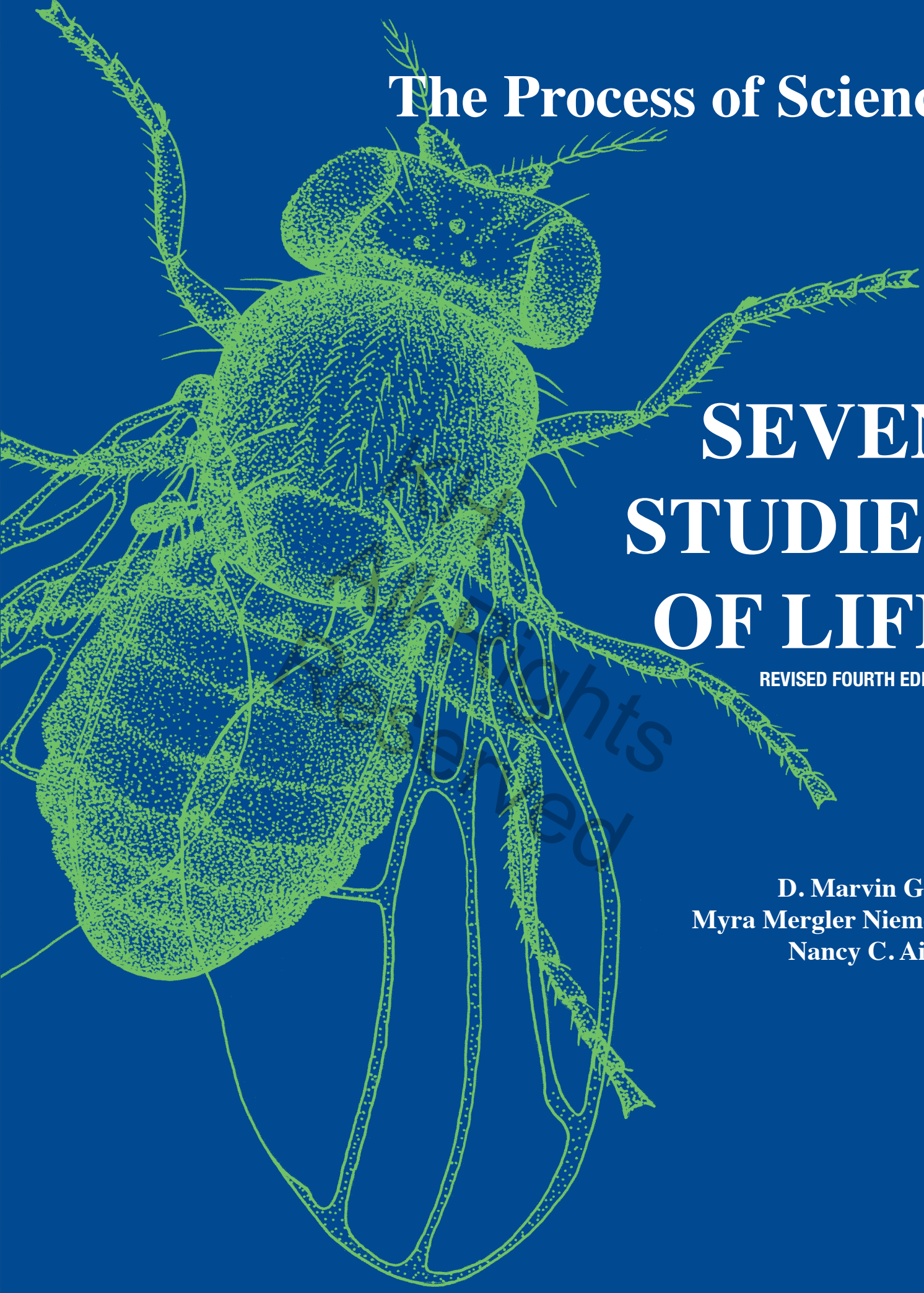
The Process of Science



SEVEN STUDIES OF LIFE

REVISED FOURTH EDITION

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The Chemistry of Life

Required Knowledge

- Definition of hydrolysis
- Structure of protein molecules
- Relationship of wavelength to color

Introduction

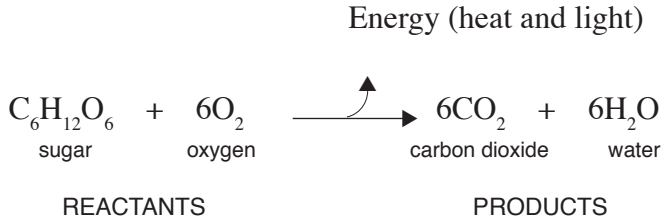
In the early 1960s, a song from the movie *Carnival!* included the lyrics "love makes the world go 'round." As popular as the tune was, it seems that the lyrics were badly flawed. We now know that it is not love that makes the world "go 'round," but enzymes! If there is any shred of truth to the song's lyrics, it is that enzymes make love and *all* of life "go 'round."

So, what is life after all? What separates a star from a rock star, a grain of sand from a sand flea, or a chocolate turtle from a painted turtle? Each is made of atoms, and in many cases contains the same atoms. Each item occupies space and is highly organized, though organized differently. So, what does it mean to be alive?

Living things respond to stimuli, adapt to changes in their environment, reproduce and are made of one or more cells. Cells are tiny containers whose enormously complex chemical activities are controlled by enzymes. **Metabolism** refers to all of these chemical reactions.

Some of the same chemical reactions also occur in the nonliving world. If you ignite a cube of sugar with a match in the presence of oxygen, you see a dancing flame and feel the heat as the sugar burns

to ash. The light and heat are energy released from the bonds that held the atoms of the sugar molecules together. The general reaction for the burning of sugar is shown below. Sugar and oxygen are the **reactants** in this reaction; carbon dioxide, water and energy are the **products** of this reaction.



Your body cells also use oxygen to burn sugar and release energy in the process. Where is the flame? Why is there no intense heat? Why are you not reduced to ash? It's elementary, my dear Watson… ENZYMES!

Enzymes make possible a slow, controlled burning of sugar at a relatively low temperature. This "slow burn" allows cells to capture some of the energy released from sugar for storage in ATPs, rather than having it escape as intense heat and light. Cells also release energy from sugar as heat, which accounts for body temperature.

There are many metaphors for the slow burn within us. Life and love are both sometimes referred to as a flame. We "burn the candle at both ends." Sometimes we feel "burned out." Our emotions, thoughts and body processes probably can all be reduced to chemistry. The packaging of that chemistry within cells, where it is controlled by enzymes, is unique to the living world. It is this chemistry that distinguishes a bacterium, an oyster, a rose or a racehorse from a pickle in a jar, a smooth stone in your pocket or a statue in the park.

You will study the chemistry of life over a three-week period. You will begin by learning what a catalyst is, and then learn about enzymes, the special catalysts found in cells. You will work extensively with a *group* of enzymes present in yeast. You will manipulate factors that affect how well the enzymes perform their functions.

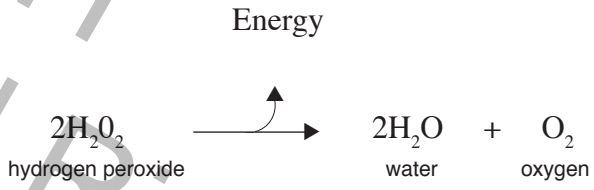
An Inorganic Catalyst

Objective 1
Define the term catalyst and describe the action of an inorganic catalyst on the decomposition of hydrogen peroxide.

Our study begins with the effect of an *inorganic* catalyst on a simple chemical reaction that involves the breakdown of hydrogen peroxide. A **catalyst** is a substance that increases the speed of a chemical reaction without being consumed in the process.

Your medicine cabinet at home probably contains a bottle of 3% hydrogen peroxide (H_2O_2). It is available over the counter at the local drug store and is frequently used to cleanse open wounds.

When hydrogen peroxide decomposes (breaks down), as shown below, it forms water and oxygen and releases energy. During World War II, the German army discovered that the rapid decomposition of concentrated hydrogen peroxide released enough energy to launch missiles!



You may have noticed that hydrogen peroxide is stored in brown bottles. Exposure to light and heat speeds up its breakdown. In fact, a solution of hydrogen peroxide decomposes entirely to water and oxygen before it comes to a boil. The dilute solution of hydrogen peroxide in the medicine cabinet breaks down much more slowly at room temperature, so you need not worry that the bottle will explode.

