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Fluidized Bed Low-Cost Desktop Learning Module (LCDM) with Heterogenous Catalytic Reaction

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Work-in-Progress: A Low-Cost Desktop Learning Module Using an Immobilized Enzyme Fluidized-Bed Reactor to Teach Kinetics and Transport

1. Introduction

Unit operations laboratories are most effective when students connect a visible experimental signal to quantitative analysis and then to an engineering interpretation. Desktop Learning Modules' experiments can strengthen this connection when they require students to make operational decisions, produce data under time constraints, and justify interpretations using established models rather than only following cookbook procedures [1], [2]. Enzyme-catalyzed model reactions are well suited for this purpose because they can provide clear time-dependent signals while remaining safe and practical for repeated instructional use.

In this work, we use a widely applied beta-galactosidase colorimetric assay to support a complete experimental workflow within an operations laboratory setting. In the ONPG assay, beta-galactosidase cleaves ONPG to form o-nitrophenol and galactose, producing a yellow product, ONP, that can be monitored by absorbance in the visible range, commonly within 410 to 420 nm [3], [4]. We extend this assay by immobilizing the enzyme inside hydrogel beads and operating the beads in a fluidized-bed column [5]. By comparing two bead diameters under a consistent workflow, students observe how transport length scale influences apparent performance in an immobilized system. This observation motivates discussion of internal diffusion limitations and their influence on apparent rate behavior, commonly framed using effectiveness-factor and reaction–diffusion concepts [6]– [8]. This module is designed as a single, low-footprint experiment that links measurement, kinetics extraction, and transport-limited reactor interpretation within the same instructional workflow. In contrast to typical unit operations labs where kinetics and transport are treated in separate activities or via pre-provided datasets, students generate a time-resolved spectrophotometric dataset, estimate kinetic parameters from a free-enzyme assay, and then interpret immobilized-bead performance using a controlled bead-size change as a transport length-scale perturbation [1], [2], [6]–[8].

2. Module overview and implementation context

The module is delivered across a multi-week sequence within an operations laboratory course. Students meet twice per week with three-hour lab periods. A manual and worksheet guide the activity from bead preparation through reactor operation and data analysis, with checkpoints designed to ensure that calculations and plots match expected physical behavior before teams proceed. Teams consist of three students. The workflow requires coordinated division of responsibilities because bead preparation, reactor operation, timed sampling, spectrophotometer measurements, and data reduction must be completed within a fixed laboratory period. Collaboration is embedded in the design through required role allocation and real-time decision-making during operation and troubleshooting. Teams discuss unexpected trends, select corrective actions, and repeat runs when outputs are not consistent with the measurement chain or model assumptions. Instructor checkpoints emphasize measurement consistency, timing discipline during sampling, and conceptual alignment of analysis outputs.

3. Apparatus and methods

3.1 Reactor configuration and operating mode

The reactor is a vertical column with a 1-in inner diameter and 12-in length, operated in recycle-batch mode using a 400-mL reservoir beaker. A pump withdraws the substrate solution from the beaker into the

column inlet, and the column outlet returns it to the same beaker. Samples are taken at 10-min intervals over a 60-min instructional window, and waste is collected in dedicated containers.

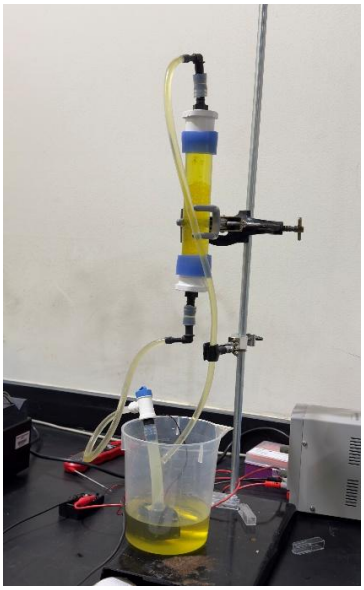

Fig. 1. Desktop fluidized-bed reactor module used for the immobilized β-galactosidase experiment. The yellow color indicates formation of o-nitrophenol (ONP) from ONPG during enzymatic hydrolysis in the fluidized hydrogel-bead bed.

3.2 Substrate preparation

For bead-size comparison runs, 0.3 g ONPG is dissolved in 400 mL buffer. Using an ONPG molecular weight of 301.25 g/mol, the initial ONPG concentration is 2.49 mM [3].

