Biodegradation of Ether-Containing Pollutants

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A presentation to the Federal Remediation Technologies Roundtable
December 6, 2006

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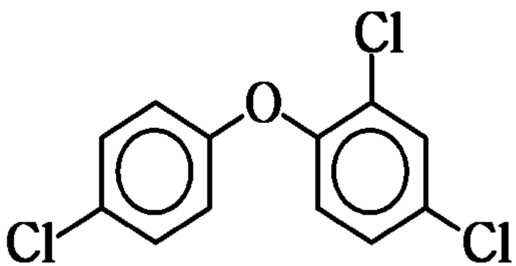
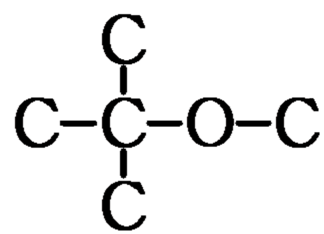
To Protect The Innocent, It Is Hereby Duly Stated That The Opinions Expressed During This Talk Are Those Of The Speaker Alone.

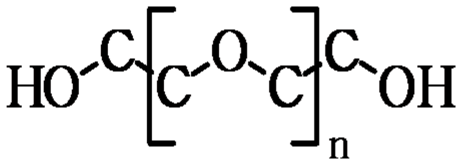
Examples of Widely Used Ethers



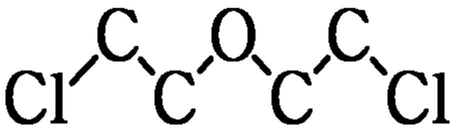
Triclosan



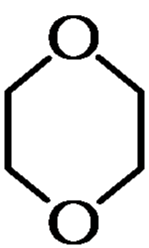
MTBE



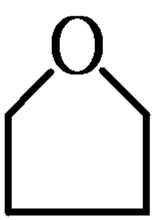
Polyethylene glycol



bis-2-chloroethylether



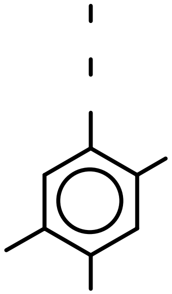
1,4-Dioxane



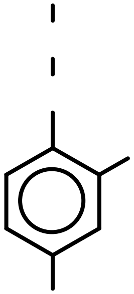
Tetrahydrofuran

Another Example

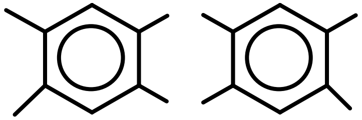
Agent Orange



2,4,5-T



2,4-D

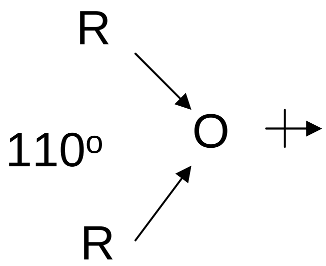


2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD

- Mimics plant hormone
- Induces uncontrolled plant growth-death
- ~20 million gallons sprayed on Vietnam, Cambodia, and Laos
- Defoliate and decrease food supply
- TCDD contamination caused cancers etc.

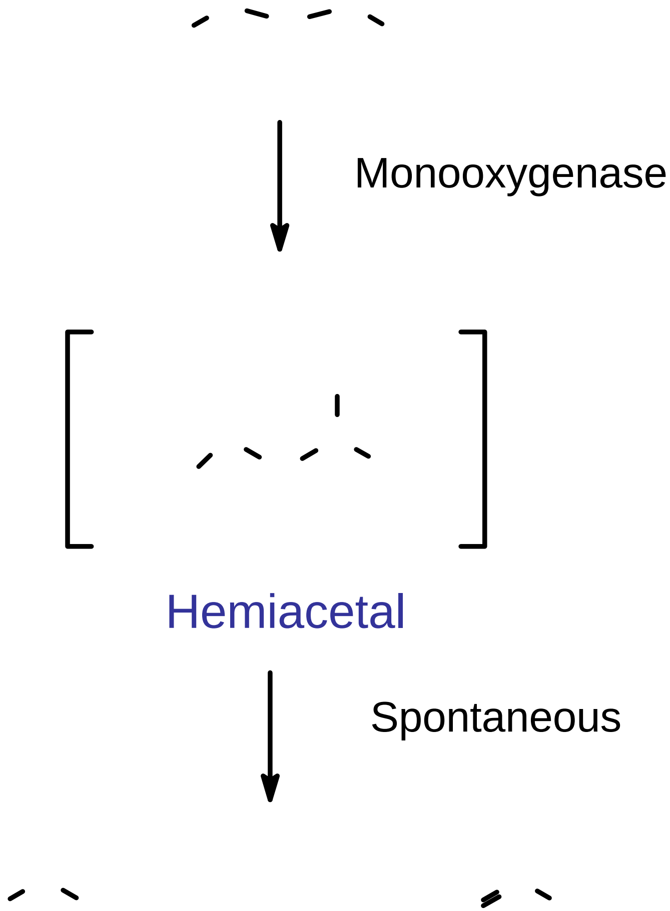
Ether Background

- R-O-R
  - R groups Aliphatic or Aromatic
  - Symmetrical or Asymmetrical
  - Linear or Cyclic
- Bond Angle of 110°
  - Weak Polarity (Small Net Dipole Moment)
  - Solubility Comparable to Alcohols
  - Boiling Point Comparable to Alkanes
  - No Hydrogen Bonding Like Seen in Alcohols
- Relatively Non-Reactive
  - Chemical Stability
  - Biological Recalcitrance



Common Alkyl Ether Degradation Mechanism

O-dealkylation

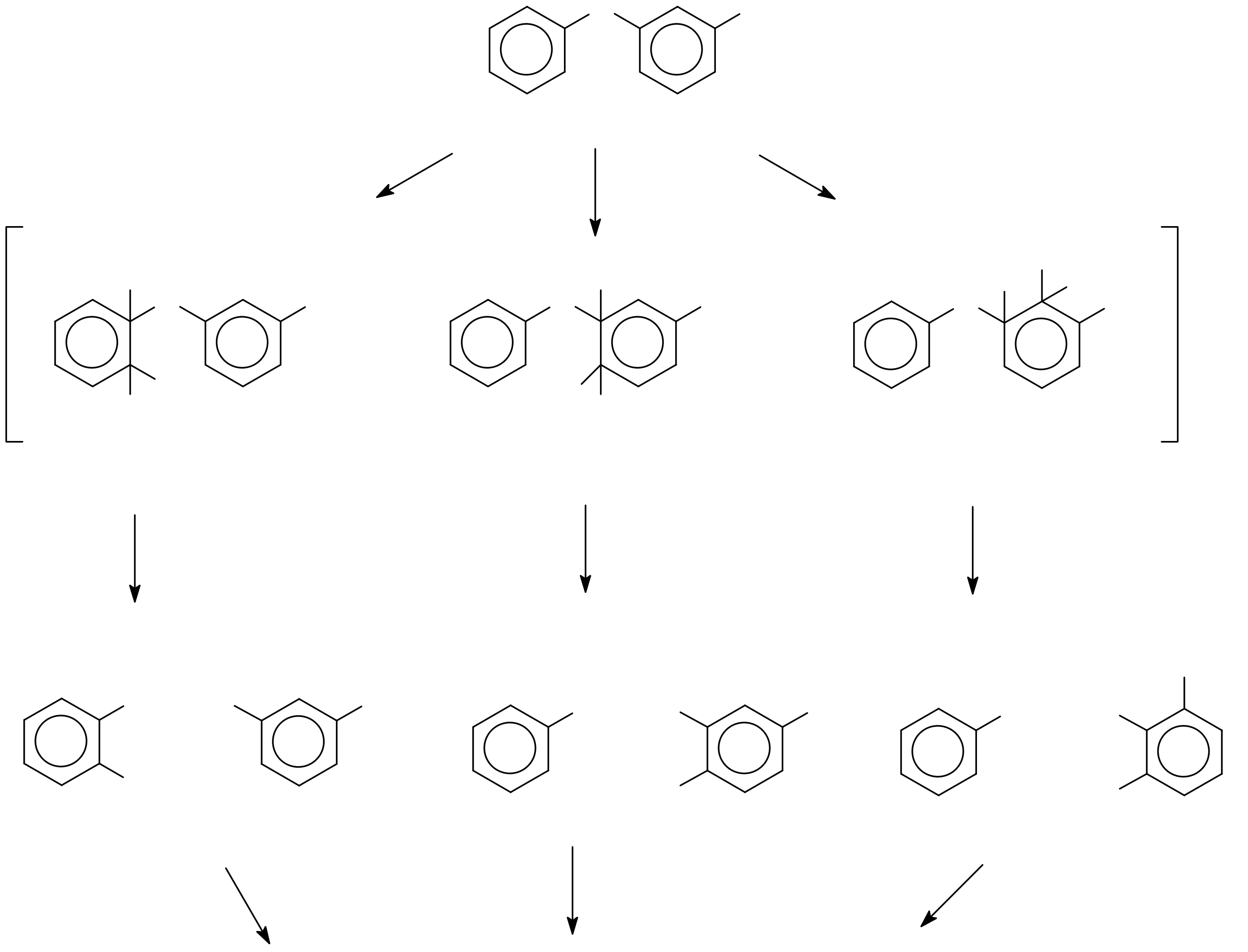


Alcohol   Aldehyde

- Aryl-O bond stronger that alkyl-O, therefore alkyl-O cleavage likely to dominate

White et al., Microbiol. Rev. 60:216-232 (1996)

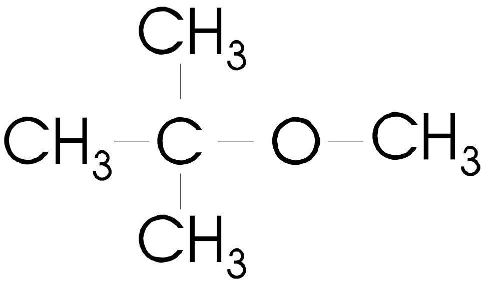
Common Aryl Ether Cleavage Mechanism



Ortho Cleavage Pathway

From: Schmidt et al., 1992. FEMs Microbiol. Lett. 96:253

Bioremediation of MTBE: An Historical Prospective



MTBE

Much Ado About Nothing???

1999 News Papers

Wall Street Journal 7/26/99

EPA Advisory Panel Urges Reduction In Use of Antipollution Fuel Additive

By John J. Fialka

Staff Reporter of The Wall Street Journal

WASHINGTON—A federal advisory panel is urging the Environmental Protection Agency to substantially reduce use of the air-pollution-fighting fuel additive MTBE because it is polluting ground water in some parts of the country.

EPA Administrator Carol M. Browner said that she would work with Congress to soften a law requiring such fuel additives in smog-prone cities. She said the shift away from MTBE (methyl tertiary butyl ether) must be carried out so that air-quality benefits provided by MTBE aren't lost.

The panel's report, which will be released today says that MTBE, a highly soluble petroleum-based substance, is being detected in drinking water, mostly in very low levels. In Maine, California and other areas, there have been reports that the additive is changing the taste and odor of water in private wells. California is in the process of banning its use.

"It's a pesky little molecule," said Jason Grumet, an environmental consultant who served on the 13-member advisory panel. "While not particularly toxic, it zips into the ground water."

That raises a second problem, he added, because MTBE causes gasoline to burn cleaner. One result is that cars that use it release as much as 35% fewer toxic substances into the air. "If we're going to give refiners the flexibility to use less of this stuff, we need to make sure they don't put more toxic additives back in," Mr. Grumet said.

According to Dan Greenbaum, chairman of the EPA advisory panel, oil companies and other manufacturers produce 269,000 barrels a day of MTBE, making it a $2.4 billion-a-year industry.

MTBE is one of a family of so-called oxygenates that refiners began adding to gasoline in the 1970s when lead additives were banned for environmental reasons. Oxygenates boost the octane rating of gasoline; a higher octane rating means better mileage. One potential substitute for MTBE is ethanol, an alcohol-based additive manufactured from corn.

The advisory panel will also rec mend that the EPA take more than 20 ot actions to reduce the spillage of gasoli especially from underground storage ta and from boats, some of which spr large amounts of unburned gasoline on surface of lakes and rivers.

"People have got to learn to ha gasoline better," said Mr. Grumet, no that a considerable amount of MTBE i leased by consumers while pouring g line into the family lawn mower.

Gasoline with MTBE and other genating additives represents about 3 the nation's gasoline market. Nine m

The Sunday Record August 8, 1999

N.J. water at risk from gas additive

Analysis points to trouble in wells, public supplies

By BRUNO TEDESCHI

Trenton Bureau

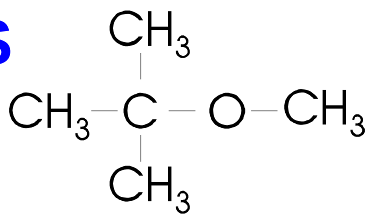
When it was added to gasoline in the early 1990s, MTBE was

mend drastically reducing the use of the additive in gasoline.

Although state environmental officials have not tracked the full extent of the problem in New Jer-

MTBE In The Environment

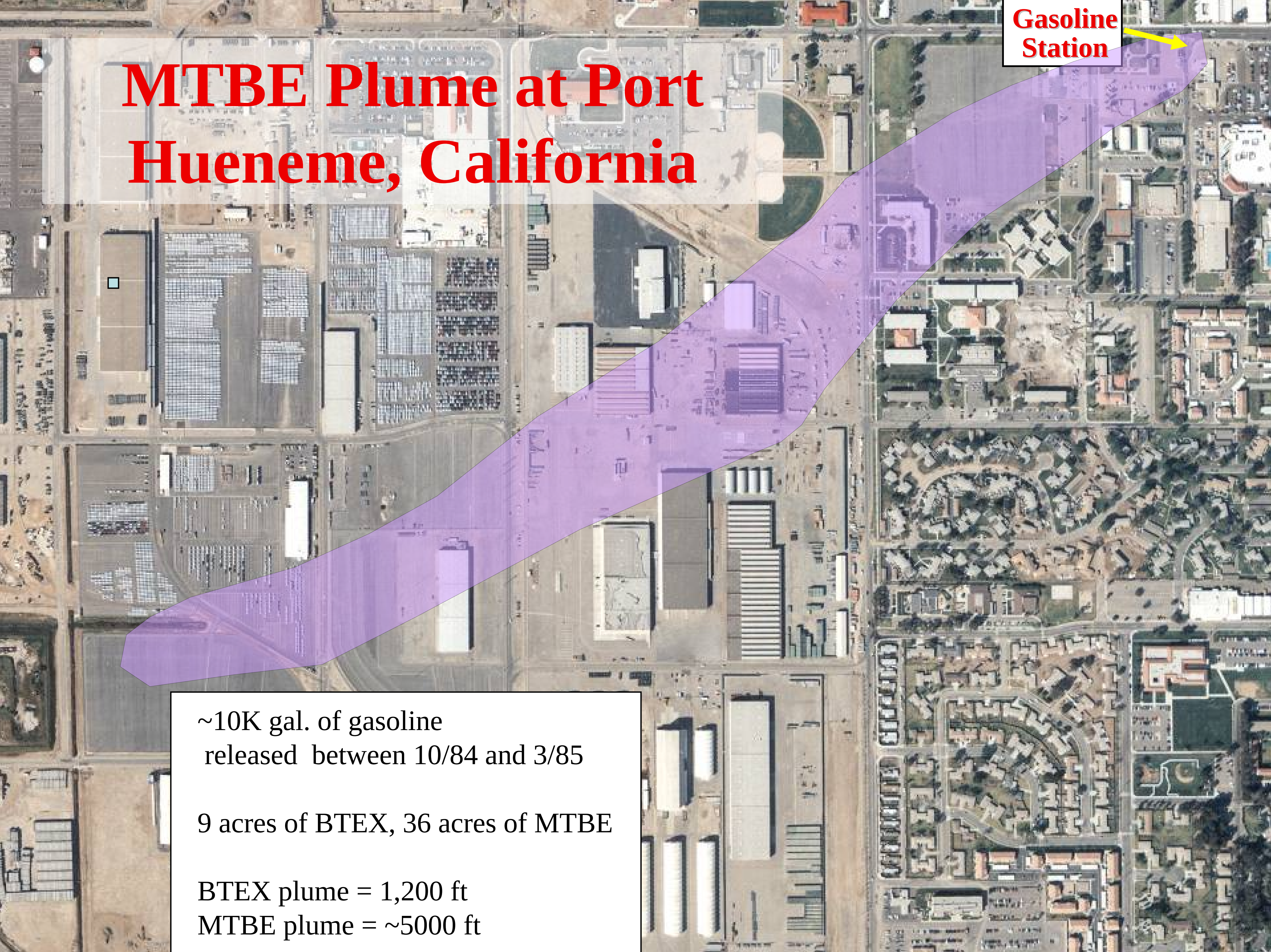
- **4 L of r-Gasoline Can Contaminate 16,000,000 L of GW to 20 μg/L**
- **Estimated >50,000 Contaminated Sites in US**
  - **>18,000 in California**
  - **>5,000 in New York**



MTBE

- **Detectable MTBE at Operating Sites (Buscheck et al., 1998):**
  - **California - 83% -- 47% >1 mg/L**
  - **Texas - 96% -- 63% >1 mg/L**
  - **Maryland - 98% -- 82% >1 mg/L**
- **48% of Sites Evaluated in Washington, Although MTBE has NOT Been Used as an Oxygenate.**

MTBE Plume at Port Hueneme, California



~10K gal. of gasoline
released between 10/84 and 3/85

9 acres of BTEX, 36 acres of MTBE

BTEX plume = 1,200 ft
MTBE plume = ~5000 ft

The Mid to Late ’90s
A Period of Hysteria

- Extreme public and regulatory interest
  - Blue Ribbon Panels
  - Law Suits
  - Groundwater Surveys (USGS, ’96; LLNL, ’95,’97)
- Ability to Treat MTBE Becomes a Technology “Gold Standard”
  - Improved ways to add oxygen
  - Advanced chemical oxidation
  - Biological treatment

MTBE Biodegradation in the Environment
Scientific Literature

The "Early Days"

- '84 No degradation in sludge (Fujiwara et al.)
- '90 No degradation in sludge, aquifer, soil (Arvin et al.)
- '93 No degradation methanogenic aquifers (Suflita et al.)
- '94 First degradation in sludge (Salanitro et al.)

