

**EDCI 786 Topics: Science Research in the Classroom
Final Report**

1. Brief Description of Research Partner’s Work (as you would describe it to your students).

Emily Ragan and the research group in Dr. Kanost’s lab are studying the genetics behind insect cuticle sclerotization (hardening). Using RNAi (RNA interference) they have already discovered which enzyme is active in the cuticle hardening process. This research may prove valuable in;

1. helping improve our understanding of insect cuticle structure and physiology
2. developing novel insect control methods that target the cuticle physiology process
3. creating new technologies for industry and medicine that mimic the sclerotization process.

2. Brief Description of Classroom Adaptation/Fit with Your Curriculum.

I added the ppt presentation, vocabulary review and lab activity to the end of my unit on biochemistry. I like using this lab as a reinforcing activity of material already covered.

3. School Environment (Rural/Suburban/Urban; Local Economy; Other relevant features)

We are a small rural community with a strong agricultural connection.

I used the agricultural connection during my presentation

- applications of this research include possible pest control methods - pesticides

4. Students Participating in This Unit:

				Ethnicity						
				Asian	Black	Hispanic	Native Am	White	Multi-Ethnic	Total
#	Students					28		28		
%	Students					100		100		

				Gender		
				Female	Male	Total
#	Students	14	14	28		
%	Students	50	50	100		

					SES			
					Free	Paid	Reduced	Total
#	Students	2	22	4	28			
%	Students	7.1	78.6	14.3	100			

			Other	
			Free/Reduced Lunch	Special Needs
#	Students	6	3 = gifted	
%	Students	21.4	10.7	

5. Description/Reflection of Classroom Implementation

a. Day Taught/Placement in the Curriculum (what students already know)

- a. Due to some purchase order delays the lab was delayed. I intended to do the lab the last week

of October but it ended up being the last week of November. Next year I plan to schedule this lesson as the final activity in the biochemistry unit and before assessment assignments.

b. General Response of Students/Points of Student Difficulty

b. The student response was great. They enjoyed the time in lab and the successful results were a plus. Six of the eight groups (four groups per period x two class periods) completed the procedure successfully with only minor additional direction. Two of the groups, however (one per class period) did not complete the lab correctly.

...One lab group erred by placing the same pH buffer solution (7.5) in all the test tubes. Result = no reaction occurred in any of the tubes.

...One lab group placed enzyme in all 8 test tubes. It is unclear if it was due to poor lab technique or to failure to follow lab procedure directions.

c. Student Outcomes Data:

c. Since the lab was delayed until after the unit test was taken, I could not evaluate the impact on their learning. I apologize and would be glad to turn in outcome data next year if you are interested.

Student #	Pre-assessment % Score	Post-assessment % Score	Learning Gain Score	Formula: $\frac{\text{Post Assessment \%} - \text{Pre Assessment \%}}{(100\% - \text{Pre Assessment \%})} = \frac{\text{Actual Gain}}{\text{Potential Gain}}$
all	NA	NA	NA	
Average	NA	NA	NA	

Student Response Data:

Student #	Enjoyed the Lesson	Learned a lot	More Excited About Science
1	3	2	2
2	2	2	1
3	2	2	2
4	2	2	3
5	2	2	2
6	3	2	1
7	2	2	1
8	2	3	2
9	2	2	2
10	2	2	2
11	2	2	3
12	3	3	2
13	2	3	2
14	2	3	3
15	2	2	2
16	3	3	2
17	3	3	2
18	3	3	3
19	3	2	1
20	3	2	2
21	2	3	2
22	1	2	1
23	1	1	1
24	2	2	2
25	1	2	1
26	1	2	1
27	1	2	1
28	abs	abs	abs
Average	57/27=2.11	61/27=2.30	49/27=1.81

Seems very few are excited about science.

1 = Disagree 2 = Neutral 3 = Agree

d. Unanticipated Problems/Successes

requisition/shipping delays

Storage of the enzyme may be a problem if you do not have designated freezer space. I have mine labeled in a

sealed container in the staff lounge freezer. Not ideal but it works.

e. Adaptations/Changes Made to Final Lesson Draft

Part 1 was completed by my Biology II class. I'm glad I didn't try to have my biology I students tackle the task of preparing the buffer solutions. It involves too many new lab techniques and would have been difficult for them to carry out the procedures accurately. And, it was a good lab for the advanced students. We also did collect quantitative data using a spetro20. It was a good reinforcement of earlier learning of quantitative vs qualitative data.

f. Recommendations for Future Users

I highly recommend this lab for use with Biology I students who are studying or have studied protein denaturation and enzyme structure and function. It is also great for

reinforcing proper scientific method... IV, DV, control, data (quantitative and qualitative), etc. I even had them write a question they thought would be answered by this lab.

6. Final Version of the Lesson

(see next page) --- Remember... only part 2 was conducted by the Biology I students.

I divided the buffer solutions into two containers and placed one set on each side of the lab table. Each group had their own tube of enzyme and substrate.

