

Effect of Temperature on Amylase activity

Aim:

To study the effect of temperature on enzyme activity and to determine the optimum temperature.

Principle:

In any enzyme catalyze reaction the velocity increases with temperature till it reaches the maximum the velocity decreases on further increase of temperature this effect of temperature may be due to several reasons.

- An effect on the stability of enzyme
- An effect on actual velocity of the breakdown of complex
- An effect on enzyme substrate complex.

Reagents:

- 0.5% Starch in 20 to 100 mill molar phosphate buffer (pH 6.8)
- **Preparation of phosphate buffer:** Dissolve 0.2M (2.7218 grams) of KH_2PO_4 in 100ml of distilled water. To this solution add 0.5M (2.8053 grams) KOH drop by drop till the pH is set to 6.8. Then make it to 200ml with distilled water. So the final concentration is 0.1M of 200 ml phosphate buffer.
- **Diluted Saliva (Enzyme source):** Saliva is the best and easily available source of amylase collect some saliva in a beaker and dilute it to 1:20 dilution with distilled water.
- **1% NaCl:** Dissolve 1 gram of Sodium Chloride in 100ml of distilled water.
- **Dinitrosalicylic acid: (DNS reagent)** Dissolve 1.6 grams of NaOH in 20 ml of distilled water. Take 1 gram of 3,5-DNS in NaOH solution. In another beaker take 30 grams of sodium potassium tartarate. Dissolve in 50 ml of distilled water. Mix the volume up to 100l with distilled water

Procedure:

- 0.5 ml of buffer substrate is taken in a series of test tubes and 0.2ml of NaCl is added.
- The reaction is started with the addition of 0.3ml of dilute saliva and incubate at different temperature (4°C , 10°C , 20°C , 40°C , 60°C , 80°C 100°C).
- The reaction is started by adding 1ml of DNS reagent. Controls are setup for each temperature.
- The contents are mixed well and the tubes are heated in a boiling water bath for 10 minutes.
- The test tubes are cooled and 10 ml of distilled water is added.
- The color developed is read at 520nm calorimetrically.
- The amylase activity is calculating using maltose standard graph.

Report:

At optimum temperature the Amylase activity is _____ grams of maltose/15 minutes/ 100ml.

The optimum temperature is _____ C.

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Tabulation:

Temperature (°C)	Absorbance at 520 nm	Concentration against Maltose standard graph
4°C		
10°C		
20°C		
40°C		
60°C		
80°C		
100°C		

Temperature (°C)	Mg of Maltose 15 minutes/ 0.3ml	Mg of Maltose 15 minutes/100ml	Mg of Maltose 15 minutes/100ml	Grams of Maltose 15 minutes/100ml
4°C				
10°C				
20°C				
40°C				
60°C				
80°C				
100°C				