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

Authors: Elliott P. Barnhart, Katherine K. Davis, Matthew Varonka, William Orem, Alfred B. Cunningham, Bradley D. Ramsay, & Matthew W. Fields

Barnhart EP, Davis KJ, Varonka M, Orem W, Cunningham AB, Ramsay BD, Fields MW, "Enhanced coal-dependent methanogenesis coupled with algal biofuels: Potential water recycle and carbon capture," International Journal of Coal Geology, 2017 February; 171:69-75. doi: 10.1016/j.coal.2017.01.001.

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

Elliott P. Barnhart, Katherine J. Davis , Matthew Varonka , William Orem, Alfred B. Cunningham, Bradley D. Ramsay, & Matthew W. Fields

Keywords:
Stimulated coal methanogenesis
Coal natural gas

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-en-hanced 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 mi-crocosms. 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 N99.5% 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).

An increasing world energy demand is creating unprecedented chal-lenges for generating power and mitigating the environmental impacts of developing energy resources. The Powder River Basin (PRB) in north-eastern Wyoming and southeastern Montana contains the largest de-posits 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 tech-nology 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 pro-duced 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 indicat-ed 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

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

changes to the bulk coal resulting from biodegradation (Table 2). Coal characterization included: total moisture, ash, sulfur, and gross caloric value (Geochemical Testing, Somerset, PA) according to American Society for Testing and Materials (ASTM) methods D2961-02, D3174-04, D4239-08, and D5865-07a, respectively. All values were reported on a dry weight basis.

3. Results

3.1. Biostimulation of the coal-dependent community

Yeast extract (YE) contains many nutrients that could be the key stimulant for enhanced methane production including complex proteins, vitamins, and amino acids. Therefore, representative factors of YE (e.g., peptone, vitamins, glutamate) were tested for stimulation of coal-dependent methane production with the microbial consortium in the absence and presence of coal. Aliquots removed from a triplicate methane-producing coal enrichment that had not previously been exposed to YE were added to the different enrichments with coal and without coal as controls. Methane was measured in the headspace of these enrichments after 60 days and 752 days to analyze short and long-term enhancement potential of the constituents.

Within 60 days, the coal only (Media + Coal) produced more methane than the no addition (Media), YE only, peptone only, glutamate only, and vitamin only treatments (Fig. 1). Only after longer incubation (752 d) did the YE-only, peptone-only, and glutamate-only treatments produce more methane than the coal-only with peptone being the highest. However, when coal was combined with YE, peptone, or glutamate (YE > Peptone > glutamate), the observed methane was higher than both coal-only and the respective stimulant-only (Fig. 1). The addition of vitamins only increased the methane in the longer-term incubation, and the additional mixed vitamins had a marginal effect on methane production (approximately one-third of the activity observed with YE). Peptone and glutamate increased methane production in coal treatments but was 86% and 65%, respectively, of that observed with YE (Fig. 1).

3.2. Algae enhanced CBM production

The CBM enhancement potential of algae extract (AE) was investigated by adding AE with lipids removed to similar enrichments in a defined medium as was done with the other stimulants (e.g., YE, peptone, glutamate, vitamins) (Fig. 2). The coal-only treatments (n = 3) averaged 56 ± 51 μg of methane within 27 d while the YE-only treatments averaged 62 ± 54 μg and the AE-only sample did not produce methane above the detection limit (Fig. 2). At 165 d, the YE-only samples averaged 648 ± 451 μg and the AE-only treatments had only 230 ± 391 μg. For the treatment with coal + stimulant at day 27, the YE + coal and AE + coal treatments averaged 385 ± 101 μg and 228 ± 26 μg of methane, respectively. Within 165 d, the coal only cultures averaged 341 ± 89 μg of methane compared to 1400 ± 313 μg and 920 ± 95 μg for YE + coal and AE + coal stimulated treatments, respectively. At this time point, the AE incubation was 65% of that with YE. After longer-term incubations (1400 d), the AE + coal incubation was 86% of that achieved with the YE + coal incubation. When comparing the cumulative methane generated from coal-only plus the stimulant-only to the actual methane generated in the presence of both (i.e., coal + stimulant), the AE treatment gave 1.6-fold increase in realized methane compared to a 1.4-fold increase in methane realized for the YE treatment (Fig. 2).

