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Biogenic coal-to-methane conversion can be enhanced with small additions of algal amendment in field-relevant upflow column reactors

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ABSTRACT

Subsurface coal environments, where biogenic coal-to-methane conversion occurs, are difficult to access, resulting in inherent challenges and expenses for *in situ* experiments. Previous batch reactor studies provided insights into specific processes, pathways, kinetics, and engineering strategies, but field-relevance is restricted due to limited substrate availability or byproduct accumulation that may influence reactions or metabolisms. In this study, continuous-flow column reactors were used to overcome some batch limitations, improve the understanding of *in situ* conditions, and increase field-relevance for subsurface engineering technology development. The bench-scale reactor system was constructed to investigate the addition of algal amendment for enhancing microbial coal-to-methane conversion previously developed in batch systems. Four reactor columns were packed with coal and inoculated with a microbial consortium from the same Flowers-Goodale coal bed. Two reactors were amended with ^{13}C-labeled algal amendment on day 0, and two were unamended. On day 61, one previously amended and one previously unamended reactor were re-amended. Produced gases were captured in a gas trap, and CH_4 and CO_2 were quantified. The reactor amended twice produced 1712.6 µmol CH_4 (4.6% as $^{13}CH_4$). The reactor amended only on day 0 produced 1485.5 µmol CH_4 (2.6% as $^{13}CH_4$). The reactor amended only on day 61 produced 278.9 µmol CH_4 (3.9% as $^{13}CH_4$). The reactor with no amendment produced no measurable gases for the duration of the 172-day experiment. Amendment increased the rate of coal-to-methane conversion and total gas production; most of the produced gases were due to coal conversion with only small contributions ($< 7\%$) from amendment conversion.

1. Introduction

Biogenic coalbed methane (CBM), produced by microbial consortia that convert coal to methane in subsurface coal seams, is an unconventional gas resource that has been extracted in many coal basins around the world [1,2]. However, commercial extraction rates often exceed the rates of microbial coal-to-methane conversion, resulting in short well lifespans [3]. To enhance coal-to-methane conversion, methods have been investigated for biostimulation using various organic and/or inorganic nutrient amendments [1,2,4–7]. Other studies have investigated methods for increasing coal bioavailability through oxidation [8,9], chemical treatment [10–13], or increasing surface area [14–16]. Studies focused on developing methods to increase total methane production and rates of microbial coal-to-methane conversion have primarily used batch systems.

Subsurface CBM-producing microbial processes require an anoxic environment and low redox potential. Batch reactors are a simple and useful tool for studying processes requiring a strict oxygen-free environment and have commonly been used to investigate microbial CBM production [4–6,17]. While batch studies are simple to use and fairly inexpensive systems for experimental purposes, their field-relevance may be limited due to potential microbial inhibition resulting from substrate depletion, gas accumulation, or byproduct inhibition [18–20] and the inability to account for important *in situ* conditions such as the effects of groundwater flow on abiotic and biotic processes. To facilitate the transition from small batch-scale experiments to field studies of biogenic CBM production and to understand whether the methane-enhancing strategies developed in batch systems can be applied *in situ*, it is

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important to scale-up laboratory work to better mimic the *in situ* environmental conditions [21]. Experiments using flow reactors can scale-up the reactor volume to investigate effects of flow and transport on the processes of interest and improve the development of strategies for field-scale experiments and technology applications. In addition to creating more field-relevant conditions, flow reactor systems can also reduce the effects of substrate depletion and accumulation of gases and other byproduct common in batch reactors [18,21]. However, to date, only two other microbial coal-to-methane conversion studies in continuous-flow systems have been published [22,23].

Challenges to flow reactor systems for biogenic coal-to-methane conversion studies include choosing an appropriate flow rate to mimic observed *in situ* conditions, reproducibility between systems, potentially higher construction and operating costs, and the requirement of maintaining an oxygen-free reactor environment. Constructing reactors and running experiments in an anaerobic chamber is one method for maintaining oxygen-free conditions. However, the difficulty of working within an anaerobic chamber, such as limitations on space and loss of dexterity, can make this option less appealing.

An upflow column reactor system was designed for laboratory bench-top studies of biogenic coal-to-methane enhancement strategies and can be run at varying flow rates while maintaining an oxygen-free environment to facilitate the desired low redox potential. Individual reactors in the system have sampling ports at the top and bottom for injection or sampling and a gas trap where the produced gases are separated and collected for sampling and analysis. The system has four reactors fed from the same liquid influent source, allowing for up to four replicates of the same treatment, or different amendments can be added to each reactor for different treatments, while still utilizing the same influent source. Previously published continuous-flow systems for studying biogenic coal-to-methane conversion had only one reactor which makes running controls problematic [22,23]. The reactor system described here was designed to study microbial CBM processes under flow conditions and compare to previously amended batch systems [5,24]. ^{13}C-labeled algae amendment was used to track the carbon source for methane production to determine whether the microorganisms were using the coal or the amendment itself to produce methane [25]. The purpose of this paper is to describe the column reactor system for studies of microbial coal-to-methane conversion processes that require an anoxic environment and to test methods for increasing the microbial coal-to-methane conversion that were previously investigated in batch systems [5,24,25]. Implementation of these methods in flow reactors allows systematic laboratory experimentation step to better understand *in situ* processes.

