Microbial Ecology

Overview: The Microbe City, or Winogradsky column, can then be observed from as little as a month to longer than a year and is easy to assemble. The purpose of creating this column is to observe the growth and development of microbes in their natural environment. When building a Winogradsky column, students can learn essential concepts related to the role of microbes in the ocean. These concepts can be taught as separate units while the Winogradsky column develops or introduced prior to building the Microbe City. Direct observations of the community will reinforce these basic ecological concepts. Different results can be seen by changing the location of the column (exposure to light etc.), origin of sand/water, and by using different sizes of Microbe Cities.

Background: Microscopic organisms (or microbes) are the most abundant life forms on Earth. Microbial oceanography is the study of the abundance, distribution, growth, metabolism, and diversity of microbes in the marine environment, from coastal regions to the open ocean. Understanding the roles that microbes play in the cycling of nutrients in the ocean is a key component of microbial oceanography. The lessons outlined below are designed to help students understand the importance that microbes play in the environment while also reinforcing ecological concepts.

What is the Winogradsky Column? Sergei Winogradsky, a Russian scientist, studied sediment microbial communities in the 1880's. He constructed a simple device to do this, which we now refer to as the Winogradsky column. These columns allow us to "see" microbes without using a microscope, creating what we call the Microbe City!

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Images and narrative courtesy of the C-MORE Education Office: http://cmore.soest.hawaii.edu/, and from http://www.brooklyn.cuny.edu/bc/ahp/CellBio/Growth/MGecol.html and http://serc.carleton.edu/microbelife/index.html

Building a Microbe City

Materials needed: House, tenants, accessories, food

House: The basic building block for the house needs to be a clear plastic container sealed on the bottom but open on the top that can stand freely (Figure A and B). A larger unit may be easier for students to observe changes through time as gradients develop. This may include having one large classroom unit and students having their own small personal cities.

- 2 clear 1-liter plastic bottles.
- A fluorescent light housing tube is a cheap option for making a large city. Microbe layers are easier to see in taller cities, but it doesn't need to be wide. Build a solid base such as a wooden board or a small dolly on wheels if you'll need to move it, remember that wet sand/mud is very heavy! Adjust the ingredients listed as needed to fill a larger space.

Accessories: Sand or Mud. Microbes thrive everywhere, but we need a place where we can see them (clear container) and distinguish where they live (pent house or basement). Any color sand or mud, regardless of how "clean" you think it is will be a good medium to fill your house.

- 1 small bucket filled with sand **and** water from the same source, enough to fill your house.

Tenants: Already there! No need to advertise; by using sand or mud found in the "wild", you've already included many different types of local organisms to live and multiply.

Food: Although the microbes are already living in the sand or mud, we need some food to support the growth of a variety of microbes. Make sure you mix up all the food listed below throughout the sand or mud before filling the house.

- 1 sheet of newspaper (as a source of carbon)
- 1 tablespoon powdered chalk (as a source of carbon)
- 1 hard-boiled egg (as a source of sulfur)

Building Supplies:

- 1 paint stirrer
- Plastic wrap and rubber band

What to do now? Follow the instructions included on the Student Handout to build the column and watch your column grow as long as you desire. To dispose of your Microbe City, pour out the water and dump the wet sand on the ground or a large garbage bag to dry out.

Figure A. Two sample Microbe Cities.

Summary

Once you fill the column, it will take some time for the distinct communities to develop. The development of different colors, smells, and layers represent the growth of different microbes. The following pages give you some background concepts that can be taught while these communities develop. These are just suggested topics and are provided only as a guide. There are many other sites that can offer more direct information if there is something that interests you, especially in regards to chemical formulas and biogeochemical cycles. Please see the Appendix websites below for ideas.

