Understanding Catalysis in Nanoscale Biofuel Cell Technologies

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Summary

Overview of Project:

This module is designed to give students hands-on experience in current bioengineering related projects. Students begin their exploration by learning about fundamental aspects of enzymes and their role as catalysts in a simple Jell-o/pineapple activity in which the enzyme properties of processed and fresh pineapple are tested. Next, the students will calculate the surface area to volume ratio using data collected from clay spheres and apply this information to better understand reaction rates within biological systems. The third activity emphasizes the effect of surface area upon reaction rates. Students will calculate reaction rates for various Alka-Seltzer particle sizes as they dissolve. The fourth activity will draw upon the concepts of catalysis and reaction rate as influenced by the concentration of substrate. Students will construct a gas collection device which demands teamwork to collect data in order to calculate a rate law equation similar to equations used by engineers in the enzyme kinetics fields. The capstone activity shows the relationship between the concepts of catalysis, reaction rate and surface area:volume, along with a display of current concepts that researchers are investigating to overcome existing obstacles within the field of nanoscale enzyme biofuel cells.

Intended Audience:

This module was designed to fit within most 9-12 biological and chemical science classes. However, it could be easily adapted to fit other classes.

Estimated Duration:

All parts of this module should take between 9 and 11 days to complete.

Prerequisite Student Skills/Knowledge:

Knowledge of lab safety protocols
Microsoft Excel (input data)
Basic graph construction and interpreting skills
For an in depth investigation of the “Modeling Enzyme Activity” lab, Students should have Algebra II skills such as:
  - Exponents
  - Natural logarithms
  - Point slope form of a line (y = mx + b)
Introduction

This module is designed to meet and exceed several standards of learning required in today’s secondary classrooms. While incorporating standard acquisition, it also utilizes cutting edge research to introduce potential science and engineering students to the professions.

The nanoscale biofuel cell focus throughout the module highlights a technology that while in its infancy stage, promises exciting developments to benefit humankind. The intent of the authors is to excite students and encourage them to consider becoming problem solvers in this rapidly changing world.

Rationale for Module:

The rationale for this module is to provide students with a brief preview of the research conducted by Dr. Su Ha in the area of nanoscale biofuel reactor cells. Dr. Ha’s research provides a great example of biological engineering and potential applications within the biomedical fields. The hands on models and activities parallel those techniques and concepts utilized in his lab.

Science:

The science behind our module includes enzyme activity and their functioning capabilities in nanoscale biofuel cells. In studying the enzymes, experimental design is fundamental to the data gathering and analysis components of the laboratory activities and provides a framework from which students can build future design components.

Engineering:

By examining the science behind enzymes and enzyme activity, students gain a background for the obstacles engineers face when trying to utilize them in new technologies. In this module, the students explore the engineered mechanisms for dealing with two major problems incurred by enzyme-based technology. One, the loss of costly enzymes with the product after the reaction on a substrate set is complete, and two, the denaturing of enzymes under stress. The students create a tangible model of a “nanoscale” biofuel cell. In the model, both problems are solved, retaining the enzymes for continuous use and preventing the denaturing process from occurring.

Goal:

Provide secondary science educators a module packed with modern engineering concepts needed to adequately introduce students to fields in which jobs are in high demand. This benefits society as a whole by providing human resources for developing solutions to currently looming problems.
HIERARCHICAL DIAGRAM

Nanoscale Enzyme Reactor Model
(The Wiffle Ball Lab)

Modeling Enzyme Activity (The Mentos Lab)

Understanding the Function of Enzymes in a System (The Pineapple Lab)

Investigating the Impact of Surface Area on Reaction Rates (The Alka-Seltzer Lab)

Surface Area to Volume Ratio Lab (The Clay Lab)
THE PINEAPPLE LAB

Understanding the Function of Enzymes in a System

(See Appendix A for teacher instructions and suggestions)

**Background:** Catalysts are substances that speed up chemical reactions without being used up. Enzymes are organic catalysts. Plants produce enzymes for a wide variety of functions. In this lab you will explore the enzyme activity of the common pineapple plant on gelatin compounds.

**Objectives:**
The student will be able to:

- Explain the function of a catalyst and define terminology relating catalysts and enzymes.
- Identify the control group and experimental group.

**Materials:**
Fresh Pineapple Chunks (Similar in size to canned pineapple chunks)
Canned Pineapple Chunks
Prepared Jell-O gelatin solution
Refrigerator
250 mL Beaker (2 per group)
Stirring Rod
Triple beam balance
Plastic Wrap
Transport Container that will fit in Refrigerator

**CAUTION!!** Do NOT eat or drink any materials in the lab. Safety goggles must be worn at all times.

**Procedure:**
Day One

1. Label your beakers using the following format:

   **Beaker One**
   - Beaker Number
   - Lab Members or Group Name
   - Date
   - Fresh Pineapple

   **Beaker Two**
   - Beaker Number
   - Lab Members or Group Name
   - Date
   - Canned Pineapple
2. Obtain one chunk of fresh pineapple and one chunk of canned pineapple of equal shape and mass. Place each chunk in their appropriately labeled beaker.  
   **Note:** Use a paper towel on the triple beam balance when finding the masses of your pineapple chunks. Use a different paper towel for each pineapple measure to avoid cross-contamination of the samples.

