Winogradsky Column Procedure

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1 MATERIALS

- Sediment and water sample
- Gloves
- Goggles
- Sharpie
- 1000 ml graduated beaker
- Mixing spoons or rods
- 250 ml tissue culture flask
- 300 ml diatomaceous earth (DE)
- 300 ml freshwater inoculum from sediment
- 0.5 g shredded paper towel (cellulose)
- 0.5 g Na_2SO_4
- 0.5 g $CaCO_3$
- 0.5 g NH_4Cl
- 0.5 g $K2PO_4$

2 PROCEDURE

1. You will be given a water and sediment sample in the laboratory that was obtained from a lake, pond, or stream. Please keep a record of the source. Once you have obtained the sample containing approximately 100 ml sediment and 400 ml water, you should gently agitate it so the microbes will be dislodged from the sediment particles. Let the large particles settle for 10 minutes before adding to beaker with the DE.
2. While you are waiting for the particles to settle, prepare your column. Add 0.5 g of paper towels (cellulose) and 0.5 g $CaCO_3$ to the tissue culture flask.
3. Measure 300 ml of DE in a graduated beaker. DE is an irritant to your eyes, skin, and lungs. Please wear goggles and gloves when handling DE. Add 0.5 Na_2SO_4, 0.5 g NH_4Cl, and 0.5 g K_2PO_4. If you are not making one of the controls, add any other additional chemicals as instructed by your TA. Slowly pour 300 ml of the prepared inoculum into the beaker, stirring frequently. Avoid pouring in the sediment. The consistency of the slurry will be similar to a milkshake. If additional water is needed, add DI water.
4. Pour the DE slurry from the beaker into the tissue culture flask containing the cellulose and the $CaCO_3$. Pause frequently to stir the slurry in the beaker so the DE remains suspended. Stop pouring when the slurry is 2 cm below the mouth of the flask. Cap the flask and tap the sides in order to release the air bubbles. The DE will compress overnight, leaving a layer of water above.
    a. Any container can be used, as long as its tall and clear (disposable water bottle, mason jar, etc).
    b. Instead of chemicals, you can use an egg yolk or Epsom salts. Add 1 egg yolk (raw or boiled) or 1 tbsp Epsom to 6 cups of mud.
5. Please label the side of your column with your name, date, lab time, day, and control **or** the experimental variable of interest.
6. Take the column home and incubate it in sunlight (unless the variable you are testing is no light). You will need to loosen the lid at home so the column does not become anaerobic. After a few weeks, you should notice a visible change in the column. You will need to upload a picture every week by the due date share with your lab section.

Adapted from: Benoit, T.G. 2015. Increase the visibility of microbial growth in a Winogradsky column by substituting diatomaceous earth for sediment. J. Microbiol. and Biol. Ed. 16:85-86.