Essentially, we would like students to understand a few key concepts:

1. Microbes are everywhere (and there's lots of them) and they're not all bad.
2. The different colors you see are different types of microbes.
3. The zones are created because there is a gradient of oxygen and sulfur in the column, creating unique habitats especially designed for certain types of microbes. In fact, the microbes themselves create these different habitats just by living and reproducing.

Appendix

Examples of websites that demonstrate or have instructions on Winogradsky columns: these sites may answer some of the questions that students have for you.

1. NASA WebQuest: Complete educators guide to building a Winogradsky column and integrating it into your curriculum, including national standards and handouts. http://quest.nasa.gov/projects/astrobiology/fieldwork/lessons/Winogradsky_5_8.pdf
2. http://www.woodrow.org/teachers/bi/2000/Winogradsky_Column/winogradsky_column.html
3. http://www.brooklyn.cuny.edu/bc/ahp/CellBio/Growth/MGecol.html
4. http://serc.carleton.edu/microbelife/index.html
5. http://www.woodrow.org/teachers/esi/2002/Biology/Projects/p5/wc.htm
6. http://dwb4.unl.edu/Chem/CHEM869P/CHEM869PLinks/helios.bto.ed.ac.uk/bto/microbes/winograd.htm

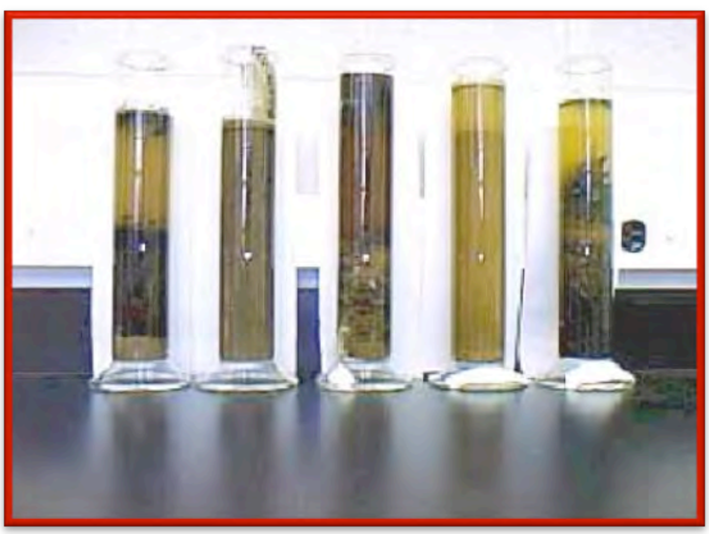

Figure B. Examples of Microbe Cities and their color gradients.

Biodiversity

1. **What are microbes?** Marine microbes are very small and have been around for a long time. Most microbes can only be seen through microscopes: they are too small to be seen by the unaided human eye. The name microbe comes from the Greek *mikro* ("small") and *bios* ("life"). Microbes are so tiny that millions of them can fit on the head of a pin.
   - Microbes were the first life forms on Earth. They began inhabiting the Earth over 3 billion years ago. Some of the oldest fossilized remains of microbes were found in the Pilbara region of Western Australia, and are estimated to be around 3.5 billion years old.

2. **How many are there?** Microbes are the most abundant and diverse biological entities in the ocean. They represent approximately 98% of the ocean's biomass. Just imagine, there are more microbes in the ocean than stars in the known universe. A single teaspoonful of sea water contains millions of bacteria and thousands of protists.

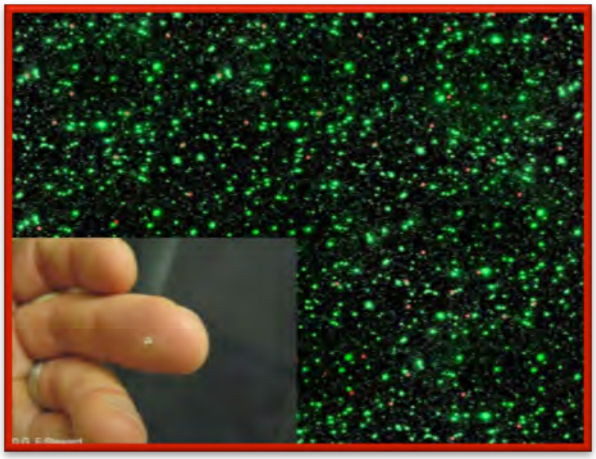

Figure C. There are more microbes than stars in the known universe.

