

An Investigation of Coal Biodegradation by *Phanerochaete chrysosporium*

Xu Jingyao* and Mingxu Zhang

School of Materials Science and Engineering, Anhui University of Science & Technology,
Shungeng Road 168, Huainan, Anhui, C.P. 232001, China.

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A mutant of *Phanerochaete chrysosporium* (*P. chrysosporium*), which is capable of degrading lignin, was bred by ultraviolet ray irradiation and this mutant strain shows high capability of degrading of lignite. The degradation ratio of original Yima lignite and nitric acid treated Yima lignite by this mutant are 37.6 % and 51.62 % respectively, whereas those by the original strain are 31.84 % and 32.55 % respectively. The degradation products were characterized by X-ray diffractograms analysis (XRD), Fourier transmission infrared (FTIR) and the thermogravimetric analysis (TG). The results show that the polymerization degree of the aromatic nucleus and the molecular weights of products are decreased obviously, and the contents of the functional groups are remarkable changed compared with the original lignite.

Key words: *Phanerochaete chrysosporium*, Ultraviolet, Mutation breeding, Biodegradation.

Lignite is rich in China; however, its utilization is limited by its high content of moisture and ash, low thermal stability and low caloric value (Tao *et al.*, 2009; Wang *et al.*, 2004). Seeking new environmentally benign processing technology to obtain clean energy and useful chemicals from lignite is the problem to be resolved (Polman *et al.*, 1995; Catchaide *et al.*, 1999).

The microorganism conversion technology of lignite is to degrade it via participation of microorganism (Zhang *et al.*, 2005). This technology has been widely investigated for its mild reaction conditions and the highly specific bio-catalysts. The biodegradation of lignite can be dated back to later 20th century. Fakoussa E V (Fakoussa. 1994) and Cohen M S (Cohen *et al.*, 1982) found that some fungus can grow on lignite bulks and convert it to black liquid. Thereafter, some progress has been made (Scott *et al.*, 1986;

Faison *et al.*, 1990; Ralph *et al.*, 1994) in this field. Some microorganisms, including bacterium and fungus have been successfully separated for oxidized low rank coal to some limited extent (Tien *et al.*, 1983; Gupta *et al.*, 1990; Han *et al.*, 1994). So seeking new effective strains is an emergent task in this field.

P.chrysosporium is a kind of the white rotten fungus, which is characterized by its capability of excreting a kind of enzyme for lignin degradation (Tien *et al.*, 1983). Lignin is a main component of lignocellulosic biomass and the precursor of coal and it is composed of condensed aromatic hydrocarbons. Due to the similar structure of lignin and coal, some components of coal can also be degraded by some white rotten fungus (Ralph *et al.*, 1994). Conversion of coal into clean energy and useful chemicals via biological method is new research orientation and which focuses on breeding high effective fungus strains. To further improve the capability of *P.chrysosporium* for degrading the lignite, a high effective strain of *P.chrysosporium* mutant has been screened out via irradiation by ultraviolet ray.

* To whom all correspondence should be addressed.
E-mail: jingyxu@sohu.com

MATERIALS AND METHODS

Materials

Lignite was come from Henan Province in China. *P.chrysosporium*: BKMF-1767, is bought from store centre of microorganism research institution of Guang dong province and the optimum temperature and pH value were 28 ° and 6.0-8.5 respectively.

Culture medium and conditions

Basic medium: 200 g extractum of potato, 20 g amylaceum, 3 g KH_2PO_4 , 1.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 8 mg vitamin B_1 , dissolved to 1000 mL.

Obliquity spore medium: 200 g extractum of potato, 20 g amylaceum, 20 g agar, 3 g KH_2PO_4 , 1.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 8 mg vitamin B_1 .

The optimal pH value, temperature and rotation speed of the culture bed were 7.0, 28 ° and 150 r/min, respectively.

