Introduction:

Enzymes are biological catalysts responsible for the speeding up of chemical reactions in organisms (Cooper, 2000).They contribute vitally to metabolic processes such as digestion, energy generation, and cell repair, among others. These proteins, synthesised by our cells, play crucial roles in transforming chemicals within the body by reducing the activation energy needed for reactions, thus facilitating interactions with reactants (Bhatia, 2018). Enzymes have defined three-dimensional structures, usually composed of proteins that take up a particular conformation into active sites, in which substrate molecules are situated and reactions take place. Enzyme activity is influenced by temperature and pH, with each enzyme having optimal levels for both. Extreme temperatures or pH can denature enzymes, impairing their function. Additionally, substrate and enzyme concentrations, along with the presence of inhibitors, activators, and cofactors, play a significant role in modulating enzyme efficiency. (Ashkan et al., 2021). Optimal pH and temperature values also vary according to the enzyme and the environment, thus showcasing the complex nature of enzymatic processes to function optimally within biological settings.

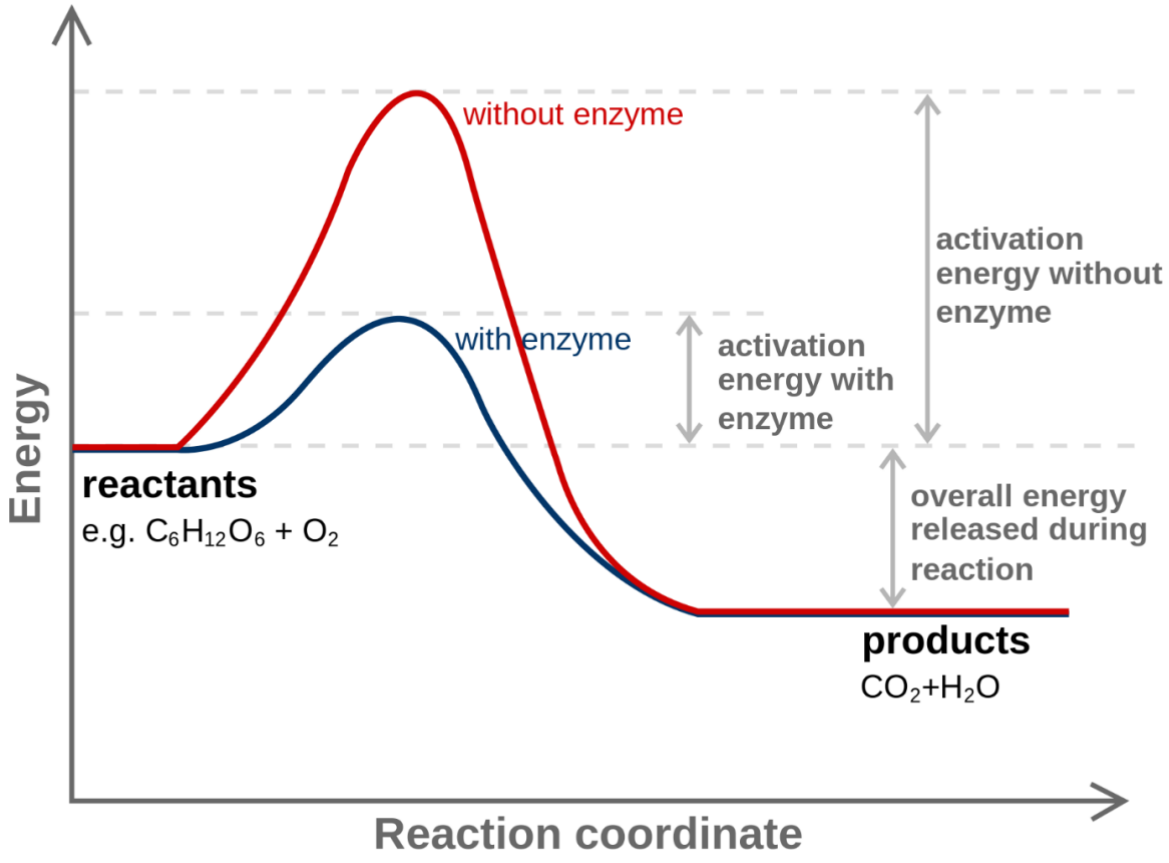

Figure 1: Energy diagram for a chemical reaction with and without the presence of an enzyme as a catalyst. (Wikipedia Contributors, 2024)

An enzyme is a substance produced by a living organism that acts as a catalyst to bring about a specific biochemical reaction. A reactant in a chemical reaction is called a substrate when it is acted upon by an enzyme. The part of the enzyme where the substrate binds is called the active site (since that's where the catalytic “action” happens). The active site is the part of an enzyme to which substrates bind and where a reaction is catalyzed. A substrate enters the active site of the enzyme. This forms the enzyme-substrate complex.

