Bioremediation

MICROBES
INVISIBLE INVADERS
AMAZING ALLIES

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What is Bioremediation??

- Using subsurface microorganisms to transform hazardous contaminants into relatively harmless byproducts, such as ethene and water
  - Biodegrade
  - Mineralize
  - Biotransform
- Techniques or types of bioremediation:
  - A component of Natural Attenuation
  - Enhanced Bioremediation
  - Bioaugmentation

Bioremediation Background

- Natural Attenuation is **Not fast enough, Not complete enough, Not frequently occurring enough** to be broadly used for some compounds, especially chlorinated solvents
- The current trend is to stimulate/enhance a site's indigenous subsurface microorganisms by the addition of nutrients and electron donor
- In some cases, bioaugmentation is necessary when metabolic capabilities are not naturally present.

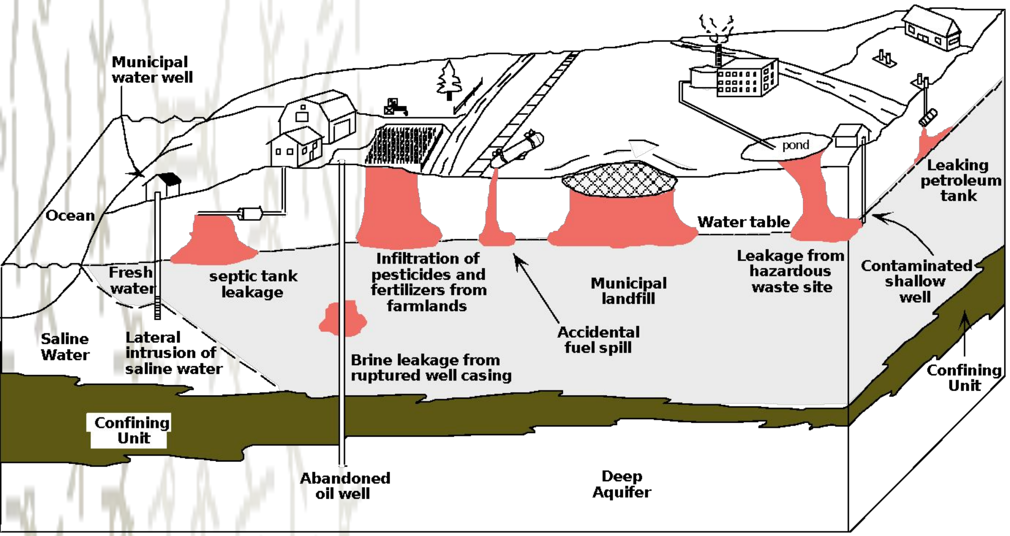
Historical Perspective

- ~1900 Advent of biological processes to treat organics derived from human or animal wastes (and the sludges produced)
- ~1950 Approaches to extend wastewater treatment to industrial wastes
- ~1960 Investigations into the bioremediation of synthetic chemicals in wastewaters
- ~1970 Application in hydrocarbon contamination such as oil spills and petroleum in groundwater
- ~1980 Investigations of bioremediation applications for substituted organics
- ~1990 Natural Attenuation of ’70 and ’90, and the development of barrier approaches
- ~2000 High-rate in situ bioremediation; source zone reduction; bioaugmentation

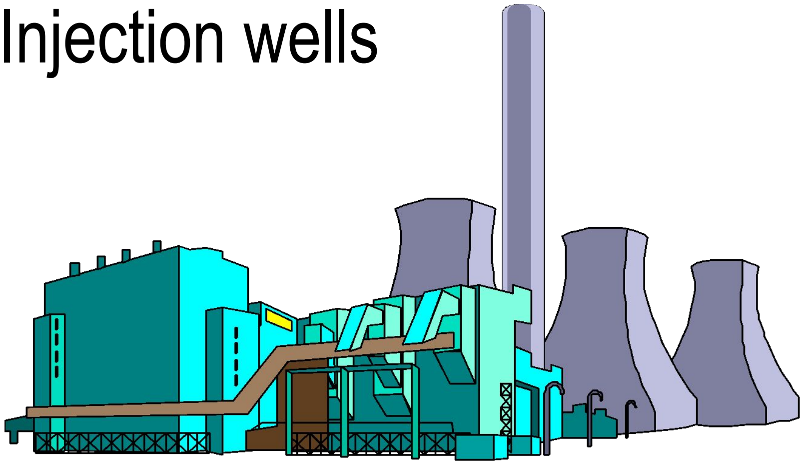
Soil and Subsurface Contaminants

- Benzene and related fuel components (BTEX)
- Pyrene and other polynuclear aromatics
- Chlorinated aromatics and solvents
- Herbicides and pesticides
- Nitroaromatic explosives and plasticizers

