Enzymes

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Objectives

- Appreciate the sensitivity of enzymatic activity to environmental conditions
- Understand what an optimal condition means for enzymatic activity
- Follow the time course of an enzymatic reaction
- Observe the effect of enzyme concentration on enzymatic reactions

Introduction

Enzymes are a class of proteins which have the amazing ability to speed up or catalyze a reaction between reactants to produce the products. As with all proteins, enzymes are composed of a chain of amino acids, twisted and bent into a specific 3-dimensional (3-D) structure. This structure is due to chemical bonds between atoms of the amino acid's backbone, the amino acid R groups, and with other amino acid chains. Thus, each enzyme with a different sequence of amino acids (its primary structure) will have different twists and bends in its 3-D structure (its secondary, tertiary, and quaternary structure). It is this precise and unique 3-D structure which gives enzymes their catalytic ability and specificity.

The strength of those chemical bonds maintaining the 3-D structure of the enzyme is dependent on environmental conditions. Temperature, pH, ion concentrations, and many other environmental conditions have the potential of weakening these bonds, upsetting the precise 3-D structure of the enzyme. As the 3-D structure of the enzyme is degraded, there is a corresponding loss of catalytic ability by the enzyme. If the catalytic ability of the enzyme has been totally lost, that enzyme is said to have been denatured.

In this exercise, you will be determining the catalytic ability of a hypothetical enzyme at a variety of environmental conditions in an effort to determine those conditions which are optimal for its catalytic ability.

Select the Enzymes simulation in the Cell Biology section on the BiologyOne DVD.

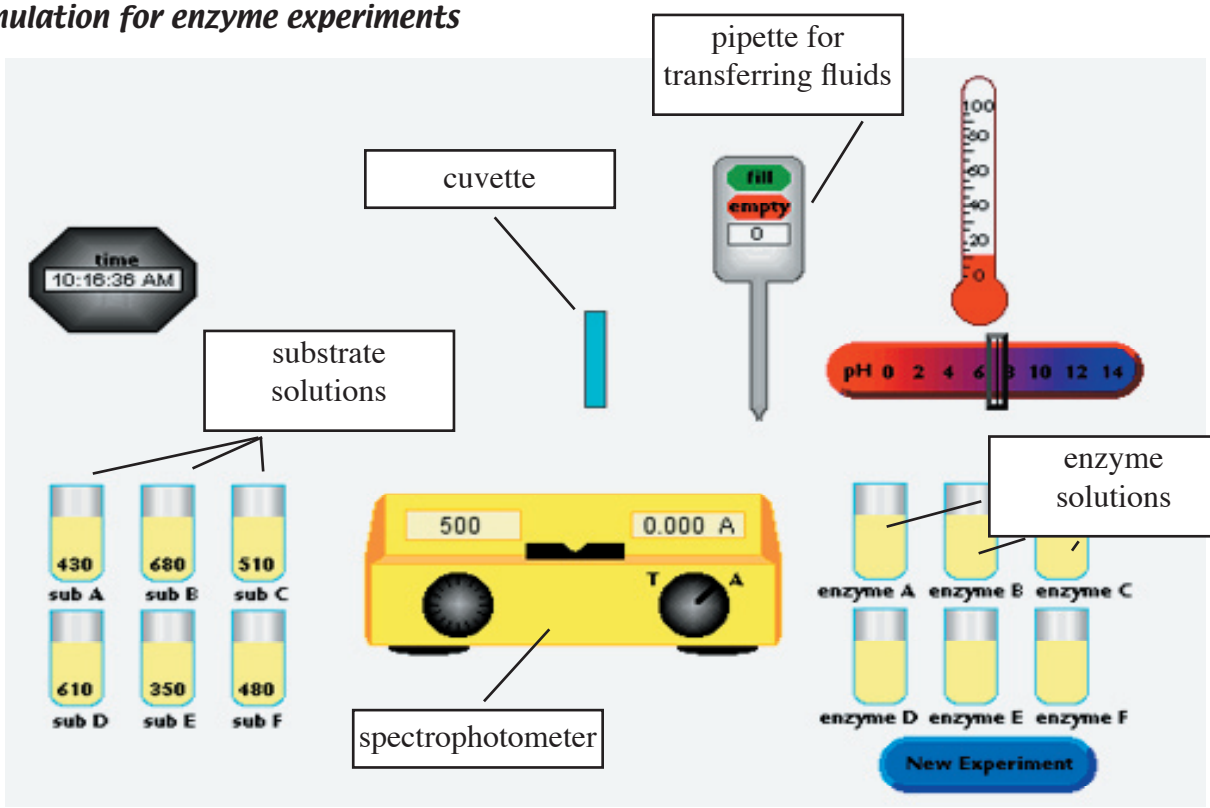
This simulation uses a spectrophotometer to measure the progression of a chemical reaction. Due to its structure, a molecule of a particular type will absorb light energy at particular wavelengths. Different molecules absorb different wavelengths. By measuring the amount of light of a particular wavelength which passes through a solution you can measure the quantity of a particular chemical. The spectrophotometer measures light absorption at specific wavelengths. In this simulation you will be measuring the amount of product produced during the course of an enzymatic reaction.

