



Algae to Energy Systems Student Lab

Your Challenge

Imagine your laboratory has just received grant funding to conduct research on the optimal growing conditions for *Chlorella*, a freshwater green algae species. Formulate at least two testable questions that you could use to drive your research.

What variable could you manipulate to examine one of your questions? Could you examine this variable at multiple levels?



Independent Variable:

What could you measure to examine the effects of your independent variable?

Dependent Variable:

Create a hypothesis that links your independent and dependent variable together in the context of algae: "If independent variable, then dependent variable."

Example: If we increase hours of light the algae receives, then the optical density will increase.



Materials and Methods

BTI Will Provide: (per class of 32 students, 8 groups of 4)

- Sterile Toothpicks
- Sterile cotton balls
- 32 air stones
- 4 packs of aquarium tubing
- 4 air pumps
- 4 four-way gang valves
- 12 T-valves
- Aquarium salt
- Urea
- Glucose
- 8 Transfer pipets
- 1 compressed air can
- 8 hemocytometers
- culture of *Chlorella protothecoides*

You will need to provide:

- Chlorox[®] disinfectant wipes – *OR* – Paper towels and a store-bought liquid disinfectant (e.g., Lysol[®] All-Purpose Cleaner, 32 fl. oz., trigger-bottle) containing the microbicide alkyl dimethyl benzyl ammonium chloride
- Power drill with 13/64 inch drill bit
- Ruler
- Scissors
- Permanent marker
- 30 or more 500 mL clear plastic unopened water bottles
- 100 mL graduated cylinder
- Balance (capable of measurement to 0.1 g)
- Weigh boats
- Light bank or fluorescent lamps
- Aluminum foil, baking soda, pH paper (optional – depends on experiment variables)
- Bleach

Safety Information

Listen to all laboratory instructions and wash your hands prior to and after working with algal photobioreactors. Review MSDS sheets for any chemicals with which you are using. **PLEASE NOTE:** *Upon completion of the laboratory, the algae cultures should be properly disposed of by adding 4.7 mL, undiluted bleach per bioreactor, swirl gently to mix, and let stand for 10 min. before pouring down the drain.*

Construction of the Photobioreactor

1. Before starting, select an appropriate workstation (e.g., benchtop or desk).
 Preferably, to avoid possible contamination of the photobioreactor during or following construction, the workstation should be removed from windows, doors, and/or overhead airconditioning and heating vents that permit airflow – and associated airborne microbes (e.g., fungi and bacteria) – over the worksurface.
2. The chosen workarea should be approximately 4 ft² (0.4 m²) and void of general clutter.
3. Wipe down the work surface thoroughly using a disinfectant wipe or the liquid disinfectant and paper towels. Discard used cleaning materials to trash.
4. Prepare a “clean zone.” Once the worksurface is clean (sanitized), arrange four, unused disinfectant wipes (2x2) on the center of the worksurface (see below).
 Alternatively, unfold 4 pieces of paper towel and moisten with the liquid disinfectant – enough to make the towels stick to the work surface. All material and tools (e.g., scissors) entering “the clean zone,” illustrated below, must be wiped down with “fresh wipes” or liquid disinfectant.

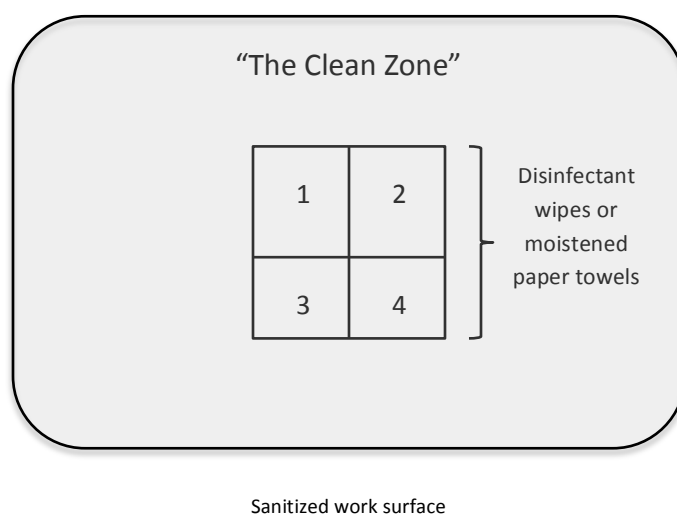


Fig 1. Illustration of a “Clean Zone” prepared for constructing the photobioreactor

