

BIL 151

Enzymes: A Sample Experiment with NaCl

Part III. Experimental Protocol

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The procedures outlined here are the same as those you used to practice during the last lab session. We reproduce them here for your convenience, but they should be familiar to you.

Experimental Protocol

Delegate duties to each team member.

While you are waiting for your samples to incubate, do the following.

1. Decant about **135 mL of stock H₂O₂** (from center tray) into the labeled beaker from your station and take it back to your station.

CAUTION: Hydrogen peroxide is somewhat caustic. It can sting skin and bleach clothing.

REMEMBER: DO NOT CONTAMINATE STOCK (OR ANY OTHER) SOLUTIONS!
Any item (syringe, graduated cylinder, beaker, etc.) that touches a solution MUST be labeled for that type of solution.

Include the name of the reagent, its concentration, and your team name.

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

2. **Open** the LoggerLite software.
3. **Connect** the O₂ 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.
4. Click "**Collect**" on the toolbar to ensure that the sensor is working.
The %O₂ should be recorded once per second in the table to the left and on the graph.
5. About five minutes before your sample is ready, prepare for an experimental reading.
 - Ready LoggerLite software
 - Team members in position and ready to do their assigned tasks
 - **Draw 20mL of stock H₂O₂ solution into a labeled syringe.**
 - When your alarm goes off, and your sample is fully incubated...
6. Pour the **15 mL yeast suspension sample** into the Vernier respiration chamber.
7. Add **20 mL of H₂O₂**

The reaction begins very quickly once H₂O₂ is added. Be quick and efficient!

8. Insert the O₂ probe to seal the chamber.
9. 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 (friction!)**
10. 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.

11. When the %O₂ readings begin to plateau, click “**Stop**.”
(This will happen automatically after 300 seconds.)
12. Click “**Save**” to save the readings from that sample.
Label the recording appropriately (T1, C1, etc.)
13. 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.)
 - **DO NOT INCLUDE AREAS OF THE CURVE WHERE THE REACTION IS STARTING UP OR LEVELING OFF. Use only the area representing the maximum rate of reaction.**
 - **Move brackets on either side of your chosen area of the curve to find the span in which “correlation” (in the pop up window) is as close to 1.0 as possible.**
 - This will give you a **best fit** reaction rate for the linear portion of your reaction.
 - **Record the rate in an appropriate spreadsheet.**
14. Remove the O₂ sensor and place it upright in its dry, labeled beaker.
15. Discard the yeast suspension in the **labeled WASTE VESSEL** at your station.

REPEAT STEPS 1 – 15 for each of your treatment and control samples.

**DECANT ALL USED REAGENTS INTO THE
LBELED 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.**