Sources of Contamination



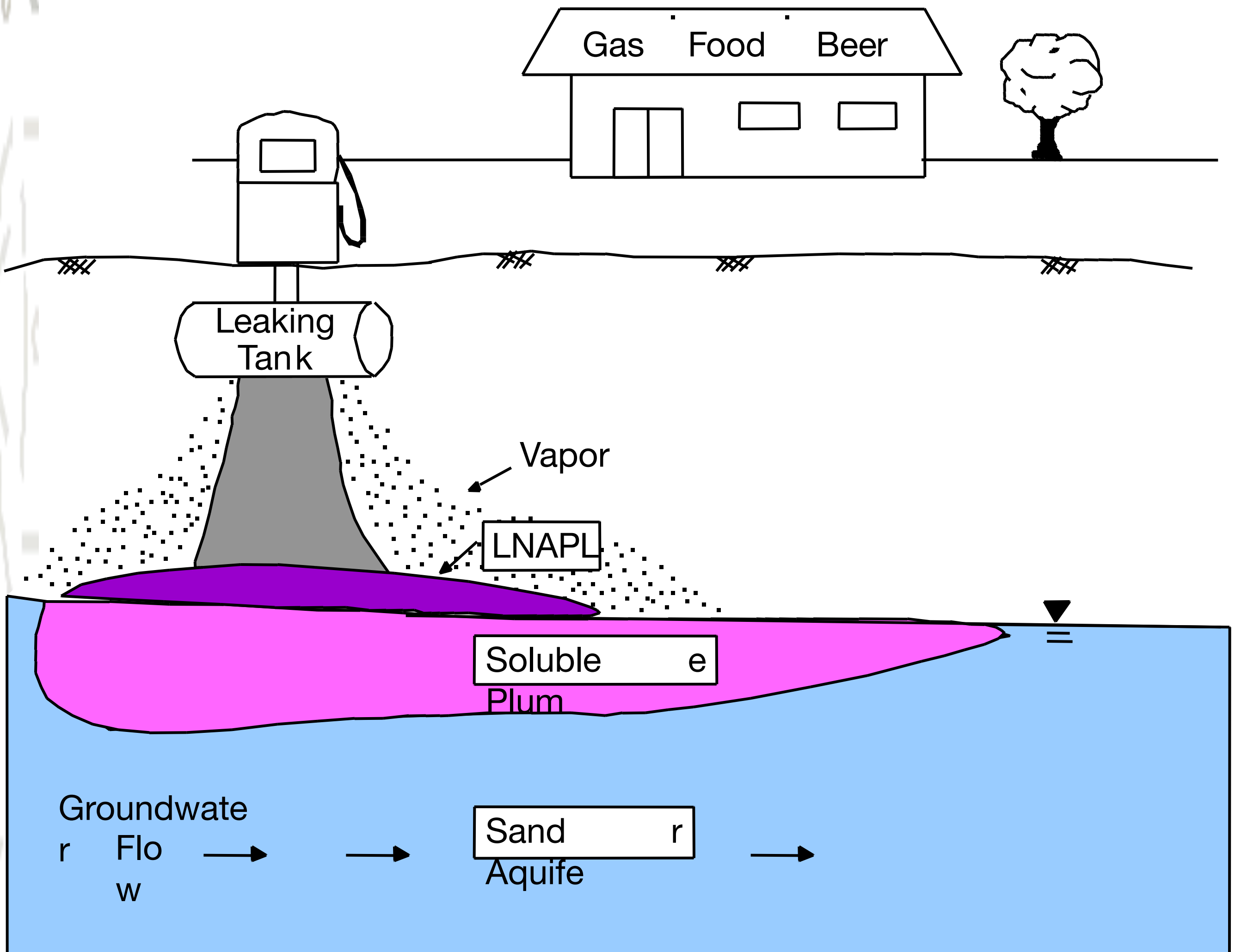
- Industrial spills and leaks
- Surface impoundments
- Storage tanks and pipes
- Landfills
- Burial areas and dumps
- Injection wells



Current Water Issues Associated with Gasoline Use

- Widespread contamination
- Major treat to drinking water resources
- Components of fuels are known carcinogens
- Current fuel oxygenate, MTBE, very mobile and not very degradable
- Ethanol is due to replace MTBE, but its behavior in the subsurface is not yet understood

