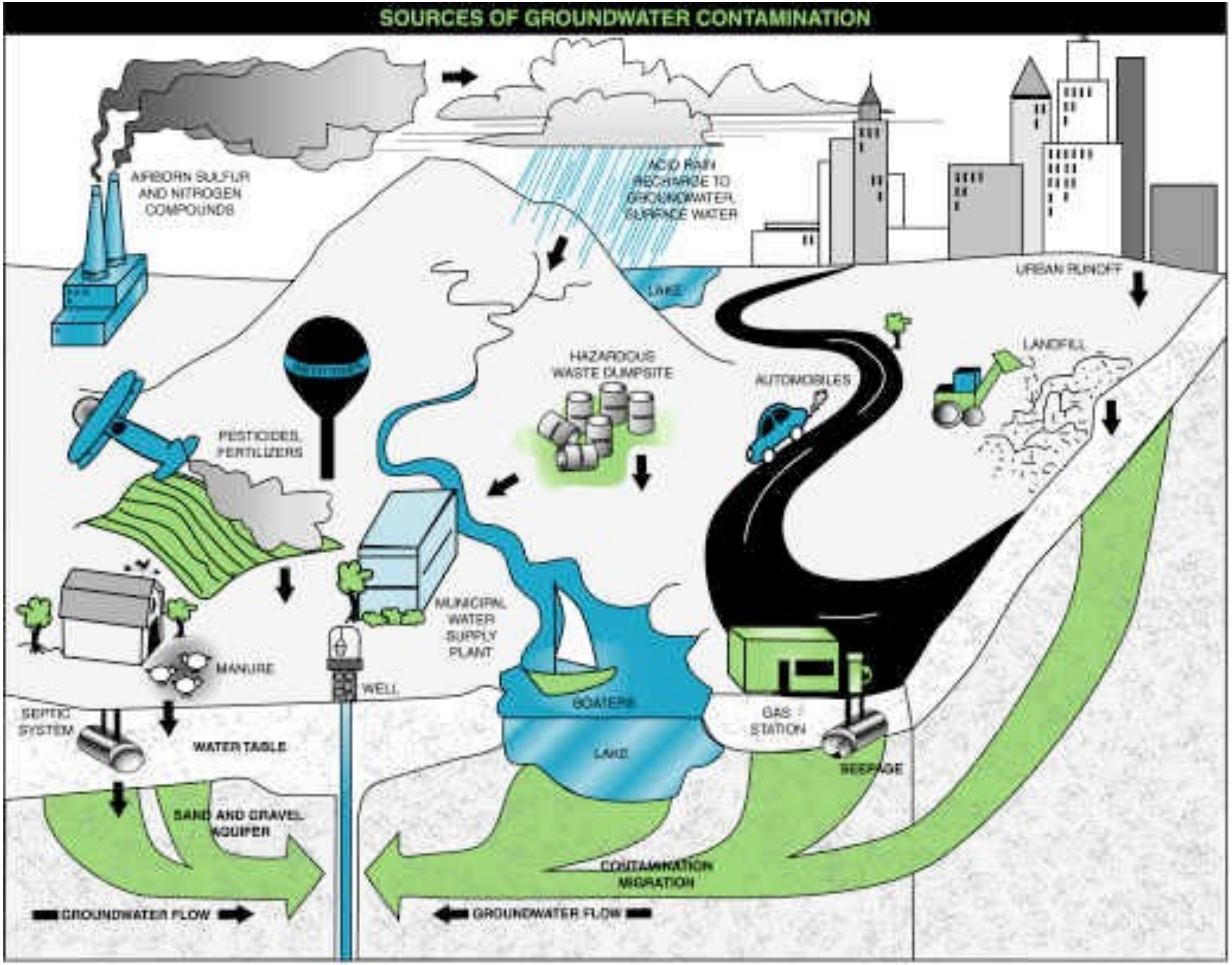
In situ bioremediation

Lecture 8

Learning outcomes

- *In situ* bioremediation groundwater
- Natural monitored attenuation

Groundwater contamination

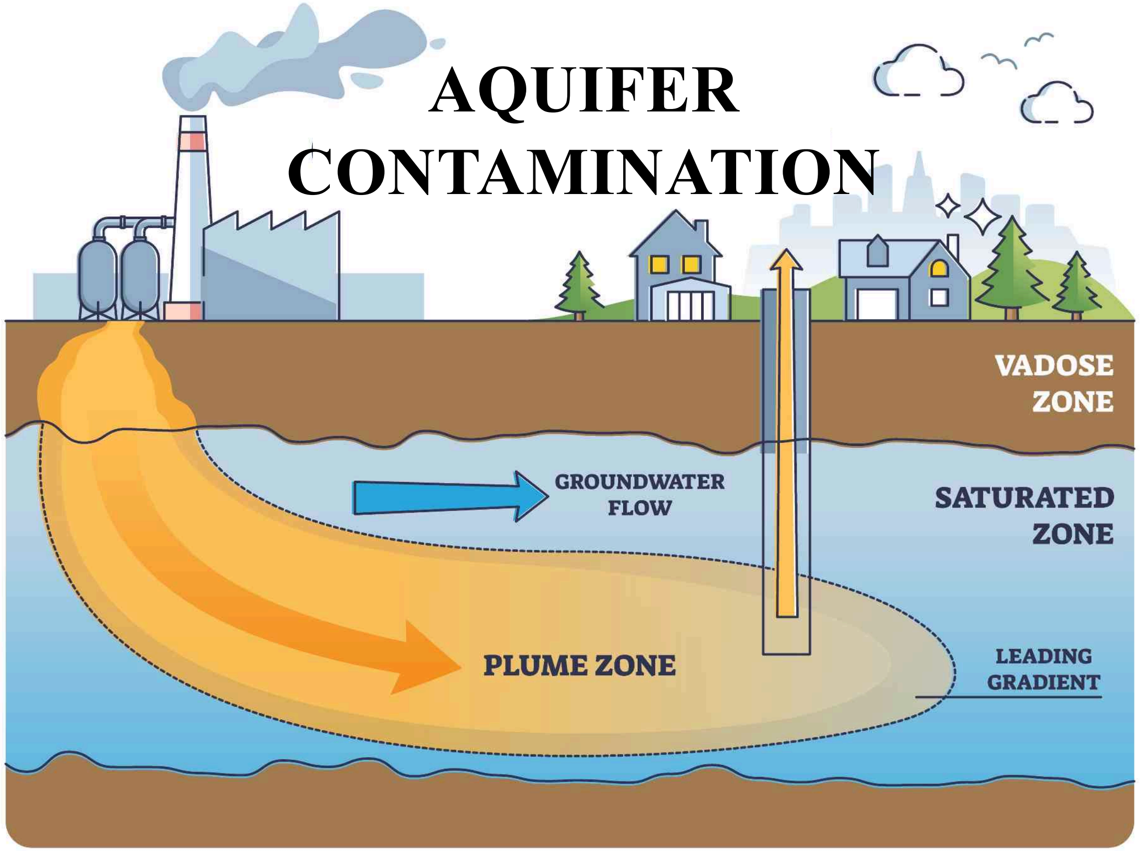


Classical in situ bioremediation

Bioremediation

- The use of microorganisms to remove or immobilize contaminants in the subsurface
- Mainly use biostimulation: amend substrates to stimulate the activity of existing microorganisms
- Sometimes use bioaugmentation: add microorganisms with specific metabolism that is not available at the site
- Metabolic or co-metabolic metabolism: the contaminant is the substrate, or the contaminant is degraded fortuitously through the transformation of another substrate

Aquifer contamination



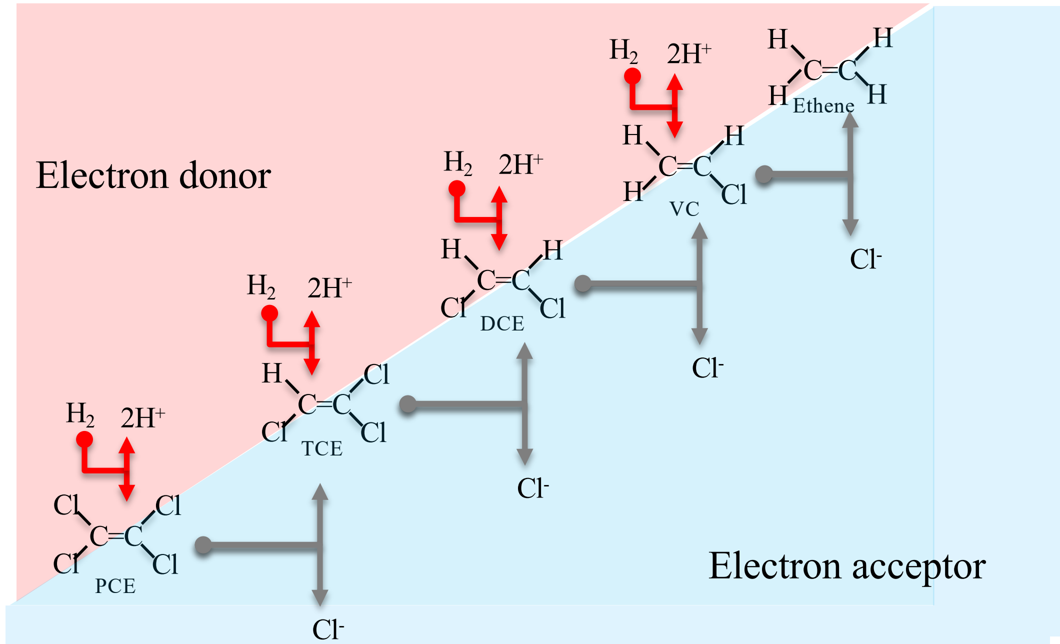
- Contamination at the surface can result in the transport of the contaminants to the subsurface

- Aquifers can become contaminated

- Groundwater flow results in the formation of a contamination plume that grows with time

- Wells or surface water can be contaminated

Halogenated compounds



PCE: tetrachloroethylene
TCE: trichloroethylene
DCE: dichloroethylene
VC: vinyl chloride

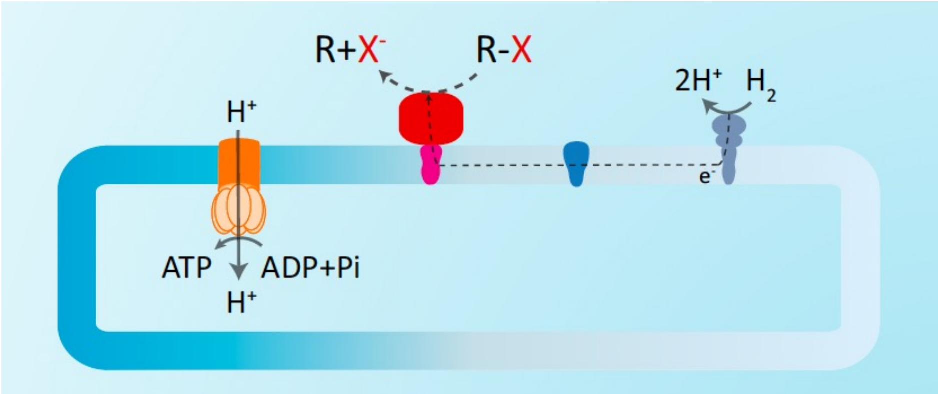
- Reductive dechlorination of chlorinated solvents
- Anaerobic microorganisms (e.g., *Dehalococcoides* sp.) able to use these chlorinated solvents as electron acceptors
- H_2 serves as the electron donor

