Delivered Cost- One Reactor, \$	Total Equipment Cost \$x10 ⁶	Capital Cost \$x10 ⁶
700	1.137×10^5	6.014×10^3	20	5685	1.142×10^6	22.84	70.347
800	1.112×10^5	8.572×10^3	29	3834	8.995×10^5	26.086	80.345

Operating Costs

Agitation Rate RPM	Utilities			Labor and Supervision		Maintenance		Taxes and Insurance	
	TP(HP)	\$x10 ⁵ /yr	\$/mmBtu	\$x10 ⁶	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶	\$/mmBtu
700	6.014×10^3	2.357	0.129	3.517	0.193	3.57	0.193	1.407	0.077
800	8.572×10^3	3.360	0.164	4.017	0.220	4.017	0.220	1.607	0.088

Agitation Rate	Depreciation		Operating Costs Without Material Costs		TV ₂ (m ³)	Material Costs		Total Operating Costs	
	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu		\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
RPM	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	TV ₂ (m ³)	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
700	5.628	0.308	16.426	0.90	9.097x10 ⁴	6.648	0.364	23.074	1.264
800	6.428	0.352	19.429	1.064	8.899x10 ⁴	6.503	0.350	25.932	1.421

Note: TVR = Total Reactor Volume
 TP = Total HP required
 TV₂ = Total Solution Volume

Table 7.22 Cost Estimation for the CSTR:

Case 2A: Methane Production for the Co-culture of 150 psi, 1.56 hr retention time

Capital Costs

Agitator size: 300 HP, $\$1.734 \times 10^4$

Agitation Rate RPM	TVR (m^3)	TP (HP)	No. of Reactors	Tank Vol. (m^3)	Delivered Cost One Reactor, \$	Total Equipment Cost $\times 10^6$	Capital Cost $\times 10^6$
700	5.57×10^4	2.947×10^3	10	5571	1.128×10^6	11.280	34.742
800	5.558×10^4	4.283×10^3	15	3705	8.637×10^5	12.956	39.904

Operating Costs

Agitation Rate RPM	TP(HP)	Utilities		Labor and Supervision		Maintenance		Taxes and Insurance	
		$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6$	$\$/\text{mmBtu}$
700	2.947×10^3	1.115	0.063	1.737	0.095	1.737	0.095	0.695	0.038
800	4.283×10^3	1.679	0.092	1.995	0.109	1.995	0.109	0.798	0.044

Agitation Rate RPM	Depreciation		Operating Costs Without Material Costs		Material Costs		Total Operating Costs		
	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	TV ₂ (m^3)	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$
700	2.779	0.152	8.1	0.443	4.457×10^4	3.287	0.178	11.357	0.622
800	3.192	0.175	9.659	0.529	4.446×10^4	3.249	0.178	12.908	0.707

Note: TVR = Total Reactor Volume
 TP = Total HP required
 TV₂ = Total Solution Volume

Table 7.23 Cost Estimation for the CSTR:

Case 2B: Methane Production from the Co-culture at 14.7 psi, 0.73 hr retention time

Capital Costs

Agitator size: 300 HP, $\$1.734 \times 10^4$

Agitation Rate RPM	TVR (m ³)	TP (HP)	No. of Reactors	Tank Vol. (m ³)	Delivered Cost One Reactor, \$	Total Equipment Cost $\times 10^6$	Capital Cost $\times 10^6$
700	2.894×10^5	1.530×10^4	51	5675	1.141×10^6	58.191	179.228
800	2.746×10^5	2.117×10^4	71	3868	9.043×10^5	64.205	197.751

Operating Costs

Agitation Rate RPM	Utilities			Labor and Supervision		Maintenance		Taxes and Insurance	
	TP(HP)	$\times 10^6$	$\$/\text{mmBtu}$	$\times 10^6$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6$	$\$/\text{mmBtu}$
700	1.530×10^4	5.997	0.329	8.961	0.491	8.961	0.491	3.585	0.196
800	2.117×10^4	8.296	0.455	9.888	0.542	9.888	0.542	3.955	0.217

Agitation Rate RPM	Depreciation		Operating Costs Without Material Costs		Material Costs		Total Operating Costs		
	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	TV ₂ (m ³)	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$
700	14.388	0.786	41.942	2.293	2.315×10^5	17.011	0.932	58.953	3.225
800	15.820	0.867	47.847	2.623	2.195×10^5	16.144	0.885	63.991	3.508

Note: TVR = Total Reactor Volume
 TP = Total HP required
 TV₂ = Total Solution Volume

Table 7.24 Cost Estimation for the CSTR:

Case 2B: Methane Production from the Co-culture at 75 psi, 0.73 hr retention time

Capital Costs

Agitator size: 300 HP, $\$1.734 \times 10^4$

Agitation Rate RPM	TVR (m^3)	TP (HP)	No. of Reactors	Tank Vol. (m^3)	Delivered Cost One Reactor, \$	Total Equipment Cost $\times 10^6$	Capital Cost $\times 10^6$
700	5.671×10^4	3.0×10^3	10	5671	1.140×10^6	11.400	35.112
800	4.784×10^4	4.148×10^3	14	3417	8.391×10^5	11.747	36.181

Operating Costs

Agitation Rate				Labor and Supervision		Maintenance		Taxes and Insurance	
	Utilities								
RPM	TP(HP)	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶	\$/mmBtu
700	3.0x10 ³	1.176	0.064	1.756	0.096	1.756	0.096	0.702	0.038
800	4.148x10 ³	1.626	0.089	1.809	0.099	1.809	0.099	0.714	0.040

Agitation Rate	Depreciation		Operating Costs Without Material Costs		Material TV ₂ (m ³)	Costs		Total Operating Costs	
	\$x106/yr	\$/mmBtu	\$x106/yr	\$/mmBtu		\$x106/yr	\$/mmBtu	\$x106/yr	\$/mmBut
RPM	\$x106/yr	\$/mmBtu	\$x106/yr	\$/mmBtu	TV ₂ (m ³)	\$x106/yr	\$/mmBtu	\$x106/yr	\$/mmBut
700	2.809	0.154	8.199	0.448	4.537x10 ⁴	3.316	0.182	11.515	0.631
800	2.894	0.159	8.862	0.486	4.106x10 ⁴	3.147	0.172	12.009	0.658

Note: TVR = Total Reactor Volume
 TP = Total HP required
 TV2 = Total Solution Volume

