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# Optimizing the Components of Active Water Fracturing Fluids for Coalbed Methane Using Biochemical Engineering Approaches

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## ABSTRACT

Hydraulic fracturing is an effective technique for enhancing coal seam permeability. While coal as a macromolecular organic material, can be biodegraded by microorganisms, limited research has explored the integration of these processes to develop bioactive fracturing fluids. This study employed Shengli lignite from Inner Mongolia as the substrate and coal seam mine water as the microbial inoculum. Through single-factor experiments and orthogonal experimental design, investigated the effects of wetting agent concentration, clay stabilizer concentration, and demulsifier concentration in bioactive fracturing fluid additives on the microbial methanogenesis of lignite. The results indicate that, in terms of their impact on gas production, the three factors rank in descending order of significance as follows: wetting agent concentration, clay stabilizer concentration, and demulsifier concentration. Orthogonal analysis identified the optimal additive combination for bioactive fracturing fluid as 0.15% wetting agent, 2% clay stabilizer, and 0.15% demulsifier by mass concentration. The optimized formulation led to a substantial 31.73% increase in biogas yield compared to the pre-optimized condition. Three-dimensional fluorescence spectroscopy revealed that the fluorescence intensity of fulvic acid-like and humic acid-like substances was higher after optimization, indicating more complete degradation of the coal samples. Furthermore, metagenomic sequencing analysis demonstrated that the optimized bioactive fracturing fluid significantly improved the microbial community structure, markedly increasing the abundance of Methanosarcina. The predominant methane metabolism pathways were acetate fermentation and carbon dioxide reduction, with a substantial increase in the gene abundance related to methanogenic pathways, thereby promoting microbial methane production. These findings elucidate the mechanistic effects of bioactive fracturing fluid composition on lignite biogasification and provid.

## KEYWORDS

Coalbed Gas Bioengineering; Active Water Fracturing; Composition Optimization; Dissolved Organic Matter; Metabolic Pathway.

## 1. INTRODUCTION

With the progress towards China’s "dual carbon" goals, the proportion of fossil energy dominated by coal is steadily decreasing, prompting a rapid growth in the demand for clean energy, particularly natural gas, which has increasingly relied on imports in recent years. Coalbed methane (CBM), as a high-quality unconventional energy source, holds significant value for its rational development, contributing to energy optimization, safety, and environmental protection. CBM development not only enhances economic benefits but also effectively prevents gas accidents and reduces greenhouse gas emissions. This makes CBM a crucial factor in optimizing energy structure, ensuring production safety, and promoting the sustainable development of the ecological environment[1,2].

Coalbed Gas Bioengineering (CGB) primarily aims to stimulate production in CBM wells by injecting growth media to activate indigenous microorganisms[3-6]. Hydraulic fracturing, an effective technology for enhancing the permeability of low-permeability reservoirs, serves as a prerequisite for the implementation of microbial production enhancement measures. Active water fracturing fluid, due to its low pollution and cost-effectiveness, has become the primary technology widely used for coalbed reservoir modification in China[7]. Combining methane-producing archaea and other microorganisms with active water fracturing fluid for coal reservoir modification allows for conventional fracturing effects, while also promoting microbial degradation of the coal reservoir. This not only increases CBM production but also enhances the commercial value of CBM wells. Studies have shown that adding the surfactant AN to microbial fracturing fluid effectively reduces capillary pressure, helping to alleviate water blocking damage. Additionally, the surfactant does not interfere with microbial methane production[8]. Hydraulic fracturing significantly increases the variety of microorganisms in the water of coal reservoirs, with the most notable changes being the increase in the abundance of Actinobacteria at the phylum level, as well as the elevated concentrations of Amicenantes and Planctomycetes[9,10]. Furthermore, research suggests that hydraulic fracturing enhances the abundance of Aminocenantes, which is beneficial for the generation of hydrogenotrophic methane[11].

Based on the existing formulation of active water fracturing fluid, this study explores the impact of the additive amounts of wetting agents, clay stabilizers, and demulsifiers on the production of biological methane from lignite. On this basis, an orthogonal experiment combined with variance analysis is conducted to determine the optimal gas production combination. The soluble organic matter changes during the biological methane production process are analyzed using three-dimensional fluorescence spectroscopy. Additionally, metagenomic analysis is employed to study the intrinsic mechanisms of biological methane conversion during anaerobic digestion, revealing changes in key metabolic functions and gene abundance throughout the process. The results confirm the feasibility of biologically active water fracturing fluid, providing a practical formulation and theoretical foundation for its application in coal reservoir modification.

## 2. MATERIALS AND METHODS

### 2.1. Sample Collection and Preparation

The coal sample was selected from the Shengli West Mine in Inner Mongolia. The sample was dried at 105°C for 8 hours and then pulverized to a particle size of 60-80 mesh for further use. The industrial and elemental analyses were conducted according to the national standards ISO 17246–2010 and ISO 17247-2013, respectively. The results are presented in Table 1.

