

Waterford Fermenters

Designed and created by C. Kohn, Dept of Agricultural Sciences, Waterford WI. Based on an original design by the Great Lakes Bioenergy Research Center of the US Department of Energy and the Madison Area Technical College, Madison, WI



Background: Waterford Fermenters are miniaturized versions of real research apparatuses that were designed to be used primarily for fermentation instruction. Because similar models from science supply catalogs cost upwards of hundreds of dollars, efforts were made to produce a similar product at a much lower cost. The Waterford Fermenter utilizes materials that are easily available to any instructor who has access to an internet connection and a hardware store. The total cost can be reduced to less than \$20 per unit (especially when materials are bought in bulk), enabling a course with typical funding to purchase and build multiple units for each student group in the class (ideally, one unit per group of 4).

This particular design is meant to be paired with Vernier hardware, but the design could easily be adjusted to fit different probes. A magnetic stirrer is necessary for many activities associated with this piece of equipment. A low cost option for building a magnetic stirrer is included if a classroom does not have an adequate supply of standard stirrers.

Waterford Fermenters were designed to allow for controlled conditions in a closed environment. This enables an instructor to create ideal conditions for students to test and investigate their hypotheses on key biological processes ranging from respiration and photosynthesis to fermentation and decomposition. The Waterford Fermenter is ideal for a wide range of model organisms, including yeast (*Saccharomyces cerevisiae*), *Daphnia magna*, *Elodea*, *Drosophila melanogaster*, Wisconsin Fast Plants, radishes, and many others.

The Waterford Fermenter design is ideal for any instructor looking for a low cost, durable option for effective inquiry-based instruction.

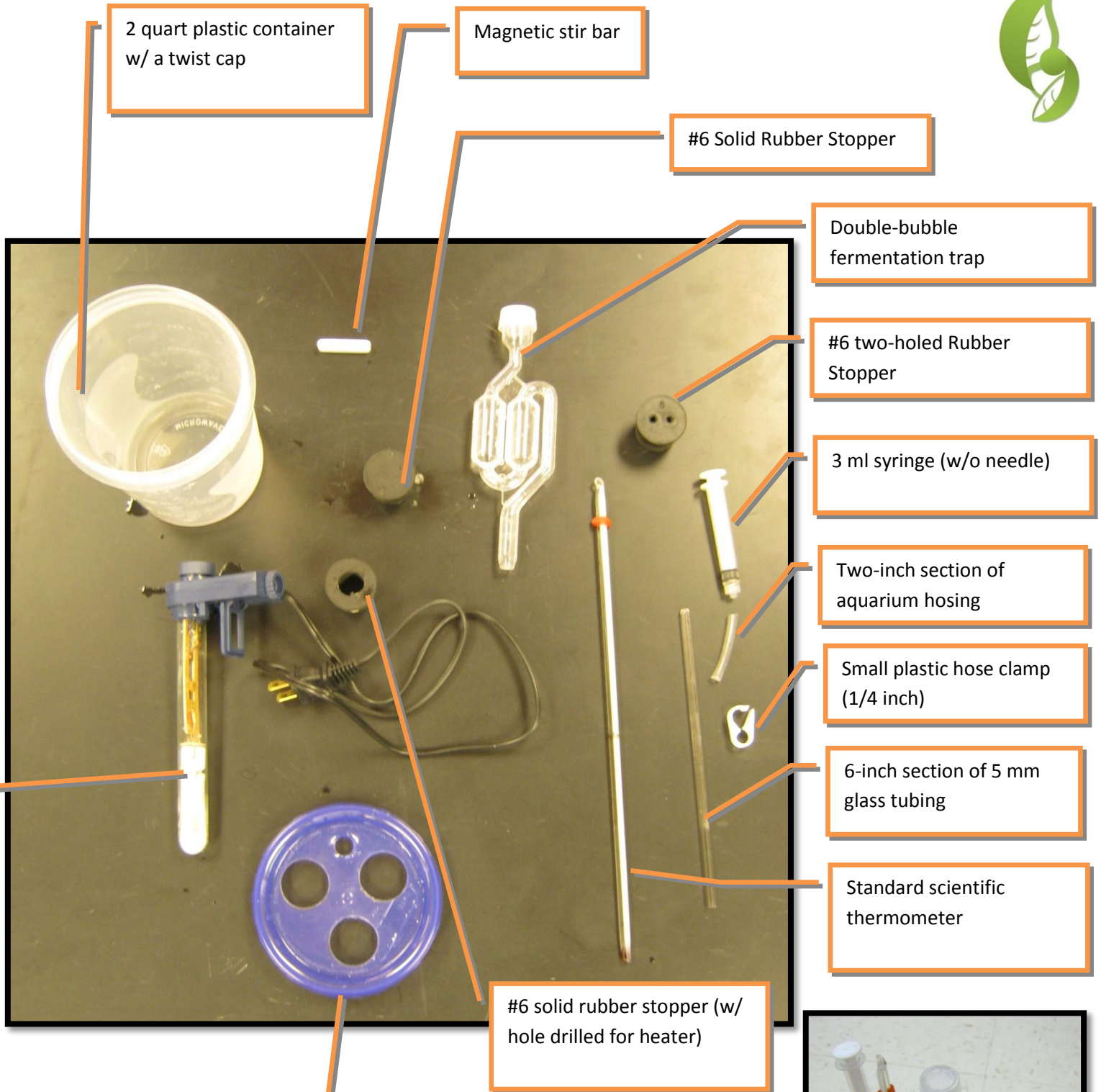
Materials:

