

The Sulfur Conversion Functional Microbial Communities in Biogas Liquid Can Participate in Coal Degradation

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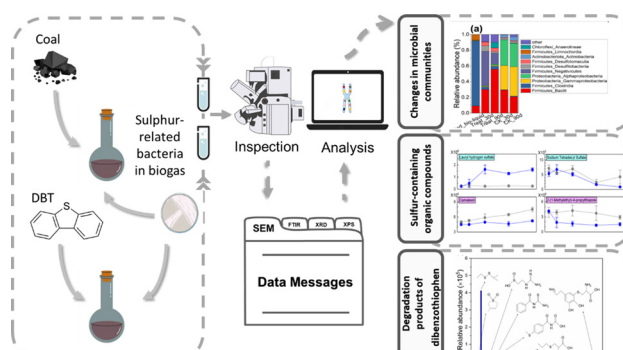
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Abstract

The addition of biogas liquid is a practical way to improve the yield of biological coalbed methane. The microbial composition in biogas liquid is complex, and whether it could participate in the sulfur conversion of coal remains unknown. In this study, sulfur conversion-related microbial communities were enriched from biogas liquid, which was dominated by genera *Anaerostipes*, *Bacillus*, *Hydrogenispora*, and *Oxobacter*. The co-culture of these groups with coal significantly changed the coal microbial community composition but did not increase the content of CH₄ and H₂S. The changed microbial communities mainly belonged to phyla Firmicutes, Proteobacteria, and Actinobacteriota, and increased the relative abundance of genera *Bacillus*, *Thermicoccus*, *Hydrogenispora*, *Oxobacter*, *Lutispora*, *Anaerostipes*, *Desulfurispora*, *Ruminiclostridium*, and *Fonticella*. From the microscopic structure of coal, an increase in the number of holes and roughness on the surface of the coal was found but the change of surface functional groups was weak. In addition, the addition of S-related microbial communities increased the contents of phoxim, methylthiobenzoylglycine and glibornuride M5 in aromatic compounds, as well as the content of lauryl hydrogen sulfate in alkyl compounds. Furthermore, the dibenzo-



thiophene degradation-related microbial communities included *Bacillus*, *Brevibacillus*, *Brevundimonas*, *Burkholderia-Caballeronia-Paraburkholderia*, and *Thermicoccus*, which can break C-S bonds or disrupt benzene rings to degrade dibenzothiophene. In conclusion, the S-related microbial communities in biogas liquid could rebuild the coal microbial community and be involved in the conversion process of organic sulfur in coal.

Key words: biogas production, biogas liquid, sulfur conversion, dibenzothiophene

Introduction

The nonrenewable nature of fossil fuels and the potential threat of pollution (Chew 2014) have fueled the rapid development of clean and low-carbon energy sources (Chai and Wang 2020). The conversion of coal into coalbed methane (CBM) has become an effective and environmentally friendly measure to utilize deep coal reserves (Park and Liang 2016). Microorganisms have great potential to degrade and gasify coal, and this increase in biogenic CBM is considered an impor-

tant addition to unconventional natural gas resources (Wang et al. 2023).

Inexpensive techniques, such as enhanced microbial activity to recover more methane, are valuable (Fakoussa and Hofrichter 1999). A number of measures have been identified that can be used to enhance the production of biological CBM, for example, chemical pretreatment with permanganate (Huang et al. 2013) and hydrogen peroxide (Liu et al. 2019), co-fermentation of coal and corn stover (Zhang et al. 2022), and the addition of yeast extract, peptone, and Fe powder (Zhang

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et al. 2018a). In addition, a large number of researchers have demonstrated that the use of exogenous additives such as biogas liquid is an effective measure to increase the production of coal methane (Marañón et al. 2012; Wang et al. 2018a). Wang et al. (2017) indicated that exogenously adapted anaerobic bacteria from biogas liquid efficiently converted coal to methane with a methane production rate of 77.68 $\mu\text{mol/g}$. Biogas liquid is a byproduct of anaerobic digestion and contains a large amount of organic matter, macronutrients (N, P and K) and micronutrients, which adjust nutrient ratios and promote the activities of microbial communities (Wang et al. 2018b). Furthermore, exogenously adapted anaerobic bacteria from biogas liquid can efficiently convert coal to methane, and many soluble substances are produced during the conversion process. In addition, the solubilization process in which organic matter is biodegraded into substrates that can be utilized by methanogens may be the rate-limiting step in the bioconversion of coal to methane (Wang et al. 2017).

Biogas liquid is a mixture that contains microbes that not only carry out methanogenic processes but also other elemental metabolisms, such as sulfur (S) transformation. Previous studies have shown that when anaerobic processes are used to treat biogas, the availability of sulfate as an alternative electron acceptor stimulates the growth of sulfate-reducing bacteria (SRB), which reduce sulfate to sulfide using H_2 and low molecular weight organic matter (VFA, ethanol, and methanol) as electron donors (Olivera et al. 2022). In addition, regarding the role of S transformation in coal degradation, Zhang et al. (2018b) found that elevated sulfate concentrations and a high relative abundance of specialized acetate-oxidizing bacteria suggest possible competition between sulfate-reducing and methanogenic archaea (Gutekunst et al. 2022). Zhang et al. (2015) found that different microbial strains are actively engaged in sulfate reduction and oxidation. In addition, high concentrations of sulfate can alter intracellular osmotic pressure or sulfate reduction could cause the release of hydrogen sulfide, affecting methane formation and causing corrosion of technological parts of facilities (Hierholtzer and Akunna 2012; Jung et al. 2019). These results suggest that various functional microbiota are able to participate in the biogeochemical processes involving S during coal degradation and release a variety of functional enzymes (Guo et al. 2015; Kotelnikov et al. 2020) that can influence coal gas production. However, it is not yet known whether the S transformation process during biogenic CBM production enhancement by biogas liquid promotes coal degradation.

In this context, the aim of this study was to investigate the effect of the S-related functional groups in the biogas liquid on the coal microbial communities and the organic sulfur components in coals.

Experimental

Materials and Methods

Collection and preparation of coal samples. The Jiaojiazhai Coal Mine (38.83°N, 112.35°E) in Xinzhou City, Shanxi Province, China, provided the coal samples used in this experiment. Coal samples (3# coal) were collected from zones without structural belts by a core drilling rig. The coal samples were quickly placed in sealed sterile bags after being extracted from the well with a sampling drill and transported in a refrigerator. After the coal samples were transported to the laboratory, the surface coal layer (approximately 2 cm thick) was removed with a sterile blade in an anaerobic glove box (TORUN VGB-3A; Changshu Tongrun Electronic Technology Co., Ltd, China), and the coal samples were crushed, ground, and sieved. The basic properties of the coal samples were as follows: 1.32% air-dried moisture (M_{ad}), 27.46% air-dried ash (A_{d}), 29.23% air-dried basis volatile matter (V_{ad}), 78.43% carbon content (C), 9.74% oxygen content (O), 5.31% hydrogen content (H), 1.59% nitrogen content (N) and 1.86 % sulfur content (S) (organic S 1.49% and inorganic S 0.37%).

