BIL 151
Enzymes: A Sample Experiment with NaCl
Part I. Experimental Protocol: Reagents and Equipment

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Today you will use the techniques you practiced last week to perform a sample experiment.
You will examine the effect of sodium chloride (NaCl) on the catalase reaction.

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

- operating the equipment confidently
- mixing reagents
- collecting data
- calculating molarities
- keeping experimental conditions consistent

Remember:

- **gram** is abbreviated **g**
- one **mole =** 6.02 x 10^{23} particles.
  - **Molar solution** (moles/liter) is abbreviated **M**
- **liter** is abbreviated as **L**
- 1 **cubic centimeter (cc)** = **1 milliliter (mL)**
- One **L** = **1000mL**
- **Molecular weight** is abbreviated as **MW**

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

4. Sodium Chloride (NaCl) solution

Sodium chloride has been shown to increase peroxidase activity in several different species.
Your stock NaCl solution is 1M NaCL in sodium phosphate buffer.

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

As before, your team's lab station should be equipped with

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

Obtain a **GoLink Vernier adaptor cable** 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.

Your lab station should be equipped with each of the following:

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

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. Your Oxygen Sensor Equipment

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.** (Do you recall why?)

3. Set up the O_2 sensor and data logger software

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

1. If you have not already done so, download the necessary software at
   http://www.vernier.com/products/software/logger-lite/#download
2. *After you have installed the software*, connect the O_2 gas sensor to your laptop USB port.
3. Click on the Logger Lite 1.4 icon to open the software.
4. The software will detect the sensor and load a data table and graph.

4. Collecting data with the O_2 sensor: Review

1. **Carefully** place the O_2 sensor into the plastic reaction chamber.
   - 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.