



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.



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

- Camera
- Gloves for each group member
- 1 x trowel
- 1 x large colander
- 1 x large container (8-10L)
- Bottle with lid

Assemble the Winogradsky Columns

- 8-10 litres of sediment from sample area (see collect the sample method)
- Water from sample area (see collect the sample method)
- Cover for bench area
- 10 x small labels
- Permanent marker
- 5 x clear bottles of the same size with lids
- 5 x medium containers
- Gloves for each group member
- Measuring cup
- 1 x cup of shredded newspaper (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
- 1 x stick or rod
- 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.
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 $\frac{3}{4}$ of the bottle it will be transferred to.
7. Add $\frac{1}{2}$ 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 $\frac{1}{2}$ 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 $\frac{1}{2}$ cup of loosely packed shredded newspaper, the yolk of an egg and $\frac{1}{2}$ 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.
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 $\frac{3}{4}$ 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.



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 - Weekly Observations

Table 2: Observations Week 0 (start of experiment) – Date _____

Column	Observations of the side of the column exposed to:	
	Light	Dark
control		
carbon		
sulfur		
calcium carbonate		
carbon + sulfur + calcium carbonate		

Table 3: Observations Week 1 – Date _____

Column	Observations of the side of the column exposed to:	
	Light	Dark
control		
carbon		
sulfur		
calcium carbonate		
carbon + sulfur + calcium carbonate		



Table 4: Observations Week 2 – Date _____

Column	Observations of the side of the column exposed to:	
	Light	Dark
control		
carbon		
sulfur		
calcium carbonate		
carbon + sulfur + calcium carbonate		

Table 5: Observations Week 3 – Date _____

Column	Observations of the side of the column exposed to:	
	Light	Dark
control		
carbon		
sulfur		
calcium carbonate		
carbon + sulfur + calcium carbonate		



Table 6: Observations Week 4 – Date _____

Column	Observations of the side of the column exposed to:	
	Light	Dark
control		
carbon		
sulfur		
calcium carbonate		
carbon + sulfur + calcium carbonate		

Table 7: Observations Week 5 – Date _____

Column	Observations of the side of the column exposed to:	
	Light	Dark
control		
carbon		
sulfur		
calcium carbonate		
carbon + sulfur + calcium carbonate		



Table 8: Observations Week 6 – Date _____

Column	Observations of the side of the column exposed to:	
	Light	Dark
control		
carbon		
sulfur		
calcium carbonate		
carbon + sulfur + calcium carbonate		

Table 9: Observations Week 7 – Date _____

Column	Observations of the side of the column exposed to:	
	Light	Dark
control		
carbon		
sulfur		
calcium carbonate		
carbon + sulfur + calcium carbonate		



Table 10: Observations Week 8 – Date _____

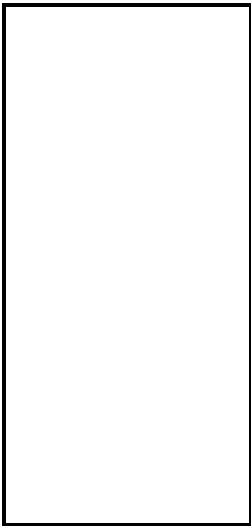
Column	Observations of the side of the column exposed to:	
	Light	Dark
control		
carbon		
sulfur		
calcium carbonate		
carbon + sulfur + calcium carbonate		



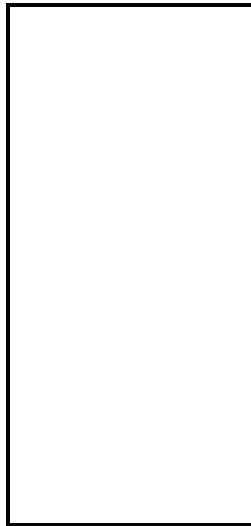
Monthly Observations – Labelled Diagrams of Columns