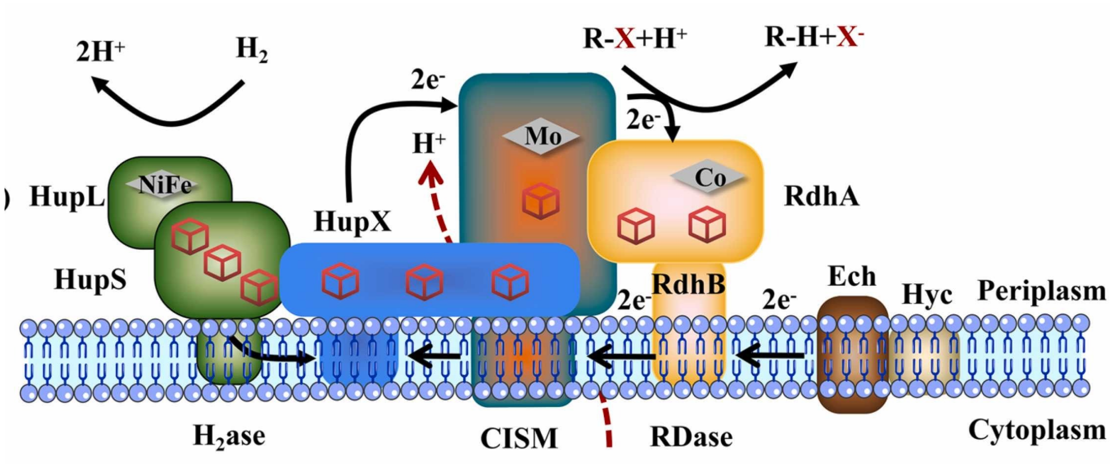
accept 2 e^-

$$Cl_2C{=}CCl_2 + H_2 \Rightarrow Cl_2C{=}CHCl + 2\,H^+ + Cl^-$$

donate 2 e^-

Dehalogenation

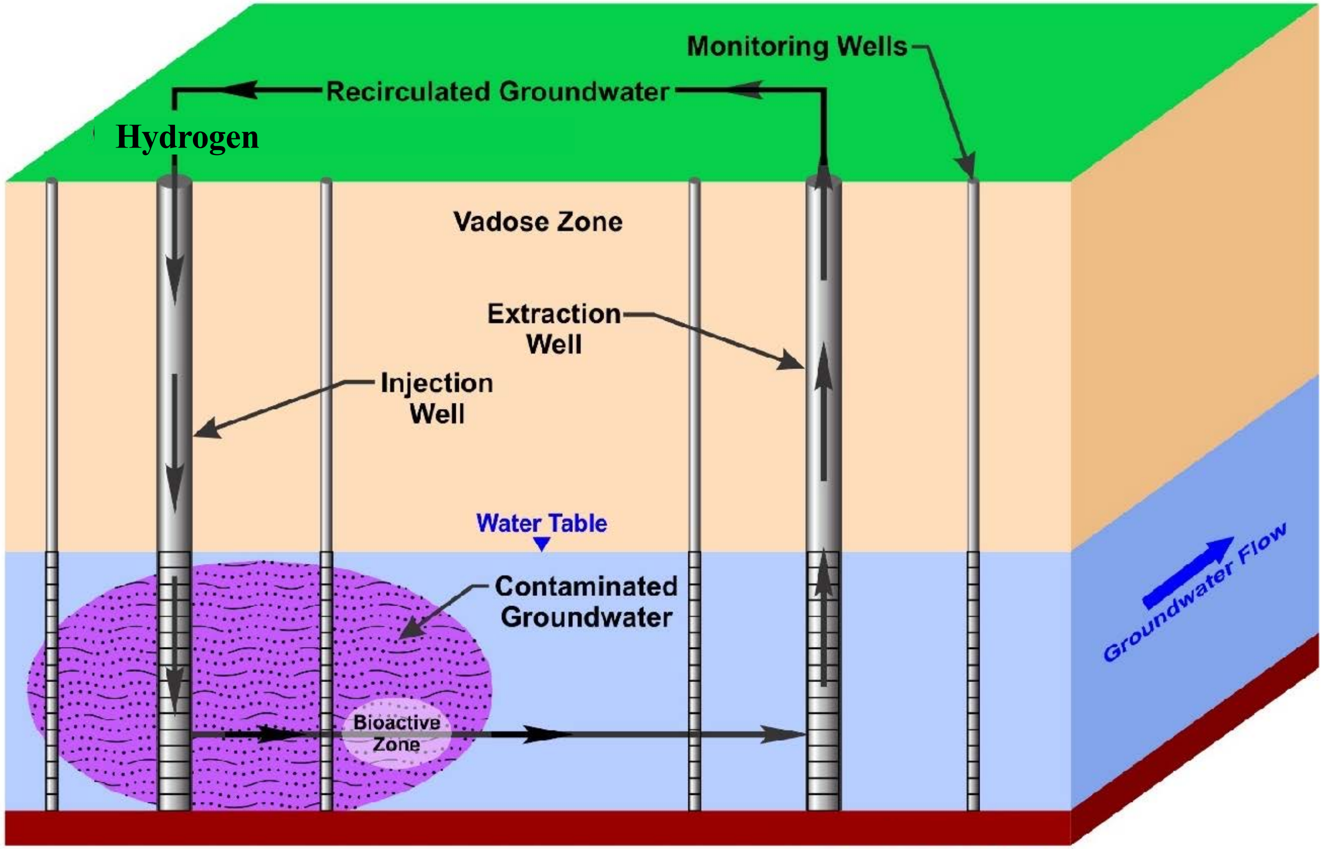




Lu et al., 2024
Yu et al., 2023

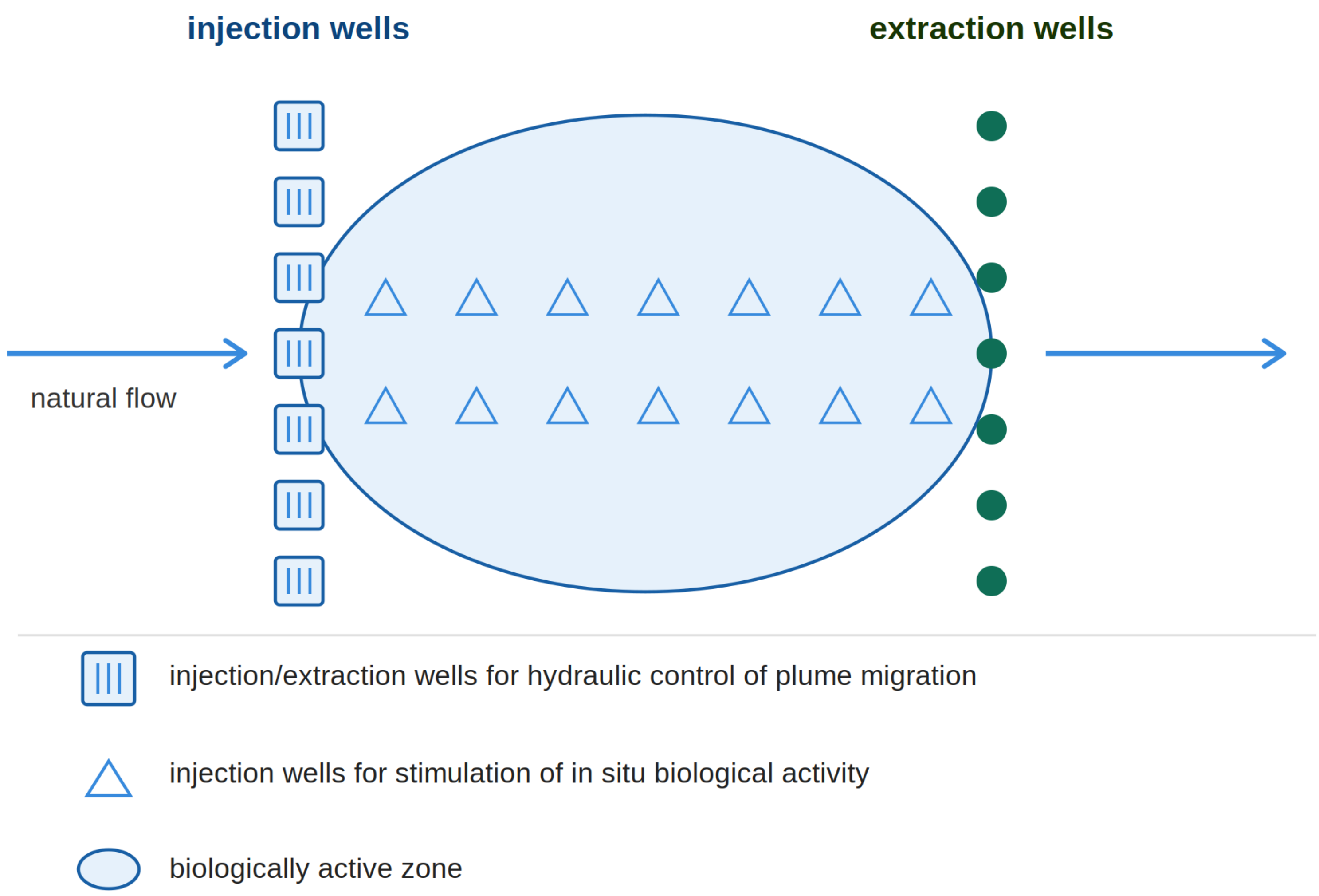
- Respiratory process by which ATP is produced from the oxidation of H_2 and reduction of chlorinated solvents
- Complex electron transport chain (with or without quinones) and the involvement of several metal centers (NiFe, Mo, Co, FeS)
- Reductive dehalogenase (RDase) is the key enzyme

Engineered remediation



- Amendment of substrate to abstracted groundwater
- Re-injection of groundwater
- Creation of a bio-active zone around the injection gallery

Engineered remediation



Classical *in situ* bioremediation

- Biostimulation
  - Anaerobic processes sometimes favorable
  - If aerobic process is sought, then O_2 saturated water is injected

Variants on classical in situ bioremediation:

- Bio-stimulation
- Solvent enhanced
- Bio-augmentation

Biostimulation

Biostimulation

- The key is to enhance activity of indigenous organisms including those able to degrade pollutant
- e- donor that will change redox potential
  - Molasses in aquifer to stimulate microbial activity and deplete O_2; may stimulate anaerobic processes such as reductive dechlorination and metal reduction
- e^- donor for cometabolism: degradation of a compound only in the presence of other organic material that serves as the primary enzyme source
- e^- acceptor amendment: O_2 most common but can amend nitrate, sulfate.
- Carbon source for inorganic pollutants (ethanol, lactate)
- Nutrient addition: N, P, K, S, Mg, Ca, Fe, trace . Can use oleophilic fertilizer in the case of hydrophobic compound.
- Surfactants to increase contact between water-soluble nutrients and oil-soluble contaminants => microemulsions

Biostimulation

	Oxic conditions	**Anoxic zone**
Goal	maintain aerobic conditions in the active zone	create a zone in which anaerobic microbes can be active
Contaminants	petroleum hydrocarbons, phenolic compounds, chlorinated compounds (chlorobenzene, methylene chloride, vinyl chloride)	Chlorinated solvents and metals
Amendments	O_2, cometabolic substrate	e^- donor, cometabolic substrate

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		✔	✔	✔	

Electron donors

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.

Use of Regenesis products: ORC and HRC

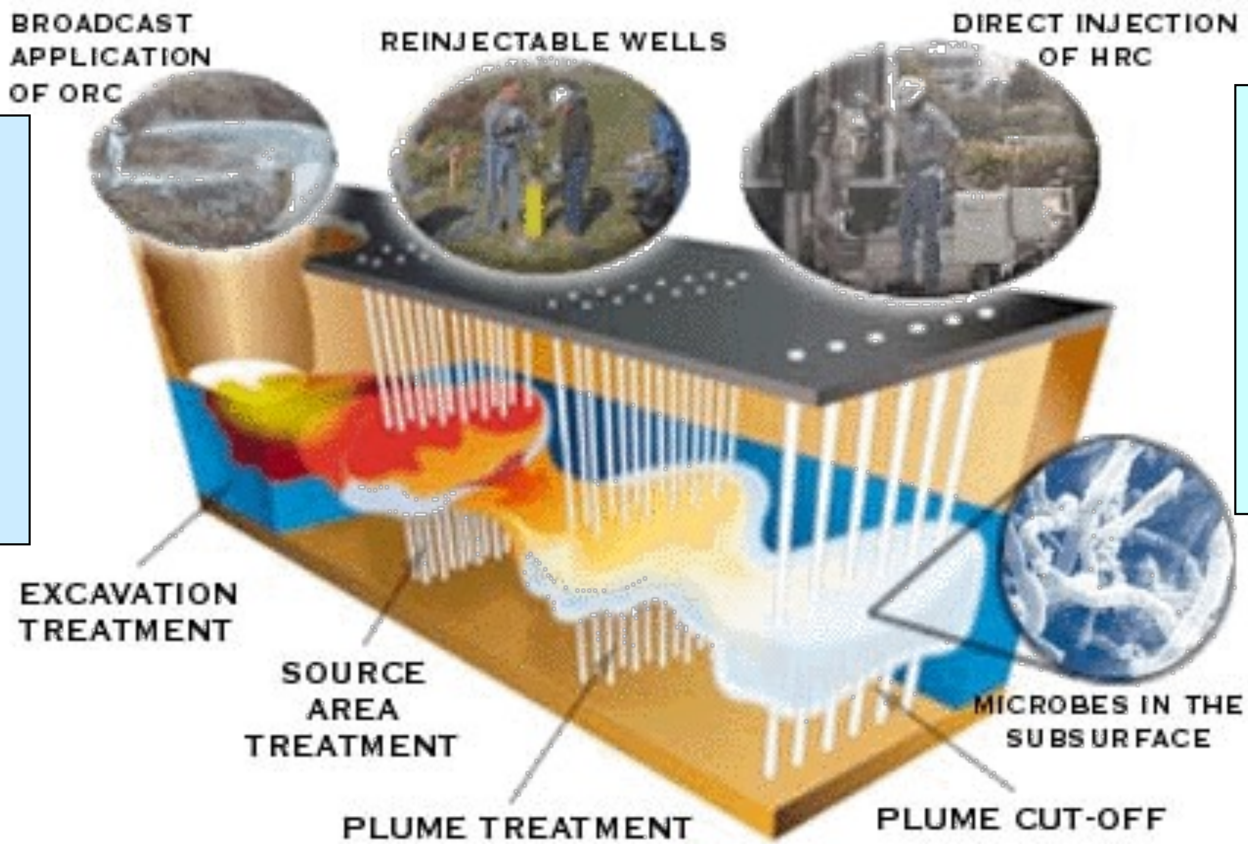
ORC = oxygen release compound

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

HRC = hydrogen release compound

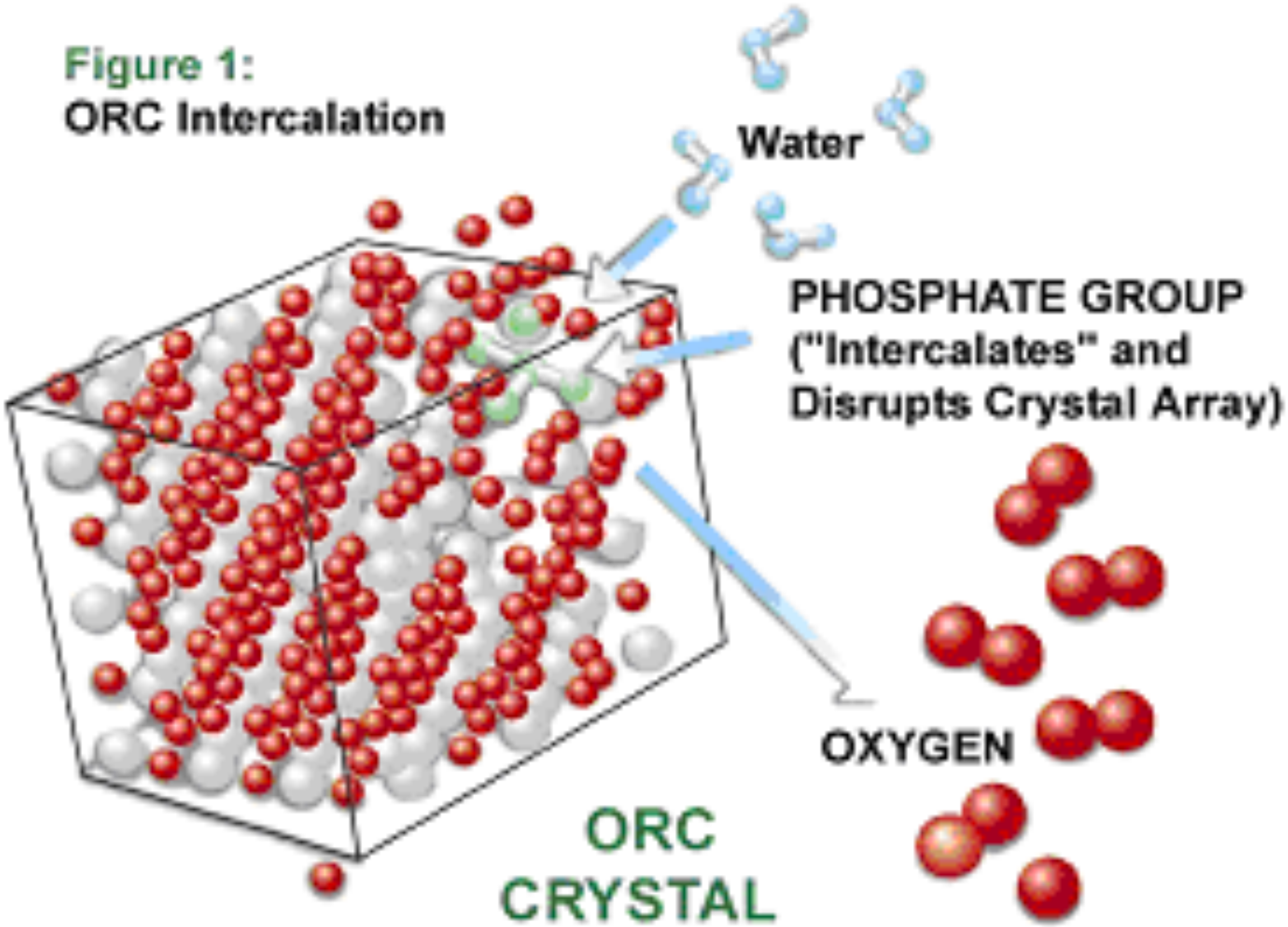
Polylactate + $H_2O \rightarrow$ lactate $\rightarrow H_2$ + acetate

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



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

ORC – How does it work?



HRC – How does it work?