3.3. BTU analysis of coal from long-term enrichments

Microcosm methane measurements indicated most of the methane stimulation occurred within the first 60 days (Table 2). Analysis of BTU content of coal from long-term incubations (1169 days) compared to unaltered coal indicated >99.5% of BTU content remained after CBM stimulation with either AE or YE (Table 2). The variation between the coal samples was within the margin of error of the analysis, and the results indicated the BTU values for the tested coal were unchanged after the stimulation of coal-dependent methanogenesis for the tested time period.

4. Discussion

Biogenic methane production, carried out by methanogenic *Archaea* (methanogens), is an essential part of the global carbon cycle that promotes the breakdown of complex organic material by *Bacteria* that would otherwise be thermodynamically unfavorable (syntrophic degradation) (McInerney et al., 2009). Previous studies have demonstrated many coal beds contain the requisite microbial consortia capable of methanogenesis (Barnhart et al., 2013, 2016; Green et al., 2008; Guo et al., 2012; Harris et al., 2008; Jones et al., 2013; Krüger et al., 2008; Penner et al., 2010; Strąpoć et al., 2008, 2011; Thielemann et al., 2004) and that the consortia are active in situ (Ulrich and Bower, 2008). With respect to the global carbon cycle, little is known about the turnover of recalcitrant organic matter in the terrestrial subsurface (i.e., coal) and the implications for CH_4 generation and the potential for CO_2 re-capture. The anaerobic breakdown of complex carbon molecules is usually very slow but can be stimulated with the addition of certain nutrients (Cunningham et al., 2001). While the process of microbial conversion of coal to methane is poorly understood, it has been hypothesized that soluble organics are released from the coal by degradative bacteria, and the organic by-products are ultimately degraded further to metabolites that can be utilized by methanogens (archaea) to produce methane (Jones et al., 2010; Orem et al., 2010; Ritter et al., 2015). Recent research has indicated in certain circumstances, a single methanogen is capable of making methane from many different

Table 2
Average methane production and coal characterization of total moisture, ash, sulfur, and gross caloric values from long-term enrichment cultures with coal-only (Coal) algae extract added (Coal AE) and yeast extract added (Coal YE) along with an uninoculated control (Neg. Control). The final methane measurement (1229 days) was from the microcosms that were not destructively sampled for coal analysis.

	Neg. control	Media + Coal	Media + Coal + AE	Media + Coal + YE
Average CH_4 produced (μg CH_4/g coal) (60 days)	0	311 ± 51	528 ± 100	928 ± 134
Average CH_4 produced (μg CH_4/g coal) (261 days)	0	282 ± 33	486 ± 200	855 ± 28
CH_4 produced (μg CH_4/g coal) (1229 days)	0	232	576	1052
Total coal moisture	4.12	4.15	4.28	4.11
Coal ash	3.20	3.32	3.37	2.95
Coal sulfur	0.32	0.34	0.40	0.35
Coal BTU/LB	11,710	11,714	11,661	11,692