You can determine whether hydrogen peroxide has decomposed by checking for the presence of the products of the reaction. What products would you look for when hydrogen peroxide decomposes? List the reactant and products associated with the decomposition of hydrogen peroxide.

Reactant:

Products:

EXERCISE 1

Decomposition of Hydrogen Peroxide with an Inorganic Catalyst (Manganese Dioxide)

▸ Work as tables.

The decomposition of hydrogen peroxide can be detected by the release of bubbles of oxygen gas or by using a glowing splint. A glowing splint of wood will continue to glow, or even burst into flames, in the presence of this oxygen.

1. Pour 3% hydrogen peroxide into a test tube to a depth of about 3 cm.

Reactant _____

Inorganic Catalyst _____

Product(s) _____

2. Observe the hydrogen peroxide in the test tube and record your observation(s). Fill in the blanks in the left margin.

3. Add a small amount of the catalyst, manganese dioxide (MnO_2), to the test tube. Record your observation(s).

4. The instructor will demonstrate a glowing splint test with a test tube of hydrogen peroxide that contains a small amount of manganese dioxide. What did the glowing splint test show?

5. Manganese dioxide is the catalyst for the decomposition of hydrogen peroxide. In your own words, explain the meaning of the previous sentence.

Hydrogen peroxide is produced commercially and is also found in plant and animal cells (including your own). It is a product of the metabolism of certain chemicals such as vitamin B_2. Because hydrogen peroxide is toxic, even at low concentrations, cells use an enzyme to break it down immediately to water and oxygen.

A Biological Catalyst

Objective 2
Define the term enzyme. State the function of catalase.

Because the temperature of living organisms is relatively low, most of their chemical reactions would occur slowly without the help of *organic* catalysts called enzymes. **Enzymes** are usually protein molecules that serve as catalysts to speed up chemical reactions. Enzymes are not consumed during a reaction. Without enzymes, cell chemistry is too slow to sustain life.

Plants and animals are protected from hydrogen peroxide toxicity by producing an enzyme in their cells that catalyzes its immediate breakdown. The enzyme is named **catalase**; the water and oxygen produced by the reaction are useful products for cells. You will observe catalase activity in the following exercise.

EXERCISE 2
Decomposition of Hydrogen Peroxide with an Organic Catalyst (the enzyme catalase)

- Work as tables.

Objective 3
Describe the effect of enzyme concentration and boiling on catalase activity. Provide evidence for the presence of catalase in selected materials.

1. Get a 1 cm slice (cross section) of banana from the refrigerator. Cut the slice into four equal pie-shaped wedges with a razor blade. Discard one wedge.

2. Near the top, label each of three *large* test tubes "B" (boiled), "M" (mashed), and "W" (whole) with a wax pencil.

   a. Drop one wedge of banana into test tube B. Place tube B into a beaker of boiling water for five minutes while you prepare tubes M and W. ***Remember to wear safety glasses when boiling water. Use a test tube holder to handle the hot tube.***

b. Place another wedge of banana into test tube M. Use a plastic rod to mash the banana.

c. Place the third wedge of banana into test tube W.

3. Draw a line 2 cm from the bottom of each test tube with a wax pencil. Fill the tubes to the line with hydrogen peroxide. Observe and immediately record your observations.

Tube B (boiled):

Tube M (mashed):

Tube W (whole):

Reactant _____

Catalyst (Enzyme) _____

Product(s) _____

Fill in the blanks in the left margin.

4. Based on the above results, identify two factors that affect enzyme activity.

5. Suggest why hydrogen peroxide bubbles when it is poured on an open wound?

6. Place each of the following in separate test tubes: sand, potato, celery and mushroom. (Potato, celery and mushroom pieces are in the refrigerator.) Make all samples equivalent to the size of a new pencil eraser. Test each substance for the presence of catalase and record the results in Table 1.

7. Drain liquid from test tubes into the sink. **Dispose of solids in the trash**. Wash test tubes with warm soapy water and a test tube brush. Rinse thoroughly.

Table 1. Presence of catalase in various substances

Substance	Catalase activity (none) o, +, ++, +++, (most)
Sand	
Potato	
Celery	
Mushroom	

Enzyme Action

Objective 4
List characteristics of enzymes.

Objective 5
Describe two ways that enzyme inhibitors work.

Objective 6
Write a paragraph that describes how an enzyme functions.

In the previous exercise, you observed the activity of the enzyme catalase in selected materials. *Catalase is only one of thousands of enzymes involved in the chemistry of life*. In this section, you will examine how catalase and other enzymes work.

You learned previously that, according to kinetic theory, molecules are constantly in random motion and may collide with each other. When molecules collide with sufficient energy, a chemical reaction may occur if the molecules are properly oriented when they make contact. **Activation energy** is the amount of energy required to initiate a chemical reaction. An enzyme has a special location on its surface, called the **active site**, where the reacting molecules fit so that they are properly oriented. By bringing reactants together efficiently, *enzymes reduce the amount of activation energy required* for a successful collision.

Enzymes share a number of characteristics.

- *Enzymes are specific* for the reactions they catalyze. For example, catalase only speeds up the decomposition of hydrogen peroxide. It is not involved in other reactions.

  The molecule(s) with which enzymes interact are called **substrates**. The generalized scheme for enzyme action is:

$$\underset{\text{enzyme}}{E} + \underset{\text{substrate}}{S} \longrightarrow \underset{\text{enzyme-substrate complex}}{ES} \longrightarrow \underset{\text{enzyme}}{E} + \underset{\text{products}}{P}$$

The conventional method for naming enzymes is to add the suffix *-ase* to the name of the enzyme's substrate. For example, the enzyme lipase acts on the substrate lipid; the enzyme maltase acts on the substrate maltose.

What do you suppose is the name of the enzyme that interacts with fructose? _____ With sucrose? _____ Unfortunately, this convention for naming enzymes is not used consistently. For example, hydrogen peroxide is the substrate for the enzyme catalase.

- *Nearly all enzymes are protein molecules.* Proteins consist of long chains of amino acids that are coiled or folded to form large molecules with unique, three-dimensional shapes. The coils or folds result from relatively weak hydrogen and ionic bonds between various amino acids along the chains.

- *An enzyme works best at an optimum temperature and pH.* An enzyme molecule may change shape and become **denatured** when temperature increases above the optimum or when the pH changes. A denatured enzyme does not work because the unique shape of the active site has changed so that it no longer accepts its substrate(s).

  For every 10 °C increase in temperature, the speed of enzyme activity doubles until it reaches the optimum temperature. The optimum temperature for most enzymes in humans is body temperature (roughly 37 °C or 98.6 °F).

- *Each enzyme has an optimum pH consistent with the pH of the location in the body where that enzyme normally functions.* For example, salivary amylase works best at about pH 7, the pH of saliva. When saliva is swallowed and reaches the stomach where the pH is roughly 2, amylase stops working. However, pH 2 is optimum for the stomach enzyme pepsin. Increasing or decreasing the pH from an enzyme's optimum pH denatures the enzyme. Refer to your text-book for graphs that illustrate the optimum temperature and pH for enzymes.

  Recall what happened to catalase in Exercise 2 when you boiled the banana wedge. Explain those results based on your knowledge of enzyme structure and function.

- *Many enzymes require nonprotein helpers called **cofactors***. A cofactor for one enzyme may be an **ion** such as Ca^{++} (calcium) or K^{+} (potassium); for another enzyme it may be a nonprotein organic molecule called a **coenzyme**. A coenzyme attaches to its enzyme and carries electrons or groups of atoms to other molecules, thereby allowing the enzyme to function properly. Coenzymes are frequently derived from vitamins, which explains why vitamins are essential to your diet.

- *Enzymes may be inactivated by inhibitors*. Inactivation occurs when substrate molecules are prevented from binding to an enzyme's "active site." This inactivation is called **inhibition**. There are two kinds of inhibition, competitive and noncompetitive. In **competitive inhibition**, a molecule has a shape so similar to the substrate that it competes directly with the substrate for the active site.

