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Biological Solubilization of Untreated North Dakota Lignite by a Mixed Bacterial and a Mixed Bacterial/Fungal Culture

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ABSTRACT

Four microbial cultures, two pure and two mixed, were examined for their abilities to solubilize chemically treated (oxidized) lignites, thermally treated (wet-carbonized) lignite, and untreated lignites. Extensive solubilization of oxidized lignites and limited solubilization of untreated North Dakota lignite was observed by three of the four cultures tested. Solubilization of wet-carbonized (a technique to reduce equilibrium moisture and oxygen contents) lignite was not demonstrated. The increase in solubilization correlated with the increase in the oxygen content of lignite and the pH of culture broths. These results also suggest that microbial solubilization of coal may involve nonlignin degrading organisms capable of producing alkaline conditions in the presence of coal.

Index Entries: Microbial coal solubilization; mixed cultures; North Dakota lignite; alkali coal solubilization.

INTRODUCTION

The microbial solubilization of low rank coals has been reported by several researchers (*1–8*). Solubilization has been observed with pure cultures of lignin degrading fungi and streptomyces (*1–3,5,6,10*). The susceptibility of coal to microbial solubilization seems to be dependent on the

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oxygen content of the coal (*3*). The greatest degree of solubilization was initially observed with a leonardite coal, which is an oxidized form of lignite (*3*). Researchers have found that increasing the oxidation state of the coals through chemical pretreatment significantly enhances the susceptibility of these coals to solubilization by microorganisms (*11*). Nitric acid and hydrogen peroxide were two chemicals used to pretreat the coals (*11*). Nitric acid pretreatments appeared to yield the greatest degree of solubilization, i.e., greater than 80%.

Microbial solubilization of coal was observed using both agar surface and submerged liquid cultures (*3,5,10*). The evidence for solubilization by agar surface cultures was the formation of discrete liquid droplets on the top surface of the coal, whereas for submerged liquid cultures, solubilization was indicated by a blackening of the culture broth (*3,10*). Preliminary chemical characterization of these liquid materials indicated that the products are water soluble, polar, alkaline, and have a molecular weight between 30,000 and 300,000 (*3,12*).

The demonstration and confirmation by several researchers that oxidized coals can be microbially solubilized has led efforts to be directed toward elucidating the mechanism(s) involved in the solubilization process (*4,6,10,13*). Most researchers suggest that microbial solubilization is an extracellular process (*6,10,13*). Strandberg and Lewis (*10*) have observed cell-free solubilizations of leonardite and acid pretreated coal in filter-sterilized broths from 7-d cultures of *S. setonii* 75Vi2. Cohen et al. (*6*) have observed solubilization of leonardite in filter-sterilized broths from cultures of *Polyporous versicolor.* Pyne et al. (*13*) have observed solubilization of leonardite with an extracellular protein fraction purified from *Polyporous versicolor.* Another mechanism proposed is that microbial solubilization of oxidized coal involves formation of alkali by the microorganisms (*4*). Quigley et al. (*4*) have examined the relationship between pH and solubilization of oxidized coal. They found that when acid treated coal was added to the microbial cultures there was a simultaneous increase in culture fluid pH and material solubilized. They extracted acid-treated coal with alkaline buffers and found that coal was susceptible to solubilization in alkali solutions.

This paper examines the abilities of four microbial cultures, both lignin degraders and nonlignin degraders, pure as well as mixed cultures, to solubilize treated and untreated coal. The microbial cultures include *Phanerochaete chrysosporium, Cunninghamella* YML-21, and two mixed cultures. Two different types of lignite, North Dakota and Texas, were examined. The oxidation state of the coals was modified to examine the role of oxygen content on solubilization. The oxygen content of the two untreated coals was similar. Both coals were chemically pretreated with either hydrogen peroxide or nitric acid to increase the oxidation state. North Dakota lignite was treated by a wet-carbonization process to reduce the moisture and oxygen content. The pH of the systems was also examined.

METHODS

Cultures

Cunninghamella YML-21 was obtained from Quigley, Idaho National Engineering Laboratory, Idaho Falls, ID. *Phanerochaete chrysosporium* BKMF 1767 was obtained from Kirk, US Dept. of Agriculture, Forest Service, Forest Products Laboratory, Madison, WI. The mixed cultures were isolated at the Institute of Gas Technology. *Cunninghamella* and *Phanerochaete* were maintained on yeast malt agar (YMA) slants (Difco, Inc., Detroit, MI) at 25 and 37°C, respectively. The mixed cultures were maintained on Sabouraud Maltose Agar (SMA) slants (Difco, Inc., Detroit, MI) at 30°C.