2. Materials & methods

2.1. Reactor description

The laboratory benchtop upflow column reactor system used in this study was designed to maintain an oxygen-free environment during flow conditions and capture produced gases to investigate the anoxic microbial processes involved in enhanced biogenic coal-to-methane conversion. A schematic of the system described is shown in Fig. 1.

Each reactor column held a total volume of 300 mL, and a total of four coal-packed reactor columns (only one is shown in Fig. 1) were used in the experiments presented here. Each reactor was equipped with sampling/injection septum ports for the influent (bottom; A) and effluent (top; B). The effluent from the reactor (C) discharged directly to the 55-mL gas trap (D) where produced gases could be collected via a septum-sealed sampling port (E) for analysis. The liquid effluent leaving the gas trap was collected in the effluent reservoir (F) for disposal. The four reactors were all fed from the same 15-liter carboy influent source (G) upstream of the peristaltic pumps. An 8-liter Tedlar® bag was placed inside the carboy to hold the liquid medium and provide an additional barrier to oxygen infiltration. Two peristaltic pumps (H) were used, and each pump provided flow to two reactors. Additional details of the reactor design and sampling are described in the Supplementary Section.

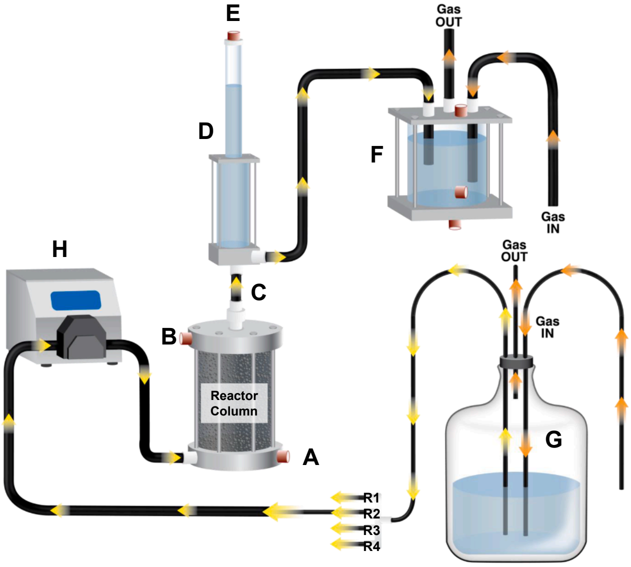

Fig. 1. A schematic of the reactor system showing all components of a single reactor system and designating the split from the influent carboy to three other identical setups: sampling/injection ports for (A) influent and (B) effluent; (C) reactor effluent; (D) 55-mL gas trap; (E) gas trap septum sampling port; (F) effluent reservoir; (G) 15-L influent carboy; and (H) peristaltic pumps.

2.2. Material sources and preparation

Coal cores from the U.S. Geological Survey field site near Birney, MT were collected in 2013 from the Flowers-Goodale (FG) coal bed (Eocene Tongue River Member of the Fort Union Formation) when three new wells were drilled and completed in this coal bed [26]. The 2-inch (~5 cm) diameter coal cores were cut to approximately 12-inch (~30.5 cm) lengths and stored at room temperature (21 ± 1 °C) in sealed polyvinyl chloride (PVC) tubes filled with FG formation water. Two months prior to the experiment, a FG core (381–382 ft, 116.1–116.4 m) was dried in the anaerobic chamber, crushed, sized to 2–4 mm, and stored in air tight glass containers. This size was chosen to reduce the coal fines that could cause blockage in the reactor plumbing. Coal density was estimated at 1.25 g/cm^3 by the water displacement method [27]. The measured density was slightly lower than the reported range of 1.44–1.54 g/cm^3 for FG coal [26]. Analysis of three FG coal samples from the same coring as the coal used in this study had an average heating value of 9079 Btu/lb (21,119 kJ/kg) on a moisture- and ash-free basis, and an average elemental composition was 49.54% C, 6.47% H, 37.67% O, 0.88% N, and 0.36% S (Table S1) [26]. The ultimate analysis and heating value indicated that the FG coal is sub-bituminous in rank.

The formation water used in this study was collected in May 2016 from the Birney field site FGM-13 well. Six-gallon plastic jugs were rinsed twice with formation water before being filled and stored at 4 °C upon return to the laboratory (Montana State University, Bozeman, MT). The microbial consortium used to inoculate the reactor system was collected from the FG-09 well in July 2016 using the subsurface microbial samplers (DMS) previously described by Barnhart et al. (2013). The slurry (formation water with high suspended solids) from the DMS (20 mL) was added to a serum bottle prepared with 5 g FG coal and 45 mL anoxic FG formation water in the field and transported to Montana State University (Bozeman, MT) where it was incubated at room temperature (21 ± 1 °C) in the dark for two months prior to inoculating the reactors.

