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Catalyzing the Knowledge of Catalase:

Investigating the Effect of Substrate Concentration on Reaction Rates Involving Catalase

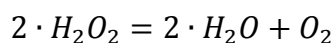3 November 2013

Mr. Allen

AP/IB Biology P.3

Data Collection and Processing:

Raw data for variation of the concentration of the substrate, H_2O_2, is displayed here:

Concentration of H2O2 (% Volume± 0.01%)	Percent Oxygen ±0.1%									
	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
	Initial		Initial	Final	Initial	Final	Initial	Final	Initial	Final
0.75%	15.4	17.5	15.5	17.1	15.3	17.3	17.2	19.4	17.4	19.4
1.50%	17.4	22.0	17.4	21.3	17.3	21.5	17.0	21.6	17.0	21.9
3.00%	18.0	24.7	18.2	26.3	17.9	25.4	17.6	24.0	17.9	25.9
4.00%	17.6	25.6	17.0	23.7	16.9	26.0	16.9	24.0	15.1	22.4
6.00%	19.2	23.7	18.9	29.0	18.2	25.8	13.9	20.9	15.7	22.2

Our goal is to process the reaction rate for the reaction:

$$2 \cdot H_2O_2 = 2 \cdot H_2O + O_2$$

In order to solve this equation, we have measured the concentration of the percent oxygen at different times, thus providing us a simple way of calculating the rate of this reaction. The kinetics of how this is possible will be discussed later on in this paper.

To find this rate, the $\Delta O_2\%$ must be found and divided by the Δt, which is 180 seconds. This is because

$$rate = \frac{\Delta O_2\%}{\Delta t}$$

Therefore, we can solve for $\Delta O_2\%$ by

$$\Delta O_2\% = O_2\%_{final} - O_2\%_{final}$$

as depicted in the following data point, from the 0.75% Trial 1 data:

$$\Delta O_2\% = 17.5\% - 15.4\%$$

$$\Delta O_2\% = 2.0\%$$

Therefore, the new data looks like:

Concentration of H2O2 (% Volume± 0.01%)	Change in Percent Oxygen (±0.1%)				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
0.75%	2.0	1.6	2.0	2.2	2.0
1.50%	4.6	3.9	4.2	4.6	4.8
3.00%	6.8	8.1	7.4	6.3	8.0
4.00%	8.0	6.8	9.1	7.1	7.4
6.00%	4.5	10.1	7.7	7.0	6.6

In order to find the average rate, we first take the average of all the $\Delta O_2\%$s. This is done by the formula for simple arithmetic mean of

$$\bar{x} = \frac{x_1 + x_2 + x_3 + x_4 + x_5}{5}$$

Using data from the 0.75% trial, we have:

$$\bar{x} = \frac{(2.0 + 1.6 + 2.0 + 2.2 + 2.0)}{5}$$

$$\bar{x} = 2.0$$

In addition to the average value, we can also calculate the standard deviation of our raw data and use it as the error value in the graph. To do so, we must know the standard deviation formula, which is:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{5}(x_i - \mu)^2}{5}} \, [1]$$

Which provides the following numbers, give the 0.75% data:

$$\sigma = \sqrt{\frac{(2-2)^2 + (1.6-2)^2 + (2-2)^2 + (2.2-2)^2 + (2-2)^2}{5}}$$

$$\sigma = \sqrt{\frac{0 + .16 + 0 + .4 + 0}{5}}$$

$$\sigma = \sqrt{.04}$$

$$\sigma = .2$$

Therefore, the following data table is produced:

Concentration of H2O2(Volume$\pm$ 0.01%)	Average % Change	% Change Error[2]
0.75%	2.0	0.18
1.50%	4.4	0.32
3.00%	7.3	0.70
4.00%	7.7	0.82
6.00%	7.2	1.82

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[1] Standard Deviation and Variance
[2] The reason for the discrepancy between this value and the value in the table is due to rounding errors. The table value is more precise and accurate.

