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AUTHOR	McConnell, Bill
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ABSTRACT

This publication is the seventh of a series of seven supplementary investigative materials for use in secondary science classes providing up-to-date research-related investigations. This unit is structured for grades 7 through 10. It is concerned with the roles of fermentation processes in the agriculture and food industry. The guide enables students to study the effect of temperature on the rate of fermentation by yeasts. The first part of this guide provides the teacher with: (1) materials needed; (2) supplementary information; (3) preparation of sugar solution and yeast suspension; and (4) suggested readings. The second part provides students with background information and one laboratory investigation - fermentation: activities of a fabulous fungus. The investigation consists of: (1) materials needed for a four-student team; (2) procedures; (3) questions for thought; (4) extending the investigation; and (5) suggested readings. (HM)

science study aids

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fermentation: activities of a fabulous fungus

Developed by Bill McConnell, a science teacher at Redwood High School in Larkspur, California. Mr. McConnell prepared the manuscript in cooperation with Agricultural Research Service (ARS) scientists, U. S. Department of Agriculture, at the Western Regional Research Center, Berkeley, California.

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TO THE TEACHER

This Science Study Aid (SSA) is structured for grades 7 through 10. It is based on research conducted by ARS scientists studying the roles of fermentation processes in agriculture and the food industry.

Activities in this SSA enable students to study the effect of temperature on the rate of fermentation by yeasts. Students will collect, record, and interpret data on the fermentation process in much the same way ARS scientists do in their studies.

The inquiry method is used to develop meaningful group or class discussions. Also, for students who show special interest and for the more able learner, extensions of the investigation are provided. The activities can be conducted using standard laboratory equipment or improvised equipment found in the home, supermarket, or hardware store.

The material will be of particular value in general science and introductory biology courses during studies of the lower plants, energy cycles, nutrition, and ecology. An essential part of this SSA is the background material. It is recommended that students receive the background information a few days prior to the laboratory activities. This will allow sufficient time for students and the teacher to survey the suggested reading material at the back of the SSA, along with teacher-assigned readings from textbooks.

Depending on the availability of equipment, students may work individually or in teams to perform the activities in this SSA.

MATERIALS LIST

For your convenience, we have listed below the materials needed to perform the Investigation in the Science Study Aid. The following list gives the quantities needed for one student or one team of two or more students:

STUDENT MATERIALS

2 600 ml beakers
2 15 x 150 mm test tubes
2 10 ml graduated cylinders
1 centigrade thermometer
2 number 4 1-hole stoppers with glass elbows
2 12" pieces of rubber tubing O.D. 1/4"
10 ml yeast suspension
50 ml 5% sugar solution
crushed ice, hot water and tap water
watch or wall clock

TEACHER MATERIALS

2 600 ml beakers
500 ml water
25 g sucrose or glucose (or 50 ml molasses)
1 g peptone
1/2 pkg. dry baker's yeast

PREPARATION OF SUGAR SOLUTION AND YEAST SUSPENSION

Approximately 500 ml of yeast suspension and 500 ml of sugar solution are needed for a class of 28 students, each doing the Investigation with a complete equipment complement. The following procedures for preparing the solutions are recommended:

YEAST SUSPENSION

500 ml water
25 g sucrose or glucose (or 50 ml molasses)
1 g peptone
1/2 pkg dry baker's yeast
Culture for at least 12 hours at 25° to 30°C.

SUGAR SOLUTION

Prepare the same as yeast suspension above; however, OMIT the yeast.

SUGGESTED READINGS FOR THE TEACHER

Fermented Foods: Current Science and Technology, New York State Agricultural Experiment Station, Geneva, N.Y., Eighth Annual Symposium, Special Report No. 16, 1974.

Preservation of Carrots by Lactic Acid Fermentaion, Niketic-Aleksic, G. K., Bourne, M.C., and Stamer, J. R., 1973, J. Food Sci. 38: 84-87.

Growth Rates and Fermentation Patterns of Lactic Acid Bacteria Associated With the Sauerkraut Fermentation, Stamer, J. R., Stoyla, B. O., and Dunckel, B. A., 1971, J. Milk and Food Technol. 34: 521-525.

Pilot Plant Studies on Tempeh, Steinkraus, K. H., Hand, D. B., Van Buren, J. P., and Hackler L. R., 1961. Proceedings of Conference on Soybean Products for Protein in Human Foods. USDA, NRRL, Peioria, Ill. Sept. 1961.

FILM

Microbiology, Part II: Metabolic Diversity, No. 11, 1961. (Sound, color, 28 min.) Purchase or rent, AIBS (McGraw-Hill).

