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# Enhanced coal-dependent methanogenesis coupled with algal biofuels: Potential water recycle and carbon capture

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ABSTRACT

Many coal beds contain microbial communities that can convert coal to natural gas (coalbed methane). Native microorganisms were obtained from Powder River Basin (PRB) coal seams with a diffusive microbial sampler placed downhole and used as an inoculum for enrichments with different nutrients to investigate microbially-enhanced coalbed methane production (MECoM). Coal-dependent methanogenesis more than doubled when yeast extract (YE) and several less complex components (proteins and amino acids) were added to the laboratory microcosms. Stimulated coal-dependent methanogenesis with peptone was 86% of that with YE while glutamate-stimulated activity was 65% of that with YE, and a vitamin mix had only 33% of the YE stimulated activity. For field application of MECoM, there is interest in identifying cost-effective alternatives to YE and other expensive nutrients. In laboratory studies, adding algal extract (AE) with lipids removed stimulated coal-dependent methanogenesis and the activity was 60% of that with YE at 27 d and almost 90% of YE activity at 1406 d. Analysis of British Thermal Unit (BTU) content of coal (a measure of potential energy yield) from long-term incubations indicated >99.5% of BTU content remained after coalbed methane (CBM) stimulation with either AE or YE. Thus, the coal resource remains largely unchanged following stimulated microbial methane production. Algal CBM stimulation could lead to technologies that utilize coupled biological systems (photosynthesis and methane production) that sustainably enhance CBM production and generate algal biofuels while also sequestering carbon dioxide ($CO_2$).


## 1. Introduction

An increasing world energy demand is creating unprecedented challenges for generating power and mitigating the environmental impacts of developing energy resources. The Powder River Basin (PRB) in northeastern Wyoming and southeastern Montana contains the largest deposits of low-sulfur subbituminous coal in the world (Scott and Luppens, 2013). Biogenic coal bed methane (CBM), natural gas found in many underground coal beds, has been harvested in the PRB since 1993 (Hower et al., 2003). CBM is microbially-generated (biogenic) in the PRB and other shallow subbituminous coal beds around the world (Strąpoć et al., 2011). The combustion of CBM produces less nitrogen oxides, carbon dioxide, sulfur dioxide, and mercury compounds per British thermal unit (BTU) than coal or oil but major sustainability issues arise from current CBM production techniques (Lueken et al., 2016; Meredith et al., 2012). The most economical and widely utilized technology for CBM development in the PRB involves pumping an average of 16,800 gal of water/day/well (Rice and Nuccio, 2000) from a CBM-producing coal bed, recovering the natural gas and disposing the produced water in holding ponds (Bank and Kuuskraa, 2006; Meredith et al., 2012). This type of production has resulted in unsustainable CBM production, with well life spans averaging less than ten years (Meredith et al., 2012) and the construction of over 4000 holding ponds in the PRB containing produced water with elevated sodium and heavy metal concentrations (Hodgskiss et al., 2016; Hower et al., 2003; Sowder et al., 2008).

Laboratory studies and pilot-scale field tests in the PRB have indicated that microbially-enhanced CBM production (MECoM) is possible with nutrient additions such as yeast extract (YE), which consists of the extracted contents of processed yeast with cell walls removed (Green et al., 2008; Ritter et al., 2015). In situ enhancement of CBM

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could extend the life of the CBM wells by increasing the rate and extent of CBM production by the microbial communities (Ritter et al., 2015). However, even the lowest-cost commercial YE ($8.50 per kilogram) can be expensive for ex-situ bioreactors (Solaiman et al., 2007; Vilcáez, 2015; Zhang et al., 2016). To apply this technology basin-wide there is a critical need to identify cost-effective alternatives to YE and other expensive nutrients for MECoM to be economical (Zhang et al., 2016). Regulatory agencies involved with permitting pilot-scale tests of nutrient injections (that included YE) in the PRB have also expressed concerns related to the impact of MECoM on coal quality in regards to BTU content since there is very little information available (Crockett and Wright, 2011). Identifying less expensive alternatives to YE and investigating the impact MECoM could have on the BTU content of coal could enhance field applications of this technology.

For the described study, a diffusive microbial sampler (DMS) was used to capture the active in situ CBM-producing microbial community from a PRB well as previously described (Barnhart et al., 2013). Laboratory enrichments were inoculated with slurry from the DMS and both short and long-term methane measurements indicated YE, and less complex components (glutamate, peptone, or vitamins), enhanced coal-dependent methane production. Algae extract (AE) from lipid-extracted *Scenedesmus* was tested as a cheaper alternative to YE for CBM stimulation by adding AE to enrichments. AE consists of high protein meal residue after lipids have been extracted for biofuel production (Ward et al., 2014). The presented results demonstrate that AE also stimulates methanogenesis in the presence of coal. In addition, the change in coal BTU content also was evaluated in long-term enrichments with both YE and AE to evaluate potential impact on coal quality.

## 2. Methods and materials

### 2.1. Sampling site

The study site was located in southeastern Montana in the PRB. A DMS was used to sample the in situ microbial community as previously described (Barnhart et al., 2013) within well HWC-O1 (45° 7′ 31″ N 106° 28′ 55″ W) which is completed in the Canyon coal seam (Scott and Luppens, 2013). Sediment within the DMS was composed of approximately 25 g of subbituminous coal particles (>2 mm and <4 mm diameter) from the Decker Coal Mine in the PRB. This coal is the same rank as most coal found throughout the PRB, including the Canyon coal seam (Scott and Luppens, 2013). The well was drilled to a depth of 70.7 m, sealed with a packer at 63.1 m, and screened from 63.7 m to 68.2 m. Complete geochemical analysis of groundwater was collected before the DMS was deployed into the well (Table 1). Prior to sampling, the well was flushed by pumping at least three well volumes of water until pH and conductivity were stable. Additional well and water analysis can be obtained from the Montana Bureau of Mines and Geology's Ground Water Information Center (GWIC) ID 8107.

