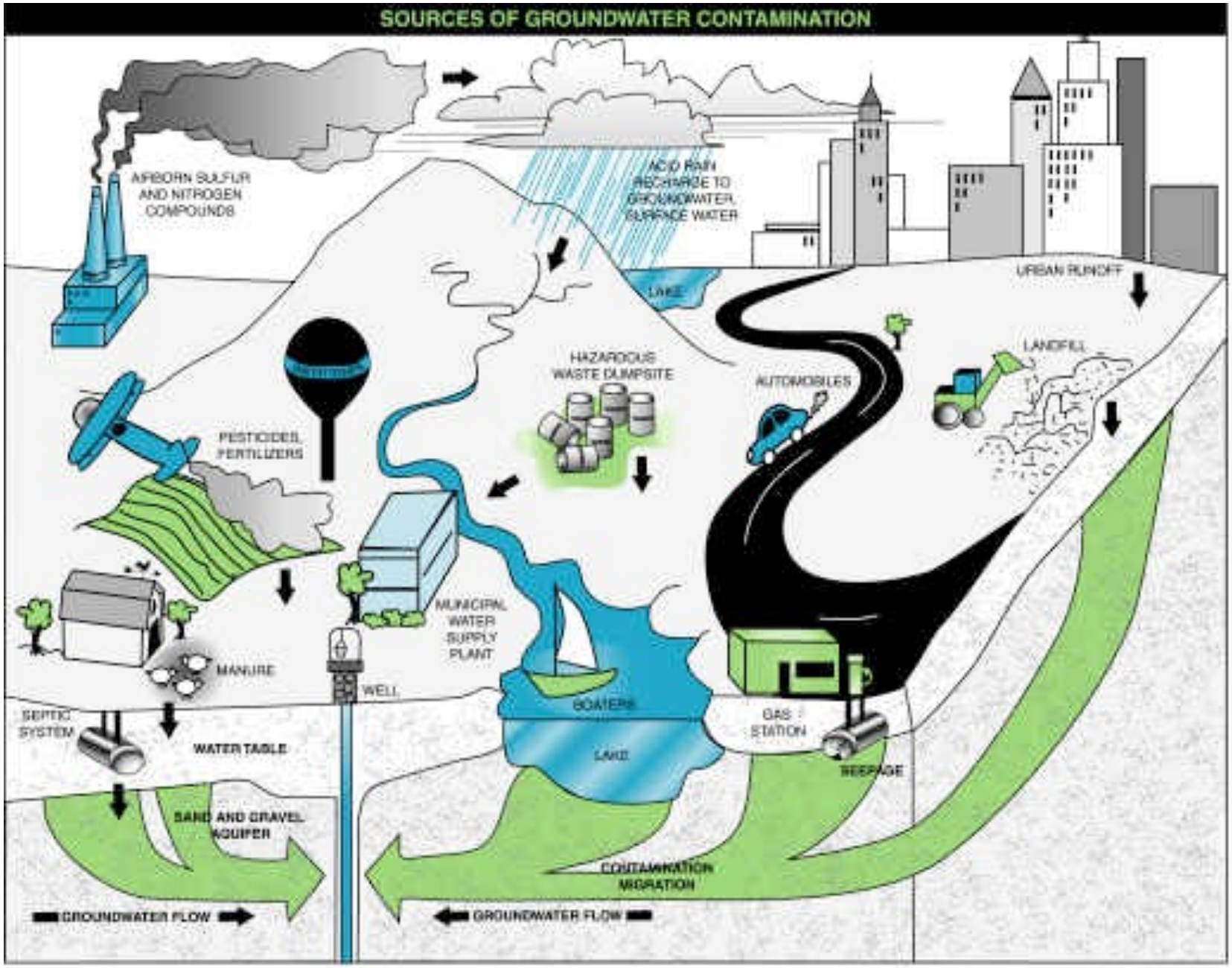
In situ bioremediation

Lecture 8

Learning outcomes

- *In situ* bioremediation groundwater
- Natural monitored attenuation

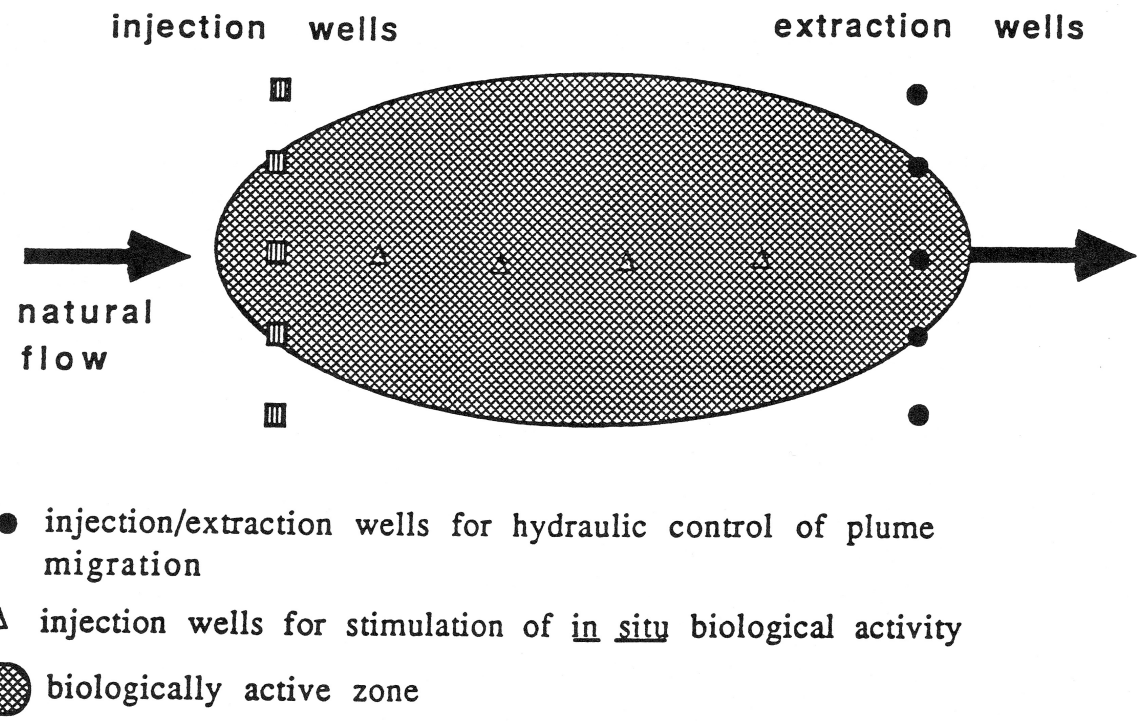
Groundwater contamination



Classical in situ bioremediation

Classical *in situ* bioremediation

Principle: pump nutrients-laden, O_2-saturated water up-gradient from plume, pump out water down-gradient and recycle



Classical *in situ* bioremediation

- Sometimes anaerobic processes favorable, injected water not O_2 saturated.
- Bio-stimulation
- Solvent enhanced
- Bio-augmentation

biostimulation

- As many bioremediation techniques, the key is to enhance activity of indigenous organisms
- Nutrient addition: combinations of N and P. Can use oleophilic fertilizer in the case of hydrophobic compound.
- e^- donor for cometabolism: degradation of a compound only in the presence of other organic material that serves as the primary enzyme source
  - TCE transformed by monooxygenase stimulated by methane or toluene
- e- donor that will change redox potential
  - Ex: molasses in aquifer to stimulate microbial activity and deplete O_2; may stimulate anaerobic processes such as reductive dechlorination and metal reduction
- Denitrifying bacteria are stimulated by C and N and that can degrade for ex. CT

Bio-stimulation

- Enhance the activity of indigenous organisms including those able to degrade pollutant
- Oxygen: important limitation
- Nutrients: N, P, K, S, Mg, Ca, Fe, trace
- Carbon source for inorganic pollutants (ethanol, lactate)
- Surfactants to increase contact between water-soluble nutrients and oil-soluble contaminants ==> microemulsions

Biostimulation

- Two types of goals:
  - create an anaerobic zone
  - maintain aerobic conditions in the active zone
- Anaerobic zone: promote reductive dechlorination and the immobilization of metals
- Aerobic zone: petroleum hydrocarbons, phenolic compounds, chlorinated compounds (chlorobenzene, methylene chloride, vinyl chloride degraded faster aerobically)

Biostimulation

- Primary substrate: can be an e^- donor (aerobically and anaerobically) or an e^- acceptor (respiration)
- Cometabolic (fortuitous): aerobically as an e^- donor (co-oxidation), anaerobically as an e^- acceptor (reductive dechlorination)
- Cometabolic reduction is catalyzed by reductases acting on other e^- acceptors

Table 4.4 Electron Donors That Have Been Used to Enhance Reductive Dechlorination and Relative Costs per lb of PCE[51–53]

Electron Donor	Bulk Price $/lb	$/lb of PCE
Soluble (Fast Release) Donors		
Methanol	0.05	0.64
Milk	0.05	0.18
Ethanol	0.20 – 0.25	NA
Molasses	0.20 – 0.35	0.16
Sugar (Corn Syrup)	0.25 – 0.30	0.40
Sodium Lactate	2.20	NA
Slow Release Donors		
Whey	0.05	0.04
Edible Oils	0.20 – 0.50	NA
Flour (Starch)	0.30	0.85
Cellulose	0.40 – 0.80	NA
Chitin	2.25 – 3.00	NA
Methyl Cellulose	4.00 – 5.00	NA
HRC™ (Regenesis Commercial Material)	12.00	NA

NA – Not Analyzed.

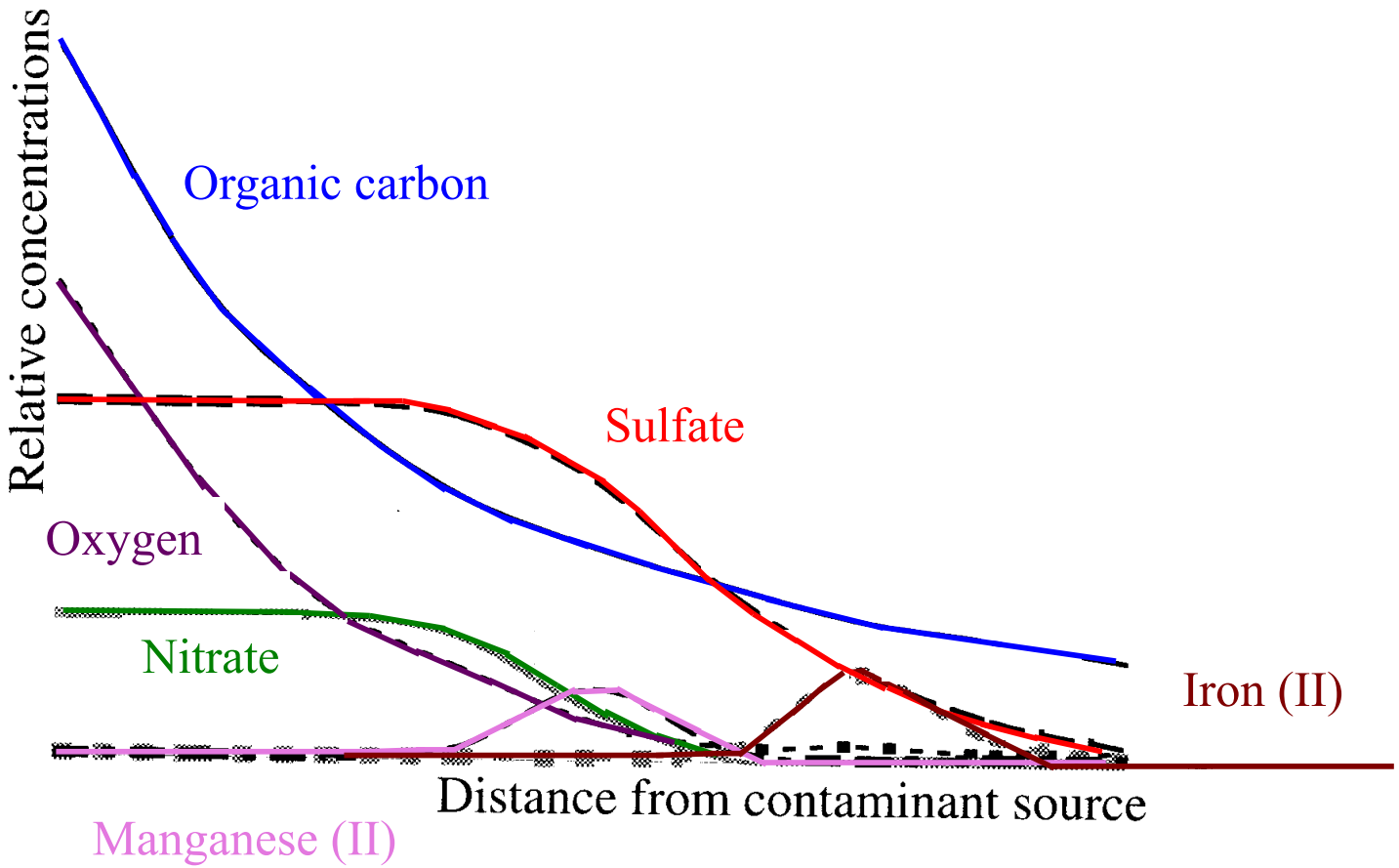
Biostimulation

	Primary substrate			Cometabolic substrate	
Chlorinated compound	Halorespiration	Direct aerobic oxidation	Direct anaerobic oxidation	Aerobic cometabolism	Anaerobic cometabolism
PCE	✔				✔
TCE	✔			✔	✔
DCE	✔	✔	✔	✔	✔
VC	✔	✔	✔	✔	✔
TCA	✔			✔	✔
DCA	✔	✔		✔	✔
CT	✔				✔
MC		✔	✔	✔	