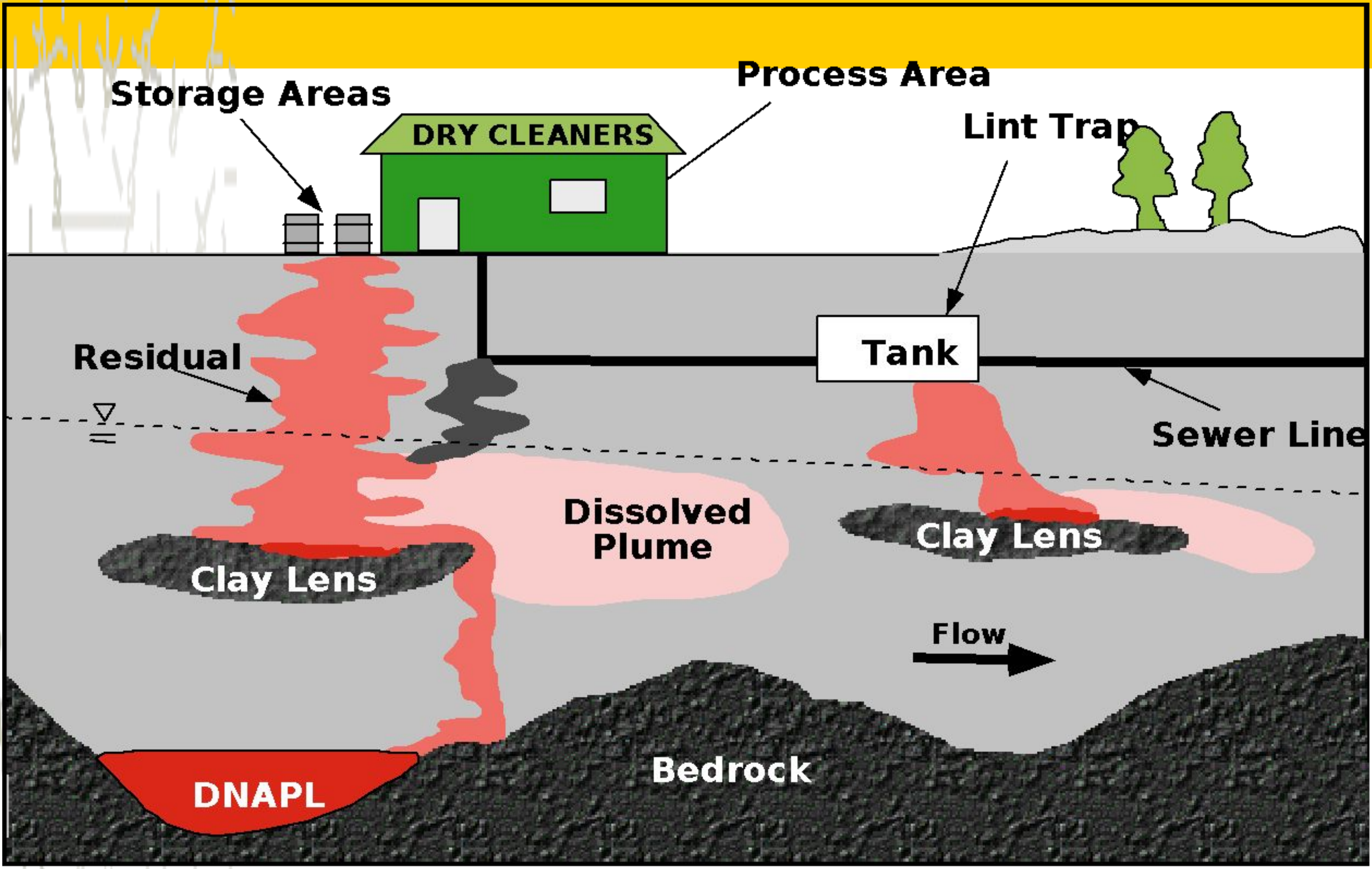
Typical Fuel (BTEX) Spill



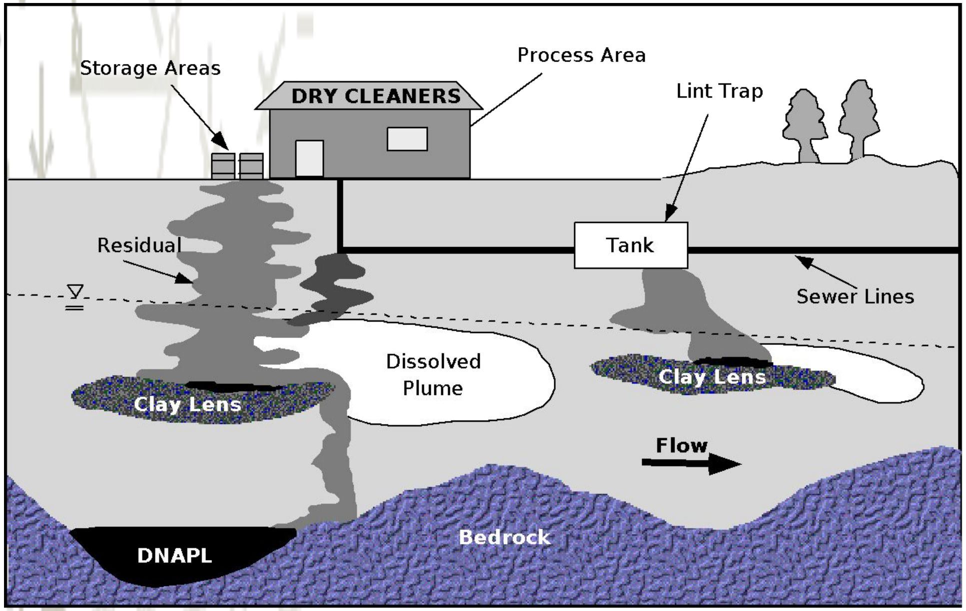
Chlorinated Background

- Groundwater plumes of chlorinated solvents are widespread due to their extensive use at industrial, DOD, and dry cleaner sites.
- Chlorinated compounds commonly exist as dense nonaqueous-phase liquids (DNAPLs) that act as long-term, continuing sources that slowly solubilize into groundwater.
- Known carcinogenic and toxic effects
- Not a primary substrate for any known bacteria

Routes of DNAPL Migration



DNAPL
Our Most Difficult Challenge



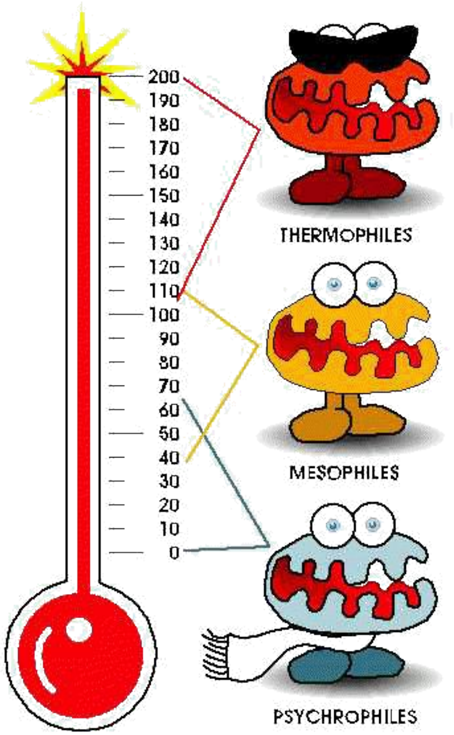
- DNAPL source
- Residual phase
- Trapped on lenses
- Pools in low areas
- Creates soluble plumes for years
- Extremely hard to remediate

