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Preliminary Study on the Surface Modification of Lignite and Bioflotation by White-Rot Fungi *Hypocrea lixii* AH

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Abstract: The efficient utilization of lignite is a crucial area of research for the sustainable management of existing coal resources. One potential technique for cost-effective and environmentally friendly coal processing is the application of microbes or their derivatives to modify the surface of lignite for bioflotation. However, the precise process of surface modification between microbes and coal remains largely unknown. In this study, we focused on the use of a white-rot fungus called *Hypocrea lixii* AH and its various components, including spores, hyphae, extracellular polymer substances (EPSs), and culture solution, as biosurfactants for lignite modification. By employing techniques such as zeta potential analysis, induction time measurement, contact angle measurement, and Fourier infrared spectroscopy, we investigated the changes in the surface properties of raw and modified lignite. Furthermore, we conducted a preliminary bioflotation test using biosurfactants as collectors in order to explore the potential application of fungal modification in this context. Our results revealed that all biosurfactants were effective in improving the surface properties of lignite, with the EPS demonstrating the most prominent effect, followed by the culture solution, hyphae, and spores. The zeta potential and induction time of the modified lignite decreased, indicating enhanced hydrophilicity, while the contact angle exhibited a slight increase, suggesting a minor increase in hydrophobicity. Analysis of the Fourier infrared spectra indicated that EPS treatment resulted in the highest abundance of functional groups, including carboxyl, hydroxyl, and amidogen groups. Although fungal cells were found to improve the hydrophobicity of coal, they did not exhibit a significant effect on the flotation of lignite. Nonetheless, our findings suggest that fungal cells and their derivatives have the potential to remove or transform minerals present in lignite, particularly those containing sulfur. While they may not serve as effective bio-collectors in microflotation, their capability in mineral alteration makes them valuable candidates for lignite processing with a focus on mineral reduction.

Keywords: lignite; surface modification; bioflotation; white-rot fungus; extracellular polymeric substances

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1. Introduction

China possesses abundant reserves of lignite. However, the utilization of lignite faces environmental pollution challenges due to its low heating value and high ash content. Therefore, enhancing the efficiency of low-rank coal utilization holds significant importance [1]. Flotation, an effective separation process, is used to remove impurities and enhance the quality of lignite. However, low-rank coal contains hydrophilic functional groups (-COOH and -OH) which can form stable hydration films that prevent the adsorption of oily collectors, resulting in low flotation efficiency [2–4]. Therefore, researchers

have adopted a series of methods, including pretreatment, dry grinding, heat treatment, microwaving, and ultrasonic treatment, to enhance the surface hydrophobicity of lignite for improving flotation [5,6]. Apart from these traditional methods, bioflotation has received increasing attention due to its strong selectivity, environmental friendliness, and economic efficiency [7]. In principle, microbial cells and their metabolites can be used as flotation collectors [8,9], foaming agents [10], inhibitors [11–13], as well as modification agents for bioflotation processes that achieve selective separation through adhesion and chemical reactions [11,14,15]. In the past decades, fungi have emerged as a potential tool in the bioflotation process, with studies highlighting the effectiveness of *Mycobacterium phlei* and *Rhodotorula mucilaginosa* as collectors, wherein their hydrophobicity, influenced by pH, dissolved ions, cell concentration, and other parameters, played a crucial role [8,16]. Furthermore, *Bacillus subtilis*, *Paenibacillus polymyxa*, and *Bacteriophage bdellovibrio* have been used as collectors or depressants in coal bioflotation [11,12].

White-rot fungi (WRF) produce lignin-degrading enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac), which have been employed in coal processing for several decades [17–19]. Certain fungi, such as *Trichoderma atroviride*, *Polyporus versicolor*, and *Phanerochaete chrysosporium*, have been proven to degrade lignin-like structures in brown coal, thus obtaining liquid fuels from brown coal and high-value chemical raw materials [20–22]. Recent studies have explored the application of WRF in coal biocoagulation and desulfurization [23–26]. The use of WRF in coal desulfurization by leaching was shown to remove 40% of sulfur from Turkish brown coal [27]. WRF can attach to the coal surfaces through electrostatic, hydrophobic, or binding forces, and they could produce hydrophobic spores and EPSs, all of which were able to affect the process of coal bioflotation or biocoagulation. Despite extensive research on the applications of white-rot fungi in coal processing, desulfurization, and bio-agglomeration, limited attention has been given to their use in flotation processes and their impact on coal modification and flotation efficiency. Several mechanisms and unknown aspects of bioflotation remain worth exploring.

