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Bioethanol Production from Sugarcane Bagasse by Fermentation Process Using *Saccharomyces cerevisiae* as Yeast Species

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ABSTRACT

Bioethanol is a renewable energy source derived from the fermentation of plant sugars and starches. It is generated using agricultural crops including maize, rice, sugarcane, potatoes, and beets, as well as waste items such as grapes, bananas, dates, and others. The majority of energy consumption in Uganda is derived from fossil fuels, including coal, crude oil, and natural gas. Regrettably, a significant issue has arisen due to the depletion of natural resources and the subsequent increase in greenhouse gas emissions, hence exacerbating global climate change. Hence, it is crucial to establish a sustainable energy source that has a reduced impact on the climate. The objective of this research was to efficiently generate bioethanol from sugarcane bagasse via the process of fermentation using Saccharomyces cerevisiae. The study also sought to assess the impact of pH and temperature on both the yield and concentration of bioethanol in water. Alpha-amylase and glucoamylase enzymes were used to degrade the cellulose present in sugarcane bagasse. In the experiment, fermentation was carried out using yeast (Saccharomyces cerevisiae) as well. Five samples were subjected to various preparation methods, with pH being altered to assess the impact of pH on bioethanol production at a consistent temperature of 350C. Another set of five samples was created to evaluate the influence of temperature on bioethanol yield while maintaining a constant pH level of 4.5. The potency of bioethanol was calculated using the formula (100-A) × 0.5714, using the standard spirit table and readings from the Sikes hydrometer and thermometer. These measurements were taken during the distillation process of the bioethanol acquired by a rotary evaporator. The findings indicated that the optimal bioethanol concentration in water was achieved at a pH of 4.5 and a temperature of 35°C. The yeast cells (Saccharomyces cerevisiae) were found to make bioethanol most effectively at a pH of 4.5 and a temperature of 35°C.

Keywords: *Saccharomyces cerevisiae*, Bioethanol, Sugarcane bagasse, Fermentation, Temperature, pH

INTRODUCTION

Uganda relies significantly on imported crude oil to fulfil its domestic energy needs for petroleum products. In 2022, Uganda used a total of 555.6 million litres of petroleum products in the road sector, 39.6 million litres in rail, and 99.2 million litres in fisheries. This represents a 0.4% increase compared to the 693.5 million litres consumed in 2021. Uganda must investigate other energy sources due to the growing dependence on petroleum for transportation. Bioethanol is becoming a favourable choice because of the diminishing supplies of fossil fuels and the increasing levels of environmental pollution. The user's text is enclosed in tags. Internationally, the significant increase in energy use, along with the gradual exhaustion of oil deposits, has stimulated the exploration for substitute energy options. The issue at hand has escalated into a significant matter as a result of heightened fuel usage, the phenomenon of global warming, the exhaustion of fossil fuel reserves, the surge in fuel costs, and the release of greenhouse gases. The given text is not complete. It appears to be a list with two elements, but the second element is missing. Biofuels, namely bioethanol derived from sustainable and renewable sources, provide a promising answer for addressing the increasing need for energy [4]. Bioethanol and other valuable bio-products may be produced by fermentation using

