Cellular Respiration

Introduction

In the process of photosynthesis, plants capture light energy and use it to make the monosaccharide glucose. The energy from the light is packaged into the chemical bonds of the glucose molecule. This glucose can be converted into a number of other molecules, which are either used or stored in the plant. For example, glucose made in the leaves is changed into sucrose, a disaccharide, before it is transported to other parts of the plant. Glucose may also be stored as starch, which is a long chain of glucose molecules. When animals eat plants, the starch and sucrose can be changed back into glucose for use in metabolism. Animals also store excess glucose in long chain molecules (glycogen) or by converting it into fat. Starch and glycogen can in turn be reconverted into glucose when energy is needed by the plant or animal.

The energy contained in the glucose molecule cannot be used directly by the cell. That energy must first be repackaged in ATP (adenosine triphosphate), the molecule that provides the energy for most cellular work. The process of **cellular respiration** transfers the energy stored in glucose bonds to bonds in ATP so that it can be used more easily by the cell. Each glucose molecule can generate as many as 38 ATP molecules through cellular respiration.

In this lab topic we will consider two methods by which cells make ATP using the energy that comes from breaking down the chemical bonds in glucose. These two methods, called **metabolic pathways,** are alcoholic fermentation and aerobic respiration.

Outline

Exercise 6.1: Alcoholic Fermentation

Exercise 6.2: Aerobic Respiration

Exercise 6.3: Designing an Experiment

Exercise 6.4: Performing the Experiment and Interpreting the Results

EXERCISE 6.1
Alcoholic Fermentation

Objectives

After completing this exercise, you should be able to

1. Describe alcoholic fermentation, naming the substrate and products.
2. Explain how alcoholic fermentation can be measured.
3. List factors that can affect the rate of alcoholic fermentation.

Alcoholic fermentation is a metabolic pathway used primarily by yeasts and some bacteria. In fermentation, glucose is broken down into ethyl alcohol (ethanol) and carbon dioxide (Figure 6.1). In the process, some of the energy that had been stored in the glucose bonds is used to form high energy bonds in ATP.

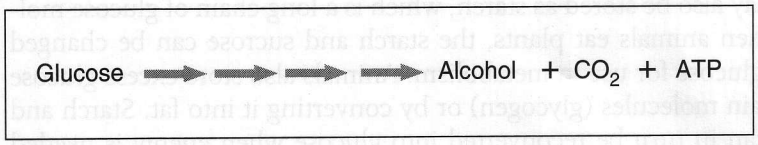

Figure 6.1.
Substrate and products of alcoholic fermentation.

The arrows in Figure 6.1 indicate that a series of enzyme-catalyzed reactions is needed to complete the conversion of glucose (the substrate) into alcohol, carbon dioxide, and ATP (the products). Therefore, any of the factors that affect enzyme activity can affect the rate at which fermentation occurs.

Name some factors that affect enzyme activity.

Although glucose is the substrate at the beginning of the pathway, keep in mind that other molecules can be changed into glucose and used in cellular respiration. For example, starch or glycogen (storage molecules) can be broken down into their many component glucose molecules. Sucrose (table sugar) can be broken down into its two component sugars, glucose and fructose. The fructose can then be converted to glucose by another enzymatic process.

In the procedure you will use, fermentation is performed by yeast, a single-celled fungus. That is, yeast contains the cellular machinery, including the **enzymes,** which is capable of breaking down glucose by alcoholic fermentation. Corn syrup, which contains sucrose and fructose, will be used as the **substrate.** You will measure the rate of alcoholic fermentation by collecting carbon dioxide (CO_2), which is one of the **products,** at intervals after fermentation has begun.

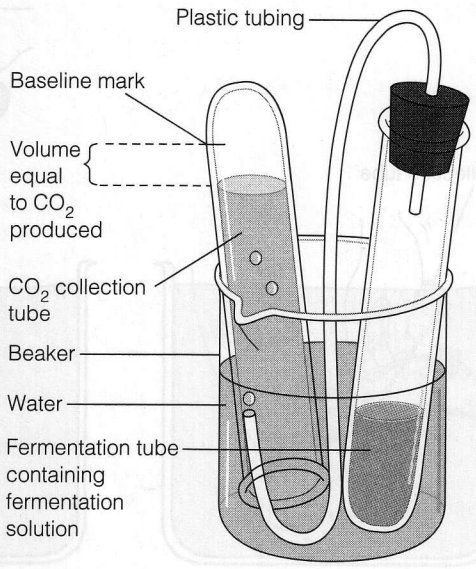

Figure 6.2.
Apparatus for measuring CO_2 production in alcoholic fermentation.