And Now…

- '95-'97 Pure co-metabolic cultures isolated (Kulpa, Envirogen, Hyman)
- '99 First growth cultures (Skow, Envirogen)
- '00 - Methanogenic conditions (Wilson et al.)
- '01 - Iron, Nitrate, Sulfate Reducing (Lovely, USGS, Häggblom)
- '02 - "Everywhere we look" (USGS)
- Today - "Just add oxygen, or not!"

A Tale of Two Cultures

- **Propane Oxidizing Bacteria (POB)**

-- Indigenous

-- Growth on Propane

-- No Apparent Growth on MTBE

-- Converts MTBE to CO_2

-- Co-Metabolism?

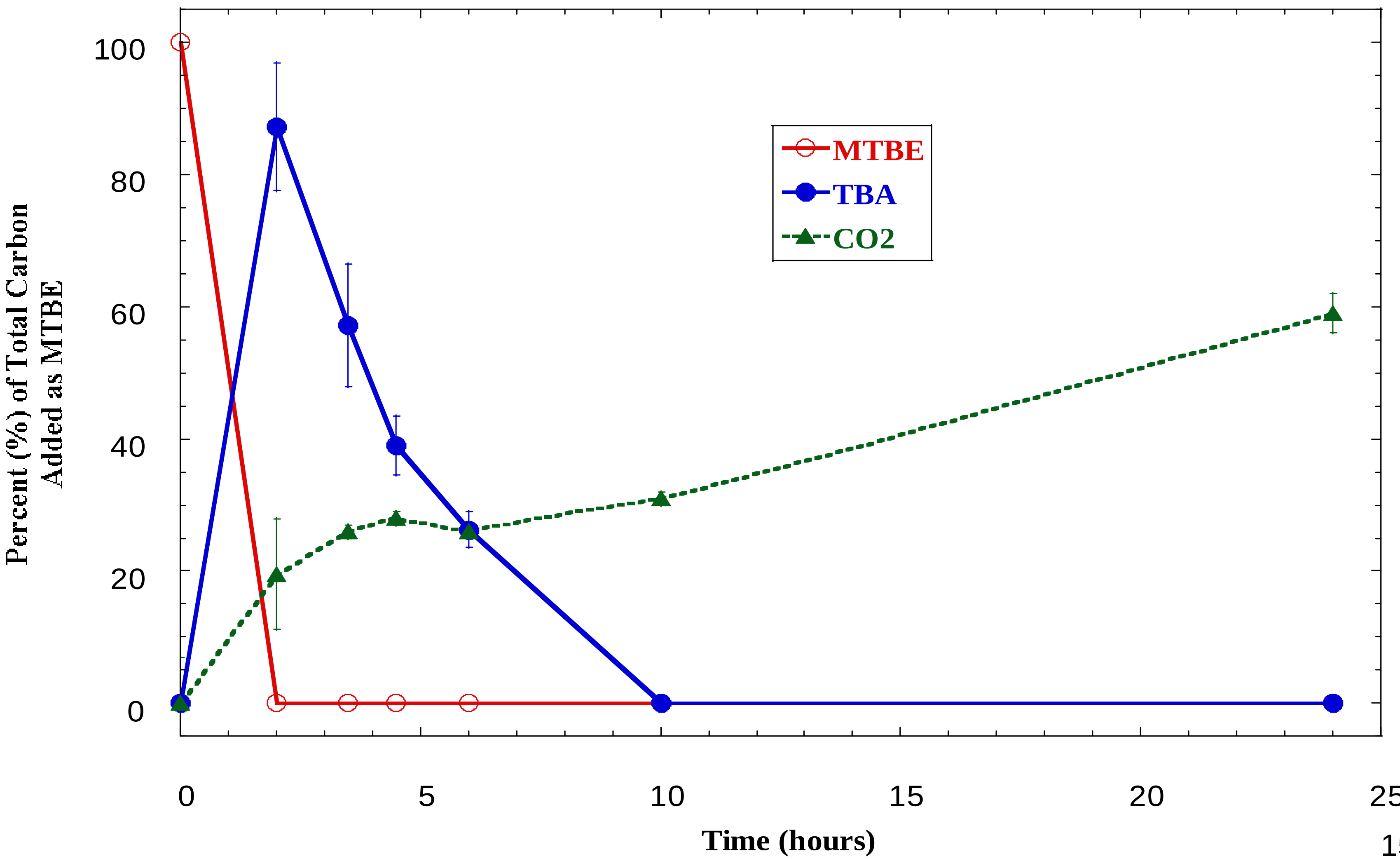
-- Steffan et al., 1997. Appl. Environ. Microbiol. 63:4216.

-- Biostimulation, Bioaugmentation

- **Strain ENV735**

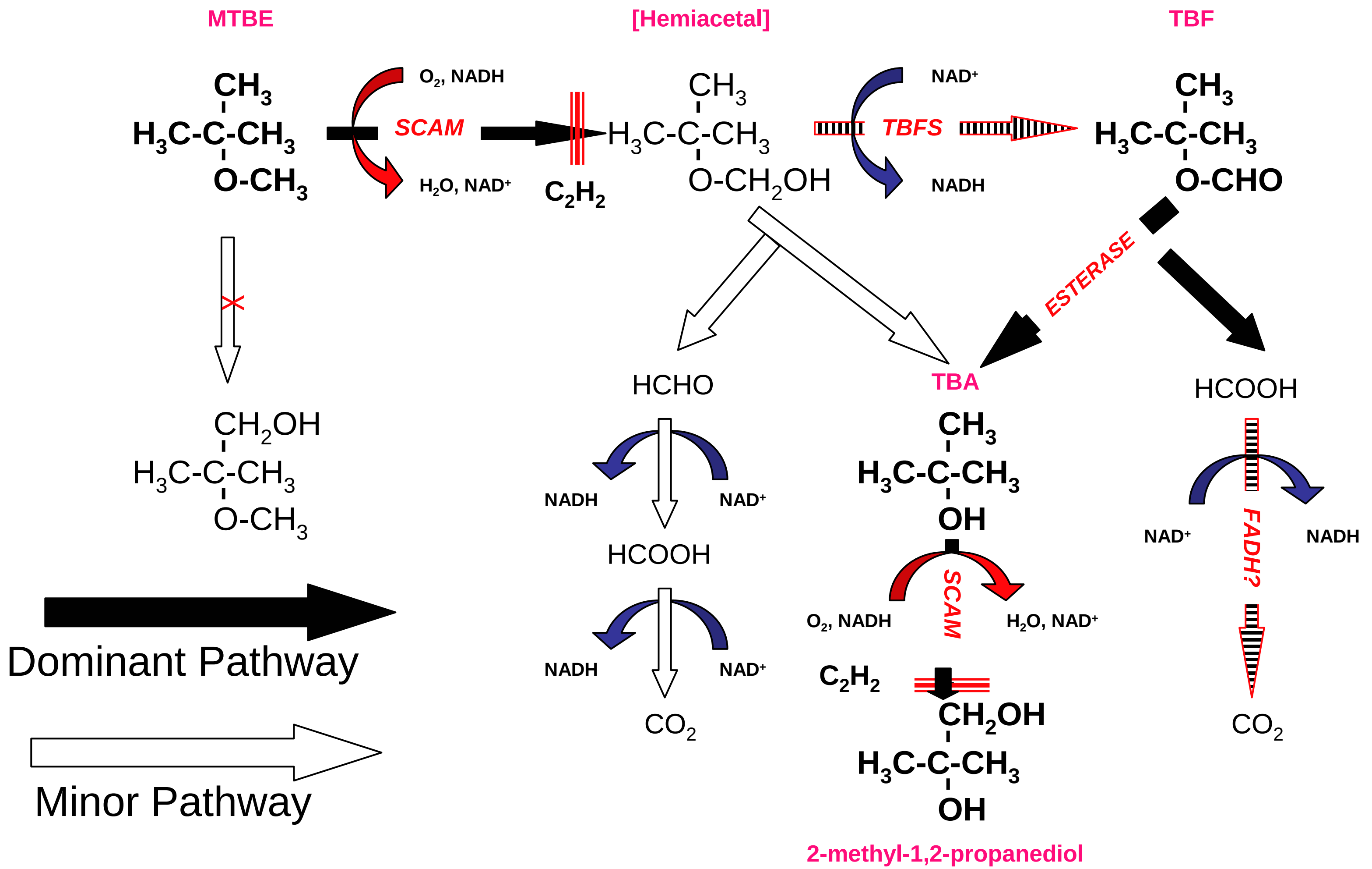
- Environmental Isolate
- Growth on H_2 and Others
- Growth on MTBE
- Inducible TBA degradation
- Converts MTBE to CO_2
- Hatzinger et al., 2001. Appl. Environ. Microbiol. 67:5601.
- Bioreactors, Bioaugmentation

Biodegradation of 20 ppm [^{14}C]MTBE by ENV425



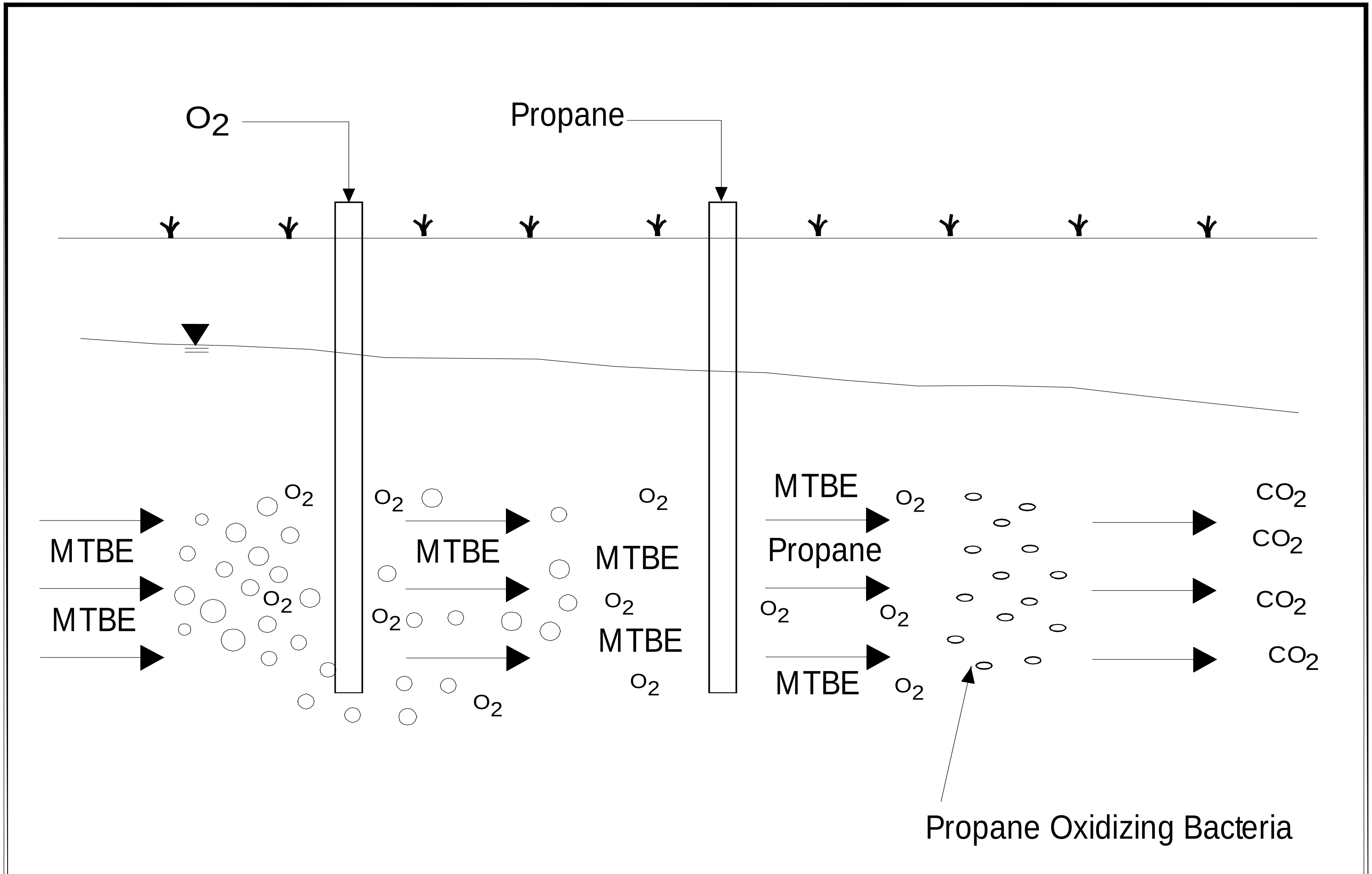
From: Steffan et al., 1997. Appl. Environ. Microbiol. 63:4216-4222

Initial Reactions in MTBE Oxidation



Slide From Michael Hyman, NC State University

Propane Biosparging for MTBE Remediation



U.S. Patent # 5,814,514, 1998

Why In Situ Biostimulation With Propane?

- Inexpensive and Non-Toxic Substrate
- Utilizes Adapted Indigenous Microbes
- Flexible Implementation
  - air sparging systems
  - permeable sparging walls
  - recirculating wells
  - existing systems and equipment
- Degrades both MTBE and TBA

In Situ Treatment

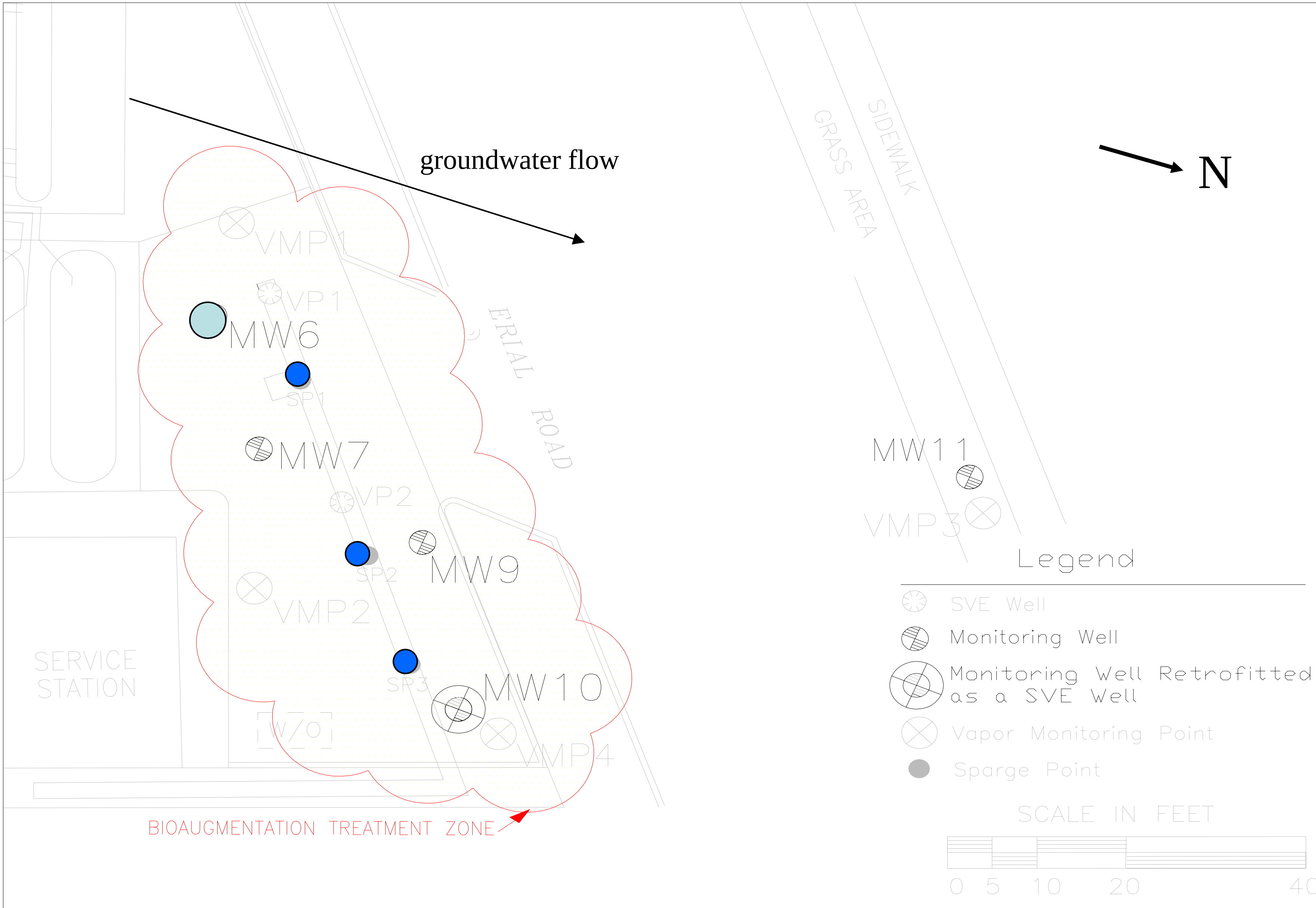
Biostimulation with Propane Oxidizing Bacteria

