Enzyme: Lactose

Ragini Chaudhari

Research Question: How do the pH levels of 3, 4, 5, 9 and 13 affect the time taken for glucose to be indicated on the test strip in a solution of enzyme Lactase, and substrate Lactose (10%)?

Variables:

<table>
<tr><td>Independent Variable</td><td>Buffer Solutions (pH 3, 4, 5, 9, 13)

Table 1: Ratio of buffer solution to lactose in each 10ml solution
<table><tr><td>Increment</td><td>pH level</td><td>Lactose (g ± 0.001g)</td><td>Concentration (%)</td></tr><tr><td>1</td><td>10ml of pH3</td><td>1</td><td>10</td></tr><tr><td>2</td><td>10ml of pH4</td><td>1</td><td>10</td></tr><tr><td>3</td><td>10ml of pH5</td><td>1</td><td>10</td></tr><tr><td>4</td><td>10ml of pH9</td><td>1</td><td>10</td></tr><tr><td>5</td><td>10ml of pH13</td><td>1</td><td>10</td></tr></table></td></tr>
<tr><td>Dependant Variable</td><td>The time it takes for glucose to be indicated on the test strip (seconds ± 0.5) after lactase is added. This is possible because when enzyme lactase is added, it results in a catabolic reaction which breaks down lactose into glucose and galactose. The time will be measured using a stopwatch.</td></tr>
<tr><td>Controlled Variables</td><td>- The amount of lactose added to the buffer solution. It has to be controlled since a different substrate concentration would alter the data. This will be ensured by using 1g of Lactose throughout the experiment.
- The test strip has to remain the same since different test strips from different time periods may have different ways of indicating the presence of glucose. Furthermore, test strips that are too old may not work. Throughout the experiment the same test strips will be used.
- The amount of lactase added to the lactase and buffer solution will remain the same. This is because a greater enzyme concentration can affect the results. Therefore, throughout the experiment, the same amount of lactase will be used.</td></tr>
<tr><td>Uncontrolled Variables</td><td>- The temperature of the room was uncontrolled. At times it is warmer and at times cooler. A substantial change in temperature can affect the data acquired since temperature affects enzymatic activity too. However, no drastic changes in the temperature should occur.</td></tr>
</table>

Materials:

Experiment:

1. 5 × 10 ml of pH 9 *Buffer Solution*
2. 5 × 2.3g lactase pills
3. 5 × 1 g lactose
4. 5 × Small Test tubes
5. 1 × 50ml beaker
6. 10 ml Graduated Cylinder (± 0.05ml)
7. Electronic Balance (± 0.001g)
8. 2 × Spoons
9. 5 × Test strip
10. Timer (seconds ± 0.5)

Safety:

1. Latex/Plastic Gloves
2. Safety goggles
3. Lab coat

Method:

1. Pour 10 ml of pH 9 buffer solution into a test tube
2. Measure 1g of lactose
3. Add the 1 g of lactose into the test tube. Mix the lactose and buffer solution well.
4. Place the *Glucose measuring paper* in the test tube. Make sure that the correct side of the paper is visible from the outside of the test tube.
5. Once the glucose paper is inside, add one lactase pill and mix thoroughly (2.3g)
6. Record the time as you pour the lactase into the solution, until the colour of the glucose paper changes to a green color.

Safety:

pH levels from 3 to 9 are on the relatively safer side. Despite this, wearing safety goggles and gloves is recommended at all times since it can cause irritation in the eyes and skin. On the other hand, pH level 13 is extremely basic. When it comes in contact with your skin it creates an oily sensation. This sensation is the top layer of skin breaking down and dissolving. Therefore, wearing gloves is crucial. Most lab equipment is made of glass, and glass breakage can cut skin if not handled properly. In the case that a test tube is broken, handle broken parts with care and discard properly.

Raw data:

Qualitative Observations:

Table 2: Qualitative observations witnessed during the experiment				
Ph 3	**Ph 4**	**Ph 5**	**Ph 9**	**Ph 13**
The color of the test strip continued to change after the measured time The translucent solution made it difficult to correctly record the time taken for the glucose paper to change color. Some lactase fell out when pouring it into the test tube → changing the mass.	The solution was a Milky-white color , which made it difficult to see the pH strip. Some lactase and lactose fell out when pouring them into the test tube altering the mass.	No data acquired due to faulty equipment.	It was challenging to pour in the lactase and start the timer at the same time. Some lactase fell out as we were trying to pour it into the test tube causing a change in mass. It was hard to know when the strip changed color due to the translucent solution.	No enzyme activity was witnessed after 13 minutes of waiting.

