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Biological Production of Alcohols from Coal Through Indirect Liquefaction

Scientific Note

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INTRODUCTION

Microorganisms may be used to convert coal to chemicals either directly or indirectly by their action on synthesis gas. Microbial processes offer certain advantages over chemical conversions. Microorganisms exist and carry out conversions at ambient temperatures and pressures, which should result in substantial energy and equipment savings. Also, yields from microbial conversions are quite high, since the microorganism utilizes only a small fraction of the substrate for energy and growth. Under proper conditions, microbial conversions are quite specific, generally converting a substrate into a single product, with perhaps a few byproducts. These advantages are offset by slower reaction rates and special reactor considerations, such as sterility, nutrient provision, and so on.

Direct Coal Conversion

Direct microbial conversion involves the selection of a microorganism to produce liquid and gaseous fuels by direct biological action on coal, perhaps *in situ*. Although the prospects for success are limited, there is tremendous potential for this type of coal conversion process. In-

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dicative of the problems associated with direct microbial action on coal is the apparent toxicity of liquefied coal products and wastewater streams from the coal conversion processes (*1–5*). Also, it has been extremely difficult to get active flora to flourish on the ground cover composed of coal mine spoil or tailings (*6,7*).

Many species of fungi and a few bacterial strains have been found to degrade lignin. These include *Coriolus versicolor, Sporotrichum pulverulentium, Phanerochaete chrysosporium, Aspergillus fumigatus, Metulius tremellosus,* and some species of *Acetiomycetes* and *Eubacteria* (*8–11*). Some lignolytic fungi and bacteria have been shown to be capable of solubilizing lignite. A fungal mat of *Polyporus versicolor* has been used to produce a black water soluble liquid from leonardite (*12*). Bacterial species of the genus *Streptomyces* have been shown to solubilize lignite in submerged culture (*13*). However, conventional liquid fuels have not as yet been produced.

Indirect Coal Conversion

A more promising biological approach, perhaps, is the indirect coal conversion of synthesis gas by microorganisms capable of producing alcohols, acids, aldehydes, and so on from CO, CO_2, H_2, and H_2O. Synthesis gas is first produced by catalytic action on coal using conventional coal gasification techniques. The synthesis gas is then converted to liquid fuels biologically. Thus, the process is an indirect liquefaction process utilizing conventional and biological techniques.

A detailed and comprehensive review of the literature has identified organisms capable of converting CO, CO_2, and H_2 in synthesis gas to organic acids such as acetate and butyrate, and to methane (*14*). The literature also indicates that acetate may be utilized to produce higher organic acids, such as citric acid and lysine. However, no source identified mixed or pure cultures capable of producing liquid fuels from CO, CO_2, H_2, or acetate. Therefore, alternate pure cultures or bacteria isolated from natural sources must be utilized to determine the feasibility of biological liquid fuel production.

Purpose

The purpose of this project is to demonstrate the feasibility of producing liquid fuels from the components of synthesis gas through biological indirect liquefaction. The results of pure culture and natural source screening studies aimed at finding organisms capable of carrying out the conversions are presented and discussed.

MICROBIOLOGY OF BIOLOGICAL CONVERSION

The major known reactions in the biological conversion of synthesis gas are the formation of methane precursors and the biomethanation re-

actions. The organisms *Rhodopseudomonas gelatinosa* and *Rhodospirillum rubrum* utilize CO to produce CO_2 and H_2 by the reaction

$$CO + H_2O \rightarrow CO_2 + H_2 \quad (1)$$

The organisms *Peptostreptococcus productus, Acetobacterium woodii,* and *Eubacterium limosum* produce acetate (CH_3 COOH) by the reaction

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

All methane bacteria utilize CO_2 and H_2 to produce CH_4 by the reaction

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (3)$$

Methanothrix soehngenii utilizes acetate to produce CH_4 by the reaction

$$CH_3COOH \rightarrow CO_2 + CH_4 \quad (4)$$

All of these organisms have been isolated from sewage sludge or animal rumin. Thus, mixed cultures from natural sources or pure cultures of the above organisms might be utilized for the desired conversion to liquid fuels.