Treatment Techniques

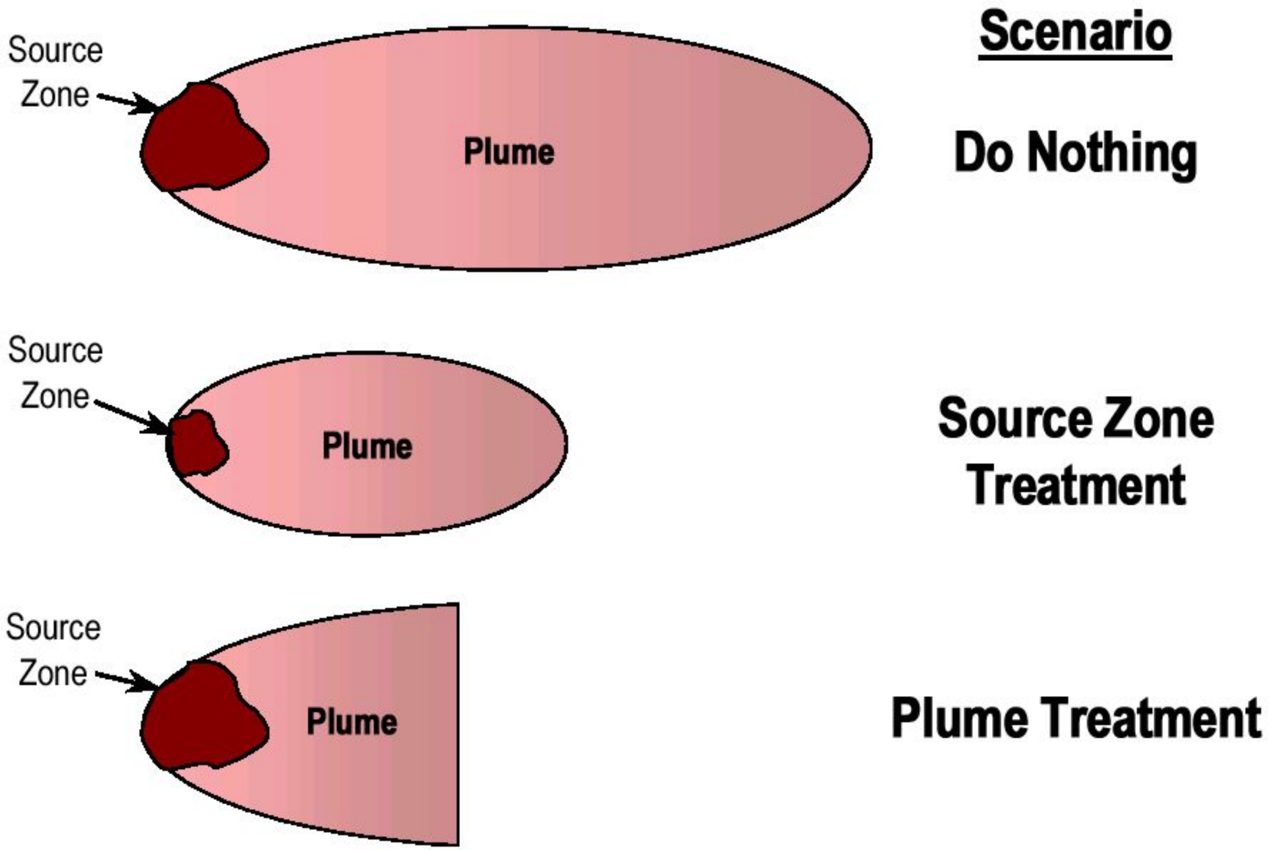
- Soil Extraction
- Pump and Treat
- Physical and/or reactive barriers
- Air and Hydrogen Sparging
- Biological (microbes)
- Chemical (surfactants)

Why use Bioremediation?

- No additional disposal costs
- Low maintenance
- Does not create an eyesore
- Capable of impacting source zones and thus, decreasing site clean-up time



Source Zone Treatment vs. Plume Treatment



Fundamentals of Biodegradation

- All organics are biodegradable, BUT biodegradation requires specific conditions
- There is no Superbug
- Contaminants must be bioavailable
- Biodegradation rate and extent is controlled by a “limiting factor”

Biotic Transformations

- Result of metabolic activity of microbes
- Aerobic and anaerobic biodegradation
- Reduces aqueous concentrations of contaminant
- Reduction of contaminant mass
- Most significant process resulting in reduction of contaminant mass in a system

Bioremediation Processes

- Conversion of contaminants to mineralized (e.g. CO_2, H_2O, and salts) end-products via biological mechanisms
- Biotransformation refers to a biological process where the end-products are not minerals (e.g., transforming TCE to DCE)
- Biodegradation involves the process of extracting energy from organic chemicals via oxidation of the organic chemicals

