June 20, 2012

On behalf of the University of Wyoming School of Energy Resources and the Center for Biogenic Natural Gas Research, I welcome you to the Secondary Biogenic Coal Bed Natural Gas International Conference.

We’re very pleased that experts and professionals from around the world will be in Laramie, Wyoming this week to take part. Our team of conference organizers have created an unprecedented opportunity for colleagues to share their experience and ideas in this very exciting area of research in a region of the United States where these clean energy technologies can be deployed to great advantage.

The two-day conference opens officially at 8:30 a.m. on Wednesday, June 20. Rob Hurless, the deputy director of the UW School of Energy Resources and energy policy advisor to Wyoming Governor Matt Mead, will give the general session welcome. The technical program follows with seven sessions and 26 platform and poster presentations. The conference concludes Thursday afternoon with a panel discussion and student award ceremony.

We owe thanks to all who have been involved in organizing this conference, including the steering committee, panel members, session chairmen and the presenters who have devoted their efforts to presenting an impressive program.

I invite you to spend some of your free time to visit the University of Wyoming campus and enjoy this beautiful corner of Wyoming.

Sincerely,

Michael Urynowicz
Director, Center for Biogenic Natural Gas Research
University of Wyoming
CONFERENCE AGENDA

Wednesday, June 20, 2012

7:30 am – 8:30 am  Registration & Breakfast

8:30 am – 9:00 am  GENERAL SESSION – WELCOME
Keynote Address – Rob Hurless, Deputy Director, School of Energy Resources, University of Wyoming

9:00 am – 10:30 am  MICROBIAL CONSORTIA IN COAL BED RESERVOIRS
Session Chair: Karen Budwill, Alberta Innovates – Technology Futures, Canada

9:00 am – 9:30 am  In-situ and Enriched Microbial Community Composition and Function Associated with Coal-bed Methane from Powder River Basin Coals
Presenter: Elliott P. Barnhart, U.S. Geological Survey, USA

9:30 am – 10:00 am  Molecular Analysis of Bacterial and Archaeal Communities Associated with Transformation of Coal into Methane in an Indian Coal Bed
Presenter: Durgesh Narain Singh, Banaras Hindu University, India

10:00 am – 10:30 am  In silico Metabolic Prediction for Biogenic Coal Bed Natural Gas
Presenter: Lawrence Wackett, University of Minnesota, USA

10:30 am – 10:45 am  Break

10:45 am - 11:45 am  BIOGENIC NATURAL GAS PRODUCTION
Session Chair: Elizabeth J.P. Jones, U.S. Geological Survey, USA

10:45 am – 11:15 am  Feasibility of Microbial Production of Natural Gas from Low-rank Non-Producing Coals
Presenter: Vincent Ren, Next Fuel, Inc., USA

11:15 am – 11:45 am  Biogenic Natural Gas Production from Coals of Different Ranks
Presenter: Patricia J.S. Colberg, University of Wyoming, USA

11:45 am – 12:45 pm  Lunch
12:45 pm – 2:15 pm  
**BIOGENIC NATURAL GAS PRODUCTION IN THE POWDER RIVER BASIN**  
Session Chair: Michael Urynowicz, University of Wyoming, USA

12:45 pm – 1:15 pm  
The Effect of Coalbed Dewatering and Partial Oxidation on Biogenic Methane Potential  
Presenter: Elizabeth J.P. Jones, U.S. Geological Survey, USA

1:15 pm – 1:45 pm  
Secondary Biogenic Gas to Enhanced Methanogenesis: The Road from Hunter Gatherer to “Gas” Farmer  
Presenter: William Mahaffey, Luca Technologies, USA

1:45 pm – 2:15 pm  
Microbial Gas Creation Technologies: Production Response of Coal Seams  
Presenter: Roland DeBruyn, Luca Technologies, USA

2:15 pm – 2:30 pm  
Break

2:30 pm – 3:30 pm  
**BIOGENIC NATURAL GAS PRODUCTION IN THE POWDER RIVER BASIN** Continued  
Session Chair: Michael Urynowicz, University of Wyoming, USA

2:30 pm – 3:00 pm  
Fermentative and Methanogenic Activity of a Coal Microbial Community  
Presenter: David M. Bagley, University of Wyoming, USA

3:00 pm – 3:30 pm  
MicGAS™ Coal Biotechnology Demonstration of Turkish Coals for Production of Gas and Organic Humic Products  
Presenter: Daman S. Walia, ARCTECH, Inc., USA

3:30 pm – 5:30 pm  
**POSTER SESSION & COCKTAIL RECEPTION**

Coalbed Natural Gas Co-Produced Water Interaction with Soils: Assessing Isotopic and Chemical Compositional Changes  
Presenter: Kyle J. Lilly, University of Wyoming, USA

Development of a Chemostat for Characterization of Coal-to-Methane Consortia  
Presenter: Samuel L. Papendick, South Dakota School of Mines and Technology, USA

Field Demonstration of Producing Biogenic Coalbed Natural Gas from Coal with No Production History  
Presenter: Jon Larsen, Next Fuel, Inc., USA

GC-MS Based Method Development for Separation and Identification of Naphthenic Acids in Environmental and Geochemical Samples  
Presenter: Jenna Milliken and Rajendra Mahat, University of Wyoming, USA

Production of Biogenic Methane Following In vivo and In vitro Enzymatic Assay of Wyodak Coal  
Presenter: Tanrui Ning, University of Wyoming, USA
Trace Elements of Stimulate Methane Production and Alter Active Microbial Communities in an Enrichment Study of Produced Water from a Coal Bed Methane Well.
Presenter: Burcu Ünal. Department of Microbiology, University of Massachusetts, USA

Thursday, June 21, 2012

7:30 am – 8:30 am Registration & Breakfast
8:30 am – 9:00 am Plenary Session – Michael Urynowicz, Director, Center for Biogenic Natural Gas Research, Department of Civil & Architectural Engineering, University of Wyoming
9:00 am – 10:30 am INTERNATIONAL PRODUCTION OF BIOGENIC NATURAL GAS
   Session Chair: Song Jin, Next Fuel, Inc.
9:00 am – 9:30 am Metagenomic Analysis of Coal Samples and Coal-Based Methanogenic Enrichment Cultures from Coalbed Methane Sites in Alberta, Canada
   Presenter: Karen Budwill, Alberta Innovates – Technology Futures, Canada
9:30 am – 10:00 am A Review of Secondary Biogenic Coal Bed Methane Occurrences in Australia
   Presenter: Mohinudeen Faiz, Origin Energy, Australia
10:00 am – 10:30 am Coalbed Methane from Eastern Australian Basins: Assessing Gas Origins and the Potential for New Biomethane
   Presenter: Patrick C. Gilcrease, South Dakota School of Mines & Technology, USA
10:30 am – 10:45 am Break
10:45 am – 11:45 am COAL SOLUBILIZATION/DEPOLYMERIZATION PROCESSES
   Session Chair: David Bagley, University of Wyoming, USA
10:45 am – 11:15 am An Evaluation of Chemical and Enzymatic Pretreatment Agents for the Stimulation of Secondary Biogenic Coal Bed Natural Gas
   Presenter: Zaixing Huang, University of Wyoming, USA
11:15 am – 11:45 am Utilization of Plant Derived Carbohydrates by Syntrophic Coal Bed Microbial Communities
   Presenter: Christine Sednek, University of Wyoming, USA
11:45 am – 12:45 pm Lunch
12:45 pm – 2:15 pm  
**COMPLICATING FACTORS, UNKNOWNS AND CHALLENGES**
Session Chair: *Mohinudeen Faiz*, Origin Energy, Australia

12:45 pm – 1:15 pm  
**Biogenic Methane from Coal: The Oxidation Factor**
Presenter: *Lisa Gallagher*, Colorado School of Mines, USA

1:15 pm – 1:45 pm  
**Ethanol Conversion to Methane by a Coal Microbial Community**
Presenter: *David M. Bagley*, University of Wyoming, USA

1:45 pm – 2:00 pm  
Break

2:00 pm – 3:00 pm  
**CURRENT AND FUTURE REGULATORY ENVIRONMENT**
Session Chair: *Crystal McDonough*, Associated Legal Group, LLC, USA

2:00 pm – 2:30 pm  
**In Situ CBM Biostimulation: An Overview of the Current Regulatory and Permitting Requirements of the Underground Injection Control Program of the State of Wyoming**
Presenter: *Kathy Shreve*, Wyoming Department of Environmental Quality, USA