**Table 1** Proximate and ultimate analysis of coal samples

| Sample source | Ro,max | Mad | Aad | Vad | Cdaf | Hdaf | Odaf | Ndaf | Sdaf |
|---|---|---|---|---|---|---|---|---|---|
| Shengli West Mine | 0.37 | 10.36 | 23.01 | 47.20 | 52.44 | 4.26 | 41.84 | 1.25 | 0.22 |

Ro,max: Maximum reflectance of vitrinites; M: Moisture content; A: Ash content; V: Volatile matter; ad: Air-dried basis; daf: Dry, ash-free basis; C: Carbon; H: Hydrogen; O: Oxygen; N: Nitrogen; S: Sulfur.

### 2.2. Methanogen Enrichment Culture

The microbial culture used in the experiment was enriched from long-term acclimatized mine water. The enrichment medium for the microbial community consists of the following components: $K_2HPO_4$

0.4 g, $KH_2PO_4$ 0.2 g, $NH_4Cl$ 1.0 g, $MgCl_2$ 0.1 g, HCOONa 0.5 g, $CH_3COONa$ 0.5 g, Yeast extract 1.0 g, Cysteine hydrochloride 0.5 g, $Na_2S$ 0.2 g, $NaHCO_3$ 2 g, Peptone 0.1 g, Trace element solution 10 mL.

The trace element solution consists of: Aminotriacetic acid (EDTA) 1.5 g, $MnSO_4 \cdot 2H_2O$ 0.5 g, $MgSO_4 \cdot 7H_2O$ 3 g, $FeSO_4 \cdot 7H_2O$ 0.1 g, NaCl 1.0 g, $CoCl_2 \cdot 6H_2O$ 0.1 g, $CaCl_2 \cdot 2H_2O$ 0.1 g, $CuSO_4 \cdot 5H_2O$ 0.01 g, $ZnSO_4 \cdot 7H_2O$ 0.1 g, $H_3BO_3$ 0.01 g, $AlK(SO_4)_2$ 0.01 g, $NiCl_2 \cdot 6H_2O$ 0.02 g, $Na_2MoO_4$ 0.01 g. These components were dissolved in 1 L of deionized water.

The medium was autoclaved at 121 °C for 20 minutes, then cooled. The pH was adjusted to 7 using 1 mol/L NaOH or HCl, and then mixed with the microbial culture in a 10:1 ratio. After nitrogen ($N_2$) flushing for 5 minutes, the mixture was sealed and incubated in a 35°C constant temperature incubator for 5 days before use.

## 2.3. Biogas Production Experiment

### 2.3.1. Single Factor Experiment

The initial formulation of the active water fracturing fluid is known to be as follows: 0.1% by mass of the wetting agent BH, 3% by mass of the clay stabilizer FP, and 0.3% by mass of the demulsifier XP. The aim is to optimize this formulation to develop a microbe-friendly active water fracturing fluid.

In the experiment, 20 g of coal sample is mixed with 500 mL of microbial culture solution and placed into a 500 mL Erlenmeyer flask. A 0.05% by mass of wetting agent BH is added to the flask. Nitrogen gas is then flushed through the flask for 5 minutes to create an anaerobic environment. The flask is sealed with a sealing film, and the entire setup is incubated in a 35°C constant temperature incubator. The gas produced during the experiment is collected using a gas collection bag, and the experiment continues until completion. The gas composition is analyzed using gas chromatography (GC9720 Plus, Fuli Analytical Instruments Co., Ltd., China).

Under the same cultivation conditions, different concentrations of the wetting agent BH, clay stabilizer FP, and demulsifier XP are varied to study the effects of each factor on the biogas production of lignite. The single-factor experimental design is shown in Table 2.

**Table 2** Single factor experiment

| Wetting agent BH mass concentration/% | Clay stabilizer FP mass concentration/% | Emulsion breaker XP mass concentration/% |
|---|---|---|
| 0.05 | 0.5 | 0.05 |
| 0.10 | 1.0 | 0.10 |
| 0.15 | 1.5 | 0.15 |
| 0.20 | 2.0 | 0.20 |
| 0.25 | 2.5 | 0.25 |
| 0.30 | 3.0 | 0.30 |

### 2.3.2. Orthogonal Experiment

Building on the results of the single-factor experiment, a three-factor, three-level orthogonal experiment is conducted, using the biological methane production as the evaluation criterion. The experiment investigates the impact of the active water fracturing fluid additives—wetting agent BH, clay stabilizer FP, and demulsifier XP—on the biogas production of lignite. The goal is to determine the optimal formulation of the biologically active water fracturing fluid. The factors and levels for the orthogonal experiment are presented in Table 3.