In our previous work, we discovered that white-rot fungi (WRF) achieved a liquefaction rate of 40% for coal, demonstrating promising liquefaction effects [28]. However, a considerable amount of residual coal remained after liquefaction. To maximize the utilization of this residual coal, we sought to investigate the potential of WRF in coal flotation. In the present work, we chose white-rot fungi and their derivates (including their spores, hyphae, extracellular polymer substance (EPS), and culture solution) as the biosurfactants for lignite bioflotation. Our objectives are to explore the potential of white-rot fungi as a bioflotation agent in lignite utilization, identify their effective components, evaluate the flotation efficiency of white-rot fungi on lignite using various methods, determine the metabolites produced by the fungi that contribute the most to the flotation effect, and investigate the underlying mechanism of bioflotation activity exhibited by white-rot fungi. This research endeavors to provide novel ideas and methods for lignite utilization and bioflotation, thus contributing to the advancement of this field.

2. Materials and Methods

2.1. Culture and Preparation of Biosurfactants

The fungal strain *Hypocrea lixii* AH (accession number in NCBI: FJ645728) was obtained from our laboratory [29]. The strain was activated and cultured in the Salt Meat Broth (SMB) medium [29] until it reached the stable phase (about 6 days). On the 6th day, the culture solution obtained was filtered with filter paper (Double ring, Shanghai China, 100 μm) to collect hyphae. The filtrate was centrifuged at 5000 rpm to precipitate the spores. The supernatant was filtered (0.45 μm, BBI, Shanghai, China) to obtain the filtrate containing the EPS. One hundred milliliters of ethanol (Molecular Biology Grade $\geq$ 99.7%, BBI, Shanghai, China) was added to the filtrate, and the mixture was stored at 4 °C for 12 h. The precipitate was designated as the crude EPS. The alcohol in the supernatant fraction was evaporated under negative pressure, and the residual solution was designated as the culture solution.

The crude EPS was purified using a 3500 Da MWCO dialysis membrane (F132590, BBI, China) for 12 h.

2.2. Lignite Sample and Biotreatment Test

The lignite was obtained from the Shengli coal mine in the eastern part of Inner Mongolia Province, China (39.4667° N, 109.9833° E). Before the experiment, the lignite was crushed, ground, and sieved to a particle size of 0.074–0.125 mm. During the biotreatment test, the prepared 1 g lignite sample was mixed with excessive biosurfactants: hyphae (1 g wet weight in 10 mL sterilized culture medium), spores (2.0×10^8 cells·mL^{-1} in 10 mL sterilized culture medium), EPS (0.001 wet weight EPS in 10 mL sterilized culture medium), and 10 mL culture solution for 1 h. To investigate the lignite's bioflotation characteristics after biotransformation, the raw lignite was cultured with WRF for 7 days according to our previous work [30], and the residual coal was collected for further testing. The obtained biotreated lignite samples were then filtered and rinsed to remove any free biosurfactants or cells. Subsequently, the biotreated coal samples were freeze-dried for 24 h before further analysis.

2.3. Surface Property Tests

2.3.1. Zeta Potential Analysis

Zeta potential measurements of the raw and biotreated lignite samples were conducted using a Zeta-meter (Nano ZS90, Malvern company, Malvern, UK). Two grams of the coal sample were added to 100 mL of a KCl solution (10^{-3} mol·L^{-1}). The pH of the solution was adjusted within the range of 2–12 using HCl (1.0 mol·L^{-1}) or KOH (1.0 mol·L^{-1}). The suspension containing lignite particles was transferred into an electrophoresis cell for measurements. The testing conditions were as follows: temperature: 28 °C, voltage: 10 V, switching time of voltage: 700 ms. Ten readings were recorded, and the mean value was used to represent each sample. For each measurement, 10 separate readings were calculated to take the average value, and the test was repeated 3 times.

2.3.2. Induction Time Analysis

The induction time measurements between air/oily bubbles and coal particles before and after pretreatment were performed with a 2015EZ Induction Timer Instrument (Edmonton, AB, Canada) as performed in our previous work [5]. A bubble was knocked out by a capillary and induced to attach to the lignite surface. A time pulse was set. If the time pulse was long enough, there would be lignite powders adhering to the bubble. Otherwise, there would be no adhesion. The shortest time pulse enabling adhesion was determined as the induction time. All measurements followed our previous work [5], and all the measurements were repeated ten times, and the average value of them was calculated and taken as the final result. The data were analyzed using IBM SPSS Statistics 27 to conduct a one-way ANOVA. The purpose was to compare the significant differences between the raw coal and the various treatments. The significance level used for assessing differences in means was set at 0.05.

2.3.3. Contact Angle Analysis

In order to accurately assess the difference in hydrophobicity of the coal samples before and after biotreatment, the raw and biotreated lignite samples were pressed for 50 s with 15 MPa of pressure into thin circular plates. The contact angle was measured with a contact angle apparatus (Zhongchen, Shanghai, China), and the detailed process followed the reference [5]. The goniometry method was used to decide the contact angle value, and both sides of the droplet were measured to take the average value. Five trials were conducted to take the average value for each measurement. The data were analyzed using IBM SPSS Statistics 27 to conduct a one-way ANOVA. The purpose was to compare the significant differences between the raw coal and the various treatments. The significance level used for assessing differences in means was set at 0.05.

