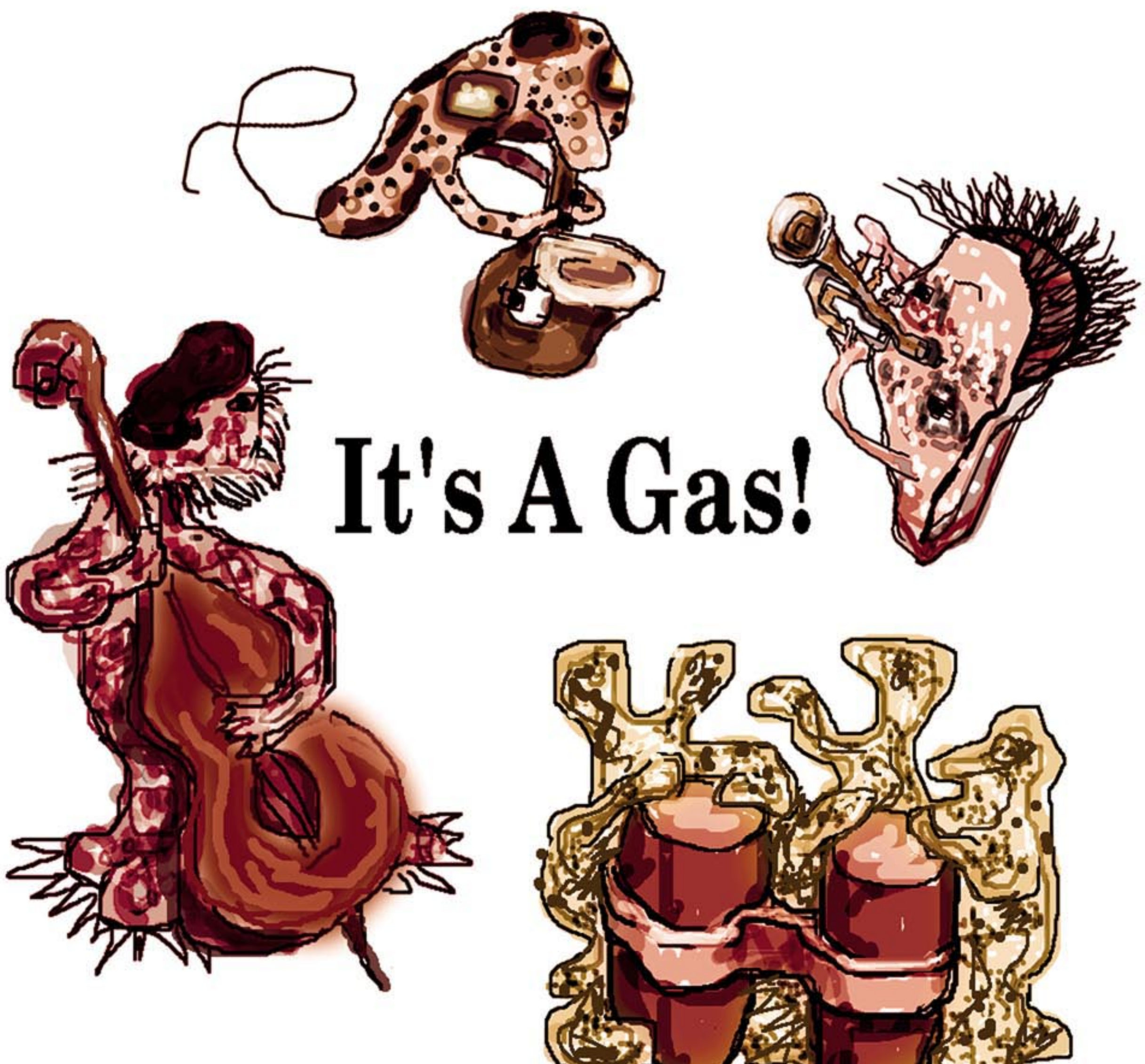
SCIENCE IN THE REAL WORLD

Microbes in Action



It's A Gas!

