

Active Biogenesis of Methane in Wyoming's Powder River Basin

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Abstract

Numerous coal cores were carefully obtained to limit exposure to air during the course of their extraction from the Powder River Basin (PRB) of northeastern Wyoming. These coal samples were examined for their indigenous microbial populations and their ability to support methanogenesis. Significant populations of anaerobic microorganisms and rates of active methanogenesis were detected in nearly all of the tested coals. Conditions were identified that stimulated or inhibited this microbial methane production. Extrapolated results suggest that a huge potential bioreservoir for natural gas production exists in these PRB coalfields.

Introduction

Natural gas is one of the most desired and environmentally benign sources of energy in today's world. While domestic production of natural gas in the United States plateaued over 30 years ago, demand continues to outpace supply. Until recently, most of the earth's methane was thought to be derived from long-buried carbonaceous materials subjected to intense heat and pressures over geologic time. More recent estimates indicate that between 20 and 65% of the earth's methane deposits, including hydrates, is biogenic (Rice and Claypool, 1981; Claypool 2004, personal communication). Biogenic methane accumulations have been detected in coal seams, sandstone, shales, oil reservoirs, and as gas hydrates. The timing of gas generation and contribution of real-time biogenic methane formation (methanogenesis) are not known. Nevertheless, recent reports detected geologically young methane in the Antrim Shale located in the Michigan Basin and significant rates of methanogenesis associated with marine gas hydrates and deep marine sediments (Wellsbury et. al, 1997) which have generated considerable interest.

The ability of microbes to survive and in some cases thrive in subsurface environments is well documented (Krumholtz et. al, 1997). However, the consortia of microorganisms responsible for biogenic methane production as well as their specific pathways/genes are largely unknown. It is likely that conversion of complex sources of hydrocarbon substrate to methane is dependent on a consortium of cross-feeding microorganisms. The activity of these subsurface microbial consortia could be controlled by a number of environmental conditions such as nutrient availability, carbon substrate heterogeneity, water availability and exchange, diffusion rates of gases and metabolic products, contact with the carbon substrates, consortium composition, communication between members of the microbial consortium, and combinations thereof.

Both geologic studies and chemical characterization of the methane in the PRB coal seams indicate that it is biogenic in nature (Law et. al, 1991). In the 1990's, geoscientists and petroleum engineers from a variety of companies began to exploit this source of natural gas. Luca Technologies has begun efforts to better understand the dynamics of biogenic gas production and characterize the microbial consortia present within various coal-beds in the PRB.

The primary goals of the research were to evaluate the recent and ongoing biogenic methane formation in PRB coal seams and to identify some of the variables that may affect the creation of biogenic gas in these coals. The sheer size of the PRB coal-bed resource as substrate for biogenic methane creation is the primary incentive. The coal-beds of the PRB are thought to contain ~580 billion tons of coal in contiguous seams at least 20 feet thick (DeBruin et. al, 2001). Only a small portion of this coal is accessible for domestic use via mining. Although substantial quantities of methane exist in the PRB coal seams (estimated total resource of ~37 TCF, DeBruin et. al, 2001), this quantity of gas likely represents a small fraction of the methane that could be created through biogenesis supported by hydrocarbon substrates within the coals. For instance, the conversion of only 1% of the known PRB coal resource above would generate approximately 86 TCF of gas (Luca estimation).

While stimulating biogenic gas production in the field is the ultimate goal of Luca's work, prudence dictates that a better understanding of biogenic methane production under laboratory conditions is necessary prior to application in the field. Experimental design, methodology development, sample characterization and research execution were performed in Luca's Golden, Colorado facilities. Luca has chosen to highlight a representative coal sample from the Dietz coal seam in this study, knowing that there are both more and less encouraging examples to cite.

Materials and Methods

Sample location. The Dietz 32D1-2183 well is located in northern Sheridan County, Wyoming at township 58N, Range 83W, Section 21, NE quadrant. The project area is situated in the northwest corner of the Tertiary Fort Union coal-bed methane-producing trend of the PRB. A geologic overview is available (De Bruin et. al, 2001).