- One standard 2 quart twist-top plastic container (available at most grocery stores)
- One 25 W adjustable aquarium heater
- One small magnetic stir bar
- One standard scientific thermometer (Celsius or Celsius and Fahrenheit)
- Two solid #6 rubber stoppers
- One two-holed #6 rubber stopper
- One double bubble fermentation trap
- A short section of aquarium hosing
- A 3 ml syringe (w/o needle)
- A 6-inch piece of 5 mm glass tubing
- A plastic clamp

If a magnetic stirrer is needed:

- A cigar box or jewelry box (like those found at a craft store)
- Four exhaust fans from a computer tower
- A 6-volt battery or solar panel
- Alligator clips
- Two strong flat magnets (such as "Super Magnets" from a hardware store)





2 quart plastic container w/ a twist cap

Magnetic stir bar

#6 Solid Rubber Stopper

Double-bubble fermentation trap

#6 two-holed Rubber Stopper

3 ml syringe (w/o needle)

Two-inch section of aquarium hosing

Small plastic hose clamp (1/4 inch)

6-inch section of 5 mm glass tubing

Standard scientific thermometer

#6 solid rubber stopper (w/ hole drilled for heater)

25 W adjustable aquarium thermometer

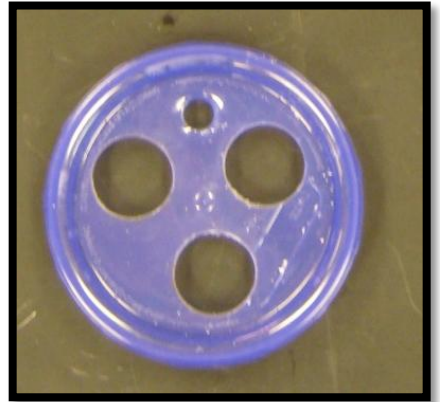
Cap to a 2-quart plastic container w/ three 1 1/8" holes and one 3/8" hole

Supplies shown here were purchased at the following locations:
Nasco – enasco.com
Pick 'N Save grocery stores
PET Discounters – PETdiscounters.com
US Plastics – usplastic.com
Science Kit – sciencekit.com
Beer & Wine Hobby - beer-wine.com