3. Using the prepared Jell-O solution from your teacher, fill each beaker to the 100 mL mark.

4. Cover your beakers with plastic wrap and place them in the designated container, which will be placed in the refrigerator by your teacher.

5. Clean up your lab station as directed by your teacher.

The remainder of the lab portion will be completed at our next class meeting. Use the remainder of this class session to complete the Extension below, due five minutes before class is out.

**Extension:**

Predict the impact of enzymes on gelatin formation in each of the beakers.

**Beaker One**

**Beaker Two**

In order to make valid comparisons in this experiment, what type of control is needed that has not yet been established? Brainstorm on possible control group requirements and prepare to communicate your thoughts to the rest of the class in the last five minutes. Be sure to write your ideas in the space below.

**EVERYONE** needs to write the ideas down.
Day Two

5. Retrieve your beakers from the container you placed them in at the end of the last class session.
6. Observe any differences in the beakers and record your observations in the space below:
    Beaker One Observations

    Beaker Two Observations

7. Communicate your findings with other groups in the class to validate your results.
8. Draw conclusions about the role of the enzymes present in pineapples.

9. List three possible causes for differences among your control and experimental groups.
    1.
    2.
    3.

Post-Lab Questions:

1. What may have happened in the canning process to change the viability of the enzymes?

2. In your own words, explain the relationship between a catalyst and an enzyme.
THE CLAY LAB
Surface Area to Volume Ratio Lab

(See Appendix B for teacher instructions and suggestions)

Background:
Three dimensional objects possess inherent properties such as shape, volume, and surface area. Enzymes are three dimensional objects. The three mentioned properties are important for enzyme function. In this lab, we will explore the relationship between surface area and volume. For correlation to enzymes, we will use the sphere as our shape, as many enzymes take on a near spherical shape. In order to calculate the surface area and volume of a sphere, we will need the following equations.

Surface Area: \[ S.A. = 4\pi r^2 \]
Volume: \[ V = \frac{4}{3}\pi r^3 \]

Objectives:
The student will:

Measure and manipulate data using the metric system.
Graph the relationship between surface area and volume in spherical shapes.
Apply knowledge of dependent and independent variables to create a graph.
Use a graph to interpret data.
Use a data table to identify trends.
Relate cell form to function.
Relate surface area to volume ratios to enzyme kinetics in engineering applications.

Materials:
Modeling Clay – Ping Pong Ball Size
Metric Ruler
Graph Paper

CAUTION!! Do NOT eat or drink any materials in the lab. Safety goggles must be worn at all times.

Procedure:
1. Using the entire lump of clay provided by your teacher, roll it into a ball. This is best accomplished by squeezing it into a near spherical shape and then rolling it between the palm of your hand and a clean smooth surface in a circular motion.

2. When the spherical ball is formed, measure the diameter using the metric ruler.

3. Using the provided equations, calculate the surface area and volume and record them in the attached data table. (Note: Be sure to use the radius (r) in your equations, NOT the diameter.)
4. Roughly divide the sphere in half. Using only one of the two halves, form a new sphere.

5. Repeat steps 2 through 4 above to fill out the attached data table. In the end, you should form a total of 5 different sphere shapes.

6. Graph the data for each sphere on the attached sheet. Make sure to identify and place the dependent and independent variables on the correct axes of the graph. (Hint: Independent Variable is placed on the X-axis.)
# THE CLAY LAB

Data and Graph Sheet

<table>
<thead>
<tr>
<th>Size of Sphere</th>
<th>Diameter (cm)</th>
<th>Radius (cm)</th>
<th>Surface Area ($\text{cm}^2$)</th>
<th>Volume ($\text{cm}^3$)</th>
<th>Surface Area:Volume Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Surface area Vs. Volume**

![Surface area Vs. Volume graph](image-url)
Post Lab Questions:
1. What do the spheres represent in this lab?

2. Using your graph, state what happens to the value of surface area as the volume is increased.

3. Using the data table, state the trend of the surface area to volume ratio as the spheres get smaller.

4. Cells require a certain amount of energy per unit of volume. The energy is taken in across the surface of the cell membrane to reach the inner cell.
   a. Explain why cells tend to be so small?

   Bonus* What allows a single-celled chicken egg to be so large?

5. WSU chemical engineer Su Ha works with enzymes in his quest to build nanoscale biofuel cells. List at least 2 advantages of using nanoscale sized particles in a fuel cell.
THE ALKA-SELTZER LAB

Investigating the Impact of Surface Area on Reaction Rates
(See Appendix C for teacher instructions and suggestions)

Background: As previously discovered in the surface area to volume exploration using clay balls, we learned that the smaller the particle size, the higher the surface area to volume ratio. Today, we will explore the impact of surface area/particle size on reaction rates using Alka-Seltzer as our substrate. A substrate is the generic term given to that substance acted upon by an enzyme or catalyst.

