Enhancement of biogenic methane production by co-degradation of coal and straw: microbial and organic analysis

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Abstract: Co-degradation of coal and straw could produce significantly higher methane which was potential to increase biogenic CBM. In this study, the success of microflora and organic compounds during co-degradation was determined by MiSeq and GC-MS, and compared with cultivations with only coal (C) and with only straw (RS). The results showed that the methane production in co-degradation was 12 times higher than that in cultivation C. A shift of dominant methanogen was caused by the addition of straw from acetoclastic *Methanosaeta* in inoculum to methylotrophic *Methanomethylovorans* in 7 days, then hydrogenotrophic *Methanobacterium*. The bacteria and fungi with ability to degrade macromolecules in coal and metabolize VFAs were enriched which would facilitate methanogenesis. VFAs, especially butanoic acid, were dominant in intermediates of co-degradation which contributed to methane production as their content were negatively corelated with methane production. The different component of intermediates and microbial communities among co-degradation, cultivations C and RS suggested that the metabolic pathway in co-degradation was distinctive and the fracture of coal molecules was almost completed in the first 7 days of cultivation. Coal might also serve as the suitable microhabitat for microorganisms to avoid the threat from environment in addition to function as methanogenic substrates.

Keywords: Biogenic coalbed methane; Co-degradation; Rice straw; Microbial communities; Organic intermediates

1. Introduction

As an important unconventional nature gas resource, coalbed methane (CBM) plays an increasing role in the improvement of energy-supplying structure all over the world. It can be divided into two types: biogenic CBM and thermogenic CBM. The former is produced by microbially degrading coal which has attracted the interest of more and more experts from CBM exploitation, biology, chemistry, and geology . The technology of microbially enhanced CBM (MECBM) has been put forward based on the formation of biogenic CBM . It can not only increase the CBM reserves and prolong the service life of CBM wells, but also improve the permeability of coal seam and enhance the recovery rate of CBM .

However, the biomethane production reported by previous studies are relatively low . It is generally believed that the main limiting factors to coal biodegradation are the complexity of coal structure . A series of measures have been carried out to increase methane production, including chemical, physical, and biological pretreatment, and supplying extra carbon resources . Among them, co-degradation of coal and straw has been reported to significantly increase methane production under different coal ranks and different types of straw . Yoon et al. first reported the feasibility of enhancing biogenic CBM by co-degradation and the mass ratio of rice to lignite needed to be more than 33.3% to increase methane production . Then, the significant increment of biomethane production was observed in co-degradation of maize straw with lignite and two bituminous coals that the maximum methane production reached 2.69 mmol/g coal which was 448.98% higher than that from only coal . The straw type, straw

part, and particle size of coal and straw were also found to affect the methane production in co-degradation of anthracite and straw, and straw probably stimulated coal degradation . It was reported that the addition of corn straw in co-degradation promoted the complementary advantages of archaeal genera and decreased the adverse bacterial genera compared with the fermentation system of only coal . These studies strongly proved that co-degradation of coal and straw is a good way to increase methane production. However, there are just a few reports until now resulting in the mechanism of co-degradation unclear.

Biomethane is one of final products of coal biodegradation by synergistic action of methanogenic archaea, bacteria, and fungi . Complex macromolecular polymers in coal are first degraded into simpler long-chain alkanes and monocyclic aromatic compounds by various bacteria and fungi, and further decomposed into simple compounds with small molecular such as CO_2, H_2, and acetic acid, which are finally utilized by methanogens to produce methane . Various methanogens with all three types of methanogenesis, more than ten bacterial phylum and diverse fungi with potential to involve in coal hydrolysis, hydrocarbon metabolism, and generation of methanogenic substrates have been found in cultures, coal mines, and groundwater . Due to the complex composition of coal, many organic intermediates are produced during biodegradation, such as alkanes, aromatics, hexadecanoic acid, long-chain fatty acids, and olefins which has been detected in produced water and culture medium .

As a mixed carbon substrate, the addition of straw would affect the microbial

structure and intermediates during methanogenic process. In this paper, the succession of microflora and intermediates during co-degradation of coal and straw were studied by MiSeq and GC-MS. And the distinct methanogenic mechanism in co-degradation was discussed by comparing with the cultivations with only coal or with only straw.

