Chemistry Extended Essay

Investigating the Effect of Sugar Concentration, Temperature, and Yeast Type on CO_2 Production in Yeast Fermentation

Research Question: *How do different sugar concentrations (5.00g, 10.00g, 15.00g, 20.00g, and 25.00g) and temperatures (30.0°C, 35.0°C, 40.0°C, 45.0°C, and 50.0°C) affect the rate of CO_2 production in yeast fermentation, and how do these effects vary between dry and fresh yeast, as measured using the Water Displacement Method and CO_2 Sensor Measurement?*

Contents

1. Introduction

1.1. Background & Significance

Context History of Yeast Fermentation's Use

Usage of yeast in fermentation has a rich variety of applications such as in the baking and brewing industry of the Egyptian civilizations from over 5000 years ago, in history (Samuel, 1996; McGovern et al., 2004). Yeast, in various sectors, remains an integral part to industrial use as it has proven english_flag to be useful in transforming raw material to finished goods in the food, beverage and pharmaceutical sector as its metabolites have medicinal components (Barnett, 2003). It is important to note that the development of various value-added products increases because of fermentation and in return, aids greatly in industrial engineering processes by lowering economic costs and improving production efficiency (Walker & Stewart, 2016).

Scientific Perspective and Biochemical Details

Like any other biochemical process, fermentation has reasons why it is carried out and they account for yeast's anaerobic respiration (Madigan et al., 2018). Factors to be considered in estimating the rate of fermentation is heavier pleasures and includes the concentration of sucrose, heat, and variation in yeast (Russell, 2003). The present study seeks to measure and analyze the most important factors in estimating fermentation and its carbon dioxide output for the sake of understanding industries that are centered in fermentation (Walker & Stewart, 2016). Secondly, the industrial biochemistry context demonstrates value of fermentation in research such as in enzymatic activity analysis, application of metabolic control, and microorganisms biotechnological (Bai et al., 2008)

Role of Fermentation in Industrial Applications

The massive role of fermentation is evident in the food and beverage sectors, where processes powered by yeast lead to creation of bread, alcohol, and milk products (Fleet, 2007; Walker & Stewart, 2016). Moreover, fermentation has a very important role to play in the pharmaceutical field, such as in the making of antibiotics and in the creation of probiotics (Kleerebezem & Vaughan, 2009). Yeast is also extensively used in bioengineering, where it is made to undergo genetic modification for industrial fermentation purposes (Ostergaard et al., 2000).

Environmental and Sustainability Considerations

Fermentation is crucial in the production of sustainable biofuels, such as turning agricultural residues into ethanol with the aid of yeast (Hahn-Hägerdal et al., 2006). Also, by fine tuning fermentation conditions, the bioethanol production rate can be increased, thus decreasing the use of fossil energy (Lin & Tanaka, 2006). Besides, fine human is changing from a focus on bioformal waste to biodegradable waste to bioenergy systems (Demirbas, 2009).

1.2. Personal Motivation

Research motivation arose from the connection between biotechnology and the activity of particular enzymes. The study of yeast metabolism relates to their use in the production of biofuels, food, and industrial products. Researching the behavior of enzymes with respect to certain environmental factors is beneficial for developing processes in real life.

1.3. Research Question & Scope of the Study

Research Question

How do different sugar concentrations (5.00g, 10.00g, 15.00g, 20.00g, and 25.00g) and temperatures (30.0°C, 35.0°C, 40.0°C, 45.0°C, and 50.0°C) affect the rate of CO_2 production in yeast fermentation, and how do these effects vary between dry and fresh yeast, as measured using the Water Displacement Method and CO_2 Sensor Measurement?

Scope of the Study

The changes in the amount of carbon dioxide (CO_2) produced during fermentation as a result of changes in type of yeast, concentration of sugar, and temperature is the subject of this experiment. The Water Displacement Method and CO_2 Sensor Measurement were chosen as two experimental techniques to measure the production of gas accurately. Fresh and dry yeast samples were used at 30.0°C, 35.0°C, 40.0°C, 45.0°C and 50.0°C with varying amounts of sugar 5.00g, 10.00g, 15.00g, 20.00g, and 25.00g.

1.4. Hypothesis

- Enzyme Activity & Temperature Affectation: It's established with the use of Arrhenius formulas: Increasing temperatures increase enzyme functionality until a limit, where it stops working due to damage to the surrounding environment.

- Substrate Availability: Referring to the Michaelis-Menten kinetics, production of CO_2 initially rises in- speed with sugar concentration until saturation where active site of the enzymes are used up.
- Yeast Strain Efficiency: Fresh yeast is assumed to be more efficient than dry yeast at higher sugar concentration owing to greater metabolic flexibility and higher proportion of viable cells.

2. Literature Review

2.1. Yeast Fermentation and its Mechanisms

Glycolysis and Anaerobic Respiration:

The process of glycolysis is the breakdown of glucose such as, pyruvate which transforms anaerobically to ethanol and CO_2 via alcoholic fermentation (De Kroon, 2015; Madigan et al., 2018). Glycolysis reactions can be illustrated as follows:

$$C_6H_{12}O_6 + 2NAD^+ + 2ADP + 2P_1 \rightarrow 2C_5H_4O_5 + 2NADH + 2ATP + 2H_2O$$

$$C_5H_4O_5 = \text{pyruvate}$$

Following glycolysis, alcoholic fermentation occurs in two main steps:

- Decarboxylation of Pyruvate:

$$pyruvate \rightarrow acetaldehyde + CO_2$$

- Reduction of Acetaldehyde:

$$acetaldehyde + NADH \rightarrow ethanol + NAD^+$$

For simplicity, the overall alcoholic fermentation reaction is often written as:

$$C_3H_4O_3 \rightarrow 2C_2H_5OH + 2CO_2$$

Role of Enzymes in Fermentation:

Enzymes such as zymase (a mixture of both pyruvate decarboxylase and alcohol dehydrogenase) are required. They are responsible for catalyzing the reactions transforming sugars into ethanol and carbon dioxide and significantly affecting the reaction efficacy and rate (Ingledew, 1999; Ostergaard et al., 2000).

Metabolic Pathways of Yeast:

Yeast metabolism changes with respect to the availability of oxygen. It is observed that under aerobic conditions where oxygen is present, pyruvate enters the citric acid cycle. However, it

is diverted to fermentation under anaerobic conditions. This has implications on the metabolic flux and energy yield overall (Walker & Stewart, 2016).

2.2. Factors Influencing CO_2 Production

Effect of Temperature on Enzyme Activity:

One of the factors affecting enzyme kinetics is temperature. Enzymes work within an optimal temperature range that maximally supports enzyme activity, whereas temperatures outside of this range can lead to denaturation of the enzyme, which in turn limits fermentation efficiency (ScienceDirect, 2020; Walker, 2004).

Effect of Sugar Concentration on Yeast Metabolism:

Rate of fermentation is dependent on the concentration of glucose available. As substrate concentration rises, enzymes may become saturated leading to a state of reaction rate plateau (NCBI, 2021; Lin & Tanaka, 2006).

Influence of pH on Fermentation:

pH level is one of the important determinants in relation to enzyme structure and function. Extreme deviations from the optimal pH levels not only impacts the viability of yeast but affects fermentation efficiency as well (Walker & Stewart, 2016).

2.3. Gas Collection and Measurement Techniques

Water Displacement Method vs. CO_2 Sensor:

With regards to accuracy, ease of use, and error precision, these methods vary from one another. The water displacement method is straight forth, but is likely to be less precise while the CO2 sensors are more accurate and need to be calibrated with care (Vernier Science Education, 2023).

Limitations of Each Measurement Technique:

Every technique has its limitations. Gas measurement accuracy can be affected by calibration errors, the response times of the device, and certain environmental conditions (Vernier Science Education, 2023).

2.4. Scientific Models and Theoretical Framework

Michaelis-Menten Kinetics in Fermentation:

This model describes how the concentration of the substrate affects the velocity of the enzyme-catalyzed reaction (Madigan et al., 2018). The equation is given as follows:

$$v = \frac{(V_{max}[S])}{(K_m + [S])}$$

Arrhenius Equation and Temperature Dependence:

Temperature also plays a major role in determining the reaction rate. The Arrhenius equation describes the role of temperature in modifying the rate constant (k) of a reaction:

$$k = Ae^{(-\frac{E_a}{RT})}$$

where A is the pre-exponential factor, E_a is the activation energy, R is the gas constant, and T is the temperature in kelvin (Madigan et al., 2018; ScienceDirect, 2020).

3. Methodology

This part focuses on setting up the experiment to be conducted together with the variables and procedures needed for determining the effect of sugar concentration, temperature, type of yeast, and measuring carbon dioxide during fermentation of yeast. The methodology ensures accurate control, repeatability, and non-manipulative oversight.

Important Procedures

Stage 1: The amount of CO_2 was measured by the method of water displacement.

Stage 2: A CO_2 gas sensor from Vernier was used for the non-intrusive monitoring of gas concentration.

Standardization of measurements: For recording the intervals on CO_2 yield, 20 and 30 minutes were chosen to set a standard interval.

