Amylase Enzyme

a) Enzymes

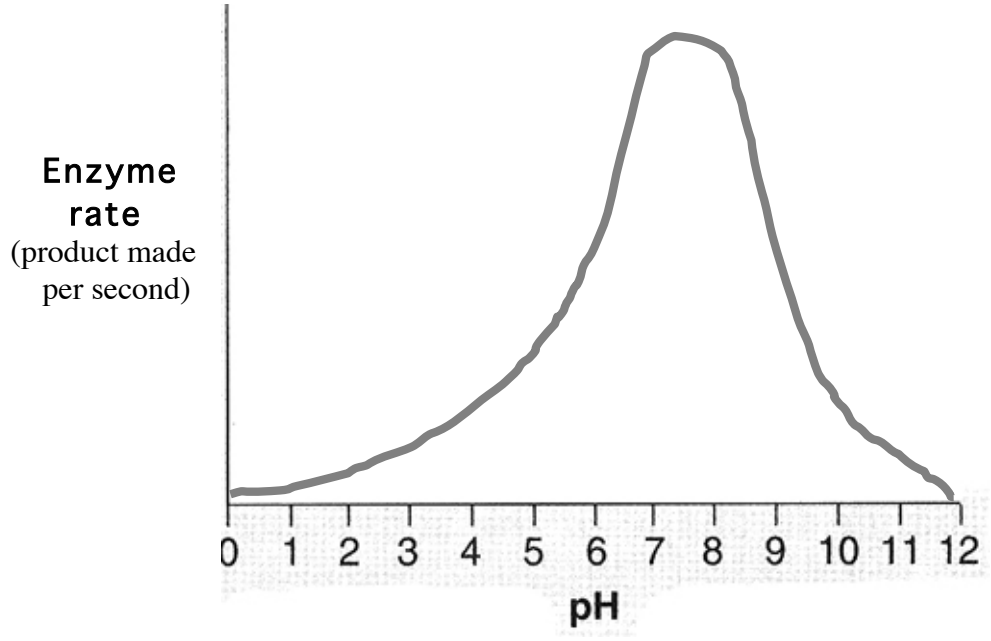
Enzymes, which are a type of protein, are found in all living things. What do enzymes do? An enzyme's job is to change molecules. An enzyme may add atoms to a molecule, remove atoms from a molecule, split a large molecule into two smaller molecules, or join together smaller molecules to form a larger molecule. But the important point is that enzymes always change molecules. The term "chemical reaction" means a change in a molecule, so the best way to define enzymes is to say **enzymes are proteins that carry out chemical reactions.**

Enzymes work in this way: They begin by binding to the molecule that they are going to change (called the **substrate** molecule). Each enzyme has a special crevice, called its **active site**, where it binds its substrate molecule. Once the substrate is in the active site, the enzyme changes it and then releases it. The changed substrate is called the **product**.

b) Optima of enzymes

The speed at which an enzyme converts substrate to product is called the **rate** of the enzyme. For example, if an enzyme converts 40 micrograms of substrate into 40 micrograms of product in one second, we say that the enzyme's rate is 40 micrograms product per second.

Biologists have found that the temperature and pH of the enzyme's environment can change its rate. For example, the graph below shows the different rates at different pH's for a certain enzyme.



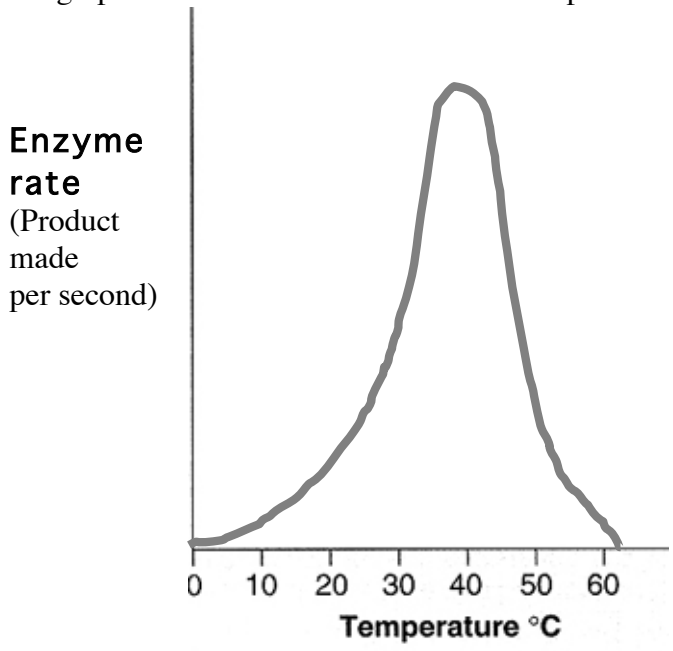
Notice that this enzyme's fastest rate occurs at pH 8. This is called the "optimum pH" of the enzyme. Not all enzymes have a pH optimum at 8, but usually an enzyme's optima