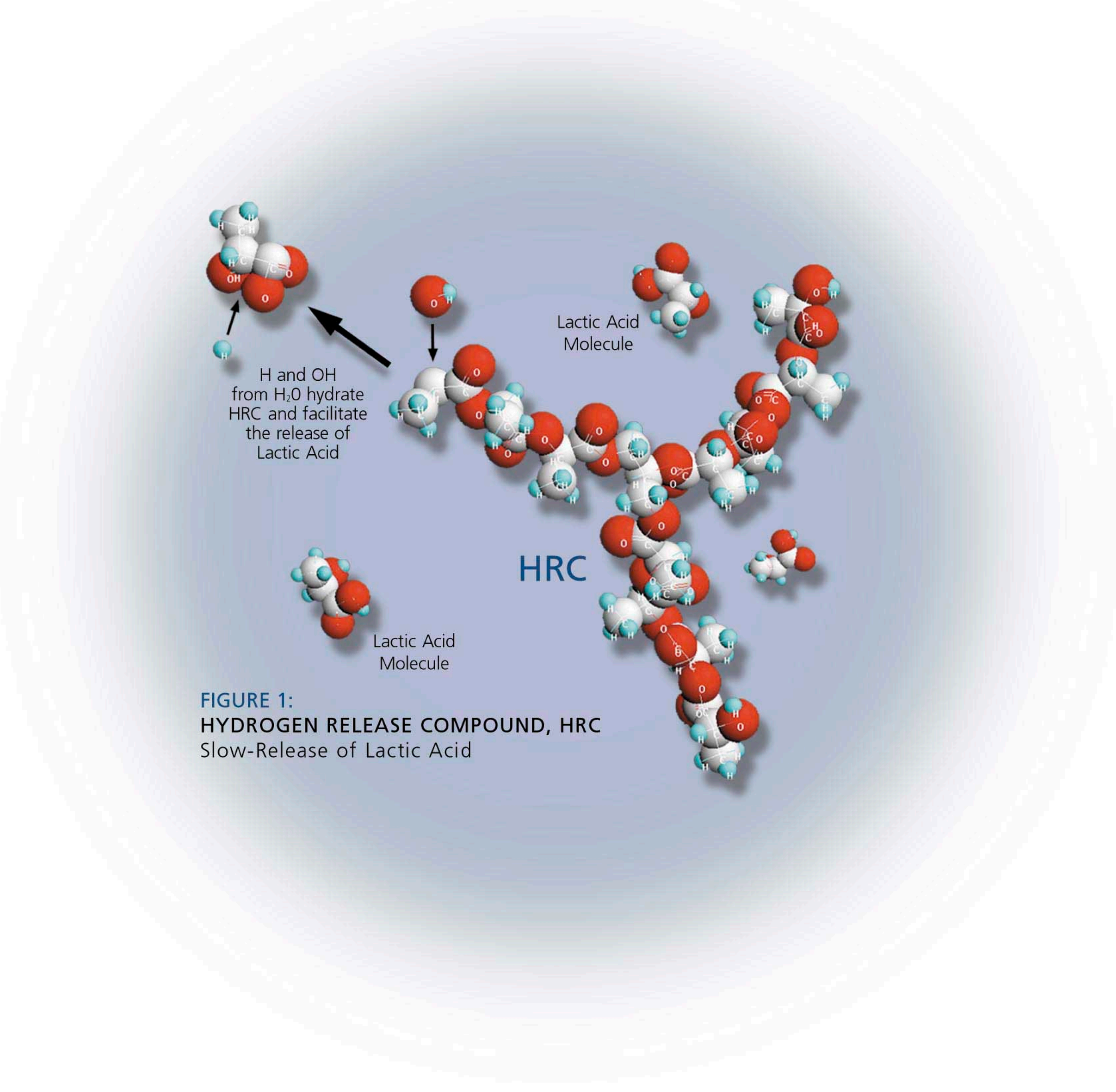
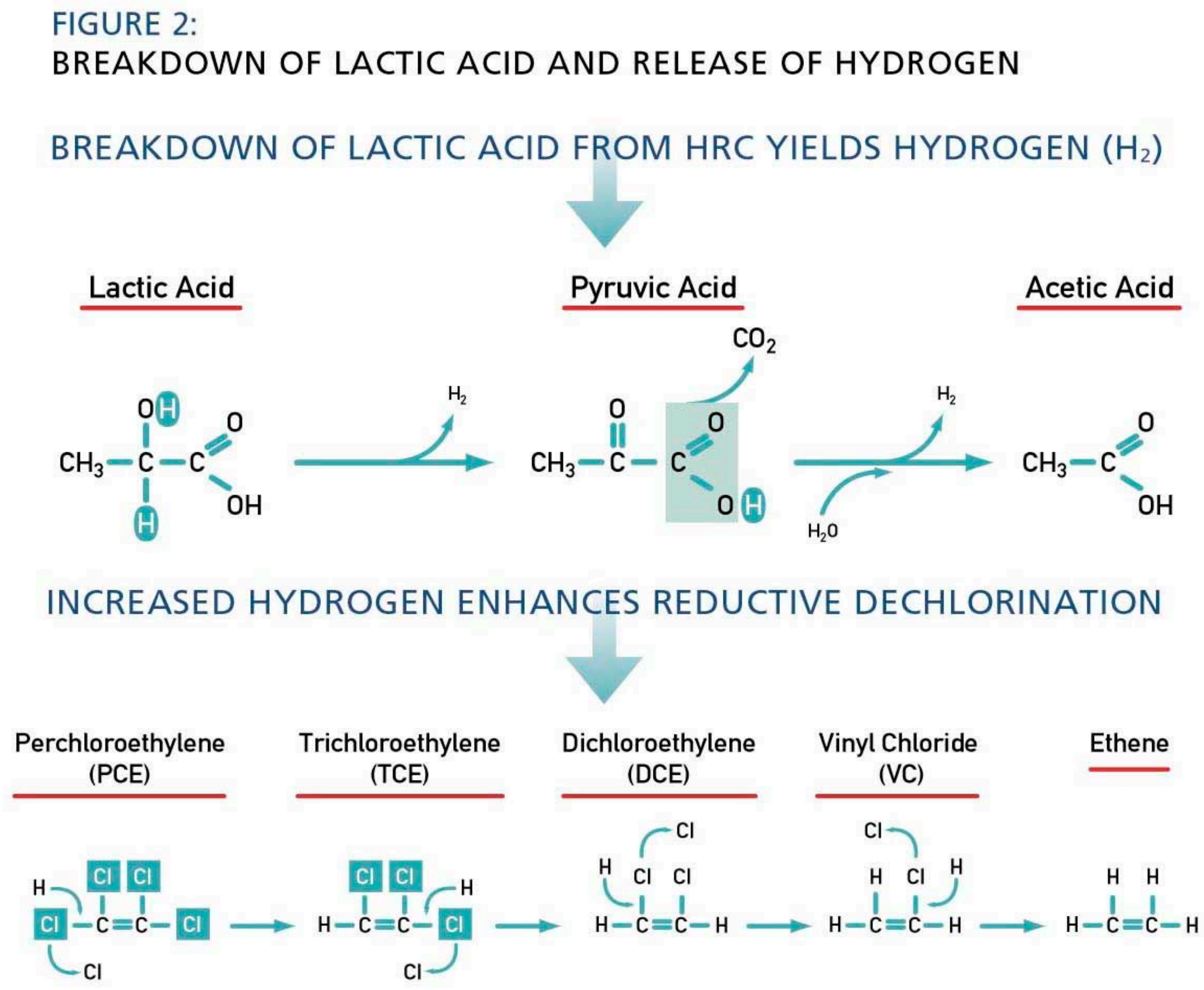


FIGURE 1:
HYDROGEN RELEASE COMPOUND, HRC
Slow-Release of Lactic Acid

HRC – How does it works?



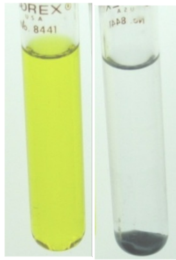
Challenges in biostimulation

- 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

Metal reduction

- Fortuitous microbial reduction of many redox active metals- Sometimes results in precipitation

- Chromate [Cr(VI)] to Cr(III)

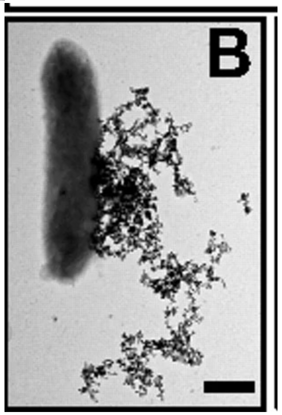


$$Cr^{VI}O_4^{2-}\,(aq) + 3\,e^- + 5\,H^+ \Rightarrow Cr^{III}(OH)_3\,(s) + H_2O$$

- Selenate to Se(0)

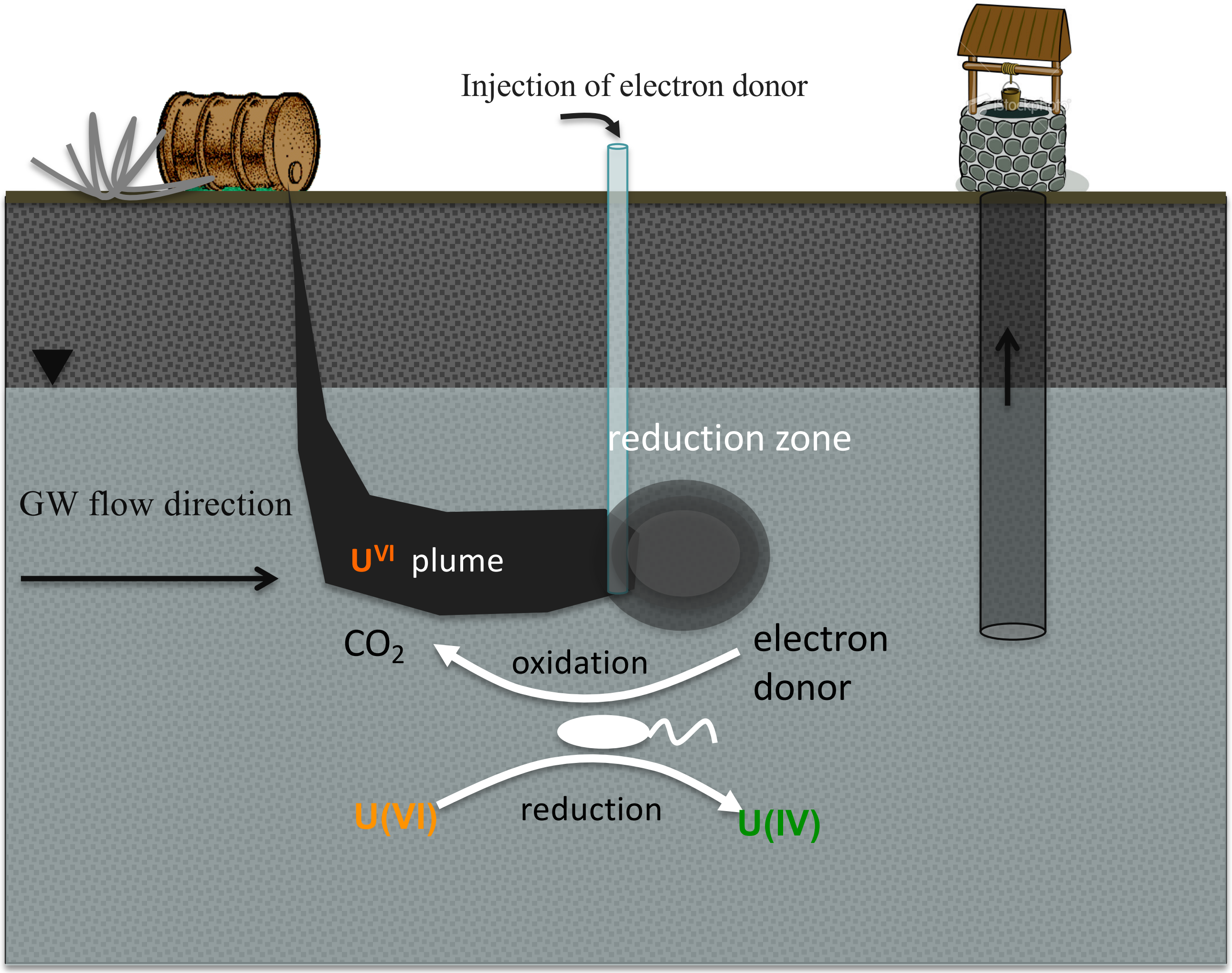
$$Se^{VI}O_4^{2-}\,(aq) + 6\,e^- + 8\,H^+ \Rightarrow Se^0\,(s) + 4\,H_2O$$

- Hexavalent U to U(IV)

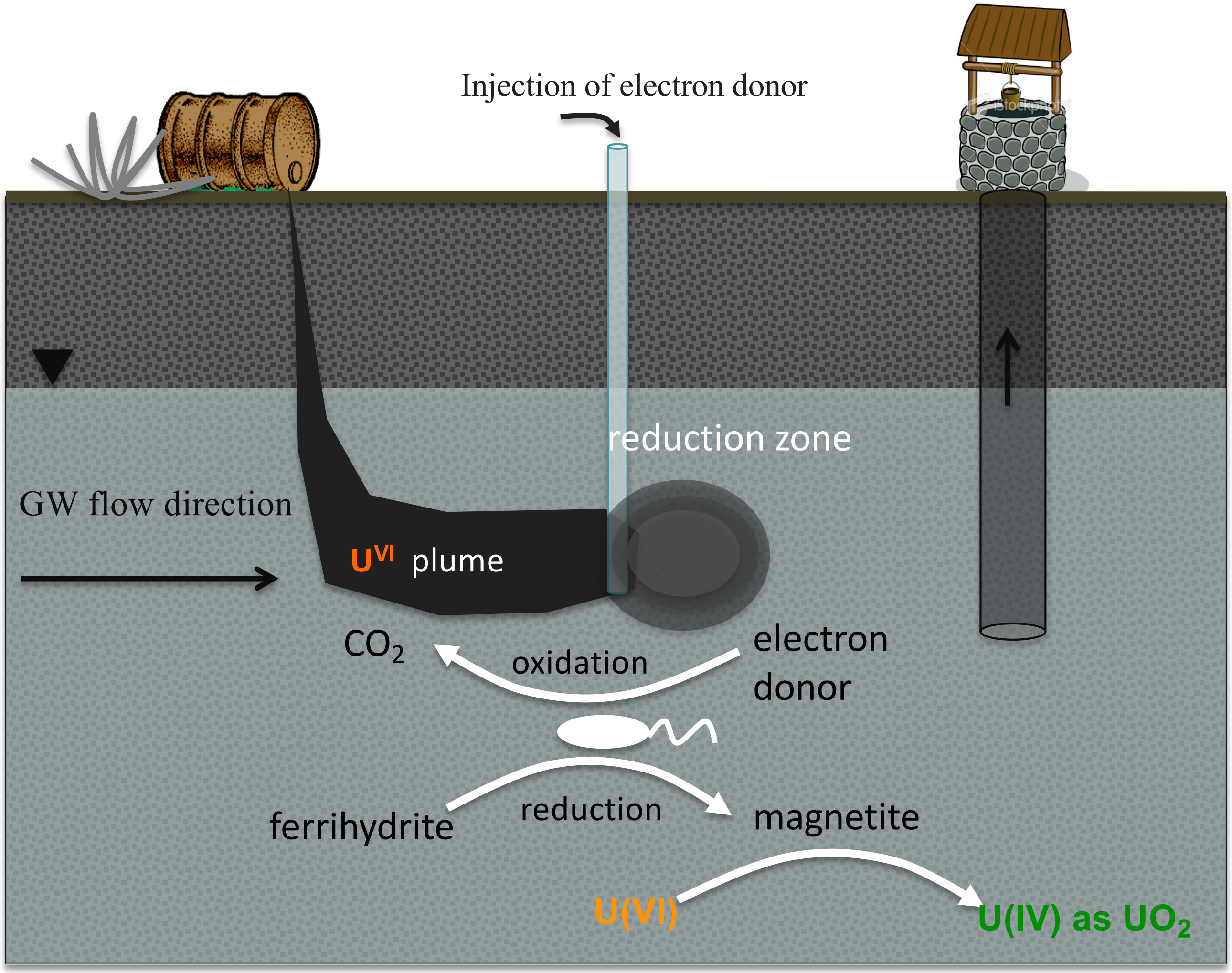


$$U^{VI}O_2^{2+}\,(aq) + 2\,e^- \Rightarrow UO_2\,(s)$$

Uranium reduction *in situ* (direct)

