Laboratory Studies of Biogenic Methane Production from Coal by Microbial Consortia: Identifying Organisms, Reactions and Intermediates*

Elizabeth J. Jones[1], Mary A. Voytek[1], William H. Orem[2], Margo D. Corum[2], Anne L. Bates[2], and Harry E. Lerch[2]

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[1]Water Resources, U.S. Geological Survey, Reston, VA (ejjones@usgs.gov)
[2]Geology, U.S. Geological Survey, Reston, VA

Abstract

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.

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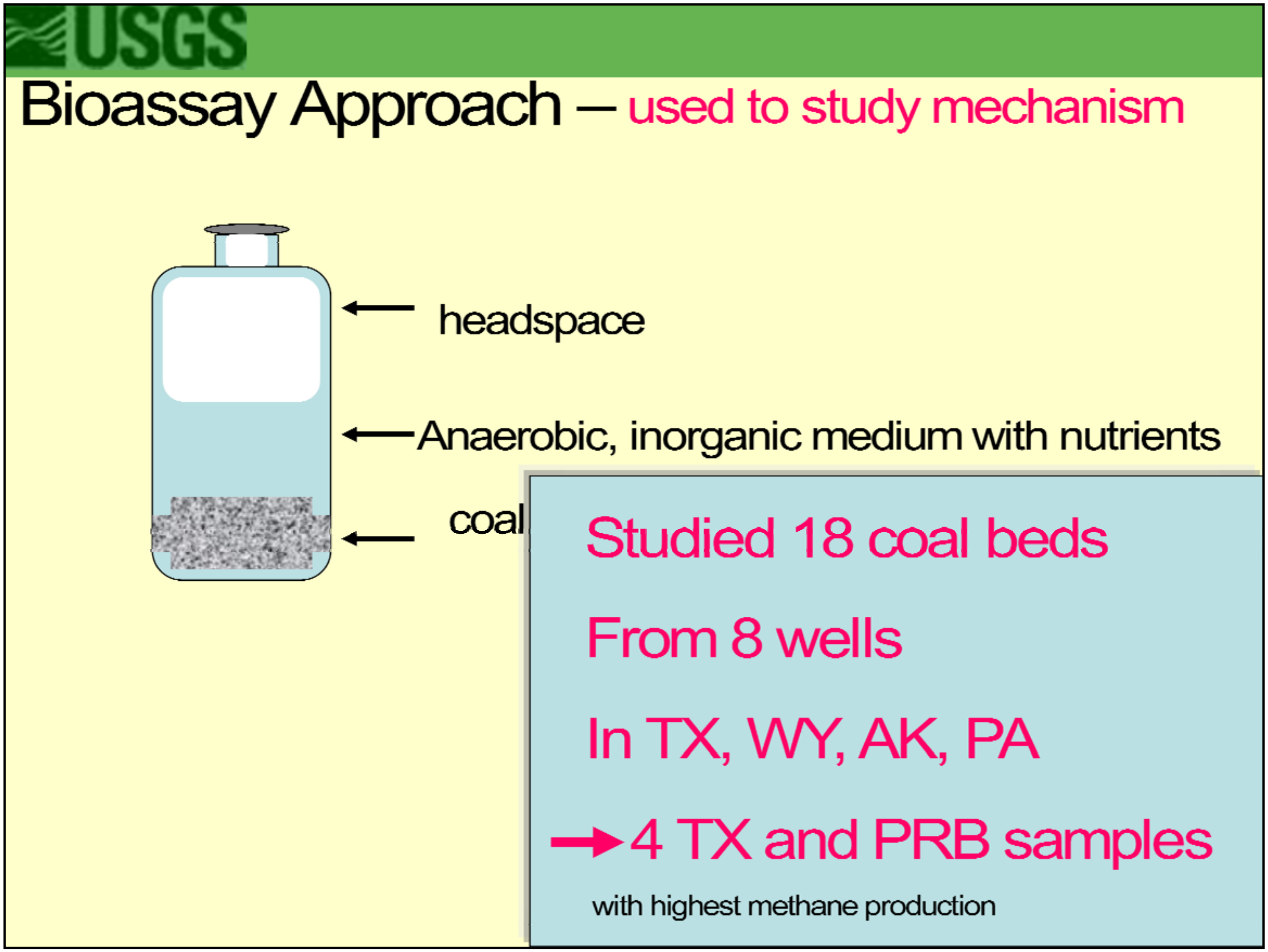
USGS looks at factors controlling new biogenic methane from coal

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

WBC-2 Bioassay

Jones et al. -IJCG special issue - 2008

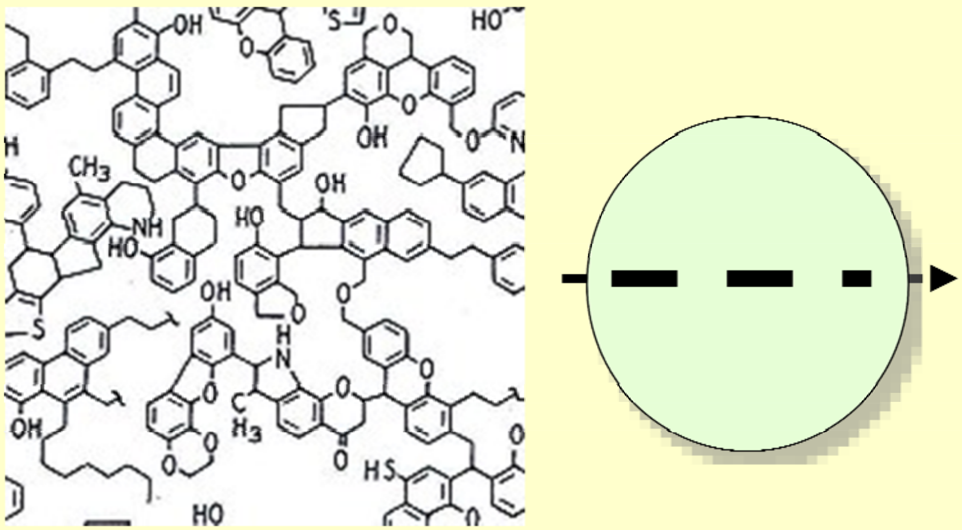


Notes by Presenter: We studied 18 coals from Texas, Wyoming, Alaska and Pennsylvania and selected the 4 with the highest methane for more detailed study.

USGS

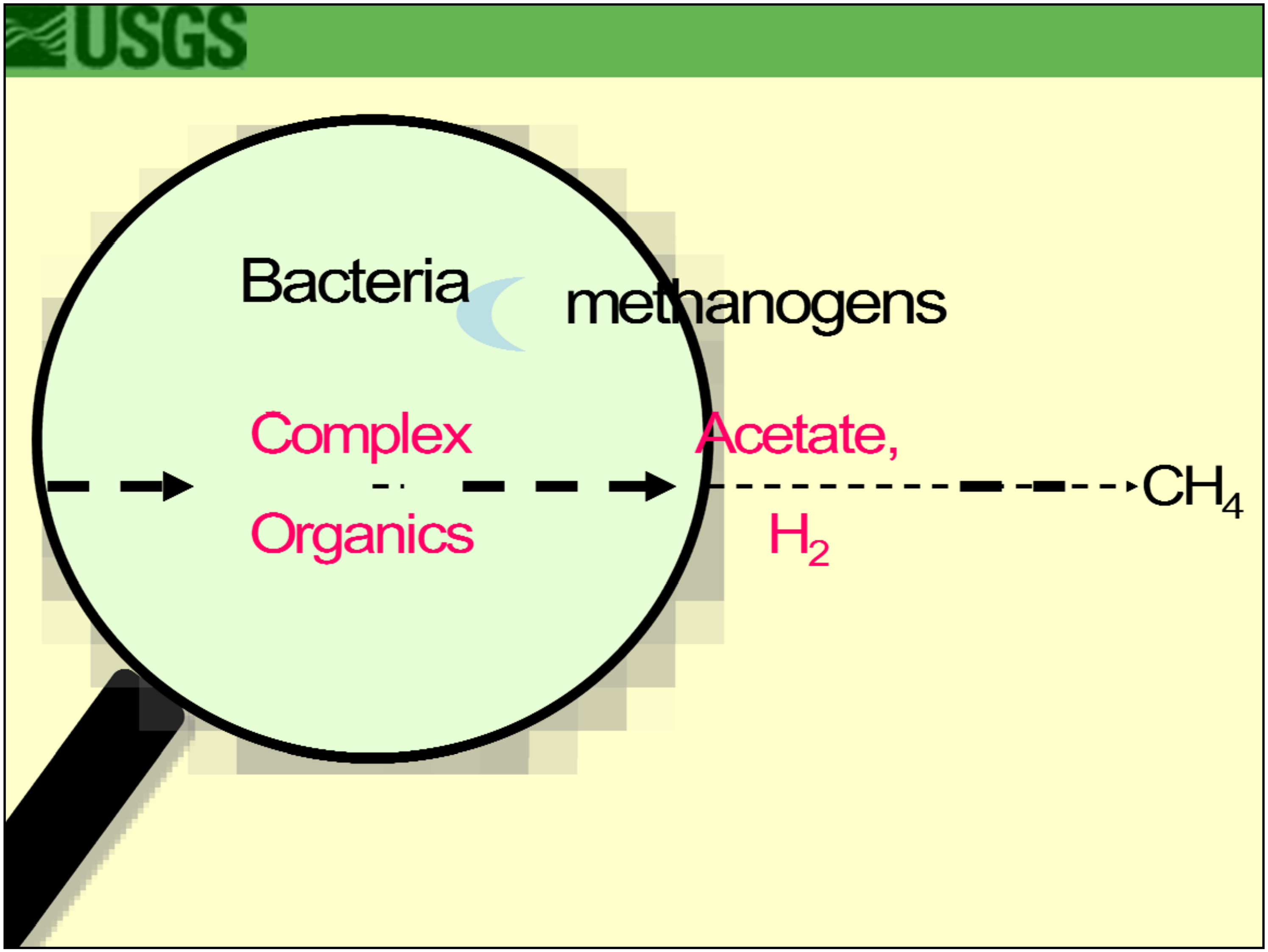
Objective:

Determine the mechanism by which biogenic methane is generated from coal.

