The effect of type of yeast and temperature on CO_2 production

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Abstract

This study investigates how the temperature impacts CO2 production in 2 different types of yeast i.e. Baker's yeast and Brewer's Yeast. Using a water displacement volumetric gasometer, the time taken for CO_2 bubbles to appear and fill up the tubes was measured at 25°C and 35°C. A two-way ANOVA was used to confirm the effects of temperature, yeast type and the interaction effect on CO_2 production. The findings and results of this experiment were supported by prior research on yeast fermentation (Zakhartsev et al. 2015).

Introduction

Yeasts are microorganisms that are widely found in nature and play essential roles in both ecological cycles and industrial applications. In the food and brewing industries, selecting the appropriate yeast species and controlling fermentation temperature are crucial factors for optimizing product quality and yield (Kuloyo et al. 2014). Fermentation is a biological process that convert sugars into ethanol and CO_2 anaerobically. The rate and efficiency of this process depend on multiple factors, with temperature being one of the most significant. Previous research has shown that increased temperature generally raises the metabolic activity rate, leading to faster CO_2 production (Zakhartsev et al. 2015).

Different yeast strains vary in their fermentation efficiency. While baker's yeast is known for rapid fermentation at higher temperatures, brewer's yeast is suited to cooler conditions, making it ideal for lager brewing. This study builds on existing research in temperature-dependent fermentation kinetics, employing a structured methodological framework to examine the temperature's effect on CO_2 production rates (Jones et al. 1970, Yamagishi et al. 2010).

We hypothesize that temperature significantly impacts CO_2 production rates, with distinct responses from baker's yeast and brewer's yeast. Specifically, we expect that CO_2 production will vary significantly between 25°C and 35°C, and that the two yeast types will demonstrate different rates of CO_2 production under these conditions. Additionally we predict an interaction effect between temperature and yeast type, where each yeast response to temperature changes will differ. By recording the time required to produce a specific amount of CO_2 at 25°C and 35°C, this experiment aims to demonstrate the effect of temperature on

CO_2 production rate using replicable methods, providing valuable data for ecological understanding and industrial applications.

Methods

The experiment is conducted at 2 different temperatures (25°C and 35°C) for baker's yeast and brewer's yeast to investigate CO_2 production by recording the time required for the CO_2 to fill the tubes while using the gasometer.

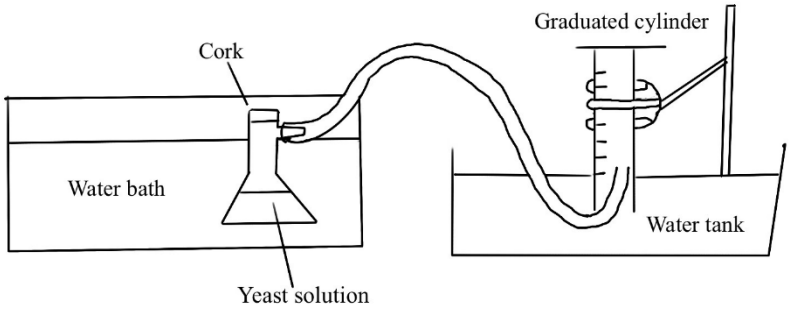


Figure-1. Experiment setup. Here is a schematic diagram showing how the experiment apparatus was set up. On the left, there is a water bath used to control the temperature of the yeast solution. Inside the water bath, a filtering flask is placed with the opening sealed with a cork; the rubber tube connects the filtering flask and extends to a graduated cylinder on the right. The graduated cylinder is inverted in the water tank with water filled to the top. As the fermentation occurs and CO_2 is produced by the yeast, the gas travels through the tube into the graduated cylinder, displacing water gradually. During the actual experiment, the setup had multiple set of gas collecting system for different yeast and trials and control.

Figure-2. Yeasts. This is the two types of yeast used in the experiment.

Preparation and sample collection:

After setting up the apparatus, we prepared yeast stock solutions. We weighed 10 grams of yeast and dissolved it in 100 mL of 0.35 M sucrose solution prepared with distilled water. We simultaneously prepared two types of yeast (baker's yeast and brewer's yeast) and allowed both stock solutions to sit for 5 minutes to activate. After 5 minutes, we ensured the stock solutions were in suspension, pipetted 30 μL of each yeast solution into an Eppendorf tube, and fixed it with 70 μL of 100% ethanol for later hemocytometer analysis.

We transferred 30 mL of each stock solution into the filtering flask and conducted a total of six trials for each type of yeast. We started the timer as soon as the solutions were submerged in the water bath, which we set to the desired temperature. When the CO_2 displaced all the water in the graduated cylinder, we stopped the timer and recorded the results. We then pipetted another 30 μL of each yeast solution into an Eppendorf tube and fixed it with 70 μL of 100% ethanol for comparison with the initial samples. We repeated these steps under other temperature conditions.

Control of variables:

We ensured consistency by conducting all trials under the same laboratory conditions, using the water bath system to maintain a stable water temperature throughout the experiment. We conducted all trials simultaneously to minimize errors caused by variations in the activation state over time. We also deployed a negative control group, using only sucrose solution in the flask connected to the graduated cylinder.

