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Biosolubilization and Liquid Fuel Production from Coal

M. D. ACKERSON,* N. L. JOHNSON, M. LE, E. C. CLAUSEN, AND J. L. GADDY

University of Arkansas, Department of Chemical Engineering, Fayetteville, AR 72701

ABSTRACT

Recently, several microorganisms have been shown to be capable of directly solubilizing low-rank coals. This bioextract has a high molecular weight and is water soluble, but is not useful as a liquid fuel. This paper presents the results of studies to biologically solubilize coal and convert the solubilized coal into more useful compounds. Preliminary experiments have been conducted to isolate cultures for the serial biological conversion of coal into liquid fuels. Coal particles have been solubilized employing an isolate from the surface of Arkansas lignite. Natural inocula, such as sheep rumen and sewage sludge, are then employed in developing cultures for converting the bioextract into fuels. This paper presents preliminary results of experiments in coal solubilization and bioextract conversion.

Index Entries: Biosolubilization; fuel production; coal; lignite.

INTRODUCTION

Microbial solubilization of coals and lignites is being developed as an alternative method of liquid fuels production. The biological approach under study offers the potential of significant cost savings by converting solid coal to a liquid product, with minimal loss in total energy content, at near ambient conditions of temperature and pressure. The phenomenon of coal utilization and liquefaction by fungal and bacterial species was first reported in the early 1980s (*1,2*). A number of strains of fungi and filamentous bacteria are now known to interact with low-rank coals, via extracellular processes, to yield a darkened medium when grown in sub-

*Author to whom all correspondence and reprint requests should be addressed.

merged culture (*3,4*), or dark droplets of liquid on the coal's surface when grown on agar surface culture (*1,3,5*).

The coal liquid produced by bioliquefaction is a mixture of water soluble, polar organic compounds with relatively high molecular weights. Ultrafiltration and gel permeation chromatography have shown that the molecular weight is in the range of 30,000 to 300,000 Dalton (*3*). The chemical structure is extremely aromatic, with a large number of hydroxyl groups. Because of the low relative volatility, analysis to quantify the product by mass spectrophotometry or gas chromatography has been difficult.

Although this technology has great potential, there are a number of serious problems to be solved. In the absence of water or suitable solvent, the product is a solid. Although the solubilized product has a reasonably high energy content and may be useful as a combustion fuel, it is not suitable as a transportation fuel. Also, most of the organisms require expensive sugars and media for growth over a period of about 2 wk. Inexpensive media and fast-growing organisms will be required for commercial application. Another serious economic problem relates to the requirement for pretreatment to achieve high yields. Highly oxidized lignites, such as leonardite, can be converted almost entirely. Higher rank coals must be chemically oxidized before significant liquefaction occurs in a reasonable time (*6,7*). Chemical treatment is likely to be cost prohibitive.

Research at the University of Arkansas has led to the isolation of several organisms, as yet unidentified, that are fast growing in inexpensive mineral salts media and that convert coal into liquid and flocculant in a few hours in surface or submerged culture. High-rank coals are converted without pretreatment. One culture is a fungus isolated from a lignite outcrop. Another is a rod-shaped bacterium that is not a *Streptomyces* and is likely a previously unknown strain with coal or lignin activity. Further study is continuing to develop and quantify coal conversion with these organisms.

It is highly unlikely that any single organism will be able to completely liquefy coal to low molecular weight fuels. However, it is likely that organisms can be utilized to convert the high molecular weight microbial products to useful fuels. Such a second-stage conversion should probably be anaerobic to avoid further oxidation of the product. The purpose of this study is to define cultures of microorganisms to degrade untreated coal and define a second-stage culture for the serial biological conversion of the coal bioextract to liquid fuels. This paper presents a brief summary of progress to biologically produce low molecular weight liquid fuels from coal.

MATERIALS AND METHODS

Solubilization Studies

The preliminary work for producing solubilized lignite was carried out in submerged culture in a 1.5 L Biostat-M stirred-tank fermenter by B.

Braun Instruments (Bethlehem, PA). The fermenter was equipped with pH and temperature control, as well as dissolved oxygen monitor. All studies were performed at 28°C and a stirring rate of 150 rpm with filtered air for aeration.

Three different organisms have been isolated that show an ability to solubilize untreated coals. The first organism isolated, H12, is a bacterium obtained from a high-rank coal mud. An inoculum of mud was taken from a drainage area of a high-rank Wyoming bituminous coal pile, and the organism was isolated from the mud by successive transfers into flasks containing Wyoming coal as the only carbon source.

