

# Effect of Impoundment Management Strategies on Microbial Communities and the Fate of Radionuclides\*

Kelvin B. Gregory<sup>1</sup>, Arvind Murali Mohan<sup>2</sup>, and Radisav D. Vidic<sup>2</sup>

Search and Discovery Article #80312 (2013)\*\*  
Posted September 11, 2013

\*Adapted from oral presentation presented at AAPG Annual Convention and Exhibition, Pittsburgh, Pennsylvania, May 19-22, 2013

\*\*AAPG©2013 Serial rights given by author. For all other rights contact author directly.

<sup>1</sup>Steinbrenner Institute, Carnegie Mellon University, Pittsburgh, PA 15213 ([kelvin@cmu.edu](mailto:kelvin@cmu.edu))

<sup>2</sup>Steinbrenner Institute, Carnegie Mellon University, Pittsburgh, PA 15213

## Abstract

Flowback is commonly impounded at the surface prior to treatment, reuse and/or disposal. During impoundment, microbial activity alters the fate of organic carbon, metals, and gives rise to odor causing compounds that complicate water and waste management, and increase production costs. Here we report on the microbial community that appears in well-head samples of flowback water as well as the microbial community that arises in flowback impoundments under various treatment regimes. We also describe the impacts of various alternate treatment regimes on the fate of uranium in flowback impoundments.

Microbial communities were examined using molecular microbial ecology techniques based on PCR amplification of 16S rRNA genes. A clone library approach in conjunction with Quantitative-PCR was used for the analyses. Results from clone libraries show that the microbial communities present in well-head samples were variable with time and distinct from each other. The majority of the flowback and produced water communities were most closely affiliated with known halotolerant, anaerobic, and sulfidogenic bacteria. Q-PCR enumeration indicated uniform 16S rRNA gene concentrations in fracturing water and flowback samples but were two orders of magnitude lower in the produced water phase.

Microbial community surveys of flowback impoundments reveal that the untreated and biocide-amended impoundment had diverse and depth-dependent bacterial communities of aerobic, fermentative, and anaerobic bacteria. In contrast, the bacterial community in the aerated impoundment was homogeneous with depth and was dominated by sequences most similar to aerobic, iodide oxidizing species. Archaea were only observed in the deeper clines of the untreated and biocide amended impoundments and all were most closely related to known methanogens. Treatment regimes were closely linked with the solubility and hence fate of uranium in flowback impoundments. The findings from these studies reveal the diversity of organisms that are present in flowback water and that environmental management strongly impacts the microbial communities and subsequent biogeochemistry in the impoundments.

## References Cited

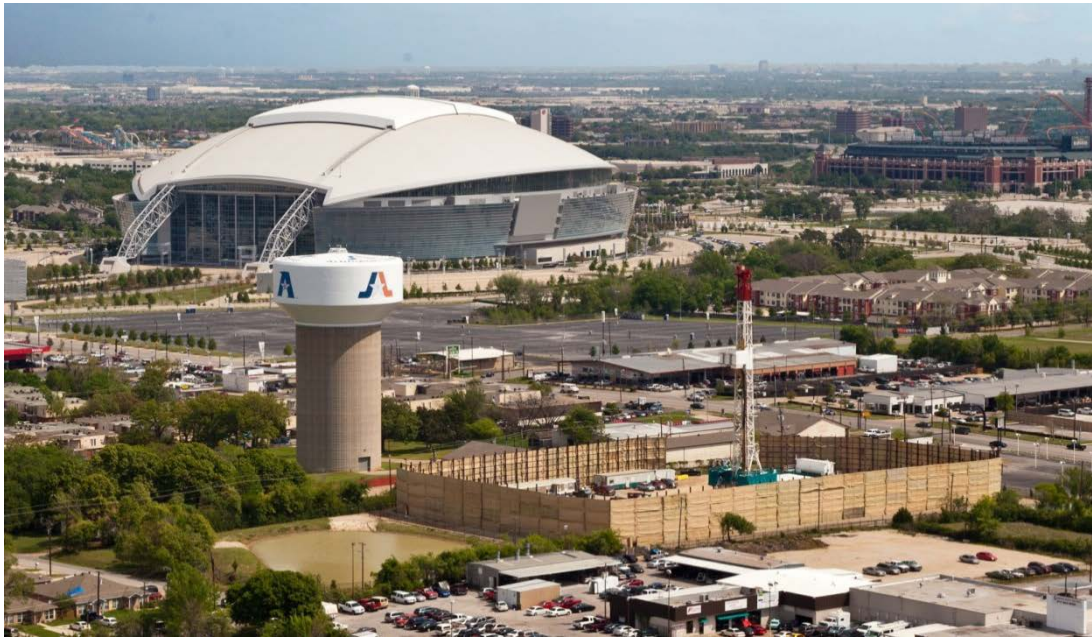
Barbot, E., N.S. Vidic, K. Gregory, and R.D. Vidic, 2013, Spatial and temporal correlation of water quality parameters of produced waters from Devonian-age shale following hydraulic fracturing: *Environmental Science & Technology*, v. 47, p. 2562-2569.

Gregory, K.B., D.A. Dzombak, and R.D. Vidic, 2011, Water management challenges associated with the production of shale gas by hydraulic fracturing: *Elements*, p. 181-186.

Holmes, D.E., K.T. Finneran, R.A. O'Neil, and D.R. Lovley, 2002, Enrichment of members of the family geobacteraceae associated with stimulation of dissimilatory metal reduction in uranium-contaminated aquifer sediments: *Applied and Environmental Microbiology*, v. 68/5, p. 2300-2306.

Lovley, D.R., E.J.P. Phillips, Y.A. Gorby, and E.R. Landa, 1991, Microbial reduction of uranium: *Nature*, v. 350, p. 413-416.

# Effect of Impoundment Management Strategies on Microbial Communities and the Fate of Radionuclides



**Kelvin B. Gregory**  
**Arvind Murali Mohan**  
**Radisav D. Vidic**



**STEINBRENNER INSTITUTE**  
for Environmental Education & Research

**Civil and Environmental**  
**ENGINEERING**

**CarnegieMellon**

# Oil/Gas Extraction: PA 1919



On the banks of the Yougheny River, Versailles, PA, 1919.  
Photo Courtesy McKeesport Historical Society. Located and Digitized by Joel Tarr, Carnegie Mellon.

# Some Produced Water Constituents



	minimum	maximum	average	number of samples
TDS (mg/L)	680	345,000	106,390	129
TSS (mg/L)	4	7,600	352	156
oil and grease (mg/L)	4.6	802	74	62
COD (mg/L)	195	36,600	15,358	89
TOC (mg/L)	1.2	1530	160	55
pH	5.1	8.42	6.56	156
alkalinity (mg/L as CaCO <sub>3</sub> )	7.5	577	165	144
SO <sub>4</sub> (mg/L)	0	763	71	113
Cl (mg/L)	64.2	196,000	57,447	154
Br (mg/L)	0.2	1,990	511	95
Na (mg/L)	69.2	117,000	24,123	157
Ca (mg/L)	37.8	41,000	7,220	159
Mg (mg/L)	17.3	2,550	632	157
Ba (mg/L)	0.24	13,800	2,224	159
Sr (mg/L)	0.59	8,460	1,695	151
Fe dissolved (mg/L)	0.1	222	40.8	134
Fe total (mg/L)	2.6	321	76	141
gross alpha <sup>a</sup> (pCi/L)	37.7	9,551	1,509	32
gross beta <sup>a</sup> (pCi/L)	75.2	597,600	43,415	32

