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Microbial Liquefaction of Lignite Pretreated with Dilute Acid at Elevated Temperature and Pressure

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ABSTRACT

Two microbial cultures—ML-13 (a *Candida* sp.) and LSC (a fungal isolate from the University of Arkansas)—have been employed in the direct liquefaction of Louisiana lignite. Lignite samples were pretreated with nitric acid and microbial culture broths at elevated temperatures and pressures. Subsequent treatment with active cultures and culture derivatives resulted in significant solubilization of the lignite. Up to 50% liquefaction of pretreated coal (20% HNO_3 at ambient temperature and pressure) was observed in 4 d with ML-13 cultures, whereas almost 80% liquefaction occurred in a similar time period when exposing pretreated lignite to an autoclaved, cell-free culture broth.

Index Entries: Lignite; coal solubilization; coal liquefaction; coal bioconversion; coal biotreatment.

INTRODUCTION

In recent years, much attention has been given to the ability of microorganisms to modify or biodegrade lignite coals. Beginning with the work by Cohen and Gabriel (*1*), numerous researchers have investigated the phenomenon of the microbial solubilization of coal (*2,3*). A number of organisms have been identified that exhibit a solubilizing capability (*3–5*) both by bringing the coal into direct contact with the organisms (*4,6–9*) and by treating the coal with cell-free extracts (*10–12*). A variety of factors influencing the degree of solubilization or biodegradation have been

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studied, including pH (*13*), the degree of chemical pretreatment by strong oxidizing or reducing agents (*2,11*), or by the introduction of nitrogen-containing functional groups (*14*) and cation removal from the coal (*15*). The microbial production of alkaline materials (*6,11,16*) and the ability of extracellular solutions to liquefy coal (in the absence of viable cells, pointing to the possible production of coal-solubilizing enzymes) (*6,10*) have each been proposed as part of the synergism of the overall biosolubilization phenomenon. Coal "bioliquefaction" has been demonstrated both with cultures growing on surface plates and in submerged culture broths (*7,9*). Process improvements in solubilization have been sought through application of specific enzyme systems (oxidoreductases) in aqueous and organic phases (*17*). Research has expanded to the study of the biosolubilized product as an intermediate for upgrading to methane or liquid fuels (*18*). Further work is ongoing for the development of assays for the quantitative determination of the degree of solubilization (*19*) and the characterization of the solubilized product (*20*).

Lignite, with its sizeable deposits in North America and its proven susceptibility to microbial liquefaction, serves as a sizeable resource for fuels and chemical feedstocks that may be recovered, in part, through the application of bioliquefaction. The presence of large lignite deposits serves as an impetus for further investigation into possible applications of the liquefaction process *in situ*.

The work herein described outlines further investigation into the degree of solubilization attainable through microbial treatment of lignite. The degree of pretreatment includes various concentrations of nitric acid reacted with coal at ambient conditions, and at elevated temperatures and pressures. The solubilizing capability of active surface and submerged cultures is compared with cell-free "bioextracts." Such effort will improve the knowledge base for *in situ* applications.

METHODS

Culture Preparation and Maintenance

The cultures used were ML-13 (a *Candida* sp.) (*4*) and LSC (a University of Arkansas fungal isolate)—both obtained from the University of Arkansas. These two cultures were selected in preliminary tests from a number of proven coal-solubilizing cultures. Cultures isolated at Louisiana Tech from a local lignite outcrop initially have exhibited nominal solubilizing capability to date (data not shown) in comparison and, at present, have been excluded from further study. All cultures were maintained on agar slants and plates of Sabauroud Dextrose Agar (SDA) at pH 8.0 and at 30°C. Cultures were also maintained in Sabauroud Dextrose Broth (SDB) at the same growth conditions as the plates with agitation on a rotary shaker at

