UNIVERSIDAD AUTÓNOMA DE SAN LUÍS POTOSÍ
Doctorado Instituto de Física

SUCCESSION AND IMPACT OF INDIGENOUS MICROORGANISMS IN COAL FLOTATION

PARA OBTENER EL GRADO DE

DOCTORADO POSGRADO EN CIENCIAS INTERDISCIPLINARIAS

QUE PRESENTA

Jianbo Li

DIRECTORES

Dr. Shaoxian Song Hu

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PATROCINADO POR CONACyT Beca número 814723

San Luis Potosí, S.L.P. Marzo 2024

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Resumen

El carbón es un combustible fósil importante, pero la liberación excesiva de bióxido de carbono durante la combustión limita su uso. Un paso crucial en el procesamiento del carbón es la técnica de flotación de carbón, mediante la cual se remueven impurezas minerales, mejora la calidad del carbón, reduce la contaminación ambiental y mejora la eficiencia del uso del carbón. A nivel industrial, la eficiencia de la flotación de carbón depende de varios factores como el tamaño de partícula del carbón, pH, agentes de flotación y los microrganismos nativos, aunque éstos no han sido considerados en mucho tiempo. Una variedad de microorganismos endémicos en las vetas de carbón pueden entrar en el sistema del proceso de la flotación, junto con el procesos del carbón, adherirse al carbón y cambiar sus propiedades de superficie afectando la eficiencia de la flotación. Sin embargo, este fenómeno no ha recibido mucha atención. En esta tesis, se analiza de manera sistemática la composición de los microorganismos nativos y sus propiedades fisicoquímicas en diferentes etapas del sistema de flotación en sitio. También, varias cepas de microorganismos en el carbón bruto fueron aisladas y varias pruebas realizadas para revelar las fuentes y descendencia de los microorganismos nativos en el sistema de flotación y sus efectos sobre la eficiencia de este proceso. Los resultados muestran que los microorganismos en la flotación de carbón corresponden a phyla 34 y genera 98, como las fuentes de las vetas de carbón. *Proteobacterias*, *Acidobacterias*, *Actinobacterias* y *Crenachaeota*, son los microorganismos dominantes a nivel de phylium. La adición de agentes de flotación pueden cambiar las propiedades del proceso, especialmente pueden aumentar el contenido de la demanda química de oxígeno, y así promover la sucesión de las comunidades de microbios y el cambio en su diversidad funcional. El análisis de la correlación éntre los microorganismos nativos y el contenido de ceniza en el sistema de flotación, revela que dichos microorganismos tienen un impacto negativo en la flotación. Los resultados experimentales del efecto de esos microorganismos en la flotación de carbón confirman esta conclusión. Los *bacilos*, que son también microorganismos nativos, pueden reducir la hidrofobicidad de la superficie del carbón mediante el incremento del número de grupos funcionales conteniendo oxígeno sobre la superficie del carbón. El rendimiento de la flotación de carbón se redujo en 15% cuando 10^8 células/ml fueron añadidas. *Escherichia coli*, como microorganismo acuático en el sistema de flotación, puede adsorberse sobre la superficie del carbón

mediante puentes de hidrógeno y reducir su hidrofobicidad. Cuando la concentración de *Escherichia coli* alcanzó 5 x 10^9 células/ml, solamente el 50.25% del carbón fue recuperado. *Sphingomonas*, también microorganismos nativos, biodegradaron el 66.4% de poliacrilamida en un tiempo de 76 h, debilitando su efecto de floculación con lo cual se redujo la razón de sedimentación del carbón afectando el proceso de flotación del carbón. Finalmente, *Arthrobacter*, que metaboliza azufre, fue aislada del carbón. Las pruebas de biodesulfurización mostraron que estos microorganismos pudieron remover 42% del azufre orgánico del carbón en un tiempo de 160 h, lo cual es beneficioso para la remoción de azufre del carbón. Diversas especies de microorganismos nativos tienen diversos efectos sobre la flotación del carbón. El presente trabajo amplia el entendimiento de la influencia de dichos microrganismos en el proceso de flotación de carbón y se espera que provea bases científicas para la tecnología de la flotación de carbón.

Palabras clave. Flotación de carbón; Agentes de flotación; Microorganisms nativos; Funciones microbianas; Adsorpción; Biodegradación; Biodesulfurización.

Abstract

Coal is an important fossil fuel, but the excessive release of carbon dioxide during the combustion process limits its utilization. Coal flotation, as a crucial step in coal processing, can remove impurity minerals, improve coal quality, reduce environmental pollution, and enhance coal utilization efficiency. In industry, the efficiency of coal flotation is influenced by various factors such as coal particle size, pH, flotation reagents, while the effect of microorganisms on coal flotation has been neglected for a long time. A variety of indigenous microorganisms in the coal seam can enter the coal flotation system along with the coal processing process, adsorb to the coal surface and change the coal surface properties, thus affecting the coal flotation efficiency. However, this phenomenon has not received much attention.

In this study, the indigenous microbial composition and physicochemical properties of various stages in the on-site coal flotation system were systematically analyzed, and also several microbial strains from the raw coal were isolated and conducted tests to reveal the sources and succession of indigenous microorganisms in the coal flotation system and their effects on the coal flotation efficiency. The results show that the microorganisms in the coal flotation are assigned to 34 phyla and 98 genera, all source from the coal seam. Proteobacteria, Acidobacteria, Actinobacteria, and Crenachaeota are the dominant microorganisms at the phylum level. And the addition of flotation reagents can change the properties of the coal flotation system, especially increasing the chemical oxygen demand content, thus promoting the microbial communities succession and changing the microbial communities functional diversity. The correlation analysis between indigenous microorganisms and ash content in the coal flotation system revealed that indigenous microorganisms have a negative impact on coal flotation. And the experimental results on the effect of indigenous microorganisms on coal flotation also confirmed this conclusion. *Bacillus*, as an indigenous microorganism, can reduce the hydrophobicity of coal surface by increasing the number of oxygen-containing functional groups on the coal surface, and the coal flotation yield was reduced by 15% when added at 10^8 cells/ml. *Escherichia coli*, as a waterborne microorganism in coal flotation systems, can adsorb to the coal surface through hydrogen bonding, thereby reducing the hydrophobicity of the coal surface. When the concentration of *Escherichia coli* reached 5×10^9 cells/ml, only 50.25% of the coal could be recovered. *Sphingomonas*, an indigenous microorganism, biodegraded 66.4% of polyacrylamide within 76 h, weakening its

flocculation effect, reducing coal settling rate and affecting the coal flotation process. In addition, *Arthrobacter* with sulfur metabolism was isolated from coal, and the results of biodesulfurization tests showed that the microorganism could remove 42% of organic sulfur from coal within 160 h, which is beneficial for sulfur removal from coal.

Different species of indigenous microorganisms have diverse effects on coal flotation. The study broaden the understanding of the influence of indigenous microorganisms in the coal flotation process and is expected to provide a scientific basis for the coal flotation technology.

Keywords: Coal flotation; Flotation reagents; Indigenous microorganisms; Microbial functions; Adsorption; Biodegradation; Biodesulfurization

Extended Abstract

Coal is an important fossil energy resource with abundant global reserves, which can be used for power generation, heat supply and industrial production. However, the combustion of coal produces a large amount of greenhouse gases such as carbon dioxide, which has a negative impact on global climate change. Therefore, developing clean coal technology and improving energy efficiency are crucial measures to mitigate the environmental impact of coal utilization. Coal flotation technology can effectively separate coal from impurities by the hydrophilic and hydrophobic differences between coal and impurities. It plays a vital role in improving coal quality, enhancing process efficiency, reducing environmental impacts, creating economic value, and optimizing the utilization of coal resources. Coal flotation efficiency is influenced by multiple factors. However, the majority of research focuses primarily on the impact of flotation reagents and equipment on coal flotation, while neglecting the influence of microorganisms on coal flotation. During the formation of coal, various indigenous microorganisms coexist, which can enter the coal flotation system during the coal processing and subsequently affect the efficiency of coal flotation. Therefore, it is essential to study the sources, composition, succession, and impact of indigenous microorganisms in coal flotation systems.

In this study, samples of raw coal, mine water, circulating water, flotation feeding, clean coal, middling, and tailing were collected from Qinxin coal processing plant in Changzhi, Shanxi, China, and the sources of indigenous microorganisms in the coal flotation system was analyzed using high-throughput sequencing technology. The effects of physical and chemical properties (pH, dissolved oxygen, chemical oxygen demand, phosphorus content, and nitrogen content) on the microbial community were also analyzed to investigate the succession of the coal flotation system. The results found 98 genera and 34 phyla of indigenous microorganisms in the coal flotation system, all originating from the coal seam. And the microbial diversity increased in the coal flotation system compared to that of the coal seam microorganisms as a result of the succession of microbial communities. The addition of flotation reagents is a significant factor in promoting the succession of microbial communities. Flotation reagents can alter the physicochemical properties of the coal flotation system, particularly by increasing the content of chemical oxygen demand. This change impairs the metabolic function of the microbial community, which in turn reduces the reproduction of Nitrospirae, Bacteroidetes, Proteobacteria, and

Actinobacteria, while promoting the growth of GAL15. As a result, the microbial composition undergoes changes, leading to the development of new microbial communities in the coal flotation system.

Furthermore, the relationship between indigenous microorganisms in the coal flotation system and flotation indicators (ash content) was established to predict the potential impact of indigenous microorganisms on coal flotation. The results demonstrated that the majority of indigenous microorganisms exhibit a positive correlation with ash content, indicating that indigenous microorganisms are detrimental to coal flotation. To confirm this conclusion, a strain of *Bacillus* sp. was isolated from raw coal and conducted to bioflotation tests. The results showed that *Bacillus*, as an indigenous microorganism in the coal flotation system, can adsorb to the coal surface, increasing the number of oxygen-containing functional groups on the coal surface and decreasing the hydrophobicity of the coal surface, thus inhibiting the coal flotation yield. When *Bacillus* sp. was added at 10^8 cells/ml, the coal flotation yield was reduced by 15%.

In addition, *Escherichia coli*, as an waterborne microorganism in coal flotation system, also affects the flotation efficiency of coal. With the increase of *Escherichia coli* addition, the coal flotation yield gradually decreased. When *Escherichia coli* concentration reached 5×10^9 cells/ml, only 50.25% of the coal could be recovered. By comparing the changes in zeta potential, scanning electron microscopy, Fourier-transform infrared spectroscopy, and contact angle before and after *Escherichia coli* adsorption onto the coal surface, it can be observed that hydrogen bonding force between *Escherichia coli* and the coal surface is greater than the electrostatic repulsion force, which results in the adsorption of *Escherichia coli* onto the coal surface and reduces the hydrophobicity of the coal surface, thus affecting the coal flotation yield.

The flotation reagents used in coal flotation systems can facilitate the succession of microbial communities, indicating a mutual relationship between indigenous microorganisms and the reagents. The results of microbial function prediction show that indigenous microorganisms possess the ability to degrade complex organic matter and xenobiotics. To confirm this prediction, a *Sphingomonas* strain, isolated from raw coal, was employed to degrade polyacrylamide that had a flocculating effect. As the microbial biomass increased, polyacrylamide concentration decreased, and the biodegradation rate reached 66.4% within 76 hours. Various techniques such as scanning electron microscopy, atomic force microscopy, UV-Vis spectrophotometry,

Fourier transform infrared spectroscopy, and high-performance liquid chromatography were used to confirm that the extracellular enzymes secreted by *Sphingomonas* can hydrolyze and oxidize the amide group and main carbon chain of polyacrylamide, breaking it down into smaller organic products. However, the biodegradation of polyacrylamide has a negative impact on coal slurry settling. The results of coal slurry settling tests show that the settling rate and settling layer height of coal slurry significantly decrease after polyacrylamide biodegradation.

Additionally, the study also found that specific microorganism is advantageous for coal processing and utilization. The results of microbial functional prediction suggested that *Arthrobacter*, as an indigenous microorganism in raw coal, has excellent sulfur metabolism and can be used for sulfur removal from coal. And the results of the desulfurization test of coal by *Arthrobacter* showed that *Arthrobacter* could remove 42% of sulfur and reduce 1.06% of ash in 160 h. The comparison of X-ray diffraction and Fourier-transform infrared spectroscopy results before and after the desulfurization of coal showed that *Arthrobacter* can not only remove organic sulfur from coal, but also interact with kaolinite to reduce the content of kaolinite during the microbial desulfurization process of coal.

In conclusion, coal flotation systems contain a wide variety of indigenous microorganisms, and their effects on coal flotation vary based on their different structures and functions. Therefore, investigating the composition, succession, functions, and impact of indigenous microorganisms in coal flotation systems can offer new insights from a microbial perspective to improve flotation efficiency and enable effective microbial management in on-site coal flotation systems.

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Chapter I. Introduction

1.1 Justification

Coal is a black solid mineral composed of carbon, hydrogen, oxygen, nitrogen and other elements, formed by ancient plants through biochemical and geological effects [1]. Coal is a critical fossil energy source, known as "industrial food", and occupies the main position in primary energy production and consumption. Coal can be used in thermal power, iron and steel, chemical industry, building materials and other industrial fields, and is an important driving force for the development of human civilization [2,3]. However, coal combustion process will produce a lot of sulfur dioxide, carbon dioxide, nitrogen oxides, carbon monoxide and other pollutants, which is the main cause of atmospheric pollution and acid rain [4]. Therefore, in order to reduce the environmental hazards of coal combustion, coal must be processed before it can be utilized. Coal processing is a technology that applies physical and chemical methods to exclude mineral impurities and harmful elements from coal and improve the comprehensive utilization of coal.

Burning 1 ton of raw coal can generate 20908 MJ of heat, but through coal processing, where mineral impurities are removed from the raw coal, 1 ton of coal can yield 28908 MJ of heat. Simultaneously, burning 1 ton of coal can release 2.66 tons of carbon dioxide. Therefore, under the condition of the same heat demand, the combustion amount of coal after coal processing is lower, and compared with the raw coal, the carbon dioxide release is reduced. The coal processing techniques can enhance coal quality and utilization efficiency, leading to a decrease in carbon dioxide emissions.

Coal flotation is a method of coal processing which utilizes the difference in physicochemical properties between the surface of coal and impurity minerals for separation, and is the most widely used in fine coal (<0.5mm) [5]. Flotation reagents are important factors that influence the efficiency of coal flotation. These reagents can improve the properties of the solid, gas, and liquid phases in the flotation system, while also modifying the surface properties of minerals, enabling the separation of coal from impurity minerals. Flotation reagents consist of collectors, frothers, and modifiers. Collectors improve the hydrophobicity of coal surfaces, frothers reduce the interfacial tension between gas and liquid, and modifiers control the interaction

between minerals and collectors. Flotation reagents are typically organic and inorganic compounds [6,7].

Some studies have proposed that microorganisms can act as flotation reagents to achieve coal flotation, and the process is known as bioflotation [8]. The peptidoglycans and lipids present in microbial cell walls can determine the microbial charge and hydrophobicity, thereby influencing the flotation behavior [9,10]. Different microorganisms exhibit distinct physicochemical properties due to variations in their cell wall composition [11]. Microorganisms can interact with coal through electrostatic forces, hydrophobic interactions, surface free energy, hydrogen bonding, surface functional groups, and alter the electrical properties and hydrophobicity of coal, achieving flotation separation effects [12]. For example, *Xanthomonas hortorum* can selectively adsorb onto the coal surface through specific bonding interactions, with its hydrophobic flagella extending into the water, increasing the hydrophobic surface area and enhancing the hydrophobicity of coal [13]. *Bacillus subtilis*, as a flocculant, can significantly accelerate the sedimentation of fine coal [14]. And the iron-sulfur metabolism-related bacteria *Acidithiobacillus ferrooxidans* and *Ferroplasma acidiphilum* can selectively adsorb onto the surface of pyrite, increasing the hydrophilicity of pyrite surface and improving the desulfurization effect of coal flotation [15]. Usually, the microorganisms in bioflotation are exogenous microorganisms, originating from air, water, etc., which need to be domesticated in co-culture with coal and then adapted to the coal slurry environment to play the role of flotation reagents [11].

Indeed, coal seams contain a variety of microorganisms due to the microbial degradation of organic matter during coal formation. These indigenous microorganisms can enter the coal flotation system during the coal processing stages. The indigenous microorganisms in the coal seam are already highly domesticated microorganisms after a long period of coexistence and selection with the coal, which have a stronger ability to interact with the coal and will have a greater impact on coal flotation [16]. Once indigenous microorganisms enter the coal flotation system, changes in environmental conditions such as moisture, oxygen, and nutrients can occur, leading to two possible outcomes. Firstly, they may die, leaving behind remnants. Hydrophilic remnants such as proteins, polysaccharides, and nucleic acids released from the shattered cells can adsorb onto mineral surfaces, thereby altering their properties. Secondly, some microorganisms may survive and proliferate, producing more microorganisms and metabolic secretions. They can proliferate and

accumulate in the coal slurry system through water recirculation, resulting in a significant increase in their population [17]. Consequently, the entry of indigenous microorganisms from coal seams into the coal flotation system, regardless of whether they die or continue to propagate, can impact coal flotation efficiency. However, this phenomenon has often been overlooked and has not been thoroughly researched.

Consequently, in this study, coal samples were collected from the on-site coal flotation system of the Qinxi Coal Preparation Plant in Changzhi, Shanxi, China. High-throughput sequencing technology was employed to analyze the microbial composition and diversity in the coal flotation system. By comparing the differences in microbial composition among various stages of the coal flotation system, the indigenous microbial sources were revealed. Furthermore, the correlation between microbial communities and environmental factors within the coal flotation system was examined to elucidate the coal succession patterns. Additionally, the relationship between microorganisms and coal flotation indicators was established to predict the impact of indigenous microorganisms on coal flotation. Moreover, multiple strains of indigenous microorganisms were isolated from the raw coal to further investigate their influence on coal flotation.

1.2 Hypothesis

Many previous microbial flotation experiments have demonstrated that microorganisms can act as various types of flotation reagents in coal flotation systems, adsorbing onto the coal surface and altering its hydrophobicity, thus affecting the coal flotation efficiency. Most of these studies have focused on exogenous microorganisms isolated from soil and water. However, it is known that coal seams harbor a substantial population of indigenous microorganisms that play a crucial role in the coal formation process. It is hypothesized that these indigenous microorganisms can enter the coal flotation system along with coal processing and undergo growth, reproduction, and death within the system. During this process, the microorganisms themselves and the extracellular polymers they secrete may influence coal flotation efficiency. The objective of this study is to analyze the microbial composition in on-site coal processing plant flotation systems, establish the relationship between microorganisms and physicochemical properties in the coal flotation system, elucidate the succession patterns of microorganisms in the system, and isolate multiple strains of indigenous microorganisms to investigate their impact on coal flotation efficiency.

1.3 Objectives

1.3.1 General objective

The study conducted a comprehensive analysis of the composition and community structure of indigenous microorganisms in the industrial on-site coal flotation system, aiming to determine their sources and patterns of succession. And further investigated the mechanisms of indigenous microorganisms impact coal flotation efficiency using correlation analysis and bioflotation experiments. The objective of the study is to offers novel approaches to enhance coal flotation efficiency from a microbial perspective.

1.3.2 Goals

1) Investigate the indigenous microorganisms composition and the physicochemical properties of coal flotation system.

2) Compare the microbial community structures of indigenous microorganisms between coal seam and coal flotation system, establish the correlation between indigenous microorganisms and environmental factors, and reveal the significant factors influencing indigenous microbial succession.

3) Isolate indigenous microorganisms from raw coal, conduct bioflotation experiments, and investigate the mechanisms of indigenous microorganisms influence coal flotation efficiency.

4) Analyze the interaction between indigenous microorganisms and reagents in the coal flotation system to explore whether indigenous microorganisms can indirectly affect coal flotation efficiency by influencing the efficacy of reagent action.

5）Indigenous microorganism with sulfur metabolism capabilities was isolated from raw coal to investigate the impact of indigenous microorganisms on coal desulfurization.

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Chapter II. Antecedents

2.1 Introduction

Coal, as an important fossil fuel, is widely used in fields such as electricity, industry, and transportation, playing a crucial role in the development of human society. Particularly in developing countries and emerging economies, coal occupies a significant position in energy supply [1]. However, coal mining and combustion have serious environmental impacts, such as air pollution and climate change [2,3]. Therefore, while ensuring energy supply, the clean utilization of coal is also essential. Coal flotation is a technology that separates coal and associated minerals based on the differences in physical and chemical properties of the surface [4]. This technology can improve the quality and value of coal, achieve diversified utilization of coal, reduce environmental pollution, and improve environmental quality, thereby achieving the goal of clean coal utilization [5]. Currently, research on coal flotation mainly focuses on three aspects: (1) the study of coal surface properties and the interaction mechanism with associated minerals [6,7]; (2) research on flotation reagents [8,9]; and (3) research on coal flotation equipment [10,11]. In fact, the study on coal flotation has been expanding. Some scholars have found that specific microorganisms can affect the efficiency of coal flotation by acting as collectors, frothers, regulators, etc [12,13]. However, most of these microorganisms are exogenous microorganisms isolated from soil and water. During coal processing, various indigenous microorganisms present in coal seams enter the coal flotation system and impact coal flotation. As these microorganisms have long used coal seams as their habitat, they have specificity with coal, and their influence on coal flotation efficiency is more significant. However, this phenomenon has not received enough attention. Therefore, this article summarizes the relevant reports on indigenous microorganisms in coal flotation systems and provides a new approach to improve coal flotation efficiency.

2.2 Coal utilization and processing

Coal plays a dominant role in primary energy production and consumption and has made important contributions to the economic and social development of China as a strategic resource. However, during the combustion of coal, all carbon atoms in coal are converted into carbon dioxide, leading to prominent carbon emissions and

bringing severe challenges to environmental governance. Coal processing technology can remove mineral impurities and harmful elements in coal, improve coal utilization efficiency, reduce air pollution, and achieve clean coal utilization.

2.2.1 Coal utilization

Energy is a vital material foundation for the survival and growth of human society. Since the beginning of the 21st century, with the aim of building a modern society, the demand for energy materials by humans has been increasing. Coal is one of the most important energy materials, and according to the World Coal Association, 37% of the world's electricity is produced from coal [14]. In addition, coal has many important applications in modern society, such as steel and cement production, coal byproduct production, rare earth element extraction, hydrogen production, etc [15]. Table 2.1 shows the total production and composition of primary energy in China. The results indicate that China is a country rich in coal, poor in oil, and lacking in natural gas. In 2020, coal accounted for 67.6% of China's primary energy production, which is much higher than oil at 6.8% and natural gas at 6.0%. Other energy sources only accounted for 19.6% [16]. This illustrates that coal plays a crucial role in China's energy and industrial sectors. According to statistics, China's total coal mining volume has continued to increase in the past five years, with a total mining volume of 4.56 billion tons in 2022. Meanwhile, in 2021, China's consumption reached 5.24 billion tons, while the total mining volume was only 4.13 billion tons, indicating that demand far exceeds supply (Fig.2.1)[16]. It can be foreseen that as a transitional energy in China's energy transition process, coal will still occupy an important position in China's energy landscape.

The widespread use of coal brings serious environmental problems. Coal combustion causes air pollution, and in 2021, coal accounted for more than 40% of the global increase in carbon dioxide emissions. Due to coal being a product of ancient plant remains buried underground and undergoing a series of biochemical processes, impurities are often present during the coal mining process. These impurities can cause incomplete combustion of coal, lower coal utilization efficiency, and generate carbon monoxide pollutants, which are the main factors affecting coal combustion efficiency [17-19]. Furthermore, due to associated ores such as pyrite present in coal, toxic and harmful substances such as sulfur dioxide are also emitted, causing secondary pollution such as acid rain and soil acidification [20,21]. In addition, impure coal is not conducive to producing modern coal-based chemical

products such as coal-to-olefins, aromatics, and high-end materials such as carbon fiber and graphene. Therefore, regardless of whether coal is used as energy or raw materials, cleaning coal is the prerequisite and foundation for the clean and efficient utilization of coal.

Table 2.1 The total production and composition of primary energy in China [16]

Time	Total primary energy production (Ten thousand tons of standard coal)	Proportion（%）			
		Coal	Oil	Gas	Other energy sources
1978	62770	70.3	23.7	2.9	3.1
1980	63735	69.4	23.8	3	3.8
1985	85546	72.8	20.9	2	4.3
1990	103922	74.2	19	2	4.8
1991	104844	74.1	19.2	2	4.7
1992	107256	74.3	18.9	2	4.8
1993	111059	74	18.7	2	5.3
1994	118729	74.6	17.6	1.9	5.9
1995	129034	75.3	16.6	1.9	6.2
1996	133032	75	16.9	2	6.1
1997	133460	74.2	17.2	2.1	6.5
1998	129834	73.3	17.7	2.2	6.8
1999	131935	73.9	17.3	2.5	6.3
2000	138570	72.9	16.8	2.6	7.7
2001	147425	72.6	15.9	2.7	8.8
2002	156277	73.1	15.3	2.8	8.8
2003	178299	75.7	13.6	2.6	8.1
2004	206108	76.7	12.2	2.7	8.4
2005	229037	77.4	11.3	2.9	8.4
2006	244763	77.5	10.8	3.2	8.5
2007	264173	77.8	10.1	3.5	8.6
2008	277419	76.8	9.8	3.9	9.5
2009	286092	76.8	9.4	4	9.8
2010	312125	76.2	9.3	4.1	10.4
2011	340178	77.8	8.5	4.1	9.6
2012	351041	76.2	8.5	4.1	11.2
2013	358784	75.4	8.4	4.4	11.8
2014	362212	73.5	8.3	4.7	13.5
2015	362193	72.2	8.5	4.8	14.5
2016	345954	69.8	8.3	5.2	16.7
2017	358867	69.6	7.6	5.4	17.4
2018	378859	69.2	7.2	5.4	18.2
2019	397317	68.5	6.9	5.6	19
2020	408000	67.6	6.8	6	19.6

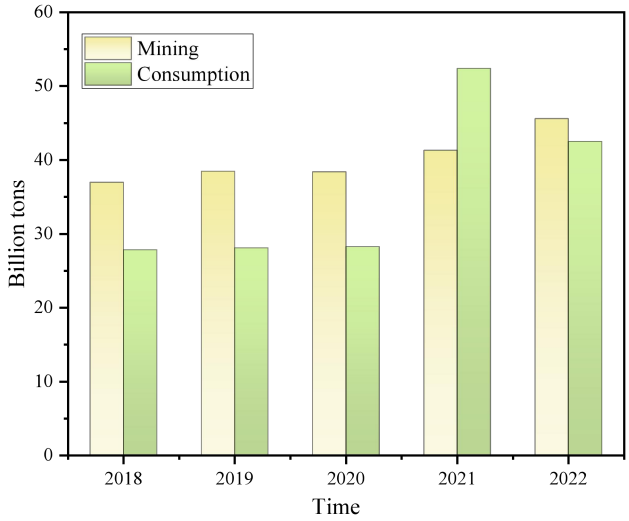

Fig.2. 1 Coal mining and consumption in China in recent 5 years [16]

2.2.2 Coal surface properties

The surface characteristics of coal are important factors that affect coal flotation. These surface characteristics include coal specific surface area, surface charge, surface wetting properties, surface active sites, and surface functional groups.

Coal specific surface area: The specific surface area of coal refers to the surface area per unit mass of coal. It is an important parameter describing the fineness and porosity of coal particles, and can also serve as an indicator for evaluating coal's adsorption capacity, reactivity, and mass transfer properties. The specific surface area of coal is closely related to coal particle size, the smaller the coal particles, the larger the specific surface area. This is because smaller particles have more surface area, providing more adsorption and reaction sites. Additionally, the presence of pore structures in coal affects its specific surface area, as the presence of pores increases the surface area of coal. The specific surface area of coal is an important parameter used to characterize coal's pore structure, adsorption performance, reactivity, and mass transfer properties.

Coal surface charge: The surface charge of coal refers to the charged properties on the surface of coal particles. Due to the structure and composition of coal, functional groups or ionic groups on the surface can induce the charging of coal particles, resulting in surface charge. The surface charge of coal can be either positive or negative, depending on the chemical functional groups present on the surface and the environmental conditions.

The generation mechanisms of coal surface charge can involve the following aspects:

Ion exchange: Ionic exchange sites present in coal can exchange ions with those in the solution, resulting in the surface charge of coal particles. For instance, cation exchange sites in coal can adsorb anions, leading to a positively charged surface; conversely, anion exchange sites can result in a negatively charged surface.

Dynamic dissociation: Some functional groups on the coal surface may possess dynamic dissociation characteristics, meaning they can lose or gain charges under specific pH conditions. This dissociation behavior can result in charged coal particles.

Chemical reactions: Chemical reactions occurring on the coal surface can generate charges. For example, oxidation reactions can introduce electron imbalances on the coal surface, leading to surface charge.

The presence of coal surface charge significantly influences the behavior and interactions of coal. It can affect the adsorption performance, dispersibility, colloidal stability, and interactions with other substances such as ions in solution, gas molecules, or other solid particles.

Coal surface wetting properties: The wetting properties of the coal surface describe the characteristics of how coal interacts with a liquid (typically water) when they come into contact. The wetting properties primarily depend on the chemical composition, surface structure, and surface energy of coal. The wetting properties of coal can be characterized by the contact angle, which is the angle formed by a liquid droplet in contact with the solid coal surface. Based on the magnitude of the contact angle, wetting properties can be classified into the following categories:

Hydrophilicity (wettability): When a liquid droplet spreads and forms a smaller contact angle on the coal surface (< 90°), it indicates good wetting properties of the liquid on the coal surface. This means that the liquid can uniformly distribute and penetrate the coal surface.

Hydrophobicity (non-wettability): When a liquid droplet forms a larger contact angle on the coal surface (> 90°), it indicates poor wetting properties of the liquid on the coal surface. This means that the liquid cannot uniformly spread on the coal surface and tends to form droplets or beads.

Neutral wetting: When a liquid droplet forms a contact angle close to 90 degrees on the coal surface, it indicates neutral wetting properties of the liquid on the coal surface. In this case, the liquid partially wets the coal surface without complete penetration.

Wetting properties have significant implications for the processing and utilization of coal.

Coal surface active sites: Coal surface active sites refer to chemical sites or functional groups present on the surface of coal particles that exhibit high reactivity or adsorption capacity. These active sites play a significant role in surface chemical reactions, gas adsorption capacity, dispersibility, and colloidal stability of coal. The properties and quantity of coal surface active sites can be influenced by various factors, including coal composition, structure, processing methods, and environmental conditions. Some common examples of coal surface active sites include:

Hydroxyl groups (-OH): Hydroxyl functional groups on the coal surface exhibit hydrophilicity and chemical reactivity, allowing them to form hydrogen bonds with water molecules or participate in acid-base neutralization reactions.

Ketone groups (C=O): Ketone functional groups on the coal surface can participate in oxidation-reduction reactions, carbonyl addition reactions, and other chemical processes.

Carboxyl groups (COOH): Carboxyl functional groups on the coal surface can participate in acid-base reactions, ion exchange, and other chemical interactions.

Amine groups (NH_2): Amine functional groups on the coal surface possess the ability to adsorb gas molecules and participate in chemical reactions involving amines.

Sulfur groups (-SH): Sulfide or thiol functional groups in coal can provide electrons during oxidation reactions, thus participating in catalytic reactions.

The presence of coal surface active sites significantly influences surface chemical reactions, gas adsorption capacity, dispersibility, and colloidal stability of coal. Therefore, understanding the properties and distribution of coal surface active sites is of great importance for studying coal reaction properties, modification methods, and optimizing coal processing processes.

Coal surface functional groups: Coal surface functional groups refer to functional groups that exhibit chemical reactivity and are present on the surface of coal particles. These functional groups have a significant influence on the surface properties and chemical reactions of coal. Here are some common coal surface functional groups:

Hydroxyl groups (-OH): Hydroxyl groups are one of the most common functional groups on the coal surface. They can interact with water molecules through hydrogen bonding, affecting the wetting properties of coal and wet processing.

Carboxyl groups (COOH): Carboxyl functional groups contain carboxylic acid groups (-COOH). They exhibit acidity and can participate in acid-base reactions, ion exchange, and coordination reactions.

Ketone groups (C=O): Ketone functional groups contain carbon-oxygen double bonds and can participate in oxidation-reduction reactions, carbonyl addition reactions, and other reactions.

Amine groups (NH_2): Amine functional groups contain nitrogen atoms and can participate in hydrogen bonding and coordination reactions. They play an important role in adsorption and catalytic reactions.

Sulfur groups (-SH): Sulfur functional groups contain sulfur atoms and can participate in oxidation reactions, sulfurization reactions, and ion exchange.

Alkene groups (C=C): Alkene functional groups contain carbon-carbon double bonds and can participate in conjugation reactions, addition reactions, and oxidation reactions.

The presence and quantity of these coal surface functional groups are influenced by coal composition, structure, and coal processing methods. They determine the surface chemical properties, adsorption capacity, reactivity, and interactions with other substances such as gases, liquids, or solids.

2.2.3 Coal processing

Coal processing is the process of separating raw coal into clean coal. Coal processing technology can improve the utilization rate of coal and reduce the emission of harmful air pollutants, which can not only alleviate China's future energy pressure but also reduce the environmental problems caused by carbon emissions. It is a research hotspot for many scholars. Currently, coal processing technologies include physical, chemical, and biological methods [22,23].

Physical methods are separation techniques based on the differences in physical properties between minerals and coal, including gravity separation, flotation, magnetic separation, etc [24]. Gravity separation is a separation method based on the difference in specific gravity between coal and minerals, and is suitable for coal with a particle size > 0.5 mm. In gravity separation technology, dense medium separation is the most widely used process. Honaker et al. increased the centrifugal force by applying high-pressure feed injection in a dense medium cyclone separator, achieving ash removal of fine coal (< 1 mm) and reducing the ash content of coal from 29% to 7% [25]. However, it is impossible to reduce the ash content to the desired level

through gravity separation alone, and it needs to be combined with flotation. Coal flotation as shown in Fig.2.2 is a separation process based on the difference in surface properties between coal and associated mineral particles, to obtain low-ash clean coal smaller than 0.5 mm. During the flotation process, coal particles attach to the surface of bubbles due to their hydrophobicity, while associated minerals cannot adsorb on the bubble surface [26,27]. The coal slurry floated essentially forms a dispersed system consisting of solid, liquid, and gas phases. The solid phase refers to coal and associated minerals, the liquid phase refers to water medium, and the gas phase is generally air [28]. Bubbles, as a selective carrier, are the medium of separation. The determining factors for coal flotation separation include the following：

(1) There are differences in surface properties between coal and associated minerals.

(2) Large amounts of stable and fine bubbles are generated in the coal slurry.

(3) Solid particles collide with bubbles, causing low-ash coal particles to adsorb on the bubbles and be recovered as clean coal, while high-ash associated minerals cannot adsorb on the bubbles and become flotation tailing.

(4) During the flotation process, bubbles as the medium of separation and also as the carrier for transporting clean coal particles.

