Enhancing Biogenic Methane Production from Coals; How the Microbial Ecology and Diversity Respond*

Karen Budwill[1], Tara Penner[2], and Julia Foght[2]

Search and Discovery Article #80032 (2008)
Posted October 29, 2008

*Adapted from oral presentation at AAPG Annual Convention, San Antonio, TX, April 20-23, 2008

[1]Alberta Research Council, Edmonton, AB, Canada. (karenb@arc.ab.ca)
[2]University of Alberta, Edmonton, AB, Canada.

Abstract

Contemporary biogenic methane is known to occur in many coal seams. We have been investigating how nutrient additions could enhance methanogenesis to economic levels in coals. However, very little is known about the types and role of microorganisms involved in this methane generation. The purpose of this study was to determine the major Bacterial and methanogenic Archaeal species detectable in coal seams by 16S rRNA gene sequencing, and determine how these microbial populations might change with additions of complex nitrogen sources. Most of the Bacterial sequences amplified from uncultured coal samples were related to the Proteobacteria including *Pseudomoas stutzeri*, *Thauera* spp., and *Acidovorax* spp. and are reported to have various physiological traits, including hydrogen utilization, nitrate reduction, and nitrogen fixation. When the coal samples were incubated in the laboratory with added complex nitrogen sources, methane was produced in significant amounts, and the Bacterial populations changed to comprise fermentative organisms within the Clostridia and the Bacteriodetes. Nearly all of the Archaeal sequences detected in methanogenic enrichment cultures inoculated with coal were closely related to *Methanosarcina* spp., which is capable of using a broad range of carbon sources for methane production, including acetate, H_2/CO_2, formate and methanol. Archaeal 16S rRNA gene sequences could not be amplified from DNA extracted from uncultured coal cores, and methane was not produced in significant amounts from coal incubated in minimal salts medium, indicating that methanogens are not present in very high numbers in the coal itself. This research has provided an insight into microbial diversity and ecology of coal beds which can be used in the development of enhanced methanogenesis as a secondary CBM recovery technology.

Enhancing Biogenic Methane Production From Coals; How the Microbial Ecology and Diversity Respond

Karen Budwill[1], Tara Penner[2], and Julia Foght[2]
[1]Alberta Research Council
[2]University of Alberta
Edmonton, Alberta, Canada

