Chapter 30-31

Environmental Microbiology

What is microbial ecology?

The study of...

- the relationships that exist between microorganisms and the environment.
- the relationships that exist between different populations of microorganisms.
- the microbially-mediated processes that sustain life on earth.
- the fate of man-made products in the environment.

Ecological Community

- Indigenous (native) organisms
  - *Spirillum volutans* - stagnant water
  - *Streptomycetes* spp. - soil
  - *Escherichia coli* - human digestive tract
- Non-indigenous organisms (temporary)
  - Organisms that appear after an oil spill

Microbial Relationships

- Symbiosis
- Mutualism
- Commensalism
- Antagonism
- Competition
- Parasitism
- Predation

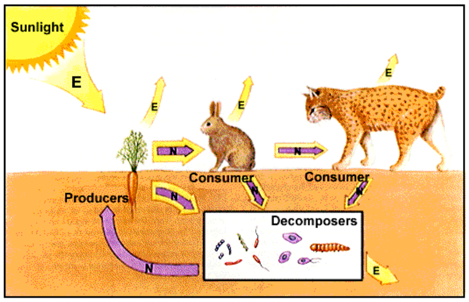
Trophic levels

- Primary producers
  - Autotrophs
- Consumers
  - Heterotrophs
    - 1° consumers
    - 2° consumers
    - 3° consumers
- Decomposers
  - Detritus

Trophic levels &Flow of energy

- The sun - ultimate source of energy
  - Exception - chemolithotrophic (energy - inorganic)
- Primary producers
  - Autotrophs - energy from the sun
    - Photosynthetic bacteria, cyanobacteria, protists and algae
- Consumers
  - Heterotrophs - Heterotophic bacteria, protists, fungi, and viruses - eat producers or consumers
    - 1° consumers
    - 2° consumers
    - 3° consumers
- Decomposers - eat dead bodies or waste products
  - Numerous fungi and bacteria
  - Detritus

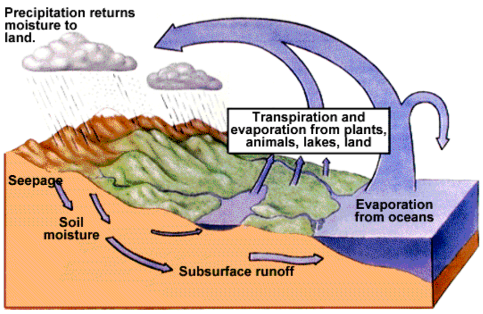
The energy cycle



Biogeochemical Cycles

Bio = living thing
Geo = earth

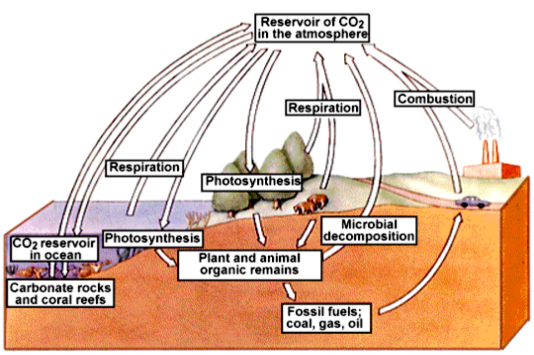
Water cycle or hydrologic cycle



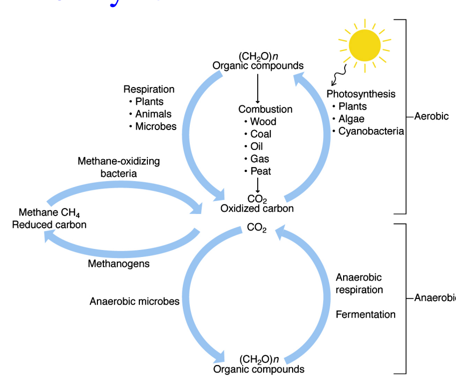
The Carbon Cycle

- Carbon dioxide fixation
  - photosynthesis
  - chemosynthesis
- Forms of carbon in the soil
  - derived from plant material--
    - cellulose and hemicelluloses
    - lignin
    - waxes
    - phenolic compounds

