Laboratory Inquiry 3: Enzymes: Planning Your Project

IMPORTANT: You must bring a laptop or other electronic device for taking notes and keeping a copy of your team’s Project Planning Worksheet and other team materials completed in this lab session.

Despite experience to the contrary you might have had in doing science fair projects, science is not about materials and methods. Science is about observing natural phenomena around us and asking questions about them. Science is the process of trying to answer those questions in a logical, rigorous fashion that helps us better understand Life, the Universe, and Everything.

Science is about ideas.

So when your team meets today to discuss options for examining the effects of environmental conditions on the activity of catalase in live yeast, refrain from making your project about the methods.

Yes, you now understand how to use the equipment and reagents. But put that in the back of your mind for now, and focus instead on what you have learned in class and from your literature search about enzymes in general, and catalase in particular. Your project is about an idea, not about how to collect data.

Organizing your ideas

Linked to the syllabus (Session 3) is a Project Planning Worksheet. All team members should open that document on a laptop or other electronic device and work together to complete it. Every team member should have an identical copy of the document by the end of lab, and your team must send (via email) the completed form to your Laboratory Instructor for approval before you are allowed to leave the lab. Any student leaving the lab before having his/her team’s protocol sheet approved and being given permission to leave by the Lab Instructor will be docked five points.

The instructions here will help guide you through your Project Planning Worksheet.

A. Observations

All members of your team have finished a literature search. From these, you should be able to glean some observations. Be aware that you might not be able to perform an experiment directly related to the research papers you have read, but you can use what you have learned from them to consider things about catalase that might be interesting to investigate. List as many facts you've learned from the literature as possible. Each one is an observation.

B. Hypothesis

Based on the observations you have listed, you should be able to formulate a question, such as "How would a change in [variable of your choice] affect the rate of O₂ molecules produced by yeast (Saccharomyces cerevisiae) in the presence of hydrogen peroxide?"

Don’t forget to include why it is interesting, important, and relevant to investigate this variable. Scientists writing research grants must justify their proposals, and you are no different. Well, you’re a little different. But we’ll forgive you. For now.

1. Material Safety Data Sheet

If you ever wish to use a particular reagent in an experiment, then before you go even one step further, you must always do an online search for the chemical’s Material Data Safety Sheet (MSDS). No chemicals that are toxic, carcinogenic, or pose other health hazards will be allowed in your experiments. Simply open your browser, and in the search engine enter the name of any
chemical reagent and “MSDS”. One of the clearest MSDS sites is Sigma-Aldrich Chemical at www.sigmaaldrich.com. Click on the tab entitled “Safety and Documentation” and you will find all the safety information about the chemical. If you click on any of the safety symbols (red diamond with a graphic inside), a window will open with a key to the meaning of all the symbols.

2. Catalase Inhibitors and Other Potentially Useful Compounds

If you did a proper literature search, you probably noticed that many catalase-inhibiting (or enhancing) compounds seem to strike everyone within 15 feet dead. This means these compounds are not particularly nice to have in a laboratory full of frisky first year students. So here is a list of compounds that we have stock. Your team should have a look at this list, and do a quick literature search on any of these compounds with the appropriate keywords, and then confer with your team to continue to design your project USING THESE AVAILABLE REAGENTS.

Ascorbic acid
Acetyl salicylic acid (in stock aqueous solution)
Copper sulfate (in stock aqueous solution of less than 0.5M for safety)
Ethanol
Isopropanol
Succinic acid (in stock aqueous solution)
Salts to make buffers of various pH

Remember: just because we provide a chemical for you does not mean that we're going to tell you how it works or why it would be interesting to test its effects on catalase. That is still up to you and your team.

3. Overall and Statistical Hypotheses

Use your observations (literature search results) to devise your overall hypothesis. It might be something like, “In the catalase reaction that results from the addition of hydrogen peroxide to yeast (Saccharomyces cerevisae) suspension, the rate of oxygen production will be significantly [higher/lower--you decide] in a suspension with [variable A] than the reaction rate in a suspension with [variable B].”

Your overall hypothesis will allow you to tailor statistical hypotheses (a null hypothesis (H₀) and an alternative hypothesis (Hₐ)) to your protocol. These are two mutually exclusive hypotheses that should state the two (opposite) possible outcomes of your experiment. The null hypothesis is essentially the opposite of your overall hypothesis, and is stated in terms of no difference between the two groups being compared.

For example, your null and alternative hypotheses for the example above would be:

H₀: “In the catalase reaction that results from the addition of hydrogen peroxide to yeast (Saccharomyces cerevisae) suspension, the rate of oxygen production will be the same in a suspension with [variable A] as the reaction rate in a suspension with [variable B].”

Hₐ: “In the catalase reaction that results from the addition of hydrogen peroxide to yeast (Saccharomyces cerevisae) suspension, the rate of oxygen production will be significantly different in a suspension with [variable A] than the reaction rate in a suspension with [variable B].”

If you specify the direction of the results in the alternative hypothesis (i.e., “higher,” “lower,” “greater than,” “less than”, etc.), it is known as a one-tailed hypothesis.

