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Evaluation of the Impact of Temperature on Ethanol Production from Molasses using *Saccharomyces cerevisiae* to Determine the Optimal Temperature for Maximum Yield

Esele Stephen and Byaruhanga Ivan

Department of Physical Sciences, School of Natural and Applied Science, Kampala International University, Uganda

ABSTRACT

Molasses is a viscous by-product of refining sugarcane into sugar, that has been considered as a cheap source of raw material for industrial ethanol production. The experiment was designed to determine the optimal temperature for ethanol production using a specific fermentation process. Fermentation experiments were conducted using a standardized procedure with varying temperature conditions. Samples were collected and analyzed to measure ethanol concentration at each temperature point. In this study, the ability of ethanol production from molasses using *Saccharomyces cerevisiae* was investigated at different temperatures (32°C, 34°C, and 36°C) on the scales of 500 ml, 2000 ml molasses solution, and 34°C was selected as the appropriate temperature to carry out the fermentation scale at 5000 ml of molasses solution. Experiments were carried out in molasses with an initial sugar concentration of 85 Brix, pH of 4.5, and fermented for 3 days (72 hours). In the fermentation of 500 ml of molasses solution, Ethanol concentrations of 8.6, 9.4, and 7.5% (v/v) were achieved at 32, 34, and 36, respectively. The results of the ethanol production at 34°C from 2000 ml of molasses solution showed that the ethanol concentration was 9.7% (v/v), the level was high compared to other treatments at two other temperature ranges. Ethanol concentrations of 8.8% and 8.2% (v/v) were obtained at 32 and 36, respectively. From the results, 34°C was selected as the optimal temperature to carry out the fermentation experiment at the scale of 5000 ml of molasses solution with an ethanol concentration of 9.8% (v/v) was achieved. The study found that the optimal temperature for *Saccharomyces cerevisiae* to make ethanol at the pH of 4.5 was 34°C, yielding the highest amount of ethanol at 9.8% (v/v).

Keywords: Ethanol, *Saccharomyces cerevisiae*, Molasses, Fermentation, Temperature

INTRODUCTION

Saccharomyces cerevisiae, commonly known as baker's yeast or brewer's yeast, is a vital microorganism utilized extensively in industrial processes, particularly in converting sugars into ethanol through fermentation [1] This ability has rendered it indispensable in sectors such as alcoholic beverage production, biofuels, and biochemical manufacturing. Ethanol, a crucial compound in both industry and consumer applications, has historically been pivotal as a lamp fuel and later gained widespread use in beverages and solvents [2] Molasses, a by-product of sugar refining, has emerged as an economically viable feedstock for ethanol production due to its high concentration of fermentable sugars like sucrose, glucose, and fructose [3] Being a by-product, molasses is readily available and cost-effective, making it a sustainable choice for ethanol production [4]. However, optimizing the conversion of molasses into ethanol depends on various factors, with temperature playing a critical role. Research has consistently shown that temperature significantly impacts ethanol fermentation efficiency when using yeast cells. The optimal temperature range for yeast fermentation typically falls between 30°C and 35°C, where yeast cells exhibit maximal metabolic activity and ethanol productivity [5] Temperature influences yeast metabolism, enzyme activity, and fermentation kinetics, directly impacting ethanol yields [6] Deviations from this optimal range can lead to reduced ethanol production and metabolic stress on yeast cells [7]. Despite the extensive use of *Saccharomyces cerevisiae* in ethanol production, understanding the specific effects of temperature on fermentation dynamics, especially in molasses-based ethanol production, remains crucial. By thoroughly investigating the influence of temperature on fermentation kinetics, yeast metabolism, and ethanol production from molasses, this study aims to provide insights that can optimize ethanol production processes. Such optimizations are vital for enhancing yields and ensuring the sustainability of ethanol production practices, which are increasingly important in the context of renewable energy initiatives [8]. Previous studies, such as those by Sharma et al. [9] and Singh et al. [10], have explored the impact of temperature on ethanol fermentation using molasses. They found that precise temperature control within the optimal range enhances ethanol productivity and purity while minimizing undesirable byproducts. These findings underscore the importance of temperature management in maximizing ethanol yield and quality during fermentation processes. The overarching objective of this research is to identify the optimal temperature range that maximizes ethanol yield from molasses fermentation by yeast cells. This investigation is crucial given the challenges in optimizing fermentation conditions for efficient ethanol production. Temperature control strategies are essential not only for maximizing ethanol production efficiency but also for maintaining consistent fermentation performance [11,12]. Temperature directly influences enzymatic activities like glucoamylase, which is crucial for efficient sugar conversion during fermentation [13]. Therefore, understanding and effectively managing temperature conditions are pivotal in ensuring sustainable and efficient ethanol production processes [14]. Improving ethanol production efficiency holds significant potential benefits, including reduced greenhouse gas emissions and mitigating climate change by providing a cleaner alternative to fossil fuels [15]. Furthermore, advancements in ethanol production technology through temperature optimization can have positive implications across various industries, including automotive, pharmaceuticals, and beverages, where ethanol serves as a crucial raw material [17]. This study aimed to contribute valuable insights into the temperature-dependent factors influencing ethanol production from molasses. By enhancing our understanding of how temperature affects yeast fermentation dynamics and ethanol yields, this research seeks to advance biotechnological and industrial microbiology knowledge. Ultimately, optimizing ethanol production processes through temperature control can lead to sustainable practices and economic benefits, further solidifying ethanol's role as a vital component of renewable energy solutions.