2.3.4. Fourier Transform Infrared Analysis

FT-IR analysis was performed on coal samples using an infrared spectrometric analyzer (Nicolet380, Nicolet Company, Madison, WI, USA). Pellets were prepared by grinding 2 mg of the samples and 100 mg of dried KBr into powder, which were then pressed into molds. Signals were acquired in the range of 400 cm^{-1} to 4000 cm^{-1} with a resolution of 4 cm^{-1}. Twenty scans were performed for each sample and background to obtain the IR spectra. The acquired spectra of lignite samples were processed using PeakFit 4.12 (SeaSolve Software Inc., San Jose, CA, USA). Additionally, the alkyl chain structure in coal was further investigated by employing the structural parameter $A(CH_2)/A(CH_3)$ to characterize the length and branching degree of the alkyl chains in the samples.

2.4. Bioflotation Test

The bioflotation experiment was conducted in a 60 mL flotation cell (XFG-63 type) at a constant impeller speed of 1800 rpm with a pulp concentration of 60 $g \cdot L^{-1}$. Initially, n-dodecane was used as the collector but it was inefficient even at high concentrations. Later, diesel oil was subsequently used as a collector with an optimized concentration of 10 kg/t. For bioflotation, the fungal cells and spores with a concentration of 2×10^8 cells$\cdot mL^{-1}$, 10 $kg \cdot t^{-1}$ EPS, and culture solution were used to replace diesel oil as the collector. Methyl isobutyl carbinol (MIBC) was used as the foam agent in all flotation experiments at a concentration of 500 $kg \cdot t^{-1}$.

In each test, the raw biotreated and bio-transformed coal samples were mixed with tap water for 2 min. After that, the collector agent was added and conditioned for another 2 min. The frother was added into the mixture and stirred for 1 min. Upon completion of the tests, the froth concentrates and flotation tailings were filtered, vacuum dried at 60 °C, weighed, and taken for ash determination to obtain the last recovery of concentrate and combustible matter.

3. Results and Discussion

3.1. Zeta Potential Results of Raw and Biotreated Lignite

Zeta potential, which represents the surface charge of particles, is an important parameter indicating the stability of colloidal dispersion systems. In this study, the zeta potential of raw and biotreated lignite samples was measured to evaluate the surface charge changes after biotreatment. As shown in Figure 1, an increase in pH led to a decrease in the zeta potential of all samples. This trend can be attributed to the replacement of hydrogen ions (H+) by hydroxide ions (OH-) on the coal surface, resulting in an increase in negative charge. The surface of lignite contains some charged chemical functional groups, such as carboxyl groups and hydroxyl groups, which contribute to its surface charge [31]. The addition of biosurfactants interacted with these functional groups, altering the surface charge density and reducing the zeta potential. Compared to the isoelectric point of the raw lignite (6.6), the samples treated with biosurfactants exhibited lower isoelectric points: 6.2 for spores, 5.6 for hyphae, 4.3 for the EPS, and 5.4 for culture solution. This observation can be attributed to the negative charges carried by the chemical substances, such as proteins, sugars, and nucleic acids, present on microbial cell surfaces or in their metabolites [32]. These substances contain functional groups with negative charges, such as acidic residues and phosphate ions, contributing to the negative charge on the surface of microorganisms.

EPS treatment resulted in the lowest zeta potential within the pH range of 2–5.5, indicating stronger adherence to the coal surface compared to the other biotreatment methods [33,34]. The EPS, containing carboxyl and phosphate groups in its polysaccharide molecules, exhibited higher electronegativity, attracting positively charged ions in the surrounding environment and increasing its negative charge. The EPS can also modify its electrical properties by altering the chemical structure of its polysaccharide molecules, such as branching, chain length, or functional group types. At pH values between 7.0 and 11.0, the EPS-treated lignite showed significantly higher negativity than untreated lignite, indicating effective adsorption between microorganisms and coal particles through

electrostatic forces. Other factors such as acid–base reactions and hydrophobicity may also play a role in the interaction between microorganisms and minerals [35,36]. Most studies have shown that good adsorption occurs at the isoelectric point, while some studies have reported that maximum adsorption can be achieved for certain microorganisms and minerals under neutral or alkaline pH conditions [11,34]. In some extreme cases, pH has shown almost no effect on adsorption [12], which could be attributed to the disruptive effects of extreme acidic or alkaline conditions on microbial structures [37].

