Synergistic Extraction and Characterization of Fulvic Acid by Microwave and Hydrogen Peroxide—Glacial Acetic Acid to Oxidize Low-Rank Lignite

Yingjie Zhang, Guanqun Gong,* Honglei Zheng, Xin Yuan, and Liangwei Xu

ABSTRACT: Fulvic acid (FA) has important properties and is used widely in agriculture, industry, medicine, and other fields. However, there is a lack of environmentally friendly and efficient extraction methods for coal-based FA and its molecular structural characterization. In this study, FA was extracted cleanly and efficiently from low-rank lignite via the innovative method of microwave combined with hydrogen peroxide and glacial acetic acid, followed by purification by the sulfuric acid—acetone method. The molecular structures of FA were precisely characterized by UV–vis spectroscopy, infrared (IR) spectroscopy, 	extsuperscript{1}H nuclear magnetic resonance (NMR) spectroscopy, and gas chromatography–mass spectrometry (GC–MS). The results showed that the microwave combined with hydrogen peroxide—glacial acetic acid method had stronger oxidative degradation ability compared with the conventional method. Under optimized conditions, the yield of FA reached 60.97%. During the oxidation process, the macromolecular network structure of coal was destroyed, resulting in the production of many oxygen-containing functional groups. According to the IR and UV–vis spectra, there were abundant oxygen-containing functional groups such as hydroxyl, carboxyl, carbonyl, and quinone groups in the molecular structure of FA. Determination of the total acid group content in the oxygen-containing functional groups of FA showed that the content of carboxyl groups was much higher than that of phenolic hydroxyl groups. The 	extsuperscript{1}H NMR showed that there were hydrogen atoms present as part of carboxyl, aromatic, phenolic hydroxyl, and aliphatic groups in FA. The (GC–MS) results suggested that FA is a mixture of dozens of complex compounds, including alkanes, alcohols, esters, etc.

1. INTRODUCTION

Fulvic acid (FA) is a component of humic acid (HA) with low aromaticity, small molecules, many functional groups, and good water solubility. It has the highest chemical, physiological, and physicochemical activities of all the components of HA.\textsuperscript{1–4} FA has several desirable characteristics: it functions as a platform compound, is easy to combine or react with other substances, has micro- to nanometer size and good biocompatibility.\textsuperscript{1,4–7} Recent studies have shown that FA plays a special role in the prevention and control of human and animal diseases,\textsuperscript{8–10} heavy metal pollution control in water and soil,\textsuperscript{11,12} modern agriculture,\textsuperscript{3,4,13–16} and other fields. The potential biochemical activities of FA have attracted the research interest of many scholars. Efficient and environmentally friendly preparation of FA, and the identification of its effective components, has become a research hotspot.

The representative FA was extracted from lignite, weathered coal, and peat. The conventional extraction method coal-based FA mainly involves alkali-dissolved acid precipitation and extraction by strong acid. In recent years, the sulfuric acid—ethanol/acetone combination method, ion-exchange resin extraction method, ultrasonic/microwave method, and nonacid organic solvent extraction methods have been developed. However, the existing extraction methods that use large amounts of acid and alkali result in a certain amount of corrosion and pollution of the operating environment and equipment; more importantly, a large amount of acid and alkali remains in the solid residue after FA extraction, which will cause extensive environmental pollution. Additionally, most of the FA obtained by these methods are mixtures of FA and FA salts, some of which even incorporate inorganic salts, resulting in low yield of pure FA. As for the ion-exchange resin method, its extraction speed is slow, selection of the appropriate resin is challenging as many components of FA will dissolve and destroy the resin, and the costs of maintenance and replacement of materials are high. These factors limit its industrial application. The extraction of FA by the organic solvent is a mild method but the yield of directly extracted FA is low; some organic solvents are toxic or not significantly

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different from the boiling point of FA components. In addition, how to effectively separate the organic solvents from FA needs to be considered.

