



Future of Agriculture Curriculum for Teaching Sustainability

FACTS Bioreactor

User Manual

by Craig A. Kohn

Model 4.0

Table of Contents

Part 1	Overview
Part 2	Getting Started
Part 3	How it Works
Part 4	Additional Needed Materials
Part 5	Fermentation Instructions (titration)
Part 6	Fermentation Instructions (probe)
Part 7	General Respiration Instructions
Part 8	General Photosynthesis Instructions
Part 9	General Eutrophication Instructions
Part 10	General Biodiesel Instructions
Part 11	Climate Change Simulation
Page 12	Tabletop Ecosystems
Part 13	Miscellaneous Notes



Introduction

Thank you for your purchase of a FACTS Bioreactor! These models were designed with teaching and instruction in mind. Built by students for students and teachers, FACTS Bioreactors exist to bring high quality, inquiry-based instruction to students in science, agricultural, and environmental classes at the high school and college levels.

1. Overview

FACTS Bioreactors are meant for a wide range of purposes. While they were designed for fermentation, FACTS Bioreactors are essentially controlled bio-reaction chambers in which experiments can be conducted on just about any biological process. From respiration to photosynthesis to biofuel production, FACTS Bioreactors are capable enabling of a wide range of activities and lessons.

2. Getting Started

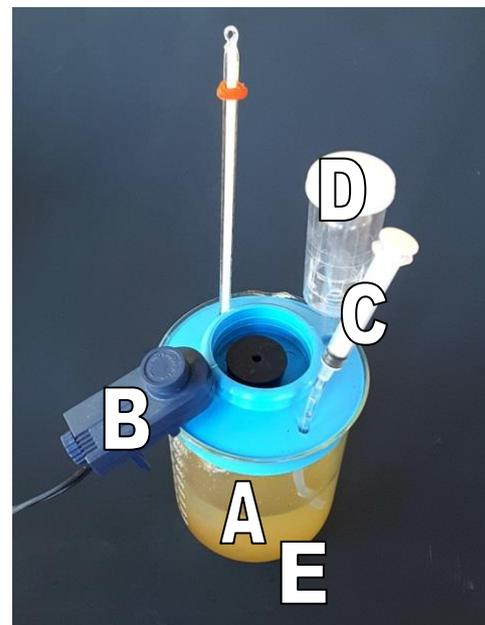
When you receive your FACTS Bioreactor, immediately inspect it for any damage. All models are inspected before they are shipped, but accidents can occur during the shipping process. If you notice any cracks or deformities, please contact us immediately to make arrangements to switch out the broken part. Please also check to make sure you have all parts.



3. How it works

Each part of your bioreactor plays an important role in its function:

- A. **The chamber:** this is where the reactions will occur.
- B. **Heater:** this allows you to control the temperature inside your chamber. The heater has only two modes: on or off. Use the dial at the top of the heater to adjust the temperature to which it will heat. Turn it clockwise to raise the temperature and counter-clockwise to lower it. If you see the orange light turned on, it is heating. If the orange light is off, it is not heating.
- C. **Sterile sampling tube:** The sampling tube allows you to collect a sample without opening the unit and risking contamination. Simply raise the stopper of the syringe to collect a sample for testing. You may also add small amounts of substances in this manner as well.
- D. **Airlock:** this apparatus is necessary for two reasons –
 1. This is how excess gas escapes your unit. NEVER block the ventilation of your bioreactor. Fermentation in a vessel that is sealed will cause it to explode. The airlock allows gas to escape without contaminating the contents inside.
 2. This is how you can measure the CO₂ production in your unit. Using a 1 molar KOH solution and strontium chloride, you can use phenolphthalein titration to measure the CO₂ produced during fermentation or respiration. More details are included later in this manual. This component can be substituted w/ a CO₂ Probe.
- E. **Magnetic stir bar:** this enables the solution in your chamber to be agitated using a magnetic stirrer (not included but available in most high schools and college labs). Position the bioreactor on the stirrer so that the magnet can spin unobstructed. Check to make sure your heater, thermometer, and sampling tube are not struck by the magnet as it spins.