Figure D. The tiny cube on the finger measures 1 mm on each side which represents a volume of 1 microliter. The main photo shows the vast numbers of microbes in 1 microliter of seawater. The big red and green dots (about 1,000) are bacteria and the very small background dots (about 10,000) are viruses.

Who are they? There are 5 main types of microbes; Archaea, Bacteria, Fungi, Protista, and Viruses. Find out more in the handout **Microbe Types.doc** and on the website http://www.microbeworld.org/ .

3. **What maintains biodiversity?** Biodiversity describes the number of species living together in a defined place. All microbes are competing with each other for resources, mostly nutrients they need to survive. Their ability to use each other's waste and take advantage of different nutrients make each microbe unique and able to survive. Nutrients are continuously cycled between and within microbes, supporting the diversity of life.
   - Oxygen, Carbon Dioxide, Nitrogen, and Phosphorus, among other nutrients, are exchanged between microbe species, creating a competition for resources. This "food web" cycle is so small it isn't something that we can observe with our eyes.
   - The diversity of microbes is most easily studied through genetic methods and culturing techniques to see which species are present.
   - There is a whole world of microbes out there waiting to be discovered.

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Habitat

Niche theory: Each species of microbe can coexist because they occupy their own niche in the use of resources and the habitat that they thrive best in. A niche can be small or large, overlap with many species, or be unique to one organism. See the following readings to understand niches in microbial oceanography.

- Ecological Niche Theory according to Dr. Seuss
  http://nsm1.nsm.iup.edu/rgendron/seuss.shtml

> ***And NUH is the letter I use to spell Nutches,***
> ***Who live in small caves, known as Niches, for hutches.***
> ***These Nutches have troubles, the biggest of which is***
> ***The fact there are many more Nutches than Niches.***
> ***Each Nutch in a Nich knows that some other Nutch***
> ***Would like to move into his Nich very much.***
> ***So each Nutch in a Nich has to watch that small Nich***
> ***Or Nutches who haven't got Niches will snitch.***

-On Beyond Zebra (1955)

- Easy background on Ecological Niche theory and how it applies to microbes in Microbial_Ecological_Niche.doc
  http://www.brooklyn.cuny.edu/bc/ahp/CellBio/Growth/MGecol.html

Environmental gradients: The Microbe City becomes stratified (different layers stacked on top each other) over time because different microbes are living in different areas of the column. Although to us it may all look like mud/sand, the amount of nutrients, temperature and oxygen differs throughout the column; these varying amounts are what define the different habitats. The top of the column has continual access to oxygen while the bottom of the column accumulates hydrogen sulfide. Just as humans preferred habitat are temperatures above freezing and fresh oxygen, each microbe species lives in its own habitat. Microbes like the surface of the ocean, others live buried deep in the mud, while some thrive on the ocean floor near hydrothermal vents! Some habitats are easy to define with our eyes, while others need careful measuring, monitoring and scientific analysis to understand.

- See The_Microbe_Zoo_Pond.pdf for more details on what the layers represent.
  http://commtechlab.msu.edu/sites/dlc-me/zoo/zwpmain.html

- The different colored zones are controlled mainly by oxygen and hydrogen sulfur availability. Each microbe has its preference/aversion to these chemicals. See Figure E for an example and read more about the resources available in each zone.