A Yeast Fermentation Lab

Science in the Real World

Microbes In Action

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At A Glance

Description
This is a two- day laboratory exercise in which the students will determine the effect of different concentrations of sugar on the respiration and fermentation rates of yeast. Students will collect data, analyze it and graph their results.

Time Requirements
This lab will require two days to complete.

Curriculum Placement
This exercise could be used as an introduction to the scientific method for solving a problem. This lab could also be done in conjunction with a unit on cellular respiration and fermentation.

Equipment
hot plate for making the sugar solution (optional)
balance

Materials (For 30 students - each doing one solution)
30 - 18 mm x 150 mm culture tubes
30 - 6 mm x 50 mm culture tubes (gas collecting tubes)
7 different glucose solutions
distilled water
yeast suspension with dropping pipette
metric rulers
test tube racks or beakers to hold tubes
10 ml graduated cylinders (one for each solution)

It's a Gas

Background
Yeast are one-celled organisms that have been used by humans for centuries in the preparation of food and drink. In fact, using yeast this way was one of the first examples of biotechnology.

The scientific name for yeast is *Saccharomyces cerevisiae*. Saccharomyces comes from two Latin words, saccharum which means sugar and myces which means fungus. So, yeast are a type of fungus that use sugar. In fact, sugar is a source of energy for many living things.

Yeast can use sugar to make energy in one of two ways. If there is oxygen present, yeast use a process called aerobic cellular respiration, the same process that occurs in each and every cell in your body. This process can be shown chemically as follows:
$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{energy}$
(glucose)

Yeast can also grow in conditions where there is no oxygen (anaerobic conditions) using a process called fermentation. The yeast still use sugar to make energy, but no oxygen is available so the chemical reaction is different;
$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + 2CO_2 + \text{energy}$
(glucose) (ethyl alcohol)

Notice that both processes have two products in common. The energy produced is ATP, which fuels all the chemical reactions in the cells. Also, CO_2 (carbon dioxide) is produced by each reaction. In your body the CO_2 you exhale comes from cellular respiration. When yeast grow in bread dough the carbon dioxide creates small bubbles that cause the bread to rise. Ethyl alcohol (CH_3CH_2OH), which is produced by yeast, is the alcohol present in beer, wines and other alcoholic drinks.

Since yeast produce CO_2 by both aerobic respiration and fermentation, this gas can be used to determine the rate of these processes. As the rate of respiration or fermentation increases, the amount of CO_2 produced will also increase.

Purpose
1. To develop and perform an experiment to test a hypothesis.
2. To collect, analyze and interpret data collected from an experiment.

Problem
Explain the problem being investigated in the space on your data sheet.

Hypothesis
Explain your hypothesis in the space on your data sheet.

Control
Describe a control for this experiment in the space on your data sheet.

Materials needed for each group
8 large culture tubes
8 small culture tubes
test tube rack to hold tubes
marking pen
parafilm
tape

Materials available at stations in the room
yeast suspension
7 different concentrations of glucose
graduated cylinder or pipette
water

Procedure-Day One
1. Each group will test seven concentrations of glucose. There should be one control tube per group.

2. You will need one large tube and one small tube for each solution to be tested. Label each large tube: put your group initials, hour and concentration of glucose solution. Also record on your data sheet which solution(s) you are testing.

3. Once you have your tube(s) labeled, add 10 ml of the correct glucose solution to each tube. Rinse the pipette or graduated cylinder before adding a different glucose concentration.

4. Mix the yeast suspension by pipetting the solution in and out several times. Add 2 drops of thoroughly mixed yeast suspension to each of your tubes.

5. Once you have added the yeast suspension, drop the small tube, called the gas collecting tube, into the large tube. The open end of the small tube goes into the large tube first. See Figure 1.

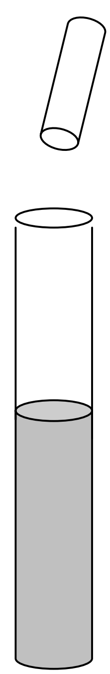


Figure 1

6. Fill the small gas collecting tube with the solution while it is inside the large tube. Your teacher will demonstrate how to do this. Use the parafilm or stopper to seal the end of the larger tube. With your thumb on the parafilm or stopper of the larger tube, turn the tube upside down and gently tap or shake the tube (Figure 2a). This will force the air out of the small tube and fill it with the solution. Slowly turn the large tube upright. Check to be certain there are no air bubbles in the small tube (Figure 2b). If there are, repeat the procedure until no air is present.