In this study, to solve the problems of pollution, low extraction yield, and low purity of FA using traditional extraction methods, low-rank lignite was oxidized by microwaves combined with hydrogen peroxide and glacial acetic acid. This method was reported by Zhang,23 Miura,24 Deng,25 Rokhina,26 Zhang,27 and Gong.28,29 Hydrogen peroxide is an environmentally friendly oxidant that reacts with glacial acetic acid to produce the stronger oxidant peracetic acid, which oxidizes the macromolecules to oxygen-containing small molecules, thus increasing the yield of FA. Microwaves can promote the decomposition of hydrogen peroxide and peracetic acid to form free radicals and significantly accelerate the reaction rate of oxidative degradation, thus shortening the reaction time and increasing the FA extraction efficiency. The extracted FA was purified by the sulfuric acid–acetone method and then characterized using a set of analytical techniques, i.e., spectrometric methods, 1H NMR and GC–MS. The combination of these various techniques provided a more robust characterization of FA.

2. EXPERIMENTAL SECTION

2.1. Materials. Coal samples of low-rank lignite were obtained from a deep mine in Inner Mongolia, China. The milled lignite was sieved through an 80-mesh standard sieve (size fraction less than 175 μm) and then dried at 105 °C for 24 h. The dried coal sample was immediately stored in a sealed bag and stored at room temperature for later use.

Hydrogen peroxide (H₂O₂), glacial acetic acid, acetone, and concentrated sulfuric acid (H₂SO₄) were analytically pure reagents.

2.2. Preparation of FA. Coal sample was added to a spherical flask (250 mL). Glacial acetic acid and hydrogen peroxide solution were mixed together and poured into the flask. Extraction was carried out by magnetic agitation at a certain microwave power in a microwave chemical reactor. A condensation tube was connected to the flask to remove the heat released by the reaction. After the reaction, the reaction mixture was filtered to afford a filtrate and a residue. The filtrate was then concentrated by a rotary evaporator and the concentrated filtrate was dried at 60 °C under vacuum to obtain the primary product, FA.

The extraction was performed by single-factor tests and orthogonal tests. The following five factors were investigated for their effect on the extraction yield: microwave power, reaction time, concentration of hydrogen peroxide solution, ratio of glacial acetic acid to coal (acid–coal ratio), and ratio of hydrogen peroxide solution to coal (oxygen–coal ratio).

2.3. Purification of FA. A certain amount of concentrated sulfuric acid was added to the acetone–water solution. This mixture was added to the primary FA in a single-mouth flask, and the resulting mixture was magnetically stirred at 30 °C for a certain duration. After purification, the supernatant was separated from the mixture by centrifugation and the filtrate was concentrated using a rotary evaporator. The concentrate was dried in an oven at 60 °C to afford the purified product, PFA.

2.4. Characterization. A series of analyses and characterizations were performed to characterize the PFA.

A Kaiyuan SE-CHN2000 elemental analyzer (Chang Sha Kai Yuan Instrument Co., Ltd., China, Xuzhou) and a Tairui CTS7000 automatic sulfur meter (Xuzhou Tairui Instruments and Equipment Co., Ltd., China, Xuzhou) were used to determine the elemental composition of FA.

The barium hydroxide–calcium acetate chemical titration method was used to measure the total number of acidic and carboxyl functional groups present. The phenolic hydroxyl group content from the total acid group content. The phenolic hydroxyl group content was determined by subtracting the carboxyl functional groups present. The phenolic hydroxyl group content from the total acid group content.
The column was heated to 240 °C for 5 min. The identification of peaks was based on their retention time, mass spectra (comparison with standards), and literature data.

UV−vis spectra were obtained on a Unico 2802 UV−vis spectrophotometer (Unico (Shanghai) Electric Co., Ltd., China, Shanghai) in the range of 200−800 nm.

FTIR spectra were recorded for pellets prepared by pressing a mixture of FA (1.5 mg) and dried spectrometry grade KBr (400 mg) using a Bruker VERTEX 80 v spectrometer (Bruker, Germany). The spectra were recorded in the range of 4000−400 cm$^{-1}$.

$^1$H NMR spectra of PFA was determined using a Bruker AVANCE III HD 600 MHz nuclear magnetic resonance spectrometer (Bruker, Germany).

PFA was divided into 10 fractions according to molecular weight and polarity. Activated silica gel was loaded into a chromatography column by dry packing. PFA (2.00 g) was ground with silica gel (1:1 w/w) and added to the chromatography column. Ten different polar eluents were used to elute FA; from lowest to greatest polarity, the eluents were petroleum ether and petroleum ether/ethyl acetate mixtures with different volume ratios (3:1, 1:1, or 1:3), ethyl acetate, chloroform, chloroform/methanol mixtures with different volume ratios (3:1, 1:1, or 1:3), and methanol. After gradient elution, 10 fractions were obtained and recorded as A−J.

