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Sustaining biogenic methane release from Illinois coal in a fermentor for one year

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ABSTRACT

To evaluate coal biogasification in a larger reactor over a longer duration as compared to studies reported so far, a 3-liter fermentor was established. During the one-year study, a nutrient recipe was added three times to sustain methane release from Illinois bituminous coal. The cumulated methane production was 5171 ft^3/ton with a methane content of 75.4% on day 365. After the fermentation was terminated, the residual coal and fermentation broth were characterized in detail. Compared to the untreated coal, the treated coal residue appeared to be finer and highly degraded with less carbon but more ash. Based on mass balance, volatile and fixed carbon decreased 15.9% and 29.6%, respectively, using the untreated coal as the baseline. According to GC/MS analysis, the fermentation broth contained mainly three groups of compounds: fatty acids and their derivatives, aromatics, and hydrocarbons. In addition, the fermentation broth was found to have effect on flocculation and contained compounds that possessed surface-active properties. Further investigations are needed to identify these chemicals responsible for these activities and develop ways to further enhance coal biogasification based upon results obtained then.

1. Introduction

Coalbed methane (CBM) is an important natural gas resource that has attracted increasing attention worldwide [1]. Generally, CBM is contributed by two processes, geological and biological. Accumulated geological data has shown that the secondary biogenic source is a more important origin of CBM [2]. Generation of biogenic methane is due to microbial activities after coalification, which indicates that coal has the potential to be converted to methane under normal ambient conditions [3]. Recently, great efforts have been extended to enhance biogenic methane production from coal in view of promising results reported in the literature [1,4].

For the purpose of enhancing methane production from coal both in situ and ex situ, different biological approaches have been tested, including adding external microbial sources- bioaugmentation and supplementing chemicals and nutrients- biostimulation. These approaches could be used separately or in combination to achieve continued generation of biogenic methane from existing CBM installations. For bioaugmentation [5], microorganisms may be added if they have demonstrated greater capability in methane production than the existing microorganisms in the coal beds, or the target coal beds lack microbial activities toward methane release. Intuitively, it seems that native microbial communities would be optimally adapted to their environment in the presence of coal and would provide higher methane production compared with the foreign microbial consortia [1]. However, in some cases, the opposite is true as evidenced by reports that some foreign microbial communities were able to produce similar or more methane from coal than native communities [5–8]. But, if legal aspects are considered, such as getting permits for injecting microbes to a given environment, bioaugmentation may face daunting challenges. Thus, a better niche for this may be for it to be used ex situ. In terms of biostimulation, numerous studies have evaluated various recipes including MS medium, trypticase soy broth, commonly used anaerobic medium, and different solvents [9–13]. However, the majority of these studies focused on short-term evaluation of methane production from different ranks of coal in small reactors. The study periods normally were 30–45 days and the reactor volume was generally less than 250 mL. Thus, at this point, it is unknown whether results obtained from short-term studies in small vessels can be extrapolated to longer term and in large scales.

In addition, even though a great number of studies have been published in the domain of coal biogasification, only a few have

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evaluated the residual coal after bioconversion. According to Barnhart et al. [14], after 1169-day bioconversion with the addition of algal extract or yeast extract, the British Thermal Unit (BTU) content of the treated coal was 99.5% of that of the untreated coal. In addition, several parameters, such as total coal moisture, coal ash and coal sulfur did not vary significantly among different treatments and controls. Thus, the coal quality remains largely unchanged following a long term stimulated microbial methane production. In another study [15], however, compared to the untreated coal, the coal residues after 30- and 60-day biogasification were found to have lower carbon content, higher sorption capacity, more pore surface area, higher gas storage capacity, and significantly enhanced diffusion rates as a result of continued bioconversion. Further test of similar samples revealed that after biotreatment, the mesopore surface area and pore volume decreased with increased average pore diameter, while the micropore surface area increased with decreased pore volume. After bioconversion, both inaccessible meso-/micropore size distributions decreased while the accessible micropore size distribution increased, making a portion of closed micropore network accessible. In addition, the methane adsorption capacities increased after bio-treatment, which was confirmed by the increase of micropore surface area [16].

Considering different results published by different research groups, the effect of bioconversion on coal structure remains to be elucidated, in particular at relatively larger scale. To fill this critical knowledge gap, this study was designed to evaluate coal biogasification in a 3-liter fermentor for a one-year duration. Besides measuring and computing methane yield, we have specifically focused on: (1) evaluating the residual coal with regard to particle size, elemental composition, and morphology and (2) studying the fermentation broth with respect to their chemical composition and potential functions as bioflocculant and biosurfactant. It needs to be noted that this study is an extension and scale up of what we have extensively studied in the past several years at the microcosm level [9–13,17,18]. At those levels, we have demonstrated through delicate experimental designs that coal is the dominant carbon source for methane detected even though the microcosms are supplemented with suitable nutrient solutions.

