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The Advantages of Methane Production by Combined Fermentation of Lignite and Wheat Straw

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Abstract

Biogasification of coal is important for clean utilization of coal. Experiments on the fermentation of single lignite, single straw and their mixture were performed to explore the variation characteristics of gas production potential, microbial community and methanogenic metabolic pathways of mixture. Research has shown that mixed fermentation of lignite and straw significantly promoted biomethane production. The abundance of hydrolytic acidifying functional bacteria genera (*Sphaerochaeta*, *Lentimicrobium*) in mixed fermentation was higher than that in the fermentation of single lignite and single straw. The abundance of some key CAZy metabolic enzyme gene sequences in mixed fermentation group was increased, which was favorable to improve methane production. Aceticlastic methanogenesis was the most critical methanogenic pathway and acetic acid pathway was more competitive in methanogenic mode during peak fermentation. Macrogenomics provided theoretical support for the claim that mixed fermentation of coal and straw promoted biomethane metabolism, which was potentially valuable in expanding methanogenesis from mixed fermentation of lignite with different biomasses.

Keywords

Lignite, Wheat Straw, Mixed Fermentation, Microbial Community, Macrogenomics

1. Introduction

China is rich in lignite resources, but the combustion and utilization quality of lignite is poor and the existing traditional extensive utilization is resulting in great environmental pollution. Upgrading and conversion of lignite are impor-

tant ways of improving the efficiency of coal resource utilization [1]. Currently, the conversion and utilization of lignite resources in China mainly include gasification, liquefaction and anaerobic fermentation. Lignite gasification and liquefaction processes produce harmful gases and suffer from the disadvantages of low yield and high energy consumption due to high temperature [2]. Since Scott proposed the anaerobic fermentation of coal [3], many researchers have studied this topic [4] [5] [6]. In anaerobic fermentation process of single coal, the organic matter in coal is difficult to be degraded by microorganisms, which affects the fermentation efficiency and leads to low methane production [7]. Common ways to improve the efficiency of coal anaerobic fermentation include pretreatment, electrochemical treatment and mixed fermentation [8] [9]. Among them, coal dissolution effect after pretreatment and electrochemical electrolysis was increased to a certain extent and the degradation rate of organic matter was increased, which significantly improved energy conversion rate [10] [11]. However, there were some difficulties such as environmental pollution and high cost. On the other hand, in mixed fermentation of coal, other wastes in addition to coal are used to degrade, which increase methane production while reducing environmental pollution [12], resulting in economic and environmental benefits.

Yoon *et al.* showed that the efficiency of lignite as a single substrate in converting methane was not high and the addition of small amounts of straw increased biomethane yield [13]. Guo *et al.* analyzed the gas production characteristics the fermentation of coal, corn straw, and their combination using methane production as an indicator. The obtained experimental results revealed that the addition of corn straw had significant effects on biological gas production [14] [15]. Esteban *et al.* studied the mixed fermentation of sewage sludge and kitchen waste and found that the addition of kitchen waste as a co-substrate enhanced the methane production of sewage sludge and applied metagenomic technology to investigate methanogenic metabolic pathway more comprehensively [16]. Many researchers have improved our understanding of the metabolic pathways of organic compounds and the metabolic networks of microbial communities through metagenomic analysis [17]. This analysis method was different from the previous molecular biological methods since it only studied a few genes, proteins or biochemical pathways, but focused on overall scientific issues such as the compositions of biological systems and the relationships among species in the community, structure and function of system, and community structure and ecosystem [18]. Few studies are available on the mixed fermentation of lignite and straw by using metagenomic technology and the relationship between the expression of functional enzymes and microbial community is yet to be studied. Based on the characteristics of metagenomics, this paper has analyzed the structure of the microbial community of lignite and straw fermentation and the variation characteristics of key metabolic enzymes. Combined with methanogenic metabolic pathway, the mixed fermentation process and the reasons for high yield were investigated, which provided theoretical support for the clean

application of lignite.

2. Materials and Methods

2.1. Sample Preparation

Fresh lignite was collected from Shengli coalfield in Inner Mongolia and wheat straw was obtained from Suqian area in Anhui. Coal samples were crushed to 100 - 150 mesh and straw was cut into pieces about 1 cm in size. Elemental analyses were performed using a Flash 2000 (Thermo Scientific, America) organic elemental analyzer (Table 1).

2.2. Enrichment Culture of Methanogens

Fresh coal samples were collected from coal areas (Henan Yima mining area, Shanxi Liulin mining area, Inner Mongolia Wulantuga mining area, etc.) using sterilized anaerobic tanks. The coal samples were crushed and added to the sterilized liquid medium in the laboratory, and cultured at 35˚C for 10 - 15 days under anaerobic conditions. The formula of bacterial liquid medium was shown in References [19].

2.3. Experimental Methods

2.3.1. Biological Gas Production Experiment

Before fermentation experiments, fermentation bacterial source was taken and the strain was reactivated and cultured. During activation period, the bacterial source: nutrient solution volume ratio of 2:3 was mixed and solution pH was adjusted at about 7. Anaerobic activation culture was carried out at 35˚C and methane production was monitored, bacterial source was found to be in the highest activity. Methane production was observed in anaerobic activated culture at 35˚C, indicating the highest activity of the bacterial source. Straw and coal samples as well as bioreactor were sterilized at 121˚C for 20 min before use for preventing exogenous microbial contamination. Coal and straw were added to the activated bacterial solution in the reactor, the reactor was filled with nitrogen for 5 min, and sealed after oxygen removal. The reactor was placed in a constant-temperature incubator and dark fermentation was carried out at 35˚C. The biogas produced during anaerobic fermentation was collected using an aluminum foil gas sampling bag and gas production was calculated using a calibrated syringe. A gas chromatograph equipped with Carbonplot column and TCD detector (Agilent 7890 GC, Agilent Technologies Inc., Santa Clara, CA, USA) was applied to determine methane concentration.

