

Chapter 2:

Bean Brew

INSTRUCTOR'S GUIDE



As with all the cases in this book, please read the preface if you have not already done so. In the preface, you will find suggestions for using Investigative Case Based Learning (ICBL) in different instructional situations such as starting a new lecture topic, assessing what students already know, setting a context for lab activities, and so on. The preface also describes ways to use cases in a variety of classroom settings and suggests multiple ways to assess learning with cases.

Bean Brew accompanies Unit Two: The Cell in *Campbell Biology*, 11th edition. The case emphasizes material covered in Chapter 9: Cellular Respiration and Fermentation, with strong links to Chapter 8: An Introduction to Metabolism and Chapter 7: Membrane Structure and Function. There are four strands in the case:

- Fermentation
- Glycolysis
- Enzyme actions
- Osmosis

Students should complete the Case Analysis immediately following the reading of the case. We strongly suggest that students work in groups to complete the Case Analysis. Actively listening to and challenging the ideas of others can help learners become aware of their own misconceptions, yet also value their own and others' prior knowledge.

Five investigations accompany *Bean Brew*. The first three are “core” investigations relating directly to the facts of the case, and two are additional investigations that extend beyond the case itself. Table IG2.1, on the next page, describes what students will gain from each investigation.

Table IG2.1 Bean Brew Case Overview

Investigation	Learning Goals	Inquiry Skills Used
Core Investigations		
I. Critical Reading	Students use Chapter 9 as their primary resource, with additional information from Chapters 7 and 8 to explain the cellular processes taking place during the production of soy sauce.	<ul style="list-style-type: none"> identifying key information applying generalized information to a specific problem following a metabolic pathway (glycolysis) and learning to analyze changes to substrates and electron acceptors interpreting written redox reactions
II. Fermentation of Grapes	Students use output of a model of grape fermentation to further extend their understanding of fermentation. They then apply this information to the case.	<ul style="list-style-type: none"> interpreting graphs applying fermentation and respiration concepts to new situations
III. Alcohol Dehydrogenase	Students explore the enzyme alcohol dehydrogenase and its mode of action in humans and in yeast. They think about reactions in both directions, and consider differences in this enzyme among species.	<ul style="list-style-type: none"> drawing two-dimensional chemical structures examining reactions to identify changes and oxidation/reduction
Additional Investigations		
IV. More Human Uses of Fermentation	Students must meet identified criteria as they prepare a paper discussing a product that undergoes fermentation.	<ul style="list-style-type: none"> identifying, evaluating, and managing information, including primary literature extending general ideas of fermentation to additional specific settings
V. Open-Ended Investigations	Students use free online models of wine fermentation to investigate variables	<ul style="list-style-type: none"> identifying variables designing experiments interpreting graphs manipulating a model

Table IG2.2 contains several resources related to *Campbell Biology*, 11th edition, that will help your students further their understanding of this case.

Table IG2.2 Campbell-Related Resources

Resource	Chapter/Activity	Topics Covered/Activity Titles
Critical Reading from <i>Campbell Biology</i> , 11th edition	Chapter 7: Membrane Structure and Function	Fluidity of membranes (Concept 7.1); effects of osmosis on water balance (Concept 7.3)
	Chapter 8: An Introduction to Metabolism	Catalysis in the active site of enzymes; effects of local conditions on enzyme activity (Concept 8.4)
	Chapter 9: Cellular Respiration and Fermentation: Harvesting Chemical Energy	Redox reactions; the stages of cellular respiration (Concept 9.1); glycolysis (Concept 9.2); fermentation (Concept 9.5); glycolysis connects to other metabolic pathways (Concept 9.6)
MasteringBiology Study Area	Chapter 7 Activity	Osmosis and Water Balance in Cells
	Chapter 7 Video	Plasmolysis
	Chapter 8 Activity	How Enzymes Work
	Chapter 9 Activities	Glycolysis, Fermentation
Morgan/Carter <i>Investigating Biology</i> , 8e	Lab Topic 3	Diffusion and Osmosis
	Additional Investigation Questions IV, V	Alcohol Fermentation, Designing and Performing Your Open-Inquiry Investigation

Case Narrative

Students were asked to underline terms or phrases in the introductory narrative that they think are important to understanding the case. Suggested terms and phrases students might have chosen are in bold type.

