

# RE5A: Hydrogen Peroxide Decomposition by Baker's Yeast

Group B5

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TKP4110 - Chemical Reaction Engineering

January 9, 2022

## 1 Introduction

Across several generations, baker's yeast has been used for baking and in fermentation processes.<sup>[1]</sup> The purpose of this experiment was to experience how baker's yeast works as a catalytic microfactory when it converts hydrogen peroxide to oxygen and water. The dependence of the rate of the  $\text{H}_2\text{O}_2$  concentration was analyzed by mixing diluted  $\text{H}_2\text{O}_2$  with a yeast solution, using different concentrations of  $\text{H}_2\text{O}_2$  while keeping the concentration of baker's yeast constant. The data was analyzed, and a Lineweaver-Burk graph was made to determine the Michaelis-Menten parameters.

## 2 Theory

### 2.1 Conversion of $\text{H}_2\text{O}_2$ to Oxygen and Water

Hydrogen peroxide is a very toxic by-product in hydrolysis and dehydrolysis. Enzymes like catalase can convert 40 million  $\text{H}_2\text{O}_2$  to  $\text{O}_2$  and  $\text{H}_2\text{O}$  in only one second. This makes catalase a very strong catalyst for hydrogen peroxide removal in liquids. Unfortunately, these enzymes are very sensitive to changes in their environment. Therefore it is necessary to use microorganisms as microfactories so that other processes can occur simultaneously, and the precise conditions are being controlled less easily. There are two important drawbacks to consider: The catalytic rate of conversion and the presence of other active enzymes.

The catalytic rate of conversion will decrease due to slow diffusion of reactants into, and products out of the cells. The presence of other active enzymes can affect the selectivity towards the wanted product because of other conversion reactions.<sup>[1]</sup> In many commercially available processes use of microorganisms are favoured. This because the microorganisms are at lower costs and easier to use than well-isolated enzymes, which are more selective and expensive.<sup>[1]</sup>

In this experiment, baker's yeast reacts with hydrogen peroxide to convert it to oxygen and water. This is the equation that describes this reaction:



It is necessary to use a low concentration of hydrogen peroxide, for having a safe experiment, and to better control the rate of formation and amount of produced oxygen. Therefore a 3 wt% solution of hydrogen peroxide, and a yeast suspension will be used (instead of e.g. a yeast slurry or yeast paste). This will also give a better control on the rate of formation and formed amount of oxygen. Since the reaction is exothermic, dilution will reduce an increase of temperature and make the reaction near-isothermal.<sup>[1]</sup>

### 2.2 The Batch Reactor

The mole balance for a batch reactor is:

$$F_{A0} - F_A + G_A = \frac{dN_A}{dt} \quad (2.2)$$

where  $F_{A0}$  is the initial flow of substance A in to the system, while  $F_A$  is the flow out of the system. The  $G_A$  shows how much of substance A that has been generated in the reactor.  $\frac{dN_A}{dt}$  shows how much substance that has been accumulated in the system over a short period of time.

In a batch reactor, the flow into the system is the same as the flow out of the system, so  $F_{A0}=F_A$ , so the mole balance can also be written like this:

$$G_A = \frac{dN_A}{dt} \quad (2.3)$$

$G_A$  can also be written like this (where  $r_A$  is the reaction rate):

$$G_A = r_A * V = \frac{dN_A}{dt} \quad (2.4)$$

It is desirable to find the reaction rate, and since the volume is constant ( $V = V_0$ ) we can find it:

$$r_A = \frac{dN_A}{V_0 dt} = \frac{dC_A}{dt} \quad (2.5)$$

where  $C_A$  is the concentration of the substrate A.

## 2.3 The Michaelis-Menten Equation

The Michaelis-Menten equation is a kinetic model used to describe the relation between the reaction rate and the concentration of the substrate and the enzyme in enzymatic reactions<sup>[1]</sup>. The general reaction mechanism for an enzymatic reaction can be expressed as:



The Michaelis-Menten equation gives an expression for the reaction rate,  $v$ ,

$$v = k_2 E_0 \frac{[S]}{K_m + [S]} \quad (2.7)$$

where  $k_2$  is the rate constant for the formation of the product,  $S$  is the substrate and  $K_m$  is the equilibrium constant for the first step of the reaction<sup>[1]</sup>. Defining the parameter  $V_m$  as the maximum rate of the reaction for a given enzyme concentration, the Michaelis-Menten equation becomes:

$$v = V_m \frac{[S]}{K_m + [S]} \quad (2.8)$$

In order to determine the Michaelis-Menten parameters,  $K_m$  and  $V_m$ , from experimental data, it is common to invert the equation:

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m} \frac{1}{[S]} \quad (2.9)$$

Plotting  $\frac{1}{v}$  as a function of  $\frac{1}{[S]}$  gives a Lineweaver-Burk plot, where  $\frac{1}{V_m}$  is the intersection with the y-axis, and  $\frac{K_m}{V_m}$  is the slope of the graph.

## 3 Experimental Procedures

### 3.1 Apparatus

The reaction was run in a 100 mL round-bottom flask, connected to a gas syringe using flexible plastic tubes. The round-bottom flask was connected to a plastic tube using a tube connector as soon as the reaction was initiated<sup>[1]</sup>.

### 3.2 Preliminary Test of the Activity of Yeast

In this experiment, yeast was used as a catalyst. Due to yeast being an organic catalyst, it is unpredictable. Because of this, it is necessary to run a preliminary test to obtain an estimate of the apparent activity of yeast. The reactions will be timed using a stopwatch<sup>[1]</sup>.

The reaction rate was estimated using the following procedure:

A 100 mL yeast suspension was prepared in a 150 mL beaker. 1.2 g of dry yeast was added. The suspension was homogenized before any samples were taken. The set-up was prepared as described above with a 30 mL gas syringe.

8 mL of the homogenized yeast suspension, and 18 mL of distilled water was added to the round-bottom flask. 4 mL of 3 wt%  $\text{H}_2\text{O}_2$  was added, and the system was closed immediately after. The timer was started.

