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Laboratory Experience: Comparison of Bovine Catalase Activity of Liver and Heart with the Support of Cell Phone Videos

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Abstract

Secondary school teachers need to acquire new tools to capture the attention of students and to be able to transmit the knowledge necessary to cover the contents of the curriculum. One of the methodologies we propose is the use of simple laboratory practices that allow teachers to demonstrate and incorporate knowledge through experimentation, trial and error. Therefore, the purpose of our work is to develop a simple laboratory experience for secondary school students. We also consider it relevant to incorporate technologies that motivate students in the study of subjects related to Biological Sciences, Physics, and Chemistry.

Subject Areas

Education Administration, Educational Technology

Keywords

Catalase, Enzyme Activity, Tissue Specificity

1. Introduction

One of the challenges facing a secondary school science teacher is the need to incorporate simple scientific practices or demonstrations. Although the Internet offers videos with laboratory experiences, these are not always adapted to the curriculum that the teacher has to face. On the other hand, from a pedagogical

point of view, the construction of learning is best achieved through hands-on experience [1]. The purpose, therefore, of the present work is to introduce a laboratory experience for secondary school students. On the other hand, we consider it relevant to incorporate technologies, such as the cell phone, that motivate students in the study of subjects related to experimental sciences.

In the case of the use of mobile phones in the classroom, they are mainly used to track information through different search engines (Google, Yahoo, etc.) [2]. It has been observed that mobile phones are associated with activities called "mobile phone addiction". This includes everything from Instagram to Youtube [3]. It is therefore relevant to find an experimental activity away from these addictive activities. In other words, taking into account Bloom's taxonomy [4], which considers cognitive, emotional, and psycho-motor aspects as the main axes, we would be in the first step of the necessary actions to establish a successful teaching-learning process.

The fact of having images or videos recorded by the mobile phone means that the student can observe the information not only in the classroom but also outside it, having the necessary time according to their cognitive needs.

Our aim in this work is to present a practical exercise to establish the activity of the enzyme catalase in two different tissues.

Following this objective, we present a practical activity with the following characteristics

a) To move from the "two dimensions" of the paper to a macroscopic observation.

b) To be able to adapt the practice to different teaching levels.

c) To access the consolidation of learning through trial and manual skills.

d) To obtain experimental results and adjust the practice according to the response of the students.

2. Development of the Experience

Day 1

a) The teacher will introduce the reaction to be tested in the context of the course of study developed. A theoretical framework of the substrate/product reaction which is being catalyzed by the enzyme should be given.

It is assumed that the students will have seen the topics of functional groups and enzyme characteristics.

The teacher in a brief introduction should make it clear that the important thing is to observe in a macroscopic way the property of the system being used [5].

Enzyme reaction:

$$\text{Substrate} \rightarrow \text{Product}$$

Catalase reaction:

$$H_2O_2 \rightarrow H_2O + 1/2\,O_2$$

b) The system to be used is as follows

Tube A: 1.0 ml of a liver homogenate (E1).

Tube B: 1.0 ml of a heart homogenate (E2).

To both tubes, 0.1 g of flour shall be added from a suspension.

Each preparation shall be contained in identical 15 ml test tubes of equal internal diameter and glass thickness.

For the preparation of each homogenate, the students shall disintegrate approximately 1.0 g of liver or heart with 10 ml of physiological solution (NaCl 0.9%) in a mortar and pestle cooled with ice (**Figure 1**).

A 1.0 ml volume of the preparation containing the enzyme is then mixed with 1.0 ml of a 1 g/10 ml flour suspension.

The tube is ready to start the reaction with the addition of the substrate (H_2O_2, 10%).

c) The teacher instructs two students to make marks on the tubes 0.5 cm apart up to the end of the tube or, if they prefer, they can place a ruler to the right of the tube rack.

For the video, students should place black cardboard behind the test tube rack for a better view.

d) A student prepares his phone to film the tubes sequentially with preparations E1 and E2.

The recording starts first and then another student adds 1.0 ml of H_2O_2 to tube E1 and subsequently 1.0 ml to tube E2.

The recording continues until no change in the levels of the suspensions in each tube is observed.

The videos are sent to the rest of the students for analysis in the next class.