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 to 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*. They put the *moromi* into **airtight containers** and let them **ferment for at least six 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.

Suggested Answers for Case Analysis

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

Biology-related topics or issues: biotechnology and new strains of fungi; production of soy sauce; fungal digestion of wheat and soy; fermentation of soy sauce, pickles, sauerkraut, and wine; handling fermented products; role of salt in fermentation

2. **What specific questions do you have about these topics?** By yourself, or better yet, in a group, make a list of 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.

There are many possible answers, depending on the experiences of your students. Below are some likely responses:

What Do I Know?	What Do I Need to Know?
<ul style="list-style-type: none">• Many Asian dishes use soy sauce.• The basic processes of making soy sauce. <i>Soy sauce is made of soybeans, wheat, water, salt, and microbes.</i>• Some students may have had experience with pickling or preparing other fermented products.• You can purchase “lite” or low-salt soy sauce.• Microorganisms are needed for fermenting.• Fermentation produces alcohol. (Note: Unless they have read Chapter 9, students are unlikely to know that there are several forms of fermentation.)	<ul style="list-style-type: none">• Does soy sauce contain alcohol?• What is the role of salt in fermentation?• Why does soy sauce need two production stages?• What is happening metabolically in each stage?• Why is it difficult for people to digest soybeans?• How does fermentation change soy and wheat into soy sauce?• Why are microorganisms needed?• Why fungi?• How could biotechnology improve the fungal strains?

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

You should expect a range of responses. Most students will use the contextual clues of being in a biology class and beginning the cell unit to identify fermentation-related questions.

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.

Accept any reasonable resource (e.g., text, other book, Internet sites, maps, data tables, and so on) that could be related to the case. The answer “the Web” is too vague. Students should explain the type of site they are looking for or search terms they might use.

Suggested Answers for 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 and Fermentation.

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?

Enzymes are proteins that contain a pocket or groove known as the active site. The active site is the area of the enzyme that binds to specific substrate molecules. When substrates are in the active site (forming the enzyme-substrate complex), the enzyme undergoes a conformational change, which brings chemical groups of the active site into positions that enhance their ability to catalyze the chemical reaction. When the reaction is complete, the resulting product molecules are released, and the enzyme returns to its previous conformation, ready for the next reaction.

Enzymes catalyze reactions through a variety of mechanisms. The active site may act as a template, helping two or more reactants become oriented favorably for a reaction to occur. Enzymes may also stretch the substrate molecules toward their transition-state conformation, stressing and bending critical chemical bonds. This reduces the amount of free energy that must be absorbed to achieve a transition state. Alternatively, amino acids in the active site may provide a microenvironment with a pH more favorable to the reaction than exists in the surrounding cell environment. Sometimes, the amino acids of the active site briefly form covalent bonds with the substrates as a way to facilitate the reaction.

2. Explain how enzymes break down macromolecules. What is the role of water? What bonds are broken, what bonds are formed? Examine Figure 8.16 as you develop your answer.

When breaking down macromolecules, the enzyme “holds” the substrate molecule so that the bond between the two building blocks is exposed to water. The enzyme may also place physical stress on these bonds. The bond between two building block molecules (for example, between the C and N of two adjacent amino acids or between the carbons of two adjacent sugars) is broken. As discussed in Chapter 5, these enzymes are involved in hydrolysis reactions. They add a molecule of water to the bond, giving an H to one side of the bond and an OH to the other side. So, at the same time the bonds between amino acid subunits are being broken, an H is added to the amino side of the peptide bond and a hydroxyl group is added to the carboxyl side of that bond. Similarly, in carbohydrates, an H is added to one side of the glycosidic bond and a hydroxyl to the other.

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?

10

- b. What types of reactions do isomerases catalyze?

Isomerases facilitate the transformation of one type of isomer to another.

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

kinases

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

The splitting of fructose-1,6-bisphosphate into two 3-carbon isomers, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, and all reactions of glycolysis subsequent to that reaction, would be unlikely to occur.

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.*)

Students should sketch a shrinking cell showing water moving out of the cell and into the hypertonic environment. Student explanation: Osmosis will occur, causing water in the hypotonic cell to move out into the hypertonic environment.

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?

The differences in osmolarity in the two environments will cause the death of microorganisms not adapted for high salt concentrations. This is likely to reduce the diversity of microbes in the moromi.