cellulose, sugar, and starch-rich substrates, particularly from renewable sources such as biomass. Developing ethanol as an alternative to fossil fuels is beneficial since biomass waste offers a plentiful and economical supply of raw materials [5]. Agricultural crop leftovers include a large amount of cellulose, which is plentiful in polysaccharides. The mentioned materials include rice, barley, wheat straws, and sugarcane bagasse [6]. Sugarcane bagasse, an agricultural waste, has attracted considerable interest in the bio-refinery field because of its high cellulose content, which makes it a promising candidate for producing fermentable sugars for biofuel generation [7]. In addition, sugarcane bagasse minimises the conflict between food production and fuel production when compared to other types of energy crops [8]. The crop has quick growth and can thrive in many production circumstances, such as different climates and soil types, with little input needs. It has the ability to produce substantial quantities of biomass [9]. Pretreatment is an essential stage in bio-refinery operations, since it improves the decomposition of biomass components and makes following stages easier [10]. The selection of pretreatment method, choice of raw material, and efficient utilisation of biomass components such as cellulose, sugar, and starch have a substantial impact on the conversion process [11]. Hydrothermal processing is a thermo-chemical pretreatment technique that utilises water at subcritical and supercritical conditions to transform biomass into biofuels or high-value goods. This process is known for its efficiency and profitability [12]. Pretreatment facilitates the conversion of cellulose into dextrin by the action of the alpha-amylase enzyme [13]. This procedure entails a sequence of stages that disassemble the resistant framework of cellulosic biomass [14], necessitating energy to elevate the temperature and pressure of the system. The effectiveness of the system is influenced by a range of factors, including different energy sources and operating variables such as temperature, pressure, particle size, water-solid ratio, pH, gravity, and residence duration [15]. Hence, the careful selection of suitable biomass, techniques, and technologies may contribute to the recycling of nutrients, reduction of waste and energy consumption, and improvement of cost effectiveness [16]. Given the increasing costs of crude oil, the depletion of resources, political instability in oil-producing nations, and environmental difficulties, biomass has considerable potential to substitute traditional energy sources in Uganda. Uganda, as a burgeoning economy, has the task of fulfilling its energy requirements to sustain its rising population, necessitating the production of two to three times the existing energy output. The use of sugarcane bagasse as a raw material for producing bioethanol offers a more secure and environmentally friendly form of waste disposal [17]. With a population of almost 45 million, ensuring food security is a topmost concern for Uganda. The government lacks the financial means to implement enzymatic hydrolysis for ethanol production, as it is commonly done in Europe and the USA. Preconditioning sugarcane bagasse using Saccharomyces cerevisiae may accelerate the fermentation process, making it suitable for bioethanol production in plants like Kakira, Kinyara, Mayuge, Kaliro, and SCOUL [18]. Bioethanol is essential for environmental preservation since it helps reduce global warming and preserve fossil fuel resources. Utilising biomass or trash to generate bioethanol results in a decrease in crude oil consumption and environmental degradation. This research has great importance for government institutions and non-governmental organisations (NGOs) that are striving to mitigate environmental pollution and enhance air quality. It demonstrates that the use of bioethanol as a fuel option results in the release of emissions that are not detrimental and also helps in reducing emissions from fossil fuels when mixed together. The objective was to generate bioethanol from sugarcane bagasse by means of fermentation with *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Preparation of Sugarcane Bagasse

The sugarcane bagasse was obtained from Sugar Corporation of Uganda Limited, Lugazi, Buikwe, and Kakira Sugar Works, Kakira, Jinja Uganda. After being carefully cleaned with tap water, the sugarcane bagasse was chopped into smaller pieces using a high-speed multi-functioning comminutor. They dried for 3 days at 60°C in an oven since the sugarcane bagasse's enzymes might be impacted by greater temperature. The sugarcane bagasse was ground using a grinding machine after it had dried, sealed in a poly bag or sealed bag, and kept at room temperature.

Buffer Preparation

Two different kinds of buffer were made, that is acetic acid with sodium acetate buffer for glucoamylase and phosphate buffer for alpha-amylase.

Phosphate Buffer Preparation

Distilled water (400 mL) was prepared in a 500 mL beaker, 10g of sodium phosphate dibasic heptahydrate and 2.5g of sodium mono basic monohydrate were added and the mixture was stirred to form a solution. The pH meter was used to ensure pH range was maintained within the range of 5.8-7.4. Distilled water was filled up to the mark.

Acetate Buffer (acetic acid with sodium acetate) Preparation

Distilled water (400 mL) was prepared in a 500ml beaker, 6g of sodium acetate and 2g of acetic acid were added, and the mixture was stirred to form a solution. The pH meter was used to check on to 5.0 and distilled water was filled up to the mark. Then, the enzymes glucoamylase and alpha-amylase were diluted using these buffers prepared.

