



## Yeast Reproduction in Sugar Substitutes

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### Experimental Procedure

#### Working with Biological Agents

For health and safety reasons, science fairs regulate what kinds of biological materials can be used in science fair projects. You should check with your science fair's Scientific Review Committee before starting this experiment to make sure your science fair project complies with all local rules. Many science fairs follow Intel® International Science and Engineering Fair (ISEF) regulations. For more information, visit these Science Buddies pages: [Projects Involving Potentially Hazardous Biological Agents](http://www.sciencebuddies.org/science-fair-projects/project_src_biological_agents.shtml) ([http://www.sciencebuddies.org/science-fair-projects/project\\_src\\_biological\\_agents.shtml](http://www.sciencebuddies.org/science-fair-projects/project_src_biological_agents.shtml)) and [Scientific Review Committee](http://www.sciencebuddies.org/science-fair-projects/project_src.shtml) ([http://www.sciencebuddies.org/science-fair-projects/project\\_src.shtml](http://www.sciencebuddies.org/science-fair-projects/project_src.shtml)). You can also visit the webpage [ISEF Rules & Guidelines](http://www.societyforscience.org/Page.aspx?pid=312) (<http://www.societyforscience.org/Page.aspx?pid=312>) directly.

### Setting Up the Gas Collection Apparatus

1. Remove the small red cap from one of the squeeze bottles. Then connect the tubing to the tip opening, as shown in Figure 2. Make sure that you have a tight fit.



**Figure 2.** Tube connected to the bottle opening.

2. You will be collecting carbon dioxide from the yeast by displacing water trapped in an inverted graduated cylinder. Here's how to set it up:
  - a. Fill your plastic dishpan (or bucket) about one-third full with water.
  - b. Fill the 100-mL graduated cylinder with water.
    - i. If your dishpan is deep enough, fill the graduated cylinder by tipping it on its side inside the dishpan. Allow any bubbles to escape by tilting the cylinder up slightly, while keeping it under water. Keeping the opening of the cylinder under water, turn it upside down and attach it to the side of the dishpan with packing tape (or have your helper hold it in place).
    - ii. If your dishpan is not deep enough, fill the graduated cylinder completely using the faucet and cover the top tightly with plastic wrap. Quickly invert the cylinder and place the opening in the dishpan, beneath the surface of the water. Remove the plastic wrap. Attach the cylinder to the side of the tub with packing tape (or have your helper hold it in place).
  - c. The graduated cylinder should now be upside down, full of water and with its opening under the surface of the water in the dishpan. Place the free end of the tubing from the plastic bottle inside the graduated cylinder. Your apparatus is now ready to trap carbon dioxide from the yeast (see Figure 3).



**Figure 3.** Picture of the inverted graduated cylinder gas collection apparatus.

d. You can test your gas collection apparatus by removing the tube from the bottle top and blowing gently into the tube. The bubbles you create should be captured inside the cylinder. (You will need to reconnect the tube to the bottle and re-fill the cylinder before starting your experiment.)

### Running the Experiment

1. Using a permanent marker, label each of the bottles with the type of solution you will be feeding the yeast (e.g., sugar, nothing, saccharin, sucralose, aspartame, acesulfame potassium). If you need more than four bottles, you can re-use them. Make sure to rinse them out thoroughly between experiments.
2. Dissolve 1 teaspoon (tsp.) of sugar in  $\frac{1}{2}$  cup of warm water (110°F–115°F). When the sugar is fully dissolved, add  $\frac{1}{2}$  teaspoon of yeast, mix and pour into the appropriate bottle. Be sure to note the actual temperature of the water in your lab notebook.
  - a. You will be making one solution at a time (unless you decide to set up more than one gas collection apparatus). It is important to use the same water

- temperature each time you make a solution, since yeast activity is temperature-dependent.
3. Cap the bottle tightly with your "tube cap," and place the open end of the tube inside your gas collecting cylinder. Note the starting time in your lab notebook.
    - a. There should be water in the tubing as soon as it is submerged in the water. The CO<sub>2</sub> gas will push some water out of the tubing before the graduated cylinder starts to fill with CO<sub>2</sub> gas.
  4. Within 5–10 minutes, the yeast solution should start foaming, and you should see bubbles collecting in the graduated cylinder. Note the time when you first start seeing bubbles in your lab notebook.
  5. Decide how long to collect CO<sub>2</sub> (somewhere between 15–30 minutes is probably good, but you may need to adjust for your particular conditions). Use the same amount of time for all of your tests.
    - a. *Note:* Do not let the graduated cylinder become completely filled with CO<sub>2</sub>, but instead stop it before this point. If you let it become completely filled, and the next condition you test makes even more CO<sub>2</sub>, this could lead to poor and inaccurate results because your graduated cylinder may fill up before your test time is over.
    - b. *Tip:* If your solution makes a large amount of CO<sub>2</sub> very quickly, you can try to make it produce less CO<sub>2</sub> by using less sugar and possibly less yeast. For example, you could repeat this step using ½ tsp. sugar (instead of 1 tsp.) and ¼ tsp. yeast (instead of ½ tsp.).
  6. When the time is up, note how much CO<sub>2</sub> was collected by observing how much water was displaced from the graduated cylinder.
  7. Re-fill your gas collection cylinder, and carefully rinse out the yeast solution from the bottle. You should run at least three separate trials for each food source.
  8. For each of the sugar substitutes, use the properly labeled bottle. When preparing your yeast solution, use the same temperature for the warm water and the same amount of yeast (½ tsp.). Use 1 tsp. of each sugar substitute instead of sugar.

## Analyzing Your Data

1. Calculate the average volume of the CO<sub>2</sub> produced for each condition you tested and write this in your lab notebook.
2. Make a graph of your results.
  - a. Write the different conditions (e.g., plain sugar, saccharin, no sugar, etc.) on the x-axis (the horizontal axis).
  - b. Plot the corresponding average volume of CO<sub>2</sub> produced on the y-axis (the vertical axis).
3. How much CO<sub>2</sub> did the yeast produce when given the sugar substitutes compared to plain sugar? Could the yeast grow and reproduce using sugar substitutes? Did some work better than others? Can you explain your results?
  - a. *Note:* Many commercial sugar substitutes are mixtures, not pure compounds. Check the labeling of your sugar substitute packaging carefully, and examine the ingredients. How might the additional ingredients affect the outcome of your experiment?