International Journal of Coal Geology 154–155 (2016) 205–212

Contents lists available at ScienceDirect

International Journal of Coal Geology

journal homepage: www.elsevier.com/locate/ijcoalgeo

The effect of coal rank on biogenic methane potential and microbial composition

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ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form 4 January 2016
Accepted 4 January 2016
Available online 5 January 2016

Keywords:
Coal bed methane
Coal rank
Acetate
16S rRNA gene
Microbial activity

ABSTRACT

Demand for natural gas is expected to increase faster than any other fossil fuel over the coming decades. Australian coal bed methane (CBM) resources are among the largest in the world and are already being utilized to meet increasing demand. The majority of methane contained within CBM producing coal beds is microbially generated and low rank coals are often associated with higher bioavailability. However, the results of previous studies are conflicting, and it is unclear how or if coal rank has an effect on microbial community structure. Here, enrichment cultures grown on coals spanning 13 different ranks (lignite to bituminous) were characterized with 16S rRNA gene amplicon sequencing and combined with volatile fatty acid (VFA) and headspace methane measurements to understand the effect of coal rank on CBM microbial communities. Our results show that there is a significant negative correlation between final methane yield and rank, suggesting that the bioavailability of the coal organic material decreases with increasing thermal maturity. The concentration of VFAs generally increased with decreasing rank and revealed that community composition was significantly correlated with the concentration of specific VFAs (e.g. acetate). These data suggest that the observed higher methane production from lower rank coals is linked to increased concentrations of low molecular weight acids desorbing from the coal. The increase in low molecular weight VFAs was associated with the enrichment of specific taxa known to specialize in the degradation of low molecular weight acids and alcohols.

1. Introduction

Coal is a complex sedimentary rock composed of plant matter that has been flooded, buried, heated, and compacted over the course of millions of years. In general, coal can be classified by several defining characteristics, which reflect its burial history and the geological forces that have acted on it over time (Diessel, 1992; O'Keefe et al., 2013; Suárez-Ruiz et al., 2012; Taylor, 1998; Ward, 1984). These characteristics include the original plant matter (peat) from which the coal is derived, the minerals incorporated into the coal, and coal rank, the extent of diagenetic transformation experienced by the coal, primarily as a result of compaction and heating (O'Keefe et al., 2013). Coals are classified into a rank system reflecting their thermal maturity, with rank designations increasing from lignite, sub-bituminous, bituminous, to anthracite. As the original peat is coalified, individual plant components of similar chemical composition are compacted into semi-discrete units within the coal called macerals. Over time, geothermal heating of the coal results in predictable geochemical changes in the organic chemistry of each maceral. For example, vitrinite, derived from woody, lignin-rich tissue of plants (Taylor, 1998), undergoes a high degree of condensation of its aromatic structure with increasing rank, resulting in an increase in shine/reflectance (O'Keefe et al., 2013; Strąpoć et al., 2011). For this reason, vitrinite reflectance, the percentage incident light reflected from the surface of the coal, is commonly used as a proxy for rank and maturity.

As coal increases in rank, heteroatoms such as oxygen, sulfur, and nitrogen are lost. At the same time, the aromatic lignin-derived structure of the coal condenses to form higher order polyaromatic compounds and eventually aromatic sheets (Strąpoć et al., 2011). The increase in aromaticity, coupled with a loss of heteroatom moieties amenable to microbial attack, is associated with a loss in bioavailability. As a result, it has been assumed that coals of higher rank are less bioavailable and will produce less methane than coals of lower rank (Fallgren et al., 2013; Strąpoć et al., 2011). To date, very few studies have been conducted to validate this assumption. While a meta-analysis of published methanogenesis rates showed support for the proposed inverse correlation between rank and methane biogenesis potential (Strąpoć et al., 2011), other studies have shown that higher rank can be associated with increased methane production (Fallgren et al., 2013) or found no correlation (Wawrik et al., 2012). In the latter cases, the authors suggested that low molecular weight hydrocarbons trapped within the coal matrix were used, rather than the coal matrix itself. The nature of the relationship between rank

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http://dx.doi.org/10.1016/j.coal.2016.01.001
0166-5162/

and methane production remains unclear. While it has been shown that coal properties can affect microbial community composition (Fry et al., 2009; Susilawati et al., 2014), the effect of coal rank on microbial community composition and function has not been fully addressed.

Here, 14 enrichment cultures grown using coals of different rank (peat to bituminous) as the sole carbon and energy source were characterized using 16S rRNA gene amplicons. Methane production, as well as volatile fatty acid composition, was coupled with microbial community composition analysis to provide insight into how CBM producing microorganisms and gas production are influenced by coal rank.

