

CHARACTERIZATION OF BIODEGRADED COALS

R. M. Bean, J. A. Franz, J. A. Campbell,
J. C. Linehan, D. L. Stewart, and B. L. Thomas

Pacific Northwest Laboratory
P. O. Box 999
Richland, WA 99352

INTRODUCTION

Microbial degradation of coals to materials that are soluble in water has been a topic of intensive research for the last few years. The potential for economical recovery of low-grade coals, coupled with possibilities for further upgrading by microbial desulfurization or methanation has spurred intensive research at a number of laboratories. Until very recently, coal biodegradation has been accomplished using low-grade, naturally oxidized coals such as Leonardite (1,2,3), or coals subjected to pretreatment with oxidizing chemicals (4). We have been able to accomplish the biodegradation of bituminous Illinois #6 coal after a pretreatment consisting of air oxidation, using a culture of the fungus *Penicillium* sp. We report in this paper results of chemical and spectrometric analyses of the starting materials and products from Illinois #6 coal biodegradation, and compare the results with those previously reported (2) from the biodegradation of Leonardite.

EXPERIMENTAL

Coal and Coal Pretreatment - Illinois #6 coal (mine-washed) was obtained from the Illinois Department of Natural Resources. Prior to microbial treatment, the coal was sized to 0.5 to 5 mm diameter, spread to a layer approximately 1 cm thick on Al foil, and heated in a forced draft oven at 150°C for 7 days. Thermocouple measurements made on the coal during pretreatment showed that the coal temperature did not deviate significantly from the oven temperature.

Coal Biodegradation - Agar plates containing Sabourauds maltose medium (Difco Laboratories, Detroit, MI) were inoculated with a strain of *Penicillium* obtained from Professor Bailey Ward of Louisiana State University. After 7 days incubation at room temperature, 0.3 to 0.4 g of pretreated Illinois #6 coal was placed on each plate, and incubation continued. Water-solubilized coal was harvested by pipette after 5 weeks, passed through 0.45 μ filters, and freeze-dried. The residual material was extracted with 50 mL portions of 0.5 N NaOH until extracts were colorless (approximately 700 mL was used per plate). The base solubilized coal was filtered (0.45 μ), acid precipitated (HCl, pH 2), and dried.

Solubilization of leonardite coal by *Coriolus versicolor* has been described elsewhere (2).

Analysis for conversion to Soluble Products - Conversion of coal to water or base solubles was determined by measurement of optical density at 680 nm after appropriate sample dilution. Standards were prepared from dry acid-precipitated subsamples of solubilized coal product.

Elemental Analysis - Samples were dried for 48 hr under vacuum at 125°C prior to analysis. Elemental analyses were carried out by Schwarzkopf Microanalytical Laboratory, Woodside, NY.

Gel Permeation Chromatography(GPC) - GPC was conducted in tetrahydrofuran solvent containing 0.1% acetic acid flowing at 1 mL/min through two μ Styragel columns (100 and 500 Å, Waters Associates (Milford, MA) in series. Detection was by ultraviolet absorbance (Waters Model 490) and by mass detector (Applied Chromatography Systems Ltd, (Bedfordshire, England). Calibration was with polystyrene standards, and with pyrene.

¹³C NMR Spectroscopy - A Varian VXR-300 equipped with an ultra high speed probe (Doty Scientific, Inc., Columbia, SC), spinning at 14 kHz, was used to obtain NMR spectra of coal and coal bioproducts. At the high spin rate, no sidebands interfere within the entire spectral window of interest. The instrument was run in the cross-polarization mode, with a 56 kHz decoupling field.

RESULTS

Recoveries of water and base soluble material from the plates after degradation for 5 weeks are shown in Table 1. In contrast to the biosolubilization of leonardite, which is rendered water soluble by *C. versicolor*, yields of water solubles from *Penicillium*-biodegraded pretreated Illinois #6 coal were very low. The total yield of solid material that could be directly pipetted from the plates was on the order of 3%. When this material was recovered from solution by acid precipitation rather than freeze-drying, less than half could be recovered, an indication that the water-solubles were contaminated by the media in which the organism was grown. Recovery of coal material by base extraction was much more satisfactory. Although recovery with 0.5 N NaOH is indicated to be 80 to 90% in Table 1, recent data has shown that after 6 weeks of incubation, over 95% can be extracted from the plates with base. The high degree of solubility in even 0.01 N base is an indication that the solubilization by base results from a metathetical exchange of sodium ions for active hydrogens, rather than solvolysis reactions.