Shake Flask Experiments

For examining the interaction between coal and microbial cultures, the organisms were grown for several days and the various coals added as described below. *Cunninghamella* was grown for 2 d in Yeast Malt Broth (Difco, 50 mL in 125 mL flasks) at 25°C on a rotary shaker (140 rpm). *Phanerochaete* was grown in a low nitrogen medium (pH 4, 50 mL in 250 mL flasks) for 4 d (*14*). Both static and shaking (rotary shaker, 125 rpm) cultures of *P. chrysosporium* were maintained at 38°C. The mixed cultures were grown in Sabouraud Maltose Broth (Difco, 50 mL in 250 mL flasks) for 5 d at 30°C on a rotary shaker (130 rpm). The sterilized (121°C, 45 min) coals (1 g) were added to the grown cultures and the cultures were then incubated. At 1–2-d intervals, 4 mL aliquots were removed from the coal containing cultures and controls. The pH of athe supernates was determined after centrifuging the samples at 10,000 rpm for 10 min to remove biomass and coal particles. Controls consisted of coal-free cultures and cell-free coal samples. UV-VIS difference spectra (Beckman, Model No. DU-65 Spectrophotometer) were obtained by subtracting the supernates of cultures without coal (control) from supernates of coal containing cultures. Both the control and sample supernates were diluted equally for spectral analysis. Replicate samples showed less than 1% error for spectral analyses.

Mixed Culture Identification

API20E strips (Analytab Products, Plainview, NY) were used for identification of the bacteria present in the mixed cultures. Additional biochemical tests included casein (Difco, Inc.) hydrolysis, tyrosine (Difco, Inc.) decomposition, starch hydrolysis (Difco, Inc.), and growth in nutrient broth/sodium chloride solutions at 2, 5, 7, and 10% salt concentrations.

Polymeric Dye Assay

The assay was performed as described by Kelley et al. (*15*). Poly R-478 and Brilliant Remazol Blue were obtained from Sigma Chemical Company (St. Louis, MO)

Coals and Coal Treatments

Indianhead North Dakota Lignite (Institute of Gas Technology), Texas lignite (provided by Quigley, Idaho National Engineering Laboratory, Idaho Falls, ID), and wet-carbonized North Dakota lignite (Institute of Gas Technology) were used in this study. Wet-carbonization is a thermochemical process for removing bound water from low-rank coals by heating the coal in the presence of water to temperatures in the range of 400–650°F (*16*). The sample used in this study was processed at 556°F for 30 min at 2300 psig. The wet-carbonization process removes a fraction of the oxygen from the coal by decarboxylation and dehydration reactions. For this specific coal sample, the O_2 content was reduced from 22.69 to 19.73%. North Dakota lignite and Texas lignite were oxidized using nitric acid or hydrogen peroxide. The nitric acid treated Texas lignite (TXL-35S) was provided by Quigley (*4*).

Acid treated North Dakota lignite was produced by treating 100 g of North Dakota lignite (> 10 mesh) with 300 mL 14% nitric acid for 8 h while stirring. Hydrogen peroxide treated North Dakota lignite was produced by treating 100 g of North Dakota lignite (> 10 mesh) with 300 mL of 10% hydrogen peroxide for 48 h while stirring. Both treated coals were recovered by centrifugation and washed with deionized water until the pH was about 5. Treated coal was then further washed using a Soxhlet extraction apparatus until no further colored material leached from the coal (1 wk). The coal was then dried at room temperature.

Buffer Preparations

Acetate (pH 4 and 5), citric acid-phosphate (pH 6), phosphate (pH 7), Tris (pH 8 and 9), and CAPS (pH 10) buffers (50 m*M*) were prepared. Coal (0.6 g) was added to the various buffers (100 mL in 250 mL flasks) and incubated at 30°C on a rotary shaker (130 rpm). The pH was determined and UV-VIS difference spectra were performed on these samples. The procedures for pH determinations and difference spectra were described above in the shake flask experiment section.

RESULTS

Identification of Bacteria

Two mixed microbial cultures were isolated from an enriched medium (Sabouraud Maltose Broth) containing untreated North Dakota lignite.

Culture one (IGT MX15) consisted of bacteria and a fungus, and culture two (IGT B1) consisted of bacteria. Based on morphological and biochemical analyses, three different bacterial isolates appeared to be present in IGT MX15, and included *Bacillus cereus, Bacillus pumilus,* and *Bacillus subtilis* (Table 1). The fungus, present in IGT MX15, appeared to belong to the subclass *Ascomycetes* and was tentatively identified as *Aspergillus* based on morphological observations. Culture two, IGT B1, the mixed bacterial culture, consisted of the three bacterial species present in IGT MX15.

Examination of Organisms for Lignin Degradation

These mixed cultures as well as the pure fungal cultures, *Cunninghamella* and *Phanerochaete,* were examined for their lignin degrading abilities. A polymeric dye (Poly R-478 and Remazol Brilliant Blue) assay was used to determine ligninolytic activity. Organisms possessing ligninolytic activity will decolorize these polymeric dyes. *Phanerochaete chrysosporium,* a known lignin degrader, tested positive on the plate assay for lignin degrading activity. However, neither *Cunninghamella* nor the two mixed cultures showed any such activity.

Interaction of Microorganisms with Coal

The mixed bacterial culture (IGTB1), the mixed bacterial/fungal culture (IGTMx15), as well as *Cunninghamella,* a known coal solubilizing fungi, and *Phanerochaete,* a known lignin degrading fungi, were examined for their abilities to solubilize treated and untreated North Dakota and Texas lignites.

