Winogradsky Column – Demonstration of Succession

Introduction

A Winogradsky column is used to illustrate how microorganisms develop in certain habitats in response to appropriate environmental conditions and how populations exhibit succession (i.e., how one group of organisms succeeds another as conditions are created which select for them). These columns are complete, self-contained recycling systems, driven only by energy from light!

Invented by Sergei Winogradsky, this method uses a deep cylindrical vessel containing soil, roots, leaves, other organic matter, and water. The column incubates under natural light or a growth lamp for several months with observations being made on a weekly basis.

Because this is a closed system (i.e. the cylinder/container is capped), it helps to illustrate the diversity of organisms present in the soil. Many of these organisms are not present in high concentrations because conditions are not right for rapid growth. Over time in this self-contained recycling system, different conditions are created which favor different groups of organisms, as described below. When conditions are unfavorable for certain organisms, these organisms will go into protected resting stages.

All the organisms are present initially in low numbers, but when the columns are incubated for 2 to 3 months, the different types of microorganisms proliferate and occupy distinct zones where the environmental conditions favor their specific activities. At first, a population of facultative heterotrophs uses the plant materials, carbohydrates, lignins, etc., for growth and in so doing creates an anaerobic environment especially at the bottom. Under these anaerobic conditions, sulfate-reducing organisms multiply using organic carbon compounds and at the same time reducing sulfate to reduce sulfur compounds such as H_2S. Such an environment now allows the development of photosynthetic bacteria requiring reduced sulfur compounds, anaerobiosis and light.

Consequently, one finds the purple sulfur bacteria and the green sulfur bacteria growing deep in the vessel while at the same time it is possible to find blue-green algae growing in the surface water since they are not strict anaerobes. The photosynthetic organisms (i.e. algae, bacteria and cyanobacteria) tend to cluster at different levels since they are motile and can swim where concentrations of nutrients and degree of illumination are most appropriate for their growth - an example of both chemotactic and phototactic response.

The column provides numerous gradients, depending on additive nutrients – types and amounts of organic matter or addition of synthetic nutrients – from which the variety of organisms can grow. The aerobic water phase and anaerobic soil phase are one such distinction. Due to low oxygen solubility in water, the water quickly becomes anoxic towards the interface of the soil and water. Anaerobic phototrophs are still present to a large extent in the soil phase; there is still capacity for biofilm creation and colony expansion. Algae and other aerobic phototrophs are present along the surface and water of the upper half of the columns. Green growth is often attributed to these organisms.

Materials

- Soil samples
- Bottles, jars or tubes with lids
- Rain or tap water
- Calcium carbonate, calcium sulfate or sodium sulfate, optional
- Shredded newspaper, hay, ground eggshell or egg yolk, optional

Method

1. Fill a clear glass or plastic cylinder or a bottle about 2/3 full with soil from different sites or adding differing amounts of residue.
2. The sample may be supplemented with ~0.25% w/w calcium carbonate and ~0.50% w/w calcium sulfate or sodium sulfate, shredded newspaper or hay (for cellulose), ground eggshell and/or egg yolk.
3. Rain or tap water is added until the soil is saturated and about half the remaining volume is filled.
4. Cap or seal the container and incubate for several months in natural light or under a growth lamp.

Observations

1. Observe growth of organisms and formation of biofilms. See Fig. 1.
2. Record the formation of layers and changes in color.
3. Take a sample from different layers and observe under the microscope. Record microscopic observations – organism type, shape, size, number, or other distinguishing characteristics.

Figure 1. Winogradsky columns made in either clear cylinders (A) or bottles (B) exhibit changes in color as different organisms grow due to changes in the oxygen concentration or carbon sources.

What is happening

Initially, the entire system is aerobic. As resources are consumed and the oxygen levels change throughout the column and over time, the following sequence of events occurs (Fig. 2):

1. The large amount of cellulose present initially promotes rapid microbial growth which soon depletes the oxygen in the sediment and in the water column. Only the very top of the column remains aerated because oxygen diffuses very slowly through water.

   organic matter + O_2 = organic acids + CO_2

2. Further down in the column, the only organisms that can grow in anaerobic conditions are those that ferment organic matter and those that perform anaerobic respiration. Fermentation is a process in which organic compounds are degraded incompletely; for example, yeasts ferment sugars to alcohol. Anaerobic respiration is a process in which organic substrates are degraded completely to CO_2, but using a substance other than oxygen as the terminal electron acceptor. Some bacteria respire by using nitrate or sulfate ions, in the same way as we use oxygen as the terminal electron acceptor during respiration. The organic acids act as electron donors for reduction of sulfates and sulfites to hydrogen sulfide (H_2S) by anaerobic sulfate reducing bacteria. The H_2S will react with any iron in the soil, producing black ferrous sulfide. However, some of the H_2S diffuses upwards into the water column, where it is utilized by other organisms.