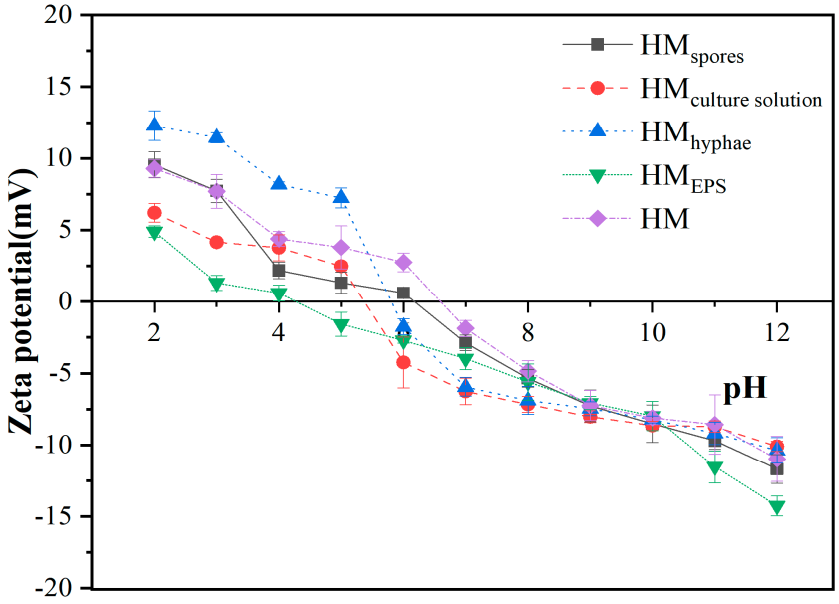

Figure 1. Zeta potential of lignite before and after processing with different biosurfactants (HMspores—lignite processed by spores, HMculture solution—lignite processed by culture solution, HMhyphae—lignite processed by hyphae, HMEPS—lignite processed by EPS, HM—unprocessed lignite). (The error bars indicate the standard error).

3.2. Contact Angle and Induction Time Results of Raw and Biotreated Lignite

As shown in Figure 2a, the contact angle of untreated coal was approximately 54°. After biotreatment, the contact angle increased to 56° for hyphae, 61° for the culture solution, and 63° for the EPS, while the contact angle of spores remained unchanged at 54°. Among the four biosurfactants, EPS treatment had the most significant effect on the contact angle, indicating that the EPS resulted in the highest hydrophobicity. This may be due to the presence of hydrophobic alkyl groups or aromatic structures in the EPS. A thinner hydration film on the surface of hydrophobic particles reduces surface tension, facilitating the attachment of particles to the bubble surface [38]. The induction time reflects the mineralization process between bubbles and coal particles, affecting the adhesion between them and ultimately influencing flotation efficiency. As shown in Figure 2b, the coal samples treated with biosurfactants exhibited decreased induction times compared to the raw coal. The induction time of raw coal was 230 ms, whereas the induction times for the EPS-treated coal, culture solution, spores, and hyphae were 117 ms, 167 ms, 200 ms, and 217 ms, respectively. EPS treatment resulted in the shortest induction time, indicating its strong surface activity and adsorption ability. The EPS rapidly interacted with the coal surface, leading to surface property changes that improved flotation efficiency. These results align with the changes observed in the zeta potential, supporting the effective adsorption of the EPS onto the coal surface.

The initial attachment phase, as discussed by Dwyer [39], represents the initial stage of interaction between biosurfactants and coal. This phase arises from a combination of attractive and repulsive forces between cells and the target surfaces [40]. The interactions are influenced by the physicochemical properties of the cell surface, where cell adhesion

primarily relies on the molecular structure and arrangement of surface molecules. The presence of adhesive molecules on the cell surface, along with extracellular matrix and glycosylation modifications, contributes to cell adhesiveness through various types of interactions upon contact with external objects. Due to the unknown chemical composition of these four biogenic surfactants, providing an accurate description of how they enhance surface performance is challenging. Nevertheless, the disparity in induction time suggests that extracellular polymeric substances (EPSs) might augment the affinity of brown coal for bubbles, possibly due to alterations in its properties.

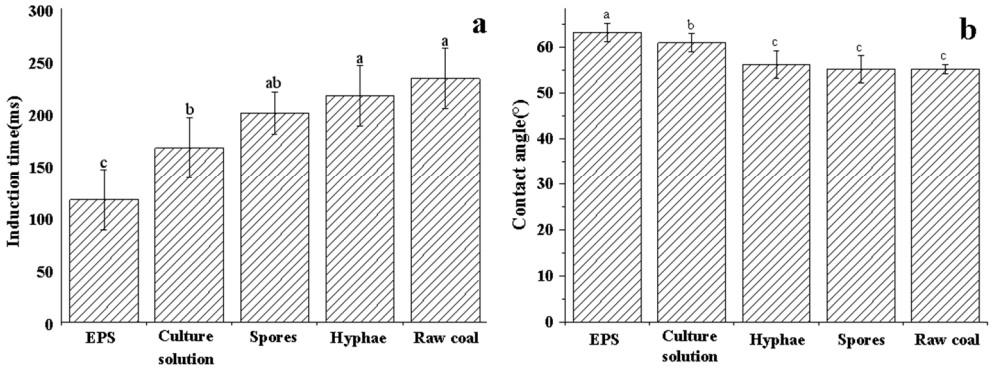

Figure 2. Induction time (**a**) and contact angle (**b**) of raw lignite and processed lignite. Different letters indicate significant differences between treatments ($p < 0.05$).

