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Molecular Structure Modification Mechanism during Biodegradation of Coal with Different Degrees of Metamorphism

Tianyao Zhou [1], Bingjun Liu [2, *]

[1] School of Safety Science and Engineering, Anhui University of Science & Technology, Huainan, Anhui Province 232001, China
[2] State Key Laboratory of Mining Response and Disaster Prevention and Control in Deep Coal Mines, Anhui University of Science & Technology, Huainan, Anhui Province 232001, China

*** Corresponding author:** Bingjun Liu

Abstract: To explore the process of molecular structure change during the biodegradation of coal, Proximate and ultimate analysis, 16S rRNA sequencing analysis and Liquid chromatography-mass spectrometry (LC-MS) were used to analyze the evolution process of molecular structure of different coal ranks (bituminous coal, anthracite coal, coking coal) in the process of biodegradation. The results indicate that the biodegradation of coal is a process of enriching carbon elements (increasing C element by 1.28%~4.04%) and removing hydrogen, oxygen, nitrogen, and sulfur elements. Biodegradation destroys the ether bond (C-O-C) and carbonyl (C=O) structures in coal, causing the breakage of its aliphatic side chains and the detachment of surface functional groups. In addition, biodegradation breaks the weak bridging bonds between aromatic layers in coal, leading to the cracking of polycyclic aromatic hydrocarbon structures and an increase in aliphatic side chains, resulting in a slight improvement in the aromaticity of coal samples. These results suggest that biodegradation contributes to the aromaticity and maturity of coal, which is similar to the peatization process of coal.

Keywords: Gasification of Coal; Coal Matrix Modification; Biogenic Coalbed Methane; Rank of Coal Metamorphism.

1. Introduction

Coalbed methane is an important renewable energy source, and its efficient extraction is currently a focus of research in the field of energy [1]. Coalbed Methane Bioengineering (CBM) uses hydraulic fracturing to inject microbial fluids and nutrients into underground coal seams [2]. By stimulating microbial degradation of coal matrix, the permeability of coal seams is changed, and the production and extraction efficiency of coalbed methane are improved [3]. This metabolic behavior relies on co-metabolism between microorganisms, where the large organic structures in coal are first degraded into liquid chemical substances, including low molecular weight aliphatic and aromatic compounds [4]. This biological behavior will improve the pore structure of coal reservoirs and contribute to the migration and enrichment of coalbed methane[5]. Meanwhile, in further metabolic processes, methanogens will utilize these low molecular weight compounds to produce a clean energy source, methane[6]. Therefore, in-depth research on the impact of microbial degradation on the organic structure of coal is of great significance for the safe extraction of coalbed methane and the improvement of coalbed methane single well production.

The above research provides a rich theoretical basis for exploring the evolution of the microstructure of middling coal in the process of biodegradation, but there is still research on the biodegradation process of the molecular structure of coal with different metamorphic degrees[7]. This study selected three types of coal, namely high volatile bituminous coal, anthracite coal, and coking coal, with domesticated mixed microbial bacterial liquid as the inoculation source. Explore the differences in organic structure and surface functional groups of coal before and after biodegradation metabolism through Proximate and ultimate analysis, and determine the characteristics of changes in coal molecular structure. The transformation pathways of various organic structures in coal under microbial action were constructed using gene sequencing and LC-MS technology. The aim of this study is to explore the changes in organic structure under the biodegradation of different coal rank coals, providing a theoretical basis for the efficient exploitation of coalbed methane in low-permeability coal seams.

2. Proximate/Ultimate Analysis

Table 1. Proximate and ultimate analysis results of coal samples

Sample	Proximate analysis (%)				Ultimate analysis (%)				
	M_{ad}	A_{ad}	V_{daf}	FC_{ad}	C	H	O*	N	S
YM	1.73	9.67	32.38	56.22	73.03	6.42	18.42	1.75	0.38
WYM	1.6	8.04	10.89	79.47	86.06	3.17	7.96	1.6	1.21
JM	0.82	6.95	23.01	69.66	76.07	5.19	16.36	1.3	1.08
YM-B	1.11	7.83	31.04	60.02	77.07	5.23	16.36	1.09	0.25
WYM-B	1.22	8.85	9.81	80.12	87.34	2.97	7.38	1.26	1.05
JM-B	0.26	6.5	22.59	70.65	79.88	3.38	15.02	1.05	0.67

O*: Differential method oxygen content.

