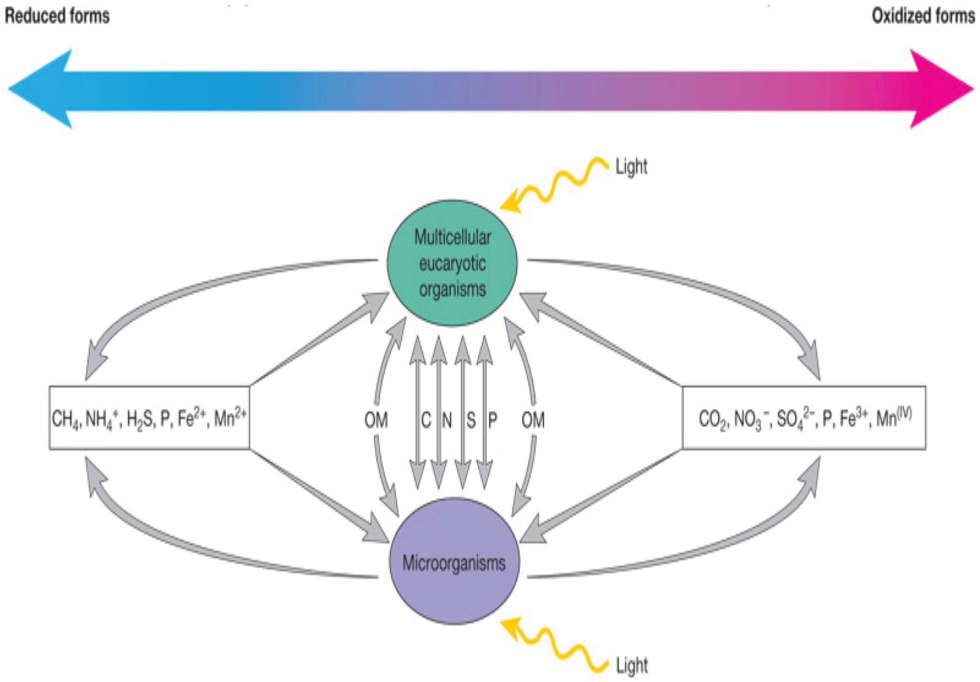
Environmental Microbiology

Nutrient Cycles, Biodegradation and Bioremediation

prepared by Prof. Bulent Icgen

The Biogeochemical Cycles



- Biogeochemical cycling of nutrients
  - involves biological and chemical processes
  - often involves oxidation-reduction reactions that change chemical and physical characteristics of nutrients
- All nutrient cycles are linked and make life on Earth possible

The Biogeochemical Cycles

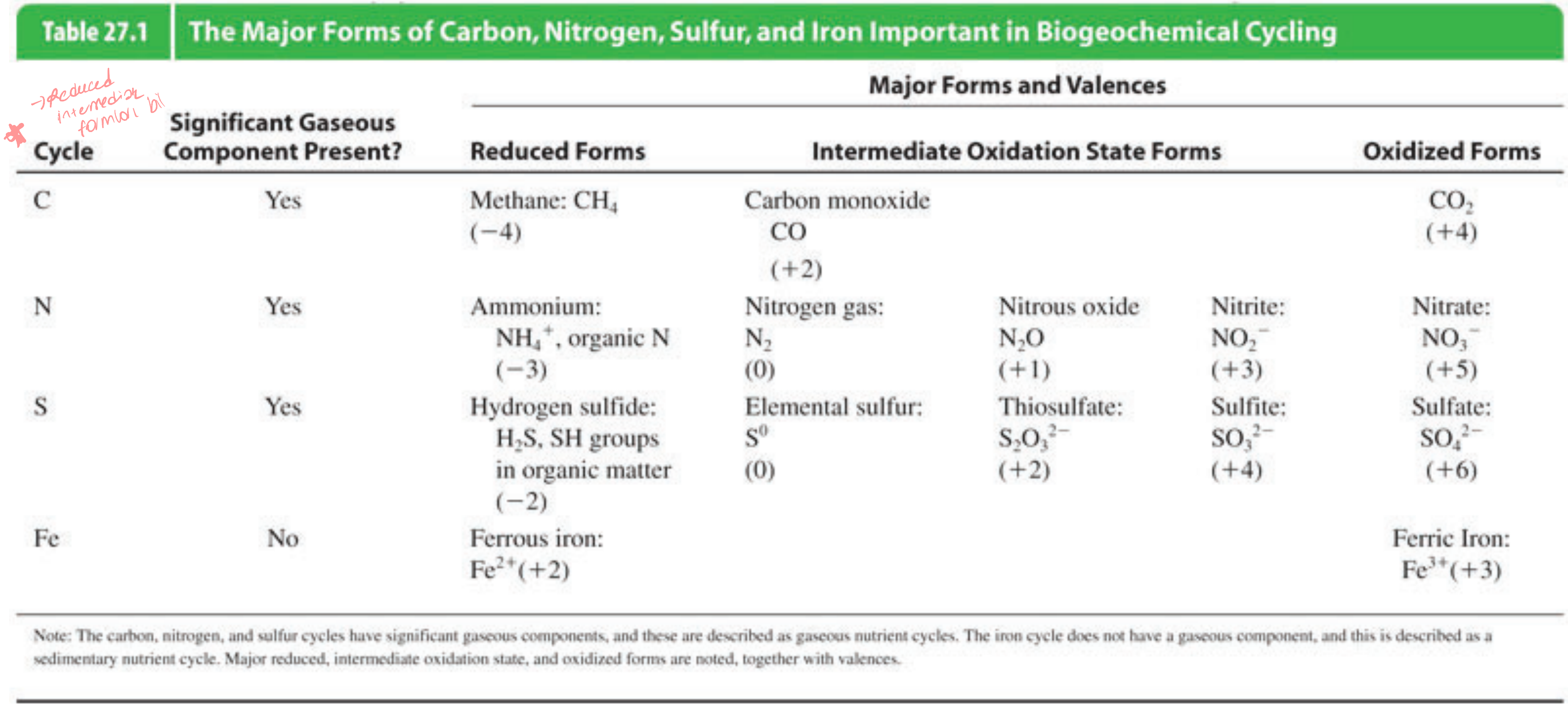


Table 27.1 The Major Forms of Carbon, Nitrogen, Sulfur, and Iron Important in Biogeochemical Cycling

→Reduced intermediar formları bil

Cycle	Significant Gaseous Component Present?	Major Forms and Valences				
		Reduced Forms	Intermediate Oxidation State Forms			Oxidized Forms
C	Yes	Methane: CH_4 (−4)	Carbon monoxide CO (+2)			CO_2 (+4)
N	Yes	Ammonium: NH_4^+, organic N (−3)	Nitrogen gas: N_2 (0)	Nitrous oxide N_2O (+1)	Nitrite: NO_2^- (+3)	Nitrate: NO_3^- (+5)
S	Yes	Hydrogen sulfide: H_2S, SH groups in organic matter (−2)	Elemental sulfur: S^0 (0)	Thiosulfate: $S_2O_3^{2-}$ (+2)	Sulfite: SO_3^{2-} (+4)	Sulfate: SO_4^{2-} (+6)
Fe	No	Ferrous iron: Fe^{2+}(+2)				Ferric Iron: Fe^{3+}(+3)

Note: The carbon, nitrogen, and sulfur cycles have significant gaseous components, and these are described as gaseous nutrient cycles. The iron cycle does not have a gaseous component, and this is described as a sedimentary nutrient cycle. Major reduced, intermediate oxidation state, and oxidized forms are noted, together with valences.

