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Oil Has a Higher Methanogenic Potential than Coal in an Oil-Bearing Coal Seam

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Cite This: *ACS Omega* 2023, 8, 23880−23888

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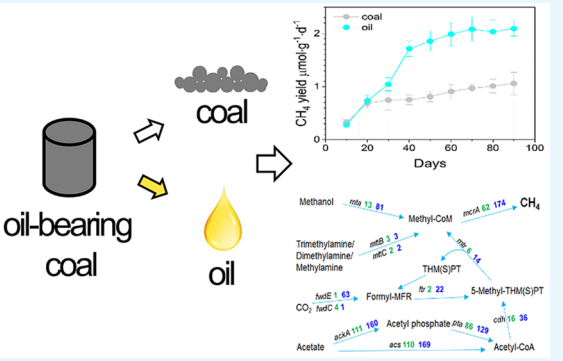
ABSTRACT: The presence of oil in coal seams from coal−oil symbiosis areas poses a serious threat to the safe and efficient mining of coal. However, the information about the application of microbial technology in oil-bearing coal seams was insufficient. In this study, the biological methanogenic potential of coal and oil samples in an oil-bearing coal seam was analyzed by anaerobic incubation experiments. The results showed that the biological methanogenic efficiency of the coal sample increased from 0.74 to 1.06 from day 20 to day 90, and the biological methanogenic potential of the oil sample was about twice as high as that of the coal sample after 40 days of incubation. The Shannon diversity and observed operational taxonomic unit (OTU) number of oil were lower than those in coal. The major genera in coal were *Sedimentibacter*, *Lysinibacillus*, *Brevibacillus*, etc., and the major genera in oil mainly included *Enterobacter*, *Sporolactobacillus*, and *Bacillus*. The methanogenic archaea in coal mainly belonged to the order *Methanobacteriales*, *Methanocellales*, *Methanococcales*, etc., and the methanogenic archaea in oil mainly belonged to the genera *Methanobacterium*, *Methanobrevibacter*, *Methanoculleus*, and *Methanosarcina*. In addition, metagenome analysis showed that functional genes belonging to processes such as methane metabolism, microbial metabolism in different environments, and benzoate degradation were in a higher abundance in the oil culture system, while genes belonging to sulfur metabolism, biotin metabolism, and glutathione metabolism were in a higher abundance in the coal culture system. The metabolites specific to coal samples mainly belonged to phenylpropanoids, polyketides, lipids, and lipid-like molecules; meanwhile, the metabolites specific to oil were mainly organic acids and their derivatives. In summary, this study has a reference value for the elimination of oil from coal in oil-bearing coal seams and can be used to separate oil from oil-bearing coal seams and reduce the hazard brought by oil for coal seam mining.



1. INTRODUCTION

Coal is the most abundant and widely distributed fossil fuel on earth, and it often exists in symbiotic form with other energy sources or resources, such as oil shale, bitumen, bauxite, germanium, paste salt, uranium, pyrite, etc.[1] Due to the similar age of mineralization, and the same ore-forming material, there are a variety of deposits coeval and have a relatively stable regional tectonic background and weak deformation. Among them, oil and coal symbiosis is common and generated by the time of oil and gas generation coinciding with the time of tectonic movement, and the main transport channel is the fault, so the coal seam is often generated by the accumulation of high oil content.[2] Coal and oil coassociated resources are widely distributed including the Shenfu coalfield in the Ordos Basin,[3] the Western edge of the Majiadan mine,[4] Shaanxi Huangling and Gansu Yaojie mining area,[5] the Lower Carboniferous Basin,[2] etc.

Oil is often present in coal seams in coal−oil symbiosis areas, and the coexistence of oil-type gas in the surrounding rocks poses a serious threat to the safe and efficient mining of coal.[6] For example, the coexistence of coal and oil in the Haishiwan mine in the Yaojie coalfield, which also involves the risk of coal and gas protrusion, may cause crude oil pollution during mining and may also generate power disasters.[7] In general, once the excavation tunnel exposes oil and gas wells, there will be major safety hazards such as water, gas, and oil gushing into the working face, causing serious production accidents.[8,9] In addition, there are many abandoned oil wells in the coal and oil resource overlap area because oil was exploited first,[3] and the presence of these abandoned wells poses significant design difficulties for the mining of adjacent coal seams.

Eliminating the impact of oil is critical to addressing the safety risks associated with the coexistence of coal and oil. Comprehensive utilization of oil is an important means to eliminate the hidden danger of oil in coal seams, including dry