Quantitative Data:

Table 3: Relationship between pH levels and the time (seconds ± 0.5) it takes for glucose to be indicated on the test strip.					
Time (s ± 0.5)	**Ph 3**	**Ph 4**	**Ph 5**	**Ph 9**	**Ph 13**
Trial 1	106	88	No data	70	No enzyme activity
Trial 2	80	42	No data	81	No enzyme activity
Trial 3	79	44	No data	62	No enzyme activity
Trial 4	82	35	No data	51	No enzyme activity
Trial 5	81	56	No data	65	No enzyme activity

Processed Data:

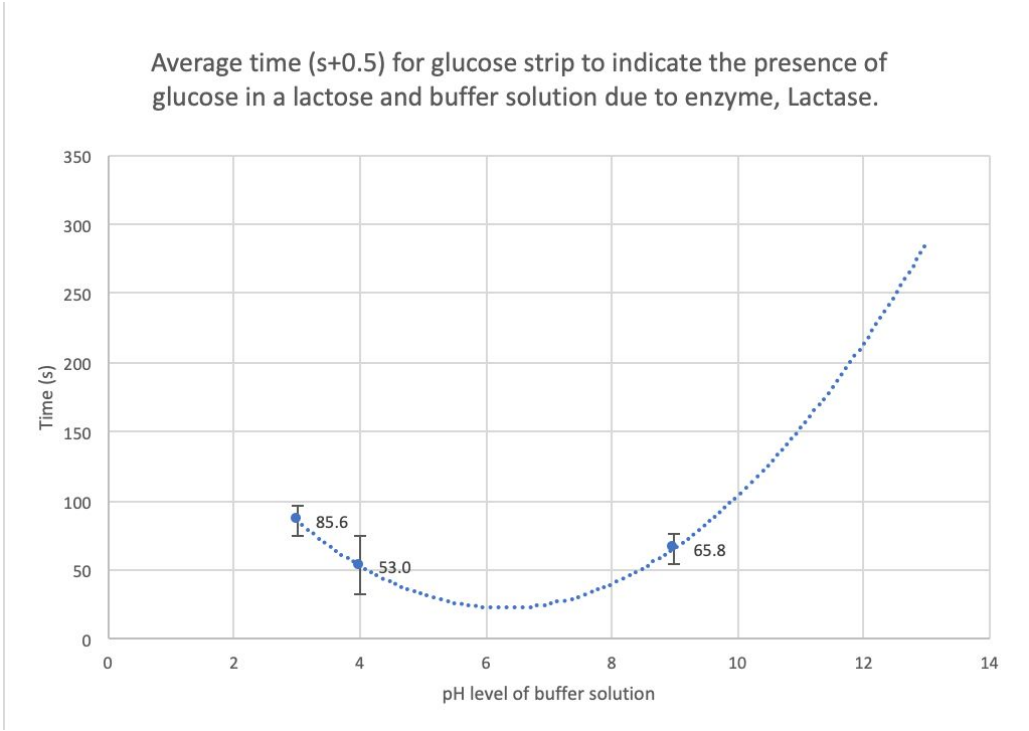
Table 4: Average times (seconds ± 0.5) taken for each pH level to indicate glucose.					
	pH 3	**pH4**	**pH5**	**pH9**	**pH13**
Average	*86*	*53*	*No data*	*65*	*No enzyme activity after 13 minutes*
Example Calculation: **Calculating Averages for pH3:** 1:46 + 1:20 + 1:19 + 1:22 + 1:21 = 428/5 = 1:26					

Figure 1: Calculations for Standard Deviation. (Calculated using Excel)

PH	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average	st
3	1:46	1:20	1:19	1:22	1:21	1:25	=STDEVA(C42:H42)
4	1:28	0:42	0:44	0:35	0:56	0:53	
5							
9	1:10	1:21	1:02	0:51	1:05	1:05	0.00762941
13							

1R x 6C (value1, [value2], ...)

Figure 2: The relationship between the average time (s ± 0.5) for the test strip to indicate the presence of glucose and pH level.



Data Analysis:

In this experiment, the independent variable, the different pH levels, impacted the dependent variable, time it takes for glucose to be detected in the solution. Based on the data collected, the time taken for the test strip to indicate the presence of glucose was faster when the pH level was at 4 (53 seconds). It took the longest to detect glucose in the pH 3 solution (86 seconds).

The results provide adequate information to answer the research question. From the data acquired, a clear relationship between the pH level and the time taken for glucose to be indicated on the test strip is witnessed. *Table 4* exhibits that pH 3 had an average time of 86 seconds, pH 4 had an average time of 53 seconds and pH 9 had an average time of 65 seconds. Therefore, the optimal pH level (the time at which glucose is indicated the quickest), can be interpreted to be around pH level 4. This can be supported by *Graph 2.* Looking at the trendline, as the values on the x-axis approach the pH level 6, the value on the y-axis gets lower, indicating that pH levels closer to 6, are suitable for lactase to function in. With this data, a conclusion can be drawn. Lactase works most efficiently at pH level 6 at which glucose is detected by the test strip in < 50 seconds. Therefore, as the pH level gets farther away from pH 6, the time taken for lactase to break down lactose into glucose and galactose would take longer.