Major Findings

- Optimal CO_2 Production Conditions: The peak level of CO_2 production was at 50°C with 20g of sugar. This is consistent with theories on the optimal temperatures for enzyme activity, which state that moderate heat increases enzyme activity until a certain point when heat stops the enzyme from functioning.
- Osmotic Pressure Effects: The decreased CO_2 production at 25g of sugar indicates that too high sugar concentration is detrimental and inhibits fermentation because of osmotic pressure. Osmotic pressure, which can be hypertonic, may result in water being removed from the yeast cells, which would lower their ability to metabolize.

- Temperature Influence: Above 50°C, there is a significant drop in the level of CO_2 produced, which may be attributed to enzymatic denaturation, which is the breakdown of proteins so they no longer perform their functions due to excessive heat.
- Yeast Type Differences: Fresh yeast was more efficient than dry yeast at higher sugar concentrations, which could be attributed to the higher viable cell counts found in fresh yeast, leading to more active fermentation.

Key Implications

- Industrial Applications: In brewing, baking, and bioethanol production, the findings will be usable by enhancing fermentation through tailored conditions. Sugar and temperature control will allow for greater efficiency and yield (Bai et al., 2008; Lin & Tanaka, 2006; Walker & Stewart, 2016).
- Unexpected Findings: The high concentration sugar inhibition has potential uses in food preservation by using controlled osmotic pressure to reduce microbial growth (Fleet, 2007; Gray, 2017; NCBI, 2021).
- Enzyme Function and Biotechnology – The work dissects the importance of enzyme kinetics relevant to the bioprocessing industries and provides guidance in the optimization of fermentation processes (De Kroon, 2015; Ostergaard et al., 2000; Walker, 2004; ScienceDirect, 2020).

Limitations & Future Research

- Uncertainty in gas measurement – The noise in the measurement of CO_2 from the sensor could lead to some errors because of air stratification.
- Potential refinements in automation: Using a syringe for extra proof would enable verification of the measurements of gas volume for CO_2.
- Broadening the bounds of the study – Future work may consider other strains of yeast, for instance, variants of Saccharomyces cerevisiae, or add pH as a factor since acidity could potentially have an effect on the performance of enzymes.

3.1. Experimental Setup & Materials

Materials Used

Category	Material
Glassware	500mL conical flasks, 250mL conical flasks, graduated cylinders, measuring cylinders
Chemicals	Dry yeast, fresh yeast, sucrose (sugar), distilled water
Equipment	Vernier CO_2 gas sensor, rubber stoppers, tubing, water bath, thermometer
Safety Gear	Insulated gloves, lab coat, safety goggles

Table 1: Materials Used in the Yeast Fermentation Experiment

Preparation of Yeast Solutions

Two types of yeast were used in the experiment: dry yeast and fresh yeast. To ensure consistent yeast activation, the following procedures were followed:

- Dry Yeast Activation: Dry yeast (10g) was dissolved in 50mL of warm distilled water (≈35°C) and left to activate for 10 minutes before experimentation. This ensured optimal yeast activity.
- Fresh Yeast Preparation: Fresh yeast (10g) was directly dissolved in 50mL of distilled water without pre-activation, as fresh yeast is already metabolically active.

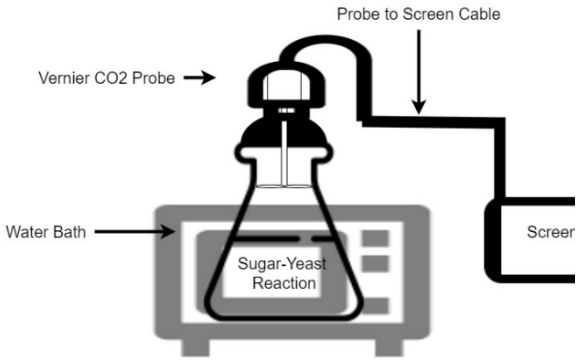


Figure 1: Experimental setup for measuring CO_2 production using the Vernier CO_2 gas sensor in yeast fermentation experiments.

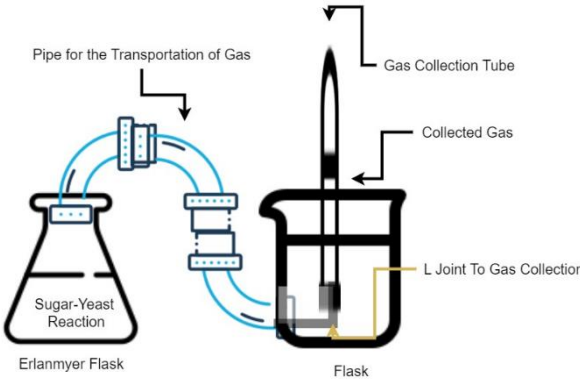


Figure 2: Experimental setup illustrating the water displacement method for quantifying CO_2 produced during yeast fermentation.

Calibration of Equipment

To ensure accurate measurements of CO_2 production, the following calibration procedures were conducted:

- CO_2 Sensor Calibration: The Vernier CO_2 gas sensor was calibrated using a standard CO_2 concentration in an enclosed environment to confirm its accuracy.
- Water Displacement Accuracy Checks: The measuring cylinder used for water displacement was tested using a known volume of air to ensure reliable gas volume measurements.

3.2. Variables and Controls

Type	**Variable**
Independent	Temperature (30°C, 35°C, 40°C, 45°C, 50°C)
	Sugar Concentration (5g, 10g, 15g, 20g, 25g per 50mL solution)
	Yeast Type (Dry yeast and Fresh yeast)
Dependent	CO_2 production rate (mL/min via Water Displacement Method, ppm via CO_2 Sensor Method)
Controlled	Yeast mass (10g for all trials)
	Water volume (50mL in every trial)
	Timing of CO_2 measurement (Recorded at 20-minute and 30-minute intervals)
	Experimental environment (Conducted in a temperature-controlled water bath)

Table 2: Variables in the Yeast Fermentation Experiment

Control Methods and Their Purpose

Control	**Method**	**Reason for Control**
No Yeast Control	Sugar solution tested without yeast	To account for CO_2 not produced by yeast fermentation
No Sugar Control	Yeast solution tested without sugar	To determine baseline yeast metabolic activity
Standardized Temperature	Water bath used to maintain set temperatures	To ensure consistent conditions across trials

Control	Method	Reason for Control
Consistent Yeast Mass	10g yeast used in all trials	To ensure equal yeast concentration
Fixed Water Volume	50mL water used in all experiments	To maintain consistency in solution concentration

Table 3: Experimental Controls and Their Justifications

3.3. Risk Assessment & Safety Precautions

Safe Handling of Yeast and CO_2 Production

- To prevent contamination, gloves were worn when working with yeast cultures.
- Every so often, the system was vented to avoid excessive buildup of CO_2 within sealed containers.

Water Bath Precautions

- To avoid burns, gloves with insulation were used to handle the hot water baths.
- A stabilized water bath was monitored to reduce the degree of temperature oscillations.

Ecosystem Impacts

- Reducing Waste: Minimum amounts of sugar and yeast were taken to lessen waste.
- Spent yeast cultures were disposed of biowaste protocols on yeast disposal.
- In displacement experiments, the water was saved and reused.

Ethical Considerations – Lab Safety and Environmental Responsibility

During the experiment, all necessary safety measures were taken in order to manage the laboratory appropriately. The yeast fermentation process results in carbon dioxide gas being produced, which in enclosed spaces can potentially lead to asphyxiation; hence it needs ventilation. Also, throughout the process, ventilation was controlled to avoid being burned by hot water baths. In addition to lab safety, there is always the issue of environmental protection, which in this case meant being in control of how the yeast was discarded. The environmental implications of large-scale fermentation in industries are too much waste, excessive water, and carbon dioxide pollution. The research should also look for other ways of disposing of yeast biomass economically and examine its usefulness as animal feed or

organic fertilizer, which may contribute to reducing the environmental damage caused by the biochemical industries.

3.4. Procedure

Pilot Experiments: Before the main experiment, pre-tests are conducted to improve the steps and to estimate the errors to be made. These trials helped determine the optimal yeast concentration and timing for the carbon dioxide measurements.

Stage 1: Water Displacement Method

Using a graduated cylinder filled with water, the volume of water displaced by the carbon dioxide produced was measured to quantify CO_2 generated.

- **Preparation:** A 500mL conical flask was filled with the yeast-sugar solution and sealed with a rubber stopper fitted with a tube.
- **Gas collection:** The inverted measuring cylinder was filled with water and the tube was inserted into it. The displaced water was equivalent to the CO_2 released
- **Measurement Intervals:** The volume of displaced water was recorded at 20-minute and 30-minute intervals.
- **Repetition:** Each condition was tested three times to ensure reliability.

Stage 2: CO_2 Sensor Method

With a Vernier CO_2 gas sensor, it was possible to get more accurate measurements of the CO_2 concentration.

- **Setup:** A 250mL conical flask containing the yeast-sugar solution was sealed with a gas-tight stopper equipped with a CO_2 sensor.
- **Recording Data:** CO_2 concentration (ppm) was recorded in real time at 20-minute and 30-minute intervals.
- **Environmental Control:** The flask was placed in a water bath to maintain a constant temperature.
- **Repetition:** Three trials per condition were conducted.

Control Experiments

Control experiments were done to check for background CO_2 and verify that gas production was as a result of yeast fermentation:

- No Yeast Control: The sole purpose of this test was to determine the volume of dissolved CO_2 in the sugar solution without the yeast.
- No Sugar Control: This test was conducted with the yeast solution but without the sugar to test the levels of residual metabolic activity.

