A Mathematical Approach Examining Characteristics Affecting Enzyme Peroxidase Activity

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Abstract

This lab seeks to prove that highest possible peroxidase concentration, temperature close below denaturization temperature, and low pH are optimal peroxidase conditions. It also sought to prove that if peroxidase is sustained at temperatures above 100^{o}C, it will become ineffective. An essential catalyst in the conversion of toxic hydrogen peroxide in water, peroxidase must be maintained within certain optimal conations to effectively catalyze. Spectrophotometric analysis of brownish-orange tetraguaiacol product was used to determine reaction progression and ultimately reaction rate, a measure of how "optimal" a given condition consisting of temperature, pH, or concentration is for peroxidase activity. Poor lab technique prevented adequate validation of these hypothesis, however extrapolative methods determined that highest possible peroxidase concentration and temperature slightly higher than room temperature are optimal for peroxidase activity. Optimal pH was not determined.

Introduction

Life is dependent on metabolism, a collection of chemical reactions that manage the chemical and energy resources of an organism. (Cain, et al., 2011) If the metabolism of an organism stopped, all cellular processes such as DNA replication, gene expression, phagocytosis, and digestion (in vertebras) would likewise cease. (Reece, J. B., et al., 2011) Protein digestion, in particular, is a very energy intensive operation. (Reece, J. B., et al., 2011) To have any meaningful value to the vertebra's cells, a protein must be broken into amino acids via hydrolysis. (Elhefnawy, M. E., 2012) Unfortunately, proteins do not spontaneously hydrolyze in water. (Reece, J. B., et al., 2011) Remarkably however, they are digested through the aid of catalysts, more specifically, enzymes. (Reece, J. B., et al., 2011) All reactions have a minimum energy requirement to proceed: the activation energy. (Brown, T. L., et al., 2012) A catalyst is a chemical that lowers the activation energy of a reaction by temporarily binding to the reactant(s). (Brown, T. L., et al., 2012) This binding step forms an intermediate and is usually the rate determining step in metabolic reactions. (Brown, T. L., et al., 2012) Next the intermediate will react with leftover ions from the first step or simply decompose to form the final product and recompose the original catalyst. (Brown, T. L., et al., 2012) The catalyst is thus able to perform the same reaction repeatedly. (Brown, T. L., et al., 2012) In biological systems, specific catalysts made from proteins govern metabolic activity known as enzymes. (Brown, T. L., et al., 2012) Most enzymes can only catalyze one type of reaction. (Cain, et al., 2011) Cellulase and lactase, for example, aid in the decomposition of cellulose and lactose respectively. (Cain, et al., 2011) In protein digestion, the enzyme pepsin lowers the activation energy of protein hydrolyzation enabling it to proceed at a much faster rate than pure water hydrolysis offers. (Reece, J. B., et al.,

2011) While many reactions simply proceed slower without a catalyst, some reactions cease altogether without lowered activation energy. (Cain, et al., 2011)

Enzymatically catalyzed reactions consist of an enzyme catalyzing the reaction of reactants known as substrates. (Coleman, 2015 Lab VII Introduction) Enzymes lower reaction activation energy by means of an active site, a region of the enzyme that holds the substrates in a particular manner so that the desired products are a result of the substrates seeking equilibrium. (Brown, T. L., et al., 2012) Only a few amino acids compose the active site; the rest of the protein is dedicated to structural features. (Reece, J. B., et al., 2011) Often when performing a reaction however, the entire enzyme changes shape to construct a product shaped differently than the substrate(s). (Reece, J. B., et al., 2011) Also, during the reaction, the active site may temporarily donate a few of its atoms or even polyatomic ions to the substrate however these are returned prior to reaction completion so that the enzyme can continue catalyzing. (Reece, J. B., et al., 2011) Just as only one kind of key operates a lock, an enzyme's active site usually only catalyzes one type of reaction. (Coleman, 2015 Lab VII Introduction) The active site only works under very specific conditions known as optimal conditions. (Coleman, 2015 Lab VII Introduction) Sufficient deviation in enzyme geometry due to applied loading results in an active site that is unable to operate. (Reece, J. B., et al., 2011) Often, in liquid phase, enzymes, being solutes, according to kinetic molecular theory exert partial pressure on neighboring enzymes which deforms them thus bounding enzyme optimal conditions at extreme concentration. (Brown, T. L., et al., 2012) More commonly however, temperature or pH changes outside optimal conditions bring these same effects. (Coleman, 2015 Lab VII Introduction)