5. Remove all paper/plastic labeling or decals from the clear plastic water bottle and, then wipe down the bottle with a “fresh wipe” or liquid disinfectant, making certain to clean the bottle cap and neck. Place bottle into your “clean zone”
6. Using permanent marker, label the neck of the water bottle with the date, assigned treatment, bottle number, and your names/initials (for example: 3/14/14, No Urea #2, JD [Jan Doe])
7. Using a 100 mL graduated cylinder, decant 70 mL from the water bottle; cap and seal immediately after decanting. Leave the remaining 430mL of water in the bottle.
8. Using a scale, weigh boat, and scoopula measure out the growth constituents (nutrients) you wish to add to the photobioreactor. We suggest:
 - a. **0.2 g aquarium salt (final concentration= 4.3×10^4 g/mL)** — Aquarium salts provides algae with the metals and ions needed for sustained growth.
 - b. **0.8 g urea (final concentration= 1.7×10^3 g/mL)** —Urea is a form of nitrogen; providing a nitrogen-rich environment necessary for the growth of algae.
9. Carefully fold the the weight boat on itself and add growth constituents to the water bottle, making sure NOT to touch the bottleneck with the weigh boat.
10. Cap and seal immediately, and shake the nutrient-water solution for at least 2 min. until the constituents have completely dissolved. Set aside and in “clean zone.”
11. Wipe down (surface sanitize) tubing for the airline and vent, and using separate wipes, the drill and drill bit. Place the tubing onto the moistened disinfectant wipes in your “clean zone.”
12. Cut-to-length (Figure 1, A):
 - a. 1, 2 ft piece of aquarium tubing (Aeration)
 - b. 1, 3 in. piece of aquarium tubing (Vent)
 - i. Wipe down each length of tubing and place in “clean zone”
13. Prepare the vent. (Figure 1, A and B). Pinch and remove a small piece of cotton, and using a sterile toothpick, force (stuff) the cotton approximately 1 in. into the tubing.
14. Drill two holes (diameter= 13/64 in.) into the cap of the bottle. Don’t worry about shavings falling into the nutrient-water solution. (Skip this step if teacher is pre-drilling the bottles)
15. Take the tip of the aeration tube and forcefully insert the tubing through one of the previously-made holes in the bottlecap and feed approximately 1.5 ft of tubing through the cap and attach a sterile airstone to the tubing inside the bottle (Figure 1, C).
16. Carefully wipe the length tubing between the cap and airstone with a “fresh wipe,” making sure keep the tubing and airstone off the work surface and away from conacting anything in the work area.
17. Carefully insert airstone-airline and seal the bottlecap. Strip out of the bottle any slack in the airline. The airstone should be gently touching the bottom-center of the bottle.

18. Insert vent tubing into the remaining hole and position the vent at a depth of no more than ¼ in. into bottle.
19. Quickly open the bottle, and using a sterile seriological or sterile transfer pipet, transfer 1 mL (2.5×10^3 cells) from the alga stock culture to the bottle – AVOID touching the pipet tip to the bottle's lip or neck.
20. Attach the free-end of the airline tubing to one of the spigots on the four-way gang valve or T-valve
21. Connect the four-way gang valve to the air pump with a 6 inch piece of airline tubing. Plug in the air pump and adjust the gang valve so that it appears that each bottle is getting equal air.
22. Place the photobioreactors in a well lit area (e.g. under two, equi-spaced lamps).
23. Follow your teachers instructions on what data to collect from your bottle, and how that is to be done. Before taking a reading, you should always swirl your sample for at least 30 seconds to ensure that all algae is mixed thoroughly throughout your sample, so your results are more consistent. Take an initial reading from your bottle and record it in your data table and in the class data table.
24. Over the next 7 days, evaluate and compare the estimated effect(s) of the individual treatments and/or treatment combinations on algae growth.

