SES Microbial Methods
Winogradsky Column

7 Sept 2017

This lab will be used to construct a Winogradsky column from sediments and water collected from Little Sippewisset Marsh, MA. Since it takes several weeks to months for the microbial communities to develop in these columns, the lab and lectures describing the microbial processes at work will be occur in Lab/Lecture 5 (week 5/6) to allow time for the community to develop.

A Winogradsky column, named after the Russian microbiologist Sergei Winogradsky, is easily constructed from lake, river, or estuarine water and sediments. As oxygen is depleted in the column by microbial respiration, microenvironments arise which foster the development of a diverse community of microorganisms that are able to catalyze extraordinary biogeochemical reactions. These columns will be used to demonstrate photoautotrophic, chemoheterotrophic, photoheterotrophic, and chemoautotrophic growth. In particular, bacteria capable of sulfate reduction, anaerobic photosynthesis, fermentation, sulfide/sulfur oxidation, iron oxidation, and methanogenesis will develop and flourish within the column. We will also use these columns as a surrogate for field-based sampling during the course.

Materials (Supplied)

- Winogradsky column, ~1400 mL, with 5 sample ports.
- Carbon sources: cotton (cellulose), wool (keratin protein) or chitin (crushed lobster shells)
- Inorganic compounds: $CaSO_4$, $CaCO_3$, NH_4Cl, PO_4
- Bucket

Field Site: Little Sippewisset marsh, Falmouth.

Procedure:

- Collect enough ***sandy*** sediments from Little Sippewisset estuary to fill ALL saltwater columns about half way (~700 mL ea) and place your sediments in ONE large container with the other student's and mix. Do not use muddy sediments, as they will plug sample ports.
- Remove ~700 mL of sediments from above and place in separate beaker with carbon source and inorganic compounds (see Treatments for amounts).
- Mix sediments and additives with enough water to produce slurry and poor/scoop into column. Shake or mix sediments to remove large air pockets from column. Sediment-water interface should be just above port #2 from top.
- Collect water sample in 1 l bottle to add to column when back at lab.
- At lab, top off column with collected water and place in light (except for dark treatments).

- Over the next five weeks, observe color development and smell. Are the anaerobic photosynthesizers visible? Can you identify the location of the aerobic-anaerobic interface?

Treatments:

1. Control, no additions
2. 15 g cotton, 5 g $CaSO_4$, 5 g $CaCO_3$, 25 mg NH_4Cl, 10 mg K_2HPO_4
3. 15 g cotton + Carbon Fiber, 5 g $CaSO_4$, 5 g $CaCO_3$, 25 mg NH_4Cl, 10 mg K_2HPO_4
4. Same as 2., but place in the dark.
5. Same as 2., but place in the dark.
6. 15 g chitin, 5 g $CaSO_4$, 5 g $CaCO_3$, 25 mg NH_4Cl, 10 mg K_2HPO_4
7. 15 g chitin, 5 g $CaSO_4$, 5 g $CaCO_3$, 25 mg NH_4Cl, 10 mg K_2HPO_4
8. 15 g wool, 5 g $CaSO_4$, 5 g $CaCO_3$, 25 mg NH_4Cl, 10 mg K_2HPO_4
9. 15 g wool + Carbon Fiber, 5 g $CaSO_4$, 5 g $CaCO_3$, 25 mg NH_4Cl, 10 mg K_2HPO_4
10. *Freshwater*: Control, no additions
11. *Freshwater*: 15 g cotton, 5 g $CaCO_3$, 25 mg NH_4Cl, 10 mg K_2HPO_4
12. *Freshwater*: 15 g cotton + Carbon Fiber, 5 g $CaCO_3$, 25 mg NH_4Cl, 10 mg K_2HPO_4

Although we will be covering this experiment in week 6, you might want to look at the following web site that describes Winogradsky columns:
http://www.biology.ed.ac.uk/research/groups/jdeacon/microbes/winograd.htm

Some results from a previous year's class can be found at:
http://ecosystems.mbl.edu/SES/MicrobialMethods/Winogradsky/default.htm