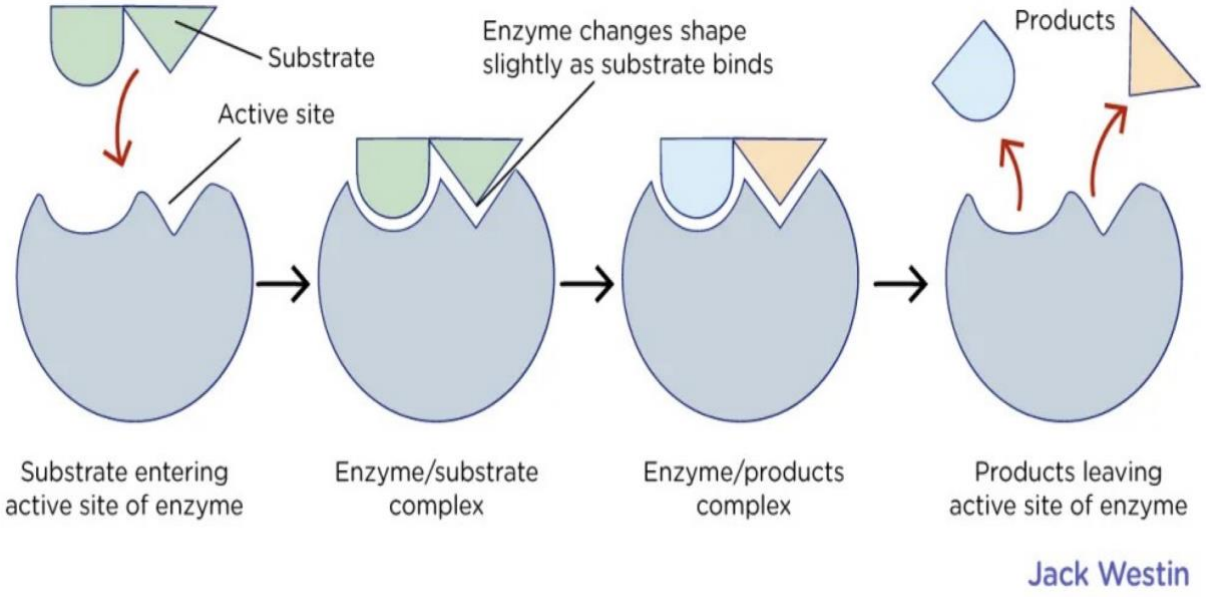

Figure 2: Example of catabolic reaction involving an enzyme (Wikipedia Contributors, 2024)

Aim: investigate how variation in pH impact the catalytic activity of catalase in the decomposition of hydrogen peroxide. A hypothesis that addresses enzyme concentration and reaction rate, and the optimum pH level is 7.

Materials, Methods, and Safety:

During this experimental study, a potato served as the source of the catalase enzyme. The independent variable was the pH of buffer solutions (ranging from pH 4 to pH 10), while the dependent variable was the duration, measured in seconds, for a standardised 10 mm-thick potato disc to rise. Precision was maintained by using a scalpel to cut 12 uniform potato discs.

Five small beakers held distinct pH buffer solutions (pH 4.0, pH 5.0, pH 6.0, pH 7.0, pH 8.0, pH 9.0, and pH 10.0). Hydrogen peroxide solution (5 cm^2) was introduced into five separate test tubes using a clean syringe. The experiment began by combining the pH 4.0 buffer solution with hydrogen peroxide in a test tube, followed by the addition of a potato disc using forceps. Stopwatch timing commenced as the potato disc entered the tube, and the rising time was recorded in seconds. This process was systematically repeated for each buffer

solution, and the results were meticulously documented. Subsequently, averaging was applied to the time measurements for each pH level based on two sets of experimental data.

Safety precautions must be observed during the experiment. The use of a sharp scalpel for potato cutting necessitates caution. Hydrogen peroxide, being corrosive, and the pH solutions, posing hazards, mandate the adoption of safety measures. Throughout the experimental procedure, the wearing of gloves, safety goggles, and a lab coat was consistently maintained to ensure participant protection.

