Winogradsky Columns and Metabolism Sustainability Activity - Eukaryotic Evolution and Endosymbiosis

Pre-lab Addition:
Although a couple bacteria formed endosymbiotic relationships with early prokaryotic cells, other bacteria did not. In fact, there are still quite a few anaerobic prokaryotic cell lineages around, and these prokaryotes, as well as other microbes (microscopic eukaryotes and aerobic prokaryotes), play an important role in keeping the environment as we know it! This is especially true in soil, where in just one handful you can find billions of prokaryotes and eukaryotes.

This clump of soil has billions of tiny microbes in it!

One great way to study microbes in soil is by making a Winogradsky Column (https://hmsc.harvard.edu/winogradsky-column#:~:text=This%20tall%20column%20of%20sediments,home%E2%80%9D%20for%20millions%20of%20microbes). A Winogradsky Column is a device used to culture and study microbes in aquatic environments. Making a column is similar to making a cake, you mix together a couple ingredients (sulfur, carbon, and mud), put them in a mold (column), and let it 'bake' in the light for a while. After some time, various microbes start to develop into a community within the column, producing a layered work of art like the column below.

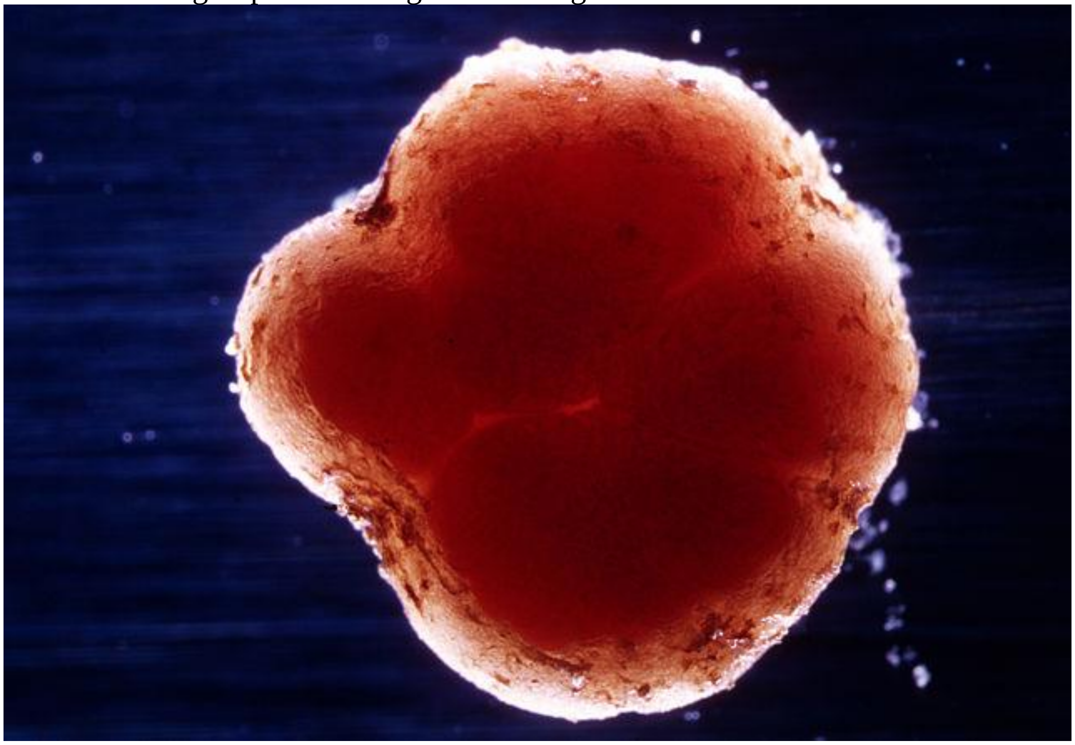
A Winogradsky Column.

To learn more about the beauty of Winogradsky Columns and what different colors mean within the column, watch the video below.
https://www.youtube.com/watch?v=FkSdgcksnz8

As stated in the video, each color represents a different type of microbe that colonizes in specific environmental conditions. This is related to how they obtain energy and carbon, also known as metabolism. **Photoautotrophs** are organisms that use light as an energy source and obtain carbon from their environment. A great example of this are plants.

Plants are photoautotrophs.

Chemoautotrophs are organisms that get energy by breaking chemical bonds and obtain carbon from their environment. An example of this is nitrogen fixing bacteria, the type of bacteria that is responsible for making impossible burgers taste so good!



Rhizobium is a nitrogen fixing bacteria that is used to make impossible burgers. This bacteria is a chemoautotroph.

Photoheterotrophs are organisms that use light as an energy source but obtain carbon by consuming other organisms. This weird type of metabolism is only seen in prokaryotes. A great example of this are purple non-sulfur bacteria, the purple bacteria from the purple-people eater column in the above video.

