

Bio-Liquefaction/Solubilization of Low-Rank Turkish Lignites and Characterization of the Products

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The effect of some white-rot fungi on the bio-liquefaction/solubilization of two low-rank Turkish coals and the chemical composition of the liquid products and the microbial mechanisms of coal conversion were investigated. Turkish Elbistan and Beypazari lignites were used in this study. The white-rot fungi received from various laboratories used in the bio-liquefaction/solubilization of the lignites were *Pleurotus sajor-caju*, *Pleurotus sapidus*, *Pleurotus florida*, *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, and *Coriolus versicolor*. FT-IR spectra of raw and treated coal samples were measured, and bio-liquefied/solubilized coal samples were investigated by FT-IR and LC-MS techniques. The *Coriolus versicolor* fungus was determined to be most effective in bio-liquefying/solubilizing nitric acid-treated Elbistan lignite. In contrast, raw and nitric acid-treated Beypazari lignite seemed to be unaffected by the action of any kind of white-rot fungi. The liquid chromatogram of the water-soluble bio-liquefied/solubilized product contained four major peaks. Corresponding mass spectra of each peak indicated the presence of very complicated structures.

Introduction

Microorganisms may either attack the carbonaceous coal matrix or the dispersed inorganic materials within the structure of coal, affording liquefaction. One possible route to liquefaction is the depolymerization of the coal and cleavage of various links. A second route could be a reduction of oxygen content via transformation of C=O to CH₂ or possibly via decarboxylation of the matrix, which in turn would improve the calorific value. A third mode of liquefaction would be the removal of sulfur, nitrogen, or metals from the coal prior to combustion, which might break up the structure as well as reduce undesired emissions.¹ Bio-liquefaction/solubilization of coals has been studied extensively in the past two decades by many research groups. Fakoussa² and Cohen and Gabriele³ were the first to report microorganisms capable of using coal material as an energy source for growth. These microorganisms possessed a particular

ability to depolymerize and thus liquefy/solubilize coal. While the molecular mechanisms of biosolubilization of brown coal by fungal attack is limited, it has been suggested that several compounds produced by microorganisms, such as alkaline substances, oxidative and hydrolytic enzymes, and chelators, could be involved in the microbial solubilization of coal.⁴ Most filamentous fungi are described as saprophytes, that is, they live on organisms that are no longer alive, such as fallen trees and leaves. These microorganisms depolymerize macromolecules from the environment and use the simple products as an energy source for growth. When fungi secrete enzymes into an extracellular medium bearing their food source, the enzymes digest the food and then the fungi absorb the breakdown products. Ligninase, Mn-peroxidase, and laccase are some of the enzymes used during lignin depolymerization.^{5,6} As coals contain some material derived from lignin or fossilized plants, it would follow that the material should be vulnerable to depolymerization by similar extracellular fungal enzymes. In fact, liquefaction/solubilization by fungi

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Table 1. Analyses of the Lignites

	Elbistan	Beypazari
elemental analysis, %, dmmf		
C	62.7	65.2
H	4.7	5.4
N	0.8	2.1
S (total)	4.0	5.4
O (by difference)	27.8	21.9
H/C	0.9	1.0
proximate analysis, %		
moisture, as received	34.6	22.3
fixed carbon, dry	27.7	25.4
volatile matter, dry	46.7	31.8
mineral matter, dry	25.6	42.8
calorific value, kJ/kg	10500	18190

yields a solution of coal macromolecules, principally humic acids.⁷ It is recognized that low rank coals and oxidized coals were more susceptible to solubilization by the wood-rot fungus *Coriolus versicolor* and by a range of other fungal species.⁸ These findings suggest that the vulnerability of coals to bio-liquefaction/solubilization decreases with an increase of rank and increases with the state of preoxidation.

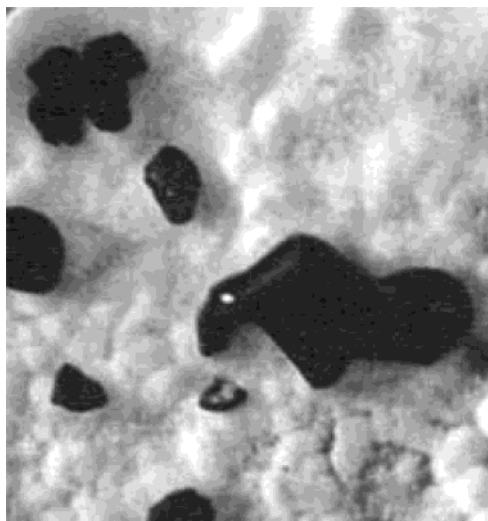
The microbial solubilization of some Turkish lignites was investigated recently.⁹ The purpose of the present work was to investigate the effect of some white-rot fungi on the bio-liquefaction/solubilization of two low-rank Turkish coals, to characterize the liquid products, and to investigate and assess the consequences of microbial coal conversion.

Experimental Section

Preparation of Lignite Samples. Turkish Elbistan and Beypazari lignites were used in this study. Elbistan lignite is in the transition zone between lignite and peat ranks (Pleistocene–Pliocene, 2–5 million years B.P.)^{10,11} and Beypazari lignite is a tertiary lignite (Late Miocene, 25–5 millions years B.P.).¹² The Afsin-Elbistan lignite field is situated about 500 km SE of Ankara.¹¹ The large Beypazari basin lies about 100 km NW of Ankara in Central Anatolia.¹³ The elemental analyses of the lignites are presented in Table 1. Lignite samples were ground in a ball mill to 3.35 mm–2.00 mm size using –6/+9 mesh sieves. Lignite samples were oxidized in 8 M HNO₃ for 48 h. Samples were washed with distilled water until the pH of the washings was 5.0, then they were dried in an oven at 70 °C for 24 h.

