Bacteria on the edge: redox and pH

- Anaerobic bacterial communities

II. Acidic environments

Anaerobic microbial communities

1) What causes anaerobic conditions (anaerobiosis)??

where rate of consumption of O_2 > rate of diffusion of O_2

Main point: in all environments "exposed" to the atmosphere, anaerobiosis requires biological activity! (specifically, O_2 respiration)

Sterile, waterlogged soils will be aerobic environments

Diffusion of O_2 is much much slower in water than in air

air: 0.205 $cm^2 s^{-1}$

water: 0.180 x $10^{-4} cm^2 s^{-1}$

Thus, water is an effective diffusion barrier to O_2 movement, but water does not create anaerobic conditions by consuming O_2 – only living things do that!

For anaerobiosis to develop, aerobes must consume O_2, allowing conditions to become reducing (anaerobic), and subsequent groups of bacteria to flourish

Examples of anaerobic microbial communities

1) gut of newborn human

Lactobacillus, E coli – facultative anaerobes, consume O_2

later, *Bacteroides* (strict anaerobe) and other obligate anaerobes become established

2) gut of newborn mouse

Flavobacterium (strict aerobes!) and enterococci – establish initially and decrease after about 2 weeks

Lactic acid bacteria (facultative anaerobes)

Bacteroides – dominant in 'climax' community

3) **Wetland soils, e.g. saltmarsh**

Because of O_2 consumption and slow diffusion from the atmosphere, marshes support communities using various electron acceptors, becoming more and more anaerobic (low Eh)

iron bacteria (Fe^{3+}/Fe^{2+})
denitrifiers (NO_3^-/N_2)
sulfur reducers (SO_4^{2-}/H_2S)
methanogens (CO_2/CH_4)

zones in the soil column with communities of each type

Oxygen-requiring bacteria will use products of anaerobic metabolism:

methanotrophs (CH_4 is energy source)
sulfur oxidizers (H_2S is energy source)

As methanogens and sulfur reducing communities develop, these will support methanotrophs and sulfur oxidizing bacteria in the aerobic zone

4) The **Winogradsky column** illustrates interdependence of different microorganisms: the activities of one organism enable another to grow, and vice-versa.

How to make one:

Collect sediment from the bottom of a lake or river

Add cellulose, sodium sulphate and calcium carbonate, to the lower one-third of the tube.

Fill tube with water from the lake or river, cap and place near a window.

Incubate for 2-3 months

1. Cellulose promotes rapid microbial growth, depletes [O_2] except in the very top of the column

2. Anaerobic organisms thrive:
   fermenters (organisms that degrade organic compounds incompletely, using organic molecules as terminal electron acceptors)

   e.g., **cellulose-degrading *Clostridium*** thrive when the [O_2] is depleted

   Clostridium spp. are strictly anaerobic; vegetative cells are killed by exposure to oxygen, but they can survive as spores in aerobic conditions (like *Bacillus* spp).

   Clostridium spp cellulose → glucose → ethanol, acetic acid, succinic acid (fermentation end products)

3. S**ulfur-reducing bacteria** (e.g., *Desulfovibrio*) use these fermentation products as energy sources (e- donors) during **anaerobic respiration**, using sulfate or other partly oxidized forms of sulfur (e.g. thiosulfate) as the terminal electron acceptor

$$SO_4^{2-} \rightarrow H_2S$$

Some H_2S reacts with Fe, producing black ferrous sulfide.

Some of the H_2S diffuses upwards into the water column, where it is used by other organisms.