Group 1	LAB TABLE One set of buffer solutions (shared)	Group 2	SINK
Drain trough			
Group 3	One set of buffer solutions (shared) LAB TABLE	Group 4	

pH and Enzyme Function Lab Activity

Emily Ragan, Kansas State University, Manhattan KS

Tina Savage, Clifton-Clyde High School, Clyde KS

Background about laccases---Laccases are enzymes that catalyze oxidation of substrates and use the electrons they acquire to reduce molecular oxygen, O₂, to water. Laccases contain 4 coppers and can oxidize a variety of substrates, especially phenolic compounds¹. Laccase was first discovered in the Japanese tree *Rhus vernicifera* in 1883². Currently we know laccases are present in plants, fungi, bacteria, and insects where they are involved in many different processes including detoxification, wound healing, cell wall synthesis, and lignin synthesis and degradation. People are interested in using laccases for a variety of purposes. In the food industry, laccases are used to reduce phenolic compounds for improving the clarity of fruit juice, beer and wine³. Laccases can be used for delignification in the paper and pulp industry and textile industries use laccases to remove dyes from waste water⁴. The Kanost Lab at Kansas State University studies the function of laccases in the mosquito *Anopheles gambiae* as well as the role of laccase in insect cuticle sclerotization (hardening). This activity introduces high school students to an enzyme activity assay in which enzyme activity can be detected by a color change. The substrate ABTS is clear in its reduced form and turns green after it is oxidized by laccase.

Instructor Notes

Time Required: Approximately two 50-min lab periods will be needed. Lab period one includes pre-lab instructions and Part 1, buffer preparation, of the lab activity. The second lab period includes all steps of Part 2, pH and Enzyme Function, of the lab activity.

Group Size: Students should work in teams of two or four. The total number of students working in the lab will depend on your lab facilities (8-24 students).

Materials Needed (see student handout) Each group will prepare one buffer solution to be used by the entire class in part 2. Plan to have at least 4 student groups.

Instructor preparation for part 2, laccase activity assay

A. Prepare 0.25 unit(U)/ul *Rhus vernicifera* laccase

NOTE: The *Rhus vernicifera* laccase may come in different units than the concentration used for these calculations. Be sure to adjust the values accordingly.

$$0.250 \text{ U/ul} = 250 \text{ U/ml} \quad 250\text{U/ml} \times 1 \text{ mg}/120 \text{ U} = 2.08 \text{ mg laccase/ml buffer.}$$

We observed that transfer pipettes typically make drops that are ~30 ul. Each group will need 4 drops of laccase. Giving each group a microcentrifuge tube with 0.5 ml will give each group over three times what they need.

To make enough laccase for 12 groups, dissolve ~25 mg of laccase in 12 ml of 100 mM citrate 100 mM sodium phosphate buffer, pH either 4 or 5.5. Shake vigorously and for a few minutes, but know IT WILL NOT ALL DISSOLVE. This is normal, the laccase from Sigma is a crude preparation. You and your students can avoid using the chunks but it will be okay if a little insoluble material gets into the reactions. Laccase should be kept in the refrigerator or on ice until it is used. For longer term storage, add 50% glycerol and store at -20 C (freezer).

B. Prepare 25 mM ABTS

¹ Piontek K. et al. (2002) *JBC* **277**, 37663-37669.

² Yoshida, H. (1883) *J. Chem. Soc. (Tokyo)* **43**, 472-486.

³ Couto S.R. and Herrera J.L.T. (2006) *Biotech Adv* **24**, 500-513.

⁴ Couto S.R. and Herrera J.L.T. (2006) *Biotech Adv* **24**, 500-513.

For 1 ml: $1 \text{ ml} \times 25 \text{ mmol}/1000 \text{ ml} = 0.025 \text{ mmol} \times 548.68 \text{ mg}/\text{mmol} = 13.7 \text{ mg ABTS}$

Each group will use 8 drops of ABTS. Giving each group 0.5 ml will give them twice as much ABTS as they'll need. We think it might be good to make up some extra, just in case of spilling. If you weigh out 200 mg of ABTS, dissolve it in 14.58 ml of buffer (pH 4 or 5.5) to make 25 mM. Store ABTS in the dark (perhaps covered with aluminum foil) until it is used. Plan to use the ABTS soon after dissolving it in buffer, certainly no longer than a week. The ABTS dissolves easily and will be a pale green color.

You might want your students to practice making drops from transfer pipettes on the same day they make buffer. It takes a little practice to consistently release just one drop, and that will be important for the enzyme assays they'll run in part 2.

Additional notes to the instructor:

If you have a spectrophotometer, students can take absorbance readings of their samples after a set length of time, perhaps 20 or 30 minutes, using light with a wavelength of 414 nm.

The detergent SDS can denature and thereby inhibit laccase. This can be worked into the experiment by having each group of students run 4 more tubes, adding buffer, ABTS, 5 drops of 20% SDS, and then enzyme. The tubes containing SDS are expected to stay clear, like the control. The instructor would need to prepare the 20% SDS (20g/100 ml); take care, SDS becomes suspended in the air and you don't want to breathe it in!

