The Effect of Temperature on the Rate of Activity of the Enzyme Catalase in Hydrogen Peroxide

Observations:

Table 1: Physical Properties of Filter Paper Disks coated with Catalase Enzyme before and after Exposure to 3% hydrogen peroxide solution in Culture Tubes

	Before Exposure to 3% hydrogen peroxide	After Exposure to 3% hydrogen peroxide
Catalase-Coated Filter Paper Disks	• White • Damp • Solid • Opaque	• White • Damp • Solid • Fuzzy with bubbles
3% hydrogen peroxide solution (H_2O_2)	• Liquid • Clear • Colourless	• Liquid • Clear • Colourless

Table 2: Time Taken for different temperature conditions of Enzyme Catalase coated onto Filter Paper Disks to travel to the top of 3% hydrogen peroxide solution in Culture Tubes

Target Catalase Enzyme Temperature (°C)	Trial	Actual Temperature of Catalase (±0.5°C)	Time (±0.1s)
0	1	0.3	4.0
	2		2.9
	3		3.5
	4		3.6
	5		4.4
20	1	22.0	3.4
	2		3.6
	3		3.1
	4		4.1
	5		3.1
40	1	39.8	3.8
	2		3.9
	3		3.6
	4		4.6
	5		6.1
60	1	60.1	16.4
	2		12.2
	3		18.8
	4		16.6
	5		7.9
80	1	81.3	NR for all trials
	2		
	3		
	4		
	5		

*NR = No Reaction

Analysis:

Sample Calculations:

To calculate Relative Rate of Enzyme activity of Catalase: E.g. For Target 20°C Enzyme Catalase Temperature, Trial 1:

$$r_1 = \frac{1}{t_1}$$

$$r_1 = \frac{1}{3.4}$$

$$r_1 = 0.29/s$$

Where:

r_1= relative rate of Enzyme activity of catalase for Trial 1 of the target 20°C temperature condition of catalase data set

t_1 = time taken for enzyme catalase to travel to the top of the 3% hydrogen peroxide for Trial 1 of the target 20°C temperature condition of catalase obtained from table 2

To calculate Mean Relative Rate of Enzyme activity of catalase: E.g. For the target 20°C Enzyme Catalase Temperature Data Set:

$$Mean\ (\bar{x}) = \frac{\sum x}{n}$$

$$Mean\ (\bar{x}) = \frac{0.29 + 0.28 + 0.32 + 0.24 + 0.32}{5}$$

$$Mean\ (\bar{x}) = 0.29/s$$

Where:

n = the number of values for the target 20°C catalase enzyme temperature data set

$\bar{x}$ = mean relative rate of Enzyme Catalase for the target 20°C temperature condition of catalase data set

x = relative rate of Enzyme Catalase for each trial of the target 20°C temperature condition of catalase data set obtained from table 3

To calculate Standard Deviation: E.g. For Mean Relative Rate of Enzyme activity of catalase for the target 20°C Temperature condition of Catalase Data Set:

$$S = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

$$S = 0.03 \ /s$$

Where:

S = standard deviation for mean relative rate of Enzyme activity of catalase for the target 20°C temperature condition of catalase data set

x = relative rate of Enzyme activity of catalase for each trial of the target 20°C temperature condition of catalase data set obtained 3

$\bar{x}$ = mean relative rate of Enzyme activity of catalase for the target 20°C temperature condition of catalase data set

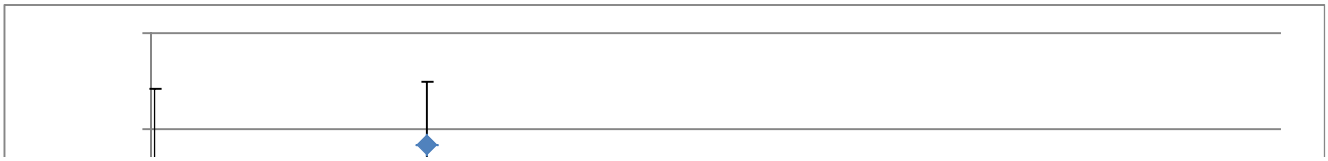
n= number of relative rates of Catalase enzyme for the target 20°C temperature condition of catalase data set

Table 3: Relative Rate of Enzyme Catalase, Mean Relative Rate of Enzyme Catalase and Standard Deviation for Mean Relative Rate of Enzyme Catalase for Filter Paper Disk coated with Catalase Enzyme exposed to 3% hydrogen peroxide Solution in Culture Tubes

