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Enzymatic Hydrolysis of Starch

Experiment 8 - 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

Mix a measured buffered starch aliquot with the assigned enzyme aliquot at the laboratory-selected pH and temperature; retain the no-enzyme comparison.

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

Record the first scheduled interval with no blue-black iodine colour. Endpoint time is an approximate comparison under identical substrate conditions, not a calibrated enzyme unit.

6. Graph

Record colour versus time and explain loss of iodine-reactive starch. Loss of colour does not establish complete conversion to glucose.

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.

- At fixed initial starch, iodine-negative endpoints of 120 and 240 seconds give comparison indicators $1/120 = 0.00833\ s^{-1}$ and $1/240 = 0.00417\ s^{-1}$. These are not enzyme activity units.
- For a calibrated initial-rate test, a change of 0.040 µmol product in 20 seconds equals 0.120 µmol/min.

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 alpha-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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