4. Additional Needed Materials

In addition to the supplies included and general laboratory glassware, you will need the following items commonly found in high school and college laboratories:

- In general: magnetic stirrer; electrical outlet; safety materials (gloves, goggles, aprons)
- CO₂ Measurement: Either a CO₂ Probe -or- 37% hydrochloric acid, phenolphthalein, strontium chloride, potassium hydroxide, and a burette or eye dropper.



5. Fermentation Instructions (using titration)

1. Set up your FACTS Bioreactor. DO NOT plug in the heater right away as the glass may crack if the heated instrument is placed in room temperature water. Check that your bioreactor has...
 - a. A heater
 - b. A thermometer and sampling syringe/tubing
 - c. A plastic airlock
 - d. A magnetic stir bar
 - e. NO cracks or deformities; clean your bioreactor if you see any residues
2. Add to the bioreactor: 3 tsp. yeast, and 500 mL warm tap water.
3. Place on magnetic 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 (so that a vortex forms) with NO heat for 8-10 minutes to activate the yeast.
5. While waiting, remove the cap of the airlock and add 20 ml of 1 M KOH solution.
 - a. To make 1 M KOH, dissolve 1.1 g KOH in 100 ml of water in an Erlenmeyer flask or beaker.
6. Replace the cap of the airlock.
7. 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.
 - a. The heaters are only on or off. If the orange light is on, the unit is heating. When you obtain the temperature you need, set the heater so that it only turns on if it falls below that temperature.
8. After 15 minutes or more, remove stopper with the airlock and carefully pour the KOH solution into a small graduated cylinder using gloved hands.
9. Add 2 ml 1 molar SrCl_2 .¹
10. Add two drops of phenolphthalein. It should turn pink. Recap your phenolphthalein bottle.
11. Take your graduated cylinder with your KOH solution to a sink.
12. Turn on the sink's faucet and let it run at a low setting.
13. Add 37% hydrochloric acid drop by drop to the KOH solution over the sink using gloved hands while wearing goggles and an apron. Gently swirl between drops.
 - a. NOTE: HCl acid is EXTREMELY dangerous!!!! Use extreme caution!
 - b. NEVER add water to acid. Always turn on the faucet and let it run before dumping your acid.
14. Count each drop until the solution turns clear and stays clear. Record the total number of drops added. The fewer drops of acid needed, the more CO₂ was produced.
 - a. The CO₂ lowers the pH of the KOH solution – the more CO₂ produced, the less acid is needed to lower the pH to the point where the phenolphthalein turns clear.
 - b. The amount of CO₂ produced is proportional to the ethanol produced.
15. Wash your bioreactor. Be careful NOT to lose your stir bar! Use soap and water – do not leave any residues!

¹ 1.6 g SrCl_2 per 10 ml of water

6. Fermentation Instructions (using a CO₂ Probe, such as those from Vernier)

1. Set up your FACTS Bioreactor. 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 bioreactor: 3 tsp. yeast, 500 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. After 15 minutes or more, compare the CO₂ levels inside the bioreactor to those of the room's air. (CO₂ levels are an indirect indicator of the production of ethanol – for every molecule of ethanol produced, one molecule of CO₂ is produced). (NOTE: 500 ml of water is necessary to keep the heaters from cracking; you may need to support the CO₂ probe with a ringstand and clamp to prevent it from reaching the water).

7. General Respiration Instructions

1. Add 20 ml of 1 M KOH solution to the airlock of the FACTS Bioreactor.
 - a. To make 1 M KOH, dissolve 1.1 g KOH in 100 ml of water in an Erlenmeyer flask.
 - b. NOTE: there is a hole in this vial to allow gas exchange! Pour the KOH over the sink and avoid letting it drip out the hole!
2. Replace the airlock's cap.
3. Using a glass beaker, measure 300 ml of water. Add 25 pea seeds (such as those from a hardware store) to the beaker and record the change in volume.
4. Add the peas (or other fast-germinating seed) and water to the bioreactor and seal.
5. After 24 hours, remove the plastic vial and (without dripping out the hole) pour the KOH solution into a small graduated cylinder.
6. Add 2 ml 1 molar SrCl₂.²
7. Add two drops of phenolphthalein. It should turn pink - see your instructor if it does not. Recap your phenolphthalein bottle.
8. Take your graduated cylinder with your KOH solution to a sink.
9. Turn on the sink's faucet and let it run at a low setting. Add 37% hydrochloric acid drop by drop to the KOH solution over the sink using gloved hands. Gently swirl between drops.
 - a. NOTE: HCl acid is EXTREMELY dangerous!!!! Use extreme caution! NEVER add water to acid. Always turn on the faucet and let it run before dumping your acid.
10. Count each drop until the solution turns clear and stays clear. Record number of drops.
11. Wash your bioreactor. Be careful NOT to lose your stir bar! Use soap and water – do not leave any residues!
12. Repeat with either an empty bioreactor or by changing a specific variable (heat, water, pH) and compare to the original results (or compare titration results to a 1 M KOH in open air).