At 10 mL of gas, the timer was stopped and the tube connector was opened. The concentration of  $\text{H}_2\text{O}_2$  in the solution was adjusted until the recorded time was within the 80-120 s interval.

### 3.3 Dependence of the Reaction Rate on $\text{H}_2\text{O}_2$ Concentration

In order to determine the reaction rate dependence on the  $\text{H}_2\text{O}_2$ , the procedure from the preliminary test was repeated, but the concentration of  $\text{H}_2\text{O}_2$  in the round-bottom flask was changed.<sup>[1]</sup> It was anticipated that the reaction produced about 40 % of the maximum theoretical  $V_{\text{O}_2}$  presented in Table A.1, and the size of the gas syringe was determined in order to get the most accurate readings possible for each sample.

A yeast suspension was prepared in a 250 mL volumetric flask. 6.0 g of yeast was added to the flask along with 100 mL of water. The suspension was homogenized before adding the remaining 150 mL. The suspension was homogenized again.

The reactions were run as described in the preliminary test. Using the compositions described in Table A.1. The  $\text{H}_2\text{O}_2$  was added lastly, the tube connector was immediately closed, and the timer was started.

During the reaction, the time at different volume intervals was written down. The intervals changed between different compositions in the round-bottom flask, depending on what gas syringe was used. The reaction was stopped at 40 % of the maximum theoretical  $V_{\text{O}_2}$ , or when the volume in the syringe did not change for 4 minutes. The timer was stopped and the tube connector was opened.

This procedure was repeated for all the compositions in Table A.1, until data from a minimum of two parallels for each composition had been collected.

## 4 Results

### 4.1 Preliminary Tests

Table 4.1 shows the data collected after the preliminary test. Sample no. 2 gave a satisfactory result, and was used to calculate the necessary concentration of yeast in the solution during the main part of the experiment.

**Table 4.1:** Collected data from the preliminary test.

| Sample no. | 3 wt% $\text{H}_2\text{O}_2$<br>[mL] | Distilled $\text{H}_2\text{O}$<br>[mL] | Yeast Suspension<br>[mL] | Time<br>[s] |
|------------|--------------------------------------|----------------------------------------|--------------------------|-------------|
| 1          | 4                                    | 18                                     | 8                        | 194         |
| 2          | 4                                    | 10                                     | 16                       | 103         |

## 4.2 Initial Reaction Rates

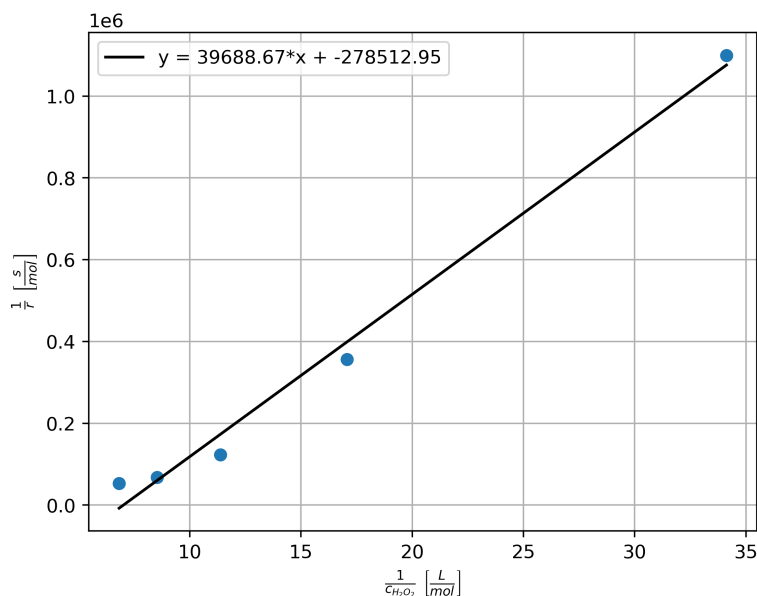
Measurements done during the main part of the experiment are presented in Appendix A.2. Plotting  $V_{O_2}$  as a function of time gave the plots presented in Appendix B. Using linear regression on the three first data points in each parallel, the slope of the line was determined. From the slope, the initial reaction rate was determined, as described in Appendix C.2, using the average of the slopes from each parallel. The slope of the regression lines, and the calculated initial reaction rates are presented in Table 4.2.

**Table 4.2:** The initial reaction rate and the initial rate of formation of oxygen gas for the different samples

| Sample no. | $\frac{dV_{O_2}}{dt}$ , parallel 1<br>[mL s <sup>-1</sup> ] | $\frac{dV_{O_2}}{dt}$ , parallel 2<br>[mL s <sup>-1</sup> ] | $\overline{\frac{dV_{O_2}}{dt}}$<br>[mL s <sup>-1</sup> ] | Initial reaction rate, $r$<br>[mol s <sup>-1</sup> ] |
|------------|-------------------------------------------------------------|-------------------------------------------------------------|-----------------------------------------------------------|------------------------------------------------------|
| 1          | 0.0195                                                      | 0.0256                                                      | 0.0225                                                    | $9.094 \cdot 10^{-7}$                                |
| 2          | 0.0673                                                      | 0.0745                                                      | 0.0696                                                    | $2.813 \cdot 10^{-6}$                                |
| 3          | 0.2254                                                      | 0.1920                                                      | 0.2013                                                    | $8.125 \cdot 10^{-6}$                                |
| 4          | 0.3779                                                      | 0.3420                                                      | 0.3640                                                    | $1.469 \cdot 10^{-5}$                                |
| 5          | 0.4159                                                      | 0.4315                                                      | 0.4661                                                    | $1.882 \cdot 10^{-5}$                                |

## 4.3 Michaelis-Menten Parameters

Figure 4.1 shows the Lineweaver-Burk plot made from the data collected during the experiment. The Michaelis-Menten parameters  $K_m$  and  $V_m$  were determined to be  $-0.142 \text{ mol L}^{-1}$  and  $-3.59 \text{ } \mu\text{mol s}^{-1}$ , respectively.