The algal amendment used to enhance the coal-to-methane conversion was ^{13}C-labeled algal biomass grown and processed as previously described [25] with a total carbon content of 46.8% (% w/w) that was 95% labeled with ^{13}C. Seventy-five mg of ground algae were added to 5 mL of degassed, reduced (by sparging and addition of Na_2S reductant, as explained in detail in Section 2.3) FG formation water in an oxygen-free serum bottle. One mL of this prepared amendment was added to the designated amended reactors (15 mg algae per reactor per amendment dose).

2.3. Reactor preparation

Prior to conducting the microbial coal-to-methane conversion studies, all Norprene tubing and sampling port septa in the reactor system were replaced. The entire system was chemically disinfected by the following protocol. Two liters of four disinfection solutions were prepared: (1) 1% v/v bleach, (2) 1% w/v sodium chloride, (3) 0.25% w/v sodium thiosulfate, and (4) 70% v/v ethanol. Each solution was pumped first into the influent bag and then into the reactor system. The entire volume of each solution was pumped through the reactor system before the next was loaded into the influent bag. After all four disinfectant solutions were pumped through the reactor system, 3 L of 0.2-μm filtered FG formation water were loaded into the influent bag and distributed through the reactor systems to remove any residual disinfection solution. All fluids were drained from the reactors, gas traps, and effluent reservoirs. The disinfected reactor columns were removed from the system and moved to a microbiological safety cabinet. Prepared coal (190 g per reactor) was loaded into each reactor column, and the columns were reinstalled.

Eight liters of FG formation water were filtered with 0.2-μm bottle top filters. Resazurin (1 mg/L) was added as a redox indicator (pink in the presence of oxygen and colorless when redox potential is < -110 mV), and the water was sparged with 5% CO_2/95% N_2 gas (for pH control) for 18 h to reduce dissolved oxygen. The formation water was reduced with sulfide (1 mM as $Na_2S{\cdot}9H_2O$) to act as a reductant and ensure the necessary low redox potential. The prepared FG formation water was pumped into the Tedlar® bag inside the influent carboy. The FG water was left in the influent Tedlar® bag overnight to ensure the water maintained the desired low redox potential as indicated by Resazurin remaining colorless.

The reactor system was purged of atmospheric air, specifically targeting the removal of O_2 by purging with 5% CO_2:95% N_2. The entire reactor system was filled (all four reactors and gas traps) with the prepared FG formation water in the influent carboy by pumping at 6 mL/min. All four reactors were filled until the FG water just reached the effluent reservoir to ensure all gases were displaced by formation water from all components except the effluent reservoir.

Using the measured coal density (1.25 g/cm^3), total reactor volume (300 mL), and mass of coal in the reactor (190 g), the pore volume and effective porosities of the coal in the reactors were calculated to be 148 mL and 0.49, respectively. The experimental flow rate of 0.005 mL/min resulted in a reactor average linear velocity of 0.022 ft/day (0.0068 m/day) and average hydraulic residence time of 20.5 days. While the Birney field site FG average linear velocity was estimated at approximately 0.00644 ft/day (0.0020 m/day) [26] using the low porosity of 1% [28–30], the experimental flow rate was chosen due to the peristaltic pump limitations for minimum reproducible flow. Thus, the reactor linear velocity was approximately 3.5 times greater than the estimated Flowers-Goodale linear velocity at the Birney field site. However, other coal beds have average linear velocities similar to or exceeding the linear velocity of this reactor system [26]. For further discussion on the experimental flow rate chosen, see Supplementary Information.

The two system pumps were run at the experimental flow rate of 0.005 mL/min for 48 h prior to inoculation to ensure all gases were removed and redox potential was maintained as indicated by no change of color of the Resazurin oxygen indicator. All four reactors were inoculated through the sampling port at the bottom (influent) of the reactor with 15 mL of the microbial consortium containing slurry (~10% v:v) collected from the FG-09 well as described above. Reactors 1 (R1) and 2 (R2) were amended with 1 mL of the prepared algae amendment, resulting in 15 mg of algae per reactor and approximating a 0.1 g/L amendment concentration. Reactors 3 (R3) and 4 (R4) were not amended on day 0.

2.4. Gas analysis

Gas samples were collected from the gas trap for quantification and analysis using a gas tight syringe when at least 2 mL of gas had accumulated in the gas trap. Methane and carbon dioxide were quantified using an SRI Instruments (Torrance, CA, USA) Model 8601C GC equipped with a thermal conductivity detector (TCD) and interfaced with PeakSimple Chromatography software. A Supelco HayeSep-D packed stainless-steel column (6 feet × 1/8″ O.D., 1.83 m × 3.175 mm) was used with ultra-high purity helium carrier gas for separation using the following conditions: 1 mL manual injection, oven temperature 40 °C, TCD temperature 150 °C, and carrier gas pressure 8 psi. An Agilent 6890 GC 5973 electron impact ionization mass selective detector (Agilent Technologies, Palo Alto, CA, USA) interfaced with Agilent Enhanced ChemStation software and operated in scan mode was used to measure isotope ratios of $^{13}CH_4$:$^{12}CH_4$ and $^{13}CO_2$:$^{12}CO_2$. A GS-Carbonplot column (60 m × 0.320 mm i.d. × 1.50 μm film thickness) was used for analysis. The following parameters were used: 500 μL manual split ratio 30:1 injection, constant flow at 1 mL/min, injector temperature of 185 °C, interface 60 °C, and scan range m/z 2–100. Ultra-high purity helium was the carrier gas. The % $^{13}CH_4$ and $^{13}CO_2$ were determined as previously described [25], and the gas chromotography0mass spectrometry (GC–MS) measurements and deconvolution calculations are shown in detail in the Supplementary Information Section 6.

