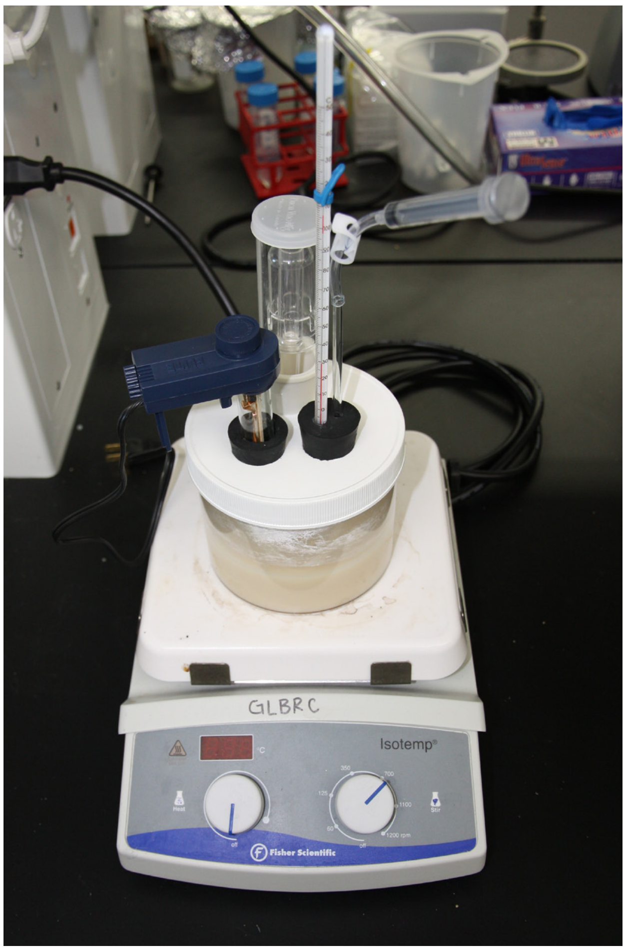
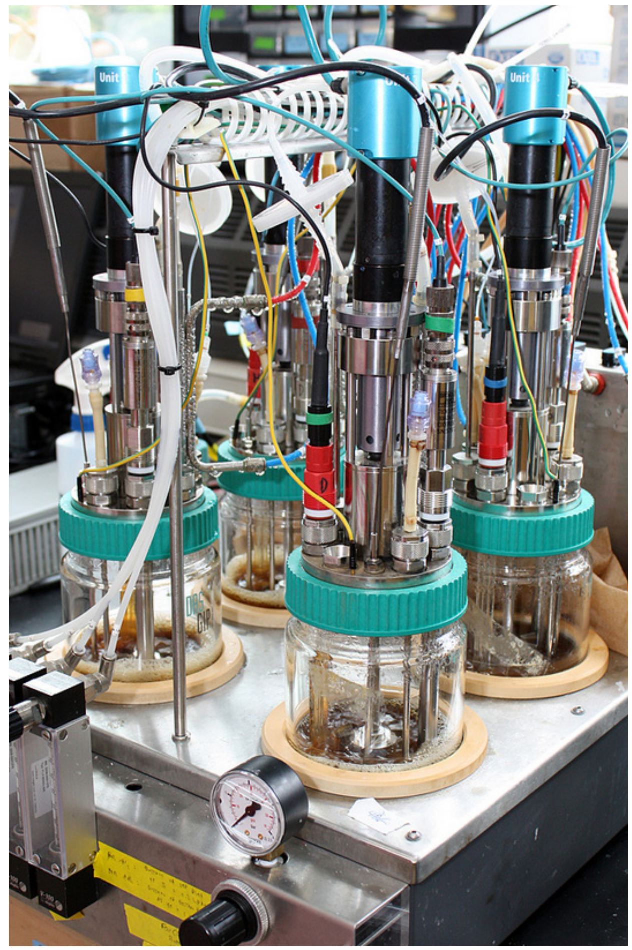
Make Your Own Mini Fermenter

This mini fermenter can be used to conduct small-scale fermentation investigations or demonstrations similar to research done by GLBRC scientists. The design allows for students to use simple techniques and classroom-grade probes to collect data during fermentation on a range of variables, such as ethanol concentration, CO_2 production, temperature and pH. The complete mini fermenter can be built with readily-available supplies for approximately $20 (detailed supplies list included with instructions).



Basic mini fermenter for classroom use.

