Enzymes Lab (Version 2)

Before you begin, save this Lab Report Template on your computer as LastNameAPBIOEnzyme2

Read all the instructions below BEFORE you start!

1. Please go to the following website: http://www.biocourse.com/mhhe/bcc/domains/biolabs.xsp
2. Scroll down and click on the link to ***Enzyme Kinetics*.** It is the 5th one down on the left.
3. Read the instructions for entering the virtual lab and then open and ***PRINT*** the Lab Manual Instructions for the Enzyme Kinetics Lab.
4. Follow all the steps for the lab in the lab manual you printed out. You will be asked to design experiments, gather data, and graph your results. YOU DO NOT NEED TO ANSWER THE ONLINE QUESTIONS, but you should think about them as you proceed thorough the lab.

Experiment 1: Control Enzyme

a. Click Clear Data
b. Select Control Enzyme from the enzyme list
c. Enter Control Temperature of 37°C and control pH of 7.0
d. Click Graph Data
e. Record reaction rate (slope of y = mx line…you can see this by rolling your mouse over the blue Trial 1 bar to the right.
f. Repeat with the temperature and pH values listed in Table 1. Record the slope for all trials!
g. Highlight the row indicating optimal temperature and pH for the control enzyme. (Hint: select the row with the highest reaction rate)

Data Table 1: Control Enzyme

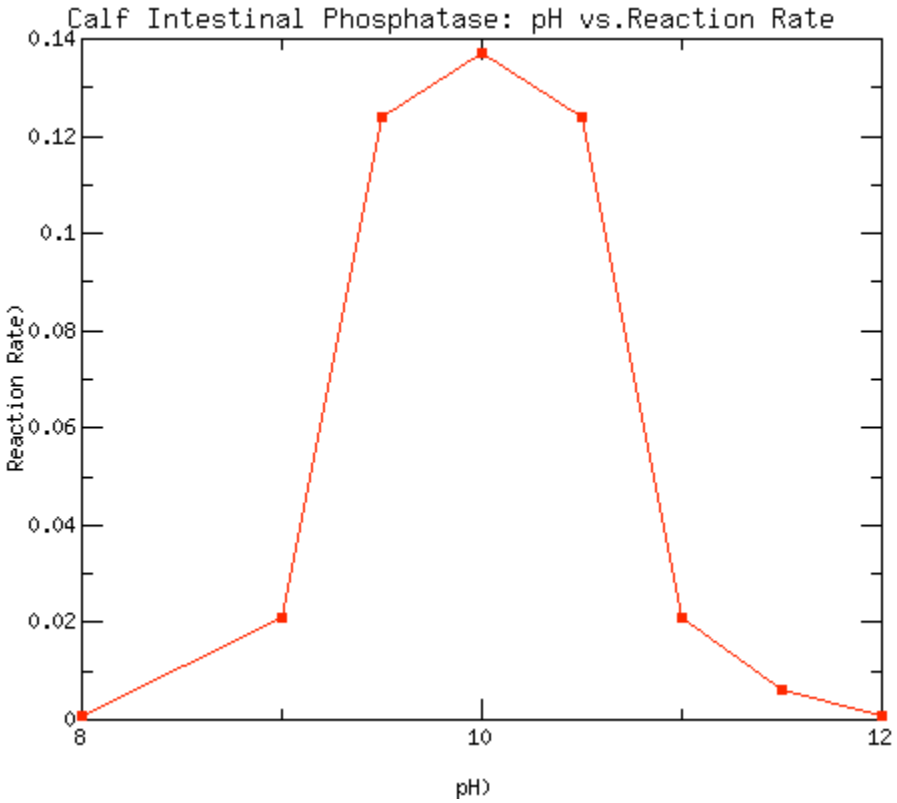
Trial	*Temperature (°C)*	*pH*	*Reaction Rate (slope) (absorbance/min)*
1	*37*	*7*	*0.137*
2	*90*	*7*	*0.057*
3	*90*	*6.5*	*0.044*
4	*90*	*7.5*	*0.044*
5	*90*	*8.0*	*0.021*
6	*90*	*8.5*	*0.006*

h. Click *Continue* to save your experiment results.
You will be asked to interpret your results after you graph them. Think about how you would answer the questions but you DO NOT have to answer them online

