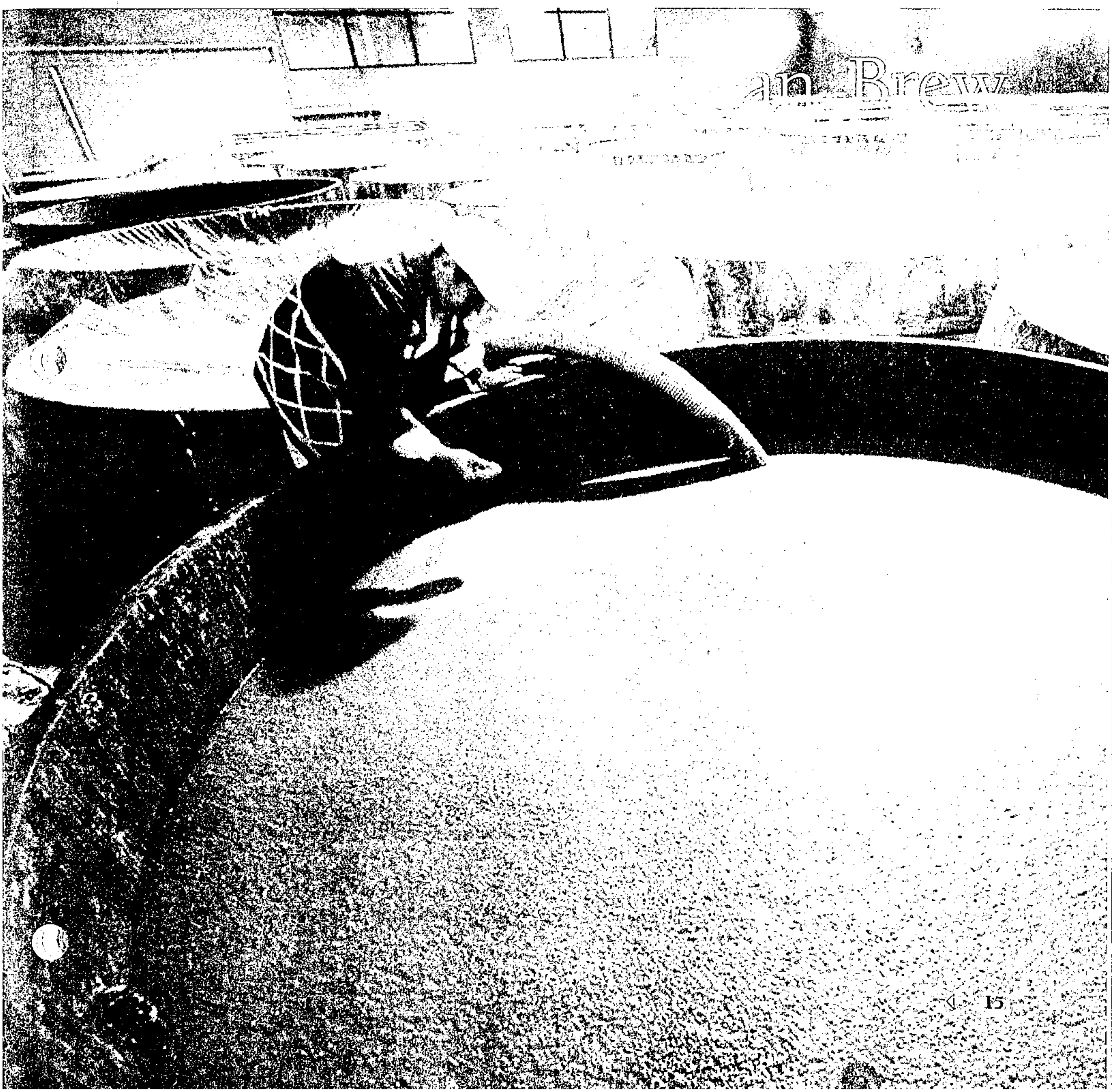
CHAPTER



Bean Brew

Henry, Edie, Taki, and Sally sat around the table at their favorite restaurant celebrating Henry's new job. "I can't believe it's already been six years since we met," Sally said.

It wasn't long before the talk around the table turned to biotechnology stocks. Edie and Taki were always well informed about the latest companies and enjoyed arguing about what products were going to be the "next big thing."

"Excuse me," Sally began with a smile when there was a break in the animated conversation. "What's all the fuss about a new strain of transgenic fungus? I can't imagine how this would affect me . . ."

