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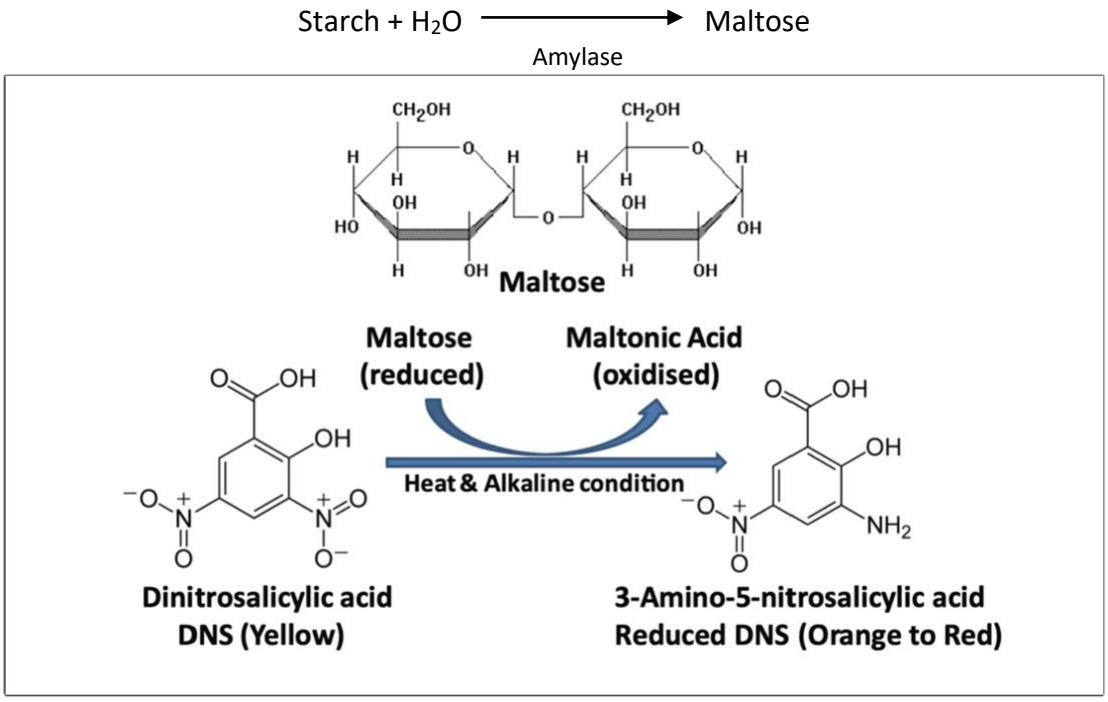
BIOL150L

# The Effect of pH on Enzyme Activity

## Introduction

Enzymes are biological catalysts that controls the rate of reactions by speeding them up, all while not being used by the reaction. Optimal conditions for an enzyme can be found in different factors such as pH level, salt concentration, temperature, and substrate/enzyme concentration. Like the story of Goldilocks, there is a happy medium for enzymes to perform their best. Take temperature; as it rises, molecular motion (kinetic energy) increases the rate of random collisions between the enzyme and its substrate, leading to more product formation. If the temperature gets too hot, though, the shape of the enzymes active site starts to morph, and product formation decreases. The enzyme is no longer in its optimum temperature. pH plays a role in how the $H^+$ and salt concentration affect the stability of secondary and tertiary structures maintained by the hydrogen bonds and disrupt salt bridges held by ionic bonds.

In this lab, amylase will be used as the enzyme. It plays the role in catalyzing the hydrolysis of starch into sugar. Upon research of the bacterial amylase to be used in the lab, it was found that a pH of 7 is the optimal range for enzyme activity and stability (de Souza & de Oliveira Magalhães, 2010, pg. 4). Dinitrosalicylic acid or DNS (Yellow) will be used to help determine the production of maltose. DNS is used as the concentration of maltose produced by hydrolysis of starch is directly dependent on the activity of the bacterial amylase (*Exercise 4B, ENZYMES, Functional and Structural Analysis*). Absorbance is used to determine what the optimal conditions for amylase activity are.




(*Exercise 4B, ENZYMES, Functional and Structural Analysis*)

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# Part I: Standard Curve for Estimation of Maltose

This part of the lab will help us create a standard curve for the estimation of maltose by carrying out the DNS assay with pre-measured amounts of maltose and read the absorbances at 540nm. The data will then be used to create a reference graph that shows the estimate of maltose concentration in a solution. This graph will be used in the next part to help determine the optimal pH level for enzyme activity of bacterial amylase.