2. Materials and Methods

2.1. Experimental samples and microflora

Anthracite sample was obtained from No.3 coal seam located at southern Qinshui Basin, China (see the location map in Guo, Yu, Thompson, Zhang [22]). Coal sample was crushed, and then sifted through 100 to 200 meshes. Rice straw sample was taken from the suburb of Jiangsu Province, China, and cut into approximate 5 mm. All samples were dried at 60 ^{o}C for 6 h before used. The results of industrial analysis and elemental analysis of coal and rice straw were shown in our previous study .

The methanogenic microflora was enriched from produced water obtained from active CBM wells in southern Qinshui Basin using anthracite as the sole carbon source. The anaerobic medium was prepared according to the previous study .

2.2. Methane production and pH determination

The anaerobic cultivations were performed in 500 mL serum bottles containing 216 mL of culture medium and 24 mL of microflora inoculums. Co-degradation of coal and straw was carried out with 8.0 g coal and 4.8 g straw (CRS). The cultivations with only 8.0 g coal (C) or only 4.8 g straw (RS) were set as controls. Methane production and pH were measured at 7-day intervals, and fermentation broth samples were also

taken at the same time to determine the microbial structure and intermediates. These samples were labeled combining substrates and sampling days as shown in Table 1. All the cultivations were performed in triplicate and cultivated at 35 °C without shaking.

2.3. DNA Extraction and MiSeq Sequencing

Fermentation broth samples were filtered with a 0.22 μm membrane filter (Millipore, United States) to collect the microorganisms. The genome DNA of each filter was extracted using the UltraClean Soil DNA Isolation Kit (Mobio, United States) according to the manufacturer's instructions. The archaea-specific primer sets 344F-915R , the bacteria-specific primer sets 515F-907R , and the fungi-specific primer sets ITS1F-ITS2R were used to respectively amplify the 16S rRNA genes of archaeal and bacterial communities, and ITS genes of fungal communities in culture solutions. The PCR conditions were set as follows: initial denaturation at 95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. The obtained PCR products were purified by AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City, CA, U.S.) after 2% agarose gel electrophoresis.

All amplified fragments were quantitatively determined using Qubit 3.0 (Life Invitrogen). Then the double-ended sequencing library was constructed and PE300 sequencing was performed on Illumina MiSeq platform. The 16S rRNA gene and ITS gene sequences derived from MiSeq sequencing were deposited in the NCBI

Sequence Read Archive with the accession number PRJNA552164.

2.4. TOC and GC-MS analysis

The obtained fermentation broth samples were filtered by 0.7 μm membrane filter (Millipore, United States) and then the content of total organic carbon (TOC) in the filtrate was determined by TOC-VCPH analyzer (TOC-VCPH, Shimadzu Corporation, Kyoto, Japan). The filtrate was extracted using CH_2Cl_2 for three times at neutral, alkaline, and acidic conditions, respectively. All the separated liquid was mixed. Excess anhydrous sodium sulfate was added in the mixed liquid, and the sealed bottle was placed at 4 °C for 12 h before rotary evaporation at a temperature of 40 °C. The obtained solution was evaporated to about 5 mL and concentrated to about 1 mL by nitrogen purge. Then, the organics in the extracted samples were analyzed by GC-MS system (7890B/5977B, Agilent, USA). The GC column was operated in a temperature programmed mode by maintaining the temperature at 60 °C for 3 min, then increasing to 150 °C with an increment of 20 °C/min, finally increasing to 230 °C with an increment of 5 °C/min, and then maintaining for 5 min. The identification of the organic compounds was undertaken with reference of National Institute Standard and Technology (NIST 14L).

3. Results and Discussion

3.1. Methane production and the success of TOC and pH

After 35 days of anaerobic cultivation, methane production in cultivation CRS reached 749.16 μmol (Fig. 1a), which was more than 12 times higher than that in

cultivation C (62.09 μmol). The maximum methane production rate of 41.22 μmol/day occurred between the 14^{th}-21^{st} day in co-degradation. These results were consistent with the previous studies and further proved the significant effect of co-degradation on biomethane production. However, there was barely methane observed in cultivation RS which was not expected, as straw has been widely utilized to generate methane and hydrogen under anaerobic condition .

