



## Windogradsky Columns: Microbial Ecology in the Classroom

### INTRODUCTION

Bacteria and Archaea are not only the most ancient forms of life, going back at least 3.5 billion years, but they are also the most diverse and numerous organisms on Earth. For the first 2 billion years of Earth's history, they were the only living things on the planet. These microorganisms exhibit astounding diversity in metabolic mechanisms, such as oxygenic and anoxygenic photoautotrophy, chemoautotrophy, and photoheterotrophy, to name a few. They also play an essential role in cycling elements that make the planet habitable for all other types of organisms. The diversity of these simple life forms is evident in the myriad of ecological niches they inhabit, from hydrothermal vents to the acidic lining of your stomach.

In this activity, you will observe a sampling of the diverse microorganisms that live in your local environment. You will set up Winogradsky columns—simple devices for constructing a stratified ecosystem—that illustrate different types of microbial metabolism in a colorful way.

The activity takes 6 to 8 weeks to complete. It will take a couple of hours to get samples and set up the experiment, and then you will need to record observations for a few minutes about once a week.

### MATERIALS

- 4 clear, 16-oz. plastic bottles. Larger containers will also work; adjust the sediment volumes accordingly.
- 1–4 disposable containers (e.g., plastic storage container or plastic baggie) for mixing sediment
- 1 trowel for digging a sediment sample
- Permanent marker
- 4 small labels (1 for each bottle)
- Water
- 1 bucket or container large enough to hold 6–10 cups of sediment
- A well-lit location where the columns can sit undisturbed for 6–8 weeks
- Digital camera or cell-phone camera
- Large measuring cup or other container for measuring sediment and mixture
- A carbon source, shredded newspaper; approximately 1 cup loosely packed
- 6–10 cups of sediment (mud)
- A sulfur source; raw egg yolk is best Large mixing spoon (optional)
- 1 funnel (optional) Colored pencils (optional)

### PROCEDURE

#### Plan the Activity

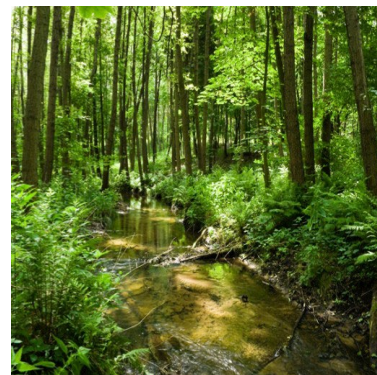
You are going to set up four (4) Winogradsky columns: a control column and columns that contain added carbon, added sulfur, and carbon *and* sulfur. Please be sure to **read through this entire document** first so that you are aware of what is expected. You will need to go on a short field trip to collect your sediment (mud) sample. Once you have the sediment, it will take approximately an hour to set up the four (4) columns. In addition, set aside a few minutes once a week during the 6- to 8-week experimental period to make

observations and take photos of your columns.

You may choose to let your columns run for longer than 8 weeks. The columns can be analyzed for several months and might reveal other changes the longer they are set up.

### Collect the Sediment Sample

1. Identify a sediment source in your area. Anywhere with dirt and water is appropriate, such as a stream, creek, marsh, pond, bay, ocean beach (sand), or even a backyard puddle (Figure 1).
2. Bring a plastic bucket or other container (with a lid, if available), a trowel, and a camera (digital or phone).
3. Take 2 or 3 photographs of your sample site to illustrate the location where your sediment was collected.
4. Collect approximately 10 cups of sediment in the plastic bucket. The sample should be wet. Avoid or pick out rocks, sticks, and leaves; include some additional water from the sample site.