Received: April 5, 2023
Accepted: June 8, 2023
Published: June 21, 2023

distillation of oil shale, semi-coke combustion, fine oil shale and gas power generation, and ash utilization.[3] The protective seam mining technology was also adopted in the Haishiwan Mine, which was used to eliminate the protrusion risk of this coal seam by selecting the oil-bearing coal seam as the protective seam and combining it with the coal seam gas extraction technology.[7] In addition, the outstanding performance of functional microbial communities in coal and oil degradation has led to widespread interest in microbial-related technologies, such as microbial-enhanced coalbed methane technology[10] and microbial oil recovery technology.[11] However, the role of microbial communities in oil-bearing coal seams was not as widely reported. Microorganisms have a long history with oil hydrocarbons and are used in oil hydrocarbon-related industries, such as environmental cleanup of oil spills and biological treatment of refinery waste, as well as oil exploration, microbial-enhanced oil recovery (MEOR), biodesulfurization and biodenitrogenation of fractions, biodemetallization, bio-classification of heavy crude oil and refinery residues, bioconversion of residual oil to methane, and control of acidification and corrosion in oil fields.[12] Among them, due to the strong ability of functional microbial communities to degrade crude oil, MEOR has become one of the most viable and profitable technologies for extracting residual oil from low-capacity reservoirs.[13]

The structures of coal and oil are very different, making the methanogenesis pathways different for these two types of substances. Coal is complex in its organic structure and not easily biodegradable, resulting in a more complex process for biomethane production. The basic structure of coal is a condensed aromatic system, and the main structures are aromatic compounds, aliphatic compounds, and heterocycles. The bioavailability and activity of these organics are a prerequisite for effective coal biodegradation,[14,15] and the dissolution of these organics is the first step in the biogenic methane formation pathway.[16,17] Accordingly, oil is composed of hydrocarbons formed by the combination of carbon and hydrogen (accounting for about 95 to 99% of the composition), mainly including alkanes, cycloalkanes, and aromatic hydrocarbons. Wang et al.[18] suggested that aliphatic compounds in oil such as olefins and alkanes can be used by a variety of bacteria, and the resulting products can be used by methanogens. Methanogens convert various low-molecular-weight compounds including amines, alcohols, organic acids, and carbon dioxide to form methane through acetic acid fermentation or carbon dioxide reduction. The biodegradation efficiency of these compounds varies widely, with aliphatic compounds, especially *n*-alkanes, being the most biodegradable and the first components to degrade to methane.[19]

Therefore, we hypothesized that coal and oil have different methane conversion efficiencies in coal−oil symbiosis resources or oil-bearing coal seams. To confirm this hypothesis, the methanogenic potential, microbial community composition, functional genes, and metabolic pathways of these two different materials need to be compared.

2. MATERIALS AND METHODS

2.1. Experimental Design. The coal sample (weakly caking coal) and oil sample were collected in Huangling Coal Mine (109°15′E, 35°34′N) at Shaanxi Huangling Mining Co., Ltd., China. Bulk fresh coal samples were taken in the working face, and crude oil was collected through the oil and gas drainage hole. The coal and oil samples were stored in sterile bags, and pure nitrogen was flushed into the collection bags to avoid prolonged contact of coal and oil samples with oxygen. The sterile bags containing coal or oil were transported to the laboratory by an ice box. The outer layer of about 2 cm of coal was removed by a sterile blade, and the inner coal sample was ground into a powder with a sterile mortar and stored at −80 °C. The basic property of the coal sample was as follows: 2.70% air-dried moisture (M_{ad}), 17.11% air-dry coal ash (A_d), 33.75% dry ash-free basis volatile matter (V_{daf}), and 4.30% oil content.

Each coal powder sample or oil sample (10 g for each) was cultured in triplicate in 500 mL sterile bottles at 37 °C with 100 mL of minimal salt media (NH_4Cl 0.3 g/L, NaCl 0.5 g/L, $MgCl_2{\cdot}6H_2O$ 0.5 g/L, $CaCl_2{\cdot}2H_2O$ 0.1 g/L, KCl 0.5 g/L, and KH_2PO_4 0.2 g/L). Each culture bottle was sealed by a sterile nitrile stopper and the headspace air was replaced with nitrogen.

2.2. Coal Methanogenic Potential Analysis. To avoid the effect of methane in coal or oil samples on methanogenic potential, control groups with sodium 2-bromoethanesulfonate (BES) were established. That is, 1.0 mmol/L BES was added in the same culture system. We calculated the methanogenic potential by comparing the methane content in the headspace of the culture system with and without BES.

The CH_4 content was detected using gas chromatography with a TDX-01 packed column every 10 days. The headspace air was replaced with nitrogen after detection. The CH_4 content was calculated according to a calibration curve. The temperatures of the inlet, column, and thermal conductivity detector were set as 105, 90, and 120 °C, respectively.

2.3. Microbial Composition Analysis. The total genomic DNA in each culture system was extracted by the MB AquaScreen Fast Extract. The 16S rRNA gene fragment was amplified using primer 341F/806R and sequenced at the HiSeq platform (BGI Genomics Co., Ltd., China). The microbial data were analyzed according to the QIIME pipeline. The chimeric sequences were identified and removed based on the UCHIME algorithm using the "identify_chimeric_seq.py" and the "filter_fast.py" commands. Operational taxonomic units (OTUs) were clustered at 3% dissimilarity, and the taxonomic annotation was performed according to the SILVA reference data (v128). Sequences classified as "chloroplast", "mitochondria" or "unassigned" were removed.

