Growing Chemosynthetic Bacteria

Overview

Students will grow and observe *succession* and *chemosynthesis* of bacterial colonies: one lighted, the other in the dark. This activity uses the concepts of the Winogradsky *column*, a device which enriches and isolates certain organisms involved in the sulfur and nitrogen cycles. The activity provides a rough analog to both processes of chemosynthesis and succession; processes which occur at deep sea *hydro-thermal vents* and form the base of the food chain in the absence of sunlight. (Note that the original Winogradsky column was made by a Russian microbiologist Sergei N. Winogradsky.)

Concepts

- Some organisms cannot draw energy from the Sun and must find other energy sources to live.
- Both *photosynthesis* (uses sunlight) and chemosynthesis are means of producing sugar (stored chemical energy)
- Photosynthetic organisms use light as their energy source; chemosynthetic organisms use chemicals. The result for both organisms is the same: energy. The by-products are different, however. In photosynthesis, oxygen is given off as a waste product. In chemosynthesis sulfate is given off.
- As organisms thrive in a given environment their by-products create a new environment where new species thrive. This is called succession.

Materials

For each group:

- Black mud (enough to fill the *graduated cylinders*). See Preparation section.
- 80 g of $CaSO_4$ (Plaster of Paris: found in any hardware store)
- 20 Jars or beakers for mixing
- Stirring rods
- Organic straw or filter paper bits. (Tear strips of lab filter paper)
- 3 Liters (about 3 quarts) of pond, salty sea water or swamp water
- 4 Grams baking soda
- 20 Multivitamin pills and something with which to crush them
- Plastic wrap
- Rubber bands
- A light source that can stay on for six weeks or longer
- Tape and markers for labeling columns
- Flashlight with red cellophane on lighted end (can be attached with rubber band)
- Two 500 milliliter graduated cylinders or columns

Preparation

Divide the class into pairs or small groups. Each pair of students will set up two identical columns. One will be kept in the dark and the other will be placed under a light source.

Obtain mud from a local lake, river, or bay or estuary. If it is not completely black let the mud sit for awhile in a jar to blacken.

PROCEDURE

Engagement

Bacteria that are chemosynthetic may be one of the oldest life forms on Earth. The classic Winogradsky column, developed long before vent ecosystems were discovered, provides an excellent illustration of bacterial growth and succession. Around seafloor hydrothermal vents, scientists have discovered communities of different bacteria that draw their energy from chemicals in Earth's crust, rather than from the Sun.

In 1880, the Russian scientist Sergei Winogradsky discovered the bacteria *Beggiatoa*. These bacteria metabolize hydrogen sulfide to produce the energy for making food (simple sugars). They do not need light as a source of energy; they instead use hydrogen sulfide. *Beggiatoa* is among the bacteria found in the deep-sea hydrothermal vent environment, but it is not the only bacteria to take advantage of this chemosynthetic process.

Activity

1. Add about four grams of $CaSO_4$ to enough mud to fill one graduated cylinder to a depth of about 8.0 cm (3.2 inches). Then dump it into a jar or beaker and mix it thoroughly with the stirring rod.
2. Place the straw or filter paper in the jar with the mud and mix gently. (It may help to add some pond or seawater here to ease stirring.)
3. Transfer the mixture back to the cylinder and add pond or seawater so that the mud is covered with at least 8 centimeters (3.2 in) of water.
4. Add to the cylinder 0.2 grams of baking soda and one crushed vitamin pill. Stir again to make sure all the air bubbles are gone.
5. Set the cylinder aside for 30 minutes to settle.
6. After 30 minutes, if more than two centimeters (0.8 in) of water have pooled at the top, pour off all but one centimeter (0.4 in). If there is less than one centimeter (0.4 cm), add more pond water.
7. Repeat steps (1) through (6) for the other graduated cylinder.
8. Label the cylinders with the students' names.
9. Place one graduated cylinder in a darkened area where it will not be disturbed for at least six weeks. Place the second cylinder under the light source. (You may wish to store both set-ups in the same area with one cylinder in a box. This will help to keep both in similar conditions.)
10. Record smell, color, number of layers of mud, or any other observations.
11. For at least six weeks, examine the columns weekly and look for signs of bacterial growth. You may use a safety light (flashlight covered with red cellophane) to examine the columns being grown in the dark. Record your observations.
12. (OPTIONAL) At the end of the third week take samples for microscopic wet mounts observation from the following locations: (1) surface layers of water, (2) surface layers of the mud, (3) colored layer from the mud. Try using a pipette and be careful not to disturb the column.
13. (OPTIONAL) Observe the wet mounts under high-power magnification, looking for cell shapes that would indicate the types of organisms present.

