Biodegradation of photo-oxidized lignite and characterization of the products

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Abstract. Biodegradation of photo-oxidized Inner Mongolia lignite by pseudomonas aeruginosa was studied and the degradation percentage reached 56.27%, while the corresponding degradation percentage of the strain degrading raw Inner Mongolia lignite is only 23.16%. The degradation products were characterized. Proximate and ultimate analyses show that the higher oxygen content increased by photo-oxidation pretreatment maybe promoted the degradation process. Ultraviolet spectroscopy (UV) analysis of the liquid product reveals that it contains unsaturated structures and aromatic rings are the main structure units. Gas chromatography-mass spectrometry (GC-MS) analysis indicates that the main components of the ethyl acetate extracts are low molecular weight organic compounds, such as ketones, acids, hydrocarbons, esters and alcohols. Infrared spectroscopy (IR) analysis of raw lignite, photo-oxidized lignite and residual lignite demonstrates that the absorption peaks of functional groups in residual lignite disappeared or weakened obviously. Scanning electron microscopy (SEM) analysis manifests that small holes appear in photo-oxidized lignite surface, which may be promote the degradation process and this is only from the physical morphology aspects, so it can be inferred from the tests and analyses results that the more important reason of the high degradation percentage is mostly that the photo-oxidation pretreatment changes the chemical structures of lignite.

1. Introduction
Lignite accounted for 13% of China's coal reserves, in which Inner Mongolia lignite accounts for more than three-quarters [1, 2]. Lignite direct combustion will release a great deal of nitrogen and sulfur compounds, which will badly pollute the environment and damage the ecology [3]. With the development of biotechnology, biodegradation of coal as a green way to coal clean processing and utilization was widely concerned. Since the 1980s, Dr. Fakoussa and professor Cohen reported that some fungi could grow on the coal and transformed coal into black aqueous solution[4], biodegradation of coal with its advantages of mild reaction condition, clean, low energy consumption and could extract high value chemicals from the products attracted the attention of many researchers in the world [5, 6]. In recent years, lots of researchers focus most of their attention on strain breeding, mechanism of coal biodegradation, the application of biodegradation products and so on, still formed
four widely accepted mechanisms of coal biodegradation, there were many successful examples for products applications as well [7, 8]. However, the studies of coal pretreatment methods, biodegradation products composition and impacts on coal quality were not deeply enough. Therefore, in-depth experimental research needs to be carried out in these fields.

In this study, the degradation of photo-oxidized Inner Mongolia lignite by *Pseudomonas aeruginosa* was carried out and the degradation products were characterized with an aim of to a more profound understanding of the biodegradation process.

2. Materials and methods

2.1. Coal sample and strain

The coal samples used in the experiments were mined from Inner Mongolia Silian coal mine, China, which was crushed, blended with the photo-catalyst (amphiphilic nano-TiO$_2$) at a mass ratio of 1: 0.002, grinded and only fragment particles of 180–106 μm was used for the experiments. The sample was photo-oxidized for 48 h in a rotary bed photochemical reactor with the UV lamp of 120 Watts, rotary speed of 60 r/min [9].

The experimental strain was *pseudomonas aeruginosa* purchased from China General Microbiological Culture Collection Center (CGMCC), which was screened from eight bacteria because of its outstanding ability to degrading lignite. *pseudomonas aeruginosa* preserved by glycerol-medium at 4°C in refrigerator was reactivated and purified, then cultured in LB medium (beef extract 5 g, peptone 10 g, sodium chloride 5 g and distilled water 1 L, pH 7.2-7.4) repeatedly for three generations, and a parent bacteria liquid was obtained for the subsequent lignite degradation experiments.

