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.	• 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.	• 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.	• 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.	• 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	• 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 *Campbell Biology,* 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 *Investigating Biology,* 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?
• *Many Asian dishes use soy sauce.* • *The basic processes of making soy sauce. Soy sauce is made of soybeans, wheat, water, salt, and microbes.* • *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.)*	• *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 NAD^1.

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.

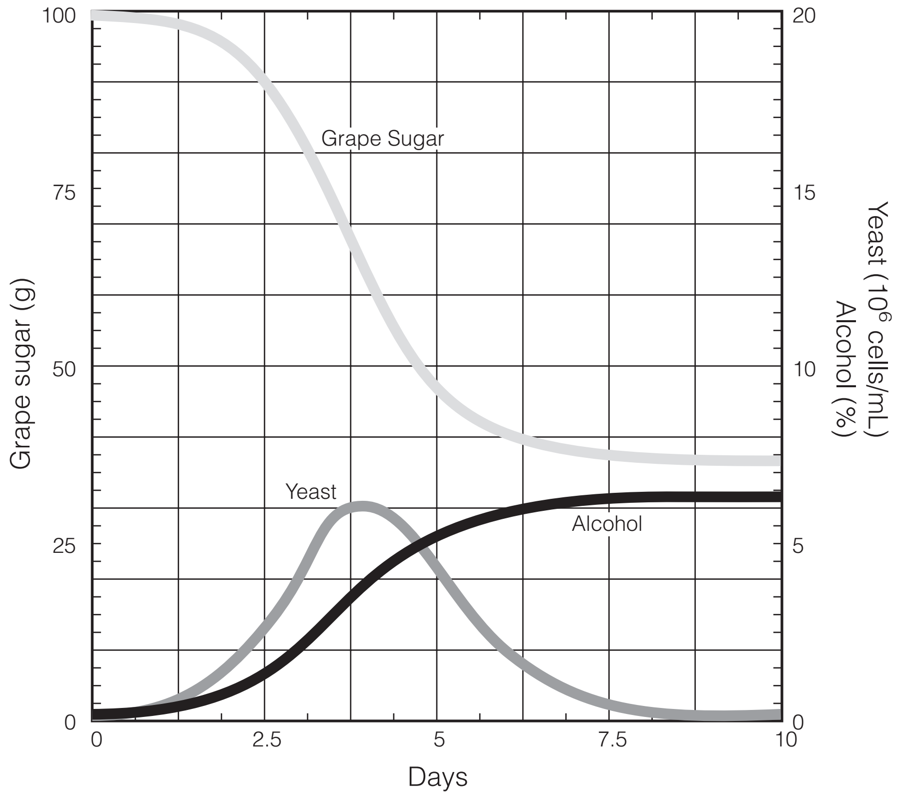


Figure 2.2 Results from a simulation of wine fermentation (Stanley et al., 2003). The graph shows changes in grape sugar, yeast population, and percentage alcohol over a 10-day period. (*Note:* Read grape sugar on the left axis. Yeast and alcohol are shown on the right axis.)

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 preceeding 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.