  In **noncompetitive inhibition**, an inhibitor molecule binds to an enzyme at a location other than the active site. When this occurs, the shape of the enzyme molecule is distorted so that the substrate no longer fits the active site.

  Now that you are familiar with the structure of an enzyme, demonstrate your understanding of how an enzyme functions by writing a paragraph that correctly incorporates the following terms: enzyme, substrate, enzyme-substrate complex, activation energy, active site, cofactor (ion and coenzyme) and inhibition (competitive and non-competitive).

Write your summary paragraph here:

A Study of Fermentation

Objective 7
Describe the overall process of fermentation in animal and yeast cells.

At any given moment in the life of a cell, thousands of different chemical reactions occur. Each reaction is catalyzed by a specific enzyme. This collection of chemical reactions is organized into groups of reactions called metabolic pathways. A **metabolic pathway** is a sequence of enzyme controlled reactions in which the product of one reaction becomes the substrate for the next reaction. A generalized metabolic pathway is illustrated below:

$$A \xrightarrow{\text{enzyme}_1} B \xrightarrow{\text{enzyme}_2} C \xrightarrow{\text{enzyme}_3} D \xrightarrow{\text{enzyme}_4} E$$

Notice that in the first reaction, A is changed to product B by enzyme 1. In the second reaction B (the product of reaction 1) is converted to C by enzyme 2. Two additional reactions complete this metabolic pathway and the final product of the pathway is E.

One major metabolic pathway in both plant and animal cells is the series of reactions by which glucose (food for cells) is broken down *in the presence of oxygen* to release energy (ATP) for cell activities. You learned in the lecture portion of this course that this metabolic pathway is called **aerobic respiration**. It includes several hundred reactions, each catalyzed by its own enzyme.

When oxygen is absent or limited, **a dramatically shortened metabolic pathway is used to release energy from food (sugar), a pathway called *fermentation* (anaerobic respiration).** The end products of fermentation are specific for different organisms. Bacteria and most animal cells produce lactate (lactic acid) as an end product of fermentation. At the end of fermentation, yeast cells produce ethanol (ethyl alcohol) and carbon dioxide.

- **Lactate fermentation** in bacteria and most animal cells:

$$\underset{\text{glucose}}{C_6H_{12}O_6} \longrightarrow\longrightarrow\longrightarrow \underset{\substack{\text{lactate}\\(\text{lactic acid})}}{2C_3H_6O_3}$$

- **Alcohol fermentation** in yeast cells:

$$\underset{\text{glucose}}{C_6H_{12}O_6} \longrightarrow\longrightarrow\longrightarrow \underset{\text{ethanol}}{2C_2H_5OH} + \underset{\text{carbon dioxide}}{2CO_2}$$

People have known about the process of fermentation for centuries. The presence of beer yeast and bread yeast in ancient Egyptian tombs suggests that fermentation was used as early as 2000 BC. In the years that followed, many cultures consumed beer and wine at meals because supplies of clean water were often limited.

Fermentation of sugar by yeast still remains the basis for making wine and beer. The carbon dioxide released during fermentation provides sparkle or a head. Fermentation is also involved in baking bread, where the carbon dioxide released by yeast causes leavening (rising) of the dough. In fact, the word "enzyme" is derived from the Greek words that mean "in yeast" or "leaven." If you bake, you now have a clue about why the dough may not rise if you use old yeast or if the water in which you mix the yeast is too hot.

In the following exercises you will study the activities of enzymes in yeast associated with fermentation. ***Understand that you are not studying one particular enzyme but the combined activities of all the enzymes involved in the fermentation pathway.***

EXERCISE 3
Effect of Different Substrates on Fermentation

▸ Work as tables.

Objective 8
Compare the amount of fermentation using different sugars.

You will explore whether fermentation is affected by the kind of sugar (substrate) made available to yeast. You will monitor the amount of fermentation by measuring the amount of carbon dioxide produced.

Ten percent glucose, 10% galactose, 10% sucrose and water will be used in your study. You will need four clean J-shaped fermentation tubes for your experiment. *Fermentation tubes are expensive. Please handle them with great care. Keep the fermentation tubes in the* ***center*** *of your table to protect them from breakage.*

1. Write a hypothesis that compares the amount of fermentation by yeast cells when placed in glucose, galactose and sucrose.

2. What is the function of the water in the fourth fermentation tube?

3. Rinse four J-tubes thoroughly with tap water to make certain that they are clean. Use a wax pencil to mark the base of each J-tube to identify its contents.

   Four graduated cylinders labeled "glucose," "sucrose," "galactose" and "water" are on your tray. Use the appropriate graduated cylinder to measure and add 15 ml of sugar solution to three J-tubes. Also add 15 ml of water to the fourth J-tube. *You will avoid contamination by using the correctly labeled graduated cylinder.*

4. Weigh **1 g** of yeast for each fermentation tube. Weigh yeast for each tube in a separate plastic weighing boat. ***Do not add yeast to the fermentation tubes until all weighing is completed.***

5. Carefully add the yeast to the liquid in each fermentation tube. Plug the opening of each tube with a small rubber cork and hold it with your thumb as you **shake the tube to thoroughly mix the yeast and sugar solution.** ***Remove the cork from each J-tube.***

6. Tip the J-tube so the yeast suspension *flows into and fills the tube*. When you return the J-tube to the upright position, there should be no air space at the top of the tube (see figure 1a). Repeat the filling procedure if necessary.

7. Observe the J-tubes every 5 minutes for at least 20 minutes. As fermentation occurs, CO_2 will accumulate at the end of the tube (see figure 1b). Use a metric ruler to measure, in millimeters (mm), the amount of carbon dioxide that accumulates at the end of the tube. As the data become available, record the results in Table 2 and plot them in Graph 1.

Set up Exercise 5 while this experiment is in progress.

Figure 1. J-shaped fermentation tubes

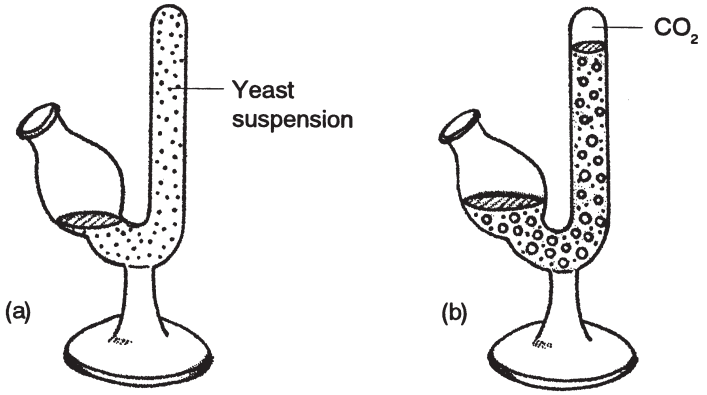
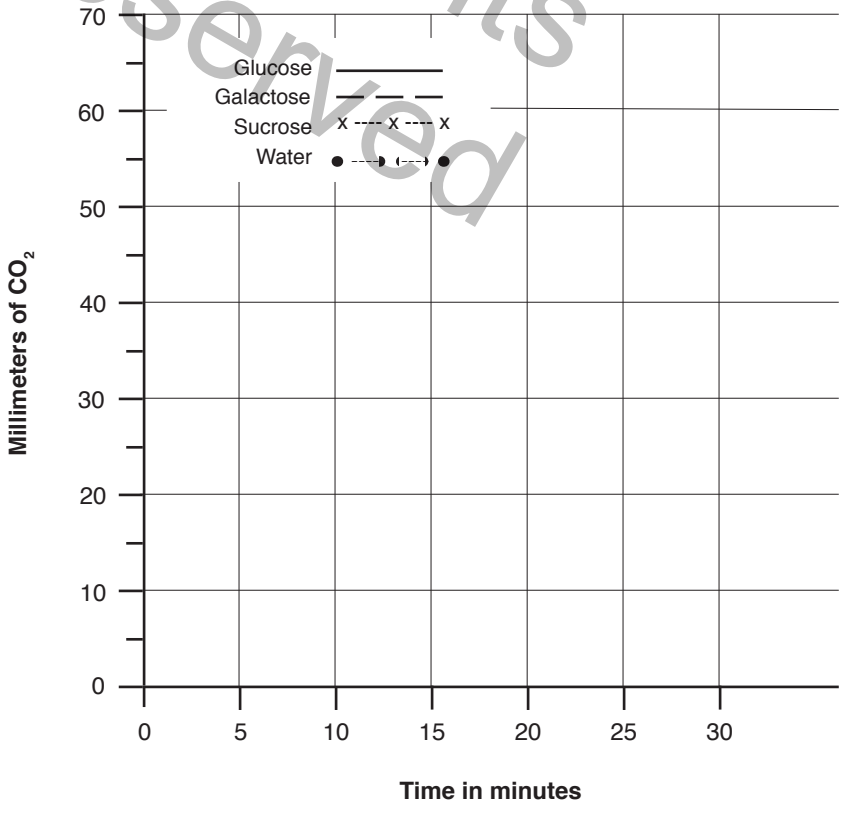