Environmental Gradients

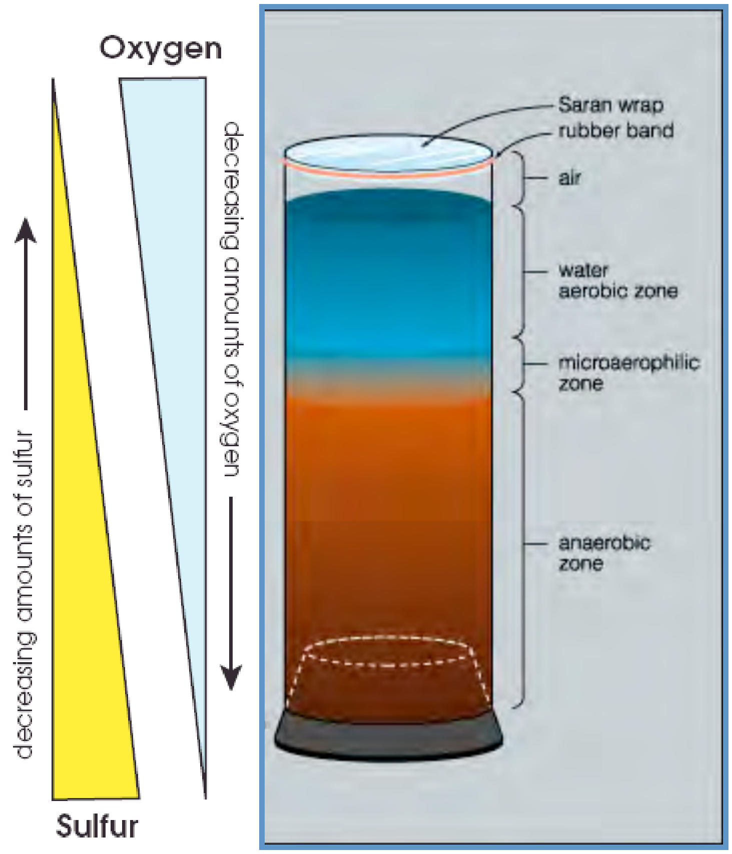


Figure E. Example of the environmental gradients present within the Microbe City.
http://www.personal.psu.edu/faculty/j/e/jel5/biofilms/winogradsky.html

Succession

Changing conditions (thru time in one place): Ecological succession is a fundamental concept in ecology that refers to the change in the composition or structure of a community. Succession may be initiated either by formation of new, unoccupied habitat (*e.g.*, a lava flow or a severe landslide) or by some form of disturbance (*e.g.* fire or logging) of an existing community. The path of ecological change is influenced by site conditions, by the interactions of the species present, and by other factors such as availability of new species or weather conditions at the time of disturbance.

- A Microbe City will change with time as the communities adjust to the disturbances created when making the columns. Light and oxygen levels control the development of other environmental factors such as hydrogen sulfide through the competition, survival and maintenance of different microbe species.

- These changes in community succession are observed by the creation and disappearance of colored zones.

Disturbance and Recovery: The column will go through the phases below within a week to a few months depending on the size of your column and the abundance of the original organisms, "food" and resources available. The following path of development is just an example of a normal recovery of the ecosystem after the initial disturbance (creation of the column). See accompanying pictures of our **Microbe City** as it developed (Winogradsky_Poster.pdf).

1. **Initial** - Sediment with lots of bacteria (microbes) & organic material (food). No color differences throughout the column.
2. **Secondary** - All the organic material helps the bacteria thrive and they deplete the oxygen throughout the tube. The column may stink and become blackish.
3. **Next** – Bacteria rapidly grow in their new environment. Colors and zones begin to appear.
4. **Then** – Environmental gradients develop based on the amount of resources available and are seen through the abundance of different microbes in zones of various colors.
5. **Continual** – Zones develop & change as conditions inside and outside the column change.

Microbe Layers and why they exist where they do…

The colored bands you see in the column are the pigments of billions of photosynthetic bacteria. As microbes grow over a couple of months, the zones become very colorful and distinct. Given enough time without disturbance, each particular organism will dominate their own niche.