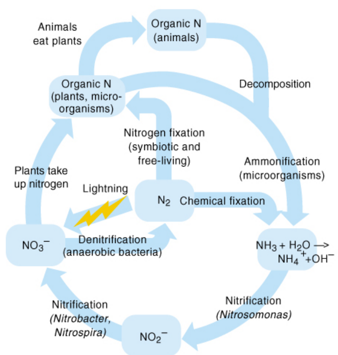
Carbon cycle



Carbon cycle



Nitrogen cycle



The Nitrogen Cycle

- Mainly a terrestrial cycle
- Nitrogen is cycled in a series of microbially-mediated oxidation and reduction reactions
- There are 4 main forms of nitrogen
  - dinitrogen gas, organic N, ammonia, and nitrate

Nitrogen Fixation

$$N_2 + 3H_2 \rightarrow 2NH_3$$

- Industrial nitrogen fixation
- Lightning nitrogen fixation
- Biological nitrogen fixation

Industrial Nitrogen Fixation

Haber-Bosch Process

- "Fixes" nitrogen under high temperatures and pressures.
- Source of most fertilizer nitrogen.
  - Main forms of fertilizer nitrogen include ammonium, nitrate, urea, and diammonium phosphates.

Lightning fixation

- Energy from lightning
  - sufficient to oxidize dinitrogen to nitrogen oxides.
  - This is a major global contributor of fixed nitrogen.

Biological Nitrogen Fixation

- biological nitrogen fixation requires a lot of energy
  - Like industrial fixation and lightning fixation,.
- It requires 12ATPs per NH_3 fixed:
  - 4 ATPs per bond broken by the enzyme nitrogenase

Biological Nitrogen Fixation

- There are 2 types of biological nitrogen fixation
  - Non-symbiotic Nitrogen Fixation
    - Free-living microorganisms
- Symbiotic Nitrogen Fixation
  - Association of a microorganism with another organism, such as a plant.

Nonsymbiotic Nitrogen Fixation

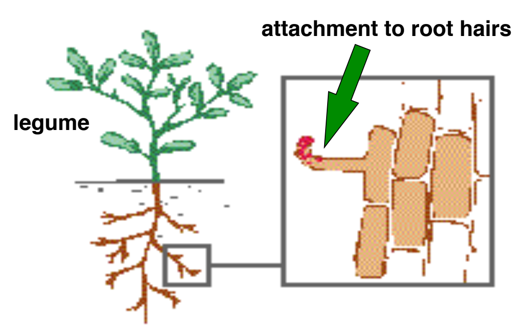
- Important in flooded rice fields, rock surfaces, and nitrogen-deficient soils.
  - Microorganisms include:
    - *Azotobacter*
    - *Clostridium*
    - cyanobacteria

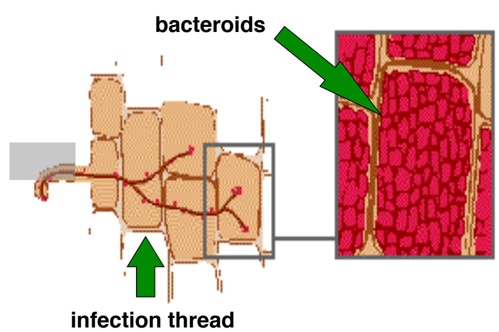
Symbiotic Nitrogen Fixation

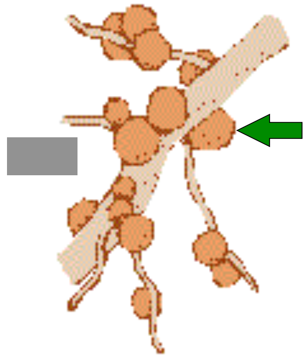
- Association - nitrogen-fixing microorganism with a plant
- Important - soils where the fixed nitrogen is insufficient for more than one growing season
- *Rhizobium* - legume
- *Anabaena* (cyanobacteria)- *Azolla* (fern)
- *Frankia* (actinomycetes) - Alder tree

Rhizobium-legume symbiosis

- Attachment of rhizobia to surface of root hair.
- Penetration of rhizobia into deformed root hair.
- Formation of an infection thread of bacteria from root hair to root cortical cells.
- Formation of a nodule containing bacteroids.