Results:

The following Table 1 shows the experiment results of time taken for potato discs to lift from bottom to the test tube.

Table 1: Experiment results for Time Taken for Potato Discs to Lift from Bottom to Tube Top

pH	**Trial 1/s**	**Trial 2/s**	**Mean**
3.0	90.10	89.60	89.85
4.0	60.50	61.00	60.75
5.0	49.80	50.40	50.10
7.0	22.80	22.60	22.70
8.0	41.00	40.30	40.65
10.0	56.20	56.90	56.55

The following Table 2 shows the rate of reaction by calculating the 1/100 of time taken for potato discs to rise at different pH levels

Table 2: Results for Enzymes Activity based on the Time Taken for Potato Discs to Rise at Different pH Level.

pH Level	Time Taken (s)
3.0	01.11
4.0	01.64
5.0	01.99
7.0	04.40
8.0	02.46
10.0	01.76

Discussion:

In this experimental study, the impact of pH on the activity of catalase sourced from potatoes was investigated, with results suggesting a distinct optimal pH for catalase activity. According to the data, catalase exhibits maximum activity at a neutral pH of 7.0, where the time taken for the potato disc to rise was the shortest (22.60 seconds). This aligns with the understanding that enzymes have an optimal pH at which their activity is highest.

Catalase, like other enzymes, has a specific three-dimensional structure that is crucial for its function. The active site of the enzyme, where substrate binding occurs, is particularly sensitive to changes in pH. At pH levels far from the enzyme's optimal range (in this case, pH 7.0), the excess of H+ ions in acidic solutions (pH 4.0 and 5.0) or OH- ions in alkaline solutions (pH 8.0, 9.0, and 10.0) can disrupt the ionic and hydrogen bonds that maintain the enzyme's tertiary structure. This disruption leads to a misshaped active site, making it less efficient at catalysing the breakdown of hydrogen peroxide, as evidenced by the increased time for the discs to rise at these pH levels. To illustrate the correlation between pH and reaction rate more effectively, Table 2 was graphed, as depicted in the figure below.

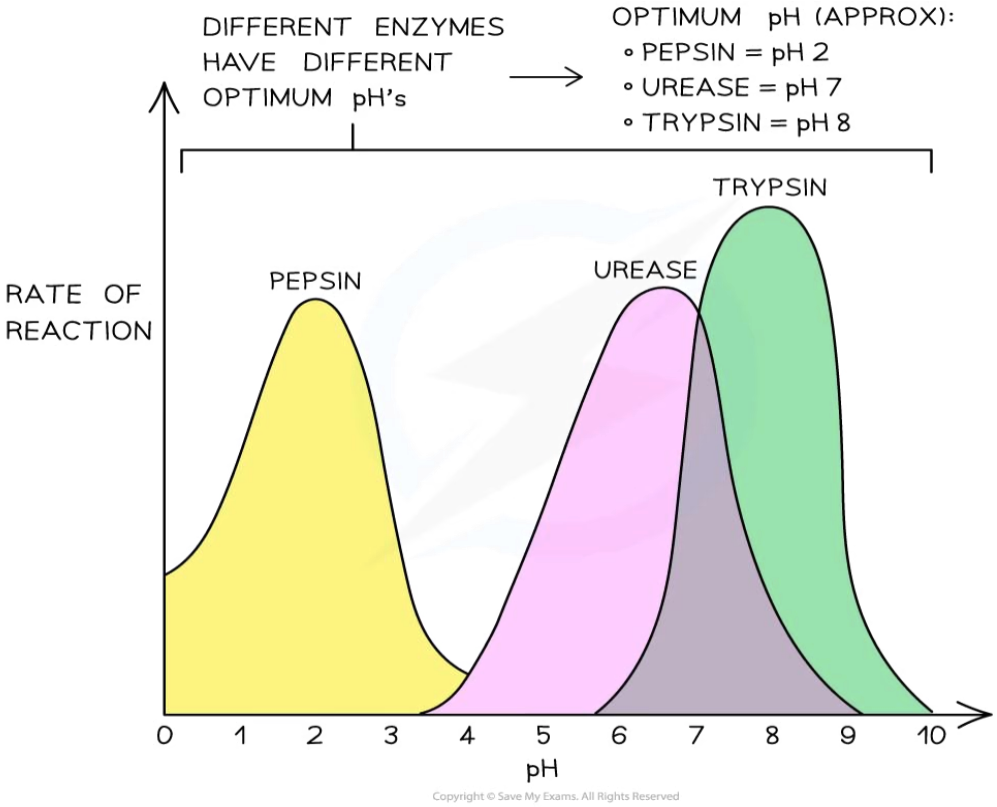

Figure 3: The effect of pH on the rate of an enzyme-catalyzed reaction for three different enzymes (each with a different optimum pH) (3.2.2 rate: PH | CIE A Level Biology Revision Notes 2022, no date)

