Amylase Enzyme

a) Enzymes

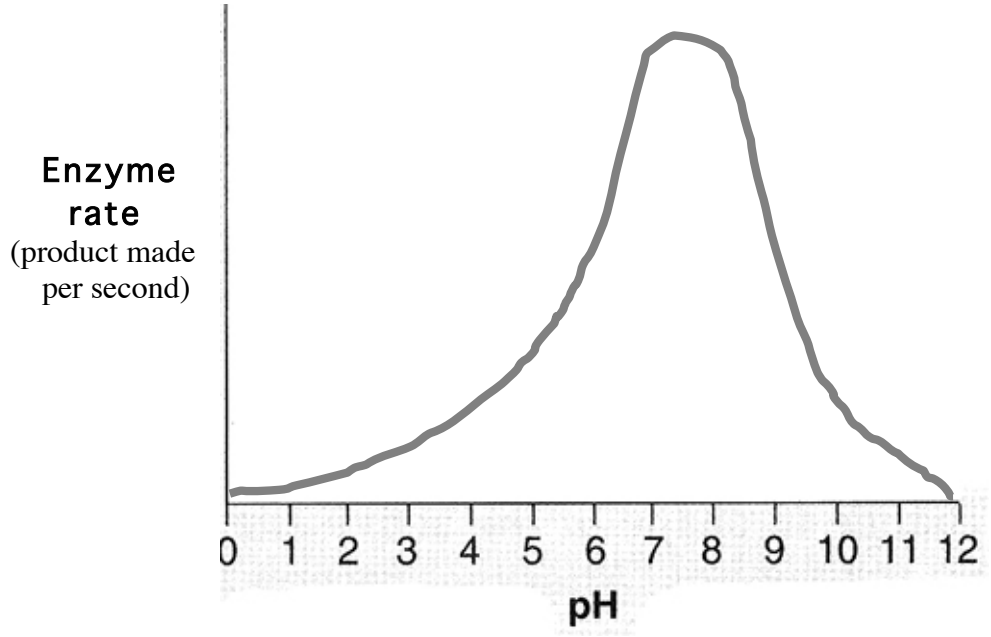
Enzymes, which are a type of protein, are found in all living things. What do enzymes do? An enzyme's job is to change molecules. An enzyme may add atoms to a molecule, remove atoms from a molecule, split a large molecule into two smaller molecules, or join together smaller molecules to form a larger 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 (**not** amylase).



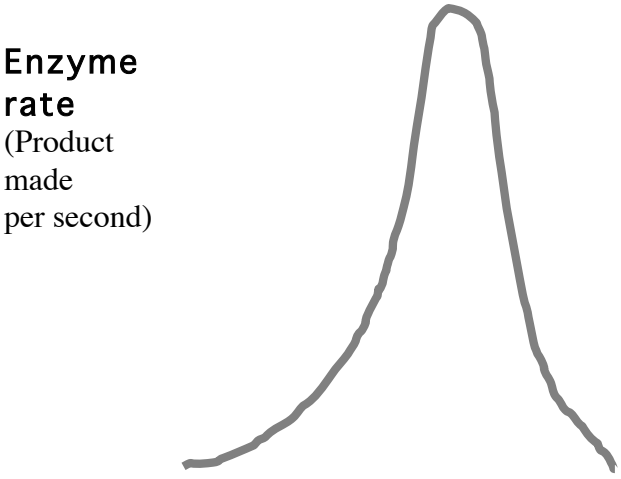
Notice that this enzyme's fastest rate occurs at pH 8. This is called the "optimum pH" of the enzyme. Not all enzymes have a pH optimum at 8, but usually an enzyme's optima

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 (**not** amylase) 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.

G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G-G

Starch

Amylase enzyme

G-G G-G G-G

G-G G-G G-G G-G

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/orange	**red-purple**	**purple**	**blue**	**dark blue**
No Starch	*Very little starch*	*Some starch*	*Much starch*	*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) A note about avoiding cross-contamination of the solutions by the pipettees

In this experiment, you will use several plastic pipettes to pipet several solutions. It is important that the pipettes do not cross-contaminate the solutions. Follow these two rules:

a) Use each 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 a 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.

e) The effect of pH on amylase rate

1) Obtain a spot plate and add four drops of iodine to each well in your group's spot plate's twelve wells. Put the plate on a white piece of paper to see the colors better.