Figure 6.2 shows the setup you will use to collect the CO_2. Fermentation will take place in the test tube on the right, which contains the yeast and corn syrup, the fermentation solution. The test tube is capped with a rubber stopper to prevent CO_2 from escaping. Plastic tubing leads from the fermentation tube to the CO_2 collection tube, which is upside down and contains water. As CO_2 is produced by fermentation, it goes through the tubing into the collection test tube, where it displaces the water. The displacement (in mm) is recorded as a measure of the amount of CO_2 produced.

Procedure

1. You will need to have three fermentation setups, so you should have six large test tubes, three pieces of plastic tubing that have been inserted into rubber stoppers, and three beakers (400 or 600 mL). Using a wax pencil, label three of the test tubes 1, 2, and 3 and set them aside. Assemble the setups one at a time following steps 2–7 (see Figure 6.3 on the next page).
2. Fill a tub or sink with *hot* water (50°–60°C).
3. Insert the end of the plastic tubing into one of the test tubes. This tube will be the CO_2 collection tube. Submerge the collection tube and plastic tubing in the tub of hot water (Figure 6.3a).
4. Submerge the beaker. Place the collection tube in the beaker in an inverted position (Figure 6.3b).
5. Bring the beaker out of the water. One end of the plastic tubing should still be inserted in the collection tube. Hold up the other end of the tubing (the one with the rubber stopper on it) so that the water won't be siphoned out.

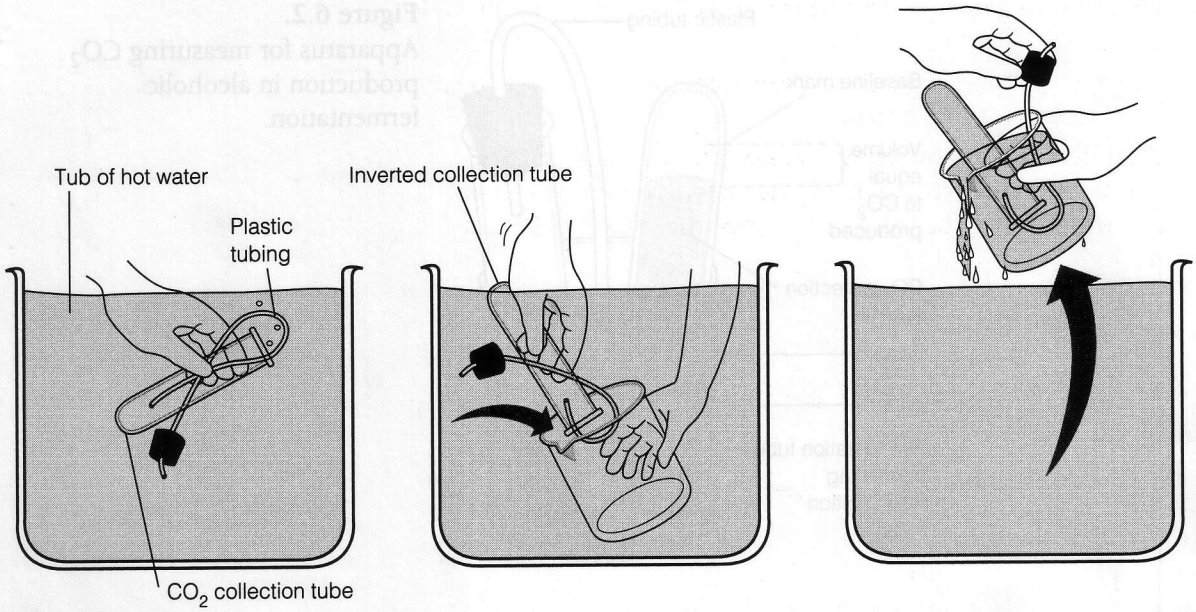

Figure 6.3.
Setting up the CO_2 collection tube.