Many different enzymes are in one cell. Because they are unchanged by chemical reactions, only a small amount of any enzyme may be present. Several factors affect the action of enzymes: salt concentration, pH, temperature, enzyme poisons, radiation, the concentration of enzymes, and the concentration of the substrate. In this lab activity, the enzyme catalase in potatoes was investigated. The substrate, the substance that is acted upon by the enzyme, was hydrogen peroxide (H2O2). The bubbling reaction observed in both cases was the metabolic process of decomposition. This reaction was caused by catalase within the potato. The observation was catalase breaking hydrogen peroxide into oxygen and water. It was noted that the boiled potato produced little bubbles. This was because the heat denatured the catalase enzyme, making it incapable of reacting with hydrogen peroxide. The room-temperature potato produced more bubbles because catalase works best at room temperature. The chemical formula for this reaction is catalase.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Catalase is found in both plant and animal tissues. It is found in the peroxisomes of liver and kidney cells of animal cells but is especially abundant in plant storage organs such as potatoes and the fleshy parts of fruits. Catalase is extremely important in the cell because it prevents the accumulation of hydrogen peroxide. Hydrogen peroxide is a strong oxidising agent that tends to disrupt the delicate balance of cell chemistry. If too much hydrogen peroxide accumulates, it will kill the cell.

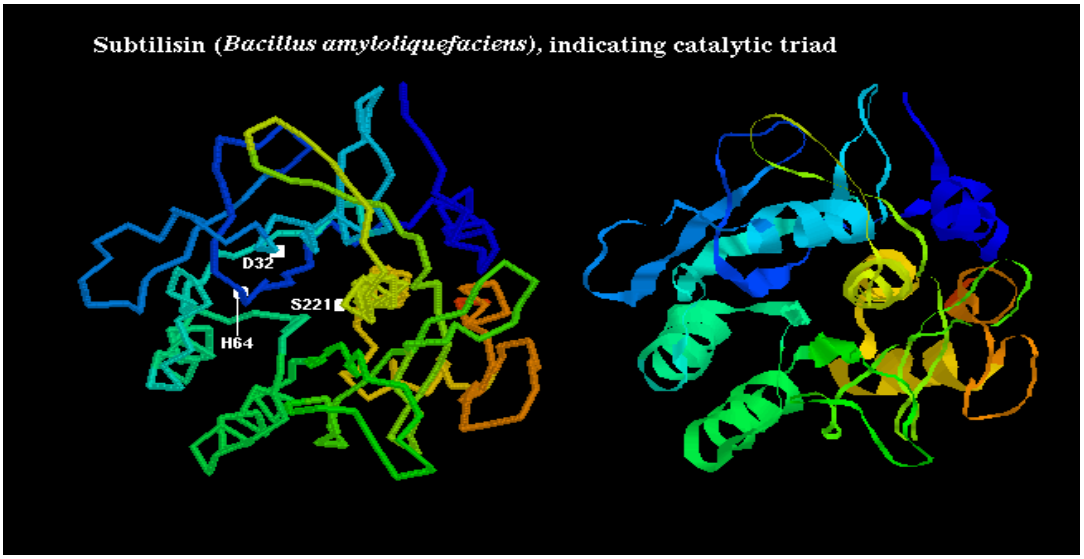

Figure 4: (Tertiary Structure- Globular Proteins, no date)

Enzymes have a tertiary structure, and enzyme activity is modulated through concentration regulation achieved by controlling synthesis, inducing or repressing enzymes, and managing levels of degrading enzymes. Individual enzyme molecules can be inhibited by inhibitors or enhanced by effectors. Inactivation occurs if the substrate cannot bind to the active site, with irreversible inhibition involving covalent bonding or tight binding leading to slow dissociation. Reversible inhibition involves a rapid equilibrium, with competitive inhibitors binding to the active site and non-competitive inhibitors binding elsewhere but reducing the rate constant. Effector molecules may have opposing effects, while in oligomeric enzymes, allosteric interactions at one site influence the binding capabilities of others.

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