Sustainable Bioethanol Production from Corn Husk using *Saccharomyces cerevisiae*: Optimization of Key Parameters for Enhanced Yield

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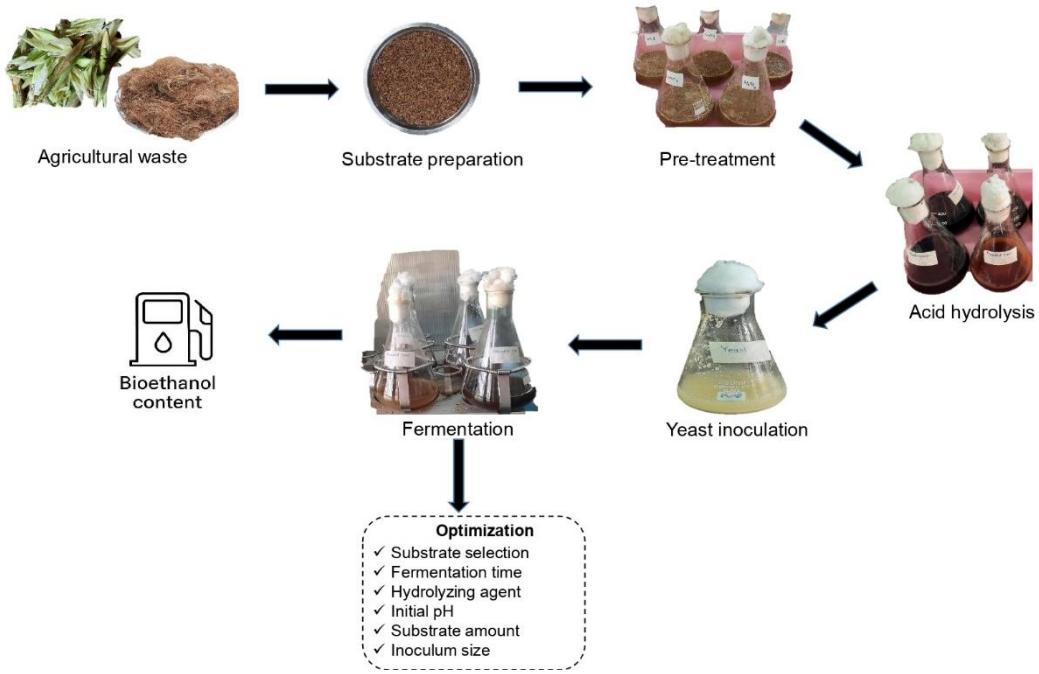

Abstract

Non-renewable fossil fuels dominate energy use globally, prompting a shift to renewable biofuels and utilizing underused agricultural residues. This study aimed to evaluate effective agricultural waste materials for biofuel production and to optimize cultivation conditions to improve yield. Among the tested agricultural wastes (arecanut husk, arecanut leaf, rice husk and corn husk) fermented with *Saccharomyces cerevisiae*, corn husk produced significantly higher bioalcohol yields. Utilizing corn husk in fermentation media (4 g/L yeast extract, 8g/L KH_2PO_4, 0.6 g/L $(NH_4)_2SO_4$, 0.3 g/L peptone, 0.6 g/L $MgSO_4.7H_2O$) with baker's yeast (25 g/L) at 30 ± 2 °C, 100 rpm for 24 hours yielded 0.3% ethanol. Sequential optimization increased yields significantly. Extending fermentation to three days raised ethanol production by 1.6 times (0.5%). Hydrolyzing corn husk with 1M H_2SO_4 improved bioalcohol yields compared to other agents. Optimizing media pH to 7.0 further enhanced production. Increasing corn husk substrate to 40g/100ml boosted ethanol content from 0.7% to 1.1%. Higher yeast inoculum (75 g/L) elevated bioalcohol yield to 1.2%, compared to non-optimized conditions (25 g/L). The findings reveal that corn husk can serve as a valuable raw material for bioalcohol synthesis, emphasizing the importance of process optimization to maximize yield.

Keywords: *Agricultural waste, Bioalcohol, Corn husk, Fermentation optimization, Lignocellulosic biomass, Saccharomyces cerevisiae*

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1. Introduction

Alcohols are organic compounds that play diverse and important roles in day-to-day life and are utilized across numerous fields. They are commonly used in the food industry for producing alcoholic beverages, in healthcare for disinfection and sterilization, and as fuels for cooking and transportation. Population growth and excessive usage have led to a growing demand for alcohol, both for human health and as an energy source. This demand has drawn the attention of scientists to find an alternative method of alcohol production using renewable raw materials at a low cost and with a reduced environmental impact (Vasic et al., 2021). Biofuels such as biodiesel, bioethanol, and biogas are produced from biomass and serve as sustainable alternatives to fossil fuels. They offer benefits including reduced greenhouse gas emissions, economic growth, and improved energy security. Its production is classified into four categories; first generation uses edible biomass, the second generation is based on non-edible biomass, the third generation is derived from microalgae, and the fourth generation involves genetically modified organisms. While the first two generations are widely implemented, the latter two are still under development (Christy et al., 2023[a]).