6. Pour some water out of the beaker so that the water level is at least 1 cm from the top of the beaker (Figure 6.3c).
7. Check the tubing for kinks. If the CO_2 can't get through the tubing, you'll have to start over.

Assemble all three setups before proceeding to mix the fermentation solutions.

8. Mix the fermentation solutions for Tubes 1, 2, and 3 according to Table 6.1.

Table 6.1
Contents of Fermentation Tubes

	Fermentation Tube (volume in mL)		
	1	2	3
Water	4	3	1
Yeast suspension	0	1	3
Corn syrup	3	3	3

9. Swirl each test tube gently to mix the reactants. Place one test tube in each beaker.
10. Put the rubber stoppers in the fermentation tubes. This will force most of the water out of the tubing.
11. After the air bubbles from inserting the stopper have cleared the tubing (half a minute to a minute), mark the water level on each collection tube with a wax pencil. This marks the baseline for your experiment.

If the water level is all the way to the top of the collection tube, where the tube is curved, you should wait until it has descended to the part of the tube where the sides are straight before you mark the level.

12. At 5-minute intervals, measure (in mm) the distance from the baseline mark to the water level. Continue taking data for at least 20 minutes. Record your data in Table 6.2.

Table 6.2
Results of Fermentation Experiment

Tube	Minutes						
	0	5	10	15	20	25	30
1 (3 mL corn syrup)							
2 (3 mL corn syrup + 1 mL yeast suspension)							
3 (3 mL corn syrup + 3 mL yeast suspension)							

While you are waiting to collect data, look again at Table 6.1 to see how the experiment was designed.

What is the independent variable?

What is the dependent variable?

What hypothesis is being tested?

Predict the outcome of the experiment in terms of your hypothesis. Explain what results would support your hypothesis.

The instructor will collect data from all the student teams. Average the data and, in Figure 6.4, plot the average water displacement for each interval against time. This shows the reaction rate (CO_2 produced/time). The steeper the slope of the line, the faster the reaction rate. Be sure to label the axes completely.

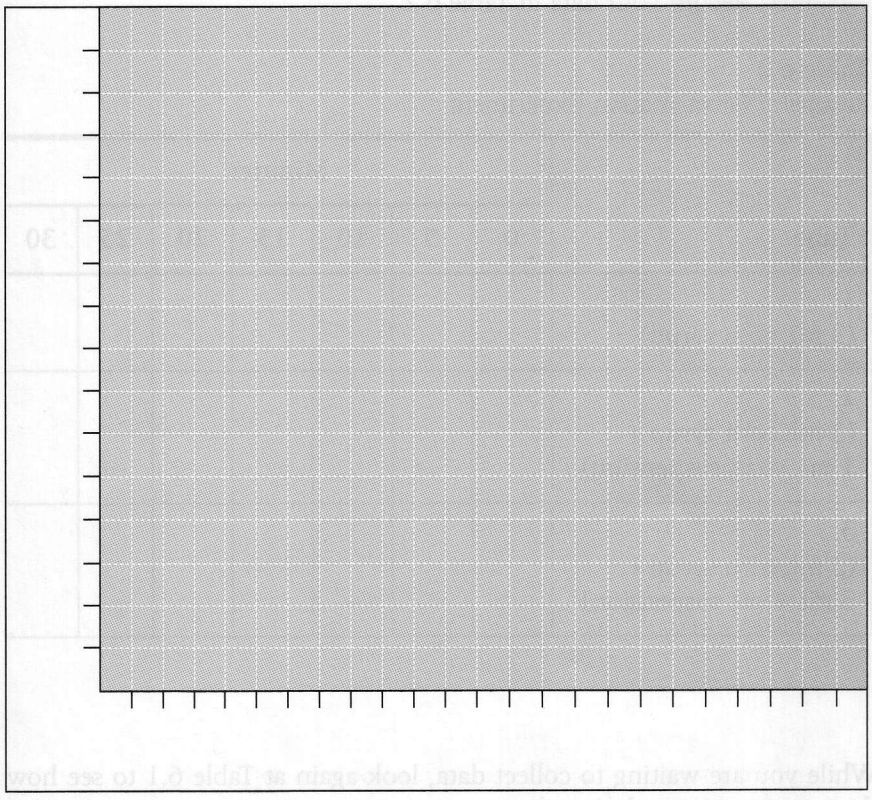

Figure 6.4.
Rate of CO_2 production by yeast fermentation.