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.

The microbes that survive are those that can produce ATP in the absence of oxygen—facultative anaerobes and microbes that are always anaerobic. During fermentation, glycolysis produces ATP. The fermentation cycle continues when pyruvate is converted to a substance that can be reduced by NADH, or pyruvate is reduced by NADH. Without a supply of NAD⁺, glycolysis will halt.

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?

Pyruvate is the molecule transformed into these waste products. In the bacteria performing lactic acid fermentation, pyruvate is reduced by NADH to form lactate. In the fungus, which performs alcohol

fermentation, pyruvate is first converted to acetaldehyde (with a release of carbon dioxide as a waste product). Then acetaldehyde is reduced by NADH to ethanol.

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

They are both oxidized, giving up electrons to NAD¹.

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.

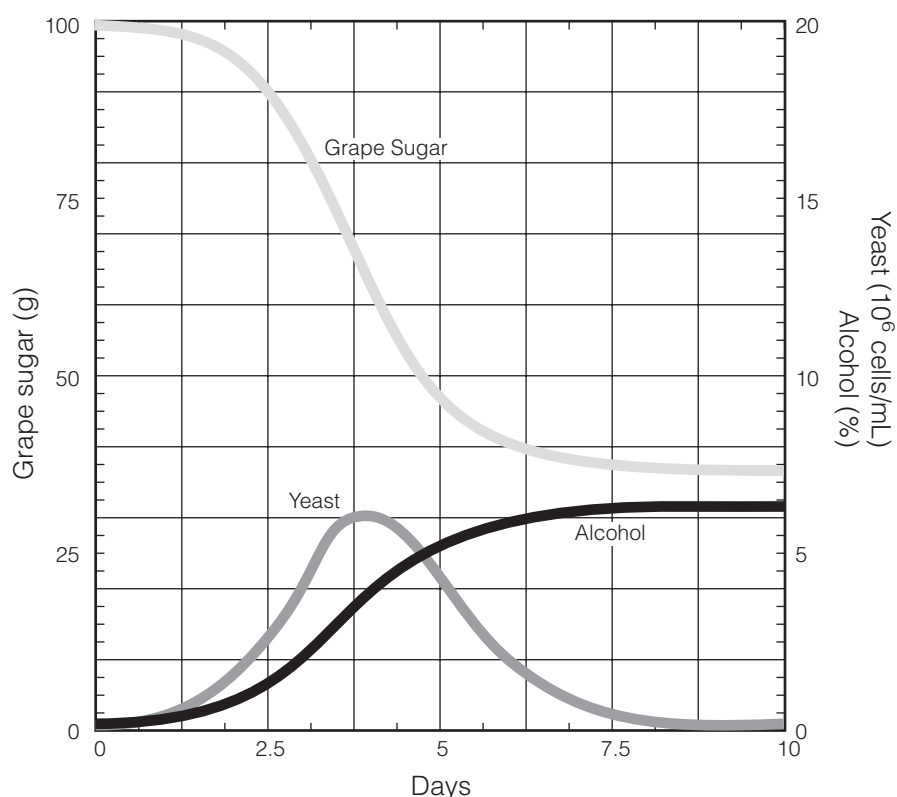


Figure 2.2 Results from a simulation of wine fermentation (Stanley et al., 2003). The graph shows changes in grape sugar, yeast population, and percentage alcohol over a 10-day period. (Note: Read grape sugar on the left axis. Yeast and alcohol are shown on the right axis.)

1. Examine Figure 2.2 and fill in the information below.

a. The grape sugar level starts at _____ g and ends at _____ g.

100 g; approximately 35 g

b. The yeast population reaches its highest level of approximately _____ on Day _____.

6×10^6 cells/mL; Day 4

c. The alcohol level starts at _____ % and ends at approximately _____ %.

0%; 6.4%

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? _____ %

4%

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

When the microorganisms die, the process of fermentation stops.

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

carbon dioxide

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.

The amount of grape sugar would decrease because high alcohol levels would not kill the yeast. If alcohol were removed, the yeast population would most likely increase until it reached the limit imposed by its food source, at which time the yeast population would decrease.

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.*)

Contamination from airborne microbes results in new metabolic activity. The grape sugar is broken down by aerobes using the citric acid cycle and oxidative phosphorylation.