First-generation bioethanol is made from food-based biomass that is rich in sugar and starch, including crops like sugarcane, corn, and sweet sorghum. It is widely used in countries such as USA, Brazil, and India because of its high sugar content and relatively low conversion costs. However, its reliance on food crops impacts food production, leading to issues like rising food prices and land competition, which are addressed by second-generation feedstocks (Sarkar et al., 2012). Second-generation bioethanol is produced from non-edible lignocellulosic biomass like wheat straw, sugarcane bagasse, and rice husk. These materials are inexpensive, readily available locally, and do not compete with food resources (Thompson and Meyer, 2013). However, the complex structure of lignocellulosic biomass requires pretreatment and hydrolysis, which increases production costs and time, despite its renewable and sustainable benefits (Christy et al., 2023[b]). Algae, including both macro and micro marine types like *Chlamydomonas*, *Chlorella*, and *Synechocystis*, offer promising alternatives to first and second generation feedstocks for bioethanol production because of their high carbohydrate, protein, and lipid content. While algae cultivation benefits from sustainable marine environments, avoiding competition with land and freshwater resources, challenges like biomass pretreatment, high operating costs, and energy consumption remain significant obstacles (Tan et al., 2020).

Fourth-generation bioethanol uses genetically modified organisms to enhance production efficiency, representing the latest technological advancement in bioethanol production. Genetic engineering is used to modify crops and algae for traits like improved sugar production, increased lipid synthesis, enhanced photosynthesis, and better carbon fixation (Cavelius et al., 2023).

Considering the increasing interest in sustainable bioethanol production, the aim of this study was to explore the potential of agricultural wastes, including arecanut leaf, arecanut husk, corn husk and rice husk as substrates for bioalcohol production using *Saccharomyces cerevisiae*. The key objectives include evaluating the fermentative performance of selected plant-based materials for bioalcohol production, identifying the substrate that yields the highest alcohol content, and optimizing culture conditions to enhance productivity. This study sought to promote a cost-effective and environmentally friendly approach to biofuel production by using agro-waste materials as an alternative to conventional methods that depend on expensive enzymatic pretreatments.

2. Materials and Methods

2.1 Chemicals and Culture Media

All chemicals used in this study were procured from standard commercial sources. A solution of 1 M NaOH was employed for pretreatment, while a solution of 1 M H_2SO_4 was used for hydrolysis. The fermentation medium contained 4 g/L yeast extract (Himedia, India), 8 g/L potassium dihydrogen phosphate (KH_2PO_4), 4 g/L ammonium sulfate ($[NH_4]_2SO_4$), 2 g/L peptone (Himedia, India), and 4 g/L $MgSO_4 \cdot 7H_2O$ (Christy et al., 2023[a]).

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2.2 Raw materials and Source of Strain

Agricultural wastes such as arecanut leaf (*Areca catechu*), arecanut husk, corn husk (*Zea mays*), and rice husk (*Oryza sativa*) were collected from the Jaffna region to be used as substrates. Commercial Baker's yeast (*Saccharomyces cerevisiae*) (DCL Instant Dry Yeast) was purchased from a local retailer.

2.3 Substrate Preparation

The collected samples were cleaned with tap water, sun-dried, and subsequently oven-dried at 50°C until a constant weight was achieved. The dried samples were ground into a fine powder and sieved to obtain particles with sizes less than 500 µm (passed through a 35-mesh sieve). The processed powders were stored in clean, airtight containers until further use (Shayanthavi and Kapilan, 2021).

2.4 Inoculum Preparation

To activate *Saccharomyces cerevisiae*, 5 g of the yeast was incubated in an activation media on an orbital shaker at 100 rpm and 30 ± 2 °C for 18 hours. The activation medium consisted of sucrose (5 g) and glucose (5 g) dissolved in 100 mL of distilled water. The medium was sterilized at 121°C and 15 psi for 15 minutes (Inparuban et al., 2009).

