Period 1 (Jan. 22)

A. Instruction in use of the laboratory (Dierdra Daniels, Kendra Custard)
B. Preparation of Winogradsky columns. (See attachments from "Brock" and Nester")

Background: Winogradsky columns are self-contained, anaerobic ecosystems that we will make from Bay Mud. Some will be made without and some with supplements.
Background: At the turn of the 20th century, Sergei Winogradsky pioneered studies of microbial autotrophy (lithotrophy), the ability of various bacteria and archaea to use CO_2 as their primary carbon source. He discovered chemoautotrophy, the capacity of some autotrophic organisms to use chemical energy as their primary energy source. Winogradsky columns are kept moist and are illuminated to encourage the growth of photosynthetic bacteria.

Materials:
1. Bay mud (high in sulfide content)
2. Bay water
3. 1 liter graduated cylinders (glass, non-sterile)
4. Petri dish lids with which to cover graduated cylinders
5. cellulose (carbon source), 2 g (non-sterile)
6. calcium carbonate (carbon source and buffer), 2 g non-sterile
7. 1 scoop/pair for transferring mud into cylinders
8. Test tubes and test tube racks for taking samples

Methods:
1. Label the base of your graduated cylinder with your name and the date. If you will add supplements (every other group), note that on the base of the cylinder.
2. For those adding supplements (every other group), add to the bottom of your cylinder: approximately 2 g (2 spoonsful) each of cellulose and calcium carbonate. You may vary this if you wish.
3. Add mud to fill your graduated cylinder between 1/2 and 3/4 full. Try not to trap large air pockets in the mud.
4. Add water to the top of the mud to keep it moist. Clean the outside of your column and cover it.
5. Incubate your column in a window or lighted room so that light energy is available for the growth of photosynthetic bacteria. IS THERE AN INTERESTING CONTROL TO BE DONE IN THIS REGARD?
6. As your column dries out, add fresh distilled water.

Analysis:
1. Make weekly observations/drawings of the appearance of your column. Keep these in a separate section of your lab notebook, so that you have a continuous log. You may turn your log in at the end of the semester for extra credit.
2. Periodically, we will examine the organisms in these columns microscopically.

Perspective 31.2

Winogradsky Column

In order to isolate bacteria that might be found in sediment samples from a lake, a simple device called a **Winogradsky column** can be set up. This technique was named for Sergei Winogradsky, a Russian microbiologist, who in addition to his studies on nitrogen fixation also studied sulfur bacteria in the 1890s.

To prepare a Winogradsky column, a large glass cylinder is filled about one-third full with an organically rich mud. Some organic material such as hay, sawdust, shredded leaves, or any other carbon-rich material is then added to the mud along with a buffer such as $CaSO_3$ and $CaSO_4$. These ingredients are mixed together and packed tightly in the glass cylinder. A small amount of unadulterated mud is added on top and then the rest of the cylinder is filled with pond water. The column is placed in a north facing window where it gets plenty of light but not direct sunlight. It is then left undisturbed for several weeks (figure 1).

Typically, algae and cyanobacteria quickly appear on the top of the water column. They produce oxygen and thus keep the area aerobic. Sulfur-reducing bacteria appear in the mud layers. As a result of the production of sulfide, photosynthetic purple sulfur bacteria and green sulfur bacteria show up as purple and green patches that can be observed in the mud layers. The different layers of the column can be sampled by inserting a long, thin pipette, removing some mud or water, and inoculating it on an enrichment media.

A Winogradsky column is useful because it is possible to isolate a particular bacterium merely by adding the compound that is being studied and then selecting the organisms that will degrade it. Winogradsky columns are closer to the natural environment than are other culturing techniques and thus scientists are able to observe a larger variety of bacteria.

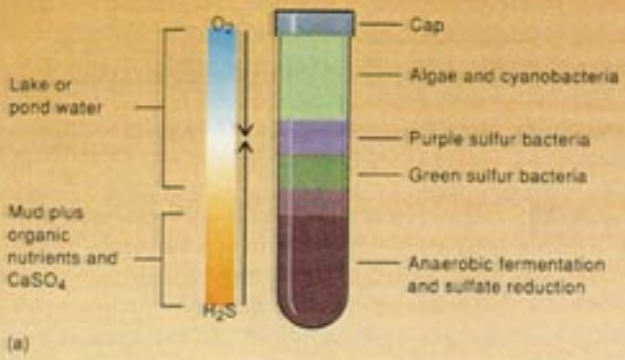


Figure 1

(*a*) Microorganisms and nutrients interact within the vertical gradient set up in the column. A typical column is exposed to light. Different microorganisms grow in specific regions of the column. (*b*) Photo of Winogradsky columns.

Nester, E. W., Roberts, C. E., and Nester, M. T. (1995). Microbiology: A Human Perspective, p. 696. Wm. C. Brown Publishers, Dubuque, IA.