Case Study 1

- New Jersey Service Station
- History of Air Sparging with Little MTBE Removal
- High Concentration of MTBE
- Low Residual BTEX
- Low pH

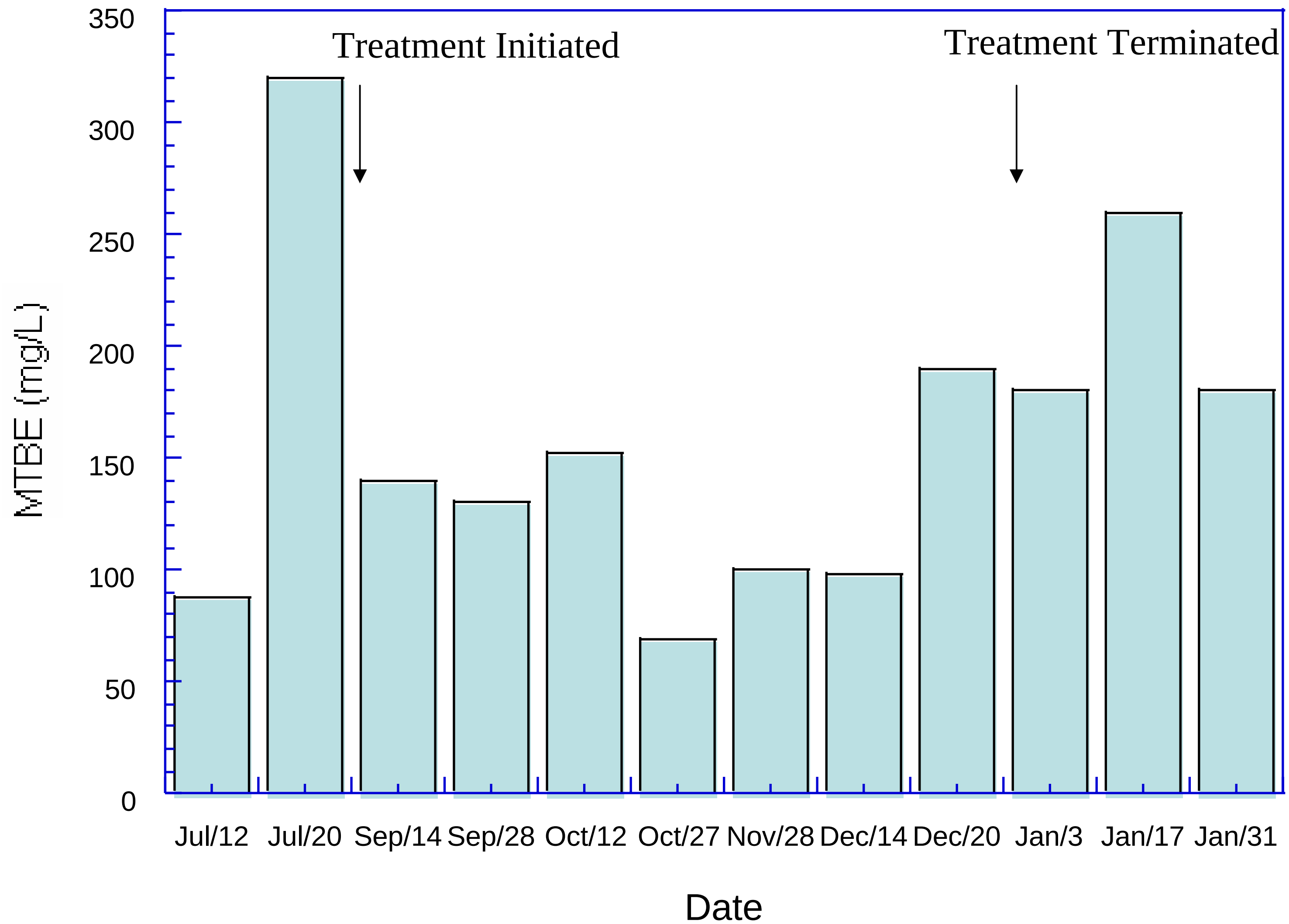
System Operating Conditions

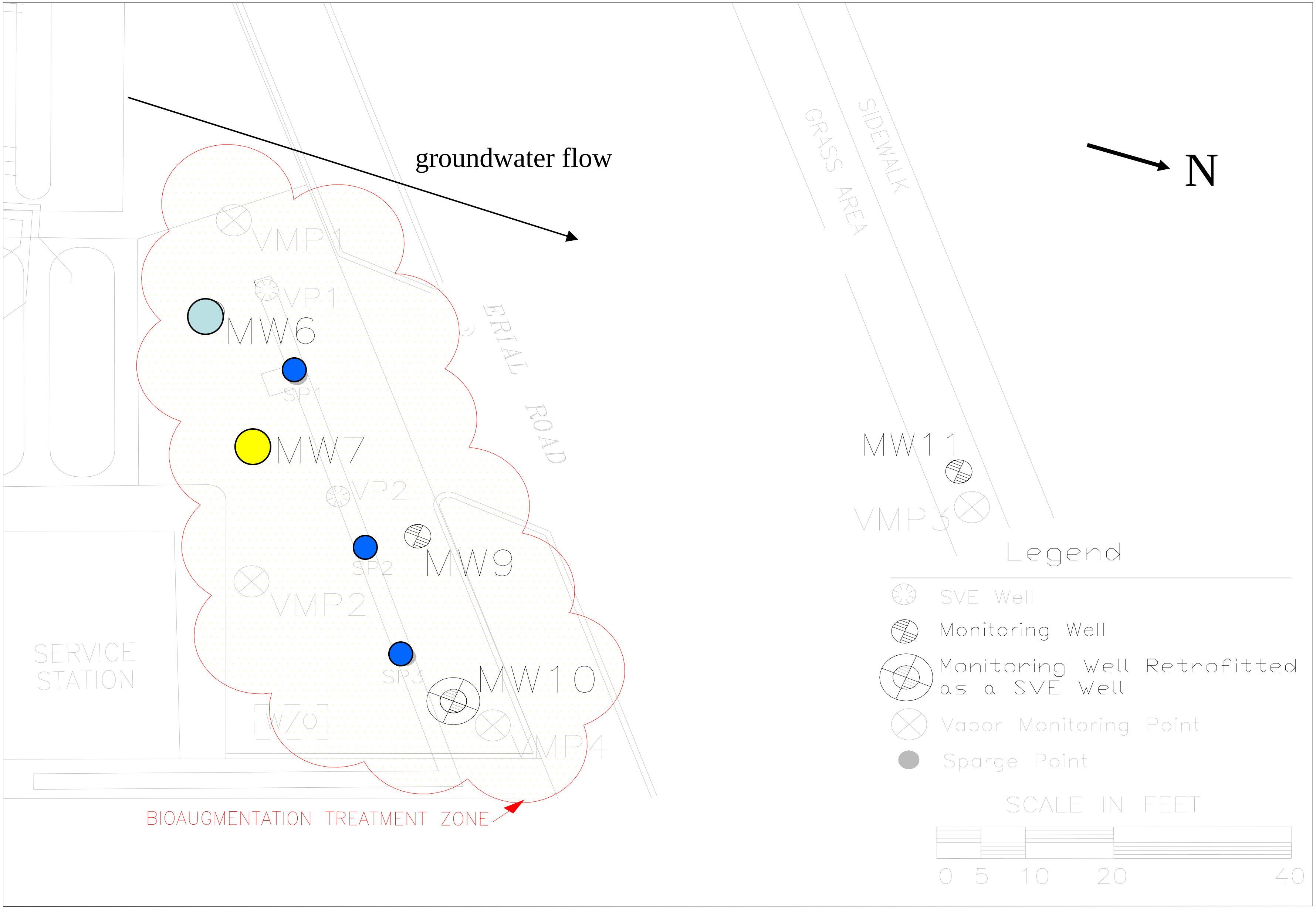
- 6 L of strain ENV425 added to each sparge point as seed culture
- Continuous air sparging at 13 SCFM
- Propane added 10 min. every 3 hrs (~0.23 kg/d; <10% LEL)
- Periodic buffering by adding sodium bicarbonate to sparging wells



groundwater flow
N
VMP1
VP1
MW6
SP1
MW7
VP2
MW9
SP2
VMP2
SP3
MW10
VMP4
SERVICE STATION
W/O
ERIAL ROAD
GRASS AREA
SIDEWALK
MW11
VMP3
BIOAUGMENTATION TREATMENT ZONE
Legend
SVE Well
Monitoring Well
Monitoring Well Retrofitted as a SVE Well
Vapor Monitoring Point
Sparge Point
SCALE IN FEET
0 5 10 20 40

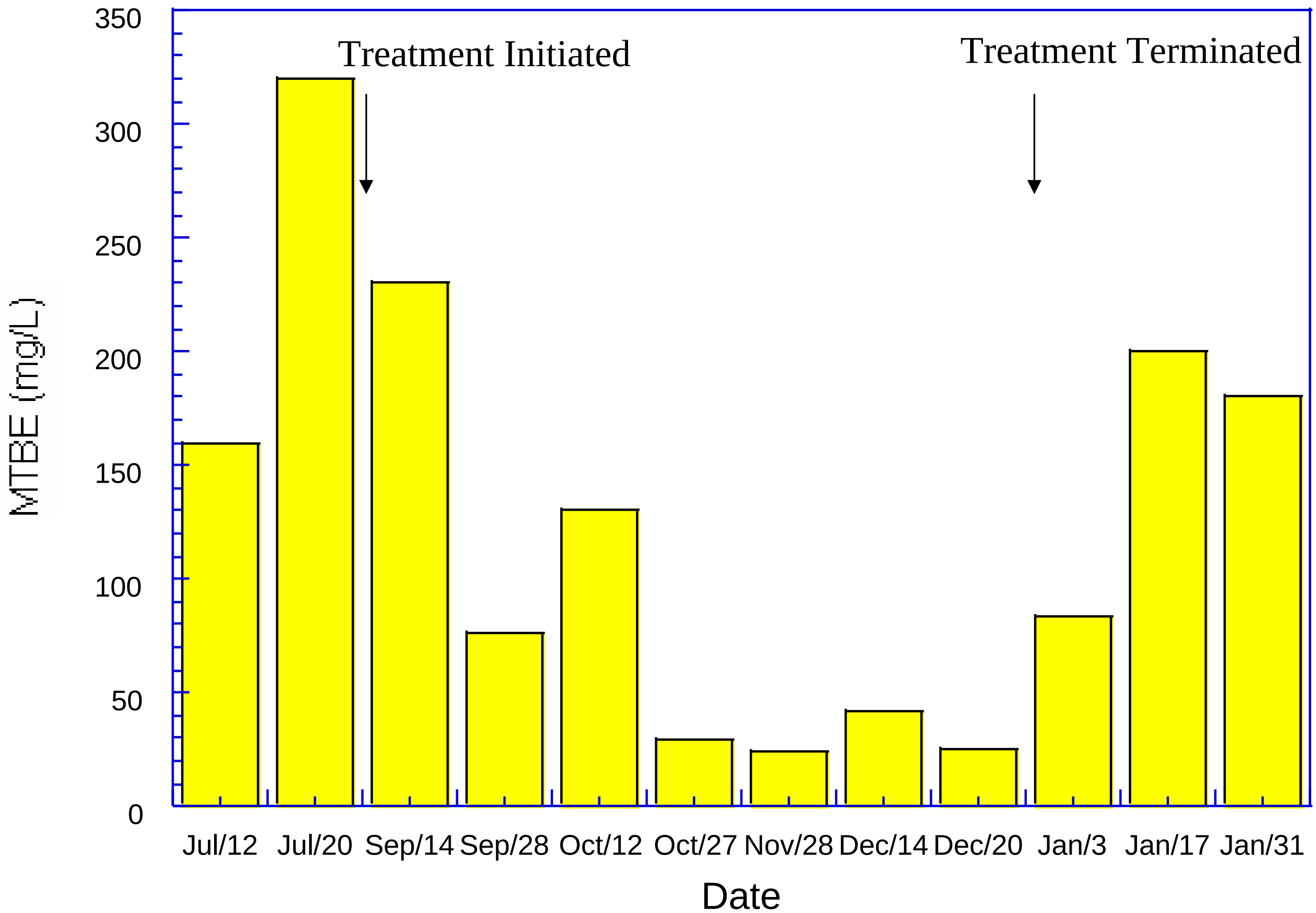
MTBE Concentration in MW6
(upgradient edge of treatment zone)

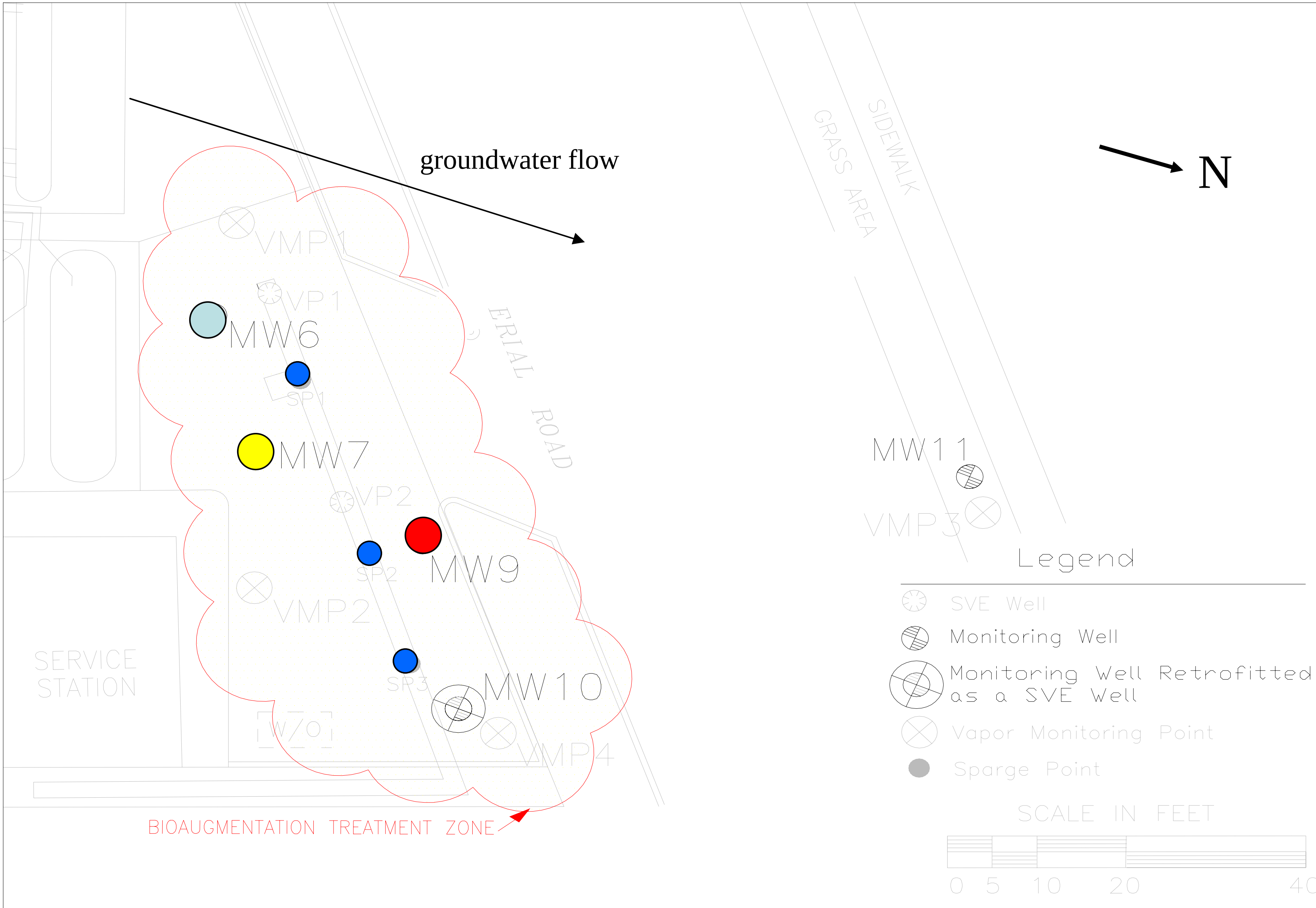



groundwater flow
N
VMP1
VP1
MW6
SP1
MW7
VP2
MW9
SP2
VMP2
SP3
MW10
VMP4
W/O
SERVICE STATION
ERIAL ROAD
GRASS AREA
SIDEWALK
MW11
VMP3
BIOAUGMENTATION TREATMENT ZONE
Legend
SVE Well
Monitoring Well
Monitoring Well Retrofitted as a SVE Well
Vapor Monitoring Point
Sparge Point
SCALE IN FEET
0 5 10 20 40

MTBE Concentration in MW7

(just upgradient of injection wells)

