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Experimental Study on Producing Hydrogen Sulfide from Different Sulfur-Containing Substrates by Methanogens

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Abstract

DMS and DMSO are common volatile organic sulfides. In order to study their degradation products under anaerobic conditions, this paper used DMS and DMSO as fermentation substrates and methanogenic mixed bacteria as strains to carry out Anyang fermentation. The experimental results show that there is no methylotroph methanogens in the fermentation system, and the degradation bacteria are *Paraclostridium*, *Aminobacterium* and *Desulfovibrio*, and the degradation product is hydrogen sulfide.

Keywords

H_2S; DMS; DMSO; Anaerobic Degradation.

1. Introduction

Organic sulfur in coal is mainly converted from proteins, mercaptans and thioethers in primitive plants and microorganisms, and is an important part of coal matrix. It exists in the form of five sulfur-containing groups, such as mercaptan, thiophene, thioether, sulfoxide and sulfone [1]. Organic sulfur in coal exists in the organic macromolecular structure of coal and is dispersed in the mineral structure at the molecular level. The existing states are generally thiophenyl (C_4H_4S-), sulfhydryl (-SH), sulfur ether (-S-) and polysulfide chain (-S-) X, etc. [2]. According to previous studies, methanogens (methyl trophic type) play an important role in the degradation of organic sulfur compounds. For example, the production and degradation of organic sulfur compounds in anaerobic digestion sludge both exist cyclic pathways. In terms of environmental protection, the degradation of volatile sulfur-containing organic compounds or post-production treatment is usually enhanced according to the regulation of substrates and microorganisms formed by organic sulfur compounds [3]. Among them, there are two main metabolic pathways of DMS (DMS): the demethylation of DMS leads to H_2S [4], or the degradation of DMS into DMSO leads to the oxidation of methyl sulfonic acid (MSA) [5]. Under anaerobic conditions, DMS can be metabolized by methanogenic bacteria and SRB, and methanogenic bacteria can degrade methyl mertan and DMS to form hydrogen sulfide. In the absence of sulfate, part of DMS will be converted into CH_4. In the presence of sulfate, SRB can also participate in the utilization [6]. Due to its thermal instability, DMSO can be disproportionated into DMS, DMS and dimethyl sulfone $DMSO_2$. Studies have shown that in active sludge, dimethyl sulfone $DMSO_2$ is degraded to SO_4^{2-} by microorganisms, and in addition, DMSO can also be directly degraded to SO_4^{2-} by bacteria [7]. In the anaerobic fermentation process of biogas, anaerobic microorganisms will decompose and transform complex organic matter such as lipids, proteins and cellulose into small molecular organic matter such as amino acid, sugar and glycerol. The transformed small molecular matter will be absorbed and utilized by microorganisms, among which sulfate-containing proteins will be decomposed and utilized to generate fatty acids, ammonia and hydrogen sulfide [8].

Therefore, this paper takes coal as carbon source, DMS and DMSO as sulfur source, and adds methanogenic bacteria that have been domesticated in the laboratory for a long time. Through

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anaerobic fermentation experiment, the production process of H_2S is identified, and combined with the change trend of sulfur-containing substances (including ions and organic matter) and the metabolic pathway analysis in the formation process of hydrogen sulfide. The formation mechanism of H_2S in anaerobic fermentation was systematically discussed.

2. Experimental Materials and Methods

2.1 Experimental Materials

The coal sample number is FK. Firstly, the coal sample is pretreated: (1) washing and drying. As coal sample is coal dust in drilling process, mixed with drilling fluid, need to be cleaned first. After ultrasonic washing with deionized water until the pH value of deionized water is neutral, the coal sample is placed in a drying oven at 200℃, and stored in a wide-mouth bottle with discolored silicone. (2) Crushing. The coal sample is put into the crusher, grinding and screening to make pulverized coal between 80 and 100 mesh. (3) sterilization. The selected coal samples were put into an autoclave for sterilization at 121℃ for 20min. (4) Coal sample test. Industrial analysis, elemental analysis, total sulfur form sulfur determination, vitrinite reflectance and other analysis tests were carried out on the screened coal sample (Table 1, 2).

Two sulfur-containing small molecules, DMS and DMSO, were selected as sulfur-containing substrates, and the bacteria used were derived from high-yield and high-efficient methanogenic bacteria domesticated in the laboratory.