Explanation

Before the experiment begins, give students a tutorial on what to look for in their cylinders. In the column left in the light students should see green-colored algae the first week. Then, over a period of six weeks, at least five different bacteria may grow in succession in both columns.

It is difficult to know what bacteria are actually growing in the students' column; however, as an example, at hydrothermal vents the first bacterium present is *Clostridium*. This is an *anaerobic heterotroph* that uses, in our simulated column, the straw or filter paper as a carbon source to produce food. A second bacterium, *Desulfovibrio*, uses the waste of the anaerobic heterotroph as its source of carbon and the $CaSO_4$ as an energy source. This bacterium produces the hydrogen sulfide required by the rest of the *ecosystem*. Three other bacteria *Beggiatoa* (white or yellow), *Chlorobium* (green), and *Chromatium* (purple and violet) use hydrogen sulfide as part or all or their energy source to make food. This process requires oxygen so you will find these bacteria near the surface of the sediments. After the original purple and green patches form, black spots will begin to appear. These black patches are deposits of H_2S (hydrogen sulfide, which has a distinct odor) that are created by the sulfur-oxidizing *aerobic* cyanobacteria. Chemosynthetic bacteria need the H_2S for energy, and grow soon after these black spots do. The bacteria that use light as their major energy source with some hydrogen sulfide are heterotrophic and the bacterium that uses hydrogen sulfide as its entire energy source (*e.g.*, *Beggiatoa*) is *chemotrophic*.

Scientists once thought that sunlight was the only source of energy for life and that photosynthesis was the only way to make food. It is now known that another source of energy for life is reduced chemicals from Earth's hot interior. In the process of chemosynthesis, bacteria oxidize chemicals and use the liberated energy to combine hydrogen and carbon dioxide. This produces sugar in a biochemical process similar to photosynthesis. Unlike photosynthesis, chemosynthesis requires no light and can occur at the extreme temperatures and high pressures characteristic of the deep ocean environment [Fig. 1].

The chemosynthetic *food web* supports dense populations of animals. Life in these communities is based on energy available from *oxidation-reduction reactions* when circulating sea water leaches minerals

