

ALGAL REGROWTH EXPERIMENT

EQUIPMENT

Field:

20 L carboy
153 μm prefilter
Van Dorn
1 L brown nalgene bottle
Small funnel

Lab:

Sputnik 90 mm filter apparatus
90 mm GF/A filter
Masterflex peristaltic pump
20 L carboy – designated regrowth carboy
3 2 L polycarbonate bottles
75 μm prefilter
1 L brown bottle (in addition to one from field)
Low temperature incubator set up with lights and air bubble lines (see appendix)

REAGENTS

Alga-Gro concentrated medium from Carolina Biological Supply

A: Collect sample water

1. Rinse the carboy 3 times with prefiltered lake water
2. Fill the carboy with ~10 L of water from the center of the lake at a depth of ~1 m using the Van Dorn and prefiltering through 153 μm mesh.
3. Fill 1L bottle with water from 1 m—no need to prefilter.

B: Filter water and inoculate

1. In the lab, use the pump and the Sputnik with a 90 mm GF/A to filter the water from the carboy and into the three 2 L polycarbonate bottles. Be sure to rinse each bottle 3 times before filling. Fill to the shoulder of the bottles. (Tubing should go from carboy into IN of pump, OUT of Pump to top of Sputnik, bottom of Sputnik to bottles.)
2. Filter water from brown bottle through a 75 μm prefilter and into a clean brown bottle (or directly into the graduated cylinder).
3. Dump the water from the 3 bottles into the designated regrowth carboy (this is different than the one you used in the field).
4. Add 14 mL of Alga-Gro to the carboy (that's about 2 mL per L).
5. Inoculate the sample with 150 mL of water from bottle in step 2.
6. Mix sample in carboy by swirling.
7. Take initial sample of Chl and dD-water.
8. Fill each 2 L bottle with water.
9. Place bottles in the incubator and insert air stones—make sure that each one is bubbling.
10. Fill out log sheet.

C: Monitoring

1. Check on the samples every day and fill out log sheet.
2. Make sure air stones are bubbling.
3. Rotate the position of the bottles (i.e. move bottle 1 to back and move 2 and 3 forward).
4. Swirl each bottle to mix the algae that have settled.
5. Every few days, sample for Chl in order to monitor the growth rate. When sampling for Chl or anything else, combine the water for all 3 bottles into the designated Regrowth carboy.
6. Check for dripping and pooled water and change the paper towels on the top shelf if necessary.

D. Finish

1. When enough algae has grown, you can end the run.
2. Combine water from bottles into the carboy and take a final sample of Chl, dD-water, and dD-POC.

Appendix (Incubator setup):

I used the Fisher low temperature incubator in the common lab, the one on the right. On the underside of the top rack, I hung 3 18" fluorescent light fixtures, one with a warm bulb, one cool bulb, and one plant growing bulb. I hung two clip lamps (one on each side) with a 100 watt compact fluorescent bulb in both. The sample bottles were placed on the second shelf under the lights. All of the lights were plugged into a power strip that was resting on top of a test tube rack (in order to keep it off of the bottom and prevent it from getting wet) on the bottom of the incubator. The power strip was plugged into an on/off timer so that the lights would atomically turn on and off at the desired times. The timer was plugged into an extension cord that was plugged into the orange outlet behind the incubator (thus giving it backup power). The two shelves were covered with aluminum foil to contain the light within the bottle area, and to help prevent water from dripping onto the lights and power strip (water typically condenses below the chiller and drips down below). On the top shelf an air pump was connected to two lines that dropped into the bottles and which had air stones attached. Each line was split once giving 4 bubbling lines, one which we didn't use. A 0.2 μ m 25mm Nalgene disposable syringe filter was connected to each air line to filter out any contaminants. The filters were connected via the anti-siphon connectors that came with the pump. Other adapters could be used but these fit and were convenient. The air pump was plugged into the outlet that is built into the incubator and not the power strip. Otherwise, the air would turn off with the lights. To prevent any water related problems, the air pump and power strip were covered with aluminum foil and plastic respectively. Also, paper towels were placed on the top shelf to absorb the water. These were changed frequently.