Objectives:
The student will:
State the importance of a standard in measurement.
Identify and state the importance of constants in controlled experiments.
Obtain data from a graph using interpolation/extrapolation.
Identify the dependent and independent variable.

Materials:
Alka-Seltzer Tablet – One per group
Stopwatch
10oz Clear Plastic Cups
Graph Paper
Graduated cylinder
Thermometer
100 mL of teacher supplied water

CAUTION!! Do NOT eat or drink any materials in the lab. Safety goggles must be worn at all times.

Group Number:_______

Particle Size Key

<table>
<thead>
<tr>
<th>Group</th>
<th>Size of pieces</th>
<th>Size of pieces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Whole</td>
<td>1</td>
</tr>
<tr>
<td>Group 2</td>
<td>Halves</td>
<td>1/2</td>
</tr>
<tr>
<td>Group 3</td>
<td>Thirds</td>
<td>1/3</td>
</tr>
<tr>
<td>Group 4</td>
<td>Quarters</td>
<td>1/4</td>
</tr>
<tr>
<td>Group 5</td>
<td>Fifths</td>
<td>1/5</td>
</tr>
<tr>
<td>Group 6</td>
<td>Sixths</td>
<td>1/6</td>
</tr>
<tr>
<td>Group 7</td>
<td>Sevenths</td>
<td>1/7</td>
</tr>
<tr>
<td>Group 8</td>
<td>Eighths</td>
<td>1/8</td>
</tr>
</tbody>
</table>
Procedures

1. In order to have results that can be compared effectively, our class must agree on the point at which the Alka-Seltzer “stops” reacting. As a class, we will all observe the reaction of the control group and establish the criteria for the “stop” time, which you are expected to adhere to in your individual experimental group.

Record your observations and stop time criteria in the space below.

2. Obtain 100 mL of water from the supplied tub using the graduated cylinder and pour it into the plastic cup.
3. Double-check with your timer before adding your Alka-Seltzer to make sure they are ready to go.
4. When your timer is ready, gently drop your Alka-Seltzer into the water and begin timing the reaction upon contact.
5. When the “stop” time criteria is achieved, stop timing and record the data below. Be sure to record your data in seconds. Share your data with the other groups by organizing it on the board at the front of the room or as directed by your instructor.
6. While waiting for other groups to finish, clean up your lab area.

Note: Pour your Alka-Seltzer/water solution in the sink
   Dispose of the plastic cup as directed by your teacher.
   Return your graduated cylinder, stopwatch, and thermometer to their proper locations
Data:

<table>
<thead>
<tr>
<th>Group</th>
<th>Size of Piece</th>
<th>Reaction Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>1/2</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>1/3</td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td>1/4</td>
<td></td>
</tr>
<tr>
<td>Group 5</td>
<td>1/6</td>
<td></td>
</tr>
<tr>
<td>Group 6</td>
<td>1/7</td>
<td></td>
</tr>
<tr>
<td>Group 7</td>
<td>1/8</td>
<td></td>
</tr>
<tr>
<td>Group 8</td>
<td>1/9</td>
<td></td>
</tr>
</tbody>
</table>

Graphing:
Graph your data using the grid below or, if available, using the graphing function in Excel.
Post-Lab Questions:

1. Why is it so important that every group use the same “stop” time criteria?

2. Why were you instructed to get the water from the tub instead of from the sink faucet?

3. Identify five constants present in the control and experimental groups:
   -
   -
   -
   -
   -

4. What represents the control group in this experiment?

   Explain:

5. According to your graph, what happens to reaction time as particle size decreases?

Extrapolation
6. According to your graph, what is the reaction time of infinitely small particles?

Interpolation
7. According to your graph, what is the interpolated reaction time of 1/5\textsuperscript{th} sized particles?

8. Identify each of the following:

   Independent variable –

   Dependent variable -
THE MENTOS LAB
Modeling Enzyme Activity
Using Diet Pepsi and Mentos to determine a Rate Law Equation

(See Appendix D for teacher instructions and suggestions)

Background:
Enzymes act upon specific substrates in biological systems. The rate at which these reactions occur is affected by several factors. Here, we focus on the effect of concentration of a substrate (CO₂ dissolved in Diet Pepsi) on the reaction rate driven by a catalyst (Mentos). In true enzyme driven reactions, such as that found in the biofuel cell reaction of glucose oxidase (an enzyme) with glucose, a rate law equation (Michaelis-Menten) has been established. The materials and equipment needed to conduct a true enzyme driven reaction are too costly. Therefore, we will use a simplified model to calculate a rate law equation using easily obtained, low cost materials.

Objectives:
The student will:
Measure and manipulate data using the metric system.
Create a gas collection system for measurement that utilizes water displacement.
State the importance of teamwork in data collection.
Use data manipulation software in analyzing results of experiments.
Create a Rate Law equation derived from collected data.
Use the derived Rate law equation to predict hypothetical values.
Use mathematics to model and explain scientific concepts.