2) 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. If you are not sure which mark on the pipette is the 1 mL mark, ask your instructor.

Using a separate pipette, add 1 mL of the starch into the glass test tube. Be sure that the starch pipette does not touch the glass test tube

3) Shake the amylase plastic test tube to resuspend any white pellet at the bottom. Now add 10 drops of amylase to the glass test tube.

Start a timer as soon as you add the amylase to the glass test tube. Mix the tube contents thoroughly by flicking the bottom of the tube.

4) Exactly 30 seconds after you added the amylase, use the new plastic dropper to transfer three drops 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 has no hint of blue color. No hint of blue shows that the enzyme completely digested all the starch (see the color table on page 3). If the wells still have some hint of blue after 6 minutes (after all twelve wells) you can stop taking samples and end the experiment.

6) In the table on page 9, 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 had 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.

If you are in one of the Right-side groups of the lab, repeat steps 1 - 6 but use pH 4 buffer instead of pH 7 buffer.

If you are in one of the Left-side groups of the lab, repeat steps 1 - 6 but use pH 9 buffer instead of pH 7 buffer.

When you have entered your pH data into the data results table on page 9, show your results to your instructor. Your instructor will then give you the results for the pH that your group didn't do. Copy that pH result into your results table.

f) The effects of cold and body temperatures on amylase rate

Do this activity only if you are in one of the Right-side groups of the lab. If you are a Left-side lab group, do activity (g) instead. But both groups should give their data to the instructor when the activity is finished.

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

2) Obtain a new glass test tube. Add 1 mL of pH 7 buffer and 10 drops of amylase.

Pack ice solidly into an empty beaker then add some water to make ice water.

Place the glass test tube into the ice water (0°) bath for 3 minutes. You will keep this test tube at 0 degrees for the rest of the experiment. In other words, **the glass test tube remains in the 0° in water bath for the rest of this experiment.**

3) Now add 1 mL of the starch into the glass 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 glass test tube. Mix the glass test tube contents thoroughly by flicking the bottom of the tube, then return the glass test tube to its ice water 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 glass 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) Staying at the ice water bath, repeat step (4) every 30 seconds until the well has no hint of blue color. No hint of blue shows that the enzyme completely digested all the starch (see the color table on page 3). If the wells still have some hint of blue after 6 minutes (after all twelve wells) you can stop taking samples and end the experiment.

6) In the results table on page 9, 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. Take the spot plate and all of your lab solutions to the 37˚ water bath on the countertop. Staying at the 37˚ water bath, repeat steps 1 – 6 but use the 37˚ degree (body temperature) water bath instead of ice-water. **In other words, the glass test tube stays in the 37˚ water bath as you run the whole experiment.**

When you have entered your 0˚ degree and your 37˚ degree temperature data into the data results table, show your results to your instructor. Your instructor will then give you the results for the boiling (100˚ degree) experiment that your group didn't do. Copy that 100˚ degree data into your table.

g) The effects of boiling temperature on amylase rate

Do this activity only if you are in one of the Left-side groups of the lab. If you are in a Right-side lab group, do activity (f) instead. But both groups should give their data to the instructor when the activity is finished.

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

2) Obtain a new glass test tube. Add 1 mL of pH 7 buffer and 10 drops of amylase. Place the test tube in a boiling water (100˚) bath for 3 minutes. You will keep this test tube at 100 degrees for the rest of the experiment. In other words, **bring your spot plate and your lab solutions to the 100˚ water bath and keep the glass test tube in the 100˚ water bath for the rest of this experiment.**

3) Now add 1 mL of the starch into the glass 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 glass test tube. Mix the tube contents thoroughly by flicking the bottom of the tube, then return the test tube to its boiling 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 glass 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) Staying at the boiling water bath, repeat step (4) every 30 seconds until the well has no hint of blue color. No hint of blue shows that the enzyme completely digested all the starch (see the color table on page 3). If the wells still have some hint of blue after 6 minutes (after all twelve wells) you can stop taking samples and end the experiment.

