

SERIAL BIOLOGICAL CONVERSION OF COAL INTO LIQUID FUELS

by

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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 convert the solubilized coal into more useful compounds. Preliminary experiments have been conducted to isolate cultures for the serial biological conversion of coal into alcohols. 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 alcohols. This paper presents preliminary results of experiments in coal solubilization and bioextract conversion.

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 1980's (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 submerged 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 (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.

While 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 two weeks. Cheap 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 a bacterium, as yet unidentified, that is fast growing in cheap mineral salts media and that converts coal into liquid and flocculate in a few hours in submerged culture. High rank coals are converted without pretreatment. This rod-shaped bacterium is not a Streptomyces and is likely a previously unknown strain with coal or lignin activity. Further study is required to develop and quantify coal conversion with this organism.

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 upgrade the initial microbial products to useful fuels. Such a second-stage conversion should probably be anaerobic to avoid further oxidation of the product. The evaluation of serial biological conversion of coal to liquid fuels is currently underway. This paper presents a brief summary of progress to biologically produce low molecular weight liquid fuels from coal.

MATERIALS AND METHODS

Substrate

The solubilized lignite used as the substrate for the anaerobic bacteria was obtained from submerged culture experiments. An organism isolated from an Arkansas lignite sample was used to solubilize the coal.

The solubilization was carried out in a 1.5 liter Biostat M stirred-tank fermentation system from B Braun Instruments. The fermenter was equipped with pH and temperature control as well as a dissolved oxygen probe. The temperature was maintained at 28°C and the agitation rate was 150 rpm.

The lignite surface culture was allowed to grow for 3 days on a media consisting of 0.5 percent glucose and 0.5 percent peptone. After 3 days, 9g of 8-20 mesh Arkansas lignite were added. Within 24 hours, 35 percent of the lignite was solubilized. The solubilized lignite was collected by filtration and precipitated by adjusting the pH of the solution to 1.0 with acid. The

precipitate was washed and dried and then redissolved in distilled water by adjusting the pH to approximately 6.5. Portions of this solution were then used as substrate in the attempts to produce alcohol fuels.

Media

The original culture media contained yeast extract (Difco), 0.1g; B-vitamins (Wolfe's), 1 ml; KH_2PO_4 , 0.6 mg; $(\text{NH}_4)_2\text{SO}_4$, 0.6 mg; NaCl , 1.2 mg; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.4 mg; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 mg; lignite, 0.03 g, added to 100 ml of deionized water, and adjusted to pH 7 with NaOH.

Media for transfers from the original cultures contained yeast extract (Difco), 2.0 g; α -D glucose (Aldrich), 5.0 g; B-vitamins (Wolfe's), 10.0 ml; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 mg; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.15 mg; H_3PO_3 , 1.5 mg; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 1.0 mg; $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 mg; Na_2MoO_4 , 0.5 g; $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 7.5 mg; Na_2SeO_3 , 0.05 mg; KH_2PO_4 , 0.5 g; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.33 g; NaCl , 0.4 g; NH_4Cl , 0.4 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 50 mg; rumen fluid (filtered and sterilized in autoclave), 100 ml; lignite, 0.3 g, per liter of water, and adjusted to pH 7 with NaOH. The control medium was of the same composition without lignite.

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 minutes. Just prior to inoculation of media, $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 0.05%, was added to each media bottle to lower the oxidation-reduction potential. Resazurin was not added as an indicator of anaerobiosis as it would interfere with spectrophotometric readings; however, experience with the anaerobic techniques used here have shown them to be reliable.

Cultures

Rumen has been used a number of times as source of bacteria capable of breaking down complex structures (8,9,10). Cultures were started with fresh rumen contents from cow 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 was 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, shaking at 100 rpm.

A mixed culture derived from sheep rumen which appeared to degrade lignite at 0.03 g% 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 inside an anaerobic chamber. Inoculated plates were incubated at 37°C in an anaerobic jar (Oxoid). 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.

Liquid samples were also analyzed on a spectrophotometer. A standard plot of optical density or absorbance at 580 nm versus lignite concentration using dilutions of lignite in water was made and a linear plot was obtained (Figure 1); the darker the solution, the higher the lignite concentration. Thus, it was concluded that spectrophotometry could be used to follow the lignite utilization of the culture. Liquid samples were centrifuged at 10,000 x g for two minutes. One ml of supernatant was diluted with 1 ml of distilled water. Readings of absorbance at 580 nm were taken on a Bausch and Lomb Spectronic 21. Control media served as reference for corresponding lignite media samples.

Results

Of the initial mixed cultures of sheep rumen 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 as seen in Tables 1 and 2, indicating a decrease in the lignite concentration of approximately 90 percent.

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 percent solubilized lignite. The results of this test are presented in Table 3. As seen in Table 3, culture 2 appears able to degrade solubilized lignite better than cultures 1 and 3.

Due 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 percent to 0.25 percent. The absorbance at 580 nm was monitored on each tube with time to determine the affect of solubilized lignite concentration on the degrading ability of the culture. The results of these experiments are shown in Table 4. Based on these results it appears that solubilized lignite concentrations of 0.1 percent or higher inhibit the cultures ability to degrade the lignite.

Further work is being done with these cultures to determine the ability to produce lower alcohols and organic acids. At this time, none of the cultures have shown the ability to produce substantially higher quantities of ethanol, propanol, butanol, acetic, propionic or butyric acids from solubilized lignite when compared to a control culture without solubilized lignite. Work is continuing, however, on the analysis of these experiments to better determine what compounds are being produced.