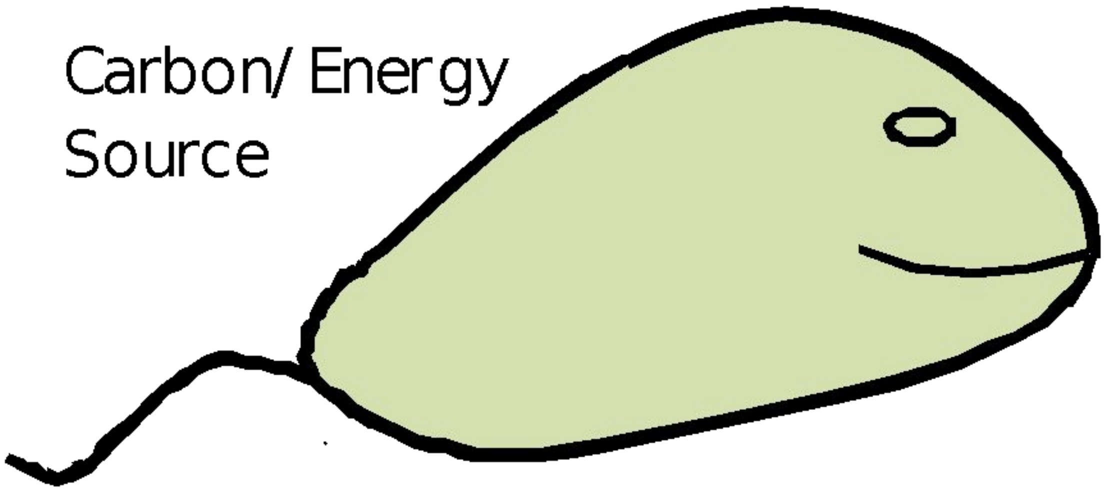
How Microbes Use the Contaminant

- Contaminants may serve as:
  - Primary substrate
    - enough available to be the sole energy source
  - Secondary substrate
    - provides energy, not available in high enough concentration
  - Cometabolic substrate
    - fortuitous transformation of a compound by a microbe relying on some other primary substrate

Requirements for Microbial Growth

Electron Acceptor
(O_2, NO_3^-, SO_4^{2-}, etc.)

Carbon/ Energy
Source

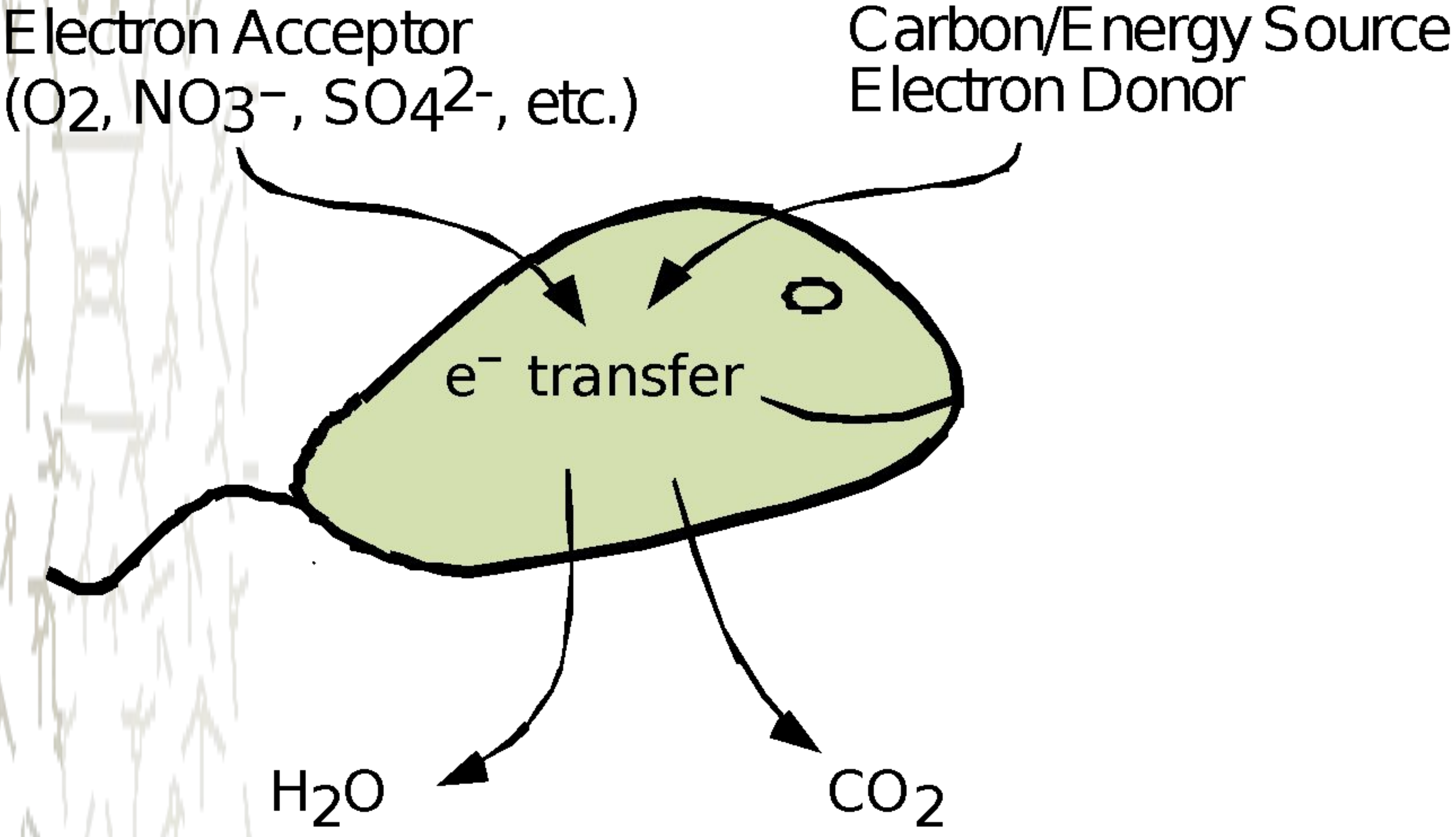


Environmental
Conditions
(Temp, pH, Eh)