Table 2. Yeast fermentation of different sugars (substrates)

Sugar	CO_2 produced in millimeters (mm)					
	5 min	10 min	15 min	20 min	25 min	30 min
Glucose						
Galactose						
Sucrose						
Water						

Graph 1. Yeast fermentation of different sugars (substrates)



8. Do the data support your hypothesis? How might you explain the differences in fermentation of the sugars you used?

9. Empty the contents of the J-tubes into the sink. *Rinse the tubes thoroughly with soap and water. Do not use a test tube brush!* ***Be sure the tubes are clean.*** You will use these tubes again in the next exercise.

EXERCISE 4

Effect of an Enzyme Inhibitor on Fermentation

▸ Work as tables. May be presented as a demonstration.

Objective 9
Describe the effect of an enzyme inhibitor on fermentation. Provide evidence for that effect from Exercise 4.

In this exercise your instructor will demonstrate the effect of sodium fluoride (NaF) on fermentation using the following procedures.

1. Add 10 ml of 10% sucrose solution to each of two clean J-tubes labeled "W" (water) and "S" (sodium fluoride). Add an additional 10 ml of water to tube W.

2. Add 10 ml of 0.2% sodium fluoride to tube S.

3. Add 1 g of yeast to each J-tube. Plug the opening of each tube with a small rubber stopper and hold it with your thumb as you shake the tube to suspend the yeast. Fill the neck of each J-tube as you did previously. Remove the stopper from the J-tube.

4. Observe the fermentation tubes after approximately 20 minutes and summarize the results. Discuss results with your partners.

   Tube W: (Be certain to comment on the function of this tube.)

   Tube S:

5. State the effect of sodium fluoride on fermentation.

EXERCISE 5
Effect of Enzyme Concentration on Fermentation

▸ Work as tables.

Objective 10
Describe the effect of enzyme concentration on fermentation.

In this exercise you will examine the effect of different concentrations of enzymes on fermentation.

1. Write a hypothesis relating enzyme concentration to fermentation.

2. Design an experiment to test your hypothesis. Discuss with other students at your table how you will vary the enzyme concentration. A 10% sucrose solution is available for your use in this exercise. Be sure to draw on knowledge from Exercise 3.

   Write the steps of your experiment in the space below. If you forgot how to design an experiment, refer to the first laboratory exercise entitled, "What Is This Thing Called Science."

   Variable being tested:

   Constant(s):

   Materials and amounts (volumes, concentrations, weights):

   Procedure:

Have your instructor check the design of your experiment before you proceed.

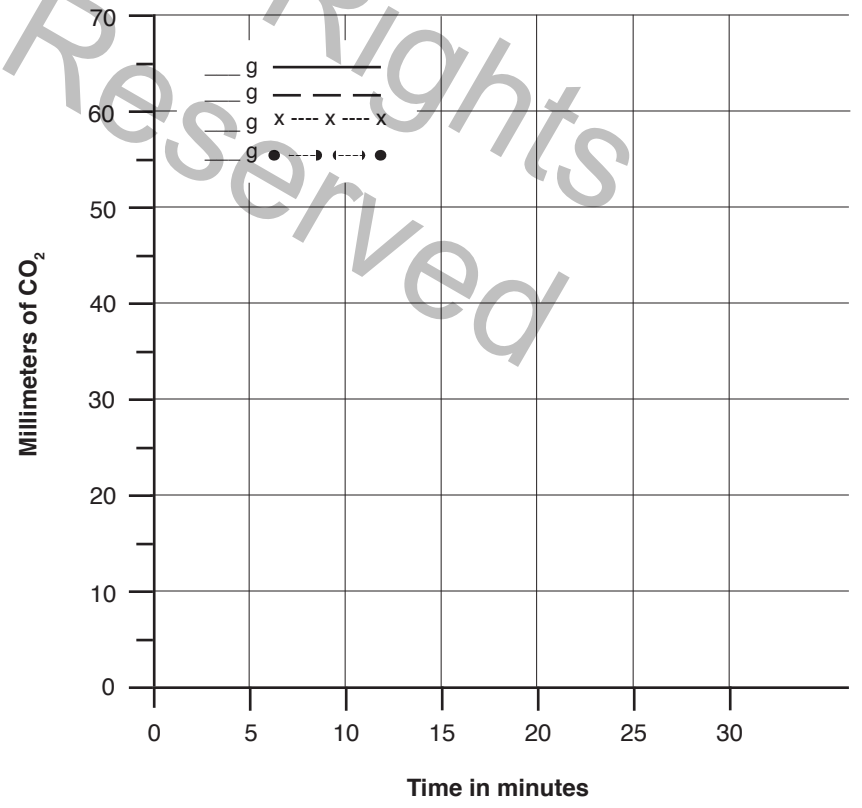
Record in the first column of Table 3 the quantity of yeast approved by your instructor.

3. Perform your experiment and record the results in Table 3. Plot the results on Graph 2 as the data become available.

Table 3. Yeast fermentation of sucrose at different concentrations of enzymes (yeast)

Yeast in grams (g)	CO_2 produced in millimeters (mm)					
	5 min	10 min	15 min	20 min	25 min	30 min
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____

Graph 2. Fermentation of sucrose at different concentrations of enzymes (yeast)



4. Interpret the results of your study and state a conclusion:

5. Complete Clean-up: Use a sponge and warm, soapy water to thoroughly clean all surfaces. Clean all J-tubes but *do not* use a test tube brush.

You will continue to explore the enzyme activity associated with fermentation for two more weeks. Instead of using fermentation tubes, you will learn to use an instrument to measure fermentation indirectly.

Week 2

As a review, write the summary reaction for the metabolic pathway by which yeast cells ferment glucose. Label the substrate, products and enzymes in the reaction. Refer to your work from last week, if necessary.

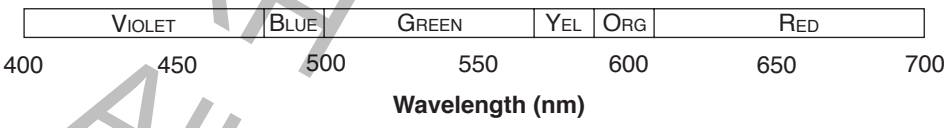
There are several ways to measure the speed of a chemical reaction. *You can measure the consumption of a substrate during the reaction or you can measure the amount of a product formed*. Previously, you monitored fermentation by measuring the quantity of carbon dioxide produced by yeast. This week you will learn how to operate a sophisticated laboratory instrument called a **spectrophotometer**. It will permit you to measure the amount of glucose (a substrate) consumed during fermentation.

Using a Spectrophotometer

Objective 11
Describe how a spectrophotometer works.

As a child you were fascinated to see a prism separate white light into a **spectrum** of colors. You learned in science courses that white light is a combination of all visible wavelengths. Each wavelength is perceived by us as a specific color, as illustrated in figure 2. For example, a wavelength of 450 nm is _____ in color, the color orange has a wavelength of _____ (include units), and a wavelength of 650 nm is _____ in color. Remember, one nm = 0.001 micrometer (μ).