Experiment 2: Calf Intestinal Phosphatase pH Optimization I and II
Read the online information about

a. Select Calf Intestinal Phosphatase from the enzymes list.
b. Select a temperature between 30—50°
   (Hint: the normal body temperature of a calf is 37°C)
c. Run the simulation for 6 trials with low, mid, and high range pH values
d. Record your data in the Data Table 2
e. Click Continue to save your data.
f. Clear data and repeat with the same temperature you used above and with pH values in the range with the highest slope from Part I. Adjust using increments of 0.5.
g. Use GraphPad (http://antoine.frostburg.edu/cgi-bin/senese/graphpad.cgi) to construct a graph of pH vs Reaction Rate.
   (Resize rows to 12. pH is on the x-axis and reaction rate on the y-axis
   Title your graph, label the curve, and connect the points.
   DO NOT use LS line or LS line & equation!)
h. Copy and paste your graph below the data chart.
i. Determine the optimum pH for calf intestinal phosphatase from the graph.

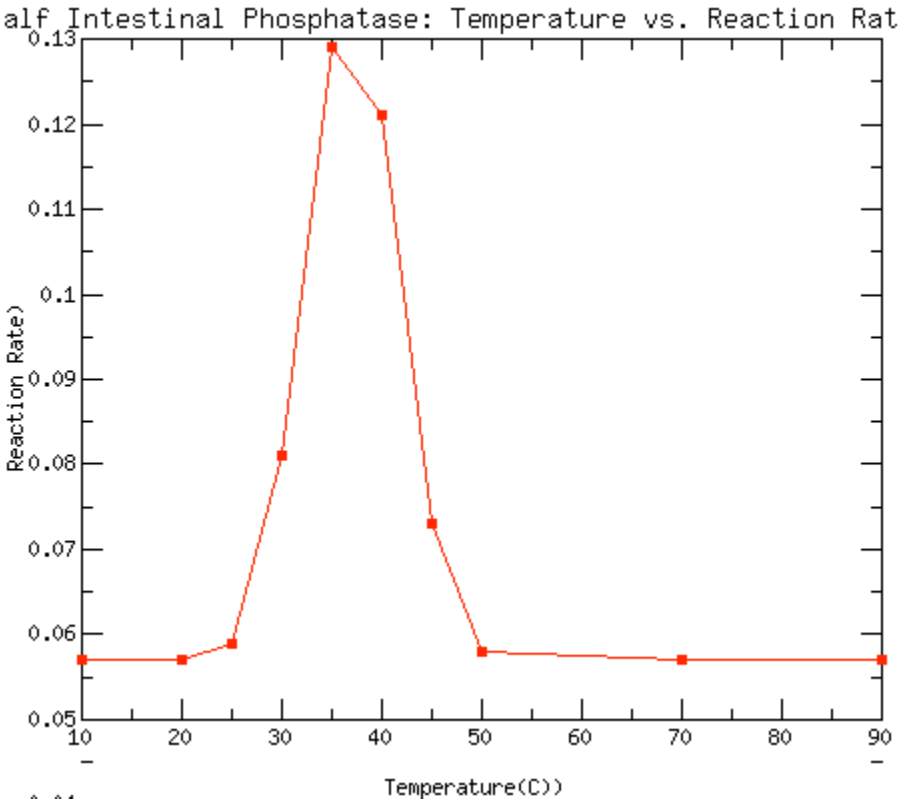
Data Table 2: Calf Intestinal Phosphatase *Temperature = _____37_____*		
Trial	*pH*	*Reaction Rate (slope) (absorbance/min)*
Part I		
1	*1.0*	*0*
2	*4.0*	*0*
3	*5.0*	*0*
4	*8.0*	*0.001*
5	*9.0*	*0.021*
6	*12.0*	*0.001*
Part II		
1	*9.0*	*0.021*
2	*9.5*	*0.124*
3	*10.0*	*0.137*
4	*10.5*	*0.124*
5	*11.0*	*0.021*
6	*11.5*	*0.006*
Optimal pH →	*10.0*	


