

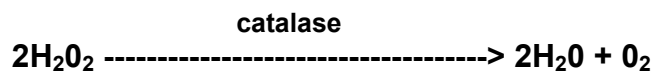
LABORATORY 3 new

Effect of Environmental Variables on Enzyme Reaction Rate in Living Cells

A **catalyst** is a substance that increases the rate of a chemical reaction without being consumed or permanently changed in the reaction. The most common catalysts in biological systems are special proteins called **enzymes**. The substance that an enzyme operates on is known as its **substrate**.

The physical and chemical properties of enzymes are affected by the conditions under which they must operate (and ultimately, under which they have evolved). Temperature, pH, relative concentrations of enzyme and substrate and other factors will affect the **rate** at which an enzyme catalyzes its particular reaction.

This week, the star of the show is the enzyme **catalase**. In aerobic bacteria, protists, fungi, plants, and animals alike, its function is to catalyze the breakdown of hydrogen peroxide (a toxic byproduct of many metabolic reactions) into harmless water and oxygen via the following reaction:



To determine how quickly this reaction proceeds under various conditions, one could measure the amount of H_2O_2 decomposed, or the amount of H_2O or O_2 produced, *per unit time* (i.e., the *rate* of this decomposition or production). We will measure the rate of the catalase reaction as the rate of O_2 production.

Objectives

After doing this lab, you should be able to:

- Design an experiment to measure the effect of environment on enzyme reaction rate.
- Mix and titrate a phosphate buffer solution.
- Collect data on enzyme reaction rate using an O_2 gas sensor and data logger.
- Analyze a rate curve to find the rate of reaction.
- Graph and compare rates of reaction caused by different values of an environmental variable.
- Explain and discuss the effect of environment on enzyme activity, on both the organismal and molecular levels.

I. About the Chemical Reagents

You will be provided with the reactant (hydrogen peroxide in aqueous solution) and the enzyme (catalase in living potato cells). You will use an electrochemical O_2 sensor (Vernier Software, www.vernier.com) to detect one of the products of the reaction, O_2 gas.

A. Potato catalase

Catalase is an antioxidant enzyme made up of four interlocked subunits, each of which consists of a polypeptide (in potato, 492 amino acid residues, $\text{MW} \approx 56,000$ g/mole) and an iron-containing heme group. Under optimum conditions, this highly efficient enzyme

catalyzes the breakdown of millions of hydrogen peroxide molecules per second (Goodsell 2004, UniProt 2008). The potato tuber, being the main food storage organ for the plant, is metabolically active. Even when dormant, it is protected by antioxidants, including catalase.

B. 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 the cell, resulting in cell damage and death. Interestingly, there is evidence that at low levels, H_2O_2 acts as a hormone in plants, and in the potato it promotes sprouting, wound healing, and the response of the plant's immune system to pathogens (Bajji *et al.* 2007, Bajji *et al.* 2008, Panina *et al.* 2004). Because catalase activity affects the concentration of H_2O_2 , catalase plays a role in regulating these functions.

C. Phosphate buffer

Buffers maintain constant pH in solution. Buffer systems are widespread in living cells and organisms, and (therefore not surprisingly) in biological experiments as well. Whether you run your experiment in a neutral (pH = 7.0), acidic (pH < 7.0) or basic (pH > 7.0) solution, you should mix your reagents in a buffer solution so that the products of your reaction will not affect the pH of the reaction's environment (which could certainly affect your results!). There are many different recipes for buffers. In this lab, you'll learn how to make and use your own phosphate buffer solutions, which are useful for many procedures in cellular and molecular biology.

II. Experimental Procedure

Before designing experiments, you should first become familiar with using the O_2 sensor and data logger, and then with the basic procedure for measuring the rate of decomposition of hydrogen peroxide by 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.

A. Setting up the O_2 sensor and data logger software

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

1. Holding it only by the edges, place the Logger Lite CD in the CD drive and follow the installation directions that appear.
2. *Only after you have installed the software*, connect the O_2 gas sensor and interface to the USB port on your computer. **Keep the O_2 sensor upright at all times! Treat it with extreme care or you may damage it and will be penalized!**
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!