Rhizobium-legume symbiosis

- **Interaction is very specific.**

Rhizobium species	Legume
Bradyrhizobium japonicum	soybeans
Rhizobium leguminosarum	pea, vetch, sweetpea
Rhizobium meliloti	alfalfa, sweet clover
Rhizobium phaseoli	beans
Rhizobium lupini	lupines
Rhizobium trifolii	clover

Biologically-fixed nitrogen

Legume	kg N $ha^{-1}y^{-1}$
Alfalfa	125-335
Red clover	85-190
Pea	80-150
Soybean	65-115
Cowpea	65-130
Vetch	90-155

Decomposition

- Decomposition
  - plant,animal and microbial residues
    - proteins into the soil.
  - Proteins undergo *proteolysis*
    - amino acids.
  - Most nutrients go through living microbial biomass before being accessible to other organisms
    - heterotrophs
  - aerobic conditions decomposition is *faster* and *more complete* than under anaerobic conditions.

Ammonification

- The degradation of amino acids
  - release of ammonia.
  - another term is *deamination*.

Immobilization or Uptake

- Plants and microbes use nitrogen
  - Ammonium NH_4
  - Nitrate (most usable to plants) NO_3^-

Nitrification (ammonia oxidation)

$$NH_3 \xrightarrow{Nitrosomonas} NO_2^- \xrightarrow{Nitrobacter} NO_3^-$$

- A two-step process
  - ammonia is converted into nitrite (NO_2^-) toxic to plants
  - then to nitrate.
- Aerobic process

Nitrification

$$NH_3 \xrightarrow{Nitrosomonas} NO_2^- \xrightarrow{Nitrobacter} NO_3^-$$

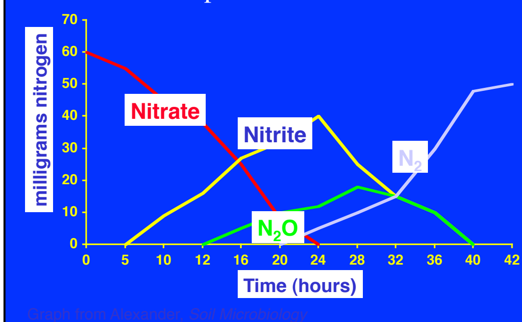
- Oxidation of nitrite is inhibited by ammonium.
- Chemolithotrophic microorganisms or some heterotrophs.

Denitrification (nitrate reduction)

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

- Mineral nitrogen (NO_3^-) is reduced to dinitrogen gas (N_2)
- Anaerobic process (oxygen poor soils)
- Heterotrophic organisms using nitrate as a terminal electron acceptor: anaerobic respiration (breath Nitrate)

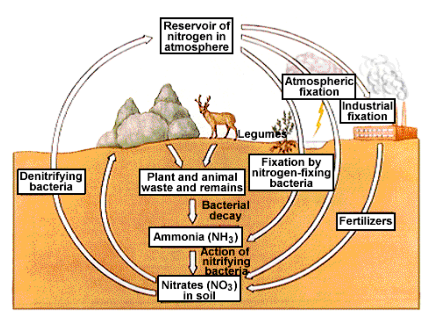
Denitrification products in Melville loam



Denitrifying microorganisms

- Organisms are aerobic, but use nitrate as an alternate terminal electron acceptor in anaerobic respiration.
- *Pseudomonas*, *Thiobacillus*, and *Micrococcus* species.
- Removes nitrates and interferes with plant growth