Table 1
Typical Analysis of Louisiana Lignite, Dolet Hills Mine

	Ultimate analysis, wt. %		
	As received		Moisture free
Moisture	35.07		
Carbon	41.37		63.71
Hydrogen	2.86		4.40
Nitrogen	0.86		1.32
Chlorine	0.03		0.05
Sulfur	0.54		0.83
Ash	8.61		13.26
Oxygen	10.66		16.42
Calorific values, Btu/lb (kJ/kg)			
As-received basis	7019	(16,328)	
Dry coal basis	10,810	(25,147)	
Dry-ash-free basis	12,463	(28,992)	

150 rpm. Both surface and submerged cultures were allowed to grow 7–21 d prior to exposure to coal. Surface-culture transfers were made either by traditional streaking techniques, or by washing fungal mats with aliquots of sterile, distilled water (typically 10 mL) and transferring this solution to the new plate. Broth cultures were maintained by transfers of growth medium (10% v/v) from active broth cultures.

Coals Used and Coal Pretreatment

The coal primarily used was lignite obtained from the active mining operation—the Dolet Hills Lignite mine operated by the Central Louisiana Electric Co. in Mansfield, Louisiana (typical analyses presented in Table 1) (*21*). Additionally, weathered lignite samples were obtained from a nearby outcrop submerged in a semiactive stream bed (e.g., typically dry during the summer months). For comparison, Leonardite, which has been shown to be readily biosolubilized (*12*), was also tested. Coals were ground (using a ball mill grinder) and sieved to uniform particle sizes (with the exception of the Leonardite samples, which immediately separated into particles of approx −50 to +100 mesh on mixing with culture medium or distilled water). Lignite samples were separated into three ranges: −4 X +10, −10 X +30, and −50 X +100 mesh.

Coal samples were exposed to microbial broths either untreated or after pretreatment with nitric acid (HNO_3) of various concentrations (*2*). Nitric acid pretreatment was conducted at ambient conditions and at elevated temperatures and pressures in a Parr High Pressure Reactor (650 mL working vol). The reactor consists of a T316 stainless-steel vessel (and

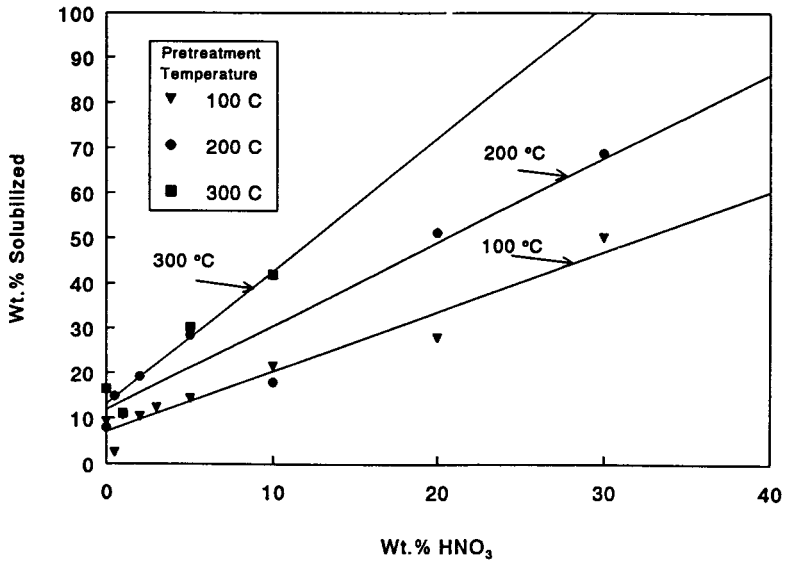

Fig. 1. Pretreatment solubilization with HNO_3 concentration and temperature.