2:30 pm – 3:00 pm  
**Secondary Biogenic Natural Gas Production: A Discussion of the Current Regulatory Framework, or Lack Thereof**
Presenter: *Joseph M. Evers*, University of Wyoming, USA

3:00 pm – 4:00 pm  
**PANEL DISCUSSION**
Session Chair: *Michael Urynowicz*, University of Wyoming
*Karen Budwill*, Alberta Innovates – Technology Futures, Canada
*Mohinudeen Faiz*, Origin Energy, Australia
*Song Jin*, Next Fuel, Inc., USA
*Elizabeth J.P. Jones*, U.S. Geological Survey, USA
*William Mahaffey*, Luca Technologies, USA
*Cristal McDonough*, Associated Legal Group, USA
*John Passehl*, Wyoming Department of Environmental Quality, USA

4:00 pm – 4:15 pm  
**Student Awards – Song Jin**, Next Fuel, Inc., USA

4:15 pm – 4:30 pm  
**Closing Comments – Robert Ettema**, Dean, College of Engineering and Applied Science, University of Wyoming

Meeting Adjourn
**In-situ and Enriched Microbial Community Composition and Function Associated with Coal-bed Methane from Powder River Basin Coals**

**Elliott P. Barnhart**
U.S. Geological Survey; Graduate Student, Center for Biofilm Engineering Montana State University, USA

**Background.** The Powder River Basin (PRB), in southeastern Montana and northeastern Wyoming, is the largest source of mineable coal in the United States, but the majority of the coal in the PRB occurs in formations too deep to be economically mined. A methane-producing microbial ecosystem provides access to energy from the deep coal seams. However, little is known about the in situ microbial community, the environmental conditions conducive to coal-bed methane (CBM) production, or the microbial community interactions that promote CBM production. An increased understanding of this microbial system, and the biotic and abiotic parameters that control activity, may result in microbiologically-enhanced CBM production that is industrially sustainable through the application of suitable strategies to stimulate in situ microbial methane production.

**Approach.** Several locations within the PRB were identified as methane-producing sites based on geochemical analysis of produced water. Core samples obtained above, within, and below a methane-producing coal seam were analyzed with 454-pyrosequencing to investigate the in-situ microbial community associated with CBM production. Diffusive microbial samplers (DMS) that had been loaded with coal and deployed on site were analyzed with 454-pyrosequencing and microscopy to determine the actively colonizing in-situ microbial community structure and composition. The composition and structure of microbial communities from the DMS were investigated by applying molecular methods in combination with cultivation techniques (with and without nutrient supplementation) to maximize methane production in batch, bench-scale incubations. Multivariate statistical methods were used to relate these community features to hydrogeochemical parameters.

**Results.** The results of this study indicate that the largest portion of the methane-producing consortia in the PRB is attached to coal particles in the subsurface. The sampling strategy developed in this study targeted an active coal-utilizing microbial consortium composed of a diverse bacterial community associated with a less diverse methanogenic community. Fluorescent in-situ hybridization (FISH) analysis of microbes within the DMS revealed that the coal was actively colonized through biofilm formation. DNA analysis of the cores, DMS coal, and laboratory enrichments identified predominant small subunit ribosomal DNA (SSU rDNA) sequences related to bacteria and archaea, providing insight into in-situ and stimulated CBM production within the PRB.
Molecular Analysis of Bacterial and Archaeal Communities Associated with Transformation of Coal into Methane in an Indian Coal Bed

**Durgesh Narain Singh**

School of Biotechnology, Faculty of Science, Banaras Hindu University, India

The analysis of methane from the coal beds has indicated that a significant proportion of it is biogenic. With a view to understand their role in methanogenesis and to eventually produce methane from coal we have analyzed the diversity of bacterial and archaeal community present in the formation water collected from coalbeds. We carried out cultivation-independent analysis of the bacterial and archaeal communities present at 600-700 m depth in a coalmine (located at Jharia, Jharkhand, India). Metagenomic DNA isolated from the formation water was used for constructing archaeal and bacterial 16S rRNA gene clone libraries, which were screened by ARDRA to generate clusters of related clones. Whereas Archaeal 16S rRNA gene clone library was dominated by taxa showing maximum similarity (98-100%) with different species of *Methanobacterium* and *Methanothermobacter*, the clones of bacterial 16S rRNA gene library were closely related to the different species of *Azonexus*, *Azospira*, *Dechloromonas*, *Pseudomonas* and *Thaurea*, which are well known for their ability to degrade aromatic compounds aerobically as well as under anaerobic conditions. Elucidation of microbial community structure suggested the existence of predominantly hydrogenotrophic methanogens and proteobacteria exhibiting nitrogen fixation, nitrate reduction and polyaromatic compound degradation. On the basis of identification of microbial community of coal-bed we established microcosms with formation water and coal as sole carbon source with different chemical amendments for enhancement of methanogenesis. Members of bacterial community were identified from the microcosm showing significantly enhanced methane generation compared to unamended control. One of the members of the bacterial community of such enrichment was a strain of *Pseudomonas stutzeri* which produced high amount of rhamnolipid biosurfactant when coal powder was supplemented in the medium. We have also attempted to characterize the role of biosurfactant producing *P. stutzeri* in coal biodegradation.
**In silico** Metabolic Prediction for Biogenic Coal Bed Natural Gas

**Lawrence P. Wackett**  
McKnight Professor of Biochemistry, University of Minnesota, USA

**Problem to be addressed.** Coal bed natural gas is generated microbiologically via initial reactions that are poorly understood at present. A greater understanding of the reactions can help identify relevant microorganisms and lead to approaches for their stimulation. The problem is difficult to address experimentally because the molecular structures within coal are complex. However, the overall structural types within coal are known and can be modeled computationally.

**Approach.** Biodegradation metabolism can be modeled by using computational tools available on the University of Minnesota Biocatalysis/Biodegradation Database (UM-BBD). The UM-BBD is freely available at: http://umbbd.msi.umn.edu. The database contains information on over 1400 biodegradation reactions. This information has been mined to extract metabolic rules describing reactions of structural types, also known as functional groups. The rules have been used to develop a metabolic prediction tool called the Pathway Prediction System (PPS), found at: http://umbbd.msi.umn.edu/predict/. The PPS can work with millions of chemicals compounds, or structures, for which metabolism has not been demonstrated yet experimentally. This system is being applied here to aid efforts in understanding microbial metabolism in coal beds.

**Results/Lessons Learned.** The major functional groups found in coal: benzenoid and heterocyclic rings, ethers, thioethers, alcohols, carboxylic acids, and others are already modeled in the PPS. Further, the PPS denotes the likelihood of different reactions in the metabolic pathways it predicts. The PPS predicted reactions also allow the user to go back to the original reaction rules. The reaction rules provide links to the scientific literature and to the microorganisms known to carry out each specific reaction. In this way, one can find known organisms carrying out the reactions and link to genomic information of those strains. The application of these methods to the common groups in coal can help provide new knowledge about the microorganisms and metabolism that transform coal to methane.
Feasibility of Microbial Production of Natural Gas from Low-rank Non-Producing Coals

Vincent Ren
Vice President - China Operations, Next Fuel, Inc., USA

Background. Natural gas (methane) from coal formations is known to have originated from both biogenic and thermogenic processes. Biogenic natural gas typically occurs in the formations where anaerobic processes such as fermentation and methanogenesis are favored. Interest in stimulating microbial natural gas production in coal seams has gained interest from industry and focus is typically on formations that have a history of biogenic natural gas production and restoring depleted wells. However, limited research and development has been conducted on initiating and sustaining microbial natural gas production from low-rank non-producing coals with no gas-producing history.

Experimental. Lignite (brown coal) samples with formation water were collected from Australia, Indonesia, and Inner Mongolia (China). The lignite samples were from seams with no baseline natural gas production. Microbial stimulation tests were conducted in microcosms, which contained mixtures of coal and formation water. One set of microcosms was amended with a proprietary mixture of nutrients that our team previously developed. The nutrients consist of inorganic salts and trace elements. The other set of microcosms served as controls with no additional amendments. Headspace methane and carbon dioxide were monitored and analyzed by a gas chromatograph with a thermal conductivity detector. Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) analysis were used to determine changes in the distribution of the bacterial populations. Quantitative PCR (qPCR) analysis was used to quantify the changes in methanogen numbers in the microcosms.