The changes of TOC and pH were shown in Fig. 1b. The highest TOC was observed in cultivation RS while the lowest was found in cultivation C. The TOC changed a little in cultivation CRS which gradually increased and then decreased with the highest of 1292 mg/L on the 21^{th} day. The TOC in cultivation RS gradually increased from 1366 mg/L to 1882 mg/L, while that in cultivation C decreased gradually over time from 123 mg/L to 21 mg/L. The change of TOC was not consistent with gas production in cultivations CRS and RS that high content of TOC remained after methane production completed. It might be due to the generation of toxic substances to methanogenesis in CRS which inhibited the degradation of intermediates and would be overcome by adding new nutrient . No methane production in cultivation RS led to the accumulation of organic matters. The value of pH in cultivations CRS and RS were both acidic and decreased with time, while that in cultivation C was alkaline. The lowest pH was observed in cultivation RS with the value of 4.11 and the highest pH was found in cultivation C with the value of 8.43.

Compared with cultivation C, the value and changing trend of TOC and pH in cultivations CRS and RS were close. But distinct methane production was observed

(Fig. 1a), which suggested that biomethane production was not positively correlated with the amount of dissolved organic carbon. And coal would be essential to methane production in co-degradation. Besides that the organic matters in coal function as methanogenic substrates, coal pore or coal surface might also serve as the suitable microhabitat for microorganisms , and the existence of such charged surface groups as carboxylic acid in coal would result in the different pH values between coal surface and fermentation broth to avoid the threat from low pH .

3.2. Evolution of microbial communities revealed by MiSeq

3.2.1. MiSeq data of 16S rRNA and ITS genes

MiSeq provided a rich data set (Table S1). For all samples, the number of bacterial OTUs was generally much higher than that of archaea, but lower than that of fungi. Consistently, the same phenomenon was observed in Shannon indexes, suggesting the highest diversity of fungi and lowest diversity of archaea. Rarefaction curves of archaeal, bacterial, and fungal sequencing results were shown in Fig. S1. With the increment of sequencing depth, the rarefaction curves became flatten, showing that the test data was reliable . Besides, the diversity of microbial community was higher on the 7^{th} day which would promote biodegradation of coal and increase biomethane production.

3.2.2. Evolution of archaeal community

All the archaea sequence reads were classified into methanogen (Fig. 2a). However, the structure of archaeal communities varied with time during methane production.

The dominant archaea in co-degradation CRS changed from acetoclastic methanogen *Methanosaeta* in sample CRS0 with 74.17% of sequence reads to methylotrophic methanogen *Methanomethylovorans* in sample CRS7 with 74.92% of sequence reads, and then to hydrogenotrophic methanogen *Methanobacterium* in samples CRS14, CRS21, CRS28, and CRS35 ranging from 68.80% to 98.53%. These results suggested that the addition of straw made a significant effect on the structure of methanogens which would lead to the variation of methanogenesis. Similar phenomenon was also found in co-digestion of vegetable and fruit residues that the dominant methanogen changed from *Methanosaeta* to *Methanobacterium* . It was a pity that archaeal 16S rRNA gene was not successfully amplified from cultivation RS which might be due to the low value of pH (Fig. 1b). It suggested that methanogens were not survive in cultivation RS which would be the reason to no methane production observed.

3.2.3. Evolution of bacterial community

Similar with the changes in archaeal communities, the dominant bacteria at genetic level in co-degradation changed significantly (Fig. 2b), from *Macellibacteroides* (23.39%) in sample CRS0 to *Paraclostridium* (29.15%) in sample CRS7, then to *Caproiciproducens* in samples CRS14, CRS21, CRS28, and CRS35 which contained 36.43% to 55.02% of sequence reads. The proportion of *Macellibacteroides* gradually decreased with time. It would syntrophically degrade coal to produce methane with *Methanosaeta* by acetoclastic methanogenesis in inoculum as it can produce acetate, lactic acid, butyrate, and isobutyrate from sugars hydrolyzed from cellulose and hemicelluloses . It is also the main component during gas production process from

lignite and the key functional genus in the treatment of textile dyeing industry wastewater . *Paraclostridium* is more likely to metabolize syntrophically with methylotrophic methanogens as *Paraclostridium* and *Methanomethylovorans* were both dominant in CRS7 and then decreased gradually. However, only a few of literatures have reported the methylotrophic methanogenesis in coal seam whose mechanisms was still unclear .