internals), agitated at a constant rate of 700 rpm with two four-blade impellers of 40 mm diameter and a 45° pitch. The impellers were placed equidistantly between the reactor bottom and the liquid surface. The reactor temperature was thermostatically controlled by use of an external heating mantle and an internal cooling water coil. Twenty-five grams of coal were added to the reactor with 200 mL of nitric acid solution (or in some case, growth medium). The reactor temperature was maintained at constant values of 100, 200, and 300°C, respectively, for various treatments. Treatment periods varied from 30 min in the Parr reactor to 48 h for pretreatment at ambient conditions. Considerable liquefaction was observed during the pretreatment step, particularly with nitric acid pretreatment at elevated temperatures and pressures in the Parr reactor. Figure 1 presents typical solubilization values for pretreatment in the Parr reactor for 30 min/run as a function of nitric acid concentration and reaction temperature. Although some scatter is evident, particularly for the elevated temperature runs at 200 and 300°C, a general trend toward a linear increase in percent solubilization with increasing nitric acid concentration (at a constant pretreatment time) is observed. Lignite treated in this manner was retained for future use until baseline bioliquefaction studies were conducted with lignite pretreated with HNO_3 at ambient conditions.

After pretreatment, the coal was washed with water (*6*) until the pH was >6. Subsequently, the coal was dried at 105°C until a constant weight was achieved. Other studies have shown that the degree of solubilization depends significantly on the type of coal used—its grade, degree of weathering or oxidation, and its elemental analysis (*2,13,15*). When compared

with published reports of other lignites (*2,14*), Louisiana lignite contains a significantly lower amount of oxygen and slightly less nitrogen—two constituents that have been shown to affect the coal's susceptibility to biosolubilization. With this observation, some type of pretreatment of the coal was expected to induce microbial solubilization. Indeed, initial studies with a number of proven coal-solubilizing organisms growing in the presence of the untreated Louisiana lignite proved fruitless. Therefore, a pretreatment regimen was established to determine the conditions needed to induce bioliquefaction. Lignite samples were pretreated with a variety of chemicals and conditions, including air oxidation at 105°C, NaOH, KOH, H_2O_2, HCl, and HNO_3 (data not shown). The nitric acid pretreatment emerged as the superior agent for this coal and was used throughout the work reported here.

RESULTS

Surface-Culture Studies

To establish baseline data, lignite ($-10 \times +30$ mesh) was pretreated by adding the lignite to 20% HNO_3 (w/w) in a 2:1 (w/v) ratio. This slurry was allowed to stand for 48 h at ambient conditions (1 atm, 25°C) with periodic stirring. The slurry was then placed in a separatory funnel, and washed with an upflow of water until the pH exceeded 6 and until discoloration of the fluidized particles ceased. Plates containing SDA were inoculated with ML-13 and LSC, and allowed to grow until the plates were completely covered (approx 14 d). Predried and preweighed coal fractions were then placed on the surface of preweighed 0.4-micron (μm) cellulose nitrate filters, which, in turn, were placed on fungal mats of the active cultures of ML-13 and LSC. With the onset of solubilization (observed by the formation of black liquid droplets on the surface of the coal particles [3]), the filters were carefully removed, the coal washed with distilled water, and the remaining coal particles dried to a constant weight to determine the degree of solubilization gravimetrically (*6,7*). The filters were then carefully replaced on the surface of the culture plates. All tests were run in duplicate. Controls were conducted by placing coal on filters without active cultures. No solubilization was noted with controls. Results are shown in Fig. 2. Each data point represents an average of three individual coal samples. Over the 5-d run, three sets of plates (LSC-1, LSC-2, and ML13-1) showed similar rates of solubilization. However, by day 3, both plates with ML-13 clearly demonstrated a higher degree of solubilization as compared to the plates with the LSC culture. The plate showing the strongest degree of coal solubility (ML13-2) reached an average solubilization of 50%, ranging from 43 to 62% for the three coal samples tested.