Students are asked to draw a graph of what is observed in both tubes for discussion on day 2.

Students should now have the "Protocol for the determination of enzyme activity": Presented below to work with, in the next class.

Day 2

The teacher will ask the students to share the graphs obtained by mobile phone.

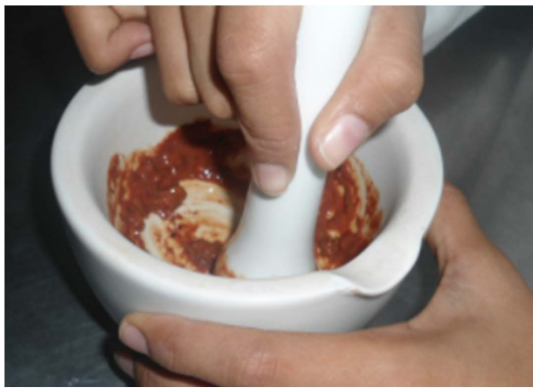

Figure 1. Preparation of homogenate to obtain the suspension containing catalase enzyme activity.

The teacher will evaluate the performance of the graphs, stressing the idea that the strength of the graph lies in its ability to reproduce or synthesize the macroscopic observation.

Speed values are calculated according to the following formula: (see **Appendix**).

$$\text{Speed: } \frac{\Delta d}{\Delta t} = \frac{df - di}{tf - ti} \frac{\left(\text{distance difference}\right)}{\left(\text{time difference}\right)} \quad (1)$$

The calculations made for the velocities of E1 and E2 will be discussed in an attempt to explain the differences, if any, between the two preparations. Reasons could be: Which was faster, why does the "tube" slow down?

In our experience with the students, issues arose that we could not be a generalized statement and say that they could occur with other students. So let us summarize our experience:

a) The students were surprised to see that for the same video they had obtained dissimilar results. "Why don't we all get the same graph? Was their cry. In our case, bearing in mind that we are in a "football" country, we asked them": Have you never seen two people arguing about a penalty that the VAR didn't validate? Haven't you both seen the same video? Here then, depending on the level of the students, we could introduce the idea that it is necessary to repeat the experiments and that through a statistical method or program the idea of which value best represents the experimental observation will be settled.

b) Some students simply joined the points each time to make the graph. The teacher should give the idea of using the most probable line through the points. The teacher can introduce the topic of linear regression or mention that an average line is drawn that passes closest to each point.

If these questions do not appear, it is not necessary to introduce them, as it would be more useful to work with the cognitive curiosities of the working group.

3. Perspectives

The present work involves introducing or taking the cell phone a few steps beyond a mere tool for searching for information, selfies, curiosities, or entertainment.

Our protocol allows us to advance to a stage of comparison between liver and heart enzyme preparation [5] [6].

The calculus of the nmol of H_2O_2 produced can be shown to the students with the following reasoning: A concentration of 10 vol. of H_2O_2 is equivalent to a 0.44 Molar solution. Besides 0.44 Molar is 0.44 mol/L. This concentration can be expressed as 0.44 mmol/mL.

Therefore 1.0 ml contains 0.44 mmol of H_2O_2.

The student can more accurately express that the preparation produced 0.22 mmol at the end of the experiment.

For a university level, the determination of proteins by Bradford [7] can be included and the Specific Activity (SA) of the preparation can be expressed. As a

representative example, we present the graph made by a student (see **Appendix**).

For the calculation of the velocity of E1 the following procedure was used:

a) The Y and X axes are plotted on graph paper. Y represents the height developed by the homogenate/flour suspension while X indicates the associated seconds. In the example, they correspond to 1, 2, 3, 4, and 5 seconds.

b) The students included points arising from the observation of the corresponding video.

c) Students drew the line that passes closest to each of the points (most probable line) starting from the origin (Y = X = 0).

d) In the example presented for 5 seconds the value of the height corresponds to 9 cm

$$\text{Velocity: } \frac{\Delta d}{\Delta t} = \frac{9\text{ cm} - 0\text{ cm}}{5\text{ s} - 0\text{ s}} \tag{2}$$

$$\text{Velocity} = 1.8\text{ cm/s}$$

For the heart homogenate (E2) a value of velocity = 0.25 cm/s was obtained (data not shown). The comparison allowed us to deduce the higher presence of catalase in the volume of the E1 (liver) homogenate preparation.