Sequence of events for a site contaminated with chlorinated compounds

1. aerobic bacteria consume nonchlorinated organics until O_2 is depleted
2. Denitrifying bacteria consume nonchlorinated organics until NO_3^- is exhausted
3. Iron reducing bacteria consume nonchlorinated organic until Fe(III) is depleted
4. Fermentation processes consume nonchlorinated organics and produce H_2
5. Dechlorinating bacteria use H_2, sulfate-reducing bacteria consume nonchlorinated organics and methanogenic bacteria consume H_2 to generate CH_4

Progression of electron acceptors



Possible reactions for benzene and toluene

Benzene= C_6H_6

Toluene= C_6H_5-CH_3

TABLE 10-13
Coupled benzene and toluene reduction/oxidation reactions

Degradation type	Electron donor	Reaction
		Benzene
Aerobic	O_2	
Denitrification	NO_3^-	
Manganese reduction	MnO_2	
Iron reduction	Fe^{+3}	
Sulfate reduction	SO_4^{-2}	
Methanogenesis	CO_2	
		Toluene
Aerobic	O_2	
Denitrification	NO_3^-	
Manganese reduction	MnO_2	
Iron reduction	Fe^{+3}	
Sulfate reduction	SO_4^{-2}	
Methanogenesis	CO_2	

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TABLE 10-13
Coupled benzene and toluene reduction/oxidation reactions

Degradation type	Electron donor	Reaction
		Benzene
Aerobic	O_2	$C_6H_6 + 7.5O_2 \Rightarrow 6CO_2 + 3H_2O$
Denitrification	NO_3^-	$C_6H_6 + H^+ + 6NO_3^- \Rightarrow 6CO_2 + 3N_2 + 6H_2O$
Manganese reduction	MnO_2	$C_6H_6 + 15Mn^{+4} + 12H_2O \Rightarrow 6CO_2 + 30H^+ + 15Mn^{+2}$
Iron reduction	Fe^{+3}	$C_6H_6 + 30Fe^{+3} + 12H_2O \Rightarrow 6CO_2 + 30H^+ + 30Fe^{+2}$
Sulfate reduction	SO_4^{-2}	$C_6H_6 + 7.5H^+ + 3.75SO_4^{-2} \Rightarrow 6CO_2 + 3.75H_2S + 3H_2O$
Methanogenesis	CO_2	$C_6H_6 + 4.5H_2O \Rightarrow 2.25CO_2 + 3.75CH_4$
		Toluene
Aerobic	O_2	$C_6H_5{-}CH_3 + 9O_2 \Rightarrow 7CO_2 + 4H_2O$
Denitrification	NO_3^-	$C_6H_5{-}CH_3 + 7.2NO_3^- + 7.2H^+ \Rightarrow 7CO_2 + 3.6N_2 + 7.6H_2O$
Manganese reduction	MnO_2	$C_6H_5{-}CH_3 + 18MnO_2 + 36H^+ \Rightarrow 7CO_2 + 18Mn^{+2} + 22H_2O$
Iron reduction	Fe^{+3}	$C_6H_5{-}CH_3 + 36Fe^{+3} + 14H_2O \Rightarrow 7CO_2 + 36Fe^{+2}$
Sulfate reduction	SO_4^{-2}	$C_6H_5{-}CH_3 + 4.5SO_4^{-2} + 3H_2O \Rightarrow 2.25H_2S + 2.25HS^- + 7HCO_3^- + 0.25H^+$
Methanogenesis	CO_2	$C_6H_5{-}CH_3 + 5H_2O \Rightarrow 2.5CO_2 + 4.5CH_4$

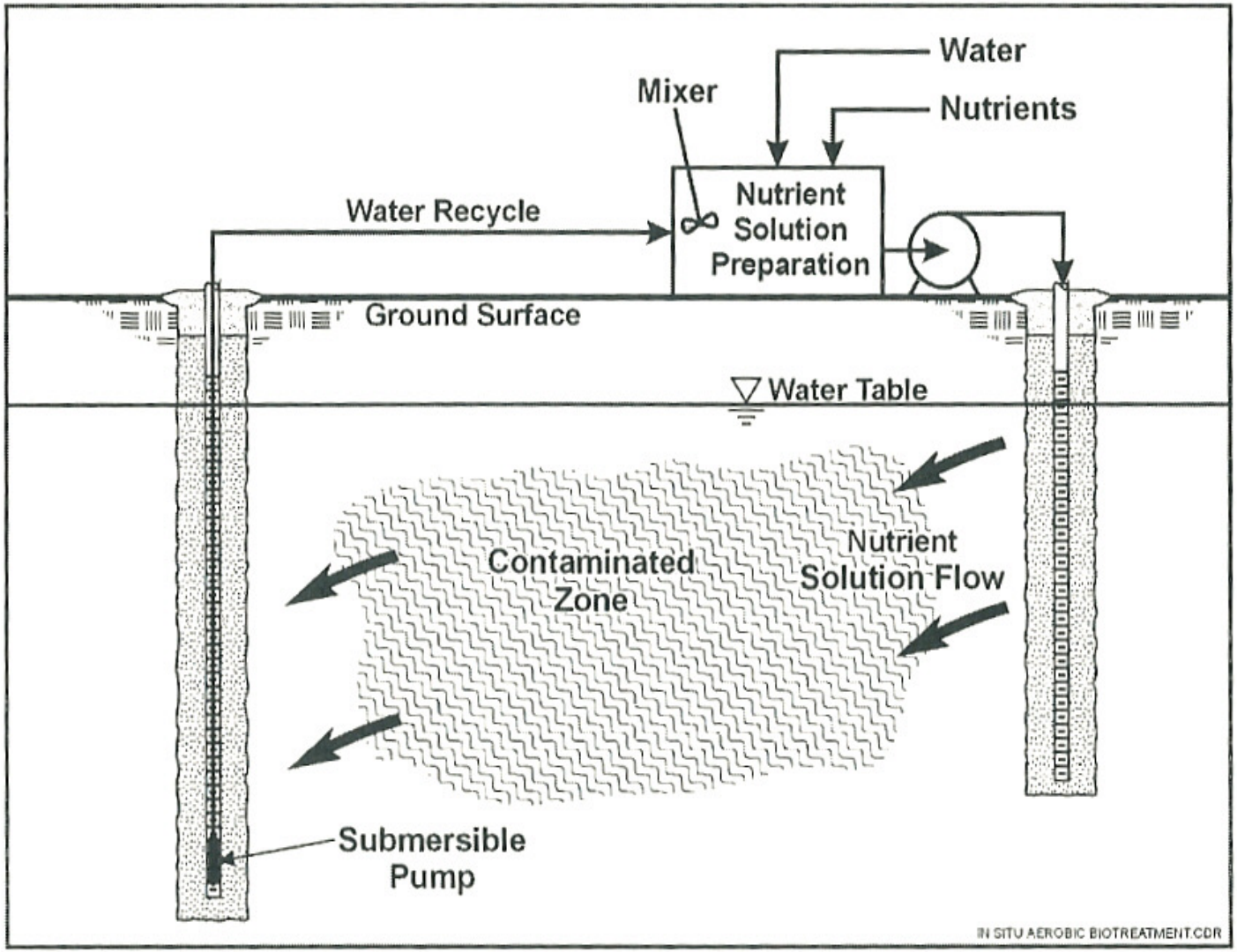
Challenges in biostimulation

- Reagent delivery
- Production of natural surfactants: use in modeling or may underestimate solution contaminant
- Fermentation: if uncontrolled can form acetone and butanone
- Overcome oxidative conditions: challenging in shallow or fast-moving aquifers
- Biofilm development: around injection wells
- Low concentration of contaminant: difficult to stimulate microbial community
- High concentration of contaminant: either toxicity or biosurfactant production

Aerobic zones

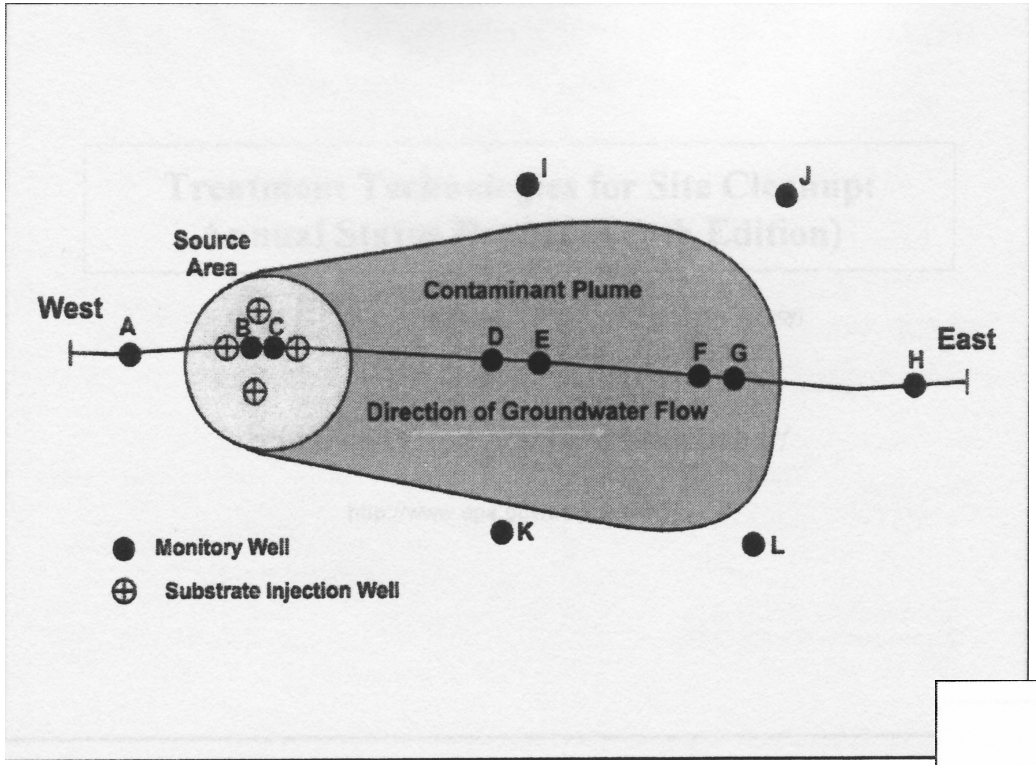
- Chloroethanes degraded by aerobes
- Cometabolic transformation: substrate (ex: toluene) induces expression of oxidase.
- Methanotrophs (oxidize CH_4) are an important group.
- Using methane monooxygenase (MMO), break down TCE, DCE
- Inject methane and oxygen in subsurface to stimulate such activity (Moffett Field, CA).