3.3 Bead formulation and sizing

Beads are prepared using a syringe pump and droplet method in 0.1 M calcium chloride with 3 percent alginate. Two bead diameters are used, nominally 2 mm and 4 mm, with curing times of 10 min and 30 min, respectively. Beads are rinsed with buffer after curing. Each bead batch is prepared from a 30 mL total bead-mixture solution. The enzyme portion is 8 mL per batch and has an activity of 2,600 U/mL. The remaining 22 mL consists of buffer and gelling components, including alginate and gelatin. Alginate entrapment is widely used because encapsulation occurs under mild conditions while retaining permeability to small substrates and products [9]. Bead size and structure depend on needle gauge, formulation, and calcium crosslinking conditions [10].

3.4 Flow-rate measurement and fluidization target

Students adjust pump flow until a stable and visually desirable bed expansion height is achieved. After stabilization, the volumetric flow rate is measured by collecting the reactor outlet and recording the time required to fill 100 mL. The measured flow rate is used in worksheet calculations related to reactor operation and interpretation.

3.5 Sampling and spectrophotometry

Samples are read immediately at 415 nm in a quartz cuvette. The procedure emphasizes consistent timing

and handling across time points. ONP concentration was calculated by converting the measured absorbance at 415 nm to concentration using the calibration relationship in Fig. 2:

$$A_{415} = m \cdot C_{ONP} + b$$

Thus, the following expression was used for all reported concentration values:

$$C_{ONP} = \frac{(A_{415} - b)}{m}$$

3.6 Safety considerations

The experiment uses dilute aqueous buffer, calcium chloride, alginate/gelatin, and ONPG/ONP at millimolar levels. Standard PPE (safety goggles) is required; gloves and additional protection are used as needed when handling hazardous materials, and solutions are handled on a spill tray to prevent staining from the yellow ONP product. Waste is collected in labeled containers and disposed of in accordance with institutional guidance for aqueous organic-containing lab waste; glass cuvettes are rinsed immediately after use to prevent residue buildup.

4. Stability and operating envelope

Bead integrity is required for instructional use because dissolution or cracking interrupts sampling and reduces data quality. Early trials showed bead dissolution within the time scale of a run, motivating adjustment of bead formulation and buffer selection to preserve bead integrity while maintaining a strong optical signal at 415 nm. Alginate bead stability can depend on ionic environment and exposure conditions, and swelling or degradation behavior has been reported in aqueous media depending on crosslinking and ion exchange [10], [11].

Enzyme stability was addressed in two ways. First, buffer and pH were selected to maintain functional activity during the instructional operating window; in less controlled conditions, including deionized water exposure, reduced activity and loss of function were observed, motivating the use of the selected buffer system. Second, enzyme retention within the bead matrix was evaluated through a qualitative leaching check. Beads were fluidized for one hour in 400 mL buffer without substrate, then an outlet sample collected at the end of the run was challenged with substrate. No color development was observed, indicating no detectable enzyme leaching into the bulk under the test conditions during the one-hour operating window. Under the current formulation and operating conditions used in the module, beads retained their shape and did not crack or dissolve during the one-hour instructional run. In additional testing under the same conditions, beads remained intact and functional under continuous fluidization for up to three hours.

5. Data analysis approach

A calibration curve is used to convert absorbance at 415 nm to product concentration and reinforce the measurement chain from instrument output to engineering interpretation. For immobilized-bead reactor runs, the bead-size comparison is intentionally reported using a slope comparison of product concentration versus time over the one-hour sampling interval.

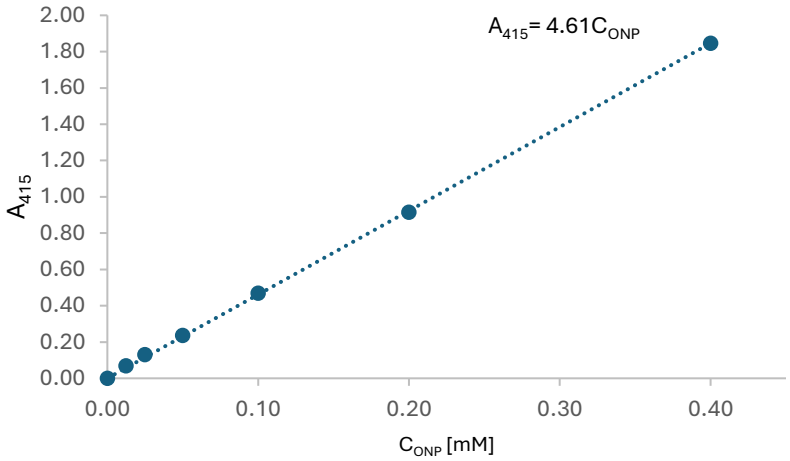

Fig. 2 Calibration curve for the ONPG assay at 415 nm. A_{415} is absorbance (dimensionless), and C_{ONP} is o-nitrophenol concentration (mM).