Results. The headspace gas composition results indicated that active microbial metabolism of coal was occurring in the microcosms. After 74 to 97 days of incubation, the methane production in the microcosms was determined to range from 5.00 to 9.00 L/kg coal (equivalent). Higher methane production was observed in microcosms containing nutrient amendments than those that were not amended. Methane production was 70, 64 – 146, and 375 – 463% higher in nutrient amended microcosms containing Australia, Indonesia, and Inner Mongolia lignite, respectively. The DGGE analysis indicate shifts in the bacteria community upon addition of nutrients, and the qPCR analysis shows the enrichment of methanogenic populations in microcosms amended with nutrients. Overall, the results demonstrate that the application of nutrients to non-producing low-rank coals can initiate and sustain substantial methane production in low-rank coal seams with no history of natural gas production.
Biogenic Natural Gas Production from Coals of Different Ranks

**Patricia J.S. Colberg**  
Associate Professor, Department of Civil & Architectural Engineering, University of Wyoming, USA

**Background.** Stimulating biogenic natural gas production from coal has gained increasing interest by industry to restore depleted gas wells and as a source of relatively cleaner energy from low-value or un-mineable coal. Typically, biogenic natural gas correlates with low-rank coals such as lignite (brown coal) and subbituminous coal, which contain higher fractions of volatile organic matter. Little is known about the potential of stimulating microbial methane production in higher-rank coals.

**Experimental.** Coals of different ranks, including lignite, subbituminous, high-volatiles bituminous, and low-volatiles bituminous coal samples were obtained and used for this study. The study was conducted in microcosms containing each type of coal, which were amended with a proprietary mixture of inorganic compounds and trace elements that were previously developed by our team to stimulate biogenic methane production. Headspace methane and carbon dioxide concentrations were monitored and analyzed by a gas chromatograph with a thermal conductivity detector.

**Results.** Headspace analysis indicates that each type of coal can yield organic compounds that can be utilized by microorganisms to produce methane. Preliminary results suggest that methane production rates were higher in the bituminous coals, which may be due to potentially trapped volatile organic compounds that were more bioavailable to the microorganisms. This finding is opposite to our hypothesis that lower rank coal would contain more bioavailable compounds that can serve as substrates for microbial pathways to produce methane. Pending results will indicate the overall correlation of coal rank and biogenic natural gas production potential.
Coal formation dewatering was associated with enhanced secondary biogenic methane measured in anaerobic lab incubations of Powder River Basin coal. We hypothesized that dewatering can stimulate microbial activity and increase the bioavailability of coal. We analyzed one dewatered and two water-saturated coals to examine possible ways in which dewatering influences CBNG biogenesis by looking at differences with respect to the native coal microbial community, coal-methane organic intermediates, and residual coal oxidation potential. Biomass did not increase in response to dewatering, but more bacterial diversity was measured in the dewatered coal. No increase was observed in the prevalence of aerobic bacteria, fungi, or methanogens in the dewatered coal. There was a higher incidence of long chain and volatile fatty acid intermediates in incubations of the dewatered coal versus the water saturated coals. This could be due to differences in microbial enzymatic activities or to chemical oxidation of the coal associated with O₂ exposure. Dilute H₂O₂ treatment of structural coal was used as a proxy for chemical oxidation by O₂. The dewatered coal had a low residual oxidation potential compared to the saturated coals. Oxidation with 5% H₂O₂ did increase the bioavailability of structural coal, and the increase in residual oxidation potential in the water saturated coals was approximately equivalent to the higher methanogenic potential measured in the dewatered coal. Evidence from this study supports the idea that coalbed dewatering could stimulate biogenic methanogenesis through partial oxidation of the structural organics in coal.
Secondary Biogenic Gas to Enhanced Methanogenesis: The Road from Hunter Gatherer to “Gas” Farmer

William Mahaffey
Chief Technology Officer, Luca Technologies, USA

Biogenic methane has enormous potential as a sustainable energy source and is found in a wide variety of subsurface, anaerobic, hydrocarbon bearing environments. An area of great interest for biogenic methane is the Powder River Basin in Wyoming, United States. This area has been previously developed for Coalbed Methane production with over 30,000 wells drilled basin wide. The PRB has been shown to be an active “geobioreactor” based on the gas isotopic signatures of produced methane (δ13C-methane -57‰, δD-Methane -320‰). That the coal beds of the PRB are “alive” with active methanogenic communities that can be stimulated to create new methane from coal has been determined by coal conversion studies in laboratory experiments. Experimental data will be presented that shows new methane being created in real-time by activating the microbial communities with activation amendment packages and observing headspace methane accumulation in excess of the stoichiometric production from the nutrients. In addition, BESA inhibited cultures exhibit an accumulation of metabolic intermediates in coal slurries prepared with live formation waters but not in controls with active formation water only. Data will be presented showing laboratory field support research and parallel field research and development, supporting commercial scale proof-of-concept. Quickly moving to projects of large scale, we have been able to demonstrate production of commercial quantities of new gas in multiple basins, using an enhanced in situ microbial based process we call “Methane Farming.”

Understanding the composition and metabolism of the methanogenic consortia has been one of several key steps in commercializing the process of sustainable biogenic methane production. In order to identify microbes present in these methanogenic consortia we performed microbial community analysis using error-correcting barcode pyrosequencing analyzed with the QIIME bioinformatic pipeline. This involved thousands of samples collected from different locations within set geographical areas over several years. Both bacterial and archaeal specific primers were used to amplify these distinct populations from our DNA samples. The QIIME pipeline was used for library demultiplexing, OTU picking, alignment, taxonomic identification, and statistical analysis of community structure using Unifrac. Visualizations of principle coordinate analyses of the QIIME results and metadata from the formation will be used to illustrate the variables most responsible for the shift in community demographics associated with successful gas production.

Here we demonstrate the large scale sampling and community analysis of coalbed methane (CBM) wells within a discrete but large area of the Powder River Basin, the data set encompassing areal as well as temporal components. The temporal component is defined as the natural baseline state, followed by a bio-stimulation phase (i.e., Restoration), dwell phase and enhanced methane production phase. Under the baseline conditions, we find distinct bacterial and archaeal populations that vary by coal seam and water chemistry, as well as over time. The community signatures help to determine areas of greatest methanogenic potential as well as identify patterns of ground water recharge, water movement, and potential metabolic bottlenecks.
Numerous laboratory studies have created new biogenic methane from coal substrates. The studies reported in the literature have typically been static microcosms of field water and crushed coal that are physically different from in situ environments.

Luca Technologies transitioned lab results to the field starting in 2006. Since that time, several hundred nutrient deployments have been completed in a number of United States basins.

This presentation will present summary results of the methane production resulting from those field treatments in three basins: Powder River Basin, Black Warrior Basin, and Uinta Basin. Discussion will include rates, impact of production operations on gas production responses, and characteristics of the various geological settings of these projects.

Biography:

Roland P. DeBruyn is Vice President, Engineering of Luca Technologies Inc. in Golden, Colorado. He has been with Luca since its founding in 2003. His work focuses on transitioning technology from Luca’s lab research to the field; project design and management; and technical support of regulatory compliance. He holds a Bachelor of Science degree in chemical engineering from the University of Calgary, and an MBA from the University of Phoenix. He has co-authored a number of technical papers in the fields of coalbed methane engineering and methane biogenesis; is co-inventor on several domestic and international patents; and has addressed meetings of the American Association of Petroleum Geologists, the Ground Water Protection Council, and Wyoming Geological Association. He is registered as a professional engineer in Alberta, Canada.
Fermentative and Methanogenic Activity of a Coal Microbial Community

David M. Bagley
Professor and Department Head,
Department of Chemical and Petroleum Engineering, University of Wyoming, USA

To determine the extent of fermentative and methanogenic activity in coal samples and to propose the limiting steps in accelerating biogenic methane production from coal, Smith coal collected from the Powder River Basin was examined. Five conditions were examined to identify the potential for methane production using Smith coal as the sole inoculum. The only condition to produce methane was Smith coal with added cysteine. The absence of methane production from Smith coal with both cysteine and sulfate suggests that sulfate reducing activity consumed electrons that would have been available for methane production. To evaluate the hydrogenotrophic methanogenesis that occurred in the cysteine-fed bottles, additional hydrogen was added. Hydrogen consumption and methane production occurred concurrently, stoichiometrically and more rapidly than previously in the same bottles. These results suggest that the coal itself was the source of the microbial community that facilitated fermentation (of cysteine) and methanogenesis. Hydrogen added to the coal cultures without cysteine also produced methane. The hydrogen consumption and biomass growth rates for the coal without added cysteine were simulated using a Monod-type model. Although the coal examined was the presumed source of fermentative and hydrogenotrophic methanogenic organisms, neither hydrogen nor methane were detected when coal was used as both the sole inoculum and substrate. Overall, the fermentative and methanogenic organisms present in the coal bed were capable of readily consuming added substrates in preference to coal constituents. The rate-limiting step for biogenic CBM production appears to be the degradation of the coal matrix itself into methanogenic substrates such as H\textsubscript{2} and acetic acid.
MicGAS™ Coal Biotechnology Demonstration of Turkish Coals for Production of Gas and Organic Humic Products

Daman S. Walia
ARCTECH, Inc.
USA

MicGAS™ Coal Biotechnology approach for bioconversion of coals was demonstrated in mobile pilot units in Turkey for production of both gas and organic humic products for agriculture and environmental applications. Techno economic analysis from the demonstration tests and integration of it for application to deep unminable colas support an approach with a potential of production of energy and non-energy products with highly favorable economic as well as utilizing vast resources of coals into environmentally safe manner.