You can also compare the effect of concentration by adding an additional drop of substrate or enzyme or half as much substrate or enzyme; (fine tipped transfer pipettes tend to have drops of ~15 ul).

Finally, you and your class can use a computer to look at the three dimensional structure of *Tremetes versicolor* laccase or some other laccases for which there are crystal structures. PDB files are available from the Protein Data Bank at www.rcsb.org. One PDB file for *Tremetes versicolor* laccase is 1GYC. Entering this code into the space at the Protein Data Bank will take you to a page with more information. On the left you can click on "Display molecule". One option is to use Jmol Viewer to move the protein around while you look at it. There are 4 small round balls representing the 4 bound copper molecules and the backbone of the protein is in gray.

pH and Enzyme Function Lab Activity

Emily Ragan, Kansas State University, Manhattan KS

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Purpose:

To test the effect of pH changes on structure and function of enzymes.

To practice lab techniques ie. measuring, observing and collecting data.

Chemicals:

Part 1-

- citric acid, sodium phosphate (dibasic, Na₂HPO₄), 5 M NaOH, 5 M HCl, distilled water

NOTE: monobasic sodium phosphate may be used but it will require more solution to adjust pH.

Equipment:

Part 1-

- Safety glasses, gloves and lab apron
- labeling tape and marking pencils or sharpies
- magnetic stirrers (or stirring rods)
- spatulas
- weigh boats or weigh paper
- 1 100 or 200-mL beaker
- 100 ml Graduated cylinder
- 100 ml glass storage bottle with lid
- Tongs (to remove magnetic stirrer from buffer solution)
- balances which can measure to the nearest 0.1 or 0.01 gram
- pH meter

Procedure:

Part 1-buffer preparation

1. Write teacher assigned pH value here. _____
2. Make the 100 mM Citrate (citric acid) 100 mM Sodium Phosphate buffer:
--For 100 ml buffer
 - a. Measure 1.92 g citric acid and place in a large beaker.
(10 mmol citric acid 0.010 mol x 192 g/mol = 1.92 g citric acid)
 - b. Measure 1.38 g sodium phosphate and place in beaker with citrate.
(10 mmol Sodium Phosphate 0.010 x 138 g/mol = 1.38 g Sodium Phosphate)
3. Add 90 ml water and dissolve on magnetic stir plate. Pour into graduated cylinder and add water to make 100 ml.
4. Check pH.
5. Adjust pH to desired value (2.5, 4, 5.5, and 7.5)
 - a. Use NaOH solution to raise pH
 - b. Use HCl solution to lower pH.
6. Pour into storage bottle.
7. Label with buffer name, pH and date. Seal and store for use in Part 2.
8. Clean up lab area.
9. Use remainder of class to practice making single drops with the micropipette.

Part 2-Enzyme activity assay

Chemicals:

- 4 buffer solutions prepared in part 1 (2.5, 4.0, 5.5, 7.5 pH).
- 25mM ABTS
- 0.25 U/ml tree laccase enzyme

Equipment:

- Safety glasses, gloves and lab apron
- Labeling tape and marking pencils or sharpies
- 6 1-mL disposable transfer pipettes
- 8 small test tubes, stoppers, and rack
- 2 small test tubes or 1.5 ml microcentrifuge tubes (so each group has their own ABTS & laccase)
- Aluminum foil
- 4 small beakers if you want groups to have their own containers of the buffers prepared in part 1. Each group will need 6 ml of each buffer. (or they can share from the large bottles)
- Optional: 8 microcentrifuge tubes for storing sample overnight.
- Distilled water washer bottle

Procedure:

1. Label 8 test tubes, 2 for each pH (2.5, 4, 5.5, 7.5).
 - a. Four of your tubes, one of each pH, will be a control. The control will have buffer and the substrate (ABTS). You may want to label the control tubes with a C.
 - b. The other tube at each pH will have buffer, substrate, and enzyme (laccase). Label these tubes so you know they contain enzyme.
2. Using a 10 ml graduated cylinder, add 3 ml of **appropriate** pH buffer solution to both the control and enzyme labeled tubes. MAKE SURE THE BUFFER YOU PUT IN EACH TUBE HAS THE CORRECT pH! 2.5 test tubes get 2.5 pH buffer, 4.0 test tubes get 4.0 pH buffer, etc. Be sure to rinse the graduated cylinder with distilled water after measuring each pH.
3. Add 1 drop 25 mM ABTS to all eight test tubes.
4. To the enzyme labeled tubes **ONLY**, add 1 drop of 0.25 U/ml tree laccase enzyme.
5. Cap your tubes and invert them to mix (turn the tubes upside down then right side up again). Try to start all the reactions (adding ABTS and laccase) at close to the same time.
6. Record the start time on your data page. Compare the intensity of color change over the next half hour. Rank each test tube (1= darkest to 4 = lightest). Every 4-5 minutes, observe and record color change data in a table.
7. If you like, leave the tubes overnight and check their color the next day. Your instructor might like to put some of your sample in a microcentrifuge tube for the overnight storage.
8. Rinse the rest of your sample down the sink and clean up your supplies and your bench space.