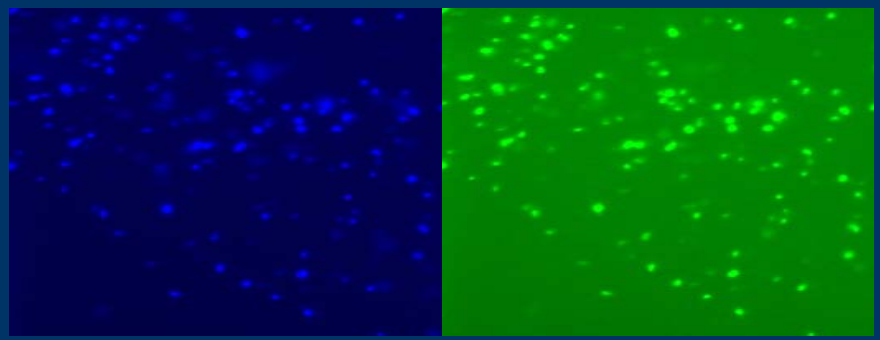
Enzyme Activity Probes



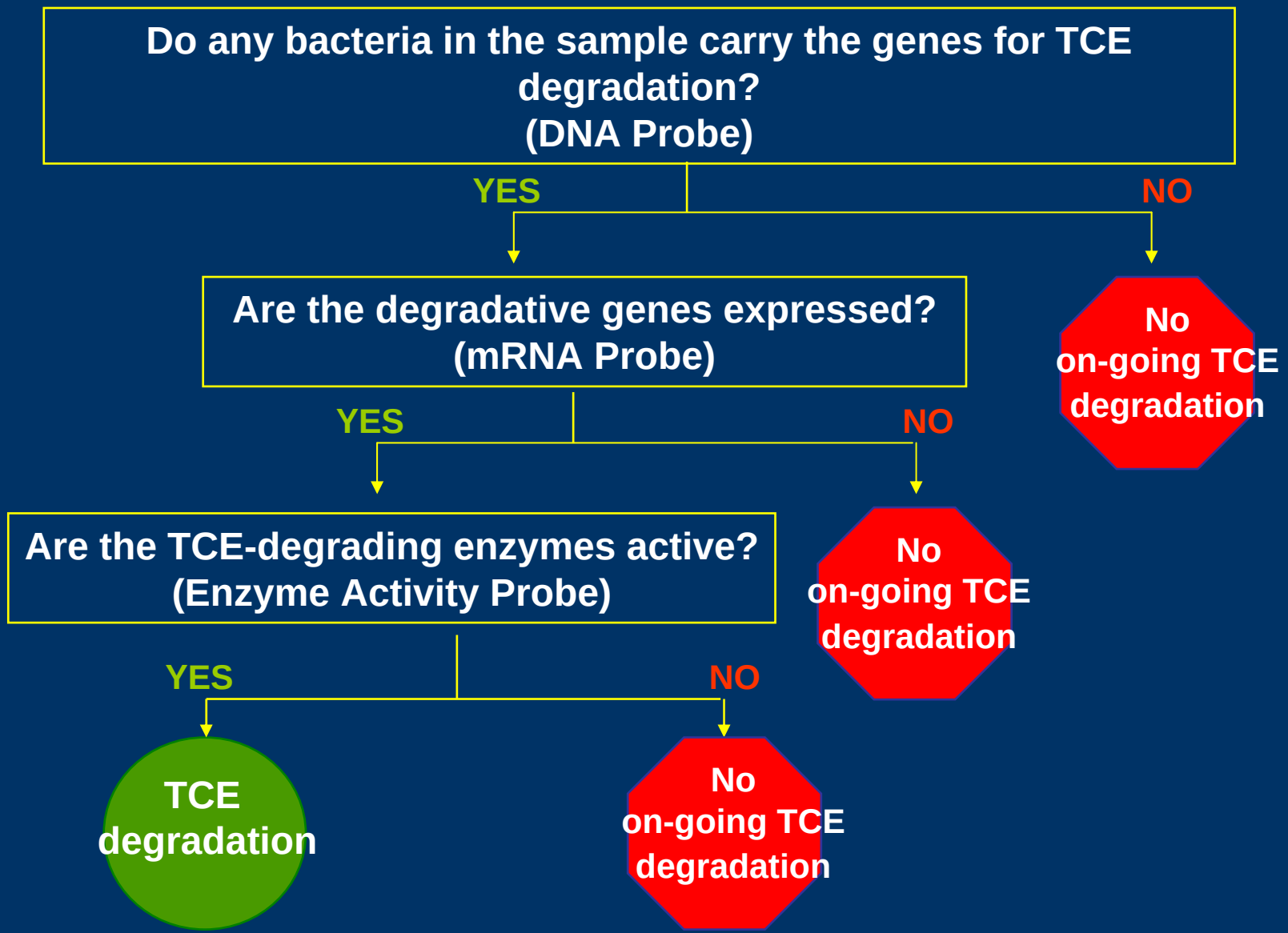
M. Hope Lee

North Wind

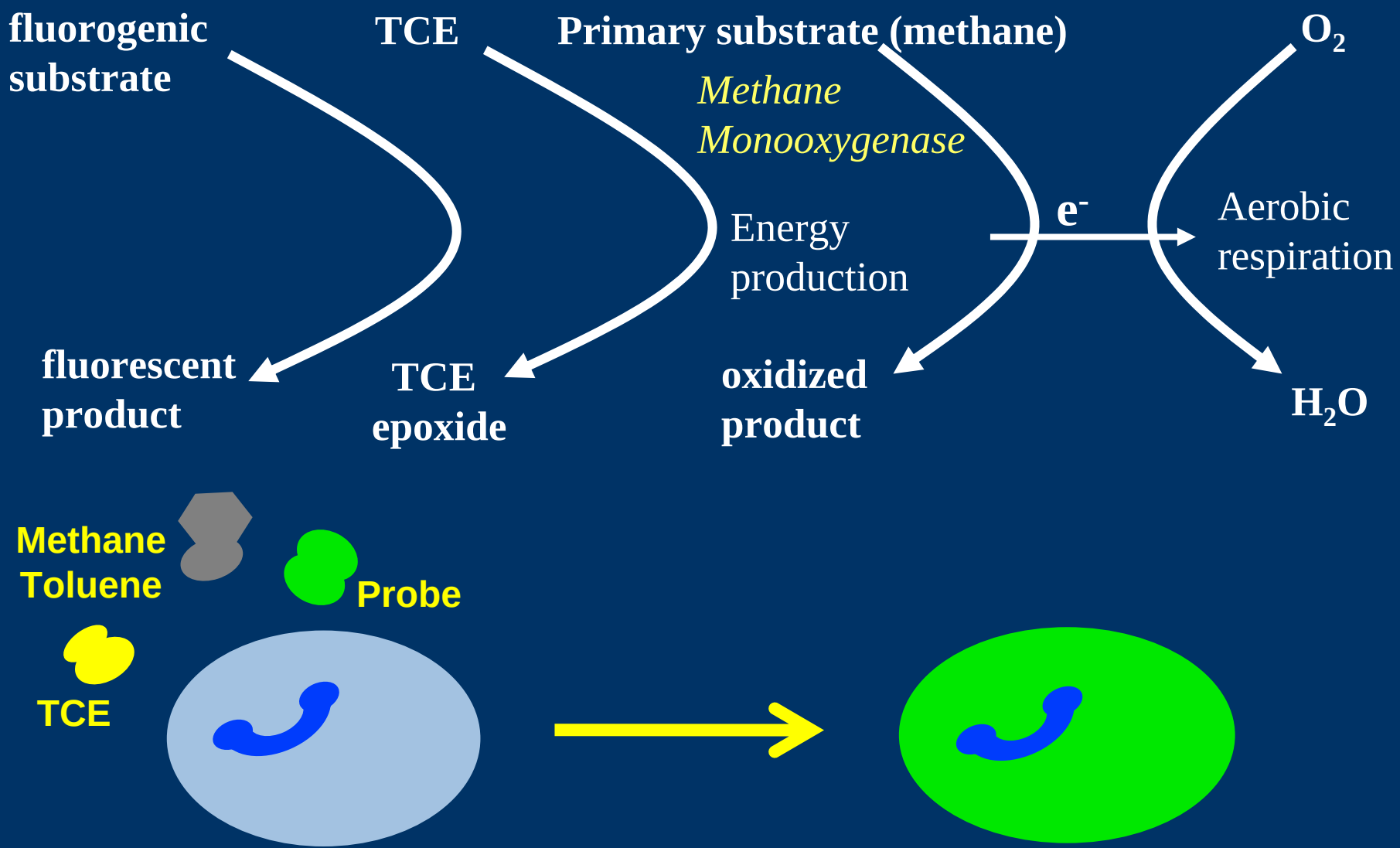
Biodegradation / Bioremediation of TCE

- TCE can be degraded by bacteria
- Several different mechanisms, including anaerobic reductive dechlorination and *aerobic cometabolic oxidation*
- Bioremediation technology can be based on microbial degradation capacity
- Tools are *needed* to detect appropriate enzyme systems and assess their *activity* in the environment

Why are Enzyme Probes Important?