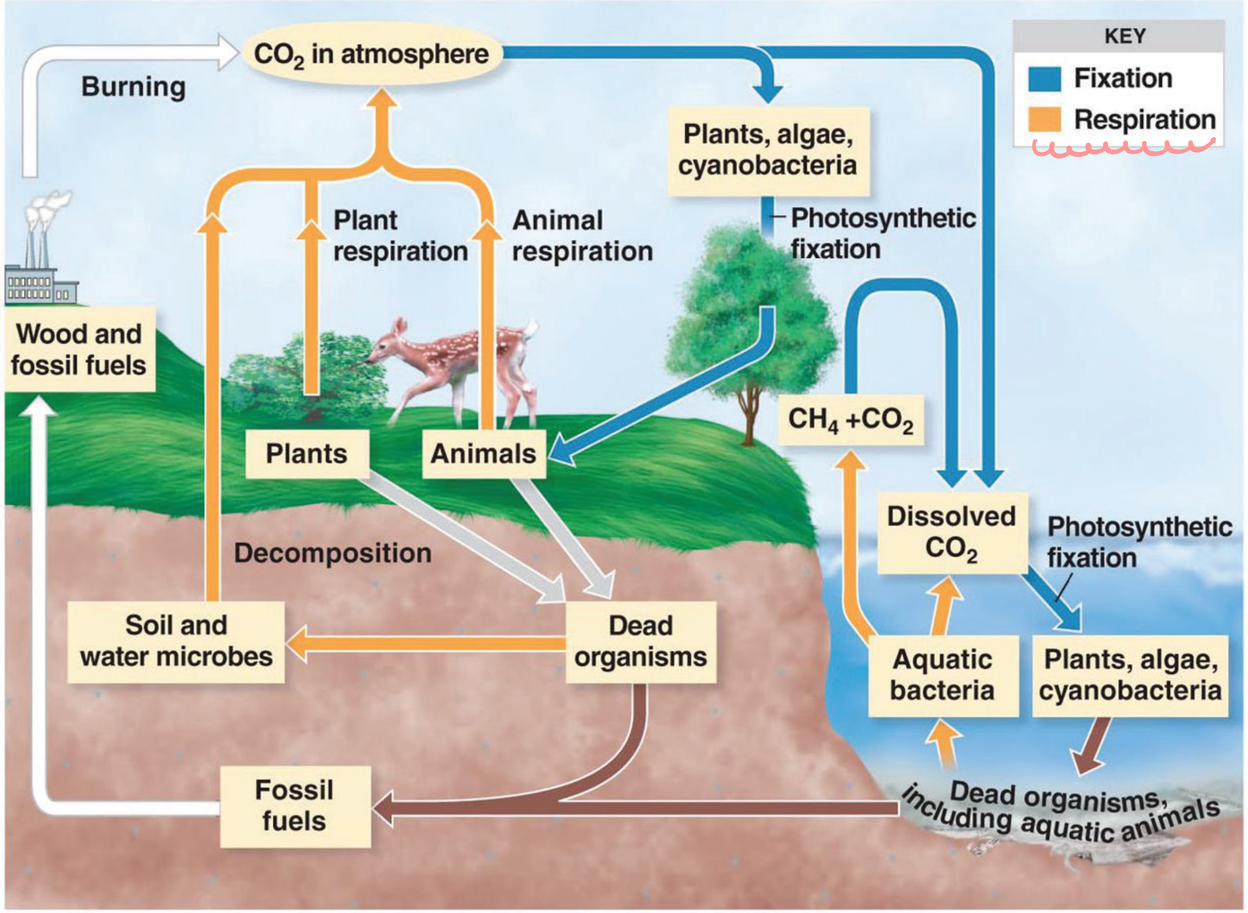
Nutrient Cycles

- The Carbon Cycle
- Syntrophy and Methanogenesis
- The Nitrogen Cycle
- The Sulfur Cycle
- The Iron Cycle
- The Phosphorus, Calcium and Silica Cycles

The Carbon Cycle

Carbon is cycled through all of Earth's major carbon reservoirs

- Includes atmosphere, land, oceans, sediments, rocks and biomass
- Reservoir size and turnover time are important parameters in understanding the cycling of elements



The Carbon Cycle

- CO_2 in the atmosphere is the most rapidly transferred carbon reservoir
- CO_2 is fixed by photosynthetic land plants and marine microbes
- CO_2 is returned to the atmosphere by respiration as well as anthropogenic activities

Microbial decomposition is the largest source of CO_2 released to the atmosphere

Carbon and oxygen cycles are linked

- Phototrophic organisms are the foundation of the carbon cycle

Oxygenic phototrophic organisms can be divided into two groups: plants and microorganisms

- Plants dominant organisms of terrestrial environments
- Microorganisms dominate aquatic environments