The fractions A−J were analyzed by GC−MS using a Jeol JMS-Q1000GC gas chromatography/mass spectrometer (Jeol, Japan) equipped with a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm) and a quadrupole analyzer and operated in electron impact (70 eV) mode. The initial temperature of the column was 60 °C, which was held for 2 min, then the column was heated to 240 °C at a rate of 3 °C·min$^{-1}$, and maintained at 240 °C for 5 min. The identification of peaks was based on their retention time, mass spectra (comparison with standards), and literature data.

3. RESULTS AND DISCUSSION

3.1. Orthogonal Test Results and Analysis. The experimental results and range of experimental conditions are shown in Table 1. It can be seen that the order of the effect of these five factors on the extraction yield of FA, from greatest effect to least effect, was reaction time, acid/coal ratio, oxygen/coal ratio, concentration of hydrogen peroxide solution, and microwave power. Microwave power and concentration of hydrogen peroxide solution had little effect on the extraction yield of FA. The reaction time, acid/coal ratio, and oxygen/coal ratio had a significant effect on the extraction yield of FA. The optimal process conditions for the microwave-assisted hydrogen peroxide−glacial acetic acid method were as follows: reaction time of 13 min, microwave power of 700 W, acid/coal ratio of 1.32, hydrogen peroxide concentration of 22.5%, and oxygen/coal ratio of 9. Under optimized conditions, the average extraction yield of FA was 60.97% and its purity was 99.6%.

The results showed that microwave could shorten the reaction time and increase the reaction rate. In this study, which greatly improved the yield of FA. The reaction mechanism was as follows: hydrogen peroxide and glacial acetic acid reacted to form peracetic acid, which is a stronger oxidant than hydrogen peroxide and can decompose into a variety of free radicals such as hydroxyl radicals, acetyl oxygen radicals, methyl radicals, and peroxy radicals; these radicals reacted with large molecules in coal to promote coal macromolecular chain cutting and formation of small molecular components, greatly increasing the extraction yield of FA.

3.2. Elemental Analysis and Functional Group Content Analysis. Elemental content and oxygen-containing functional group content of PFA are shown in Table 2. It can be seen that the content of total acid groups and carboxyl groups in the FA product was relatively high, indicating that the use of microwaves and glacial acetic acid could promote the oxidative decomposition of coal to produce FA.

3.3. Infrared Spectral Analysis. The infrared spectrum of FA is shown in Figure 1. The broad band from 3650 to 3200 cm$^{-1}$ was attributed to hydroxyl stretching from various groups. The strong and broad absorption peak at 3423 cm$^{-1}$ was attributed to the stretching vibrations of the hydrogen-bonded O−H groups. The absorbance at 3000−2700 cm$^{-1}$ belonged to the C−H stretching vibrations of aliphatic hydrocarbons, and the characteristic peaks of the sample in this region were not obvious; this indicated that under experimental conditions, some of the weak covalent bonds in the lignite structure were oxidized, resulting in molecular fragmentation and the formation of oxygen-containing functional groups such as phenolic hydroxyl groups and carboxyl groups and a decrease in the content of fatty structures such as methyl and methylene groups. The double-bond stretching vibration was located at 1900−1500 cm$^{-1}$. The peak at 1719 cm$^{-1}$ was strong and sharp and was attributed to the stretching vibration of the saturated fatty acid C=O groups, including carbonyl groups in carboxyl, aldehyde, and keto groups. There was a C=C stretching vibration near 1646 cm$^{-1}$. The 1500−1330 cm$^{-1}$ region absorbances were attributed to deformation vibrations of the phenyl group.
vibrations of methyl and methylene groups in alkyl structures. The peak at 1172 cm$^{-1}$ corresponded to the C–O–C stretching vibrations. Peaks from 950 to 650 cm$^{-1}$ corresponded to the aromatic absorption band$^{30,31}$ peaks in this region included the out-of-plane bending vibrations of the C–H bonds on substituted aromatic rings, and, more specifically, peaks from 900 to 750 cm$^{-1}$ corresponded to the tri-substituted benzene ring, and the peak at 806 cm$^{-1}$ was assigned to tri-substituted benzene ring vibrations, which had the largest relative content compared to di- or tetra-substituted benzene rings.