Purple non-sulfur bacteria are photoheterotrophs.

Chemoheterotrophs are organisms that get energy by breaking chemical bonds and obtain carbon by consuming other organisms. You, as well as many other animals, use this type of metabolism!

Squirrels are an example of a chemoheterotroph.

In this lab, you will learn more about microbes in the environment by working with your group to develop an experiment using a Winogradsy column that you will observe over the course of the semester.

Activity:

Part II: Winogradsky Column

While you just found microbes within the building, microbes can literally be found everywhere! In fact, microbes play an important role in aquatic ecosystems (https://education.nationalgeographic.org/resource/ecosystem) because they recycle nutrients back into the environment to be used by other organisms.

Recall from your prelab that one way scientists study microbes in aquatic ecosystems is by building Winogradsky Columns. This is done by mixing a sulfur and carbon source (microbe food!) with dirt and water collected from a river, pond, or marsh, pouring this mixture into a clear container and letting it sit for months to see how the microbes interact and develop. Overtime, various colored microbes reproduce and create populations in different areas of the column, leaving the column with a layered appearance.

Layering of three Winogradsky Columns.

Overall, there are five main colors you may see develop in your column (clear, light brown, rust, green/purple, and black). Each color represents a different type of environment that microbes grow in. See the chart below for explanations of each color and what type of environment this represents.

Color	Environment	Types of Microbes
Clear or Light Green	This is an oxygenated zone with access to lots of light. Microbes that are aerobic and use light as an energy source will live here. Some of these microbes will also produce oxygen as a leftover product of photosynthesis, so this zone will remain oxygenated.	Protozoans, Fungi, Cyanobacteria, Green algae - All Metabolic Types
Light Brown	This is an oxygenated zone, but only has some access to light. In addition to some sunlight, sulfur can be found in this region, so microbes that use light or sulfur for energy will reproduce here. These microbes will get their carbon from their environment.	Aerobic photoautotrophs and chemoautotrophs.
Rust or Reddish Brown	This region contains lower levels of oxygen but still has some access to light. Because of this, the microbes use light as an energy source but must 'eat' carbon from other organisms.	Anaerobic or aerobic photoheterotrophs
Green or Purple	This region contains no oxygen but does have some access to light. These microbes do photosynthesis; however, they do this process using sulfur compounds instead of water, so sulfur must also be present. Like chlorophyll (where plants do photosynthesis and makes them appear green), these microbes have bacteriochlorophyll, making them appear green or purple.	Anaerobic photoheterotrophs
Black	This region contains no oxygen and has little access to light. However, there is plentiful access to carbon and some sulfur. Therefore, the microbes that survive in this region are anaerobic and use carbon or sulfur as an energy source. They must also obtain their carbon from other organisms.	Anaerobic chemoheterotrophs

Now that you have learned a little more about these columns, it is time for you to develop one of your own! Much like the microbial scavenger hunt, start off by brainstorming with your lab table what sulfur and carbon source(s) you want to use in your column. The amount and type of each source will influence which microbes thrive within your column. Some environmental conditions, such as light and temperature, will remain consistent between all of the columns. In addition, also discuss a hypothesis for what your will look like after four months based on what sources you chose and the environmental conditions for your experiment.

Once your group is ready, follow the directions below to build your column.

1. Begin by scooping out two scoops of soil and placing this into a bowl.
2. Next, mix in your groups sources of carbon and sulfur. Make sure that this mixture is not more than the amount of dirt that is in the bowl.
3. Now add the source water into the mixture until the dirt has turned into the consistency of a thick mud. Spoon the mixture into your cylindrical tube until it is about ⅔ full.
4. Afterwards, fill the top part of the tube with more source water until you are about ½ inch away from the top.
5. Next, create a wet mount using a drop of water from the top of your column. This will be used to make base observations later.

6. To finish up with your column, place plastic wrap over the opening and secure it with a rubber band. Make sure to label your column with your groups initials, your lab section, and the date.

Over the next couple of months you will be observing changes within your column. For now, it is important to take down some base observations that you can reference throughout the semester.

First, record what your column looks like. Make sure to write down the color and the noticeable texture differences within your column.

Second, record and discuss with your group what your column smells like. Try to be as descriptive as possible (avoid using 'it smells like dirt.' What does dirt smell like? Feel free to reference a fragrance wheel (https://en.wikipedia.org/wiki/Fragrance_wheel) for ideas).

Third, see how many microbes you can find in the wet mount of the water in your column. How many different organisms can you find? What is the abundance of microbes in the water? Use the posters on the side bench to help identify different organisms.

Once you have finished with your observations, place your column on the side bench. Your column will be stored until the next time you complete observations. Now, move on to part three.