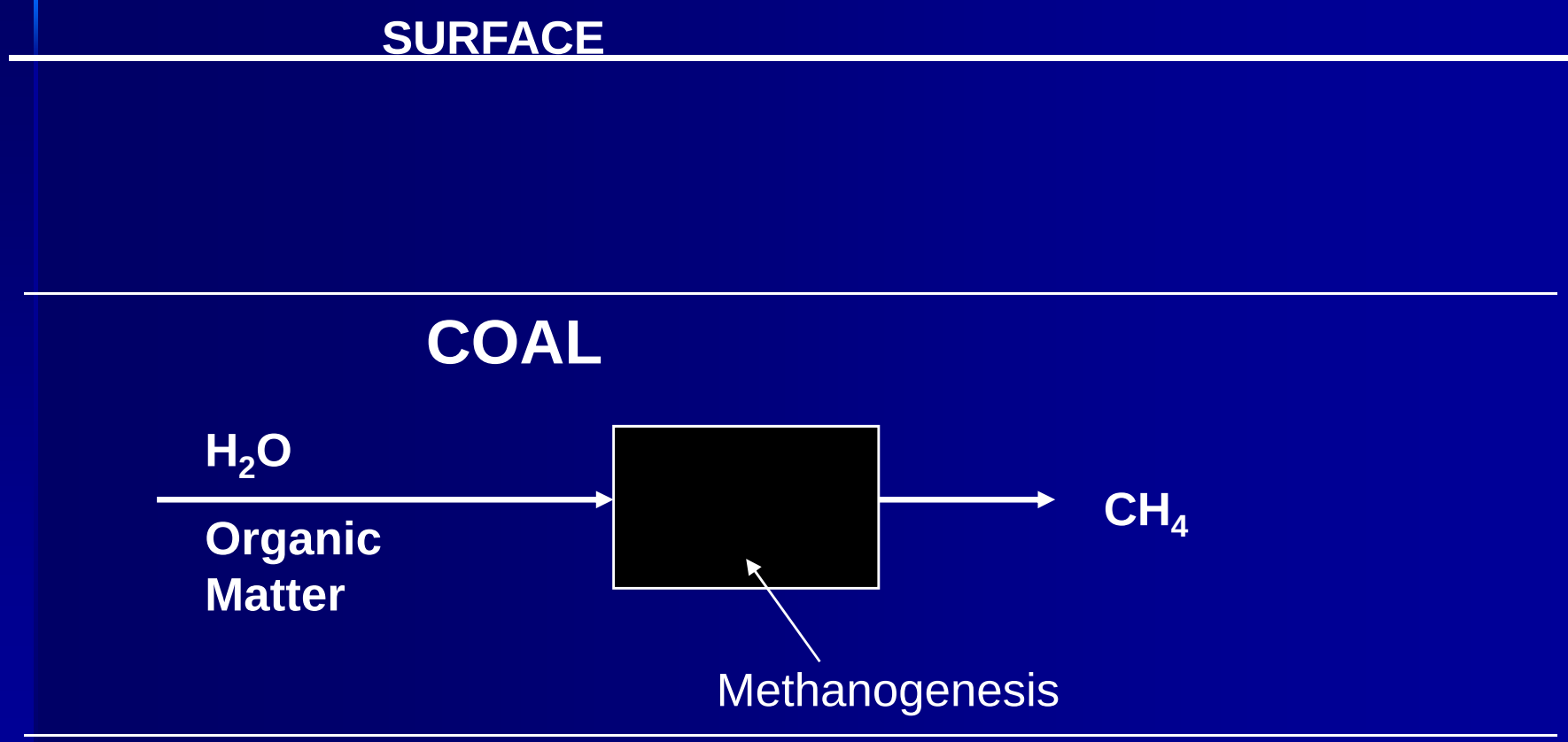
Acknowledgements

- **Alberta Energy Research Institute**
- **Natural Resources Canada**
- **University of Alberta**

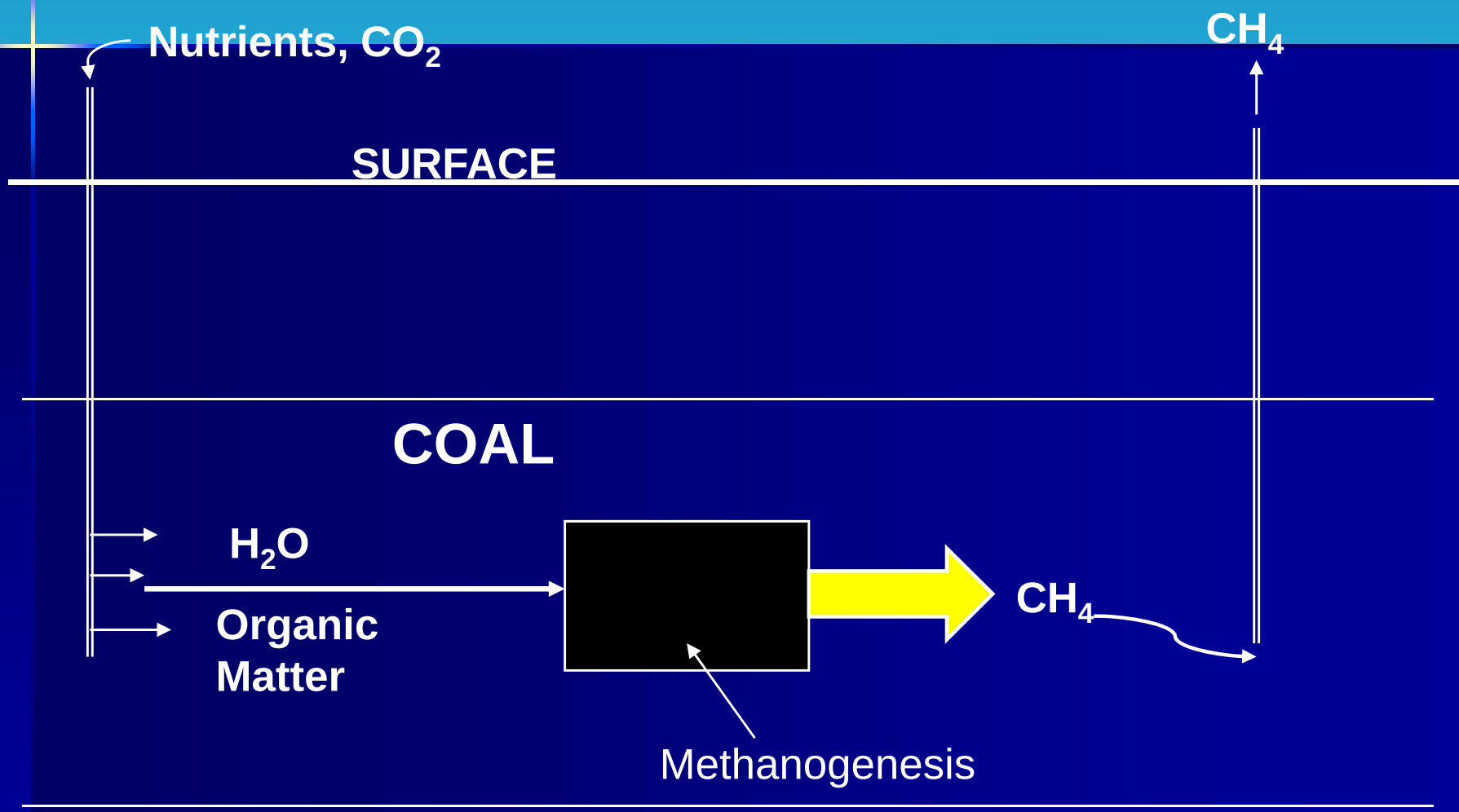
Outline of Presentation

- **Introduction**
- **Experimental methods (sources of coal and cultures)**
- **Results**
- **Conclusions**
- **Future work**

Introduction: Enhanced Biogenic Methane Production in Coal Beds



Introduction: Enhanced Biogenic Methane Production in Coal Beds



Past Research

- Growth studies using coal enrichment cultures.
- Effects of nutrient additions, coal rank, salinity, high pressures on methane production.