Was your hypothesis proven false or supported by the results? Use data to support your answer.

Which fermentation tube was the control?

Why were different amounts of water added to each fermentation solution?

What are some other independent variables that could affect alcoholic fermentation?

EXERCISE 6.2
Aerobic Respiration

Objectives

After completing this exercise, you should be able to

1. Name the three phases of aerobic respiration.
2. Explain how aerobic respiration can be measured using DCPIP and the succinate → fumarate reaction.
3. List factors that can affect the rate of aerobic respiration.

Most organisms, including humans, produce most of their ATP by using the process of **aerobic respiration** rather than by fermentation. Aerobic respiration begins with glucose, the same substrate that is used in fermentation. However, aerobic respiration extracts much more of the energy stored in the bonds of each glucose molecule. A single glucose molecule can yield as many as 38 ATP molecules through aerobic respiration, compared with a yield of two ATP per glucose molecule from alcoholic fermentation.

Like fermentation, aerobic respiration begins with the metabolic pathway (series of enzyme-catalyzed reactions) called **glycolysis.** In aerobic respiration, however, pyruvate (which is the product of glycolysis) enters another metabolic pathway called the **Krebs cycle.** Some of the reactions in the Krebs cycle release protons and electrons, which then enter the **electron transport system.** Most of the ATP molecules that are gained in aerobic respiration are produced by the electron transport system.

Glycolysis takes place in the cytoplasm, but the Krebs cycle and electron transport occur within a specific organelle, the **mitochondrion.** Mitochondria are found in all eukaryotic organisms (organisms whose cells have a true nucleus and membrane-bound organelles). You will use a suspension made from ground-up lima beans to study aerobic respiration. The suspension contains mitochondria, which, under our experimental conditions, will continue to carry out aerobic respiration as if they were in intact

cells. Sucrose has been added to the mitochondrial suspension as a source of glucose for respiration, just as corn syrup was used in alcoholic fermentation.

The rate of aerobic respiration can be measured by studying the activity of one particular enzyme in the Krebs cycle. This enzyme catalyzes a reaction that converts succinate, which is the substrate for the reaction, to fumarate (Figure 6.5).

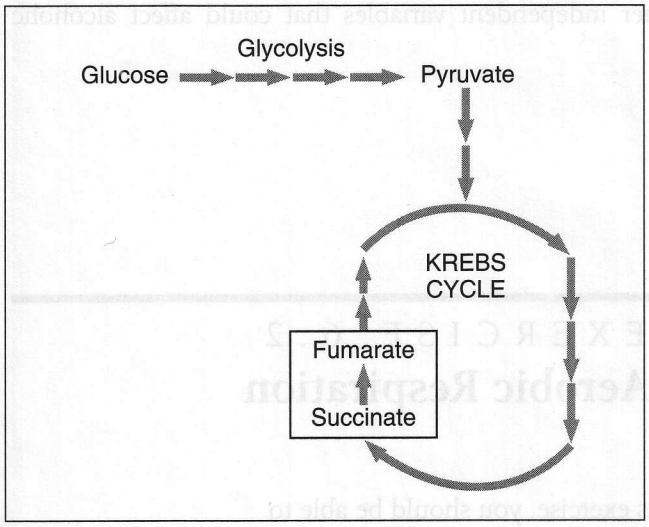

Figure 6.5.
Breakdown of glucose by glycolysis and the Krebs cycle. The enzyme-catalyzed conversion of succinate to fumarate is one step in the Krebs cycle.

As the enzymatic reaction occurs, protons and electrons are released from the succinate molecule. In the living mitochondrion, these protons and electrons are carried to the electron transport system and used to make ATP. In order to investigate aerobic respiration, you will add a blue compound, DCPIP (di-chlorophenol-indophenol) to the reaction mixture. DCPIP intercepts the protons and electrons before they get to the electron transport system. When a DCPIP molecule picks up a proton and electron, its blue color disappears and it becomes colorless. We can use this color change as a measure of the enzymatic reaction rate, which in turn is a measure of the rate of aerobic respiration. As succinate is converted to fumarate (and as aerobic respiration proceeds), the solution in the test tube gradually turns from blue to colorless. The reaction is summarized in Figure 6.6.