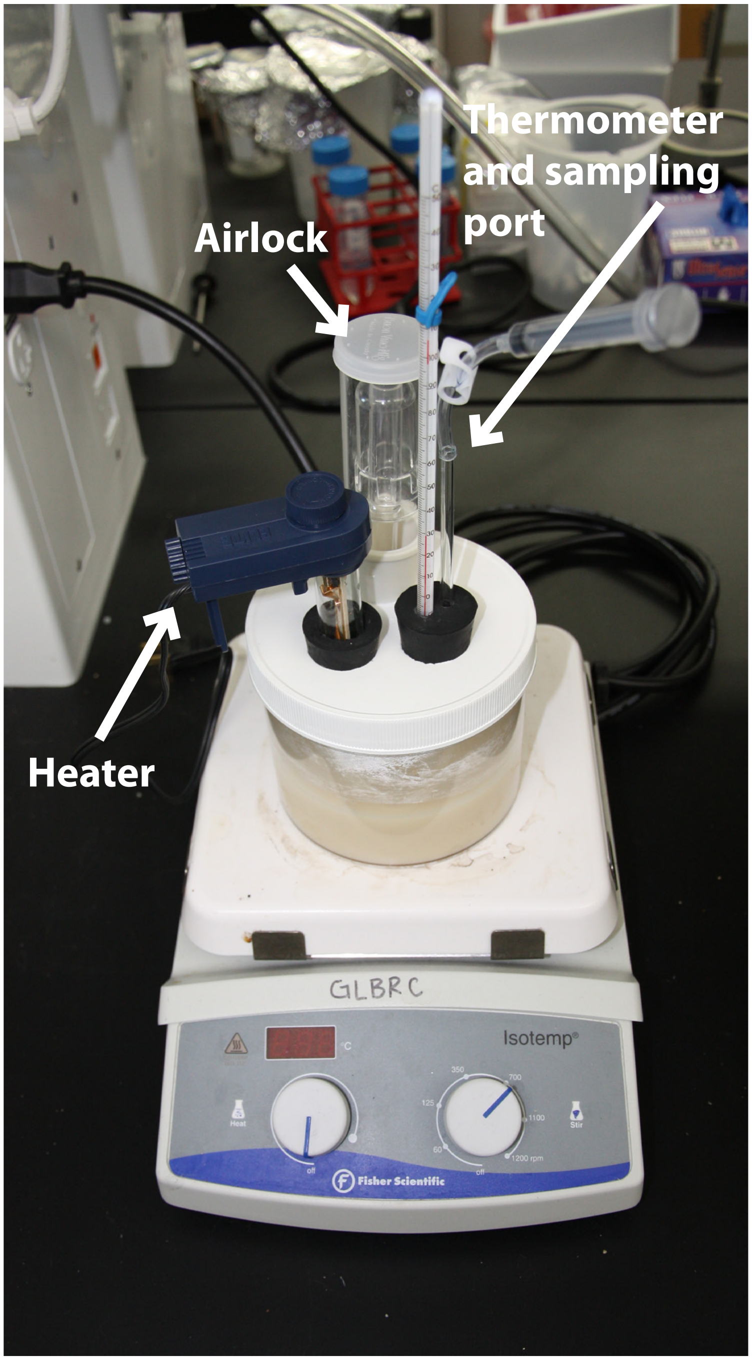

Fermenters used in GLBRC research.

This design and instructions are based largely upon the mini-fermenter model developed by Craig Kohn, Agriscience Instructor at Waterford Union High School. Partially assembled kits can also be purchased from the Agricultural Sciences Department for $40 each, plus shipping. Instructions for other experiment ideas using the mini fermenter chamber can also be found on their website, including topics like eutrophication, biodiesel, and climate change. Please see http://www2.waterforduhs.k12.wi.us/staffweb/ag/Website/Kohn-Fermenter.html for further information.

Options:

Depending on the supplies you have on hand and the methods and accuracy you wish to use with your mini fermenter, there are two mini fermenter options for you to choose from.

Version 1: Basic Mini Fermenter



This mini fermenter follows Craig Kohn's model and is available for purchase from his website. It can also be built using the supplies listed below. It is the most simple version, containing a heater, airlock for ventilation, thermometer and sampling port. Fermentation can be viewed and CO_2 measurements can be taken using titration methods (see instructions on page 8).

Notes on Version 1:

- The tube with the syringe attached is your sampling port. This allows for easy collection of samples or addition of substances without removing the jar lid and contaminating the chamber. To do this, unlock the clamp and pull back on the syringe.
- The thermometer can be difficult to move after assembly. Use caution when adjusting.
- CO_2 can be measured using titration. This process requires the use of 37% hydrochloric acid, phenolphthalein, strontium chloride, potassium hydroxide, and a dropper. KOH and hydrochloric acid can produce strong fumes and chemical burns. Operate with caution and always wear gloves and goggles. See page 8 for further instructions.

