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Tips and Tools

Educational activity of enzyme kinetics in an undergraduate biochemistry course: invertase enzyme as a model

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ABSTRACT This article aims to simplify and facilitate the process of practical teaching of enzyme kinetics by utilizing minimal teaching laboratory requirements. Simultaneously, it ensures that students comprehend the enzyme kinetics experiment effectively. The focus is on teaching students how to estimate the maximum velocity (Vmax) and Michaelis constant (Km) of β-fructofuranosidase enzyme (also known as invertase) isolated from dry yeast. The invertase enzyme catalyzes the hydrolysis of sucrose substrate into glucose and fructose, employing the Michaelis-Menten approach of evaluating invertase enzyme kinetics as well as Lineweaver-Burk linear graphic approach of evaluating the Michaelis-Menten enzyme kinetics. The practical experiment seeks to reinforce the concepts of initial velocity dependence on substrate concentration. The data presented in the work were generated from a genuine practical biochemistry course enrolled by second-year undergraduate students in the Department of Pharmacy and the Department of Medical Laboratory Science. While there were minor variations in the invertase enzyme kinetic parameters among students, they successfully carried out the experiment. The students accurately estimated the Vmax and Km of the invertase enzyme in the sucrose hydrolysis chemical reaction. Moreover, they demonstrated an understanding of the meanings of the kinetic parameters (Km and Vmax) and the utility of the Lineweaver-Burk plot.

KEYWORDS education, practical biochemistry, invertase enzyme, enzyme kinetics, Vmax and Km

Enzymology is a significant area of biochemistry (1). It encompasses the study of enzyme structure, function, regulation, and kinetics (2, 3). Enzymes, specialized proteins serving as biological catalysts in living organisms (4), are a focal point in biochemistry education. Undergraduate practical biochemistry courses often introduce students to the basics of enzyme kinetics (5), emphasizing a hands-on approach for a better understanding of enzyme reactions and kinetics (4).

However, incorporating enzyme kinetics experiments into undergraduate education poses challenges for many tertiary institutions, whether due to limited resource availability or inadequately equipped laboratories, particularly in newly developed settings. Nevertheless, the teaching methodology plays a crucial role in effectively conveying the practical objectives to undergraduate students (6). In such cases, refining the educational methodology of enzyme kinetic experiments becomes essential.

This experimental tip addresses the challenge by providing affordable and readily available materials and instruments. Designed to be used with minimal hazards manageable through basic safety procedures, these resources facilitate the execution of the experiment in any teaching laboratory setting.

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Background

This enzyme kinetics experiment utilized invertase enzyme as a model to determine the maximum velocity (Vmax) and Michaelis constant (Km) of the enzyme. Invertase enzyme, also known as β-fructofuranosidase, is a globular protein that catalyzes the hydrolysis of sucrose disaccharide (table sugar) into glucose and fructose (7).

$$\text{Sucrose} + H_2O \xrightarrow{\text{invertase}} \text{glucose} + \text{fructose}$$

Invertase is naturally produced in various plants and microorganisms, including *Saccharomyces cerevisiae* (8). In this practical procedure, students track the sucrose hydrolysis reaction facilitated by invertase enzyme by measuring the concentration of glucose produced in the reaction tubes. The reaction conditions of invertase enzyme hydrolysis of sucrose in this procedure were set at a temperature of 30°C and a neutral pH. The reaction is allowed to proceed for a time interval of 20 min. Students measured the glucose concentration at different time points using a glucometer.

Intended audience

The current tip seeks to streamline the instruction of enzyme kinetics in a practical experiment to first- or second-year undergraduate students of allied health majors as well as non-major biochemistry undergraduate students from diverse backgrounds. Students in this level normally have no previous experience and require laboratory skills. The objective of this experiment is to facilitate a straightforward and comprehensive understanding of the enzyme kinetics topic. However, by the end of this activity, students should be able to define the different terms of enzyme kinetics. In addition, students should be able to estimate the kinetic parameters Vmax and KM from enzyme saturation data using direct fitting and Lineweaver-Burk methods.

The approach involves employing invertase enzyme as a model in the experiment to estimate both Vmax and Km, using the Lineweaver-Burk method to evaluate Michaelis-Menten kinetics.

