MMG 301 Lec. 29 Bioremediation (and other Biotech applications)

Questions for Today:

1. What is *bioremediation* and what are some examples?
2. What is *bioleaching* and what is an example?
3. What are *biosensors* and how are they used?

Bioremediation: some important terms

The conversion of toxic substances to less toxic or non-toxic substances through microbial metabolism.

For many organic toxins the process involves *biodegradation* of the compounds to safer chemical forms, including *mineralization* (their conversion to CO_2).

Transformations of *xenobiotic* (man-made) compounds may occur by *co-metabolism*, using enzymes designed to function with natural products. (No energy derived.)

Recalcitrant molecules are stable (difficult to decompose) and may require very long time periods for elimination.

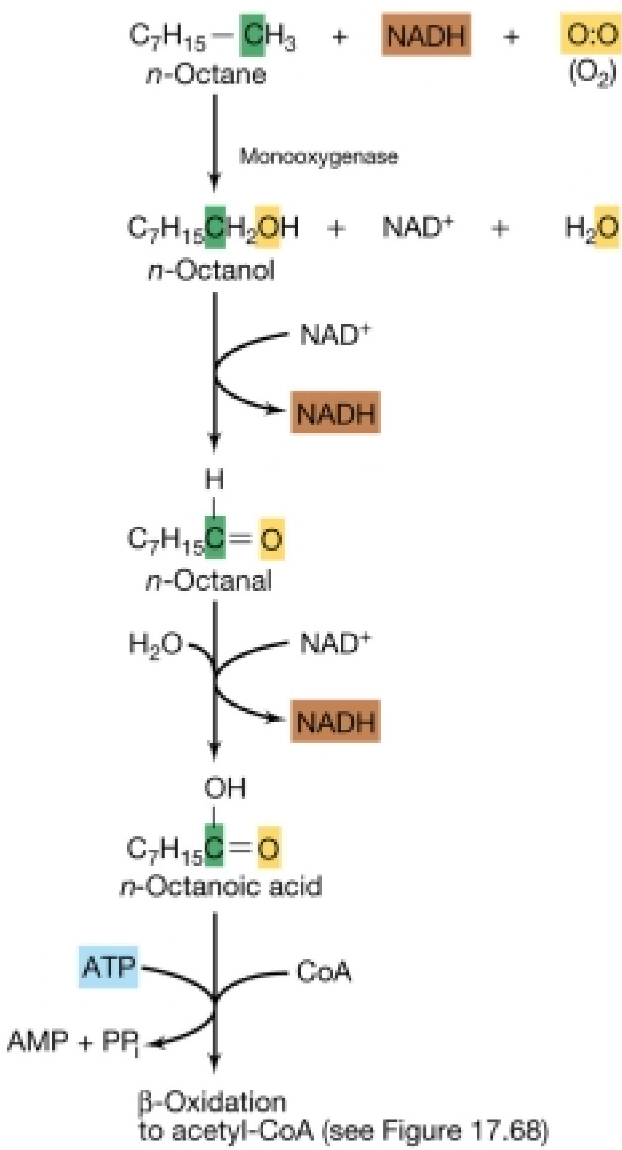
Bioremediation often involves complex *microbial communities*, but can sometimes occur by single species.

We will consider examples involving hydrocarbons, chlorinated aromatic compounds, polymers, and methyl mercury.

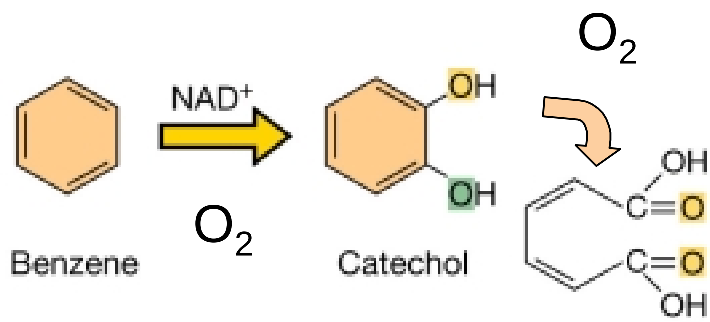
Bioremediation of hydrocarbons

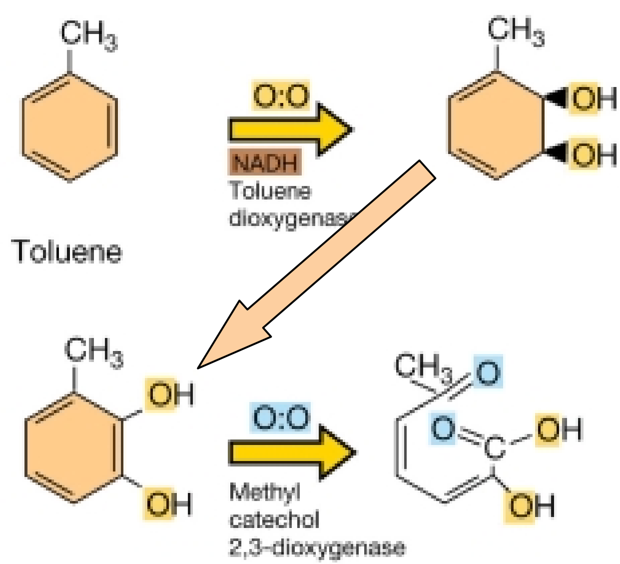
Hydrocarbons such a octane, benzene, and toluene occur naturally, but are toxic if present in excessive levels (e.g., an oil spill).

