EXERCISE 6 - Postlab

Part I

1. Plot a scatter diagram showing how the A_{510} values changed over time when glucose oxidase was added to the solution containing 30 mg/dL of glucose. Use an entire sheet of paper for your scatter diagram and show the independent variable along the x axis and the dependent variable along the y axis.

2. Now, on the same graph, plot scatter diagrams showing how the A_{510} values changed over time when the enzyme was mixed with the 5 other diluted glucose solutions. Use points of different shapes (e.g. triangles, squares) or colors to distinguish between the different substrate concentrations. Make sure that your axes are clearly labeled and include units of measurement.

3. Examine the graph you just prepared (containing the 6 scatter diagrams). For each substrate concentration, determine the time interval where the relationship between A_{510} values and time appears to be linear. Circle those points. Use linear regression to determine the equation for the best-fit straight line. This line is called the "**enzyme progress curve**". The **slope** of the line tells you the rate of the reaction, and is a measure of "**enzyme activity**". Finally, prepare a table like the one below and fill in the required information.

Substrate Concentration (mg Glucose/dL)	**Equation for "best fit" straight line**	**Correlation Coefficient (r)**	**Slope of line, or enzyme activity (ΔA_{510}/sec.)**

4. Plot the six best-fit lines on your graph. Label each line or include a legend to indicate the glucose (substrate) concentrations in mg/dL for each line. Title your graph "Progress Curves for Glucose Oxidase at Different Substrate Concentrations".

5. Create a second graph by plotting a scatter diagram showing how enzyme activity (the slopes of your progress curves) varies with substrate concentration. Use the entire sheet of paper for your scatter diagram and show the independent variable along the x axis and the dependent variable along the y axis. Label the axes appropriately, including the units of measurement, and title your figure.

6. Note that the slope of each enzyme **progress curve** is a measure of the rate at which the product accumulates over time (i.e. the slope equals change in concentration of product divided by change in time). This rate is referred to **enzyme activity.** How does the slope of the enzyme progress curve change as the substrate concentration changes? What does this indicate about how substrate concentration affects enzyme activity?

7. V_{max} refers to the maximum enzyme activity that is approached as substrate concentration is increased. Estimate V_{max} for your solution of glucose oxidase.

Part II

8. Create a third graph by plotting a scatter diagram of A_{510} values versus time for each temperature tested. Use an entire sheet of paper for your graph and plot the data for all 4 temperatures on the same graph. For each scatter diagram, determine the time interval where the relationship between A_{510} values and time appears to be linear. Circle those points and use linear regression to determine the equation of the "best fit" straight line for these points. Plot the 4 "best fit" straight lines on your graph. Label each line or include a legend to indicate the temperature at which the enzyme activity was measured.

9. In an appropriately labeled table, record the equation for the "best fit" straight line, the correlation coefficient for this line, and slope of the line for each temperature.

10. Create a fourth graph by plotting a scatter diagram showing how enzyme activity (the slopes of your progress curves) varies with temperature. Use the entire sheet of paper for your scatter diagram and show the independent variable along the x axis and the dependent variable along the y axis. Label the axes appropriately, including the units of measurement, and title your figure.

11. At what temperature does glucose oxidase have the greatest activity? Compare enzyme activity at room temperature (indicate actual room temperature) with enzyme activity at 37 ^{o}C. At which of these temperatures did you observe the most enzyme activity? Explain why you think this was so.

12. Describe the effect that each of the temperature extremes—0 ^{o}C and 65 ^{o}C-- had on enzyme activity. Explain why you think these temperatures would have these effects.

Part III

13. Create a fifth graph by plotting a scatter diagram of A_{510} values versus time for each pH tested. Use an entire sheet of paper for your graph and plot the data for all 4 pH values on the same graph. For each scatter diagram, determine the time interval where the relationship between A_{510} values and time appears to be linear. Circle those points and use linear regression to determine the equation of the "best fit" straight line for these points. Plot the 4 "best fit" straight lines on your graph. Label each line or include a legend to indicate the pH at which the enzyme activity was measured.

14. In an appropriately labeled table, record the equation for the "best fit" straight line, the correlation coefficient for this line, and slope of the line for each pH.

15. Create a sixth graph by plotting a scatter diagram showing how enzyme activity (the slopes of your progress curves) varies with pH. Use the entire sheet of paper for your scatter diagram and show the independent variable along the x axis and the dependent variable along the y axis. Label the axes appropriately, including the units of measurement, and title your figure.

16. At what pH does glucose oxidase have the greatest activity? At what pH did the enzyme have the least activity? Explain your results.

Part IV

17. Prepare a table in which you record the observations that you made of the effects of different substrates on the activity of glucose oxidase.

18. Draw the structures of β-D-glucose, β-D-mannose, and β-D-galactose. In what ways are their structures the same? In what ways are their structures different?

19. Based on your observations of the activity of glucose oxidase with these 3 substrates, would you classify this enzyme as highly specific? What do you suppose can cause this level of enzyme specificity?

Part V

20. The potato juice used in part IV contains catecholase (enzyme) and a small amount of catechol (substrate.) Adding additional catechol allows you to see a stronger reaction. Explain the design of this experiment by briefly describing the purpose of each tube (i.e. why it is included in the experiment):

    Tube 1 _____

    Tube 2 _____

    Tube 3 _____

    Tube 4 _____

    Tube 5 _____

21. Based on your results, does catecholase need any cofactors? If so, what cofactor(s) does it require?

22. Based on all the observations you made during this lab, do you think refrigeration could slow the browning of bruised or cut produce? Explain why or why not.

23. Cooks sometimes sprinkle sliced fruits with lemon juice to slow browning of the surface. Based on all the observations you made during this lab, propose a mechanism by which the browning of fresh fruits is delayed by lemon juice.