RE5: Hydrogen peroxide decomposition by Baker's yeast

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1 Introduction

The goal of this experiment is to gain insight into the kinetics of the degradation of hydrogen peroxide, H_2O_2, by enzymatic reactions inside of the microorganism Saccharomyces cerevisae, commonly known as Baker's yeast [1]. By studying the decomposition reaction in a batch reactor, the Michaelis-Menten parameters of the reaction can be retrieved from a Lineweaver-Burk plot. As H_2O_2 is a toxic compound obtained as a by-product in many reactions in living organisms, it is useful to understand its degradation process. In this experiment, the reaction rate dependency of the H_2O_2 concentration will be examined. Commercially, It is possible to utilize the yeast bacteria's intracellular enzymes for degradation of toxic H_2O_2. It is more practical to use the living organisms as a black box wherein the reaction happens rather than operating with enzymes outside of the cells, due to their dependency of factors as pH and temperature. For studying this reaction, Baker's yeast will be put in solutions with different H_2O_2 concentrations, and the development of O_2 gas over time will give the necessary data to determine the desired parameters.

2 Theory

2.1 Batch reactor

From the general mole balance for a defined system,

$$F_{i0} - F_i + G_i = \frac{dN_i}{dt}, \tag{2.1}$$

where F_{i0} indicates the rate flow of species i into the system, F_i the flow out of the system, G_i the rate of generation of of i and $\frac{dN_i}{dt}$ the rate of accumulation, the design equation of a batch reactor [2]. In a batch reactor, there is no flow in or out. Thus, $F_{i0} = F_i = 0$. With a constant reaction volume V in the reactor, the generation of species i can be written as $G_i = r_i V$ where r_i is the rate of formation for species i given in $\text{molL}^{-1}\text{s}^{-1}$. The mole balance for the system becomes

$$r_i V = \frac{dN_i}{dt}. \tag{2.2}$$

This equation assumes a constant reaction volume and an ideal mixture. If r_i is to be expressed in mols^{-1}, the equation becomes

$$r_i = \frac{dN_i}{dt}. \tag{2.3}$$

2.2 Decomposition of H_2O_2

Hydrogen peroxide is formed as a by-product in many living organisms [3]. This toxic compound is quickly decomposed to oxygen and water by the reaction

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2 \tag{2.4}$$

catalyzed by the enzyme catalase. Since hydrogen peroxide is a thermodynamically unstable compound, it decomposes by the same equation spontaneously. The rate of decomposition depends on factors such as temperature and pH, and increases drastically when exposed to sunlight. As the reaction volume in a batch reactor is constant, the formation rate of oxygen gas, r_{O_2}, can be expressed in mols^{-1} from equation (2.2),

$$r_{O_2} = \frac{dN_{O_2}}{dt}. \tag{2.5}$$

2.3 Michaelis-Menten kinetics

Michaelis-Menten kinetics is a common model for biochemical enzyme kinetics [2]. The model connects reaction rates, concentration of the relevant compounds and rate constants. For the generic enzymatic reaction

$$E + S \rightleftharpoons ES \xrightarrow{k_2} E + P, \tag{2.6}$$

the Michaelis-Menten equation gives the relation

$$r = k_2 E_0 \frac{[S]}{K_m + [S]}. \tag{2.7}$$

Here, S is the substrate, E is the enzyme, E_0 is the total enzyme concentration, k_2 is the rate constant for the reaction where the products are made, r is the reaction rate and K_m is the Michaelis constant. The Michaelis constant is the same as the equilibrium constant for the formation of the enzyme substrate complex [ES] given by

$$K_m = \frac{[ES]}{[E][S]}. \tag{2.8}$$

By introducing the variable $V_m = k_2 E_0$, which denotes the maximum reaction rate when all of the enzymes are occupied by substrates, and inverting equation (2.7), we get

$$\frac{1}{r} = \frac{1}{V_m} + \frac{K_m}{V_m}\frac{1}{[\mathrm{S}]}. \tag{2.9}$$

2.4 Lineweaver-Burk plot

A Lineweaver-Burk plot is constructed by plotting $\frac{1}{[\mathrm{S}]}$ against $\frac{1}{r}$. Seen from equation 2.9, the Michaelis-Menten parameters can be calculated from the resulting plot as the slope of the linear graph is $\frac{K_m}{V_m}$, the intersection with the y-axis is $\frac{1}{V_m}$, and the intersection with the x-axis is $-\frac{1}{K_m}$.