The data in Table 2 indicates that the biodegraded material is different from materials derived from base solubilization of the

air-oxidized coal. Base solubilization of air-oxidized Illinois #6 coal is only accomplished to a significant extent from 24-hr treatment with 2.4 N base. Conditions producing high yields of solubilized material from the biotreated coal give only 6% solubles from the undegraded pretreated coal. In addition, the biodegraded material has significantly different molecular weight properties than the undegraded, base solubilized oxidized coal (Table 2). Although the molecular weight (MW) ranges are similar, the base soluble biodegraded coal exhibits a weight-average MW only one-sixth that of the base solubilized oxidized coal. After recovery by acid precipitation, the water-soluble biodegraded Illinois #6 coal is essentially indistinguishable in MW properties from the corresponding base-soluble material. For comparison, the molecular weight characteristics of biodegraded leonardite are included; there is a significant difference in both weight average MW and MW range between the Illinois #6 product and the leonardite product.

Ash and elemental analyses of the Illinois #6 coal, before and after pretreatment, and the coal-biodegraded products are presented in Table 3. It is clear from the data that the pretreatment caused profound changes in the coal composition. Weight loss upon heating in air was 10%; essentially the same as when the coal is vacuum dried at 125°C. However, Table 3 shows that there was a substantial loss in both carbon and hydrogen after heating, offset by a large increase in oxygen content. The elemental compositions of the biodegraded materials were lower in sulfur, presumably because of pyrite losses during the solubilization and filtration processes. Nitrogen was somewhat elevated, probably due to some contamination with protein material. Oxygen was appreciably elevated in the water soluble product, and oxygen/carbon ratios were elevated in the products over the starting pretreated coal. Low material balances in the acid-precipitated products resulted from relatively high quantities of chlorine were present (2.6% in the water soluble product, and 7.4% in the base soluble product). Chlorine (as chloride ion) would displace metallic oxide oxygen or hydroxyl during the acid precipitation process.

¹³C NMR spectra of untreated Illinois #6 coal, 150°C air-treated Illinois #6 coal, and the base soluble biodegradation product are shown in Figure 1. Chemical shifts obtained from carbon in different chemical environments have been well-documented in coal samples (5). The chemical shift region from 0 to 75 ppm includes resonance from carbon in aliphatic linkages; the region from 90 to 155 ppm contains resonance from carbon present in aromatic rings; from 155 to 215 ppm is found signal from carbon present in carbonyl structures. Specifically, chemical shifts in the region 165-185 ppm are assigned to carbon in carboxyl groups (6). From Figure 1, it can be seen that upon air treatment at 150°C, the aliphatic region is reduced in intensity relative to the aromatic region, and the aromatic region somewhat broadened. Upon biodegradation, the aliphatic peak is even

further reduced, while a distinct peak in the carboxyl region is produced. For the three Illinois #6 samples, quantitative determinations of the relative abundances of different carbon obtained by integration of the three chemical shift areas are given in Table 4, together with data obtained from leonardite and biodegraded leonardite.

DISCUSSION

Recent studies of the air oxidation of coal between ambient and 150°C (7, 8, 9) indicate that the incorporated oxygen is largely present in ether-type linkages rather than in carboxyl groups as might be expected. Infrared studies of coal during oxidation at temperatures between 25 and 100°C (7,8) have indicated initial formation of carboxyl groups, followed by their disappearance (presumably through thermal decarboxylation), and the evolution of ether bonds. A study of the oxidative weathering of freshly mined Illinois #6 coal (9) in which an additional 26% oxygen was incorporated over 2 months under ambient conditions, found that no carbonyl groups were present. The NMR spectrum obtained from our pretreated coal sample indicates that carboxyl groups are not in high concentration. The broadening of the peak containing chemical shifts from aromatic carbon toward higher chemical shifts in the pretreated sample (Figure 1), may be an indication of carbon involved in ether bonds, since the C-O chemical shift is in the region 148-158 ppm (5). We thus have evidence through the literature and through the NMR spectra that oxygen incorporation into Illinois coal during pretreatment is largely through ether formation, and that losses of carbon and hydrogen are through CO and CO₂ evolution, as well as losses of other volatiles.