The organism used in most of the solubilization experiments presented in this paper is a lignite surface culture, LSC, isolated from a weathered outcrop of Arkansas lignite collected from Saline County, AR. This same lignite was used as the coal source for the solubilization experiments. Another organism has been isolated that grows on coal as the sole carbon source.

Inocula Cultures

The inocula cultures were grown on 4% Sabouraud Maltose Broth (Difco, Detroit, MI) for 2–3 d at 30°C and shaking at 150 rpm in a G-30 Shaking Incubator (New Brunswick Scientific, New Brunswick, NJ) until the dry weight of the culture was approximately 1.5 g/L. The pH of these cultures was approximately 8.0.

Solubilization with Cell Extract

The medium used for the cell extract solubilization experiments consisted of 6.5 g glucose and 6.5 g peptone (Difco) in 1300 mL distilled water. The media was sterilized by autoclaving at 120°C for 25 min. The pH of the medium was maintained at 9 and controlled automatically by the addition of 2*N* NaOH or HCl.

After sterilization, the fermenter was inoculated with 50 mL of seed culture. The culture was allowed to grow without the presence of lignite, and samples were withdrawn every 12–24 h. The samples were analyzed for glucose concentration and cell dry weight. These samples were then centrifuged at 2000*g* for 10 min to remove the cells. After the cells were removed, 0.3 g Arkansas lignite (60–100 mesh) was added to 15 mL of the cell-free culture broth. These samples were maintained at 30°C in a shaker incubator (150 rpm) for 28 h, at which time they were removed and analyzed for lignite solubilization.

Determination of Solubilizing Activity

Arkansas lignite (60–100 mesh, no pretreatment) was used for all solubilization assays. The lignite concentration was held constant at 2% (w/v). Normally, 0.30 g Arkansas lignite was added per 15 mL of cell-free (by centrifugation @2000*g*, 10 min), pH adjusted (NaOH or HCl) broth in a screw cap culture tube lying horizontally in a 30°C shaking incubator (150

Table 1
Media for Submerged Culture Solubilization

Nutrient	g/L
$(NH_4)_2\ SO_4$	2.6
K_2HPO_4	1.0
KH_2PO_4	0.5
$MgSO_4 \cdot 7H_2O$	0.2
$CaCl_2 \cdot 2H_2O$	0.01
Yeast extract (Difco)	1.0
Glucose	2.5

rpm). The activity was measured by filtering undissolved lignite and cells from the sample and acidifying the filtrate to pH 0.97 with 4*N* HCl to precipitate the solubilized coal, then centrifuging at low speed (500 rpm, 5 min) and drying the pellet in a tared aluminum pan at 80°C overnight. The weight of the pellet represented the recovered solubilized coal.

Submerged Culture Solubilization

The media used in the direct submerged culture solubilization of lignite is shown in Table 1. The media was sterilized by autoclaving for 25 min. The pH was maintained at 9 and controlled by the addition of 2*N* NaOH or HCl. After sterilization, the fermenter was inoculated with 50 mL of seed culture.

Approximately 2 h after autoclaving, 12.5 g of 60–100 mesh untreated Arkansas lignite were added to the fermenter. The lignite had been sterilized by heating in a 150°C oven for 24 h. Samples were withdrawn from the fermenter every 24 h and analyzed for glucose and solubilized lignite concentration.

Owing to the mixture of cells and unsolubilized lignite, the cell concentration could not be determined. To determine the lignite solubilization, the samples were centrifuged at 2000*g* for 10 min, and the absorbance was measured at 580 nm, or the lignite was precipitated and weighed. A wavelength of 580 nm was chosen, based on the ability to develop a standard curve at this wavelength.

Nutrient Studies

Work is presently being conducted to develop media that can produce rapid growth of the organisms, without the use of expensive nutrients. Experiments are being performed in which the organisms are grown in media containing only mineral salts and ground corn cobs as the carbon source. This media produces fast growth of each of the coal solubilizing organisms, and preliminary work has shown that the solubilization capabilities are retained when the organisms are grown in this media.

Conversion of Solubilized Lignite to Liquid Fuels

Preliminary experiments have been conducted to determine the feasibility of serial conversion of coal to alcohol fuels. The experiments have used sewage sludge, animal rumen fluid, or soil environments as natural inocula.