B. Collecting data with the O₂ sensor

Let's compare the O₂ concentration of your exhaled breath with that of the atmosphere.

1. **Carefully and gently**, place the O₂ gas sensor into the plastic reaction chamber as shown in figure 3-1. Gently push the sensor down until it stops. The sensor is designed to seal the chamber without unnecessary force.

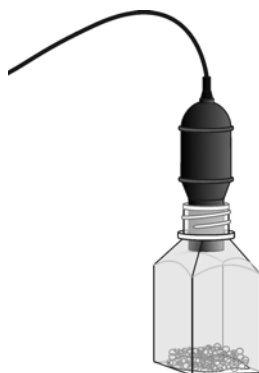


Figure 3-1. O₂ gas sensor and reaction chamber ready to collect data.

2. Click "**Collect**" on the toolbar at the top of the Logger Lite window. The sensor will now start measuring, 1x per second, the O₂ concentration (as %O₂) of the air in the chamber. Note that the current %O₂ 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 %O₂ value has stabilized, click "**Stop**" on the toolbar.
4. Record the %O₂ value.
5. Click "**Store**" on the toolbar to store this data run, and to prepare the software for collecting new data.
6. Gently remove the O₂ sensor and place it upright in its dry 250 mL 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 O₂ 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 O₂ sensor from the chamber and place it upright in its beaker.

Results:

What is the % concentration of O₂ in the atmosphere? _____

What is the % concentration of O₂ in your exhaled breath? _____

How long did it take the O₂ sensor to detect fully the %O₂? _____

C. Customizing data collection and graphical display

To make your subsequent work easier, let's now get familiar with some of the features of 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." The first page will tell you that a Go Temp probe should be attached. Ignore this; you will still be able to use the information in the tutorial. 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 can 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

D. Variability in enzyme activity data

Now you are ready to study the rate of reaction of catalase in potato! You'll do three preliminary trials under the same conditions, both to gain experience with the method and to study experimental variability.

1. Click "New" on the top toolbar to open a new data table and graph.
2. Prepare 6 test tubes. Label 3 tubes as potato #1, #2, and #3 and 3 tubes as H₂O₂ #1, #2, and #3.
3. Take a small beaker each of water and 6% H₂O₂ to your workspace. Using the syringes provided, add 10 mL of water to each of the potato tubes, and 5 mL of water + 5 mL of 6% H₂O₂ solution to each of the H₂O₂ tubes, as shown in table 3-1. **Be careful with the H₂O₂—do not splash it! Avoid contact with eyes!**
4. Using a #2 cork-borer, cut 0.5 cm diameter cores from a potato. Cut off the skin and cut the cores into uniform 1 cm pieces. Place the pieces in a beaker and cover with a watch glass to keep them from drying out.
5. Weigh (together) and add 20 potato pieces to each potato tube as shown in Table 3-1.
6. Pour the contents of H₂O₂ tube #1 into potato tube #1. Cover the opening with a finger + a small piece of parafilm and invert 2 times to mix and start the reaction. Quickly pour the combined contents into the reaction chamber.
7. Quickly but gently place the O₂ sensor into the chamber. Gently push the sensor down until it stops.

Table 3-1. Reagent quantities and reaction rates for the breakdown of hydrogen peroxide by potato.

tube #	# of potato pieces	Weight of potato pieces (g)	mL H ₂ O for potato	mL H ₂ O ₂	mL H ₂ O to add to H ₂ O ₂	Rate (%O ₂ /s)	Adjusted rate (mLO ₂ /g/s)
1	20		10	5	5		
2	20		10	5	5		
3	20		10	5	5		
						mean	
						standard deviation	