Figure 2. Relationship of wavelength of light to color

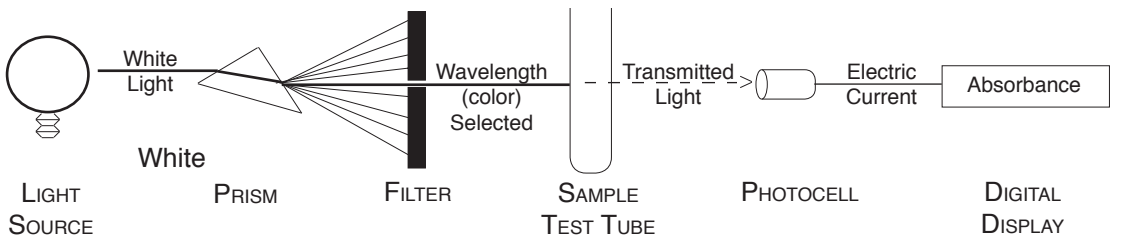


Three things can happen when light strikes a solution (or object). Often, wavelengths that are **reflected** by a solution are responsible for its color. Other wavelengths may be **transmitted** (passed) through the solution and some may be **absorbed** by it.

A spectrophotometer can measure the amount of light absorbed by a solution. The spectrophotometer separates white light into a spectrum of colors (wavelengths). It then directs a specific wavelength of light at a tube that contains a solution. The spectrophotometer calculates the amount of light the solution absorbs. The amount of light absorbed by a solution depends on the wavelength of light and on the concentration of the solution.

The essential parts of a spectrophotometer and their functions are described in Box 1.

Box 1. Components of a spectrophotometer



Light Source: Produces white light that is a combination of all visible wavelengths (colors).

Prism: Separates white light into a rainbow of colors (spectrum).

Filter: Permits the wavelength (color) selected to shine through it.

Sample Test Tube: Small test tube that holds a solution through which a selected wavelength of light shines; some of the light is absorbed by the solution, some is reflected and the rest passes through it as transmitted light.

Photocell: Receives transmitted light and converts it to an electrical current; the amount of electricity produced is proportional to the amount of transmitted light, i.e., the more light transmitted the more electricity produced.

Digital Display: Displays the amount of light absorbed by the sample (determined by measuring the electrical current produced by the transmitted light, and converting it to an absorbance value).

Blank Test Tube: Used to calibrate the spectrophotometer; identical to the sample test tube but contains only a clear fluid. We will use distilled water.

EXERCISE 6

Develop an Absorption Spectrum for a Red Solution

▸ Work as tables.

Objective 12
Use a spectrophotometer to develop an absorption spectrum and interpret the results.

In this exercise you will learn to use a spectrophotometer. It will project different wavelengths (colors) of light at a red solution. The spectrophotometer will display a number that indicates the amount of light absorbed by the solution at each wavelength. You will record and graph these data. The graph, called an **absorption spectrum**, shows how much of each wavelength was absorbed by the solution.

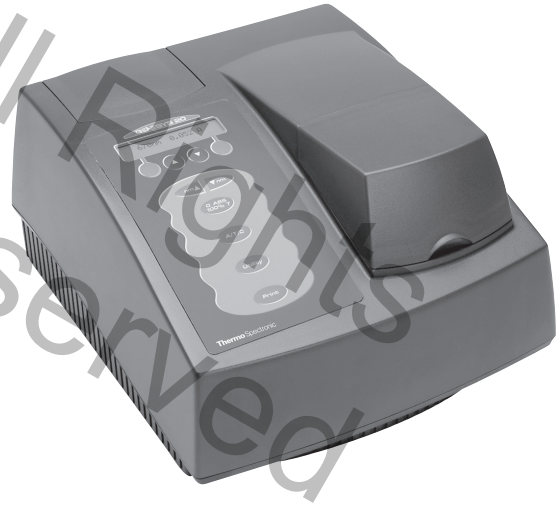
Several small test tubes are in your lab tray. Handle these sample tubes carefully and only at the top. The tubes have special optical qualities and should not be scratched or smudged because that will affect the transmission of light through them. Use only KimWipes to dry spectrophotometer tubes.

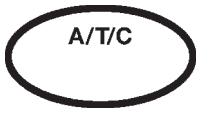
Notice that each tube has a white square with a vertical line above it. The BLANK tube contains clear fluid. The SAMPLE tube contains a red dye. (**Disregard tubes labeled with letters of the alphabet; they will be used in the next exercise**.)

1. Because each student is responsible for knowing how to operate the spectrophotometer, students at your table should divide the work in the following way.

   Student #1 reads the directions in Steps 2-6 aloud for student #2.
   Student #2 operates the spectrophotometer to obtain data for wavelengths 400 nm through 520 nm (see Table 4).
   Student #3 records absorbance values in Table 4.
   Student #4 plots the absorbance values for each wavelength (color) of light directed at the red sample in Graph 3.

Figure 3. 20 Genesys spectrophotometer





2. Press **A/T/C** to select the absorbance mode. (An "A" appears on the display.)

3. Press **nm** ▲ or **nm** ▼ to select the first wavelength shown in Table 4.

4. Lift the sample compartment door and insert the BLANK tube into the square holder. *The tube will fit in the square holder.* **Note:** *Position the tube so the white square faces you*. Close the door.

5. Press 0 ABS/100% T to "zero" the spectrophotometer (sets the BLANK to 0% absorbance).

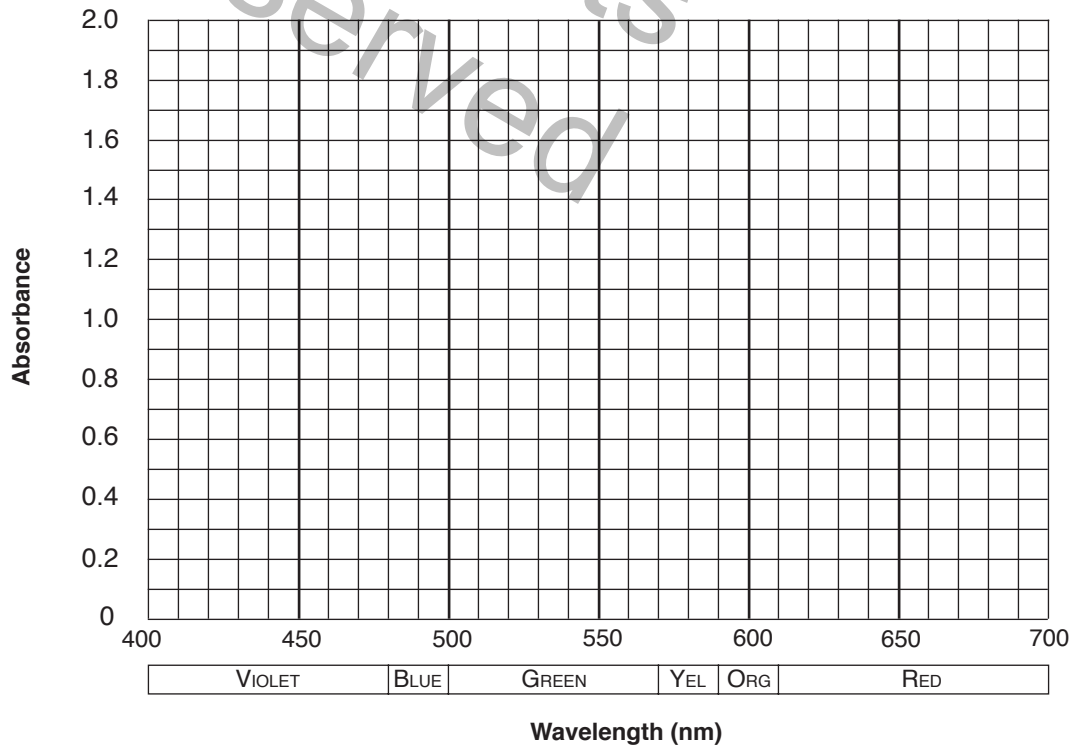
6. Remove the BLANK tube, insert the SAMPLE tube and close the door. Record the absorbance value in Table 4. **Repeat steps 3-6 for each new wavelength in Table 4.**

7. Students #1 and #2 and Students #3 and #4 exchange roles for wavelengths 550 nm through 680 nm (see Table 4).

Table 4. Absorbance values for a red dye solution

Wavelength	Absorbance	Wavelength	Absorbance
Nanometers (nm)	Red dye	Nanometers (nm)	Red dye
400		550	
420		580	
440		600	
460		620	
480		640	
500		660	
520		680	

Graph 3. Absorption spectrum for a red dye solution



8. Use the absorption spectrum in Graph 3 to help you respond to the following questions.

   a. Which wavelength of light was absorbed most by the red sample? Which wavelength (color) was absorbed least?

   b. Explain why the color of the solution is red, rather than some other color.