Table 1. Element content analyses of samples.

Sample	C_d/%	H_d/%	O_d/%	N_d/%	S_d/%	*C*: *N*/%
Lignite	63.27	4.56	22.22	1.28	0.32	49.43
Wheat straw	46.22	6.03	38.70	0.51	0.16	90.63

2.3.2. Macrogenome Test

Whole genome shotgun (WGS) strategy was applied to extract total metagenomic DNA or cDNA double strands synthesized by metatranscriptome using mRNA as template from fermentation broth at biogas production peak. Gene fragment library was constructed by Shanghai Persino Corporation after cDNA double strands were randomly cut into short DNA fragments. Paired-end (PE) sequencing was performed on these libraries using MiSeq Reagent Kit V3 (600 cycles) on a MiSeq machine at 2 × 300 bp.

3. Result and Discussion

3.1. Analysis of Gas Production Results

The biogas production of control group without substrate (Control), single lignite (LIG), mixed fermentation of lignite and wheat straw (LIG-WS) and single wheat straw (WS) were carried out. The gas production results are summarized in Table 2.

It was seen from the subtract blank group that in the process of lignite and wheat straw fermentation, methane production of LIG-WS was 183.78 and 124.87 mL higher than those of LIG and WS, which accounted for 462.69% and 126.60% increase, respectively. Compared with the sum (138.35) of LIG (39.72) and WS (98.63) methane production, LIG-WS (223.50) increased by 85.15 mL and increased by 61.19%. By comparing gas production effects, the methane production of the mixed fermentation of lignite and wheat straw was found to be significantly increased and the effect of biological methane production was obvious.

3.2. Comparative Analysis of Microbial Structure

3.2.1. Microbial Diversity and Abundance

In order to further explore the internal mechanism of the effect of wheat straw on the methane production of lignite anaerobic fermentation, the liquid products of methane production peak were analyzed for microbial diversity (Table 3) and abundance (Figure 1).

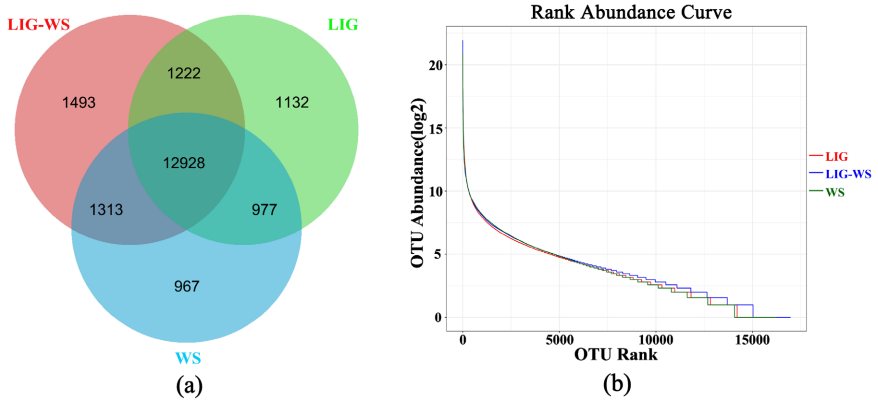

Figure 1. Differences of various substrate flora. (a) Species Wayne diagram; (b) Abundance grade curve.

Table 2. Biological gas production of different substrates.

Numbering	Coal quality/g	Wheat straw quality/g	Bacteria source/mL	Biogas production/mL	Methane production/mL	Subtract blank group/mL
Control	0	0	200	66.00	22.12	0
LIG	20	0	200	205.60	61.84	39.72
LIG-WS	20	6.25	200	536.70	245.62	223.50
WS	0	6.25	200	365.00	120.75	98.63

Table 3. Alpha diversity index.

Sample	Simpson	Chao1	ACE
LIG	0.930	17795.411	17542.955
LIG-WS	0.832	18353.263	18076.135
WS	0.931	17801.368	17527.765

Generally, using a variety of indicators to reflect microbial diversity level, different indexes focus on different. Chao1 and ACE indices reflect the richness of flora. Increased values of this index indicated higher richness of flora. Simpson indice shows the evenness of flora and is more sensitive to rare and dominant species in flora. Greater diversity indices revealed lower community diversity [20]. It was seen from the table that Chao1 and ACE indices of LIG-WS were the highest, indicating that the richness of the species was increased by the mixed fermentation of lignite and wheat straw. In addition, Simpson indice of LIG-WS was lower than those of LIG and WS, indicating that the evenness of flora was decreased and new dominant species could appear in mixed fermentation group.

As shown in **Figure 1(a)**, the number of species detected by LIG, LIG-WS and WS was 16,259, 16,956 and 16,185, respectively. Also, the number of common species was 12,928 and those of endemic species of LIG, LIG-WS and WS were 1132, 1493 and 967, respectively. The results revealed that the number of common species in the three groups accounted for the vast majority of their respective species, and the number of species and endemic species in mixed fermentation group were the largest. **Figure 1(b)** shows the abundance grade curve of the three sample groups, intuitively reflecting high abundance of flora and rare species number. Longer lines meant that more species were represented and smoother lines indicated higher evenness of community composition. Compared with LIG and WS, the line of LIG-WS was longer and its reduction process was relatively gentle, indicating that LIG-WS had the highest number of species and species evenness, which might be one of the reasons for better gas production in mixed fermentation group.