Table 7.25 Cost Estimation for the CSTR:

Case 2B: Methane Production from the Co-culture at 150 psi, 0.73 hr retention time

Capital Costs

Agitator size: 300 HP, $\$1.734 \times 10^4$

Agitation Rate RPM	TVR (m^3)	TP (HP)	No. of Reactors	Tank Vol. (m^3)	Delivered Cost One Reactor, \$	Total Equipment Cost $\times 10^6$	Capital Cost $\times 10^6$
700	2.794×10^4	1.500×10^3	5	5598	1.132×10^6	5.660	17.433
800	2.693×10^3	2.076×10^3	7	3847	9.014×10^5	6.310	19.435

Operating Costs

Agitation Rate		Utilities		Labor and Supervision		Maintenance		Taxes and Insurance	
RPM	TP(HP)	\$x106/yr	\$/mmBtu	\$x106	\$/mmBtu	\$x106/yr	\$/mmBtu	\$x1-6	\$/mmBtu
700	1.500x10 ³	0.588	0.032	0.872	0.048	0.872	0.048	0.349	0.019
800	2.076x10 ³	0.814	0.045	0.972	0.053	0.972	0.053	0.389	0.021

Agitation Rate	Depreciation		Operating Costs With-out Material Costs		Material Costs	Total Operating Costs			
	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$		$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$
RPM	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\text{TV}_2(\text{m}^3)$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$
700	1.395	0.076	4.076	0.223	2.269×10^4	1.658	0.091	5.734	0.314
800	1.555	0.085	4.702	0.257	2.154×10^4	1.574	0.086	6.276	0.344

Note: TVR = Total Reactor Volume
 TP = Total HP required
 TV2 = Total Solution Volume

Table 7.26 Cost Estimation for the CSTR:

Case 3: Methane Production from the Mixed Culture at 14.7 psi

Capital Costs

Agitator size: 300 HP, $\$1.734 \times 10^4$

Agitation Rate RPM	TVR (m ³)	TP (HP)	No. of Reactors	Tank Vol. (m ³)	Delivered Cost One Reactor, \$	Total Equipment Cost \$x10 ⁶	Capital Cost \$x10 ⁶
500	7.081×10^5	1.498×10^4	50	14162	1.993×10^6	99.65	306.920
600	6.646×10^3	2.316×10^4	78	8521	1.461×10^6	113.958	350.991

Operating Costs

Agitation Rate		Utilities		Labor and Supervision		Maintenance		and Insurance	
RPM	TP(HP)	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶	\$/mmBtu
500	1.498x10 ⁴	5.783	0.322	15.346	0.841	15.346	0.841	6.138	0.336
600	2.316x10 ⁴	9.078	0.497	17.550	0.962	17.550	0.962	7.020	0.385

Agitation Rate	Depreciation		Operating Costs Without Material Costs		Material Costs			Total Operating Costs	
	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	TV ₂ (m ³)	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
500	24.554	1.345	67.257	3.685	5.665x10 ⁵	41.400	2.268	108.657	5.954
600	28.079	1.539	79.277	4.345	5.317x10 ⁵	38.857	2.129	118.134	6.473

Note: TVR = Total Reactor Volume
 TP = Total HP required
 TV₂ = Total Solution Volume

Table 7.27 Cost Estimation for the CSTR:

Case 3: Methane Production from the Mixed Culture at 75 psi

Capital Costs

Agitator size: 300 HP, $\$1.734 \times 10^4$

Agitation Rate RPM	TVR (m ³)	TP (HP)	No. of Reactors	Tank Vol. (m ³)	Delivered Cost One Reactor, \$	Total Equipment Cost $\times 10^6$	Capital Cost $\times 10^6$
500	1.388×10^5	2.936×10^3	1C	13880	1.969×10^6	19.690	60.645
600	1.303×10^5	4.539×10^3	1E	8144	1.422×10^6	22.752	70.076

Operating Costs

Agitation Rate RPM	Utilities	Labor and Supervision		Maintenance		Taxes and Insurance	
	TP(HP)	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$
500	2.936×10^3	1.151	0.063	3.032	0.166	3.032	0.166
600	4.539×10^3	1.779	0.097	3.504	0.192	3.504	0.192

Agitation Rate RPM	Depreciation		Operating Costs Without Material Costs		Material Costs		Total Operating Costs	
	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	TV ₂ (m ³)	$\times 10^6/\text{yr}$	$\$/\text{mmBtu}$	$\times 10^6/\text{yr}$
500	4.852	0.266	13.280	0.727	1.110×10^5	8.112	0.444	21.392
600	5.606	0.307	15.795	0.865	1.042×10^5	7.615	0.417	23.410

Note: TVR = Total Reactor Volume
 TP = Total HP required
 TV₂ = Total Solution Volume

Table 7.28 Cost Estimation for the CSTR:

Case 3: Methane Production from the Mixed Culture at 150 psi

Capital Costs

Agitator size: 300 HP, $\$1.734 \times 10^4$

Agitation Rate RPM	TVR (m^3)	TP (HP)	No. of Reactors	Tank Vol. (m^3)	Delivered Cost One Reactor, \$	Total Equipment Cost $\times 10^6$	Capital Cost $\times 10^6$
500	6.943×10^4	1.469×10^3	5	13886	1.969×10^6	9.845	30.323
600	6.516×10^4	2.270×10^3	8	8145	1.422×10^6	11.376	35.038

Operating Costs

Agitation Rate RPM	Utilities	Labor and Supervision	Maintenance	Taxes and Insurance
	TP(HP) $\times 10^6/\text{yr}$ $/\text{mmBtu}$	$\times 10^6$ $/\text{mmBtu}$	$\times 10^6/\text{yr}$ $/\text{mmBtu}$	$\times 10^6$ $/\text{mmBtu}$
500	1.469×10^3 0.576 0.032	1.516 0.083	1.516 0.083	0.606 0.033
600	2.270×10^3 0.890 0.049	1.752 0.096	1.752 0.096	0.701 0.038

Agitation Rate RPM	Depreciation	Operating Costs Without Material Costs	Material Costs	Total Operating Costs
	$\times 10^{-6}/\text{yr}$ $/\text{mmBtu}$	$\times 10^{-6}/\text{yr}$ $/\text{mmBtu}$	TV ₂ (m^3) $\times 10^6/\text{yr}$ $/\text{mmBtu}$	$\times 10^6/\text{yr}$ $/\text{mmBtu}$
500	2.426 0.133	6.64 0.364	5.554×10^4 4.059 0.222	10.699 0.586
600	2.803 0.154	7.898 0.433	5.213×10^4 3.810 0.209	11.708 0.642

Note: TVR = Total Reactor Volume
 TP = Total HP required
 TV₂ = Total Solution Volume

As shown in the tables, the total capital costs range from \$17 million for methane production from the co-culture at a 0.73 hour retention time, 150 psi and 700 rpm, to \$413 million for the P. productus culture at 700 rpm and atmospheric pressure. The operating costs per million Btus ranged from 22¢ for methane production from the co-culture at a 0.73 hour retention time, 150 psi and 700 rpm, to \$5.41 for the co-culture at atmospheric pressure, a 1.56 hour retention time and 800 rpm.