Table 1. The Proximate analysis and Ultimate analysis

Coal sample	R_O/%	Proximate analysis				Ultimate analysis				
		M_{ad}/%	A_{ad}/%	V_{daf}/%	FC_{ad}/%	C%	H%	N%	O%	S%
FK	0.68	0.99	29.93	32	38.08	55.92	4.321	0.95	9.803	0.166

Note: M_{ad} is the moisture of air-dried coal samples; A_{ad} is the ash content of air-dried coal samples; V_{daf} is the volatile matter of coal; FC_{ad} is the carbon content; R_O is the vitrinite reflectance.

Table 2. The sulfur form analysis

Coal sample	$S_{t,d}$/%	$S_{s,d}$/%	$S_{s,d}$ accounts for/%	$S_{p,d}$/%	$S_{p,d}$ accounts for/%	$S_{o,d}$/%	$S_{o,d}$ accounts for/%
FK	0.509	0.012	2.35	0.183	35.95	0.314	61.69

Note: $S_{t,d}$ is full sulfur; $S_{s,d}$ is sulfate sulfur; $S_{p,d}$ is iron sulfide; $S_{o,d}$ is organic sulfur.

2.2 Experimental Process

In the experimental design, a 2L conical flask was selected as the fermentation vessel. 200mL of laboratory long-term acclimated bacteria solution was added into the culture medium after autoclaving at 120℃ for 20min with a pipetting gun, and then 100gFK coal was added into the bacterial solution in a ratio of 1:20. DMS and DMSO groups were divided into three groups according to the ratio of 1:10 to add 200mL sulfur-containing substrate, and the anaerobic culture was conducted at 35℃, and the gas production data were recorded every 2 days. The gas produced by fermentation was collected by a 200mL air bag. The gas composition content was analyzed by a gas chromatograph, and the volume of the gas produced was recorded. At the same time, the fermentation liquid samples were taken every 2 days and the pH value, SO_4^{2-} concentration, SO_3^{2-} concentration, $S_2O_3^{2-}$ concentration, HS^- concentration, S^{2-} concentration, CO_3^{2-} concentration and HCO_3^- concentration of the liquid samples were tested. The liquid phase organic matter and flora in the fermentation system were detected and analyzed every 6 days.

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2.3 Test Method

2.3.1 Gas Phase Analysis

In this experiment, Agilent gas chromatography (Agilent6820) was used to analyze the H_2S and other gas components produced in the fermentation process.

2.3.2 Analysis of Each Ion Content in Liquid Phase

The content of each ion in the experiment was measured by titration. Excessive iodine titration was used to determine SO_3^{2-}, $S_2O_3^{2-}$, HS^-, S^{2-}, EDTA titration to determine SO_4^{2-}, two indicator titration to determine CO_3^{2-} and HCO_3^-.

2.3.3 Microbial Diversity Test

The fermented bacterial solution in the system was sampled regularly and sent to Shanghai Meiji Biomedical Technology Co., Ltd. every 6 days for microbial diversity test.

3. Experimental Results

3.1 Gas Generation Characteristics of Anaerobic Fermentation System

In the process of anaerobic fermentation, the hydrogen sulfide production capacity of bacteria was the strongest at 0-6 d, and the change trend was consistent. The gas production peak was reached at 3d, and the gas production decreased rapidly at 3-9d and basically stabilized after 9d, with the gas production capacity being DMS>DMSO. When DMS was used as substrate for fermentation, the highest stage yield was 5.25 mL/g, and the cumulative gas production reached 12.71 mL/g. When DMSO was used as substrate for fermentation, the highest stage yield was 2.53 mL/g, and the cumulative gas production reached 5.79 mL/g. The production of nitrogen lasted for 15-18 days, and the ability of bacteria to produce nitrogen reached the highest value on day 3, with DMS at 0.29 mL/g and DMSO at 0.12 mL/g. The second highest value of DMSO was 0.09 mL/g on day 15. The methanogenic capacity is relatively weak on the whole, but the peak time of CH_4 is consistent with H_2S and N_2, both in the early stage (Fig. 1).