Fermenter Construction

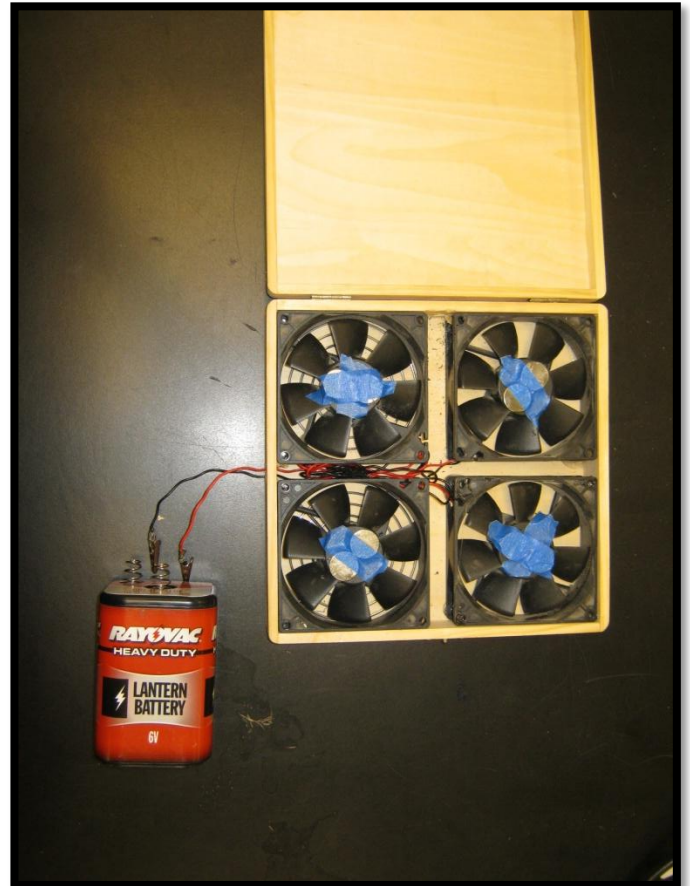
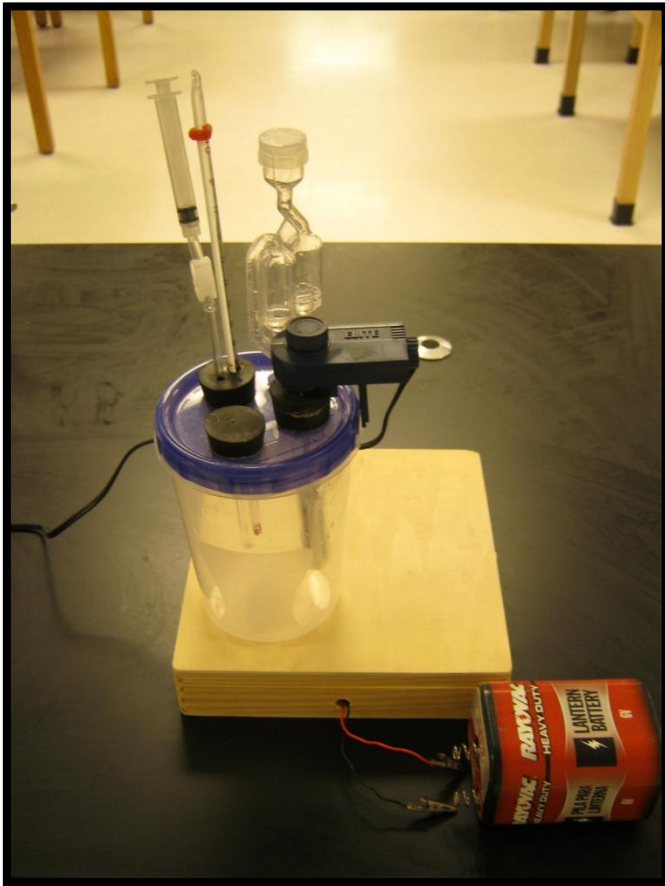
- Using a drill press (preferably) or cordless drill, drill three 1 1/8" holes equidistantly spaced into the top of the plastic container.
 - Be sure to have a small block of wood under the lid or it will crack under the pressure of the drill
 - Use steady even pressure, slowly increasing pressure to prevent shattering. Use a file or sander to smoothen rough edges.
 - Each hole will support a #6 rubber stopper.
- Drill a 3/8" hole centered between two of the larger holes. This will be used for the fermentation trap.
 - If your fermentation trap has a different diameter, change the size of this hole to fit.
 - Fit the trap into the hole. It will require some gentle force if it is sized to create an airtight seal.
 - The trap must create an airtight seal in this hole. If the seal is not tight, fix with hot-glue.
- Carefully drill a hole into one of the solid rubber stoppers. This hole should be the same diameter as your aquarium heater.
 - Be careful! Use a slower drill speed if necessary. Smaller guide holes may help. Be sure to have a holder or a helper. A similar-sized plastic test tube can give you more of a grip.
- Insert the heater into the drilled #6 rubber stopper. Use petroleum jelly if needed. Be sure that the fit is airtight.
- Insert your thermometer into one of the holes of the two-holed rubber stopper. Add your section of 5 mm glass tubing to the other hole.
 - Test your stopper with the thermometer and tubing for fit in the container.
 - You want to be about an inch off the bottom of the container when sealed. The same should be true for your heater.
- Fit the piece of aquarium hosing over the top of the glass tubing. Place the plastic hose clamp over the tubing and close. Insert the syringe into the tubing.
- Add your magnetic stir bar to the container.
- Seal the lid onto the container. Add water to your fermentation trap.
- Check to see that the fermenter is airtight by removing the syringe, opening the clamp, and blowing into the container. The water in the fermentation trap should bubble from escaping air.
- Rinse and wash both containers using warm soapy water. Dry.