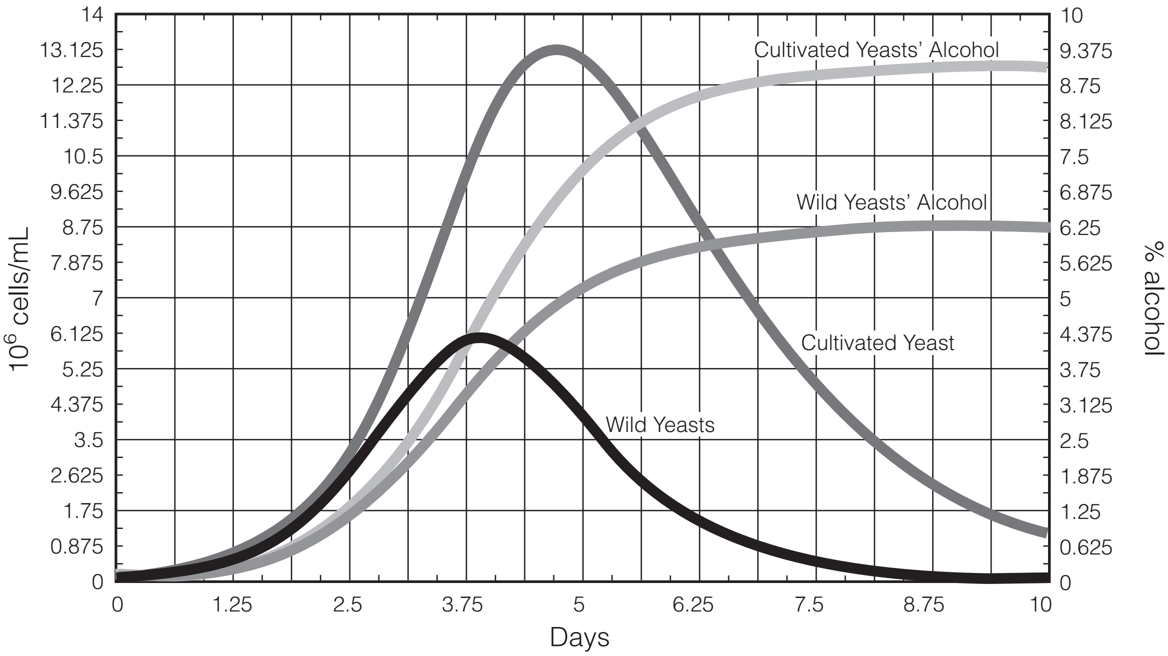


Figure 2.3 Simulated fermentation by wild and cultivated yeasts (Stanley et al., 2003). (*Note:* Read population size on the left axis. Alcohol production is shown on the right axis.)

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

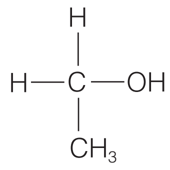
Ethanol, which is toxic to yeast cells, is also toxic to human cells. We can consume alcohol due to alcohol dehydrogenase, an enzyme produced by humans and many other animals that catalyzes the oxidation of alcohols to aldehydes. In this reaction, nicotinamide adenine dinucleotide (NAD^+) is used as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

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

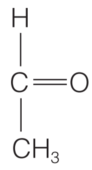
Not only does alcohol dehydrogenase allow humans to detoxify (within limits!) the ethanol that we consume, it also detoxifies the alcohol produced by certain fermentative microbes that reside in our small intestine and colon.

1. Draw molecules of ethanol and acetaldehyde.

Student sketches should appear as follows:



Ethanol



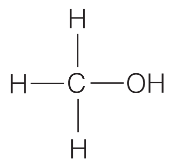
Acetaldehyde

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

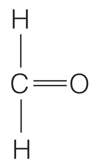
Ethanol loses electrons when it is converted to acetaldehyde. The carbon atom in ethanol that is bonded to the hydroxyl group is the same as the carbon atom in acetaldehyde that is double-bonded to oxygen.

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.

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

methanol → formaldehyde

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.

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

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

NADH is oxidized to NAD^1. 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
Chocolate
Coffee
Sourdough bread
Cheeses
Tempeh
Kimchee
Sauerkraut
Citric acid (widely used as an ingredient)
Dental caries (product is the decayed tooth)
Vinegar
Yogurt

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.

Notes to Instructors

Chapter 2 The Chemical Context of Life
Chapter 3 Water and the Fitness of the Environment

What is the focus of these activities?

Living organisms function in the real world, so they are subject to all the laws of chemistry and physics. In addition, biological organisms and systems are variable. No two organisms are exactly alike, and no two systems are identical in form or function. As a result, our analysis of such systems tends to deal with statistical averages or probabilities. This means that it is difficult to understand biological systems without having a good basic understanding of chemistry, physics, and math (including probability and statistics).

The vast majority of introductory biology students have studied inorganic chemistry in their high school and first-year college chemistry courses. Many students compartmentalize their knowledge, however. In some cases, the compartmentalization is so extreme that the students feel uncomfortable dealing with chemical formulas and ideas outside of chemistry classes. Therefore, it is generally useful to review some of the basic ideas in chemistry and, at the same time, demonstrate how they can be applied to understanding biological systems.

What are the particular activities designed to do?

Activity 2.1 A Quick Review of Elements and Compounds
The questions in this activity are designed to help students review and understand:

- atomic/molecular number, mass number, and atomic/molecular weight and how they can be used to determine the reactivity of elements;
- various types of chemical bonds and how they affect the structure and energetics of molecules; and
- the difference between a mole and a molar equivalent and how knowledge of these can be used in biological applications.