6) In the results table on page 9, 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.

When you have entered your 100˚ degree temperature data into the data results table, show your results to your instructor. Your instructor will then give you the results for the ice water (0˚ degree) and body temperature (37˚ degree) experiments that your group didn't do. Copy those two temperature data into your table.

h) The effect of a 0˚ to 37˚ degree temperature change on amylase enzyme

Do this activity only if you are in one of the Right-side groups of the lab. If you are in a Left-side lab group, do activity (i) instead. But both groups should give their data to the instructor when the activity is finished.

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

2) Obtain a new glass test tube. Add 1 mL of pH 7 buffer and 10 drops of amylase. Place the test tube in ice water (0˚) bath for 3 minutes. After 3 minutes, move the glass test tube into the 37˚ degree water bath for one minute. (This will shift the enzyme from 0˚ to 37˚ degrees). You will keep this test tube at 37 degrees for the rest of the experiment. In other words, **bring your spot plate and your lab solutions to the 37˚ water bath and run the rest of this experiment at the 37˚water bath.**

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 glass 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) Staying at the water bath, repeat step (4) every 30 seconds until the well has no hint of blue color. No hint of blue shows that the enzyme completely digested all the starch (see the color table on page 3). If the wells still have some hint of blue after 6 minutes (after all twelve wells) you can stop taking samples and end the experiment.

6) In the data table on page 9, record the number of seconds it took the enzyme to completely digest the starch. (Record 10,000 seconds if the wells never turned yellow).

When you have entered your 0˚ degree →37˚ degree temperature change data into the data results table, show your results to your instructor. Your instructor will then give you the results for the 100˚ degree →37˚ degree temperature change that your group didn't do. Copy that data into your results table.

7) 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).

i) The effect of a 100° to 37° degree temperature change on amylase enzyme

Do this activity only if you are in one of the Left-side groups of the lab. If you are in a Right-side lab group, do activity (h) instead. But both groups should give their data to the instructor when the activity is finished.

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

2) Obtain a new glass test tube. Add 1 ml of pH 7 buffer and 10 drops of amylase. Place the test tube in boiling water (100°) bath for 3 minutes. After 3 minutes, move the glass test tube into the 37° degree water bath for one minute. (This will shift the enzyme from 100° to 37° degrees). You will keep this test tube at 37 degrees for the rest of the experiment. In other words, **bring your spot plate and your lab solutions to the 37° water bath and run the rest of this experiment in the 37° water bath.**

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 glass 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) Staying at the water bath, repeat step (4) every 30 seconds until the well has no hint of blue color. No hint of blue shows that the enzyme completely digested all the starch (see the color table on page 3). If the wells still have some hint of blue after 6 minutes (after all twelve wells) you can stop taking samples and end the experiment.

6) In the data table on page 9, record the number of seconds it took the enzyme to completely digest the starch. (Record 10,000 seconds if the wells never turned yellow).

When you have entered your 100° degree →37° degree temperature change data into the data results table, show your results to your instructor. Your instructor will then give you the results for the 0° degree →37° degree temperature change that your group didn't do. Copy that data into your results table.

7) 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).

Results table

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

Sections (f) and (g)

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

Sections (h) and (i)

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.