2.4. Untargeted Metabolomics Analysis. The aqueous-phase samples from coal and oil culture systems were collected on the 0th and 90th day, which was used to compare the changing organic composition associated with the decomposition of coal and oil samples during culturing. The liquid samples were extracted by dichloroalanine and methanol and analyzed by a Xevo G2-XS QTOF (Waters, U.K.). The missing values were filled and the low-mass ions were removed by the KNN method in metaX software.[20]

The missing values were filled and the low-mass ions were removed by the KNN method in metaX software,[20] and the data correction was performed by quality control-based robust LOESS signal correction (QC-RSC).[21] In addition, the ions with relative standard deviation (RSD) >30% were removed. The metabolites were identified according to the KEGG database, after peak alignment, peak extraction, normalization, and deconvolution in Progenesis QI (v 2.2).

2.5. Metagenomic Analysis and Assembly Genomics Analysis. The total genomic DNA was sequenced at the DNBSEQ platform. The high-quality data were assembled by

https://doi.org/10.1021/acsomega.3c02303
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MEGAHIT[22] after filtering by SOAPnuke.[23] The metagenomic genes were predicted using MetaGeneMark (v1), clustered by CD-HIT, and annotated by Diamond[24] according to the KEGG database (https://www.kegg.jp/).[25] The abundance of metagenomic genes was calculated by Salmon (v1.9.0).

Metagenome-assembled genomes (MAG) were performed in a MetaWRAP pipeline.[26] The high-quality data were binned by MetaBAT2 and MaxBin2 and concocted after assembling by MEGAHIT. Each bin was classified by Taxater-tk according to the NCBI_nt and NCBI_tax databases and annotated by Prokka. The phylogenetic tree was constructed by the Genome Taxonomy Database Toolkit (GTDB-Tk, v1.3.0) based on a concatenated set of 120 bacterial proteins.[27]

3. RESULTS

3.1. Changes in Microbial Diversity. Both coal and oil samples can produce CH_4 under culture conditions (Figure 1).

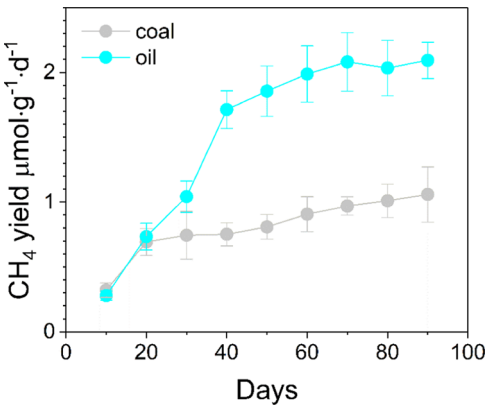

Figure 1. Changes in the biological methanogenic potential between coal and oil culture systems.

The coal biological methanogenic potential increased slowly after the 20th day and increased from 0.74 to 1.06 from the 20th day to the 90th day, respectively. The biological methanogenic potential of the oil sample increased significantly in the first 40 days and then flattened after the 40th day. The biological methanogenic potential of the oil sample was 1.98−2.29 times higher than that of coal after the 40th day.

A total of 441,351 high-quality microbial sequences were obtained. The read number for each sample was normalized to 72,000. The microbial diversity indices of coal and oil samples were significantly different (Figure 2). The observed OTU number and the Shannon diversity index in coal (observed OTUs 1033 ± 36; Shannon diversity 3.93 ± 0.04) were significantly higher than those in oil (observed OTUs 211 ± 8; Shannon diversity 1.42 ± 0.13). The Bray−Curtis dissimilarities between and within groups were analyzed to compare the difference in the microbial phylotype composition, and the intergroup difference of the Bray−Curtis dissimilarities (0.99 ± 0.01) between the coal and oil samples was much higher than those of the intragroup differences of the Bray−Curtis dissimilarities in the coal (0.06 ± 0.01) and oil (0.05 ± 0.02) samples.

3.2. Changes in Microbial Composition. The microbial compositions of coal and oil samples were quite different (Figure 3). The major genera included *Sedimentibacter*, *Lysinibacillus*, *Brevibacillus*, *Bacillus*, *Nocardiopsis*, *Paenibacillus*, *Mobilitalea*, *Garciella*, *Sporolactobacillus*, *Domibacillus*, and *Ruminiclostridium_1*. The major genera in oil included *Enterobacter*, *Sporolactobacillus*, *Bacillus*, *Klebsiella*, *Lysinibacillus*, *Escherichia-Shigella*, and *Domibacillus*. The relative abundance of methanogenic archaea in the microbial community was low. The metagenomic taxonomic annotation revealed that methanogenic archaea in coal mainly belonged to orders Methanobacteriales, Methanocellales, Methanococcales, Methanobacteriales, Methanomicrobiales, and Methanosarcinales (0.022 ± 0.003%); meanwhile, the methanogenic archaea in oil mainly belonged to genera *Methanobacterium*, *Methanobrevibacter*, *Methanoculleus*, and *Methanosarcina* (0.003 ± 0.001%).

