Enzymes and Bioprocessing AAC – Sample Report C

Title: Comparing the Efficiency of a Packed-Bed Bioreactor and a Batch Reactor Using Immobilised Amylase

Section 1 – Title and Introduction

Bioprocessing industries often employ continuous reactors to achieve higher product yield and enzyme stability. Immobilising enzymes enables reuse and allows for continuous flow operation, improving sustainability and cost-effectiveness.

In this investigation, a simple packed-bed reactor (PBR) built from a separating funnel containing immobilised amylase beads was compared to a batch reactor using the same enzyme and substrate under identical conditions.

Research question:
Does a packed-bed reactor containing immobilised amylase produce starch hydrolysate more efficiently than a batch reactor under the same conditions?

Hypothesis:
It is predicted that the packed-bed reactor will deliver a steadier and more consistent throughput of starch breakdown over time than the batch reactor, even if initial rate is slightly lower due to diffusion limitations within beads.

Section 2 – Background Research

In continuous bioprocessing, substrate passes through a column containing immobilised enzyme. Mass-transfer limitations may reduce the instantaneous rate, but product can be collected continuously (Doran, 2020). Batch systems, by contrast, rely on mixing enzyme and substrate in one vessel, leading to a fall-off in rate as substrate is depleted (Najafpour, 2015).

Entrapment of enzymes in calcium-alginate beads is a well-established immobilisation technique; beads are chemically inert and allow repeated use (Bickerstaff, 2022). Industrial packed-bed reactors are used in the production of glucose syrup, lactose-free milk, and biofuels.

Secondary data show that immobilised amylase retains > 80 % activity after multiple cycles, while batch free enzyme loses most activity after one run (Chaplin & Bucke, 2020).

Quality of sources:
Peer-reviewed journals and textbooks (2015–2024) were consulted; all agree on enhanced stability but lower diffusion-controlled rate, confirming reliability.

Section 3 – Designing and Planning

Table of Variables

Variable Type	Variable	How Controlled/ Measured
Independent	Reactor mode (batch vs packed-bed)	Constructed as two systems using same enzyme amount
Dependent	Rate of starch hydrolysis (mL hydrolysed per min) and degree of conversion (qualitative iodine test)	Collected fraction volumes / colour change time
Controlled	Substrate (1 % starch), pH 7 buffer, temperature 37 °C, enzyme loading, bead volume, flow rate (≈ 1 mL min^{-1}), reaction time (10 min per run)	Kept constant for fairness
Safety	Hot water, glassware breakage, enzyme contact	Goggles and lab coats worn; retort stand secured

Equipment & Materials

Separating funnel (250 mL) with stopcock, beakers, conical flasks, tubing, retort stand, thermometer, graduated cylinders, droppers, water bath (37 °C), ice bath, 1 % sodium alginate, 0.15 M calcium chloride, 1 % amylase solution, 1 % starch solution, phosphate buffer pH 7, iodine solution, stopwatch.

Method Summary

1. Prepare alginate beads: Mix 10 mL 2 % sodium alginate with 10 mL amylase; drop into 100 mL 0.15 M $CaCl_2$; cure 10 min; rinse.
2. Assemble packed-bed: Place cotton plug in stopcock end of separating funnel; fill with equal volume of beads (~50 mL); mount on retort stand above collection flask.
3. Maintain temperature: Immerse column in 37 °C water bath.
4. Run packed-bed: Pour 100 mL 1 % starch into column; open stopcock to maintain constant flow (~1 mL min^{-1}). Collect 10 mL fractions each minute for 10 min.
5. Batch run: Place equal volume of beads and 100 mL starch in a conical flask; keep in same water bath; stir gently. Take 1 mL samples each minute for iodine testing.
6. Qualitative test: Add 1 drop sample to iodine on spotting tile; record colour (blue-black = starch, brown/yellow = hydrolysed).
7. Replicate: Repeat each reactor type three times.me.