Shown in Table 5 are the empirical formulas, based on 100 carbon atoms, obtained from elemental analyses of Illinois #6 and leonardite coals and coal products. In terms of elemental composition, the effect of the microbial action on the oxidized coal does not appear to be great. For the Illinois #6 coal 4 oxygens, 11 hydrogens, and 1 nitrogen were added per 100 carbon atoms; for the leonardite case, 2 oxygens, 7 hydrogens, and 2 nitrogens were added per 100 carbons. Although stoichiometry is not precise, the addition of the elements of water during the microbial degradation indicates that hydrolysis is involved in the biodegradation of oxidized coal. Further, an oxidative hydrolysis mechanism is suggested by the reduction in molecular weight of the microbial product, the appearance of carbonyl in the ¹³C NMR spectra after biodegradation, the facile solubility of the product in weak base, and the finding in our laboratories that soluble enzymes produced by the coal-degrading organisms readily hydrolyze benzyl ethers and oxidize aromatic hydrocarbons (J. A. Campbell et al., presented at this symposium).

The oxidation of lignin materials has been reported to occur through mechanisms involving cleavage of beta-aryl ethers (10,11), and

aromatic ring cleavage (12). In addition, parallel pathways of side chain degradation, decarboxylation, and aromatic ring opening have been described for the degradation of lignin model compounds (13). From our ^{13}C NMR data (Table 4), leonardite loses 10% aromatic carbon while gaining 10% C=O carbon, which is consistent with a mechanism of aromatic oxidative hydrolysis; however, there does not appear to be a loss of aryl carbon in the case of the Illinois #6 coal (Table 4). For Illinois #6 coal, ether cleavage and side chain degradation seems to be favored, since loss of aliphatic carbon is observed.

REFERENCES

- (1) Cohen, M. J.; Gabriel, P. D. Appl. Environ. Microbiol. (1982), 44, 23-27.
- (2) Wilson, B. W.; Bean, R. M.; Franz, J. A.; Thomas, B. L.; Cohen, M. S.; Aronson, H.; Gray, E. T., Jr. Energy and Fuels (1987) 1, 80-84.
- (3) Pyne, J. W., Jr.; Stewart, D. L.; Fredrickson, J.; Wilson, B. W. Appl. Environ. Microbiol. (1987), 53, 2844-2848.
- (4) Quigley, D. R.; Wey, J. E.; Breckinridge, C. R.; Stoner, D. L. In: Processing and Utilization of High Sulfur Coals II, Y. P. Chugh and R. C. Caudle, Eds. Elsevier Science, pp 316-322.
- (5) Snape, C. E.; Ladner, W. R.; Bartle, K. D. Anal. Chem., 1979, 51, 2189.
- (6) Levy, G. C.; Nelson, G. L. Carbon-13 Nuclear Magnetic Resonance for Organic Chemists, Wiley-Interscience, NY, NY, p 117.
- (7) Bouwman, R.; Freriks, I. L. C. Fuel (1980), 59, 315-322.
- (8) Gethner, J. S. Appl. Spect. (1987), 41, 50-63.
- (9) Liotta, R.; Brons, G.; Isaacs, J. Fuel (1983), 62, 781-791.
- (10) Miki, K.; Renganathan, V.; Gold, M. H. Biochem. (1986), 25, 4790-4796.
- (11) Enoki, A.; Goldsby, G. P.; Gold, M. H. Arch Microbiol. (1981), 129, 141-145.
- (12) Umezawa, T.; Higuchi, T. FEBS (1985) 182, 257-259.
- (13) Haider, K; Trojanowski, J. Arch. Microbiol. (1975), 105, 33-41.

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Table 1. Yields of *Penicillium*-Degraded Illinois #6 Coal

<u>Extracting Solvent</u>	<u>% of Biodegraded Illinois #6 Coal Extracted</u>
Water	3.0 ±0.8 (s.d., n=6)
0.50 N NaOH	79.0 ±10.9 (range, n=2)
0.05 N NaOH	89.4 ±2.2 (range, n=2)
0.01 N NaOH	51.0 ±6.5 (range, n=2)

Table 2. Yields and GPC-Determined Molecular Weights of Soluble Coal Fractions

<u>Sample</u>	<u>Solvent Treatment</u>	<u>% Soluble</u>	<u>Wt. Av. Mole Weight</u>	<u>MW Range (THF Sol*)</u>
Biodeg. Ill #6 (Insol. H ₂ O, sol. base)	0.05-0.5 N	100	900	200 - 100,000
H ₂ O-Sol Ill#6 (Acid pptd)	H ₂ O	100	1000	200 - 100,000
Air Ox. Ill #6 (150°C, 7 days)	2.4 N NaOH	25	6,000	200 - 100,000
Air Ox. Ill #6	0.5 N NaOH	6	-	-
Biodegraded Leonardite	H ₂ O	100	2,000	200 - 20,000