4. Conclusion

The proposed test was highly motivating for the students who carried it out. The homogenate/flour ascending column makes it possible to "visualize" the catalase enzyme activity and to easily calculate the speed values for each tissue. The present assay is operationally simple but provides a suitable framework for discussing enzyme activity and tissue specificity.

Conflicts of Interest

The authors declare no conflicts of interest.

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Appendix

Protocol for the determination of enzyme activity:

a) Watch the videos sent by the Watsapp application for the enzyme reaction in which a liver homogenate suspension (E1) or a bovine heart homogenate (E2) was used as an enzyme source. In both cases, the reaction was initiated by the addition of 1.0 ml of 10 vol H_2O_2 (substrate) solution.

b) From each video analysis: determine the time taken for the flour column to rise from the lower limit of the test tube to the upper limit of the enzyme reaction for preparation E1 and E2 (Table A1 and Figure A1).

From the data obtained in the video, make a single graph showing the height developed for each column of flour versus time (Figure A2).

Table A1. Students complete the table of time and height of the homogenate/flour suspension column based on the observation of the videos made with the cell phone.

Time (seconds)	E_1 (liver) (height-cm)	E_2 (heart) (height-cm)
t_1		
t_2		
t_3		
t_4		

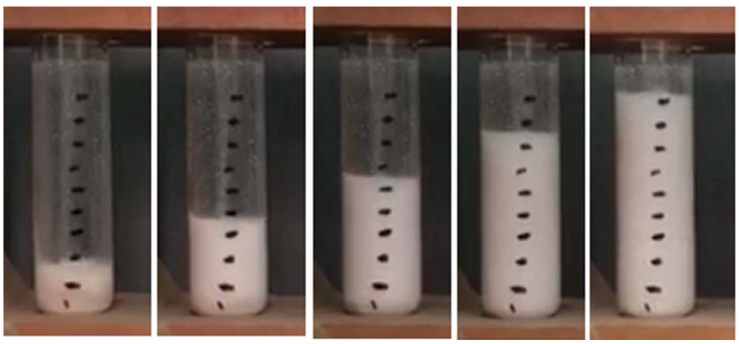

Figure A1. Representative example: the increase in the reaction column for E1. The video was captured at different time intervals by the students.

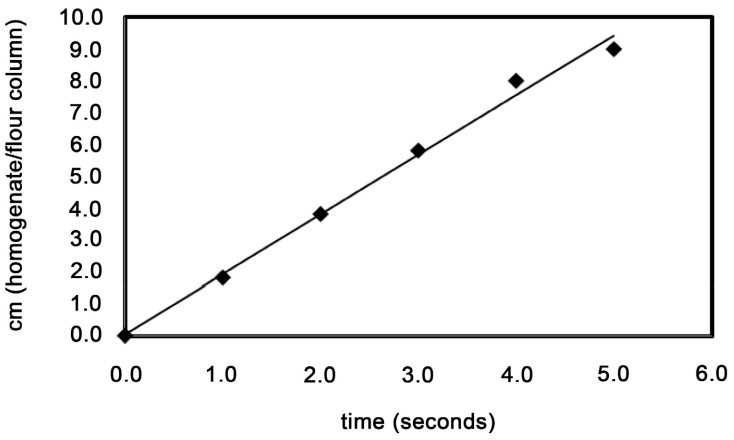

Figure A2. Graph made by the students from the video capture [8].

c) Calculate the reaction rate for each test tube using the following formula:

$$\text{Speed: } \frac{\Delta d}{\Delta t} = \frac{df - di}{tf - ti} \frac{\left(\text{distance difference}\right)}{\left(\text{time difference}\right)} \quad (1)$$

Consider the initial part of the graph before the level of foam generated by the presence of flour remains constant in both homogenates.

d) Reflect on why the reaction you have performed in both homogenates reaches a maximum level (hint: The substrate is exhausted!). Justify if you have observed differences in the rates of the two enzyme preparations (hint: The tissues differ in their activities which is reflected in different enzyme activities!)