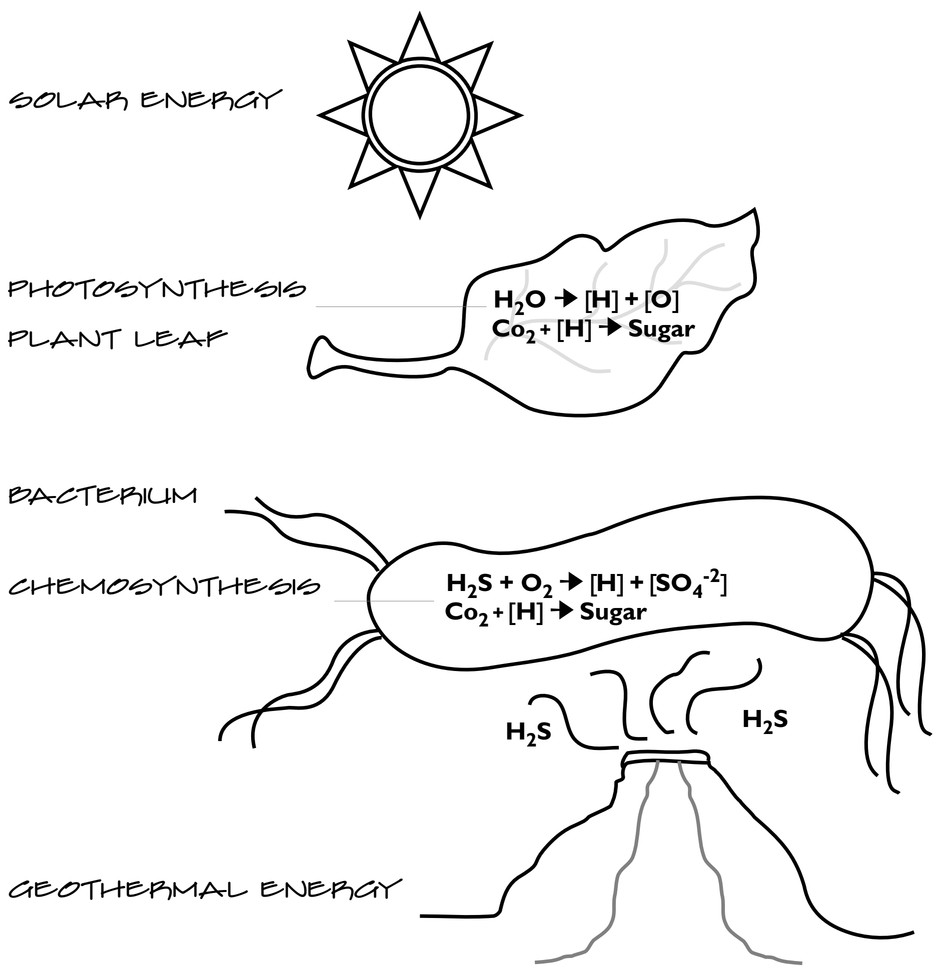

Figure 1. **Photosynthesis and chemosynthesis**. Both processes use energy to make sugar. Photosynthesis uses sunlight to produce sugar in plant leaves (top). Chemosynthesis is carried out by bacteria and its energy source is from hydrothermal vents (bottom).

from the hot rock. High temperatures and high concentrations of dissolved minerals in the seawater cause the formation of compounds such as hydrogen sulfide. Sulfur bacteria are the most prominent chemosynthetic bacteria in the region of hydrothermal vents.

Extension

This activity roughly simulated the development of succession of bacterial communities found around hydrothermal vents. The specific bacteria may not be the same but the scientific principles of succession and chemosynthesis have been demonstrated. Have students view video of hydrothermal vents on the Juan de Fuca Ridge and see for themselves evidence of bacterial succession.

Closely examine the spreading center smokers. Does the color of the bacteria change with distance from the vents? Why or why not?

Sulfur bacteria that carry out these sulfur-oxidizing reactions live in one of three habitats associated with hydrothermal vents. Some live in the hydrothermal vent community, inside tubeworms, clams and mussels and maintain a *symbiotic* relationship with them. Some live in the hydrogen sulfide-rich waters that flow out of the vent. Others live on the surface of rocks and animals wherever there is sufficient hydrogen sulfide to support chemosynthesis. The sugar that these bacteria manufacture supports the entire *hydrothermal* vent community. Look at the video of seafloor hydrothermal vents and the image of tube worms near a hydrothermal vent. For these, can you find any/or all of these three bacteria habitats in the hydrothermal vent community: symbiotic, vent outflow, and rock/animal surface? What characteristics distinguish these habitats from one another?

An alternate way to conduct the experiment is to add a third cylinder, but keep its contents mixed (oxygenated) throughout the six-week period. This would demonstrate what happens if animals that mix, or “bioturbate,” the sediments which are present.

Links to Related CD Activities, Images, and Movies

Image of *Tubeworms near hydrothermal vent*
Movie of *Seafloor hydrothermal vent smokers*

Vocabulary

aerobic
anaerobic
chemosynthesis
chemotrophic
column
ecosystem
food web
graduated cylinder
heterotroph
hydrothermal vent
oxidation-reduction reaction
photosynthesis
succession (cycle of)
symbiosis

Source

Adapted from Orange County Marine Institute / San Juan Institute Activity Series.