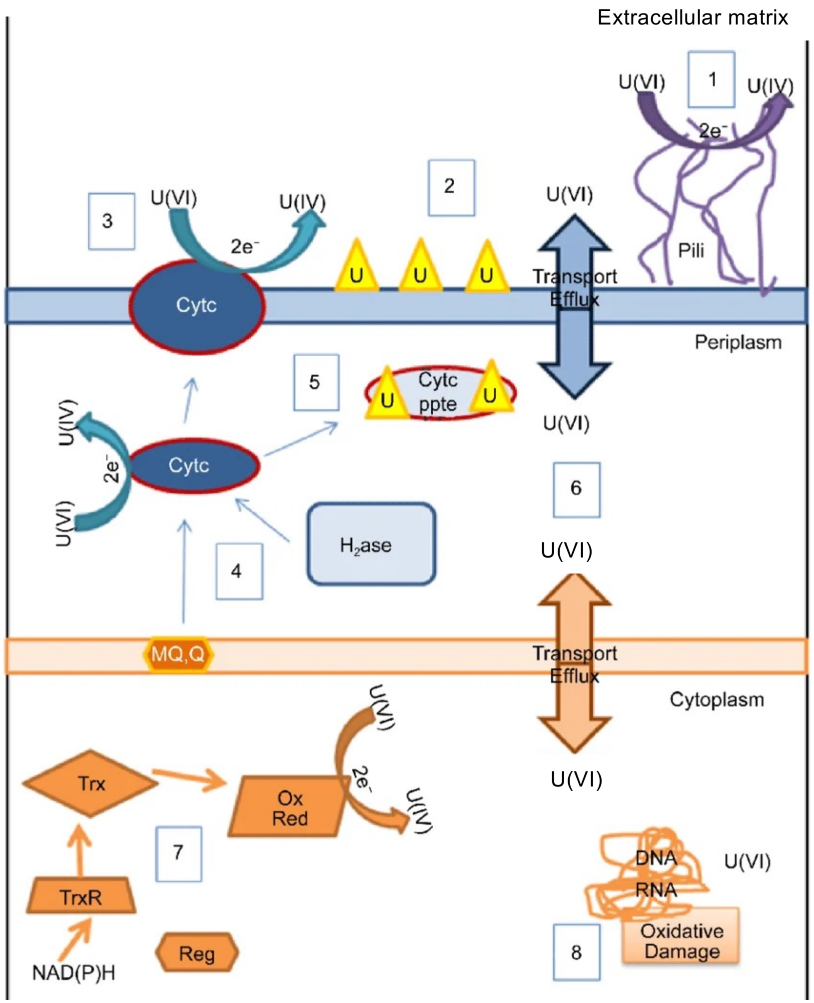


Uranium reduction *in situ* (indirect)



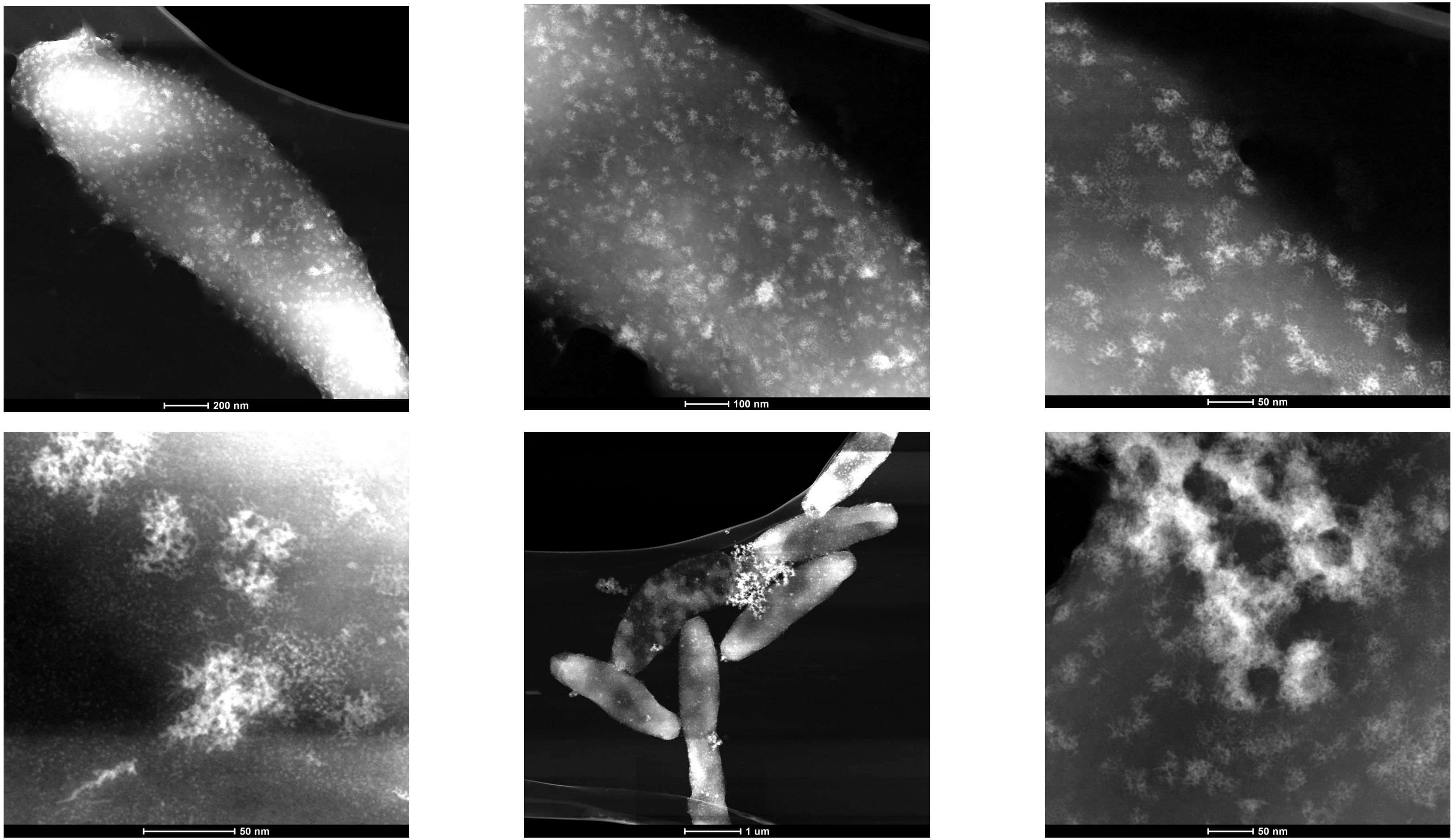
Uranium bioremediation

- Fortuitous reduction of U(VI) to U(IV) by reductases
- Anaerobes capable of U(VI) reduction to U(IV):
  - Iron-reducing bacteria
  - sulfate-reducers
  - Fermenters
  - Anoxygenic phototrophs (?)
  - Denitrifiers (?)
- U(IV) in the form of UO_2 is highly insoluble and precipitates



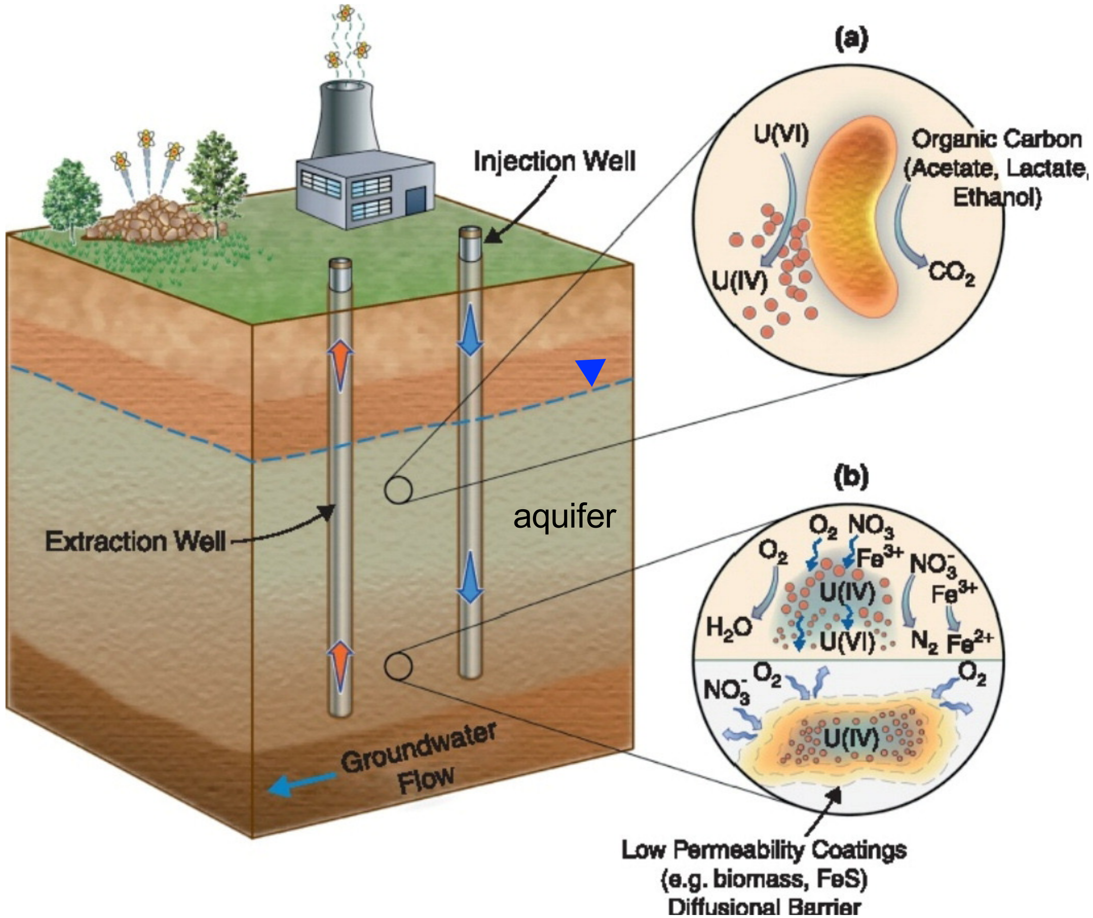
Examples of U(VI) reduction pathways

Shewanella oneidensis MR-1 CryoTEM images with U(VI) reduced to U(IV)



Uranium bioremediation

- Amendment of electron donor (e.g., lactate, acetate) to the subsurface (Bio-stimulation)
- Consumption of O_2 (if present), formation of low permeability zones in which reduction can take place
- Sulfate-reducing conditions and iron-reducing conditions established



Uranium bioremediation

- Amendment of electron donor (e.g., lactate, acetate) to the subsurface

- Consumption of O_2 (if present), formation of low permeability zones in which reduction can take place

- Sulfate-reducing conditions and iron-reducing conditions established forming Fe(II), S(-II) and FeS (mackinawite)

  - $UO_2^{2+} + HS^- \Rightarrow UO_2 + SO_4^{2-} + H^+$
  - $UO_2^{2+} + Fe(II) \Rightarrow UO_2 + Fe(III)$
  - $UO_2^{2+} + FeS \Rightarrow UO_2 + Fe(II) + SO_4^{2-} + H^+$

⇨ Likely a mixture of biotic and abiotic U(VI) reduction

Example 1

A subsurface is impacted by toluene ($C_6H_5CH_3$). 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

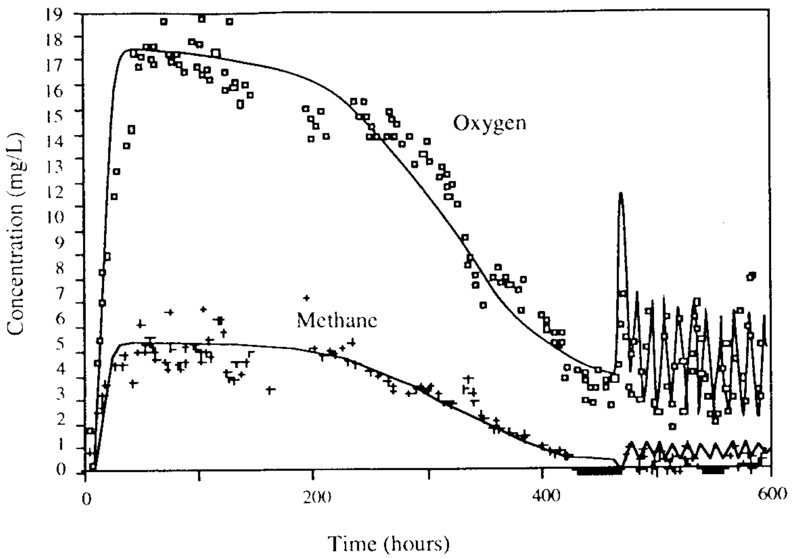
$K_D = 6.76 * 10^{-6}$ m^3/g

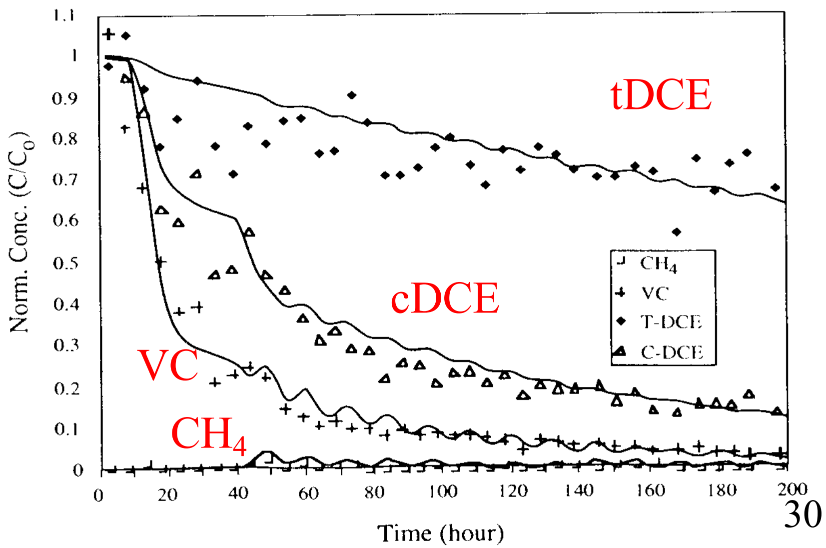
Assume 20 ºC and DO saturation= 9 mg/L

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

Field-scale study-aerobic

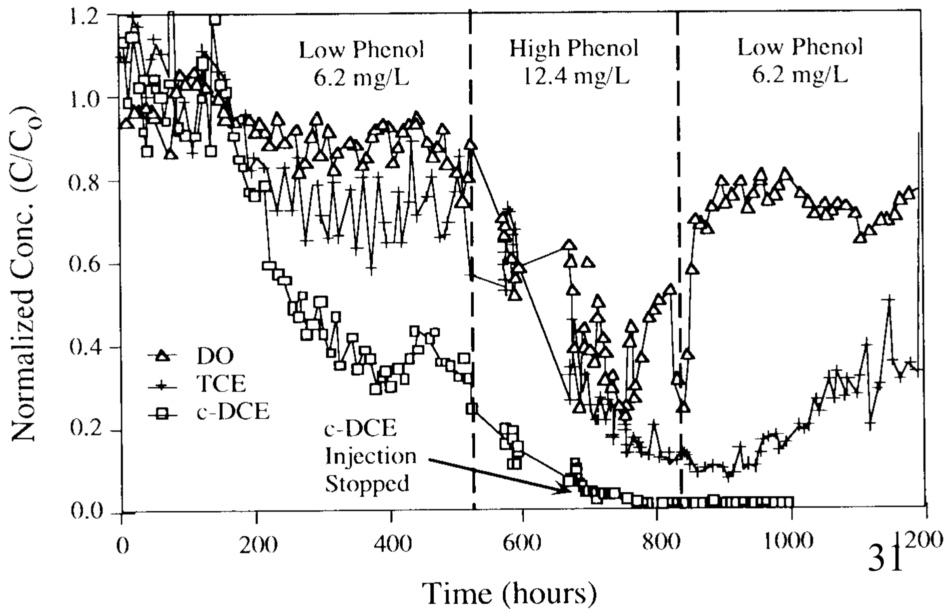
- Moffett field in CA
- Groundwater extracted from treatment zone, amended with substrate+ O_2 and reinjected
- Inject methane and O_2 => stimulate methanotrophs => cometablic degradation of chlorinated hydrocarbons
- Late in injection, pulse methane and O_2 to minimize clogging
- Conclusions:
  – Stimulation 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





