

Biogenic Coal-to-Methane Conversion Efficiency Decreases after Repeated Organic Amendment

Katherine J. Davis,^{*,†,‡,§,||} Elliott P. Barnhart,^{§,†} Matthew W. Fields,^{†,||} and Robin Gerlach^{*,†,‡,§,||}

[†]Center for Biofilm Engineering, Montana State University, Bozeman, Montana 59717, United States

[‡]Department of Chemical and Biological Engineering, Montana State University, Bozeman, Montana 59717, United States

[§]U.S. Geological Survey, Helena, Montana 59601, United States

^{||}Department of Microbiology and Immunology, Montana State University, Bozeman, Montana 59717, United States

Supporting Information

ABSTRACT: Addition of organic amendments to coal-containing systems can increase the rate and extent of biogenic methane production for 60–80 days before production slows or stops. Understanding the effect of repeated amendment additions on the rate and extent of enhanced coal-dependent methane production is important if biological coal-to-methane conversion is to be enhanced on a commercial scale. Microalgal biomass was added at a concentration of 0.1 g/L to microcosms with and without coal on days 0, 76, and 117. Rates of methane production were enhanced after the initial amendment but coal-containing treatments produced successively decreasing amounts of methane with each amendment. During the first amendment period, 113% of carbon added as amendment was recovered as methane, whereas in the second and third amendment periods, 39% and 32% of carbon added as amendment was recovered as methane, respectively. Additionally, algae-amended coal treatments produced ~38% more methane than unamended coal treatments and ~180% more methane than amended coal-free treatments after one amendment. However, a second amendment addition resulted in only an ~25% increase in methane production for coal versus noncoal treatments and a third amendment addition resulted in similar methane production in both coal and noncoal treatments. Successive amendment additions appeared to result in a shift from coal-to-methane conversion to amendment-to-methane conversion. The reported results indicate that a better understanding is needed of the potential impacts and efficiencies of repeated stimulation for enhanced coal-to-methane conversion.

1. INTRODUCTION

Natural gas found in many of the world's coal reserves is commonly referred to as coalbed methane (CBM). It can be formed by thermogenic or biogenic processes and is commercially produced in some regions. Unlike thermogenic CBM, formed by heat and pressure on geologic time scales, biogenic CBM is formed by microbial processes that convert coal to methane and occur on shorter time scales, thus providing an opportunity for enhancement strategies for increased production.^{1–4}

The Powder River Basin (PRB) in Montana and Wyoming is the largest known U.S. coal reserve and a site of commercial CBM collection. Previous studies have shown that the methane collected in the PRB is almost exclusively of biogenic origin.^{5–7} Commercial collection of biogenic CBM often exceeds the rate of microbial methane production; therefore most production wells have short lifespans (7–10 years).^{8,9} When wells no longer produce commercially viable gas quantities to justify continued pumping, wells are often abandoned, leaving coal and valuable infrastructure in place. In the Montana portion of the PRB alone, there were 1046 shut-in or abandoned wells and an additional 597 with expired permits in 2015. Thus, the PRB is potentially a lucrative environment for application of strategies to increase the rates and amounts of microbially produced methane utilizing the already in place infrastructure for collection.

Previous studies have tested methods for enhancing biogenic coal-to-methane conversion in the laboratory, demonstrating enhanced biogenic methane production from coal using a variety of inorganic and organic nutrient additions^{7,10,11} and coal oxidation pretreatments.^{12–15} However, most of these methods could be too costly to implement *in situ* due to the investment required for production, transport, and application of the amendment. The use of yeast extract or tryptic soy broth, which are commonly used nutrients for the cultivation of diverse microorganisms, have been implemented as methane-enhancing amendment strategies to reduce the necessity of determining the exact “recipe” of nutrients needed, making it a potentially less expensive addition.^{4,16–18} However, the application of even the lowest-cost commercial-grade yeast extract (\$8.50/kg) to *in situ* coalbeds could be prohibitively expensive compared to use in *ex situ* bioreactors, and less-expensive alternatives need to be investigated.^{16,19,20}

In an effort to address potential costs associated with enhanced CBM production by amendment addition, the use of algal extract as an alternative amendment for increasing microbial coal-to-methane conversion has been introduced.¹⁸ Microalgae can grow in CBM production water ponds, and a *Neosporangiococcus* spp. isolated from a production water pond

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has been shown to accumulate lipids.²¹ In addition to lipids for potential biofuel production, microalgae have also been grown commercially for production of nutritional supplements, food additives, fertilizer, aquaculture feedstock, and other high-value chemicals.²² Potential amendment transportation costs could be reduced by growing algae on or near the CBM collection site, while providing alternative economic benefits in the form of biofuels or other value-added products.^{16,18} Thus, the costs associated with CBM enhancement could be reduced by using microalgal amendments. The methane enhancement effect of organic amendments (microalgae, cyanobacteria, and yeast cells and extract) was investigated at two concentrations and demonstrated similar methane production with all amendments.¹⁷

Most CBM enhancement studies have been performed using batch systems, and it has been observed that methane production rates and amounts can be increased for a period up to 80 days before methane production slows down or ceases completely in nearly all studies.^{11,14,17,23,24} The purpose of this study was to determine whether methane production can be increased repeatedly when systems are reamended after enhanced methane production has slowed down. The studies presented here focus on microalgal amendment at a low amendment concentration (0.1 g/L) which was shown to better maintain the coal-to-methane converting microbial community structure compared to higher amendment concentrations.¹⁷

For a long-term application of enhanced CBM strategies *in situ*, it is necessary to ascertain the potential benefits and challenges arising from repeated amendments of coal systems and the potential economic impacts on methane enhancement strategies. The results of this study build on previous work¹⁷ and contribute toward a better understanding of the ability of complex organic amendments to increase microbial production of coalbed methane with repeated amendment addition.

2. MATERIALS AND METHODS

2.1. Site and Sample Collection. The sampling site, located near Birney, Montana, in the PRB, was thoroughly described previously.²⁵ Water from the Flowers-Goodale (FG) coal bed was pumped and retrieved in May 2016 from the FGP-13 well. Two well volumes were pumped prior to formation water collection. Plastic jugs (6-gal volume) were rinsed twice with formation water before being filled and stored at 4 °C prior to microcosm set up. Formation water characterization for the FG coal bed has been previously reported.²⁵ Coal cores were collected during the July 2013 drilling of the FG monitoring wells (FGM-13 and FGP-13). Twelve-inch sections of the 2-in. diameter cores were placed in polyvinyl chloride (PVC) tubes that were filled completely with formation water from the FG-11 well and sealed with flexible rubber caps. Microbes were collected from FGM-13 in September 2015 using microbial samplers similar to those previously described.²⁶ Liquid from the FGM-13 sampler was added to 3 serum bottles prepared with 5 g of FG coal and 45 mL of anoxic FG formation water before being incubated at room temperature (21 ± 1 °C) in the dark. The three serum bottles were combined prior to use as inoculum for the described studies.

2.2. Amendment Growth and Preparation. A *Chlorella* microalga species, strain SLA-04, was grown in photobioreactors for biomass accumulation as previously described.¹⁷ The biomass was lyophilized and stored at -20 °C before being used in microcosm studies. The algal amendment was ground to a fine powder with a ceramic mortar and pestle. A 1g/L stock solution (dry w/v) was prepared in 0.2 μ m filtered and degassed FG formation water and sealed in oxygen-free serum bottles using anoxic methods.

2.3. Microcosm Setup and Reamendment. Microcosms were set up anoxically in 26 mL Balch tubes with butyl rubber stoppers and

aluminum crimp seals. The FG coal core (depth 374–376 feet [~ 114 m]) was opened in an anaerobic glovebag, and the core material was dried, crushed, and sieved to an effective size range (0.85–2.0 mm). The prepared coal was stored in oxygen-free glass bottles until microcosms were established. Borosilicate glass beads (1 mm) were autoclaved and used in lieu of coal to provide a carbon-free solid substrate as appropriate controls. Each Balch tube received 1 g of prepared coal or glass beads (GB). The formation water was filtered with 0.2 μ m bottle top filters and sparged for 5 h with an oxygen-free gas mixture (5% CO₂/95% N₂). Sodium sulfide (1 mM as Na₂S·9H₂O) was used as an oxygen scavenger to ensure low redox conditions. All amended treatments received 1 mL of the prepared amendment concentrate. The initial pH of the degassed and reduced formation water was 7.6, similar to what has been observed in the FG formation water.²⁵ All inoculated treatments received 1 mL of inoculum from the combined serum bottles of the previously enriched FG microbial consortium. The initial total liquid volume of all microcosms was 10 mL. Inoculum slurry was frozen and lyophilized for carbon analysis (5 mL). All microcosms were incubated in the dark at room temperature (21 ± 1 °C), approximately 3 °C warmer than the *in situ* (~ 18 °C) temperature.²⁵ Headspace gas was sampled and analyzed approximately every 2 weeks.