Gregory et al, Elements 2011; Barbot et al, ES&T 2013

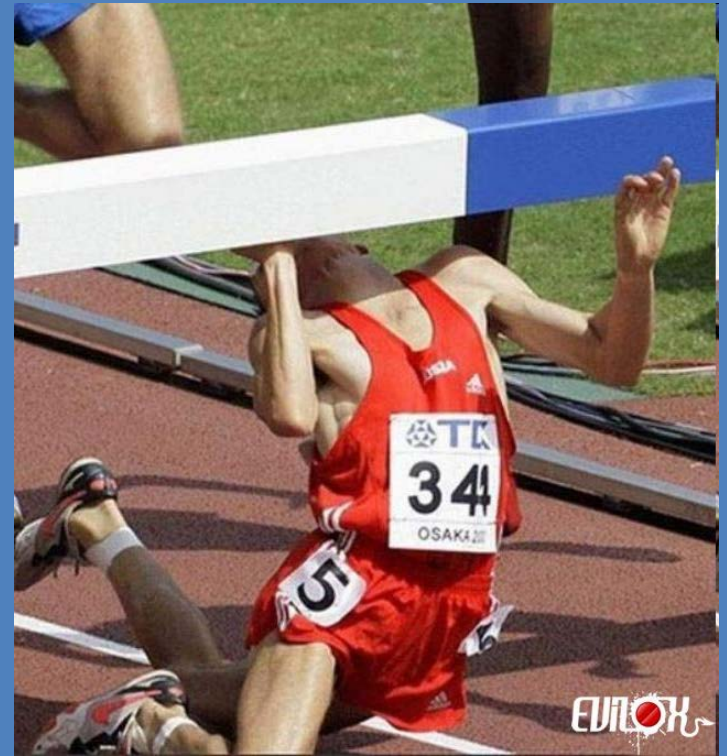
# Water Management Hurdles in Pennsylvania

## Disposal

- Deep-Well Reinjection → Few in PA
- Dilution → Waterway Contamination
- Ag Reuse → Too salty

## Treatment

- Membrane Technology → \$\$\$
- Thermal Distillation → \$\$\$\$
- Freeze Thaw Evaporation → Bad Climate
- Artificial Wetlands → Too salty



# Water Management Hurdles in Pennsylvania

## Disposal

- Deep-Well Reinjection → Few in PA
- Dilution → Waterway Contamination
- Ag Reuse → Too salty

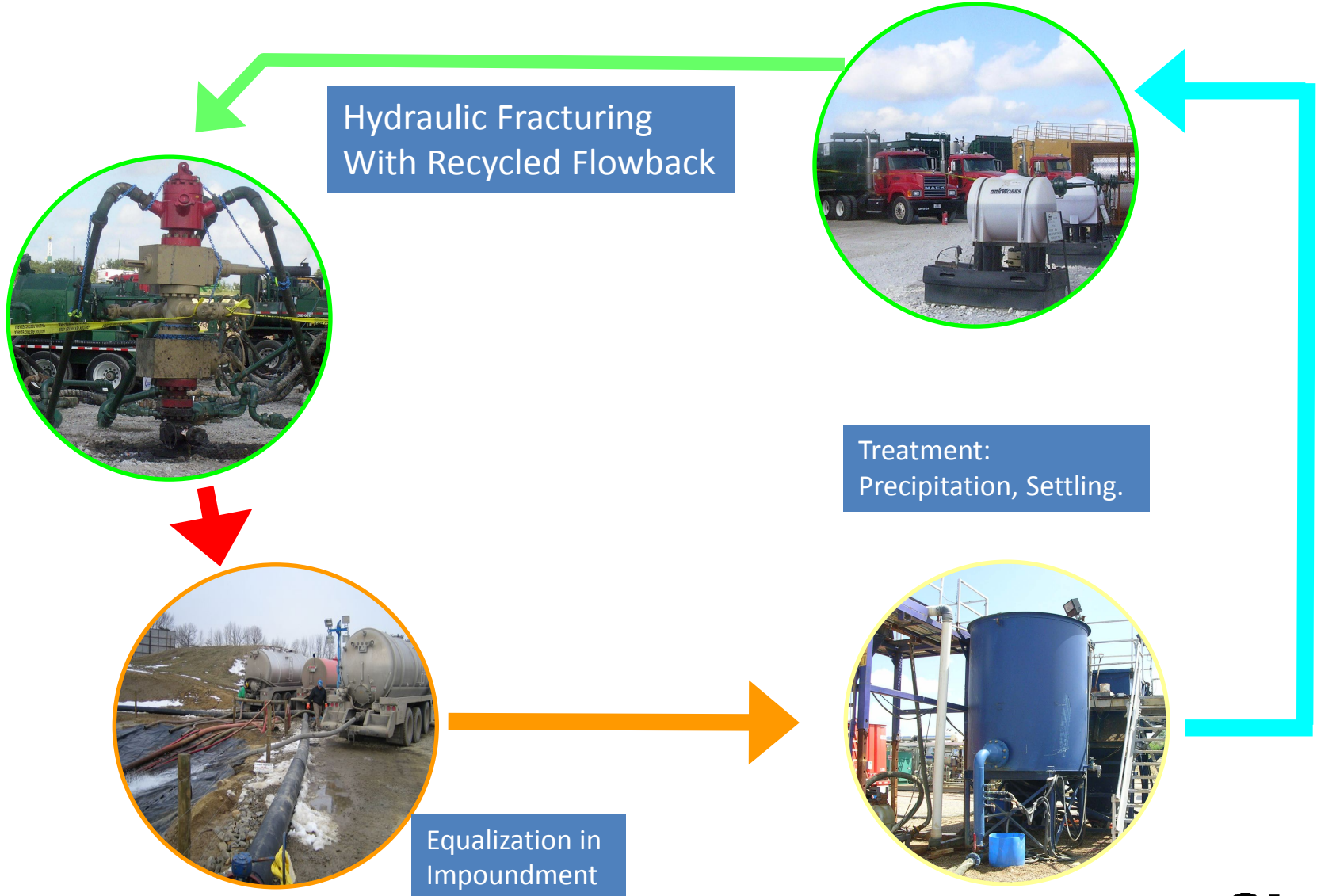
## Treatment

- Membrane Technology → \$\$\$
- Thermal Distillation → \$\$\$\$
- Freeze Thaw Evaporation → Bad Climate
- Artificial Wetlands → Too salty