Liquification of Sugarcane Bagasse

Sugarcane bagasse (120 g) was measured in a 2500 mL conical flask. Distilled water (2000 mL) was added too to form a slurry. Sodium hydroxide (1.0 mL) was added to reduce pH to 4.5. Subsequently, 0.2 uL of enzyme alpha-amylase was added to the blend using a micropipette, before being introduced to the slurry, the alpha-amylase was diluted with 20mls of phosphate buffer, and the concoction was raised to 50°C. The cellulose was broken down into a small substance called dextran by alpha-amylase enzyme.

Saccharification of Sugarcane Bagasse

At 40°C, the mixture was cooled and then supplemented with 0.2 ul of enzyme glucoamylase. Before being put into the slurry, glucoamylase was diluted with 20mls of acetic acid with sodium acetate buffer, while the glucoamylase degraded the dextrin to fermentable glucose, the mixture was kept at 50°C. After cooling the mixture to 32°C, 20mls of saccharomyces cerevisiae (baker's yeast) was added and the mixture was then transferred to a flat-bottomed flask of 5000 mL.

Fermentation of Sugarcane Bagasse

Saccharomyces cerevisiae often known as baker's yeast was utilized in the fermentation process to convert simple sugar into ethanol and carbon dioxide, to ascertain the impact of pH on ethanol yield, a constant temperature of 37°C was maintained while the pH was adjusted to 3, 3.5, 4, 4.5, and 5, then, the pH was maintained at 4.5 to ascertain how temperature affected the amount of ethanol produced when adjusted to 25°C, 30°C, 35°C, 40°C, and 45°C. For 48 hours, the fermentation process went on.

Distillation of Ethanol

What man filter paper was used to filter the sample 48 hours later to remove the residue and isolate the ethanol, the rotary evaporator was used to distill the bioethanol, and at 80°C, the mixture was heated to extract the bioethanol.

Effects of pH and Temperature on the ethanol concentration (%) in water

Ten (10) samples were made from a mixture in a 5000 ml flat-bottomed flask of 200 ml in 200 ml flat-bottomed flasks. 5 of 10 samples of 200 ml in flat-bottomed flasks were adjusted at different pH of 3.0, 3.5, 4.0, 4.5, and 5.0 using phosphoric acid at 37°C maintained. The samples were heated in an oven, the fermentation process was allowed to take place for 48 hours. The remaining 5 samples of 200mls in flat-bottomed flasks were adjusted to different temperatures of 25°C, 30°C, 35°C, 40°C and 45°C using an oven as the heat source, the PH of 4.5 was maintained, the fermentation process was allowed to occur for 48 hours.

Determination of Bioethanol Concentration (%) in water

Each 200 mls of fermented mixture sample was transferred into a 500 mls flat-bottomed flask, 200 mls of distilled water was added, and a few drops of antifoam. The flask was connected to the distillation assembly, heated using a heating mantle, and distillation was carried out, initial 200 ml distillate was collected in a 200 ml flask. The flask with distillate was placed in cold water for cooling after it was poured into the measuring cylinder. The temperature and strength of the distillate were checked using a thermometer and a standard Sikes hydrometer. Both were dipped at once, and the percent under the proof of the distillate was determined from the standard spirit table using temperature and strength by the formula $(100\text{-}A) \times 0.5714$, where 100 and 0.5714 are constants, A- is the distillate percent under proof (indication) at the distillation temperature.

Strength of Bioethanol

The remained mixture in the 5000 ml flask was transferred into a 500 ml flat-bottomed flask. The pH was maintained at 4.5 and the temperature was also at 37°C for the effective fermentation process. For 48 hours, the fermentation process was allowed to take place. A What man filter paper was used to filter the sample 48 hours later to remove the residue and isolate ethanol, the filtrate was distilled using the rotary evaporator, at 80°C, the filtrate was heated to extract the bioethanol, and the obtained distilled bioethanol was cooled underwater. A sample volume of the bioethanol sample was poured into a glass cylinder to rinse it out. Then full whole bioethanol sample was poured into the cylinder and the glass alcohol meter was dipped in it. The alcohol meter was allowed to float freely in the cylinder. The indication was taken on the scale of the alcohol meter. Simultaneously, the thermometer was dipped too in the cylinder and its reading was taken. The strength of the bioethanol was obtained using the standard spirit table by the formula (100-A) × 0.5714. Where A= Is the distillate percent over proof and 100 & 0.5714 are constants.