An anomaly witnessed in this experiment was that no data was acquired for pH level 5 and pH13 despite waiting for glucose to be detected for 10+ minutes. Theoretically, pH 5 should be a reasonable pH level for lactase to breakdown lactose, and should catabolize lactose fairly quickly. Additionally, pH13, although it will take longer, shouldn't exceed the 10 minute mark. However, in this experiment, no data was collected. This was due to faulty equipment. Not being able to obtain this information drastically affects the accuracy of our data since rather than having 5 data points, it results in only 3. This affects the reliability of the data.

The Error bars were caused due to a series of reasons. A few systematic errors occurred. Firstly, when the lactose was being transferred into the test tubes, there was a lot of spilling of the lactose thus changing the mass. This changes the substrate concentration in the solution. This alters the results since changes in substrate concentration affect the speed of the reaction as well. Another cause of the uncertainty was due to the translucent solution. As mentioned in *Table 2,* the translucent color of the solution made it challenging to correctly record the time taken for the glucose paper to change color. Moreover, there was no particular shade of green that was being looked for. Consequently, some strips may have been removed when they were a light green whereas others when they were dark green. Another uncertainty in our data was due to a systematic error. It was extremely challenging to pour in the lactase into the test tube and start the timer simultaneously. Therefore, at times the timer started earlier or later. This results in an unreliability in the data. Moreover, measurement uncertainties also contribute to the error bars. When pouring 10ml of the buffer solution into the graduated cylinder, often it wasn't exactly 10ml. It was slightly over and below 10ml, altering the volume of the buffer solution. This affects the

From the data, as well as the graph we can conclude that pH 6 is the optimal pH level for enzyme, Lactase. This is because the minimum point, where the y-axis (time) is at its lowest, of the graph is at pH level 6. This can be supported by research from other students. An experiment in California State Science Fair verifies that the optimum pH of Lactase is pH 6-7. "Lactase worked the best in a neutral (7 pH) or slightly acidic (6.5 pH) environment" (2009, J. N. Ho). Therefore, the data acquired can be verified.

Conclusion

Table 5: Improvements for Future Replications of this experiment.		
Problem	Why the Problem is Significant	Solution
The difference in pH level between each increment was different. It didn't go up consistently.	In order to understand the effects of substrate concentration on enzymatic reaction rate, making sure the concentration increases at the same rate between increments could help collect more supportive results.	In the future, this will be done by ensuring that in the independent variable section, each increment is increasing at a consistent rate.
When transfering the lactose into the test tube a lot of lactose fell out. This altered the mass causing it to be < 1g.	The mass of the lactose should be 1g or the same for each increment as any less would result in a decreased number of substrates. This alters the efficiency of the enzyme.	To prevent a change in mass when transporting the lactose, a funnel can be used. This would allow more precision and ensure that 1g of lactose is being transported into the solution.
The translucent color of the solution made it challenging to read when the test strip changed.	The change in color signifies the presence of glucose. Therefore, being able to see when the strip changes color is critical to collecting reliable results.	In order to make the process easier, using a larger beaker would allow for more control in placing the strip in a more legible location (i.e. closer to the edge of the beaker).
The small rim of the test tube made it difficult to pour in the lactase and start the timer at the same time.	The time, our dependent variable, plays a larger role in the data collected. Therefore, due to this, errors in the data were present since the timers were started later or sooner resulting in inaccurate data.	Using a beaker in the future would resolve the problem. The larger circumference allows the lactase to be transported with more ease as well as make it easier to see when the lactase hits the solution. This will make it easier to see when to start the timer.

In order to extend this experiment, you could attempt to find out how enzyme lactase reacts to different substrate concentrations. For example, How does the concentration of lactose solution affect the time it takes for the test strip (seconds) to detect the presence of glucose in a solution of lactase and the lactose. In this experiment, each increment would have a different concentration of lactose. Another extension would be to immobilize the enzymes prior to the experiment. This would allow one to discover if the concentration affects the enzymatic reaction. For example, How does the concentration of hydrogen peroxide solution affect the enzymatic reaction rate between catalase and hydrogen peroxide by measuring the time in seconds for a reaction to occur. It would be interesting to identify the optimal substrate concentration.

<u>Bibliography:</u>

Ho, J. N. (2009, April 9). The Effect of pH on Lactase. Retrieved February 25, 2020, from http://csef.usc.edu/History/2009/Projects/S0410.pdf