Addition of nitric acid or hydrogen peroxide treated lignites resulted in extensive color change (from yellow to brown) of the culture broth supernates from *Cunninghamella* and both mixed cultures. Generally, the initial pH was about 5.5 and the final pH was about 8. It is believed that the color change of the broths was the result of coal being solubilized. No color change of *Phanerochaete* culture broth supernates was observed. Difference-spectra (test cultures minus controls) of the materials from the chemically treated coals were generally characterized by a broad absorption peak from 350 to 550 nm that intensified as the culture aged. A typical difference-spectra of the materials produced by microbial solubilization is presented in Fig. 1. Difference-spectra of the products from the solubilization of hydrogen peroxide treated North Dakota lignite by both mixed cultures and *Cunninghamella* were characterized by an absorption maximum at 375 nm that intensified with time. Difference-spectra of the product from the solubilization of nitric acid treated Texas lignite by both mixed cultures were characterized by an absorption maximum at 425 nm that increased with time.

Addition of untreated North Dakota lignite to both mixed cultures as well as *Cunninghamella* resulted in the slow color change of the culture

Table 1
Biochemical Identification of Bacterial Species
Present in the Mixed Cultures Isolated from North Dakota Lignite

Biochemical tests	Isolate 1	Isolate 2	Isolate 3
Gram stain	gram positive rods	gram positive rods	gram positive rods
Spore-formers	+	+	+
Motile	+	+	+
Oxygen requirement	aerobic	aerobic	aerobic
Acid from glucose	+	+	+
Acid from arabinose	+	+	–
Acid from mannitol	+	+	–
Gelatin liquefaction	+	+	+
Starch hydrolysis	+	–	+
Tyrosine decomposition	–	–	+
Casein hydrolysis	+	+	+
Growth in sabouraud dextrose broth (pH 5.7)	+	+	+
Growth in 2% NaCl	+	+	+
Growh in 5% NaCl	+	+	+
Growth in 7% NaCl	+	+	+
Growth in 10% NaCl	–	–	–
Catalase	+	+	+
Reduction of nitrate to nitrite	+	–	+
Bacteria identified	*B. subtilis*	*B. pumilus*	*B. cereus*

+Growth
–No growth

broth supernates. The change in color of the supernates was observed after 2 wk in the presence of coal. Addition of untreated North Dakota lignite to *Phanerochaete* cultures did not result in solubilization. Difference-spectra of the products obtained from *Cunninghamella* had an absorption peak at 360 nm that increased with time. Difference-spectra of the products obtained from the two mixed cultures were similar. A comparison of the

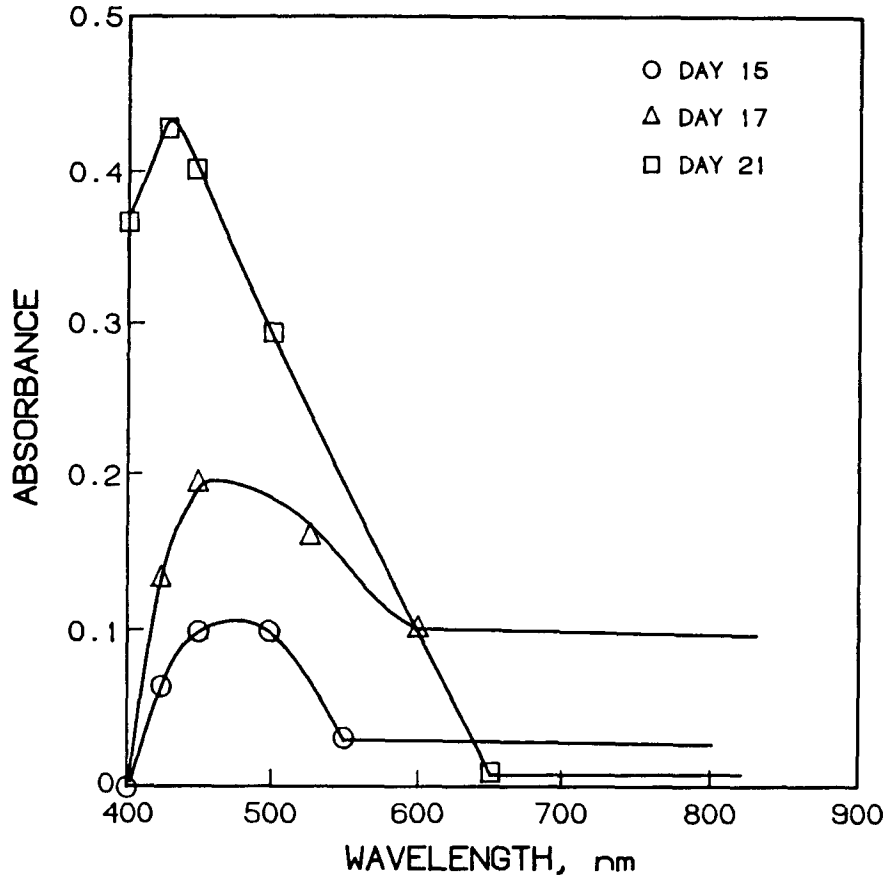

Fig. 1. Difference spectra of product from the solubilization of acid treated North Dakota lignite by a mixed bacterial/fungal culture. Samples and controls diluted 1:50.

difference-spectra of the products from the solubilization of untreated and hydrogen peroxide treated North Dakota lignite by the mixed bacterial/fungal culture is presented in Fig. 2. The spectra of the two products are different from each other. The product from the solubilization of hydrogen peroxide treated lignite is characterized by an absorption maximum at 375 nm. The product from the solubilization of untreated lignite is characterized by an absorption maximum at 425 nm. Also, solubilization of hydrogen peroxide treated lignite is much more extensive based on absorbance than solubilization of untreated lignite.