8. **Wait 60 seconds**, then click “**Collect**” to begin collecting data.
9. If %O₂ readings start to plateau, click “**Stop**.” Data collection will automatically stop after 300 seconds (unless you change this setting).
10. Click “**Store**” to store this data run.
11. Gently remove the O₂ sensor and place it upright in its beaker.
12. Discard the contents of the chamber in a labeled “waste” beaker (*not the sink!*), and rinse the chamber with tap water. Shake out excess water and dry the neck of the chamber.
13. Repeat steps 6-12 above with tubes #2 and #3.
14. Thoroughly rinse your potato tubes with water and keep all tubes for reuse.
15. Find the rate of reaction for each trial using the Logger Lite software as explained below. Express the rate of reaction as %O₂/s, and after correction for the wet weight of potato and volume of the reaction chamber as mLO₂/g/s.
16. Find the mean rate of reaction and the standard deviation for the 3 trials.

E. Calculating rate of reaction

Now let’s use the software to calculate and compare the rates of reaction for the three trials you just did. Examine the curves on your graph. Each curve shows the %O₂ as it changes over time—in other words, the time course of the reaction. At first, there may be a lag period, as 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 %O₂ increase over time will slow, and the curve will gradually flatten. We are interested only in the initial linear portion. Follow the steps below for each curve.

1. Highlight the initial linear 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.
3. Look at the equation of the best fit line you chose. The *slope*, or m , shows the change in %O₂ over the change in time—in other words the rate of the reaction! Record this value as the rate (%O₂/s) in the appropriate cell of Table 3-1.
4. To account for differences in the amount of tissue used in each trial, calculate the reaction rate per gram of tissue (wet weight) using the weight of the potato pieces you measured for this trial. Your units should now be %O₂/g/s.
5. The working volume of the reaction chamber, with the O₂ gas sensor inserted, is estimated to be 280 mL. Now you can calculate the mL O₂/g/s produced as a percentage of the total working volume. Record this adjusted rate as mL O₂/g/s in Table 3-1.
6. Cut and paste your data table and graph to Word or similar program. Be sure each group member gets a copy.

F. Discussion

You 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? Note: in this and all future discussions of experimental variability (also known as *experimental error*), do not dwell on mistakes you may have made. **Experimental error ≠ mistakes!** If you think you made non-random mistakes that could affect your results, you should do the experiment again. When contemplating your results, your fellow scientists will assume you have done your experiments as carefully as possible, and have minimized mistakes.

III. The fun part: Designing your own experiment!

Like any enzyme, catalase operates most efficiently under a certain set of environmental conditions. Reaction rate depends on many different factors, including concentration of enzyme, concentration of substrate, and the pH, osmolarity, and temperature of the reaction environment. As you’ve seen, the catalase you used for your preliminary trials was inside the cells of a potato. Consider the environment that a potato lives in (underground at a farm wherever potatoes are grown, or eventually, under the sink in your apartment!). Potatoes have coevolved with humans to function well under certain conditions. Think about what those conditions might be. Also think about how, on the molecular level, different enzyme or substrate concentrations, enzyme inhibitors, pH, or temperature might affect the functioning of catalase.

Now you are ready to design and perform your own experiment. The materials available to you include:

- Catalase in living potatoes
- 6% Hydrogen peroxide in water
- Phosphate buffers of varying pH
- Thermometers, ice and hotplates, and a heated waterbath
- One or more potential inhibitors of catalase, such as salicylic acid, acetylsalicylic acid (aspirin), succinic acid, or ascorbic acid (vitamin C)

On the following pages are suggestions for altering a single *independent variable* in the catalase environment. Your group of four should select (or will be assigned) ONE of these variables. Each group should test a different independent variable. You'll devise null and alternative hypotheses about how that variable might affect the reaction rate of catalase, design an experiment to test your hypotheses, and then perform the experiment. Before your group begins its experiment, be sure to have your instructor approve your design and make helpful suggestions. Note that whatever your independent variable is, you must still record the other variables (enzyme and substrate concentrations, temperature, pH) that you hold constant. Because of time constraints, we suggest that you initially try no more than five different treatments of your selected independent variable. Do one trial of each treatment first, then do more trials as time permits to give the most meaningful results.