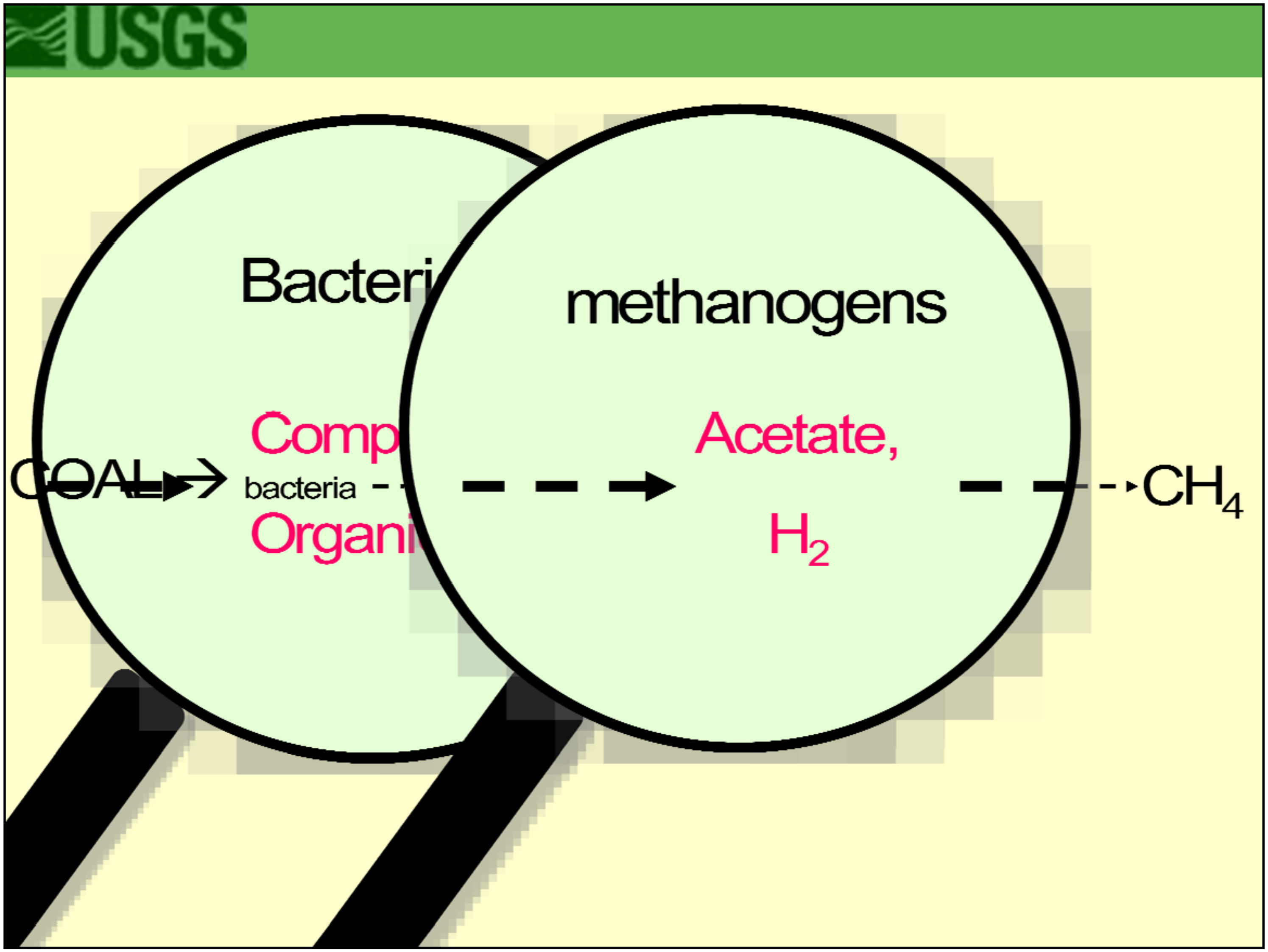


CH_4

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.

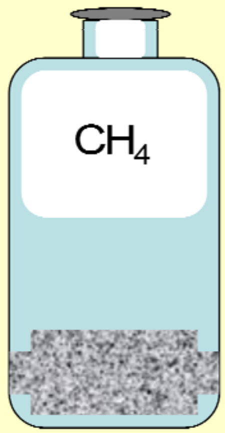


Notes by Presenter: It takes a microbial consortium to generate methane from coal.



Notes by Presenter: It takes a microbial consortium to generate methane from coal.

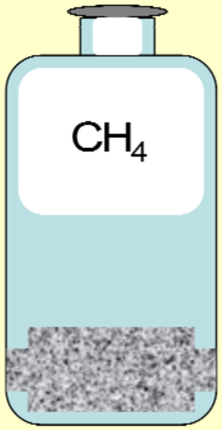
Approach 1



Treatments

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

Approach 1



Analyzed organics:

Solvent extractable organics GC-MS

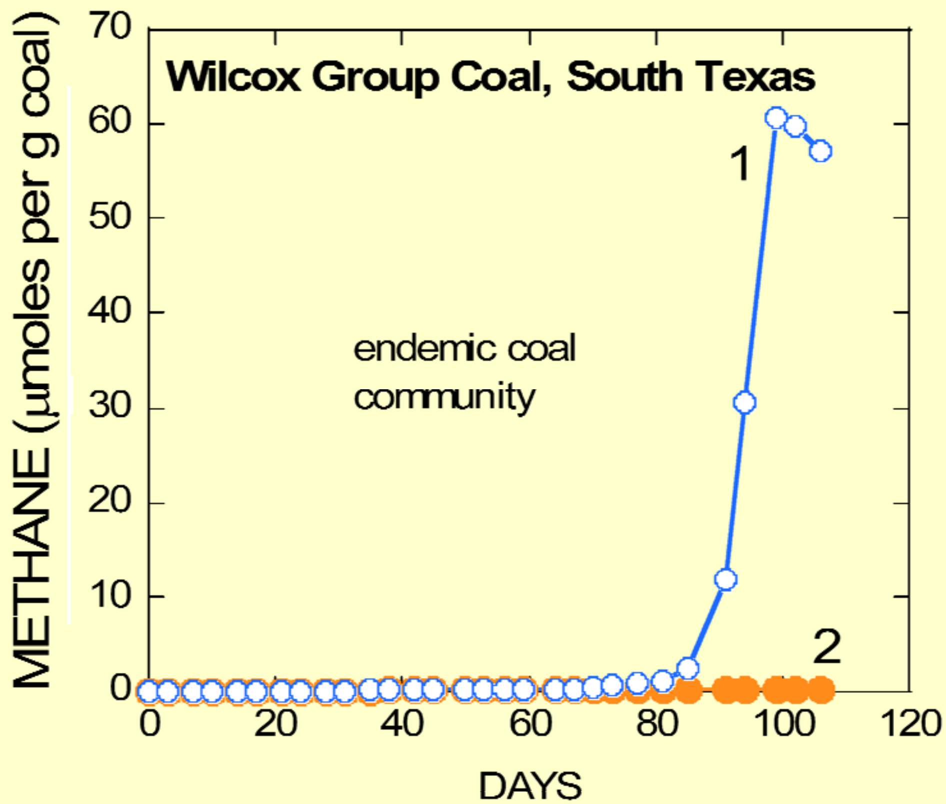
Volatile Fatty Acids - IC

Analyzed DNA:

Bacterial fingerprint - TRFLP

Methanogens - qPCR

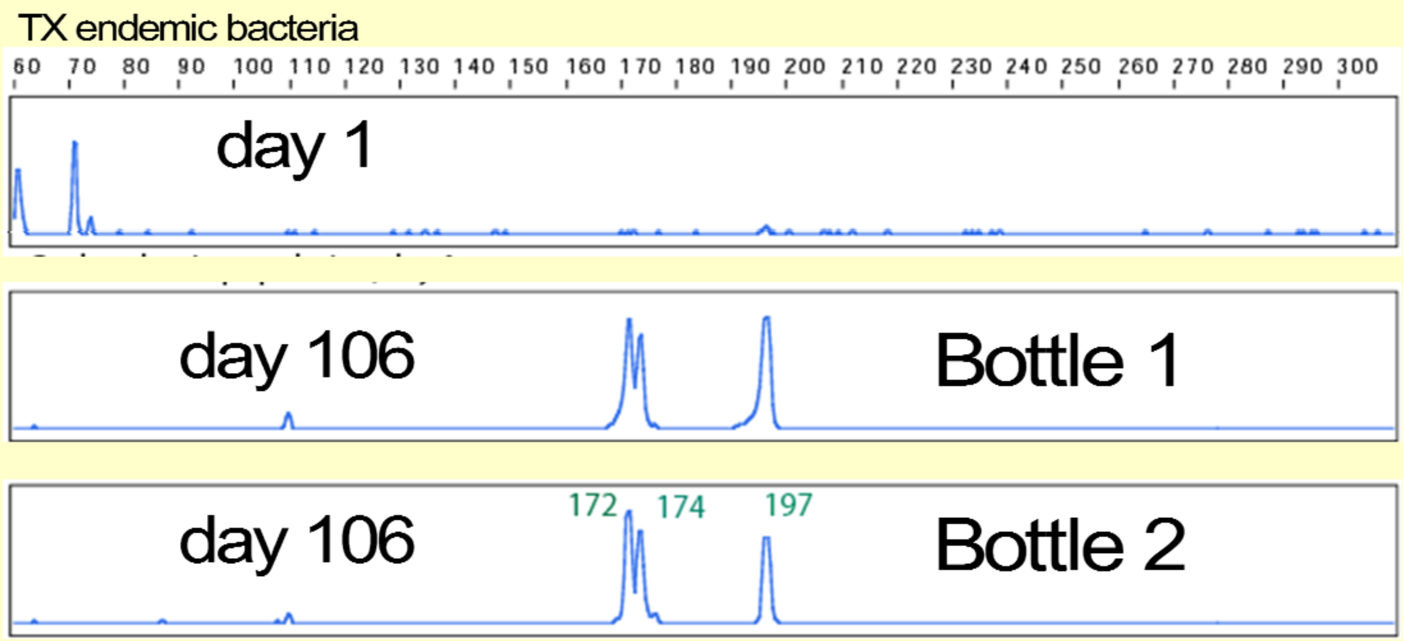
Duplicate “endemic” treatments



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.