How do enzyme activity probes work?



Common Myths, dismissed…

Early studies (1980-mid-90s) determined that:

(a) Cometabolism requires a natural substrate induction (methane, phenol, other aromatic)

(b) Cometabolic degradation of TCE results in TCE epoxides and/or oxygen radicals which inactivate the active site of the oxygenase

(c) Growth on non-inducing substrates will result in an enzyme that will not cometabolize chlorinated solvents (TCE)

Recent studies have shown that under natural conditions:

(a) Non-aromatic substrates can induce activity (naturally occurring phenolic compounds e.g. humics); TCE itself can induce cometabolic activity

(b) Studies have shown that TCE epoxides do not cause significant decreases in TCE cometabolizing abilities or rates

(c) Growth on non-inducing substrates results in TCE degradation

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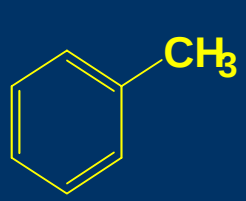
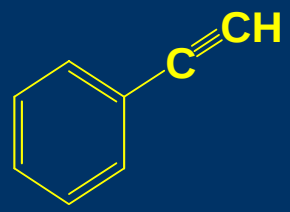
Heald and Jenkins, 1994; McClay et al., 1995; Leahy et al., 1996; Shingleton et al., 1998; Ryoo et al., 2000, 2001; Lee et al., 2002; Yeager et al., 2004

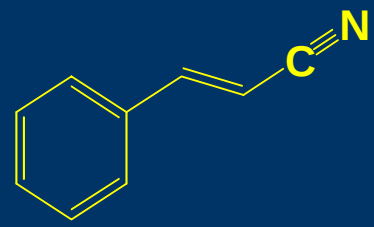
Representative Enzyme Probes and Target



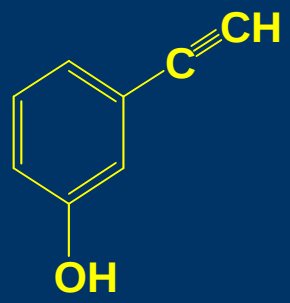
toluene
(natural
substrate)



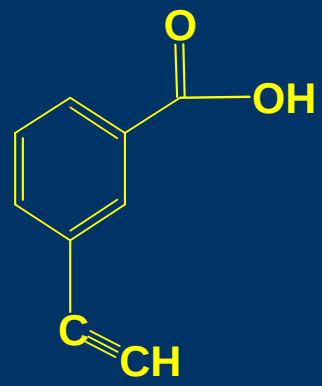
phenylacetylene
(2,3-TDO)



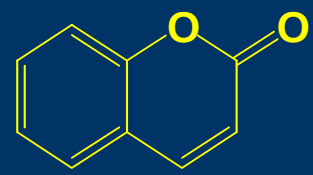
trans-cinnamonitrile
(2,3-TDO)



3-hydroxy-phenylacetylene
(2-TMO)

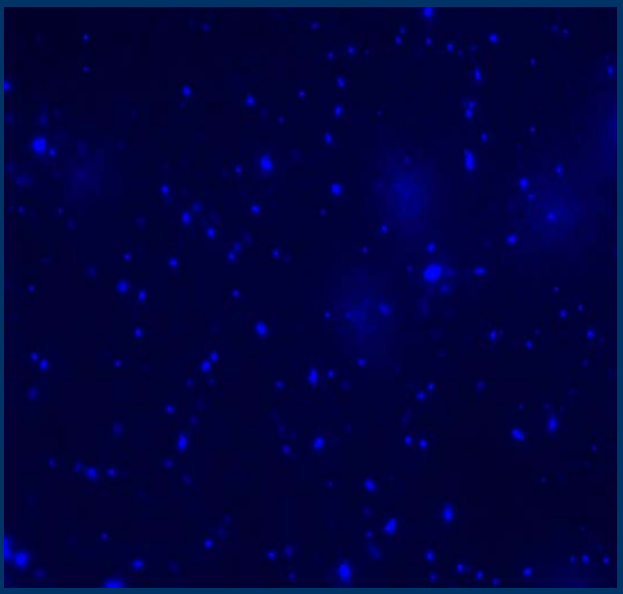


3-ethynyl-benzoate
(side chain MO)