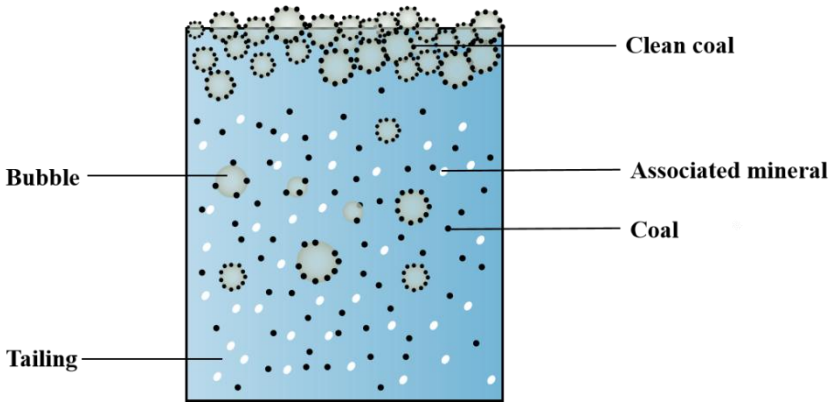

Fig.2. 2 Coal flotation process

Chemical methods of coal processing use strong acids and bases to thoroughly remove inorganic minerals, pyrite sulfur, and organic sulfur from coal, and can be used to produce ultra-clean coal [29]. Specifically, it can be divided into acid leaching, alkali leaching, and alkali-acid leaching [30]. Acid leaching can effectively dissolve metal ions to remove impurities in coal minerals. The research of Fan has shown that hydrofluoric acid can effectively dissolve quartz and kaolin to remove inorganic

minerals, and hydrogen iodide can remove almost all pyrite sulfur under certain conditions [31]. At the same time, acid combination and continuous leaching processes have been proposed to improve acid leaching efficiency and shorten reaction time. For example, the combination of 30% hydrochloric acid and nitric acid can achieve a desulfurization rate of 75.4% under mild conditions (90°C, 90 min). The use of nitric acid leaching after hydrofluoric acid leaching can reduce ash content to 0.63% [32]. The alkali leaching process mainly converts kaolin and quartz in coal into hydrated alkaline silicates, sodium alumino-silicate compounds, and other aluminum silicate complexes [33]. Compared with acid leaching, the pressure and temperature conditions required for alkali leaching are relatively mild, and it can also remove some pyrite and organic sulfur. Kara used sodium hydroxide to treat Turkish lignite and removed 91% of inorganic minerals [34]. Balaz studied the chemical treatment of coal by grinding and alkali leaching, reducing the sulfur content by 50% [35]. Low-cost calcium hydroxide has also been applied to the de-mineralization of coal. For example, Wang et al. reduced the ash content of Newstan coal from 9.2% to 2.2% at 340℃ and 5% CaO for 120 min [36]. Combining alkali leaching and acid leaching has also been widely studied. The advantage of strong alkali is that it can effectively remove kaolin and quartz, as well as some pyrite and organic sulfur. However, the solubility of alkali-treated products is weak, and further treatment with dilute acid is needed. Yang et al. used a three-stage leaching process (sodium hydroxide-sulfuric acid-nitric acid) to treat raw coal, and the coal quality was significantly improved (ash content <0.6%, iron content <0.03%, silicon content <0.02%) [37]. Chemical methods are necessary for producing ultra-clean coal. However, chemical methods also have obvious limitations, and expensive chemical reagents and secondary pollution are still unsolved problems. Therefore, they are currently rarely applied in industry.

Biological methods mainly include microbial flotation and microbial leaching. Microbial flotation refers to the method of using microorganisms instead of chemical agents in traditional flotation. Numerous microorganisms have been found to have excellent flotation separation effects. Amini et al. used *Acidithiobacillus ferrooxidans* as an inhibitor for pyrite in coal flotation, reducing the sulfur content of pyrite by 14% under kerosene flotation conditions [38]. Abdel et al. used *Bacillus Subtilis* as an inhibitor, removing more than 70% of sulfur and ash content in coal [39]. Ramos-Escobedo et al. used *Staphylococcus carnosus* as a collector, achieving a coal recovery rate of 90% under pH 9, even higher than the coal recovery rate in traditional

chemical flotation [40]. However, research on microbial flotation is still in the laboratory flotation stage, and the uncontrollability of microorganisms, as well as the harsh operating conditions, hinder its application in the flotation field. Microbial leaching refers to the process of using the characteristics of microbial metabolism to oxidize or reduce some components in coal and separate them in the form of an aqueous solution or ion. Liu et al. found that after treatment with domesticated *Acidithiobacillus ferrooxidans* and *Pseudomonas sp.*, the ash content of low-rank brown coal decreased from 7.11% to 6.31%, while the sulfur content decreased by 2.76% to 3.23%, and the heating value of coal increased from 6219 cal/g to 6406-6315 cal/g [41]. Kargi et al. used the thermophilic microorganism *Sulfolobus acidocaldarius* to remove 44% of organic sulfur in four days at 70℃ [42]. Although biological treatment has a high desulfurization rate and less coal waste, its high cost and processing time make it difficult to be widely used in industry.

Currently, physical methods still dominate coal processing, and with the mechanization of mining processes, coal particles are becoming increasingly finer. Therefore, flotation technology is also increasingly being valued.

2.3 Indigenous microorganisms in coal flotation system

Currently, research on coal flotation mainly focuses on flotation agents and equipment, while ignoring the influence of indigenous microorganisms in the coal flotation system. In fact, a large number of indigenous microorganisms in coal seams can enter the coal flotation system along with the coal processing, which may have a potential impact on the efficiency of coal flotation.

2.3.1 Indigenous microorganisms in coal seams

Coal is a sedimentary organic mineral formed by the biological and physicochemical transformation of plant remains, composed of a mixture of various high molecular weight compounds and minerals. The process of coal formation is divided into two stages: peatification and coalification [43]. Indigenous microorganisms play an important role in coal formation, particularly in the peatification stage. Microorganisms can degrade organic matter and transform it into raw materials for coal formation, while also accelerating coalification. Therefore, as shown in Fig.2.3(a), the coal seams contain a variety of microorganisms, and the number of microorganisms changes with the depth of the coal seams [44,45]. And Nur Hazlin Hazrin-Chong observed indigenous microorganisms on the coal surface

using scanning electron microscopy (Fig.2.3(b)) [46]. The widespread application of high-throughput sequencing technology has provided the possibility for further research on indigenous microorganisms in coal seams. Silas used 16S rDNA to study the microbial community composition of Bowen Basin, Sydney Basin, and Surat Basin in Eastern Australian coal seams. The study revealed that coal seams contain a significant amount of microorganisms. The most dominant communities were Proteobacteria, Firmicutes, Euryarchaeota, and Bacteroidetes, with relative abundances of 44.2%, 17.9%, 17.7%, and 8.8%, respectively. Meanwhile, Silas combined with other research data on microbial communities in coal seams to construct the Coal Seam MicroBiome (CSMB) dataset. The CSMB contains microbial community information from 97 coal seams worldwide and provides a reference dataset for subsequent analysis of indigenous microorganisms in coal seams [47]. Mine water is the water entering the shaft, roadway, and working face during coal mining, and also exists in coal seams. In a study conducted by IIunga Kamika et al., it was discovered that mine water contains a total of 6 phyla and 10 classes. The most abundant phyla found were Proteobacteria (58.33%) and Cyanobacteria (36.25%) [48]. Similarly, Florentino et al. found that certain families in the class Bacteroidia, such as Porphyromonadaceae, Prolixibacteraceae, and GU454901 dominate the coal mine water [49]. Also, a study by Ganiyu et al. found that coal mine water contains 10 phyla, including Pseudomonadaceae, Sphingomonadaceae, and Anaeromyxobacteria [50].In summary, many research results have proven that coal seams do indeed contain a diverse range of microorganisms.

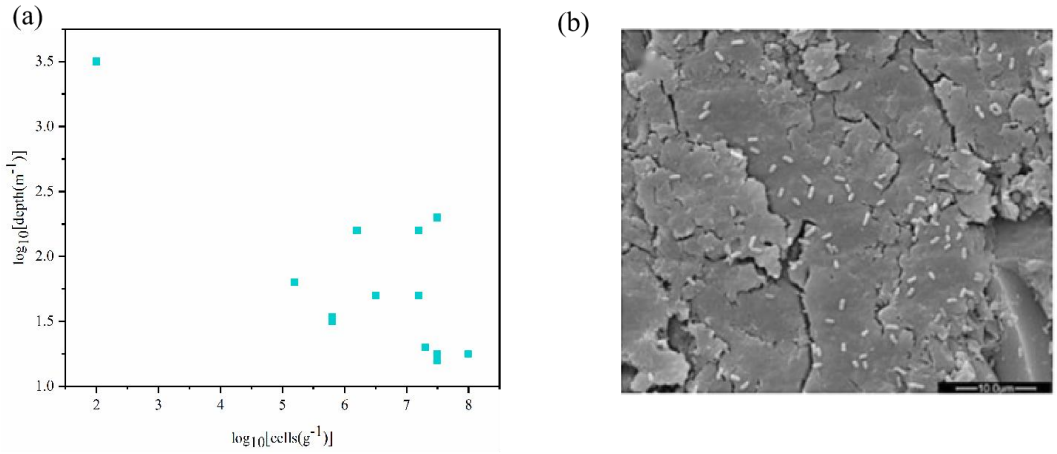

Fig.2. 3 Numbers of indigenous microorganisms in coal seams and SEM images ((a) The number of indigenous microbes in coal seams; (b) .SEM of indigenous microorganisms in coal seams)[46]

2.3.2 Indigenous microorganisms in coal flotation system

Raw coal and mine water in coal seams are the raw materials and water mediums of the coal flotation process [51]. Coal seams contain various microorganisms, which can enter the coal flotation system during coal processing (Fig.2.4). And the coal flotation process uses a large number of organic reagents such as collectors, frothers, and flocculants, which accumulate in the closed-circuit water medium of the coal flotation system and provide nutrients for microbial growth. Therefore, it is inferred that the coal flotation system contains a variety of microorganisms, but research in this area is relatively scarce. Recently, Wang and Li conducted a detailed study on indigenous microorganisms in the flotation system of the Shanxi Qinxin coal preparation plant using 16S rDNA. They obtained a total of 57,760 gene sequences from 27 coal samples and found 34 phylum-level and 98 genus-level microorganisms in the coal flotation system. The results of they studies pointed out that microorganisms in the coal flotation system are all derived from indigenous microorganisms in the coal seam, but there are significant differences in their abundance due to changes in environmental factors. The dominant microorganisms at the phylum level in the coal flotation system are Proteobacteria (15.99% ~ 36.06%), Acidobacteria (10.80 ~ 24.07%), and Actinobacteria (5.30% ~ 24.57%) [52,53]. This is similar to the microbial species previously reported in coal, and many microorganisms in Proteobacteria and Acidobacteria can degrade lignin, hydrocarbons, polycyclic aromatic hydrocarbons, and other large molecular compounds in coal [54,55]. Actinobacteria and Acidobacteria are also common in sewage treatment systems. The dominant microorganisms at the genus level are *Nitrososphaera* (5.19% ~ 17.98%), *Kaistobacter* (0.86% ~ 4.78%), and *Arthrobacter* (0.28% ~ 8.90%). *Nitrososphaera* is a typical ammonia-oxidizing archaea and is closely related to nitrogen cycling. Meanwhile, Li used PICRUSt2 to predict the function of indigenous microorganisms in the coal flotation system as shown in Fig.II.5 and found that the metabolism function of the microbial community is strong, including the degradation of aromatic hydrocarbons, fatty acids, and polycyclic aromatic hydrocarbons, as well as carbon and nitrogen metabolism. The metabolism function of the microbial community is closely related to the main components of coal and the organic agents in the flotation system [52,53]. If calculated based on 10^6 cells/g of indigenous microbial biomass in the coal seams, the number of microbial cells entering the coal flotation system of a 4 Mt/a coal preparation plant per day would reach 10^{16} cells [56]. After entering the coal flotation system, these

microorganisms will experience changes in growth conditions such as water content, oxygen, and nutrients, resulting in two possible outcomes. One result is indigenous microorganisms die and leave behind remnants, or their cells break apart, releasing hydrophilic remnants such as proteins, polysaccharides, and nucleic acids [57]. And the other result is the microorganisms may survive and grow, producing more microorganisms and metabolites and continuously proliferating and enriching in the circulating water of the coal slurry system to reach a huge number [58]. The activity of indigenous microorganisms in the coal flotation system can have a certain impact on the flotation efficiency, making research on indigenous microorganisms succession in the coal flotation system extremely necessary.

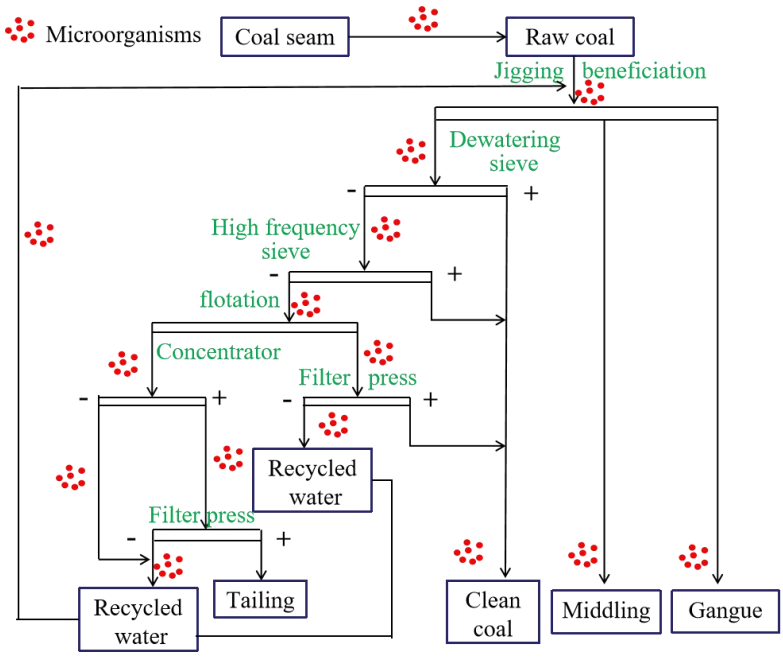

Fig.2. 4 Coal processing process flow chart

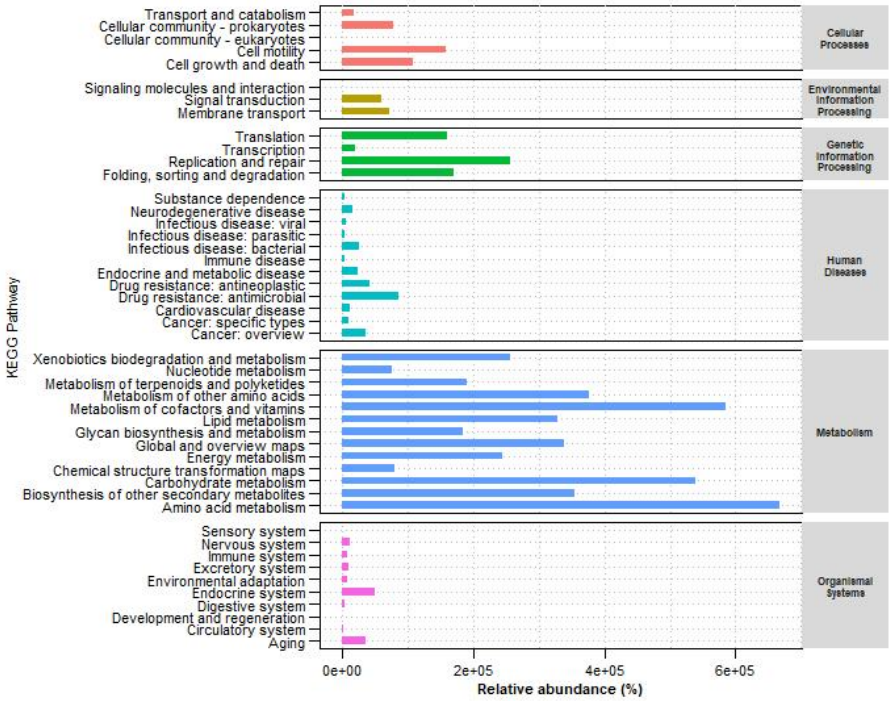

Fig.2. 5 The function of indigenous microorganisms in the coal flotation system

2.4 The impact of flotation agents on indigenous microorganisms

Various flotation agents are added during the coal flotation process to improve flotation efficiency. These agents can alter the growth environment of indigenous microorganisms in the coal flotation system, which has a significant impact on the succession and activity of indigenous microorganisms.

2.4.1 Flotation agents in coal flotation

The addition of collectors, frothers, and modifiers can improve the coal flotation efficiency during the coal flotation process [59,60]. Collectors are organic substances that selectively act on the coal surface, making the coal surface hydrophobic, such as non-polar hydrocarbons like kerosene and diesel [61,62]. Frothers are chemicals that work on the interface between water and air. They disperse air into small bubbles in the slurry, which can improve the degree of bubble mineralization and stability during the flotation process. Some examples of frothers commonly used in the industry include pine alcohol oil, secoctyl alcohol, and methyl isobutyl carbinol (MIBC) [63,64]. Modifiers can adjust the interaction between other agents and the coal surface, adjust the properties of coal slurry, and improve selectivity towards coal, such as pH adjusters, activators, and dispersants [65]. With in-depth research on flotation agents, the use of mixed reagents has become more common, and new agents such as amines

and quaternary ammonium salts have also been used in coal flotation [66,67]. Flotation agents have a high content of organic matter, and long-term use can significantly alter the growth environment and nutritional conditions of indigenous microorganisms in the flotation system, thereby affecting the structure and function of the microbial community.

2.4.2 Flotation agents affect the growth of indigenous microorganisms

During the coal flotation process, the water medium used is closed-loop circulating water, and the residual flotation agents can continuously accumulate in the water circulation process, thereby increasing the content of organic compounds. Organic compounds have carboxyl groups, hydroxyl groups, phenolic aldehydes, aromatic groups, quinone structures, nitrogen, various aromatic rings, and weak fatty acids, which can bring more metabolic pathways and substrate types to indigenous microorganisms, thereby affecting their growth [68,69,53]. Many studies have also confirmed the idea that flotation agents can increase the content of organic compounds in circulating water. The results of S.R. Rao found that the circulating water in flotation systems contains many dissolved compounds that can fundamentally change the chemistry of the system [70]. And the research of G. Levay pointed out that the existence of circulating water depressants, dispersants, flocculants, and collector decomposition products will increase the organic carbon content [71]. Furthermore, Li's research showed that the residual flotation agents in the coal flotation system increase the content of chemical oxygen demand (COD). COD can be used to measure the quantity of organic substances in water. A higher COD value indicates a greater degree of water pollution caused by organic matter. In Li's study, it was found that the COD in the flotation feeding is 380-390 mg/L higher than that in the mine water. And Li investigated the effect of adding different concentrations of kerosene and MIBC on the growth of indigenous microorganisms in coal slurry. The results showed that the addition of kerosene and MIBC can promote the growth of bacterial biomass. At a dosage of 120 mg/L to 480 mg/L, the bacterial biomass increased by 1.2 to 2 times compared with the blank group [53]. In addition, Li also studied the effect of polyacrylamide on the growth of *Sphingomonas* which was isolated from raw coal. The results showed that *Sphingomonas* can use the amide groups and main carbon chains in polyacrylamide as nitrogen and carbon sources for cell growth. Therefore, the accumulation of residual agents in the coal flotation system can provide nutrients for microorganisms, promote their growth and

reproduction, increase the number of microorganisms in coal flotation systems, and thus affect the coal flotation process [72].

2.4.3 Flotation agents regulate the structure of indigenous microbial community

Various residual agents in coal flotation systems can indirectly affect microbial community structure by directly altering environmental factors, leading to changes in microbial function. The functional diversity of the microbial community in the coal flotation system is closely related to coal flotation efficiency. Research by Wang has also shown that the diversity of the microbial community in coal slurry (Shannon index 6.85-7.16) increases to a certain extent compared to flotation feeding (Shannon index 6.73) after the addition of agents, indicating that the addition of agents can increase the microbial diversity of the flotation system and alter community structure [52]. Furthermore, Li explored the sources and succession of microbial communities in coal preparation plants and found that indigenous microbial communities in the coal flotation system originate from coal seams but underwent structural changes due to the addition of agents. Compared with the indigenous microbial community in coal seams, the abundance of Proteobacteria, Bacteroidetes, and Nitrospirae decreased, while the abundance of GAL15 increased in the coal flotation system. Additionally, analysis of microbial community function changes by PICRUSt2 revealed that the metabolism functions of the microbial community in the coal flotation system, such as Xenobiotics biodegradation and metabolism, Nucleotide metabolism, Metabolism of cofactors and vitamins, and lipid metabolism, are significantly enhanced. Changes in function can affect the impact of microorganisms on coal flotation efficiency [53]. Therefore, the relationship between flotation agents and microbial community structure can be used to control microbial community structure and reduce negative impacts on coal flotation. For example, the introduction of amine agents can increase the abundance of nitrifying and denitrifying functional bacteria while affecting the growth of species that have competitive or synergistic relationships. Excessive foaming agents can enhance the activity and reproductive ability of aerobic bacteria, promoting the increase of relevant microbial abundance. Acid-base regulators can selectively promote the increase of acidophilic or alkaliphilic microbial abundance while being unfavorable to the growth of other species. This information makes it possible to regulate the impact of microbial communities by changing the types and ratios of agents.

2.5 Interaction of microorganisms with coal

Indigenous microorganisms in the coal flotation system can adsorb onto the coal surface through interactions with the surface of coal, which can alter the physical and chemical properties of the coal surface and play a role in the coal flotation process, thereby affecting the efficiency of the coal flotation.

2.5.1 Microbial adsorption on the coal surface

Previous studies have shown that microorganisms can influence coal flotation. The adsorption of microorganisms on the coal surface is the first step in the microbial influence on coal flotation. The cellular structure of microorganisms is crucial in the adsorption process. The cell walls of microorganisms contain various components that influence their surface chemistry, charge, and behavior in flotation. Differences in microbial cell membrane proteins cause differences in biochemical properties. Microbial cell membrane polysaccharides determine the microbial surface charge, and the negative charge is determined by peptidoglycan. And the lipids in the microbial cell wall are responsible for the hydrophobic properties of microorganisms [73-75]. The microbial structure is complex, and when the microorganisms and extracellular polymeric substance (EPS) adsorb to the coal surface through a variety of mechanisms, they will change the physicochemical characteristics of the coal surface and affect the efficiency of coal flotation [76,77]. At present, many studies have proposed that microorganisms can adsorb to the coal surface through electrostatic interactions and hydrophobic interactions as shown in Fig.2.6.

In electrostatic interactions, several studies have found that the surface charge of microorganisms plays a very important role in the adsorption process with coal surface. The results of Ramos-Escobedo showed that *S. carnosus* can adsorb to the coal surface and act as a collector during coal flotation. The adsorption process is physical adsorption, and the electrostatic force plays a major role[40]. Further, Vijayalakshmi found the isoelectric point (IEP) around pH 4.8 of *Bacillus subtilis* and the IEP around pH 3 of coal. When the pH level of a solution is higher than the isoelectric point (IEP) of both bacteria and coal, the surfaces of both become negatively charged. This can result in reduced adhesion between the two surfaces. However, the addition of electrolytes can help improve the adsorption of *Bacillus subtilis* on the surface of coal [78]. In addition, the study of Yu also showed that *Rhodotorula mucilaginosa* can adsorb to the coal surface through electrostatic

interactions and change the charge properties on the coal surface [79]. Raichur pointed out that the Illinois #6 coal has a positive potential in the pH region below 6, and *Mycobacterium phlei* has a negative potential in the widest range of pH greater than 1.5. Therefore, the obvious electrostatic interactions operate between the *Mycobacterium phlei* and Illinois #6 coal in the pH range of 1.5 - 6.0 [80]. In summary, the surface charge of coal is a critical factor in coal flotation, which can affect the dispersion and condensation of the suspension, and affect the adsorption of collectors on the coal surface. However, microorganisms can introduce charged groups to the coal surface or modify the charge of existing functional groups, alter the surface charge, and affect the efficiency of coal flotation.

Another mechanism of adsorption is through hydrophobic interactions. The coal surface is known to be highly hydrophobic, which can result in the adsorption of hydrophobic microbial cells onto the coal surface. This is because that hydrophobic interactions between the coal and microbial cell surfaces are stronger than the repulsive forces between the microbial cell surface and water molecules. According to certain studies, *Mycobacterium phlei*'s surface contains cyclanes, alicyclic hydrocarbons, aromatic nuclei, and various types of organic functional and ionizing groups. Due to its high hydrophobicity, it can be selectively adsorbed to coal surfaces through hydrophobic interactions, which facilitates the flocculation of fine coal. [81-85]. In addition, the adsorption of *Bacillus polymyxa* on the surface of three different Indian coal samples was explored by Raichur. The results showed that in the adsorption experiment, both coal samples and *Bacillus polymyxa* surfaces are negatively charged, so it is difficult to achieve electrostatic adsorption, while the adsorption of *Bacillus polymyxa* and coal samples is completed by hydrophobic interactions [86].

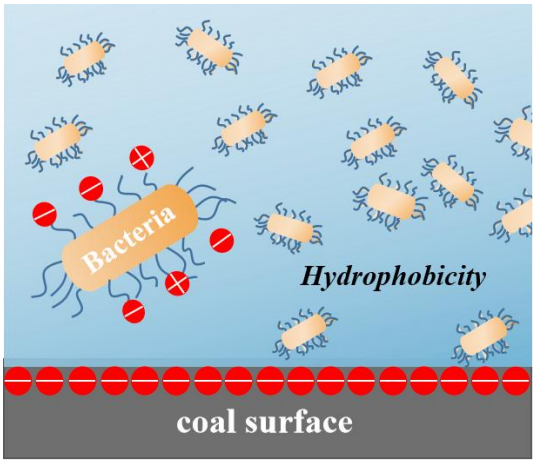

Fig.2. 6 Microbial adsorption on the coal surface

2.5.2 Effect of microorganisms on coal flotation

Microorganisms with different properties adsorb to the surface of coal and associated minerals through electrostatic attraction and hydrophobic interactions, and change the physicochemical properties of coal and associated minerals' surface, which further affects the coal flotation efficiency. The conclusion has been confirmed by many studies. Amini et al. used *Acidithiobacillus ferrooxidans* as an inhibitor of pyrite in coal flotation and reduced pyrite sulfur by 14% under flotation conditions with kerosene as a collector [38,87-89]. Abdel et al. used *Bacillus Subtills* as an inhibitor and removed more than 70% of the sulfur and ash content from the coal [90]. Ramos-Escobedo used *Staphylococcus carnosus* as a collector and achieved 90% coal recovery at pH 9, even higher than that of coal in conventional chemical flotation [40]. According to Fazaelipoor's results, *Pseudomonas aeruginosa* has a good ability to create froth by decreasing surface tension and maintaining its stability. It has been effectively used as a frother in coal flotation, resulting in 72-79% recoverable coal, 10-15.5% ash content, and a separation efficiency of 55-57.5% [91]. The results of Li found that *Escherichia coli* has the ability to stick to the surface of coal through hydrogen bonding. This, in turn, reduces the coal surface's hydrophobicity, leading to lower coal flotation efficiency. When added at a concentration of 5×10^9 cells/ml, the coal recovery rate was only 43% [92]. Therefore, there are differences in the effects of microorganisms with different characteristics on coal flotation efficiency. Some microorganisms are beneficial to the efficiency of coal flotation. They can improve the efficiency of coal flotation by interacting with associated minerals and acting as collectors and frothers. However, some microorganisms are not conducive to coal flotation efficiency. They can directly adsorb to the surface of coal, reduce the hydrophobicity of coal, and thus inhibit the efficiency of coal flotation.

2.6 Effect of indigenous microorganisms on coal flotation

Coal contains many indigenous microorganisms, which can also adsorb to the coal surface, change the physicochemical properties of the coal surface, and affect the coal flotation efficiency. Although relatively few studies have been conducted on the effects of indigenous microorganisms on coal flotation, several studies have shown that indigenous microorganisms have a more significant effect on coal flotation than exogenous microorganisms. Yu isolated a bacterium from Wuhai fertilizer coal and was identified as *Xanthomonas*, which showed a higher adsorption rate to raw coal

compared with the exogenous microorganism *M. phlei*. The adsorption rate of *Xanthomonas* on the coal surface could reach 84.18% at a coal slurry concentration of 50 g/L and a cell addition of 120 ppm. The fine coal adsorbed by *Xanthomonas* can form hydrophobic agglomerates, which can be more easily trapped by bubbles and improve flotation efficiency. *Xanthomonas* recognized receptors on the coal surface through specific adhesin proteins on the cell wall surface, which is selective and special. After adhesion, *Xanthomonas* firmly binds to the coal surface through extracellular polysaccharides, making the adhesion stronger and irreversible, while the bacterium uses the surface properties of the cell wall, extracellular material, or bacterial hairs to change the surface properties of the coal through electrostatic attraction or hydrophobic interaction [79]. The strain of *Sphingomonas* was isolated by Li from coking coal and found that the strain could degrade polyacrylamide with a degradation rate of 66.4% in 76 h. Contrast the biodegradation rate of polyacrylamide by exogenous microorganisms, the indigenous microorganism has a high biodegradation rate. As shown in Fig.2.7, polyacrylamide can enhance the settling rate of coal slurry. However, *Sphingomonas* produces extracellular enzymes that can degrade polyacrylamide by hydrolyzing and oxidizing its amide group and main carbon chain. This biodegradation process leads to the formation of smaller molecular weight organic products, which ultimately inhibit the settling rate of coal slurry [72]. Wang successfully isolated *Bacillus* from coking coal and investigated the effect of *Bacillus* on coal flotation. The results showed that *Bacillus* is hydrophilic and adsorbs onto the coal surface, which increases the number of oxygen-containing functional groups on the coal surface and decreases the hydrophobicity of the coal surface, and the coal flotation yield decreased by 10% when *Bacillus* was added at 10^8 cells/g [93]. Based on the research results, the indigenous microorganisms in coal can promote or inhibit the coal flotation efficiency. Indigenous microorganisms can affect the coal flotation efficiency by adsorption to the coal surface, changing the physicochemical properties of the coal surface, and can also affect the coal flotation efficiency through the interaction with agents.

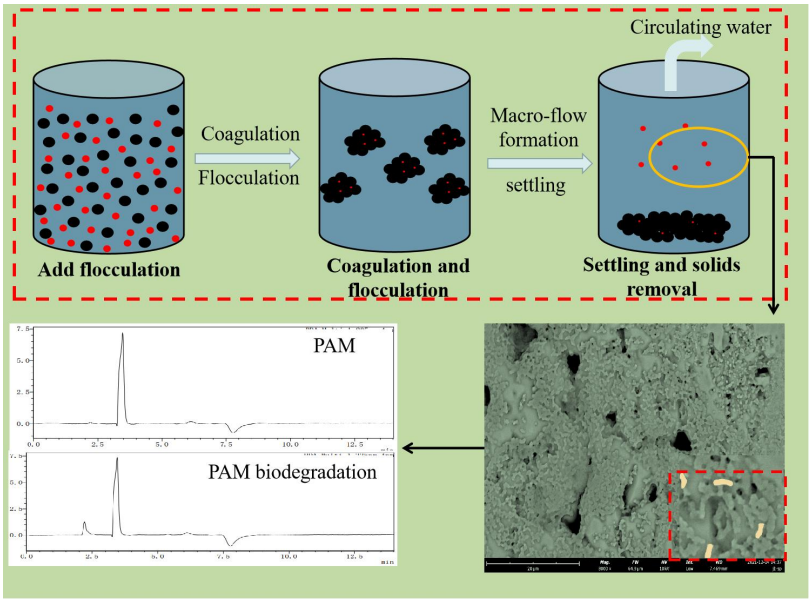

Fig.2. 7 Sphingomonas isolated from raw coal biodegradable polyacrylamide

2.7 Conclusion

During the coal formation process, microorganisms can promote organic matter degradation and drive coal conversion. These indigenous microorganisms coexist with coal and can enter the coal flotation system during coal processing, affecting coal flotation efficiency. Microorganisms have different physicochemical properties due to differences in cell wall composition, which affect the coal surface physicochemical properties by adsorption on the coal surface, thus affecting the coal flotation efficiency. Moreover, the indigenous microorganisms in the coal flotation system can also indirectly affect coal flotation efficiency through interactions with flotation reagents. Therefore, indigenous microorganisms play an important role in the coal flotation process.

Nowadays, global environmental pollution is serious, and the efficient and clean utilization of coal resources is highly valued. Coal flotation technology is the key to the clean utilization of coal. Indeed, indigenous microorganisms as an integral part of coal flotation should be given enhanced attention. In the future, more research should be focused on systematically investigating the composition, community structure, correlations with environmental factors, and the impact of microorganisms in coal flotation systems. A new path for improving coal flotation efficiency from the perspective of microbial management.

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Chapter III. Sources and succession of microorganisms in industrial coal flotation system

3.1 Introduction

Coal flotation can recover coal (< 0.5 mm) and increase economic efficiency based on differences in surface properties between coal and inorganic minerals [1-2]. The flotation reagents play a significant role in the flotation process [3,4]. Some studies have pointed out that microorganisms or extracellular polymeric substances (EPS) could act as surface modifiers, depressants, collectors, frothers, or dispersants in coal flotation [5-8]. Because microorganisms could rapidly adsorb to mineral surfaces through electrostatic interaction and hydrophobicity. During the adsorption process, molecularly mediated binding and microbially EPS formed from polysaccharides, proteins, nucleic acids, and lipids continuously affected mineral flotation behavior [9-14]. Fazaelipoor et al. reported that *Pseudomonas aeruginosa* had good froth ability in terms of reducing surface tension and maintaining froth stability. In addition, it had been successfully used as a frother for coal flotation with 72-79% combustible recovery, 10-15.5% ash content, and 55-57.5% separation efficiency [5]. Chen demonstrated that *Rhodotorula mucilaginosa* could be selectively adhered to the surface of fine coal through electrostatic interaction and hydrophobic interaction to enhance the hydrophobicity of the coal surface and improve the coal flotation efficiency [15]. And G.T. Ramos-Escobedo showed that the recovery of fine coal could be increased by more than 40% by using *Staphylococcus carnosus*, which had hydrophobic properties and changed the surface charge of coal by excreting some exopolymers [16]. Therefore, the interaction between microorganisms and the coal surface can affect the coal flotation efficiency.

In biosurfactants, almost all microorganisms are external microorganisms. In fact, the flotation system contains internal microorganisms. Päivi Kinnunen pointed out that microorganisms could be introduced into the flotation system from the intake water and ore material [17]. During the coal flotation process, raw coal in the coal seams is used as the material, and mine water as the intake water. As microorganisms participate in coal formation, a variety of microorganisms with high content have been found in underground coal seams [18]. Magnabosco et al. demonstrated that

microorganisms were observed at different depths in underground mineral layers, including coal, and the number of cells could reach up to 10^6 cells/g [19]. Yang et al. explored the bacteria in coal seams and found that *Oceanospirillales* and *Pseudomonadales* were dominant bacteria [20]. In the process of coal mining, the water entering the shaft, roadway and working face is mine water. IIunga Kamika et al. proposed that a total of 6 phyla with 10 classes were identified in the mine water. In general, Proteobacteria (58.33%) and Cyanobacteria (36.25%) were the most abundant phyla [21]. In addition, Florentino et al. found that families in the class Bacteroidia, such as Porphyromonadaceae, Prolixibacteraceae, and GU454901 were dominant in coal mine water [22]. Ganiyu et al. explained that the bacterial community spanned 10 phyla in coal mine water, including Pseudomonadaceae, Sphingomonadaceae, and Anaeromyxobacteracea [23]. Therefore, it can be reasonably assumed that microorganisms in raw coal and mine water in coal seams can enter the coal flotation system with the coal processing, and then adsorb on the coal surface as biosurfactants to affect the coal flotation efficiency. However, previous studies have mostly focused on the effect of external microorganisms as biosurfactants on coal flotation, and the role of internal microorganisms has not been fully explored. The widespread use of high-throughput sequencing technology offers the possibility to explore the internal microorganisms in coal flotation system, which allows parallel sequence determination of a large number of nucleic acid molecules at a time to reveal the characteristics of the microbial community [24,25].

In this study, raw coal, mine water, and flotation feeding samples were collected from the coal seam and coal preparation plant in Shanxi Province, China. The 16S rDNA was used to investigate the composition and diversity of the microbial community in the samples and determined the sources of microorganisms in the coal flotation system. The sustained growth of strain QX4 in media containing organic reagents and the correlation between the microbial community and environmental factors in flotation feeding indicated the succession of the microbial community in the flotation system. In further, the predicted microbial community function and correlation network of environmental factors with the microbial function was analyzed to reveal the mechanism of microbial community succession in the flotation system.

3.2 Materials and methods

3.2.1 Samples

The raw coal, mine water, and flotation feeding (RC, MW, FF) were obtained from coal seams in Qinxin Coal Preparation Plant at Changzhi (36°57'N, 112°19'E), China (Fig.3.1). Three parallel samples were taken for each sample and labeled as RC_1, RC_2, RC_3, MW_1, MW_2, MW_3, FF_1, FF_2, and FF_3. The coal type was coking coal.

The pH and dissolved oxygen (DO) content of samples were measured by a portable pH meter and dissolved oxygen meter (AZ8403, CHN) on site and the temperature was 21.3 °C. The acid potassium dichromate oxidation method was used for the determination of chemical oxygen demand (COD) in the coal slurry. Nitrogen and phosphorus always play important roles in the shaping microbial community [26-29], which also exists in coal slurry. Therefore, total nitrogen (TN) was detected by the alkaline persulfate dissolving ultraviolet spectrophotometry method using the ultraviolet photo spectrometer (UV-5260, Unico, CHN) at the wavelengths of 220 and 275 nm, and total phosphorous (TP) was measured by the molybdenum antimony colorimetric method using the ultraviolet photo spectrometer at a wavelength of 700 nm [30].