EXERCISE 7
Develop a Standard Curve

▸ Work as tables.

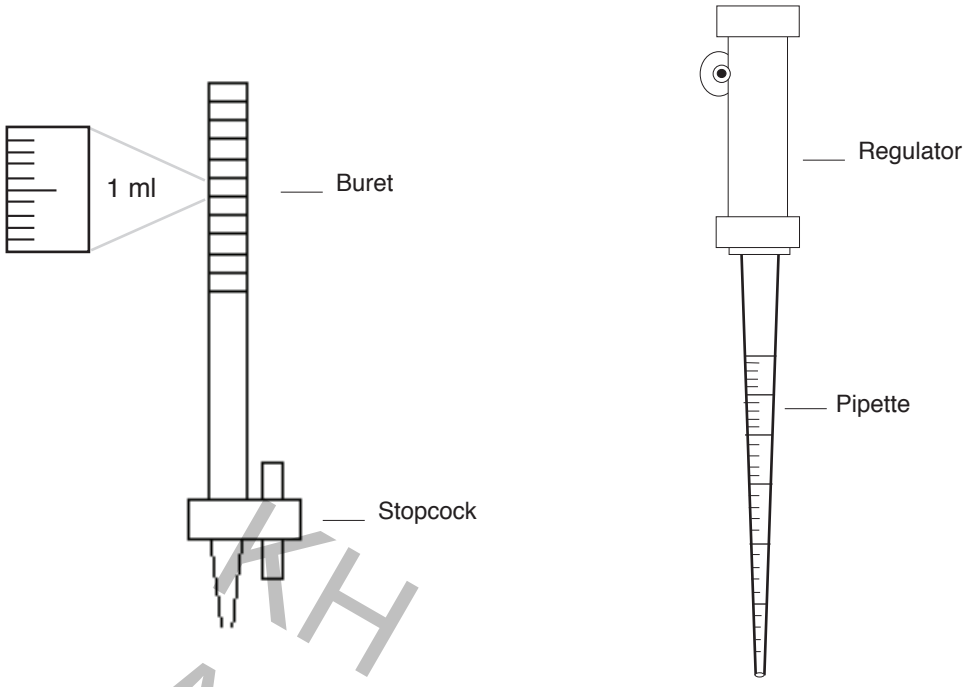
Objective 13
Develop a standard curve for red dye and use it to determine the concentration of red dye in unknown solutions.

A **standard curve** is a line on a graph that shows the relationship between the absorbance value for a solution (vertical axis) and the concentration of solute it contains (horizontal axis). To construct a standard curve, a series of solutions is prepared with different, known concentrations of solute. The absorbance for each solution is measured with a spectrophotometer. The absorbance values for all the solutions are plotted on the graph to form the standard curve. This line is then used to determine the *unknown* concentration of solute in a solution after determining its absorbance value.

Careful measurements are needed to make an accurate standard curve for a given solute. The **buret** and **pipette** (see figure 4) measure volume, but are very precise because of their small diameters. A valve (stopcock) controls the flow of liquid leaving the buret. The pipette is filled and emptied using a hand held regulator. You will use both a buret and a pipette in this exercise.

The purpose of this exercise is for you to learn how to construct and use a standard curve. You will prepare six solutions with different concentrations of a red dye, measure the absorbance of each solution and plot these values on a graph. You will then use this graph, called **a standard curve**, to determine the unknown concentration of this red dye in various solutions.

Figure 4. Buret and pipette with regulator



1. Label six test tubes with a wax pencil as indicated below.

   Accurate measurements are essential!

   To prepare the solution for each tube, obtain red dye solution from the buret at the side counter. Use the pipette with the regulator at your table to measure water. *Ask your instructor to demonstrate how to attach a pipette to a regulator to avoid personal injury due to broken glass*.

Test tube	Contents
W	5 ml distilled water
1	1 ml red dye + 4 ml distilled water
2	2 ml red dye + 3 ml distilled water
3	3 ml red dye + 2 ml distilled water
4	4 ml red dye + 1 ml distilled water
5	5 ml of red dye

2. Refer to Graph 3 to help you select the best wavelength of light to use for measuring absorbance by red dye. *Note: After setting the wavelength, remember to zero the spectrophotometer with a "BLANK" (test tube W)* ***before*** *you measure the absorbance of the solutions in the other tubes*. The wavelength best absorbed by *this* red dye is _____ (include units).

3. Measure the absorbance of the solution in each tube with a spectrophotometer and record the absorbance values in Table 5.

   You will need to pour the solutions into spectrophotometer tubes. *Rinse the tubes with distilled water before adding a new solution.*

Table 5. Absorbance values for specific concentrations of red dye

Test tube	Concentration milliliters dye/5 ml	Absorbance
W	0 ml/5 ml	
1	1 ml/5 ml	
2	2 ml/5 ml	
3		
4		
5		

4. The absorbance values in Table 5 represent the data points used to draw a standard curve for red dye. Plot these values on Graph 4. Draw a line of "best fit."

In the laboratory the concentration of a solute is often expressed in milligrams/milliliter (mg/ml). **Each ml of red dye solution in the buret contains 10 mg of red dye.**

5. Table 6 shows how to convert the concentration of red dye in each test tube to mg of dye/ml of solution. Complete Table 6 before you proceed to the next step.

Table 6. Conversion of ml red dye/5 ml to mg red dye/ml

Tube number	Test tube contains				
	ml dye/5 ml		mg dye/5 ml		mg dye/ml*
W	0	x 10 mg dye/ml =	0	÷ 5 ml =	0
1	1				
2	2				
3	3				
4	4				
5	5				

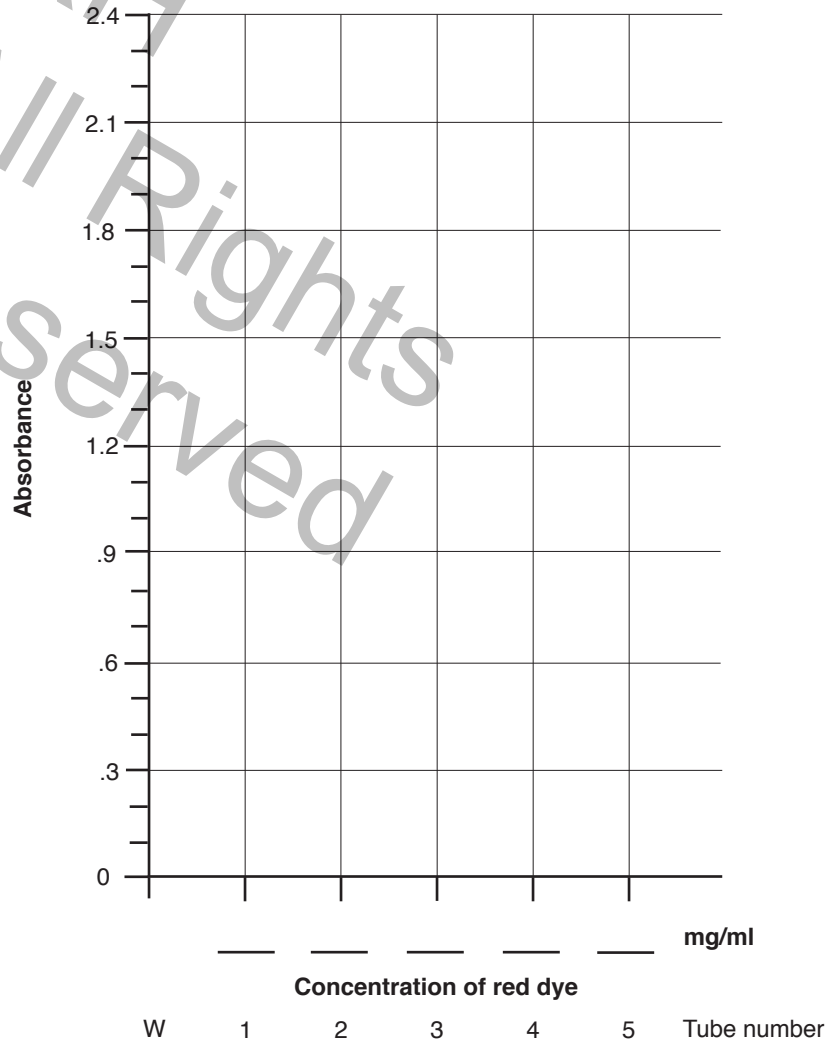
*Enter these values in blanks at the bottom of Graph 4.