3 Experimental procedures

The experiment consisted of two parts, one preliminary test to determine an optimal yeast concentration, and the main part where the reaction rate of H_2O_2 was to be determined from the evolution of O_2 gas.

3.1 Preliminary test

1.2 g dry yeast was mixed with 100 mL water to a homogeneous suspension. 8 mL of the suspension was mixed with 18 mL distilled water and 4 mL 3wt% H_2O_2, and the produced gas from the reaction was trapped in a low friction gas syringe. The time it took for 10 mL gas to be created was recorded. As long as the recorded time was outside a 80-120s window, the yeast concentration was adjusted until a satisfactory gas evolution rate was obtained.

3.2 Primary experiment

Yeast suspension (250 mL) with the concentration found in the preliminary test was prepared. Yeast suspension, distilled water and H_2O_2 were mixed in five different ratios such that the total reaction volume was 30 mL in each case. The different mixtures are shown in table 3.1. For each of the mixtures, two parallels were run. As in the preliminary test, the time was recorded when the reaction started, and noted at regular volume intervals.

Table 3.1: Volumes used in the different tests

	Test 1	Test 2	Test 3	Test 4	Test 5
Yeast suspension [mL]	15.0	15.0	15.0	15.0	15.0
H_2O_2, 3wt% [mL]	1.0	1.6	2.2	2.8	3.4
H_2O [mL]	14.0	13.4	12.8	12.2	11.6
Total volume V[mL]	30.0	30.0	30.0	30.0	30.0

4 Results

4.1 Preliminary test

As the evolution of gas was faster than desired with the initial concentration of yeast-suspension (10 mL gas produced in 26 s when mixed with 0.12 mmol L^{-1} H_2O_2), the yeast concentration for the main part of the experiment was halved to 0.6 g per 100 mL. This gave 10 mL of gas produced in 118 s when mixed with with 0.12 mmol L^{-1} H_2O_2.

4.2 Main experiment

The collected data from the experiment (volume O_2 versus time) is shown in tables A.1 through A.10. Some of the data points for each parallel were used to construct linear graphs for approximating the derivative $a \approx \frac{dV_{O_2}}{dt}$. The calculated derivatives from the linear regression are shown in table 4.1.

Table 4.1: Calculated slopes from the linear regressions for each parallel

Mixture	$a \approx \frac{dV_{O_2}}{dt}$[mLs^{-1}] for parallel 1	$a \approx \frac{dV_{O_2}}{dt}$[mLs^{-1}] for parallel 2
1	0.009	0.011
2	0.032	0.037
3	0.055	0.057
4	0.093	0.108
5	0.141	0.112

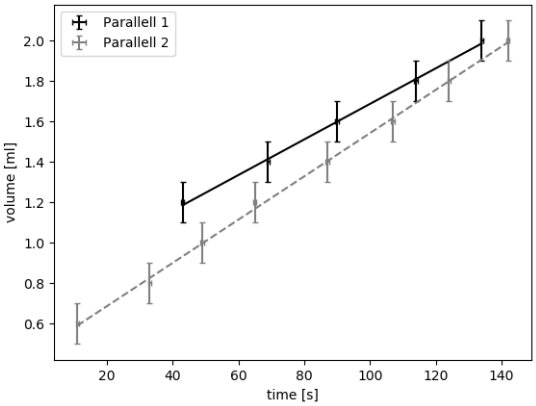

Figure 4.1: Volume O_2[mL] gas produced plotted against time[s] for both parallels of mixture 1