Naturally occurring enzymes are present in some microbes to mineralize these compounds while providing energy to the cells.



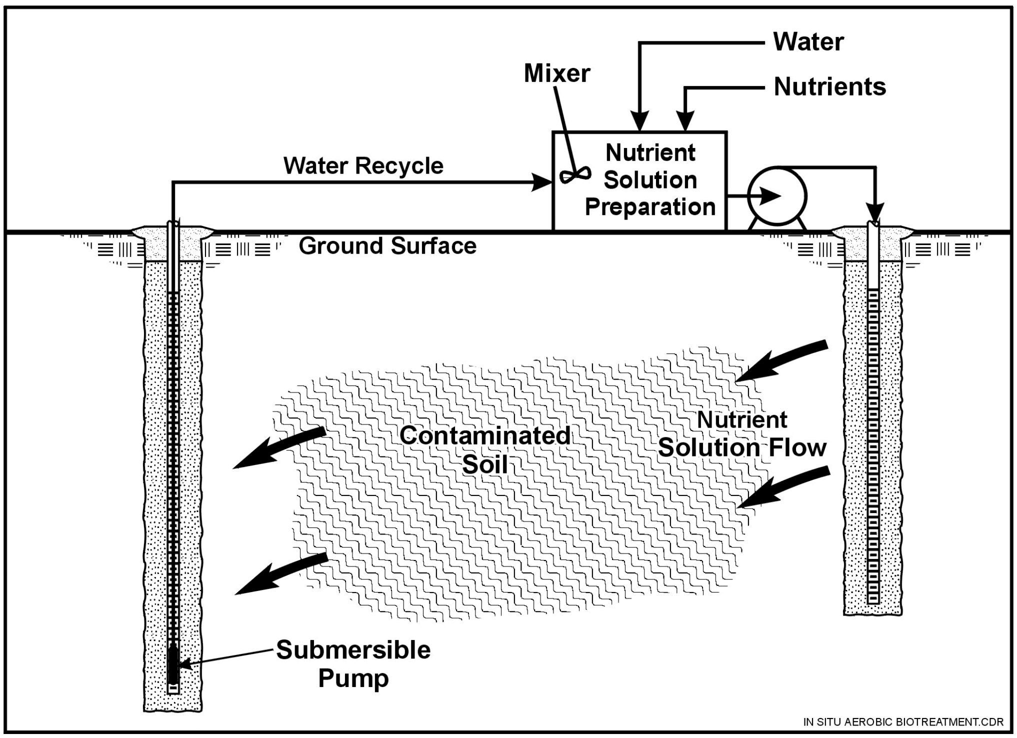
Oxygen is often used to metabolize hydrocarbons. This includes linear and ring structures:



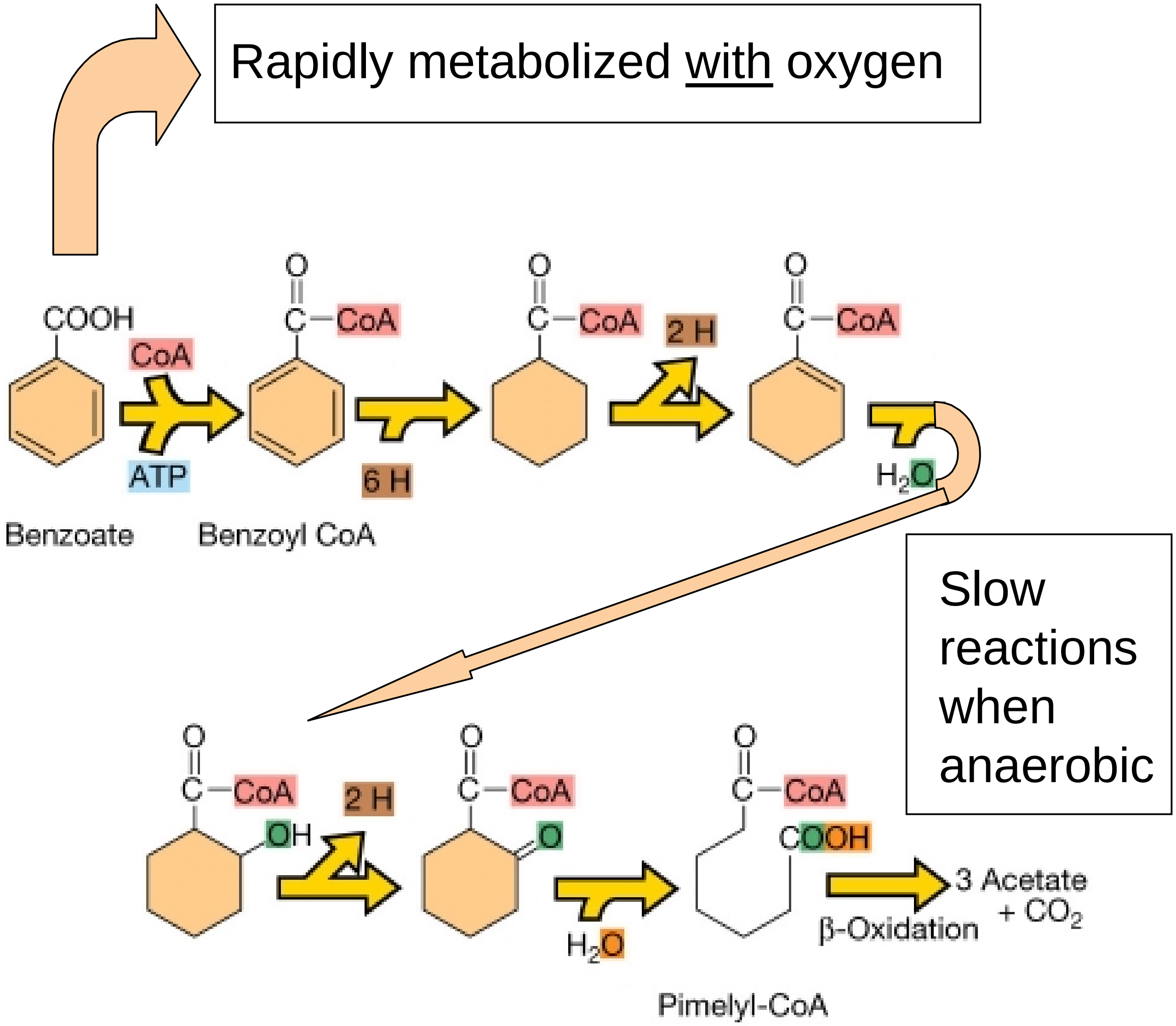


Small scale bioremediation can be carried out in reactor tanks.

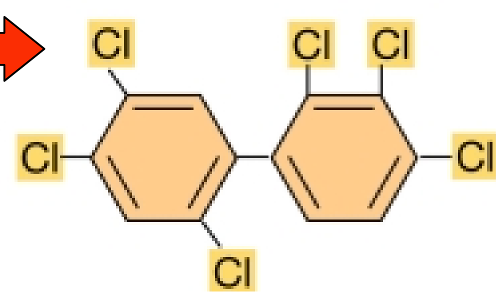
When encountering a large hydrocarbon spill (e.g., Exxon Valdez), *in situ* bioremediation works best. Provide oxygen, a nitrogen source, and other nutrients to stimulate growth.



In the absence of oxygen bioremediation may still take place, but usually on a much slower timescale.



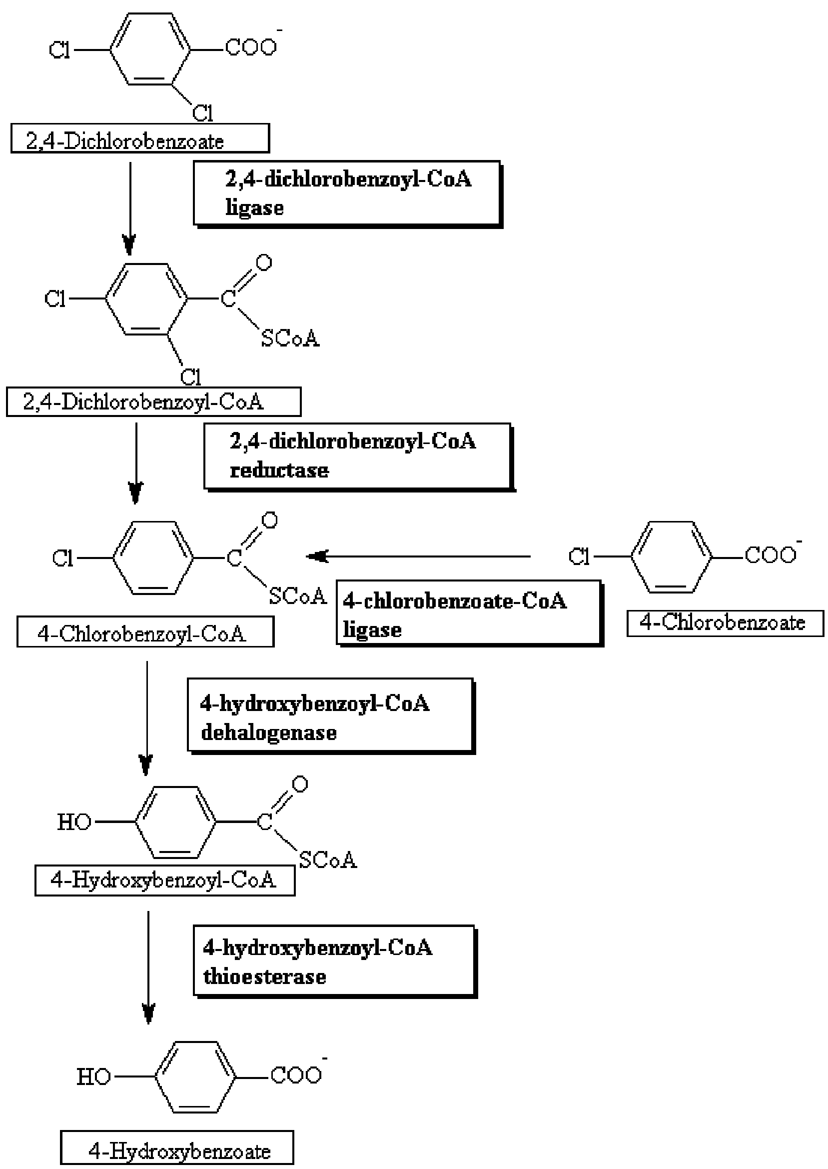
Some compounds (e.g., highly chlorinated PCBs) must undergo anaerobic transformations before oxygenases can participate.



