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Effect of Temperature on Salivary Amylase Activity

Experiment 9 - 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

Set matched reaction tubes in baths at 20, 37, 50 and 70°C, with at least duplicate tubes; pre-equilibrate enzyme and substrate separately.

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

Plot temperature against the measured rate indicator and discuss increased reaction rate followed by loss of activity at sufficiently high temperatures.

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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