3.2.2. Analysis of Dominant Bacteria of Different Substrates

The community differences of bacteria and archaea in the mixed fermentation process of lignite and straw were analyzed by using liquid products at gas pro-

duction peak. In this research, *Bacteroidetes*, *Spirochaetes* and *Proteobacteria* were the main dominant phyla. *Bacteroidetes* are mainly involved in degrading macromolecules to produce formic acid, H_2 and CO_2 [21]. *Spirochaetes* are mainly involved in carbohydrate degradation to produce ethanol, acetic acid, H_2 and CO_2 [22]. *Proteobacteria* are a class of aerobic facultative anaerobic bacteria, which mainly ferment glucose to acetic acid and CO_2 [23]. Compared with LIG and WS, *Spirochaetes* had much higher abundance in LIG-WS mixed fermentation group and *Bacteroidetes* had almost the same abundance in the three groups. This indicated that carbohydrate degradation in mixed fermentation group produced more small molecular organic matters and had the highest methane production.

As shown in **Figure 2(a)**, bacterial flora was mainly consisted of *Sphaerochaeta*, *Lentimicrobium*, *Proteiniphilum*, *Desulfovibrio*, *Petrimonas* and *Aminobacterium*. In various fermentation groups, the abundances of *Sphaerochaeta* and *Lentimicrobium* were increased in LIG-WS compared with those in LIG and WS. The abundance of *Sphaerochaeta* increased most significantly, and LIG-WS increased by 37.49% and 20.17% compared with LIG and WS, respectively. *Sphaerochaeta* mainly produced ethanol, acetic acid, H_2 and CO_2 by degrading carbohydrates [24]. Compared with LIG and WS, the abundance of *Lentimicrobium* in LIG-WS increased by 11.48% and 8.79%, respectively. *Lentimicrobium* is a member of strictly anaerobic fermentation bacteria, which can employ macromolecular glucose to produce acetic acid and propionic acid [25]. Both *Sphaerochaeta* and *Lentimicrobium* are hydrolytic acidification functional bacteria. During fermentation process, the functional enzyme abundance of LIG-WS hydrolysis and acid production in the mixed fermentation group were higher than those of single lignite LIG and single straw WS; therefore, mixed fermentation group could use more substrates and produce the highest amount of methane in methane production process.

As shown in **Figure 2(b)**, archaeal flora was consisted of *Methanosarcina*, *Methanoculleus*, *Methanobacterium*, *Methanohalophilus*, *Methanofollis*, etc., among which *Methanosarcina* and *Methanoculleus* were dominant. The abundance of *Methanosarcina* in LIG, LIG-WS and WS was 95.15%, 91.82% and 80.63%, and that of *Methanoculleus* was 3.64%, 6.70% and 17.80%, respectively. *Methanosarcina* is an extremely strict anaerobic bacterium capable of producing methane by either acetate or CO_2 reduction pathways [26]. *Methanosarcina* can use H_2, CO_2, acetate, methanol and other substrates to produce methane. *Methanoculleus* is a hydrogenotrophic methanogen which can produce methane using HCO_3^- [27]. On the other hand, LIG contained higher amounts of *Methanosarcina* and lower amounts of *Methanoculleus* while WS contained lower amounts of *Methanosarcina* and higher amounts of *Methanoculleus*. Mixed fermentation group LIG-WS increased the amount of *Methanosarcina* compared with the straw alone fermentation group WS and *Methanosarcina* use various approaches to produce methane, which result in gas production.