are the same as its natural environment. For example, enzymes that are found in the stomach (an acidic environment) have optima at acidic pH's (usually pH 2 or 3).

Why does the enzyme's rate decrease if it is not at its pH optimum? The answer is that the enzyme becomes **denatured** (unfolded). Denaturing an enzyme disrupts its active site, and therefore the enzyme loses the ability to bind (and change) its substrate.

Enzyme Denatured enzyme (not functional)

The pH is not the only environmental factor that can change an enzyme's rate. The temperature also has an effect. The effect of temperature on a certain enzyme is shown on the graph below. Notice that there is a "temperature optimum" (a temperature where the enzyme has its highest rate). Usually this is the temperature of the enzyme's natural environment. For example, most enzymes in the human body have a temperature optimum at 37 ºC because that is body temperature.

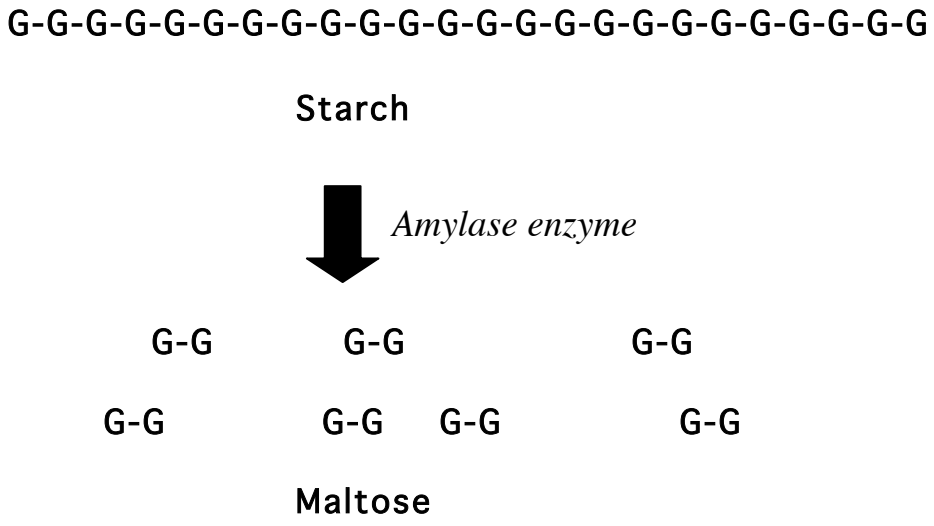


Why does the enzyme rate decrease when its temperature is above its optimum? For the same reason explained for pH: The enzyme is denatured. In other words, high temperatures unfold enzymes.

Why does the enzyme rate decrease when its temperature is below its temperature optimum? You might think at first that the enzyme is denatured, but that is not correct. The reason for the rate decrease is that molecules move slower at colder temperatures. You can think of the enzyme molecule and its substrate molecules going into "slow motion" at colder temperatures. They are not denatured, they are simply slowed.

c) Amylase enzyme

In today's laboratory exercise you will find the pH and temperature optima of an enzyme called amylase, which is a starch-digesting enzyme. Recall that starch is a polysaccharide molecule made of hundreds of glucose sugars linked together. In the diagram of starch below, each G represents a glucose sugar. Amylase enzyme digests starch by breaking it into the disaccharide maltose.



Many different organisms, from bacteria to human beings, make amylase enzyme. In humans, it is made in the salivary glands and the pancreas.