**Figure 4.1:** The Lineweaver-Burk plot of the experimental data collected during the experiment.

## 5 Discussion

From the data in Table 4.2, it is clear that the initial reaction rate, and the rate of formation of oxygen gas, increase with increasing concentration of  $\text{H}_2\text{O}_2$ . This is due to that the active sites of the enzymes are able to access more substrate with higher concentrations of  $\text{H}_2\text{O}_2$ , which increases the speed of the reaction. The results make it reasonable to assume that  $\text{H}_2\text{O}_2$  is the limiting reactant of the reaction.

$V_m$  is the maximum reaction rate, and  $K_m$  is the equilibrium constant for the formation of the enzyme substrate complex. The Michaelis-Menten parameters  $K_m$  and  $V_m$  were found to be negative, which does not make any physical sense, as this would imply that reaction (2.1) would run from right to left, which does not match the observations made in the laboratory. This means that the Michaelis-Menten equation was an inaccurate model for the experiment.

The biggest source of errors are human errors during the timing and measuring of the formation of oxygen gas. Another source of errors is the setup of the equipment. The syringes might not have been entirely horizontal, and they were assumed to be friction-less, which does not match the observations made during the experiment. It was assumed that the oxygen behaved like an ideal gas, which is impossible, and will have caused some error. All of these sources of errors were constant during the experiment, and because of that, can be neglected, as we measured the relative development of oxygen gas.

## 6 Conclusion

In this experiment yeast has been observed to be an efficient way to decompose hydrogen peroxide into oxygen and water. A higher concentration of  $\text{H}_2\text{O}_2$  gives a higher initial reaction rate, since it allows enzymes to access the substrate, which gives a faster production of oxygen gas. The Michaelis-Menten parameters,  $K_m$  were found to be  $-0.142 \text{ mol L}^{-1}$ , and  $V_m$  were found to be  $-3.59 \mu\text{mol s}^{-1}$  by the Lineweaver-Burk plot. The values are negative, this does not make sense, which implies that the Michaelis-Menten equation is a poor model for this experiment.

Trondheim, January 9, 2022

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## References

- [1] Heinz Preisig. Hydrogen peroxide decomposition by baker's yeast. *Felleslab*, 2021.
- [2] Wiley. *Aylward, G.H and Finlay, SI Chemical Data 7th edt.* Australia, 2014. ISBN 978-0-7303-0246-9.

# Appendices

## A Tables

### A.1 Composition of the Samples During the Main Experiment

Table A.1 shows the compositions of the solution in the round-bottom flask during the main part of the experiment.

**Table A.1:** Composition of the samples used to determine the dependence of the rate on  $\text{H}_2\text{O}_2$  concentration.  $V_{\text{O}_2(\text{g})}(\text{max})$  was calculated using the method described in Appendix C.

| Sample no. | 3 wt% $\text{H}_2\text{O}_2$<br>[mL] | Distilled $\text{H}_2\text{O}$<br>[mL] | Yeast suspension<br>[mL] | $V_{\text{O}_2(\text{g})}(\text{max})$<br>[mL] |
|------------|--------------------------------------|----------------------------------------|--------------------------|------------------------------------------------|
| 1          | 1                                    | 14                                     | 15                       | 9.8                                            |
| 2          | 2                                    | 13                                     | 15                       | 19.7                                           |
| 3          | 3                                    | 12                                     | 15                       | 29.5                                           |
| 4          | 4                                    | 11                                     | 15                       | 39.3                                           |
| 5          | 5                                    | 10                                     | 15                       | 49.2                                           |

### A.2 Data Collected During the Main Experiment

Table A.2 shows the data that was collected from the first sample, using 1 mL 3 %  $\text{H}_2\text{O}_2$ :

**Table A.2:** Data collected from sample 1

| Volume $\text{O}_2(\text{g})$<br>[mL] | Time used in Parallel 1<br>[s] | Time used in Parallel 2<br>[s] |
|---------------------------------------|--------------------------------|--------------------------------|
| 1                                     | 26                             | 28                             |
| 1.4                                   | 45                             | 41                             |
| 1.8                                   | 67                             | 59                             |
| 2.2                                   | 140                            | 120                            |

Table A.3 shows the data that was collected from the second sample, using 2 mL 3 %  $\text{H}_2\text{O}_2$ :

**Table A.3:** Data collected from sample 2

| Volume $\text{O}_2(\text{g})$<br>[mL] | Time used in Parallel 1<br>[s] | Time used in Parallel 2<br>[s] |
|---------------------------------------|--------------------------------|--------------------------------|
| 1                                     | 14                             | 13                             |
| 1.5                                   | 22                             | 19                             |
| 2                                     | 30                             | 26                             |
| 2.5                                   | 36                             | 33                             |
| 3                                     | 45                             | 43                             |
| 3.5                                   | 55                             | 61                             |

Table A.4 shows the data that was collected from the third sample, using 3 mL 3 %  $\text{H}_2\text{O}_2$ :

**Table A.4:** Data collected from sample 3

| Volume $\text{O}_2(\text{g})$<br>[mL] | Time used in Parallel 1<br>[s] | Time used in Parallel 2<br>[s] |
|---------------------------------------|--------------------------------|--------------------------------|
| 1                                     | 10                             | 8                              |
| 2                                     | 14                             | 14                             |
| 3                                     | 19                             | 19                             |
| 4                                     | 22                             | 24                             |
| 5                                     | 28                             | 29                             |
| 6                                     | 36                             | 36                             |
| 7                                     | 44                             | 44                             |
| 8                                     | 55                             | 54                             |
| 9                                     | 68                             | 68                             |
| 10                                    | 88                             | 92                             |
| 11                                    | 111                            | 118                            |
| 12                                    | 140                            | 150                            |

