BIL 151 Laboratory
Enzymes: Planning Your Project

Science is a process we use to answer questions about the natural world in a logical, rigorous fashion that helps us better understand Life, the Universe, and Everything.

Despite what you've seen at many high school science fairs, **science is not about methods**. When your team discusses options for examining the effects of environmental conditions on the activity of catalase in yeast, **DO NOT FOCUS ON METHODS**. (Example: You are using yeast as a *model organism* to explore the peroxidase system, *not* to explore the effect of variables on yeast.)

In short, your project is not about how to collect data. Your project is about an *idea*.

I. Organizing your ideas

As you did last week in the sample experiment, you must consider what you are trying to do before you begin designing your experiment and protocols.

A. Observations

As a team, **discuss your literature search results**. List as many facts you've learned from the literature search as possible (see the sample experiment from last week for guidance).
Each of these facts is an **OBSERVATION**.

B. Question, Hypotheses, and Predictions

Based on your observations, formulate a **QUESTION**.

> "How would a change in [variable of your choice] affect the rate of O_2 molecules produced by yeast (*Saccharomyces cereviseae*) in the presence of hydrogen peroxide?"

Include reasons why your question is interesting, important, and relevant to this system. (Scientists writing research grants must justify their proposals. So should you.)

1. Overall and Statistical Hypotheses

Rephrase your question as an overall hypothesis. For example:

> "In the catalase reaction resulting from the addition of hydrogen peroxide to yeast (*Saccharomyces cereviseae*) suspension, rate of oxygen production will be significantly [different/higher/lower--you decide] in a suspension with [variable A] than the reaction rate in a suspension with [variable B]."

Now tailor statistical hypotheses (**null (H_0)** and **alternative (H_A)**) to your protocol.
These should be two mutually exclusive hypotheses that state the two (opposite) possible outcomes of your experiment. For example:

> **H_0:** "In the catalase reaction that results from the addition of H_2O_2 to yeast (*Saccharomyces cereviseae*), the rate of O_2 production in a suspension with [variable A] **will not differ** from the rate of O_2 production in a suspension with [variable B]."
>
> **H_A:** "In the catalase reaction that results from the addition of H_2O_2 to yeast (*Saccharomyces cereviseae*), the rate of O_2 production in a suspension with [variable A] **will be significantly different/higher/lower** than the rate of O_2 production in a suspension with [variable B]."

Your team must decide whether a one-tailed or two-tailed hypothesis is more appropriate.

2. Prediction

Your overall hypothesis should parallel your **prediction.** It can be likened to an educated guess. Explain and record your reasoning and justifications for your prediction.
For clarity, you may wish to state your prediction as an if/then statement. For example:

> "If we add hydrogen peroxide to yeast (*Saccharomyces cereviseae*) suspension in the presence of [variable A], the rate of oxygen production will be [different/greater than/less than] in the presence of [variable B]."

When making your prediction, determine

a. What will define 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)?

II. Experimental Design

Although materials and methods are not the focus of your research, they are certainly critical to obtaining accurate results. Success requires planning.

Project Protocol Worksheet – Planning (PPW1) is linked to the syllabus in the section for today's lab session. PPW1 will become your best friend, as you will be

1. completing it
2. revising it
3. following it to perform your experiments

All team members should open PPW1 on [electronic device] and work together to complete it.
By the end of today's session, every team member should have an identical copy of PPW1.
Send a copy of your completed PPW1 to your instructor before you leave lab.

Any student leaving the lab
- **before having his/her team's PPW1 approved and**
- **being given permission to leave by the Lab Instructor**

...will be docked five points.

A. Materials and Methods

Various reagents can serve as peroxidase enhancers or inhibitors. Environmental factors such as temperature and pH also can affect enzyme activity. Your team should decide what variable to manipulate and *why* (in a greater context than just a simple lab experiment. In other words, why should we care about your experiment?)

1. Material Safety Data Sheet

If you wish to use a particular reagent in an experiment, *always* do an online search for the chemical's **Material Safety Data Sheet (MSDS**) (Figure 1).

A useful site for obtaining MSDS documents is **https://www.msds.com/**

1. You will be required to register to use the site, but just select the FREE plan.
2. A free plan includes five SDS views. Since your team has four members, that's 20 freebies.
3. Enter the name of the desired chemical in the site's search engine.
4. When the list of .pdf SDS choices appears, be sure to choose the language of your choice.
5. Use the SDS to complete your protocol sheet, as appropriate.

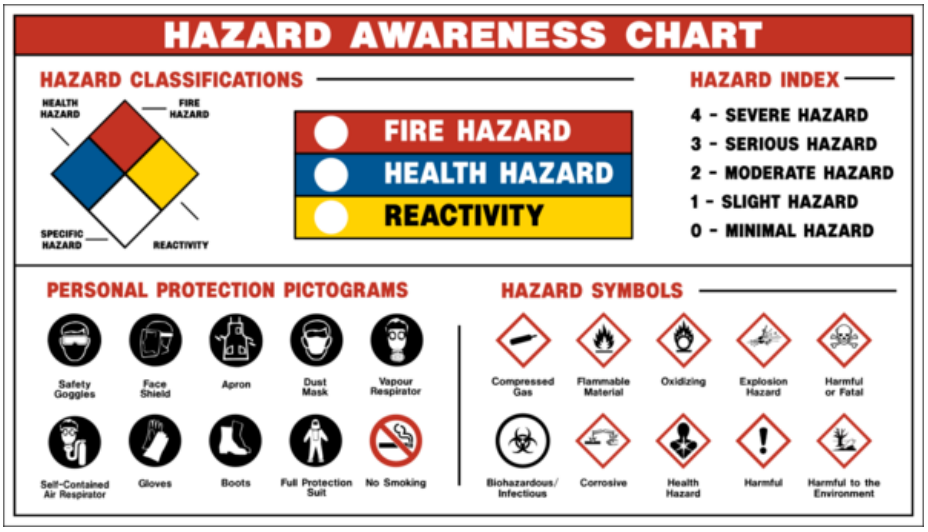

Figure 1. Meaning of symbols and colors on a Material Safety Data Sheet (MSDS).

