Catalase Enzyme Reaction Student Experiment - Eden Tomes

# Rationale:

Cells, the fundamental units of life, depend on a multitude of biochemical reactions for survival and function, collectively known as metabolism (*Biology LibreTexts*, 2016). Many of these essential reactions would occur too slowly to sustain life without biological catalysts called enzymes (*Cooper*, 2000). Enzymes, primarily proteins, accelerate reaction rates by lowering the activation energy. They achieve this by binding to specific reactant molecules, or substrates, at a region called the active site, facilitating their conversion into products (*Robinson*, 2015).

Catalase is a crucial enzyme found in nearly all aerobic organisms, playing a vital protective role. It catalyses the decomposition of hydrogen peroxide (H₂O₂), a toxic byproduct of cellular metabolism, into harmless water (H₂O) and oxygen (O₂) (*Zamocky et al.*, 2008). This detoxification prevents oxidative damage to cellular components, a key aspect of how cells maintain homeostasis (*Nandi*, 2019).

The rate of enzyme-catalysed reactions is influenced by factors such as temperature, pH, substrate concentration, and enzyme concentration (*Robinson*, 2015). While a previous experiment explored temperature effects, this investigation focuses on how varying enzyme concentration impacts reaction rates. According to established enzyme kinetics, when substrate is abundant, the initial reaction rate is directly proportional to the enzyme concentration (*Aebi*, 1984). This is because more enzyme molecules mean more active sites are available to process the substrate, leading to faster product formation (*Robinson*, 2015).

This experiment aims to systematically investigate how altering the volume of catalase solution, thereby changing its effective concentration, affects the initial rate of oxygen production from hydrogen peroxide decomposition. By keeping substrate concentration, volume, and temperature constant, the study will isolate the influence of enzyme quantity on reaction rate. This will provide insight into how cells can regulate metabolic pathways by controlling enzyme availability.

# Research Question:

How does increasing the volume of catalase solution (representing different enzyme concentrations) affect the rate of oxygen gas production when reacting with a constant volume and concentration of hydrogen peroxide?

# Methodology:

## Original Experiment

The original experiment investigated enzyme activity by reacting 1mL of stock catalase solution (yeast) with a set volume of hydrogen peroxide (10mL) in a reaction chamber. The chamber is submerged in a room-temperature water bath. Oxygen gas, the product of the reaction, is collected over water in an inverted measuring cylinder, and its volume is recorded at regular time intervals (every 30 seconds for 5 minutes) to determine the rate of reaction.

## Modifications

The original experimental design was modified to specifically address the research question concerning the effect of enzyme concentration on reaction rate. These modifications involved refinement for improved data quality and redirection to investigate the chosen variable.

The experimental procedure was refined to enhance the reliability of the collected data by conducting three independent trials for each of the tested volumes of catalase solution (1mL, 2mL, and 4mL). This replication improves the precision of the results by allowing for the calculation of mean oxygen production volumes and standard deviations. Averaging the outcomes from multiple trials helps to minimize the impact of random errors, such as slight variations in measurement or inconsistencies in bubble collection, thereby providing a more consistent and trustworthy dataset for each enzyme concentration.

The experiment was redirected from its original focus to directly investigate the new research. This was achieved through the systematic variation of the enzyme quantity. Instead of using a single volume, three different volumes of catalase solution were tested: 1mL (as a baseline, identical to the original method), 2mL, and 4mL. Since the stock catalase solution's concentration is constant, altering its volume directly changes the total amount of enzyme, and thus the effective enzyme concentration in the reaction mixture.

## Safety & Ethical Considerations

|  |  |  |
| --- | --- | --- |
| **Hazard** | **Identified Risk(s)** | **Control Measure(s)** |
| Hydrogen Peroxide (H202) | * Skin irritation or mild chemical burns. * Eye irritation or damage. | * Wear safety goggles and a lab coat at all times. * Handle H2O2 with care to avoid spills. * If H2O2 contacts skin, rinse immediately with plenty of water |
| Glassware (test tubes, beakers) | * Cuts from breakage. | * Handle carefully and avoid excessive force. * Keep glassware away from the edge of the bench. * Dispose of any broken glassware in a designated broken glass bin. |
| Chemical Spills | * Slip hazard on the floor. * Contamination of the work area or equipment. | * Clean up any spills immediately with paper towels as per teacher instructions. * Work on a clear, uncluttered bench space. |