**Table 3** Orthogonal experimental factors and levels

| Level | Considerations | | |
|---|---|---|---|
| | Wetting agent BH mass concentration A/% | Clay stabilizer FP mass concentration B/% | Emulsion breaker XP mass concentration C/% |
| 1 | 0.10 | 1.0 | 0.05 |
| 2 | 0.15 | 1.5 | 0.10 |
| 3 | 0.20 | 2.0 | 0.15 |

### 2.4. Three-Dimensional Fluorescence Analysis

The three-dimensional fluorescence analysis was performed using a Hitachi F-7000 fluorescence spectrophotometer. The system used a 150 W xenon lamp as the light source, with the photomultiplier tube voltage set at 700 V. The excitation and emission slit widths were both 10 nm, with a scanning speed of 1200 nm/min. The excitation wavelength range was set from 200 to 420 nm with a step size of 5 nm, and the emission wavelength range was from 240 to 600 nm with a step size of 2 nm.

### 2.5. Metagenomic Sequencing Analysis

Total DNA was extracted from the fermentation liquid at peak production and the bottom coal sample using the FastDNA® Spin Kit for Soil. The integrity of the extracted genomic DNA was verified by 1% agarose gel electrophoresis. The DNA was then fragmented to approximately 350 bp using the Covaris M220 ultrasonicator. The PE library construction was carried out using the NEXTFLEX Rapid DNA-Seq Kit. Metagenomic sequencing was performed by Shanghai Meiji Biotechnology Co., Ltd., using the Illumina NovaSeq sequencing platform.

## 3. RESULTS AND DISCUSSION

### 3.1. Analysis of Single-Factor Experimental Results

The results of the single-factor experiments on the effect of fracturing fluid additives on biological methane production from lignite coal are shown in Fig.1. As shown in Fig.1(a), the concentration of the surfactant has a clear effect on biological methane production. Initially, methane production increases with the surfactant concentration, reaching its peak at a concentration of 0.15%. This can be attributed to the fact that lower surfactant concentrations enhance the coal sample's solubility, facilitating better contact between the coal and the microbial culture, thereby promoting microbial degradation of the coal. However, when the surfactant concentration becomes too high, it exerts toxicity on the microorganisms, thereby reducing microbial activity and subsequently decreasing methane production. In Fig.1(b), the clay stabilizer concentration also shows a significant effect on methane production. The highest methane yield is observed at a concentration of 1.5%. Further increases in the clay stabilizer concentration lead to higher salinity, which reduces microbial activity and suppresses microbial reproduction, ultimately causing a decline in methane production. As seen in Fig.1(c), increasing the demulsifier concentration results in little to no change in biological methane production. This suggests that within the tested concentration range, the demulsifier does not significantly affect microbial activity, and therefore, there is minimal impact on methane production.

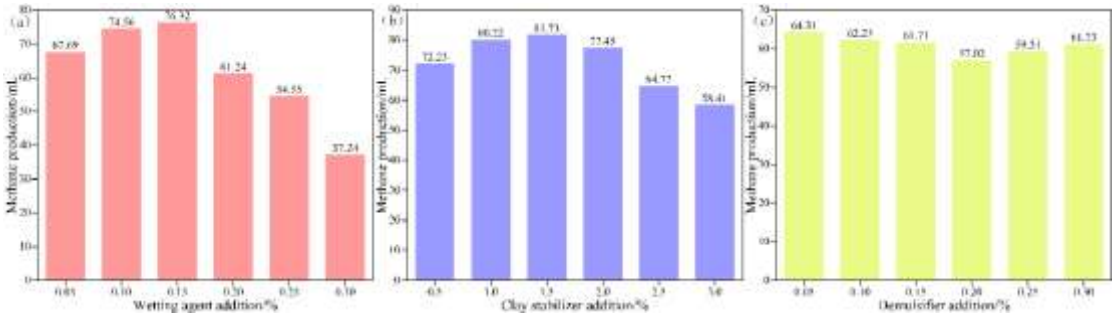

**Fig. 1** Single factor experimental results of fracturing fluid additives on methane produced by lignite (a) wetting agent (b) clay stabilizer (c) demulsifier

## 3.2. Orthogonal Experiment Analysis

The results of the orthogonal experiment on the effect of fracturing fluid additives on biological methane production from lignite coal are shown in Table 4. The calculation of the range values (R) in Table 4 reveals that the factors influencing biological methane production, in descending order of their impact, are the surfactant (BH) concentration, the clay stabilizer (FP) concentration, and the demulsifier (XP) concentration. Among these, the concentrations of the surfactant and clay stabilizer have a significantly greater effect on methane production than the demulsifier. Both the surfactant (BH) and clay stabilizer (FP) concentrations have a marked impact on the biological methane production process. This is because these additives can influence the activity of methane-producing microorganisms, which require a period to adapt to the environmental conditions of the system. The experimental results show that after optimization, the optimal combination of additives for the bioactive water fracturing fluid is A2B3C3, where the surfactant (BH) concentration is 0.15%, the clay stabilizer (FP) concentration is 2%, and the demulsifier (XP) concentration is 0.15%. Under this optimal formulation, the biological active water fracturing fluid achieves a maximum methane production of 94.48 mL.