**Independent variable:** Concentration of maltose
**Dependent variable:** Absorbance at 540nm
**Experimental group:** solutions with included volumes of maltose
**Control group:** solution with absolutely no maltose in it and just water

**Equipment and Materials required:**

- Maltose solution (2.5mg/mL)
- Deionized water
- Six test tubes
- DNS reagent (yellow solution)
- Micropipettes & appropriate tips
- 10mL glass pipette and pipet pump
- Spectrophotometer and pair cuvettes
- 85°C water bath

**Procedure:**

1. Add 0uL, 200uL, 400uL, 600uL, 800uL, and 1000uL of a standard maltose solution (5mg/mL) to each test tube. Add distilled water to make up the total volume to 1000uL in all 6 tubes.
2. Add 1000uL (1.0mL) of DNS reagent (yellow) into each tube.
3. Place the tubes into the 85°C water bath for 5 minutes to speed up the redox reaction between maltose and DNS. A color change should occur across the tubes.
4. Carefully remove the tubes and place them on a test tube rack. Add 8.0mL of deionized water to dilute the reaction mix before reading the absorbance, using the 10mL glass pipette and pipet pump.
5. Mix the contents of each tube by pipetting up and down if you have enough 10mL glass pipettes. Change pipettes to avoid contamination, but if you do not have that available then swirl and flick the tube to mix.
6. Measure the absorbance of the reduced DNS at 540nm and record the absorbance for each tube.
7. Calculate the final concentration of maltose ($C_2$) using the following formula:

$$\boldsymbol{C_1V_1 = C_2V_2}$$

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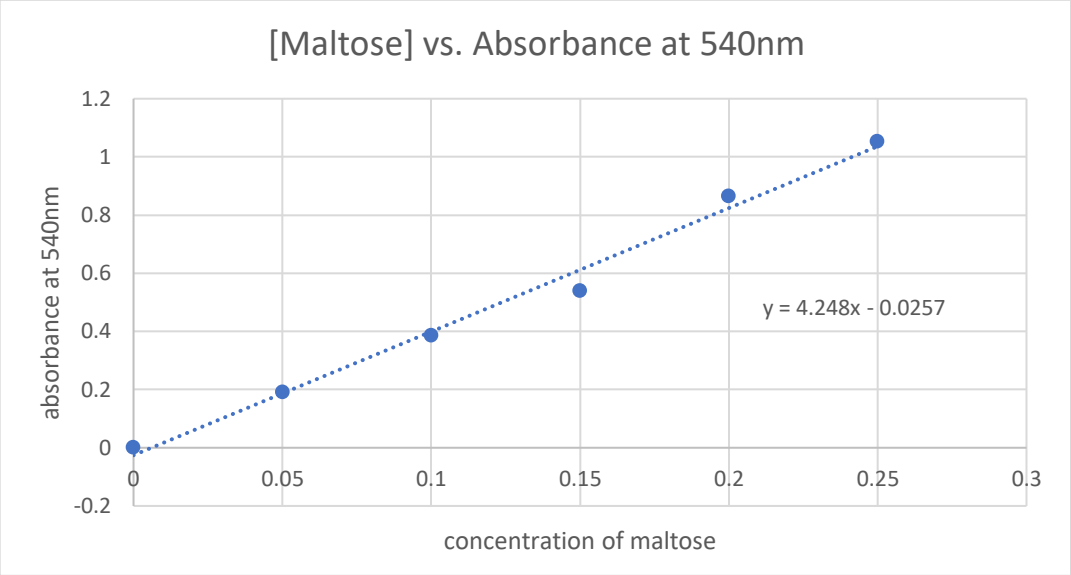
Table 1

| tube number | volume (mL) of standard maltose solution ($V_1$) | volume (mL) of water added to make up to 1.0mL | concentration of maltose (mg/mL) ($C_2$) | absorbance at 540nm |
|---|---|---|---|---|
| 1 | 0.0 | 1.0 | 0.00 | 0.000 |
| 2 | 0.2 | 0.8 | 0.05 | 0.191 |
| 3 | 0.4 | 0.6 | 0.10 | 0.386 |
| 4 | 0.6 | 0.4 | 0.15 | 0.538 |
| 5 | 0.8 | 0.2 | 0.20 | 0.865 |
| 6 | 1.0 | 0.0 | 0.25 | 1.052 |