**Figure 1.** A possible source for collecting sediment.

### Assemble the Winogradsky Columns

5. If you don't have a funnel, you can make one by cutting off the top three inches of one of your plastic bottles (Figure 2).
6. Write "control" on one label sticker. On the other three (3) labels, write "carbon," "sulfur," and "carbon and sulfur." Stick the labels on the sides of the four 16-oz. plastic bottles near the bottom.
7. Label four disposable containers: control, carbon, sulfur, and carbon + sulfur. Add approximately 1.5 cups of the sediment sample that you collected to each of the disposable containers (i.e., plastic storage container or plastic baggie). You may reuse a single mixing container if you rinse it with tap water between uses.
8. For the control container, skip to step 10.
9. For the other three (3) containers, follow the instructions below for each column:
  - a. For the "carbon" column: Add  $\frac{1}{2}$  cup of shredded newspaper (loosely packed) to the sediment (step 7 above) and mix with a mixing spoon or trowel. (The newspaper contains cellulose, a source of carbon.)
  - b. For the "sulfur" column: Add the yolk of an egg (raw is best) to the sediment in the container labeled "sulfur" and mix with a mixing spoon or trowel. If using hard-boiled eggs, crumble the yolks. (The egg yolk is a source of calcium sulfate in the column.)
  - c. For the "carbon and sulfur" column: Add both nutrients as described in steps **a** and **b**.
10. Mix each of the samples thoroughly. Make sure to remove any large debris such as leaves, rocks, or sticks. Slowly mix in water (either water that you collected or tap water) until the mixture has the consistency of a milk shake.
11. Using a large spoon, slowly add approximately 1 cup of the mixture to the appropriately labeled plastic column (bottle). You may wish to use a funnel or an inverted, cut bottle top to add the sediment mixture



**Figure 2.** Funnel

(Figure 1). As you add the sample, tap the column on a counter or table to release any trapped air in the column. The sediment should fill  $\frac{1}{2}$  to  $\frac{3}{4}$  of the column.

12. Add water from the sediment collection source (or tap water) on top of the sediment until there is a 2-cm layer of water on the surface. (There should be air at the top of the column.)
13. Place the lid on each column and turn the lid a  $\frac{1}{2}$  turn. **DO NOT** tighten the lid! If you cut the bottle top, place plastic wrap over your column and hold the wrap in place with a rubber band.
14. Place all four columns in a well-lit space such as a windowsill.
15. Using a camera or phone, take a photograph of the columns to document week 0 of the experiment.

### Make and Report Observations

The following items explain the observation and data-collection portions of this activity.

#### A. Weekly Observations

You should observe your Winogradsky columns for at least 8 weeks--longer is better (Figure 3). Spend a few minutes each week recording your visual observations of the columns. Try to make observations on the same day each week. For example, if you set up your columns on a Sunday, check them every Sunday. A table is provided for you to record your observations, but you may choose to make your own table on a computer.

**Hint:** Consider these questions when recording observations:

- Has the color of the sediment changed? If so, note the color(s).
- Has the color of the water changed? If so, note the color(s).
- What differences between the columns do you observe?
- Does the sediment appear to have settled? Shifted?
- Do you see any layers forming in the sediment? In the water?
- How does the thickness of the layers change from week to week?
- Are there differences between the side facing the light and the side away from the light?



Figure 3. Examples of columns.

#### B. Take Weekly Photographs

Using a digital camera or cell phone, you are encouraged to take weekly photographs of your columns side by side with the labels showing. As you save your photos, we recommend that the file names include your initials and the week the photo was taken. (For example, "skn\_week1" would be an appropriate photo file name.) Make sure that the labels on the columns are clearly visible in the photos.

**Hint:** Your columns should always face in the same direction in relation to the light source. If you must move the columns to take the photographs, be sure to place the columns back in the same orientation relative to the light.

#### C. Once a Month, Sketch the Columns

Sketch the columns, in color, 3 times during the course of the 8-week experiment. Consider sketching once during each calendar month of the experiment. Sketching encourages deeper observation of the columns. Blank images on the attached work sheet are for your use.

Winogradsky Columns: Microbial Ecology in the Classroom**QUESTIONS**

During the course of the experiment, spend some time considering the following questions. Some of these questions may require extra research. Feel free to consult a microbiology textbook (e.g., to learn about the sulfur cycle).