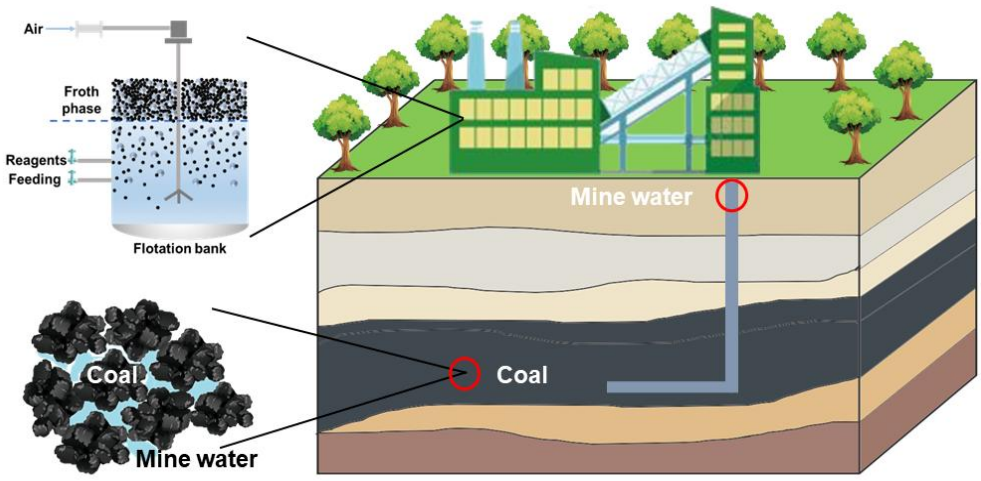

Fig.3. 1 The location of the sampling
(The red circles represent the sampling points)

3.2.2 DNA extraction, PCR amplification, sequencing, and data processing

The total genomic DNA of the samples was extracted using a Power Soil DNA extraction kit following the manufacturer's instructions. Thermo NanoDrop One was used to quantify the extracted DNA. PCR primer pairs 515F and 806R targeting the V4 hypervariable region of the 16S rDNA gene were used for DNA amplification. The PCR reaction mixture consists of 2x Premix Taq (25 μl), Primer-F (10 μM) (1 μl), Primer-R (10 μM) (1 μl), DNA (50 ng), Nuclease-free water (50 μl). PCR amplification cycling conditions were as follows: initial denaturation at 94 ℃ for 5 min, followed by 30 cycles of denaturing at 94 ℃ for 30 s, annealing at 52 ℃ for 30 s, and extension at 72 ℃ for 30 s, and single extension at 72 ℃ for 10 min, and end at 4 ℃ (BioRad S1000, Bio-Rad Laboratory, CA). PCR products were detected on 1% agarose gels, and concentration was determined with Gene Tools Analysis Software (Version 4.03.05.0, SynGene). The PCR mixed products were recovered by E.Z.N.A ® Gel Extraction Kit (Omega, USA) and eluted with TE buffer. The library construction was performed according to the standard procedure of NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, USA). The constructed amplicon library was subjected to PE250 sequencing using the Illumina Nova 6000 platform （Guangdong Magigene Biotechnology Co., Ltd. Guangzhou, China）

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina Nova 6000 PE250 platform (Guangdong Magigene Biotechnology Co.， Ltd. Guangzhou， China). Raw FASTQ files were de-multiplexed using an in-house Perl script, and then quality-filtered and merged by FLASH version 0.14.1. UPARSE with a 97% sequence similarity level was used to group the optimized sequences into operational taxonomic units (OTUs). The most abundant sequence for each OTU was selected as a representative. Finally, QIIME2 was used to generate the OTU table based on 97% sequence similarity.

The strain was identified by 16S rDNA, and the phylogenetic tree of strain was constructed by the neighbor-joining method using the MEGA software. The vegan package in R (version 4.1.1) was used to calculate the alpha diversity index, and the Venn diagram (version 1.7.1) package was used to analyze and visualize community differences between groups. QIIME software was used to calculate the distance between samples by the bray-Curtis method, and the beta diversity index was analyzed by Principal Coordinate analysis in R (version 4.1.1). Redundancy analysis (https://www.bioincloud.tech) was applied to analyze the correlation between

environmental factors and microbial community. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2) was used to determine the microbial community function of samples. PICRUSt2 can predict functional profiles using community-based 16S rDNA data. Correlation network analysis was performed using the OmicStudio tools at https://www.omicstudio.cn/tool.

3.2.3 Effect of organic reagents in coal flotation on microbial growth

Luria-Bertani (LB) medium was used to isolate bacteria from raw coal and to study the growth of bacteria in coal slurry containing organic reagents. LB was composed of 10 g/l tryptone, 5 g/l yeast extract, and 10 g/l NaCl. The bacteria were isolated at 32℃ on a rotary shaker for 130 rpm. The 3 ml coal slurry and 100 ml LB were mixed in a 250 ml conical flask and stirred for 48 h at 32℃ on a rotary shaker at 130 rpm to enrich bacteria in the coal slurry. Then the bacterial solution (5 mL) was inoculated into solid LB for culture to obtain the isolated strain QX4. To study the effect of organic reagents on bacterial growth, seven 250 ml conical flasks were set up, 200 ml LB culture solution was added to each conical flask, 10 ml of strain QX4 was inoculated, 120 mg/l, 240 mg/l, 480 mg/l kerosene was added to three conical flasks, 120 mg/l, 240 mg/l, 480 mg/l Methyl Isobutyl Carbinol (MIBC) was added to the other three conical flasks, and one conical flask was used as a blank sample. All samples were incubated at 32℃ and 130 rpm, and OD600 values (GENESYS 150, Thermo Scientific) were measured periodically. Meanwhile, the growth tests of strain QX4 in coal slurry containing different organic reagents were further investigated. Five 250 ml conical flasks were added with 250 ml LB medium and 10 ml strain QX4, and four of them were added with 25 g coal. In four conical flasks containing coal, one was added with 480 mg/l kerosene, one with 480 mg/l MIBC, one with 480 mg/l kerosene and 480 mg/l MIBC, and the last one was used as a blank control. The growth of strain QX4 was compared after all conical flasks were set at 32℃ and 130 rpm for 30 h.

3.3 Results and discussion

3.3.1 Sources of microorganisms in coal flotation system

3.3.1.1 Number of OTU

OTU is an artificial taxonomic grouping mark (phylum, species, genus, etc.) [31,32]. A group is an OTU through the classification operation [33]. In this study, bioinformation statistical analysis was carried out on more than 97% of OTU of a similar level. Fig.3.2(a) shows that the number of OTU treated by flotation feeding, mine water, and raw coal samples is 8058, 10231, 9733, and Fig.3.2(b) shows that the same OTU number of three samples is 2181, among which the overlap number of raw coal and mine water samples is the largest, which is 3388. The microbial community of raw coal and mine water is highly similar. Because mine water is a source of water for coal seams during coal mining, the microbial community of raw coal will naturally enter mine water.

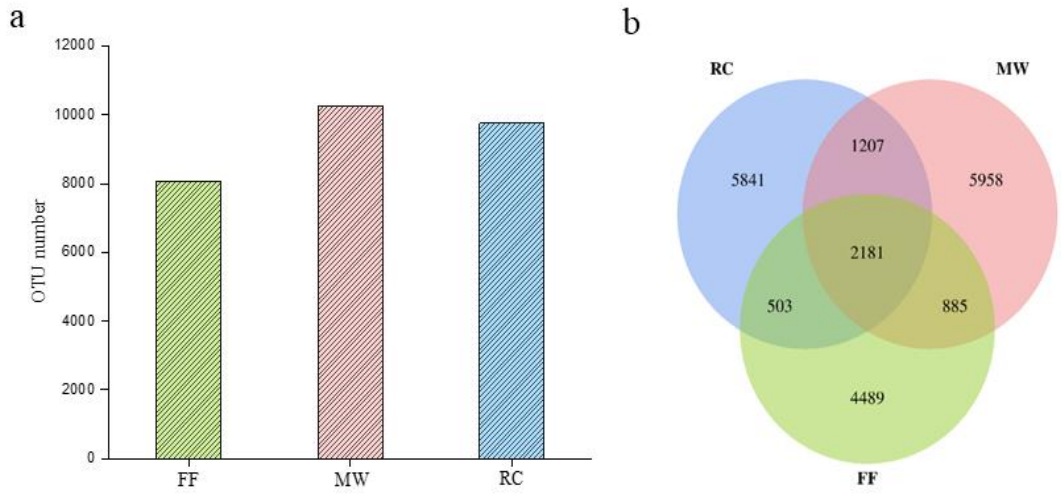

Fig.3. 2 OTU number of microorganisms in different samples
(a.Total OTU number in different samples; b. Venn diagram)

3.3.1.2 Microbial community composition

The microbial species of raw coal, mine water, and flotation feeding are the same at the phylum level which are all assigned to 34 phyla and 98 genera. The samples of raw coal and mine water were from the coal seam, indicating that the microorganisms in the coal slurry are from the coal seam and enter into the coal flotation system.

Fig.3.3(a) shows the top 10 microorganisms and their abundances at the phylum level. At the phylum level, the dominant microorganisms of flotation feeding, mine water, and raw coal are the same, including Proteobacteria, Acidobacteria, Actinobacteria, and Crenarchaeota, where Crenarchaeota is archaea and the others are bacteria. In Fig.3(b), the comparison of the top 10 microorganisms at the phylum level and the coal seam microbiome (CSMB) are shown. The CSMB is a reference OTU sequence set containing microbial data from 28 coal seams in three coalfields in eastern Australia [34]. The results of Fig.3.3(b) present that nine of the top 10 microorganisms are matched in the CSMB reference set, indicating that the microorganisms in the sample are universal. The results of Wei and Ma also indicated that coal contained a variety of microorganisms, including Actinobacteria, Bacteroidetes, Proteobacteria, Nitrospirae, Planctomycetes, and Acidobacteria [35,36]. The highest percentage of Proteobacteria is found in the microbial community composition of all samples. Priyanka found that the Proteobacteria could degrade the aromatics of organic matter in coal [37]. Coal is a heterogeneous and complex hydrocarbon made up of polycyclic aromatic hydrocarbons, alkylated benzenes, and long and short-chain n-alkanes, and despite its recalcitrance, microbial consortia have been shown to degrade it in a range of coal formations [38,39]. And the results show that the abundance of Proteobacteria, Bacteroidetes, and Nitrospirae in the raw coal and mine water samples is significantly higher than that in the flotation feeding. But the abundance of GAL15 in the raw coal and mine water samples is obviously lower than that in the flotation feeding.

Based on the microbial community composition of raw coal, mine water, and flotation feeding, it can be found that different samples contain the same microbial species, but the microbial abundance has changed, indicating that the microorganisms in flotation feeding come from the coal seam, but the microbial abundance has changed with environmental changes.

3.3.1.3 Microbial alpha diversity

Microbial alpha diversity can assess the degree of microbial diversity within a sample [40]. The abundance of OTU can determine the accuracy of the test results. The flatter the dilution curve, the more reliable the sequencing results [41]. The dilution curve in Fig.3.4(a) tends to flatten with the increase of sample sequences, indicating that most microorganisms in the samples fall within the range of sequencing. The Shannon index can reflect the diversity of microorganisms, and the

Chao1 index can reflect the richness of microorganisms [42,43]. In Fig.3.4(b), the Shannon index and Chao1 index values for different samples are relatively close, which indicates no significant differences in the abundance and diversity of the microbial community of flotation feeding, raw coal, and mine water samples. Therefore, it can be assumed that microorganisms in raw coal and mine water can enter the coal flotation system during coal preparation.

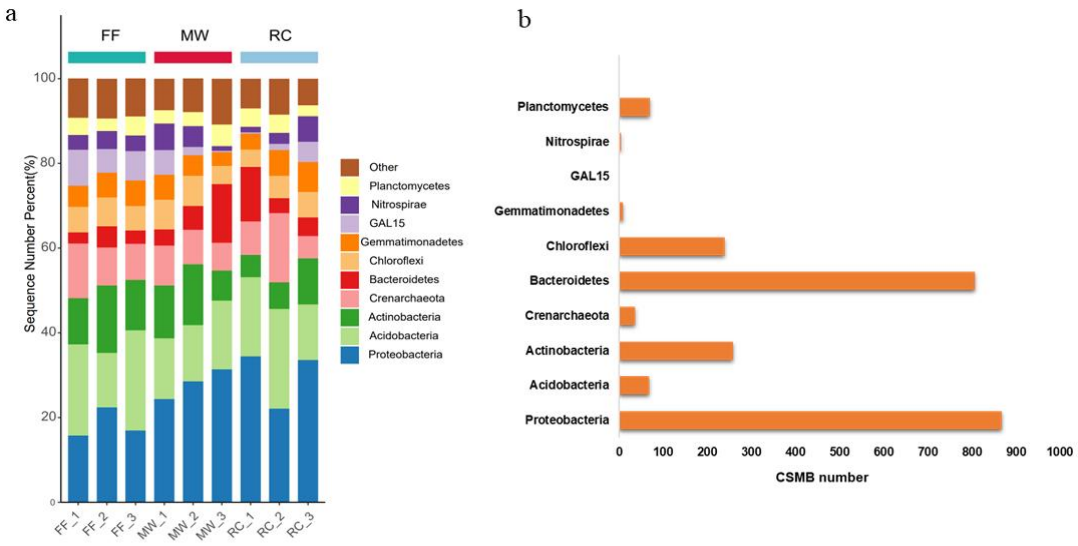

Fig.3. 3 Distribution of microorganisms in samples at phylum levels and comparison with CSMB(a. Distribution of microorganisms in samples at phylum levels;b. Comparison of microorganisms in samples with CSMB)

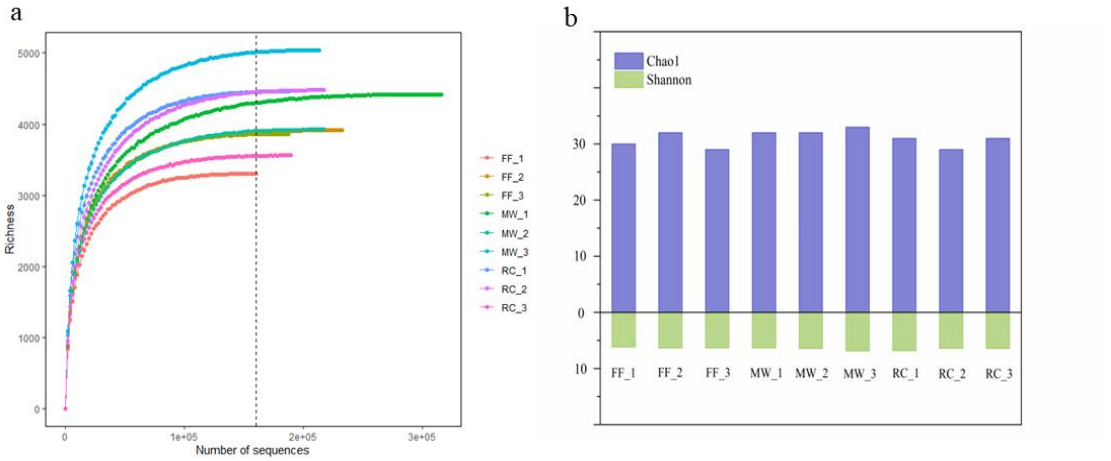

Fig.3. 4 The microbial alpha diversity index in different samples dilution curves; b. the diversity index of microorganisms)

In conclusion, the results of microbial community composition and microbial alpha diversity show that the microbial species in the raw coal, mine water, and

flotation feeding samples are the same and the microbial richness is close. Moreover, raw coal and mine water samples come from the coal seam, so it can be inferred that microorganisms in flotation feeding come from the coal seam.

3.3.2 Succession of microorganisms in coal flotation system

3.3.2.1 Microbial beta diversity

Microbial beta diversity allows the comparison of microbial diversity in different samples [40]. A principal coordinate analysis (PCoA) is a non-constrained data dimension reduction method that can be used to examine similarities and differences in microbial community composition.

The results of Fig.3.5 show that the PC1 and PC2 axes explain 33.7% and 20.75% of microbial community changes, respectively, and 54.45% of microbial community changes in total. The PC1 and PC2 axes did not show a significant difference between raw coal and mine water samples, so it is difficult to distinguish between them. Despite the proximity between the flotation feeding samples and the other two types of samples on the PC1 axes, they can be distinguished on the PC2 axis. Therefore, the microbial community structure of raw coal and mine water samples are similar but different from flotation feeding samples.

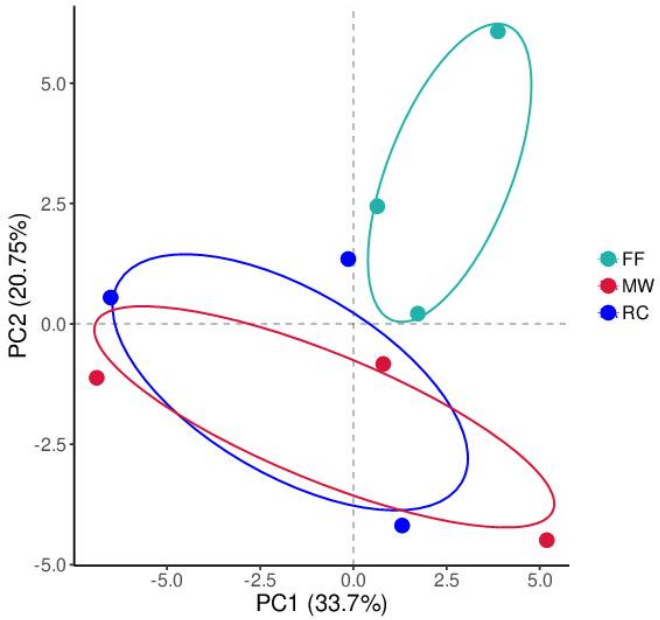

Fig.3. 5 The principal co-ordinates analysis of microorganisms based on OTU levels with different samples

3.3.2.2 Relationship between microbial community and environmental factors

The environment is the great factor determining how diverse microorganisms are, and microbial community is closely related to and restricted by environmental factors. To investigate the causes of changes in the microbial community, the changes in environmental factors in different samples were analyzed and the relationship between the microbial community and environmental factors was established. And the correlation network of environmental factors with microorganisms in the flotation feeding and the growth of indigenous microorganisms with changes in environmental factors were studied to explain the microbial community succession. Since the microbial community of raw coal and mine water samples is similar and the microbial community of flotation feeding samples is prominently different, the relationship between microbial community and environmental factors of raw coal and flotation feeding was compared.

The pH, dissolved oxygen (DO), chemical oxygen demand (COD), phosphorus content (TP), and nitrogen content (TN) of the raw coal and flotation feeding samples were measured to compare changes in environmental factors. From Fig.3.6(a) (b), it can be seen that compared with the environmental factors in the raw coal, the pH value of the flotation feeding is relatively lower, and the values of TN, and TP are slightly changed, while the values of COD and DO are significantly changed. In raw coal, the contents of COD and DO are 40 ~ 50 mg/L and 14 mg/L respectively, but in flotation feeding, the contents of COD and DO increase to 380 ~ 390 mg/L and 19 mg/L. COD can measure the content of organic substances in water. The higher the COD, the more serious the pollution of water by organic matter. Due to the coal preparation process being closed-circuit, organic reagents such as collectors, frothers, and flocculants in the flotation process will remain in the coal slurry, thereby increasing the organic matter content of the flotation feeding [44]. Therefore, compared with raw coal, the residual flotation reagents in flotation feeding lead to the change in environmental factors, especially the increase of COD and DO contents.

Canonical correspondence analysis (CCA) and Redundancy analysis (RDA) are sequential analyses that can be used to reflect the relationship between microbial communities and environmental factors. CCA and RDA are respectively correspondence analysis and principal component analysis. Detrended correspondence analysis (DCA) determines which method is used to analyze the relationship between the microbial community and environmental factors. In DCA results with axis length > 4.0, CCA is used, 3.0 - 4.0, RDA and CCA are both available, and < 3.0,

RDA is used. [45]. A correlation analysis was conducted based on the changes in environmental factors such as pH, DO, COD, TP, TN, and microbial community of raw coal and flotation feeding. For DCA analysis, based on the OTU table with a similar level of 97%, the axis length had a value of 0.75004, which was < 3.0, so RDA analysis was chosen. Fig.3.6(c) shows the RDA ($p < 0.05$) analysis results. The values of RDA1 and RDA2 in Fig.III.6(c) represent the proportion of changes in the microbial community explained by environmental factors. The RDA1 and RDA2 explain 55.16% and 26.54% of the changes in the microbial community, respectively, and the two ranking axes explain 81.70% of the changes in the microbial community together. The environmental factors and the microbial community of the samples are located in the same direction, indicating that the environmental factors are positively correlated with the changes in the microbial community, and the opposite is a negative correlation. Therefore, DO, COD, TP, and TN contribute to microbial community changes in flotation feeding and are negatively correlated with microbial community changes in raw coal, while pH is the opposite.

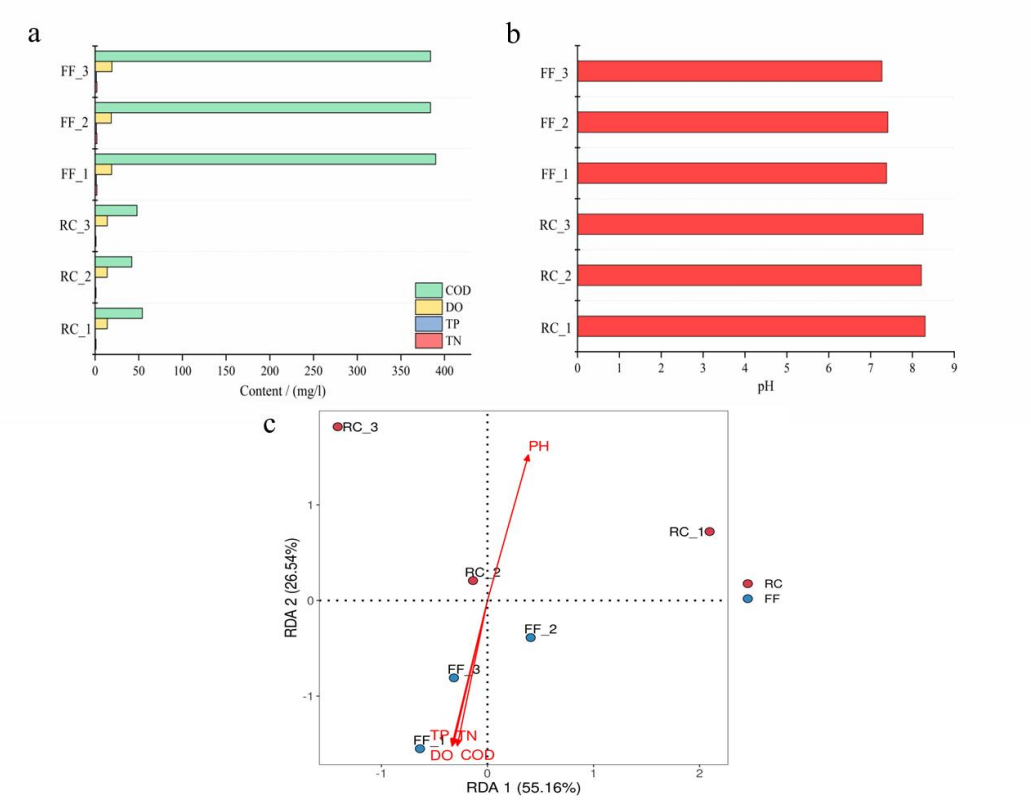

Fig.3. 6 Variations in environmental factors and RDA analysis
(a.Variations in environmental factors (COD, DO, TP, TN) in raw coal and flotation feeding; b. variations in pH in raw coal and flotation feeding; c. RDA analysis of the influence of environmental factors on microbial community of raw coal and flotation feeding)

In further, the correlation network of environmental factors with microorganisms in the flotation feeding was studied to explain the microbial community changes. In Fig.3.7, the gray dotted line represents a negative correlation, and the yellow solid line represents a positive correlation. The wider the line is, the stronger the correlation is. The results of Fig.3.7 show that COD and DO are important factors affecting the changes in the microbial community. COD and DO contents are significantly negatively correlated with Nitrospirae, Bacteroidetes, Proteobacteria, and Actinobacteria, and COD contents are positively correlated with GAL15. The phenomenon can also be found in the microbial community composition in Fig.3.3(a). Compared with raw coal, the abundance of Proteobacteria, Bacteroidetes, and Nitrospirae decrease, while the abundance of GAL15 increases obviously.

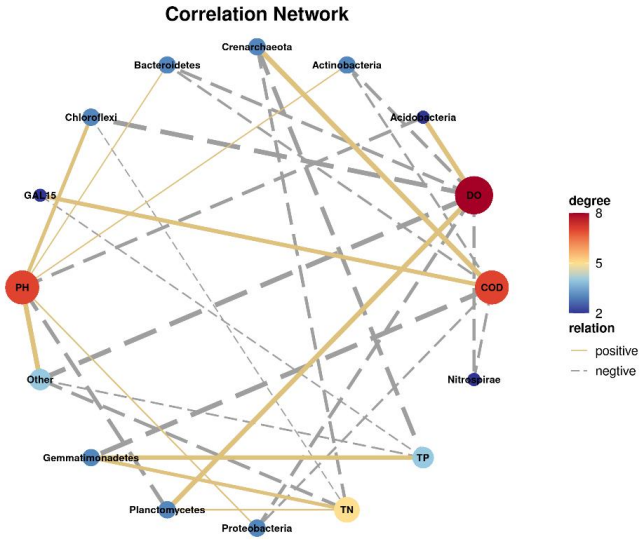

Fig.3. 7 Correlation network of environmental factors with microorganisms in flotation feeding

To confirm the effect of environmental factors on the growth of indigenous microorganisms in the coal flotation system, a strain named QX4 was isolated from raw coal to investigate the effect of organic reagents on the growth of QX4 in coal flotation. Fig.3.8(a) is the phylogenetic tree which was obtained by PCR amplification of the full length 16S rDNA sequence of strain QX4 and comparison with GenBank data. In Fig.3.8(a), the strain QX4 and Bacillus infantis strain SMC 4352-1 are grouped together. Kerosene and MIBC are commonly used as collector and frother reagents in coal flotation [46-48]. Therefore, in Fig.3.8(b), the effect of environmental factors on the growth of indigenous microorganisms was studied by adding different concentrations of kerosene and MIBC to the LB medium inoculated with QX4.

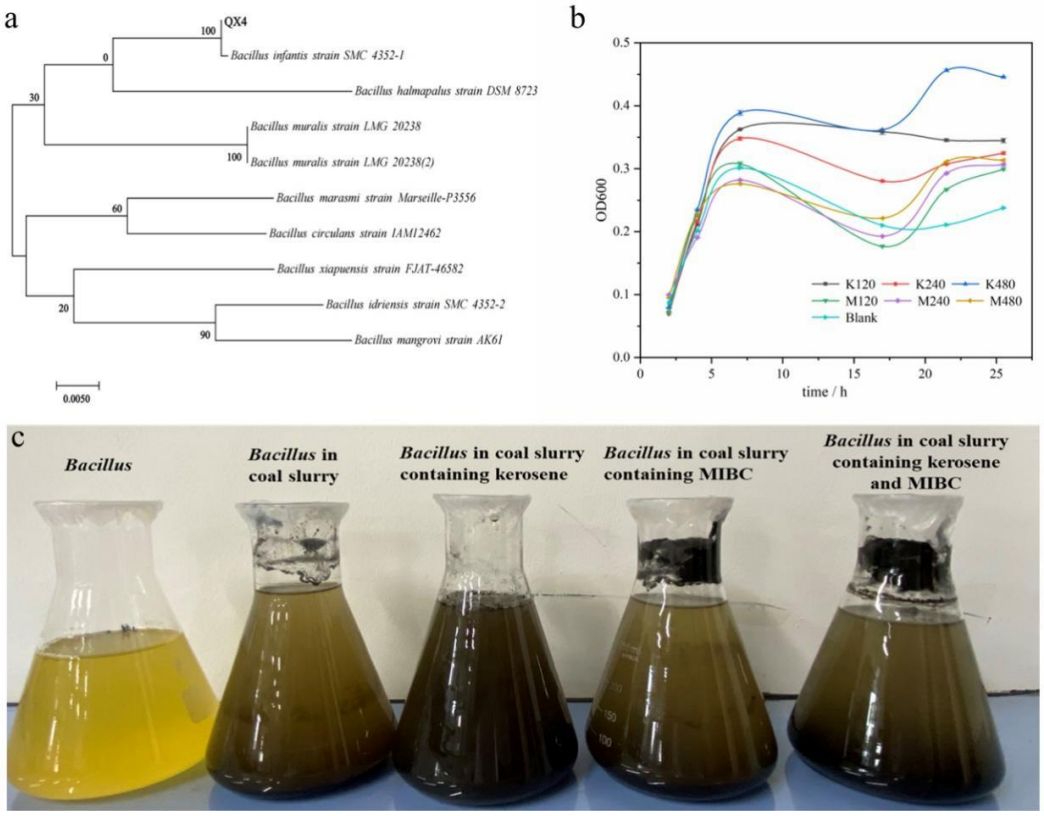

Fig.3. 8 Effect of organic reagents on the growth of Bacillus in coal flotation system (a. Phylogenetic tree of bacteria based on 16S rDNA sequence (The scale bar represents 0.005 substitutions per nucleotide site); b. Effect of kerosene and MIBC on the growth of Bacillus; c.The growth of Bacillus in coal slurry)

The results of Fig.3.8(b) show that strain QX4 undergoes logarithmic growth, stable stage, and decay stage, and the addition of kerosene and MIBC can change the growth of strain QX4. The addition of 120 mg/l, 240 mg/l, and 480 mg/l MIBC can inhibit the growth of strain QX4 within 7-17 h, but after 17 h, the promotion effect is prominent, and the higher the MIBC content, the more biomass of the strain QX4. The addition of kerosene can stimulate the growth of the strain QX4 at all stages. And when kerosene is added at 480 mg/l, the biomass of strain QX4 increases the most, followed by 120 mg/l and 240 mg/l. And, in 4 - 25.5 h, kerosene is better than MIBC in promoting the growth of strain QX4. Further, the effect of kerosene and MIBC on the growth of the strain QX4 was tested in the coal slurry system, and the results are shown in Fig.3.8(c). The presence of coal did not affect the growth of the strain QX4, and the strain QX4 can grow in the coal slurry system containing 480 mg/l kerosene, 480 mg/l MIBC, and 480 mg/l kerosene + 480 mg/l MIBC, respectively. The strain QX4, as an indigenous microorganism in coal, can grow normally in coal slurry containing collector and frother, and its biomass is obviously affected by organic

reagents. Therefore, the test results prove that the change of environmental factors in the coal flotation system can directly affect the growth of indigenous microorganisms, thus indirectly realizing the continuous succession of microorganisms in the coal flotation system.

In summary, the relationship between microbial community and environmental factors and the sustained growth of strain QX4 within the organic reagents medium can demonstrate that changes in environmental factors in the flotation feeding can stimulate the succession of the microbial community.

3.3.2.3 Relationship between microbial community and function

The microbial community function is the vital cause of changes in the microbial community. The correlation between microbial community and function was analyzed to further explore the causes of the microbial community succession. The function of microorganisms in raw coal and flotation feeding was investigated using PICRUSt2. Using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, the sequence data were compared [49]. Primary (KEGG L1) and secondary (KEGG L2) functional layers were obtained to predict gene abundance.

Fig.3.9(a) shows the KEGG L1 comparison results of microorganisms in raw coal and flotation feeding. The primary functions of microorganisms of raw coal and flotation feeding contain six types (average relative abundance > 1%): cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organic systems. And the main function of microorganisms in raw coal and flotation feeding is metabolism, accounting for about 73% of both.

To deeply investigate the relationship between microorganisms and metabolism functions of raw coal and flotation feeding, correlation network diagrams (Fig.3.9(b)(c)) between microorganisms in raw coal and flotation feeding and sub-functions (KEGG L2) of metabolism were created which reflect the relationship between microorganisms and sub-functions of metabolism. In Fig.3.9(b)(c), the red color represents a positive correlation, the green color represents a negative correlation, and the * represents a strong correlation ($p < 0.05$).

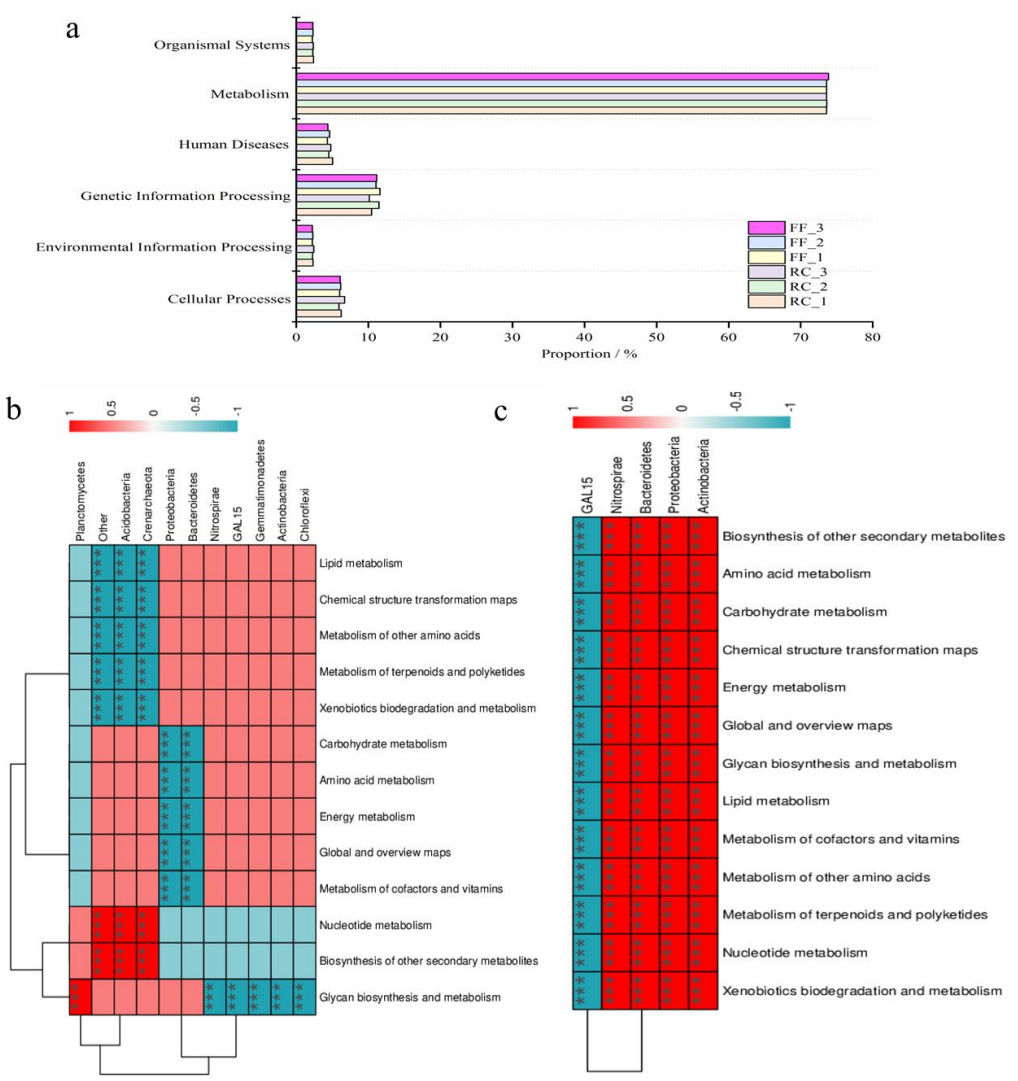

Fig.3. 9 Relationship between microbial community and function
(a. Predicted microbial community function in raw coal and flotation feeding
b. Correlation network between microorganisms and sub-functions of metabolism in raw coal; c. Correlation network between microorganisms and sub-functions of metabolism in flotation feeding)

Fig.3.9(b) is the correlation network between microorganisms and sub-functions of metabolism in raw coal. In Fig.3.9(b), the negative correlation between microorganisms and metabolism function in raw coal is significantly stronger than the positive correlation. And Planctomycotes has outstanding Glycan biosynthesis and metabolism function. Acidobacteria and Crenarchaeota can prominently promote the Nucleotide metabolism and Biosynthesis of other secondary metabolites. Acidobacteria, Crenarchaeota, Proteobacteria, and Bacteroidetes have significant inhibitory effects. Acidobacteria and Crenarchaeota can inhibit lipid metabolism,

chemical structure transformation maps, metabolism of other amino acids, metabolism of terpenoids and polyketides, and xenobiotics biodegradation and metabolism. And Proteobacteria and Bacteroidetes can inhibit carbohydrate metabolism, amino acid metabolism, energy metabolism, global and overview maps, and metabolism of cofactors and vitamins. Nitrospirae, GAL15, Gemmatimonadetes, Actinobacteria, and Chloroflexi all can inhibit glycan biosynthesis and metabolism. Fig.3.9(c) is the correlation network between microorganisms and sub-functions of metabolism in flotation feeding. In Fig.3.9(c), Nitrospirae, Bacteroidetes, Proteobacteria, and Actinobacteria show a strong positive correlation with all sub-functions of metabolism, while GAL15 is negatively correlated with the sub-functions of metabolism.

