International Journal of Coal Geology 165 (2016) 121–132

Contents lists available at ScienceDirect

International Journal of Coal Geology

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

Factors controlling the co-occurrence of microbial sulfate reduction and methanogenesis in coal bed reservoirs

Andrew W. Glossner [a], Lisa K. Gallagher [b], Lee Landkamer [b], Linda Figueroa [b], Junko Munakata-Marr [b], Kevin W. Mandernack [a,*,1]

[a] Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, CO 80401, USA
[b] Department of Civil and Environmental Engineering, Colorado School of Mines, Golden, CO 80401, USA

ARTICLE INFO

Article history:
Received 11 May 2016
Received in revised form 5 August 2016
Accepted 9 August 2016
Available online 12 August 2016

Keywords:
Sulfate reduction
Methanogenesis
Coal
Microbial
Powder River Basin

ABSTRACT

Sulfate-reducing microorganisms (SRM) and methanogenic archaea have been previously observed in coal bed methane reservoirs, suggesting that the model for separation of these organisms based on sulfate concentration may not apply to such reservoirs. Using a methanogenic consortium enriched from coal, microcosm experiments showed simultaneous activity of methanogens and sulfate reducers at sulfate concentrations ranging from 50 to 1000 μM when coal was the sole substrate. These experiments revealed no apparent correlation between methanogenic potential and sulfate concentration. In other microcosm experiments with varying acetate amendments, concentrations of the phospholipid fatty acids (PLFAs) 14:0, 16:1ω5, 16:1ω7*cis*, 16:1ω7*trans*, and *cy*17:0 correlated strongly with the initial acetate concentration in microcosms with 500 μM sulfate, while *i*17:0 correlated strongly in microcosms with 200 μM sulfate. A significant portion of the acetate in these experiments went to microbial metabolisms other than dissimilatory sulfate reduction or methanogenesis, suggesting that some of these PLFAs were likely produced by some other unknown acetate-consuming micro-organisms. Copies of the *dsrA* gene increased at least 10-fold over initial levels in samples without molybdate (MoO_4^{2-}) across all experiments, indicating that SRM were active when not inhibited by MoO_4^{2-}. In experiments with <300 μM acetate, copies of the *mcrA* gene increased over 49 days regardless of sulfate concentration. These results suggest that both SRM and methanogens are active at low acetate concentrations and may compete for available acetate with other acetate-consuming bacteria in coal bed methane reservoirs.

1. Introduction

Coal bed methane (CBM) has become an important energy resource, comprising roughly 7% of natural gas production (Annual Energy Outlook, 2014), with approximately 40% of this gas being microbial in origin (Strąpoć et al., 2011). From laboratory and field studies, some basins, such as the Powder River Basin (PRB) in Wyoming and Montana, USA, have been shown to harbor active microbial communities capable of ultimately converting coal to methane (Harris et al., 2008; Jones et al., 2010; Ulrich and Bower, 2008). There is much commercial interest in developing microbial consortia or other technologies to enhance this process (Ritter et al., 2015), but doing so requires a fundamental understanding of the interactions of the entire microbial community and its metabolisms in coal bed reservoirs.

The process of microbial methanogenesis from coal is complex, but it is thought to be enhanced with introduction of microbes and nutrients through meteoric water recharge (Strąpoć et al., 2011). Such recharge is a common feature at many of the world's largest reserves of microbial CBM (Flores et al., 2008; Martini et al., 1998, 1996; McIntosh et al., 2008; Schlegel et al., 2011a,b; Scott et al., 1994; Tseng, 1997; Walvoord et al., 1999; Zhou and Ballentine, 2006). Strąpoć et al. (2011) provide a thorough review of the pathways of methanogenesis from coal. Basically, fermentative, anaerobic microorganisms degrade the large geopolymers to form long chain organic acids, which are then further broken down to monomers and oligomers and ultimately to the substrates necessary for methanogenesis, mainly hydrogen and acetate. In many anoxic environments, sulfate-reducing microorganisms (SRM) and methanogenic archaea compete for these latter substrates.