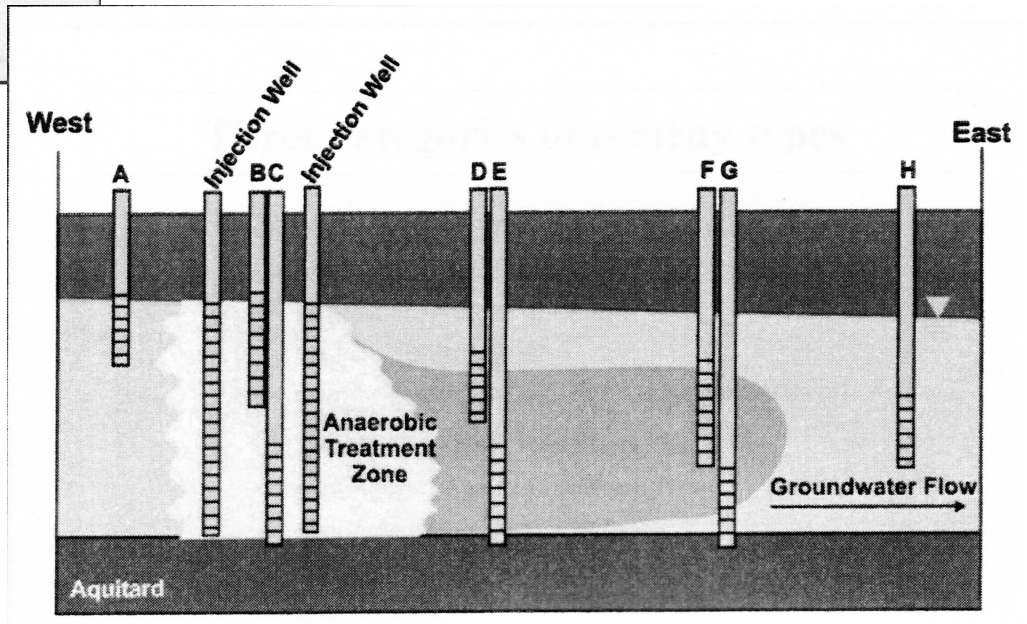
Enhanced *in situ* aerobic bioremediation



Conceptual Diagram of Typical Enhanced In Situ Aerobic Biodegradation System

Enhanced *in situ* anaerobic bioremediation





Use of Regenesis products
ORC and HRC

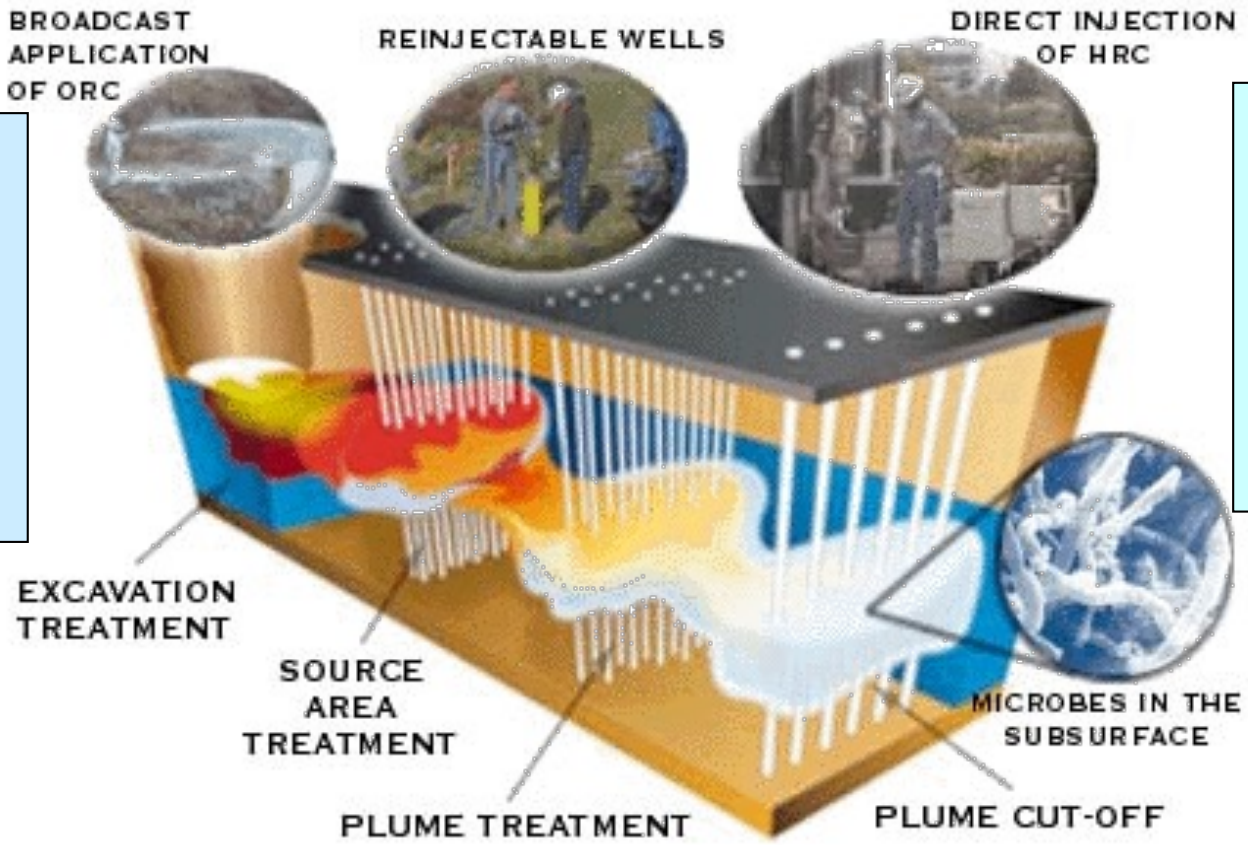
ORC = oxygen release compound

$MgO_2 + H_2O \rightarrow$ 1/2 O_2 + $Mg(OH)_2$

HRC = hydrogen release compound

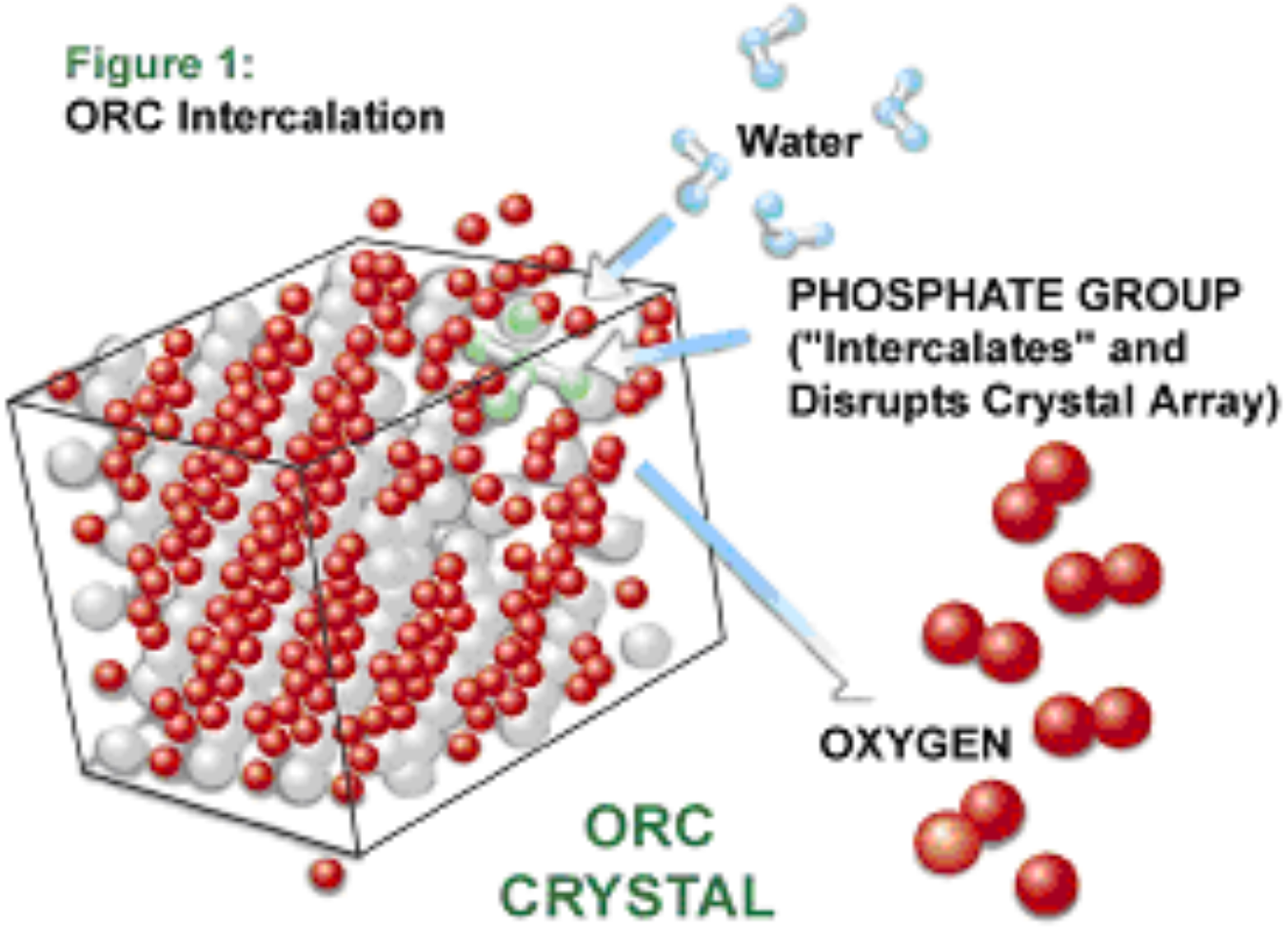
Polylactate + $H_2O \rightarrow$ lactate $\rightarrow$ H_2 + acetate

<u>oxidation of:</u>
BTEX
alcohols
ketones
MTBE
vinyl chloride



<u>reduction of:</u>
PCE, TCE,
nitrate
chromium
perchlorate
explosives

ORC – How it works



HRC – How it works (1)