*Note: columns highlighted in purple are what is graphed below in Graph 1*

Graph 1




**Calculating for $C_2$:**

Concentration of maltose solution ($C_1$) = 2.5mg/mL
Volume of standard solution ($V_1$) = 200uL = 0.2mL
Concentration of maltose solution after dilution ($C_2$) = unknown
Volume of maltose solution after dilution ($V_2$) = 10.0mL

$$C_2 = \frac{C_1 V_1}{V_2} = \frac{2.5\frac{mg}{mL}\, x\, 0.2mL}{10.0mL} = 0.05mg/mL$$

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# Part II: Testing the Effect of pH on Enzyme Activity

This part of the lab was designed by the group of students: Bernice, Tiffany, and Jazleen to study the effect of pH level on bacterial enzyme activity.

**Question:** At what pH level would enzyme activity be at its most optimal?
**Hypothesis:** Bacterial amylase will undergo optimal enzyme activity in a pH level 7 environment
**Prediction:** If bacterial amylase is put in an optimal pH level of 7 then its enzyme activity will be at its highest.
**Independent variable:** pH level
**Dependent variable:** Concentration of maltose
**Experimental group:** solutions with pH solutions and amylase
**Control group:** solution of only water and starch and *no* amylase

**Equipment and Materials required:**

- Spectrophotometer and cuvettes
- 85°C water bath
- Test tubes
- DNS reagent (yellow solution)
- Pipettes with tips for each
- 10mL glass pipette and pipet pump
- Deionized water
- Bacterial amylase enzyme
- Buffers for pH 1.0, 3.0, 5.0, 7.0, 9.0, and 12.0

**Procedure:**

1. Gather 7 tubes and label them: B (blank), 1.0, 3.0, 5.0, 7.0, 9.0, and 12.0
2. Add 450uL of each buffer into tubes 1.0-12.0 (make sure pH corresponds correctly).
3. Add 450uL or the starch to each buffer into tubes 1.0-12.0.
4. In tube B, add 550uL of water and 450uL of starch. The blank will have no buffer.
5. Add 100uL of bacterial amylase to each tube with buffer and agitate for a few seconds to ensure it's well mixed. Do **NOT** add amylase to tube B as it is the blank.
6. Keep all tubes on the rack on your bench for 5 minutes to allow the amylase to catalyze the hydrolysis of the starch then add 1000uL of DNS to all the tubes.
7. Place the test tubes in the 85°C water bath for 5 minutes to speed up the redox reaction between maltose and DNS. A color change should occur because of the DNS.
8. Remove the tubes and put them back into the test tube rack.
9. Add 8mL of deionized water to dilute each tube using the 10mL glass pipette and a plastic pump. Agitate the tubes/flick the tubes for proper mixing.
10. Use the blank tube to calibrate the spectrophotometer and measure the absorbance of the reduced DNS at 540nm and record the absorbance for each tube.

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Table 2

| tube # | pH | volume of pH buffer (uL) | volume of starch (uL) | volume of water | volume of bacterial amylase (enzyme) (uL) |
|---|---|---|---|---|---|
| B (blank) | **none** | 0 | 450 | 550 | 0 |
| 1 | **1** | 450 | 450 | 0 | 100 |
| 2 | **3** | 450 | 450 | 0 | 100 |
| 3 | **5** | 450 | 450 | 0 | 100 |
| 4 | **7** | 450 | 450 | 0 | 100 |
| 5 | **9** | 450 | 450 | 0 | 100 |
| 6 | **12** | 450 | 450 | 0 | 100 |

Table 3

| tube # | pH | absorbance at 540nm | concentration of maltose | rate of amylase activity = [maltose]/7 |
|---|---|---|---|---|
| B (blank) | none | 0 | 0.0000 | 0 |
| 1 | 1.00 | 0.003 | 0.0068 | 0.00096516 |
| 2 | 3.00 | 0.014 | 0.0093 | 0.00133508 |
| 3 | 5.00 | 0.054 | 0.0188 | 0.00268025 |
| **4** | **7.00** | **0.116** | **0.0334** | **0.00476527** |
| 5 | 9.00 | 0.04 | 0.0155 | 0.00220944 |
| 6 | 12.00 | -0.007 | 0.0044 | 0.00062887 |

*Note: columns highlighted in purple are what is graphed in Graph 2*

**Calculating concentration of Maltose using line of best fit from Graph 1:**

$$y = 4.248x - 0.0257$$

$y =$ absorbance at 540nm for the concentration of maltose you are looking for
For example, we'll use 0.003 for $y$ and solve for $x$ which is [maltose]

$$x = \frac{y + 0.0256}{4.248} = \frac{0.003 + 0.0257}{4.248} = 0.0068$$

**Rate of amylase activity = concentration of maltose/# of minutes you waited for the reactions to take place.** We used 7 as we went over the 5 minutes written in the instructions.

