

TEACHER REFERENCE PAGES - AMYLASE ACTIVITY LAB

INTRODUCTION

Enzymes are of fundamental importance in many of the chemical reactions which take place in living organisms. When digestion occurs, enzymes released into the mouth, stomach, and intestines *catalyze* or accelerate reactions which result in the breakdown of large food molecules into small 'building block' molecules.

For example:

salivary amylase: starch --> maltose (a disaccharide)
gastric pepsin: protein --> smaller peptides
pancreatic chymotrypsin: protein --> smaller peptides
pancreatic lipase: fats --> fatty acids

Enzymes are protein molecules. The molecules upon which an enzyme acts are called the substrates. Any environmental conditions which destroy protein molecules will also abolish enzymatic activity. For example, when a chicken egg is cooked the color and consistency of the white and yolk change.

The *rate of the reaction (or enzyme activity)* can be changed. The most effective way to change enzyme activity is to alter the concentration of *substrates, products, or enzymes* themselves. The relationship between substrates, enzymes and products can be represented by the equation:



The enzyme promotes conversion of the substrate into the product, but is not used up during the reaction.

Salivary amylase: Approximately one liter of saliva is secreted into the human mouth each day by three pairs of salivary glands. Saliva contains many enzymes, including salivary amylase, an enzyme which catalyzes the breakdown of starch (a polysaccharide) into smaller molecules as follows:



The substrate for amylase is starch, a polysaccharide composed of amylose + amylopectin. The product of the amylase reaction is maltose, a disaccharide (made from two glucose molecules).

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EXPERIMENTAL GOALS

Each of the following experiments can be done separately to study a different aspect of enzyme activity.

In this experiment you will do the following:

- A. Establish a method to measure the amount of maltose produced.
- B. Determine the effect of different amounts of enzyme on the rate of reaction.
- C. Determine the effect of different amounts of substrate on the rate of the reactions.
- D. Determine the effect of pH and temperature on the rate of the reaction

EQUIPMENT

- 2 UV-Vis Spectrophotometer
- 2 Water bath
- 4 Vortex mixers
- 4 Pipet pump dispensers

SUPPLIES (for 2 lab groups each/maximum of eight groups)

PART A-MALTOSE STANDARDS

- 10 pyrex test tubes (1.5 X 13 cm)
- 2 test tube racks
- 2 200-1000 μ L pipettors with tips
- 2 50 mL beakers (for standard maltose solutions, on ice)
 - maltose solution
 - dns solution
 - amylpectin solution
 - NaK Tartrate solution
 - DI Water
- 2 Styrofoam cups with ice

PART B-AMYLASE CONCENTRATION

- 10 pyrex test tubes (1.5 X 13 cm)
- 2 test tube racks
- 2 200-1000 μ L pipettors with tips
- 1 40-200 μ L pipettors with tips
- 2 50 mL beakers (for amylase solution, on ice)
 - amylase solution
 - dns solution

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- amylopectin solution
- NaK Tartrate solution
- DI Water
- 2 Styrofoam cups with ice

PART C-AMYLOPECTIN (SUBSTRATE) CONCENTRATION

- 10 pyrex test tubes (1.5 X 13 cm)
- 2 test tube racks
- 2 200-1000 μ L pipettors with tips
- 1 40-200 μ L pipettors with tips
- 2 50 mL beakers (for amylopectin solution, on ice)
 - amylase solution
 - dns solution
 - amylopectin solution
 - NaK Tartrate solution
 - DI Water
- 2 Styrofoam cups with ice

PART D-pH, TEMPERATURE EFFECTS

- 10 pyrex test tubes (1.5 X 13 cm)
- 2 test tube racks
- 2 200-1000 μ L pipettors with tips
- 3 50 mL beakers
 - pH 4 Citrate buffer
 - pH 7 Phosphate buffer
 - pH 10 Borate buffer
 - amylase solution
 - dns solution
 - amylopectin solution
 - NaK Tartrate solution
- 2 Styrofoam cups with ice

NOTE: Test tubes will need to be stored in a refrigerator after the first day of the lab.

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SOLUTIONS: All solutions will be provided for van visits, ready to use. The following solutions have an extended shelf-life.

To prepare buffers: Weigh out the appropriate amount of the buffer salt. Dissolve in one half the volume of DI water. Titrate to the desired pH with NaOH or HCl. Dilute to final volume with DI water.

Borate buffer: 0.2 M at pH 10.0

38.1 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$

500 mL DI water

Titrate with NaOH to pH 10.0. Note: This is very near saturation; adjust the pH to dissolve completely.

Citrate buffer: 0.2 M at pH 4.0

29.41 g sodium citrate

500 mL DI water.

Titrate with HCL to pH 4.0

Phosphate buffer: 0.2 M at pH 7.0

17.4 g Potassium Phosphate (K_2HPO_4) in 500 mL DI water

Titrate with H_3PO_4

Phosphate buffer: 0.02 M at pH 7.0

Dilute 0.2 M buffer.

DNS solution

Saturated DNS (3,5-dinitrosalicylic acid)

1 L 2.0 M NaOH

Tartrate solution

500 g Na-K tartrate

Add DI water to dilute to 1 L. Note: This is near saturation, and endothermic. Allow to warm to room temperature to dissolve completely.