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.

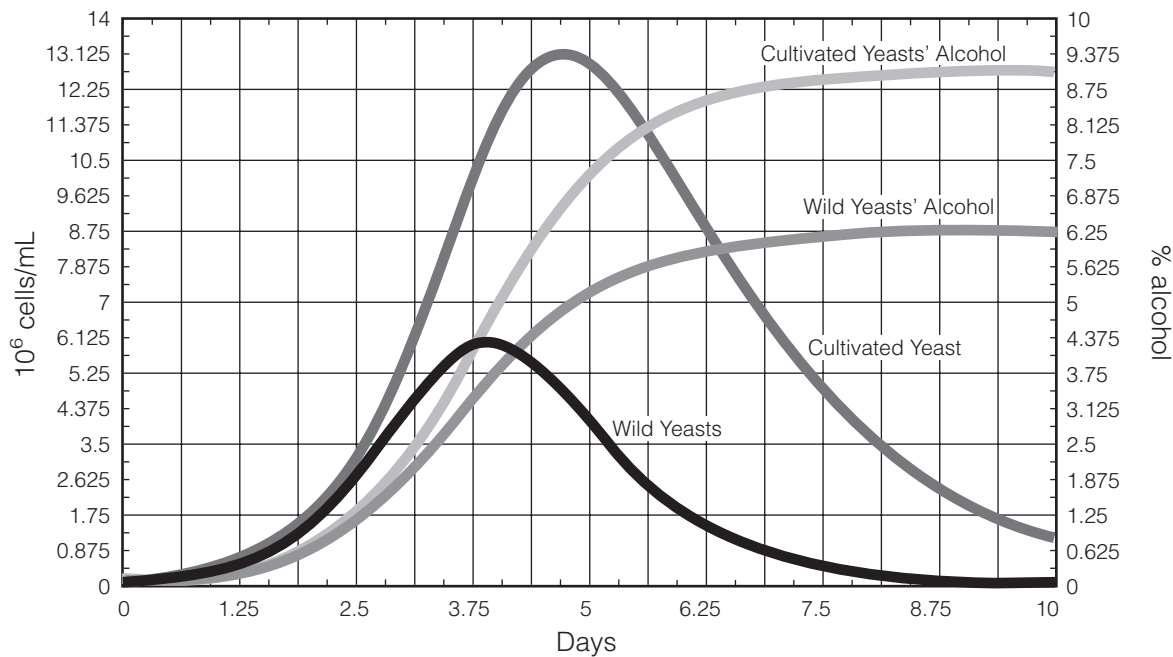


Figure 2.3 Simulated fermentation by wild and cultivated yeasts (Stanley et al., 2003). (Note: Read population size on the left axis. Alcohol production is shown on the right axis.)

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?

The cultivated yeast tolerates alcohol at a higher level of about 7.5% alcohol before its population begins to decline. The wild yeast begins to die at about 4% alcohol.

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.

More alcohol is produced the greater the number of yeast cells present. If you compare the two containers at Day 3.75, the number of cultivated yeast cells is approximately 10×10^6 as compared to wild yeasts at approximately 6×10^6 .

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.

Boiling the soy kills the majority of remaining organisms, putting a stop to metabolism. Making the bottles airtight prevents any additional microbes from entering and breaking down molecules in soy sauce for food. Further, any aerobic microorganisms remaining in the soy sauce die once the bottle is sealed. Some fermentation will continue if any anaerobic microbes survive the boiling process.

In barrels, the soy sauce was only filtered, so there would have been a greater population of surviving microbes, and more microbes could have entered the barrels during shipping. In barrels, the soy sauce would be used for cellular respiration and perhaps further fermentation. For shorter trips within Asia, this method would have worked because the soy sauce would be delivered before the actions of the microbes could cause it to spoil.

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?

If a large population of anaerobic organisms were still alive and fermenting, carbon dioxide would build up inside the bottles. The pressure exerted on the bottles by the gas could cause weak or damaged bottles to explode.

