

## Laboratory Inquiry 2: **Enzymes: Materials and Methods**

**All students should bring a laptop, electronic pad, or other USB-equipped device for recording and storing data and for taking notes. This is good practice for all lab sessions for this semester.**

Before you begin designing your own project, you should become familiar with the equipment and experimental methods you will use. Today's lab session will be devoted to your team's rehearsal for the real thing. Before you start, be sure you are familiar with the information about enzymes from the previous lab, with the reagents and equipment you will have available in this lab period (see this lab chapter), the basic types of data you will be collecting, and the statistical methods appropriate for analyzing your data.

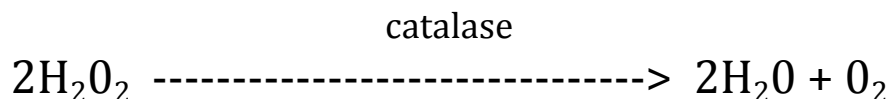
### **I. Experimental Protocol: Reagents and Equipment**

An **enzyme** is a biological catalyst that speeds up reactions in a living cell. The molecules upon which an enzyme acts are called its **substrates**. The molecules resulting from an enzymatic action on a substrate are called its **products**. Any particular enzyme, depending on its identity and function, can either build up a product from its substrates (**anabolism**), or break down a substrate into its components (**catabolism**).

All organisms produce large quantities of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as a byproduct of many metabolic reactions. Hydrogen peroxide is highly oxidative, so living cells must produce antioxidant enzymes (**peroxidases**) that break down peroxide into harmless water and oxygen, thereby preventing oxidative damage. In animals and fungi, one such peroxidase enzyme is known as **catalase**.

We will use live **yeast (*Saccharomyces cerevisiae*)** in an aqueous suspension as our model organism. Yeast are living, metabolically active fungi. Even while dry and dormant, yeast cells are protected by antioxidants, including catalase.

The equation showing the catabolysis of hydrogen peroxide by catalase is as follows:



One simple way to determine how well an enzyme is working is to measure the rate of its reaction. To determine catalase's reaction rate, one could measure the amount of H<sub>2</sub>O<sub>2</sub> decomposed or, equally well, the amount of H<sub>2</sub>O or O<sub>2</sub> produced, *per unit time* (i.e., the *rate* of this decomposition). Since O<sub>2</sub> is a gas, we can conveniently calculate the rate of its production with an oxygen gas sensor. And we just happen to have some.

In today's lab, your team will practice the methods you will use for your own original research project. By the end of this session, you should be able to (1) operate the equipment confidently, (2) know how to mix reagents suitable for your study, and (3) understand how to collect raw data.

The following section is an overview of the chemical reagents and equipment you will have available for your experiments.

#### **A. Chemical Reagents**

You will be provided with reactant (hydrogen peroxide in aqueous phosphate buffer) and enzyme (catalase in living yeast cells). NOTE: A **solution** is a liquid in which a solute is dissolved. A **suspension** is a liquid in which solid particles are floating. *Suspensions always should be mixed thoroughly before use, to ensure an accurate concentration of reactant throughout the liquid.*

## 1. Catalase (in living yeast cells)

**Catalase** is type of peroxidase enzyme made up of four interlocked polypeptide subunits linked to an iron-containing heme complex. Yeast catalase has a total molecular weight of 248,000 g/mole (Seah and Kaplan, 1972). Under optimum conditions, this highly efficient enzyme catalyzes the breakdown of millions of hydrogen peroxide molecules per second.

The stock yeast suspension we have provided for you contains 70g of yeast per 1L of stock pH 7.0 sodium phosphate buffer solution.

## 2. Hydrogen peroxide

**Hydrogen peroxide** ( $H_2O_2$ ) is a powerful oxidizing agent produced as a toxic byproduct of aerobic metabolism. Without rapid enzymatic catalysis,  $H_2O_2$  would quickly destroy essential biomolecules in a living cell, resulting in cell damage and death.