**Local Challenges → Innovation & Local Solutions**

# Local Solutions Arise: Reuse of Flowback for HF





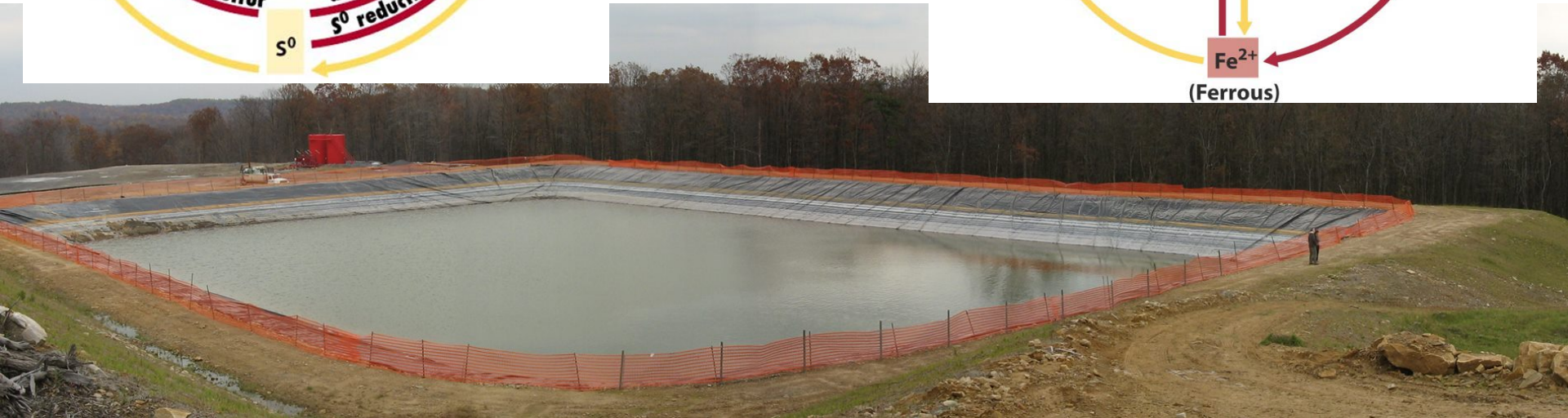
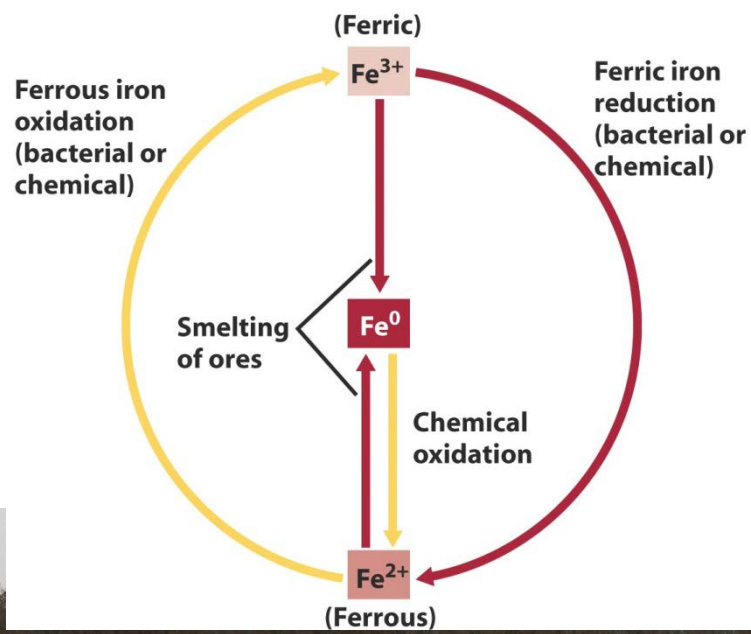
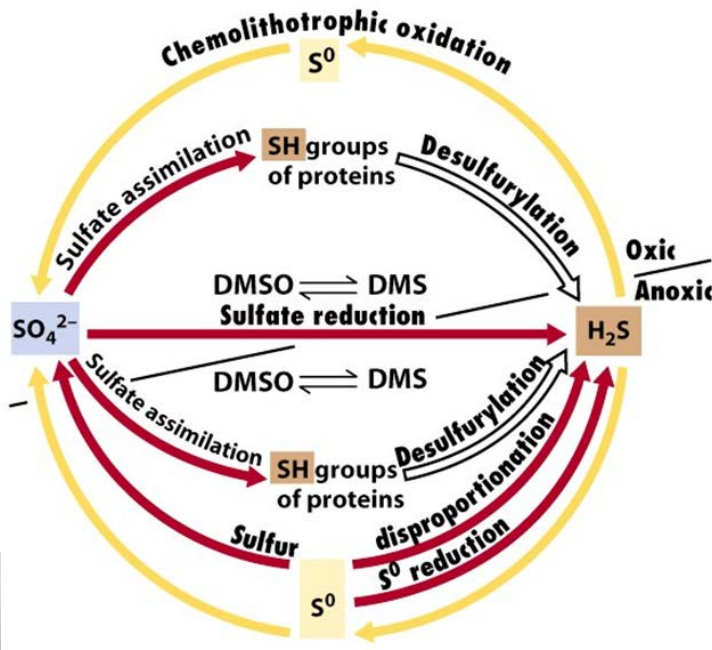
# Recycling: Larger and Lengthy Impoundment



- Mixed flowback and produced fluids
- Large Impoundments
- Lengthy Impoundment time
- Evolving biogeochemistry

What is happening in the impoundments w.r.t. biogeochemistry, metals and NORM ?