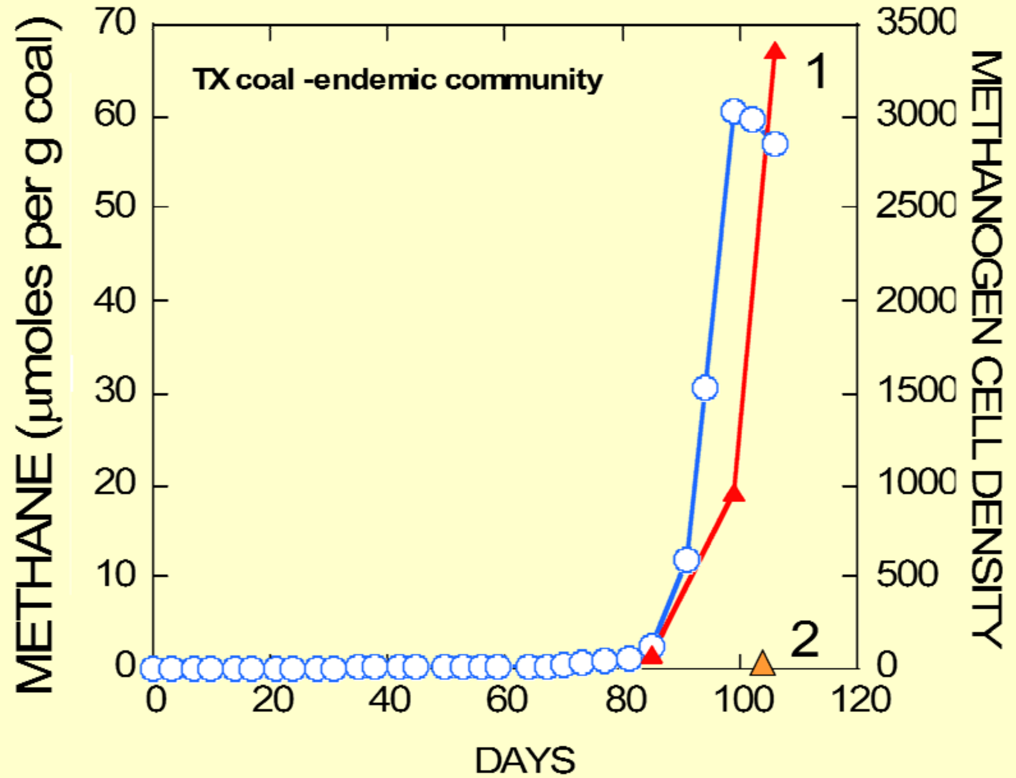
Bacterial DNA fingerprint



TRFLP 16S rDNA – mnl-I

Notes by Presenter: Describe DNA profile

methanogen growth

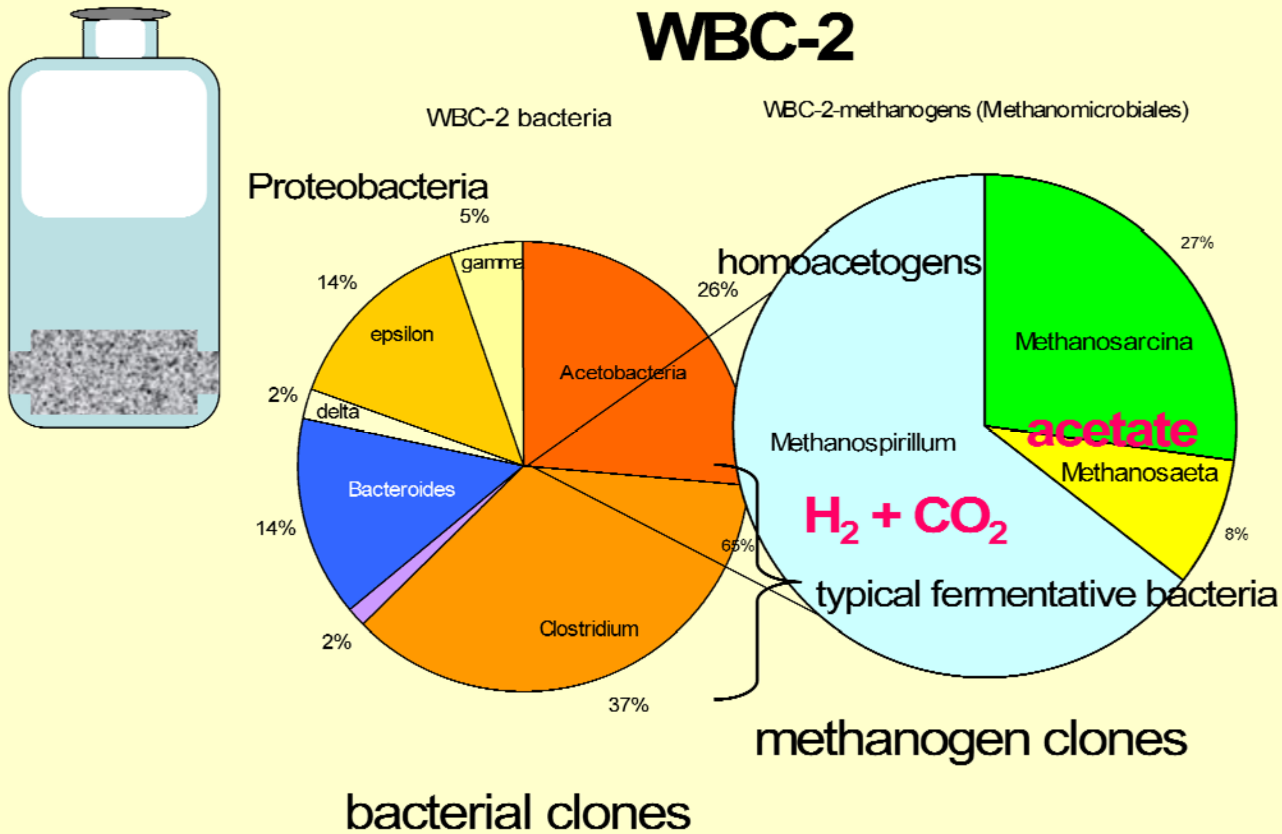


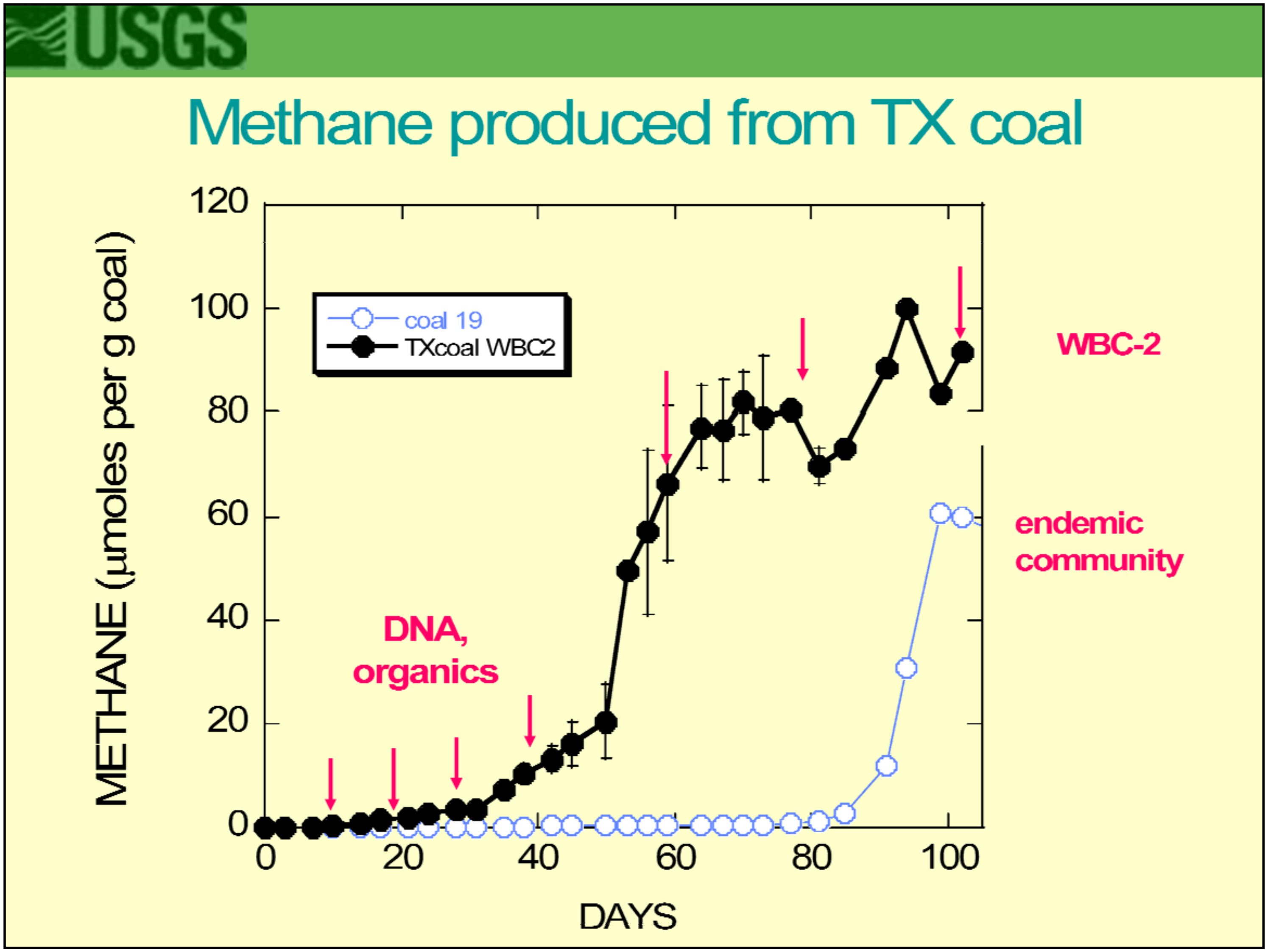
msarc

Approach

•Standardized microbial consortium

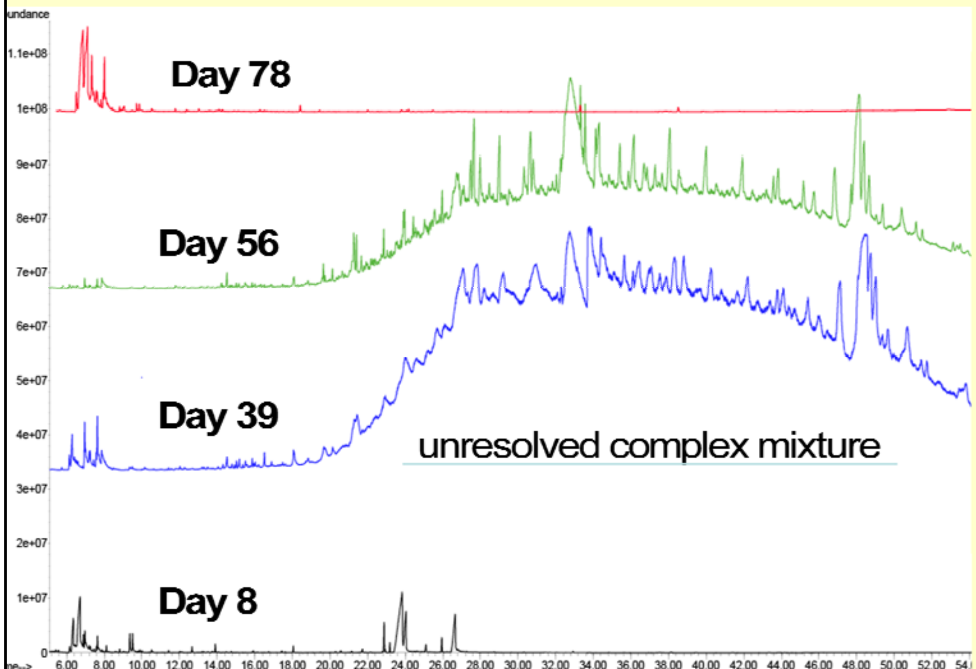
WBC-2





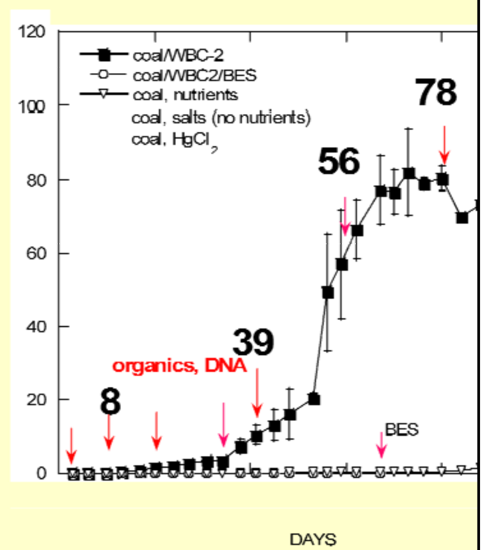
Notes by Presenter: Only 2 of the 18 coal samples studied exhibited endemic activity; endemic activity was difficult to predict or reproduce. By adding a culture with similar behavior we were able to sacrifice bottles from replicates to analyze solvent extractable organics, VFA and DNA.

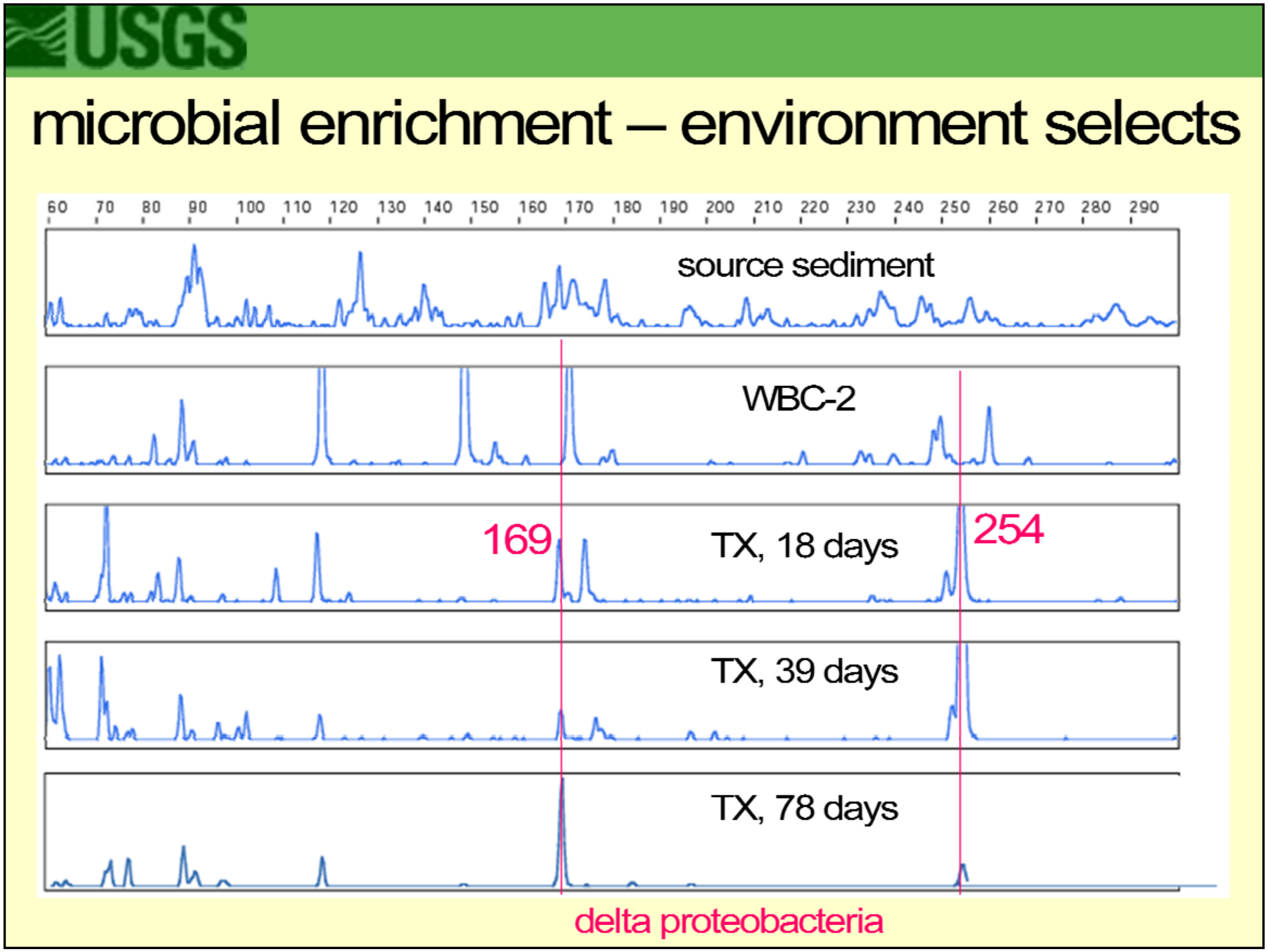
Buildup and Depletion of Solvent-Extractable Organics Over Time



GC-MS

Methane production from TX coal





Notes by Presenter: New environment selects new organisms.