3.3. FT-IR Spectra of Raw and Biotreated Lignite

Figure 3 shows the FT-IR spectra of the raw coal and biotreated coal samples with four different biosurfactants. The absorption band at 1000–1800 cm^{-1} corresponds to oxygen-containing functional groups, mainly including -OH (alcohol and phenol hydroxyl groups), -COOH, C=O, etc. The presence of these functional groups contributes to the hydrophilicity of lignite. Upon contact with surfactants, the hydrogen bonding between the oxygen-containing functional groups enhances the adhesion between surfactants and coal, resulting in increased hydrophobicity [41]. The intensity of the absorption bands slightly decreased after biotreatment, suggesting potential decomposition of aromatic compounds or chemical reactions between microbial metabolites and lignite [42,43].

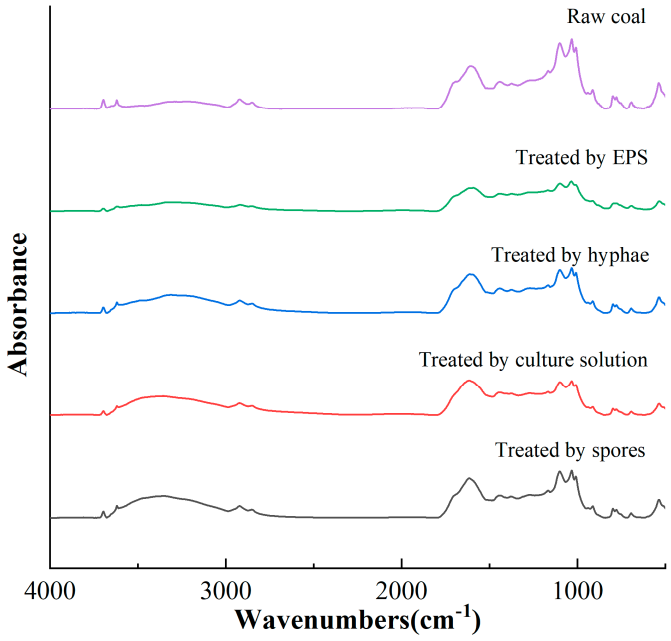

Figure 3. FT-IR spectra of raw and biotreated lignite samples.

The FT-IR analysis suggests that the biotreated lignite exhibited an increased presence of polar groups, particularly -OH and -COOH, compared to raw lignite. These polar functional groups, formed by organic acids in the culture solution or EPS, can impart negative charges to the lignite surface, leading to changes in its zeta potential. Additionally, these functional groups can also interact with water molecules through hydrogen bonding, enhancing the hydrophilicity of the lignite surface.

Coal contains various functional groups, making it challenging to accurately determine peak positions and heights in the FT-IR spectrum due to spectral overlap. However, peak fitting was performed on selected absorption bands to analyze specific functional group changes. Sub-peak fitting revealed changes in the relative content and position of functional groups.

The range of 3650–3000 cm^{-1} is assigned to the absorption vibration zone of -OH, which includes various hydroxyl structures in addition to free hydroxyls between 3650–3560 cm^{-1}. This range also includes the -OH stretching vibration absorption peak of adsorbed water as the experimental wafer inevitably absorbs moisture from the air during testing. Therefore, we performed peak fitting on the absorption peaks between 3650–3000 cm^{-1} while disregarding any interference caused by adsorbed water.

As shown in Figure 4(a_1,a_2), the content of free hydroxyls is relatively low in both coal samples, and the relative content of free hydroxyls significantly decreased in the sample treated with the EPS from 4.96% to 1.97%. It might be caused by the hydroxyl's modification on the coal surface by the EPS. In addition, EPS treatment may stimulate the chemical interaction on the coal surface, leading to changes in functional group structures. Cyclically bound hydroxyl hydrogen bonds are the main type of hydroxyl hydrogen bonds in raw coal, accounting for 83.03%. In the EPS-treated coal sample, a specific peak (3339.7 cm^{-1}) in the raw coal disappeared, and the frequency of 3339.7 cm^{-1} usually corresponds to the -OH vibration formed by hydrogen bonding with hydroxyls. This disappearance suggests that the compound represented by the peak may have undergone conversions during the biotreatment process. The alterations in the surface groups of the EPS-treated coal samples coincide with the previously mentioned changes in zeta potential and contact angle of the EPS-treated lignite. While a direct correlation between individual observations cannot be established, the consistency between these results implies a change in the surface chemistry of coal induced by the EPS, particularly in terms of hydroxyl content and hydroxyl–hydrogen bonding.

