
Biological and Geochemical Context for Monitored Natural Attenuation

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Biological Processes

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Natural Attenuation of Petroleum Hydrocarbons in Ground Water

John T. Wilson

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National Risk Management Research Laboratory
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Patterns of Natural Bioremediation

- Limited by supply of a soluble electron acceptor
 - Aerobic respiration
 - Nitrate reduction
 - Sulfate reduction
- Controlled by mixing processes (bioplume)

Patterns of Natural Attenuation

- Limited by biological activity
 - Iron reduction
 - Methanogenesis
 - Sulfate reduction
- First-order kinetics

Patterns of Natural Attenuation

- Limited by supply of electron donor
- Reductive dechlorination
- Controlled by supply of electron donor

Lines of Evidence

- Documented loss of contaminants at the field scale
- Geochemical indicators
- Laboratory microcosm studies, accumulation of metabolic end-products, volatile fatty acids, FAME

Documented Occurrence of Natural Attenuation

- Use geochemical data to support natural attenuation
- Trends during biodegradation (plume interior vs. background concentrations)
 - Dissolved oxygen concentrations below background
 - Nitrate concentrations below background
 - Iron (II) concentrations above background
 - Sulfate concentrations below background
 - Methane concentrations above background

Total Assimilative Capacity

Calculation of BTEX destroyed from changes in the concentrations of :

Oxygen
Nitrate
Iron II
Sulfate
Methane

Total Assimilative Capacity

Calculations are most appropriately used to rationalize degradation of BTEX that appears to have already happened in the field

Calculations are usually not appropriate to predict future degradation of BTEX in existing contamination

Total Assimilative Capacity

Calculations reveal:

Assimilative Capacity that was used

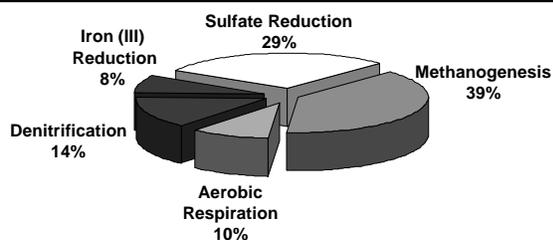
Not Assimilative Capacity remaining

Total Assimilative Capacity

Oxygen	=	1,920 µg/L
Denitrification	=	1,680 µg/L
Iron Reduction	=	2,550 µg/L
Sulfate reduction	=	21,000 µg/L
Methanogenesis	=	2,560 µg/L

Total Assimilative Capacity = 29,710 µg/L

Relative Importance of Biodegradation Mechanisms at 25 Fuel Spill Sites



Total Assimilative Capacity

Greatest sources of error:

Under-estimates contribution of iron reduction.

Assumes all the electron acceptor demand is BTEX.

Native organic matter (TOC) may have an important electron acceptor demand.

Natural Attenuation of Oxygenates in Ground Water

John T. Wilson

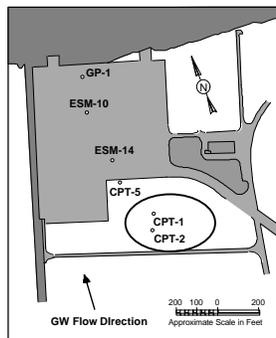
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Natural Attenuation of MTBE in Ground Water

Natural Attenuation of MTBE in Ground Water
under methanogenic conditions

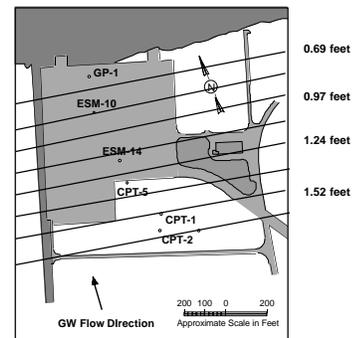
Depletion of MTBE and Benzene down gradient
of the source area at the U.S. Coast Guard
Support Center at Elizabeth City, N.C.

The source is a spill of JP-4 jet fuel from an old
fuel farm in the flood plain of the Pasquotank
River. The source area is located on the
following map



Elizabeth City, North Carolina

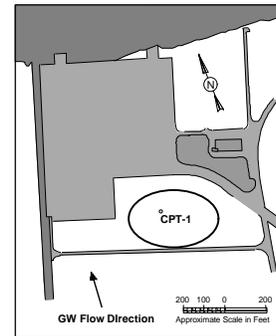
Source



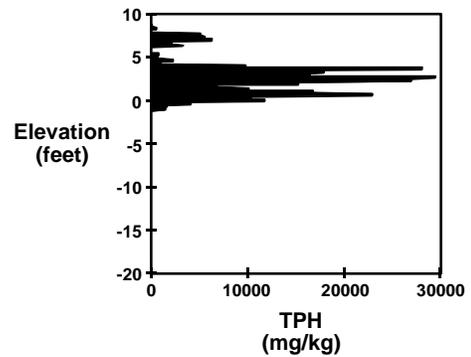
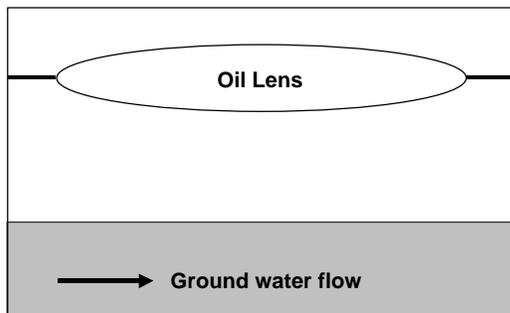
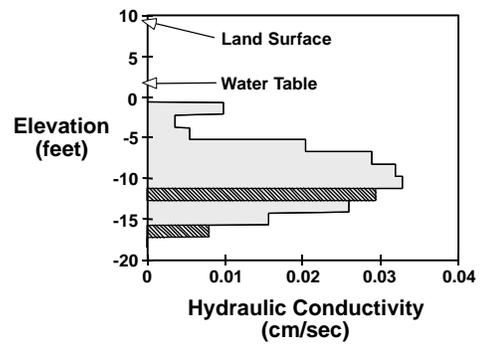
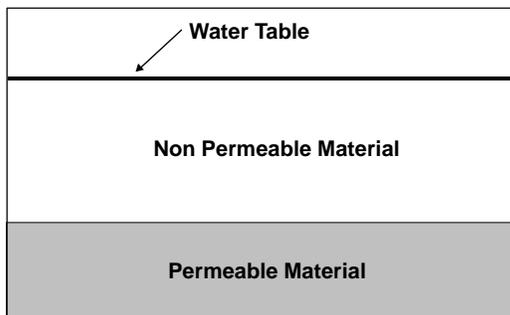
Elizabeth City, North Carolina