2. Materials and methods

2.1. Coal samples

For this study, the coal sample used was the same as what has been investigated and reported before [9–11,13,15,18]. Briefly, coal blocks were collected from the Herrin Seam, # 6 in the Illinois basin. This coal contained 70.1% of carbon, 1.4% of nitrogen, 5.2% of hydrogen, 0.6% of sulfur, 15.4% of oxygen, and 7.5% ash (dry weight basis). Contents of volatile matter and fixed carbon were 49.9% and 42.6% (dry weight basis), respectively. Immediately before use, a block of coal was broken into lumps approximately 1.3 cm in size. The coal lumps were subsequently ground and sieved to obtain coal samples less than 200 mesh (74 μm). This particle size was chosen based upon our previous observation that among different particle sizes, biogasification of coal < 74 μm led to the highest methane yield for this Illinois coal [10]. Ground coal samples were stored in re-sealable ziploc bags at room temperature in order to prevent moisture loss and oxidation.

2.2. Formation water collection

Formation water used in this study was collected from an established coal-bed methane (CBM) well as described in our reported study [10,17]. At the sampling site, the formation water was retrieved from a depth of around 850 ft. The in situ temperature was measured immediately after the formation water came to the surface. For those dedicated to experimental setup as described below, the water samples in half-gallon containers were supplemented with sodium sulfide (Na_2S) at 0.25 g/L and resazurin at 1 mg/L to maintain anaerobic conditions. Once sealed tightly, these containers were brought back to our laboratory where they were immediately stored in a −20 °C freezer for later use. Fresh formation water without the addition of these two chemicals was analyzed thoroughly in terms of its chemical composition as reported already [17].

2.3. The microbial community

The microbial community used in this study was that initially present in the formation water aforementioned above. Upon arrival in our laboratory, the formation water was concentrated 80 times through high-speed centrifugation at 10,000*g* force for 30 min. The resulting concentrate was used to make glycerol frozen stocks. Based on next-generation 16S rDNA sequencing, this community comprised a total of 231 Bacterial species and 33 species of Archaea [18]. The Bacteria were distributed among 24 phyla. The dominant three were Proteobacteria, 40.8 ± 0.0%; Bacteroidetes, 22.9 ± 2.0%; and Firmicutes, 17.9 ± 0.1%. In terms of Archaea, the majority (89.8 ± 0.7% of the total) fell within the order of Methanobacteriales within the phylum of Euryarchaeota.

2.4. Experimental setup and monitoring

Most biogasification studies have been conducted in small serum bottles lasting for a few months or shorter. To understand how biogasification performs in a larger reactor over a longer duration, a 3-liter fermentor (Eppendorf, Hauppauge NY, USA) was used. The testing conditions were the same as the optimal conditions gained from our previous study [10]. Specifically, the coal loading was 200 g/L, the temperature was 32 °C, and the coal particle size was < 200 mesh (74 μm). The recipe used in this study was developed from our previous work targeting in situ biogasification [17]. This recipe contained Fe-powder at 74 mM (particle size: 80 nm–100 nm); methanol at 97.9 mM; ethanol at 100 mM, and a trace mineral solution at 100%. For the trace mineral solution, a 100% supplement was used to ensure that the formation water, after external trace minerals were added, had the same composition of trace metals as in a standard MS medium [19]. Specifically, a trace mineral stock solution was made containing $CoCl_2{\cdot}6H_2O$ at 1.3 mg/L, $ZnCl_2$ at 0.76 mg/L, $Na_2WO_4{\cdot}2H_2O$ at 0.26 mg/L and H_2SeO_3 at 0.01 mg/L. For each recipe addition, 10 mL of the trace mineral stock solution was added to one liter of formation water. It needs to be noted that the formation water used in this study was filtered through 0.45 μm filters to minimize impacts of suspended solid in the water. The fermentation system was started by adding 100 g of coal samples together with 500 mL filtered formation water, and 50 mL of inoculum developed from the glycerol frozen stocks. In light of the fact that yeast extract and trypticase peptone are important nitrogen sources and their demonstrated effect on stimulating coal bioconversion [9], these two ingredients were added at 2 g/L for each. After all ingredients were added, the fermentor was sealed and purged with N_2 completely to remove oxygen. It is noteworthy that the developed recipe was not supplemented on day 0 and the fermentor had an approximately 2-liter headspace at the beginning together with the fermentor, three replicate uninoculated control microcosms were established. These microcosms included coal at 200 g/L, the filtered formation water and the same amendments as those added to the fermentor and at the same concentrations, but not the inoculum. These controls were set up in the same way as described in Zhang et al. [17].

Starting from day 10, the headspace gas in the fermentor was released and collected in a 3-L airbag. The fermentor was then purged entirely by at least six liters of N_2 gas to ensure a zero concentration of methane in the 1-atm headspace. The volume of the released gas together with gas content measured by a Gas Chromatography (GC) at different time points were recorded. On day 31, day 121, and day 300, the developed recipe described above was injected into the fermentor following nitrogen purging to supplement what was consumed by the

microbial community. At the end of one year, the fermentation was terminated and the final samples were collected and analyzed.

The whole content of the reactor was centrifuged at 5000 × *g* for 20 min to separate the liquid and residual coal. The solid fraction was washed with deionized and distilled water 3–5 times to remove medium and cells associated with coal. The washed coal was dried in an oven at 105 °C until the solid weight was constant, then was kept at a 4 °C refrigerator for later use. The liquid sample which was referred to as fermentor broth, were kept in the same refrigerator and were analyzed extensively as described in the following. The same procedures were performed on the three controls.