# Processed Data + Calculations:

Table 1: Sample Calculations

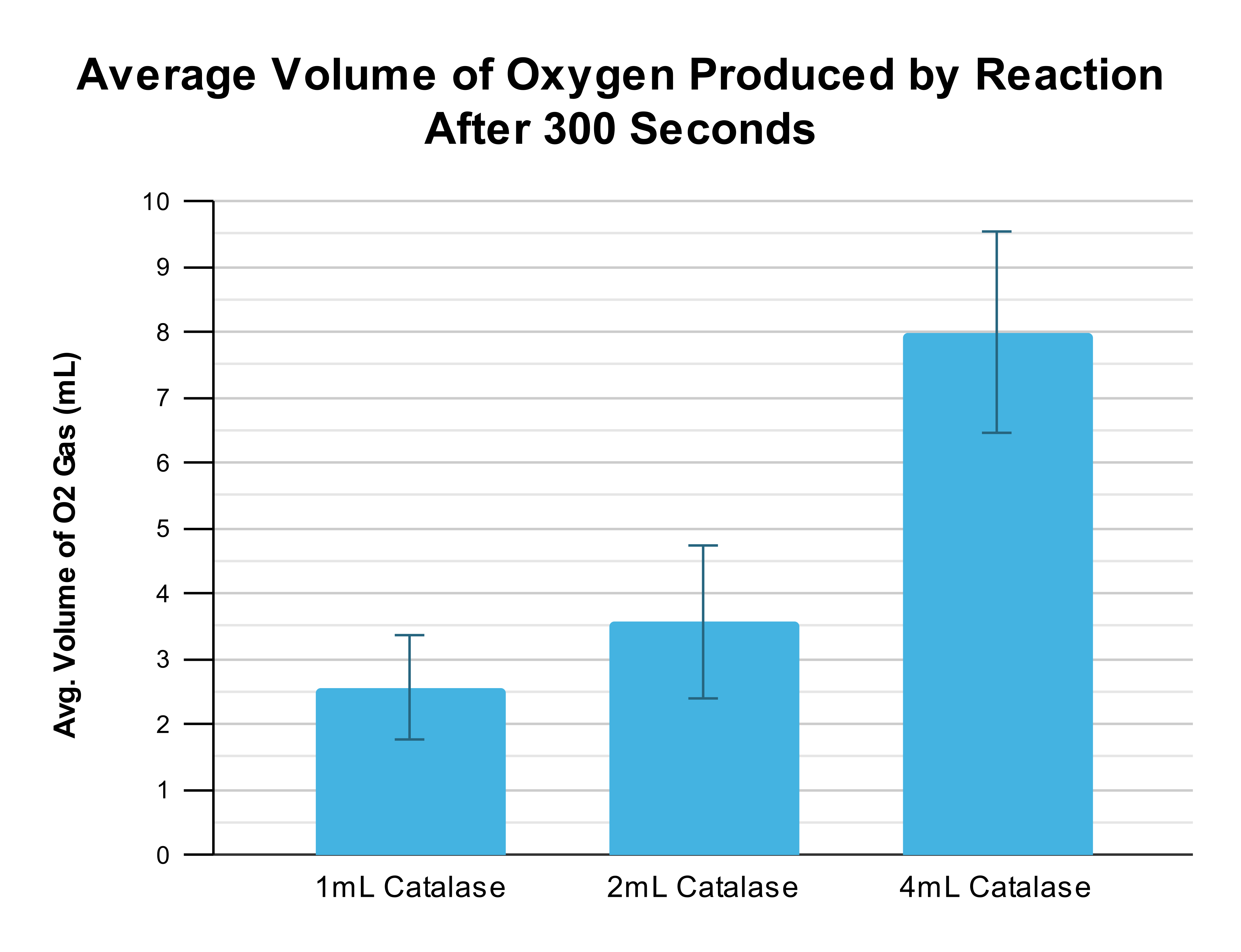
|  |  |
| --- | --- |
| **Calculation** | **Example** |
| **Mean oxygen volume** |  |
| **Standard deviation (SD) for a sample population** | Standard deviation was calculated in excel by using the STDEV.S and the three trial measurements for each catalase volume. For 1mL: |
| **Standard error** | Standard error was calculated in excel by dividing the standard deviation by the square root of the sample size. For 1mL: |
| **Upper/lower limit** | The upper and lower limit for each catalase volume was calculated as follows: |