Fairness, Accuracy, and Safety

- Identical enzyme and substrate volumes ensured valid comparison.
- Flow rate monitored using graduated cylinder and stopwatch.
- Water-bath kept ± 0.5 °C.
- Safety: secured glassware, goggles, avoided CaC_2 (replaced by $CaCl_2$ for safety).

Section 4 – Conducting the Experiment

The experiment was performed under supervision in the biology laboratory. The packed-bed operated smoothly, giving a steady drip rate of 1 mL min^{-1}. Batch reactions showed visible clearing of starch after several minutes.

Minor adjustments: increased bead curing to 15 min to improve strength and prevent compression in column. All primary data recorded in the lab book and photographed for authentication.

Section 5 – Data and Analysis

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

Reactor Type	Time (min)	Volume Collected (mL)	Iodine Colour Score (0 = no starch, 5 = deep blue)	Mean Rate (mL min^{-1})
Packed-bed	1 – 10	10 per min (steady)	4 → 0 gradually	1.0 ± 0.05
Batch	1 – 10	– (mixed system)	5 → 0 by 7 min	Initial rate ≈ 1.3 ± 0.07 mL min^{-1}

(Representative summary – full tables logged in appendix)

Graph 1 – Rate of Product Formation vs Time
Batch curve = rapid increase then plateau after 7 min; packed-bed = linear steady rate across 10 min.

Graph 2 – Colour Score vs Time for Packed-Bed
Score declined uniformly from 4 → 0 over 10 min, showing gradual conversion.

Analysis
•Both reactors achieved full starch hydrolysis (iodine = brown/yellow).
•Packed-bed produced consistent throughput (1 mL min^{-1}) – no drop in rate over time.
•Batch showed higher initial rate but complete exhaustion after ~7 min.
•Reusability test (three runs): packed-bed retained ~90 % rate after third use; batch activity decreased to < 60 %.
•Standard deviations < 0.07 mL min^{-1} → high precision.

Interpretation: Steady output from packed-bed confirms efficiency for continuous processing. Diffusion within beads limits instantaneous speed, but enzyme protection and reusability outweigh this drawback.

Accuracy: Flow rate constant; equal enzyme loading validated; anomalies (slight flow fluctuation ± 0.05 mL min^{-1}) logged as within tolerance.

Statistical summary: Two-sample t-test ($p < 0.05$) shows significant difference between mean rates, confirming distinct reactor behaviours.

Section 6 – Conclusion and Evaluation

Conclusion

The packed-bed bioreactor provided a steady, reproducible throughput of starch hydrolysis, whereas the batch reactor showed faster initial reaction but rapid decline as substrate was consumed. The packed-bed system therefore demonstrated superior operational stability and reusability, supporting the hypothesis.

Evaluation

•Results aligned with industrial literature showing continuous reactors give greater productivity over time.
•Strength: identical enzyme quantities and temperature control ensured valid comparison.
•Limitation: qualitative colour test subjective; no direct quantification of maltose produced.

Suggested Improvements

1.Use a colorimeter to measure absorbance of starch-iodine complex for quantitative rate.
2.Incorporate flow-meter to measure precise residence time and volumetric productivity.
3.Investigate different bead sizes or packing densities to optimise mass transfer.
4.Evaluate long-term operation (> 30 min) to model industrial continuous processes.

Quality of Evidence

Triplicate runs and low variance indicate good precision. Data trend matches theoretical expectation and published studies (Bickerstaff, 2022; Doran, 2020). Minor uncertainties do not alter overall interpretation.

Section 7 – Reflection and Societal Context

This investigation deepened my understanding of bioprocess design and the importance of variable control and replication. Constructing a functional column from basic equipment demonstrated how scientific principles can be applied creatively to real-world problems.

I learned that continuous processes reduce energy waste and enzyme loss, supporting sustainable manufacturing and circular-bioeconomy goals. Understanding the advantages of packed-bed systems connects biology with engineering and green technology.

If I repeated the work, I would extend it by measuring glucose output using test strips and compare with a computer-controlled flow system, bringing it closer to industrial practice.

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.
Doran, P. M. (2020) Bioprocess Engineering Principles. 3rd edn. Amsterdam: Elsevier.
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