This lab investigates the optimal concentration, pH, and thermal conditions of peroxidase, an important enzyme in metabolic activity. (Coleman, 2015 Lab VII Introduction) A toxic

byproduct of many metabolic processes capable of drastically raising pH, hydrogen peroxide must be safely regulated by the cell to maintain homeostasis. (Kareska, S., 2010; Shu, Z., 2013) Beneficially, peroxisomes host peroxidase which catalyzes hydrogen peroxide's reaction into water assisted by a hydrogen acceptor. (Kareska, S., 2010) Like all enzymes though, peroxidase has optimal conditions where it operates best – and in some cases, at all. (Elhefnawy, M. E., 2012) This lab seeks to prove that peroxidase concentration, environmental pH, peroxidase boiling, and system temperature directly affect peroxidase reaction rate. (Coleman, 2015 Lab VII Introduction) It tests whether reaction rate is proportional to enzyme concentration raised to a natural power, lower acidity results in a faster reaction rate, heating peroxidase to temperatures above 100^{o}C will reduce peroxidase's ability to catalyze by denaturization, and if sub-boiling temperature increase reduces the additional activation energy required for hydrogen peroxide to react forming water as seen by mean reaction time. (Coleman, 2015 Lab VII Introduction)

Materials and Methods

At the UT Tyler Biology Laboratory on Monday October 16 at 2 P.M., 2017, 8 grams (g) of tissue from the internal portion of a turnip was weighed out on a digital scale and then homogenized with 300 milliliters (mL) of cold (4^{o}C) 0.1 molar (*M)* phosphate buffer solution at pH 7 (BpH7; henceforth, phosphate buffer of pH X, BpHX) by blending for 20 seconds (s) at high speeds in a laboratory blender. The blended mixture was filtered through several layers of cheesecloth so that an isotropic solution resulted. A pipette labeled "extract" was placed in the 500mL beaker containing homogenized turnip tissue suspended in an ice bath at near 0^{o}C temperatures. (Coleman, 2015 Lab VII Part A)

Using a clean curvet containing a control group solution of 4mL 0.1*M* phosphate buffer at pH 5.0 (henceforth, BpH5), 1mL BpH7, 1mL guaiacol, and 2mL of hydrogen peroxide, the spectrophotometer was blanked at 500nm. Next two test tubes – one containing 1mL BpH5, 2mL hydrogen peroxide, and 1mL guaiacol and the other containing 2mL BpH5, 1mL BpH7, and 1mL extract – were prepared for the experimental group. The text tube containing the reactants was then poured into the other containing the extract and quickly placed into a clean curvet for spectrophotometric absorbance observation. The absorbance at 20s intervals was recorded for a duration of 120s at 500 nanometers (nm). If a linear pattern of absorbance from 0 to 1 was not observed in 120s, the experimental group mixture was repeated with more extract if the reaction fell short of an absorbance of 1 by 120s or less extract if spectrophotometric absorbance values were approached 1 before 120s. Before reperforming the reaction, BpH7 volumes were adjusted in the test tube containing extract so that total test tube volume still equaled 4mL. If it was necessary to repeat the reaction, new absorbance values were observed at 15s intervals for 120s. The final trial necessary to achieve total linear absorbance change from 0 to 1 was recorded in

15s increments until only 60s. The final amount of enzyme extract used was then recorded as the enzyme standard amount which was used in all subsequent experiments. (Coleman, 2015 Lab VII Part B)

A control test tube next prepared containing 4mL BpH5, 1mL BpH7, 2mL hydrogen peroxide, and 1mL guaiacol was used as a control group for determining the effects extract concentration play in peroxidase activity. This control was the same as in the one used in the previous enzyme standardization activity for blanking the spectrophotometer at 500nm. Two experimental groups were then tested, each consisting of the mixture of two separate test tubes – one containing the reactants, the other containing the catalyst. Both the first and second experimental group's reactant test tubes contained 1mL BpH5, 2mL hydrogen peroxide, and 1mL guaiacol. The first experimental group's catalyst test tube contained half the amount of standard extract determined in the previous activity, an amount of BpH7 that equals 1mL plus half the adjustment made from 1mL of BpH7 volume determined in the previous activity, and a variable amount of BpH5 chosen so that the total volume is 4mL. The second experimental group's catalyst test tube contained double the amount of standard extract determined in the previous activity, an amount of BpH7 that equals 1mL plus double the adjustment made from 1mL to BpH7 volume in the previous activity, and a variable amount of BpH5 chosen so that the total volume is 4mL. The absorbance values of both experimental groups were recorded in 15s increments for 60s. This was done by pouring the reactants test tube into the extract test tube and then quickly pouring the combined 8mL in a clean curvet. (Coleman, 2015 Lab VII Part C)