   organic acids + $S0_4$ = H_2S + CO_2

3. The diffusion of H_2S from the sediment into the water column enables anaerobic photosynthetic bacteria to grow. They are seen usually as two narrow, brightly colored bands immediately above the soil - a zone of green sulfur bacteria then a zone of purple sulfur bacteria (Fig. 1). The green and purple sulfur bacteria gain energy from light reactions and produce their cellular materials from CO_2 in much the same way as plants do. However, there is one essential difference: they do not generate oxygen during photosynthesis because they do not use water as the reductant; instead they use H_2S. The following simplified equations show the parallel:

   $6\ CO_2 + 6\ H_20 = C_6H_{12}O_6 + 6\ O_2$ (plant photosynthesis)

   $6\ CO_2 + 6\ H_2S = C_6H_{12}O_6 + 6\ S$ (bacterial anaerobic photosynthesis)

4. Most of the water column above the photosynthetic bacteria is colored bright red by a large population of purple non-sulfur bacteria. These bacteria grow in anaerobic conditions, gaining their energy from light reactions but using organic acids as their carbon source for cellular synthesis and are called photoheterotrophs. The organic acids that they use are the fermentation products of other anaerobic bacteria, but the 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.

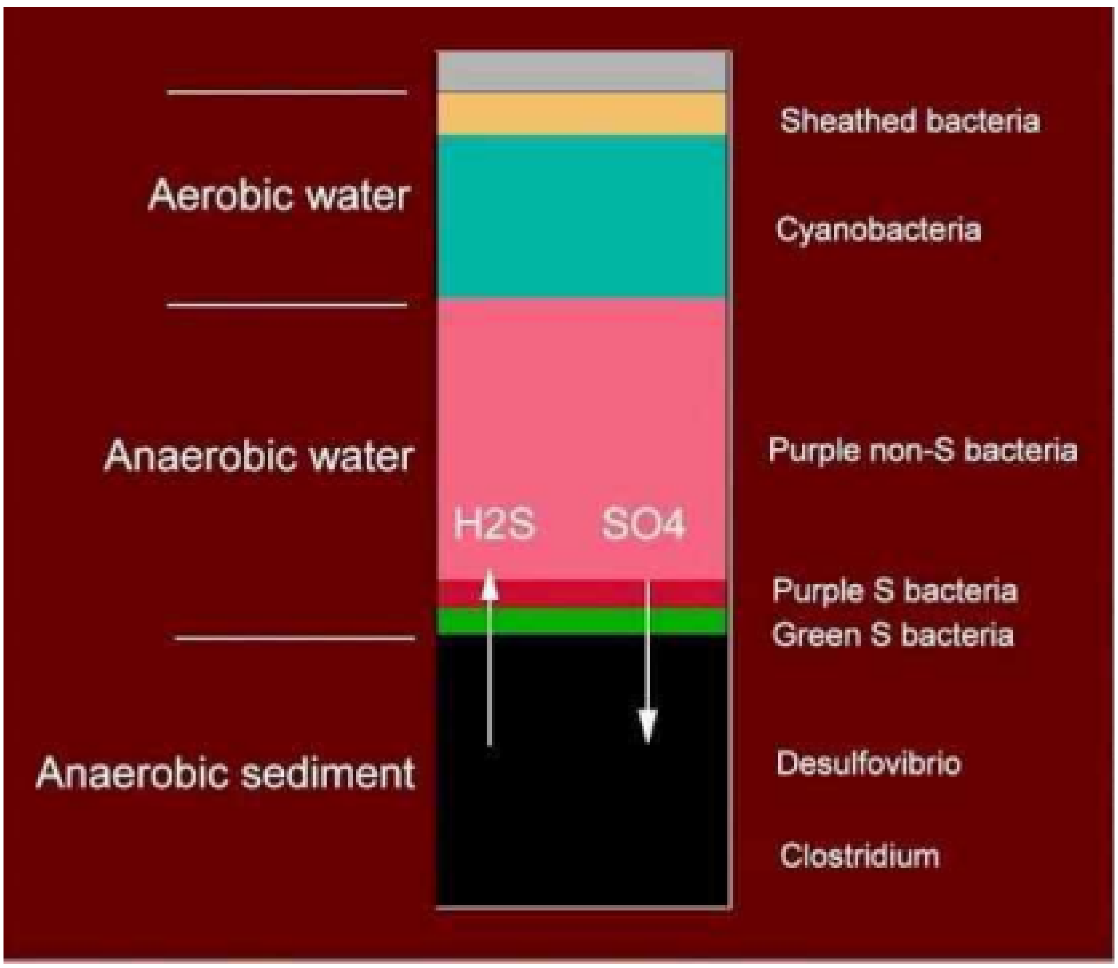

Figure 2. In a Winogradsky column, microbial habitats change as oxygen and carbon sources change. These changes are exhibited in the formation of different colored layers as illustrated above.