In addition, alcohols can, theoretically, be produced from carbon monoxide, carbon dioxide, and hydrogen or acetate as indicated by the free energies of reaction. Table 1 presents a list of possible reactions utilizing these substrates as reactants, with the associated free energies. Reactions with highly negative free energies have the most potential as being viable reactions. As noted, there is strong likelihood that methanol (CH_3OH), ethanol (C_2H_5OH), and butanol (C_4H_9OH) may be produced from synthesis gas. CO, CO_2 and H_2 appear to be better feedstocks than acetate.

Table 1
Theoretically Feasible Reactions Using CO, CO_2, and H_2 or Acetate

ompound	Net Reactions	Δ G^0, kcal/mol of product formed[a]
lethanol	$CO + 2H_2 \rightarrow CH_3OH$	−6.99
	$3CO + 2H_2O \rightarrow CH_3OH + 2CO_2$	−16.64
	$CO_2 + 3H_2 \rightarrow CH_3OH + H_2O$	−2.23
	Acetate + $4H^+ \rightarrow 2CH_3OH$	10.31
thanol	$2CO + 4H_2 \rightarrow C_2H_5OH + H_2O$	−32.83
	$6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2$	−51.93
	$2CO_2 + 6H_2 \rightarrow C_2H_5OH + 3H_2O$	−23.31
	Acetate + $2H_2 \rightarrow C_2H_5OH + H_2O$	−2.3
utanol	$4CO + 8H_2 \rightarrow CH_3(CH_2)_2CH_2OH + 3H_2O$	−77.86
	$12CO + 5H_2O \rightarrow CH_3(CH_2)_2CH_2OH + 8CO_2$	−116.16
	$4CO_2 + 12H_2 \rightarrow CH_3(CH_2)_2CH_2OH + 7H_2O$	−58.62

[a]Calculated from Thauer et al. (*15*).

BACTERIAL SCREENING

Natural sources of bacteria such as sewage sludge, animal wastes, and muds serve as a final repository for a consortium of bacteria, capable of converting a wide variety of substrates to many different products. Almost all of the pure culture bacteria found in culture collections today originated from a mixed bacterial population isolated from such natural environments. The natural carbon flow in the mixed cultures in sewage sludge and animal rumin is the metabolism of substrate to methane. Thus, methane inhibitors must be added to samples from these sources in order to stop methane production and to allow intermediates to form. Acclimation to CO, CO_2, and H_2 must be achieved over an extended period of time, during which the cultures are supplemented with basal salts, vitamins, and minerals.

Procedures

Several experimental studies were initiated in an attempt to produce alcohols from CO, acetate, and synthesis gas. Three natural sources of inocula were utilized, sewage sludge, chicken waste, and coal/coal mud samples. One mL of natural inocula sample was added to 9 mL of basal media containing acetate (80 m*M*), Co (52–55%, 2 atm), or synthetic synthesis gas as the primary carbon sources. Although the synthesis gas did not contain contaminants such as H_2S, COS, or SO_2, previous studies with other organisms in the presence of these contaminants showed no effect on CO uptake of sulfur gas concentrations of 2% or less. Similar studies are planned for organisms isolated in these studies. Monensin (1 μg/mL) and 2-bromoethanesulfonic acid (BESA) (20 μM/mL) were used as methanogenic inhibitors. Anaerobic media were prepared using the Hungate method and were placed in serum tubes sealed with butyl rubber stoppers. Samples were incubated at 37°C with the pH between 4 and 7. Gas samples were analyzed for the consumption of synthesis gas and liquid samples were analyzed for the production of acids and alcohols.

The gas phase composition was measured by gas-solid chromatography using a Porapak QS column. The liquid phase composition was measured by gas-solid chromatography using separate procedures for alcohol and acid analysis.

A large number of these samples were inoculated at various conditions in an attempt to produce alcohols. All experiments were conducted in triplicate. After a 3–4 wk period, the cultures were transferred with fresh media and gases and the experiments repeated. Successive transfer of the best cultures allows the mixed population to evolve to utilize the desired substrate and produce the desired products.

Results—Inhibition of Methanogenesis

As mentioned previously, the natural tendency for most mixed bacterial cultures is to produce methane from organic substrates. In order to

produce alcohols, which are intermediates in methane synthesis, inhibitors must be added to the cultures to block methane production. Therefore, the first step was to determine whether methanogenesis could be blocked with certain chemical inhibitors.