After 76 and 117 days, appropriate treatments were reamended as shown in Figure 1. The amendment was prepared identically to the

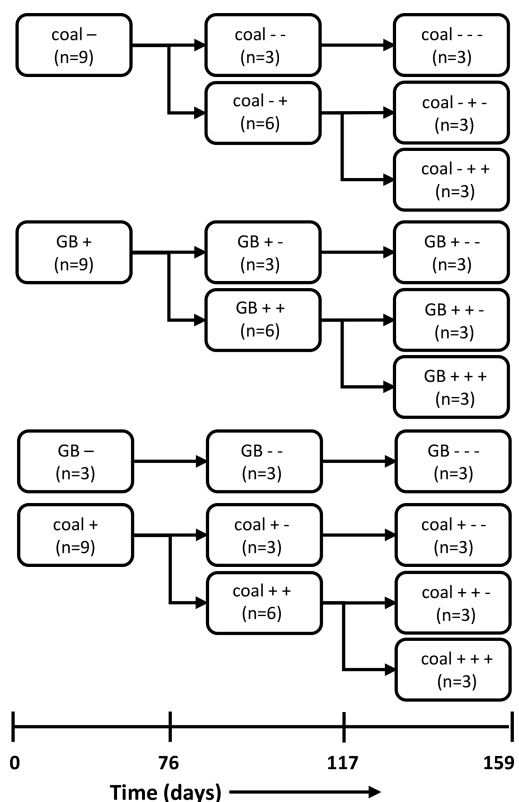


Figure 1. Experimental treatments (FG coal and glass bead (GB)) for each amendment time period. Amendment addition is indicated for each amendment time as “+” (addition of algal amendment in FG formation water) or “-” (addition of FG formation water only). The number of replicates is indicated as “n=#”.

initial microcosm set up and 1 mL of prepared amendment was added to each of the reamended treatments. Treatments not reamended with additional algae amendment received 1 mL of degassed FG formation water to account for volume changes and potential nutrient addition from the formation water added with the algal amendment.

2.4. Gas Analysis. Methane and carbon dioxide were monitored using an SRI Instruments (Torrance, CA, USA) Model 8610C gas chromatograph (GC) equipped with a thermal conductivity detector

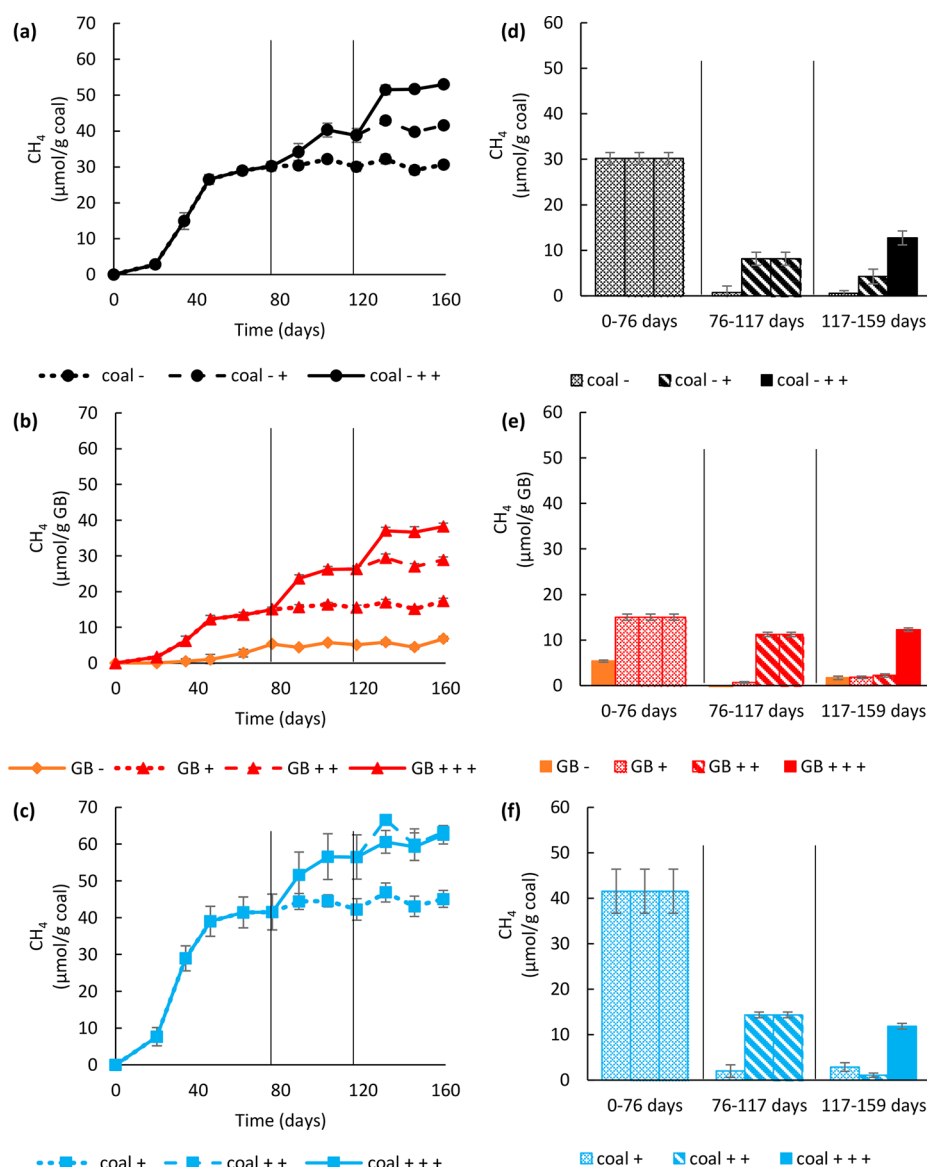


Figure 2. Methane production shown as a time series (a–c) and as production per amendment period (d–f) for initially unamended coal (a, d), glass bead (b, e), and initially amended coal (c, f) treatments. Error bars represent one standard deviation of 3–9 replicates (see Figure 1). Vertical lines indicate time of reamendment (where appropriate). Amendment addition for each of the three amendment periods are indicated with “+” for addition and “–” for no addition.

(TCD) interfaced with PeakSimple Chromatography software. A Supelco HaySep-D packed stainless-steel column (6 feet × 1/8 in. O.D.) was used with ultrahigh purity helium carrier gas for separation using the following conditions: manual injection, oven temperature 40 °C, TCD temperature 150 °C, and carrier gas pressure 8 psi. Gas (1 mL) was collected from the microcosm headspace for GC injection. To prevent creating a negative pressure in the tubes, 1 mL of anoxic 5% CO₂/95% N₂ gas was injected to replace the sample volume removed. After the initial 20-day incubation period, reactors were sampled approximately every 2 weeks for gas analysis for the duration of the 159-day study.

2.5. Carbon Analysis. Dissolved inorganic carbon (DIC) and nonpurgeable organic carbon (NPOC) were measured using a Formacs^{HT/TN} instrument (Skalar, Inc., Breda, The Netherlands). Samples were centrifuged for 10 min at 4700 rpm and 4 °C and filtered through 0.7 μm GD/X filters. NPOC samples were acidified with 3 N HCl to decrease the pH below 2 and purged with oxygen gas for 180 s prior to analysis. DIC and NPOC measurements were made every 4 weeks and on each reamendment day. Because these analyses required destructive sampling of reactors, only one reactor per treatment was sampled for

each sampling date except at the end of the experiment on day 176 when DIC and NPOC measurements were taken by destructively sampling the three remaining samples of each treatment. Carbon, nitrogen, hydrogen, and sulfur analysis were performed on the dry algal biomass and lyophilized inoculum using a Thermo Scientific (Waltham, MA, USA) CE Elantech Flash 2000 CHNS-O Analyzer.