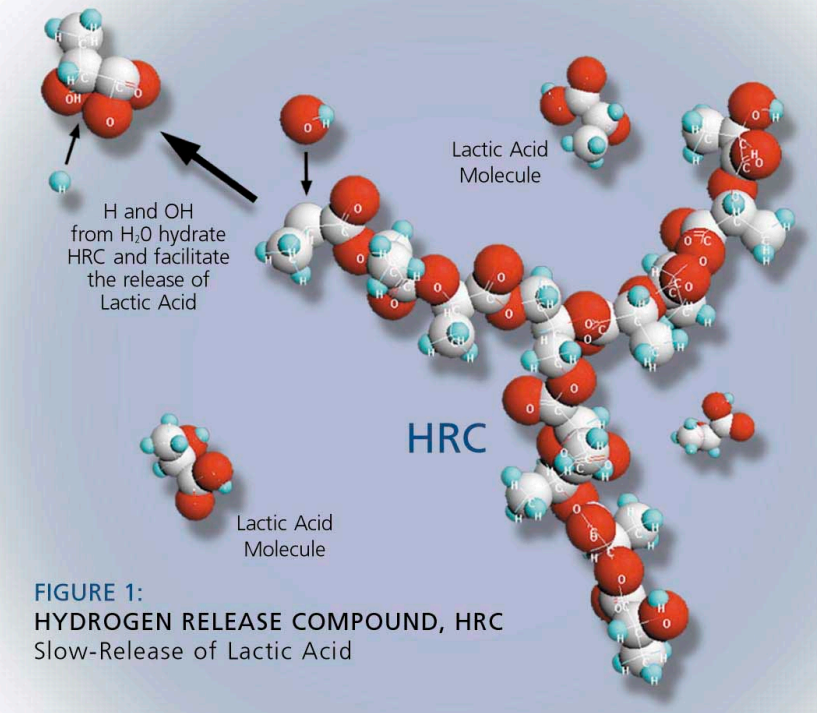
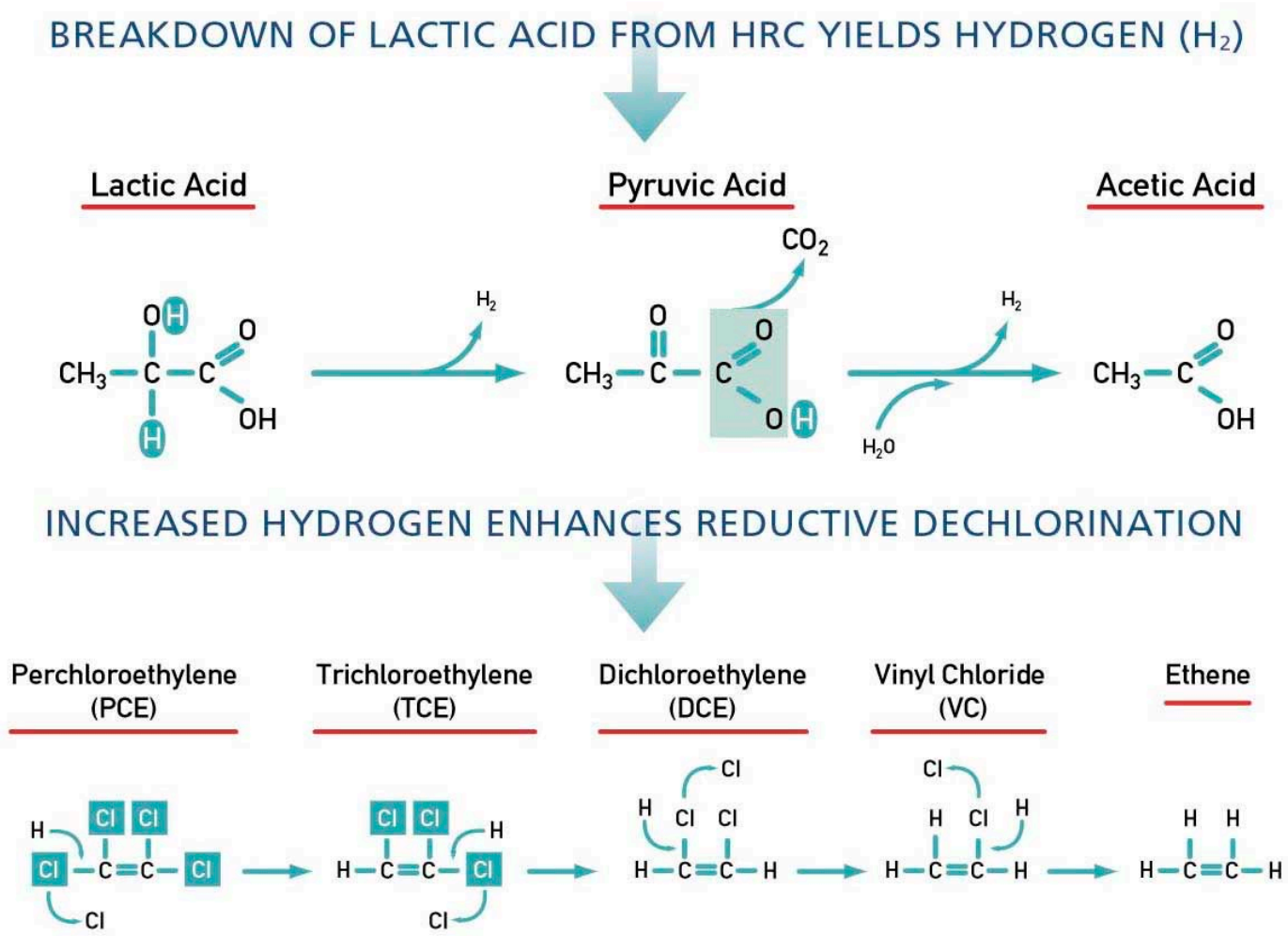


FIGURE 1:
HYDROGEN RELEASE COMPOUND, HRC
Slow-Release of Lactic Acid

HRC – How it works (2)

FIGURE 2:
BREAKDOWN OF LACTIC ACID AND RELEASE OF HYDROGEN



Example 1

A subsurface is impacted by gasoline. The average dissolved toluene concentration of the groundwater samples is 20 mg/L. In situ bioremediation is being considered for aquifer restoration. The aquifer has the following characteristics:

Porosity: 0.35

Organic content: 0.02

Dry bulk density: 1.6 g/cm^3

DO concentration: 4 mg/L

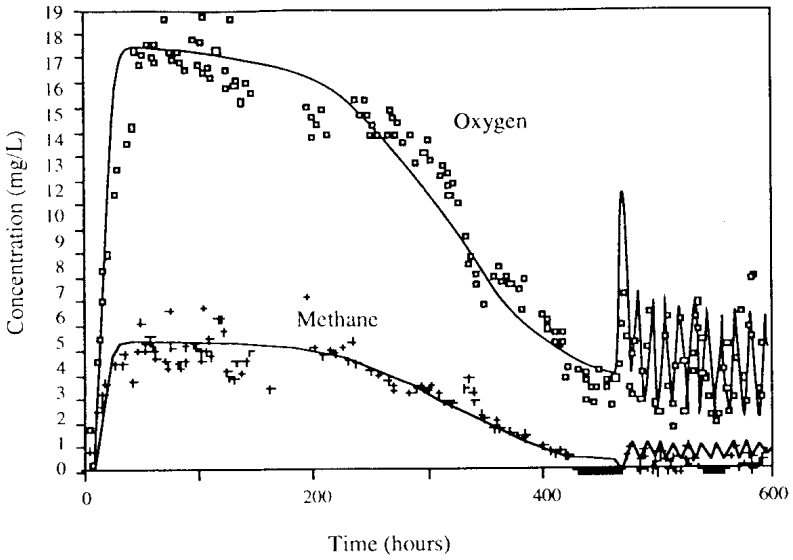
K_D= 6.76 * 10^{-6} m^3/g

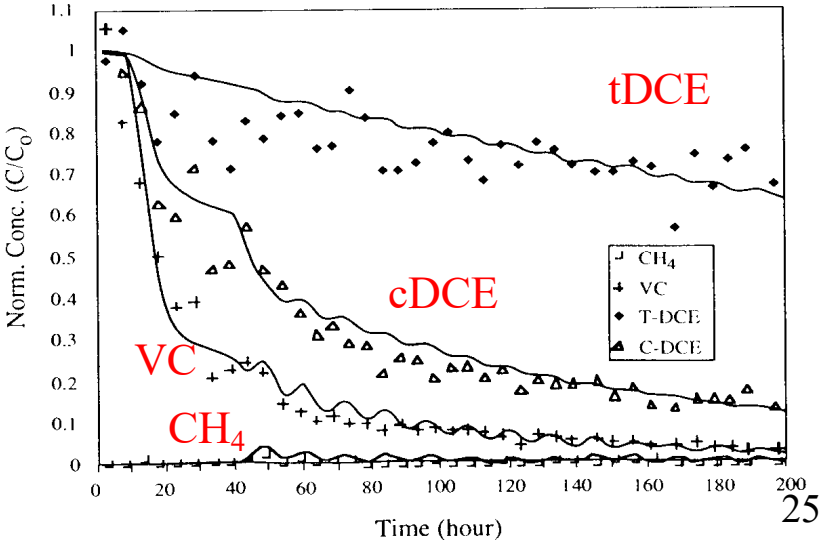
Assume 20 ºC and DO saturation= 9 mg/L

Is the addition of oxygen necessary to support the biodegradation of toluene?

Field-scale study-aerobic

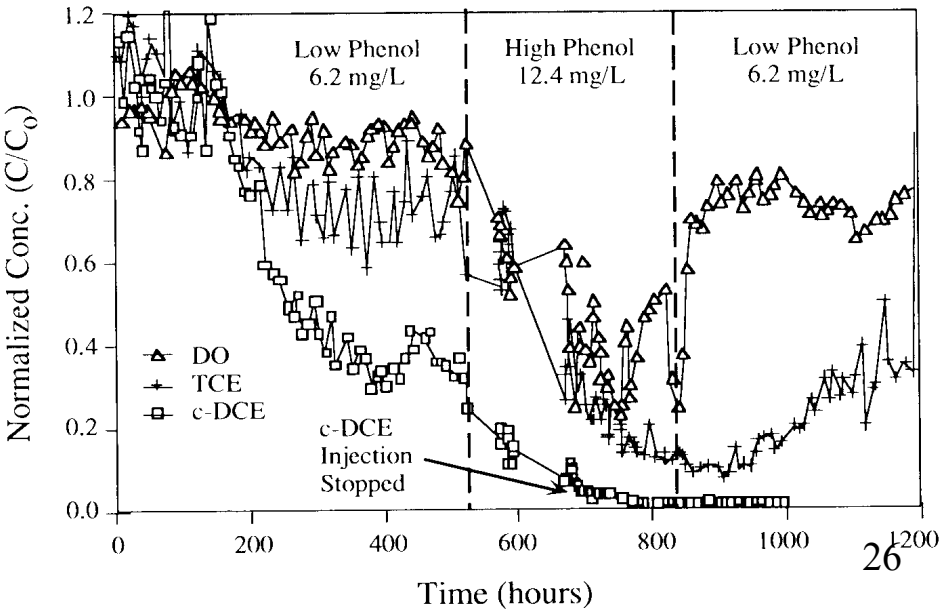
- Moffett field in CA
- Groundwater extracted from treatment zone, amended with substrate+ O_2 and reinjected
- Inject methane and O_2 => stimulate methanotrophs => cometablic degradation of chlorinated hydrocarbons
- Late in injection, pulse methane and O_2 to minimize clogging
- Conclusions:
  - Stimulation of indigenous methanotrophs possible
  - Model needed to account for competitive inhibition and rate-limited desorption (tDCE and VC compete)
  - Decrease in VC concentration limited by rate of desorption.
  - Limited TCE degradation (not shown)
  - tDCE degraded rapidly but much sorbed so apparent rate slower than VC





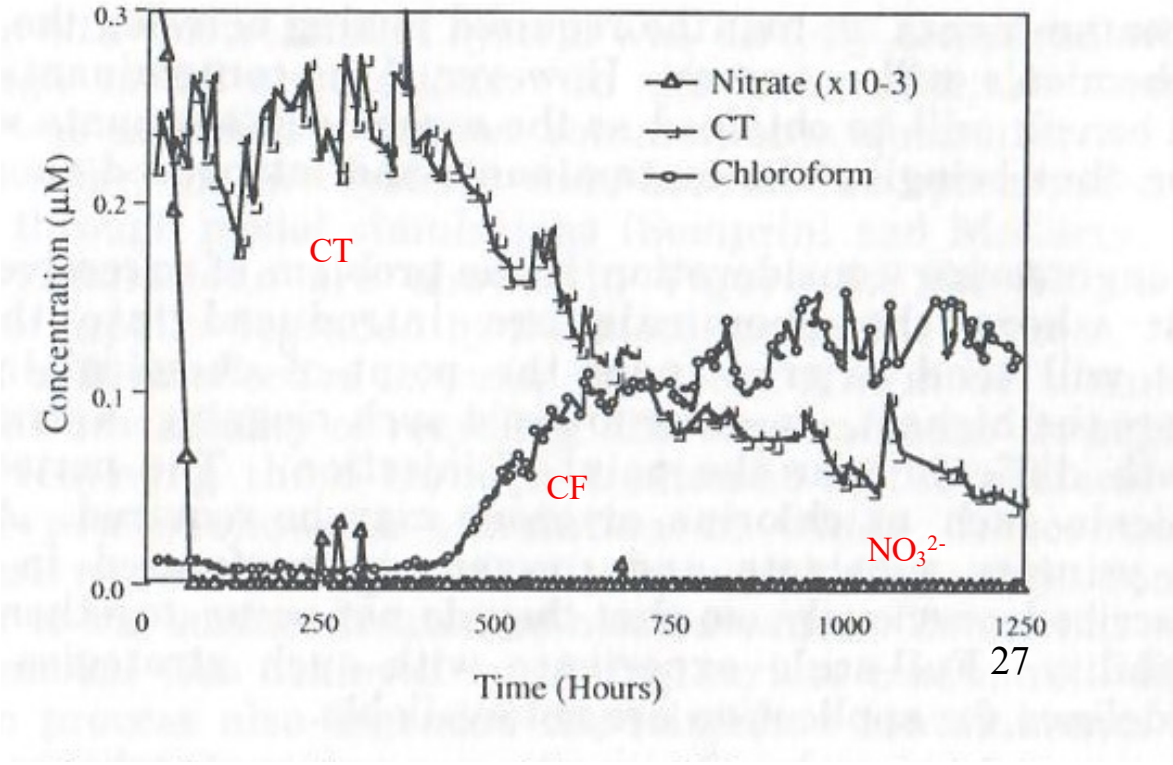
Field-scale study-aerobic

- Introduce phenol as a primary growth substrate and O_2
- Pulsing of phenol 6-12 mg/L
- Decrease in cDCE and TCE correspond to decrease in DO: result from biostimulation
- Conclusions:
  - Stimulated indigenous phenol utilizing bacteria
  - TCE 80% degraded in 2m zone and cDCE 95% degraded
  - Phenol degraders better at degrading TCE and cDCE and methane degraders better at VC