j) Graphing and analysis of data

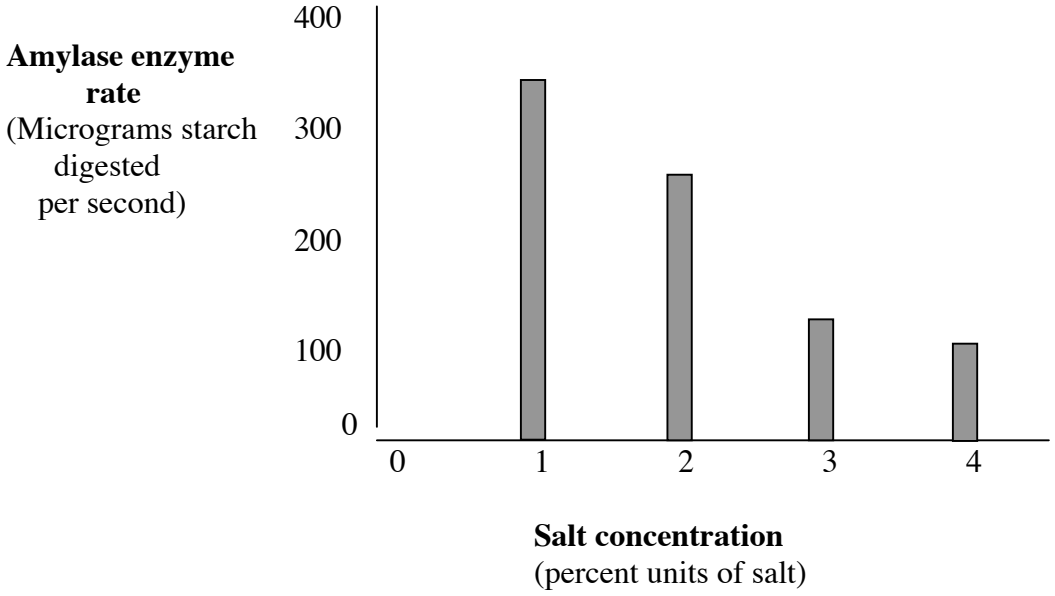
Now that you have your data, it's time to graph it and see what the temperature and pH optima of amylase enzyme are.

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.**



k) 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 as it was defined in this handout.

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

l) Review question answers

a) Starch
Maltose
7
37˚

b) Amylase would digest starch best in the intestines. The experiment shows that amylase's rate of starch digestion is lower at an acidic pH (pH 4) than at neutral pH (pH 7). Therefore there would be better starch digestion in the neutral pH intestines than in the acidic stomach.

c) Enzymes denature (become unfolded) when they are far outside of their optimum pH. The enzyme's optimum pH is pH 7, so at pH 9 the enzyme was denatured. When an enzyme is denatured it becomes unfolded. Without being properly folded, the enzyme's active site (where it binds its substrate molecule) cannot form. Therefore, at pH 9 the enzyme could not bind to its substrate. That is why it had a very low rate at ph 9.

d) The answer is similar to the answer above, but for temperature instead of pH. Enzymes denature (become unfolded) when they are far above their optimum temperature The enzyme's optimum temperature is 37 degrees C, so at 100 degrees C (boiling) the enzyme was denatured. When an enzyme is denatured it becomes unfolded. Without being properly folded, the enzyme's active site (where it binds its substrate molecule) cannot form. Therefore, at 100 degrees C the enzyme could not bind to its substrate. That is why it had a very low rate when boiled.

e) Unlike the previous two answers, the explanation is **not** that the enzyme denatured. Temperatures that are below an enzyme's optimum temperature do not denature the enzyme. In other words, the enzyme does not unfold or lose its active site. But low temperatures do slow the movement of molecules. So at low temperature the enzyme continued to perform its reaction on its substrate molecule but much more slowly than its rate at its optimum temperature.

f) The amylase that was put in ice water was able to regain its normal function (digesting starch) when returned to 37 degrees C. The boiled amylase was not able to regain its formal function when returned to 37 degrees C.

As was described in answer (d) above, boiling an enzyme denatures the enzyme. Denaturing is a permanent change in an enzyme. In other words, returning an enzyme from boiling to its optimum temperature does not "repair" the denatured enzyme.

g) The enzyme at pH 9 would not regain its normal activity if returned to pH 7. As was explained in answer (c) above, the enzyme is denatured at pH 9. And as was explained in answer (f) above, denaturing an enzyme permanently unfolds it. Therefore, returning the pH 9 enzyme to its optimum pH 7 environment would not allow the enzyme to regain its activity.

h) Denature (in regard to enzymes) means that the enzyme is permanently unfolded. It can no longer perform its chemical reaction on its substrate molecule because the unfolding has caused it to lose its active site (where it binds its substrate).

i) Enzymes cannot refold themselves after being denatured. This was shown in activity (i), where the amylase enzyme was denatured by boiling then returned to its optimum temperature of 37 degrees C. The enzyme did not regain activity.