254 delta Proteobacteria (Geobacter), related to bacteria from

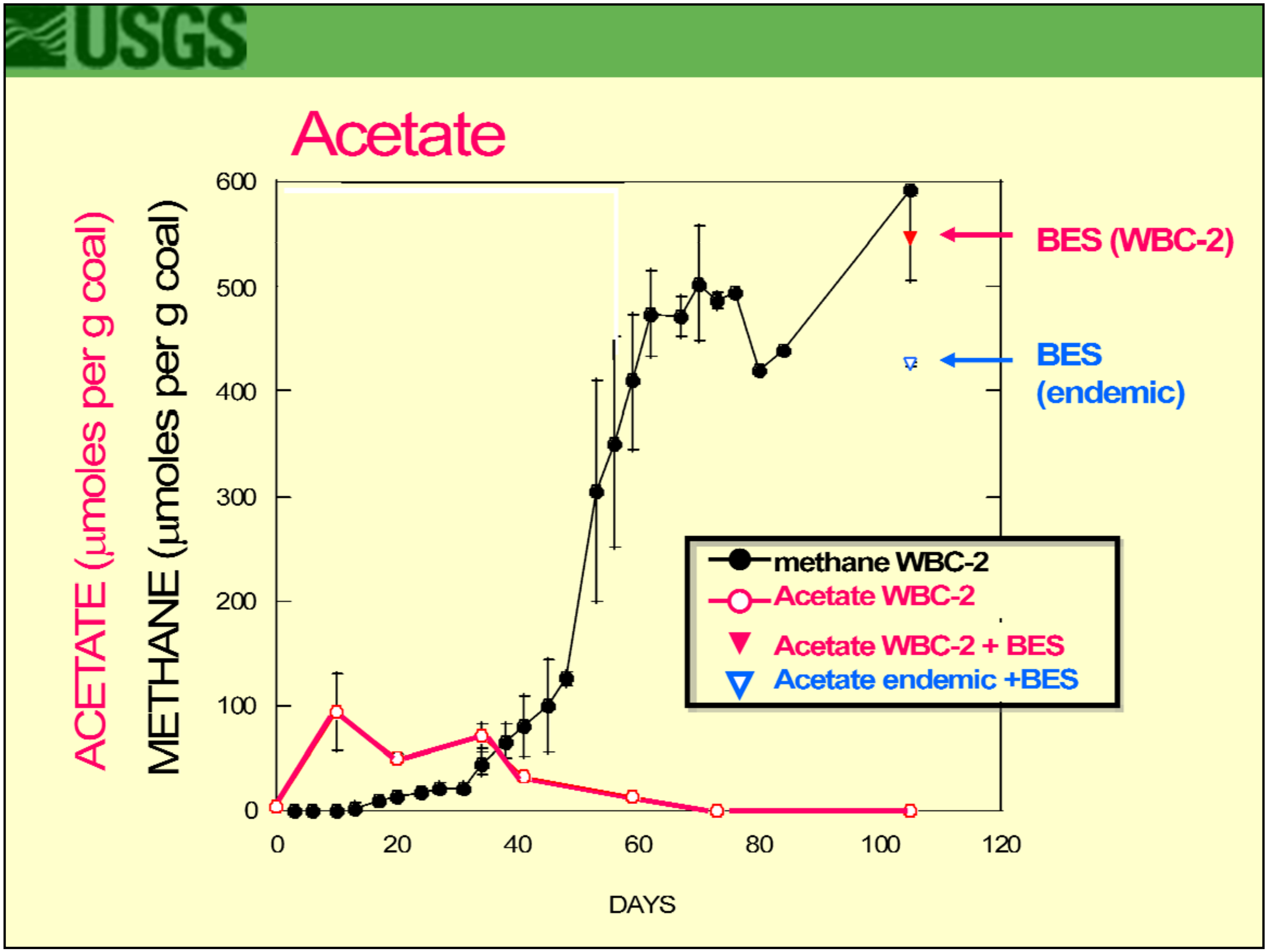
- methanogenic consortium
- deep coal seam
- holocene sediment
- oilfield

169 delta Proteobacteria

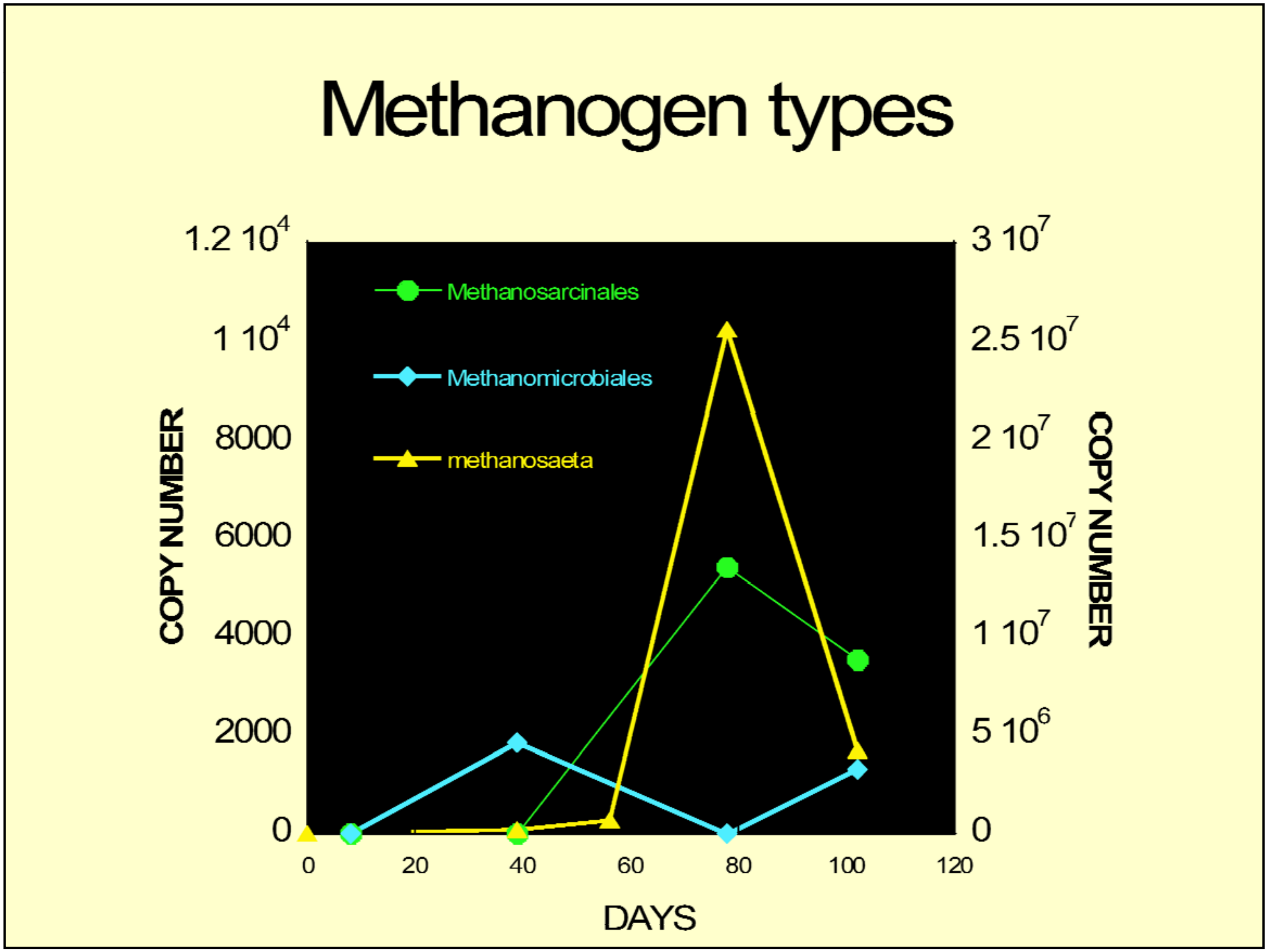
- Methanogenic environment

Syntrophic?