Bio-Liquefaction/Solubilization. Malt extract agar was used as the growth medium. The composition of the malt extract agar is presented in Table 2. Malt extract agar solutions (100 mL) were sterilized in Erlenmeyer flasks placed in an autoclave at 120 °C for 15 min and then poured into Petri dishes for solidification.

The subsequent white-rot fungi received from various laboratories used in the bio-liquefaction/solubilization of the lignites were *Pleurotus sajor-caju*, *Pleurotus sapidus*, *Pleurotus*

**Figure 1.** Complete bio-liquefaction/solubilization of the nitric acid-treated Elbistan lignite as observed on the 15th day.**Table 2. Composition of Growth Media**

maltose, g	12.75
dextrine, g	2.75
bactoglycerol, g	2.33
bactopeptone, g	0.78
bactoagar, g	15.00
water, mL	1000

florida, *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, and *Coriolus versicolor*. The fungi were grown on malt extract agar slants. Mycelia from solid cultures were removed and suspended in 10 mL of sterilized distilled water and were homogenized. Constant volumes of homogenate in test flasks were incubated aerobically using an orbital shaker with a shaking rate of 150 rpm at 30 °C for 7 days. Inocula of 2-mL size were drawn from this homogenate poured onto solid growth media in Petri dishes and used for the bio-liquefaction/solubilization of the lignites. Lignite particles previously treated with nitric acid solution were manually distributed into the Petri dishes as to be 1 lignite particle/cm² area of the Petri dish. Petri dishes were placed into a constant-temperature and air-ventilated furnace. Bio-liquefaction/solubilization experiments were conducted at 30 °C. The time of addition of the lignite particles into the Petri dishes was recorded as the zero time for the bio-liquefaction/solubilization experiments. After incubation, the remaining solid coal was separated and dried at 80 °C. Liquefied material was kept under nitrogen atmosphere for further analyses.

Chemical Characterization of the Raw and Treated Coals. FT-IR spectra of raw and treated coal were measured with a Bruker EQUINOX 55 FT-IR spectrometer. Coal samples were dried under a nitrogen atmosphere at 110 °C for 24 h. KBr pellets were prepared by grinding 2.5 mg of dry coal sample with 200 mg of dried KBr. Spectra were obtained with 200 scans at a resolution of 2 cm⁻¹.

The liquid chromatogram of the bio-liquefied/solubilized product was measured using a Shimadzu LC-MS-2010 HPLC system.

LC Parameters. Pump flow rate: 0.2 mL/min; oven temperature: 45 °C; LC column: Macherey-Nagel C18; mobile phase: 80% water, 15% acetonitrile, 5% methanol; injection volume: 5 µL.

PDA (Photodiode Array Detector) Parameters. Lamp: deuterium; wavelength: 200–300 nm; sampling rate: 0.64 s.

MS parameters. Ionization mode: ESI (Electrospray Ionization); acquisition mode: scan negative; probe voltage: –3.5 V;

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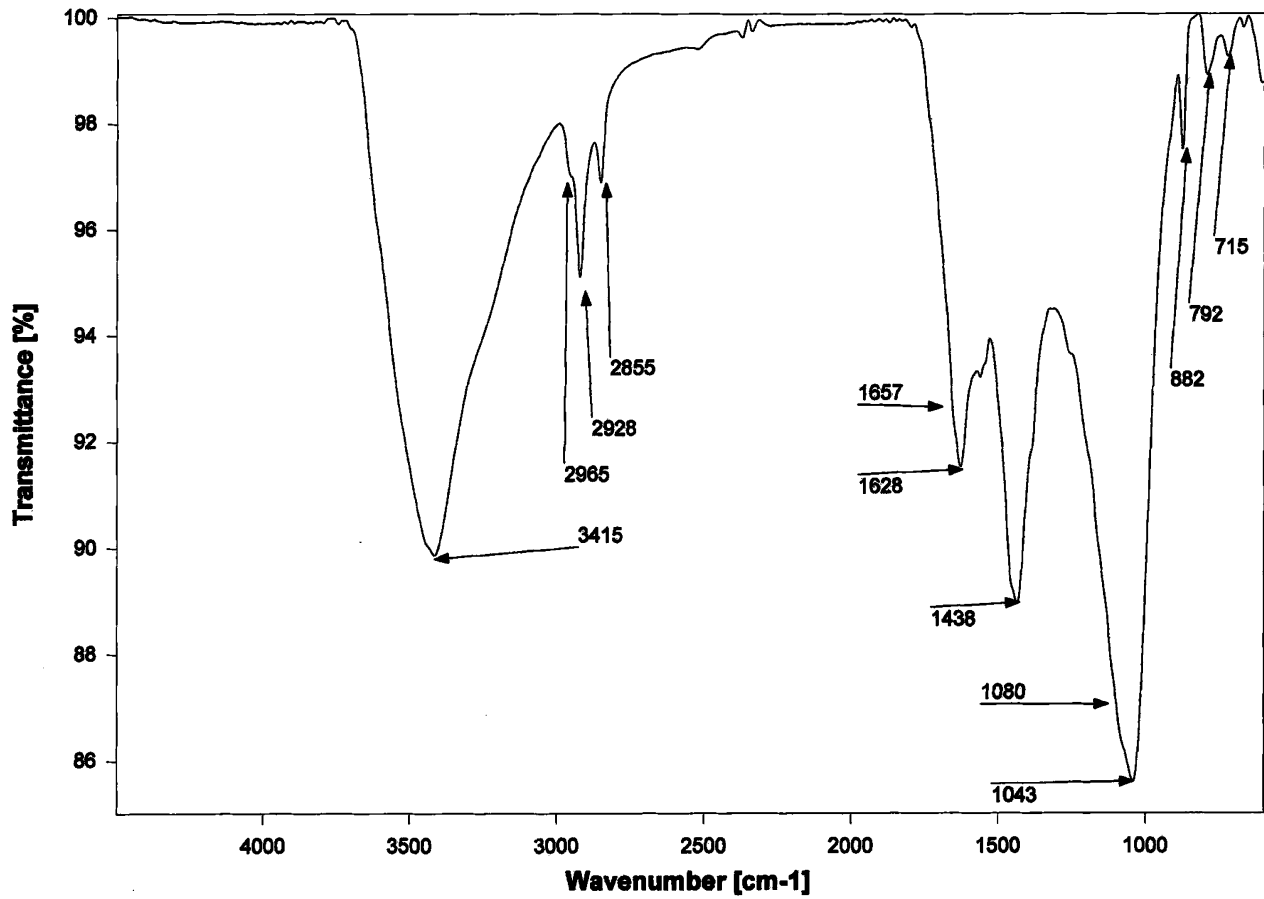


