

Factors Affecting the Rate of an Enzyme-Catalyzed Reaction

Teacher Resource

This activity covers the following prescribed learning objective from BC Biology 11 & 12 Integrated Resource Package 2006:

B11 Analyze the roles of enzymes in biochemical reactions: apply knowledge of proteins to explain the effects on enzyme activity of pH, temperature, substrate concentration, enzyme concentration, competitive inhibitors, and non-competitive inhibitors including heavy metals.

Time Required: 60-70 minutes

Assigning pre-reading about factors that affect the rate of an enzyme catalyzed reaction (e.g. substrate concentration, enzyme concentration, temperature, pH, and inhibitors) and providing access to the internet may reduce the time required to complete the activity.

Answer Key:

1. For each of the following changes in trial condition, predict its effect on the reaction rate and expected results and explain your reasoning.

Condition	Effect on Reaction Rate & Expected Results	Reasoning for Expected Results
Increasing temperature to 60°C	No reaction occurs so no change in absorbance (remains colorless). <i>or</i> Significant decrease in reaction rate so slow gradual increase in absorbance occurs.	No enzyme activity because the high temperature denatures amylase by breaking hydrogen and disulfide bonds in its secondary and tertiary structure. It alters the shape of its active site and the substrate cannot bind to the active site.
Doubling concentration of CNPG-3	Significant increase in the reaction rate so absorbance increases more rapidly. <i>or</i> No increase in reaction rate, but rate remains steady or decreases more slowly over time.	Amylase activity is proportional to the substrate concentration so increased concentration increases reaction rate. If amylase is already saturated with the substrate, then an increased concentration does not increase reaction rate (no increase in effective collisions) but maintains high activity until it is no longer saturated.
Adding 100µL 1M HCl	No reaction occurs so no change in absorbance (remains colorless).	Increased concentration of H ⁺ alters the structure of amylase by interacting with charged side chains of amino acids and alters the shape of its active site.

2. Explain why the slope decreases as the reaction proceeds.

Decreasing slope indicated that the reaction rate decreased. Reaction rate is proportional to the substrate concentration. The substrate concentration decreases as the reaction proceeds so the reaction rate also decreases over time as fewer effective collisions occur.

3. The buffered, salt solution was used to dissolve and dilute the CNPG-3 and amylase. Explain why a buffer and salt are used instead of water.

Enzymes require an optimal pH and salt ion concentration to help maintain an optimal structure. The buffer helps to maintain an optimal pH as reaction proceeds, while salt ions interact with charged side chains on amino acids and stabilizes the structure of amylase.

Claim: For the following trials, identify which factor was tested. Describe how this factor affects the reaction rate.	Evidence: Describe the evidence that supports your claim.	Reasoning: Apply concepts from this unit to explain the connection between your claim & supporting evidence.
Trials 1 & 2 <i>e.g. Control trials: Negative control verifies that amylase is required to catalyze hydrolysis of substrate, CNPG-3. Positive control verifies that amylase catalyzes hydrolysis of substrate and provides baseline results for comparison with factors that affect the reaction rate.</i>	<i>e.g. No change in absorbance (remained colorless) indicates that no products were produced so no reaction occurred, while gradual increase in absorbance (increasing intensity of yellow) indicates increasing product concentration as reaction occurred.</i>	<i>e.g. Results verify that the substrate remains unreacted (stable) until it gets hydrolyzed by an enzyme. Hydrolysis of the substrate into products only occurs in the presence of the appropriate enzyme.</i>
Trials 3 & 4 - Decreased substrate concentration - Reaction rate decreased	Decreased reaction rate was indicated in both trials by the: 1) lower average slopes (absorbance increased more gradually) so the product formed slower. 2) lower final absorbance so less product formed after five minutes. Trial 4 had a lower substrate concentration than trial 3 and its reaction rate was slower than trial 3.	With a lower substrate concentration, the number of substrate molecules available decreases so the frequency of collisions between the substrate and enzyme decreases. The reaction rate decreases as fewer active sites are occupied by substrate molecules.
Trials 5 & 6 - Decreased substrate concentration - Reaction rate decreased	Similar to trials 3 and 4: Decreased reaction rate was indicated in both trials by the: 1) lower average slopes (absorbance increased more gradually) so the product formed slower. 2) lower final absorbance so less product formed after five minutes. Trial 6 had a lower enzyme concentration than trial 5 and its reaction rate was slower than trial 5.	With a lower enzyme concentration, the number of enzyme molecules available decreases so the frequency of collisions between the substrate and enzyme decreases. The reaction rate decreases as fewer active sites are available to catalyze the reaction.
Trials 7 & 8 - Change in temperature - Reaction rate decreased with lower temperature and increased with higher temperature	Similar to trials 3 and 4: In trial 7 with a decreased temperature, decreased reaction rate was indicated by the: 1) lower average slope (absorbance increased more gradually) so the product formed slower. 2) lower final absorbance so less product formed after five minutes. Vice versa for trial 8 with an increased temperature.	With a higher temperature, the substrate molecules move faster and the reaction rate increases as: - the frequency of collisions between the substrate and enzyme increases. - more collisions are effective because more energy is available to overcome the activation energy barrier. With a lower temperature, the reaction rate decreases because there are fewer successful collisions.
Trial 9 - Addition of inhibitor - No reaction occurred	No change in absorbance (straight, horizontal line on graph) indicates that reaction did not proceed so no product formed in the presence of Montbretin A.	Certain molecules can stop an enzyme catalyzed reaction by preventing the substrate from binding to the active site such as acids, salts, and inhibitors. Montbretin A must be an inhibitor because it is a flavonol that does not affect pH or ion concentration. Note: Montbretin A is a competitive inhibitor that binds the active site and blocks the substrate.