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Effect of Substrate Concentration on Salivary Amylase Activity

Experiment 10 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To demonstrate starch hydrolysis by amylase and investigate the named reaction condition.

Principle

Amylase cleaves internal alpha-1,4 glycosidic bonds in starch, producing smaller carbohydrates. Iodine-reactive chains diminish during hydrolysis. pH, temperature, enzyme concentration and substrate concentration must be controlled to make comparisons meaningful.

Requirements

- Assigned amylase, buffered starch, dilute iodine, water blanks and controlled baths.
- Matched tubes, pipettes, well plate, timer and calibrated colour measurement when required.

Procedure

1. Prepare controls

Use instructor-provided human salivary amylase or a characterised teaching preparation. Prepare a no-enzyme starch control and a no-starch blank. Keep total reaction volume, buffer pH and enzyme amount fixed unless that is the stated variable.

2. Prepare the comparison

Prepare starch concentrations such as 0.10, 0.25, 0.50 and 1.00% at equal final reaction volume; keep enzyme, pH and temperature fixed.

3. Start together

Add enzyme last and start the timer. Mix uniformly without adding iodine to the reaction tube; iodine belongs in the separate test wells.

4. Follow hydrolysis

Withdraw equal small aliquots into iodine-containing wells at fixed intervals and record the colour change from blue-black towards the iodine blank. For quantitative substrate comparisons, use a calibrated remaining-starch or reducing-sugar measurement during the initial linear interval.

5. Record and compare

Measure the endpoint time only at identical starting substrate concentration. For varied substrate concentrations, calculate initial rates from matched calibrated readings; 1/time alone is not a valid Michaelis-Menten comparison.

Biochemistry**6. Graph**

Plot initial rate against final substrate concentration. Discuss saturation without claiming an exact K_m or V_{max} from sparse data.

Observations and Record

Condition / replicate	Time or initial-rate reading	Control / observation
_____	_____	_____
_____	_____	_____
_____	_____	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- If calibrated product rises from 0.010 to 0.050 μmol in the first 20 seconds, initial rate = $(0.050 - 0.010) / (20/60) = 0.120 \mu\text{mol}/\text{min}$.
- A plateau at higher substrate may support saturation only when enzyme amount, reaction interval and conditions remain identical.

Result

Record the observed outcome and calculated value, with units. State whether the stated laboratory specification was met; do not copy the worked example as your result.

Precautions

- Do not share saliva or mouth-pipette. Use assigned preparations and dispose of biological material through the laboratory system.
- Take equal aliquots at scheduled times. Keep iodine out of the main reaction and use controls for colour comparisons.
- Heating can change starch behaviour as well as enzyme activity; interpret controls and repeat with a fresh enzyme aliquot.

Viva Questions and Answers**1. What bonds does salivary amylase mainly cleave?**

Internal α -1,4 bonds; it does not directly hydrolyse every starch linkage.

2. What does an iodine-negative result establish?

The tested aliquot no longer contains enough iodine-reactive starch chains to give the observed colour.

3. Why distinguish initial rate from endpoint time?

Initial rate evaluates reaction progress before major substrate depletion; endpoint time depends on starting substrate amount.

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