As part of today's experiment, you will find the rate at which amylase enzyme digests starch. You will do this by mixing amylase and starch in a test tube, and then timing how long it takes for the enzyme to completely digest the starch. How can you tell when all the starch is gone? Recall from the Carbohydrates laboratory the iodine test for starch. To perform the test, the iodine solution is mixed with a substance that might contain starch. If no starch is present, the iodine solution remains its original color (yellow). If a large amount of starch is present, the iodine solution turns a black color. Colors between yellow and black indicate various amounts of starch:

Yellow *No Starch*	**gray-yellow** *Very little starch*	**gray** *Some starch*	**blue** *More starch*	**black** *Very much starch*

When you mix the amylase and the starch together, at first the iodine test will show a black color because the enzyme has not had time yet to digest the starch. The more time the enzyme has, the more starch it will digest, so the less dark the iodine test will show. When the iodine test shows yellow color, it means the enzyme has completely digested all the starch. Note that if the color is orange or light orange-brown, that is the same as yellow (no starch).

d) The experimental controls

Remember that controls are the parts of an experiment that show that the experimental procedure is working correctly. A positive control shows that your experiment gives a positive result when the thing that you are detecting is present, and a negative control shows that your experiment gives a negative result when the thing that you are detecting is absent.

1) Obtain a plastic spot plate. Put it on a white piece of scratch paper to see it better. In two of the wells, put a drop of iodine. Also, obtain a flask containing 20 ml of starch solution. Put a clean pipette into the starch flask. From here on, this pipette should only touch the starch solution.

An important note about pipettes: In this experiment, the plastic pipettes will be used many times so it is vital that they do not become contaminated. Follow these two rules:

a) Use the pipette only for the solution that it first touches. For example, the starch pipette should only go into the flask with the starch.

b) When delivering solution using the pipette, make sure the pipette doesn't touch the test tube that it is delivering the solution to. Do this by holding the pipette above the test tube it is delivering into.

2) To the first well, add one drop of the starch to the iodine drop. It should turn black. This is the experiment's positive control.

3) In the second well, add a drop of deionized water (from a beaker on the front desk) to the iodine drop. It should remain yellow. This is the experiment's negative control.

4) If the wells were the correct colors described above, record their colors in the table on page 7, then wash out the spot plate with water.

e) The effect of pH on amylase rate

1) Add three drops of iodine to each well in your group's spot plate's twelve wells.

2) Shake the amylase plastic test tube to resuspend the white amylase pellet at the bottom. Obtain a glass test tube. Add 1 ml of pH 7 buffer. The top mark on the side of the plastic pipettes is the 1 ml mark. enzyme. If you are not sure which mark on the pipette is the 1 ml mark, ask your instructor. Now add 1 drop of amylase.

3) Now add 1 ml of the starch into the test tube. Start a timer as soon as you add the starch to the test tube. Be sure that the starch pipette does not touch the test tube. Mix the tube contents thoroughly by flicking the bottom of the tube.

4) Exactly 30 seconds after you added the starch, use the new plastic dropper to transfer one drop from the test tube to the first well of iodine on the spot plate. Gently shake the plate to mix the drop with the iodine in the well. The well should turn black or blue or gray (showing that starch is still present in your test tube).

5) Repeat step (4) every 30 seconds until the well stays yellow or brown-yellow (showing that the enzyme has completely digested all the starch), or until you run out of the twelve wells. In other words, stop the experiment if the wells are still blue but you run out of wells.

6) In the table on page 7, record the number of seconds it took the enzyme to completely digest the starch. If the starch was never completely digested (in other words, if all 12 wells remained blue) then enter 10,000 seconds as the time of the digestion.

7) Wash out the spot plate. Get a new glass test tube, and a new plastic dropper. Repeat steps 1 – 6 but use the pH 4 buffer instead of pH 7.

9) Wash out the spot plate. Get a new glass test tube, and a new plastic dropper. Repeat steps 1 – 7 but use the pH 9 buffer. **Show your instructor your results before continuing.**

f) The effect of temperature on amylase rate

1) Add three drops of iodine to each of the spot plate's twelve wells.

2) Get a new glass test tube. Add 1 ml of pH 7 buffer and 1 drop of Amylase. Place the test tube in ice water for 5 minutes.

3) Now add 1 ml of the starch into the test tube. Start a timer as soon as you add the starch to the test tube. Be sure that the starch pipette does not touch the test tube. Mix the tube contents thoroughly by flicking the bottom of the tube. but return the test tube to its bath while you run the experiment. In other words, **you run this whole experiment in ice water.**

4) Exactly 30 seconds after you added the starch, use the new dropper to transfer one drop from the test tube to a well of iodine on the spot plate. The well should turn black or blue or gray (showing that starch is still present).