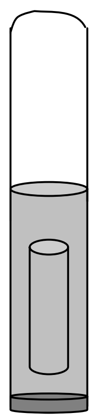


Figure 2a

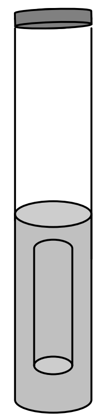


Figure 2b

7. Place your tube(s) in a test tube rack with the rest of the group. Then place the rack of test tubes in the location suggested by your teacher.

Procedure-Day Two

1. Measure the amount of CO_2 collected in the small tube. Use a metric ruler to measure in millimeters the amount of CO_2 in the small tube. Do not take the small tube out of the larger one. Measure from the outside of the large tube. See Figure 3.

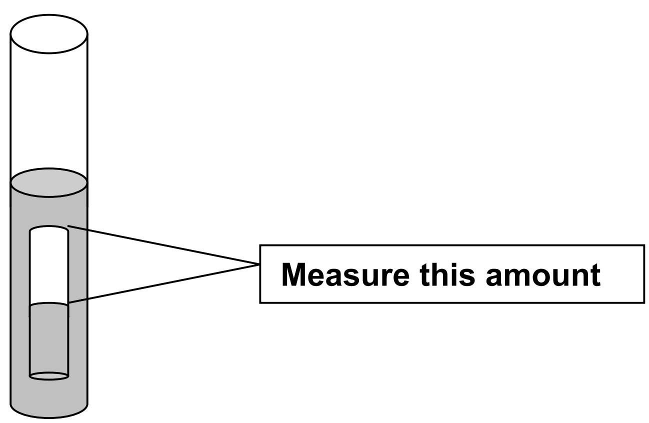


Figure 3

2. Record your measurement in Data Table A on the Data Sheet. Also record your data in the appropriate space on the data table on the classroom chalkboard.

3. Copy the data from the board in Data Table B on your Data Sheet. Next, find the average amount of CO_2 produced in each sugar solution. Record the averages in Data Table B.

Name ___________________________

Date_____________________

Data Sheet

Day 1
Problem: Explain the experimental problem.

____

Hypothesis: Explain your hypothesis in the space below, using an "If...then..." statement.

____

Control: Describe a control for this experiment.

____

Concentration(s) tested ______________________________________

Day 2
Table A- Group Data

Sugar Concentrations (%)

	0	0.5	1	2	4	8	16	32
mm of CO_2 in tube								

Table B- Class Data

Sugar Concentrations (%)

	0	0.5	1	2	4	8	16	32
mm of CO_2 in tube								
class average								

1. Graph your group results and the class data using different colors or symbols for each data set. Include a legend to identify each set of data.

2. Clearly label the x-axis and the y-axis.

3. Provide a title for the graph that briefly describes the experiment.

Name ______________________

Date ___________________

Analysis

1. What was the control used in this experiment? Explain your answer.

2. Does the experiment support your hypothesis? Explain your answer.

3. Using your graph, compare your group's data to the class average. What is the advantage of using class data?

4. Refer to your explanation of the problem being investigated and write a conclusion based upon the results of the lab.

5. Based on your data, do you think yeast would grow well in pancake syrup that was about 32% sugar? Can high concentrations of sugar be used to preserve foods and if so, can you think of any foods that may use high sugar concentration to prevent growth of microbes?

Teacher Guide

Instructional Objectives

At the end of this unit the student should be able to:

1. demonstrate the methods of scientific inquiry by:
    a. stating a problem,
    b. writing a hypothesis,
    c. performing an experiment according to directions,
    d. gathering and organizing data,
    e. analyzing data.

2. demonstrate the following laboratory skills:
    a. using a graduated cylinder to measure volume,
    b. using a metric ruler to measure length,
    c. preparing fermentation tubes and
    d. graphing data.