TO THE STUDENT

BACKGROUND

If you were to eat at Fisherman's Wharf in San Francisco, California, chances are you would be served some of the city's famous San Francisco sourdough French bread. Like many others, you might wonder about its unique and intriguing flavor. Agricultural Research Service (ARS) scientists at the Western Regional Research Center (WRRC) in Berkeley, California, also were curious about this unique flavor. Their research efforts resulted in the discovery that bacteria and yeast were responsible for the sour but pleasant taste of this famous bread. The scientists named the bacteria Lactobacillus san francisco. To complete this research, the scientists had to be aware of the important biological process known as fermentation. During the leavening (raising) of the bread, prior to baking, fermentation occurs and chemical substances known as by-products are produced by the bacteria which account for the flavor.

Like the research on the flavor of San Francisco sourdough French bread, many other interesting and exciting research projects are being carried out by ARS scientists involving fermentation processes; for example, ARS studies on the use of fermentation processes to improve the feed value of waste products from agriculture and industry. Research is being conducted on the use of the fermentation activities of microorganisms to convert waste materials such as paper, straw, corn cobs, and cannery wastes into protein that can be used for food - - a fantastic way to utilize wastes that, in some cases, are now being dumped into our environment as pollutants.

Here's how you can learn about the important biochemical process known as fermentation.

Yeasts are among the more important microbes that ferment carbohydrates (sugars). A most useful species of yeast is Saccharomyces cerevisiae, called baker's yeast, or wine yeast. The yeast is useful because it has the ability to live anaerobically, that is, in the absence of oxygen. From glucose (grape sugar), yeasts can derive energy and manufacture important by-products as indicated in the reaction below.

Anaerobic Fermentation by Yeasts

$C_6H_{12}O_6$	$2\ CO_2$ +	$2\ C_2H_5OH$ +	energy
(sugar)	(carbon dioxide)	(ethyl alcohol)	(2 ATP)

In this process, the energy obtained from the reaction is used for growth and cell division. The total anaerobic process is called fermentation. You will notice that, during the fermentation of glucose, ethyl alcohol (C_2H_5OH) is produced and, as you know, the effects of this substance on human behavior are well-known. What product of this reaction is given off as a gas?

Although the first interest in fermentation came from an interest in alcoholic beverages, today the greater part of fermentations carried out in our country are directed at the production of alcohol for other purposes. For example, in the production of industrial alcohol, the by-products of fermentation that are so important for the taste in wines and distilled drinks are of no importance; therefore, many kinds of waste material can be used as the source of fermentable carbohydrates. Some of these are potatoes, sugarcane waste, cannery waste, and soybean waste. In some cases, not yeasts, but bacteria are used as fermenting agents. For example, lactic acid is a by-product of the fermentation of glucose by lactic acid bacteria. This process is best known for its souring effect on milk. Other familiar and more desirable products of bacterial fermentations are cheeses, buttermilk, and yogurt. Can you think of, or suggest, other by-products of fermentation?

A good researcher always prepares for future experiments by seeking out and reading available reference material. How about you? Do you think the reference materials in your room or library might give you a better understanding of the biochemical events that occur during fermentation? Assigned reading on the subject from your textbook can be enlightening, so remember to review this material.

Like ARS scientists, you will consider problems dealing with fermentation. You will be provided with the opportunity to study fermentation by yeasts and to record, think about, and interpret your data. Remember, use the Investigation as a springboard to the additional experiments suggested or to new ideas of your own.

INVESTIGATION 1 - FERMENTATION: ACTIVITIES OF A FABULOUS FUNGUS

Before you begin the activities in this Investigation, read through the procedure and see if you can predict what will happen. Write your predictions in your notebook and then design your own graph and data table. The graph (Figure 1) and the data table (Table 1) might suggest ideas as to how you can do this. As you progress through the activities in this Investigation, be sure to record your data. These data will assist you in answering the questions at the end of the Investigation.

MATERIALS

2 600 ml beakers
2 15 x 150 mm test tubes
2 10 ml graduated cylinders
1 centigrade thermometer
2 #4, 1-hole stoppers
  with glass elbows
2 12" pieces of rubber tubing
  O.D. 1/4"
10 ml yeast suspension
50 ml 5% sugar solution
crushed ice, hot water and tap water
watch or wall clock

PRELIMINARY PROCEDURE

1. Study the procedure. Write your predictions concerning what you think will happen and record this in your notebook.
2. Design your own graph and data table based on the procedure.
3. Study the equipment design shown in Fig. 2. Keep this in mind as you prepare to carry out the activities in this Investigation.

PROCEDURE

1. Pour approximately 250 ml of cold tap water into one 600 ml beaker. Add crushed ice. With your thermometer, take temperature readings of the water until the temperature stabilizes at approximately 5^{o} C. Fill the second beaker two-thirds full of tap water. Set the beakers aside until you have prepared the yeast suspension tube and the sugar solution tube.