Calf Intestinal Phosphatase: pH vs.Reaction Rate
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Reaction Rate)
8
10
12
pH)

Experiment 3: Calf Intestinal Phosphatase Temperature Optimization I and II

a. Clear Data
b. Select Calf Intestinal Phosphatase from the enzymes list.
c. Select the optimal pH you determined from the previous experiment.
d. Run the simulation for 6 trials with low, mid, and high range temperature values
e. Record your data in the Data Table 2
f. Click Continue to save your data.
g. Clear data and repeat with the same pH you used above and with temperature values in the range with the highest slope from Part I. Adjust using increments of 0.5.
h. Use GraphPad (http://antoine.frostburg.edu/cgi-bin/senese/graphpad.cgi) to construct a graph of Temperature vs Reaction Rate.
(DO NOT use LS line or LS line & equation!)
i. Copy and paste your graph below the data chart.
j. Determine the optimum temperature for calf intestinal phosphatase from the graph.

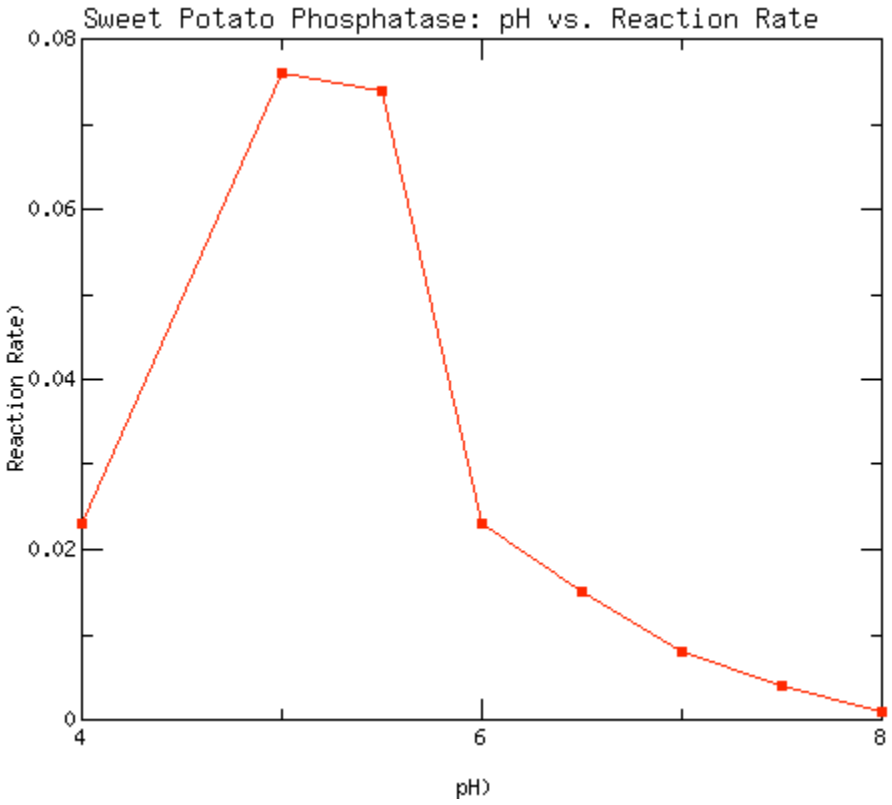
Data Table 2: Calf Intestinal Phosphatase *pH = _____10.0_____*		
Trial	*Temperature (^{o}C)*	*Reaction Rate (slope) (absorbance/min)*
Part I		
1	*10*	*0.057*
2	*20*	*0.057*
3	*30*	*0.081*
4	*50*	*0.058*
5	*70*	*0.057*
6	*90*	*0.057*
Part II		
1	*20*	*0.057*
2	*25*	*0.059*
3	*30*	*0.081*
4	*35*	*0.129*
5	*40*	*0.121*
6	*45*	*0.073*
Optimal Temperature → 35		


alf Intestinal Phosphatase: Temperature vs. Reaction Rat
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
Reaction Rate)
10
20
30
40
50
60
70
80
90
Temperature(C))