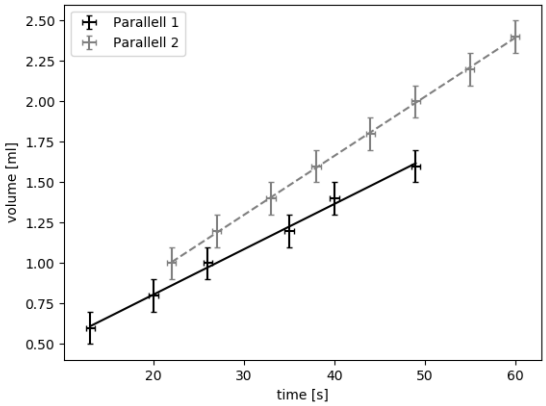

Figure 4.2: Volume O_2[mL] gas produced plotted against time[s] for both parallels of mixture 2

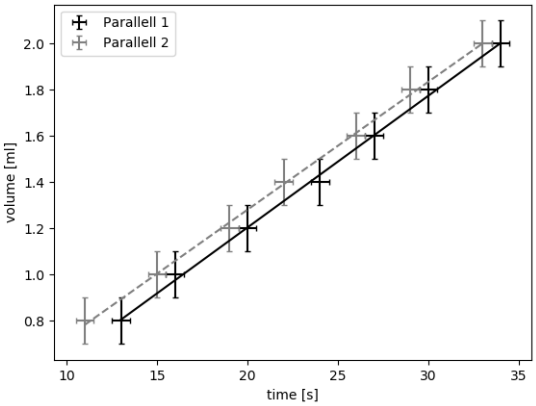

Figure 4.3: Volume O_2[mL] gas produced plotted against time[s] for both parallels of mixture 3

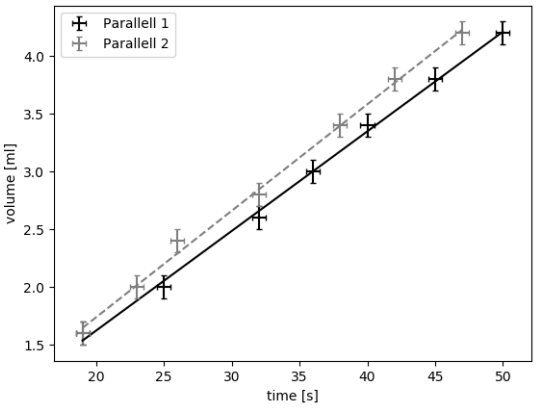

Figure 4.4: Volume O_2[mL] gas produced plotted against time[s] for both parallels of mixture 4

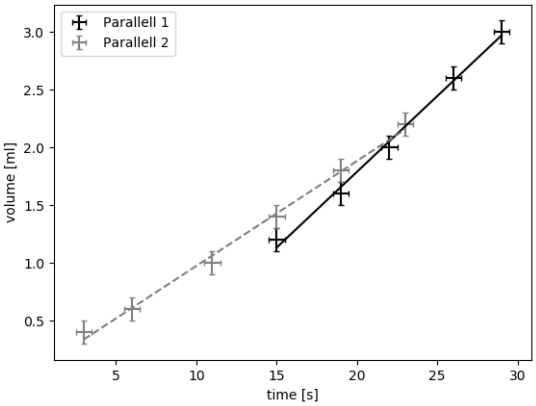

Figure 4.5: Volume O_2[mL] gas produced plotted against time[s] for both parallels of mixture 5

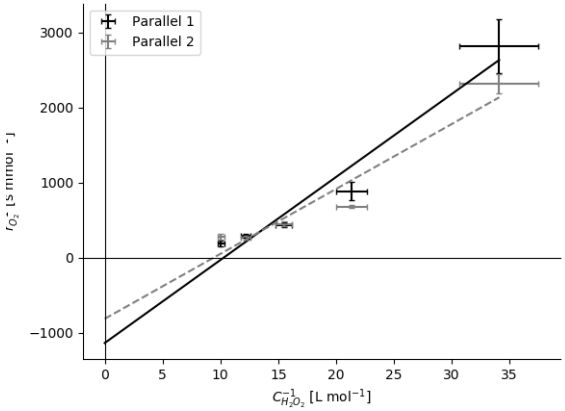

Figure 4.6: A Lineweaver-Burk from all the mixtures using all data points, where parallels 1 and 2 are held separate