Enrichment of microbial communities. The biogas liquid was an anaerobic mixture prepared by mixing raw biogas liquid (10 ml) (collected from the methane-generating pit from Henan Yunferment Biotechnology Co., Ltd., China) and a medium solution (100 ml). The mixture was sealed in a nitrogen atmosphere in 250 ml sterile bottles and incubated in a constant temperature incubator at 37°C. The sterilized acclimatization medium solution was prepared by mixing KH_2PO_4 0.5 g/l, NH_4Cl 1.1 g/l, Na_2SO_4 1.1 g/l, Na_2S 1.1 g/l, and dibenzothiophene (DBT) 2 g/l.

Acclimatization was carried out by anaerobic culturing for two months, and the medium was changed for subculture when the optical density (OD) reached 0.1 at wavelength 600 nm by a spectrometer (Shanghai Spectral 751, China). Microbial enrichment was performed at the 50th incubation. To avoid interference from solid DBT particles and culture medium, the solution was left undisturbed, and the suspension was taken when the OD value reached 0.6. The microorganisms were collected in an anaerobic chamber after suspension filtration through a 0.22 μm filter membrane, and the microbes on the filter were washed with sterilized water three times to obtain the mixed flora for the experiments (Fig. 1).

Experiments on the sulfur conversion of coal. Fifty grams of coal sample, 20 ml of resuspended mixed microbiota, and 200 ml of minimal salt media were added to autoclaved 500 ml flasks, and these three flasks were set up as the Treatment groups. The control (CK) groups were prepared using the same method without the addition of the mixed microflora. The six culture

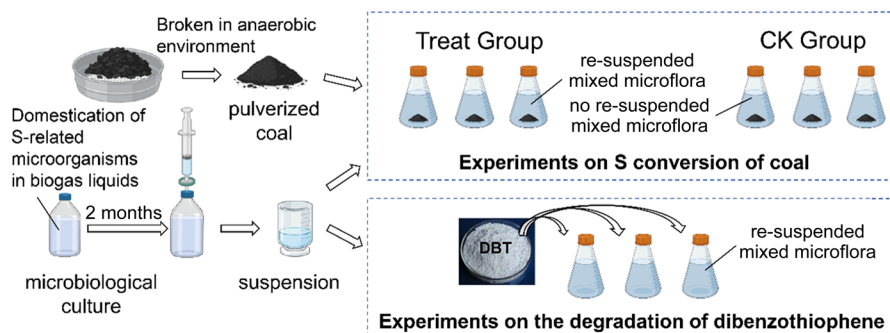


Fig. 1. Flow chart for experimental studies.

bottles were repeatedly vacuumed, and nitrogen was used to replace the headspace gas. This was repeated three times, and the bottles were incubated at 37°C in a constant temperature incubator. The composition of the minimum salt medium was as follows: NH_4Cl 0.3 g/l, NaCl 0.5 g/l, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 0.5 g/l, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1 g/l, KCl 0.5 g/l, and KH_2PO_4 0.2 g/l.

Culture fluid samples were collected at 1d, 2d, 4d, 7d, 10d, 15d, 20d, 30d, 40d, 50d, 60d, 70d, 80d, and 90d of incubation, and samples of culture fluid were collected at 30 and 90 days were further analyzed to determine the composition of the microbial community. At the end of the incubation period, the degradation solution in the bottles was filtered through a 0.22 μm membrane, and the residual coal was collected.

CH_4 content in the headspace was determined using gas chromatography with a TDX-01 packed column. The temperatures of the inlet, column, and thermal conductivity detector (TCD) were set as 105°C, 90°C, and 120°C, respectively. H_2S in the headspace was detected by a gas chromatography with a flame photometric detector (FPD) detector and GDX-303 packed column. The temperatures of the inlet, column, and FPD detector were set as 150°C, 70°C, and 200°C, respectively. However, the CH_4 and H_2S contents were below the detection limit.

Experiments on the degradation of dibenzothiophene. DBT is a typical S-containing organic compound in coal. The degradation process of DBT and its main microbiota were analyzed by using DBT as the sole carbon source in laboratory cultures. Approximately 20 ml of resuspended mixed microflora were placed in triplicate into 500 ml sterile bottles containing 200 ml of minimum salt medium (DBT 2 g/l, NH_4Cl 0.3 g/l, NaCl 0.5 g/l, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 0.5 g/l, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1 g/l, KCl 0.5 g/l, and KH_2PO_4 0.2 g/l). Each bottle was sealed with a sterile nitrile rubber stopper, the headspace air was replaced with nitrogen, and the bottles were incubated at 37°C for 90 days.

Analysis of main physical and chemical properties. The changes in the surface morphology of the coal samples were observed by SEM-EDS. A small amount

of coal powder sample was taken to evenly sprinkle on the conductive adhesive, and then was sprayed gold for about 10s before blowing away the unstuck sample. It used a Thermo Scientific™ Apreo™ 2S+ (Thermo Fisher Scientific, Inc., USA) instrument and an OXFORD Ultim® Max 65 spectrometer (Oxford Instruments, UK), and the test mode was secondary electronic mode, where the working distance was 10 mm and the shooting multiple range was 1–200 μm . X-ray photoelectron spectroscopy (XPS) with a Thermo Scientific™ ESCALAB™ Xi+ (Thermo Fisher Scientific, Inc., USA) instrument was used to study the surface composition of the coal, and the energy spectra of C, N, O, and S were mainly monitored. Fourier transform infrared spectroscopy (FTIR) by a Thermo Scientific™ Nicolet™ iS™ 5 FTIR spectrometer (Thermo Fisher Scientific, Inc., USA) was used to scan the coal samples in the wavenumber range of 4000–400 cm^{-1} . X-ray diffraction (XRD) analysis by a Japanese Rigaku-Smartlab type instrument was used to scan coal samples at a rate of 5° per minute in two regions, 16–34° and 39–49°.