Target Catalase	Trial Number	Relative Rate	Mean Relative	Standard Deviation

Enzyme Temperature (°C)		of Enzyme activity of catalase (/s)	Rate of Enzyme activity of catalase (/s)	for Mean Relative Rate of Enzyme activity of catalase (/s)
0	1	0.25	0.28	0.044
	2	0.34		
	3	0.29		
	4	0.28		
	5	0.23		
20	1	0.29	0.29	0.033
	2	0.28		
	3	0.32		
	4	0.24		
	5	0.32		
40	1	0.26	0.24	0.046
	2	0.26		
	3	0.28		
	4	0.22		
	5	0.16		
60	1	0.061	0.077	0.030
	2	0.082		
	3	0.053		
	4	0.060		
	5	0.13		
80	1	0	0	0
	2	0		
	3	0		
	4	0		
	5	0		

Figure 1: Actual Temperature of Enzyme Catalase vs. Mean Relative Rate of Enzyme activity of catalase for Filter Paper Disk coated with Catalase Enzyme exposed to 3% hydrogen peroxide Solution in Culture Tubes



*Error Bars represent ±1 Standard

T-Test:

H_{o1}: There will be no significant difference between mean relative rate of Enzyme activity of catalase for the target 0°C catalase temperature data set and the target 20°C enzyme catalase temperature data set.

H_{o2}: There will be no significant difference between mean relative rate of Enzyme activity of catalase for the target 20°C catalase temperature data set and the target 40°C enzyme catalase temperature data set.

H_{o3}: There will be no significant difference between mean relative rate of Enzyme activity of catalase for the target 40°C catalase temperature data set and the target 60°C enzyme catalase temperature data set.

H_{o4}: There will be no significant difference between mean relative rate of Enzyme activity of catalase for the target 60°C catalase temperature data set and the target 80°C enzyme catalase temperature data set.

H_{o5}: There will be no significant difference between mean relative rate of Enzyme activity of catalase for the target 20°C catalase temperature data set and the target 60°C enzyme catalase temperature data set.

H_{o6}: There will be no significant difference between mean relative rate of Enzyme activity of catalase for the target 0°C catalase temperature data set and the target 40°C enzyme catalase temperature data set.

H_{o7}: There will be no significant difference between mean relative rate of Enzyme activity of catalase for the target 0°C catalase temperature data set and the target 80°C enzyme catalase temperature data set.

H_{o8}: There will be no significant difference between mean relative rate of Enzyme activity of catalase for the target 0°C catalase temperature data set and the target 60°C enzyme catalase temperature data set.

Table 4: Sample size, Mean relative rate of enzyme activity of catalase, Range of values, Standard deviation, T-value, Degrees of freedom and critical value for a comparison of mean relative rate of enzyme activity of catalase and enzyme catalase temperature of filter paper disks coated with enzyme catalase exposed to 3% hydrogen peroxide solution in culture tubes,

Target Enzyme Catalase Temperature (°C)	Sample Size	Mean Relative Rate of Enzyme activity of catalase (/s)	Range of Values (/s)	Standard Deviation (/s)	t-value	Degrees of Freedom	Critical Value for p=0.05
0	5	0.28	0.23-0.34	0.044	0.501	8	2.308
20	5	0.29	0.24-0.32	0.033			
20	5	0.29	0.24-0.32	0.033	2.08	8	2.308
40	5	0.24	0.16-0.28	0.046			
40	5	0.24	0.16-0.28	0.046	6.21	8	2.308
60	5	0.077	0.053-0.13	0.030			
60	5	0.077	0.053-0.13	0.030	5.51	8	2.308
80	5	0	0	0			
20	5	0.29	0.24-0.32	0.033	10.4	8	2.308
60	5	0.077	0.053-0.13	0.030			
0	5	0.28	0.23-0.34	0.044	1.48	8	2.308
40	5	0.24	0.16-0.28	0.046			
0	5	0.28	0.23-0.34	0.044	14.8	8	2.308
80	5	0	0	0			
0	5	0.28	0.23-0.34	0.44	8.55	8	2.308
60	5	0.077	0.053-0.13	0.030			

<u>Sample Calculations:</u>

T-Test: For target 20°C Enzyme Catalase Temperature Data set and the target 40°C Enzyme Catalase Temperature Data set

$$t = \frac{(\bar{x}_A - \bar{x}_B)}{\sqrt{\frac{S_A^2}{n_A} + \frac{S_B^2}{n_B}}}$$

Where: t = t-test for the target 20°C enzyme catalase temperature data set and the target 40°C enzyme catalase temperature data set

$\bar{x}_A$ = mean relative rate of enzyme activity of catalase for the target 20°C temperature data set

$\bar{x}_B$ = mean relative rate of enzyme activity of catalase for the target 40°C temperature data set

S_A = standard deviation for mean relative rate of enzyme catalase activity for the target 20°C catalase temperature data set