An example of the inhibition of methanogenesis using CO the substrate with a sewage sludge inoculum is shown in Table 2. Without an inhibitor, methane concentrations as high as 10% were found in 14 d, and over 20% in 35 d. In the presence of monensin, the maximum methane concentration produced was 9% in 35 d. In the presence of BESA, the maximum methane concentration was less than 5% in 35 d. Both monensin and BESA appear to be good inhibitors of methane production in a mixed culture.

Results—Initial Alcohol Production

After several months of culture transfer and acclimation, small quantities of acids and alcohols began to appear in some of the cultures from the conversion of CO in synthesis gas. Table 3 shows qualitative results for acid and alcohol production from CO and acetate as a function of pH using a sewage sludge culture in the presence of the two methane inhibitors. As noted, C_1–C_4 alcohols were produced, as well as C_2–C_4 organic acids.

Small quantities of acetic, propionic, *n*-butyric and *i*-butyric acids were found in essentially all of the samples, with acetic acid the predominant acid produced. Methanol was found in approximately half of the

Table 2
Inhibition of Methanogenesis from CO Using Sewage Sludge as Primary Inoculum

Substrate and Inhibitors	pH	Mol % Day 14 CO	Day 14 CH_4	Day 35 CO	Day 35 CH_4
CO[a]	4	51.7	.6	47.3	.25
	5	39.7	9.0	17.3	20.9
	6	41.9	7.7	21.5	16.4
	7	39.5	10.6	15.4	20.5
CO	4	53.7	0	46.3	0.47
Monensin[b]	5	42.0	9.3	0	25.1
	6	48.8	4.9	47.1	6.1
	7	49.4	3.2	38.0	9.2
CO	4	50.8	.3	27.5	1.2
BESA[c]	5	46.7	.8	23.8	3.2
	6	44.1	1.1	30.3	2.3
	7	37.8	2.7	5.1	4.8

[a]CO level was 52–55%, 2 atm on 0 d.
[b]1 μgram/mL (μg/mL) filter sterilized.
[c]20 μM, filter sterilized.

Table 3
Production of Alcohols and Acids by Bacterial Enrichments Obtained from Sewage Sludge at Various Levels of pH and in the Presence of Methane Inhibitors

pH	Inhibitor	+BESA, 20 μM	+Monensin, 1 μg/mL
CO as carbon source[a]			
4		E,B,A,PR,1B,NB	M,E,B,A,PR,1B,NB
5		M,E,B,A,PR,1B,NB	M,E,A,PR,NB
6		A,PR,1B,NB	M,E,B,A,PR,1B,NB
7		A,PR,1B,NB	M,A,PR,1B,NB
Acetate as carbon source[b]			
4		M,P,A,PR,1B,NB	A,PR,1B,NB
5		M,P,A,PR,1B,NB	A,PR,1B,NB
6		A,PR,1B,NB	A,PR,1B,NB
7		A,PR,1B,NB	M,E,A,PR,1B,NB

[a]Gas phase contained 60% CO by volume (2 atm); balance 80:20 N_2, CO_2
[b]8m*M*/mL concentration. All samples were taken after 28 d of incubation.Samples were transferred at least 5 times before these analyses were done.

M Methanol
E Ethanol
P Propanol
B *n*-butanol
A Acetic acid
PR Propionic acid
1B iso-butyric acid
NB *n*-butyric acid

Values for alcohols are less than 1g/L. Values for acids are more than 1g/L.

samples. Propanol was produced only in the samples fed acetate at pH 4 and 5 using a BESA inhibitor. Ethanol and *n*-butanol were produced only from CO, and most prominantly at pH 4 and 5. The most predominant alcohol produced was ethanol. Although quantitative results are not given, the typical concentration levels were .01–.5 g/L, with acids being more prevalent than alcohols.

Similar results were obtained from the inoculum derived from chicken waste. The coal mud samples were much less productive and were abandoned. From the preliminary screening data, mixed cultures were shown to produce alcohols when CO, CO in synthesis gas, or acetate was used as the sole carbon source. In general, more alcohols were detected in the samples with lower pH and in the presence of methanogenic inhibitors such as BESA and monensin.