The addition of untreated Texas lignite and wet-carbonized North Dakota lignite to *Phanerochaete, Cunninghamella,* or both mixed cultures did not result in any solubilization.

The pH of the coal containing cultures and controls was monitored. For *Cunninghamella* and both mixed cultures containing the various coals, as well as the coal-free cultures, the pH values increased with time as the culture aged. Generally, the initial pH was about 5.5 and the final pH was about 8. For the *Phanerochaete* cultures, the pH did not increase as the

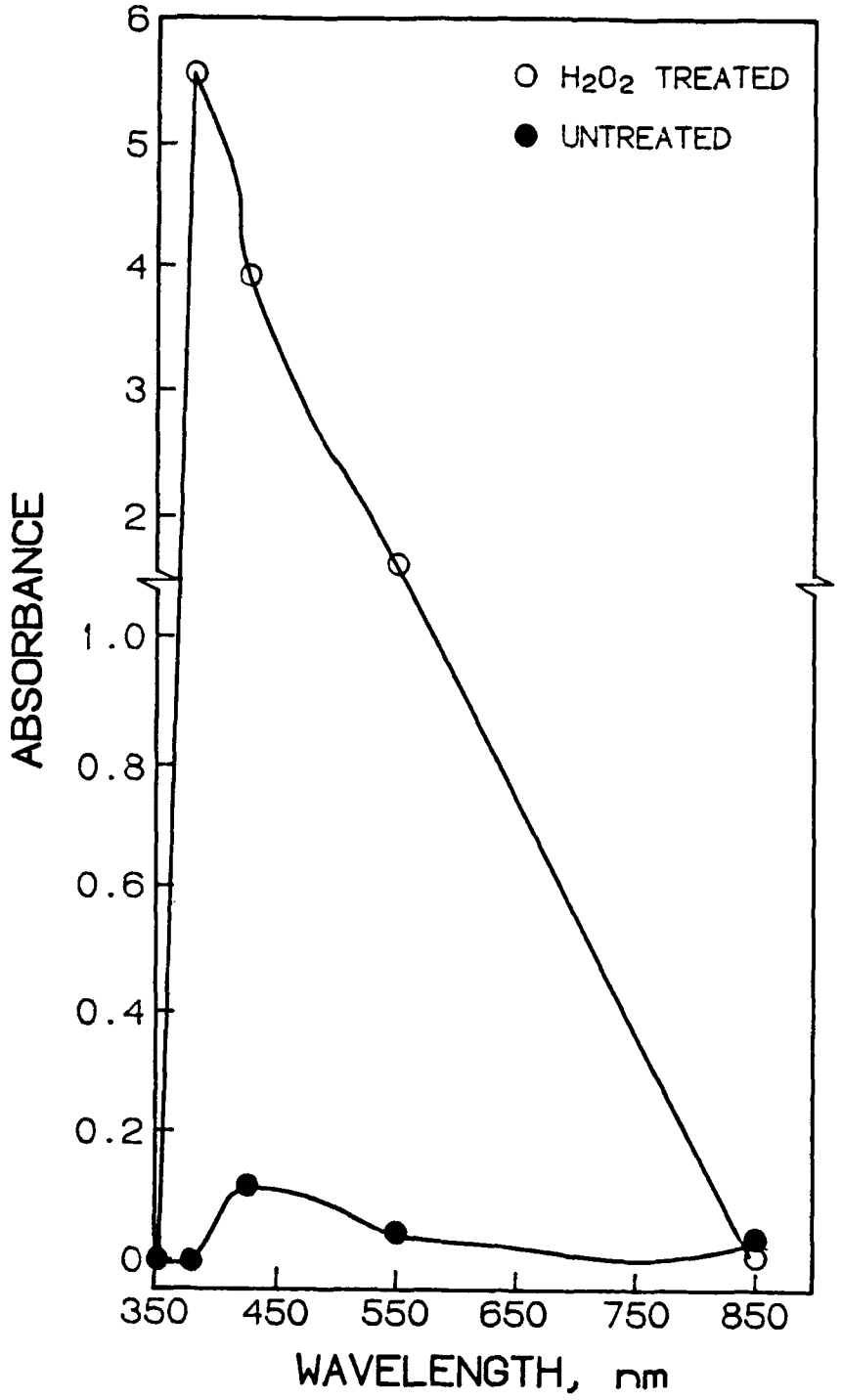

Fig. 2. Comparison of difference spectra of products from the solubilization of untreated and H_2O_2 treated North Dakota lignite by a mixed bacterial/fungal culture. Values are corrected for dilution.