A clean curve identical in composition to the spectrophotometric blank in the standardization activity was used as a control group to determine the effects of pH on enzymatic activity. Four control groups each consisted of a reactants test tube containing 2mL hydrogen

peroxide and 1mL guaiacol and a catalyst test tube containing the standardized amount of extract and an amount of phosphate buffer so that the catalyst test tube's total volume was 5mL. The first experimental group's phosphate buffer pH was 3, the second experimental group's phosphate buffer pH was 5, the third experimental group's phosphate buffer pH was 7, and the forth experimental group's phosphate buffer pH was 9. The reactant and catalyst's test tubes were poured together and quickly inserted into the spectrophotometer, and each experimental group's absorbance readings were recorded at 15s intervals for 60s. All spectrophotometric readings were taken at 500nm. (Coleman, 2015 Lab VII Part D)

The effects enzymatic denaturization on enzyme activity were determined recording the effect peroxidase sustained at above boiling temperatures had on reaction rate in water and tetraguaiacol production by hydrogen peroxide and guaiacol. The absorbance readings of a control group consisting of 5mL BpH5, 1mL guaiacol, the standardized amount of extract, and an amount of BpH7 close to 1mL determined so that total volume is 8mL were used to blank the spectrophotometer at 500nm. The experimental group consisted of two test tubes – one containing the reactants and another holding the catalyst. In this trial, 3mL of extract placed in a test tube were suspended in a boiling water bath for 5 minutes and then allowed to cool to room temperature. The reactant test tube contained 1mL BpH5, 2mL hydrogen peroxide, and 1mL guaiacol. 2mL BpH5, the standardized amount of preboiled extract, and a variable amount of BpH7 were used to compose the catalyst test tube with a total volume of 4mL. The reactant test tube was then poured into the catalyst test tube and quickly inserted into the blanked spectrophotometer so that absorbance readings at 15s intervals for 60s could be recorded. (Coleman, 2015 Lab VII Part E)

The effects of temperature on enzyme activity were observed by comparing the absorbance of a control group with four experimental groups, each at progressively increasing temperatures within the liquid temperature range of water: 4ºC, 23ºC, 32ºC, and 48ºC. The control group used to blank the spectrophotometer at 500nm consisted of 1mL guaiacol, the standardized amount of extract, and an amount of BpH5 measured out to complete the control group's total volume at 8mL. Each experimental group consisted of two test tubes incubated at their respective group temperature for 900s. The first test tube for all experimental groups contained 1mL BpH5, 2mL hydrogen peroxide, and 1mL guaiacol. 2mL BpH5, the standardized amount of extract, and a variable amount of BpH7 were held in the second test tube of all experimental groups so that their total volume was 4mL. Both test tubes of each experimental group were then combined into one per experimental group and placed in a clean curvet where absorbance data was recorded at 15s intervals for 60s. (Coleman, 2015 Lab VII Part F)

Data collected from all experiments and standardization activities were graphed and qualitatively analyzed to determine statistical fidelity and experiment trends.

RESULTS

Homogenized turnip tissue was standardized at 1mL and subsequently used in four experiments. The homogenized turnip tissue and other reactants used were clear except hydrogen peroxide which turned slightly greyish clear after 2 minutes of exposure to light. Within 5s of mixing in a curvet however, color was discernably brown throughout the entire 8mL curvet solution. After 120s, all mixtures transmitted deep brown-orange hues. Except for trial 2 of the extract standardization and the readings at -1°C of Part F (Table 1; Table 5), all other brown-orange product solutions floated above the line of the spectrophotometer absorbance detection. Instead, a mostly clear solution with only slight orange hues was recorded. (Figure 2) These improperly recorded solutions generally exhibited a zero slope of their scatterplots with a low R^2 value seeming to indicate no spectrophotometrically detected reaction. That tetraguaiacol product was not properly recorded is further confirmed by the impossible-to-exist negative absorbance reading found in Part E. (Table 4; Figure 5) By contrast, properly recorded solutions showed a square-root function-like appearance in their scatterplots that started at a positive y-intercept and tapered off to a zero slope over time with a near 100 R^2 value. (Table 1; Table 5) The final 23°C standardization proceeded slightly faster than the -1°C trial in Part F. (Figure 1; Figure 6)

Table 1. Turnip extract standardization absorbance readings taken at 500nm recorded against seconds elapsed since reactant mixing for up to 120s for Part B. (A_x = absorbance readings at nanometer wavelength x; * = adjusted recording time from lab manual specifications; ** = not recorded) Spectrophotometric absorbance readings are output in triple digit precision. (0.000)

Trial 1		Trial 2	
Time	A_{500}	Time	A_{500}
10*	0.500	0	0.500
20	0.180	15	0.735
40	0.159	30	0.899
60	0.159	45	0.974
80	0.151	60	1.094
100	0.118	75	1.176
120	0.106	90	1.225
**	**	105	1.260
**	**	120	1.280