Coring and field sampling. Surface casing for the 32D1-2183 was set at a depth of 60 ft. The well was then deepened to the top of the Dietz coal-bed which was encountered at 296 ft. Casing was run to the top of the coal-bed and cemented. Plug and cement were drilled out and the Dietz coal was cored with a 3 ½ inch diameter by 20 ft in length Baker Hughes core barrel with a PVC inner sleeve using Dietz formation water produced from a nearby well as the drilling fluid. 19.5 ft of core was cut of which 17.5 ft was recovered. Core was retrieved by tripping the entire assembly and the sleeve was laid on the ground and marked. Marked intervals were cut with a chop saw and placed in canisters pre-charged with argon, which were then sealed. The core was cut from 8:08 to 8:18 am, laid out on the surface at 8:38 am and completely canistered by 9:05 am on June 9, 2001.

Preparation of methanogenic activity experiments. The containers of core were opened inside an anaerobic glove bag filled with 98% nitrogen and 2% hydrogen under slightly positive pressure with controlled temperature (22C) and humidity (95%). Geological characterization, photography, and sample preparation all occurred inside the glove bag. Core samples had their external core surfaces (which were briefly exposed to air at the rig-site) pared away inside the glove bag using sterile knives. Mechanical processing (such as crushing) of solid substrates was also performed inside the glove bag using sterile mortar and pestle.

Slurry bottles were prepared within the glove bag by placing ~5 grams of coal into each experimental bottle. Weights of coal are done without adjustment for water or ash content, although all core samples were subjected to vacuum extraction prior to commencement of experimentation to desorb endogenous methane. Anoxic formation water was added via a sterile 10 ml pipette using an automated dispenser. Sodium sulfide (to 0.5 mM) was added to the water to ensure strict anoxic conditions in the coal slurries during long-term incubation. The coal slurries were sealed with sterile butyl rubber stoppers, removed from the glove bag, vacuumed and purged with helium for three cycles. A small helium overpressure (~ 3 psi) was left in the headspace of the bottles.

Nutritional supplements were added to the coal slurries from anoxic sterile stock solutions. Most of the nutritional supplements tested are inorganic compounds needed in very low or trace concentrations. The purity of chemicals used was generally over 99.9%. Selected coal slurries were amended with ¹⁴C-bicarbonate (2 uCi/bottle, ICN Biomedicals) to track methanogenic activity supported by carbon dioxide reduction and to distinguish between biogenic methane formation and possible methane desorbing from the coal samples.

Sterile controls consisted of samples that were autoclaved (121 C for 20 minutes, three times). Bromoethanesulfonic acid (BESA, Aldrich), a methanogenesis inhibitor, was added to selected incubations. Oxygen treated samples had their headspaces purged with pure oxygen and were left with a slight (~3 psi) oxygen overpressure in the bottles. Unamended coal slurries (without chemical additions) were also prepared.

Sampling and analysis of methanogenic activity. Over the course of an approximate five month incubation period the aqueous phase of the coal slurries were sampled for organic acid analysis and the headspace sampled for gas analysis (including methane, carbon dioxide, radioactive methane, and radioactive carbon dioxide) using sterile helium purged syringes and needles. Methane and carbon dioxide concentrations were determined with a Hewlett Packard 5890 gas chromatograph (GC) equipped with a thermal conductivity detector. Radiolabeled methane and carbon dioxide were determined using a gas proportional counter (Innus Systems, Tampa Fl.) in-line with the GC. Organic acids were determined by gas chromatography (Ram Model 3 detector with Alltech 267 column) from the aqueous phase of samples removed from the bottles.

Results & Discussion

Laboratory evidence for recent biogenic methane formation in the PRB

Luca's research demonstrates that coal seams in the PRB are living bioreactors. Unamended coal samples in formation water produced methane in real-time. Adding certain compounds to either the liquid slurry or the gas headspace can accelerate or inhibit methane production. These results are discussed below.