# Motivations: Microbial communities drive evolution of impoundment chemistry, impact management

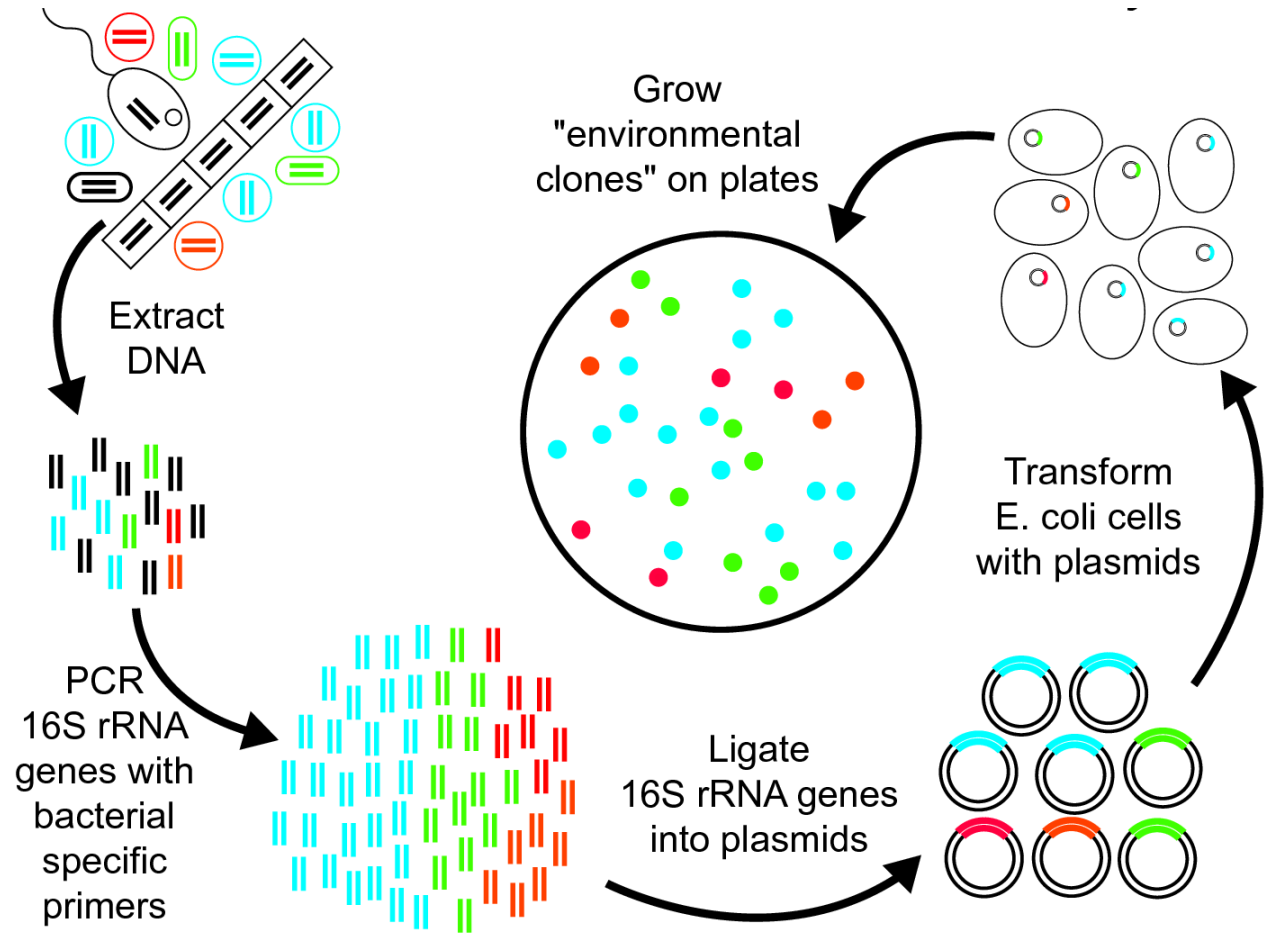


Volatile Sulfur Compounds  
Volatile Fermentation Products

Hydrocarbon  
biodegradation

Metals Solubility  
Fate of NORM

# Microbial Ecology: Clone Library Method

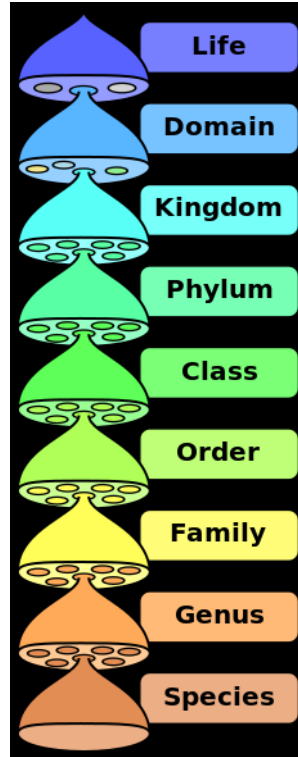
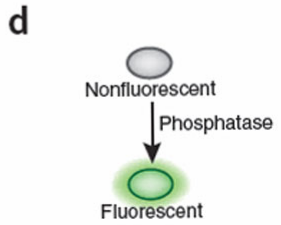
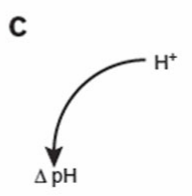
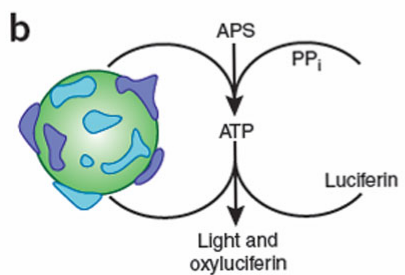
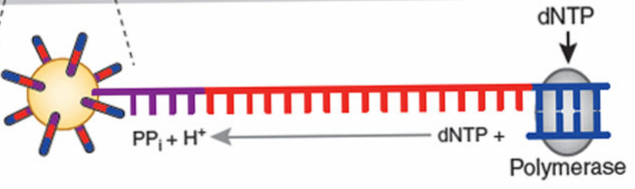
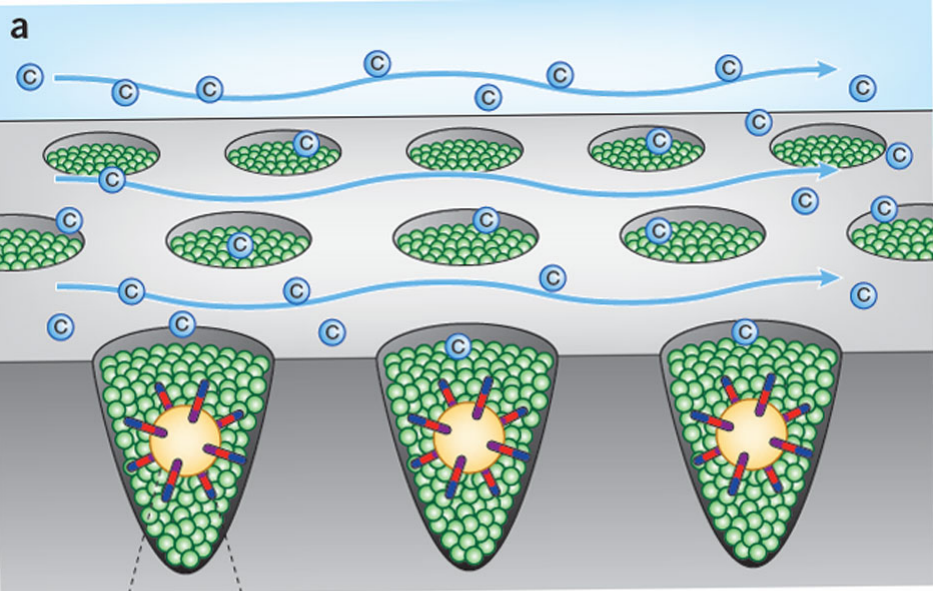


- Sequence the cloned DNA from each colony
- Compare these sequences to that of identified species
- Group like sequences and enumerate

- Yields ~100+ sequences
- ~500 bp per sequence
- Reveals species-level information about each clone

# Microbial Ecology: Pyrosequencing Method

- Less intuitive, much higher throughput
- Sequence the cloned DNA from the entire community
- Compare these sequences to that of identified species
- Group like sequences and enumerate
- Yields 5,000 (or much more) sequences
- 250 bp each
- Can reveal class and order-level information
- Can “see” organisms in very low copy numbers



Important differences in metabolic capabilities revealed with higher levels of specificity

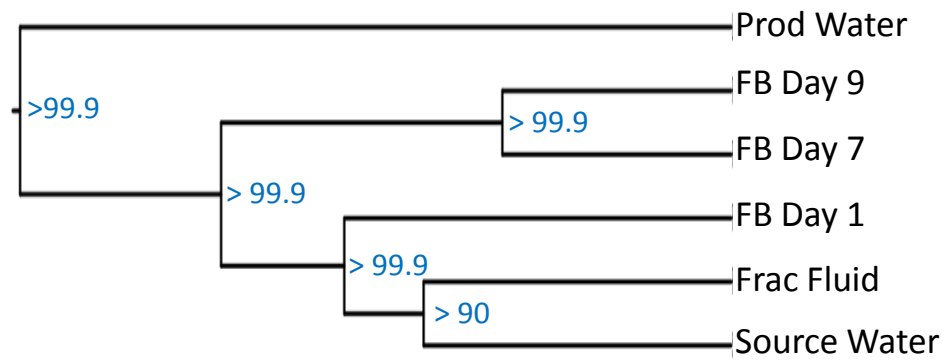
# Wellheads



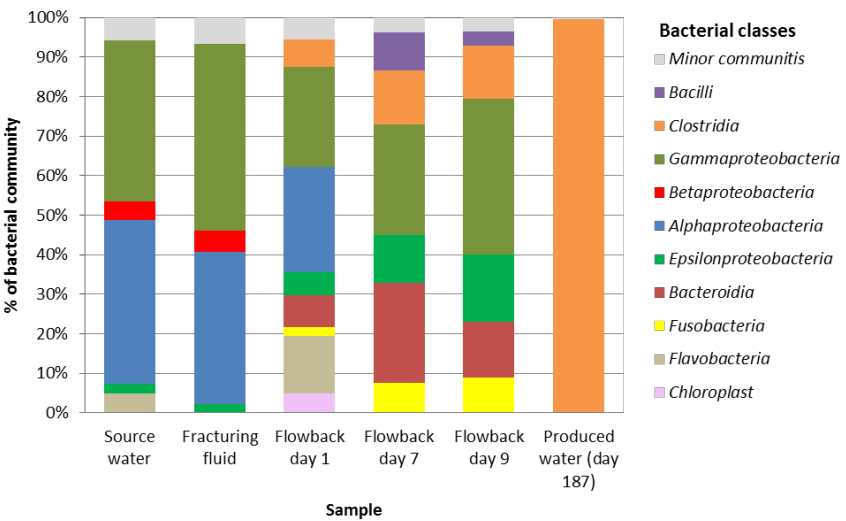
# Results at the Wellheads

Analyte	Chemical Constituents of Water Samples (mg/L)					
	Source water	Fracturing fluid	FB day 1	FB day 7	FB day 9	Produced water (day 187)
Ba <sup>2+</sup>	71.9	110	473	2118	2077	3169
Sr <sup>2+</sup>	126	209	473	1859	1910	2687
Ca <sup>2+</sup>	522	866	1885	6179	6071	9994
Cl <sup>-</sup>	3635	5980	18626	63596	63106	91800
Br <sup>-</sup>	35.3	56.6	161	485	492	876
Mg <sup>2+</sup>	48.3	78.8	182	690	699	1255
Na <sup>+</sup>	2953	4541	13899	42146	43094	44770
K <sup>+</sup>	26.3	47.5	158	246	251	294
Total Fe	ND <sup>2</sup>	0.7	4.2	83	82	109
Total S	7.4	12	32	57	56	51.7
NO <sub>3</sub> <sup>-</sup>	4.7	7.6	14	11	13	18.1
SO <sub>4</sub> <sup>2-</sup>	6.2	9.1	32	11	9.3	9.5
Rad (pCi/L)	171	198	3062	8634	9031	18300