However, we are not looking for the average % change, but instead, the rate of reaction. In order to approximate, we will divide the average % change by the time it took for this change, as discussed in the beginning of the DCP. This results in, for the 0.75% data,

$$rate = \frac{2.0}{180} = 0.011$$

Therefore, our data becomes:

Concentration of H2O2(Volume $\pm\,0.01\%$)	Rate of reaction (%O2 / second)	Rate Error(ppt %O2 /second)
0.75%	0.011	0.00099
1.50%	0.025	0.00177
3.00%	0.041	0.00386
4.00%	0.043	0.00456
6.00%	0.040	0.01009

While it should be fine to proceed from these values, there is more to do in order to gain a more complete understanding of the data. The first to cover is to find a more precise approximation for the concentration of Hydrogen Peroxide. While it is seen as a volume derivative at the moment, it would be better suited to be used as a molar concentration in the form of mole/L, or M.

In order to calculate this value, an understanding of chemistry and molarity is required, in order to gain the following derivation:

Assuming x is the volume of H_2O_2, for the 0.75% data:

$$\frac{x}{1+x} = 0.75\% = 0.0075$$

$$x = 0.0075 + 0.0075x$$

$$(1 - 0.0075) \cdot x = 0.0075$$

$$x = \frac{0.0075}{1 - 0.0075}$$

$$x = 0.00755 \, L = 7.55mL$$

Assuming that the density of Hydrogen Peroxide is 1.45 g/mL[3], we find that

$$mass \, H_2O_2 = 7.55mL \cdot \frac{1.45 \, g}{mL}$$

$$mass \, H_2O_2 = 10.95g$$

Because the molar mass of H_2O_2 can be found via a periodic table, and is calculated to be

18.02g/mol, we find that the amount of H_2O_2 is:

$$mol \, H_2O_2 = 10.95g \cdot \frac{18.02g}{mol}$$

$$mol \, H_2O_2 = .6075 \, mol$$

Calculating the concentration of Hydrogen Peroxide would thus be:

$$[H_2O_2] = \frac{.6075 \, mol \, H_2O_2}{1.00755 \, L}$$

$$[H_2O_2] = 0.60294M$$

By doing all of these steps for each of the percent volumes, as well as for the uncertainty value in

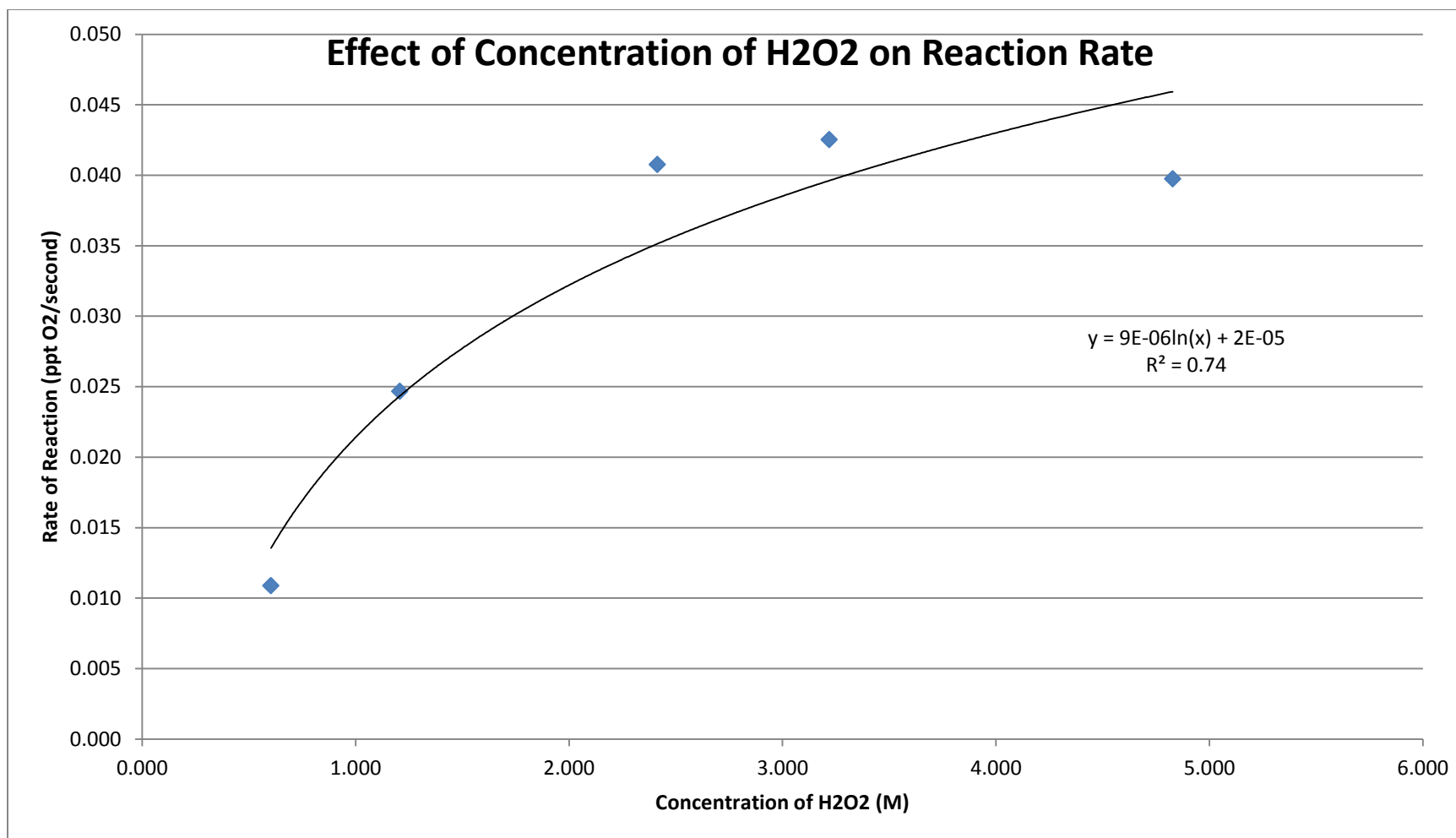the instrument (given as 0.1%)[4], we obtain the following data:

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[3] ChemSpider
[4] Allen, Phillip

Concentration of H2O2(+- 0.00847M)	Rate of reaction (%O2 / second)	Rate Error (ppt %O2 / second)[5]
0.603	0.011	0.00001
1.207	0.025	0.00001
2.414	0.041	0.00002
3.219	0.043	0.00003
4.828	0.040	0.00006

The Oxygen probe measures the pressure of the Oxygen in the meter, so that in order to find the reaction rate, merely dividing the pressure of the oxygen by the time is sufficient. Therefore, no further refining is required of this data.

With this final data, we are ready to see the true graph:

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[5] Note: Parts per trillion is an equivalent notation to %O2, with a conversion factor of 1 %O2 = 100 ppt O2.

Effect of Concentration of H2O2 on Reaction Rate

x-axis: Concentration of H2O2 (M)

y-axis: Rate of Reaction (ppt O2/second)

$y = 9E{-}06\ln(x) + 2E{-}05$

$R^2 = 0.74$

Note: There are most certainly error bars in both the x and y direction included on this graph, but due to the very small values for both x and y error, they are not visible in any way.

For this graph, a natural log regression was used, primarily due to the tendency of the graph to level off as the concentration of hydrogen peroxide increased. However, this is not entirely true and valid.

This general trend seen in this graph is that after some concentration of Hydrogen Peroxide, the reaction rate does not increase any further. This is reasonable, as seen in the Enzyme activity done in class, we observed that at some point, the concentration of the substrate is so great that the limiting factor for the reaction is the speed of the enzyme.
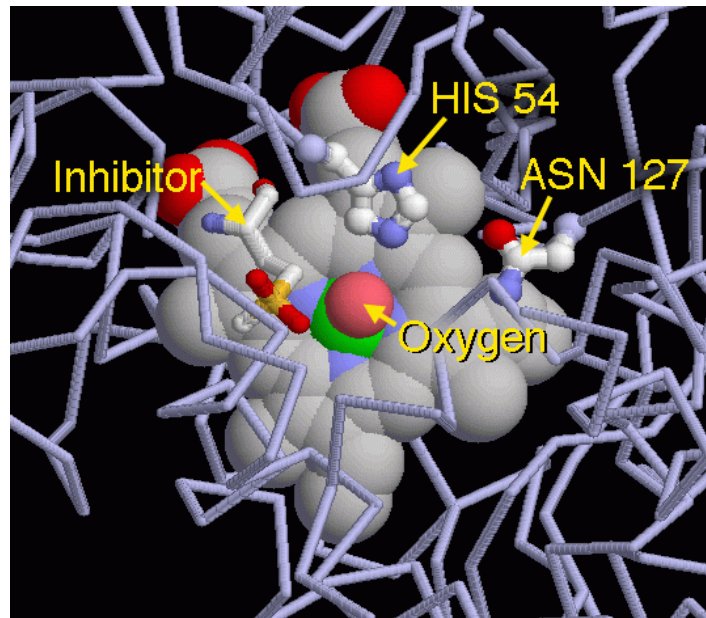
The x and y intercept makes sense, at (0.0) , as when there is no hydrogen peroxide in a solution of the catalase, the catalase is obviously not going to be making the reaction move forward in any rate!