The competition between SRM and methanogens in anoxic environments is governed mainly by sulfate, hydrogen, and acetate concentrations. However, at both freshwater (Lovley and Klug, 1986, Lovley and Klug, 1983) and marine sulfate concentrations (Middelburg et al.,

* Corresponding author at: Department of Earth Sciences, Indiana University–Purdue University, 723 West Michigan Street, SL118B, Indianapolis, IN 46202, USA.
E-mail addresses: andy.glossner@gmail.com (A.W. Glossner), llandkam@mines.edu (L. Landkamer), lfiguero@mines.edu (L. Figueroa), jmmarr@mines.edu (J. Munakata-Marr), kevinman@iupui.edu (K.W. Mandernack).
[1] Present address: Department of Earth Sciences, Indiana University–Purdue University, 723 West Michigan Street, SL118, Indianapolis, IN 46202, USA.

http://dx.doi.org/10.1016/j.coal.2016.08.012
0166-5162/

1993; Mitterer, 2010), sulfate reducers are known to outcompete methanogens due to their higher affinity and lower threshold concentrations for hydrogen and acetate (Muyzer and Stams, 2008; Schönheit et al., 1982). In marine sediments the commonly accepted model for the distribution of these two processes holds that methanogenesis will not emerge as the dominant terminal electron accepting process until the sulfate has been depleted (Jørgensen and Kasten, 2006; Martens and Berner, 1974; Oremland and Taylor, 1978). Because the concentration of sulfate in marine sediments is typically much higher (10–50 mM) than in freshwater sediments (10–500 μM), sulfate reducers often account for the majority of carbon mineralized under high sedimentation rates in marine sediments (Canfield et al., 2005). Under freshwater sulfate conditions SRM can still thrive at the lower sulfate concentrations by having much higher affinity for sulfate (Ingvorsen et al., 1984) and therefore rapidly deplete the available sulfate in freshwater sediments (Canfield et al., 2005; Lovley and Klug, 1983).

While freshwater bogs, lake sediments, and surface waters illustrate the fundamental relationships between SRM and methanogens, deep subsurface basins are different because physicochemical parameters, such as groundwater recharge or mineral dissolution, result in limited amounts of sulfate and low rates of organic matter degradation due to the recalcitrant nature of coal (Lovley and Chapelle, 1995). Subsurface coal bed methane reservoirs can be viewed as essentially closed systems over short time periods because the rates of groundwater recharge and organic matter degradation are slow relative to surface sites (Bates et al., 2011). Schlegel et al. (2011b) argue that in parts of the Illinois Basin sulfate reduction occurred at some point in the past 10 ka, imparting a lighter isotopic signature on the DIC and enriching the remaining sulfate pool in ^{34}S. The authors argue that methanogenesis occurred subsequent to sulfate reduction and that methanogenesis had not yet overprinted the isotopic signature of the DIC that sulfate reduction had generated. In this model sulfate reduction and methanogenesis are mutually exclusive processes separated by time rather than geochemical zonation as occurs in sediments. Other studies have found 16S rRNA gene sequences indicative of SRM in coal bed methane reservoirs (Green et al., 2008) as well as in abandoned mines with active methanogenic populations (Beckmann et al., 2011). It is therefore possible that sulfate reduction and methanogenesis may occur contemporaneously in such reservoirs, albeit at very low rates due to the limiting amount of labile carbon.

The availability of substrates necessary for both sulfate reduction and methanogenesis in CBM reservoirs will also influence their relative activities. The produced water from the Wyodak-Anderson coal zone of the PRB generally has low sulfate concentrations of around 1.6 μM (Rice et al., 2008), though localized regions within the basin can have much higher concentrations up to 40 mM (Ulrich and Bower, 2008). Acetate is another potential limiting substrate for SRM and methanogens in coal beds, and is generally at low levels in produced waters from the PRB (<2 μM) (Ulrich and Bower, 2008). Acetate is an important intermediate in the degradation of complex organic matter in environments such as peat bogs (Metje and Frenzel, 2007), oil reservoirs (Bonch-Osmolovskaya et al., 2003), lacustrine sediments (De Graaf et al., 1996; Winfrey and Zeikus, 1979), and various organic-rich shales, clays, and mudstones (Jones et al., 1989; McMahon et al., 1992; Routh et al., 2001). In these environments, it is generally maintained at very low concentrations (<10 μM) by active microbial consumption, including methanogenesis. Acetate has been shown to be an important intermediate in coal degradation in abandoned coal mines (Beckmann et al., 2011) and in the Forest City Basin CBM reservoir (McIntosh et al., 2008). Given the variability of sulfate concentrations and the limited metabolic activity of the microbial community in CBM wells, it is possible that sulfate reduction and methanogenesis co-occur in CBM reservoirs like the PRB, with both being limited by the availability of acetate.