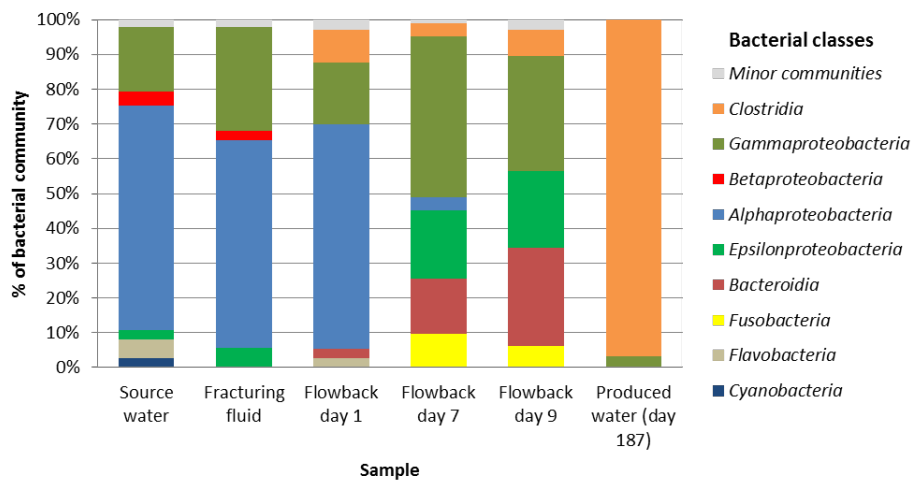
Similarity between communities in each sample



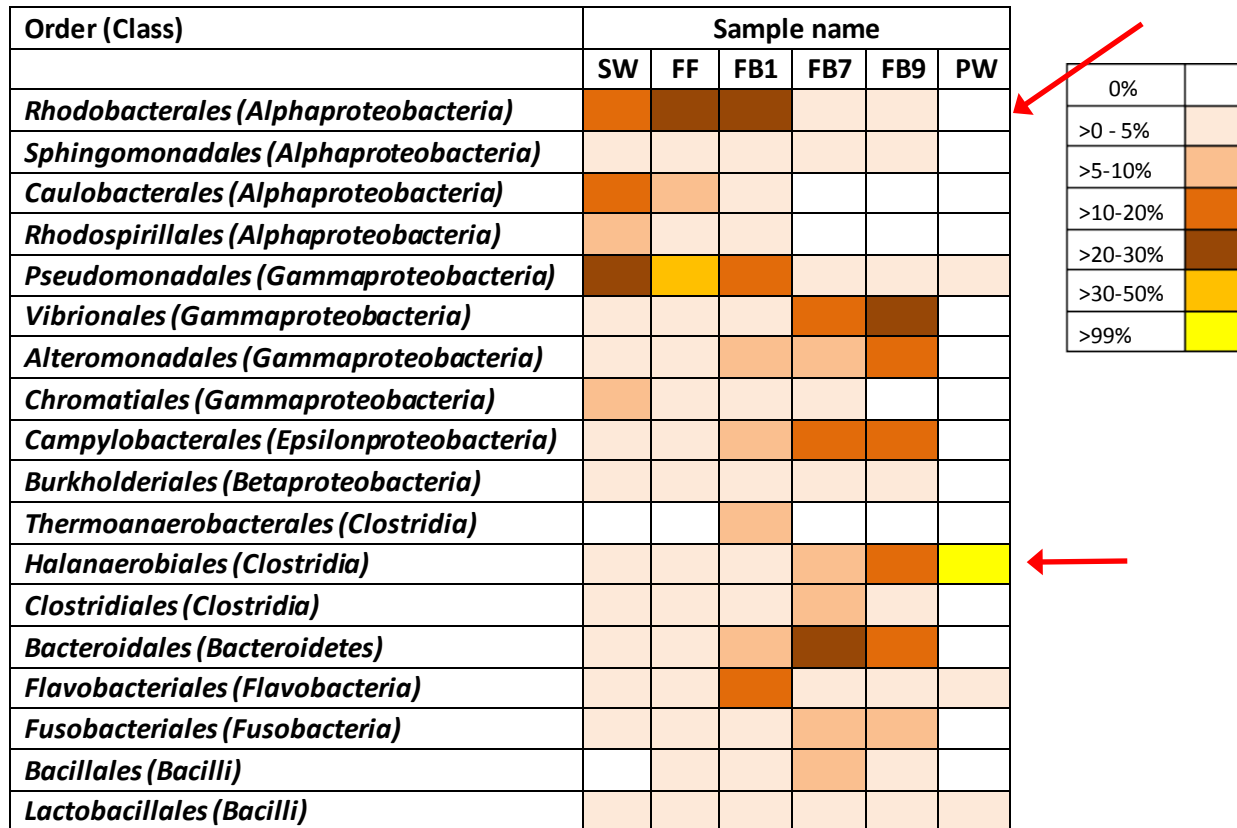
## Pyrosequencing



## Clone Library



# Graphical Relative Abundance of Important Orders at the Wellheads



# Impoundments





# Results in the Impoundments

## Chemical Constituents of Water Samples (mg/L)

Element	Untreated			Biocide Amended			Treated-Aerated		
	Surface	Middle	Bottom	Surface	Middle	Bottom	Surface	Middle	Bottom
Ba <sup>2+</sup>	277	339	418	63	175	228	10	9.5	9.5
Ca <sup>2+</sup>	6150	8818	8679	7526	10790	8167	5780	5733	5253
Total Fe	0.3	4.4	64.9	21.6	14.9	14.2	0.1	0.1	0.1
K <sup>+</sup>	190	224	261	351	410	420	161	188	201
Na <sup>+</sup>	14250	20910	20410	19930	26500	17590	12810	12940	11120
Sr <sup>2+</sup>	894	1296	1256	1058	1493	1082	800	803	707
Cl <sup>-</sup>	35100	51000	50900	43500	55400	56800	32300	32100	32500
Br <sup>-</sup>	371	552	549	461	597	615	345	344	346
I <sup>-</sup>	5.6	9.8	10.3	7.2	9.1	9.3	4.4	4.4	4.4
NO <sub>3</sub> <sup>-</sup>	ND <sup>b</sup>	ND	ND	ND	ND	ND	ND	ND	ND
SO <sub>4</sub> <sup>2-</sup>	15.1	25.7	16.5	43.7	49.9	37.3	234	234	236
Acetate	ND	32.6	75.9	39.4	46.5	62	ND	ND	ND

# Bacterial Communities in Impoundments

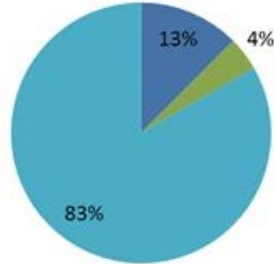
Untreated

Biocide Amended

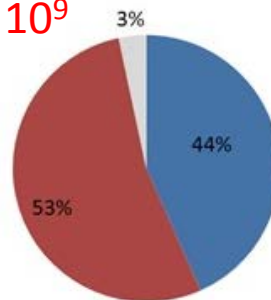
Aerated

Surface

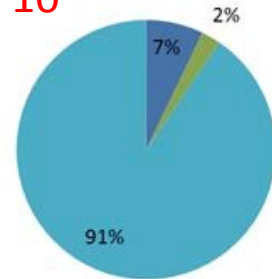
$10^5$



$10^9$



$10^6$



Taxonomic Classification

$\gamma$ -proteobacteria

Clostridia

Bacteroidetes (Phylum)

$\alpha$ -proteobacteria

$\delta$ -proteobacteria

Thermotogae

Synergistia

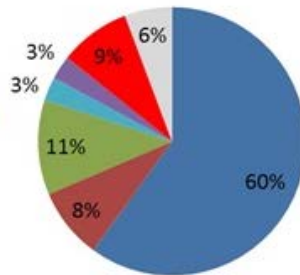
Spirochaetes

Planctomycetes (Phylum)

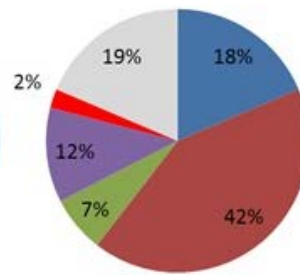
Other

Middle (1.5m)