A. Controlling pH: Phosphate buffers

In the previous preliminary trials of the catalase reaction in potato, we ignored possible pH effects. However, pH often strongly influences biological reactions. Using a buffer system will ensure that pH is kept constant while you are altering some other variable. Buffers also allow you to adjust pH, as the independent variable, to whatever values you may choose. For example, by mixing various proportions of monobasic (acid) and dibasic (base) sodium phosphate, you can make phosphate buffers for any pH between 5.7 and 8.0. To hold pH constant while changing another variable, you would usually make only a pH 7.0 phosphate buffer, unless you have a rationale for using another pH (for example, if the intracellular pH of your organism is not 7.0). To vary pH as the independent variable, make a wide range of buffers.

You will have available stock solutions of:

0.1 M sodium phosphate, monobasic

0.1 M sodium phosphate, dibasic

1. To make 60 mL of 0.1 M sodium phosphate buffer, combine the quantities of each stock solution for the desired pH, as shown in Table 3-2 (modified from Sørensen 1986).
2. Check the pH with a pH meter and add, drop by drop, sodium phosphate monobasic solution to lower the pH, or dibasic to raise it. Stir with a stirring rod and continue this *titration* until the desired pH is reached.
3. To use your phosphate buffer(s) in your experiment, add buffer (instead of water) to each potato tube.

Table 3-2. Quantities of 0.1 M sodium phosphate monobasic and dibasic to combine to make phosphate buffers of desired pH.

monobasic (mL)	dibasic (mL)	pH
56.1	3.9	5.7
55.2	4.8	5.8
54	6	5.9
52.6	7.4	6.0
51	9	6.1
48.9	11.1	6.2
46.5	13.5	6.3
44.1	15.9	6.4
41.1	18.9	6.5
37.5	22.5	6.6
33.9	26.1	6.7
30.6	29.4	6.8
27	33	6.9
23.4	36.6	7.0
19.8	40.2	7.1
16.8	43.2	7.2
13.8	46.2	7.3
11.4	48.6	7.4
9.6	50.4	7.5
7.8	52.2	7.6
6.3	54.3	7.7
5.1	54.9	7.8
4.2	55.8	7.9
3.2	56.8	8.0

B. The effect of enzyme concentration on reaction rate

As more enzyme molecules become available to collide with molecules of substrate, how might the rate of reaction change? Design an experiment in which you examine the effect of enzyme concentration on rate of reaction. Before you begin, construct null and alternative hypotheses.

H₀: _____

H_A: _____

rationale for H_A: _____

Helpful tips:

- Although you will not measure the enzyme concentration directly, you may assume that it is proportional to the amount (weight) of potato that you use.
- *Alternatively*, you may increase the effective enzyme concentration by cutting a given amount of potato into smaller pieces of known size. Express enzyme concentration as the total surface area of the potato pieces for each treatment.
- Add the potato pieces to a phosphate buffer to control pH.
- The pH, buffer volume, H₂O₂ volume, and total volume must be the same in all treatments.
- Measure and record the temperature of the system after each trial.

Table 3-3. Reagent quantities and reaction rates for the breakdown of hydrogen peroxide by potato under controlled conditions.

Number of trials per treatment: _____

# or size of potato pieces	Mean wt. of potato pieces (g)	pH of buffer	mL buffer for potato	mL H ₂ O ₂	mL H ₂ O to add to H ₂ O ₂	Mean temp (°C)	Mean rate (%O ₂ /s)	Adjusted mean rate (mLO ₂ /g/s)

C. The effect of substrate concentration on reaction rate

As more substrate molecules become available to collide with enzyme molecules, how might the rate of reaction change? What about when the enzyme molecules are all working at their maximum rate? Design an experiment in which you vary the concentration of substrate. Before you begin, construct null and alternative hypotheses.