MATERIALS AND METHODS

Sample Collection

The sample (sugarcane molasses) was obtained from the factory molasses tank of the Sugar Corporation of Uganda Limited (SCOUL), located at Lugazi town council in Buikwe district. It was placed in a clean beaker and kept at a room temperature of 25°C before being used. The yeast cells (*Saccharomyces cerevisiae*) used were obtained from the distillery main laboratory at the Sugar Corporation of Uganda Limited (SCOUL).

Sample Pretreatment

The sample of molasses was treated to remove dirt, sludge, sand, and other unwanted impurities. The substance for treatment was concentrated H_2SO_4 to remove other unwanted microorganisms.

Mash Preparation and Fermentation

Ethanol production from 500 mLs of molasses solution

Molasses (50 g) and distilled water (450 mL) were measured and mixed in an Erlenmeyer flask to create the culture medium. 10 g of yeast cells were weighed out and added to the culture medium in the Erlenmeyer flask. Sterilization and adjustment of nutrient levels were performed to optimize conditions for fermentation. The initial pH of the culture medium was determined. Phosphoric acid was then added as needed to adjust the pH to the desired level for fermentation. Three separate Erlenmeyer flasks were set up for fermentation at 32°C, 34°C, and 36°C. Portions of the culture medium were transferred into each flask. The flasks were sealed with stoppers or cotton plugs to allow for gas exchange while preventing contamination. The flasks were placed in temperature-controlled environments set to 32°C, 34°C, and 36°C, respectively. Fermentation was allowed to proceed for 72 hours. The fermentation process was regularly monitored for parameters such as pH, and temperature. At the end of the 72-hour fermentation period, fermented wash was taken from each flask. The samples were analyzed for various parameters, including pH, ethanol concentration, and residual sugars. The results obtained from fermentations conducted at different temperatures were compared. The efficiency of fermentation at each temperature was evaluated based on the analyzed parameters. All observations, measurements, and analysis results were recorded. Any deviations from expected outcomes and potential reasons for these differences were documented.

Ethanol Production from 2000 mLs of Molasses Solution

Molasses (200 g) and distilled water (1800 mL) were measured and mixed in an Erlenmeyer flask to create the culture medium. 25g of yeast cells were weighed out and added to the culture medium in the Erlenmeyer flask. All necessary pretreatment procedures, such as sterilization and adjustment of nutrient levels, were performed to optimize conditions for fermentation. The process continued as in the case (500 mL) above.

Ethanol Production from 5000 mLs of Molasses Solution

Molasses (500 g) and distilled water (4500 mL) were measured and mixed in an Erlenmeyer flask to create the culture medium. 50g of yeast cells were weighed out and added to the culture medium in the Erlenmeyer flask. All necessary pretreatment procedures, such as sterilization and adjustment of nutrient levels, were performed to optimize conditions for fermentation. The process continued as in 500 mL above. The methods used for the determination of ethanol concentration and residual sugars were distillation and titration respectively [18].