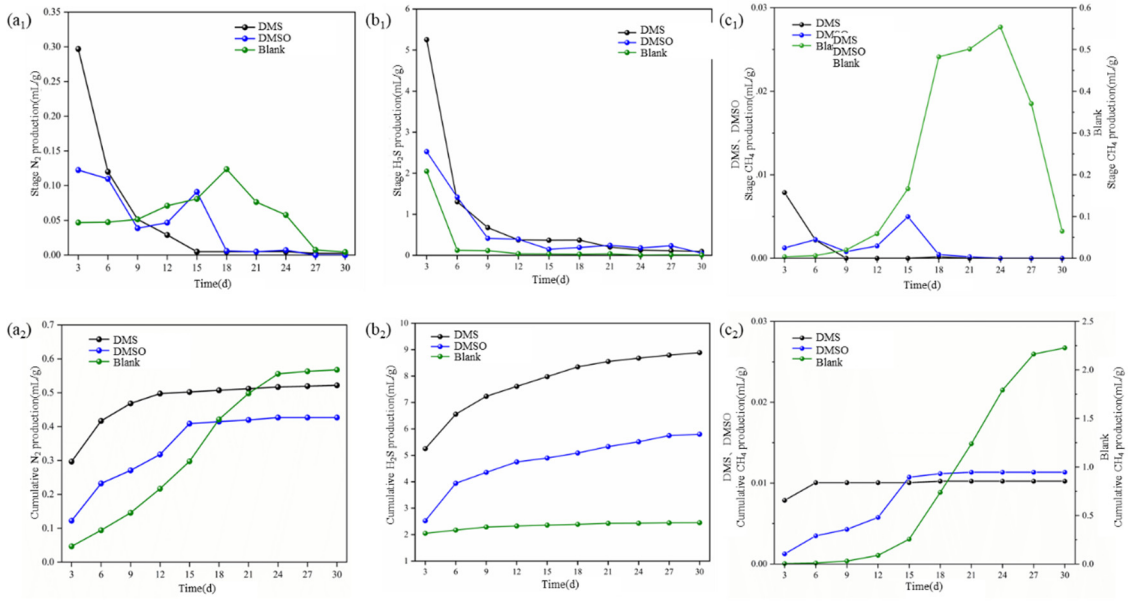

Fig 1. Phased output of N_2, H_2S and CH_4 (a_1, b_1, c_1) and total output (a_2, b_2, c_2) in anaerobic fermentation process

3.2 Results of Ion Content in Fermentation Process

Firstly, the SO_4^{2-} concentration in the fermentation system with DMS as sulfur-containing substrate showed an overall trend of increasing first and then decreasing, increasing to 252.8mg/L at 0-15 days and then continuously decreasing to 46.08mg/L at 27 days, and increasing to 76.8mg/L at the end of fermentation. The ion concentration of SO_3^{2-} showed a trend of dynamic stability, and decreased at

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9d and 15d respectively. $S_2O_3^{2-}$ ion concentration dropped steadily to 44.8mg/L in 12d after gas generation, then rose to 100.8mg/L and then kept its downward trend to 23.6mg/L in 24-30d, and then increased to 89.6mg/L in 24-30d (Fig. 2a). The concentration of SO_4^{2-} in the fermentation system with DMSO as a sulfur-containing substrate decreased from 207.36mg/L to 92.16mg/L during 6-12 d, then increased to 138.24mg/L, and then continued to decrease to 38.4 mg/L during 12-21 d, then increased and remained stable. The ionic concentration of SO_3^{2-} increased to 200mg/L at 0-12 d and then decreased to 80mg/L, increased to 200mg/L at 12-18 d, decreased to 160mg/L at 18-21 d and then increased to 240mg/L at 27d, and then decreased to 120mg/L. $S_2O_3^{2-}$ Ion concentration is basically stable (Fig. 3a). The total amount of H_2S was represented by the sum of dissolved H_2S (HS^-, S^{2-}) in the gas phase and water, and showed an overall increasing trend.

Secondly, the change trend of HCO_3^- and CO_3^{2-} ion concentration is consistent on the whole. In the fermentation system with DMS as sulfur-containing substrate, the concentration of HCO_3^- and CO_3^{2-} ions showed a dynamic increasing trend on the whole, with the variation range of CO_3^{2-} ranging from 510.17 to 576.19mg/L and that of HCO_3^- ranging from 915.3 to 1044.66mg/L (Fig. 2b). In the fermentation system with DMSO as a sulfur-containing substrate, the concentration of HCO_3^- and CO_3^{2-} ions on the whole showed an increasing - decreasing - increasing trend, and the concentration of CO_3^{2-} continued to increase from 456.15 mg/L at the beginning to 528.17 mg/L at the 9th day and then decreased to 456.15 mg/L at the 21st day. 24d is stable after its value rises to 546.18 mg/L; HCO_3^- continued to rise from 817.67 mg/L at the beginning to 878.69 mg/L at day 9d, then dropped to 724.92 mg/L at day 21d, and stabilized after its rise to 969.0 mg/L (Fig. 3b).