Activity 3.1 A Quick Review of the Properties of Water
The questions in this activity are designed to help students review and understand the properties of water and how they support life. Students are asked to review these key properties:

- H_2O molecules are cohesive; they form hydrogen bonds with each other.
- H_2O molecules are adhesive; they form hydrogen bonds with polar surfaces.
- Water is a liquid at normal physiological (or body) temperatures.
- Water has a high specific heat.
- Water has a high heat of vaporization.
- Water's greatest density occurs at 4°C.

In addition, students review pH and how it is related to both the ionization constant of pure water and the concentration of H^+ ions in a solution.

What misconceptions or difficulties can these activities reveal?

Activity 2.1

Question 1: Many students don't understand that nutrients for plants are inorganic and most nutrients for animals (heterotrophs) are organic.

Questions 2 and 3: Most students know how to balance a chemical equation. Fewer understand the relationship between molecules of a substance and moles of that substance. Similarly, most students can recite what a mole is; however, the majority have not thought about how that knowledge can be applied. Therefore, much of this first activity is devoted to making it clear that a balanced equation indicates not only the number of molecules required but also the number of moles required. It also explains why moles can be substituted for molecules in such equations.

Question 4: Some students have difficulty understanding that a solution's concentration or molarity does not change if you aliquot, or subdivide, the solution into smaller volumes. To test this, ask your students: "There is 10% sugar in this solution. If I pour half of it into one beaker and the other half into another beaker, what percent sugar will I have in each beaker?" More than half of the students will automatically answer 5%.

Questions 5 and 6: These questions are designed to help students understand how a knowledge of balanced equations and molar equivalents can be useful in biology.

Questions 7 and 8: The answers go into a little more detail than does *Campbell Biology*, 11th edition. Students obviously shouldn't be asked to know the specific electronegativity of each of the elements. However, using concrete numbers may help students understand how their electronegativity is related to the type of bonds formed between elements.

Activity 3.1

Most students have no difficulty stating the properties of water and the definition of pH. On the other hand, not all of them have a good understanding of how these properties are related to biological and other phenomena. Therefore, some questions ask students to relate pH values to actual concentrations of H^+ ions in solution and to relate the properties of water to common experiences they have had in class or in life.

Answers

Activity 2.1 A Quick Review of Elements and Compounds

1. Table 2.1 (page 29) lists the chemical elements that occur naturally in the human body. Similar percentages of these elements are found in most living organisms.

a. In what abiotic (nonlife) chemical forms are these elements often found in nature?	b. In what chemical form(s) do animals need to obtain these elements?	c. In what chemical form(s) do plants need to obtain these elements?
These elements are most commonly found as CO_2, N_2, and O_2 in the atmosphere and as H_2O, PO_4, and S compounds on Earth.	With the exception of oxygen and water, animals obtain the majority of these elements in the form of organic compounds.	Plants can obtain C as CO_2, N as ammonia or nitrite or nitrates, phosphorus as phosphates, sulfur as sulfides or sulfates, and so on. In other words, plants obtain these elements as inorganic compounds.

2. A chemical element cannot be broken down to other forms by chemical reactions. Each element has a specific number of protons, neutrons, and electrons.

   a. What is the name of the following element, and how many protons, neutrons, and electrons does it have?

11
Na
23

Name	Number of protons	Number of neutrons	Number of electrons
Sodium	11	12	11

b. What information do you need to calculate or determine the following?

The atomic number of an element	The mass number of an element	The weight in daltons of one atom of an element
The atomic number is equal to the number of protons (or electrons).	The mass number is equal to the number of protons plus the number of neutrons.	You can estimate the weight in daltons as 1 dalton per proton or neutron. Therefore, the weight in daltons of an element is approximately equal to the number of protons plus the number of neutrons.

c. What are the atomic number, mass number, and weight in daltons of the element shown in part a?