Natural Attenuation of MTBE in Ground Water under methanogenic conditions

Conditions in the source area (CPT-1)

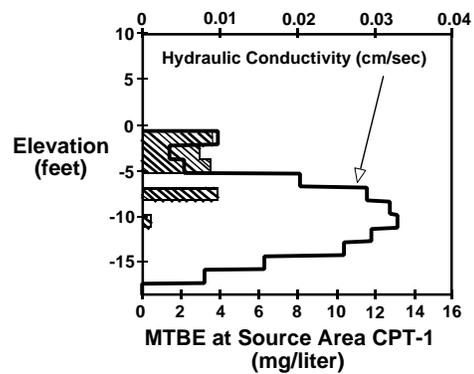
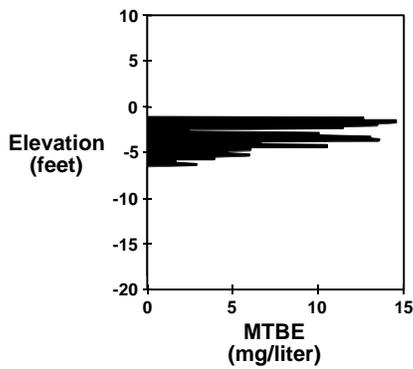
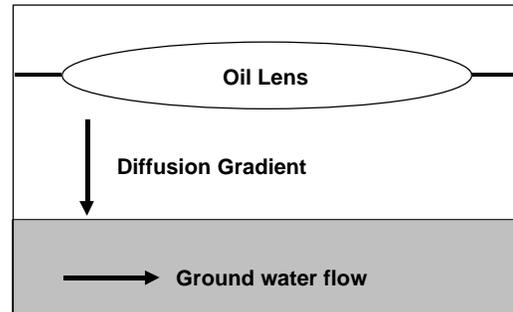


Elizabeth City, North Carolina



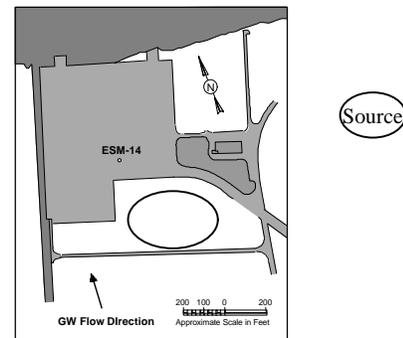
In many floodplain landscapes, the most important transfer of contaminants from LNAPL to ground water is through diffusion from the LNAPL to transmissive layers in the aquifer, rather than through dissolution and direct advection.

This suggests an approach to estimate the impact of spills of petroleum hydrocarbons on ground water.

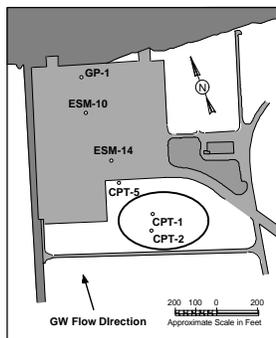
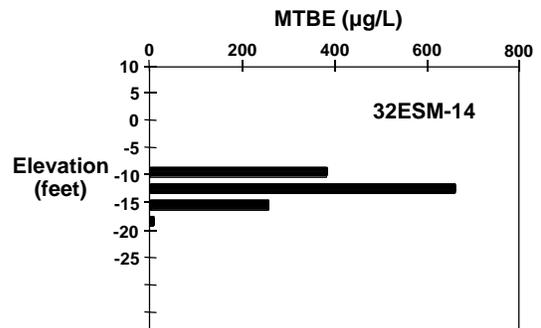
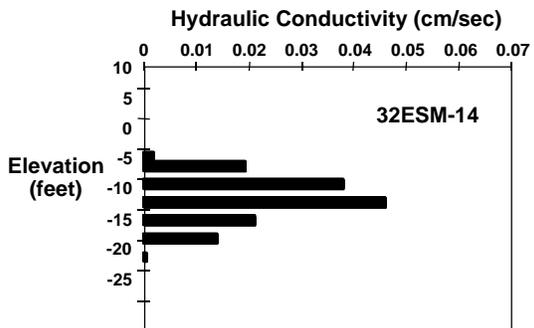


Natural Attenuation of MTBE in Ground Water under methanogenic conditions

Conditions down gradient of the source area, beyond the edge of the LNAPL at ESM-14