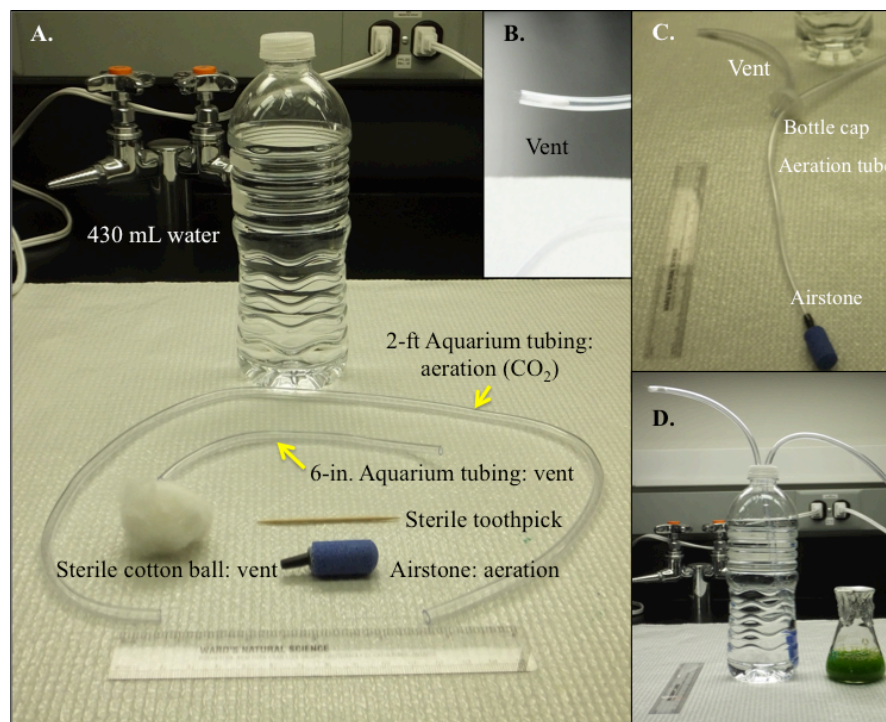


Figure 1. Construction basics for an algal bioreactor: (A) materials per reactor; (B) vent; (C) assembled components – vent, bottle cap, and aeration tubing with attached airstone; and, (D) bioreactor upon completion, ready for algae.

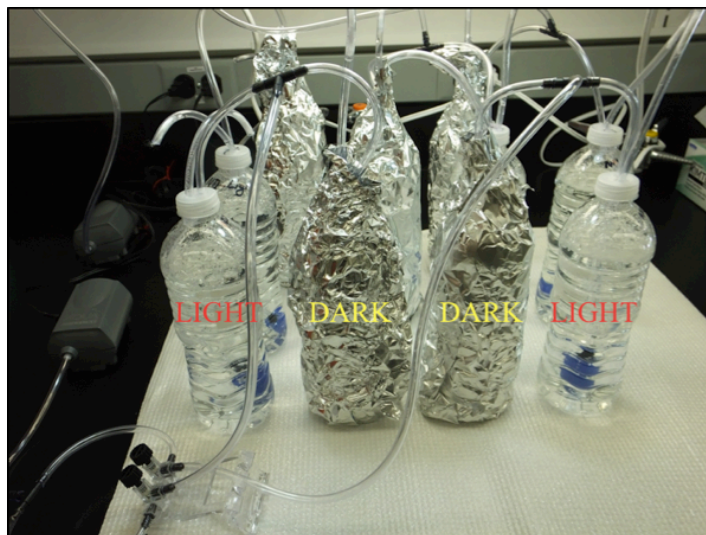


Figure 2 . General setup of a 4-bottle bioreactor system, varying only light regime(24:24 h, light:dark) across reactors.

Observations & Data Collection

Condition	Bottle	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Notes:
A=	1								
	2								
	3								
	4								
B=	1								
	2								
	3								
	4								
C=	1								
	2								
	3								
	4								
D=	1								
	2								
	3								
	4								



Questions

How many algal cells did you have on day 1 for bottle number _____?

How many algal cells did you have by day 7 for bottle number _____?

Graph & Analyze

Create a graph that displays your results.

Do you notice any trends in your data? Which condition supported the greatest growth? Which supported the least growth? Why might this be?

How much variability was there among the bottles in the same condition? What may have led to variability among the replicate bottles? How might this influence your understanding of your results?

If you were to perform this lab again, what changes would you make to explore your independent variable more fully? Propose an idea for future research.



Conclusions

Based on the results of your research, what recommendations could you make to a company asking you about the growing requirements of the green algae *Chlorella*? Provide evidence to support your claim.

How might this information impact the company's ability to make biodiesel sustainably when thinking about the environmental or economic impacts of production?