3. demonstrate an understanding of respiration and fermentation by:
    a. identifying the gas produced in the reaction,
    b. identifying respiration and fermentation as cellular processes and
    c. describing the process of respiration and fermentation.

Background

A. An introduction to the scientific method:
For many students the first unit in biology consists of a discussion of the scientific method. This activity will enable the students to actively work through the scientific method to solve a problem.

You could initiate the discussion by asking the students if yeast are living organisms. After the students reach the conclusion that they are, you might ask, "What do all living things require to stay alive?" (food or a source of energy). You could then ask, "What do yeast use for a source of energy?" (sugar or carbohydrates). Finally, show students containers with different concentrations of sugar and ask, "How does sugar concentration effect the growth of yeast?" This is the problem students will be investigating in this laboratory.

At this point, you could have a package of yeast to show the students. Repeat that the yeast are living but inactive. Ask, "What could we add to make them active?" (add sugar, or a source of energy). Pour some of the yeast into a beaker and add some sugar. Ask, "Will they become active now?" (most students will probably say no). Ask, "What else should we add?" (water).

At this point indicate to the students that they have now covered the first two steps of the scientific method. They have the problem and together they have gathered some background information or research. The next step is to develop a hypothesis. Based on the small amount of knowledge they have about yeast, they should develop a hypothesis, or an answer to the problem. Many people use the “If...then...” form for writing a hypothesis. For this problem, you may want to give the students some guidance. Tell the students that you have sugar solutions ranging from plain water to 32% sugar. Students could then propose a hypothesis something like this, “If the sugar concentration increases, then the yeast will grow better.” This leads to the next part of the scientific method, the experiment. A good hypothesis will suggest how the experiment will be carried out and what to look for in the results.

Another important component of the Scientific Method is the control. Ask the students, “Why will you need plain water for this experiment?” (control).

You may want to have the students break into their lab groups for a period of time to design an experiment to test their hypothesis. After they have worked for a while, bring them together and discuss their experiments. Guide the students to the experimental design outlined in this activity.

One question that might arise is how to determine how active the yeast are, or how fast the yeast are growing. Ask the students “What happens when you become more active?” (breathe faster, give off more carbon dioxide). Yeast are the same; as they become more active, they produce more carbon dioxide.

Given the preceding information, the students should be ready to do the lab.

For homework, the students could write down the problem and hypothesis on the student data sheet.

B. Cellular respiration/fermentation:
Yeast can be used to demonstrate aerobic respiration and/or fermentation. Most accurately, the yeast in this lab are primarily using fermentation. The yeast will use the oxygen present in the solution fairly rapidly; as a result they must switch to fermentation to survive. Since both processes produce carbon dioxide, the amount of carbon dioxide produced is an indication of the activity of the yeast. In this lab the students will measure the amount of carbon dioxide produced by yeast in different concentrations of sugar.

During class discussions of cellular respiration and fermentation students learned that cells must have a source of energy; in this case, sugar. Many students will believe that the greater the amount of sugar, the greater the rate of respiration or fermentation. By measuring the amounts of carbon dioxide produced overnight in various concentrations of sugar, students will determine whether this is true.

Sources of Supplies

Packages of active dry yeast were purchased at the supermarket. Fleischmann's Rapid Rise Yeast was used in the development of the lab. Any type of active yeast should work.

Carolina Biological Supply
2700 York Road
Burlington, NC 27215
800-334-5551

Description	Stock Number	Quantity
18 X 150 culture tubes	K3-73-1464	case of 500
6 X 50 culture tubes	K3-73-1402A	72
glucose (dextrose)		

Equipment
hot plate (optional)
balance

Materials
glucose
distilled water
graduated cylinders
dropping pipettes
Per class: 16 flasks or beakers - two for each sugar solution and two for distilled water

For each group*
8- 16 mm x 150 mm culture tube
8- 6 mm x 50 mm culture tube (gas collecting tube)
marker to label tubes
yeast suspension
sugar solutions and distilled water

*Decide how you want to group the students. If you have a class of 30 and each student tests one solution, you have about 4 experiments with each concentration. If your students work in groups of 2 and each group does all 8 solutions, you will need 120 tubes.