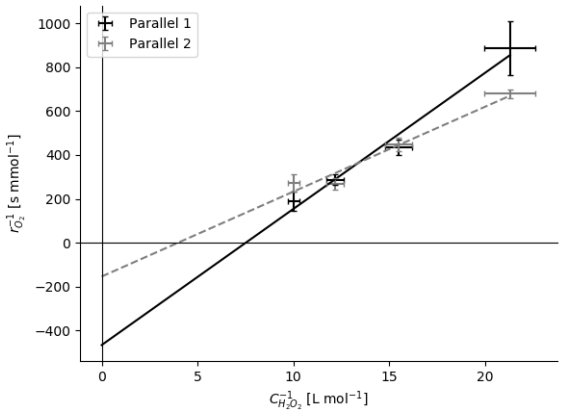

Figure 4.7: A Lineweaver-Burk from all the mixtures without the data point containing the lowest concentration, where parallels 1 and 2 are held separate.

5 Discussion

The most important sources of error in the experiment are assumption of standard temperature and pressure, lack of continuous mixing, the reaction mixture being exposed to light and high relative error in the lower concentration mixtures of H_2O_2. The results obtained from the Lineweaver-Burk plot shown in Figures 4.6 and 4.7 are non-physical as they show a negative maximum rate of reaction. The errors in inverse concentration are very high. As shown in Appendix C, error in the inverse concentration increases with c^{-2}. To illustrate this, there are two Lineweaver-Burk plots in section 4. It is clear that removing the data point containing the lowest H_2O_2 concentration changes the overall graph significantly. It would therefore have been reasonable to work at higher concentrations of H_2O_2 and lower concentrations of yeast-suspension to minimize error while at the same time keeping reaction rate at a reasonable level for making measurements. Lack of mixing in the reactor became especially clear at larger volumes of H_2O_2 solution, as shown in Figures A.1 to A.5. Here, the reaction rate often increased the first seconds after adding H_2O_2 to the reactor. This is likely due to the H_2O_2 solution taking a few seconds to mix with the yeast suspension.

None of the calculations made take into account that H_2O_2 spontaneously decomposes when exposed to light. This is a clear source of error, and there has not been done anything to quantify it. The size of the total calculated error, and the large number of factors not taken into account give a very low confidence in the results. At a given temperature, O_2 gas has a given solubility in water. This means that some of the produced oxygen from the reaction was dissolved in the water rather than collected in the gas syringe. Although, since the solubility

of oxygen in water is constant at a given temperature, and that the reaction mixture didn't have a significant temperature difference for the different H_2O_2 concentrations (even though the decomposition reaction is exothermic), this factor most likely didn't affect the results.
Measures that could be taken to reduce the error are: Measuring temperature and pressure in the lab, continuously mixing the reactor, letting the reaction occur in a dark environment, closing the system before mixing H_2O_2 and yeast and using larger volumes of H_2O_2 solution or more precise equipment for measuring the volume of H_2O_2 added to the reactor.

6 Conclusion

The rate of O_2 production by enzyme catalysed decomposition of H_2O_2 was measured for a series of different concentrations of H_2O_2. This data was used to make a Lineweaver-Burk plot from which it was possible to extract kinetic data for the decomposition-reaction. Due to several factors that were not accounted for beforehand, the calculated kinetic data was nonphysical. If the experiment is to be repeated, measures should be taken to minimize decomposition of H_2O_2 from exposure to light and to better the mixing of the reactor. It would also be reasonable to use larger volumes of H_2O_2 solution or more precise measuring equipment to minimize error in the inverse concentration used in the Lineweaver-Burk plot.

A Measured data

Table A.1: Data table for mixture 1, parallel number 1.