We have provided pre-mixed  $H_2O_2$  stock solution consisting of 33ml of 30-volume (9.1%)  $H_2O_2$  added to 1L of pH 7.0 sodium phosphate buffer. *You will need to calculate the percentage  $H_2O_2$  of this phosphate/peroxide solution to present in your presentation.*

## 3. Phosphate buffer

The pH of a solution expresses its acidity or alkalinity on a logarithmic scale. A solution with a pH of 7.0 is neutral. Values higher than 7.0 are alkaline/basic, and values lower than 7.0 are acidic. The higher the pH, the more alkaline/basic the solution, and the lower the pH, the more acid the solution.

**Buffers** maintain constant pH in solution. Buffer systems are widespread in living cells and organisms, where they help maintain homeostasis. Buffers are often used in experiments examining biological functions, to mimic living cell conditions as closely as possible.

You will be provided with a stock solution of pH 7.0 (neutral) phosphate buffer for your experiments.

## 4. Sodium Chloride (NaCl) solution

Sodium chloride has been shown to increase peroxidase activity in several different species. As a practice exercise, your team will explore the effects of sodium chloride concentration on the activity of yeast catalase. We have provided you with a stock solution of 1M NaCl in water.

## **B. Experimental Workstation and Equipment**

Before you begin, check your laboratory workstation for cleanliness, and to be sure all the materials listed below are present and in good working order. If something is not right, check with your laboratory instructor for replacements.

### **1. Your Lab Workstation**

Check your lab workstation to be sure all the following equipment is present.

**Consider: Do you trust the students who used this workstation before you to have left it perfect for the next team? Neither do we. So before using any of the equipment, wash every piece well, and rinse with distilled water. This is a good rule of thumb for lab work in most situations.**

Stock solutions of yeast suspension, hydrogen peroxide ( $H_2O_2$ ) solution, pH 7 phosphate buffer, and 1M sodium chloride (NaCl) solution can be found in the center of each lab bench. These are in labeled beakers, covered with a watchglass. Cover reagents after taking your share.

Two stirring rods are available for stirring stock yeast suspension. Always replace stirring rods in their labeled containment beaker when you are finished stirring the yeast.

## Your Team's Research Station

At your team's lab station (one station is located at each end of each lab bench), there should be one Vernier O<sub>2</sub> sensor probe in its box (stored UPRIGHT), one plastic Vernier respiration chamber (Figure 1), closed with a plastic cap, and one clean, dry beaker labeled "probe" for storing your O<sub>2</sub> probe upright.

To connect the probe to your laptop computer, you will need the Vernier adaptor cable. This is either in the box with your probe, or available at the TA desk at the front of the lab.

Your lab station should also be equipped with a tray bearing each of the following:

- 100ml beaker labeled "yeast"
- 250ml beaker labeled "H<sub>2</sub>O<sub>2</sub>"
- 10cc syringe labeled "yeast"
- 20cc syringe labeled "H<sub>2</sub>O<sub>2</sub>"
- 100ml graduated cylinder labeled "yeast"
- 100ml graduated cylinder labeled "H<sub>2</sub>O<sub>2</sub>"
- 100ml graduated cylinder labeled "H<sub>2</sub>O"
- one 1cc syringe labeled "NaCl"
- one 1cc syringe labeled "H<sub>2</sub>O"
- two glass stirring rods
- three clean, empty unlabeled 100ml beakers
- one roll of labeling tape
- one Sharpie labeling marker (or China marker)
- one small pair of scissors
- graduated cylinders labeled
  - "H<sub>2</sub>O<sub>2</sub>"
  - "Yeast"
  - "H<sub>2</sub>O"

Before you leave lab today, be sure all the items listed above are neatly replaced on your tray and that all spills are wiped up. **TEAMS WHO LEAVE LAB WORKSTATIONS DIRTY WILL BE DOCKED FIVE POINTS.**

## 2. Your Oxygen Sensor Equipment

You will use an electrochemical O<sub>2</sub> sensor (Vernier Software, [www.vernier.com](http://www.vernier.com)) to detect one of the products of the H<sub>2</sub>O<sub>2</sub> hydrolysis reaction, O<sub>2</sub> gas. Before designing experiments, you must first become familiar with using the O<sub>2</sub> sensor and data logger, and then with the basic procedure for measuring the rate of decomposition of hydrogen peroxide by yeast catalase.