Calculations.

The method used in calculating was the centesimal reduction method as described by Fritz Plato in the late 19th Century

Glass alcohol meter reading = 45

Thermometer reading. = 25.3°C.

25.3°C was changed to °F

= 32×1.8 + 25.3

= 82.9°F

= 83°F

A page with 83°F was checked for in the standard spirit table.

A column of indication was followed to look for 45 and the corresponding value in the percent over proof column was taken i.e., 12.0% which was then substituted in the formula.

=(100-12.0) × 0.5714

= 50.3% strength of bioethanol

Comparison of Standards

According to the standards, there are different kinds of alcoholic drinks, that is; un-distilled and distilled drinks. This study dealt with distilled alcohol known as "Rum". Rum is distilled alcohol from sugarcane bagasse also known as bioethanol. According to standards, the strength of Rum or bioethanol ranges from 40% to 57.5% v/v, the range within which 50.3% lies. Thus, a bioethanol strength of 50.3%v/v is of standard.

RESULTS

Table 1:The effects of PH on the bioethanol concentration (%) in water for SCOUL

pH	Bioethanol Concentration in water (%)	Sikes hydrometer indication	Thermometer reading (°C)	(°F)
3.0	10.7	8.2	25	83
3.5	11.6	79.7	24.5	82
4.0	11.9	79.1	23.5	81
4.5	14.8	74.1	24	82
5.0	00	100	23	81

Table 2:The effects of temperature on the bioethanol concentration (%) in water for SCOUL

Temperature (°C)	Bioethanol Concentration in water (%)	Sikes hydrometer indication	Thermometer reading (°C)	(°F)
25	0.0	100	24.5	82
30	0.0	100	24	82
35	13.8	75.8	23.5	81
40	12.2	78.6	25	83
45	11.1	80.5	23	81