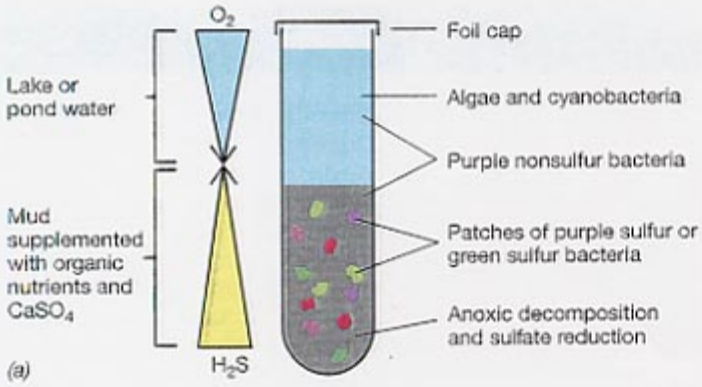
The Winogradsky Column

For isolation of purple and green phototrophic bacteria and other anaerobes, the **Winogradsky column** has traditionally been used. Named for the famous Russian microbiologist Sergei Winogradsky (☞ the box, Winogradsky's Legacy, Chapter 15 and Section 1.9), the column was devised by him in the 1880s to study soil microorganisms. The column is a miniature anaerobic ecosystem that can be an excellent long-term source of all types of prokaryotes involved in nutrient cycling.

A Winogradsky column can be prepared by filling a large glass cylinder about one-third full with organic-rich, preferably sulfide-containing, mud (Figure 16.5). Carbon substrates are first mixed into the mud; organic additions that have been successfully used in the past include hay, shredded newsprint, sawdust, shredded leaves or roots, ground meat, hard boiled eggs, and even dead animals! The mud is also supplemented with $CaCO_3$ and $CaSO_4$ as a buffer and as a source of sulfate, respectively. The mud is packed tightly in the container, care being taken to avoid entrapping air (Figure 16.5). The mud is then covered with lake, pond, or ditch water, and the top of the cylinder covered with aluminum foil. The cylinder is placed in a north window so as to receive adequate (but not excessive) sunlight and left to develop for a period of weeks (in the Southern Hemisphere use a south window).

In a typical Winogradsky column a mixture of many different types of organisms develops. Algae and cyanobacteria appear quickly in the upper portions of the water column and by producing O_2 help to keep this zone oxic. Fermentative decomposition processes in the mud quickly lead to the production of organic acids, alcohols, and H_2, suitable substrates for sulfate-reducing bacteria. As a result of the production of sulfide, purple and green patches appear on the outer layers of the mud exposed to light, the purple patches, consisting of purple sulfur bacteria, frequently developing in the upper layers, and the green patches, consisting of green sulfur bacteria, in the lower layers nearest the source of sulfide (this occurs because of differences in sulfide tolerance between green and purple bacteria). At the mud–water interface the water is frequently quite turbid and may be colored as a result of the growth of purple sulfur and purple nonsulfur bacteria (Figure 16.5). Sampling of the column for phototrophic bacteria is performed by inserting a long, thin pipet into the column and removing some colored mud or water. These can be used to inoculate enrichment media (Table 16.1).

Winogradsky columns have been used to enrich for a variety of prokaryotes, both aerobes and anaerobes. The great advantage of a column, besides the ready availability of inocula for different enrichment cultures, is that it can be spiked with a particular compound whose degradation one wishes to study and then allowed to select from the inoculum for an organism or organisms that can degrade it. In addition, because the Winogradsky column more nearly resembles the natural environment than do culture media, a variety of each physiological type of microorganism is more frequently observed in Winogradsky columns than in enrichments where a liquid culture medium is inoculated directly with a natural sample. In the latter approach, rapidly growing species frequently rise to the forefront, leaving the slower growing species behind.



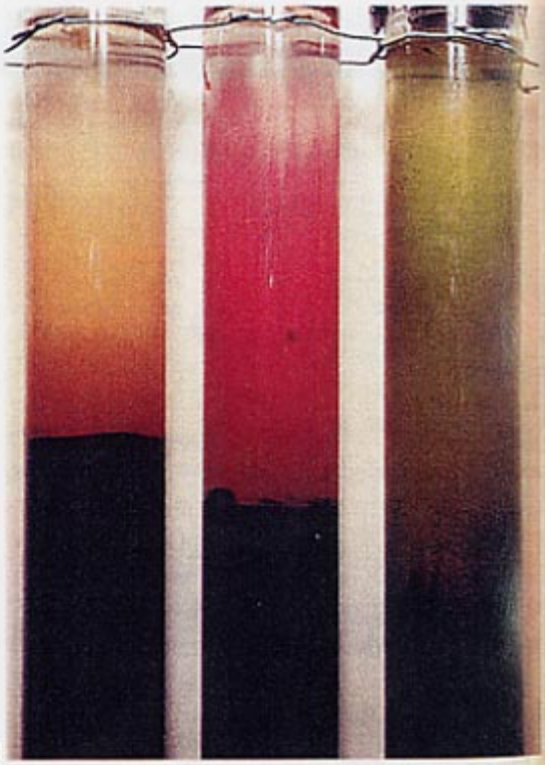


FIGURE 16.5 The Winogradsky column. (a) Schematic view of a typical column. The column is placed to receive subdued sunlight. Chemoorganotrophic bacteria grow throughout the column, aerobes and microaerophiles in the upper regions, anaerobes in the zones containing H_2S. Anoxic decomposition leading to sulfate reduction creates the gradient of H_2S. Green and purple sulfur bacteria stratify according to their tolerance for H_2S. (b) Photo of Winogradsky columns that have remained anoxic up to the top where blooms of three different phototrophic bacteria have occurred in the mud and up into the water column. Left to right: *Thiospirillum jenense*, *Chromatium okenii*, and *Chlorobium limicola*.