Month 1: Date _____

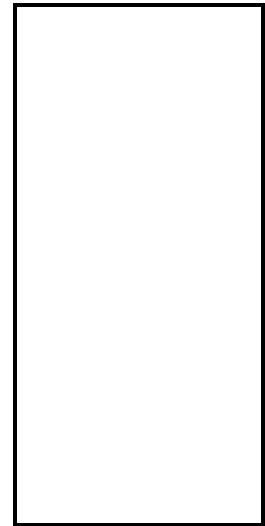
Side of column exposed to the light



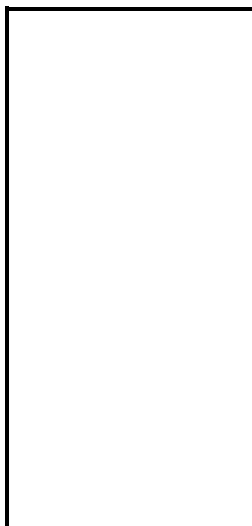
control



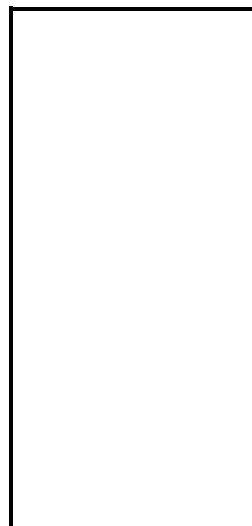
carbon



sulfur



calcium
carbonate



carbon, sulfur
and calcium
carbonate

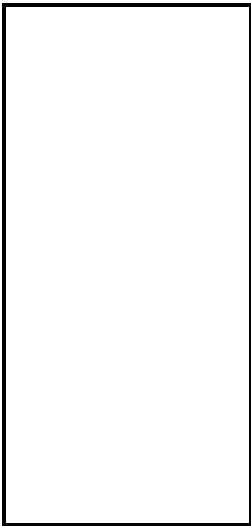
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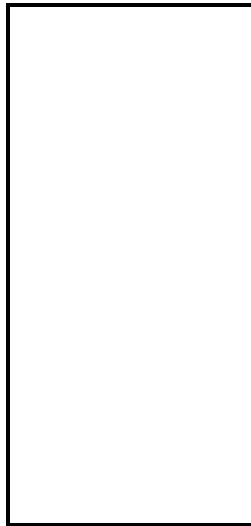