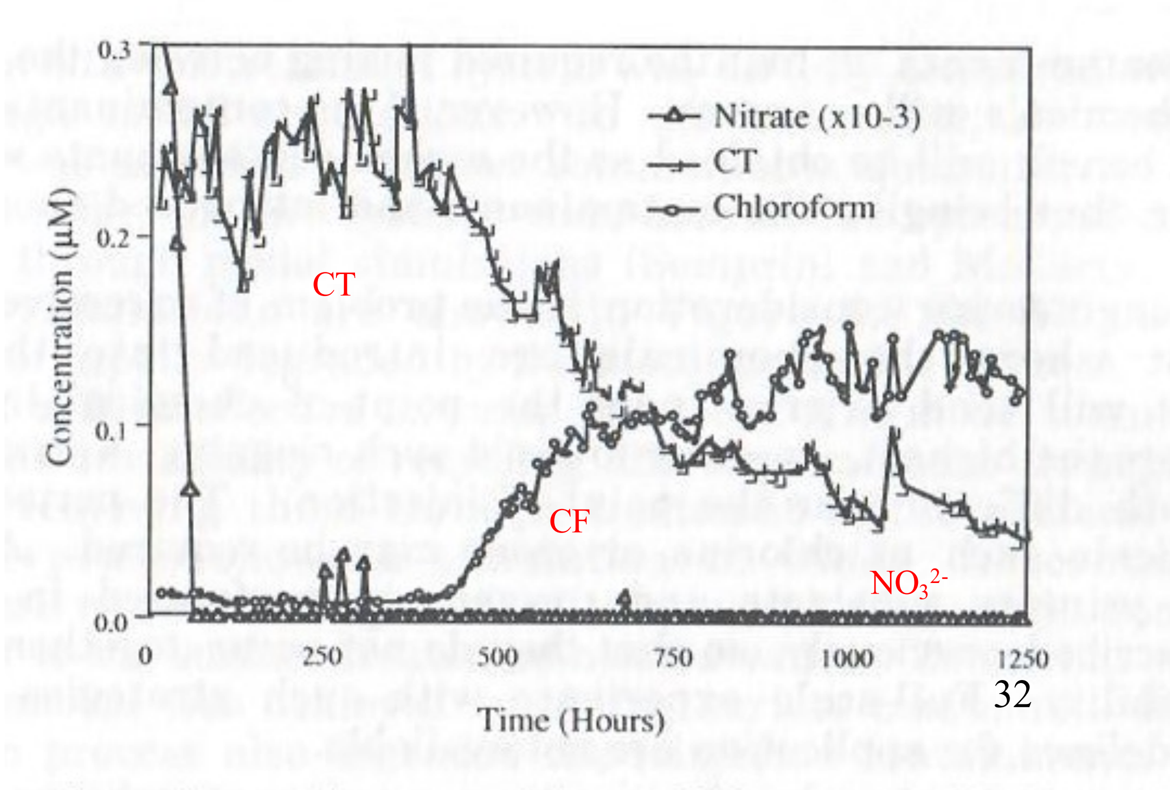
Field-scale study-aerobic

- 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



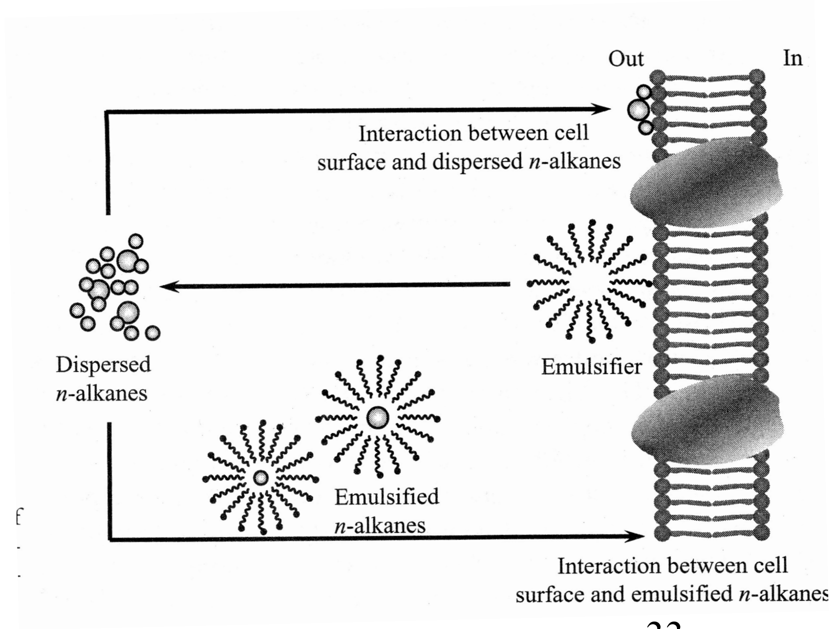
Field-scale study-anaerobic

- 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

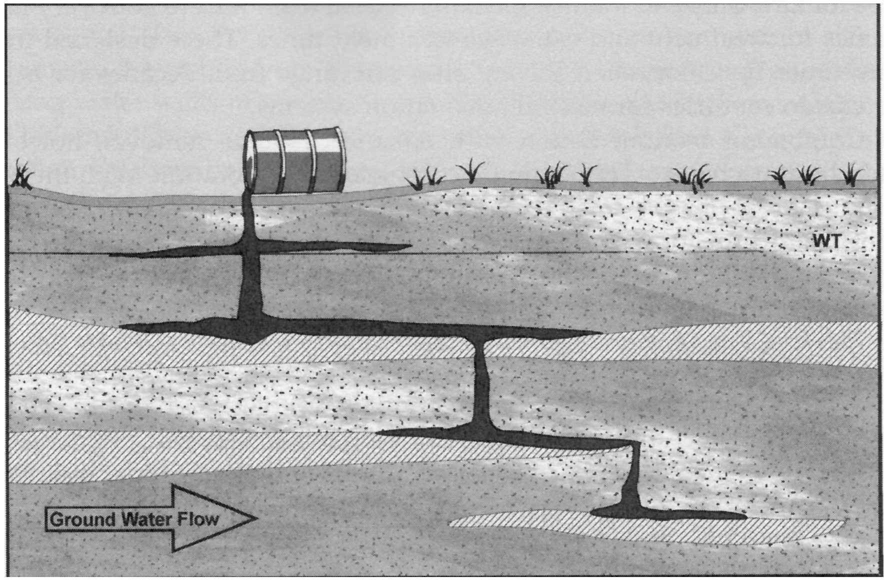


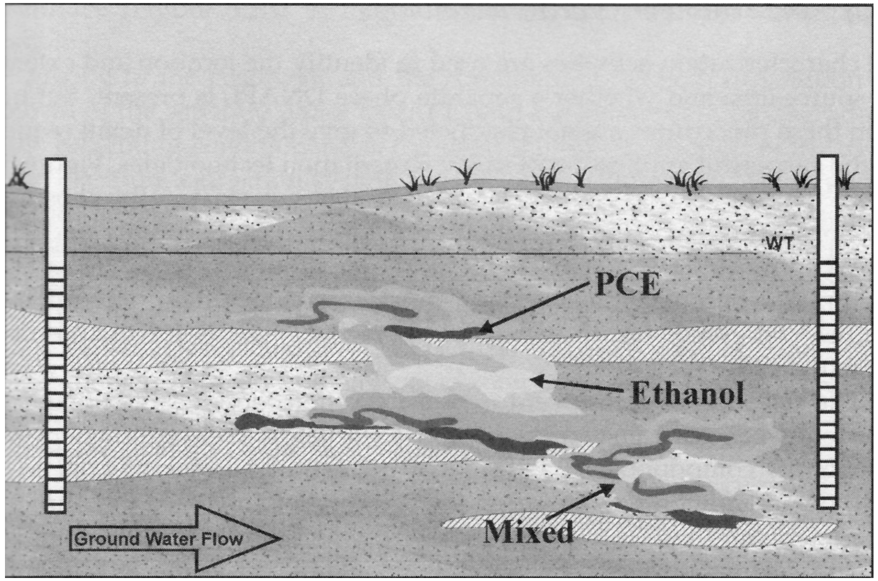
Enhancing bioavailability

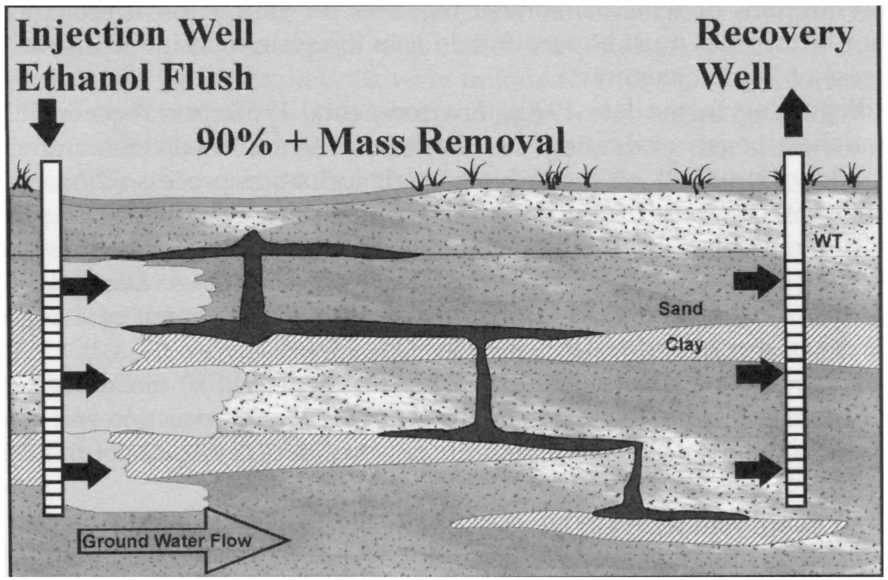
- Biggest obstacle to effective bioremediation is sorption of contaminants to soil
- Improve bioavailability by solubilizing organic compounds associated with OM using surfactants
- Issue of toxicity and resistance to degradation
- Biosurfactants:
  - Produced as a response to low water solubility of 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

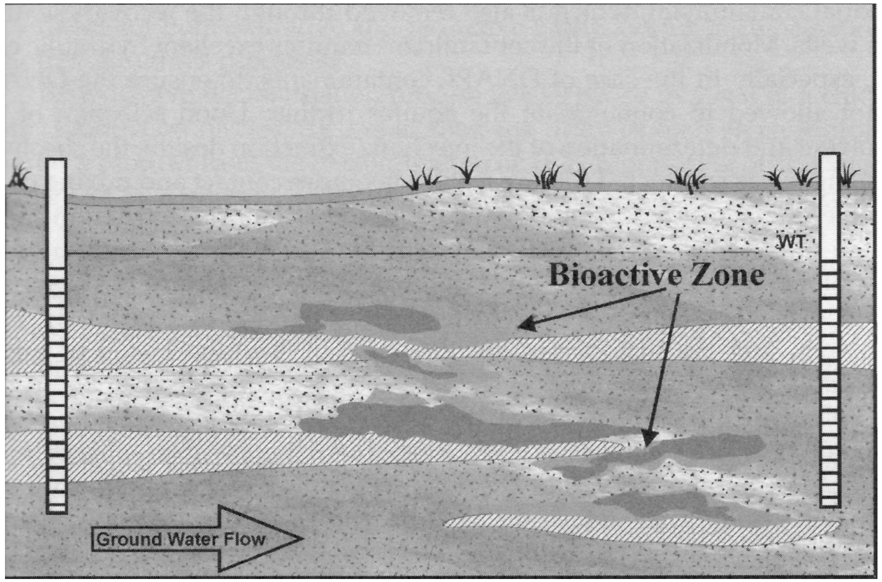


Solvent extraction+bioremediation









Bioaugmentation

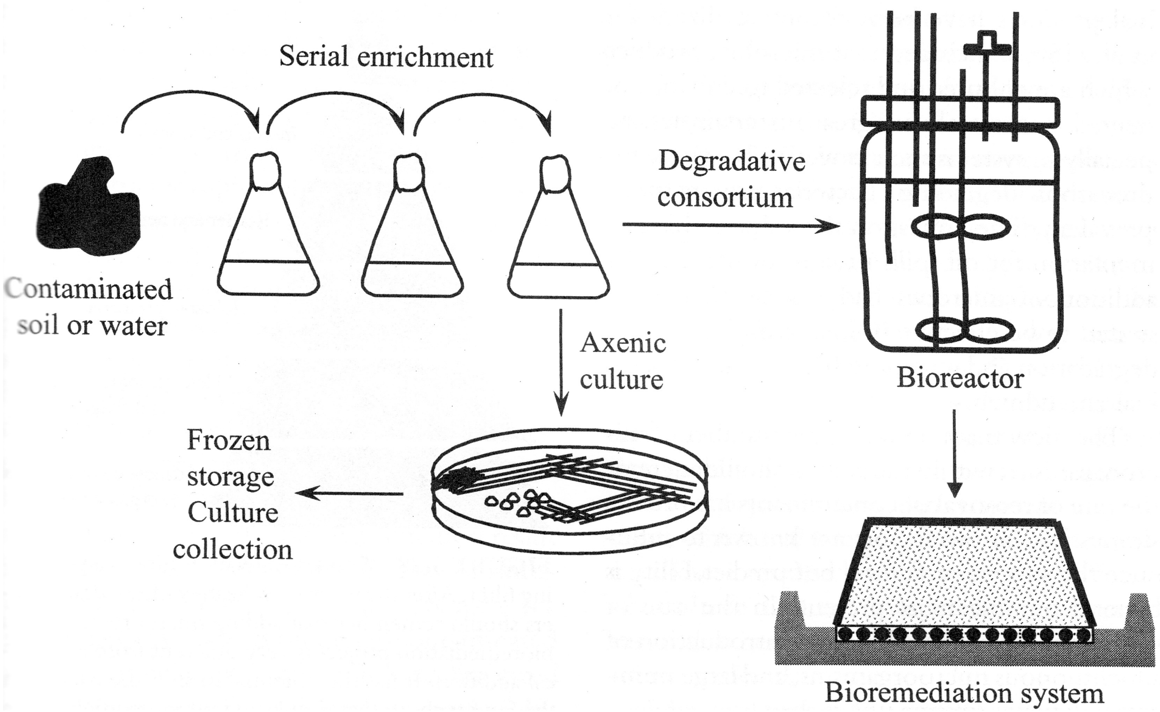
Bioaugmentation strategies

- Reason for bioaugmentation:
  - Indigenous organisms deemed unable to degrade contaminant
  - Need to amend specific microbial strains or consortia to ensure (or improve) the biodegradation of a compound
  - Usually applied for recalcitrant compounds
  - Limitation: decline of the introduced population is rapid due to competition and predation
- Two strategies:
  - *Strategy 1*: degradative microorganisms complement native population.
    - Selected for ability to survive or occupy a specific niche.
    - Stimulants or selective substrate can be added to improve survival.