**Table 4** Range analysis results of orthogonal experiment

| Serial number | Considerations | | | | Methane production/mL |
|---|---|---|---|---|---|
| | A | B | C | Inaccuracies | |
| 1 | 1 | 1 | 1 | 1 | 68.55 |
| 2 | 2 | 2 | 1 | 2 | 87.69 |
| 3 | 3 | 3 | 1 | 3 | 78.73 |
| 4 | 3 | 2 | 2 | 1 | 64.84 |
| 5 | 2 | 1 | 2 | 3 | 79.22 |
| 6 | 1 | 3 | 2 | 2 | 81.56 |
| 7 | 1 | 2 | 3 | 3 | 69.48 |
| 8 | 2 | 3 | 3 | 1 | 94.48 |
| 9 | 3 | 2 | 3 | 2 | 54.23 |
| K1 | 219.59 | 202.00 | 234.97 | 227.87 | |
| K2 | 261.39 | 222.01 | 225.62 | 223.48 | |
| K3 | 197.80 | 254.77 | 218.19 | 227.43 | |
| k11 | 73.1967 | 67.3333 | 78.3233 | 75.9567 | |
| k22 | 87.1300 | 74.0033 | 75.2067 | 74.4933 | |
| k33 | 65.9333 | 84.9233 | 72.7300 | 75.8100 | |
| R | 21.1967 | 17.5900 | 3.1167 | 0.1467 | |
| Weighting order | | | A＞B＞C | | |
| Optimal combination | | | A2B3C3 | | |

The variance analysis of the orthogonal experiment data for biological methane production from lignite coal using bioactive water fracturing fluid is presented in Table 5. Variance analysis is commonly used to evaluate the variability of experimental data. The data in the table were computed using IBM SPSS Statistics 27 software. In the analysis, Adj SS represents the adjusted sum of squares, Adj MS represents the mean square, F-value is the statistic used for hypothesis testing, and P-value indicates the significance of the results. The P-value reflects the likelihood of randomness in the data—lower P-values suggest a smaller chance of randomness, indicating stronger statistical significance. Additionally, the $R^2$ value represents the coefficient of determination, while the adjusted $R^2$ ($R^2$(adj)) accounts for the number of predictors in the model. In this analysis, $R^2$ = 99.7% and $R^2$(adj) = 98.7%, which indicates a very strong relationship between the variables and confirms the high reliability of the data. From the variance analysis table, it can be observed that the concentrations of surfactant (BH) and clay stabilizer (FP) had a significant impact on biological methane production from lignite coal. In contrast, the demulsifier (XP) showed no significant effect on methane production during the experiment.

**Table 5** Orthogonal experimental variance analysis of methane produced by lignite in active water fracturing fluid

| Considerations | Degrees of freedom | Adj SS | Adj MS | F value | P value |
|---|---|---|---|---|---|
| A | 2 | 696.192467 | 348.096234 | 178.672763 | 0.005566 |
| B | 2 | 473.143400 | 236.571700 | 121.428833 | 0.008168 |
| C | 2 | 47.132867 | 23.566434 | 12.096309 | 0.076357 |
| Inaccuracies | 2 | 3.896467 | 1.948234 | | |

## 3.3. Biological Methane Production Before and After Optimization of Active Water Fracturing Fluid

The results of biological conversion of lignite coal before and after the optimization of the active water fracturing fluid are presented in Table 6. The experimental group HX-H exhibited a cumulative gas production of 261.3 mL, with a cumulative methane yield of 94.48 mL, accounting for 36.07% of the total gas produced. In comparison, the experimental group HX-Y showed a cumulative gas production of 230.4 mL, a cumulative methane yield of 71.72 mL, and a methane percentage of 31.13%.

The cumulative methane production of the HX-H group was 31.73% higher than that of the HX-Y group, indicating a significant improvement. Furthermore, compared to the KB group, the HX-H group showed an 11.86% increase in methane production.

These experimental data indicate that the optimized active water fracturing fluid significantly enhanced biological adaptability, improving methane production potential. This demonstrates the feasibility of using bioactive water fracturing fluid in lignite coal bioconversion processes.

**Table 6** The results of methane produced by lignite before and after fracturing fluid optimization

| Sample number | Substrate composition | Before and after fracturing fluid optimization | Cumulative gas production(mL) | Cumulative methane production(mL) | Percentage of methane(%) |
|---|---|---|---|---|---|
| KB | lignite 20 g | / | 247.30 | 84.46 | 34.15 |
| HX-H | lignite 20 g+0.15% BH+2% FP+0.15% XP | post-optimization | 261.90 | 94.48 | 36.07 |
| HX-Y | lignite 20 g+0.1% | pre-optimization | 230.40 | 71.72 | 31.13 |

BH+3%
FP+0.3% XP

### 3.4. Analysis of Soluble Organic Matter Changes at Different Stages of the Digestion Process

Soluble organic matter (DOM) is a complex, stable high-molecular organic compound that serves as a direct nutrient source for microorganisms. Changes in the content and composition of DOM can influence the microbial community structure and its activity [12].