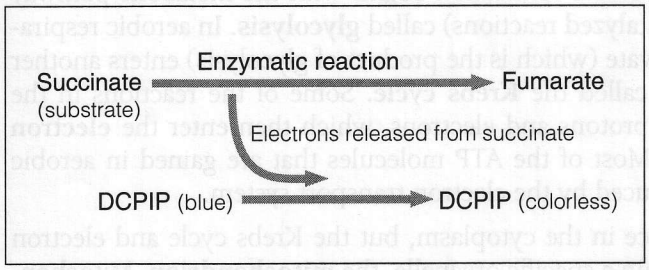

Figure 6.6.
Color change of DCPIP caused by the conversion of succinate to fumarate.

The color change that DCPIP undergoes thus provides a way to visualize aerobic respiration as it occurs. We now need a method of measuring how much color change occurs. An instrument called a spectrophotometer can be used. A common model (the one referred to in this lab topic) is

the Spectronic 20 (Spec 20). You may have a different model in your laboratory.

If spectrophotometers are not available, you can use a color chart to determine how much color change has taken place. Compare your samples with Plate 2 and record the number for the color intensity that matches the sample most closely.

As shown in Figure 6.7, the Spec 20 shines light through a sample of reactants in a tube and measures the amount of light that penetrates through the tube. In Lab Topic 5 (Enzymes), you recorded data from the absorbance scale: The data showed how much light had been absorbed by each sample. In this experiment you will record data from the % transmittance scale.

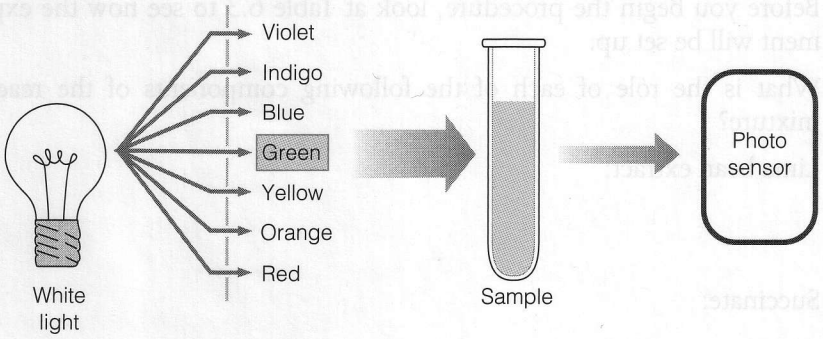

Figure 6.7.
Diagrammatic representation of how a Spec 20 works. Like a prism, a spectrophotometer divides light into its component wavelengths.

Your data will show how much light has been transmitted by the sample. The more intense the color of the sample, the less light it transmits. Percent transmittance measures how much of the blue color has disappeared. The blue DCPIP becomes colorless as aerobic respiration proceeds, so the higher the transmittance reading the more mitochondrial activity has been observed.

On the axes in Figure 6.8, sketch the relationship between % transmittance or color intensity and mitochondrial activity (aerobic respiration). Label both axes.

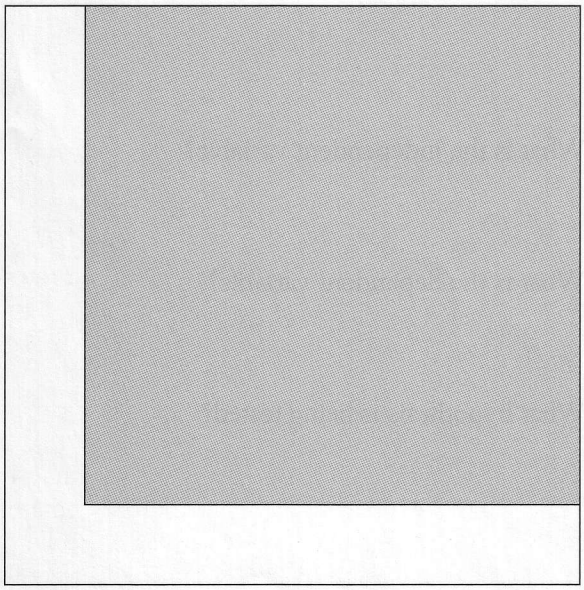

Figure 6.8.
Relationship of % transmittance or color intensity to mitochondrial activity.