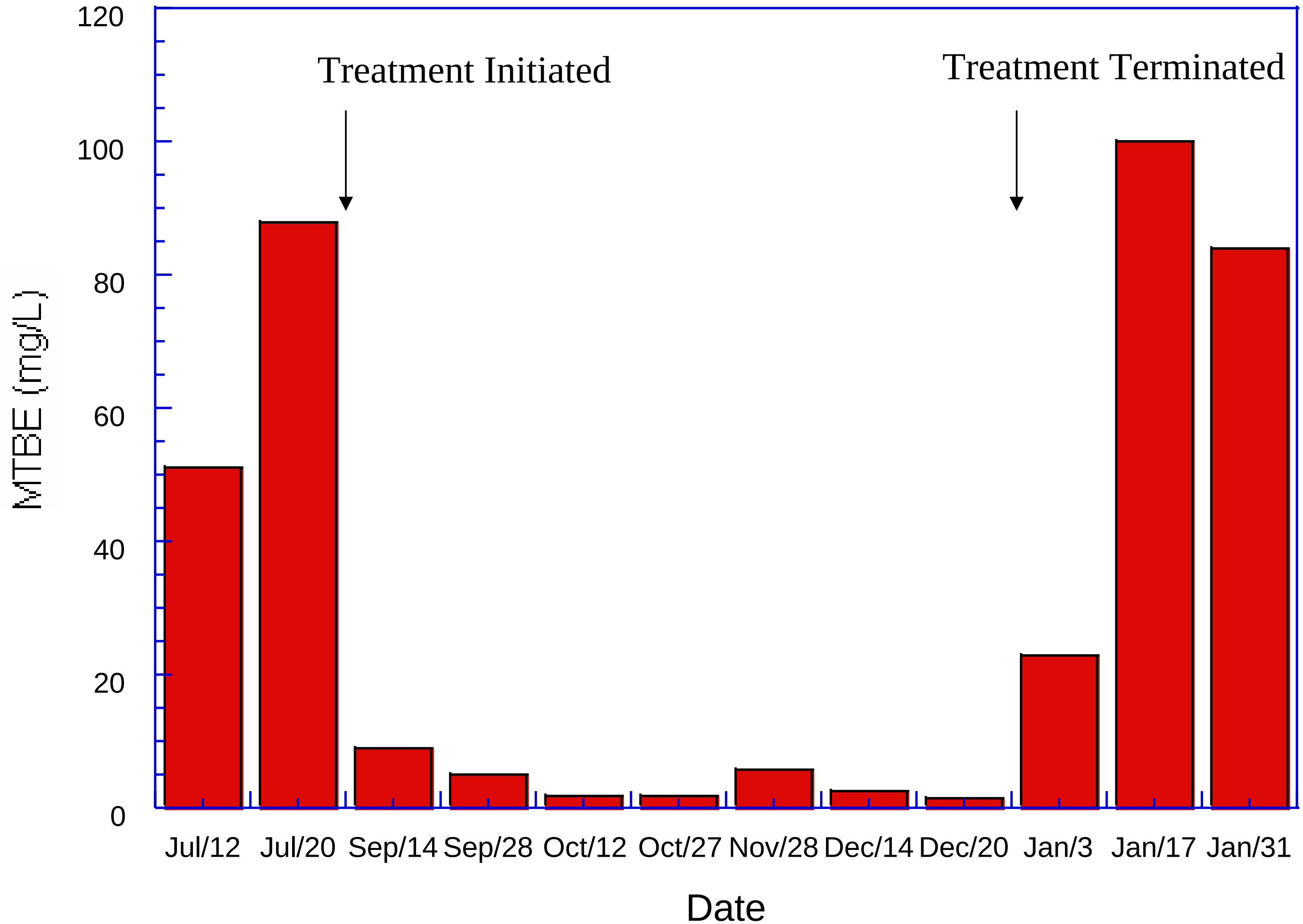


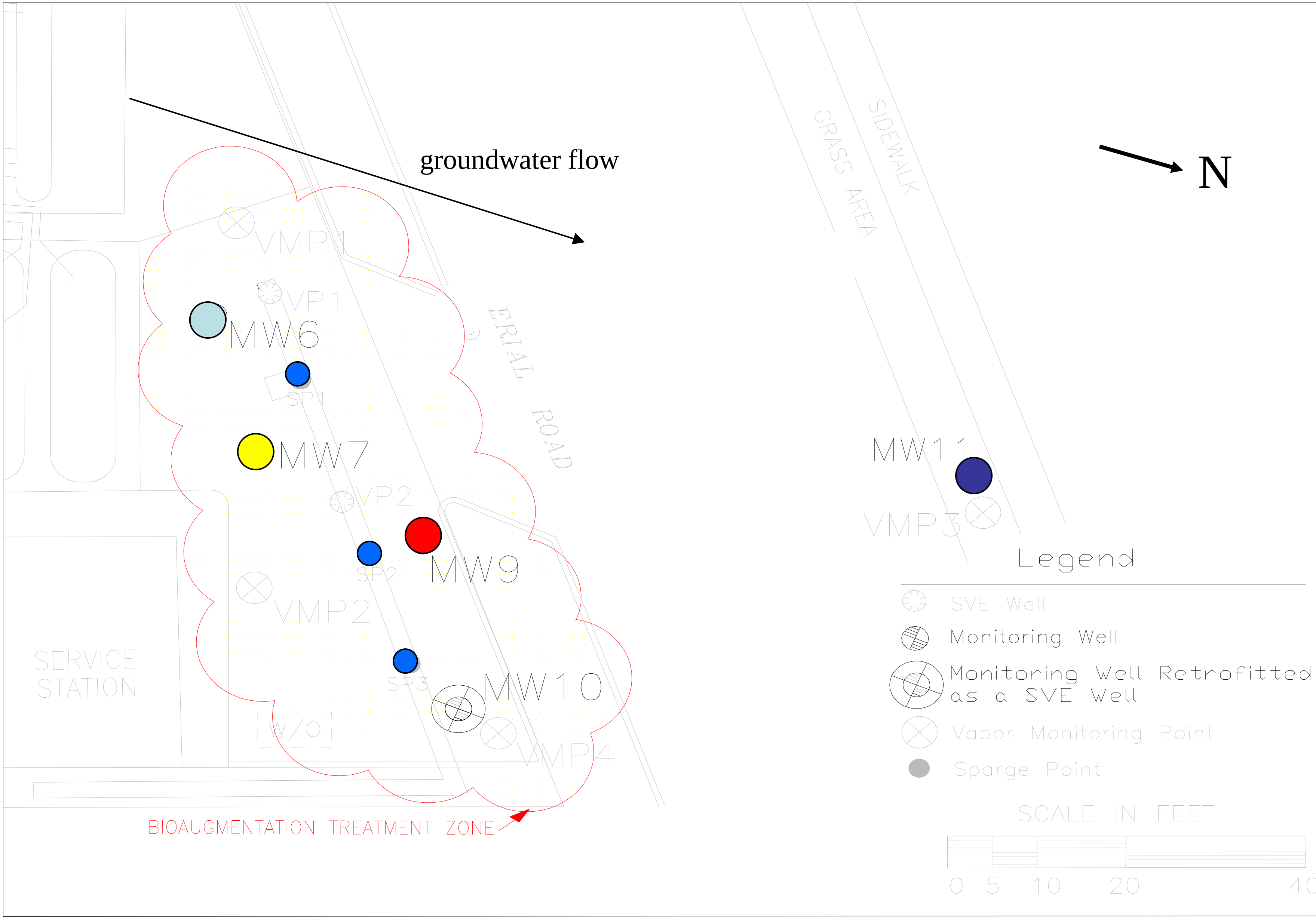


groundwater flow
N
VMP1
VP1
MW6
SP1
MW7
VP2
MW9
SP2
VMP2
SP3
MW10
VMP4
SERVICE STATION
W/O
ERIAL ROAD
GRASS AREA
SIDEWALK
MW11
VMP3
BIOAUGMENTATION TREATMENT ZONE
Legend
SVE Well
Monitoring Well
Monitoring Well Retrofitted as a SVE Well
Vapor Monitoring Point
Sparge Point
SCALE IN FEET
0 5 10 20 40

MTBE Concentration in MW9

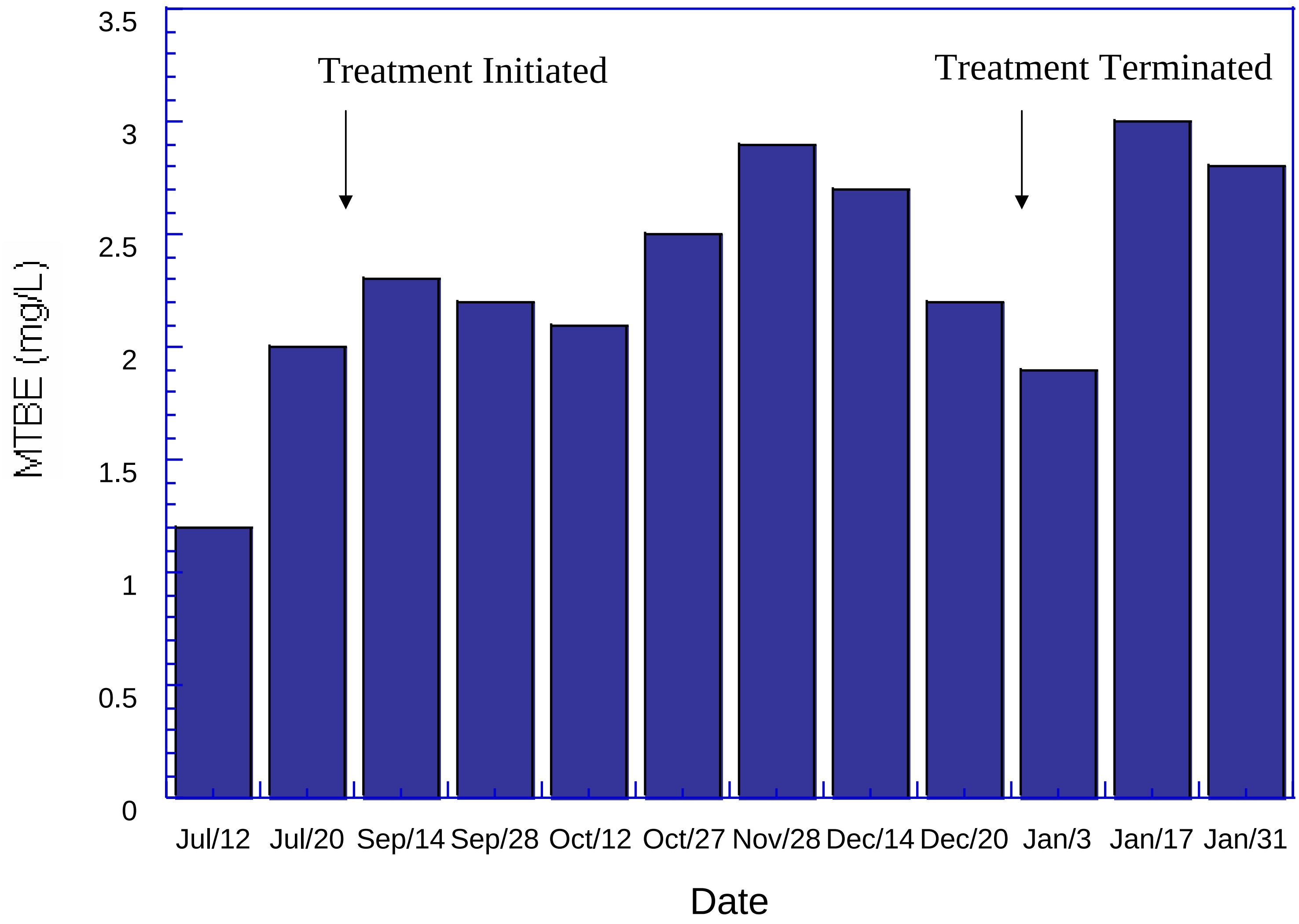
(just downgradient of injection system)





groundwater flow
N
VMP1
VP1
MW6
SP1
MW7
VP2
MW9
SP2
VMP2
SP3
MW10
VMP4
W/O
SERVICE STATION
ERIAL ROAD
GRASS AREA
SIDEWALK
MW11
VMP3
BIOAUGMENTATION TREATMENT ZONE
Legend
SVE Well
Monitoring Well
Monitoring Well Retrofitted as a SVE Well
Vapor Monitoring Point
Sparge Point
SCALE IN FEET
0 5 10 20 40

MTBE in MW11 (down gradient)



Case 1 Conclusions

- MTBE concentrations were reduced 93% at MW-9, 76% at MW-7, and 40% at MW-6.
- Minimal stripping of MTBE and propane
- MTBE rebound due to continuous MTBE source
- Propane cost -- $240 (5 months); Bacteria added -- 18L

The Early 2000s

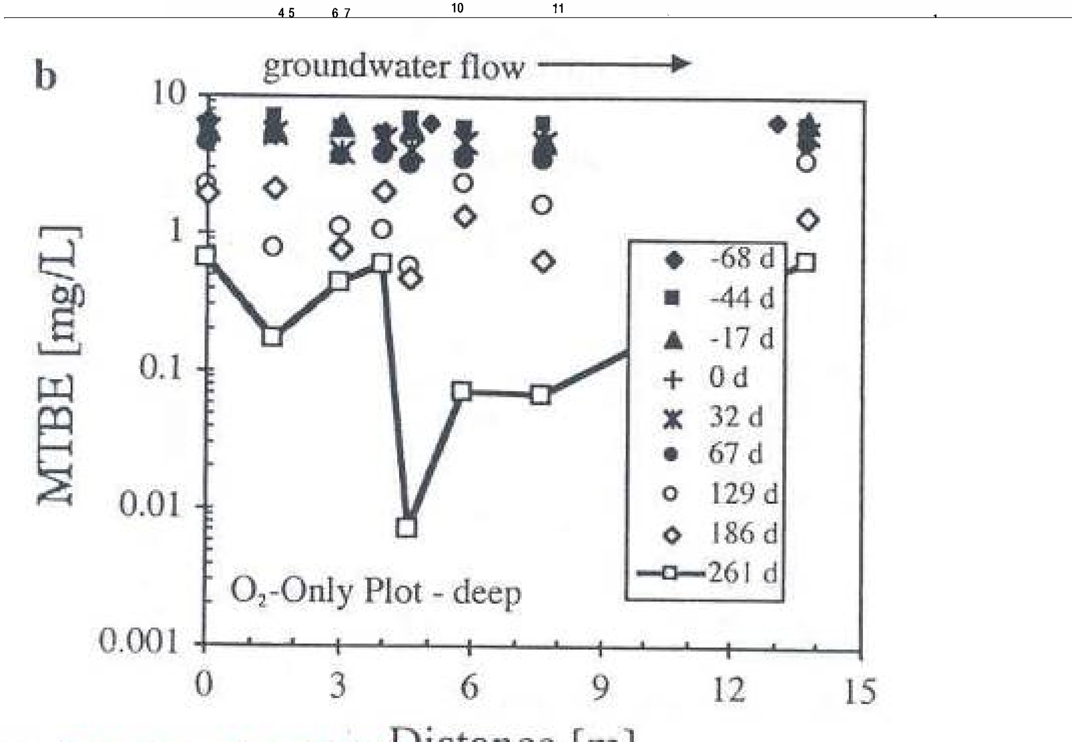
"MTBE? Who cares about MTBE?"

- Apparent easing of public and regulatory hysteria
- MTBE disappearing at many sites
- Natural attenuation a possibility
  - Questions remain about TBA
- Oxygen addition is sufficient at many sites

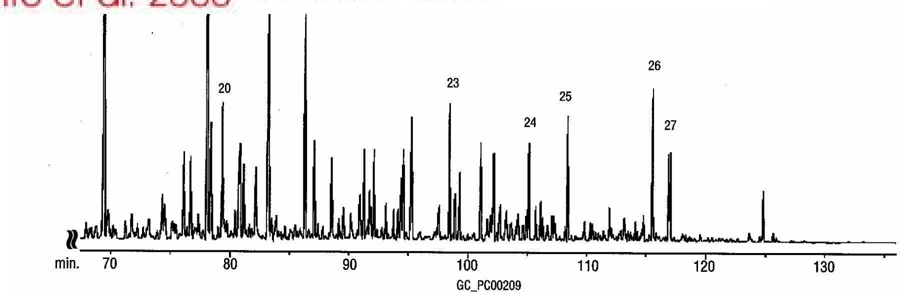
So Where's the MTBE Going?

Envirogen/Shaw Demonstration at PH 2001

Shell Oil MTBE Demonstration at PH ca. 1998



From Salanitro et al. 2000



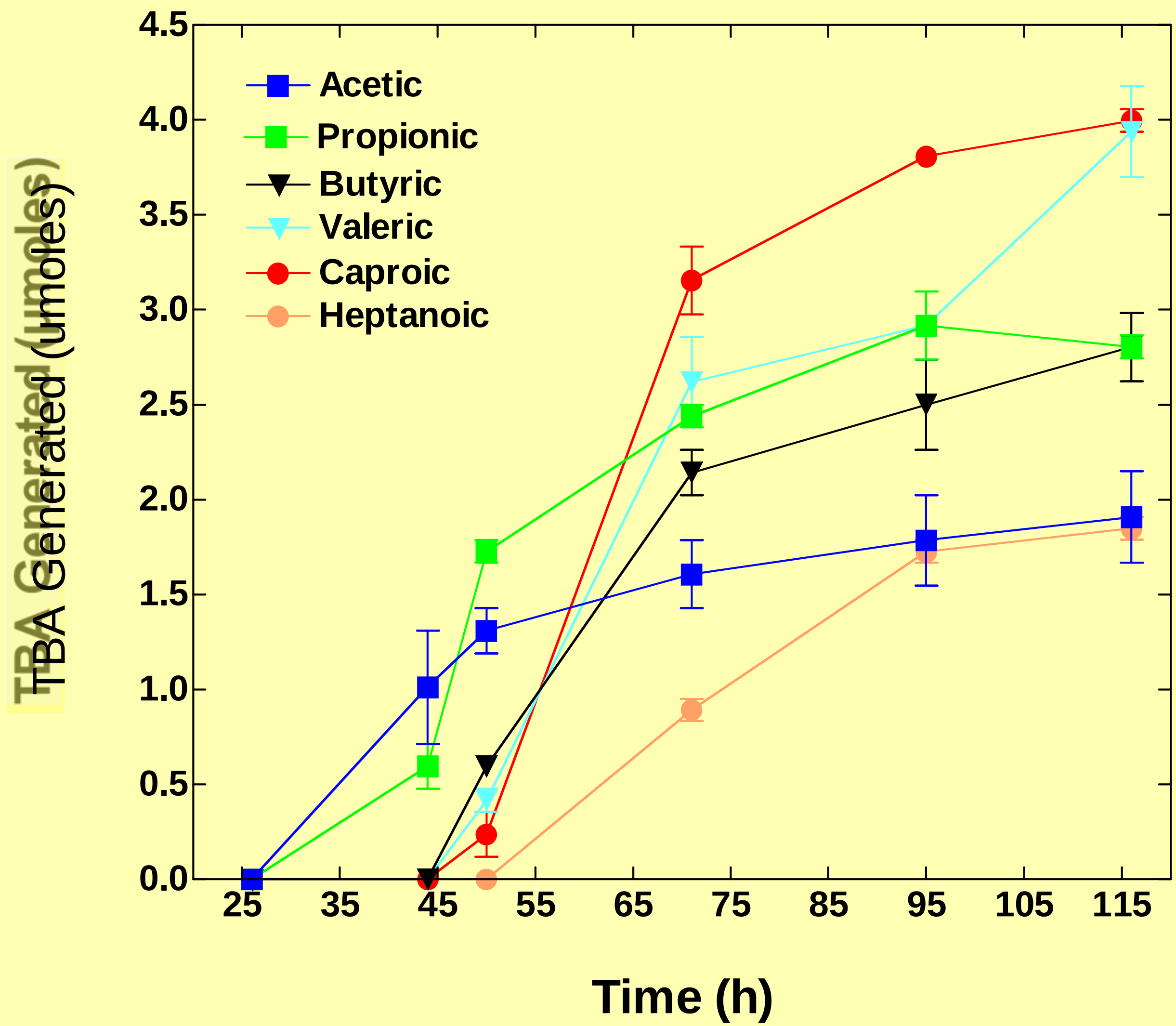
The Better Bacteria Model

- Naturally-occurring MTBE degrading bacteria have evolved, been recruited, or been enriched in contaminated aquifers.