Version 1: Materials and Supplies

Tools for Construction

- Drill
- Spade or round saw drill bits
- Sandpaper or round bastard file
- Glass tubing cutter or triangular file
- Petroleum jelly or glycerol

Materials and Lab Supplies

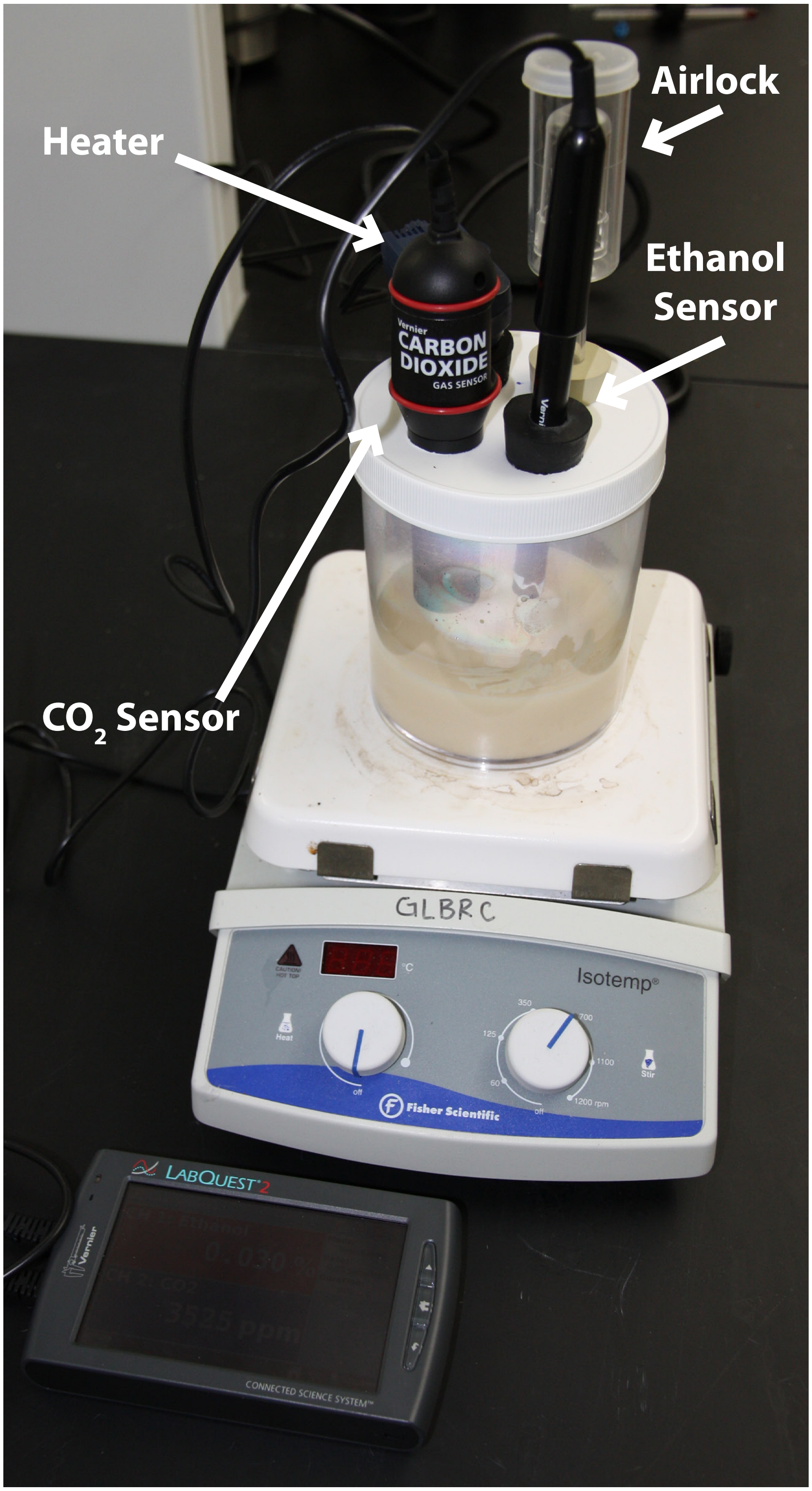
Some of these items you may already have for use in your lab. If not, see the chart below to find the recommended vendor.