Materials (per group of 3):
6 Mentos
1 591mL (20 FL OZ) room temperature Diet Pepsi
50cm of 5mm inside diameter rubber tubing
1 plastic disposable pipette with a long tip
1 ring stand
2 test tube clamps
250 mL Erlenmeyer flask
Single hole rubber stopper to fit 250ml Erlenmeyer flask
Two hole rubber stopper to fit 100 ml graduated cylinder
Fitting to attach stopper to rubber tubing
~0.5 m of 18mm wide masking tape
Fine tipped felt marker
Stopwatch with split / lap function
2 – 100ml graduated cylinders
1 – 1000ml container (used for water displacement)

CAUTION!! Do NOT eat or drink any materials in the lab. Safety goggles must be worn at all times.
**Procedure:**
1. Construct the gas collection apparatus as shown in Figure 1-1 below.

![Figure 1-1](image1.png)

**Figure 1-1**

Note: Use a scissors to remove the bulb from a micropipette. Use the tip and insert it into the end of the rubber hose as shown in Figure 1-2 below.

![Figure 1-2](image2.png)

**Figure 1-2**

2. Measure out 100ml of water with a graduated cylinder and pour it into the 250 ml Erlenmeyer flask.

3. Using a fine tipped water resistant marker, mark the meniscus of the water height and label this mark “100 ml”.

4. Pour the water down the drain.
Gas collection apparatus setup instructions (Note: you will do this for each trial)

1. Fill up the graduated cylinder with water, cover it with your hand, and invert it so that the top is under the surface of the water in the 1000 ml container.

2. Position the graduated cylinder into the test tube clamp and have your partner fasten it in place. Next remove your hand from the top of the graduated cylinder.

Note: Try to minimize any air bubbles in the graduated cylinder (practice this a few times)

3. Place the pipette tip into one of the two holes of the rubber stopper. Then place the 2-hole stopper into the mouth of the inverted graduated cylinder. (See Figure 1-1) If water begins to drain from the graduated cylinder, have one group member pinch the hose.

4. Place ~10 cm of masking tape along the length of the inverted graduated cylinder, starting at the 0 ml mark and working towards the mouth of the graduated cylinder. (Do NOT cover up the ml numbers.)

5. Using the graduated cylinder, measure the required amount of water (see Table 1-1 below for each trial) and pour this into the 250ml Erlenmeyer flask.

<table>
<thead>
<tr>
<th>Trial #</th>
<th>ml Diet Pepsi</th>
<th>ml Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>70</td>
</tr>
</tbody>
</table>

6. Carefully pour diet pepsi into the flask until the volume reaches the “100 ml” mark you made in step 3.

Note: As you proceed through this lab, your effort to standardize procedures between each trial will improve your data’s validity. (ie: how you dispense the diet pepsi)

7. Designate each of the following roles to members of your group:
   - Timer (needs to be familiar with lap / split function)
   - Mentos inserter / stopper placer
   - Volume marker
8. The Mentos inserter will drop 1 Mentos into the 250ml flask and quickly place the stopper into the mouth of the flask to form a seal. The stopper will seal with very little pressure. The Timer needs to start the stopwatch at the same time the stopper forms a seal. Note: If the hose was pinched, it must be released at the same time that the stopper is inserted.

9. The Volume marker will use the fine tipped marker to mark the volumes of gas at ~3ml – 5ml increments. Each time the volume is marked, a verbal cue signals the Timer to push the lap button on the stopwatch. Continue collecting data until six volume marks have been made/timed. (See Figure 1-3)

10. Record the time intervals for the current trial into the appropriate data table located on Student Data Sheet 1.
Note: Record the cumulative time for each data point, NOT the interval time.

11. Record Volume readings for each time interval into the appropriate data table located on Student Data Sheet 1.

12. Repeat steps 5-15 for each trial (Note: you will only change the amounts of Diet Pepsi and Water for each trial)

13. Transfer your data from Student Data Sheet 1 into the Excel file designated by your instructor.
Post Lab Questions:
1. In the space below, diagram the lab setup used in this experiment.

2. Explain why it was necessary to perform this lab as a team.

Extension:
3. Write the Rate law derived from your data in the space below. The information needed to determine this rate law equation is found by creating a “Best Fit Line” in the graph on the Excel sheet titled “Linearized Rate vs. Concentration Data”.

4. Of what use is a Rate law equation to scientists and engineers working with enzymes?

5. What is the value of K in your rate equation? (Hint: use $e^x$ on your calculator for your ln K value)

6. Suppose you are a fan of Diet Pepsi, but only if its concentration is at or above 140 micromoles CO$_2$/ml Diet Pepsi. One day while walking across the Gobi Desert, you come upon a tent which contains 1 bottle of 22 degree C Diet Pepsi. The problem is that the seal on the bottle is broken. This is a problem for you because you are not sure if the CO$_2$ concentration meets your requirements for drinking. Fortunately, this special edition Gobi Desert Diet Pepsi bottle has a Built In Mentos Driven Rate Sensor (BIMDRS). You activate the BIMDRS and get a reading of 0.6 micromoles CO$_2$/s

Using your Derived Rate law from Question 3 above, Determine whether or not you will drink the Diet Pepsi based only upon the concentration requirement. (Show all calculations for credit) Enjoy your Diet Pepsi…. Or maybe not.
# STUDENT DATA SHEET 1