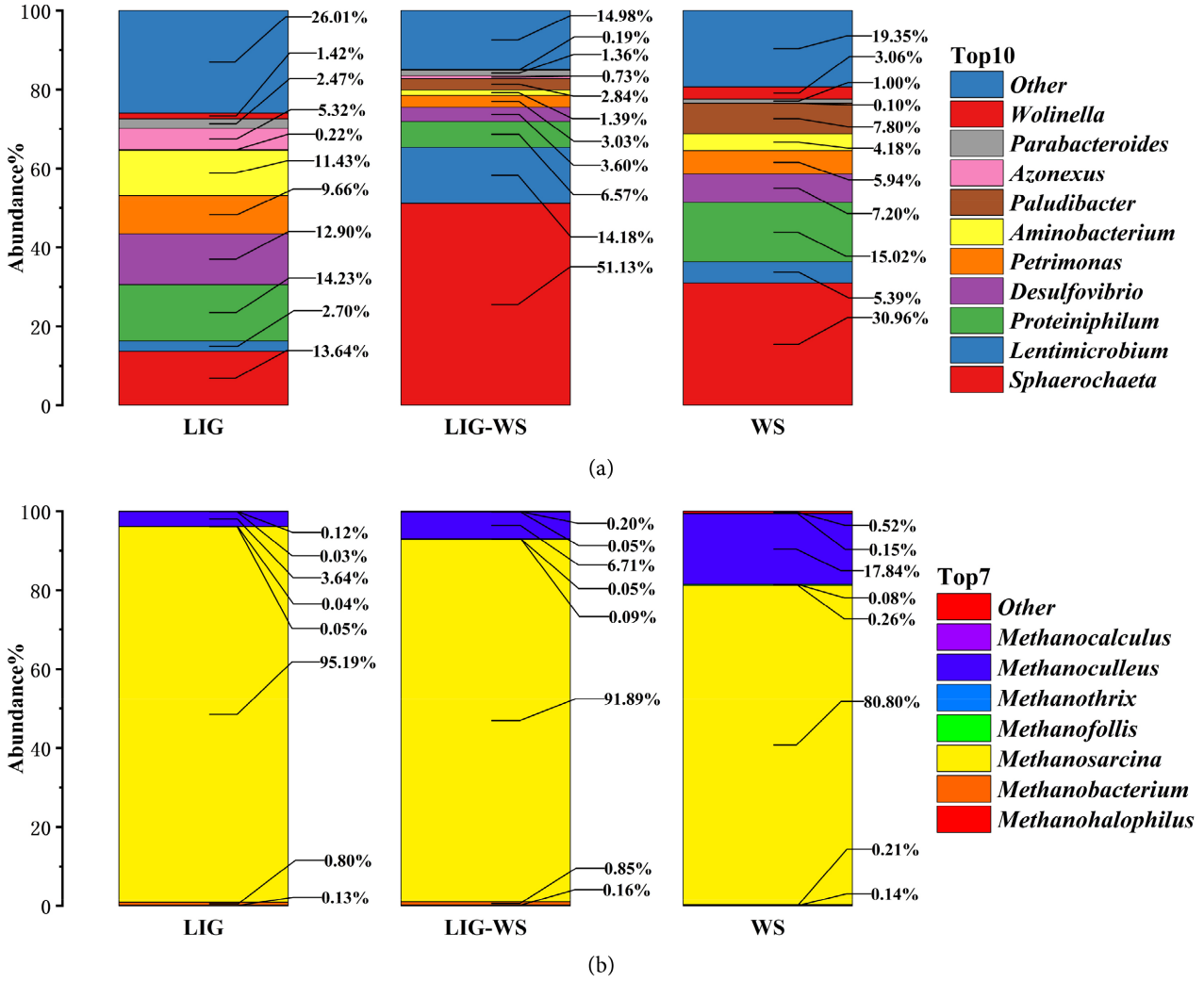

Figure 2. Dominant microorganisms at different substrate levels. (a) Dominant bacteria at genus level (Top 10). (b) Dominant archaea at genus level (Top 7).

3.3. Process Analysis of Biomethane Metabolism in Different Substrates

3.3.1. Comparison of Flora Metabolic Function Genes

Based on KEGG functional classification, the first class mainly includes metabolism, environmental information processing, genetic information processing and cellular processes. As shown in **Figure 3**, the number of genes with metabolism function in the three groups of samples was the largest, among which the number of genes with carbohydrate metabolism function was the highest, followed by amino acid metabolism. The main role of acid production is carbohydrate metabolism in anaerobic fermentation conditions, acid metabolism in pyruvate can be converted to ethanol, propanol, acetate and so on [28]. Amino acid metabolism decomposes amino acids into α-keto acids, amines and carbon dioxide through deamination and decarboxylation. α-keto acids can be converted into sugars and lipids and generate CO_2 and H_2O through tricarboxylic acid cycle. The quantities of carbohydrate and amino acid metabolism in LIG-WS

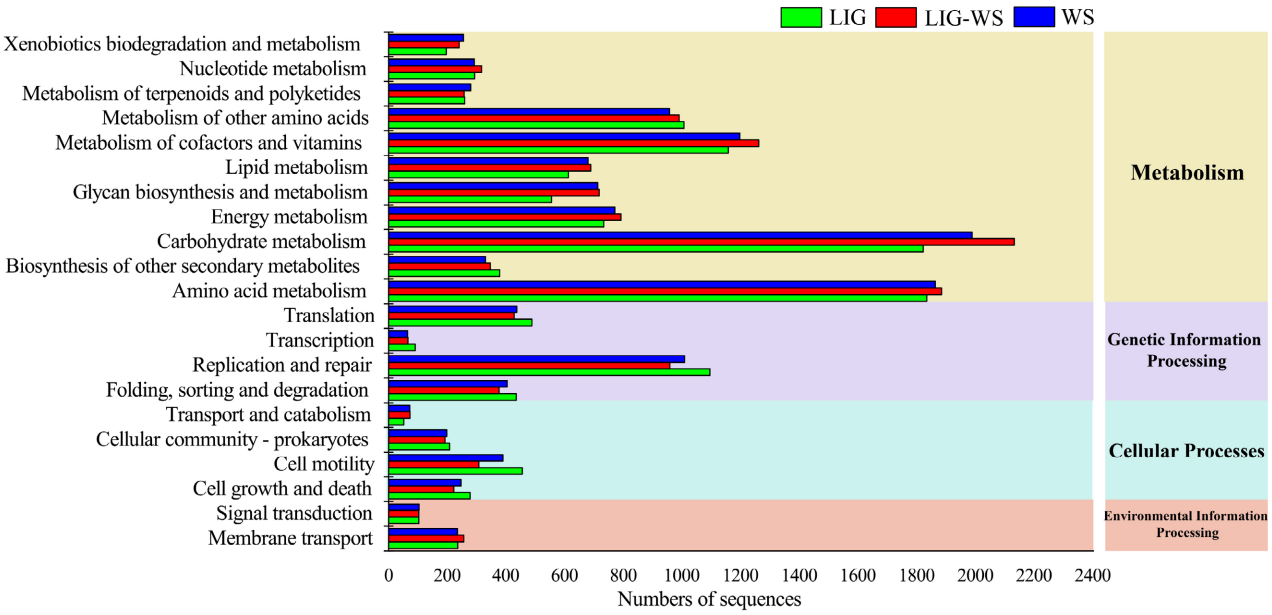

Figure 3. Comparison diagram based on KEGG functional classification.

were higher than those in LIG and WS. It was seen that the mixed fermentation group of lignite and straw had stronger acid production and metabolism functions, which could produce higher amounts of substrates for methanogens to use and promote the increase of methane production.

3.3.2. Comparison of the Number of Sequences Related to Enzyme Genes

The activity of microorganisms runs through the entire process of anaerobic fermentation. When the sequencing data set was encoded as a carbohydrate active enzyme, mixed fermentation group exhibited different functional characteristics than the two single fermentation groups. CAZy enzymes could be divided into six functional modules according to their functions: auxiliary activities (AAs), carbohydrate-binding modules (CBMs), carbohydrate esterases (CEs), glycoside hydrolases (GHs), glycosyl transferases (GTs), and polysaccharide lyases (PLs).