2.5. Reactor breakdown and sampling

The reactor system ran for 172 days, or 8.4 retention times, before destructively sampling R1 for desorption studies. The reactor column was first isolated and removed from the rest of the system. The liquid contents were pushed out of the column into a Tedlar® bag by purging with nitrogen gas from the top of the column. The liquid contents were placed on a rocker table for 24 h to allow for gas dissolution. The resulting gas was collected and analyzed. The inlet to the column was plugged and a second Tedlar® bag was attached to the top of the column for 7 h to collect desorbed gases. Two vacuum desorption tests were run using an ISCO D1000 pump (1000 mL) (Teledyne, Nebraska, USA). The first test applied a vacuum of −7 psi on the reactor column. The reactor column was isolated under vacuum and the gases collected in the pump were collected in a third Tedlar® gas sampling bag. A second vacuum of −10 psi was drawn on the reactor column and maintained for 18 h. These gases were collected in a fourth gas sampling bag. All gases were analyzed analogously to the gas samples collected from the gas trap during the reactor incubation period.

3. Results and discussion

3.1. Gas production

The amendment schedule and observation of first produced gas are summarized in Table 1. On day 0, R1 and R2 were amended with 1 mL of the prepared ^{13}C-labeled algae amendment. The mass of amendment added per reactor was 15 mg, resulting in an approximate concentration of 0.1 g/L previously shown to enhance biogenic coal-to-methane conversion while minimizing microbial community changes [5]. R3 and R4 were not amended on day 0. Gas was first observed in the gas trap of

Table 1
Summary of amendment strategy and first observed gas for all reactors.

Reactor	Initial algae amendment (mg)	Day 61 amendment (mg)	First gas observed (day)
R1	15	15	27
R2	15	0	33
R3	0	15	142
R4	0	0	n/a

Table 2
Summary of gas production for the three gas-producing reactors during the period after each amendment addition.

Reactor	Days 0–61		Days 61–172	
	Total CH_4 (% as $^{13}CH_4$) µmol (%)	Total CO_2 (% as $^{13}CO_2$) µmol (%)	Total CH_4 (% as $^{13}CH_4$) µmol (%)	Total CO_2 (% as $^{13}CO_2$) µmol (%)
R1	740.3 (2.6%)	62.0 (1.0%)	972.3 (6.1%)	66.6 (1.5%)
R2	463.5 (2.6%)	41.2 (1.0%)	1022.3 (2.6%)	71.8 (1.0%)
R3	0	0	278.9 (3.9%)	25.1 (1.2%)

R1 on day 27 and R2 on day 33. On day 61, R1 was re-amended with 1 mL of the prepared algae amendment. R3, which was initially unamended and had not yet produced any measurable gas, was also amended with 1 mL of the prepared algae amendment. Continued gas production was observed for both R1 and R2 (initially amended reactors) for the duration of the study. Gas production was first observed in R3 on day 142, 81 days after algae amendment addition. R4, which was never amended, produced no measurable gas at any point during the 172-day study.

The methane and carbon dioxide production curves (Fig. 2) reflect measurements of gases collected from the gas trap during the 172-day experiment. These production curves do not account for dissolved methane and CO_2, dissolved inorganic carbon (DIC), or gases sorbed to the coal. During the 172-day study, R1 produced a total of 1712.6 µmol of CH_4 and 128.6 µmol of gaseous CO_2; ^{13}C-label gases were measured at 4.6% $^{13}CH_4$ and 1.2% $^{13}CO_2$. R2 produced a total of 1485.8 µmol of CH_4 and 113.0 µmol of CO_2. Of these total produced gases 2.6% was measured as $^{13}CH_4$ and 1.0% as $^{13}CO_2$. R3 produced 278.9 µmol of CH_4 and 25.1 µmol of gaseous CO_2 with initial gas production observed on day 142; 3.9% of the methane was $^{13}CH_4$ and 1.2% of the CO_2 was