The goal of this study was to determine the effect of varying sulfate and acetate concentrations on the competing processes of sulfate reduction and methanogenesis in coal from the PRB. To investigate this, a series of microcosm experiments was undertaken with a microbial consortium enriched and maintained on PRB coal. These experiments were conducted with variable concentrations of sulfate (50–1000 μM) and acetate (250–1000 μM) that might be expected in produced waters in CBM reservoirs (Orem et al., 2007; Rice et al., 2008). The effects of these variations on the bacterial community structure were analyzed using microbial membrane phospholipid fatty acids (PLFAs) and real-time quantitative polymerase chain reaction (qPCR). PLFA analysis is a commonly accepted method for determining both living microbial biomass (Balkwill et al., 1988; Boschker et al., 1998; Mills et al., 2010) as well as community structure when PLFAs can be putatively assigned to specific metabolic groups (Dowling et al., 1986; Vainshtein et al., 1992). To better understand the energy and carbon constraints in each microcosm experiment, we measured sulfate and acetate concentrations, methane production, and concentrations of PLFAs. Functional genes for methanogens and sulfate reducers, methyl coenzyme-M reductase (*mcrA*) and dissimilatory sulfite reductase (*dsrA*), respectively, were measured by qPCR to further assess how SRM and methanogens in these microcosm experiments responded to changing sulfate and acetate concentrations.

2. Methods

2.1. Experimental design

Coal was collected in September 2009 by straining cuttings from the effluent of working drill rigs in the PRB. Three seams were sampled from the Wyodak-Fort Union formation, including the Big George, Smith, and Felix seams at depths ranging from 240 to 610 m. Coal cuttings were immediately rinsed with sterile deionized water in the field to remove drilling fluids, placed in sterile whirl-pak bags, sealed in vacuum bags with chemical oxygen scrubbing packets (OxyFree 504), and stored at 4 °C until use. The coal was rinsed again in the lab in the anaerobic chamber with sterile, anoxic deionized water (described below) and then crushed using a sterile mortar and pestle before being rinsed again over a sterile 80 mesh (0.177 mm) sieve prior to use.

2.2. Preparation of enrichment culture and lab microcosm experiments

A mixed consortium of microorganisms, whose growth was dependent on coal provided as a substrate and which contained both SRM and methanogens, was enriched from the coal cuttings (Gallagher et al., 2013). The enrichment consortium was incubated at 30 °C and continually maintained by transferring every 60 days inocula from the microcosms that previously produced the most methane to new microcosms with fresh coal. The anoxic nutrient medium was modified (Tanner, 2006) to exclude sulfate by replacing $MgSO_4$ with $MgCl_2$, and sulfate was added to the desired concentration as Na_2SO_4. Medium was prepared by flash-autoclaving deionized water to reduce oxygen saturation, then sparging with 4:1 N_2:CO_2 for 15 min before adding 1 g/L $NaHCO_3$ just before sealing under N_2:CO_2 and autoclaving. The trace-vitamin, -mineral and -metal solutions (Tanner, 2006) were filter-sterilized (0.22 μM pore size) and added to the anoxic bicarbonate solution after it had cooled in an anaerobic chamber.

All experiments utilized the microbial consortium noted above and were prepared in an anaerobic chamber with an atmosphere of 5% H_2, 5% CO_2, and a balance of N_2. Experiments were initiated by adding 10 g coal and 50 mL of the sterile, anoxic medium to sterile 200 mL serum bottles. All microcosms were inoculated with 0.5 mL (1%) of the microbial consortium. Upon sealing with butyl rubber stoppers, the headspace of each serum bottle was purged with 4:1 N_2:CO_2 for at least 5 min and pressurized to 1.1 atm. Prior to the initiation of the sulfate and acetate experiments described here, a series of inhibition experiments were conducted to test whether or not hydrogenotrophic methanogenesis was a major source of methane for our consortium.