Experiment 4: Sweet Potato Phosphatase pH Optimization I and II

a. Select sweet potato phosphatase from the enzymes list.
b. Select a temperature between 15—35° C.
   (Hint: Sweet potatoes are specialized roots that provide nutrient storage for the sweet potato plant and contain enzymes associated with cellular metabolism. The normal temperature during the sweet potato growing season is 15—35° C)
c. Run the simulation for 6 trials with low, mid, and high range pH values
d. Record your data in the Data Table 2
e. Click Continue to save your data.
f. Clear data and repeat with the same temperature you used above and with pH values in the range with the highest slope from Part I. Adjust using increments of 0.5.
g. Use GraphPad (http://antoine.frostburg.edu/cgi-bin/senese/graphpad.cgi) to construct a DO NOT use LS line or LS line & equation!)
h. Copy and paste your graph below the data chart.
i. Determine the optimum pH for sweet potato phosphatase from the graph.

Data Table 2: Sweet PotatoPhosphatase Temperature = _____25_____		
Trial	*pH*	*Reaction Rate (slope) (absorbance/min)*
Part I		
1	*1*	*0*
2	*4*	*0.023*
3	*5*	*0.076*
4	*8*	*0.001*
5	*9*	*0*
6	*12*	*0*
Part II		
1	*5*	*0.076*
2	*5.5*	*0.074*
3	*6*	*0.023*
4	*6.5*	*0.015*
5	*7*	*0.008*
6	*7.5*	*0.004*
Optimal pH →	*5*	

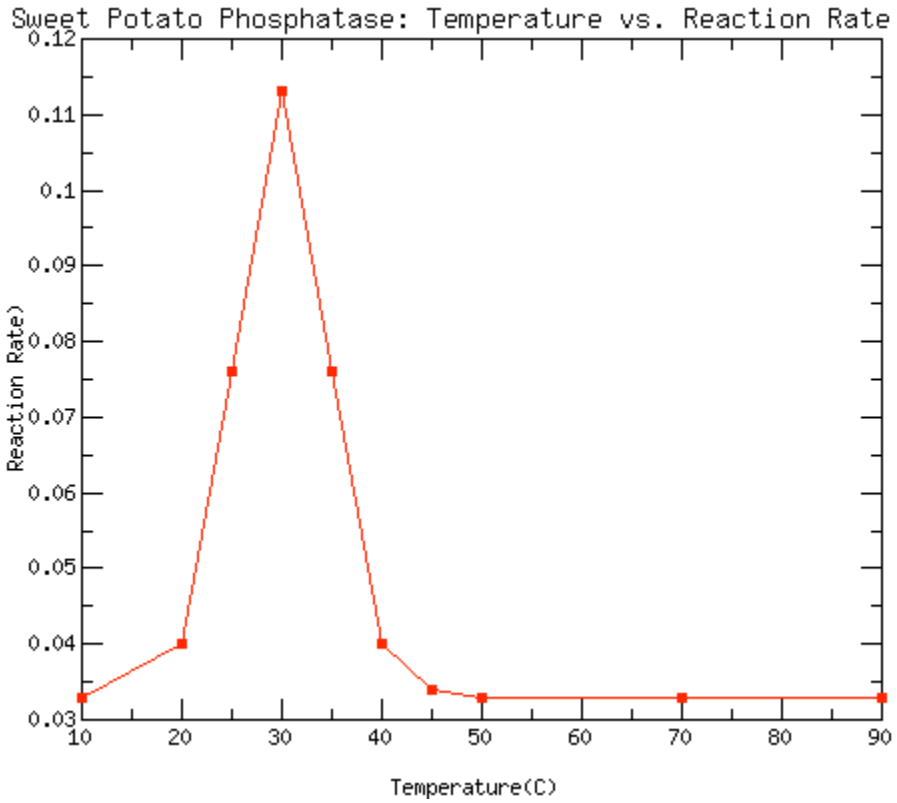

Sweet Potato Phosphatase: pH vs. Reaction Rate
0.08
0.06
0.04
0.02
0
Reaction Rate)
4
6
8
pH)