2-step mineralization of PCBs

Anaerobic transformations of highly chlorinated PCBs leads to dechlorination (a process that can provide energy to some cells!) yielding less chlorinated PCBs (chloro-biphenyls).

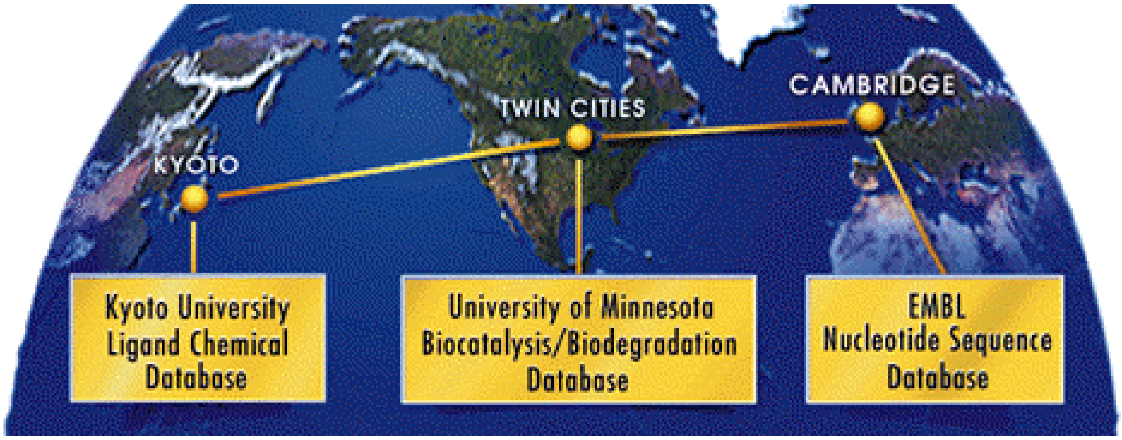
Oxygenases decompose lightly chlorinated biphenyls to produce various chlorobenzoates. An oxidative pathway for degrading 2,4-dichlorobenzoate (and 4-chlorobenzoate) is shown below:



A web site is available to examine what is known about your favorite chemicals:

http://umbbd.ahc.umn.edu/index.html

The University of Minnesota Biocatalysis/Biodegradation Database



Combines three databases: information on DNA sequences, info on enzymatic reactions, and info on binding properties of proteins.

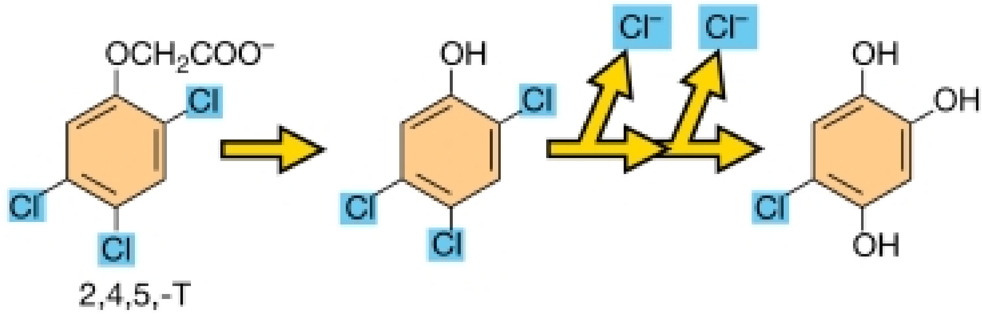
As of 3/17/03, the database contains information on 131 biodegradative pathways, 785 compounds, 831 reactions, and 530 enzymes.