Month 1: Date _____

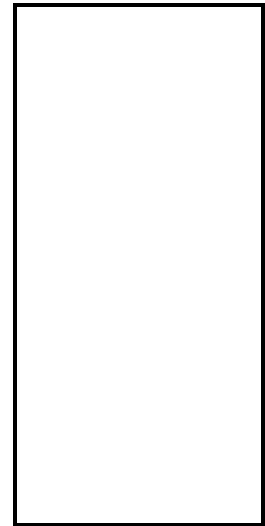
Side of column exposed to the dark



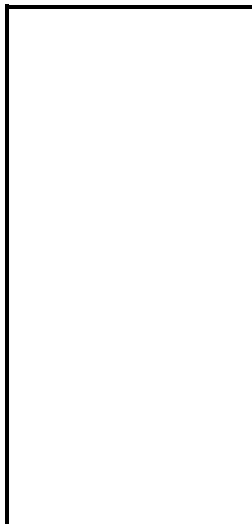
control



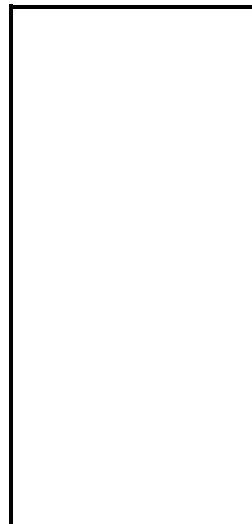
carbon



sulfur



calcium
carbonate



carbon, sulfur
and calcium
carbonate

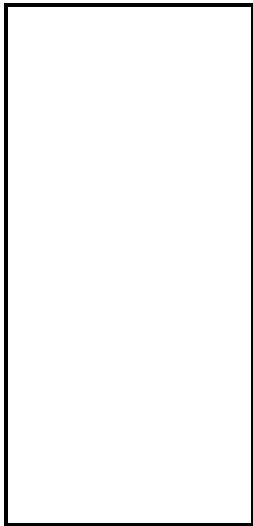
Resourced by



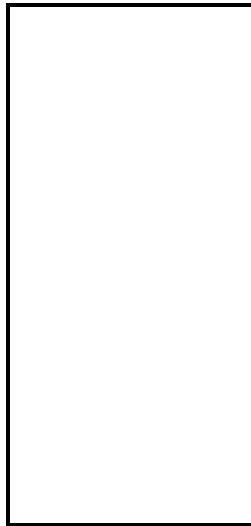


Month 2: Date _____

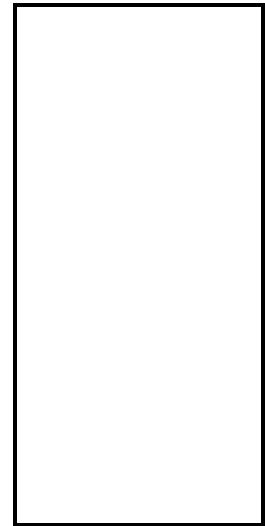
Side of column exposed to the light



control



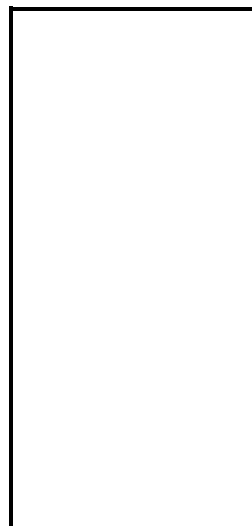
carbon



sulfur



calcium
carbonate



carbon, sulfur
and calcium
carbonate

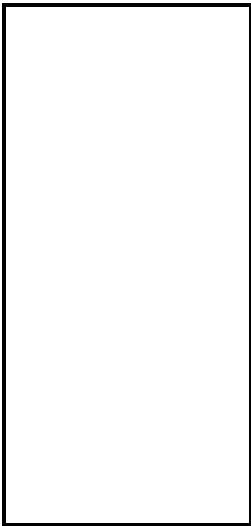
Resourced by



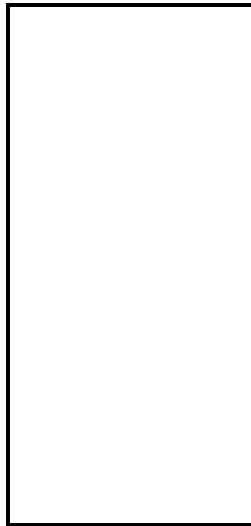


Month 2: Date _____

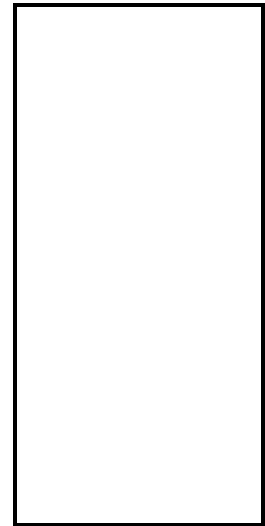
Side of column exposed to the dark



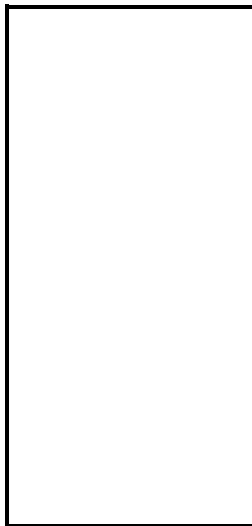
control



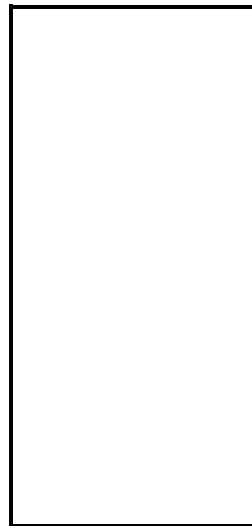
carbon



sulfur



calcium
carbonate



carbon, sulfur
and calcium
carbonate

Resourced by





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 differences and similarities observed in the columns.

2. Explain the differences and similarities observed in the columns.

3. Explain the need for the control column and any changes observed in it.

4. Oxygen concentration gradients form in Winogradsky columns. Predict the distribution of oxygen throughout the entire column (air, water and sediment).

5. Explain where oxygenic photosynthetic organisms, such as cyanobacteria, could be expected to be found in the columns.



6. Explain where anoxygenic photosynthetic organisms, such as purple sulfur bacteria, could be expected to be found in the columns.

7. Explain the role of microorganisms, such as those in the Winogradsky columns, in underwater hydrothermal vent and underwater volcanic ecosystems.

8. Explain how the Winogradsky columns can be used as a model of life on early Earth.