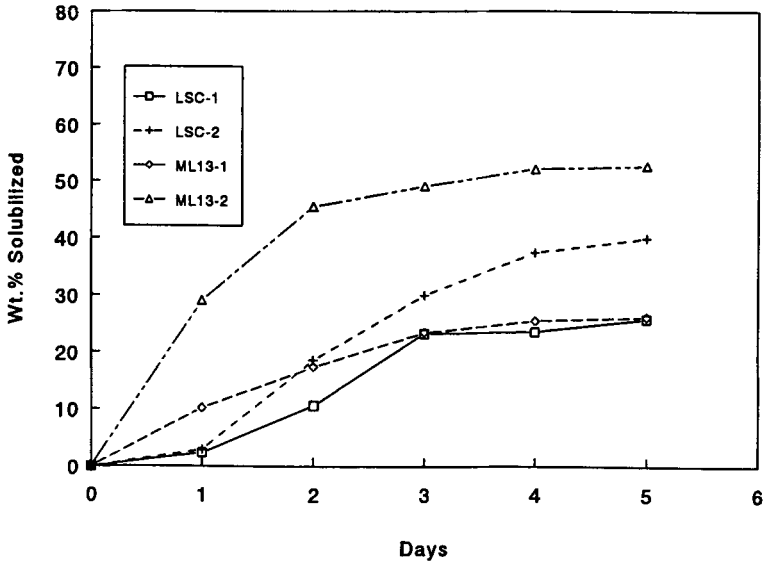

Fig. 2. Surface-culture liquefaction of coal. Pretreatment with 20% HNO_3 at ambient conditions.

Active Submerged Cultures and Cell-Free Broths

The solubilizing activity of some cultures has been shown to remain in varying degrees after autoclaving the broth (*11*). The surface-culture studies confirmed observations made by previous researchers that solubilization may be induced by cellular excretions when the coal is not in direct contact with the organisms (*3*). A second baseline study was conducted with submerged cultures by placing coal into flasks containing filtered, then autoclaved (30 min at 15 psig and 121°C) broth from active cultures of ML-13 and LSC. The cultures were inoculated into SDB and allowed to grow for 10 d. The growth media were then filtered through Whatman No. 1 filters and autoclaved. Ten-millileter aliquots of medium were mixed with 1-g quantities of HNO_3 pretreated coal (of various concentrations) in 20-mL culture tubes. As described with the surface-culture study, lignite fractions were pretreated with HNO_3 at ambient conditions. Tubes were placed in a stationary incubator at 30°C. Duplicate tubes were harvested every 24 h with the remaining tubes inverted to mix at each harvest period. The ML-13 broth showed a significantly higher degree of solubilization over the 5-d experimental period. The solubilization of the coal as a function of HNO_3 concentration is shown in Fig. 3. Again, as with acid pretreatments, the yield of biosolubilized coal increased with the concentration of HNO_3 in a somewhat linear fashion. The highest level of solubilization at 5 d occurred with 35% HNO_3 pretreated coal (36% biosolubilized).

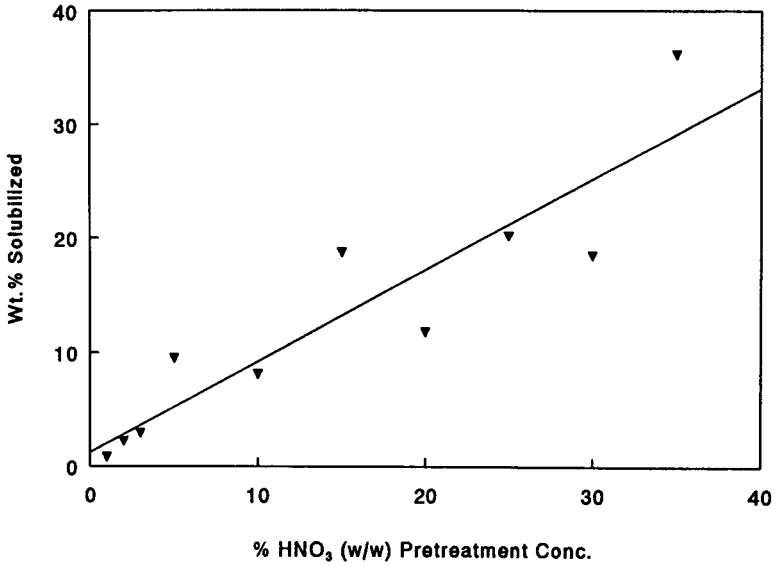

Fig. 3. Submerged culture liquefaction as a function of HNO_3 pretreatment concentration.