2.5. Sample analysis

2.5.1. GC analysis

The content of methane, nitrogen, and CO_2 in the fermentor headspace was analyzed through a 17A GC (Shimadzu, Columbia, MD, USA). This GC was equipped with a 60 m × 0.53 mm RT-Msieve 5A porous layer molecular sieve (Restek, Bellefonte, PA, USA) and a flame ionization detector with argon being the carrier gas with a flow rate of 10.1 mL/min. The isothermal zone temperatures for the injector and detector were set at 75 °C and 310 °C, respectively. Calibration curves for methane, nitrogen, and CO_2 (5–99%) were established using standard gases (Air Liquide, Plumsteadville, PA, USA).

2.5.2. GC/MS analysis

To understand the chemical composition of the fermentor broth, nonadecanoic acid ($C_{19:0}$) (98%, Acros Organics, New Jersey, USA) was used as an internal standard for the GC/MS analysis. In short, three replicates of 1.5 mL of the liquid sample were transferred into 15-mL centrifuge tubes. After the pH was adjusted to 2.0 using concentrated HCl, 5 mL dichloromethane (DCM) (99.9%, Fisher Chemical, Pittsburgh, Pennsylvania, USA) was added. The glass tubes were then vortexed vigorously for 10 min. When phase separation was complete, the DCM layer was withdrawn from the bottom of the tubes. The remaining aqueous phase was extracted by DCM twice. All DCM fractions were pooled together, passed through dried sodium sulfate powder, and evaporated to dryness under a gentle stream of nitrogen. Methanol (1.0 mL) was then added to dissolve the dried powder followed by transferring the whole content to a GC vial where 0.1 mL tetramethylammonium hydroxide (TMAH) stock solution (20 g/L) was added for derivatization.

GC/MS analysis (Agilent 7890A/5975C) was performed using helium (1 mL/min) as the carrier gas and a capillary column, HP-5MS (30 m 5% phenyl methyl siloxane * 0.25 mm i.d.; 0.25 μm film thickness, Agilent). The GC oven was heated to 50 °C for 1 min then to 300 °C at a rate of 4 °C/min with an isothermal period of 5 min. Spectra were recorded in the EI mode (electron energy = 70 eV), with a scan range from 33 to 650 *m/z* in 0.42 s/scan. The injection volume was 1 μL. The identification of each compound was achieved by matching each peak's mass spectrum with that in the spectral library (NIST 11 database).

2.5.3. Particle size, proximate and ultimate analyses of the coal samples

To facilitate discussion, the residual coal samples from the three uninoculated controls were referred to as untreated and those from the fermentor were termed as treated. In this study, both untreated and treated coal samples were subject to: (1) sieve analysis to determine their particle size distribution according to a standard operating procedure [20]; (2) proximate analysis conducted by using a LECO TGA701 instrument according to manufacturer recommended procedures. The moisture, volatile matters and ash were analyzed at different temperature until weight was no longer changed; and (3) ultimate analyses by using a Thermo Flash 2000 Elemental Analyzer (Hudson, New Hampshire, USA) following manufacturer recommended protocols.

2.5.4. Bioflocculant test

To test whether the culture broth from the fermentor could act as a flocculant, a flocculating activity test was set up following reported procedures [21,22]. Briefly, 0.1 mL of culture broth, the liquid phase after centrifuging the whole fermentor content, was added into 5 mL kaolin (Fisher Scientific, USA) suspension at 4 g/L. The mixture was vortexed for 30 s and then kept still for 5 min. The absorbance of the supernatant and the blank control without culture broth was measured at 550 nm (as OD_{550} and OD_{blank}, respectively) using a spectrophotometer. The aluminum sulfate (Acros Organics, USA) solution at 10 mg/L was used as a positive control and the liquid samples from the uninoculated controls were used as negative controls for this test.

The flocculating activity was defined and calculated as follows:

$$\text{Flocculating activity}(\%) = (OD_{blank} - OD_{550})/OD_{blank} \times 100$$

2.5.5. Biosurfactant test

Subsamples of the aqueous phase from the fermentor and the three controls were also subject to surfactant test to see whether surface active chemicals were present. For this test, approximately 3 mL of the sample was divided into three aliquots. For each aliquot, three surface tension measurements using a tensiometer (Kibron U-troughs) were observed for a total of nine measurements. These measurements were based on the Wilhelmy plate method [23,24]. The tensiometer was calibrated using a clean tungsten wire probe and double distilled water and the same wire probe was used for all of the measurements taken. In between each measurement within a sample, the wire probe was cleaned by dipping it into double distilled water baths and then wiped clean to remove any residue and, between each sample, the wire probe was thoroughly cleaned by a flaming process.

2.5.6. SEM observation

A FEL Corp. Quanta FEG 450 scanning electron microscope (SEM) was used for observing coal samples from the fermentor and the controls. The samples were coated with gold for 20 min, and then imaged following the manufacturer recommended procedures.

3. Results and discussion

As described above, this study is an extension of what we have studied in the last couple of years. Through our past extensive investigations, we have demonstrated that: (1) coal can be biogasified to methane by studied microbial communities [11,15,18]; (2) with nutrient supplementation, rate of methane release from coal can be enhanced significantly [9,10,13]; (3) even though the developed nutrient recipe contains organic carbon, the majority of methane observed is from coal [12,17]. These solid conclusions were reached through delicate experimental designs where multiple positive and negative controls are included. Based upon all insights we have gained so far, this study was designed to evaluate effect of biogasification on coal in a larger scale and over a longer duration through comparing results from biotic and uninoculated conditions.