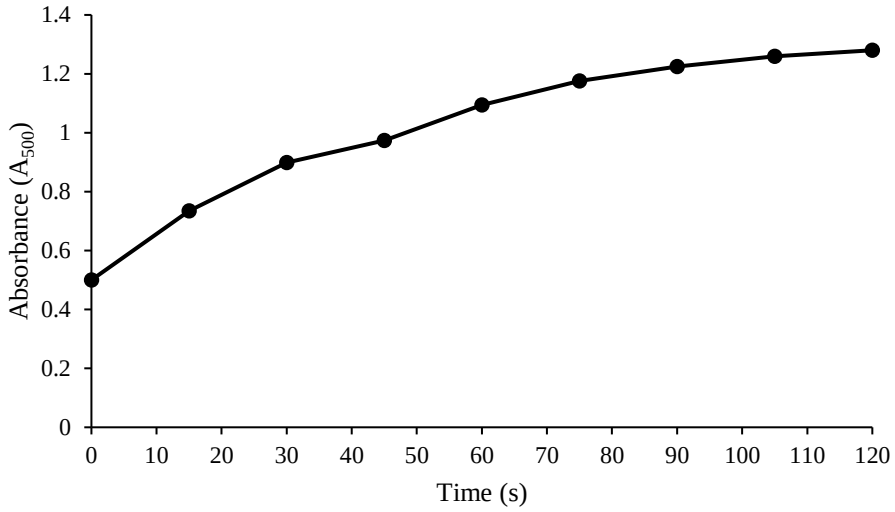

Figure 1. Final extract standardization absorbance readings at 500nm over 120s from standardization trial 2.

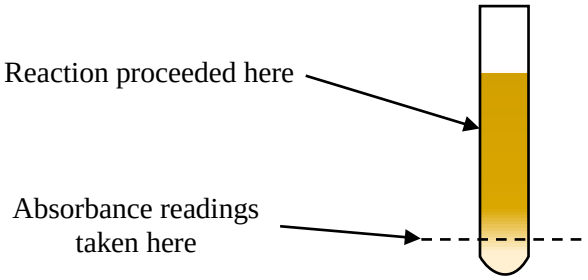

Figure 2. Fluid equilibrium in curvets after most trials as viewed from the side shows absorbance reading location not to always be the primary site of reaction activity.

Table 2. Tetraguaiacol production for varying turnip extract concentration absorbance readings taken at 500nm recorded against seconds elapsed since reactant mixing for 60s for Part C. (A_x = absorbance readings at nanometer wavelength x) Times are logged within 2s accuracy. Spectrophotometric absorbance readings are output in triple digit precision. (0.000)

Time	½x extract concentration A_{500}	2x extract concentration A_{500}
0	0.326	0.167
15	0.320	0.132
30	0.318	0.116
45	0.319	0.114
60	0.319	0.113

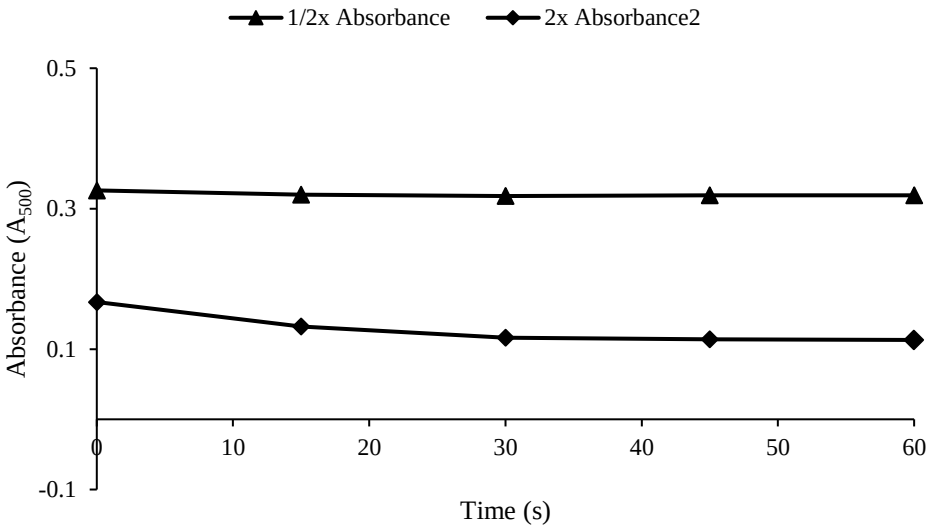

Figure 3. Part C tetraguaiacol production absorbance readings at 500nm over time for half and double enzyme peroxidase concentration using standard conditions.