5) Repeat step (4) every 30 seconds until the well stays yellow or brown-yellow (showing that the enzyme has completely digested all the starch), or until you run out of wells. In other words, stop the experiment if the wells are still blue but you run out of wells.

6) In the table on page 7, record the number of seconds it took the enzyme to completely digest the starch. (Put 10,000 seconds if the wells never turned yellow). Also, record the temperature of the bath.

7) Wash out the spot plate. Get a new glass test tube, and a new plastic dropper. Repeat steps 1 – 6 but use a 37 degree water bath instead of ice-water. **In other words, you run the whole experiment at 37 degrees.**

8) Wash out the spot plate. Get a new glass test tube, and a new plastic dropper. Repeat steps 1 – 6 but use a boiling water bath instead of ice-water. **In other words, you run the whole experiment at boiling temperature.**

Show your instructor your results before continuing.

g) The effect of temperature changes on amylase enzyme

1) Add three drops of iodine to each of the spot plate's twelve wells.

2) Obtain a test tube. Add 1 ml of pH 7 buffer and one drop of amylase. Place the test tube in a boiling water bath for 5 minutes, then place it in the 37 degree water bath for one minute. (This will shift the enzyme to 37 degrees). You will keep this test tube at 37 degrees for the rest of the experiment. In other words, **the rest of this experiment is run at 37 degrees.**

3) Now add 1 ml of the starch into the test tube. Start a timer as soon as you add the starch to the test tube. Be sure that the starch pipette does not touch the test tube. Mix the tube contents thoroughly by flicking the bottom of the tube. but return the test tube to its bath while you run the experiment.

4) Exactly 30 seconds after you added the starch, use the new plastic dropper to transfer one drop from the test tube to a well of iodine on the spot plate. The well should turn black or blue or gray (showing that starch is still present).

5) Repeat step (4) every 30 seconds until the well stays yellow or brown-yellow (showing that the enzyme has completely digested all the starch), or until you run out of wells.

6) In the table on page 7, record the number of seconds it took the enzyme to completely digest the starch. (Record 10,000 seconds if the wells never turned yellow).

7) Wash out the spot plate. Get a new glass test tube, and a new plastic dropper. Repeat steps 1 – 6, but use an ice water bath instead of the boiling water. In other words, you put the tube with amylase in ice water for 5 minutes, then return it to 37 degrees, then you run the rest of the experiment at 37 degrees.

8) When done, wash the spot plate and put it back where you obtained it. The pipettes can be disposed of in the trash. The test tube contents can be dumped down the sink and then the tubes can go into the GLASS WASTE BOX (**not** the trash).

Show your instructor your results before continuing.

Results tables

Section (d)

Color of iodine mixed with starch: _____
Color of iodine mixed with water: _____

Section (e)

Time for amylase to digest starch at pH 4: _____ seconds
Rate: _____ ug starch per second

Time for amylase to digest starch at pH 7: _____ seconds
Rate: _____ ug starch per second

Time for amylase to digest starch at pH 9: _____ seconds
Rate: _____ ug starch per second

Section (f)

Time for amylase to digest starch in ice water: _____ seconds
(Temperature = _____ degrees C) Rate: _____ ug starch per second

Time for amylase to digest starch at room temperature: _____ seconds
Temperature = _____ degrees C) Rate: _____ ug starch per second
(for the room temperature, use your pH 7 data from section (e), above).

Time for amylase to digest starch at 37 degrees C: _____ seconds
(Temperature = _____ degrees C) Rate: _____ ug starch per second

Time for amylase to digest starch in boiling water: _____ seconds
(Temperature = _____ degrees C) Rate: _____ ug starch per second

Section (g)

Time for amylase to digest starch at 37 degrees, after amylase was in boiling water for five minutes: _____ seconds

Time for amylase to digest starch at 37 degrees, after amylase was in ice water for five minutes: _____ seconds

$$\text{Enzyme rate} = \frac{\text{Amount of substrate converted to product}}{\text{seconds}}$$

The volume of substrate (which is starch) you put into each tube was 1 ml. But at the starch concentration that was used in this experiment, one ml is equal to 10,000 micrograms of starch. This is the amount of substrate the enzyme converted to product. The seconds it took for the amylase enzyme to do this are the seconds you recorded for each tube in the results table.