culture aged because of the buffer in the medium. The pH of all *Phanerochaete* coal cultures remained between 4 and 5. The pH of the cell-free coal controls did not change throughout the experiment. Generally, the increase in pH observed in experiments that contained both coal and organisms correlated with the increase in intensity of the absorption peaks with time (Fig. 3). However, in the presence of wet-carbonized lignite, an increase in pH did not correlate with solubilization. Figure 4 presents a comparison of pH and solubilization of wet-carbonized, untreated and hydrogen peroxide treated North Dakota lignite. The pH of the culture supernates increased to 8 in the presence of untreated and wet-carbonized lignite. In the presence of hydrogen peroxide treated lignite, the pH of the culture supernates increased to 7. There is no solubilization of the wet-carbonized coal, limited solubilization of the untreated lignite and extensive solubilization of the hydrogen peroxide treated lignite. The increase in solubilization correlates with the increase in the oxidation state of the coals. This correlation will be elucidated later.

Effect of pH on Solubilization

The effect of pH on solubilization of untreated North Dakota lignite was examined. The pH and solubilization were monitored during the course of the experiment. Coloring of the culture broth supernates at pH 8, 9, and 10 was observed. At pH 10, coloring was observed within 24 h, within 72 h at pH 9, and within 96 h at pH 8. Below pH 8, the supernate samples remained relatively colorless. Difference-spectra of the chemically solubilized material at pH 10 was characterized by an absorption maximum at approximately 230 nm that intensified with time. The difference-spectra of the material solubilized at pH 9 were characterized by an absorption maximum at approximately 220 nm that increased with time and the material at pH 8 was characterized by an absorption maximum of approximately 210 nm that intensified with time. The spectra of the materials produced from chemical solubilization of untreated North Dakota lignite differ from the spectra of the biologically solubilized materials. A comparison of the difference-spectra of the materials from biological and chemical solubilization is presented in Fig. 5. The chemically solubilized product was obtained at pH 8. The spectrum of the chemically solubilized material was characterized by an absorption maximum at approximately 210 nm, whereas the spectrum of the biological solubilized material was characterized by an absorption peak at 425 nm. For the chemically solubilized material, the majority of the absorbance occurred between 200–400 nm, where there was minimal to no absorption of the biologically solubilized material.

As the pH increased, the amount of chemically solubilized material also increased. The highest amount of solubilization occurred at pH 10 and was about 10-fold greater than that at 9 and pH 8. There was also slightly more solubilization at 9 than at pH 8 (Fig. 6).