The following solutions may be made in advance and **stored in the refrigerator for up to two weeks.**

Potato amylopectin solution

500 mL 0.02 M PO_4 buffer, pH 7.0

0.2 g NaCl

1.7 g amylopectin

Maltose solution (1.0 mg/ml)

500 mg maltose

500 mL double distilled water

Amylase stock solution

1.0 g barley amylase

500 mL 0.02 M PO_4 buffer, pH 7.0

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UV-Vis Spectrophotometer Operating Instructions

Turn on computer, load program.

1. **Plug in the main power cord. Attach the keyboard and mouse if necessary.**
2. **Turn on the power box switch, UV-Vis spectrophotometer and the printer.** The UV-Vis switch is at the back left corner; the printer switch is at the front left. The computer will self boot and run through self tests; the UV-Vis will also power up and run through self tests. Clicking sounds coming from UV-Vis are normal. **Wait until busy light on the UV-Vis goes out.**
3. **When Windows icons appear, double click on the UV-Vis icon.** This will load the operating software. You will not need the mouse after this point to operate the UV-Vis.
4. **Choose General Scanning (tap F1 softkey at top of keyboard).**

Set up the computer for this lab:

5. **Choose Individual wavelength (tap F1 to give a red background to “individual”).**
6. **Set wavelength to 540 nm. (tap F2 softkey, enter 540 and enter.** If necessary, change additional wavelengths to 0).
7. **Scan Blank (tap the F8 softkey).** The UV-Vis should be empty. You will hear a clicking sound. A table will appear.

Scan samples:

8. Fill cuvette 2/3 full. Do not touch the clear sides. If there are fingerprints on the clear sides, wipe them off with Kimwipes. **Insert the cuvette into the UV-Vis with the clear sides oriented to the light beam (ridges in front and back).** Lock into place gently. Remember to unlock the holder before removing sample.
9. **Choose Scan (F1 key).** Sample number and absorbance will appear. Note that the sample numbers are consecutive; they may not match the numbers you have recorded for each sample.
10. **Repeat Steps 4 & 5 for each of your remaining samples to record data for each sample.**
11. **Record the absorbance for each sample in the table on your data sheet.**

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12. Choose Hardcopy (F9 Key) to print a hardcopy (optional.)

Prepare for next lab group:

13. Press Delete (F3 Key) to clear data. Press "A" and enter to clear all.

14. Repeat from step 3 for each group of samples. If you are not using the computer to create the graphs, go to step 30 when finished for shut down instructions.

Graphing the data:

15. Return to the Windows Program Manager by holding down the "Alt" key and hitting "Tab", then double click on Quattro Pro icon.

16. Click on "File", then click on "Open".

17. When the dialog box appears, click on "tops" folder to highlight and click "OK".

18. Find the file for the lab you are running: amylase1.wb1 and click to highlight. Click "OK" to open. The ...2.wb1 and ...3.wb1 files are copies of the same files, in case one is corrupted in some way. Once you have the appropriate file opened, continue with step 15.

Input data:

19. Select the column which matches the particular section/conditions for which you have data (i.e., your data is for part B, with amounts of amylase, absorbance, and corresponding amounts of maltose, so you move to columns G&H, row 3). Use the arrow keys to move to the appropriate column and row and enter the absorbances from your data sheet. Be sure you are opposite the correct volume of amylase. If you have not already done so, use the standard curve you produced in part A to convert absorbance to volume of maltose and also enter that data.

20. Continue until you have entered all your data.

21. Repeat this process for each set of conditions for which you have data.

Graph data:

22. Click on the small white arrow at the bottom center of the screen. This will take you to the pre-programmed graphing files for this lab.

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23. Double click on the icon for the lab section which you want to graph (Part A, Part B, etc). You will see a view of your graph. There are two sets of graphs for Parts B, C, D. Those labeled Pt. B/Enzyme, etc. are plotted vs. Volume of Maltose as determined from the standard absorption curve obtained in Pt. A. If time does not permit this conversion to be done, you can plot vs. Absorbance instead. In that case, use Graph Part B, etc.

24. With the mouse, click on “File”, then on “Print”.

25. When the “Graph Print” window appears, click on the printer icon (be sure the printer is turned on first). Your graph will be printed automatically.

26. Click on the “Close” button at the bottom of the box to close the graphing window.

27. If you need to graph another section, choose that icon and continue as above.

28. Click on the small white arrow at bottom center again to return to the data window.

Prepare for next group:

29. Delete your data by placing cursor on the first reading in the first column. Hold down the left mouse button and drag the mouse to the last entry in the last column. Release the mouse button and tap “delete” to erase all data. You will need to do this separately for each part of the lab.

You can toggle back and forth from the UV-Vis to Quattro Pro by holding down “Alt” key and tapping the “Tab” key.

To Exit when finished:

30. Choose Exit (F10 key).

31. Choose Exit General Scanning Mode (F10 key).

32. Choose Exit to Toplevel (T key + enter).

33. Choose Exit to MS-DOS (F10 key).

34. Answer Y(es) to "Are you sure you want to exit?"

35. Close UV-Vis window (double click top left corner).

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36. Close Program Manager window (double click top left corner). If Quattro Pro is open, answer “NO” when asked if you want to save changes to file.

37. Exit Windows (click "OK").

38. When C:\> prompt appears, **turn off transformer switch.**