Results of demonstration tests and applicability tests of organic humic products for meeting large needs of agriculture, environmental and wastes recycling in Turkey were evaluated. A comparison of the products from the Turkish lignite with the ARCTECH’s current commercial organic humic products will be presented.
Metagenomic Analysis of Coal Samples and Coal-Based Methanogenic Enrichment Cultures From Coalbed Methane Sites in Alberta, Canada

Karen Budwill
Senior Researcher, Environment and Carbon Management Division, Alberta Innovates – Technology Futures, Canada

Objectives. Geochemical and isotopic evidence suggest that biogenic methane production occurred and is likely still occurring, albeit at very slow rates, in many coal beds in Alberta. The microbes involved in this biogenic methane production could potentially be stimulated to increase methane yields in older or low gas content coalbed methane (CBM) wells and could be used to augment traditional CBM operations. Investigations of the microbial ecology and associated methanogenesis processes in coal seams using a metagenomics-based approach contribute to our understanding of how to increase biogenic methane production to economic levels.

Methodology. To date, we have collected 17 coal cuttings and 2 produced water samples from different cone zones and depths in Alberta. We have also obtained 12 core samples from different depths taken from 3 different well sites. Whole community DNA has been extracted from the majority of the coal samples and the 16S rRNA gene sequenced by 454 pyrosequencing. Metagenomic sequencing of select cuttings and core samples has also been carried out. We have also enriched for methanogenic communities from the coal cuttings samples and investigated the effects of different nutrient additions to the cultures on enhancing methane production rates.

Results. Sequencing of the 16S rRNA genes of various coal-related samples has revealed that distinct and unique microbial communities exist in Alberta coal formations. The microbial community compositions appear to be strongly influenced by the geochemical environment such as depth, coal rank and salinity. The metagenomic analysis of a microbial community associated with a cutting sample confirmed the presence of genetic potential for several key steps of coal biomethanization. This potential includes enzymes with putative roles in various coal solubilization and depolymerization mechanisms, and others known for their involvement in the anaerobic degradation of polyaromatics, aromatics, aliphatics, and in acetogenesis and several methanogenic pathways (acetilastic, methylotrophic, CO2 reduction). All enzymes were assigned a taxonomic identity, and the resulting profiles were compared to profiles obtained from other North American coalbeds. Biogenic generation of methane from coal can be greatly enhanced from 5- to 25-fold through the addition of a dilute organic nitrogen-based nutrient. Evidence from DNA sequencing and GC-MS analysis suggests the nutrient stimulates the growth of fermentative bacteria which activate the coal structure. These active coal-based methanogenic enrichment cultures are also being investigated using metagenomics analyses to gain a greater understanding of the roles and functions of the methanogenic community in converting coal to methane.
A Review of Secondary Biogenic Coal Bed Methane Occurrences in Australia

Mohinudeen Faiz
Principal Geologist, Origin Energy, Australia

Australia is one of the largest coal bed methane (CBM) producing countries in the world with total proven and probable reserves of approximately 40 trillion cubic feet. All of these reserves are contained in the Permian and Jurassic coal bearing basins in Queensland and New South Wales. In addition to these reserves a larger volume of unproven resources exist in other basins in Queensland, New South Wales, Victoria, South Australia and Western Australia. The coals in the CBM producing fields in Queensland and New South Wales range from sub-bituminous to low volatile bituminous in rank whereas those in Victoria are lignite (Brown coal) in rank.

Stable carbon and hydrogen (2H) isotopes of gas in coals have been studied in Australia since the late 1970s to understand the source of CH4 and CO2 in underground coal mines. The 13C values for CH4 in these coals range from about -75 to -25‰ (VPBD) with majority of the values for CH4 desorbed from high rank coals (vitrinite reflectance ~1 to 1.3%) in underground coal mines (depth ~300 to 500m) ranging between -65 and -50‰. Due to the association of this CH4 with high rank coals it was interpreted as thermogenic gas, typically generated at temperatures of about 120°–160°C. However, in the early 1990s Smith et al. (1992) re-interpreted the isotope and gas composition data and, perhaps controversially, suggested that CH4 in most of the deep coal seams is of biogenic origin. This hypothesis was later confirmed by the identification and culturing of methanogenic consortia and other microorganisms from several deep coals (both high rank and low rank) in various basins in Australia. More recent geochemical studies including stable isotope composition of CH4, C2H6 and CO2, molecular composition of desorbed gas and solvent extract chemistry of coals have confirmed that a significant proportion of CBM is secondary biogenic in origin where pre-existing thermogenic hydrocarbons and carbon dioxide occluded in coal have been degraded to methane by micro-organisms.

Microorganisms in Australian CBM reservoirs were identified and cultured for the first time in the mid 2000s; a preliminary culture-independent survey of prokaryotic microbial communities was undertaken for a series of water and coal samples from CBM reservoirs and coal mines in eastern Australia (Li et al., 2008). For the first time in Australia methanogenic consortia were identified and cultured from CBM formation water samples from the Gippsland Basin (Midgley et al., 2010). Subsequently methanogenic consortia have been identified and cultured from formation waters from various CBM reservoirs in the Surat, Bowen and Sydney basins in eastern Australia.

Recent studies using both traditional, culture-based approaches and genomic methods, diverse bacterial lineages have been identified from coal seam formation water samples. Reservoirs with temperatures up to 45°C, typically host an abundance of deltaproteobacterial taxa along with clostridia from the order Clostridiales. At higher temperatures, Thermoaerobacterales clostridia, rather than Clostridiales, tend to be more frequently detected. Archaeal taxa are also abundant in coal-seam formation water, most commonly detected taxa are methanogens from the classes Methanobacteria and Methanomicrobia. Intriguingly, the microbiology of the coal itself appears to be distinct from the associated formation water. Indeed, methanogenic archaea which are abundant in the water are very infrequently detected on coal. Similarly, while Clostridiales and Thermoaerobacterales clostridia are still detected on coal, they are typically at much lower abundance. In their place, there appears to be a much greater diversity of Alpha-, Beta-, Delta- and Gammaproteobacteria.


Coalbed Methane from Eastern Australian Basins: Assessing Gas Origins and the Potential for New Biomethane

Patrick C. Gilcrease
Associate Professor, Chemical and Biological Engineering, South Dakota School of Mines & Technology, USA

Background. Between 1996 and 2008, coalbed methane (CBM) production in Queensland increased from 1 petajoule (PJ) per annum to 167 PJ per annum, with an even more spectacular rise in certified 2 P (proven and probable) reserves of CBM to some 28000 PJ. Stable carbon and hydrogen isotope data confirm the microbial origins of methane in lower rank coals of the Surat Basin and the presence of secondary microbial methane at shallow to intermediate depths (< 500m) in higher rank uplifted coals of the Bowen and Sydney Basins. Produced water samples collected from Surat Basin coal seams in eastern Queensland, Australia were shown to contain viable microbial consortia with the ability to convert native Walloon coal into methane. This suggests an opportunity to enhance coalbed methane reserves via in-situ stimulation of indigenous microbial consortia, but such a process will likely be limited by the bioavailability and/or biodegradability of coal substrates.

Approach. Because coal seam gases are generally dryer than natural gases, the distinction between thermogenic and biogenic coal seam methane is heavily reliant on carbon isotope ratio. Hydrogen isotope compositions are used to determine whether biogenic methane is the product of microbial CO2 reduction or methyl-type fermentation.

An enrichment culture technique was used for detecting and characterizing methanogenic consortia present in coal seam gas formation waters. To determine the effect of coal surface area, enrichment cultures were amended with native Walloon coal of three particle size fractions (<300 µm, 300–600 µm, and 600–850 µm).