7.6.3 Cost Estimation for the Immobilized Cell Reactor

The cost of the immobilized cell reactor using Case 1 only operated at 1 atm, 75 psi, and 150 psi for both a mass transfer optimized reactor size and a 1000 M³ reactor size are listed in Tables 7.29-7.34. In these analyses, the cost of the wood chips packing material is neglected. As with the CSTR, the total capital costs are tabulated at 2 retention times, including the number of reactors. The operating costs are also tabulated at the two retention times, including utilities, labor and supervision, maintenance, taxes and insurance, depreciation, and material costs. The total operating costs are again presented with and without media supplementation.

Total capital costs range from \$36 million for reactors based on a 1000M³ size at 150 psi and a 0.5 hour retention time to \$3.8 billion for reactors based on mass transfer optimized size at 1 atm and a 0.83 hour retention time. Operating costs range from \$0.40/million Btu to \$42/million Btu for the same conditions, respectively. Costs for immobilized cell operation are higher than CSTR operation because in the laboratory studies a high cell density could not be obtained to yield high conversions at low retention times. A priority item for future study is the attainment of higher cell densities in the immobilized cell reactors.

7.7 Discussion of Results

The analyses presented in the previous chapters are based on the results of the laboratory feasibility on the potential of biological methanation. No optimization attempt has been carried out at this point. Therefore, the values presented in this report are only potential values, which could improve with further optimization. Secondly, there is no information available on the life of the media or the substitution of a cheaper medium. This of course, results in uncertainty in calculating any materials costs. Thus, the discussions in this chapter will be limited to operating costs without material costs included. Thirdly, the simulated coal gas composition used in this analysis is based on 65 percent CO, 22 percent H₂, 11 percent CO₂ and 2 percent CH₄. This composition has a higher carbon monoxide to hydrogen ratio than the usual coal gas. For a gas containing a one to one ratio of CO to H₂, 0.5 ml methane can be produced instead of 0.3346 mole methane per mole CO converted. Fourthly, high pressure operation must be demonstrated for all systems up to 150 psi; this is under investigation in the laboratory. Fifthly, it is realized that scale-up from laboratory scale to industrial scale without a piloting the process is speculative.

In spite of the above concerns, the scale-up to a 5.0x10¹⁰ Btu/day size plant has been performed. In general, the high retention time used

Table 7.29 Cost Estimation of the Immobilized Cell Reactor Based on Mass Transfer Optimized Size at 14.7 psi

Capital Costs

Retention Time hr	Reactor Size m ³	No. of Reactors	Delivered Cost One Reactor \$	Total Equip. Cost \$x10 ⁶	Capital Costs \$x10 ⁶
0.5	110.01	2844	9.806x10 ⁴	278.883	858.960
0.8333	8.355	62425	1.994x10 ⁴	1244.754	3833.842

Operating Costs

Retention Time hr	Utilities		Labor and Supervision		Maintenance		Taxes and Insurance		Depreciation	
	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁻⁶ /yr	\$/mmBtu
0.5	0	0	42.948	2.353	42.948	2.353	17.179	0.941	68.717	3.765
0.8333	0	0	191.692	10.504	191.692	10.504	76.677	4.201	306.707	16.806

Retention Time	Operating Costs Without Material Costs		Material Costs			Total Operating Costs	
	\$x106/yr	\$/mmBtu	TV ₂ (m ³)	\$x106/yr	\$/mmBtu	\$x106/yr	\$/mmBtu
hr							
0.5	171.792	9.413	1.738x10 ⁵	12.701	0.696	184.493	10.109
0.8333	766.768	42.015	2.898x10 ⁵	21.179	1.160	787.947	43.175

Note. TV₂ = Total Solution Volume

Table 7.30 Cost Estimation of the Immobilized Cell Reactor Based on Mass Transfer Optimized Size at 75 psi

Capital Costs

Retention Time hr	Reactor Size m ³	No. of Reactors	Delivered Cost One Reactor \$	Total Equip. Cost \$x10 ⁶	Capital Costs \$x10 ⁶
0.5	110.01	533	9.806x10 ⁴	54.227	167.019
0.8333	8.355	12237	1.994x10 ⁴	244.006	3833.842

Operating Costs

Retention Time hr	Utilities		Labor and Supervision		Maintenance		Taxes and Insurance		Depreciation	
	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
0.5	0	0	8.351	0.458	8.351	0.458	3.340	0.183	13.362	0.732
0.8333	0	0	37.577	2.059	37.577	2.059	15.031	0.824	60.123	3.294

Retention Time hr	Operating Costs Without Material Costs		Material Costs			Total Operating Costs	
	\$x10 ⁶ /yr	\$/mmBtu	TV ₂ (m ³)	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
0.5	33.404	1.830	3.376x10 ⁴	2.467	0.135	35.871	1.966
0.8333	150.308	8.236	5.680x10 ⁴	4.151	0.227	154.459	8.464

Note. TV₂ = Total Solution Volume

Table 7.31 Cost Estimation of the Immobilized Cell Reactor Based on Mass Transfer Optimized Size at 150 psi

Capital Costs

Retention Time hr	Reactor Size m ³	No. of Reactors	Delivered Cost One Reactor \$	Total Equip. Cost \$x10 ⁶	Capital Costs \$x10 ⁶
0.5	110.01	277	9.806x10 ⁴	27.163	83.662
0.8333	8.355	6118	1.994x10 ⁴	121.993	375.738

Operating Costs

Retention Time hr	Utilities		Labor and Supervision		Maintenance		Taxes and Insurance		Depreciation	
	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
0.5	0	0	4.183	0.229	4.183	0.229	1.673	0.092	6.693	0.367
0.8333	0	0	18.787	1.029	18.787	1.029	7.515	0.412	30.059	1.647