Objectives

- **To detect and identify major Bacterial and Archaeal species associated with coal sampled from CBM sites in Alberta, Canada.**
- **To study the effect on microbial diversity when nutrient amendments were used to stimulate methanogenic consortia.**

Experimental Methods

Coal Samples

Sample	Depth (m)	Comments
Tri7 (Trident Rowley)	258.7	Cuttings (CH4 generated)
KB2 (Grande Cache)	126.7	
B88 (Bashaw)	145.0	Shale present
B93 (Bashaw)	457.0	Shale present
SH2 (Swan Hills)	1235.9	Crushed coal

Enrichment Culture Samples

Culture	Temp. (C)	Source	Age
S24C160	30	CBM Desorption Canister	2 years
S32C169	30	CBM Desorption Canister	2 years
Obed Mine Sludge	30	Coal mine tailings pond	3 years
ARC Therm	50	CBM Desorption Canister	4 years
KB2-C	30	Grande Cache coal sample KB2	5 months
B93-C	30	Bashaw coal sample B93	5 months
Tri7-C	30	Trident coal cuttings	6 months

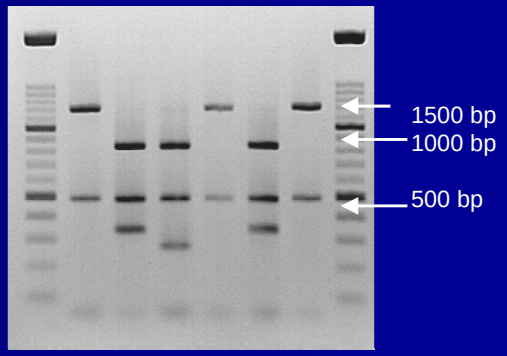
DNA Extraction

- DNA was extracted by beadbeating directly from cultures or from macerated coal samples.
- Extracted DNA was purified by precipitation as described by Fogt et al., 2004. Microbial Ecology 47:329-340.

Cloning and RFLP of 16S rRNA genes.

- 16S rRNA gene fragments were amplified from genomic DNA using Bacterial[1]- or Archaeal[2]-specific primers and used to construct 16S rRNA gene clone libraries.
- Cloned inserts were screened by digestion with either HaeIII or CfoI.
- One representative from each pattern group was selected for sequencing.
- Patterns represented by only one clone were not sequenced.

[1]Foght et al., 2004. Microbial Ecology 47:329-340.
[2]Delong 1992. Proc. Natl. Acad. Sci. USA 89:5685-5689.