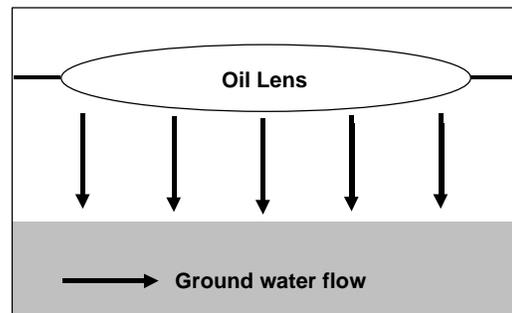
Elizabeth City, North Carolina

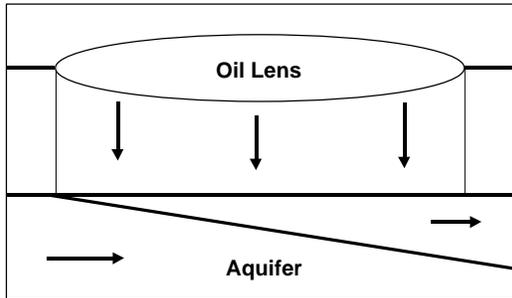


Elizabeth City, North Carolina

Location	MTBE	Benzene	Methane
	------(mg/liter)-----		
CPT-2	0.47	0.033	0.57
CPT-1	3.9	2.3	6.1
CPT-5	0.71	1.6	10.6
ESM-14	0.38	0.39	9.2
ESM-10	0.024	0.47	8.5
GP-1	0.001	0.015	2.3

Location	DO	Sulfate	Nitrate	Iron II
	------(mg/liter)-----			
CPT-2	1.3	35.3	<0.1	2.6
CPT-1	0.0	10.9	<0.1	22.8
CPT-5	0.0	<0.1	<0.1	47.3
ESM-14	0.1	<0.1	<0.1	91.3
ESM-10	1.1	<0.1	<0.1	68.8
GP-1	0.1	<0.1	<0.1	91.5





Natural Attenuation of MTBE in Ground Water under methanogenic conditions

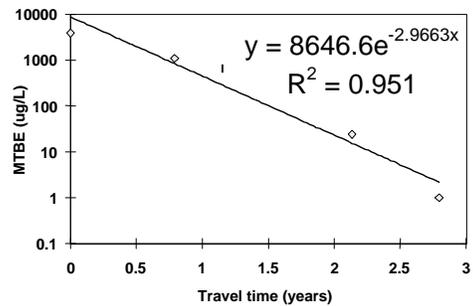
By the time ground water had moved entirely underneath the LNAPL, soluble electron acceptors were depleted, Methane and Iron II were accumulating, and the ground water contained high concentrations of MTBE and BTEX.

Natural Attenuation of MTBE in Ground Water under methanogenic conditions

The highest hydraulic conductivity and the hydraulic gradient were used to estimate travel time between monitoring locations along the flow path.

A linear regression of the Natural Logarithm of MTBE concentration against time of travel predicts a first order rate in the field of

-3.0 per year.



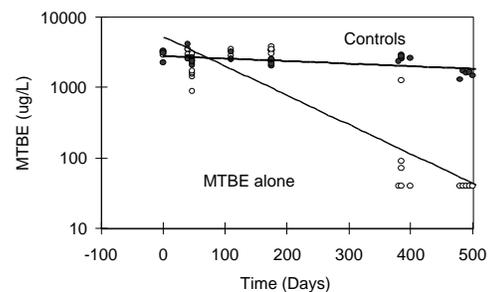
Natural Attenuation of MTBE in Ground Water under methanogenic conditions

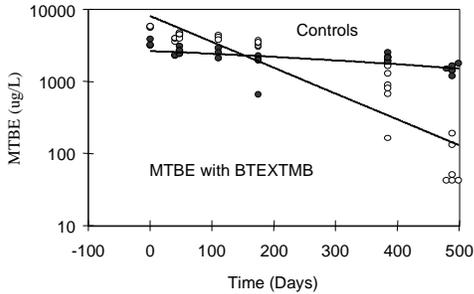
Core material was acquired from the more conductive depth intervals at location MW-14.

Microcosms were constructed with:

MTBE alone, and an autoclaved control

MTBE plus BTEX, and an autoclaved control





Rate of Natural Biodegradation of MTBE under methanogenic conditions in microcosms

Treatment	Rate	Upper 95%	Lower 95%
MTBE alone	-3.21	-3.72	-2.70
MTBE plus BTEXTMB	-2.62	-2.95	-2.30

Rates of removal in controls subtracted

Natural Attenuation of MTBE in Ground Water under methanogenic conditions

The rate of attenuation in the field is in good agreement with the rate in laboratory.

At this site, the rate of attenuation was rapid.

Elizabeth City, N.C., Old Fuel Farm
Exposure: Decades

Geochemistry Strongly Methanogenic

MTBE Degradation rate 2 to 3 per year

Elizabeth City, N.C. Fire Station Spill

A leak from a buried pipeline, about 1/2 mile from the fuel farm site.