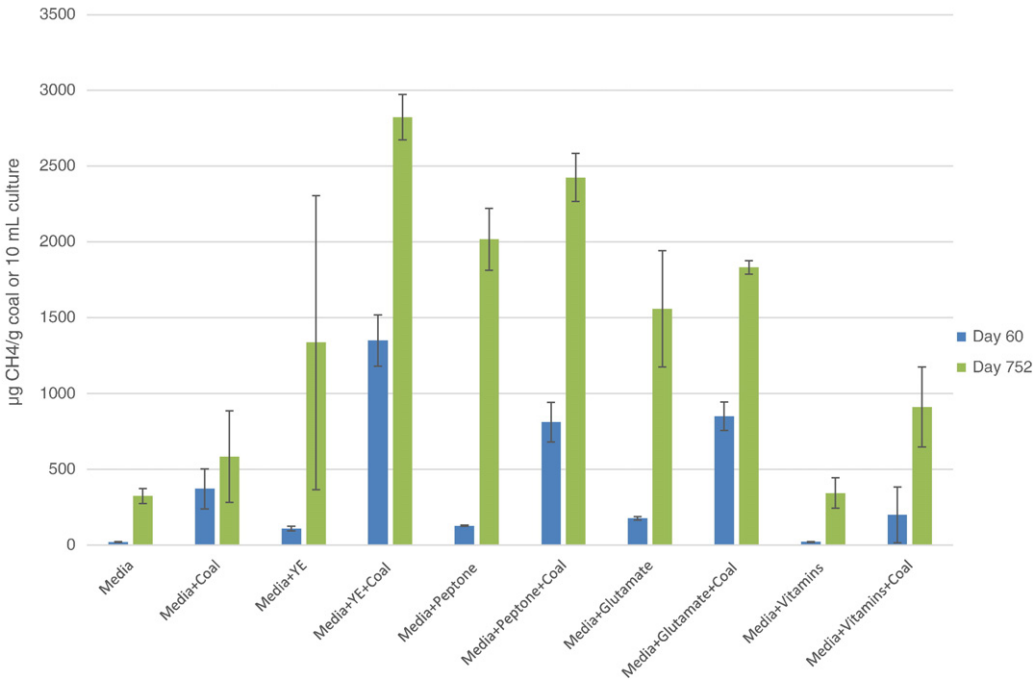

Fig. 1. Short and long-term CBM enhancement of yeast extract components: short and long-term (60 and 752 days) methane measurements indicate individual components of yeast extract stimulate CBM production. Peptone enrichments represent the protein fraction of yeast extract and glutamate enrichments represent the major amino acid in yeast extract. Error bars represent 1 standard deviation for the triplicate enrichments.

methoxylated aromatic compounds commonly observed in coal (Mayumi et al., 2016); and therefore, unique metabolism(s) are likely to be discovered. However, given the recalcitrant nature of coal and the potentially limiting levels of available nitrogen and phosphorus, overall microbial activity can be limited. Nutrient supplementation, for example with YE, has been demonstrated to trigger additional methane production in laboratory coal experiments (Barnhart et al., 2013; Beckmann et al., 2011; Faiz and Hendry, 2006; Flores et al., 2008; Green et al., 2008; Guo et al., 2012; Jones et al., 2010; Klein et al., 2008; Penner et al., 2010; Strąpoć et al., 2008, 2011; Ulrich and Bower, 2008; Unal et al., 2012), but little is known about specific compounds or the relationship to more natural components that may act in situ.

Nutrient additions with YE have also been previously shown to stimulate acetate and biosurfactant production by bacteria (Konishi et al., 2011; Liria et al., 1998; Qazi et al., 2013) and this may indirectly stimulate overall methanogenesis. In the described work, YE, peptone and glutamate (a major component in YE) increased methane production in the presence of coal (Fig. 1). YE comprises the water-soluble components of the lysed yeast cell, and is primarily composed of amino-acids, peptides, carbohydrates and salts. However, when coal is incubated with YE, observed methane is higher than with coal-only or YE-only, and these data suggest a synergistic and stimulatory effect that is not merely methanogenic activity from the stimulant alone. Enrichments were used to evaluate the methane stimulating contribution of different components in YE: peptone added to enrichments to represent the peptides and carbohydrates, glutamate represented a predominant amino acid, and mixed vitamins signified the contribution of added trace elements/vitamins. The ability of glutamate and peptone alone to stimulate CBM production suggested that nitrogenous compounds or proteins could stimulate coal-dependent methanogenesis. The low level of observed stimulation with vitamins alone suggested that nitrogen contributed significantly to the stimulatory effect of YE. Previous enrichment studies suggest complex organic nutrients such as YE and tryptone, stimulate CBM production, whereas the addition of non-nitrogenous

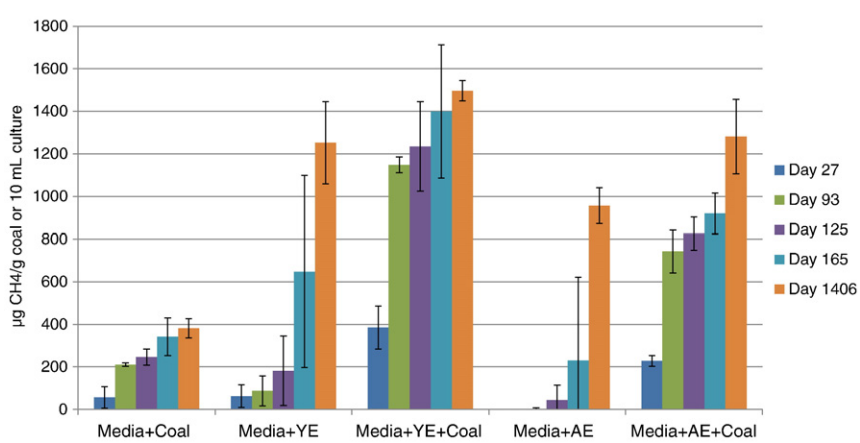

Fig. 2. Algae stimulated CBM: methane measurements indicate algae extract (AE) with lipids removed increased CBM production compared to the control without nutrients added (Bicarb coal). CBM production from AE was slightly less than with yeast extract (YE) but the trend was very similar.

nutrients failed to stimulate CBM production (Barker and Dallegge, 2006; Green et al., 2008; Penner et al., 2010). Yeast extract is a heterogeneous mixture of biomass, and further work is needed to further discern other components of YE that may contribute to stimulation of coal-dependent methanogenesis.