Caproiciproducens, a strict anaerobic bacterium, might be the key bacteria in co-degradation as it was the most abundant genus since the 14th day of cultivation when methane production increased rapidly and hydrogenotrophic methanogen dominated. *Caproiciproducens* can use glucose and galactitol to produce acetic acid, butanoic acid, hexanoic acid, and H_2 . *Clostridium* also maintained a high abundance throughout co-degradation process. The presence of *Clostridium* might promote the enrichment of *Caproiciproducens* as the growth of *Caproiciproducens* was reported to be enhanced when co-cultured with other anaerobic strains that produced ethanol, acetic acid or butanoic acid . *Clostridium* can grow in a wide range of pH from 3.7 to 6.9 with the final products of butyrate, lactate, acetate, formate, H_2 and CO_2 and oxidize acetic acid to generate CO_2 and H_2 in the presence of hydrogenotrophic methanogens . The two bacteria might have a joint relationship during degradation, and facilitate methane production.

The bacterial community in sample RS35 was different from that in inoculum and co-degradation. *Paraclostridium* was the most abundant genus accounting for 39.28% of the sequence reads, following by *Clostridium* (35.49%), *Bacillus* (10.02%),

Caproiciproducens (6.52%), and *Desulfotomaculum* (5.77%).

3.2.4. Evolution of fungal community

Diverse fungal communities were detected (Fig. 2c). The structure of fungal communities, in general, did not change significantly during co-degradation. At the end of co-degradation, *Cryptococcus*, *Cladosporium*, *Fusarium*, *Petriella, Aspergillus, and Mortierella* were mainly enriched, which was close to that in cultivation RS, but a little different from that in inoculum. *Cryptococcus* is known as a crude oil degrading fungus for lipid production . *Cladosporium* is good at degrading polycyclic aromatic hydrocarbons (PAHs) . *Fusarium* can effectively degrade PAHs and is widely used in soil treatment in coal mining areas . The enrichment of them suggested that co-degradation enhanced coal biodegradation especially the degradation of aromatics in coal which is consistent with our previous study . The other genera detected in co-degradation were also able to degrade compounds with high molecular weight. *Aspergillus* has been reported to degrade plant-derived carbohydrates and effectively produce organic acids . *Mortierella* produces microbial lipids using a variety of substrates, including monomeric sugars, glycerol, and lignocellulosic biomass hydrolysates .

3.3. The success of intermediates during methane production detected by GC-MS

The GC-MS chromatograms of organics in cultivations CRS, C, and RS were shown in Fig. S2, S3, and S4, respectively. The corresponding compounds with serial number were described in detail in Table 2, S2, and S3, respectively. Only a small number of

257organics were detected in samples CRS0, C0, and RS0 with the dominance of 258aromatics (36.22-58.61%) and aliphatics (33.50-54.09%). In the process of methane 259production, VFAs were important intermediates regardless of the substrates were coal 260and straw, or only coal, or only straw. The success of VFAs in cultivations CRS, C, 261and RS was shown in Fig. 3. A large amount of VFAs were produced on the 7^{th} day 262which accounted for 88.52%, 57.47%, and 70.31% of organics in CRS, C, and RS, 263respectively. Then, it decreased gradually to 80.11% at the end of cultivation CRS 264(Fig. 3a) while even disappeared in cultivation C (Fig. 3b), which were negatively 265corelated with methane production (Fig. 1). On the contrary, it increased to 82.95% in 266RS14 and remained about 79.5% for the rest of cultivation (Fig. 3c) which was 267consistent with that no methane production was observed in cultivation RS (Fig. 1). 268These results suggested that VFAs contributed to methane generation which might 269provide substrates for methanogens. The higher content of VFAs in cultivation CRS 270and the different changes during methane production between cultivations CRS and 271RS demonstrated that part of VFAs in cultivation CRS would come from coal.