1. How do your columns differ? How are they the same? Explain the differences you see.
2. Did you observe changes in the control column? If so, explain why they occurred.
3. Winogradsky columns form oxygen concentration gradients. Predict the distribution of oxygen throughout the column. (Consider the entire column: the sediment, the water, and the air.)
4. Winogradsky columns form sulfide concentration gradients as well. In the columns that contain egg yolk, predict how sulfide will be distributed throughout the column. (Consider the entire column: the sediment, the water, and the air.)
5. Sulfur reduction is a form of anaerobic respiration. *Desulfovibrio* are an example of bacteria that reduce sulfur as a way of respiring in the absence of oxygen and release sulfide. Where in the columns would you expect to find them?
6. Purple sulfur bacteria and green sulfur bacteria are two types of bacteria that use sulfide to support photosynthesis. In general, green sulfur bacteria tolerate higher levels of sulfide than purple sulfur bacteria do. Predict where the green and purple sulfur bacteria would be in relation to each other. Also predict where in the column the purple sulfur bacteria would be in relation to the *Desulfovibrio* bacteria.

7. If samples were extracted from the various layers of all the columns, where would you find photosynthetic organisms such as cyanobacteria and algae? Explain why.
  
  
  
  
  
  
  
  
  
  
8. Explain how Winogradsky columns illustrate the diversity of microorganisms found on Earth today in terms of the diversity of niches they occupy.
  
  
  
  
  
  
  
  
  
  
9. Explain what the Winogradsky columns illustrate about life on early Earth.

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**Weekly Observations**

Observations During the Week Of:	Control Column	Carbon Column	Sulfur Column	Carbon and Sulfur Column
<p><b>Week 0</b></p> <p>Actual Date: _____</p>				
<p><b>Week 1</b></p> <p>Actual Date: _____</p>				
<p><b>Week 2</b></p> <p>Actual Date: _____</p>				
<p><b>Week 3</b></p> <p>Actual Date: _____</p>				
<p><b>Week 4</b></p> <p>Actual Date: _____</p>				

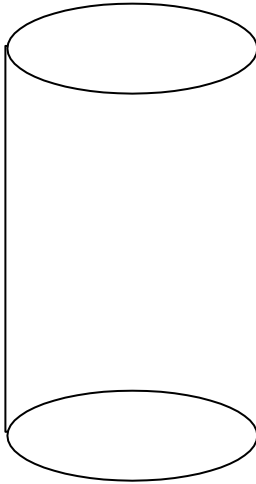
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Observations During the Week of:	Control Column	Carbon Column	Sulfur Column	Carbon and Sulfur Column
<b>Week 5</b> Actual Date: _____				
<b>Week 6</b> Actual Date: _____				
<b>Week 7</b> Actual Date: _____				
<b>Week 8</b> Actual Date: _____				

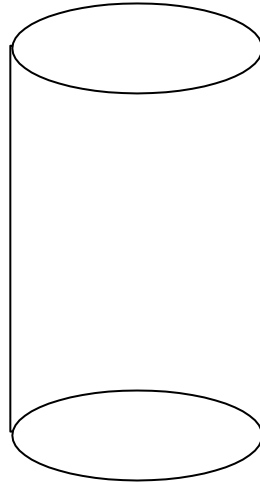
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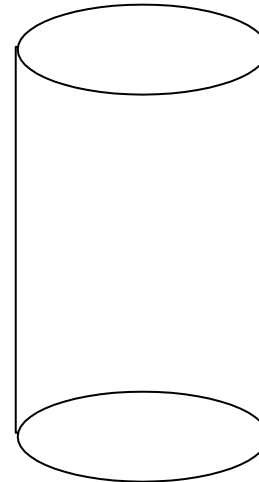
Month 1: Date \_\_\_\_\_