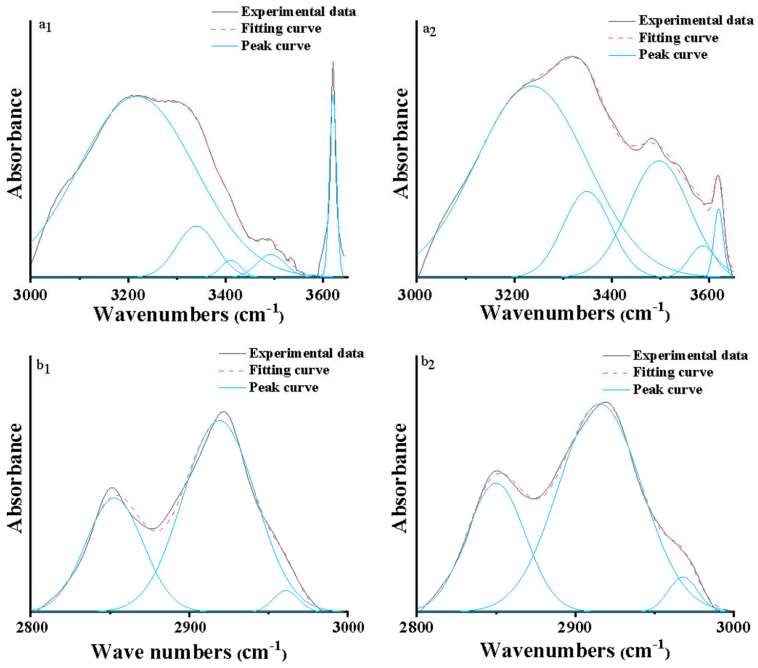

Figure 4. Peak fit of raw coal ($\mathbf{a_1}$,$\mathbf{b_1}$) and EPS-treated coal ($\mathbf{a_2}$,$\mathbf{b_2}$).

The absorption band of coal samples within the 3000–2800 cm^{-1} range is assigned to the stretching vibration band of aliphatic hydrocarbon structures. The fitting results are shown in Table 1 and Figure 4(b_1,b_2). The relative contents of these functional groups did not exhibit obvious changes between the raw coal and the EPS-treated coal samples, although variations were observed in the peak positions. In particular, the appearance of the 2968 cm^{-1} peak suggests that the biotreated coal might contain more aromatic structures. -CH_3 and -CH_2 are usually considered hydrophilic functional groups, while aromatics are considered hydrophobic. Therefore, based on the relative content and position changes of the CH groups in the raw coal and the EPS-treated coal samples, it is possible that EPS treatment decreased the content of hydrophilic functional groups in coal and increased the content of hydrophobic functional groups. The structural parameter $A(CH_2)/A(CH_3)$ was used to evaluate the length of aliphatic chains and degree of branching in coal samples. The EPS-treated lignite exhibited a higher $A(CH_2)/A(CH_3)$ value (2.44) than raw lignite (2.13). This suggests that EPS treatment resulted in longer aliphatic chains and a higher degree of branching.

Table 1. Relative content of aliphatic hydrocarbon structure sub-peaks in raw coal and EPS-treated coal samples.

Samples	Antisymmetric Stretching Vibration of CH_3 (%)	CH Stretching Vibration (%)	Symmetric Stretching Vibration of CH_2 (%)
Raw coal	66.22	2.60	31.18
Treated by EPS	66.91	3.99	29.10

The 1800–900 cm^{-1} absorption band of coal samples is assigned to the absorption vibration zone of oxygen-containing functional groups, which includes stretching vibrations of -COOH, -C=O, -OH, and ether bonds, as well as the bending vibration of -CH_3 and -CH_2, the C=C stretching vibration of aromatic or fused rings, and the absorption peaks of Si-O-Si and Si-O-C (ash content). Within this range, both coal samples were divided into 13 sub-peaks (Figure 5(a_1,a_2)), indicating similar chemical structures and compositions. However, notable differences were observed between the raw coal and the EPS-treated coal sample. In the raw coal, prominent peaks appear at 1098.9 cm^{-1} and 1611.9 cm^{-1}, indicating the presence of a large number of carboxylic acid and aromatic ring structures. In contrast, the most obvious feature in the EPS-treated coal was the appearance of a new peak at 1256.9 cm^{-1}, potentially indicating the formation of new chemical bonds or functional groups in the coal.

Additionally, in the raw coal, the two highest relative peak intensities were 21.08% (1611.9 cm^{-1}) and 18.32% (1098.9 cm^{-1}), indicating a substantial presence of aromatic ring structures and -COOH in the coal. Conversely, in the EPS-treated coal, the peak with the highest relative intensity is 48.73% (1708.6 cm^{-1}), corresponding to the stretching vibration of the C=O bond in ketones and aldehydes. This suggests that, during the treatment process, some important components in the coal may have been removed or decomposed. Furthermore, it is evident that the content of most functional groups decreased in the EPS-treated coal, including a reduction in Si-O-C (ash content). This can be attributed to the capability of EPS treatment to remove undesirable impurities and inorganic substances from coal, thus improving its quality.

