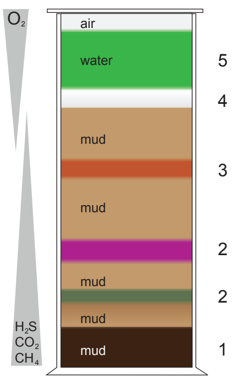
MiSACactivities 3

PRODUCED BY THE **MICROBIOLOGY IN SCHOOLS ADVISORY COMMITTEE**

The wonderful Winogradsky Column



The Winogradsky Column illustrates the wide diversity of different types of physiological activity that is found in the microbial world. It was devised in the 19th century by the Russian soil microbiologist Sergei Winogradsky (1856-1953) who was a pioneer in research on bacteria of the nitrogen and sulfur cycles.

A range of different ecological conditions is established by allowing vertical gradients of hydrogen sulfide and oxygen concentrations to develop through the length of a column. Each condition promotes the development of a different physiological group of microbe, the growth of which can be seen by the naked eye and is distinguished by its characteristic colour (see diagram).

The many references to the Winogradsky Column on the internet include diagrams, photographs and animated tutorials, e.g. www.sumansinc.com/webcontent/animations/content/winogradsky.html. It is interesting to note that some of these are educational materials produced by the US National Aeronautics and Space Administration (NASA) Astrobiology Institute.

Procedure

The procedure described here is derived from several published procedures that differ in detail but all follow the same principles. Dimensions, weights and volumes are given as guidelines only.

COMPONENT	PROVISION	SPECIFICATION
Vessel	Tall transparent container, e.g. glass or clear plastic measuring cylinder, gas jar, clear plastic drinks bottle	Transparency and 15-20 cm minimum height of vessel required; 0.5 litre measuring cylinder is ideal
Inoculum	Mud from pond or lake and overlying water	Mud:water:head space, 5:1:1 by volume
Organic carbon	Filter paper or newspaper	2 circles of 7 cm Whatman No 1 filter paper, or equivalent, cut into about 0.5 cm wide strips
Sulfur	Calcium sulfate ($CaSO_4$)	Calcium sulfate, 1% (w/v) total mud
Carbon dioxide (CO_2)	Microbial action; calcium carbonate ($CaCO_3$) supplement (optional)	Calcium carbonate, 1% (w/v) total mud
Oxygen (O_2)	Microbial action, diffusion	Reduced at depths by consumption by aerobic microbes; increased at top by diffusion from head space
Evaporation control	Cover top of vessel	Lid or base of Petri dish, plastic film, metal foil, greaseproof paper or similar
Incubation	Room temperature on a window sill, not in direct sunlight; supplementary illumination by artificial light optional	Low intensity lamp (40-60 w), about 500 cm away so as not to heat the column
Observation	Growth is visible to the naked eye as coloured bands or patches	Examine weekly for a month or more; can then be maintained as a permanent museum specimen

Add filter paper/newspaper, calcium sulfate and (optional) calcium carbonate to a sufficient amount of mud in a bowl to make a thick slurry and mix well. Add the slurry to the transparent vessel and compact the mixture to remove pockets of air. Gently add the remainder of the mud and then the overlying water, taking care to disturb the mud layer as little as possible. Cover the top of the vessel and place it in an illuminated position. Add water from time to time to replace any lost by evaporation.

The Winogradsky Column: theory

The sequence of events begins with the carbon cycle in the anaerobic conditions of the mud in the depths of the column where **chemotrophic*** bacteria, e.g. the strictly anaerobic bacterium *Clostridium*, gain energy from anaerobic fermentation of the cellulosic material (filter paper or newspaper). Products of fermentation include alcohols, organic acids, carbon dioxide (CO_2) and methane (CH_4). Some are used as electron donors in the sulfur cycle by another chemotroph (*Desulfovibrio*) in the reduction of sulfate (electron acceptor) to sulfide (H_2S) which reacts with iron in the mud, producing black patches of iron sulfide, see diagram (**1**).

Coloured **phototrophic*** (photosynthetic) sulfur-metabolising bacteria develop higher up the column using H_2S as electron donor, CO_2 from anaerobic fermentations as their main source of carbon, and energy from light. Microbes for which CO_2 is the main source of carbon are known as **autotrophs***. These anaerobic photosynthetic microbes are the green and purple sulfur bacteria, i.e. respectively *Chlorobium* and *Chromatium*, the former being the more tolerant of high concentrations of H_2S (**2**).