B: Representing biodegradation

The Proximate/Ultimate analysis data of raw coal and biodegradable coal are shown in Table 1. After 40 days of microbial degradation, the V_{daf} content of YM, WYM, and JM coal samples decreased by 1.34%, 1.08%, and 0.42%, respectively, while the relative content of fixed carbon increased by 3.8%, 0.65%, and 0.99%, respectively. The elemental analysis results indicate that the biodegradation of coal is a process of fixing carbon elements and removing hydrogen, oxygen, nitrogen, and sulfur elements.

3. Sequencing Analysis

Six microbial communities classified at the phylum level were detected in the YM, WYM, and JM culture systems (Fig.1a). The dominant phyla of the cultivation system are Firmicutes (73.05% -97.18%) and Proteobacteria (2.12%-25.68%), which are further classified into microbial communities at the genus level of 60 species (Fig.1b). In addition, Verrucomicrobiota, Bacteroidota, Actinobacteriota, and Cyanobacteria were also present in the culture system, but their relative abundances were all below 1%. This is similar to the microbial communities obtained from previous enrichment and domestication for coal degradation. Firmicutes are the main hydrolyzing and acid-producing bacteria in anaerobic degradation, decomposing cellulose and starch polysaccharides and producing short-chain fatty acids, and Proteobacteria are mainly involved in the decomposition of soluble sugars into monosaccharides and short-chain fatty acids. In addition, due to the presence of elemental sulfur in coal, Sulfate-Reducing Bacteria (SRB) utilizes citric acid to reduce the sulfate structure in coal to H_2S. Principal component analysis (PCA) was used to demonstrate the succession patterns of microbial communities in different culture systems (Fig.1c). The results showed that when the same exogenous strains were added, the strains in the three groups of culture systems did not undergo obvious succession, Firmicutes and Proteobacteria were the main dominant strains in the coal biodegradation process.

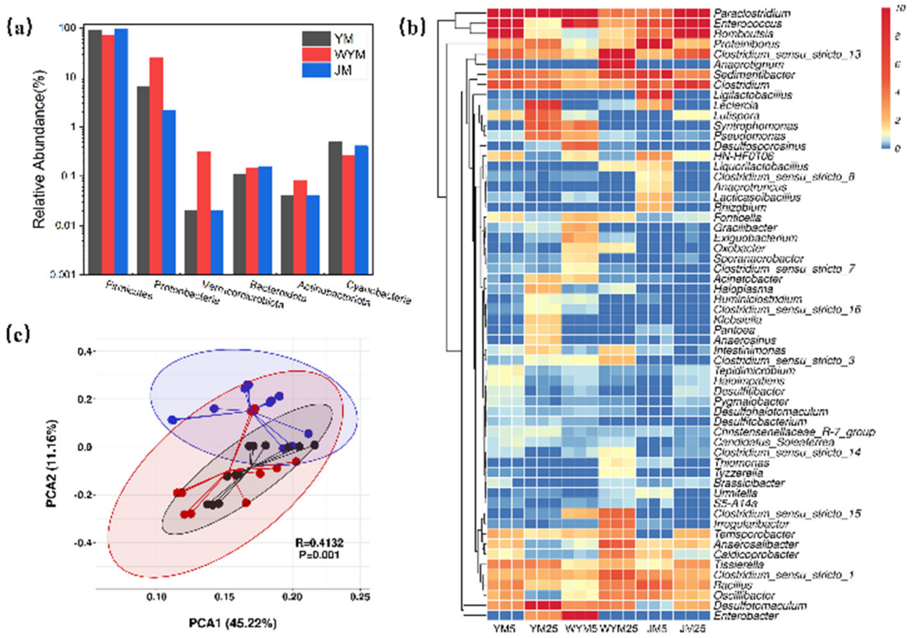

Fig 1. a) Microbial community composition at the phylum level. b) Microbial community composition at the genus level. Numbers represent days of incubation. c) PCA analysis based on the level of OTUs

To explore the degradation pathways of various organic structures by the above-mentioned microbial communities, the gene function analysis of the microbial community was featured by KEGG pathways according to PICRSt. Fatty acid degradation, Glycolysis, Benzoate degradation, Methane metabolism, TCA cycle, Sulfur metabolism, and Phenylalanine metabolism were the main three culture system microbial metabolic pathways (Fig. 2). The biodegradation of coal depends on the hydrolysis and acidification ability of microorganisms. In the process of coal biodegradation and biogasification, all kinds of organic matter (including alkanes, aromatics, benzenes, cellulose, proteins, etc.) in coal will be decomposed into precursors or building block molecules (CO_2, Pyruvate, Succinyl-CoA and Acetyl-CoA) necessary for cell biosynthesis, and microorganisms synthesize nutrients needed for each component of the cell by synthesizing small molecule organic matter, which is rejoined into the carbon cycle of the coal seam by carbon metabolism.

