Work Plan - Experiment RE5A, part 2

1 Introduction

The purpose of this experiment is 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 H_2O_2 concentration will be focused on in the experiment.

2 Theory

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

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2 \tag{2.1}$$

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 there will be used a 3 wt% solution of hydrogen peroxide, and just a yeast suspension (instead of e.g. a yeast slurry or yeast paste). Since the reaction is exothermic, dilution will reduce an increase of temperature and make the reaction near-isothermal. [1]

3 Experimental Procedures

3.1 Apparatus

The reaction is run in a 50 mL round-bottom flask, connected to a 30 mL gas syringe using flexible plastic tubes. The round-bottom flask is connected to the plastic tube using a tube connector as the reaction is initiated. [1]

3.2 Preliminary test of the activity of yeast

In this experiment, yeast will be 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. [1]

The following procedure will be used to estimate the reaction rate:

- Prepare a 100 mL yeast suspension in a 150 mL beaker. Add 1.2 g of dry yeast, or 5.0 g per 100 mL of water. Homogenize the suspension before taking samples.
- Before starting the experiment, prepare the set-up as described above.
- Add 8.0 mL of the homogenized yeast suspension, and 18.0 mL of distilled water to the round-bottom flask.
- Add 4.0 mL of 3 wt% H_2O_2 and close the system immediately. Start the timer.
- At 10 mL of gas, stop the timer and open the tube connector.
- The final time should be about 90 seconds. If the time is outside the 80-120 seconds range, the concentration of yeast need to be readjusted until it does.

3.3 Dependence of the rate on H_2O_2 concentration

In order to determine the reaction rate dependence on the H_2O_2, we will repeat the procedure from the preliminary test, but change the concentration of H_2O_2 in the round-bottom flask. [1]

- Prepare a yeast suspension in a 250 mL volumetric flask. Add 2.5 times the amount of yeast determined in the preliminary test. Add 100 mL of water to the yeast. Homogenize the suspension as good as possible before adding the remaining 150 mL. Homogenize the suspension again.
- Prepare the same setup as in the preliminary test, but with a 100 mL gas syringe.
- Run the reactions as described in the preliminary test. Using the compositions described in Table A.2. Add the H_2O_2 last, and immediately close the tube connector and start the timer.
- During the reaction, note down the time at different volume intervals. The intervals will change between different compositions in the round-bottom flask.
- At 10 mL of gas, stop the timer and open the tube connector.
- Repeat for all the compositions in table A.2. Minimum two parallels for each composition.

4 Data analysis

The experimental data will be analyzed in order to estimate the initial reaction rate and find the Michaelis-Menten parameters.

The experiment occurs in a batch reactor. This means that the concentration of hydrogen peroxide changes over time. To estimate the reaction rate, $\frac{dV}{dt}$, the amount of oxygen will be plotted as a function of time. Using linear regression, the initial reaction rate will be approximated for each parallel.

Michaelis-Menten kinetics is an easy kinetic model. Kinetic models determines the relation between the reaction rate, ν, the concentration of the reactant, $[S]$, and product, $[P]$, in addition to intrinsic parameters. This is the version of the Michaelis-Menten that we use in this experiment:

$$\frac{1}{\nu} = \frac{1}{V_m} + \frac{K_m}{V_m} * \frac{1}{[S]} \tag{4.1}$$

The data from the experiment will be plotted in a Lineweaver-Burk plot, that is, $\frac{1}{\nu}$ plotted as a function of $\frac{1}{[S]}$. Using linear regression, we will obtain the Michaelis-Menten parameters, K_m and V_m.[1]

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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.

A Tables

Table A.1 shows what data will be collected during the preliminary test.

Table A.1: Data to be collected from the preliminary test.

Sample no.	3 wt% H_2O_2 $[mL]$	Distilled H_2O $[mL]$	Yeast suspension $[mL]$	Time [s]
1	4	18	8	
2	4	18	8	
3	4	18	8	

Table A.2 shows the compositions of the liquid in the round-bottom flask during the reactions in the main part of the experiment.

Table A.2: Composition of the samples used to determine the dependence of the rate on H_2O_2 concentration. $V_{O_2(g)}$ (max) was calculated using the method described in Appendix B.

Sample no.	3 wt% H_2O_2 $[mL]$	Distilled H_2O $[mL]$	Yeast suspension $[mL]$	$V_{O_2(g)}$ (max) $[mL]$
1	1	14	15	9.9
2	2	13	15	19.7
3	3	12	15	29.6
4	4	11	15	39.5
5	5	10	15	49.3

Table A.3 shows what data will be collected during the main part of the experiment for each parallel.

Table A.3: Data to be collected from the reactions of each parallel described in Table A.2

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

B Calculations

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 byproducts
- 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:

$$m_{H_2O_2} = V_{H_2O_2} * 0.03\,\mathrm{g\,mL^{-1}} \tag{B.1}$$

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

$$n_i = \frac{m_i}{M_i} \tag{B.2}$$

where M i the molar mass of the molecule. Furthermore, the molar balance from Reaction (2.1) gives

$$n_{O_2} = \frac{n_{H_2O_2}}{2} \tag{B.3}$$

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

$$V_i = \frac{m_i}{\rho_i} \tag{B.4}$$

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

$$V_{O_2} = \frac{M_{O_2} * V_{H_2O_2} * 0.03\,\mathrm{g\,mL^{-1}}}{2 * M_{H_2O_2} * \rho_{O_2}} \tag{B.5}$$

Using $M_{O_2} = 32.00\,\mathrm{g\,mol^{-1}}$, $M_{O_2} = 34.02\,\mathrm{g\,mol^{-1}}$ and $\rho_{O_2} = 1.43 * 10^{-3}\,\mathrm{g\,mL^{-1}}$.[2]
For $V_{H_2O_2} = 4\,\mathrm{mL}$,

$$V_{O_2} = \frac{32.00\,\mathrm{g\,mol^{-1}} * 4\,\mathrm{mL} * 0.03\,\mathrm{g\,mL^{-1}}}{2 * 34.02\,\mathrm{g\,mol^{-1}} * 1.43 * 10^{-3}\,\mathrm{g\,mL^{-1}}} = 39.5\,\mathrm{mL}$$

C Health, Safety and Environment

A hazard is something that possibly can cause harm to an object or a person. A risk is the probability to get harmed, and the it is being calculated based on the exposure of the harmful specie/event.

The largest hazard in this experiment is the hydrogen peroxide. This can be corrosive and it is dangerous if it is in contact with eyes, or is being inhaled. The risk of the hazard is low if we use the right protection, and the fume hood. If a harmful event occurs, we will contact the supervisor and follow the protocol that is required for the specific event, and then consider if the doctors or anybody else should be contacted. If chemicals are spilled in the eyes - we will use the eye washing station for several minutes. If chemicals get spilled on a person, the clothes will be removed, and the skin will be washed. In the case of inhaling chemicals, the individual needs to be moved to a well ventilated area for a while. In the case of cuts, it needs to be considered how severe the cut is. If it is a small cut, it needs to be cleaned and disinfected.

The largest risk with this experiment is that the glass tubing of the reactor can explode because of overpressure. This can happen suddenly, and with no warning.

To reduce this risk, the experiment will take place behind a screen, that will absorb the glass if it explodes. It is also important to use a gas syringe that is not too small.

According to the MSDS we have to use safety googles, that is fitting tightly for eye/face protection. For skin protection, we are obligated to use gloves, that has been inspected before they are being used. For body protection we have to use a complete suit that is protecting against chemicals (in our case we just need a lab coat). We are going to use a fume hood, so respiratory protection is not necessary.