Biology A Name_____ Per _____

Enzyme Simulation Lab: Tubease

Introduction:

Enzymes are proteins that are used as catalysts in biochemical reactions. A **catalyst** is something that controls the rate of a reaction without itself being used up. Often enzymes are used to speed up the rate of a reaction by reacting with a substrate. There are at least two things that can affect the rate of a reaction: 1) **substrate concentration**, and 2) **temperature**. Here is a set of activities designed to simulate how substrate concentration and temperature affect enzyme function.

In those activities that follow:

- one person is the enzyme **TUBEASE,** and his/her fingers are the **ACTIVE SITE**
- the microcentrifuge tubes are the **SUBSTRATE**
- to react with the tubes, the enzyme picks up a tube with one hand and presses the cap in with the other. Press the cap into the tube until it clicks into place.

Prelab:

1. What do you predict will happen in the following parts of the lab?

    a. Part B: The rate will be (greater than/less than/equal to) the rate in Part A. ***Explain.***

    b. Part C: The rate after the ice will be (greater than/less than/equal to) the initial rate. ***Explain.***

    c. Part D: The rate after the denaturing will be (greater than/less than/equal to) the initial rate. ***Explain.***

Procedure Part A: Rate of Product Formation

1. Select 50 tubes and place them in a shallow bowl.

2. In your group, one person will be the timer, one will record the data, one will be the enzyme, tubease, and the other(s) will be a counter. The enzyme is to put the cap on **ALL** the tubes *without looking* and all of the products (capped tubes) must remain in the bowl. Tubes may only be metabolized (capped) once. Only one will fit in the active site at a time.

3. The experiment is conducted in 20 second intervals. The timer calls out start and then marks each 20 second interval. The recorder tallies the **cumulative** number of tubes capped as each interval is announced by the timer. (e.g. if the enzyme caps 10 tubes in the first interval and 8 in the second, the recorder will tally 18 after 40 seconds.) If the enzyme is unable to find substrates to break for 3 consecutive intervals (one minute), you may choose to consider the trial completed.

4. Record your results for Part A in the data table labeled Fig 1 in the results section.

5. Graph the results plotting Total Product Formed (the cumulative number of tubes capped at each 20s interval) vs. Time on a separate sheet of graph paper and attach to the back of this lab packet.

6. Calculate the rate of enzyme action in tubes per second for each 60s interval How many tubes were capped after 1 minute, 2 minutes, 3 minutes, etc. Record the rates in the table labeled Fig 2 in the Results section.

Procedure Part B: Reaction Rate vs. Substrate Concentration

1. Remove the tubes from the bowl and uncap all the tube substrate molecules.

2. Place 50 beads in the bowl. The beads represent a "solvent" in which the tube substrate molecules are "dissolved". Mixing different numbers of tubes in with the beads simulates different concentrations.

3. For the first trial, place 10 uncapped tubes in the bowl with the beads. The enzyme has 20 seconds to react (cap as many possible, *again while not looking*, again putting the capped tubes back in the bowl). In the results section, record the number capped at a concentration of 10 tubes per 50 beads in the table labeled Fig 3.

4. Remove the capped tubes and repeat with concentrations of 20, 30, 40, and 50 tubes per beads (i.e., generate 5 data points). Use 50 beads for each of the five trials; you are increasing the concentration of tubes. Record your results in Fig 3.

5. Calculate reaction rate at each concentration and record in Fig 3.

6. Graph the results by plotting Reaction Rate vs. Substrate Concentration on a separate sheet of graph paper and attaching it to the back of this lab packet

Procedure Part C: Reaction Rate vs. Temperature: Cold

1. Select 10 uncapped substrate molecules. Time how long it takes to metabolize the 10 tubes as fast as you can. Record the results in the table labeled Fig 4.

2. Place your hands in the pail of iced water for about two minutes. WARNING: Pull your hands out and stop if you get uncomfortable!

3. Repeat step 1 for a second set of data. Record results in Fig 4.

4. Calculate the rate of enzyme action in substrate metabolized per second for each set of Part C data. Record in Fig.4.

Procedure Part D: Reaction Rate vs. Temperature: Denature

When proteins are heated above a certain critical temperature, the amino acids begin permanently changing their covalent bonds. This is called denaturing. (Think about what happens to the albumin protein in an egg white when it is heated in a frying pan). You will simulate the effect of high temperatures rearranging protein bonds by taping your fingers.

1. The person who will be acting as tubease will have one finger on each hand taped to another finger. The group may decide which fingers will be taped. Use a few pieces of tape to simulate the denaturing.

2. Select 10 uncapped substrate molecules. Time how long it takes to metabolize the 10 tubes as fast as you can.
3. Calculate the rate of enzyme action for Part D. Compare the rate of enzyme action before denaturing (see Part C – room temp data) with the rate after denaturing. Record this data in the table labeled Fig 5.

Results:

Fig 1:

Part A: Enzyme Action per 20 second interval

Time (sec)	Total Tubes Capped
20	
40	
60	
80	
100	
120	
140	
160	
180	

Fig 2:

Part A: Rate of Enzyme Action Over Time

Time Interval	Reaction Rate (tubes/sec)
First 60 sec	
Second 60 sec	
Third 60 sec	

Fig 3:

Part B: Reaction Rate vs. Substrate Concentration

Substrate Concentration (tubes per 50 beads)	Number of Tubes Capped in 20 seconds	Reaction Rate (tubes/sec)
10		
20		
30		
40		
50		

Fig 4:

Part C: Reaction Rate vs. Temperature

Temperature	Time Required to Cap 10 Tubes (sec)	Reaction Rate (tubes/sec)
Room Temp		
Cold		

Fig 5:

Part D: Reaction Rate: Denatured Enzyme vs. Normal Enzyme

Reaction Rate of Room Temp Enzyme from Part C (tubes/sec)	Reaction Rate of Denatured Enzyme (tubes/sec)

Analysis:

1. How do you explain the change in rate during successive intervals in Part A?

2. Did you find a relationship between the substrate concentration and the enzyme reaction rate in Part B? If so, describe the relationship and explain why this relationship might exist.

3. Explain the results in Part C in terms of kinetic energy.

4. Describe the relationship between active site and substrate shape. Explain why a denatured enzyme loses its functionality.

Notes to the teacher:

- It is recommended that the class do the prelab the day before carrying out the simulation. Check on the lab day that each group has tables.

- Because kids don't read, you may need to remind students that they may use two hands to cap the tubes, but they may only cap one tube at a time. They will try to put capped tubes out of the bowl. They will look while capping. They will misunderstand that the enzyme is to just keep working and they will try to work in 20-second trials rather than just cap tubes continuously.

- Give the kids a cautionary tale about being in the ice bath too long.