FIG. 1

EFFECT OF TEMPERATURE ON CO_2 PRODUCTION DURING SUGAR FERMENTATION BY YEASTS

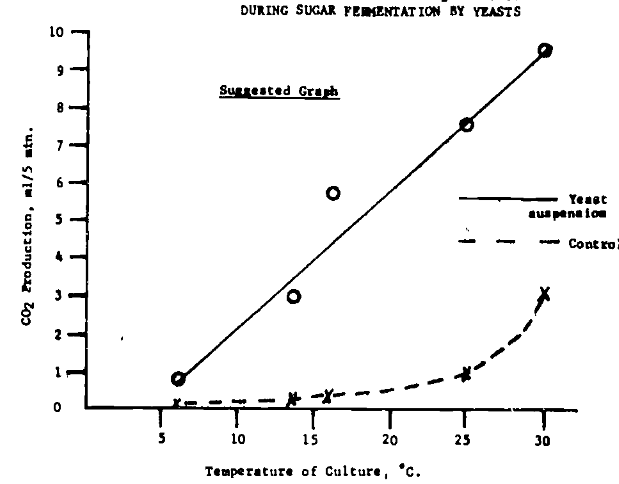

TABLE 1

EFFECT OF TEMPERATURE ON CO_2 PRODUCTION DURING SUGAR FERMENTATION BY YEASTS

Run	Time	Temp. (°C.)	CO_2 Production	Change in Control
1	5 min.	7° C.	.75 ml	0
2	"	13° C.	2.9 ml	.2 ml
3	"	17° C.	5.8 ml	.5 ml
4	"	25° C.	7.7 ml	1.0 ml
5	"	30.5° C.	9.6 ml	3.0 ml

(Suggested Data Table)

2. Into one test tube, pour approximately 8 ml of yeast suspension. Be sure to swirl and mix the culture before pouring it into the test tube. Can you think of a reason for doing this?

   Into the second tube, pour an equal amount of sugar solution. Be sure to check the volumes in each tube and make sure they are equal. Stopper the tubes and attach the rubber tubing to the glass elbow inserted into the stopper. (CAUTION: don't break the glass elbow.) At the same time, place both tubes in the ice water bath. Check the temperature and record this in your data table.

3. Allow the tubes to remain for 2 or 3 minutes in the ice water bath and then proceed to step 4. Can you suggest why you should wait?

4. Fill the graduated cylinder with tap water. Cover the mouth of the cylinder with your fingers. Carefully invert the cylinder and lower it into the beaker filled with tap water. Check the cylinder carefully and make certain that no air is trapped at the top. The cylinder must be completely filled with water. Now repeat the same procedure using the other cylinder. Label one cylinder control and the other experimental.

5. Place the two beakers side by side and carefully lift both cylinders in one hand until you can insert the ends of the rubber tubing into the cylinders. This must be done at the same time and without taking the cylinders out of the water. Which tube is inserted into the cylinder labeled control? Which one experimental?

   Lower the cylinders to the bottom of the beaker and check the time on your watch or wall clock. Allow the system to run for five minutes and then remove the tubes from the cylinders. With the cylinders still in the beaker, measure the number of milliliters of gas produced in each tube. Record these data in your data table.

6. Now, repeat steps 1 through 5, only increase the temperature by 10 degrees in the water bath. This can best be accomplished by slowly adding small quantities of warm water to the beaker. Again, allow the system to run for 5 minutes and measure the amount of gas produced at this new temperature. Be sure to record your data.

   Repeat steps 1 through 5 a third and fourth time, increasing the temperature in the water bath by 10 degrees each time. When you have finished your fourth and last test, clean up your equipment and work area.