As shown in **Figure 4**, CBMs, GHs and GTs were the main enzymes in CAZy enzymes, accounting for more than 90% of the total amount of enzyme. The main function of CBMs was to improve the catalytic efficiency of enzyme and enhance enzyme activity by targeting the substrate to close the distance between the enzyme and the substrate and promoting the interaction with the substrate [29]. GHs can reveal the ability of microbial community to hydrolyze carbohydrates during anaerobic fermentation with its main function being hydrolyzing cellulose, oligosaccharides and peptidoglycan [30] [31] Moreover, GHs account for almost half of CAZy enzyme family and are important for the metabolism of microbial community [32]. GTs mainly have catalytic activation functions that connect sugars to various receptor molecules such as proteins, nucleic acids, sugars, and lipids [33]. The abundance of GH13 in LIG-WS group was 0.04%

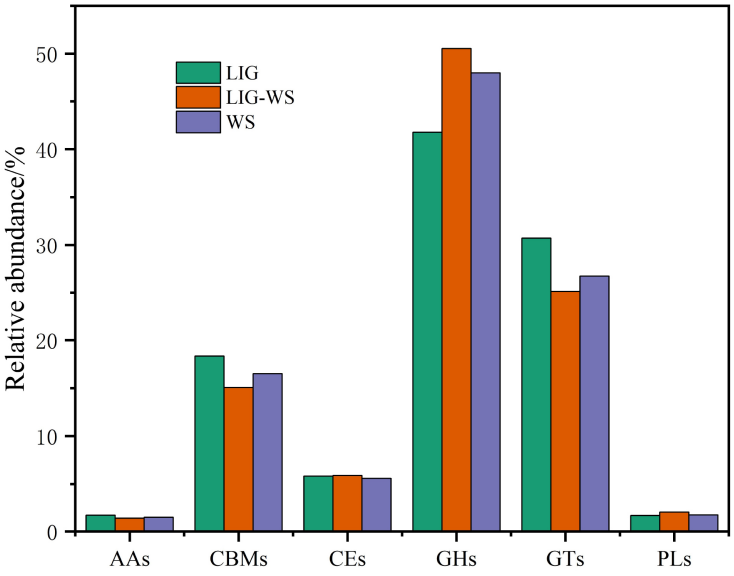

Figure 4. Comparative analysis of the number of sequences related to enzyme genes.

higher than that in WS group. GH13 is a hydrolase, mainly involved in glycosidic bond hydrolysis, and can destroy cell structure, which is a critical functional enzyme in carbohydrate metabolism [34].

Therefore, the gene abundance of CAZy metabolizing enzyme in the mixed fermentation of lignite and straw was changed, the enzyme activities of partially hydrolyzed cellulose and carbohydrates were increased, and carbohydrate enzyme metabolism products were increased, which increased methane production.

3.3.3. Methane Metabolic Pathway

In anaerobic fermentation process, methanogenic stage is the final stage and the metabolism of methanogenic bacteria is obviously different from those of other bacteria. Variations of methanogenic metabolic pathways during anaerobic fermentation could be analyzed based on KEGG database. The genes encoding key methanogenesis-related enzymes are shown in **Table 4**.

As shown in **Figure 5(a)**, methanogenic metabolic pathway is mainly consisted of three pathways: *i.e.*, CO_2 reduction, acetic acid nutrition and methyl nutrition pathways. In CO_2 reduction methanogenesis pathway, the functional gene abundance of LIG was higher than those of LIG-WS and WS (**Table 4**), indicating that the key enzymes of CO_2 reduction pathway had the strongest metabolic function in single lignite fermentation group. In acetate methanation pathway, the key enzymes contributed to the conversion of acetate to acetyl-CoA are acetate kinase (K00925), phosphate acetyltransferase (K00625), and acetyl-CoA synthetase (K01895) [35]. Acetate kinase is a carboxyl-based phosphotransferase that can transfer phosphorus-containing groups. Phosphate acetyltransferase is an acyltransferase that converts acetylphosphoric acid to acetate in the presence of two NDP + molecules [36]. The abundances of these two enzymes in LIG-WS were increased compared to LIG and WS groups, verifying that lignite and straw

mixed fermentation promoted the metabolic activity of acetic acid methanogenesis. Acetyl-CoA synthetase is an acid thiol ligase that acts on propionates and acrylates [37]. In methylotrophic methanogenesis pathway, the functional gene abundance of LIG was the highest (Table 4), showing that the key enzymes in methyl reduction pathway had the strongest metabolic function in the single lignite fermentation group. It was seen from Figure 5(b) that the abundance of key enzyme genes in acetate reduction pathway (76.38% - 93.43%) was significantly higher than those in CO_2 (4.72% - 25.52%) and methyl (5.61% - 12.12%) reduction pathways. On the whole, acetate-nutritive approach to produce methane was more competitive and the gene abundances of other key enzymes in methanogenesis were lower. In addition, relative proportion of acetic acid in mixed fermentation group LIG-WS was higher than that in single lignite group LIG, revealing that acetic acid methanogenesis metabolic pathway was enhanced by the addition of straw to single lignite substrate, which was beneficial to methane production.