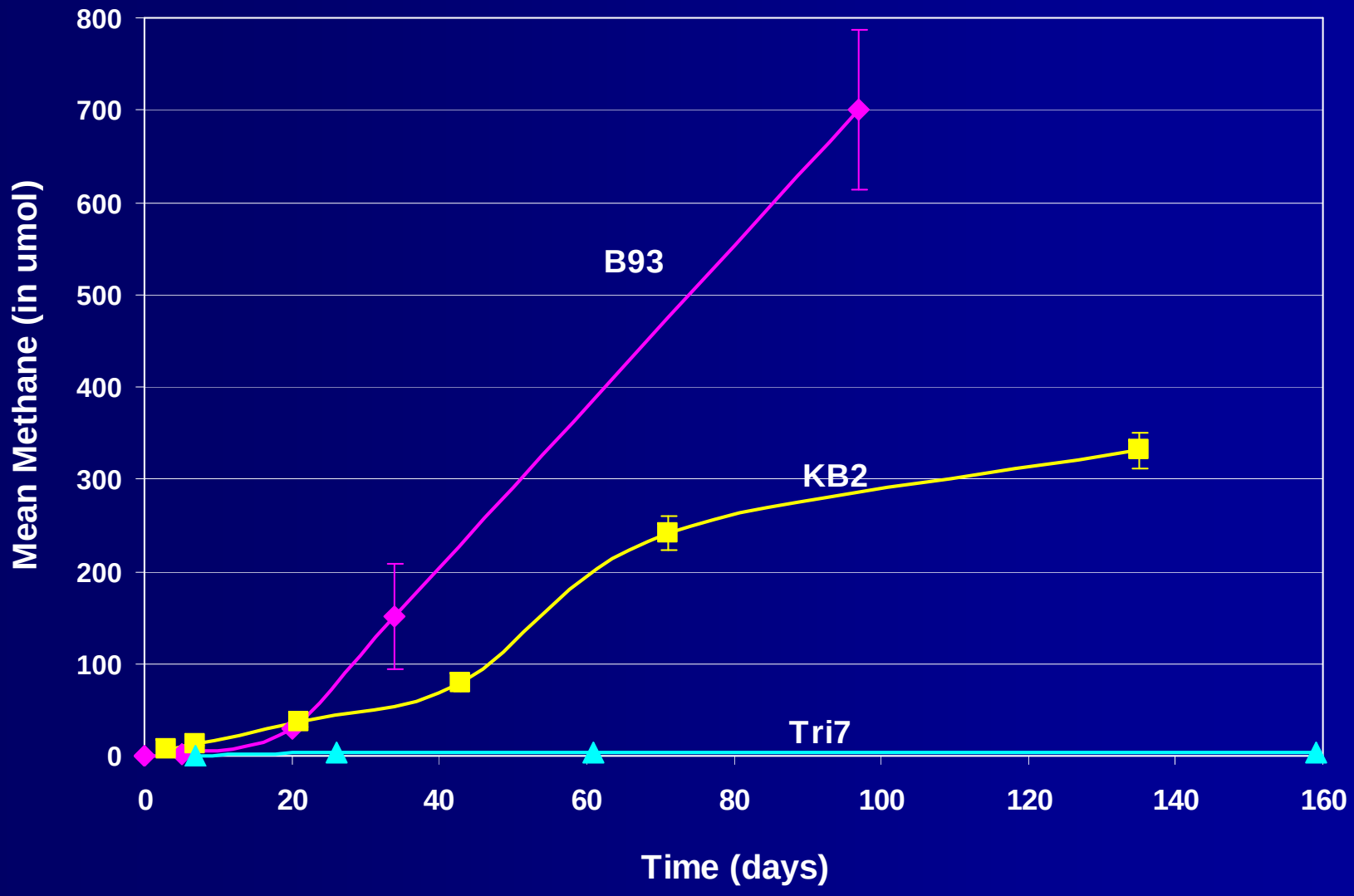


Results

Phylogenetic groups represented by Bacterial 16S rRNA gene clone sequences amplified from uncultured coal samples.

	Number of Clones (% of Library)				
Closest Match	**B88**	**B93**	**KB2**	**Tri7**	**SH2**
γ-Proteobacteria					
Pseudomonas sturzeri	**55 (66)**	**49 (56)**		**3 (3)**	**9 (10)**
Pseudomonas fluorescens			**12 (14)**		
Aeromonas		**2 (2)**			
Acinetobacter				**3 (3)**	
Halomonas					**5 (6)**
Marinobacter					**2 (2)**
β-Proteobacteria					
Acidovorax spp.		**5 (6)**	**55 (63)**		
Hydrogenophaga spp.	**9 (11)**				**3 (3)**
"Thiobacillus" Q				**23 (26)**	
Thauera aromatica		**6 (7)**			**28 (32)**
Janthinobacterium spp.			**1 (1)**		
Massilia spp.			**1 (1)**		
Aquaspirillum		**12 (14)**			
Uncultured	**2 (2)**				
α-Proteobacteria					
Agrobacterium spp.				**15 (17)**	
Catellibacterium					**6 (7)**
Uncultured *Rhizobiales*					**5 (6)**
Mesorhizobium					**2 (2)**
Roseobacter					**2 (2)**
Sphingomonas spp.			**1 (1)**		
Actinobacteria					
Arthrobacter spp.			**7 (8)**		
Uncultured Bacteriodetes				**12 (13)**	**2 (2)**
Unsequenced singletons	**17 (20)**	**13 (15)**	**10 (11)**	**34 (38)**	**24 (27)**
Total Clones	**83 (100)**	**87 (100)**	**87 (100)**	**90 (100)**	**88 (100)**

Methane production in short-term enrichment cultures from fresh coal samples.