Atomic number	Mass number	Weight in daltons
11	23	23

3. One mole of an element or compound contains 6.02×10^{23} atoms or molecules of the element or compound. One mole of an element or compound has a mass equal to its mass number (or molecular weight) in grams. For example, 1 mole of hydrogen gas (H_2) contains 6.02×10^{23} molecules and weighs 2 g.

a. What is the weight of 1 mole of pure sodium (Na)? 23 g	b. How many molecules of Na are in 1 mole of Na? 6.02×10^{23}

c. How would you determine how many grams are in a mole of any chemical element or compound?
A mole of any chemical element or compound is equal to the mass number in grams of that mole or compound. For example, the mass number of Na is 23; therefore, a mole of Na has a mass of 23 g. The mass number of water is 18; therefore, a mole of water has a mass of 18 g.

4. One atom of Na can combine with one atom of Cl (chlorine) to produce one molecule of NaCl (table salt).

a. If Cl has 17 electrons, 17 protons, and 18 neutrons, what is its mass number? 35	b. What is the mass number of NaCl? $23 + 35 = 58$	c. How many grams of NaCl equal a mole of NaCl? 58 g

d. If you wanted to combine equal numbers of Na and Cl atoms in a flask, how much Cl would you have to add if you added 23 g of Na? (Include an explanation of the reasoning behind your answer.)

23 g of Na is equal to 1 mole of Na. A mole contains 6.02×10^{23} molecules of the substance. To add an equal number of molecules of Cl, you need to add 1 mole of Cl, or 35 g.

e. To make a one-molar (1 *M*) solution of NaCl, you need to add 1 mole of NaCl to distilled water to make a final volume of 1 L (1,000 mL). A 1 *M* solution is said to have a molarity of 1. If you added 2 moles of NaCl to 1 L of distilled water, you would make a 2 *M* solution and its molarity would equal 2. You make up a 1 *M* solution of NaCl.

How many molecules of NaCl are in the 1 *M* NaCl solution?	How many molecules of NaCl are there per mL of the solution?
If you used 1 L of water to make the 1 *M* solution, you would have 6.02×10^{23} molecules in the liter.	To calculate the number of molecules per mL, divide 6.02×10^{23} by 1,000 = 6.02×10^{20} molecules/mL.

f. Next, you divide this 1 *M* solution of NaCl into four separate flasks, putting 250 mL into each flask.

How many grams of NaCl are in each flask?	How many molecules of NaCl are in each flask?	How many molecules of NaCl are there per mL of distilled water?	What is the molarity of NaCl in each of the four flasks?
58/4 = 14.5 g	6.02×10^{23} divided by 4 = 1.51×10^{23}	6.02×10^{20}	1 *M*

5. The summary formula for photosynthesis is

$$6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2$$

a. How many molecules of carbon dioxide and water would a plant have to use to produce three molecules of glucose ($C_6H_{12}O_6$)?	b. How many moles of carbon dioxide and water would a plant have to use to produce 2 moles of glucose?
For each molecule of glucose produced, 6 molecules of carbon dioxide and 6 molecules of water are consumed. Therefore, the plant would need to use 18 molecules of each.	Because a mole of anything contains the same number of molecules, the plant would need to use 6 times as many moles of carbon dioxide and water, or 12 moles of each.

c. Refer to the summary formula for photosynthesis. If you know the number of molecules or moles of any of the reactants used (or products produced), how would you calculate the number of molecules or moles of all of the other reactants needed and products produced?

If the formula is balanced and if it is a true representation of the overall reactions that occur, then the numbers in front of each reactant and product indicate the molecular or molar equivalents required for the reactions.

Note: To represent the actual reactants required and products produced, the overall formula for photosynthesis is more correctly stated as:

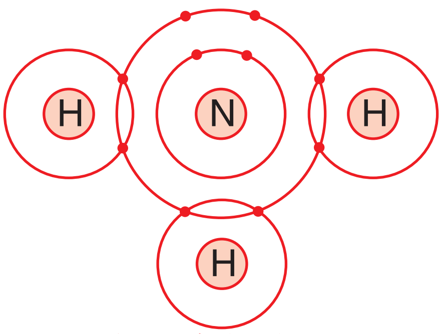
$6\ CO_2 + 12\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O$

In most texts, however, this is reduced to $6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2$

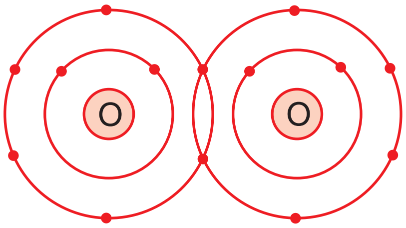
6. A biologist places a plant in a closed chamber. A sensor in the chamber maintains the carbon dioxide level at the normal atmospheric concentration of 0.03%. Another sensor allows the biologist to measure the amount of oxygen produced by the plant over time. If the plant produces 0.001 mole of oxygen in an hour, how much carbon dioxide had to be added to the chamber during that hour to maintain the atmospheric concentration of 0.03%?