Retention Time	Operating Costs Without Material Costs		Material Costs			Total Operating Costs	
	\$x10 ⁶ /yr	\$/mmBtu	TV ₂ (m ³)	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
hr							
0.5	16.732	0.917	1.687x10 ⁴	1.233	0.068	17.965	0.984
0.8333	75.148	4.118	2.839x10 ⁴	2.075	0.114	77.223	4.231

Note. TV₂ = Total Solution Volume

Table 7.32 Cost Estimation of the Immobilized Cell Reactor Based on
1000m³ Reactor Size at 14.7 psi

Capital Costs

Retention Time hr	Reactor Size m ³	No. of Reactors	Delivered Cost One Reactor \$	Total Equip. Cost \$x10 ⁶	Capital Costs \$x10 ⁶
0.5	1000	313	3.836x10 ⁵	120.067	369.806
0.8333	1000	512	3.836x10 ⁵	200.239	616.736

Operating Costs

Retention Time hr	Utilities		Labor and Supervision		Maintenance		Taxes and Insurance		Depreciation	
	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
0.5	0	0	18.490	1.013	18.490	1.013	7.396	0.405	29.584	1.621
0.8333	0	0	30.837	1.690	30.837	1.690	12.335	0.676	49.339	2.704

Retention Time	Operating Costs Without Material Costs		Material Costs			Total Operating Costs	
	\$x10 ⁶ /yr	\$/mmBtu	TV ₂ (m ³)	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
hr							
0.5	73.960	4.053	1.730x10 ⁵	12.701	0.696	86.661	4.749
0.8333	123.348	6.759	2.890x10 ⁵	21.179	1.160	144.527	7.919

Note: TV₂ = Total Solution Volume

Table 7.33 Cost Estimation of the Immobilized Cell Reactor Based on 1000m³ Reactor Size at 75 psi

Capital Costs

Retention Time hr	Reactor Size m ³	No. of Reactors	Delivered Cost One Reactor \$	Total Equip. Cost \$x10 ⁶	Capital Costs \$x10 ⁶
0.5	1000	62	3.836x10 ⁵	23.783	73.252
0.8333	1000	103	3.836x10 ⁵	39.511	121.694

Operating Costs

Retention Time hr	Utilities		Labor and Supervision		Maintenance		Taxes and Insurance		Depreciation	
	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
0.5	0	0	3.663	0.201	3.663	0.201	1.465	0.080	5.860	0.321
0.8333	0	0	6.085	0.333	6.085	0.333	2.434	0.133	9.736	0.533

Retention Time hr	Operating Costs Without Material Costs		Material Costs			Total Operating Costs	
	\$x10 ⁶ /yr	\$/mmBtu	TV ₂ (m ³)	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
0.5	14.651	0.803	3.376x10 ⁴	2.467	0.135	17.118	0.938
0.8333	24.34	1.334	5.608x10 ⁴	4.151	0.227	28.491	1.561

Note. TV₂ = Total Solution Volume

Table 7.34 Cost Estimation of the Immobilized Cell Reactor Based on
1000m³ Reactor Size at 150 psi

Capital Costs

Retention Time hr	Reactor Size m ³	No. of Reactors	Delivered Cost One Reactor \$	Total Equip. Cost \$x10 ⁶	Capital Costs \$x10 ⁶
0.5	1000	31	3.836x10 ⁵	11.892	36.627
0.8333	1000	52	3.836x10 ⁵	19.947	61.437

Operating Costs

Retention Time hr	Utilities		Labor and Supervision		Maintenance		Taxes and Insurance		Depreciation	
	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
0.5	0	0	1.831	0.100	1.831	0.100	0.733	0.040	2.930	0.161
0.8333	0	0	3.072	0.168	3.072	0.168	1.229	0.067	4.915	0.269

Retention Time hr	Operating Costs Without Material Costs		Material Costs			Total Operating Costs	
	\$x10 ⁶ /yr	\$/mmBtu	TV ₂ (m ³)	\$x10 ⁶ /yr	\$/mmBtu	\$x10 ⁶ /yr	\$/mmBtu
0.5	7.325	0.401	1.687x10 ⁴	1.233	0.068	8.558	0.469
0.8333	12.288	0.673	2.839x10 ⁴	0.075	0.114	14.363	0.787

Note: TV₂ = Total Solution Volume
p

gives unusually large reactor sizes. This results in a high capital investment, and thus, a high capital related operating cost. More detailed discussions are presented in the following paragraphs.

7.7.1 CSTR

A summary of CSTR cost comparisons is shown in Table 7.35. As described in Tables 7.17-7.28, the operating pressure can reduce both the capital costs and operating costs (without material cost) significantly. The reduction of capital cost is related to nearly the ratio of operating pressure to atmosphere pressure. By operating at 150 psia, the capital costs are reduced from several hundred million dollars to less than 100 million dollars. However, the operating cost (without material cost) is reduced by a smaller ratio due to an increase in utilities with increasing pressure. The operating costs (without material cost) are still reduced from about four dollars per million Btu to 0.5 dollars per million Btu at 150 psi since the utilities are a small portion of the total operating cost (without material costs).

As expected, even though the increase in agitation speed can reduce the overall reactor volume, the higher power input per unit volume results in a higher number of reactors and thus higher capital and operating costs. By comparing Cases 2A and 2B, changing the retention time from 1.56 to 0.73 hour reduces both the capital and operating costs (without material cost) by one-half.

Since each of the cases are run at different retention times, it is difficult to tell which case is the best. However, it seems that retention time is a much more important factor than which case was used. The best CSTR performance investigated in the study was Case 2B, the co-culture operated at 700 rpm and 150 psia. This case required five reactors for a total capital cost of 17.433 million dollars and an operating cost excluding material cost of 0.223 dollars per million Btu.

7.7.2 Immobilized Cell Reactor

A summary of immobilized cell reactor cost comparisons is shown in Table 7.36. In this category, only Case 1 has been studied. Again, using high operating pressures to increase the overall mass transfer rate can reduce the size of the reactor and, thus the capital and operating costs. The capital costs are reduced by the pressure ratio as above. For a 0.5 hour retention time and 1000 M³ volume reactor, the capital costs are reduced from \$369.806 million to \$36.627 million, and the operating costs (without material cost) are reduced from \$4.053 to \$0.401 per million Btu at 150 psia. It should be noted that these cost comparisons are based upon only preliminary data in the laboratory. Higher conversions are expected with increased cell density.