	Item	Manufacturer Number	Vendor
1.	32 oz polystyrene container[1]	70225	US Plastic Corp (usplastic.com)
2.	120/400 cap (for container)[1]	66215	US Plastic Corp
3.	1/4" plastic tubing clamp	59199	US Plastic Corp
4.	3 cc syringes	C15780(A)N (pack of 10)	Nasco Science (enasco.com)
5.	Glass thermometer	SB15198(X)M	Nasco Science
6.	Magnetic stir bar	SB07308M	Nasco Science
7.	Aquarium tubing	SB07971M (10 ft)	Nasco Science
8.	Rubber stopper, size 6, 2 hole	S08521M (pack of 17)	Nasco Science
9.	Rubber stopper, size 6, 1 hole	S08511M (pack of 17)	Nasco Science
10.	5 mm glass tubing	CE-TUBEG12 (one 12" piece)	Home Science Tools (hometrainingtools.com)
11.	Elite 6" mini thermostatic heater, 25-watt	B0002AQF84	Elite (Amazon.com)
12.	Airlock, 3 piece type	6005	Midwest Supplies (midwestsupplies.com)
13.	Matching 1 hole size 6 stopper for airlock	6100x	Midwest Supplies
14.	Magnetic stir station[2]	STIR	Vernier Science (vernier.com)

[1] The container and its cover do not come together as one entity. Be sure to order the cover that matches the container.

[2] The estimated price to build a mini fermenter does not include purchase of a stir station.

Version 2: CO_2 and Ethanol Sensor Mini Fermenter



Version 2 requires two essential changes to the basic model. In order to incorporate all the necessary tools, **four** identical holes are drilled. This allows room for the heater, airlock, an ethanol probe and a CO_2 probe. This model also requires a **taller** container. To use the CO_2 probe properly, all the sensors must be inside the jar but above the liquid substance. The basic fermenter jar is too short for this measurement. Must have interface for use with probes (such as a Vernier LabQuest).

Notes on Using Version 2:

- Remember to use the taller container when building this model. The greater height is essential for the CO_2 sensor to be fully submerged in the container and also far enough away from any liquid to avoid damaging the probe.
- Before placing the probes, use a thermometer or temperature probe to adjust the aquarium heater to the appropriate level. Set the dial so the solution will be stable and stay between 35-40 °C.
- Position the sensors above the solution. During fermentation, the mixture will foam and rise a few centimeters. Take this into consideration when placing the probes so they do not get wet during the experiment.
- This model can be used with multiple variations. For example, a pH probe could be substituted for the ethanol or CO_2 sensor to observe how pH changes during fermentation.

Version 2: Materials and Supplies

Tools for Construction

- Drill
- Spade or round saw drill bits
- Sandpaper or round bastard file
- Glass tubing cutter or triangular file
- Petroleum jelly or glycerol

Materials and Lab Supplies

Some of these items you may already have for use in your lab. If not, see the chart below to find the recommended vendor.

	Item	**Manufacturer Number**	**Vendor**
1.	32 oz Clear Plastic Jar -PET 89/400	2852B15-CLR	Freund (freundcontainer.com)
2.	Cap, White PP 89/400 (INCLUDED WITH PURCHASE OF ITEM #1)[1]	LP89	Freund
3.	Magnetic stir bar	SB07308M	Nasco Science (enasco.com)
4.	2 rubber stoppers, size 6, one hole	S08511M (pack of 17)	Nasco Science
5.	25-watt, 6 inch mini thermostatic heater	B0002AQF84	Elite (Amazon.com)
6.	Airlock, 3 piece type	6005	Midwest Supplies (midwestsupplies.com)
7.	Matching 1 hole size 6 stopper for airlock	6100x	Midwest Supplies
8.	Magnetic stir station[2]	STIR	Vernier Science (vernier.com)
9.	Sensor interface (LabQuest)[2]	LABQ2	Vernier Science
10.	Ethanol sensor[2]	ETH-BTA	Vernier Science
11.	CO_2 gas sensor[2]	CO2-BTA	Vernier Science
12.	pH sensor[2, 3]	PH-BTA	Vernier Science

[1]This item comes with the jar. When checking out, the shopping cart will show the 32 oz plastic jar with a row underneath it stating "INCLUDED WITH THE ITEM ABOVE" and shows an image of the cap.

[2]The estimated price to build a mini fermenter does not include purchase of these items.

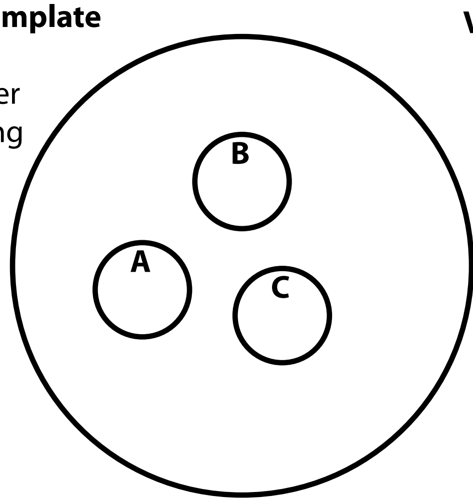
[3] Not needed for Version 2, but can be substituted for other probes to find supplemental measurements.