4. Analysis of Gas Products

Test the headspace gas components every five days, and the main gas phase products during coal biodegradation/ gasification are CH_4 and CO_2(Fig.3). During the 40-day degradation process, the YM methane production of high volatile bituminous coal was the highest, with the cumulative yields of 231.9, 224.56 and 216.3 μmol/g, respectively. The WYM methane production of anthracite coal was the middle, with the cumulative yields of 177.8, 169.7 and 179.7μmol/g, respectively. The JM methane production of medium volatile bituminous coal was the lowest, with the cumulative yields of 206.3, 191.6 and 207.1μmol/g, respectively. The carbon dioxide concentration in the YM, WYM, and JM culture systems reached its peak on the 10th day, reaching 17.16%, 13.78%, and 12.74%, respectively. Within 10 to 40 days of the experiment, the CO2 concentration began to decrease and remained in a dynamic equilibrium of 4.67% ± 1.04%. The coal quality of coal has a significant effect on the efficiency of its biogasification and shows a trend that the higher the coal quality, the lower the degradation and gasification efficiency.

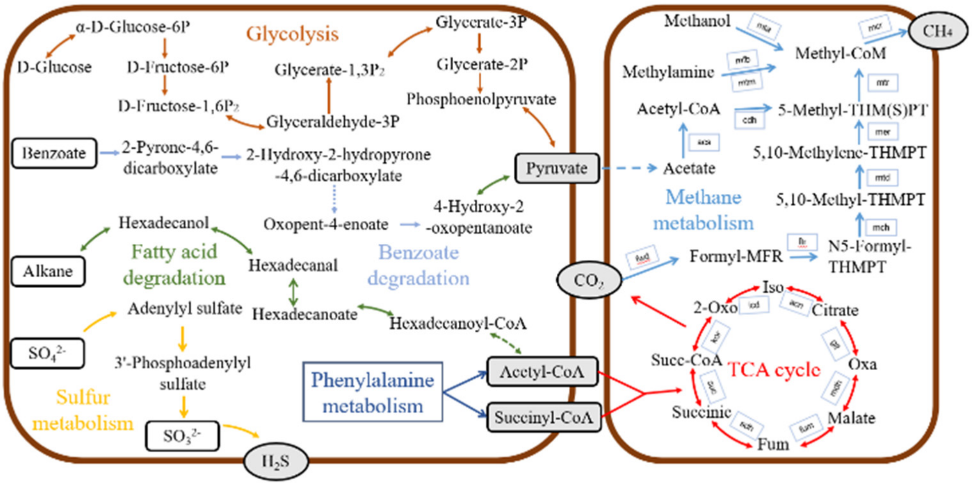

Fig 2. Significant metabolic pathways associated with coal biogasification

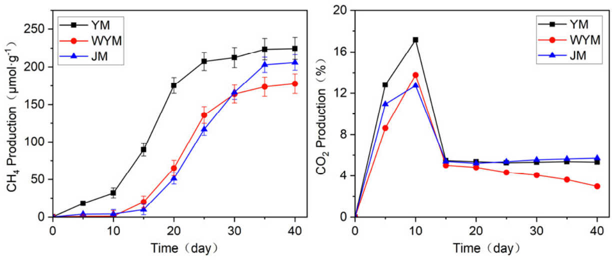

Fig 3. (a) Methane production between different samples. (b) CO2 production between different samples.

