Lithotrophic Bacteria - Rock Eaters
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So far in catabolism we have spent our time looking at microbes that use organic compounds as their source of food (energy and building blocks for making cells). Interest in these species is understandable since our metabolism is very similar, but this ignores a large group of microbes that are capable of using inorganic substances as their source of energy. They are termed lithotrophs, literally meaning rock eaters.

General Concepts

- Common habitats of lithotrophs include waste water, volcanoes, deep sea ocean vents, the atmosphere, mines, seawater, fresh water. Basically they can be found everywhere.

- Energy is generated from reduced inorganic molecules. These molecules have high potential electrons that can be used to drive ETS. Typical energy substrates include, H2, CH4, CO, S, H2S, NH4, NO2, N2O, Fe+2 and Mn+2.

- Oxygen is often, though not always, the terminal electron accepter. Due to its willingness to accept electrons, oxygen gives the largest energy gain and is often employed by lithotrophs when available.

- Cell carbon is often from CO2 frequently using the Calvin Cycle. There are also microbes that can grow heterotrophically.

- Energy yields from lithotrophy are low per substrate oxidized and a large amount of substrate has to be metabolized per cell.

Their appetite for substrate makes lithotrophs important in the global cycling of elements that they attack. Lithotrophs have a major impact on the movement of nitrogen, sulfur and carbon though the biosphere.
To help the reader get a general idea of what these microbes are like, we look at two interesting lithotrophs. These are just two examples in a sea (pun intended) of many aquatic and terrestrial lithotrophs.

**Nitrifying Bacteria.**

Degradation of organic material typically results in the release of ammonia (NH₃) into the environment. and nitrifying bacteria are more than willing to make a living oxidizing ammonia. They are ubiquitous in the environment and have even been discovered living in the sandstone of the Cologne Cathedral in Germany - The bacteria penetrate to depths of 15 cm in the sandstone blocks. These bacteria generate energy by oxidizing reduced forms of nitrogen to NO₂ or NO₃. The concentration of nitrifiers depends upon the rate of NH₃ production in the surrounding environment. The faster the rate, the higher the population of microbes. We will briefly look at the biochemistry of two nitrifiers, *Nitrosomonas* and *Nitrobacter*

*Nitrosomonas* oxidizes ammonia in the following reaction...

\[ \text{NH}_3 + \frac{1}{2} \text{O}_2 \rightarrow \text{HNO}_2 + \text{H}_2\text{O} \]

Figure 1 - The reduction of ammonia by nitrifying bacteria

Extracted electrons are donated directly to an ETS and no carrier is involved. The result of running the ETS is that a proton gradient is formed which can then synthesize ATP using ATP synthase.
Figure 2 - Energy generation in *Nitrosomonas*. Only two enzymes, ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) are involved in the oxidation of ammonia to nitrite.

Nitrite can be further acted on by another nitrifying bacteria, *Nitrobacter*. This microbe oxidizes nitrite to nitrate using oxygen as the terminal electron accepter. A proton gradient is established with resultant synthesis of ATP. *Nitrobacter* is often found in tandem with *Nitrosomonas* since the end product of *Nitrosomonas* metabolism is the energy substrate for *Nitrobacter*. This type of loose association is probably common in the environment and in this case benefits both organisms. *Nitrobacter* is provided with substrate and *Nitrosomonas* has its end product removed, which helps drive its metabolism.

**Tube worms in the deep sea**

The deep ocean was once thought to be a lifeless place. Lacking sunlight, which can only penetrate the first few 100 meters of water, the
only living organisms were thought to be scavengers that feed off the organic material that trickled down from the ocean surface. In 1977 a geological deep sea expedition was begun to investigate areas of active volcanism deep beneath the ocean at depths of 1500 to 3700 meters. To the scientists great surprise a thriving community of clams, mussels, worms, shrimps and crabs was discovered surrounding these areas of active volcanism. This created a sensation in the scientific community. How did this community survive? What was the basis of the food chain? It couldn't be photosynthesis - everyone was intrigued.

The communities encircled areas where heated water was being ejected from "black smokers" so named because the liquid coming out of the vents was black due to rapid precipitation of black polymetal sulfides. These hydrothermal vents come about by sea water penetrating the ocean floor and passing over molten lava where it becomes superheated and loaded with reduced minerals. This hydrothermal fluid (350°C) then percolates up to the sea floor where it flows into the surrounding ocean (4°C) forming black smokers. (Remember that water at this depth is under great pressure and will not boil until it reaches temperatures of 450°C) The resulting temperature gradient and load of reduced minerals provides an ample source of energy for lithotrophs, mainly sulfur oxidizing bacteria, which are the primary producers in the deep sea food chain.

Some organisms in this biosphere feed on the microbial mats of bacteria that grow around the hydrothermal vents, but other organisms form symbiotic relationships with the primary producers.

One of the more interesting lithotrophs investigated is a bacterial species I will call the symbiont, reasons for which you will see in a minute (keep reading!).
Figure 3 - Tube worms in the deep sea. Click on the image to go to a 1.7 MB video of tubes worms produced by the NOAA/VENTS research group. You need to have the ability to play mpeg movies from your browser. One of the easiest ways to accomplish this is with the QuickTime plugin from Apple Computer.

This symbiont takes hydrogen sulfide, produced out of the black smokers and oxidizes it to sulfate using oxygen as the terminal electron accepter. The electrons extracted are used in an ETS to form a proton gradient which drives the synthesis of ATP. Cell carbon is obtained via the Calvin cycle. Its cell shape and coccoid morphology suggest it may be from the genus Thiovulum, but this association is tenuous at best. Analysis of the 16S ribosomal RNA will be required to correctly place it in the phylogenetic tree. The symbionts characteristics in itself are not unique, but where is makes its home is. It is contained in a tissue called the trophosome inside the tube worm Riftia pachytila.

A large tube worm is in a close symbiotic relationship with the symbiont. The worm provides a home for the microbe, giving it space to grow and providing both hydrogen sulfide and oxygen for the symbionts
metabolism. These gases are delivered via a circulatory system that contains specialized hemoglobins that are capable of binding oxygen and hydrogen sulfide. The CO2 content of the worms blood is also high providing a carbon source for the assimilation into cell material. In return the symbiont provides a portion of its production from the Calvin cycle to the tube worm.

![Electron transport system](image)

**Figure 4** - The electron transport system in sulfide oxidizing bacteria. The endosymbiont of *Riftia pachytila* probably has a similar system for energy generation. flavoprotein (FP), cytochrome b (cyt b), cytochrome c (cyt c).

It has not been possible to bring the endosymbiont into culture for in depth analysis and this has hindered research into its metabolism.

The existence of life on our planet that does not depend on the sun, but on terrestrial energy, has sparked the exciting possibility of life on other bodies in our solar system. Europa, a moon of Jupiter is a cold white sphere that may contain water several kilometers below its icy crust. If there is water, there may be life, at least microbial life. NASA's Galileo spacecraft has just completed its mission at Europa and soon scientist will have more information about the existence of liquid water on Europa.