To deeply explore the succession mechanism of microbial community, the relationship between microbial community function and environmental factors in the flotation feeding was established in Fig.3.10 to reveal the essential reason for changing the microbial community and promoting microbial community succession. The results in Fig.3.10 show that the microbial function in flotation feeding is limited by COD and DO, which are negatively correlated with all sub-functions of metabolism, and the inhibition of COD is higher than DO. In Fig.3.9(c), the enhancement of sub-functions of metabolism can promote the growth of Nitrospirae, Bacteroidetes, Proteobacteria, and Actinobacteria, while inhibit the growth of GAL15. Therefore, compared with raw coal, the increase of COD and DO content in the flotation feeding will weaken the sub-functions of metabolism, further inhibit the reproduction of Nitrospirae, Bacteroidetes, Proteobacteria, and Actinobacteria, and promote the growth of GAL15. This conclusion is also confirmed by the results in Fig.3.3(a).

In conclusion, the increase of COD and DO content in flotation feeding can weaken the metabolism function of the microbial community, further affect the reproduction of specific microorganisms, and change the microbial community composition, so as to realize the succession of the microbial community in the coal flotation system. In the process of microbial community succession, the change of environmental factors is the external factor, the change of microbial function is the internal factor, and the microbial community composition is the result of succession.

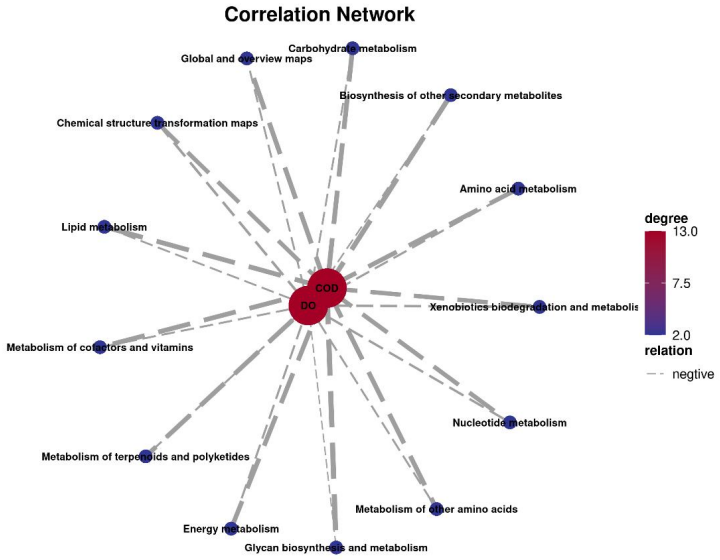

Fig.3. 10 Correlation network of environmental factors with microbial sub-functions of metabolism

3.4 Conclusions

In this study, the characterization of the microbial community in raw coal, mine water, and flotation feeding and the growth of microorganisms in environments containing organic reagents were analyzed to illustrate the sources and succession of microorganisms in the industrial coal flotation system. The results of microbial community composition, diversity of raw coal, mine water, and flotation feeding show that the microorganisms in the coal flotation system come from the coal seam. Additionally, the correlation network of environmental factors with microorganisms in the flotation feeding and the growth of strain QX4 with changes in environmental factors points to the succession of microbial community after the microorganisms enter the flotation system from raw coal as a result of environmental factor changes. Further, the change of microbial community function in flotation feeding and the correlation with environmental factors explain the reason for microbial community succession. The increase of COD and DO content in flotation feeding can weaken the metabolism function of the microbial community, further inhibit the reproduction of Nitrospirae, Bacteroidetes, Proteobacteria, and Actinobacteria, and promote the growth of GAL15, so as to change the microbial community composition and realize the succession of the microbial community in coal flotation system. In short, environmental factors in flotation systems modify microbial function, which changes microbial composition and leads to the succession of microbial community.

A variety of indigenous microorganisms in the raw coal and mine water can enter the flotation system and carry out succession, which affects coal flotation. Changes in coal flotation efficiency can significantly affect the calorific value of coal, which can cause changes in CO2 emissions and affect the air environment. However, this phenomenon had been ignored by practical production. Therefore, it is important to investigate the sources and succession of microorganisms in coal flotation systems for theoretical knowledge and production of coal preparation. Further, the indigenous microorganisms in the flotation systems can be separated, the advantages and disadvantages of microorganisms on coal flotation can be studied, the microorganisms that promote the efficiency of coal flotation can be protected, and the microorganisms that inhibit the efficiency of coal flotation can be removed, so that the control of microorganisms in the coal flotation system can be realized.

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Chapter IV. Impact of indigenous microorganisms on coal flotation

4.1 Introduction

Coal is one of the most important energy feedingstock, producing 27.2% of the global primary energy [1]. Coal cleaning production is particularly important for the utilization of coal, which can not only improve the efficiency of coal utilization but also reduce the emission of hazardous substances [2]. Given the characteristics of high ash [3] and fine particles [4] of raw coal in modern coal mining production, flotation technology is usually used in coal preparation plants to separate coal and gangue [5]. Various methods including equipment update [6] and process transformation [7] were used to improve coal recovery in the coal preparation plant, however, no attention has been paid on indigenous microorganisms as an influencing factor at the source.

Raw coal contains a large number of microorganisms, generally 10^4-10^5cells/ml in coal seam [8,9]. In flotation plants, the usage of circulation water accumulates kinds of inorganic ions and some organic agents [10-12], which provides vital conditions for the growth of microorganisms. The indigenous microorganisms in raw coal, not in the water, will boost due to the improvement of nutrient conditions [13], and due to long-term coexistence and domestication with coal, they will also have a greater impact on the surface properties of coal [14]. It has been reported that the number of microorganisms in the flotation system can reach to 10^8cells/ml [15]. It is well known that microorganisms grow by attachment, so the existence of microorganisms will change the surface properties of coal. Microbial flotation, based on the above theory, has been researched for several decades. A variety of bacteria have been proven to have positive or negative effects on flotation, depending on the microbial taxa and quantity, for example, *Thiobacillus ferrooxidans* can inhibit 59% of pyrite at a concentration of 4.4×10^8 cells/ml [16], the recovery of coal can reach 90% when using *Staphylococcus carnosus* with a concentration of 10^9 cells/ml [17], and an 83% flocculation rate was achieved under at the dosage of 80 mg/L *Azotobacter chroococcum* [18]. However, these microorganisms were exogenous and usually added into the flotation systems as dead biomass reagents of a single genus, the actual influence way of indigenous microorganisms cannot be reflected.

In this study, raw coal, flotation feeding, mine water, circulating water, clean coal, middling, and tailing were collected from a coal preparation plant in Shanxi Province, China. The on-site microbial community structure was analyzed, and the relationship between slurry microorganisms and coal ash was further established to for predicting the effect of indigenous microorganisms on coal flotation. To confirm the prediction, an indigenous microbial strain was isolated from the raw coal to investigate the actual effect of indigenous microorganism on coal flotation. And the impact mechanism of microorganism was explored using contact angle and X-ray photoelectron spectroscopy.

4.2 Materials and methods

4.2.1 Study site and sampling

The composition of microbial community in the flotation environment is the basis for further analysis, so raw coal (RC), flotation feeding (FF), mine water (MW), and circulating water (CW) were sampled, and a total of 12 samples were collected in three parallel samples at each location of coal preparation plant in Changzhi, Shanxi, China. The 27 coal slurry samples were filtrated through a 0.22 μm membrane. The obtained sediment was used for high-throughput sequencing analysis.

4.2.2 Raw coal characterization

The rank of the raw coal is coking coal, the composition of coal was determined by proximate and ultimate analysis. X-ray diffraction (D8 Advance) was used to detect the major gangue impurities in the raw coal. Vario EL cube (Elementat, GER) was used to determine the contents of C/H/N/S/O element.

4.2.3 High-throughput sequencing analysis

High-throughput sequencing, as an emerging research method, is able to construct genomic libraries to obtain comprehensive information on the microbial community structure and species composition in a given ecosystem, avoiding the shortcoming of traditional biological research that can only study individual cultureable species. By combining correlation analysis and other common bioinformatics tools, the study can also construct correlations between microorganisms and industrial indicators.

DNA of the filtered samples was extracted by the MagaBio DNA Kit

BSC48L1E-G (Bioer Technology, CHN). The concentration and purity of DNA were checked with NanoDrop One (Thermo Fisher Scientific, USA). The V4 region of bacteria 16S rDNA gene was subsequently amplified by polymerase chain reaction (PCR) using the primer pairs of 515F (3'-GTGCCAGCMGCCGCGGTAA-5') and 806R (3'-GGACTACHVGGGTWTCTAAT-5') with barcode. PCR was performed in triplicate in BioRad S1000 (Bio-Rad Laboratory, CA). The concentration and length of the PCR products was examined by 1% agarose gel electrophoresis, the fragments were controlled to an average size of about 290-310 bp. E.Z.N.A.® Gel Extraction Kit (Omega, USA) was used to recover PCR mixed products, and TE buffer was used to elution and recover the target DNA fragments. The database building was carried out according to the standard process of NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, USA). The amplicons were sequenced using a PE250 (Illumina Nova 6000, USA).

After obtaining the raw data, qimme2 was used to remove the primer sequences from the sequences. The quality numbers were selected for denoise according to the evaluation on the http://view.qiime2.org, OTUs were assigned according to 97% of sequence similarity, and then each OTU was compared with the Silva database to generate species information tables. Spearman correlation analysis was used to establish the relationship between bacteria and coal ash. Significance analysis of the data was made by IBM SPSS 26, and the results were visualized using ggplot2 package in R (version 4.1.1).

4.2.4 Isolation and identification of indigenous microorganism

To ensure the stability of the experiment, single microorganism rather than a microbial consortium was selected to explore the effects of microorganisms. The strain named QX4, was isolated from coal by LB medium (10g tryptone, 5 g yeast extract, 10 g NaCl, and 15 g agar dissolved in 1000ml ultrapure water). The morphology of microorganism was characterized by a CX22 microscope (JPN, Olympus).The strain was identified by 16S rDNA, and the result sequences were compared on NCBI (http://www.ncbi.nlm.nih.gov). According to the result of blast, the species with high similarity were selected to conduct a phylogenetic tree by MEGA7. The distribution of strain QX4 in coal seam was investigated by CSMB (coal seam microbiome). Generally, the concentration of microorganism is linearly related to the optical density (OD) at a certain wavelength range. In this study, the concentration of QX4 was determined by measuring the optical density of 600 nm via

Genesys 150 ultraviolet photospectrometer (ThermoFisher Scientific, U.S.) using Eq.(1). The linear relationship is only true when the OD_{600} is between 0.2 and 1.0.

$$Concentration = OD600 \times 22.47\ (R^2 = 0.99786)\ (1)$$

4.2.5 Flotation test

An orthogonal experiment is to use part of the level combination instead of all the level combinations to carry out the experiment, the purpose is to select the best level combination or the most important influencing factors. In addition, the response surface methods can also be constructed according to the orthogonal experiment results for prediction. The flotation performance of coal samples under different agents and microorganism concentrations was studied by central composite experiment.

The dosage of flotation agents is one of the most important factors in coal flotation. Collector dosage, frother dosage, and bacteria concentration were chosen as the three factors in the orthogonal test. And to ensure the stability of the experiment, the collector and frother were using kerosene and MIBC standard substances, which dosage level was referred to in the previous work [19]. In general, microorganisms in flotation plants derive from the raw coal itself (bacterial content is generally 10^4cells/ml [20-22]), and the water environment of flotation provides a more nutritious environment for microorganisms with a higher number of microorganisms, so the -1 level of microbial concentration was set at 10^5cells/ml. Then, the indigenous microorganisms would be gradually enriched due to aeration, reagent addition, and water circulation [23], it had been reported that the microbial content in a flotation environment could be up to 10^8 cells/ml [24], which was already a high concentration. Besides, microorganisms are greatly affected by seasonal changes and nutrient changes, with concentrations fluctuating by up to 2-3 orders of magnitude [25]. In conclusion, the concentrations of bacteria in industrial water in flotation plants generally range from 10^5 to 10^8 cells/ml, with upper and lower limits corresponding to -1 and +1 levels in orthogonal tests, respectively (as shown in Table 4.1).

A central composite experimental design, the most commonly used in response surface design methods, was used for the experimental design to facilitate the response surface analysis. With the number of factors at 3, the axial test point was taken as 1.682. To ensure the prediction accuracy, the central test point was subjected to three parallel experiments for a total of 17 trials.

Flotation tests were carried out in a XFG II 100 ml flotation tank (Tankuang, CN), and the process could be briefly described as follows: 1. 100 g/L slurries were configured with different concentrations of the bacterial solution, and the coal was stirred at an impeller speed of 1600 rpm for three minutes to ensure that the coal was fully mixed with the cells; 2. Different doses of agents were added, and the coal was also stirred at an impeller speed of 1600 rpm for three minutes to ensure that the coal was fully mixed with the agents; 3. The air was introduced at a rate of 0.4 m^3/h and the froth would be collected. The yield of flotation products, combustible recovery, and ash content of concentration were measured, and the water consumption of the flotation process was also recorded:

$$Yield = \frac{M_{dry}}{10} \times 100\% \#(2)$$

where M_{dry} is the dry weight of flotation products.

Table 4. 1 The experimental conditions of the orthogonal test. For microorganisms, the experimental conditions were calculated by linearizing the values using a logarithmic scale

Factor	Experimental conditions				
	-1.682	-1	0	+1	+1.682
Collector, mL/kg	0.064	0.3	0.65	1	1.24
Frother, mL/kg	0.0064	0.03	0.065	0.1	0.124
Microorganism, M	4	5	6.5	8	9

4.2.6 The characterization of surface properties

The hydrophobicity of coal plays a crucial role in the adhesion to air bubbles and affects floatability, which can be characterized by the contact angle [26]. The contact angle measurements in this study were accomplished by a JC2000C1 contact angle meter (Powereach, CN). After individual and joint action with collector (1 ml/kg) and microorganisms (10^8 cells/ml), the raw coal samples were extracted and fixed on the filter membrane and air-dried in a sterile chamber to remove water, and the surface contact angle of coal after different treatments was measured.

The surface chemical properties of coal are the key factors in the flotation effect [27]. EscaLab 250Xi X-ray photoelectron spectroscopy (ThermoFisher Scientific, U.S.) was used to detect the functional groups on the coal surface before and after

interaction with strain QX4, in an ultra-high vacuum system at 20°C, setting the energy to 100 eV and passing through the energy at 20 eV in steps of 0.05 eV. The binding energy was corrected by setting the C1s peak at 284.8 eV.

4.3 Results and discussion

4.3.1 Coal characterization

The ultimate analysis of the coal and the proximate analysis results based on moisture is given in Table 4.2, with the ash of 22.52%, fixed carbon of 40.54% and sulfur content of 0.306%. The results of the mineral composition analysis of the coal are shown in Fig.4.1. It can be seen that the main components of gangue impurities in this coal are quartz and kaolinite.

Table 4. 2 The proximate and ultimate analysis of coal

Parameter	Component	Value
Proximate analysis (%)	Inherent moisture	3.14
	Ash	22.52
	Volatile matter	33.77
	Fixed carbon	40.54
Ultimate analysis(%)	S	0.306
	N	0.96
	C	65.26
	H	3.32
	O	5.05

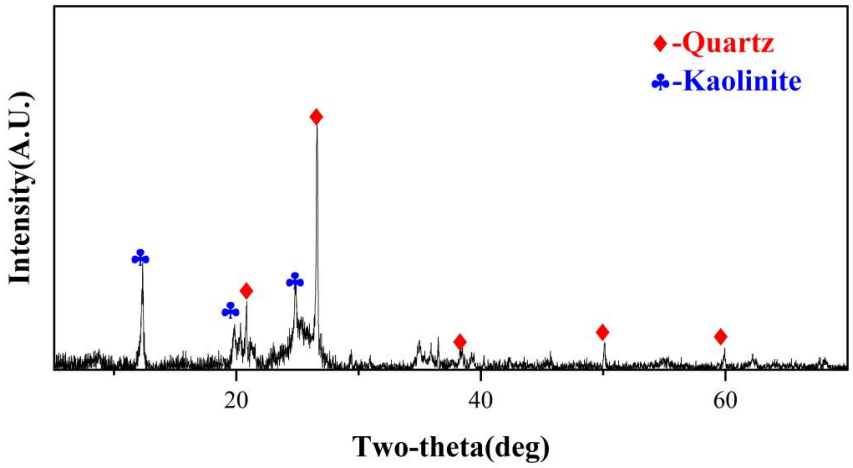

Fig.4. 1 The mineral composition of coal

4.3.2 Indigenous microbial analysis

4.3.2.1 Microbial composition

A total of 57760 sequences were obtained from the 27 samples. All sequences ranged from 200 to 386 bp in length, with an average length of 252 bp. The results of

microbial community composition as shown in Fig.4.2. A total of 56 bacterial phyla were identified in all samples. The microbial species in coal, process water, and slurry were almost the same, with the dominant microoganisms at the phylum level of Proteobacteria (15.99%-36.06%), Acidobacteria (10.80-24.07%) and Actinobacteria (5.30%-24.57%) (Fig.4.2(a)); at the genus level, *Nitrososphaera* (5.19%-17.98%), *Kaistobacter* (0.86%-4.78%), and *Arthrobacter* (0.28%-8.90%) (Fig.4.2(b)). The relative abundance of the various dominant species did not change much during flotation process, proving that most of indigenous bacteria survive and sustain.

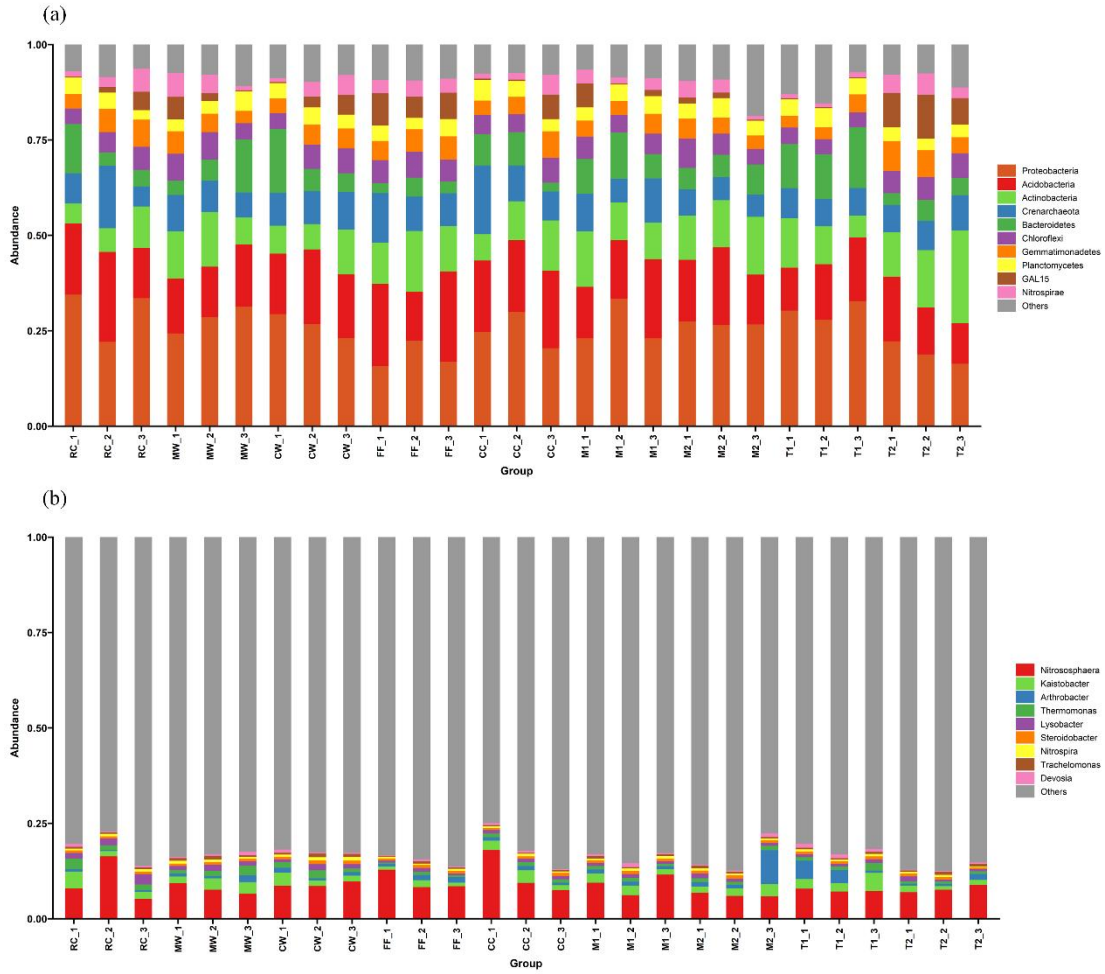

Fig.4. 2 Taxonomic composition of bacteria at the phylum level (a) and genus level(b) in coal, process water, and slurry of coal flotation system)

4.3.2.2 Correlation of microbial community and ash content

Spearman correlation analysis was used to establish the relationship between the top 200 microorganisms in abundance and typical coal flotation indicators (ash content) [28,29], and the results showed that 8 genera had a strong and significant correlation with ash content (Fig.4.3). In which *Acanthamoeba*, *Bacillus*, *Bdellovibrio*, *Nonomuraea*, *Pseudomonas*, *Solibacillus*, *Virgisporangium* showed a significantly positive correlation with ash content, indicating that these indigenous microorganisms may negatively affect coal flotation, as reflected by the increase in ash content. However, *Nitrososphaera*, the most abundant microorganism at the genus level, showed a significant negative correlation with ash content.

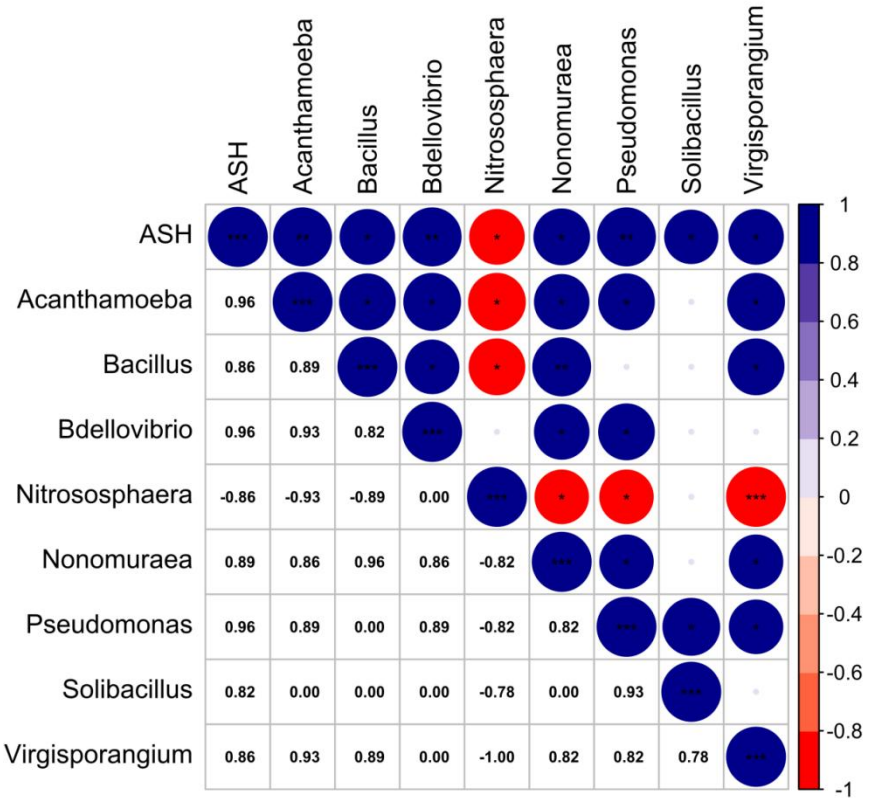

Fig.4. 3 Correlation of microorganisms and flotation indicators (ash content). The blue color indicates a positive correlation, while the red color denotes a negative correlation. The numbers in the lower left part represent the Spearman correlation coefficients. The symbols *, **, *** represents statistically significant level at the 0.05, 0.01, 0.001

4.3.3 Impact of indigenous microorganism on coal flotation

4.3.3.1 Isolation of indigenous microorganisms from coal

The strain named QX4 was isolated from coal, and the phylogenetic tree by MEGA7 showed that strain QX4 was more than 97% similar to several strains of *Bacillus* sp., so it was named *Bacillus* sp. QX4 (Fig.4.4(b)). The strain QX4 was rod-shaped with a 2-4 μm length (Fig.4.4(a)). The distribution of *bacillus* in coal seams was investigated by CSMB (coal seam microbiome), and the result was shown in Fig.1(c). *Bacillus* was found in many coal seams and has a worldwide distribution [30], so it is representative to select *Bacillus* to explore the impact of indigenous microorganisms on flotation.

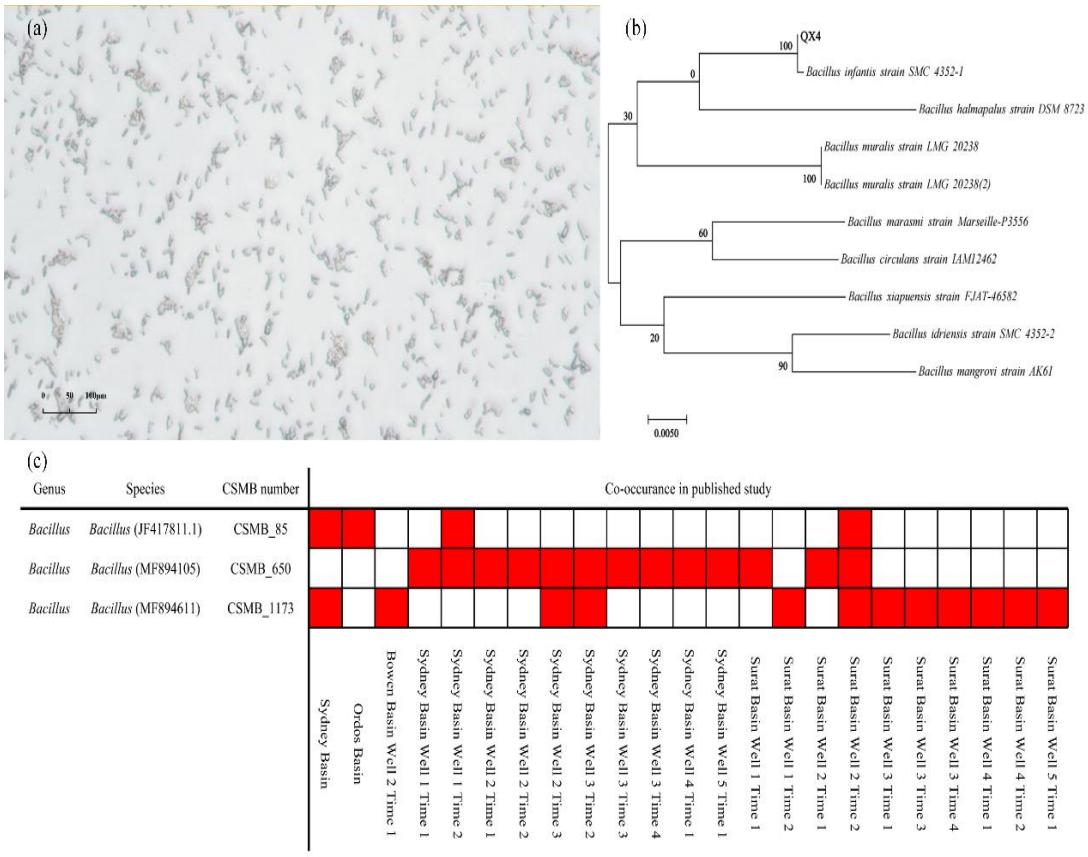

Fig.4. 4 (a) the microscope image of QX4; (b) the phylogenetic tree of QX4; (c) the distribution of Bacillus in CSMB

4.3.3.2 Impact of indigenous microorganisms on coal flotation yields

Fig.4.5 shows the main effects of the three factors, the interaction between

microorganisms and agents, and the correlation coefficient values between the three factors and the indicator. The dosage of the collector (Fig.4.5(a)) and frother (Fig.4.5(b)) showed a positive correlation trend with the flotation yield, consistent with common sense. A significant negative correlation occurred between the concentration of strain QX4 and the yield (Fig.4.5(c)) ($p=0.0367$), which meant that microorganism is harmful to coal flotation. Surprisingly, the microbial concentration was the most critical factor influencing the coal flotation yield (Fig.4.5(f)), even more than the influence of the collector. This result was beyond expectation and needed to be further explained in the context of characterization.

The interaction can be inspected in Fig.4.5(d) and Fig.4.5(e). Whether at the agent level of -1 and +1, the effect of microorganism on the yield was basically the same (the red line and blue line did not cross), which meant that there was no obvious interaction between strain QX4 and agents on the yield, the influence of microorganism would not change with the dosage changes of the agents. This is a disappointing result, which means that it is impossible to regulate the influence of microorganisms by changing the agents dosage in actual production, but it is still a feasible way to control the growth of microorganisms by changing the kind of microorganisms, which of course requires more in-depth research.

Response surface analysis was used to predict the optimum point (saddle point) of coal flotation in the range of pharmaceutical dosage and microbial concentration in actual flotation as shown inf Fig.4.6. However, there was no curved surface in the response surface constructed by strain QX4 and reagent factors, and saddle points could not be found. The prediction of the response surface indicates that there is no optimum condition for coal flotation yield in the range of microorganisms in the actual coal preparation plant. The above results mean that in actual coal preparation plants, the existence of microorganisms may only have a negative effect on the yield. In addition, the reduction in yield due solely to the increase in microorganism content was about 10% - 15%. However, from the collector level of -1 to +1, the yield only increased by 7%. If the results are reflected in the actual production, it can be predicted that the coal preparation plant will blindly increase the dosage of agents in order to improve the yield, which will lead to an increase in operating costs and waste of agents.

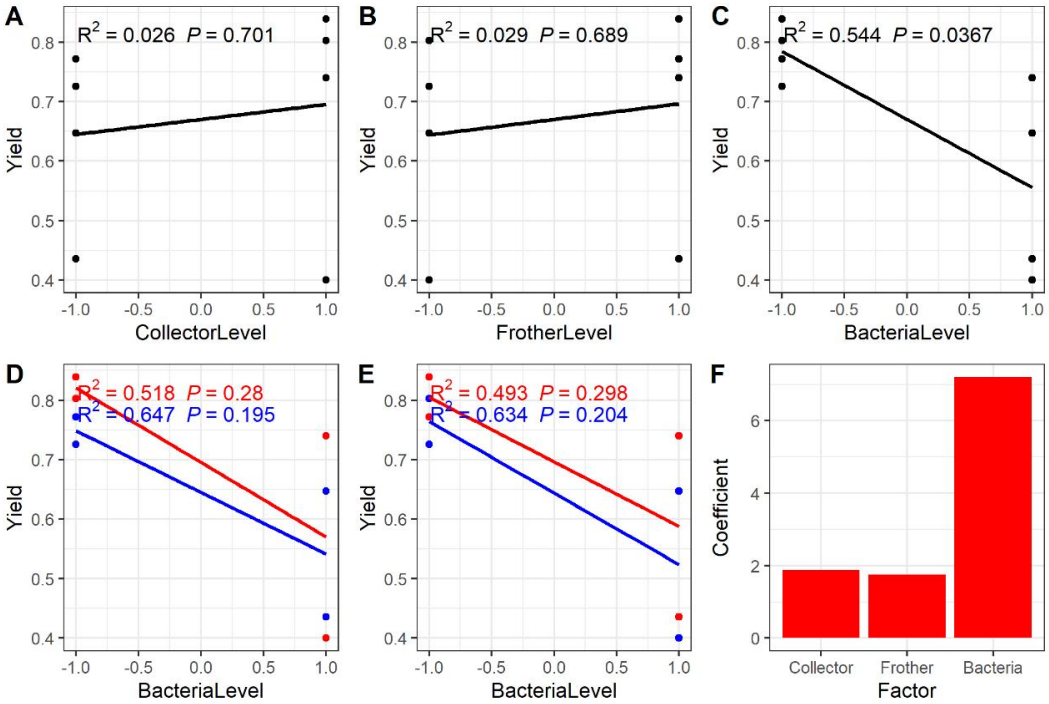

Fig.4. 5 The effect of different factors on the yield based on -1 and +1 level
((a) the main effect of the collector, (b) the main effect of frother, (c) the main effect of bacteria, (d) the interaction between bacteria and collector (blue line represents -1 level of collector and red line represent +1 level), (e) the interaction between bacteria and collector, (f) absolute values of the correlation coefficient between the three factors and the indicator obtained by response surface analysis in Design Expert)

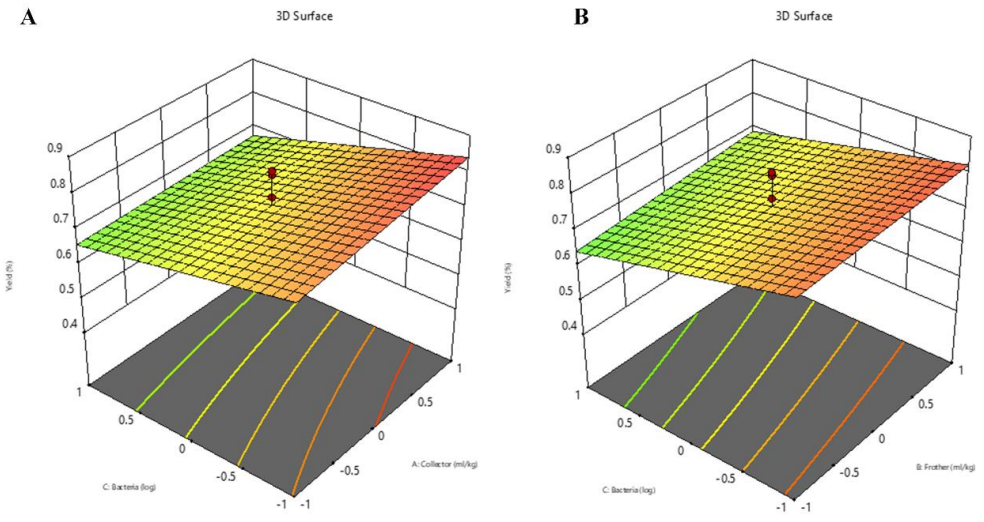

Fig.4. 6 Response surface of the yield as a function of reagent and microorganisms construct by designexpert
((a) collector and microorganisms; (b) frother and microorganisms)

4.3.4 The impact of indigenous microorganism on the surface properties of coal

The results of the contact angle (Fig.4.7) showed that the contact angle of the coal used in the experiment was 79.5°, which is inherently hydrophobic, and after the addition of the collector, the contact angle of the coal increased to 116.99°. The strain QX4 used in the experiment was hydrophilic with a contact angle of 37°, so the contact with it led to an increase in coal's hydrophilicity. However, it was rather surprising that the effect of microorganism on the hydrophobicity of coal overrides that of the collector at a concentration of 10^8 cells/ml, and even the hydrophobicity of this sample was not as good as that of the original coal. It is speculated that coal particles were difficulty binding to the collector molecules due to the attachment of microorganism. Besides, the increase of hydrophilicity will prolong the adhesion time of coal and froth, which is not conducive to the flotation recovery [31]. The above results indicated that the presence of a high concentration of microorganisms in industrial production might lead to lower yield and increased agents consumption.

As shown in Fig.4.8, the atomic carbon content of untreated coal was not significantly different from that of the samples treated by microorganisms, but the atomic oxygen content and the relative proportion of oxygen of the samples treated by strain QX4 were significantly increased, which was due to a large number of oxygen-containing functional groups caused by microorganisms [32-34]. The C1s spectrum of coal is shown in Fig.4.8. The peaks at 284.8 eV, 286 eV, and 287 eV correspond to the following groups: C-C/C-H, C-O, and C=O. There were few oxygen-containing peaks in coal, only 5.78% in C-O and 2.22% in C=O, the vast majority of C1s belong to C-C/C-H, which derived from the adsorption of aromatic compounds and alkanes on coal surface [35]. After treatment with strain QX4, the oxygen content peak of coal increased significantly, mainly in the increase of C=O from 2.22% to 9.61%. The source of these carbonyl groups may be the extracellular secretion of microorganism [36,37]. The above results showed that the oxygen-containing functional groups on the coal surface increased due to the existence of microorganism, which was the reason for the decrease in coal hydrophobicity.