Results—Experiments to Quantify Alcohol Production

The next step in manipulating the mixed cultures for enriching specific microflora in producing alcohols is the quantification of the timing of alcohol production. Selected experiments were carried out at pH levels from 4 to 7, monitoring gas concentration, alcohol concentration and acid concentrations on a daily basis. The criteria used in selecting inocula for these experiments was based upon previous results where

sampling had taken place on a weekly to biweekly basis. These inocula consisted of both sewage sludge and chicken waste inocula at various pH levels and had showed either the best utilization of CO or CO in synthesis gas, or the best production of alcohols of all samples at a given pH in the previous studies.

Five mL aliquots of inocula were anaerobically transferred into 20 mL of liquid media in 65 mL stoppered bottles. The liquid media contained yeast extract, vitamins, basal salts, and either monensin or BESA as the methane inhibitor. The pH was adjusted to the desired level by the addition of acid or base as needed. The gas phase was initially at a pressure of 1 atm, and consisted of either CO in CO_2 and nitrogen (N_2) or a synthetic synthesis gas mixture of CO, H_2, CO_2, and CH_4. The gas phase with N_2 consisted of approximately 64 mol% CO, 24% N_2, and 12% CO_2. The synthesis gas phase consisted of approximately 15 mol% H_2, 73% CO, 2% CH_4, and 10% CO_2.

Representative results of CO and CO in synthesis gas utilization studies are shown in Table 4 and Fig. 1. In this experiment, a chicken waste inoculum was utilized in the presence of BESA inhibitor. The initial pH of the experiment was 5.0. Only alcohol production is shown, although organic acids were also produced. The ratio of total acids to total alcohols was approximately 8:1. As noted in the Figure and Table, the carbon monoxide concentration of the gas phase decreased from 73 to 39% CO in 14 d. Very little H_2 or CH_4 was utilized. The CO_2 concentration increased from 9 to 39% during the experiment, indicating the production of CO_2 with alcohol and acid production. The predominant alcohol produced during the experiment was ethanol, reaching a concentration of .12 g/L after 13 d. Lesser amounts of methanol, propanol, methyl propanol, and *n*-butanol were also produced.

It was found through continued experimentation that the culture isolated from chicken waste at pH 5 with a BESA inhibitor outperformed the other culture with regard to CO utilization and alcohol production. At pH 4, alcohol and acid production was limited to background production from yeast extract. At pH 6 and 7, alcohol production was negligible, whereas acid production was quite high. The results with the BESA inhibitor appeared to be better than with monensin. Media manipulation studies were thus carried out on the preferred culture to learn more about alcohol production from synthesis gas.

CULTURE OPTIMIZATION STUDIES

Although alcohol production had been demonstrated from coal synthesis gas, several questions remained with regard to the culture and its use in a process scheme.