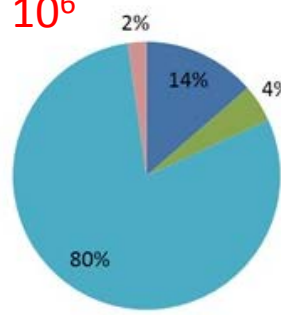
$10^7$



$10^9$

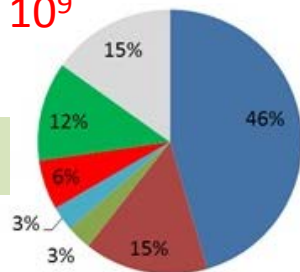


$10^6$

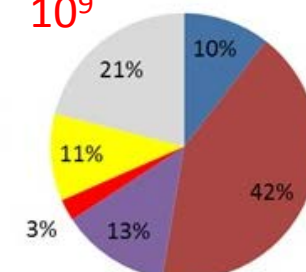


Bottom (3.5m)

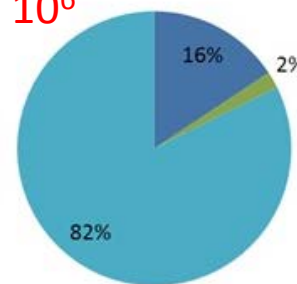
$10^9$



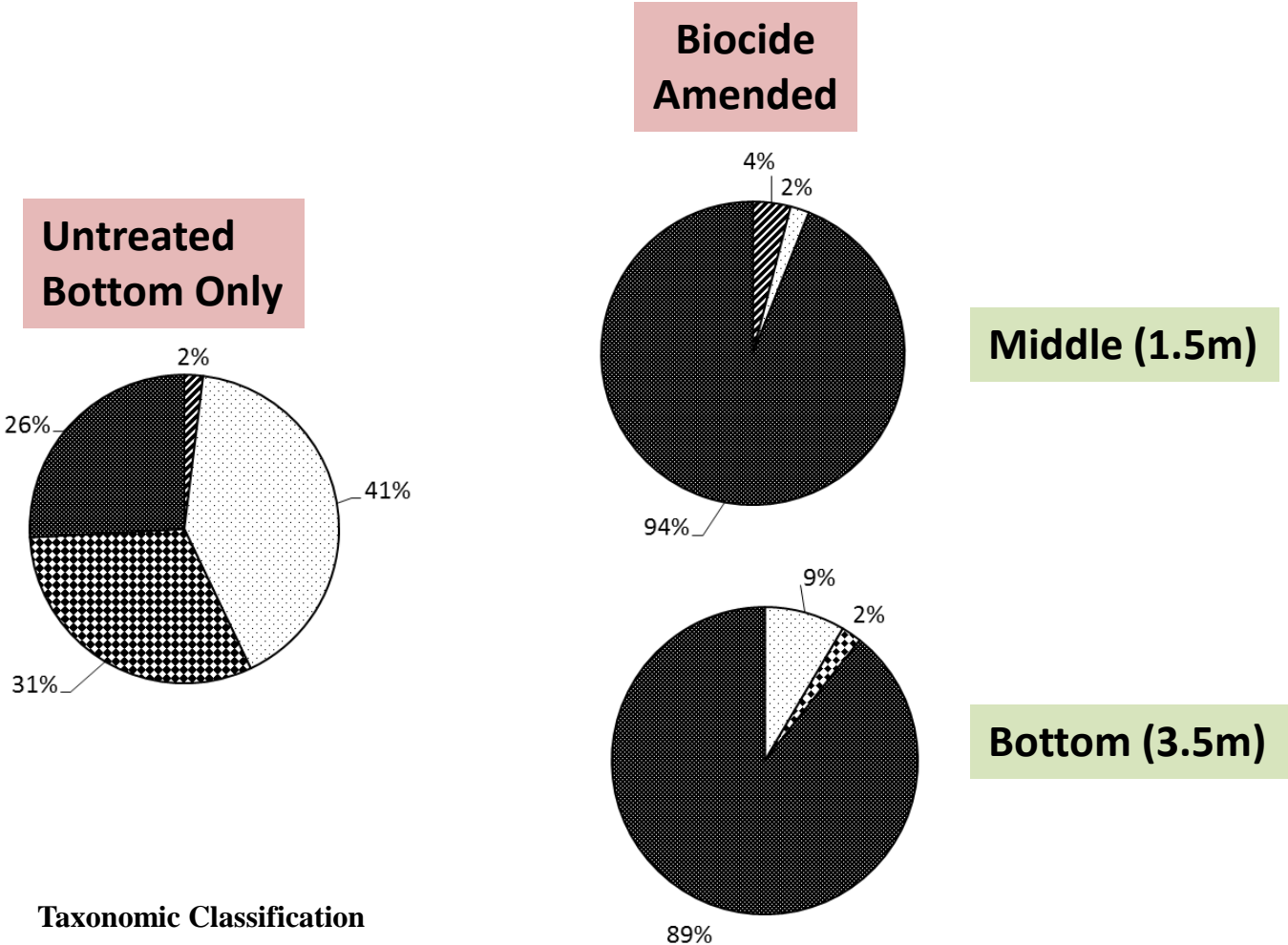
$10^9$



$10^6$



# Methanogenic Communities in Impoundments



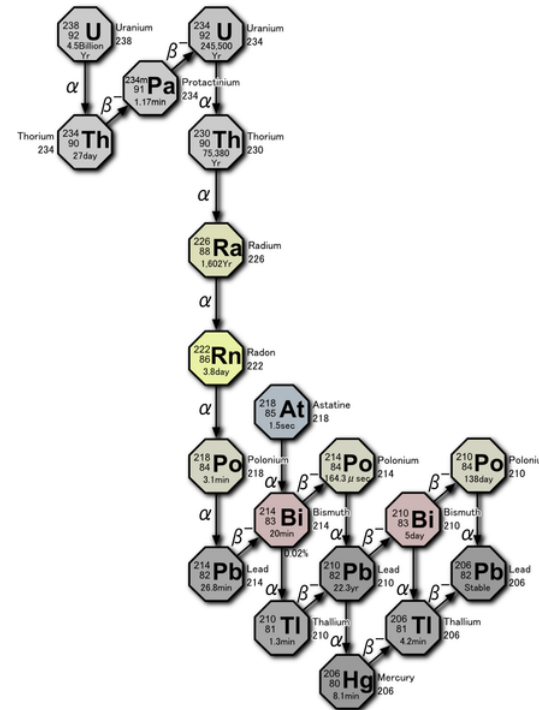
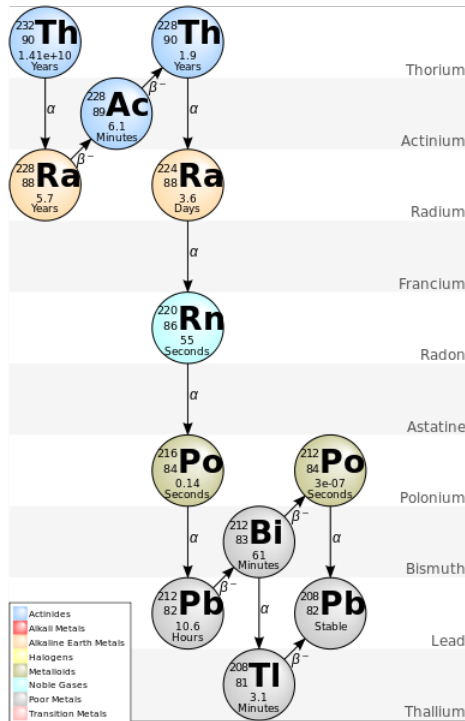
### Taxonomic Classification