Results. The carbon and hydrogen isotope compositions of methane from exploration and production wells in the Bowen and Surat Basins commonly plot in the mixed origins field and display linear trends in carbon isotope composition, reflecting a continuum from shallow, isotopically light biogenic gas to isotopically heavy thermogenic gas at depth. Moreover, methane production rates are highest at intermediate depths in a number of gas fields where carbon isotope data show the gases to be of mixed thermogenic and microbial origin. The hydrogen isotope data indicate that microbial CO2 reduction is the main process generating biogenic methane in the eastern Australian coal basins.

In laboratory cultures, enhanced methanogenesis rates and yields were observed with decreasing particle size, suggesting that that surface mass transfer is the rate-limiting step, and that only surface substrates are bioavailable during limited culture periods. To estimate field in-situ methane production potential, laboratory rates and yields were extrapolated based on the ratio of estimated in-situ cleat surface area to laboratory coal particle surface area. With laboratory to field coal surface area ratios of 40 to 1, our maximum laboratory culture rate of 1.0 m3/t/day (30 scf/ton/day) would correspond to an in-situ rate of 0.025 m3/t coal/day (0.81 scf/ton/day). At this rate it would take 140 days to replenish the coal seam gas content (3.5 m3 of methane per metric ton of coal), which is still significant from a production standpoint.
Coal bed methane is considered an underexploited source of clean energy. With the realization of its biogenic nature it has attracted increasing interest in recent decades. Most of the published studies on biogenic coal bed natural gas have focused either on biostimulation (the addition of nutrients to stimulate the native microbial populations) or bioaugmentation (the addition of both nutrients and nonnative consortia of microorganisms from outside the coalbed) methods. Although these approaches have shown promise, they only address the assumptions that the microorganisms living within the coal seam are nutrient limited or that the existing microbial communities are not optimized for the biogenic conversion of coal to natural gas. We have hypothesized that the organic matter present within the coal matrix is not readily available to the microorganisms living within the coal seam. For the purpose of this study chemical treatment agents including acid (HNO3), base (NaOH), and oxidants (catalyzed H2O2 and KMnO4) as well as a fungal enzyme (manganese peroxidase) were evaluated for enhancing the solubility and biodegradability of subbituminous coal from the Powder River Basin. Although potassium permanganate (KMnO4) was shown to produce only a moderate amount of dissolved organic carbon, based on the results from biometer and anaerobic respirometer tests the solubilized coal derived compounds proved to be more bioavailable than those derived from the other chemical treatments. In addition, the chemically treated coal was shown to be more susceptible to further solubilization by subsequent treatment with manganese peroxidase.
Utilization of Plant Derived Carbohydrates by Syntrophic Coal Bed Microbial Communities

Christine Sednek
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Furthering the discovery of biogenic natural gas within the Powder River Basin, this study assesses the ability of coal derived microbial communities to utilize plant-derived carbohydrates. Biomass has the potential to supply vast quantities of renewable biogenic coal bed natural gas. The approach would have numerous advantages over more conventional energy production methods. For example, it would produce methane using natural processes that occur within the coal seam. Consequently, there would be few if any emissions. In addition, since the microorganisms would utilize biomass as a food source instead of coal, it has the added advantage of being nearly “closed loop” with respect to carbon. Conventional biochemical conversion processes typically involve the conversion of biomass to sugar or other fermentation feedstock through pretreatment, conditioning, enzymatic hydrolysis, and fermentation, followed by the processing of fermentation products to produce fuel-grade methanol, ethanol, butanol and other fuels. Since vast coal seams would serve as an infinitely scalable reactor and the indigenous microorganisms within would transform simple sugars into natural gas, the need for capital-intensive biomass conversion plants would be eliminated. Moreover, transforming biomass into natural gas rather than ethanol has the added advantage that the gas can be readily and inexpensively transported long distances in existing natural gas pipelines.

This study explores the syntrophic microbial communities living in coal seams by evaluating their potential for utilizing cellulose and various five- and six-carbon sugars as potential substrates for the production of methane. The chosen substrates are readily used by fermentative bacteria, which initiate the syntrophic degradation process which culminates with the production of methane (methanogenesis). Coal from the Wyodak formation served as the inoculum for the study which included ten (10) enrichment cultures. High performance liquid chromatography (HPLC) was used to analyze for volatile fatty acid intermediates and gas chromatography (GC) was used to determine the rate and magnitude of methane production. Preliminary results suggest that the coal derived syntrophic microbial community is well adapted for the utilization of plant derived carbohydrates.
Biogenic Methane from Coal: The Oxidation Factor

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Background/Objectives. Vast reserves of coal represent an untapped resource that can be used to produce methane gas, a cleaner energy alternative compared to standard fossil fuels. Microorganisms have demonstrated the ability to utilize coal as a carbon source, producing biogenic methane. With increasing demand for cleaner energy resources, understanding and enhancing biogenic methane production has become an area of active research. The conversion of coal to methane by microorganisms has been demonstrated experimentally by a number of research groups, but the state of the coal used as a substrate has not always been reported and may impact biogenic methane production.

Approach/Activities. Microcosm experiments were designed in order to assess how prior exposure of coal to oxygen might influence methane production (e.g. as in a dewatered coal-bed natural gas system). Oxidized and un-oxidized coal samples from the Powder River Basin were incubated in microcosms inoculated with an enrichment culture that was derived from coal. Microcosms were characterized by headspace gas analysis, organic acid production, functional gene abundance (qPCR), and pyrosequencing of the 16S rRNA gene.

Results/Lessons Learned. Although the microbial consortium demonstrated the ability to utilize both oxidized and un-oxidized coal as a sole carbon source to generate methane, it was produced in higher quantities from the un-oxidized coal. This microbial community was dominated by Methanobacteriaceae (45%), epsilon-Proteobacteria (32%) and delta-Proteobacteria (13%). The results of this study provide a basis to develop strategies to enhance biogenic methane production from coal, as well as demonstrate the need for careful substrate preparation for inter-study comparisons.
Ethanol Conversion to Methane by a Coal Microbial Community

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The objectives of this study were to determine the capability of a native coal microbial community to convert ethanol to methane and to identify whether ethanol addition stimulated degradation of the coal matrix itself. Big George coal collected from the Powder River Basin was exposed to different concentrations of ethanol, in the absence of any other additives. Hydrogen was produced during ethanol exposure for 24 hours, indicating the presence and activity of fermentative microorganisms. Methane was also produced during 36 days of incubation from Big George coal that was provided 1, 5 or 10 mg ethanol. The coal cultures were then supplied with medium that included inorganic nutrients but no additional methane was detected during the following 18 days of incubation. Acetic acid and iso-propanol were detected on Day 54 in the cultures that had received 5 or 10 mg ethanol. The chemical oxygen demand recoveries of ethanol in the coal cultures receiving 1, 5 and 10 mg ethanol were 7%, 34.3±7.6% and 38.3±2.9%, respectively. The pHs of these coal cultures were near neutral, indicating that pH did not inhibit the activity of microbial community. While ethanol conversion to methane occurred in cultures containing only Big George coal, the conversion was incomplete. Therefore, another series of cultures received both medium and inoculum immediately after the coal samples were exposed to different concentrations of ethanol for 1 hour. The ethanol recoveries from the cultures exposed to 5 and 10 mg ethanol were 96.1±0.4% and 98.1±4.7%, respectively, higher than from cultures exposed to 1 mg ethanol (74.5±2.4%) and ethanol control cultures (containing inoculum only but no coal). The native microbial community of the Big George coal was able to degrade ethanol to produce methane even when not supplied medium containing inorganic nutrients but showed increased activity when medium was supplied immediately after exposure to ethanol. The methane produced from coal never exceeded that which could be produced solely from the added ethanol, even though there was a distinct pattern of initial rapid methane production followed by slow methane production. This suggests that the added ethanol did not stimulate degradation of the coal matrix itself, at least not within the time limits of this experiment.
In Situ-CBM Biostimulation: An Overview of the Current Regulatory and Permitting Requirements of the Underground Injection Control Program of the State of Wyoming

**Kathy Shreve**

Environmental Program Principal, Wyoming Department of Environmental Quality, Underground Injection Control Program, USA

**Overview.** This presentation describes the current permitting approach used by the Underground Injection Control Program (UIC Program), Water Quality Division, Wyoming Department of Environmental Quality, and discusses applicable state environmental regulations that operators must comply with when applying for In-Situ CBM Biostimulation permits.