The 'machine' in the middle of the screen is the spectrophotometer (see diagram below). On the left hand side of the spectrophotometer is a knob and above that, a numerical reading. The left-hand numerical reading is the wavelength of light the machine is measuring. This wavelength can be adjusted by clicking on the knob. Clicking on the left side of the knob lowers the wavelength, clicking on the right side of the knob increases the wavelength. The range of the machine is between 300 and 700 nm.

On the right side of the spectrophotometer is a numerical reading followed by either a 'T' or an 'A'. If the 'T' is present, the reading is the % light transmitted through the sample. If the 'A' is present, the reading is the absorption of light by the sample. Clicking on the knob under this reading will toggle between the T and A readings. You will be measuring the absorption of light by the sample.

At the center of the spectrophotometer is the sample holder represented by a black bar with a notch. This is the sample holder for a special vial called a cuvette. When you start the program, the cuvette is the blue rectangle located above the spectrophotometer. You can move the cuvette around the screen by clicking on it and dragging. When you get in the area of the sample holder, the cuvette will 'pop' into position. You can easily drag the cuvette out of the spectrophotometer.

Simulation for enzyme experiments



Activity 7.1
Optimal pH

To the left of the spectrophotometer are 6 different substrates labeled A through F. On the vial for each substrate is a number which is the wavelength setting you should use to measure the product formed from that substrate.

To the right of the spectrophotometer are 6 different enzymes labeled A through F. These correspond with the substrates A through F (enzyme A reacts with substrate A, etc.).

Above the vials of substrates is a clock which you will use in following the progress of the reaction. Above the enzyme vials are sliding scales you can use to set the temperature and pH at which the reaction is occurring.

At the start of the simulation, to the right of the cuvette is the pipette to transfer solutions from their vials to the cuvette. The numerical value shown here represents the ml of solution within the pipette.

Finally, in the lower right corner is a blue button which will clean the cuvette and pipette so that you can conduct another experiment.

1. Select one of the substrate/enzyme pairs you wish to investigate. Set the wavelength on the spectrophotometer to the value indicated on the substrate vial. Set the spectrophotometer to read light absorption. Record these in the Results Section.

2. Set the temperature to 25 degrees. Set the pH to 2.

3. Using the pipette, transfer 25 ml of substrate into the cuvette.

4. Be ready to record your data before you begin the next step. Complete this next step at a time on the clock which is convenient to determine when 1 minute has passed.

5. Using the pipette, transfer 5 ml of enzyme into the cuvette. As quickly as possible, move the cuvette into the sample holder of the spectrophotometer. Note the time in the Results section.

6. At the 1 minute mark, record the absorption value.

7. Start a new experiment by removing the cuvette from the spectrophotometer and clicking on the ‘New Experiment’ button.

8. Set the pH to 4 and repeat steps 3 through 7. Continue this experiment so you have readings for pH values of 2, 4, 6, 8, and 10.

9. Graph the data putting the pH value on the x axis and the absorption value on the y axis as shown on the graph in the Results Section. Draw a smooth curve through your data points. The peak of this curve represents the optimal pH value for this enzyme. (You may wish to collect data for pH values between those collected above to get a more precise peak or curve. Add those to your graph.)

Activity 7.2
Optimal Temperature

1. Using the same substrate/enzyme pair, be sure the wavelength on the spectrophotometer is set to the correct wavelength.

2. Set the pH to the value you determined to be optimal in Part 1. Set the temperature to 10 degrees.

3. Using the pipette, transfer 25 ml of substrate into the cuvette.

4. Be ready to record your data in the Results Section before you begin the next step. Complete this next step at a time on the clock which is convenient to determine when 1 minute has passed.

5. Using the pipette, transfer 5 ml of enzyme into the cuvette. As quickly as possible, move the cuvette into the sample holder of the spectrophotometer. Record the time.

6. At the 1 minute mark, record the absorption value.

7. Start a new experiment by removing the cuvette from the spectrophotometer and clicking on the 'New Experiment' button.