For possible large-scale application, YE could be potentially cost-prohibitive and carbon intensive. However, the use of AE has several advantages that promote economic feasibility and reduces the carbon/water footprint of CBM operations. Algae can be cultivated in low-quality water, utilize sunlight for energy, and incorporate CO_2 as a carbon source (Fields et al., 2014). In addition, some algae accumulate lipid/oil as a carbon/energy reserve, and the lipid/oil can be utilized for biodiesel production (Fields et al., 2014). In order to ascertain the feasibility of AE for the stimulation of coal-dependent methanogenesis, we compared the ability of AE to stimulate methane production with YE and major components of YE.

The presented results demonstrated that AE could stimulate coal-dependent methanogenesis in a similar fashion to YE. In addition, lipids were first extracted from the algal biomass so that the AE represented residual biomass that would remain after processing for biodiesel production. Several studies have investigated the use of residual algal biomass for anaerobic digestion (Kinnunen et al., 2014; Rashid et al., 2013), but the use as a stimulant for coal-dependent methanogenesis has not been previously reported. In addition, our results indicated the BTU values for the tested coal were unchanged after the stimulation of coal-dependent methanogenesis for the tested time period with either YE or AE.

We recently reported the growth of a lipid-accumulating alga in CBM production water that could promote on-site growth of algal biomass directly in CBM production water (Hodgskiss et al., 2016). Therefore, the described research demonstrates that the production of domestic natural gas could be coupled with phototrophic biofuels while at the same time recycling wastewater, nutrients, and CO_2 thereby substantially reducing impacts to the atmosphere, surface waters, and subsurface environments associated with CBM production. Water management is one of the largest expenses involved with CBM production in the Powder River Basin (PRB) of Montana and Wyoming (Brinck et al., 2008). The CBM produced water ponds naturally grow algae which could be harvested for biofuels and used to stimulate further CBM production with existing infrastructure (Hodgskiss et al., 2016; Sowder et al., 2008). Fig. 3 outlines the conceptual integration of CBM production activities and phototrophic biofuel generation by showing links between these seemingly disparate research and technology development areas. The proposed integration would transform CBM production by mitigating environmental impacts and producing value added products. In addition to providing a bioavailable substrate for acetate production, lysed algal biomass could also provide a nitrogen source based on an average composition of microalgae given by $C_{2.08}H_{3.81}O_{1.0}N_{0.2}P_{0.02}$ (Grobbelaar, 2004; Heaven et al., 2011). A thorough investigation of many production wells throughout the PRB provided a positive correlation between nitrogen levels and methane production, but the source of the nitrogen was not determined (Meredith et al., 2011). A recent study detected a high proportion (>15%) of phototrophic DNA sequences in a well on the western margin of the PRB (Barnhart et al., 2013). Algae and cyanobacteria percolating into subsurface coal beds with recharge could provide an allochthonous source of carbon and nutrients which could be used to enhance methanogenesis just as yeast cell components stimulate methanogenesis in laboratory enrichments. However, further work is needed to discern the potential impact of phototrophic biomass associated with re-charge from surface waters.

The described research provides a link between green energy development and CBM production while providing a beneficial use for the water produced from CBM development. Large scale production of algae for biofuels is unsustainable with existing technologies (National Research Council, 2012). Algal biofuel production has been limited, partially due to water access for algal growth (NRC, 2012). The mostly undeveloped Montana portion of the PRB is much smaller than the Wyoming portion but still could have almost 25,000 CBM production wells at full capacity (Bureau of Land Management, 2007), which could produce >1.100,000 ha-m of groundwater (ALL, 2001; Meyers, 2009). High concentrations of sodium bicarbonate are the geochemical signature of CBM water in the PRB (Van Voast, 2003). Although sodium bicarbonate can negatively impact irrigated soils, it may provide an excellent media for algal biofuel production because of the recently documented ability of sodium bicarbonate to stimulate lipid production in algae (Gardner et al., 2012). In addition to biofuels, algae also offer a diverse spectrum of valuable products such as food, nutritional compounds, omega-3 fatty acids, and animal feed that could provide additional revenue for this energy generating system. The addition of AE to laboratory CBM-producing enrichments greatly enhanced CBM production in this study. Further work is needed to better understand