Liquid organic matter analysis, including sulfur-containing organic matter. Each liquid sample was extracted with methanol. Extracts were analyzed by an LC-qTOF system (6530 Q-TOF LC/MS; Agilent, USA) with electrospray Jet Stream Technology. Isolates were injected to facilitate chromatographic separation by an InfinityLab Poroshell 120 EC-C18 column (3.0 $\times$ 100 mm) (Agilent, USA). Fragmented ions were scanned in the quadrupole analyzer in the mass range of m/z 100–3000. For quality assurance and control, the stability of mass accuracy was checked daily, and if values were above 2 ppm error, the instrument was recalibrated. Ions with a relative standard deviation (RSD) greater than 30% were filtered out from the collected data. Peak alignment, peak extraction, normalization, deconvolution, and compound identification were performed using MassHunter (Agilent, USA), and products with sulfur-containing organic matter were identified according to the METLIN database. These biomarker constituents were characterized by their retention times and responses in diagnostic mass chromatograms.

Table I
The relative abundance of dominant phyla (> 1%) in the samples, different lowercase letters indicate a significant difference (ANOVA with Tukey’s post hoc test, $p < 0.05$) among samples.

	Acclimated biogas liquid	CK_30d	CK_90d	Treat_30d	Treat_90d
Firmicutes	99.23 ± 0.18% a	32.75 ± 19.80% b	25.11 ± 3.87% b	98.32 ± 0.31% a	90.65 ± 5.36% a
Proteobacteria	0.61 ± 0.12% b	63.55 ± 20.87% a	67.22 ± 1.41% a	1.47 ± 0.38% b	2.32 ± 0.69% b
Actinobacteriota	0.03 ± 0.02% c	3.14 ± 0.95% b	5.71 ± 1.51% a	0.03 ± 0.02% c	0.12 ± 0.06% c
Chloroflexi	0.00 ± 0.00% b	0.09 ± 0.03% ab	0.92 ± 0.85% ab	0.09 ± 0.10% ab	6.81 ± 5.56% a
Bacteroidota	0.07 ± 0.03% a	0.37 ± 0.36% a	0.42 ± 0.27% a	0.04 ± 0.02% a	0.03 ± 0.01% a

Microbial composition analysis. Total genomic DNA was extracted from the culture samples using the Aqua-Screen® FastExtract method (Minerva Biolabs GmbH, Germany), and then genomic DNA was amplified using primers 515F/806R (Kuczynski et al. 2012) targeting 16S rRNA gene fragments (515F: 5'-GTGCA GCMGCCG-CGGTAA-3'; 806R: 5'-GGACTACNVGGGT WTCTA-AT-3'). These products were sequenced on the DNB-seq platform of the Beijing Genome Research Institute Ltd. (China). Raw data were processed and analyzed using QIIME2 Quality control, noise reduction, and chimera removal were performed using the DADA2 plug-in, and amplicon sequence variants (OTUs) were obtained (Callahan et al. 2016; Bolyen et al. 2019). OTU representative sequences were annotated based on the SILVA reference database (version 138) (Quast et al. 2013). The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in the National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA013685) that are publicly accessible at <https://ngdc.cncb.ac.cn/gsa>.

Data analysis. A heatmap of the dominant genera (>0.5%) was plotted in the R environment (version 4.1.2) with the heatmap package, and the complete method was used for clustering (R Core Team 2021). Bray-Curtis’s distance was calculated according to the relative abundance of the OTU matrix by the Vegan

package in the R environment (version 4.1.2). Non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity was used to visualize the ordering of community composition by Vegan’s metaMDS function. Comparison of the distributions and abundances of individual compounds within each sulfur-containing organic matter for the Treatment and CK groups enabled the determination of the relative extent of biodegradation of specific components. The relative abundance of each sulfur-containing organic matter between different groups was compared by peak area. One-way analysis of variance (ANOVA, Tukey’s HSD test with significance at $p < 0.05$) was used to analyze the differences in the Shannon diversity and relative abundance of microbes in sulfur-containing organic matter.

Results

Community structure and changes in microbial communities. A total of 876,938 high-quality bacterial sequences were obtained by 16S rRNA gene fragment sequencing, which ranged from 53,314 to 63,613. The readings for each sample were normalized to 53,000 for subsequent analysis. Firmicutes were the predominant group in acclimated biogas liquid (Table I), which mainly contained classes Bacilli, Limnochordia and Clostridia (Fig. 2a), among them genus *Anaeroso-*

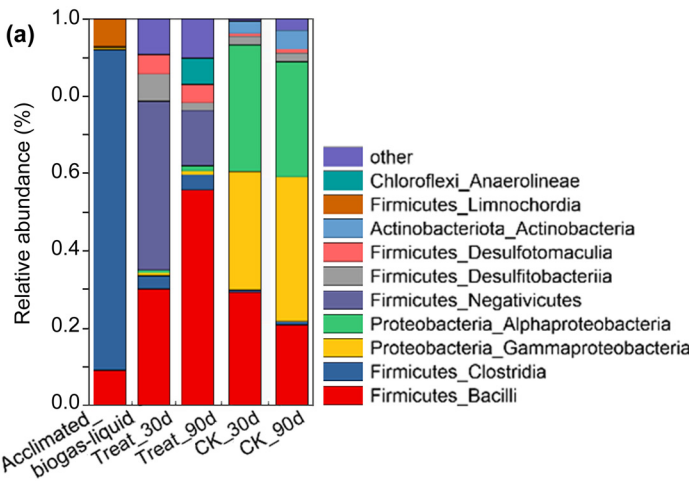


Fig. 2 Changes in microbial diversity in samples.
a) Relative abundance of major bacterial phyla in biogas liquid (relative abundance > 1%).

