IFT Experiments in Food Science Series

Microbiology in Food Systems

Activity #1

What Affects Yeast Growth?

A Science Unit for Secondary School Curriculum

Institute of Food Technologists
The Society for Food Science and Technology

TEACHER ACTIVITY GUIDE

What Affects Yeast Growth?

EXPECTED OUTCOMES

This experiment will illustrate that there are several factors which affect the growth of yeast. A beneficial microorganism, yeast has been used in the fermentation of foods for thousands of years. Bread, wine, beer, and fruit are processed and/or preserved using yeast in fermentation. The by-products of the fermentation process are carbon dioxide and ethyl alcohol (ethanol).

ACTIVITY OBJECTIVE

A yeast population is affected by a number of factors, the control of which is essential for optimal activity. These factors include pH, temperature, nutrient availability, and the concentration of available nutrients. By determining which factors affect the yeast activity, these variables can be controlled in the fermentation process.

This experiment will illustrate to the student that the growth of yeast is affected by pH, temperature, and nutrient level and that one natural by-product of this fermentation process is carbon dioxide.

ACTIVITY LENGTH

It is suggested that students be put into collaborative groups for this activity for several reasons. First, this limits the amount of materials needed. Second, more activities can be conducted in a shorter amount of time. And third, students will experience working as a team. Depending on the amount of teacher preparation done prior to the activity, all of the lab experiments can be done in a typical classroom period of 30–45 minutes.

This activity can be conducted in several ways—with all students in groups repeating all tests; dividing the class into four groups, with each group conducting one section of the tests; or dividing the class into groups of four students, with some groups repeating tests.

Solutions may be prepared by the students, or ahead of time by the teacher.

SCIENTIFIC PRINCIPLES

All living organisms, large and small, have one thing in common: the cell. This is a tiny living factory capable of converting simple food substances into energy and new cell material and of reproducing itself.

Microorganisms are made up of a very few cells, or even a single cell, capable of carrying on all of life's processes. A basic understanding of cell structure and function is essential to understanding the actions of bacteria, yeast, and molds. Since the cell is the basic unit of all living things, one might think it is a simple structure. Nothing could be farther from the truth. The cell is complex in its makeup and its function. Many scientists have spent their lives studying it. The main parts of the cell are the nucleus, cytoplasm, and cell wall. The nucleus is the control center—it directs cell division, the formation of new cells. The cytoplasm contains the parts which convert food material into energy and new cell materials. The cell wall or membrane holds everything together and controls the passage of material into and out of the cell.

Yeasts are small, single-celled plants. They are members of the fungus family (plural = fungi), which also includes mushrooms. Fungi differ from other plants in that they have no chlorophyll.

While bacteria thrive on many different types of food, yeasts require carbohydrates, such as sugar and starch. (However, the yeast used in this experiment, *Saccharomyces cerevisiae*, cannot utilize starch.) From these, they produce carbon dioxide (CO_2) gas and alcohol. Thus, they have been useful to man for centuries in the production of certain foods and beverages. They are responsible for the rising of bread dough and the fermentation of wines, whiskey, brandy, and beer. They also play the initial role in the production of vinegar.

Some yeasts are *psychrotrophs*, meaning that they can grow at relatively low temperatures. In fact, the fermentation of wines and beer is often carried out at temperatures near 7°C. This also means that they can create a spoilage problem in meat coolers and other refrigerated storage areas.

Unlike bacteria, which multiply by binary fission (cell division), yeasts reproduce by a method called *budding*. A small knob or bud forms on the parent cell, grows, and finally separates to become a new yeast cell. Although this is the most common method of reproduction, yeasts also multiply by forming spores.

Because they can grow under conditions of high salt or sugar content, yeasts can cause the spoilage of certain foods in which bacteria would not grow. Examples are honey, jellies, maple syrup, and sweetened condensed milk. Foods produced by the bacterial fermentation process, such as pickles and sauerkraut, can also be spoiled by yeasts which interfere with the normal fermentative process.

VOCABULARY

Aerobic—requiring oxygen or air for growth
Anaerobic—growth in the absence of oxygen
Budding—reproduction in which a small part of the cell wall swells out and a wall of cellulose soon shuts off this new growth from the parent plant. It becomes an independent cell, soon growing other buds.
By-product—one result of a chemical process.
Facultative anaerobes—microorganisms which can grow with or without oxygen
Fermentation—the process by which compound sugars and some starches are converted into simple sugars, which are further hydrolyzed into alcohol and CO_2.
Generation time—the time it takes for a microbial cell to double
Psychrotrophs—microbes which grow at refrigeration temperature
Yeasts—tiny, one-celled, oval-shaped, microscopic plants which can be found naturally in the nectar of flowers, on the surface of fruits, and in the soil.