Taki reached for the small container of soy sauce on the table and held it up. "It turns out that this fungus will increase the efficiency of the first stage of brewing soy sauce. Did you know that brewing soy sauce is one of the original biotech industries? They were shipping the stuff in barrels in Asia over 500 years ago and in bottles to Europe by the 1600s. Now most of the world uses soy sauce."

The friends settled in; considering Taki's usual attention to detail, this would be a long story. "About 5,000 years ago in China," he began, "people grew soybean crops for food and animal feed. Storing beans was risky because of spoilage. Salt was added as a preservative, but over time the beans fermented."

"Like pickles and sauerkraut?" Henry asked.

Taki nodded and continued, "Except the beans softened as they fermented. This paste was easier to digest, so people started to eat it. It's called miso today. Then, about 500 years ago, someone discovered that the liquid in the bottom of the barrel could be used for cooking. And so, soy sauce was invented!"

"Is this fermentation process similar to making wine from grapes?" Sally asked.

"Well, soy sauce brewing is actually done in two stages. In Japan soy sauce is called *shoyu*. To make it, you first steam the soybeans and mix them with toasted, crushed wheat. Then add the fungi *Aspergillus oryzae* and *Aspergillus sojae*. The new mixture, called *koji*, is left uncovered for a couple of days, while the fungi partially digest the soy and wheat."

"So, is the transgenic fungus you were talking about *Aspergillus*?" asked Sally.

"Exactly," Taki replied. "Okay, in the next stage, you mix the koji with water and a lot of salt to form a mash called *moromi*. Then put the moromi into airtight containers and let them ferment for at least 6 months. Squeeze this mash to get the liquid soy sauce, which is filtered, pasteurized, and tightly bottled. So that's it—soybeans, wheat, water, salt, and microbes. Back in the days of the empire, they even had special recipes that they made only for the emperor by adding extra flavors."

"So what kind do we have here?" asked Edie.

"Oh, an emperor's brew, for sure," asserted Henry. They all laughed.

Figure 2.1 Commercial production of soy sauce involves the extended fermentation of moromi, a mash of soybeans and wheat in a salt solution.

CASE ANALYSIS

1. **Recognize potential issues and major topics in the case.** What is this case about? Underline terms or phrases that seem to be important to understanding this case. Then list **three or four** biology-related topics or issues in the case.

2. **What specific questions do you have about these topics?** By yourself, or better yet, in a group, list what you already know about this case in the "What Do I Know?" column. List questions you would like to learn more about in the "What Do I Need to Know?" column.

What Do I Know?	What Do I Need to Know?

3. Put a check mark by **one to three** questions or issues in the "What Do I Need to Know?" list that you think are most important to explore.

4. **What kinds of references or resources would help you answer or explore these questions?** Identify two different resources and explain what information each resource is likely to give that will help you answer the question(s). Choose specific resources.

Core Investigations

I. Critical Reading

To complete this investigation, you should have already read Chapter 7: Membrane Structure and Function (specifically Concepts 7.1 and 7.3); Chapter 8: An Introduction to Metabolism (specifically Concept 8.4); and Chapter 9: Cellular Respiration.

A. The Koji Phase. In the koji phase of soy sauce production, fungi produce enzymes that break down the carbohydrate and protein in the soybeans and wheat, thereby obtaining energy and molecules for fungal growth. Recall that koji is left uncovered for a few days, which allows many other types of microbes to enter the soybean-and-wheat mixture.

1. Describe a typical enzyme-substrate complex. What mechanisms do enzymes use to lower activation energy and speed up a reaction?

2. Explain how enzymes break down macromolecules. What is the role of water? What bonds are broken, what bonds are formed? Examine Figure 8.15 as you develop your answer.

3. In the koji stage of soy sauce production, *Aspergillus* fungi digest soybeans and wheat. *Aspergillus* uses some of the glucose produced by the breakdown of the carbohydrates to generate ATP through cellular respiration or fermentation. Examine Figure 9.9 and answer the following questions about glycolysis, the first stage of respiration.

   a. How many different enzymes shown in Figure 9.9 are used to transform glucose into pyruvate?

b. What types of reactions do isomerases catalyze?

c. What kinds of enzymes catalyze reactions that transfer a phosphate group from ATP to another molecule?

d. If you added an aldolase inhibitor, what key reaction would be unlikely to occur? Explain.

B. The Moromi Phase. Once *Aspergillus* has broken down the macromolecules in the soybeans and wheat into monomers, the koji phase ends. Moromi is then made by mixing the koji with water and enough salt to make a 16–20% concentrated salt solution, or brine.

1. In the moromi phase of soy sauce production, the osmotic conditions for microbes are drastically changed. Sketch a generic cell showing what happens to most cells when they are placed in brine. Explain your sketch. (Hint: Consider the movement of water.)