UV mutagenesis method

Preparation of spore liquid: Take 10 mL physiology saline water to test tube obliquity bacterium cultured for 5 days under the 28 ° temperature. Using sterilization inoculator shaves the spore to triangle bottle under 28 temperature, number agreement 200 r/min for 5 h, to make spore disperse and activation. The spore potency is diluted to 10^6 /mL when it is mutagenesised.

UV mutagenesis: Turn on ultraviolet light (30 W), heat up for 20 min, take 5mL bacterium liquid to glass garden of diameter 9 cm, and move the glass garden to rotating disk.(33 r/min) about 30 cm from ultraviolet light. Treating time is 20 s, 40 s, 80 s, 120 s, 160 s, 200 s, 250 s, 300 s. Take it to dull place after ultraviolet irradiation preventing light restoration.

Elementary selection and reselection

Dilute the spore liquid to fixed potency, spread on improved PDA culture, culture it under 28 ° for 3 d, count the living bacterium and death rate. Select big ratio bacterial colony for 3 generation, then carry on coal degradation experiment.

Biodegradation process of Lignite by *P.chrysosporium*

Method of coal degradation: Some 250 mL taper bottle is putted in 100 mL liquid culture and sterilized coal. Then it is putted to, culture

box, vibrate for 1 h, place still for 1 h, adjust pH to 7.0, added *P.chrysosporium* 10 mL/100 mL, cultured for 10 d under 28 °C 0150 r/min, filter and centrifuge. The light liquid subsides after adding acid (alkali). Precipitant is dried under 70 °C, weighed to count the degradation ratio of coal. (Fig. 1).

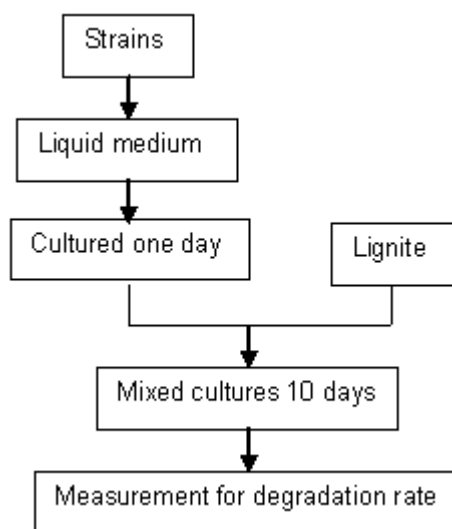


Fig. 1. Process flow diagram of Lignite biodegradation

Test method of coal biodegradation: The precipitate adding sour (alkali) gains with filtrate of the coal degradation queen as a result of being at odds with the community the day after tomorrow dries queen's mass, ratio of being the outcome mass and the coal putting in-like mass of degradation is the coal degradation productivity.

$$\eta = \frac{M_1}{M_0} * 100\%$$

M_0 -adding coal mass (g) M_1 - degradation product mass (g); η -ratio of coal degradation (%).

In the experiment, centrifugation of Henan YiMa City former the azabache degradation queen liquid adopts 5M NaOH solution. The centrifugation liquid after the Henan YiMa City azabache degradation queen that hydrogen nitrate handles adopt 3 M H_2SO_4 solution.

Characterization

The structures of the original lignite, lignite pretreated with nitric acid, precipitating product and residue were characterized by using WQF-510 FTIR spectrometer (Ruili, China), X-ray diffractograms (XRD; PANalytical, Holland) and

SDT2960 thermal analysis (TG-DTA; TA, USA), respectively.

The thermal stabilities of original lignite, lignite pretreated with nitric acid, precipitating product and residue were analyzed by using a thermogravimetric analysis with a heating rate of 10°C/min in the range from 20°C to 700°C under nitrogen atmosphere.

RESULTS

Lignite characterization

The lignite sample was soaked in a 5 M nitric acid for two days, and then it was filtered and washed with distilled water until pH value approaching 7. Finally the filter was dried and disinfected and proximate analysis of the sample (as shown in Table 1).