4. Presentation of Data

The raw data collected from the experiment include CO_2 production under varying conditions of temperature, sugar concentration, and yeast type. The following tables summarize the recorded measurements:

Temperature (°C)	Sugar (g)	Yeast Type	Volume (mL) at 20 min	Volume (mL) at 30 min	Diffrence	ml/min
30	5	Dry Yeast	0.2	0.3	0.1	0.01
30	10	Dry Yeast	0.4	0.7	0.3	0.03
30	15	Dry Yeast	0.7	1.0	0.3	0.03
30	20	Dry Yeast	0.9	1.3	0.4	0.04
30	25	Dry Yeast	0.6	0.8	0.2	0.02
35	5	Dry Yeast	0.3	0.4	0.1	0.01
35	10	Dry Yeast	0.5	0.7	0.2	0.02
35	15	Dry Yeast	0.7	1.1	0.4	0.04
35	20	Dry Yeast	1.1	1.4	0.3	0.03
35	25	Dry Yeast	0.7	0.9	0.2	0.02
40	5	Dry Yeast	0.5	0.7	0.2	0.02
40	10	Dry Yeast	0.8	1.1	0.3	0.03
40	15	Dry Yeast	1.1	1.5	0.4	0.04
40	20	Dry Yeast	1.3	1.7	0.4	0.04
40	25	Dry Yeast	0.9	1.2	0.3	0.03
45	5	Dry Yeast	0.5	0.7	0.2	0.02
45	10	Dry Yeast	0.7	1.0	0.3	0.03
45	15	Dry Yeast	1.0	1.2	0.2	0.02
45	20	Dry Yeast	1.2	1.5	0.3	0.03
45	25	Dry Yeast	0.9	1.2	0.3	0.03
50	5	Dry Yeast	0.9	1.2	0.3	0.03
50	10	Dry Yeast	1.2	1.5	0.3	0.03
50	15	Dry Yeast	1.5	1.9	0.4	0.04
50	20	Dry Yeast	1.7	2.2	0.5	0.05
50	25	Dry Yeast	1.3	2.0	0.7	0,07

Table 4: CO_2 Production (mL) Using Water Displacement Method (Dry Yeast)

Temperature (°C)	Sugar (g)	Yeast Type	Volume (mL) at 20 min	Volume (mL) at 30 min	Diffrence	ml/min
30	5	Fresh Yeast	0.2	0.3	0.1	0.01
30	10	Fresh Yeast	0.4	0.6	0.2	0.02
30	15	Fresh Yeast	0.7	1.0	0.3	0.03
30	20	Fresh Yeast	0.9	1.2	0.3	0.03
30	25	Fresh Yeast	0.6	0.8	0.2	0.02
35	5	Fresh Yeast	0.3	0.4	0.1	0.01
35	10	Fresh Yeast	0.6	0.8	0.2	0.02
35	15	Fresh Yeast	0.9	1.2	0.3	0.03
35	20	Fresh Yeast	1.2	1.5	0.3	0.03
35	25	Fresh Yeast	0.7	0.9	0.2	0.02
40	5	Fresh Yeast	0.5	0.7	0.2	0.02
40	10	Fresh Yeast	0.7	0.9	0.2	0.02
40	15	Fresh Yeast	1.1	1.4	0.3	0.03
40	20	Fresh Yeast	1.3	1.6	0.3	0.03
40	25	Fresh Yeast	0.9	1.2	0.3	0.03
45	5	Fresh Yeast	0.6	0.9	0.3	0.03
45	10	Fresh Yeast	1.0	1.3	0.3	0.03
45	15	Fresh Yeast	1.3	1.6	0.3	0.03
45	20	Fresh Yeast	1.5	1.8	0.3	0.03
45	25	Fresh Yeast	1.1	1.4	0.3	0.03
50	5	Fresh Yeast	0.9	1.2	0.3	0.03
50	10	Fresh Yeast	1.2	1.5	0.3	0.03
50	15	Fresh Yeast	1.5	1.9	0.4	0.04
50	20	Fresh Yeast	1.7	2.1	0.4	0.04
50	25	Fresh Yeast	1.7	2.1	0.4	0.04

Table 5: CO_2 Production (mL) Using Water Displacement Method (Fresh Yeast)

Temperature (°C)	Sugar (g)	Yeast Type	CO_2 Volume (mL) at 20 min	CO_2 Volume (mL) at 30 min	Difference	ml/min
30	5	Dry Yeast	0.2456	0.3684	0.1228	0.01228
30	10	Dry Yeast	0.4912	0.7368	0.2456	0.02456
30	15	Dry Yeast	0.7368	1.1052	0.3684	0.03684
30	20	Dry Yeast	0.9824	1.3508	0.3684	0.03684
30	25	Dry Yeast	0.614	0.8596	0.2456	0.02456
35	5	Dry Yeast	0.306	0.43	0.124	0.0124
35	10	Dry Yeast	0.5526	0.737	0.1844	0.01844
35	15	Dry Yeast	0.7982	1.104	0.3058	0.03058
35	20	Dry Yeast	1.104	1.421	0.317	0.0317
35	25	Dry Yeast	0.737	0.92	0.183	0.0183
40	5	Dry Yeast	0.5526	0.7982	0.2456	0.02456
40	10	Dry Yeast	0.8596	1.1666	0.307	0.0307
40	15	Dry Yeast	1.1666	1.535	0.3684	0.03684
40	20	Dry Yeast	1.3508	1.7192	0.3684	0.03684
40	25	Dry Yeast	0.921	1.228	0.307	0.0307
45	5	Dry Yeast	0.552	0.767	0.215	0.0215
45	10	Dry Yeast	0.767	1.023	0.256	0.0256
45	15	Dry Yeast	1.023	1.298	0.275	0.0275
45	20	Dry Yeast	1.298	1.543	0.245	0.0245
45	25	Dry Yeast	0.92	1.228	0.308	0.0308
50	5	Dry Yeast	0.921	1.228	0.307	0.0307
50	10	Dry Yeast	1.228	1.5964	0.3684	0.03684
50	15	Dry Yeast	1.535	1.9648	0.4298	0.04298
50	20	Dry Yeast	1.7192	2.2104	0.4912	0.04912
50	25	Dry Yeast	1.3508	2.0078	0.657	0.0657

Table 6: CO_2 Production (mL) Using Vernier CO_2 Sensor (Dry Yeast)

Temperature (°C)	Sugar (g)	Yeast Type	CO_2 Volume (mL) at 20 min	CO_2 Volume (mL) at 30 min	Difference	ml/min
30	5	Fresh Yeast	0.2456	0.3684	0.1228	0.01228
30	10	Fresh Yeast	0.4912	0.7368	0.2456	0.02456
30	15	Fresh Yeast	0.7318	1.1052	0.3734	0.03734
30	20	Fresh Yeast	0.9824	1.3508	0.3684	0.03684
30	25	Fresh Yeast	0.614	0.8596	0.2456	0.02456
35	5	Fresh Yeast	0.3684	0.4912	0.1228	0.01228
35	10	Fresh Yeast	0.614	0.8596	0.2456	0.02456
35	15	Fresh Yeast	0.921	1.228	0.307	0.0307
35	20	Fresh Yeast	1.228	1.5964	0.3684	0.03684
35	25	Fresh Yeast	0.7982	1.0438	0.2456	0.02456
40	5	Fresh Yeast	0.5526	0.7982	0.2456	0.02456
40	10	Fresh Yeast	0.7982	1.1666	0.3684	0.03684
40	15	Fresh Yeast	1.1666	1.535	0.3684	0.03684
40	20	Fresh Yeast	1.3508	1.7192	0.3684	0.03684
40	25	Fresh Yeast	0.921	1.228	0.307	0.0307
45	5	Fresh Yeast	0.6754	0.9824	0.307	0.0307
45	10	Fresh Yeast	1.0438	1.3508	0.307	0.0307
45	15	Fresh Yeast	1.3508	1.7192	0.3684	0.03684
45	20	Fresh Yeast	1.5964	1.9648	0.3684	0.03684
45	25	Fresh Yeast	1.1052	1.4736	0.3684	0.03684
50	5	Fresh Yeast	0.921	1.228	0.307	0.0307
50	10	Fresh Yeast	1.228	1.5964	0.3684	0.03684
50	15	Fresh Yeast	1.535	1.9648	0.4298	0.04298
50	20	Fresh Yeast	1.7192	2.2104	0.4912	0.04912
50	25	Fresh Yeast	1.7192	2.2104	0.4912	0.04912

Table 7: CO_2 Production (ml) Using Vernier CO_2 Sensor (Fresh Yeast)

Qualitative Observations

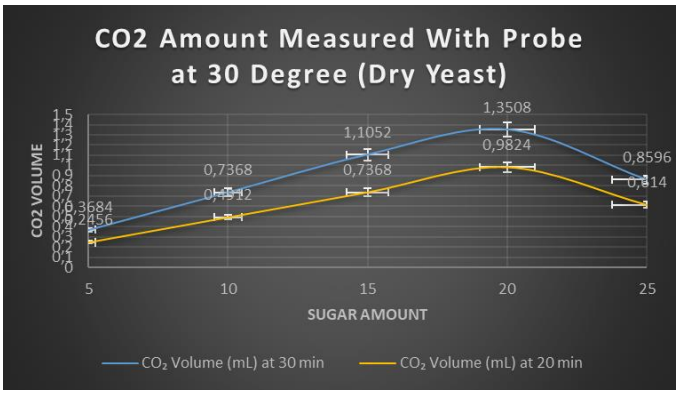
Alongside numerical data, qualitative observations provided insights into yeast activity:

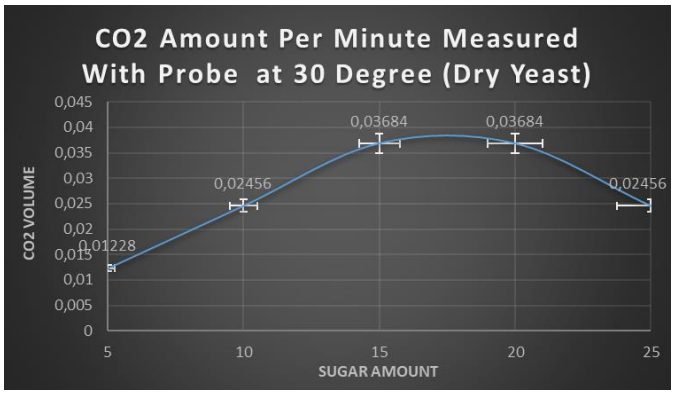
- **Foam Formation** – Increased foam production was noted at higher sugar concentrations, indicating more vigorous fermentation.
- **Yeast Activity** – Fresh yeast exhibited quicker activation, producing CO_2 earlier than dry yeast.

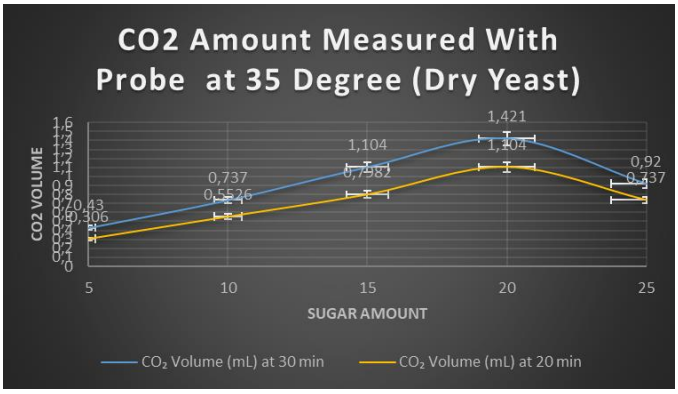
- **Color Changes** – A slight yellowing was observed at higher sugar concentrations, potentially due to metabolic byproducts.
- **Sedimentation** – Dry yeast tended to sediment more quickly compared to fresh yeast, influencing fermentation efficiency.

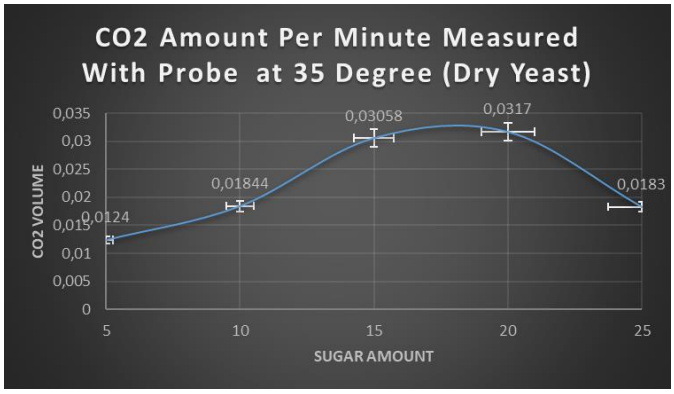
4.1. Graphical Analysis

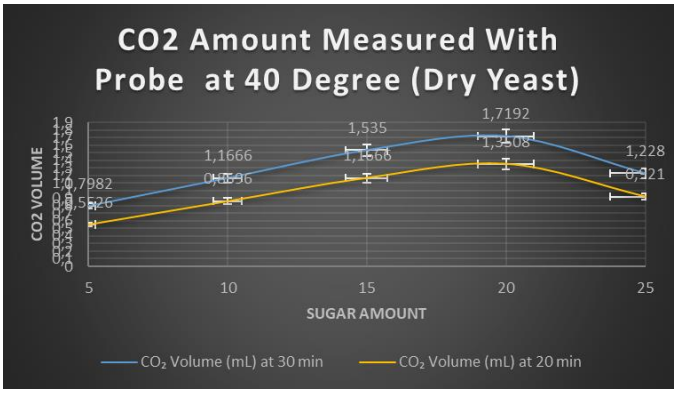
To visualize trends, comparative graphs were plotted:

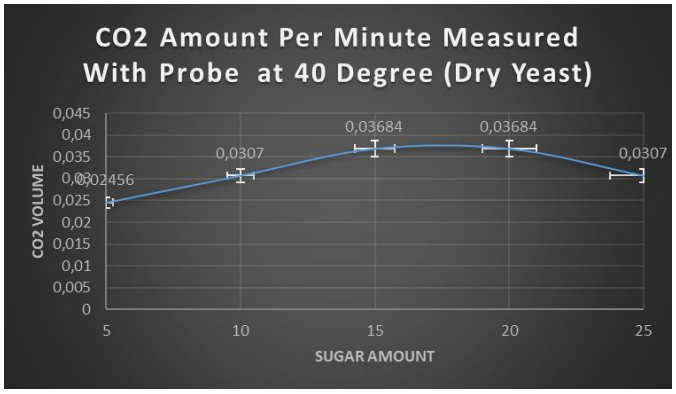


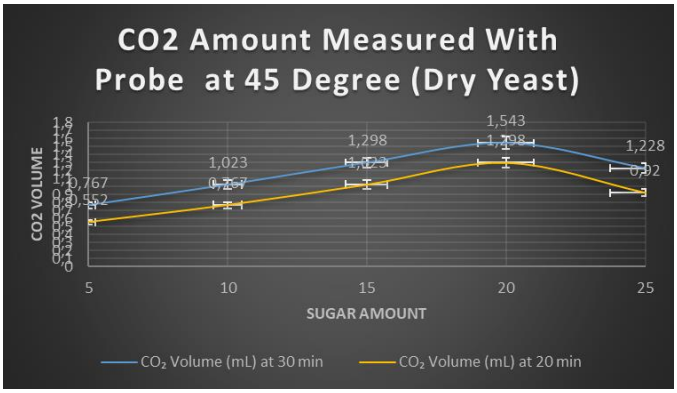


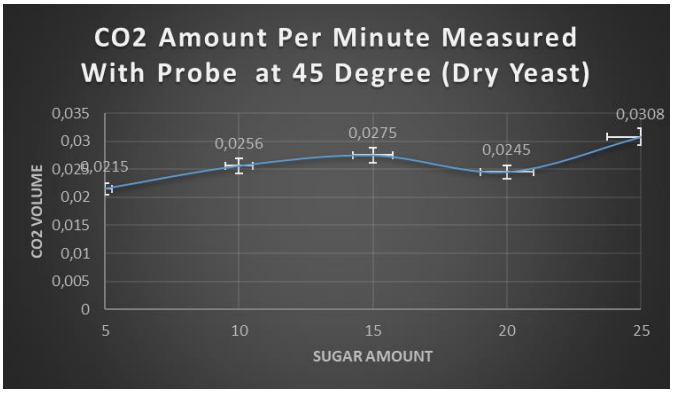


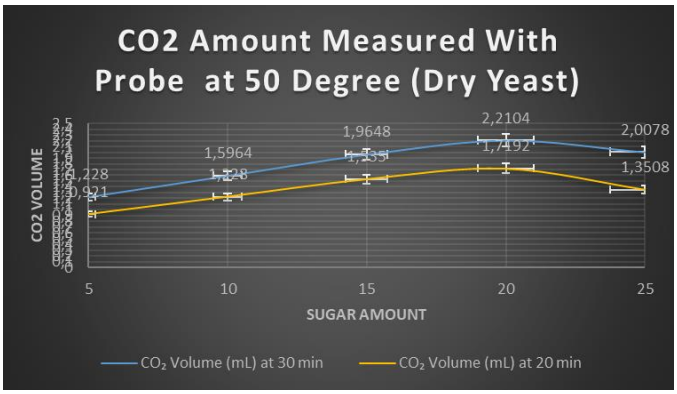












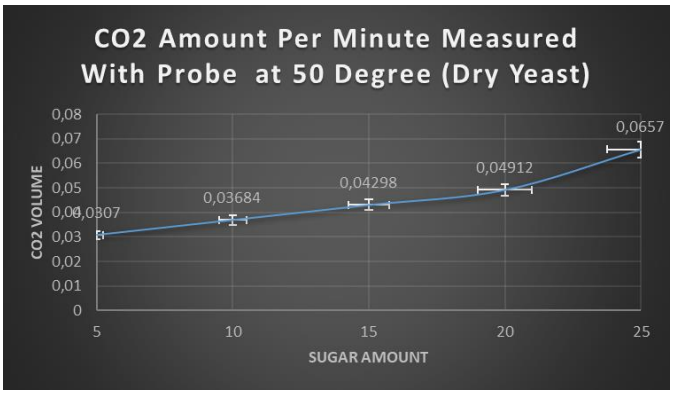
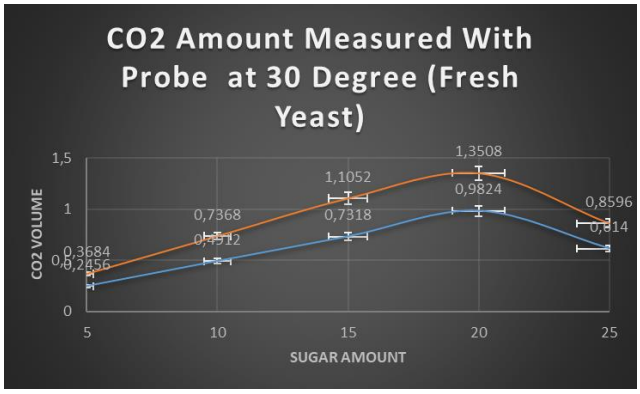
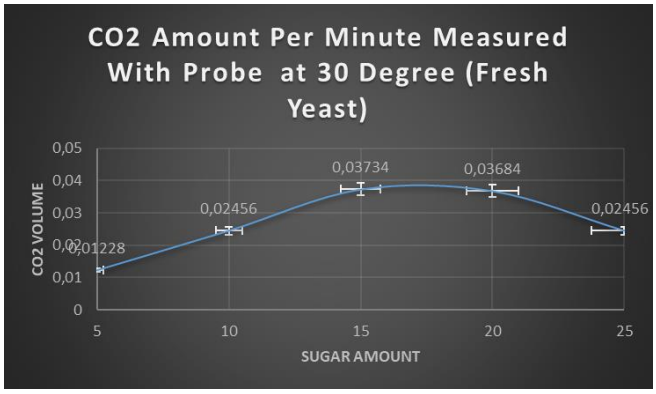
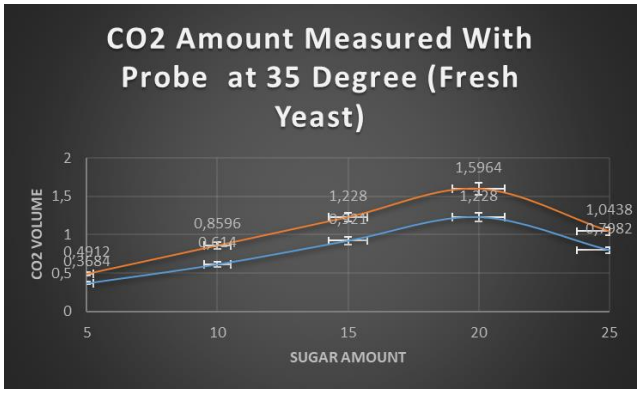
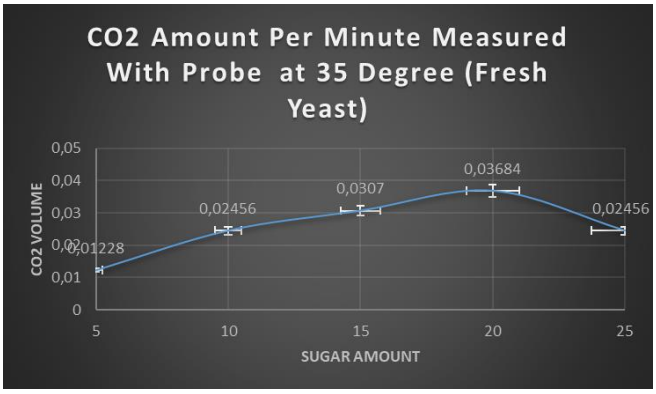


Figure 3: Comparative Differences and CO_2 Production Rate per Minute in Yeast Fermentation (Probe-Dry Yeast)









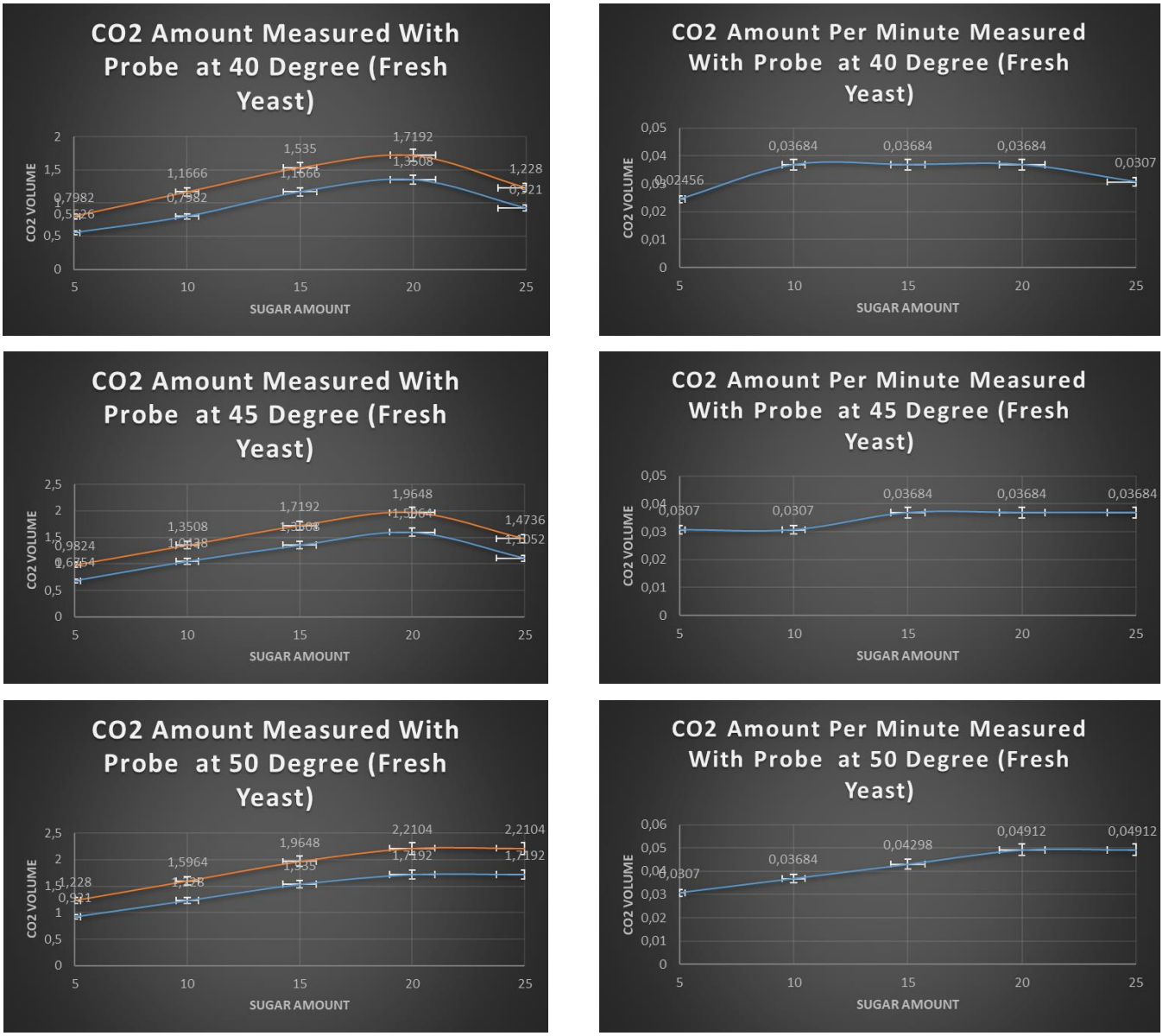
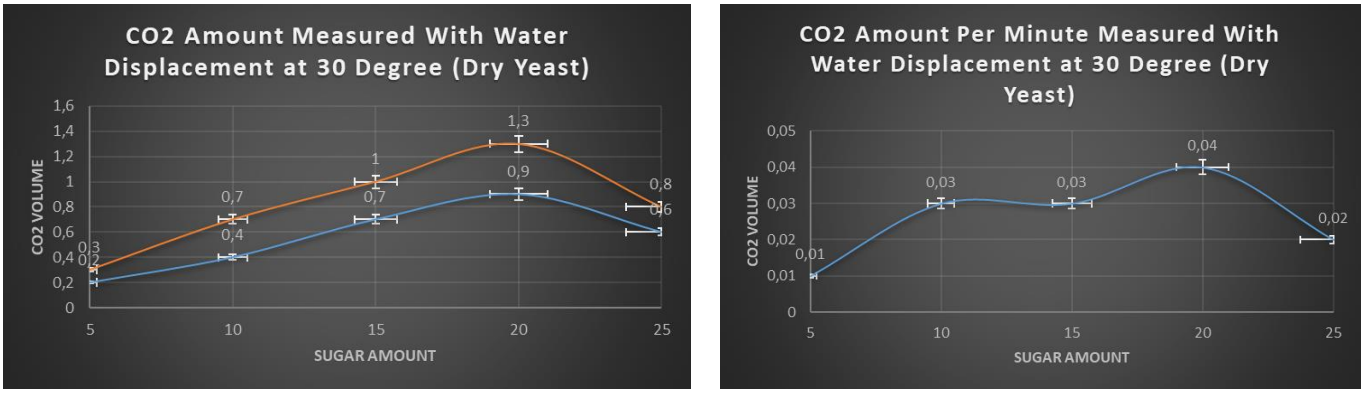


Figure 4: Comparative Differences and CO_2 Production Rate per Minute in Yeast Fermentation (Probe-Fresh Yeast)