For every mole of oxygen produced, 1 mole of carbon dioxide had to be consumed. Therefore, 0.001 mole of carbon dioxide had to be added to maintain a constant level of CO_2 in the chamber.

7. O_2 and NH_3 are both small covalent molecules found in cells. NH_3 is extremely soluble in the aqueous environment of the cell, while O_2 is relatively insoluble. What is the basis for this difference in solubility between the two molecules? In reaching your answer, draw the structures of the molecules as valence shell diagrams (as in Figure 2.12). Given these diagrams, consider the types of interactions each molecule could have with water.



Ammonia (NH_3)

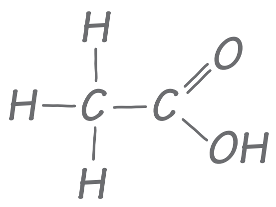


Oxygen (O_2)

Ammonia is a polar molecule much like water. The N in it is relatively negative, and the Hs are relatively positive. Polar substances tend to be more soluble in water. O_2, on the other hand, is not polar.

8. Refer to Chapter 2 of *Campbell Biology*, 11th edition, which describe these types of chemical bonds: nonpolar and polar covalent bonds, ionic bonds, hydrogen bonds, and van der Waals interactions.

The molecule diagrammed here can also be represented by the formula CH_3COOH.



Explain how you could determine which of the bonds between elements in this molecule are polar or nonpolar covalent bonds, ionic bonds, hydrogen bonds, and van der Waals interactions.

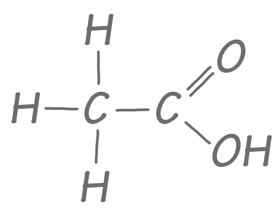
The best way to determine the bond types is to determine each atom's electronegativity, or its attraction for electrons. As a general rule, the more filled the outer electron shell of an atom is, the higher is its electronegativity. In addition, the fewer electron shells, the greater the electron negativity. As a result, an atom's attraction for electrons increases as you go from left to right in the periodic table. Electronegativity values tend to decrease as you go from top to bottom of the periodic table. To determine whether bonds are ionic, polar covalent, or nonpolar covalent, you need to determine the difference in electronegativity between the atoms that make up a molecule. If the difference in electronegativity is small, the bond is likely to be nonpolar covalent. If the difference is very large, the bond is likely to be ionic. Intermediate differences produce polar covalent bonds. The following table lists specific electronegativity values for selected elements.

H = 2.1						
Li = 1.0	Be = 1.5	B = 2.0	C = 2.5	N = 3.0	O = 3.5	F = 4.0
Na = 0.9	Mg = 1.2	Al = 1.5	Si = 1.8	P = 2.1	S = 2.5	Cl = 3.0

Using specific electronegativity values, you can determine the type of bond: If the difference in electronegativity between two atoms in a compound is less than 0.5, the bond is nonpolar covalent. If the difference is between 0.5 and 1.6, the bond is polar covalent. If the difference is greater than 1.6, the bond is ionic.

Activity 3.1 A Quick Review of the Properties of Water

1. Compounds that have the capacity to form hydrogen bonds with water are said to be hydrophilic (water loving). Those without this capacity are hydrophobic (water fearing).

	Is the molecule on the left hydrophilic or hydrophobic? Explain your answer. This molecule is acetic acid. The −COOH group is polar, which makes this molecule hydrophilic.

2. In addition to being polar, water molecules can dissociate into hydronium ions (H_3O^+, often described simply as H^+) and hydroxide ions (OH^-). The concentration of each of these ions in pure water is 10^{-7}. Another way to say this is that the concentration of hydronium ions, or H^+ ions, is one out of every 10 million molecules. Similarly, the concentration of OH^- ions is one in 10 million molecules.

   a. The H^+ ion concentration of a solution can be represented as its pH value. The pH of a solution is defined as the negative $\log_{10}$ of the hydrogen ion concentration. What is the pH of pure water?