V_{O_2}[mL]	1.0	1.2	1.4	1.6	1.8	2.0
t[s]	27.0	43.0	69.0	90.0	114.0	134.0

Table A.2: Data table for mixture 1, parallel number 2.

V_{O_2}[mL]	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0
t[s]	11.0	33.0	49.0	65.0	87.0	107.0	124.0	142.0

Table A.3: Data table for mixture 2, parallel number 1.

V_{O_2}[mL]	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4
t[s]	4.0	13.0	20.0	26.0	35.0	40.0	49.0	53.0	58.0	64.0	68.0

Table A.4: Data table for mixture 2, parallel number 2.

V_{O_2}[mL]	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4
t[s]	4.0	14.0	22.0	27.0	33.0	38.0	44.0	49.0	55.0	60.0

Table A.5: Data table for mixture 3, parallel number 1.

V_{O_2}[mL]	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4
t[s]	4.0	13.0	16.0	20.0	24.0	27.0	30.0	34.0	38.0	42.0

Table A.6: Data table for mixture 3, parallel number 2.

V_{O_2}[mL]	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.8
t[s]	3.0	11.0	15.0	19.0	22.0	26.0	29.0	33.0	36.0	38.0	44.0

Table A.7: Data table for mixture 4, parallel number 1.

V_{O_2}[mL]	0.6	0.8	1.0	1.6	2.0	2.6	3.0	3.4	3.8	4.2	4.6	5.0
t[s]	4.0	11.0	16.0	19.0	25.0	32.0	36.0	40.0	45.0	50.0	54.0	59.0

Table A.8: Data table for mixture 4, parallel number 2.

V_{O_2}[mL]	0.6	0.8	1.0	1.2	1.6	2.0	2.4	2.8	3.4	3.8	4.2	4.6
t[s]	3.0	8.0	11.0	15.0	19.0	23.0	26.0	32.0	38.0	42.0	47.0	52.0

Table A.9: Data table for mixture 5, parallel number 1.

V_{O_2}[mL]	0.6	0.8	1.0	1.2	1.6	2.0	2.6	3.0	3.6	4.0	4.4	4.8
t[s]	3.0	8.0	11.0	15.0	19.0	22.0	26.0	29.0	35.0	38.0	41.0	44.0

Table A.10: Data table for mixture 5, parallel number 2.

V_{O_2}[mL]	0.4	0.6	1.0	1.4	1.8	2.2	2.8	3.2	3.8	4.2	4.8	5.2
t[s]	3.0	6.0	11.0	15.0	19.0	23.0	26.0	29.0	35.0	39.0	43.0	48.0

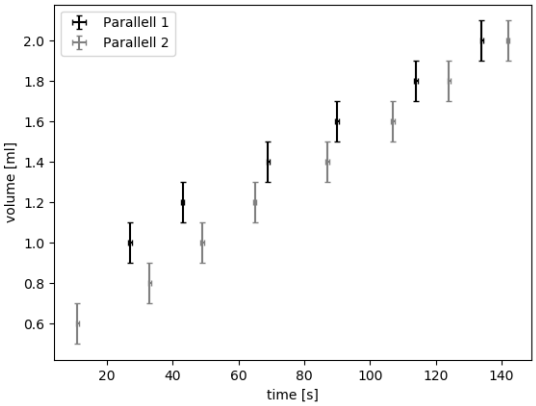

Figure A.1: All measured data points V_{O_2}[mL] versus time [s] for both parallels for mixture 1

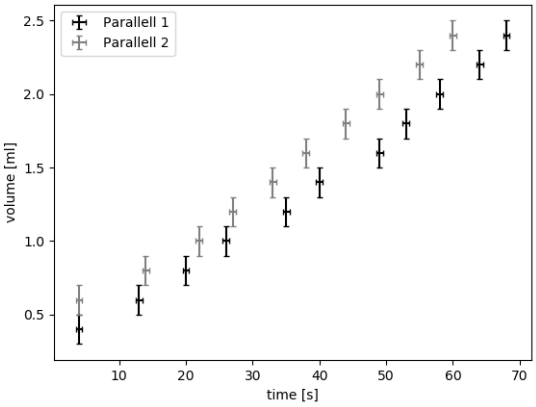

Figure A.2: All measured data points V_{O_2}[mL] versus time [s] for both parallels for mixture 2