**You will work in teams of four.** Each team member should participate in all aspects of all activities, and should be able to explain the rationale for each step of the methods. Delegate jobs to each team member (such as measuring substrate or enzyme, rinsing glassware, ordering pizza, etc.), but you are wise to have the same team member perform the same duties each time. Why do you think this is important? \_\_\_\_\_

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## 3. Setting up the O<sub>2</sub> sensor and data logger software

One team member with a laptop computer will kindly volunteer it for data collection.

1. Go to <http://www.vernier.com/products/software/logger-lite/#download> to download the software you will need on your laptop to complete this experiment. (If this lab chapter is in .pdf format, you may need to cut and paste this URL into your browser.)
2. *Only after you have installed the software*, connect the O<sub>2</sub> gas sensor and interface to the USB

port on your computer. **Keep the O<sub>2</sub> sensor upright at all times! Treat it with extreme care or you may damage it. Students who damage probes through negligence will be penalized by losing points.**

3. Start the Logger Lite 1.4 software by clicking on the icon on the desktop.
4. The software will detect the sensor and load a data table and graph. You are now ready to collect data!

#### **4. Collecting data with the O<sub>2</sub> sensor**

As an exercise to practice using the O<sub>2</sub> sensor, use the following instructions to compare the O<sub>2</sub> concentration of your exhaled breath with that of the atmosphere.

1. **Carefully and gently**, place the O<sub>2</sub> gas sensor into the plastic reaction chamber as shown in Figure 1. Gently push the sensor down until it stops. The sensor is designed to seal the chamber without unnecessary force.
2. Click **“Collect”** on the toolbar at the top of the Logger Lite window. The sensor will start measuring, 1x per second, the O<sub>2</sub> concentration (as %O<sub>2</sub>) of the air in the chamber. Note that the current %O<sub>2</sub> is displayed in the lower left corner of the window, while the readings over time are displayed on the data table and graph.
3. When the %O<sub>2</sub> value has stabilized, click **“Stop”** on the toolbar.
4. Record the %O<sub>2</sub> value.
5. Click **“Store”** on the toolbar to store this data run, and ready the software for the next.
6. Gently remove the O<sub>2</sub> sensor and place it upright in its dry holding beaker.
7. Breathe several times into the reaction chamber. Try to replace the air in the chamber with your exhaled breath.
8. Quickly, but still carefully and gently, place the O<sub>2</sub> sensor into the chamber as in step #1 above.
9. Collect data as in steps 2-6 above.
10. Click **“Save”** to save the results of this exercise.
11. Gently remove the O<sub>2</sub> sensor from the chamber and place it upright in its beaker.

#### **Results:**

What is the % concentration of O<sub>2</sub> in the atmosphere? \_\_\_\_\_

What is the % concentration of O<sub>2</sub> in your exhaled breath? \_\_\_\_\_

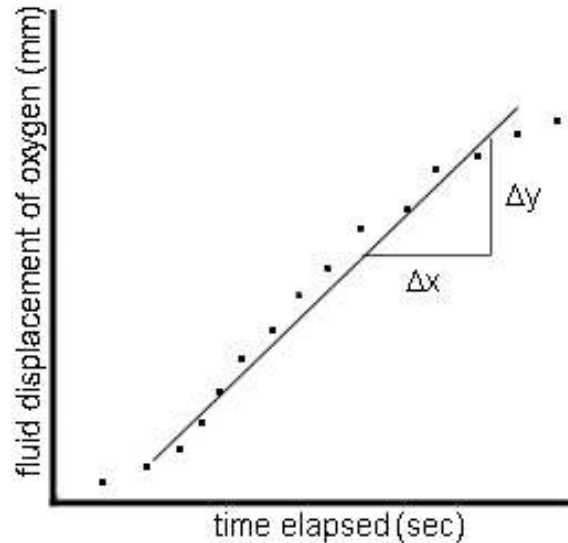
How long did it take the O<sub>2</sub> sensor to detect fully the %O<sub>2</sub>? \_\_\_\_\_



**Figure 1. Vernier O<sub>2</sub> gas sensor probe and reaction chamber ready to collect data.**

### C. Performing a Practice Experiment (designed by Alesia Sharber)

You are now ready to study the rate of reaction of catalase in living yeast cells. First, you will do three preliminary (**control**) trials, both to gain experience with the method and to study **experimental variability** (which is NOT the same as human error!). The data you collect will be in the form of a rate curve similar to the one shown in Figure 2. (*Note how the axes in Figure 2 are properly labeled. Whenever you create a figure, be sure the axes are properly labeled with units of measure!*)



**Figure 2. Oxygen generated by the catalysis of hydrogen peroxide by catalase**

Recall that yeast use catalase (a type of peroxidase) to break down toxic hydrogen peroxide into harmless water and oxygen. Over the course of your project, you may use the terms “catalase” and “peroxidase” interchangeably, but understand that while catalase is a peroxidase, not all peroxidases are catalase. There. We feel better about that now.

