AUSTRALIAN EARTH SCIENCE EDUCATION

Modelling Life in the Archaean
Teacher Notes

Introduction

Archaea and bacteria are ancient forms of life that evolved at least 3.5 billion years ago. For the first 2 billion years of Earth's history, they were the only living things on the planet and they are now the most numerous and diverse organisms on Earth. Archaea and bacteria use a range of metabolic mechanisms, such as oxygenic and anoxygenic photoautotrophy, chemoautotrophy, and photoheterotrophy. They play a key role in cycling elements in biogeochemical cycles, such as the carbon, sulfur and nitrogen cycles. They occupy a range of ecological niches such as hydrothermal vents, deep ocean sediments and mangrove muds.

This activity takes 6 to 8 weeks to complete. It will take a couple of hours to collect samples and set up the experiment. Recording observations will take a few minutes, about once a week.

Winogradsky columns on a windowsill.

Aim

To build a Winogradsky column using sediments from a creek, bay, beach or mangrove to observe diverse modes of metabolism in a stratified microbial ecosystem of bacteria and Archaea.

Equipment (per group)

Collect the Sediment Sample

- Digital or phone camera
- Gloves for each group member
- 1 x trowel to dig a sediment sample
- 1 x large colander to sieve sediment
- 1 x large bucket with a lid or container large enough to hold 8-10 litres of sediment
- Bottle with lid, to collect water from the same source as the sediment

Assemble the Winogradsky Columns

- 8-10 litres of sediment (dependent on size of bottles) from sample area (see collect the sample method)
- Water from sample area (see collect the sample method)
- Newspaper or plastic to cover bench area
- 10 x small labels (1 for each bottle and mixing container)
- Permanent marker
- 5 x clear bottles of the same size with lids (1.25 L plastic soda bottles work well)
- 5 x medium containers for mixing sediment (ice-cream containers work well)
- Gloves for each group member
- Large measuring cup or other container for measuring sediment and mixture
- 1 x cup of shredded newspaper or paper (cellulose = a carbon source)
- 1 x raw egg (calcium sulfate source)
- 1 x cup of crushed eggshell (calcium carbonate source)
- 5 x large mixing spoons

- 1 x large funnel with a wide spout
- 1 x stick or rod to fit through funnel spout (to push sediment through funnel)
- Optional = black cardboard and masking tape
- Well-lit location where columns can sit undisturbed for 6-8 weeks

Table 1: Risk Assessment

Risk	Control
Microbes or pollution in sediments	Wear gloves and wash hands thoroughly after handling sediments
Sharp objects in sampled area	Wear fully enclosed shoes whilst collecting sediment
Allergies to raw egg	Avoid egg or sediments containing egg
Bottles exploding	Loosen lids on bottles to allow gases to escape

Method

Collect the Sediment Sample

1. Identify a sediment source in the local area, avoiding protected habitats such as seagrass meadows, nature or marine reserves and National Parks. Anywhere with dirt and water is appropriate, such as a creek, marsh, pond, dam, bay, ocean beach (sand) or mangrove area.
2. Take several photos of the sample site to illustrate the location of the sediment sample.
3. Dig sediment with the trowel and place into a colander over the large container.
4. Sieve sediment into the large container, using the colander, to remove rocks, leaves and sticks.
5. Collect enough sediment to fill all clear bottles.
6. Add some additional water from the sample site to the sediment in the bucket.
7. Collect a bottle of water from the sample site.

Sieving sediment to remove rocks and twigs.

Assemble the Winogradsky Columns

1. Cover your bench area
2. Write two of each of the following labels:
    a. CONTROL
    b. CARBON
    c. SULFUR
    d. CALCIUM CARBONATE
    e. CARBON + SULFUR + CALCIUM CARBONATE.

3. Stick one set of the labels to the sides of the five bottles, near the bottom.
4. Stick the second set of labels to the five medium containers.
5. Put on your gloves.
6. Add enough sediment to each medium container to fill at least ¾ of the bottle it will be transferred to (for a 1.25 L bottle add at least 1 L of soil).
7. Add ½ cup of loosely packed shredded newspaper to the sediment in the container labelled CARBON and mix thoroughly with a clean large mixing spoon. Slowly add water from the sediment collection site and mix until it has the consistency of a thick milkshake.
8. Add the yolk of an egg to the sediment in the container labelled SULFUR and mix thoroughly with a clean large mixing spoon. Slowly add water from the sediment collection site and mix until it has the consistency of a thick milkshake.
9. Add ½ cup of loosely packed crushed eggshell to the sediment in the container labelled CALCIUM CARBONATE and mix thoroughly with a clean large spoon or trowel. Slowly add water from the sediment collection site and mix until it has the consistency of a thick milkshake.
10. Add ½ cup of loosely packed shredded newspaper, the yolk of an egg and ½ cup of loosely packed crushed eggshell to the sediment in the container labelled CARBON + SULFUR + CALCIUM CARBONATE and mix thoroughly with a clean large mixing spoon. Slowly add water from the sediment collection site and mix until it has the consistency of a thick milkshake.
11. For the medium container labelled CONTROL, slowly add water from the sediment collection site and mix using a clean large mixing spoon until it has the consistency of a thick milkshake.


Nutrients added to sediment.