Table 1. Industry analysis of -0.2mm original coal and coal pretreated with nitric acid

Coal	Mad (%)	Ad (%)	Vad (%)	St.ad (%)
Yima lignite	19.52	18.90	42.34	0.64
Yima lignite treated by nitric acid	10.30	15.22	39.51	0.37

Impair the lethality rate of *P.chrysosporium* by Ultraviolet ray

Handled by Ultraviolet radiation, we discover death ratio reaches 89.5 % dealt for 160 s, 100 % for 200 s. The changes of relation of spore lethality rate can reflect the mechanism and radiation time (Fig. 2).

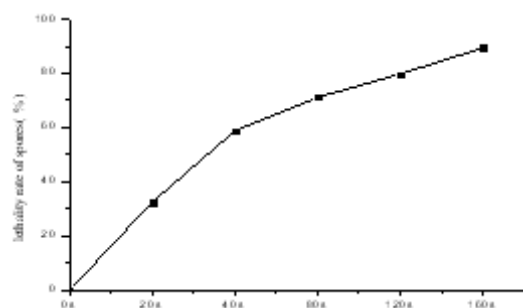


Fig. 2. Lethality rate of spores of *P.chrysosporium* by Ultraviolet ray

Lignite biodegraded by *P.chrysosporium*

From mutagenesis result we know that mutation ratio increase with the increase of radiation time. When radiation time is less than 40 s, mutation ratio increase with the radiation time. When radiation time is 40 s, mutation ratio becomes the highest and when radiation time is more than 40 s, mutation ratio increase with the radiation time, but the good mutation ratio decrease, which lead to reduce the coal degradation ratio. The good mutation ratio is the highest. Its biodegradation of lignite increases from 31.84 % to 37.6 % and lignite treated by nitric acid infusion was increases from 32.55 % to 51.62 %. (Fig. 3).

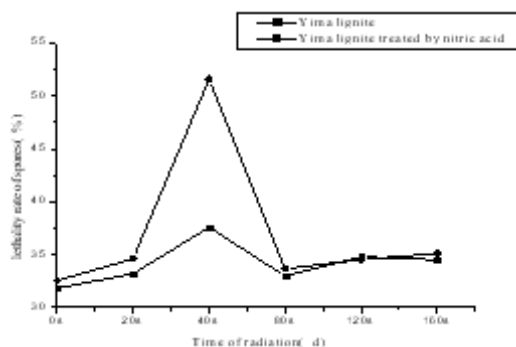


Fig. 3. Relationship of degradation and irradiation time

XRD characterization

The structure of Sample #1: The alkaline precipitating product from *P.chrysosporium* treated original lignite; Sample #2: the residue of lignite after *P.chrysosporium* degradation; Sample #3: The alkaline precipitating product from *P.chrysosporium* treated lignite (nitric acid treated) is tested by XRD analysis (Fig. 4).

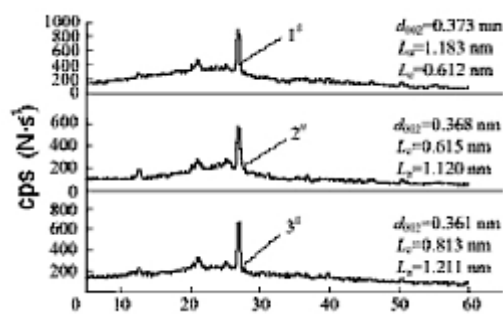


Fig. 4. Relationship among L_a , L_c and d_{002}

FTIR characterization

The functional groups of lignite (F), lignite treated by nitric acid (B), cinder of lignite (C), deposit degraded after 10 days (D) and deposition degraded after 7 days (E) were measured by FTIR analysis