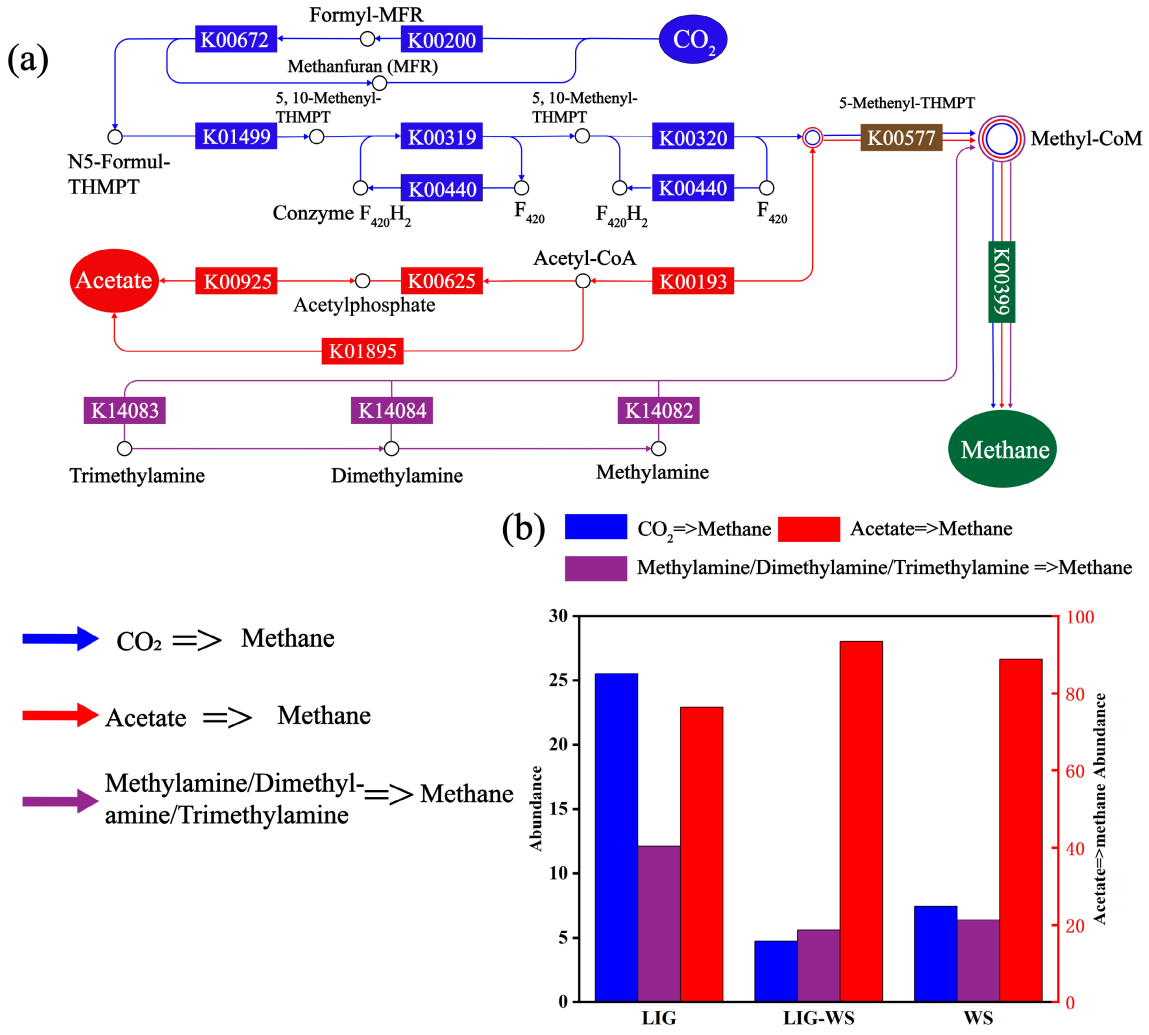

Figure 5. Analysis of methanogenic pathway based on KEGG (a) Methane pathway metabolic pathway map (b) Gene abundance of key enzymes in different substrates.

Table 4. Gene and relative abundance of enzymes related to methanogenic metabolic pathway.

K number	Gene abundance	Relative abundance/%		
		LIG	LIG-WS	WS
K00193	acetyl-CoA decarbonylase/synthase, CODH/ACS complex subunit beta	0.84	0.44	0.07
K00200	formylmethanofuran dehydrogenase subunit A	3.86	0.82	1.86
K00319	methylenetetrahydromethanopterin dehydrogenase	1.20	0.00	0.09
K00320	5,10-methylenetetrahydromethanopterin reductase	1.03	0.03	0.08
K00399	methyl-coenzyme M reductase alpha subunit	4.17	1.41	0.90
K00440	coenzyme F_{420} hydrogenase subunit alpha	1.64	0.17	0.59
K00577	tetrahydromethanopterin S-methyltransferase subunit A	5.68	0.92	0.86
K00625	phosphate acetyltransferase	14.65	23.58	20.24
K00672	formylmethanofuran-tetrahydromethanopterin N-formyltransferase	2.62	0.10	0.56
K00925	acetate kinase	24.94	39.10	38.39
K01499	methenyltetrahydromethanopterin cyclohydrolase	5.32	1.27	2.50
K01895	acetyl-CoA synthetase	26.10	27.98	28.40
K14082	[methyl-Co(III) methylamine-specific corrinoid protein]: coenzyme M methyltransferase	0.18	0.54	0.00
K14083	trimethylamine-corrinoid protein Co-methyltransferase	6.68	2.74	5.27
K14084	trimethylamine corrinoid protein	1.09	0.92	0.20

4. Conclusion

In this paper, the methanogenic performance of lignite and straw mixed fermentation and their methanogenic metabolic pathways were studied. The obtained results revealed that mixed fermentation of lignite and straw significantly promoted biomethane production. Regarding differences in microflora characteristics, the highest number of species and endemic species were detected in the mixed fermentation group. The abundance of hydrolytic acidifying functional bacteria genus (*Sphaerochaeta* and *Lentimicrobium*) in mixed fermentation was the highest, and its hydrolytic acidifying function was stronger. Macrogenomic analyses revealed that increased abundance of gene sequences of some key CAZy metabolic enzymes in the mixed fermentation group improved methane production. Among the above three methanogenic metabolic pathways, acetate trophic was the most critical methanogenic pathway, while microbial community in mixed fermentation broth presented increased carbohydrase activity, higher amounts of organic matter small molecules produced by the degradation of carbohydrates which ultimately promotes the increase of methane production.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Du, F., Ren, Z.X., Deng, W.A., Li, Z., Wang, X.Z., Wang, H.M. and Fu, X.Y. (2020) Effect of Dry Dewatering on Physicochemical Structure and Hydro-Conversion Performance of Lignite. *Journal of China Coal Society*, **45**, 778-785.

[2] Jin, X.L. (2018) Analysis on Effective Utilization Technology of Lignite. *Coal Science and Technology*, **46**, 260-263, 226.