Figure 2. FTIR spectra of Beypazari lignite.

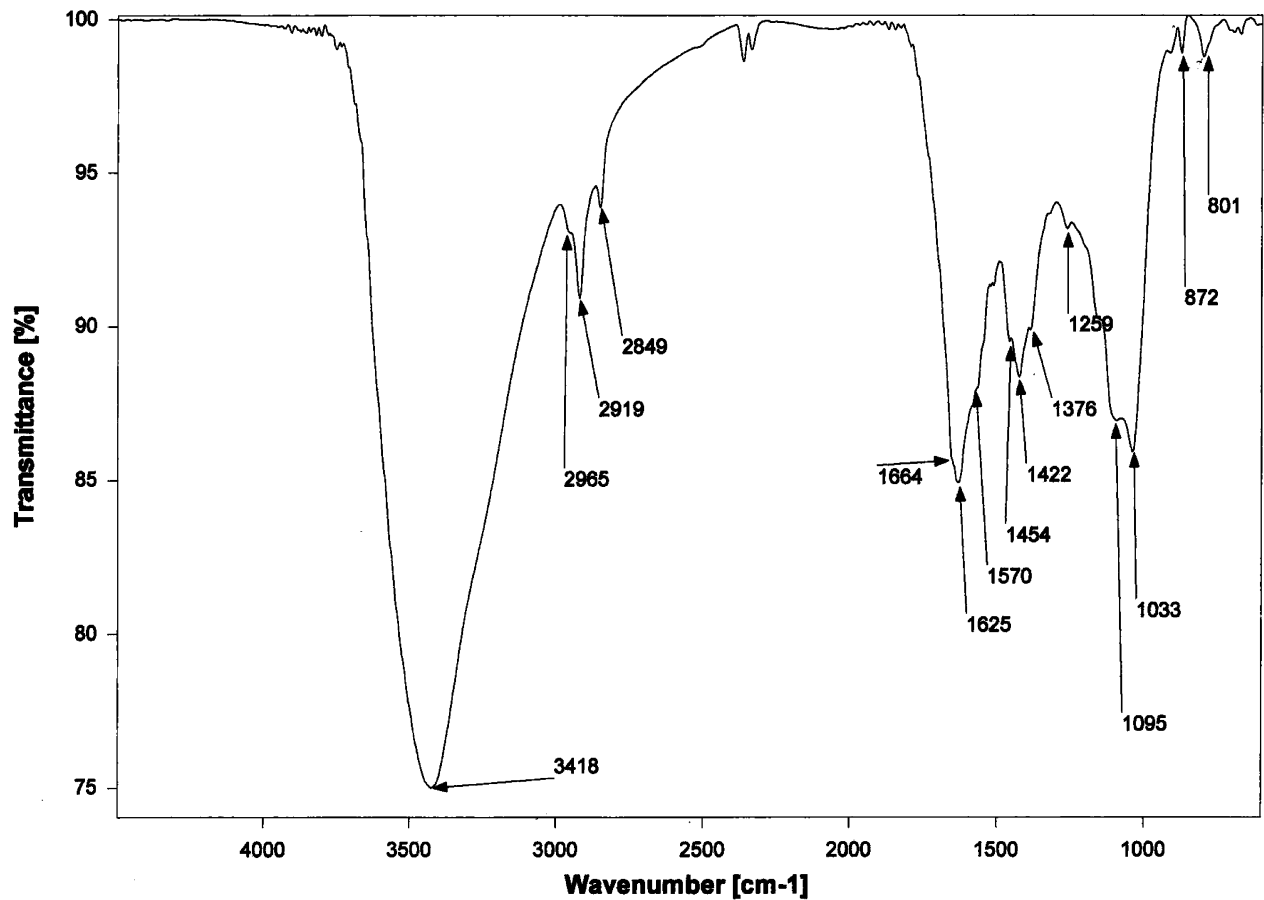


Figure 3. FTIR spectra of Elbistan lignite.