- Microbe Website.pdf explains how the Microbe City grows and briefly describes each of the zones below. There are some really involved chemical reactions that control this process as well as interactions between species.
 http://www.sumanasinc.com/webcontent/animations/content/winogradsky.html
- To understand what type of organism dominates each zone you can culture a sample and send it to a lab to analyze (ask someone at CMORE for help)!

Three main zones are created:

1. **Aerobic** (oxygen present): The top of the water column can contain large populations of diverse bacteria. These aerobic organisms are found in organic-rich freshwater habitats such as shallow ponds, polluted streams, etc. These are generally flagellated which allows the bacteria to move 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.

   a. **Algae or 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.

   b. **Aerobic decomposers** - From the mud source below, sulfur will diffuse upward into the aerobic zone and be oxidized to sulfate by bacteria such as *Beggiatoa* and *Thiobacillus*. These bacteria gain energy from oxidation of hydrogen sulfide (H_2S), and synthesize their own organic matter from carbon dioxide (CO_2). They are termed chemoautotrophs.

2. **Microaerophilic** (oxygen scarce): In this zone, oxygen diffuses down from the surface but is limited in concentration. Sulfur from the lower part of the column has begun to move up in the form of H_2S. This diffusion of H_2S from the sediment into the water column enables anaerobic photosynthetic bacteria to grow. *The H_2S is the chemical that causes the column to smell.*

   a. **Purple and Green sulfur bacteria**: They are seen usually as two narrow, brightly colored bands immediately above the sediment - a zone of green sulfur bacteria, such as *Chlorobium*, characterized by a green/olive color indicative of growing anaerobic conditions, then a zone of purple sulfur bacteria, such as *Rhodospirilum* and *Rhodopseudomonas*, which takes on a red/ orange or rust color.

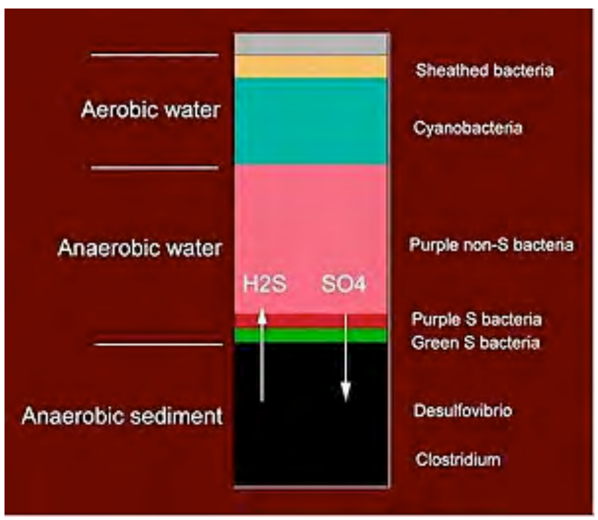
3. **Anaerobic Zone** (Oxygen depleted): The only organisms that can grow in anaerobic conditions are those that ferment organic matter and those that perform anaerobic respiration. Fermentation is a process in which organic compounds are degraded incompletely; for example, yeasts ferment sugars to alcohol. Anaerobic respiration is a process in which organic substrates are degraded completely to CO_2.

a. **Sulfate reducing decomposers**: There are three basic levels that form in the lower level of the column. At one level, purple sulfur bacteria such as *Chromatium*, in a red to purple layer, are processing Sulfates into Sulfur. At another point *Gallionella*, a stalked bacteria, processes iron to help create the black layer that forms just below. This level is marked by a strong rust/orange color.

b. **Fermentative decomposers**: Some cellulose-degrading *Clostridium* species start to grow when the oxygen is depleted in the sediment. All *Clostridium* species are strictly anaerobic because their vegetative cells are killed by exposure to oxygen, but they can survive as spores in aerobic conditions. They degrade the cellulose to glucose and then ferment the glucose to gain energy.