Table A.5 shows the data that was collected from the fourth sample, using 4 mL 3 %  $\text{H}_2\text{O}_2$ :

**Table A.5:** Data collected from sample 4

| Volume $\text{O}_2(\text{g})$<br>[mL] | Time used in Parallel 1<br>[s] | Time used in Parallel 2<br>[s] |
|---------------------------------------|--------------------------------|--------------------------------|
| 2                                     | 9                              | 8                              |
| 3                                     | 12                             | 11                             |
| 4                                     | 14                             | 14                             |
| 5                                     | 16                             | 16                             |
| 6                                     | 20                             | 20                             |
| 7                                     | 24                             | 24                             |
| 8                                     | 28                             | 28                             |
| 9                                     | 32                             | 32                             |
| 10                                    | 36                             | 36                             |
| 11                                    | 43                             | 42                             |
| 12                                    | 50                             | 48                             |
| 13                                    | 58                             | 56                             |
| 14                                    | 71                             | 66                             |
| 15                                    | 85                             | 80                             |
| 16                                    | 106                            | 98                             |

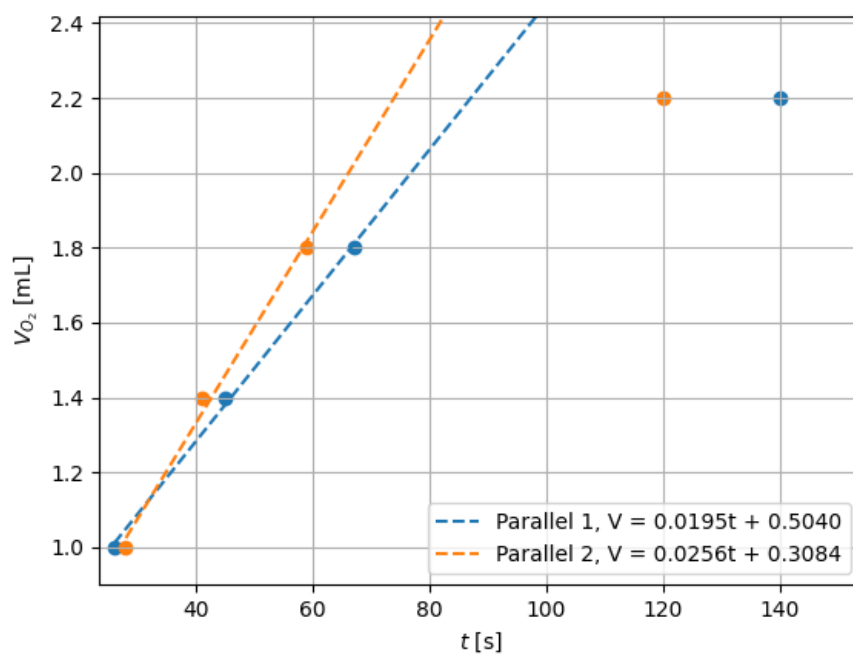
Table A.6 shows the data that was collected from the fifth sample, using 5 mL 3 %  $\text{H}_2\text{O}_2$ :

**Table A.6:** Data collected from sample 5

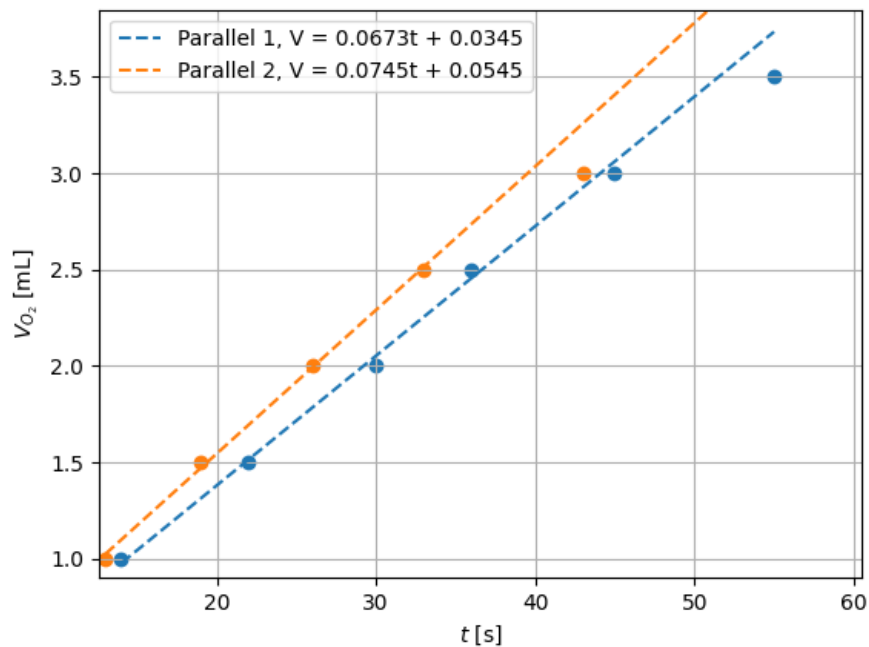
| Volume O <sub>2</sub> (g)<br>[mL] | Time used in Parallel 1<br>[s] | Time used in Parallel 2<br>[s] |
|-----------------------------------|--------------------------------|--------------------------------|
| 2                                 | 7                              | 9                              |
| 4                                 | 11                             | 12                             |
| 6                                 | 16                             | 17                             |
| 8                                 | 21                             | 22                             |
| 10                                | 26                             | 27                             |
| 12                                | 33                             | 33                             |
| 14                                | 42                             | 41                             |
| 16                                | 52                             | 51                             |
| 18                                | 69                             | 67                             |
| 20                                | 97                             | 93                             |