Fermentation liquids from the pre-gas production, middle, and post-gas production stages of the HX-H and HX-Y groups were collected for soluble organic matter testing. The fluorescence spectrum primarily consists of tyrosine-type proteins (Region I), tryptophan-type proteins (Region II), fulvic acids (Region III), soluble microbial metabolic products (Region IV), and humic acids (Region V) [13].

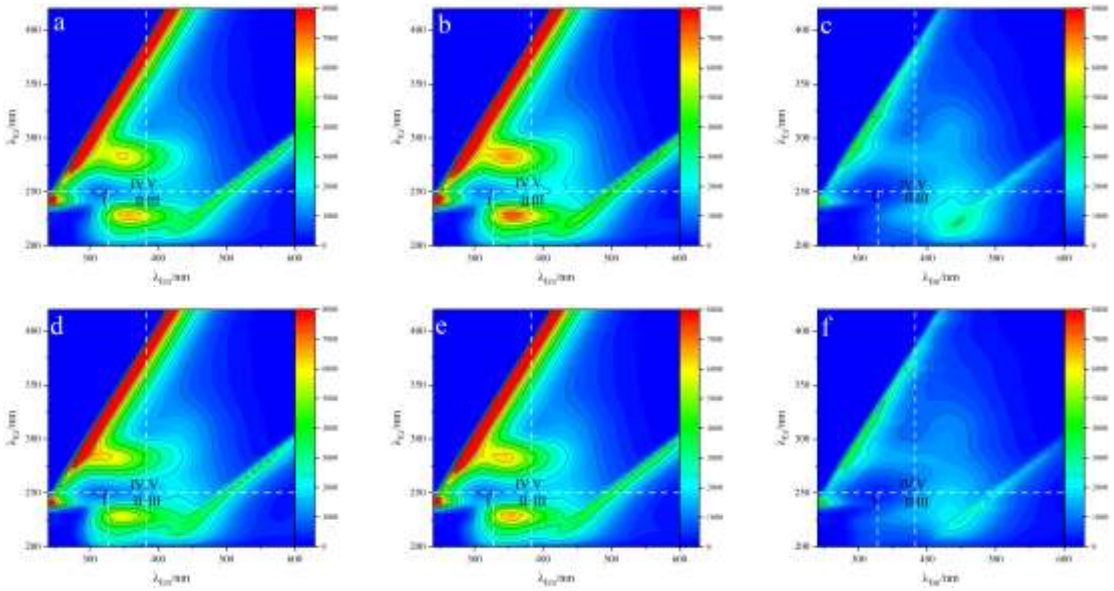


**Fig. 3** Characteristics of three-dimensional fluorescence changes in different stages (a) HX-H early stage (b) HX-H peak stage (c) HX-H end stage (d) HX-Y early stage (e) HX-Y peak stage (f) HX-Y end stage

According to the results shown in Fig.3, the DOM in each group of samples is primarily distributed in Region II and Region IV, with tryptophan-type proteins and soluble microbial metabolic products being the dominant components. The content of fulvic acid substances in Region III is relatively low, while the contents of tyrosine-type proteins in Region I and humic acid substances in Region V are the lowest. As the biological methane production experiment progresses, the fluorescence intensity of tyrosine-type proteins (Region I), tryptophan-type proteins (Region II), and soluble microbial metabolic products (Region IV) initially increases and then decreases. This indicates that these three types of substances are involved in the biological methane production process, and they are associated with microbial metabolic activity. In the later stage of gas production, the fluorescence intensity of Region III is significantly higher than that of the other regions. Under the action of microorganisms, fulvic acid substances are released from the coal sample. These substances contain a large number of groups such as phenolic hydroxyl groups and ketone carbonyl groups. However, these substances have a low microbial utilization rate and tend to accumulate in the liquid phase. In Region V, the fluorescence intensity of HX-H samples is noticeably higher than that of HX-Y samples. The fluorescence in this region originates from the humic acid organic matter in the coal, which is decomposed and transferred to the liquid phase under the influence of microorganisms. This suggests that the biological degradation effect of the HX-H coal sample is better. The optimized active water

fracturing fluid exhibits better biological adaptability, confirming the feasibility of the biological active water fracturing fluid.

## 3.5. Microbial Community Composition and Metabolic Pathway Analysis

### 3.5.1. Microbial Community Changes

To explore the impact of fracturing fluid additives on the microbial community structure in the reaction system, the microbial populations of archaea and bacteria were identified at the peak gas production period in experimental groups HX-H and HX-Y. The microbial community types and abundances are shown in Fig. 4.