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 …



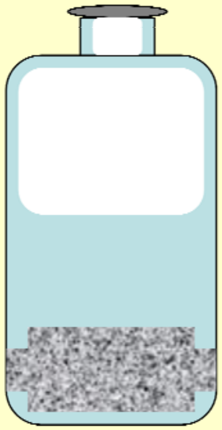
Methanogen types
Methanosarcinales
Methanomicrobiales
methanosaeta
COPY NUMBER
COPY NUMBER
DAYS
1.2 10^4
1 10^4
8000
6000
4000
2000
0
3 10^7
2.5 10^7
2 10^7
1.5 10^7
1 10^7
5 10^6
0
0
20
40
60
80
100
120

Approach

1

treatments

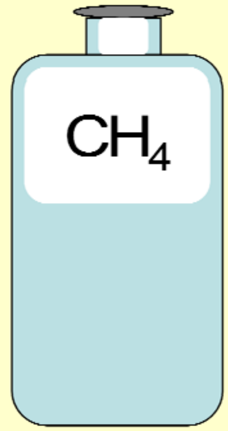
•with coal



→ Identified

organics in solution

2



Test microbial consortium with potential intermediates*

*off the shelf

Coal intermediates identified with GC-MS

tested with WBC-2

Long Chain Fatty Acids

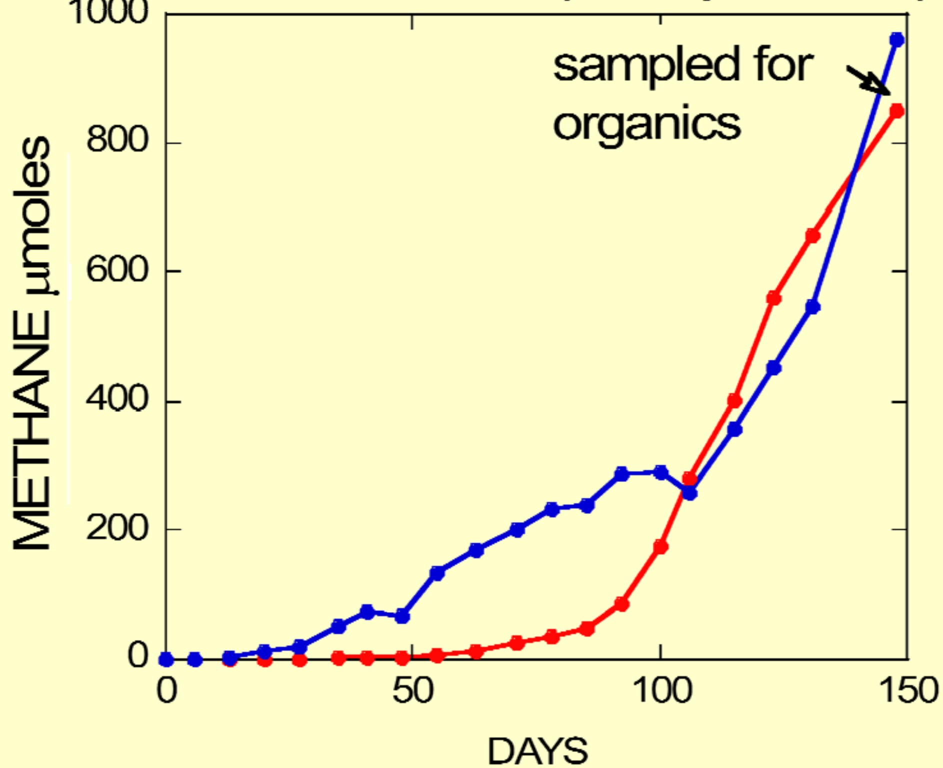
- -Octadecanoic
- -Hexadecanoic
- -dodecanoic
- -decanoic

Single-ring Aromatics

Long-Chain Alkanes

Sterolic structures

Methane from octadecanoic and hexadecanoic (100 µmoles)



Notes by Presenter: Long lag followed by vigorous growth.

CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2- ...COOH

Fatty acids → CH_4

from μmoles **octadecanoic acid (18C)**

18C ⟶ 16C ⟶ 14C ⋯⋯→ acetate ⟶ CH_4

from μmoles **hexadecanoic acid (16C)**

16C ⋯⋯→ butyrate ⟶ acetate ⟶ formate ⟶ CH_4

Coal intermediates tested with WBC-2

Long Chain Fatty Acids

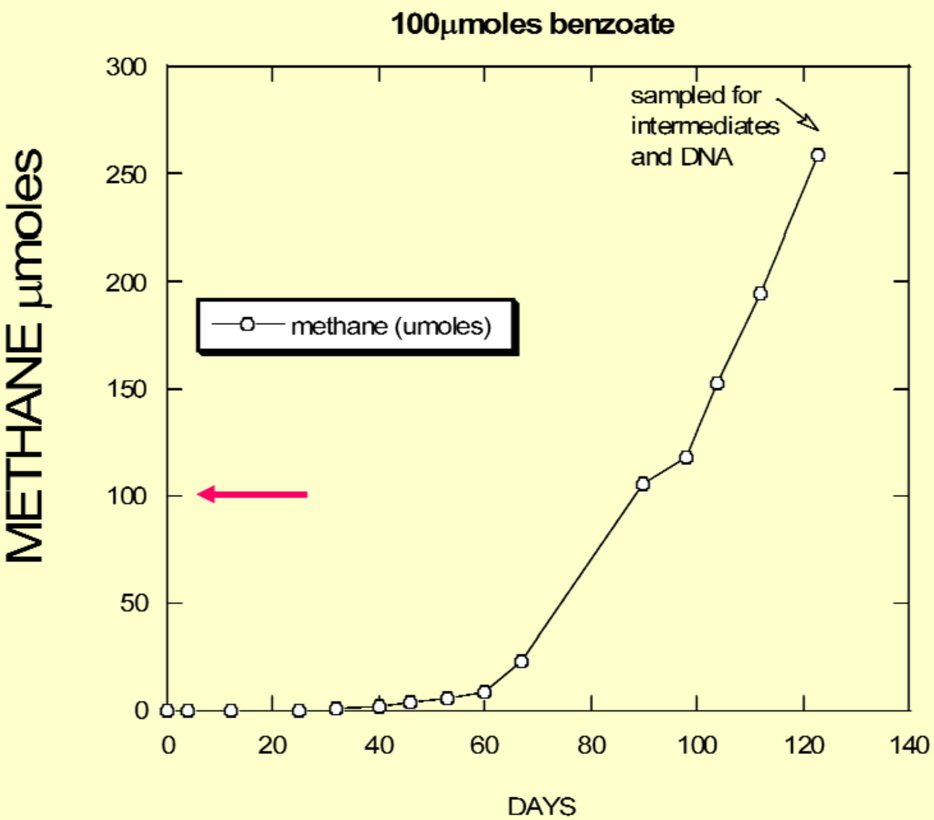
Single-ring Aromatics

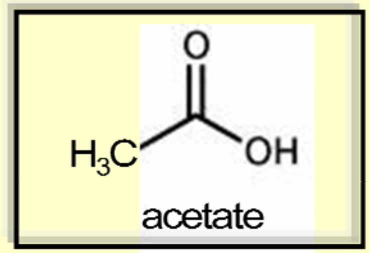
- -Phenol ✗
- -Vanillic acid ✓
- -Caffeic acid ✗
- -Benzoic acid ✓