The Hyman Model

-Gasoline components and their oxidation products support co-metabolic MTBE degradation.

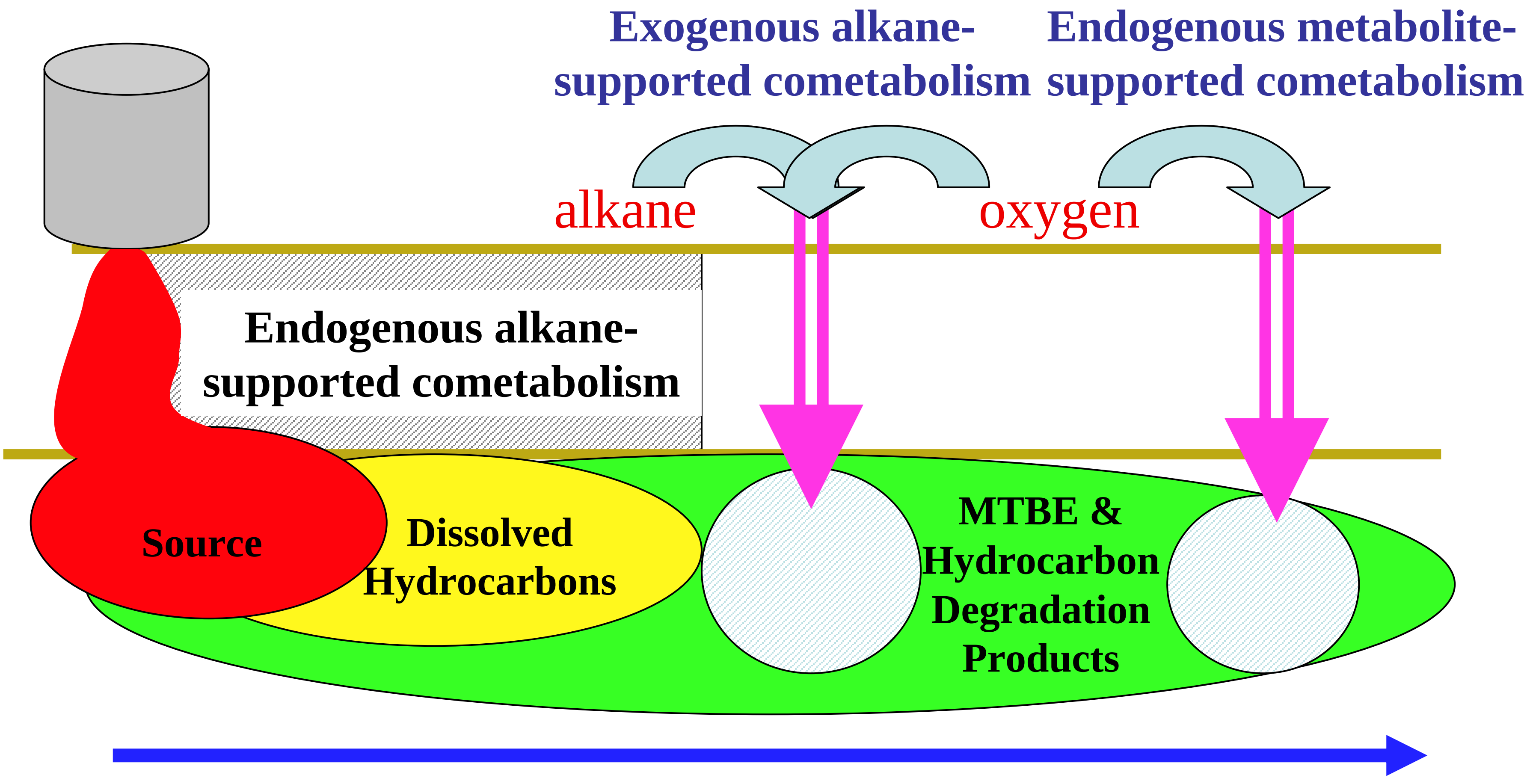
MTBE Degradation by Propanotrophs Grown on n-Acids



n-Acids can induce MTBE oxidation activity

Slide courtesy of Dr. Mike Hyman; NCSU

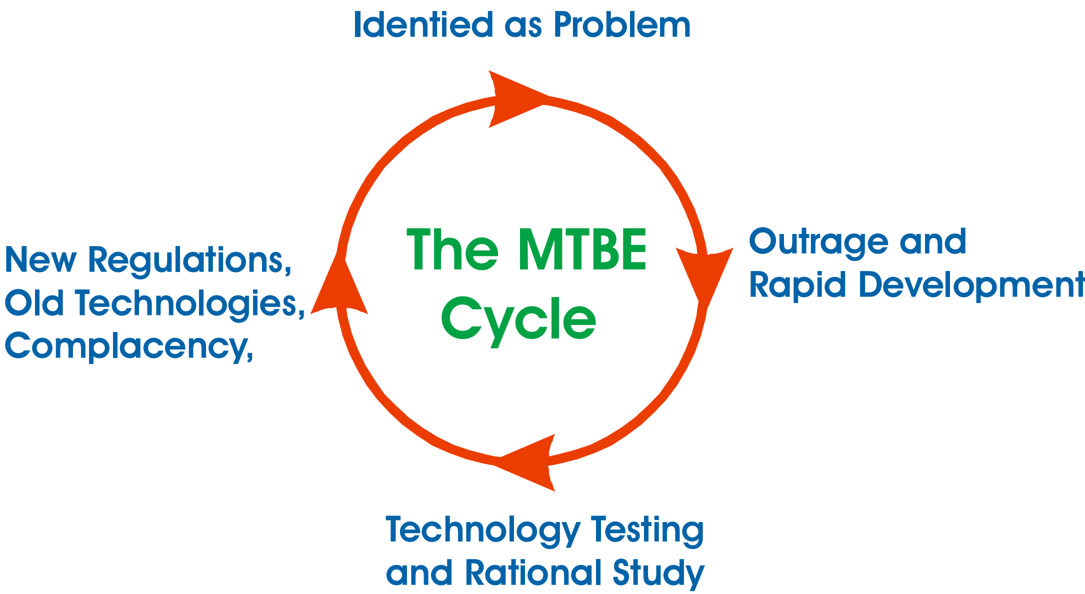
Potential Roles for Cometabolism in Natural Attenuation/Remediation of MTBE



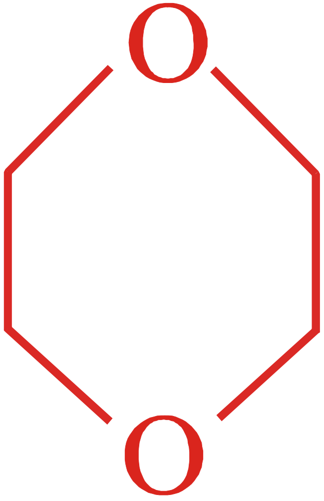
Slide courtesy of Dr. Mike Hyman; NCSU

MTBE Summary

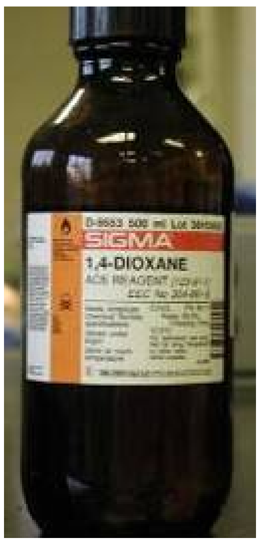
- The identification of MTBE as a groundwater contaminant in the mid 1990s led to great public and regulatory concern
- The biodegradability of MTBE was confirmed in laboratory and field studies
- Most MTBE sites are now treated with traditional remedial technologies
  - MNA, oxygen stimulation, air sparging/stripping



Biodegradation of 1,4-Dioxane



To Learn More About Solvent Stabilizers



1,4-Dioxane

SOLVENT STABILIZERS

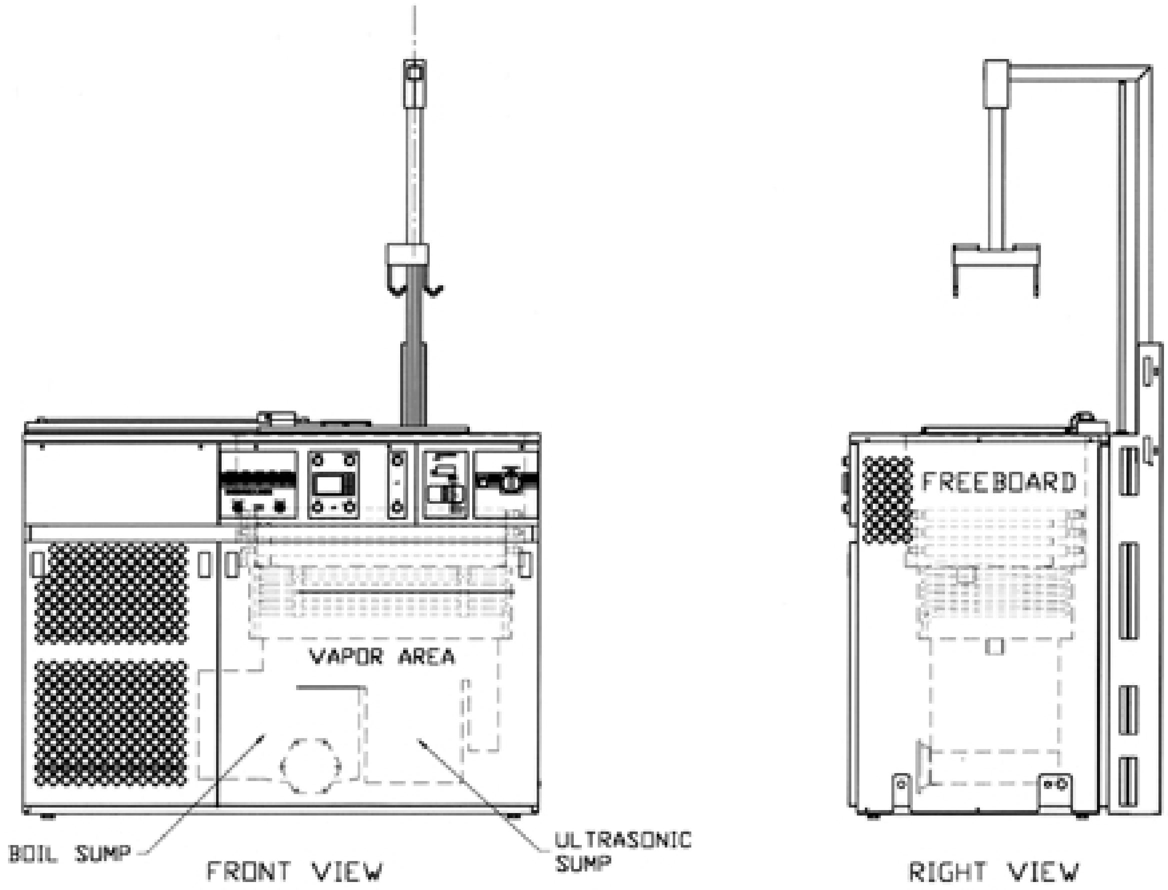
WHITE PAPER
PREPUBLICATION COPY
June 14, 2001

Thomas K. G. Mohr, R.G., C.E.G., C.H.
Associate Engineering Geologist
Underground Storage Tank Program - Water Supply Division
Santa Clara Valley Water District
5750 Almaden Expressway, San Jose, California, 95118
tommohr@scvwd.dst.ca.us 408-265-2607 x 2626

1,1,1-Trichloroethane

http://www.ValleyWater.org

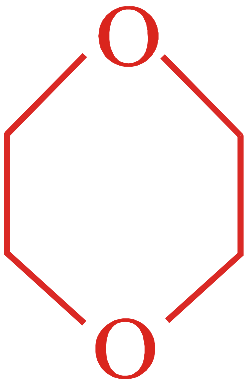
Vapor Degreaser



Characteristics of 1,4-Dioxane

Chem/Phys/Tox

- -- Cyclic Ether
- -- High Miscibility in Water
- -- Low Henry's Law Coefficient -- 4.9×10^{-6} atm/m^3/mol
- -- Low Partitioning Coefficient -- $K_{oc} = 1.23$
- -- Probable Human Carcinogen

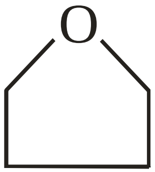


The Result

- -- Chemically Stable
- -- Low Odor and Taste Threshold
- -- Difficult to Biodegrade
- -- Difficult to Remove by Air Stripping or Carbon Sorption
- -- Very Mobile in Groundwater
- -- Recently Identified as a Contaminant of Concern

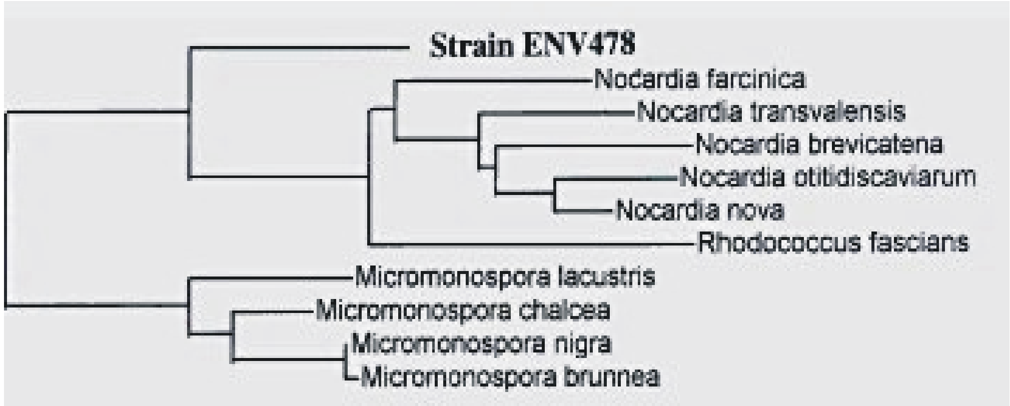
Isolation of a New 1,4-Dioxane Degrader

Primary Enrichment on Tetrahydrofuran



Tetrahydrofuran (THF)

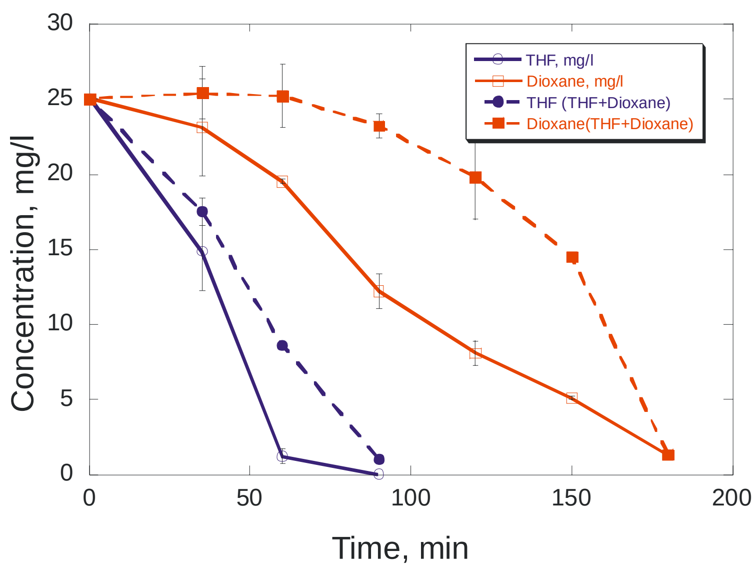
Molecular Characterization



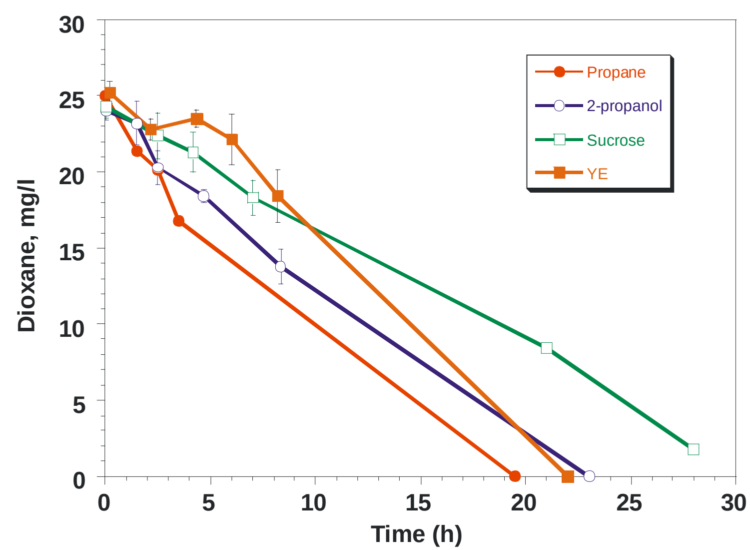
16s rDNA sequence alignment for strain ENV478. The sequence differed by >4.8% from *Nocardia farcinica*, and did not closely match any other sequences in the Genbank DNA database, indicating that it is a new 1,4-dioxane degrading isolate.