      The hydrogen ion concentration of pure water is 10^{-7}. The $\log_{10}$ of 10^{-7} is -7. The negative $\log_{10}$ of 10^{-7} is therefore +7.

   b. Refer to the diagram of the molecule of acetic acid in question 1. The COOH group can ionize to release a H^+ ion into solution. If you add acetic acid to water and raise the concentration of H^+ ions to 10^{-4}, what is the pH of this solution?

      The pH of a solution with a H^+ ion concentration of 10^{-4} is 4.

3. Life as we know it could not exist without water. All the chemical reactions of life occur in aqueous solution. Water molecules are polar and are capable of forming hydrogen bonds with other polar or charged molecules. As a result, water has the following properties:
   A. H_2O molecules are cohesive; they form hydrogen bonds with each other.
   B. H_2O molecules are adhesive; they form hydrogen bonds with polar surfaces.
   C. Water is a liquid at normal physiological (or body) temperatures.
   D. Water has a high specific heat.
   E. Water has a high heat of vaporization.
   F. Water's greatest density occurs at 4°C.

Explain how these properties of water are related to the phenomena described in parts a–h below. More than one property may be used to explain a given phenomenon.

a. During the winter, air temperatures in the northern United States can remain below 0°C for months; however, the fish and other animals living in the lakes survive.

Water's greatest density occurs at 4°C. In a lake, the 4°C water sinks below the water that is colder (or warmer). As a result, 0°C water is less dense than 4°C water. In addition, as it freezes, water takes on a crystalline structure and becomes ice. Ice has a density of about 0.92 g/cm^3, pure water at 0°C has a density of about 0.99 g/cm^3, and pure water at 4°C has a density of 1.0 g/cm^3. The ice on top of a lake acts like insulation and, as a result, most deep lakes do not freeze to the bottom.

b. Many substances—for example, salt (NaCl) and sucrose—dissolve quickly in water.

Water is very polar. The attraction of the polar water molecules for the Na^+ and Cl^- ions of NaCl is strong enough to allow them to dissociate and interact with water molecules (dissolve).

c. When you pour water into a 25-mL graduated cylinder, a meniscus forms at the top of the water column.

Water is attracted to the polar molecules that make up the glass (or plastic) cylinder. At the same time, they are attracted to each other. As a result, some of the water molecules associate with the polar molecules of the cylinder and are apparently "pulled up" the inside edge of the cylinder.

d. Sweating and the evaporation of sweat from the body surface help reduce a human's body temperature.

Water has a high specific heat. The specific heat of water is 1 cal/g/°C. In other words, it takes 1 calorie of heat to change the temperature of 1 g of water 1°C. In addition, water has a high latent heat of vaporization (540 cal/g at 100°C). This can be thought of as the additional heat required to break apart polar water molecules so that they can move from the liquid to the gaseous state. As a result, evaporation (change of water from liquid to gaseous state) carries with it large amounts of heat.

e. A bottle contains a liquid mixture of equal parts water and mineral oil. You shake the bottle vigorously and then set it on the table. Although the law of entropy favors maximum randomness, this mixture separates into layers of oil over water.

The oil molecules are nonpolar and hydrophobic. The water molecules are polar and cohesive. As a result, the water molecules tend to interact strongly with each other and exclude the oil molecules. The oil layers on top of the water because it is less dense than water.

f. Water drops that fall on a surface tend to form rounded drops or beads.

Water molecules are cohesive and form hydrogen bonds with each other. As a result, a drop of water tends to bead up or become rounded.

g. Water drops that fall on your car tend to bead or round up more after you polish (or wax) the car than before you polished it.

The wax (or polish) is hydrophobic and therefore less polar than the surface was likely to be before you polished it. Because the adhesion between the surface and the water molecules is lower, the cohesion of the water molecules for each other appears even more dramatic.

h. If you touch the edge of a paper towel to a drop of colored water, the water will move up into (or be absorbed by) the towel.

The polar water molecules adhere to the cellulose in the paper towel and cohere to each other. As a result, they are drawn up into the towel. The same mechanism accounts for the movement of water molecules up capillary tubes.