Today you’re going to conduct an experiment to determine how catalase functions at different ion concentrations. Below, you will find an example of a proper scientific proposal and protocol. After you complete this sample experiment, you will be better prepared to design your own experiment to perform in the next lab session. **Remember, this is only an example of how to create your own experiment. You will be choosing a parameter other than salt tolerance when you develop your own original projects.**

#### 1. Building a Hypothesis

Before you begin any experiment, you must collect **observations** in your area of research already made by scientists and published in peer-reviewed literature. For our sample experiments, we will study the effects of changing sodium chloride (NaCl) concentration on the activity of catalase. A literature search reveals that some research already has been done in this area, and we learn that

- Increased NaCl concentration has been shown to increase peroxidase activity in the halophytic archaen, *Halobacterium halobium* (Brown-Peterson 1993).
- Many plants show an increase in peroxidase activity when experiencing salt stress. In one study on rice (*Oryza sativa*), salt stress was induced by immersing the plant’s roots in 0.1M NaCl for 48 hours (Swapna 2003).

- Wild-type *Saccharomyces cerevisiae* does have some degree of salt tolerance. Concentrations of 0.3M NaCl induced stress responses after 45 minutes of exposure (Lewis 1995).

Based on this information, we ask the **question**, “How would an increase in NaCl concentration (from 0M to 0.3M) affect the rate of O<sub>2</sub> molecules produced by yeast (*Saccharomyces cerevisiae*) in the presence of hydrogen peroxide?”

Because we already have learned that increased NaCl concentration increases catalase activity, our proposed answer to this question--our **hypothesis**--is, “In the peroxidase reaction that results from the addition of hydrogen peroxide to a yeast (*Saccharomyces cerevisiae*) solution, the rate of oxygen production will be significantly higher in a solution with a 0.3M NaCl concentration when compared to that in a solution with a 0M NaCl concentration.” (More simply stated, we are **predicting** that increasing NaCl concentration in a yeast suspension from 0M to 0.3M will increase the rate of the peroxidase reaction.)

The above hypothesis is based on some known facts and some educated assumptions. Here is the **reasoning** we might use to justify the hypothesis and experimental design:

- Although the structure of peroxidases varies across different groups (bacteria, fungi, plants), all peroxidases display the same basic four-heme structure (Brown-Peterson 1993). Since the structure is similar between groups and multiple groups display the same increase in peroxidase activity when exposed to salt, peroxidase activity in *Saccharomyces cerevisiae* should follow this same trend.
- Former studies exposed their study organism to a NaCl solution for some time before observing a stress response or measuring increased peroxidase activity, so we will do the same. (Swapna 2003, Lewis 1995)

We now must define what outcomes will support the hypothesis. We **predict** that, “If ion concentration positively affects the peroxidase reaction, then the observed maximum rate of oxygen production will be higher in the 0.3M NaCl solution than in the 0M NaCl solution.”

## **2. Experimental Design – Measuring Reagents in Advance**

Before starting an experiment, always develop a solid plan to follow. Poor preparation leads to mistakes, which leads to having to throw out collected data and start over. All calculations needed for mixing reagents should be done in advance so that the protocol will be complete before beginning any step of the experiment.

- Stock solution was made by adding 70g of yeast to 1L of pH 7 sodium phosphate buffer solution.
- Stock solution of hydrogen peroxide solution was made by adding 33mL of 30-volume (9.1%) hydrogen peroxide to 1L of pH 7 sodium phosphate buffer solution.
- Each reaction will require 10mL of yeast suspension and 20mL of H<sub>2</sub>O<sub>2</sub>.