Nutrients (N, P)

Trace Elements

Electron Exchange



Aerobic v. Anaerobic

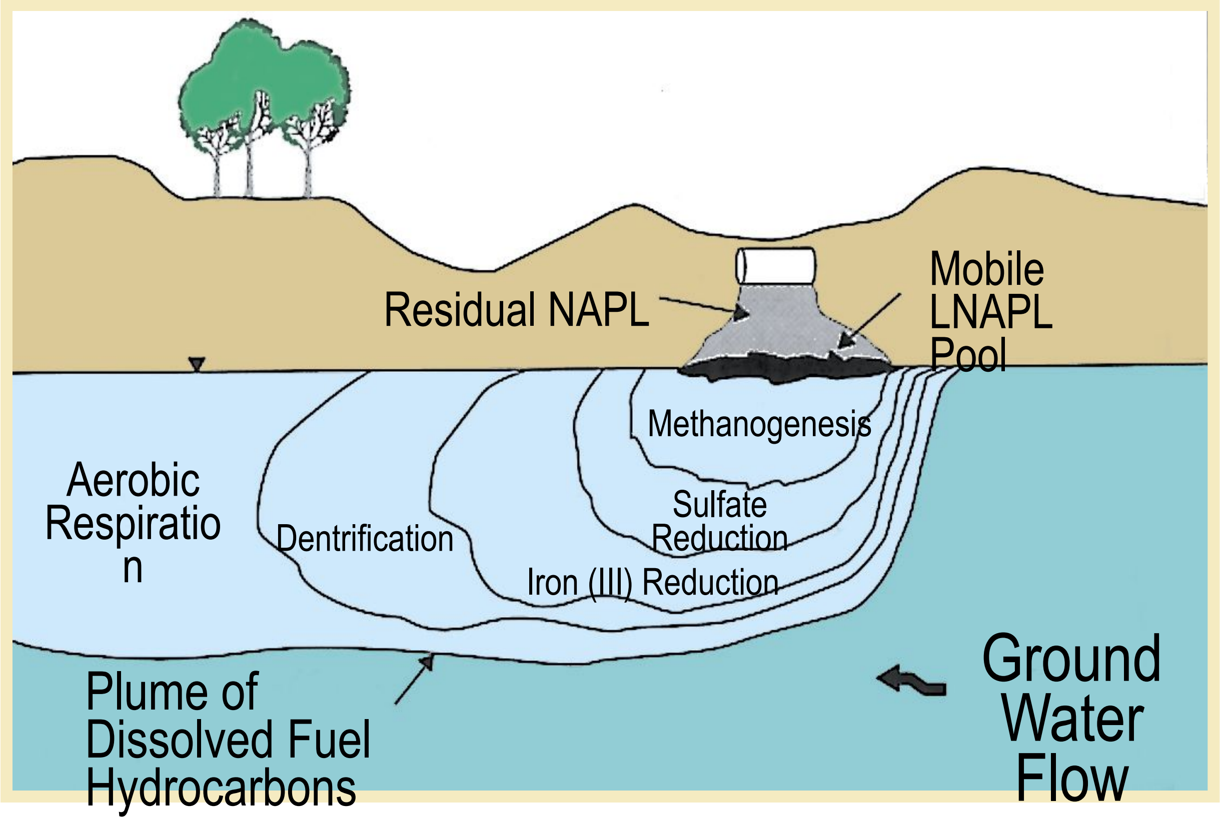
- If oxygen is the terminal electron acceptor, the process is called aerobic biodegradation
- All other biological degradation processes are classified as anaerobic biodegradation
- In most cases, bacteria can only use one terminal electron acceptor
- Facultative aerobes use oxygen, but can switch to nitrate in the absence of oxygen

Bacterial Metabolism

Aerobic	Anaerobic
Oxidation	Denitrification
Cometabolism	Manganese reduction
	Iron reduction
	Sulfate reduction
	Methanogenesis

Electron Acceptor Zones

- After O_2 is depleted, begin using NO_3^-
- Continue down the list in this order
  - O_2 —> NO_3^- —> Fe^{3+} —> SO_4^{2-} —> CO_2



Electron Acceptor Condition

Compound(s)	Aerobic	Anaerobic
Acetone	1	1
BTEX	1	2 to 4
PAH's	1	3 to 4
PCB's		
highly substituted	4	2
minimally substituted	2	4
Chlorinated ethenes		
PCE	4	1 to 2
TCE	3	1 to 2
DCEs	3	2 to 3
Vinyl chloride	1 to 2	3 to 4

1 Highly biodegradable 2 Moderately biodegradable
3 Slow biodegradation 4 Not biodegraded

Bioremediation Practice

- Understand physical and chemical characteristics of the contaminants of interest
- Understand the possible catabolic pathways of metabolism and the organisms that possess that capability
- Understand the environmental conditions required to:
  - Promote growth of desirable organisms
  - Provide for the expression of needed organisms
- Engineer the environmental conditions needed to establish favorable conditions and contact organisms and contaminants

Oxygen is of Primary Importance

- Most of the time oxygen is the primary factor limiting *in situ* biodegradation
- In most cases if adequate oxygen can be supplied then biodegradation rates are adequate for remediation
- Other limiting factors exist, but are usually secondary to oxygen