- ▨ *Methanocalculus*
- *Methanoplanus*
- ▩ *Methanolobus*
- *Methanohalophilus*

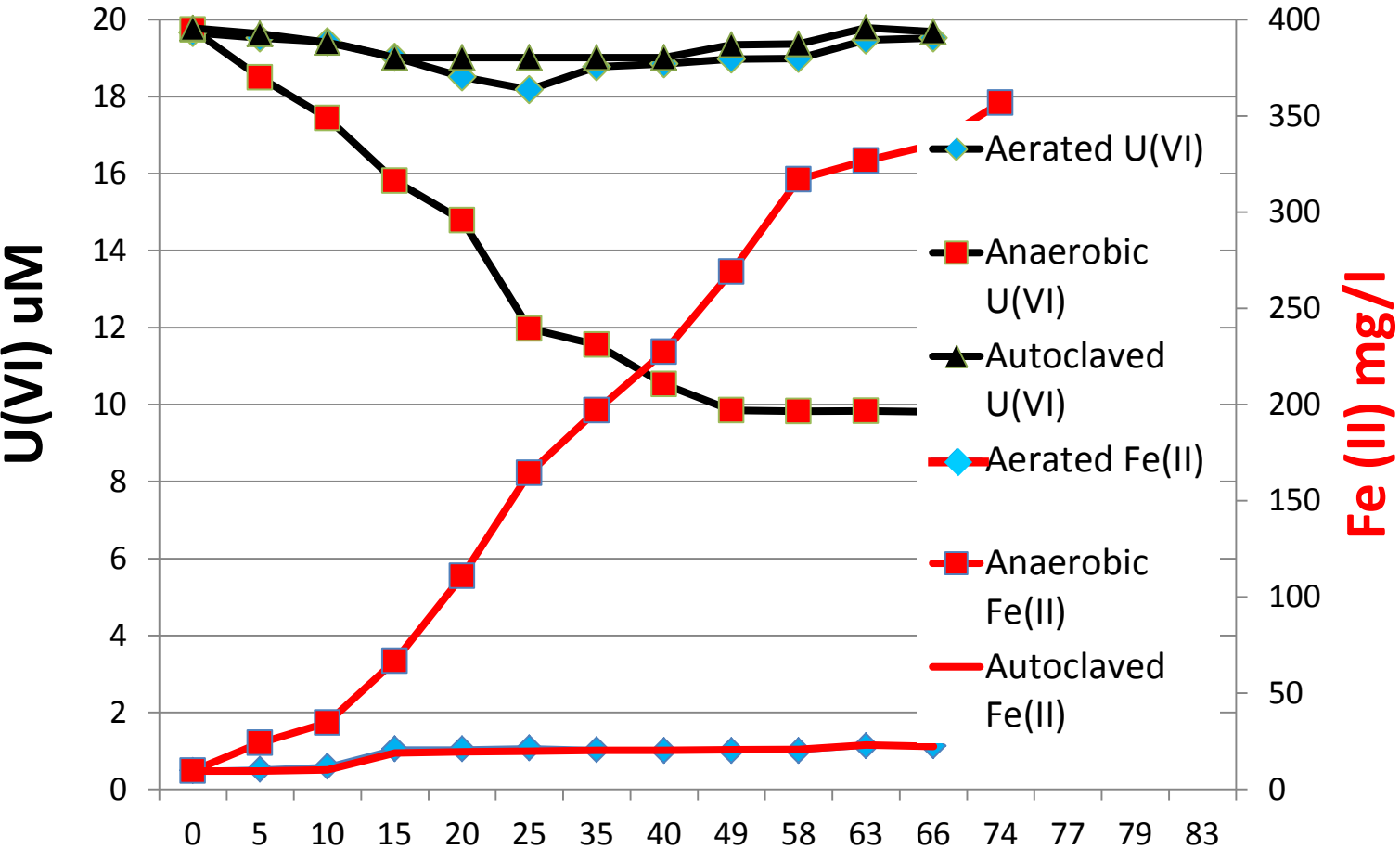
# Species-level Information on Bacteria

OTU name <sup>a</sup>	No. of seq.	Class	Species	Info, Isolation source	Untreated			Biocide amended			Pretreated-aerated		
					Surf.	Mid.	Bot.	Surf.	Mid.	Bot.	Surf.	Mid.	Bot.
s1sur_18p	131	α-proteobacteria	Roseovarius sp (99%)	Iodide-oxidizing oil brine	75%	3%					74%	68%	65%
s2sur_7A	51	Clostridia	Halanaerobium congolense sp. (99%)	Anaerobic Fermentative Sulfur reducing Oil field		5%	3%	53%	42%	37%			
s1mid_11H	34	γ-proteobacteria	Marinobacterium sp. (99%)	Hydrocarbon oxidizing, Sulfide oxidization Oil field produced fluid		52%	42%						
s2sur_9A	19	γ-proteobacteria	Marinobacter hydrocarbonoclasticus sp. (99%)	Hydrocarbon oxidizing Sea water	2%			30%	16%	5%			
s2bot_7C	17	Uncultured bacteria	Uncultured bacterium (93%)	Sediment, Japan sea		3%	9%		14%	18%			
s2bot_6D	7	δ-proteobacteria	Desulfobacter halotolerans sp.(99%)	Sulfur-reducing Great salt lake (NR_026439)					9%	8%			
s2bot_3A	7	Thermotogae	Thermotogae/Geotog a petraea sp.(99- 100%)	Oil well (EU721761, HM037999)		8%	6%		2%	3%			
s1bot_10C	4	Spirochaetes	Spirochaetes sp. (99- 100%)	Oil well and produced fluids (GU179808, HM041923, AY800103)			12%						
s2bot_10B	4	Synergistia	Aminobacter colombiense sp. (90%)	Wastewater plant (CP001997)						11%			
<b>Combined minor OTUs (OTUs that represent on average &lt;5% of bacterial population)</b>					<b>21%</b>	<b>29%</b>	<b>28%</b>	<b>17%</b>	<b>17%</b>	<b>18%</b>	<b>21%</b>	<b>27%</b>	<b>27%</b>

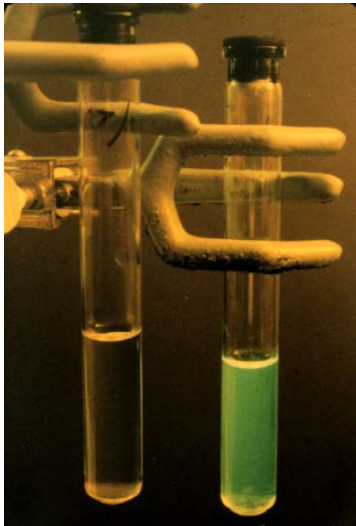
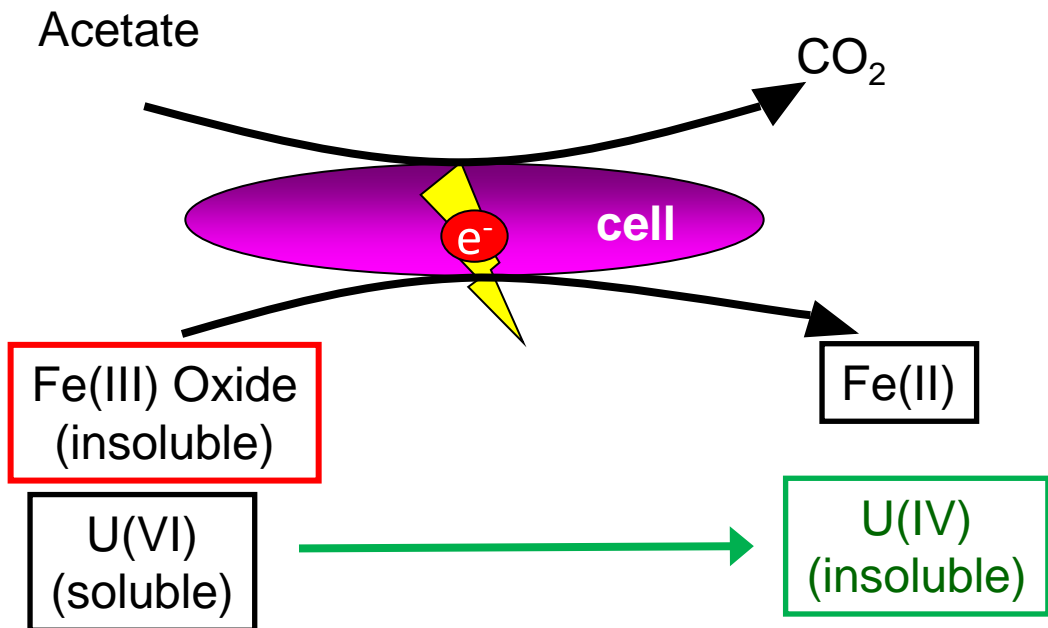
# Fate of NORM