Magnetic Stirrer Construction

1. Drill a small (3/8") hole in the side of the box for wires.
2. Remove any unnecessary guards or parts from the exhaust fans and secure with screws or glue in the jewelry box. The fans should be installed blades-up.
3. Glue two magnets onto the fan blade of each fan. Be sure that the magnets face opposite directions (one with the positive side up, the other positive-side-down). Tape may be necessary to hold the magnets in place until the glue dries.
4. Work the wires of the fan through the hole on the box. Attach the positive and negative wires to alligator clips.
5. When the magnets are secured to the fan blades, attach the alligator clips to the terminals of the battery to ensure that the fans operate smoothly.
6. Keep the alligator clips unattached until the stirrer is needed.



Fermentation Recipe (courtesy of GLBRC)



Basic Fermentation Recipe

1. DO NOT plug in the heater right away as the glass may crack if the heated instrument is placed in room temperature water.
2. Add to the fermenter: 3 tsp. yeast, 300 mL warm tap water, small stir bar.
3. Place on stirplate. Cover with lid, including heater. DO NOT plug heater in yet – just let it acclimate to the warm tap water.
4. Allow mixture to incubate, stirring very fast (700 rpm) with NO heat for 8-10 minutes to activate the yeast.
5. Add 3 tsp. sugar. Reduce stirring speed to medium (350 rpm). Plug in heater, set to lowest heat setting that will maintain a water temperature of 38-40 degrees C.
6. Mixture may run for 1-2 hours. Refresh when needed.



A word on the Great Lakes Bioenergy Research Center, Madison WI – glbrc.org

Located on the University of Wisconsin campus in Madison, the Great Lakes Bioenergy Research Center is committed to helping to advance the science, application, and implementation of bioenergy. The mission of the Great Lakes Bioenergy Research Center of the US Department of Energy is to ...

- *Apply cutting-edge research to help create a new generation of sustainable bioenergy feedstocks, processing technologies and fuels*
- *Evaluate the economic and environmental impacts of these new technologies, and use the results to guide research activities*
- *Bring technological advances to other academic scientists, the private sector and the marketplace*
- *Balance mission-driven project management and evaluation with the creative milieu of its academic, private sector and national laboratory research partners*
- *Recruit broad segments of the academic, industrial and national laboratory communities to develop and participate in relevant research programs*
- *Provide a training program for future leaders of the biofuels industry*
- *Inform a diversity of audiences on the scientific and societal issues associated with biofuels*

The Great Lakes Bioenergy Research Center is a great source for a wide variety of high quality, instructor-designed educational materials. All materials have a strong inquiry component and tend to be highly popular with students. Furthermore, these activities are standards based and are high in both rigor and relevance.

Activities from GLBRC can be accessed at <http://glbrc.org/education/educationalmaterials> . The Waterford Fermenter designed is 100% compatible with all related activities on this site. All GLBRC materials are used with permission.

Lab Activity Ideas - Waterford Fermenters

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The ideal lab activity for a fermenter requires students to form hypotheses prior to any instruction and any lab setup. By having students incorporate prior knowledge, they will gain a stronger understanding not only of the content, but also of the gaps and misconceptions of their prior knowledge (problems that otherwise may not be realized until after an assessment). Students should be given time and opportunity to predict what will happen, analyze the data after it is collected, and defend or reject their original hypothesis. As an instructor, emphasis should be placed as much on *how* a student learns as *what* they learn. By treating students like scientists, and by placing them in situations in which they must apply their knowledge of a topic to real question, students will develop a much stronger level of comprehension of the material. In addition, they will be markedly more engaged in the lesson. Use the mysteries of science to engage.