Table 2: Processed data table for volume of Oxygen After 300 Seconds  
(values are in mL and rounded to 2 decimal places)

|  |  |  |  |
| --- | --- | --- | --- |
|  | **1mL Catalase** | **2mL Catalase** | **4mL Catalase** |
| **Trial 1** | 4.00 | 0.70 | 11.00 |
| **Trial 2** | 3.00 | 5.00 | 8.50 |
| **Trial 3** | 0.70 | 5.00 | 4.50 |
| **Mean** | 2.57 | 3.57 | 8.00 |
| **Std. Deviation** | 1.69 | 2.48 | 3.28 |
| **Std. Error** | 0.98 | 1.43 | 1.89 |
| **Upper Limit** | 5.95 | 8.53 | 14.56 |
| **Lower Limit** | 0.00 | 0.00 | 1.44 |

## Interpretation:

The data shows the mean oxygen volume produced after 300 seconds was 2.57 ± 0.98 mL for 1mL catalase, 3.57 ± 1.43 mL for 2mL catalase, and 8.00 ± 1.89 mL for 4mL catalase. The standard error has been used as a measure of the uncertainty associated with these averages (±SE). The progressively larger standard errors across treatments (0.98 → 1.43 → 1.89 mL) suggest decreasing precision with higher enzyme concentrations, potentially due to more vigorous reactions being difficult to measure consistently or high biological variation in enzyme activity at different concentrations.