A high-quality metagenome-assembled genome (MAG) relating to *Enterobacter* (the genus that showed the highest relative abundance in the oil culture system) was successfully reconstructed. The phylogenetic tree based on a concatenated set of 120 bacterial proteins showed that the genome was different from the known *Enterobacter* species (Figure S1). Based on the UniProt-DB protein database, *Enterobacter* MAG possessed the major metabolic pathways including glycolysis, *β*-oxidation, TCA cycle, oxidative phosphorylation, nitrogen metabolism, and sulfur metabolism (Figure S2). This *Enterobacter* bin possessed urease subunits (*ure* genes) and was

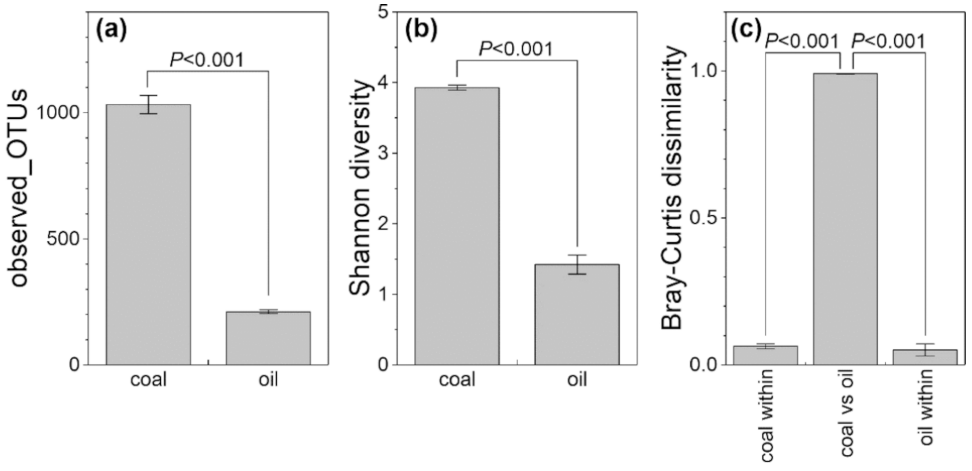

Figure 2. Changes in microbial diversity. (a) Observed OTU number, (b) Shannon diversity, and (c) Bray−Curtis dissimilarity between and within groups.

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ACS Omega 2023, 8, 23880−23888

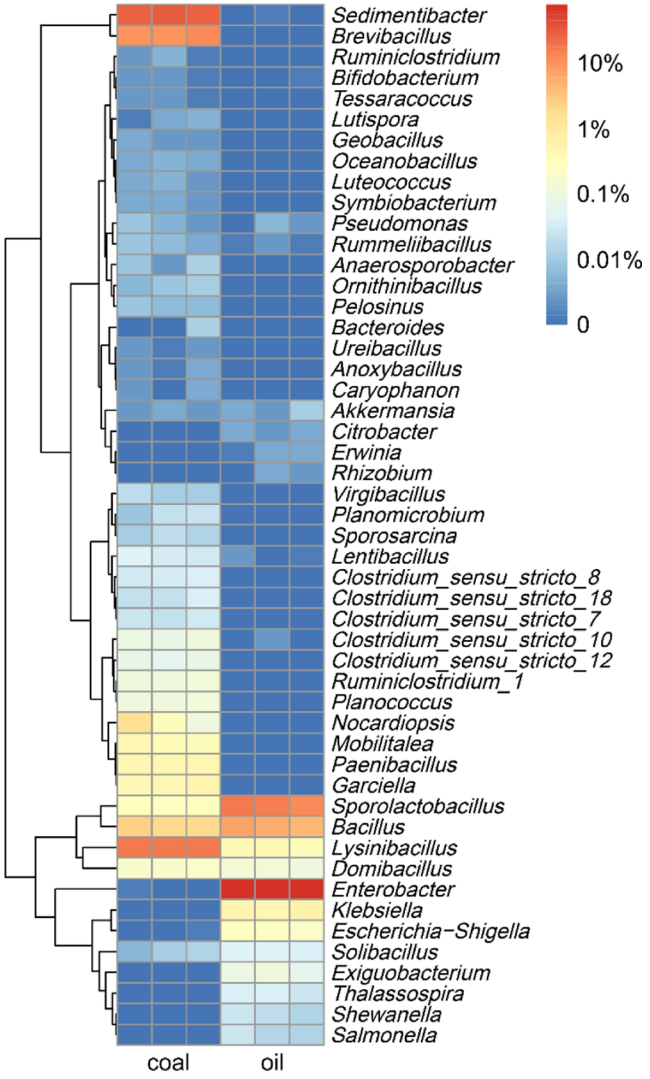

Figure 3. Heatmap for the relative abundance of the main microbial genera.

involved in the supply of N and S elements in the biosynthesis possess of acetate via serine. For example, this bin possessed urease subunits (*ure*), glycine dehydrogenase (*gcv*), glycine hydroxymethyltransferase (*gly*), serine *O*-acetyltransferase (*cysE*), sulfate reductase (*cys*), and cysteine synthase (*cysK*). The flagellar movement of *Enterobacter* bin could be ascertained by the presence of genes related to flagella assembly and motor proteins (*mot*, *flh*, *fli*, and *flg*).