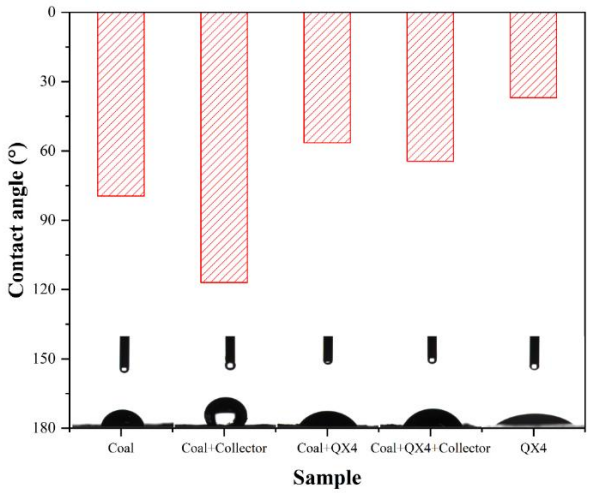

Fig.4. 7 Contact angles of coal treated by QX4 or collector

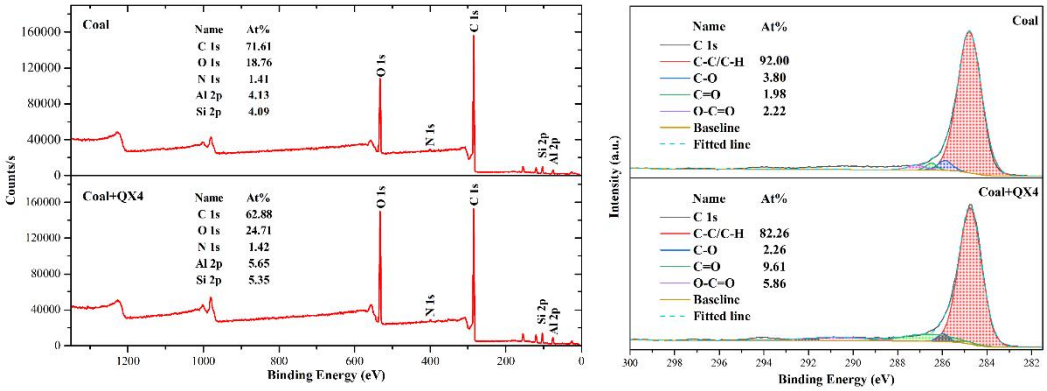

Fig.4. 8 XPS wide scan result and C1s scan results for Coal and Coal + QX4

4.4 Conclusion

It is the first study to comprehensively investigate the microbial community structure and their potential impact on coal flotation in a coal preparation plant using high-throughput sequencing techniques. And an indigenous microorganism identified as *Bacillus* was isolated from coal for microbial flotation tests, and the impact of indigenous microorganism on coal flotation was analyzed in depth.

Coal slurry contains many kinds of indigenous microorganisms which sustain after entering into the flotation system and affect the ash content of coal direct or indirectly. The effect of strain QX4 on coal flotation yield showed that the microbial addition of 10^8cells/ml reduced the coal flotation yield by 15%. Further mechanisms resulting from contact angle and XPS showed that microorganisms affect coal

flotation by decreasing the hydrophobicity of the coal surface. This study broadened the understanding of the influence of indigenous microorganisms in the coal flotation process and was expected to provide a scientific basis for the makers of clean coal technology and policy.

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Chapter V. Impact of *Escherichia coli* on coal flotation

5.1 Introduction

Coal accounts for 30% of the global primary energy, and it generates 37% of electricity [1]. In the process of coal utilization, coal preparation can remove inorganic minerals, reduce coal ash and sulfur content. Therefore, coal preparation plays an important role in coal utilization, which can improve coal quality and reduce air pollution [2]. Coal flotation is an important coal preparation technology based on differences of surface properties between hydrophobic low-ash coal particles and hydrophilic high-ash [3]. Coal flotation is the highest water-consuming unit process in coal preparation where water mainly comes from rivers and mines [4]. The river and mine water contains a lot of bacterial spp. The typical bacterial number in river waters can reach 10^6 to 10^7 cells/ml [5]. Acidic mine waters were shown to contain higher diversity as microbial numbers reach 10^6 - 10^7 16S rRNA gene copies/ml [6]. *Escherichia coli* (*E.coli*) is a water-borne bacteria. In pure water bodies, the number of *E.coli* can reach 10^2 to 10^3 cells/ml [7]. But in typical untreated municipal wastewater or groundwater, due to the increase of TDS, Ca and Mg, *E.coli* concentration is at a magnitude of 10^5 cell/ml [8-11]. Since river and mine water is more complex than municipal wastewater or groundwater, higher amounts of *E.coil* are expected. Therefore, when river or mine water is used as the water source in the flotation system, a large amount of *E.coli* is also introduced. Furthermore, coal flotation with water recirculation can provide favorable conditions for *E.coli* growth, given that there is potential nutrition from reagent addition, appropriate oxygen levels and suitable temperature [12]. The accumulation of *E.coli* cells in the water recirculation is expected to result in a negative effect on flotation performance [13].

Some studies have explored the role of *E.coli* in the mineral flotation process. Wenying Liu studied the influence of *E.coli* on the flotation of chalcopyrite and found that the attachment of *E.coli* to the surface of chalcopyrite affected the attachment of the collector to the particles, resulting in a decrease in the hydrophobicity of the mineral surface, and ultimately reducing the recovery rate and flotation efficiency [14]. At the same time, Wenying Liu found that disrupted *E.coli* had a negative effect on copper as well as gold flotation [15]. Tao wu and Mohsen Farahat studied the adsorption of *E.coli* onto quartz and found that *E.coli* confer hydrophobic properties to quartz and the biotreated quartz was positively charged, so a large amount of the

collector was adsorbed and the recovery increased [16,17]. Due to the different effects of *E.coli* on minerals, *E.coli* can act as a collector for specific minerals, separating the target mineral from impurities. Mohsen Farahat's research found that using *E.coli* could separate quartz from a hematite–quartz mixture [18].

Compared with the study of *E.coli* on the flotation of minerals such as quartz and chalcopyrite, there are relatively few studies on coal flotation. In coal bioflotation, a number of studies focused on the use of bacteria as surface modifiers on coal flotation such as *Paenibacillus polymyxa*, *Rhodotorula mucilaginosa*, etc [19]. However, far less is known about the impact of water-borne bacteria on coal flotation. To understand this, we investigated the effect of *E.coli* concentration and pH on the recovery of coal flotation. The adsorption of *E.coli* on the coal surface was confirmed by Scanning electron microscopy (SEM) and adsorption mechanism was studied by Contact angle measurements, Zeta potential and Fourier transform infrared spectroscopy (FTIR).

5.2 Experimental

5.2.1 Coal sample

The coal sample was obtained from Taixi, China. The coal sample was crushed and wet ground with a ball mill. The -75 + 38 μm fraction was used for floatation tests, SEM, Contact angle and FTIR measurements. The -10 μm fraction was used for Zeta potential tests.

5.2.2 The bacteria strain

A pure culture of E.coli strain was cultured in Luria-Bertani (LB) medium (tryptone (10 g/l), yeast extract (5 g/l), NaCl (10 g/l)) and grown in flasks for 16 h at 130 rpm, 37 °C. Then the cells were harvested by Heraeus multifuge X1R centrifugation (Thermo Scientific) at 5000 r for 5 min. Finally the cell pellets were suspended in the sterilized distilled water after being washed three times. The bacterial cell concentration was measured by optical density (OD600) using an Orion Aquamate 8000 (Thermo Scientific), where 1 OD equals 1×10^9 cell/ml.

5.2.3 Flotation experiments

The RK/FGC35 laboratory flotation machine (Rock Machine Factory, Wuhan, China) was used in flotation tests. The flotation cell was 0.1 L. In each experiment, 4

g of coal particles and a known concentration of *E.coli* were conditioned in 80 ml of distilled water at a specific pH value for 15 min. The desired pH value was regulated by HCl (0.1 mol/l) and NaOH (0.1 mol/l). Then the frother Methyl Isobutyl Carbinol (MIBC) (200 g/t) was added to the flotation cell and stirred for 3 min at 1600 r/min. The flotation concentrates were filtered, dried at 60 °C in a dryer and weighed for analysis.

5.2.4 Zeta potential measurements

Malvern Zetasizer Zeta-Nano (Malvern, UK) was used to test the Zeta potential of the coal and *E.coli* at the same ionic strength (10^3 M KNO_3). Zeta potential was measured by the combination of electrophoresis and laser Doppler velocimetry. In order to measure zeta potential of coal after interaction with *E.coli*, the sample was first conditioned with *E.coli* under the required conditions (pH, adsorption time and cell concentration).

5.2.5 FTIR measurements

Fourier transform infrared spectroscopy (FTIR) measurements of the coal and *E.coli* was conducted using a Nicolet FTIR-6700 spectrometer (Thermo USA). All measurements were conducted at room temperature (25 ± 1 ℃) and the background spectrum was obtained using KBr pellets. The coal sample was conditioned with *E.coli* under the required conditions (pH, adsorption time and cell concentration), dried in the air and used for measurement.

5.2.6 Scanning electron microscopy

SEM studies were carried out to observe *E.coli* attachment to the coal surface. After adsorption under suitable conditions (pH, adsorption time and cell concentration), air drying and coating with gold under vacuum using an ion coater, images were then acquired using a Phenom Prox (Phenom Scientific, Netherlands) scanning electron microscope.

5.2.7 Contact angle measurements

The contact angle measurements were carried out through a Contact angle detector (JC2000C1) using the captive bubble method. The coal was taken before and after interaction with the *E.coli* suspension at the required conditions (pH, adsorption

time and cell concentration), dried in the air. Then the contact angle measurements were carried out.

5.3 Results and discussion

5.3.1 Flotation

Fig.5.1 shows the effect of *E.coli* concentration on the flotation recovery of coal. The experiments were carried out at pH 6 in the presence of distilled water for a conditioning time of 15 min and a flotation time of 3 min. Due to the great hydrophobicity and high flotation recovery of coal, and in order to further study the effect of *E.coli* on coal surface without considering the effect between *E.coli* and the reagent, no collector was added in this test. In Fig.5.1, the flotation recovery of coal decreased as the concentration of *E. coil* increased. The recovery of coal could reach 84.25% in the absence of *E. coli* from the flotation process, but only 50.25% of the coal could be recovered when 5×10^9 cells/ml existed. The increase in cell concentration decreased the flotation recovery of coal. This result was similar to the effect of *E.coli* on the flotation of other minerals. Wenying Liu studied the quantitative relationship between the *E.coli* concentration and the flotation performance of chalcopyrite and pyrite， and found that chalcopyrite and pyrite recovery decreased with increasing bacterial concentration [12].

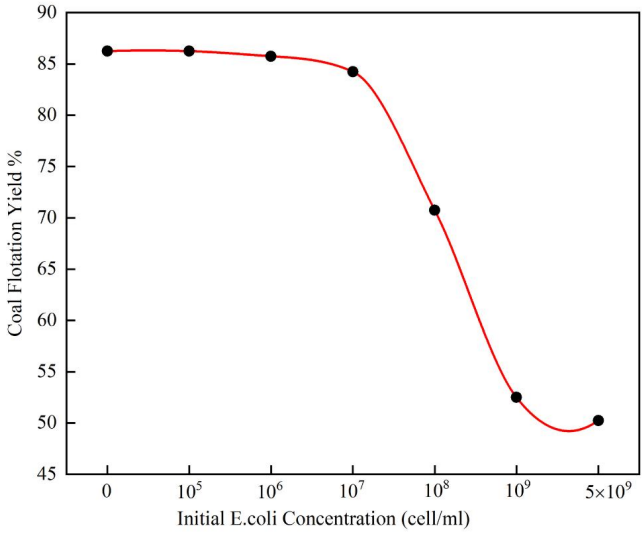

Fig.5. 1 Effect of *E.coli* concentration on flotation recovery of coal

In order to explore the effect of pH on coal bioflotation, bioflotation tests at different pH values were carried out, and the results are shown in Fig.5.2. Experiments were carried out at an *E.coli* concentration of 5 × 10^9 cells/ml in the presence of distilled water for a conditioning time of 15 min at different pH values and a flotation time of 3 min. The results showed that the pH value had little effect on the bioflotation of coal. When the pH = 2 - 8, the bioflotation recovery of coal was about 40%, when pH = 10, the bioflotation recovery of coal was 47%. Therefore, compared with pH = 2 - 8, the adverse effect of *E.coli* on coal flotation was relatively reduced when pH was 10, which may be due to the fact that the high pH is not conducive to the growth of *E.coli*.

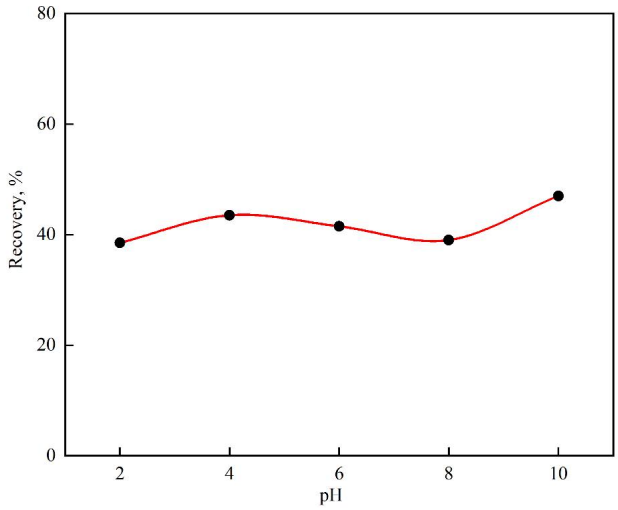

Fig.5. 2 Effect of pH on flotation recovery of coal with *E.coli* at concentration of 5 × 10^9 cells/ml

5.3.2 Scanning electron microscopy

The adsorption of *E.coli* on coal surface can be observed by SEM. Fig.5.3(a) shows a SEM image of *E.coli* cells. It revealed that *E.coli* is a rod-shaped bacteria of 3μm length. Fig.5.3(b) shows a SEM image of coal after biotreatment with 5 × 10^9 cells/ml of *E.coli* at pH 6. In Fig.5.3(b), the orange and green parts show the areas where coal surface is adsorbed and not adsorbed with *E.coli* respectively. It could be seen from Fig.5.3(b) that the coal surface was basically covered by *E.coli*. Generally, the bacterial adsorption is based on the electrical potential and hydrophobicity [20]. And the adsorption of bacteria starts with transportation, followed by contact and initial adhesion, firm attachment and surface colonization [21]. When bacteria was

adsorbed to the mineral surface, the electrokinetic and hydrophobicity properties of the mineral surface were subsequently changed, and that affected the flotation effect [22].

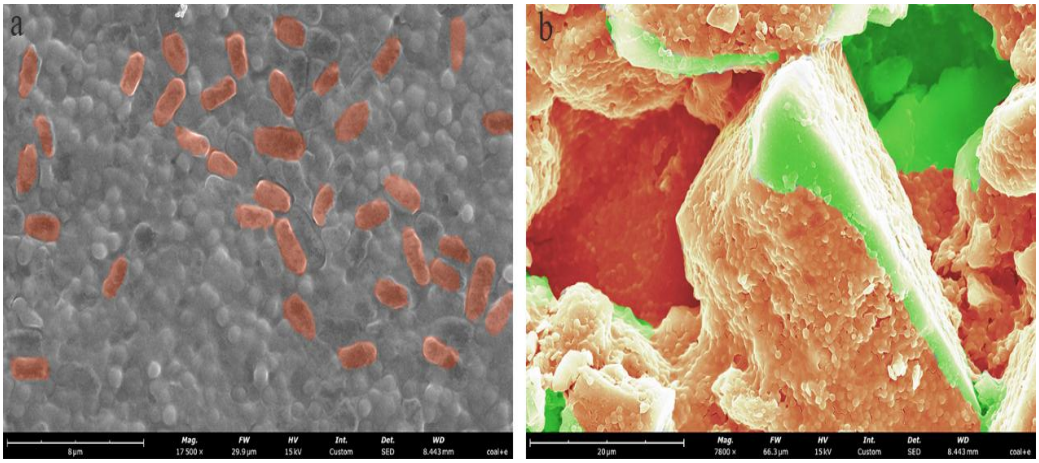

Fig.5. 3 Scanning electron micrograph
((a) *E.coli* cells and (b) coal interaction with *E.coli*)

5.3.3 Contact angle

The contact angle can reflect the surface characteristics of *E. coli* and coal. Fig.5.4 shows the contact angles of coal before and after interaction with *E.coli* (5.0×10^9 cells/ml) at pH 6. The surface of coal was hydrophobic prior to interaction with *E.coli*, and its contact angle was 81°. Due to the high hydrophobicity of the coal surface, the recovery of coal could reach 84.25% under the condition of flotation without collectors. The contact angle of *E.coli* was 9.5°, which implied that its surface had strong hydrophilicity. However, after interaction with *E.coli*, this coal gained hydrophilic characteristics, and its contact angle became17.9°. This can be attributed to the adsorbed bacterial cells onto the mineral surface, as they formed a biofilm that imparted their own surface properties to the mineral. [23].

5.3.4 Zeta potential measurements

E.coli is a Gram-negative bacteria with cell wall, cell membrane, cytoplasm, ribosomes, plasmids, and nucleoids. The cell wall plays an important role in bacterial cells adsorbed onto the mineral surface. The anionic and cationic functional groups in the macromolecular molecules of cell wall gives the bacteria amphoteric characters which affect the bacterial adsorption [24].

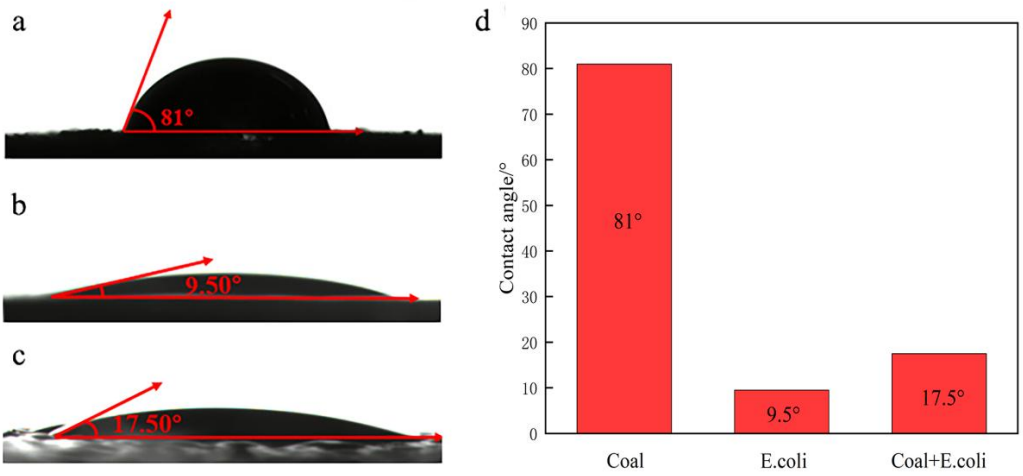

Fig.5. 4 The contact angles of coal before and after interaction with *E.coli* ((a) coal, (b) *E.coli*, (c) coal after interaction with *E.coli*, (d) The difference in contact angle before and after the interaction of coal with *E. coli*)

Zeta potential as a function of pH for coal before and after interaction with *E.coli* was tested, and the results are shown in Fig.5.5. When pH < 3, *E.coli* was positively charged. At PH 3, the anionic and cationic charges were in balance, which was the isoelectric point (IEP) of *E. coli*. When pH ranged from 3-10, *E.coli* was negatively charged, and the surface charge increased with increasing pH. At pH 10, the amount of charge reached a maximum value of 12 mV. According to the report, the negative charge of *E.coli* is primarily due to the presence of amino, carboxyl and phosphate groups in peptidoglycan and the IEP of *E. coli* has been shown to result from cell wall gluconic acids or other polysaccharide-associated carboxyl groups [25,26].

The IEP of coal was pH 2.8, when pH was 2.8, coal was positively charged, while at pH 2.8, coal was negatively charged, which agreed with previously reported data [27]. Under strong acidic, neutral and alkaline conditions, both *E.coli* and coal had the same electrical properties, so electrostatic interaction did not play a major role in the adsorption of *E.coli* on the coal surface. After interaction with *E.coli*, the IEP and zeta potential of the coal was close to that of *E.coli*, especially at pH 2 - 8. This indicated that the adsorption of *E.coli* onto the coal surface conferred the *E.coli* surface properties to the coal surface through the formation of a biofilm. At pH = 10, zeta potential of the coal after interaction with the *E.coli* was close to that of the coal. This may be due to the fact that the suitable pH range for the survival of *E.coli* is 4 - 8. At pH = 10, *E. coli* died and cannot be adsorbed on the surface of the coal. This showed that in a strong alkaline condition, *E.coli* was difficult to be adsorbed to the coal surface, but the extracellular polymeric substances (EPS) of bacteria could still

change the hydrophobicity of the mineral and affect the flotation recovery [28]. The result of zeta potential could explain the effect of pH on the flotation recovery of coal with *E.coli*.

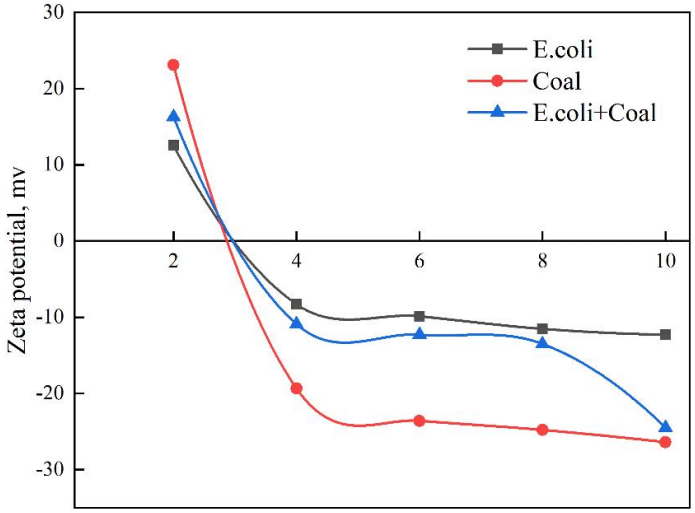

Fig.5. 5 Zeta potential of coal before and after interaction with *E.coli* as a function of pH

5.3.5 FTIR analysis

Fig.5.6 shows the FTIR spectra of coal before and after interaction with *E.coli*. The intracellular and extracellular structures of microbial cells contain biological macromolecules such as polysaccharides, proteins and nucleic acids [22]. The information of the macromolecular structure in the cell can be characterized by infrared spectroscopy, and the adsorption of *E.coli* on the coal surface can be inferred [29]. In the spectrum of *E.coli*, the characteristic peaks at 3645cm^{-1} were due to the O-H stretching vibration of hydrogen bonding and -OH stretching vibration peak of sugar C-OH. The peaks at 3421cm^{-1} were due to the NH_2 symmetrical stretching in protein molecules. The characteristic peaks at 2959, 2925 and 2854 cm^{-1} were due to CH_3 asymmetric stretching fatty acids, CH_3 symmetric stretching proteins, and CH_2 symmetric stretching lipids respectively. The characteristic peaks at 1648, 1546 and 1453 cm^{-1} were due to amide I C=O stretching proteins, amide II N-H bending, C-N stretching proteins and CH_2 bending lipids. The characteristic peaks at 1398 cm^{-1} were due to COO- symmetric stretching of amino acid side chains and fatty acids. The characteristic peaks at 1236 and 1064 cm^{-1} were due to C-O-C, C-O, C-O-P and P-O-P vibrations of polysaccharides [30].

The results of coal interaction with *E.coli* showed that the new peaks appeared

near 3645, 3421, 1236, and 1064 cm^{-1}, which were attributed to the characteristic peaks of *E.coli*. This indicated that when *E.coli* had an interaction with the coal, the groups in the polysaccharide and protein molecules in the cell wall of *E.coli* could form hydrogen bonds with the coal surface, so that *E.coli* could be adsorbed on the coal surface.

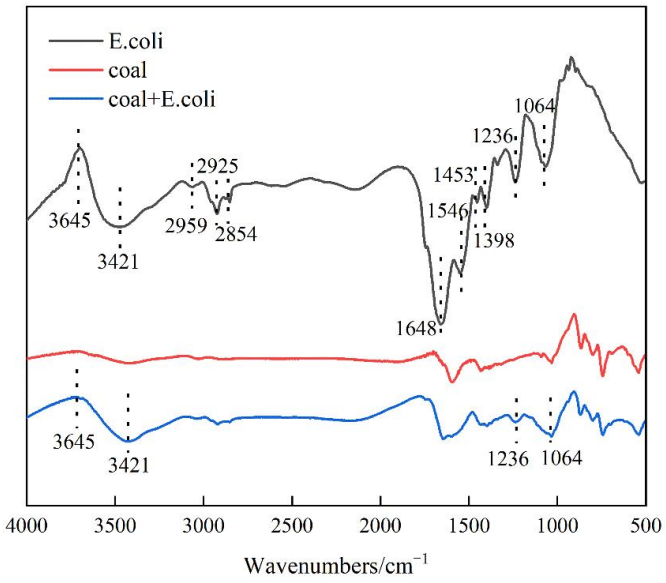

Fig.5. 6 FTIR of coal and *E.coli* before and after their interaction

5.4 Conclusions

This study assessed the adverse effect of *E.coli* on coal flotation, the results showed that *E.coli* concentration affected the flotation recovery of coal. When *E.coli* concentration reached 5×10^9 cells/ml, only 43% of the coal could be recovered. The pH value had little effect on the bioflotation of coal. Zeta potential results showed that under strong acidic, neutral and alkaline conditions, both *E.coli* and coal had the same electrical properties, so there was electrostatic repulsion between *E.coli* and coal surface which would hinder *E.coli* adsorption. But SEM, FTIR and Contact angle results showed that *E.coli* could be adsorbed to coal surface through hydrogen bonding, and then conferred its own surface characteristics to the coal surface by forming a biofilm. So it could be inferred that the hydrogen bonding force when *E.coli* adsorbed to the coal surface was greater than the electrostatic repulsion force. As the surface of coal was hydrophobic, and the surface of *E.coli* was hydrophilic, during the flotation process, *E.coli* adsorbed to the coal surface, which changed the hydrophobicity of the coal surface and affected the recovery of coal flotation. Many

investigations have used known external bacteria as flotation reagents for bioflotation. In this study, the impact of internal bacteria of recirculation water on coal flotation was researched. Due to the negative effects of *E.coli* on coal flotation, it is necessary to remove *E.coli* from recirculation water before flotation.

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Chapter VI. First insight into indigenous microorganisms in coal slurry involved in polyacrylamide biodegradation

6.1 Introduction

Coal is an important fuel and raw material for the power and coal chemical industries. The coal preparation process is necessary for improved quality, and efficient and clean production [1]. In the coal preparation process, coal slurry is an inevitable by-product; however, the fine coal particles in the coal slurry cause dewatering and solid-liquid separation problems, affecting economic value and leading to the failure of the coal preparation process [2,3]. The addition of flocculants (long-chain polymers) to coal slurry can bridge fine coal particles, forming highly porous and irregularly shaped flocs that enhance the coal slurry settling rate [4]. Polyacrylamide (PAM), a linear water-soluble polymeric formed by the polymerization of acrylamide monomers, is the most widely used flocculant [5-7].

The complete linear PAM structure is vital for flocculation. However, many previous studies have shown that microorganisms can biodegrade PAM by destroying its molecular structure [8-11]. The prior work showed that PAM could be biodegraded both aerobically and anaerobically, and Chang et al. found that the amide group could be completely degraded under anaerobic conditions, whereas it could only be partially degraded under aerobic conditions [12]. In most studies, PAM was biodegraded under aerobic conditions. Kunichika and Kinoshita first reported PAM biodegradation. They isolated two strains from the soil and identified them as *Enterobacter agglomerans* and *Azomonas macrocytogenes,* which could degrade PAM with a biodegradation rate of 15–20% [13]. And Matsuoka et al. proposed that *Bacillus sphaericus* and *Acinetobacter* in the soil could degrade PAM with biodegradation rates of 16% and 19%, respectively [14]. Qin used *Bacillus cereus*, isolated from an activated sludge sample, to degrade PAM. The results suggested that 70% of PAM was removed at the end of the seventh day after a single inoculation [15]. The available studies provided seemingly contradictory results regarding the PAM biodegradation mechanism under aerobic conditions. Kunichika and Kinoshita found that *Enterobacter agglomerans* and *Azomonas macrocytogenes* could use amid and main chain of PAM as the sole nitrogen and carbon source to biodegrade the PAM [13]. Kay-Shoemake et al. proposed that indigenous bacteria in soil could use the polymer

as a nitrogen source via inducible amidase activity to biodegrade PAM [16,17]. Wen et al. observed that *Bacillus cereus* and *Bacillus flexu* were PAM-degrading bacterial strains isolated from activated sludge and soil in an oil field respectively. Both strains could use PAM as the sole carbon source to remove the amide group from the PAM main chain [18]. Therefore, the pathway of PAM biodegradation is not clear.

The coal slurry may hold different kinds of microorganisms, which can degrade the PAM. Because some studies have shown that the raw coal contains indigenous microorganisms and these microorganisms can enter the coal slurry with the raw coal [19]. Magnabosco et al. demonstrated that microorganisms were observed at different depths in underground mineral layers, including coal, and the number of cells could reach up to 10^2 cells/g [20]. Silas et al. studied the 28 coal seam samples across three Eastern Australian coal basins and provided the Coal Seam MicroBiome (CSMB) reference set [21]. Raudsepp et al. observed that the coal seam contained some microorganisms, including the genera *Pseudomonas* and the family Rhodocyclaceae [22]. Kruger's study exhibited the presence of a diverse archaeal community in coal, such as *Methanosarcina barkeri* and *Methanosarcina lacustris* [23]. Shao and Colmer successfully isolated the microorganism strains *Acinetobacter* and *Tk. thiooxidans* from coal mines [24, 25]. Miettinen found that the microbial community of the mineral slurry system was dominated by bacteria, such as *Thiobacillus*, and *Halothiobacillus* [26]. Therefore, when the microorganisms in raw coal enter the coal slurry system, they may biodegrade PAM and affect the coal slurry settling rate; however, this phenomenon has been ignored. Limited studies have been conducted on indigenous microorganisms in coal slurry systems and the effect of microorganisms on coal slurry settling.

To study the indigenous microorganisms in coal slurry involved in PAM biodegradation and explore the PAM biodegradation mechanism under aerobic conditions, we analyzed the microbial community composition in coal slurry and predicted microbial community function. PAM biodegradation experiments were performed using microorganism isolated from coal slurry under aerobic conditions, and the effect of PAM biodegradation on coal slurry settling was studied. Scanning electron microscopy, atomic force microscopy, UV-Vis spectrophotometry, Fourier transform infrared spectroscopy and high-performance liquid chromatography were used to examine the morphology and structural changes of PAM before and after biodegradation and explain the mechanism of PAM biodegradation.

6.2 Materials and methods

6.2.1 Samples

The coal slurry samples were obtained from flotation tailings of four different flotation systems (D, F, M, and N) at the Qinxin Coal Preparation Plant, Changzhi (36°57'N, 112°19'E), China. The sampling time was July 23, 2021, the temperature was 21.3°C, and the coal type was coking coal. The flotation process and reagent dosage were the same for all four systems. Three parallel samples were collected from each system, and a total of 12 coal slurry samples (D-TC1, D-TC2, D-TC3, F-TC1, F-TC2, F-TC3, M-TC1, M-TC2, M-TC3, N-TC1, N-TC2, and N-TC3) were used for microbial analysis, isolation, and coal slurry settling experiments. Samples for microbial analysis and isolation were kept at −20℃, and samples for coal slurry settling were kept at 25℃ temperature.

The PAM sample was obtained from the Aikeda Chemical Reagent Company (Chengdu, China). PAM is anionic with a molecular weight of 1.2×10^7 Da.

6.2.2 Bacteria incubation, isolation, and domestication

Luria-Bertani (LB) medium and the basic medium (BM) were used for isolation, domestication and PAM biodegradation, respectively. LB was composed of 10 g/l tryptone, 5 g/l yeast extract, and 10 g/l NaCl. The BM composition was 0.1 g/l yeast extract, 0.5 g/l PAM, 0.5 g/l KH_2PO_4, 0.5 g/l K_2HPO_4, 0.2 g/l $MgSO_4$, 5 g/l NaCl. The pH values of the two liquid media were neutral.

The bacteria isolation was performed at 32℃ on a rotary shaker at 130 rpm. Three milliliters of coal slurry were mixed with 100 ml LB in a 250 ml conical flask at 37℃ on a rotary shaker at 130 rpm. The bacteria in the coal slurry were enriched under the culture conditions for 48 h. The bacterial solution (5 mL) was inoculated into 100 mL of BM. After two days of cultivation under the same conditions, 5 mL of the domestication solution was absorbed and transferred to solid BM for cultivation. The domestication cycle lasted for two days. The following culture at 30℃ for 24 - 48 h, different colonies growing on the plate were selected for streak purification, and the isolated strains were preserved.

6.2.3 DNA extraction, PCR amplification, sequencing, and data processing

The total genomic DNA of the coal slurry samples and microbial isolates was

extracted using a Power Soil DNA extraction kit, following the manufacturer's instructions. Quantification of the extracted DNA was performed using Thermo NanoDrop One. The PCR primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') targeting the V4 hypervariable region of the 16S rDNA gene were used for DNA amplification. The PCR mixture included 2 × Premix Taq (25 μl), Primer-F (10 μM) (1 μl), Primer-R (10 μM) (1 μl), DNA (50 ng), and nuclease-free water (50 μl). PCR amplification cycling conditions were as follows: initial denaturation at 94℃ for 5 min, followed by 30 cycles of denaturation at 94℃ for 30 s, annealing at 52℃ for 30 s, extension at 72℃ for 30 s, and single extension at 72℃ for 10 min, and finally at 4℃ (BioRad S1000, Bio-Rad Laboratory, CA). The PCR amplification products were detected on a 1% agarose gel, and the concentration was compared using Gene Tools Analysis Software (Version 4.03.05.0, SynGene). PCR products were recovered using the E.Z.N.A ® Gel Extraction Kit (Omega, USA) and eluted with TE buffer. Library construction was performed according to the standard procedure of the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, USA). The constructed amplicon library was subjected to PE250 sequencing using an Illumina Nova 6000 platform（Guangdong Magigene Biotechnology, Guangzhou, China）

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina Nova 6000 PE250 platform (Guangdong Magigene Biotechnology). Raw FASTQ files were de-multiplexed using an in-house Perl script, quality-filtered, and merged using FLASH version 0.14.1. The optimized sequences were then clustered into operational taxonomic units (OTUs) using UPARSE with a 97% sequence similarity level. The most abundant sequence for each OTU was selected as a representative sequence. Finally, QIIME2 was used for OTU taxonomic annotation with a confidence threshold of 70%.

The 16S rDNA sequences in coal slurry were compared with the (CSMB) (https://data.csiro.au/dap/landingpage?pid=csiro:26472) which is a dataset of OTU sequences specific to the coal seam environment to investigate the relationship between microorganisms in coal slurry and coal seam microbiome [21,27]. The phylogenetic tree of microbial isolates was constructed by the neighbor-joining method using the MEGA software. Microbial community composition and network analyses were computed in R using the vegan R package. The microbial community function of the coal slurry was determined using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2). PICRUSt2

enables the prediction of functional profiles using community-based 16S rDNA data. The correlations between dominant microorganisms and functions in the samples were calculated using Spearman's rank correlation analysis, and the groups and indicators with correlation coefficients > 0.6 and corrected $P < 0.05$ were selected, and the correlation network diagram was drawn using R.

6.2.4 Polyacrylamide concentration determination, biodegradation, and coal slurry settling experiments

Varying PAM concentrations (10, 50, 100, 250, and 500 mg/l) were prepared, and the turbidity method was used to detect the PAM concentration. The method principle is that acetic acid reacts with sodium hypochlorite to generate Cl_2, which then reacts with PAM to generate insoluble ammonium chlorate, making the solution cloudy. Five milliliters of PAM were placed in a 50 ml beaker, and 5 ml of 5 ml/L acetic acid and 1.31% NaClO were added in sequence. The precipitation reaction was completed after approximately 20 min, and the solution became cloudy. A UV-Vis spectrophotometer (GENESYS 150, Thermo Scientific) was used to measure absorbance at 470 nm.