## Trial 1

<table>
<thead>
<tr>
<th>Conc</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (s)</td>
<td>Volume of CO₂ (ml)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

## Trial 2

<table>
<thead>
<tr>
<th>Conc</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (s)</td>
<td>Volume of CO₂ (ml)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

## Trial 3

<table>
<thead>
<tr>
<th>Conc</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (s)</td>
<td>Volume of CO₂ (ml)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

## Trial 4

<table>
<thead>
<tr>
<th>Conc</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (s)</td>
<td>Volume of CO₂ (ml)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

## Trial 5

<table>
<thead>
<tr>
<th>Conc</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (s)</td>
<td>Volume of CO₂ (ml)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

## Trial 6

<table>
<thead>
<tr>
<th>Conc</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (s)</td>
<td>Volume of CO₂ (ml)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
THE WIFFLE BALL LAB

Nanoscale Enzyme Reactor Model

(See Appendix C for teacher instructions and suggestions)

Background:
Several biofuel processes currently rely upon costly enzymes which must be continuously replaced in order to get the desired energy products. Current research employs the idea of confining enzymes in an enclosed space which serves two major functions. The first is the ability to reuse expensive enzymes in multiple reactions as they are not discarded from the system with the product. The second is the prevention of the unwinding (denaturing) of the enzymes due to the confined space of several enzymes at some optimal packing density within the space. This nanoscale engineering has the potential to provide a power source for several human health monitoring devices which are impractical using current technology.

This lab provides the opportunity for students to model a nanoscale enzyme reactor; guaranteed to capture your attention. You will discover how an optimal packing of enzymes prevents denaturing while confining the expensive enzymes for continuous use.

Objectives:
The student will:
Use an “enzyme” model to illustrate denaturing.
Construct a model to represent the prevention of the denaturing of enzymes in a nanoscale enzyme reactor vessel.
Calculate the packing density of enzymes
Predict relative efficiency in a nanoscale enzyme reactor using the concepts of packing density.
Work in an engineering team to design a mechanism that illustrates the importance of crosslinking enzymes in a nanoscale enzyme reactor.

Materials (per group of 3):
Hot plate
6 prepared “enzyme” models
Ice cubes
~400mL beaker
1000 mL container for ice water bath
1 jumbo paper clip
1 wiffle ball (golf ball size)
thermometer
CAUTION!! Do NOT eat or drink any materials in the lab. Safety goggles must be worn at all times.

Procedure:

1. Fill the 400mL beaker to ~350 mL and begin heating on high using the hot plate. Adjust heat to achieve 95 C water temp.

2. Take the prepared “enzyme” models provided by your instructor and submerge them in an icewater bath.

3. While submerged in the icewater bath, form all six enzymes (nitinol-bead complex) into spherical shapes (Note: Do NOT tie wires into knots as this will prevent the desired results from occurring). Each wire sphere should be separate from the other “enzymes”. Measure and note the average diameter here: ________cm

4. Insert five enzymes into the wiffle ball (nanoscale reactor vessel).

5. Shake the wiffle ball to observe the loss of enzymes. This would be a problem, as enzyme loss = money loss. NOT GOOD.

6. To reduce enzyme loss, create cross linking sites among enzymes using magnets or any other instructor approved methods that your group engineers. It is your responsibility to determine how and where these cross-linking sites are fastened to the enzymes.

7. Return the cross-linked ready enzymes to the nanoscale reactor vessel. Note the effectiveness of cross-linking.

8. Using the last enzyme (the one that is outside of the nanoscale reactor vessel). Gently drop it into the 95 C water within the 400mL beaker. Note your observations in the space below.
9. Modify the jumbo paperclip into a shape capable of suspending the nanoscale reactor vessel. (See Figure 1-4)

![Figure 1-4](image)

10. Suspend the nanoscale reactor vessel from the paperclip, and slowly lower the vessel into the 95 C water within the 400mL beaker. Note your observations in the space below.

11. Remove the enzymes from the nanoscale reactor vessel and return all of them to your instructor.

**Post lab questions:**

1. What happened to the single enzyme exposed to the stress of heat in the water?

2. Describe the effectiveness of an enzyme that has experience stress similar to the above enzyme model.

3. Describe the importance of cross-linking enzymes in the nanoscale reactor vessel.
4. Engineers working with enzymes often calculate an enzyme packing density. This equation, stated below, allows engineers to select optimal packing densities for their nanoscale reactor vessels. Use the equation to calculate the packing densities for nanoscale reactors containing 1, 2, 3, 4, and 5 enzymes with diameters equal to the value measured in step 8 of the procedure.

Enzyme Packing Density = \frac{\text{Total volume of enzymes}}{\text{Total volume of reactor vessel}}

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<thead>
<tr>
<th># Enzymes</th>
<th>Volume of Enzymes</th>
<th>Volume of Reactor Vessel</th>
<th>Enzyme Packing Density</th>
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5. How does an engineer determine the optimal packing density?