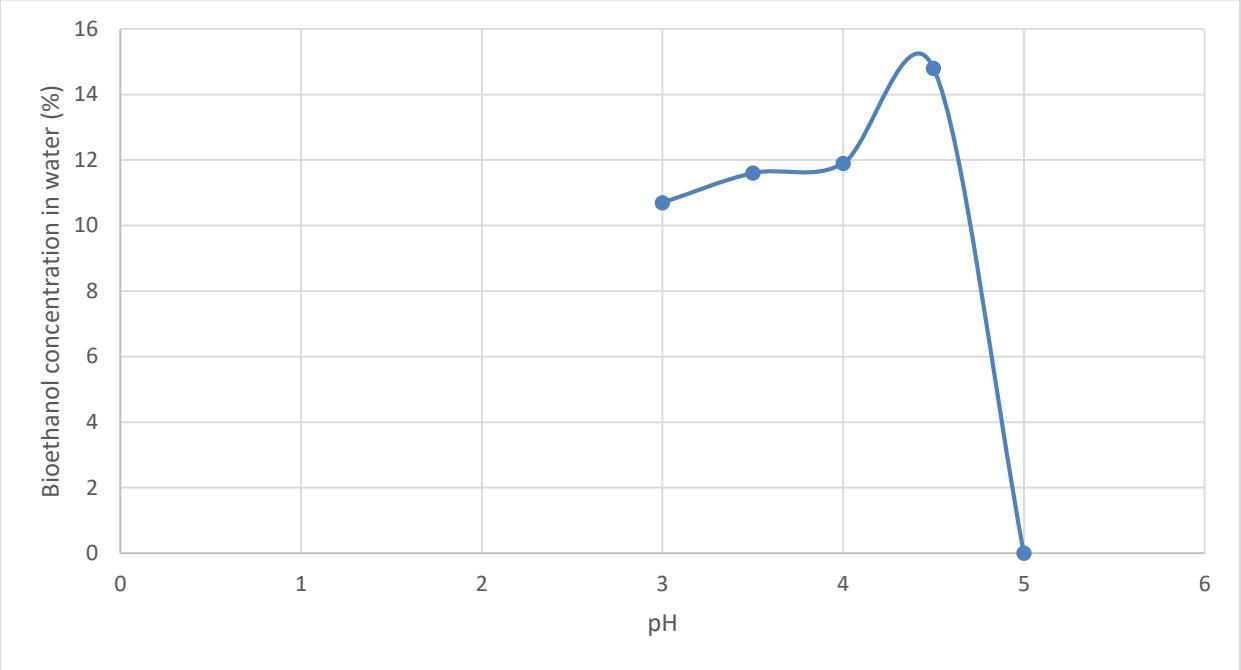

Figure 1: showing the effects of PH against bioethanol concentration (%) in water for SCOUL

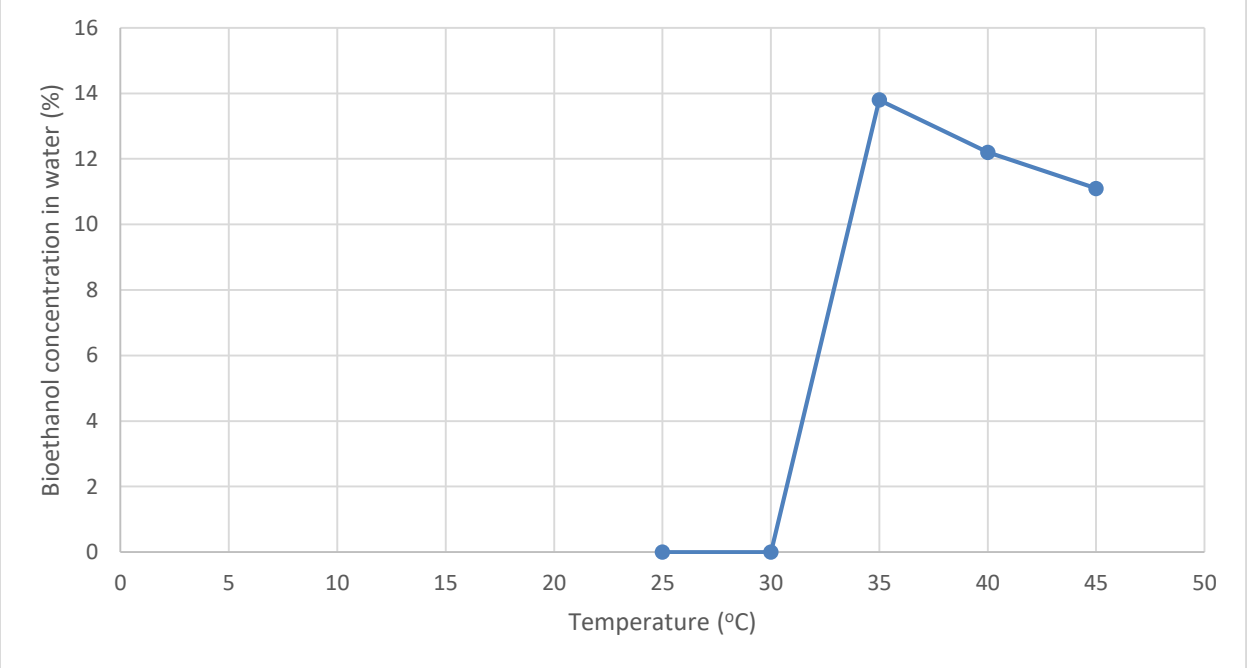

Figure 2: showing the effects of temperature against bioethanol concentration (%) in water for SCOUL

Table 3: The effects of PH on the bioethanol concentration (%) in water for Kakira Bagasse

pH	Bioethanol Concentration in water (%)	Sikes hydrometer indication	Thermometer reading (°C)	(°F)
2.0	0.0	100	24.5	82
2.5	8.6	84.9	24	82
3.0	10.9	80.9	25	83
3.5	11.8	79.3	25	83
4.0	12.4	78.3	23	81
4.5	15.2	73.4	24	82
5.0	0.0	100	25	83