Gas production in unamended slurry bottles. Direct measurements of methanogenesis in carefully handled and processed coal cores from the Dietz coal seam within the PRB indicate that an active consortium of microorganisms capable of converting coal to methane exists in this coal seam. In Figure 1, methane production in coal slurries containing just formation water and crushed coal (unamended) peaked at about 3% of headspace after 135 days and remained essentially level for the remainder of the experiment.

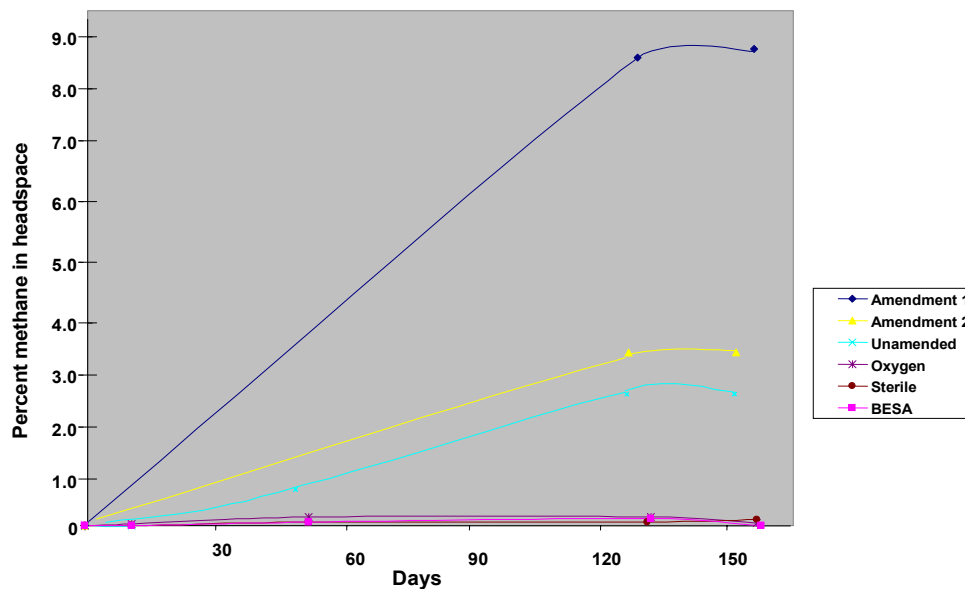


Figure 1. Comparison of methanogenic activities observed in Dietz coal samples under varying conditions.

While the reason for the leveling off of methane production after 135 days is not known at this time, one could surmise that nutrient limitation or inhibitor build-up may be the cause. The cessation of microbial activities is commonly observed in closed system experiments. Encouragingly, in the absence of any potentially costly amendments, methane production was significant under imperfect conditions. We have made similar

findings from approximately one dozen coal samples collected from four PRB coal seams.

The sorbed gas content of PRB coals is typically in the range of 25 to 50 scf per ton of coal, generally increasing with depth. Using a correlation developed from public data for PRB coals (Crockett, 2003, personal communication), the Dietz coal seam from which this sample was taken would be estimated to contain 17 scf per ton of sorbed gas *in situ*. The rates of methanogenesis observed in these unamended slurries would generate this volume in 2.3 years. The same rates of methanogenesis would produce 50 scf per ton in 6.8 years.

Methane production in amended slurry bottles. Luca's work in stimulating and inhibiting methanogenesis from PRB coals is still in its early stages. Figure 1 shows results from several experiments in which a variety of compounds were added to the slurry bottles. Amendment 2 had a modest effect above the unamended sample whereas Amendment 1 had a significant stimulatory impact on methanogenesis.

Negligible methane formation was detected in the coal slurry incubations that were sterilized, treated with BESA, or had oxygen added. Sterilization of coal samples kills all living microorganisms within the sample. BESA is a known inhibitor of methanogenesis (Muller et. al, 1993), and oxygen is a known inhibitor of methanogens (Zinder, 1993). Importantly, methanogens are considered among the most oxygen sensitive of the anaerobes and short exposures have been shown to efficiently kill several species (Zinder, 1993). Therefore it is not surprising that sterilization, BESA treatments, or exposure to oxygen will inhibit and perhaps destroy biogenesis.