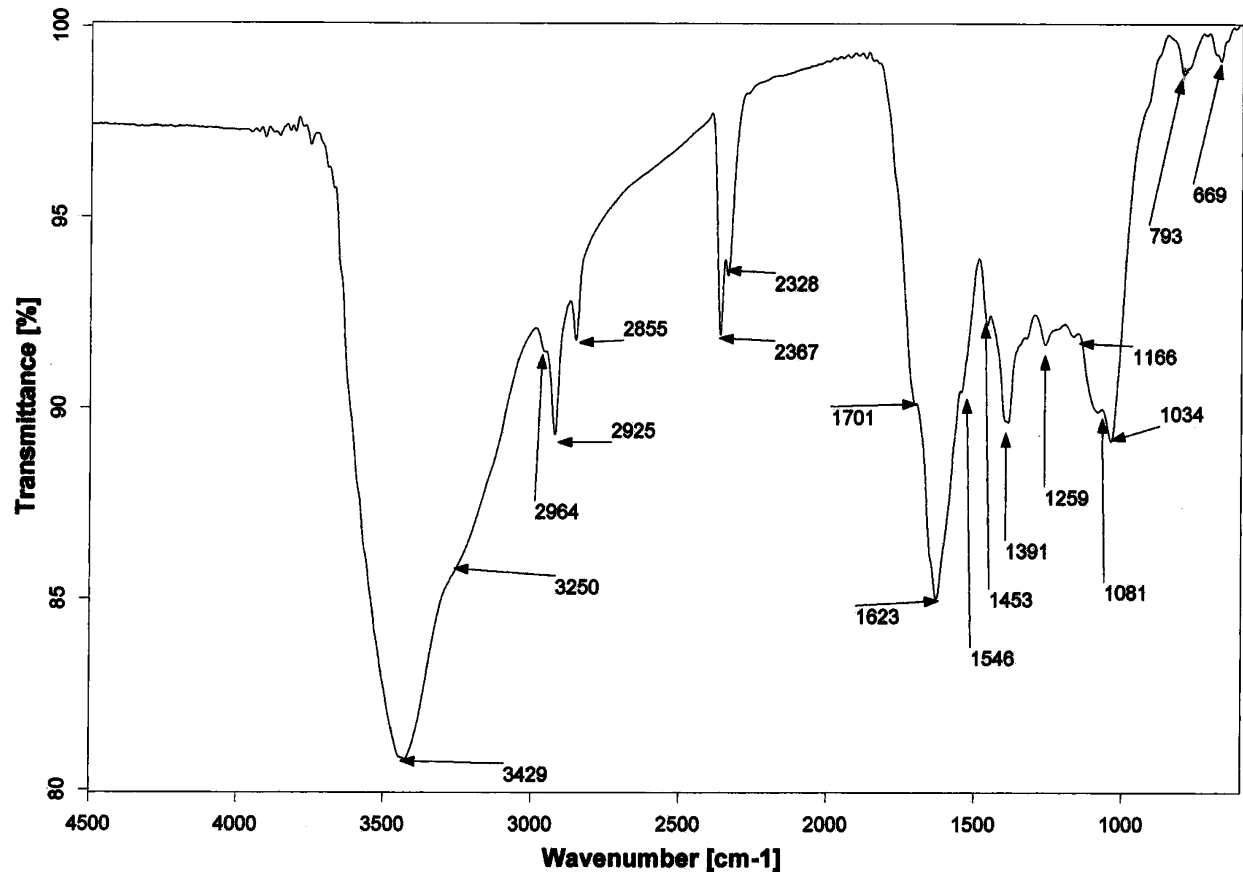


Figure 4. FTIR spectrum of the bio-liquefied/solubilized product obtained from Beypazari lignite.

PDA Chromatogram

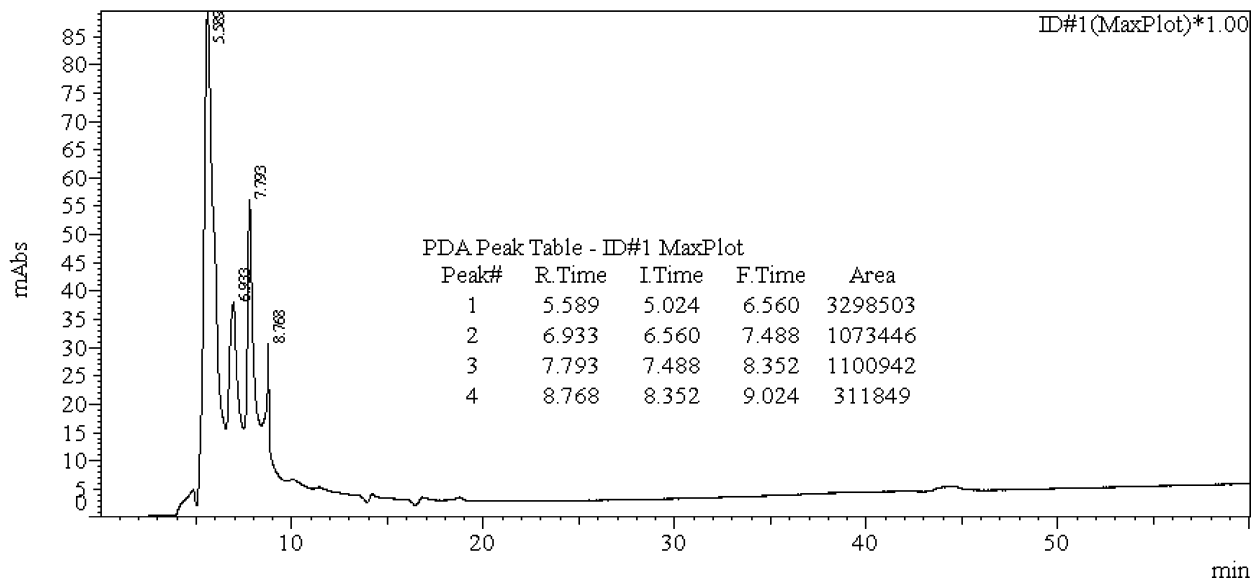


Figure 5. HPLC of the water-soluble material obtained from the raw Elbistan lignite.