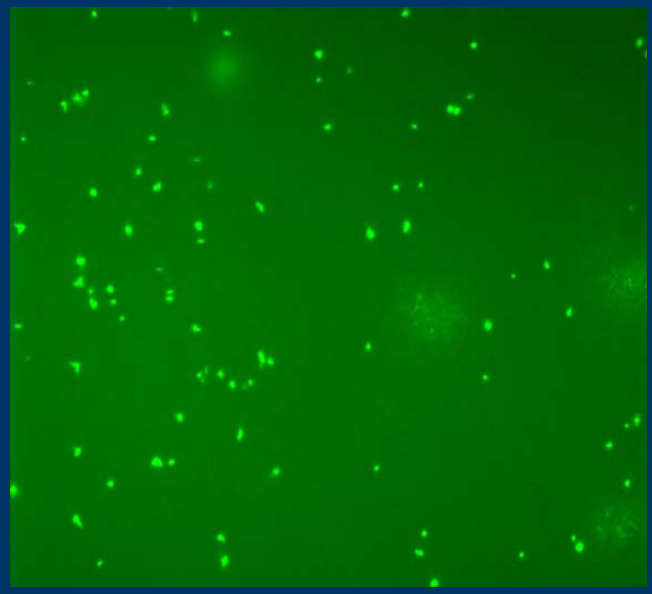


coumarin
(sMMO)

Enzyme Activity Probes: groundwater



Total bacterial cell count



Positive probe response

Negative probe response

1. Serve as alternative substrates for TCE cometabolizing enzymes
2. Are transformed by enzymes into a quantifiable product, i.e. direct evidence of activity.
3. Represent one of only a few technologies that have the capability of measuring *activity in situ*

Control Assays

Purpose: To ensure that the measured degradation is attributable to the organisms of interest; *verify that either the sMMO or toluene enzymes are responsible for any observed positive response to the assay.*

(a) Acetylene: irreversible inhibitor of sMMO
(b) Methane: competitive reversible inhibitor
(c) 1-pentyne (3.5%): irreversible inhibitor for the 2-monooxygenase pathways
(d) 3-hexyne (2%): irreversible inhibitor for the 3-and 4-monooxygenases
(e) Phenylacetylene (10-15%): dioxygenase
(f) DNA…

Additional Control Assays

To offer supporting evidence for the enzyme activity probes.

PCR *characterize the potential of the microbial community*

TOD: toluene 2,3-dioxygenase
TOL: xylene monooxygenase
RMO: toluene-3,-4-monooxygenase
PHE: toluene-2, -3, -4-monooxygenase
sMMO: mmoX (f882 & r1403)
Universal: (8F and 907R).