  <u>Goal</u> => achieve prolonged survival and growth of amended organism
  - *Strategy 2*: large amounts of bacterium added
    - Single or multiple amendment(s) of concentrated biomass with cells already primed for activity

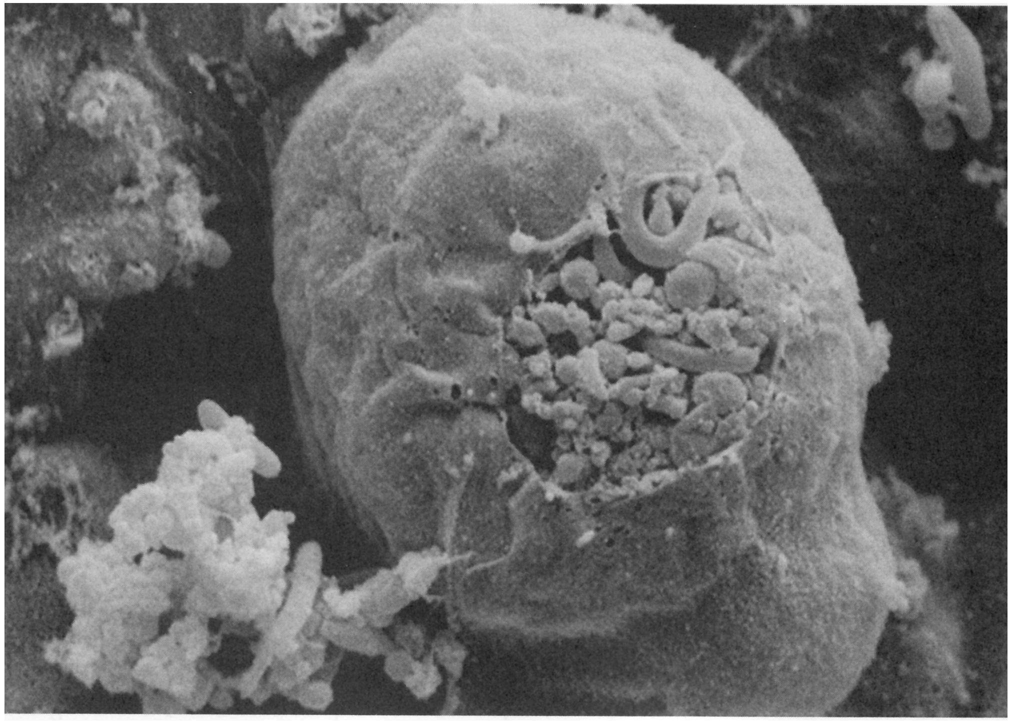
  <u>Goal</u> => Degradation of large amounts of contaminant before becoming inactive

Identifying the right microbes for bioaugmentation



- Two approaches to select microorganisms to amend:
  1. Use well characterized isolates with specific pollutant degradation activity
  2. Take samples from site and use them for serial enrichment with contaminant and build a degradative consortium

Improving the odds of success for bioaugmentation



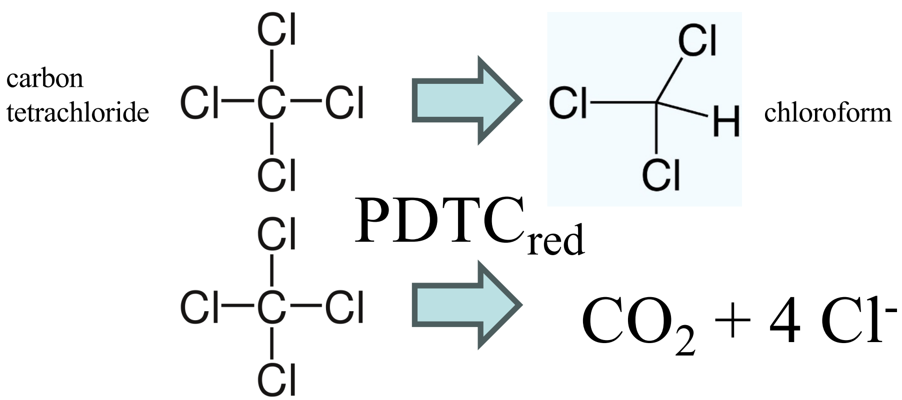
- Amended microorganisms may not be viable in sediments (predation, competition, inadequate growth conditions)
- Limited mobility of amended microbes (remain around the well)
- Novel delivery systems such as encapsulation in hydrogel (e.g., polyvinyl alcohol (PVA)) allow:
  - Survival of competition, predation, chemical extremes
  - Travel further in the aquifer (low adsorption to the porous medium)

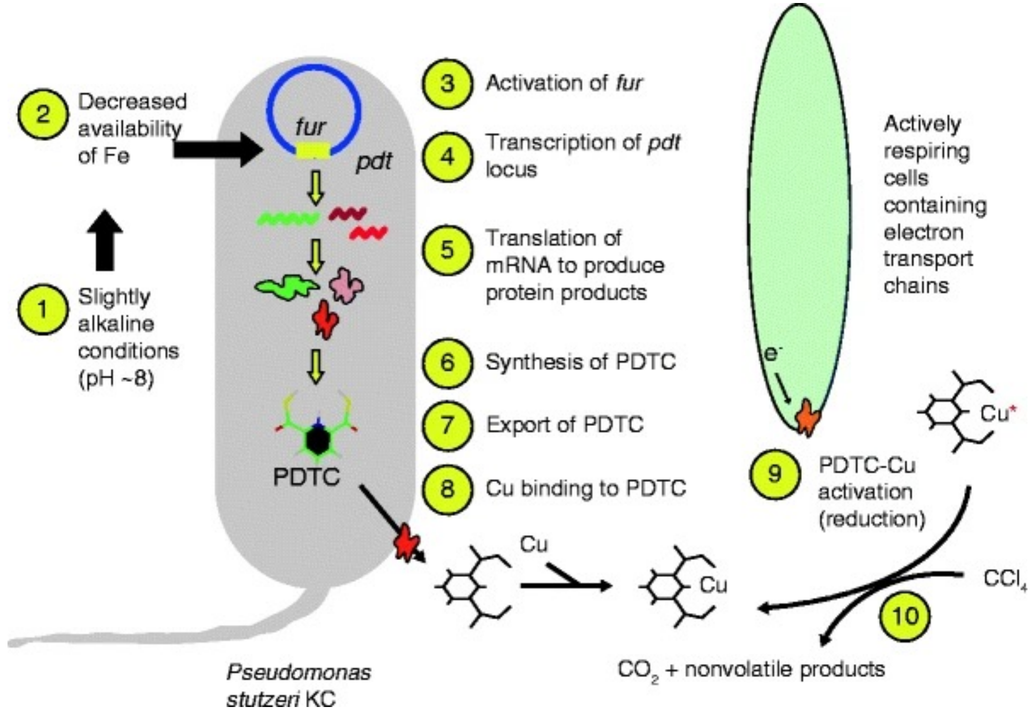
Examples of bioaugmentation

1. Co-metabolism of carbon tetrachloride
2. Low adhesion cell for toluene degradation
3. Degradation TCE and cDCE to ethene

Carbon tetrachloride co-metabolism

- Carbon tetrachloride dechlorination results in the formation of chloroform (more toxic than CT)
- Strain KC produces a compound PDTC (pyridine-2,6-bis-thiocarboxylate) that complexes with Cu
- The complex reacts with CT and leads to its oxidation to CO_2

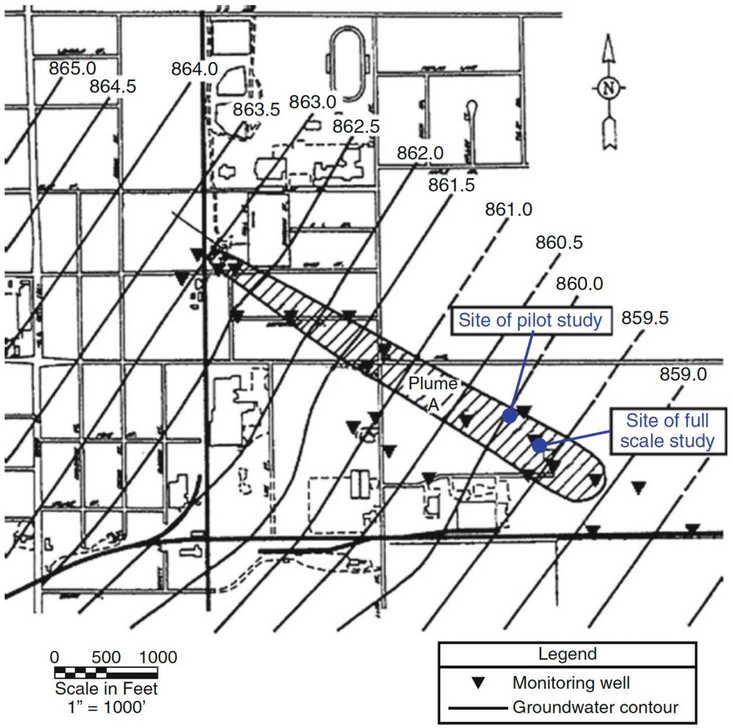


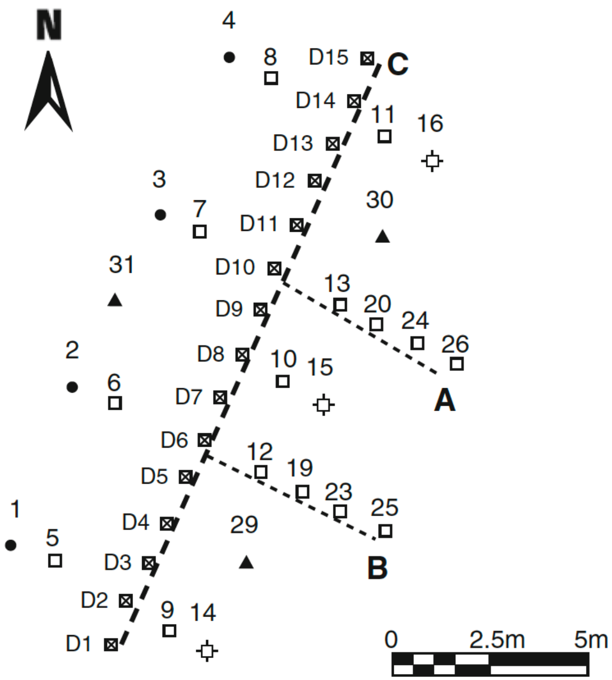


Criddle et al., 2012

In situ bioremediation

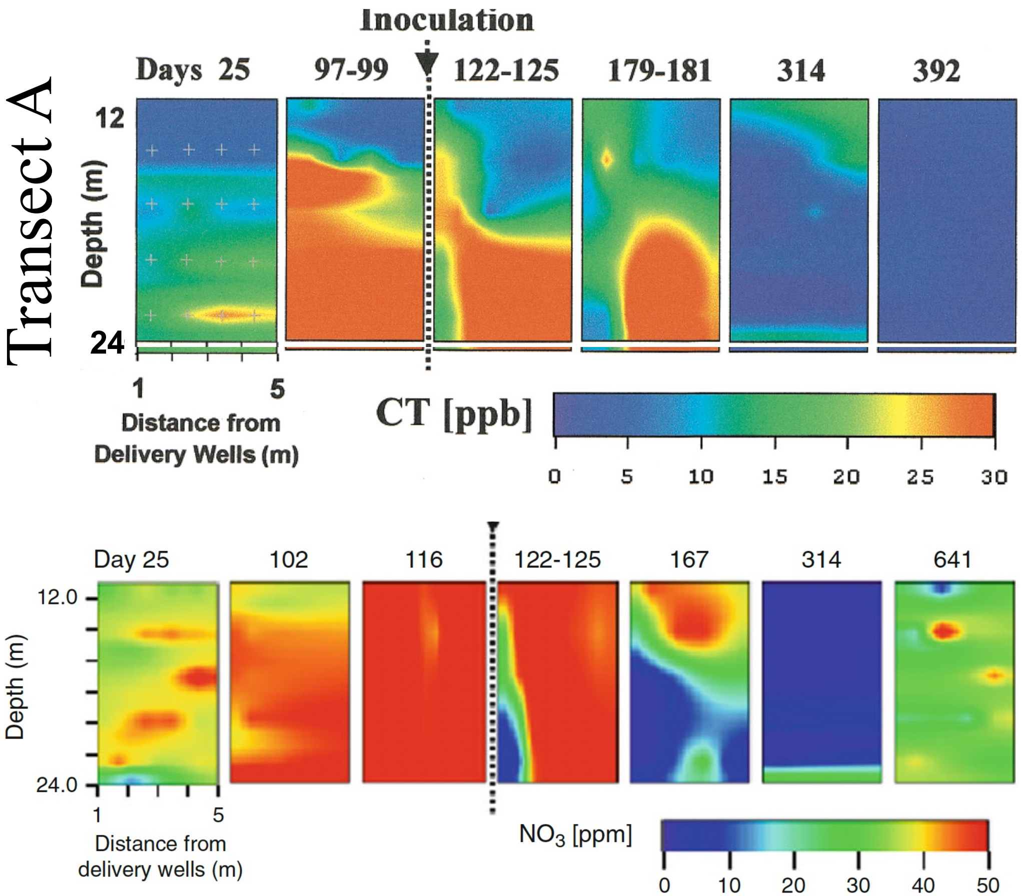
- Carbon tetrachloride plume
- Transect C is a series of wells that deliver substrates, including cells
- Transects A and B are downgradient monitoring wells





Dybas et al., 2002

In situ bioremediation



Dybas et al., 2002

- Adjust the pH (favor Strain KC)
- Amend trace metals (Fe, Cu, Zn, Ni, Mn)
- Bio-augmentation with Strain KC
- Amendment of acetate weekly (100 mg/L)