* Base soluble fractions were 70 to 90% soluble in the GPC solvent

Table 3 Elemental Analysis of Illinois #6 Coal
Biodegradation Products: Comparison with Starting
Materials and Leonardite Products

Sample	% C	% H	% O	% N	% S	% Ash	Total
ILL #6 Coal	67.43	4.08	11.8	1.5	4.35	14.16	103.32
Air Ox Coal	58.53	2.13	23.42	1.16	4.36	14.42	104.02
Base Sol Prod	52.85	2.41	23.74	1.77	2.57	10.15	93.49
H ₂ O Sol Prod (acid precip)	56.12	2.14	29.75	3.04	2.59	2.03	95.67
Leonardite	54.95	3.6	30.24	0.8	1.06	8.32	98.97
Leon Prod (acid precip)	53.53	3.83	30.7	2.07	1.06	3.48	94.67

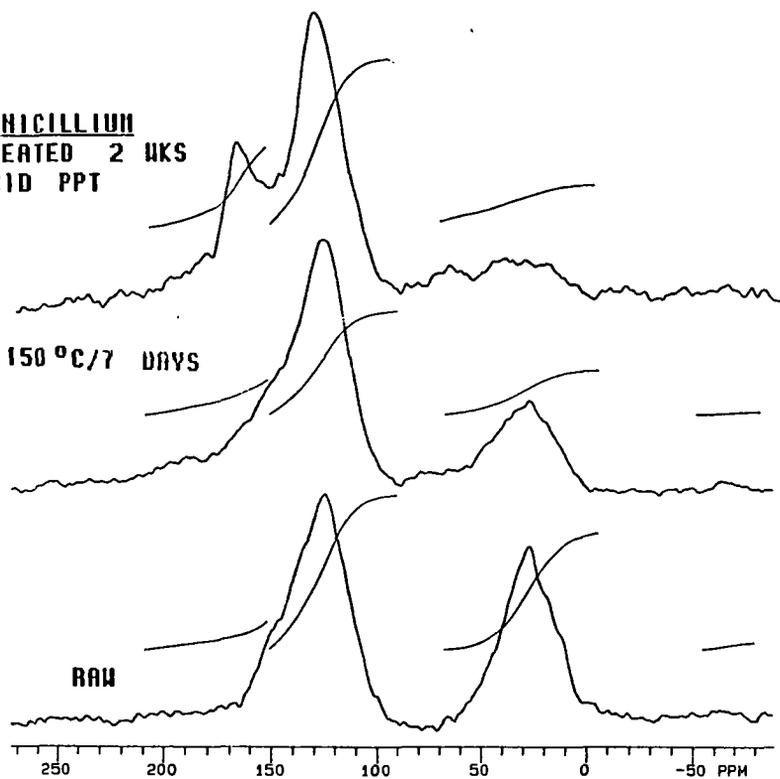
Table 4. Carbon-Type Analysis of Coal and Biodegraded Coal
Samples by ¹³C NMR

	% C=O	% Aromatic C	% Aliphatic C
Illinois #6	5	55	40
Oxidized Illinois #6	17	58	25
Biodegraded Ill #6	28	60	12
Leonardite	10	45	45
Biodegraded Leonardite	20	35	45

Table 5. Empirical Formulas Calculated for Illinois #6 Coal,
Leonardite, and Their Biodegradation Products

Illinois #6 Coal	C ₁₀₀ H ₇₃ O ₁₃ N _{1.9} S _{2.4}
Heat-Treated Illinois #6 Coal	C ₁₀₀ H ₄₄ O ₃₀ N _{1.7} S _{2.8}
Base Soluble, Acid Precipitate	C ₁₀₀ H ₅₅ O ₃₄ N _{2.9} S _{1.8}
Leonardite	C ₁₀₀ H ₇₉ O ₄₁ N _{1.2} S _{0.7}
Water Soluble, Acid Precip.	C ₁₀₀ H ₈₆ O ₄₃ N _{3.3} S _{0.7}

**PENICILLIUM
TREATED 2 WKS
ACID PPT**



150 °C / 7 DAYS

RAW

Figure 1. ^{13}C NMR spectrum of Illinois #6 coal and coal products. Bottom, untreated Illinois #6 coal; Middle, coal pretreated by heating at 150°C in air; Top, pretreated coal after degradation by the fungus *Penicillium* sp.