Exposure < 10 years

Geochemistry is Sulfate Reducing, no Methane

MTBE Degradation in Field 0.47 per year

East Patchogue, NY

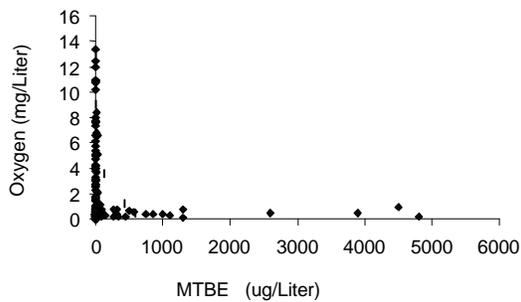
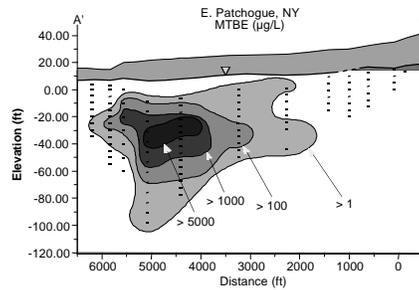
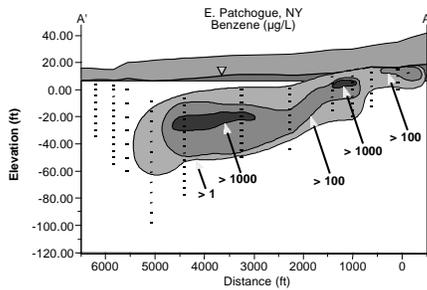
Glacial Sands on Long Island

Hydraulic Conductivity 0.05 to 0.10 cm/sec, or 40 to 80 feet/day

Release after 1979, tanks removed 1988

Geochemistry No Oxygen where MTBE is present, little Methane

MTBE is persistent



East Patchogue, NY
 Glacial Sands on Long Island
 Where oxygen is present in the ground water (>1.0 mg/L), MTBE is absent (<20 ug/Liter)
 MTBE exists in a "shadow" of depleted oxygen, down gradient from the spill.
 No Oxygen, No Methane, No MTBE degradation

Location CFB, Ontario
 Exposure A few years
 Geochemistry No Oxygen
 No Nitrate
 MTBE Degradation None apparent

Location CFB, Ontario
 Exposure A few more years
 Geochemistry Mixed in Oxygen
 MTBE Degradation Gone?

Location CFB, Ontario

Exposure A few more years

MTBE Degradation at Field Scale
 0.44 per year

MTBE Degradation in Aerobic
Microcosms
 2.4 per year

Location Sampson Co, N.C.

Exposure Many years

Geochemistry Iron Reducing
 No Methane

MTBE Degradation in Field
 0.0, 0.3 and 0.4 per year

MTBE Degradation in Aerobic Microcosms
 2.4 per year

Aerobic Degradation of MTBE in Microcosms is much more Rapid than at Field Scale

Aerobic Degradation may be controlled by the Kinetics of Re-oxygenation, not the Kinetics of Biodegradation.

Kinetics of Aerobic Biodegradation may be Specific to the Geochemistry and Geometry of the MTBE plume.

Location Sampson Co, N.C.

Exposure Many years

Geochemistry Iron Reducing
 No Methane

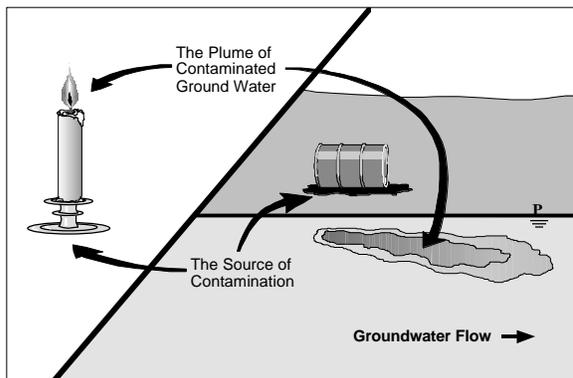
MTBE Degradation in Field
 0.0, 0.3 and 0.4 per year

MTBE Degradation in Aerobic Microcosms
 2.4 per year

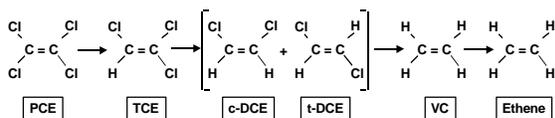
Natural Attenuation of Chlorinated Solvents in Ground Water

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Mechanism of Chloroethene Biotransformation



Reductive dehalogenation:

- Oxidation/reduction reaction where electrons are transferred from donor to chlorinated hydrocarbon acceptor

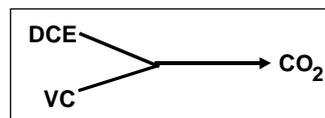
Co-metabolic process:

- Organisms growing on alternate carbon sources

Primary substrates:

- Potential for natural (soil organic matter) and anthropogenic sources

Alternate Pathways for Chloroethene Biotransformation



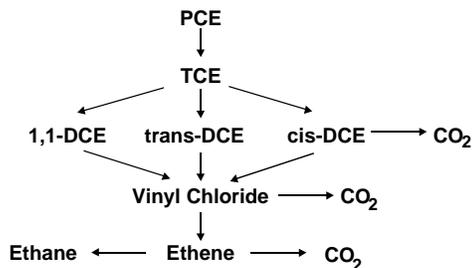
Oxidative biodegradation:

- Vinyl chloride shown to biodegrade under aerobic conditions
- Fe reducers may also oxidize vinyl chloride

Supporting evidence:

- Transport properties (migration) of DCE and VC relative to TCE
- Aerobic biodegradation of vinyl chloride to CO₂ demonstrated in microcosms

Native Biotransformations for Chloroethenes



Requirements for Reductive Dechlorination

- Primary substrate
 - Native organic carbon, BTEX, landfill leachate, etc.
- Strongly reducing conditions
 - Generally need methanogenic conditions

Behavior of Chlorinated Solvent Plumes

- Type 1 Behavior
 - Primary substrate is anthropogenic organic carbon
 - Solvent plume degrades
- Type 2 Behavior
 - Primary substrate is native organic carbon
 - Solvent plume degrades
- Type 3 Behavior
 - Low native organic carbon concentrations
 - Low anthropogenic organic carbon concentrations
 - PCE, TCE and DCE? do not degrade

Type 1 Behavior

- Primary substrate is anthropogenic organic carbon
 - BTEX, landfill leachate, etc.
- Anthropogenic organic carbon drives dechlorination
- Questions
 - Does electron acceptor supply exceed demand? (i.e., is electron acceptor supply adequate?)
 - Will plume strangle before it starves?
 - What is role of competing electron acceptors?
 - Do PCE, TCE and DCE dechlorinate?
 - Is vinyl chloride oxidized?
 - Is biodegradation rate adequate?