The solubilized lignite used as the substrate for the anaerobic bacteria was obtained from submerged culture experiments. LSC was used to solubilize the coal, as in the direct solubilization method above. The solubilized coal was recovered by filtering the media after several days of fermentation and precipitated by adjusting the pH of the solution to 0.97 with HCl. The precipitate was washed and dried and then redissolved in distilled water by adjusting the pH to 6.5. The concentration of the redissolved lignite was approximately 50 g/L. Portions of this solution were then used as substrate in attempts to produce alcohol fuels.

Rumen Fluid Inocula

In addition to using sewage sludge as a source of alcohol-producing bacteria, rumen fluids from cows and sheep were used as inocula. Rumen fluid has been used frequently as a source of bacteria capable of breaking down complex structures (*8–10*). Cultures were started with fresh rumen contents from cows and sheep. Only sheep rumen cultures showed lignite-degrading potential and were studied further. Following growth of serial dilutions of a sheep rumen culture, the dilution tube showing the greatest color change from baseline, as measured on the spectrophotometer by its absorbance at 580 nm, were transferred to fresh media; subsequent sheep rumen cultures were all derived from this dilution tube. Cultures were started generally with a 10% inoculum to fresh media. Incubation was at 37°C and shaking at 100 rpm.

The original rumen fluid culture media is shown in Table 2. The pH was adjusted to 7 with NaOH. Transfer media is shown in Table 3. The pH of the transfer media was also adjusted to 7 with NaOH.

Media solutions were made anaerobic by briefly boiling, then cooling under 80% N_2/20% CO_2; the gases passed over heated copper to remove any oxygen. Anaerobic media was transferred to 100 mL stoppered serum bottles and autoclaved at 15 psig for 20 min. Just prior to inoculation of media, $Na_2S \cdot 9H_2O$, 0.05%, was added to each media bottle to lower the oxidation-reduction potential. Resazurin was not added as an indicator of anaerobiosis since it would interfere with spectrophotometric readings; however, experience with the anaerobic techniques used here have shown them to be reliable.

A mixed culture derived from sheep rumen, which appeared to degrade lignite at 0.03 wt%, was streaked onto plates; solid media had the same composition as the liquid media with glucose, with the addition of 2.0% agar (Difco). Plates were poured, and all inoculating was done in-

Table 2
Original Media for Anaerobic Fuels Production

Nutrient	Amount
Yeast extract	0.1 g
B-vitamins (Wolfe's)	1.0 mL
$KH_2\ PO_4$	0.6 mg
$(NH_4)_2\ SO_4$	0.6 mg
NaCl	1.2 mg
$MgCl_2 \cdot 6H_2O$	0.4 mg
$MgSO_4 \cdot 7H_2O$	0.3 mg
Lignite	0.03 g
Distilled water	100 mL

Table 3
Transfer Media for Anaerobic Fuels Production

Nutrient	Amount
Yeast extract	2.0 g
Glucose	5.0 g
B-vitamins (Wolfe's)	10.0 mL
$ZnSO_4 \cdot 7H_2O$	0.5 mg
$MnCl_2 \cdot 4H_2O$	0.15 mg
H_3PO_3	1.5 mg
$CoCl_2 \cdot 6H_2O$	1.0 mg
$NiCl_2 \cdot 6H_2O$	0.1 mg
$Na_2\ Mo\ O_4$	0.5 g
$FeCl_2 \cdot 4H_2O$	7.5 mg
$Na_2\ Se\ O_3$	0.05 mg
$KH_2\ PO_4$	0.5 g
$MgCl_2 \cdot 6H_2O$	0.33 g
NaCl	0.4 g
$NH_4\ Cl$	0.4 g
$CaCl_2 \cdot 2H_2O$	50 mg
Rumen fluid (filtered and sterilized)	109 mL
Lignite	0.3 g
Distilled water	1000 mL

side an anaerobic chamber. Inoculated plates were incubated at 37°C in an anaerobic jar (Oxoid, Columbia, MD). Three different colonies were transferred to solid media in slant tubes and later to liquid anaerobic media in flasks.

Microscopically, the isolated cultures did not look pure. One showed growth of large rods with endospores, but it appeared there were also smaller rods present. The other two cultures showed growth of at least two types of rods—a long, slender rod and a shorter, more refractile one.

Table 4
Seed Culture Medium for Soil Inocula

Nutrient	Amount
Pfennig's Mineral	5.0 mL
Pfennig's Metals	0.1 mL
B-vitamins	0.5 mL
Yeast extract	0.2 g
Resazurin (0.1%)	0.1 mL
Water	100 mL

Sewage Sludge Inocula

Experiments were conducted utilizing fresh sewage sludge as a source of bacteria to convert the solubilized lignite to alcohols. These experiments were conducted in 125 mL Wheaton serum bottles. Each bottle contained 20 mL of media, consisting only of minimal methanogen medium–mineral salts, and 5 mL fresh sewage sludge. The initial pH was adjusted to 7. One milliliter solubilized lignite solution was added to one serum bottle (A) and 10 mL of solubilized lignite solution were added to a second serum bottle (B). The bottles were incubated at 37°C and agitated at 150 rpm on a New Brunswick Shaker Incubator.