MATERIALS REQUIRED

Safety goggles
125-mL Erlenmeyer flasks or small (8-oz) glass soft-drink bottles
Balloons, 7.8-cm (7-inch) size
Table sugar (sucrose)
Fructose, lactose, and glucose from science supply catalog or health food store
pH paper
Wax pencil or marker
Masking tape
Large bottle or 16 packages of rapid-rise yeast
Vinegar

Ammonia
Clock or stopwatch
Warm water bath (40°C and 80°C)
Triple-beam balances or scales
100-mL graduated cylinders
Eyedropper
Thermometers

STUDENT EXPERIMENTAL PROCEDURE

Group 1—Temperature Experiment

Label flasks A through D. Add 80 mL of tap water (neutral pH only) to each flask and place the flasks in the following conditions:

Flask A—in ice bath.
Flask B—at room temperature.
Flask C—in 40°C water bath.
Flask D—in 80°C water bath.

Dissolve 5 g of sucrose in each flask. Add 4 g of rapid-rise yeast to each flask and stir. Then place a balloon on each flask and seal it securely with masking tape. Periodically stir the contents by spinning the flask slowly.

Group 2—Water Activity Experiment

Label flasks E through H. Add 80 mL of 40°C water (neutral pH only) to each flask and dissolve the following amounts of sucrose in each:

Flask E—0 g (water only)
Flask F—5 g
Flask G—30 g
Flask H—50 g

Add 4 g of rapid-rise yeast to each solution and stir. Then place a balloon on each flask and seal it securely with masking tape. Periodically stir the contents by spinning the flask slowly.

Group 3—pH Experiment

Label flasks I through L. Add 80 mL of tap water (neutral pH only) to each flask and add vinegar or ammonia to adjust the pH as shown below. Use pH paper to verify the pH.

Flask I—add vinegar to adjust the pH to 3.
Flask J—add vinegar to adjust the pH to 5.
Flask K—add vinegar or ammonia to adjust the pH to 7.
Flask L—add ammonia to adjust the pH to 10.

Dissolve 5 g of sucrose in each flask and warm the solutions to 40°C. Add 4 g of rapid-rise yeast to each solution and stir. Then place a balloon on each flask and seal it securely with masking tape. Periodically stir the contents by spinning the flask slowly.

Group 4—Nutrient Experiment

Label flasks M through P. Add 80 mL of tap water (neutral pH only) at 40°C to each flask and dissolve 5 g of each of the following sugars:

Flask M—fructose
Flask N—glucose
Flask O—sucrose
Flask P—lactose

Add add 4 g of rapid-rise yeast to each solution and stir. Then place a balloon on each flask and seal it securely with masking tape. Periodically stir the contents by spinning the flask slowly.

All Groups—Observations

1. After 15 minutes, record initial observations in the table provided for each test. Then make additional observations at 10-minute intervals, and final observations.

These observations should include a description of the fermentation activity and a measure of the amount of gas produced, either by measuring the actual volume of gas produced (see ancillary activities below) or by measuring the circumference of the balloon. To measure the circumference, wrap a string around the balloon at its widest point, then measure the length of the string.

2. Prepare bar graphs of balloon circumference (or cm^3 of gas produced) against each of the following:
   - Temperature
   - pH
   - Type of sugar
   - Water activity

TEACHING TIPS

- Warm water baths (40°C and 80°C) should be set up prior to the experiments for the placement of the flasks (except for the temperature experiment). If a commercial water bath is not available, a Styrofoam chest or bucket can be used. A pot of hot water will need to be available to add water as needed to maintain the water temperature in the water baths.

- There is a tendency for the flasks to float in the water bath during incubation. To prevent this, the students can physically hold down the flasks, use lead "donuts" around the neck of the flasks, tape the flasks to the side of the water bath, or add enough marbles to the flasks prior to water addition to keep the flasks from floating.

- **Caution:** Remind students to be careful when attaching balloons to the flasks. They must carefully tape the balloon securely to the flask to avoid any leaks. Also, they must remove the balloons **very carefully,** as the foam, which will rise into the balloons, will be expelled under great pressure on removal. It is suggested that the balloons remain attached to the flasks until the next class period to allow the CO_2 gas to escape overnight.

- It is important that the tap water be warmed to about 40°C for each test prior to adding the sugar and yeast. Keep enough water available at this temperature for all tests.

- Students may use a lighted match to demonstrate that CO_2 is present in the balloons, as follows: Trap some of the gas from the flask into a test tube. (See ancillary activities below.) Place the lighted match into it. The match should be immediately extinguished. (Here is an opportunity to discuss the use of CO_2 in fire extinguishers).

- In the temperature experiment (Group 1), Flask C should show the best production of CO_2, as 40°C is the ideal temperature for yeast growth. There should be little or no production of CO_2 in Flasks A, B, and D. Students should make observations regarding the fact that there is no foam in Flask D. They may be confused about the slight expansion of the balloon in Flask D. This is a result of air being heated inside the flask, which causes slight expansion of the balloon.