2. Some microbes have adaptations for osmoregulation in order to live successfully in high-salt environments. When the brine is added, the populations of bacteria and fungi found in the koji change. Do you expect greater or lesser microbial diversity? Why?

3. Yet another challenge faces the microbes in moromi. After the brine is added, workers place the moromi in airtight containers for several months. Which types of microbes will survive under these conditions? Explain how they will obtain energy for life processes.

4. *Tetragenococcus halophilus* (a bacterium) and *Zygosaccharomyces rouxii* (a fungus) are two facultatively anaerobic species that thrive in moromi. Through fermentation, *Tetragenococcus* produces lactic acid (lactate in its ionized form) and *Zygosaccharomyces* produces ethanol. What molecule is transformed into these waste products? Describe the two processes. What other waste products are produced?

5. Are ethanol and lactate oxidized or reduced in these reactions?

II. Fermentation of Grapes

A. Yeast and Rising Alcohol Concentrations. One of the oldest uses of fermentation by people is to make alcoholic beverages such as wine. However, fermentation also occurs without human intervention. Once grapes ripen on the vine, tiny breaks in the skin of the fruit enable the entry of microbes such as bacteria and fungi. The interior of the grape provides both a high concentration of sugars and low pH. Fermentative yeasts thrive in this environment and metabolize the grape sugars for energy. The products carbon dioxide and ethanol are rapidly transported out of the cells as wastes.

When people make wine by fermenting grapes, the process occurs within an airtight container. Alcohol continues to build up in the container until the alcohol tolerance level of the specific yeast population is reached, ending the fermentation cycle. Figure 2.2 shows the results from a simulation of wine fermentation over a 10-day period.

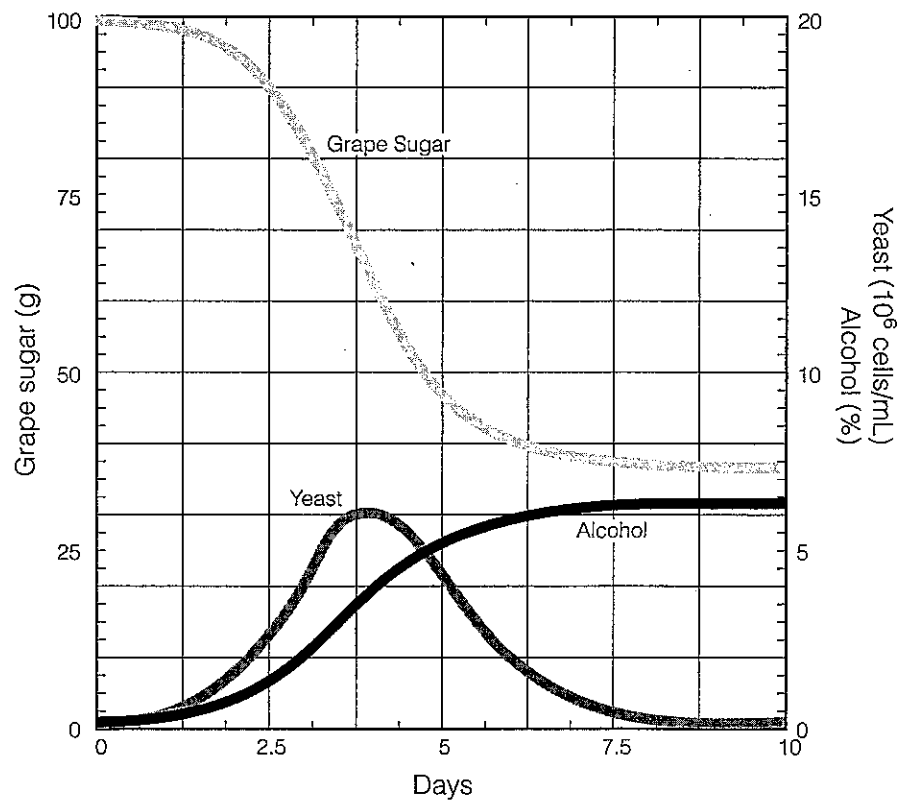


Figure 2.2 Results from a simulation of wine fermentation (Stanley et al., 2003). The graph shows changes in grape sugar, yeast population, and percentage alcohol over a 10-day period. (*Note:* Read grape sugar on the left axis. Yeast and alcohol are shown on the right axis.)