Further up the column, other types of anaerobic phototrophic bacteria develop by using organic products of fermentation as principal source of carbon and electron donors, i.e. they are **photoheterotrophs***. They produce a rust-coloured zone and are described as purple non-sulfur bacteria, e.g. *Rhodopseudomonas* and *Rhodospirillum*. Although being anaerobic, they can tolerate small concentrations of oxygen which may diffuse from above. Microbes that have this type of relationship with oxygen are known as 'microaerophiles' (**3**).

In the top layers of mud there are good supplies of dissolved oxygen from the air and, from microbial action, carbon dioxide and small residual amounts of hydrogen sulfide. In these conditions, there is growth of **chemoautotrophic*** colourless sulfur bacteria, e.g. *Thiobacillus* and *Beggiatoa*, which oxidise sulfide to sulfuric acid (!) and sulfur; the latter substance gives a white colour to the wispy growth in the water immediately above the mud (**4**).

Finally, aerobic photosynthesis occurs in the overlying water which becomes green from the growth of algae and cyanobacteria (**photoautotrophs***) that flourish in the good supplies of dissolved oxygen and carbon dioxide from the air, the latter enriched by microbial action from below (**5**).

***Nutritional categories**

The diversity of the different types of physiological activity that is found among microbes is illustrated by considering their various types of nutrition. A simple approach takes into account the nature of two major requirements, i.e. the source of energy and the principal source of carbon.

REQUIREMENT	SOURCE	TYPE
Source of energy	Light	Phototroph
Principal source of carbon	Organic or inorganic compounds	Chemotroph
	Carbon dioxide	Autotroph

The following 4 nutritional categories are obtained by combining these two major requirements and their sources:

- **Photoautotrophs**, e.g. green and purple sulfur bacteria, algae;
- **Photoheterotrophs**, e.g. purple non-sulfur bacteria;
- **Chemoautotrophs**, e.g. colourless sulfur bacteria, nitrifying bacteria in the nitrogen cycle;
- **Chemoheterotrophs**, e.g. protozoa, fungi, most other bacteria.

HEALTH & SAFETY NOTES

1. The use of anaerobic conditions is admissible here because: (a) the system remains closed throughout; (b) the investigation is of a natural fermentation; (c) the conditions are not conducive to the proliferation of pathogenic microbes, i.e. the absence of a rich source of organic nutrients and incubation at room temperature.
2. Disposal: contents can be poured onto a garden. Wear eye protection and gloves. The contents have a putrid smell.

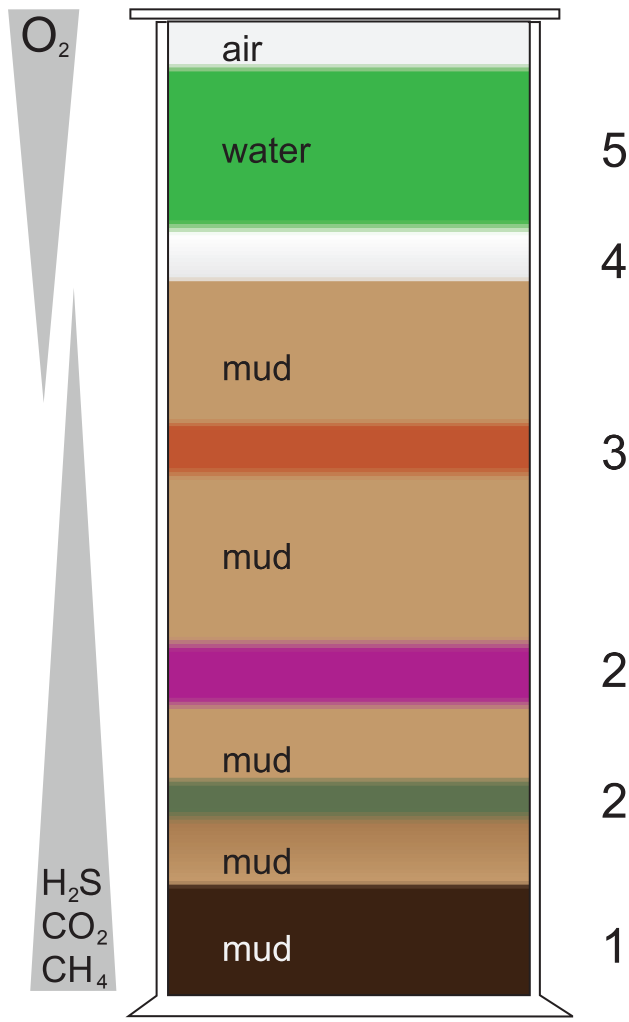


Diagram of a Winogradsky Column
Note: in practice the layers are not as clearly defined as in the diagram.