In the FT-IR spectrum of the coal sample, the absorption vibration region of aromatic hydrocarbon structures is observed between 1000–700 cm^{-1} (Figure 5(b_1,b_2)). Changes in the peak positions and relative intensities indicate alterations in the aromatic hydrocarbon structures in the coal sample after EPS treatment. The dominant absorption peak observed in the unprocessed coal sample, which was detected at a wavenumber of 797.82 cm^{-1}, corresponds to the stretching vibration of the C-H bonds within the aromatic ring structure. Conversely, in the coal sample treated with EPS, the most intense absorption peak shifts to a lower wavenumber, specifically 763.13 cm^{-1}, suggesting the presence of newly formed functional groups that exhibit distinct vibrational characteristics. Furthermore, an increase

in the absolute content of distinct sub-peaks, accounting for 46.77% as opposed to 31.05% of the total peak area, suggests modifications within the aromatic hydrocarbon framework following EPS treatment. These alterations may involve molecular restructuring or the conversion of specific functional groups, such as phenols, ethers, or carboxylic acids.

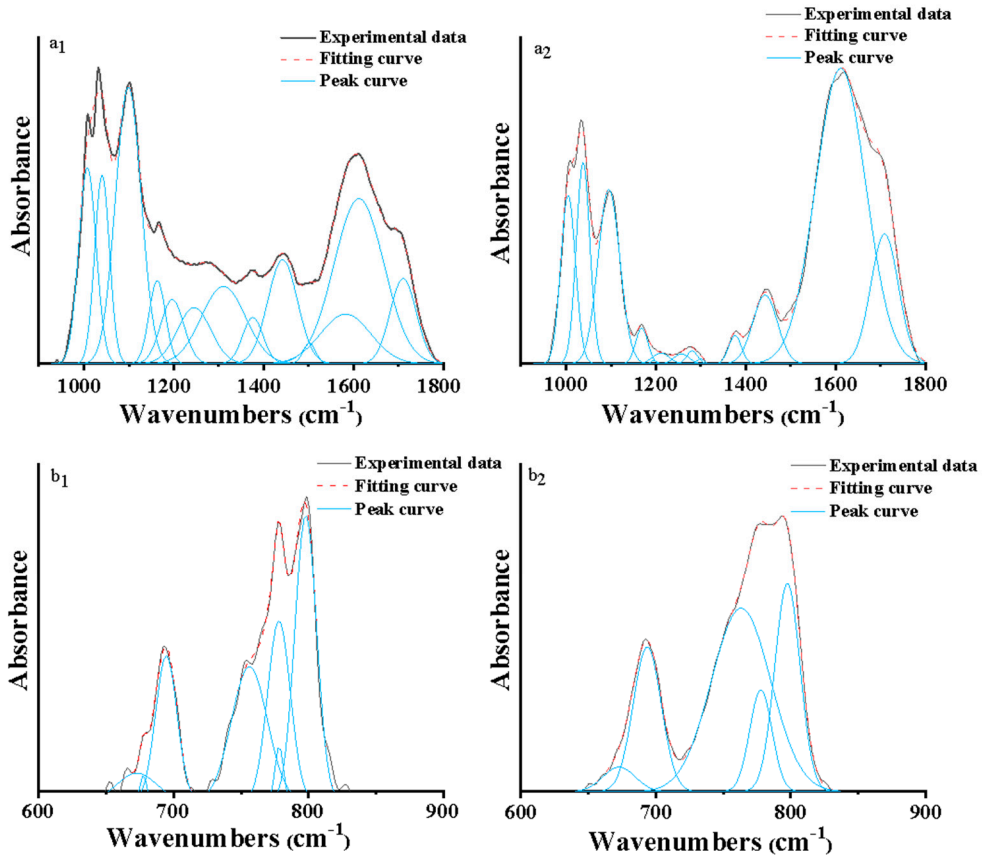

Figure 5. Peak fit of raw coal (**a1**,**b1**) and EPS-treated coal (**a2**,**b2**).

As a surfactant, EPSs can improve the wetting effect between foam and coal particles, thereby improving flotation efficiency. The aromatic hydrocarbon structure in coal is often closely related to the hydrophobicity/hydrophilicity of coal. If EPS treatment strips benzene rings and increases the surface hydrophobicity, it may reduce the difficulty of coal flotation. Therefore, appropriate EPS treatment processes should be selected according to specific conditions and optimized in combination with other flotation reagents.

3.4. Bioflotation Tests of Raw and Biotreated Lignite

The results presented in Table 2 demonstrate the low flotability of lignite, with all flotation recoveries below 33%, even with the addition of excessive diesel oil. The maximum concentrate coal recovery rate (31.95%) was achieved with a diesel oil concentration of 10 kg·t^{-1}. Interestingly, the fungal cells did not show an improvement in flotation efficiency and even hindered the flotation process, resulting in negligible concentrate coal recovery when hyphae were used as the collector. Among the biosurfactants, the EPS showed the most promising results as a bio-collector with a recovery rate of 11.30%, lower than diesel oil. Surprisingly, even the control using no collector has a higher recovery (16.39%). However, it should be noted that the ash content of the concentrate was higher (16.01%) when the EPS was used as the collector compared to the control without a collector (16.39%).