The Carbon Cycle

- Photosynthesis and respiration are part of redox cycle
- Photosynthesis

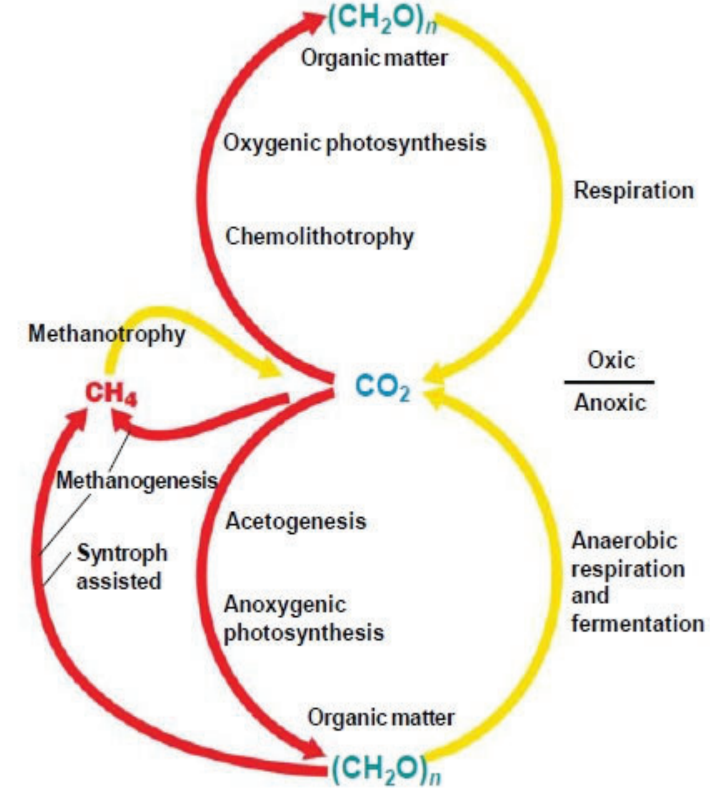
$CO_2 + H_2O\ (CH_2O) + O_2$

- Respiration

$(CH_2O) + O_2\ CO_2 + H_2O$

- The two major end products of decomposition are CH_4 and CO_2

Redox cycle for carbon



The diagram contrasts autotrophic processes (CO_2 → organic compounds) and heterotrophic processes (organic compounds → CO_2).
Yellow arrows indicate oxidations; red arrows indicate reductions.

Syntrophy and Methanogenesis

→ All of this steps are anaerobic → Done by anerobic bacteria and for the last step done by archea

Hydrolisis
↓
Acidogenesis (fermentation)
↓
Acetogenesis
↓
Methanogenesis

Also used in anerobic digester → To produce the methane. (as a end product)

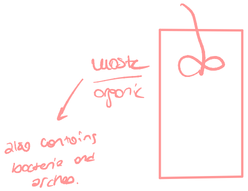
- Methanogenesis is central to carbon cycling in anoxic environments → anaerobic
- Most methanogens reduce CO_2 to CH_4 with H_2 as an electron donor; some can reduce other substrates to CH_4 (e.g., acetate)
- Methanogens team up with partners (syntrophs) that supply them with necessary substrates Sulfate reducers

Hydrolysis → larger molecules become monomers

(fermentation) Acidogenesis → This monomers become Acetate, H_2, CO_2

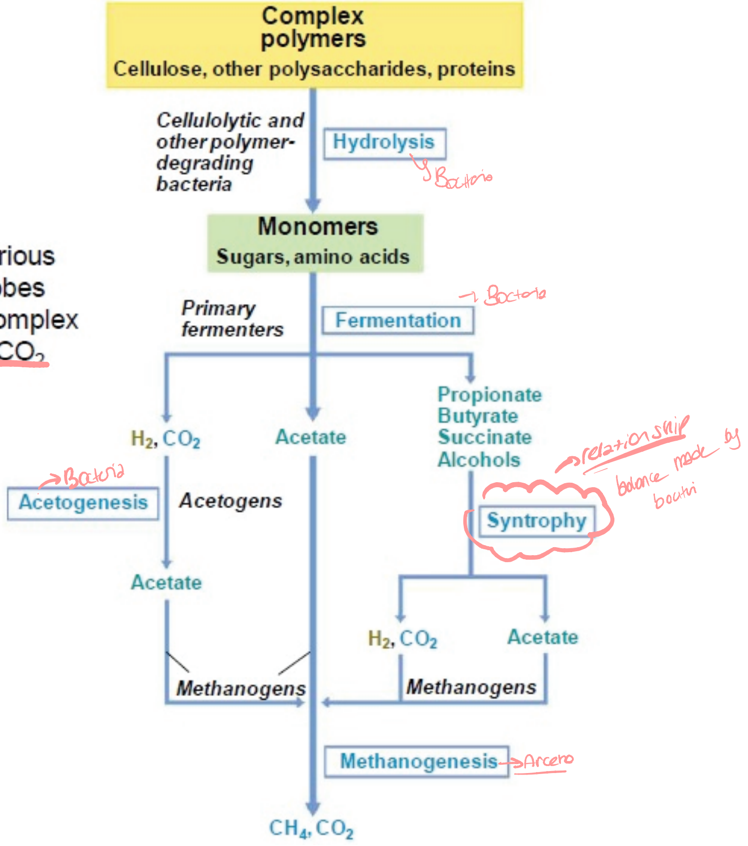
Acetogenesis

Methanogenesis → Acetate → CH_4



Anaerobic decomposition

In anaerobic decomposition various groups of fermentative anaerobes cooperate in the conversion of complex organic materials to CH_4 and CO_2



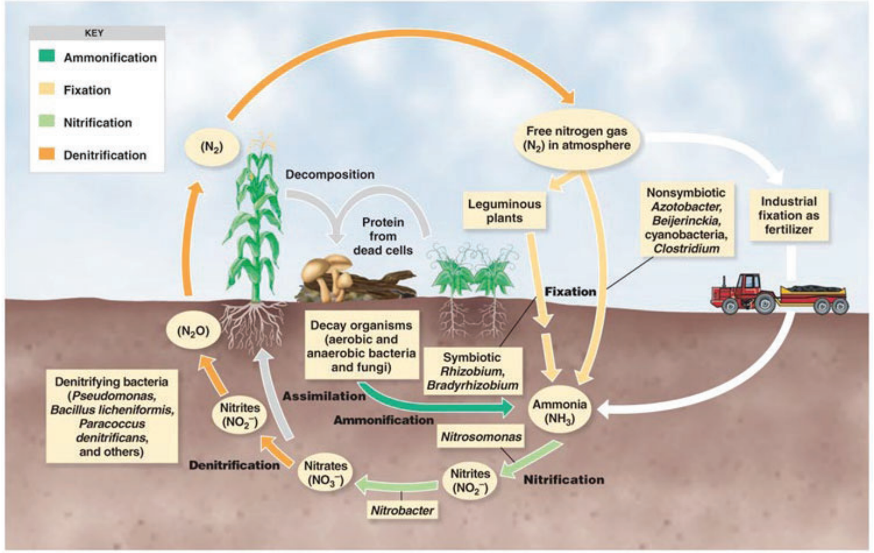
Syntrophy and Methanogenesis

- Methanogens can be found in some protists (e.g. within the cells of protists inhabiting the termite hindgut)
- Possible that endosymbiotic methanogens benefit protists by consuming H_2 generated from glucose fermentation
- On a global basis, biotic processes release more CH_4 than abiotic processes
- Acetogenesis is another H2-consuming process competing with methanogenesis in some environments
- Occurs in termite hindgut, permafrost soils
- Methanogenesis is energetically more favorable than acetogenesis
- Acetogens can ferment glucose and methoxylated aromatic compounds, whereas methanogens cannot
- Sulfate-reducing bacteria outcompete methanogens and acetogens in marine environments