² 1.6 g SrCl₂ per 10 ml of water

8. General Photosynthesis Instructions (multiple bioreactors and an O₂ probe are needed)

1. *One week prior to experiment:* start radish seedlings in paper Dixie cups or 6-pack seedling trays (available at gardening supply stores).
2. Add 300 ml of general topsoil to your bioreactor's chamber.
3. Add a radish seedling to the soil in your chamber.
4. Add 100 ml of water.
5. Remove the components from the lid of your bioreactor. Add the lid to your chamber; carefully adjust the heater and thermometer as needed.
6. Repeat with a second bioreactor.
7. Place near a source of light (such as a window or light bulb. Make sure that light can reach the plant inside the chamber (light directly overhead will not reach the plant in sufficient quantities; a grow light placed on its side will work just fine).
8. Select one bioreactor as a control and change one variable in the other bioreactor (temperature, moisture levels, color of light, ventilation, etc.).
9. Typically, photosynthesis rates can be compared by measuring O₂ rates. The simplest method of measuring O₂ rates is with an O₂ probe (such as those from Vernier). Take daily readings over a predetermined period of time and compare the impact of the changed variable on O₂ release. The more O₂ released, the greater the rates of photosynthesis.

9. General Eutrophication³ Instructions (multiple bioreactors and an O₂ or D.O. probe are needed)

1. Add 300 ml of water to your chamber.
2. Add an individual *Elodea* plant to the water (*Elodea* is a great model organism and can be purchased from general science supply companies such as Nasco). You could also use algae from a supply company or collected from a body of water.
3. Repeat with the second bioreactor.
4. Choose one bioreactor to be a control that will remain unchanged. To the second bioreactor, increase the nutrient levels using a fertilizer, manure, or pure supply of a nutrient.
5. Measure the O₂ in the air or the dissolved oxygen in the water using a probe. When not measuring, keep a #6 rubber stopper in the access port so that the container is sealed. The higher the O₂, the greater the risk of eutrophication.
6. You can also test methods of controlling eutrophication, such as physical removal of the algae, dying the water red or blue, or by testing commercial methods of algae control.

³ Eutrophication is when excess nutrient levels cause an increase in plant and algae growth; while oxygen levels increase initially, levels decrease as plant/algae dies and as plant growth blocks light to the water below.



10. General Biodiesel Instructions (*use in a very well ventilated area or fume hood – use extreme caution at all times and have access to extinguishers and fire blankets*)

Day 1: Transesterification of the oil (you will need gloves, goggles, and aprons for this entire portion).