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

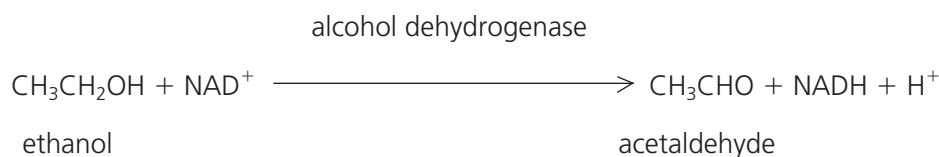
Boiling the soy sauce for bottling may destroy some of the molecules that enhance the flavor of soy sauce. The possibility of continued fermentation exists because boiling may not have killed all of the organisms. Anaerobes may continue to grow and make products that affect the taste.

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?

Pasteurization raises the temperature to only 60°C, which is sufficient to kill off many microbes, but not all. Once the soy sauce bottle has been opened, other microbes can get in, and those not killed by pasteurization will continue fermenting. This will occur only if the remaining microbes survive the anaerobic environment. As discussed in Chapter 7, enzymatic proteins may become inactivated at cold temperatures, slowing down both the growth of the microorganisms and the rate of fermentation.

III. Alcohol Dehydrogenase

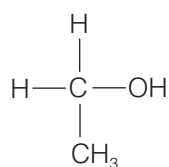
Ethanol, which is toxic to yeast cells, is also toxic to human cells. We can consume alcohol due to alcohol dehydrogenase, an enzyme produced by humans and many other animals that catalyzes the oxidation of alcohols to aldehydes. In this reaction, nicotinamide adenine dinucleotide (NAD⁺) is used as an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



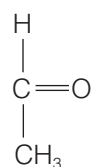
Not only does alcohol dehydrogenase allow humans to detoxify (within limits!) the ethanol that we consume, it also detoxifies the alcohol produced by certain fermentative microbes that reside in our small intestine and colon.

1. Draw molecules of ethanol and acetaldehyde.

Student sketches should appear as follows:



Ethanol



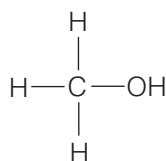
Acetaldehyde

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

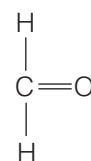
Ethanol loses electrons when it is converted to acetaldehyde. The carbon atom in ethanol that is bonded to the hydroxyl group is the same as the carbon atom in acetaldehyde that is double-bonded to oxygen.

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.

Student sketches should appear as follows:

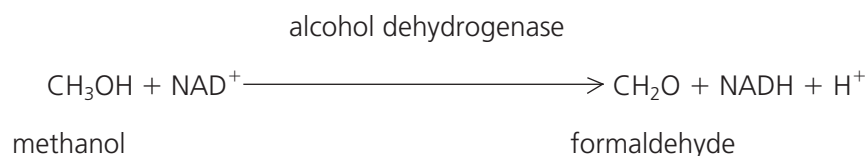


Methanol



Formaldehyde

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



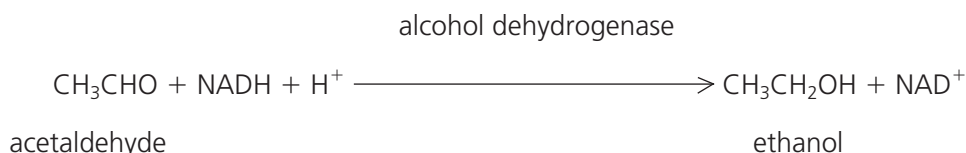
Methanol is oxidized and NAD^+ is reduced.

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

The conversion of methanol to formaldehyde is prevented because the enzyme necessary to catalyze the reaction is not available.

(Note: The methanol eventually is excreted via the urinary system.)

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.



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

NADH is oxidized to NAD^+ . Acetaldehyde is reduced to ethanol.

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

The toxic alcohol is rapidly transported from the yeast cell to the external environment.

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

This oxidizing agent is used in other redox reactions within the cell.

Additional Investigations

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

Students, working individually or in groups, should submit a paper of one to three pages on how fermentation is involved in the production of one of the products listed in the student version. The paper

should be based on reliable sources. Consider asking students for at least one primary literature article. The paper should address the following items:

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

As an alternative to a paper, you could ask students to create a poster.

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

References

- Noda, F., K. Hayashi, and T. Mizunuma. Antagonism between osmophilic lactic acid bacteria and yeasts in brine fermentation of soy sauce. *Applied and Environmental Microbiology*, 40(3):4452–457, 1980.
- 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.