12. For each trial: CONTROL, CARBON, SULFUR, CALCIUM CARBONATE and CARBON + SULFUR + CALCIUM CARBONATE:
    a. Place a large,cleaned, funnel in the mouth of the matching labelled clear bottle and slowly add the mixture until the bottle is ¾ full of sediment. A stick or rod may help to push sediment through the funnel spout.
    b. As the sample is added, periodically tap the bottle on the bench to release any trapped air in the column of sediment.
    c. Add water from the sediment collection site to the bottle until there is a 2 cm layer of water above the surface of the sediment.
    d. Ensure all sediment has settled and trapped air is released then mark the top of the sediment on the side of the bottle (using the permanent marker).
    e. There should be space for air at the top of the column.
    f. Place a loosely fitted lid on each column to allow gases to escape and reduce evaporation, DO NOT tighten it fully.

13. Optional - cover half the bottle from top to bottom with black cardboard to observe the effect of light and dark on microbes in the column.
14. Place all columns in a well-lit area, such as a windowsill. If a side is covered with cardboard face this side away from the light.
15. Use a camera to take photos of the columns at Week 0, ensure the labels are visible in photographs.

Make Observations

1. Take photos of the columns, ensuring the labels can be read in the photographs. If present, carefully open the cardboard section and take photos that compare the section exposed to light with the section that was covered.
2. Use results table 2 to record visual observations such as:
    - The colour of the sediment and water
    - Any layering present (colour, thickness, texture)
    - Settling or raising of the sediment, relative to the marked line
    - Differences between the side facing the light and the side facing away.
3. Replace the cardboard (if used) and return the columns to their location, ensuring they are set up with the cardboard covered side away from the light.
4. Observe the columns weekly for at least 8 weeks. Try to make your observations at the same time each week (recording results in tables 3-10 – creating and attaching any extra tables if observing beyond 8 weeks).
5. Make a labelled diagram of the columns at least once per month.

Results

Photos taken and observations recorded, in student worksheet results tables, from experiment start onward. There are enough tables on the student worksheet for 8 weeks of observation. Labelled diagrams to be completed at weeks 4 and 8, again, space provided on the student worksheet.

Explore

Use the virtual Winogradsky Column: Microbial Ecology in a Bottle interactive to learn about the processes that might be occurring in a Winogradsky column.

Analysis

1. Explain the similarities observed in the columns.
   Similarities may be that all sediment in the columns exposed to no light (dark) will appear grey or black indicating buildup of hydrogen sulfide. Oxygenic organisms will be found in the upper sediments and anoxygenic in the lower sediments.

2. Explain the differences observed in the columns.
   Differences seen are based on the nutrients available. The columns with high sulfur, from egg yolk, have red colours indicating the presence of purple sulfur bacteria (these only grow in the light). The columns with calcium carbonate generally have high amounts of green

cyanobacteria. Some of the green may also be green sulfur bacteria. Orange sediments are caused by the formation of iron oxide by iron-oxidising bacteria. Microbial mats may also form on the surface of the sediment or billow up into the water.

3. Explain the need for the control column and any changes observed in it.
   The control column is a reference to compare the changes observed in other columns. It has no added nutrients, so any changes are due to nutrients present in the original sediment.

4. Oxygen concentration gradients form in Winogradsky columns. Predict the distribution of oxygen throughout the entire column (air, water and sediment).
   Oxygen levels would be higher in the water and top layers of sediment. The deeper into the sediment the less oxygen there would be. At the bottom of the columns, it is possible that there would be no oxygen and that it would be anoxic sediment.

5. Explain where oxygenic photosynthetic organisms, such as cyanobacteria, could be expected to be found in the columns.
   Oxygenic photosynthetic organisms, such as cyanobacteria, would be found on the surface of the sediments, in the top zones exposed to the light and where ample oxygen is available.

6. Explain where anoxygenic photosynthetic organisms, such as purple sulfur bacteria, could be expected to be found in the columns.
   Anoxygenic photosynthetic organisms, such as purple sulfur bacteria, would be found in areas exposed to the light and in the mid parts of columns where it is usually the boundary between anoxic and oxygenic layers. Some are more tolerant of hydrogen sulfide so will be able to survive in the lower parts of the column where hydrogen sulfide levels are higher.

7. Explain the role of microorganisms, such as those in the Winogradsky columns, in underwater hydrothermal vent and underwater volcanic ecosystems.
   Hydrothermal vents and underwater volcanoes release lots of minerals and inorganic nutrients into the environment. Microbes convert these minerals and nutrients into forms that other organisms can use.

8. Explain how the Winogradsky columns can be used as a model of life on early Earth.
   In the environments of the early Earth the only living things were Archaea and bacteria. Many of the bacteria found in the Winogradsky columns are direct descendants of those ancient microbes. The processes that they use in the Winogradsky columns are the same processes that would have been used in early Earth environments. Before there was a buildup of oxygen in the atmosphere many of the organisms now found in the anoxic layers would have been able to survive close to the surface, in and on sediments. The sulfate reducers would have reduced sulfate into hydrogen sulfide, via anaerobic respiration. This was an important form of microbial metabolism on Earth up to around 2.5 billion years ago

before there was oxygen in the atmosphere. Today they are found deep in sediments or at the bottom of the Winogradsky columns. Once the cyanobacteria evolved, producing oxygen as a byproduct of photosynthesis, oxygen would have accumulated in upper sediments, water and the atmosphere. This would have caused stratification in the environment similar to that observed in the Winogradsky columns. Oxygenic microbes at the top and anoxic microbes at the bottom. The Winogradsky columns are self-contained ecosystems where there are a range of microbes converting and cycling nutrients.

Variations

- Use other combinations of nutrients – egg yolk + newspaper, egg yolk + eggshell, newspaper + eggshell
- Increase the iron available to encourage iron-oxidising bacteria by using iron-rich sand or by adding iron sulfate
- Use different sources of sediments
- Change the pH outside the normal range of pH 6-8 for most sulfur reducing bacteria
- Increase the temperature to encourage thermophiles