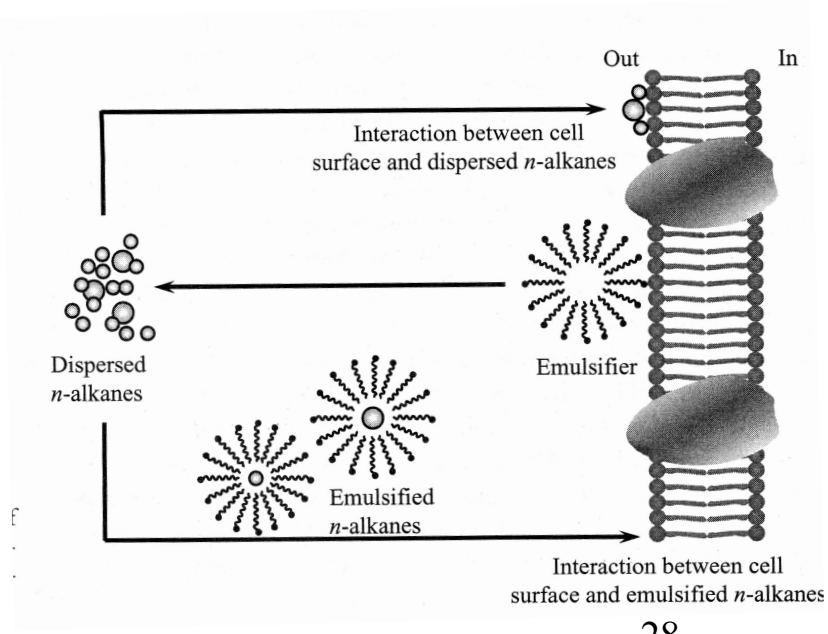
Field-scale study-anaerobic

- CT degradation under denitrifying conditions
- Acetate amended (nitrate and sulfate present)
- Decrease in CT but formation of chloroform
- Conclusions:
  - Stimulated indigenous community utilized acetate to reduce nitrate
  - Reductive dechlorination of CT

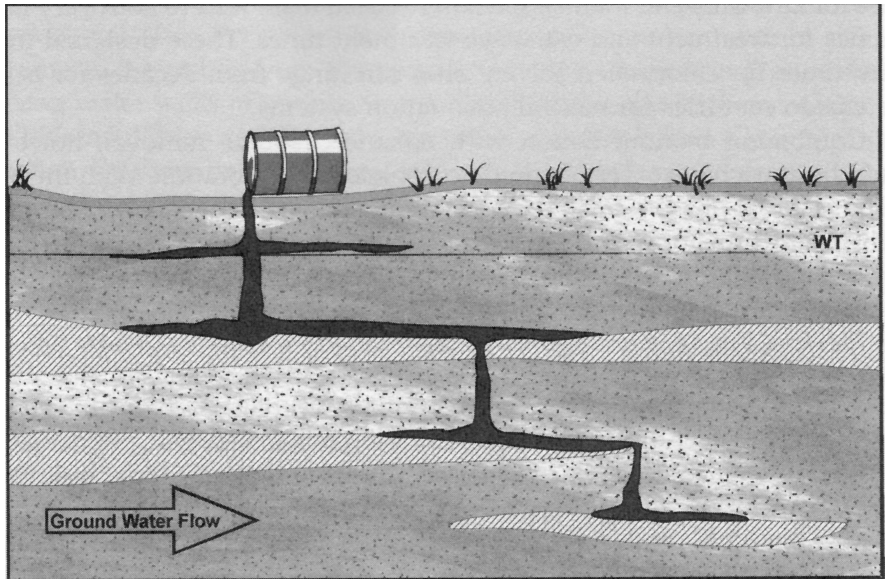


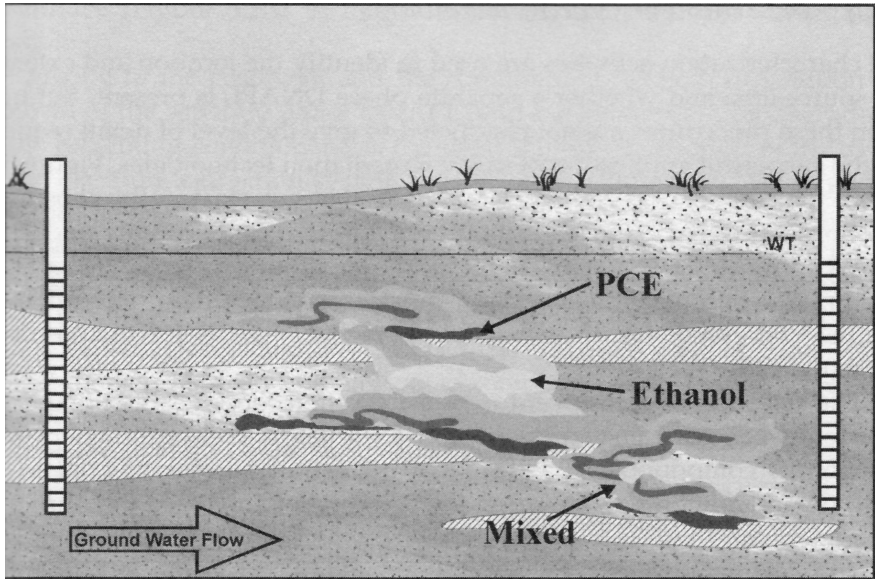
Enhancing bioavailability

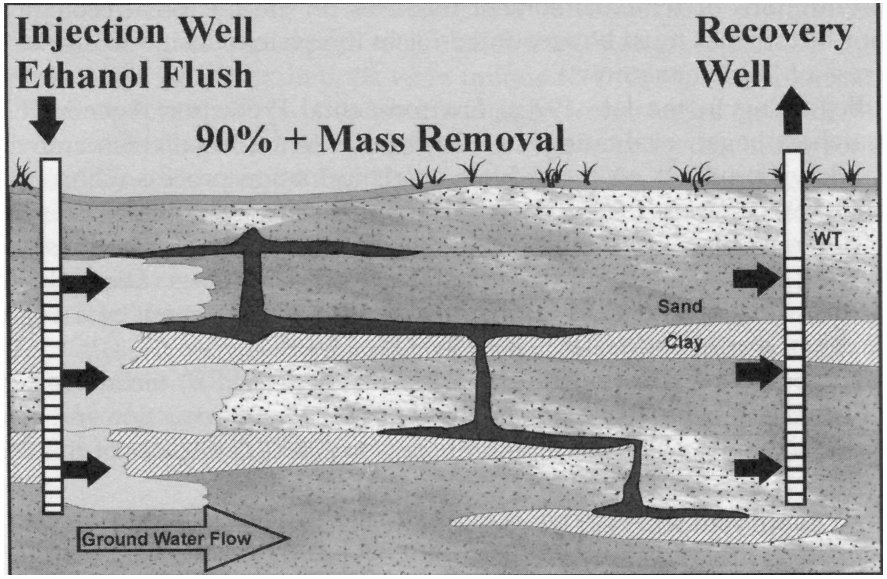
- Biggest obstacle to effective bioremediation is sorption of contaminants to soil
- Improve bioavailability by solubilizing organic compounds associated with OM using surfactants
- Issue of toxicity and resistance to degradation
- Biosurfactants:
  - Produced as a response to low water solubility of n-alkanes as growth substrates
  - Less toxic, more biodegradable than synthetic surfactants
  - Effective and many are produced
  - Limitation is production cost for use in bioremediation- currently too expensive for large scale use

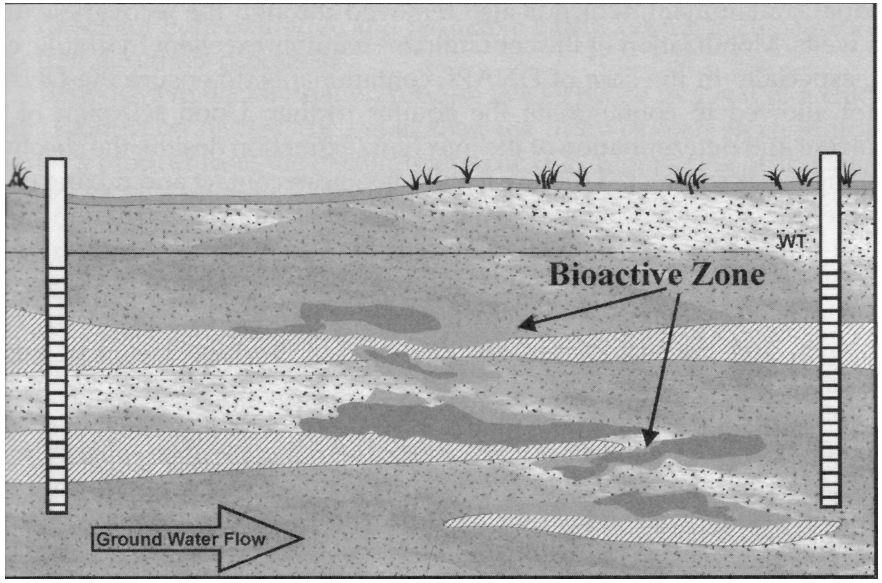


Solvent extraction+bioremediation







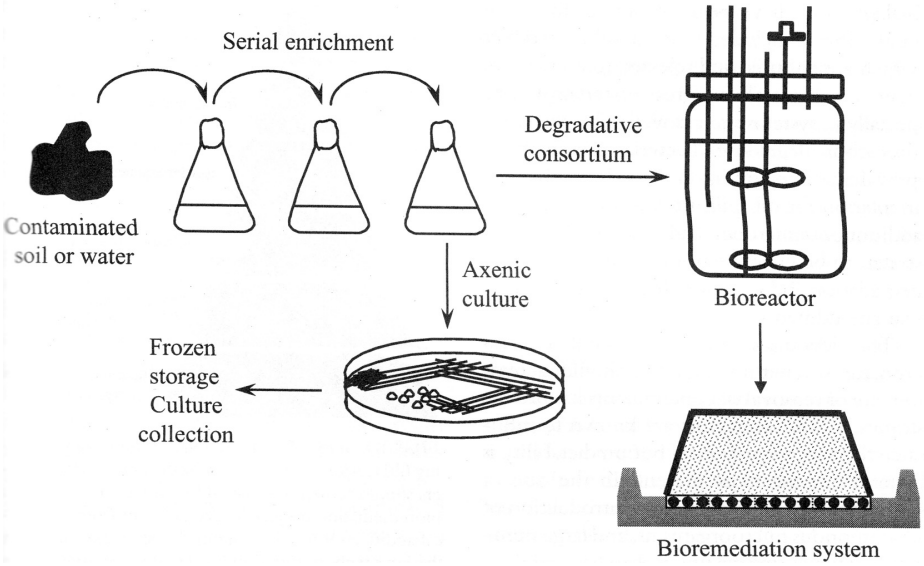


Bio-augmentation strategies

- Strategy 1: degradative organisms added to complement or replace native population. Selected for ability to survive or occupy a specific niche. Stimulants of selective substrate can be added to improve survival. Goal achieve prolonged survival and growth of amended organism
- Strategy 2: large amounts of bacterium added in the hope that will degrade large amounts of contaminant before becoming inactive.