The Nitrogen Cycle

Nitrogen

- A key constituent of cells
- Exists in a number of oxidation states



SINAV

Proteins and waste products $\xrightarrow{\text{Microbial decomposition}}$ Amino acids

Amino acids ($-NH_2$) $\xrightarrow{\text{Microbial ammonification}}$ Ammonia (NH_3)

Ammonium ion (NH_4^+) $\xrightarrow{\text{Nitrosomonas}}$ Nitrite ion (NO_2^-)

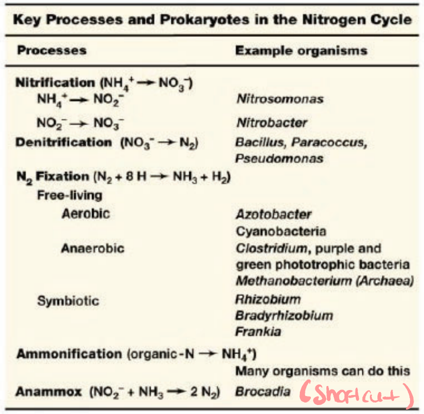
Nitrite ion (NO_2^-) $\xrightarrow{\text{Nitrobacter}}$ Nitrate ion (NO_3^-)

Nitrification

Nitrate ion (NO_3^-) $\xrightarrow{\text{Pseudomonas}}$ N_2 denitrification

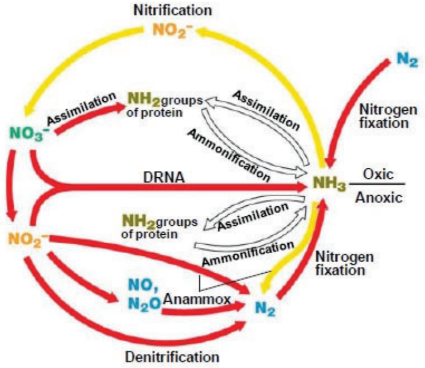
N_2 $\xrightarrow{\text{Nitrogen fixation}}$ Ammonia (NH_3)

Redox cycle for nitrogen



Key Processes and Prokaryotes in the Nitrogen Cycle

Processes	Example organisms
Nitrification ($NH_4^+ \rightarrow NO_3^-$)	
$NH_4^+ \rightarrow NO_2^-$	*Nitrosomonas*
$NO_2^- \rightarrow NO_3^-$	*Nitrobacter*
Denitrification ($NO_3^- \rightarrow N_2$)	*Bacillus, Paracoccus, Pseudomonas*
N_2 Fixation ($N_2 + 8\,H \rightarrow NH_3 + H_2$)	
Free-living	
Aerobic	*Azotobacter* Cyanobacteria
Anaerobic	*Clostridium*, purple and green phototrophic bacteria *Methanobacterium (Archaea)*
Symbiotic	*Rhizobium* *Bradyrhizobium* *Frankia*
Ammonification (organic-N $\rightarrow NH_4^+$)	
	Many organisms can do this
Anammox ($NO_2^- + NH_3 \rightarrow 2\,N_2$)	*Brocadia* (shortcut)



Oxidation reactions are shown by yellow arrows and reductions by red arrows. Reactions without redox change are in white. DRNA, dissimilative reduction of nitrate to ammonia
The anammox reaction is $NH_3 + NO_2^- + H^+ \rightarrow N_2 + 2H_2O$

The Nitrogen Cycle

N_2 is the most stable form of nitrogen and is a major reservoir

- Only a few prokaryotes have the ability to use N_2 as a cellular nitrogen source (*nitrogen fixation*)
- *Denitrification* is the reduction of nitrate to gaseous nitrogen products and is the primary mechanism by which N_2 is produced biologically
- Ammonia produced by *nitrogen fixation* or *ammonification* can be assimilated into organic matter or oxidized to nitrate

Anammox is the anaerobic oxidation of ammonia to N_2 gas

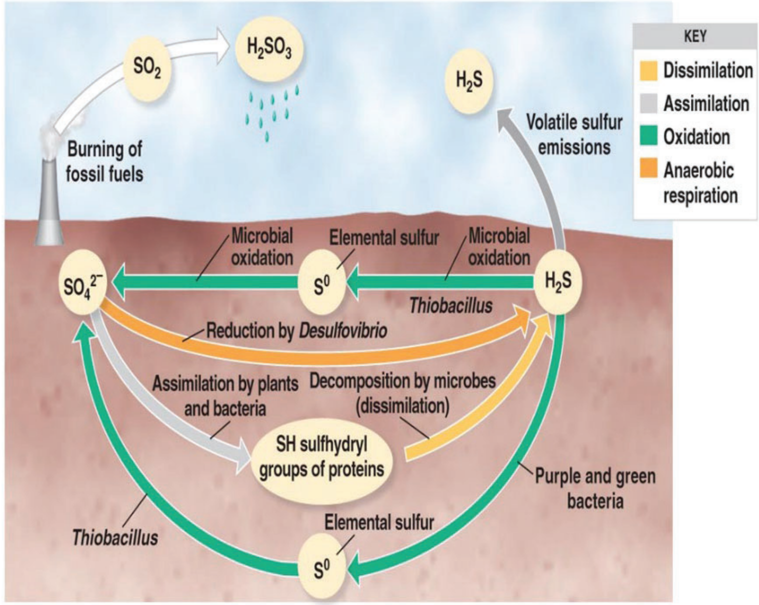
$NH_3 + NO_2^- + H^+ \rightarrow N_2 + 2H_2O$

- Denitrification and anammox result in losses of nitrogen from the biosphere

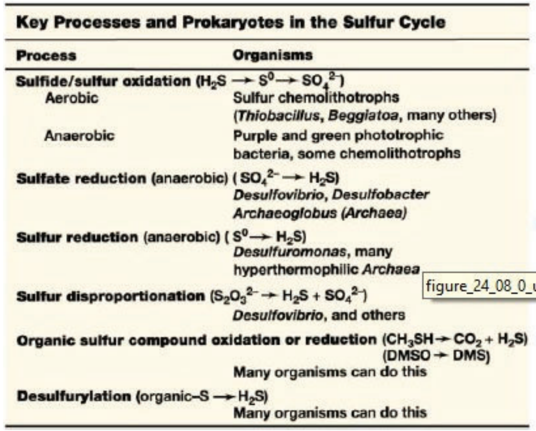
The Sulfur Cycle

- Sulfur transformations by microorganisms are complex
- The bulk of sulfur on Earth is in sediments and rocks as sulfate and sulfide minerals (e.g., gypsum, pyrite)
- The oceans represent the most significant reservoir of sulfur (as sulfate) in the biosphere