**Background/Objectives.** The UIC Program is the regulatory authority of the state of Wyoming that is tasked with the protection of the state’s groundwater resources from materials injected into the subsurface. Beginning in the summer of 2010, the UIC Program has received applications for In-Situ CBM Biostimulation facilities, a type of injection facility that had not previously been permitted within the state of Wyoming. This presentation discusses the UIC Program’s current permitting approach, the regulatory basis for this approach, permitting challenges, how In-Situ CBM Biostimulation facilities fit into the state’s current permitting framework, and the status and history of In-Situ CBM Biostimulation permitting in the state of Wyoming.

**Approach.** Currently, operators desiring to obtain a permit for In-Situ CBM Biostimulation facilities in the state of Wyoming are required to collect a large amount of baseline groundwater quality data prior to commencement of biostimulation activities. The purpose of baseline groundwater quality data collection is to provide a basis for groundwater classification in the proposed project area, and a subsequent framework for establishing permit injectate limits and monitoring requirements. In addition, the operator is required to provide a detailed list describing the amendments they are proposing to inject, including proposed concentrations, which the UIC Program evaluates to determine compliance with applicable groundwater regulations.

**Results/Lessons Learned.** The UIC Program has, as of the date of this abstract, permitted two In-Situ CBM Biostimulation Projects. However, should this technology prove economically viable, the UIC Program expects to receive many more applications for facilities of this type, and hopes to permit these facilities in a more timely and less resource-intensive manner by informing potential permittees of the current permitting requirements.
Secondary Biogenic Natural Gas Production: A Discussion of the Current Regulatory Framework, or Lack Thereof

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Background/Objectives. Legislators and policy makers have a tendency to force new energy technologies and developments into existing legal regimes and established regulatory frameworks. More often than not however, new energy technologies do not fit neatly into the existing legal regime, making the implementation of new technologies difficult. This paper examines the regulatory uncertainty that faced Luca Technologies, Inc. (Luca) from 2006-2012 regarding its proposed “methane farming” development in the Powder River Basin (PRB) of Wyoming. Further, this paper discusses potential improvements that may be made to existing regulatory regimes that may serve to encourage future growth and expansion of secondary biogenic natural gas development across the United States.

Luca uses its proprietary technology and processes to stimulate native microorganisms within the coal seam to produce coalbed methane (CBM). Until recently, the Bureau of Land Management (BLM) and Luca were at odds over how the owner of the coal estate should be compensated for the use of its coal in the production of biogenic CBM. This paper discusses the existing legal regime that guides CBM development, the differences between Luca’s process and traditional CBM development, how the BLM will regulate Luca, concerns of the BLM in managing the coal estate for the benefit of the public, the possible legal arguments for classifying secondary biogenic CBM as pertinent to the oil and gas estate or coal estate, and the potential need for improving the legal/regulatory regime for secondary biogenic coal bed natural gas development on a national scale.

Approach. The remaining regulatory uncertainty surrounding allocating rights to the coal estate or oil and gas estate may hamper the future growth of secondary biogenic CBM development on a national scale. This paper considers the economic, environmental, and regulatory efficiency of existing and potential regulatory schemes based on case law and policy analysis through Lasswell’s seven decision functions: planning, promotion, prescription, invocation, application, appraisal, and termination.

Results. This paper presents possible recommendations for the improvement of the existing legal regime surrounding the development of secondary biogenic coal bed natural gas.
**Poster Presentation Abstracts**

**Coalbed Natural Gas Co-Produced Water Interaction with Soils:**
Assessing Isotopic and Chemical Compositional Changes

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University of Wyoming, USA

**Background.** Recovery of natural gas from coalbeds is commonly associated with co-production of water (i.e., CBNG water). This water can be an environmental concern. In order to mitigate potential environmental consequences, it may be important to use a tracer to study discharged water as it interacts with soils and the atmosphere.

**Approach.** A study was conducted to evaluate stable C isotope signatures of dissolved inorganic C ($\delta^{13}$C-DIC) after contact of CBNG water with soils from the Powder River Basin, WY. A CBNG water sample was mixed with a PRB soil and allowed to react for a one month period. Gas and water samples were taken at six different time periods and analyzed for carbon isotopes. Other geochemical proxies like major cations, electrical conductivity (EC), and pH were used in conjunction with isotope water quality measurements to understand geochemical changes of CBNG water as it interacted with soils.

**Results.** CBNG water was found to be isotopically and chemically different than surface stream waters after interacting with soils over the course of one month. The $\delta^{13}$C of DIC in the CBNG water decreased over time (from 5‰ to -13‰), and ECs of CBNG water increased with time when mixed with soil (from 1.64 to 3.26 dS m$^{-1}$). Variable pH values (7.5 to 8.4) were observed in the CBNG water interacting with soil over time, and no trends were visible. Calcium, magnesium, and potassium concentrations increased in the soil-water solution. Sodium concentrations remained the same over time. The $\delta^{13}$C-CO$_2$ isotopic signature in the headspace of CBNG water decreased with time when mixed with soil (from -5‰ to -28‰), and $\delta^{13}$C-DIC values correlated well with the $13$C-CO$_2$ values. Results from this study demonstrate that these contrasting $\delta^{13}$C-DIC signatures coupled with standard geochemical measurements can be used to distinguish CBNG produced water from natural stream waters as the waters interact with soils.
Development of a Chemostat for Characterization of Coal-to-Methane Consortia

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Background. Coal reservoir depth and limited well access make in-situ microbial studies difficult. While limited field tests have demonstrated the presence of viable methanogens in coal seams, most research to date has used batch culturing techniques to study coal biomethanogenesis in-vitro. Low cell numbers found in formation waters and coal provide poor phylogenetic results; enrichment cultures provide sufficient DNA for extraction, but they select for a subset of the original population. Generally, 25-160 ml airtight tubes or bottles are sealed with a combination of coal, formation water, and nutrient media, with microbial growth correlated to methane production. Such batch enrichment cultures can provide data on phylogeny and detectable intermediates, and allow for some optimization under ideal conditions. That said, batch culture environments transition from nutrient-excess to nutrient-limited growth conditions; the resulting relative abundance of consortium members will then depend on when the cultures were sampled, as different members will be better adapted to certain conditions. As such, batch enrichment cultures provide limited insight into in-situ microbial activity in an underground, open system.

Approach. Continuous culturing is another technique for studying microbial systems, where fresh sterile feed is introduced to a chemostat at the same rate an effluent is collected. When coupling mixed culture results to genomic data, various growth conditions can characterize consortia adaptability to each environment. One technique increases the feed rate to elevate non-coal nutrient concentrations; in an unstimulated coal seam, substrate availability will initially be low until nutrient injection begins and a new nutrient-rich environment is established. Understanding how microbial communities adapt to the changing environment will assist in proper underground stimulation. Another advantage over batch culturing is the continual monitoring and control of pH and temperature. These conditions affect metabolic activity and consortia dynamics; with the correct environmental conditions, methane production may be optimized with respect to carbon dioxide and cell-mass production.

Results. When studying coal seam microbial activity, the chemostat is a valuable bridge between small-scale batch cultures and true in-situ conditions. It provides the benefit of varying one environmental condition while maintaining others constant. For example, other research has indicated fatty acid toxicity toward microbial growth, but it was unclear if this represents inherent compound toxicity, or if this was simply caused by a drop in pH. Our work focuses on the change in relative microbial members with respect to coal/nutrient availability, and how this affects coal biodegradation rates/yields. Data showing the effect of dilution rate and coal solids loading on overall methane productivity will be presented.
Field Demonstration of Producing Biogenic Coalbed Natural Gas from Coal with No Production History

Jon Larsen
VP of Field Operations, Next Fuel, Inc., USA

Background. Enhancing biogenic natural gas production from coal has gained interest over the past decade as a method to extend the life of CBM wells. A number of laboratory researchs have demonstrated that indigenous microorganisms can be stimulated to produce methane from coal. Since 2001, our group has been conducting R&D of real-time biogenic methane production from coal. Based on results obtained from laboratory studies, our process was established on the alteration and optimization of in situ geochemical conditions, without any introducing any microbial species. Our first field pilot test was completed in 2007 in the Powder River Basin, Wyoming, with fresh biogenic methane being produced within 29 days after the injection of the proprietary nutrients were injected in a coal seam of negative gas pressure. In 2011, we completed a field demonstration in Indonesia, and achieved commercial scale gas production within 2 months. The field demo was performed on a lignite seam with no history of methane production. To our knowledge, this was the first field application that successfully generated fresh biogenic natural gas from a virgin coal seam of zero background gas production.

