INQUIRY & INVESTIGATION

Heating Up Enzyme Kinetics: A Safe, Inexpensive & Quantitative Inquiry Activity

RECOMMENDED FOR *AP Biology*

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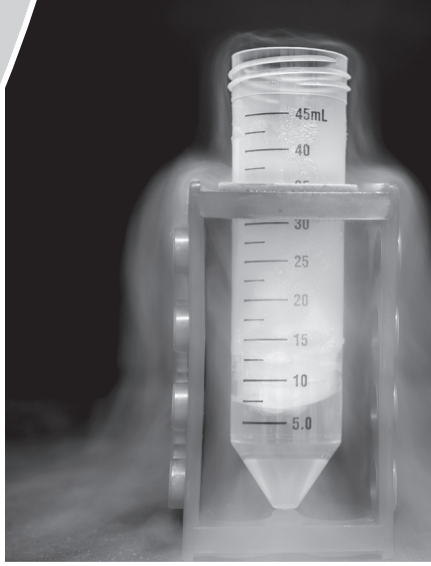

ABSTRACT

Enzyme kinetics is an essential process of life, but it is a concept that challenges introductory biology students. Most enzyme kinetics laboratory activities either use hazardous chemicals to visualize the enzyme product or do not afford quantitative analysis at all. We have designed an inquiry-based activity that provides insight into enzyme function and that is budget friendly, safe, and produces quantitative results. The materials required are household products, so this activity can be adapted even for online classes. Furthermore, we suggest options for a sequential, experiential experiment-design activity for students.

Key Words: *Experiential learning; inquiry-based; quantitative analysis; safe.*

○ Introduction: Inquiry Learning in the Biology Classroom

Scientific organizations such as the National Academy of Sciences (NAS) and the American Association for the Advancement of Science (AAAS), as well as educational organizations such as the College Board, affirm the importance of inquiry in science education (AAAS, 2011; National Research Council, 2012; College Board, n.d.). Inquiry-based, or experiential, learning involves the creation of new knowledge by the students themselves through an active process (Lawson et al., 1990). Inquiry learning can occur at a variety of levels, with each successive level involving less control by the instructor. While open or minimally guided inquiry is often touted as an ideal methodology in science education, attempts to implement this approach have had disappointing results, particularly for novice learners (Kirschner et al., 2006; Eastwell, 2009). In practice, guided inquiry, in which students explore a question scaffolded by the instructor, is much more common (Beck et al., 2014), and studies have shown superior learning gains for guided inquiry versus open or minimally guided inquiry (Lazonder & Harmsen, 2016). The nature of guided inquiry also makes it a good fit for the teaching laboratory. Recent meta-analyses have found a positive impact of inquiry-based practices on student learning across a number of studies at multiple institutions (Beck et al., 2014; Lazonder & Harmsen, 2016). Just as importantly, the use of inquiry learning methods also has a significant positive effect on student attitudes toward science, and this attitude improvement does not come at the cost of gains in student achievement (Beck et al., 2014; Savelsbergh et al., 2016).

A budget-friendly, nonhazardous process can be employed to quantitatively study enzyme kinetics.

○ Importance of Quantitative Skills in Modern Biology

The latest position papers from NAS, AAAS, and the Association of American Medical Colleges (AAMC) have all affirmed the central importance of quantitative skills in 21st-century biology education (AAMC-HHMI Committee, 2009; Labov et al., 2010; Woodin et al., 2010). The *Next Generation Science Standards* (NGSS) from the NAS list data analysis, data interpretation, and mathematical reasoning as crucial practices for the modern science classroom (National Research Council, 2012). Furthermore, it has been shown that integrating quantitative skills into the curriculum of a biology course has numerous positive outcomes. Students in such courses improved both their skill in applying mathematics to biological problems and their confidence in their own mathematical abilities (Rheinlander & Wallace, 2011; McCright, 2012; Hoffman et al., 2016). Furthermore, Hester et al. (2014) found that an increased

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emphasis on quantitative skills did not reduce students' mastery of basic biological content. Thus, it is essential that science educators make a concerted effort to provide students the opportunity to acquire necessary skills in numeracy and data analysis.