From Fig.4(a), it can be observed that the bacterial genera in HX-H are dominated by Lysinibacillus (23.37%), Lascolabacillus (9.69%), Stutzerimonas (8.36%), Acinetobacter (5.49%), and Aminobacterium (3.80%). Among these, Lysinibacillus is the most abundant genus in the HX-H group. This genus primarily participates in the breakdown of organic matter, producing various beneficial substances and highly active enzymes. These enzymes help rapidly consume the residual $O_2$ in the system, creating a favorable anaerobic environment for subsequent anaerobic digestion[14]. Lascolabacillus is involved in the degradation of proteins, sugars, and other substances during anaerobic digestion, producing $H_2$, $CO_2$, acetic acid, and butyric acid[15,16]. Stutzerimonas and Acinetobacter are capable of degrading proteins and fats. Stutzerimonas is a denitrifying bacterium[17] and has a broader substrate utilization range than methane-producing bacteria, with the ability to degrade both short- and long-chain alkanes as well as aromatic hydrocarbons[18-21]. Additionally, denitrifying bacteria compete with methane-producing bacteria for carbon sources, with denitrifying bacteria having a higher competitive ability. Methanogens begin to metabolize and produce $CH_4$ only when the carbon source ratio is sufficient[22,23]. Aminobacterium can degrade amino acids into short-chain and branched volatile acids during anaerobic digestion, along with $CO_2$ and $H_2$. The deamination of amino acids also releases $NH_4^+$, which can further increase the pH of the system[24].

In HX-Y, the bacterial genera are primarily Lascolabacillus (10.31%), Stutzerimonas (10.13%), Aliarcobacter (7.17%), Aminobacterium (5.30%), and Pseudomonas (5.29%). Aliarcobacter generates organic acids, $CO_2$, $CH_4$, and other metabolic products through its metabolic activity, providing energy and material for its growth and reproduction. From Fig.4(b), it can be observed that in both reaction systems, the archaeal communities are predominantly composed of Methanosarcina and Methanothrix, confirming that the optimization of the active water fracturing fluid did not alter the archaeal species in the reaction systems. Methanosarcina is a versatile methanogen capable of utilizing acetate, $H_2$, $CO_2$, and methyl compounds to produce $CH_4$[25]. Methanothrix, a strict acetoclastic methanogen, degrades acetate to produce $CH_4$ and $CO_2$[26]. In the HX-H group, Methanosarcina is the dominant genus (71.27%), while Methanothrix is present at a lower relative abundance (27.13%). In the HX-Y group, Methanosarcina (53.44%) is still the dominant genus, but the relative abundance of Methanothrix (43.97%) is higher compared to HX-H.

In summary, the relative abundance of denitrifying bacteria in the HX-H experimental group decreased by 17.47% compared to HX-Y. The decrease in denitrifying bacteria helped alleviate the suppression of methane-producing bacteria and other bacteria, promoting a more favorable environment for methanogenesis.Furthermore, the relative abundance of Methanosarcina in HX-H increased by 33.36%, while the relative abundance of Methanothrix decreased by 38.30%. In the same anaerobic digestion environment, Methanosarcina grows faster and can utilize multiple substrates to generate methane. In contrast, Methanothrix grows more slowly and can only utilize acetate as a substrate for methane production.Considering growth rate and substrate utilization diversity, Methanosarcina demonstrates superior methanogenic capacity compared to Methanothrix. Therefore, the optimization of the active water fracturing fluid improved the microbial composition

within the anaerobic digestion system, enhancing methane production potential and making the optimized fracturing fluid more biologically adaptive.