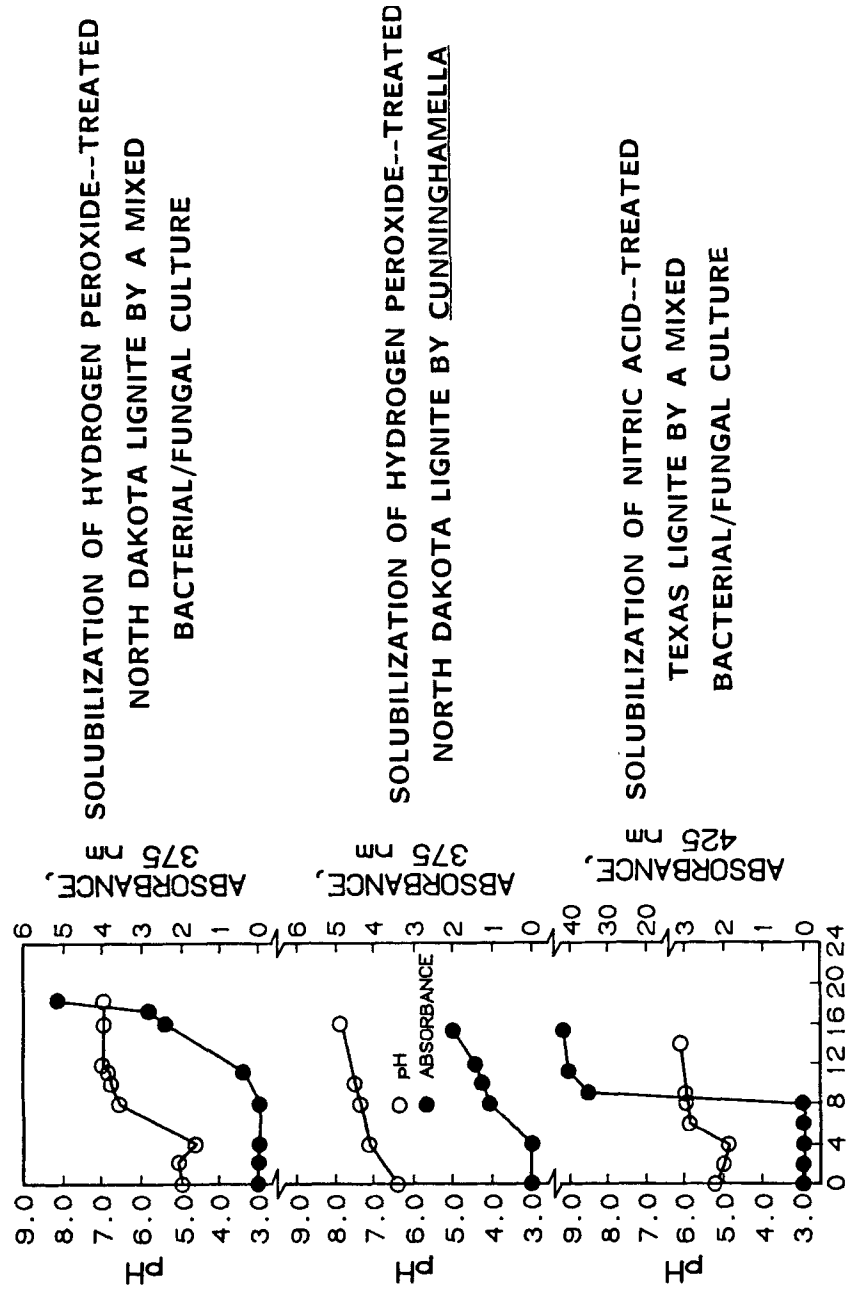

Fig. 3. Comparison of pH and microbial coal solubilization is indicated by an increase in absorption maximum as determined by difference spectra. Absorbance values are corrected for dilution.

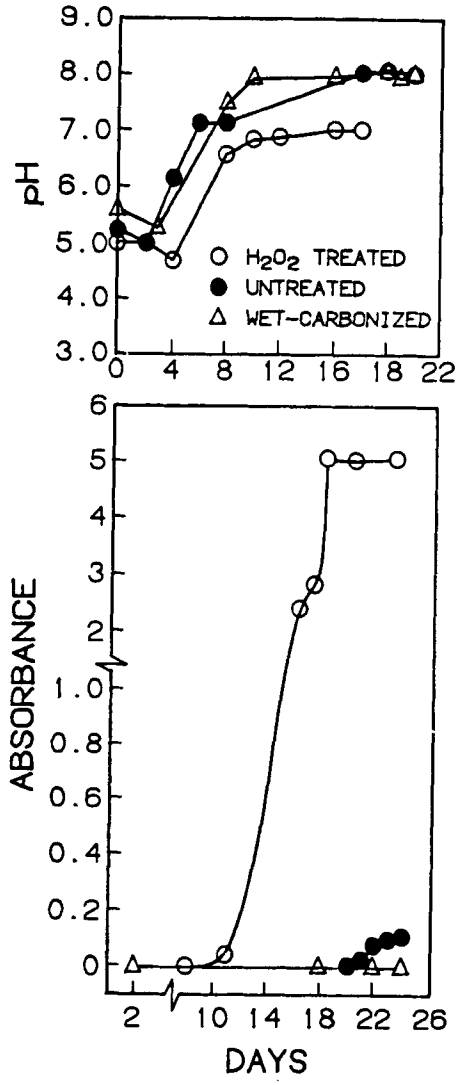

Fig. 4. Comparison of pH and solubilization of untreated, wet-carbonized, and H_2O_2 treated North Dakota lignite by a mixed bacterial/fungal culture. A) pH comparisons, B) solubilization comparisons—solubilization is indicated by an increase in absorbance maximum (425 nm for the solubilized product of untreated lignite and 375 nm for the solubilized product of H_2O_2 treated lignite). Absorbance values corrected for dilution.

DISCUSSION

Three of the four organisms examined in this study were able to solubilize coal. Extensive solubilization was observed in the presence of treated (oxidized) coals, whereas very little solubilization was observed with untreated coals. As the pH of the culture media increased, there was also an increase in the degree of solubilization (Fig. 3). In the presence of the three microbial cultures, the pH increased from approximately 5 to be-