One can easily access information on the chemical properties of a pollutant, the types of microorganisms known to catalyze its decomposition, the specific enzymes and intermediates involved, and the published literature related to the topic.

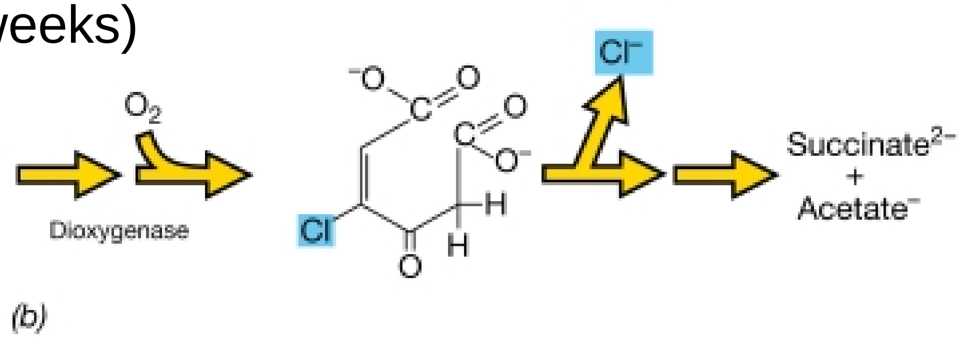
You could sign up for a web-based graduate course that requires students to add new pathways to the web site while learning about biodegradation.

The 2,4-dichlorobenzoate pathway is taken directly from this site. Other examples of compounds included are shown on the next page.

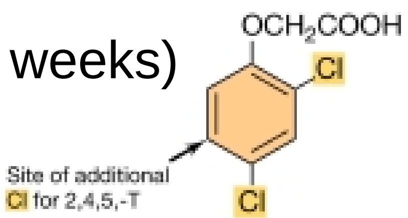
Mineralized by Co-metabolism (**times = <u>persistance</u>**)



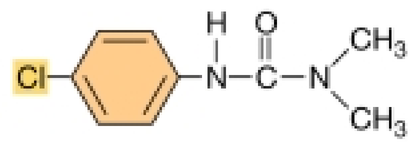
(20 weeks)



(4 weeks)

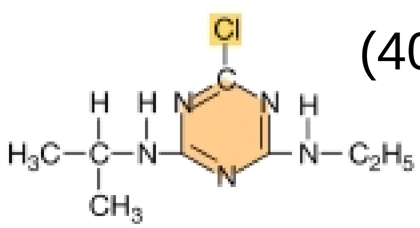


2,4-D; 2,4-dichlorophenoxy acetic acid
(a chlorophenoxy acetic acid derivative)

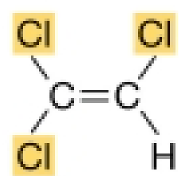


Monuron; 3-(4-chlorophenyl)-1,1-dimethylurea
(a substituted urea)

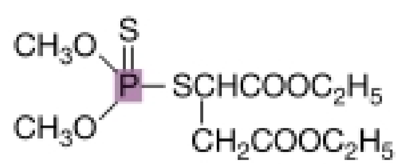
(40 weeks)



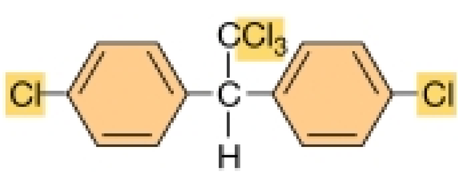
Atrazine, 2-chloro-4-ethylamino-6-isopropylaminotriazine
(a triazine derivative)



Trichloroethylene



Malathion; mercaptosuccinic acid diethyl ester
(an organophosphate)