Type 2 Behavior

- Primary substrate is native organic carbon
- Native organic carbon drives dechlorination
- Questions
 - Does electron acceptor supply exceed demand? (i.e., is electron acceptor supply adequate?)
 - Will plume strangle before it starves?
 - What is role of competing electron acceptors?
 - Do PCE, TCE and DCE dechlorinate?
 - Is vinyl chloride oxidized?
 - Is biodegradation rate adequate?

Type 3 Behavior

- Low native organic carbon concentrations
- Low anthropogenic organic carbon concentrations
- Dissolved oxygen (and nitrate) concentration(s) greater than 1.0 mg/L (oxygenated system)
- Reductive dechlorination will not occur
 - Highly halogenated compounds such as PCE and TCE will not degrade
- DCE (?) and VC may be oxidized

Natural Attenuation of Metals in Ground Water

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Factors Affecting the Concentration of Metals in Solution

ion exchange and adsorption
oxidation or reduction reactions
precipitation and dissolution of solids
acid-base reactions
complex formation

Factors Affecting the Concentration of Metals in Solution

ion exchange and adsorption

Cadmium	Copper
Lead	Mercury I and II
Nickel	Zinc

Factors Affecting the Concentration of Metals in Solution

ion exchange and adsorption
relative order of sorption, in general

Lead > Copper > Zinc > Cadmium > Nickel
Sandy Aquifers are particularly vulnerable to Cadmium and Nickel

Concentration of Metal in Solution

In the most simple form, described by
Distribution Coefficient

$K_d = \frac{\text{Concentration on Solids}}{\text{Concentration in water}}$

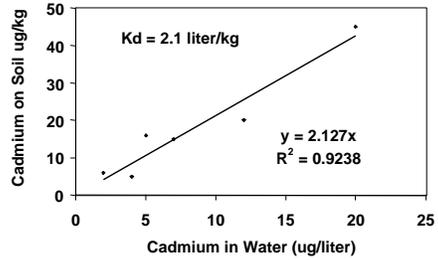
Cadmium and Nickel Distribution Coefficients for Sandy Aquifer Materials

Christensen et al, Journal of Contaminant Hydrology 24(1996):75-84

Sorption isotherms for Cadmium and Nickel in 18 samples of sandy aquifer material from 12 locations in Denmark, at pH ranging from 4.9 to 8.9

Concentration of Metals in Solution

Example sorption isotherm for Cadmium in Sandy aquifer material from Denmark, pH 4.9

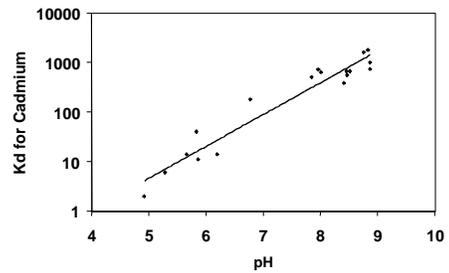


Factors Affecting the Concentration of Metals in Solution

ion exchange and adsorption

K_d is sensitive to the pH of the Ground Water

Effect of pH on K_d for Cadmium in core material from 28 sandy aquifers in Denmark

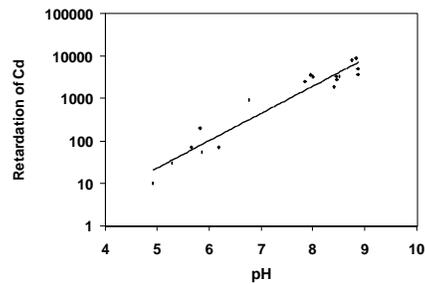


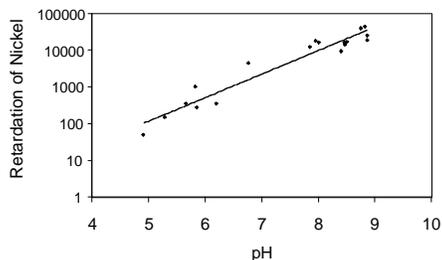
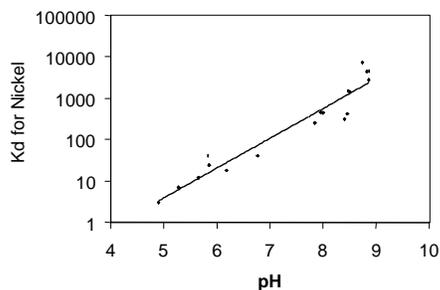
Concentration of Metal in Solution

$$K_d = \frac{\text{Concentration on Solids}}{\text{Concentration in water}}$$

If bulk density = 1.6 kg/liter
and water-filled porosity = 0.32
and $K_d \gg 1.0 \text{ liter/kg}$;

$$\text{Retardation} = 5 (K_d)$$





Factors Affecting the Concentration of Metals in Solution

ion exchange and adsorption

In neutral or alkaline ground water, simple sorption makes a substantial contribution to natural attenuation of metals that are multivalent cations, even in sandy aquifers

Factors Affecting the Concentration of Metals in Solution

oxidation or reduction reactions

Particularly important for Arsenic, Chromium and Manganese

Factors Affecting the Concentration of Metals in Solution

oxidation or reduction reactions

Under anaerobic conditions, Arsenic V (AsO_4^{-3} or Arsenate) may serve as an alternate electron acceptor and be reduced to Arsenic III (AsO_2^{-1} or Arsenite) by natural biological activity.