6. Transfer the data from the last (shaded) column of Table 6 to the blanks at the bottom of Graph 4.

7. You will now determine the concentration (mg/ml) of red dye in two solutions. Discuss with your partners how you will do this.

   Determine the concentration of red dye in two spectrophotometer tubes labeled with letters of the alphabet. The absorbance value for solution _____ is _____. Therefore, its dye concentration is _____ mg/ml. The absorbance value for solution _____ is _____. Therefore, its dye concentration is _____ mg/ml. **Do not empty tubes labeled with letters.** *Check your responses with the instructor.*

Graph 4. Standard curve for red dye solution



8. Clean the test tubes you used in this exercise with soapy water and a brush. *Rinse the spectrophotometer tubes (no brush), and return them, inverted, to the test tube rack beside the spectrophotometer.*

During the next lab period, you will use a spectrophotometer and a standard curve to monitor the effect of temperature on the enzymes that control the fermentation of yeast.

Week 3

Previously, you developed a standard curve for a red dye. You then used a spectrophotometer and the standard curve to determine the concentration of dye in several solutions. This week, you will use a spectrophotometer and a standard curve to determine the concentration of glucose in two solutions. You will then use these skills to determine the effect of temperature on glucose consumption during fermentation in yeast.

Because glucose is invisible when it dissolves in water, special techniques are required to determine its concentration. *You will work with alkaline ferricyanide, a* ***yellow*** *reagent that reacts with glucose to form glucose-ferricyanide.*

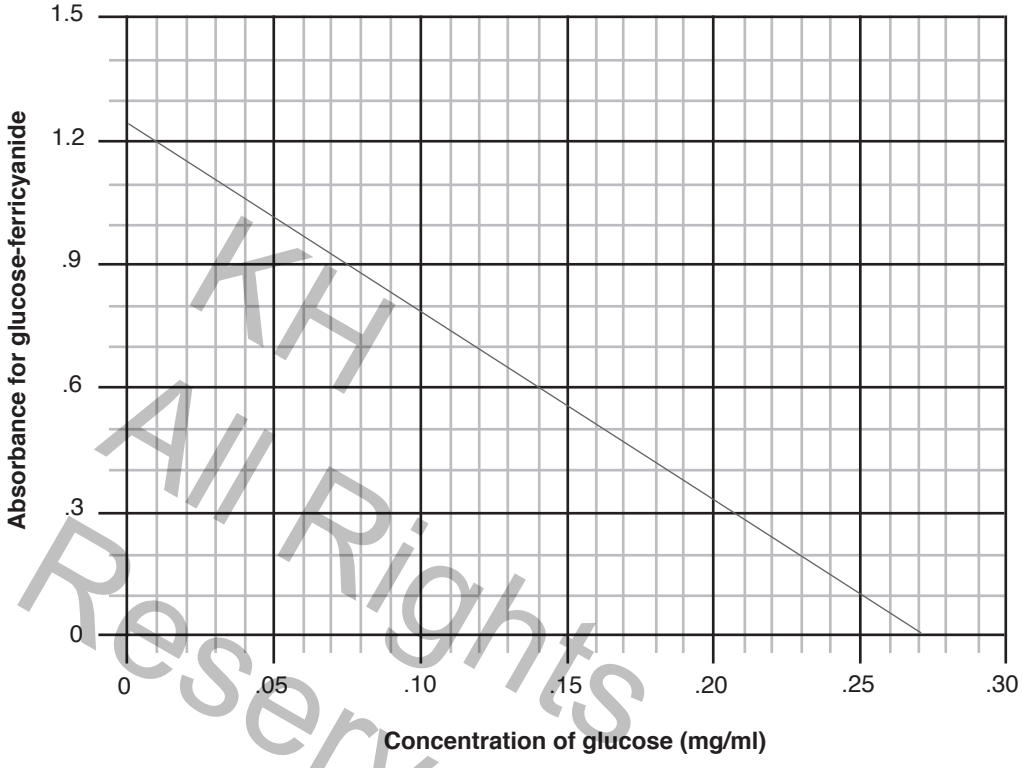
You can use a spectrophotometer to measure the absorbance of a glucose-ferricyanide solution. **The *more glucose* present, the *lighter* yellow the solution, the *less light it absorbs* and the *lower the absorbance value*.** By comparing the absorbance value with a standard curve for glucose-ferricyanide, you can determine the amount of glucose present in the solution.

One way to remember the relationship between glucose concentration, color of solution, light absorption and absorbance value is the four L's:

Lots of glucose
Light yellow
Less light absorbed
Low absorbance value

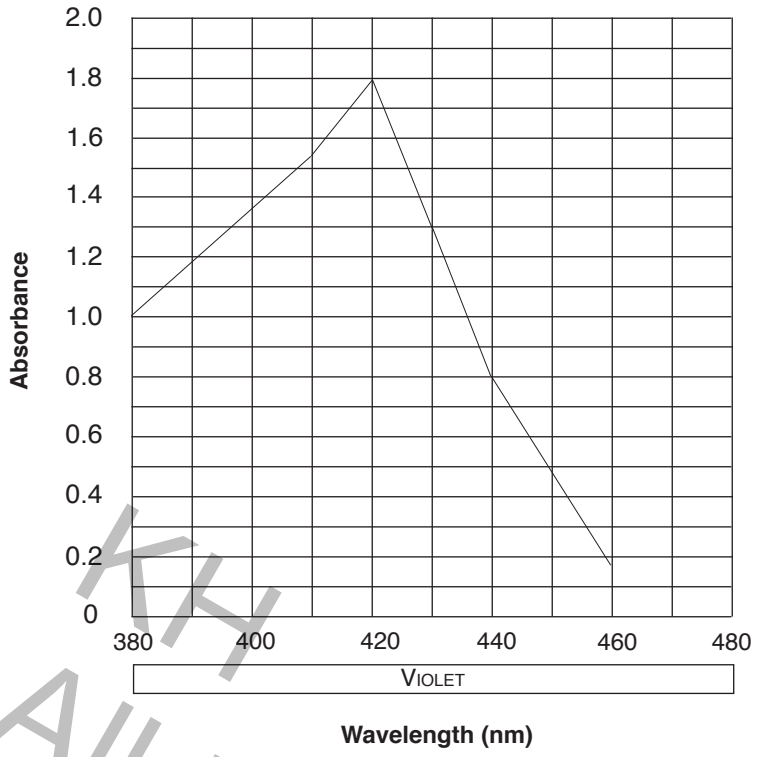
Graph 5 shows a standard curve for glucose-ferricyanide. *Notice the slope of the curve*. The lower the absorbance, the _____ (more/less) glucose is present. For example, a solution with an absorbance value of 0.9 has a glucose concentration of _.075_ mg/ml, while a solution with an absorbance value of 0.7 has a glucose concentration of _____ mg/ml.

Graph 5. Standard curve for glucose concentration



Graph 6 shows an absorption spectrum for glucose-ferricyanide. Examine the absorption spectrum to determine the optimum wavelength of light to use for measuring absorbance by glucose-ferricyanide. The optimum wavelength to use for measuring glucose-ferricyanide absorbance = _____ nm.

Graph 6. Absorption spectrum for glucose-ferricyanide



EXERCISE 8

Procedure for Measuring Glucose Concentration in Solutions

▸ Work as tables.

Objective 14
Determine the concentration of glucose in solutions by using alkaline ferricyanide and a spectrophotometer.