PROCEDURE

Safety issues

These lab procedures adhere to the recommendations set by the ASM Guidelines for Biosafety in Teaching Laboratories. No hazardous materials were involved in this experiment; however, students were advised to wear personal protective equipment and adhere to safety measures throughout the procedure, as outlined in the orientation session conducted at the onset of their undergraduate studies.

Materials and chemicals

The following materials and chemicals are required in order to perform the experiment.

- Six test tubes and a rack
- Graduated cylinders and beakers
- Spatula
- 0.4 M sucrose stock solution
- Dry yeast
- Distilled water
- Different ranges of automatic micropipettes and tips
- Microscopic slides and disposable droppers
- Glucometer and strips
- 30°C Water bath

- Timer

Experimental methodology

Step-by-step students' instructions are provided in the worksheet supplementary file number (1) (Data not shown).

Preparing of invertase enzyme solution (Appendix: Part A)

The invertase enzyme solution is prepared in advance by the technical staff. This was done by weighing 0.25 g of dry yeast into a 500-mL glass beaker. The dry yeast was suspended in 250 mL warm distilled water (30°C). The suspension was left in the water bath of 30°C for 20 min with periodic stirring. The suspension was stored at 30°C in the water bath to serve as an invertase enzyme solution.

Substrate solutions (Appendix: Part B)

Students use automatic micropipettes to prepare various concentrations of sucrose substrate solutions by diluting a 0.4 M sucrose stock solution, as detailed in Table 1.

Preparing the reactions (Appendix: Part C)

Substrate solutions are pre-incubated in a warm water bath at 30°C for 10 min to bring the solutions to the reaction temperature.

To initiate the reactions, while the substrate solutions are in the water bath, students use an automated micropipette to add 1 mL of the invertase enzyme solution to each substrate reaction tube at a specific time, as outlined in Table 2. This time point marks the start of the chemical reaction of sucrose hydrolysis by invertase enzyme.

Reading glucose concentration (Appendix: Part D)

After 20 min from the initiation of the chemical reactions, the concentration of glucose produced in the reaction tubes due to sucrose hydrolysis by invertase enzyme is measured. Using a glucometer and glucometer strips, one drop from each reaction tube is placed on a microscopic slide. A glucometer strip was inserted into the glucometer and the strip was touched to the reaction solution on the microscopic slide in order to read the glucose concentration. The glucometer readings were recorded as per the time points outlined in Table 3.

CONCLUSION

The V_o of the invertase enzyme in each reaction tube, representing a specific sucrose concentration, was calculated based on the glucose concentration readings provided in Table 4.

To estimate the Vmax and Km of the invertase enzyme from the Michaelis-Menten curve (Fig. 1) using the Excel application, students Plot the sucrose concentrations on the *x*-axis and the reaction velocity on the *y*-axis. They estimated the Vmax from the *y*-axis

TABLE 1 Sucrose substrate solutions with various concentrations

Tube #	d. H_2O (mL)	Sucrose solution volume (mL)	Final concentration (M)
1	1	1 mL from 0.4 M sucrose stock solution	0.2
2	1	1 mL from tube 1	0.1
3	1	1 mL from tube 2	0.05
4	1	1 mL from tube 3	0.025
5	1	1 mL from tube 4	0.0125
6	1	1 mL from tube 5	0.00625

TABLE 2 Time point of starting the invertase chemical reaction

Elapsed time (min)	Tube #	Enzyme volume
10	1	1 mL
11	2	1 mL
12	3	1 mL
13	4	1 mL
14	5	1 mL
15	6	1 mL

TABLE 3 Time point for reading glucose concentration in the reaction tubes

Elapsed time (min)	Tube #	Enzyme volume	Glucometer reading (mg/dL)
30	1	1 mL	674
31	2	1 mL	537
32	3	1 mL	425
33	4	1 mL	288
34	5	1 mL	198
35	6	1 mL	162

TABLE 4 Average V_o calculation in the reaction tubes

Tube #	[Sucrose] (µmol/mL)	Average [glucose] (mg/dL)	[Glucose] (mmol/L) = mg/dL × 0.0555	[Glucose] (µmol/mL) = µmol/mL	Average V_o (µmol/min/mL)
1	2×10^{-4}	674			1.87
2	1×10^{-4}	537			1.49
3	0.5×10^{-4}	425			1.18
4	0.25×10^{-4}	288			0.80
5	0.12×10^{-4}	198			0.55
6	0.06×10^{-4}	162			0.45

value corresponding to the flat part of the curve. The Km is estimated from the sucrose concentration on the *x*-axis corresponding to half of the Vmax.