2.6. Statistical Analysis. A two-way ANOVA was fit to the amount of methane produced and the maximum methane production rate for each amendment period with a factor for treatment (coal or GB) and a factor for the amendment condition over time (i.e., +, +, +, +, +, etc.). The ANOVA and Tukey tests of the interaction were performed using statistical software R.²⁷ A *p*-value < 0.05 determined statistical significance.

3. RESULTS

3.1. Methane Production. All treatments, regardless of solid substrate (coal, glass beads [GB]) or algal amendment regimen, produced methane during the 159-day study (Figure 2a–c), and the largest amount of methane was produced during

Table 1. Rates of Methane Production ($\pm$ One Standard Deviation of 3–9 Replicates, Depending on the Period) Calculated for Each Treatment As an Average for Each Time Period between Gas Analyses^a

treatment	maximum methane production rate ($\mu\text{mol CH}_4/(\text{g coal d})$)		
	days 0–76	days 76–117	days 117–159
coal – – –	0.97 $\pm$ 0.21 ($n = 9$)	0.12 $\pm$ 0.12 ($n = 3$)	0.15 $\pm$ 0.05 ($n = 3$)
coal – + –		0.44 $\pm$ 0.08 ($n = 6$)	0.40 $\pm$ 0.12 ($n = 3$)
coal – + +			0.80 $\pm$ 0.14 ($n = 3$)
GB + – –	0.50 $\pm$ 0.07 ($n = 9$)	0.06 $\pm$ 0.02 ($n = 3$)	0.16 $\pm$ 0.01 ($n = 3$)
GB + + –		0.66 $\pm$ 0.03 ($n = 6$)	0.20 $\pm$ 0.03 ($n = 3$)
GB + + +			0.79 $\pm$ 0.01 ($n = 3$)
GB – – –	0.18 $\pm$ 0.06 ($n = 3$)	0.10 $\pm$ 0.01 ($n = 3$)	0.05 $\pm$ 0.02 ($n = 3$)
coal + – –	1.52 $\pm$ 0.13 ($n = 9$)	0.32 $\pm$ 0.02 ($n = 3$)	0.33 $\pm$ 0.06 ($n = 3$)
coal + + –		0.73 $\pm$ 0.10 ($n = 6$)	0.31 $\pm$ 0.06 ($n = 3$)
coal + + +			0.70 $\pm$ 0.08 ($n = 3$)

^aMaximum methane production rates for the 10 inoculated treatments for each amendment period are shown.

the first amendment period (days 0–76). During the second and third amendment periods (days 76–117 and 117–159, respectively), less methane was produced than during the initial amendment period, but more methane was always produced by treatments that were reamended compared to treatments that were not reamended (Figure 2d–f) ($p < 0.05$). Tukey comparison groupings are shown in Supplementary Table S1. After the initial amendment period, treatments that were not reamended produced little additional methane.

3.1.1. Initially Unamended Coal Treatments. During the first amendment period (days 0–76), the unamended coal treatments (coal –) produced $30.2 \pm 1.3 \mu\text{mol CH}_4/\text{g coal}$ ($n = 9$) (Figure 2d). The methane production during the second amendment period (days 76–117) for unamended coal treatments (coal –) was $0.7 \pm 1.5 \mu\text{mol CH}_4/\text{g coal}$ ($n = 3$). Initially unamended coal treatments that were amended on day 76 (coal – +) produced an additional $8.2 \pm 1.4 \mu\text{mol CH}_4/\text{g coal}$ during the second amendment period ($n = 6$). During the third and final amendment period from days 117 to 159, the unamended coal treatments (coal – –) produced $0.6 \pm 0.6 \mu\text{mol CH}_4/\text{g coal}$ ($n = 3$); coal – + – treatments that were amended on day 76 but not reamended on day 117 produced an additional $4.3 \pm 1.6 \mu\text{mol CH}_4/\text{g coal}$ ($n = 3$). Coal treatments amended on both days 76 and 117 (coal – + +) produced $12.7 \pm 1.5 \mu\text{mol CH}_4/\text{g coal}$ during amendment period 3 ($n = 3$).

3.1.2. Glass Bead Treatments. During the first amendment period (days 0 to 76), the unamended GB treatment (GB –) produced $5.4 \pm 0.2 \mu\text{mol CH}_4/\text{g GB}$ ($n = 3$), whereas the initially amended GB treatments (GB +) produced $15.0 \pm 0.7 \mu\text{mol CH}_4/\text{g GB}$ ($n = 9$) (Figure 2e). On day 76, six of the original GB + treatments were reamended. Between days 76 and 117, unamended GB treatments produced $-0.2 \pm 0.1 \mu\text{mol CH}_4/\text{g GB}$ (GB –) and $0.7 \pm 0.2 \mu\text{mol CH}_4/\text{g GB}$ (GB + –) ($n = 3$, both). The six reamended GB treatments (GB + +) produced $11.2 \pm 0.5 \mu\text{mol CH}_4/\text{g GB}$. During the third amendment period (days 117–159), GB amendments not reamended on day 117 produced $1.7 \pm 0.4 \mu\text{mol CH}_4/\text{g GB}$ (GB – –), $1.8 \pm 0.3 \mu\text{mol CH}_4/\text{g GB}$ (GB + –), and $2.2 \pm 0.4 \mu\text{mol CH}_4/\text{g GB}$ (GB + + –) ($n = 3$, each). GB + + + treatments were reamended on day 117 and produced an additional $12.3 \pm 0.4 \mu\text{mol CH}_4/\text{g GB}$ ($n = 3$).

3.1.3. Initially Amended Coal Treatments. During the first 76-day amendment period, the initially amended coal treatments (coal +) (Figure 2f) produced $41.5 \pm 4.9 \mu\text{mol CH}_4/\text{g coal}$ ($n = 9$). Between days 76 and 117, coal + – treatments (n

= 3), which were not amended on day 76, produced an additional $2.0 \pm 1.4 \mu\text{mol CH}_4/\text{g coal}$. Coal + + treatments (reamended on day 76) produced $14.3 \pm 0.6 \mu\text{mol CH}_4/\text{g coal}$ ($n = 6$) during the second amendment period. During the third amendment period (days 117–159), treatments not reamended on day 117 produced $2.9 \pm 0.9 \mu\text{mol CH}_4/\text{g coal}$ (coal + – –) and $1.1 \pm 0.5 \mu\text{mol CH}_4/\text{g coal}$ (coal + + –) ($n = 3$, both). Coal treatments (coal + + +), reamended on both day 76 and 117, produced $11.8 \pm 0.6 \mu\text{mol CH}_4/\text{g coal}$ during the third amendment period.

3.1.4. Comparisons of Amendment Regimens. During the first amendment period, the amended coal (coal +) treatments produced significantly more methane than both the unamended coal (coal –) treatments ($p < 0.05$) and the amended glass bead (GB +) treatments ($p < 0.05$), 1.4 and 2.8 times, respectively. However, during the second amendment period, the coal + + produced 1.7 times more methane than the coal – + ($p < 0.05$) and 1.3 times the amount of methane produced by the GB + + treatments ($p = 0.574$). During the last amendment period, the coal + + + produced a similar amount of methane as the coal – + + ($p = 1$) and GB + + + ($p = 1$) treatments, 0.93 times and 0.96 times, respectively. When comparing the coal treatments only amended on day 0 (coal + – –) to coal treatments never amended (coal – – –), coal + – – produced significantly more (1.4 times) methane than coal – – – during amendment period 1 ($p < 0.05$). However, while coal + – produced 2.9 times more methane during amendment period 2 than coal – – and coal + – – produced 3.0 times more than coal – – – during amendment period 3, these small amounts of additional methane production were not statistically significantly different ($p = 1$) (Figure 2).

3.2. Methane Production Rates. Maximum rates of methane production for all treatments during each amendment period are shown in Table 1.

