



Development and Implementation of a Remote Enzyme Kinetics Laboratory Exercise

Abhishek Bhattacharjee,^a Eileen Johnson,^a Pablo Perez-Pinera,^{a,b,c,d,e} and  Karin Jensen^a

^aDepartment of Bioengineering, University of Illinois Urbana-Champaign, Urbana, Illinois, USA

^bCarl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana, Illinois, USA

^cCancer Center at Illinois, University of Illinois Urbana-Champaign, Urbana, Illinois, USA

^dDepartment of Biomedical and Translational Sciences, Carle-Illinois College of Medicine, University of Illinois Urbana-Champaign, Urbana, Illinois, USA

^eDepartment of Molecular and Integrative Physiology, University of Illinois Urbana-Champaign, Urbana, Illinois, USA

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INTRODUCTION

Online course offerings for undergraduate degrees have been increasing in recent years. In 2019, prior to the abrupt onset of the COVID-19 pandemic, the U.S. Department of Education reported that 32.9% of undergraduate students had been enrolled in at least one online course at some point in their college careers (1). This trend reached an inflection point at the beginning of the COVID-19 pandemic in 2020, when the unprecedented disruption of traditional in-person learning models increased the demand for online course offerings beyond our capacity to create and deliver high-quality instructional materials remotely. In particular, the development of online biology laboratory courses, which often require costly equipment and pose biological safety risks, presents a formidable challenge resulting in a very limited number of available tools or online activities to teach introductory lab skills and concepts. It has been shown qualitatively that traditional classroom settings are well-suited for laboratory courses (2, 3) because they provide a setting where students value highly the contribution of practical applications to their learning experience (4), which is very difficult to recreate in online courses. Further, online courses without haptic feedback (5) or collaborative (active) learning (6) can result in decreased student engagement (4–6). Nonetheless, it is crucial to overcome these obstacles and provide students

with activities to nurture the development of technical skills, which are a critical part of a biology student's education (2).

To address these challenges, we developed an at-home enzyme kinetics lab for students enrolled in an online introductory biology lab course. The experiment consists of the digestion of lactose by the enzyme lactase that produces glucose, a molecule that can be easily and precisely quantified using a glucose meter, an economical and widely accessible measuring instrument (Fig. 1). Students first mixed lactose and lactase solutions and measured the production of glucose at seven time points. Lactose concentrations over time were then calculated using known glucose concentrations. Using product (glucose) and substrate (lactose) concentrations over time, lactase kinetic parameters could be computed. Here, we describe the equipment and protocols used by the students as well as sample data, instructor reflections, and implementation tips.

PROCEDURE

Materials and supplies

The reagents, supplies, and instructions for instructors to prepare for the lab are provided as Appendix 1 in the supplemental material while the protocol that students followed is provided as Appendix 2. Briefly, the equipment necessary for this activity includes a glucose meter and a set of micropipettes. Supplies included microcentrifuge tubes, 15- and 50-mL tubes, micropipette tips, and a tube rack. Provided reagents included 15 mL of 400 mg/dL lactose solution in 5% wt/vol bovine serum albumin (BSA) and two different brands of lactase tablets containing the same amount of lactase (IUs/tablet). The use of 5% wt/vol BSA allows for accurate detection of glucose by our glucose meter (OhCare

Address correspondence to Department of Bioengineering, University of Illinois Urbana-Champaign, Urbana, Illinois, USA. E-mail: pablo@illinois.edu, or kjens@illinois.edu.