Acid Rains



Redox cycle for sulfur



Key Processes and Prokaryotes in the Sulfur Cycle

Process	**Organisms**
Sulfide/sulfur oxidation ($H_2S \rightarrow S^0 \rightarrow SO_4^{2-}$)	
Aerobic	Sulfur chemolithotrophs (*Thiobacillus*, *Beggiatoa*, many others)
Anaerobic	Purple and green phototrophic bacteria, some chemolithotrophs
Sulfate reduction (anaerobic) ($SO_4^{2-} \rightarrow H_2S$)	*Desulfovibrio*, *Desulfobacter* *Archaeoglobus* (*Archaea*)
Sulfur reduction (anaerobic) ($S^0 \rightarrow H_2S$)	*Desulfuromonas*, many hyperthermophilic *Archaea*
Sulfur disproportionation ($S_2O_3^{2-} \rightarrow H_2S + SO_4^{2-}$)	*Desulfovibrio*, and others
Organic sulfur compound oxidation or reduction ($CH_3SH \rightarrow CO_2 + H_2S$) ($DMSO \rightarrow DMS$)	Many organisms can do this
Desulfurylation (organic–S $\rightarrow H_2S$)	Many organisms can do this

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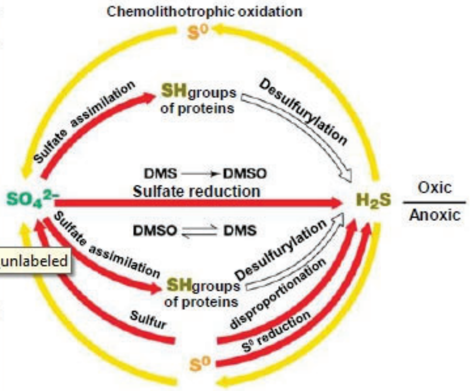


Oxidations are indicated by yellow arrows and reductions by red arrows. Reactions without redox changes are in white. DMS, dimethyl sulfide; DMSO, dimethyl sulfoxide

The Sulfur Cycle

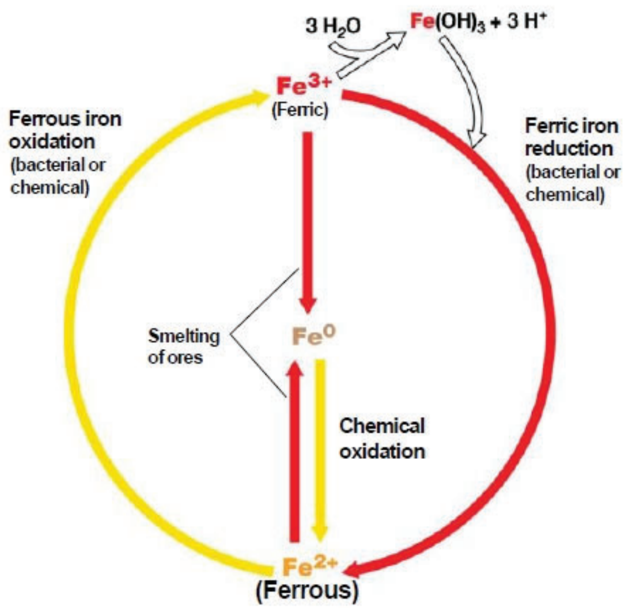
- Hydrogen sulfide (H_2S) is a major volatile sulfur gas that is produced by bacteria via sulfate reduction or emitted from geochemical sources
- Sulfide (S^{2-}) is toxic to many plants and animals and reacts with numerous metals
- Sulfur-oxidizing chemolithotrophs can oxidize sulfide (S^{2-}) and elemental sulfur (S^0) at oxic/anoxic interfaces
- Organic sulfur compounds can also be metabolized by microorganisms
- The most abundant organic sulfur compound in nature is dimethyl sulfide (DMS)
- Produced primarily in marine environments as a degradation product of dimethylsulfoniopropionate (an algal osmolyte)
- DMS can be transformed via a number of microbial processes

The Iron Cycle

Iron is one of the most abundant elements in Earth's crust

- On Earth's surface, iron exists naturally in two oxidation states:
- Ferrous (Fe^{2+})
- Ferric (Fe^{3+})
- The redox reactions in the iron cycle include both oxidations and reductions

Redox cycle for iron



The major forms of iron in nature are Fe^{2+} and Fe^{3+}; Fe^0 is primarily a product of smelting of iron ores. Oxidations are shown by yellow arrows and reductions by red arrows. Fe^{3+} forms various minerals such as ferric hydroxide, $Fe(OH)_3$

Leaching (Bioleaching)
↳ Low grade ores (Bakr, Athn), closure of mining area.

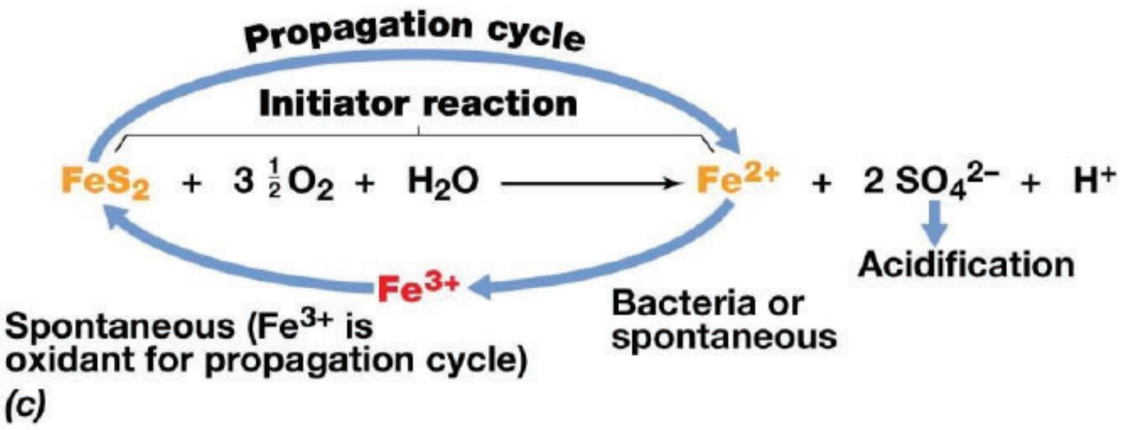
The Iron Cycle

- Fe^{3+} can be used by some microorgansims as electron acceptors in anaerobic respiration
- In aerobic acidic pH environments, acidophilic chemolithotrophs can oxidize Fe^{2+} (e.g., *Acidithiobacillus*)

Pyrite (FeS_2)

- One of the most common forms of iron in nature
- Its oxidation by bacteria can result in acidic conditions in coal-mining operations

Reactions in pyrite degradation



The primarily abiotic initiator reaction sets the stage for the primarily bacterial oxidation of Fe^{2+} to Fe^{3+}. The Fe^{3+} attacks and oxidizes FeS_2 abiotically in the propagation cycle.

