Experiment 2. On the kinetics studies of catalase enzymatic activity

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Abstract. Both hydrogen peroxide and catalase are two very common molecules inside organisms. The function of catalase is to decompose the harmful hydrogen peroxide into water and oxygen. It's known that different concentrations of these two substances give different reaction rates. In this experiment the decomposition kinetics of hydrogen peroxide by catalase were studied through the monitoring of oxygen pressure of various solutions inside a fixed volume vessel. The software LoggerPro and specialized compatible hardware were used to measure, control and compile data. The inhibition effects of ethanol were also explored. It was possible to find a decomposition rate and order of reaction specific to each solution.

I. INTRODUCTION

Enzymes are biological molecules found in most living bodies. They are tools that work as catalysts, speeding up reactions by providing an alternative reaction pathway of lower activation energy. [1]. Early enzymatic studies were started around 1833 [2] and since then it has been of keen interest for scientists to describe their functionality within a body. One enzyme in particular, catalase, is of interest within this paper.

Catalase is commonly found in all living organisms exposed to oxygen and it aids in the degradation of hydrogen peroxide (H_2O_2). Hydrogen peroxide is hazardous when in contact with tissues and therefore it is quickly decomposed into water and oxygen. Even though it's an unstable molecule inside the body, the natural decomposition rate of H_2O_2 is relative slow and that's where catalase comes in. Catalase has a certain 3D structure when it is active, which contains a channel into which the hydrogen peroxide can diffuse. In the channel is a heme group which is a iron molecule bound to the center of a ring-like structure called a porphyrin ring. Here the reaction between H_2O_2 and the heme group give rise to a modified catalase and water. This second version of catalase can later interact with yet another H_2O_2 molecule and produce back the original catalase plus water and oxygen.

It has been proposed that the rate of reaction r for hydrogen peroxide is equal to

$$r = -\frac{\mathrm{d}[H_2O_2]}{\mathrm{d}\,t} = k[H_2O_2]^{\alpha} \tag{1}$$

where k is a proportionality constant and α is the order of reaction.

Thanks to initial reaction rate conditions it's possible to arrange this equation as

$$-\frac{\mathrm{d}[H_2O_2]}{\mathrm{d}\,t} = \frac{\mathrm{d}(P_{O_2})}{\mathrm{d}\,t} \tag{2}$$

$$\ln\left(\frac{P_{O_2}}{t}\right) = \ln(k) + \alpha\ln([H_2O_2]) \tag{3}$$

where P_{O_2} is pressure in Pa and time t in seconds.

II. EXPERIMENTAL PROCEDURE

Experiments were performed using Hydrogen peroxide (3% in volume), Monobasic potassium phosphate, Dibasic potassium phosphate, Ethanol and Catalase, using a Vernier pressure sensor and a thermometer to measure the change in pressure caused by oxygen.

Buffer solution was prepared using 0.54 g of K_2HPO_4 and 0.25 g of KH_2PO_4 in distilled water (50mL of solution) .Catalase was prepared with 1.08 g of K_2HPO_4 and 0.49 g of KH_2PO_4 in distilled water (100mL of solution). 1.5% and 1% Peroxide was obtained adding water proportionally. Pure ethanol as well as 50%,30%, and 10% ethanol was used.

A total of 22 measurements were made corresponding to the 11 tests on table 1 with a repetition in each. In each measurement the substances were heated in water to reach 37 Celsius for at least 2 min. Finally substances were mixed and the test tube was closed, measurements were recorded with the sensor.

TABLE I: Substances used per test

Experiment	H_2O_2[mL]	Cat.[mL]	Buf.[mL]	Ethanol[mL]
1	1 (3.0% in v.)	1	2	0
2	1 (3.0% in v.)	2	1	0
3	1 (3.0% in v.)	3	0	0
4	1 (3.0% in v.)	1	0	0
5	1 (0.5% in v.)	1	0	0
6	1 (1.0% in v.)	1	0	0
7	1 (1.5% in v.)	1	0	0
8	1 (3.0% in v.)	1	0	1 (100.0% in v.)
9	1 (3.0% in v.)	1	0	1 (050.0% in v.)
10	1 (3.0% in v.)	1	0	1 (030.0% in v.)
11	1 (3.0% in v.)	1	0	1 (010.0% in v.)