## B Plots

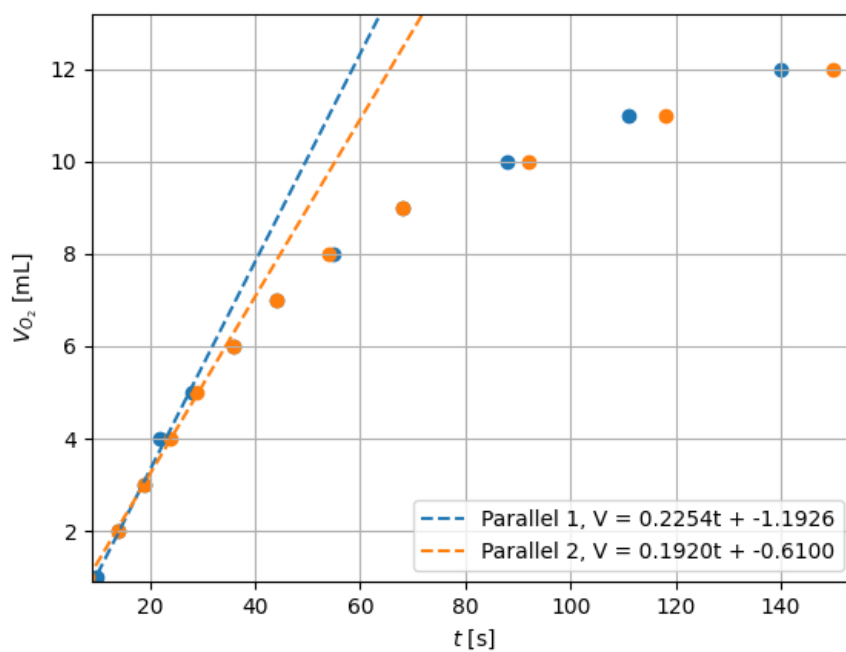
Figures B.1-B.5 shows the volume of oxygen gas generated during the experiment as a function of time for the different samples, in two parallels.



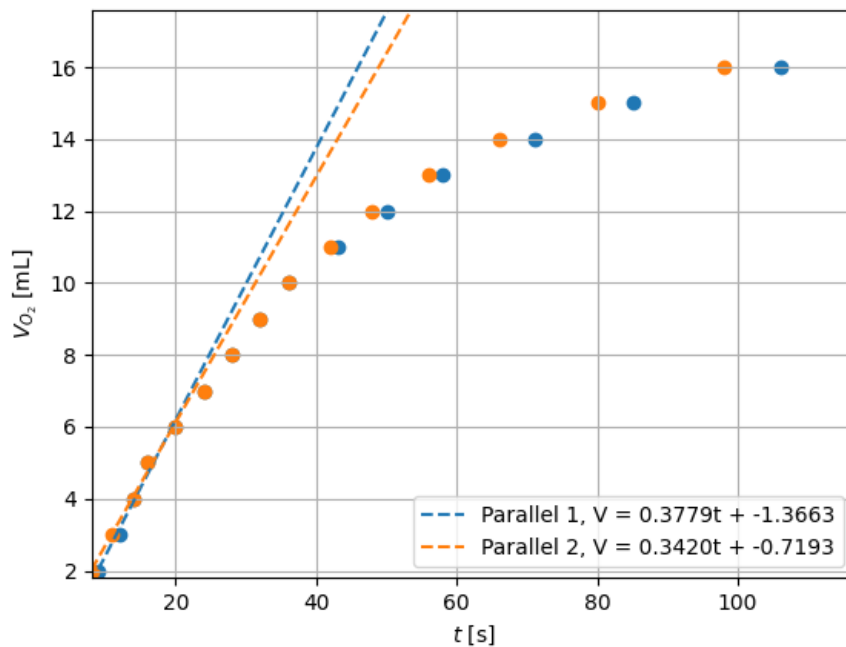
**Figure B.1:** The generated volume of oxygen gas plotted as a function of time for sample 1.



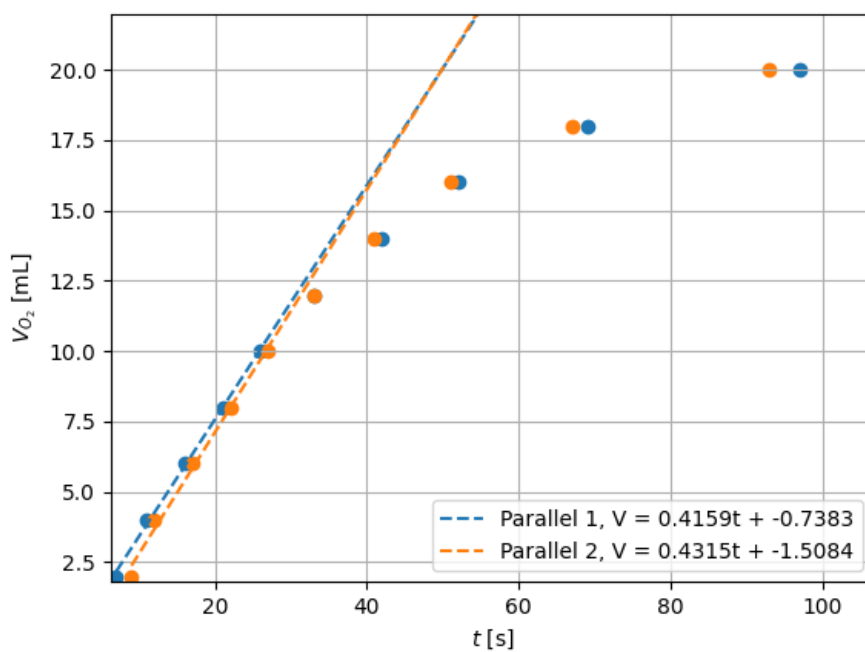
**Figure B.2:** The generated volume of oxygen gas plotted as a function of time for sample 2.



**Figure B.3:** The generated volume of oxygen gas plotted as a function of time for sample 3.



**Figure B.4:** The generated volume of oxygen gas plotted as a function of time for sample 4.



**Figure B.5:** The generated volume of oxygen gas plotted as a function of time for sample 5.

## C Calculations

Table C.1 shows physical data used in the calculations.