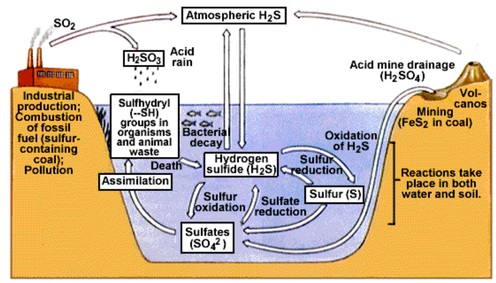
Nitrogen cycle summary



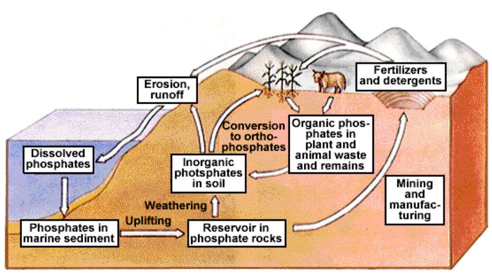
The Sulfur Cycle

- Mainly aquatic
- Sulfate-reducing bacteria, *Desulfovibrio*, *Desulfomonas*, *Desulfotomaculum*
  - Reduce SO_4^{2-} to H_2S
  - Anaerobic process - SO_4^{2-} is the final electron acceptor
- Sulfur-reducing bacteria, *Desulfonema*
  - reduce sulfate to H_2S
  - Anaerobic process, sulfur is the final electron acceptor
- Sulfur-oxidizing bacteria, *Thiobacillus ferroxidans*
  - Elemental S is oxidized to sulfate
  - Plants use sulfate
    - amino acid biosynthesis.
- Some phototrophic bacteria can oxidize H_2S to elemental sulfur

Sulfer cycle



Phosphorus cycle



Atmosphere Microbiology

Microorganisms in the atmosphere

- Air is very inhospitable for microorganisms
  - dust particles, water, sputum, or aerosol droplets.
  - Some droplets (droplet nuclei) may exist for hours or days
  - Spores - Mold and bacteria, *Bacillus subtilis*

Factors affecting the fate of microorganisms in the atmosphere

- Humidity
- Temperature
- Amount of sunlight
- Size of particles harboring the microorganisms.
- Formation of cysts and spores

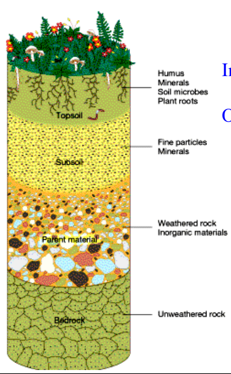
Where do microorganisms in the atmosphere come from?

- Soil (winds pick up dust particles)
- Oceans (winds create sprays)
- Industry, agriculture, municipal facilities can create aerosols
  - water treatment plants
  - irrigation

Controlling air-borne microbes

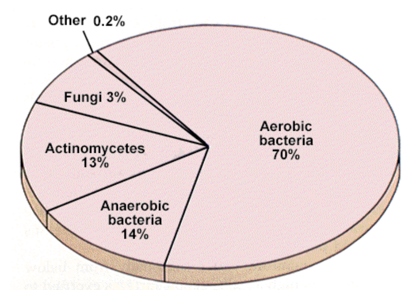
- Triethylene glycol, resorcinol, and lactic acid
- UV radiation
- Filtration (laminar airflow)
  - HEPA filters

Soil horizons



Inorganic components
rock, minerals, water, & gases
Organic component
humus & living organisms

Microorganisms in soil



Factors affecting soil microbes

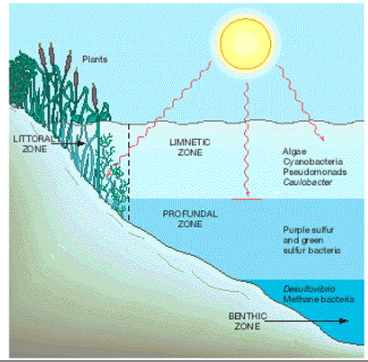
- Moisture
- Oxygen
- pH
  - Optimum 6-8
- Temperature

Soil pathogens

- *Clostridium tetani*
  - tetanus
- *Clostridium botulinum*
  - botulism
- *Clostridium perfringens*
  - Gas gangrene
- *Bacillus anthracis*
  - anthrax