III. RESULTS

For the experiment there was a total of 22 measurements. Table 1 shows measurements were divided in three sections that had different objectives: Hydrogen peroxide concentration effect (section A), catalase concentration effect (section B), and inhibition effects from ethanol (section C). Figure 1,2 and 3 shows the evolution of pressure while the degradation of H_2O_2 took place.

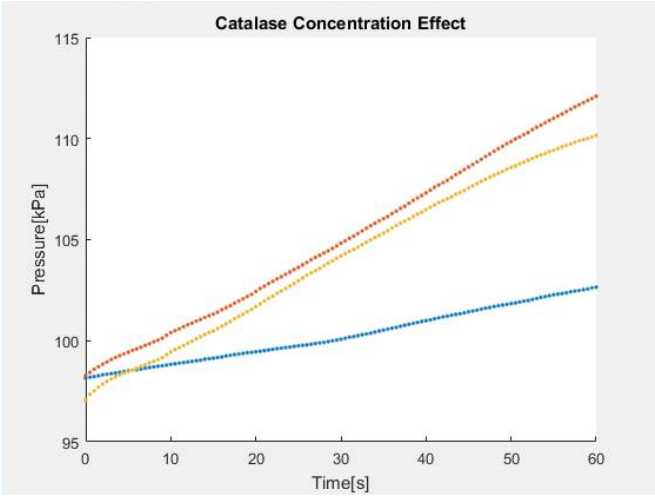

FIG. 1: Pressure vs. Time graph for section a). From bottom to top Test 1, Test 3, Test 2

Table 1 gives a synthesis of individual results per repetition, showing the value for the initial reaction rate, the coefficient k and order of reaction. Figure 4 through 7 show the evolution of the rate of reaction as hydrogen peroxide's concentration changed. Results from these are shown in a logarithmic scale.

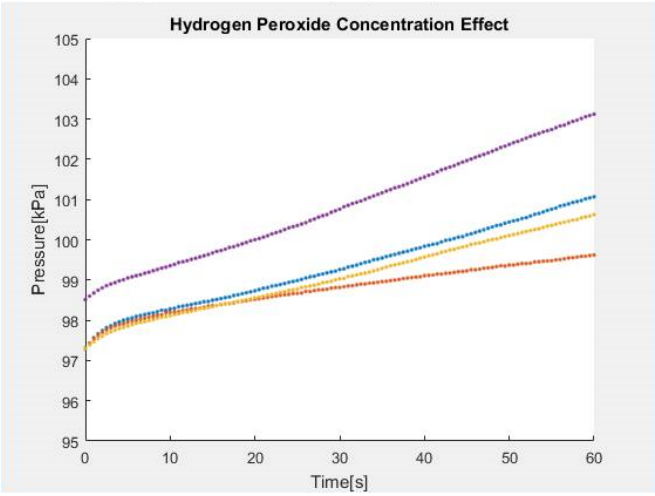

FIG. 2: Pressure vs Time graph for section b. From bottom to top Test 5, Test 6, Test 4, Test 7.

Figures 1 through 3 show pressure as a function of time for 1) Catalase Concentration Effect, 2) Hydrogen Peroxide Concentration Effect, 3) Inhibition effect of elcohol. As expected under the presence of Catalase the rate of production of oxygen is greater. A drop in reaction rate can be better observed in Figure 3.

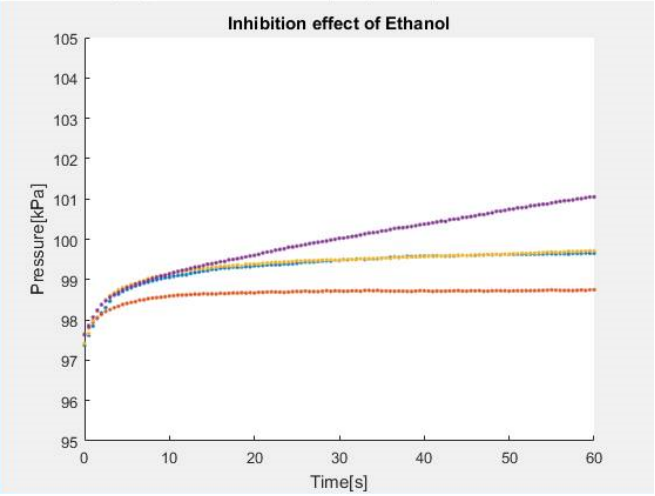

FIG. 3: Pressure vs Time graph for section b. From bottom to top Test 9, Test 10, Test 8, Test 11.