Table 6.3
Reaction Mixtures for Mitochondrial Activity Experiment

	Tube (volume in mL)		
	1	2	3
Buffer	4.4	4.3	4.2
DCPIP	0.3	0.3	0.3
Mitochondrial suspension	0.3	0.3	0.3
Succinate	0	0.1	0.2

Before you begin the procedure, look at Table 6.3 to see how the experiment will be set up.

What is the role of each of the following components of the reaction mixture?

Lima bean extract:

Succinate:

DCPIP:

Buffer:

Why should there be a different amount of buffer in each tube?

What is the independent variable?

What is the dependent variable?

What hypothesis is being tested?

Predict the outcome of the experiment in terms of your hypothesis. Explain what results would support your hypothesis.

Procedure

Zeroing the Spec 20 (skip steps 1–7 if you are using the color chart)

When you look at the mitochondrial suspension, you will notice that it is cloudy. So even though it is colorless, transmittance of light through the sample will already be less than 100% before any DCPIP is added. If you are using a Spec 20, you will therefore prepare a "blank" tube containing mitochondrial suspension but no DCPIP and use that tube to set the transmittance at 100%. This is called zeroing the machine.

1. Get a clean test tube (either a small test tube or a special Spec 20 tube) and use a wax pencil to label it B. When you put the tube into the sample holder, it should always be inserted with the B facing front.
2. Measure 4.6 mL buffer, 0.3 mL mitochondrial suspension, and 0.1 mL succinate into Tube B.
3. Cover the Tube B tightly with Parafilm and invert it to mix the reactants thoroughly.
4. Set the wavelength of the Spec 20 to 600 nm (Figure 6.9).
5. Use the knob on the left to set % transmittance (top scale) to 0.
6. Wipe the blank tube (B) with a cleaning tissue and insert it into the sample holder.
7. Use the knob on the right to set % transmittance to 100%.
8. Set Tube B aside. You will need to rezero the Spec 20 later.

The Spec 20 is now zeroed and ready to measure sample tubes for the experiment.

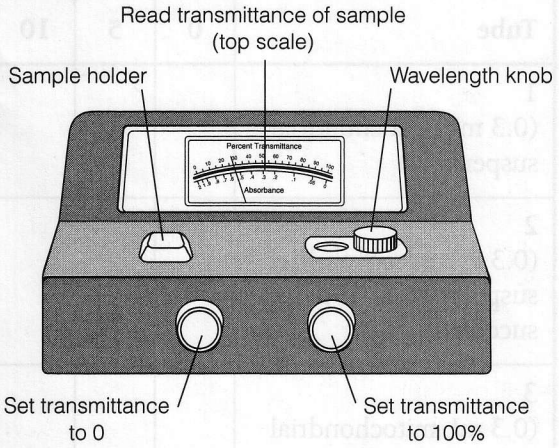

Figure 6.9.
Spec 20.

Preparing Reaction Mixtures for the Experiment

9. Get three Spec 20 tubes or small test tubes and use a wax pencil to label them 1, 2, and 3.
10. Measure the buffer, DCPIP, and mitochondrial suspension into Tubes 1, 2, and 3 as specified in Table 6.3, but it is important not to add the succinate until the other ingredients are in the tubes.
11. As quickly as possible, add the succinate to each tube.
12. Cover each tube tightly with Parafilm and invert to mix.

Spec 20 Method

13. As you complete each tube, insert it into the sample holder of the Spec 20 with its number facing to the front and record the % transmittance in Table 6.4 at time 0.
14. After 5 minutes, use the blank to rezero the Spec 20 (steps 5–7). Invert each tube to remix its contents and read and record the % transmittance.
15. Continue to record the % transmittance in the tubes at 5-minute intervals for at least 20 minutes or until Tube 3 is nearly colorless (% transmittance is near 100%). You should rezero the Spec 20 before each reading and always insert the tubes with the number facing the front.