Free-enzyme assays at multiple substrate concentrations are used to estimate kinetic parameters and reinforce rate analysis. Initial rates are obtained from early-time spectrophotometric trends and analyzed using Michaelis–Menten kinetics. A Lineweaver–Burk linearization is used by plotting 1/v versus 1/[S], allowing estimation of $Vmax$ and Km from the intercept and slope [12], [13].

5.1 Relevant equations

ONP calibration (linear):

$$A_{415} = m \cdot C_{ONP} + b$$

ONP concentration from absorbance:

$$C_{ONP} = \frac{(A_{415} - b)}{m}$$

Initial rate:

$$V_0 = \frac{dC_{ONP}}{dt} |t_{\to 0}$$

Michaelis–Menten:

$$V_0 = \frac{Vmax \cdot C}{Km + C}$$

Lineweaver–Burk:

$$\frac{1}{V_0} = \frac{Km}{V\max} \cdot \frac{1}{C} + \frac{1}{V\max}$$

where A415 is the absorbance measured at 415 nm, m is the calibration-curve slope, CONP is the o-nitrophenol concentration, and b is the calibration intercept. In the kinetic expressions, V0 is the initial reaction rate, t is time, and C is the initial ONPG substrate concentration. $Vmax$ is the maximum reaction rate, and Km is the Michaelis constant. For the Lineweaver–Burk plot, $Km/Vmax$ corresponds to the slope, and $1/Vmax$ corresponds to the y-intercept.

6. Representative results and discussion

6.1 Bead-size comparison in the recycle-batch reactor

Figure 3 shows product concentration, C_{ONP}, as a function of time for 2 mm and 4 mm bead conditions over the first 60 min. The two bead sizes were evaluated in separate runs under the same workflow, and the 60-min interval is reported because it matches the laboratory sampling and measurement window. Linear fits yield slopes of 0.0009 mM/min for 2 mm beads and 0.0003 mM/min for 4 mm beads, with enzyme amount, pH, temperature, and all other conditions held constant. The slope ratio is 3.0, indicating that the smaller bead produced the product approximately three times faster than the larger bead over the reported interval.

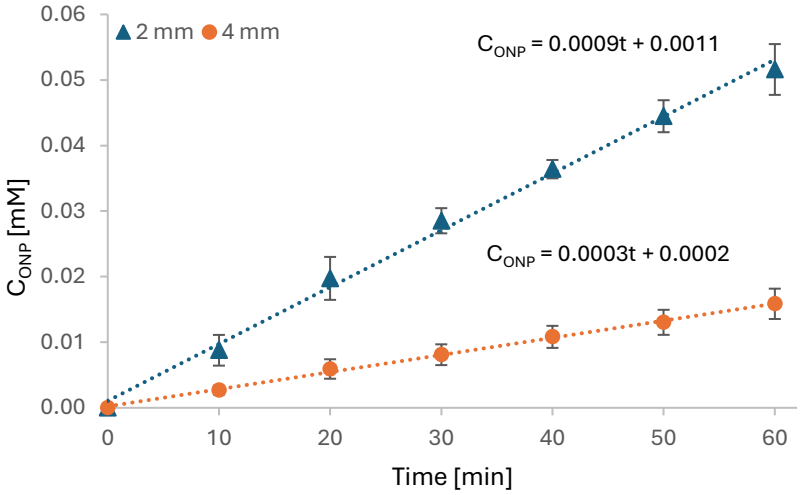

Fig 3. Product concentration versus time in the recycle-batch fluidized-bed reactor for 2 mm and 4 mm beads. C_{ONP} is the concentration of o-nitrophenol (mM). Points show mean ± SD (n = 3).

This contrast provides a clear basis for discussing how particle size can influence observed performance in immobilized enzyme systems. Internal diffusion can reduce apparent rates relative to intrinsic kinetic rates, and effectiveness-factor frameworks connect the magnitude of this reduction to particle size, diffusivity, and kinetic behavior [6]–[8]. The bead-size comparison, therefore, creates a direct, measurable link between a physical design variable and a time-series reactor signal suitable for interpretation in a unit operations laboratory setting.