Water and Wastewater Microbiology

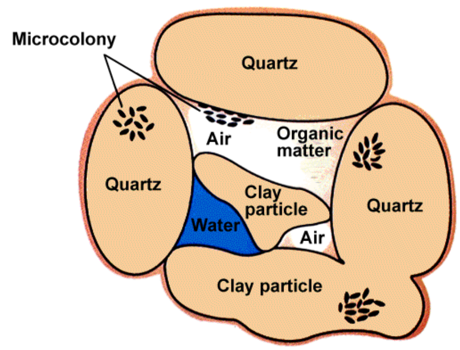
Zonation in a lake



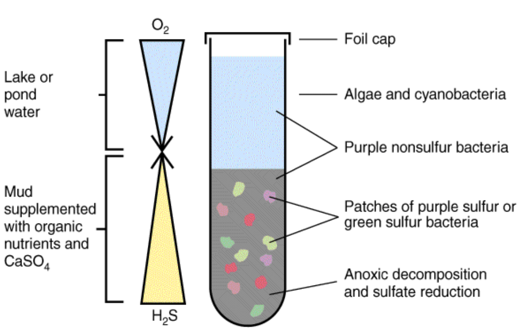
Factors affecting microorganisms in aquatic environments

- Temperature
- Hydrostatic Pressure
- Light
- Salinity
- Turbidity
- pH
  - Fresh 2-9
  - Ocean 6.5-8.3
- Nutrients

Oxygen and water relationship



Oxygen vs hydrogen sulfide



Freshwater environments

- Limnology--the study of freshwater environments.
- Freshwater environments include:
- lakes
- ponds
- streams
- rivers
- estuaries

Marine environments

- phytoplankton, cyanobacteria, algae, diatoms, dinoflagellates
- Gram negative bacteria
  - *Pseudomonas, Vibrio, Achromobacter, Flavobacterium*
- Marine fungi and protozoa
- The ocean environment is oligotrophic.
  "Oligotrophic" means that there are few nutrients available for organisms to use for growth.

Drinking Water

- Rivers, streams, and lakes
- Groundwater
- All of these water sources can become chemically and biologically polluted.

Water purification

- Alum (aluminum potassium sulfate)
  - Flocculation
- Filtration
  - Beds of sand or charcoal
- Chlorination
  - Kills bacteria, some viruses

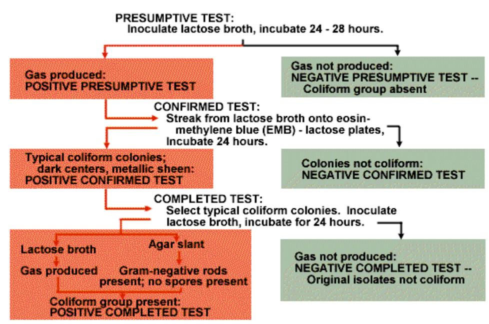
Testing water for potential pathogens

✓ Use "indicator microorganisms"
✓ Present in polluted water
✓ Present when pathogens are present
✓ Quantity correlates with number of pathogens
✓ Survives better than pathogens
✓ Has uniform and stable properties
✓ Generally harmless to humans
✓ Present in greater numbers than pathogen
✓ Easily detected in the lab

Human pathogen transmitted in water

ORGANISMS	DISEASES CAUSED
Salmonella typhi	Typhoid fever
Other Salmonella species	Salmonellosis (gastroenteritis)
Shigella species	Shigellosis (bacillary dysentery)
Vibrio cholerae	Asiatic cholera
Vibrio parahaemolyticus	Gastroenteritis
Escherichia coli	Gastroenteritis
Yersinia enterocolitica	Gastroenteritis
Campylobacter fetus	Gastroenteritis
Hepatitis A virus	Hepatitis
Poliovirus	Poliomyelitis
Giardia intestinalis	Giardiasis
Balantidium coli	Balantidiasis
Entamoeba hist	Amoebic dysentery

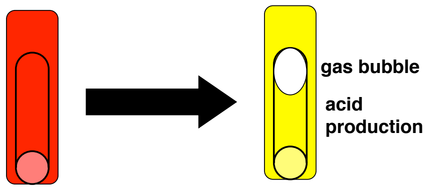
Multiple tube fermentation test



Multiple-tube test, Part 1

- Presumptive test

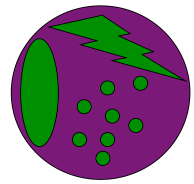
  + gas production in lactose broth.