272Specifically, VFAs in sample CRS7 was dominated by butanoic acid with 57.42%. 273These VFAs might be produced by the oxidative breakdown of various functional 274groups . The increment of relative abundance of acid-producing bacteria in sample 275CRS7 including *Paraclostridium* , *Caproiciproducens* , *Clostridium* , 276*Lachnoclostridium* , *Anaerotruncus* , and *Anaerocolumna* (Fig. 2b) would contribute 277to the high yields of VFAs. The large amount of VFAs in culture might be the reason 278of the accumulation of *Cryptococcus* as it can use VFAs to increase cell quality and

279produce microbial lipids . In sample CRS14, butanoic acid decreased to 36.82% when
280acetic acid increased to 17.82% from 8.94% in sample CRS7 and methane production
281began to sharply increase, suggesting that butanoic acid might contribute significantly
282to biomethane formation by converting to acetic acid to supply substrate for
283methanogens. The change of acetic acid was consistent with the trend of *Firmicutes*
284(Fig. 2b). *Firmicutes* could produce extracellular enzymes, including cellulases,
285lipases, and proteases, to hydrolyze cellulose, proteins, lignin, and lipids, and use
286butanoic acid and its analogs for acetic acid production (Garcia-Peña et al. 2011).
287After 14 days, acetic acid continued to decrease while the methanogenic pathway was
288changed to hydrogenotrophic methanogenesis. Acetic acid would be oxidized by such
289acetic acid-consuming bacteria as *Clostridium* to H_2 and CO_2 which was utilized by
290*Methanobacterium* to produce methane.

291In cultivation C, propanoic acid dominated in samples C7, C14, and C21 with
29226.93%, 26.27%, and 24.56%, respectively, and disappeared in sample C28.
293Isohexanoic acid, pentanoic acid, and isobutanoic acid were exhausted in sample C21.
294Isopentanoic acid, butanoic acid, and acetic acid were accumulated before 28th day
295and used up on the 35th day. In general, the VFAs with high molecular weight were
296gradually converted into low molecular compounds which was more likely to produce
297acetic acid. The low content of acetic acid would be due to the utilization by
298*Methanosaeta*. Similar phenomenon was observed previously that *Methanosaeta* was
299dominate when acetic acid concentration was low , and anaerobic microorganisms
300began to convert propanoic acid and butanoic acid into acetic acid when acetic acid

concentration was low, and continued to use acetic acid to produce methane . VFAs in sample RS7 were dominated by butanoic acid (39.77%). It significantly increased in sample RS14, and remained unchanged after 14 days (Fig. 3c). The other VFAs also remained almost constant after 14 days.

3.4. The unique metabolic characteristics in co-degradation

PCA distributions of organic compounds in all samples were shown in Fig. 4a. The cumulative variance contribution rates for the first two principal components were 60.2% and 14.3%, respectively. It was shown that three groups from the 7^{th} day to 35^{th} day were well distinguished as shown in red, blue, and green circles. The samples CRS7 and RS7 were overlapped. The samples of cultivations CRS and RS were respectively clustered in a small range after 14 days, indicating that the organics did not changed significantly which was consistent with the composition of organic compounds (Table 2 and S3). However, the samples of cultivation C were not clustered tightly. They could be divided into three subgroups including C7 and C14, C21 and C28, and C35, which indicated a significant change of metabolic pathway in methane production from coal and was consistent with methane production (Fig. 1a).

The difference of organic compounds was further analyzed by heatmap clustering (Fig. 4b). Four groups were detected. The first group included samples CRS0, C0, RS0 and C35. The second group included samples C7, C14, C21, and C28. The third group included samples CRS14, CRS21, CRS28, CRS35, and RS7. The fourth group included samples RS14, RS21, RS28, RS35, and CRS7. The samples were grouped

322according to the substrates except samples CRS7 and RS7, which was consistent with
323the PCA results (Fig. 4a).