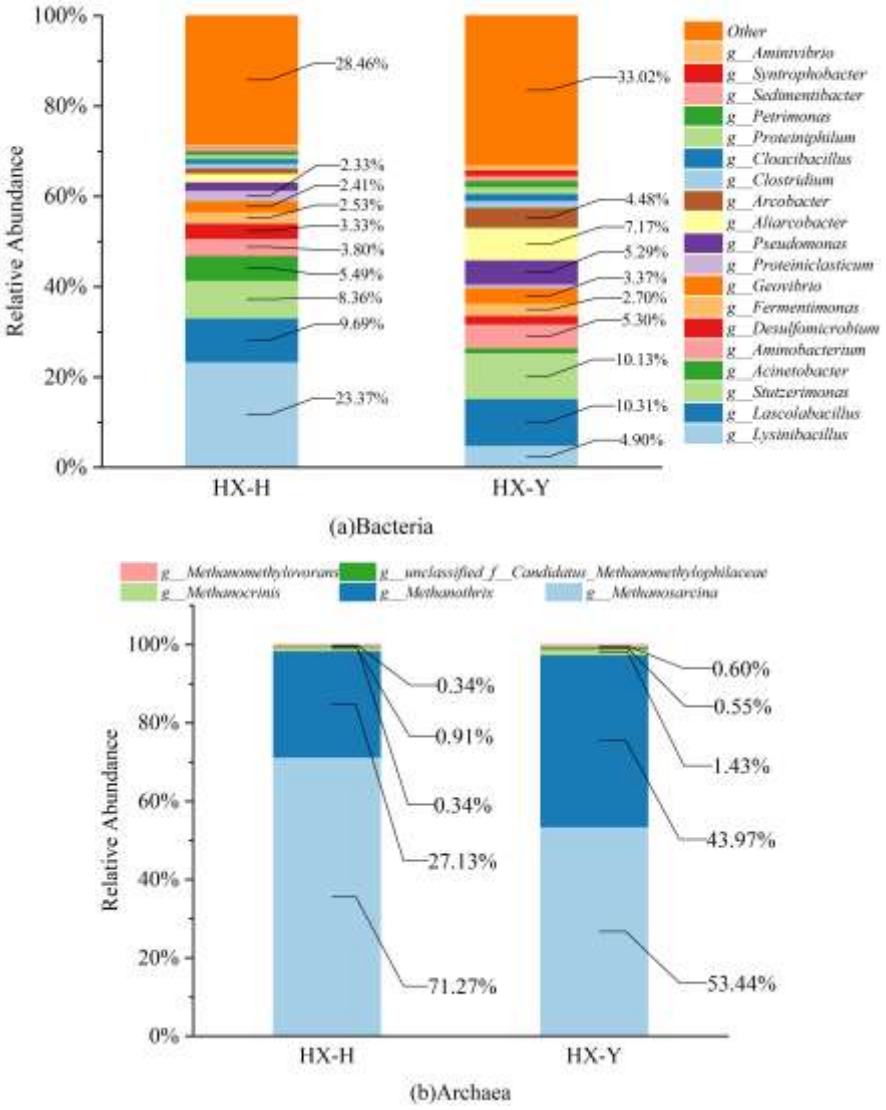


**Fig. 4** Microbial community structure at genus level at the peak of gas production

### 3.5.2. Methane metabolism pathway

**Table 7** The abundance of genes related to methane metabolic pathway based on KEGG

| Pathway | EC Number | K Number | Abundance | |
|---|---|---|---|---|
| | | | HX-H | HX-Y |
| M00567 | 1.2.7.12 | K00200 | 0.018507 | 0.014100 |
| | | K00201 | 0.021260 | 0.016923 |
| | | K00202 | 0.013204 | 0.010457 |
| | | K00203 | 0.009560 | 0.007073 |
| | | K00204 | 0.000040 | 0.000046 |
| | | K00205 | 0.014580 | 0.011052 |
| | | K11261 | 0.040740 | 0.042203 |
| | | K11260 | 0.010098 | 0.008357 |
| | 2.3.1.101 | K00672 | 0.006958 | 0.005419 |
| | 3.5.4.27 | K01499 | 0.006374 | 0.005691 |
| | 1.5.98.1 | K00319 | 0.006316 | 0.004442 |
| | 1.5.98.2 | K00320 | 0.007663 | 0.005523 |
| | 1.12.98.1 | K00440 | 0.003979 | 0.002701 |
| | | K00441 | 0.024291 | 0.023198 |
| | | K00442 | 0.002446 | 0.004320 |
| | | K00443 | 0.003863 | 0.002296 |
| M00357 | 2.7.2.1 | K00925 | 0.043255 | 0.042926 |
| | 6.2.1.1 | K01895 | 0.124654 | 0.103214 |
| | 2.3.1.8 | K00625 | 0.021156 | 0.018802 |
| | | K13788 | 0.011949 | 0.007449 |
| | 2.3.1.169 | K00193 | 0.015477 | 0.013505 |
| | 2.1.1.245 | K00197 | 0.018716 | 0.017114 |
| | | K00194 | 0.012915 | 0.011174 |
| M00356 | 2.1.1.90 | K04480 | 0.014193 | 0.008091 |
| | 2.1.1.246 | K14080 | 0.018334 | 0.012666 |
| | MtaC | K14081 | 0.012383 | 0.006553 |
| M00563 | 2.1.1.250 | K14083 | 0.011185 | 0.006293 |
| | MttC | K14084 | 0.010468 | 0.005905 |
| | 2.1.1.247 | K14082 | 0.006038 | 0.003644 |
| | 2.1.1.249 | K16178 | 0.013140 | 0.007415 |
| | MtbC | K16179 | 0.007987 | 0.006113 |
| | 2.1.1.248 | K16176 | 0.016154 | 0.009387 |
| | MtmC | K16177 | 0.005107 | 0.002955 |