Degradation for Benzene: $C_6H_6 + 7.5O_2 \longrightarrow 6CO_2 + 3H_2O$

Oxygen Supply is the Key to Aerobic In Situ Bioremediation

- Two ways to introduce oxygen in situ
- Dissolved in water :
  - Actively pumped: H_2O_2 , aerated water
  - Passively: ORC ® , membrane, aeration
- In gaseous form, usually air
  - Bioventing above the water table
  - Air sparging below the water table

Dehalogenation

- Stripping halogens (generally Chlorine) from an organic molecule
- Generally an anaerobic process, and is often referred to as reductive dechlorination

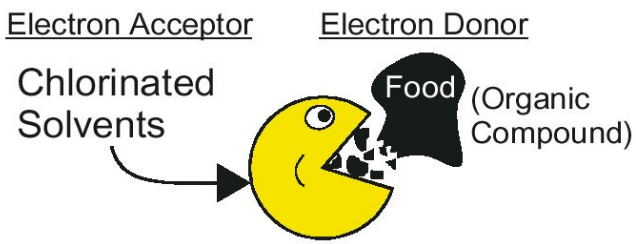
$$R{-}Cl + 2e^{-} + H^{+} \longrightarrow R{-}H + Cl^{-}$$

- Can occur via
  - Dehalorespiration (anaerobic)
  - Cometabolism (aerobic)

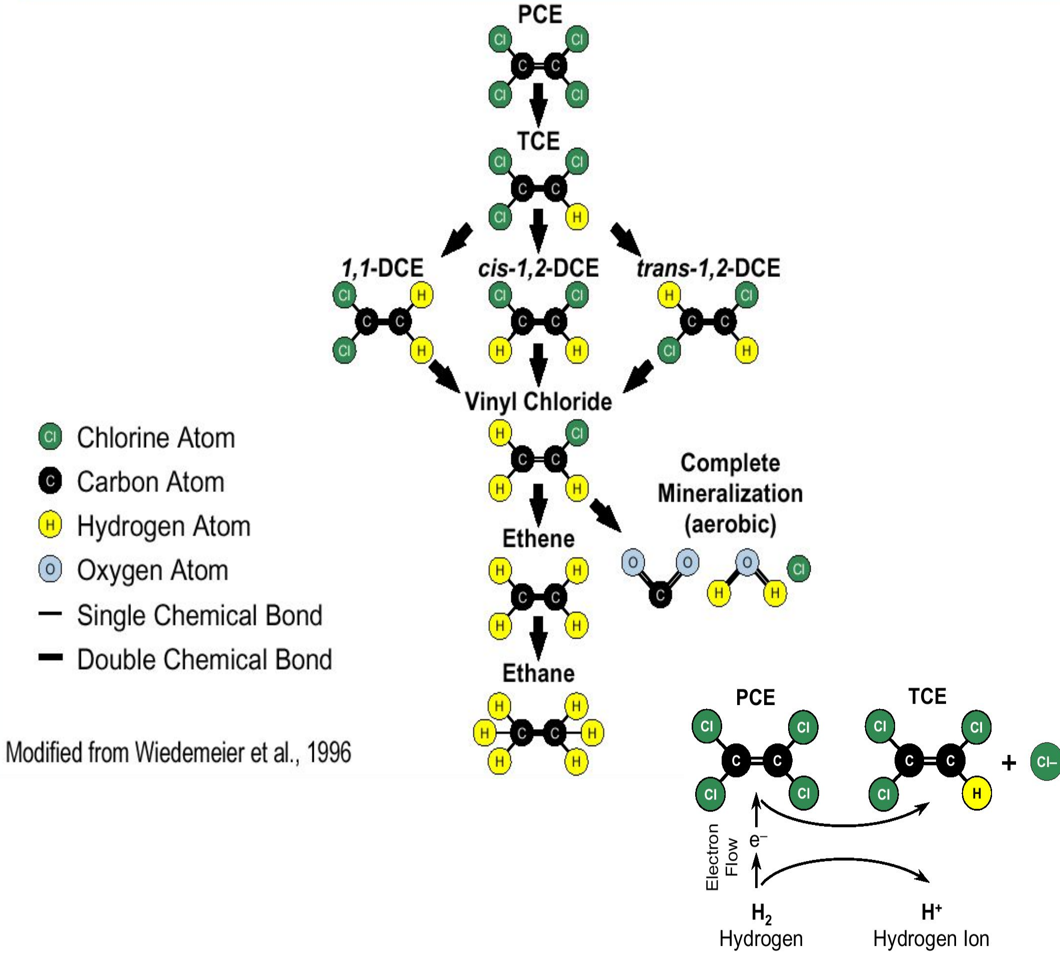
Dehalorespiration

- Certain chlorinated organics can serve as a terminal electron acceptor, rather than as a donor
- Confirmed only for chlorinated ethenes
- Rapid, compared to cometabolism
- High percentage of electron donor goes toward dechlorination
- Dehalorespiring bacteria depend on hydrogen-producing bacteria to produce H_2, which is the preferred primary substrate

Reductive Dechlorination



An electron donor, such as hydrogen, and an electron acceptor is needed to transfer from one product to the next



Added Danger

- Dechlorination of PCE and TCE should be encouraged, but monitored closely
- The dechlorination products of PCE are more hazardous than the parent compound
- DCE is 50 times more hazardous than TCE
- Vinyl Chloride is a known carcinogen