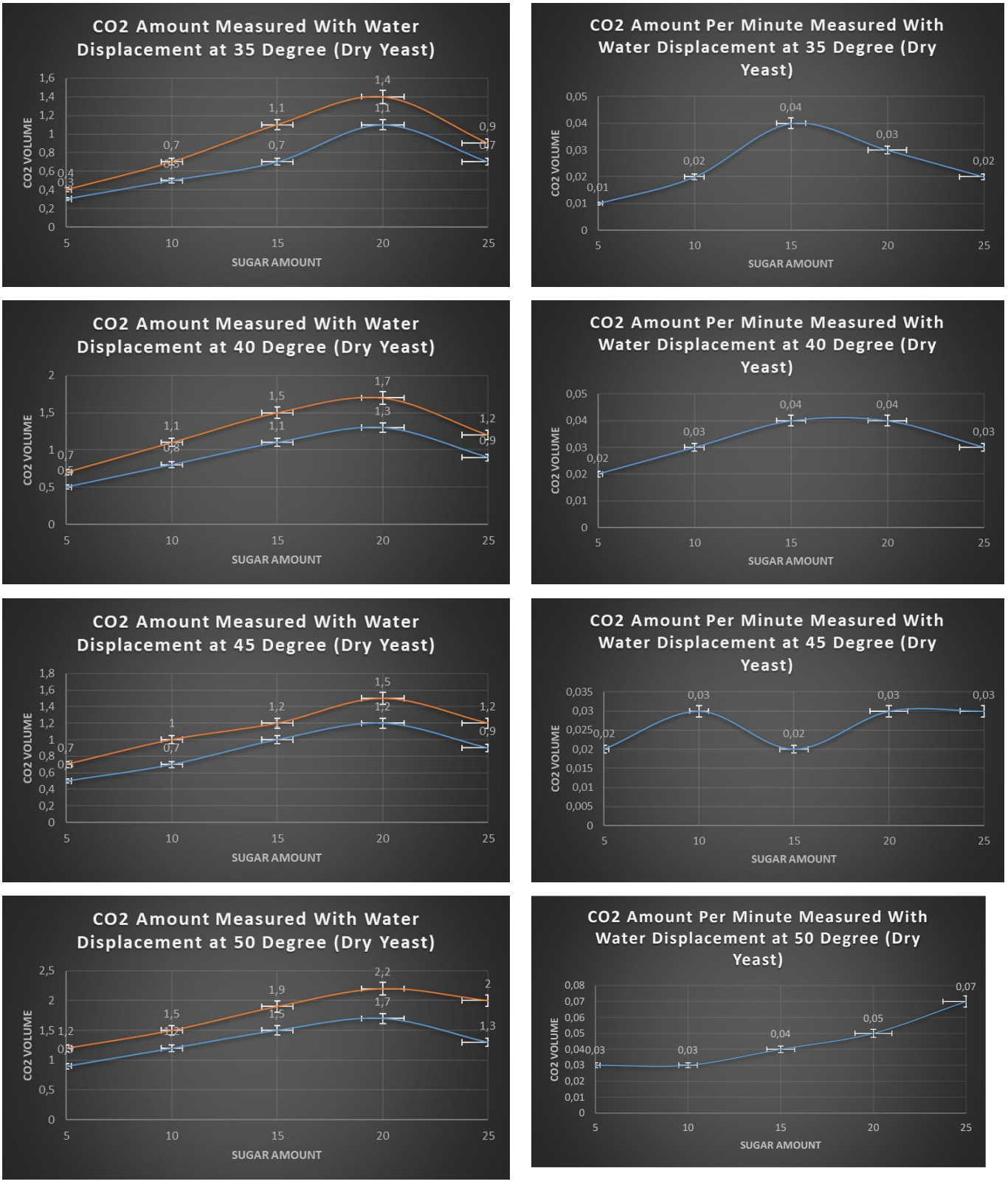
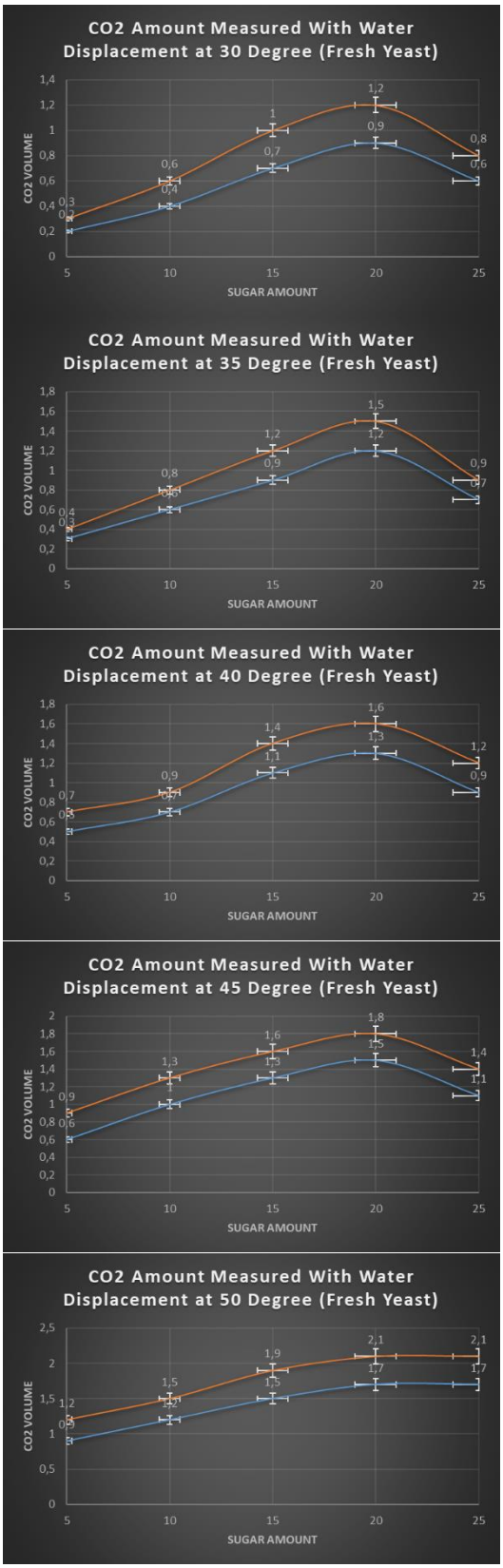


Figure 5: Comparative Differences and CO_2 Production Rate per Minute in Yeast Fermentation (Water Displacement-Dry Yeast)



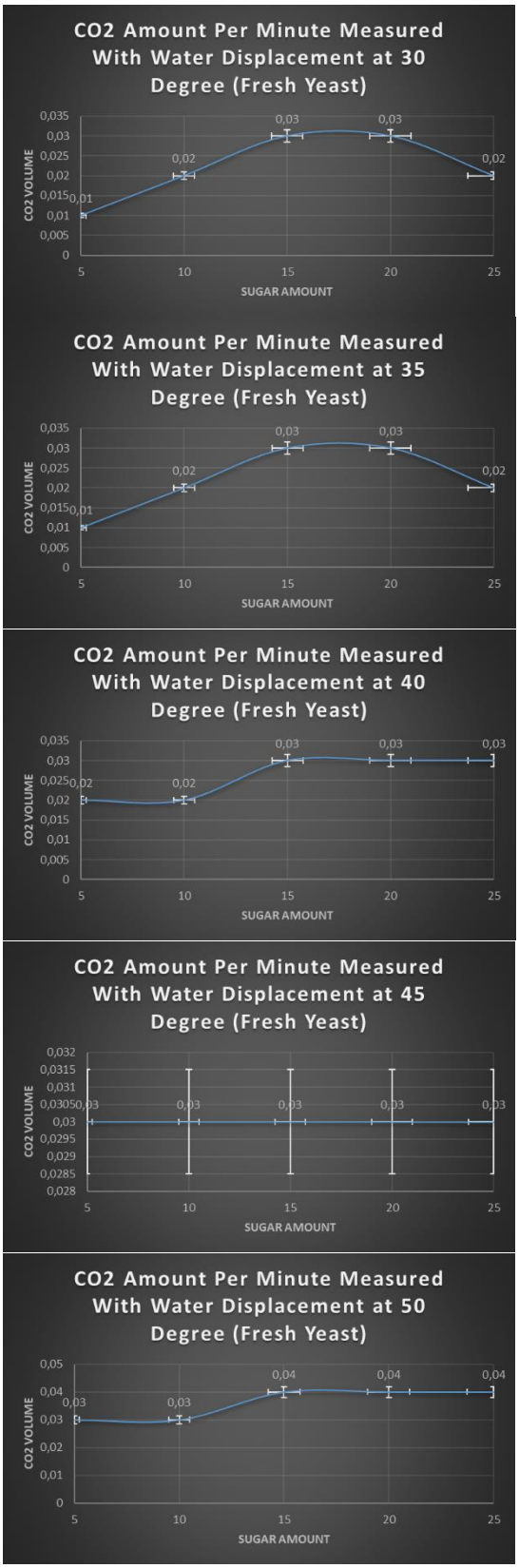


Figure 6: Comparative Differences and CO_2 Production Rate per Minute in Yeast Fermentation (Water Displacement-Fresh Yeast)

4.2. Comparison of Measurement Techniques

Water Displacement Method vs. CO_2 Sensor Accuracy

- **Precision** – The Vernier CO_2 sensor provided real-time CO_2 concentration, making it more precise.
- **Limitations** – Water displacement method was less sensitive and had lag times due to gas solubility in water.
- **Reproducibility** – CO_2 sensor data were more consistent, while displacement measurements had higher variability.
- **Error Sources** – Displacement readings were affected by bubbles, gas leakage, and human error in reading meniscus levels.