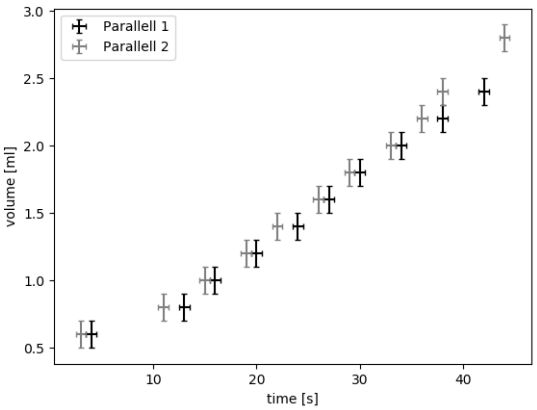

Figure A.3: All measured data points V_{O_2}[mL] versus time [s] for both parallels for mixture 3

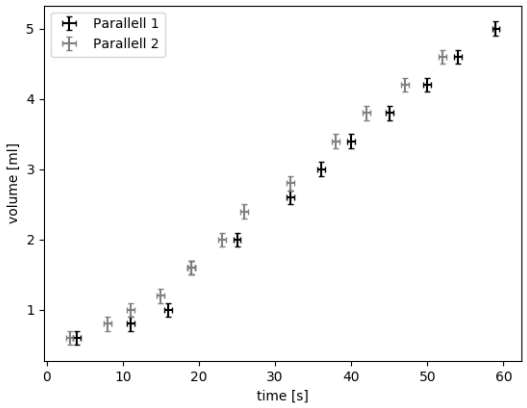

Figure A.4: All measured data points V_{O_2}[mL] versus time [s] for both parallels for mixture 4

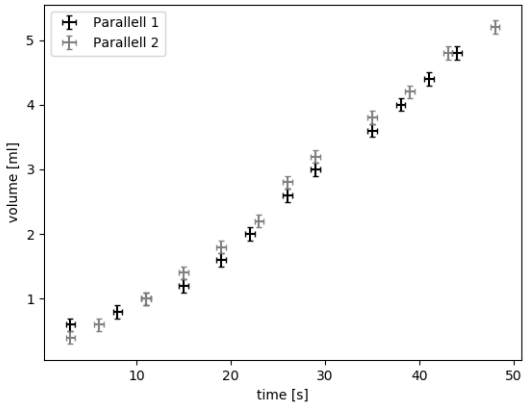

Figure A.5: All measured data points V_{O_2}[mL] versus time [s] for both parallels for mixture 5

B Calculations

B.1 Inverse concentration of H_2O_2

Due to the low concentration of H_2O_2, the density of 3wt% H_2O_2 can be approximated with the density of H_2O. This gives

$$\begin{aligned} \omega_{H_2O_2} &= \frac{m_{H_2O_2}}{m_{H_2O_2} + m_{H_2O}} \\ m_{H_2O_2} &\approx \omega_{H_2O_2} \rho_{H_2O} V_{sol} \\ N_{H_2O_2} &\approx \frac{\omega_{H_2O_2} \rho_{H_2O} V_{sol}}{M m_{H_2O_2}} \end{aligned} \tag{B.1}$$

where ρ_{H_2O} is the density of water, V_{sol} is the volume of the hydrogen peroxide solution, $\omega_{H_2O_2}$ is the weight fraction of H_2O_2 and $Mm_{H_2O_2}$ is the molar mass of hydrogen peroxide.