Experimental. A site in Indonesia with a shallow lignite seam was selected for implementing the demonstration project. The lignite seam at this site was 30 m below ground surface, and had no observable natural gas. Wells, a production/injection system, and other infrastructure components were established at the site. A proprietary mixture of nutrients and other enhancement materials were injected into the seam, and water was pumped out of production wells and recycled back to the injection well. This circulation method was utilized to maximize contact with the coal and minimize loss of nutrients. Water quality, gas production and composition were monitored.

Results and Discussions. The results and observations from the demonstration project include: (1) ammonium concentrations substantially increased after nutrient injections, indicating that the lignite was permeable and the enhancements were transported within the coal; (2) continuous production of combustible gas was observed approximately 47 days after the enhancements injection; (3) the total combustible gas production rate was determined to be approximately 18 m³/d (or approximately 400 m³/ha/d) before the end of the project. This project was the first known successful field demonstration of biogenic methane production from a coal with no history of gas production. The successful field demonstration made a significant contribution to the application of biotechnology in clean energy industry.
GC-MS Based Method Development for Separation and Identification of Naphthenic Acids in Environmental and Geochemical Samples

**Jenna Milliken**  
Graduate Student, University of Wyoming, USA

Naphthenic acids (NAs) are a class of surfactant like compounds that are found in small amounts (~ 4%) in crude oils and coals. Usually, these are alicyclic (5 or 6 membered ring) mono carboxylic acids with the general molecular formula $C_nH_{2n+z}O_2$, where $z$ is 0 or a negative even integer (-2, -4, -6 etc) representing hydrogen deficiency due to presence of rings. NAs are a class of compounds holding great commercial, environmental and hygienic significances. Mass Spectrometry-based methodology is one of the most prevalent techniques to characterize and classify naphthenic acids present in a given sample based on their composition and origin. Direct analysis of NAs with a MS based technique like GC-MS is not amenable because of the low volatility of NAs. So, a GC-MS method will be developed to derivatize a synthetic sample containing 17 individual NAs and a commercial NA mixture. Then, this method will be applied to collected samples from the environment. Two different approaches viz. silylation and esterification will be adopted to derivatize NAs to their corresponding product.

Silylation is a derivatization process in which the active hydrogen ion in the NA is replaced by a bulky tert-butyldimethylsilyl group through reaction with N-(tert-butyldimethylsilyl)-Nmethyltrifluoroacetamide (MTBSTFA). Both full scan and SIM method will be employed to “catch” the most abundant fragment ion of silylated NA, i.e. molecular weight of NA+57. A negative temperature oven program will also be used to improve baseline separation of all co-eluted NA peaks in chromatogram.

Another approach of derivatization involves the select esterification of the carboxylic acid present on the NA with a heavy ester “mass tag.” The derivatized NA sample is mass analyzed in a precursor ion scan mode (a.k.a., parent ion scan) in the first quadrupole and then dissociates due to collision in the second quadrupole. The dissociated “mass tag” fragment is mass analyzed by the third quadrupole. By devoting the third quadrupole to scan exclusively for the charged mass tag, the sensitivity of the precursor mass is increased. It will be possible to classify the naphthenic acid by charting the precursor ions against the intensity of the tagged species.

**Rajendra Mahat**  
Graduate Student, University of Wyoming, USA
Production of Biogenic Methane Following *In vivo* and *In vitro* Enzymatic Assay of Wyodak Coal

Yanrui Ning  
MS Candidate, University of Wyoming, USA

**Objectives.** The conventional approach for enhancing the production of secondary biogenic coal bed natural gas (i.e., methane farming) is through the introduction of growth limiting nutrients into the coal seam. The nutrients allow for greater microbial activity resulting in increased methanogenesis. However, the production of biogenic natural gas can also be enhanced using *in-situ* treatments that increase the bioavailability of the coal. In recent years fungal enzymes have attracted much attention with respect to the degradation of cellulose, hemicellulose, and lignin. Although significant technical challenges exist, including the multiplicity of enzymes and the complexity of the substrate, fungal enzymes can also be used to solubilize coal. The objective of this study is to assess the *in vivo* and *in vitro* enzymatic solubilization of Wyodak Coal. The bioavailability of the treated coal and aqueous coal derived compounds will be further assessed using biometer and anaerobic microcosm approaches. Various fungi, including *Nematoloma frowardii* b19, *Trichoderma atroviride* ES11, and *Phanerochaete chrysosporium*, have been shown to depolymerize/solubilize coal. This study will investigate the activity of fungi recently discovered growing on coal samples collected from the Wyodak formation. The capacity of the treated coal and coal derived compounds for methane production using microorganisms indigenous to the coal will also be assessed.

**Experimental design.** The genus of the unknown fungi will be determined using genomic DNA analysis by the universal primer 18S rDNA. *In vivo* and *in vitro* enzymatic assays will be performed with various peroxidases, oxidases and hydrolases to determine the extent of coal solubilization.
Trace Elements Stimulate Methane Production and Alter Active Microbial Communities in an Enrichment Study of Produced Water from a Coal Bed Methane Well

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PhD Candidate, Department of Microbiology, University of Massachusetts, USA

**Background/Objectives.** Biodegradation of coal organic deposits into methane is limited to anoxic and reduced conditions, and by the interaction of at least four metabolically diverse groups of microorganisms; hydrolytic, fermentative, acetogenic bacteria, and methanogenic archaea. The current understanding suggests that the rate-limiting step for coal biodegradation is the initial solubilization of a macromolecular, polycyclic, lignin-derived aromatic network into utilizable substrates by bacteria. Here we argue that a subsequent limitation in coal biodegradation to methane is methanogenic growth and activity, and both are restrained if the amount of trace elements is limited. Trace elements are micronutrients cells need for their metabolic activity, essential components of enzymes or cofactors of metabolic pathways associated with methanogenesis.

**Approach/Activities.** We investigated laboratory enrichment cultures obtained from coal bed methane well produced water samples from the Powder River Basin, Wyoming and amended them with trace elements. This study examined the effects of eight trace elements (iron, nickel, cobalt, molybdenum, zinc, manganese, boron, and copper) on methane production, on transcription levels for the marker gene (*mcrA*) for methanogenic activity, and on microbial community structure.

**Results/Lessons Learned.** Methane production was shown to be significantly limited both by a lack of additional trace elements as well as by the addition of an overly concentrated trace element mixture. Addition of trace elements at optimized concentrations enhanced methane production by 37%. Total cell numbers were 125% higher in trace element amended than in unamended enrichment cultures after 5 weeks of incubation. After seven days of incubation, the levels of *mcrA* transcripts in enrichment cultures with trace element amendment were significantly higher than in cultures without amendment. Transcript levels of *mcrA* correlated positively with elevated rates of methane production in trace element amended enrichments ($R^2=0.95$). Metabolically-active methanogens, identified by clone sequences of *mcrA* mRNA retrieved from enrichment cultures, were closely related to *Methanobacterium subterraneum* and *Methanobacterium formicicum*. Enrichment cultures were dominated by *M. subterraneum* and had slightly higher predicted methanogenic richness, but less diversity than enrichment cultures without amendments. An archaeal 16S rRNA gene clone library from produced water samples also identified *Methanobacterium sp.* (60%) as the most dominant species. Furthermore, metabolically-active bacterial community structure shift was observed in trace element amended enrichments. These results suggest that varying concentrations of trace elements in produced water from different subsurface coal wells may cause changing levels of coal bed methane production and alter the composition of the active microbial communities.
KEYNOTE SPEAKER AND MEMBERS OF THE PANEL DISCUSSION

Karen Budwill, Alberta Innovates – Technology Futures, Canada

Budwill has been a research scientist at Alberta Innovates – Technology Futures since 1996. She received her BSc in biology at the University of Waterloo and her PhD in microbiology from the University of Alberta. She specialized in anaerobic degradation processes including methanogenesis during her graduate work. She obtained further experience in anaerobic microbiology and molecular microbial ecology while at the University of Idaho as a post-doctoral fellow. While at AITF, she has investigated the use of bacteria in various environmental remediation technologies. Since 2000, Budwill has focused her research on biogenic methane production from deep subsurface coal seams. Investigations include understanding the microbial ecology of coal beds (e.g. identification of bacteria and stable isotope analyses of generated gas), and the effects of coal rank and nutrient additions on methane generation.