Data recording and analysis:

The time required to displace all the water from the graduated cylinder by CO_2 was recorded for each trial. The data were analyzed using ANOVA (Analysis of Variance) to assess the effects of temperature and yeast types on CO_2 production rates. ANOVA was chosen as it allows for comparison of multiple groups and interactions between factors, helping to determine whether temperature and yeast type have statistically significant effect on fermentation rates. Hemocytometer analysis of the initial and final samples was conducted to measure any changes in yeast concentration.

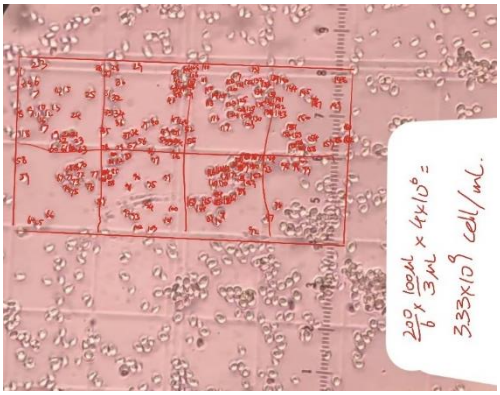


Figure-3. Hemocytometer. This image shows the use of a hemocytometer to count cells in a yeast sample. The cells within the highlighted grid squares have been counted and the final concentration calculation is shown on the right side of the image. Here the dilution factor is 100 μL/ 3μL, because the fixed sample (30 μL yeast and 70 μL ethanol) is diluted again with 90 μL of distilled water and 10 μL of fixed sample; the cell count was using the smallest gird, therefore the grid-specific factor is 4×10^6, results in an estimated cell concentration of 3.33×10^9 cells/mL

Results

Table-1. Time (s) Required to Displace All Water from the Graduated Cylinder under Different Yeast and Temperature Conditions.

Temperature (°C)	Baker's yeast	Brewer's yeast
25	252	1100
	285	1020
	N/A*	993
35	126	480
	108	504
	106	491

*Data missing due to equipment malfunction.

Table-2. Two-way ANOVA Results for Temperature and Yeast Type Interaction on CO_2 Production

	Df	Sum Sq	Mean Sq	F value	P-value
Temperature	1	498426	498426	480.01	1.04e-07***
Yeast type	1	822811	822811	792.42	1.83e-08***
Temperature: Yeast type	1	101834	101834	98.07	2.283e-05***

This table presents the results of a two-way ANOVA, which evaluates the effects of temperature, yeast type, and their interaction on CO_2 production.

Df (degrees of freedom) indicates the number of levels for each factor minus one. For example, temperature and yeast type each have one degree of freedom because there are two levels for each factor (e.g., 25°C vs. 35°C, baker's yeast vs. brewer's yeast).

Sum Sq (sum of squares) represents the variation in CO_2 production attributed to each factor. Larger values indicate a greater contribution of the factor to the observed variation.

Mean Sq (Mean Squares) is calculated by dividing the Sum of Squares by the Degrees of Freedom. It reflects the average variation attributed to each factor.

The F value tests whether the variation attributed to a factor is significantly greater than random variation. Higher F values suggest a stronger effect. For instance, yeast type has the

highest F value (792.42), indicating a very strong effect on CO_2 production.

P-value shows the probability of observing the data if the null hypothesis (no effect) is true. Values below 0.05 (commonly used threshold) indicate statistically significant effects.

Table-3. Cell Count (cell/mL) Comparison of Baker's and Brewer's Yeast at 25°C and 35°C

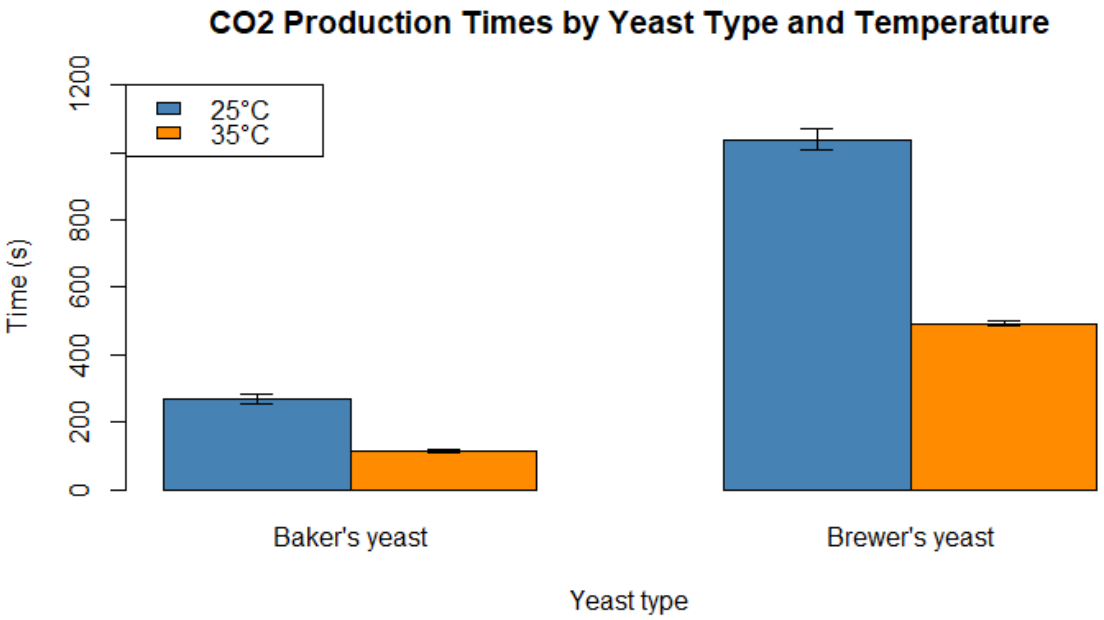
Cell count (cell/mL)	Baker's yeast		Brewer's yeast	
Temperature (°C)	Initial	Final	Initial	Final
25	2.05×10^9	3.33×10^9	4.44×10^9	3.81×10^9
35	2.05×10^9	3.81×10^9	4.44×10^9	5.33×10^9