Next, 1, 3, and 5 ml domesticated bacterial liquid (OD_{600} = 0.7) was inoculated into sterilized BM containing 500 mg/l PAM . The PAM biodegradation was performed at 32℃ on a rotary shaker at 130 rpm under aerobic conditions. Ten milliliters of the biodegradation solution were collected every 3 h. The cells were harvested using Heraeus multifuge X1R centrifugation (Thermo Scientific) at 5000 rpm for 5 min. The supernatant was filtered by a 0.25 μm membrane twice by adding acetic acid and NaClO. Bacterial growth and PAM concentration were determined using a UV-VIS spectrophotometer (GENESYS 150, Thermo Scientific) three times and averaged.

In the coal slurry settling experiments, 3 ml samples of PAM before and after biodegradation were added to 500 ml of 35 g/L coal slurry, and the settling compression layer height of coal slurry was observed within 2 and 30 min respectively. Three repetitions of the experiment were conducted.

6.2.5 Characterization of PAM biodegradation

UV-Vis spectrophotometry (GENESYS 150, Thermo Scientific), Fourier transform infrared spectroscopy (FTIR, Nicolet FTIR-6700, Thermo Scientific), Scanning electron microscopy (SEM, Phenom Scientific, Netherlands), Atomic force

microscope (AFM, Brucker Multimode 8 system), High-performance liquid chromatography (HPLC, LC - 20AD, Agilent) were used to determine the changes of PAM before and after biodegradation. In the UV-Vis spectrophotometry and HPLC measurements, the PAM solution after biodegradation was filtered by the filterable membrane (0.25-μm pore size). In FTIR, SEM, and AFM measurements, the solution had to be further dried in the air. UV measurements were performed to determine the absorbance at 470 nm. The FTIR measurements were conducted at room temperature (25 ± 1℃), and the background spectrum was obtained using KBr pellets in the range of 4000 to 400 cm^{-1}. The mobile phase for HPLC measurements consisted of a water-methanol (50:50) mixture with a flow rate of 0.25 ml/min and a detection wavelength of 210 nm.

6.3 Results and discussion

6.3.1 Microbial community composition and function

6.3.1.1 Microbial community composition

In coal slurry samples, the microorganisms are assigned to 34 phyla and 98 genera. Fig.6.1 shows the top 20 microorganisms and their abundances at the phylum and genus levels. According to the analysis of microbial community composition, the dominant microorganisms at the phylum level are Bacteroidetes (53%), Proteobacteria (15%), Acidobacteria (8%), Actinobacteria (6%), Crenarchaeota (4%), accounting for more than 86%; the dominant microorganisms at the genus level are Candidatus_*Nitrososphaera* (28%), *Kaistobacter* (8%), *Arthrobacter* (5%), *Thermomonas* (4%), *Lysobacter* (4%), *Steroidobacter* (3%), accounting for more than 52%.

In Fig.6.2, the comparison of the top 10 microorganisms at the genus level from coal slurry and assigned CSMB numbers is shown. Eight of the top 10 microorganisms are matched in the CSMB reference set co-occurred in the Ordos Basin, Bowen Basin, and Sydney Basin, including Candidatus_*Nitrososphaera, Arthrobacter, Thermomonas, Lysobacter, Flavobacterium, Pseudomonas, Devosia, Bacillus*. The results suggest that coal slurry and subsurface coal seams contain similar indigenous microorganisms. Some studies have confirmed that microorganisms can enter coal slurry from raw coal [28-32]. In indigenous microorganisms, *Bacillus* and *Pseudomonas* have been reported to degrade PAM [33-34]. Therefore, it can be speculated that indigenous microorganisms in coal slurry

may be able to biodegrade PAM.

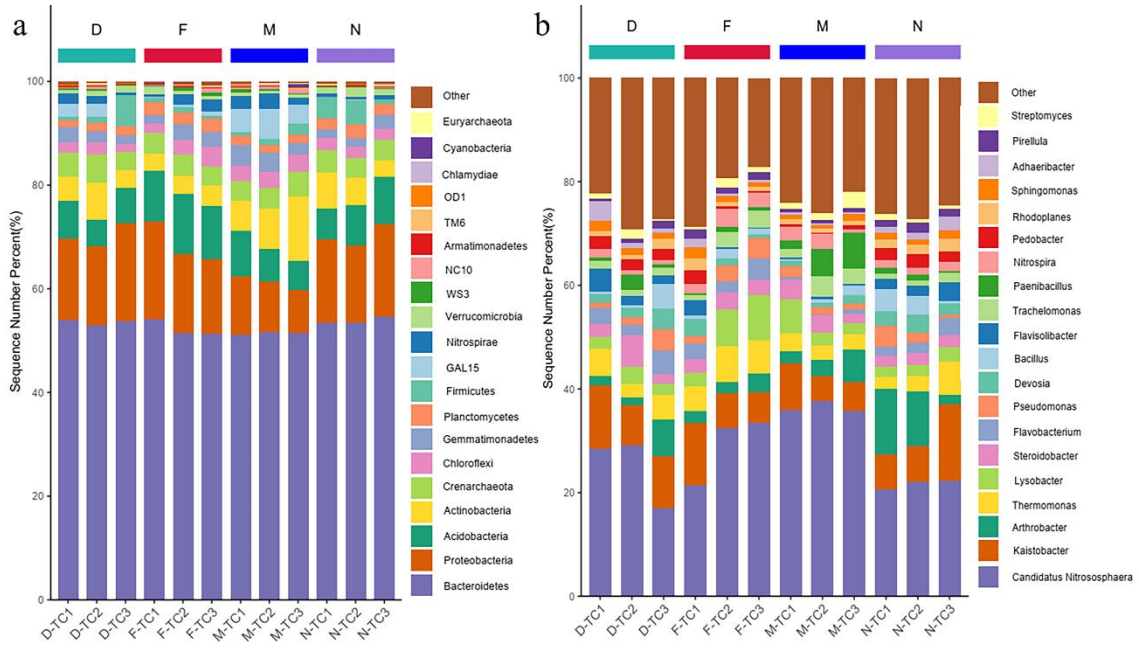

Fig.6. 1 Distribution of microorganisms in coal slurry at phylum and genus levels (a. phylum level; b. genus level)

Dominant microorganism	CSMB number
Candidatus_Nitrososphaera	CSMB_132
Arthrobacter	CSMB_3 CSMB_11
Thermomonas	CSMB_2058 CSMB_2231
Lysobacter	CSMB_189 CSMB_739
Flavobacterium	CSMB_90 CSMB_117 CSMB_3208 CSMB_4360
Pseudomonas	CSMB_20 CSMB_582 CSMB_1260 CSMB_2000
Devosia	CSMB_49 CSMB_125 CSMB_2634 CSMB_3604
Bacillus	CSMB_85 CSMB_650 CSMB_1173 CSMB_2100

Fig.6. 2 The comparison of the top 10 microorganisms from coal slurry and assigned CSMB numbers

6.3.1.2 Microbial community function

The predicted function of dominant microorganisms can be used to understand the role of microorganisms in coal slurry. Fig.6.3 shows the 21 functions of the dominant microorganisms in coal slurry. All functions are involved in Xenobiotics biodegradation and metabolism which has been reported to participate in the biodegradation of complex organic matter [35]. In general, *Devosia*, *Bacillus*, *Pseudomonas*, and *Arthrobacter* promote the Xenobiotics biodegradation and metabolism, while Candidatus *Nitrososphaera*, *Thermomonas*, and *Lysobacter* inhibit

the Xenobiotics biodegradation and metabolism. The relationship between *Kaistobacter*, *Flavobacterium*, and Xenobiotics biodegradation and metabolism is weak. Among them, *Devosia* and Candidatus *Nitrososphaera* play an important role in Xenobiotics biodegradation and metabolism, presenting an opposite relationship. Metabolism of Xenobiotics by Cytochrome P450, Drug Metabolism - Cytochrome P450, Caprolactam degradation, and Bisphenol degradation are positively correlated with *Devosia*, but negatively correlated with Candidatus *Nitrososphaera*. Therefore, the dominant microorganisms are closely related to the organic matter biodegradation in coal slurry. During the coal slurry settling, PAM as the organic matter is added to accelerate the settling rate of fine-grained coal. Based on the predicted function of microorganisms in coal slurry, it is possible that some microorganisms can biodegrade PAM.

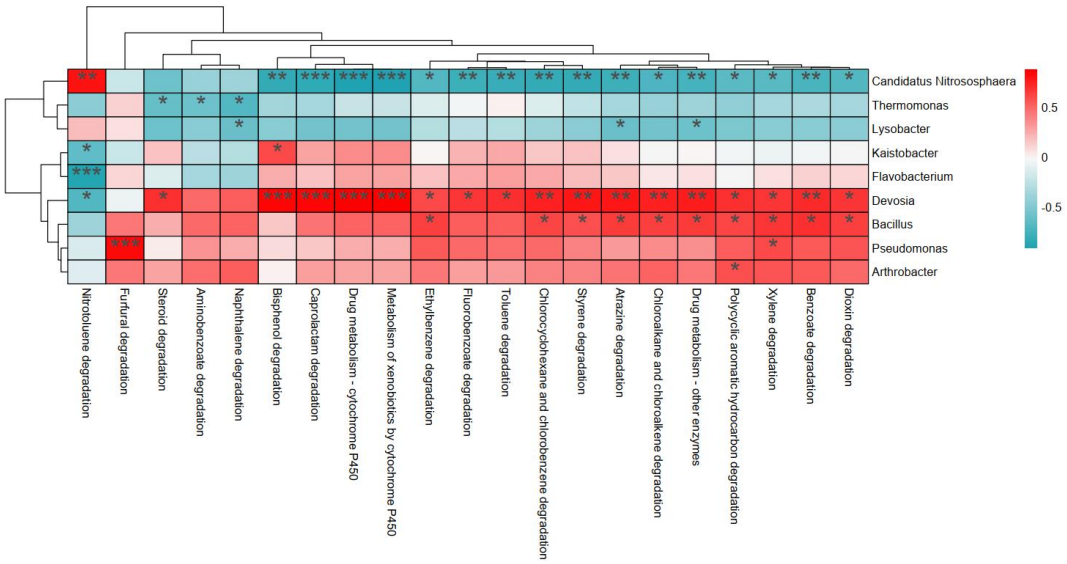

Fig.6. 3 Correlation network diagram between dominant microorganisms in coal slurry and functional classes

6.3.2 PAM biodegradation affects the coal slurry settling

6.3.2.1 Identification of isolated bacteria strain, PAM biodegradation, and coal slurry settling

To confirm the organic matter degradation function of microorganisms in coal slurry, a variety of strains were isolated from coal slurry, and the full-length 16S rDNA sequence of the strain named DBY was amplified by PCR and compared with the data from the GenBank to construct a phylogenetic tree, as shown in Fig.6.4. The bacteria BRY and *Sphingomonas aquatilis strain JSS-7* are grouped together. And

Fig.6.1(b) shows that *Sphingomonas* is an indigenous microorganism in coal slurry. *Sphingomonas* is widely present in the environment and has a strong degradation function, and can break down aromatic hydrocarbons and methylene blue [36,37].

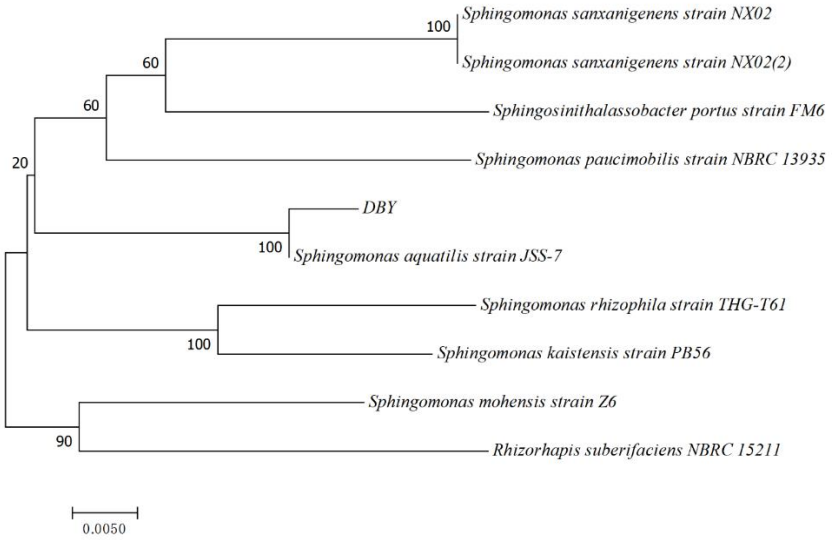

Fig.6. 4 Phylogenetic tree of bacteria based on 16S rDNA sequence (The scale bar represents 0.005 substitutions per nucleotide site)

The PAM concentration determined by turbidimetry is linearly related to OD_{470} [38]. The higher the PAM concentration, the more turbid generated by the insoluble chloramine, and the OD_{470} value is shown in Fig.6.5(a). The standard curve was calculated using the following equation:

$$y = 0.051 + 1145.777\,x \qquad R^2 = 0.999$$

where y is the PAM concentration (mg/l) and x is the absorbance.

The growth and PAM biodegradation curves of *Sphingomonas* with different inoculum amounts are shown in Fig.6.5(b) and (c). *Sphingomonas* mainly undergoes logarithmic growth and decay phases, and the stable phase is relatively short. The logarithmic growth period of *Sphingomonas* is 0–12 h for 3 ml and 5 ml inoculation amounts and 0–22 h for 1 ml inoculation amounts. The highest biomass is obtained at an inoculation volume of 5 ml, indicating that increased inoculation amount can increase bacterial biomass. During the logarithmic growth period, the *Sphingomonas* grows rapidly and metabolizes vigorously, and the PAM concentration decreases significantly. However, with the increase of time, the PAM concentration increases at 12–25 h. The increase in PAM concentration may be due to the production of NH_3-N. The extracellular enzyme secreted by *Sphingomonas* during the biodegradation process can use the amide group of PAM to produce a large amount of NH_3-N, which reacts with acetic acid and sodium hypochlorite, making the solution cloudy and

affecting the measurement of PAM concentration [39,40]. At 25–76 h, although the growth rate of *Sphingomonas* slows down, various secreted enzymes can continuously biodegrade PAM. At 76 h, the PAM concentration is 168 mg/l, and the biodegradation rate reaches 66.4%. As shown in Table 6.1, previous work has studied PAM biodegradation by different microorganisms under aerobic conditions. Compared with the previous studies, the microorganism isolated from coal slurry has a high PAM biodegradation rate [15,33,41-43].

To explore the influence of PAM biodegradation on coal slurry settling, 3 mL of PAM before and after biodegradation was added to 35 g/L coal slurry, and the settling results are shown in Fig.6.6. Before biodegradation, PAM promotes the coal slurry settling through efficient flocculation (Fig.6.6(a)). A prominent settling layer of coal slurry appears within 2 min, whereas flocculation weakened after PAM biodegradation, and the coal slurry settling rate significantly decreases (Fig.6.6(b)). After 2 min, no noticeable settling of coal slurry is observed. After standing for 30 min, the settling layer volume of the coal slurry with PAM is 50 ml, whereas that of the coal slurry with PAM after biodegradation is 40 ml. The suspension clarity and settling compression layer height can reflect the settling effect of coal slurry. In this study, the suspension of coal slurry with PAM after biodegradation is turbid, and the height of settling compression layer h_2 is evidently lower than h_1 (Fig.6.6(c), (d)). Therefore, the PAM biodegradation by indigenous microorganisms in the coal slurry seriously inhibits the coal slurry settling and affects the coal preparation process.

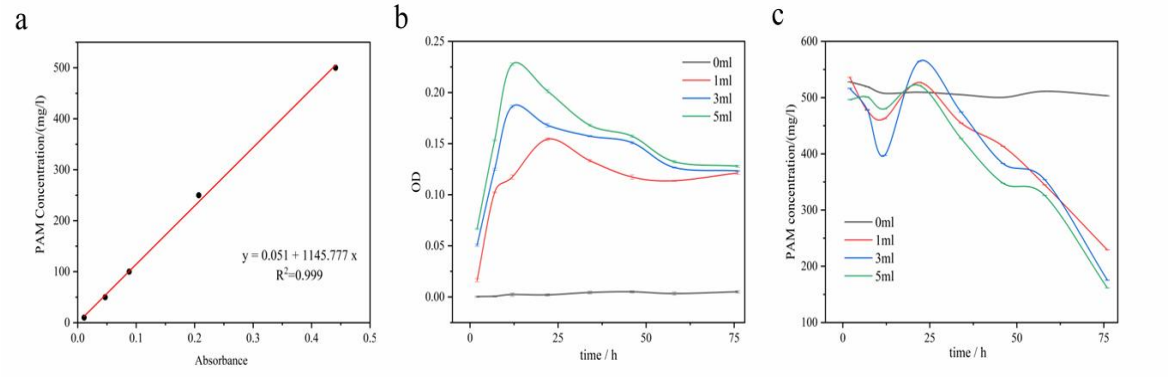

Fig.6. 5 Growth and PAM biodegradation curves of *Sphingomonas*
(a. PAM concentration-absorbance standard curve; b. Growth curve of *Sphingomonas* with different bacterial inoculum amounts; c. Biodegradation curve of PAM with different bacterial inoculum amounts)

Table 6. 1 Biodegradation rate of PAM by different bacteria

Bacterial strains	source	Molecular weight / Da	Conditions	Degradation time / day	Degradation rate
Bacillus cereus	oil	2×10^7	aerobic / 40℃	7	33.7%
Bacillus sp.	oil	2×10^7	aerobic / 40℃	7	29.1%
Bacillus cereus	sludge	1.6×10^7	aerobic / 20℃	7	70%
Bacillus sp.	oil	2×10^7	aerobic / 37℃ / pH = 8.0	9	59.9%
Chelatococcus sp.	oil	2×10^7	aerobic / 40℃ / pH = 7.1	9	58.4%
Bacillus sp.	soil	2.3×10^6	aerobic / 40℃ / pH = 7.1	14	16%
Acinetobacter sp.	soil	2.3×10^6	aerobic / 37℃ / pH = 8.0	14	19%
Pseudomonas putida	sludge	1.6×10^7	aerobic / 39℃ / pH = 7.2	7	31.1%
Sphingomonas	coal	1.2×10^7	aerobic / 30℃ / pH = 7.2	3.2	66.4%

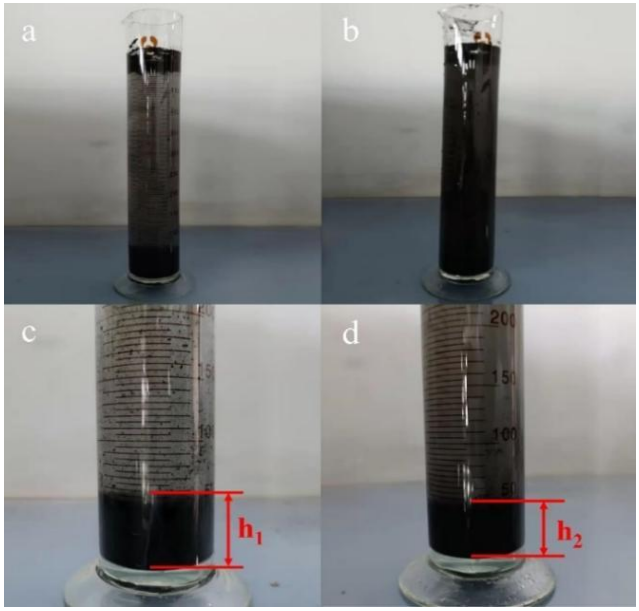

Fig.6. 6 Effects of PAM before and after biodegradation on coal slurry settling (a. Settling of coal slurry within 2 min before PAM biodegradation. b. Settling of coal slurry within 2 min after PAM biodegradation; c. Height of settling compressed layer (h1) formed by coal slurry settling within 30 min before PAM biodegradation; d. Height of settling compressed layer (h2) formed by coal slurry settling within 30 min after PAM biodegradation)

6.3.2.2 Morphology and structure changes before and after PAM biodegradation

The structure of PAM before and after biodegradation was studied using SEM analysis. Fig.6.7(a) shows that the shape of PAM before biodegradation is cuboid at 800 × magnification. Further, at 8000 × magnification (Fig.6.7(b)), the PAM surface is dense, flat, and smooth, showing a tight chain shape, which agrees with previous reports [32,44]. As shown in Fig.6.7(c) and (d), after the PAM biodegradation, irregular pores are observed on the PAM surface at 800 × magnification, and the PAM surface is covered with rod-shaped, 2 μm-long *Sphingomonas* at 8000 × magnification. The SEM results suggest that microbial activities can cause PAM biodegradation.

To further study the molecular structure of PAM after biodegradation, we used AFM to observe the structural changes in PAM before and after biodegradation, and the results are shown in Fig.6.8. PAM presents a chain structure before biodegradation (Fig.6.8(a)). After the biodegradation of *Sphingomonas*, the PAM chains are broken, and the PAM molecules are cleaved into small molecular particles (Fig.6.8(b)).

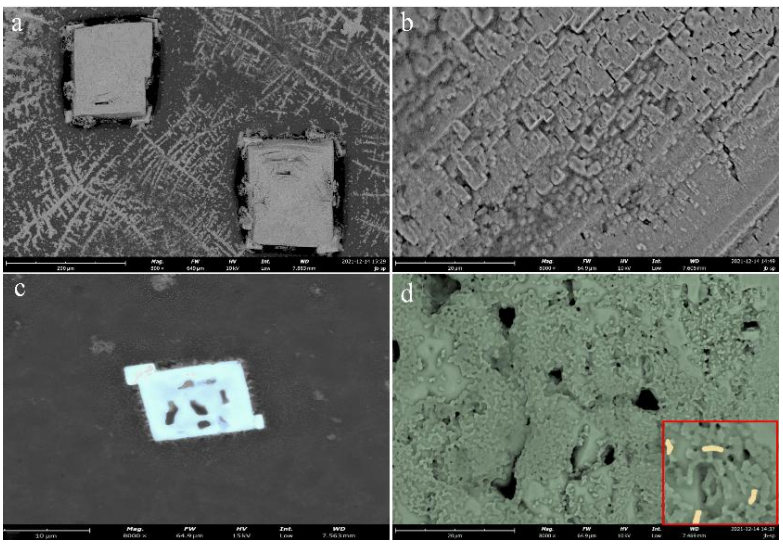

Fig.6. 7 Scanning electron microscope (SEM) images of PAM before and after biodegradation (a,b. SEM images of PAM before biodegradation; c,d. SEM images of PAM after biodegradation)

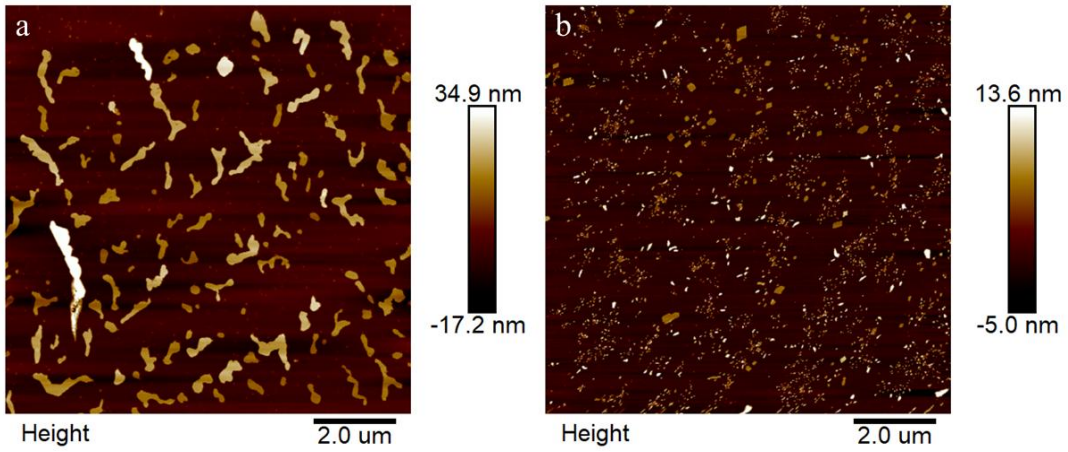

Fig.6. 8 Atomic force microscope images of PAM before and after biodegradation (a. AFM image of PAM before biodegradation; b. AFM image of PAM after biodegradation)

The UV and FTIR spectra of the PAM before and after biodegradation were measured at wavelengths of 190–400 nm and 400–4000 nm, respectively, as shown in Fig.6.9(a) and (b). In the UV spectra, there is a strong absorption peak at 212 nm before and after biodegradation of PAM, which is the ultraviolet absorption generated by the polyene and amide group-conjugated system. After PAM biodegradation, a weak absorption band at 265 nm is observed, which may be due to the UV absorption generated by the carboxyl group, indicating that PAM is partially biodegraded.

In the FTIR spectra, PAM is the primary amide corresponding to the characteristic absorption peak of free -NH_2 at 3411 cm^{-1}. The characteristic absorption peak of the antisymmetric stretching vibration of the methylene group is 2958 cm^{-1}, and 1667 cm^{-1} corresponds to the symmetrical stretching of C=O in the amide group vibration peaks; 1451 cm^{-1} is the characteristic peak of methylene deformation, and 1149 cm^{-1} is the characteristic peak of the stretching vibration of the CN bond. Comparing the infrared spectra before and after PAM biodegradation, in the 3100–3500 cm^{-1} region, the characteristic peak of -NH_2 at 3411 cm^{-1} in the spectrum becomes a broad peak, which is affected by the O-H stretching vibration [45]. A new characteristic absorption peak appears at 2451 cm^{-1} after PAM biodegradation, which may be a ketene substance. A strong ketene characteristic peak also appears at 1120 cm^{-1}. The peak at 1671 cm^{-1} corresponds to the blue shift of the characteristic peak of C=O (1671 cm^{-1}). The deformation characteristic peak of methylene at 1451 cm^{-1} is significantly weakened after biodegradation, which may have been utilized by the microorganisms. From the infrared spectra of PAM before and after biodegradation, it can be observed that some distinct groups of PAM are changed after biodegradation.

This indicates that the amide group is biodegraded into a carboxyl group.

Further studies were carried out on PAM before and after biodegradation by HPLC. Fig.6.9(c) shows the HPLC results. There are two peaks in the HPLC before biodegradation, and the corresponding retention times are 3.50 min and 6.18 min. In the HPLC of PAM after biodegradation, the peaks at 3.50 min and 6.18 min still existed. However, a new peak appears at 2.25 min, indicating that the PAM biodegradation produces new organic matter. Because PAM is a copolymer of acrylamide, it can be degraded into a small molecule, acrylamide. Some studies have shown that the retention time of acrylamide monomers is approximately 4 min [32]. Thus, no acrylamide monomer is generated during PAM biodegradation.

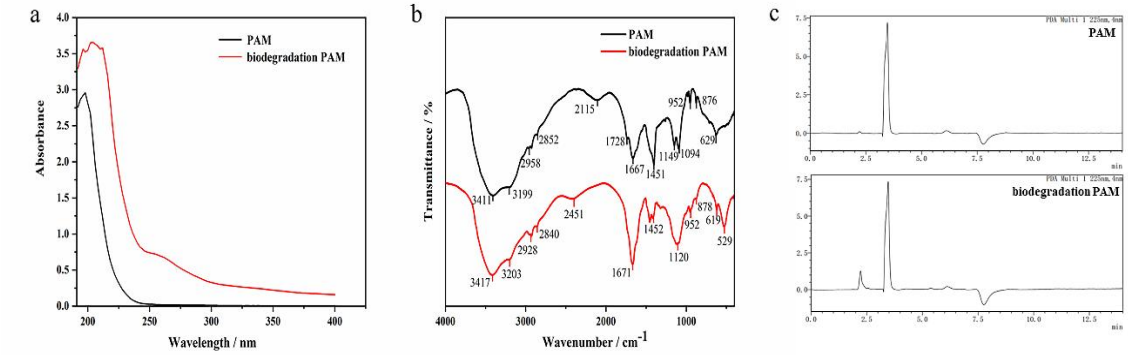

Fig.6. 9 UV, FTIR, and HPLC of PAM before and after biodegradation
(a. UV spectra of PAM before and after biodegradation; b. FTIR spectra of PAM before and after biodegradation; c. HPLC peaks of PAM before and after biodegradation)

6.3.2.3 Mechanism of PAM biodegradation

Most previous studies have focused on the PAM and hydrolyzed form of polyacrylamide (HPAM) biodegradation mechanisms. Kay-Shoemake et al. [16] proposed that microorganisms could destroy the side chains of HPAM because microbial amidase enzymes produced nitrogen by hydrolyzing amide groups without damaging the main carbon chain. However, Ma et al. [46] found that the side and main carbon chains of HPAM could be decomposed by microorganisms simultaneously. HPAM provided nitrogen and carbon sources for the growth of microorganisms, and enzymes secreted by microorganisms broke down HPAM into small molecules but not acrylamide. Furthermore, the studies of Song, Zhao, and Dai et al. [47-49] also agreed with the results. They observed that amidase hydrolyzed the amide group of HPAM, resulting in side-chain fracture and HPAM degradation to

polyacrylate (PAA). The oxidases then catalyzed the cleavage of the PAA carbon chain. To further understand the mechanism of PAM biodegradation, WANG et al. [50] used a docking study to identify the binding modes and interaction details for the degradation of HPAM and PAA. The results suggested that PAA was much more difficult to biodegrade than HPAM.

In this study, the SEM and AFM results show significant PAM biodegradation, and the UV spectrometry, FTIR, and HPLC results indicate possible biodegradation pathway for PAM as shown in Fig.6.10. In the PAM biodegradation process, the amidase secreted by microorganisms provide nitrogen for microorganisms by hydrolyzing the PAM amide group, during which the -COOH- group replace the -NH_3- group. Simultaneously with hydrolysis, monooxygenases provide carbon for microorganisms by oxidating the main carbon chain. The -CH- group on the PAM carbon chain is oxidized to the -COH- group by monooxygenase, then transfer to the -CHO- group, and is further oxidized to the -COOH- group. During the hydrolysis and oxidation process, PAM is broken down into small molecules such as acrylic acid, which can be further biodegraded.

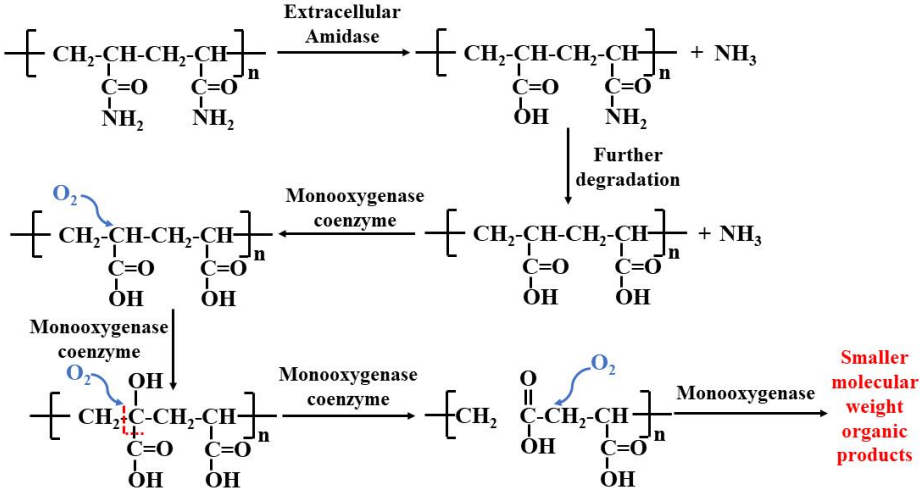

Fig.6. 10 Hypothetical mechanism of PAM biodegradation

6.4 Conclusions

This study explored the microbial community composition and function in coal slurry and explained the relationship between indigenous microorganisms and coal slurry. The results show that indigenous microorganisms in the coal slurry can biodegrade PAM to inhibit the coal slurry settling.

In coal slurry samples, the microorganisms are assigned to 34 phyla and 98 genera, and Candidatus_*Nitrososphaera, Arthrobacter, Thermomonas, Lysobacter, Flavobacterium, Pseudomonas, Devosia, Bacillus* are matched in the CSMB reference set co-occurred in the Ordos Basin, Bowen Basin, and Sydney Basin. The predicted function results present that microorganisms in coal slurry are closely related to the Xenobiotics biodegradation and metabolism, and can degrade complex organic matter. To confirm this prediction, the *Sphingomonas* isolated from coal slurry was used to biodegrade PAM. With an increase in microbial biomass, the concentration of PAM decreased, and the biodegradation rate reached 66.4% within 76 h. The results of SEM, AFM, UV spectrometry, FTIR, and HPLC suggest that the extracellular enzymes secreted by *Sphingomonas* can hydrolyze and oxidize the amide group and main carbon chain of PAM and biodegrade it into smaller molecular weight organic products. The PAM biodegradation has a negative effect on coal slurry settling. The results of coal slurry settling tests demonstrate that the settling rate and settling layer height of coal slurry markedly decrease after PAM biodegradation.

In this study, a strain of bacteria was isolated from coal slurry for PAM biodegradation, which can promote the study of organic pollutant biodegradation. Moreover, it reveals the influence of indigenous microorganisms on coal slurry settling and inspires the importance of microorganisms in the practical production process. However, this research only explored the biodegradation effect of one type of microorganisms in coal slurry on PAM. Previous studies have shown that mixed microorganisms could accelerate PAM biodegradation. In coal slurry, various indigenous microorganisms can simultaneously act on PAM, stimulate the PAM biodegradation, and significantly affect the coal slurry settling. Therefore, studying and managing indigenous microorganisms in coal slurry is necessary. This will not only make the coal processing process run smoothly, but also make effective use of coal resources, reduce carbon emissions and protect the environment.

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Chapter VII. Study on desulfurization effect of indigenous microorganisms on coal

7.1 Introduction

Coal, as a primary source of energy and raw material for the chemical industry, plays a significant role in global economic and social development [1]. While it provides energy for industrial production and human life, it also causes serious environmental pollution, the most prominent of which is the harm caused by the burning of sulfur in coal to form acid rain [2,3]. The sulfur in coal is divided into organic and inorganic sulfur. Organic sulfur is the sulfur combined with the organic structure of coal, and inorganic sulfur is mainly from various sulfur-containing compounds in minerals [4]. Most of the inorganic sulfur and organic sulfur in coal are combustible sulfur, usually accounting for about 90% of the total sulfur, which can generate sulfur dioxide and a small amount of sulfur trioxide after combustion [5]. It is the main factor causing air pollution and the formation of acid rain. Therefore, in order to effectively control the pollution of the environment by sulfur dioxide generated in the process of coal utilization, it is necessary to desulfurize coal.

The inorganic sulfur in coal is mainly pyrite, which can be removed by physical coal beneficiation process [6,7]. In contrast, organic sulfur in coal is symbiotic with organic matter, knitted together, evenly distributed and not easily removed [8]. At present, the methods to remove organic sulfur include chemical and microbial methods. Chemical methods include hot alkali leaching, oxidation, chlorination, etc [9,10]. Although they can remove organic sulfur from coal, the reaction conditions are strong, the macromolecular structure of coal and the bonding of coal are destroyed, and the calorific value is lost. Microbial desulfurization technology with mild reaction conditions, low cost and little environmental pollution is an environmentally friendly new technology for coal desulfurization. Many studies have confirmed the feasibility of microbial desulfurization of organic sulfur.

The organic sulfur in coal is found as refractory polynuclear aromatic sulfur heterocyclics (dibenzothiophene (DBT)). Many studies have confirmed the biodegradation of DBT by microorganisms. Bahuguna isolated a strain of *Lysinibacillus sphaericus* from diesel-contaminated soil, which could remove DBT with 2-hydroxybiphenyl (2HBP) as the desulfurization product, and the efficiency of

DBT desulfurization could reach 60% within 15 days [11]. Papizadeh explored the effect of *Enterobacter sp. NISOC-03* on DBT removal in the presence of organic sulfur and glucose as sulfur and carbon sources, showing that *Enterobacter sp. NISOC-03* consumed 63.9% of DBT in 10 days, and after 96 hours, there was 2HBP production [12]. Seo isolated the *Arthrobacter* sp. P1-1 from a polycyclic aromatic hydrocarbon (PAH)-contaminated site, and found that the strain could degrade approximately 82% of DBT in 14 days. Dibenzothiophene-5,5'-dioxide was formed from the sulfur oxidation of DBT. Dibenzothiophene diols underwent ortho- and meta-ring cleavages to produce benzo[b]thiophene-2,3-dicarboxylic acid and hydroxyl-benzo[b]thiophene carboxylic acid, respectively, after transformation from 1,2- and 3,4-dioxygenations of DBT [13]. Gun suggested that *S. solfataricus P2* had a potential DBT - desulfurization ability, and it could utilize DBT - containing and its derivatives organosulfur compounds through specific metabolic pathways [14]. And Bhanjadeo explored the microbial type culture collection strains *Rhodococcus rhodochrous*, *Arthrobacter sulfureus*, *Gordonia rubropertincta*, and *Rhodococcus erythropolis* for the desulfurization of DBT by C-S bond cleavage assay, and the results showed that all strains were > 99% efficient in desulfurizing DBT within 10 days [15]. Therefore, it can be presumed that microorganisms have the advantage of removing organic sulfur in coal. In the process of coal organic sulfur desulfurization, the selection of efficient desulfurizing bacterial strains is the most crucial step. Currently, strains are typically obtained from the natural environment and domesticated using DBT.