Multiple-tube test, Part 2

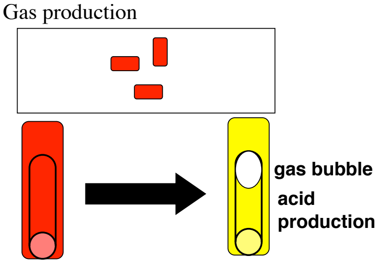
- Confirmed test

  metallic green colonies on EMB agar.



Multiple-tube test, Part 3

- Completed test
  - growth of Gram negative, non-spore forming rods on agar slant
  - Acid & Gas production



Testing water purity

- Multiple-tube fermentation method
- Membrane filter method
  - 1 cfu/100 ml of water
- ONPG and MUG test
  - Enzyme that convert reduce dyes

Municipal Wastewater

Where does it originate?

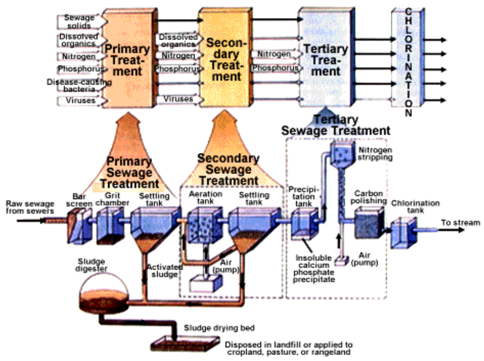
- domestic sources (human wastes, and wash water)
- industrial sources
- ground, surface, and atmospheric water

Municipal Wastewater

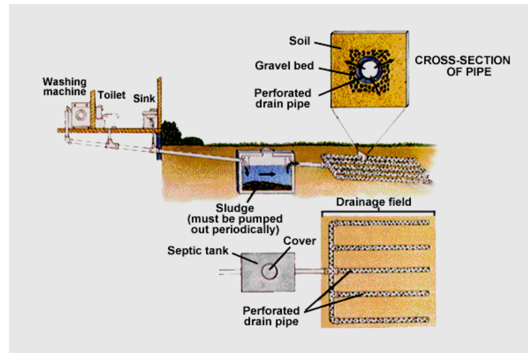
Three main steps:

1. sedimentation
2. filtration
3. chlorination

Sewage treatment



Septic tank



Pesticide and Pollutant Degradation

What are pesticides?

- Natural and man-made substances used to control pests.

- They include:
  - herbicides--control of weeds
  - insecticides--control of insects
  - fungicides--control of fungi
  - nematicides--control of nematodes

The Fate of Pesticides in the Environment

Bioremediation

Depends on:

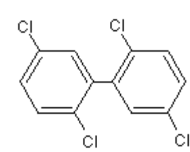
- Complexity of the molecule
- Presence of halogens, such as chlorine atoms in the molecule
- Presence and number of microorganisms capable of degrading the pesticide
- Presence of other nutrients
- Concentration of the pesticide

Pollutants we might be concerned about...

- polychlorinated biphenyls (PCBs)
- pesticide residues in foods and drinking water.
- ozone, nitrogen and sulfur oxides.
- heavy metals

- *Phanerochaete chrysosporium*
  - White rot fungus

An example of a polychlorinated biphenyl



2,2',5,5'-tetrachlorobiphenyl

Degradation of PCBs by microorganisms

Chlorinated biphenyls

↓ *Acinetobacter*

Chlorobenzoic acid

↓ *Pseudomonas*

Cells + Cl^-

Some pesticides