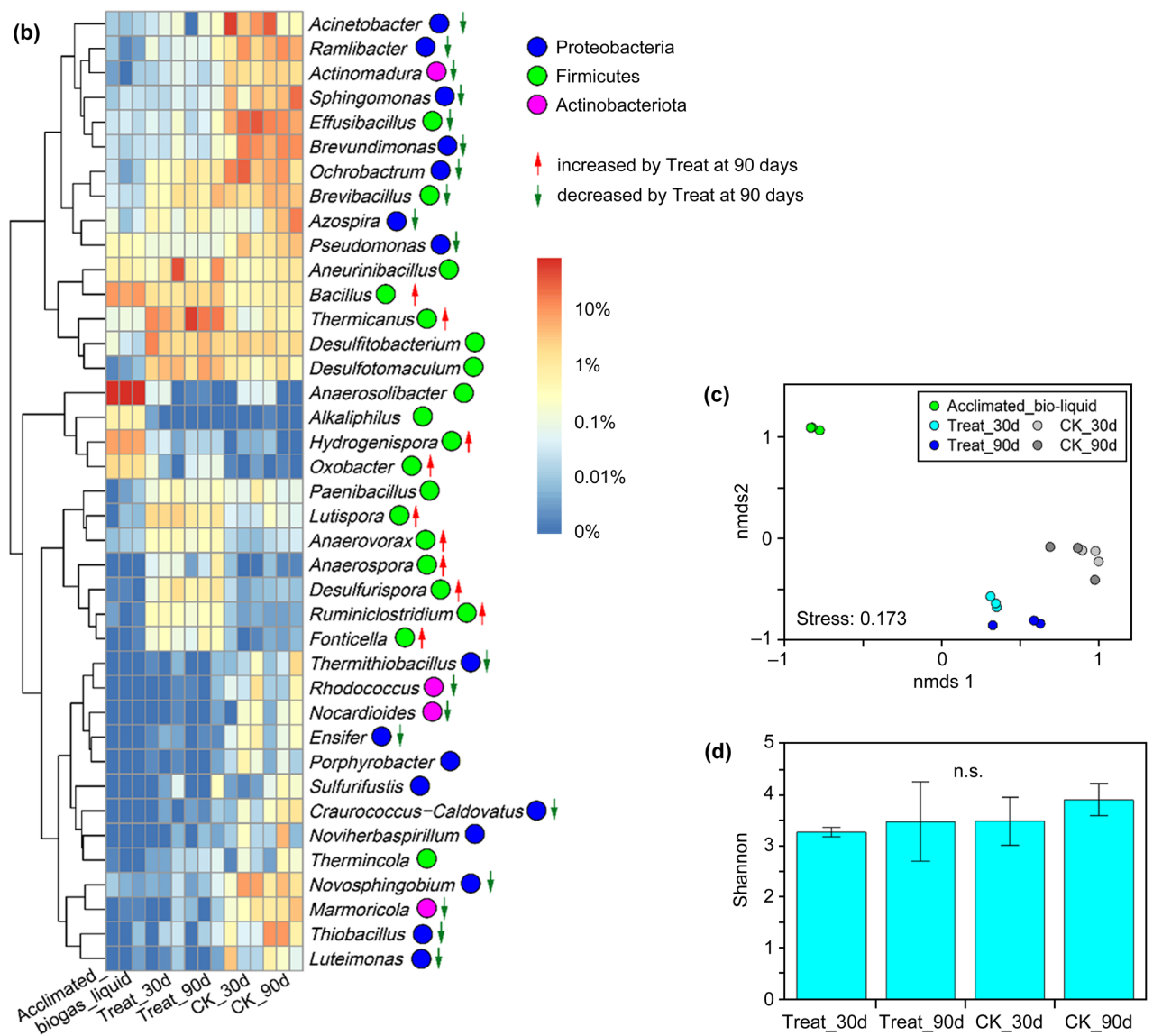


Fig. 2 Changes in microbial diversity in samples.

b) heatmap for relative abundance of major microbial genera in samples (relative abundance > 0.05%), and red and green arrows indicated significant differences between Treat and CK groups at 90 days (t-text, $p < 0.05$); c) ordering of microbial community composition by nonmetric multidimensional scaling (NMDS) using the Bray-Curtis distance; d) Shannon diversity among samples, n.s. indicates no significant difference (ANOVA with Tukey's post hoc test, $p < 0.05$) among treatments.

libacter ($79.95 \pm 1.71\%$) dominated the S conversion-related acclimated biogas liquid, followed by *Bacillus* ($8.12 \pm 1.34\%$), *Hydrogenispora* ($7.03 \pm 0.42\%$) and *Oxobacter* ($1.81 \pm 0.27\%$).

The addition of acclimated biogas liquid resulted in great changes in microbial composition. For example, the relative abundance of phylum Firmicutes increased, and that of phylum Proteobacteria decreased in the Treat group (Table I). In addition, the classes Alphaproteobacteria, Anaerolineae, Bacilli, Clostridia, Desulfotobiaria, Desulfotomaculia, Negativicutes, and Limnochordia showed visual changes in relative abundance between Treat and CK groups (Fig. 2a).

The dominant genera (> 0.5%) mainly belonged to the phyla Proteobacteria, Firmicutes and Actinobac-

teriota. The addition of acclimated biogas liquid also significantly increased the relative abundance of genera *Bacillus*, *Thermicanus*, *Hydrogenispora*, *Oxobacter*, *Lutispora*, *Anaerovorax*, *Desulfurispora*, *Ruminiclostridium* and *Fonticella*, as well as decreased the relative abundance of genera *Acinetobacter*, *Ramlibacter*, *Actinomadura*, *Sphingomonas*, *Effusibacillus*, *Brevundimonas*, *Ochrobactrum*, *Brevibacillus*, *Azospira*, *Pseudomonas*, *Thermithiobacillus*, *Rhodococcus*, *Nocardioides*, *Ensifer*, *Craurococcus-Caldovatus*, *Novosphingobium*, *Marmoricola*, *Thiobacillus*, and *Luteimonas* (Fig. 2b).

Nonmetric multidimensional scaling (NMDS) based on the Bray-Curtis similarity of phylotypic compositions showed a visual separation between Treat and CK groups in the microbial community structure both