Table 3. Tetraguaiacol production for varying pH values for Part D. (A_x = absorbance readings at nanometer wavelength x) Times are logged within 2s accuracy. Spectrophotometric absorbance readings are output in triple digit precision. (0.000)

Time	pH 3 A_{500}	pH 5 A_{500}	pH 7 A_{500}	pH 9 A_{500}
0	0.311	0.164	0.927	0.039
15	0.287	0.065	0.614	0.036
30	0.291	0.055	0.743	0.030
45	0.307	0.043	0.804	0.023
60	0.307	0.037	0.763	0.027

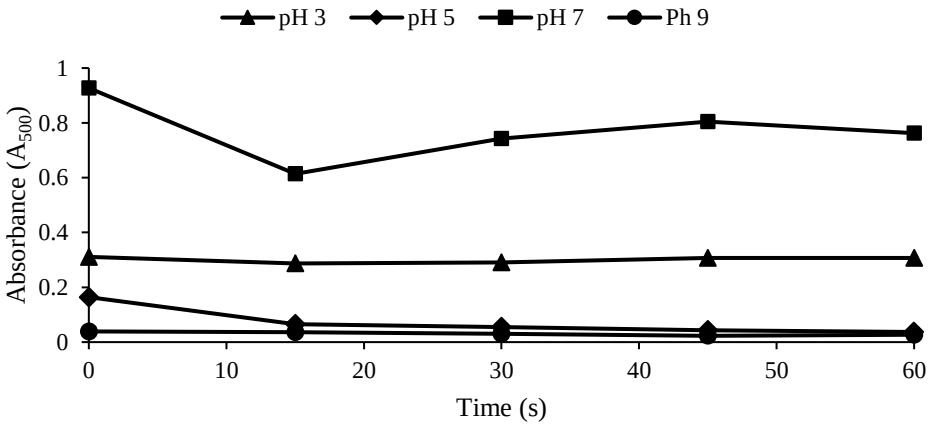

Figure 4. Part D tetraguaiacol production absorbance readings at 500nm over time for four pH values.

Table 4. Tetraguaiacol production with denatured peroxidase for Part E. (A_x = absorbance readings at nanometer wavelength x) Times are logged within 2s accuracy. Spectrophotometric absorbance readings are output in triple digit precision. (0.000)

Time	A_{500}
0	0.161
15	0.000
30	-0.010
45	-0.012
60	-0.006

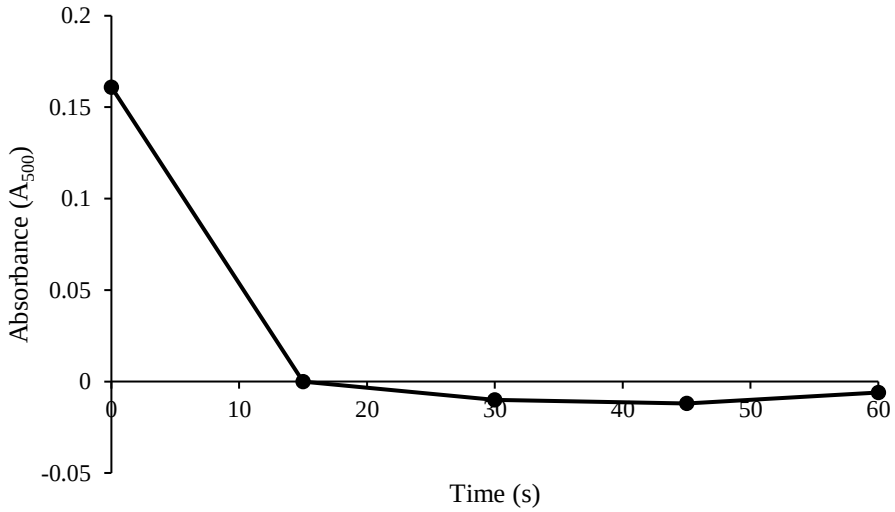

Figure 1. Final extract standardization absorbance readings at 500nm over 120s from standardization trial 2.

Table 5. Tetraguaiacol production at varying temperatures for Part F. (A_x = absorbance readings at nanometer wavelength x) Times are logged within 2s accuracy. Spectrophotometric absorbance readings are output in triple digit precision. (0.000)

Time	-1°C A_{500}	23°C A_{500}	55°C A_{500}	70°C A_{500}
0	0.602	0.729	0.995	0.317
15	0.882	0.379	0.820	0.229
30	0.983	0.355	0.791	0.221
45	1.049	0.330	0.793	0.198
60	1.079	0.311	0.793	0.191