5. Analysis of Liquid Products

Based on LC-MS unbiased detection of metabolites (mainly small molecule compounds with a relative molecular weight of less than 1000 Da). As illustrated in Fig.6, these metabolites are mainly composed of Organoheterocyclic compounds, Organic oxygen compounds, Organic acids and derivatives, Benzenoids, Lipids and lipid-like molecules. This study used principal component analysis (PCA) for multivariate statistical analysis and utilized scatter plots to visualize multidimensional data, with the aim of visually representing the differences in metabolites among different coal qualities (Fig.4d). The model indicates good separation among the three samples, and there are significant differences in the total metabolites of YM, WYM, and JM. Metabolic pathway enrichment analysis of the metabolites using the KEGG database elucidated the degradation pathways of the compounds, thus helping to explain the biodegradation process of coal. This study annotated 18 metabolic pathways (Fig.6f). The existence of pathways such as Napoleon degradation and Chlorocyclohexane and Chlorobenzene degradation may indicate the involvement of microorganisms in the degradation of aromatic and cycloaliphatic compounds in coal. In these pathways, in the presence of enzymes such as 4-hydroxybenzoyl-CoA reductase subunit gamma [EC:1.1.7.1], naphthalene 1,2-dioxygenase subunit alpha [EC:1.14.12.12], benzene and its derivatives will be degraded into small molecule compounds such as Pyruvate, Succinyl-CoA and Acetyl-CoA.

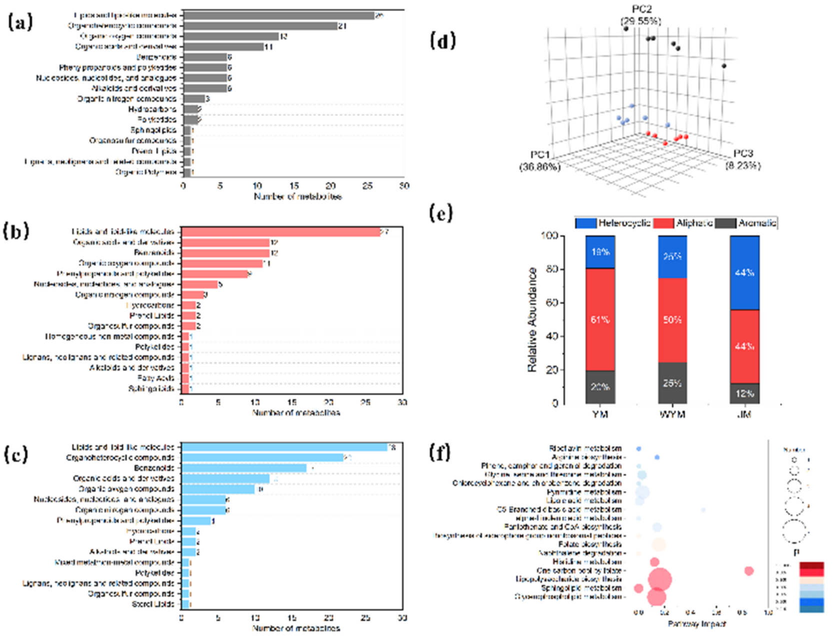

Fig 4. (a) Classification of compounds detected in sample YM. (b) Classification of compounds detected in sample WYM. (c) Classification of compounds detected in sample JM. (d) Principal component analysis of total metabolites in different samples. (e) Classification of compounds based on carbon skeleton. (f) Enrichment analysis of metabolic pathways of metabolites

Based on the classification method of organic carbon skeleton of the compounds, the compounds detected in this study were categorized into three groups, including: aliphatic compounds, aromatic compounds, heterocyclic compounds (Fig. 4e). Aliphatic compounds are the main degradation products in coal. They exist in various forms such as alkanes, alkenes, alcohols, phenols, ethers, aldehydes, ketones, carboxylic acids, esters, etc. Specific substances include: Phytophingosine, 10,20-Dihydroxyeicosanoic acid, 2,6-Dimethyl-1,8-octanedioic acid, etc. The content of aromatic compounds is second, mainly existing in the form of aromatic hydrocarbons, polycyclic aromatic hydrocarbons, and benzene derivatives containing heteroatom side chains. Specific species include Chamuvaritin, Clausarinol, Debenzoylzucchini factor B, etc. In addition, with the continuous improvement of coal quality, the types and contents of aromatic compounds in coal show an increasing trend, and a small amount of polycyclic aromatic compounds appear. The heterocyclic compounds detected in this study are mainly nitrogen-containing heterocyclic compounds and oxygen-containing heterocyclic compounds, including Clausarinol, dubamine, D-Fructosazine, etc. The detection of metabolites showed the process of coal biodegradation, and the large amount of long-chain fatty compounds (61%) in the YM group indicated that microbial biodegradation of low rank coal mainly acts on the fatty side chains in the coal structure. As the coal quality increases, aromatic compounds and heterocyclic compounds begin to increase, which may be due to the low content of aliphatic compounds in high-order coal causing the degradation target of microorganisms to change to the weak bonds that destroy the coal aromatic layer.