Table B.1: Values[4] used for calculating $c_{H_2O_2}$

Variable	Value
ρ_{H_2O}	$997\,\mathrm{kg\,m^{-3}}$
$Mm_{H_2O_2}$	$34\,\mathrm{g\,mol^{-1}}$
$\omega_{H_2O_2}$	0.03
R	$8.314\,\mathrm{J\,mol^{-1}\,K^{-1}}$
T	298 K
p_{atm}	1 bar

Because we are only looking at the initial reaction rate, the volume of the liquid in the batch reactor can be assumed to be approximately constant. This gives an inverse concentration of H_2O_2

$$c^{-1} = \frac{V_{rx}}{N_{H_2O_2}}. \tag{B.2}$$

Inserting the expression for $N_{H_2O_2}$ in equation B.1 yields

$$c^{-1} = \frac{M m_{H_2O_2} V_{rx}}{\omega_{H_2O_2} \rho_{H_2O} V_{sol}}. \tag{B.3}$$

Inserting the volumes in the different mixtures gives the inverse concentrations shown in table B.2. Calculation of errors is shown in Appendix C.

Mixture	V_{sol} [mL]	c^{-1} [L mol^{-1}]
1	1.0 ± 0.1	34.1 ± 3.41
2	1.6 ± 0.1	21.31 ± 1.33
3	2.2 ± 0.1	15.5 ± 0.71
4	2.8 ± 0.1	12.18 ± 0.44
5	3.4 ± 0.1	10.03 ± 0.3

Table B.2: Inverse concentrations with calculated error

B.2 Formation rate of O_2

From a set of the collected data points of each parallel, a linear regression method in Python was used to obtain an approximation of $dV_{O_2}/dt = a$. By assuming the ideal gas law holds for the produced gas,

$$pV = nRT, \tag{B.4}$$

that the trapped gas in the syringe was in mechanical equilibrium with the surrounding air (i.e. $p_{O_2} = p_{\text{atm}}$), and using equation 2.3, we get

$$\frac{1}{r_{O_2}} = \frac{dt}{dN_{O_2}} = \frac{dt}{d\left(\frac{pV_{O_2}}{RT}\right)} = \frac{RT}{pa}. \tag{B.5}$$

C Error analysis

Error propagation from measured to calculated values was calculated with Gauss' error propagation law, given by

$$\Delta f(x_1, x_2, ..., x_n) = \sqrt{\sum_{i=1}^{n} \left(\frac{\partial f}{\partial x_i}\Delta x_i\right)^2}, \tag{C.1}$$

where f is a function of x_n, Δf is the error in f and Δx_i is the error in x_i. With inverse concentration of H_2O_2 calculated as shown in Appendix B, this gives an error in $\frac{1}{c}$ given by

$$\begin{aligned} \Delta c^{-1} &= \sqrt{\left(\frac{\partial c^{-1}}{\partial V_{\text{rx}}}\Delta V\right)^2 + \left(\frac{\partial c^{-1}}{\partial V_{\text{sol}}}\Delta V\right)^2} \\ \Delta c^{-1} &= \frac{M m_{H_2O_2}}{\omega_{H_2O_2}\rho_{H_2O}}\Delta V\sqrt{\left(\frac{1}{V_{\text{sol}}}\right)^2 + \left(\frac{V_{\text{rx}}}{V_{\text{sol}}^2}\right)^2} \end{aligned} \tag{C.2}$$

The volumes of 3wt% H_2O_2 in the different parallels and their corresponding errors are shown in table B.2.

Temperature and pressure were not measured in the lab, so they have been assumed exactly equal to standard temperature and pressure. This is an obvious source of error, but also one that is hard to quantify. The aim of the following analysis is to show the error resulting from the standard deviation in the slope coefficient from the linear regressions shown in Figures 4.1 through 4.5. Using Gauss' error propagation law on the expression for r^{-1} given in equation (B.5) yields:

$$\begin{aligned} \Delta r^{-1} &= \sqrt{\left(\frac{\partial r^{-1}}{\partial a}\sigma_a\right)^2} \\ \Delta r^{-1} &= \frac{RT}{pa^2}\sigma_a \end{aligned} \tag{C.3}$$

Where a is the slope-coefficient and σ_a is its standard deviation, returned from numpy.polyfit(). Inserting the calculated values and standard deviations for a yields the uncertanties shown in table 4.1.

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