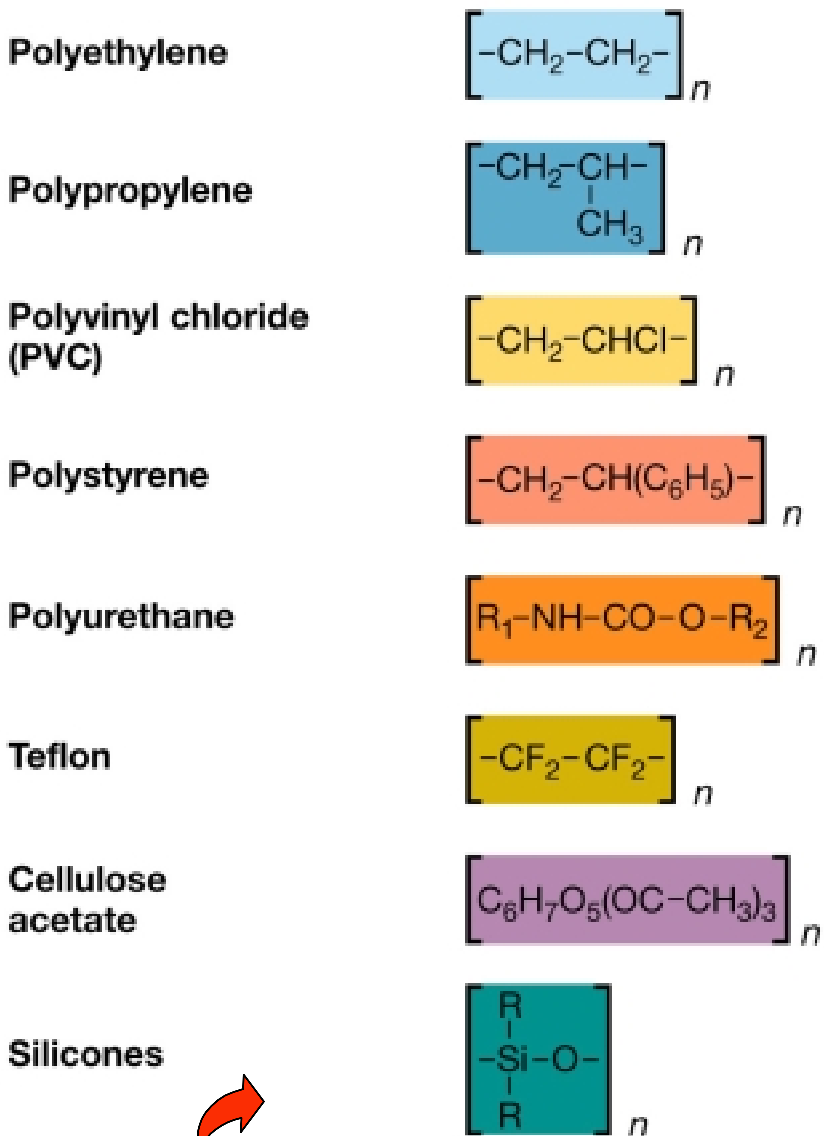


DDT; dichlorodiphenyltrichloroethane
(an organochlorine)

(4 years)

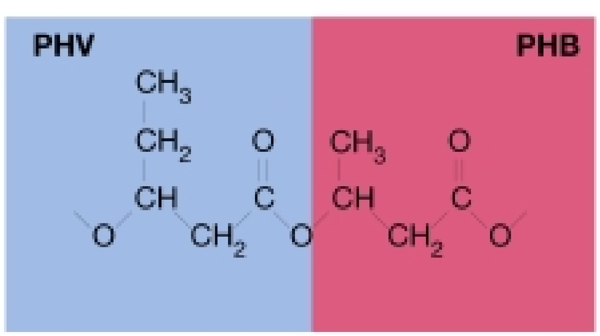
(1 week)

Synthetic polymers are often highly recalcitrant:



Microbial produced plastics are biodegradable:

These last in landfills for <u>very</u> long times



(a)

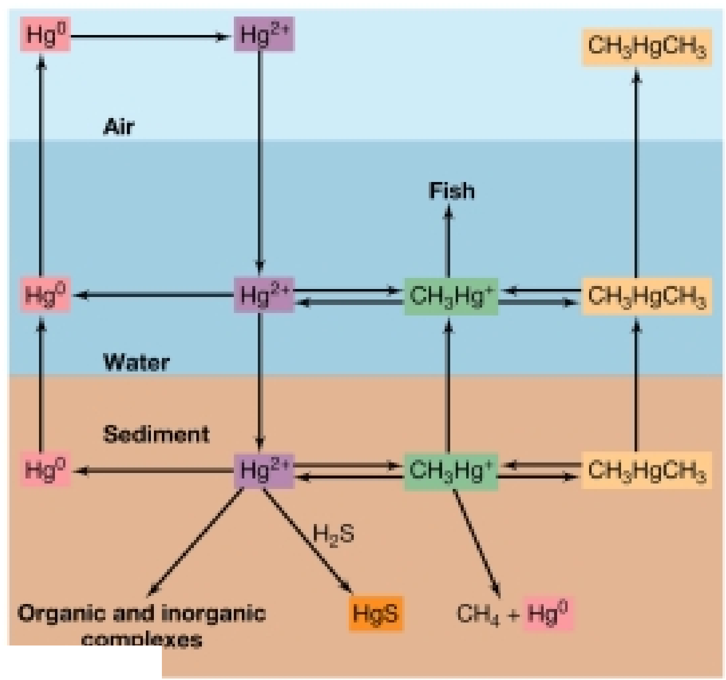
Co-polymer of poly-β-hydroxyvalerate and poly-β-hydroxybutyrate