4. H_2S diffuses into the water column, supporting **anaerobic photosynthetic bacteria**.

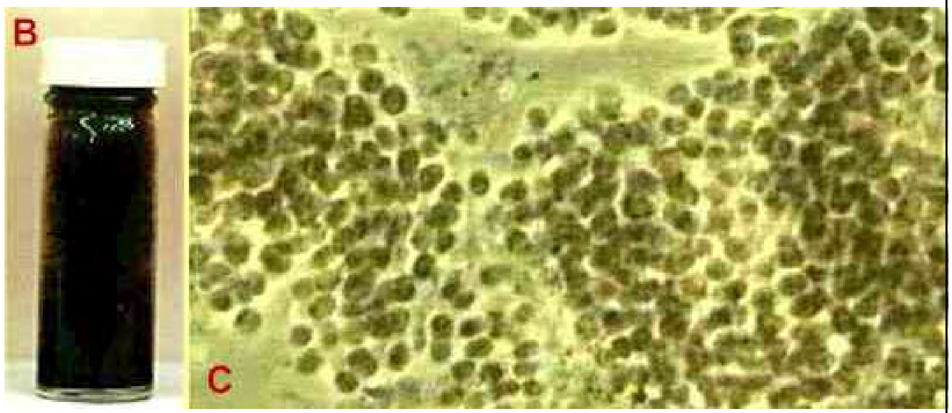
$$6\ CO_2 + 12\ H_2S \rightarrow C_6H_{12}O_6 + 6\ H_20 + 12S$$

All are strictly anaerobic

purple sulfur bacteria

green sulfur bacteria

sulfate-reducing bacteria



Sediment culture of *Desulfovibrio* spp, typical of the lowest (most reduced) zone of a Winogradsky column. Black color due to ferrous sulfide.

Phase-contrast photomicrograph of a species of *Thiocapsa* (**purple sulfur bacterium**) from a Winogradsky column. (Squint a little, and the purple color can be seen where the cells are very dense.)

5. The water column above the sulfur bacteria is bright red, inhabited by a large population of

Facultative anaerobes

purple non-sulfur bacteria

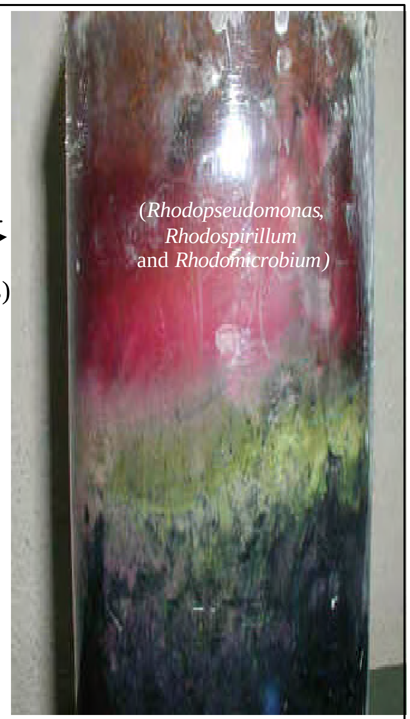
(+ other facultative anaerobes)

All are strictly anaerobic

purple sulfur bacteria

green sulfur bacteria

sulfate-reducing bacteria



Purple non-sulfur bacteria are intolerant of high H_2S concentrations, so they occur above the zone where the green and purple sulfur bacteria are found.

They can, however, use H2S as an electron donor

Pure culture of a purple non-sulfur bacterium

6. In the upper-most, oxygenated zone of the column, there are

(i) **Aerobic sulfur-oxidizing bacteria**

oxidize H_2S (energy source)

synthesize their own organic matter from CO_2 (C source)

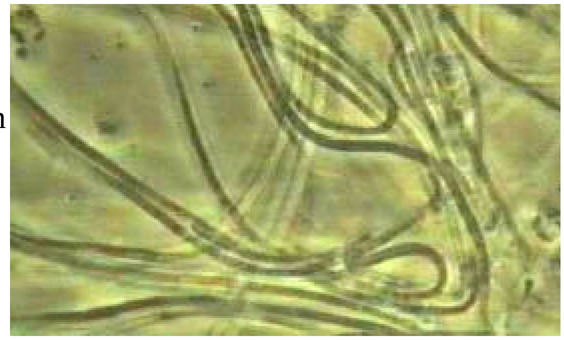
use O_2 (electron acceptor)

\ **chemoautotrophs**.