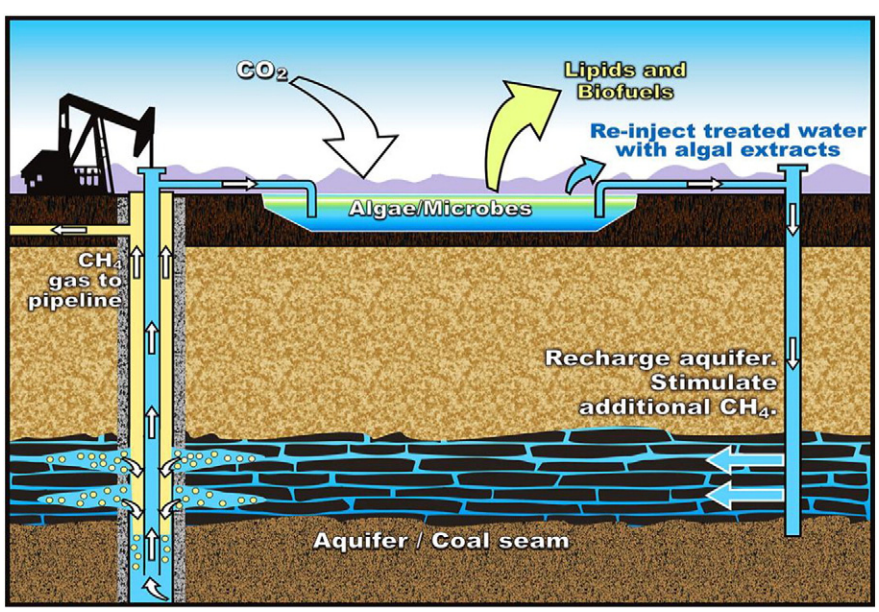

Fig. 3. Conceptual model of coal bed methane (CBM) stimulation with algae. The growth of algae for CBM stimulation and lipid production could productively utilize the CBM wastewater ponds already in place in the PRB from previous development. Algal residues remaining after lipid extraction could stimulate CBM production similarly to yeast extract.

how the different components of algal biomass can impact coal-dependent methanogenesis both ex situ and in situ.

Acknowledgements

The authors would like to thank Peg Dirkx for generating the conceptual model figure. The authors would also like to thank Dr. Robin Gerlach for computational assistance and methodological guidance. The authors would also like to thank Dr. Elizabeth Meredith, John Wheaton, Simon Bierbach and Erika Peters at the Montana Bureau of Mines and Geology for providing access to well HWC-01, groundwater geochemistry data and field assistance. The authors would like to thank Leslie Ruppert and Arthur Clark for insightful discussions about field applications. The authors would like to thank Drs. Richard Macur and Jacob Valenzuela for providing the lipid-extracted algae and Jacob Valenzuela. The authors would also like to thank Dr. Kristen Brileya for culturing assistance. The presented work was partially supported by the Department of Energy under Award Number DE-FE0024068 and by the U.S. Geological Survey Energy Resources Program. This project was also supported in part by DOE ZERT Program under grant no. DE-FC26-04NT42262.

References

ALL, 2001. Water resources technical report. Montana statewide oil and gas environmental impact statement and amendment of the Powder River and Billings resource management plans. Prepared for US Bureau of Land Management, Miles City, Montana. All Consulting, Tulsa.

Bank, G.C., Kuuskraa, V.A., 2006. The Economics of Powder River Basin Coalbed Methane Development: DOE Report 1–6.

Barker, C.E., Dallegge, T., 2006. Secondary gas emissions during coal desorption, Marathon Grassim Oskolkoff-1 Well, Cook Inlet Basin, Alaska: implications for resource assessment. Bull. Can. Petrol. Geol. 54, 273–291.

Barnhart, E.P., De Leon, K.B., Ramsay, B.D., Cunningham, A.B., Fields, M.W., 2013. Investigation of coal-associated bacterial and archaeal populations from a diffusive microbial sampler (DMS). Int. J. Coal Geol. 115, 64–70.

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., Fields, M.W., 2016. Hydrogeochemistry and coal-associated bacterial populations from a methanogenic coal bed. Int. J. Coal Geol. 162:14–26. http://dx.doi.org/10.1016/j.coal.2016.05.001.