Factors Affecting the Concentration of Metals in Solution

oxidation or reduction reactions

Manganese salts of Manganese IV may also be reduced to Manganese II (Mn^{+2}).

Factors Affecting the Concentration of Metals in Solution

oxidation or reduction reactions

Arsenite and Mn^{+2} are more toxic than Arsenate or Mn^{+4} , are more soluble, and more mobile in ground water.

Factors Affecting the Concentration of Metals in Solution

oxidation or reduction reactions

Under aerobic conditions, Arsenic III (AsO_2^{-1} or Arsenite) and Manganese II (Mn^{+2}) may be oxidized back to Arsenic V (AsO_4^{-3} or Arsenate) and Manganese IV by natural biological activity.

Factors Affecting the Concentration of Metals in Solution

oxidation or reduction reactions

Chromium VI exists as an oxyanion, as bichromate $HCrO_4^-$ below pH 6.5
chromate CrO_4^{-2} near pH 6.5
and dichromate $Cr_2O_7^{-2}$ at concentrations greater than 10 mM.

Factors Affecting the Concentration of Metals in Solution

oxidation or reduction reactions

Chromium VI is mobile in ground water, and is a greater health hazard than Chromium III

Factors Affecting the Concentration of Metals in Solution

oxidation or reduction reactions

Chromium III is a cation, that tends to bind strongly to aquifer material

Factors Affecting the Concentration of Metals in Solution

oxidation or reduction reactions

Dissolved Organic Matter in the ground water will reduce Chromium VI to Chromium III, making it effectively immobile.

Factors Affecting the Concentration of Metals in Solution

oxidation or reduction reactions

Oxidized forms of Manganese in the aquifer matrix material will oxidize Chromium III back to Chromium VI

Factors Affecting the Concentration of Metals in Solution

oxidation or reduction reactions

The equilibrium concentration of Chromium VI, and therefore the natural attenuation of chromium, is controlled by the competition between the oxidation and reduction reactions.

Factors Affecting the Concentration of Metals in Solution

oxidation or reduction reactions

The natural attenuation of chromium, is site specific, and must be confirmed by monitoring

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Geochemical Processes

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Geochemical Processes and Natural Attenuation

U.S. Geological Survey

Why is Geochemistry Important to Natural Attenuation?

- ❖ Ground-water geochemistry is a record of ongoing chemical, physical, and microbial processes.
- ❖ Ergo: The efficiency of natural attenuation can often be determined from ground-water chemistry information (redox conditions).

What is a redox process?

- ❖ Electrons are transferred in chemical or biochemical reactions.
- ❖ Benzene + O₂ → CO₂ + e⁻

*In a redox reaction, one
compound donates an electron
and another compound accepts
an electron:*

- ❖ Benzene + O₂ → CO₂ + e⁻ (Benzene is electron donor)
- ❖ e⁻ + TCE → DCE + Cl⁻ (TCE is electron acceptor)

*The flow of electrons from donors
to acceptors is capable of doing
work.*

- ❖ Microorganisms (and everybody else) uses the work done by flowing electrons to sustain life functions.

*Biodegradation of Petroleum
Hydrocarbons are electron-
donating processes.*

- ❖ Benzene → CO₂ + e⁻ (benzene donates e⁻)
- ❖ 2e⁻ + O₂ → 2H₂O (Oxygen accepts e⁻)
Electron
Acceptor

Because the biodegradation of petroleum hydrocarbons are electron donating processes:

- ❖ The availability of electron acceptors determines the rate and extent of biodegradation.

- Oxygen
- Fe(III)
- sulfate
- CO₂
- Chlorinated solvents

Biodegradation of Benzene Consumes Dissolved Oxygen

- ❖ Low concentrations of dissolved oxygen are associated with benzene biodegradation coupled to oxygen reduction.

Biodegradation of Benzene Produces Dissolved Iron

- ❖ High concentrations of dissolved iron are associated with benzene biodegradation coupled to iron reduction.

Benzene Oxidation Aerobic Respiration

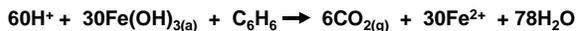


$${}^2G_r = -3566 \text{ kJ/mole benzene}$$

$$\text{Mass Ratio of O}_2 \text{ to C}_6\text{H}_6 = 3.1:1$$

$$0.32 \text{ mg/L C}_6\text{H}_6 \text{ degraded per mg/L O}_2 \text{ consumed}$$

Benzene Oxidation Iron Reduction



$${}^2G_r = -2343 \text{ kJ/mole benzene}$$

$$\text{Mass Ratio of Fe}(\text{OH})_3 \text{ to C}_6\text{H}_6 = 41:1$$

$$\text{Mass Ratio of Fe}^{2+} \text{ produced to C}_6\text{H}_6 \text{ degraded} = 15.7:1$$

$$0.06 \text{ mg/L C}_6\text{H}_6 \text{ degraded per mg/L Fe}^{2+} \text{ produced}$$

Benzene Oxidation Sulfate Reduction



$${}^2G_r = -340 \text{ kJ/mole benzene}$$

$$\text{Mass Ratio of SO}_4^{2-} \text{ to C}_6\text{H}_6 = 4.6:1$$

$$0.22 \text{ mg/L C}_6\text{H}_6 \text{ degraded per mg/L SO}_4^{2-} \text{ consumed}$$

Biodegradation of Benzene Consumes Sulfate

- ❖ Low concentrations of dissolved sulfate are associated with benzene biodegradation coupled to sulfate reduction.
- ❖ High concentrations of H₂S

Biodegradation of Benzene Produces Methane

- ❖ High concentrations of methane are associated with benzene biodegradation coupled to methanogenesis.