3.3. Untargeted Metabolomics Revealed the Shifts in Organic Degradation. According to the criteria of fold change (FC) >2 and variable importance in projection (VIP) ≥1, anaerobic culture increased 1549 metabolites in the coal sample (Table S1). These metabolites can be divided into 19 super classes including benzenoids (108 metabolites), organoheterocyclic compounds (125 metabolites), phenylpropanoids and polyketides (48 metabolites), lipids and lipid-like molecules (48 metabolites), fatty acyls (16 metabolites), etc. Anaerobic culture increased 836 metabolites (Table S2) in the oil sample including 82 organoheterocyclic compounds, 58 benzenoids, 46 organic acids and derivatives, and 18 fatty acyls. In addition, a total of 129 metabolites in the aqueous phase of the oil culture system were significantly higher than those of the coal culture system (Table S3); meanwhile, 196 metabolites in the coal culture system were higher than those in the oil culture system (Table S4). Among them, we further screened the 29 metabolites (19 in the oil culture system and 10 in the coal culture system) related to methane metabolism, degradation of aromatic compounds, fatty acid degradation, polycyclic aromatic hydrocarbon degradation, and other benzene series degradation (Figure 4). The benzenoids with a higher abundance in the oil culture system included hydroquinone, 1,3,5-trihydroxybenzene, 2,5-dichlorophenol, 1,3-benzenediol, α-oxo-benzeneacetic acid, and toluene, and organic acids and derivatives with a higher abundance in the oil culture system included acetoacetate, acetic acid, and 2-methylpropanoate.

3.4. Changes in Functional Genes. ReporterScore[28] was applied to analyze the difference in KEGG orthologs (KOs). It showed that the abundance of functional genes belonging to methane metabolism, microbial metabolism in diverse environments, benzoate degradation, porphyrin metabolism, xylene degradation, styrene degradation, butanoate metabolism, nitrotoluene degradation, and fluorobenzoate degradation was higher in the oil culture system than that in the coal culture system (Figure 5a). In parallel, the abundance of functional genes belonging to sulfur metabolism, biotin metabolism, and glutathione metabolism was higher in the coal culture system than that in the oil culture system. The difference in the abundance of functional genes belonging to the degradation of aromatic compounds, carbon metabolism, and microbial metabolism in diverse environments showed that the oil culture system had a higher level in the KOs (at level 3) belonging to nitrogen fixation, dissimilatory sulfate reduction, thiosulfate oxidation by the SOX complex, acetyl-CoA pathway, propanoyl-CoA metabolism, incomplete reductive citrate cycle, ethylmalonyl pathway, methanogenesis (including methylamine/dimethylamine/trimethylamine to methane, CO_2 to methane, methanol to methane, and acetate to methane), homoprotocatechuate degradation, benzoate degradation, and catechol meta-cleavage (Figure 5b).

Based on the KEGG functional gene annotation, the microbial methanogenic pathway was reconstructed (Figure 6). The results showed that the functional genes for the methanogenic model of acetate to methane showed the highest relative abundance among the four pathways, and most of the functional genes in the four pathways had a higher relative abundance in the oil culture system.

4. DISCUSSION

In this study, samples from oil-bearing coal seams in China were used, and it is the first to survey a higher biological methanogenic potential of oil than that of coal in an oil-bearing coal seam. Coal and oil coassociated resources are widespread, and nowadays, the impact of oil on coal mining is mainly reduced by the protective layer mining technology and oil comprehensive utilization, but these do not eradicate its hidden danger. The results of this study have a reference value for the elimination of oil from coal in oil-bearing coal seams, namely, the oil fraction can be converted to methane and extracted by microbial technologies.

The biological methanogenic efficiency of coal in this study increased from 0.74 to 1.06 from day 20 to day 90, respectively, and the biological methanogenic potential of the oil sample was about twice that of the coal sample after 40 days of incubation. In a previous study of anaerobic fermentation experiments on coal and oil, the biological methanogenic yields