H₀: _____

H_A: _____

rationale for H_A: _____

Helpful tips:

- Vary the concentration of H₂O₂ by varying the proportion of H₂O₂ and water that you add to the H₂O₂ tube. Use a 1 mL syringe for volumes < 1 mL.
- Add the potato pieces to a phosphate buffer to control pH.
- The amount of potato, pH, buffer volume, and total volume must be the same in all treatments.
- Measure and record the temperature of the system after each trial.
- Your stock substrate solution is 6% H₂O₂ by volume. The end of this chapter has a section on calculating the final % H₂O₂ of the solution in the reaction chamber.

Table 3-4. Reagent quantities and reaction rates for the breakdown of hydrogen peroxide by potato under controlled conditions.

Number of trials per treatment: _____

# of potato pieces	Mean wt. of potato pieces (g)	pH of buffer	mL buffer for potato	mL H ₂ O ₂	mL H ₂ O to add to H ₂ O ₂	Mean temp (°C)	Mean rate (%O ₂ /s)	Adjusted mean rate (mLO ₂ /g/s)

D. The effect of temperature on reaction rate

As temperature rises, molecular motion increases (by definition). How might this affect the rate of reaction of the enzyme? What about the effect of temperature on the secondary, tertiary, and quaternary bonding and shape of the enzyme, and thus on its activity? Design an experiment to test how temperature changes might affect the rate of reaction of catalase.

H₀: _____

H_A: _____

rationale for H_A: _____

Helpful tips:

- You will need to actively monitor and maintain the temperature of your treatments. Think about the best ways to do this before you begin.
- Add the potato pieces to a phosphate buffer to control pH.
- The amount of potato, pH, buffer volume, H₂O₂ volume, and total volume must be the same in all treatments.

Table 3-5. Reagent quantities and reaction rates for the breakdown of hydrogen peroxide by potato under controlled conditions.

Number of trials per treatment: _____

# of potato pieces	Mean wt. of potato pieces (g)	pH of buffer	mL buffer for potato	mL H ₂ O ₂	mL H ₂ O to add to H ₂ O ₂	Intended temp (°C)	Actual mean temp (°C)	Mean rate (%O ₂ /s)	Adjusted mean rate (mLO ₂ /g/s)

E. The effect of pH on reaction rate

Enzymes are charged and usually polar, especially at the active site. How might the pH (concentration of H^+) affect the shape and charge of the enzyme, particularly at the active site, and thus its affinity for the substrate? How might these changes in the enzyme in turn affect the rate of reaction? Design an experiment to test the activity of catalase under varying conditions of pH.

H₀: _____

H_A: _____

rationale for H_A: _____

Helpful tips:

- Choose a wide range of pH treatments. Follow the directions in section III A to make the phosphate buffers.
- Add the potato pieces to the various phosphate buffers (instead of water).
- The amount of potato, buffer volume, H₂O₂ volume, and total volume must be the same in all treatments.
- Measure and record the temperature of the system after each trial.

Table 3-6. Reagent quantities and reaction rates for the breakdown of hydrogen peroxide by potato under controlled conditions.

Number of trials per treatment: _____

# of potato pieces	Mean wt. of potato pieces (g)	pH of buffer	mL buffer for potato	mL H ₂ O ₂	mL H ₂ O to add to H ₂ O ₂	Mean temp (°C)	Mean rate (%O ₂ /s)	Adjusted mean rate (mLO ₂ /g/s)

F. The effect of inhibitors on reaction rate

Some types of inhibitor molecules may compete with substrate molecules for binding to the enzyme's active site. Others may change the shape of the enzyme and slow its rate of reaction non-competitively. How might you discern the type or degree of inhibition? Design an experiment in which you explore inhibition of the catalase reaction.