FISH *characterize the activity of the microbial community*

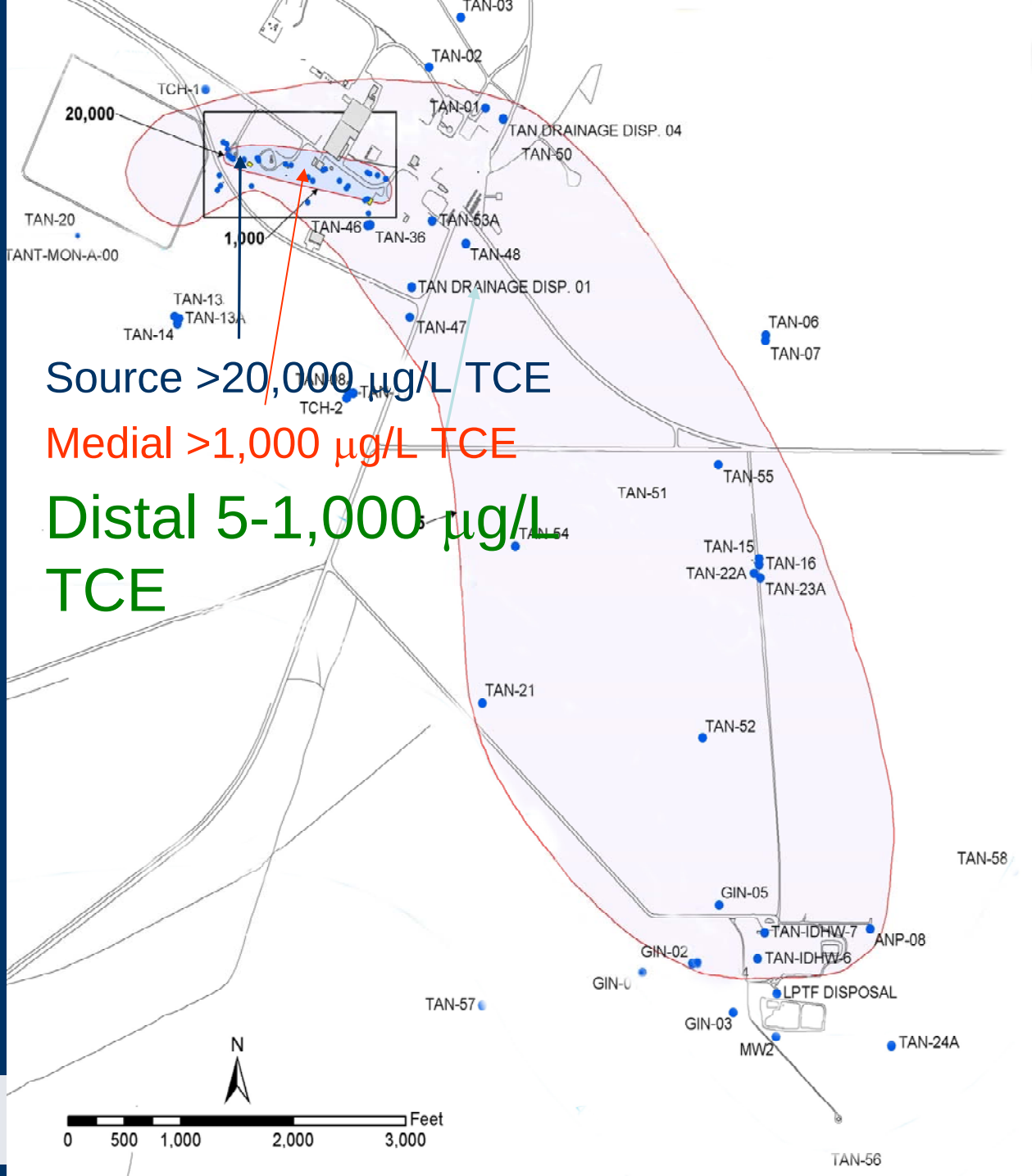
Eubacteria
Cytophaga-Flavobacterium (most common toluene degrading organisms), type I and type II methanotrophs
component B of the sMMO

Test Area North Background

- Past waste injections into the deep, fractured basalt aquifer have resulted in a nearly 2-mile long TCE plume at the Test Area North (TAN) facility of the Idaho National Laboratory (INL).

- 1995 ROD selected 30 years of pump and treat as the default remedy, but allowed for innovative technology evaluation.

- Monitored natural attenuation was evaluated as a remedy for the distal zone of the plume.


Source >20,000 µg/L TCE
Medial >1,000 µg/L TCE
Distal 5-1,000 µg/L TCE
20,000
1,000
TAN-03
TAN-02
TCH-1
TAN-01
TAN DRAINAGE DISP. 04
TAN-50
TAN-20
TANT-MON-A-00
TAN-46
TAN-36
TAN-53A
TAN-48
TAN DRAINAGE DISP. 01
TAN-13
TAN-13A
TAN-14
TAN-47
TAN-06
TAN-07
TCH-2
TAN-55
TAN-51
TAN-54
TAN-15
TAN-16
TAN-22A
TAN-23A
TAN-21
TAN-52
TAN-58
GIN-05
TAN-IDHW-7
ANP-08
GIN-02
TAN-IDHW-6
LPTF DISPOSAL
TAN-57
GIN-03
MW2
TAN-24A
TAN-56
N
0
500
1,000
2,000
3,000
Feet

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MNA Field Evaluation

- Studied all attenuation mechanisms for TCE in groundwater
  - Anaerobic reductive dechlorination
  - Aerobic cometabolism
  - Non-degradative mechanisms (e.g. dispersion)

Indirect Evidence: Aerobic TCE Degradation

- TCE concentrations decrease with distance from the source area in relation to PCE and tritium with a half-life of 9-21 years.
- A numerical model generates a plume that more closely matches field data when the model incorporates a TCE degradation term.
- Laboratory studies have shown that organisms capable of aerobic cometabolic oxidation of TCE are native to TAN.