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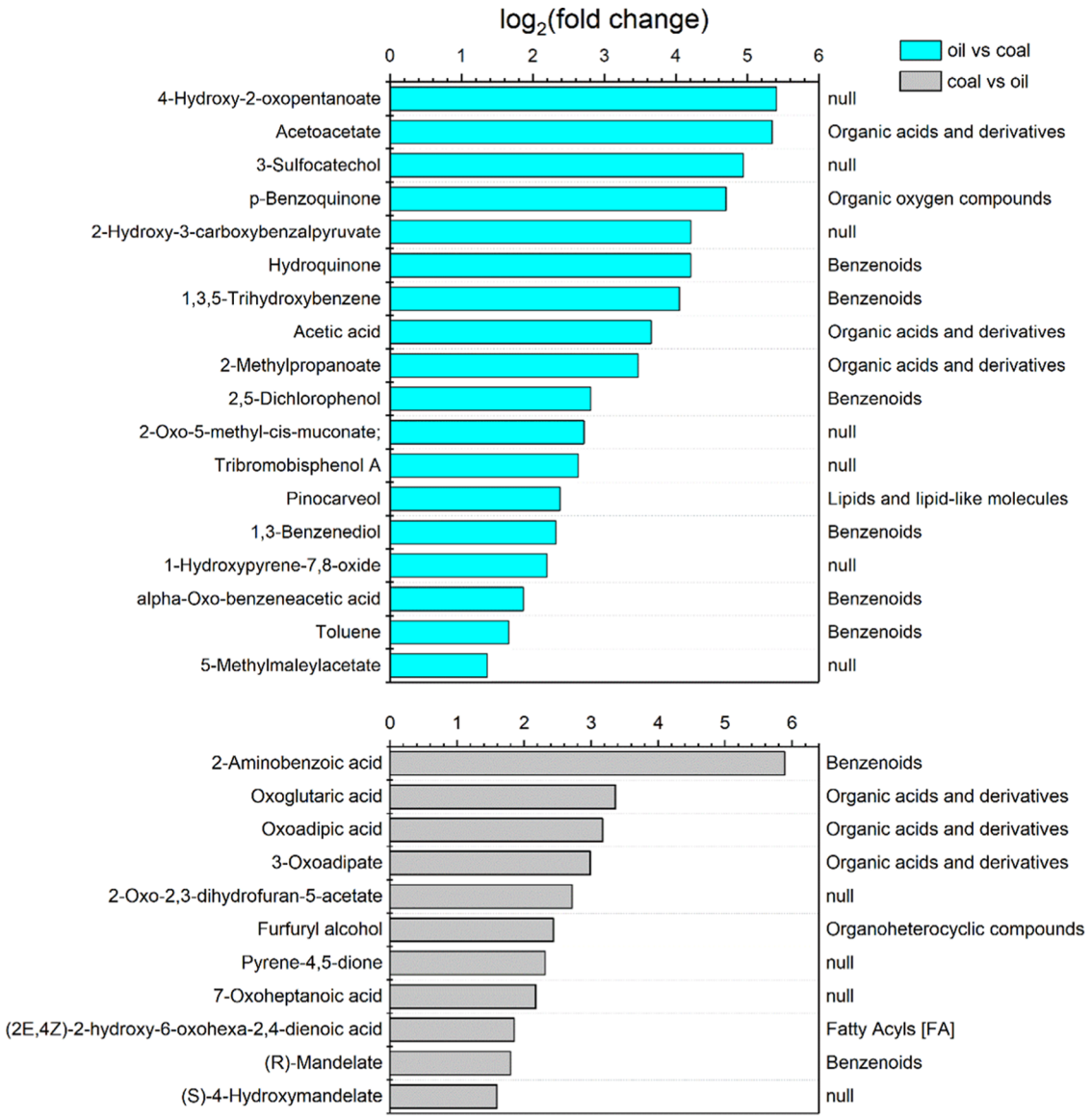

Figure 4. Differential metabolites related to methane metabolism, degradation of aromatic compounds, fatty acid degradation, polycyclic aromatic hydrocarbon degradation, and other benzene series degradation.

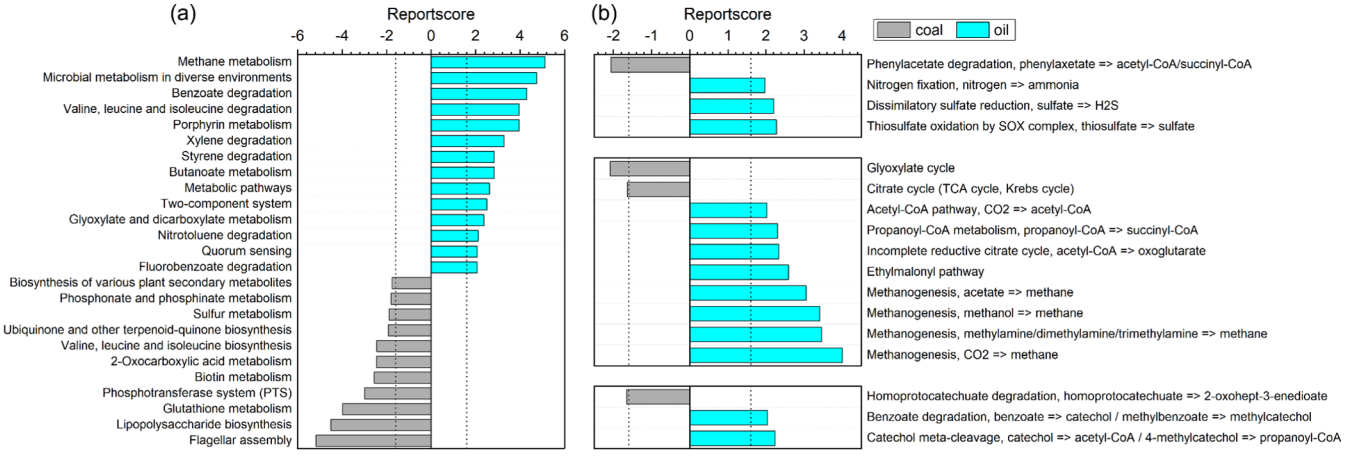

Figure 5. Functional gene difference among samples. (a) The ReporterScore of the methanogenic pathway at level 2 in coal and oil samples. (b) The ReporterScore of methanogenic pathways for the degradation of aromatic compounds, carbon metabolism, and microbial metabolism in diverse environments at level 3 in coal and oil samples.