Table 7.35 CSTR Cost Comparisons

Case	Retention Time, hr	Pressure, psi	Capital Costs	Operating Costs
			(\$x10 ⁶)	(excluding materials \$/mmBtu)
1		14.7	357	3.444
		75	72	0.890
		150	35	0.438
2A	1.56	14.7	360	4.608
		75	70	0.900
		150	35	0.443
2B	0.73	14.7	179	2.293
		75	35	0.448
		150	17	0.223
3		14.7	307	3.685
		75	60	0.727
		150	30	0.364

TABLE 7.36

Immobilized Cell Reactor Cost Comparisons

Reactor Size	Pressure, psi	Capital Cost	Operating Costs
		(\$ x 10 ⁶)	(without materials costs) \$/mmBtu
Mass transfer optimized	14.7	859	9.413
	75	167	1.830
	150	84	0.917
1000 M ³	14.7	370	4.053
	75	73	0.803
	150	37	0.401

As mentioned previously, retention time is the major factor in determining the cost. By changing the retention time from 0.5 hour to 0.8333 hour, the capital and operating costs are increased by 70 percent. As discussed in Section 7.4.2, $k_L a$ can be increased as the scale up. This means that the immobilized cell reactor has the potential to be operated at a retention time smaller than 0.5 hour if a proper scale-up ratio is given.

7.7.3 Comparison of the CSTR and Immobilized Cell Reactor

In the CSTR, the utilities make up less than 20 percent of the total operating costs. Thus, because of performance, the immobilized cell reactors are expected to yield a higher cost at the same retention time. For the same retention time, the immobilized cell reactor has 44 percent more working volume than the CSTR reactor. This will give higher capital costs and capital-related operating costs excluding material cost. On the other hand, the consumption of nutrients is expected to be less in the immobilized cell reactor. Also, as mentioned in the above section, the retention time of the immobilized cell reactor can be reduced by a suitable scale-up ratio. If a higher cell density can be obtained in the ICR, much lower capital and operating costs are expected.

8.0 CONCLUSIONS

Single stage biomethanation has been studied in a single mixed culture isolated from sewage sludge and acclimated to coal gas by gradual introduction of the gas over several months. Experimental studies were undertaken to measure the effects of gas flow rate (retention time) and agitation on CO and H₂ conversion ultimately to methane.

Agitation rate studies indicate that agitation rates of 500-600 rpm are required in stirred-tank vessels to yield sufficient mass transfer of coal gas into the liquid cultures. At an agitation rate of 500 rpm and a 1.94 hr gas retention time (based upon liquid volume) the conversions of both CO and H₂ reach 100 percent.

Retention time studies at a constant agitation rate of 500 rpm showed that a retention time of about 2 hours was required to reach over 90 percent conversion of CO at atmospheric pressure. Higher pressures up to 50 psig brought about no decrease in conversion. An analysis of the data shows that the mass transfer coefficients for both H₂ and CO can be represented as a function of the gas flow rate in cm³/hr by the equation:

$$k_{1a} = 6.66 F^{0.0066} \quad (2.5)$$

One interesting result arose when comparing the conversions of H₂ and CO after 8 months of operation in the mixed culture. The performance of the culture measured in terms of its ability to convert coal gas to methane increased dramatically with time. The retention time to achieve 90 percent conversion of both CO and H₂ was decreased from 4 hours to 2 hours. The apparent reason for this increase lies in the ability of the culture to produce additional cells due to increased acclimation. Increasing the number of cells increases the chemical enhancement of mass transfer.

Five anaerobic bacteria have been compared for their ability to convert CO to methane intermediates. Screening experiments comparing maximum optical density, conditions for growth, doubling time, the time required to completely utilize a given quantity of CO, and the maximum CO tolerance were compared. Based upon these studies, P. productus was chosen as the bacteria for acetate production from CO and H₂O. This bacteria grows best at 37°C and has a maximum CO tolerance of 90 percent. The doubling time of P. productus on 50 percent CO was 4-6 hours, producing a maximum optical density of 0.83. P. productus can completely utilize 50 percent CO in 4 days in a batch reactor without agitation.

R. Rubrum (II) was chosen as the bacteria for CO₂ and H₂ production from CO and H₂O. This bacteria requires tungsten light for growth at 30°C and has a maximum CO tolerance of 60 percent. The doubling time of R. rubrum (II) on 50 percent CO was 24 hours, producing a maximum optical density of 1.0. R. rubrum (II) can completely utilize 50 percent CO in 10 days in a batch reactor without agitation.

The optimum growth requirements for both Peptostreptococcus productus and Rhodospirillum rubrum (II) have been determined. Only slight media changes from the original recommended media have been made during the

optimization studies. Both organisms are able to utilize up to 90 percent CO which may become important to decrease acclimation times when using other CO-rich coal gases as the feed gas. Both organisms can also utilize CO in the presence of other coal gas constituents such as CH₄, CO₂, and H₂. The pH, temperature, and anaerobicity requirements of the organisms are as documented in the literature. The major end-products are as expected. P. productus was also shown to utilize CO₂ and H₂ in the production of acetate. This reaction, shown in Equation (3.2), occurs only after the CO has been depleted from the media.

R. rubrum unfortunately requires a tungsten light source for growth. This requirement could be a major disadvantage in utilizing the bacteria in larger-scale experimentation.

From the experiments with stagewise upgrading of coal gas, it can be seen that various types of reactors can be used in the bio-conversion of coal gas to methane. A mixed culture and P. productus were found to be extremely important bacteria which could be used in the industrial conversion of coal gas to methane or methane precursors. Reactor studies indicate that P. productus metabolized as high as 29.58 millimoles of CO/gm dry cell weight, when a gas retention time of 0.63 hour and an agitation of 500 rpm were provided. This rate could be elevated, although bacterial conversion of CO was dependent on mass-transfer coefficients. CO conversions as high as 100 percent could be obtained using proper agitation rates and gas retention times. Parr pressure studies also indicated that bacteria could convert coal gas under reactor pressures at least up to 100 psi. The maximum rate of CO conversion at 40 psig was 21.25 mmoles of CO/hour/liter; but this rate decreased at higher pressure. In situations where only acetate was produced during coal gas conversion, methanogenic reactors could be used to metabolize acetate to CH₄. The feasibility of bacterial immobilization and the use of the immobilized cell reactor were studied using both mixed culture and P. productus. Preliminary data indicated that the bacteria could be immobilized using various bio-support materials and cross-linkage methodology. Although, the present method had various short comings, a CO conversion up to 45 percent and 76.6 percent were obtained when a mixed culture and P. productus were used in an ICR. Further improvements in the immobilization techniques could demonstrate the ICR as a potential means of bacterial conversion of coal gas at higher reaction rates.