1. Pour 200 ml of new vegetable oil into a clean glass beaker (500 ml). Heat to 55° C using a hot plate (not the bioreactor heater!) and stir using the magnetic stir bar.
2. While the oil is heating, measure 40 ml of methanol and pour into a clean Erlenmeyer flask. Stopper the flask.
3. Using the scales at your stations and the paper holders, measure 1.3 g of potassium hydroxide (KOH).
 - a. It takes 6.5 g per liter of new vegetable oil to cause a complete reaction. Because we are working with 200 ml instead of 1000 ml, we will divide 6.5 by 5 to get 1.3 g/L.
4. After checking to ensure that the room exhaust fans are running, add your 1.3 g KOH to your flask of methanol and quickly stopper the flask (do this away from your face).
 - a. Use a fume hood for this step if possible.
5. Swirl your flask until all of the KOH dissolves (it will take some time; be persistent with your swirling).
 - a. CAUTION: this solution is extremely caustic and produces toxic fumes. Keep your flask sealed until you are ready to add your solution to your oil. Once the solution is in the heated oil, its danger is reduced by the transesterification reaction.
6. When the oil is between 55-57° C, quickly but carefully pour your 40 ml of methoxide into the oil. Re-cap your flask. Continue stirring – stirring should be vigorous but not so fast that it forms a vortex.
7. Keep your reaction between 55-57° C; shoot for 56° C. Allow your mixture to stay at this temperature for 45-60 minutes. Keep the solution mixing using the magnetic stir bar.
8. After 45-60 minutes, turn off your heat source and stop the mixing. Allow the mixture to cool and settle overnight (or at least 2 hours).
9. Rinse your flasks in a well ventilated area. Wash with soap, water, and a bottle brush. Rinse well and hang on the drying rack.



Day 2: Separation and washing (you will need gloves, goggles, and aprons for this entire portion).

1. If your reaction was performed correctly, you should now see a dark layer of glycerine on the bottom of the flask with the clearish yellow biodiesel on top.
2. Carefully pour your light yellow biodiesel into a separatory funnel (NOTE: make sure it is closed before your pour!). Try to not transfer any of the dark glycerine at the bottom. Dispose of the glycerine as instructed.
3. Add 50 ml of fresh water to the separatory funnel with your biodiesel.
4. Gently swirl the funnel to mix the diesel and water. Allow the mixture to settle (this may take a few minutes). After the water has settled to the bottom, drain into a collection jar under the funnel. Stop draining when all of the water has been removed.
5. Add 50 ml of fresh water again and repeat the two steps above. You should notice the wash water getting clearer each time and that it should separate more quickly from the biodiesel each time.
6. Add 50 ml of fresh water for a third time and drain when it settles to the bottom.
7. Allow your wash water to stand in the well-ventilated lab for a day to allow the excess methanol to evaporate.
8. Allow your biodiesel to dry open overnight in the separatory funnel.
9. After ensuring that no glycerine remains in your beaker, rinse your beaker in a well ventilated area (glycerine will clog drains – be sure none remains before you rinse!). Wash your beaker with soap, water, and a bottle brush. Rinse well and hang on the drying rack.

Day 3: Collection (you will need gloves, goggles, and aprons for this entire portion).

1. Remove any impurities in your biodiesel by draining them from the separatory funnel.
2. Remove your waste collection jar and dispose of your waste as instructed.
3. Place a clean dry mason jar (or other closable non-plastic container) under your separatory funnel and slowly transfer your biodiesel into your jar. Seal your jar.
4. Carefully wash your separatory funnel and collection jar and hang on the drying rack.
5. Label your jar of biodiesel with your group names and date. Place your jar in the location provided by your instructor.

11. Tabletop Climate Change Simulation

1. Use two FACTS Bioreactors. Be sure that your airlock has water in it to prevent gas from escaping.
2. Fill each bioreactor with 400 ml of tap water. Seal the containers.
3. After ensuring that both containers are completely sealed and are air-tight, remove the stopper with the airlock from the access port of one of the bioreactors and add one Alka-Seltzer tablet. Immediately reinsert the stopper and seal tightly. Allow the tablet to react completely. Be sure to keep track which bioreactor was treated with the tablet.
 - a. The tablet will add CO₂ to the air of your bioreactor and increase CO₂ levels above normal atmospheric levels.
4. Turn on both of your heaters by plugging them into an outlet. Ensure that the orange light inside the glass tubing of the heater turns on. If it does not, turn the heater onto a higher setting using the knob on top (arrows point which direction to turn).
5. Keep the heaters on until both reach 40-45° C. If one reaches before the other, unplug or adjust the knob so that it stays at the same temperature.
6. Keep both bioreactors within a degree of each other for at least 2 minutes. When you have balanced the temperature of both bioreactors, unplug the heaters. Note the time.
7. Record the temperature of the air at 5 minute intervals for 30 minutes or until the temperature levels out (whichever comes first)
8. After you have completed recording the temperatures at each interval, begin graphing your results.
9. During or after your graphing, rinse out each bioreactor chamber in a sink with tap water and dry with a paper towel. Return your bioreactor to the location specified by your instructor.