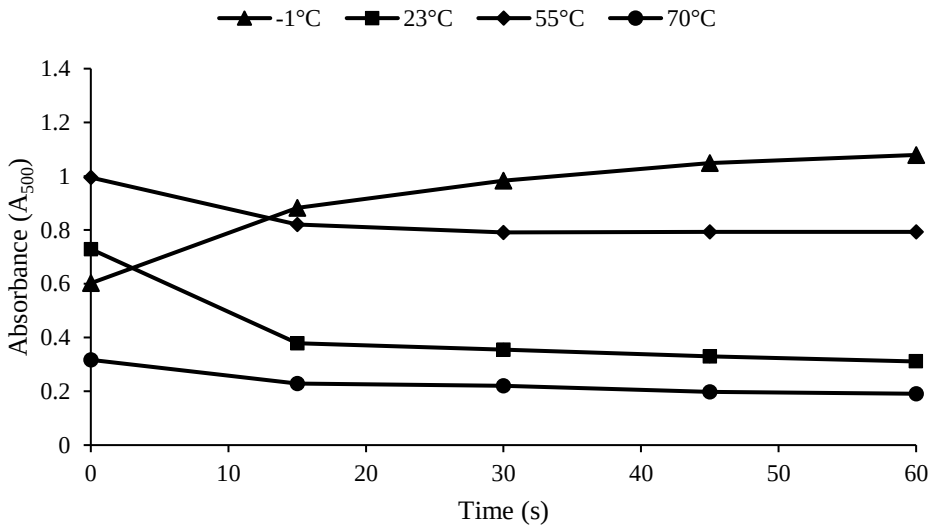

Figure 6. Part F tetraguaiacol production absorbance readings at 500nm over time at four different temperatures: -1°C, 23°C, 55°C, and 70°C.

Discussion

This lab sought to prove that peroxidase concentration, environmental pH, peroxidase boiling, and system temperature directly affect peroxidase reaction rate. More specifically, whether reaction rate is proportional to enzyme concentration raised to a natural power, lower acidity results in a faster reaction rate, heating peroxidase to temperatures above 100^{o}C reduces peroxidase's ability to catalyze by denaturization, and if sub-boiling temperature increase reduces the additional activation energy required for hydrogen peroxide to react forming water were seen by examining mean reaction time. Four experiments followed a standardization activity to test these hypotheses. Reaction progress was determined by the spectrophotometric absorbance readings of tetraguaiacol, a product of peroxidase catalyzed activity. Poor lab technique resulted in data showing no quarrelation between time, extract concentration, temperature, pH, or other variables and absorbance, yet noncorrupt data demonstrated remarkably smooth, nonlinear reaction procession.

A major problem first encountered in extract standardization and further in all parts of the lab was buoyant separation of the brownish-orange tetraguaiacol indicator product and colorless reactants. These clear reactants sunk below the product and resulted in spectrophotometric readings taken at a point usually interpolated between the two solutions or entirely in the clear reactant solution. Since the less dense product was produced over time, fluid equilibrium as depicted in the curvet diagram was not instantly reached. (Figure 2) The less dense product produced at the bottom rose to the upper portion of the curvet over time. Any reactants left to react that were at the bottom of the curvet where their absorbance could be recorded were clear. As soon as they reacted to form a brownish-orange product, the product floated upward and its absorbance readings were not taken. While after 20s, much of the bottom of the curvet was

opaque product, after 60s, most of that product had floated upwards to the top of the curvet. That is why most trials show a general negative trend in absorbance as fluid equilibrium was reached. (Table 1 trial 1; Table 2; Table 3; Table 4; Table 5 23°C, 55°C, 70°C) Two trials, however, did not follow this trend. (Table 1 trial 2; Table 5 -1°C)

Standardization trial 2 and the -1°C trial of Part F both exhibited a regular nonlinear increase over time. Figure 1 graphs the standard pattern also followed by the -1°C trial of Part F. (Figure 1) This graph of reaction progress over time shows a trend seen by functions of the form $y(x) = ax^n + b$ where a and b are arbitrary constants and $0 < n < 1$. Since any expression $ax^n + b$ has a corresponding derivative $\frac{d}{dx} ax^n + b = anx^{n-1}$ which given aforementioned constraints is negative for all positive domain, it follows that $\lim_{x \to \infty} anx^{n-1} = 0$. Thus, the reaction never reached completion with any finite time such as 60s.

Reaction speed of reaction $guaiacol + H_2O_2 \xrightarrow{peroxidase} tetraguaiacol + H_2O$ is determined by the rate law $r = k[guaiacol]^x[H_2O_2]^y[peroxidase]^z = -\frac{d[reactants]}{dt} = +\frac{d[products]}{dt}$. (Brown, T. L., et al., 2012) Under standard conditions, $[guaiacol]^x[H_2O_2]^y$ can be simplified into $[reactants]^u$ where $reactants$ and u are arbitrary constants. Then, by equality, $-\frac{d[reactants]}{dt} = k[reactants]^u[peroxidase]^z$. Applying algebraic identities, $[reactants]^{-u}d[reactants] = -k[peroxidase]^z dt$. Integrating as a separable differential equation, an explicit formula is derived for tetraguaiacol: $\int_0^t [reactants]^{-u}d[reactants] = -k[peroxidase]^z \int_0^t dt = \frac{[reactants]_t^{1-u}}{1-u} - \frac{[reactants]_o^{1-u}}{1-u} = k[peroxidase]^z t$

$\Rightarrow [reactants](t) = \sqrt[1-u]{(1-u)k[peroxidase]^z t + [reactants]_o^{1-u}} = c_1[product](t) = c_1[tetraguaiacol](t)$ where c_1 is another experimentally determined constant and $u \neq 1$.