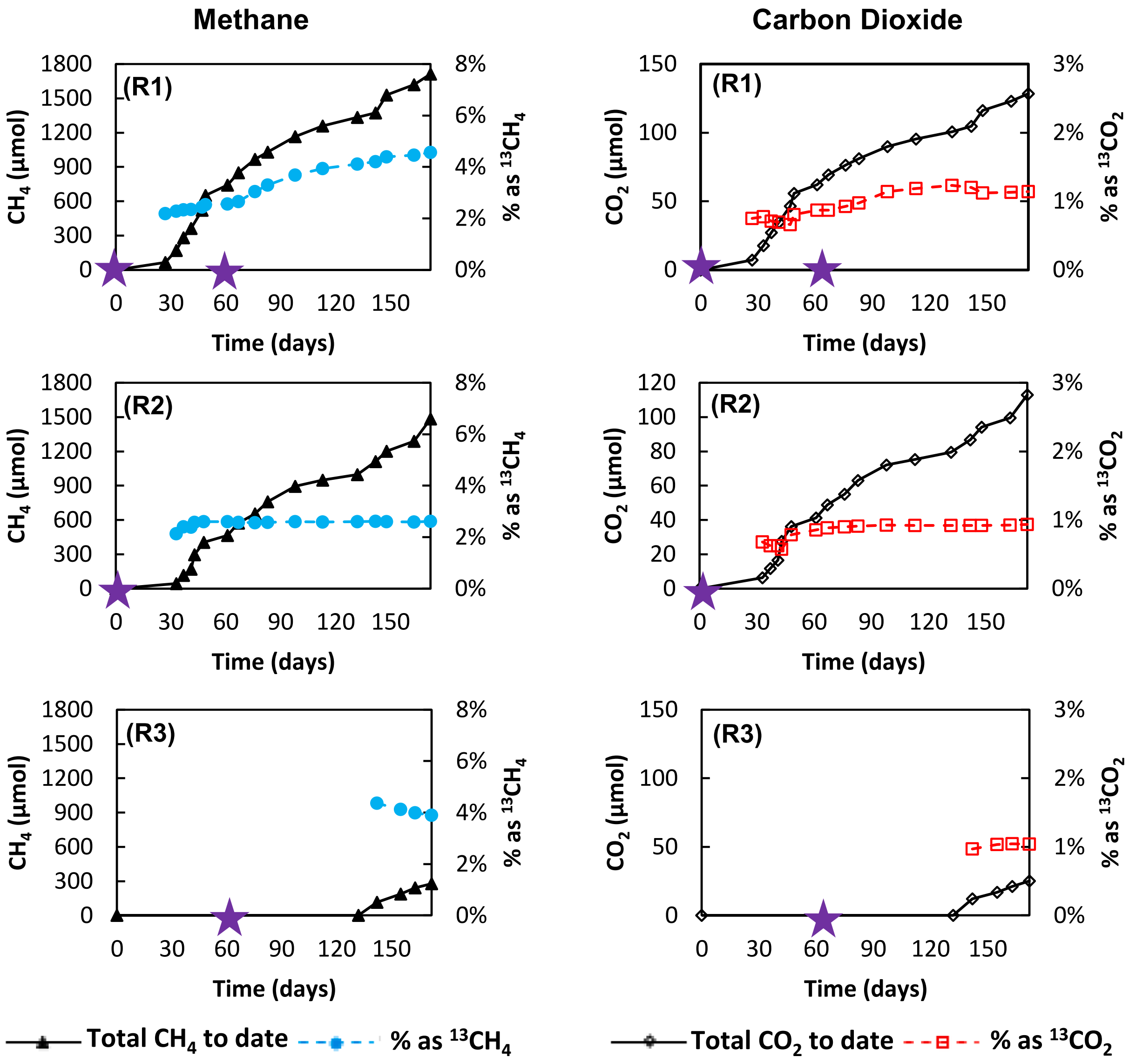

Fig. 2. Total CH_4 and CO_2 production over time for R1, R2, and R3 (solid line) and cumulative % as ^{13}C-labeled. Time of algae amendment is designated by stars (★). Note: Results for R4 are not shown because this reactor produced no measurable gas during the 172-day study.

$^{13}CO_2$. R4 produced no gases during the 172-day study period and is not shown in Fig. 2 or other following figures and tables.

Table 2 summarizes the gas production in the three producing reactors. During the first 61 days, neither of the unamended reactors (R3 and R4) produced measurable gases. In contrast, R1 produced 740.3 μmol CH_4 and 62.0 μmol of CO_2, and R2 produced 463.5 μmol of CH_4 and 41.2 μmol of CO_2. Both amended reactors produced 2.6% of total methane as labeled $^{13}CH_4$. The CO_2 as $^{13}CO_2$ detected in both reactors was approximately 1.0%, near the natural abundance of ^{13}C (1.1%). By day 83, R2 had produced a total of 760.5 μmol of CH_4 and 63.1 μmol of gaseous CO_2, reaching the 61-day totals for R1. These differences in gas production between the amended reactors could be due to differences between the columns, such as preferential flow paths, minor variation in flow rates between pumps (though not observed to an appreciable amount over the duration of the study), or possible small leaks. However, it is also possible that the differences in gas production observed in R1 and R2 were due to variations in microbial community structure, resulting in different metabolic rates, or heterogeneity of the coal in the reactor.