3.1. Methane production

As shown in Fig. 1, methane content reached 32% with a methane yield of 188.32 ft^3/ton at day 30, which was very close to our pervious study [18] where only yeast extract and peptone were the key ingredients in the nutrient solution. It needs to be noted that no recipe was added to the fermentor during the first month of operation. On day 31, the entire headspace gas was purged with N_2 and one dose of the developed recipe was added. As a result, the headspace methane content increased rapidly from 0.0% to 61.2% on day 60 and 67.2% on day 90, but dropped to 62.7% on day 120. Similar trend was observed again on day 121 and day 300 as a result of recipe supplementation. The final methane content at the end of one year was 75.4%.

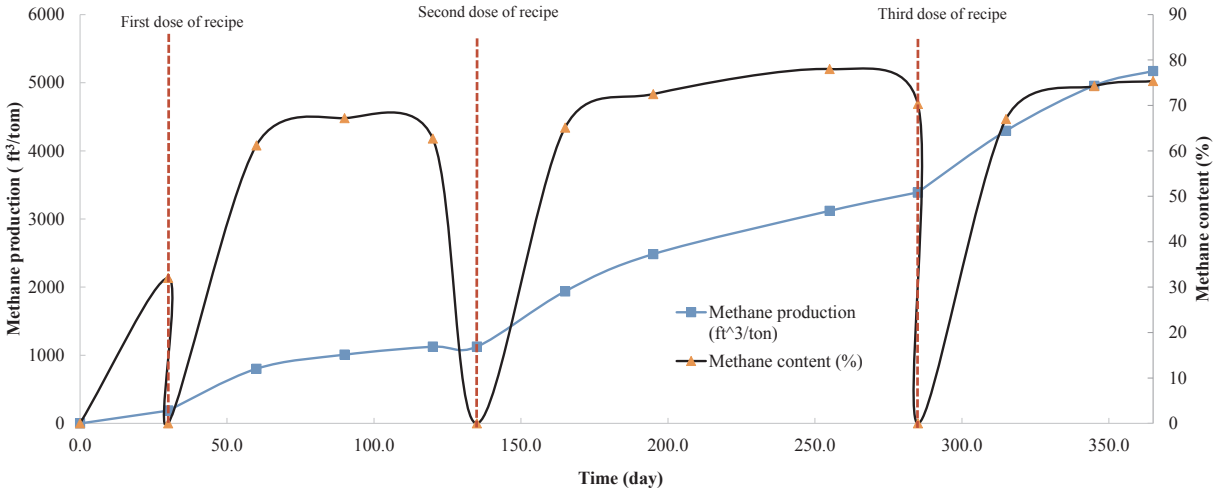

Fig. 1. Results of the methane yield and content responding to recipe addition.

Regarding methane production, even though there were times when methane content dropped in the headspace, the cumulative methane yield has been increasing all the time and reached 5171 ft^3/ton at the conclusion of this one-year biogasification process. Corresponding to recipe addition, a repeating trend was that rapid increase of methane release was followed by slow increase. The rapid methane generation phase typically lasted for 30 days after recipe addition. This is in agreement with what we have observed before [9,13,18] and confirmed that: (1) coal was still bioavailable; (2) the degradation products from coal were not inhibitory to biogasification. Specific to this study, the liquid in the fermentor was not withdrawn at all during the one-year experimental period; and (3) nutrients were the rate-limiting factor. The added nutrients included methanol, ethanol, yeast extract and peptone. Based on our previous studies [12], the alcohols alone did not contribute much to methane release. The same is true for the two nitrogen sources. Even though nitrogen and phosphorous themselves were not limiting, some compounds within these new nutrient sources appear to have significant effect on methane release or microbial activities.

Interestingly, methane production rate (ft^3/ton-day) in 30 days increased with time (Table 1). After the first dose of recipe addition, the immediate methane release rate was 20.5 ft^3/ton-day. After the second and third dose, it was 27.0 and 30.1 ft^3/ton-day, respectively. Thus, it seems that the microbial community adapted to coal, the recipe, and the fermentor environment better with time. It could be assumed that coal biogasification would continue and higher rate would be detected if the experiment was allowed to last longer. This hinted that large scale coal biogasification either in situ or ex situ can be potentially sustained over a long period of time if suitable nutrients are added at different time intervals. This assumption, however, would need to be validated by data from field test. Throughout the whole experimental period, no methane was observed from the three controls. Thus, it is obvious all methane released was due to biogasification of coal.

3.2. Coal particle size

To understand effect of biogasification on coal structures, the particle size of untreated and treated coal was compared. As shown in Fig. 2, as a result of one year treatment, the fraction of coal with particle sizes less than 500 mesh (25 μm) increased from 25.7 ± 1.85% in untreated coal to 52.8 ± 0.21% in treated coal. On the contrary, the fractions with particle sizes larger than 400 mesh (37 μm) dropped from 33.84 ± 0.94% in untreated to 16.65 ± 0.04% in treated coal. Thus, the biogasified coal was much finer than those untreated.

