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The effect of coal oxidation on methane production and microbial community structure in Powder River Basin coal

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ABSTRACT

Vast reserves of coal represent a largely untapped resource that can be used to produce methane gas, a cleaner energy alternative compared to burning oil or coal. Microorganisms are able to utilize coal as a carbon source, producing biogenic methane. The conversion of coal to methane by microorganisms has been demonstrated experimentally by a number of research groups, but coal handling and treatment prior to incubation often goes unreported and may impact biogenic methane production. Microcosm experiments were designed to assess how prior exposure of coal to oxygen might influence methane production (e.g., as in a dewatered coal-bed natural gas system). Microcosms containing oxidized and un-oxidized coal samples from the Powder River Basin were incubated with and without inoculation with an enrichment culture derived from coal. Gas chromatography and pyrosequencing of the 16S rRNA gene were used to assess how coal oxidation affects methane production and microbial community structure within microcosm samples. Although the magnitude of methane production differed between experiments, the oxidized coal microcosms consistently produced between 50 and 100 micromoles less methane per gram of coal than the un-oxidized microcosms. Additionally, un-inoculated microcosms produced levels of methane comparable to their inoculated counterparts, demonstrating the importance of native, coal-associated microbial assemblages in biogenic methane production. Specific methanogens were identified in the different treatments and their relative prevalence supported the relative level of methane production. Common coal-associated bacterial groups such as δ- and γ-Proteobacteria, Spirochaetes and Firmicutes were prevalent in different microcosms, though the presence of specific bacteria was not correlated with methane production. These data suggest that while coal oxidation decreased methane production, oxidation was not a primary factor in the variation between microcosm community structures.

1. Introduction

Vast reserves of coal represent an untapped resource that can be used to produce methane gas, a cleaner energy alternative compared to burning oil and coal. According to the last estimates made by the United States Geological Survey (USGS), the total coal resources in the United States are approximately 4 trillion short tons. Of this total, only about 260 billion short tons are viable to mine using current technology (USEIA, 2012). If even a tiny fraction of the remaining coal could be converted to natural gas, then large energy reserves could be generated from previously underutilized material.

Consumption of natural gas in the U.S. is currently increasing (24.1 trillion cubic feet (tcf) in 2010 to 26.6 tcf by 2035 (Conti et al., 2012)), and in order to meet expected demand, unconventional natural gas reservoirs such as subsurface coalbeds are being explored. Coalbed methane (CBM) is methane gas associated with coal in the subsurface, held in place by hydrostatic pressure. Two types of CBM are characterized by their respective formation processes. The first type is thermogenic CBM, which is formed by high pressure and temperature through the coalification process. The second type is biogenic CBM, which is formed via the metabolic activity of microorganisms.

Microorganisms are able to utilize coal as a sole carbon source, producing biogenic methane (Harris et al., 2008; Jones et al., 2010). Methanogenic archaea carry out the process of methanogenesis, generating methane from precursors such as acetate or hydrogen and carbon dioxide. In order to break down a complex substrate such as coal a metabolically diverse microbial community is necessary to generate these precursors from the macromolecules comprising the coal structure. Because of the complexity of this system, the process is not well characterized. The increasing demand for cleaner energy resources has led to enhanced biogenic methane production from coal becoming an active area of research.

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The conversion of coal to methane by microorganisms has been demonstrated by a number of research groups that have handled and maintained anaerobic conditions appropriately (Harris et al., 2008; Jones et al., 2008, 2010; Li et al., 2008); however, coal handling and treatment prior to incubation often goes unreported and may impact biogenic methane production. Additionally, coalbed dewatering (which exposes the coalbed to oxygen) has been suggested to negatively impact the native microorganisms carrying out the process of methanogenesis from coal (Green et al., 2008; Jones, 2012; Klein et al., 2008). To determine the effects of coal oxidation on biogenic methane production, microcosm experiments were conducted using oxidized and un-oxidized coal from the Powder River Basin (PRB).

2. Materials and methods

2.1. Coal sampling and preparation

Powder River Basin (Wyoming and Montana, USA) coal samples were collected from the Big George coal seam (sub-bituminous rank) by filtration from the drill effluent during the process of drilling a CBM well. Once collected, the cuttings were rinsed with sterile, anaerobic de-ionized water and vacuum-sealed in airtight bags with oxygen scrubbers. The samples were kept on ice and transported back to the lab, where they were stored at 4 °C.

Microcosm experiments were designed to assess how exposure of coal to oxygen prior to incubation might influence methane production and the microbial community structure. Oxidized coal was prepared by crushing with a sterile mortar and pestle and exposing to air at room temperature for 48 hours. The oxidized coal was then placed in an anaerobic chamber, with a gas composition of 5% CO_2, 5% H_2 and 90% N_2, for 48 hours to remove residual oxygen. Un-oxidized coal was prepared by crushing, similarly to the oxidized coal, using a sterile mortar and pestle inside the anaerobic chamber. Five grams of oxidized or un-oxidized coal and 50 mL of carbon-free medium were added to 120 mL serum bottles. Medium was prepared as described by Tanner (1997), with the exception of substituting chloride salts when sulfate salts were specified. Each microcosm was inoculated (except un-inoculated controls) using an enrichment culture that was derived from coal (described below). Autoclaved control microcosms were autoclaved twice at 121 ° C and 15 psi for 30 minutes, after all components, including inoculum, were added. All treatments were run in triplicate and are described in Table 1. Microcosms were provided a 4:1 N_2 to CO_2 headspace by flushing for three minutes then pressurizing to 14 kPa. Microcosms were incubated at 30 °C in the dark.