The Iron Cycle

Acid Mine Drainage ! (AMD)

- An environmental problem in coal-mining regions
- Occurs when acidic mine waters are mixed with natural waters in rivers and lakes
- Bacterial oxidation of sulfide minerals is a major factor in its formation

Acid mine drainage from a surface coal-mining operation

T. D. Brock

The yellowish-red color is due to the precipitated iron oxides in the drainage

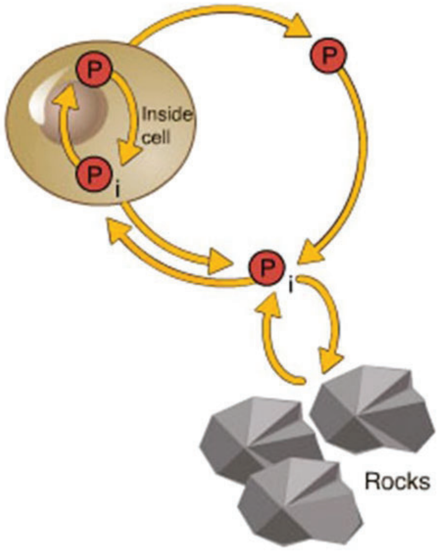
The Phosphorous, Calcium, and Silica Cycles

Phosphorous Cycle

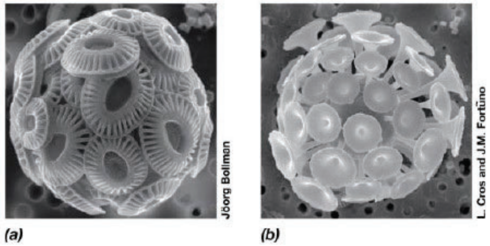
- Organic and inorganic phosphates
- Cycles through living organisms, water and soil (rock)

Calcium Cycle

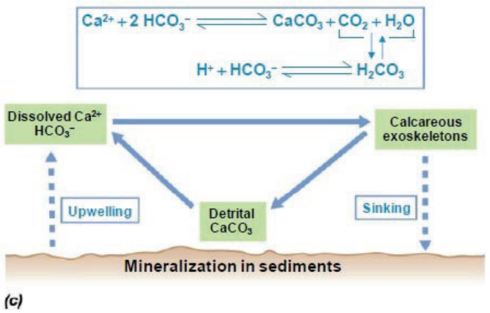
- Reservoirs are rocks and oceans
- Marine phototrophic microorganisms use Ca^{2+} to form exoskeleton



The marine calcium (Ca) cycle



Scanning electron micrographs of cells of the calcareous phytoplankton (a) *Emiliania huxleyi* and (b) *Discophaera tubifera*. The exoskeletons of these phytoplanktons are made of $CaCO_3$



The marine calcium cycle; dynamic pools of Ca^{2+} are shaded in green. Detrital $CaCO_3$ is that in fecal pellets and other organic matter from dead organisms. Note how H_2CO_3 formation decreases ocean pH.

The Phosphorous, Calcium, and Silica Cycles

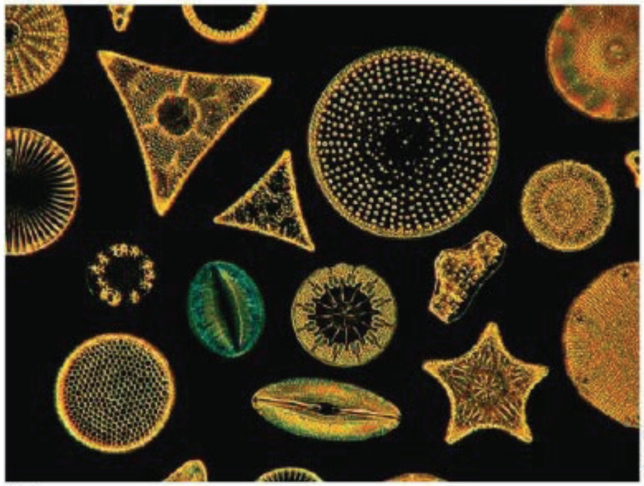
Silica Cycle

The marine silica cycle is controlled by unicellular eukaryotes that build cell skeletons called frustules

frustules

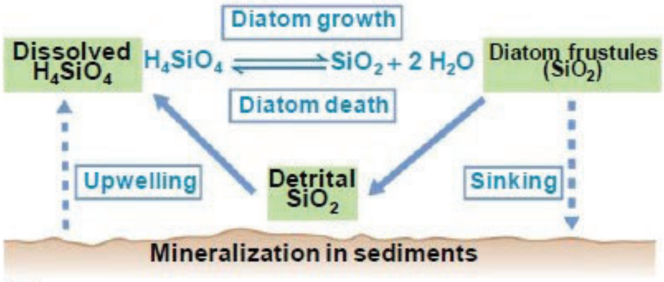
- Examples: diatoms, dinoflagellates, and radiolarians

The marine silica cycle


Jörg Piper

(a)

(a) Dark-field photomicrograph of a collection of diatom shells (frustules). The frustules are made of SiO_2.



(b)

(b) The marine silica cycle dynamic pools of Si are shaded in green

Biodegradation and Bioremediation

- Microbial Leaching
- Mercury Transformations
- Petroleum Biodegradation and
- Bioremediation
- Xenobiotics Biodegradation and Bioremediation

Microbial Leaching

cells itself (bacteria fungi)
part of the cell (enzymes)
plants (phytoremediation) ⇒ grow the plant in the contaminated area
↳ non-polluting plants

↳ plants absorb the contaminant by their root, stock in their leaves and bar.
leaves ⇒ cut leaves
↳ place called rhizosphere (part of the soil around the root) where microbe-plant interaction take place.