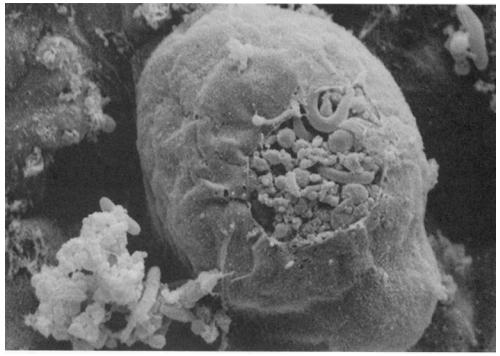
Bioaugmentation

- Inoculation of contaminated soil or groundwater with specific strains or consortia of microorganisms to improve biodegradation of a compound
- usually done for recalcitrant compounds
- May have value if indigenous organisms deemed unable to degrade contaminant and active microorganisms are introduced
- Limitation: decline of the introduced population is rapid due to competition and predation
- Two approaches:
    - Increase genetic diversity by adding mixture of microorganisms
    - Take samples from site and use them as serial enrichment with contaminant and return to site



Bio-augmentation

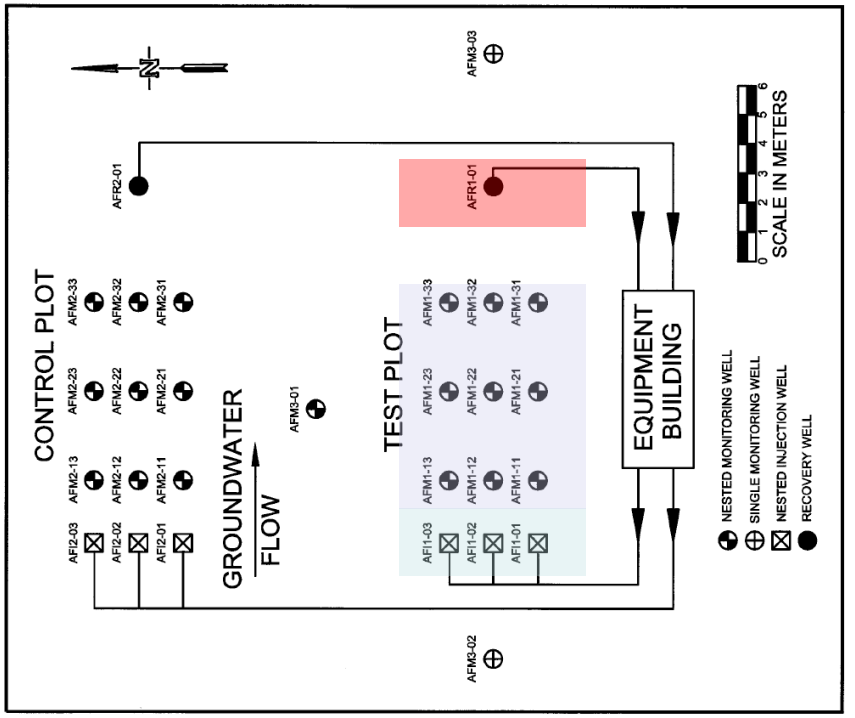
- For specific pollutants, indigenous microbial population insufficient
- Add a bacterial culture capable to catalyzing the reaction of interest
  - Ex: Carbon tetrachloride degraded to CO_2 only by *Pseudomonas stutzeri*- other bacteria produce chloroform



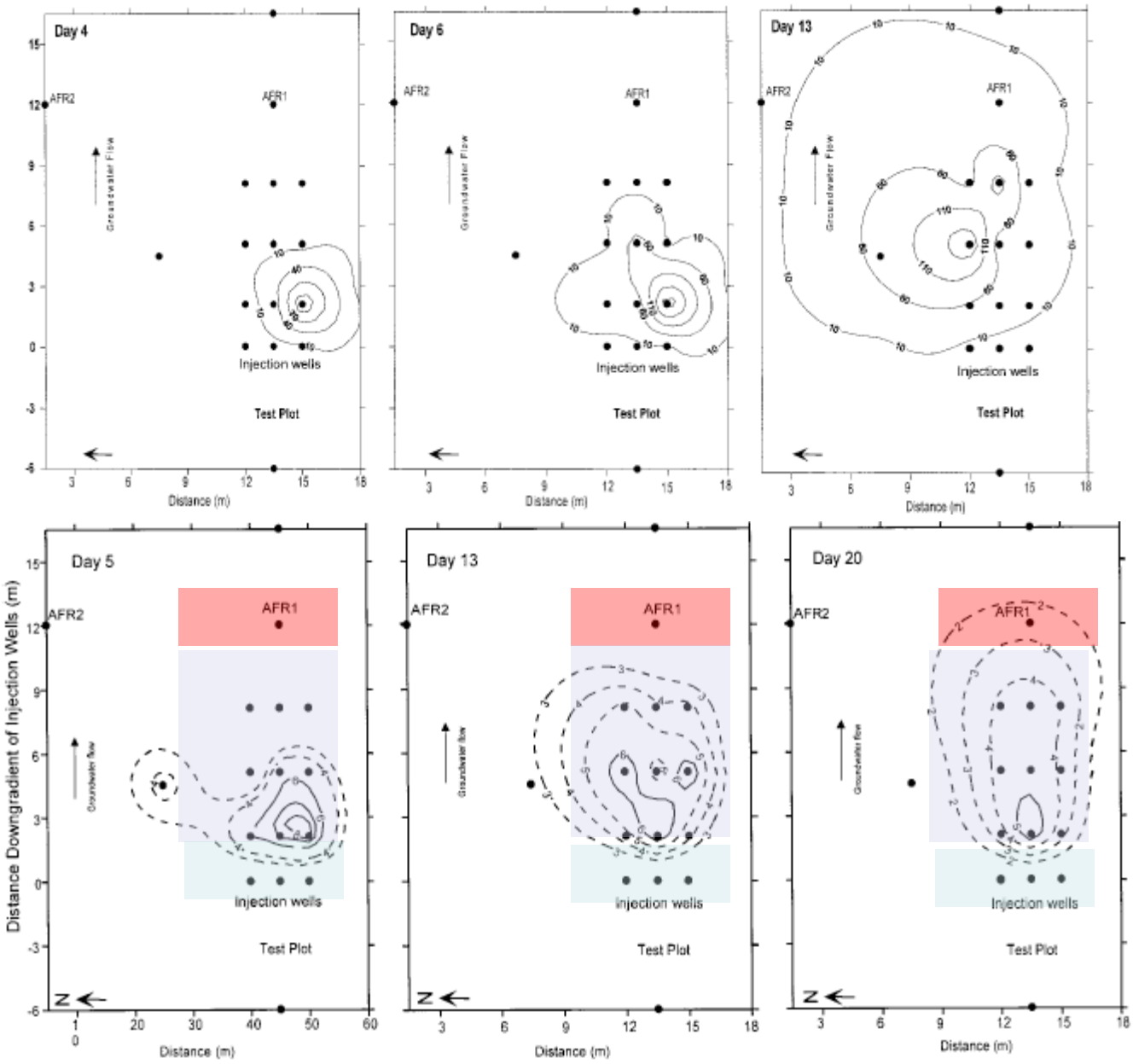
- May not be viable in sediments (predation, competition, adequate growth conditions)
- Novel delivery systems:
  - Do not travel great distances if injected as culture
  - If immobilized, cells survive competition, predation, extremes
  - Entrapment in polyvinyl alcohol (PVA) hydrogel

Pennsauken, NJ

- *Burkholderia cepacia* ENV435: low adhesion capacity and constitutive expression of toluene monooxygenase
- Can grow in large fermentors (750 L) to high cell density
- Accumulate large amounts of internal energy which can prolong degradation activity under non-optimal conditions
- Two trials: (1) injected bacteria in injection wells and (2) injected bacteria mixed with recirculating water into monitoring wells
- Injected O_2 with and after bacteria injection
- Site contaminated with PCE, TCE and VC