In summary, the use of bacterial cultures grown in various kinds of reactor systems including the ICR have tremendous potential in the future industrial conversion of coal gas. The use of series reactor systems showed great promise in the bacterial conversion of synthesis gas to high Btu methane.

From the experiments with culture enhancement, it can be concluded that the biological conversion of coal gas to gaseous fuels can be a practical and important means to derive energy from coal synthesis gas. Based on CO tolerance studies, both P. productus and R. rubrum are suitable to be used in the industrial conversion of coal gas to methane precursors. P. productus produces acetate and R. rubrum converts CO and H₂O to CO₂ and H₂, which are the principal energy substrates for most methanogenic bacteria. It is estimated that almost 70 percent of the methane produced in

nature is derived from acetate, indicating the importance of P. productus in the biological conversion of waste coal gas or carbon monoxide.

Reactor studies indicate the versatility of P. productus in the metabolism of CO at high gas flow rates. For example, at 500 rpm and a 0.63 hour gas retention time, P. productus could metabolize 29.58 m moles of CO/gm dry cell weight/hour. These rates could be elevated with higher cell densities, and if problems with CO-mass transfer-coefficients are resolved. As predicted, the bacterial conversion of CO is dependent upon agitation: the higher the agitation, the higher the CO conversion. However, a 100 percent conversion of CO could be achieved using proper agitation rates and gas retention times. A co-culture of P. productus with H₂-CO₂ utilizing and acetate-cleaving bacteria is possible and could lead to coal gas conversion in a single-stage reactor system. A suitable tri-culture system could be more beneficial in converting CO, H₂ and CO₂ contained in coal gas to methane, which can contribute more than 50 percent of the outlet gas.

In summary, P. productus and R. rubrum are potential microorganisms which can be used in the biological conversion of coal gas to CH₄ precursors. Reactor studies with P. productus involving CH₄ producing bacteria show that biological process can be a viable alternative to the catalytic conversion of coal gas to methane.

The effects of sulfur gases (H₂S, COS and SO₂) on bacterial growth, CO metabolism and product formation have been determined. Two pure cultures, P. productus and R. rubrum and a mixed culture known to metabolize CO were used in this study. The order of sulfur gas toxicity on bacterial cells was found to be H₂S < COS < SO₂. Among the bacterial cultures, the mixed culture was relatively more resistant to the sulfur gases than P. productus and R. rubrum. Both P. productus and R. rubrum showed almost equal tolerances during growth and metabolism of CO in the presence of sulfur gases. However, lower concentrations of sulfur gases had no harmful effects on the bacterial growth; but their presence at high concentrations reduced bacterial cell density or the cultures experienced a long lag. Similarly, the metabolism of CO was unaffected in the presence of less than 2 percent sulfur gases. Bacterial conversion of CO was more than 95 percent, when the cultures were grown with 50 percent CO and lower concentrations (up to 2 percent) of sulfur gases. Interestingly, the addition of sulfur gases did not alter the pathway of bacterial metabolism of CO and therefore, no change occurred in the formation of methanogenic precursors such as acetate, H₂ and CO₂. The sulfur gases exhibited potential harmful effects on the methanogens as methane formation were lower when the cultures were grown with H₂S and COS. Further studies using pure cultures of methanogens are needed to determine the effects of sulfur gases on bacterial growth and transformations of acetate, H₂ and CO₂ to CH₄. The experiments with pre-grown cultures of R. rubrum suggest that the toxic effects of certain sulfur-containing gases can be overcome through culture manipulations.

As the total concentrations of sulfur gases present in the coal gas does not exceed more than 1 percent, no problems are anticipated in growing bacterial cultures in the presence of sulfur-contaminated coal gas. Therefore, the use of these bacterial cultures are recommended in

the biomethanation processes of coal gas contaminated with small amounts of H_2S , COS and SO_2 .

The conclusions of this economical analysis study is that the biological methanation plant with a size of 5.0×10^{10} Btu/day can be economically attractive. The best case in this study requires a fixed capital investment of 17.433 million dollars and 0.257 dollar per million Btu operating cost excluding material cost. This can be compared to 1.5 dollars per million Btu operating cost estimated for the catalytic methanation process. The best CSTR utilizes a co-culture of P. productus and a methanogen, operated at 150 psia and a 0.73 hour gas retention time (based on liquid volume). The agitation power input is 0.06 HP per M^3 of solution.

9.0 RECOMMENDATIONS

During this contract, the technical and economic feasibility of biologically converting coal synthesis gas to a higher value fuel has been demonstrated in mixed, pure and co-culture and in stirred-tank and immobilized cell reactors. The potential of utilizing pressure to increase reaction rate for these gas phase reactions has been explored.

Future work should concentrate on optimizing reactors and reactions, reactor scale-up and process demonstration. The following recommendations are listed for future study:

1. Determine the best performance for stirred-tank and immobilized cell reactors using mixed, pure and co-cultures at a variety of pressures.
2. Demonstrate the performance of the cultures in continuous gas flow in the presence of H_2S , COS and SO_2 .
3. Determine the major contributing bacteria present in the mixed culture for possible improvement in pure and co-culture systems.
4. Determine the effects of pressure, acetate and CO on mixed and pure culture performance.
5. Determine the necessary mass transfer and kinetic parameters for successful scale-up of these biological processes.
6. Demonstrate the suitability of scale-up parameters by operating an optimized bench-scale biological reaction system.
7. Perform economic evaluations for each of the optimized reactor systems.

In addition, the following recommendations are suggested for a complete economic analysis including optimization:

1. Study how long the nutrients can last in both the CSTR and immobilized cell reactor and search for a cheaper source of media.
2. Determine complete power input and mass transfer rate correlations for the CSTR reactor having a configuration similar to a commercial reactor.
3. For the CSTR, study the mass transfer rate correlation in the process of scale-up based on the same retention time instead of the same superficial gas velocity.
4. For the immobilized cell reactor, find a mass transfer correlation and confirm the concept that scale-up based on the same retention time can increase the mass transfer rate.
5. Study at what mass transfer rate the overall reaction is no longer controlled by mass transfer.