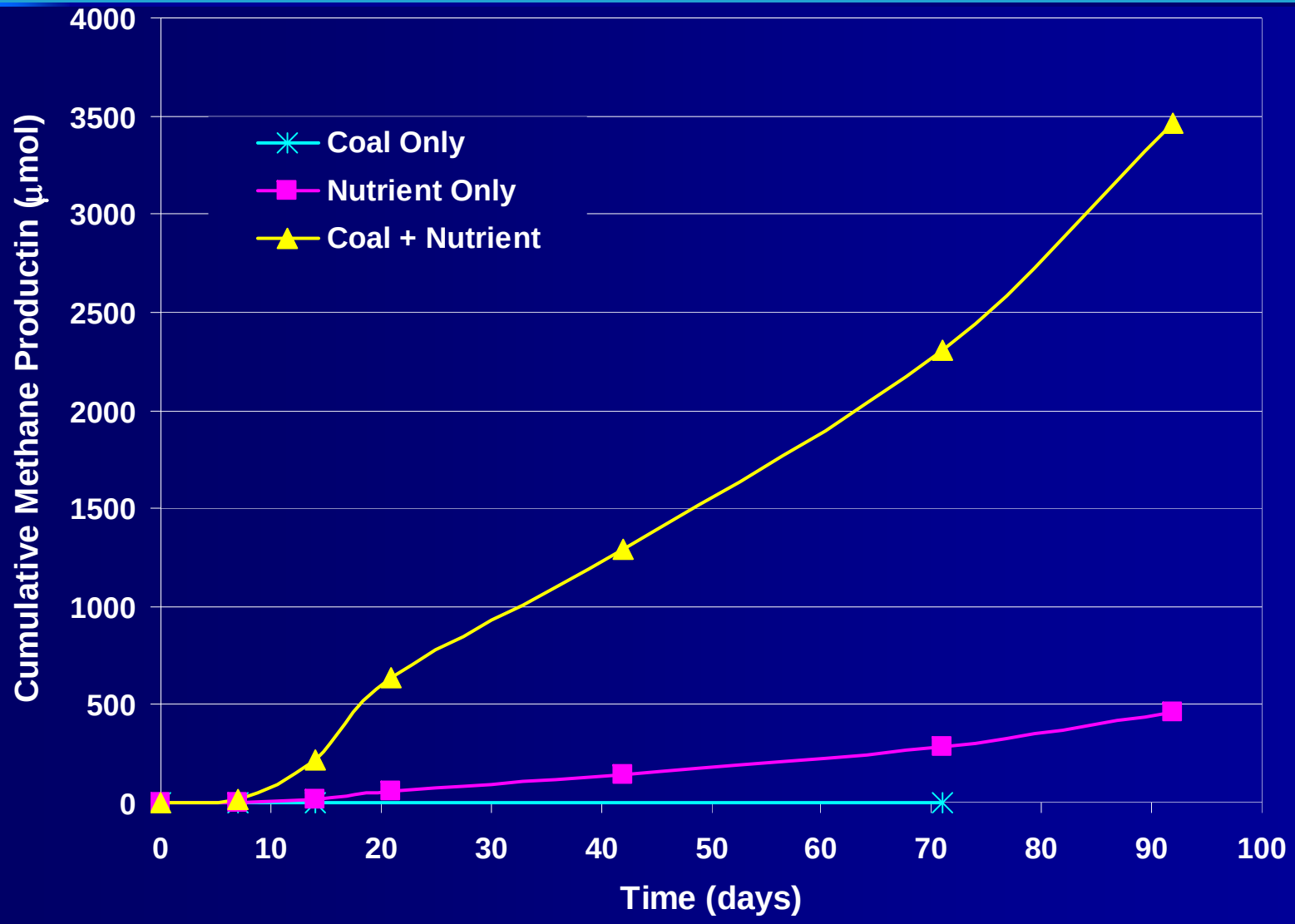


Phylogenetic groups represented by Bacterial 16S rRNA gene clone sequences amplified from short-term coal enrichment cultures.

	Number of Clones (% of Library)	
Closest Match	**B93-C**	**KB2-C**
γ-Proteobacteria		
Pseudomonas sturzeri	**3 (4)**	**49 (56)**
Aeromonas	**4 (5)**	**10 (12)**
Citrobacter	**11 (13)**	**22 (26)**
Shewanella	**2 (2)**	
β-Proteobacteria		
Delftia		**7 (8)**
Uncultured Bacteriodetes	**17 (20)**	
Firmicutes		
Clostridium	**21 (25)**	
Lactobacillales		**29 (35)**
Unsequenced singletons	**26 (31)**	**16 (19)**
Total Clones	**84 (100)**	**84 (100)**

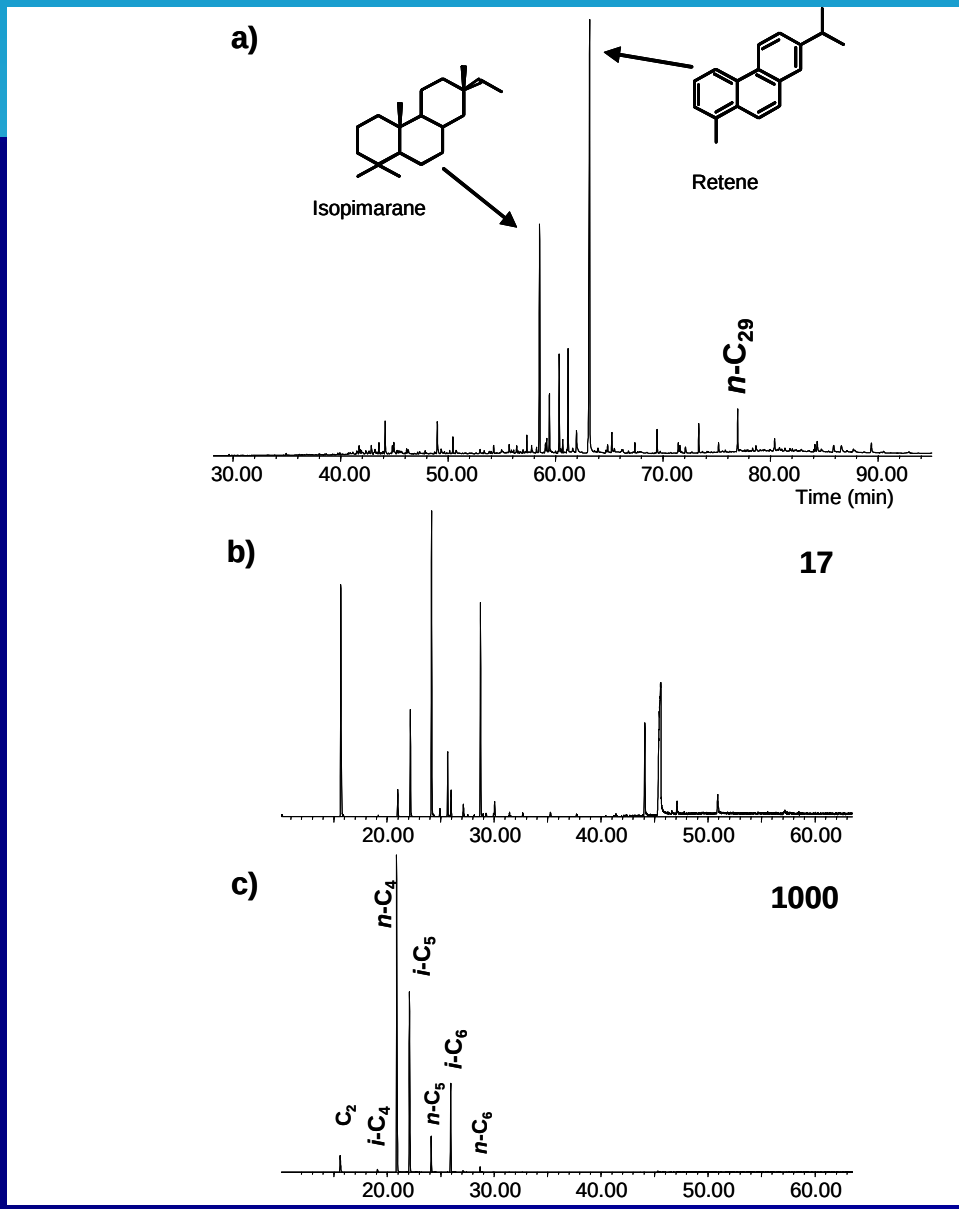
Archaeal results: 74 clones in B93-C were 98% similar to *Methanosarcina* spp.

