

Labs & Demos
Educator Materials

OVERVIEW

This activity complements the 2012 Holiday Lectures on Science, "Changing Planet: Past, Present, Future." Winogradsky columns provide a visual example of various modes of metabolism in the microbial world. It takes approximately 6 to 8 weeks to see layers of microbial growth, but the experiment can be conducted for a longer time period.

KEY CONCEPTS

- Microbes have a wide variety of metabolic capabilities, many examples of which can be found in a generic sample from the environment.
- A combination of microbial metabolism and physical parameters (such as light availability and diffusion) lead to ecosystem stratification.
- The metabolic requirements of one group of organisms can be provided by the byproducts of another group.
- While different microbes are adapted to different ecological niches, they also play a role in forming those niches.
- Microbes play a role in elemental cycling.

STUDENT LEARNING TARGETS

- Make observations on an ongoing experiment.
- Make inferences based on observations.

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)

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ANSWER KEY

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. *Answers will vary.*
- 2. Did you observe changes in the control column? If so, explain why they occurred.

 Answers will vary. Gradients may also form in control column because there are natural forms of carbon, sulfur, and other nutrients in the sediment. All the same microbial processes will occur but maybe to a lesser extent (depending on the sediment source).
- 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.)

 Over time, an oxygen gradient develops from high at the top to completely anoxic (no oxygen) at the bottom of the column. The overlying air has the highest oxygen concentration, and the concentration decreases as you move down through the water and sediment to the bottom of the column.
 - Extra detail: When the column is prepared, oxygen is evenly distributed throughout the column. It is consumed by respiration throughout the column, but it is only produced in the photosynthetic layer at the top. Any oxygen that diffuses down into the sediment from the topmost layer reacts with chemical compounds in the anoxic layer.
- 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.)
 - Sulfide will be highest in the bottom (anoxic) part of the column and will decrease upward, with no sulfide at the top of the sediment or in the overlying water. Extra detail: When the column is prepared, sulfur will be distributed throughout the column by mixing. Sulfur is converted to sulfide by anaerobic respiration, which will only occur in the bottom of the column. Any sulfide that diffuses upward will react with oxygen (either abiotically or through microbial metabolism).
- 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?
 - Desulfovibrio would be found in the bottom parts of the columns where there is no oxygen.
- 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.
 - Purple sulfur bacteria will be concentrated in a layer above the green sulfur bacteria because that is where there is less sulfide. In addition, both the green and purple sulfur bacteria will be layered above the Desulfovibrio bacteria. Extra detail: Since Desulfovibrio can reduce sulfur, they produce sulfide that supports the metabolism of green and purple sulfur bacteria. However, Desulfovibrio do not require light, so they will be found lower in the column.

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- 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.

 Photosynthetic cyanobacteria and algae will most likely be sampled from the water at the top of each column because they require only water, carbon dioxide, and light, which is most intense at the top of the column (assuming an overhead lightsource).
- 8. Explain how Winogradsky columns illustrate the diversity of microorganisms found on Earth today in terms of the diversity of niches they occupy.

 Different layers form in the column based on the availability of oxygen and other nutrients. Different groups of organisms occupy each of these layers, but they all came from the original sample. This illustrates the point that there is a rich diversity of organisms in very common environments such as your backyard stream. Furthermore, the gradients themselves are a product of microbial metabolism. This illustrates that microbes don't just adapt to their environment; their metabolisms actually create chemical niches with the environment.
- 9. Explain what the Winogradsky columns illustrate about life on early Earth.

 Most of the strata in the columns are anoxic, yet life abounds. Similarly, Earth's early atmosphere was devoid of oxygen, but microfossils and geochemical evidence suggest that life was ubiquitous. A large diversity of microbes are adapted to life without oxygen.

Extra Detail: Winogradsky columns are ecosystems in which the byproducts from one group of organisms support the growth of another (e.g., Desulfovibrio produce sulfide, which supports the growth of green and purple sulfur bacteria). In this way, microbes shape the environment to create ecological niches for other organisms. On a global scale, cyanobacteria are the organisms responsible for the oxygenation of Earth. Only after oxygen accumulated (through photosynthesis) could larger and more complex life forms evolve9. Explain what the Winogradsky columns illustrate about life on early Earth.

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