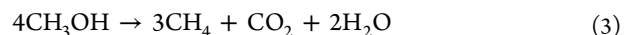
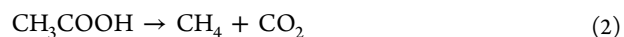
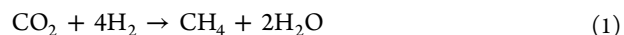
3.2.1. Initially Unamended Coal Treatments. The maximum rate of methane production observed during the first amendment period for unamended coal treatments (coal –) was $0.97 \pm 0.21 \mu\text{mol CH}_4/(\text{g coal d})$ and occurred between days 34 and 46. During the second amendment period, both coal – – and coal – + treatments had lower maximum methane production rates compared to the first amendment period ($p < 0.05$). Coal – – was not amended on day 76 and had a methane production rate of $0.12 \pm 0.12 \mu\text{mol CH}_4/(\text{g coal d})$. Coal – + treatments were amended at the beginning of the second amendment period and had maximum methane production rates for this period of $0.44 \pm 0.08 \mu\text{mol CH}_4/(\text{g$

coal d). During the third amendment period (days 117–159), treatments not reamended on day 117 had maximum methane production rates of 0.15 ± 0.05 and $0.40 \pm 0.12 \mu\text{mol CH}_4/(\text{g coal d})$ for coal – – – and coal – + –, respectively. Coal – + + treatments that were reamended on day 117 had a maximum methane production rate of $0.80 \pm 0.14 \mu\text{mol CH}_4/(\text{g coal d})$ for the third amendment period. For all initially unamended coal treatments, the maximum methane production rates were significantly higher during the first amendment period ($p < 0.05$) except for coal – + + treatments which had a rate not statistically significantly different from the coal treatments ($p = 0.890$). With amendment addition on days 76 and/or 117, maximum methane production rates were higher for coal – + than coal – – ($p < 0.05$) and higher for coal – + + than both coal – – – and coal – + – ($p < 0.05$).

3.2.2. Glass Bead Treatments. The maximum rate for unamended GB treatments (GB –) between day 0 and 76 was $0.18 \pm 0.06 \mu\text{mol CH}_4/(\text{g GB d})$ and occurred between days 62 and 76. The maximum rate for amended GB treatments occurred between days 34 and 46 and averaged $0.50 \pm 0.07 \mu\text{mol CH}_4/(\text{g GB d})$ for all nine samples (GB +). For the GB treatments during the second amendment period, the maximum rates for GB treatments not reamended during the second amendment period were $0.10 \pm 0.01 \mu\text{mol CH}_4/(\text{g GB d})$ (GB – –) and $0.06 \pm 0.02 \mu\text{mol CH}_4/(\text{g GB d})$ (GB + –). The maximum rate for GB treatments amended initially and reamended on day 76 (GB + +) was $0.66 \pm 0.03 \mu\text{mol CH}_4/(\text{g GB d})$, an increase from the $0.50 \pm 0.07 \mu\text{mol CH}_4/(\text{g GB d})$ rate observed for initially amended GB treatments during the first period. Maximum methane production rates observed during the third amendment period for GB treatments not amended on day 117 were $0.05 \pm 0.02 \mu\text{mol CH}_4/(\text{g GB d})$ for GB – – –, $0.16 \pm 0.01 \mu\text{mol CH}_4/(\text{g GB d})$ for GB + – –, and $0.20 \pm 0.03 \mu\text{mol CH}_4/(\text{g GB d})$ for GB + + –. Only the GB + + + treatments were reamended on day 117 and had a maximum rate of methane production of $0.79 \pm 0.01 \mu\text{mol CH}_4/(\text{g GB d})$, a possible increase over both of the previous amendment periods ($p > 0.542$).

3.2.3. Initially Amended Coal Treatments. During the first amendment period, the maximum rate for the amended coal treatments (coal +) was significantly higher ($p < 0.05$) than for all other amended coal treatments at later times and occurred between days 20 and 34. This maximum rate averaged across all nine samples was $1.52 \pm 0.13 \mu\text{mol CH}_4/(\text{g coal d})$ and was the highest methane production rate observed for all treatments over the duration of the study. During the second amendment period (days 76–117), treatments not amended on day 76 (coal + –) had a maximum methane production rate of $0.32 \pm 0.02 \mu\text{mol CH}_4/(\text{g coal d})$. The maximum rate for coal treatments amended initially and reamended on day 76 (coal + +) was $0.73 \pm 0.10 \mu\text{mol CH}_4/(\text{g coal d})$ ($n = 6$). These rates were lower than the maximum rate observed for the coal + treatments during the first amendment period ($p < 0.05$) but higher than the rates for coal + – ($p < 0.05$). During the third amendment period (days 117–159), the maximum methane production rates for treatments not amended on day 117 were 0.33 ± 0.06 and $0.31 \pm 0.06 \mu\text{mol CH}_4/(\text{g coal d})$ for coal + – – and coal + + –. On day 117, only the coal + + + treatments were reamended and had a maximum methane production rate of $0.70 \pm 0.08 \mu\text{mol CH}_4/(\text{g coal d})$, similar to the rate observed for coal + + treatments during the second amendment period ($p = 1$). Tukey comparison groupings for methane production rates are provided in [Supplementary Table S2](#).

3.3. Inorganic carbon (IC) Production. Methanogenic biochemical pathways (eq 1–3) can produce or utilize carbon dioxide (CO_2). Hydrogenotrophic methanogenesis (eq 1) consumes CO_2 while acetoclastic (eq 2) and methylotrophic (eq 3) methanogenesis produce CO_2 . In addition to methanogenesis, coal degradation reactions can produce or utilize CO_2 .^{28,29}



CO_2 is not the only form of inorganic carbon (IC) in these systems. Increased CO_2 concentrations also result in increased bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) concentrations and the ratio of these components of the carbonic acid system are pH-dependent. Thus, dissolved inorganic carbon must be tracked in order to obtain a more complete carbon balance. Lastly, CO_2 sorption to coal can occur.

3.3.1. Dissolved Inorganic Carbon (DIC) and CO_2 Production. Dissolved inorganic carbon (DIC) was measured every 2–4 weeks during the 159-day study. The initial DIC for the prepared formation water used to set up all microcosms was $284 \pm 0.8 \mu\text{mol C/g coal or GB}$. Because destructive sampling was required for measurement, only one sample for each treatment was analyzed for each time point except for the final sampling on day 159 when triplicate samples were analyzed. Therefore, the variability between equivalent treatments was assessed for the final measurement only. When the DIC was measured on day 7, the GB treatments exhibited a potentially small increase to $287 \mu\text{mol C/g GB}$ while coal treatments displayed a decrease in DIC to $224 \mu\text{mol C/g coal}$ for unamended coal treatments (coal –) and $229 \mu\text{mol C/g coal}$ for amended coal treatments (coal +) ([Figure 3a](#)). These initial decreases in DIC observed in the coal treatments but not in GB treatments could be the result of CO_2 sorption to the coal but not to GB. Apart from the initial decrease in DIC detected in all coal treatments, a general trend toward increasing DIC was observed for all treatments for the rest of the 159-day study. All GB treatments were associated with higher DIC concentrations than all coal treatments for the duration of the study. The observed increase in DIC may indicate a prevalence of acetoclastic and methylotrophic methanogenesis.

Headspace CO_2 concentrations were measured for all treatments approximately every 2 weeks during the 159-day study ([Figure 3b](#)). The initial CO_2 in the headspace of all treatments was $28.0 \pm 0.0 \mu\text{mol/g coal or GB}$. By the first sampling on day 20, unamended coal treatments (coal –) had greater headspace CO_2 concentrations ($45.5 \pm 3.6 \mu\text{mol/g coal}$); amended GB treatments (GB +) had a CO_2 concentration of $25.8 \pm 1.8 \mu\text{mol/g GB}$; and amended coal treatments (coal +) had a headspace CO_2 concentration of $49.7 \pm 3.0 \mu\text{mol/g coal}$. From day 20 to the end of the study, headspace CO_2 concentrations changed little for all treatments, and in contrast to the DIC concentrations, headspace CO_2 concentrations were higher for the coal treatments than for the GB treatments. One possible explanation for this observation is small differences in pH: the coal treatments had a slightly lower day 159 pH (7.54–7.61) on day 159 while the GB treatments had a higher pH (7.90–8.10) ([Supplementary Table S3](#)).