Summary of MNA Field Evaluation

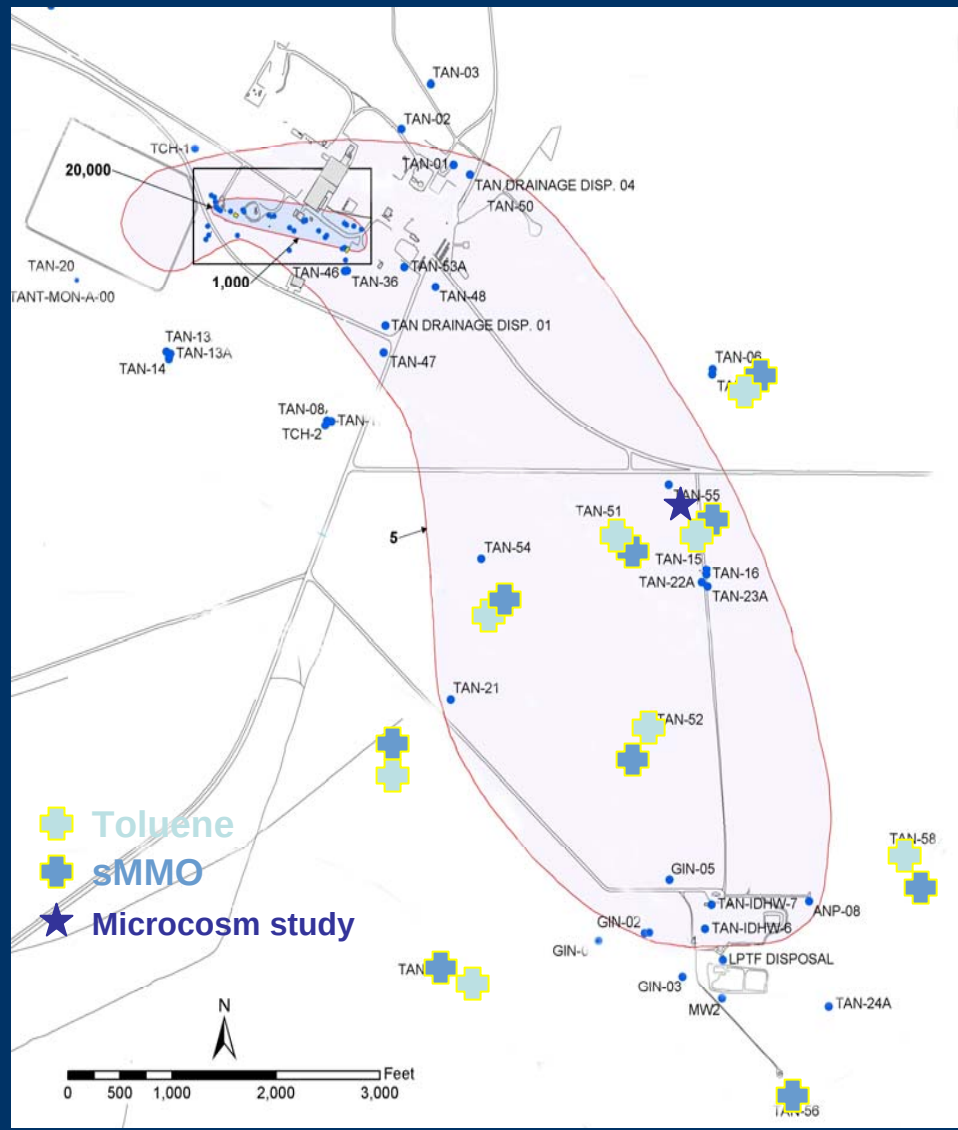
- The multiple lines of indirect evidence showed that TCE degradation was occurring and suggested that the mechanism was aerobic cometabolic oxidation.
- This led to the selection of MNA as the remedy for the distal portion of the plume (DOE-ID, 2001).
- However, direct evidence for the actual degradation mechanism was needed…

Field application of probes

2001: 4 wells sampled: 3 inside the plume, 1 outside

2002: 6 wells sampled: 3 inside the plume, 3 outside

- sMMO
- All ‘toluene’ probes
- Controls



FLUTe/Enzyme Probe Sampling Strategy

- Sample all depths in each of the five FLUTe liners to generate water chemistry profiles
- Collect triplicate enzyme probe samples at two non-FLUTe wells
- Collect enzyme probe and/or DNA samples from three discrete intervals in three of the five FLUTe wells

