The **Winogradsky Column** is a simple device for culturing a large diversity of [microorganisms](http://en.wikipedia.org/wiki/Microorganism). Invented by [Sergei Winogradsky](http://en.wikipedia.org/wiki/Sergei_Winogradsky), the device is a column of pond mud and water mixed with a carbon source such as newspaper (containing [cellulose](http://en.wikipedia.org/wiki/Cellulose)) or egg-shells (containing [calcium carbonate](http://en.wikipedia.org/wiki/Calcium_carbonate)) and a [sulfur](http://en.wikipedia.org/wiki/Sulfur) source such as gypsum ([calcium sulfate](http://en.wikipedia.org/wiki/Calcium_sulfate)) or egg-yolk. Incubating the column in sunlight for months results in an [aerobic](http://en.wikipedia.org/wiki/Aerobic)/[anaerobic](http://en.wikipedia.org/wiki/Hypoxia_(environmental)) gradient as well as a sulfide gradient. These two gradients promote the growth of different micro-organisms such as [clostridium](http://en.wikipedia.org/wiki/Clostridium), [desulfovibrio](http://en.wikipedia.org/wiki/Desulfovibrio), [chlorobium](http://en.wikipedia.org/wiki/Chlorobium), [chromatium](http://en.wikipedia.org/wiki/Chromatium), [rhodomicrobium](http://en.wikipedia.org/w/index.php?title=Rhodomicrobium&action=edit&redlink=1), [beggiatoa](http://en.wikipedia.org/wiki/Beggiatoa), as well as many other species of bacteria, cyanobacteria, and algae.

The column provides numerous gradients, depending on additive nutrients, from which the variety of aforementioned organisms can grow. The aerobic water phase and anaerobic mud or soil phase are one such distinction. Due to low oxygen solubility in water the water quickly becomes anoxic towards the interphase of the mud and water. Anaerobic phototrophs are still present to a large extent in the mud 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.

**http://en.wikipedia.org/wiki/Winogradsky\_column**

<http://www.woodrow.org/teachers/bi/2000/Winogradsky_Column/winogradsky_column.html>

**Methodology**Building the Basic Column

* A glass or plastic container minimally15 cm in height and 5 cm in diameter is ideal but any size container will do.  Plastic bottles are ideal because they can be manipulated easily to allow for extraction of species of culturing. If possible, the container’s sides should also be smooth to make observing the bacterial growth easier.  As well, very tall and wide containers take longer to grow and are more difficult from which  to extract bacteria.
* Clear plastic film and a rubber band
* A long wooden dowel
* Cellulose source--Shredded paper, grass, leaves, lettuce, sawdust or wood chips
* Sulfur source--calcium sulfate, magnesium sulfate, egg yolk.  This should be added to be about 1-2% approximately of the weight.
* Carbon dioxide source(optional)--calcium carbonate – 1-2%.  Both of the previous sources can be approximate
* A selection of soils--pond mud, river mud, saturated soil
* Water from the source of the mud

**Procedure**

1. Check the assigned soil sample for clumps that will prevent packing. Break up the soil if necessary.  It can be stirred up to gain a uniform consistency.
2. The amount of mud you add will depend on the size of the container.  As well, it’s important to keep the type of mud consistent with the water (fresh with fresh, marine with marine, etc.)
3. Mix the sulfur source and carbon dioxide source (optional) in with the mud.  They should be 1 to 2% of the final mass.
4. Then mix in an equal volume of cellulose. The cellulose should be near the bottom but it can be in the middle if a variation is desired.  However, it can’t be near the top since the bacteria involved are anaerobic.
5. Add the mud and pack this material into layers of 2-3 cm.  Use the dowel or something equivalent to tamp down the mud to force out trapped air.  Your last layer (water) should be about 5 cm from the top.
6. Cover the top with plastic film and secure with a rubber band.   Place the column next to a low heat/high intensity light source probably less than 60 watts.  It is important that it doesn’t over heat.
7. Examine the columns weekly for at least a month, recording changes in color and depth as they occur.
8. Sampling can occur at weekly intervals to check succession or can be done at the end of the month to see the final flora of bacteria that develops.

**Variations**If a freshwater model as described above is used, this is the standard Winogradsky Column.  However, with just a few changes, some different columns can be created to compare for growth rates, etc.

1. sodium sulfide can be added in place of a sulfate.  This may inhibit growth of the sulfur reducing bacteria bringing about different species of bacteria.
2. Changing the pH may effect which species grow. Many of the standard sulfur reducers are comfortable in a pH of 6-8 (Brock 2000)  Creating a more acidic or alkalinic environment may change the species diversity as well.
3. Enriching for Extremophiles.  There are three basic types that may be created:
   * Thermophiles – these species are very tolerant of high heat and if you put the column in front of a light source that creates more heat, thermophiles may be developed.
   * Acidophiles – will develop when the pH is significantly acidic.
   * Halophiles – If you opt to create a marine environment, Halophilic bacteria will predominate. The same can occur if your soil is inoculated with a 10% NaCl solution.  Pickles or Sauerkraut in the substrate would work as well (Levandowsky, oral communication)

**General Biology of the Column**After a month to six weeks, the column should stabilize into three distinct environments and develop communities of bacteria specific to their environmental requirements and should resemble Figure 1.

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| http://www.woodrow.org/teachers/bi/2000/Winogradsky_Column/winogradskyp6.gif |

**Aerobic Zone (Oxygen Rich)**The top of the water column can contain large populations of diverse bacteria.  These are aerobic organisms that are found in organic-rich freshwater habitats such as shallow ponds, polluted streams, etc.  These are generally flagellated which allows the bacteria to migrate and establish themselves in new areas.   In addition, there may be a diverse phototrophic fauna as well from the original water and mud source. At the very top of the zone the mud is characterized by a light brown color.  This is the most oxygen  rich part of the mud and the most sulfur poor.

Photosynthetic **cyanobacteria** can grow in the upper zones. This area is characterized by a Grass green color. These are the only bacteria that have photosynthesis like that of plants. In fact, there is very strong evidence that the chloroplasts of plants were originally ancestral cyanobacteria that established themselves as symbionts inside the cells of a primitive eukaryote. Similarly, there is equally strong evidence that the mitochondria of present-day eukaryotes were derived from purple bacteria.

From the mud source below, H2S  will diffuse upward into the aerobic zone and can be oxidized to sulfate by the sulfur-oxidizing bacteria such as Beggiatoa and Thiobacillus. These bacteria gain energy from oxidation of H2S, to elemental sulfur and they synthesize their own organic matter from CO2. So they are termed **chemoautotrophs**.