S_B = standard deviation for mean relative rate of enzyme catalase activity for the target 40°C catalase temperature data set

n_A = number of relative rates of enzyme activity for the target 20°C catalase temperature data set

n_B = number of relative rates of enzyme activity for the target 40°C catalase temperature data set

$$t = \frac{|0.292 - 0.236|}{\sqrt{\frac{0.033^2}{5} + \frac{0.046^2}{5}}}$$

$$t = 2.08$$

*See Sample Calculation for t-test attached to back of lab

Degrees of Freedom: For target 20°C Enzyme Catalase Temperature Data Set and the target 40°C Enzyme Catalase Temperature Data set

$$d_f = n_A + n_B - 2$$

$$d_f = 5 + 5 - 2$$

$$d_f = 8$$

Where d_f = degrees of freedom for the target 20°C catalase temperature data set and the target 40°C catalase temperature data set

n_A = number of relative rates of enzyme activity for the target 20°C enzyme catalase temperature data set

n_B = number of relative rates of enzyme activity for the target 40°C enzyme catalase temperature data set

Conclusion:

In this study of increasing target temperature of enzyme catalase coated onto paper filter disks reacting through 3% hydrogen peroxide solution in culture tubes, there was an increase in amount of time taken for the enzyme catalase coated filter paper disks to float to the top of the 3% hydrogen peroxide solution in culture tubes (table 2) as the temperature of the catalase enzyme passed the 40° mark. All of the paper filter disks coated with enzyme catalase were placed in the same amount and temperature (in mL and filled to the same level in every culture tube with 2cm gap from top of culture tube) of 3% Hydrogen peroxide solution in culture tubes. All the filter paper disks coated with enzyme catalase were damp, opaque, white and solid (table 1) before exposure to 3% hydrogen peroxide solution and after exposure to 3% hydrogen peroxide they were fuzzy with bubbles on them, solid, damp and white (table 1). The fuzziness and bubbles indicate the reaction of the enzyme catalase coated onto the filter paper disk occurred with the 3% hydrogen peroxide in the culture tube and that oxygen was produced, which were the bubbles as it should according to the literature decomposition balanced equation of 3% hydrogen peroxide where enzyme catalase is the catalyst: $2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$. The 3% hydrogen peroxide was clear, colourless and liquid before and after the reaction with the filter paper disk coated with enzyme catalase (table 2) in the culture tubes. The observations and analysis (table 2 & 3 and figure 1) support the hypothesis of the relative reaction rate of catalase enzyme increasing up to a certain temperature (around 37°C for humans) then decrease past the optimal temperature (around 37°C in humans) because the enzyme begins to denature. Firstly, from temperatures 0-40°C (table 2) of the enzyme catalase coated onto filter paper disk there was a small increase in time taken (about 0.2-0.5 for each trial) to rise the top of the 3% hydrogen peroxide solution in the culture tube and then a huge increase in time (about 10 seconds increase for each trial) from 40-60°C (table 2) of the enzyme catalase coated onto filter paper disk. Then from 60-80°C temperature of the enzyme catalase coated onto filter paper disk it was an almost infinite time increase (from about 15 seconds to no reaction which could be almost infinite) as no reaction was occurring in the 80°C temperature condition (table 2). Additionally there is a huge increase from 0°C to 60°C and 80°C (from about 3.5 seconds of 0°C to about 15 seconds for 60°C and to almost infinite for 80°C). Secondly, Table 3 shows that the that the relative enzyme catalase reaction rate increases from 0.227/s to 0.292/s for temperature 0°C and 20°C respectively. This shows that the relative reaction rate for the enzyme catalase is increasing as it's getting closer to its' optimal temperature (around 37°C in humans) which is where the enzyme functions most efficiently. For temperature of 40°C the relative reaction rate of catalase enzyme is beginning to decrease (goes from 0.292/s of 20°C to 0.236/s for 40°C temperature). This suggests that the highest relative reaction rate for enzyme catalase is in between 20°C to somewhere before 40°C (around the literature value of 37°C) as the relative reaction rate begins to drop at 40°C. For temperatures past 40°C which are 60°C and 80°C the relative reaction rate of enzyme catalase decreases incredibly (0.077/s and 0/s respectively). Thirdly, Figure 1 Illustrates that as the temperature is increased the mean relative reaction rate of enzyme catalase increases up to about 37-39°C (up to around 0.3/s mean relative reaction rate). Then

past the 37-39°C mark, the mean relative reaction rate of enzyme catalase begins to drop from 40°C and onwards. The mean relative reaction rate is about 0.24/s for 40°C and about 0.08/s for 60°C, both of which are smaller than the mean relative reaction rates of 0°C and 20°C (0.28/s and 0.29/s respectively).When it reaches 80°C there is no reaction occurring meaning a mean relative reaction rate of 0/s. Additionally, the error bars (based on the standard deviation) only overlap for temperatures 0°C, 20°C and 40°C which are below/near (respectively) to the optimal temperature of enzyme catalase, which suggests that the mean relative reaction rates of those temperature condition do fall within the same range. The error bars however do not overlap at temperatures 60°C and 80°C with each other and with any other temperature condition (0°C, 20°C and 40°C), which suggests that the mean relative reaction of 60°C and 80°C do no fall within the same range of each other's mean relative reaction rates and any other conditions mean relative reaction rate.