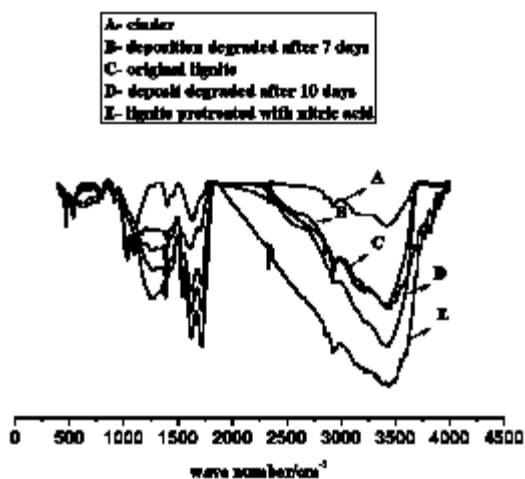


Fig. 5. FTIR spectra of original lignite, lignite pretreated with nitric acid and deposit

(Fig. 5 and in Table 2)

TG characterization

Results of the lignite in thermal stability are investigated by TG analysis (Fig. 6).

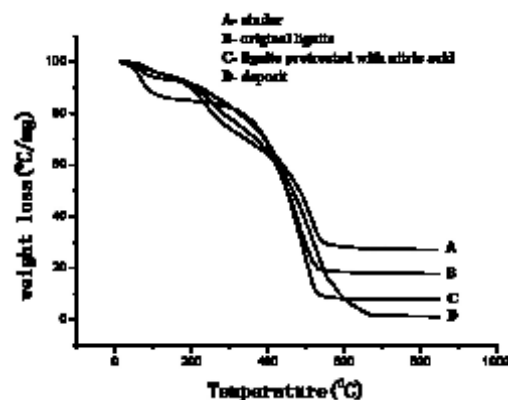


Fig. 6. TG curves of original lignite, lignite pretreated with nitric acid, cinder and deposit

Table 2. FTIR absorption peaks of original lignite, lignite pretreated with nitric acid and deposit

Wave numbers (cm ⁻¹)					Functional group affiliation
B	C	D	E	F	
3437	3423	3407	3422	3414	phenolic hydroxyl of -RH ₂ or Ar-NH ₂
2928	2923	2921	2928	2921	cycloalkane or alicyclic hydrocarbon of -CH ₃
2339	2342	2339	2339	2855	X-H (X=P, Si) stretching vibrations -CH in aldehyde group bending vibrations
1713		1719	1719		stretching vibrations of carbonyl or heterocyclic
1623	1615	1623	1623	1630	stretching vibrations of aromatic
1383	1399	1383	1383	1398	stretching vibrations of aromatic
		1265	1265		CyO stretching vibrations of phenol, alcohol, ether, ester
1108	1101			1101	C-O stretching vibrations of phenol, alcohol, ether, ester
1040	1042			1033	C-O, C=S and S=O stretching vibrations
	796				-CH in aromatic group bending vibration
		630	630		-CH in aromatic group stretching vibration
541	542			541	S-S stretching vibration

DISCUSSION

In this study, analysis of lignite we can seen lignite was composed of large amount of rotten

plant acid, water and ash. The conversion of lignite might be hampered by some metal ions combined with coal via acidic functional groups. The metal ions might be dissolved via acid washing or

soaking to set free the carboxyl groups, thus reducing cross linking between the structures units, which were good to the microbe degradation of lignite. In addition, acid washing is also an ordinary way of removing the ash and sulfur (Liu *et al.*, 1996).

P.chrysosporium handled by Ultraviolet radiation we know that the longer the radiation time, the high the death ratio is when the time is 200 s, the death ration is 100 %, which show *P.chrysosporium* is sensitive to Ultraviolet radiation.

From mutagenesis result we know that mutation ratio increase with the increase of radiation time may be that physiological reaction caused by short time radiation can not make large of mutation. However, physiological reaction caused by long time can damage DNA, leading high mutation ratio and low good mutation. When radiation time is 40 s, physiological reaction is mild and DNA can not be damaged. So the good mutation ratio is the highest and its biodegradation of lignite increases.