○ Practical Considerations for Laboratory Exercises

Investigation of the actions of enzymes is an essential part of an introductory biology laboratory course. Unfortunately, many of the available procedures possess certain drawbacks. One standard method of investigating enzyme function in a laboratory classroom is through the use of an artificial substrate, which produces a color change that can be measured by spectrophotometry. For example, many teaching laboratories examine the activity of a peroxidase using the artificial substrate guaiacol (Weinheimer & White, 2003). While this method provides abundant quantitative data, it requires that the instructor has access to expensive spectrophotometry equipment and supplies. Additionally, the artificial substrate may be costly, particularly for a larger course. Specialized organic reagents can also necessitate special handling; for example, the U.S. Environmental Protection Agency lists guaiacol as a skin, eye, and respiratory irritant, and it is a volatile chemical that produces an unpleasant odor in the laboratory. Special disposal procedures are also required for this chemical. At a time when budget shortfalls at the state and federal level have reduced educational funding, it has become especially important for educators to find ways to reduce costs in classroom laboratories without sacrificing the quality of students' experiences.

On the other hand, procedures that do not require such equipment and reagents may provide qualitative, subjective, or only semi-quantitative data (Clariana, 1991; Shmaefsky, 1993). Such results can still enhance student understanding of the nature of enzymes, an essential biological concept. However, they provide little or no potential for mathematical analysis. Instructors then lose a valuable opportunity to help students improve mastery of quantitative skills, such as the preparation and interpretation of graphs, in a biological context.

○ Rationale for the New Procedure

Here, we present a novel procedure for investigating enzyme activity in a classroom laboratory. This procedure takes advantage of the fact that the yeast species *Saccharomyces cerevisiae* produces a highly active catalase enzyme. Cleavage of hydrogen peroxide (H_2O_2) by this enzyme is an exothermic reaction, and the increase in temperature is readily measurable and is an indirect indicator of enzyme activity. Equipment and supplies required for the proposed experiment are minimal and nonhazardous. The experiment renders reliable quantitative data. Moreover, the initial procedure can be followed by a second class where it is extended into a guided inquiry. In the experiential activity, the instructor would provide only the baseline protocol and students would then design their own investigation of factors influencing enzyme function.

○ Methods & Materials

Before-Class Preparation

Activated Yeast Solution: Fleischmann's Active Dry Yeast obtained from a convenience store was used for the experiment. A 10% (w/v) suspension of yeast in 2% (w/v) sucrose solution was prepared by dissolving 0.5 g of sucrose in 25 mL of water and adding 2.5 g of yeast. This suspension was activated by incubating for 30 minutes at room temperature prior to performing the procedures. This volume of yeast suspension is sufficient to perform one set of the procedures described below. Fresh yeast suspension must be prepared and activated each time these procedures are performed.

Hydrogen Peroxide (H_2O_2): A freshly opened bottle of 3% hydrogen peroxide solution from a medical store was used as a substrate for the enzymatic reaction.

Thermometer: For measurement of temperature, a cylindrical Fisher Scientific digital thermometer (catalog no. S01595) was used. Similar results were obtained for baseline with a Carolina Biological digital pocket thermometer (catalog no. 745360). For data collection, temperatures were measured in degrees Fahrenheit because the temperature change is more pronounced compared to Celsius and therefore easier for students to note.

pH: 1 M NaOH solution (Carolina Biological, catalog no. 889573) was used to modify the pH for one of the experiments.

In-Class Procedure

A summary of the laboratory procedures can be found in Table 1.

Baseline: 0.5 mL of 3% H_2O_2 and 0.5 mL of water was added to a 50 mL graduated plastic tube (United Lab Plastics, catalog

Table 1. Summary of catalase enzyme activity laboratory procedures.

Procedure	Substrate		Enzyme		Total Volume (mL)
	3% H_2O_2 (mL)	H_2O (mL)	10% Yeast (mL)	H_2O (mL)	
Baseline	0.5	0.5	4	0	5
Temperature	0.5	0.5	4	0	5
2× Substrate	1	0	4	0	5
0.5× Enzyme	0.5	0.5	2	2	5
pH	0.5	0.5	4	0	5

Table 2. Temperature (°F) change indicating enzyme activity.