Preparation

Sugar Solutions:
At least one day before the lab the sugar solutions should be prepared. The amount of solution needed will depend upon how you want to set up the student groups. One test requires 10 ml of a sugar solution. The concentrations given here are based upon using glucose. Other sugars could be used. In fact, this would be a good project for more advanced students. If you choose to use another sugar, amounts of sugar and yeast may differ from those given here.

First decide how you will group the students and how many concentrations each student or group will do. For example, if you have 150 students and you want each student to test one concentration, divide 150 students by 8 different solutions. This tells you that you will need enough of each solution to run about 19 tests. Each test requires 10 ml of solution. So about 250 ml of each solution should be sufficient. Proceed as follows to make the solutions. First add 160 g of glucose to about 350 ml of distilled water* (using warm water helps to dissolve the sugar). When the sugar is completely dissolved, pour it into a 1,000 ml graduated cylinder and add distilled water to bring the volume to 500 ml. You now have the starting solution of 32%. Get seven 500 ml flasks or beakers and add 250 ml of distilled water to each one. Label one flask 16%, another 8%, then 4%, 2%, 1%, .5% and finally label the last one 0% or distilled water. To the flask labeled 16%, add 250 ml of the 32% solution and mix thoroughly. Now add 250 ml of the 16% solution to the flask labeled 8% mix and continue this procedure through the 0.5% flask (the 0.5% flask will end up with 500 ml of solution). Do not add anything to the distilled water flask. You should now have 7 containers of different sugar solutions and a container with distilled water. If you do this more than one day before the lab, store solutions in the refrigerator.

It is important to use distilled water, as the results will vary greatly if tap water is used. Several tests showed that the yeast grow much better in tap water. Since tap water varies widely from location to location, distilled water will give more consistent results. If you must use tap water, you will need to decrease the amount of yeast used. If the rate of respiration and fermentation is too high, the gas collecting tubes will be filled to overflowing and a comparison of volumes of gas will not be possible.

Yeast Suspension:
The yeast suspension is prepared by adding 1 gram of active dry yeast to each 10 ml of distilled water. You will need approximately 0.25 ml of yeast suspension per tube. For a class of 30, with each student testing one concentration, 10 ml of yeast suspension should be sufficient. Once you have made the solution, divide it into several containers so all of the students do not have to share one container.

Timeline

Day One of Lab

1. The morning of the lab, make the yeast suspension as directed. Decide how many stations you want and divide the suspension into that many beakers. Mix the solution before dividing it to be certain the suspension is dispersed evenly. The yeast settle fairly quickly; therefore, remind the students to always swirl the beaker to resuspend the yeast. Place a dropping pipette into each container.

2. Divide the sugar solutions into the number of flasks appropriate for the number of stations you have decided to use. Have a 10 ml graduated cylinder or pipette labeled for each sugar solution and distribute to the stations around the room. Have the two different size culture tubes available. Also, have test tube racks or large beakers to hold the tubes. Markers should also be available for the students to label the tubes.

3. Assign students to groups and tell them which solutions they will use in this lab.

4. Demonstrate how to fill the small gas collecting tube inside the larger tube with the solution. To do this, first add the small tube to the larger tube containing the sugar and yeast mixture. Emphasize to the students that the small tube is inserted open end down. Then use a stopper, piece of parafilm or even your thumb to cover the opening of the larger tube. Now invert the larger tube and gently tap the covered opening onto your other hand. This will force the air out of the small tube and fill it with the yeast and sugar solution. Slowly turn the tube upright and check the small tube. If there is still air trapped in the small tube repeat the inversion and tapping.