Experiment 5: Sweet Potato Phosphatase Temperature Optimization I and II

a. Clear Data
b. Select sweet potato phosphatase from the enzymes list.
c. Select the optimal pH you determined from the previous experiment.
d. Run the simulation for 6 trials with low, mid, and high range temperature values
e. Record your data in the Data Table 2
f. Click Continue to save your data.
g. Clear data and repeat with the same pH you used above and with temperature values in the range with the highest slope from Part I. Adjust using increments of 0.5.
h. Use GraphPad (http://antoine.frostburg.edu/cgi-bin/senese/graphpad.cgi) to construct a graph of Temperature vs Reaction Rate.
(DO NOT use LS line or LS line & equation!)
i. Copy and paste your graph below the data chart.
j. Determine the optimum temperature for sweet potato phosphatase from the graph.

Data Table 2: Sweet Potato Phosphatase
pH = _____5.0_____

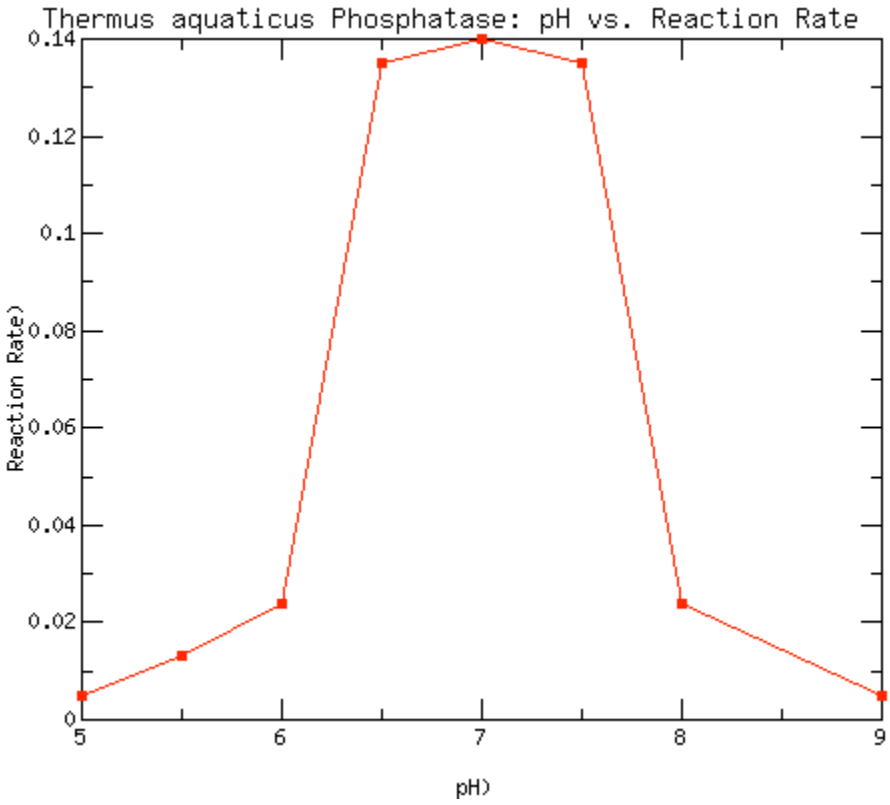
Trial	*Temperature (^{o}C)*	*Reaction Rate (slope) (absorbance/min)*
Part I		
1	*10*	*0.033*
2	*20*	*0.04*
3	*30*	*0.113*
4	*50*	*0.033*
5	*70*	*0.033*
6	*90*	*0.033*
Part II		
1	*20*	*0.04*
2	*25*	*0.076*
3	*30*	*0.113*
4	*35*	*0.076*
5	*40*	*0.04*
6	*45*	*0.034*
Optimal Temperature →30		