Typical methane production in long-term enrichment cultures



GCMS analyses of free hydrocarbons/extractable organic matter from coal

- A) Extractable organic matter from coal.
  - Series of conifer-derived saturated and aromatic diterpanoids with sub-ordinate waxy n-alkanes.
- B) Coal culture given tryptone: series of low molecular weight (C2-C9) monocarboxylic acids (low abundance)
- C) Culture with tryptone (NO coal): monocarbonxylic acids concentrated by over 50 fold.



Work performed by Dr. C. Boreham, GeoScience, Australia

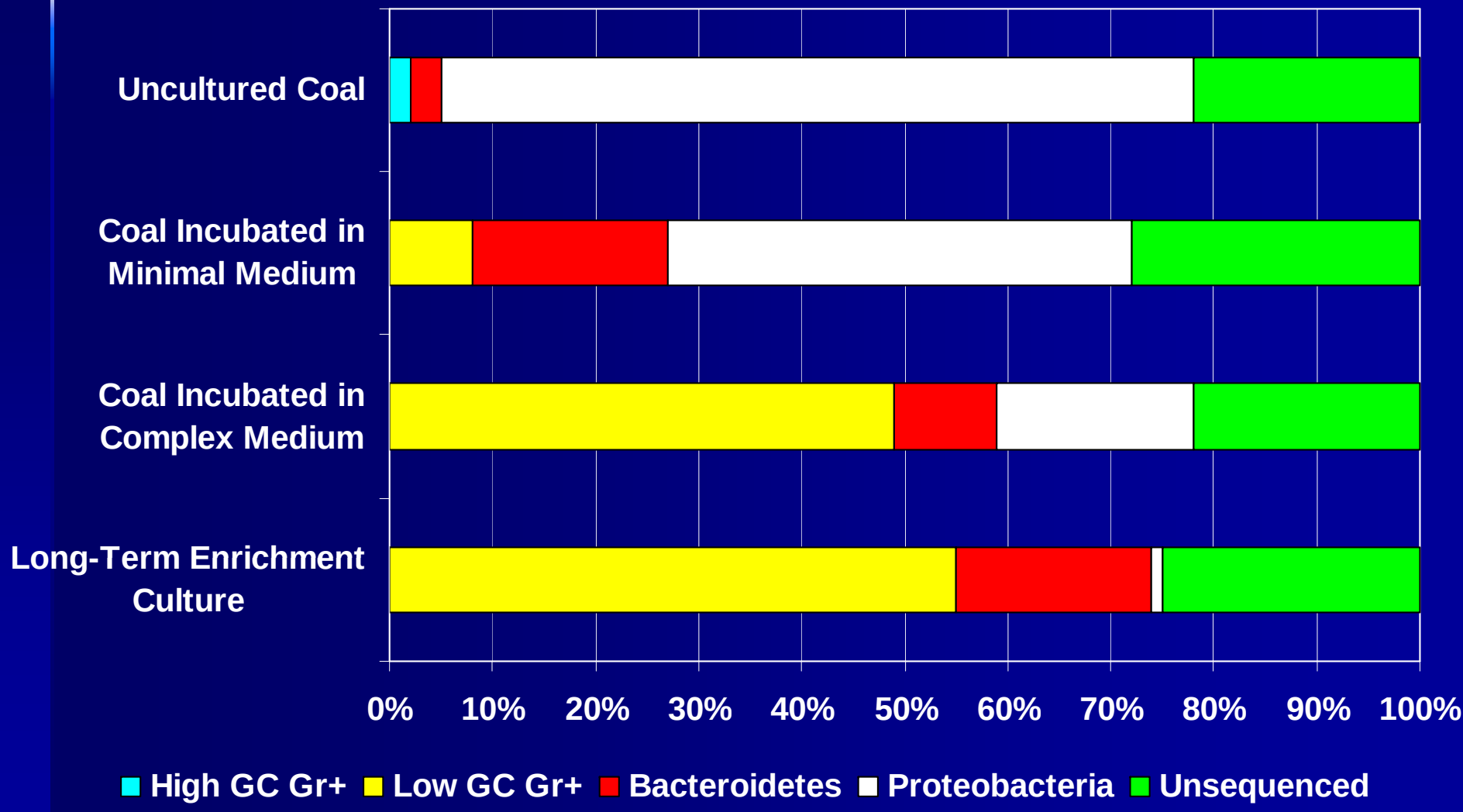
Phylogenetic groups represented by Archaeal 16S rRNA gene clone sequences amplified from long-term coal enrichment cultures.