6. Biological Transformation Pathways of Coal Organic Structure

Based on the above analysis results, this study constructed a schematic diagram of the organic structure biodegradation process of high metamorphic coal and low metamorphic coal (Fig.5). Biodegradation first acts on the intermolecular bridging structure of coal. Firstly, the chemical structure of coal with medium to low degree of metamorphism was established based on the Wiser model, and the chemical structure of coal with high degree of metamorphism was established based on the Spiro model. Under the action of extracellular enzymes secreted by microorganisms, coal is hydrolyzed into aromatic oxygen-containing derivatives, aromatic nitrogen-containing derivatives, long-chain alkane derivatives, long-chain alkene derivatives, and polycyclic aromatic hydrocarbon derivatives. These polymers and organic compounds fall off from the coal and dissolve in the culture medium or aggregate on the surface of the coal, which is one of the main factors affecting the pore structure and chemical structure of the coal through microbial degradation. At the same time, the detachment of these compounds also leads to hydroxylation of coal aromatic structures and an increase in the elongation of carbon atom grids. As the degradation process continues, aromatic hydrocarbons and alkanes with high bioavailability are first degraded into short chain fatty acids or fatty alcohols. The target of biodegradation has shifted towards aromatic oxygen-containing derivatives and aromatic nitrogen-containing derivatives, which will cause some weak bond breaks in these compounds and directly degrade nitrogen-containing heterocyclic organic compounds to obtain nitrogen sources. In addition, the presence of polycyclic aromatic hydrocarbon structures in liquid-phase products and the lack of gene functional expression for polycyclic aromatic hydrocarbon degradation detected in gene sequencing in this study require further exploration of the degradation of these polycyclic aromatic hydrocarbon structures.

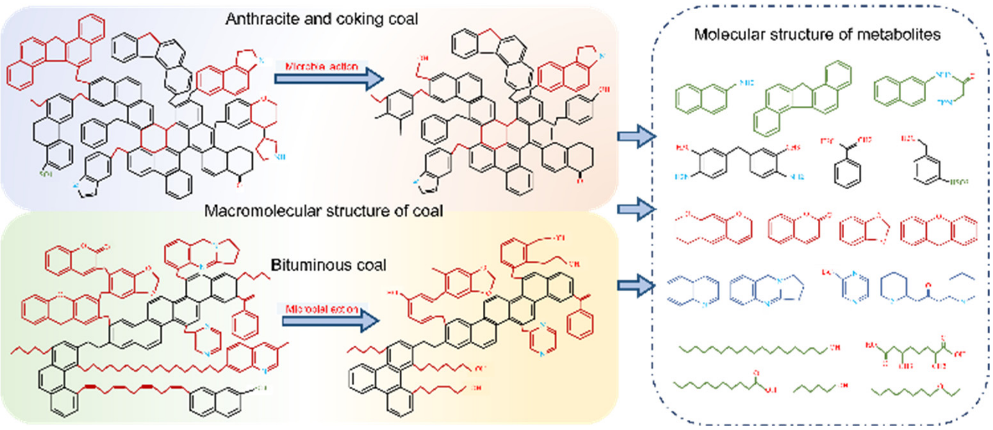

Fig 5. Biodegradation pathways of coal macromolecular organic structures

7. Conclusion

This study investigated the evolution patterns of the organic structures of three types of coals: bituminous, anthracite, and coking coal before and after biodegradation/biogasification using Proximate and ultimate analysis, LC-MS, and 16S rRNA sequencing. The main conclusions are as follows: The biodegradation process of low-rank coals is similar to that of peatization, including shortening of aliphatic side chains and shedding of surface functional groups, changes that result in a decrease in volatile matter and an increase in carbon content of the coal. Biodegradation of higher-order coals differs in that the breaking of intermolecular bridge bonds and degradation of thick cyclic aromatic structures will result in an increase in the coal's aliphatic side chains and a slight increase in aromaticity. Gene sequencing and LC-MS data indicate that the biodegradation of coal involves substitution reactions of methyl groups in the aliphatic structure, breakage of ether bonds, and detachment of aromatic and heterocyclic functional groups. Firmicutes and Proteobacteria are the main dominant bacterial species in coal degradation, participating in the degradation of cellulose and soluble glycosides in coal. Based on the above conclusion, a biodegradation pathway was constructed for the transformation of high and low rank coal macromolecular organic structures into soluble small molecule structures. The research results will provide a theoretical basis for the modification of middling coal matrix in the process of coal biodegradation.

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