Isotopic studies have indicated that some coal beds produce methane of biogenic origin. Little is known, however, about the organisms, reactions, and intermediates involved in the process of biogenic methane formation from geopolymers like coal. Laboratory microcosms of coal and nutrients, some with an added microbial consortium (WBC-2) enriched from a modern wetland, were used to study the production of methane from coal. Subbituminous coal samples from the Wilcox Group (Zavala County, Texas) and the Fort Union Formation (Campell County, Wyoming), both known to produce biogenic methane in the field, released 56 and 16 scf/ton of methane, respectively, in laboratory experiments with WBC-2 added. Generation of methane from coal involves a consortium of microorganisms that ferment complex organics to methanogenic substrates which are then converted to methane. Microbial biomass in the coals was low and methane generation was limited by methanogen growth, which did not always occur. When WBC-2 was added, organics released from the coal [predominantly long chain fatty acids (LCFAs) and alkanes] were degraded over about 70 days. Acetate accumulated initially, then decreased as methane was formed. WBC-2 also produced methane in coal-free treatments with organics, such as octadecanoic, hexadecanoic, benzoic, and vanillic acids, confirming that coal intermediates can be fermented to methane precursors. There was a shift in the WBC-2 microbial population grown in coal microcosms, indicating growth of some new dominant members specific to coal fermentation. Phylogenetic identification of community members in the coal incubations will be useful for understanding and manipulating in situ coalbed populations.
Laboratory studies of biogenic methane production from coal by microbial consortia: Identifying organisms, reactions and intermediates

Elizabeth Jones, Mary Voytek, William Orem, Margo Corum, Anne Bates, Harry Lerch
USGS looks at factors controlling new biogenic methane from coal

- Presence of endemic microorganisms
- Favorable environmental conditions
- Bioavailability of coal organics

WBC-2 Bioassay

Jones et al. - IJCG special issue - 2008
Notes by Presenter: We studied 18 coals from Texas, Wyoming, Alaska and Pennsylvania and selected the 4 with the highest methane for more detailed study.
Notes by Presenter: This talk focuses on the process of converting coal to methane. Notice that coal is a complex geopolymer composed primarily of polyaromatic hydrocarbons with various functional groups.
Notes by Presenter: It takes a microbial consortium to generate methane from coal.
Notes by Presenter: It takes a microbial consortium to generate methane from coal.
Approach 1

**Treatments**

- Endemic coal population
- Standardized microbial consortium (WBC-2)
- Methanogen Inhibitor BES
Approach

**Analyzed organics:**
- Solvent extractable organics GC-MS
- Volatile Fatty Acids - IC

**Analyzed DNA:**
- Bacterial fingerprint - TRFLP
- Methanogens - qPCR
Duplicate “endemic” treatments

Notes by Presenter: There must be something different between the microbial communities in these two bottles. This gave us an opportunity to look at differences in the microbial community.
Bacterial DNA fingerprint

TX endemic bacteria

day 1

day 106  Bottle 1

day 106  Bottle 2

TRFLP 16S rDNA – ml-l

Notes by Presenter: Describe DNA profile
methanogen growth

TX coal - endemic community

METHANE (µmoles per g coal)

DAYS

METHANOCOGEN CELL DENSITY

0 10 20 30 40 50 60 70

0 100 200 300 400 500 600 700

0 1000 2000 3000 4000 5000 6000

msarc
Approach

- Standardized microbial consortium

**WBC-2**

- **Proteobacteria**
  - gamma
  - epsilon
  - delta
  - Bacteroides
  - Clostridium

- **Acetobacteria** (26%)

- **Homoacetogens** (27%)
  - Methanosarcina
  - Methanosphaera
  - Methanomassilis

- **H₂ + CO₂**

- **Typical fermentative bacteria**

- **Bacterial clones**

- **Methanogen clones**
Notes by Presenter: Only 2 of the 18 coal samples studied exhibited endemic activity; endemic activity was difficult to predict or reproduce. By adding a culture with similar behavior we were able to sacrifice bottles from replicates to analyze solvent extractable organics, VFA and DNA.
Buildup and Depletion of Solvent-Extractable Organics Over Time

Methane production from TX coal

Day 78
Day 56
Day 39
Day 8
unresolved complex mixture
microbial enrichment – environment selects

source sediment

WBC-2

169 TX, 18 days 254

TX, 39 days

TX, 78 days

delta proteobacteria

Notes by Presenter: New environment selects new organisms.
Notes by Presenter: Drop in Geobacter is an artifact of the way % calculated. We know that acetate is important intermediate.
Notes by Presenter: We did not measure H2, however …
Approach