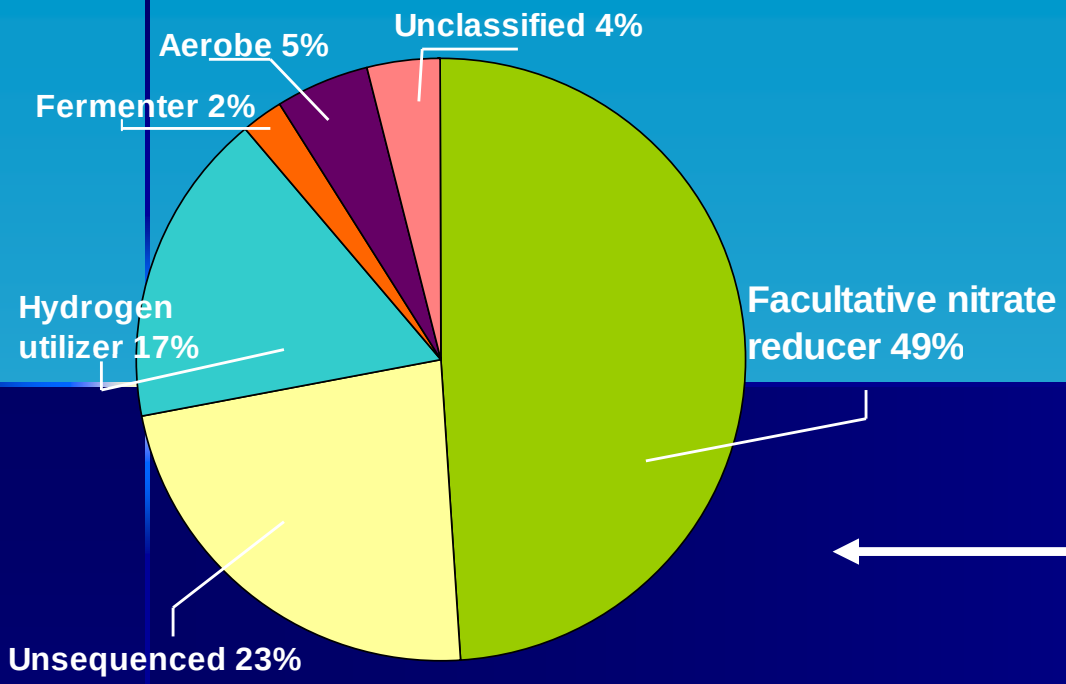
	Number of Clones (% of Library)			
Closest Match	**S24C160**	**S32C169**	**Obed Sludge**	**ARC Therm**
Methanosarcinacese				
Methanosarcina	**62 (77)**	**75 (85)**	**70 (88)**	**20 (22)**
Methanobacteriales				
Methanothermobacter				**58 (64)**
Methanobrevibacter		**2 (2)**		
Methanobacterium		**2 (2)**	**10 (11)**	
Methanomicrobiales				
Methanoculleus		**3 (3)**		**9 (10)**
Methanocalculus				**2 (2)**
Unclassified Methanomicrobiales				
Unsequenced Archaeal clones	**19 (23)**	**6 (7)**		**1 (1)**
Total Archaeal Clones	**81 (100)**	**88 (100)**	**80 (100)**	**90 (100)**

Phylogenetic groups represented by Bacterial 16S rRNA gene clone sequences amplified from long-term coal enrichment cultures.

	Number of Clones (% of Library)			
Closest Match	**S24C160**	**S32C169**	**Obed Sludge**	**ARC Therm**
Clostridia				
Sedimentibacter	**15 (19)**	**43 (49)**	**5 (6)**	
Thermophilic clostridia				**30 (39)**
Other *Clostridia*	**14 (18)**	**26 (30)**	**41 (46)**	**2 (3)**
Uncultured Bacteroidetes	**37 (46)**	**12 (14)**	**14 (16)**	
Bacillaceae			**4 (4)**	
Proteobacteria				**2 (2)**
Pseudomonas stutzeri		**2 (2)**		
Uncultured Spirochaetes			**8 (9)**	
Unsequenced Bacterial clones	**14 (18)**	**4 (5)**	**17 (19)**	**44 (58)**
Total Bacterial Clones	**80 (100)**	**87 (100)**	**89 (100)**	**76 (100)**