   i. Deeper in the column, the sulfur-reducing bacteria, marked by a deep black layer and typified by *Desulfovibrio* can utilize these fermentation products by anaerobic respiration, using either sulfate or other partly oxidized forms of sulfur generating large amounts of H_2S by this process. The H_2S will react with any iron in the sediment, producing black ferrous sulfide. This is why lake sediments (and our household drains) are frequently black. However, some of the H_2S diffuses upwards into the water column, where other organisms utilize it.

   ii. Finally, at the bottom, depending on the source of the mud, a pink layer will develop due to purple sulfur bacteria with gas vesicles. A characteristic species is *Amoebobacter*. This environment is very high in H_2S and more tolerant of air and light.



Microbe City

The Microbe City you are going to make will have lots of different kinds of microbes (tiny plants and animals that are too small to see unless you have a microscope). If all goes well, your Microbe City will develop several layers with different colors. The colors identify the type of microbe that is living in that neighborhood of the city. You are going to give the microbes a variety of food so that each one has their favorite food.

What you need:

- 2 clear 1-liter plastic bottles
- 1 small bucket filled with equal parts sand and water (enough to fill both bottles)
- 1 cup
- 1 paint stirrer
- 1 sheet of newspaper (as a source of carbon)
- 1 tablespoon powdered chalk (as a source of carbon)
- 1 teaspoon mashed hard-boiled egg yolk (as a source of sulfur)
- 1 spoon
- Plastic wrap and rubber band

What to do now?

1. Mix up the sand and water in your bucket and remove any sticks, leaves, or rocks.
2. Shred a full sheet of newspaper into very small pieces. Add the shredded newspaper to the sand mixture.
3. Add the whole bag of powdered chalk to the mixture.
4. Peel the hard-boiled egg and carefully remove the yolk (the yellow part). Mash the hard-boiled egg yolk. Crush the egg shell into small pieces. Add the yolk and shell to the sand mixture.
5. Stir the mixture using the paint stirrer.
6. Pour or scoop a small amount of the mixture into the base of each bottle.
7. Pack the mixture to remove trapped air, by placing your hand over the top of the bottle and tapping the bottom of the bottle firmly on the table.
8. Repeat the two previous steps until the bottle is about 90% full.
9. Stir the mixture in the bottle with the spoon or paint stirrer to remove any air bubbles.
10. You should have a small layer of water (1 inch deep) above the sand. Add/remove the water in your bottle as needed.
11. Cover the bottle with plastic wrap and a rubber band.
12. Place the ***Microbe City*** in a well-lit place away from direct sunlight or intense heat. Make sure the ***Microbe City*** is outside because foul-smelling hydrogen sulfide (H_2S) gas will likely be produced. If there is not enough water covering the sand, add a little water from the tap. Once a suitable place has been found, do not move or re-orient the column.

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What's next?

- In your **Microbe City**, different colored layers (indicative of different microbial groups) will eventually develop due to differences in the amount of oxygen at the top and the bottom of your bottle. The top of the column has lots of oxygen from the atmosphere and supports microbes that like oxygen (these are called aerobes).
- In the bottom of the column, all of the oxygen gets used up so there is no oxygen. The microbes that don't like oxygen live here (these are called anaerobes). Sulfur develops and diffuses from the bottom of the column, creating a stink smell. Oxygen and sulfur are the 2 main sources of energy in the bottle.
- There are tables for you to record the changes you see over the next couple of months in your **Microbe City**. Make visual observations about the color of the sand and water and how bright the light is when you make your observations. Once a week (or month), make a colored drawing of the column.

Questions to think about:

1) How long do you think it will take for you to see different colors?

2) How many types of bacteria do you think are in your column?

3) Will Microbe City smell like mud? When did you start to smell it and was causes this?

4) How many layers of color did you see while your microbes were growing? Did the layers get thicker or thinner over time?

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