Finally, according to the t-tests shown in table 4, the t-values (6.21, 5.51, 10.4, 14.8 and 8.55) are much greater than the critical values (2.308 for all comparisons at p=0.05) for every comparison except for the 0°C to 20°C, 20°C to 40°C and 0°C to 40°C comparisons where the t-values are below (0.501, 2.08 and 1.48 respectively) the critical values (2.308, 2.308 and 2.308 respectively at p=0.05). This indicates that the null hypotheses (except for the 0°C to 20°C, 20°C to 40°C and 0°C to 40°C comparisons) are incorrect meaning the differences in the mean relative reaction rates of enzyme catalase are significant. These significant differences further support the hypothesis as it illustrates that temperatures above the optimal (37°C) by a huge amount (ex. Temperatures that are greater than 40°C) incredibly lower and/or even completely stop enzyme reaction rate activity due to denaturation of high temperatures. These t-tests show that any differences between the means have a less than 5% chance of simply being due to random variation. For the t-test values (0.501, 2.08 and 1.48) below the critical values (2.308 for all comparisons at p=0.05), the difference in mean relative reaction rates are not significant which supports the null hypotheses. These non-significant differences further support the hypothesis as it illustrates that temperatures around and below (down to 0°C) the optimal 37°C (in humans) have a high enzyme reaction rate. These t-tests show that any differences between the means have a greater than 5% chance of simply being due to random variation.

The observations and calculated values support the hypothesis, illustrating that the relative reaction rate of enzyme catalase increases until it reaches an optimal temperature of approximately 35-40°C which is close to the human body temperature (Campbell and Reece, 2002). Any temperature above the optimal temperature of enzymes causes denaturation to occur to the enzyme due to the increase in the motion of molecules which increases heat that causes breakage of bonds (which maintain the structure of the enzyme) in the enzyme. (Allott, 2007) The denaturation thus causes a decrease in the mean relative reaction rate of catalase enzyme because the substrate (in this investigation is hydrogen peroxide) does not fit the active site of the enzyme (in this investigation is catalase enzyme). The huge difference in mean relative reaction rate of catalase enzyme between 0°C to 80°C explains the denaturation of the catalase at high temperatures. And the little variation of the mean relative reaction rate at the

lower temperatures of catalase enzyme of 20°C and 40°C support the hypothesis as they are close to the 35-40°C optimal temperature. Overall the data and values support the hypothesis.

Evaluation:

The filter paper disks were drying up quickly after being patted with a paper towel to remove excess liquid. The dried filter paper disks become less sticky and fall off the rubber stopper (when trying to insert them into culture tubes) into the 3% hydrogen peroxide solution before the culture tube was inverted. This caused the production of oxygen (bubbles) to happen sooner and since both sides of the filter paper disks are reacting in the beginning (as compared to only one side reacting at the beginning until it somewhat rises into the 3% hydrogen peroxide solution) it resulted in a decrease in amount of time taken to rise to them top of 3% hydrogen peroxide solution which increases relative reaction rate of the enzyme catalase. To fix this, dip only one paper filter paper disk into catalase enzyme solution at a time, and tap each side carefully using tweezers on paper towel and immediately place onto rubber stopper so they are still sticky.

The enzyme catalase extract solution was obtained from a beaker. For the temperatures of 40°C, 60°C and 80°C the beakers were placed on hot plates to keep the temperature consistent. Since the beakers were left on the hot plates for a while, at high temperatures especially the 80°C condition, the water in the solution of the enzyme catalase can begin to evaporate and thus increasing the enzyme concentration in the solution. This causes the relative rate of enzyme activity of catalase to increase (meaning takes less time to flout to top of 3% hydrogen peroxide solution) as there are more enzymes to decompose hydrogen peroxide for the 40°C, 60°C and 80°C temperature conditions. To fix this problem, heat up the catalase enzyme extract solution to the desired temperature condition and use that catalase enzyme extract solution immediately to minimize evaporating of water.

References:

Campbell N, Reece J. 2002. Biology. San Francisco: Pearson/Benjamin Cummings. Pg.100.

Allott A. 2007. Biology. Oxford: Oxford University Press. p19.