Each team will perform 3 trials each of both the **control** and **experimental/treatment** group. Given this information, how much stock solution will you need?

In case you have forgotten:

- **gram** is abbreviated **g**
- one **mole** =  $6.02 \times 10^{23}$  particles. **Molar solution** (moles/liter) is abbreviated **M**

- **liter** is abbreviated as **L**.
- one **cubic centimeter (cc)** is equal to **one milliliter (mL)**.
- One **L = 1000mL**.

**Yeast suspension:** You will perform six trials in all, each using 10mL of yeast suspension. Therefore, you will need at least 60mL yeast suspension. You'll need to **decant** (Note: to "decant" means to carefully pour) around 70mL of the stock suspension into your team's yeast beaker to allow for measurement error, but do not pour much more than you will need. It is wasteful!

**IMPORTANT:** Never measure reagents in beakers or flasks. Only graduated cylinders or syringes are accurate enough for proper measurement of reagents in any experiment.

**Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) solution:** Since you will perform six trials using 20mL/trial, you will need a total of 120mL of H<sub>2</sub>O<sub>2</sub> solution. You'll need to decant around 135mL to allow for measurement error.

**Sodium chloride (NaCl) solution:** For your experiment today, you will need to add solid salt or a saline solution to your treatment yeast suspension (NOT your controls!) and allow it to sit for 30 minutes to allow the yeast to react to the increased salt concentration.

### **3. Practice Calculating and Mixing Reagents**

To practice calculating how to make solutions of particular concentrations, complete the following three exercises. Solve for x, giving a number and units.

1. To calculate what mass (**g**) of a **solid chemical** to use to make a particular volume of a particular concentration of Chemical X solution, use this formula:

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

For example, if you wish to make 200mL (200mL = 0.2L) of 0.5M NaCl (molecular weight = 58.4 g/mole) solution:

$$0.5 \text{ mole/L} \times (0.2 \text{ L}) \times (58.4\text{g/mole}) = \text{g of NaCl}$$

**Solve:** How many g of NaCl must you add to to 0.2L of water to make a 0.5M solution? \_\_\_\_\_

2. Thus, if you wish to add solid Chemical X to your yeast suspension to bring it to a particular molarity, use this formula:

$$(\text{desired final concentration in moles per L}) \times (\text{desired total volume in liters}) \times (\text{molecular weight of Chemical X})$$

For example, if you wish to bring 10mL of yeast suspension from 0M NaCl to 0.3M:

$$(0.3 \text{ mole/L}) \times (0.01\text{L}) \times (58.4 \text{ grams/mole}) = \text{g of NaCl}$$

**Solve:** How many g of NaCl must you add to 0.2L of water to make a 0.5M solution? \_\_\_\_\_



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

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

For example, if you wish to achieve a 0.3M solution of NaCl in 15mL of yeast suspension, and have a stock solution of 1M NaCl, solve for  $x$ :

$$(1 \text{ mole/L}) (x) = (0.3\text{mole/L}) \times (0.15\text{mL})$$

**Solve:** How many L of 1M NaCl to make 15mL of 0.3M NaCl/yeast? \_\_\_\_\_

Note: If the amount of yeast suspension and the value of  $x$  do not add up to your desired final volume, you can add deionized water to your suspension to achieve your desired final volume. In the above example, we wish to achieve a final volume of 15mL. Since  $x = 4.5$  in the example above, we would add 0.5 deionized water to make up the difference.

#### **4. Performing a Sample Experiment**

***If you find that any steps are unclear today, write yourself notes so that you will have a personalized protocol nearly complete by the time you have to turn one in next week with your original project design.***

1. Check workstation and equipment. Clean any dirty items with deionized water and drain on a paper towel. (Check the list of supplies, above, that should be at your station. These items must be clean and back at your station when you check out for the day or your grade will be docked. When you are ready to leave, ask your Undergraduate Teaching Assistant (UGTA) to check your station and approve it before you go.)

2. Using labeling tape and a marker, re-label syringes and other supplies, as necessary.

**Remember: If an item is clean, it doesn't need a label until you have contaminated it with a reagent. If any item (syringe, graduated cylinder, beaker, etc.) touches a solution, it MUST be labeled with the type of solution (concentration and chemical) and your team name (in case items may get mixed up between neighboring groups). Clean and re-label items throughout the lab as necessary.**

3. Open the LoggerLite program on the laptop your team will use for data collection. (The download link is on page 3 of this chapter.)