Time (sec)	Baseline	Temperature	pH	2× Substrate	0.5× Enzyme
0	72.5	72.3	72.0	72.5	72.3
10	75.5	72.3	72.5	77.1	74.0
20	75.5	72.3	72.5	77.9	74.7
30	76.0	72.3	72.5	78.0	74.9
40	76.1	72.3	72.5	78.0	75.0
50	76.1	72.3	72.5	78.3	75.0
60	76.2	72.3	72.5	78.2	75.0
70	76.2	72.3	72.5	78.2	75.0
80	76.2	72.3	72.5	78.2	75.0
90	76.2	72.3	72.5	78.1	75.0
100	76.2	72.3	72.5	78.1	75.0

no. UP2261). The thermometer was allowed to equilibrate with the temperature of this solution for 1 minute. Once the temperature became steady, 4 mL of activated yeast solution was added. The temperature of the solution was recorded immediately upon addition of yeast and then every 10 seconds for a total of 100 seconds (Table 2).

Temperature: To test the effect of temperature on the activity of the enzyme, 4 mL of the yeast solution was heated in a dry heat block at 100°C for 10 minutes; after heating, it was placed on ice for 4 minutes to rapidly cool, then finally equilibrated to room temperature for 5 minutes. This treated yeast mixture was then used in place of the 4 mL of activated yeast solution in the baseline procedure described above.

pH: To investigate the effect of pH on the activity of the enzyme, the pH of 4 mL of the yeast suspension was raised to pH 12.0 by the dropwise addition of 1 M NaOH. The pH was monitored using pH test strips. This mixture was equilibrated at room temperature for 2 minutes and then used in place of the 4 mL of activated yeast solution in the baseline procedure described above.

2× Substrate: To investigate the effect of changing substrate concentration on the activity of the enzyme, 1 mL of 3% H_2O_2 was substituted for the 0.5 mL of 3% H_2O_2 + 0.5 mL water. This substitution doubles the concentration of substrate in the test tube. The digital thermometer was allowed to equilibrate with the temperature of this solution by placing the thermometer in the solution for 1 minute. Once the temperature became steady, 4 mL of activated yeast solution was added as before, and the temperature change was recorded as described above.

0.5× Enzyme: To investigate the effect of changing enzyme concentration on the activity of the enzyme, 2 mL of activated yeast solution was added to 2 mL of water. The substitution of 4 mL of activated yeast solution with 2 mL of yeast solution and 2 mL of water effectively dilutes the enzyme in the reaction to half of the original concentration. This mixture was used in place of the 4 mL of activated yeast solution in the baseline procedure described above.

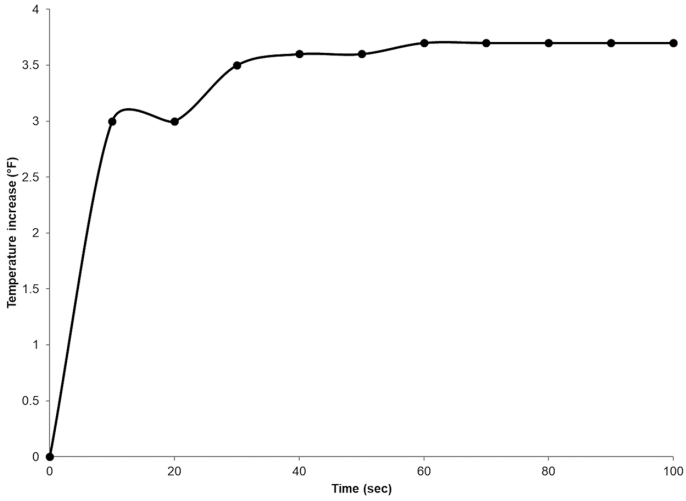

Figure 1. Temperature increase over time as a result of the exothermic reaction of yeast peroxidase. A suspension of yeast activated with sucrose was mixed with H_2O_2, and temperature increase was measured at 10-second intervals for 100 seconds.

Results

The 3°F increase in temperature in the baseline experiments confirmed that the experimental setup was efficient in capturing the indirect enzyme activity. Plotting the change in temperature as a function of time for the baseline procedure exhibits a classic enzyme activity graph (Figure 1). The reaction is quite rapid. Temperature increases 3°F within the first 20 seconds, and the reaction reaches completion less than 1 minute after addition of substrate. Results of all experiments are listed in Table 2.