RESULTS

The fermented wash was obtained and analyzed to determine the ethanol content using the distillation method at different experiments.

Ethanol Production in 500ml of Molasses Solution using Yeast Cells (*Saccharomyces cerevisiae*)

Table 1: Ethanol Production in 500ml of Molasses Solution using Yeast Cells (*Saccharomyces cerevisiae*)

Temperature (°C)	pH before Fermenta	Total Reducing Sug	Ethanol Concentrat	Residual Sugar (RS)
32	4.5	46.51	8.6	2.18
34	4.5	46.51	9.4	1.96
36	4.5	46.51	7.5	2.35

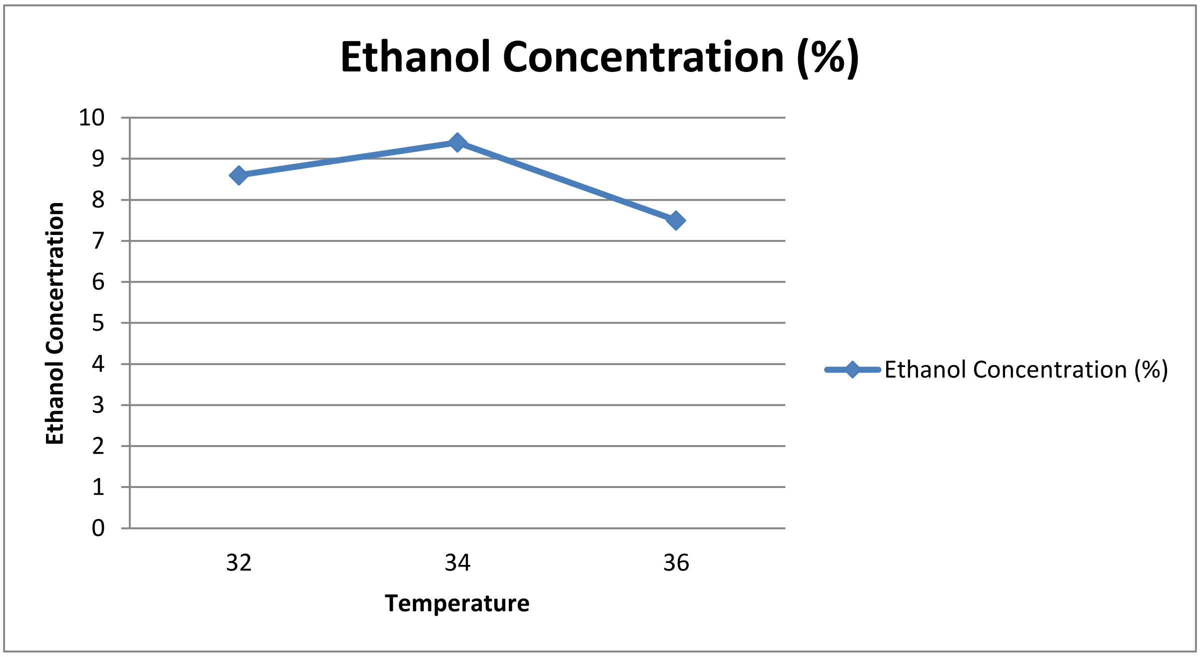

Figure 1: Ethanol Concentration

Fig 1: Shows a graph of ethanol concentration at 500 ml of molasses using *Saccharomyces cerevisiae* at 32°C, 34°C and 36°C

Ethanol Production from Molasses Solution at the Scale of 2000 mL

Molasses was prepared and fermented at 2000 mL. After 3 days of fermentation, samples were taken to measure residual sugar, Brix, and ethanol concentration.