6. Study the performance of the organisms at high mass transfer rates.
7. Optimize the performance of the mixed culture system.

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11.0 APPENDICES

APPENDIX A

ANALYTICAL PROCEDURES AND CULTURING CONDITIONS

ANALYTICAL PROCEDURES

Bacterial Growth

Bacterial growth was determined by measurement of optical density (OD) using a Bausch and Lomb Spectronic-20 spectrophotometer. The greater the optical density, the greater the number of cells in the culture. This procedure was necessary to monitor the viable cells in the culture. Growth of the mixed culture, P. productus and A. woodii were measured at 580 nm whereas the optical densities of R. gelatinosa and R. rubrum (Strains I and II) are taken at 600 nm.

Ammonia and pH

An Orion Research Ionalyzer (model 407A) was used for measuring ammonia in the bacterial culture. To 99 ml of an NH_4^+ standard (24-26.0 mg per liter) solution, 1 ml of 10 N NaOH was added to obtain a background reading. One ml of the sample was then added to this solution and the increase in ammonia concentration was recorded. The total ammonia concentration was then calculated after subtracting the background values.

Adenosine Triphosphate (ATP) Analysis

Cellular ATP was measured by luminescence techniques using a Pico-Lite Luminometer Analyzer (Packard Instruments Company). Bacterial cultures (25 μl) were first diluted with Picochec buffer (1:4) and then 10 μl samples were treated with 40 μl solution of Picozyme F (Luciferase). A 10 μl solution of 1×10^{-5} M ATP was used as an internal standard. Picoex-B (10 μl) was used for extracting ATP from bacterial cells. The cellular ATP concentration was determined after subtracting background values from the total ATP concentration.

Gas Analysis

Gas compositions were determined by gas-solid chromatography using a Perkin-Elmer Sigma 300 gas chromatograph equipped with a hot wire detector. A 6' x 1/8" stainless steel column packed with 60-80 mesh Carbosphere, was used for this purpose. Samples (0.15 ml) were injected using a Pressure-LOK syringe and the composition of gases were calculated using a LCI 100 Perkin-Elmer Laboratory Computing Integrator System. The operating conditions of the gas chromatograph were: Oven temp. 120°C, Injector/Detector temp. 175°C, carrier gas (He) flow rate 40 ml/min. Under these conditions, the retention times for the gases were: H_2 0.55 min, N_2 2.46 min, CO 3.54 min, CH_4 6.69 min and CO_2 10.08 min.

Volatile Fatty Acids Analysis

The fermentation products were determined as free acids using a Perkin-Elmer Sigma 3B gas chromatograph fitted with a FID detector. The

samples (0.5 ml) were acidified using 50% phosphoric acid (1 part acid to 10 parts sample) before the gas chromatographic analyses. One μ l of the sample was injected into a 2' x 1/8" teflon column packed with Poropak QS and the amount of the acids were calculated using an area percent concentration standard curve for the individual acids. Other operating conditions were: Oven temp. 200°C, and flow rate 45 ml/min. Helium was used as carrier gas. Under these conditions, the retention times for acetic acid, propionic acid and butyric acid were 1.29, 2.46 and 4.7 min, respectively.

MEDIA AND CONDITIONS OF CULTIVATION FOR PURE CULTURE BACTERIA

The anaerobic techniques for the preparation and use of media were essentially those of Hungate⁴⁰ as modified by Bryant⁴¹ and Balch and Wolfe⁴².

The basal salts media used to grow the bacterial cultures are listed in Tables A.1, A.2, A.3, and A.4. For the preparation of the media, basal salts, yeast extract and other constituents in appropriate amounts were taken in a round bottomed flask and were boiled for 2-3 minutes under a N₂-CO₂ (80:20) atmosphere. NaHCO₃ (0.35 g/100 ml) was added when the media was cooled to about 55°C. Media, in appropriate amounts, was then pipetted anaerobically into serum stoppered tubes (18 x 150-mm) or serum bottles (ca. 120 or 160 ml) and crimp sealed. The tubes and bottles were then autoclaved at 121°C for 20 min.

Sodium sulfide (2.5%) was used to reduce the media prior to use. The media was always inoculated (5-10% inoculum) with a log phase culture. A CO or coal gas mixture was added using either a sterile syringe fitted with a one-way valve or a gas manifold in connection with a vacuum pump. The final pressure for the tubes or the bottles was 2 atm. The gas manifold was used for adjusting pressure (15-50 PSI) in the bottles inoculated with the organisms during pressure studies. Different percentages of CO (20-90%) or coal gas were also introduced using this manifold.

Control tubes without CO or coal gas were run simultaneously with each experiment. The cultures of P. productus were incubated (flat) in the dark at 37°C, and A. woodii was incubated at 30°C. In some experiments, P. productus tubes were agitated at 200 rpm using a temperature controlled New Brunswick Incubator Shaker (Model G-25). All incubations of R. rubrum (Strains I and II) and R. gelatinosa were performed under tungsten light at 30°C.

TABLE A.1

Composition of Media for the Growth of Rhodopseudomonas gelatinosa
and Rhodospirillum rubrum, Strain I

per 100 ml

Mineral #1*	50 ml
EDTA (0.425%)	0.1 ml
Pfenning's Trace Metal **	0.1 ml
B-Vitamins ***	0.5 ml
Trypticase	0.1 g
Resazurin (0.1%)	0.1 ml
Sodium Bicarbonate	0.35 g
Sodium Sulfide (2.5%)	2.0 ml
Gas Phase - N ₂ :CO ₂	60%-40%

* Contained per liter: K₂HPO₄ 9.0 g; KH₂PO₄, 9.0g; (NH₄)₂SO₄, 9.0 g; NaCl, 18.0 g; MgSO₄·7H₂O, 3.6 g; CaCl₂·2H₂O, 2.4 g; and NH₄Cl, 0.2 g.

** Contained per liter: ZnSO₄·7H₂O, 0.1 g; MnCl₂·4H₂O, 0.03 g; H₃BO₃, 0.3 g; CoCl₂·6H₂O, 0.2 g; CaCl₂·H₂O, 0.01 g; NiCl₂·6H₂O, 0.02 g; Na₂MoO₄·2H₂O, 0.03 g, and FeCl₂·4H₂O, 1.5 g.