Clearly linearization does not make sense, and therefore, we are finished with the preliminary data collection and processing.

Conclusion & Analysis:

Although we have completed the preliminary data collection, given an additional knowledge of chemistry and reaction rates, we are able to further our understanding of this topic.

As we saw in our lab, there was an eventual leveling off in the reaction rate after a certain concentration of H2O2, which in our data set, seems to be approximately 2.5M of H2O2. This can be especially seen in how for the 2.414, 3.219, and 4.828M data, there is no real difference in rate. The reaction rate seems relatively stable at approximately $\frac{.045 \ kPa \ O_2}{second}$. Through our understanding of how an enzyme works, we know that the substrate, H2O2 in this case, must enter the enzyme's active site, undergo induced fit, and then come out as two products.

Fig 1.[6]

Because it takes some amount of time for a hydrogen peroxide molecule to come into the active

site of the catalase, this amounts for the slower reaction rate observed at low concentrations of

hydrogen peroxide. However, after about 2.5 M H2O2, there is a saturation of hydrogen peroxide

within the solution such that as soon as the catalase enzyme completes the catalysis of one

hydrogen peroxide molecule, another molecule will immediately be available as a substrate.

At this point, increasing the concentration of hydrogen peroxide will not have any effect, as the

current rate is dependent on the speed that the catalysis reaction operates, as determined by the

catalase enzyme.

In a chemistry term, this means that the reaction within the catalase is the new rate-limiting step.[7]

[6] Goodsell, David
[7] Biotech Environ

This is a commonly known concept in organic biology, and two additional terms are used to describe this relation. A natural logarithm graph is not the best way to model this data, but it is possible to instead describe the relationship in terms of V_{max} and K_m, where V_{max} is the maximum reaction rate for the catalase[8], and K_m is the concentration of substrate at which the reaction rate is ½ of the maximum reaction rate.[9]

In order to determine V_{max} and K_m, a common technique used is the Lineweaver-Burk double reciprocal plot[10], which rearranges the equation to be:

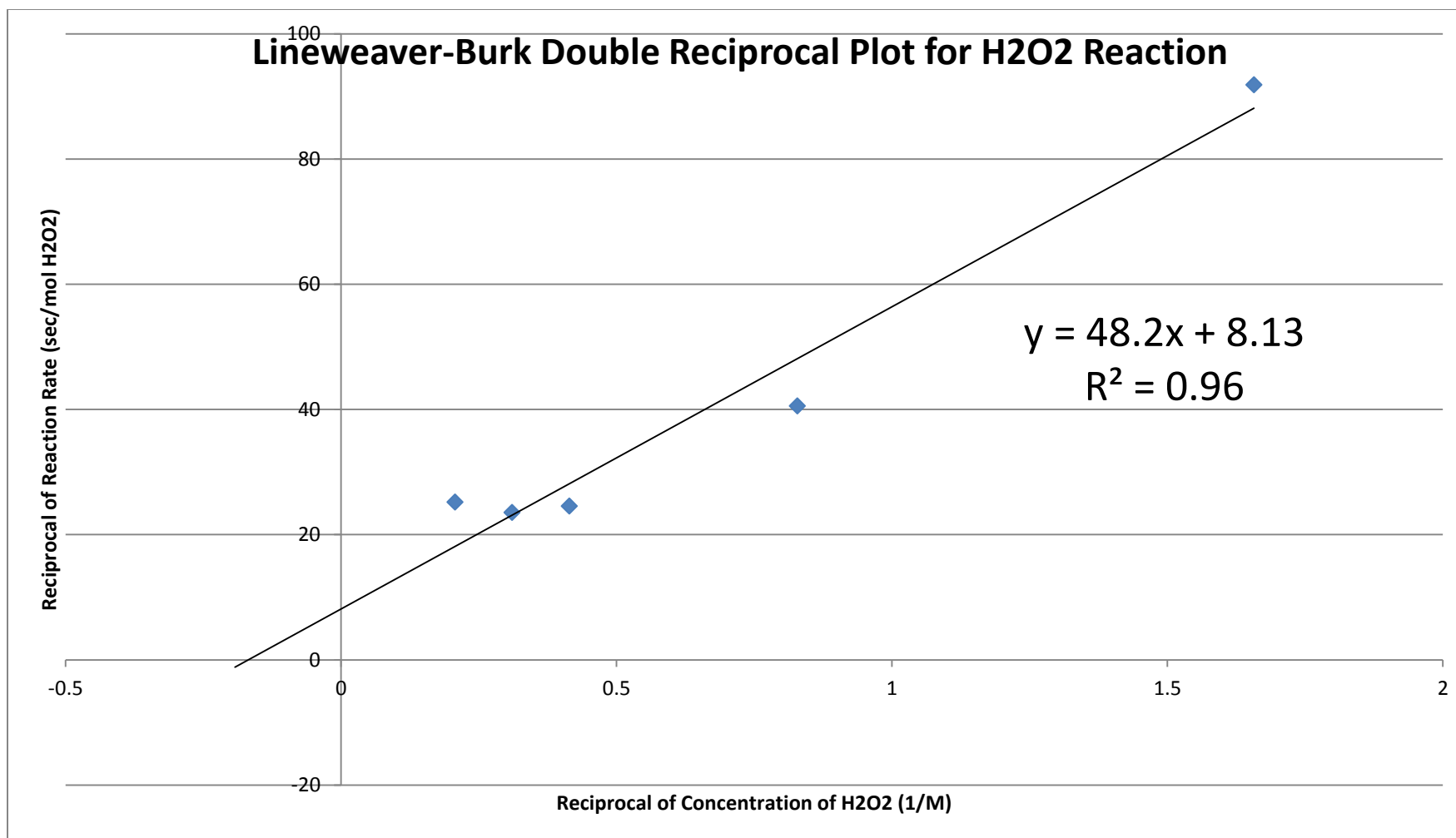$$\frac{1}{v} = \frac{1}{V_{Max}} + \frac{K_m}{V_{Max}} \cdot \frac{1}{[s]}$$

Where [s] is the concentration of the substrate and v is the reaction rate.

This produces the following graph:

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[8] "Introduction to Enzymes"
[9] Bender, David A.
[10] Perryman, Lauren et. al.

Lineweaver-Burk Double Reciprocal Plot for H2O2 Reaction

$y = 48.2x + 8.13$

$R^2 = 0.96$

X-axis: Reciprocal of Concentration of H2O2 (1/M)

Y-axis: Reciprocal of Reaction Rate (sec/mol H2O2)

Note: There are most certainly error bars in both the x and y direction included on this graph, but due to the very small values for both x and y error, they are not visible in any way.

Although the lab question was to determine the effect of increased concentration on the reaction rate, which we have already answered, it is more interesting to calculate and find the K_m value, commonly known as the Michaelis Constant[11].