1. Examine Figure 2.2 and fill in the information below.
   a. The grape sugar level starts at ______ g and ends at ______ g.
   b. The yeast population reaches its highest level of approximately ______ on Day ______.
   c. The alcohol level starts at ______% and ends at approximately ______%.
   d. Look at the graphs showing the correlation between yeast population and percentage alcohol. At what percentage alcohol does this yeast population begin to decline? ______%

2. Why isn't the remaining grape sugar converted to ethanol and carbon dioxide?

3. What product of alcohol fermentation is not shown in the graph in Figure 2.2?

4. If you removed the alcohol as it was produced, would you predict an increase or a decrease in the amount of grape sugars at 10 days? An increase or decrease in the population of yeast at 10 days? Explain.

5. A bottle of wine may spoil if it is allowed to sit for some time after being opened or if its cork does not form a tight seal. Explain what causes the wine to spoil under these conditions. (*Hint:* Available grape sugar declines.)

B. **Fermentation with Wild and Cultivated Yeasts.** In an experiment to identify differences in fermentation carried out by wild and cultivated yeasts, a batch of grapes was divided in two. One batch of grapes was treated with sulfur dioxide to kill wild yeasts before the juice was extracted. The other batch was left untreated, allowing wild yeasts to survive.

Fermentation of grape juice extracted from these two groups was carried out in separate containers. In the first container, the juice from the treated grapes was inoculated with a special cultivated strain of yeast. The untreated juice in the second container was inoculated with only wild yeast populations. Both containers were allowed to ferment for 10 days. Samples were removed daily to estimate the number of yeast cells and the level of alcohol in each container. Results are shown in Figure 2.3.

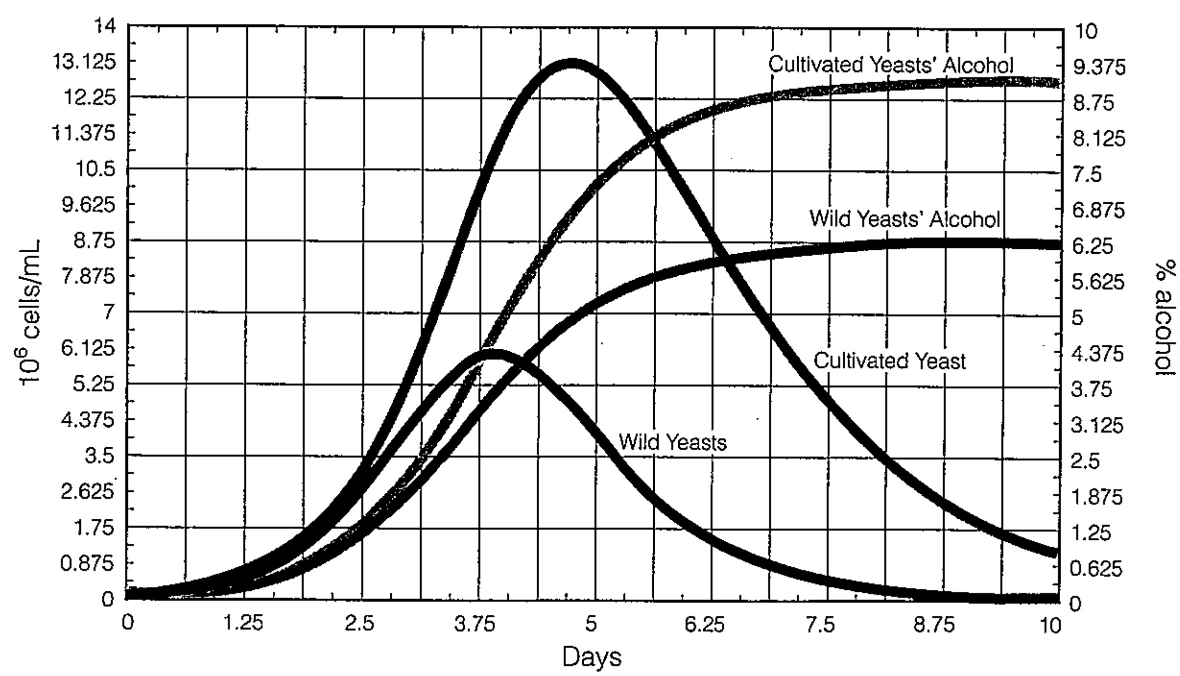


Figure 2.3 Simulated fermentation by wild and cultivated yeasts (Stanley et al., 2003). (*Note:* Read population size on the left axis. Alcohol production is shown on the right axis.)

1. Assuming alcohol level affects the growth of yeast, which yeast has a higher tolerance for alcohol? At approximately what percentage alcohol do the two yeast populations in the different containers begin to decline?

2. Why do you think the alcohol levels increase more rapidly in one of the containers? Use data from Day 3.75 to support your hypothesis.