Methyl mercury bioremediation

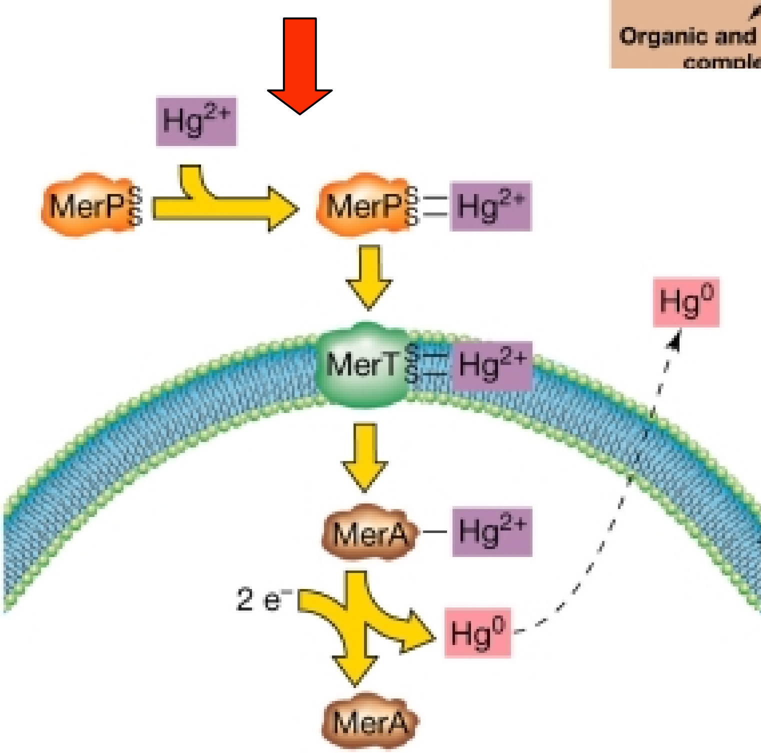
Mercury enters the environment during mining and as byproducts of the electronics and chemical industries.

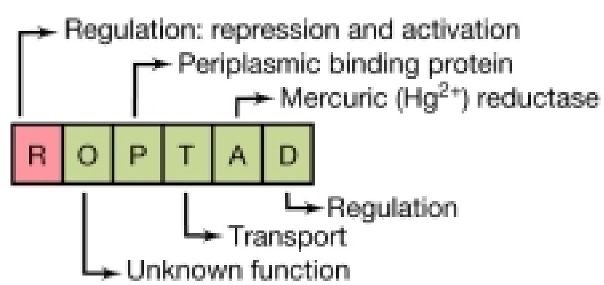
Hg exists in several forms, with the most toxic being methylmercury or dimethylmercury (accumulate in fish and humans).

Microbes catalyze several transformations of Hg compounds, and can be used to remediate a site.