2.2. Experimental methods

Conical flask containing 50 mL LB medium was inoculated with 5 mL parent liquid, and added 0.5000±0.0002 g coal samples, the flask was then moved to a thermostat incubator set to 30 °C and oscillation speed of 160 r/min, for 15 days. Each experiment was done in three parallel while making a blank control (without bacteria, another with the raw lignite). In the end of the experiments, the degradation product was filtered to obtain liquid product, and the filter residue washed some times to remove the mycelia and dried. The biodegradation percentage, $P$, was determined by the net weight loss of the coal samples, and was calculated as (1) [10]:

$$ P = \left(1 - \frac{W_1}{W_0}\right) \times 100\% $$

Where $P$ is biodegradation percentage (%); $W_0$ is the initial weight of photo-oxidized lignite (g); $W_1$ is the weight of the residual lignite (g).

3. Results and discussion

3.1. Proximate and ultimate analyses

Proximate and ultimate analyses of raw, photo-oxidized and residual lignite are presented in Table 1. It is found that the contents of C, H, N and S in the photo-oxidized are lower than in the raw lignite and the oxygen content is increased, which show that the raw lignite is oxidized and photo-degraded under the combined actions of light, photo-catalyst and oxygen of the air. The increase of ash content, the decreases of volatile and fixed carbon indicate that the organic compounds in the photo-oxidized lignite are further degraded into a soluble substance dissolving in the degradation liquid or producing a gas molecule to escape. The contents of C, N and S in residual lignite are reduced, and the organic compounds of lignite may be degraded or utilized to produce soluble matter or gas molecules, this is consistent with the results of proximate analysis. The further increase of H and O contents probably
because the degradation process may be accompanied by oxidation and hydrolysis reactions, so that the oxygen of air or hydrogen and oxygen in water are taken into the system. It can be inferred that the higher oxygen content increased by photo-oxidation pretreatment maybe promote the degradation process [11].

**Table 1.** Proximate and ultimate analyses of raw, photo-oxidized and residual lignite

| Coal samples          | Proximate analysis (ad) wt% | Ultimate analysis (ad) wt% |
|-----------------------|-----------------------------|---------------------------|
|                       | M | A | V | FC | C | H | N | S | O |
| Raw lignite           | 12.16 | 9.67 | 25.08 | 53.10 | 57.52 | 4.82 | 0.69 | 0.65 | 14.53 |
| Photo-oxidized lignite| 13.70 | 10.31 | 21.52 | 52.44 | 52.13 | 3.22 | 0.63 | 0.41 | 19.82 |
| Residual lignite      | 11.94 | 18.76 | 18.68 | 50.73 | 42.86 | 5.19 | 0.52 | 0.26 | 20.47 |

3.2. UV spectrum analysis

The biodegradation liquid product was diluted for 20 times with deionized water and scanned with a light wave lengths from 190-600 nm, using a spectrophotometer (TU-1900).

The result of UV spectroscopy for biodegradation liquid product is shown in Figure 1. Absorbance in 270-310 nm range indicates that unsaturated structures exist in the liquid and aromatic ring is the main structure unit [12]. Absorbance in 220-270 nm range shows that unsaturated groups, such as aldehydes or ketones exist in the liquid as well.

![Figure 1. UV spectroscopy of liquid product](image1)

**Figure 1.** UV spectroscopy of liquid product  **Figure 2.** TIC of liquid product extracts by ethyl acetate

3.3. GC-MS ions chromatogram analysis

Gas chromatography (GC) was used to separate the chemical components in the liquid product and the chemical structure of each component was subsequently identified by mass spectroscopy (MS) [10]. Different organic solvents, such as ethyl acetate, carbon tetrachloride, petroleum ether and methyl benzene were tried to extract the components from the liquid with the volume ratio of 10 (organic solvent) to 1 (black liquid) for 4 h in a 100 mL separating funnel, respectively. The extracts were analyzed using GC-MS instrument (Agilent7890A) which was equipped with a capillary column with HP-5MS (crosslink 5% PH ME Siloxane, 30m×0.25 mm i.d., 0.25 um film thickness). The mass scanned ranged from 30-500 amu. Data were acquired and processed using Chemstation (software), and the compounds were analyzed by comparing mass spectra with NIST05 database.