324The results of PCA and heatmap analysis on intermediates showed that the
325components and succession of intermediates in CRS were distinct from that in
326cultivations C and RS, especially after 14 days. Combing with the success of
327microbial communities (Fig. 2), these results suggested that the metabolic
328characteristics of co-degradation was unique. The structure of archaeal communities
329showed that methane in co-degradation CRS was mainly produced by
330hydrogenotrophic methanogenesis, while acetoclastic methanogenesis was dominant
331in inoculum and no methanogens survived in cultivation RS. The structures of
332bacterial and fungal communities were changed in CRS comparing with those in
333cultivation C. Although their roles in methane production were similar that they were
334all potential to function in the fragment of coal molecule and fermentation of
335intermediates, the ability of fungi to degrade macromolecular compounds and the
336ability of bacteria to syntrophically ferment with methanogen were enhanced in CRS
337as its methane production was much higher than that in cultivation C.

338The results of microbial communities and intermediates also suggested that the first 7
339days were essential to methane production. A large amount of organic matters were
340produced on the 7^{th} day of cultivations CRS, RS, and C as shown by the results of
341TOC. After then, the content of TOC either changed a little or declined with time. At
342the same time, various organic compounds were generated in all three groups which
343mainly distributing in the retention time of 7-11 minutes (Figs. S2, S3, S4). The

content of VFAs reached the peak on the 7th day while no VFAs was detected at the beginning of cultivation. Accordingly, methane production was also accelerated and hydrogenotrophic methanogenesis became dominant in co-degradation from the 7th day (Fig. 1a and 2). It seems that the fragment of macromolecules in coal and straw was mostly implemented in 7 days by diverse bacteria and fungi. Then, microbes mainly fermented the intermediates and generated methane. Thus, the first 7 days would be the crucial period as the fragment of coal structure was considered as the limited step in coal biodegradation .

4. Conclusions

A significant methane production was observed in co-degradation of coal and straw which was 12 times higher than that in cultivation with only coal. The structure of archaea, bacteria, and fungi were altered by the addition of straw resulting in the change of methanogenic pathway to hydrogenotrophic methanogenesis in co-degradation. The bacterial and fungal communities were also changed by the enrichment of *Caproiciproducens*, *Clostridium*, *Cryptococcus*, *Thielavia*, *Cladosporium*, and *Fusarium*, which were all potential to function in the fragment of coal molecule and fermentation of intermediates. VFAs were the dominant intermediates whose contents were negatively corelated with methane production, suggesting that VFAs contributed to methane generation. The intermediates generated in co-degradation were different from cultivations with only coal and with only straw which suggested together with the results of microbial communities that the metabolic pathway in co-degradation was distinctive. Besides of functioning as methanogenic

substrates, coal might also serve as the suitable microhabitat for microorganisms to avoid the threat from environment. And the fracture of coal molecules was almost completed in the first 7 days of co-degradation.

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Table 1 The labeled names for samples obtained from anaerobic cultivations at different time [a]

Substrates \ Time (days)	0	7	14	21	28	35
Coal and Rice Straw	CRS0	CRS7	CRS14	CRS21	CRS28	CRS35
Coal	C0	C7	C14	C21	C28	C35
Rice Straw	RS0	RS7	RS14	RS21	RS28	RS35

[a] The naming rule is that letter C represents coal, letter RS represents rice straw, and the number represents the sampling days (0 days, 7 days, 14 days, 21 days, 28 days and 35 days)

Table 2 Organic compounds identified by GC-MS and their relative abundances (%) at different stages of co-degradation of coal and straw (CRS).