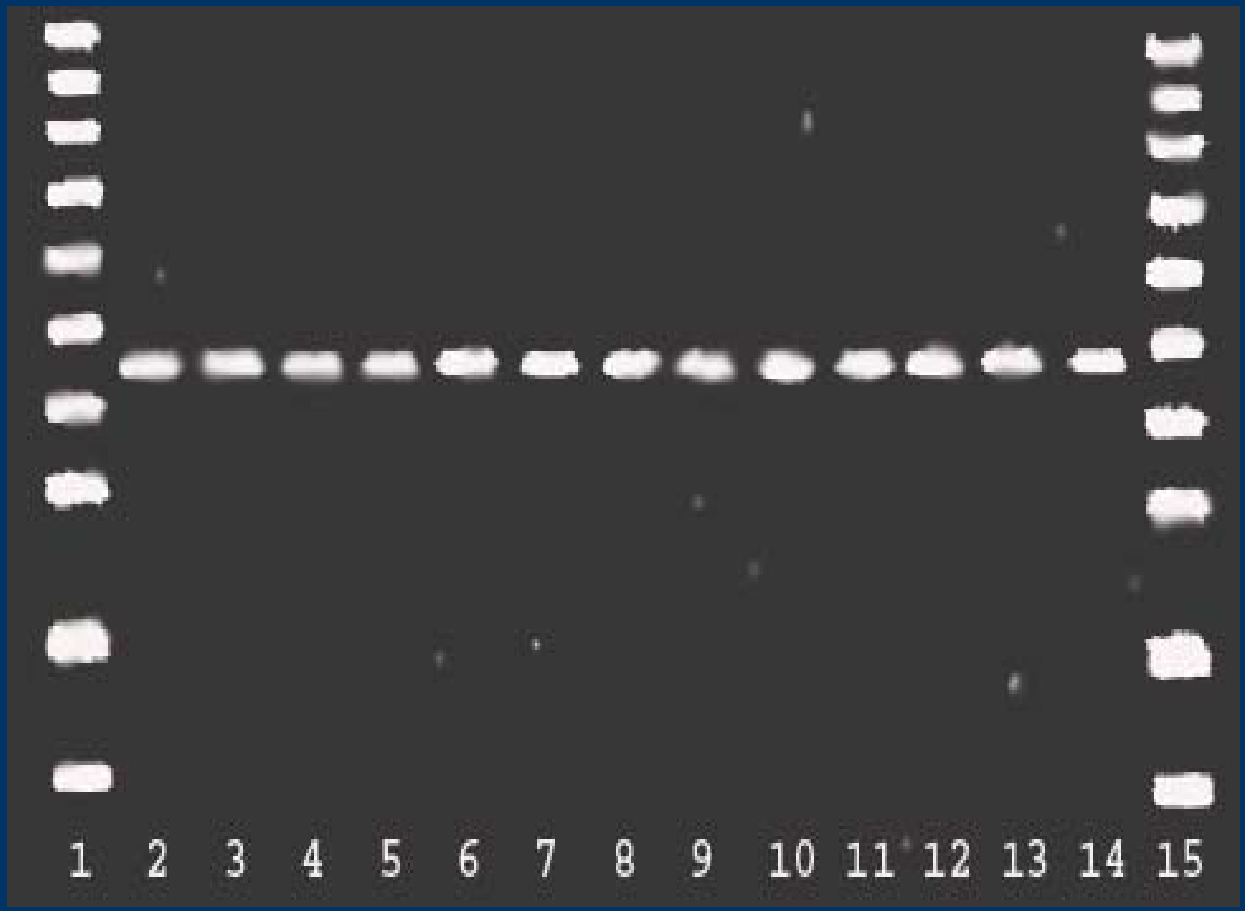
9/11/02	TAN 7	Filter 1, 50L		Filter 2, 20 L		Filter 3, 60L	
eb		0	2.8E+04	6	4.E+05	55	5.5E+05
hpa		79	1.1E+06	34	5.E+05	15	8.4E+05
cinn		70	7.8E+05	63	3.E+05	0	4.7E+05
pa		76	4.3E+04	49	4.E+05	63	4.8E+05

9/16/02	TAN 55	317 FT		424 FT		461 FT	
eb		60	2.5E+05	53	3.7E+05	50	8.7E+05
hpa		20	2.4E+05	10	4.0E+05	49	4.8E+05
cinn		20	2.4E+05	39	5.8E+05	16	4.8E+05
pa		33	4.7E+05	24	2.7E+05	48	5.3E+05