Table 2. Flotation test results.

Tests	Ash Content of Concentrate (%)	Ash Content of Tailings (%)	Recovery of Concentrate (%)	Recovery of Combustible (%)
L*	14.72	15.67	16.39	16.58
L* + diesel oil	15.52	16.01	31.95	32.14
L* + EPS	19.82	15.45	11.92	11.30
L* + spores	15.10	15.67	3.96	3.99
L* + culture solution	16.18	15.73	9.66	9.60
Liquefied L* + diesel oil	11.04	15.30	43.65	45.85

* L means lignite.

Regarding EPS treatment, two possibilities may account for the increased ash content in the concentrate. (1) The EPS tends to adhere to the mineral surface, enhancing its hydrophobicity and facilitating flotation. (2) The EPS adheres to the surface of both lignite and minerals, acting as a flotation inhibitor, with a greater inhibition effect on lignite than on minerals. Considering the reduced flotation recoveries observed when bio-collectors were used, it is more likely that fungal cells functioned as depressants in the flotation process. Microflotation tests by Govender [33] showed that EPSs of the tested bacteria could improve the flotation of $CuFeS_2$, with free EPSs proving more effective than cells with bound EPSs. These findings support the notion that the fungal cells served as flotation depressants.

Several factors contribute to the low recovery of concentrate coal in the bioflotation tests. Firstly, the fungal cells, being larger than bacteria, tend to form large aggregates that are difficult to float. Secondly, the presence of EPSs and other cellular substances can inhibit bubble formation and weaken the effective flotation force. Additionally, the relatively hydrophilic nature of *Hypocrea lixii* AH cells limits their adhesion to bubbles as their structure aggregates with hydrophilic parts facing outward. The limited time allocated for irreversible adhesion and the pH conditions may also affect the flotation process.

WRF (white-rot fungi) has gained widespread use in the desulfurization of coal. Studies by Gonsalvesh et al. [44,45] have reported that WRF can remove up to 90% of sulfur in coal. The application of FT-IR analysis also indicates that this fungus and its EPS have the potential to decrease the sulfur and other mineral contents in lignite. In the flotation test conducted in this research, the EPS was employed as a collector, and it was mixed with lignite for only 5 min, which may have been insufficient for it to fully impact the lignite. In a subsequent experiment involving liquefaction, the lignite sample was mixed with the fungal culture for two weeks. Following this, the coal underwent flotation using diesel oil as the collector and MIBC as the frother. The results show an increase in recovery and a decrease in the ash content of the concentrate (Table 2). It is conceivable that hydrophilic functional groups such as hydroxyls and carboxyls were decomposed during this process, evident from the increased contact angle of the residual lignite. This finding suggests a potential transformation of surface properties that contributed to improved flotation characteristics.

Considering the moderate hydrophobicity of the white-rot fungus AH observed under the current experimental conditions, it may not be an optimal collector for the flotation of lignite. However, it does show potential applications in the field of flocculation, an area that has not been previously explored. Additionally, the white-rot fungus AH may serve as a depressant in the reverse flotation of lignite [46], as it has demonstrated specific interactions with both lignite and impurities.

4. Conclusions

In summary, biosurfactants derived from *Hyprocrea lixii* AH were investigated for their effects on lignite flotation. Based on the results obtained, the following conclusions can be drawn:

1. All tested biosurfactants exhibited the ability to enhance the surface properties of lignite, with EPS demonstrating the most pronounced effect, followed by the culture solution, hyphae, and spores. Notably, the most significant change observed was the reduction in induction time when lignite was treated with EPS.

2. Lignite displayed poor inherent flotability. While the fungal cells improved the hydrophobicity of the coal, they did not contribute to improved flotation. Conversely, the EPS demonstrated the capacity to either depress the concentrate or facilitate the flotation of impurities present in lignite.
3. Although fungal cells were not found to be effective bio-collectors in microflotation tests, they exhibited the ability to remove or modify minerals in lignite, particularly those containing sulfur. This suggests a potential for the fungal cells to play a role in desulfurization or mineral transformation processes within lignite.

Overall, these findings highlight the distinctive effects and potential applications of the biosurfactants derived from the fungus *Hyprocrea lixii* AH in lignite treatment, shedding light on their ability to alter surface properties, affect flotation behavior, and interact with minerals present in lignite. Future studies could focus on optimizing biotreatment methods and explore alternative strategies for bioflotation or other applications, such as flocculation or coal desulfurization.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. As this study is a preliminary exploration of the role of white rot fungi on coal modification, we will follow up with in-depth processing and exploration of the data, so the data cannot be made public.

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