It is interesting to note that the aforementioned relationship $y(x) = ax^n + b$ while adequately defining a reaction progress graph does not fit the form of the equation derived $c_1[tetraguaiacol](t) = \sqrt[1-u]{(1-u)k[peroxidase]^z t + [reactants]_o^{1-u}}$ or more generally, $y(x) = \sqrt[n]{\frac{x}{a} + \frac{b}{a}}$ which is also the inverse of $y(x) = ax^n + b$. Using algebraic identities, $n = 1 - u$, $\frac{x}{a} = (1-u)k[peroxidase]^z t$, and $\frac{b}{a} = [reactants]_o^{1-u}$. Since this is a function of product concentration over time, $x = t$ and $y(x) = c_1[tetraguaiacol](t)$. Therefore, $a = \frac{1}{(1-u)k[peroxidase]^z}$ and $b = \frac{[reactants]_o^{1-u}}{(1-u)k[peroxidase]^z}$. Since $A_{500} \propto [solution]$, $A_{500} = c_2[solution]$ therefore $c_2^{-1}A_{500} = [solution]$. Finally, a triple, strong, and explicit definition can be made between absorbance, time, and tetraguaiacol concentration:

$$[tetraguaiacol](t) = c_2^{-1}A_{500} = c_1^{-1}\sqrt[1-u]{(1-u)k[peroxidase]^z t + [reactants]_o^{1-u}} \quad \blacksquare$$

While much data recorded held no meaningful information, the two well defined trials provide enough data to formulate an explicit equation with absorbance and product concentration both functions of time. This formula allows extrapolation of data for other trials not successfully conducted. Additional comparison is made between this and similar labs.

Since catalyst concentration raised to a natural power is directly proportional to t, increasing extract concentration increases reaction rate thus decreasing time required to reach a given absorbance compared to a reaction of lower extract concentration. It is expected then that noncorrupt data in the 2x trial using twice as much extract would have a higher final absorbance reading than the ½x trial in Part C. (Table 2) The optimal concentration of peroxidase, then, is the highest possible concentration of it that can be contained within a solution.

A near identically performed lab by Susan Kareska found that production rate increased with peroxidase concentration. (Kareska, S., 2010) However, while doubling enzyme concentration, reaction rate increased by 35%, quadrupling concentration only increased reaction rate by 58%. (Kareska, S., 2010) If concentration was linear with reaction rate, quadrupling concentration according to $2\times[enzyme] = 35\%$, then $4\times[enzyme]$ would have been 70%. However, actual enzyme reaction rate was 12% lower than linear predictions on an absolute scale of 100% and 83% less than linear predictions comparatively. Therefore, reaction rate must be proportional to enzyme concentration raised to a power on the open interval $(0,1)$. In other words, $0 < \frac{z}{1-u} < 1$ which given the preceding data implies z is slightly less than $1 - u$. The second intraradical term, $[reactants]_o^{1-u}$ indicates, then, that u is close to 0. To realize this in terms of a single expression then, $reaction\ rate \propto [peroxidase]^z$ where $z \lesssim 1$.

The above formula makes no comment on hydronium ion concentration. However, an examination of intermolecular enzyme forces provides a small basis for determining effects of pH deviation. Since neutral water in any sufficiently defined space dV has a net charge $q_{total} = \int q_{ion} dV = 0$, addition of hydronium, which is charged, results in a non-balanced charge. Electric charge produces a corresponding force according to Coulomb's law $F = k\frac{q_1 q_2}{r^2}$. (Brown, T. L., et al., 2012) Given the minute distances between hydronium ions and enzymes in solution, any unbalanced electric charges easily produce a very large force. Enzymes have a very delicate active site which only functions under optimal conditions. (Reece, J. B., et al., 2011) By increasing electric force loading on the enzyme however, its molecular geometry is altered yielding an inactive site. (Reece, J. B., et al., 2011) As a result, deviations form optimal pH result in a decrease in reaction rate. (Reece, J. B., et al., 2011) If peroxidase's optimal pH is around pH 9, then reactions at pH 7, 5, and 3 all would have progressively proceeded slower over time, yet

corrupt data in Part D prevents any optimal pH from being determined. (Table 3) Similar labs performed present more useful information. Studies by Susan Kareska, Zongmei Shu, et al., and Mohamed E. Elhefnawy indicate pH values around 7, 7.6, and 7.4 respectively to be most optimal conditions for peroxidase activity. (Kareska, S., 2010; Shu, Z. et al., 2013; Elhefnawy, M. E., 2012)