1. Are alcohols and acids produced by a single organism, or is production by a group of organisms?

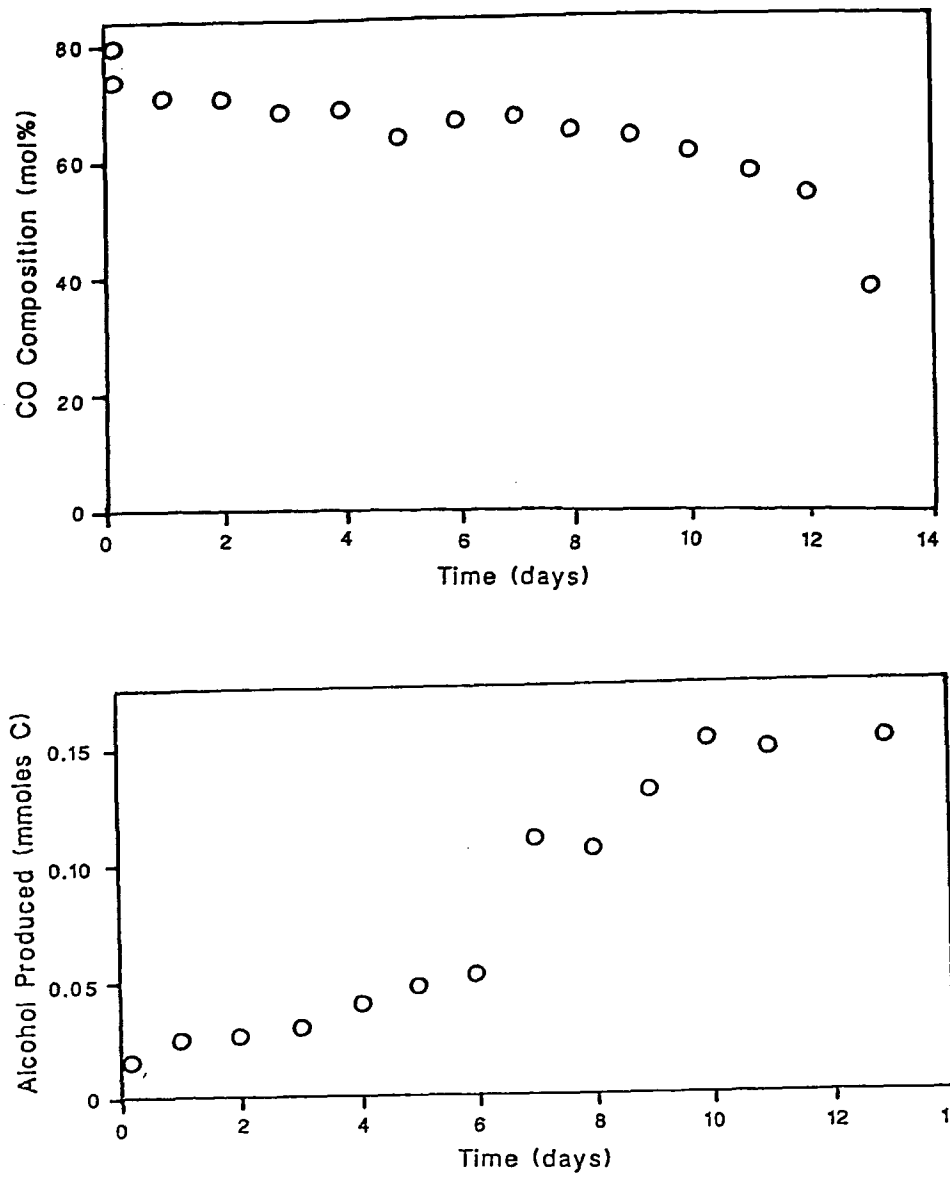

Fig. 1. Synthesis gas consumption and alcohol production at pH 5 -- chicken waste inoculum, BESA inhibitor.

2. What media factors influence alcohol and acid production, and how can alcohol production be maximized while minimizing acid production?
3. Can a single alcohol be produced from synthesis gas?
4. What substrates are utilized under various conditions?

The answers to these questions are under study in the University of Arkansas laboratories. Qualitative results are presented in the following paragraphs, addressing several of these points.

Table 4
Synthesis Gas Utilization at pH5: Chicken Waste Inoculum, BESA Inhibitor

	Gas phase composition, mol %					Alcohol production, g/L				
Day	H_2	N_2	CO	CH_4	CO_2	MeOH	EtOH	PrOH	MePrOH	BuOH
0	14.6	.9	73.1	2.0	9.0	.004	.001	—[a]	—	—
1	14.4	1.0	71.3	2.0	11.1	.013	.011	—	—	.002
2	14.2	1.5	70.3	1.9	11.9	.010	.016	.001	—	—
3	13.4	.9	68.8	2.8	14.0	—	.015	.002	.008	—
4	14.1	.8	69.1	2.2	14.5	.017	.017	.006	—	—
5	20.0	1.4	63.7	1.7	13.0	.010	.026	.004	.004	—
6	13.6	1.3	68.8	1.9	15.8	.009	.032	.007	.002	.002
7	13.5	1.1	67.8	2.1	15.6	.012	.045	.010	.010	.019
8	13.9	1.3	64.9	2.1	17.3	—	.070	.015	—	.008
9	13.4	1.3	64.1	1.9	19.2	.012	.078	.021	—	.008
10	13.0	1.9	61.5	2.1	21.4	.013	.099	.024	—	.004
11	12.6	1.6	58.1	1.9	25.7	.016	.105	.017	—	—
12	12.8	2.3	54.1	2.1	28.7	.011	.067	.019	.003	—
13	11.9	1.3	50.8	2.7	33.2	.013	.117	.013	—	—
14	10.8	9.6	38.6	2.3	38.6	—	—	—	—	—

[a]None detected.