- In the water activity experiment (Group 2), Flask H should show little growth. In this solution, enough sugar is present to tie up the water molecules so that the yeast growth is inhibited. Flask E is the control in this test and should show no growth. Any inflation is due to air being heated inside the flask. As an additional experiment, have students try other sugar concentrations.

- In the pH experiment (Group 3), there should be the most growth in flask K because the ideal pH for yeast growth is around 7. There should be little or no growth in flasks I, J, and L. As an additional experiment, have students try additional pH levels.
- In the nutrient experiment (Group 4), Flask M should have the fastest-expanding balloon, since fructose is a simple sugar and therefore requires fewer steps to hydrolyze it to alcohol and CO_2. Flasks N and O will produce expanded balloons that are almost equal in size; however, since glucose is a simpler sugar than sucrose, the balloon on flask N may expand faster. There should be no expansion in Flask P, since baker's yeast does not produce the enzyme lactase needed to hydrolyze lactose during fermentation.

QUESTIONS & ANSWERS

1. What observations did you make about the flasks prior to the addition of the yeast?

Ans. Most sugar is dissolved, though some undissolved sugar may remain visible.

2. Which flasks showed the greatest yeast growth, i.e., most production of CO_2 gas?

Ans. Answers may vary, but should generally be: Flask C in test 1; Flask G in test 2; Flask K in test 3; and Flask M in test 4.

3. Did the contents of the flasks look the same at the end of the test time? Why or why not?

Ans. No, there should be a good deal of foaming present in many of the flasks. These changes are due to fermentation's having taken place to yield CO_2 and alcohol.

4. Knowing what you have learned about yeast "food," do you think yeast will hydrolyze gelatin or fat?

Ans. No. Yeast will not ferment fat or protein. The only nutrients yeast uses for energy are sugars and starches.

5. Which were the most favorable conditions for growth?

Ans. The most favorable conditions were 40°C (slightly warm temperature), neutral pH (pH 7), and the presence of sucrose and fructose.

6. Which were the least favorable conditions for growth?

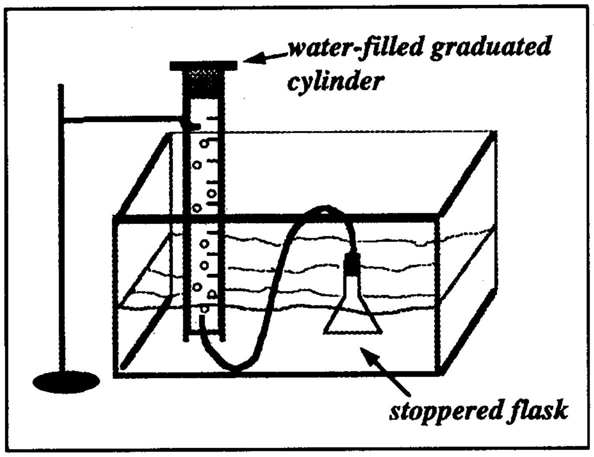
Ans. The least favorable conditions were too high or low pH, too hot or cold temperature, too much or too little sugar, and the presence of lactose.

ANCILLARY ACTIVITIES

1. To quantify the amount of CO_2 produced, a flask containing the test solutions may be stoppered with a glass tube extending from the stopper, as shown in Figure 1. Rubber or plastic tubing is connected to the glass rod on the flask and then placed inside a 250-mL graduated cylinder which has been filled with water and turned upside down in a water bath. As the CO_2 is expelled from the flask, it travels through the tubing and into the graduated cylinder. The water in the cylinder is displaced into the water bath at the same rate as the gas is expelled.

2. In place of the flasks and cylinders, 15-mL graduated conical centrifuge tubes with caps (Corning #25319) can be used. For

Figure 1—Setup for Measuring Amount of CO_2 Produced



further information, see "Fermentation, Respiration, and Enzyme Specificity: A Simple Device and Key Experiments with Yeast," by L. Reinking, J. Reinking, and K. Miller, *The American Biology Teacher,* March 1994, pp. 164–168.)

3. Incubate at different temperature.

4. Replace sugar with 5 g of cooking oil, corn starch, or gelatin. These variations will illustrate that yeast is unable to utilize protein or fat.

5. Grind one Lactaid or Dairy Ease tablet into a flask containing 5 g of lactose and 80 mL of water. Incubate at 40°C for 5 minutes. After the addition of the yeast, fermentation will occur because the Lactaid or Dairy Ease has broken down (hydrolyzed) the lactose into a mixture of glucose and galactose. While yeasts are unable to utilize the disaccharide lactose, they are able to ferment the monosaccharides glucose and fructose. This would be an ideal time to discuss lactose intolerance in humans.