An enzyme must have a specific shape to catalyze. (Reece, J. B., et al., 2011) After sustaining temperature for several minutes above boiling, many enzymes lose their specific shape and are said to be denatured. (Reece, J. B., et al., 2011) They are no longer effective. Of corse, deviations in temperature exist even within objects at room temperature. (Brown, T. L., et al., 2012) Simply because the average energy is a given temperature does not mean that all components of an object share that exact same thermal energy. These deviations in thermal energy may be modeled by a statistical bell curve of temperature where standard deviation increases with temperature. (Brown, T. L., et al., 2012) An enzyme solution therefore at 100ºC is much more likely to have components above denaturization temperature than an enzyme solution at room temperature. That is why tetraguaicol production in Part E likely would have been close to 0 and may even resemble closely the data listed in table 4 if negative values hadn't been reported. (Table 4)

Using the Arrhenius equation $k = Ae^{-\frac{E_a}{RT}}$ (Brown, T. L., et al., 2012), it becomes apparent that any increase in temperature produces an exponential increase in reaction rate. Decreasing temperature lowers reaction rate. If spectrophotometric records provided meaningful information, the 23ºC, 55ºC, and 70ºC trials of Part F would have generally shown increasing final absorbance readings as temperature increases. (Table 5) This general trend is confirmed by extraUTT experimental observations where reaction rate increase was observed for increasing

temperatures of 4°C, 23°C, and 38°C. (Kareska, S., 2010) Since as discussed in the preceding paragraph, simply having an average solution temperature below peroxidase denaturization temperature does not mean that all molecules in the solution are below denaturization temperature, it cannot simply be asserted that increasing temperature up to but not including denaturization temperature means faster reaction rates. That is why many optimal temperature graphs show a skewed bell curve that falls off faster closer to denaturization temperature than away from optimal temperature. Peroxidase optimal temperature is likely close to 50ºC ± 25ºC.

Optimal conditions are absolutely essential to living systems. If a eukaryotic cell is sustained at subfreezing conditions, its metabolism will slow to a halt. Not only will hydrogen peroxide build up without being converted back into water but all enzymes will stop functioning. That cell will eventually die. At a more macroscopic scale, if a human is left uninsulated in a subfreezing environment, he or she will die of hypothermia. Simply increasing temperature though does not avert death; humans can also die of hyperthermia. It becomes evident then, that neither too hot nor too cold of an environment can host human activity. There is an optimal temperature where humans best live. Likewise, with chemical concentration, human blood must ferry a variety of nutrients at optimal concentrations. An important characteristic of blood routinely examined in hospital patients is red blood cell count. (Mayo Clinic Staff, 2016) Higher than permissible red blood cell count is often the cause or effect – certainly an indicator – of dehydration. (Mayo Clinic Staff, 2016) However, less than necessary red blood cell count also brings with it failure to supply sufficient oxygen. (Mayo Clinic Staff, 2016) It is generally recognized that strong epidermal acidic or basic exposure causes corrosive injury. (Deptartment of Chemistry and Biochemistry, 2016) Thus, human epithelial tissue has an optimal pH – close to 7. (Reece, J. B., et al., 2011) Examining optimal concentration, pH, and temperature with the

human body highlights the importance of understnading optimal conditions as this lab examined *en vitro* with peroxidase.

Conclusion

A fundamental process in animal metabolism, conversion of toxic hydrogen peroxide into water is metabolized by peroxidase. In this lab, optimal conditions of peroxidase were investigated under experimentation. Spectrophotometric observations were recorded of peroxidase catalyzing hydrogen peroxide and guaiacol reaction into tetraguaiacol and water. While most of the trials resulted in inaccurate spectrophotometric readings, valid data was used to determine general trends in temperature and concentration with reaction rate. Optimal peroxidase concentration is determined to be as much as could possibly be contained within a solution. Optimal pH could not be determined. Peroxidase optimal temperature is guesstimated around $50^{o}C \pm 25^{o}C$. This validated the hypotheses that increasing concentration increases reaction rate, that sustaining temperature above aqueous boiling temperature hampers peroxidase's ability to catalyze, and that increased temperature increases reaction rate up to a certain point. However, the hypothesis that higher acidity accompanies faster reaction rate could not be tested and is unknown. The optimal conditions determined in this lab are likely the same found *in vivo* peroxidase catalysis.

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