Media Background Studies

An experiment was performed using the mixed culture without CO or synthesis gas as the carbon source. Only background media (salts, vitamins, yeast extract) were present. A summary of the results of this experiment are shown in Table 5. As noted, essentially no alcohol production occurred from the media at pH 5. However, significant levels of organic acids were produced, probably using yeast extract as the carbon source. This result could be important in eliminating or minimizing acid production from CO in synthesis gas.

The Effects of Agitation

Preliminary experiments were carried out in batch bottles at 37°C without agitation. Since the utilization of synthesis gas components requires transfer of a marginally soluble gas into the liquid phase, agitation may prove to be beneficial in improving mass transfer and thus improving reaction rate. Studies carried out in biologically converting carbon monoxide to acetate and methane have shown dramatic improvements in rate with agitation (*16*).

Experiments were carried out with gentle agitation in a shaking incubator at 37°C. Table 6 shows the time for a given CO conversion with and without agitation. As noted, the rate of CO uptake increased dramatically with agitation requiring only 2 d for 90% CO utilization, as opposed to 15–20 d in previous experiments. If more cells are present, the rate increases further. The ratio of alcohol to acid did not improve, but it was noticed that hydrogen was utilized after all of the CO had been exhausted from the culture. If the CO and H_2 levels were allowed to fall to zero, the culture died.

The Role of BESA

In metabolizing CO in synthesis gas to alcohols and acids, it was noticed that the levels of methane in the gas phase were essentially con-

Table 5
Production of Alcohols and Acids from Background Media, pH5

	Total Concentration, g/L	
Day	Alcohol	Acid
1	.047	.440
3	.009	.544
5	.021	.711
7	—	.710
9	.078	.745
11	.036	.032
13	—	.840
15	.001	.737

Table 6
CO Uptake by the Mixed Culture With and Without Agitation, 10% Inoculum

Percent CO conversion	Time required to reach conversion, h	
	Without agitation	With agitation
3	24	2.5
6	72	6
10	120	22.5
15	240	24
20	264	27.1
30	312	30.2
50	340	35.6
70	"	42.5
0	"	46

"Blanks indicate no sample taken.

stant at a level of 2% or less. BESA is known to be a strong inhibitor of several biological reactions. Since BESA was added for the sole purpose of inhibiting methane production, there may be benefits to inhibitor removal from the enriched culture.

Experiments were carried out using the enriched culture without the presence of the BESA inhibitor. The results of these experiments are shown in Table 7, where ethanol and acetate concentrations are shown with time for experiments with and without the addition of BESA. The times shown in the table reflect the fact that multiple gas additions were employed to replenish the CO supply since the total time for the experiments was 300 h. When BESA was excluded from the media, methane

Table 7
Production of Ethanol and Acetate from CO With and Without BESA Inhibitor

Time, hr	Concentration, g/L			
	Ethanol		Acetate	
	W/BESA	W/O BESA	W/BESA	W/O BESA
0	.07	.06	"	"
27	.08	.08	.41	.23
67	.09	.21	.52	.24
75	.13	.40	.55	.22
100	.35	.91	.76	1.84
125	.66	1.86	2.76	1.95
150	1.10	2.54	3.25	2.22
200	1.12	3.67	3.85	3.44
300	1.14	4.12	4.62	3.86

"Blanks indicate no sample taken.

production levels remained low. In addition, the levels of ethanol increased, whereas the acetate levels decreased slightly.

The Role of Yeast Extract

Previous experiments indicated that the culture evidently uses yeast extract in producing organic acids. No alcohols, however, were produced without the presence of carbon monoxide. It was decided to determine the necessary levels of yeast extract to maximize ethanol production, while minimizing acid production.

When yeast extract was eliminated from the media, the ratio of ethanol to acetate improved by 300% (data not shown) (*17*). However, organic acids remained in the media. Subsequent experiments without yeast extract using the same culture were not as successful, and indicated that a minimum level of yeast extract (approximately .01%) is necessary to provide trace nutrients.

Media Optimization Results

The results of an experiment without inhibitors is shown in Table 8 and Fig. 2. Gentle agitation was provided to increase the rate of transfer of CO from the bulk gas phase to the liquid phase. A rather long lag phase of 76 h was required because of a low inoculum level of 2% instead of the usual 10%. The dashed lines in the upper plot of Fig. 2 indicate multiple gas additions. No liquid media additions were made.