Building Instructions

1. Measure and drill three (four if building Version 2) 1 ⅛ inch holes in the lid using a drill. Smooth rough edges with sandpaper or a file. If the foam seal on the inside of the cover is difficult to drill through it can be removed without causing any significant defects to the mini fermenter.
2. In one of the #6 rubber stoppers, drill a ⅝ inch hole for the heater. Using petroleum jelly or glycerol, lubricate the heater and insert it into the stopper until approximately three inches can be seen from the bottom. This process tends to stretch the rubber stopper and may cause it to become too big for the hole in the container. If necessary, file down the outside of the stopper with a triangle file to ensure the stopper properly fits in the hole.
3. VERSION 1 ONLY: Using the same lubrication procedure, insert the thermometer into one hole on the two holed #6 rubber stopper. Using a glass tubing cutter or file, cut the 5 mm glass tubing to approximately 6 inches. Insert this piece of glass tubing in the remaining hole of the rubber stopper, next to the thermometer.
4. VERSION 1 ONLY: Cut a 2 inch piece of aquarium tubing. Add a plastic tubing clamp to this cut piece. Slide the plastic tubing onto the glass tubing inserted into the stopper. Add the 3 cc syringe to the other end of the aquarium tubing. This setup can be used to take small samples from the chamber.
5. VERSION 2 ONLY: Insert the ethanol sensor into a #6 rubber stopper. Vernier ethanol sensors come with a corresponding stopper to fit the probe which can be used for this experiment. Otherwise, a one holed #6 rubber stopper can be used in its place. Using a ½ inch drill bit, drill a hole into the center of one stopper. Insert the ethanol probe through the stopper, using petroleum jelly or glycerol for lubrication if necessary.
6. Insert the 3 piece airlock into its corresponding rubber stopper.
7. You should now have three assembled probing devices. Insert the probes and their stoppers into the holes drilled on the cover.
8. Place the magnetic stir bar into the container and twist on the lid. Your mini fermenter is now assembled and ready for use!

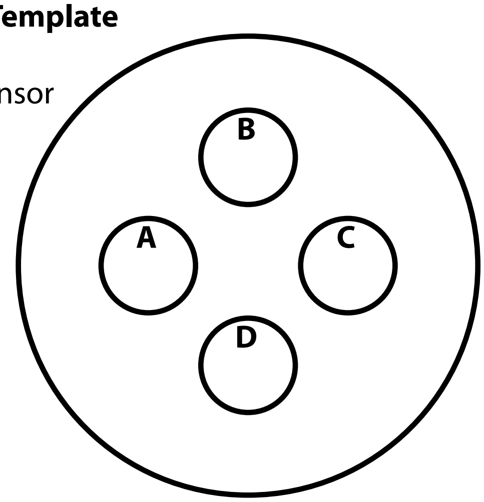
Version 1 Cover Template

A. Thermometer and Sampling Port
B. Airlock
C. Heater



Version 2 Cover Template

A. Ethanol Sensor
B. CO_2 Sensor
C. Airlock
D. Heater



Not scaled to size. All holes should be approximately 1 ⅛ inches in diameter.

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. Only plug in the heater when it is submerged in the liquid.
2. Add to the fermenter: 3 teaspoons yeast and 300 mL warm tap water (approx 35-40° C).
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 or until a vortex forms) with NO heat for 8-10 minutes to activate the yeast.
5. Reduce stirring speed to medium (350 rpm). Plug in heater and set to lowest heat setting that will maintain a water temperature of 35-40° C. Add 3 teaspoons sugar or biomass.
6. Mixture may run for 1-2 hours. Refresh when needed.

Notes on Fermentation:

- Do not heat the container using a hotplate – it will melt! Instead, use the aquarium heater to adjust the temperature of the reaction. Fermentation works best at a temperature of 35-40° C.
- The heater is heating when the orange light is on. Adjust the dial as necessary to obtain a relatively stable temperature as indicated by your thermometer or temperature probe.
- Before beginning fermentation, check to ensure the motion of the stir bar does not hit any of the probes or sensors.