H₀: _____

H_A: _____

rationale for H_A: _____

Helpful tips:

- To vary the concentration of a potential inhibitor, vary the proportion of inhibitor and water that you add to the H₂O₂ tube. Use a 1 mL syringe for volumes < 1 mL.
- Add the potato pieces to a phosphate buffer to control pH.
- The amount of potato, pH, buffer volume, H₂O₂ volume, and total volume must be the same in all treatments, unless your question and design dictate otherwise.
- Measure and record the temperature of the system after each trial.
- Note the molarity of your stock solution of inhibitor(s). The end of this chapter has a section on calculating the final molarity of inhibitor in the reaction chamber.

Table 3-7. Reagent quantities and reaction rates for the breakdown of hydrogen peroxide by potato under controlled conditions.

Number of trials per treatment: _____

potential inhibitor	# of potato pieces	Mean wt. of potato pieces (g)	pH of buffer	mL buffer for potato	mL H₂O₂	mL H₂O to add to H₂O₂	mL of potential inhibitor to add to H₂O₂	Actual mean temp (°C)	Mean rate (%O₂/s)	Adjusted mean rate (mLO₂/g/s)

G. The effect of enzyme localization on reaction rate

If catalase functions mostly to regulate sprouting, wound healing, or immunity, where in the potato might it be most abundant? What about if it functions mostly as an antioxidant for metabolizing stored food? Might there be more need for catalase in old (stem end) or young (bud end) tissue? Design an experiment in which you test different locations within the potato for relative catalase activity.

H₀: _____

H_A: _____

rationale for H_A: _____

Helpful tips:

- To test tissue near the outside of the potato, cut pieces from just inside the skin, but avoid including the skin itself.
- To test stem end vs. bud end tissue, be sure you can tell the two ends apart!
- Add the potato pieces to a phosphate buffer to control pH.
- The amount of potato, pH, buffer volume, H₂O₂ volume, and total volume must be the same in all treatments.
- Measure and record the temperature of the system after each trial.

Table 3-8. Reagent quantities and reaction rates for the breakdown of hydrogen peroxide by potato under controlled conditions.

Number of trials per treatment: _____

Location within potato	# of potato pieces	Mean wt. of potato pieces (g)	pH of buffer	mL buffer for potato	mL H ₂ O ₂	mL H ₂ O to add to H ₂ O ₂	Actual mean temp (°C)	Mean rate (%O ₂ /s)	Adjusted mean rate (mLO ₂ /g/s)

IV. Data analysis and presentation

A. Experimental variability in enzyme activity

1. The tables and graphs generated by the Logger Lite software are raw (uncorrected) data, which would usually not be considered ready for a presentation or report. However, because the data you collected in section II D represent preliminary trials, you may use the graph mostly as is. Be sure to increase the size of the font on the axis labels and key to at least 28-point, so that it is readable when projected.
2. Write a figure legend for this set of *rate curves*. For advice on how to do this, and for making your graphs ready for presentation, see the appendix [How to Create Figures and Tables](#).
3. Be sure you are able to illustrate or explain, if asked:
 - which part of the graph represents the initial linear rate curve (remember: even a straight line is a type of "curve") for each of your preliminary trials.
 - what a best fit line is.
 - what the statistic *r* says about the fit of the line to your data.
 - how to determine the slope of the best fit line, should you have to do so by hand (see [How to Create Figures and Tables](#)).
 - how to calculate adjusted rates of reaction from the slopes, and find the mean.
 - reasons (excluding mistakes) for the variation among the set of curves.
4. Everyone in the room has done essentially the same exercise here. Keep this in mind and minimize the time you spend on this part in your presentation.