Table 4: The effects of temperature on the bioethanol concentration (%) in water for Kakira bagasse

Temperature (°C)	Bioethanol Concentration in water (%)	Sikes hydrometer indication	Thermometer reading (°C)	(°F)
25	0.0	100	25	83
30	0.0	100	24.5	82
35	14.6	74.4	23	81
40	12.8	77.6	24	82
45	10.2	82.1	25	83
50	8.4	85.3	25	83

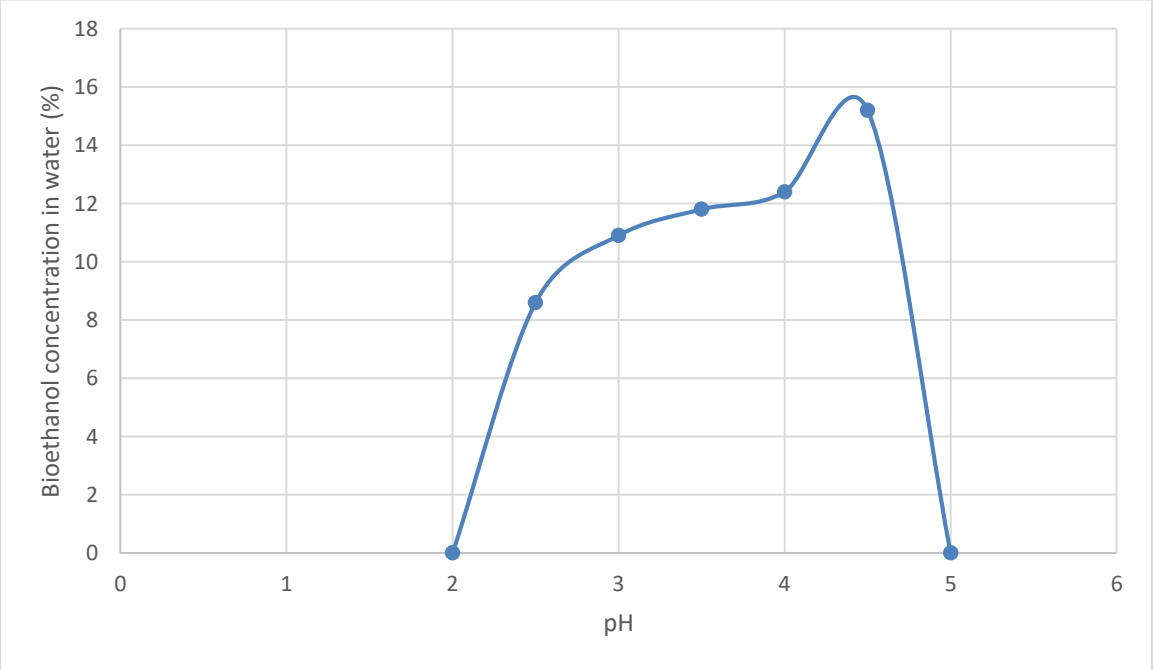

Figure 3: showing the effects of pH against bioethanol concentration (%) in water for Kakira bagasse