After day 61, when R1 was re-amended with a second algae addition and R3 was amended with a first algae addition, until the end of the 172-day study, the four reactor systems represented different treatments, and thus, no replicates existed. Between day 61 and day 172, R1 produced an additional 972.3 μmol of CH_4 (6.1% as $^{13}CH_4$) and 66.6 μmol of CO_2 (1.5% as $^{13}CO_2$). In contrast, R2, which was only amended on day 0, produced an additional 1022.3 μmol of CH_4 (2.6% as $^{13}CH_4$) and 71.8 μmol of CO_2 (1.0% as $^{13}CO_2$). Between day 61 and day 172, R3 produced measurable gas 81 days after amendment on day 61. R3 produced 278.9 μmol of CH_4 (3.9% was $^{13}CH_4$) and 25.1 μmol of CO_2 (1.2% as $^{13}CO_2$). As stated previously, R4 did not produce any measurable gas throughout the 172-day study.

While R1 produced the greatest sum of CH_4 and CO_2 gases of all the reactors, R2 produced 50 μmol more CH_4 than R1 during the last 111 days of the study, even though R1 received twice the amendment of R2. This suggests that the amendment serves to "jump-start" the microbial processes leading to increased rates of coal-to-methane conversion. Additional amendment, at least on the time scale used in this study, may be unnecessary for increasing coal-to-methane conversion once the "jump-start" of microbial coal-to-methane processes has occurred.

On a moles-produced basis, the production ratio of CH_4 to CO_2 differed between the amended reactors: R1 produced 13.3 times, R2 produced 13.1 times, and R3 produce 11.1 times more CH_4 than CO_2. This observation, combined with the higher fraction of ^{13}C in the produced gases in R1, suggests that the utilization of the algal amendment itself results in a slightly higher CH_4 to CO_2 ratio than coal-to-methane conversion processes.

3.2. Analysis of substrates for gas production

The total amount of carbon produced as CH_4 and CO_2 (C_{out}) was compared to the total amount of carbon added to the system in the form of algal amendment (C_{in}). Table 3a summarizes the comparison of C_{out}/C_{in} for days 61 and 172. A C_{out}/C_{in} ratio of greater than 1 indicates definitive conversion of coal to CH_4 and CO_2 beyond complete conversion of amendment carbon to CH_4 and CO_2 (see [5] for extensive analysis details).

In this study, R3, which was amended only on day 61 and did not begin gas production until day 132, did not produce enough CH_4 and CO_2 gases to exceed the gas production potential of amendment conversion alone. In contrast, the C_{out}/C_{in} ratio for R1 was greater than 1 on both days 61 and 172, indicating that R1 produced more carbon in the form of methane and CO_2 than could have been attributed to amendment conversion alone. Thus, coal-to-methane conversion in this column indisputably occurred. The C_{out}/C_{in} for R2 was less than 1 on day 61 of the experiment, and therefore it cannot be concluded with certainty that coal-to-methane conversion occurred in this reactor during the first 61 days. However, on day 172, C_{out}/C_{in} for R2 was 2.73, the highest of all three gas producing reactors for both time points assessed. This supports coal-to-methane conversion in R2 and indicates that at least 72% of the total gases produced were certainly from the degradation of coal and not from amendment conversion.

Table 3
Comparison of (a) total carbon produced as CH_4 and CO_2 gases as a fraction of the carbon content of the algae amendment and (b) carbon-13 produced as $^{13}CH_4$ and $^{13}CO_2$ gases as a fraction of the carbon-13 content of the algae amendment.

(a)	Day 61			Day 172		
Reactor	1	2	3	1	2	3
CH_4 (μmol C)	740	464	0	1713	1486	279
CO_2 (μmol C)	62	41	0	129	113	25
Algae (μmol C)	585	585	0	1170	585	585
C_{out}/C_{in}	1.37	0.86	n.a.	1.57	2.73	0.52

(b)	Day 61			Day 172		
Reactor	1	2	3	1	2	3
CH_4 (μmol C)	19.0	12.0	0	78.2	38.8	10.9
CO_2 (μmol C)	0.5	0.4	0	1.5	1.1	0.3
Algae (μmol C)	556	556	0	1112	556	556
$^{13}C_{out}/^{13}C_{in}$	0.034	0.022	n.a.	0.070	0.070	0.020

While a direct comparison of C_{out}/C_{in} is useful for assessing the possible carbon source for gas production, this method assumes complete conversion of the algae amendment to CH_4 or CO_2. It should be noted that because not all the algae amendment was converted to CH_4 and CO_2, this method underestimates the contribution of coal conversion for gas production.