Effect of water on methanogenesis. Standard experimental conditions utilized sample bottles containing 14 ml of formation water to prepare coal slurries. Table 1 identifies several types and quantities of water that were used with unamended coal samples in addition to the standard conditions. "Native" moisture samples denote slurry bottles containing coal samples without any additional water. Other experimental conditions include slurry bottles with deionized water or reduced amounts of formation water. Clearly formation water is more supportive of methanogenesis compared to deionized water. A threshold amount exceeding 0.2 ml of formation water per 5 gram sample is required for moderate methanogenesis. Two ml to fourteen ml of formation water yielded essentially identical amounts of methane.

Overcoming potential moisture and free water deficiencies in the Dietz coal is not the primary mechanism by which the addition of formation water stimulates methanogenic activity since deionized water is a poor substitute. A critical volume of formation water is necessary for biogenesis to take place and a yet to be understood interaction between coal, formation water, and the indigenous microbial consortia is required for productive methanogenesis. It should be noted that negligible methanogenic activity has been observed in experiments consisting of formation water samples without coal (data not shown).

Table 1. Average methane production after 159 days from incubations containing 5 g samples of crushed coal. Units of methane production in the coal samples is extrapolated to standard cubic feet (scf) of methane per ton of coal.

<u>Treatment</u>	<u>% methane in headspace</u>	<u>Avg. $\mu\text{mol/bottle}$</u>	<u>Avg. ml/bottle</u>	<u>Scf CH₄/ton coal</u>
Unamended (14 ml formation water)	3.04%	20.29	0.49	3.2
Unamended (14 ml deionized water)	0.63%	4.08	0.10	0.6
<u>Effect of process inhibitors</u>				
Sterilized by heat	0.17%	1.26	0.03	0.2
Oxygen in headspace	0.08%	0.15	0.00	0.0
BESA treatment	0.00%	0.00	0.00	0.0
<u>Effect of water in slurry</u>				
Native moisture only	0.13%	1.53	0.04	0.2
Unamended (0.2 ml formation water)	0.52%	5.85	0.14	0.9
Unamended (2.0 ml formation water)	1.91%	20.76	0.51	3.2
<u>Effect of nutritional amendments</u>				
Amendment 2	3.61%	24.39	0.60	3.8
Amendment 1	8.48%	65.00	1.59	8.93

Figure 2 compares headspace methane content with headspace radioactive methane in individual replicates of the Dietz coal methanogenesis experiments after approximately four months of incubation. The experiments were designed to identify variables that support and/or stimulate methanogenic activity over a wide range of conditions. For reference, the headspace methane concentration in unamended incubations averaged ~3%, which corresponds to 20 μmol of methane. Several amendments (some not shown in Fig. 1) increased methanogenic activity significantly above the unamended slurries while others decreased biogenic methane formation. A strong correlation between headspace methane and radioactive methane produced from added radioactive carbon dioxide (as $\text{H}^{14}\text{CO}_3^-$) attests to the biogenic methane production in these experiments and confirms that carbon dioxide reduction is an important pathway for methanogenesis in these coal samples. These findings are consistent with the isotopic studies of formation water and gas samples, which also attest to recent methanogenesis via the carbon dioxide reduction pathway *in-situ*.