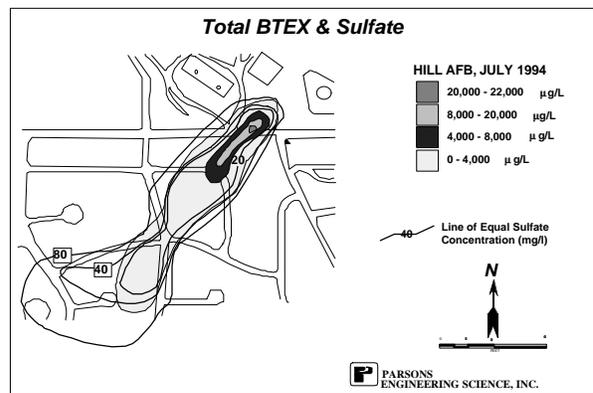
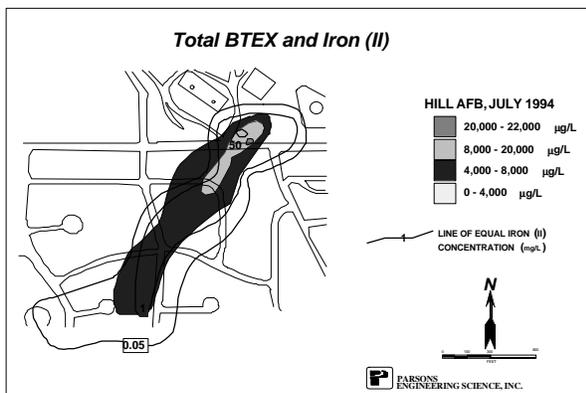
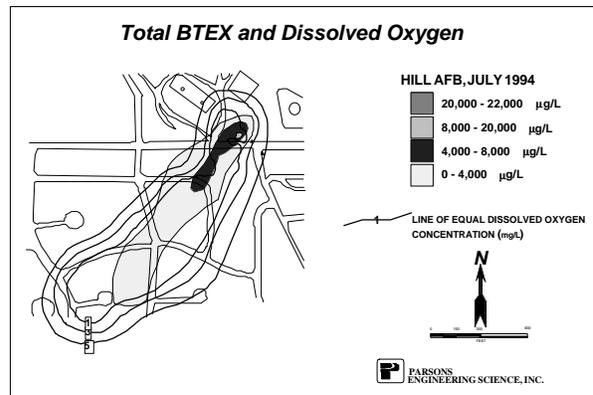
Benzene Oxidation Methanogenesis

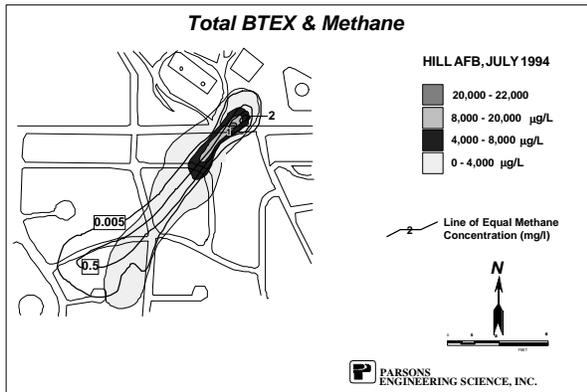


$$\Delta G^\circ_r = -135.6 \text{ kJ/mole benzene}$$

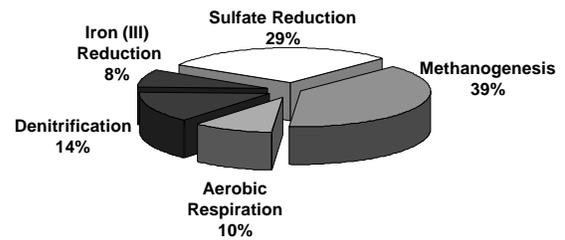
Mass Ratio of CH₄ produced to C₆H₆ = 0.8:1

1.25 mg/L C₆H₆ degraded per mg/L CH₄ produced





Relative Importance of Biodegradation Mechanisms at 25 Sites



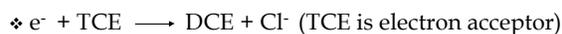
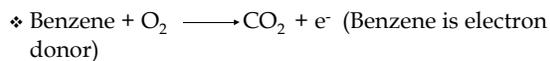
Geochemical Data Can Indicate:

- ❖ If biodegradation is occurring.
- ❖ If biodegradation has occurred in the past.
- ❖ If electron acceptors are available to support biodegradation in the future!

Redox Zonation and Biodegradation Efficiency

U.S. Geological Survey

*In a redox reaction, one
compound donates an electron
and another compound accepts
an electron:*



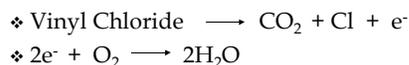
*The flow of electrons from donors
to acceptors is capable of doing
work.*

- ❖ Microorganisms (and everybody else)
uses the work done by flowing
electrons to sustain life functions.