Alterations in optimum temperature and pH of the enzyme substantially affect the progression of an enzyme-catalyzed reaction. Heating the enzyme to 100°C (Figure 2) or increasing the enzyme

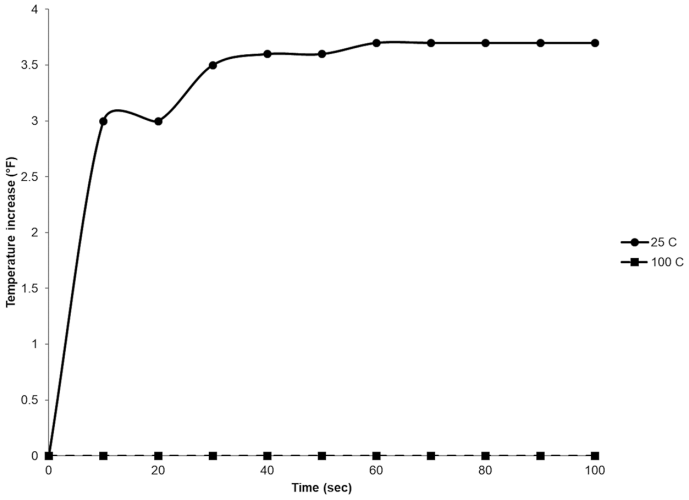

Figure 2. Effect of elevated temperature on the activity of yeast peroxidase. When yeast is heated to 100°C prior to conducting the enzymatic reaction, no enzyme activity is observed. Circles: baseline reaction. Squares: yeast heated to 100°C.

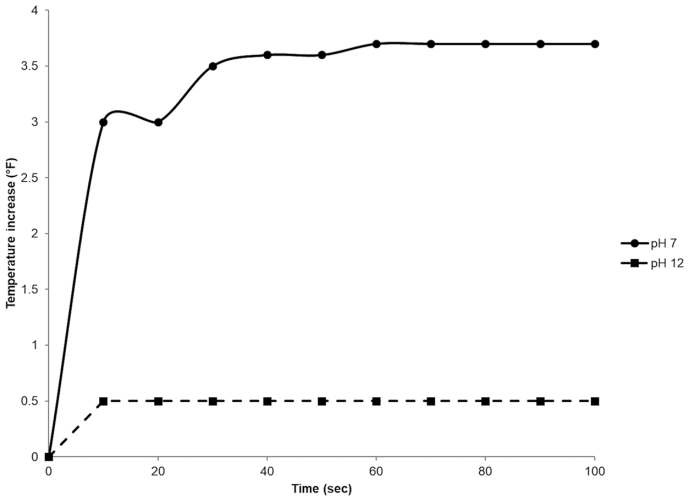

Figure 3. Effect of increased pH on the activity of yeast peroxidase. When pH is adjusted higher than optimal for this reaction, minimal enzyme activity is observed. Circles: baseline reaction. Squares: pH 12.

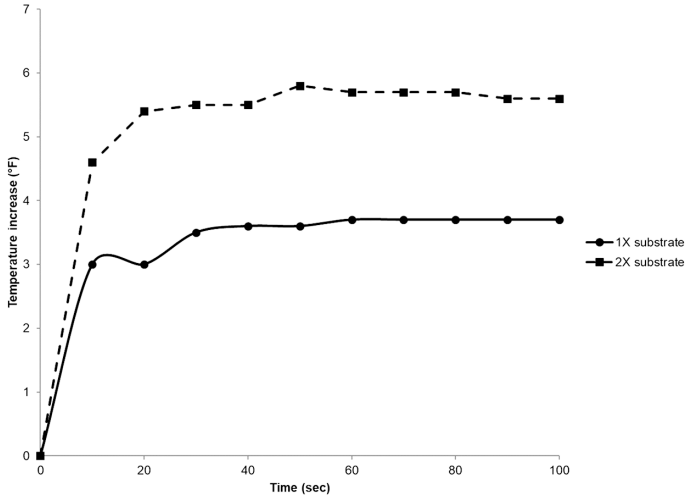

Figure 4. Effect of substrate concentration on the activity of yeast peroxidase. Increasing concentration of substrate results in an increase in enzyme activity. Circles: baseline reaction. Squares: increased substrate concentration.