4. Keeping the O<sub>2</sub> sensor probe UPRIGHT in its box, connect it to the laptop using the adaptor cable. **Remember to keep the probe dry and upright in its labeled beaker at all times when not in use.**

5. To ensure that the sensor is working correctly, click "Collect" on the toolbar. The %O<sub>2</sub> should be recorded once per second in the table to the left and on the graph. Try breathing into the dry plastic respiration chamber and putting the sensor in the top. The oxygen readings should go down. Now click "Stop." You can click "store" on the toolbar to save one data run and "save" to save an entire batch. To open a fresh data table and graph, click "New."



6. Before beginning the experiment, delegate duties to everyone in the team. Every person must know how to do every task, but it will be easiest to assign duties such as manning the computer, cleaning glassware, recording times, and so on amongst the team members.

#### **4a. Preparing your Treatment Samples**

Since the **treatment groups** will need time for the salt to induce a response in the yeast, those solutions should be set up first.

1. Label three beakers Treatment 1, 2, and 3
2. Find the stock yeast suspension on a tray at the center of your lab table. Mix the contents to ensure that yeast are evenly distributed and then decant 70mL into a labeled beaker. Stir the suspension with a stirring rod often to keep yeast from settling.
3. Add 10 mL of yeast suspension to each Treatment beaker, using the labeled syringe.
4. Add 4.5 mL of 1M NaCl and 0.5 mL of water to the first beaker and **record the time on the beaker label**. Set a timer or alarm on your phone for 30 minutes. (When the alarm goes off, it's time to use Treatment 1 for an experimental run.)
5. Wait five minutes. Then repeat steps 2, 3, and 4 to prepare Treatment 2.
6. Wait an additional five minutes. Then repeat steps 2 and 3 again to prepare Treatment 3.  
(Waiting five minutes between each treatment preparation will allow you to stagger the times when the samples are ready to be used, and still have the same NaCl exposure time for all treatment samples.)

#### **4b. Preparing and Using your Control Samples**

After the treatment groups are set up, the **control groups** can be prepared. Since they do not need to be incubated, you may place each control sample directly into the Vernier plastic respiration chamber and run the experiment immediately, without placing your samples in separate beakers.

1. Mix your remaining yeast suspension to ensure that yeast are evenly distributed.
2. Add 10 mL of yeast suspension to the plastic Vernier respiration chamber.
3. Add 5 mL of deionized water to ensure equal volumes between the treatment and control groups.
4. Find the stock hydrogen peroxide solution on the tray at the center of your lab table. Decant about 135 mL into a labeled beaker. Even at this low concentration, **hydrogen peroxide is slightly caustic, and can bleach clothing. Don't splash it!**
5. Back at your station, draw 20mL of your H<sub>2</sub>O<sub>2</sub> solution into a labeled syringe.
6. Prepare everything for an experimental reading, making sure your LoggerLite software is ready, that everyone is in position and ready to do his/her job.
7. When everyone is ready to start the experiment, quickly and carefully squirt 20 mL of H<sub>2</sub>O<sub>2</sub> solution into the plastic Vernier chamber already containing your yeast suspension.
8. Quickly and carefully insert the O<sub>2</sub> probe to seal the chamber.