Example: removal of divalent Hg

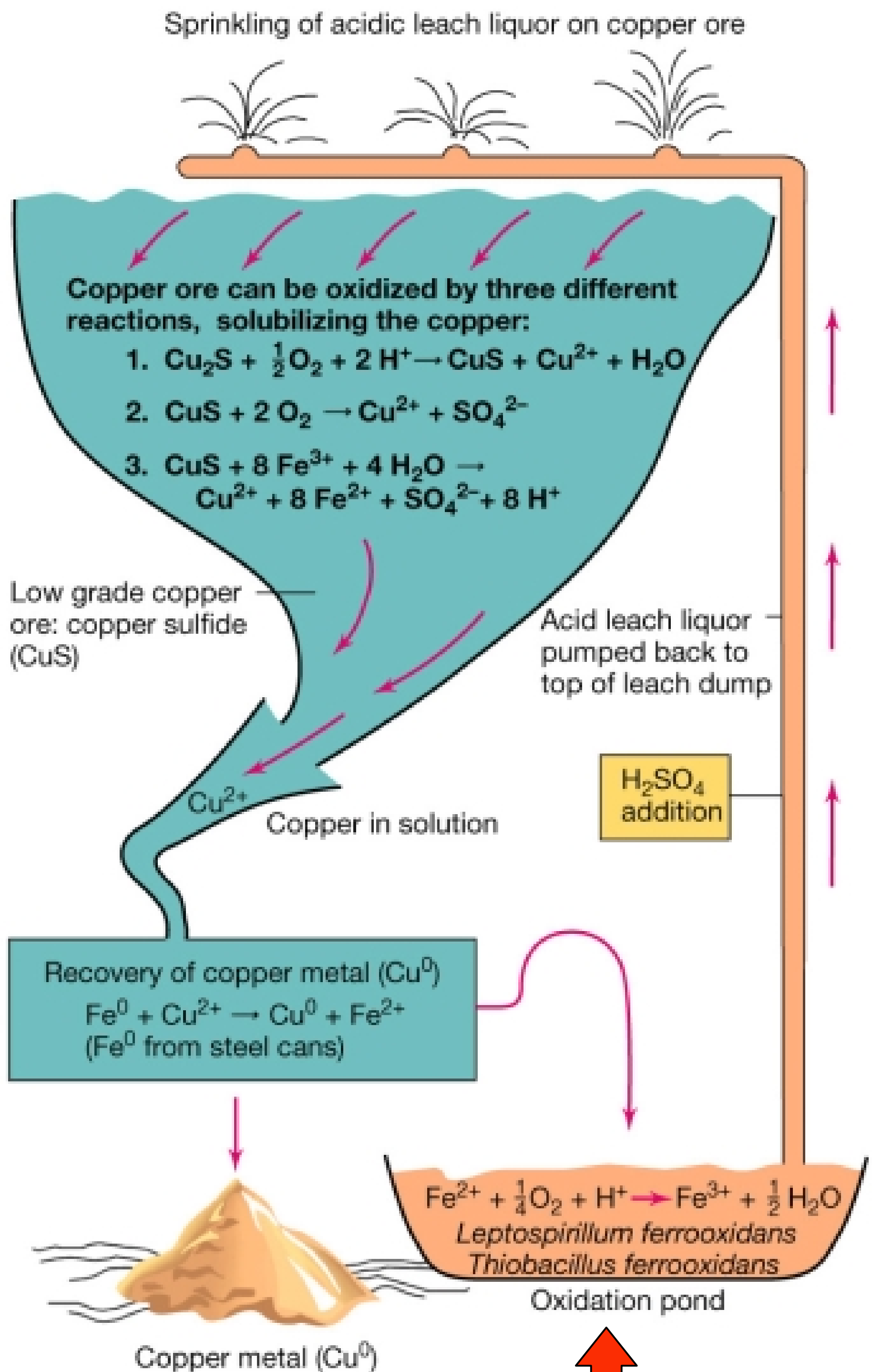




(other genes are needed to convert methylmercury to Hg^{2+})

Bioleaching

Bioleaching – the use of microorganisms for recovery of metal from ore. Common microbes used: *Thiobacillus ferrooxidans* or *Leptospirillum ferrooxidans*



Cu reduction coupled to Fe oxidation to recover Cu^0 metal

Fe^{2+} reoxidized by *Thiobacillus* to be recycled

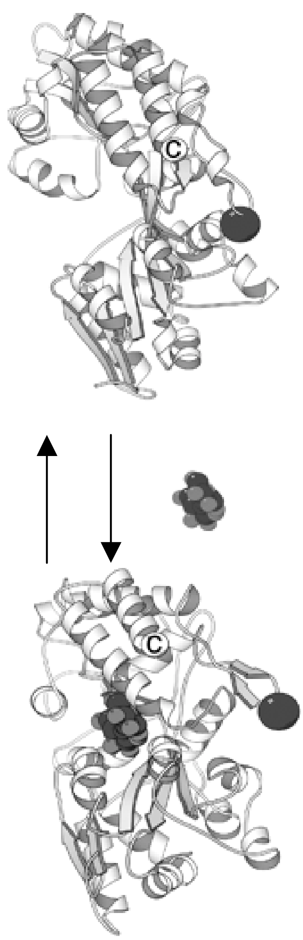
Similar processes used for uranium and gold leaching

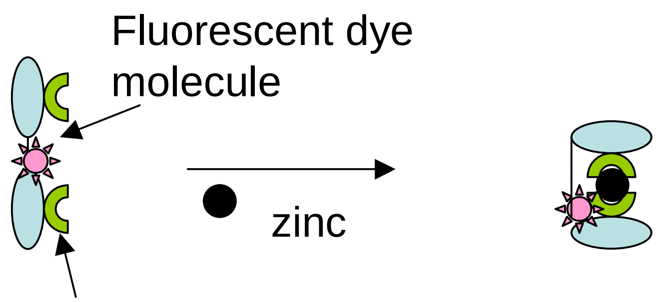
Emerging technologies: biosensors

Biosensor: biomolecule (often a product of “protein engineering”) or whole organism used to monitor a chemical compound or process whereby some type of signal is produced.

Two examples of a biosensor molecules

1. Detection of zinc in beer using a <u>genetically altered</u> maltose binding protein





Recombinant DNA methods to add zinc binding site (e.g., proper placement of His residues)

Change in fluorescence

Maltose binding protein

This method was published in *Science* in 2001.

2. Whole cell sensor for TNT (trinitrotoluene explosives); studied at Los Alamos National Laboratory

Start with a strain of *Pseudomonas putida* that degrades TNT.

Genes for TNT degradation are regulated such that expression increases when cells detect TNT in the soil.

Genetically manipulate *P. putida* to insert a fluorescent protein into a TNT-degradation gene.

Production of the fluorescent protein will commense when cells sense a TNT-containing environment.

Disperse cells on ground, allow to grow, then use UV light to detect fluorescent glow on ground.

A commonly used fluorescent marker is the Green Fluorescent Protein (GFP).

An alternative marker is to monitor light production by *luciferase*, an enzyme isolated from bioluminescent bacteria (e.g., *Photobacterium*).

$$FMNH_2 + O_2 + R\text{-}CHO \rightarrow FMN + H_2O + R\text{-}COOH + \text{Light}$$