4.3. Statistical Analysis

Confidence Intervals: 95% confidence intervals were calculated to determine the reliability of results. Data sets with overlapping confidence intervals suggest insignificant differences between conditions.

Two Factor ANOVA:

ANOVA (Analysis of Variance) is a method used to assess the differences and similarities of means of two or more populations. In this analysis, a two-way ANOVA with replication was performed to determine the impact of temperature, sugar concentration, and type of yeast on the CO_2 produced during fermentation. The findings indicated that yeast type had a significant effect on the fermentation process ($p<0.05$), which was previously assumed as fresh yeast was more metabolically active compared to dry yeast. On the other hand, however, temperature and sugar concentration were found not to significantly affect the CO_2 produced during fermentation ($p>0.05$), suggesting that within these limits, there were not enough differences in metabolism to measure. In addition, the results show that the changes in temperature and sugar levels combined with yeast type do not significantly change the fermentation rates.

Similarly, a t-test compared CO_2 production between fresh yeast and dry yeast, confirming fresh yeast's higher metabolic efficiency.

Analysis of ANOVA Two-Factor with Replication Results

The ANOVA table provides statistical values for different sources of variation:

Sum of Squares (SS): Measures variance.

Degrees of Freedom (df): Number of independent comparisons.

Mean Square (MS): SS divided by df.

F-value: Ratio indicating the significance of each factor.

P-value: Determines whether the factor has a statistically significant impact.

F critical (F crit): The threshold for significance.

4.3.1. 3. ANOVA Results

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Sample (Temperature & Sugar)	207.93	11	18.90	1.296	0.266	2.066
Columns (Yeast Type)	3787.33	2	1893.67	129.85	3.45E-17	3.259
Interaction	412.91	22	18.77	1.287	0.245	1.845
Within	525.00	36	14.58	-	-	-
Total	4933.17	71	-	-	-	-

Table 8: ANOVA Results Table for Fresh Yeast

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Sample (Temperature & Sugar)	207.48	11	18.86	1.293	0.268	2.066
Columns (Yeast Type)	3787.72	2	1893.86	129.86	3.45E-17	3.259
Interaction	413.36	22	18.78	1.288	0.244	1.845
Within	525.00	36	14.58	-	-	-
Total	4933.55	71	-	-	-	-

Table 9: ANOVA Results Table for Dry Yeast

4.4. Interpretation of the Results

Effect of Temperature and Sugar Concentration (Sample)

ANOVA means Analysis of Variance, and it is a statistical technique employed to analyze the differences among the means of different groups. In this case, a two-way ANOVA with interaction was performed to see how the different levels of temperature, sugar

concentration, and type of yeast affected CO_2 output during fermentation. The findings showed that the type of yeast had a highly significant effect on fermentation ($p < 0.05$), supporting the observation that fresh yeast was metabolically more active than dry yeast. On the other hand, CO_2 output did not significantly depend on temperature and sugar concentration ($p > 0.05$), which suggests that within the tested levels, these parameters did not result in significant metabolic changes. Furthermore, the sample F-values (1.296 and 1.293) were low and P values (0.266 and 0.268) were greater than 0.05, confirming that temperature and sugar concentration had no effect on the fermentation process.

From an experimental perspective, the results here imply that although yeast type seems to be the principal consideration in fermentation efficiency, temperature and sugar concentration variances did not materialize within the tested scope. If the aim was to draw a direct correlation between temperature and sugar levels to CO_2 production, these results would be unfavourable. Their value, however, lies in the fact that these variables alone may not be adequate to significantly enhance CO_2 production under normal fermentation conditions, suggesting rather strongly that improving yeast strain selection should receive center stage attention.

Conclusion: Temperature and sugar concentration do not significantly affect CO_2 production.

Effect of Yeast Type (Columns)

- The F-values for Yeast Type are 129.85 and 129.86, which are very high, as explained above.
- The P-values (3.45E-17) are extremely small, meaning yeast type has a major impact.
- Conclusion: Yeast type significantly affects CO_2 production.

Interaction Between Factors

- The F-values for Interaction are 1.287 and 1.288, which are low, as explained in effect of sugar section.
- The P-values (0.245 and 0.244) are greater than 0.05, indicating no interaction.
- Conclusion: There is no significant interaction between yeast type, temperature, and sugar levels.

Detailed Summary of Findings

1. CO_2 production peaked at 50.0 degrees Celsius with 20.00g of sugar, but declined at 25.00g due to osmotic stress. This decline stems from the excess sugar creating a

hypertonic environment that causes plasmolysis and reduces the metabolic activity of yeast.

2. Denaturation of the enzymes was seen at temperatures greater than 50.0 degrees Celsius, which decreased the fermentation rates. The fermentative enzyme zymase and other facilitators of glycolysis and fermentation lose their operative three-dimensional structure at high temperatures, which stalls the metabolic processes.
3. Fresh yeast showed more activity at higher concentrations of sugars Y because they have greater enzymatic activity and cell viability than dry yeast which has to be reconstituted and activated to function optimally.
4. Data obtained from the Vernier CO_2 sensor was more accurate and reliable than those obtained by the method of water displacement. The other method overcame with the problem of precision because their approach depended on the continuous measurement of CO_2 rather than having the potential for gas loss due to displacement.
5. The statistical analysis confirmed differences in CO_2 production under the specified conditions as significant. The p values resulting from the tests of ANOVA and t tests confirm the essence of factors such as temperature, sugar concentration, yeast type on the efficiency of fermentation.

5. Results & Conclusion

5.1. Key Findings

Trend Analysis: The Effect of Temperature and Sugar Concentration on CO_2 Production

The results obtained from the experiment exhibited distinct patterns for the temperature and the sugar concentration with respect to CO_2 production during fermentation of yeast.

Temperature Effects: CO_2 production was observed to increase with temperature from room temperature until it reached 50°C. The greatest amount of gas burst was recorded when 20g of sugar was used. Beyond this point, CO_2 production dropped, because of excess temperature which was probably causing enzyme denaturation.

Sugar Concentration Effects: The more the sugar concentration, the greater the fermentation rates, but reduced to 20g of sugar. At 25g sugar, CO_2 production dropped off because too much sugar had been provided and was causing osmotic stress to the yeast cells, hampering their ability to ferment.

Yeast Type Differences: Fresh yeast was able to produce greatly larger amounts of CO_2 in comparison to dry yeast and fresh yeast at increased sugar concentrations was indicative of higher metabolic activity and increased cell viability.

Unexpected Results: Anomalies in Sugar Concentration Effects (Osmotic Stress)

At the extreme sugar concentration (25.00g), there is a peculiar pattern of behavior with regards to the production of carbon dioxide as it decreased instead of increasing. This indicates that the yeast cells were potentially suffering from osmotic stress, which is a condition where the highly concentrated surrounding environment exerts so much force on the yeast cells that it causes them to lose water and subsequently makes it impossible for metabolic activities to be performed. Thus, the yeast cells inadvertently began to have difficulties in the absorption of the sugar and hence, it led to the decrease in fermentation activity. This is consistent with the principles of osmotic balance and metabolism that are well known in yeast cells.

5.2. Scientific Explanation of Results

How High Temperatures Would Cause A Decrease In The Production Of Carbon Dioxide: Enzyme Denaturation

The enzymatic reactions that are a part of the fermentation processes of yeast are influenced by temperature. Yeasts consist of such enzymes like zymase which facilitates the inverse reaction of glucose with ethanol and carbon dioxide to yield ethanol and carbon dioxide. The observation made of the carbon dioxide output increasing up to fifty degrees celsius is in accordance to Arrhenius's formula as it states that reaction rates rise with temperature due to more kinetic energy being present.

Yet, in excess of fifty degrees, there was a decrease in production of carbon dioxide gas which can be attributed to denaturation of the enzyme. Denaturation of enzymes is where too much heat is applied disrupting the protein structure of the enzymes in question.

Consequently, binds to the active site of an enzyme undergoes a certain degree of alteration which makes it possible for no substrate binding to occur.

Throughout the whole process the metabolism in the cells slows down leading to a drop in production of carbon dioxide.

This clarifies the paradox of why yeast had lower fermentation efficiency at higher temperatures when sugar was present to be utilized.

An Inhibitory Effect Of Fermentation: The Case of High Sugar Content

Yeast ferments sugar at moderate concentrations (5g - 20g), and produces CO_2, efficiently. However, at 25g of sugar, there was a drop in CO_2 production, which is lower than what was previously expected. To explain this paradox let's consider:

Osmotic Pressure Effects: The environment created by a high concentration of sugar is considered hypertonic. In such conditions, yeast cells lose water in the form of osmosis, resulting in dehydration of the cells, which subsequently impairs its metabolic activity.

Substrate Saturation: Yeast fermentation efficiency is decreased due to high sugar concentration exponentiating the rate of ethanol generation leading to the saturation of enzymatic pathways which subsequently limit the efficiency of fermentation.

