Amylase: temperature and reaction time

Student activity • practical and reasoning

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The question

How does temperature affect the time for starch to become undetectable?

Investigate one assigned temperature in pairs, repeat it three times and pool the class results across five temperatures. The endpoint is a detection threshold, not proof that every starch molecule has disappeared.

Equipment

- Eye protection; two reaction tubes plus control tubes, rack, 2 cm^3 syringes, 1 cm^3 measure and pipette
- Spotting tile or white well plate, timer and thermometer
- Supplied 1% starch, pilot-checked amylase solution, pH 7 buffer, dilute iodine and water
- Access to assigned thermostatic bath; no flame

Wear eye protection; iodine stains and can irritate eyes. Avoid contact with buffer and enzyme solutions. Hot water and glass can burn: use racks and holders at the warmer baths. Students do not handle enzyme powder or saliva.

Course coverage: Shared Biology / Double Award: 2.12. Core practical: temperature and enzyme activity.

Plan, model or apparatus sketch

Draw and label the apparatus or biological model; identify one limitation.

Method and reasoning

Read the whole sequence before starting

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1. Wear eye protection. Work at the assigned 20, 30, 40, 50 or 60 °C bath; record its actual temperature. Prepare separate iodine wells and a starch-positive and water-negative reference.

2. Measure 2 cm^3 starch into one tube. In a second tube combine 2 cm^3 supplied amylase with 1 cm^3 pH 7 buffer. Stand both in the bath for five minutes; keep volumes and stock concentrations constant.

3. Combine the solutions, mix gently and start the timer immediately. Keep the reaction tube in the bath. Use a clean pipette to add one drop to a fresh iodine well every 20 seconds.

4. Record the last blue-black sample time and the first sample that retains the negative-reference colour. Never return iodine to the reaction tube. Stop at 300 s if no endpoint occurs and write >300 s.

5. Repeat twice with fresh solutions. Run a no-enzyme control with 2 cm^3 water replacing amylase. Keep pH, volumes, equilibration and sampling unchanged; pool repeats from the same measured temperature.

6. For completed runs calculate 1/t in s^{-1}, summarise repeats and plot activity against measured temperature. Keep >300 s results separate: they are below the measurable endpoint-rate limit, not zero. Evaluate sampling uncertainty and temperature spacing.

Cleanup

- Collect iodine and buffer mixtures as directed by the school’s disposal instructions.
- Rinse equipment promptly, return it safely, clean the bench and wash hands.

Record your evidence

Blank spaces are for your own work

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Reference and task data

Record your own times, including last-positive and first-negative bounds. Example calculation only: an endpoint at 100 s gives 1/t = 0.010 s^{-1}. A result >300 s gives an upper bound below about 0.0033 s^{-1}; do not enter 300 as an exact time or plot a zero rate. Use the same enzyme batch throughout; its optimum depends on its source.

Trial	Actual °C	Last + / s	First – / s	1/t / s^{-1}
1				
2				
3				
No enzyme				

Show your calculations, labelled model or correction here.

Explain and evaluate

Use evidence and show calculation steps where relevant

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1. Identify the independent and dependent variables.

2. Why equilibrate the two solutions separately?

3. Calculate 1/t for an endpoint time of 80 s.

4. How should a >300 s result be treated?

5. What does the no-enzyme control check?

6. Why is the exact optimum not established by five temperatures?