Enrichment cultures were established using coal from the PRB. Five to ten grams of coal were crushed and added, along with 50 mL of the carbon-free medium previously described, to a 120 mL serum bottle. The bottles were sealed and provided an anaerobic headspace of 80% N_2 and 20% CO_2. One milliliter of coal slurry was transferred to fresh coal and medium after incubating for a minimum of 30 days. Enrichment cultures demonstrated the ability to generate methane using coal as a carbon source and were selected as inocula based on methane production values. Inoculum 1 was the first generation of enrichment from BG-1 coal. Inoculum 2 was the third generation enrichment from BG-1 coal, undergoing two transfer events after it was used as Inoculum 1. Inoculum 1 was incubated for 30 days prior to use, while inoculum 2 was incubated for 95 days prior to use.

Table 1
Summary of microcosm test conditions for experiments one and two. All microcosms contained 5 g coal, 50 mL medium and 4:1 N_2/CO_2 headspace. All coal was from the Big George (BG) coal seam (same well and depth), but different sample bags (i.e., BG-1 and BG-2) were used as substrate in each experiment.

Experiment	ID	Coal ID	Coal condition	Inoculum
1	Un-ox.1	BG-1	Un-oxidized	0.5 mL of Inoc.1
	Ox.1	BG-1	Oxidized	0.5 mL of Inoc.1
	Ctrl.1	BG-1	Un-oxidized & Autoclaved	0.5 mL of Inoc.1
2	Un-ox.2	BG-2	Un-oxidized	0.5 mL of Inoc.2
	Ox.2	BG-2	Oxidized	0.5 mL of Inoc.2
	Ctrl.2	BG-2	Un-oxidized & Autoclaved	0.5 mL of Inoc.2
	No-Inoc.2	BG-2	Un-oxidized	No Inoc

2.2. Gas chromatography

A Shimadzu GC-17A was used to measure cumulative headspace methane concentrations. This instrument used a Molsieve with a 5 Å pore size, a HayeSep Q column (2-m × 2-mm i.d.) and a flame ionization detector (FID). The oven and injector remained at 100 °C, while the FID temperature was 200 °C. The helium carrier gas flow rate was 17 mL/min. Methane was detected using the FID, with a retention time of 3.2 minutes. Microcosms were shaken for 30 seconds prior to sampling to promote consistent sample collection. A gas-tight syringe needle was flamed and allowed to cool before collecting 0.1 mL of headspace gas, which was directly injected into the gas chromatograph. Calibration standards of 100 ppm or 1% methane (Scotty Analyzed Gases) were injected at atmospheric pressure for standard curve generation.

2.3. DNA extraction and sequencing

One milliliter of slurry sample was collected for each microcosm at each time point (as displayed in Fig. 1) of the experiments and stored in cryovials at −80 °C until DNA was extracted. Bulk DNA was extracted from coal slurry samples using a MoBio Powersoil® DNA Isolation Kit (MoBio Laboratories). To increase DNA recovery, the method was modified by utilizing all of the supernatant generated (900 μL) after bead beating and centrifuging rather than the 400–500 μL of supernatant specified in the method. The liquid content of the slurry samples was high compared to typical soil samples, so larger volumes of supernatant were generated at this step. This resulted in two “replicates” processed for each extraction, resulting in elution from two filters into one final pool.

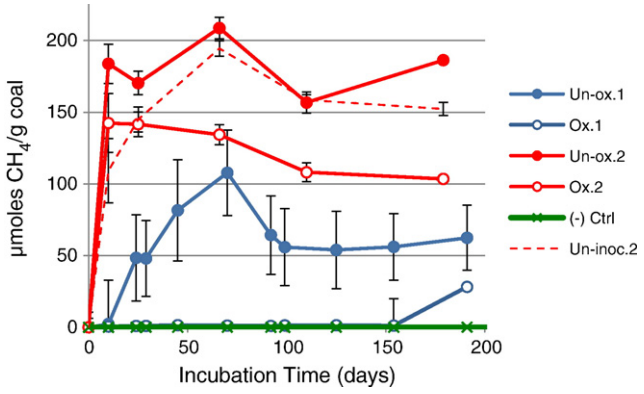

Fig. 1. Cumulative methane production for first (blue) and second (red) oxidized coal experiments. Each point represents an average of three microcosms; error bars represent standard error of triplicate microcosm samples. Oxidized microcosms are represented by open markers; un-oxidized microcosms by closed markers. The dashed line represents un-oxidized, un-inoculated microcosms. Green represents six autoclaved control microcosms from experiments one and two.