GC-MS results indicate that the extracts by ethyl acetate containing the most kinds of organic compounds. GC-MS spectrometry for extracts by ethyl acetate is presented in Figure 2. There are over 35 kinds of components detected from the GC-MS results including ketones, acids, hydrocarbons,
esters and alcohols, and the respective contents calculated by the peak area of each compound listed in the table 2. The results are consistent with the results of UV spectrum and infrared spectrum analyses.

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\begin{array}{|c|c|c|c|c|c|c|}
\hline
& \text{ketones} & \text{acids} & \text{hydrocarbons} & \text{esters} & \text{alcohols} & \text{others} \\
\hline
\text{Percentage/\%} & 44.06 & 27.78 & 15.05 & 2.69 & 1.21 & 9.21 \\
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Table 2. Compounds detected in extracts by ethyl acetate

3.4. IR spectroscopy analysis

Infrared spectroscopy analysis can determine the specific organic chemical groups in the raw, photo-oxidized and residual lignite. For the infrared spectroscopy, 2.0 mg dried coal samples and 300 mg dried KBr mixed together and ground to powder making a pellet for test. The pellets were tested using an infrared spectrum analyzer (Perkin Elmer GXIV-5.0.1) with light wave lengths from 4000-400 cm\(^{-1}\).

The infrared spectra of raw lignite, photo-oxidized lignite and residual lignite are presented in Figure 3. The major absorbance bands in infrared spectra for the three samples are hydroxyl vibration peak (3300-3130 cm\(^{-1}\)), naphthenic base vibration peak (2910 cm\(^{-1}\)), benzene skeleton vibration peak (1580-1640 cm\(^{-1}\)), ether bond vibration peak (1260 cm\(^{-1}\)) and aromatic vibration peak (900-650 cm\(^{-1}\)). In addition, there are C=O stretching vibration (1730-1705 cm\(^{-1}\)) and C=N stretching vibration (1560-1520 cm\(^{-1}\)) exist in the photo-oxidized lignite, which indicate that the photo-oxidation pretreatment enhanced the unsaturated structures of lignite [12]. It is found that most of the peaks of residual lignite corresponding to the raw and photo-oxidized lignite disappeared or weakened obviously, which indicates that the structure of photo-oxidized lignite was changed by bacteria actions.

![Figure 3. The IR spectra of raw, photo-oxidized and residual lignite](image)

3.5. SEM images analysis

SEM images analysis helps to observe the microstructure characteristics of raw lignite, photo-oxidized lignite and residual lignite, which were tested using SEM instrument (Thenom Pro) with 5 kV and 5000 times.
Figure 4. The SEM images of raw lignite (a), oxidized lignite (b) and residual lignite (c)

Figure 4 shows the SEM images, the surface of raw lignite is loose with cracks, Figure 4 (a). The surface morphology of photo-oxidized lignite appears holes in some of the small protrusions, Figure 4 (b). And the surface of residual lignite becomes rough and with gap structures shown in Fig.4 (c). It can be seen that the photo-oxidation and bacteria actions modified the surface of the lignite, and we can deduce that the small holes in the photo-oxidized surface maybe promote the degradation process and this is only from the physical aspects of the surface. From the above tests and analyses results can be inferred that the more important reason for the high degradation percentage is mostly that the photo-oxidation pretreatment changes the chemical structure of lignite.

4. Conclusion
Biodegradation of photo-oxidized Inner Mongolia lignite by *Pseudomonas aeruginosa* has a high degradation percentage reached 56.27%. The coal pretreatment method of photo-oxidization using a rotary bed photochemical reactor can efficiently promote the biodegradation process. The results of products characterization indicate that photo-oxidation pretreatment can increase the oxygen content and the porosity of lignite, which make lignite more susceptible to bacterial actions. The absorption peaks of characteristic functional groups in IR has appeared or weakened shows that the lignite was degraded by *Pseudomonas aeruginosa* efficiently. The ethyl acetate extracts of liquid product is rich in organic compounds, such as ketones, acids, hydrocarbons, esters and alcohols.

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