8. Set the temperature to 20 and repeat steps 3 through 7. Continue this experiment so you have readings for temperature values from 10, 20, 30, 40, 50, 60, 70, and 80.

9. Graph the data putting the temperature value on the x axis and the absorption value on the y axis. Draw a smooth curve through your data points. The peak of this curve represents the optimal temperature value for this enzyme. (You may wish to collect data for temperature values between those collected above. Add those to your graph.)

Activity 7.3
Reaction Rates

1. Using the same substrate/enzyme pair as above, set the wavelength to the correct setting, set the pH to the optimal pH you found in experiment 1, and set the temperature to the optimal temperature you found in experiment 2.

2. Using the pipette, transfer 25 ml of substrate into the cuvette.

3. Be ready to record your data before you begin your next step. Complete the next step as quickly as possible at a time which is convenient to monitor 10 second intervals.

4. Using the pipette, transfer 5 ml of enzyme into the cuvette. As quickly as possible, move the cuvette into the sample holder of the spectrophotometer. Note the time and record the absorption value. This is your absorption at time '0'.

5. At 10 second intervals, record the absorption value. Continue collecting readings until you have readings through 2 minutes.

6. Graph the data by putting time on the x axis and absorption on the y axis. Draw the best fitting straight line through your data points.

7. Repeat steps 2 through 5 but add 15 ml enzyme into the cuvette instead of 5.

8. Plot this new data on the graph you created in step 6. Visually compare the slope of the lines. Which reaction occurred at a faster rate?

