Sample

Course Code:

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Introduction and Aim

Enzymes are biochemical catalysts that are essential for enabling a range of metabolic reactions in living things without being eaten themselves. Among these, amylase stands for a class of enzymes that are especially important because of their function in breaking down starch into less complex sugars like maltose. Amylases are widely distributed and play a crucial function in the digestive system; examples of these sources include human saliva and the pancreas. (Butterworth et al., 2011). The following Figure 1 shows the three dimensional structures of α-amylases.

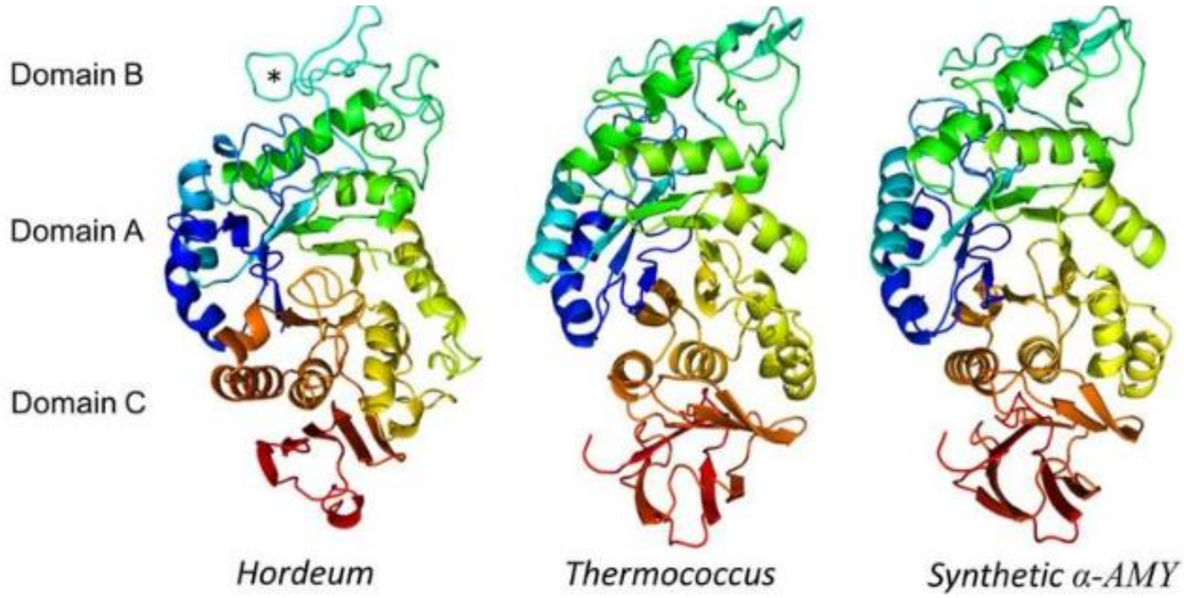

Figure 1: Three Dimensional Structures of α-Amylases (Lévêque et al, 2000).

The structure of amylase, in three dimensions is crucial for its role in breaking down starch into sugars. In Figure 1 we can see the design of an amylase molecule focusing on its site where substrate molecules bind and react chemically. The specific arrangement of acids within the site enables it to interact effectively with starch molecules leading to the efficient conversion of polysaccharides into maltose units. Understanding the configuration of amylase its site, is key to grasping how changes, in pH can impact the enzymes shape and function. Structural studies, such as those conducted by (Nielsen et al 2001) have shown that slight alterations in the enzyme's environment, including pH shifts, can lead to conformational changes that affect its catalytic efficiency. The activity of amylase is not constant and can be influenced by several factors, including temperature, enzyme concentration, substrate concentration, and particularly pH levels

The acidity level of the surroundings where an enzyme functions can have an impact, on how it's structured and how well it works. Each enzyme has a pH range in which it functions best showing its level of activity. Any deviations, from this pH range can lead to reduced enzyme performance. May even cause the enzyme to lose its functional shape, a process known as denaturation (Singh et al, 2015). For example human salivary amylase works best at a pH of 6.8 to 7.0 which matches the slightly alkaline conditions found in the mouth. On the hand pancreatic amylase performs optimally at a higher pH level reflecting the more basic environment of the small intestine (Sky Peck et al, 1977).

Understanding the pH dependence of amylase is not only of academic interest but also has practical implications in industries where amylases are employed, such as in food processing, textile, and paper industries, as well as in medical diagnostics (Saini et al, 2017). Therefore, investigating the effect of pH on amylase activity can provide valuable insights into optimizing conditions for its industrial applications and understanding digestive health issues related to enzyme malfunction.

This experiment aims to study the impact of acidity (pH) on the ability of amylase to break down starch into maltose. We want to find out how well amylase works at different pH levels and determine the pH conditions that allow it to work best.