nebulizer gas (N_2) flow rate: 4.50 L/min; block temperature: 250 °C; CDL temperature (Heated Capillary): 250 °C; CDL voltage: 25 V; Q-array voltage: 40 V.

Results and Discussion

Effect of the Fungi. *Pleurotus sajor-caju*, *Pleurotus sapidus*, *Pleurotus florida*, *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, and *Coriolus versicolor* white-rot fungi were used in the bio-liquefaction/solubilization

of Beypazari and Elbistan lignites. Among these, *Coriolus versicolor* fungi were found to be the most effective in bio-liquefaction/solubilization of nitric acid-treated Elbistan lignite. All of the fungi other than *Coriolus versicolor* fungi were not effective in the bio-liquefaction/solubilization of nitric acid-treated Elbistan lignite, and the solid lignite particles obtained using these fungi were not investigated afterward. The raw Elbistan lignite could not be bio-liquefied/solubilized by

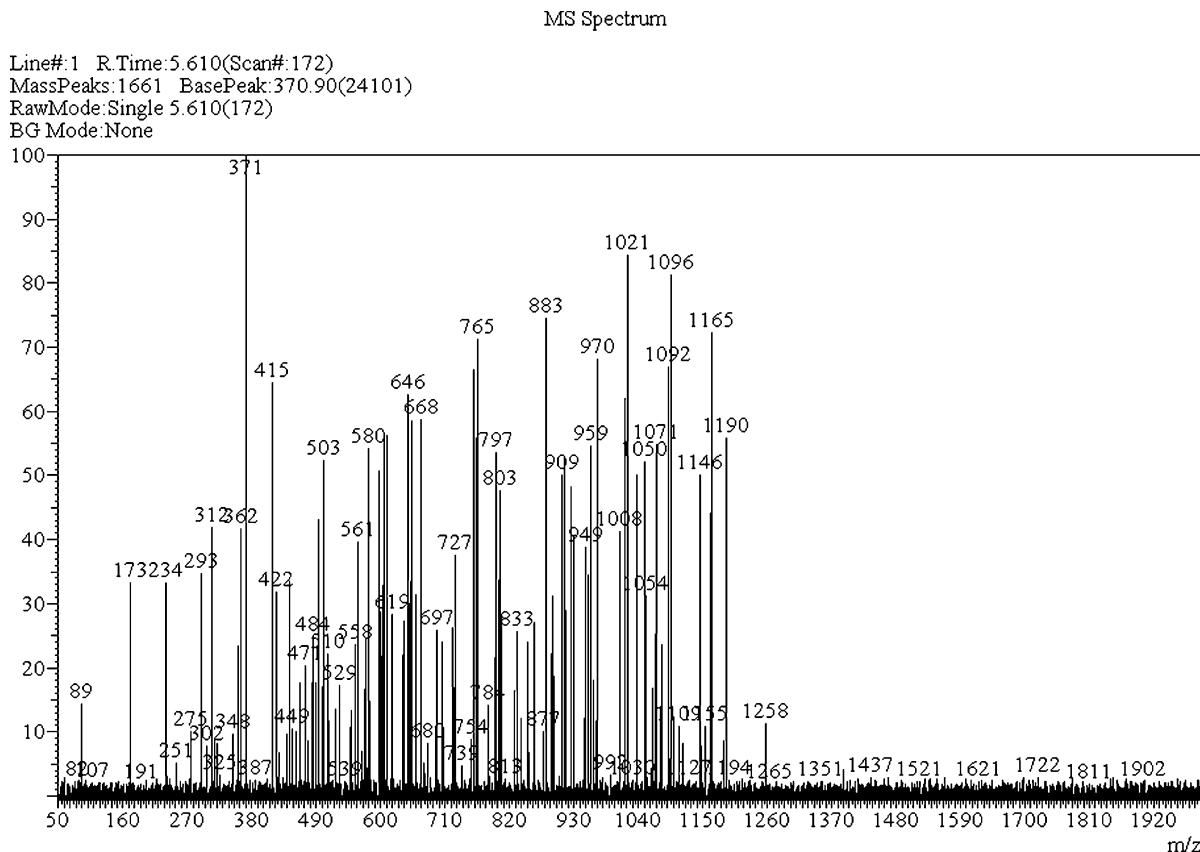


Figure 6. Mass spectrum of peak 1 in the HPLC of the bio-liquefied/solubilized product obtained from the Elbistan lignite.

