

# BIL 151

## Enzymes: A Sample Experiment with NaCl

### Part IV. Data Analysis

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We now must determine whether there is a significant difference in reaction rate between our treatment and control samples.

Use the Vernier software to calculate the **rates of reaction** of your treatment and control trials. Remember that each curve shows the **%O<sub>2</sub> in the chamber** as it changes over time.

The S-shaped curve represents

- Slight catalase lag time at the start of reaction
- Leveling off of reaction as substrate is used up

The early, *linear* portion of each curve reflects the **maximum reaction rate**.

*It is these portions of the curve for each experimental run that should be compared.*

1. Use your mouse to **highlight the maximum rate of reaction region** of the curve.
2. Click on “**Analyze**” on the top menu, then “**Linear Fit**”.
3. In the dialog box that appears, **check the curve** you’ve just highlighted.
4. A **best fit linear regression line will appear** for the points on the curve you highlighted.
5. There will also be a box containing the line’s equation.
6. The **correlation statistic (*r*)** shows how well your actual data points fit the line.
7. A correlation of 1.0 indicates a perfect fit.
  - Using the mouse, grab and move brackets and change highlighted points, to find a better fit.
  - The line, correlation and equation will automatically update as you move the brackets.

**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. This is the **rate of reaction**. Record this value as the rate (%O<sub>2</sub>/sec) in a table like that shown below (Table 1).

**Adjust reaction rate to account for actual O<sub>2</sub> collection volume.**

**Logger Lite records the rate of reaction in %O<sub>2</sub>/sec.**

However, this does not take into account the volume of the chamber taken up by solutions. By adjusting the rate to account for this, the rates will be more meaningful for comparison

1. The total volume of your reaction chamber is 280mL.
  - Some of this volume (10mL) is occupied by yeast suspension.
  - Some of this volume (20mL) is occupied by H<sub>2</sub>O<sub>2</sub> solution.
  - Some of this volume (5mL) is occupied by water or NaCl solution.
2. Subtract liquid volume (35mL) from total volume (280mL), to get the adjusted **245mL** of space.
3. Multiply %O<sub>2</sub>/sec x (245mL) to adjust to **mL O<sub>2</sub>/sec**.

**Calculate mL O<sub>2</sub>/g/sec generated by the yeast.**

Recall that your stock yeast suspension contains 70g/L of yeast.

Use this information to calculate the **mL O<sub>2</sub>/g/sec** generated by the yeast:

4. Record this final adjusted rate for each sample in a table like that shown below (Table 1).

**Be sure each team member has a copy of the data before you leave lab today. Never have just 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.**

| Sample #    | Yeast suspension volume (mL) | H <sub>2</sub> O <sub>2</sub> volume (mL) | Reaction rate (% O <sub>2</sub> /sec) | Adjusted Reaction Rate (mL/O <sub>2</sub> /g/sec) |
|-------------|------------------------------|---|---------------------------------------|---|
| Treatment 1 |                              |   |                                       |   |
| Treatment 2 |                              |   |                                       |   |
| Treatment 3 |                              |   |                                       |   |
| Control 1   |                              |   |                                       |   |
| Control 2   |                              |   |                                       |   |
| Control 3   |                              |   |                                       |   |

Calculate a **mean** and **standard deviation** for the rates of your Treatment and Control samples. Record them in a table like the one shown below (Table 2).

**Table 1. Rate of O<sub>2</sub> generation by peroxidase catabolysis of H<sub>2</sub>O<sub>2</sub> in treated and untreated yeast.**

| Group     | Mean Reaction Rate | Standard Deviation |
|-----------|--------------------|--------------------|
| Treatment |                    |                    |
| Control   |                    |                    |

## Statistical Analysis

Today's sample experiment provided only three treatment and three control rates of reaction. This is a very small sample size, and not enough to confidently analyze statistically. But we'll let you do it, anyway, just for practice.

### The Student's t test

**The student's t test compares the means of two groups, in our case, treatment and control.**

For the sake of expediency, you may use this convenient t-test calculator:

<http://graphpad.com/quickcalcs/ttest1.cfm>

1. Under "Choose Data Entry Format", choose "**enter up to 50 rows**".
2. **Enter** your three **control** values in Column 1.
3. **Enter** your three **treatment** values in Column 2.
4. Under "Choose a Test", select "**Unpaired t test**".
  - A paired t test compares the same individuals, before and after
  - An unpaired t test compares two different groups of individuals subjected to one altered variable.
5. Click "**Calculate Now**"

As if by magic, the calculator will generate for your data:

- **P value** and its statistical significance.
  - Recall that a P value of  $< 0.05$  is considered statistically significant.
- **confidence interval** between 0 – 99.99%
- **t statistic**
- **Degrees of freedom**
- **Standard error of difference**

(When I was an undergraduate, I had to walk five miles through the snow to buy a calculator and figure out my own t statistic.)

Do your t statistic and its associated P value indicate **rejection** of the **null** hypothesis? \_\_\_\_\_

Do your t statistic and its associated P value indicate **rejection** of the **alternative** hypothesis? \_\_\_\_\_

**Based on this, draw conclusions and explain the results of your experiment.**

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

Your team will now proceed to design an experiment to test hypotheses based on observations gleaned from your literature searches.

You will be using essentially the same techniques and protocols you used today, but modifying them to fit the factor you have chosen to manipulate.

You will perform your team experiment for two weeks, starting two lab sessions from now (see syllabus). But there's no need to wait until next week to start thinking of experiments your team could design. Before you leave lab today, your team should discuss your literature search results and consider some interesting ideas for an experiment. The more planning, the better your research!

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

Except for your single variable, your team used the same quantities of reagents in each trial, and (presumably) ran each trial under the same physical and environmental conditions.

Did you get exactly the same reaction rate for all control trials?

Did you get exactly the same reaction rate for all treatment trials?

What might account for slightly different results among trials that should be exactly the same?

Slight variation in carefully run trials is known as **experimental variability** or **experimental error**.

**The natural variability of experimental error is *not* the same as variability caused by actual *mistakes* (human error) made during the experiment.**

**NEVER CITE HUMAN ERROR AS A REASON FOR UNEXPECTED RESULTS!**

**Accidental mistakes that could affect results require that the investigator start over and do the experiment correctly. Citing human error as an acceptable reason for bad results is like saying, "Oops! I'm terrible at science. But meh...I don't really care enough to do it right."**

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.

## Homework: Customizing Data Collection and Graphical Display

Before you perform your own experiment, take time to go through the LoggerLite tutorials. They will help you view your data more easily and organize it more clearly.

### To view the tutorials (and make your future work easier:

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 tutorial information without it.)
4. Click on "**Pages**" on the toolbar to go to other pages.
5. Using tutorial information you should be able to modify your saved graphs by
  - Changing the rate and duration of data collection
  - Giving the graph a title
  - Un-connecting the points of the graph
  - ...and more.
6. Next, open "**Tutorial 4 - Working with graphs**". This will teach you to
  - Change the scale of the x and y axes
  - Stretch the x and y axes
  - Autoscale the axes

By the time you're done, you'll want your own LoggerLite to keep as a pet.