3.3.2. Total System Inorganic Carbon. System IC was calculated by summing the headspace CO_2 and dissolved

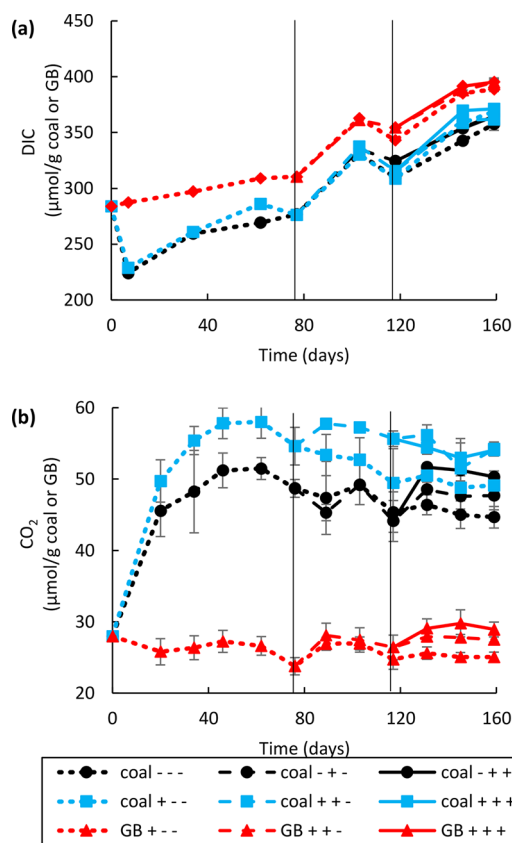


Figure 3. (a) Dissolved inorganic carbon (DIC) and (b) headspace CO_2 concentrations in treatments. Reamendment (where appropriate) is indicated by vertical lines. DIC measurements are from single replicates, except on day 159. CO_2 measurements with error bars indicating one standard deviation of 3–9 replicates (depending on the period, as per Figure 1)

inorganic carbon (DIC) concentrations. For these analyses, the sorption of IC to coal was assumed to have no effect on the measurable IC in the system. Produced amounts of IC for all systems are shown in Figure 3. DIC for unamended GB treatments (GB $-$) was not measured during the study because destructive sampling required more replicates than were initially set up. IC changes are reported here as “production”, and a negative value for production would indicate a potential utilization of IC. However, because it was possible to only measure IC for one sample for each treatment for all time points except the final measurement on day 159, some of the negative “production” values may be attributed to naturally occurring differences between samples. All systems had a cumulative IC production for the 176-day study (Figure 4a–c).

During the first amendment period (days 0–76), unamended coal treatments (coal $-$) produced $13.3 \mu\text{mol IC/g coal}$. Amended GB treatments (GB $+$) produced $18.7 \mu\text{mol IC/g GB}$ while amended coal treatments (coal $+$) produced $38.6 \mu\text{mol IC/g coal}$. During the second amendment period, coal $-$ treatments produced $29.9 \mu\text{mol IC/g coal}$ while coal $-$ + treatments amended on day 76 produced $45.1 \mu\text{mol IC/g coal}$. GB $+$ treatments produced $37.6 \mu\text{mol IC/g GB}$, and GB $+$ + treatments produced $48.6 \mu\text{mol IC/g GB}$ during amendment period 2. Initially amended coal treatments produced $14.0 \mu\text{mol IC/g coal}$ for treatments not amended on day 76 (coal $+$ $-$) and $24.8 \mu\text{mol IC/g coal}$ for amended treatments (coal $+$ $+$).

During the third amendment period, coal treatments that were never amended (coal $-$ $-$) produced $1.7 \pm 3.2 \mu\text{mol IC/g coal}$ ($n = 3$). IC production by coal $-$ + $-$ treatments was $-1.2 \pm 3.9 \mu\text{mol IC/g coal}$. Coal $-$ + + treatments amended on day 76 and day 117 produced $5.8 \pm 7.3 \mu\text{mol IC/g coal}$. Glass bead treatments, during the third amendment period, produced $-13.2 \pm 2.8 \mu\text{mol IC/g GB}$ for GB $+$ $-$ $-$ treatments, $-15.0 \pm 3.1 \mu\text{mol IC/g GB}$ for GB $+$ $+$ $-$ treatments, and $-10.9 \pm 5.2 \mu\text{mol IC/g GB}$ for GB $+$ $+$ + treatments. IC produced during the third amendment period by initially amended coal treatments was $11.6 \pm 4.6 \mu\text{mol IC/g coal}$ for coal $+$ $-$ $-$ treatments, $1.9 \pm 2.3 \mu\text{mol IC/g coal}$ for coal $+$ $+$ $-$, and $9.8 \mu\text{mol IC/g coal}$ for coal $+$ $+$ $+$.

3.4. Dissolved Nonpurgeable Organic Carbon. Non-purgeable organic carbon (NPOC) was also measured every 2–4 weeks during the study. The initial NPOC for the prepared formation water used to set up microcosms was $57.9 \pm 0.8 \mu\text{mol C/g coal or GB}$. The initial increase in NPOC observed on day 7 (Figure 5a) to 187, 162, and $228 \mu\text{mol C/g coal or GB}$ for unamended coal (coal $-$), amended GB (GB $+$), and amended coal (coal $+$), respectively, can potentially be attributed to NPOC carryover from the inoculum, dissolved organics in the algae amendment, and/or dissolution of coal organics after microcosm set up. After this initial increase, NPOC decreased to 35, 14, and 26 mg C/L for coal $-$, GB $+$, and coal $+$ treatments, respectively, on day 35. After this time, the NPOC decreased to less than $20 \mu\text{mol C/g coal or GB}$ for all treatments and remained below $20 \mu\text{mol C/g coal or GB}$ for all subsequent time points (Figure 5b).

3.5. Carbon Produced per Carbon Added. The total amount of carbon produced as methane and IC was compared to the total amount of carbon added as algae amendment ($C_{\text{out}}/C_{\text{in}}$) during the 159-day study (Figure 6). $C_{\text{out}}/C_{\text{in}}$ ratios greater than 1 indicate that the amount of carbon converted to CH_4 and IC exceeded the amount of carbon added with the algal amendment.

Comparing treatments of the same initial condition (coal $-$, GB $+$, or coal $+$), regardless of solid substrate, $C_{\text{out}}/C_{\text{in}}$ ratios decreased with each subsequent amendment (i.e., coal $+$ $-$ $-$ $>$ coal $+$ $+$ $-$ $>$ coal $+$ $+$ $+$). Unamended coal treatments (coal $-$ $-$) were not amended during the study ($C_{\text{in}} = 0$) and therefore cannot be analyzed with this method. However, coal $-$ + $-$ treatments had a $C_{\text{out}}/C_{\text{in}}$ ratio of 2.7 ± 0.1 while coal $-$ + + treatments had a lower ratio of 1.6 ± 0.1 . For initially amended GB treatments, GB $+$ $-$ $-$ treatments had a $C_{\text{out}}/C_{\text{in}}$ ratio of 1.6 ± 0.1 whereas reamendment on day 76 resulted in a ratio of 1.1 ± 0.0 for GB $+$ + $-$ treatments and reamendment on both day 76 and 117 resulted in a ratio of 0.9 ± 0.1 for GB $+$ + + treatments. The $C_{\text{out}}/C_{\text{in}}$ ratio for initially amended coal treatments was 3.0 ± 0.1 for coal $+$ $-$ $-$ treatments, 1.7 ± 0.1 for coal $+$ + $-$ treatments, and 1.2 ± 0.0 for coal $+$ + + treatments. The observation of decreasing $C_{\text{out}}/C_{\text{in}}$ ratios with sequential amendment addition suggests that reamendment results in a greater portion of produced methane being derived from the conversion of the amendment itself and less from other carbon sources including the conversion of coal to methane.