Color Chart Method

13. Compare each tube to the right chart on Color Plate 1. Observe the intensities of the colors. In Table 6.4, record the number of the color that most closely matches the color intensity in each tube at time 0. At the beginning of the experiment all the tubes should be the same color.

Table 6.4
Results of Mitochondrial Activity Experiment
(% transmittance or color intensity)

	Minutes						
Tube	**0**	**5**	**10**	**15**	**20**	**25**	**30**
1 (0.3 mL mitochondrial suspension)							
2 (0.3 mL mitochondrial suspension + 0.1 mL succinate)							
3 (0.3 mL mitochondrial suspension + 0.2 mL succinate)							

Look for a similarity in color intensity. The exact color does not have to match.

14. After 5 minutes, shake each tube gently to mix its contents and compare it to Color Plate 1. In Table 6.4, record the number of the color that most closely matches the color intensity in each tube.
15. Continue to record the color in the tubes at 5-minute intervals for 20 minutes or until Tube 3 is colorless (0).

The instructor will collect data from all the student teams. Average the data and plot the average % transmittance (or color intensity) for each interval against time in Figure 6.10. This shows the reaction rate for aerobic respiration. The steeper the slope of the line, the faster the reaction rate. Be sure to label the axes completely.

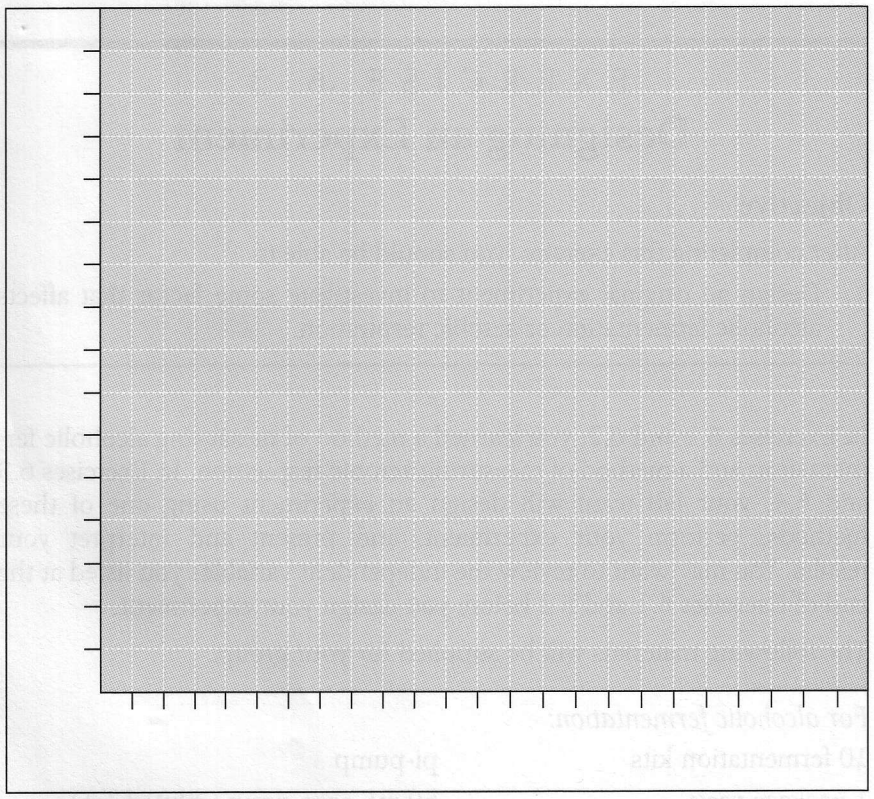

Figure 6.10.
Rate of aerobic respiration in mitochondria.

Was the hypothesis proven false or supported by the results? Use data to support your answer.

Which reaction tube was the control?

Why should the succinate be added to the reaction tubes last?

What are some other independent variables that could affect aerobic respiration?

When you design your own experiment to investigate mitochondrial activity using the Spec 20, be sure to include a blank tube for zeroing the instrument.

