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Type and amount of organic amendments affect enhanced biogenic methane production from coal and microbial community structure

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ABSTRACT

Slow rates of coal-to-methane conversion limit biogenic methane production from coalbeds. This study demonstrates that rates of coal-to-methane conversion can be increased by the addition of small amounts of organic amendments. Algae, cyanobacteria, yeast cells, and granulated yeast extract were tested at two concentrations (0.1 and 0.5 g/L), and similar increases in total methane produced and methane production rates were observed for all amendments at a given concentration. In 0.1 g/L amended systems, the amount of carbon converted to methane minus the amount produced in coal only systems exceeded the amount of carbon added in the form of amendment, suggesting enhanced coal-to-methane conversion through amendment addition. The amount of methane produced in the 0.5 g/L amended systems did not exceed the amount of carbon added. While the archaeal communities did not vary significantly, the bacterial populations appeared to be strongly influenced by the presence of coal when 0.1 g/L of amendment was added; at an amendment concentration of 0.5 g/L the bacterial community composition appeared to be affected most strongly by the amendment type. Overall, the results suggest that small amounts of amendment are not only sufficient but possibly advantageous if faster *in situ* coal-to-methane production is to be promoted.

1. Introduction

Coalbed methane (CBM) is an unconventional natural gas resource formed in subsurface coal seams by thermogenic and biogenic processes. In 2015, the United States had 12,520 billion cubic feet of proven CBM reserves, and CBM production provided approximately 4% of the total annual natural gas requirement [1,2]. The coal beds of the Powder River Basin (PRB) in southeastern Montana and northeastern Wyoming accounted for 16.3% of the CBM produced in the U.S. in 2015 [1,2], and the PRB CBM has been shown to be primarily or completely of biogenic origin [3–5].

Biogenic CBM is the result of coal-to-methane conversion by a diverse, natural microbial community [3,6–8]. Methanogenic archaea produce biogenic methane through a limited number of pathways utilizing simple substrates (H_2/CO_2, acetate, and methyl-compounds). However, generation of these simple substrates from coal requires a diverse microbial consortium, containing both archaeal and bacterial members, with interactive metabolic strategies for sequential fermentative processes to degrade coal to simpler fermentation by-products [3,6,7]. Biogenic methane is produced continuously in active coal basins, and methods have been proposed for increasing the rate and volume of microbially-produced methane [9].

Rates of commercial biogenic gas removal often exceed the rates of microbial production, resulting in reduced gas extraction. Thus, gas production from many wells is no longer economically viable [10], especially with decreased prices for natural gas due to increases in shale gas production in the 2000s. This has increased the cost-to-profit ratio for CBM retrieval, and many wells have been abandoned. The existing infrastructure creates an ideal opportunity for microbially-enhanced CBM (MeCBM) methods to increase coal-to-methane conversion rates and CBM volume thus extending the lifespan of current and future wells.

Ritter et al. summarized laboratory and *in situ* commercial applications of MeCBM techniques using various amendment methods [9]. While some studies have shown the potential for increased biogenic methane production with coal pre-treatment for enhanced

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bioavailability [11–13], significant potential exists for MeCBM strategies that stimulate microbial populations through nutrient addition [3,9,14,15]. Some previous CBM stimulation studies have used simple carbon substrates, such as acetate [8] or formate [16] to enhance methane production. While adding simple carbon substrates alone does increase methane production, it is unclear whether these additions enhance the coal-to-methane conversion or merely supply a more easily metabolized substrate for bacteria and/or methanogens. Inorganic limiting nutrients for biogenic coal-to-methane conversion include nitrogen, phosphorus, trace elements, and/or vitamins, and addition of these inorganic nutrients resulted in increased biogenic methane production in coal microcosms [14,17]. Determination of the specific nutrients needed for each potential *in situ* application could be costly and time-consuming.

A review of microbial life under extreme energy limitations suggested that microbial communities in subsurface environments can meet organic and inorganic nutritional requirements through biomass turnover [18]. Using this principle, it can be assumed that additions of complex nutrient sources (such as biomass) to the coal environment could provide the combination of nutrients necessary to encourage microbial growth and enhance coal degradation without the need for determining the exact amounts of specific nutrients required. Previous studies have supplemented limiting nutrients and successfully enhanced CBM production through the addition of yeast extract in conjunction with other organic or inorganic nutrient additions [9,14,19,20]. Barnhart et al. introduced the use of yeast and algal extracts as sole amendments to stimulate biogenic methanogenesis from coal and demonstrated a production increase with both amendments [21]. Biostimulation with yeast extract or other biomass-derived nutrient sources could provide the limiting nutrients needed for both the bacterial and archaeal populations. This could result in increased coal-to-methane conversion with potentially reduced cost and without the need to determine the exact nutrient additions needed for *in situ* CBM stimulation.

The goals of this study were as follows: (1) assess the potential of using four different biologically produced multi-nutrient amendments (algae, cyanobacteria, yeast cells, and granulated yeast extract) to enhance methane production from coal, (2) track carbon inputs and outputs to determine whether amendments are indeed stimulating coal-to-methane conversion or merely providing an alternative carbon source for methane production, and (3) determine whether amendment addition causes significant shifts in the microbial community involved in coal-dependent methanogenesis.