**Table 1**
Geochemistry analysis of groundwater from well HWC-O1 where the DMS was deployed. Values were obtained from the Montana Bureau of Mines and Geology's Groundwater Information Center (GWIC).

| Major ion results | | | |
|---|---|---|---|
| | mg/L | | mg/L |
| Calcium (Ca) | 10.7 | Bicarbonate ($HCO_3$) | 1749.50 |
| Magnesium (Mg) | 2.35 | Carbonate ($CO_3$) | 0 |
| Sodium (Na) | 590 | Chloride (Cl) | 21.32 |
| Potassium (K) | 5.76 | Sulfate ($SO_4$) | <12.5 |
| Iron (Fe) | 0.124 | Nitrate (as N) | <0.25 |
| Manganese (Mn) | 0.005 | Fluoride (F) | 4.19 |
| Silica ($SiO_2$) | 8.63 | Orthophosphate (as P) | <0.25 |

### 2.2. Microbial enrichments

Slurry (3 mL) from the DMS was added to modified anaerobic co-culture medium (47 mL) (CCM). CCM is a defined medium for the growth of methanogens and anaerobic bacteria that would allow the direct comparison of nutrient additions. The modified CCM contained (per liter) 3.86 mg $MgCl_2 \cdot 6H_2O$, 5.21 mg $CaCl_2 \cdot 2H_2O$, 0.5 g $NH_4Cl$, and 5 mg KCl and was buffered with 1.1 mM $K_2HPO_4$ and 1.04 g/L $NaHCO_3$. One milliliter per liter of 1000 × nonchelated trace elements and 1 mL per liter of 1000 × vitamin solution amended with 2.0 g/L choline chloride were added as growth supplements as previously described (Walker et al., 2009). L-Cysteine·HCl (1 mM) and sulfide (1 mM as $Na_2S \cdot 9H_2O$) were added as reducing agents. Resazurin (1 mg/L) was added as a redox indicator. Stock solutions of $K_2HPO_4$ (1 M), $NaHCO_3$ (6.0 M), L-cysteine·HCl (1 M), $Na_2S \cdot 9H_2O$ (1 M), and the nonchelated trace element and vitamin mixtures were prepared under anoxic conditions as previously described (Walker et al., 2009). The coal used in the experiments was obtained from the Decker Coal Mine in the PRB (MT) by the Montana Bureau of Mines and Geology. The enrichments were incubated in the dark at 25 °C and methane production was monitored via a direct injection onto a SRI 8610C gas chromatograph (GC) with a thermal conductivity detector (TCD) and a stainless-steel molecular sieve 13× packed column (6 ft × 1/8″ O.D.) with helium as the carrier gas. After methane production was detected, 1 mL of inoculated media was used to inoculate fresh modified CCM media amended with 1 g/L YE along with controls with and without 1 g of coal. When methane production was detected, 1 mL of inoculated media from a coal-only enrichment was used to inoculate 9 mL of modified CCM media containing 1 g coal in triplicate with either: 1 g/L peptone, 0.63 g/L sodium glutamate (calculated based on 10.4% of total amino acid analysis of YE) (BD, 2006), 1 g/L YE or 2 mL/L vitamin solution (Walker et al., 2009) along with controls to investigate components of YE that stimulate methane production.

### 2.3. Preparation of algal extract

*Scenedesmus* WC-1 was grown in a low-density polyethylene bag reactor (6 mil wall thickness) containing 20 L of Bolds media (Nichols and Bold, 1965) under 14/10 h light/dark with approximately 75 mol photons $m^{-2}$ $s^{-1}$. The reactor was continuously bubbled with air. Cells were harvested after two weeks of growth using centrifugation at 4000 ×*g* followed by lyophilization. "Lipid-free" biomass was prepared using sonication-assisted solvent extraction. Briefly, 100 mg portions of dry cell mass were suspended in 5 mL triple solvent (1:1:1, chloroform:tetrahydrofuran:hexane) and sonicated three times for 20 s with a Branson S-450D Sonicator® equipped with a microtip probe set to 80 W (Branson, Danbury, CT). The disrupted cell suspension was centrifuged at 3000 ×*g* for 30 s and the supernatant was removed. Extraction of the remaining biomass was repeated two more times using 5 mL of fresh triple solvent for each cycle. The residual cellular material was air dried and stored at −20 °C prior to use in the growth experiments. 1 mL of media from a coal-only enrichment from modified CCM media (Walker et al., 2009) was used to inoculate enrichments containing either 1 g/L YE or 1 g/L algae extract (AE) along with coal-only controls.

### 2.4. BTU analysis of coal from enrichments

Slurry from the DMS (3 mL) was added to triplicate microcosms containing 5 g of coal with previously described modified anaerobic co-culture medium (CCM). The triplicate microcosms were stimulated with either 0.5 g YE or 0.5 g AE along with unstimulated and uninoculated controls. Methane production was monitored as previously described and coal from one of the long-term enrichments from each treatment and unaltered coal (e.g. original coal that was placed in media but not inoculated with microorganisms) was characterized to evaluate any