After allowing these bottles to acclimate for several weeks, two more bottles were prepared exactly as above, one containing 1 mL solubilized lignite solution (A1) and the other containing 10 mL (B1). Two milliliters of solution were withdrawn from bottle 1A and used as inoculum for A1, whereas 2 mL were withdrawn from bottle B and used as inoculum for bottle B1. Samples were periodically withdrawn from these bottles and analyzed for liquid fuels.

Soil Inocula

An organism was also previously obtained from an inoculum of soil. The organism was stored on slants in the refrigerator. The media for the slants consisted of YM broth (Difco). A seed culture was prepared by transferring a loop of cells into 10 mL of media (shown in Table 4) and incubating at 30°C for 3 d. After 3 d, 1 mL of the seed culture was transferred into 10 mL basic salts media (shown in Table 5). The media was not prepared anaerobically; however, it was placed in a stoppered serum bottle for incubation. The culture was incubated at 30°C with shaking for several days. Every 24 h, a 0.6 mL sample was withdrawn for alcohol and acids analysis.

Analytical

Liquid samples were prepared as follows for analysis: 1 mL of medium was removed from the serum bottle, centrifuged at 10,000*g* for 2 min: 400 μL supernatant was combined with 40 μL internal standard in 3% HCl for organic acid and alcohol/hydrocarbon analysis. Organic acid and alcohol

Table 5
Basic Salts Media For Soil Inocula

Nutrient	Concentration
KH_2PO_4	1.0 g/L
K_2HPO_4	3.0 g/L
$(NH_4)_2SO_4$	0.5 g/L
Na_2SO_4	0.1 g/L
$MgSO_4 \cdot 4H_2O$	1.0 mg/L
$MnSO_4 \cdot 4H_2O$	1.0 mg/L
$FeSO_4 \cdot 7H_2O$	1.0 mg/L
$CaCl_2$	0.5 mg/L
Pretreated Texas Lignite	10.0 g/L

analyses were performed on a Varian 3400 gas chromatograph equipped with an 8035 Autosampler and DS-604 Data System. The chromatography was performed on 2 ft × 0.125 in nickel columns packed with 80/100 Poropak QS (Alltech, Deerfield, IL). GC conditions were maintained as follows: oven temperature 170°C, helium flow rate 40 cc/min, H_2 flame detector.

RESULTS AND DISCUSSION

Solubilization Studies

H12 Culture

This culture has the special capability of liquefying and modifying the crystalline structure of nonpretreated coals. The organism is a motile, short rod. Colonies are slightly pigmented. The bacterium is obviously not a *Streptomyces* (filamentous), but has not been further identified at this time.

This organism is fast growing and fast acting, bringing about degradation of untreated coal within 24 h. The organism has been tested in surface culture. Low-rank, Arkansas lignite (Saline County) was used. The culture was grown on agar (Difco) slants at 30°C for 24 h. Lignite particles were placed on the surface on the slants and, after 12 h, liquefaction was observable.

Experiments were also conducted in submerged culture with Wyoming bituminous coal and untreated Arkansas lignite. The culture was placed in 250 mL Erlenmeyer flasks and incubated at 30°C with shaking. Within 24 h, the medium had turned dark and foam was produced. Liquefied coal and modified solid coal particles were evident from micrographs of the medium. A material balance of the coal particles showed that 30% of the coal had been solubilized. The remainder had been converted into a

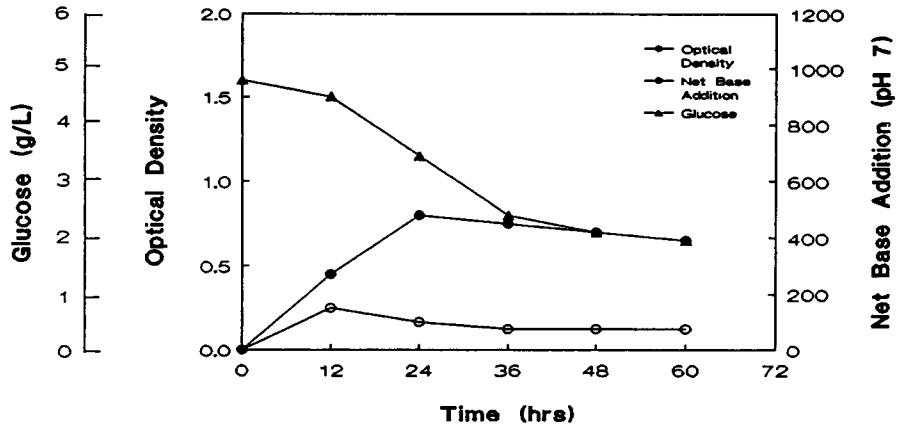

Fig. 1. Growth of LSC in submerged culture.