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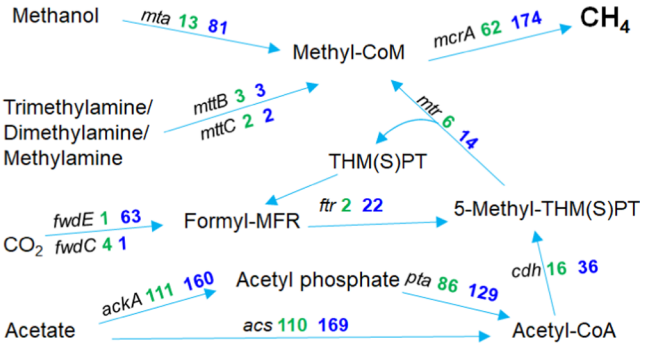

Figure 6. Overview of the anaerobic methanogenic metabolic pathway in coal and oil culture systems. Gene abundance was normalized as the read number per one hundred thousand genes. The numbers in green and blue indicate the abundance of genes detected in the coal and oil culture systems, respectively.

ranged from 0.005 to 0.437 μmol $CH_4 \cdot g^{-1}$ coal$\cdot d^{-1}$[29,30] and 3 to 5.8 μmol $CH_4 \cdot g^{-1}$ oil$\cdot d^{-1}$, respectively.[31,32] In contrast, the biological methanogenic yields of oil samples were much higher than that of coal samples. This difference is inseparable from microbial diversity, composition, and functional gene abundance.

In this study, the α-microbial diversity including Shannon diversity and observed OTU numbers in oil were lower than those in coal. In previous studies, the observed OTU number in coal samples ranged from 861 to 2789 in different basins such as the Bowen Basin, the Sydney Basin, and the Surat Basin;[33] meanwhile, the average values of OTU numbers in oil samples ranged from 199.9 to 292.4 in the Shengli oil field, the Xinjiang Karamay oil field, and the Daqing oil field.[34] It is known that for oil and coal samples, the microbial diversity of the former is generally lower than that of the latter. Therefore, it suggested that higher biodiversity does not indicate a higher methanogenic capacity in coal−oil symbiosis areas.

The microbial composition of the two materials differed significantly. The main genera in coal included *Sedimentibacter*, *Lysinibacillus*, *Brevibacillus*, etc. These taxa were widely distributed in coal seams in the Powder River Basin of the United States,[35] the Bowen Basin, Australia,[30] the San Juan Basin,[29] the Surat Basin,[33] etc. The main genera in the oil included *Enterobacter*, *Sporolactobacillus*, *Bacillus*, *Klebsiella*, etc. These taxa were the major taxa in reservoirs such as North Sea crude oil,[36] Xinjiang Karamay and Daqing oil fields,[34] and Wilcox Group wells in the United States.[37] *Bacillus* can activate hydrocarbon degradation functional genes to promote hydrocarbon degradation and methane metabolism;[3838] anaerobic hydrocarbon degradation occurs through hydrocarbon activation by carboxylation, hydroxylation, methylation, and reverse methanogenesis. Besides, these *Bacillus*-like genera in oil can produce alkane monooxygenase, alcohol dehydrogenase, and other oxidative enzymes, which are responsible for degrading long-chain hydrocarbons and facilitating the biodegradation of crude oil under aerobic conditions,[39] and can also enrich other petroleum hydrocarbon-degrading bacteria and activate hydrocarbon-degrading functional genes, which can reduce crude oil impurities including PAHs, chlorinated hydrocarbons, and chlorinated olefins.[38] In addition, the genus with the highest relative abundance in the oil was *Enterobacter*, and one of the bins was analyzed by assembling the genome, and the main metabolic pathways of the bin were found to be glycolysis, β-oxidation, TCA cycle, oxidative phosphorylation, nitrogen metabolism, and sulfur metabolism, which may be critical taxa in the degradation of petroleum hydrocarbons. Straight-chain alkanes can form carboxyl groups through β-oxidation, and fatty acids are also degraded to acetyl coenzyme A through β-oxidation and enter the tricarboxylic acid cycle or other biochemical processes.[40] Also, this taxon can use nitrate and sulfate for acetate synthesis.[34] These bacteria were also widespread in other samples from previous studies and were involved in the degradation of oil to produce methane.[37] For example, the analysis of the genomes of other strains of the genus *Enterobacter* identified several oxygenase genes that may serve as key enzymes in the hydrocarbon pollutant degradation pathway, including quercetin 2,3-dioxygenase, which can biotransform aromatic hydrocarbons to salicylic acid and cause oxidative decomposition of alkanes through the action of a series of enzymes.[41] Methanogenic archaea in coal and oil were low in relative abundance. The methanogenic archaea in coal mainly belonged to the orders Methanobacteriales, Methanocellales, and Methanococcales, which were common taxa in coals.[42,43] The methanogenic archaea in oil mainly belonged to the genera *Methanobacterium*, *Methanobrevibacter*, *Methanoculleus*, and *Methanosarcina*, which were widely present in oil reservoirs.[11,44] These taxa may have more methanogenic genes in the oil samples, and the metagenome data show that the methanogenesis-related functional genes in oil were generally higher than those in coal (Figure 6).