Mohinudeen Faiz, Origin Energy, Australia

Mohinudeen Faiz is a Principal Geologist at Origin Energy, Brisbane, Australia. Origin is a leading integrated energy company which, in association with ConocoPhillips and Sinopec, is developing Australia’s largest coal seam gas to LNG project in Queensland. Faiz’s current projects include the application of geochemistry for petroleum exploration, microbiological enhancement of coal seam gas reservoirs and evaluating new venture opportunities. Faiz graduated with a BSc (Hons) from the University of Peradeniya, Sri Lanka in 1984. He holds MSc (1990) and PhD (1993) degrees from the University of Wollongong, Australia. He has been working as a geologist for over 25 years in various fields including, ground water exploration, coal seam gas research, CO₂ sequestration and petroleum source rocks studies. In the early 2000s, while working at the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Faiz instigated research on microbiology of coal seam gas reservoirs and initiated the first research project on microbiological enhancement of coal seam methane (MECSMTM) in Australia. Faiz has extensively researched and published numerous papers on the application of stable isotopes to determine the origin of gas and identification of “sweet-spots” for coal seam gas production. Some of this early research enabled the identification of biogenically enhanced CBM “sweet-spots” in the Sydney Basin. His other research interests include organic petrology and biomarker studies of petroleum source rocks and hydrocarbons. Faiz is a member of the American Association of Petroleum Geologists, International Committee for Coal Petrologists and the Petroleum Exploration Society of Australia.

Rob Hurless, School of Energy Resources, University of Wyoming, USA

Rob Hurless is currently a Deputy Director at the School of Energy Resources at the University of Wyoming. He is on loan to Governor Mead and works on Energy Strategy. Prior to this position he served as Energy and Telecommunications Advisor to Wyoming Governor Dave Freudenthal. He came to this position from a tour as Chairman of the Wyoming Public Service Commission. For the bulk of his career Hurless was Publisher of the Casper Star-Tribune in Casper, Wyoming. He served as an officer aboard the USS Quapaw, ATF 110, Pearl Harbor, Hawaii. He has a BS in Chemistry and BA in History from Montana State University, an MBA from Harvard Business School and Masters in Applied Economics from Stanford University. He has three grown children and enjoys fly fishing and open source software.

Song Jin, Next Fuel, Inc., USA

Song Jin is the President and Chief Technology Officer of Next Fuel Inc. He also serves as the Associate Director of Center for Biogenic Natural Gas Research at the University of Wyoming. His expertise includes innovative remediation of environmental contaminants and biological conversion of carbonaceous wastes to energy. Jin is one of the first investigating and implementing biochemical techniques to stimulate production of fresh biogenic natural gas from coal, oil shale, and other carbonaceous deposits. He is the leading inventor of the biogenic coal to gas (“BCTG” or “CTG”) technology. Jin and his
team at Next Fuel, for the first time in the world, successfully conducted BCTG field pilot tests in Asia, and produced fresh natural gas from coals of no gas-producing history. He is currently leading the commercial scale application of the BCTG technology.

Jin also pioneered the R&D of bioelectrochemical systems (BECs) in treating contaminated water, soils, and sediments. BECs was acknowledged by the USPEA as a sustainable remedy for treating recalcitrant contaminants. His recent collaborative research with Peking University identifies a novel pathway that centers around natural minerals and facilitates the transfer of photon energy to non-phototrophic microorganisms, which otherwise cannot harvest light energy.

Jin holds 27 patents, and has published 120+ scientific papers in peer review journals and technical conferences. He is an adjunct professor at the University of Wyoming and visiting professor at Peking University, China. Jin received his PhD in 1997 from the University of Wyoming.

Elizabeth J.P. Jones, U.S. Geological Survey, USA

Elizabeth Jones joined the National Research Program of the US Geological Survey in 1977 (as E.J.P. Phillips) studying novel microbial processes under Derek Lovley. Their studies included ground breaking work on metals [Fe(III), Mn(IV), Cr(VI), U(VI)] as terminal electron acceptors (TEA), as well as anaerobic reduction of aromatic compounds with metal reduction, and finally humic acids as TEA. Jones added molecular biology methods to her tool box in research under Mary Voytek. She joined the USGS Energy Team studying coal bed methanogenesis in 2005. Jones is very interested in the microbial organisms involved in the release of organics from coal and the conversion of those organics to methane, as well as environmental constraints on the process.

William Mahaffey, Luca Technologies, USA

Bill is the Chief Technology Officer of Luca Technologies in Golden, Colorado. Serving as the senior technical advisor on all innovation and core technology initiative’s, his focus is on the rapid transition of lab research and development to field demonstration and optimization. His role is to ensure that research goals support strategic corporate objectives, with a focus on transformational innovations that accelerate creation of natural gas from subsurface hydrocarbon deposits. To facilitate the delivery of relevant technologies, operational solutions, and quality data generation, he focuses on functional-multidisciplinary bridging between RD&D, geosciences, engineering and the operations divisions within the company, as well as forming strategic external collaborations. Founder/President of Pelorus EnBiotech (1994-2009), a soil and ground water remediation company focused on the development, design and implementation of in situ bioremediation, chemical oxidation, surfactant enhanced processes and hydraulic fracturing emplacement of reactive propsants. From 1988 to 1994, he was Ecova Corporation’s lead research scientist for in situ bioremediation of petroleum contaminated sites. He became Vice President of R&D in 1992 and facilitated sale of the company to Amoco Oil. At Phillips Petroleum’s (1986-1988) he was responsible for fermentation process development of recombinant strains of Pichia pastoris and research to improve microbial oil sensing technology (MOST). He obtained a Bachelor's Degree in Biology & Chemistry from SUNY Brockport (1976) performing two years of independent research on Microbial Degradation of Crude Oil Spills in Multistage Continuous Culture Systems. His Masters degree in Microbiology (1978) from SUNY Brockport was on the microbial ecology of pollutant discharge to estuarine sediments (EPA Gulf Breeze, FL). In 1986 he received a Doctorate (UT, Austin) for research defining oxygenation reactions for Benz[a]anthracene biodegradation and metabolic pathways for mineralization.

Crystal McDonough, Associated Legal Group, USA

Crystal McDonough is an Associate Attorney with Associated Legal Group, LLC in Cheyenne, WY. Ms. McDonough works with clients on a variety of natural resource and energy related issues, including federal and state regulatory matters.

Crystal McDonough is a 2011 graduate of the University of Wyoming, College of Law and the Haub School of Environment and Natural Resources. Ms. McDonough’s unique experiences as a student relate to natural resources, including renewable energy, oil and gas, coal, water, land use, NEPA, CAA, FERC, Wyoming PSC and Wyoming DEQ. She was a Graduate Assistant with the School of Energy Resources, working with former Gov-
Governor Dave Freudenthal, the Haub School of Environment and Natural Resources, the University of Wyoming Extension Office, the Wyoming State Legislative Service Office, the Joint Interim Wind Energy Task Force, and the Joint Interim Judicial Committee. Ms. McDonough produced two research reports for the Wind Task Force. Additionally, she supervised law students, assisted in researching legal issues regarding pore space, carbon sequestration, water, nuclear energy, and air quality under federal and Wyoming regulatory framework.


John Passehl, Wyoming Department of Environmental Quality, USA

John Passehl P.G. is currently the Department of Environmental Quality Underground Injection Control (UIC) Geology Supervisor for the Water Quality Division. He has managed the UIC program since 2004 which includes permit evaluation, approval/denial, enforcement, and inspection. His previous experience includes whole and sidewall core analysis for the Oil and Gas industry in Texas and Louisiana, and writing water use permits for the South Florida Water Management District and the Northwest Florida Water Management District. He currently holds the rank of MAJ with the Wyoming Army National Guard as the State Preventive Medicine Officer.

Michael Urynowicz, University of Wyoming, USA

Michael Urynowicz is an Associate Professor of Environmental Engineering within the Department of Civil & Architectural Engineering at the University of Wyoming, Director of the Center for Biogenic Natural Gas Research and a Licensed Professional Engineer. He is also Co-founder and President of Wyoming-based EnWyo, LLC, a technology start-up company at the University of Wyoming. Urynowicz received his Bachelor of Science in Chemical Engineering from Michigan State University, his Master of Science in Civil & Environmental Engineering from the University of Wisconsin, and his PhD in Environmental Science & Engineering from the Colorado School of Mines. Prior to joining the faculty at the University of Wyoming, he worked in the manufacturing industry and as an environmental consultant. He was also the Founder, Managing Director and Principal Engineer at Envirox LLC, an environmental company specializing in the remediation of complex hazardous waste sites. During his career, Urynowicz has been involved in a wide range of research related to secondary biogenic coalbed natural gas, in situ chemical oxidation and the remediation of contaminated sites. He has published numerous technical papers and reports and has given invited lectures across the United States and abroad.