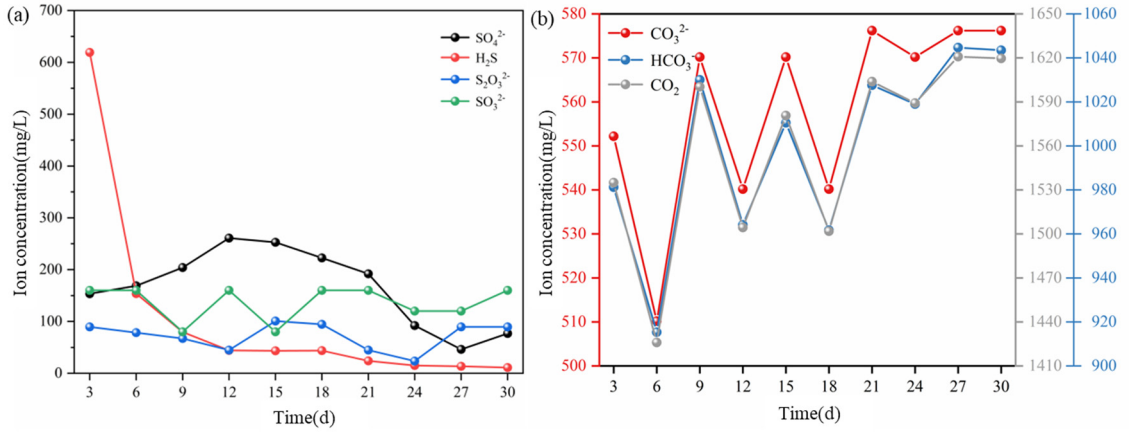

Fig 2. Changes of sulfur ion content, total H_2S (a) and contents of CO_2, CO_3^{2-} and HCO_3^- (b) in anaerobic fermentation of DMS

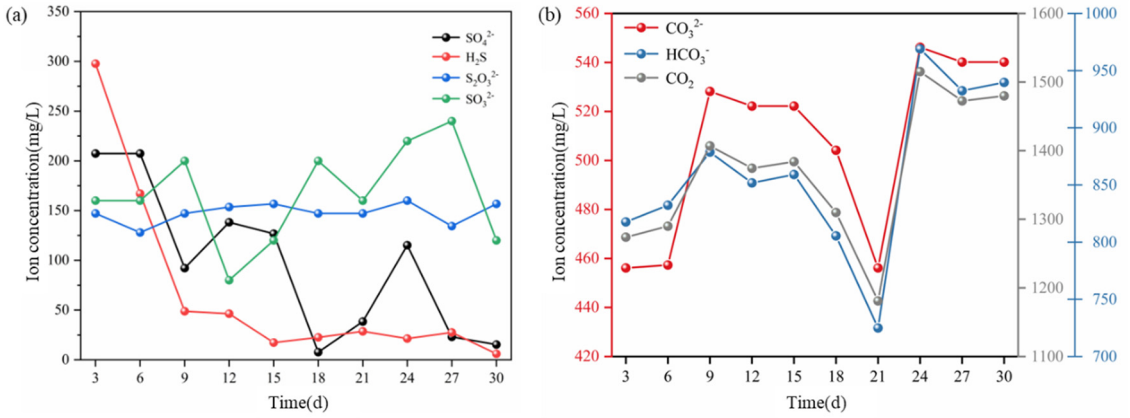

Fig 3. Changes of sulfur ion content, total H_2S (a) and contents of CO_2, CO_3^{2-} and HCO_3^- (b) in anaerobic fermentation of DMSO

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3.3 Characteristics of Microbial Community Structure in Anaerobic Fermentation System

In anaerobic fermentation with DMS as substrate, the dominant bacterial genera were *Paraclostridium* (23.93%), *Aminobacterium* (39.49%), *Clostridium-sensu-stricto-1* (5.03%). After 30 days, the dominant bacteria were *Paraclostridium* (26.89%), *Aminobacterium* (25.42%), *Clostridium-sensu-stricto-1* (4.39%), *Sedimentibacter* (4.04%), *Desulfitobacterium* (5.93%) (Fig.4a). In the early stage of fermentation, the dominant archaea were *Methanobacterium* (97.95%), *Methanosaeta* (1.57%) and *Methanoculleus* (0.11%). After 30 days of fermentation, The relative abundance of the dominant genus *Methanobacterium* decreased from 97.95% to 97.29%, that of *Methanosaeta* decreased from 1.57% to 0.86%, and that of *Methanoculleus* increased from 0.11% to 0.62% (Fig.5a).