C. Bottling Soy Sauce. Now apply some of the concepts you learned about grape fermentation to the Bean Brew case. When soy sauce was first shipped to Europe, Asian soy sauce producers tried the same method they had used for shipping shorter distances within Asia—simply filtering the soy sauce and placing it in non-airtight containers. However, the soy sauce always spoiled before it reached its European destinations! The spoilage problem was solved when the producers started to boil the soy sauce first and then place it in airtight bottles.

1. Explain why placing soy sauce in airtight bottles was more successful for long-distance shipping than simply placing the sauce in barrels.

2. When the soy sauce was not boiled before it was bottled, the bottles sometimes burst during the voyage. What do you think caused this?

3. Bottled soy sauce does not taste the same as fresh soy sauce. What do you think causes this change?

4. To preserve flavor in modern times, brewed soy sauces are not boiled but are pasteurized (heated to a temperature of about 60°C [140°F]) before being bottled. Pasteurized soy sauce tastes better than boiled soy sauce. What does pasteurization do? Why should opened bottles of soy sauce be stored in the refrigerator?

III. Alcohol Dehydrogenase

Ethanol, which is toxic to yeast cells, is also toxic to human cells. We can consume alcohol due to alcohol dehydrogenase, an enzyme produced by humans and many other animals that catalyzes the oxidation of alcohols to aldehydes. In this reaction, nicotinamide adenine dinucleotide (NAD^+) is used as an oxidizing agent.

$$\underset{\text{ethanol}}{CH_3CH_2OH} + NAD^+ \xrightarrow{\text{alcohol dehydrogenase}} \underset{\text{acetaldehyde}}{CH_3CHO} + NADH + H^+$$

Not only does alcohol dehydrogenase allow humans to detoxify (within limits!) the ethanol that we consume, but also it detoxifies the alcohol produced by certain fermentative microbes that reside in our small intestine and colon.

1. Draw molecules of ethanol and acetaldehyde.

Ethanol Acetaldehyde

2. Explain why ethanol is considered an electron donor in this redox reaction.

3. Consumption of methanol can be fatal because alcohol dehydrogenase converts methanol to formaldehyde, a highly toxic substance that can cause the death of cells in the human body. Formaldehyde is the substance once commonly used to preserve animal and plant tissues; however, due to its cancer-causing properties, its use is restricted. Draw molecules of methanol and formaldehyde.

Methanol Formaldehyde

4. What is oxidized and reduced in this reaction? Explain.

$$CH_3OH + NAD^+ \xrightarrow{\text{alcohol dehydrogenase}} CH_2O + NADH + H^+$$

methanol — formaldehyde

5. Treatment for methanol ingestion involves giving the patient an alcohol dehydrogenase inhibitor. Explain why this is helpful.

6. During fermentation in yeast, alcohol dehydrogenase catalyzes a reaction that breaks down acetaldehyde into ethanol and regenerates NAD^+. Note that this is the reverse of the reaction catalyzed by the enzyme in humans and other animals.

$$CH_3CHO + NADH + H^+ \xrightarrow{\text{alcohol dehydrogenase}} CH_3CH_2OH + NAD^+$$

acetaldehyde — ethanol

a. What is oxidized and reduced in this reaction? Explain.

b. What happens to the ethanol after it is produced?

c. What happens to the NAD^+ after it is produced?

Additional Investigation

IV. More Human Uses of Fermentation

Things are fermenting everywhere! Choose a product from the list below. Use your text and other resources, including primary sources, to find out how fermentation is used to make this product. Write a paper of one to three pages, based on reliable sources, indicating:

- the organism(s) doing the fermenting
- the metabolic pathway(s) used
- substrates
- fermentation products
- how the fermentation is accomplished
- how the product is prepared for consumption

Products

Sausage
Chocolate
Coffee
Sourdough bread
Cheeses
Tempeh
Kimchee
Sauerkraut
Citric acid (widely used as an ingredient)
Dental caries (product is the decayed tooth)
Vinegar
Yogurt

V. Open-Ended Investigations

Use the working wine model (available at http://bioquest.org/icbl/casebook/wine) to conduct your own investigations of factors involved in wine fermentation.

Additional pairs of graphs (A+B or C+D) are available on the same website for further practice in interpreting graphs, making inferences, and drawing conclusions.

References

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Stanley, Ethel D., Howard T. Odum, Elisabeth C. Odum, and Virginia G. Vaughan. Modeling wine fermentation, pp. 85–92, and software on CD-ROM. In J. R. Jungck, M. F. Fass, and E. D. Stanley, *Microbes Count!* Beloit, WI: BioQUEST Curriculum Consortium and American Society for Microbiology Press, 2003.