**Table C.1:** Physical data used in the calculations<sup>[2]</sup>.

| Component                     | $M_m$ [g mol <sup>-1</sup> ] | $\rho$ [g mL <sup>-1</sup> ] |
|-------------------------------|------------------------------|------------------------------|
| H <sub>2</sub> O <sub>2</sub> | 34.0                         | -                            |
| H <sub>2</sub> O              | -                            | 0.997                        |
| O <sub>2</sub>                | 32.00                        | 1.43 * 10 <sup>-3</sup>      |

### C.1 The Maximum Theoretical Volume of Oxygen Gas

In order to estimate the needed size of the gas syringe and to find proper volume intervals between measurements, an estimate of the volume of oxygen gas formed,  $V_{O_2(g)}$ , is needed. Calculating the maximal theoretical  $V_{O_2(g)}$  will give an upper bound. Though the values measured during the experiment are expected to be lower due to using yeast as a catalyst. The following assumptions were made during the calculations:

- Ideal gas
- Room temperature in the system
- The amount of oxygen gas dissolving into the liquid can be neglected
- The reaction produces no by-products
- The densities of water and hydrogen peroxide are equal ( $\rho_{H_2O} = \rho_{H_2O_2}$ )

The mass of hydrogen peroxide,  $m_{H_2O_2}$ , added to the round-bottom flask can be found using the following equation:

$$m_{H_2O_2} = V_{H_2O_2} 0.03 * \rho_{H_2O} \quad (C.1)$$

The amount of moles of any molecule  $i$ ,  $n_i$ , can be found from

$$n_i = \frac{m_i}{M_i} \quad (C.2)$$

where  $M_i$  is the molar mass of the molecule. Furthermore, the molar balance from Reaction (2.1) gives

$$n_{O_2} = \frac{n_{H_2O_2}}{2} \quad (C.3)$$

The volume of gas for any molecule  $i$ ,  $V_i$  can be found using

$$V_i = \frac{m_i}{\rho_i} \quad (C.4)$$

Using equations (C.1)-(C.4), an expression for the maximal theoretical volume of oxygen gas produced during the experiment can be found:

$$V_{O_2} = \frac{M_{O_2} * V_{H_2O_2} \rho_{H_2O} 0.03}{2 * M_{H_2O_2} * \rho_{O_2}} \quad (C.5)$$

Using the data from Table C.1, For  $V_{\text{H}_2\text{O}_2} = 4 \text{ mL}$ ,

$$V_{\text{O}_2} = \frac{32.00 \text{ g mol}^{-1} * 4 \text{ mL} * 0.997 \text{ g mL}^{-1} * 0.03}{2 * 34.02 \text{ g mol}^{-1} * 1.43 * 10^{-3} \text{ g mL}^{-1}} = 39.3 \text{ mL}$$

## C.2 The Initial Reaction Rate

From linear regression of the first 3 points used to plot the volume of formed oxygen gas,  $\frac{dV_{\text{O}_2}}{dt}$  was obtained.

The reaction rate of oxygen is given by

$$r_{\text{O}_2} = \frac{dN_{\text{O}_2}}{dt} \quad (\text{C.6})$$

Assuming ideal gas,

$$r_{\text{O}_2} = \frac{d\left(\frac{pV}{RT}\right)}{dt} = \frac{dV_{\text{O}_2}}{dt} \frac{p}{RT} \quad (\text{C.7})$$

Using rate laws, the reaction rate of the reaction is obtained,

$$r = \frac{r_{\text{O}_2}}{1} = \frac{dV_{\text{O}_2}}{dt} \frac{p}{RT} \quad (\text{C.8})$$

Assuming  $T = 298 \text{ K}$  and  $p = 1 \text{ bar}$ . For sample 4, using  $\overline{\frac{V_{\text{O}_2}}{dt}}$  (from Table 4.2), the reaction rate becomes,

$$r = 0.3640 \text{ mL s}^{-1} \frac{1 \text{ bar}}{8.314 * 10^{-2} \text{ L bar K}^{-1} \text{ mol}^{-1} 298 \text{ K}} = 1.469 * 10^{-5} \text{ mol s}^{-1}$$

## C.3 The Concentration of Hydrogen Peroxide in the Reaction

Making the same assumptions as in Appendix C.1, using equation (C.1) and equation (C.2), the number of moles of hydrogen peroxide is given by:

$$N_{\text{H}_2\text{O}_2} = \frac{0.03 * V_{\text{H}_2\text{O}_2} \rho_{\text{H}_2\text{O}}}{M_{\text{H}_2\text{O}_2}} \quad (\text{C.9})$$

Dividing by the total volume of the solution used in the round bottom flask gives the concentration of  $\text{H}_2\text{O}_2$

$$c_{\text{H}_2\text{O}_2} = \frac{0.03 * V_{\text{H}_2\text{O}_2} \rho_{\text{H}_2\text{O}}}{M_{\text{H}_2\text{O}_2} V_{\text{rx}}} \quad (\text{C.10})$$

The total volume of the solution,  $V_{\text{rx}} = 30 \text{ mL}$ . The concentration of hydrogen peroxide in sample 4, with 4 mL 3 wt%  $\text{H}_2\text{O}_2$  added is

$$c_{\text{H}_2\text{O}_2} = \frac{0.03 * 4 \text{ mL} * 0.997 \text{ g mL}^{-1}}{34.02 \text{ g mol}^{-1} * 30 \text{ mL}} = 0.117 \text{ mol L}^{-1} \quad (\text{C.11})$$

## D Answers to questions in the experiment description

Most of the reactions showed a small delay before the evolution of oxygen gas. There are many reasons for the delay. One of them is that there was not any oxygen in the solution in the beginning. When the reaction begins, some of the oxygen dissolves into the solution instead of escaping as gas through the tube - which could have caused the delay. If the reaction bottle was not stirred properly, there would be a delay before the  $\text{H}_2\text{O}_2$  is completely mixed in the solution.