12. Tabletop Ecosystems

Introduction: Bioreactor Ecosystems use a very simple model of an ecosystem to help students understand the relationships between different species and nonliving resources that enable an ecosystem to function. In this case, brine shrimp (*Artemia salina*) and phytoplankton (*Tetraselmis* sp.) represent the two primary living species. The phytoplankton serve as the basis of the food chain as the photosynthesizing producers, while the brine shrimp are the consumers. Bacteria and other microbes naturally present in the water serve as the decomposers.

Materials: FACTS bioreactor (or a sealed 1000 ml Pyrex beaker or glass jar), artificial seawater (e.g. *Instant Ocean* and unchlorinated water such as spring water), phytoplankton solution (e.g. *Kent Phytoplankton*), brine shrimp (e.g. *Decapsulated Brine Shrimp Eggs*), pipette or syringe, stir plate or aerator.

Directions: To set up your ecosystem, start the following steps at least a couple of days prior to the class in which this set-up will be used.

1. Obtain a clean FACTS bioreactor (or sealable jar or beaker).
2. Add 400 ml of artificial seawater (this can be created using a product such as *Instant Ocean*; follow the instructions on the packet).
3. Add 5 ml of the phytoplankton to the bioreactor.
4. Add a small pinch of the brine shrimp cysts (eggs) to the bioreactor (if possible, add the eggs to an aerated flask of water a day or two earlier so that they are hatched and ready).
5. Place bioreactor in a sunny, well-lit location at constant room temperature (or place a fluorescent bulb near the bioreactors to provide a source of light).
6. Aeration and/or gentle stirring may be necessary initially to enable the ecosystem to get started.
7. Bioreactor ecosystems will need to be checked daily to ensure that they have sufficient levels of algae and shrimp. Add more algae if it cannot be detected. Add more shrimp cysts if none can be seen.

Amazon Prime Purchasing Keywords

- Artificial Seawater: “Instant Ocean Sea Salt for Marine Aquariums, Nitrate & Phosphate-Free”
- Phytoplankton: Kent PhytoPLEX Plankton
- Brine Shrimp: Decapsulated Brine Shrimp Eggs (*Artemia* Cysts) (2 oz.)



13. Miscellaneous Information

Safety: these are all potentially dangerous lab experiments!

Methanol is very flammable – keep away from flames and sparks and avoid inhaling fumes. Methanol can be hazardous and will be easily absorbed through the skin – avoid contact at all times and do not remove gloves, eyewear, or aprons at any point while performing the lab.

Potassium hydroxide (KOH) and hydrochloric acid will cause chemical burns if inappropriately handled. NEVER remove gloves or goggles when handling a strong acid or base.

Mixing KOH with methanol will produce fumes. Open hydrochloric acid bottles will also produce dangerous fumes. Perform only in a well-ventilated area or fume hood. If you feel faint or dizzy, immediately ask for help, leave the lab, and go into another room or hallway.

DO NOT wear sandals, flip flops, or other open toed shoes during laboratory situations. Spills can be hazardous on exposed skin. If you do spill KOH, methanol, methoxide, or acid onto exposed skin, immediately flush with copious amounts of water.

YOU ARE RESPONSIBLE FOR PREVENTING ANY AND ALL PROBLEMS. SAFETY SHOULD NOT HAPPEN BY ACCIDENT!



More materials are available online. Visit the FACTS Website (www.factsnsf.org) to find more materials and information.

The information included in this packet is meant to be an introduction to the capabilities of this unit. You are encouraged to explore additional uses and options for this bioreactor. You could also encourage students to develop their own experiments using this equipment. Inquiry-education is a phenomenal tool for exposing students to lessons in critical thinking, group work, application of science to real-world situations, and development of independent analytical skills.

These models were developed in cooperation with the US Department of Energy's Great Lakes Bioenergy Research Center on the University of Wisconsin – Madison and Michigan State University campuses. Many materials and laboratory procedures can be found on their website: www.glbrc.org.