BD, 2006. In: Third Ed. Revised (Ed.), Bionutrients Tech. Manual. Becton, Dickinson Company, pp. 1–68.

Beckmann, S., Lueders, T., Krüger, M., Von Netzer, F., Engelen, B., Cypionka, H., 2011. Acetogens and acetoclastic methanosarcinales govern methane formation in abandoned coal mines. Appl. Environ. Microbiol. 77, 3749–3756.

BLM, 2007. State Oil and Gas Final Environmental Impact Statement and Amendment of the Powder River and Billings Resource Management Plan. Bureau of Land Management, Miles City, Montana.

Brinck, E.L., Drever, J.I., Frost, C.D., 2008. The geochemical evolution of water coproduced with coalbed natural gas in the Powder River Basin, Wyoming. Environ. Geosci. 15, 153–171.

Crockett, F.J., Wright, S.S., 2011. Analysis of Impacts of Methane Farming on Federal Coal in the Powder River Basin, Wyoming. https://www.blm.gov/style/medialib/blm/wy/information/NEPA/bfodocs/rough-draw.Par.59112.File.dat/Exh4-methane.pdf.

Cunningham, J.A., Rahme, H., Hopkins, G.D., Lebron, C., Reinhard, M., 2001. Enhanced in situ bioremediation of BTEX-contaminated groundwater by combined injection of nitrate and sulfate. Environ. Sci. Technol. 35, 1663–1670.

Faiz, M., Hendry, P., 2006. Significance of microbial activity in Australian coal bed methane reservoirs — a review. Bull. Can. Petrol. Geol. 54:261–272. http://dx.doi.org/10.2113/gscpgbull.54.3.261.

Fields, M.W., Hise, A., Lohman, E., Bell, T., Gardner, R., Corredor, L.F.A., Moll, K., Peyton, B.M., Characklis, G.W., Gerlach, R., 2014. Sources and Re-sources: importance of nutrients, resource allocation, and ecology in microalgal cultivation for lipid accumulation. Appl. Microbiol. Biotechnol. 98, 4805–4816.

Flores, R.M., Rice, C.A., Stricker, G.D., Warden, A., Ellis, M.S., 2008. Methanogenic pathways of coal-bed gas in the Powder River Basin, United States: the geologic factor. Int. J. Coal Geol. 76:52–75. http://dx.doi.org/10.1016/j.coal.2008.02.005.

Gardner, R.D., Cooksey, K.E., Mus, F., Macur, R., Moll, K., Eustance, E., Carlson, R.P., Gerlach, R., Fields, M.W., Peyton, B.M., 2012. Use of sodium bicarbonate to stimulate triacylglycerol accumulation in the chlorophyte *Scenedesmus* sp. and the diatom *Phaeodactylum tricornutum*. J. Appl. Phycol. 10:569–572. http://dx.doi.org/10.1007/s10811-011-9782-0.

Green, M.S., Flanegan, K.C., Gilcrease, P.C., 2008. Characterization of a methanogenic consortium enriched from a coalbed methane well in the Powder River Basin, U.S.A. Int. J. Coal Geol. 76:34–45. http://dx.doi.org/10.1016/j.coal.2008.05.001.

Grobbelaar, J.U., 2004. Algal Nutrition: Mineral Nutrition, Handbook of Microalgal Culture: Biotechnology and Applied Phycology.

Guo, H., Yu, Z., Liu, R., Zhang, H., Zhong, Q., Xiong, Z., 2012. Methylotrophic methanogenesis governs the biogenic coal bed methane formation in Eastern Ordos Basin, China. Appl. Microbiol. Biotechnol. 96:1587–1597. http://dx.doi.org/10.1007/s00253-012-3889-3.

Harris, S.H., Smith, R.L., Barker, C.E., 2008. Microbial and chemical factors influencing methane production in laboratory incubations of low-rank subsurface coals. Int. J. Coal Geol. 76:46–51. http://dx.doi.org/10.1016/j.coal.2008.05.019.

Heaven, S., Milledge, J., Zhang, Y., 2011. Comments on "anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable". Biotechnol. Adv. 29: 164–167. http://dx.doi.org/10.1016/j.biotechadv.2010.10.005.

Hodgskiss, L.H., Nagy, J., Barnhart, E.P., Cunningham, A.B., Fields, M.W., 2016. Cultivation of a native alga for biomass and biofuel accumulation in coal bed methane production water. Algal Res. 19:63–68. http://dx.doi.org/10.1016/j.algal.2016.07.014.