In this study, a new way for the screening of efficient desulfurization bacteria is provided by establishing the correlation between microorganisms and microbial functions. The strain with sulfur metabolism function was found in raw coal, and the strain was isolated and explored for organic sulfur removal tests in coal. In addition, the mechanism of organic sulfur removal by this strain was analyzed using Scanning electron microscope, Energy dispersive spectrometer, X-ray diffraction, and Fourier transform infrared spectroscopy.

7.2 Materials and methods

7.2.1 Samples

Coal samples were collected from the Qinxin coal preparation plant in Shanxi Province, China. The rank of coal is coking coal. After collection, all samples were

stored in airtight plastic bags to minimize oxidation, part samples were stored at -20℃ for microbial separation. The coal was crushed in a hammer mill and passed through a 45μm mesh sieve for organic sulfur removal tests. The Dibenzothiophene sample was obtained from the China National Pharmaceutical Company Limited, purity > 99%.

7.2.2 Bacteria incubation, isolation, and identification

The strain of bacteria, named DBW, was isolated from coal by LB medium (10g tryptone, 5 g yeast extract, and 10 g NaCl dissolved in 1000ml ultrapure water). The strain was identified by 16S rDNA, and the result sequences were compared on NCBI (http://www.ncbi.nlm.nih.gov). According to the result of blast, the species with high similarity were selected to conduct a phylogenetic tree by MEGA7.

7.2.3 DNA extraction, PCR amplification, sequencing, and data processing

The total genomic DNA of the coal slurry samples and microbial isolates was extracted using a Power Soil DNA extraction kit, following the manufacturer's instructions. Quantification of the extracted DNA was performed using Thermo NanoDrop One. The PCR primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') targeting the V4 hypervariable region of the 16S rDNA gene were used for DNA amplification. The PCR mixture included 2 × Premix Taq (25 μl), Primer-F (10 μM) (1 μl), Primer-R (10 μM) (1 μl), DNA (50 ng), and nuclease-free water (50 μl). PCR amplification cycling conditions were as follows: initial denaturation at 94℃ for 5 min, followed by 30 cycles of denaturation at 94℃ for 30 s, annealing at 52℃ for 30 s, extension at 72℃ for 30 s, and single extension at 72℃ for 10 min, and finally at 4℃ (BioRad S1000, Bio-Rad Laboratory, CA). The PCR amplification products were detected on a 1% agarose gel, and the concentration was compared using Gene Tools Analysis Software (Version 4.03.05.0, SynGene). PCR products were recovered using the E.Z.N.A ® Gel Extraction Kit (Omega, USA) and eluted with TE buffer. Library construction was performed according to the standard procedure of the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, USA). The constructed amplicon library was subjected to PE250 sequencing using an Illumina Nova 6000 platform（Guangdong Magigene Biotechnology, Guangzhou, China）

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina Nova 6000 PE250 platform (Guangdong Magigene Biotechnology). Raw FASTQ files were de-multiplexed using an in-house Perl script, quality-filtered,

and merged using FLASH version 0.14.1. The optimized sequences were then clustered into operational taxonomic units (OTUs) using UPARSE with a 97% sequence similarity level. The most abundant sequence for each OTU was selected as a representative sequence. Finally, QIIME2 was used for OTU taxonomic annotation with a confidence threshold of 70%.

The phylogenetic tree of microbial isolates was constructed by the neighbor-joining method using the MEGA software. Microbial community composition was computed in R using the vegan R package. The microbial community function was determined using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2). PICRUSt2 enables the prediction of functional profiles using community-based 16S rDNA data. The correlations between dominant microorganisms and functions in the samples were calculated using Spearman's rank correlation analysis, and the groups and indicators with correlation coefficients > 0.6 and corrected $P < 0.05$ were selected, and the correlation network diagram was drawn using R.

7.2.4 Organic sulfur removal tests and DBT concentration determination

The biodegradation tests of DBT by strain DBW was conducted to investigate the removal test of organic sulfur from coal by indigenous microorganisms in raw coal. The 2 ml strain DBW liquid (OD_{600} = 0.3) was inoculated into sterilized basic medium containing 0.2 mmol/l DBT. The concentration of DBT was determined by extraction method. The reaction cultures were taken at different times, acidified with 10% hydrochloric acid until the pH value was less than 2.0, added an equal volume of ethyl acetate extractant, and extracted by shaking fully with a partition funnel. The mixture was transferred to a centrifuge tube and centrifuged for 10 min at 5000 r/min (Thermo Scientific). The upper organic phase was taken to detect the OD_{330} value by UV spectrophotometer (GENESYS 150, Thermo Scientific). Meanwhile, the desulfurization tests of coal samples by strain DBW was carried out. In a 100 ml conical flask, 20 g of coal sample was added and 10 ml of strain DBW (OD_{600}=0.3) was added, and the desulfurization tests were carried out for 160 hours.

7.2.5 Characterization of DBT biodegradation

X-ray diffraction (XRD, D8 Advance), Fourier transform infrared spectroscopy (FTIR, Nicolet FTIR-6700, Thermo Scientific), Scanning electron microscopy, and Energy dispersive spectrometer (SEM, Phenom Scientific, Netherlands) were used to

determine the changes of coal before and after biodesulfurization. In all measurements, the solution had to be further dried in the air. The XRD measurements were performed with Cu target Kα radiation, tube voltage of 40 kV, current of 15 mA, qualitative angular scan range of 5°- 85°, and scan rate of 12°/min. The FTIR measurements were conducted at room temperature (25 ± 1℃), and the background spectrum was obtained using KBr pellets in the range of 4000 to 400 cm^{-1}.

7.3 Results and discussion

7.3.1 Microbial composition and function of coal

In raw coal samples, the microorganisms are assigned to 34 phyla and 98 genera.

Fig.7.1 shows the top 20 microorganisms and their abundances at the genus levels. The dominant species at the genus level include *Candidatus Nitrososophaera*, *Kaistobacter*, *Thermomonas*, and *Lysobacter*. *Candidatus Nitrososophaera* falls under the phylum Thaumarchaeota and the class Nitrososphaeria. And it plays a crucial role in converting ammonia (NH_3) into nitrite (NO_2^-) through the process of ammonia oxidation, which is the first step of nitrification and is closely related to the nitrogen cycle [16-18]. And *Kaistobacter*, *Thermomonas*, and *Lysobacter* also were found in other coal mines [19-21]. These microorganisms are involved in the degradation, transformation and formation processes of coal.

The functions of microorganisms in raw coal were investigated using PICRUSt2 as shown in Fig.7.2. The sequence data were compared using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [22]. Gene abundance was predicted using primary (KEGG L1) and secondary (KEGG L2) functional layers. The results of Fig.7.2 shows that the primary functions of microorganisms in raw coal contain six types (average relative abundance > 1%): cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organic systems. And the main function of microorganisms in raw coal is metabolism. The metabolism function consists of 13 sub-functions: xenobiotics biodegradation and metabolism, Nucleotide metabolism, Metabolism of terpenoids and polyketides, metabolism of other amino acids, metabolism of cofactors and vitamins, lipids metabolism, glycan biosynthesis and metabolism, global and overview maps, energy metabolism, chemical structure transformation maps, carbohydrate metabolism, biosynthesis of other secondary metabolism, amino acid metabolism. And sulfur metabolism is a function of energy metabolism as shown in

Fig.7.3(a). The results of Fig.7.3(b) shows the correlation between microorganisms and energy metabolism in coal. The colors red and green respectively indicate positive and negative correlations, while the symbol * denotes a strong correlation ($p < 0.05$). In Fig.7.3(b), *Arthobacter* and *Flavobacterium* have a close relationship with sub-functions of energy metabolism. They exhibit a negative correlation with methane metabolism, carbon fixation pathways in prokaryotes, and photosynthesis-antenna proteins. On the other hand, they show a positive correlation with photosynthesis, nitrogen metabolism, oxidative phosphorylation, carbon fixation in photosynthesis organisms, sulfur metabolism. And it is evident that *Arthobacter* and *Flavobacterium* have a strong positive correlation with sulfur metabolism. Therefore, it is indicated that *Arthobacter* and *Flavobacterium*, as indigenous microorganisms in raw coal, are beneficial to the removal of sulfur from raw coal.

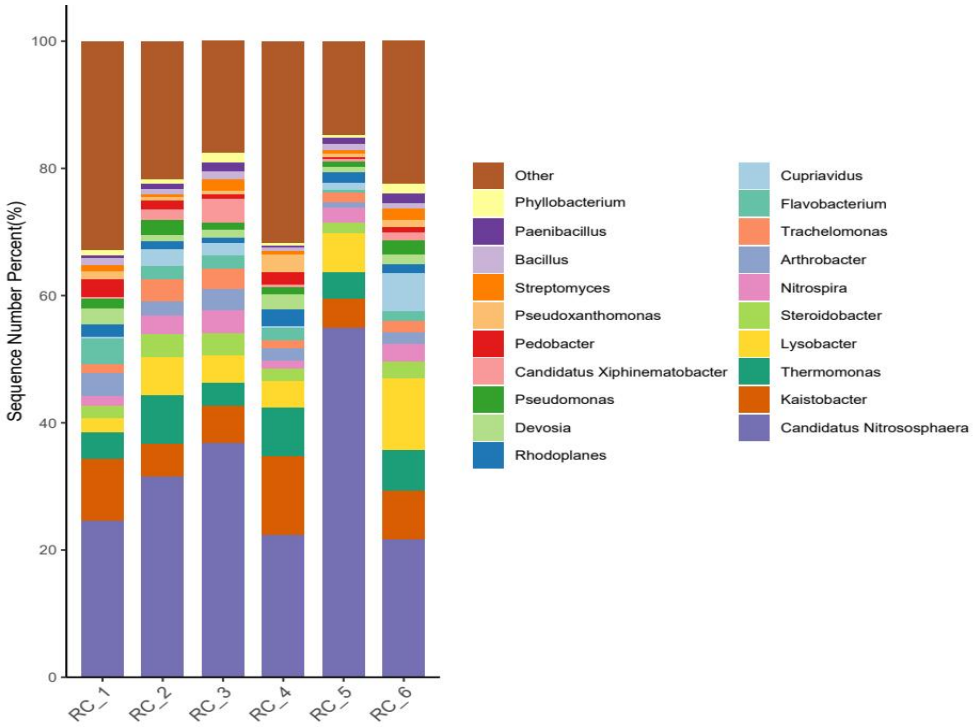


Fig.7. 1 Microbial composition of raw coal at genus level

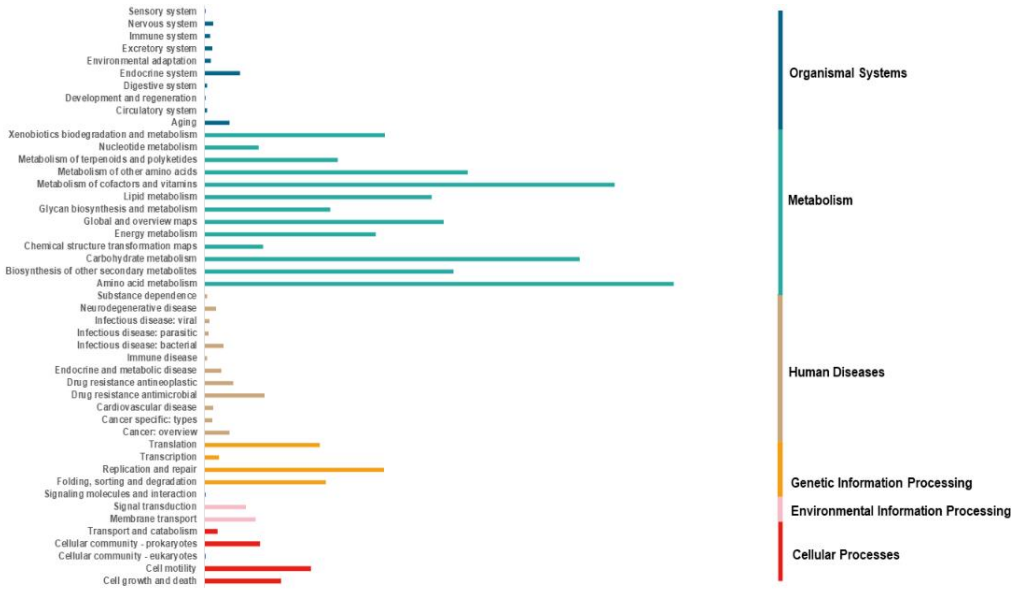


Fig.7. 2 Microbial functions of coal

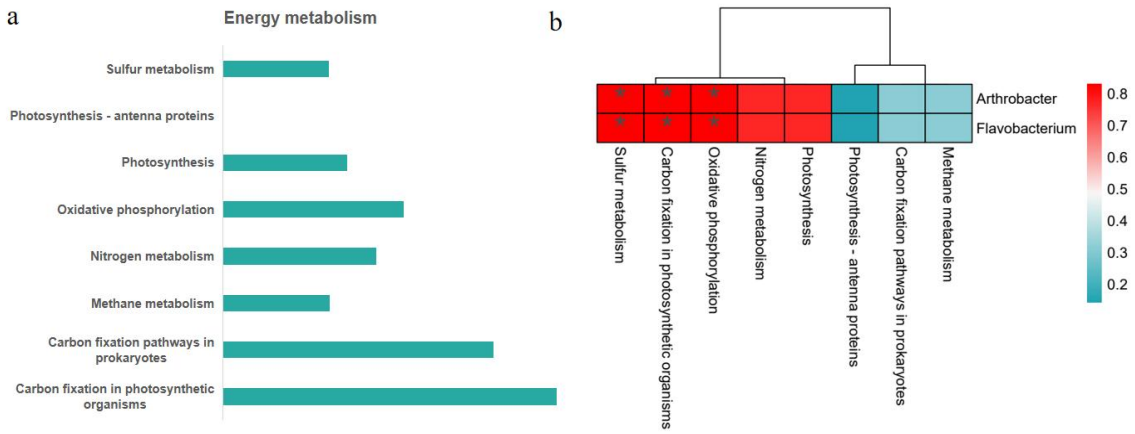


Fig.7. 3 Correlation between microorganisms and energy metabolism in coal Energy metabolism contains sub-functions; b. Correlation between microorganisms and energy metabolism in coal)

7.3.2 Coal Biodesulfurization

7.3.2.1 Identification of isolated bacteria strain

To confirm the desulfurization capability of the indigenous microorganisms，the strain DBW was isolated from the raw coal and the identification result is shown in Fig.7.4. Fig. 7.4(a) shows the phylogenetic tree obtained by comparing the full-length

16S rDNA sequence of strain DBW with GenBank data through PCR amplification. And the result shows that the strain DBW, *Arthrobacter globiformis strain JCM 1332*, and *Arthrobacter globiformis strain DSM 20124* are grouped together. Therefore, the strain DBW isolated from raw coal is identified as *Arthrobacter*. In addition, the SEM results show that *Arthrobacter* is rod-shaped, with a length of about 5 μm (Fig.7.4(b)).

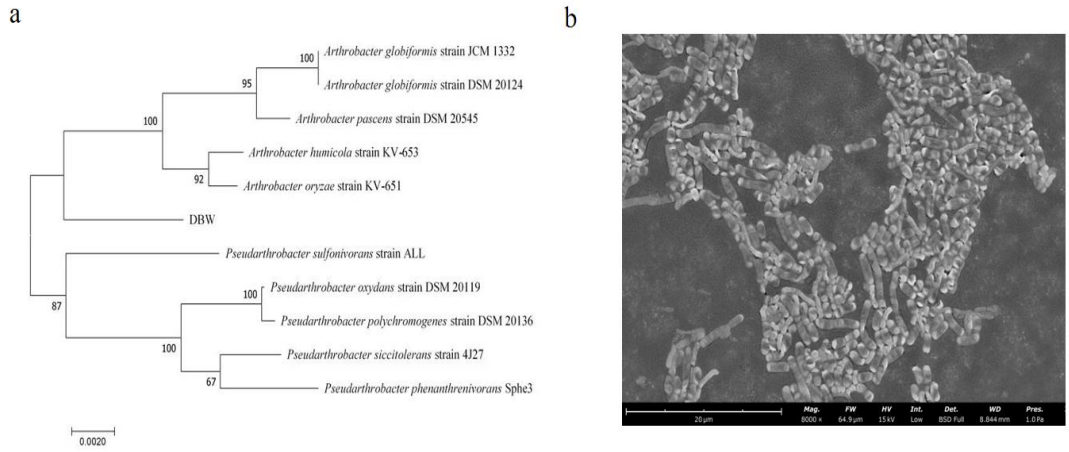


Fig.7. 4 Identification and morphology of microorganisms
(a. Phylogenetic tree of strain DBW based on 16S rDNA sequence
b. Scanning electron micrographs of strain DBW)

7.3.2.2 Coal Biodesulfurization tests

The DBT concentration is linearly related to OD_{330} value as shown in Fig.7.5(a). The standard curve was calculated using the following equation:

$$OD_{330} = 0.7327C + 0.0799 \qquad R^2 = 0.9987$$

where C is the DBT concentration (mmol/l) and OD_{330} is the absorbance.

The results of DBT biodegradation in Fig.7.5(b) show that *Arthrobacter* can grow continuously in the solution containing DBT, and the concentration of DBT gradually degrade with time. At 100 h, the degradation rate reach 50%. Comparing the reported biodegradation rates of DBT by other microorganisms as shown in Table.7.1 [11,12,23-25], it is found that the biodegradation of DBT by *Arthrobacter* isolated from raw coal is significantly superior. Therefore, it can be presumed that the strain can remove organic sulfur from coal.

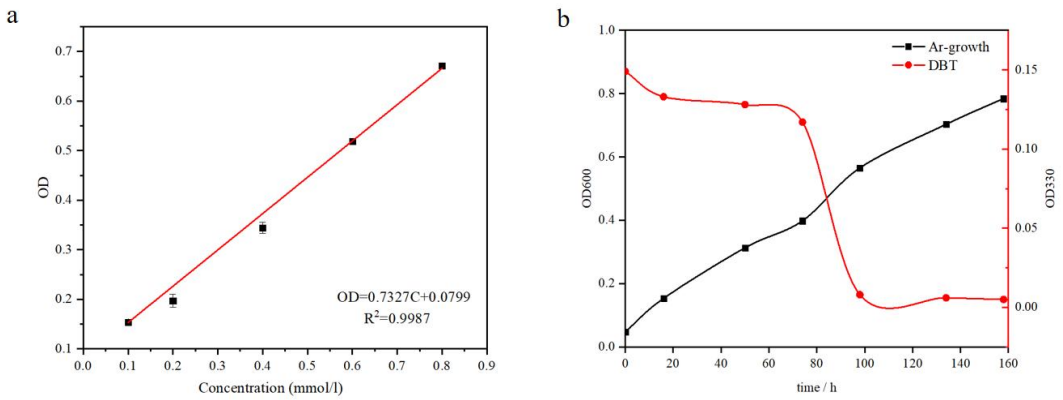


Fig.7. 5 Biodesulfurization of DBT by strain DBW

(a. DBT concentration standard curve; b. Biodesulfurization of DBT by strain DBW)

Table 7. 1 Biodegradation rate of DBT by different bacteria

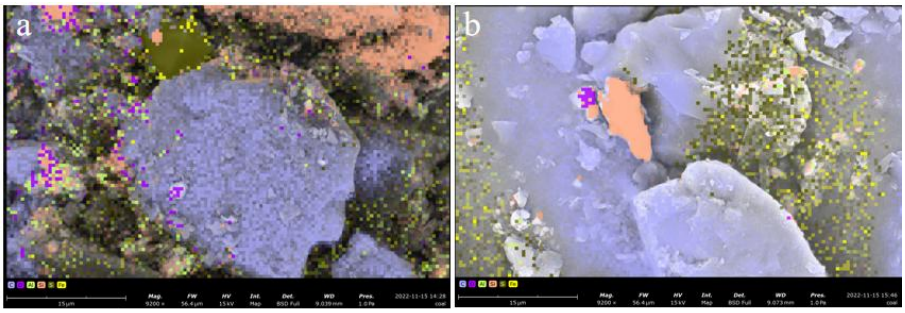
Strains	Desulfurizing Efficiency	Day of Incubation
Arthrobacter sulfureus	50%	15
Lysinobacillus sphaericus	60%	15
Enterobacter spp. NISOC-03	64%	10
Microbacterium strain ZD-M2	70%	5
Gordonia alkanivorans 1B	77%	7
Arthrobacter sp. DBW	**50%**	**4**

The *Arthrobacter* was mixed with the coal sample for a 160 h desulfurization experiment. The comparative results of coal samples before and after biodesulfurization are shown in Fig.7.6, Fig7.7, and Fig.7.8.

Fig.7.6 presents the results of elemental content comparison before and after coal biodesulfurization. Compared to the coal sample before biodesulfurization, the S content of coal after biodesulfurization is significantly reduced and the desulfurization rate is 42%. Meanwhile, the content of Al , Si, and O decreases and the content of C increases. Additionally, the results of mineral composition before and after coal biodesulfurization show that pyrite still remains in the coal after coal biodesulfurization, while the content of kaolinite decreases (Fig.7.7(a)). The results suggest that *Arthrobacter* can remove organic sulfur from coal, but not inorganic sulfur, and it interacts with kaolinite during the biodesulfurization process, resulting in the reduction of kaolinite content. And the results can explain the decrease of Al, Si, O content after coal biodesulfurization. Moreover, the results are supported by the findings in Fig.7.7(b). Fig.7.7(b) presents the comparative results of ash content before and after coal biodesulfurization, which indicate a decrease in ash content of

1.06% after biodesulfurization, likely attributed to the decrease in kaolinite content. The changes of surface functional groups before and after coal biodesulfurization were analyzed using FTIR. The peak intensities at 1097 cm^{-1}, 1031 cm^{-1},and 914 cm^{-1} are significantly reduced after coal biodesulfurization compared with those before coal biodesulfurization (Fig.7.8). The characteristic peaks at 1097 and 1031 cm^{-1} are due to S=O stretching and S-O stretching respectively. And the characteristic peak at 914cm^{-1} is due to -CH3 bending which is characteristic peak of kaolinite. Therefore, the FTIR results also suggest that *Arthrobacter* can effectively remove organic sulfur from coal, while also leading to a reduction in kaolinite content during the biodesulfurization process.

Several studies have also identified microbial interactions with kaolinite. When Ye explored the biodesulfurization of high sulfur fat by exotic microorganisms, it was found that exotic microorganisms could react with O-H groups in kaolinite, causing structural damage [26]. And some microorganisms, such as *Pseudomonas aeruginosa* could bond with kaolinite [27], and assimilated the Si and Al of kaolinite [28]. Therefore, the results of this study are consistent with the previous studies.



Element Symbol	Atomic Conc.
C	82.946
O	11.695
Al	1.497
Si	2.348
S	1.175

Element Symbol	Atomic Conc.
C	87.005
O	10.604
Al	0.429
Si	1.235
S	0.681

Fig.7. 6 Comparison of elemental content of coal before and after biodesulfurization (a. Elemental content of coal before biodesulfurization; b. Elemental content of coal after biodesulfurization)

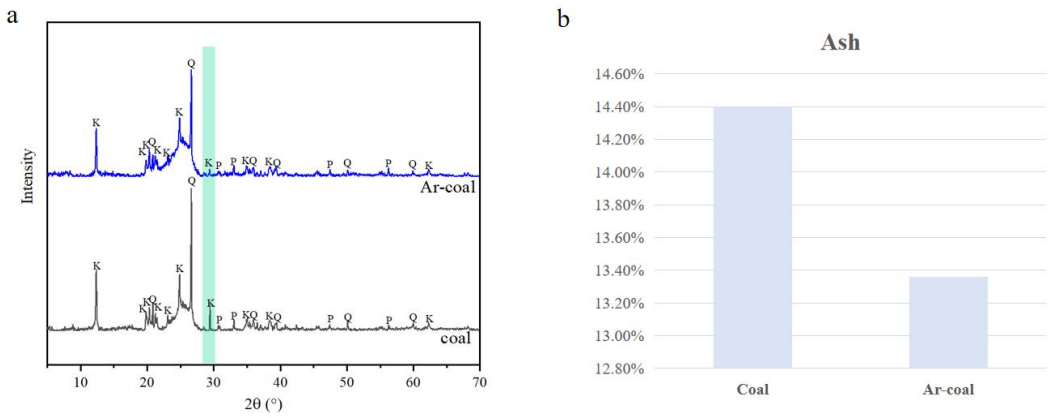
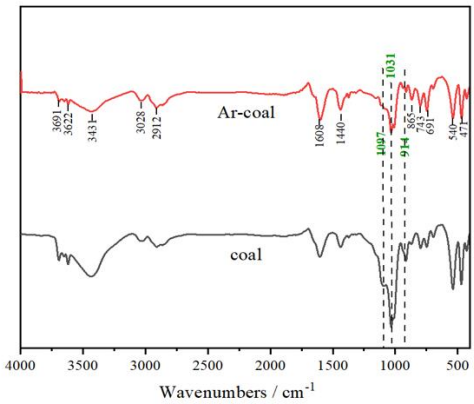


Fig.7. 7 Comparison of mineral composition and ash content of coal before and after biodesulfurization

(a. Comparison of mineral composition of coal before and after biodesulfurization; b. Comparison of ash content of coal before and after biodesulfurization)



Suggested assignment	Peak position (cm^{-1})
O - H stretching	3691, 3622, 3431
C - H stretching	3028, 2912, 2863
C = C stretching vibration aromatic	1608
C = C stretching (alkene)	1440
Amorphous Silica, - S = O stretching	**1097**
S - O stretching or SO_2 symmetric stretching	**1031**
- CH_3 bending	1003, **914**, 865
Silicate minerals with Si - O - Si bending vibration	743, 691, 540
Si - O bending vibration	471

Fig.7. 8 Changes in surface functional groups of coal before and after biodesulfurization

7.4 Conclusions

In this study, the microbial composition in raw coal was analyzed, an indigenous microorganism with sulfur metabolism was identified and successfully isolated from raw coal by analyzing the correlation between microorganisms and functions in raw coal. And the effect of this indigenous microorganism on organic sulfur removal from coal was investigated by biodesulfurization tests.

Raw coal contains a variety of microorganisms, and the dominant microorganisms at the genus level are *Candidatus Nitrososophaera*, *Kaistobacter*, *Thermomonas*, and *Lysobacter*. The main function of microorganisms in raw coal is metabolism function, and the correlation results between energy metabolism and

microorganisms show that *Arthobacter* and *Flavobacterium* have strong sulfur metabolism function. The strain DBW was isolated from the raw coal and identified as *Arthrobacter*. Biodegradation experiments of dibenzothiophene (DBT) using *Arthrobacter* were conducted to investigate the efficiency of indigenous microorganisms in removing organic sulfur from coal. The results show that at 100 h, the biodegradation rate of DBT reach 50%, indicating superior removal of organic sulfur. Further, the desulfurization tests of coal by *Arthobacter* show that the sulfur removal efficiency reach 42% and the ash content is reduced by 1.06% in 160 h. The reason for this phenomenon is that microorganisms can not only remove organic sulfur from coal, but also interact with kaolinite to reduce the content of kaolinite during the microbial desulfurization process of coal.

This study combines microbial functional analysis with the removal of organic sulfur from coal, resulting in the isolation of a highly efficient desulfurizing strain from the raw coal. This finding is beneficial for the removal of organic sulfur from coal and reducing the emission of sulfur dioxide during coal utilization, thereby achieving environmental protection. Moreover, this study provides a new approach for the development and utilization of microbial resources. By focusing on microbial functional analysis, it is possible to explore microorganisms with specific functions.

7.5 References

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Chapter VIII. Discussion

According to this study, the coal flotation system of industrial coal processing plant contains multiple indigenous microorganisms, all of which originate from the coal seam. However, with changes in the growth environment, the microorganisms in the coal flotation system undergo continuous growth and succession. In the coal flotation system, microorganisms can impact flotation efficiency and alter coal sulfur content through behaviors such as adsorption and biodegradation. Therefore, an in-depth study of the composition, succession pattern, and influence on the coal flotation efficiency of indigenous microorganisms can better guide the industrial coal flotation process and improve the quality and yield of clean coal.

1. Microorganisms composition in coal flotation system.

In this study, the dominant microorganisms at the phylum level are similar to previously reported microbial species found in coal, including *Proteobacteria* [1], *Acidobacteria* [2], and *Actinobacteria* [3]. Many bacteria in *Proteobacteria* and *Actinobacteria* can degrade lignin [4], hydrocarbons [5], polycyclic aromatic hydrocarbons, and other macromolecular compounds in coal [6]. *Actinobacteria* and *Acidobacteria* are two types of bacteria that are frequently found in wastewater treatment systems [7,8]. At the genus level, *Nitrososphaera* is the most abundant bacteria. *Nitrososphaera* is a typical ammonia-oxidizing archaea [9]. The nitrogen residue caused by explosives [10]and the occurrence of nitrogen in coal may explain.

2. Environmental factors in coal flotation systems promote microbial community succession

In the coal flotation process, reagents such as collectors, frothers, and modifiers are introduced [11]. These reagents comprise organic and inorganic compounds that can modify environmental factors within the coal flotation system, including chemical oxygen demand (COD), dissolved oxygen (DO), nitrogen content, phosphorus content, and other parameters. The results of Meng and Lu's study showed that residual chemicals from the flotation process increase the COD [12,13]. Besides, the use of mixed reagents is very common to improve flotation efficiency [14]. And the mixed-use of nitrogenous reagents such as waste engine oil [15], biomass oil [16] and amine surfactants (alkyl ether amine, amino acid surfactant [17], dodecyl amine [14] can increase nitrogen content in coal flotation system. In this study, the addition of flotation reagents increase the COD by 340 mg/L, which significantly altered the microbial growth environment. COD content has significant negative correlation with

Nitrospirae, Bacteroidetes, Proteobacteria, and Actinobacteria while positively correlated with GAL15. Therefore, environmental factors in coal flotation systems can change the structure of microbial communities by promoting or inhibiting the growth of specific microorganisms, achieving microbial succession.

3. Microorganisms impact coal flotation efficiency

Microorganisms with different properties adsorb to the surface of coal and associated minerals through electrostatic attraction and hydrophobic interactions, and change the physicochemical properties of coal and associated minerals' surface, which further affects the coal flotation efficiency. The conclusion has been confirmed by many studies. Amini et al. used *Acidithiobacillus ferrooxidans* as an inhibitor of pyrite in coal flotation and reduced pyrite sulfur by 14% under flotation conditions with kerosene as a collector [18,19-21]. Abdel et al. used *Bacillus Subtills* as an inhibitor and removed more than 70% of the sulfur and ash content from the coal [22]. Ramos-Escobedo used *Staphylococcus carnosus* as a collector and achieved 90% coal recovery at pH 9, even higher than that of coal in conventional chemical flotation [23]. In this study, the *Bacillus* isolated from raw coal can adsorb on the coal surface, increasing the number of oxygen-containing functional groups and reducing the hydrophobicity of the coal surface, thereby inhibiting coal flotation efficiency. With an increase in microorganisms from 10^5 to 10^8 cells/ml, the yield of clean coal decreased by 15%.

4. Microorganisms biodegradable flotation reagents

Many organic flotation reagents are added to the coal flotation process, which contain carbon, nitrogen, phosphorus and other elements that can provide the necessary nutrients for the growth of microorganisms. In this process, microorganisms will also destroy the structure of organic chemicals, affecting the efficiency of coal flotation. In this study， the *Sphingomonas* isolated from the raw coal has significant Xenobiotics biodegradation and metabolism function, which can hydrolyze and oxidize the amide group and main carbon chain of polyacrylamide and biodegrade them into smaller molecular weight organic products, thus weakening the flocculation effect of polyacrylamide and affecting the coal slurry settling rate. And many studies have confirmed the biodegradation of organic compounds by microorganisms. The results of Leahy have shown that microorganisms can remove oil and other hydrocarbon-based pollutants from the environment through biodegradation [24]. And Singh's study confirmed microorganisms can degrade organophosphorus compounds

through biodegradation [25]. In addition, the results of Vogt showed that microbial communities degrade organic pollutants in soil and water bodies [26]

5. Microbiological removal of organic sulphur from coal

The organic sulfur in coal is found as refractory polynuclear aromatic sulfur heterocyclics (dibenzothiophene (DBT)). Many studies have confirmed the biodegradation of DBT by microorganisms. Bahuguna isolated a strain of *Lysinibacillus sphaericus* from diesel-contaminated soil, which could remove DBT with 2-hydroxybiphenyl (2HBP) as the desulfurization product, and the efficiency of DBT desulfurization could reach 60% within 15 days [27]. Papizadeh explored the effect of *Enterobacter sp. NISOC-03* on DBT removal in the presence of organic sulfur and glucose as sulfur and carbon sources, showing that *Enterobacter sp. NISOC-03* consumed 63.9% of DBT in 10 days, and after 96 hours, there was 2HBP production [28]. Seo isolated the *Arthrobacter* sp. P1-1 from a polycyclic aromatic hydrocarbon (PAH)-contaminated site, and found that the strain could degrade approximately 82% of DBT in 14 days. In this study, *Arthrobacter* with sulfur metabolism was isolated from coal, which can remove organic sulfur from coal, and 42% of sulfur can be removed in 160 h. In the process of desulfurization, *Arthrobacter* can interact with kaolinite and reduce the kaolinite content in coal [29].

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Chapter IX. Conclusions

The coal flotation system contains a variety of indigenous microorganisms, all of which originate from the coal seam and whose growth and reproduction are limited by the flotation reagents. Some specific indigenous microorganisms can impact the coal flotation efficiency through coal surface adsorption and biodegradation reagent. And indigenous microorganism with strong sulfur metabolism can remove organic sulfur from coal. The conclusions of thesis are as follows:

1. The indigenous microorganisms in the coal flotation system come from the coal seam and are assigned to 34 phyla and 98 genera. Proteobacteria, Acidobacteria, Actinobacteria, and Crenachaeota are the dominant microorganisms at the phylum level, *Nitrososphaera*, *Kaistobacter*, and *Arthrobacter* are the dominant microorganisms at the genus level.

2. Compared with raw coal, the content of chemical oxygen demand (COD) and dissolved oxygen (DO) in the flotation feeding increase by 340 mg/l and 5 mg/l respectively, which inhibit the metabolism of Nitrospirae, Bacteroides, Proteobacteria, and Actinobacteria, while improving the metabolism of GAL15, and change the microbial community and promote the microbial succession in the coal flotation system.

3. The correlation analysis reveal a significant association between indigenous microorganisms and ash contents, implying an adverse effect on flotation. And the *Bacillus* isolated from raw coal can adsorb on the coal surface, increasing the number of oxygen-containing functional groups and reducing the hydrophobicity of the coal surface, thereby inhibiting coal flotation efficiency. With the amount of microorganisms increased from 10^5 to 10^8 cells/ml, clean coal yield decreased by 15%.

4. The *Escherichia coli* as water-borne bacteria can adsorb to the coal surface by hydrogen bonding, which change the hydrophobicity of the coal surface and then reduce the recovery of coal flotation. When *E.coli* concentration reached 5×10^9 cells/ml, only 50.25% of the clean coal could be recovered.

5. The *Sphingomonas* isolated from the raw coal has significant Xenobiotics biodegradation and metabolism function, which can hydrolyze and oxidize the amide group and main carbon chain of polyacrylamide and biodegrade them into smaller

molecular weight organic products, thus weakening the flocculation effect of polyacrylamide and affecting the coal slurry settling rate.

6. *Arthrobacter* with sulfur metabolism was isolated from coal, which can remove organic sulfur from coal, and 42% of sulfur can be removed in 160 h. In the process of desulfurization, *Arthrobacter* can interact with kaolinite and reduce the kaolinite content in coal.