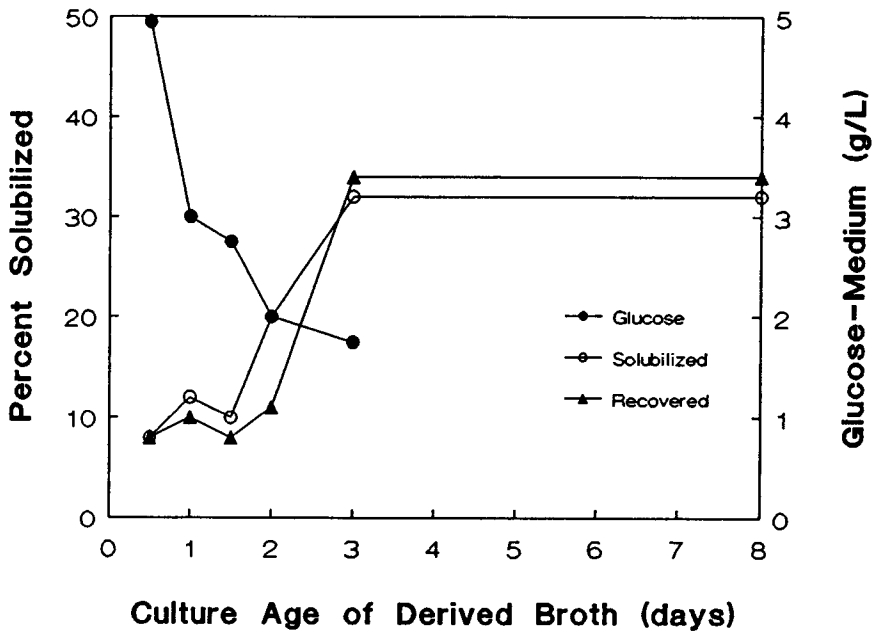

Fig. 2. Solubilization with LSC extract (28 h exposure of Arkansas Lignite, pH 9).

flocculate, with altered particle size and physical characteristics. The flocculate is much smaller and less dense than the original coal particles. The flocculate does not settle, but stays in suspension.

LSC Culture

The results of the cell extract solubilization using LSC are shown in Figs. 1 and 2. Figure 1 shows the cell growth (as OD) and glucose utilization with time for the LSC culture, as well as the net base addition to main-

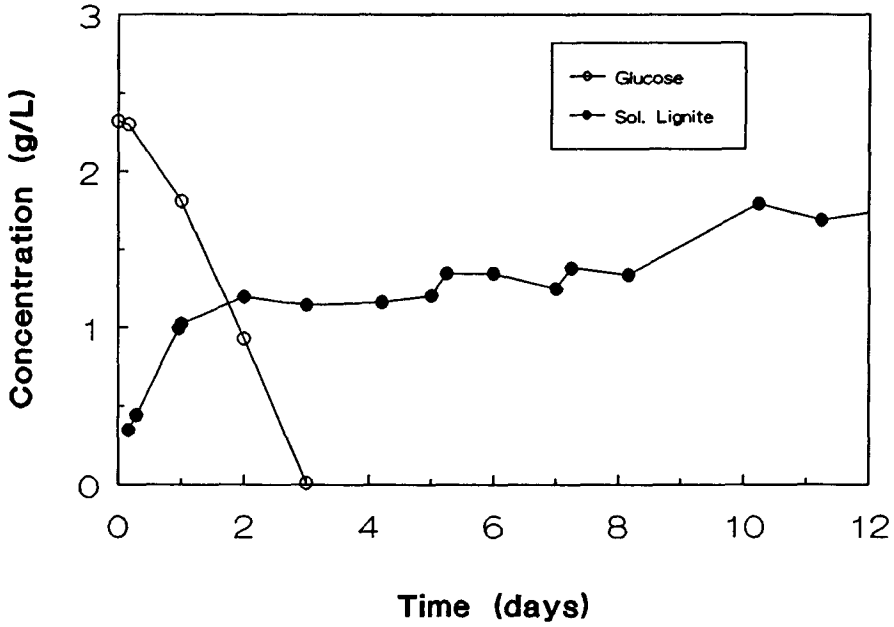

Fig. 3. Submerged culture solubilization with LSC.