All amended coal and GB treatments except the GB $+$ + + treatments had a $C_{\text{out}}/C_{\text{in}}$ ratio greater than 1. Therefore, all treatments except for the GB $+$ + + treatments produced more carbon as methane and IC than was added as algal amendment. Coal is indeed a significant carbon source for methane production in treatments containing coal. However, a $C_{\text{out}}/$

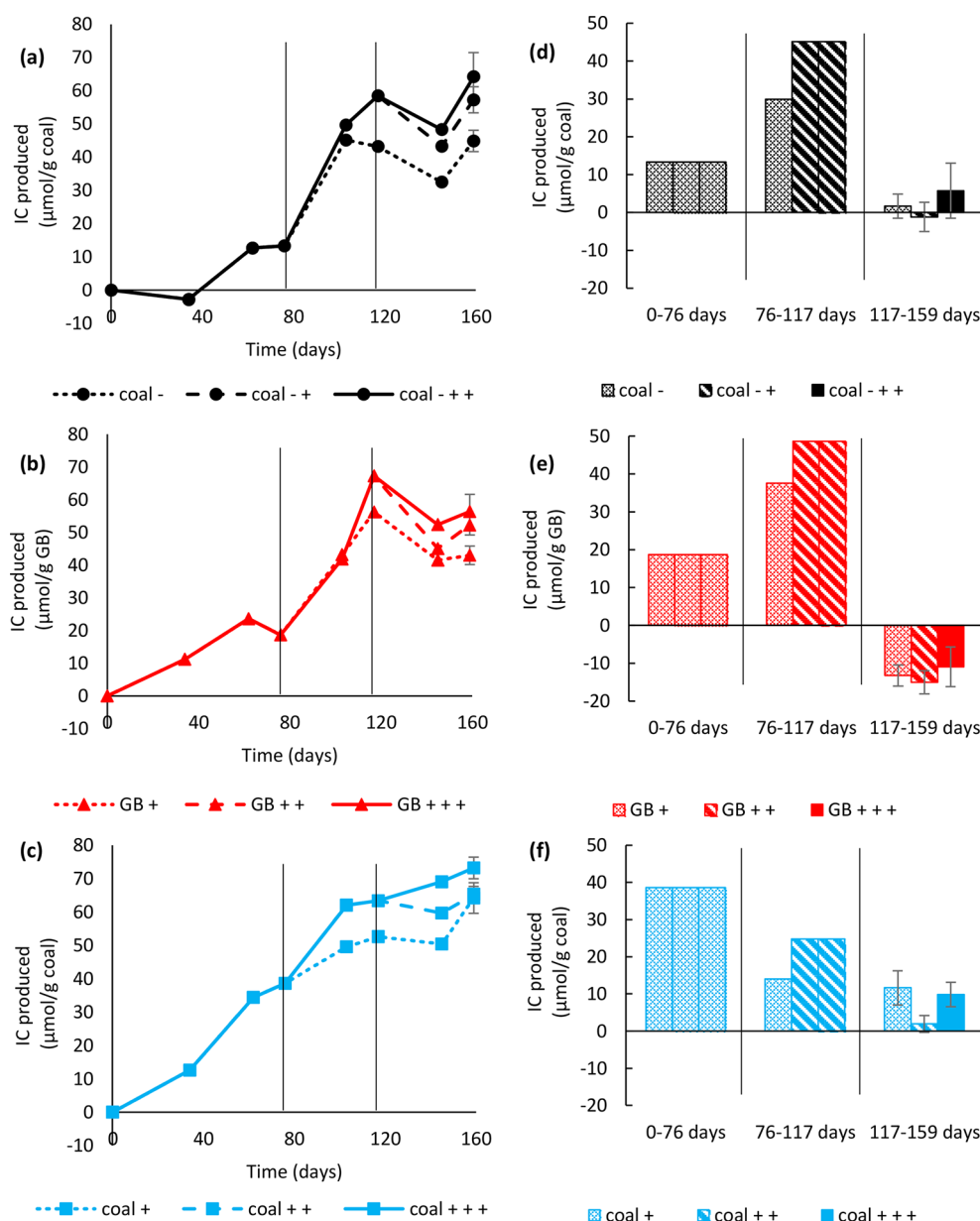


Figure 4. Inorganic carbon (IC) produced as time series (a–c) and as production per amendment period (d–f) for initially unamended coal (a, d), glass beads (b, e), and initially amended coal (c, f) treatments. Error bars on day 159 represent one standard deviation for three replicates. Vertical lines designate amendment addition days.

C_{in} ratio greater than 1 was also observed in the GB + – – and GB + + – treatments which did not have coal as an alternative carbon source (except for possible carry-over with the inoculum). Other sources of carbon in all systems potentially contributing to methane and IC production include initial dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), carbon carried over with the inoculum, or biomass turnover of the initial microbial members.

4. DISCUSSION

During the initial amendment period, coal treatments produced more methane than the corresponding GB treatments for both amended and unamended treatments. These data corroborate the conclusion from previous studies that coal can be an important substrate for methane production.^{17,18} However, the amount of methane produced by amended coal treatments (coal +) was 2.8 times the amount produced by the amended

GB treatments (GB +) during the first amendment period, but the amount of methane produced by reamended coal treatments (coal + +) was only 1.3 times the amount produced by the reamended glass bead treatments (GB + +) during the second amendment period. During the third amendment period, the coal + + + treatments produced approximately the same amount of methane as the GB + + + treatments ($p = 1$). Additionally, while the coal + treatments had a higher methane production rate in the first amendment period ($p < 0.05$), during the second and third amendment periods the average methane production rate for amended coal treatments was not significantly different than for amended GB treatments (coal + + vs GB + +, GB + + + vs coal + + +) ($p = 1$). These results indicate that the fraction of coal that can be easily converted into methane by the microbial community over the tested time period had been depleted or the microbial community had shifted away from coal utilization. These

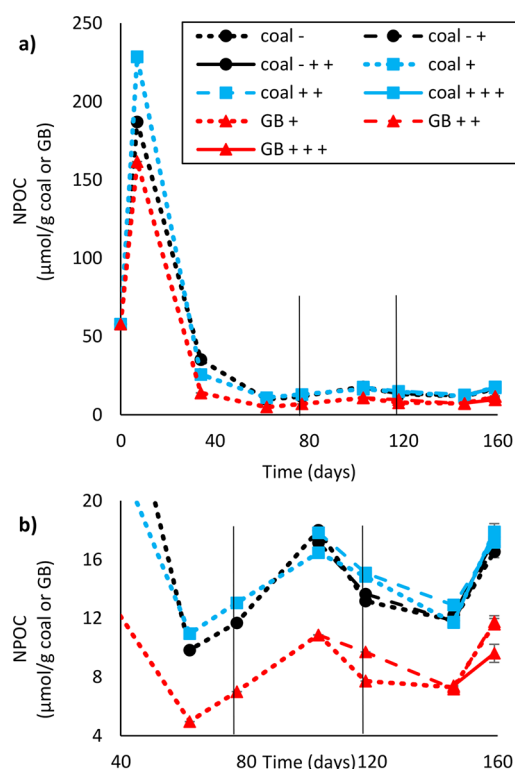


Figure 5. (a) Nonpurgeable organic carbon (NPOC) for treatments for all measured time points. (b) Days 40–159 to more clearly resolve the NPOC variations between treatments. Days of amendment addition, where appropriate, are indicated by vertical lines.

observations support the hypothesis that short-term reamendment of batch coal systems results in a resource utilization shift from the coal to the amendment itself and could be the result of a microbial community shift similar to that demonstrated previously with higher amendment concentrations.¹⁷

Previous research indicates coal-to-methane conversion in batch studies levels off after an initial production period.^{10,11,14,17,30} This can likely be attributed to common limitations of batch systems such as substrate depletion or byproduct inhibition.³¹ In batch systems that contain a limited amount of coal, it is likely that the most bioavailable coal will be degraded first to produce CH₄, IC, and other byproducts. Once the easily degraded coal fraction has been utilized, the rate of methane production appears to decrease as the microbial community begins to degrade the more recalcitrant components of the coal matrix. In addition to the possible limitation of bioavailable coal substrate, the accumulation of degradation

byproducts could become inhibitory for some microbial processes and result in the cessation of coal-dependent methanogenesis.^{16,24}

Reamended treatments appear to produce similar amounts of methane, regardless of the solid substrate present (coal or glass beads). This observation suggests amendment-to-methane conversion is the primary source for methane production in these reamended systems. Amendment addition, after methane production has slowed, facilitates more methane production, and thus reamended systems, which are no longer substrate-limited due to reamendment, do not appear to be inhibited by byproduct accumulation for amendment utilization during this study (i.e., methanogenesis is still possible in the presence of amendment).