9. Gently swirl the container (hold it by the neck, not the body) to stir. Do not rub the bottom of the chamber on the tabletop, to avoid friction. Swirl just enough to keep the mixture moving, but **don't wet the probe!**
10. The reaction begins very shortly after the introduction of hydrogen peroxide, so click "Collect" in the LoggerLite program once you have begun swirling your suspension. **Be very careful to keep the probe dry or your readings will become erratic. If this happens, use one of the ringstand/fan assemblies set up along the back table to dry your probe. Do not clamp the probe tightly, to avoid damaging it.**
11. When the %O<sub>2</sub> readings begin to plateau, click "Stop." This will happen automatically after 300 seconds.
12. Click "Store" to save the readings from that sample.
13. Gently remove the O<sub>2</sub> sensor and place it upright in its dry, labeled beaker.
14. Discard the yeast suspension in the front or rear sink and wash the chamber well with tap water until it is completely rinsed and clear. Give it a final, all-over rinse with deionized water three times to ensure that no residue is left behind. **DO NOT POUR YEAST SUSPENSION INTO THE LAB BENCH SINKS! THESE HAVE BEEN DE-COMMISSIONED DUE TO YEARS OF ABUSE BY NEGLIGENT STUDENTS, AND YEAST CANNOT BE RINSED DOWN. THEY WILL SIMPLY SIT AND FERMENT IN THE PLUMBING, CAUSING HORRIBLE STENCH, AND GREAT WAILING AND GNASHING OF TEETH.**
15. Repeat Steps 10-14 twice more for the remaining control groups. Work quickly and carefully in order to be ready for the treatment groups when their 30 minute incubation time is up (i.e., when your first alarm goes off).
16. Follow Steps 1-13 for each of your **treatment groups**, substituting your NaCl samples for the stock yeast suspension. All other steps in the protocol should be the same as for the control groups. (Note: Do not add 0.5mL of DI water to treatment samples; only controls.)

When all groups have finished running their trials, your lab instructor will pool the data from the entire class and demonstrate how to run a **Student's t-test**. This statistical test will allow you to determine whether the mean rates of reaction in your control and treatment groups are significantly different. In your future original experiments, this analysis and your resulting interpretation will be extremely important.

**Before your team leaves for the day (and every day), ensure that every team member has a copy of all data, protocols, or articles collected for that day.**

## **5. Data Analysis**

Use the Vernier software to calculate and compare the rates of reaction for the five trials you just ran. Examine the curves on your graph. Each curve shows the %O<sub>2</sub> as it changes over time (the time course of the reaction). At first, there may be a lag period, as substrate molecules enter the cell and enzyme molecules in the cells gradually come into contact with the substrate. Then there is an initial, steadily rising linear portion of the curve that represents the maximum rate of reaction. As substrate is used up, the increase in %O<sub>2</sub> will slow over time, and the curve will gradually flatten, forming an S-shaped curve. This means that all the H<sub>2</sub>O<sub>2</sub> has been broken down, and the reaction has stopped. No more oxygen is being generated.

Only the early, linear portion of the curve reflects the maximum reaction rate; this is the region of the curve you should use to compare your different reaction rates. Follow the steps below for each curve.

1. Highlight the maximum rate of reaction portion of the curve using the mouse.
2. Click on “Analyze” on the top menu, then “Linear Fit.” In the dialog box that appears, be sure to check the curve you’ve just highlighted. A best fit linear regression line will now appear for your highlighted points, along with a floating box containing the equation of the line. The *correlation* statistic (*r*) shows how well your actual data points fit the line, with a correlation of 1.0 showing a perfect fit. Using the mouse, you can grab and move the brackets to change the highlighted points, to see if there is a better fit. The line, correlation and equation will automatically update as you move the brackets.
3. Examine the equation of the best fit line you chose. The **slope (m)** shows the change in %O<sub>2</sub> over the change in time, which is the **rate of reaction**. Record this value as the rate (%O<sub>2</sub>/sec) in the appropriate cell of Table 1.
4. The volume of the reaction chamber, with the O<sub>2</sub> gas sensor inserted, is approximately 280 mL. Your solutions take up about 30mL of this space. Use this information to calculate the mL O<sub>2</sub>/sec produced as a percentage of the total working volume.
5. Recall that your stock yeast suspension contains 70g/L of yeast. With this information, you can calculate the mL O<sub>2</sub>/g/sec generated by the yeast:
  - a. Logger Lite records the rate of reaction in %O<sub>2</sub>/sec. However, we wish to calculate an adjusted rate that is more meaningful for comparison, and takes into account the volumes of the materials we are using.
  - b. The total volume of your reaction chamber is 280mL.
  - c. Some of this volume (10mL) is occupied by yeast suspension.
  - d. Some of this volume (20mL) is occupied by H<sub>2</sub>O<sub>2</sub> solution.
  - e. Some of this volume (5mL) is occupied by water or NaCl solution.
  - f. Subtract the liquid volume (35mL) from the total volume (280mL), and you have 245mL of space in the container.
  - g. Multiply %O<sub>2</sub>/sec x (245mL) = mL O<sub>2</sub>/sec
  - h. Record this adjusted rate in Table 1.
6. Cut and paste your data table and graph into a Word, Excel or similar document of your choice.