A second method was used to analyze the substrates for gas production comparing the amount of ^{13}C produced as $^{13}CH_4$ and $^{13}CO_2$ produced relative to the ^{13}C added as amendment. These comparisons are summarized in Table 3b, and $^{13}C_{out}/^{13}C_{in}$ was much less than 1, indicating that only a small amount of amendment ($\leq 7\%$) was converted to measurable gas in R1, R2, and R3. In the amended reactors for most time points, $^{13}CH_4$ and $^{13}CO_2$ were measured at higher than the expected environmental concentration of 1.1%, indicating that some of the produced gases were the result of the conversion of the amendment itself. However, due to the low $^{13}C_{out}/^{13}C_{in}$ ratios, it is apparent that most gas production in these systems was due to coal conversion. However, any increase in ^{13}C gases indicates that the microbial conversion of ^{13}C-labeled amendment to methane and CO_2 is measurable. While it was apparent that CH_4 production was higher with the addition of algal amendment, the low amounts of $^{13}CH_4$ and $^{13}CO_2$ show that coal, not algal amendment, is the primary carbon source for gas production in these coal reactor systems, supporting the hypothesis that the amendment plays an important role in enhancing the microbial coal-to-methane conversion.

3.3. Desorption analysis

On day 172, reactor R1 was taken off-line for gas desorption analysis. Fig. 3 shows the amounts and % ^{13}C-label for CH_4 and CO_2 recovered from (i) the gas trap during the 172-day experiment, (ii) the gases recovered from the reactor fluids, and (iii) from three desorption steps. R1 produced 1712.6 μmol of CH_4 captured in the gas trap during the 172-day study (Fig. 2), which was 74.2% of the total methane recovered from the system. An additional 78.5 μmol of CH_4 was detected in the reactor fluids at the end of the experiment. The initial desorption, lasting for 6.5 h with no vacuum applied (0 psi desorption in Fig. 3), resulted in a recovery of 9.6 μmol of CH_4, which was equivalent to about 0.4% of the total methane recovered. The first vacuum desorption

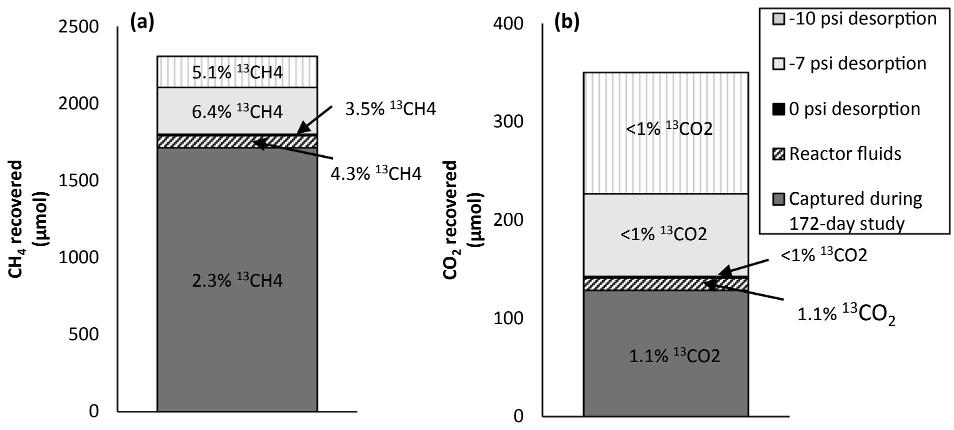

Fig. 3. (a) Methane and (b) carbon dioxide in R1 recovered during the experiment and during desorption treatment. Percentages of the total gas measured as ^{13}C labeled gases are shown for each phase. (Note: the 0 psi desorption gases were so small relative to the other gases that they appear only as a line between the −7 psi desorption and reactor fluids in this graph.)

step (−7 psi) desorbed 304.4 µmol of CH_4, while the second vacuum desorption (−10 psi for 18 h) desorbed an additional 201.5 µmol of CH_4, 13.2 and 8.7%, respectively, of the total methane recovered (Fig. 3a). The $^{13}CH_4$ contribution of each fraction is shown in Fig. 3a as a percentage of the total CH_4.

Reactor R1 produced 128.6 µmol of CO_2 recoverable in the gas trap during the 172-day study, which was 36.7% of the total CO_2 recovered. An additional 12.3 µmol of CO_2 (3.5% of the total CO_2) gas were detected in the reactor fluids at the end of the experiment. The 6.5-hour, 0 psi vacuum desorption resulted in an additional 1.81 µmol of CO_2 (0.5% of the total CO_2). During the −7 psi and −10 psi desorption phases, 83.9 and 123.4 µmol of CO_2 were recovered, respectively, and these amounts accounted for 24 and 35.3%, respectively, of the total CO_2 retrieved in this study. Almost 60% of the total CO_2 retrieved from the system was the result of the two vacuum desorption phases. Fig. 3b shows these values and the percentage of each as $^{13}CO_2$. All retrieved CO_2 fractions were near natural abundance ^{13}C (1.1%), which is similar to what was observed in all reactors during the course of the 172-day study.

More CH_4 was retrieved on a molar basis than CO_2 (Table 2, Fig. 3). Most of the CH_4 was produced and removed while fluids flowed through the reactors (~75%) whereas just over a third of the total CO_2 was detected in the gas traps. While the fraction of methane desorbed decreased during the two sequential vacuum desorption phases, the amount of CO_2 desorbed increased. It has been shown previously that CO_2 has a higher affinity for adsorption to coal than CH_4 [31,32], and methods for enhanced CBM recovery by injection CO_2 have been previously utilized [33–36].