[3] Scott, A.R. and Kaiser, W.R. (1994) Thermogenic and Secondary Biogenic Gases, San Juan Basin, Colorado and New Mexico—Implications for Coalbed Gas Producibility. *AAPG Bulletin*, **78**, 1186-1209. https://doi.org/10.1306/A25FEAA9-171B-11D7-8645000102C1865D

[4] Ning, J., Zhu, G.F., Lv, N., Wang, X.F., Wang, T., Li, J.J. and Zhou, M.D. (2018) Effects of C/N Ratio on Biogas Production by Anaerobic Co-Digestion of Pig Manure and Corn Straw. *Transations of the Chinese Society of Agricultural Engineering*, **34**, 93-98.

[5] Zhu, X.P., Yellezuome, D., Liu, R.H., Wang, Z.Z. and Liu, X. (2021) Effects of Co-Digestion of Food Waste, Corn Straw and Chicken Manure in Two-Stage Anaerobic Digestion on Trace Element Bioavailability and Microbial Community Composition. *Bioresource Technology*, **346**, Article ID: 126625. https://doi.org/10.1016/j.biortech.2021.126625

[6] Yan, Y.L., Du, Z.W., Zhang, L.Q., Li, F. and Smith, J.A. (2019) Identification of Parameters Needed for Optimal Anaerobic Co-Digestion of Chicken Manure and Corn Stover. *RSC Advances*, **9**, 29609-29618. https://doi.org/10.1039/C9RA05556H

[7] Zhang, D., He, H., Ren, Y., Rizwan, H., Michael, U., Song, J., Ishtiaq, A.M., Asif, J., Adnan, S.M., Guo, H.G., Liu, F.J. and Huang, Z.X. (2022) A Mini Review on Biotransformation of Coal to Methane by Enhancement of Chemical Pretreatment. *Fuel*, **308**, Article ID: 121961. https://doi.org/10.1016/j.fuel.2021.121961

[8] Pan, Y.X., Liu, S.Q. and He, Y.Q. (2020) Methane Production Performance of Semi-Continuous Anaerobic Fermentation with Rice Straw Pretreated by Microbial Community. *Transations of the Chinese Society of Agricultural Engineering*, **36**, 261-266.

[9] Dong, X.L., Lin, Q.S. and Lin, Y.N. (2022) Effects of Electrolytes on Acidogenic Fermentation of Waste Activated Sludge for Volatile Fatty Acids Production via Electrochemical Pretreatment. *Environmental Engineering*, **40**, 71-78.

[10] Xia, D.P., Chen, X., Wang, C. and Su, X.B. (2017) Experimental Study on the Production of H_2-CH_4 from Lignite Jointly with Acid-Alkali Pretreatment-Microbial Gasification. *Journal of China Coal Society*, **42**, 3221-3228.

[11] Haider, R., Ghauri, M.A., Sanfilipo, J.R., Jones, E.J., Orem, W.H., Tatu, C.A. and Akhtar, N. (2013) Fungal Degradation of Coal as a Pretreatment for Methane Production. *Fuel*, **104**, 717-725. https://doi.org/10.1016/j.fuel.2012.05.015

[12] Guo, H.G., Chen, Q.L., Hu, H.W. and He, J.Z. (2020) High-Solid Anaerobic Co-Digestion of Pig Manure with Lignite Promotes Methane Production. *Journal of Cleaner Production*, **258**, Article ID: 120695. https://doi.org/10.1016/j.jclepro.2020.120695

[13] Yoon, S.P., Jeon, J.Y. and Lim, H.S. (2016) Stimulation of Biogenic Methane Generation from Lignite through Supplying an External Substrate. *International Journal of Coal Geology*, **162**, 39-44. https://doi.org/10.1016/j.coal.2016.05.009

[14] Guo, H.Y., Dong, Z.W., Su, X.B., Liu, S., Jia, J.B., Yu, H.F. and Xia, D.P. (2018) Synergistic Biodegradation of Coal Combined with Corn Straw as a Substrate to Methane and the Prospects for Its Application. *Energy Fuels*, **32**, 7011-7016. https://doi.org/10.1021/acs.energyfuels.8b01120

[15] Guo, H.Y., Zhang, M.L., Dong, Z.W., Wang, Q., Xia, D.P., Lv, J.H. and Yu, H.F. (2019) The Mechanisms of Biogenic Methane Metabolism by Synergistic Biodegradation of Coal and Corn Straw. *Bioresource Technology*, **298**, Article ID: 122577. https://doi.org/10.1016/j.biortech.2019.122577

[16] Orellana, E., Davies-Sala, C., Guerrero, L.D., Vardé, I., Altina, M., Lorenzo, M.C., Figuerola, E.L., Pontiggia, R.M. and Erijman, L. (2019) Microbiome Network Analysis of Co-Occurrence Patterns in Anaerobic Co-Digestion of Sewage Sludge and Food Waste. *Water Science & Technology*, **79**, 1956-1965. https://doi.org/10.2166/wst.2019.194

[17] He, Q., Li, L. and Zhao, X.F. (2022) Metagenomic Analysis of Microbial Characteristics in Over-Acidification Systems during Anaerobic Digestion of Food Waste. *Acta Scientiae Circumstantiae*, **42**, 374-384.

[18] Luo, X. (2015) Research and Application of Metagenomic Classification and Analysis Method. Master's Thesis, Southeast University, Nanjing.

[19] Su, X.B., Zhao, W.Z. and Xia, D.P. (2018) The Diversity of Hydrogen-Producing Bacteria and Methanogens within an *in Situ* Coal Seam. Biotechnol. *Biotechnology for Biofuels*, **11**, Article No. 245. https://doi.org/10.1186/s13068-018-1237-2

[20] Su, X., Wang, J.H. and Zhang, F.Z. (2020) Microbial Community Succession Associated with Corn Straw Degradation in a Bacterium Consortium. *Acta Microbiologica Sinica*, **60**, 2675-2689.