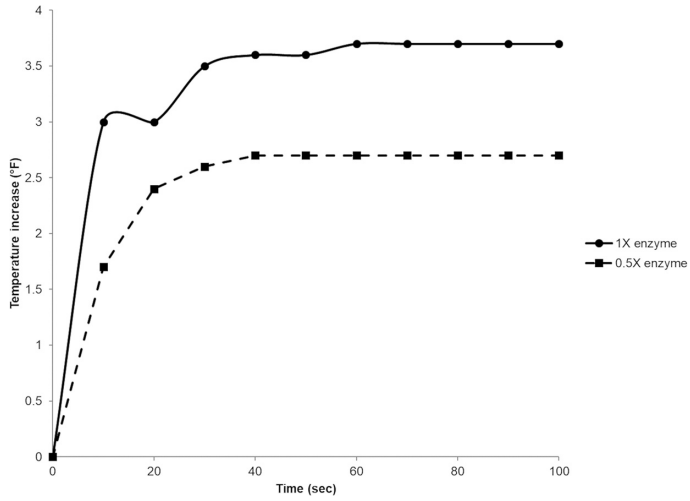

Figure 5. Effect of enzyme concentration on the activity of yeast peroxidase. Reducing concentration of enzyme results in a reduction in enzyme activity. Circles: baseline reaction. Squares: reduced enzyme concentration.

environment to pH 12 (Figure 3) prior to conducting the procedure eliminated measurable enzyme activity.

In addition to changes in temperature and pH, the concentrations of enzyme and substrate had an effect on enzyme activity. Substrate concentration was adjusted by altering the proportions of substrate (H_2O_2) and water that are added to the reaction, as described in Table 1.

The temperature increase was nearly double that observed at baseline (Figure 4) when substrate concentration was doubled. Likewise, when the yeast suspension containing the enzyme was diluted, the temperature increase was substantially less (Figure 5 and Table 2). In both cases, the change in enzyme activity, as measured by temperature increase, is directly proportional to the adjustment in substrate or enzyme concentration.

○ Discussion

Enzyme activity indirectly measured by increase in temperature followed a standard enzyme kinetics pattern as expected. We believe that the laboratory procedures presented here resolve several challenges for the teaching of enzyme kinetics in the introductory biology laboratory. First, the procedures are simple and inexpensive. The required equipment and supplies can be easily obtained, and

no special training is required for their use. Hence, these exercises can be used with learners at many different levels and at diverse types of institutions. Second, no hazardous materials are used in these procedures, eliminating risks to students and the necessity for specialized disposal practices. Finally, these procedures provide robust and highly replicable quantitative data that students can use for graphical analysis.

Graphs can be initiated in class with instructor guidance or given as homework, depending on the academic level of students. We conclude that these procedures align with the current emphasis on the development of quantitative skills in biology education (AAMC-HHMI Committee, 2009; Labov et al., 2010; Woodin et al., 2010).

The activities proposed here can be followed up by a guided-inquiry exercise in which students are provided the baseline procedure, and then they design an investigation to measure alteration of enzyme activity by variations on the factors examined in the first week. Student understanding of the baseline procedure can be assessed with a brief quiz or class discussion, allowing the instructor to clarify any points of confusion. Teams of students can then be tasked with designing procedures to examine the effects of various manipulations on the activity of this enzyme. For example, students can choose to evaluate the effect of 10× substrate or 0.25× enzyme concentration or pH 9. In this situation, the baseline procedure serves as a starting point, and students must apply their understanding of both enzyme kinetics and scientific practices in order to solve the problem.

For younger students or those who have limited experience with experimental design, it may be necessary for the instructor to either suggest or directly assign the design of certain procedures to the groups. For instance, one group could be tasked with investigating the effect of changing pH and another with investigating the effect of changing substrate concentration. More mature students can be offered more freedom to design procedures according to their own knowledge and interests. Additionally, more advanced students may also choose to investigate new factors, such as competitive or noncompetitive inhibitors. The instructor will be available for guidance during the class period, but the students themselves are responsible for selecting appropriate experimental parameters, including time, temperature, pH, sample numbers, and controls. Once their experimental design is approved by the instructor, the students can actually perform their procedures and share their experimental design and data obtained with other groups in the class. This presents a peer teaching opportunity, and hence a student engagement tool. Because the results are quantitative, they can undertake graphical analysis of their findings. As a final assessment, students can complete a written assignment such as a lab report. Rissing and Cogan (2009) reported significant learning gains in students who participated in a similar guided-inquiry laboratory module, compared to students who participated in a standard, "cookbook" style laboratory exercise.