The amounts of produced CH_4 and CO_2 reported for this study reflect only what could be collected as gas. Some of the produced methane and CO_2 was dissolved in the reactor fluids and not captured or quantified in this study. Due to the speciation of dissolved inorganic carbon (DIC) (CO_2, H_2CO_3, HCO_3^-, CO_3^{2-}), the production of CO_2 during microbial processes could also cause shifts in the concentrations of the other DIC species. DIC was not measured in this study, and therefore, the amounts of CO_2 reported do not represent the production or utilization of the total inorganic carbon present in the system.

Busch et al. observed that not all methane and CO_2 could be desorbed from Wyodak coal during adsorption and desorption studies [37]. Subbituminous Wyodak coal is part of the Tongue River Member of the Fort Union Formation in the Powder River Basin and similar to the Flowers-Goodale coal used in this study (Supplementary Table S1) [26,38]. Thus, it is likely that in the reactor system described here, some methane and CO_2 remained sorbed to the coal even after vacuum desorption and therefore was not quantifiable.

4. Summary & conclusions

While the reactor system described here was used to study biogenic coal-to-methane enhancement strategies, it could also be applied for studies of any biogenic subsurface process, particularly those requiring strict redox potential or gas-infiltration control or those producing gases or containing volatile compounds. The total system contains four individual reactors, each with a total volume of 300 mL that can be packed with any desired porous medium (coal in the studies described here) for studies of anaerobic subsurface processes. The single influent source for the four reactors allows for treating all reactors similarly but reactors can also be run under different hydraulic regimes (e.g. though the use of different pumps or flow rates) or different amendment strategies (as described here) to increase the understanding of subsurface processes. By providing an increased volume relative to batch experiments, introducing flow, and allowing multiple reactors to be run in parallel, the system described here is a useful tool for testing strategies developed in batch systems prior to implementing more expensive meso-scale or pilot-scale demonstrations. This scale-up of small laboratory experiments to larger systems prior to field trials has proven to be a successful strategy to decrease costs while facilitating successful implementation of a biomineralization sealing technology, developed in the laboratory before transferring these methods into the field to seal fractures around a well in a leaking gas well scenario [39–42].

The experiments described here were designed to examine strategies for enhancing microbial coal-to-methane conversion and applied methods previously developed in batch studies to a more field-relevant continuous-flow system. Amendment strategies developed in batch systems [5] were applied to enhance the biogenic coal-to-methane conversion. Re-amendment strategies were scaled up for further investigation and to determine applicability of re-amending coal beds in future field studies [24]. Additionally, ^{13}C-labeled algae amendment, previously used in batch experiments to trace the origins of enhanced methane production [25], was utilized to determine the viability of this method in flow systems, demonstrating its potential use in the development of analyses for use in *in situ* applications. From these experiments, it can be concluded that:

1) As in previous batch studies [5,24], the addition of algal amendment to flow reactors results in an increase in CH_4 gas production, suggesting that algal amendment could also be used to enhance the biogenic coal-to-methane conversion *in situ*.
2) More CH_4 and CO_2 gases were produced in the initially amended reactors (R1 and R2) than could be attributed to amendment-to-gas conversion alone, indicating coal-to-gas conversion was occurring. R3 (initially unamended and amended on day 61) began producing measurable gas later in the study and was still producing gas when the experiment was terminated. It is hypothesized that, given enough time, R3 would also have produced enough gas to exceed the amount expected for full amendment-to-gas conversion.
3) ^{13}C gas analyses suggested that only a very small amount ($\leq$7%) of the labelled algal amendment was converted to CH_4 and CO_2, and

these observations support the conclusion that the vast majority of the gases produced during the experimental duration were due to amendment-enhanced microbial coal-to-methane conversion.

4) The desorption studies showed that appreciable amounts of produced CH_4 and CO_2 can be sorbed to the coal, and thus, to accurately measure the amount of biogenic CH_4 produced in laboratory reactors and in the subsurface environment, sorption of produced gases cannot be ignored. More of the recoverable CO_2 was sorbed to the coal (~60%) than captured as gas during the flow phase of the experiment, while only ~25% of the recoverable CH_4 was sorbed to the coal. This result supports previous research indicating that CO_2 has a higher affinity for sorption to coal than CH_4 [43–45].
5) While amendment of coal systems in previous batch studies increased the rates of production and total methane produced in the initial 60–80 days, little methane production was observed with longer incubations [5,24]. The flow reactors here continued to produce gases for the entire 172-day study without indication of slowing production. Batch systems were likely inhibited due to substrate depletion or accumulation of byproducts, resulting in cessation of growth and methane production [18,20]; by amending flow reactor systems, these potential issues were circumvented and a more realistic field-like scenario indicated that methane production does not cease as early as indicated during the previous batch studies [5,24].

This study and potential future studies utilizing the flow reactor system described here are useful in designing a potential field-scale application of enhanced coal-to-methane conversion in subsurface coal beds.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fuel.2019.115905.

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