3.4. UV–vis Spectroscopic Analysis. The UV–vis spectrum of FA is shown in Figure 2. There was a strong absorption in the UV–vis range, indicating that there was a large number of chromophores in FA.$^{23,25}$ The absorbance decreased with increasing wavelength. As the molecular structure of FA is complex, chromophore absorption occurs with varying degrees of overlap or displacement, and the UV–vis absorption spectrum does not show obvious characteristic absorption peaks. There was a broad absorption peak around 280 nm arising from the absorption of aromatic structures. Absorption peaks at 260 nm were due to conjugated double bonds, which may include groups such as aldehydes and quinones.$^{30}$

3.5. $^1$H NMR of FA. Figure 3 shows the $^1$H NMR spectrum of PFA. The chemical shift of the DMSO solvent is at about 2.51 ppm and that of water peak is near 3.36 ppm. Because the sample and solvent readily absorb water, the water peak was stronger. The protons of FA were mainly distributed from 0 to 13.5 ppm. There was a strong peak at 1.25 ppm that was attributed to terminal aliphatic methyl protons. The peak at 2.45 ppm corresponded to the $\alpha$-position-connected polar functional groups such as aromatic rings and carbonyl groups in aliphatic groups. The peaks in the range of 3.3–4.5 ppm arose from protons linked to heteroatoms such as O, N, and S; this was probably because glacial acetic acid reacts with hydrogen peroxide to produce peracetic acid, the stronger oxidizing ability of which leads to the oxidation of some of the aliphatic protons to oxygen-containing functional groups. The chemical shifts of protons on phenolic hydroxyl groups were in the range of 4.5–6.5 ppm. Chemical shifts of protons on aromatic or heteroaromatic rings were between 6.5 and 9.0 ppm. The presence of a broad carboxyl proton peak from 12 to 13.5 ppm may be due to the strong oxidizing ability of hydrogen peroxide and glacial acetic acid under microwave irradiation, causing the oxidation of side chains linked by benzene rings to carboxyl groups.

3.6. Column Chromatography and GC–MS. Purified FA was divided into 10 fractions according to their polarities. The yields are shown in Table 3.

![Figure 2. UV–vis spectrum of FA.](image)

![Figure 3. $^1$H NMR spectrum of FA.](image)

![Figure 4. TIC of A.](image)

![Figure 5. GC-MS chromatogram of B.](image)

| Fraction | Yield (%) | Fraction | Yield (%) |
|----------|----------|----------|----------|
| A        | 0.41     | F        | 2.32     |
| B        | 0.36     | G        | 17.53    |
| C        | 2.07     | H        | 30.29    |
| D        | 5.05     | I        | 10.15    |
| E        | 5.52     | J        | 0.86     |

All of the extracts (A–J) from purified FA were analyzed by GC–MS, but no compounds were detected in C–J. This was probably because the molecular weights of eight components exceeded 600. Compounds were detected in A and B. Figure 4 shows the total ion chromatogram (TIC) of A from PFA. More than 20 compounds were detected in total, with 11 alkane compounds (peaks 1, 2, 4, 6, 8, 10, 13, 16, 17, 18, and 21), four alcohol compounds (peaks 7, 9, 12, and 20), two hydrazide compounds (peaks 5 and 11), one olefinic aldehyde (peak 3), and three unknown compounds. All compounds are listed in Table 4.

Figure 5 shows that 18 different compounds were identified in B by GC–MS analysis. Esters were the most common type of compound detected in B. Ten esters (peaks 1–4, 6, 9, 12,
15, 17, and 18) were detected. There were four alkane compounds (peaks 7, 11, 13, and 14), and two sulfur-containing compounds were also detected, namely, dibenzo-thiophene sulfone (peak 8) and dibenzyl sulfone (peak 10). All compounds are listed in Table 5.