6. Predict which enzyme packing density will maximize yield while minimizing enzyme loss. (Explain your reasoning in preparation for a class discussion)
Appendix A

The Pineapple Lab

This lab is a part of the “Understanding Catalysis in Nanoscale Biofuel Cell Technologies” module. It provides an introduction to the functioning of enzymes on a substrate.

Notes to Instructor:

Purchase gelatin packages (flavor not important), canned pineapple, and fresh pineapple. Prior to student arrival in class, prepare the gelatin solution. You will need 200 mL per Lab Group, extra is not a bad idea. Prepare the fresh pineapple in chunks similar in size to the canned pineapple.

Prepare transport container for transporting the student gelatin/pineapple complexes to a refrigerator.

Extension Question: In order to make valid comparisons in this experiment, what type of control is needed that has not yet been established?

100 mL of Jell-O without the addition of pineapple chunks

Post-Lab Questions and Answer:

1. What may have happened in the canning process to change the viability of the enzymes?
   *Heat used in the canning process may have decreased the viability of the enzymes*

2. In your own words, explain the relationship between a catalyst and an enzyme.
   *Enzymes are a type of catalyst*
Appendix B

The Clay Lab

This lab is a part of the “Understanding Catalysis in Nanoscale Biofuel Cell Technologies” module. It provides students a chance to investigate the relationship between surface area and volume, a key concept in nanoscale biofuel cells. It also provides a strong tie to the integration of math and science to engineering by using surface area and volume equations along with graphical analysis opportunity.

Notes to Instructor:

To increase the precision of measurement, calipers may be substituted for the metric ruler. Students with access to Microsoft Excel may be given the chance to graph their data electronically. A quick review of proper graphing techniques, i.e. the independent variable and dependent variable location will standardize graphs, making grading much easier for you.

It is wise to keep track of the clay and recollect as soon as possible to prevent potential custodial problems.

Post-Lab Questions and Answer:

1. What do the spheres represent in this lab?  
   *Enzymes*

2. Using your graph, state what happens to the value of surface area as the volume is increased.  
   *Increases*

3. Using the data table, state the trend of the surface area to volume ratio as the spheres get smaller.  
   *Increases*

4. Cells require a certain amount of energy per unit of volume. The energy is taken in across the surface of the cell membrane to reach the inner cell.
   a. Explain why cells tend to be so small?  
      *It allows them to get enough “energy” in and “waste” out*

      Bonus* What allows a single-celled chicken egg to be so large?  
      *It does not need to transport waste and energy in and out of the egg cell*
5. WSU chemical engineer Su Ha works with enzymes in his quest to build nanoscale biofuel cells. List at least 2 advantages of using nanoscale sized particles in a fuel cell.

- Their small size allows for small devices
- High surface area to volume ratio maximizes energy output potential

Answers may vary
Appendix C

The Alka-Seltzer Lab

This lab is a part of the “Understanding Catalysis in Nanoscale Biofuel Cell Technologies” module. It provides students a first hand look at the effects of surface area on reaction rates using common materials.

Notes to Instructor:

Group numbers need to be assigned and labeled on page one of the lab handouts. Do NOT assign a group number for 5, as students will interpolate this data point in post-lab question number 7.

When establishing the stop time criteria, make sure each group timer understands and writes the criteria in their laboratory handout for reference.

All fluid materials are safe to dispose of down the sink.

Post-Lab Questions and Answer:

1. Why is it so important that every group use the same “stop” time criteria?  
   *This allows for useful comparison of data between groups*

2. Why were you instructed to get the water from the tub instead of from the sink faucet?  
   *Sink faucets may not provide uniform water temperatures among groups, introducing too many variables.*

3. Identify five constants present in the control and experimental groups:  
   - Alka-Seltzer mass  
   - Water temperature  
   - Beaker size  
   - Timing criteria  
   - Volume of water

4. What represents the control group in this experiment?  
   *Reaction with one “unbroken” Alka-Seltzer tablet*  
   Explain: *Answers will vary*

5. According to your graph, what happens to reaction time as particle size decreases?  
   *It decreases*
Extrapolation
6. According to your graph, what is the reaction time of infinitely small particles?
   *Infinitely small*

Interpolation
7. According to your graph, what is the interpolated reaction time of 1/5\textsuperscript{th} sized particles?
   *Based on graphed data*

8. Identify each of the following:
   - Independent variable – *Particle size*
   - Dependent variable - *Reaction time*
Appendix D

The Mentos Lab

This lab provides a protocol and Excel worksheet to calculate a rate law equation of the system. This rate law equation provides students with a tool to solve rate problems similar to those used by engineers. If you have students with a strong math background, you may want to derive the rate law equation, which is calculated in the provided Excel File for you.

Notes to Instructor:

If the water is displaced to quickly, for a variety of reasons (temp, pressure, etc), consider using a larger diameter graduated cylinder for collecting the gas.

Reminder: Please stress in step 12, the Mentos inserters should lightly seal the Erlenmeyer flask with the rubber stopper to prevent excessive pressure build-up in the vessel.