2. Catalase Inhibitors and Other Potentially Useful Compounds

You may notice that many catalase-interacting compounds have the power to strike dead anyone standing within 15 feet. They are not nice to have in a lab full of frisky students.
For safety, we have limited available catalase-interacting reagents to those that are not overtly dangerous (at least at the low concentrations we will use).

The following reagents will be available.

- **acetyl salicylate solution (10mM) (100mL in covered glass vessel)**
- **ascorbate solution, 0.1M (100mL brown or foil-wrapped bottle)**
- **copper sulfate solution (0.1M) (100mL covered glass vessel)**
- **sodium chloride solution (1M) (100mL in covered glass vessel)**
- **succinate solution (0.04M) (100mL covered glass vessel)**
- **95% ethanol in covered glass vessel**
- **100% isopropanol in covered glass vessel**
- **salts to make buffers of various pH (recipes linked to syllabus in Enzymes, Session 3)**
  - **sodium phosphate buffer**
  - **glycine-sodium hydroxide buffer**
  - **sodium citrate buffer**

REAGENTS: DILUTIONS ONLY!

Note the concentrations of stock solutions above.
You will not be able to use concentrations *higher* than these.
You may only *dilute* the solutions to obtain lower concentrations.
Make a note of this when you select the reagent concentrations you will use for your experiment.

Be sure you understand concentrations.

- One molar (1.0M) solution contains 6.02×10^{23} molecules/L of solvent.
- One **millimolar** (mM) solution is 10^{-3}M.
- One **micromolar** (μM) solution is 10^{-6}M

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 use. That is still up to you and your team.

3. Planning Your Procedures

You should already be familiar with the Vernier system for measuring oxygen.
Now your team must decide how to employ it to address your hypotheses. Consider:

a. How many trials each of control and treatment will you run?
(A *minimum* of six control and six treatment trials will give you a statistically valid sample size.)

b. 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% H_2O_2/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 (PPW1) to itemize reagents your team will require to mix its own solutions.

Remember that each **control trial** is run with

- 10mL of standard yeast suspension
- 20mL of H_2O_2 in pH 7 buffer
- DI water to maintain constant volume in treatment and control reactions

Knowing this, calculate *in advance* the exact amount of all reagents you will need for all your treatment and control trials. Handy formulas:

To determine the solid mass (g) of Chemical Z needed to make a specific volume of specific concentration of Chemical Z use this formula:

[desired volume (L)] x [desired concentration (moles/L] x [MW of Chemical Z (g/mole)]
= g of Chemical Z needed (←this is your unknown)

To determine the solid mass of Chemical Z to add to a yeast suspension to bring it to a desired concentration, use this formula:

[desired concentration (moles/L)] x [(desired total volume (L)] x (MW of Chemical Z)
= g of Chemical Z needed (←this is your unknown)

To determine the volume (L) of known molarity NaCl stock solution to add to yeast suspension to attain a desired NaCl molarity of a desired volume of yeast suspension, use this formula:

[stock NaCl solution conc. (moles/L)] x [stock NaCl solution volume (L)] (←this is your unknown)
=
[desired NaCl concentration in yeast suspension) x (desired final volume of yeast suspension)

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c. What equipment will you need?

Each lab station will be equipped with the following standard supplies:

• 250 ml beaker labeled "Buffer pH 7" • 150 ml beaker labeled "Yeast" • 250 ml beaker labeled "H_2O_2" • 150 ml beaker labeled "1 M NaCl" • 10 x plastic Tri-pour beakers • 2 x 50 ml plastic graduated cylinders • 10 cc syringe labeled "yeast" • 1 cc syringe labeled "water" • 5 cc syringe labeled "water" • 1 cc syringe labeled "NaCl" • 2 glass stirring rods • 1 roll of labeling tape • 1 Sharpie marker • 1 small pair of scissors	• 1 DRY 400 ml beaker labeled "O_2 Sensor" / "Keep Dry" • 1 box of Kimwipes • 1 plastic Vernier respiration chamber with cap • 1 Vernier O_2 probe, upright, in its cardboard box • 2 CHEMICAL WASTE CONTAINERS

If you need equipment not on this list, use the table at the end of your Project Protocol Worksheet (PPW1) to itemize extra equipment or supplies your team will require.

B. 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.

Refer to previous lab manual chapters for experimental protocols, if you need a refresher.

C. Data Analysis

How will you analyze and 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** as you did for your sample experiment with NaCl.)

Write this down in the appropriate spot on your Project Planning Worksheet.

By the time you reach this point, you should have a completed PPW1 document.

Before you leave lab today, you must electronically submit your team's PPW1 and PPW2 forms to your laboratory instructor.
Do not leave lab until your instructor has approved the documents and given you permission to leave.
STUDENTS WHO LEAVE WITHOUT INSTRUCTOR PERMISSION WILL BE DOCKED FIVE (5) POINTS.