1,4-Dioxane Degradation by Strain ENV478

1,4 Dioxane Degradation by THF-Grown ENV478
(VSS = 0.38 mg/L)

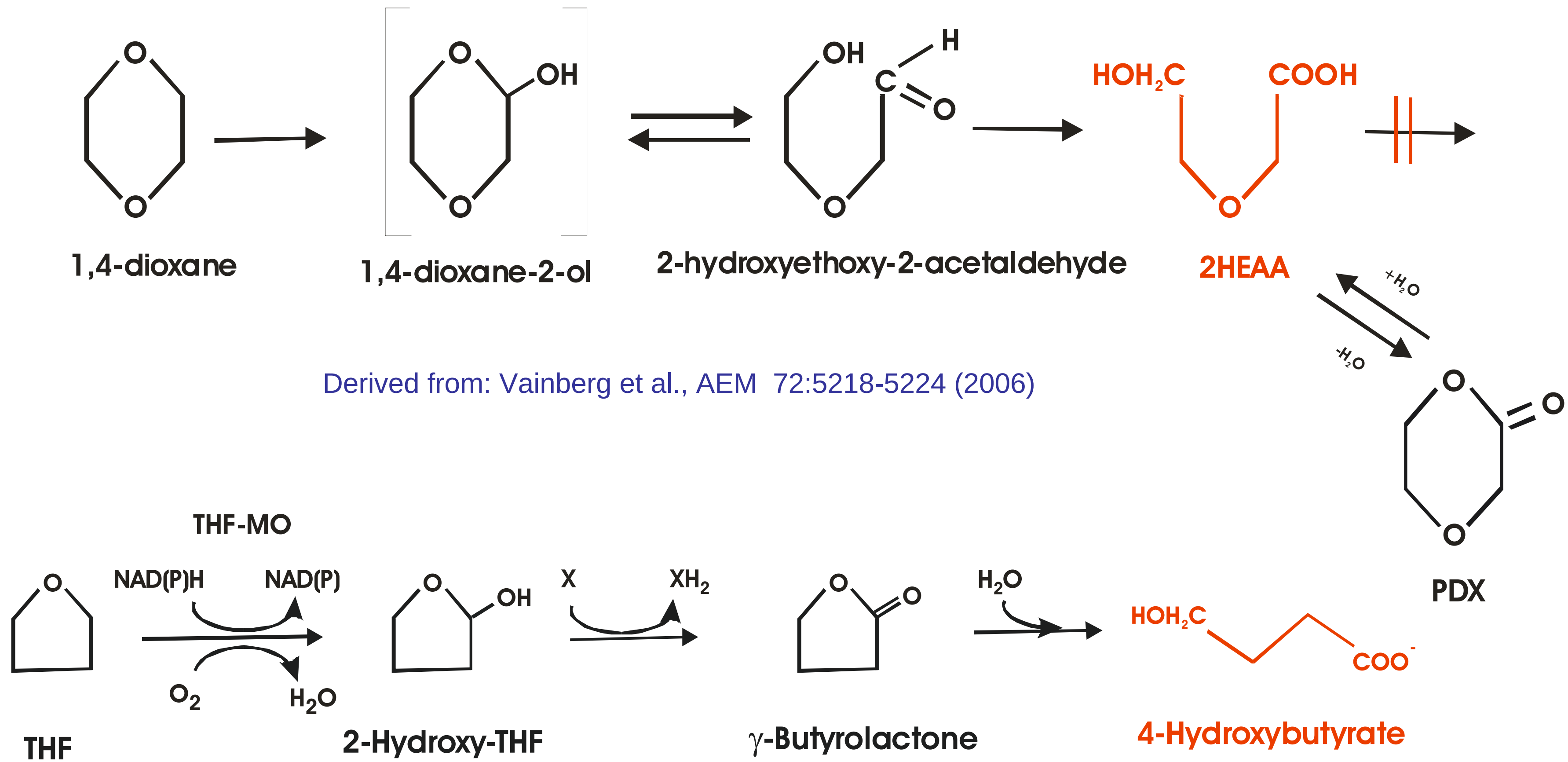


1,4 Dioxane Degradation by ENV478 After Growth on Different Substrates



DoD EPA DOE SERDP
Strategic Environmental Research and Development Program

ENV478 1,4-Dioxane Biodegradation Pathway Analysis



Derived from: Vainberg et al., AEM 72:5218-5224 (2006)

- **Strain ENV478 degrades 1,4-dioxane to 2-hydroxyethoxyacetic acid (2HEAA)**
- **Does not degrade 2HEAA**
- **Inability to grow on 1,4-dioxane likely related to inability to metabolize 2HEAA**
- **Experiments underway to generate strain that can metabolize this compound**

Environmental Analysis

1. **Collected Samples from Two 1,4-Dioxane-Contaminated Aquifers**
   - **Elkton, MD (collected by Solutions IES)**
   - **New York Commercial Site**

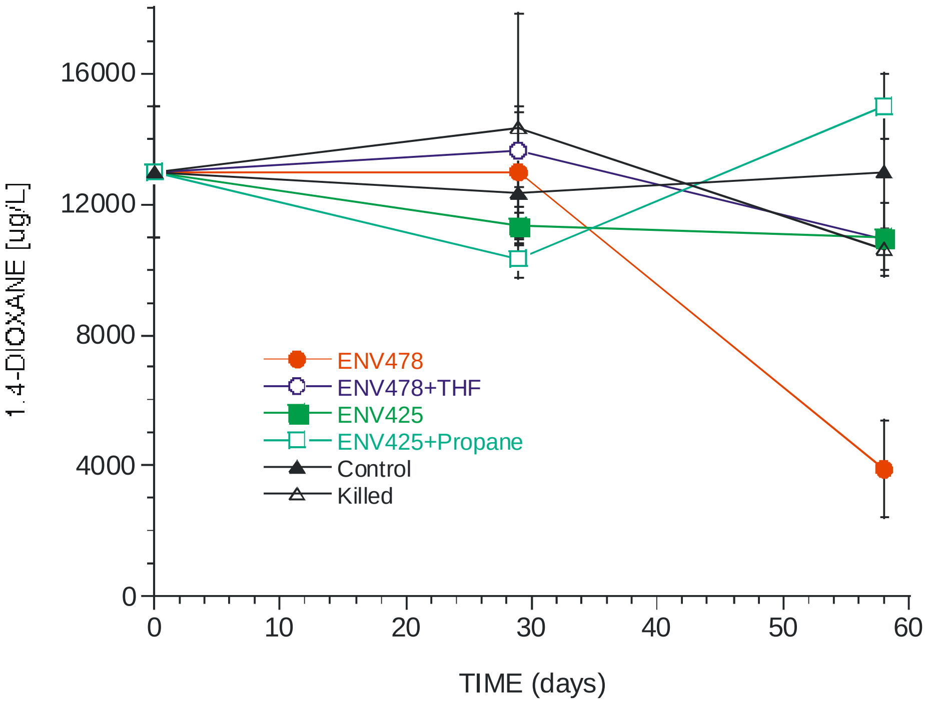
2. **Established Microcosms (n=170)**
   - **Aerobic**
   - **Nitrate reducing**
   - **Iron Reducing**
   - **Sulfate reducing**
   - **Methanogenic**
   - **Biostimulation**
     - **THF, Propane, Lactate**
   - **Bioaugmentation (strain ENV478)**
   - **Controls (no addition, poisoned)**

Site	1,4-Dioxane	1,1,1-TCA	cVOCs	Other
Bio-barrier GP-1	130 ug/L	250 ug/L	1,726 ug/L	NA
Bio-barrier GP-5	100 ug/L	600 ug/L	1,056 ug/L	NA
New York	13,000 ug/L	83 ug/L	577 ug/L	590 ug/L sVOC

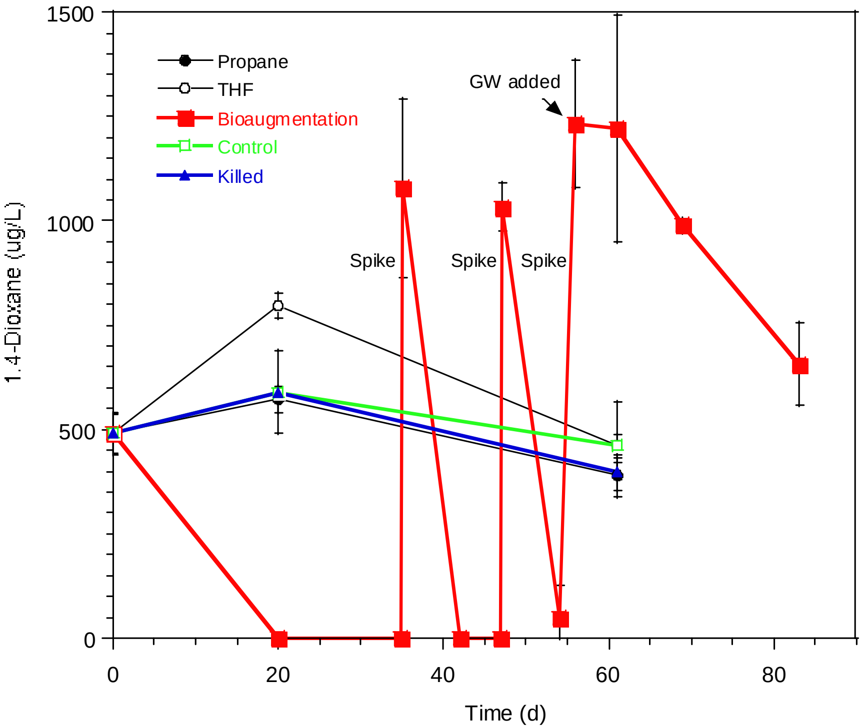
Note: New York samples sparged with nitrogen