→ pesticide herbicide
→ generally chemicals (man made = Xenobiotics)
↳ persistent in the environment ⇒ soil / water / groundwater / air pollutant

Bioremediation ⇒ remove pollutants / contaminants

- Refers to the cleanup of oil, toxic chemicals, or other pollutants from the environment by microorganisms
- Often a cost-effective and practical method for pollutant cleanup

↳ How? ⇒ By microorganisms enzyme, they degrade pollutant. (means biodegradable) → something we prefer
→ biodegradable
↳ non-biodegradable
↳ End product ⇒ CO_2 and H_2O (mineralization ✓)

if not biodegradable = Recalcitrant

mother pollutant (toxic)
↓ mineralization
end-product (non-toxic)

mother pollutant
↓ → Transformation
less toxic end product

mother pollutant
↓
more toxic
(rare situation) → mercury transformation!
↳ happens in nature. (aquatic env)

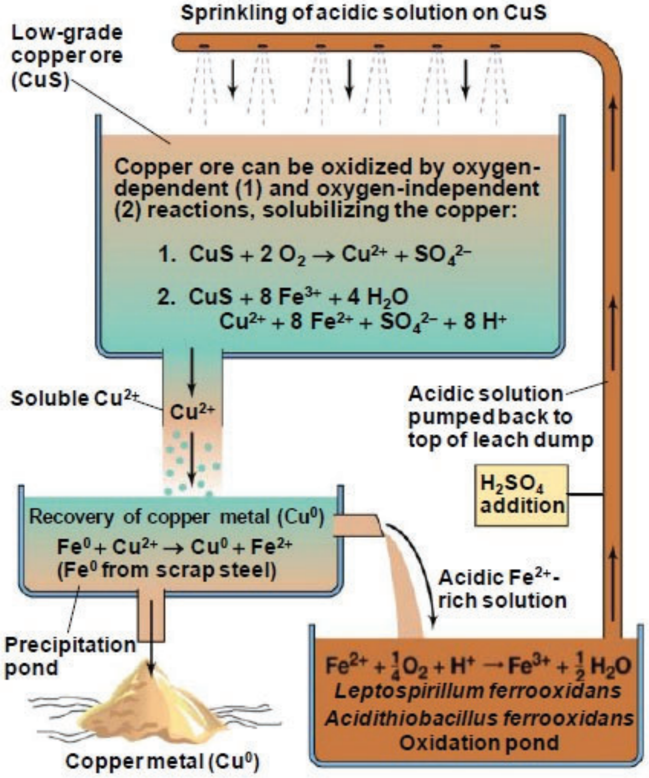
Microbial leaching

- The removal of valuable metals, such as copper, from sulfide ores by microbial activities
- Particularly useful for copper ores

Microbial Leaching

- In microbial leaching, low-grade ore is dumped in a large pile (the leach dump) and sulfuric acid is added
- The liquid emerging from the bottom of the pile is enriched in dissolved metals and is transported to a precipitation plant
- Bacterial oxidation of Fe^{2+} is critical in microbial leaching as Fe^{3+} itself can oxidize metals in the ores

Arrangement of a leaching pile and reactions in the microbial leaching of copper sulfide minerals to yield metallic copper



- Reaction 1 occurs both biologically and chemically.
- Reaction 2 is strictly chemical and is the most important reaction in copper-leaching processes.
- For reaction 2 to proceed, it is essential that the Fe^{2+} produced from the oxidation of sulfide in CuS to sulfate be oxidized back to Fe^{3+} by iron chemolithotrophs.

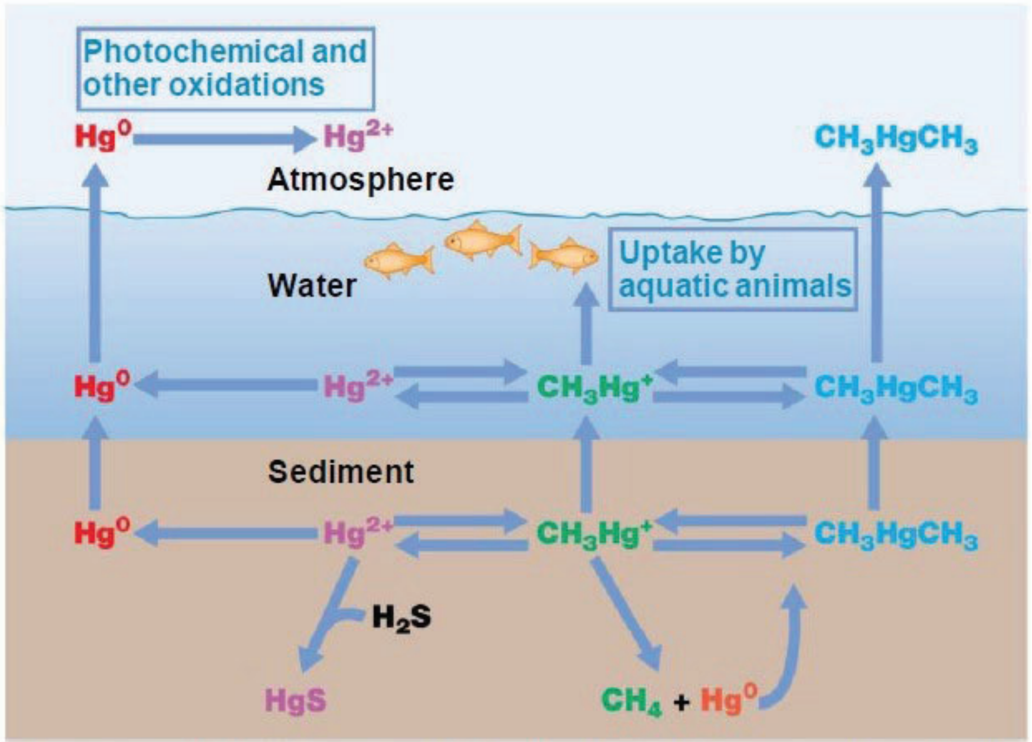
Microbial Leaching

- Microorganisms are also used in the leaching of uranium and gold ores.
- Some bacteria are able to reduce U^{6+} to U^{4+}
- U^{4+} forms an immobile uranium mineral, uraninite, thus limiting the movement of uranium into groundwater.

Mercury Transformations

- Mercury has tendency to concentrate in living tissues and it is highly toxic
- The major form of mercury in the atmosphere is elemental mercury (Hg^0), which is volatile and oxidized to mercuric ion (Hg^{2+}) photochemically
- Most mercury enters aquatic environments as Hg^{2+}

Biogeochemical cycling of mercury



The major reservoirs of Hg are water and sediments. Hg in water can be concentrated in animal tissues; it can be precipitated as HgS from sediments. The forms of mercury commonly found in aquatic environments are each shown in a different color.

Mercury Transformations

- Hg^{2+} readily absorbs to particulate matter where it can be metabolized by microorganisms
- Microorganisms form methylmercury (CH_3Hg^+), an extremely soluble and toxic compound
- Several bacteria can also transform toxic mercury to nontoxic forms
- Bacterial resistance to heavy metal toxicity is often linked to specific plasmids that encode enzymes capable of detoxifying or pumping out the metals

Petroleum Biodegradation and Bioremediation

Prokaryotes have been used in bioremediation of several major crude oil spills

Environmental consequences of large oil spills and the effect of bioremediation

US Environmental Protection Agency

(a) A contaminated beach along the coast of Alaska in 1989.