Pennsauken, NJ



Tracer

Br- in mg/L

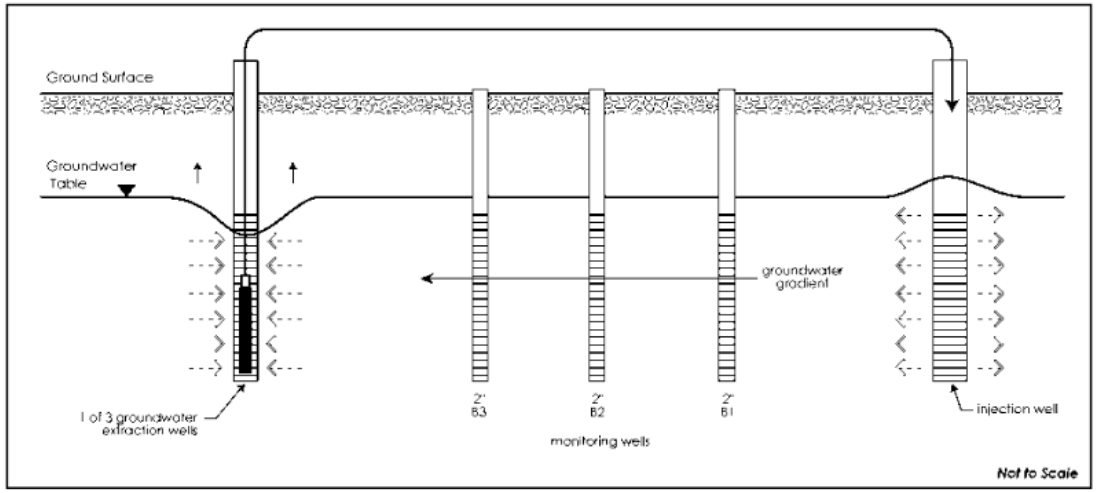
B. cepacia

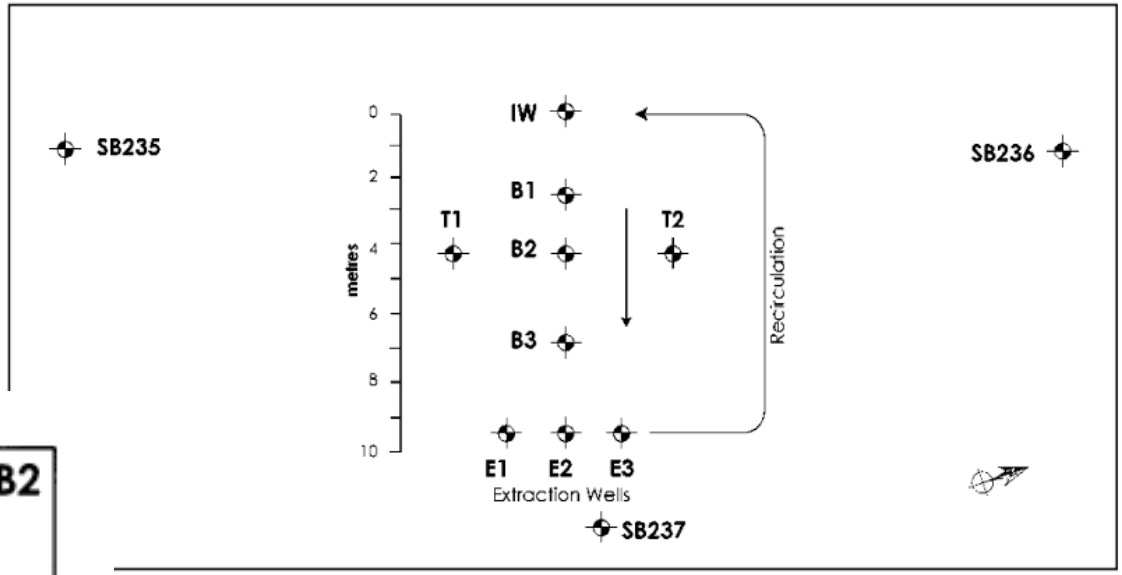
Log CFU/mL

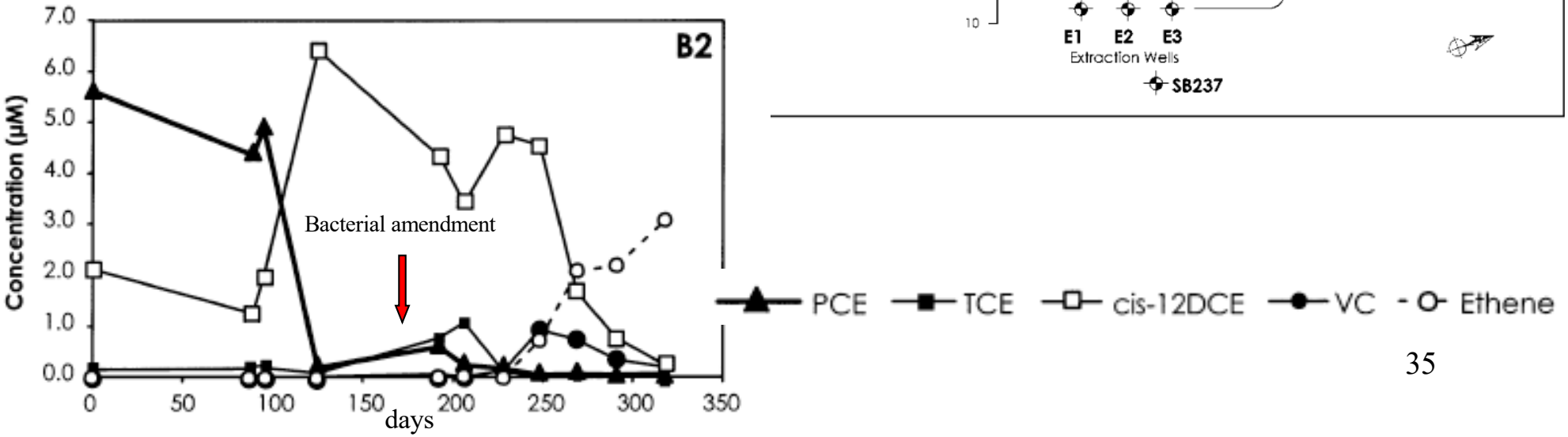
Ratio of tracer velocity to bacteria velocity was 1.2-1.4
Half-life of cells about 2 days
Trial 2 was more successful than trial 1: splitting the injection over several wells more effective

Kelly AFB

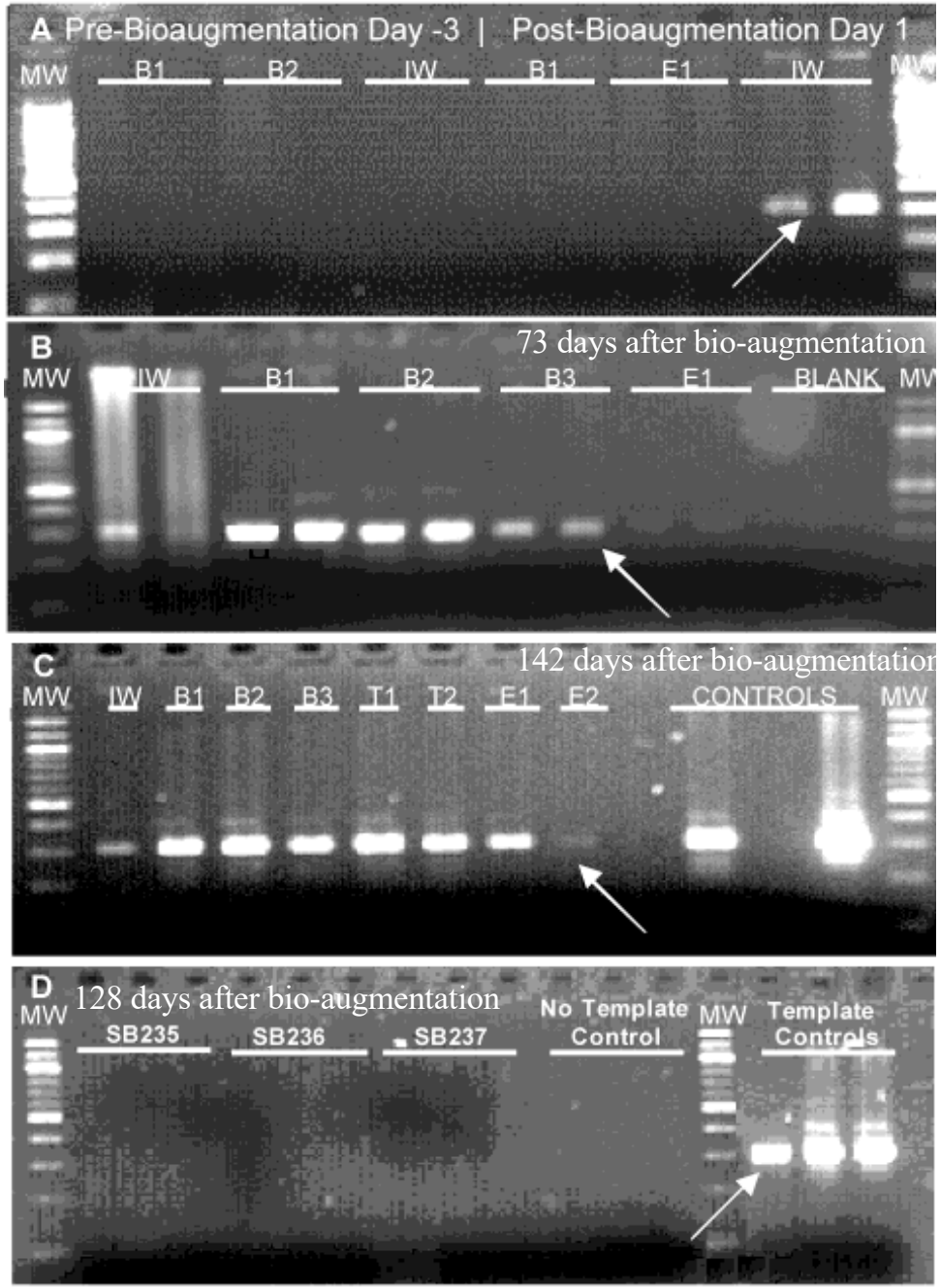
- Contaminated with PCE
- Biostimulation lead to accumulation of cDCE (methanol and acetate)
- Bio-augmentation with bacterium *Dehalococcoides sp.* KB-1 able to degrade TCE and cDCE to ethene
- 13 L of bacterial culture added anaerobically to injection well while recirculation was stopped







Kelly AFB



- Use molecular techniques to look specifically for the presence of strain KB-1 away from injection well
- After 73 days, see that strain KB-1 has reached wells B1 and B2 and B3 but not E1
- After 142 days, has moved laterally and reached T1 and T2 and has moved forward to E1
- control wells show no evidence of SB-1 after 128 days.

Example 2

A groundwater is contaminated with TCE. Either methanotrophic cometabolism or halorespiration are considered. The contaminated zone is a cylinder 25 m in diameter and 10 m tall and received 100 moles of TCE (MW= 131.4 g/mol) . The sediment has the following characteristics:

Porosity: 0.3; mass fraction of soil carbon:0.2; logKow TCE= 2.42; dry bulk density= 1.5 g/cm^3

For halorespiration: endogenous decay (0.01 d^{-1}); half-velocity coefficient (3 mg/L); Growth yield coefficient (0.6 mg biomass/mg substrate); degradation rate constant (0.4 mg substrate/(mg biomass. d))

Karickoff relation: $K_D \left[\frac{m^3}{g}\right] = 0.63 * 10^{-6} * f_{oc} * K_{ow}$

Can we use halorespiration?

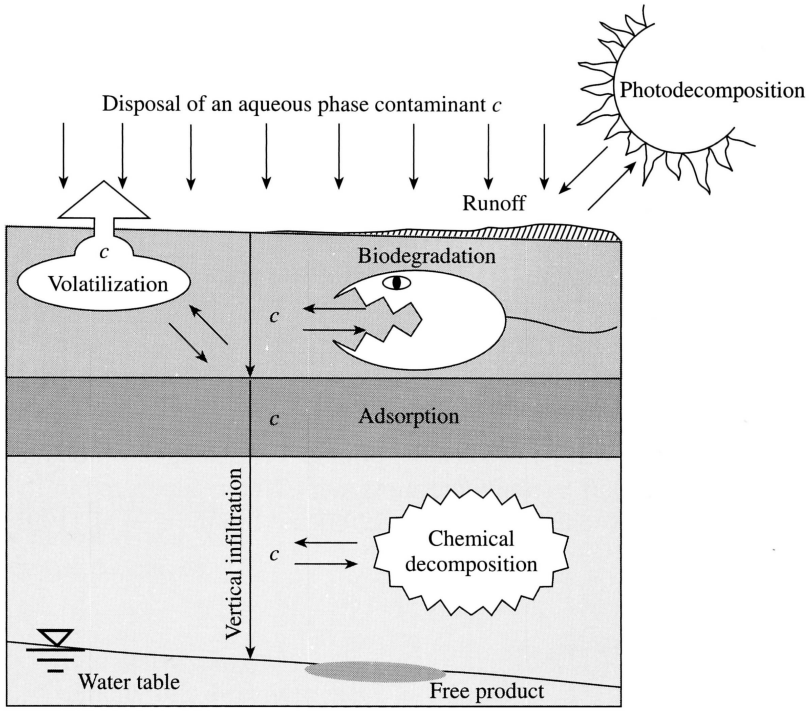
If methanotrophic cometabolism, assuming ratio of 5 CH_4 moles to one TCE, what total amount of dissolved O_2 needed?

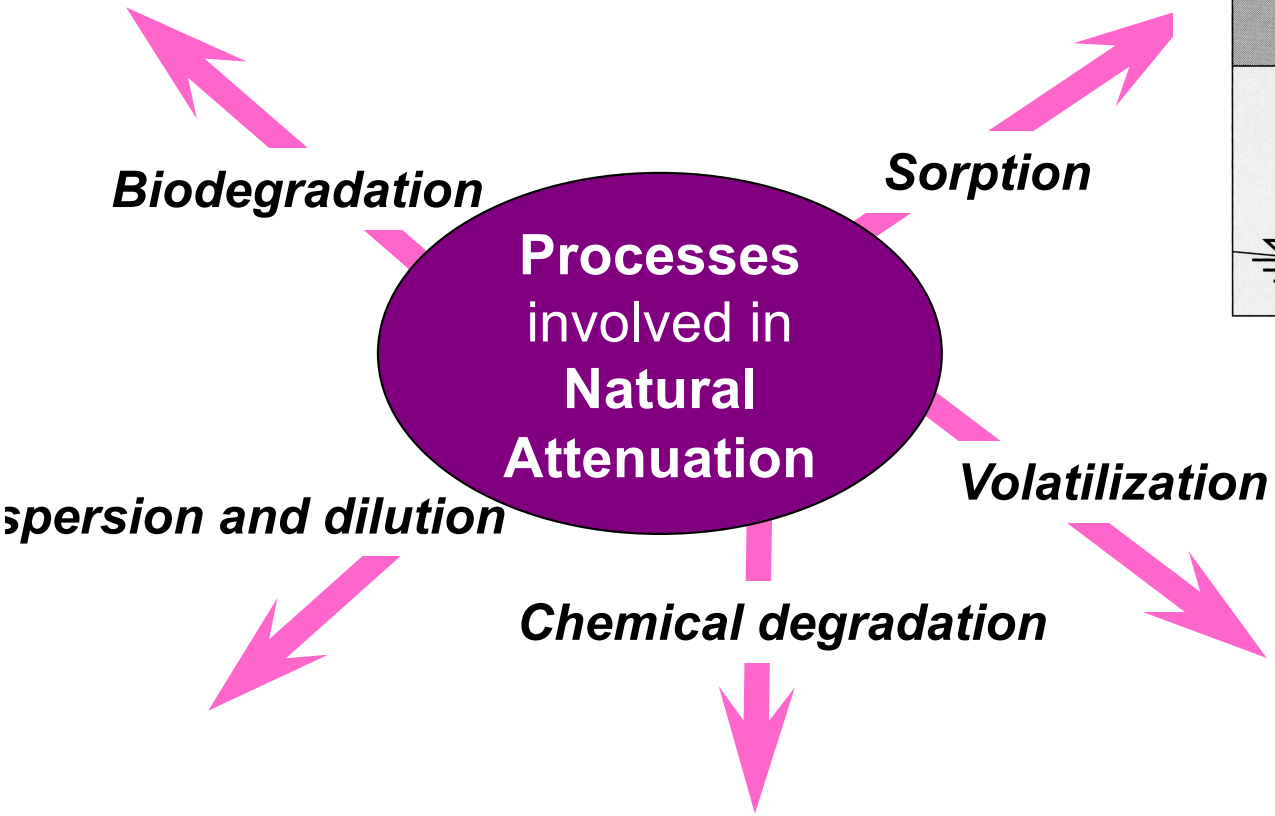
Monitored Natural Attenuation

Monitored Natural attenuation

Definition:

Reliance on natural processes to achieve site-specific remedial objectives (aka intrinsic bioremediation)





Indicators of microbial activity

Geochemical parameters, role in biodegradation, and indication of biodegradation at a site

Geochemical parameters	Role in biodegradation	Indication of biodegradation
Oxygen	Determines aerobic/anaerobic/anoxic conditions Consumed during respiration	Lower than background
Nitrate	Consumed during respiration	Lower than background
Sulfate	Consumed during respiration	Lower than background
Reduction oxidation potential	Determine reductive or oxidative conditions	Lower than background
Iron (II)	Produced during respiration	Higher than background
Manganese	Produced during respiration	Higher than background
Methane	Produced during respiration	Higher than background
Chloride	Produced from dechlorination of chlorinated organics	Higher than background
Alkalinity	Increases as a result of respiration (denitrification)	Higher than background
CO_2 or dissolved inorganic carbon	Produced during respiration	Higher than background
Reduced transformation products	Produced from dechlorination of chlorinated organics	Present
Ethane or ethene	Produced from dechlorination of chlorinated organics	Present

Monitored Natural attenuation

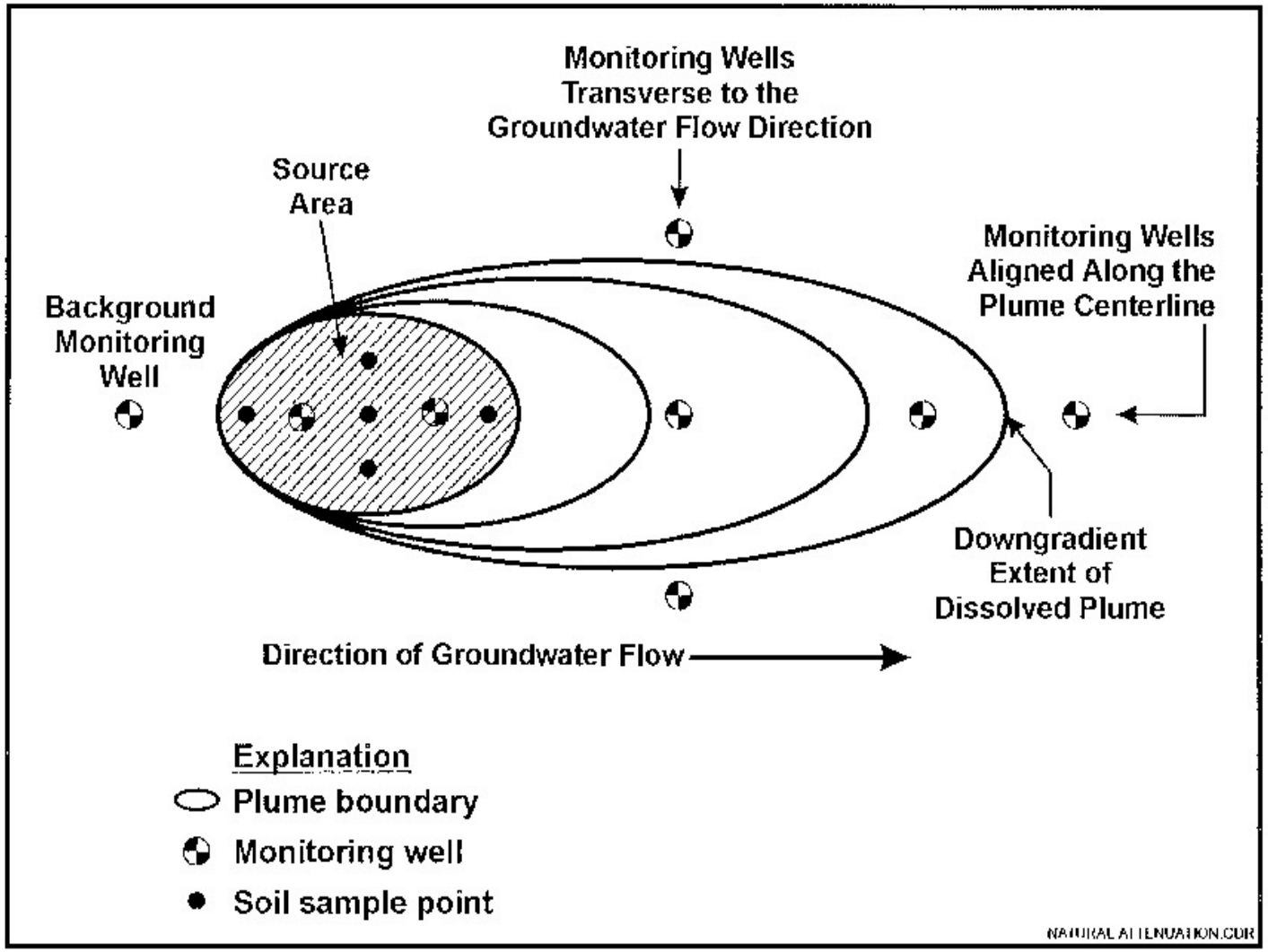


Diagram of Subsurface Natural Attenuation of a Contaminant Plume

Monitored natural attenuation

- Alternative to achieving remediation goals appropriate under limited set of circumstances
- Reliance on natural processes and monitoring to achieve site specific remediation objectives within a time frame comparable to that of other alternatives
- Biodegradation is the dominant (but not the only) process
- Important parameter is contaminant transport: it remediation occurs before exposure then risk is mitigated
- NRC: (1) demonstrate loss of contaminant in site
  (2) demonstrate that field microorganisms carry out process
  (3) show evidence of biodegradation in the field -most difficult

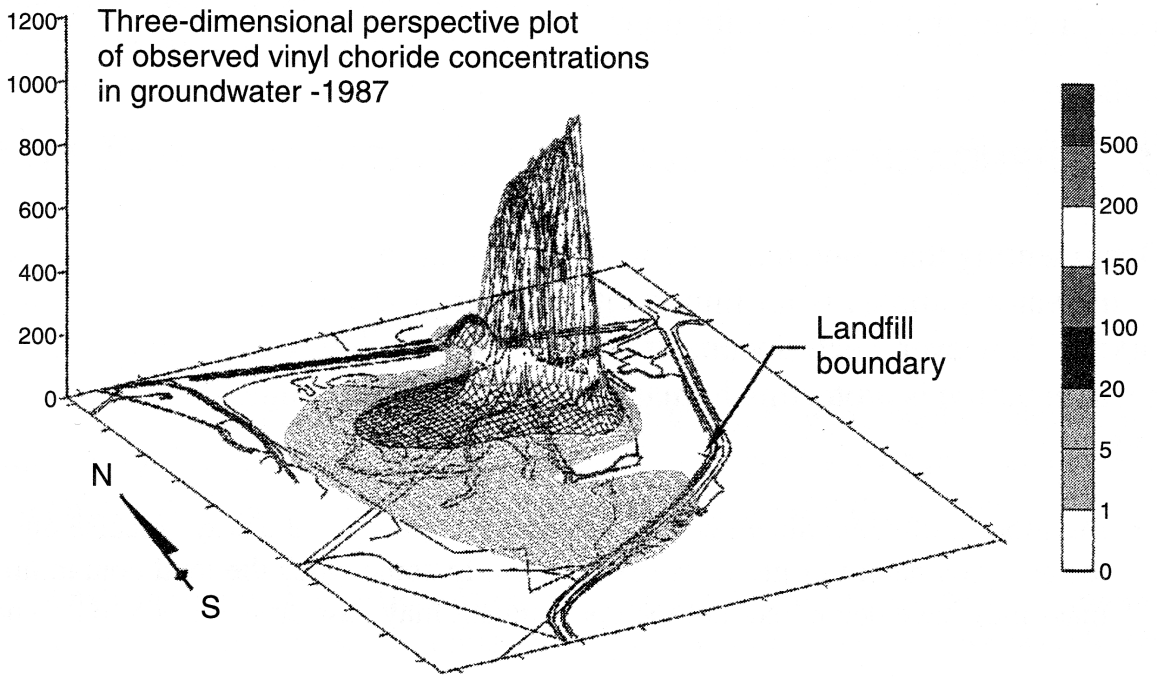
Monitored natural attenuation

- Relies on naturally-occurring supplies of e^- donor, acceptor and nutrients to develop BAZ and prevent migration
- No engineered measures (except when used as mop-up)
- Long term monitoring
- Suitable for lower contaminant concentrations in sites with high conc. e^- acceptor (shallow aquifer), nutrients, consistent gw flow and natural buffering capacity
- Careful site characterization:

  (1) extent of contamination

  (2) presence of microorganisms

  (3) rate of supply and consumption of e- donor, acceptor, nutrients


Three-dimensional perspective plot
of observed vinyl choride concentrations
in groundwater -1987
1200
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Landfill
boundary
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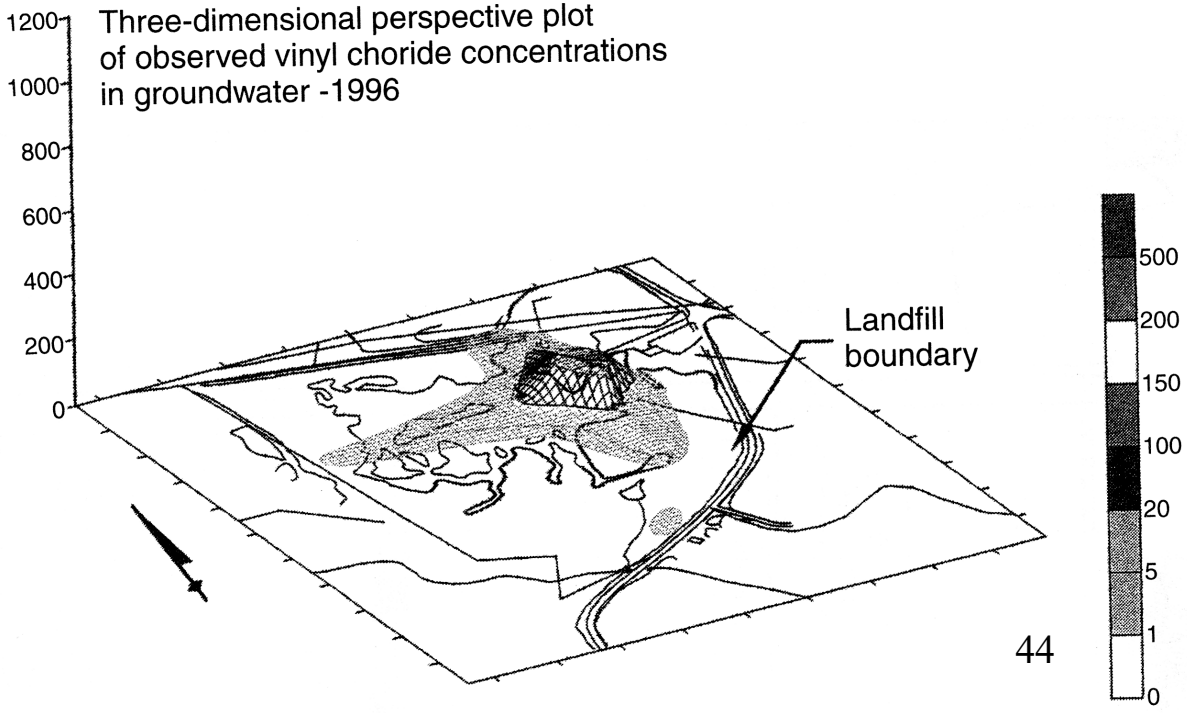
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