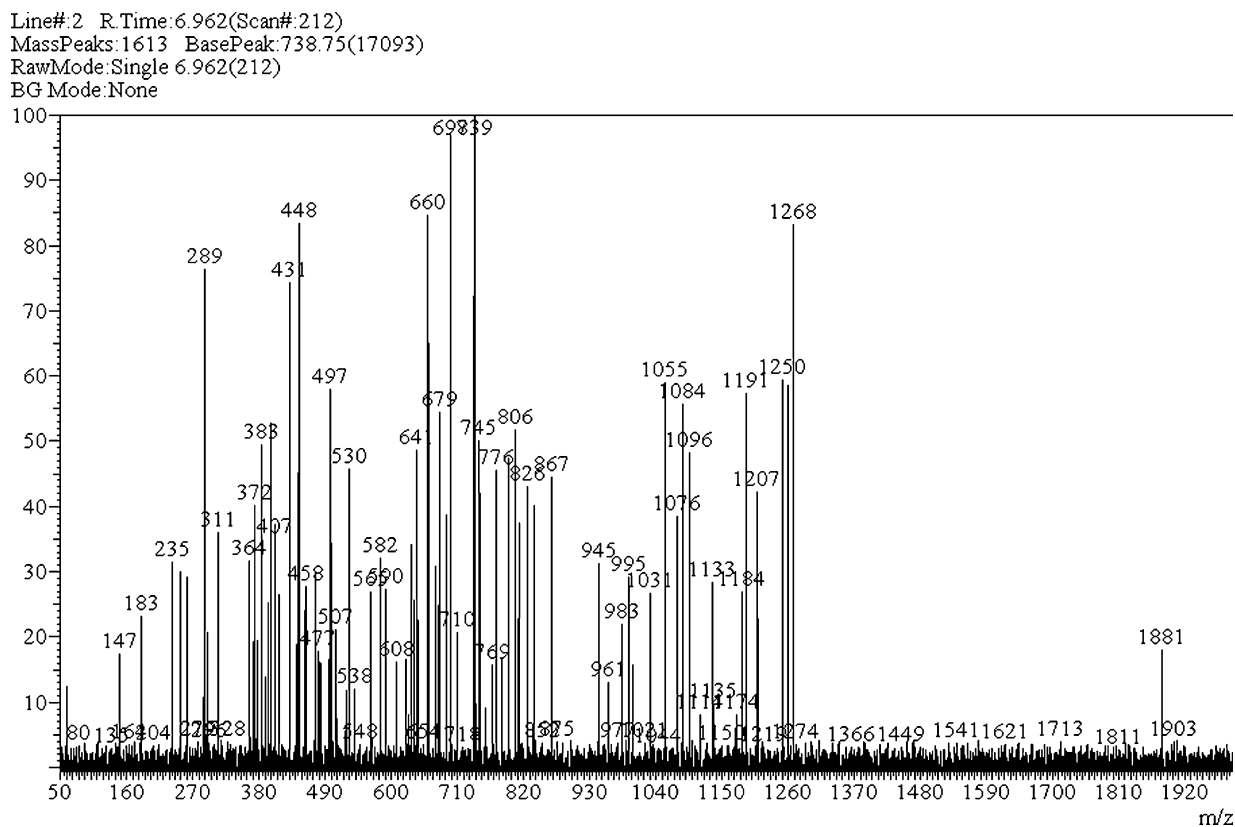


Figure 7. Mass spectrum of peak 2 in the HPLC of the bio-liquefied/solubilized product obtained from the Elbistan lignite.

the action of the *Coriolus versicolor* fungi. Similarly, raw and nitric acid-treated Beypazari lignite seemed to be unaffected by the action of any kind of white-rot fungi other than *Coriolus versicolor* fungi.

Complete bio-liquefaction/solubilization of the nitric acid-treated Elbistan lignite was observed on the 15th day of incubation of the coal particles and the *Coriolus versicolor* fungi (Figure 1). It seemed that the selective