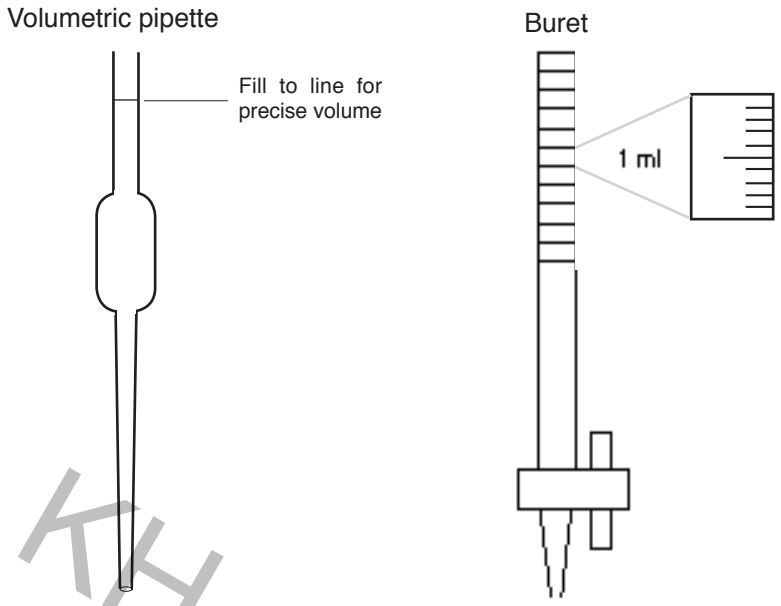
In this exercise, you will learn how to use alkaline ferricyanide to determine the concentration of glucose in solutions.

If you missed the previous demonstration of how to attach a regulator to a pipette, ask your instructor to demonstrate the procedure. If the regulator is attached improperly, you risk injury from broken glass.

1. Accuracy of measurement is very important. Use a clean 2 ml volumetric pipette with a regulator (see figure 5) to remove 2 ml of liquid from a glucose solution labeled "unknown A." Transfer the liquid to a separate, clean test tube and label the test tube with a wax pencil. ***Rinse the pipette.*** Repeat the above process with another glucose solution labeled "unknown B." Place both newly prepared tubes in a test tube rack.

2. The alkaline ferricyanide solution is in a buret at the supply counter. From the buret (see figure 5), add 3 ml of alkaline ferricyanide solution to each test tube.

Figure 5. Volumetric pipette and buret



3. Place both test tubes in a boiling water bath for 5 minutes. ***Wear safety glasses when boiling water.*** The high temperature is required to complete the glucose-ferricyanide reaction.

4. Cool the test tubes by setting them in a test tube rack in a cold tap water bath. *With a clean volumetric pipette, add 10 ml of distilled water to each test tube. Cover each tube with Parafilm and gently invert to mix.*

5. Pour the contents from each test tube into separate, clean spectrophotometer tubes. The tubes should be 2/3 full. Place distilled water into a third spectrophotometer tube that will serve as the BLANK.

6. Set the wavelength of a spectrophotometer to the value determined earlier for optimum absorbance by glucose-ferricyanide. Use the BLANK tube of water to zero the machine. *Without making further adjustments to the spectrophotometer,* measure the absorbance of both glucose solutions. Record their absorbance values in Table 7.

7. Use the standard curve on Graph 5 to determine the concentration of glucose in the two solutions. Record their concentrations in Table 7.

Table 7. Glucose concentration of unknown solutions

Solution	Absorbance at _____ nm	Glucose concentration include units
Unknown A	_____	_____
Unknown B	_____	_____

8. Check your results with the instructor before you proceed with Exercise 9.

EXERCISE 9

Effect of Temperature on Enzyme Activity

▸ Work as tables.

Objective 15
Analyze the effect of temperature on fermentation by measuring the amount of glucose (substrate) consumed.

In this exercise you will conduct an experiment to determine the effect of temperature on the enzymes that control fermentation.

Refer to the general reaction for fermentation in yeast found in Week 1 of "The Chemistry of Life." During that week, you determined the amount of fermentation in yeast by measuring carbon dioxide (product) *production*. Today, you will determine the amount of fermentation by measuring glucose (substrate) *consumption* at three different temperatures.

Week 1 you measured fermentation by measuring **carbon dioxide (product) production.**

Today you will measure fermentation by measuring **glucose (substrate) consumption.**

1. Given what you have learned about enzymes, write a hypothesis about the relationship between the amount of enzyme activity and temperature.

2. Place **0.1 g** of yeast in each of three test tubes. With a wax pencil label the tubes "R" (refrigerator), "RT" (room temperature), and "W" (water bath = 37 °C). Be sure that your label is dark and at the top of the tube. A 0.29 mg/ml stock glucose solution is being held at each of these temperatures.

3. ***You must complete this step QUICKLY so that the temperatures of the stock glucose solutions you add to the test tubes do not change.***

   Using a *clean* 10 ml volumetric pipette with a regulator, add 10 ml of stock glucose solution held at each temperature to its corresponding test tube. Mix the yeast and sugar solution in each tube by plugging the tube with a *clean* rubber stopper and vigorously shaking it for about 15 seconds, or until dissolved. ***Remove the rubber stoppers.***

4. Place each test tube at the appropriate temperature for 30 minutes. **Shake each tube at the end of the first ten minutes.**

5. While the experiment is in progress, complete steps 7a, 8a and 8b.

6. After 30 minutes, pour the contents of each tube into three separate, *clean* centrifuge tubes. Place these tubes in a centrifuge, a device that will spin them at high speed.

   Caution! ***Wear safety glasses when using the centrifuge.*** *The tubes in the machine must be balanced. If improperly balanced, the centrifuge will vibrate and may "walk" off the counter!*

   Turn on the centrifuge. After the centrifuge has reached full speed, spin the tubes for 2 minutes. The spinning action forces yeast and debris in the solutions to settle at the bottom of the tubes.

   Why should sediment be removed from a solution prior to making a spectrophotometric reading?

7. Use the following procedures to determine the concentration of glucose in the three centrifuge tubes that contain yeast.

   a. Label three clean test tubes "R," "RT" and "W." Add 3 ml of alkaline ferricyanide solution from a buret to each test tube.

   b. Use a separate, clean pipette to transfer 2 ml of liquid from the *top* of each centrifuge tube to the appropriate test tube. **Be sure to rinse the inside and outside of the pipette before taking each sample.**

   c. Place all three test tubes in a boiling water bath for 5 minutes. *Wear safety glasses when boiling water.*

   d. Cool the test tubes by setting them in a test tube rack in a cold tap water bath. With a *clean* volumetric pipette, add 10 ml of distilled water to each test tube. *Cover each tube with Parafilm and gently invert to mix*.

   e. Pour the contents from each test tube into separate, clean, labeled spectrophotometer tubes. The tubes should be 2/3 full. Place distilled water into a fourth spectrophotometer tube that will serve as the BLANK.

   f. Set the wavelength of a spectrophotometer to the value determined earlier for optimum absorbance by glucose-ferricyanide. Use the BLANK tube of water to zero the machine. *Without making further adjustments to the spectrophotometer,* measure the absorbance of the three glucose solutions. Record their absorbance values in Table 8.

Table 8. Glucose consumed by yeast at different temperatures

Temperature	Absorbance at ____ nm	Glucose (mg/ml)		
		Stock −	Remaining =	Consumed
____° C R	_____	0.29	_____	_____
____° C RT	_____	0.29	_____	_____
37 ° C W	_____	0.29	_____	_____

g. Use a **straight edge** with the standard curve on Graph 5 to determine the concentration of glucose (mg/ml) remaining in each tube. Record the concentration of glucose remaining in each tube in Table 8.

h. Calculate the glucose consumed (mg/ml) by the yeast in each tube and record it in Table 8.

i. Complete Graph 7. Place values on the vertical axis, and add bars to show the glucose consumed (mg/ml) by yeast at each temperature.

j. Clean the test tubes and centrifuge tubes with soapy water and a brush. Rinse the spectrophotometer tubes (no brush). Invert all tubes in the test tube rack in your tray. *No tubes should be left uncleaned in the lab sink*.

8. Summary of your experiment.

a. Variable tested

b. List the constants

Graph 7. Glucose consumed by yeast at different temperatures

Note: Be sure to label the axes and include units

c. What conclusion can you draw about the relationship between temperature and fermentation? Do the data support your hypothesis? How much confidence do you have in your conclusion? What would increase your level of confidence?

References

Eberhard, C. 1988. Experiments in biology. Saunders College Publishing, New York.

Forsee, W. T. 1938. Determination of sugars in plant materials: a photocolorimetric method. Industrial and Engineering Chemistry, Analytical Edition. 10:411-412.

Samuels, E. 1974. Biotutorial, Houghton Mifflin, Princeton.