EXPERIMENT 2: Maximizing and Collecting Algae Biomass for Fuel Production
modified for this workshop from “Creating Diesel from Algae” by Mark Townley,
(http://kenanfellows.org/curriculum)

Introduction:

In this activity, we will look at the growth of algae as a potential source of significant lipid production to consider it as a biofuel option, and utilize your understanding of cell parts and plant cell function to learn why organisms make fatty acids that can be converted into a biodiesel. We will be using (1) a common classroom algae, freshwater Chlorella, as well as (2) algae collected from the Charles River or other fresh water sources to practice the techniques used to grow and process algae for possible use as a biofuel. A different salt water algae, Dunaliella salina, has been used in a current study underway at North Carolina State University that looks at the growth of that organism as a biofuel for jets.

There are two options for the media that you will use to grow the algae in:
The first option is to use the treated wastewater from the Deer Island Treatment Plant to show how growing biomass from this water source could also help to further filter local lakes or rivers.
The second option, and the method we will be demonstrating and using to produce our biomass, is to use a sterile high quality media (alga-gro) commonly used for growing fresh water algae.

In your classroom, this activity would be completed over a period of 10 days, and includes initial set-up, periodic observations, and harvesting the algae product. The best time to harvest is between days 8-10, depending upon whether the green color is still darkening. To speed up the process for this workshop, stock Chlorella samples were set up 5 days ago in sterile media for each team. You will observe the algae growth for the first three days of the workshop and you will also be setting up bottles using the sterile technique described so that you can take them home to try to maximize the algae production over time, or use as a stock source for your classroom. Harvesting occurs over two days with the first day (day 4 or workshop) taking between 45-60 minutes depending on how fine the filter is and how well it drains. Once dry, massing and burning the algae and the control take approximately 20 minutes on the second day (day 5 of workshop).

Visit the link for a science article on the topic: www.tamu.edu/faculty/tpd8/BICH407/AlgaeBiodiesel.pdf

Learning Objectives:

• Students will gain a deeper understanding of the functions of the cell’s organelles and their role in the production of lipids
• Students will discuss how the production of algae as a biofuel could reduce our reliance on foreign oil and neutralize the carbon emissions from burning gasoline.
• Students will apply their knowledge of energy production during photosynthesis and aerobic respiration.

Materials: Per team of three
• Sterile stock of Chlorella grown in medium.
• 500mL clear plastic water bottle (Aquafina brand bottles work best with aquarium hood overhead lighting)
• Light source
• Fertilizer (Miracle Grow or Schultz’s fertilizer)
• Charles river water, or sludge water or other water sources
• Net tow (for plankton-enriched samples)
• Aquarium with light in hood or other fluorescent light source
• Timer to plug light source into for 16 hour light/8 hour dark cycle
• Glass stirring rods
• Digital balance
• Note: Make sure the aquarium you choose is large enough to provide space between the water bottles to allow for the most light to come in, and place white paper around the tank to reflect light throughout. Adding more light will maximize growth. A metal grate shelf can allow for more space between bottles.

Growth Options:

There are two media options for this lab.

Option 1: non-sterile water
This option requires the collection of water from a local Wastewater Treatment plant. The students will use 500ml (16 oz.) water bottles filled with the treated wastewater to create a photo bioreactor to grow algae in. The merits of a “closed” system can be considered here: algae would use the nutrients from the wastewater that would further filter the water before it is returned to out lakes and rivers.

Algae have been successfully grown without sterile technique being used at any stage of the creation of media or the growth process. Simply add the algae from the container to the water bottle using a 10mL pipette and the hand crank or automatic pump. You may want to look at the algae under a microscope when harvesting to see how much, if any, contamination occurred.

Non-sterile option
1. Treated Wastewater
2. Distilled Water (tap water is okay)
3. 1 M NaCl (14.612g in 250 mL)
4. Epsom Salts (MgSO4) 0.3g
5. Hand crank pump for pipettes
6. 10 ml pipette
7. 2 Molar Sodium hydroxide
8. Filter paper or paper towels
9. Ring Stand
10. O-ring
11. Funnel
12. Large Beaker