1 treatments
• with coal

2 Test microbial consortium with potential intermediates*

→ Identified
organics in solution

*off the shelf
<table>
<thead>
<tr>
<th>Coal intermediates identified with GC-MS</th>
<th>tested with WBC-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long Chain Fatty Acids</td>
<td>Octadecanoic ✔</td>
</tr>
<tr>
<td></td>
<td>Hexadecanoic ✔</td>
</tr>
<tr>
<td></td>
<td>Dodecanoic ✔</td>
</tr>
<tr>
<td></td>
<td>Decanoic ✔</td>
</tr>
<tr>
<td>Single-ring Aromatics</td>
<td></td>
</tr>
<tr>
<td>Long-Chain Alkanes</td>
<td></td>
</tr>
<tr>
<td>Sterolic structures</td>
<td></td>
</tr>
</tbody>
</table>
Methane from octadecanoic and hexadecanoic (100 µmoles)

Notes by Presenter: Long lag followed by vigorous growth.
Fatty acids $\rightarrow$ \( \text{CH}_4 \)

*from \( \mu \text{moles octadecanoic acid (18C)} \)*

\( 18\text{C} \rightarrow 16\text{C} \rightarrow 14\text{C} \rightarrow \text{acetate} \rightarrow \text{CH}_4 \)

*from \( \mu \text{moles hexadecanoic acid (16C)} \)*

\( 16\text{C} \rightarrow \text{butyrate} \rightarrow \text{acetate} \rightarrow \text{formate} \rightarrow \text{CH}_4 \)
Coal intermediates tested with WBC-2

Long Chain Fatty Acids

Single-ring Aromatics
- Phenol
- Vanillic acid
- Caffeic acid
- Benzoic acid

Long-Chain Alkanes

Sterolic structures
benzoic acid

100 µmoles benzoate

METHANE µmoles

sampled for intermediates and DNA

acetate

long chain alkanes

acetate

DAYS
benzoic acid fermentation pathway (methanogenic consortium)

methylcyclohexanone

heptanoate

VFA, H₂ → CH₄

cyclohexanone

caproate

after Evans and Fuchs, 1988
Vanillic acid, 100 µmole

Vanillic acid - gone

Intermediates:
- 1,2 benzenediol

METHANE µmoles

500
400
300
200
100
0

DAYS

0 20 40 60 80 100

- carbon in side groups
- sampled organics DNA

Long chain alkanes
Volatile fatty acids
- butyrate
- acetate
benzoate enrichment

benzoate, first enrichment

benzoate after third transfer

Upper Wyodak bioassay

Texas bioassay
Identifications using
- *in silico* digests of WBC-2 clone sequences
- magnetic beads

Notes by Presenter: Different selective pressures in different coals.
summary

- We were able to use WBC-2 bioassay to assess bioavailable coal and to study mechanisms.
  - Identify possible pathways
  - Identify possible key organisms

- Compound specific experiments selected for new bacteria that may be associated with specific pathways.
Future Work

Lots of it!

Acknowledgements

- Peter Warwick, Art Clark, USGS
- Energy and National Research Programs
  - Genesis Gas & Oil LLC

Elizabeth Jones 703-648-5840
terminal restriction fragment length polymorphism (TRFLP)

1- target DNA segment and tag terminal end

2- enzyme recognizes cut sites

3- analyze terminal fragments

[mixture of DNA] → community profile

• semi-quantitative
• cloning and sequencing for id
Quantitative PCR: measure abundance of organisms of interest

- Add fluorescent indicator to PCR reaction

- Run standards with known abundances of targeted gene alongside samples

- Plot standards and samples to determine abundances
<table>
<thead>
<tr>
<th></th>
<th>Stable isotopes del C-13</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CO₂ Reduction</strong></td>
<td>-80</td>
<td>-200</td>
</tr>
<tr>
<td><strong>Acetate Fermentation</strong></td>
<td>-50</td>
<td>-300</td>
</tr>
<tr>
<td><strong>TX coal</strong></td>
<td>-55</td>
<td>-310</td>
</tr>
</tbody>
</table>
Toxic effects ...

Graph showing the effect of calcium chloride (CaCl₂) and hexadecanoic on methane production over days. The x-axis represents days, the y-axis represents methane (in µmoles). Two concentrations are shown: 0.2 mM and 1 mM.