BIL 151 Laboratory
Enzymes: Practicing the Protocol

Bring a laptop, electronic pad, or other USB-equipped device for recording and storing data.
Today you will learn a technique for measuring the rate of an enzymatic reaction, the breakdown of **hydrogen peroxide** by **catalase**. Becoming familiar with the experimental protocol will help you and your team to design an original project.

I. Experimental Protocol: Reagents and Equipment

By the end of this session, you should be proficient at

- operating the equipment confidently
- collecting data with the Vernier O_2 sensor system
- maintaining a clean, efficient workspace
- leaving your workspace clean and tidy for the next team

A. Chemical Reagents

You will be provided with

- **Hydrogen peroxide** (H_2O_2) in aqueous phosphate buffer (**substrate**)
- **Catalase** (the **enzyme** that breaks down H_2O_2, in live yeast)

A **solution** is a liquid in which a solute is dissolved.
A **suspension** is a liquid in which solid particles are floating.

1. Hydrogen peroxide (H_2O_2)

Hydrogen peroxide is a powerful **oxidizing agent** produced as a toxic byproduct of aerobic metabolism. H_2O_2 can quickly destroy living cells.

Your stock H_2O_2 suspension contains 33mL of 9.1% H_2O_2 per 1L of phosphate buffer.

You must calculate the percentage H_2O_2 of this phosphate/peroxide solution to include in your presentation.

2. Catalase (in living yeast cells)

Catalase is a **peroxidase** that

- consists of four polypeptide subunits and an iron-containing heme complex
- catalyzes breakdown of millions of hydrogen peroxide molecules per second
- has a molecular weight = 248,000 g/mole

Your stock yeast suspension contains 70g of yeast per 1L of phosphate buffer.

Always use a glass stirring rod to stir stock yeast suspension before use.
Rinse and replace stirring rods in their labeled containment beaker after use.

3. Phosphate buffer

Buffers maintain constant pH in solution, and are widespread in living cells.
Buffers are used in the lab to mimic living cell conditions as closely as possible.

Your stock buffer solution is pH 7.0 (neutral) phosphate buffer.

If you wish to use buffers of different pH,
you will be provided with instruction on how to mix them.

B. Workstation and Equipment

Put on your Personal Protective Equipment (PPE).

Check your laboratory workstation for cleanliness.
If something is not right, ask your laboratory instructor for replacements.

1. Check Your Lab Workstation Supplies

Your team's lab workstation should be equipped with

- one **Vernier O_2 sensor probe** in its box (stored UPRIGHT)
- one plastic **Vernier respiration chamber** (Figure 1) with plastic cap
- one clean, dry **beaker labeled "probe"** for upright storage of your O_2 probe

You will need a **GoLink Vernier adaptor cable** to connect the system to your laptop.
This is available at the instructor's desk.

Stock solutions are located on trays in the center of each lab bench

- in labeled beakers
- covered with a watchglass

Always use the properly labeled equipment to take stock solutions.
Be careful not to contaminate solutions!
Always re-cover reagents after taking your share.

Check to be sure your lab station is equipped with the following:

- 250 ml beaker labeled "Buffer pH 7"
- 150 ml beaker labeled "Yeast"
- 250 ml beaker labeled "H_2O_2"
- 3 x 100 ml glass beakers
- 2 x 50 ml plastic graduated cylinders
- 10 cc syringe labeled "yeast"
- 1 cc syringe labeled "water"
- 5 cc syringe labeled "water"
- 2 glass stirring rods
- 1 DRY 400 ml beaker labeled "O_2 Sensor" and "Keep Dry"
- 1 box of Kimwipes
- 1 plastic Vernier respiration chamber with cap
- 1 Vernier O_2 probe, upright, in its cardboard box
- 1 x 1 L flask labeled "WASTE" and a blank piece of tape above it (to indicate what type of chemical waste)
- 1 roll of labeling tape
- 1 Sharpie marker
- 1 small pair of scissors

Do you trust the students who used this workstation before you to have left it perfect for the next team? You shouldn't.

Before using equipment, wash and rinse glassware with distilled water.
Cleaning equipment before use is a good rule of thumb for lab work in most situations.

2. Meet Your Oxygen Sensor Equipment

You will use an electrochemical O_2 sensor (Vernier Software, www.vernier.com) to detect O_2 gas generated from decomposition of hydrogen peroxide by catalase. Each team member should participate in all aspects of the experiment, and be able to explain the rationale for all methods. Delegate tasks to different team members, but **the same team member should perform a particular task throughout the data collection period.**

Why do you think having the same person do the same job in each experimental run is important?_

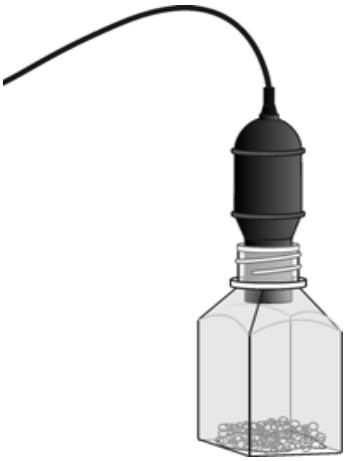

Figure 1. Vernier O_2 gas sensor probe and reaction chamber ready to collect data.

3. Set up the O_2 sensor and data logger software

Use a laptop (one per team) to collect data.

1. Go to **http://www.vernier.com/downloads** to download Logger Lite software.
2. *Only after you have installed the software*, connect the O_2 gas sensor and interface to the USB port on your computer with the adapter cable.
3. Start the Logger Lite 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. Practice using the O_2 sensor

Quick exercise: compare O_2 concentration of your exhaled breath with that of the atmosphere.