Materials and Methods

Materials used in determining the effect of pH on the digestion of starch by amylase included an agar plate infused with starch, a 3 ml pipette with its end truncated to facilitate the creation of wells in the agar, a Bunsen burner for sterilization purposes, distilled water, an incubator to maintain a controlled environment for the experiment, a marker pen and ruler for precise division and measurement on the agar plate, adhesive tape for sealing the plate, iodine solution to test for the presence of starch, sterile pipette tips to avoid contamination, and a 0.5% solution of amylase enzyme adjusted to four different pH levels: 2, 4, 7, and 13, representing a range from highly acidic to highly alkaline conditions.

Following methodology was adopted for determining the effect of pH on the digestion of starch by amylase,

1. Bunsen burner was carefully ignited adhering to all the required safety protocols.
2. Marker pen was use to carefully divide the agar plate into four equal quadrants, with each section designated to test the amylase activity at a different pH level.
3. A circular portion was removed from each section of agar using cut pipette and additional well was made in the middle of each plate for control.
4. For each section, 150 µl of the amylase solution corresponding to its assigned pH was carefully dispensed into the well using a sterile pipette tip, ensuring precision and avoiding cross-contamination.
5. After pouring the amylase solution, the plate was sealed with tape and kept horizontal.
6. After incubation period, few drops of iodine solution were poured on the top of the agar plate and plate was kept at an angle such that the iodine drops covered the surface.
7. Diameter of the clear area surrounding the well was recorded for analysis.

Results

The following Table 1 shows the measurements of the diameter of the clear area surrounding the wells.

Table 1: Measurement of Diameter of Surrounding Area

pH	Diameter				Average
	R1	R2	R3	R4	
2	0.00 cm	0.00 cm	0.00 cm	0.00 cm	0.00 cm
4	1.70 cm	1.50 cm	1.20 cm	1.80 cm	1.55 cm
7	3.00 cm	2.60 cm	2.80 cm	3.00 cm	2.85 cm
13	0.00 cm	0.00 cm	0.00 cm	0.00 cm	0.00 cm

The following Figure 2 shows the variation of the diameter surrounding the area with the pH:

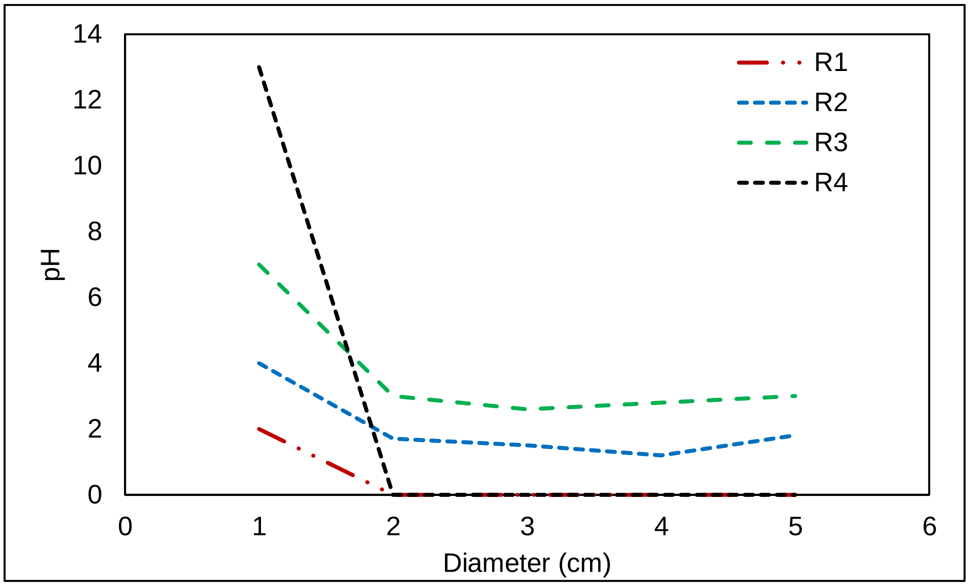

Figure 2: Trend of Diameter of Surrounding Area

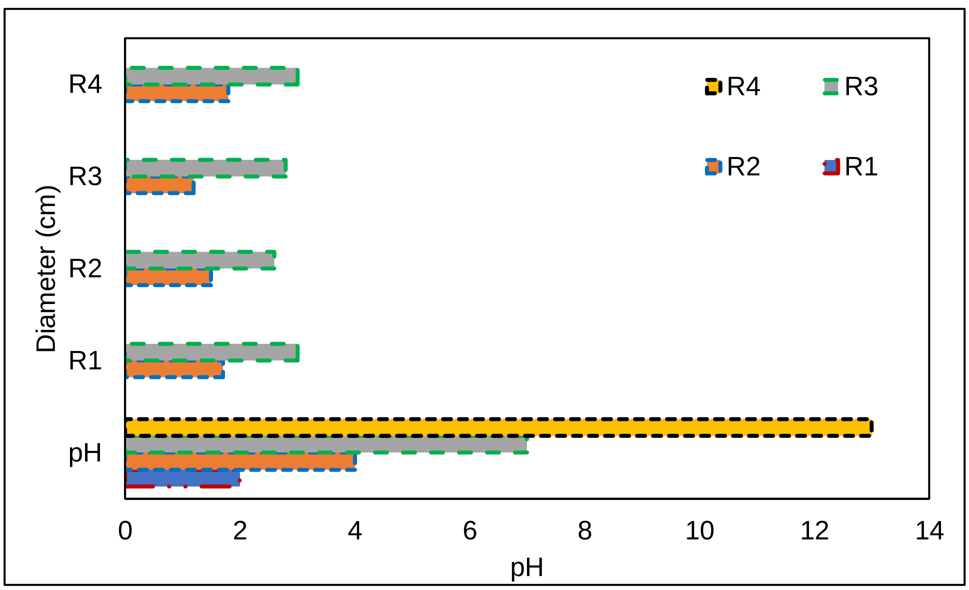

Figure 3: Bar Chart of Diameter of Surrounding Area

At extreme pH values of 2 and 13, the diameters of the clear areas were consistently 0.00 cm across all replicates (R1 to R4). This indicated that no enzymatic activity of amylase at these pH levels was observed. At a mildly acidic pH of 4, there was a noticeable increase in the diameter of the clear areas, with measurements ranging from 1.20 cm to 1.80 cm, and an average diameter of 1.55 cm. This demonstrates that amylase retained some level of activity in mildly acidic conditions, though not optimal. The most significant enzymatic activity is observed at pH 7, with clear area diameters ranging from 2.60 cm to 3.00 cm and an average of 2.85 cm indicating maximum enzymatic activity of amylase in neutral pH conditions.

Discussion

The experiment results quantified the activity of amylase in the digestion of starch across a spectrum of pH levels. At neutral pH (7), amylase exhibited its highest activity, as evidenced by the largest diameters of clear areas around the wells. This optimal activity aligns with the physiological conditions under which human salivary amylase operates, indicating a tailored evolutionary adaptation to the neutral pH of the oral cavity. Conversely, at the extremes of the pH spectrum (2 and 13), amylase activity was virtually nonexistent, highlighted by the absence of clear zones, which suggests a complete denaturation or significant loss of enzyme functionality. The moderate activity observed at pH 4, while significantly lower than at pH 7, underscores some level of enzyme resilience or partial functionality outside its optimal pH range. Amylase's maximum activity occurs at neutral pH because its structure is sensitive to the chemical environment. Like other proteins, enzymes have specific shapes that are important for their function, especially at the active site where molecules bind. Extreme pH levels can disrupt the ionic and hydrogen bonds that hold the enzyme in its proper shape. This can change the geometry of the active site, making it less likely to bind with starch molecules and reducing the enzyme's ability to catalyze reactions. (Kaiser et al, 1985). Similarly, at extreme pH values, these structural alterations can be so severe that the enzyme's active site no longer retains the correct shape to bind with the substrate, effectively halting the catalytic process. This phenomenon explains the observed inactivity of amylase at pH 2 and 13, as the enzyme is likely denatured or significantly altered in these conditions.

When it comes to human health, the optimal activity of amylase at neutral pH shows the critical importance of maintaining a balanced pH within the digestive system for efficient nutrient breakdown and absorption. Any deviations from this delicate balance, potentially caused by gastrointestinal disorders or dietary imbalances, could impair starch digestion, leading to nutritional deficiencies and digestive discomfort. In industrial applications, the sensitivity of amylase to pH conditions must be carefully managed to optimize the enzymatic processes involved in food production, biofuel development, and the pharmaceutical industry, among others. The efficiency of starch breakdown in these processes can be significantly enhanced by maintaining the pH at or near the enzyme's optimal level, thereby increasing product yield and reducing waste. Furthermore, the findings of this experimental activity serve as a foundational knowledge base for the biotechnological engineering of amylase variants with pH alteration. Such modifications can lead to the development of enzyme formulations tailored for specific industrial processes that operate outside the natural pH range of amylase, potentially opening new application of this crucial enzyme

Conclusion

This experiment has significantly contributed to our understanding of how pH levels influence amylase activity, offering a clear depiction of the enzyme's optimal performance in neutral pH conditions and its pronounced decline in activity under extreme acidic or alkaline environments. The findings not only validate the critical role of pH in enzymatic processes but also underscore the importance of maintaining appropriate pH levels for efficient starch digestion, both within biological systems and in industrial applications. By highlighting the enzyme's sensitivity to pH, the study provides essential insights that could guide the improvement of industrial processes involving amylase, as well as inform dietary and health-related recommendations to support digestive efficiency. Furthermore, this research lays the groundwork for future studies aimed at exploring enzyme behavior under varying conditions, fostering advancements in enzyme technology and biotechnological applications. Through a detailed examination of amylase activity across different pH levels, the experiment underscores the intricate interplay between enzyme structure, environmental conditions, and functional capacity, contributing valuable knowledge to the fields of biochemistry and applied sciences.

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