Tips for Using Ethanol Sensor:

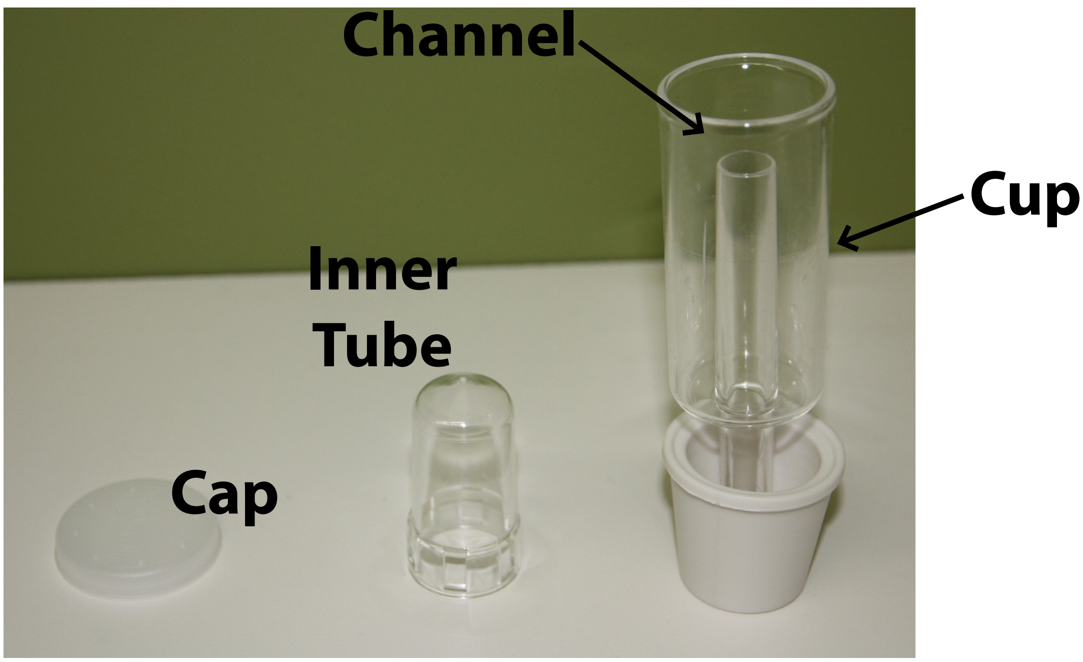
- Allow several minutes for the probe to stabilize. Calibrate if necessary.
- Vernier and PASCO ethanol sensors are accurate within a range of 0-3% ethanol concentrations. Keep this in mind when performing the experiment and use dilution techniques if necessary.
- The ethanol sensor is sensitive to temperature and pressure. Readings from the ethanol sensor can detect relative changes in ethanol production over time and demonstrate how ethanol is produced by yeast during fermentation. For more accurate measurements of ethanol take samples of the fermentation broth at regular time intervals. Take ethanol readings on each sample in a small beaker.

Tips for Using CO_2 Sensor:

- Allow several minutes for the probe to stabilize. Calibrate if necessary.
- Switch the CO_2 sensor to the higher range to read from 10,000 to 100,000 ppm.
- When placing the CO_2 probe, ensure that all sensor pores are located inside the container. If the hole drilled is 1 ⅛ inches in diameter, the container should cover all the pores.
- Basic sugar fermentation will reach and exceed the maximum reading of the CO_2 sensor (100,000 ppm) within 10 minutes.

Using the Airlock

Fermentation produces CO_2 gas. If excess gas is not allowed to escape, the container will build up pressure which can inhibit fermentation. The airlock acts as an exhaust port and allows the gas to escape while preventing contamination. It can also be used to measure the amount of CO_2 expelled during fermentation through titration (see instructions below). If you are not using the airlock to measure CO_2 with titration, it should be filled with water. To do this, remove the cap and inner tube. Fill the cup with water approximately ¾ full. Drop the inner tube over the channel and replace the cap. This forces any CO_2 particles to travel up the channel and into the water. The water may bubble slightly during fermentation. This is excess CO_2 escaping the solution.



Titration Instructions for Measuring CO_2 Production

- Instead of water, add 20 mL of 1 M KOH solution to the airlock. This can be made by dissolving 1.1 g KOH into 100 mL of water.
- After fermentation, remove the stopper and airlock and pour the KOH into a graduated cylinder.
- Add 2 mL of 1 M $SrCl_2$. This solution can be made by adding 1.6 g $SrCl_2$ for every 10 mL of water.
- Add 2 drops of phenolphthalein. The solution should turn pink.
- Add 37% hydrochloric acid drop by drop to the solution, counting each drop. Swirl between drops.
- Add drops until the solution turns and stays clear. The fewer drops of acid needed to reach this step, the more CO_2 was produced. This is because the CO_2 forms carbonic acid in water lowers the pH of the KOH solution. If there are higher concentrations of CO_2, the solution is more acidic and requires less acid to bring the pH of the solution to the point when the phenolphthalein will turn clear.
- The amount of CO_2 produced is directly proportional to the amount of ethanol produced.