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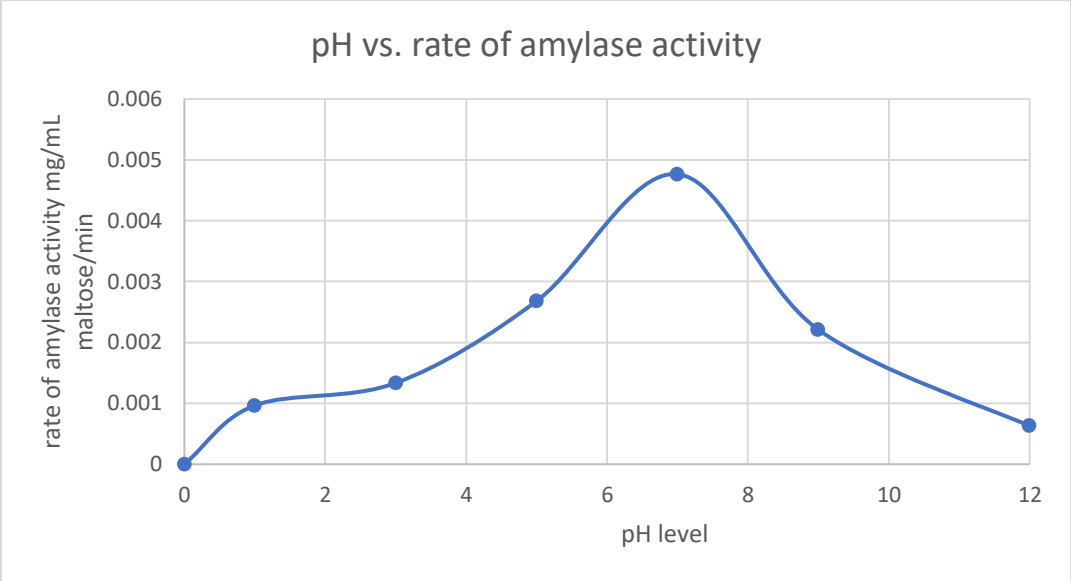
Graph 2




## Conclusion, Discussion, and Applications:

Our hypothesis stated that bacterial amylase will undergo optimal enzyme activity in a pH level 7 environment. According to our data, our experiment supports this hypothesis. If we look at Graph 2 above, we see that our highest rate of activity among all our prepared solutions is 7. This is also supported by research done before the start of lab based off a previous study done that indicated their findings also found an optimal enzyme activity level at a pH of 7.

The rate of amylase activity was determined using Part I of the experiment where we found a line of best fit using the concentration of maltose, versus the absorbance using a spectrophotometer. After graphing, we were able to find the equation to be $\boldsymbol{y = 4.248x - 0.0257}$. Using this equation, we can go back to Part II replace the absorbances we found at specified pH levels in the "y" part of the equation to solve for "x", which would be the concentration of maltose. From there, the rate is the determined by taking concentration solved from the equation and dividing it by the number of minutes used in step 7 to speed up the redox reaction. In our case, I used 7 minutes as we went over time a little due to talking during the experiment. 5 should have been used, though, but 7 will be used for accuracy purposes.

There are endless examples of enzymes and their optimal enzyme levels helping biological life. Without enzymes doing their jobs properly and being in the proper conditions to do them, we would have a hard time surviving and staying alive, or even worse, not being able to eat dairy. The horror! We call this lactose intolerance. It occurs due to the lack of the enzyme, lactase, that is needed to digest the sugars in milk and dairy (*Lactose intolerance* 2022). Without it, cursed dairy lovers suffer with diarrhea, bloating, and gas after eating dairy. I dislike that I cannot enjoy a simple ice cream but understanding the science behind it gives me closure.

References

de Souza, P. M., & de Oliveira Magalhães, P. (2010, October). *Application of microbial α-amylase in industry - A Review*. Brazilian journal of microbiology: [publication of the Brazilian Society for Microbiology]. Retrieved November 9, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3769773/

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