The curve exhibits the expected hyperbolic shape consistent with the Michaelis-Menten kinetic mechanism, reflecting the influence of substrate concentration on enzyme kinetics. However, accuracy in estimating enzyme kinetics may be compromised due to the continuous increase in reaction velocity in the flat part of the curve.

To estimate the Vmax and Km of the invertase enzyme from the Lineweaver-Burk curve (Fig. 2) using Microsoft Excel, students plot the inverse of sucrose concentrations on the *x*-axis and the inverse of reaction velocity on the *y*-axis. Then they estimated the Vmax from the *y*-intercept (equal to 1/Vmax), and Km is estimated from the *x*-intercept (equal to −1 /Km) of the generated straight line.

This enzyme kinetic practical experiment introduced the students to the basics of enzyme kinetics in an easy manner. Students were able to comprehend the different terms of enzyme kinetics through hands-on experiments and were able to estimate the Vmax and Km of invertase enzyme from the Michaelis-Menten curve and Lineweaver-Burk plot. These achievements were evident in the laboratory report the students submitted to the instructor in the following week. Individual reports were required by every student which include the Vmax and Km values estimated from the Michaelis-Menten curve and Lineweaver-Burk plot constructed manually on a graph paper or by spreadsheet.

All reports were marked and corrected to maximize students' learning outcomes.

Upon reflecting from students, they seem to have gained the understanding of the basic enzyme kinetics and were able to use spreadsheet to estimate the Vmax and Km of invertase enzyme employed in the experiment. Many students scored high marks in the lab report assessment.