*Biodegradation of Chlorinated ethenes
can be electron-accepting processes
(ie., reductive dechlorination).*



*Biodegradation of chlorinated
ethenes can also be electron-
donating processes (oxidation).*



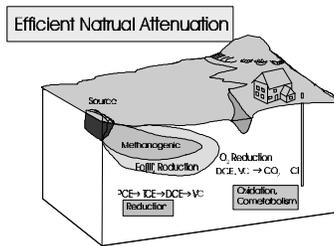
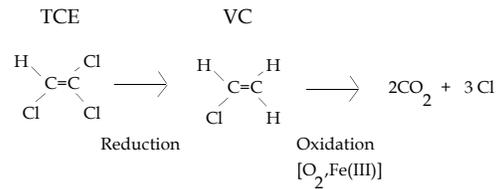
*Because of this complexity,
chlorinated ethenes do not behave
uniformly in ground-water
systems*

- ❖ Poly-Chlorinated ethenes will reduce
under reducing conditions.
❖ DCE and VC will oxidize under
oxidizing conditions.

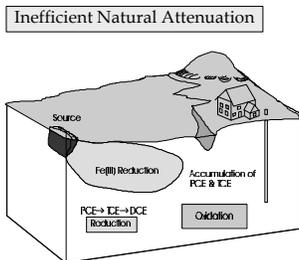
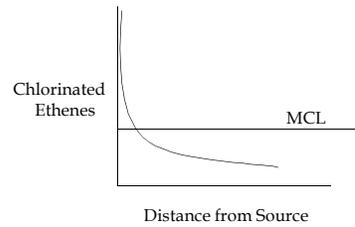
The Rate and Extent of Biodegradation Processes at any Given Site Depends Upon:

- ❖ Ambient Redox Conditions
- ❖ The Succession of Redox Conditions

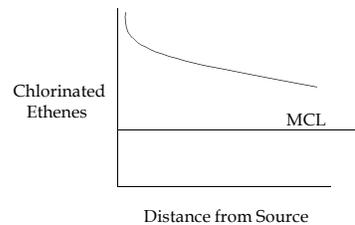
EXAMPLE
Sequential Reduction/Oxidation



Efficient NA leads to rapid decrease of contaminants away from source area.



Inefficient NA leads to gradual decrease of contaminants away from source area.



How can we quickly screen water chemistry data from a site in order to determine if chlorinated solvent biodegradation is possible?

Initial Screening Process

The screening process is designed to recognize reductive dechlorination of chlorinated solvents.

It presupposes that natural attenuation of chlorinated solvents in most plumes will be not be important unless the solvents are initially dechlorinated.

Analytical Parameters and Their Weighting for Preliminary Screening

Analysis	Condition	Value
Oxygen	< 0.5 mg/L	3
Oxygen	> 1.0 mg/L	-3
Nitrate	< 1 mg/L	2
Iron II	> 1 mg/L	3

Analytical Parameters and Their Weighting for Preliminary Screening

Oxygen is toxic to the organisms that carry out reductive dechlorination.

If it is present reductive dechlorination cannot occur.

Analytical Parameters and Their Weighting for Preliminary Screening

Analysis	Condition	Value
Sulfate	< 20 mg/L	2
Sulfide	> 1 mg/L	3
Methane	> 0.1 mg/L	2
	> 1.0 mg/L	3
Redox(Eh)	< +50 millivolts	1
	< -100 millivolts	2

Analytical Parameters and Their Weighting for Preliminary Screening

Analysis	Condition	Value
DOC	> 20 mg/L	2
Temp	> 20°C	1
CO ₂	> 2x background	1
Alkalinity	> 2x background	1

Analytical Parameters and Their Weighting for Preliminary Screening

Analysis	Condition	Value
Chloride	> 2x background	2
Hydrogen	> 1 nanomolar	3
VFA	> 0.1 mg/L	2
BTEX	> 0.1 mg/L	2

Analytical Parameters and Their Weighting for Preliminary Screening

Analysis	Condition	Value
Reduced daughter products TCE, DCE, vinyl chloride, chloroethane, chlorobenzene		2
Ethene	> 0.01 mg/L	2
	> 0.1 mg/L	3

Hypothetical Site #1

Analysis	Condition	Score
DO	0.1 mg/L	3
Nitrate	0.3 mg/L	2
Iron II	10 mg/L	3
Sulfate	2 mg/L	2

Hypothetical Site #1

Analysis	Condition	Score
Methane	5 mg/L	3
Redox	-190 millivolts	2
Chloride Background	45 mg/L 10 mg/L	2

Hypothetical Site #1

Analysis	Condition	Score
PCE (spilled)	1,000 µg/L	0
TCE (not spilled)	1,200 µg/L	2
cis-DCE	500 µg/L	2
Vinyl chloride	50 µg/L	2

Hypothetical Site #2

Analysis	Condition	Score
DO	3.0 mg/L	0
Nitrate	0.3 mg/L	2
Iron II	Not Detected	0
Sulfate	10 mg/L	2

Hypothetical Site #2

Analysis	Condition	Score
Methane	Not Detected	0
Redox	+100 millivolts	0
Chloride	15 mg/L	
Background	10 mg/L	0

Hypothetical Site #2

Analysis	Condition	Score
TCE (spilled)	1,200 µg/L	0
cis-DCE	< 1 µg/L	0
Vinyl chloride	< 1 µg/L	0

Interpretation of Results from Preliminary Screening

Total Score	Interpretation
0 to 5	Inadequate evidence
6 to 15	Limited evidence
16 to 20	Adequate evidence
over 20	Strong evidence

Interpretation of Results from Preliminary Screening

Hypothetical Site #1

23 total points - strong evidence

Hypothetical Site #2

4 total points - inadequate evidence

The Rate and Extent of Chlorinated Ethene Biodegradation Processes Depends Upon:

- ❖ Ambient Redox Conditions
- ❖ The Succession of Redox Conditions