tain the pH of 9 in the fermenter. Figure 2 shows the solubilizing activity of the cell-free culture broth at various stages of the fermentation. It can be seen from Fig. 2 that, after 3 d fermentation, the broth reached a maximum solubilizing capability, approximately 32% of the untreated lignite converted in 28 h. This compares to approximately 12% of the lignite being solubilized at 2 d. Aging the culture beyond 3 d did not provide any additional solubilization.

The results of the direct solubilization of lignite are shown in Fig. 3. This figure shows only the glucose concentration and lignite solubilization with time. Cell concentration could not be measured owing to the unsolubilized coal being present in the culture. As in the indirect method, the direct solubilization showed a significant increase in solubilization capabilities at 3 d. The amount of lignite solubilized continued to increase slightly, however, and reached a maximum value at 10 d.

It is apparent from these results that the age of the culture has a significant effect on the ability to solubilize lignite. At this time, the exact mechanism by which the solubilization takes place is not known. Experiments are currently being conducted in an attempt to determine the mechanism of solubilization.

Solubilization/Nutrient Studies

Experiments have been conducted in which the coal solubilizing organisms were grown on relatively inexpensive carbon sources, such as

corn stalks, corn cobs, and crushed barley hulls, as substitutes for expensive media. These experiments were performed in an attempt to minimize the cost of the initial coal-solubilizing step.

In one experiment, the lignite surface culture, LSC, was inoculated onto crushed barley hulls. The organism was allowed to grow for 2 d, after which time particles of pretreated Texas lignite were added to the cell mat and allowed to incubate at 30°C. No solubilization had taken place after several days of incubation. It was determined that the pH of the coal was too low after the pretreatment and, therefore, solubilization could not occur.

The experiment was discontinued, and several milliliters of distilled water were added to the flask prior to its heating to 100°C for 10 minutes. After 10 min, the water was observed to be very dark, indicating a significant amount of solubilization had taken place. This observation was unexpected, and samples of the solubilized product were taken for the determination of solubilized coal.

The samples of solubilized coal were collected and analyzed for pH and concentration of solubilized coal. The pH was found to be 6.1. This is the lowest pH at which significant solubilization has occurred. It is important to note that this also occurred in the presence of distilled water only. No other nutrients were present in the flask except for the barley hulls. The concentration of solubilized coal was found to be 34 g/L, as determined by precipitation at pH 1, drying, and weighing.

Based upon the results of the previous experiment, a more controlled experiment was conducted. In this experiment, the LSC cells were again grown on crushed barley hulls. After several days growth, a quantity of cells (0.072 g) was scraped from the surface of the hulls and placed in a flask containing 5.0 mL distilled water. Pretreated Texas lignite (1.0 g) was added to the flask. A control was also run in which 1.0 g of pretreated Texas lignite was added to 3.0 mL distilled water containing 1.0 g of crushed barley hulls. The flasks were heated to 100°C for 10 min. After 10 min, the flasks were cooled, and samples were removed for analysis of solubilized coal.

The control flask without cells showed no solubilization at all. The flask containing 0.072 g of LSC cells, however, was very dark, indicating solubilization had occurred. The sample was centrifuged at 20,000 rpm for 2 min to remove cells and any unsolubilized coal. The solubilized product was then removed by precipitation at pH 1, centrifuged, dried, and weighed. The quantity of coal recovered was 0.447 g, which resulted in a solution containing 89.4 g/L of solubilized coal. The percent of the Texas lignite solubilized in this manner was 44.7% in 10 min.

Again, it is important to note that the flask in which solubilization occurred contained no nutrients. This represents a potentially rapid and economical method for solubilizing coal. Studies are continuing in this area in which both pretreated and untreated coals will be investigated.