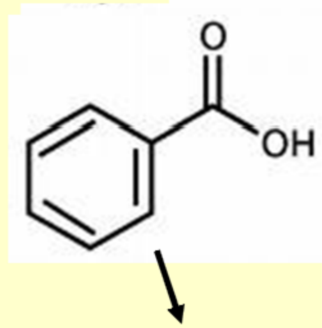
Long-Chain Alkanes

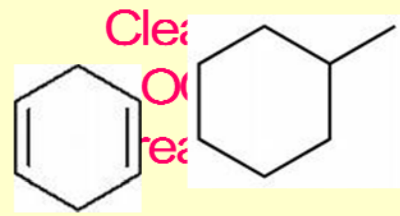
Sterolic structures

benzoic acid





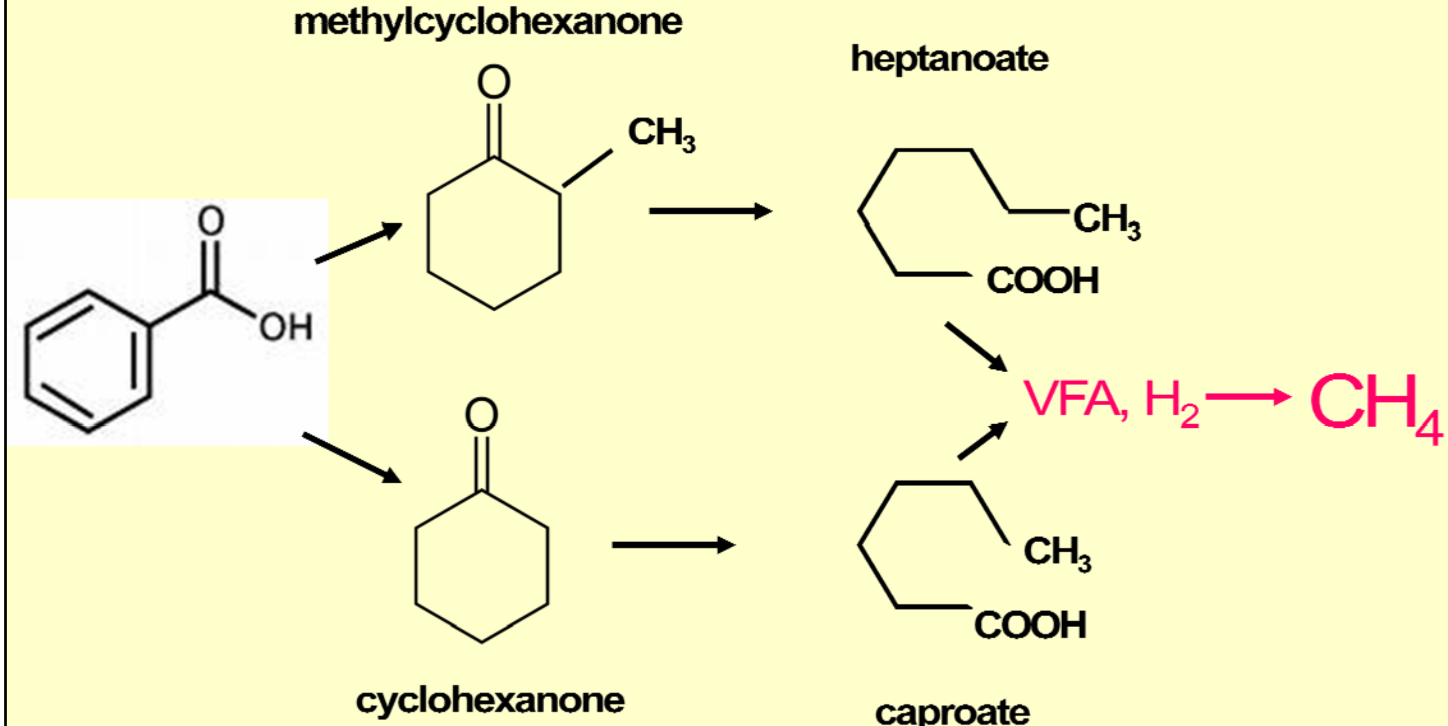




long chain alkanes

acetate

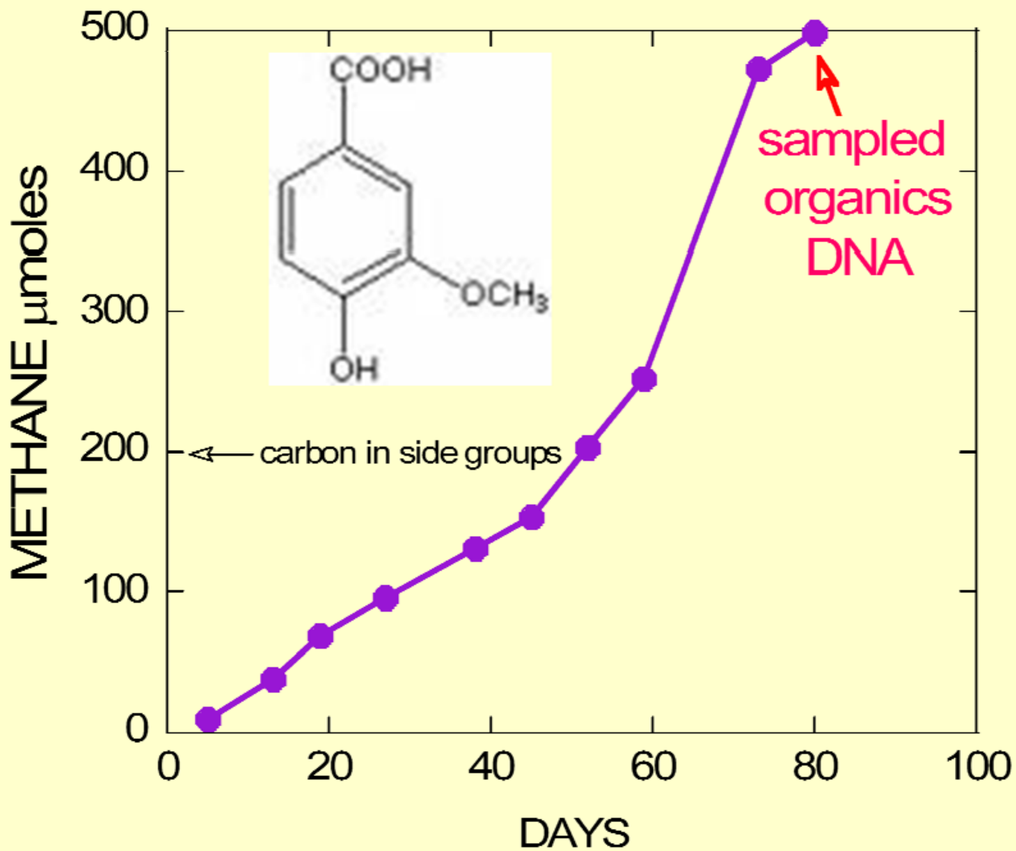
benzoic acid fermentation pathway (methanogenic consortium)



after Evans and Fuchs, 1988

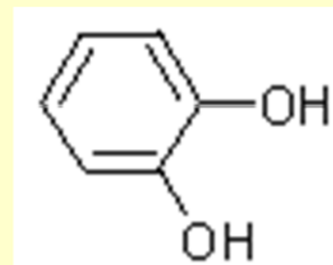
Vanillic acid, 100 μmole

vanillic acid - gone



intermediates:

1,2 benzenediol



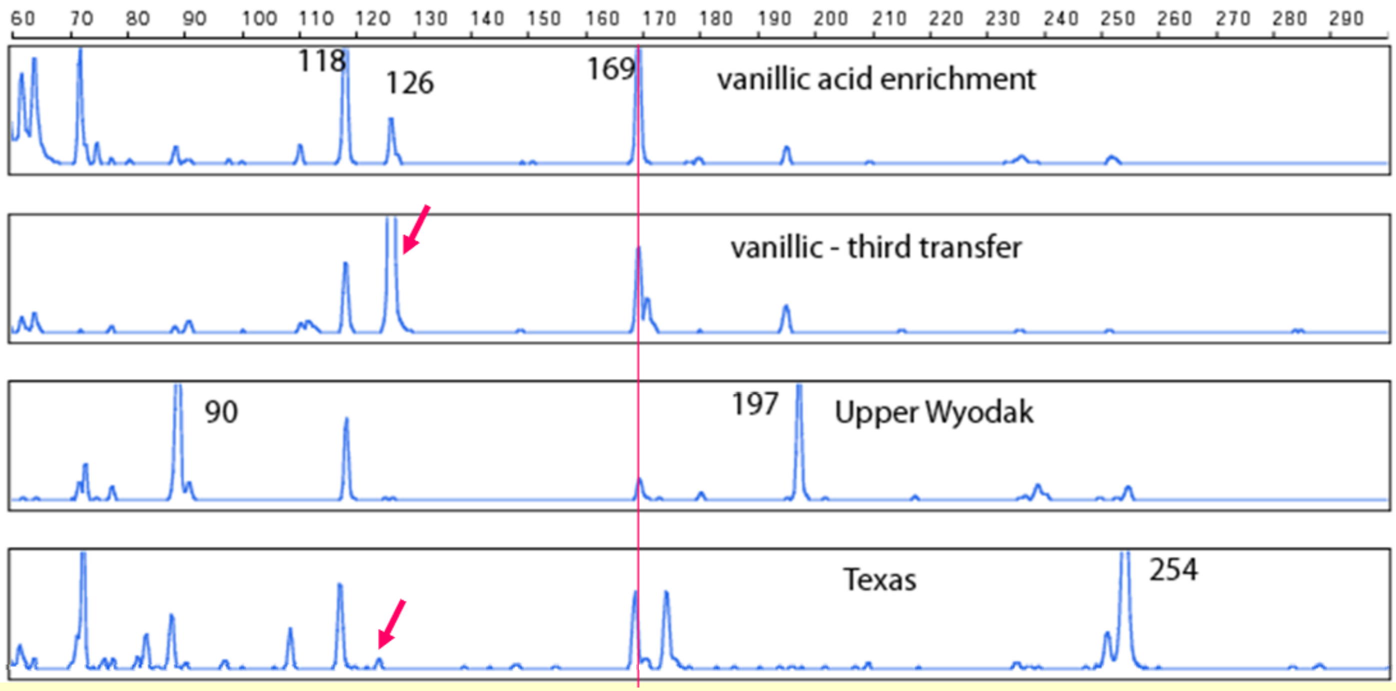
long chain alkanes

volatile fatty acids

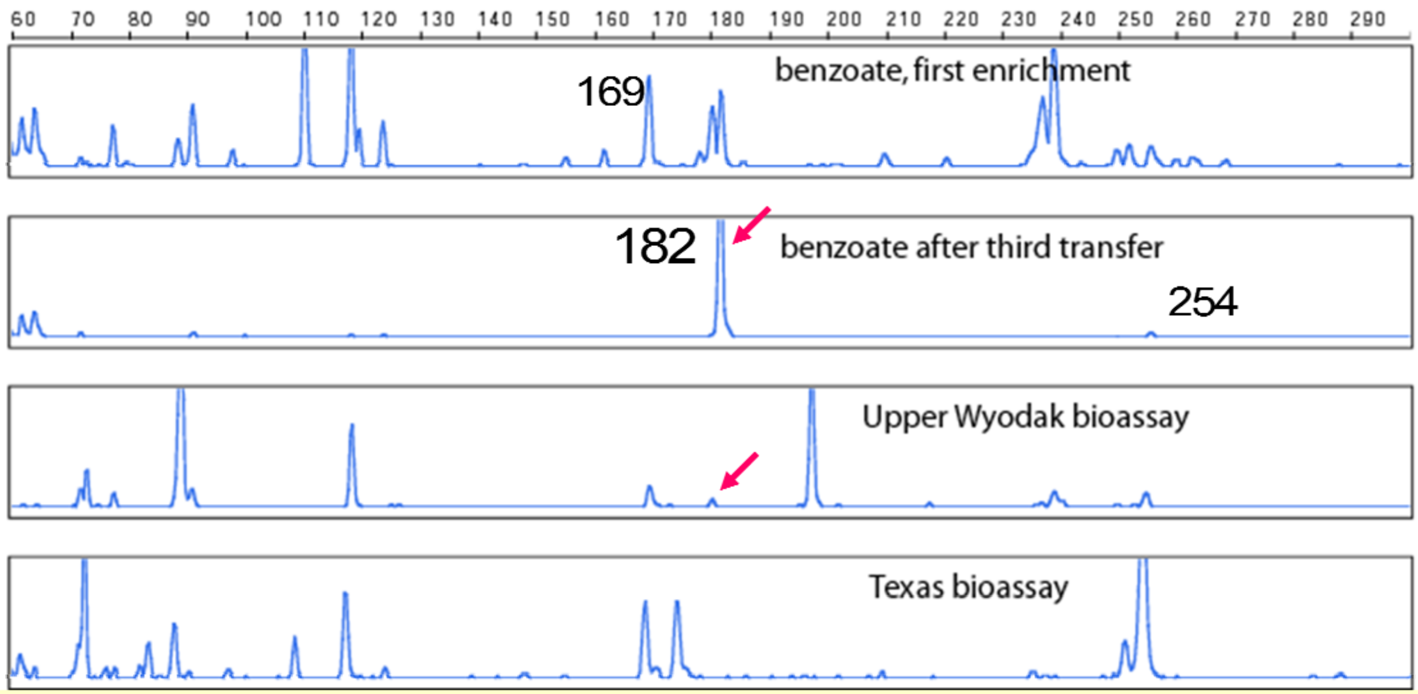
butyrate

acetate

vanillic acid enrichment

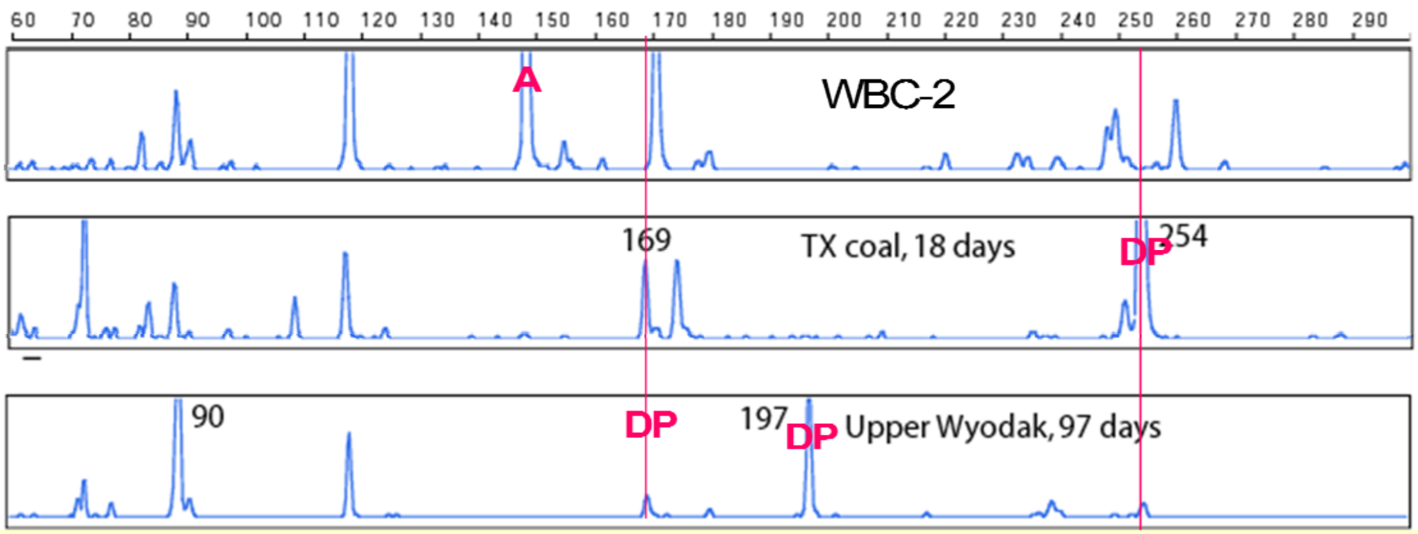


benzoate enrichment



Identifications using

- *in silico* digests of WBC-2 clone sequences
- magnetic beads



DP = delta Proteobacteria

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

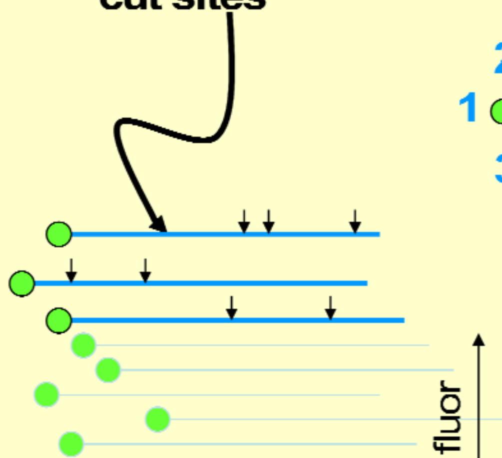
USGS

terminal restriction fragment length polymorphism (TRFLP)

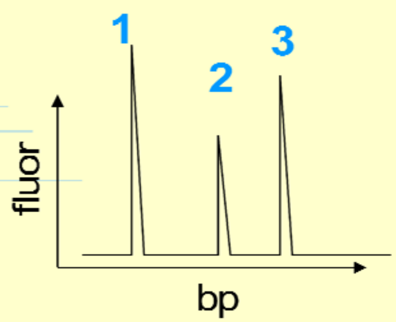
1- target DNA segment and tag terminal end

Bacterial 16s rDNA

2- enzyme recognizes cut sites



3- analyze terminal fragments



- semi-quantitative
- cloning and sequencing for id

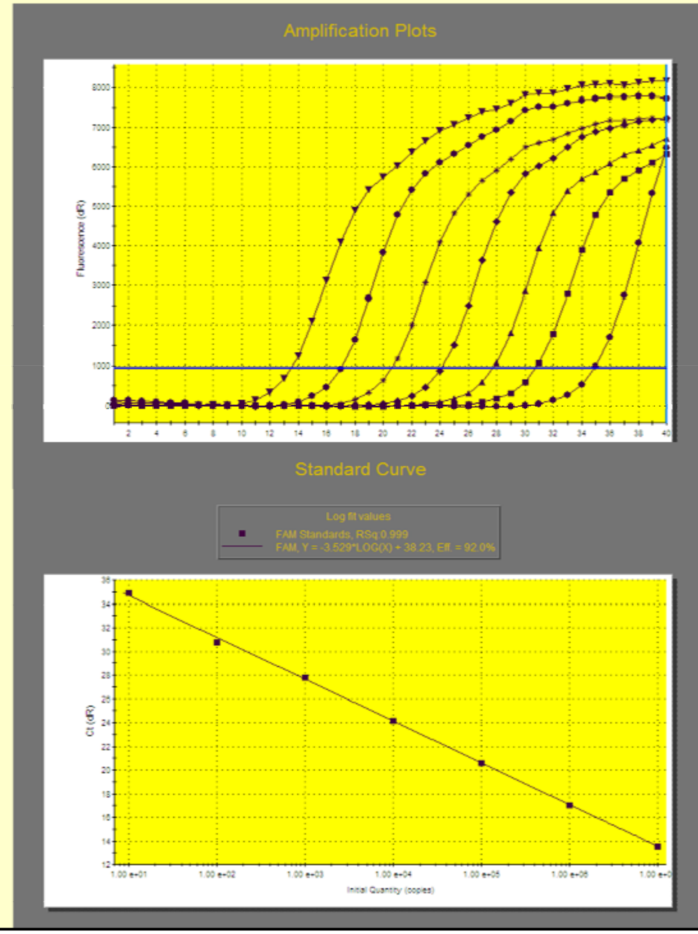
[mixture of DNA] → community profile

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



Stable isotopes

	del C-13	D
CO_2 Reduction	-80	-200
Acetate Fermentation	-50	-300 -400
TX coal	-55	-310

Toxic effects ...