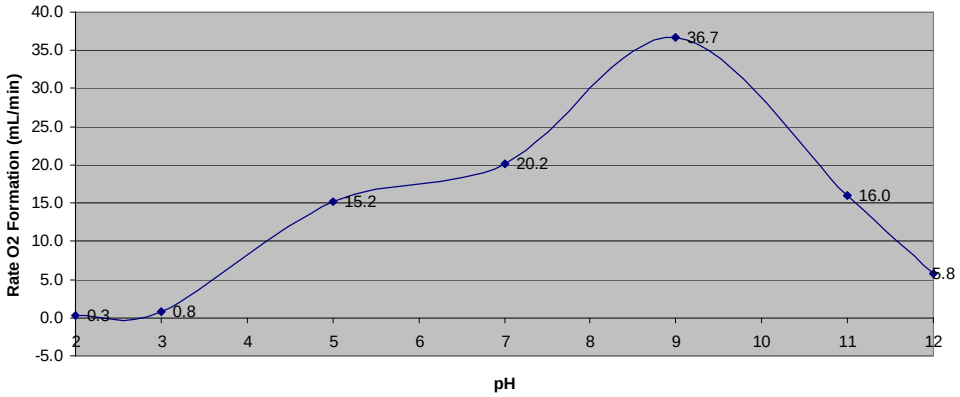
Effects of pH Levels on Catalase Enzyme Activity

Summary: My group and I observed the mixture of catalase enzyme with hydrogen peroxide solution in varying pH levels to determine whether reaction rates benefited from acidic, basic or neutral environments. We did this by testing the reactivity at pH 12 and then pooling our results with data from other groups in our class who were responsible for testing the remaining pH levels (ranging from two to 11). Our collective data showed that the catalase enzyme is most effective in catalyzing the reaction $H_2O_2 \longrightarrow H_2O + O_2$ when it is occurring in a basic environment where the pH is about 9. Our results are supported by the knowledge that catalase is more active in basic environments and at specific temperatures *(Caridis et al., 1990)*. Also, pH has an effect upon the nature of enzymes, their compounds, and the rates at which new compounds are formed through biochemical reactions *(Chance, 1951)*.

Methods & Results: My group and I began the experiment by constructing a respirometer out of a 150mL Erlenmeyer flask, a rubber stopper with a hole in it, and a 5mL pipette. The flask was filled with 100mL of 3% hydrogen peroxide (H_2O_2) solution in water and then placed in a ~22°C water bath to keep it at a relatively constant temperature throughout. A 5.00% sample of liver homogenate was used as our source of catalase enzymes. These preparations were made twice: once to test and confirm reaction rates under standard conditions, with pH at ~7, and a second time with a pH 12 tablet added to the hydrogen peroxide solution. Other groups in our class followed the same setup, but used different pH tablets to test for pH levels ranging from two to 11.

For each test, a ~1mL amount of dilute liver homogenate was added to the flask, after which it was sealed with the rubber stopper and pipette. Oxygen gas began to form, which increased the pressure in the flask and caused the mixture to rise inside the pipette. The flask was "burped" as the liquid reached the 5+1mL mark on the pipette and then allowed to drain back into the flask. Because of the loss of oxygen during this time, we decided to add 1mL extra to the measured O_2 for each "burp", meaning that a total amount of ~7mL of total gas was measured. We continued this process for three minutes and recorded the number of burps and/or mL readings on the pipette individually for each minute. A rate was determined for each concentration by dividing the total mL of oxygen gas produced by three minutes. Results of these two tests were shared and combined among the different groups in the class.

For standard conditions, we found the rate to be 14mL/min, which was lower than the class average of 18.5mL/min. The recorded reaction rates for different pH levels from the experiments performed by each group in the class produced the following graph of data:



As the orange line on the graph shows, the reaction rate is at its highest when the pH of the environment is about 9, showing that the catalase enzyme is most effective for this reaction when it is in a rather basic environment. It becomes less active in pH levels less than seven or greater than 10.5.

References:

Caridis, K. & Christakopoulos, P. & Macris, B., 1990. Simultaneous Production of Glucose Oxidase and Catalase by Alternaria Alternata, *Applied Microbiology Biotechnology*, 34(6), pp. 794-797.

Chance, B., 1951. Effect of pH Upon the Reaction Kinetics of the Enzyme-Substrate Compounds of Catalase, *Journal of Biological Chemistry*, 194, pp. 471-481.