Table 6
Optical Density or Absorbance at 580 nm
of Original Cow Rumen Culture

	A_{580}	
Days	Sample – Control	Control
0	0.202	0.360
2	0.310	0.198
4	0.293	0.205
6	0.280	0.280
10	0.285	0.208

Table 7
Optical Density or Absorbance at 580 nm
of Original Sheep Rumen Culture

	A_{580}	
Days	Sample – Control	Control
0	0.305	0.213
1	0.278	0.210
2	0.235	0.238
3	0.158	0.263
6	0.047	0.335

Conversion of Solubilized Lignite to Liquid Fuels

Rumen Fluid Inocula

Of the initial mixed cultures of sheep and cow rumen, the sheep rumen culture was the only one to show a substantial visual decrease in color during fermentation, with a decrease in the absorbance at 580 nm from 0.305 to 0:047. These data are shown in Tables 6 and 7, which indicate a decrease in the lignite concentration of approximately 90 percent. However, alcohol or acid production has not been quantified at this time.

The three cultures derived from the original sheep rumen culture were also tested for their ability to degrade solubilized lignite, based on the decrease in absorbance at a wavelength of 580 nm of a culture containing 0.03% solubilized lignite. The results of this test are presented in Table 8. As seen in Table 8, culture 2 appears able to degrade solubilized lignite better than cultures 1 and 3.

Owing to the apparent ability of culture 2 to degrade solubilized lignite, it was chosen for further work. This culture was inoculated into five culture tubes with the media for transfers described above, except the solubilized lignite concentration varied from 0.03 to 0.25%. The absorbance at 580 nm was monitored for each tube with time to determine the effect

Table 8
Absorbance at 580 nm of Cultures
Derived from Sheep Rumen

Culture	Day	A_{580}
1	0	0.328
	10	0.240
2	0	0.328
	10	0.170
3	0	0.328
	17	0.225

of solubilized lignite concentration on the degrading ability of the culture. The results of these experiments are shown in Table 6. Based on these results, it appears that solubilized lignite concentrations of 0.1% or higher inhibit the culture's ability to degrade the lignite.

These experiments show that organisms are capable of degrading solubilized lignite, although not necessarily to alcohols. Efforts to quantify alcohol production with these rumen cultures, over control cultures without lignite, are inconclusive. Further work is being done with these cultures to determine the ability to produce lower alcohols and organic acids. Work with other inocula, such as sewage sludge and lignin degrading organisms, is more promising.

Sewage Sludge Inocula

The results of the experiments, using sewage sludge inocula, are shown in Tables 9–11. Table 9 shows the results of alcohol and organic acids analysis after several weeks of acclimating the cultures to the solubilized coal. As seen in the table, the bottle containing the most solubilized lignite produced the most alcohols. However, because the bottles also contained sewage sludge (5 mL), it is not possible to determine if all of the alcohols were actually produced from the solubulized coal.

Table 10 shows the alcohol and acids production from reactors (average of two reactors) containing 1 mL solubilized coal after the first transfer, and Table 11 shows the alcohols and acids produced in the bottles with 10 mL solubilized coal after the first transfer. Because only 2 mL of liquid was withdrawn from the original bottles and used as inocula, the alcohols produced in these bottles should result from the solubilized coal added. Comparing Tables 10 and 11, it can be seen that the bottles containing the 10 mL of solubilized lignite did produce more alcohols. The increase in ethanol concentration when 10 mL solubilized lignite were used was 0.18 g/L in 10 d whereas the increase in ethanol concentration was only 0.07 g/L when 1 mL solubilized lignite was used. Work is continuing in this area with longer fermentation times being allowed after transferring and using varying amounts of solubilized lignite.

Table 9
Alcohols and Acids Production from Solubilized Lignite

Bottle	Concentration, g/L			
	EtOH	1-PrOH	HAc	pH
A (1 mL lignite)	0.0469	-	0.4572	8.0
B (10 mL lignite)	3.3616	0.2915	3.4647	5.5

Table 10
Alcohol and Acids Produced from Culture Containing 1 mL Solubilized Lignite[a]

Time, d	Concentration, g/L		
	EtOH	1-PrOH	HAc
0	-	-	0.060
6	0.0686	0.0057	0.6818
10	0.0716	0.0040	0.8303

[a]Average of two bottles.

Table 11
Alcohol and Acids Produced from Culture Containing 10 mL Solubilized Lignite

Time, d	Concentration, g/L		
	EtOH	1-PrOH	HAc
0	0.2522	0.0874	0.2578
6	0.3536	0.0340	0.6362
10	0.3861	0.0326	0.6424

Soil Inocula

The ethanol and acetic acid analysis of two experiments, using cultures derived from soil, are shown in Fig. 4 and 5. Figure 4 shows the results of a mixed culture of GNO2 and MLB grown in basic salts media with 1% Texas lignite. Figure 5 shows the results of a mixed culture using MLB and MLF.