[21] Hahnke, R.L., Meier-Kolthoff, J.P. and García-López, M. (2018) Genome-Based Taxonomic Classification of Bacteroidetes. *Frontiers in Microbiology*, **9**, Article 354825. https://doi.org/10.3389/fmicb.2018.00304

[22] Dong, X.Y., Greening, C., Brüls, T., Conrad, R., Guo, K., Blaskowski, S., Kaschani, F., Kaiser, M., Laban, N.A. and Meckenstock, R.U. (2018) Fermentative Spirochaetes Mediate Necromass Recycling in Anoxic Hydrocarbon-Contaminated Habitats. *The ISME Journal*, **12**, 2039-2050. https://doi.org/10.1038/s41396-018-0148-3

[23] Dai, Z.M., Su, W.Q., Chen, H.H., Barberán, A., Zhao, H.C. and Yu, M.J. (2018) Long-Term Nitrogen Fertilization Decreases Bacterial Diversity and Favors the Growth of *Actinobacteria* and *Proteobacteria* in Agro-Ecosystems across the Globe. *Global Change Biology*, **24**, 3452-3461. https://doi.org/10.1111/gcb.14163

[24] Miyazaki, M., Sakai, S. and Ritalahti, K.M. (2014) *Sphaerochaeta multiformis* sp. nov., an Anaerobic, Psychrophilic Bacterium Isolated from Subseafloor Sediment, and Emended Description of the Genus *Sphaerochaeta*. *International Journal of Systematic and Evolutionary Microbiology*, **64**, 4147-4154. https://doi.org/10.1099/ijs.0.068148-0

[25] Liu, G.H., Wang, J. and Qi, L. (2022) Study on Regulating Anaerobic Fermentation for Producing Short-Chain Fatty Acids from Primary Sludge in WWTPs by Different Bio-Enzymes. *China Environmental Science*, **42**, 2195-2203.

[26] Zhang, L. and Loh, K.C. (2019) Synergistic Effect of Activated Carbon and Encapsulated Trace Element Additive on Methane Production from Anaerobic Digestion of Food Wastes—Enhanced Operation Stability and Balanced Trace Nutrition. *Bio-*

resource Technology, **278**, 108-115. https://doi.org/10.1016/j.biortech.2019.01.073

[27] Thommes, M., Katsumi, K. and Alexander, V. (2015) Physisorption of Gases, with Special Reference to the Evaluation of Surface Area and Pore Size Distribution (IUPAC Technical Report). *Pure and Applied Chemistry*, **87**, 1051-1069. https://doi.org/10.1515/pac-2014-1117

[28] Chen, Y., Luo, J. and Yan, Y. (2013) Enhanced Production of Short-Chain Fatty Acid by Co-Fermentation of Waste Activated Sludge and Kitchen Waste under Alkaline Conditions and Its Application to Microbial Fuel Cells. *Applied Energy*, **102**, 1197-1204. https://doi.org/10.1016/j.apenergy.2012.06.056

[29] Najmudin, S., Guerreiro, C.I. and Carvalho, A.L. (2006) Xyloglucan Is Recognized by Carbohydrate-Binding Modules That Interact with β-Glucan Chains. *Journal of Biological Chemistry*, **281**, 8815-8828. https://doi.org/10.1074/jbc.M510559200

[30] Vanwonterghem, I., Jensen, P.D. and Rabacy, K. (2016) Genome-Centric Resolution of Microbial Diversity, Metabolism and Interactions in Anaerobic Digestion. *Environmental Microbiology*, **18**, 3144-3158. https://doi.org/10.1111/1462-2920.13382

[31] Hanreich, A., Schimpf, U. and Zakrzewski, M. (2013) Metagenome and Metaproteome Analyses of Microbial Communities in Mesophilic Biogas-Producing Anaerobic Batch Fermentations Indicate Concerted Plant Carbohydrate Degradation. *Systematic and Applied Microbiology*, **36**, 330-338. https://doi.org/10.1016/j.syapm.2013.03.006

[32] Cantarel, B.L., Coutinho, P.M. and Corinne, R. (2009) The Carbohydrate-Active EnZymes Database (CAZy): An Expert Resource for Glycogenomics. *Nucleic Acids Research*, **37**, 233-238. https://doi.org/10.1093/nar/gkn663

[33] Zheng, M.J., Li, X.Y. and Li, L. (2013) Homology Modeling of Glucosyltransferases from *Pueraria lobata* (Willd.) Ohwi and the Analysis of the Active Motif. *China Journal of Bioinformatics*, **11**, 287-292.

[34] Stam, M.R., Danchin, E.G.J. and Rancurel, C. (2006) Dividing the Large Glycoside Hydrolase Family 13 into Subfamilies: Towards Improved Functional Annotations of alpha-Amylase-Related Proteins. *Protein Engineering, Design and Selection*, **19**, 555-562. https://doi.org/10.1093/protein/gzl044

[35] Yin, Q., Yang, S. and Wang, Z. (2018) Clarifying Electron Transfer and Metagenomic Analysis of Microbial Community in the Methane Production Process with the Addition of Ferroferric Oxide. *Chemical Engineering Journal*, **333**, 216-225. https://doi.org/10.1016/j.cej.2017.09.160

[36] Thapa, L.P., Lee, S.J. and Park, C. (2017) Production of L-Lactic Acid from Metabolically Engineered Strain of *Enterobacter aerogenes* ATCC 29007. *Enzyme and Microbial Technology*, **102**, 1-8. https://doi.org/10.1016/j.enzmictec.2017.03.003

[37] Zhang, M.L., Guo, H.Y. and Xia, D.P. (2022) Metagenomic Insight of Corn Straw Conditioning on Substrates Metabolism during Coal Anaerobic Fermentation. *Science of the Total Environment*, **808**, Article ID: 152220. https://doi.org/10.1016/j.scitotenv.2021.152220