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Enhancing biogenic methane generation from a brown coal by combining different microbial communities

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ABSTRACT

The potential of microbial enrichment for enhancing methanogenesis in brown coal was investigated by using microbial communities from coal formation water and a mangrove swamp as treatments. After 30 days of incubation with a 'mixed' culture, both the rates and yields of methane generation were enhanced compared to microbial enrichment cultures having just a single origin. The microbial community derived from a mangrove swamp alone, appeared to lack the ability to degrade coal. The pH of the mixed origin treatment was favourable for growth of the mangrove derived microbial community possibly explaining the higher gas yield observed with this culture.

1. Introduction

Methane is commonly generated under anaerobic conditions (Bohutskyi and Bouwer, 2013) in environments such as rice fields (Chen et al., 2013; Neue et al., 1996), wetlands, the gastrointestinal tract of animals, and within coal and oil deposits (Jones et al., 2008; Moore, 2012). Concerns about climate change have seen rising interest in methane as a 'bridge' fuel, spanning the gap between coal-fired electricity generation and a low-carbon renewable energy (Flores et al., 1997; Flores, 1998; Hamawand et al., 2013; Park and Liang, 2016; Ritter et al., 2015; Wang et al., 2011). Coal seam methane (CSM) accounted for approximately 40% of total production (by volume) from all gas wells in the United States in 2011 (Strapoc et al., 2011). CSM is produced by both thermogenic and microbial processes, the latter often termed biogenic production. Biogenic methane represents approximately 30% of all CSM (Flores, 2008; Strapoc et al., 2011) and its production may occur in coal irrespective of its rank. Given the importance of CSM as an energy source, techniques that have the potential to increase microbial generated CSM production are of great economic and environmental interest. Previous published studies have attempted to stimulate methane production by manipulating native microbial communities using techniques such as adjusting pH, temperature, coal surface area and adding nutrients (Green et al., 2008; Jin et al., 2009; Liu et al., 2013; Midgley et al., 2010; Opara et al., 2012; Pfeiffer et al., 2011; Shumkov et al., 1999; Unal et al., 2012; Wawrik et al., 2012). All of these studies showed shifts in the microbial communities that resulted in changes in gas production, though the underlying mechanisms remain largely unknown.

Intuitively it seems likely that the native microbial communities found in coal seams would be optimally adapted to that environment, and that introduction of exogenous bacterial consortia would not result in improved gas production. In 2010 however, Jones et al., demonstrated that adding exogenous methanogenic communities to a non-CH_4-producing coal microbial community resulted in higher rates of methane generation than the addition of nutrients (Jones et al., 2010). Similarly, a microbial community derived from an American wetland sediment was demonstrated to be more efficient at the conversion of organic matter in brown coal to methane than the coal's indigenous community (Opara et al., 2012). The present study sought to determine whether a composite microbial community, consisting of a coal seam microbial community mixed with mangrove sediment, would be able to improve gas yields compared to the coal seam community alone, in an *in vitro* system.

2. Materials and methods

2.1. Samples

The coal seam microbial community was collected in formation water from a medium volatile bituminous rank Permian coal (~700 m subsurface) in the Sydney Basin, Australia (−34.111478° S, 150.737096° E). The sample was collected in pre-sterilised, 1 L bottles, to which a reductant and indicator solution were added as described in Midgley et al. (2010). Within an hour, the sample was transferred

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to an anaerobic glovebox with an atmosphere of 95% Ar and 5% H_2 and then degassed under this atmosphere.

The mangrove swamp microbial community was obtained by collecting ~200 g of sediment, from a depth of 10–15 cm, from a site near Meadowbank, Sydney (−33.819865° S,151.091569° E), using a clean, surface sterilised trowel. The collected sediment was then transferred to an anaerobic glove box under the conditions described above. The physicochemical characteristics of the formation water and swamp sediment were measured by National Measurement Institute (Sydney, Australia) (Table S1).

2.2. Feedstock coal and characterisation

A homogenised and sieved (<6 mm) sample of Cenozoic brown coal from the Latrobe Valley was used as the carbon source for experiments. The 'run-of-mine' sample was taken from the Loy Yang open cut located in the Gippsland Basin, Victoria, Australia. A representative subsample of the coal was characterised using conventional organic petrological methods, according to the Australian Standard AS2856.2 (1998) and AS2856.3 (2000). For the maceral analysis ~600 point-counts were carried out, with examinations using both reflected white light and incident ultraviolet light (UV)/blue light excitation for fluorescence mode. About 60 measurements were made for vitrinite reflectance analysis. The proximate analysis was carried out by Australian Laboratory Services (ALS), Carrington, New South Wales, Australia, according to Australian Standard AS1038.3-2000. Before the coal was used as a carbon source, it was transferred to the anaerobic chamber under conditions described previously and allowed to degas.