Keep the O_2 sensor upright at all times! Treat it with extreme care and handle gently.
Students who damage probes through negligence will be penalized with loss of points.

1. **Carefully** place the O_2 sensor into the plastic reaction chamber (Figure 1).
   - Gently push the sensor down until it stops.
   - **The sensor is designed to seal the chamber without undue force.**
2. Click "**Collect**" on the toolbar at the top of the Logger Lite window.
   The sensor will start measuring the O_2 concentration (as $\%O_2$) in the chamber once per second.
   The current $\%O_2$ is displayed in the lower left corner of the window.
   The $\%O_2$ over time is displayed on the data table and graph.
3. When the $\%O_2$ value stabilizes, click "**Stop**" on the toolbar.
4. **Record** the $\%O_2$ value.
5. Click "**Store**" on the toolbar to save this data run, and ready the software for the next run.
6. Remove the O_2 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) insert the O_2 sensor into the chamber as before.

9. Collect data as in steps 2-6 above.

10. Click "**Save**" to save all the results of this exercise.

11. Remove the O_2 sensor from the chamber and place it upright in its beaker.

Record Results:
What is the % concentration of O_2 in the atmosphere? _____
What is the % concentration of O_2 in your exhaled breath? _____
How long did it take the O_2 sensor to detect fully the $\%O_2$? _____

5. Practicing with Live Yeast

Delegate duties to each team member. Everyone should know how to do every task, but the same person should do the same task for all experimental runs.

1. **Inventory** your workstation to be sure you have everything you need.
   Wash any used/dirty items with deionized (DI) water and drain on a paper towel.

2. **Label** measuring vessels (syringes, graduated cylinders, etc.) with the reagent they will contain.
   Use labeling tape and your Sharpie/lab marker.

DO NOT CONTAMINATE STOCK (OR ANY) SOLUTIONS!
A clean/washed item needs a label only when you know what reagent it will be used to collect.

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.

Wash and re-label items throughout the lab, as necessary.

3. **Open** the LoggerLite software.

4. **Connect** the O_2 sensor probe to the laptop with the adaptor cable. Always hold it upright.
   Keep the probe dry and upright in its labeled beaker at all times when it is not in use.

5. Click "**Collect**" on the toolbar to ensure that the sensor is working.
   The $\%O_2$ should be recorded once per second in the table to the left and on the graph.

6. **Stir the stock yeast suspension well to ensure yeast are evenly distributed**.

8. To the plastic Vernier respiration chamber add:
   - **10 mL of yeast suspension**
   - **5 mL of deionized (DI) water**

9. Decant about **135 mL of stock H_2O_2** (from center tray) into a labeled beaker from your station.
   CAUTION: Hydrogen peroxide is somewhat caustic. It can sting skin and bleach clothing.

11. Back at your station, **draw 20mL of the stock H_2O_2 solution into a labeled syringe**.

12. Prepare everything for an experimental reading.
   - Ready LoggerLite software
   - Team members in position and ready to do their assigned task
   - When everything is completely ready….

13. Add to the plastic Vernier respiration chamber containing yeast suspension and water
   - **20 mL of H_2O_2**
   - **The reaction begins very quickly once H_2O_2 is added. Be quick and efficient!**

14. Insert the O_2 probe to seal the chamber.

15. *Gently* swirl the container. Heat will affect rate of reaction. Therefore:
    - **Hold the chamber by the neck, not the body.**
    - **Do not rub the bottom of the chamber on the tabletop**

Swirl just enough to keep the mixture moving, but *do not wet the probe!*
THE PROBE WILL MALFUNCTION IF IT IS WET.

16. Click "**Collect**" in LoggerLite as soon as you begin swirling your suspension.

IT IS CRITICALLY IMPORTANT TO KEEP THE PROBE DRY. *DO NOT SWIRL VIGOROUSLY.*
If readings become erratic, the probe is wet and must be replaced.

If your probe malfunctions, give it to your instructor to exchange for a new probe.

17. When the $\%O_2$ readings begin to plateau, click "**Stop**."
    (This will happen automatically after 300 seconds.)
18. Click "**Store**" to save the readings from that sample.
19. In the pull-down menu at the top labeled "**Analyze**", choose "**Linear fit**".
    - Choose the **straightest part of your reaction rate curve** with your cursor.
    - (Your instructor can demonstrate this for you if it's not intuitively clear.)
    - This will give you a (best fit) reaction rate for the linear portion of your reaction.
    - **Record the rate (V_o for this experimental run) in an appropriate spreadsheet.**
20. Remove the O_2 sensor and place it upright in its dry, labeled beaker.
21. Discard the yeast suspension in the **labeled WASTE VESSEL** at your station.

DECANT ALL USED REAGENTS INTO THE LABELED WASTE CONTAINER AT YOUR STATION.
DO NOT POUR YEAST SUSPENSION INTO THE SINKS!

IT WILL SIT AND ROT IN THE PLUMBING, CAUSING HORRIBLE STENCH,
AND GREAT WAILING AND GNASHING OF TEETH.

Repeat the above experimental procedure twice more so that you have practiced three times. If you feel the need for more practice, you may do a few additional runs.

Check supplies at your station with the list in Section B1 of this chapter.
All items must be clean and restored to your station before you check out for the day.
Ask your instructor to check and approve your station before you leave.

TEAMS LEAVING DIRTY WORKSTATIONS WILL BE DOCKED FIVE POINTS.

Next week, you will use these same procedures to perform a sample experiment in which you will explore the effects of sodium chloride on catalase activity. Read ahead and be ready!

Additional Reading

Seah, T.C.M, and Kaplan, J.C. 1972. Purification and Properties of the Catalase of Bakers' Yeast. *Journal of Biological Chemistry* 248, 2889-2893.