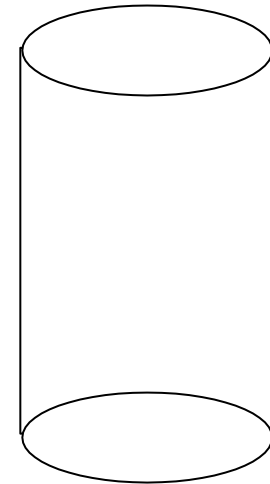
**Control**



**Carbon**



**Sulfur**

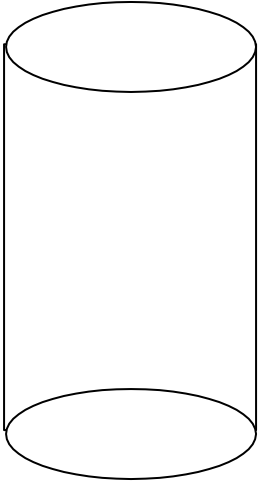
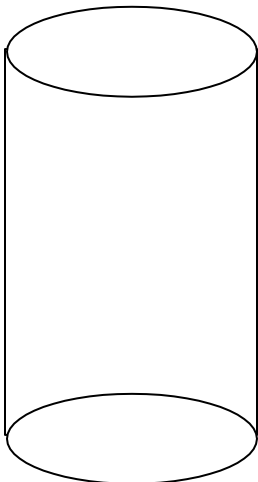
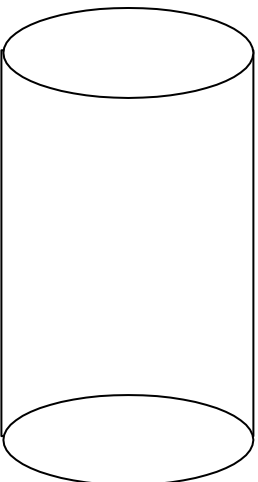
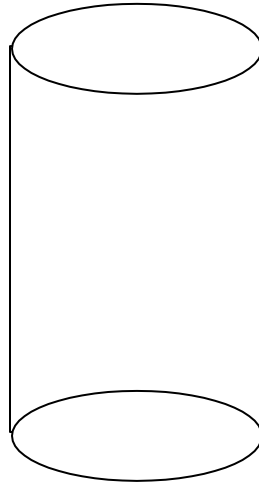


**Carbon/Sulfur**



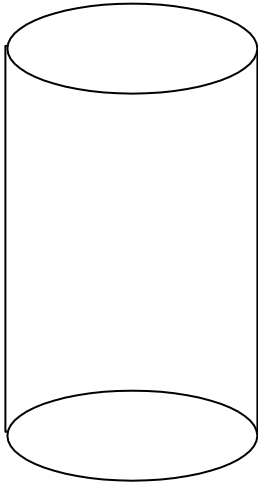
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Month 2: Date \_\_\_\_\_

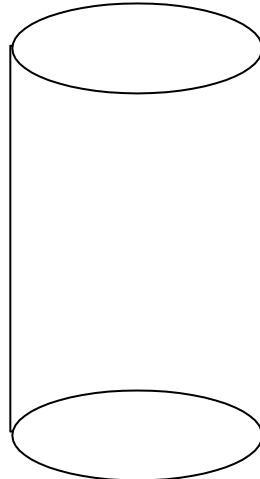
			
Control	Carbon	Sulfur	Carbon/Sulfur

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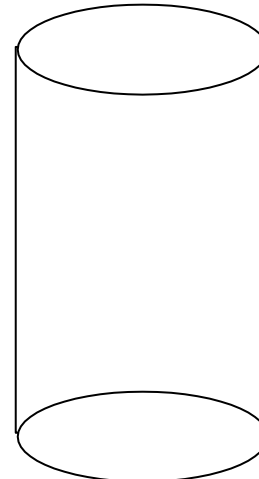
Month 3: Date \_\_\_\_\_



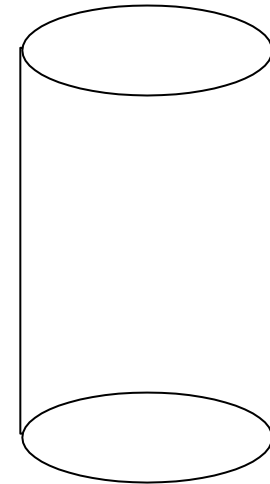
**Control**



**Carbon**



**Sulfur**



**Carbon/Sulfur**