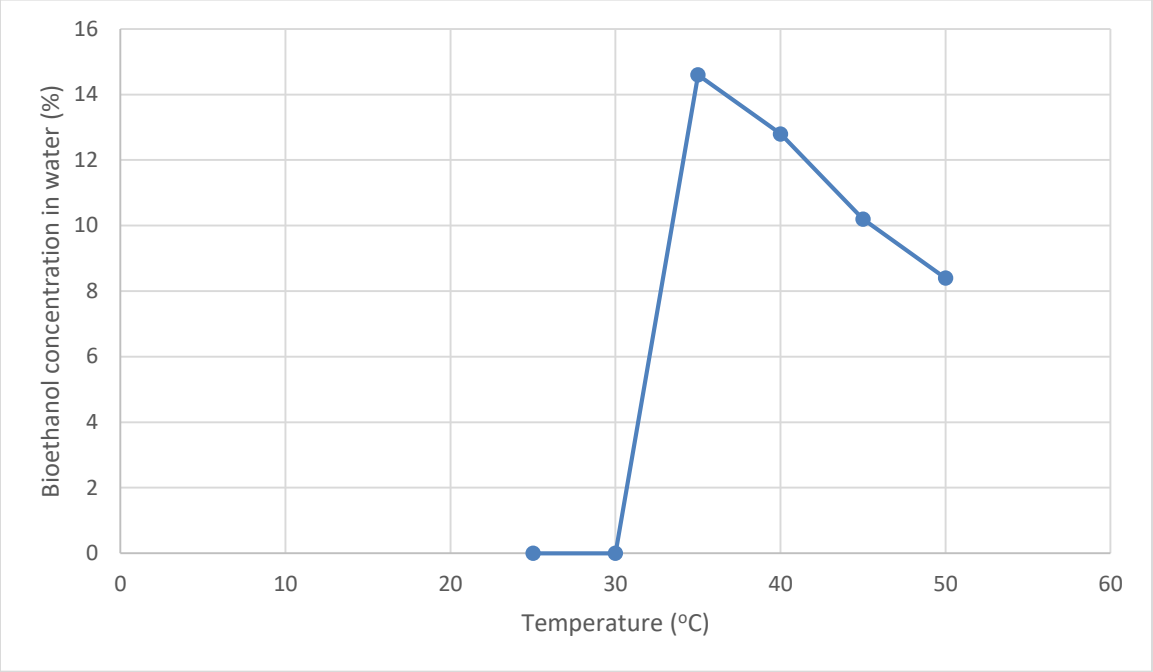

Figure 4: showing the effects of temperature against bioethanol concentration (%) in water for Kakira bagasse.

DISCUSSION

The objective of this research was to determine the primary impacts of pH on fermentation in order to maximise bioethanol production. The results indicates that the highest concentration of bioethanol in water was seen at pH 4.5, with a value of 14.8%. This was followed by pH 4.0 at 11.9%, pH 3.5 at 11.6%, and pH 3.0 at 10.7%. Table 1 and Figure 1 demonstrate that the lowest concentration of bioethanol in water was achieved at a pH of 5.0. The maximum percentage of bioethanol production is achieved at a pH of 4.5. After that, the production starts to decline. The lower concentration of bioethanol in water at different pH values indicates a decrease in yeast cell activity. The maximum concentration of bioethanol in water at pH 4.5 reflects the functionality of enzymes in that particular environment. The pH range of 4.2 to 4.5 exhibited the highest bioethanol production. In addition, increasing the pH from 4.0 to 4.5 enhances the efficiency of increasing the concentration of bioethanol in water. The optimal pH range for *Saccharomyces cerevisiae* was found to be between 4.0 and 4.5. Yeast thrives in acidic environments due to its acidophilic nature [19]. The optimal pH range for yeast cell development varies between 4.0 and 6.0, depending on factors such as yeast strain, temperature, and oxygen levels. The findings of this investigation are consistent with those of Salihu et al [20], who reported that a pH of 4.5 resulted in the greatest yield of 81% ethanol generation using *Saacharomyces cerevisiae*. Enzymes and transport proteins that are bound to the plasma membrane must operate at their optimum pH levels. The yeast must maintain a constant intracellular pH throughout its development.

During the process of growth and metabolism, many enzymes are active inside yeast cells. Due to its acidophilic nature, each enzyme in the yeast operates most effectively at its optimal acidic pH. The yeast cells must expend energy to actively transport hydrogen ions either into or out of the extracellular enzyme when the enzyme's pH deviates from the optimal level in order to maintain the desired intracellular pH. If the extracellular pH deviates much from the optimal pH range, the yeast cells may struggle to maintain a stable intracellular pH, which may impair the proper functioning of the enzyme. In addition, if the enzymes are disabled, the yeast cells will not be able to multiply and efficiently create bioethanol [21]. The observed reduction in bioethanol production may be most plausibly attributed to the starting pH of 3.0. Due to the acidic nature of a low pH, the formation of acid rather than alcohol was favoured, resulting in a reduced generation of carbon dioxide at pH 3.0. However, this research suggests that water with a pH of 5.0 contains the least amount of bioethanol. This might be due to the yeast strain's inability to