Hower, T.L., Jones, J.E., Goldstein, D.M., Harbridge, W., 2003. Development of the Wyodak coalbed methane resource in the Powder River Basin. Soc. Pet. Eng. J. 1–9 SPE Annual.

Jones, E.J.P., Voytek, M.A., Corum, M.D., Orem, W.H., 2010. Stimulation of methane generation from nonproductive coal by addition of nutrients or a microbial consortium. Appl. Environ. Microbiol. 76, 7013–7022.

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., 2013. The effect of coal bed dewatering and partial oxidation on biogenic methane potential. Int. J. Coal Geol. 115:54–63. http://dx.doi.org/10.1016/j.coal.2013.03.011.

Kinnunen, H.V., Koskinen, P.E.P., Rintala, J., 2014. Mesophilic and thermophilic anaerobic laboratory-scale digestion of *Nannochloropsis* microalga residues. Bioresour. Technol. 155, 314–322.

Klein, D.A., Flores, R.M., Venot, C., Gabbert, K., Schmidt, R., Stricker, G.D., Pruden, A., Mandernack, K., 2008. Molecular sequences derived from Paleocene Fort Union Formation coals vs. associated produced waters: implications for CBM regeneration. Int. J. Coal Geol. 76:3–13. http://dx.doi.org/10.1016/j.coal.2008.05.023.

Konishi, M., Nagahama, T., Fukuoka, T., Morita, T., Imura, T., Kitamoto, D., Hatada, Y., 2011. Yeast extract stimulates production of glycolipid biosurfactants, mannosylerythritol lipids, by *Pseudozyma hubeiensis* SY62. J. Biosci. Bioeng. 111:702–705. http://dx.doi.org/10.1016/j.jbiosc.2011.02.004.

Krüger, M., Beckmann, S., Engelen, B., Thielemann, T., Cramer, B., Schippers, A., Cypionka, H., 2008. Microbial methane formation from hard coal and timber in an abandoned coal mine. Geomicrobiol J. 25:315–321. http://dx.doi.org/10.1080/01490450802258402.

Liria, C.W., Kilikian, B.V., Sua, D.C., 1998. Effect of yeast extract on *Escherichia coli* growth and acetic acid production. J. Microbiol. 14, 331–335.

Lueken, R., Klima, K., Griffin, W.M., Apt, J., 2016. The climate and health effects of a USA switch from coal to gas electricity generation. Energy 109, 1160–1166.

Mayumi, D., Mochimaru, H., Tamaki, H., Yamamoto, K., Yoshjoka, H., Suzuki, Y., Kamagata, Y., Sakata, S., 2016. Methane production from coal by a single methanogen. Science 222–225.

McInerney, M.J., Sieber, J.R., Gunsalus, R.P., 2009. Syntrophy in anaerobic global carbon cycles. Curr. Opin. Biotechnol. 20, 623–632.

Meredith, E., Kuzara, S., Wheaton, J.R., Bierbach, S., Chandler, K., Donato, T., Gunderson, J., Schwartz, C., 2011. 2010 Annual Coalbed Methane Regional Groundwater Monitoring Report: Powder River Basin, Montana. MBMG, p. 600.

Meredith, E., Wheaton, J., Kuzara, S., 2012. Coalbed-Methane Basics: Ten Years of Lessons from the Powder River Basin. 6. Mont. Bur. Mines Geol. Inf. Pam.

Meyers, T., 2009. Groundwater management and coal bed methane development in the Powder River of Montana. J. Hydrol. 368, 178–193.

Nichols, H.W., Bold, H.C., 1965. *Trichosarcina polymorpha* Gen. et sp. Nov. J. Phycol. 1, 34–38.

NRC, 2012. National Research Council: Sustainable Development of Algal Biofuels in the United States. Natl. Acad. Press.

Orem, W.H., Voytek, M.A., Jones, E.J., Lerch, H.E., Bates, A.L., Corum, M.D., Warwick, P.D., Clark, A.C., 2010. Organic intermediates in the anaerobic biodegradation of coal to methane under laboratory conditions. Org. Geochem. 41, 997–1000.

Penner, T.J., Foght, J.M., Budwill, K., 2010. Microbial diversity of western Canadian subsurface coal beds and methanogenic coal enrichment cultures. Int. J. Coal Geol. 82:81–93. http://dx.doi.org/10.1016/j.coal.2010.02.002.