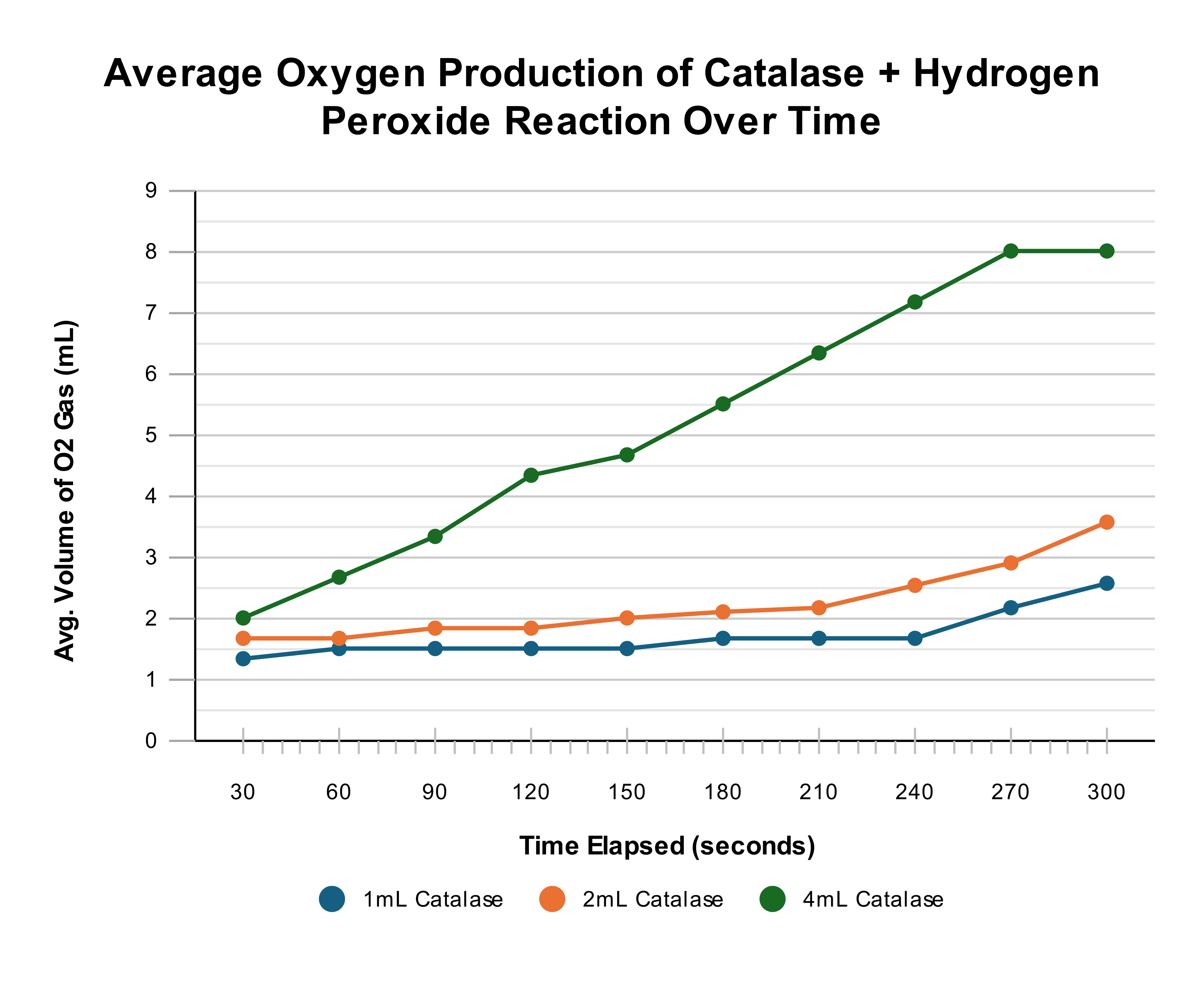
  
Graph 1: Column Graph (standard deviations represented by error bars)

## Interpretation:

The column graph shows the mean volume of oxygen gas produced after 300 seconds for each catalase volume treatment. The 4mL catalase treatment produced the highest mean oxygen volume (8.00 mL), followed by 2mL catalase (3.57 mL), and 1mL catalase (2.57 mL). This demonstrates a general positive relationship between enzyme volume and oxygen production, with the 4mL treatment producing approximately 3.1 times more oxygen than the 1mL baseline and 2.2 times more than the 2mL treatment.

## Analysis:

The data indicates substantial variability within each treatment group, as evidenced by the large standard deviations represented by the error bars (1mL: ±1.69 mL, 2mL: ±2.48 mL, 4mL: ±3.28 mL). The overlapping error bars between the 1mL and 2mL treatments suggest these results may fall within the same range, indicating no statistically significant difference between these enzyme concentrations. However, the 4mL treatment shows minimal overlap with the other treatments, suggesting a potential significant difference. The increasing magnitude of standard deviations with higher enzyme concentrations indicates decreasing precision in measurements, possibly due to more vigorous reactions being harder to measure consistently or substrate limitation effects occurring at higher enzyme concentrations.

  
Graph 2: Line Graph - Mean oxygen volume over a 300 second period

## Interpretation:

The line graph demonstrates the cumulative oxygen production over the 300-second experimental period for each catalase treatment. All treatments show the characteristic pattern of enzyme kinetics, with an initial rapid rate of oxygen production that gradually levels off as the reaction progresses. The 4mL catalase treatment exhibits the steepest initial slope and maintains the highest oxygen production throughout the time period, reaching approximately 8.00 mL by 300 seconds. The 2mL treatment shows a moderate rate of production, while the 1mL treatment displays the slowest rate, reaching only 2.57 mL after 300 seconds.

## Analysis:

The initial reaction rates (represented by the steepness of the early slopes) follow the expected pattern where higher enzyme concentrations produce faster initial rates of oxygen evolution. This aligns with enzyme kinetics theory, where increased enzyme concentration provides more active sites for substrate binding when substrate is in excess. However, the leveling off observed in all treatments, particularly pronounced in the 4mL condition, suggests that substrate depletion may be occurring before the 300-second endpoint. The plateau effect is most evident in the 4mL treatment, indicating that the 10mL of hydrogen peroxide substrate becomes limiting when enzyme concentration is high, preventing the reaction from maintaining its initial rate throughout the entire observation period. This plateau effect compromises the ability to accurately assess the true relationship between enzyme concentration and reaction rate, as the measured values at 300 seconds may not reflect the initial rate conditions where enzyme kinetics principles are most applicable.

# Evaluation:

## Limitations Of Evidence

Standard deviation, standard error, and confidence intervals are all examples of the uncertainty and limitations observed from an analysis of the evidence. This can be explained by a lack of reliability and validity in the experimental process.