2. Materials and methods

2.1. Site and sample collection

The sampling site, located near Birney (Montana, USA) in the Powder River Basin, was thoroughly described by Barnhart et al. [22]. Water from the subbituminous Flowers-Goodale (FG) coalbed was pumped and retrieved in July 2014 from the FG-11 well. Six-gallon plastic storage 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) until microcosm set up. Coal cores were collected during the July 2013 drilling of FG monitoring wells (FGM-13, FGP-13). The 2-inch diameter cores were cut into approximately 12-inch long sections and placed in polyvinyl chloride (PVC) tubes. These tubes were completely filled with formation water pumped from the FG-11 well, and sealed with flexible rubber caps to allow room for gas desorption. A detailed description of the FG coal bed samples was given by Barnhart et al. [22]. Microbial cultures were collected from two FG wells (FGM-13 and FGP-13) in November 2014 using the diffusive microbial samplers (DMS) described by Barnhart et al. [8]. The slurry (formation water with high suspended solids) from the FGP-13 DMS (13 mL) and FGM-13 DMS (7 mL) were added to a serum bottle prepared with 5 g FG coal and 45 mL anoxic FG formation water before being allowed to incubate at room temperature in the dark for 5 months prior to being used to inoculate the studies described here.

2.2. Amendment growth and analysis

The microalga, *Chlorella* sp. strain, SLA-04 (isolated from Soap Lake, WA, USA), was cultured for biomass accumulation at 20 °C in Bold's Basal Medium [23] in tube photobioreactors using methods previously described [24]. *Anabaena cylindrica* strain UTEX 1611, a nitrogen-fixing cyanobacterium, was cultured using methods similar to SLA-04 cultivation using Blue-Green Medium (BG-11) [25] without the nitrogen source. For both SLA-04 and UTEX 1611, daily cell counts were used to determine stationary phase when the cell counts were highest, 6.0×10^7 and 4.0×10^7 cells/mL respectively. A yeast, *Saccharomyces cerevisiae* strain EtOH-Red, was cultured in 100 mL of Yeast Extract Peptone Dextrose (YPD) medium [26] in 250 mL flasks at 37 °C and shaken at 100 rpm to keep cells in suspension. Optical density (OD) at 600 nm was measured daily. Yeast culture (5 mL) was collected in 26 mL Balch tubes, and OD was measured with Unico 1100RS tube spectrophotometer (Dayton, NJ, USA). OD increased from an initial OD of 0.77–1.97 OD at stationary phase. The biomass from all three cultures was concentrated by centrifugation, dried by lyophilization, and stored at −20 °C. SLA-04, UTEX 1611, and EtOH-Red biomass as well as granulated yeast extract (EMD Millipore Corporation) (known hereafter as algae, cyanobacteria, yeast, and YE, respectively) were sent to the Iowa State University Soil and Plant Analysis Laboratory (Ames, Iowa) for elemental analysis (Supplementary Table S1) [27].

2.3. Microcosm set up

All microcosms were set up anoxically in 26 mL Balch tubes with butyl rubber stoppers and aluminum crimp seals (Supplementary Fig. S1). The FG coal core (depth 374–375′) was opened in an anaerobic glove bag where it was dried, crushed, and sieved to an effective size range of 0.85–1.19 mm. The prepared coal was stored in oxygen-free glass bottles until microcosm set up. Borosilicate glass beads (GB) (1 mm diameter) were autoclaved for controls and used in lieu of coal to provide a carbon-free solid substrate. Each Balch tube received 1 g of prepared coal or GB. The formation water was sparged for 5 h with anoxic nitrogen gas and reduced with sulfide (1 mM as $Na_2S{\cdot}9H_2O$). Resazurin (1 mg/L) was used as a visual redox indicator. The amendments (algae, cyanobacteria, yeast, and YE) were ground to a fine powder with a ceramic mortar and pestle. Two concentrations of each amendment were prepared at 10× desired concentration (0.1 and 0.5 g/L final concentration) in degassed FG formation water and sealed anaerobically in serum bottles. All amended treatments received 1 mL of this prepared amendment concentrate as appropriate. The headspace of all tubes was replaced with 5% CO_2, 95% N_2. pH was tested to ensure a range of 7.5–8.5 as observed in the FG formation water [22] and adjusted with 1 M HCl as necessary. All inoculated treatments received 1 mL of the inoculum described above; 3 mL of the inoculum slurry was stored at −80 °C for microbial community analysis. All microcosms were incubated at room temperature (21 ± 1 °C) in the dark for 111 days, and headspace gas was sampled and analyzed approximately every 2 weeks.

2.4. Gas analysis

Methane production was monitored 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 Molecular Sieve 13X packed stainless steel column (6 feet × 1/8″ O.D.) was used with ultra-high purity helium carrier gas for separation using the following conditions: manual injection, oven temperature 40 °C, TCD temperature 150 °C, and carrier gas pressure 18 psi. Gas samples (1 mL) were taken from the