These results prompted an investigation into the possibility of actually enhancing solubilization by autoclaving active cultures prior to filtering, potentially releasing intracellular constituents that would contribute to the overall process. For autoclaved cultures, the broth was filtered to remove cellular particulates prior to coal addition. It should be noted that coal samples were added only after the culture broth was autoclaved and filtered, and that broth pH remained virtually constant during autoclaving. Tests were conducted with 30% HNO_3 pretreated coal (4 × 10 mesh) mixed in the Parr reactor at 200°C for 30 min. For comparison, samples of coal were treated using both active ML-13 and LSC culture broths and autoclaved volumes of the same culture broths. Samples of dried coal were placed in culture tubes with 40 mL of broth. Tubes were then placed in a stationary incubator at 30°C. Controls consisted of similarly pretreated coal placed in sterile, uninoculated broth and in distilled water—both of which showed no solubilization. At periodic intervals, the solid coal was filtered, washed, and dried to a constant weight to determine the percent solubilization. After weighing, the dried coal was returned to the culture tubes, and remixed with "fresh" broth either containing active cultures or having been autoclaved and filtered. Results are shown in Fig. 4. For coal samples mixed with autoclaved broth, a much greater degree of microbial solubilization was observed. Initially mixed with active culture broths, these coal samples experienced a relatively low degree of solubilization (0–4%) over a 6-h period. When mixed with autoclaved broths, however, a significantly higher rate and extent of solubilization were observed.

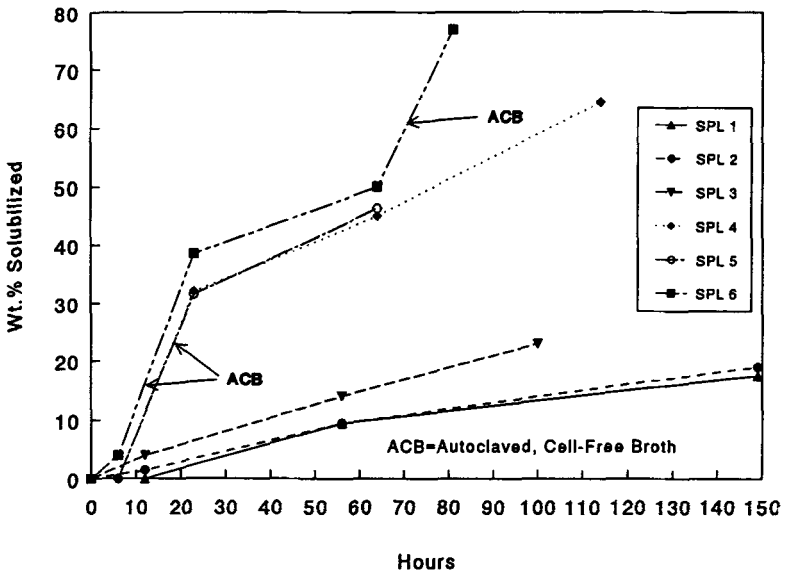

Fig. 4. Comparison of bioliquefaction in cell-free broth with active broth cultures.