*** Contained per 100 ml: B6 (pyridoxine) HCl, 1.9 mg, B1 (thiamine) HCl, 0.95 mg, Nicotinic acid, 0.95 mg; P-aminobenzoic acid, 0.195 mg, Biotin, 0.38 mg; Folic acid, 0.38 mg; B12 crystalline, 0.16 mg; B2 (riboflavin), 0.95 mg; Lipoic acid, 0.95 mg; and Pantothenic acid, 0.95 mg.

TABLE A.2

Composition of Media for the Growth of Acetobacterium woodii

	<u>Per liter</u>
Yeast extract	1.0 g
NH ₄ Cl	1.0 g
Mg SO ₄ ·7H ₂ O	0.1 g
KH ₂ PO ₄	0.4 g
K ₂ HPO ₄	0.4 g
Wolfe's Vitamin*	10.0 ml
Wolfe's Mineral **	10.0 ml
Fructose (25 ml of 20%)*	5.0 g
Resazurin (0.1%)	0.1 ml
Sodium Sulfide (2.5%)	20.0 ml
NaH CO ₃	3.0 g
Gas Phase N ₂ :CO ₂	80%-20%
Adjust pH to 6.7	

* Contained per liter: Folic acid, 2.0 mg, Pyridoxine Hydrochloride, 10.0 mg, Riboflavin, 5.0 mg; Biotin 2.0 mg; Thiamine, 5.0 mg; Nicotinic acid, 5.0 mg; Pantothenic acid, 5.0 mg; Vitamin B12, 0.1 mg; PABA, 5.0 mg; and Thiocetic acid, 5.0 mg.

** Contained per liter: Nitriloacetic acid, 1.5 g; MgSO₄, 3.0 g; MnSO₄, 0.5 g; NaCl, 1.0 g; FeSO₄, 0.1 g; CaCl₂, 0.1 g; CoCl₂, 0.1 g; ZnSO₄, 0.1 g; CuSO₄, 0.01 g; AlK(SO₄)₂, 0.01 g, H₃BO₃, 0.01 g; and Na₂ MoO₄, 0.01 g.

*** Filter sterilized and added to sterilized media.

TABLE A.3

Composition of Media for the Growth
of Rhodospirillum rubrum, Strain II

	<u>Per liter</u>
Malic Acid	2.5 g
Yeast Extract	1.0 g
(NH ₄) ₂ SO ₄	1.25 g
Mg SO ₄ ·7H ₂ O	0.2 g
CaCl ₂ ·2H ₂ O	0.07 g
EDTA (0.425%)	1.0 ml
KH ₂ PO ₄	0.6 g
K ₂ HPO ₄	0.9 g
Pfennig's Mineral*	1.0 ml
B-Vitamins**	7.5 ml
Resazurin (0.1%)	1.0 ml
Sodium Sulfide (2.5%)	20.0 ml
Gas Phase N ₂ -CO ₂	80%-20%
pH adjust to	7.0

* See footnotes of Table A.1 for details.

** See footnotes of Table A.1 for details.

Table A.4

Composition of Media for the Growth
of Peptostreptococcus productus

	<u>Per 100 ml</u>
Pfennings Mineral*	5.0 ml
Pfennings Trace Metal**	0.1 ml
B-Vitamins***	0.5 ml
Resazurin (0.1%)	0.1 ml
Yeast Extract	0.2 g
Sodium Bicarbonate	0.35 g
Sodium Sulfide (2.5%)	2.0 ml
Gas phase: N ₂ -CO ₂	80%-20%

* Contained per liter: KH₂PO₄, 10.0 g; MgCl₂·6H₂O, 6.6 g; NaCl, 8.0 g; NH₄Cl, 8.0 g; and CaCl₂·2H₂O, 1.0 g

** See footnotes of Table A.1 for details.

*** Contained per liter: Biotin, 20 mg; Folic acid, 20 mg; Pyridoxal hydrochloride, 10 mg; Lipoic acid, 60 mg; Riboflavin, 50 mg; Thiamine hydrochloride, 50 mg; Calcium-D-Pantothenate, 50 mg; Cyanocobalamin, 50 mg; PABA, 50 mg; Nicotinic acid, 50 mg; and Na₂SeO₃, 10 mg.

APPENDIX B

CALCULATION OF CO PRODUCTIVITY (VVD)

In order to determine the CO productivity for the reactor under study the volumetric flow rate of the gas through the reactor was measured, with the results being expressed in units of liters per day. The volumetric flow rates were then converted to standard temperature and pressure (STP) according to the equation:

$$V_{stp} = \frac{(P_{at} - P_{H_2O})}{P_{stp}} \times \frac{(T_{stp})}{T} \times (V) \quad (B1)$$

Where:

V_{stp} = gas flow in liter per hour at stp
 P_{at} = atmospheric pressure (mm Hg)
 P_{H_2O} = water vapor pressure (mm Hg)
 P_{stp} = standard pressure (760 mm Hg)
 T = operating temperature of the reactor ($^{\circ}K$)
 V = gas flow rate in liter per hour

CO productivity (VVD) was then calculated using the following equation:

$$VVD_{CO} = V_{stp} \times \frac{\% \text{ CO converted}}{100\%} \times \frac{24 \text{ hour}}{\text{day}} \times \frac{1}{V} \quad (B2)$$

Where:

VVD_{CO} = volume of CO converted per volume of the reactor or per volume of the culture (depending on the desired results) per day.

V = volume of the reactor or the volume of the culture.

APPENDIX C

SAMPLE CALCULATION OF METHANE PRODUCTION PER MOLE OF CO CONVERTED

Case 3 Mixed Culture

Feed Composition 65% CO and 22% H₂

The reactions of P. productus and Methanothrix can be combined as

Assume there are reactions as above for P. productus and Methanothrix,
Y reaction of R. rubrum and Z reaction of the Methanogen:

$$\text{From the feed composition, let } 4X + Y = 65 \quad (\text{C6})$$

$$4Z - Y = 22 \quad (\text{C7})$$

$$\text{Summary of (B6) and (B7), } X + Z = 21.75 \quad (\text{C8})$$

$$3X + Y - Z = (4X + Y) - (X + Z) = 43.25 \quad (\text{C9})$$

$$2X + Y - 2Z = (4X + Y) - Z (X + Z) = 21.5 \quad (\text{C10})$$

The final overall reaction equation is

Therefore, for every mole of CO converted, 21.75/65 moles of CH₄ are produced.