The mixed culture of GNO2 and MLB shows very little alcohol production; however, the acetic acid concentration reached 1.16 g/L after 48 h. The mixed culture of MLB and MLF shows both alcohol and acetic acid production. The ethanol concentration in this culture increased from 0.14 to 0.49 g/L after 48 h, whereas the acetic acid concentration increased from 0.05 to 0.45 g/L over the same time period. Pure culture experiments using these same organisms showed essentially no production of acids or alcohols. The results of these experiments are preliminary, and additional experiments are being conducted, both pure and mixed cultures, to develop and characterize these cultures.

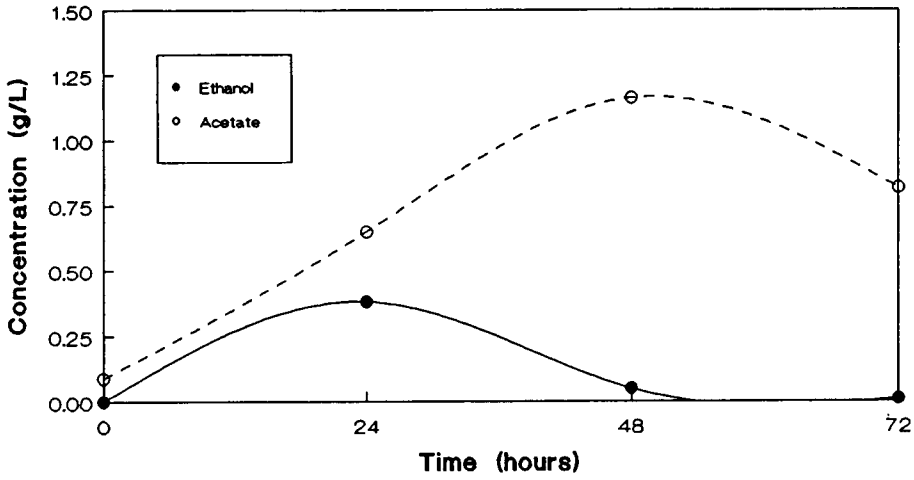

Fig. 4. Ethanol and acetate production using mixed culture of GN02 and MLB.

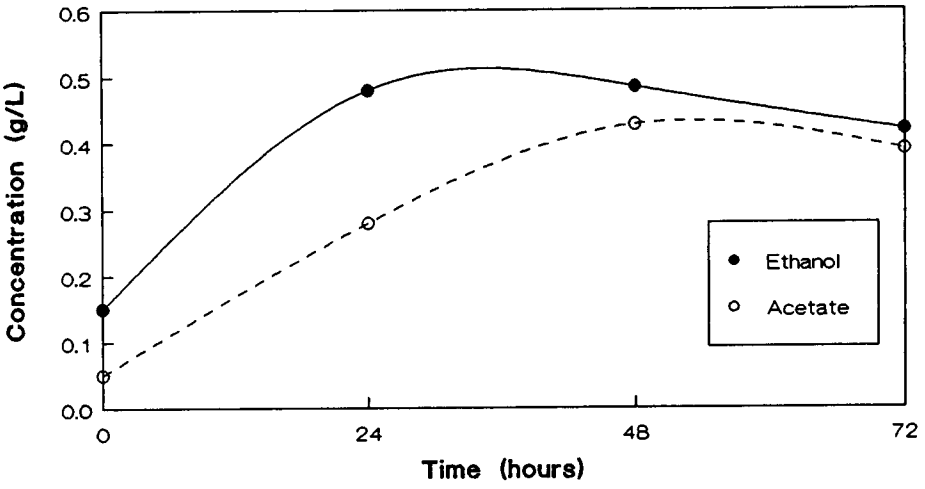

Fig. 5. Ethanol and acetate production using mixed culture of MLB and MLF.

CONCLUSIONS

Preliminary results have been reported showing the ability of cultures, isolated from natural coal environments, to solubilize untreated coal and lignite. The cultures have been shown to solubilize more than 30% of untreated lignite samples when contacted for only 28 h. When cells are grown on inexpensive carbon substrate and placed in distilled water with pretreated Texas lignite, solubilization occurs in less than 10 min at 100°C.

Preliminary results have also been reported showing that sewage sludge, and sheep rumen cultures and soil isolates have the ability to degrade the solubilized lignite produced by the LSC culture. Cultures from sheep rumen have been shown to be able to degrade solubilized lignite, as shown by a decrease in absorbance at 580 nm. Preliminary studies with cultures from a sewage sludge and soil inocula have shown alcohol production in low concentrations. Future work will include continuation of the studies with these cultures to increase alcohol yields.

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