Each section shows different behaviors regarding the evolution of oxygen generation. Higher values were reported for section A (variation of catalase) while lowest values were shown by section C (inhibition of ethanol).

TABLE II: Initial rate of reaction, coefficient k and order of reaction for each of the experiments (Sample 1 and sample 2.)

Experiment	IRR (Pa/s)	k	α
1	1.0303	96.697	-0.99020
	1.0508	95.698	-0.98336
2	1.1461	92.746	-0.95551
	1.1100	92.564	-0.96501
3	1.1100	92.564	-0.96501
	1.1469	91.445	-0.95683
4	1.0452	95.570	-0.98640
	1.0205	96.980	-0.99370
5	1.0227	97.275	-0.99357
	1.0198	96.654	-0.99462
6	1.0297	95.959	-0.99124
	1.0305	96.562	-0.99035
7	1.0552	96.296	-0.98260
	1.0283	97.148	-0.99091
8	1.0312	98.347	-0.99362
	1.0115	97.829	-0.99827
9	1.0093	97.659	-0.99868
	1.0096	98.690	-0.99825
10	1.0213	97.586	-0.99620
	1.0184	98.914	-0.99645
11	1.0343	97.353	-0.99132
	1.0280	97.499	-0.99244

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The previous reaction rates were used as data to determine linear fits of the information, and are shown in figures 4 trough 7.