From our graph, we know that the x-intercept is equivalent to $-\frac{1}{K_M}$, so if we calculate the x-intercept, we can calculate K_m. Therefore,

$$0 = 48.2 + 8.13$$

$$-\frac{8.13}{48.2} = x$$

$$x = -.169$$

$$K_m = 5.93M$$

Clearly, this value is nowhere close to what was observed. The most likely cause for this error is that the Lineweaver-Burk Double Reciprocal Plot is overly dependent on the reaction rates determined when the substrate concentration was low.
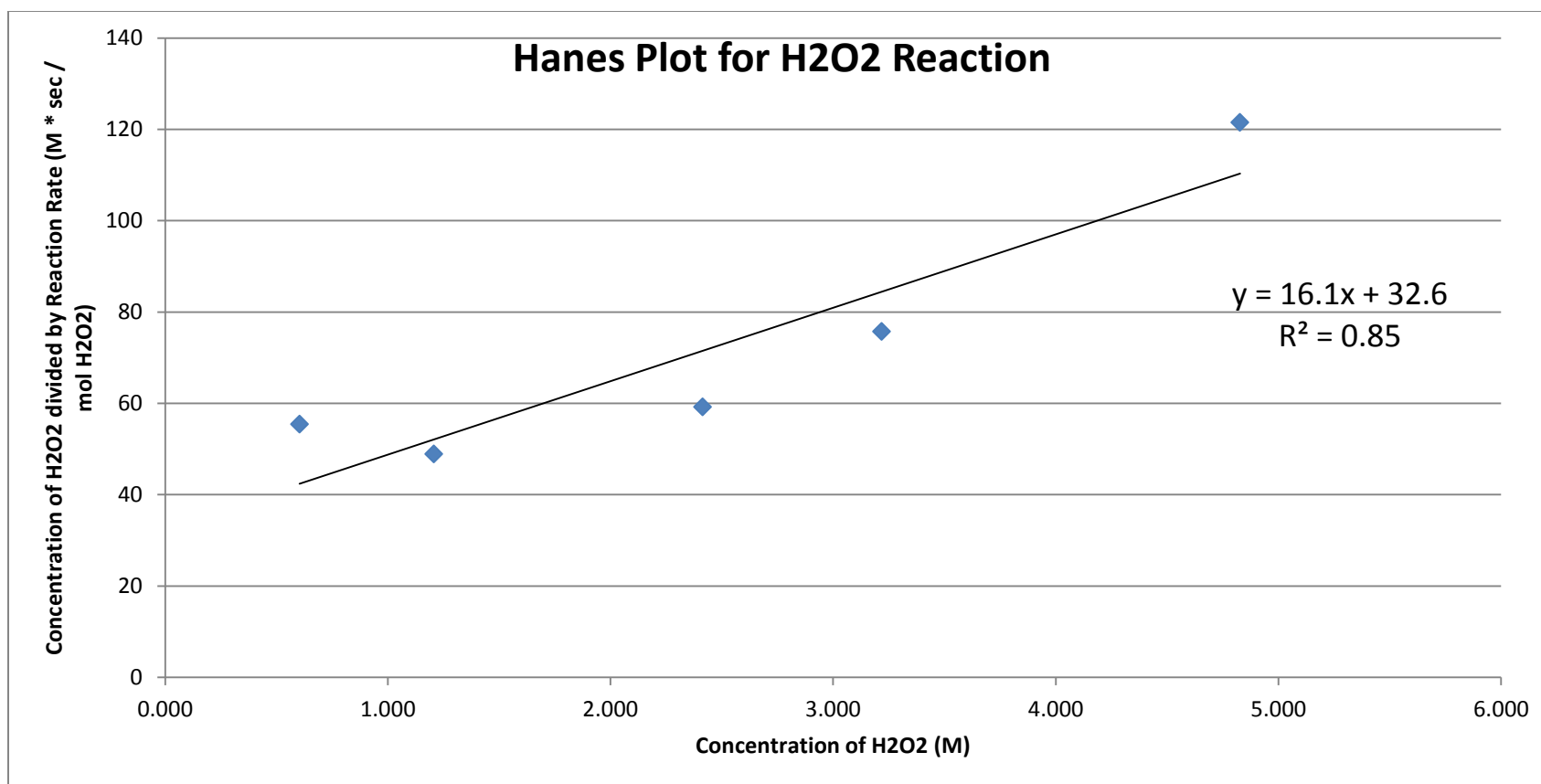
An alternative processing manner is the Hanes Plot, which resembles:

$$\frac{[s]}{v} = \frac{K_m}{V_{Max}} + \frac{[S]}{V_{Max}}\,[12]$$

So that the x intercept is equivalent to $-K_m$. This graph looks like the following:

[11] Hardy Research Group
[12] Bender, David A.