All treatments were inoculated with 1 mL of the FG microbial consortium. Each milliliter of inoculum contained a measured 39.3 ± 0.0 μmol C as biomass, DOC, DIC, and coal microparticles. It is unlikely that all of this carbon was converted to CH₄ or IC, but it cannot be completely ruled out as a potential carbon source for methane or IC production. As shown in Figure 5, the measured NPOC, after an initial spike on day 7, decreased, and a comparison of NPOC measurements between day 0 and day 159 indicate a net loss of NPOC during the experiment: 41 μmol C for coal treatments and 47 μmol C for GB treatments. This net loss could be attributed to microbial processes resulting in methane or IC production. Because the NPOC measurement quantifies the total dissolved organics and does not identify specific organic compounds present, it is likely that some of these organics are potential methanogenic substrates or easily converted to known methanogenic substrates such as formate, acetate, or methyl-compounds.^{29,30,32,33} The decrease in NPOC also could be due to consumption of organic carbon by microbes for biomass growth or sorption of organic carbon to coal which would not contribute to CH₄ or IC production.

All estimates of methane and IC production made in the described studies assume the effects of sorption of CH₄ and CO₂ to coal are either negligible or affect all systems equally. However, it is possible that neglecting sorption of both CH₄ and CO₂ results in an underestimation of the amount of CH₄ and/or IC present in the system due to the inability to measure the amounts of sorbed CH₄ and CO₂ in these systems without complete destructive sampling.

Coal sorption studies have shown preferential sorption of CO₂ over CH₄, and estimates for the relative amount of CO₂ sorbed to coal compared to CH₄ vary from 2:1 to 10:1.³⁴ It is hypothesized that this preferential sorption can be attributed to several differences. The higher boiling point of CO₂ and

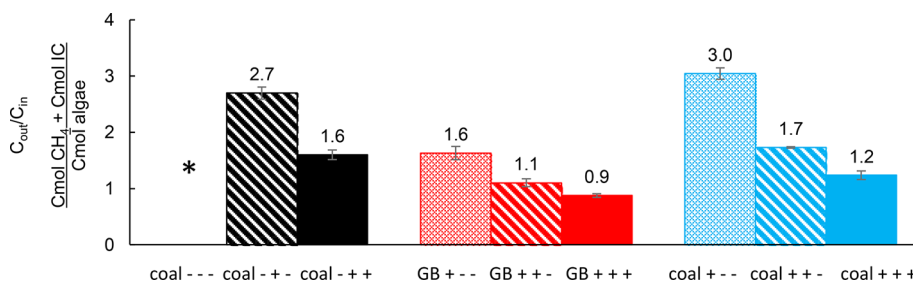


Figure 6. Ratio of produced methane and IC to algae added (normalized to the amount of carbon) for the entire 159-day study. Ratios greater than 1 indicate carbon production (as methane and IC) in excess of the carbon added as algae amendment. The initial amendment condition is labeled below each group. (*) Coal --- treatments do not have data because algal amendment was not added.

variations in physicochemical sorption processes result in increased adsorption energy of CO₂. In addition, CO₂ has a smaller molecular diameter than CH₄ and thus can diffuse into smaller pores not accessible to CH₄.³⁴ Many coal sorption studies have used dry coal. However, studies considering the effect of moisture on CH₄ and CO₂ sorption have shown that moisture reduces the sorption capacity of coal though CO₂ is still preferentially sorbed.^{35,36}

Adsorption and desorption studies were performed on dry Wyodak coal from the Powder River Basin.³⁷ This subbituminous coal is from the Tongue River Member of the Fort Union Formation as is the Flowers-Goodale coal used in this study and is thus more relevant to the potential sorption effects in this study. The ratio of CO₂ sorption relative to CH₄ was estimated at 2.69 for Wyodak coal. Additionally, the adsorption and desorption isotherms indicated that even at atmospheric pressure, not all CH₄ and CO₂ can be desorbed from the Wyodak coal.³⁷ The results suggests that the systems presented here, at near atmospheric pressure, are likely to have some amount of sorbed CH₄ and CO₂.

A decrease in DIC from day 0 to day 7 (Figure 3a) was observed in all coal treatments: −59.9 and −55.0 μmol C/g coal for unamended and amended coal treatments, respectively. Over the same time period, amended GB treatments had a possibly small increase in DIC of +3.4 μmol carbon. During the rest of the study, all samples had an overall trend of increasing DIC. While a few small decreases in DIC were observed later in the study, none were as large as the decrease observed in the first 7 days in the coal treatments. This initial decrease in DIC observed in coal treatments (but not in noncoal treatments) could be attributed to the sorption of CO₂ to coal. After day 7, the rate of IC production likely exceeded the sorption rate or produced methane sorbed to the coal to an extent that displaced some of the sorbed CO₂ resulting in an increase in DIC. With an estimated CO₂ to CH₄ sorption ratio between 2:1 and 4:1,^{34,35} it is likely that CH₄ sorption to coal is indeed a factor in these systems and suggests that CH₄ production could be greater than measured.

5. SUMMARY AND CONCLUSIONS

The results presented here indicate that the amount and rate of biogenic CH₄ production can be increased by the addition of algal amendments. However, in batch systems, after 60 to 80 days, CH₄ production slows down or ceases completely, as demonstrated in our previous work.¹⁷ For potential long-term commercial applications, it is necessary to determine the potential benefits, challenges, and overall feasibility of reamending coal systems to achieve repeated enhancement of CH₄ production and increase the total recoverable CH₄. In addition, flow conditions at larger scales will most likely impact the half-life of the amendment as well as the ratio of amendment to overall coal. Future work is needed at meso- and field-scales to determine the impact of repeated amendment.

Reamendment, after CH₄ production slowed, resulted in a temporary increase in CH₄ production rates and amounts before CH₄ production again declined. However, the rate and extent of methane production declined with each subsequent reamendment. After the third amendment, the additional CH₄ production of amended coal treatments was equivalent to reamended glass bead systems (*i.e.*, coal-dependent methanogenesis did not appear to be significant anymore). These results suggest a greater contribution of amendment-to-methane conversion in the tested batch coal systems with each

subsequent reamendment. The apparent shift from coal-to-methane conversion to amendment-to-methane conversion after repeated amendments results in diminishing returns on investment and is similar to a previous assessment indicating that high amounts of amendment result in greater amendment-to-methane conversion instead of coal-dependent methanogenesis.¹⁷ For potential long-term commercial applications, it is necessary to determine the potential benefits, challenges, and feasibility of adding additional amendment to coal systems to prolong enhanced CH₄ production and increase the total recoverable CH₄ with existing infrastructure and wells.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.energy-fuels.7b03426.

Additional methods information for gas content measurement and calculation. Tukey comparison groupings for amounts and rates of methane production as well as final pH measurements (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Tel.: 1-406-270-3192. Fax: 1-406-994-6098. E-mail: katherine.jn.davis@gmail.com (K.J.D.).

*Tel.: 1-406-994-1840. Fax: 1-406-994-6098. Email: robin_g@montana.edu (R.G.).

ORCID

Katherine J. Davis: 0000-0002-0562-7035

Robin Gerlach: 0000-0002-7669-3072

Notes

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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■ REFERENCES

- (1) Colosimo, F.; Thomas, R.; Lloyd, J. R.; Taylor, K. G.; Boothman, C.; Smith, A. D.; Lord, R.; Kalin, R. M. Biogenic methane in shale gas and coal bed methane: A review of current knowledge and gaps. *Int. J. Coal Geol.* **2016**, *165*, 106–120.
- (2) Moore, T. A. Coalbed methane: A review. *Int. J. Coal Geol.* **2012**, *101*, 36–81.
- (3) Park, S. Y.; Liang, Y. Biogenic methane production from coal: A review on recent research and development on microbially enhanced coalbed methane (MECBM). *Fuel* **2016**, *166*, 258–267.
- (4) Ritter, D.; Vinson, D.; Barnhart, E.; Akob, D. M.; Fields, M. W.; Cunningham, A. B.; Orem, W.; McIntosh, J. C. Enhanced microbial coalbed methane generation: A review of research, commercial activity, and remaining challenges. *Int. J. Coal Geol.* **2015**, *146*, 28–41.