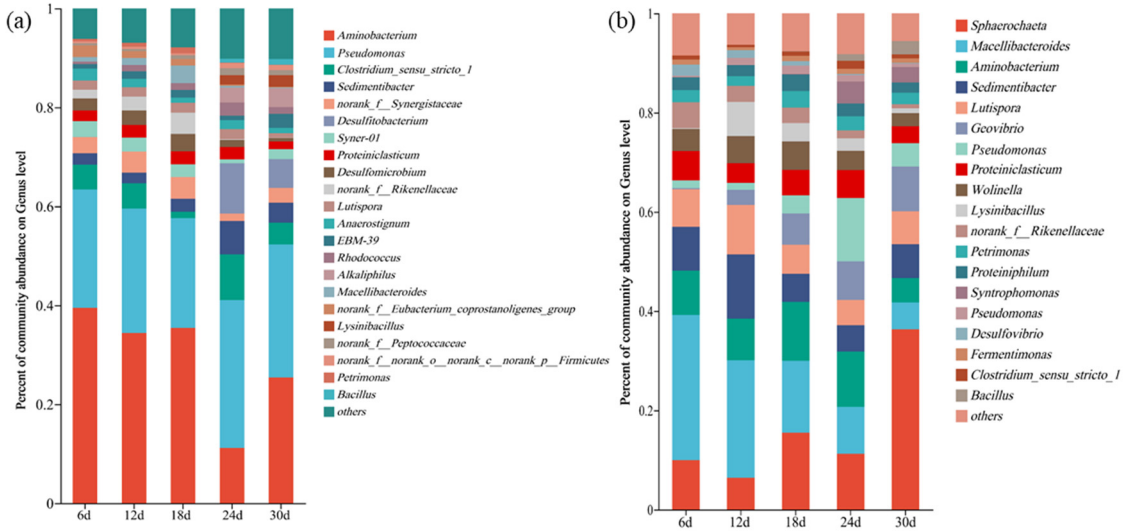

Fig 4. Structural characteristics of bacterial flora in anaerobic fermentation system DMS(a), DMSO(b)