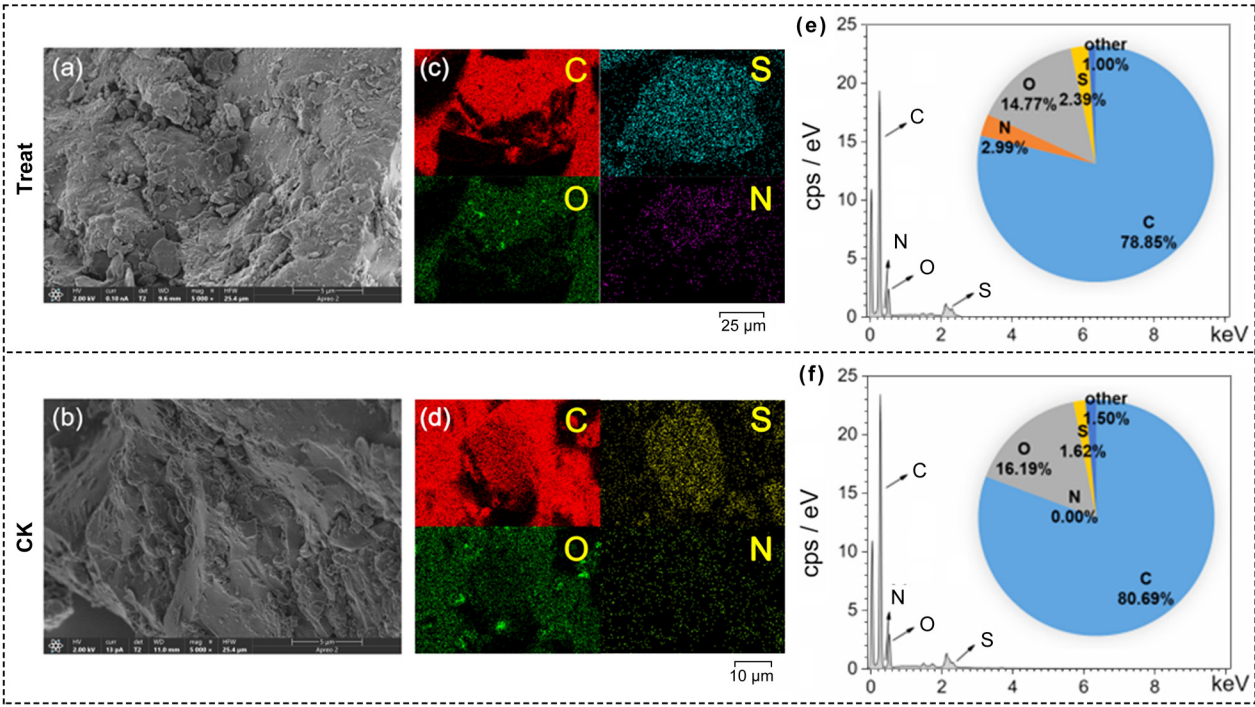


Fig. 3. Images of SEM, EDS energy spectra, and count per second (cps) data of coal samples from different treatment groups.

in 30 days and 90 days (Fig. 2c). However, there was no significant difference in Shannon diversity among samples (Fig. 2d).

Changes in the morphology of coal surfaces. The effects of microbial communities on the coal surface were investigated using SEM-EDS (Fig. 3). The surface morphology of the coal samples was scaly, according to scanning electron microscopy results. In comparison to the CK group, the surface of the coal samples treated with the domesticated bacterial solution was noticeably rougher, more irregular, and more porous. It is evident from the EDS layered images that C, N, O, and S were more prevalent in the Treatment group than in the CK group. The contents of S and N increased, and the contents of C and O decreased in the Treatment group according to a comparison of the counts per second (cps) between the CK and Treatment groups.

Changes in the crystal structure and surface functional groups of coal. The XRD spectra (Fig. 4a) of the Treatment and CK groups showed that both the Treatment and CK groups had obvious peaks at 29°, but the peak in the Treatment group was significantly higher than that in the CK group. There were also several groups of higher peaks at 20–27° in the Treatment and CK groups. According to the spectra, the main sulfate-related crystalline phases contained in the samples were related to $\text{Al}_4(\text{OH})_8(\text{Si}_4\text{O}_{10})$, $\text{Ca}(\text{CO}_3)$, MgSO_4 , and CaSO_4 .

Chemical band changes during the incubation of the Treatment and CK groups were investigated using FTIR (Fig. 4b). The main peaks in the Treatment group

belonged to O-H telescoping vibrations at 3695 cm^{-1} and 3618 cm^{-1} ; NH_2 telescoping vibrations at 3387 cm^{-1} ; and C-H broadening vibration of unsaturated hydrocarbons at 3046 cm^{-1} ; CH_2 antisymmetric and CH_2 symmetric telescoping vibrations of alkyl groups at 2921 cm^{-1} and 2855 cm^{-1} ; NH_2 angular vibration/C=C telescoping vibration at 1597 cm^{-1} ; superposed antisym-

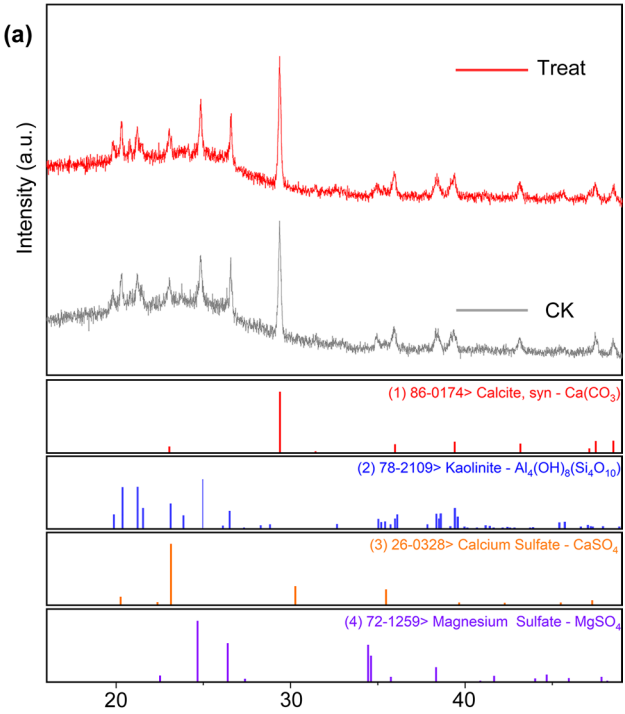


Fig. 4. XRD, FTIR, and XPS spectra of coal samples. a) XRD spectra.

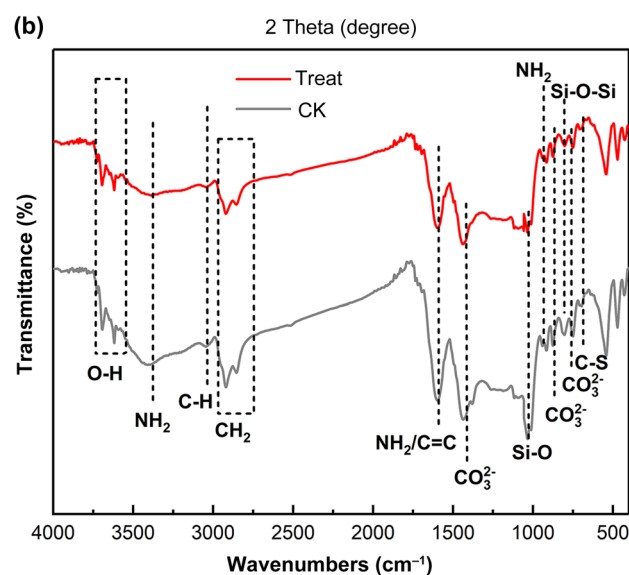


Fig. 4. XRD, FTIR, and XPS spectra of coal samples.
b) FTIR spectra.

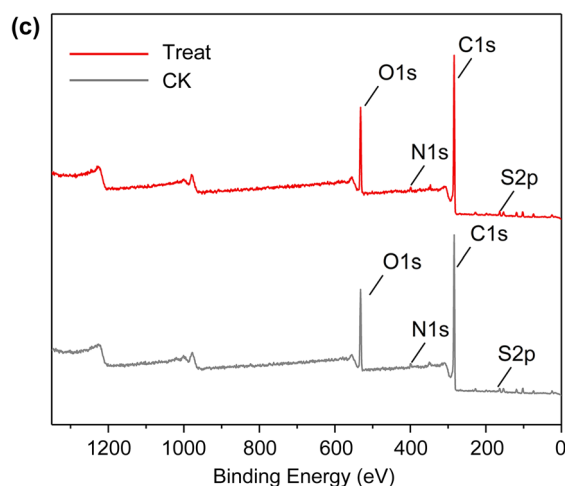


Fig. 4. XRD, FTIR, and XPS spectra of coal samples.
c) XPS spectra.