The relationship between the diameter of the microcrystallite La, the average stacking height of the microcrystallite Lc and the distance between the microcrystallite d_{002} can be found that the La and Lc of the water soluble microcrystallite from biodegrading of lignite were decreased while d_{002} is decreased (Fig. 4 in "Relationship among La, Lc and d_{002} "). This indicates that the polymerization degree of the water soluble degrading product is decreased, and the stacking height and the macromolecular have been degraded into smaller one.

The FTIR spectra of lignite before and after biodegradated of *R. spheroides* are shown that all the peak shapes are almost equal (Figure 5 in "FTIR spectra of original lignite, lignite pretreated with nitric acid and deposit"). The obvious difference peaks were at $1\,096\text{ cm}^{-1}$ and $1\,044\text{ cm}^{-1}$. The peak at $1\,096\text{ cm}^{-1}$ corresponds to phenol, alcohols, ethers and the absorption peak of C-O bond of esters, the peak at $1\,044\text{ cm}^{-1}$ corresponds to the absorption peak of CH- bond which was substituted aromatic. It can be seen that the absorption peaks at $1\,096\text{ cm}^{-1}$ and $1\,044\text{ cm}^{-1}$ are disappeared in FTIR spectra(B), which indicates that the phenol, alcohols, ethers and aromatic substances in degradation products of

lignite has reduced, but the structure of degradation products is similar to the original lignite. The FTIR spectra(C,D) also shows that the overall trend of the peak shape of degradation products through 7 days or 10 days are almost similar, basically identical, which indicates that no significant change in structure of degradation products of lignite though the degradation time is prolonged.

The thermal stability of original lignite has two obvious weight losses. The previous 13.7 % weight loss is in $12\text{--}100\text{ }^{\circ}\text{C}$ which attributes to the loss of the absorbed water. The second stage is in $300\text{--}550\text{ }^{\circ}\text{C}$ with the weight loss of 61.01 %. To the lignite pretreated with nitric acid, coal cinder and deposit, there are no obvious peaks of weight losses. With the increasing of the temperature, the weight of the samples reduces slowly. The overall weight loss of the deposit is higher than all the weight loss of the other three samples, which suggesting the maximal volatile content in the deposit. The order of the weight loss is deposit>lignite pretreated with nitric acid>original lignite>cinder. The decomposition temperatures of all the samples are low, which is attributed to the low stability of them. The terminate decomposition temperature of the deposit ($672\text{ }^{\circ}\text{C}$) is higher than those three (about $550\text{ }^{\circ}\text{C}$), which suggesting the longer decomposition time of the deposit. There are some changes in the composites and the structures of the original lignite, deposit, lignite pretreated with nitric acid and cinder. The lignite has a degree of decomposition.

In conclusion, UV breeding with clean, simple equipment, simple, safe, reliable, efficient, easy way to overcome the toxicity of the chemical mutagens such as features, broad application prospects. The UV mutagenesis mechanism is UV mainly to ensure that the DNA linked with the adjacent pyrimidine forming covalent combination of thymine dimer, which weakens the hydrogen bonds between double-stranded DNA, the double-stranded structure was twisted, impeding the normal pair, which led to microbe's mutant.

The experimental results show that the best mutagenic effects in the death rate of 40%, lignite and acid dealing with lignite dissolution rates were 37.6% and 51.62%. After mutation spore germination time in advance, the ball diameter reduced but the number increased. And the XRD, FTIR testing study of coal microbial degradation

conversion products, the coal water-soluble substances of microbial transformation is a complex mixture which aromatic polymer, molecular weight have a high degree reduced, functional group content has changed, phenol hydroxyl, hydroxyl and carbonyl type substances has increased.

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