B. The effect of environment on enzyme activity

1. You should copy and paste the tables and graphs generated by the Logger Lite software for later reference, but they represent raw (uncorrected) data, which are not yet ready for your presentation or report. You will use the equations and statistics from these graphs to calculate your results and make a new graph.
2. Using the slopes of the rate curves, calculate the adjusted rate of reaction in mL O₂/g/s for each of your trials as in Part II E above. Then calculate the mean rate of reaction for each treatment, if you did more than one trial of each.
3. Create a new figure for your presentation using graphing software such as Excel. Label the x-axis with the units of your independent variable (enzyme concentration, substrate concentration, temperature, pH, inhibitor concentration, or location within the potato) and the y-axis with units of reaction rate (mL O₂/g/second). Make either a line graph or a bar graph—whichever shows the trends in your results most effectively. You must use a bar graph if your independent variable consists of attribute data. Plot a single point or bar representing the *mean* reaction rate for each of your experimental treatments against the value of your independent variable for that treatment.
4. If your independent variable consists of continuous numerical data, attempt to graph a best-fit curve for your reaction rate vs. independent variable data points. (HINT: It won't necessarily be a straight line). You can do this on your graphing software

using a linear regression if the points appear linear, or (for example) using a parabolic (second order) regression if the points appear to describe a parabola. *Alternatively*, you can just connect the points, if that is more appropriate.

5. *Optional*: Represent experimental variation using error bars for each of your data points. Your TA will show you how to make standard error bars on Excel, if she or he would like you to do this.
6. During or following your presentation, be sure you are able to clearly illustrate and explain (note that your TA may ask *anyone* in the group to do this!):
 - your rationale for the way you graphically represented your data.
 - how the rate of reaction is affected by your independent variable.
 - how your results relate to your hypotheses. Say “supported” or “did not support” the hypothesis, *not* “proved” or “rejected” the hypothesis.
 - what your results mean in terms of the adaptation of the living potato to its environment, as well as the functioning of the enzyme on the molecular level.
 - ideas on what the *experimental unit* (also known as *unit of replication*) is in your experiment.

V. A Bit of Help for the Chemistry Challenged

A. Molarity Calculations

Because we cannot (in the time available) find the concentration of catalase in the living potato, we will not express its concentration in terms of molarity (moles/liter, M), as would usually be done. However, you should know what molarity means, and how to make stock solutions such as the sodium phosphate solutions you used, given the formula weight and the volume needed. For example, to make 100 mL each of 0.1 M sodium phosphate monobasic (NaH_2PO_4 , formula weight = 119.98) and dibasic (Na_2HPO_4 , formula weight = 141.96) solutions, how many grams of each reagent should you weigh out?

Monobasic: _____

Dibasic: _____

B. How to Calculate % Volume of Hydrogen Peroxide

1. Our stock H_2O_2 solution is 6% H_2O_2 by volume. This means that each 1.0 mL of stock solution contains 0.06 mL of pure H_2O_2 .
2. To calculate how many mL of pure H_2O_2 you used in any given trial, simply multiply the # of mL of stock H_2O_2 solution you used by 0.06. For example, in your preliminary trials, you used 5.0mL of the stock solution. This means that $0.06 \times 5.0 \text{ mL} = 0.3 \text{ mL}$ of that solution is pure H_2O_2 .
3. Once you know how many mL of pure H_2O_2 are in the amount of stock H_2O_2 solution you used, you can calculate what percentage this is of the total volume you used in the reaction chamber. For example, if the total volume of solution in the reaction chamber is 20 mL, and 0.3 mL of this was H_2O_2 , then the concentration of H_2O_2 is

0.3 mL/20 mL = 0.015 mL H₂O₂/mL solution. Multiplying by 100, the final H₂O₂ concentration is 1.5% (volume/volume or v/v). *Everyone* should be able to do this type of calculation, so practice it! For example, what is the final percent H₂O₂ if you used 2.0 mL of the 6% stock solution, and the total volume of solution in the reaction chamber is 20 mL?

Final % H₂O₂: _____

4. If your group varied the substrate concentration, label your graph with units of reaction rate (mL O₂/g/second) on the y-axis against units of substrate final concentration (% H₂O₂ by volume) on the x-axis.