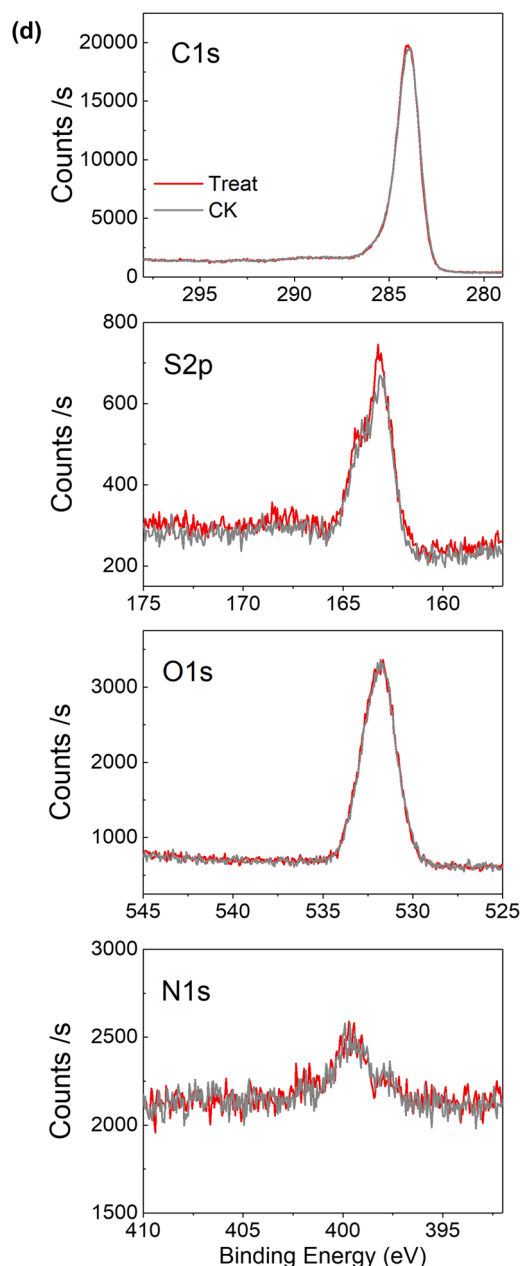


Fig. 4. XRD, FTIR, and XPS spectra of coal samples.
d) XPS spectra of C, N, O, and S, respectively.

metric telescoping vibration, out-of-plane bending vibration, and in-plane bending vibration of CO_3^{2-} at 1438 cm^{-1} , 876 cm^{-1} , and 750 cm^{-1} , respectively; Si-O antisymmetric telescopic vibration at 1033 cm^{-1} ; NH_2 symmetric variable-angle vibration at 916 cm^{-1} ; possible C-S telescopic vibration at 708 cm^{-1} ; and possible Si-O-Si symmetric telescopic vibration at 802 cm^{-1} . The infrared profiles of the Treatment and CK samples were similar, and the number of absorption peaks, peak positions, and peaks were similar to those of the CK sample.

The XPS spectra of the surface of the coal samples (Fig. 4c) showed that C1s peaked at 284.13 eV , O1s peaked at 531.89 eV , S2p peaked at 163.36 eV , N1s peaked at 399.23 eV , and P2p peaked at 133.26 eV .

The intensity of the S2p peaks increased after the addition of the domesticated bacterial solution for incubation (Fig. 4d), and the intensity of the C1s, N1s, and O1s peaks did not change significantly. The contents of C, O, N, and S on the surface of the coal samples could also be calculated using XPS broad energy spectrum analysis. The contents of C, N, and S decreased from 77.11% , 1.55% , and 1.98% to 76.44% , 1.32% , and 1.56% , respectively, which showed that treatment had little effect on the element contents of the coal surface.

Biotransformation processes of organosulfur compounds. Organosulfur compounds were annotated according to the METLIN database, and 764 sulfur-containing compounds were found overall, 23 of