Lab Exercise 7

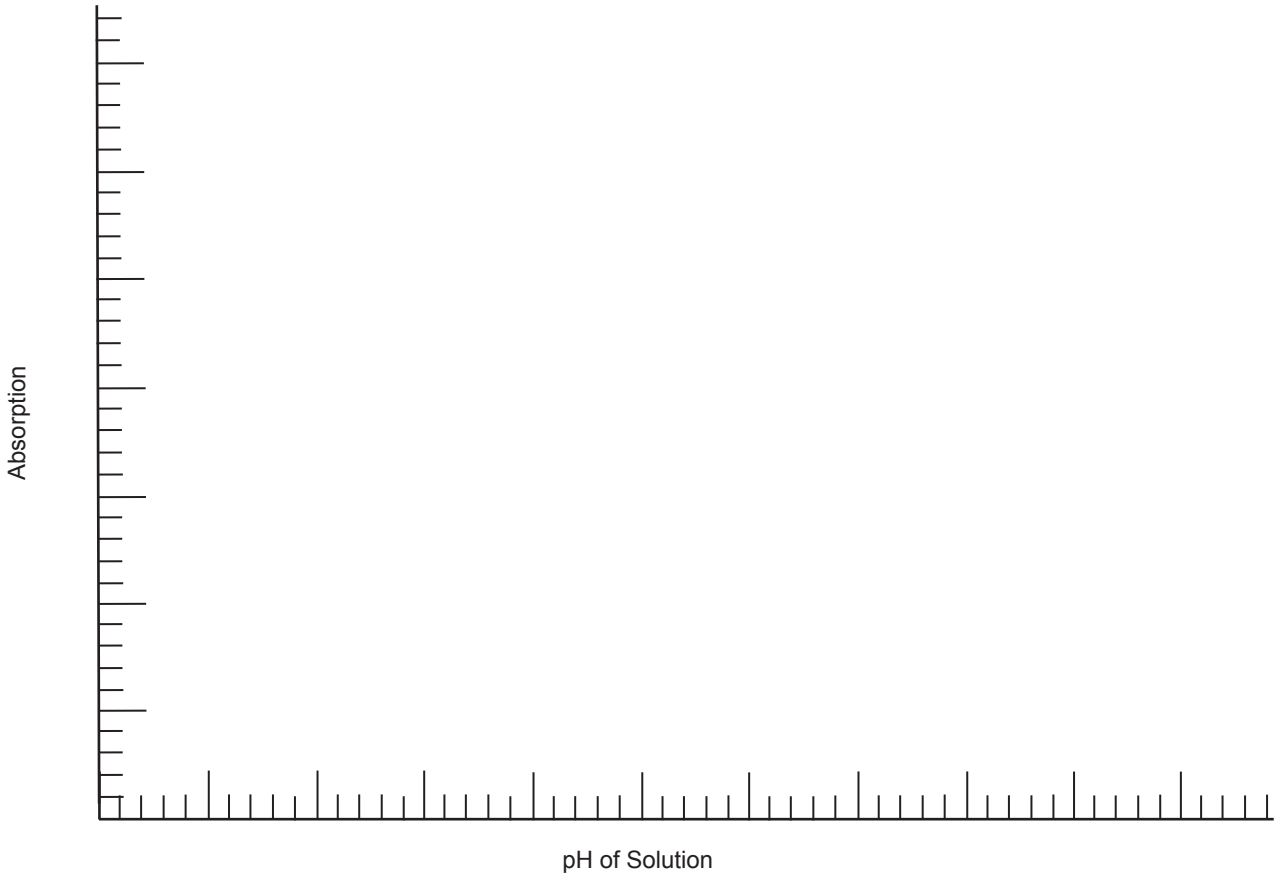
Name _____

Results Section

Activity 7.1
Optimal pH

Enzyme/Substrate Pair Tested: _____

Experimental pH	Start time	Finish time	Absorbance at 1 min.
2	_____	_____	_____
4	_____	_____	_____
6	_____	_____	_____
8	_____	_____	_____
10	_____	_____	_____

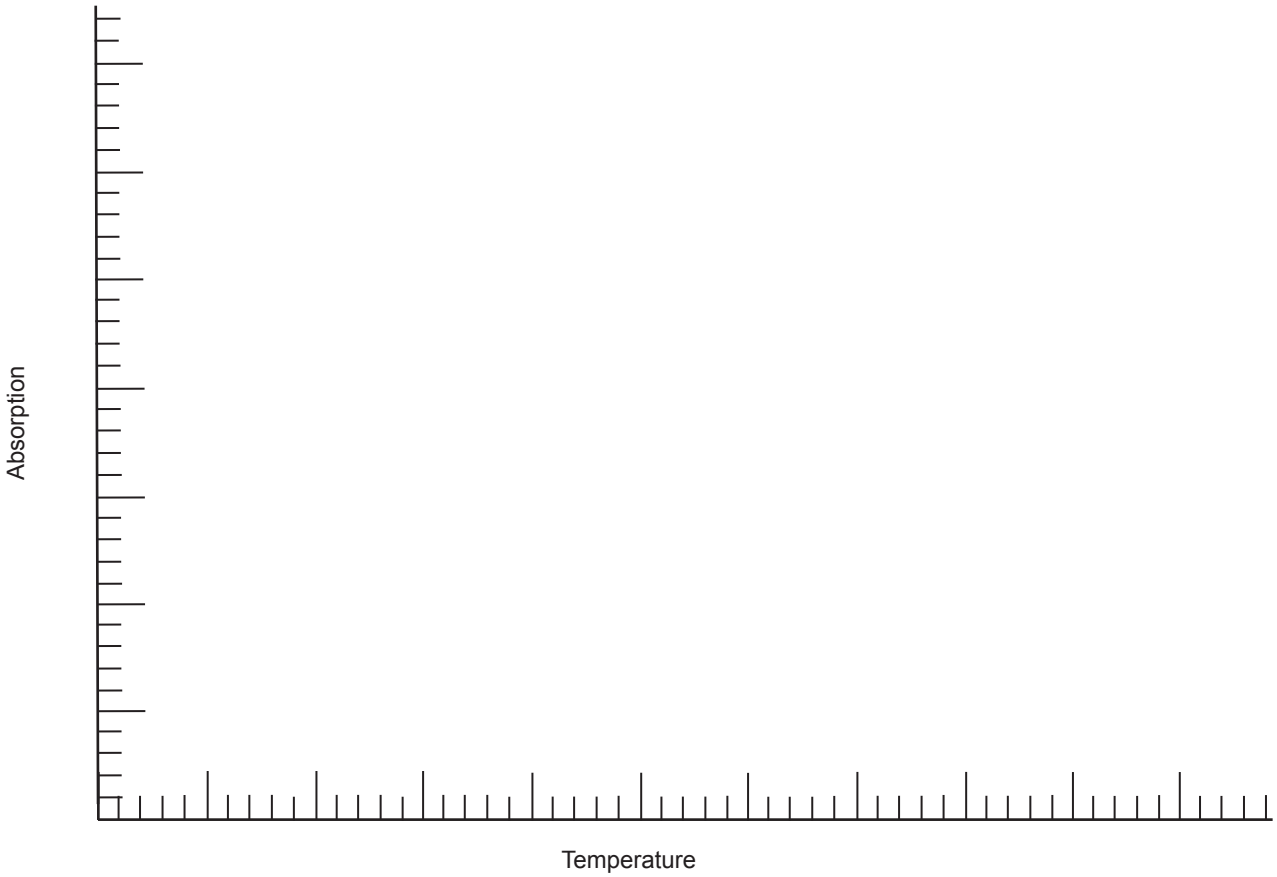


Based on results, estimate of optimal pH is _____

Activity 7.2
Optimal Temperature

Enzyme/Substrate Pair Tested: _____

Experimental Temp.	Start time	Finish time	Absorbance at 1 min.
10	_____	_____	_____
20	_____	_____	_____
30	_____	_____	_____
40	_____	_____	_____
50	_____	_____	_____
60	_____	_____	_____
70	_____	_____	_____
80	_____	_____	_____