Microcosm Results

New York Site

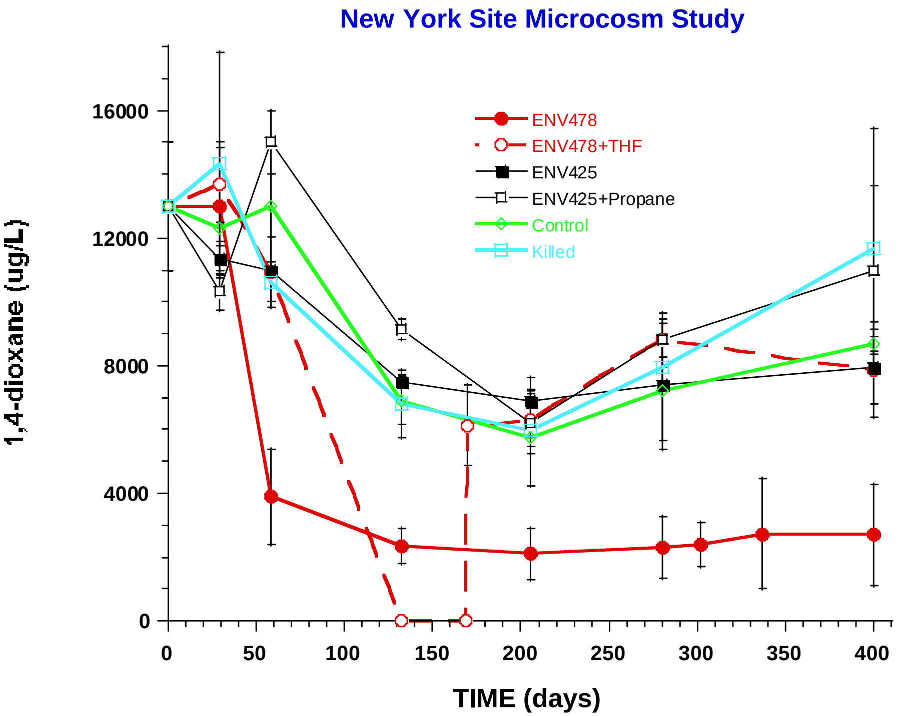


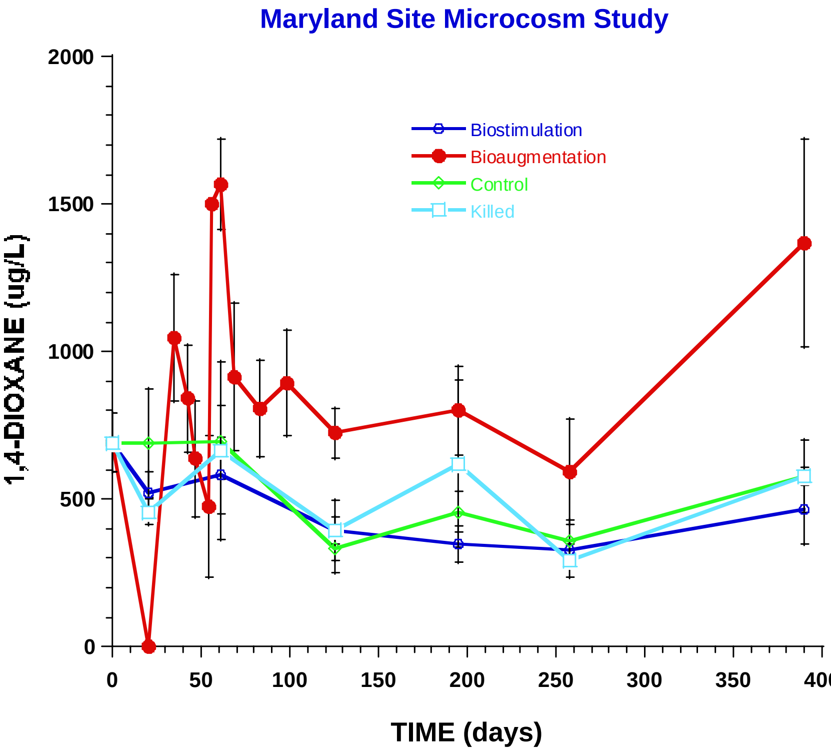
Maryland Site



1,4-dioxane is being degraded only in microcosms augmented with strain ENV478

Extended Microcosm Studies



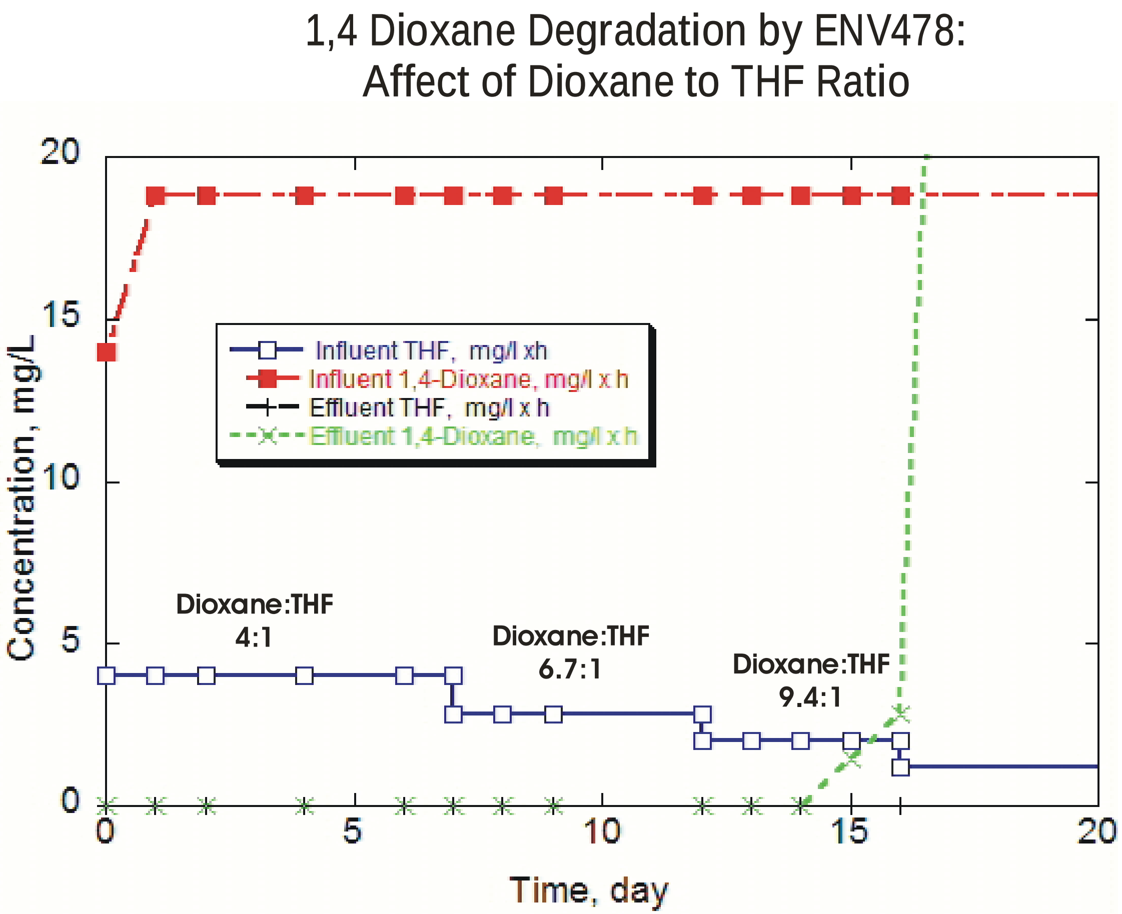


No Degradation Under Anaerobic Conditions

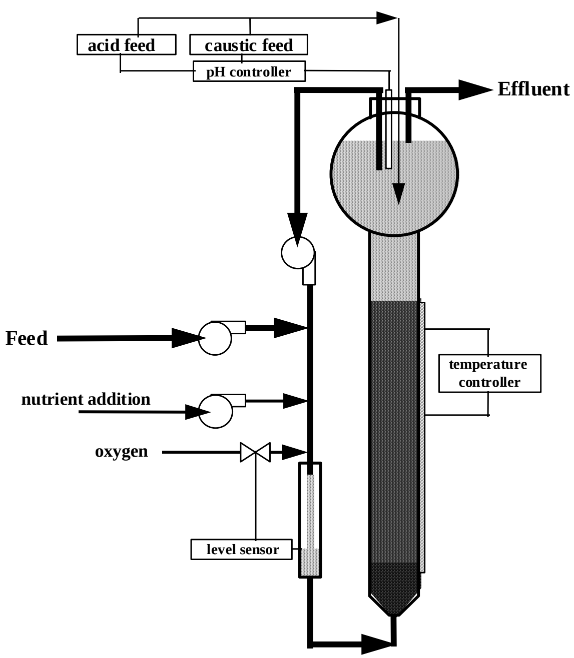
No Degradation by Native Microbes

Some Degradation with Bioaugmentation**

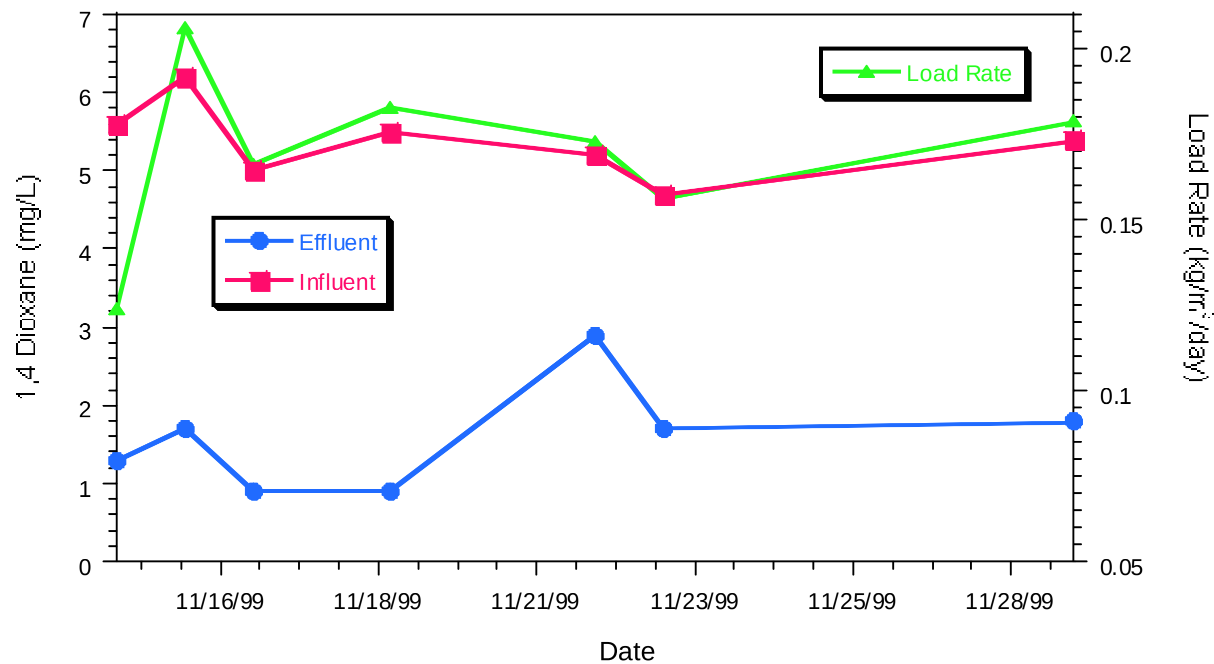
Degradation of 1,4 Dioxane in a 7-L Bioreactor



1,4-Dioxane Treatment in a Fluid Bed Bioreactor



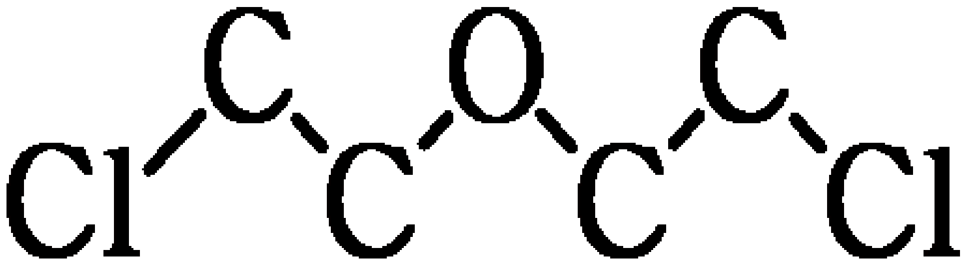
Biological Treatment of 1,4-Dioxane in a FBR
43 min. HRT



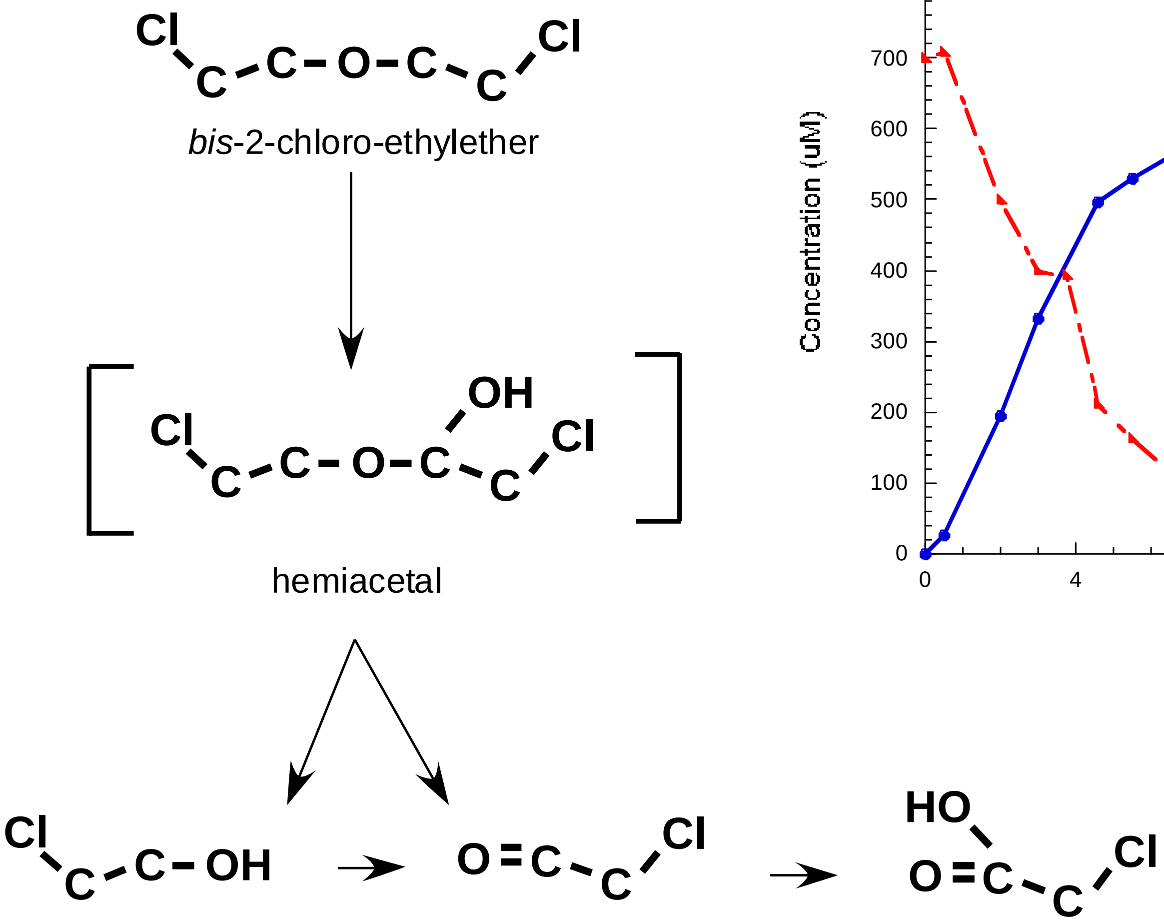
Conclusion

- Biological treatment of 1,4-dioxane is possible, but not yet proven
- -- Ex situ treatment in bioreactors
- -- In situ treatment via co-metabolism
- -- In situ treatment via bioaugmentation

Biodegradation of BCEE



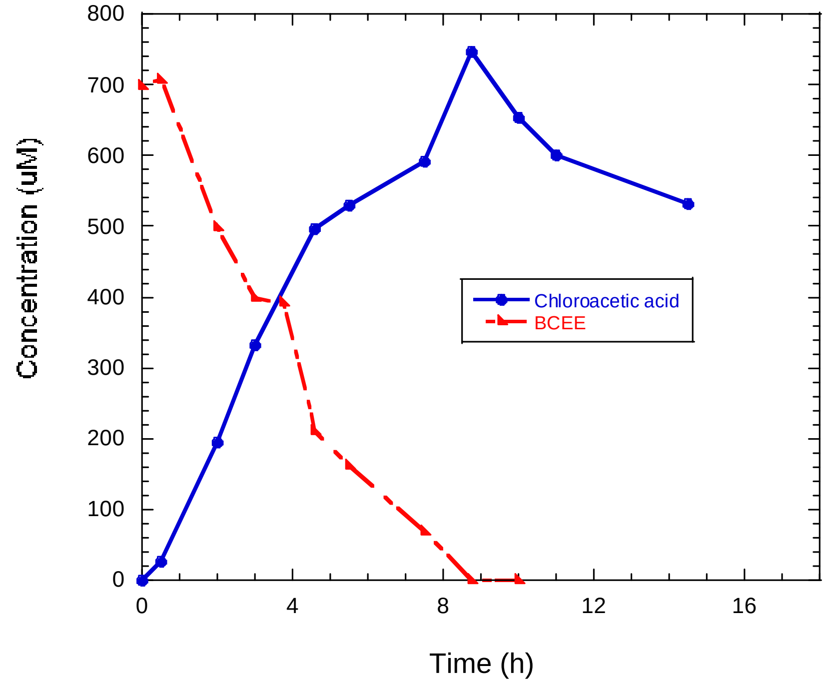
BCEE Degradation by THF-Grown ENV478



2-chloroethanol

2-chloroacetaldehyde

2-chloroacetate



ENV478 Results

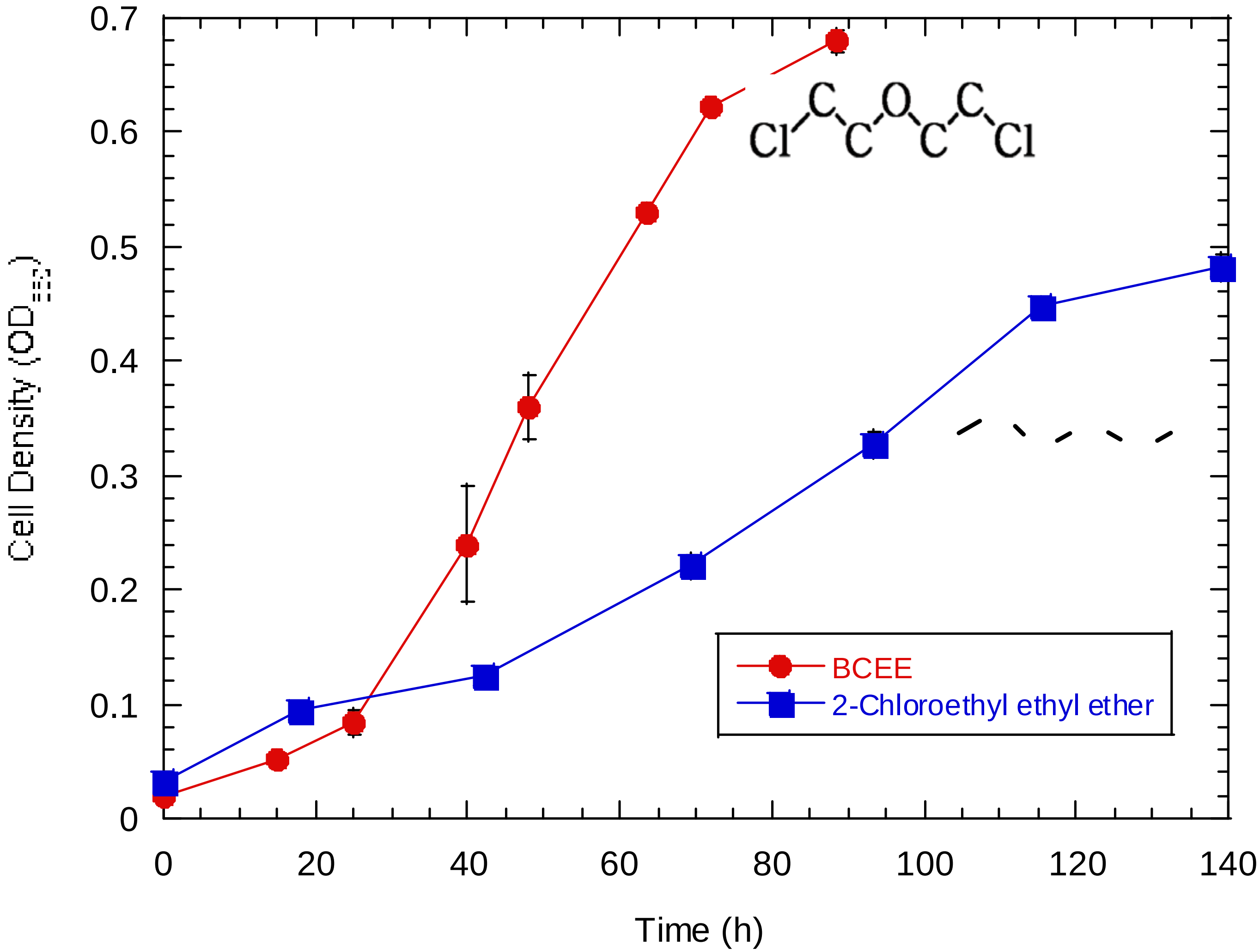
- *Pseudonocardia* sp. strain ENV748 uses an expected monooxygenase pathway to degrade BCEE

- Strain ENV478 does not grown on BCEE

Search for a Better BCEE Degrader

- Microcosm studies from a NJ Superfund Site
  - Aerobic and anaerobic BCEE degradation
  - BCEE degraded only after aromatics
- Enrichment culturing led to strain ENV481
  - *Xanthobacter* by 16S rRNA