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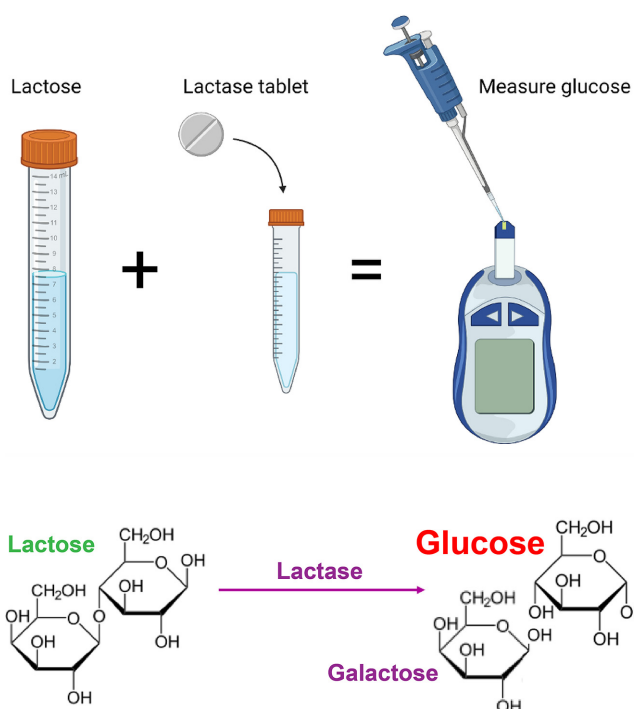


FIG 1. Schematic representation of the enzyme kinetics lab. Lactose solution is digested by lactase to generate glucose and galactose. The concentration of the glucose generated in the reaction is detected using a glucose meter. Created with BioRender.com.

Lite system). The lactose concentration was chosen to ensure student data falls within the detection range of the glucose meter (20 mg/dL–600 mg/dL). The materials and supplies were shipped to students in a cardboard box at ambient temperature, but students were instructed to refrigerate their lactose solution upon receipt. A return shipping label and instructions for returning materials and supplies were provided to students.

Remote laboratory session

The activity aimed to meet the learning objectives outlined in Table I. In weeks prior to this exercise, students were trained on how to use micropipettes (7) as well as best practices for recording data and observations in electronic lab notebooks. Before the lab, students were required to watch a prerecorded lecture that introduced enzymes, their use in biotechnology, and Michaelis-Menten kinetics. Students were also required to complete a prelab quiz on core concepts and comprehension of methodology. Then, students were provided with a protocol (Appendix 2) to measure the conversion of lactose into glucose

TABLE I
Lab activity learning objectives

1) Understand the Michaelis-Menten Kinetics Model
2) Design and conduct an experiment
3) Document lab data and observations
4) Use data to explain an experimental outcome

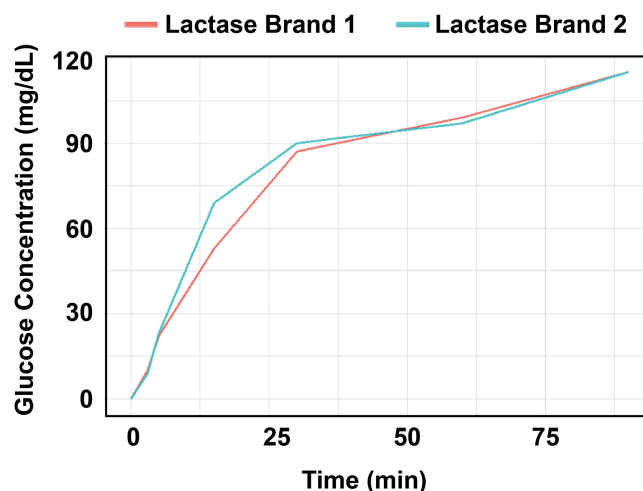


FIG 2. Typical concentrations of glucose over time measured by students. After mixing lactose and lactase, students used their glucose meters to record glucose concentration, the product of the enzymatic reaction, at time points 0, 3, 5, 15, 30, 60, and 90 min. Students were not required to generate this plot but were required to tabulate their data.