Table 2: Ethanol Production using Yeast Cells at 2000 mL of Molasses Solution

Temperature (°C)	pH of Fermentation	Total Reducing Sug	Ethanol Concentratio	Residual Sugar (RS)
32	4.5	43.4	8.8	2.12
34	4.5	43.4	9.7	1.88
36	4.5	43.4	8.2	2.33

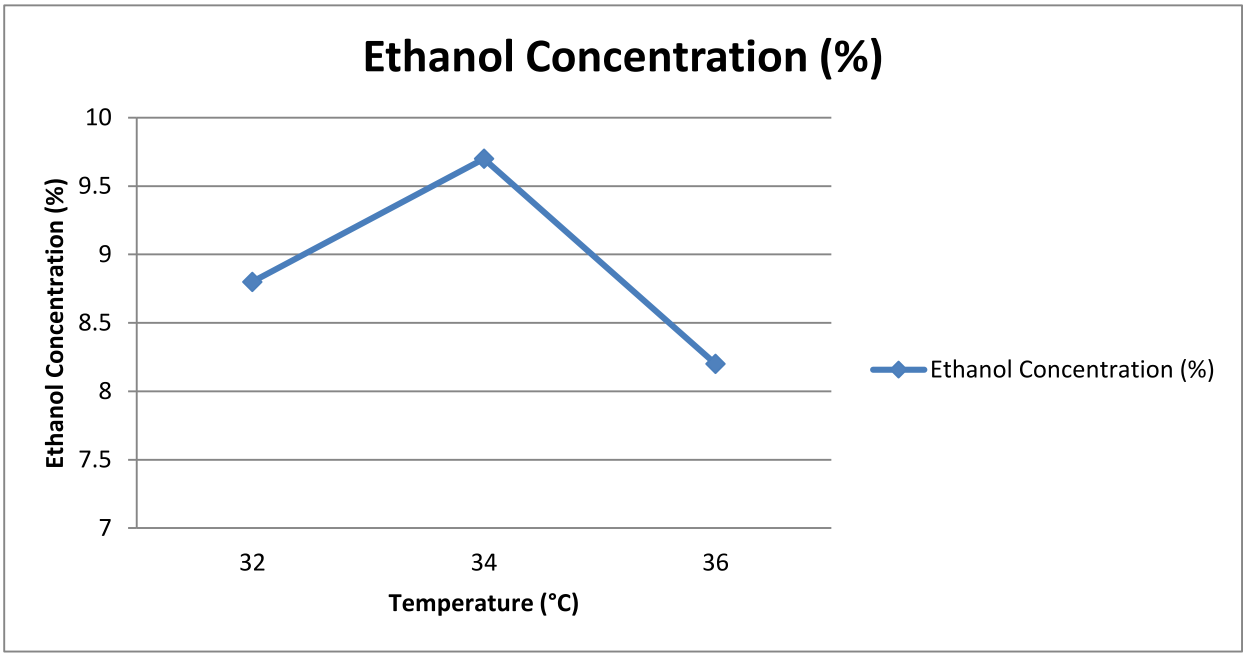

Figure 2: Shows a graph of ethanol concentration at 2000ml of molasses solution

Ethanol Production at 5000ml (5L) of Molasses solution at 34°Cusing *Saccharomyces cerevisiae*

Table 3: Ethanol Production at 5000 mL (5L) of Molasses solution at 34°Cusing *Saccharomyces cerevisiae*

pH	Volume (ml)	Temperature (°C)	Total Reducing Sugar	Ethanol Concentratio	Residual Sugar
4.5	5000	34	47.9	9.8	1.45

DISCUSSION

Ethanol Production at the scale of 500 mL of Molasses Solution

After the fermentation of molasses solution by *Saccharomyces cerevisiae*, the samples were taken to measure residual sugar and ethanol content. The results in Table 1 shows that *Saccharomyces cerevisiae* had a good ability to produce ethanol from molasses, especially at 34°C, where a high ethanol concentration of 9.4 % was obtained. At 32°C, most of the sugar was used for the growth of yeast instead of fermentation, so the resulting ethanol concentration was lower.

High temperatures inhibited yeast growth and fermentation activity which resulted to a low Ethanol concentration at 36°C since the activity of the *Saccharomyces cerevisiae* was slowed down due to higher temperature. Stressful conditions such as high temperature easily affect the action of the organism adversely [19].