h) Graphing and analysis of data

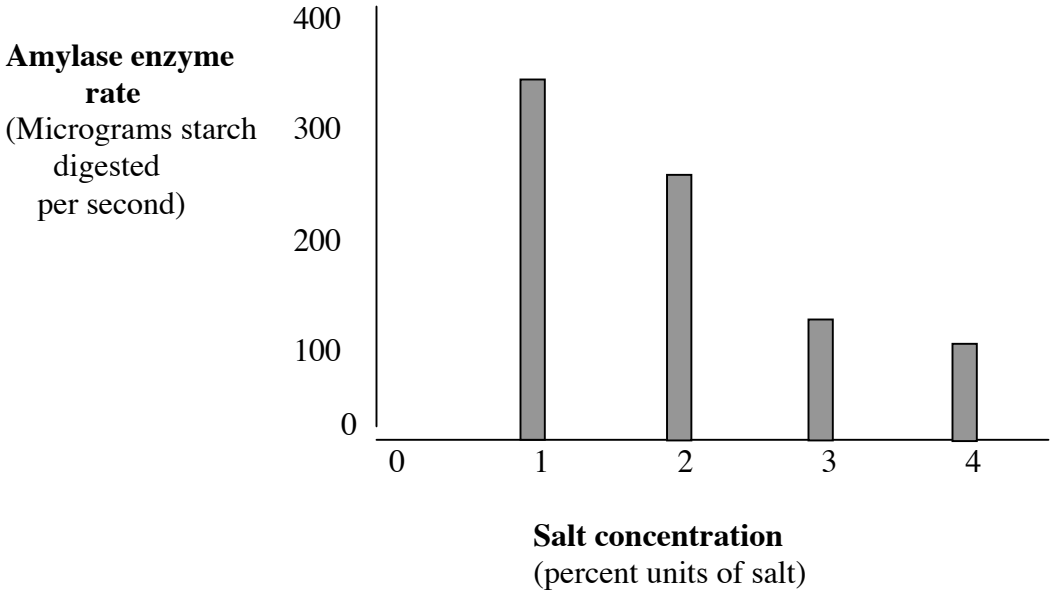
Now that you have your data, it's time to graph it and see what the temperature and pH optima of amylase enzyme are.

Make two graphs n the graph paper at the end of this handout. The first graph is amylase enzyme rate vs. pH. The second graph is amylase enzyme rate vs. temperature. When you make the graphs, be sure to do the following:

(a) Each axis must be labeled so that the reader knows what data is on that axis

(b) Each axis must state the units of the data

If you are not sure how to properly make your graphs, please ask your instructor for assistance. To help you make your graphs correctly, a sample graph is shown below. This graph is from a hypothetical experiment where the salt concentration of the enzyme's environment was changed. **Show your instructor your graph when completed.**



i) Review questions

a) Answer the following questions about amylase enzyme:
What is its substrate? _____
What is its product? _____
What is its pH optimum? _____
What is its temperature optimum? _____

b) Judging from its pH optimum, do you think amylase enzyme digests starch in the stomach (a very acidic organ) or in the intestines (which are neutral in pH). Justify your answer:

c) Explain why the enzyme's rate decreased at pH 9 compared to pH 7. What exactly changed (in terms of the enzyme and substrate) that caused the rate to decrease?

d) Explain why the enzyme's rate decreased at the highest temperature. What exactly changed (in terms of the enzyme and substrate) that caused the rate to decrease?

e) Explain why the enzyme's rate decreased at the lowest temperature. What exactly changed (in terms of the enzyme and substrate) that caused the rate to decrease?

f) In the last part of the experiment, you put one amylase sample in ice water and then returned it to 37 degrees. You put another amylase sample in boiling water and then returned it to 37 degrees. Which of the two samples was able to regain the function of the enzyme? _____

Explain, at a molecular level, why one amylase sample was able to regain function and one was not.

g) If you had put the amylase at pH 9 for five minutes, then returned it to pH 7, do you think it would have regained the function of an enzyme at pH 7? Why or why not?

h) Define "Denature" in regards to enzymes.

i) Judging from your results, especially section (g), do you think enzymes can refold themselves after being denatured? Justify your answer.

j) State the exact purpose of the two control wells in section (d).