Metagenome analysis further revealed significant differences in the functional genes of oil and coal culture systems and showed that functional genes belonging to the processes of methane metabolism, microbial metabolism in different environments, and benzoate degradation had a higher abundance in the oil culture system. Correspondingly, functional genes belonging to sulfur metabolism, biotin metabolism, and glutathione metabolism were in a higher abundance in the coal culture system. The difference in the abundance of functional genes in different degradation metabolic processes showed that higher levels belong to nitrogen fixation, isotropic sulfate reduction, etc., in the oil culture system. The fundamental reason for this difference was the different methanogenesis pathways of coal and oil. Fermentation was an important step in the biological methanogenesis of coal, where fermentative bacteria activate complex macromolecular compounds and then degrade less complex compounds into various fatty acids, CO_2, and H_2.[45] Bacteria in oil initiated the metabolism of available compounds, ranging from monosaccharides, fatty acids, alcohols to complex hydrocarbons and aromatic compounds, eventually producing intermediates such as acetate, formate, and hydrogen.[46] Genes related to benzyl succinate synthase and ethylbenzene dehydrogenase are present in coal and are associated with the activation and degradation of aromatic substrates such as toluene, xylene isomers, and ethylbenzene. Enzymes encoding enzymes involved in the anaerobic breakdown of benzoate, a key intermediate in the anaerobic degradation of aromatic hydrocarbons, are also widely present,[29] and the methanogenic mode in which alkanes are converted to acetate, by β-oxidation, and acetate is converted to methane is the main pathway by which oil is degraded to produce methane.[47,48] Meanwhile, most of the functional genes in the four methanogenic pathways possessed high relative abundance in the oil culture system. These might be the underlying reason for the higher biological methanogenic efficiency of oil.

https://doi.org/10.1021/acsomega.3c02303
ACS Omega 2023, 8, 23880−23888

The difference in methanogenesis between oil and coal can also be reflected by the change in metabolites. The total class of metabolites in this study was higher in coal samples rather than in oil samples, and the metabolites specific to coal samples belonged to phenylpropanoids, polyketides, lipids, and lipid-like molecules, while the metabolites specific to oil mainly belonged to organic acids and their derivatives. The reason for this difference was the difference in methanogenic pathways between the two types of substances. For example, the degradation of coal was carried out by primary fermentation of high-molecular-weight coal components and smaller monomers to intermediates (e.g., fatty acids, organic acids, alcohols, hydrogen, and carbon dioxide) that were used as substrates for syntrophic, homoacetylic, and methanogenic bacteria.[29] Biodegradation of aliphatic and cyclic hydrocarbons might be a source of metabolite fatty acids, metabolites can be further oxidized to methanogenic substrates, and benzoates were central intermediates in the anaerobic degradation of many natural and xenobiotic aromatic compounds.[45] Bacteria in the oil can produce low-molecular-weight organic acids such as formic acid and propionic acid during fermentation.[49] Aromatic hydrocarbons can be converted to salicylic acid by the action of enzymes.[41] In addition, the biodegradation of ethylbenzene into benzoylacetic acid was driven by a series of enzymes including ethylbenzene dehydrogenase, dehydrogenase and carboxylase.[39]

In conclusion, this paper used typical oil-bearing coal seam samples from China, and the results of the study indicated that the methane production of the oil samples was much higher than that of the coal samples, but their microbial diversity was lower than that of the coal samples. Functional genes for processes such as methane metabolism, microbial metabolism in different environments, and benzoate degradation had a higher abundance in oil, while in coal, the abundance of functional genes belonging to sulfur metabolism, biotin metabolism, and glutathione metabolism was higher. The reason for these differences lies in the different methanogenic pathways between the oil and coal samples, which also lead to differences in metabolites. It is worth noting that the exogenous interventions in this study were smaller for coal and oil samples, while the magnitude of the methanogenic potential of both samples under conditions of biostimulation and microbial enhancement has not been investigated in detail. The findings of this study facilitate the separation of oil samples from oil-bearing coal seams, reducing the hazards brought by oil for coal seam mining and improving the safety of resource extraction and resource utilization.

■ ASSOCIATED CONTENT

Data Availability Statement

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

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02303.

Phylogeny of reconstructed *Enterobacter* MAG and the overview of metabolic potentials in *Enterobacter* MAG; and metabolites in coal and oil (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was funded by the National Natural Science Foundation of China (52274171) and the Independent Research Fund of the State Key Laboratory of Mining Response and Disaster Prevention and Control in Deep Coal Mines at Anhui University of Science and Technology (SKLMRDPC20ZZ08).

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