The oxygen volumes recorded for each catalase concentration were highly inconsistent (refer to Table 2, see standard deviations of 1.69 mL, 2.48 mL, and 3.28 mL) hence the average oxygen production is calculated from data that lacks reliability, as indicated by the large standard errors. This suggests that not all variables were fully controlled. Also, the standard error of the 4mL catalase treatment data was higher than the lower concentration treatments (refer to Graph 1) which may suggest low precision in the gas collection method or high variation in reaction conditions at different enzyme concentrations.

The small sample size of this experiment (n=3 for each treatment) is a major factor in determining the width of the confidence intervals. Consequently, the evidence is limited in its ability to be used to extrapolate the findings of the experiment to the broader understanding of catalase enzyme kinetics.

# Sources Of Error:

## Affecting Reliability

* The gas collection apparatus lacks precision in measuring oxygen volume. The water displacement method and visual reading of gas volume contributes to imprecision in the data (±0.5 mL reading uncertainty). However, the variation in the data is greater than this measurement uncertainty; therefore, there must be other sources of imprecision.
* The samples were not standardized for reaction initiation timing. Random variation exists in the sealing and submersion process of the reaction chamber. This could explain some of the remaining imprecision in the data, as inconsistent reaction start times would lead to variable gas loss.
* The catalase solution homogeneity was not verified prior to conducting the experiment. Therefore, it is not known whether each aliquot contained identical enzyme concentrations, leading to random biological variation affecting the reaction rates.

## Affecting Validity

* The water displacement method leads to underestimation of actual gas volume due to oxygen dissolution. Therefore, the volume of oxygen produced is systematically underestimated. In addition, the oxygen volume is determined indirectly which could lead to greater variability in the data.
* The experimental design does not maintain constant substrate concentration across treatments. The varying total reaction volumes (11mL, 12mL, 14mL) dilute the hydrogen peroxide to different concentrations, introducing a confounding variable that affects the validity of isolating enzyme concentration effects.
* The gas collection method does not account for substrate depletion at higher enzyme concentrations. The 300-second endpoint measurement does not represent true initial rates, as substrate limitation occurs before completion, particularly in the 4mL treatment. Therefore, this could contribute to the data being inaccurate for determining the enzyme concentration-rate relationship.

# Suggested Improvements & Extensions:

## Suggested Improvements:

Reducing the random error in the experimental process would improve its reliability. In this experiment, the reliability of the data could be improved by increasing the number of repeat readings of each treatment (minimum n=5), standardizing the reaction initiation procedure, and running multiple independent trials to decrease standard error.

To address the imprecision in gas measurement, a gas syringe should be used instead of water displacement, which would eliminate oxygen dissolution effects and provide direct, more accurate volume readings. This would improve both the reliability of data and validity of the experimental process.

Maintaining constant total reaction volume across all treatments by adding distilled water to equalize volumes would ensure identical substrate concentrations, allowing the results to isolate the effect of enzyme concentration. In addition, ensuring homogeneity of the catalase stock solution through thorough mixing before each aliquot would reduce biological variation.

## Suggested Extensions:

* Redirect the experiment by investigating a wider range of enzyme concentrations with smaller incremental increases to better define the enzyme concentration-rate relationship and identify saturation points.
* Extend the experiment by investigating the effect of substrate concentration while keeping enzyme concentration constant, providing a comprehensive exploration of enzyme kinetics.

# Conclusion:

In conclusion, the evidence suggests that increasing the volume of catalase solution does increase the rate of oxygen gas production when reacting with a constant volume and concentration of hydrogen peroxide. The mean oxygen production increased from 2.57 mL (1mL catalase) to 8.00 mL (4mL catalase) over the 300-second period, indicating a positive relationship between enzyme concentration and reaction rate.

However, there are significant limitations to the experimental design including high variability between trials, potential substrate depletion at higher enzyme concentrations, and confounding variables such as varying total reaction volumes. Further statistical analysis and methodological refinements would be required to establish a definitive quantitative relationship and support the theoretical expectation of direct proportionality between enzyme concentration and initial reaction rate.

# Reference List:

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# Appendix:

## Raw Data

## A