Experiment

Effect of Substrate Concentration on Salivary Amylase Activity

Aim

To study the effect of substrate concentration on salivary amylase activity using starch.

References

Robert K. Murray et al., Harper's Illustrated Biochemistry, McGraw Hill Education 31th Edition, 59-62.

Requirements

- **Glassware Requirement:-** Test tubes, test tube stand, pipette/dropper, water bath (37°C), stopwatch, Thermometer, Stopwatch
- **Chemical Requirement:-** Saliva (diluted), starch solutions (0.5%, 1%, 1.5%, 2%, 2.5%), iodine solution.

Principle

Salivary amylase hydrolyses starch into maltose. As substrate concentration increases, enzyme activity increases until saturation occurs.

Procedure

1. Label 5 test tubes with different starch concentrations: **0.5%, 1%, 1.5%, 2%, 2.5%**
2. Add 2 ml starch + 1 ml saliva in each test tube.
3. Incubate all test tubes at **37°C for 10 minutes**.
4. After incubation, take a drop from each tube and add iodine solution.
5. Note the time taken for disappearance of blue color.

Observation

Starch Concentration (%)	Time for starch digestion (min)	Observation	Enzyme Activity
0.5%	3 min	Color disappears quickly	Low
1%	5 min	Faster reaction	Moderate
1.5%	7 min	Moderate digestion	High
2%	9 min	Slower	Higher
2.5%	9–10 min	No significant increase	Maximum (plateau)

Result

Enzyme activity increases with substrate concentration and reaches a maximum at saturation.

NOTE

Preparation of Starch Solutions

Test Tube	Required Concentration	Stock Solution (2.5%)	Distilled Water	Final Volume
1	0.5%	2 ml	8 ml	10 ml
2	1%	4 ml	6 ml	10 ml
3	1.5%	6 ml	4 ml	10 ml
4	2%	8 ml	2 ml	10 ml
5	2.5%	10 ml	0 ml	10 ml