**Option 2:** Sterile solution of Alga-Gro medium for freshwater algae

**Procedure for adding algae with sterile technique to the water bottle photo bioreactor:**

1.) See video of adding algae with sterile technique if you would like to see this procedure being performed.
2.) Spray closed container of algae, water bottles, and all necessary equipment (including hands) with 70% ethanol and place in sterile zone.
3.) Place all the water bottles and equipment nearby a Bunsen burner and then light the Bunsen burner to create a 10cm diameter sterile zone. **CAUTION: Do not spray ethanol around a lit Bunsen burner!**
4.) Loosen cap of water bottle but do not remove.
5.) Swirl the algae container so that each group is getting the same approximate amount of algae. Loosen the top of the algae without removing it.
6.) Open the top of the sterile pipette packaging where you will be attaching the pipette to the hand crank pump. **DO NOT TOUCH THE PIPETTE OR LAY IT DOWN.** Keep the pipette in the sterile field.
7.) Open algae container in the sterile field and use 10mL sterilized pipette and a hand crank pump pipette to withdraw 13mL of algae (max that 10mL pipette will hold).
8.) Take the cap off of the water bottle and release the algae into the bottle from the pipette. Hold the cap near the bottle in the sterile field with one hand while you release the algae into the bottle.
9.) Seal the bottle without touching inside the cap and gently swirl the algae to spread them throughout the medium.
10.) Place in aquarium and loosen the cap WITHOUT REMOVING to allow for venting of gas exchange.

Note: Algae have been successfully grown without sterile technique being used at any stage of the creation of media or the growth process. Simply add the algae from the container to the water bottle using a 10mL pipette and the hand crank pump. You may want to look at the algae under a microscope when harvesting to see how much, if any, contamination occurred.
Procedure for growing the algae:

1.) The timer for the fluorescent lighting should be set on a 16 hour light/8 hour dark schedule. Make sure that the light schedule is during daylight hours as well to take advantage of as much light as possible.
2.) Place the water bottles in an aquarium with a fluorescent hood light. If possible, place the lights on the back of the tank so that the light is not blocked by the caps on top. Feel free to experiment with different lighting options to see what light amounts grow the most algae. A typical aquarium starter kit should be sufficient for growing enough algae to harvest.
3.) Students should tighten cap, gently swirl, and then loosen the cap again daily in order to redistribute the algae and the medium.
4.) Record daily re-suspension and observations on the attached Algae Photo-bioreactor Daily Checklist. Observations should include color changes and settling or appearance changes from one day to the next.

Procedure for removing and drying the algae (Harvesting):

1.) Re-suspend the algae by swirling the algae in the bottle. Make any final observations and record them on your growing log.
2.) Remove the cap on the water bottle and add a drop at a time of Sodium hydroxide 2M solution with a plastic disposable pipette until the algae has flocculated into visible clumps. If you are using the treated wastewater, then 26-30 drops of 2M NaOH will be sufficient to make the algae flocs large enough to be caught by the paper towel filter. If you are using the high quality media, it will take 90 drops because the media is buffered. Place the algae aside and let it settle and clump as much as possible on the counter without re-suspending it.*
3.) Once the algae have flocculated it should be large enough for it to be filtered using a paper towel or coffee filter. Students can make a funnel shape while holding the paper towels over a large beaker, and simply pour the water through the paper towel slowly. Dip a clean coffee filter into the filtered media below to be used as a control. If you would like to determine how much algae each group grew, then follow instructions 4-9 below. Otherwise you may skip to number 9.
4.) Set up a ring stand with an o-ring large enough to hold a funnel.
5.) Place a funnel in the o-ring and raise it high enough to place a jar below the funnel to catch the medium as it filters.
6.) Fold the round piece of filter paper in half and then half again to make it pie-shaped. Open an end of the filter to make a cone shape and place that inside of the funnel hanging from the o-ring.
7.) Very slowly pour the algae media into the filter cone.
8.) Dip a clean piece of filter paper into the filtered media that is in the jar below to be used as a comparison for mass. Let the algae and the control filter paper dry in an oven at 30-40 degrees Celsius for a few hours, or on the counter over a couple of days and then mass the filter paper again. Subtract the mass from the clean filter paper to the algae-coated filter to figure out the mass of algae.
9.) Once the algae coated paper and the control paper has dried completely, you are ready to burn. Using a fume hood or by taking the class outside, the teacher should place the control paper and the algae coated paper in separate glass jars. Light the paper with a long match or with a fireplace lighter. Have the students make observations of the appearance of the different papers before, during, and after burning and the color of the flame. When the control paper is lit, you will notice that it burns extremely quickly and usually leaves a whole black piece behind. When the algae paper is burned, the flame is much slower and steadier and leaves behind a brittle, gray ash. This means that the algae coated paper burns much hotter than the control paper which suggests that the algae is producing lipids and starches that are maintaining and fueling the initial flame.

Flocculation is a necessary step in the large scale production of algae as a biofuel. Salt water algae shows much promise as a biofuel option because the salt water is higher in Magnesium ions which cause the algae to flocculate naturally once the pH rises above 10 without the need for chemical additive. This could save millions of dollars in the production cost of algae as a biofuel.

Link to the organisms we found in the algae samples that we grew this week (using pond water source)
http://www4.uwm.edu/fieldstation/naturalhistory/bugoftheweek/semi-aquatic-springtails.cfm