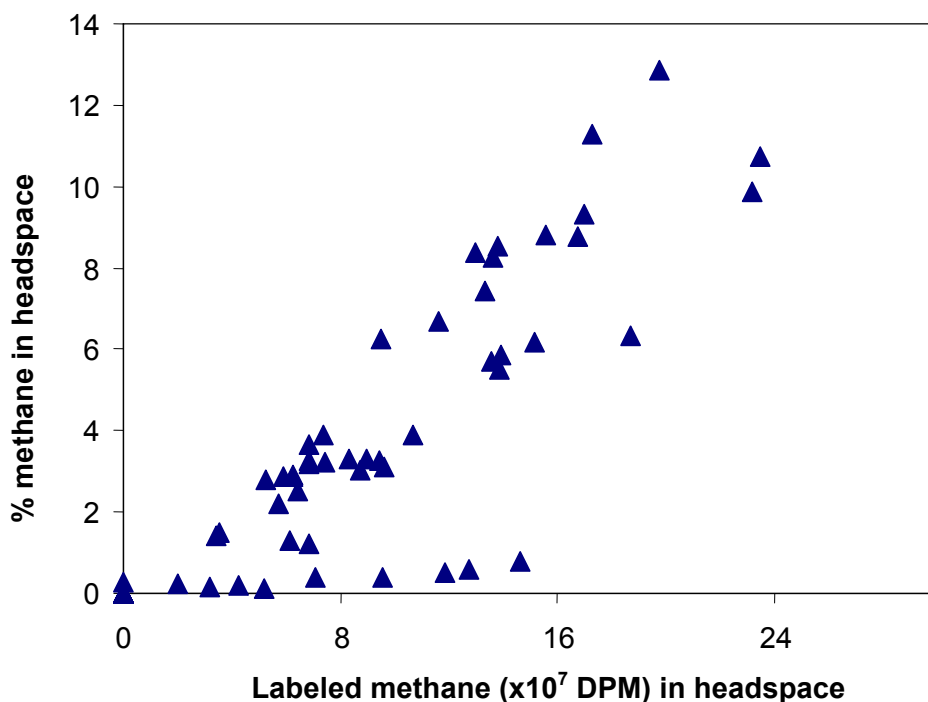


Figure 2. Correlation between methane and radioactive methane formation in coal slurry experiments.

Phylogenic inferences

Culture-independent identification of microbes provides a powerful tool to identify microorganisms in their natural environment. Polar (or phospo) lipid fatty acids (PLFA) have been elucidated for the measurement of microbial biomass, community structure, and metabolic status of environmental samples (Ringelberg et al., 1988). This method has become a benchmark indicator of general archae, bacterial, and eukaryotic families. 16S rDNA sequencing can help the phylogenic placement as homologues of this gene are present in all known organisms. However, these techniques have limited applications in determining specific microbial activities. Microbial Insights™ has provided initial data binning the PLFA from Deitz coal-beds. The PLFA profiles revealed relatively high microbial biomass ($10^6 - 10^7$ cells/g) and diverse microbial communities in the four tested PRB coals. Large proportions of anaerobic biomarkers were detected in all of the samples, although significant differences in the major groups of microorganisms and their physiological status were observed between diverse samples. On the basis of PLFA content, the microorganisms in the coals were stressed to varying degrees. Interestingly, methanogenesis was detected at significant rates in the sample that showed the least stress. The detection of a significant population of diverse microorganisms including viable methanogens is consistent with laboratory experiments that illustrate active methanogenesis in the PRB coals.

Conclusions

Coal is the substrate for the microbial gas production observed in the above experiments. The biodegradable hydrocarbon substrate in these coals is adequate to support generation of significant volumes of newly formed methane (DeBruin et. al, 2001, and Luca calculations). Although the attainable coal conversion efficiencies to methane are not yet known, there is, in theory, enough hydrocarbon in these coals seams to make a significant impact on U.S. domestic needs for the foreseeable future if this existing *in situ* bioreactor can be stimulated. Biogenic methane production can be rapidly and substantially increased by the addition of specific nutrients and other amendments in laboratory experiments. In addition, laboratory results suggest that formation water has an as yet unidentified role in stimulating biogenesis – perhaps acting as a conduit of nutrients to entrenched microbes within the coal. Data in Figure 1 also illustrate how methanogenic activity can be rapidly and completely inhibited upon exposure to oxygen. These results may have implications on production practices that produce water from coal-beds or utilize vacuum extraction techniques that may irreversibly damage coal biodegrading methanogenic consortia in PRB coal.

A better understanding of how this microbial community interacts with its environment and how we can effect it's well-being through prudent production practices is paramount in our efforts to produce clean energy from this vast methanogenic bioreactor.

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