The results from table-1 indicate that the CO_2 production time varied significantly between 25°C and 35°C for both types of yeast. Baker's yeast showed a faster CO_2 production rate at higher temperatures. Table-2's ANOVA results further confirm that both temperature and yeast type, as well as their interaction, had statistically significant effects on CO_2 production time ($p < 0.001$). In table-3, it is observed that cell count also increased with higher temperatures for both types of yeast.

Discussion:

The results of this study demonstrate that both temperature and yeast type significantly impact CO_2 production rates during fermentation, with an observable interaction effect between these two variables. This data aligns well with our hypothesis, higher temperatures (35°C) generally led to faster CO_2 production compared to lower temperature (25°C) for both yeast types. The baker's yeast population at higher temperature outgrew the population at lower temperature by 4.8×10^8 cell/mL with about half of the growing time, and the brewer's yeast also had a similar result. This supports the idea that increased temperatures enhance yeast metabolic activity, aligning with previous research showing that temperature is a critical factor in optimizing fermentation rates (Kuloyo et al. 2014).

The statistical analysis further supports these findings. The two-way ANOVA revealed significant effects of both temperature and yeast type on CO_2 production rates, as well as a significant interaction effect between the two factors. Given the p-values for all factors and their interaction (all < 0.05), we can confidently reject the null hypothesis, which stated that temperature and yeast type have no significant effect on CO_2 production rates and that there is no interaction effect between them.



Graph-1. CO_2 production times by yeast type and temperature, a visual representation of table-1.

Interestingly, the two yeast strains—baker's yeast and brewer's yeast—exhibited distinct responses to temperature variations. At 35°C, baker's yeast showed a significant increase in CO_2 production rate compared to 25°C, with a 2.37-fold increase, reflecting its optimal activation temperature as suggested on the product label. Brewer's yeast, however, displayed a smaller increase in CO_2 production rate, only 2.11 times faster at 35°C than at 25°C. This difference suggests that baker's yeast may be better adapted to higher temperature, whereas brewer's yeast maintains a steadier fermentation rate at specific temperature.

The cell counts further support this observation: at lower temperatures, the cell counts of brewer's yeast decreased compared to its initial count, potentially due to the Crabtree effect. This effect occurs when environmental conditions, such as a lower temperature, shift the balance between respiration and fermentation, favoring respiration over fermentation in the presence of abundant substrate (Deken 1966). These findings align with previous observations on yeast metabolism, highlighting how different yeast strains adapt their metabolic processes under varying environmental conditions (Hommes 1966).

The significant interaction effect between temperature and yeast type further highlights that these factors do not operate independently. Instead, specific combinations of yeast strain and temperature lead to different fermentation efficiencies. This finding is particularly relevant for industries relying on precise control of fermentation conditions, as it emphasizes the importance of selecting yeast types best suited for specific temperature ranges to

maximize CO_2 production (Steensels et al, 2014).

However, several limitations of this study should be considered. First, the experiment was conducted under controlled laboratory conditions, which may not fully replicate the complexities of industrial environments. Additionally, only two temperature points (25°C and 35°C) were tested, which limits our ability to determine the precise optimal temperature for each yeast type. Future studies could explore a wider range of temperatures to better identify these optimal conditions. Furthermore, conducting experiments with lower yeast concentrations and extending the observation period may provide deeper insights into the dynamic aspects of yeast metabolism. This approach would allow for a slower rate of CO_2 production, enabling CO_2 output to be plotted against time to observe production trends more accurately.

Conclusion

In conclusion, this study enhances our understanding of fermentation kinetics by offering useful information on how temperature and yeast type affect CO_2 production. By revealing the distinct responses of baker's and brewer's yeast to temperature variations, these findings have practical implications for optimizing fermentation processes in both the food and brewing industries. Future research could expand on this work by investigating other variables, such as pH levels and sugar concentrations, to refine our understanding of the factors influencing yeast fermentation.

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