Enzymes and Bioprocessing AAC – Sample Report B

Title: Investigating the Effect of Temperature on the Rate of Amylase Activity

Section 1 – Title and Introduction

Enzymes are biological catalysts that control metabolic reactions in living organisms and in industry. Their activity depends strongly on temperature: as temperature increases, enzyme and substrate molecules gain kinetic energy, increasing collision frequency until the enzyme denatures.

Amylase catalyses the hydrolysis of starch to maltose and is essential in food and beverage industries such as brewing and glucose-syrup production. Determining its temperature optimum informs industrial process control and energy efficiency.

Research question:
How does temperature affect the rate of starch breakdown by amylase?

Hypothesis:
It is hypothesised that the rate of reaction will increase from 10 °C to approximately 40 °C and then decrease at higher temperatures due to denaturation of the enzyme's active site.

Section 2 – Background Research

Each enzyme has an optimum temperature where catalytic efficiency is greatest. Below this, molecular motion is limited; above it, hydrogen and ionic bonds maintaining tertiary structure break, causing denaturation (Nelson & Cox, 2023).

Amylase isolated from human saliva and Bacillus sp. typically shows an optimum near 37–45 °C and rapid decline beyond 55 °C (Reed et al., 2020). In industrial reactors, thermostable amylases are chosen for high-temperature starch liquefaction (~90 °C).

At school level, reaction rate can be followed by the disappearance of starch using the iodine test—blue-black colour indicates presence of starch; loss of colour marks completion. Measuring time to endpoint gives an inverse estimate of rate (1/time).

Quality of secondary data:

Sources used were a biochemistry textbook, peer-reviewed article, and enzyme-supplier data sheet. They were published within 5 years and clearly referenced, ensuring validity and reliability.

Section 3 – Designing and Planning

Table of Variables

Variable Type	Variable	How Controlled/ Measured
Independent	Temperature (10 °C – 60 °C)	Maintained using ice bath, water bath, and incubator (± 0.5 °C)
Dependent	Rate of starch hydrolysis	1 / time to loss of blue-black colour
Controlled	pH (7 buffer), substrate conc. (1 %), enzyme conc. (1 %), total volume (10 mL), mixing rate, iodine test procedure	Kept constant for fairness
Safety	Hot water handling, eye protection, glass care, enzyme contact avoidance	Monitored by teacher

Equipment & Materials

Beakers, conical flasks, graduated cylinders, droppers, thermometer, retort stand, water baths (10–60 °C range), ice bath, incubator, 1 % starch solution, 1 % amylase solution, phosphate buffer (pH 7), iodine solution, stopwatch, spotting tiles, glass rods.

Method Summary

1. Label five water baths or beakers for 10 °C, 25 °C, 37 °C, 50 °C and 60 °C.
2. Equilibrate 5 mL starch and 5 mL amylase in separate tubes at each temperature for 5 min.
3. Combine solutions at the target temperature; start the stopwatch.
4. Sample 1 drop every 30 s onto a spotting tile with iodine; record the time when colour remains orange-brown (no starch).

5. Repeat each temperature three times for reliability.
6. Record all raw times, calculate mean time ± SD, and derive rate = 1 / time.

Fairness, Accuracy, and Safety

•Temperature monitored continuously; samples kept in bath to prevent cooling.
•Equal enzyme volumes used (checked with graduated pipette).
•Stopwatch started immediately after mixing.
•Replication (n = 3) provided mean reliability.
•Safety: handled hot water with tongs; goggles worn; all waste disposed in sink with running water.

Section 4 – Conducting the Experiment

The investigation was conducted over two double periods under supervision. The method ran smoothly; temperature control within ± 1 °C was achieved. Iodine testing was clear except at 10 °C, where reaction was very slow.

Minor procedural adjustments were logged: samples at 60 °C were cooled briefly before iodine testing to avoid temperature interference with colour. Raw data were entered in the laboratory notebook and photographed for authentication.

Section 5 – Data and Analysis

Raw & Processed Data (means ± SD, n = 3)

Temperature (°C)	**Mean Time to No-Blue (s) ± SD**	**Rate (1 / time s^{-1})**	**Relative Rate (%)**
10	820 ± 35	0.00122	18
25	390 ± 20	0.00256	38
37	210 ± 9	0.00476	71
50	150 ± 8	0.00667	100
60	360 ± 25	0.00278	42

(Representative data set)

Graph 1 – Effect of Temperature on Amylase Rate

A bell-shaped curve was obtained, peaking near 50 °C.

Analysis

•Rate increased about 5.5-fold from 10 °C → 50 °C, demonstrating strong kinetic influence.

•A sharp decline beyond 50 °C indicates partial denaturation.

•Relative rate at 60 °C dropped > 50 %, supporting hypothesis.

•Standard deviations < 10 % of mean confirm precision.

Interpretation:

Between 10–37 °C, kinetic energy increases collisions between enzyme and substrate (Arrhenius behaviour). Above 50 °C, hydrogen bonds within amylase's tertiary structure break, distorting the active site and lowering catalytic activity (Nelson & Cox, 2023).

Accuracy:

Temperature control and precise timing improved validity. The subjective colour endpoint could introduce slight error (~ ± 5 s).

Statistical consideration:

Pearson correlation (temperature 10–50 °C vs rate) = +0.99 (strong positive).

Section 6 – Conclusion and Evaluation

Conclusion

Temperature strongly affects the rate of amylase activity. The optimum observed was ~50 °C, after which rate declined rapidly due to enzyme denaturation. The hypothesis is accepted.

Evaluation

•Results match published data for Bacillus amylase (optimum 50–55 °C).

•Experimental reliability confirmed by small variation among replicates.

•Limitations: manual timing; visual endpoint; potential uneven mixing.

Suggested Improvements

1. Use a colorimeter (620 nm) to measure absorbance objectively over time.
2. Narrow temperature intervals (5 °C steps) around the optimum for finer resolution.
3. Employ a thermostatically controlled water jacket to maintain constant temperature throughout each run.
4. Quantify maltose produced using Benedict's reagent or glucose strips.

Quality of Evidence

The trend was clear and consistent with secondary literature, supporting reliability. Minor uncertainties do not alter the overall conclusion.

Section 7 – Reflection and Societal Context

Completing this investigation enhanced my understanding of enzyme kinetics and industrial bioprocess optimisation. I improved practical skills in controlling variables, recording accurate data, and analysing quantitative trends.

The experiment demonstrates how understanding temperature effects allows industries to balance efficiency and enzyme stability, reducing energy use and cost—key aspects of sustainable bioprocessing.

I also learned that enzymes can be engineered for higher thermostability through biotechnology, linking this practical investigation to current advances in sustainable manufacturing and the circular bio-economy.

Future extensions could explore immobilised amylase under varying temperatures to evaluate combined effects on stability, integrating this investigation with Report A.

Section 8 – References

Nelson, D.L. and Cox, M.M. (2023) Lehninger Principles of Biochemistry. 9th edn. New York: W.H. Freeman.

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Bickerstaff, G. (2022) Enzyme Technology. 3rd edn. Cambridge: Cambridge University Press.

Irish State Examinations Commission (2024) Biology – Assessment of Additional Component Guidelines. Athlone: SEC.

Novozymes (2021) 'Thermostable Amylases for Industrial Starch Processing'. Available at: https://www.novozymes.com (Accessed 6 October 2025).