These observations agree well with the report that microbes can biochemically modify the coal to reduce the size of coal particles and incorporate functionality to allow coal to be readily dispersed [25]. Considering this feature, biogasification may be used as an approach for

Table 1
Results of methane yield, content and production rate responding to time.

Day	Action	Methane content (%)	Overall methane production (ft^3/ton)	Methane production rate in 30 days (ft^3/ton-day)
0.0	Setup the fermentor	0	0	0.0
30	Collected the gas in the gas bag	32	188.32	6.3
30	Added nutrients to the fermentor and purged the reactor with N_2	0	188.32	
60	Collected the gas in the gas bag	61.2	802.02	20.5
90		67.2	1008.76	13.7
120		62.7	1126.56	10.4
135	Added nutrients to the fermentor and purged the reactor with N_2	0	1126.56	
165	Collected the gas in the gas bag	65.1	1937.66	27.0
195		72.5	2484.62	22.6
155		78	3119.15	16.6
285		70.3	3390.00	15.1
285	Added nutrients to the fermentor and purged the reactor with N_2	0	3390.00	
315	Collected the gas in the gas bag	67	4294.28	30.1
345		74.24	4956.22	26.1
365		75.36	5171.71	25.5

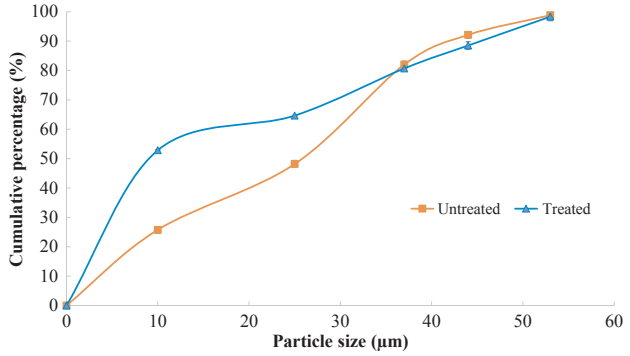

Fig. 2. Cumulative fractional distribution of untreated and treated coal.

coal particle size reduction. Compared to conventional milling techniques, such as ball milling, jet milling, and other mechanical impingement methods, which tend to be energy intensive, coal biogasification consumes much less energy since it can be conducted at ambient conditions.

3.3. SEM investigation of coal surface morphology

Results from the particle size measurements were supported by SEM observation. As shown in Fig. 3, surface of the untreated coal was relatively smooth. After one year biogasification, however, the treated coal, when observed at a magnification of 1254×, appeared to be severely degraded and eroded. At higher magnifications, such as 2514× and 4668×, the original coal particle appeared to be broken and fragmented. It needs to be noted that the SEM images of coal residues resembled nothing of biofilm like what we observed in another already [26]. To explain this severe fragmentation, we speculate that the microbes could: (1) utilize coal components as substrates or (2) secrete chemicals that could assist in coal depolymerization. Another explanation could be that intermediates from coal degradation helped dissolve coal further and the alcohols included in the recipe may act as a solvent. Even though the exact reason for this highly disrupted coal structure is unclear at this point, it does prove that coal, as a solid substrate, can be structurally changed dramatically.

3.4. Chemicals identified by GC/MS analysis

To understand whether chemicals in the liquid phase of the fermentor facilitated coal dissolution, we used GC/MS to profile and identify these compounds. As shown in Table 2 and Fig. 4, a total of 19 compounds with confidence level > 90% were identified and divided into three main categories. The first group is fatty acids and their derivatives. Among this group, seven, such as dodecanoic acid, pentadecanoic acid, hexadecanoic acid, 9-cctadecenoic acid (Z), stearic acid, tetradecanoic acid, and 13-methyltetradecanoic acid were quantified. The hexadecanoic acid and stearic acid had the highest concentration of 38.9 and 23.7 mg/L, respectively. Some of these acids, such as n-hexadecanoic and n-octadecanoic acid were also observed from degradation of sub-bituminous coal in bioaugmented microcosms [5,27]. The second group is aromatic compounds. Among this group, concentration of phenol, 4,6-di(1,1-dimethylethyl)-2-methyl was 45.1 mg/L followed by benzene, 1-methoxy-4-methyl with a concentration of 34.8 mg/L and benzoic acid at 10.5 mg/L. Benzoic acid has been demonstrated to be one of the key intermediates in anaerobic aromatic compound metabolism [28,29] and it could be converted further to methane and carbon dioxide [30,31]. The presence of aromatic compounds confirmed the pathway proposed by Strapoc et al. [32] where coal defragmentation by fermentation releases oxygen-containing single or polyaromatic chemicals.