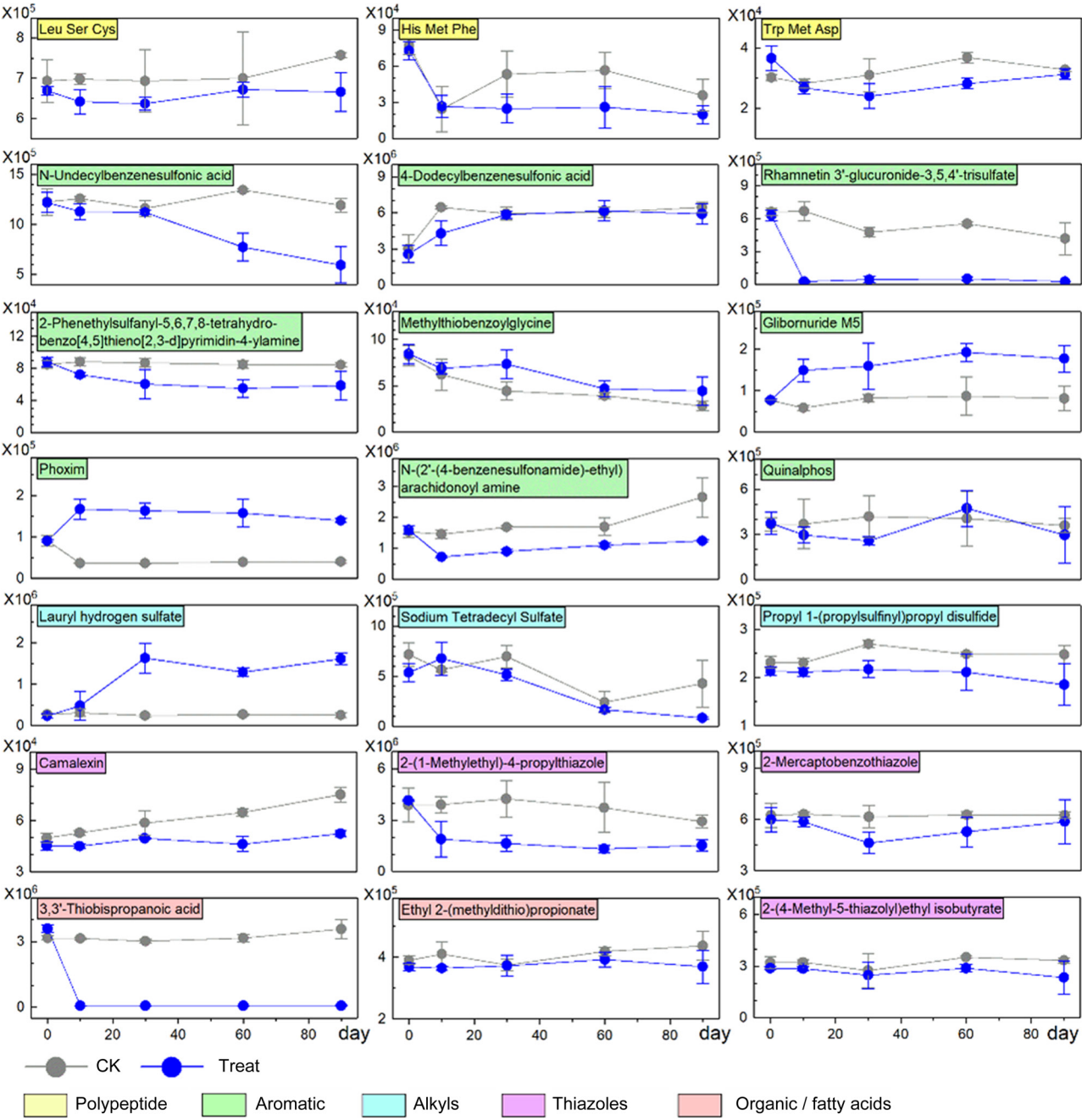


Fig. 5. Main sulfur-containing organic compounds in coal samples.

which were found in every sample (Fig. 5). Among them, three peptides, nine aromatic compounds (two of which contained sulfuric acid and one benzenesulfonic acid), three alkyl compounds (two of which contained sulfates), four thiazoles, one organic acid, one fatty acid and one pyrimidine were identified.

The content of peptides and thiazoles in the Treatment group was less than that in the CK group, which indicated that the domesticated bacteria in solution could degrade peptides and thiazoles. Among the aromatic compounds, the contents of phoxim, methylthiobenzoylglycine and glibornuride M5 were higher in the Treatment group than in the CK group, while

N-undecylbenzenesulfonic acid, rhamnetin 3'-glucuronide-3,5,4'-trisulfate, 2-phenethylsulfanyl-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-ylamine, and *N*-(2'-(4-benzenesulfonamide)-ethyl) arachidonoyl amine showed the opposite trend. The contents of 4-dodecylbenzenesulfonic acid and quinalphos were similar in both groups, probably because microbial degradation of organosulfur compounds does not lead to the production of these two aromatic compounds. The content of different alkyl analogues in the liquid phase was also different in the Treatment and CK groups. Propyl 1-(propylsulfinyl)propyl disulfide increased in the Treatment group, while lauryl hydrogen sulfate increased in

has been shown to play a significant role in organic substrate hydrolysis and methanol production at mesophilic and thermophilic temperatures (Botta et al. 2020). Genera *Lutispora* and *Fonticella* have been reported to have many functions, such as syntrophic acetate oxidation, syntrophic alcohol- and lactate-degradation, complex-compound-degradation, and proteinaceous-substance-degradation (Jiang et al. 2020). In addition, *Anaerovorax* can participate in the metabolism of volatile fatty acids (FitzGerald et al. 2019) and *Ruminiclostridium* is essential for signalization, uptake, and catabolism of the degradation products of cellulose hydrolysis (Fosses et al. 2017).

The addition of exogenous sulfur-metabolizing strains changed the structure of microbial communities but not the microbial diversity index. This may have occurred due to the resistance in the microbial community to structural changes (Allison and Martiny 2008), in which resistance to disturbance is dependent on the relative abundance and contribution of specific functional groups and their life history strategies (Wallenstein and Hall 2012).

The addition of domesticated S-related microbial communities also significantly increased the porosity and roughness of coal (Fig. 3), which increased the contact surface area between microorganisms and coal. However, these S-related microbial communities were not directly involved in the formation of CH_4 and H_2S , suggesting that these groups have no obvious promoting effect on pipeline corrosion. In addition, microbial degradation can improve the porosity and desorption capacity of methane and enhance the diffusion capacity of methane, improving the physical properties of coal reservoirs and increasing the production of coalbed methane (Xia et al. 2023). XRD was used to identify the crystal structure of the samples (Rompalski et al. 2019) and FTIR was used to provide surface functional group information on molecular structures (Meng et al. 2014), both of which indicated a weak effect of the treatment on the crystal structure and surface functional groups of coal (Fig. 3a and 3b). XPS showed that the S2p peaks increased in the presence of the domesticated S-related strains, and the S2p signal could be resolved as species that corresponded to pyrite, sulfide, thiophene, sulfoxide, sulfone, and sulfonate/sulfate (Huang et al. 2014). These physicochemical changes indicate that the microorganisms in the marsh liquid played a dominant role in the transformation of S species during the degradation of coal.

Sulfur-containing organic substances are mainly classified into broad categories that include peptides, aromatic compounds, alkyl compounds, thiazoles, organic acids, fatty acids, and pyrimidine compounds. Microorganisms can release active peptides via the production of complex enzymes and the synthesis and secretion

of peptide substances (Feng et al. 2022). Domesticated S-related strains may not participate in the production of sulfur-containing peptides, leading to a decrease in their content. In addition, strains can disrupt branched or cyclic structures and promote the decomposition of thiazoles, which are large heterocyclic compounds with branched chains (Gao et al. 2019). For S-related aromatic compounds, there was an increase in the content of aromatic compounds such as phoxim, which destroy branched chains. However, such compounds are unable to destroy aromatic rings and cannot carry out further decomposition. In contrast, there was a decrease in the content of aromatic compounds such as *N*-undecylbenzenesulfonic acid, which is considered more complex than the aforementioned compound. *N*-undecylbenzenesulfonic acid is a known antimicrobial compound (Chua et al. 2023), and the S-related microorganisms in the biogas liquid can decompose these compounds, which is conducive to the stability of the microbial communities. The content of the nonaromatic organic acid 3,3'-thiobispropanoic acid was significantly reduced, indicating that it is only an intermediate product and not the final product of transformation.

