**Soil microbiology**

**Lab. 12:**

**Isolation and culture of specialized groups of bacteria (Winogradsky column)**

The microbial populations living in a given habitat are a reflection of the environmental parameters of that habitat. The microorganisms that do not possess the physiological adaptations to survive in habitat cannot be members of the autochthonous.

However, in less extreme environments, where natural selection pressures are less severing, the diversity of microbial community makes it difficult to isolate and investigate the autochthonous microbial populations in a sample using standard plating and growth conditions. It is possible to create culture conditions that favor the growth of a particular microorganisms of interest. For example; *Sulfolobus* and *Chloroflexis* are found to dominate the communities of hot sulfur springs where other bacteria are killed by the ambient conditions. This is achieved by using enrichment culture techniques, which create a selective environment that encourages the most rapid growth rate of particular microorganisms.

**Enrichment for bacteria involved in the sulfur cycle using a Winogradsky column**

Sulfur is an essential element in biological material and is found in compounds such as vitamins, enzymes and other proteins, where it occurs as a constituents of the amino acids cysteine and methionine. The cyclical transformation of sulfur in the biosphere is carried out by diverse groups of microorganisms. Many of these microorganisms can be conveniently studied by establishing a small ecological system that simulate the sulfur cycle. This technique was first introduced by Sergei Winogradsky and so is called the **winogradsky column**.

This technique creates a model of ecosystem (microcosm) in the laboratory. The top of column is exposed to air; there is oxygen concentration gradient through the

column, reaching an obligate anaerobic zone. For the enrichment of microorganisms involved in the sulfur cycle, a tall glass cylinder is filled with mud or soil, sulfate, carbonate, phosphate and a source organic material (usually cellulose). Water from the mud is added to provide an additional source of inoculum. By controlling the level of illumination, the winogradsky column can be used to enrich for different microbial populations involved in sulfur transformation.

## Bacterial populations involved in various transformations of sulfur

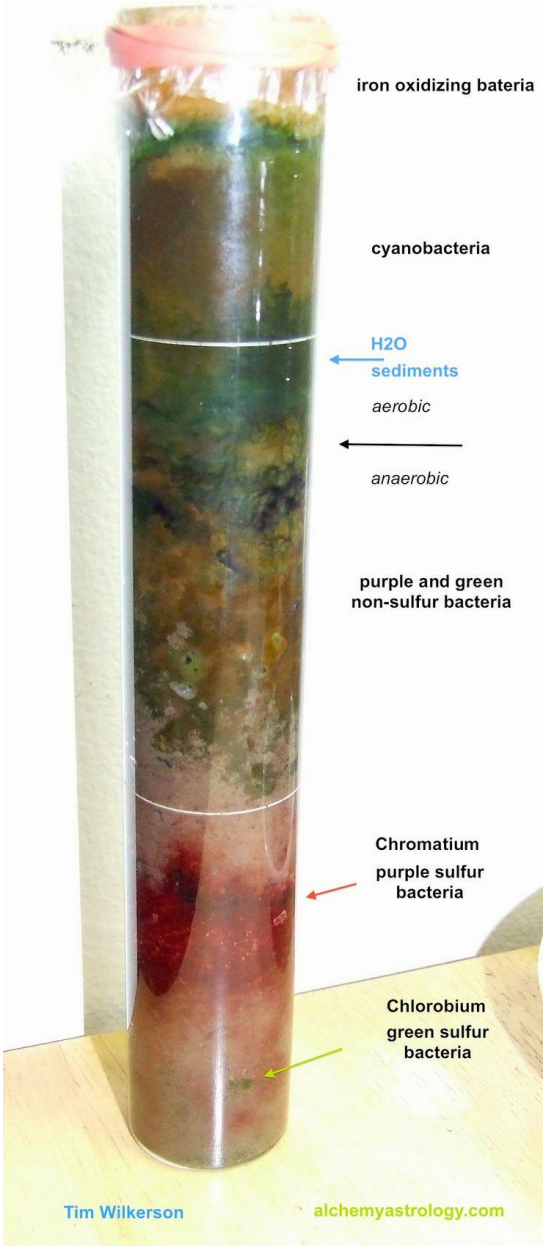
**Anaerobic**, sulfate-reducing bacteria, such as *Desulfovibrio*, occur in mud and sediment and **reduce** $SO_4^{-2}$ to $H_2S$ by **sulfate respiration**.

**Anaerobic**, purple-sulfur bacteria, such as *Chromatium*, **oxidize** $H_2S$ to $H_2SO_4$. These bacteria occur in the anaerobic (anoxic) regions of the soil column that are illuminated.

**Anaerobic**, green bacteria, such as *Chlorobuim*, **oxidize** sulfide. Thses populations also grow in the illuminated anoxic regions of the soil column. The green and purple sulfur bacteria oxidize $H_2S$ to element sulfur, which can accumulate as intracellular or extracellular globules.

**Aerobic**, sulfide-oxidizing bacteria, such as *Thiothrix* and *Beggiatoa*, are unique forms that occur at the surface of the soil and form filamentous trichomes.

**Sulfur-oxidizing bacteria**, such as *Thiobacillus*, convert sulfur or sulfide to $H_2SO_4$ and thiosulfate. They occur at the surface of the soil. Because *Thiobacillus* oxidizes sulfur to $H_2SO_4$, these bacteria can grow at very low pH values of about 1.0.



**Materials:**

Test tubes with caps, beakers, glass rods, watch glass, aluminum foil.

Cellulose powder, strips of filter paper or paper towels, calcium sulfate, calcium carbonate, dipotassium phosphate, freshly collected mud and water sample.

**Procedure**:

1. Prepare a slurry of cellulose powder or shredded filter paper.

2. Fill two large test tubes about one third full each with cellulose slurry.

3. Add 400 g of freshly collected mud with 4 g each of calcium sulfate, calcium carbonate and dipotassium phosphate to a beaker and mix. Pour this mixture into the test tubes containing the cellulose slurry until approximately two thirds to three fourth of each tube is filled.

4. Gently pack the columns with a glass rod. As the mud-slurry packs, you may find that you need to add small amounts of water. Make sure that any entrapped air bubbles are released. Allow the column to settle. Top off the column by adding pond water until the column is at about 80% capacity.

5. Cover the tubes with a watch glass to prevent evaporation and to allow evolved gases to escape.

6. Cover both tubes completely with aluminum foil to exclude light.

7. Incubate at room temperature. Note the appearance of the column when you first set it up, i.e., turbidity of the water and color of the mud. Periodically add distilled water to replenish the evaporation.

8. After two weeks' incubation, remove the aluminum foil from one of the columns. Observe the appearance of the column at this point. Decomposition of the organic materials (cellulose) and fermentation of the carbohydrates to organic acids by various anaerobic bacteria result in the growth of *Desulfovibrio*. This bacterium grows by anaerobic respiration, reducing sulfate to sulfide, which precipitates as metal sulfides and blackening of the mud in the column.

9. Incubate the uncovered column in the light. This promotes the rapid development of the photosynthetic bacteria. Continue incubating the second column covered with foil.

10. Examine the column periodically, e.g., at each laboratory period. Look for the development of green and red-purple areas in the blackened areas of the mud. These are blooms of the various photosynthetic bacteria. Also note changes in the turbidity and/or color of the water above the mud where the photosynthetic will also grow. Note uncovered columns.