catalyzed by lactase (Fig. 1). Overall, the protocol described how to prepare a 7-point time course to measure production of glucose over 90 min. In a first synchronous laboratory session (10–20 students per section), students met with an instructor to review the protocol and discuss the experiment followed by a demonstration by the instructor to emphasize how the glucose meter could be used to measure the concentration of glucose in a sample. Students then met in breakout rooms to discuss possible variables to study and design of experiments to test these variables. Next, students completed a laboratory exercise aimed at comparing glucose formation over time using lactase from two different suppliers (Fig. 2). Students utilized their raw data to create two plots: (i) substrate concentration (mg/dL lactose) versus time (minutes) and (ii) reaction velocity (mg/dL/min) versus substrate concentration (mg/dL lactose). Plots were created using a custom Michaelis-Menten program that also reported K_m and V_{max} values for each of the two enzymes (8) that was adapted to use mg/dL concentrations compatible with the glucose meter readouts (Fig. 3). Students saved all their data, observations, and calculations in electronic laboratory notebooks, which were accessible to instructors for feedback and grading purposes.

Challenge laboratory exercise

After completing the comparison of the two different brands of lactase, students were challenged with an open-ended exercise to alter one variable in the experiment and record how the enzyme behavior varies. Most students completed the challenge laboratory exercise immediately after their main lab experiment. Common student alterations were boiling or freezing of the enzyme, resulting in a reduction of enzyme activity (Fig. 4). In a final synchronous session with instructors, students presented one-slide summaries of their experimental designs and results.

Enzyme Kinetics Fitting Tool

Time [min]	Substrate 1 [mg/dL]	Substrate 2 [mg/dL]
0	115	115
3	105	107
5	93	92
16.5	50	46
30	28	25
60	16	18
90	0	0

Fit Data

Solver:
 Nonlinear
 Absolute

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Enzyme 1
  Km = 29.465 mg/dL
  Vmax = 3.477 mg/dL/min

Enzyme 2
  Km = 31.352 mg/dL
  Vmax = 3.628 mg/dL/min
    
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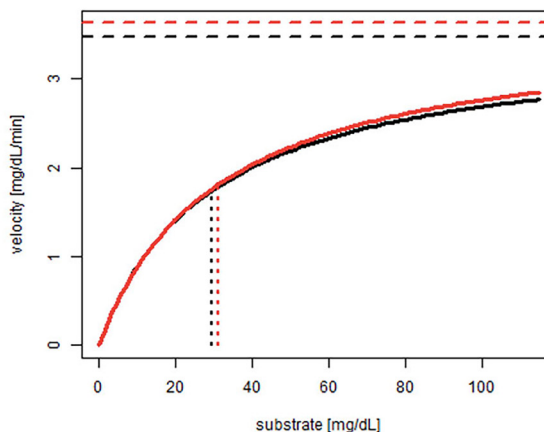
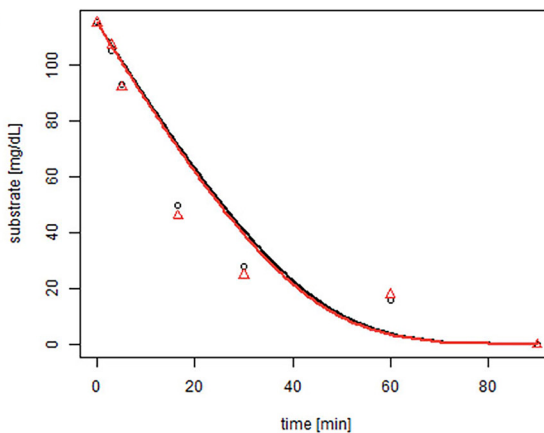


FIG 3. Michaelis-Menten program output. Students entered their lactose concentration calculations into the webtool along with the time points. The webtool then generated K_m and V_{max} values for both enzymes as well as substrate versus velocity and substrate versus time charts. Students used the K_m and V_{max} values to compare the properties of lactase enzymes from different brands and to determine whether modifications in the enzyme environment altered the kinetics of the reaction.