For the third group of hydrocarbon, only one compound, undecane was quantified at 9.0 mg/L with high confidence. Interestingly, these three groups of compounds are also identified from coal depolymerization using potassium permanganate [33]. Thus, it hints that the microbial actions on coal degradation may follow similar course as the chemical process. In the study of sub-bituminous coal, single-chain aromatics, long-chain alkanes, and long-chain fatty acids were observed to accumulate during the first 39 days and then decreased their concentrations with time [5]. This indicated that these compounds can be utilized by that microbial community. In this study, the presence of

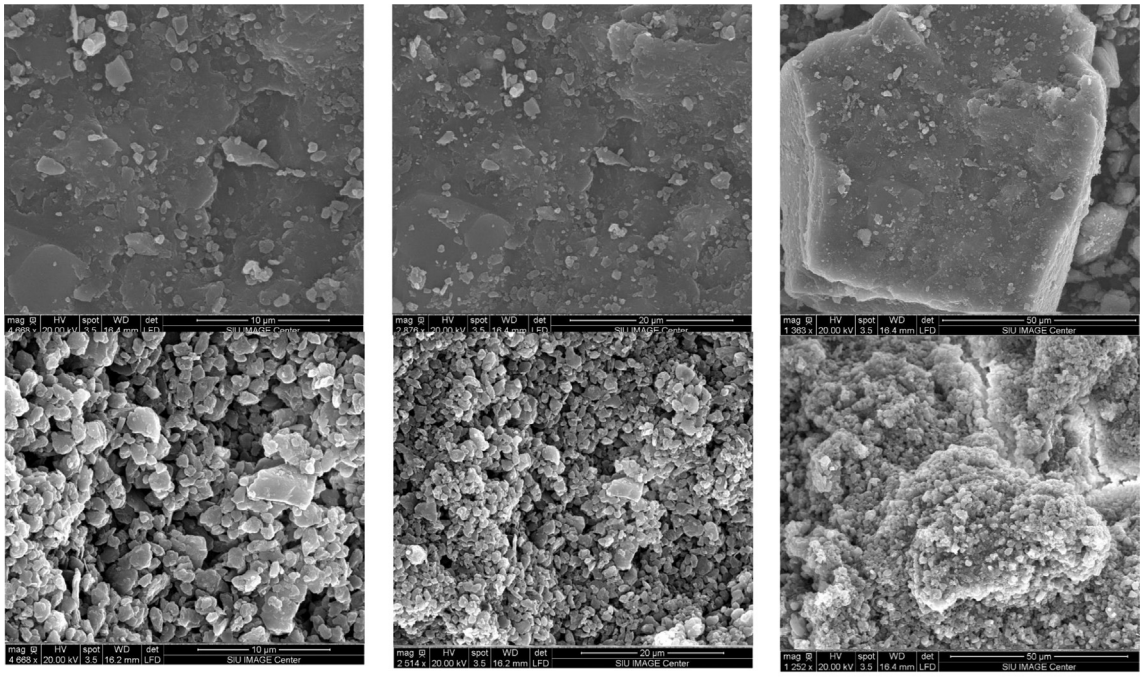

Fig. 3. Scanning electron microscopy (SEM) images of untreated coal (top) and treated coal (bottom), (a) 10 μm; (b) 20 μm; (c) 50 μm.

Table 2
DCM soluble compounds identified by GC/MS.

Group	Compound number (#)	RT (min)	Conc. (mg/L)	Hit Name	Quality
Fatty acids and derivatives	12	26.034	0.6	Dodecanoic acid	93
	14	31.669	3.0	Tetradecanoic acid	99
	15	33.331	0.7	Pentadecanoic acid	91
	16	33.543	1.6	13-Methyltetradecanoic acid	93
	17	36.784	38.9	Hexadecanoic acid	98
	18	40.861	4.7	9-Octadecenoic acid (Z)	90
	19	41.465	23.7	Stearic acid	99
Aromatics	1	9.481	34.8	Benzene, 1-methoxy-4-methyl-	97
	2	11.382	6.9	Benzaldehyde, 4-methyl-	96
	3	11.832	10.5	Benzoic acid	95
	5	20.294	0.5	Benzoic acid, 3-methoxy-,	90
	7	23.953	1.1	Phthalic acid	96
	8	24.303	2.1	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	94
	9	24.891	6.1	1,3-Benzenedicarboxylic acid	97
	10	25.288	4.5	1,4-Benzenedicarboxylic acid	97
	13	29.349	2.8	3,5-Di-*tert*-butyl-4-hydroxyanisole	98
	6	23.445	45.1	Phenol, 4,6-di(1,1-dimethylethyl)-2-methyl-	91
	11	25.674	4.5	Phenol, 2,4-bis(1,1-dimethylethyl)-	96
Hydrocarbon	4	12.129	9.0	Undecane	94

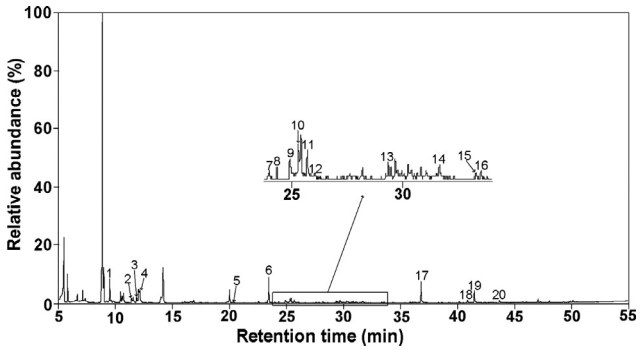

Fig. 4. The GC chromatogram of the DCM solubles in the fermentor.

these chemicals after one-year fermentation suggests that coal degradation is a continuous process. But to truly understand the change of these compounds and their degradability during coal bioconversion, time series data are needed. In addition, since only DCM solubles were analyzed in this study, other non-DCM solubles and those remaining in the aqueous phase need to be characterized. Furthermore, whether these compounds are just transient degradation intermediates or they can assist in coal dissolution is unclear at this point and deserves to be investigated further. For samples derived from the three uninoculated controls, no compounds above the detection limits were observed. Thus, the appearances of these identified compounds were due to interactions between coal and the microbes.