- (5) Flores, R. M.; Rice, C. A.; Stricker, G. D.; Warden, A.; Ellis, M. S. Methanogenic pathways of coal-bed gas in the Powder River Basin, United States: The geologic factor. *Int. J. Coal Geol.* **2008**, *76* (1–2), 52–75.
- (6) Golding, S. D.; Boreham, C. J.; Esterle, J. S. Stable isotope geochemistry of coal bed and shale gas and related production waters: A review. *Int. J. Coal Geol.* **2013**, *120*, 24–40.
- (7) Strapoć, D.; Mastalerz, M.; Dawson, K.; Macalady, J.; Callaghan, A. V.; Wawrik, B.; Turich, C.; Ashby, M. Biogeochemistry of Microbial Coal-Bed Methane. *Annu. Rev. Earth Planet. Sci.* **2011**, *39* (1), 617–656.
- (8) Meredith, E.; Wheaton, J.; Kuzara, S. *Coalbed Methane Basics: Ten Years of Lessons from the Powder River Basin*; Information Pamphlet 6; Montana Bureau of Mines and Geology, MT, 2012.
- (9) Rice, C. A.; Nuccio, V. *Water Produced with Coal-Bed Methane*, FS 156-00; U.S. Geological Survey Fact Sheet, 2000.
- (10) Green, M. S.; Flanagan, K. C.; Gilcrease, P. C. Characterization of a methanogenic consortium enriched from a coalbed methane well in the Powder River Basin, U.S.A. *Int. J. Coal Geol.* **2008**, *76* (1–2), 34–45.
- (11) Ulrich, G.; Bower, S. Active methanogenesis and acetate utilization in Powder River Basin coals, United States. *Int. J. Coal Geol.* **2008**, *76* (1–2), 25–33.
- (12) Gallagher, L. K.; Glossner, A. W.; Landkamer, L. L.; Figueroa, L. A.; Mandernack, K. W.; Munakata-Marr, J. The effect of coal oxidation on methane production and microbial community structure in Powder River Basin coal. *Int. J. Coal Geol.* **2013**, *115*, 71–78.
- (13) Huang, Z.; Urynowicz, M. A.; Colberg, P. J. S. Stimulation of biogenic methane generation in coal samples following chemical treatment with potassium permanganate. *Fuel* **2013**, *111*, 813–819.
- (14) Jones, E. J. P.; Harris, S. H.; Barnhart, E. P.; Orem, W. H.; Clark, A. C.; Corum, M. D.; Kirshtein, J. D.; Varonka, M. S.; Voytek, M. A. The effect of coal bed dewatering and partial oxidation on biogenic methane potential. *Int. J. Coal Geol.* **2013**, *115*, 54–63.
- (15) Huang, Z.; Urynowicz, M. A.; Colberg, P. J. S. Bioassay of chemically treated subbituminous coal derivatives using *Pseudomonas putida* F1. *Int. J. Coal Geol.* **2013**, *115*, 97–105.
- (16) Zhang, J.; Park, S. Y.; Liang, Y.; Harpalani, S. Finding cost-effective nutrient solutions and evaluating environmental conditions for biogasifying bituminous coal to methane ex situ. *Appl. Energy* **2016**, *165*, 559–568.
- (17) Davis, K. J.; Lu, S.; Barnhart, E. P.; Parker, A. E.; Fields, M. W.; Gerlach, R. Type and amount of organic amendments affect enhanced biogenic methane production from coal and microbial community structure. *Fuel* **2018**, *211*, 600–608.
- (18) Barnhart, E. P.; Davis, K. J.; Varonka, M.; Orem, W.; Cunningham, A. B.; Ramsay, B. D.; Fields, M. W. Enhanced coal-dependent methanogenesis coupled with algal biofuels: Potential water recycle and carbon capture. *Int. J. Coal Geol.* **2017**, *171*, 69–75.
- (19) Solaiman, D. K. Y.; Ashby, R. D.; Zerkowski, J. A.; Foglia, T. A. Simplified soy molasses-based medium for reduced-cost production of sphorolipids by *Candida bombicola*. *Biotechnol. Lett.* **2007**, *29* (9), 1341–1347.
- (20) Vilcáez, J. Stimulating effect of protein-rich matter on the biogenic conversion of CO₂ to CH₄. *J. CO₂ Util.* **2015**, *10*, 60–66.
- (21) Hodgskiss, L. H.; Nagy, J.; Barnhart, E. P.; Cunningham, A. B.; Fields, M. W. Cultivation of a native alga for biomass and biofuel accumulation in coal bed methane production water. *Algal Res.* **2016**, *19*, 63–68.
- (22) Priyadarshani, I.; Rath, B. Commercial and industrial applications of micro algae—A review. *J. Algal Biomass Util.* **2012**, *3* (4), 89–100.
- (23) Guo, H.; Zhang, J.; Han, Q.; Huang, Z.; Urynowicz, M. A.; Wang, F. Important Role of Fungi in the Production of Secondary Biogenic Coalbed Methane in China's Southern Qinshui Basin. *Energy Fuels* **2017**, *31* (7), 7197–7207.
- (24) Zhang, J.; Liang, Y. Evaluating approaches for sustaining methane production from coal through biogasification. *Fuel* **2017**, *202*, 233–240.
- (25) Barnhart, E. P.; Weeks, E. P.; Jones, E. J. P.; Ritter, D. J.; McIntosh, J. C.; Clark, A. C.; Ruppert, L. F.; Cunningham, A. B.; Vinson, D. S.; Orem, W.; et al. Hydrogeochemistry and coal-associated bacterial populations from a methanogenic coal bed. *Int. J. Coal Geol.* **2016**, *162*, 14–26.
- (26) Barnhart, E. P.; De León, K. B.; Ramsay, B. D.; Cunningham, A. B.; Fields, M. W. Investigation of coal-associated bacterial and archaeal populations from a diffusive microbial sampler (DMS). *Int. J. Coal Geol.* **2013**, *115*, 64–70.
- (27) R Core Team. *R: A Language and environment for statistical computing*; R Foundation for Statistical Computing: Vienna, Austria, 2017.
- (28) Schink, B. Syntrophic Associations in Methanogenic Degradation. In *Molecular Basis of Symbiosis*; Overmann, P. D. J., Ed.; Progress in Molecular and Subcellular Biology; Springer: Berlin Heidelberg, 2005; pp 1–19.
- (29) McInerney, M. J.; Struchtemeyer, C. G.; Sieber, J.; Mouttaki, H.; Stams, A. J. M.; Schink, B.; Rohlin, L.; Gunsalus, R. P. Physiology, Ecology, Phylogeny, and Genomics of Microorganisms Capable of Syntrophic Metabolism. *Ann. N. Y. Acad. Sci.* **2008**, *1125* (1), 58–72.
- (30) Harris, S. H.; Smith, R. L.; Barker, C. E. Microbial and chemical factors influencing methane production in laboratory incubations of low-rank subsurface coals. *Int. J. Coal Geol.* **2008**, *76* (1–2), 46–51.
- (31) Doran, P. M. *Bioprocess Engineering Principles*, 1st ed.; Academic Press Limited: London, UK, 1995.
- (32) Wawrik, B.; Mendivelso, M.; Parisi, V. A.; Suflita, J. M.; Davidova, I. A.; Marks, C. R.; Nostrand, J. D.; Liang, Y.; Zhou, J.; Huizinga, B. J.; et al. Field and laboratory studies on the bioconversion of coal to methane in the San Juan Basin. *FEMS Microbiol. Ecol.* **2012**, *81* (1), 26–42.
- (33) Singh, D. N.; Kumar, A.; Sarbhai, M. P.; Tripathi, A. K. Cultivation-independent analysis of archaeal and bacterial communities of the formation water in an Indian coal bed to enhance biotransformation of coal into methane. *Appl. Microbiol. Biotechnol.* **2012**, *93* (3), 1337–1350.
- (34) Harpalani, S.; Prusty, B. K.; Dutta, P. Methane/CO₂ Sorption Modeling for Coalbed Methane Production and CO₂ Sequestration. *Energy Fuels* **2006**, *20* (4), 1591–1599.
- (35) Busch, A.; Gensterblum, Y. CBM and CO₂-ECBM related sorption processes in coal: A review. *Int. J. Coal Geol.* **2011**, *87* (2), 49–71.
- (36) Merkel, A.; Gensterblum, Y.; Krooss, B. M.; Amann, A. Competitive sorption of CH₄, CO₂ and H₂O on natural coals of different rank. *Int. J. Coal Geol.* **2015**, *150–151*, 181–192.
- (37) Busch, A.; Gensterblum, Y.; Krooss, B. M. Methane and CO₂ sorption and desorption measurements on dry Argonne premium coals: pure components and mixtures. *Int. J. Coal Geol.* **2003**, *55* (2–4), 205–224.