Biotechnology and Renewable Energy (also see <http://glbrc.org/education/educationalmaterials>)

1. **Fermentation and Feedstocks** – use the Fermenter to compare the CO₂ production as a function of different feedstocks such as sugar, corn meal, flour, whole-wheat flour, and ground corn stover or grass.
2. **Metabolism as a Function of Temperature:** compare rates of CO₂ production from sugar under varying temperatures to investigate the role of heat as a factor in the rate of metabolism.
3. **Fermentation and pH** – allow a control to run under sealed, anoxic conditions. Pump air into a separate fermenter through the sample port (syringe and hose clamp) using an aquarium air pump. Compare the pH differences between the aerobic and anoxic conditions.
4. **Root Beer** – add 150 ml of sugar and 1 ml of powdered baker's yeast to a sanitized fermenter. Mix together for one minute and add 7 ml (half a tablespoon) of root beer extract. Fill the fermenter 3/4ths full with fresh water. Place on a stirrer and rapidly stir for 2 minutes. Store in a dark cooler at room temperature for 3-4 days. (Note: student consumption is not recommended and should be avoided due to potential alcohol formation).
5. **Enzymatic Hydrolysis** – using 50 ml of cellulase enzyme (such as the Accelerase enzyme, potentially available as a donation from Genencor), add varying cellulosic substances (grass, corn stover, corn meal, wood chips). After at least 24 hours, measure the rate of hydrolysis from cellulose to glucose using a blood glucose meter (such as those used to test the blood of diabetes patients). Possible experimental investigations include...
 - a. The effect of temperature on enzymatic hydrolysis.
 - b. The effect of acid-pretreatment on enzymatic hydrolysis.
 - c. Varying values of feedstocks as a function of the average rate of hydrolysis of each kind

Environmental Science

1. **Carrying capacities and yeast "habitats"** – remove the water from the fermentation trap and seal a balloon over the top. The balloon will capture CO₂ and inflate. The fermenter can serve as a "micro-habitat" for yeast. Demonstrate the role of habitat carrying capacities by increasing or decreasing the availability of food (sugar). Change conditions by altering the pH, temperature, salinity, or other environmental conditions. Determine if the availability of food has a direct or indirect relationship to the carrying capacity, and the limit of this phenomenon. Instead of a balloon, CO₂ can also be measured with a CO₂ probe.

2. **Nutrient Runoff and Aquatic Dead Zones** - Fill one fermenter with tap water (control) and fill two fermenters with water from a lake, river, or stream. Add 100 mg of fertilizer to one fermenter and dissolve using the stir bar. Leave the other fermenter without fertilizer. Mark all fermenters appropriately and place in a well-lit windowsill or next to grow-lights for at least a week. Using a dissolved oxygen sensor, obtain readings (ideally during peak sunlight) to determine the effect of nutrient runoff on algal growth. Plot changes in the dissolved oxygen over the course of 24 hours.
3. **Climate Change and Carbon Dioxide Sequestration in Water** – fill a fermenter half full of ice water. Use an air stone (for aquariums; found at a pet supply store) and hose to add CO₂ to the water. Dip the stone with the hose attached into the water. Blow through the stone for at least 3 minutes. Test the pH with a probe or pH paper. Seal the fermenter and allow the heater to adjust to the colder temperature. Replace the plain solid rubber stopper with a CO₂ probe. Turn on the heater and watch the changes in the CO₂ levels of the air in the fermenter. Regularly take samples using the syringe sampler and test the pH of the water.
4. **River Health and Thermal Pollution** – measure the impact of thermal pollution on dissolved oxygen by heating the water to varying temperatures and measuring the dissolved oxygen. Graph the results to determine the impact of heat on dissolved oxygen. Hypothesize the impact of thermal pollution on an aquatic habitat and model this impact using *Daphnia magna* (see below).
5. **Eutrophication and Dye** – using *Elodea* as a model organism, test the possibility of environmentally-friendly dyes as a way to control eutrophication. Because plants primarily use both red and blue light for photosynthesis, dyeing the water may have an impact on their rates of photosynthesis. Fill fermenters half full of water and add similarly-sized *Elodea* plants (note – *Elodea* is invasive; other aquatic plants may suffice if local or state restrictions are in place). Dye the water different colors (red, blue, green, purple) and measure the impact on photosynthesis by measuring oxygen, dissolved oxygen, or both. If only measuring oxygen, be sure to disturb the water using the stir bar or heat the water prior to measuring (heating the water lowers the solubility of dissolved oxygen).
6. ***Daphnia magna* and Water Health** – *Daphnia magna* are a model wetland organism. Often called the “water flea”, *D. magna* can be used to measure the deleterious impacts of pollution and other ecological disturbances. Because *D. magna* is at the base of the food chain, its change in population will be felt throughout an ecosystem. If ecological conditions become unfavorable, the sexual cycle will be induced and the diploid females will produce eggs. By placing *D. magna* in the fermenters at a rate of 4 individuals per 40 ml of synthetic hard water and inducing a change (light, temperature, pH, toxins, nutrients, turbulence, turbidity, UV light increases from a depleted ozone layer, etc.), the ecological impact or lack thereof can be assessed in one of several ways. Simply survivorship can be counted after an exposure period of 24 hours, or offspring produced can be measured after 8 days. Due to their transparent bodies, heart rate can also be assessed. Finally, the ratio of males to females can be an assessment of ecological impact (again, males are only produced when situations become unfavorable; otherwise females will reproduce asexually). The Waterford Fermenters are ideal for these lab experiments because of their closed-system nature and ability to introduce substances with a sterile technique using the syringe access ports.