3.5. Ultimate and proximate analyses of coal

As shown in Fig. 5, there were apparent changes in elemental contents between the untreated and treated coal. After one-year biogasification, the residual treated coal samples contained less carbon, but more sulfur and ash. The contents of nitrogen, hydrogen, and oxygen did not change much after bioconversion. In terms of carbon, its content dropped from 70.1% ± 0.08% to 58.78 ± 0.17%, which proved that the detected methane was indeed from carbon in the coal. This agrees well with our previous observation that carbon content in treated coal is lower than that in the untreated even only after 30-day biogasification of the same coal [15]. It needs to be noted that the lost carbon may have three forms: biogenic gas (methane and CO_2) in the gas phase, gas absorbed to the remaining coal, and those dissolved in the aqueous phase, which were removed by a washing step. Based on the elemental composition, the BTU content of the remaining coal was 10,474 ± 33 BTU/lb which was 83.5% of the BTU content of the untreated coal. This result is different from what was reported by another study where 99.5% of the BTU content was retained in the residual coal after 1169-day bioconversion supplemented with either algal extract or yeast extract once [14]. It is difficult to explain the difference since different coals, microbial communities, and biogasification conditions were studied. According to results from the proximate analysis (Fig. 6), content of fixed carbon decreased from 52.6% in untreated to 43.6% in treated coal. The content of volatile carbon appeared to be similar. However, if mass balance is considered, then content of fixed carbon and volatile carbon decreased 29.6% and 15.9%, respectively. Thus, both forms of carbon were utilized by the studied microbial community.

As a result of carbon loss during the treatment, the residual coal had higher contents of sulfur and ash. This may suggest that sulfur in the studied coal are mainly associated with fractions that are not or at least not readily biodegradable. Content wise, the fraction of ash increased more than two folds from 9.25 ± 0.01% to 18.66 ± 0.12%. Considering mass balance, the mass of ash in treated coal was 1.7 times higher than that in untreated coal. This may be explained by cell absorption of metals from the formation water used in coal biogasification. This assumption, however, needs to be further explored.

3.6. Fermentation broth: flocculating and surface active activities

The reason for testing whether the fermentation broth has activities toward flocculation is that the broth appeared to be viscous. As shown in Table 3, when 0.1 mL of fermentor broth was added to the kaolin solution, the flocculating activity was 65.59 ± 0.01%. According to a linear relationship between alum stock solution concentration and flocculating activity (Fig. 7), the fermentation broth had the same flocculating activity as an alum stock solution at 0.74 g/L. The fermentation broth contained cells in the spent formation water. After centrifuging the broth at 4500*g* for 10 min, the flocculating activity was 65.40 ± 0.03%. After further centrifugation at 10,000*g* for 10 min, the activity was 65.89 ± 0.06%. Thus, the compounds that were responsible for the flocculation, termed as the bioflocculant were water soluble.

To put things into perspective, alum is the most commonly used coagulant for water treatment. Depending on the source water quality, alum is generally used between 10 and 50 mg/L of water being treated. Thus, either the fermentation broth itself or compounds extracted from the broth can be used to replace alum used in coagulation. This possible

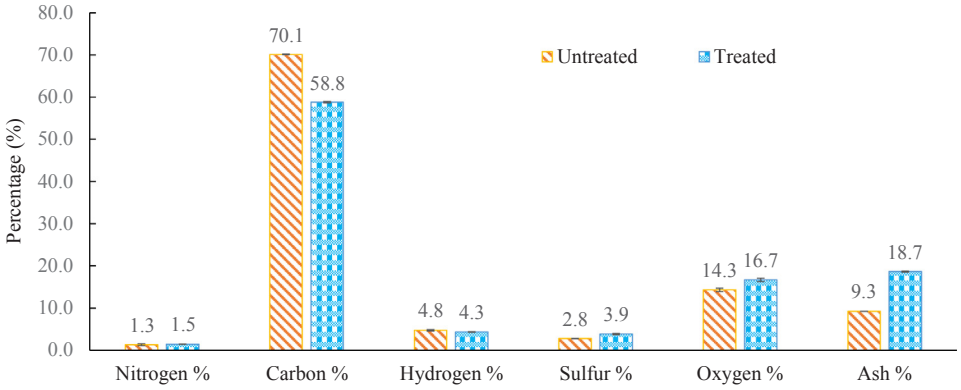

Fig. 5. Elemental composition of untreated and treated coal.

replacement is considering concerns raised from reports where a correlation between high consumption of aluminum from drinking water and Alzheimer's disease is proven from long-term studies [34,35]. But whether the fermentation broth can be used for this purpose awaits further investigation.