- Nitrate-containing groundwater

- CT removed within ~1 year of remediation

$CH_3COOH + NO_3^- => N_2$

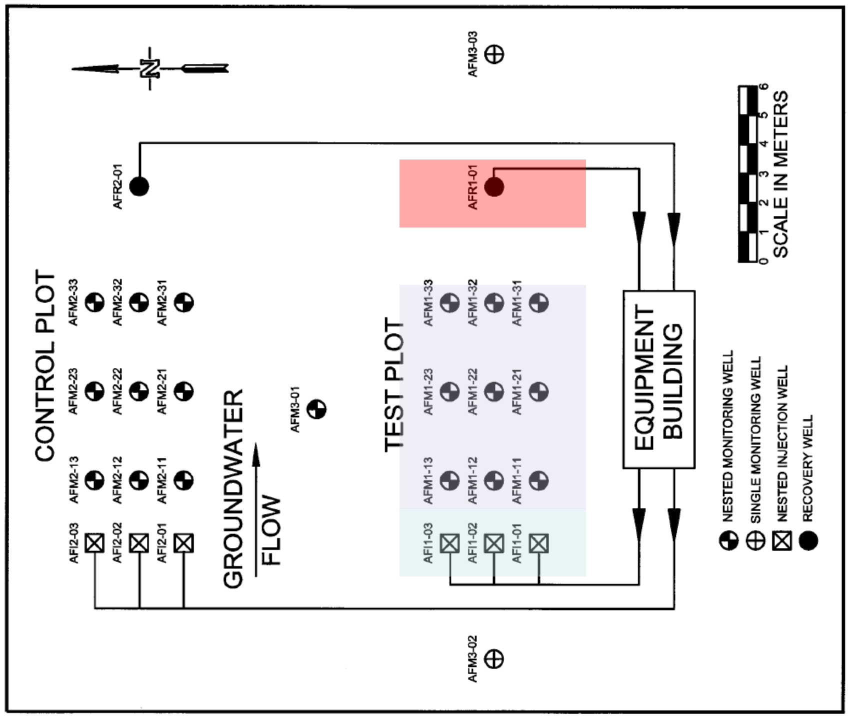
Production of PDTC

PDTC reduced during denitrification

Reduced PDTC breaks down CT

Low adhesion cells

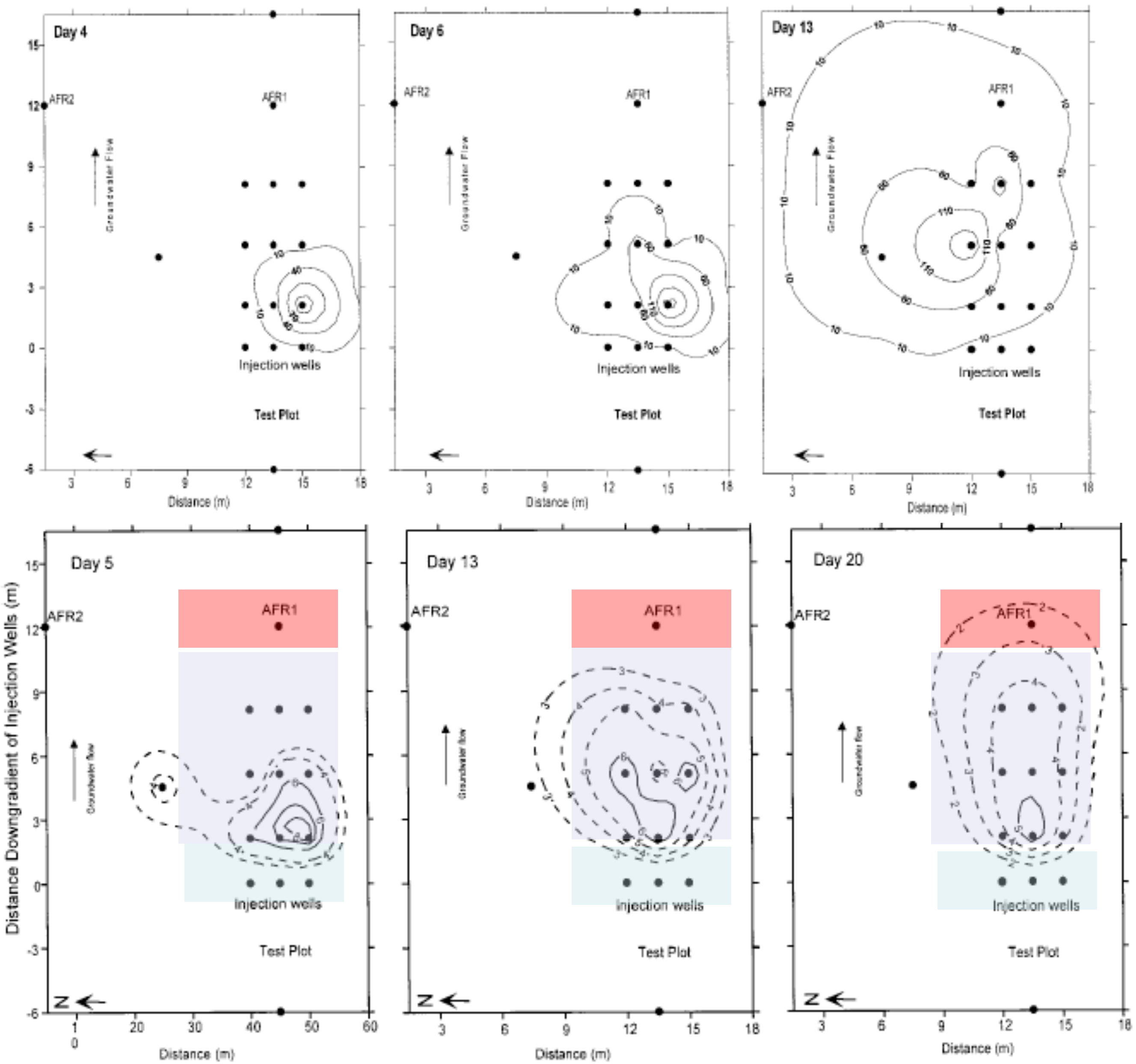
- *Burkholderia cepacia* ENV435: low adhesion capacity and constitutive expression of toluene monooxygenase
- Can grow in large fermentors (750 L) to high cell density
- Accumulate large amounts of internal energy which can prolong degradation activity under non-optimal conditions
- Two trials: (1) injected 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



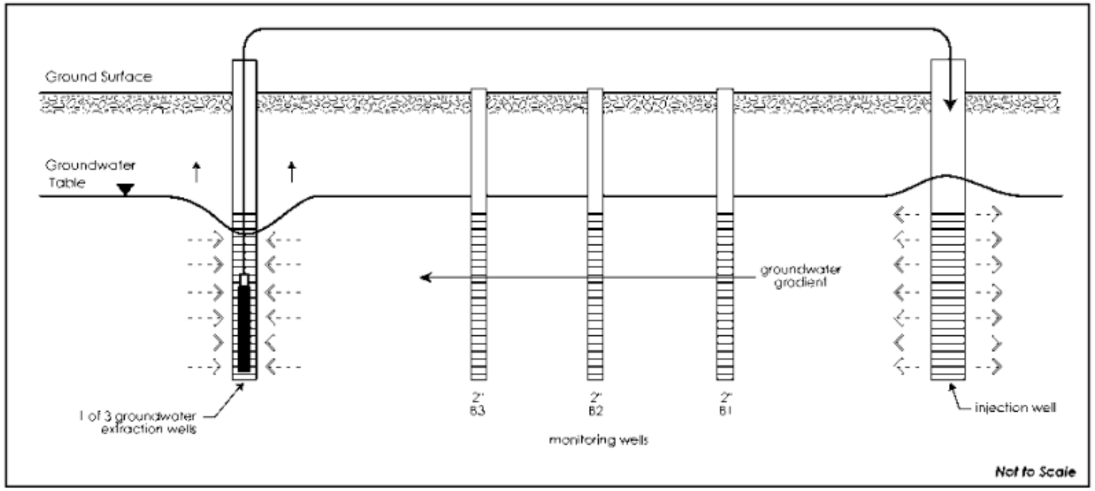
B. cepacia

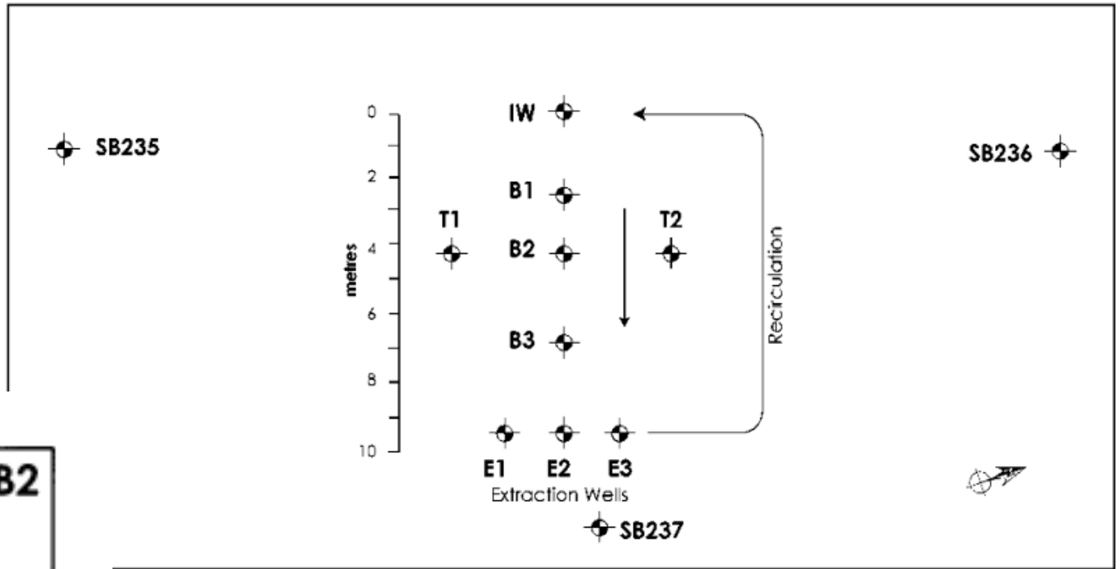
Log CFU/mL

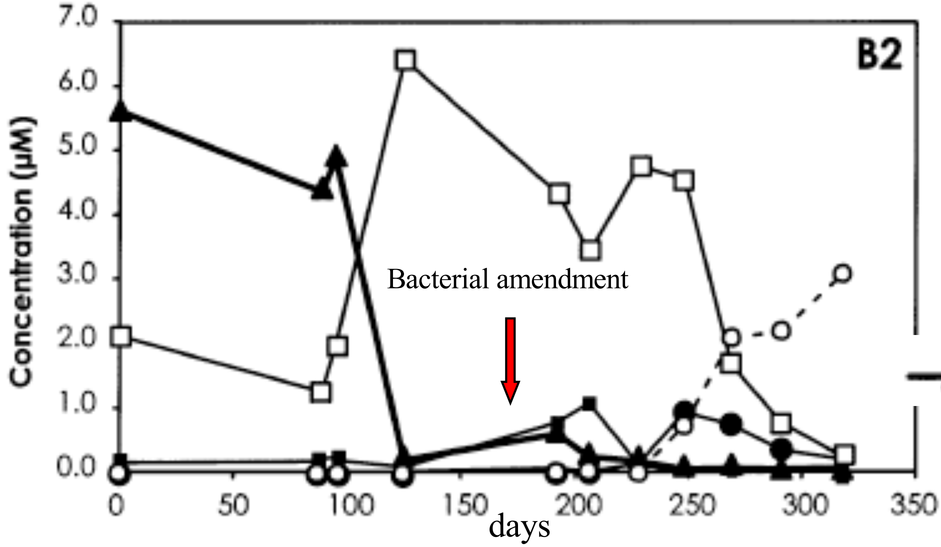
Ratio of tracer velocity to bacteria velocity was 1.2-1.4
Half-life of cells about 2 days
Trial 2 was more successful than trial 1: splitting the injection over several wells more effective

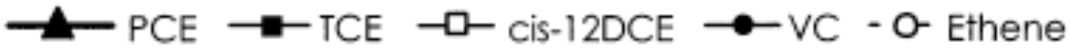
Reductive dechlorination

- 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

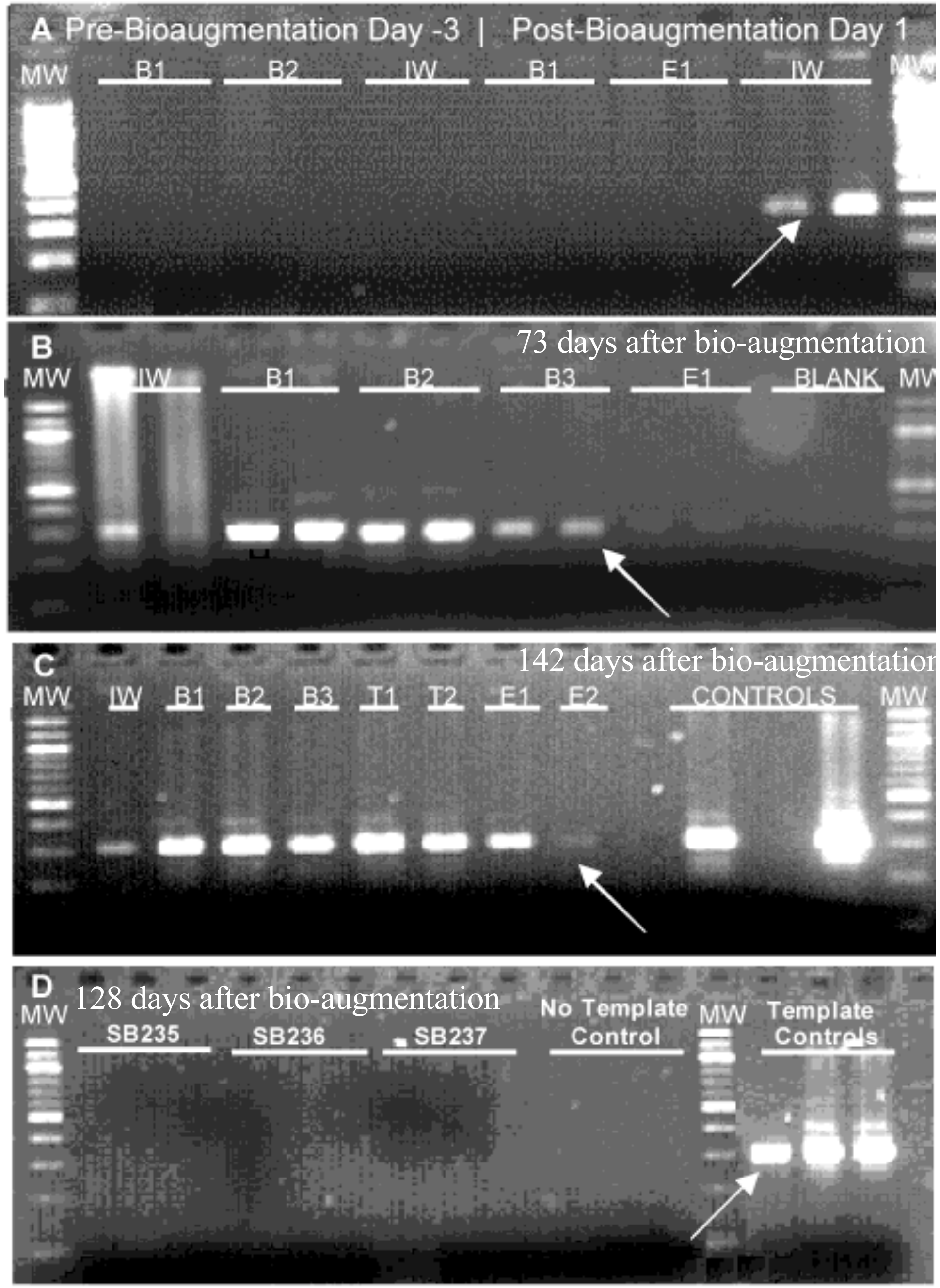








Kelly AFB



- 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?