2.3. Generation and characterisation of enrichment cultures

Two initial enrichment cultures were established, one from the Permian coal seam formation water and one from the mangrove sediment. The coal seam water enrichment culture was established by adding 20 mL of the formation water to 1 L of minor modified minimal salts (MS) medium (as described in Midgley et al., 2010) with 10 g of the Loy Yang coal. One millilitre of filtered sterile 100 μM Na_2S solution was used as a reductant. The mangrove swamp enrichment culture was established by adding 10 g of fresh sediment to 1 L of MS medium. After two weeks, 50 mL of this initial culture was transferred to 1 L of fresh, degassed MS medium supplemented with 10 g of Loy Yang coal and 0.5 g of yeast extract. Both the formation water and the mangrove sediment enrichment culture were incubated with non-sterile feedstock coal, as autoclaved coal did not support the growth of microbes. Both enrichment cultures were incubated at 33 °C for a minimum of four weeks prior to the start of the gas production tests. All culturing work was undertaken inside the anaerobic chamber under an atmosphere of 95% Ar and 5% H_2 atmosphere.

2.4. Gas production tests

To investigate whether the two enrichment cultures enhanced methane production, 2 g of coal and 50 ml of MS medium were added to 120 ml sterilised serum vials, and degassed in the anaerobic chamber. After degassing, either 1 ml of formation water enrichment culture, 1 ml mangrove sediment enrichment culture or 0.5 mL of both were added. An un-inoculated control was also included. The vials were sealed using butyl-rubber septa and aluminium crimps inside the anaerobic chamber and removed for incubation. The incubation took place at 33 °C in the dark, with the vials in an inverted position. Triplicates were established in all treatments, including controls. The headspace gases of all bottles were assayed at 10, 20 and 30 days using a gas-tight syringe as described in Midgley et al. (2010). After gas sampling (which occurred inside the anaerobic chamber), the bottles were unsealed and allowed to equilibrate for 3 min in the atmosphere of the anaerobic chamber, prior to being resealed with a new butyl-rubber septa and aluminium crimps and returned to the 33 °C incubator. This procedure effectively reset the headspace gas to that present in the anaerobic chamber.

Sampled gas was analysed on an Agilent Technologies 490 Micro Gas Chromatograph (Micro-GC). The samples were injected into the front injection port of the Micro-GC using a gas-tight syringe and a motorised syringe pump. The Micro-GC is equipped with four column modules: a 10 m Molesieve 5 Å column for separating O_2/Ar, N_2, CH_4 and CO, a 10 m Pora Plot Q column for separating CO_2, C_2H_6 and C_3H_8, a 10 m CP-Sil-5CB column for separating C_4–C_5 hydrocarbon gases and H_2S and a 20 m Molesieve 5 Å column for separating H_2 and He using argon carrier gas. The temperature of these four columns were set to 90 °C, 70 °C, 60 °C and 90 °C, respectively. A typical analysis time was 3 min for a single sample injection.

2.5. Statistical analysis

One-way ANOVA and the Tukey HSD post hoc test (Viotti et al., 2009) were used for testing for the significance of the differences observed in the CH_4 and CO_2 concentrations between each time point and different treatments. All statistical analyses were performed in R (version 3.1.0; R Development Core Team, Vienna, Austria).

3. Results and discussion

3.1. Coal petrology

The Loy Yang brown feedstock coal comprised mainly of telovitrinite and detrovitrinite, in near equal abundances, with major amounts of liptinite and minor amounts of inertodetrinite, funginite and minerals (Table 1). The liptinites mainly comprised sporinite and liptodetrinite with lesser amounts of cutinite, suberinite and resinite (Table 1, Fig. 1). The mean random vitrinite reflectance, including measurements on both telovitrinite and detrovitrinite was 0.35%. Consistent with this brown coal rank, the moisture content was ~39%, the volatile matter yield was ~33% (dry basis) and the fixed carbon content was ~28%. This high moisture content may facilitate the growth of microbial biofilms (Faison, 1991).

3.2. Gas production results

Taken across the entire time course, significantly greater total yields of methane ($p < 0.0001$) were generated from the mixed origin enrichment culture (~162 μmol CH_4 g^{-1} coal) than from either the formation water or mangrove enrichment cultures alone (~126 μmol CH_4 g^{-1} coal and ~13 μmol CH_4 g^{-1} coal, respectively; see Fig. 2a). Only trace amounts of methane (<0.1 μmol CH_4 g^{-1} coal) were observed in desorption controls,

Table 1
Maceral composition for Loy Yang brown coal.

Maceral	Volume %	Maceral group	Volume %	Volume % (mineral free)
Telovitrinite	20.5			
Detrovitrinite	33.8	Vitrinite	58.1	81.9
Gelovitrinite	3.8			
Sporinite	3.6			
Suberinite	0.9			
Resinite	0.5	Liptinite	9.6	13.5
Cutinite	<0.2			
Alginite	0			
Liptodetrinite	4.6			
Semifusinite	0			
Fusinite	0			
Macrinite	0	Inertinite	3.2	4.6
Micrinite	0			
Funginite	1.5			
Inertodetrinite	1.7			
Minerals	29.1	Minerals	29.1	–
Total	100		100	100