Because of the agitation, 72% of the CO from synthesis gas was utilized in 16.7 h. In this study, the maximum ethanol concentration reached 4.3 g/L. The maximum acetic acid concentration reached 3.9 g/L, yield a ratio of the maximum acetic acid concentration to maximum ethanol concentration of only .9. It thus appears quite possible to increase the ethanol concentration, while at the same time decreasing the ratio of acid/alcohol. The removal of the inhibitors benefited alcohol production.

CULTURE DEVELOPMENT

Several dilution experiments were performed in order to purify the highly enriched culture. The culture was diluted up to 10^{10} times with media and incubated at 37°C. Bacterial growth, CO utilization, and product formation were monitored as needed. Bacterial growth occurred in all tubes up to a dilution of 10^8, and occurred in some tubes at 10^9 and 10^{10} dilution.

The product from all of the experiments contained both acetate and ethanol. It thus appears that a single culture produces both of these products, perhaps through acetyl-coA.

Table 8
CO Consumption, Acid and Alcohol Production from Synthesis Gas: Gentle Agitation, No Inhibitor

	Gas composition, volume percent					Alcohol production, g/L					Acid production, g/L			
Time, h	H_2	N_2	CO	CH_4	CO_2	MeOH	EtOH	PrOH	MePrOH	BuOH	HA_c	HP_r	HBu	IBuH
0	13.9	5.0	69.1	1.4	10.2	.027	.064	—[b]	.001	.001				
17.1	13.8	4.4	68.6	1.9	11.3	.032	.061	—	.001	.001	.231	.007	—	—
27.5	13.5	4.2	66.9	1.7	13.6	.017	.075	—	—	—				
42.25	13.7	3.9	69.3	1.7	11.1	.021	.075	—	—	—				
52.	13.8	4.2	67.8	1.7	12.3	.019	.085	—	—	—				
66.2	14.3	4.1	63.5	1.8	16.2	.029	.205	—	—	—				
75.3	14.3	4.6	50.9	2.0	26.9	.032	.404	—	.001	.002	.223	.006		
90.5	10.9	7.4	17.9	3.4	60.3	.032	.491	—	.001	.002				
99.5	13.0	4.3	29.6	2.9	50.1									
New Gas														
91	13.3	1.4	66.2	3.2	15.7	.031								
100.6	13.3	1.7	37.6	2.4	44.8	.031	.909		.001	.001	1.843	—	—	—
New Gas														
103.3	13.0	.3	68.3	2.1	16.1									
115	10.4	.7	27.6	3.4	57.8	.032	1.338	.001	.001	.001				
New Gas														
116.55	13.7	.4	68.1	1.8	15.7									
127	11.6	.5	34.8	2.7	50.3	.031	1.858	.001	.001	.001	—			
New Gas														
128.5	13.1	1.4	68.4	1.7	14.5									
145.2	7.4	1.8	19.4	3.3	67.9	.030	2.135	.001	.001		2.054	—	—	—
New Gas														
147.25	13.4	.8	67.8	1.9	15.9									
165.25	16.4	.9	25.7	2.4	54.6	.041	3.733	.001	.008	.004				
New Gas														
166.75	13.1	1.5	66.7	2.0	16.7									
190.25					.038	3.673	.001	.002	.002	3.441	—	—	—	
210.25	15.2	1.5	48.4	2.0	33.7	.043	4.302	.001	.008	.003				
New Gas														
211.0	12.3	8.2	16.1	2.2	16.6									
338	15.7	4.1	46.0	2.6	31.6	.035	3.859	.001	.002	.001	3.049	—	-	—
261	16.2	4.6	34.0	2.8	41.7	.027	4.118	.001	—	—	2.788		.010	
262	13.6	.8	69.2	2.3	14.1									
285	14.3	.9	65.1	1.9	17.6	.037	4.086	.001	.001	.003				
312	14.6	.9	61.7	1.9	20.6	.026	4.118	.001	—	.001	3.855	—	—	—
366.5	14.7	.9	59.6	2.3	22.5						3.382	—	—	—

[a]Blanks indicate no sample taken.
[b]— None detected.