EXERCISE 6.3

Designing an Experiment

Objective

After completing this exercise, you should be able to

1. Design an original experiment to investigate some factor that affects alcoholic fermentation or aerobic respiration.

In Exercises 6.1 and 6.2, you learned a method of measuring alcoholic fermentation and a method of measuring aerobic respiration. In Exercises 6.3 and 6.4, your lab team will design an experiment using one of these methods, perform your experiment, and present and interpret your results. You may want to review the independent variables you listed at the end of Exercises 6.1 and 6.2 before you design your experiment.

The following materials will be supplied for your group.

For alcoholic fermentation:

10 fermentation kits
1 package yeast
150-mL beaker
glass stir rod
3 5-mL pipets
pi-pump
50 mL corn syrup (diluted 1:1)
bottle of water
wax marker
ruler

For aerobic respiration:

20 test tubes
test tube rack
3 1-mL pipets
1 5-mL pipet
pi-pump for 1-mL pipet
pi-pump for 5-mL pipet
20 Parafilm squares
25 mL DCPIP
25 mL succinate
100 mL buffer solution

Your instructor will be able to tell you what additional materials will be available.

Describe your experiment below.

Question or Hypothesis

Dependent Variable

Independent Variable

Explain why you think this independent variable will affect alcoholic fermentation or aerobic respiration.

Control(s)

Replication

Brief Explanation of Experiment

Predictions

What results would support your hypothesis? What results would prove your hypothesis false?

Method

Include the levels of treatment you plan to use. It might be helpful to make a table showing the contents of each reaction vessel.

Design a Table to Collect Your Data

List Any Additional Materials You Will Require

EXERCISE 6.4

Performing the Experiment and Interpreting the Results

Objectives

After completing this exercise, you should be able to

1. Perform the experiment your lab team designed.
2. Present and interpret the results of your experiment.

Before you do the experiment, be sure that everyone on your lab team understands the techniques that will be used. You may want to divide up the tasks before you begin work. Since it is important to measure the volumes of reactants accurately, you may want to ask your instructor to review pipet use with you.

Be thorough in collecting data. Don't just write down numbers; record what they mean as well. Don't rely on your memory for information you will need when reporting on your experiment later! If you have any questions, doubts, or problems during the experiment, be sure to write them down, too.

Results

Before you begin to prepare your results for presentation, decide on the best format to use. Remember, you want to give the reader a clear, concise picture of what your experiment showed. Refer to the data presentation section of Appendix A (Tools for Scientific Inquiry) for help. If you are drawing graphs, use graph paper. Complete your tables and/or graphs before attempting to interpret your results.

Write a few sentences *describing* the results (don't explain why you got these results or draw conclusions yet).

Discussion

Look back at the hypothesis or question you posed in this experiment. Look at the graphs or tables of your data. Do your results support your hypothesis or prove it false? Use your data to support your answer.

Did your results correspond to the prediction you made? If not, explain how your results are different from your expectations and why this might have occurred.

Describe how your data are supported by information from other sources (for example, textbooks or other lab teams working on a similar problem).

If you had any problems with the procedure or questionable results, explain how they might have influenced your conclusion.

If you had an opportunity to repeat and extend this experiment to make your results more convincing, what would you do?

Summarize the conclusion you have drawn from your results.

Questions for Review

1. What is the importance of cellular respiration to living organisms?

2. Yeast is used to make bread rise. How might this work?

3. Which of the following tubes would you expect to produce the highest initial rate of CO_2 production? Explain your answer.

   Tube 1: 5 mL corn syrup, 10 mL yeast, 5 mL water

   Tube 2: 10 mL corn syrup, 10 mL yeast, 0 mL water

   Tube 3: 0 mL corn syrup, 10 mL yeast, 10 mL water

4. Suppose you do an experiment on aerobic respiration using lima bean mitochondria as described in Exercise 6.2 and the results show a final % transmittance reading of 42% in Tube 1 and 78% in Tube 2. Both tubes had an initial reading of 15%. In which tube did the greatest amount of aerobic respiration occur? Explain your answer in terms of the changes that take place in DCPIP.

Acknowledgment

The procedure used to assay mitochondrial activity was based on a procedure from Succinic Acid Dehydrogenase Activity of Plant Mitochondria in F. Witham, D. Blaydes, and R. Devlin, *Exercises in Plant Physiology*, Boston: Prindle, Weber & Schmidt, 1971.