Dibenzothiophene (DBT) and its derivatives are the major polycyclic aromatic sulfur heterocyclics in coal, crude oil, and sedimentary organic matter (Ji et al. 2021). It has excellent resistance to microbial degradation and high thermal stability (Li et al. 2019) and has been widely used as a prototypical model for sulfur transformation (Mishra et al. 2016). In the process of the DBT degradation study, the acclimatization medium changed to a minimum salt medium with DBT, which resulted in the main S-related microbial communities changing to DBT degradation-related microbial communities. The main microorganisms involved in DBT degradation were *Burkholderia-Caballeronia-Paraburkholderia*, *Bacillus*, *Brevibacillus*, *Brevundimonas*, *Thermicanus*, *Pseudoxanthomonas*, and *Acinetobacter*. The degradation products of dibenzothiophene included 3-methyl sulfolene ($\text{C}_5\text{H}_8\text{O}_2\text{S}$), *N*-benzoylthiourea ($\text{C}_8\text{H}_8\text{N}_2\text{OS}$), methylthiobenzoylglycine ($\text{C}_9\text{H}_9\text{NO}_3\text{S}$), 5-S-cysteinyl-dopamine ($\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_4\text{S}$), four aromatic compounds, ethyl isopropyl disulfide ($\text{C}_5\text{H}_{12}\text{S}_2$), hypotaurocyamine ($\text{C}_3\text{H}_9\text{N}_3\text{O}_2\text{S}$), S-propyl-L-cysteine ($\text{C}_6\text{H}_{13}\text{NO}_2\text{S}$), and three ring-opening compounds. This result indicated that microbial degradation of dibenzothiophene in this experiment progressed via the Kodama pathway and the 4S pathway, which resulted in the cleavage of both carbon-sulfur and carbon-carbon bonds (Wang et al. 2019). The most abundant product was ethyl isopropyl disulfide ($\text{C}_5\text{H}_{12}\text{S}_2$), and 5-S-cysteinyl-dopamine ($\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_4\text{S}$) was the least abundant. It is speculated that dibenzothiophene was degraded to an intermediate product such as 5-S-cysteinyl-dopamine ($\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_4\text{S}$), and the added microorganisms could continue to

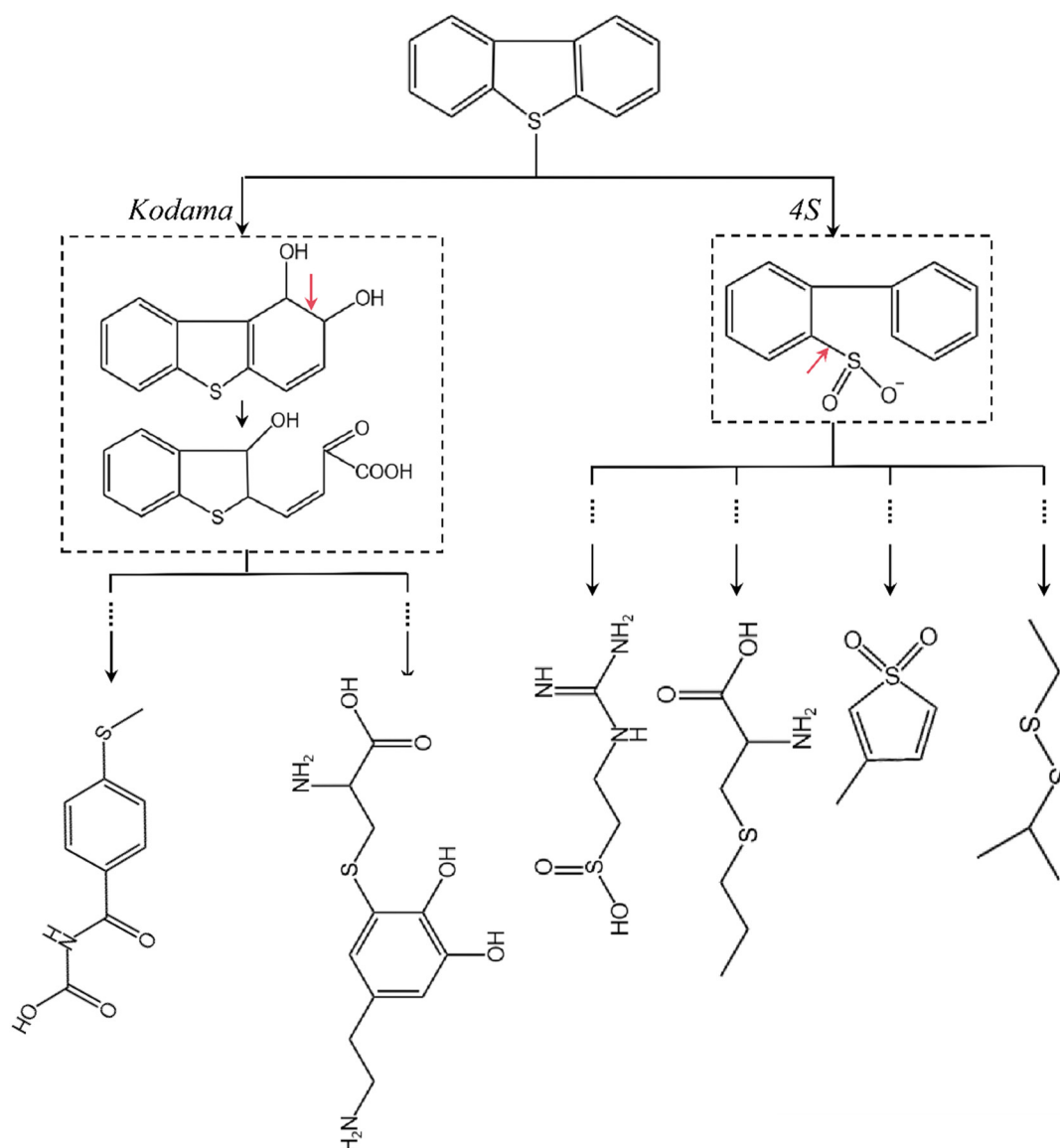


Fig. 7. Predicted degradation pathways of dibenzothiophene (DBT).

degrade the product until it decomposed into the final product, ethyl isopropyl disulfide ($C_5H_{12}S_2$) (Fig. 7).

In summary, the sulfur transformation-dominated microbial taxa present in biogas liquid can reconstruct the microbial community of coal and enhance the biodegradation of coal. This study highlighted the importance of sulfur transformation in coal biodegradation, and the interconversion of sulfur species ultimately affects the microbial homeostatic environment of coal seams, which in turn has a significant impact on biogeochemical cycles.

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Availability of data and material

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Author contributions

Yang Li: conceptualization, data curation, formal analysis, methodology, writing – original draft, funding acquisition. Zhong Liang: data curation, formal analysis. Xinyue Yan: formal analysis, writing – original draft, writing – review and editing. Tianqi Qin: data curation, formal analysis, writing – original draft. Zhaojun Wu: formal analysis. Chunshan Zheng: writing – review and editing.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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