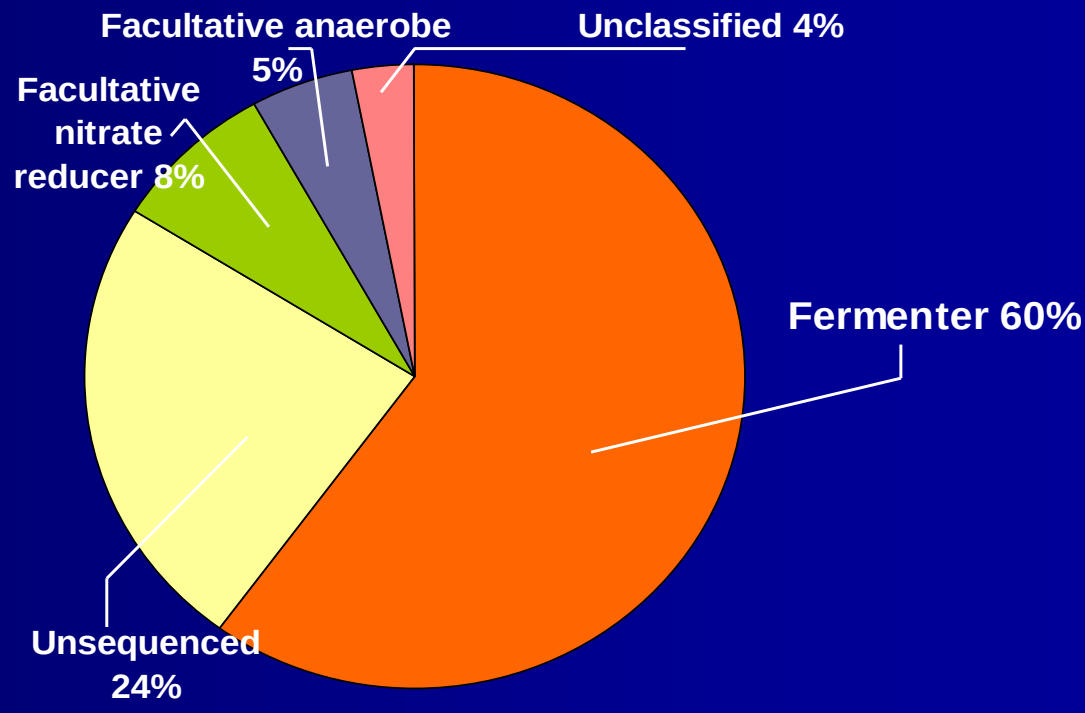
Uncultured coal comprised of sequences related to the Proteobacteria, whereas enrichment cultures were comprised of sequences related to the Clostridia and Bacteroidetes.





Uncultured coal samples were comprised mainly of facultative nitrate reducers and hydrogen utilizers

Coal enrichment cultures were comprised mainly of fermenters (such as Clostridia) and homoacetogens



Summary/Conclusions

- Methanogens are present in coal seams but probably in low numbers and exhibiting very slow growth rates.
- More sensitive methods may be needed to detect methanogens in uncultured coal.
- More rigorous sampling methods need to be used/developed to account for the presence of any contaminating bacteria.

- Bacteria in the uncultured coal are nitrate reducers and hydrogen utilizers and may have out-competed methanogens for molecular hydrogen in situ.

Summary/Conclusions

- It is the Bacteria providing the precursors for methanogenesis that are directly affected by the available nutrients and growth conditions.
- Populations shift in favor of fermentative bacteria when grown in complex medium.
  - Perhaps selected against genera potentially involved in cycling of inorganic nitrogen species (NO_3^-, NO_2) and H_2 gas.
- Organic nutrients provide some carbon substrates for methanogens, but likely stimulate growth and activity of fermentative bacteria to use coal as the main carbon source.

Future Work

- Develop sampling techniques to reduce and account for contamination from non-indigenous organisms.
- Sample more coal seams and groundwater – obtain a greater sample size.
- Link types of bacteria and methanogens with coal rank, location, geochemistry.
- Apply molecular biology techniques for monitoring progress/success of field application
  - Monitor movement and activity of microbes in coal seam.
  - Monitor changes in microbial diversity upon perturbation of a coal seam (fracing, CO_2 flood, nutrient addition).
  - Customize the type of nutrient amendment to the types of microbes present in the coal seam.

References

Boreham, C.J., B. Horsfield, and H.J. Schenk, 1999, Predicting the quantities of oil and gas generated from Australian Permian coals, Bowen Basin using pyrolyticmethods: Marine and Petroleum Geology, v. 16/2, p. 165-188, doi: 10.1016/S0264-8172(98)00065-8.

Delong, E.F., 1992, Archaea in coastal marine environments: PNAS, v. 89/12, p. 5685-5689.

Foght, J., J. Aislabie, S. Turner, C.E. Brown, J. Ryburn, D.J. Saul, and W. Lawson, 2004, Culturable bacteria in subglacial sediments and ice from two Southern Hemisphere glaciers: Microbial Ecology, v. 47/4, p. 329-340.