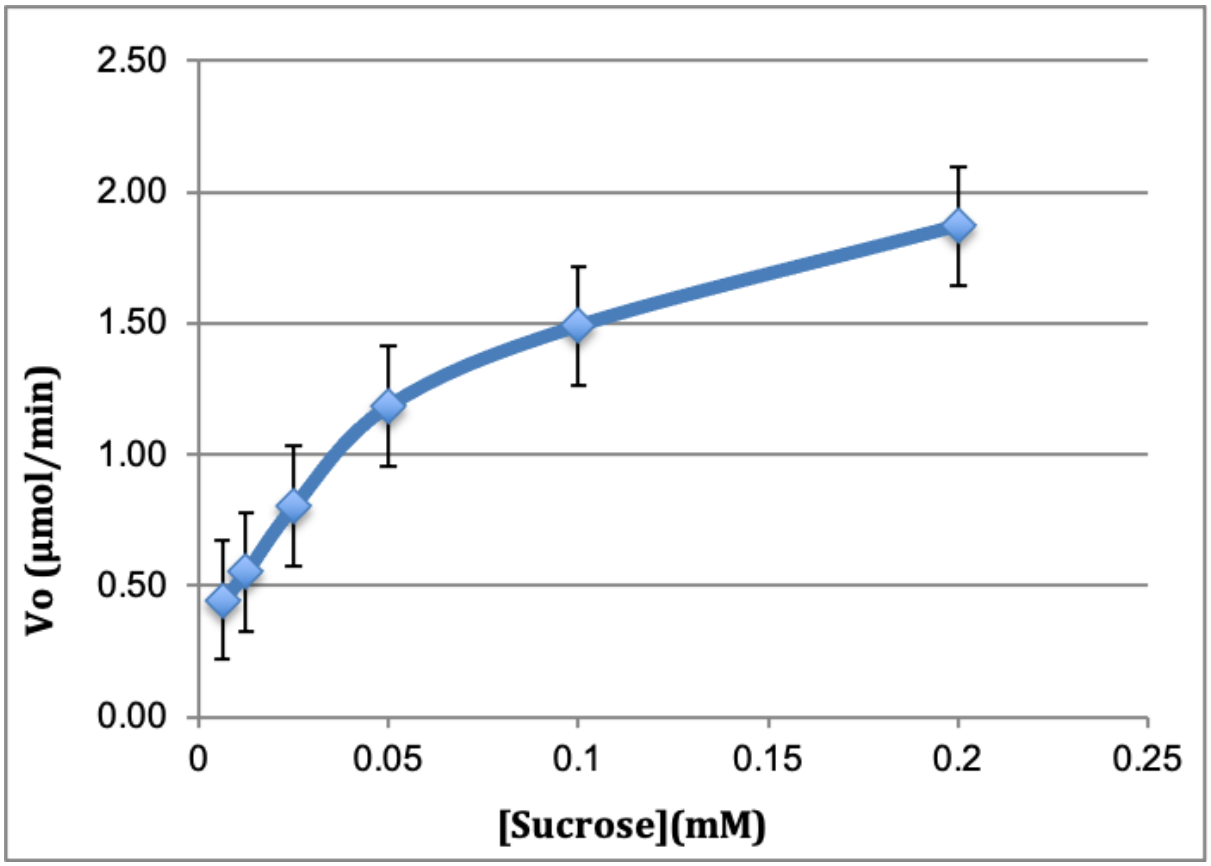

FIG 1 Michaelis-Menten curve of invertase enzyme.

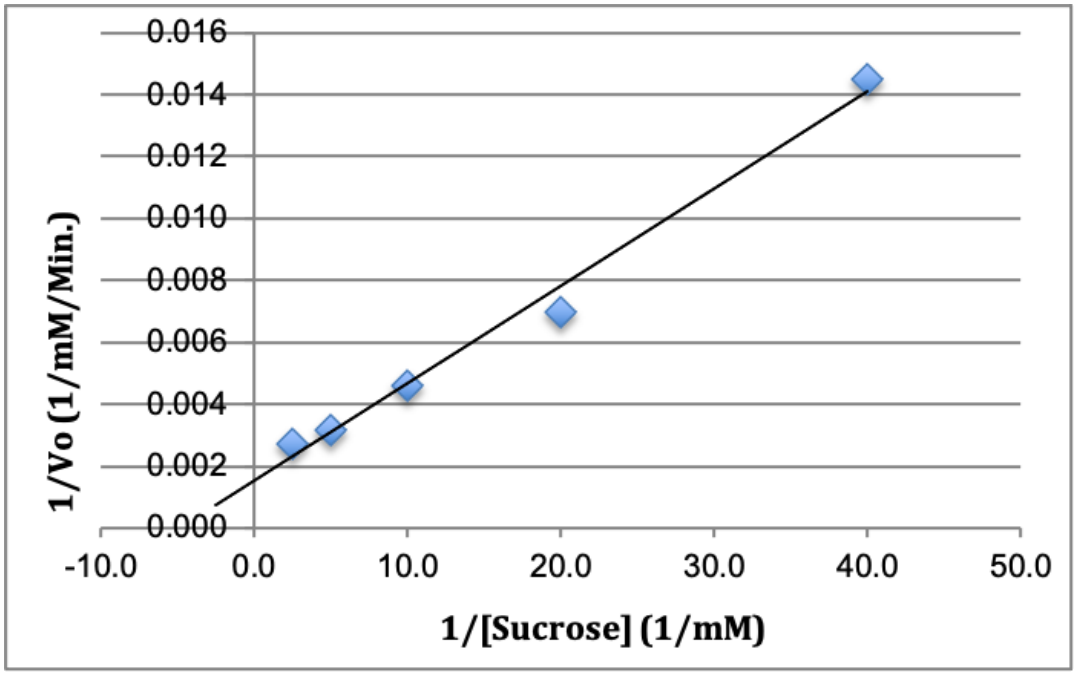

FIG 2 Lineweaver-Burk plot of invertase enzyme. $y = 0.0003\,x + 0.0015$. $R^2 = 0.9893$.

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ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental Material (jmbe00050-24-s0001.docx). Supplemental appendix (laboratory worksheet: enzyme kinetics).

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