Bioflocculant is a biodegradable polymer secreted by microorganisms with high particle flocculating capability. Salehizadeh and Shojaosadati reported that *Bacillus firmus* could produce a strongly acidic, polysaccharide flocculating agent, which acts on both inorganic and organic suspension, such as activated carbon, yeast, and kaolin [36]. In addition, bioflocculant can be released by *Sorangium* spp. and *Corynebacterium* spp. [37,38]. Based on our next generation 16S rDNA sequencing results [18], strains similar to *Bacillus firmus, Sorangium* spp. and *Corynebacterium* spp. may be present in the inoculum.

Besides the flocculating test, we also conducted tests to evaluate whether the fermentation broth had surface-active compounds. According to surface tension measurements detailed above, the surface tension of the fermentor broth was 54.5 ± 2.2 mN/m, which is significantly lower than pure water (72 mN/m). For the liquid samples collected from the uninoculuated controls, the average measurement was 70.2 ± 1.2 mN/m. Thus, the fermentor broth does have chemicals that have the function as biosurfactant. It is known that a great number of microorganisms can produce biosurfactant, such as glycolipids, lipopeptides, lipopolysaccharides, and lipoproteins [39]. Specific to coal biogasification, biosurfactant-producing Actinomycetales were detected in coal beds in the Powder River Basin and were speculated to have roles in increasing coal bioavailability [40].

Compared with inorganic and organic flocculants and surfactants, bioflocculants and biosurfactants have great advantages, such as being safe and easy to be degraded in the environment to avoid secondary pollution. They are, thus, environmentally friendly and have no harmful effects to humans [41]. The exact compounds that had these roles, however, cannot be identified due to the limit of instrumentation. Once they are confirmed, it may be worthwhile to promote the production of these biocompounds for the purpose of enhancing coal biogasification or simply producing these chemicals from low value coal resources for commercial uses.

4. Conclusion

This study showed that the IL coal could be converted to methane continuously if optimal conditions were maintained and nutrients provided. At least for a one-year duration, this statement is true. As a result of adding a nutrient solution at three intervals, the overall methane production from coal reached 5171 ft^3/ton with a final methane content of 75.4%. As a consequence of coal bioconversion, compared to the untreated coal, the residual treated coal had smaller particle sizes, appeared to be finer and highly eroded, had less content of carbon, but higher content of ash. The fermentation broth contained a wide variety of chemicals dominated by fatty acids and aromatic compounds according to analysis by GC/MS. In addition, the broth comprised compounds that could act as bioflocculant and biosurfactant. All of these suggest that coal biogasification is a highly complex process with little known thus far. Therefore, further investigation is warranted for improving the efficiency of coal biogasification and for obtaining valuables from low value and abundant coal resources.

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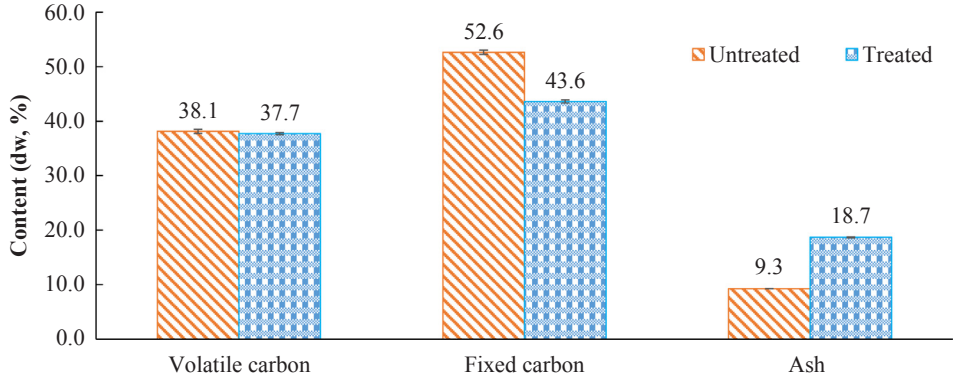

Fig. 6. Proximate analysis of untreated and treated coal.

Table 3
Flocculating activity of the fermentor broth after different centrifugation treatments.

Sample	Absorbance at 550 nm	Flocculating activity (%)
Positive control (alum solution at 10 mg/L)	2.04	8.07 ± 0.01
Negative control (liquid from the abiotic controls)	2.22	0
Fermentor broth before centrifugation	0.74	66.59 ± 0.01
Fermentor broth after centrifugation (4500g for 10 min)	0.769	65.40 ± 0.03
Fermentor broth after centrifugation (10,000g for 10 min)	0.758	65.89 ± 0.06

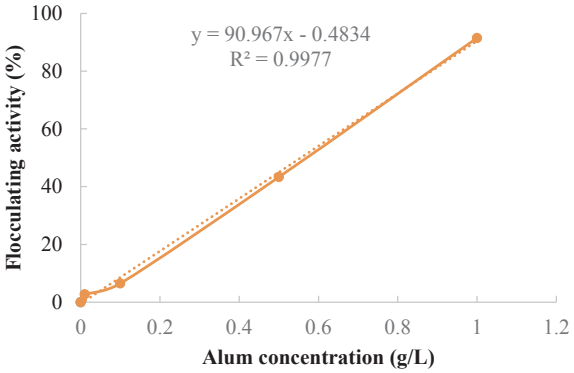

Fig. 7. The linear relationship between alum concentration and the flocculating activity.

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