Methane metabolism is the final stage of anaerobic fermentation and the microorganisms that play a major role in this stage are methanogenic archaea. The process consists of four main pathways: the M00567 (carbon dioxide reduction), M00357 (decarboxylation of acetic acid), M00356 (methanol methane conversion), and M00563 (methylamine/dimethylamine/trimethylamine methane conversion)[27]. The results of the analysis of different metabolic pathways of the two groups of samples according to the KEGG database are shown in Table 7 and Figure 5.The results showed that in the digestive system of the two groups of experiments, the acetic acid fermentative methanogenic pathway (24.81%, 21.42%) accounted for the highest percentage of methanogenesis, which was the most competitive way of methanogenesis, followed by the carbon dioxide reduction pathway (18.99%, 16.38%), which was much higher than that of the methanolic methanolysis pathway ( 4.49%, 2.73%) and methylamine/dimethylamine/trimethylamine methanation pathways (7.01%, 4.17%). It can be seen that in the anaerobic digestion process, the expression of the archaea using acetic acid and $CO_2$

as substrates was the highest, and both methane metabolism pathways were higher in the HX-H group than in the HX-Y group, indicating that the optimized activated water fracturing fluid system was more conducive to the metabolism of archaea using acetic acid and $CO_2$ as nutrients, which was beneficial to the production of material methane.

In the acetic acid metabolic pathway, the key enzymes involved in the conversion of acetic acid are acetate kinase (ack, EC:2.7.2.1), phosphate acetyltransferase (pta, EC:2.3.1.8), acetyl-CoA synthase (acs, EC:6.2.1.1) and acetyl CoA synthase (ACDS), acetic acid undergoes two processes: the conversion of acetic acid to acetyl-coenzyme A and the decarboxylation of acetyl-coenzyme A to the methane precursor (5-methyl-THMPT) by ACDS, which is finally converted to methane[28, 29]. Among them, the gene abundance of acs system (12.47%, 10.32) was higher compared to the abundance of acs-pta system (6.44%, 6.17%), indicating that the main pathway for the conversion of acetic acid to acetyl-coenzyme A during the reaction is the pathway involved in acs. And in the CO2 reduction pathway, CO2 will be reduced to a series of intermediates such as formyl, methylene and methyl groups by a variety of enzymes, and finally the methyl group is transferred to CoM to form Methyl-CoM, which finally catalyzes the reduction to $CH_4$[30, 31]. In summary, the methane pathway of lignite from activated water fracturing fluid mainly uses acetic acid and $CO_2$ as substrates for fermentation, and the dominant genera Methanosarcina and Methanothrix play an important role, and the optimized methane system of lignite from activated water fracturing fluid performs better in the decarboxylation pathway of acetic acid and the reduction pathway of $CO_2$.

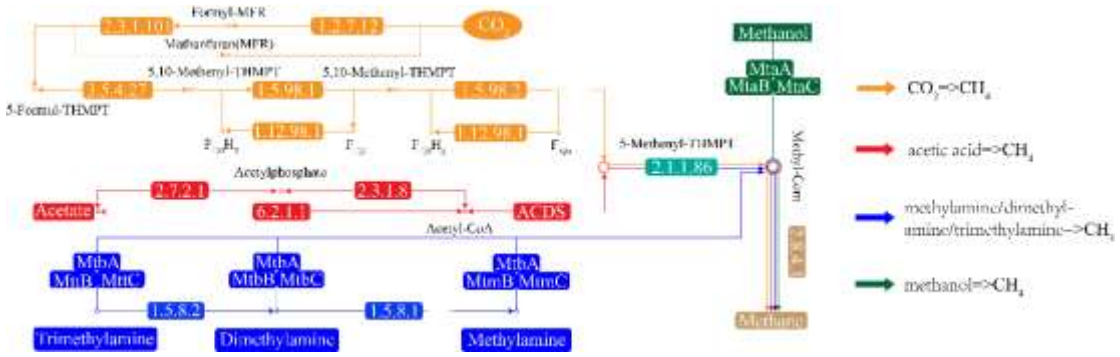


**Fig. 5** Methane metabolic pathway map

## 4. CONCLUSIONS

a. The optimized active water fracturing fluid was formulated with a wetting agent BH at a mass concentration of 0.15%, a clay stabilizer FP at a mass concentration of 2%, and an emulsion breaker XP at a mass concentration of 0.15%. The optimized active water fracturing fluid improved the biomethane conversion efficiency and significantly increased the biomethane production. The optimized active water fracturing fluid enhanced the microbial degradation of coal, and more fulvic and humic acids were dissolved into the fermentation fluid.

b. The optimized active water fracturing fluid enhanced the microbial degradation of coal, and more fulvic and humic acids were dissolved into the fermentation fluid.

c. Methanosarcina and Methanothrix became the dominant genera, which lowered the redundant microbial number and abundance, and increased the abundance of key genes of the methane metabolic pathway, thus promoting the production of biomethane.

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