Chapter X. Perspective

Global coal reserves amount to 861 billion tonnes, with low-quality coal constituting more than 50% of this total. Consequently, an increasing amount of coal needs to undergo coal flotation processes to enhance coal quality and reduce carbon dioxide emissions during combustion. This study has revealed the presence of various indigenous microorganisms within the industrial coal flotation system, which proliferate using flotation reagents as a source of nutrients. These microorganisms also adhere to the coal surface, altering its hydrophobic properties and consequently affecting coal flotation efficiency.

Building upon the existing theoretical research, this study will further delve into the relationship between microorganisms and coal flotation processes. The goal is to enhance industrial coal flotation efficiency through microorganism control, with the aim of increasing coal heating value, decreasing carbon dioxide emissions during coal combustion, and mitigating environmental pollution.

Appendix I

1. Articles published in international journals during the Ph.D. studying

1) **Jianbo Li**, Delong Meng, Xizhuo Wang, Zijing Lu, Cui Zhang, Yonghe Peng, Bernardo José Luis Arauz Lara, Shaoxian Song, Ling Xia. "First insight into indigenous microorganisms in coal slurry involved in polyacrylamide biodegradation". *Fuel* 2023;332:126006. (IF 8.035)

2) **Jianbo Li**, Delong Meng, Xizhuo Wang, Bernardo José Luis Arauz Lara, Shaoxian Song, Ling Xia. "Sources and succession of microorganisms in industrial coal flotation syetem". *Fuel* 2023;342:127917. (IF 8.035)

3) **Jianbo Li**, Xizhuo Wang, Delong Meng, Ling Xia, Shaoxian Song, Bernardo José Luis Arauz Lara. "Mechanism study of the impact of *Escherichia coli* on coal flotation". *PLOS ONE* 2022;17(8):e0272841. (IF 3.752)

4) Zhen Wang, Jinhui Chen, Jiaqi Tan, Zijing Lu, Xizhuo Wang, **Jianbo Li***. "The bio-immobilization of Pb(II) induced by the Chlorella-montmorillonite composite in the Ca(II) environment". *Frontiers in Environmental Science* 2022;10:983430. (IF 5.411)

5) Hongliang Li, Wen nan Xu, Feifei Jia, **Jianbo Li***, Shaoxian Song, Yuri Nahmad. "Correlation between surface charge and hydration on mineral surfaces in aqueous solutions: A critical review". *International Journal of Minerals, Metallurgy and Materials*, 2020;27(7):857. (IF 3.850)

6) Xizhuo Wang, **Jianbo Li**, Yinta Li, Shaoxian Song, María Eugenia Farías, Rosa María Torres Sanchez, Ling Xia. "The impact of indigenous microorganisms on coal flotation: A new perspective on water consumption". *Fuel* 2023;337:126848. (IF 8.035)

7) Xizhuo Wang, Delong Meng, **Jianbo Li**, Zijia Zhang, Cui Zhang, Shaoxian Song, Yonghe Peng, Ling Xia. "Composition and dynamics of bacterial communities during flotation in a coal preparation plant". *Journal of Cleaner Production* 2023;385: 135691. (IF 11.072)

8) Yafeng Fu, Hongliang Li, Hao Mei, Zeyu Feng, Ruxia Chen, **Jianbo Li**, Yongmei Wang, Wenbiao Fu. "Organic contaminant removal with no adsorbent recycling based on microstructure modification in coal slime filtration". *Fuel* 2021;288:119630. (IF 8.035)

2. Articles submitted to international journals during the Ph.D. studying

1) **Jianbo Li**, Zi Jing Lu, Xizhuo Wang, Bernardo José Luis Arauz Lara, Shaoxian Song, Ling Xia. "Review of indigenous microorganisms effects on coal flotation". *Minerals and Mineral Materials*, in submission.

2) **Jianbo Li**, Jingwen Pan, Zijing Lu, Zhixiang Wang, Bernardo José Luis Arauz Lara, Shaoxian Song, Ling Xia. "Effect of bentonite on soil formation in copper tailing site". *Science of the total environment*, in submission.

Appendix II

Experimental datas

Table II. 1 Microbial composition of coal samples at the phylum level

Phylum	RC_1	RC_2	RC_3	FF_1	FF_2	FF_3	MW_1	MW_2	MW_3
Caldithrix	0	10	0	12	10	0	31	0	0
Parvarchaeota	11	52	16	58	63	60	50	60	8
Thermi	2	12	3	38	134	57	14	7	7
Acidobacteria	35122	51162	24835	34427	29720	44332	45318	28702	34636
Actinobacteria	9843	13530	20668	17375	36989	22203	39214	31095	15108
AncK6	0	0	0	0	0	2	0	0	0
Armatimonadetes	865	992	955	382	576	783	1133	876	679
Bacteroidetes	24186	7511	8179	4142	11558	5764	12015	12113	29472
BHI80-139	10	0	0	0	0	0	0	0	0
BRC1	168	50	8	34	47	30	26	45	140
Chlamydiae	235	829	161	277	363	523	386	313	447
Chlorobi	452	304	87	62	131	68	160	65	470
Chloroflexi	7660	11618	11430	9664	15808	10818	22026	15531	9223
Crenarchaeota	14989	35668	9998	20741	20975	16041	29873	17808	13992
Cyanobacteria	69	143	106	780	1767	1590	317	216	131
Elusimicrobia	161	328	200	153	196	202	306	199	223
Euryarchaeota	26	111	110	396	621	247	1081	408	7
FBP	33	35	31	0	3	0	10	19	51
FCPU426	0	4	3	0	0	15	7	9	0
Fibrobacteres	188	31	63	31	26	29	28	56	147
Firmicutes	2121	3647	2736	2523	4722	2694	4930	3212	8727
Fusobacteria	2	0	0	0	0	7	0	0	0
GAL15	648	3134	9066	13562	12976	12909	18472	4389	696
Gemmatimonadetes	7084	13231	13461	8003	13625	11352	18708	10433	6877
GN02	22	45	34	6	9	32	79	53	35

GN04	13	51	28	52	134	7	51	51	14
GOUTA4	0	0	0	0	3	0	0	0	0
Kazan-3B-28	3	0	0	0	0	0	0	0	5
Lentisphaerae	0	5	0	0	0	0	0	0	0
MVP-21	3	0	0	0	0	0	0	0	5
NC10	145	284	653	2045	3691	731	2950	2135	175
Nitrospirae	2407	5664	11534	5651	9772	6918	19681	10599	2363
NKB19	3	7	3	0	0	0	3	4	24
OD1	148	445	413	141	235	398	663	559	320
OP11	37	44	29	37	26	13	56	112	91
OP3	29	257	201	133	116	270	247	187	80
PAUC34f	0	0	0	0	0	0	5	0	0
Planctomycetes	8172	9258	4779	6468	6877	8456	9927	7263	10893
Proteobacteria	64869	48025	63620	25185	52040	31601	76839	61952	66827
SBR1093	37	178	49	95	126	129	124	87	29
Spirochaetes	0	0	0	0	8	0	0	0	0
Synergistetes	0	0	2	0	2	0	0	0	0
Tenericutes	4	0	9	5	112	9	6	0	15
Thermotogae	0	0	0	0	2	0	0	0	0
TM6	454	779	436	499	730	291	800	608	531
TM7	99	63	128	80	265	124	224	233	284
Unassigned	2529	3297	1932	2446	2210	2945	3507	2655	2666
Verrucomicrobia	4397	3791	1740	2766	3442	3587	3726	2894	6694
WPS-2	0	0	5	0	15	4	5	0	10
WS2	53	4	0	4	13	3	2	14	44
WS3	964	2758	1829	1726	2144	1877	2723	2040	1081

Table II. 2 Microbial composition of coal samples at the genus level

Genus	RC_1	RC_2	RC_3	FF_1	FF_2	FF_3	MW_1	MW_2	MW_3
Arthrobacter	1265	606	800	1100	3167	2613	2412	1352	3863
Lysobacter	2788	3828	5082	781	2398	788	3447	3480	2470
Trachelomonas	915	916	869	348	1536	674	2153	2161	1038
Thermomonas	5075	2688	2906	510	1889	947	2787	2862	5024
Steroidobacter	1263	1111	1133	931	1756	1277	1926	1604	1138
Kaistobacter	8082	2952	3442	1363	4193	1768	5417	6389	6371
Massilia	712	209	169	143	785	172	322	236	702
Cupriavidus	159	716	2737	247	342	350	462	886	314
Devosia	1555	516	626	257	956	379	1011	684	1848
Candidatus Xiphinematobacter	212	312	547	643	1189	1235	1437	960	345
Pseudoxanthomons	1690	345	496	46	1132	100	1559	341	966
Flavobacterium	884	252	621	46	145	73	163	179	1695
Phyllobacterium	226	290	733	190	702	334	699	905	512
Pedobacter	1162	171	319	35	369	86	195	1619	1834
Aromyces	225	137	263	193	711	239	782	824	527
Nitrospira	804	1355	1228	755	811	999	2234	1324	946
Pseudomonas	659	518	948	246	496	223	1648	699	1431
Paenibacillus	160	420	588	598	1369	771	1691	594	239
Streptomyces	408	339	648	654	985	648	886	677	348
Flavisolibacter	1136	187	222	134	495	119	360	302	1265
Sinorhizobium	130	215	420	185	208	389	445	370	136
Bacillus	291	658	384	301	434	303	474	393	1401
Nitrosovibrio	237	133	0	56	89	51	104	93	321
Rhizobium	148	193	217	65	113	97	238	220	207
Chryseobacterium	61	40	133	30	71	17	75	86	145
Adhaeribacter	808	86	159	136	451	121	248	221	1017
Mesorhizobium	291	247	470	180	648	335	749	671	374
Pontibacter	690	241	109	61	523	134	187	125	717
Arenimonas	741	123	180	30	108	48	106	145	931
Rhodoplanes	1377	931	425	242	343	276	467	422	1621

Kribbella	104	75	226	308	659	394	1098	1476	114
Sphinomonas	802	83	229	135	366	214	555	260	680
Balneimonas	281	81	66	59	160	100	123	116	438
Arobacterium	271	115	89	31	91	48	103	95	448
Leptolynbya	16	90	45	515	1272	1029	128	25	23
Duanella	36	102	686	23	106	50	395	394	61
Sphinopyxis	249	90	139	18	85	42	36	35	322
Pirellula	643	326	63	238	276	213	289	279	779
Hyphomicrobium	246	231	212	210	337	254	480	505	266
Flavihumibacter	309	211	205	70	112	71	174	143	304
Mycobacterium	120	93	173	85	193	142	230	209	159
Azospirillum	0	0	0	0	0	0	0	0	0
Mycoplana	392	175	116	69	169	76	150	161	451
Phenylobacterium	158	55	135	52	83	71	203	193	184
Rubrivivax	247	0	0	0	75	0	108	109	242
Stenotrophomonas	651	114	59	16	52	13	38	31	720
Solitalea	21	81	397	78	49	17	799	329	113
Janthinobacterium	52	0	0	0	242	21	0	32	48
Cohnella	55	142	172	102	83	51	137	48	43
Rubrobacter	235	269	48	100	71	61	31	89	240
Nonomuraea	103	32	25	28	34	27	26	20	120
Promicromonospora	39	13	23	19	61	59	28	40	65
emmata	124	62	39	118	63	149	50	34	103
Opitutus	308	62	35	58	55	40	59	48	394
Solirubrobacter	109	60	48	19	85	25	28	43	93
Cellvibrio	168	53	32	18	56	0	32	70	138
Variovorax	79	28	62	0	0	18	108	110	127
Aeromicrobium	262	139	155	61	156	104	136	197	332
Soranium	143	46	51	12	11	10	66	24	94
Nocardioides	144	28	82	29	122	77	39	54	191
Candidatus Solibacter	150	80	55	101	112	44	100	82	135
Cellulomonas	85	25	21	21	33	26	31	34	128
Acidovorax	66	80	64	62	60	65	95	64	52

Voesella	18	11	80	59	140	0	361	211	11
Skermanella	258	44	90	0	88	78	106	89	441
Achromobacter	89	0	37	13	22	0	27	11	190
Aquicella	78	158	68	76	83	129	187	137	88
Ardenscatena	138	5	27	5	32	6	20	27	213
Luteolibacter	245	72	74	73	71	76	119	91	752
Niastella	35	24	60	26	31	100	26	22	51
Lacibacter	31	24	0	10	11	37	124	34	34
J37-A-70	16	10	90	74	204	44	252	80	9
Planctomyces	412	174	91	60	129	103	63	77	379
Chthoniobacter	164	43	15	13	23	16	25	13	262
Woodsholea	59	94	75	12	48	47	73	45	60
Pedomicrobium	143	80	87	27	34	51	129	63	47
Lentzea	32	43	50	29	58	47	70	29	0
Dokdonella	247	0	20	15	83	0	29	29	144
Chitinophaa	158	0	131	7	150	148	161	140	155
OR-59	33	79	41	103	133	108	145	116	64
Brevundimonas	120	17	26	0	43	23	13	14	237
Ammoniphilus	24	156	0	51	43	0	0	0	32
Novosphinobium	39	54	50	31	29	42	44	37	36
Dactylosporanium	48	0	0	0	0	20	0	0	38
Chelativorans	47	0	20	0	43	0	21	0	58
Ramlibacter	68	17	26	17	31	0	37	0	30
Candidatus Entotheonella	116	165	14	65	0	32	20	13	125
Methylotenera	24	0	0	0	10	0	15	11	32
Afifella	66	60	13	33	11	0	8	16	37
Ochrobactrum	63	0	0	0	0	0	0	13	23
Iamia	61	66	61	71	31	49	11	85	163
Cellulosimicrobim	49	0	0	14	18	106	46	47	43
Olivibacter	10	9	5	0	7	0	10	4	37
Plesiocystis	30	137	27	0	17	17	12	18	45
Saccharothrix	58	18	28	0	11	0	20	31	24
Bosea	18	0	52	0	81	0	158	70	30

Fimbriimonas	68	0	0	8	20	0	11	15	71
Ralstonia	0	10	25	20	50	24	49	59	12
Reyranella	62	24	36	0	0	22	32	14	55
Azoarcus	25	0	0	19	0	0	0	0	64
Caulobacter	0	0	36	0	22	0	0	0	0
Waddlia	35	33	10	31	0	9	0	12	19
Dyadobacter	90	33	34	0	24	0	15	53	291
Catellatospora	23	21	63	33	128	43	88	95	42
Inquilinus	12	9	11	8	50	46	65	30	24
Paraseitibacter	23	22	6	17	110	40	12	0	11
A17	26	26	31	25	0	0	27	29	31
Shinella	58	0	0	0	0	0	0	0	46
Hydroenophaa	63	0	0	0	0	0	0	0	52
Candidatus Rhabdochlamydia	0	183	41	46	74	67	76	51	0
Streptosporanium	26	0	0	0	9	0	0	0	44
Methylibium	111	0	18	0	0	0	79	0	54
Virisporanium	0	0	0	0	0	0	0	0	0
Niabella	60	72	0	0	0	0	0	0	104
Salinibacterium	84	0	0	0	0	0	0	0	165
Sphinobium	51	0	0	6	8	0	0	0	34
Candidatus Koribacter	7	78	0	0	12	0	0	0	21
Pseudonocardia	26	0	0	0	0	0	0	0	41
Chondromyces	40	11	0	0	0	0	0	0	34
lycomyces	0	0	0	15	17	20	20	11	0
Sinulisphaera	29	0	0	26	26	25	0	28	33
Peredibacter	0	25	74	0	64	0	19	55	27
Mucilainibacter	29	9	21	0	0	0	0	10	39
Candidatus Nitrososphaera	14974	35575	9840	20564	19242	15949	29516	16495	13958

Table II. 3 Microbial function prediction at KEGG1 level

Taxonomy	RC_1	RC_2	RC_3	FF_1	FF_2	FF_3	MW_1	MW_2	MW_3
Cellular Processes	355855.5266	343285.73	389064.57	250682.546	396929.841	310044.616	544780.629	385764.266	408796.718
Environmenal Information Processing	132116.2682	130375.88	140187.44	91873.3010	146348.812	113299.199	197721.071	141950.806	153365.713
Genetic Information Processing	595149.469	666040.68	587101.08	483020.814	710954.255	567126.380	961830.305	670188.817	677588.575
Human Diseases	285942.8572	261954.70	277780.89	179362.618	296440.471	222004.625	398292.436	294827.450	324059.123
Metabolism	4197015.531	4279149.7	4260866.6	305880 6.79	473210 6.91	375023 6.01	636822 5.36	457391 1.73	482649 6.21
Organismal Systems	136378.4803	132027.43	136191.82	92864.5588	148615.873	115832.506	196484.934	143686.285	157845.904

Table II. 4 Microbial function prediction at KEGG2 level

Taxonomy	RC_1	RC_2	RC_3	FF_1	FF_2	FF_3	MW_1	MW_2	MW_3
Cell growth and death	111032.335	106444.82	110153.403	76895.9743	120935.836	95973.287	166031.33	120326.060	125198.59
Amino acid metabolism	657168.336	692921.024	674769.652	495719.156	754431.832	599021.85	1017173.3	724801.352	755358.74
Biosynthesis of other secondary metabolites	356383.268	365377.095	349476.439	263711.321	400989.647	324579.63	541066.51	383732.681	406083.09
Carbohydrate metabolism	524514.168	550935.083	533705.253	398803.402	608701.005	487516.65	816974.61	581444.246	610931.75
Chemical structure transformation maps	83570	68912	88543	51832	95803	67776	128999	10155	90928

Energy metabolism	23869 0.399	25810 6.340	24094 6.365	18716 6.848	28136 5.043	22499 4.840	3771 01.88	26496 2.237	2727 28.75
Global and overview maps	33200 3.022	35530 2.224	34255 2.508	25813 7.157	38655 7.844	31179 9.550	5242 65.33	36875 2.552	3822 23.14
Glycan biosynthesis and metabolism	19651 2.792	17617 3.104	16602 4.776	12320 7.155	19395 5.014	15621 7.660	2574 57.80	19002 8.853	2236 14.69
Lipid metabolism	33190 9.519	31432 3.871	33983 8.853	21520 6.732	35071 2.672	27239 9.819	4696 53.09	34802 3.489	3870 02.08
Metabolism of cofactors and vitamins	57443 2.113	61022 6.598	57526 6.920	44506 5.900	66667 0.834	53698 9.133	9021 99.30	63288 5.505	6626 96.87
Metabolism of other amino acids	37595 6.607	37460 8.105	38020 5.485	26789 7.450	42014 4.442	33068 6.132	5655 48.87	40892 4.506	4317 23.84
Metabolism of terpenoids and polyketides	18735 3.058	17961 7.771	20430 1.620	12876 6.126	21330 5.417	16342 6.059	2838 62.99	20980 4.291	2184 89.08
Nucleotide metabolism	75771 .2439	83021 .0009	75073 .5065	59987 .0672	89011 .9096	71100 .1872	1201 17.05	84074 .3907	8680 6.499
Xenobiotics biodegradation and metabolism	26275 1.000	24962 5.489	29016 2.296	16330 6.472	27045 8.248	20372 8.499	3638 13.56	27492 2.629	2979 09.62

Table II. 5-1 Physical and chemical characteristics of coal flotation system

Samples	COD/(mg/L)		
M_1	138	150	150
M_2	36	42	30
M_3	54	60	54
M_4	72	66	60
M_5	78	78	84
T_1	24	24	24
T_2	0	6	6
T_3	138	132	138
T_4	36	30	42
CC_1	2500	2300	2500

CC_2	228	222	228
CC_3	1950	1950	1925
RR_1	180	180	174
RR_2	84	78	90
RR_3	390	384	384
MW	54	42	48
CW	30	30	30

Table II. 5-2 Physical and chemical characteristics of coal flotation system

	NH_4^+-N/(mg/L)			NO_2^--N/(mg/L)			NO_3^--N/(mg/L)		
M_1	1.3354	1.3021	1.2689	0.179	0.17725	0.17725	0.670	0.678	0.658
M_2	0.3982	0.3676	0.4289	0.20814	0.20659	0.20505	0.506	0.513	0.501
M_3	0.3370	0.3063	0.3370	0.21431	0.21277	0.21123	0.700	0.707	0.700
M_4	0.1225	0.0919	0.1531	0.20041	0.19887	0.19732	0.771	0.778	0.766
M_5	0.7352	0.7046	0.7659	0.28382	0.28227	0.28073	0.186	0.194	0.181
T_1	0.2451	0.2144	0.2757	0.25293	0.25138	0.24984	0.980	0.987	0.975
T_2	0.2451	0.2144	0.2757	0.24057	0.23903	0.24057	0.854	0.862	0.849
T_3	0.7046	0.6740	0.7046	0.29772	0.29617	0.29463	0.041	0.049	0.037
T_4	0.2451	0.2144	0.2757	0.21586	0.21431	0.21277	0.651	0.658	0.646
CC_1	0.1838	0.1531	0.2144	0.13400	0.13246	0.13091	0.565	0.572	0.560
CC_2	0.1531	0.1225	0.1838	0.09230	0.09075	0.08921	0.444	0.452	0.439
CC_3	0.3370	0.3063	0.3676	0.05523	0.05369	0.05214	1.314	1.321	1.309
RR_1	0.1838	0.1531	0.2144	0.17107	0.16952	0.16798	0.604	0.611	0.599
RR_2	0.2451	0.2144	0.2757	0.17107	0.16952	0.16798	0.582	0.589	0.577
RR_3	0.1531	0.1225	0.1838	0.14327	0.14172	0.14018	0.5208	0.5282	0.5159
MW	0.0306	0.0612	0.0612	0.00117	0.00117	0.00426	0.2556	0.2630	0.2507
CW	0.3063	0.2757	0.3370	0.18497	0.18342	0.18188	0.6313	0.6313	0.6264

Table II. 5-3 Physical and chemical characteristics of coal flotation system

Samples	TN/(mg/L)			PO_4^{3-}-P/(mg/L)			TP/(mg/L)		
M_1	3.432	3.438	3.4550	0.1787	0.23545	0.2	0.567	0.57298	0.57871
M_2	3.272	3.278	3.2954	0.2081	0.18137	0.15477	0.586	0.60084	0.5865
M_3	3.824	3.830	3.8474	0.2143	0.20637	0.17091	1.068	1.08788	1.10700
M_4	2.433	2.439	2.4561	0.2004	0.09191	0.07063	0.813	0.8133	0.83625
M_5	2.809	2.814	2.8317	0.2838	0.15573	0.13445	1.375	1.38684	1.39831

T_1	3.811	3.817	3.8343	0.2529	0.11488	0.08829	0.371	0.38576	0.40010
T_2	3.618	3.624	3.6409	0.2405	0.08829	0.075	0.471	0.48613	0.50047
T_3	1.224	1.230	1.2470	0.2977	0.16637	0.14509	1.742	1.75389	1.76536
T_4	2.005	2.011	2.0280	0.2158	0.10254	0.08127	1.203	1.21478	1.22625
CC_1	4.142	4.147	4.1647	0.1340	0.12818	0.10159	0.228	0.24238	0.25672
CC_2	2.298	2.304	2.3210	0.0923	0.09191	0.07063	1.031	1.04272	1.0313
CC_3	3.898	3.903	3.9207	0.0552	0.09191	0.07063	0.228	0.23979	0.25126
RR_1	2.719	2.724	2.7415	0.1710	0.11488	0.08829	1.289	1.30340	1.31774
RR_2	4.121	4.127	4.1441	0.1710	0.12818	0.10159	0.328	0.34275	0.35702
RR_3	2.035	2.041	2.0581	0.1432	0.09191	0.07063	1.421	1.43272	1.44419
MW	0.972	0.978	0.9954	0.0011	0.17091	0.13545	0.304	0.32318	0.34230
CW	3.748	3.753	3.7704	0.1849	0.14148	0.11488	0.228	0.24238	0.25672

Table II. 5-4 Physical and chemical characteristics of coal flotation system

Samples	pH	Temperature/℃	Dissolved oxygen/mg/l
FF_1	7.38	26.8	18.9
FF_2	7.6	26.1	13.01
FF_3	8.10	26.0	13.51
CC_1	7.79	26.6	13.45
CC_2	8.28	23.0	14.51
CC_3	7.65	22.3	14.23
M_1	8.03	27.5	13.23
M_2	8.03	27.6	12.64
M_3	7.91	26.0	13.95
M_4	7.96	25.9	14.05
T_1	8.06	26.0	14.30
T_2	8.10	26.0	14.44
T_3	8.17	27.6	14.46
T_4	8.16	27.2	14.04
CW	8.05	25.7	14.41
MW	8.30	23.7	13.89

Table II. 6 Effect of *E.coli* concentration on flotation recovery of coal

Amount of cell addition/ cells/ml	Coal flotation recovery/%
0	86.25
10^5	86.25
10^6	85.75
10^7	84.25
10^8	70.75
10^9	52.5
5×10^9	50.25

Table II. 7 Effect of pH on flotation recovery of coal with *E.coli* at concentration of 5 $\times 10^9$ cells/ml

pH	Coal flotation recovery/%
2	38.5
4	43.5
6	41.5
8	39
10	47

Table II. 8 PAM concentration-absorbance standard curve

PAM concentration/(mg/l)	OD470
10	0.011
50	0.047
100	0.088
250	0.207
500	0.441

Table II. 9 Growth curve of *Sphingomonas* with different bacterial inoculum amounts

	OD600			
Time/h	0ml	1ml	3ml	5ml
2	0	0.018	0.05	0.067
7	0.001	0.102	0.126	0.153
12	0.004	0.118	0.187	0.227
22	0.001	0.155	0.169	0.201

34	0.005	0.134	0.157	0.168
46	0.006	0.118	0.15	0.158
58	0.005	0.114	0.127	0.133
76	0.005	0.12	0.123	0.129

Table II. 10 Biodegradation curve of PAM with different bacterial inoculum amounts

	PAM concentration/(mg/l)			
Time/h	0ml	1ml	3ml	5ml
2	528.25381	536.27425	516.79604	496.17206
7	519.0876	477.83962	478.9854	501.90094
12	507.62983	462.94452	397.63523	480.13118
22	509.92138	527.10803	564.91868	521.37915
34	505.33827	454.92408	474.40229	427.42543
46	500.75516	413.67611	382.74013	348.36682
58	511.06716	344.92949	354.09571	326.59706
76	503.04672	229.20601	175.35449	161.60517

Table II. 11 DBT concentration standard curve

DBT concentration/(mmol/l)	OD330		
0.1	0.156	0.153	0.153
0.2	0.184	0.196	0.211
0.4	0.332	0.346	0.354
0.6	0.513	0.519	0.525
0.8	0.664	0.674	0.675

Table II. 12 Biodesulfurization of DBT by strain DBW

Time/h	OD600	OD330
0	0.047	0.149
16	0.153	0.133
50	0.313	0.128
74	0.398	0.117
98	0.565	0.008
134	0.703	0.006
160	0.784	0.005

Strain DBY gene sequence

GGATCCAGCACCGTGGTCGCCTGCCTCTCTTGCGAGTTAGCGCAACG
CCTTCGGGTGAACCCAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGC
CTGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCC
GCCTTCATGCTCTCGAGTTGCAGAGAACAATCCGAACTGAGACGGCTTTT
GGAGATTAGCGCACTCTCGCGAGTTTGCTGCCCACTGTCACCGCCATTGTA
GCACGTGTGTAGCCCAGCGCGTAAGGGCCATGAGGACTTGACGTCATCCC
CACCTTCCTCCGGCTTATCACCGGCGGTTCCTTTAGAGTCCCCAACTAAAT
GATGGTAACTAAAGGCGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACA
TCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTTCCAGTCCC
CGAAGGGAAGAGATCCATCTCTGGAAATCGTCCGGACATGTCAAACGCTG
GTAAGGTTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTG
CAGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAG
GCGGATAACTTAATGCGTTAGCTGCGCCACCCAAAGACCAAGTCCCCGGA
CAGCTAGTTATCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGT
TTGCTCCCCACGCTTTCGCACCTCAGCGTCAATACCAGTCCAGTGAGCCGC
CTTCGCCACTGGTGTTCTTCCGAATATCTACGAATTTCACCTCTACACTCG
GAATTCCACTCACCTCTCCTGGATTCAAGCGATGCAGTCTTAAAGGCAGTT
CTGGAGTTGAGCTCCAGGCTTTCACCTCTAACTTACAAAGCCGCCTACGTG
CGCTTTACGCCCAGTAATTCCGAACAACGCTAGCTCCCTCCGTATTACCGC
GGCTGCTGGCACGGAGTTAGCCGGAGCTTATTCTCCCGGTACTGTCATTAT
CATCCCGGGTAAAAGAGCTTTACAACCCTAAGGCCTTCATCACTCACGCG
GCATTGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCC
TCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTC
TCAGACCAGCTAAGGATCGTCGCCTTGGTGCGCCTTTACCACACCAACTA
GCTAATCCTACGCGGGCTCATCCTCTGGCGATAAATCTTTGGACTTTCGTC
ATCATCCGGTATTAGCAGTCGTTTCCAACTGTTATTCCGAACCAAAGGGCA
GATTCCCACGCGTTACGCACCCGTGCGCCACTAGACCCGAAGATCTCGTT
CGACTGCATGGTAGGCATCGCACGCC

Strain DBW gene sequence

GGTTAGGCCACCGGCTTCGGGTGTTACCAACTTTCGTGACTTGACGGGCG
GTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGCTGATCTGCGA
TTACTAGCGACTCCGACTTCATGGGGTCGAGTTGCAGACCCCAATCCGAA
CTGAGACCGGCTTTTTGGGATTAGCTCCACCTCACAGTATCGCAACCCTTT

GTACCGGCCATTGTAGCATGCGTGAAGCCCAAGACATAAGGGGCATGATG
ATTTGACGTCGTCCCCACCTTCCTCCGAGTTGACCCCGGCAGTCTCCCATG
AGTCCCCGCCATTACGCGCTGGCAACATGGAACGAGGGTTGCGCTCGTTG
CGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACC
ACCTGTAAACCGACCACAAGTGGGGCGCCTGTTTCCAGACGTTTCCGGTT
CATGTCAAGCCTTGGTAAGGTTCTTCGCGTTGCATCGAATTAATCCGCATG
CTCCGCCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAGCCTTGCG
GCCGTACTCCCCAGGCGGGGCACTTAATGCGTTAGCTACGGCGCGGAAAA
CGTGGAATGTCCCCCACACCTAGTGCCCAACGTTTACGGCATGGACTACC
AGGGTATCTAATCCTGTTCGCTCCCCATGCTTTCGCTCCTCAGCGTCAGTT
AATGCCCAGAGACCTGCCTTCGCCATCGGTGTTCCTCCTGATATCTGCGCA
TTTCACCGCTACACCAGGAATTCCAGTCTCCCCTACATCACTCTAGTCTGC
CCGTACCCACCGCAGATCCGGAGTTGAGCCCCGGACTTTCACGGCAGACG
CGACAAACCGCCTACGAGCTCTTTACGCCCAATAATTCCGGATAACGCTT
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GTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAG
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CACAAAAAGCTTTCCACCCCCCACCATGCGATGAGGAGTCATATCCGGTA
TTAGACCCAGTTTCCCAGGCTTATCCCAGAGTCAAGGGCAGGTTACTCAC
GTGTTACTCACCCGTTCGCCACTAATCACCGGTGCAAGCACCGGATCATC
GTTCGACTG

Appendix III

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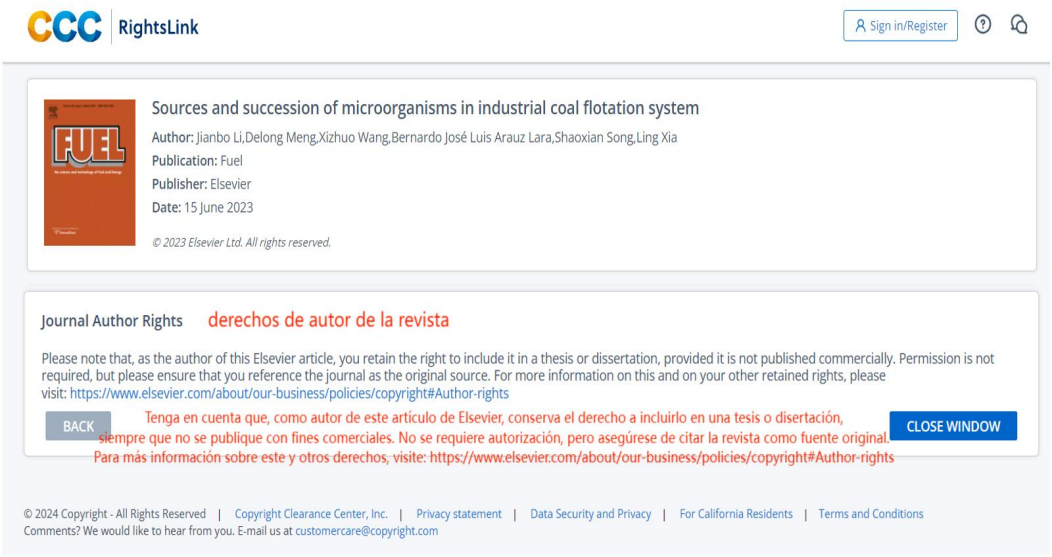

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RESEARCH ARTICLE

Mechanism study of the impact of *Escherichia coli* on coal flotation

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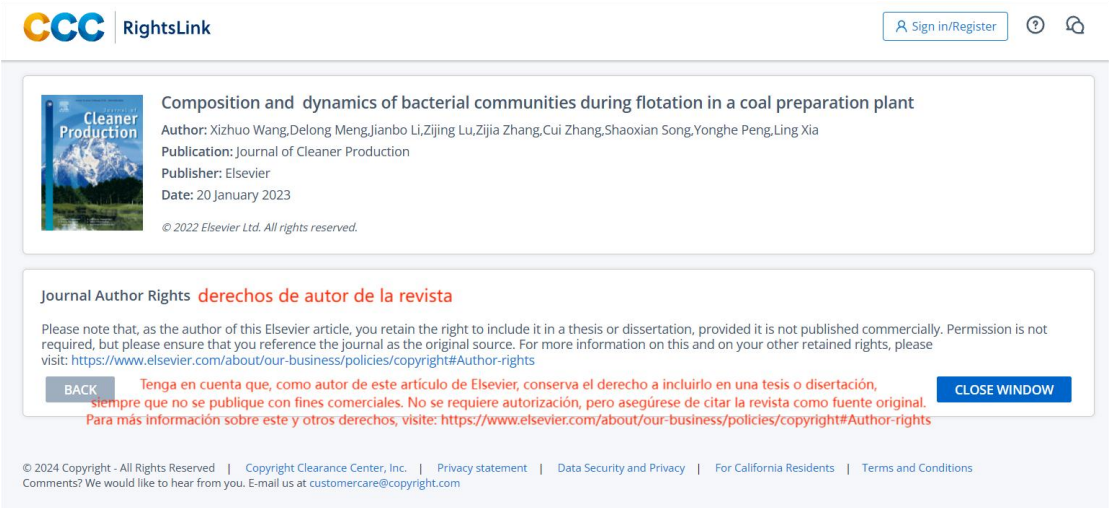
Abstract

Escherichia coli as water-borne bacteria exists in the recirculation water of coal flotation and affects the recovery of coal flotation. In order to study the effect of *Escherichia coli* on coal flotation, we changed the concentration of *Escherichia coli* and pH in the coal flotation system and found that *Escherichia coli* had an adverse effect on coal flotation. The concentration of *Escherichia coli* was negatively correlated with the recovery of coal. When the concentration of *Escherichia coli* reached 5.0×10^9 cells/ml, the recovery of coal flotation was 50.25%, and the change of pH basically did not affect the adverse effect of *Escherichia coli* on coal flotation. The mechanism was studied through Zeta potential, Fourier transform infrared spectroscopy, Scanning electron microscopy and Contact angle measurements. The results revealed that *Escherichia coli* could be adsorbed to the coal surface by hydrogen bonding, which changed the hydrophobicity of the coal surface and then reduced the recovery of coal flotation.

4. Xizhuo Wang, Delong Meng, **Jianbo Li**, Zijia Zhang, Cui Zhang, Shaoxian Song, Yonghe Peng, Ling Xia. "Composition and dynamics of bacterial communities during flotation in a coal preparation plant". *Journal of Cleaner Production* 2023;385: 135691.



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6. Fig.2.3 from Hazrin-Chong N H, Das T, Manefield M. Surface physico-chemistry governing microbial cell attachment and biofilm formation on coal. *International Journal of Coal Geology* 2020;236(3):103671, and I have got a permission to publish paper (Jianbo Li, Review of indigenous microorganisms effects on coal flotation.). All other figures and tables in chapter II are from my own study.

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