Cometabolism

- Fortuitous transformation of a compound by a microbe relying on some other primary substrate
- Generally a slow process - Chlorinated solvents don’t provide much energy to the microbe
- Most oxidation is of primary substrate, with only a few percent of the electron donor consumption going toward dechlorination of the contaminant
- Not all chlorinated solvents susceptible to cometabolism (e.g., PCE and carbon tetrachloride)

Selective Enhancement of Reductive Dechlorination

- Competition for available H_2 in subsurface
- Dechlorinators can utilize H_2 at lower concentrations than methanogens or sulfate-reducers
- Addition of more complex substrates that can only be fermented at low H_2 partial pressures may provide competitive advantage to dechlorinators

Electron Donors

- Alcohols and acids
- Almost any common fermentable compound
- Hydrogen apparently universal electron donor, but no universal substrate
- Laboratory or small-scale field studies required to determine if particular substrate will support dechlorination at particular site

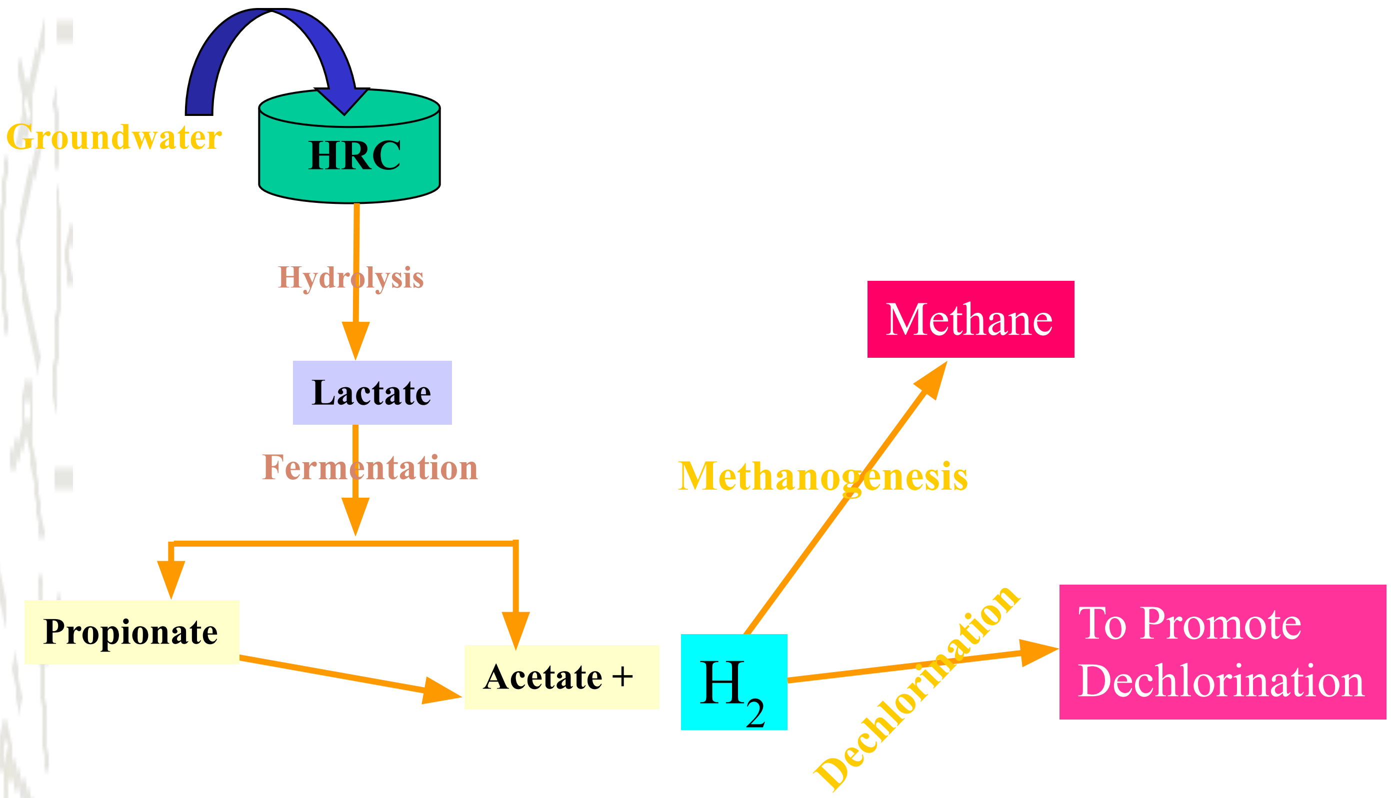
Electron Donors

Acetate Hydrogen - Pickle liquor
Acetic acid biochemical Polylactate esters
Benzoate electrochemical Propionate
Butyrate gas sparge Propionic acid
Cheese whey Humic acids - Sucrose
Chicken manure naturally occurring Surfactants -
Corn steep liquor Isopropanol Terigitol5-S-12
Ethanol Lactate Witconol 2722
Glucose Lactic acid Tetraalkoxsilanes
Hydrocarbon Methanol Wastewater
contaminants Molasses Yeast extract
Mulch

Enhanced Bioattenuation

Technology	Petroleum Hydrocarbons (e^- acceptor)	Chlorinated Solvents (e^- donor)
Liquid Delivery	Oxygen Nitrate Sulfate	Benzoate Lactate Molasses Carbohydrates
Biosparge	Air (oxygen)	Ammonia Hydrogen Propane
Slow-release	Oxygen (ORC)	Hydrogen (HRC)

Formation of a Usable Form of Electron Donor



COD=Lactate + Acetate + Propionate

Case Study

- Phoenix Site