In conclusion, a budget-friendly, nonhazardous process can be employed to quantitatively study enzyme kinetics. Furthermore, this process can be augmented to increase student investment if students are given the opportunity to design their own experiments and to evaluate parameters beyond those dictated by a lab manual. The activity serves as an experiential learning activity since the students are learning by editing the experimental design, by performing their own experiments, and by presenting the results in graphical form. For this team-based activity to be performed successfully the students have to think critically, solve issues with the experiment, and be open to and utilize feedback from the guiding instructor.

References

AAAS (2011). *Vision and Change in Undergraduate Biology Education: A Call to Action*. Washington, DC: American Association for the Advancement of Science.

AAMC-HHMI Committee (2009). *Scientific Foundations for Future Physicians*. Washington, DC: Association of American Medical Colleges.

Beck, C., Butler, A. & Burke da Silva, K. (2014). Promoting inquiry-based teaching in laboratory courses: are we meeting the grade? *CBE–Life Sciences Education*, *13*, 444–452.

Clariana, R.B. (1991). pH & rate of enzymatic reactions. *American Biology Teacher*, *53*, 349–350.

College Board (n.d.). Inquiry instruction in the AP science classroom: an approach to teaching and learning. https://apcentral.collegeboard.org/pdf/ap-biology-inquiry-statement.pdf?course=ap-biology.

Eastwell, P. (2009). Inquiry learning: elements of confusion and frustration. *American Biology Teacher*, *71*, 263–264.

Hester, S., Buxner, S., Elfring. L. & Nagy, L. (2014). Integrating quantitative thinking into an introductory biology course improves students' mathematical reasoning in biological contexts. *CBE Life Sciences Education*, *13*, 54–64.

Hoffman, K., Leupen, S., Dowell, K., Kephart, K. & Leips, J. (2016). Development and assessment of modules to integrate quantitative skills in introductory biology courses. *CBE Life Sciences Education*, *15*, 1–12.

Kirschner, P.A., Sweller, J. & Clark, R.E. (2006). Why minimal guidance during instruction does not work: an analysis of the failure of constructivist, discovery, problem-based, experiential, and inquiry-based teaching. *Educational Psychologist*, *41*, 75–86.

Labov, J.B., Reid, A.H. & Yamamoto, K.R. (2010). Integrated biology and undergraduate education: a new biology education for the twenty-first century? *CBE–Life Sciences Education*, *9*, 10–16.

Lawson, A.E., Rissing, S.W. & Faeth, S.H. (1990). An inquiry approach to nonmajors biology: a big picture, active approach for long-term learning. *Journal of College Science Teaching*, *19*, 340–346.

Lazonder, A.W. & Harmsen, R. (2016). Meta-analysis of inquiry-based learning: effects of guidance. *Review of Educational Research*, *86*, 681–718.

McCright, A. (2012). Enhancing students' scientific and quantitative literacies through an inquiry-based learning project on climate change. *Journal of the Scholarship of Teaching and Learning*, *12*, 86–102.

National Research Council (2012). *A Framework for K–12 Science Education: Practices, Crosscutting Concepts, and Core Ideas*. Washington, DC: National Academies Press.

Rheinlander, K. & Wallace, D. (2011). Calculus, biology, and medicine: a case study in quantitative literacy for science students. *Numeracy*, *4*, article 3.

Rissing, S.W. & Cogan, J.G. (2009). Can an inquiry approach improve college student learning in a teaching laboratory? *CBE–Life Sciences Education*, *8*, 55–61.

Savelsbergh, E.R., Prins, G.T., Rietbergen, C., Fechner, S., Vaessen, B.E., Draijer, J.M. & Bakker, A. (2016). Effects of innovative science and mathematics teaching on student attitudes and achievement: a meta-analytic study. *Educational Research Review*, *19*, 158–172.

Shmaefsky, B.R. (1993). Potato peroxidase for the study of enzyme properties. *American Biology Teacher*, *55*, 300–301.

Weinheimer, T. & White, D. (2003). Using peroxidase to demonstrate enzyme kinetics. *American Biology Teacher*, *65*, 116–121.

Woodin, T., Carter, V.C. & Fletcher, L. (2010). Vision and change in biology undergraduate education, a call for action – initial responses. *CBE Life Sciences Education*, *9*, 71–73.

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