Sweet Potato Phosphatase: Temperature vs. Reaction Rate
Reaction Rate)
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
10
20
30
40
50
60
70
80
90
Temperature(C)

Experiment 6: *Thermus aquaticus* Phosphatase pH Optimization I and II

a. Select *Thermus aquaticus* phosphatase from the enzymes list.
b. Select a temperature between 70--90° C.
(Hint: Thermus aquaticus go4rs in hydrothermal vents at the bottom of the ocean where the temperature can vary between 70—90 °C.
Ignore the instructions for selecting temperature at the online site because there are several typos there!)
c. Run the simulation for 6 trials with low, mid, and high range pH values
d. Record your data in the Data Table 2
e. Click Continue to save your data.
f. Clear data and repeat with the same temperature you used above and with pH values in the range with the highest slope from Part I. Adjust using increments of 0.5.
g. Use GraphPad (http://antoine.frostburg.edu/cgi-bin/senese/graphpad.cgi) to construct a DO NOT use LS line or LS line & equation!)
h. Copy and paste your graph below the data chart.
i. Determine the optimum pH for *Thermus aquaticus* phosphatase from the graph.

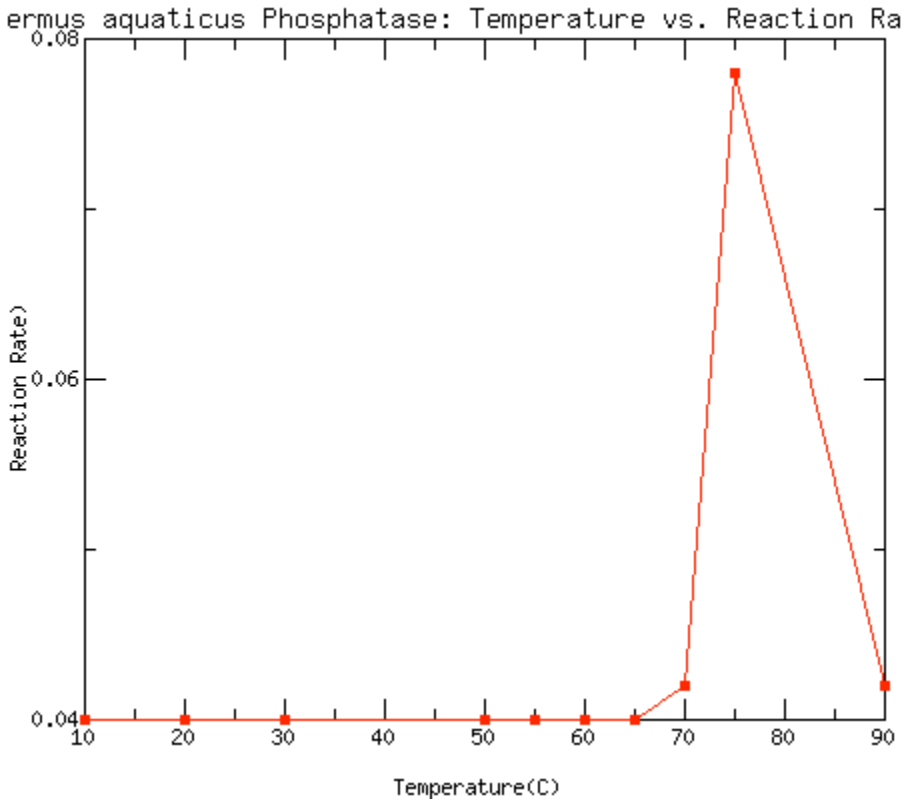
Data Table 2: Thermus aquaticus Phosphatase *Temperature = _____80_____*		
Trial	*pH*	*Reaction Rate (slope) (absorbance/min)*
Part I		
1	*1*	*0*
2	*4*	*0*
3	*5*	*0.005*
4	*8*	*0.024*
5	*9*	*0.005*
6	*12*	*0*
Part II		
1	*5*	*0.005*
2	*5.5*	*0.013*
3	*6*	*0.024*
4	*6.5*	*0.135*
5	*7*	*0.14*
6	*7.5*	*0.135*
Optimal pH →	*7*	