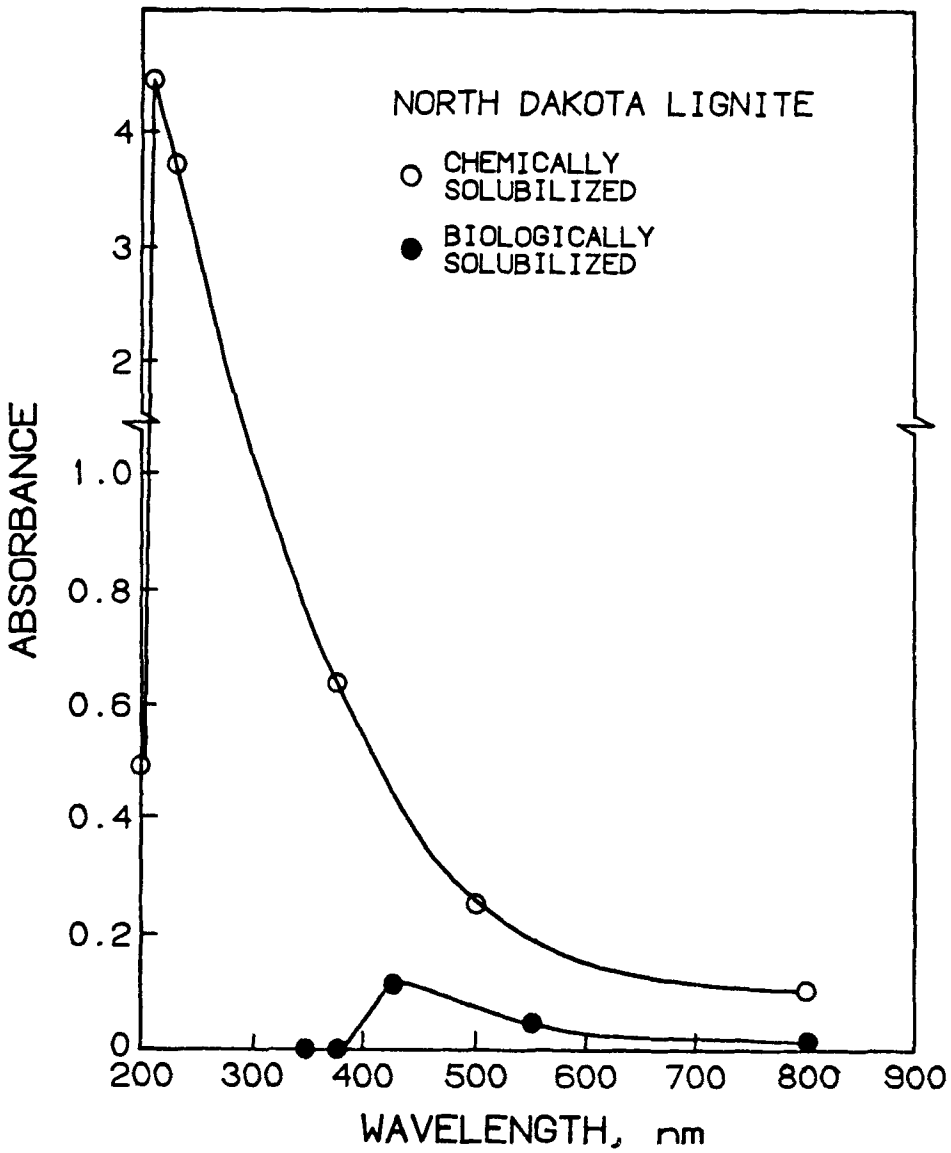

Fig. 5. Comparison of difference spectra of biologically and chemically solubilized untreated North Dakota lignite. Chemical solubilization was at pH 8.

tween 6 and 7, before solubilization of the nitric acid or hydrogen peroxide treated coals was observed. However, in the case of untreated North Dakota lignite, the pH increased to 8 before solubilization was observed.

Experiments determining pH effects on solubilization showed that the solubilization occurs only under alkaline conditions (Fig. 6). Similar to biological solubilization, untreated North Dakota lignite was soluble at pH 8, with an increase in the amount of lignite solubilized with time. The

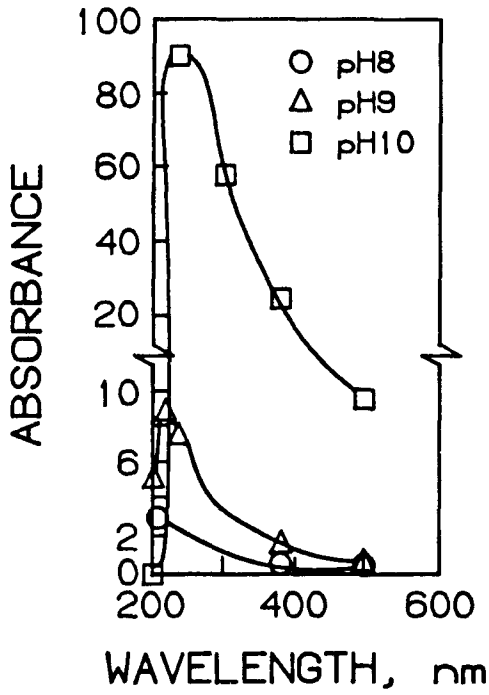

Fig. 6. Comparison of difference spectra of chemically solubilized untreated North Dakota lignite at pH 10, 9, and 8.

spectra of the material from chemical solubilization differed from the spectra of the biologically solubilized material. This observation suggests that chemically different materials are produced during microbial vs chemical solubilization. It is very likely that during microbial solubilization, products are generated by both biological interactions with coal as well as the chemical effect resulting from the natural rise in pH. Also, during both microbial and chemical solubilization, the amount of product increased with time. Chemical solubilization, however, was more rapid (occurring within 24–96 h) than the microbial solubilization that occurred as early as d 4 and as late as d 15. This variability may be caused by the different pH conditions of the systems. During microbial solubilization, there was a naturally occurring but slow pH increase, whereas for chemical solubilization, alkaline pH was set at the start of the experiment and maintained throughout the incubation period.

It was also observed that the spectra of the material from the microbially solubilized treated (oxidized) coals differed from the spectra of the material from the microbially solubilized untreated coal (Fig. 2). This suggests that materials produced by microbial solubilization may differ from each other and may be dependent on the coal, whether it is treated or

not, that is being subjected to microbial solubilization. To confirm that a variety of materials may be produced, further studies would be undertaken to characterize the materials. Studies would include infrared spectroscopy and molecular weight determinations. If, after thorough chemical characterization, different useful materials are identified, it may be feasible to select those conditions that produce the desired materials.

The correlation between increase in pH and microbial solubilization suggests that the microbial solubilization of coal may involve the production of alkaline conditions by the microorganisms and the alkali thereby solubilizes the coal. Quigley et al. (*4*) obtained similar results and suggested that alkali production is the key mechanism by which microorganisms solubilize oxidized coals. They showed that the solubilization of oxidized Texas lignite occurred by simply increasing the pH. They found a correlation between increases in culture fluid pH and solubilization.