Every time the system was closed prior to starting the measurement, a small amount of air was trapped.<sup>[1]</sup> It was impossible to start the timer at the exact same time for the different parallels, and it was difficult to read the gas syringe accurately. However, determining the initial rates will not be affected by this. The trapped air will not change throughout all the samples. So it has the same effect on all of the data and can be ignored. Since it was the same person who started and read the times on the stopwatch, the human factor is irrelevant.

It is better to measure time at regular volume intervals and not the opposite, because if we would have measured the volume at a regular time, the volumes would be difficult to read off the syringe. Because of that it is much simpler to read the time off at an exact volume.

It is favorable to keep the total liquid volume constant. If the volume is constant, the quantity of oxygen that will dissolve into the solution stays the same throughout all the samples, and will have a constant impact on all the measurements, therefore, it does not disturb the results too much, as the data is analyzed using relative changes, and not absolute changes.

## E Python Code

### E.1 Volume of Generated Oxygen Gas as a Function of Time

Below is the code used to plot  $V_{O_2}$  as a function of time, and to determine  $\frac{dV_{O_2}}{dt}$  using linear regression.

```
import numpy as np
import matplotlib.pyplot as plt

#The function collects the data from the datafiles.
def import_experimental_data(datafile1, datafile2):
    V1, t1 = np.loadtxt(datafile1, usecols=(0, 1), skiprows=1, unpack=True)
    V2, t2 = np.loadtxt(datafile2, usecols=(0, 1), skiprows=1, unpack=True)
    return V1, t1, V2, t2

def make_regression_list(datalist, n_regression):
    return np.array([datalist[i] for i in range(n_regression)])

#The function plots the data from the datafiles. n_regression is how many datapoints will be used in
#the linear regression. This is decided by looking at when the points stops being linear.
def plot_initial_rates_from_experimental_data(datafile1, datafile2, n_regression, samplenummer):
    V1, t1, V2, t2 = import_experimental_data(datafile1, datafile2)
    #Extract the values to be used for linear regression
    V1regression = make_regression_list(V1, n_regression)
    t1regression = make_regression_list(t1, n_regression)
    V2regression = make_regression_list(V2, n_regression)
    t2regression = make_regression_list(t2, n_regression)
    #Linear regression
    params1, cov1 = np.polyfit(t1regression, V1regression, 1, cov=True)
    params2, cov2 = np.polyfit(t2regression, V2regression, 1, cov=True)
    #Plot the results
    plt.figure()
    plt.scatter(t1, V1)
    plt.scatter(t2, V2)
    plt.plot(t1, t1 * params1[0] + params1[1], linestyle="--",
             label = f"Parallel 1, V = {params1[0]:.4f}t + {params1[1]:.4f}")
    plt.plot(t2, t2 * params2[0] + params2[1], linestyle="--",
             label = f"Parallel 2, V = {params2[0]:.4f}t + {params2[1]:.4f}")
    plt.xlim(min(t1)*0.9, max(t1)*1.1)
    plt.ylim(min(V1*0.9), max(V1)*1.1)
    plt.xlabel(r'$t$ [s]')
    plt.ylabel(r'$V_{O_2}$ [mL]')
    plt.grid()
    plt.legend()
    plt.show()
    #plt.savefig(f"Sample{samplenummer}")
    return samplenummer, params1[0], params2[0]

r1 = plot_initial_rates_from_experimental_data("Datafiles/Sample1_1.txt", "Datafiles/Sample1_2.txt", 3, 1)
r2 = plot_initial_rates_from_experimental_data("Datafiles/Sample2_1.txt", "Datafiles/Sample2_2.txt", 3, 2)
r3 = plot_initial_rates_from_experimental_data("Datafiles/Sample3_1.txt", "Datafiles/Sample3_2.txt", 3, 3)
r4 = plot_initial_rates_from_experimental_data("Datafiles/Sample4_1.txt", "Datafiles/Sample4_2.txt", 3, 4)
r5 = plot_initial_rates_from_experimental_data("Datafiles/Sample5_1.txt", "Datafiles/Sample5_2.txt", 3, 5)

r_array = np.array([r1, r2, r3, r4, r5])

np.savetxt("Datafiles/Reactionrates.txt", r_array, fmt='%.18e', delimiter=' ', newline='\n', header='',
          footer='', comments='# ', encoding=None)
```

## E.2 Lineweaver-Burk Plot and Michaelis-Menten Parameters

Below is the code used to create the Lineweaver-Burk plot and determine the Michaelis-Menten parameters.

```
import numpy as np
import matplotlib.pyplot as plt

R = 0.08314 #  $L \cdot \text{bar} / K \cdot \text{mol}$ 
T = 298 #  $K$ 
M = 34.02 #  $g / \text{mol}$ 
Vrx = 30 #  $\text{mL}$ 
wt = 0.03
rho = 0.997 #  $g / \text{mL}$ 
p = 1 #  $\text{bar}$ 

VH2O2, dVdt1, dVdt2 = np.loadtxt("Datafiles/Reactionrates.txt",
                                   usecols=(0, 1, 2), unpack=True) #  $\text{mL}$ ,  $\text{mol/s}$ ,  $\text{mol/s}$ 

r1 = dVdt1*10**(-3)*(p/(R*T)) #  $10^{**(-3)}$  is to convert from  $\text{mL}$  to  $L$ 
r2 = dVdt2*10**(-3)*(p/(R*T))
r = (r1+r2)/2

c_1 = VH2O2*wt*rho/(M*Vrx)*1000
x = 1/c_1
y = 1/r

params = np.polyfit(x, y, 1)
plt.scatter(x, y)
plt.plot(x, params[0]*x + params[1], c="black", label=f"y = {params[0]:.2f}*x + {params[1]:.2f}")
plt.grid()
plt.xlabel(r' $\frac{1}{c_{H_2O_2}}$  $ $\left[\frac{L}{\text{mol}}\right]$')
plt.ylabel(r' $\frac{1}{r}$  $ $\left[\frac{s}{\text{mol}}\right]$')
plt.legend()
plt.savefig('MMplot', dpi=400)
#plt.show()

#Finding the Michaelis-Menten parameters
Vm = 1/params[1]
Km = params[0]*Vm

print(f"Vm = {Vm} [mol/s]")
print(f"Km = {Km} [mol/L]")
```

