Controlling the Chemistry of Life

Background:

To start with it is important that you know the following facts:

1) All activity that occurs within an organism is chemical.
2) Life is possible because of controlled chemical reactions.
3) The chemical reactions of life require energy.
4) The energy for life is contained with the organic molecules consumed or made by the organism.
5) In order for the chemical reactions of life to occur, organisms use their stored energy to drive these reactions.

How much energy is needed? We have all learned about the need for energy conservation. In our daily lives we have been made painfully aware of the cost of energy. Many of us strive to conserve energy. Organisms must do the same. Organisms that are the most successful are those that conserve energy, another words, that get the greatest bang for their buck.

Most of us are aware that chemical reactions that take place outside of organisms require a large amount of energy to initiate the reactions and, once initiated, they often proceed in an uncontrolled manner. Did you know that wood is a carbohydrate called cellulose? To prove this, take a piece of paper (made from wood pulp) and test it. Ask your professor for iodine and test the paper for starch. How can you get this carbohydrate to decompose? If your answer is to add a large amount of heat (fire), you are correct. Igniting the paper causes it to decompose into carbon dioxide and water. As this chemical reaction proceeds large amounts of stored energy are released as heat and light energy (fire). To summarize, this decomposition reaction requires high heat energy to start and proceeds in an uncontrolled manner releasing larger amounts of stored energy.

Within organisms, chemical reactions are constantly occurring. The difference between these internal reactions and those that occur in the environment is that within the organism the reactions require far less energy to get started and, once started, proceed in a controlled manner. The reason for this is that living organisms contain thousands of specialized proteins called enzymes. Enzymes lower the energy needed to start a reaction. They also control the rate of the reaction. Enzymes are usually large, specifically shaped protein molecules that bind specific molecules called substrate, and form an enzyme-substrate complex. In this complex the enzyme pressures the substrate molecule to undergo a reaction and produce a product. Figure 1 illustrates an enzyme-controlled reaction.
Enzymes are highly specialized proteins that have specific shapes. The area of the enzyme that holds the substrate is the active site.

The shape of the active site determines the substrate the enzyme will bind. Different enzymes have different active sites. That is why each enzyme is specific for a particular substrate. Environmental factors can alter the shape of the active site and thereby effect the activity of the enzyme. For instances, high heat will distort the shape of an enzyme and thus destroy its activity. When this happens we say that the enzyme has been denatured. A seriously denatured enzyme is one that has been destroyed. It can no longer function as an enzyme.

In this lab you will examine factors that affect the rate of an enzyme-controlled reaction. The enzyme you will use is peroxidase, an enzyme found in many living tissues. Peroxidase breaks down peroxide into water and oxygen. This reaction is illustrated below.

Peroxide $\xrightarrow{\text{peroxidase}}$ water + oxygen gas

$$2\text{H}_2\text{O}_2 \xrightarrow{\text{peroxidase}} 2\text{H}_2\text{O} + 2\text{O}_2$$
Peroxide is a poison produced by living cells during their normal metabolic reactions. Since it is a poison, the cells, which contain peroxidase, destroy the peroxide as illustrated above. The peroxide starts to bubble as it changes to oxygen gas and water. Can you explain why we put peroxide on a cut or abrasion? Think about what we are trying to kill.

In this experiment you will use the enzyme peroxidase found in a turnip. Your professor has mashed up a turnip and filtered it through a coffee filter. The fluid that passes through the filter contains the enzyme peroxidase. You will now test the peroxidase to see what factors will effect the rate of the enzyme-controlled reaction. You will measure the reaction by measuring the amount of oxygen gas released when peroxide decomposes.

Procedure:

A. **The effect of temperature on an enzyme controlled reaction.**
   In this part of the experiment you try to determine how temperature affects the rate of an enzyme-controlled reaction.
   1. Prepare three water baths. One should be at room temperature, one should be very cold (use ice) and the third should be hot to the touch about 40°C Celsius. You can either use hot water from a faucet or your professor will have a supply of hot water for you.
   2. You will begin this experiment by filling three 15 ml tubes with peroxide.
   3. Cap each of the tubes and put one of each into the three water baths.
   4. In table 1 record the temperature of each of your water baths. Leave the tubes in the baths for about 10 minutes to allow the peroxide to reach the temperature of the bath.
   5. Now open one of the tubes and add three drops of the turnip extract to that tube. Quickly add a piece of parafilm tightly to the tube to seal it and poke a hole through the parafilm. Quickly invert the tube and place it back into its water bath. Note the time in table 1. Your tube will remain in the water bath for 30 minutes.
   6. Repeat this procedure with each of the other two tubes.
   7. After 30 minutes measure the amount of oxygen gas that has collected in the tube and record that volume in table 1.

<table>
<thead>
<tr>
<th>water bath</th>
<th>temperature °C</th>
<th>start time</th>
<th>finish time</th>
<th>ml gas produced</th>
<th>rate (ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cold</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>room temp.</td>
<td></td>
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</tr>
<tr>
<td>hot</td>
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</tbody>
</table>
B. The effect of pH on an enzyme controlled reaction

In this part of the experiment you will try to determine the effect pH has on an enzyme controlled reaction.

1. Prepare three 15 ml tubes and one room temperature water bath.
2. To each of the tubes add about 20 drops of one of the buffers. Record the pH of the buffers that you added to table 2.
3. Now fill the tubes with peroxide
4. Add 3 drops of turnip extract to one of the tubes.
5. Quickly seal the tube with parafilm and poke a hole in the parafilm.
6. Quickly invert the tube into a beaker of room temperature water.
7. Note the time on your table.
8. Repeat steps 4-7 for the other two tubes.
9. Leave the tubes in the solution for 30 minutes.
10. After 30 minutes measure the amount of oxygen gas that has collected in the tube and record that volume in table 2.

Table 2. The effect of pH on an enzyme controlled reaction.

<table>
<thead>
<tr>
<th>pH</th>
<th>pH start</th>
<th>finish time</th>
<th>ml gas produced</th>
<th>rate (ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>acidic</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>neutral</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>basic</td>
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</tbody>
</table>

Homework:

1. a. Using Excel, prepare a spreadsheet that shows the relationship between temperature and the rate of the enzyme controlled-reaction.
   b. Describe the effect of temperature on an enzyme-controlled reaction.

2. a. Using Excel, prepare a spreadsheet that shows the relationship between pH and the rate of the enzyme controlled-reaction.
   b. Describe the effect of pH on an enzyme-controlled reaction.

3. A student performed the experiment in Part A, however the student wondered if boiling the enzyme would speed up the experiment. Much to the surprise of the student, no gas was collected at the end of the thirty minutes. The student re-examined the boiled enzyme and found that instead of a clear liquid, the enzyme had tuned into solid flecks.
   a. What effect did the boiling have on the enzyme? Think about the effect of boiling on egg white.
   b. Explain why the boiled tube produced no gas.
4. Why do we become concerned when a person runs a very high fever? Explain your answer in terms of enzyme-controlled reactions.

5. Why is acid rain harmful to organisms living in a lake? Explain your answer in terms of enzyme-controlled reactions.