If you do not specify the direction of the results in the alternative hypothesis, it is known as a two-tailed hypothesis.
You will learn more about this in the Statistics Primer (Session 5 Required Reading) and when you do your Data Analysis.

C. Prediction

Your overall hypothesis is parallel to your **prediction**. It can be likened to an educated guess. As your team develops its research idea, be sure to explain (and record!) the reasoning you have used to justify your prediction. For clarity, state your prediction as an if/then statement. For example, using the hypothesis above as a template, rephrase as: “If we add hydrogen peroxide to yeast (*Saccharomyces cerevisiae*) suspension in the presence of [variable A], the rate of oxygen production will be [greater/less] than in the presence of [variable B].”

When making your prediction, consider

a. What will be your **control** and **treatment** groups?
b. What is your **independent variable** (the variable not affected by other variables)?
c. What is your **dependent variable** (the variable that changes in response to the independent variable, and is the one you will measure)?

D. Experimental Design

Although materials and methods are not the focus of your research, they are certainly critical to your obtaining accurate results. So it is imperative that you create a very complete protocol for your experiment before you begin. Once you have devised on your hypothesis and made a prediction, it’s time to outline your experimental protocols on the [Project Planning Worksheet](#) linked to the syllabus (Session 3).

1. How will you measure your dependent variable?

2. How many trials each of control and treatment will you run? (To allow for more accuracy in statistical testing, we will require a *minimum* of six control and six treatment runs.)

3. What equipment will you need?

   **Each lab station will be equipped with the following standard supplies on a tray:**
   - 100ml beaker labeled “yeast”
   - 250ml beaker labeled “H₂O₂”
   - 10cc syringe labeled “yeast”
   - 20cc syringe labeled “H₂O₂”
   - two 50mL graduated cylinders
   - two 25mL graduated cylinders
   - one 1cc syringe labeled “H₂O”
   - two glass stirring rods
   - six clean, empty unlabeled 100ml beakers
   - one roll of labeling tape
   - one Sharpie labeling marker (or China marker)
   - one small pair of scissors
   - one Vernier O₂ sensor probe in its box (stored UPRIGHT)
   - one plastic Vernier respiration chamber
   - one clean, dry beaker labeled "probe" for storing your O₂ probe upright

   *If you need equipment not on this list, use the table at the end of your Project Planning Worksheet to itemize extra equipment or supplies your team will require.*

4. What chemical reagents will you need?

   **The center of each lab table will be equipped with the following standard reagents:**
   - Stock **yeast** suspension (70g yeast/L pH 7 sodium phosphate buffer)
• Stock **hydrogen peroxide** (33mL of 9.1% H₂O₂/L pH 7 sodium phosphate buffer)
• Stock **sodium phosphate buffer** (0.05M, pH 7)
• Deionized (DI) water

*If you need reagents not on this list, use the table at the end of your Project Planning Worksheet to itemize extra reagents your team will require to mix its own solutions.*

Remember that each **control reaction** is run with 10mL of standard yeast suspension and 20mL of H₂O₂ in pH 7 buffer. Knowing this, calculate the exact amount of all reagents you will need for

### Helpful formulas for determining your reagent needs

To calculate the mass (g) of a **solid chemical** needed to make a solution of a desired volume and concentration of Chemical X, use this equation:

\[
(\text{desired concentration in moles/L}) \times (\text{desired volume in L}) \times (\text{molecular weight of Chemical X in g/mole}) = \text{g of Chemical X needed}
\]

If you have a stock solution of known molarity and you want your final yeast suspension to be a different molarity, use this equation:

\[
(\text{concentration of stock solution in moles/L})(\text{volume of stock solution in L}) = (\text{desired final concentration of NaCl in your yeast suspension})(\text{desired final volume})
\]

Remember: If the amount of yeast suspension and the value of x do not add up to your desired final volume, you should add deionized water to your suspension to achieve the desired final volume so that treatment and control are the same.

### E. Data Collection

Decide which team member will be responsible for each aspect of the experimental runs and write that down on your Project Planning Worksheet.

Decide how you will collect your data. **If you are using a spreadsheet, create one now** and decide how to label columns and rows to best organize your data.

Decide **now** how you will share data among group members, and assign one team member to ensure that this is done before anyone leaves the lab next week when you have finished your experiments.

### F. Data Analysis

How will you present your data? Decide what types of figures or tables would best display your data and clearly show differences between your controls and treatments.

Will you perform statistical analysis? (We’ll give you this one: You will analyze your results using a Student’s t test.) Write this down in the appropriate spot on your Project Planning Worksheet.

**By the time you reach this point, you should have a completed Project Planning Form.**

**BEFORE YOU LEAVE LAB TODAY, YOU MUST SUBMIT YOUR PROJECT PLANNING FORM TO YOUR LABORATORY INSTRUCTOR IN ELECTRONIC FORM, AND WAIT FOR THE LAB INSTRUCTOR TO APPROVE IT AND GIVE YOU PERMISSION TO LEAVE. STUDENTS WHO LEAVE WITHOUT INSTRUCTOR APPROVAL WILL BE DOCKED FIVE (5) POINTS.**