9/23/02	TAN 52	266 FT		373 FT		456 FT	
eb		0	3.4E+05	0	3.0E+05	0	7.3E+05
hpa		0	3.0E+05	0	2.8E+05	0	2.8E+05
cinn		0	1.8E+05	0	3.6E+05	0	2.3E+05
pa		13	2.9E+05	18	3.6E+05	14	1.2E+05

9/25/02	TAN 51	263 FT		342 FT		460 FT	
eb		0	4.8E+05	0	1.4E+06	0	5.2E+05
hpa		10	2.7E+05	32	7.5E+05	0	3.4E+05
cinn		0	2.7E+05	7	8.8E+05	0	3.8E+05
pa		4	3.7E+05	4	1.2E+06	0	7.0E+05

Amplification of sMMO gene directly from TAN groundwater

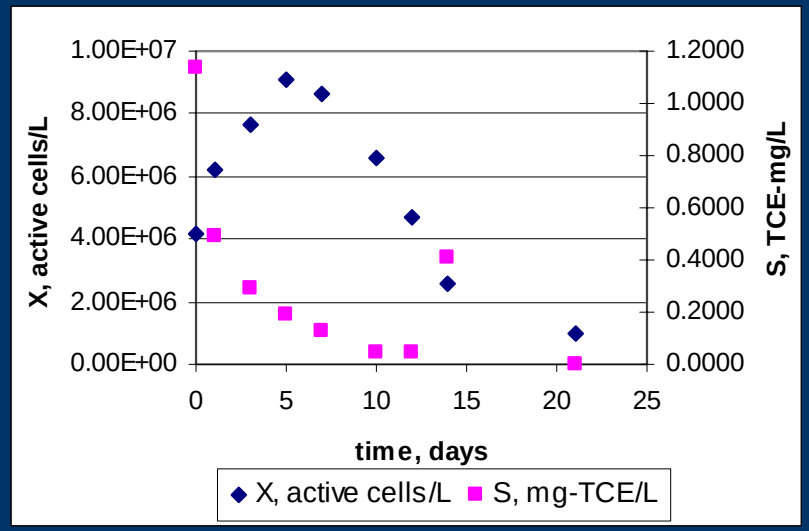


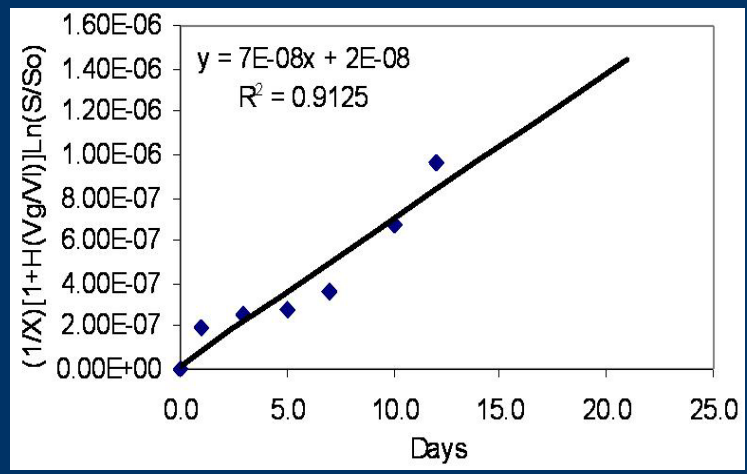
1,15	Ladder
2	9/11 #1
3	9/11 #2
4	9/11 #3
5	9/16 #1
6	9/16 #2
7	9/16 #3
8	9/23 #1
9	9/23 #2
10	9/23 #3
11	9/25 #1
12	9/25 #2
13	9/25 #3
14	Ob3B

Results of two-year study

- *In situ* enzyme activity measurement, coupled with genetic assessment, demonstrates methanotrophic and toluene-oxygenase activity at TAN
- sMMO and toluene oxygenase activity were noted for both wells both inside and outside of TCE plume
- *Based on this two year study, our results confirm that the degradation mechanisms includes aerobic cometabolism by indigenous subsurface microbial communities*

- Distal aerobic portion of the Test Area North TCE plume, Idaho (<100 μg L^{-1}); simultaneously measured TCE degradation and enzyme probes over three week period
- First order decay previously described and validated; Unique attribute of the work described is the replacement of the total concentration of cells (x) with active cells.
- Half-life determined 22.3 years (compared to 25 yr relative to PCE and 13 yr relative to tritium based on tracer-corrected method)





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