Ethanol Production from Molasses Solution at the Scale of 2000 mL

Molasses were prepared and fermented at 2000 mL. After 3 days of fermentation, fermented wash was taken to measure residual sugar, Brix, and ethanol concentration at respective temperatures. It was found that the ethanol content at 34°C was 9.7% (v/v) compared to ethanol contents at 32°C and 36°C which were 8.8 and 8.2 % (v/v) respectively, this meant that the ethanol concentration was high at 34°C the reason at 32°C is that the yeast cells used the sugars for growth and expansion instead of fermentation. At 36°C, the ethanol concentration was low due to high-temperature inhibiting yeast growth and fermentation.

Ethanol Production using Yeast Cells at the Scale of 5000 mL (5L) at 34°C

Molasses were prepared and fermented at the scale of 5000 mL (5L). After 72 hours of fermentation, the fermented wash was obtained and measured for Brix, Ethanol concentration, and residual sugars. Results showed that the ethanol concentration increased by increasing the fermentation scale, with the highest ethanol concentration of 9.8% (v/v) obtained with 5000ml of molasses solution. The results in Table 3 show that at 34°C, the ethanol concentration was 9.8% (v/v), indicating it was the optimum temperature from the experiments performed. Therefore, the best yield was found at 34°C out of the three temperature ranges used. Proper temperature control is essential for yeast to react throughout the fermentation process. Yeast is killed by excessive heat, whereas yeast activity is slowed by low temperatures. Therefore, maintaining a precise temperature range is essential. Asmamaw et al [20] earlier reported 34°C as the ideal temperature for optimum ethanol production by *Saccharomyces cerevisiae.*

CONCLUSION

Ethanol fermentation using yeast cells (*Saccharomyces cerevisiae*) at the scale of 500ml of molasses solution showed that a fermentation temperature of 34°C was selected as the optimum temperature for the fermentation process, with a concentration of 9.4% (v/v) ethanol obtained. Similarly, ethanol fermentation at the scale of 2000 mL of molasses solution demonstrated that the fermentation temperature of 34°C yielded 9.7% (v/v) ethanol. The highest concentration of 9.8% (v/v) ethanol was achieved at 34°C at the scale of 5000 mL of molasses solution. The results of this study demonstrate that sugarcane molasses is a good source of ethanol, as indicated by the high yields obtained, with the optimum temperature found to be 34°C at a pH of 4.5. Yeast, the microorganism responsible for fermenting sugars in molasses into ethanol and carbon dioxide, has an optimal temperature range for its enzymatic activity. At lower temperatures, yeast activity slows down, leading to slower fermentation rates and potentially lower ethanol production. Conversely, at higher temperatures, yeast can become stressed or even die, reducing its ability to ferment sugars effectively. At 34°C, the temperature likely falls within the optimal range for yeast activity during molasses fermentation. This temperature allows yeast to efficiently convert sugars into ethanol while maintaining its metabolic functions. As a result, the highest concentration of ethanol is achieved at this temperature compared to 32°C and 36°C, where yeast activity may be suboptimal or inhibited.

RECOMMENDATIONS

Temperature Control: Ensure precise temperature control during fermentation processes. Use appropriate heating or cooling systems to maintain a consistent temperature of around 34°C throughout the fermentation tanks or vessels. Monitoring and Adjustment: Regularly monitor the temperature during fermentation and make necessary adjustments to maintain it within the optimal range. Small fluctuations in temperature can affect yeast activity and ethanol production. Choose yeast strains that are known to perform well at temperatures around 34°C. Different yeast strains have varying temperature tolerances and optimal ranges for fermentation. Optimize fermentation conditions such as pH, nutrient levels, and oxygenation to complement the optimal temperature range for ethanol production. A well-balanced fermentation environment can enhance yeast performance and ethanol yield. Implement quality control measures to ensure consistent fermentation outcomes and ethanol quality. Regularly test samples for ethanol concentration, fermentation efficiency, and any potential contaminants. Invest in ongoing research and development efforts to explore ways to further enhance ethanol production efficiency and yield at the optimal temperature of 34°C. This could involve testing new yeast strains, fermentation techniques, or additives. Implementing these recommendations, can maximize ethanol production and ensure consistent quality during molasses fermentation processes.

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