Wet-carbonization processed North Dakota lignite was not solubilized by these cultures, although the increase in pH was observed (Fig. 4). This suggests that microorganisms produced chemicals responsible for creating the alkaline conditions, but because of the nature or state of the coal, no solubilization could occur. As mentioned earlier, the wet-carbonization process decreases the O_2 content of the coal, and, as discussed earlier, the susceptibility of coal to microbial solubilization seems to be dependent on the O_2 content of the coal (*3,4,11*). The extensive solubilization of treated (oxidized) coals, minimal solubilization of untreated coals, and no solubilization of the wet-carbonized coal strongly suggest that the microbial solubilization of coal is dependent on the oxidative state of the coal. As the oxygen content of the coal is increased, the extent of solubilization also increases.

Because of the high lignin content of low-rank coals, researchers have often used lignin degradability as a selection criterion in searching for organisms capable of coal solubilization. Ligninase enzyme is considered responsible for coal solubilization (*1*). In our study, *Phanerochaete chrysosporium*, which decolorized the polymeric dyes (indicating ligninase activity), did not demonstrate coal solubilizing activity. This suggests that there may not be a correlation between lignin degradation and coal solubilization. However, microbial solubilization, as observed by nonlignin degrading organisms, involves breakdown of the lignite to water soluble intermediate compounds. It may be assumed that with the lignin degraders, the ligninase enzyme is releasing parts of the lignite molecule that are readily metabolized and no intermediate compounds are found. This is typical of the breakdown of lignin in which no intermediate compounds are usually found.

In conclusion, these results suggest that microbial solubilization of coal is dependent on the oxidation state of the coal and may involve nonlignin degrading organisms capable of producing alkaline conditions in the presence of coal and causing solubilization. Therefore, the microbial solubilization of coal may be an indirect chemical process.

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REFERENCES

1. Scott, C. D. and Faison, B. D., "Biological Solubilization of Coal in Aqueous and Nonaqueous Media." Paper presented at the Workshop on Biological Treatment of Coals, Washington, DC, July 8–10, 1987.
2. Scott, C. D. and Lewis, S. N., "Biological Solubilization of Coal Using Both *In Vivo* and *In Vitro* Processes," Paper presented at the Ninth Symposium on Biotechnology for Fuels and Chemicals, Boulder, CO, May 5–8, 1986.
3. Scott, S. D., Strandberg, G. W., and Lewis, S. N. (1986), *Biotechnol. Prog.* **6,** pp. 131–139.
4. Quigley, D. R., Wey, J. E., Breckenridge, C. R., and Stoner, D. L. *Appl. and Environ. Microb.* In review.
5. Cohen, M. S. and Gabrielle, P. D., (1982), *Appl. and Environ. Microb.* **1,** pp. 23–27.
6. Cohen, M. S., Bowers, W. C., Aronson, H., and Gray, E. T. (1987), *Appl. and Environ. Microb.* **12,** pp. 2840–2842.
7. Ward, B. "Biodegradation and Bioconversion of Coals by Fungi," US DOE/PC/80913. Quarterly Progress Report, No. 3 for the Period April–June 1986.
8. Ward, B. "Biodegradation and Bioconversion of Coals by Fungi," US DOE/PC/80913. Quarterly Progress Report, No. 2 for the Period Jan.–March 1986.
9. Ward, B. "Biodegradation and Bioconversion of Coals by Fungi," US DOE/PC/80913. Quarterly Progress Report, No. 1 for the Period Oct.–Dec. 1985 (1986).
10. Strandberg, G. W. and Lewis, S. N. (1987), *J. Indust. Microb.* **1,** pp. 371–375.
11. Strandberg, G. W. and Lewis, S. N. "A Method to Enhance the Microbial Liquefaction of Lignite Coals." Paper presented at the Eighth Symposium on Biotechnology for Fuels and Chemicals, Oak Ridge, TN, May 13–16, 1986.
12. Wilson, B. W., Bean, R. M., Franz, J. A., and Thomas, B. L. (1987), *Energy and Fuels* **1,** pp. 80–84.
13. Pyne, J. W., Stewart, D. C., Fredrickson, J., and Wilson, B. W. (1987), *Appl. and Environ. Microb.* **12,** pp. 2844–2848.
14. Kirk, T. K., Schultz, E., Connors, W. J., Lorenz, L. F., and Zeikus, J. G. (1987), *Arch. Microbiol.* **117,** pp. 277–285.
15. Kelley, R. L., Ramasamy, K., and Reddy, L. A. (1986), *Arch. Microbiol.* **4,** p. 254.
16. Lau, F. S., Robert, M. J., and Janulis, J. A. "Wet Carbonization of Lump-Size Low-Rank Coals by a Novel Non-Slurry Approach," US DOE/DE-AC21-84MC21225 Topical Report for Task 3. PDU-Scale Wet-Carbonization Tests, Oct. 1987.
17. Lavine, I. (1940), *Fuel Sci. Prac.* **4,** pp. 78–81.