US Environmental Protection Agency

(b) The rectangular plot (arrow) was treated with inorganic nutrients to stimulate bioremediation of spilled oil by microorganisms, whereas areas above and to the left were untreated.

metals $\xrightarrow{\text{biomass}}$ biosorption
(recalcitrant)
adsorption

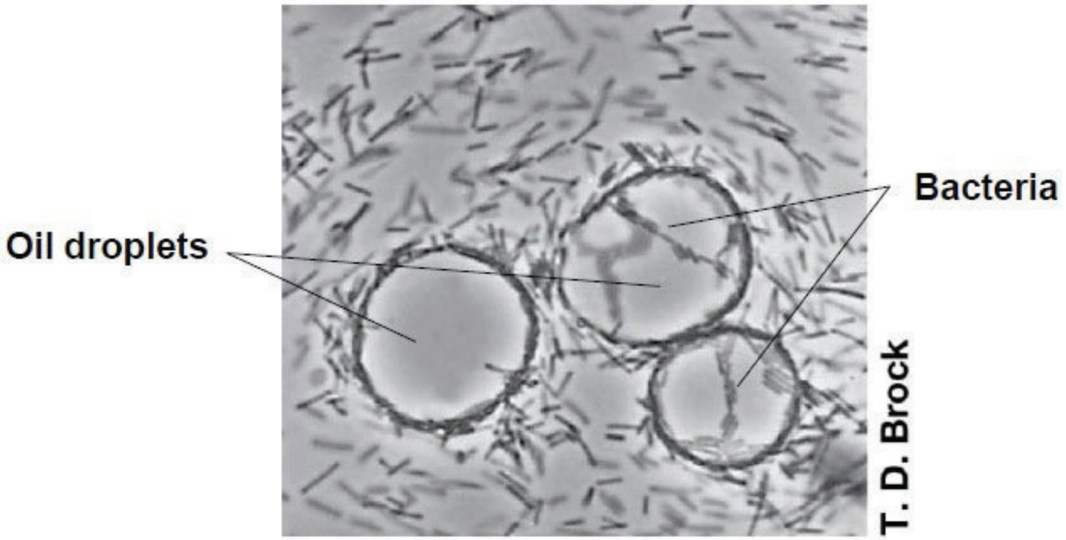
metallothionines (protein)
↓
attach the metal
↓
inside of the cell
↳ remove e cell → remove metal

petroleum (hydrocarbons) → soil pollution

Petroleum Biodegradation and Bioremediation

- Diverse bacteria, fungi and some cyanobacteria and green algae can oxidize petroleum products aerobically
- Oil-oxidizing activity is best if temperature and inorganic nutrient concentrations are optimal
- Hydrocarbon-degrading bacteria attach to oil droplets and decompose the oil and disperse the slick

Hydrocarbon-oxidizing bacteria in association with oil droplets



The bacteria are concentrated in large numbers at the oil-water interface, but are actually not within the droplet itself

Petroleum Biodegradation and Bioremediation

- Gasoline and crude oil storage tanks are potential habitats for hydrocarbon-oxidizing microbes
- If sufficient sulfate is present, sulfate-reducing bacteria can grow and consume hydrocarbons

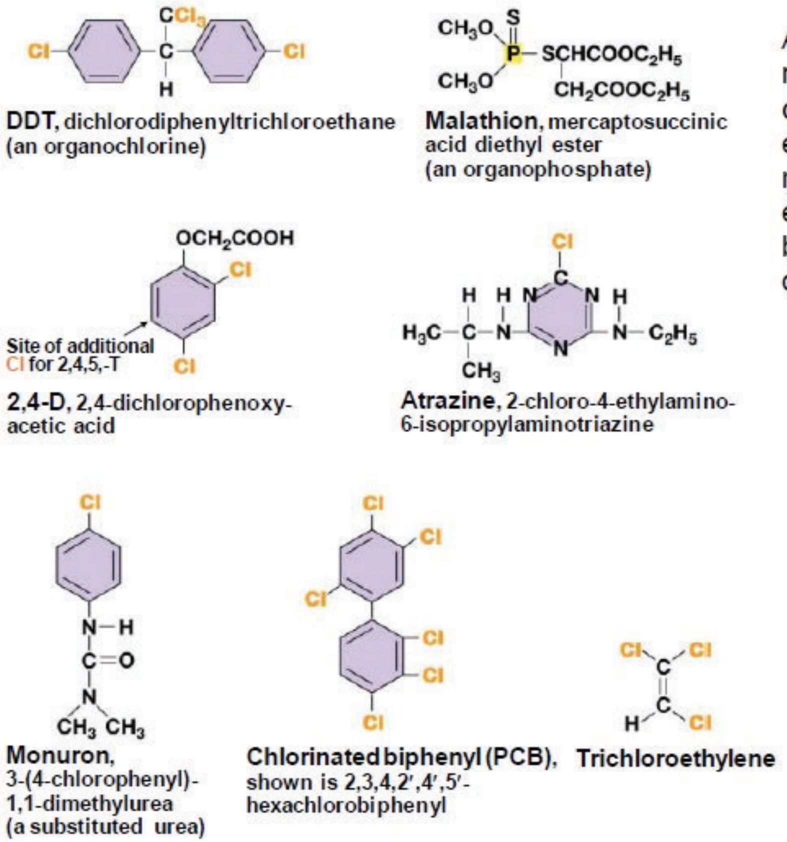
Xenobiotic Biodegradation and Bioremediation

- Synthetic chemicals that are not naturally occurring

Examples: pesticides, polychlorinated biphenyls, munitions, dyes, and chlorinated solvents

- Many degrade extremely slowly

Examples of xenobiotic compounds



DDT, dichlorodiphenyltrichloroethane (an organochlorine)

Malathion, mercaptosuccinic acid diethyl ester (an organophosphate)

2,4-D, 2,4-dichlorophenoxy-acetic acid

Atrazine, 2-chloro-4-ethylamino-6-isopropylaminotriazine

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

Chlorinated biphenyl (PCB), shown is 2,3,4,2′,4′,5′-hexachlorobiphenyl

Trichloroethylene

Although none of these compounds exist naturally, microorganisms exist that can break them down.

Xenobiotic Biodegradation and Bioremediation

Pesticides

- Common components of toxic wastes
- Include herbicides, insecticides, and fungicides
- Represent a wide variety of chemicals
- Some can be used as carbon sources by microorganisms
- Some can be used as electron donors

Xenobiotic Biodegradation and Bioremediation

- Some xenobiotics can be degraded partially or completely if another organic material is present as a primary energy source (cometabolism)
- Chlorinated xenobiotics can be degraded anaerobically (reductive dechlorination) or aerobically (aerobic dechlorination)
- Reductive dechlorination is usually a more important process as anoxic conditions develop quickly in polluted environments
- Plastics of various types are xenobiotics that are not readily degraded by microorganisms
- The recalcitrance of plastics has fueled research efforts into a biodegradable alternative (biopolymers)