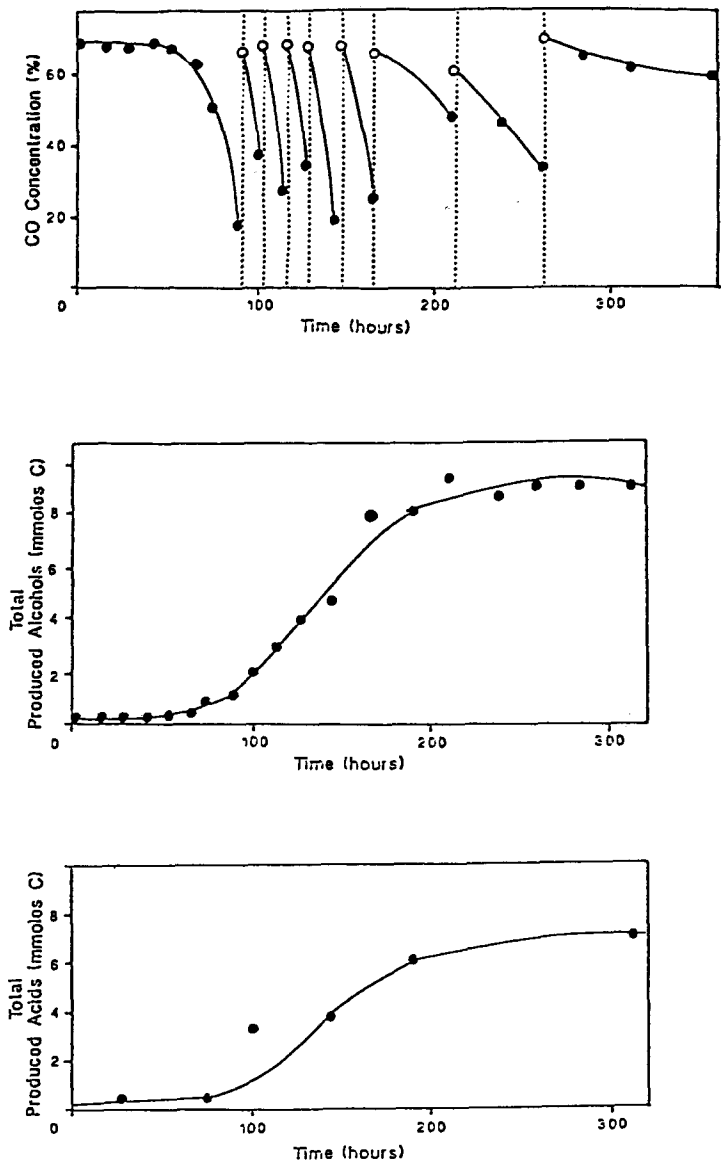

Fig. 2. CO conversion, alcohol and acids production from synthesis gas: pH 5, BESA culture, gentle agitation, no inhibitor.

Aliquots from the third dilution experiment were spread onto agar plates and incubated anaerobically at 37°C. Colonies were picked from the plates and inoculated into fresh media. These experiments are in progress, monitoring growth, substrate utilization and product yield.

Preliminary observations indicate that the culture is a *Clostridium*. More specific identification techniques are needed for positive identification and characterization.

CONCLUSIONS

A bacterial culture has been isolated from animal waste that is capable of converting CO in synthesis gas to ethanol and acetate. The culture requires a yeast extract level of approximately .01 g/L, and the conversion is enhanced by agitation. The culture produces a higher yield of ethanol and ratio of ethanol to acetate when BESA and excess yeast extract are removed from the media. An ethanol concentration of 4.3 g/L has been obtained in batch screening experiments.

The culture has been purified by successive dilution and tentatively identified as a member of the *Clostridium* species. Further experimentation is required for positive identification.

FUTURE WORK

Experiments in the near future will be concerned with further culture and media optimization in an effort to maximize ethanol levels and minimize acid levels. Preliminary reactor feasibility experiments will begin shortly. Culture identification experiments to isolate and positively identify the culture will also be initiated shortly. The long-term goal of these experiments is to obtain a culture capable of converting CO in synthesis gas to ethanol. Reaction kinetic and mass transfer studies will be carried out in the future to define design parameters for scaleup.

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