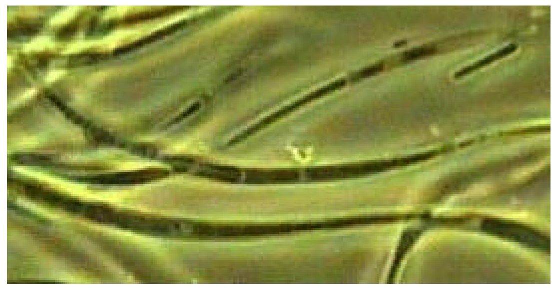
(ii) **Cyanobacteria:** the only bacteria that have **oxygen-evolving photosynthesis** like that of plants. Photoautotrophs.
(C source = CO_2; energy source = light; electron acceptor = O_2)

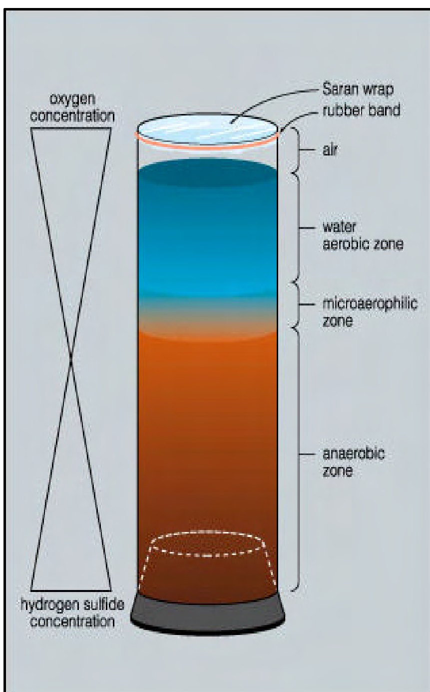
(iii) **Sheathed bacteria:** aerobic heterotrophs, but unusual because they synthesize a rigid tubular sheath from which individual cells can escape and swim away to establish new colonies.

Sheathed bacteria from Winogradsky column



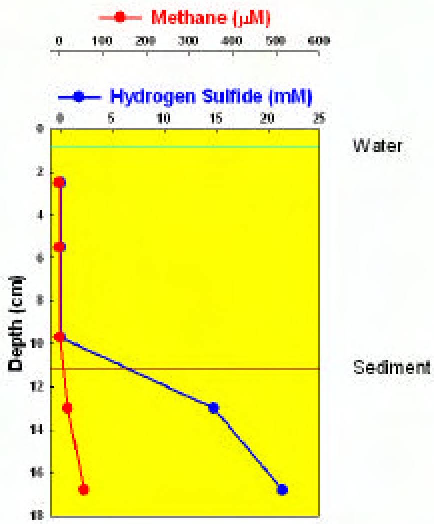
Cyanobacteria from Winogradsky column





Cyanobacteria Aerobic heterotrophs	$CH_2O \rightarrow CO_2$, $CO_2 \rightarrow CH_2O$ $H_2O \rightarrow O_2$, $O_2 \rightarrow H_2O$
H_2S oxidizers Facultative anaerobes	$H_2S \rightarrow S^o$, $CO_2 \rightarrow CH_2O$
Nonsulfur purple bacteria	$CO_2 \rightarrow CH_2O$
Purple sulfur bacteria	$H_2S \rightarrow S^o$, $CO_2 \rightarrow CH_2O$
Green sulfur bacteria	$H_2S \rightarrow S^o$, $CO_2 \rightarrow CH_2O$
Sulfate-reducing bacteria Fermentative heterotrophs	$SO_4^{2-} \rightarrow H_2S$, $CH_2O \rightarrow CO_2$ $CH_2O \rightarrow CO_2 + H_2$

Methane production and consumption can also occur in a Winogradsky column…



Methanogenesis I

Mierle Laderman Ukeles, official artist of the New York Sanitation Department

Methanogenesis II

Mierle Laderman Ukeles, official artist of the New York Sanitation Department

Methanogenesis III

Mierle Laderman Ukeles, official artist of the New York Sanitation Department

Methanogenesis IV

Mierle Laderman Ukeles, official artist of the New York Sanitation Department

II. Acidic environments

- Created naturally and anthropogenically
  - natural sources
    - production of sulfurous gases in hydrothermal vents and some hot springs
    - metabolic activities of certain acidophiles themselves.
  - Anthropogenic sources: coal mining debris.

