Enzymes and Bioprocessing AAC – Sample Report A

Title: Investigating the Effect of Enzyme Immobilisation on Amylase Activity and Reusability

Section 1 – Title and Introduction

Enzymes are biological catalysts that speed up reactions without being consumed. In industry, enzymes are widely used for bioprocessing because they reduce energy costs and increase yield. However, free enzymes are expensive to replace after each batch. Immobilisation, trapping enzymes in an inert matrix such as calcium-alginate beads, allows reuse and improves stability.

This investigation explores how immobilising amylase affects its reaction rate and reusability when breaking down starch. Amylase catalyses the hydrolysis of starch to maltose and is central to brewing and syrup manufacture.

Research question:
Does immobilising amylase in sodium-alginate beads change the rate of starch breakdown and improve reusability compared with free enzyme?

Hypothesis:
It is predicted that immobilised amylase will have a slightly lower initial rate due to diffusion limits but will retain higher activity after repeated use than free amylase, which denatures more quickly.

Section 2 – Background Research

Enzyme immobilisation techniques include adsorption, covalent bonding, entrapment, and encapsulation. Entrapment in calcium-alginate beads is inexpensive and safe for school use. The pores of alginate allow small substrate and product molecules to diffuse while preventing enzyme leakage (Bickerstaff, 2022).

Previous studies show that diffusion barriers can reduce the apparent rate of immobilised enzymes (Vasilev et al., 2019). However, immobilisation increases thermal and pH stability and enables column-reactor reuse (Chaplin & Bucke, 2020).

Industrial starch-processing reactors rely on immobilised amylases to convert starch to glucose syrup at ~40 °C and pH 7 (Novozymes, 2021).

Summary of secondary data quality:
Peer-reviewed and textbook sources were prioritised; all agree on reduced initial rate but improved reusability. These sources are credible and current (< 5 years).

Section 3 – Designing and Planning

Table of Variables

Variable Type	Variable	How Controlled/ Measured
Independent	Enzyme state (free vs immobilised)	Two treatments tested at 37 °C
Dependent	Rate of starch breakdown (s^{-1}) and % activity retained across cycles	Time to iodine colour loss (blue-black → orange)
Controlled	Substrate conc. (1 % starch), pH (7 buffer), enzyme units, temperature (37 °C water bath), total volume (10 mL), mixing rate, timing	All fixed for fairness
Safety	Eye protection; no ingestion; glass handled carefully; calcium-alginate substitution for calcium-carbide justified for safety	Risk assessment sheet appended

Equipment & Materials

Beakers, conical flasks, graduated cylinders, droppers, retort stand, thermometer, water bath (37 °C), ice bath, sodium alginate (2 %), calcium chloride (0.15 M), amylase solution (1 %), starch solution (1 %), phosphate buffer (pH 7), iodine solution, glass rods, stopwatch, mass balance, weigh boats.

Method Summary

1. Preparation of alginate beads:
   Mix 10 mL of 2 % sodium alginate with 10 mL amylase.
   Drip mixture through a dropper into 100 mL 0.15 M $CaCl_2$ to form beads (~3mm diameter).
   Cure 10 min, then rinse in deionised water.
2. Equilibration:
   Pre-warm equal volumes of starch and buffer to 37 °C. Prepare one beaker with immobilised beads (10 mL) and another with free amylase (1 mL enzyme + 9 mL buffer).
3. Reaction start:
   Add 10 mL starch to each flask simultaneously. Swirl gently and start timer.
4. Sampling:
   Every 30 s remove 1 drop, mix with iodine on a spotting tile. Record time until blue-black colour disappears.
5. Reusability test:
   Decant and rinse beads. Repeat steps 2–4 for five fresh starch batches using the same beads.
6. Controls & repeats:
   Run each treatment in triplicate for reliability.
7. Data recording:
   All raw times logged in laboratory notebook; mean values calculated.

Fairness, Accuracy, and Safety

•Temperature monitored continuously with digital thermometer (±0.5 °C).
•Equal enzyme activity volumes verified by measuring mass of bead aliquots.
•Stopwatch started consistently by the same operator.
•Use of $CaCl_2$ instead of CaC_2 eliminated production of acetylene gas (safety improvement).
•Replicates ensured reliability; anomalies retained for reflection.