Safety issues

This lab conforms to the recommendations set by the ASM Guidelines for Biosafety in Teaching Laboratories. Students were required to complete online laboratory safety and biosafety training before completing the lab. Glucose and BSA solutions are nonhazardous, but students were still advised to wear gloves to encourage aseptic technique. Safety recommendations were also made for the equipment; specifically, it was advised that the glucose meter be kept away from small children due to its small lithium battery. Students were instructed to dispose of reagents in the regular trash. Returned equipment was tested for functionality and sanitized with 70% isopropanol.

Instructor reflection

Based on limited informal feedback from students (e.g., discussions with the teaching assistant during office hours), we believe that the remote enzyme kinetics laboratory

activity was received well. Several students reported that the enzyme kinetics lab was one of their favorite parts of the course. Students' presentations of the challenge activity results to the class reinforced core concepts and provided students an opportunity to practice presentation skills. Another aspect that worked well was the added competition; in the second synchronous lab session with the instructor, students presented their experimental design and results and were judged on the creativity selecting a variable to test and experimental design, by a team of two instructors. Prizes were awarded to the winning students. Additional gamification of learning activities has been shown to have positive effects of motivating students and improving learning outcomes (9). In addition to the class presentations, the breakout sessions where students discussed potential variables and their intended experimental design also increased student engagement during the synchronous sessions. Students found the glucose meters easy to use, and measurement by the glucose meters proved to be reliable in repeated tests (7). Overall, student data was consistent

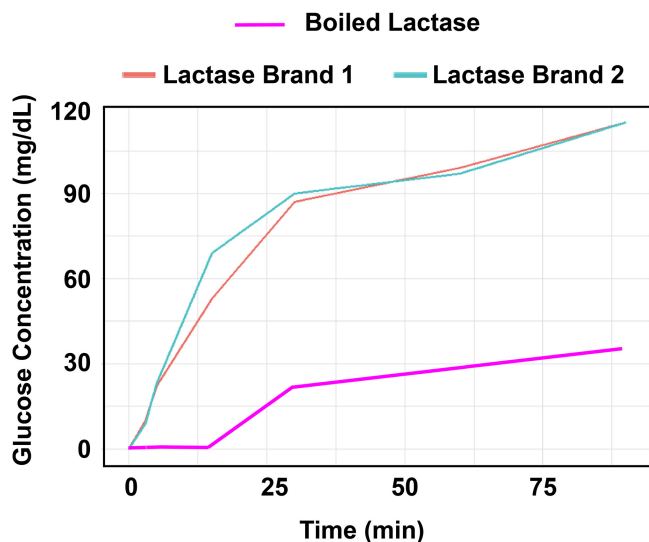


FIG 4. Students studied the kinetics of the reaction after modifying various parameters. In this example experiment, the enzyme was prepared with boiling water instead of room temperature water. This modification resulted in reduced enzymatic activity.

and reproducible. Based on these observations, the instructors recommend gamification and synchronous sessions to discuss experimental designs and results when implementing this activity.

DISCUSSION

Here, we report the development of a laboratory activity that can be completed remotely and provides students with hands-on experience involving enzyme kinetics using an at-home laboratory kit. Importantly, this activity takes advantage of readily available experimental components, such as measurement devices (glucose meter) and materials (lactase pills), which provide a low-cost alternative to traditional enzyme kinetics instructional labs. Additionally, students gained experience evaluating data and designing experiments. For example, students were able to analyze the kinetics of each enzyme at different concentrations to determine whether one has better kinetics or if the change is only due to differing amounts of enzyme. Students were excited to work with data generated by experiments they designed. Anecdotally, more students were willing to participate in discussions during the enzyme kinetics lab than during regular lecture/discussion time. The versatility of this activity and its associated kit make it usable in other educational settings, such as high school classrooms and science camps, where laboratory space may not be available. Due to the nature of the tools supplied in the lab kit, it can also be used to support multiple different lab activities (7), making it an economical option for a packaged online lab or remote science camp activities. An exit survey will also be

administered to students to gain their feedback and quantify their overall engagement, learning outcomes, and satisfaction.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.7 MB.

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