CONCLUSIONS

Preliminary results have been obtained in the second step of the serial biological conversion of coal to liquid fuels. Solubilized lignite, derived from the action of a lignite surface culture on Arkansas lignite, was used as the substrate in anaerobic culture with organisms derived from sheep rumen.

In mixed culture, the potential for degrading lignite was shown by the decrease in color and absorbance at 580 nm during fermentation. Analysis of the fermentation products of these cultures, however, has not shown substantial production of lower alcohols. The search for an anaerobic organism capable of producing higher quantities of alcohols is continuing. Work is also being conducted in an attempt to find aerobic organisms capable of degrading solubilized lignite to compounds which can then be converted to alcohols by anaerobic organisms.

REFERENCES

- (1) Fakavsa, R. M., "Coal as a Substrate for Microorganism: Investigation with Microbial Conversion of National Coals," Ph.D Thesis, Friedrich Wilhelms University, Bonn, Federal Republic of Germany (1981).
- (2) Cohen, M. S. and Gabriele, P. D., "Degradation of Coal by the Fungi Polyporus versicolor and Poria placenta," Appl. Environ. Microbiol. 44(1), 23-27 (1982).
- (3) Scott, C.D., Standborg, G. W. and Lewis, S. N., "Microbial Solubilization of Coal," Biotech. Progress 2(3), 131-139 (1986)
- (4) Standborg, G. W. and Lewin, S. N., "Solubilization of Coal by an Extracellular Product from Streptomyces setonii 75 Vi2," J. Industrial Microbiol. 1 370-375 (1987).
- (5) Ward, B., "Biodegradation and Bioconversion of Coals by Fungi," US DOE/PC80913 (1987).
- (6) Cohen, M. Biological Treatment of Coals Workshop, Proceedings U. S. DOE/ENTEEL, Herndon, VA (June 1986).
- (7) Dahlberg, M., Biological Treatment of Coals Workshop, Proceedings U. S. DOE/ENTEEL, Herndon, VA (June 1986).
- (8) Cheng, K. J., Jones, G. A., Simpson, F. J., and Bryant, M. P., "Isolation and Identification of Rumen Bacteria Capable of Anaerobic Rutin Degradation. Can. J. Microbiol. 15 1365-1371 (1969).
- (9) Simpson, F. J., Jones, G. A., and Wolin, E. A., "Anaerobic Degradation of Some Bioflavinoids by Microflora of the Rumen. Can. J. Microbiol. 15 972-974 (1969).
- (10) Tsai, C. G., and Jones, G. A., "Isolation and Identification of Rumen Bacteria Capable of Anaerobic Phloroglucinol Degradation. Can. J. Microbiol. 21 794-801 (1975).

Table 1
Optical Density or Absorbance at 580 nm of
Original Cow Rumen Culture

days	A580 (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 2
Optical Density or Absorbance at 580 nm of
Original Sheep Rumen Culture

days	A580 (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

Table 3

Absorbance at 580 nm of Cultures
Derived from Sheep Rumens

Culture	day	A580
1	0	0.328
	10	0.240
2	0	0.328
	10	0.170
3	0	0.328
	17	0.225

Table 4

Optical Density or Absorbance at 580 nm of Culture
With Varying Lignite Concentrations

Lignite Concentration	days	(Sample)-(Control)* ^{A580}	Control**
0.03%	0	0.263	0.310
	3	0.085	0.277
	7	0.057	0.267
0.05%	0	0.344	0.310
	3	0.203	0.277
	7	0.033	0.267
0.10%	0	0.740	0.310
	3	0.788	0.277
	7	0.768	0.267
0.15%	0	1.050	0.310
	3	1.013	0.277
	7	1.033	0.267
0.25%	0	--***	0.310
	3	--	0.277
	7	--	0.267

* average of two samples

** average of three samples

*** sample reading was off instrument's scale

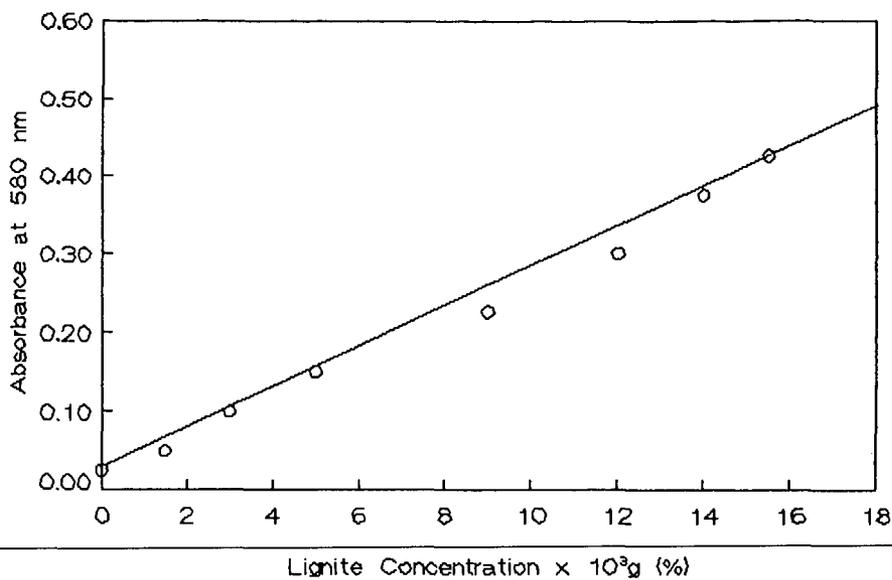


Figure 1. Calibration curve for solubilized lignite concentration using absorbance.