Error bars are the same as above.

Clearly, this plot is very much more inaccurate, and we will discuss sources of error later on in this lab. However, when calculating the x intercept by:

$$0 = 16.1 \cdot x + 32.6$$

$$x = \frac{-32.6}{16.1}$$

$$x = -2.028$$

$$K_m = 2.03 \ M$$

This value is considerably closer to the observed value in the graphs, and it is reasonable to say that this is the true Michaelis Constant for this reaction.

Error Analysis:

Through this lab, we have thoroughly processed the enzyme-catalyzed reaction of Hydrogen Peroxide by the enzyme catalase. However, as the final results reveal, there is a huge amount of error in the final conclusion. This error can come from many different sources, which will be discussed below.

The instruments used to conduct this experiment were Vernier O2 Gas Sensors, which have a measurement range between 0-27% O2 and have an accuracy of +/- 1% O2.[13] Due to the sensitive nature of our experiment, with many of the ΔO_2% under 10% and some at only 2% difference, it is very likely that the inaccuracy from the probe was greater than expected. In addition, for the 6.0% condition, the graph for the change in O2 pressure was very much erratic,

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[13] Vernier.com

which implied that the sensor was not reliable for that trial. While it was not enough error to be tossed out as an outlier, it does put that trial in question.

This instrumental systematic error is best completed by conducting even greater number of trials of data, as that would result in the systematic error better canceling itself out. Gathering additional data points would be very beneficial in this scenario.

However, the current method of collecting data is also flawed. In this experiment, the entirety of Mr. Allen's AP/IB Biology class pitched in to collect data, and the data that this paper uses draws from 2 groups in 3rd period as well as 1 group in 4th period. It is unknown if the catalase was manipulated in between the time, or if the same procedure was used by all of the groups. Therefore, additional experimenters introduce a new, human random error.

The best solution to this problem is for the entire lab to be done by a single group, thus perfectly controlling the human error throughout the experiment. However, barring that, the next best method would be for each period to only attempt one of the experiments (%substrate, pH, or conc. Catalase) and for each group to be responsible for one trial of each of the experimental conditions. Therefore, it is possible for human errors to be caught and then averaged out, increasing the accuracy of the human gathered data.

Finally, another error is in the method that the start of the experiment is carried out. Because we have a 250 mL bottle that we pour the reactants into and plop the Vernier Oxygen sensor on top of, it is possible for some amount of the O2 gas to escape, or for the sensor to be messed up by the sudden pressurization of the bottle due to forcing the sensor on.

Therefore, a proposed change would be to use a different flask, more like the side arm flask that the oxygen sensor can attach on. In this manner, there could be catalase already in the flask, and

the sensor already attached onto the top of the flask. When ready, hydrogen peroxide can be poured in via the side arm and the arm quickly sealed in order to prevent an escape of O2 gas. This would ensure the best way of collecting accurate O2 pressure data.

Hydrogen Peroxide, however, is only catalyzed by catalase; this does imply that it is possible for the spontaneous decomposition of hydrogen peroxide when left in the open. Therefore, by the time that the hydrogen peroxide is used in the experiment, it is possible that the concentration of hydrogen peroxide is radically different than the given concentration that was initially prepared.

In order to mitigate this error, the hydrogen peroxide solution should be immediately prepared before each trial. However, this is extremely difficult to do, so an alternative would be to chill the hydrogen peroxide solution as much as possible. This is because the regular reaction of hydrogen peroxide includes an activation energy, and energy is directly related to temperature. Therefore, if we reduce the temperature of the solution, by a Maxwell-Boltzman diagram it is possible to see that there would be lesser particles that would have enough energy to complete this reaction, therefore guaranteeing the concentration of hydrogen peroxide to be used.

This experiment has been very rewarding in an increased understanding of the connections between reaction rates in chemistry and in biology, and determining what effect that a catalyst such as catalase may have. Additional topics to consider are the temperature and pH conditions for such a reaction, as covered by many other students.

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