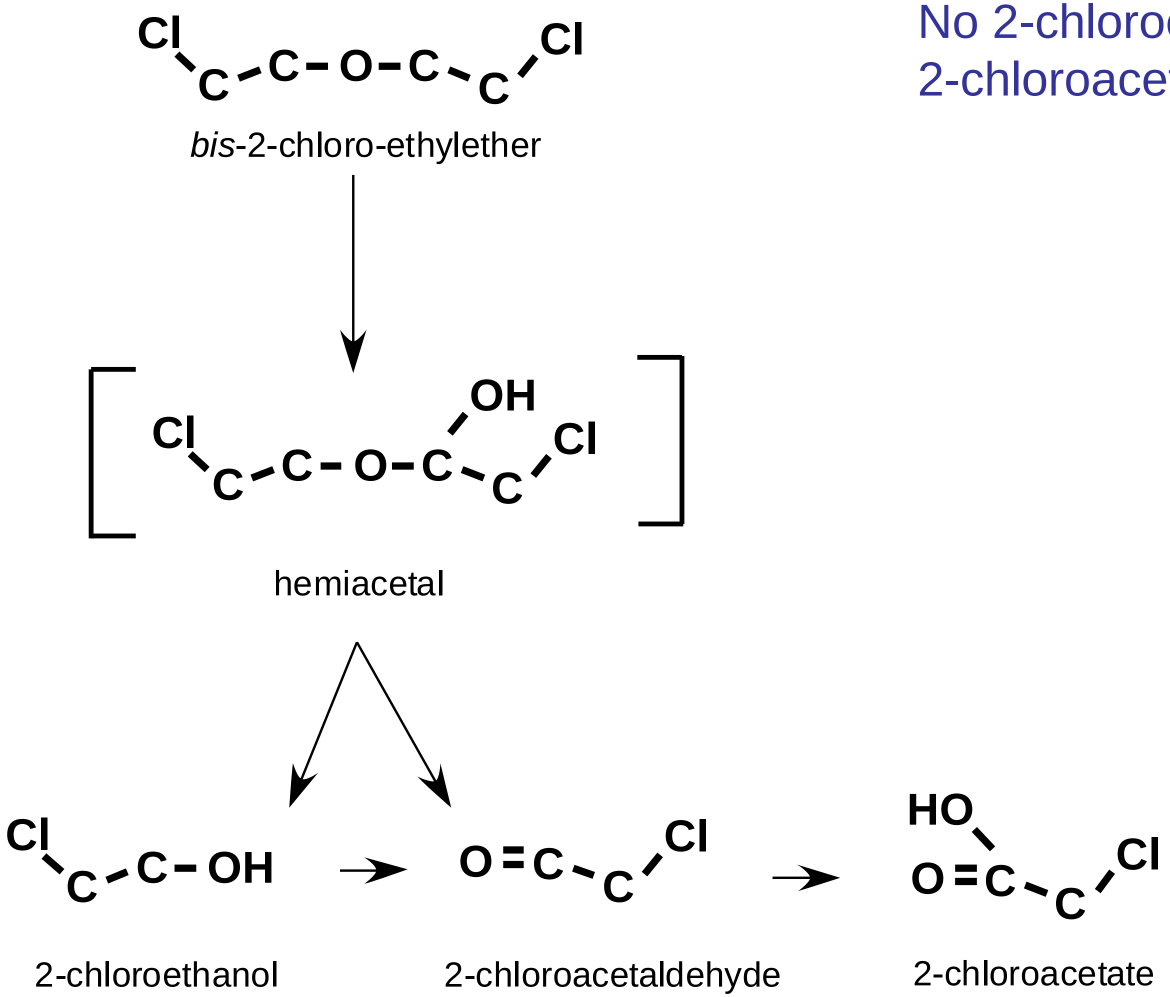
Growth of ENV481 on BCEE



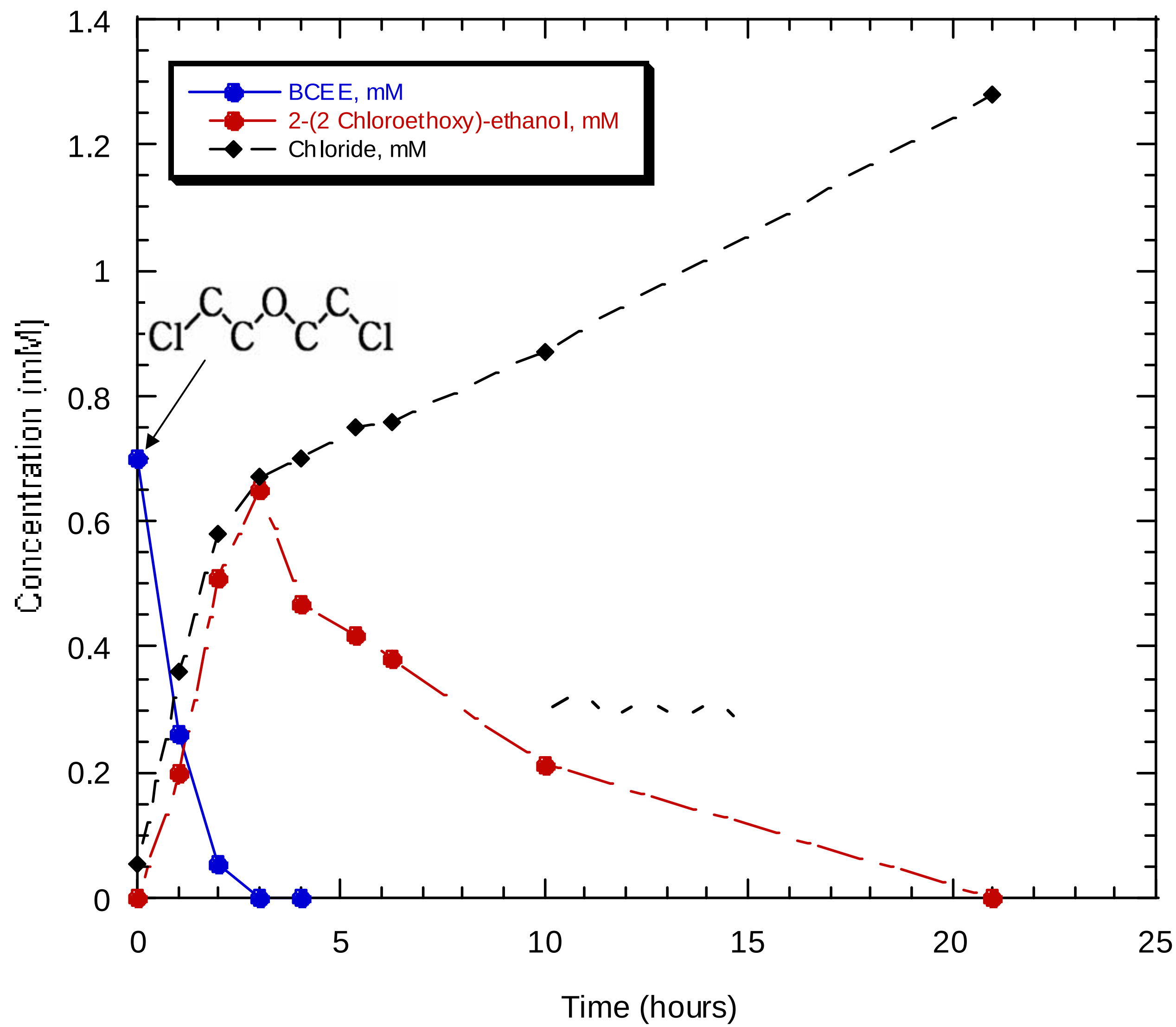
Possible Pathways for BCEE Degradation by *Xanthobacter* sp. ENV481

Monooxygenase pathway

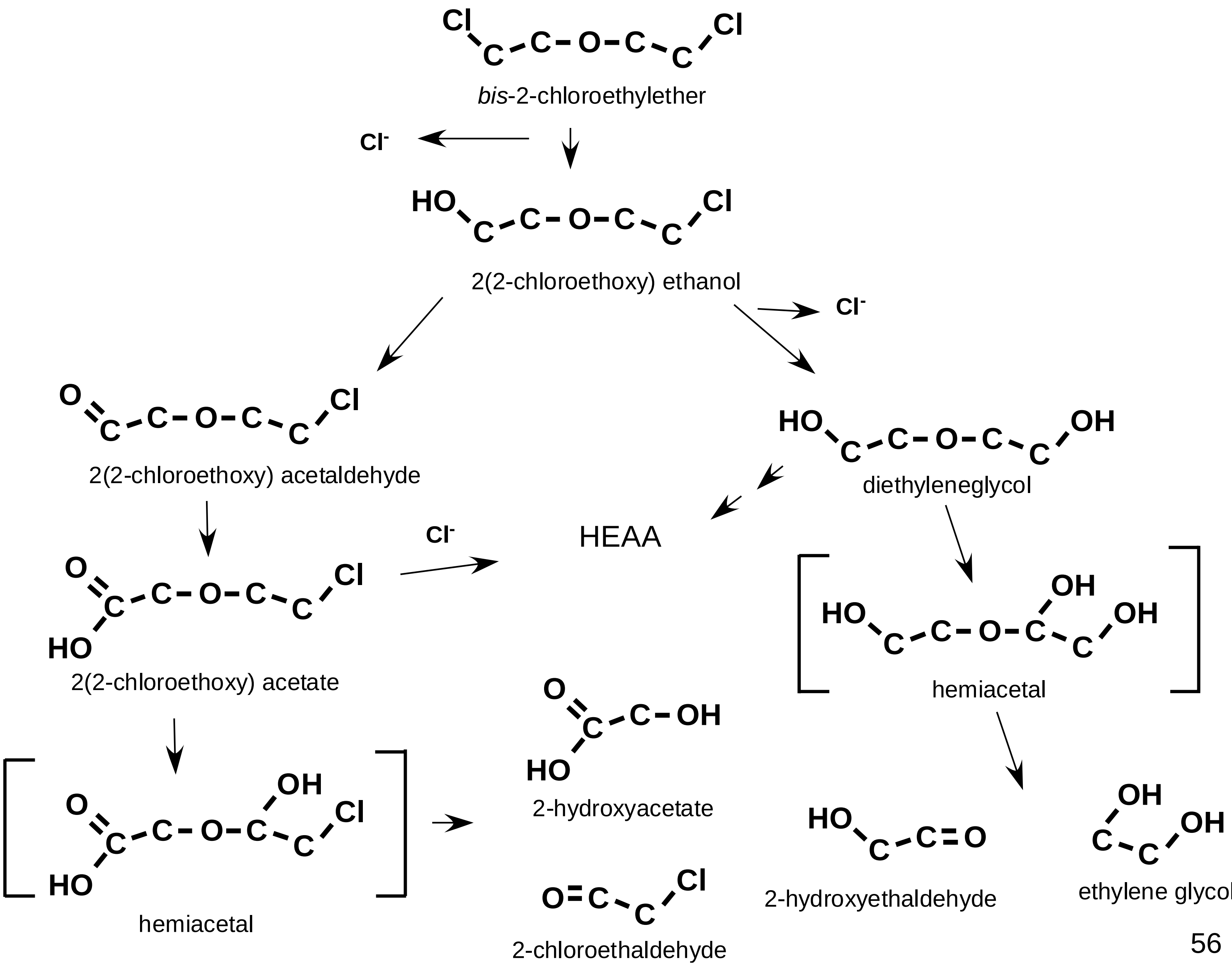
No 2-chloroethanol or
2-chloroacetaldehyde produced



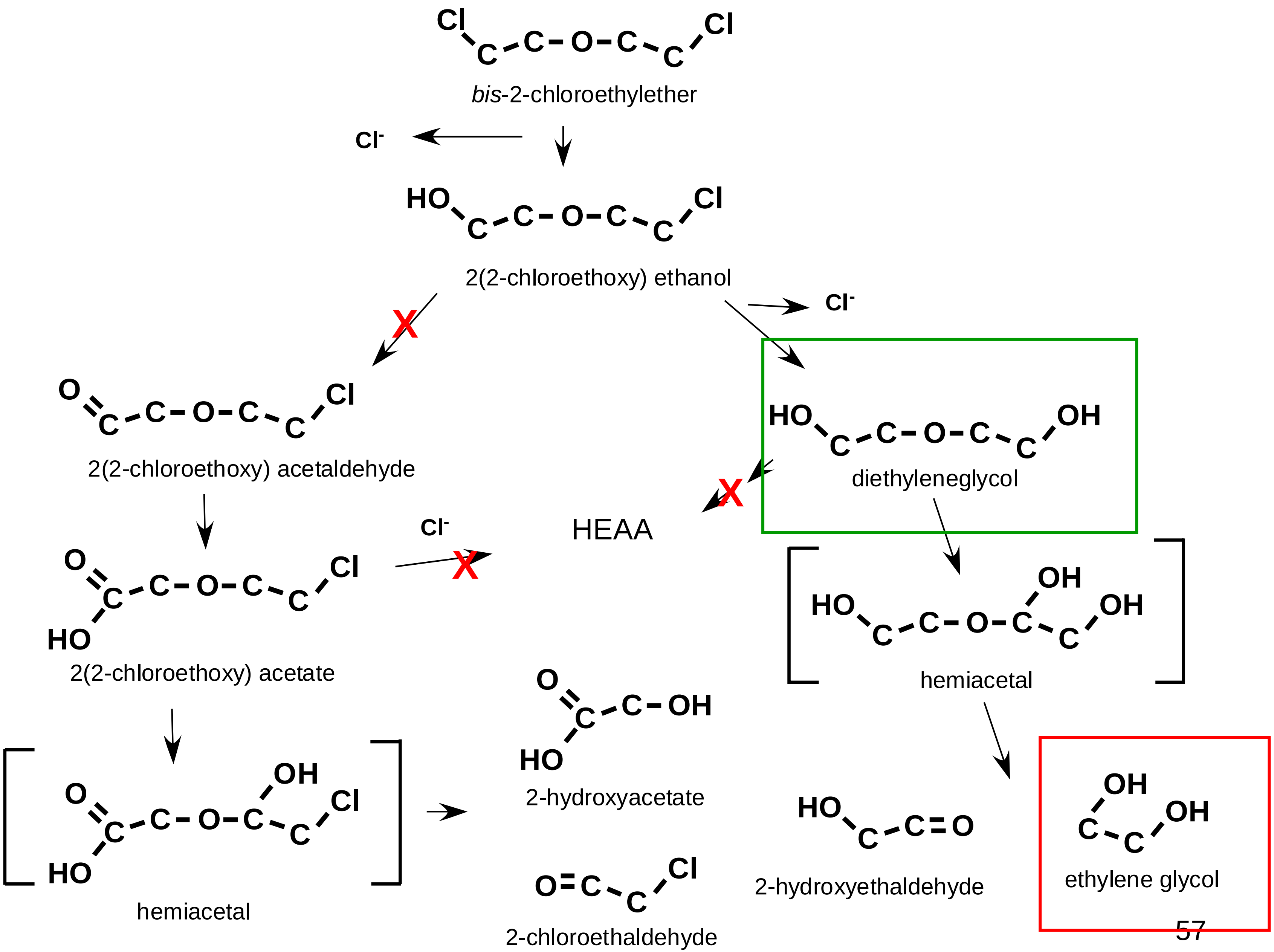
BCEE Degradation Product Analysis



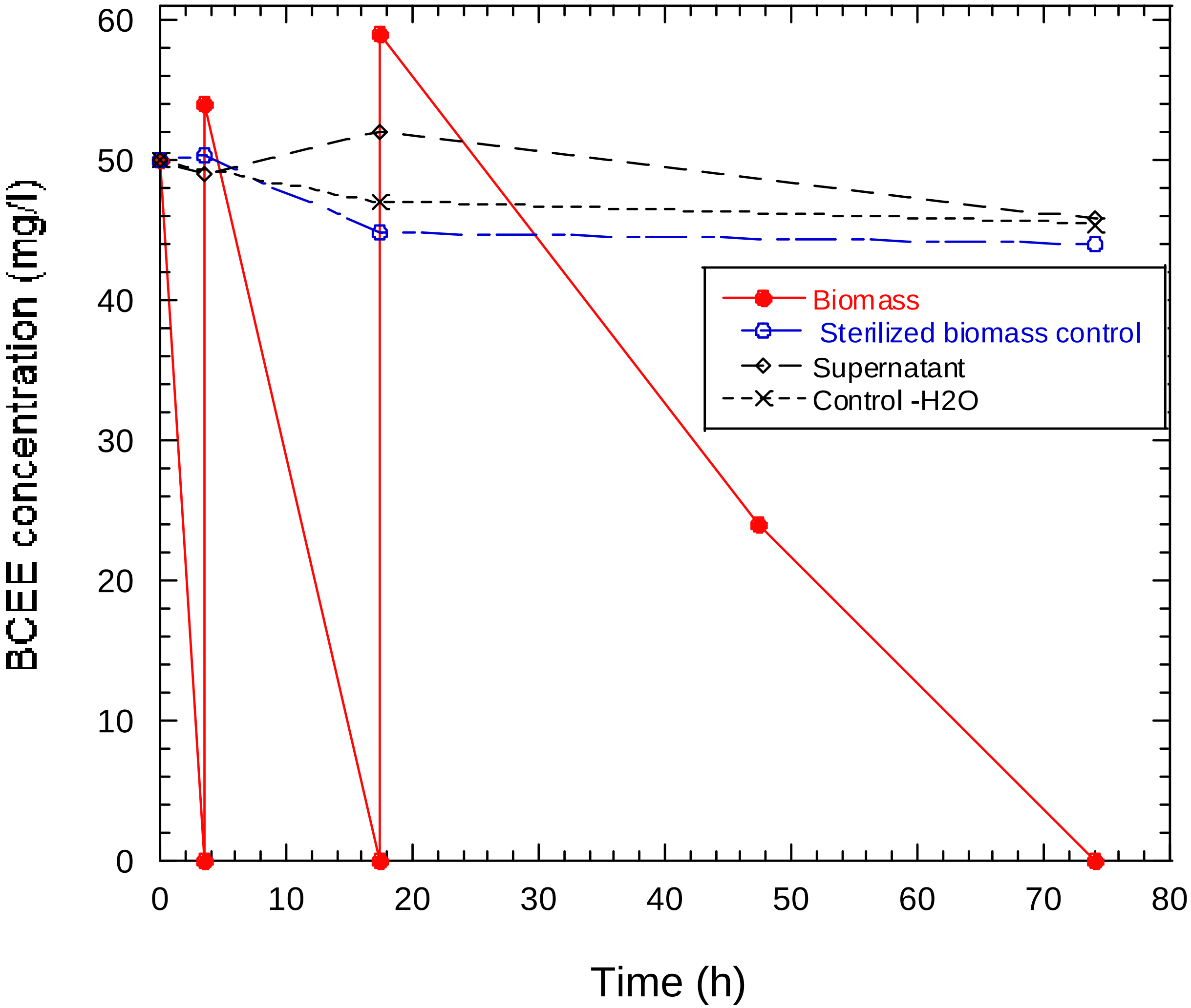
Possible Pathways for BCEE Degradation by *Xanthobacter* sp. ENV481



Possible Pathways for BCEE Degradation by *Xanthobacter* sp. ENV481



Degradation of BCEE by ENV481: Anaerobic



2(2-chloroethoxy) ethanol and diethylene glycol detected at 35 hr.

BCEE Summary

- Degraded by *Xanthobacter sp.* strain ENV481
  - Apparent hydrolytic dehalogenase
  - Aerobic or anaerobic

- Degraded by *Pseudonocardia* sp. strain ENV478
  - Unknown Monooxygenase
  - O-dealkylation mechanism

Ether Conclusions

- Many Xenobiotic and Natural Ethers
- A Few Common Degradation Mechanisms
- Anaerobic Mechanisms Less Understood

Questions?

"If I know the answer, I will tell you the answer.
If I don't, I will just respond, cleverly"

Donald Rumsfeld