7. Graph the resulting data from your investigation.

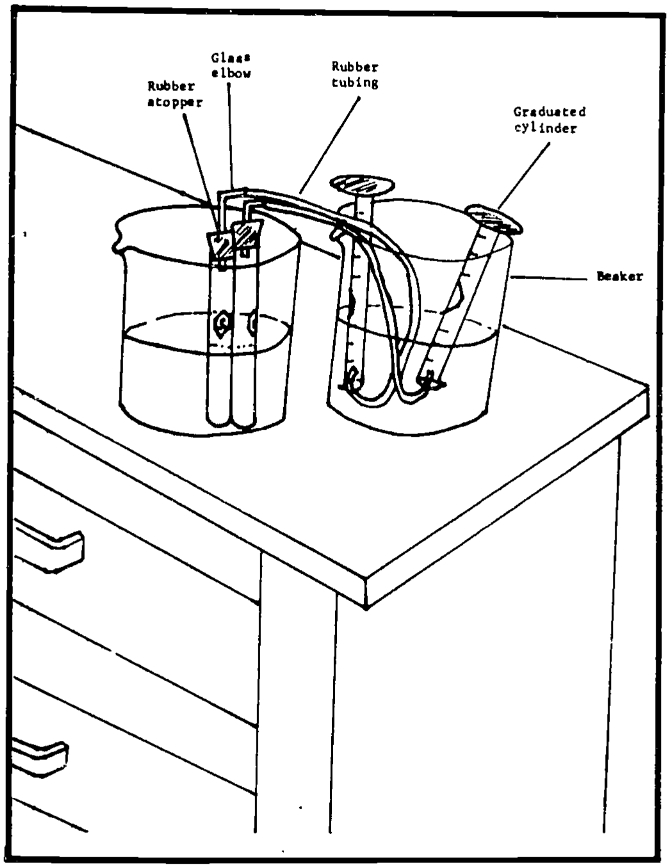

FIG. 2

QUESTIONS FOR THOUGHT

1. Write the reaction for anaerobic fermentation by yeasts. Which product did you measure in this Investigation?

2. A scientist would refer to the experimental procedure that you have just completed as being a "controlled quantitative experiment". What do you think the meaning of this would be? What or where is the control? What part of your data would you consider to be quantitative? Why?

3. Discuss the relationship between the rate of anaerobic fermentation and temperature. Base your discussion on the data collected during the Investigation.

4. What do you think would happen if you continued to increase the temperature at 10 degree intervals until your tubes reached 100° C.? Design a graph that illustrates your predictions.

5. Scientists often refer to units, like the tube with the sugar solution, as being thermobarometers. Why do you think your sugar solution tube would be called a thermobarometer? Did this tube produce any measurable gas? If your answer is "yes", how might this data help you to more accurately calculate the volume of gas produced in the experimental tube?

6. Study the reaction for anaerobic fermentation. What product of the fermentation process was not measured in this investigation? Under ideal conditions, the yeast cells in the tube could produce only about 15% by volume of this product. Can you suggest reasons why this is true?

EXTENDING THE INVESTIGATION

1. Design an experiment to show that heat energy results from the fermentation of carbohydrates by yeasts. Hint: Incorporate into your experimental design a 1-pint vacuum bottle! Most school lunch boxes have a vacuum bottle in which students bring milk or other beverages to school.

2. Using materials such as paper, cornmeal, grape juice, and apple juice, design an experiment to provide data on fermentation rates by yeasts using these substances.

3. Design an experiment to compare and to contrast the fermentation by yeasts and by bacteria of various carbohydrate (sugars) substrates. Use your data to demonstrate the nature of anaerobic fermentation.

4. First research and then design an experiment to indicate whether water sources like lakes, streams, rivers, bays, and the ocean, are polluted or nonpolluted. Many water quality control agencies use fermentation processes to determine pollution in water samples!

5. If you live in an area where wine is made, see if you can find out what is done with the tons of yeast cells after the grapes have been fermented.

6. If your school has access to bacteria cultures, try the following experiment. First, innoculate fresh (additive-free) apple juice with yeast and allow the juice to ferment anaerobically. What is the product of this fermentation process? Then, innoculate the juice with <u>Acetobacteria</u> <u>aceti</u> and bubble air through the juice using an aquarium pump. What is the product of this fermentation?

7. Find out which microorganisms, by the fermentation process, produce consumer products like cottage cheese, yogurt, buttermilk, sauerkraut, olives, and pickles

SUGGESTED READING FOR STUDENTS

<u>Textbook of Microbiology</u>, Burrow, W., 1963, 18th ed. W. B. Saunders, Philadelphia, Pennsylvania, Chap. 5.

<u>The Vital Wheel: Metabolism</u>, Haffner, Rudolph, 1963, American Education Publications, Inc., New York (Pamphlet).

<u>Our Smallest Servants: The Story of</u>

Fermentation, Pfizer, Charles, 1963, Charles Pfizer & Co., Inc., New York.

Production of Manufactured Dairy Products, 1972, DA 2-1 (73), June 20, 1973, U. S. Department of Agriculture - Statistical Reporting Service.

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are a series of supplementary investigative materials for use in secondary science classes, grades 7 - 12. The materials are based on federal and private research programs. They are written by secondary science teachers working with scientists at research facilities throughout the country. Before being published, they are tested in the laboratory and in classrooms of cooperating teachers.

Several times during the year, new SSA's are developed. If you want to be notified of their availability, request that your name be added to Wordwork's mailing list. Because we cannot provide enough copies for students, we have designed SSA's so that teachers can easily reproduce the student portion for their classes.

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