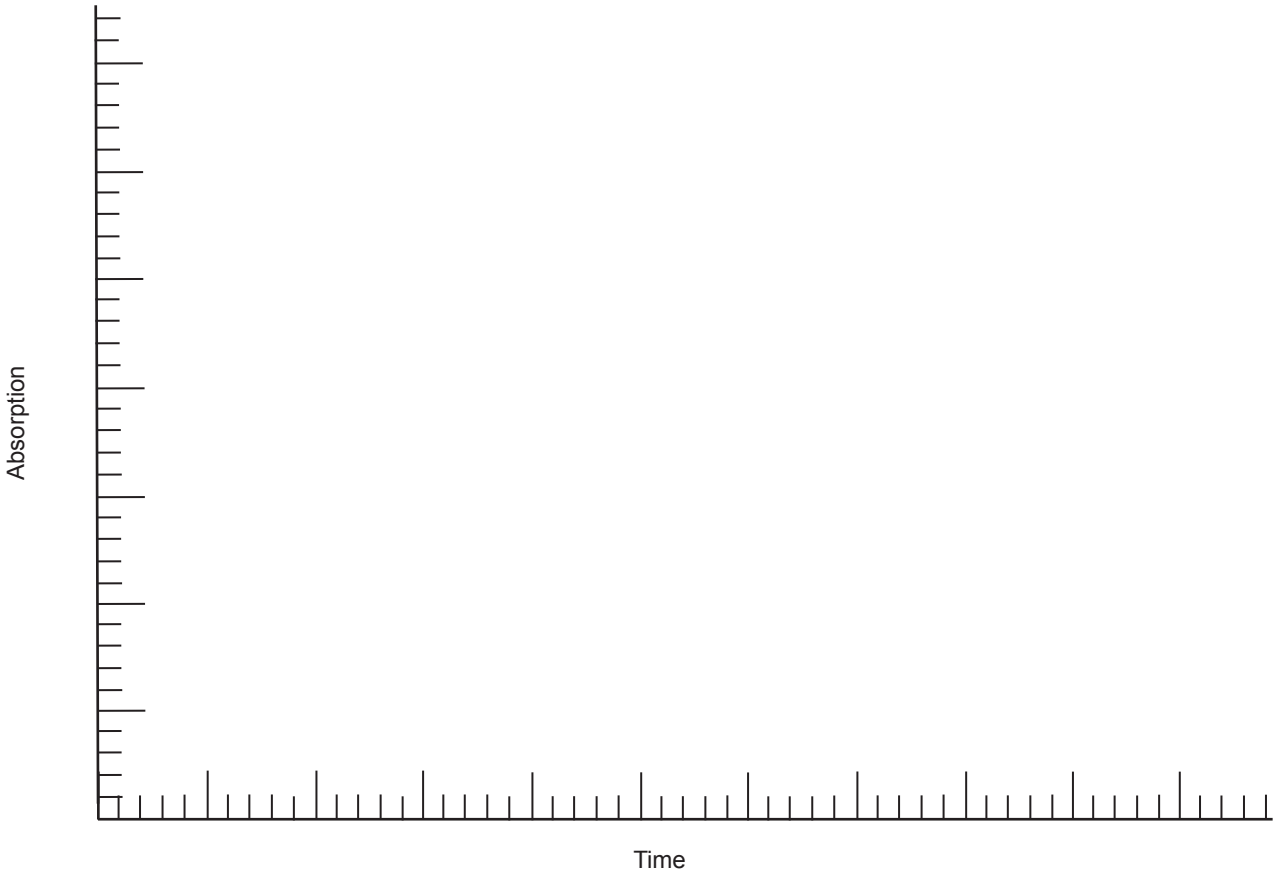


Based on results, estimate of optimal Temperature is _____

Activity 7.3
Reaction Rates

Enzyme/Substrate Pair Tested: _____

Time	5 ml enzyme	15 ml enzyme
:10	_____	_____
:20	_____	_____
:30	_____	_____
:40	_____	_____
:50	_____	_____
1:00	_____	_____
1:10	_____	_____
1:20	_____	_____
1:30	_____	_____
1:40	_____	_____
1:50	_____	_____
2:00	_____	_____



How does the concentration of enzyme influence the rate of reaction? Why do you think this occurs?