Day Two of the Lab

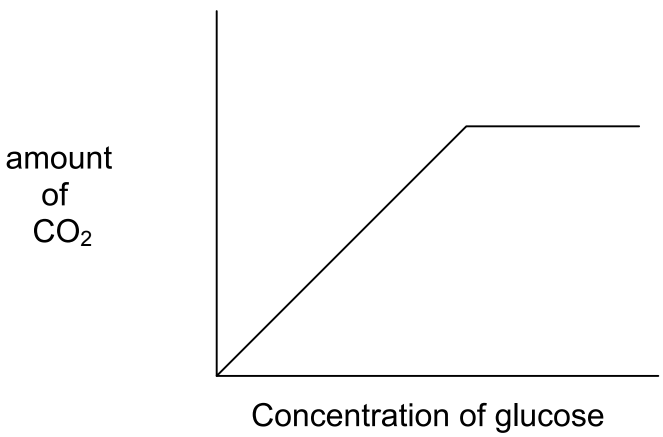
1. Demonstrate for the students how to measure the air space in the small tube while it is still inside the larger tube. Gently tip the larger tube and use the metric ruler to measure the air space inside the tube.
2. Once the students have recorded their measurements, they can record their data on the data table drawn on the board.
3. Instruct students to graph the data.

Data Sheet Answer Key

Problem
How does the concentration of sugar affect the growth of yeast?

Hypothesis
If the concentration of sugar increases, the yeast will grow faster (be more active).

Graph class data
The graph should be similar to this.



Interpretation of data
A typical pattern of gas production is low production at lower concentrations of sugar and increasing production at moderate concentrations of sugar. The initial increase in gas production reflects the increased uptake and metabolism of sugar by the yeast. At moderate concentrations of sugar, the uptake and metabolism have reached a maximum, therefore there is no further increase and there is a plateau in the graph. Occasionally at higher concentrations of sugar, growth of yeast may be inhibited by the osmotic effect of high sugar concentration.

Analysis Answer Key

1. What was the control used in this experiment? Explain your answer.
The distilled water was used as the control to show that sugar is necessary for the activity of the yeast.

2. Does the experiment support your hypothesis? Explain your answer.
The students should report that the graph shows a higher production of CO2 as the concentrations of sugar increases. Depending on their hypothesis the experiment may, or may not, support it.

3. Using your graph, compare your group's data to the class average. What is the advantage of using class data?
Students should say that it will help even out high and low values. It is also a way to repeat the experiment.

5. Refer to your explanation of the problem being investigated. Write a conclusion based upon the results of the lab.
In their conclusion students should refer to the hypothesis and describe the effect of sugar concentration on the growth of yeast.

6. Based on your data, do you think yeast would grow well in pancake syrup that was about 32% sugar? Can high concentrations of sugar be used to preserve foods and if so, can you think of any foods that may use high sugar concentration to prevent growth of microbes?
Students should answer this question based on their data. This question is designed to encourage students to make a connection between this lab and their experiences in real life. In jellies, jams and syrups, high concentrations of sugar act as a preservative.

Teacher Hints & Troubleshooting

1. The quantities and volumes used in this lab are based upon using distilled water for preparing the solutions. Experience has shown that yeast grow much better in tap water. If the yeast are too active, they produce enough carbon dioxide to completely fill the small gas collecting tube. If that should happen, you cannot measure the amount of gas produced because excess gas escapes from the tube. Different regions will vary in the composition of the tap water, so distilled water is recommended. If you use tap water you should set up the experiment to find the appropriate volumes of yeast. Generally, you should decrease the amount of yeast added to the tubes to one half.

2. The culture tubes are supposed to be disposable but they can be washed. Culture tubes are not Pyrex, so do not mix them with regular test tubes. Small test tubes can be used instead of culture tubes. Other sizes of tubes could be used but volumes will need to be adjusted accordingly.

3. Because yeast quickly settle to the bottom, be sure students vigorously mix or stir the yeast suspension each time they use it.

4. Other sugars can be used, but results could be quite different. Test various amounts of yeast if you use a sugar other than glucose (dextrose). Sucrose (table sugar) has been tested and works quite well.

Dear Parents:

Two industries that have been important in all of human history and are still important today are baking and brewing. In each of these industries, small organisms called yeast are at the very heart of the business. Yeast produce the gas bubbles that make bread light and airy. They also produce the alcohol and carbonation found in beer and other alcoholic beverages.

Ask your child about the concepts learned during this unit. It might be a fun project for your family to try their hand at making homemade bread using yeast while we are watching yeast "do their thing" in the lab.

As always, if you feel you have any expertise that could enhance the lesson, please feel free to contact me or make an appointment to come to school to visit us!