Triplicate samples (Nos. 4–6) showed an increase of over 30% solubilization (t = 6–17 h in Fig. 4). When again mixed with active culture broth, the rate of solubilization declined to a level near that of samples mixed only with active cultures throughout the experiment (Nos. 1–3) (t = 17–64 h). A last comparison was made by returning one coal sample to active culture broth and one to autoclaved broth. The coal mixed with the active culture broth experienced a rate of solubilization similar to Samples 1–3, whereas the coal mixed with the autoclaved broth again exhibited a higher rate and ultimately a much greater extent of solubilization reaching 77% ($t \geq 64$ h). This yield can be compared to only 20% solubilization of lignite pretreated with 30% HNO_3 at ambient conditions and mixed with the submerged cultures in the previous experiment described above. For comparison of both the pretreatment temperature and the autoclaved vs active culture broths, triplicate samples of coal pretreated with 30% HNO_3 at 100°C in the Parr reactor for 30 min were mixed with active ML-13 cultures. Over a 5-d period (comparable with the results described above), these samples exhibited an average of only 6% solubilization. At the completion of this experiment, however, samples containing active cultures were autoclaved and filtered. Solubilization had increased to 22% as a result of autoclaving.

Influence of pH

The influence of base constituents generated microbially on the solubilization of coal has been demonstrated (*3,13,16*). However, further evidence suggests the participation of other mechanisms, including the enzyme-

Table 2
Comparison of Coal Solubility
in Cell-Free Broth and Alkaline Solutions
32-H Test Duration

Solution conc.	Final pH	Wt.% solubilized
ML-13 Broth	6.90	17.36
0.01*N* NaOH	11.81	0
0.05*N* NaOH	12.40	2.94
0.1*N* NaOH	12.71	3.88
0.5*N* NaOH	13.02	3.92
0.01*N* NH_4OH	10.19	0
0.05*N* NH_4OH	10.69	3.92
0.1*N* NH_4OH	10.88	6.93
0.5*N* NH_4OH	11.37	0.71

catalyzed cleavage of carbon–carbon bonds and the complexation and removal of metal ions from the coal (*12,15,19*). To examine the contribution of factors other than alkalinity, pretreated samples of lignite were mixed with dilute concentrations of NaOH and NH_4OH (0.01, 0.05, 0.1, and 0.5*N*) and with autoclaved, cell-free ML-13 broth. The pretreatment procedure consisted of mixing 30 g of 4 × 10 mesh coal with 350 mL of 5% HNO_3 in the Parr reactor at 200°C for 1.5 h. After pretreatment, the moisture-free lignite was placed in culture tubes with 40 mL of solution and placed on a shaker/incubator bed operating at 150 rpm and 30°C. Table 2 shows that, although solubilization was slight for all samples, the culture broth, at a significantly lower pH than the NaOH solutions, solubilized the coal to a greater degree. Further tests are underway to verify these observations. Although ultimate and proximate analyses have not yet been performed on the coal before and after various treatments, the likelihood of participative mechanisms other than OH^--mediated solubility exists.

DISCUSSION

The biosolubilization of coal has been shown to be functionally dependent on the degree of pretreatment and confirms earlier work (*2,3,11*). Although the rate and extent of biosolubilization increased with the severity of pretreatment conditions (e.g., HNO_3 concentration and temperature), the ability to solubilize lignite with milder chemical pretreatments, but at elevated temperatures and pressures has been demonstrated. With all pretreatment schemes, variations in particle size distributions were observed to be functionally dependent on the severity of pretreatment conditions

(e.g., increasing the severity of pretreatment conditions proportionally decreases coal particle size). Additional study of the pretreatment effects on the three-dimensional and surface structure of the coal and the subsequent effects of coal particle size on its susceptibility to bioliquefaction is necessary. The preliminary observations implying a superior activity of autoclaved, filtered culture broth when compared to active culture broth will be studied further. The ability to utilize a cell-free bioextract rather than maintaining a viable culture with the inherent transport problems associated with a high cell density offers a significant improvement in the bioliquefaction process, particularly when applied to *in situ* treatment of coal deposits.

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