# Impoundments: Fate of NORM Linked to Microbiology

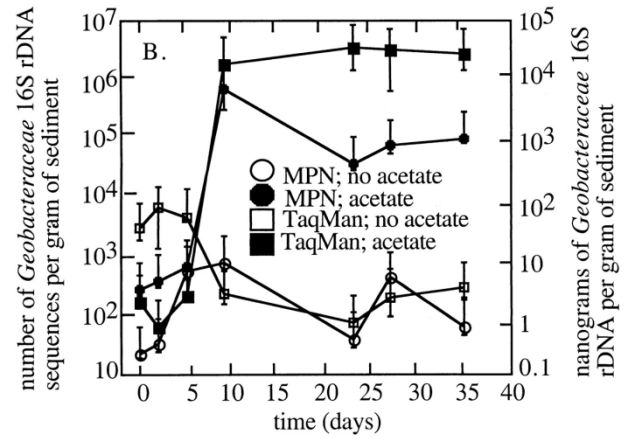
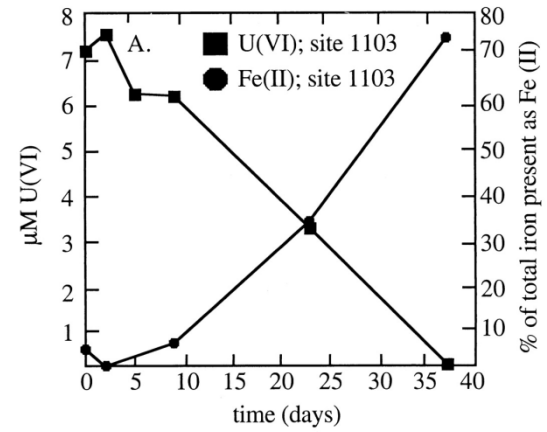


# Microbial Respiration of Iron and Uranium Precipitation



Lovley DR, et al. Nature (1991)

Iron reduction and uranium reduction coupled to growth of iron-reducing bacteria



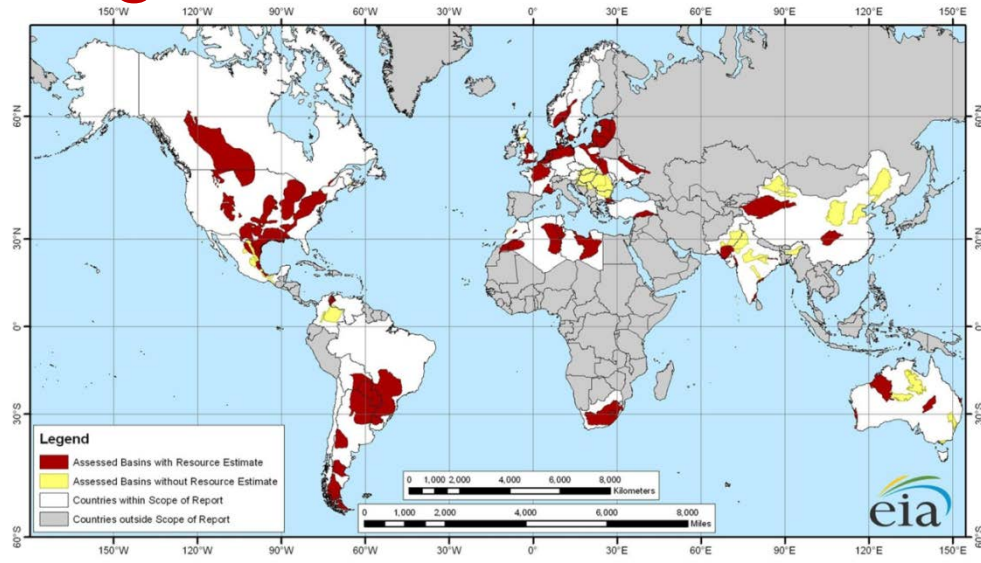
Holmes et al Appl Environ Microbiol (2002)

# Impoundments: Community independent of treatment

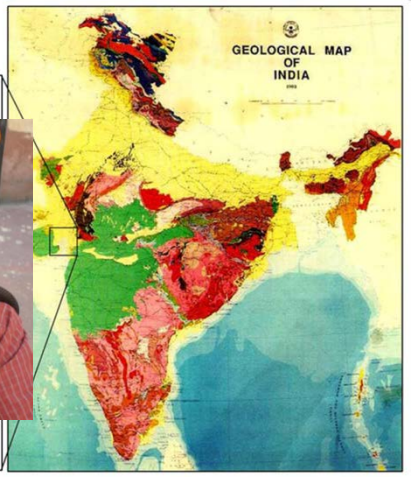
Species <sup>b</sup>	Isolation source and Accession no.	Source water (Day 0)	Anaerobic Reactor		Aerobic reactor Day 66
			Day 16	Day 66	
<i>Halomonas taeanensis</i> isolate RF32 (99%); <i>Halomonas taeanensis</i> strain NY-3 (99%)	Hypersaline lake (HE655447); Saltern (JN903897)	58%	76%	69%	68%
<i>Marinobacter hydrocarbonoclasticus</i> strain P721(1) (99%)	Northern China sea (GU370116)	18%	13%	17%	23%
<i>Halomonas alimentaria</i> T8-28M (97-98%); <i>Halomonas fontilapidosi</i> strain R086 (97-98%)	Salt from saltern (AB617543); Solar saltern lake (HM179201)	12%	4%	6%	2%
<i>Idiomarina ramblicola</i> YCSA51-1(99%); <i>Idiomarina loihiensis</i> isolate RB2 (99%)	Salt farm sediment (GQ131634); Hypersaline lake (HE655437)	8%	6%	8%	2%
Other bacterial species that represented minority of the bacterial community		5%	1%	1%	4%



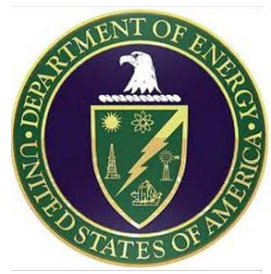
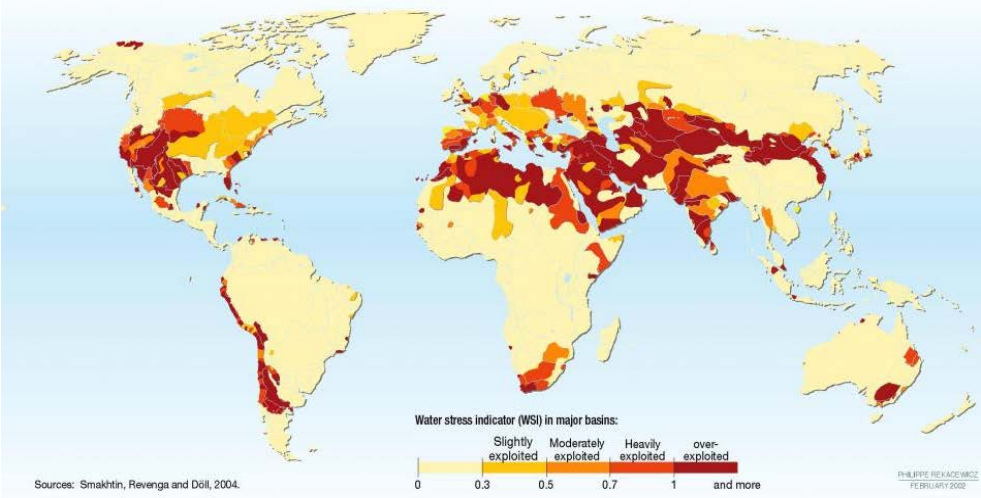
# Final Note Global Shale Plays in Water Stressed Regions



Cambay Basin



Water management problems are local  
Solutions arise locally but have global impacts.



# Disclaimer

**Disclaimer:** This project was funded by the Department of Energy, National Energy Technology Laboratory, an agency of the United States Government, through a support contract with URS Energy & Construction, Inc. Neither the United States Government nor any agency thereof, nor any of their employees, nor URS Energy & Construction, Inc., nor any of their employees, makes any warranty, expressed or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.