live in an acidic environment with a pH of 5.0. Each yeast strain needs specific pH ranges for activation and bioethanol production. Another possible reason is that the yeast cells used in the experiment were stale, meaning they were not as fresh as the yeast cells used in previous experiments. Stale yeast cells may not be able to properly carry out the fermentation process, resulting in the cessation of bioethanol production at pH 5.0 [21]. Temperature is a key factor that influences the production of bioethanol concentration in water. Figure 2 displays the percentage of bioethanol concentration in water produced at different temperatures. Based on the findings, there was an absence of bioethanol concentration in water at temperatures of 25°C and 30°C. However, when the temperature was above 30°C, there was a noticeable augmentation in bioethanol production. The water sample exhibited the greatest bioethanol content of 13.8% at a temperature of 35°C, while the lowest concentration of 11.1% was seen at a temperature of 40°C. Similarly, Salihu et al. [22] also found that the greatest ethanol concentration was achieved at a temperature of 35°C. The fermentation process requires an optimal temperature at which yeast cells undergo a chemical reaction. Therefore, it was essential to maintain a certain temperature range. However, at a temperature of 45°C, there was a decrease in the concentration of bioethanol in water. These findings indicate that the optimal temperature for bioethanol synthesis is 35°C. This finding aligned with previous studies on the impact of temperature on the concentration of bioethanol in water [12]. The results of this investigation contradicted the conclusions of Yah et al (2010), who determined that a temperature of 25°C was optimal for bioethanol synthesis [23]. Based on the findings, it can be deduced that the concentration of bioethanol declined as the temperature climbed. Additionally, the rate of a process catalysed by enzymes increased with temperature, reaching a peak at a certain temperature, beyond which the enzymes began to denature. Elevated temperature had a substantial negative impact on fermentation and hindered cell growth [24]. The research found that the concentration of bioethanol in water reduced dramatically at a temperature of 40°C, indicating that higher temperatures had a noticeable effect on inhibiting cell development. The ribosome and enzyme may undergo denaturation as a result of the increased temperature. Furthermore, increased temperature may modify the structure of the membrane and diminish its functioning. The enzyme undergoes denaturation when exposed to temperatures outside of the optimal range, resulting in a significant decrease in the enzyme's reaction rate. The user's text is "[24]". Enzymes may be influenced by temperature fluctuations due to their sensitivity. Above 40°C, the rate of respiration falls and decelerates. This is because enzymes are comprised of protein chains made up of amino acids, which adopt a helical structure with hydrogen bonds that hold the strands together. Upon the application of heat, the enzyme underwent denaturation due to the distortion of its active cell and the disruption of hydrogen bonds, resulting in the release of energy. This process is often known as denaturation. The optimal temperature for yeast enzyme activity is around 35°C. Below this temperature, the rate of reaction is slow, while temperatures over 45°C would cause the yeast enzyme to denature. The yeast cells exhibited no bioethanol concentration at low temperatures, which may be attributed to the enzymes' inability to produce bioethanol at lower temperatures. In addition, the enzymes become inactive at low temperatures, resulting in a decrease or complete halt of the process. The molecules exhibit reduced velocity at lower temperatures compared to higher temperatures. These statements indicate that the enzyme may lack the necessary energy to conduct a chemical reaction. In summary, it can be inferred that a temperature of 35°C was found to be the most favourable for the generation of bioethanol.

CONCLUSION

This study successfully demonstrated that bioethanol can be produced from sugarcane bagasse through fermentation using *Saccharomyces cerevisiae*. The research highlighted the significance of pH and temperature in optimizing bioethanol yield. The highest bioethanol concentration of 14.8% was achieved at a pH of 4.5 and a temperature of 35°C. These conditions were found to be optimal for the enzymatic activity and yeast fermentation process, providing the most efficient bioethanol production. The findings suggest that maintaining specific environmental conditions is crucial for maximizing bioethanol yield, which could significantly contribute to sustainable energy production and reduce dependence on fossil fuels in Uganda.

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