2. Materials and methods

2.1. Core collection and processing

Thirteen coal samples spanning a range of ranks from lignite to high volatile bituminous were collected from sites located in the Bowen Basin (Australia), Kutai Basin (Indonesia), Ohai Basin (New Zealand), and Greymouth Basin (New Zealand) as described in Table 1. All samples were collected and characterized as part of previous studies (Anggara et al., 2014; Dmyterko, 2014; Rahmat, in progress). Coals were obtained for processing as small blocks (≤2 cm^3) and stored in air for several months prior to use in these experiments. Due to the small size of each coal block, the outer core could not be removed. Preparation of core material for use as an enrichment culture substrate was carried out in an anaerobic chamber (COY Laboratory Products, MI, USA) under an atmosphere containing 95% nitrogen and 5% hydrogen. To ensure that the coal did not become contaminated with volatile substances during storage or preparation, the chamber atmosphere had been fully purged several months before the commencement of this experiment, and no volatile substances (e.g. ethanol) were used in the chamber. The core material was ground using a bur-style coffee grinder and then a mortar and pestle. The resulting granules were sieved to between 150 and 500 μm in size, and maceral analysis and vitrinite reflectance measurements were performed at the School of Earth Sciences (University of Queensland) in accordance with Australian Standards AS 2856.2-1998 and AS 2856.3-2000, respectively (Table 2). A wood sample of Huon Pine (HP) was included in the analysis as it has a rank of approximately 0.1.

2.2. Establishment of enrichment cultures

Enrichment cultures were prepared using each coal as the dominant carbon and energy source as previously described (Green et al., 2008; Papendick et al., 2011). In total, 3 g/L 2-((1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)amino)ethanesulfonic acid (TES) and 1 g/L sodium bicarbonate were added to the growth medium (Tanner, 2007) and the pH was adjusted to 7.4. Resazurin (1 g/L stock) was added as a redox indicator to 50 μg/L final concentration. Media was boiled for 2 min and cooled to room temperature under a stream of oxygen-free nitrogen with sodium sulfide nonahydrate reductant added to a final concentration of 0.3 mM. The cooled anaerobic medium (9.8 ml) was dispensed into 36 ml serum bottles (Wheaton, USA) containing 0.25 g of coal granules. Serum bottles were sealed with butyl rubber stoppers and aluminum crimps. The headspace was exchanged with oxygen-free nitrogen gas and serum bottles were autoclaved for 20 min at 120 °C and 1 atm of pressure. Prior to inoculation, the volatile fatty acid composition (VFA) of the supernatants of three un-inoculated enrichments containing each coal were measured using standard methods (Eaton et al., 2005) in order to determine the extent of labile carbon desorbing from the coal, as well as to ensure that ethanol was not present in these enrichments. VFAs measured include ethanol, propanol, and butanol, as well as acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, and hexanoic acids.

To create a diverse inoculum capable of degrading a wide range of organic substrates, biomass from termite gut contents (degutted and homogenized using sterile instruments), digester fluid, koala feces, sediment from the University of Queensland Lake, and the PK18 enrichment culture were combined. The PK18 enrichment community is derived from formation waters obtained from a CBM production well located in the Surat Basin, Australia that was previously shown to produce methane using coal as the dominant carbon and energy source (unpublished data). To ensure that equal cell densities from each sample were present in the inoculum, the samples were diluted to remove debris or decrease the cell density, and estimates of cell density were made using Thoma slide count. After sample mixing, approximately 1×10^7 cells in total from each source were combined into a master inoculum. One milliliter of the master inoculum was stored at −80 °C for subsequent 16S rRNA gene profiling. Enrichment cultures containing each of the 13 coals or Huon Pine sample were inoculated in triplicate with 0.2 ml of the master inoculum and incubated without shaking at 37 °C for 50 days. Cumulative headspace methane concentration was measured for each enrichment culture at regular intervals until methane production had ceased, at which point microbial biomass was harvested for 16S rRNA gene sequencing. Methane generated from the degradation of organic matter from the inoculum was determined using controls containing only the inoculum without coal. Uninoculated controls containing each coal served as negative control for methane desorbing from the coal.

2.3. Methane measurement

Cumulative headspace methane concentrations were measured using a Shimadzu GC-2014 gas chromatograph (Shimadzu, Japan) fitted

Table 1
Provenance, depth, and vitrinite reflectance of one wood (Huon Pine, HP) and 13 coal samples. Samples listed as having "No depth" were taken from open cut mines where the coal was positioned 40–60 m below surface. Basin, formation, and depth measurement are not applicable (NA) for Huon Pine. Vitrinite reflectance was measured as maximum telovitrinite reflectance and is here reported as Rmax for all coal regardless of rank for consistency.

Sample ID	Country	Basin	Coal Measures/formation	Depth (meters)	Vitrinite reflectance (Rmax)
HP	Australia	NA	NA	NA	0.1
JE2	New Zealand	Ohai	Morley coal measures	34 m	0.5
KSA	Indonesia	Kutai	Balikpapan formation	No depth	0.5
ST7	Indonesia	Kutai	Balikpapan formation	58–60 m	0.5
JE4	New Zealand	Ohai	Morley coal measures	804 m	0.5
ST3	Indonesia	Kutai	Balikpapan formation	134–136 m	0.5
JE9	New Zealand	Greymouth	Rewanui coal measures	Outcrop	0.7
IP	Australia	Bowen Basin	Rangal coal measures	No depth	0.9
JE10	New Zealand	Greymouth	Rewanui coal measures	outcrop	0.9
BM	Australia	Bowen Basin	Moranbah coal measures	No depth	1.1
CD	Australia	Bowen Basin	Rangal coal measures	No depth	1.3
JE11	New Zealand	Greymouth	Rewanui coal measures	Outcrop	1.5
MVL	Australia	Bowen Basin	Rangal coal measures	No depth	1.5
CP	Australia	Bowen Basin	Rangal coal measures	No depth	1.9