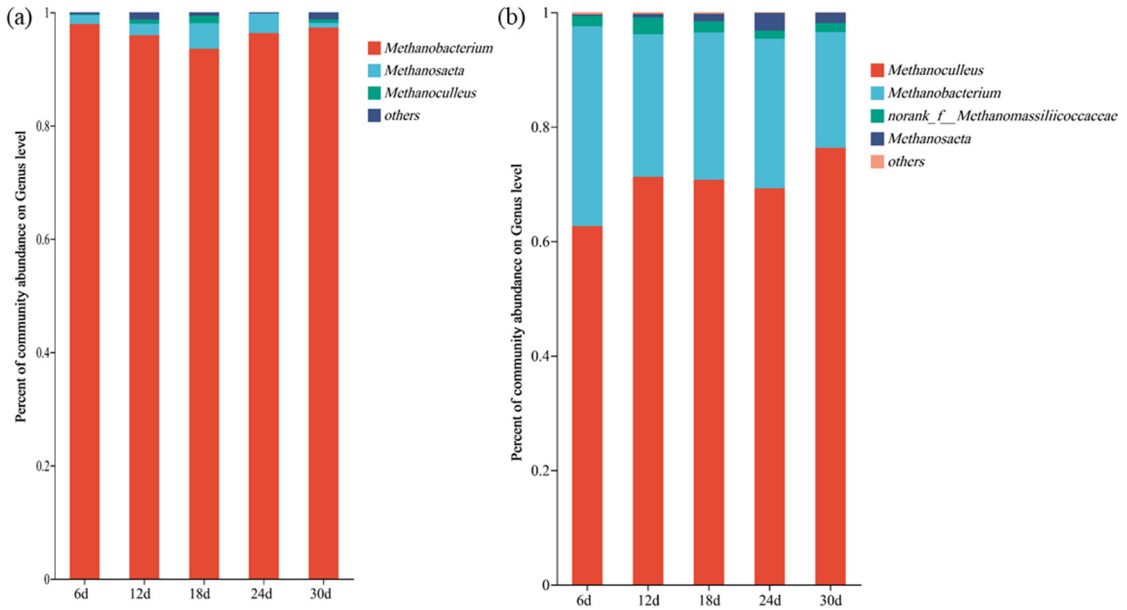

Fig 5. Structural characteristics of archaea flora in anaerobic fermentation system DMS(a), DMSO(b)

In the anaerobic fermentation process where DMSO is used as fermentation substrate, At the early stage of fermentation, the dominant bacteria were *Macellibacteroides* (29.24%), *Aminobacterium* (8.96%), *Sedimentibacter* (8.81%), *Lutispora* (7.59%) and *Proteiniclasticum* (5.9%), *Sphaerochaeta*

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(9.96%), *Desulfovibrio* (2.27%), After 30 days, the dominant bacteria genera were *Macellibacteroides* (5.42%), *Aminobacterium* (4.89%), *Sedimentibacter* (6.82%), *Lutispora* (.65%), *Proteiniclasticum* (3.4%), *Sphaerochaeta* (36.34%), *Desulfovibrio* (0.13%) (Fig.4b). The dominant archaea in early fermentation were *Methanobacterium* (34.91%), *norank-f-Methanomassiliicoccaccae* (1.82%), *Methanosaeta* (0.28%) and *Methanoculleus* (62.62%). After 30 days of fermentation, the relative abundance of the dominant genus *Methanobacterium* decreased from 30.91% to 20.21%, and that of *Methanosaeta* increased from 0.28% to 1.85%. The relative abundance of *Methanoculleus* increased from 62.62% to 76.3% (Fig.5b).

4. Analysis and Discussion

Under anaerobic conditions, sulfur-containing organic compounds are degraded to produce H_2S (HS^-) by methanogenic bacteria (Equation 1), sulfate reducing bacteria (Equation 2) and denitrifying bacteria (Equation 3) in enzymatic reaction [9], and DMSO (DMSO) can be reduced to DMS (DMS) to participate in the reaction (Equation 4) [10]. In addition, a large amount of nitrogen was produced in the early stage of the fermentation system, indicating that denitrifying bacteria used DMS to generate sulfate ions in the early stage of fermentation, and the presence of sulfate would stimulate the degradation of DMS by sulfate reducing bacteria. From an ecological point of view, these higher sulfate and H_2S concentrations may promote the degradation of DMS by sulfate-reducing bacteria, and the disappearance of degradative methyl-trophic methanogens in the fermentation system also indicates that the presence of sulfate inhibits the degradation of DMS by methanogens (Figure 8) [11]. DMSO is a precursor substance of DMS and can be reduced to DMS in Marine algae, but the enzymes involved have yet to be determined. In bacteria, DMSO is reduced to DMS by the DMSO reductase DMSOR [12].

$$2C_2H_6S+2H_2O\rightarrow 3CH_4+CO_2+2H_2S \quad (1)$$

$$C_2H_6S+SO_4^{2-}\rightarrow CO_2+HS^- \quad (2)$$

$$C_2H_6S+NO_3^-\rightarrow N_2+CO_2+SO_4^{2-} \quad (3)$$

$$(CH_3)_2SO+2H^++2e^-\leftrightarrow(CH_3)_2S+H_2O \quad (4)$$

5. Conclusion

In the process of anaerobic fermentation, coal as a carbon source decomposing into alcohols and fatty acids under the action of *Paraclostridium* and *Aminobacterium*, etc. These metabolites can be further degraded to form simple compounds required by sulfate-reducing bacteria and methanogens. In this way, sufficient substrate is provided for the survival of *Desulfovibrio* sulfate-reducing bacteria and the growth of *Methanobacterium*, *Methanosaeta* and *Methanosarcina*, enabling full metabolism of sulfate in the system to produce H_2S, while also promoting the generation of CH_4. The cyclic metabolism of sulfur includes two ways: reduction of dissimilated sulfate and conversion of sulfide to cysteine. Methane metabolism mainly includes acetic acid fermentation and carbon dioxide reduction.

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