In step 14, explain to the students that each recorded time is a measure from the beginning of data collection, NOT from the last lap time. Have each group make sure they understand how to run the split/lap function of their stopwatch prior to running their first trial. This includes both collecting the data and recalling the data from the stopwatch. A common error with this function is the resetting of the stopwatch to zero prior to acquisition of the lap data!!

Post Lab Questions:
1. In the space below, diagram the lab setup used in this experiment.

2. Explain why it was necessary to perform this lab as a team.
   
   *The timing coordination is impossible to achieve with one person*
Extension Questions:  These questions should be used for advanced math students only!

3. Write the Rate law derived from your data in the space below.

\[ \ln(\text{Rate}) = m(\ln[\text{CO}_2]) + \ln(K) \]

\( \ln(K) \) is the y-intercept value on the \( \ln(\text{Rate}) \) vs. \( \ln[\text{CO}_2] \) “Trend Line” for the graph in Excel. This equation can be displayed on the graph using the “Trend Line” function. The \( m \) (slope) is equal the slope of the line in the same graph.

So:

\( \ln(\text{Rate}) \) – To be given or solved for
\( m \) – slope of the trend line in the \( \ln(\text{Rate}) \) vs. \( \ln[\text{CO}_2] \) graph from Excel
\( \ln[\text{CO}_2] \) – To be given or solved for
\( \ln(K) \) – y-intercept of the trend line in the \( \ln(\text{Rate}) \) vs. \( \ln[\text{CO}_2] \) graph from Excel

Question six provides the rate data and requires students to solve for the \( \text{CO}_2 \) concentration using their derived rate law equation.

4. Of what use is a Rate law equation to scientists and engineers working with enzymes?

It allows them to optimize the product formation in a controlled system

5. What is the value of \( K \) in your rate equation? (Hint: use \( e^x \) on your calculator for your \( \ln K \) value)

Answers will vary based on collected data.

6. Suppose you are a fan of Diet Pepsi, but only if its concentration is at or above 140 micromoles \( \text{CO}_2 \) / ml Diet Pepsi. One day while walking across the Gobi Desert, you come upon a tent which contains 1 bottle of 22 C Diet Pepsi. The problem is that the seal on the bottle is broken. This is a problem for you because you are not sure if the \( \text{CO}_2 \) concentration meets your requirements for drinking. Fortunately, this special edition Gobi Desert Diet Pepsi bottle has a Built In Mentos Driven Rate Sensor (BIMDRS). You activate the BIMDRS and get a reading of 0.6 micromoles \( \text{CO}_2 \) / s

Using your Derived Rate law from Question 3 above, Determine whether or not you will drink the Diet Pepsi based only upon the concentration requirement. (Show all calculations for credit) Enjoy your Diet Pepsi…. Or maybe not.

Answers will vary
Appendix E

The Wiffle Ball Lab

This lab ties the concepts of previous labs into a modern day model of a nanoscale biofuel cell. The accompanying PowerPoint provides a conceptual framework into which several of the concepts unite. Students will construct a model of both a nanoscale biofuel cell vessel and “viable” enzymes. Students will see the benefits of containment vessels as unconfined enzymes become “unviable” when stressed. The stress is represented by high temperature, which also causes true enzymes to denature.

Notes to Instructor:

Nitinol wire can be purchased from vendors on the internet using “Nitinol wire” as a search term.

Materials needed for “enzyme” models:
Six - 15cm pieces .015mm diameter Nitinol wire
150 – 4mm diameter bracelet beads
Wire cutting pliers or scissors
magnets

1. Grab one of the 15 cm wires approximately 3cm from one end.

2. Bend the last 3cm of a nitinol wire back onto itself (see Figure 1-1).

3. Thread the first bead onto the short end of the wire.

4. Thread the second bead onto the long end of the wire, inserting the short end of wire through the bead as you approach the terminal end. (see Figure 1-2).
5. Continue threading successive beads onto the long end of the wire. Insert the short end into as many beads as possible.

6. When you reach the final 4 cm of bare wire, utilize the same fastening concept that was utilized at the start of the process.
   Note: This will require you to thread the wire into the beads (see Figure 1-3).

7. Repeat steps 1-6 for the remaining 5 wires.

Basic wire training and manipulation techniques:

To form the wire into spherical shapes, submerse the wire into ice water while bending. If you don’t use ice water for forming the wire, the desired outcome will not be achieved.

The authors found that differing Nitinol wire sources may require protocol adjustments. We suggest you run this lab with your materials prior to student participation.

Care should be taken when placing the unconfined “enzyme” in the hot water in step 13. The length of the “enzyme” must not exceed the inside diameter of the beaker, as the
wire straightens forcefully, with a potential to break the beaker upon expansion in a confined space.

For question 4, the equation for volume of a sphere is $V = \frac{4}{3} \pi r^3$

**Post Lab Questions:**

1. What happened to the single enzyme exposed to the stress of heat in the water?  
   *It rapidly expanded (denatured)*

2. Describe the effectiveness of an enzyme that has experienced stress similar to the above enzyme model.  
   *It becomes ineffective as it lost its three dimensional structure*

3. Describe the importance of cross-linking enzymes in the nanoscale reactor vessel.  
   *Cross-linking reduces costly enzyme loss*

4. As you learned in the PowerPoint prior to this lab, engineers working with enzymes often calculate an enzyme packing density. This equation, stated below, allows engineers to select optimal packing densities for their nanoscale reactor vessels. Use the equation to calculate the packing densities for nanoscale reactors containing 1, 2, 3, 4, and 5 enzymes with diameters equal to the value measured in step 8 of the procedure.