Qazi, M.A., Malik, Z.A., Qureshi, G.D., Hameed, A., Ahmed, S., 2013. Yeast extract as the most preferable substrate for optimized biosurfactant production by rhlB gene positive *Pseudomonas putida* SOL-10 isolate. J. Bioremed. Biodegr. 4:204. http://dx.doi.org/10.4172/2155-6199.1000204.

Rashid, N., Rehman, M.S.U., Han, J.I., 2013. Recycling and reuse of spent microalgal biomass for sustainable biofuels. Biochem. Eng. J. 75, 101–107.

Rice, C., Nuccio, V., 2000. Water Produced with Coal-bed Methane: U.S. Geological Survey Fact Sheet 156-00. USGS.

Ritter, D., Vinson, D., Barnhart, E., Akob, D.M., Fields, M.W., Cunningham, A.B., Orem, W., McIntosh, J.C., 2015. Enhanced microbial coalbed methane generation: a review of research, commercial activity, and remaining challenges. Int. J. Coal Geol. 146:28–41. http://dx.doi.org/10.1016/j.coal.2015.04.013.

Scott, D.C., Luppens, J.A., 2013. Assessment of Coal Geology, Resources, and Reserve Base in the Powder River Basin, Wyoming and Montana: U.S. Geological Survey Fact Sheet 2012–3143. USGS.

Solaiman, D.K.Y., Ashby, R.D., Zerkowski, J.A., Foglia, T.A., 2007. Simplified soy molasses-based medium for reduced-cost production of sophorolipids by *Candida bombicola*. Biotechnol. Lett. 29, 1341–1347.

Sowder, J.T., Kelleners, T.J., Reddy, K.J., 2008. The origin and fate of arsenic in coalbed natural gas-produced water ponds. J. Environ. Qual. 39, 1604–1615.

Strąpoć, D., Picardal, F.W., Turich, C., Schaperdoth, I., Macalady, J.L., Lipp, J.S., Lin, Y.-S., Ertefai, T.F., Schubotz, F., Hinrichs, K.-U., Mastalerz, M., Schimmelmann, A., 2008. Methane-producing microbial community in a coal bed of the Illinois Basin. Appl. Environ. Microbiol. 74, 2424–2432.

Strąpoć, D., Mastalerz, M., Dawson, K., Macalady, J., Callaghan, A.V., Wawrik, B., Turich, C., Ashby, M., 2011. Biogeochemistry of microbial coal-bed methane. Annu. Rev. Earth Planet. Sci. 39:617–656. http://dx.doi.org/10.1146/annurev-earth-040610-133343.

Thielemann, T., Cramer, B., Schippers, A., 2004. Coalbed methane in the Ruhr Basin, Germany: a renewable energy resource? Org. Geochem. 35:1537–1549. http://dx.doi.org/10.1016/j.orggeochem.2004.05.004.

Ulrich, G., Bower, S., 2008. Active methanogenesis and acetate utilization in Powder River Basin coals, United States. Int. J. Coal Geol. 76, 25–33.

Unal, B., Perry, V.R., Sheth, M., Gomez-Alvarez, V., Chin, K.-J., Nüsslein, K., 2012. Trace elements affect methanogenic activity and diversity in enrichments from subsurface coal bed produced water. Front. Microbiol. 3:175. http://dx.doi.org/10.3389/fmicb.2012.00175.

Van Voast, W., 2003. Geochemical signature of formation waters associated with coalbed methane. Am. Assoc. Pet. Geol. Bull. 87:667–676. http://dx.doi.org/10.1306/10300201079.

Vilcáez, J., 2015. Stimulating effect of protein-rich matter on the biogenic conversion of CO_2 to CH_4. J. CO_2 Util. 10, 60–66.

Walker, C.B., He, Z., Yang, Z.K., Ringbauer, J.A., He, Q., Zhou, J., Voordouw, G., Wall, J.D., Arkin, A.P., Hazen, T.C., Stolyar, S., Stahl, D.A., 2009. The electron transfer system of syntrophically grown *Desulfovibrio vulgaris*. J. Bacteriol. 191, 5793–5801.

Ward, A.J., Lewis, D.M., Green, F.B., 2014. Anaerobic digestion of algae biomass: a review. Algal Res. 5, 204–214.

Zhang, J., Park, S.Y., Liang, Y., Harpalani, S., 2016. Finding cost-effective nutrient solutions and evaluating environmental conditions for biogasifying bituminous coal to methane ex situ. Appl. Energy 165, 559–568.