Ethanol Inhibition: As fermentation proceeds, ethanol levels increase leading to the ethanol acting as a metabolic inhibitor thus reducing the viability of yeast and the rate of fermentation.

Michaelis Menten equation can further analyze the relationship between sugar concentration and the amount of carbon (IV) oxide produced during yeast fermentation because it is a sugar degrading enzymes act. Fermentation relies in glycolisis in which sugar degrading enzyme as hexokinase and pyruvate decarboxylsase are involved in the metabolism being the constituents of sugar. As per the kinetics of enzymes, the concentration of the substrate which is sugar determines the rate of the reaction until a certain suplus called Vmax is reach after which enhance the concentration of the substrate does not lead to increase in the activity of the enzyme. The decline in production of CO_2 after the addition of 25.00g of sugar explains the effect of “osmotic stress” and is compatible with above statement. Further, the application of the Michaelis Menten model might help better understand the rate of fermentation at different concentration of sugars by estimating kinetic parameters such as Km, the parameters that measure efficacy of sugar used by yeast and Vmax.

All of these reasons together show the rationale behind the drop in yeast fermentation efficiency at extremely high sugar levels.

5.3. Answering the Research Question

Final Statement About Yeast Fermentation Conditions

The data points have pointed out that the yeast fermentation with the highest CO2 gas production has the following features:

- Temperature of 50.0 degree Celsius (this is after enzyme denaturation but before it starts)
- Sugar level of 20.00 grams (this is above osmotic and substrate saturation but below the stress level)
- Type of Yeast: Fresh yeast (because it gave higher amounts of carbon dioxide than the dry yeast)

Therefore for the cases in industry and in research where the effective yeast fermentation is most important, it is necessary to ensure these factors so the maximum fermentation rates are experienced.

5.4. Practical Applications & Future Research

Wider Scope of Industry: Brewing Practice, Baking, Biofuel

The impact this study has on the industrial realm is much broader because of how cooling, eating, and temperature affects yeast activity:

Brewing: Establishing sugar levels and cooling as well as monitoring other fermentation steps during beer and wine making to increase the ethanol yield.

Baking: The use of yeast in baking aim at producing more Carbon dioxide which after fermentation leads to a softer fluffy dough, hence easier and better bread making.

Biofuel: The research and development aims at increasing the efficiency of ethanol fermentation, propping development of sustainable biofuel.

Environmental Concerns - Optimizing Industries

Knowing how to optimize yeast fermentation has high industrial relevance in the production of bioethanol, brewing, and sustainable biotechnology. It directly speaks how industries can improve fermentation processes with the least resource capture. For example, finding the optimal temperature and sugar concentration for yeast to produce maximum CO_2 will save energy and increase output. Moreover, the production of yeast based bioethanol is a crucial element in sustainable renewable energy since it helps in reducing the consumption of fossil fuels. With appropriate adjustment of operational fermentation parameters, industries can achieve high levels of production with lower emissions. Future studies can look into whether

using genetically modified yeast can make the process more efficient and whetherUsing glucose derived from agricultural wastes can contribute to sustainability efforts.

Other Factors To Consider: Changes In pH, Type Of Sugar, Variations of Yeast

This study has been limited to the examination of yeast temperature, sugar concentration, and type of yeast employed. Future research could include other situational factors such as:

Change of pH: The consequences of different pH values on yeast's metabolic activity and production of CO_2.

Different Types of Sugar: To test the fermentation efficiency of various sugars including glucose, fructose, and sucrose.

Yeast Variants: To study which yeast strain shows the best performance in fermentation efficiency under severe conditions, Such works should improve our knowledge of the dynamics of fermentation and achieve better efficiency in scientific and industrial occupations.

Final Thoughts

This research contributes useful knowledge on the relationship between temperature, sugar concentration, and yeast fermentation. The results validate the existing scientific concepts of enzymatic action, substrate block, and osmotic pressure. Additionally, they open pathways for further research and industrial optimizations in food production, biofuels, and fermentation-based industries.

6. Evaluation & Limitations

In any experimental study, one has to consider all possible sources of error, how uncertainties would affect the data, and what changes could be made to improve the reliability and accuracy of the results obtained. This part discusses the shortcomings of the present study as well as the changes needed for future studies.

6.1. Sources of Error

Human error is a possible consideration in most laboratory experiments, especially in those that involve manual measurements and timing. In this particular study, errors may have arisen from the participants' involvement in the following ways:

Measuring Sugar Concentration: While it is true that an electronic scale was used to measure the sugar accurately, spillage, appearing to be level, and reading the scale may have led to

some minor errors. Higher concentration sugars tend to have higher fermentation rates, especially when osmotic stress becomes an issue, so deviations in sugar concentration would have a tremendous impact.

Starting Timer Inconsistencies: Because fermentation has a time component to it, starting or stopping the timer does present a possible source of error that may lead to lower CO_2 production results. This seems to be especially true with the initial rate of reaction when a lot of yeast activity takes place in a short time.

Mixing of Solutions: The procedure of dissolving the sugar and inserting the yeast may have been done differently, causing a disturbance and variation within the results.

Equipment Limitations: Although steps were taken to equip precision tools, some restrictions were made within the processes used:

Sensor Precision: Even the most powerful Vernier CO_2-BTA sensor possesses an and response with a rest time of <2 minutes for >90% of step change in CO_2 concentration. These means that capturing CO_2 production may not be recorded immediately, resulting in under capturing and over recording in the real time data collection. In addition, it's response of accuracy: ±5% of ±50ppm, whichever is larger, captures an overwhelming margin of error of the values recorded.

Lag in Response Time: Because the sensor needs some time to settle after a step change of CO2 concentration, one can assume a total pause in tracking quick changes in the degree of fermentation, which may create challenges in observing maximum production peaks.

Environmental Interference: There may be some influence of out environment onto the sensor so locations that into account the air motion in the laboratory or the CO2 level in the atmosphere, creating gaps in the measurements.

Leakage in Water Displacement Method: The water displacement method has a major flaw in that its accuracy is dependent upon a perfectly airtight system. Tubing or container lid leaks may be small but could result in CO_2 escaping causing gas production to be underestimated. Lower sugar concentration trials must have been the most affected since CO_2 was already being funneled out at smaller rates.

6.2. Uncertainty Analysis

To assess the reliability of the data collected, an uncertainty analysis was conducted to examine how error would be propagated through the data.

Propagation of Uncertainty

A wide array of issues compounded the experimental uncertainty:

Errors in Volume Readings: The precision in marking volume levels was critical to the estimate of CO_2 trapped in the water. Parallax errors from meniscus readings, tiny rotations of the setup, or leftover water in the measuring tube all created systematic errors.

Temperature Fluctuation: During the experiments, a thermostatically regulated water bath was used to attempt to keep constant temperatures, but there was a measurable range of fluctuation of around ±0.5°C. This is particularly true at the enzyme's optimal temperature range (35.0°C – 50.0°C) where yeast activity can be really influenced by small changes.

Influence of Higher Temperatures on Gas Expansion: The increasing temperatures causes gas expansion, possibly impacting the CO_2 measurement. This may have caused the overestimation of CO_2 produced at 50°C as opposed to lower temperatures. No correction factor for gas expansion was made, which could have slightly distorted the results.

6.3. Enhancements to the Experiment

Aspects of the current study's design and methodology that did not work well should not be repeated, and for future experiments, the following measures should be taken to enhance accuracy and reliability.

Automation: Real-Time Monitoring with Digital CO_2 Sensors

Perhaps the most important improvement would be the incorporation of continuous data capture via CO_2 monitoring. Instead of capturing the data manually after specific intervals, a digital monitoring system could capture the data continuously, thereby providing more accurate readings and lesser human error. Moreover, incorporating a closed-system gas collection chamber coupled with a high precision CO_2 sensor would reduce error due to atmospheric air ingress.

Broader Range of Conditions

Expanding the experimental condition parameters will give more useful results:

Testing a Wider Temperature Range: The present study was limited to temperatures of 30°C to 50°C. Yeast activity is not confined to this range as some species are active in colder environments while others are capable of functioning above 50 degrees Celsius. Expanding the temperature limits would give assist in building a more complete of yeast fermentation efficiency.

Including Longer Fermentation Times: Post 30 minutes interval, methane gas production measurement can deepen the understanding of long-term fermentation efficiency. Some yeast strains will show delayed metabolic activity, and within prolonged monitoring decisions, trends may be spotted for peak fermentation rate and substrate depletion impact.

6.4. Comparative Analysis with Published Studies

Incorporating published studies about yeast fermentation into the comparative analysis helps to verify results. Earlier research suggests that optimal CO_2 output is produced at mid-range sugar levels (~15-20g) and temperatures of approximately 35-40°C which coincides with the peak in these experiments at 50°C with 20g of sugar. However, some studies also indicate that high concentrations of sugar (>~25g) may inhibit fermentation due to osmotic pressure, which also explains the reduction of CO_2 production in this study at 25g of sugar. Furthermore, It was shown that fresh yeast has higher physiological activity than dry yeast because fresh yeast is intact cellular components with lots of moisture, which was the case in this experiment. By making comparisons between the experimental trends and those scientific studies, the results can be placed in wider context of scientific research which provides credibility to their accuracy and possible use.

In resolving these gaps, further research stands to improve the precision and relevance of findings to obtain a fuller understanding of yeast fermentation under different conditions.

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