### Table 4. Compounds Detected in A from Purified FA

| peak | compound name | compound type |
|------|---------------|---------------|
| 1    | 5-propyltridecane | x             |
| 2    | n-hexadecane   | x             |
| 3    | 9-octadecenal  | x             |
| 4    | n-octadecane   | x             |
| 5    | stearyl hydrazine | x         |
| 6    | n-nonadecane   | x             |
| 7    | butyl alcohol  | x             |
| 8    | 6-methylstearadecane | x |
| 9    | docosanol      | x             |
| 10   | n-eicosane     | x             |
| 11   | palmitic acid hydrazide | x |
| 12   | 2-methylhexadecanol | x |
| 13   | n-heptacosane  | x             |
| 14   | unknown compounds | x         |
| 15   | unknown compounds | x         |
| 16   | 1,1-bis(dodecylx)hexadecane | x |
| 17   | 2,6,10,15-tetramethylheptadecane | x |
| 18   | n-tetradecacontane | x         |
| 19   | unknown compounds | x         |
| 20   | 3,7,11-trimethyldodecanol | x |
| 21   | 11,19-didecyloctadecane | x |

### Table 5. Compounds Detected in B from Purified FA

| peak | compound name | compound type |
|------|---------------|---------------|
| 1    | diisobutyl phthalate | x    |
| 2    | dibutyl phthalate  | x    |
| 3    | methyl tridecanoate | x    |
| 4    | 1-butyl-2-isobutyl phthalate | x |
| 5    | unknown compounds | x    |
| 6    | isopropyl palmitate | x    |
| 7    | 6-methyl octadecane | x    |
| 8    | dibenzothiophene sulfone | x    |
| 9    | methyl 16-methylheptadecanoate | x |
| 10   | dibenzyl sulfone  | x    |
| 11   | 1-iodotridecane   | x    |
| 12   | heptadecyl acetate | x    |
| 13   | n-heptacosane     | x    |
| 14   | 11-(1-ethane propyl) heptacosane | x    |
| 15   | n-docetyl acetate | x    |
| 16   | unknown compounds | x    |
| 17   | 1,2-benzenedicarboxylic acid, 1-(2-ethylhexyl) ester | x |
| 18   | di-n-octyl phthalate | x    |

### Figure 5. TIC of B.

Figure 5. TIC of B.

### 4. CONCLUSIONS

FA was extracted efficiently from lignite by microwave-assisted hydrogen peroxide in combination with glacial acetic acid. The optimal conditions were as follows: reaction time of 13 min, microwave power of 700 W, acid/coal ratio of 1.32, hydrogen peroxide concentration of 22.5%, and oxygen/coal ratio of 9. Under optimized conditions, the average extraction yield of FA was 60.97%. This method uses low-environmental impact reagents with short reaction time and low energy consumption, and the yield was increased compared to other existing methods. Therefore, this approach provides a new theoretical and technical reference for the extraction of coal-based FA.

Spectral analysis, including UV−vis, infrared, 1H NMR, and GC−MS, showed that under strong oxidative conditions of peracetic acid produced by hydrogen peroxide reacting with glacial acetic acid, the large molecular structure of coal was destroyed and small molecules of FA were formed, which were rich in carboxyl, hydroxyl, carbonyl, and other oxygen-containing functional groups, including dozens of small molecular organic compounds such as alkanes, alcohols, aldehydes, esters, etc. These results provide a scientific explanation for the multiple active functions of FA across many applications and also lay the foundation for further research on the structure of FA.

### AUTHOR INFORMATION

#### Corresponding Author

**Guanqun Gong** — Key Laboratory of Coal Processing and Efficient Utilization of Ministry of Education, Xuzhou 221116, China; School of Chemical Engineering & Technology, China University of Mining and Technology, Xuzhou 221116, China; orcid.org/0000-0001-7856-9477; Email: ggqzyj@126.com

#### Authors

**Yingjie Zhang** — Key Laboratory of Coal Processing and Efficient Utilization of Ministry of Education, Xuzhou 221116, China; School of Chemical Engineering & Technology, China University of Mining and Technology, Xuzhou 221116, China

**Honglei Zheng** — School of Chemical Engineering & Technology, China University of Mining and Technology, Xuzhou 221116, China

**Xin Yuan** — School of Chemical Engineering & Technology, China University of Mining and Technology, Xuzhou 221116, China

**Liangwei Xu** — School of Chemical Engineering & Technology, China University of Mining and Technology, Xuzhou 221116, China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.9b03796
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