## F Labjournal

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- Preliminary test

| Measurement number | 1   | 2   | 3 | 4 | 5 |
|--------------------|-----|-----|---|---|---|
| Time [s]           | 194 | 103 |   |   |   |
| $V_{O_2}$ (g) [mL] | 10  | 10  |   |   |   |

Double the concentration  
 $\Rightarrow 2.4 \text{ g} / 100 \text{ mL}$   
 $\Rightarrow 6.0 \text{ g} / 250 \text{ mL}$

- Sample 1 - Parallell 1

| Measurement number | 1  | 2   | 3   | 4   | 5   | 6 |
|--------------------|----|-----|-----|-----|-----|---|
| Time               | 26 | 45  | 67  | 140 |     |   |
| $V_{O_2}$          | 1  | 1.4 | 1.8 | 2.2 | 2.4 |   |

- Sample 1 - Parallell 2

| Measurement number | 1  | 2   | 3   | 4   | 5   |
|--------------------|----|-----|-----|-----|-----|
| Time               | 28 | 41  | 59  | 120 |     |
| $V_{O_2}$          | 1  | 1.4 | 1.8 | 2.2 | 2.4 |

- Sample 2 - Parallell 1

| Measurement number | 1  | 2   | 3  | 4   | 5  | 6   |
|--------------------|----|-----|----|-----|----|-----|
| Time               | 14 | 22  | 30 | 36  | 45 | 55  |
| $V_{O_2}$          | 1  | 1.5 | 2  | 2.5 | 3  | 3.5 |

~~- Sample 2 - Parallell 2~~

| Measurement number | 1  | 2   | 3  | 4   | 5  | 6   | 7  | 8   | 9  | 10  |
|--------------------|----|-----|----|-----|----|-----|----|-----|----|-----|
| Time               | 10 | 15  | 19 | 25  | 30 | 35  | 43 | 52  | 75 | 98  |
| $V_{O_2}$          | 1  | 1.5 | 2  | 2.5 | 3  | 3.5 | 4  | 4.5 | 5  | 5.5 |

- Sample 3 - Parallell 1

| Measurement number | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11  | 12  |
|--------------------|----|----|----|----|----|----|----|----|----|----|-----|-----|
| Time               | 10 | 14 | 19 | 22 | 28 | 36 | 44 | 55 | 68 | 88 | 111 | 140 |
| $V_{O_2}$          | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11  | 12  |

- Sample 3 - Parallell 2

| Measurement number | 1 | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11  | 12  |
|--------------------|---|----|----|----|----|----|----|----|----|----|-----|-----|
| Time               | 8 | 14 | 19 | 24 | 29 | 36 | 44 | 54 | 68 | 92 | 118 | 150 |
| $V_{O_2}$          | 1 | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11  | 12  |

- Sample 4 - Parallel 1

|                    |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
|--------------------|---|----|----|----|----|----|----|----|----|----|----|----|----|----|
| measurement number | 1 | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 |
| Time               | 9 | 12 | 14 | 16 | 20 | 24 | 28 | 32 | 36 | 43 | 50 | 58 | 71 | 85 |
| Vol                | 2 | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 |

- Sample 4 - Parallel 2

|                    |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|--------------------|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Measurement number | 1 | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Time               | 8 | 11 | 14 | 16 | 20 | 24 | 28 | 32 | 36 | 42 | 48 | 56 | 66 | 80 | 98 |    |
| Vol                | 2 | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 |    |

~~- Sample 5 - Parallel 1~~

|                    |   |    |    |    |    |    |    |    |    |    |    |
|--------------------|---|----|----|----|----|----|----|----|----|----|----|
| Measurement number | 1 | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |    |
| Time               | 6 | 11 | 15 | 19 | 30 |    |    | 38 | 48 | 64 | 90 |
| Vol                | 2 | 4  | 6  | 8  | 10 | 12 | 14 | 16 | 18 | 20 |    |

- Sample 5 - Parallel 2

|                    |   |    |    |    |    |    |    |    |    |    |
|--------------------|---|----|----|----|----|----|----|----|----|----|
| Measurement number | 1 | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |
| Time               | 7 | 11 | 16 | 21 | 26 | 33 | 42 | 52 | 69 | 97 |
| Vol                | 2 | 4  | 6  | 8  | 10 | 12 | 14 | 16 | 18 | 20 |

~~- Sample 1 - Parallel 3~~

|                    |    |    |    |     |    |
|--------------------|----|----|----|-----|----|
| Measurement number | 1  | 2  | 3  | 4   | 5  |
| Time               | 22 | 36 | 64 | 125 |    |
| Vol                | 1  | 14 | 18 | 22  | 24 |

Sample 2 - parallel 3

|                    |    |     |    |     |    |     |   |   |   |
|--------------------|----|-----|----|-----|----|-----|---|---|---|
| Measurement number | 1  | 2   | 3  | 4   | 5  | 6   | 7 | 8 | 9 |
| Time               | 13 | 19  | 26 | 33  | 43 | 61  |   |   |   |
| Vol                | 1  | 1,5 | 2  | 2,5 | 3  | 3,5 | 4 |   |   |

- Sample 5 - Parallel 3

|      |   |    |    |    |    |    |    |    |    |    |
|------|---|----|----|----|----|----|----|----|----|----|
| Time | 9 | 12 | 17 | 22 | 27 | 33 | 41 | 51 | 67 | 93 |
| Vol  | 2 | 4  | 6  | 8  | 10 | 12 | 14 | 16 | 18 | 20 |