Thermus aquaticus Phosphatase: pH vs. Reaction Rate
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Reaction Rate)
5
6
7
8
9
pH)

Experiment 7: *Thermus aquaticus* Phosphatase Temperature Optimization I and II

a. Clear Data
b. Select *Thermus aquaticus* phosphatase from the enzymes list.
c. Select the optimal pH you determined from the previous experiment.
d. Run the simulation for 6 trials with low, mid, and high range temperature values
e. Record your data in the Data Table 2
f. Click Continue to save your data.
g. Clear data and repeat with the same pH you used above and with temperature values in the range with the highest slope from Part I. Adjust using increments of 0.5.
h. Use GraphPad (http://antoine.frostburg.edu/cgi-bin/senese/graphpad.cgi) to construct a graph of Temperature vs Reaction Rate.
(DO NOT use LS line or LS line & equation!)
i. Copy and paste your graph below the data chart.
j. Determine the optimum temperature for *Thermus aquaticus* from the graph.

Data Table 2: Thermus aquaticus Phosphatase *pH = _____7_____*		
Trial	*Temperature (°C)*	*Reaction Rate (slope) (absorbance/min)*
Part I		
1	*10*	*0.04*
2	*20*	*0.04*
3	*30*	*0.04*
4	*50*	*0.04*
5	*70*	*0.042*
6	*90*	*0.042*
Part II		
1	*50*	*0.04*
2	*55*	*0.04*
3	*60*	*0.04*
4	*65*	*0.04*
5	*70*	*0.042*
6	*75*	*0.078*
Optimal Temperature →75		


ermus aquaticus Phosphatase: Temperature vs. Reaction Ra
0.08
0.06
0.04
Reaction Rate)
10
20
30
40
50
60
70
80
90
Temperature(C)

Discussion:

a. In order to do this lab, one has to have Internet access in order to access the online lab. The online lab helped you investigate the action of enzymes and the factors that affect their activity. One had to determine the rates of the enzyme reactions through a stimulated laboratory experiment. By comparing the rates of reactions under different environmental conditions, the optimal circumstances under which particular enzymes function best were also determined. All the data was recorded and then graphed.

b. Phosphatases are a group of enzymes which release groups for cell metabolism. The resulting phosphates are used for a lot of purposes. For example, they can be used for incorporation into the nucleotides of DNA and RNA and to make phospholipids, which are a component of cell membranes and make ATP. Phosphatase enzymes are found in many organisms because they are part of the making of the structure of cell membrane and make ATP, which helps run processes within the cell.

c. The optimum pH and temperature for the calf intestinal phosphatase was 10 and 35 degrees Celsius. The optimum pH and temperature for the sweet potato phosphatase was 5 and 30 degrees Celsius. The optimum pH and temperature for the Thermus aquaticus was 7 and 75 degrees Celsius.

d. The pHs of these three organisms are around the same range. The sweet potato and the *Thermus aquaticus* are really close, while the calf intestinal has a little higher pH. Looking at the temperature, it is a different story. The calf intestinal and the sweet potato react at close temperatures, while the *Thermus aquaticus* reacts at a very high temperature of 75 degrees Celsius. There was an overlap of pH with the sweet potato and the *Thermus aquaticus*, and there was an overlap of the temperature of the calf intestinal and the sweet potato. The huge difference in temperature is that the normal temperature of the sweet potato and the calf intestinal are in the same range, whereas the *Thermus aquaticus* normal temperature is higher because of it's surroundings in the ocean.

Conclusion:

The optimum pH and temperature for the calf intestinal phosphatase was 10 and 35 degrees Celsius. The optimum pH and temperature for the sweet potato phosphatase was 5 and 30 degrees Celsius. The optimum pH and temperature for the Thermus aquaticus was 7 and 75 degrees Celsius.

Reflection:

This lab required a lot of data collecting and graph making. This lab helped me learn that if I am not organized with my data that it could ruin the whole lab. I also learned more about the optimal circumstances in which enzymes function best by looking at three different enzymes.