Standards

Next Generation Science Standards (2013)

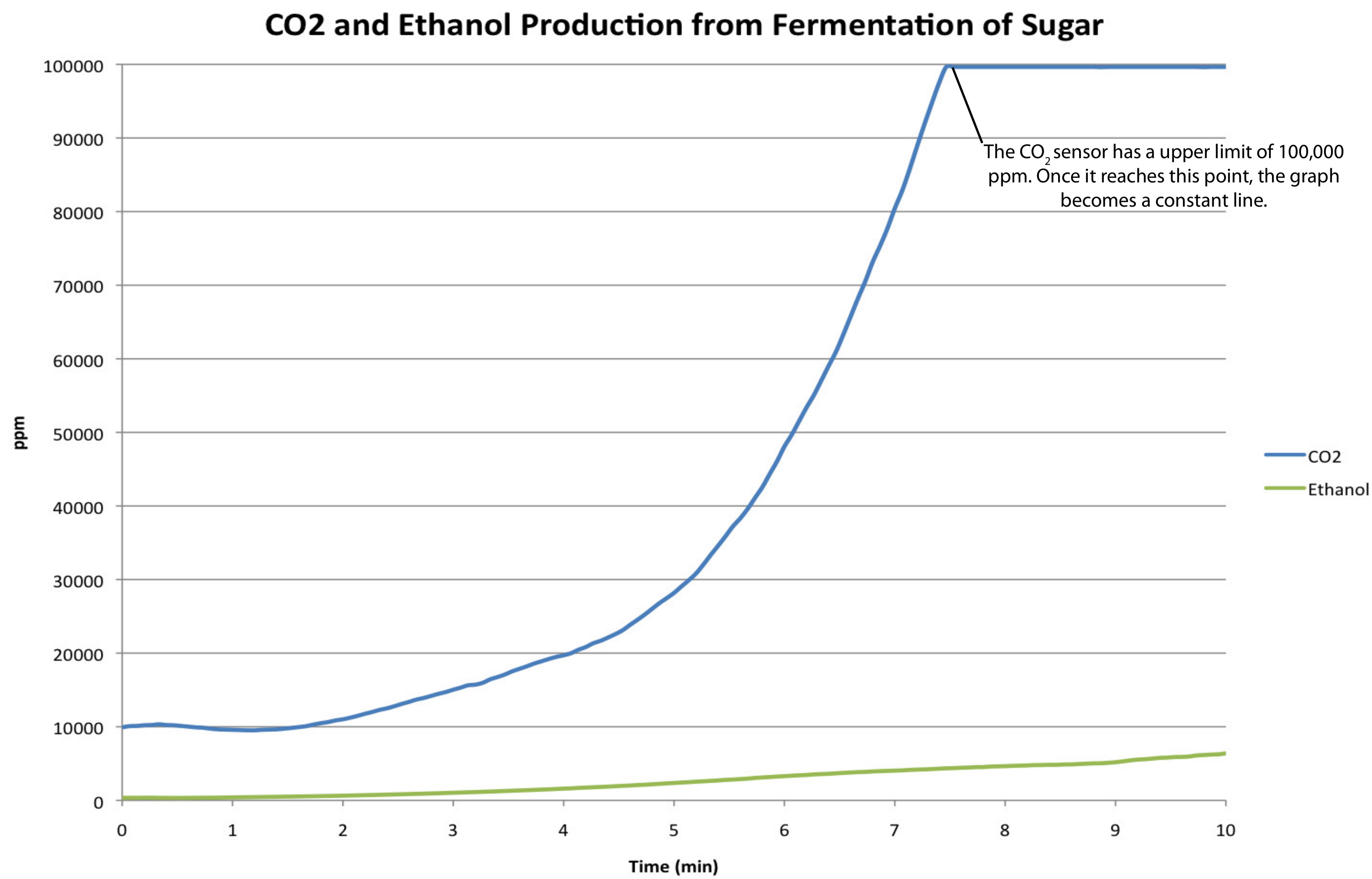
Performance Expectations

High School:

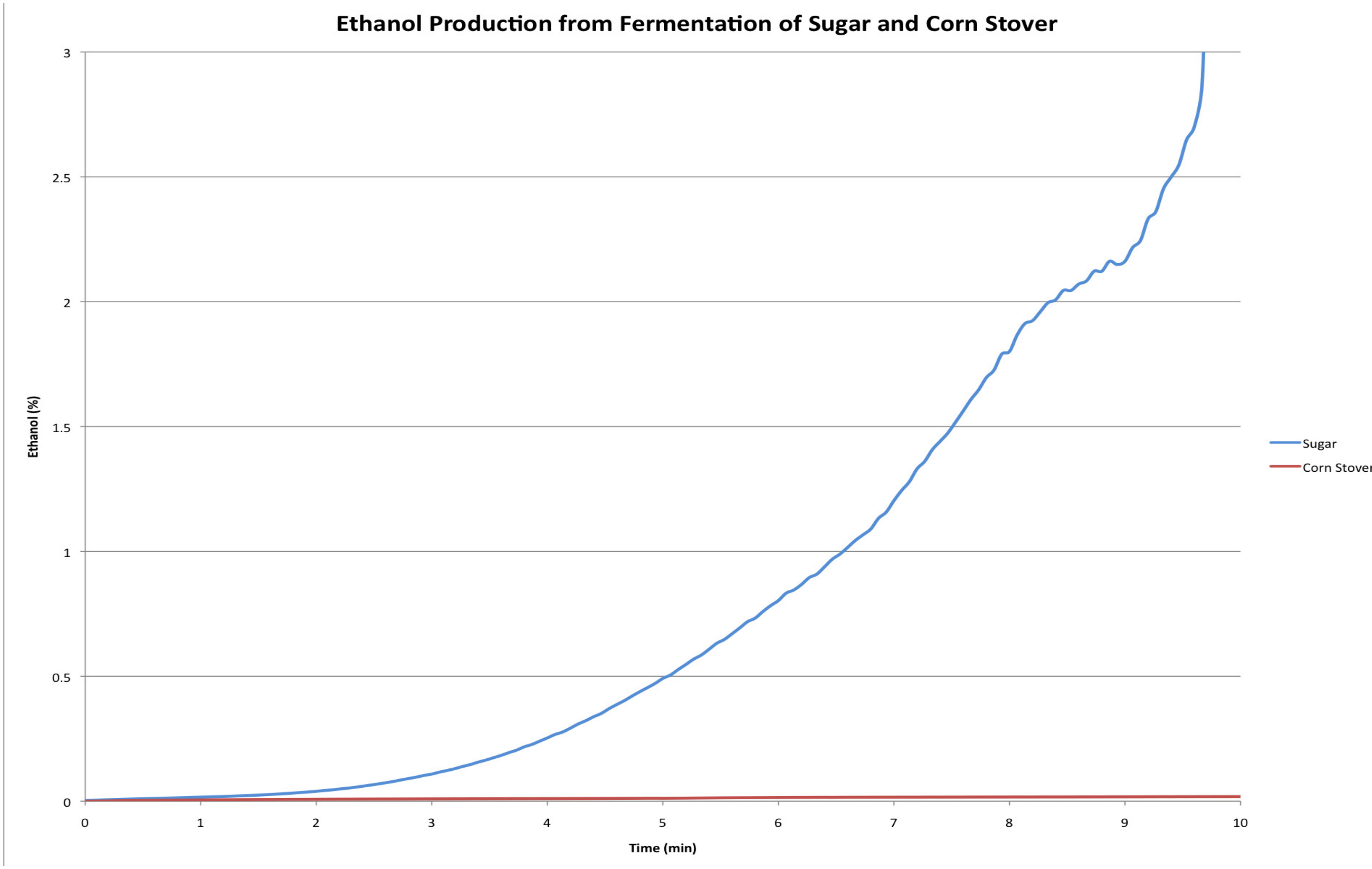
- **HS-LS2-3.** Construct and revise an explanation based on evidence for the cycling of matter and flow of energy in aerobic and anaerobic conditions.
- **HS-LS2-7.** Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.

Scientific and Engineering Practices	Disciplinary Core Ideas	Crosscutting Concepts
Asking questions and defining problems Planning and carrying out investigations	**LS2**: Ecosystems: Interactions, energy, and dynamics	Cause and effect: Mechanism and explanation Energy and matter: Flows, cycles and conservation

Example Mini Fermenter Data



Summary of experiment: Three teaspoons of yeast were added to 300 mL warm tap water. After the yeast was activated and the chamber had reached a stable temperature, 3 teaspoons of sugar were added. The experiment was allowed to run for 10 minutes while ethanol and CO_2 readings were recorded.



Summary of experiment: Fermentation trials were run in two fermenters - one with sugar the other with corn stover as the food source. Three teaspoons of yeast were added to 300 mL warm water in each mini fermenter chamber. After 10 minutes, the yeast was active and a stable temperature was reached. Three teaspoons of sugar or corn stover was then added. Fermentation ran for 10 minutes while ethanol readings were recorded.