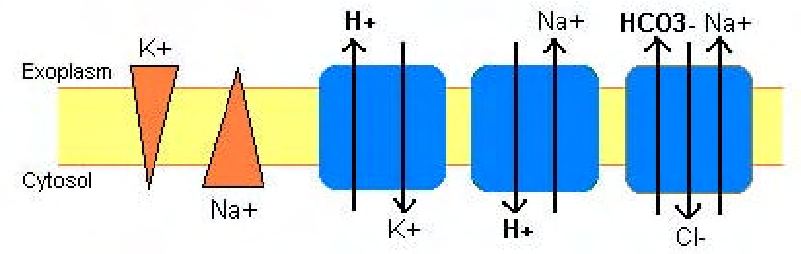
Sulfur Geyser

physiology

- acidophiles cannot tolerate great acidity inside their cells, where it would destroy such important molecules as DNA.
- They survive by keeping the acid out.
- But the defensive molecules that provide this protection, as well as others that come into contact with the environment, must be able to operate in extreme acidity.
- Extremozymes that are able to work at a pH below one--more acidic than even vinegar or stomach fluids--have been isolated from the cell wall and underlying cell membrane of some acidophiles.

Acidophiles have membrane-bound enzymes to transport H^+ out of the cell

Acidophile antiporter enzyme



Alkaliphile antiporter enzyme

- Other mechanisms for pH regulation by acidophiles:
  - Decarboxylation of amino acids (raises pH)
  - Passive regulation through buffers
  - Creation of biofilms and modified microenvironments

Acid Mine Drainage

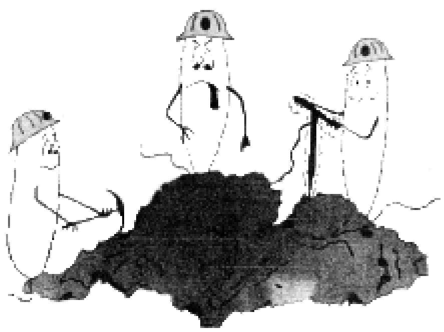
ubiquitous problem in areas where there has been a history of coal or hard rock mining.

oxidation of exposed sulfide minerals in mine tailings releases toxic heavy metal ions and acidic hydrogen into surface and ground waters.

Resulting water pollution problems are very difficult to clean up.

Acid mine drainage will go on for thousands of years once the chemical and microbial processes that create acid mine drainage are set into motion.

Thiobacillus ferrooxidans



This bacterium pollutes streams emanating from active and abandoned mining operations.

Abiotic oxidation of pyrite is slow.

T. ferrooxidans can oxidize pyrite much more quickly, producing ferric ions and hydrogen ions.

Electron micrograph of *T. ferrooxidans* cell suspension

T. ferrooxidans obtains its energy by the oxidation of either iron or sulfur according to the following reactions[(9)]:

$$Fe^{2+} + 0.25\ O_2 + H^+ \text{--->} Fe^{3+} + 0.5\ H_2O \quad (1)$$

$$H_2S + 2\ O_2 \text{--->} SO_4^{2-} + 2\ H^+ \quad (2)$$

$$S^o + H_2O + 1.5\ O_2 \text{--->} SO_4^{2-} + 2\ H^+ \quad (3)$$

$$S_2O_3^{2-} + H_2O + 2\ O_2 \text{--->} 2\ SO_4^{2-} + 2\ H^+ \quad (4)$$

under anaerobic conditions, ferric iron is an alternate e^- acceptor

In addition to Fe^{2+} and H_2S, may use Cu^+, Se^{2-}, H_2, formic acid, antimony compounds, uranium compounds, and molybdenum compounds as e^- donors

extremely versatile! Potential for cleanup