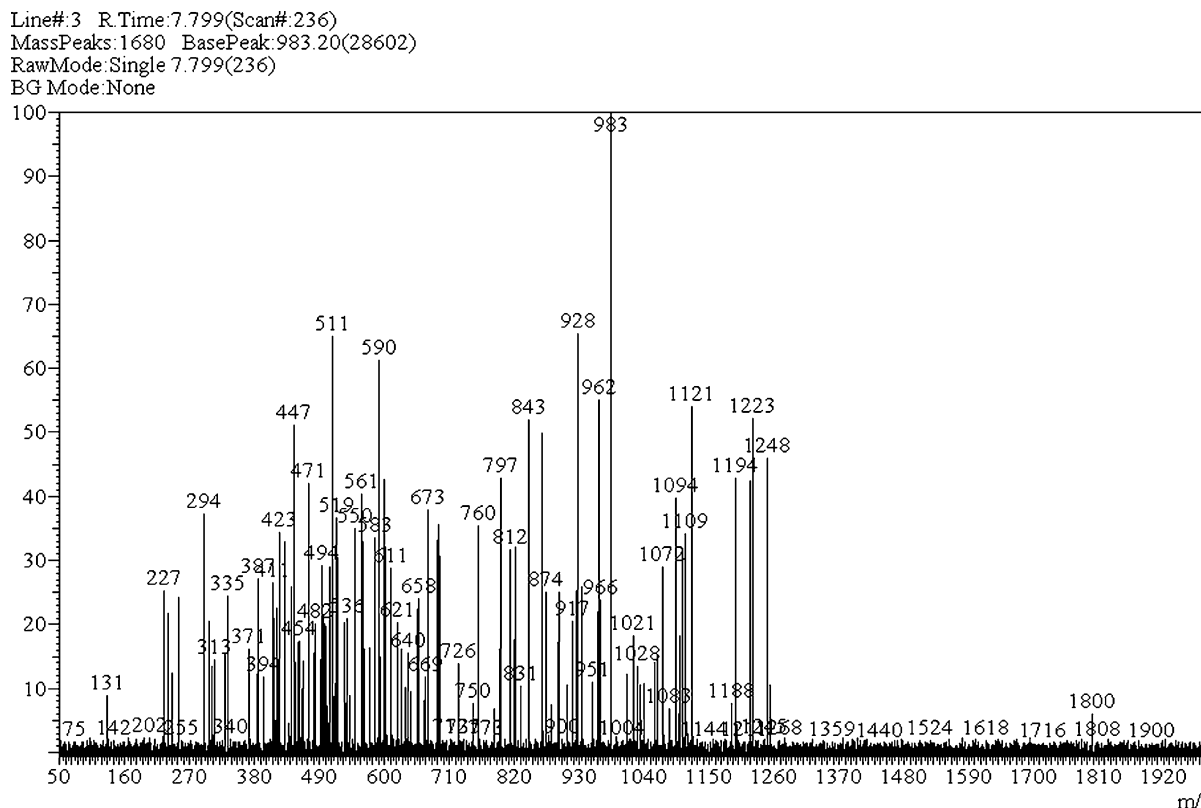


Figure 8. Mass spectrum of peak 3 in the HPLC of the bio-liquefied/solubilized product obtained from the Elbistan lignite.

efficacy of *Coriolus versicolor* fungi on immature lignites such as Elbistan lignite was probably related to the composition of the coal, as described by its phenolic, carboxylic, and carbonylic moieties, methylene, oxygen and sulfur bridges, exchangeable cations, and noncondensed aromatic rings. It follows that an increased number of aromatic rings in the structure of higher rank coals may have induced resistance of the structure toward the action of microbial actions.

In addition to the resistance of more complex organic structures present in the coal, the amount of inorganic material may also affect the interaction of microorganisms with coal. In a very recent study¹⁴ it was found that, at any given time and pH, the microorganism adhered more to the coal with the least ash content. The amount of adhering decreased with an increase in ash content. The percentage of mineral matter in Beypazari lignite and Elbistan lignite samples used in the present work were 42.8 and 25.6, respectively. Higher content of mineral matter in the Beypazari lignite sample might have been effective for a low degree of adhesion of the microorganisms to the coal that added to the inertness of the Beypazari lignite in the bio-liquefaction/solubilization reactions.

FT-IR Spectra of the Raw Lignite Samples and Bio-Liquefied/Solubilized Product. The major absorption bands in the FT-IR spectra of raw lignites were attributed to hydroxyl group stretching vibrations ($3670\text{--}3230\text{ cm}^{-1}$, $1410\text{--}1310\text{ cm}^{-1}$), aliphatic CH stretching vibrations ($2980\text{--}2845\text{ cm}^{-1}$), carbonyl group stretches ($1850\text{--}1650\text{ cm}^{-1}$), aromatic C=C stretching vibrations ($1635\text{--}1600\text{ cm}^{-1}$), asymmetric CH_2 and CH_3 bending

vibrations ($1485\text{--}1430\text{ cm}^{-1}$), C–C out-of-plane bending vibrations ($715\text{--}680\text{ cm}^{-1}$), C–H and C–C out-of-plane bending vibrations ($900\text{--}600\text{ cm}^{-1}$).^{15,16} Most of the bands mentioned were present in the spectra of the raw Beypazari and Elbistan lignite samples (Figures 2 and 3). In addition, the FT-IR spectra of Beypazari lignite (Figure 2) contained features that were found in relatively mature coals. The intensity of the phenolic hydroxyl band (3414 cm^{-1}) in Figure 2 was low when compared with that of Elbistan lignite (Figure 3). In addition to this, a strong band near 1085 and 1042 cm^{-1} due to C–OH stretching of aromatic rings indicated the presence of an intense aromatic matrix in Beypazari lignite. The presence of moderately strong bands in the region of $900\text{--}600\text{ cm}^{-1}$ due to aromatic C–C out-of-plane stretching vibrations supported the preceding statement. The FT-IR spectra of Elbistan lignite showed all of the characteristics expected of a young lignite in that the intensity of the phenolic hydroxyl band was very strong, and the profile contained relatively weaker bands near 1107 and 1035 cm^{-1} , due to aromatic C–OH stretching vibrations. The presence of bands due to carboxylate ions near 1564 and 1421 cm^{-1} showed that the Elbistan lignite contained some ligninic material, which is normally observed in immature coals.¹⁷

The FT-IR spectrum of the bio-liquefied/solubilized product is presented in Figure 4. The spectrum is generally very similar to that of the raw lignite, but there are some additional bands at 1701 and 1391 cm^{-1} .