Code	RT (min)	molecular formula	Compound	CRS0	CRS7	CRS14	CRS21	CRS28	CRS35
1	4.69	$C_4H_{10}O$	1-Butanol	-	-	1.59	0.77	1.06	0.74
2	7.43	$C_2H_4O_2$	Acetic acid	-	8.94	17.82	18.26	14.85	9.59
3	8.12	$C_3H_6O_2$	Propanoic acid	-	2.06	5.65	6.84	6.06	6.04
4	8.33	$C_4H_8O_2$	Isobutanoic acid	-	1.54	3.81	4.26	4.03	4.59
5	8.76	$C_4H_8O_2$	Butanoic acid	-	57.42	36.82	35.01	33.24	36.18
6	9.03	$C_4H_8O_3$	Butanoic acid, 4-hydroxy-	-	-	-	-	0.63	-
7	9.19	$C_5H_{10}O_2$	Isopentanoic acid	-	4.72	10.64	13.11	11.99	13.99
8	9.83	$C_5H_{10}O_2$	Pentanoic acid	-	-	0.70	0.55	0.97	0.94
9	10.51	$C_6H_{12}O_2$	Isohexanoic acid	-	11.84	5.63	4.78	5.20	4.45
10	10.97	$C_6H_{12}O_2$	Hexanoic acid	-	2.00	3.65	4.02	7.27	5.27
11	11.21	$C_7H_8O_2$	Phenol, 2-methoxy-	-	0.75	-	-	-	-
12	11.69	$C_{15}H_{24}O$	Butylated Hydroxytoluene	8.81	-	0.38	-	-	0.77
13	14.87	C_5H_9NO	2-Piperidinone	-	-	0.46	0.38	-	0.62
14	17.13	$C_{23}H_{44}O_2$	Methacrylic acid,	-	-	-	-	0.41	-

			nonadecyl ester						
15	17.38	$C_4H_8O_2S$	Propanoic acid, 3-(methylthio)-	-	-	1.12	0.72	0.41	1.02
16	17.44	$C_{14}H_{22}O$	2,4-Di-tert-butylphenol	45.81	1.65	3.04	3.03	3.01	1.79
17	18.69	$C_{24}H_{44}O_6$	Pentanoic acid, 2,2-dimethyl-, 1,2,3-propanetriyl ester	-	0.67	0.61	0.44	0.54	0.50
18	20.18	$C_5H_8O_3$	5-Hydroxymethyldihydrofuran-2-one	24.52	-	-	-	-	-
19	20.25	$C_7H_{10}O_4$	5-Oxotetrahydrofuran-2-carboxylic acid, ethyl ester	-	1.36	2.53	3.17	2.78	6.49
20	21.41	$C_8H_8O_2$	Benzeneacetic acid	-	-	2.14	1.71	1.21	1.98
21	22.00	$C_7H_{13}NO_4$	Propanedioic acid, amino-, diethyl este	9.37	-	-	-	-	-
22	22.07	$C_4H_6O_3$	2(3H)-Furanone, dihydro-4-hydroxy-	-	0.62	0.99	1.23	1.31	2.89
23	22.34	$C_9H_{10}O_2$	Hydrocinnamic acid	-	3.86	0.91	0.61	1.42	0.73
24	26.05	$C_{16}H_{33}NO$	Hexadecanamide	11.47	-	-	-	-	-

25	26.96	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	-	0.72	0.91	0.70	-	1.42
26	27.99	$C_{28}H_{46}O_4$	1,2-Benzenedicarboxylic acid, bis(8-methylnonyl) ester	-	1.19	-	-	2.55	-

Figure Captions:

Fig. 1 Methane productions and the success of TOC and pH in co-degradation of coal and straw (CRS), cultivation with only coal (C), and cultivation with only straw (RS). (a) methane productions; (b) TOC and pH. The columnar represents the change of TOC while the line represents the change of pH.

Fig. 2 Phylogenetic composition of archaeal (a), bacterial (b), and fungal (c) communities on the 0^{th} (CRS0), 7^{th} (CRS7), 14^{th} (CRS14), 21^{st} (CRS21), 28^{th} (CRS28), and 35^{th} (CRS35) day of co-degradation of coal and straw, and at the end of cultivation with only straw (RS35) at the genetic level.

Fig. 3 The success of VFAs in co-degradation of coal and straw (CRS, (a)), cultivation with only coal (C, (b)), and cultivation with only straw (RS, (c)).

Fig. 4 PCA distributions and heatmap of samples obtained on the 0^{th}, 7^{th}, 14^{th}, 21^{st}, 28^{th}, and 35^{th} day of co-degradation of coal and straw (CRS), cultivation with only coal (C), and cultivation with only straw (RS). (a) PCA distributions; and (b) heatmap.