Agronomy and Agricultural Science

- 1. Soil Temperature and Rate & Pace of Germination** – fill a fermenter with 250 ml of top soil. Add radish seeds and cover with a layer of vermiculite. Moisten the soil/vermiculite. Remove the rubber stoppers with the heater and thermometer/glass tubing. Screw on the cap of the fermenter. Position the thermometer and glass tubing so that the thermometer is in the soil but the tubing is above the soil. Insert the stopper with the thermometer/tubing. Insert the stopper with the heater so that that heater is partially immersed in the soil. Place the fermenters with the radish seeds by a sideways grow light (a windowsill can work too, but it may negate the effects of the heater due to a miniature greenhouse effect). Measure the changes in germination (rate and number) due to warmer soil. Note: the radishes can be watered through the syringe port.
- 2. Oxygen and Radish Productivity** – transport a week-old radish seedling to a fermenter filled with 250 ml of soil. Position the thermometer and glass tubing so that they will be above the soil. Insert the stopper with the thermometer/tubing. Because the heater is not needed, it can be replaced with a solid #6 rubber stopper. Weigh each apparatus (with the radish inside; be sure that each has the same equipment). Leave some fermenters as they are; these will be the controls. For the others, replace the syringe with aquarium hosing attached to an aquarium pump. Place next to a sideways grow light. After a week or two, measure the change in weight and/or height between the control seedlings and the seedlings that received the pumped air. Note: consider the impact of having a fully closed vs. a partially closed system.
- 3. Light and Radish Productivity** – Using the same procedure as above, expose the radish seedlings to white, red, and blue light. Compare both the changes in appearance of the radishes after a week or two, as well as their change in weight.
- 4. Natural Insecticides** – Using the same procedure as in #2, treat 1/3 the radish seedlings with a homemade pesticide consisting of 1 clove garlic, 1 small hot pepper, and one quart of water blended, strained, and added to a spray bottle. Treat another 1/3 of the plants with a conventional pesticide from a garden supply store (Note: use extreme caution and take all necessary precautions to ensure student safety!). Leave the remaining 1/3 untreated as a control. Add a “safe” pest such as *Drosophila melanogaster* (fruit flies) in equal amounts to each fermenter and seal (it may help to cool the flies first so that they can be handled). Record visual observations and changes in height. Note: it will be necessary to anesthetize the flies at the end of the experiment. CO₂ can be pumped into the fermenter to kill just the flies. If not available, the fermenter can be frozen for 8 hours; this will kill both the radish and the flies.
- 5. Soil Carbon Sequestration** – add 250 ml of topsoil to all fermenters. Add 50 ml of tap water to ¼ of the fermenters. Turn on the heaters for ¼ of the fermenters. Turn on the heater and add 50 ml of tap water to another ¼ of the fermenters. Leave the remaining ¼ of the fermenters untreated as a control. Add the covers and close tightly. After at least a day, compare the differences in the production of carbon dioxide. Additional treatments include disturbing the soil, changing the pH of the soil, and add fertilizer to the soil. This lab is ideal for topics regarding agricultural methods that address climate change and carbon sequestration.