***Be sure each team member gets an electronic copy of the data before you leave lab today. Never have only one person in charge of storing the only copy of your team's data! The reason for this should be obvious.***

**Table 1. Reagent quantities and reaction rates for catalysis of H<sub>2</sub>O<sub>2</sub> by yeast.**

tube #	Volume of yeast suspension	mL % H <sub>2</sub> O <sub>2</sub>	rate (%O <sub>2</sub> /sec)	adjusted rate (mL O <sub>2</sub> /g/sec)
1	10	20		
2	10	20		
3	10	20		
4	10	20		
5	10	20		
			mean	
			standard deviation	

When your team designs and executes its original research project, you will be measuring and calculating reaction rates as described above. Because you will run experimental trials multiple times for each of your two variables (i.e., treatment and control), your data will ultimately consist of multiple reaction rates (one for each experimental trial).

You will calculate a **mean** reaction rate for each of your two variables (i.e., treatment and control), and then use a statistical test known as the student's t test to determine whether the two means are sufficiently different from one another to allow your team to reject your null hypothesis. You will learn about this in greater detail once your research project data collection is complete.

## **6. Experimental Error vs. Human Error**

Your team used the same quantities of reagents in each trial, and (presumably) used the same temperature and pH—but did you get exactly the same reaction rate each time? What might account for slightly different results among trials?

Slight variation in results in carefully run trials is known as **experimental variability** or **experimental error**. Note that this natural variability is NOT the same as variability caused by actual mistakes (**human error**) made during the experiment.

**DO NOT CITE HUMAN ERROR AS A REASON FOR UNEXPECTED RESULTS IN YOUR EXPERIMENT! THAT IS SIMPLY UNPROFESSIONAL.** If you make accidental mistakes that could affect your results, you should re-do the experiment, not simply explain away those mistakes as “human error.” Citing human error as a good reason for your results is about as good as saying, “Oops! We are terrible at science. But we don't really care enough to do it right.” NEVER include human error in this or any future discussions of experimental variability. **Experimental error ≠ mistakes!** When contemplating your results, your fellow scientists will assume you have done your experiments as carefully as possible, and have minimized inaccuracies due to human error.

## **7. Translating These Techniques to Your Own Experiment**

In the next session, your team will design an experiment to test hypotheses based on an observation you have gleaned from your literature search. You will use the same the procedures described above for all of your experimental runs, manipulating only one variable.

You will be able to analyze the data from your own original experiment only if you understand (1) how to calculate the rate of any given trial, (2) how to calculate the mean rate of multiple trial runs under the same environmental conditions, and (3) how to meaningfully compare two or more means calculated for reaction rate under different environmental conditions.

In preparation for next week's lab, your team should discuss your literature search results and consider interesting ideas for an experiment to begin the following week. No need to wait until next week. Once you've completed your trials today, start thinking of experiments your team could design.

## **8. To Do At Home: Customizing data collection and graphical display**

Before you perform your own experiment, take time to go through the tutorials in the LoggerLite program. They may help you view and organize your data more clearly. To view these tutorials, click “Open” on the toolbar, then “Tutorials” in the “Experiments” folder. Tutorials may be most helpful. To make your subsequent work easier, familiarize yourself with the Logger Lite software.

1. Click “Open” on the toolbar at the top of the window.
2. Click “Tutorials” in the “Experiments” folder that opens.
3. Click on “Tutorial 3 – Customizing.” (Ignore the message about the Go Temp probe; you will still

be able to use the information in the tutorial without it.) Click on “Pages” on the toolbar to go to other pages.

4. Using the information in this tutorial, be able to do the following on your saved graph (your instructor may check to be sure that you know how to do this):
  - Change the rate and duration of data collection
  - Give the graph a title
  - Un-connect the points of the graph
5. Now open “Tutorial 4 – Working with graphs.” Be able to:
  - Change the scale of the x and y axes
  - Stretch the x and y axes
  - Autoscale the axes

### **Literature Cited**

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