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Line# 5 R.Time: 8.828(Scan#: 268)
 MassPeaks: 1671 BasePeak: 1029.85(22096)
 RawMode: Single 8.828(268)
 BG Mode: None

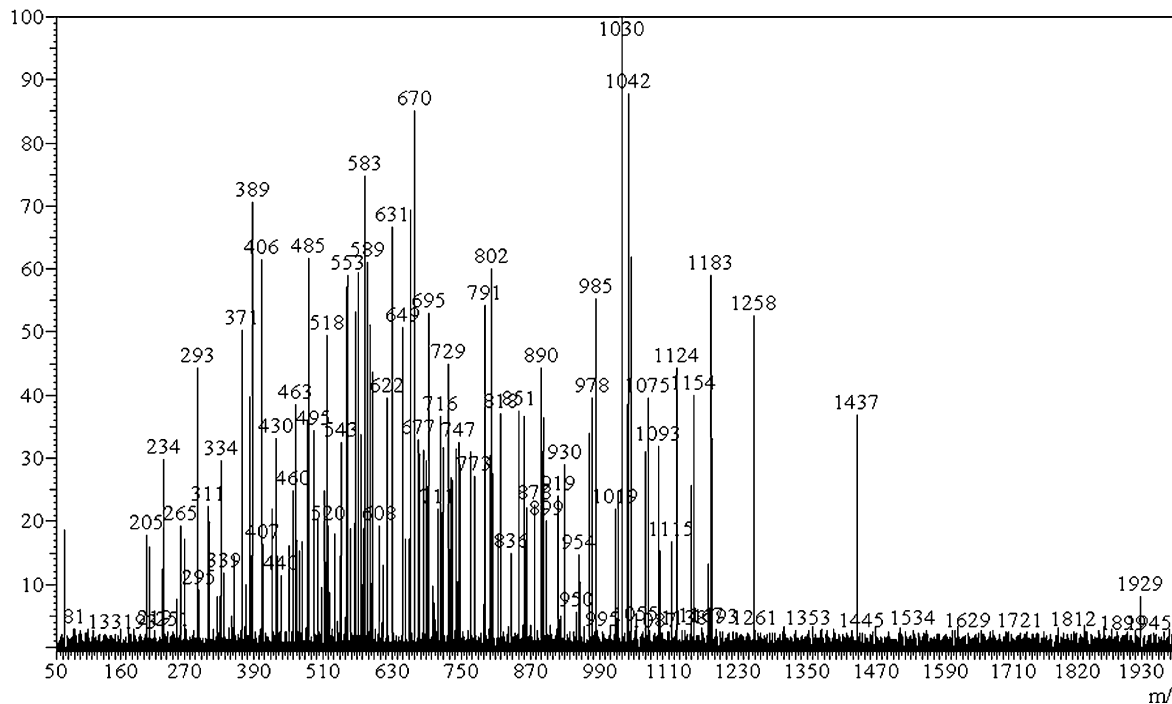


Figure 9. Mass spectrum of peak 4 in the HPLC of the bio-liquefied/solubilized product obtained from the Elbistan lignite.

The band at 1701 cm^{-1} is very specific to carbonyl group absorption, indicating the presence of some oxidation products in the liquefied sample. The band at 1391 cm^{-1} may be due to absorption of methyl symmetric bending vibrations in tertiary butyl groups.

HPLC-MS of the Bio-Liquefied/Solubilized Elbistan Lignite. A liquid chromatography technique was employed in order to gain insight into the complex structure of the liquid product. HPLC experiments performed with the material leached with water from Elbistan lignite did not produce any peaks in the liquid chromatograms. The chromatogram of the water-soluble bio-liquefied/solubilized product was presented in Figure 5. The chromatogram contained four major peaks. The retention times, initial and final elution times, and peak areas were also presented. Each profile seemed to describe an envelope containing several unresolved peaks of compounds presumably very similar in physicochemical character. Mass analyses of each peak are presented in Figures 6–9. Due to the co-elution of multiple components, the mass spectral profile of each peak was very complicated. Accordingly, the ability to analyze these spectra and to understand the type of fragments present in each sample was limited. The m/z values for the base peaks measured in the mass spectra for peaks 1, 2, 3, and 4 were 370.90, 738.75, 983.20, and 1029.85, respectively. The highest m/z value that was easy to discern in the spectra for peaks 1, 2, 3, and 4 were 1258, 1268, 1268, and 1437, respectively. More importantly, the m/z values describing fragments possibly common to all spectra were 293, 371, 447, 590, 726, 983, 1030, and 1190. While a more careful assessment of the spectra is possible, any information derived at this time would be speculative. One matter to emphasize, however, is that these spectra do in fact describe stable, soluble, and liquefied fragments of lignite.

Clearly, in the interest of future developments, further studies are required and warranted to understand the structure of the material produced by bio-liquefaction/solubilization and to comprehend the mechanism of bio-liquefaction/solubilization.

Conclusions

Coriolus versicolor fungi were found to be the most effective in bio-liquefaction/solubilization of nitric acid-treated Elbistan lignite. The raw Elbistan lignite could not be bio-liquefied/solubilized by the action of the *Coriolus versicolor* fungi. Raw and nitric acid-treated Bey pazari lignite seemed to be unaffected by the action of any kind of white-rot fungi. *Coriolus versicolor* fungi were effective only in the case of immature lignites such as Elbistan lignite, which probably contained phenolic, carboxylic, and carbonylitic functional group types, methylene, oxygen and sulfur bridges, exchangeable cations, and noncondensed aromatic rings. An increased aromatic content, characteristic of higher rank coals, probably increased the resistance of the coal structure toward the action of microbial organisms.

The liquid chromatogram of the bio-liquefied/solubilized product contained four major peaks. Mass spectral measurements of each peak indicated the presence of very complicated structures. The m/z values for the base peaks measured in the mass spectra for peaks 1, 2, 3, and 4 were 370.90, 738.75, 983.20, and 1029.85, respectively. The maximum m/z values observed in the spectra for peaks 1, 2, 3, and 4 were 1258, 1268, 1268, and 1437, respectively. The m/z values of some of the fragments common to all of the spectra were 293, 371, 447, 590, 726, 983, 1030, and 1190.