   Enzyme Packing Density = \frac{\text{Total volume of enzymes}}{\text{Total volume of reactor vessel}}

   *Answers will vary – Changes with size of beads and length of wire used*

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5. How does an engineer determine the optimal packing density?  
   *They run experimental tests and compare product amounts for different packing density setups.*

6. Predict which enzyme packing density will maximize yield while minimizing enzyme loss. (Explain your reasoning in preparation for a class discussion)

   *Students will likely predict that the highest packing density will maximize yield; however, a discussion of accessible surface area between enzymes and substrate, especially of those enzymes in the center of the reactor vessel, will affect the product yield.*
Idaho State Standards Correlation Data

Pineapple Lab
8-9.PS.1.2.1 Use observations and data as evidence on which to base scientific explanations. (648.02a)
8-9.PS.1.3.1 Measure changes that can occur in and among systems. (648.03b)
8-9.PS.1.3.2 Analyze changes that can occur in and among systems. (648.03b)
8-9.PS.1.3.3 Measure and calculate using the metric system. (648.03c)
8-9.PS.1.6.3 Use appropriate technology and mathematics to make investigations. (649.01c)

Clay Lab
8-9.PS.1.2.1 Use observations and data as evidence on which to base scientific explanations. (648.02a)
8-9.PS.1.2.2 Develop models to explain concepts or systems. (648.02b)
8-9.PS.1.3.1 Measure changes that can occur in and among systems. (648.03b)
8-9.PS.1.3.2 Analyze changes that can occur in and among systems. (648.03b)
8-9.PS.1.3.3 Measure and calculate using the metric system. (648.03c)
8-9.PS.1.6.3 Use appropriate technology and mathematics to make investigations. (649.01c)
8-9.PS.1.8.1 Analyze technical writing, graphs, charts, and diagrams. (658.02a)
9-10.B.3.3.1 Identify the particular structures that underlie the cellular functions. (651.01a)

Alka-Seltzer Lab
8-9.PS.1.2.1 Use observations and data as evidence on which to base scientific explanations. (648.02a)
8-9.PS.1.3.1 Measure changes that can occur in and among systems. (648.03b)
8-9.PS.1.3.2 Analyze changes that can occur in and among systems. (648.03b)
8-9.PS.1.3.3 Measure and calculate using the metric system. (648.03c)
8-9.PS.1.6.3 Use appropriate technology and mathematics to make investigations. (649.01c)
8-9.PS.1.8.1 Analyze technical writing, graphs, charts, and diagrams. (658.02a)

Mentos Lab
8-9.PS.1.2.1 Use observations and data as evidence on which to base scientific explanations. (648.02a)
8-9.PS.1.2.3 Develop scientific explanations based on knowledge, logic, and analysis. (648.02c)
8-9.PS.1.3.1 Measure changes that can occur in and among systems. (648.03b)
8-9.PS.1.3.2 Analyze changes that can occur in and among systems. (648.03b)
Mentos Lab - Continued
8-9.PS.1.3.3 Measure and calculate using the metric system. (648.03c)
8-9.PS.1.6.1 Identify questions and concepts that guide scientific investigations. (649.01a)
8-9.PS.1.6.2 Utilize the components of scientific problem solving to design, conduct, and communicate results of investigations. (649.01b)
8-9.PS.1.6.3 Use appropriate technology and mathematics to make investigations. (649.01c)
8-9.PS.1.6.4 Formulate scientific explanations and models using logic and evidence. (649.01d)
8-9.PS.1.8.1 Analyze technical writing, graphs, charts, and diagrams. (658.02a)
8-9.PS.5.2.1 Explain how science advances technology. (655.01a)

Wiffle Ball Lab
8-9.PS.1.2.1 Use observations and data as evidence on which to base scientific explanations. (648.02a)
8-9.PS.1.2.2 Develop models to explain concepts or systems. (648.02b)
8-9.PS.1.2.3 Develop scientific explanations based on knowledge, logic, and analysis. (648.02c)
8-9.PS.1.3.1 Measure changes that can occur in and among systems. (648.03b)
8-9.PS.1.3.2 Analyze changes that can occur in and among systems. (648.03b)
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8-9.PS.1.6.4 Formulate scientific explanations and models using logic and evidence. (649.01d)
8-9.PS.1.6.5 Analyze alternative explanations and models. (649.01e)
8-9.PS.1.6.6 Communicate and defend a scientific argument. (649.01f)
8-9.PS.1.8.1 Analyze technical writing, graphs, charts, and diagrams. (658.02a)
8-9.PS.5.2.1 Explain how science advances technology. (655.01a)
8-9.PS.5.2.2 Explain how technology advances science. (655.01a)