Section 4 – Conducting the Experiment

The experiment was completed over two 40-minute sessions. Beads formed successfully and were uniform. At 37 °C, free amylase completed starch digestion in ~150 s, while immobilised amylase required ~210 s on the first run. Across five reuse cycles, immobilised beads retained activity; free amylase could not be reused as denaturation was evident (loss of activity).

All work was supervised. Raw data sheets were photographed and attached for authentication. Minor method adjustments (increasing curing time to 15 min) were logged, improving bead strength and consistency.

Section 5 – Data and Analysis

Raw & Processed Data (means ± SD)

Treatment	**Cycle #**	**Mean Time (s) ± SD**	**Rate (1 / time s^{-1})**	**% Activity Retained**
Free amylase	1	150 ± 6	0.0067	100 (once-off)
Immobilised	1	210 ± 5	0.0048	100
Immobilised	2	215 ± 8	0.0047	98
Immobilised	3	225 ± 9	0.0044	92
Immobilised	4	240 ± 11	0.0042	87
Immobilised	5	260 ± 13	0.0038	79

(n = 3 per cycle)

Graph 1 – Initial Rate Free vs Immobilised (Bar Chart)

Graph shows free amylase has a higher initial rate, consistent with diffusion limitation in beads.

Graph 2 – % Activity Retained vs Cycle (Line Graph)

Line descends gradually from 100 % → ~80 % by cycle 5, confirming reusability.

Analysis

•Immobilisation reduced initial rate by ~28 %.

•Over five cycles, beads lost ~21 % activity, average decline ≈ 5 % per cycle.

•Standard deviations small (≤ 13 s), showing reproducibility.

•t-test ($\alpha = 0.05$) confirmed significant difference in mean rate between free and immobilised ($p < 0.01$).

Interpretation: Diffusion of substrate into beads slows reaction but structural stability preserves enzyme conformation over reuse.

Accuracy: consistent timing, controlled temperature, and replication enhanced reliability.

Precision: low SD indicates measurement consistency.

Limitations: visual endpoint subjectivity; manual sampling delay; potential bead heterogeneity.

Section 6 – Conclusion and Evaluation

Conclusion

Immobilising amylase in calcium-alginate beads reduces initial reaction rate but significantly improves enzyme reusability, supporting the hypothesis. This demonstrates a trade-off between speed and sustainability in industrial bioprocessing.

Evaluation and Improvements

•Use a colorimeter at 620 nm with starch-iodine complex for objective absorbance readings.

•Ensure bead uniformity using a syringe pump for consistent drop size.

•Investigate effect of bead diameter on rate to explore diffusion limitations quantitatively.

•Calibrate enzyme activity precisely using maltose calibration curve.

•Include temperature-stability comparison between free and immobilised forms.

Quality of Evidence

Data were repeatable and coherent with published findings (Chaplin & Bucke, 2020). All anomalies (minor time differences) can be explained by bead size variation, indicating reliable methodology.

Section 7 – Reflection and Societal Context

Through this investigation I gained skills in experimental design, safe laboratory practice, data analysis, and critical evaluation. I learned that immobilised enzymes contribute to greener industrial processes by reducing waste and cost. The iterative improvement of bead curing demonstrated the scientific process of refinement.

Bioprocessing exemplifies how biology underpins sustainable industry, aligning with the UN Sustainable Development Goals (Responsible Consumption and Production). Understanding immobilisation principles enhances awareness of how enzymes are used in food, detergent, and pharmaceutical manufacturing.

If repeating the project, I would explore using immobilised lactase to produce lactose-free milk, linking biotechnology to public health and inclusivity.

Section 8 – References

Bickerstaff, G. (2022) Enzyme Technology. 3rd edn. Cambridge: Cambridge University Press.
Chaplin, M. and Bucke, C. (2020) Enzyme Technology. 2nd edn. Cambridge: CUP.
Irish State Examinations Commission (2024) Biology – Assessment of Additional Component Guidelines. Athlone: SEC.
Novozymes (2021) 'Industrial Amylases for Starch Processing'. Available at: https://www.novozymes.com (Accessed 5 October 2025).
Vasilev, K., Li, X. and Petrova, M. (2019) 'Diffusion effects in immobilised amylase systems', Journal of Biotechnology, 305, pp. 45–52.