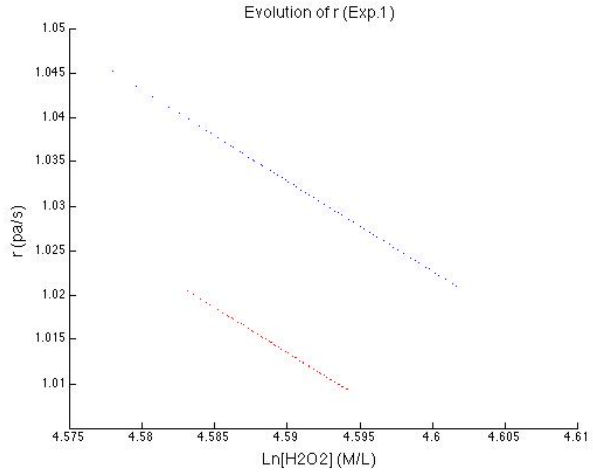

FIG. 4: Evolution of r for experiment 1 of section B of experiment. H_2O_2 3%

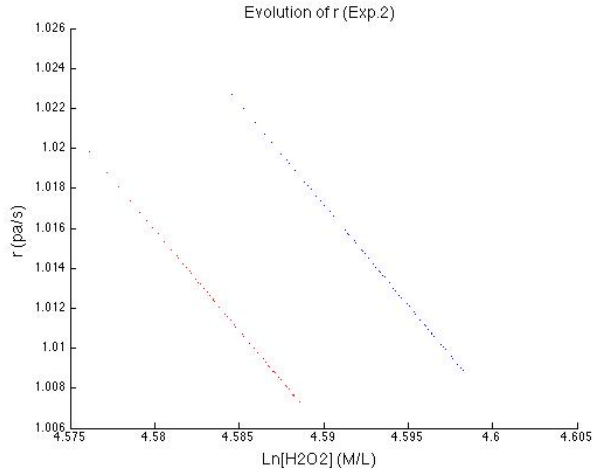

FIG. 5: Evolution of r for experiment 2 of section B of experiment. H_2O_2 .5%

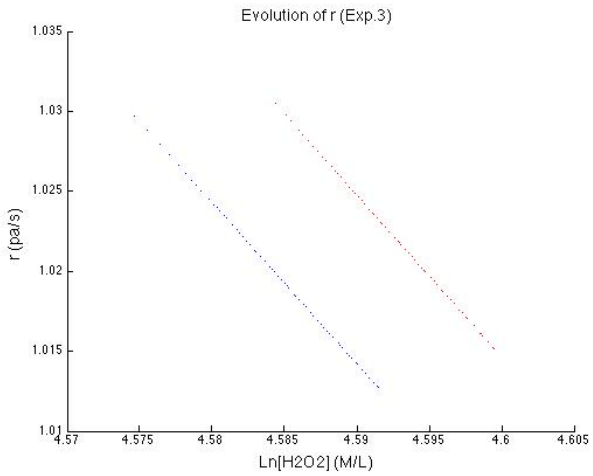

FIG. 6: Evolution of r for experiment 3 of section B of experiment. H_2O_2 1%

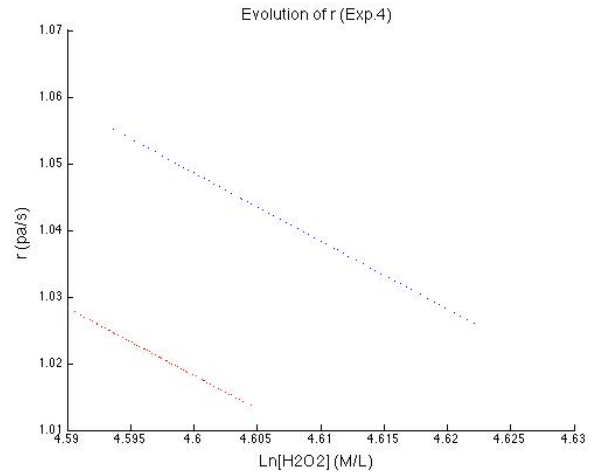

FIG. 7: Evolution of r for experiment 4 of section B of experiment.H_2O_2 1.5%

IV. DISCUSSION OF RESULTS

Catalase and hydrogen peroxide concentration effects are show during section A and B. Behavior of pressure is seen to increase in a non proportional way compared to increasing values of catalase. This can be explained by the fact that enzymatic aid in peroxidation reaches a limit as the number enzyme active sites becomes way bigger than the number of substrate molecules. [2]

Section c) results show a drastic reduction in peroxidation as the ethanol present during reaction are increased. Oxidation rates reduce to a point where they stop and they're contributed to the complete blocking from ethanol molecules as they structurally bind to catalase, partially or totally reducing H_2O_2 enzymatic interactions. [11]

The higher the concentration of ethanol is, the greater the decomposition finds itself inhibited. The rate of reaction decreases. Seen in FIG 3.
A least squares fitting was used to find values for k and α. Regression models show high resemblance to data measured during the experiment. H_2O_2 reduces to water and oxygen continually, and therefore the rate of reaction also reduces, describing a degradation event that is every time slower and in a logarithmic fashion. Therefore is useful to plot results for the rate of reaction in a logarithmic scale and expecting linear behavior (see figures 4 through 7 and equation 1 and 3). Each of the figures show two lines that correspond to the samples for each change in concentration of hydrogen peroxide. Difference between samples (horizontal translations) of the same concentration is related to experimental procedure variables such as fluctuations within the quantities composing the solution, mixing process, or the time when measurement started relative to the time that degradation event started.

V. CONCLUSION

In this laboratory experiment the decomposition rate of hydrogen peroxide was determined through the use of pressure as a variable. Using the LoggerPro application and dedicated specialized hardware it was possible to monitor the build-up of oxygen pressure inside a fixed volume vessel. Different combinations of hydrogen peroxide, catalase and ethanol were created in order to observe, write down and analyze the variations.

Through the utilization of easy-to-use software and plug-and-play hardware it was possible to do successive, fast repetitions of the experiment; which in turn allowed to the collection and storage of a relatively large volume of data. The usual paper-and-pencil approach of data collection pales in comparison to the benefits in versatility, speed and thoroughness offered by these modern technologies.

VI. REFERENCES

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