6.2 Free-enzyme kinetics and parameter estimation

Free-enzyme assays were conducted at multiple ONPG concentrations using spectrophotometric measurements at 415 nm. Initial rates were obtained from early-time trends and analyzed using a Lineweaver–Burk linearization. In this form, V_0 represents the initial reaction rate, and S represents the initial substrate concentration, so Figure 4 plots $1/V_0$ versus $1/C_{ONPG}$. From the linear regression, the intercept equals $1/Vmax$, and the slope equals $Km/Vmax$, consistent with the Lineweaver–Burk form of the Michaelis–Menten equation [4]. Using these relationships, the estimated free-enzyme parameters were $Vmax$ = 0.09 mM/s and Km = 12.2 mM.

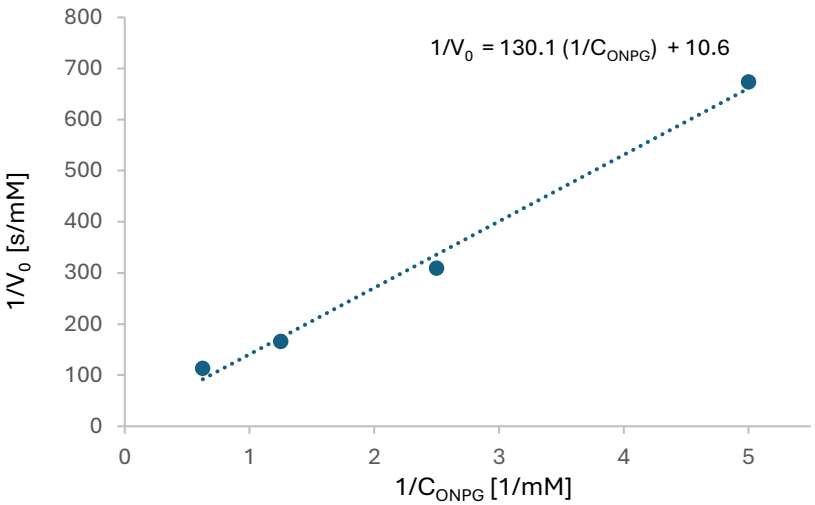

Fig 4. Lineweaver–Burk plot for free-enzyme kinetics. V_0 is the initial reaction rate (mM/s), and C is the initial ONPG concentration (mM); the plot shows $1/V_0$ versus 1/C.

In this module, the analysis reinforces how kinetic parameters are extracted from experimental data and how models are directly connected to measured time-series behavior. The free-enzyme dataset also provides a baseline kinetic reference for discussing why the behavior of immobilized-bead reactors can differ under transport-limited conditions. The free-enzyme assay provides an intrinsic kinetic baseline, $Vmax$ and Km, without internal diffusion resistance, whereas the immobilized-bead reactor exhibits an apparent rate that is sensitive to bead size, consistent with transport limitations. In the module, students use the free-enzyme kinetics as a reference and then interpret differences in reactor time-series behavior as a combined effect of immobilization and diffusion length scale (bead diameter) within the beads.

6.3 Student Feedback from Initial Implementation

Student feedback from the initial implementation indicated increased understanding of fluidized-bed enzymatic reaction behavior, with students specifically noting gains in enzyme kinetics and the role of diffusion/mass-transfer limitations alongside familiar hydrodynamic concepts (e.g., fluidization behavior). Students also reported improved confidence in core laboratory skills (solution preparation, temperature control/gelation, bead synthesis, and reactor start-up) and identified bubble control during alginate–enzyme preparation as a key practical challenge, as bubbles can compromise bead structure and fluidization.

7. Conclusions and next steps

This Work-in-Progress paper presents a low-cost desktop learning module using the ONPG to o-nitrophenol reaction in a recycle-batch fluidized-bed reactor with immobilized beta-galactosidase to teach reaction engineering. Results show a bead-size effect over one hour, with smaller beads producing product three times faster than larger ones, based on concentration versus time slopes. Free-enzyme assays yield $Vmax$ = 0.09 mM/s and Km = 12.2 mM using Lineweaver–Burk analysis. Ongoing refinements aim to clarify measurement steps and reduce instrument errors. Future work will fit reactor data to a reaction–diffusion model in MATLAB to estimate an internal effectiveness factor and quantify bead-size impacts beyond slope comparison.

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