DATA TABLE (Typical Results)

Test/Flask	Conditions	Fermentation observed	Gas produced[a]
Temperature A	Sucrose + Ice bath	*None*	*Little or none*
B	Sucrose + Room temperature	*Some initially*	*Some*
C	Sucrose + 40°C	*The most*	*The most*
D	Sucrose + 80°C	*None*	*Slight*
Water activity E	40°C + No sucrose	*None*	*Little or none*
F	40°C + 5 g sucrose	*Some initially*	*Some*
G	40°C + 30 g sucrose	*The most*	*The most*
H	40°C + 50 g sucrose	*Little*	*Little*

[a] **Balloon circumference in cm or volume of gas produced in cm^3**

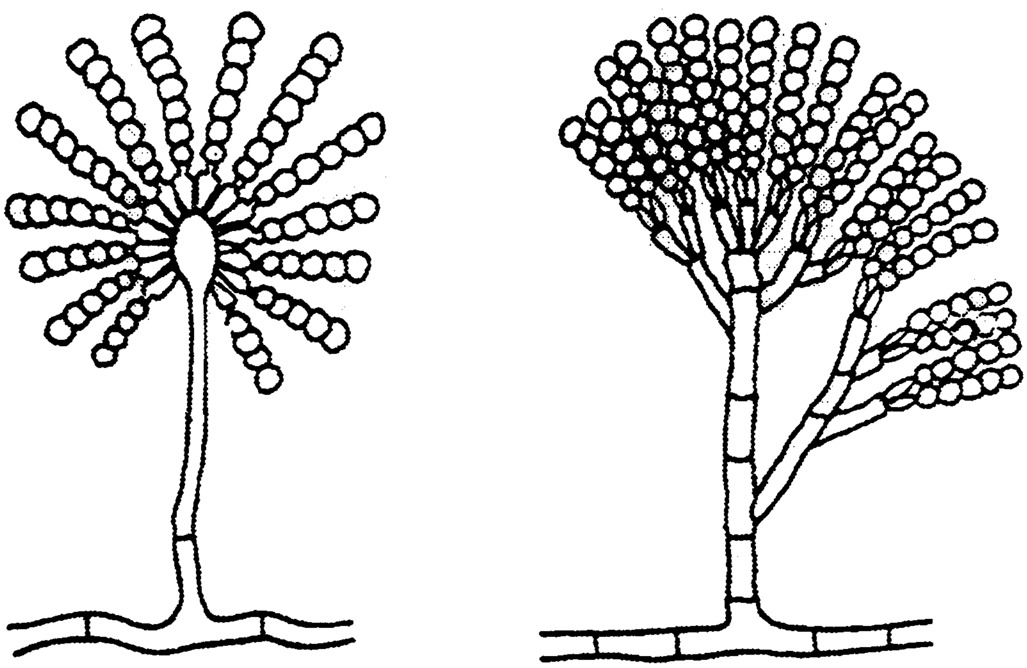
DATA TABLE (Typical Results), continued

Test/Flask	Conditions	Fermentation observed	Gas produced[a]
pH I	40°C + pH 3	None	Little or none
J	40°C + pH 5	Some initially	Some
K	40°C + pH 7	The most	The most
L	40°C + pH 10	None	Little or none
Nutrient M	40°C + Fructose	The most	The most
N	40°C + Glucose	Some	Some
O	40°C + Sucrose	Some	Some
P	40°C + Lactose	None	Little or none

[a] **Balloon circumference in cm or volume of gas produced in cm^3**

Visual Master 5

Mold Fruiting Body



Visual Master 6

Some Food Products Which Involve Mold Fermentation

Raw material	Microorganisms involved	Foods produced	Where produced
Milk	*Penicillium roqueforti,* lactic acid bacteria (LAB)	Blue (bleu) cheese	France, U.S.
	Penicillium camemberti, LAB	Brtie, Camembert cheese	France
	P. Roqueforti, LAB	Gorgonzola cheese	Italy
	Geotrichum spp., LAB, yeasts	Port Salut cheese	France
	P. Roqueforti, LAB	Stilton cheese	England
Beef, pork	*Scopulariopsis, Aspergillus. Penicillium* spp., LAB	Aged dry salami	Europe
Pork hams	*Aspergillus, Penicillium* spp	Country-cured hams	Southern U.S.
Rice, soybeans	*Aspergillus oryzae,* LAB, yeasts	Miso	Japan, China
Corn	*Aspergillus, Penicillium*, LAB	Ogi	Nigeria, West Africa
Taro corms	*Geotrichum candidum*, LAB, yeasts	Poi	U.S. (Hawaii)
Soybeans, wheat	*A. oryzae* or *A. soyae*, LAB, yeasts	Soy sauce	Japan, China
Soybeans	*Rhizopus oligosporus*	Tempeh	Indonesia