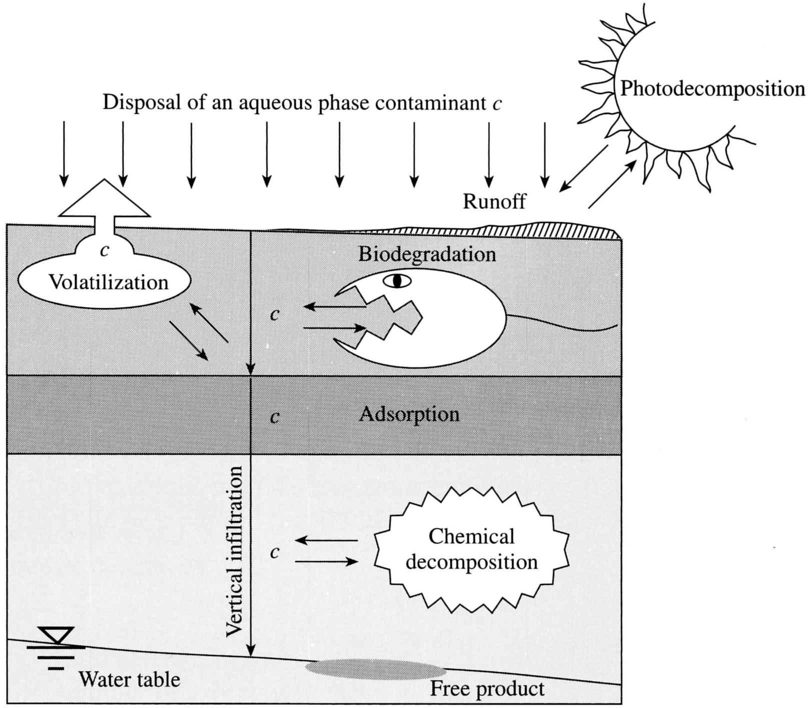
If methanotrophic cometabolism, assuming ratio of 5 CH_4 moles to one TCE, what total amount of dissolved O_2 needed?

Monitored Natural Attenuation

Monitored Natural attenuation

Definition:

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



Processes involved in **Natural Attenuation**

Biodegradation

Sorption

Volatilization

Chemical degradation

Dispersion and dilution

Indicators of microbial activity

Geochemical parameters to monitor	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

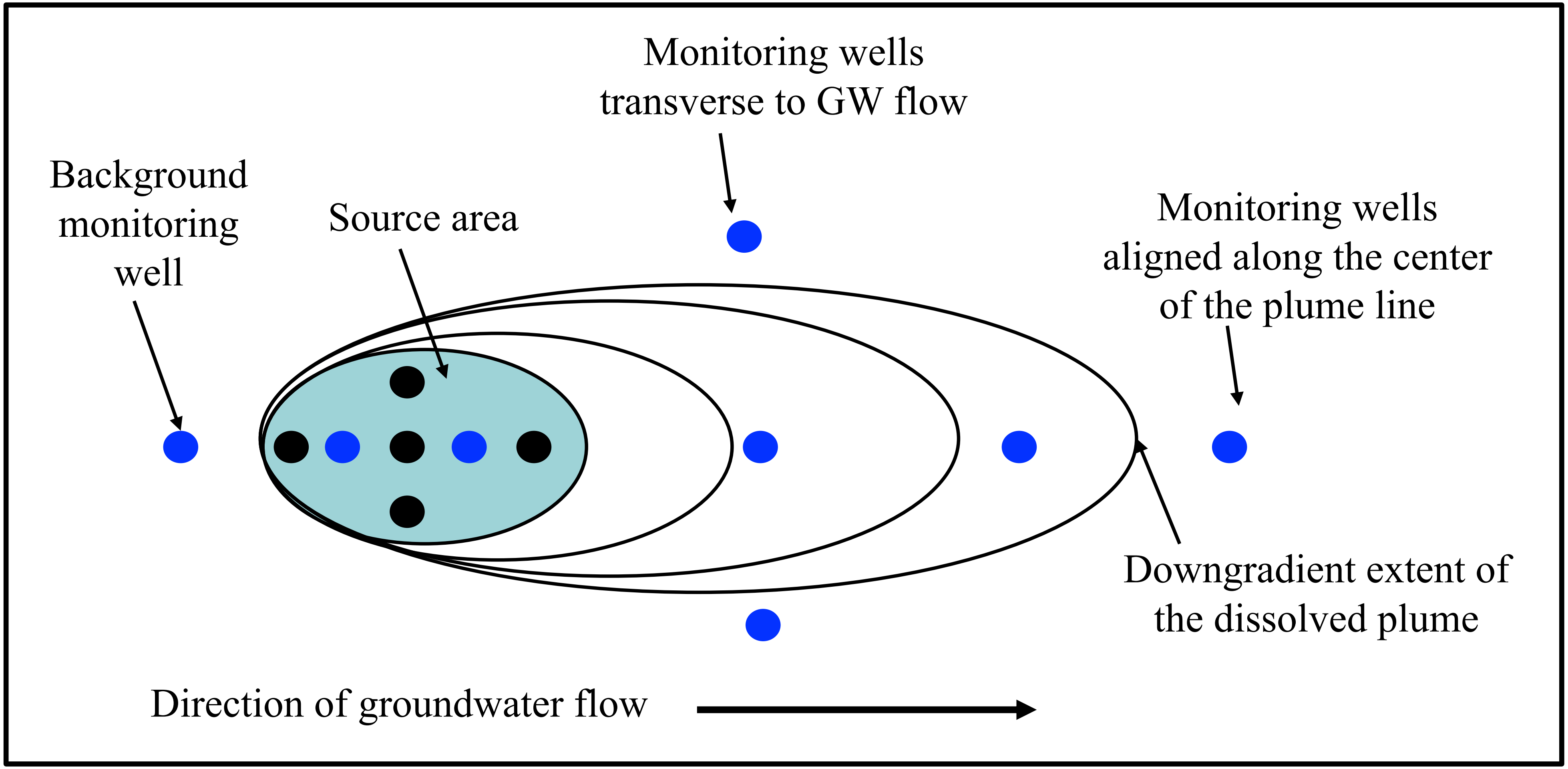


Diagram of monitoring of subsurface natural attenuation of a contaminant plume

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
- National Research Council of the US:

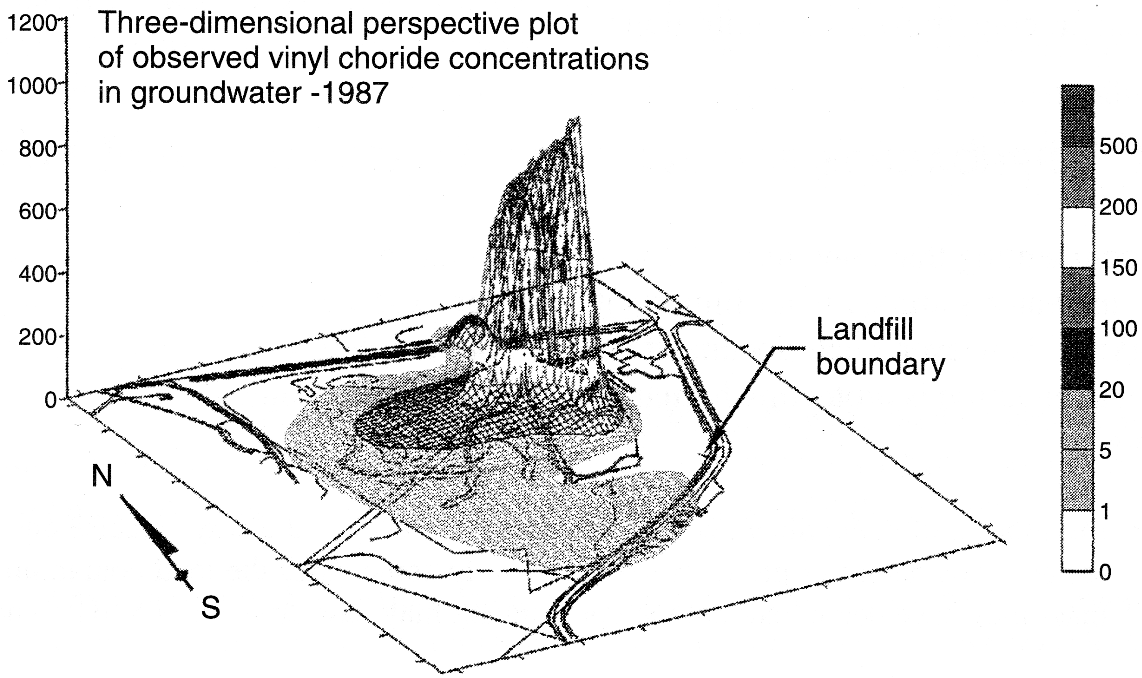
  (1) demonstrate loss of contaminant in site

  (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 a biologically active zone (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


1200
1000
800
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Three-dimensional perspective plot
of observed vinyl choride concentrations
in groundwater -1987
Landfill
boundary
N
S
500
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150
100
20
5
1
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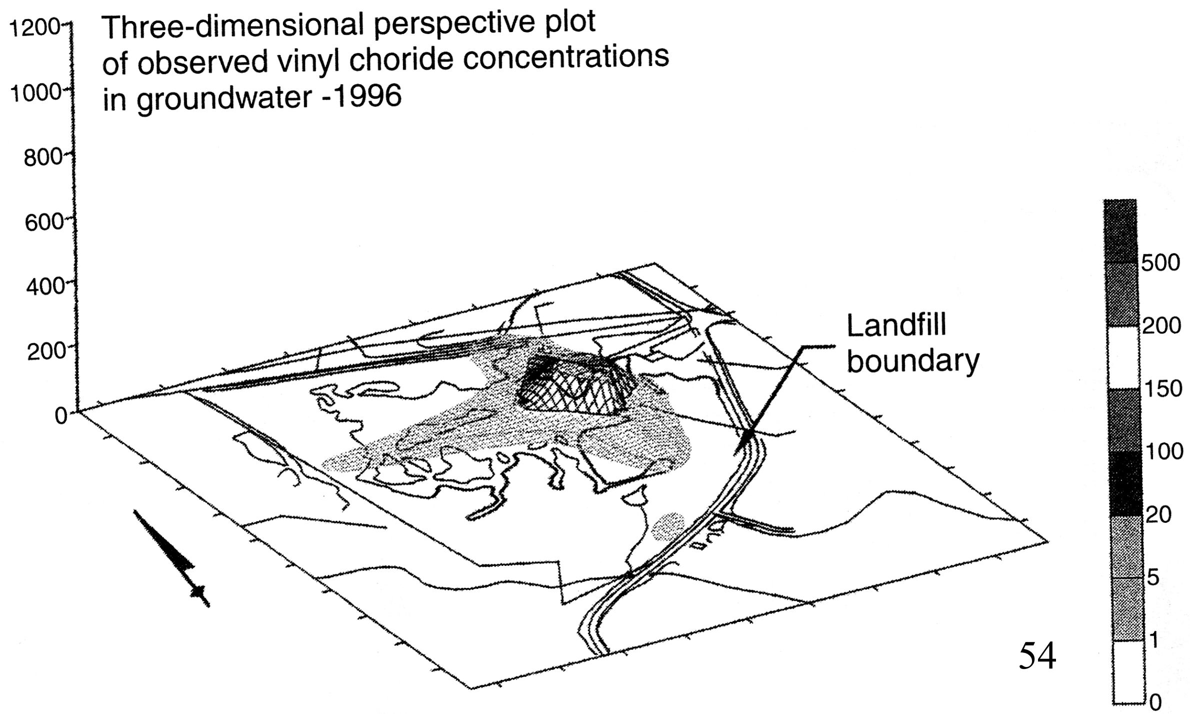
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Three-dimensional perspective plot
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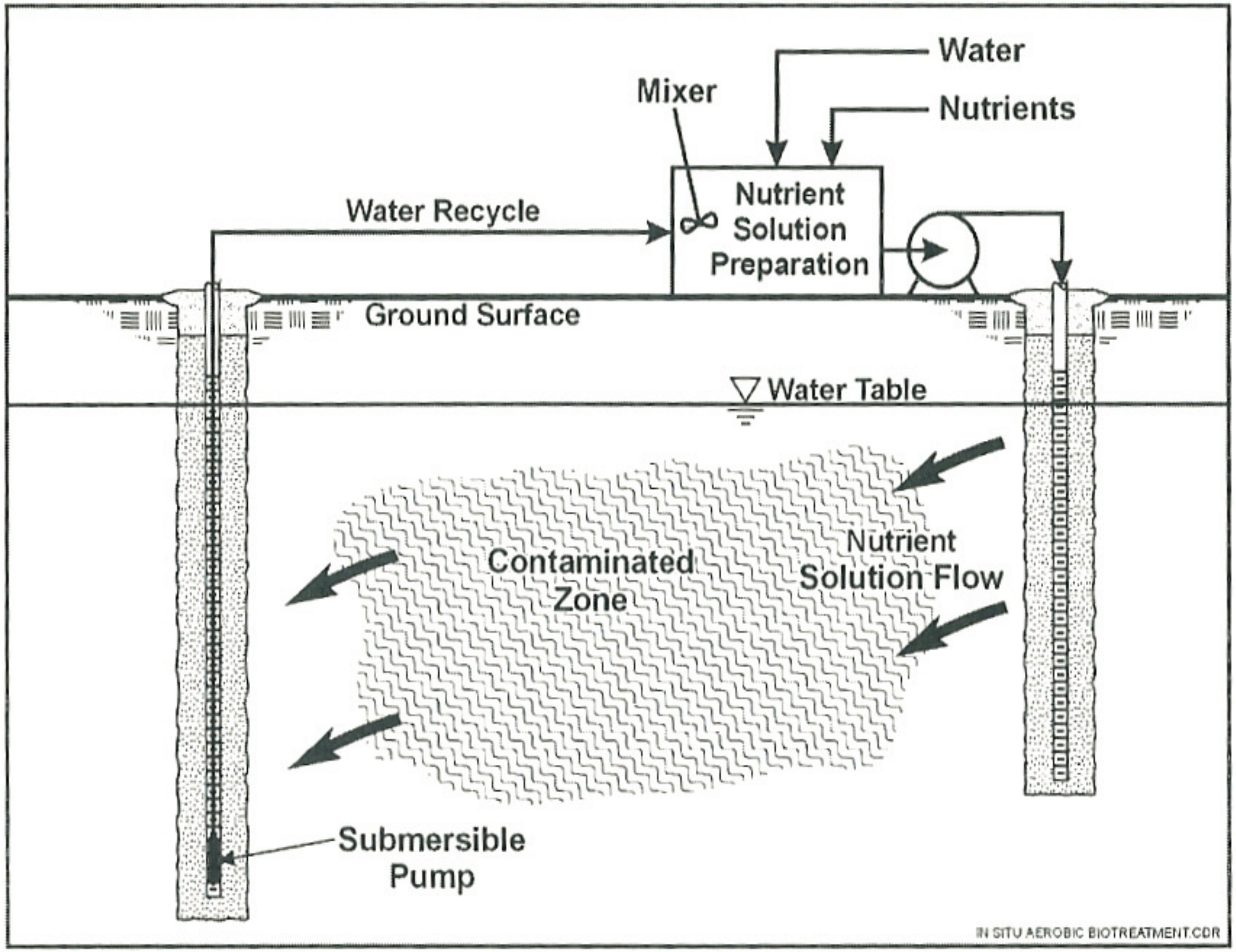
EXTRA SLIDES

Enhanced *in situ* aerobic bioremediation



Conceptual Diagram of Typical Enhanced In Situ Aerobic Biodegradation System

Enhanced *in situ* anaerobic bioremediation