C. How to Calculate Molarity of Inhibitor

1. Be sure to note the molarity of the stock solution(s) of inhibitor. This may vary with different substances.
2. Use the dilution proportion $M_1V_1 = M_2V_2$, where M_1 and V_1 are the molarity and volume (respectively) of the stock solution, and M_2 and V_2 are the final molarity and volume of the solution in the chamber. Solve the proportion for M_2 for each of your treatments. Be sure to think through how the calculation makes sense, and you will remember how to do it more easily. *Everyone* should be able to do this type of calculation, so practice it! For example, what is the final molarity of the potential inhibitor salicylic acid (SA), if you use 4.0 mL of a 0.01 M stock solution of SA, and the final volume of solution in the reaction chamber is 20 mL? Express your answer both as a decimal and in scientific notation.

Final molarity of SA (decimal): _____

Final molarity of SA (scientific notation): _____

3. If your group varied the concentration of inhibitor, label your graph with units of reaction rate (mL O₂/g/second) on the y-axis against units of inhibitor final concentration (M) on the x-axis.

VI. Statistical Tests

To analyze trends in data such as rate differences, you would normally use statistical techniques like regression analysis or ANOVA. A regression analysis, for example, can tell you the probability, expressed as a P value, that there was a change in the dependent variable (for example mL O₂/g/second) over a range of values of a continuous independent variable. It also tells you how well the actual data points correspond to the best fit line, expressed as an r or r^2 value. If you did a regression analysis on your data, you may state the P and r^2 values, but they are not required for this lab. Just know that they are there! For now, unless your TA says otherwise, you will only be asked to make a subjective judgment about whether the reaction rates you observed in your treatments show a trend, and what that trend says to you about how an enzyme might work under varied conditions. Without statistical analysis, you cannot evaluate a hypothesis about a complex system with confidence. However, you can subjectively discuss the apparent effect of a variable, keeping in mind that statistical analysis would yield a more meaningful comparison.

VII. Words to the Wise

Rendering your data into clear, analyzable form is only the beginning of a scientific presentation or report. There are no "right" or "wrong" results in an experiment—only valid and invalid explanations for the observations. The most important part of this lab is to explain your results logically, even if they are not what you expected. Think about your results and discuss them intelligently. To improve your presentation, you may use the resources available in the library or online to learn more about catalase and its reaction. Try Google Scholar for relevant journal articles. Be sure to cite and list all references properly (for example, as we have done here), lest you accidentally become guilty of plagiarism. Read the Appendices [How to Write a Scientific Paper](#) and [Creating Figures and Tables](#) before beginning to make your presentation. In addition, the next lab chapter gives an overview of what is expected of your PowerPoint presentation, and how best to create an effective one. Read that chapter completely before you begin your presentation. You are responsible for everything in Chapters 3 and 4 of this two-part lab exercise, so be prepared!

References:

- Bajji, M., M. M'Hamdi, F. Gastiny, J. Rojas-Beltrán, and P. Du Jardin. 2007. Catalase inhibition accelerates dormancy release and sprouting in potato (*Solanum tuberosum* L.) tubers. *Biotechnologie, Agronomie, Société et Environnement* 11(2): 121-131.
- Bajji, M., P. Delaplace, P. Du Jardin, M-L. Fauconnier, F. Gastiny, and M. M'Hamdi. 2008. Catalase inhibition alters suberization and wound healing in potato (*Solanum tuberosum*) tubers. *Physiologia Plantarum* 129(3), 472-483.
- Goodsell, D. S. 2004. Catalase. RCSB Protein Data Bank, http://www.rcsb.org/pdb/static.do?p=education_discussion/molecule_of_the_month/pdb57_1.html.
- Panina, Y., N. Vasyukova, and O. Ozeretskovskaya. 2004. Inhibition of activity of catalase from potato tubers by salicylic and succinic acids. *Doklady Biological Sciences* 397(1): 131-133.
- Sörensen 1986. In Hyatt. In Phosphate Buffer. OpenWetWare, http://openwetware.org/wiki/Phosphate_buffer. (the original reference was cited incompletely by the website)
- UniProt Consortium 2009. Catalase isozyme 1, *Solanum tuberosum*. The Universal Protein Resource (UniProt), <http://www.uniprot.org/uniprot/P49284>.