2.5 Pretreatment, Acid Hydrolysis, and Fermentation

Powdered substrate (30 g) was mixed with 100 mL of distilled water in a 500 mL conical flask and autoclaved at 121 °C and 15 psi for 15 minutes as a physical treatment. This process sterilized the mixture and partially disrupted the lignocellulosic structure, thereby facilitating acid hydrolysis. After cooling to room temperature, the physically pretreated substrate underwent chemical pretreatment by adding 100 mL of 1 M H_2SO_4, followed by autoclaving again at 121 °C and 15 psi for 15 minutes to hydrolyze hemicellulose and cellulose into fermentable sugars. After cooling, the liquid phase was separated from the solid residue using muslin cloth, and the filtrate was centrifuged at 8000 rpm for 15 minutes. Thereafter, the supernatant was neutralized with 1 M NaOH (Christy et al., 2021).

For fermentation, the hydrolyzed substrate (200 mL) was mixed with 25 mL of sterilized fermentation medium in a conical flask. The mixture was autoclaved, cooled, and inoculated with 20 mL of the prepared *Saccharomyces cerevisiae* inoculum. The flasks were sealed with cotton plugs and incubated in an orbital shaker at 100 rpm and 30 ± 2 °C for 24 hours (Gnanasegaram and Kapilan, 2024). After incubation, 50 ml of the fermented sample was centrifuged at 8000 rpm, and the supernatant was collected. The ethanol concentration was then determined using an ebulliometer (Christy et al., 2023a).

2.6 Optimization of Bioethanol Production

Optimization experiments were conducted by varying key parameters such as substrate concentration, pH, temperature, and inoculum size to enhance bioethanol yield. No specific positive or negative controls were included in this study.

2.6.1 Selection of suitable substrate

Various substrates were subjected to pretreatment, acid hydrolysis, and fermentation as outlined in Section 2.5. Ethanol content in the fermented broth was quantified using an ebulliometer (detection range: 0–20% v/v, accuracy: ±0.1%). The substrate yielding the highest ethanol content was selected for further optimization steps.

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2.6.2 Optimization of fermentation time

Fermentation was carried out using the substrate selected in Section 2.6.1 in an orbital shaker at 100 rpm and 30 ± 2 °C for a period of four days. Ethanol concentration was monitored at 24-hour intervals to determine the optimal fermentation time.

2.6.3 Optimization of hydrolyzing agent

To identify the most effective hydrolyzing agent, the selected substrate (30 g) was treated with 100 mL of 1 M solutions of different acids (H_2SO_4, HCl, HNO_3) and bases (NaOH, KOH). Hydrolysis was performed through autoclaving at 121 °C, 15 psi for 15 minutes. After cooling, the hydrolysates were neutralized to pH 7 using NaOH or HCl. Then fermentation was carried out using the previously optimized fermentation time and ethanol yield was measured. The hydrolyzing agent producing the highest ethanol concentration was selected for further optimization.

2.6.4 Optimization of initial pH

After selecting the optimal hydrolyzing agent, the initial pH of the hydrolyzed substrate was adjusted to 5.0, 5.5, 6.0, 6.5, and 7.0 using 1 M HCl or 1 M NaOH, depending on the required pH level. Then fermentation was carried out under optimized conditions and ethanol content was measured to determine the optimal initial pH for fermentation.

2.6.5 Optimization of substrate quantity

Different amounts of the selected substrate (25 g, 30 g, 35 g, 40 g, and 45 g) were processed using the optimized hydrolysis and fermentation conditions to assess the impact of substrate concentration on ethanol yield. Ethanol content was then measured to identify the optimal substrate quantity for maximum production.

2.6.6 Optimization of inoculum size

The effect of varying yeast inoculum concentrations (25 g/L, 50 g/L, 75 g/L, 100 g/L, and 125 g/L) on ethanol production was tested. Fermentation was conducted using the previously optimized parameters, including substrate type and amount, hydrolyzing agent, initial pH, and fermentation time. The inoculum concentration that produced the highest ethanol yield was identified as the optimal level.

2.7 Statistical Analysis

All experiments were conducted in triplicate. Mean values were calculated and presented in graphs. Statistical significance was determined using one-way ANOVA and Tukey's multiple comparison tests at a 95% confidence interval, performed with Minitab 17.0 software.

3. Results and Discussion

3.1 Effect of different substrates on bioethanol production

In this study, arecanut leaf (*Areca catechu*), arecanut husk, corn husk (*Zea mays*), rice husk (*Oryza sativa*) were evaluated as potential substrates for bioethanol production. The alcohol yield from each substrate was measured, and the results revealed that corn husk produced the highest ethanol content, with a peak alcohol concentration of 0.5% on the third day of fermentation (Figure 1). Based on these findings, corn husk was selected as the most promising substrate for further optimization studies.

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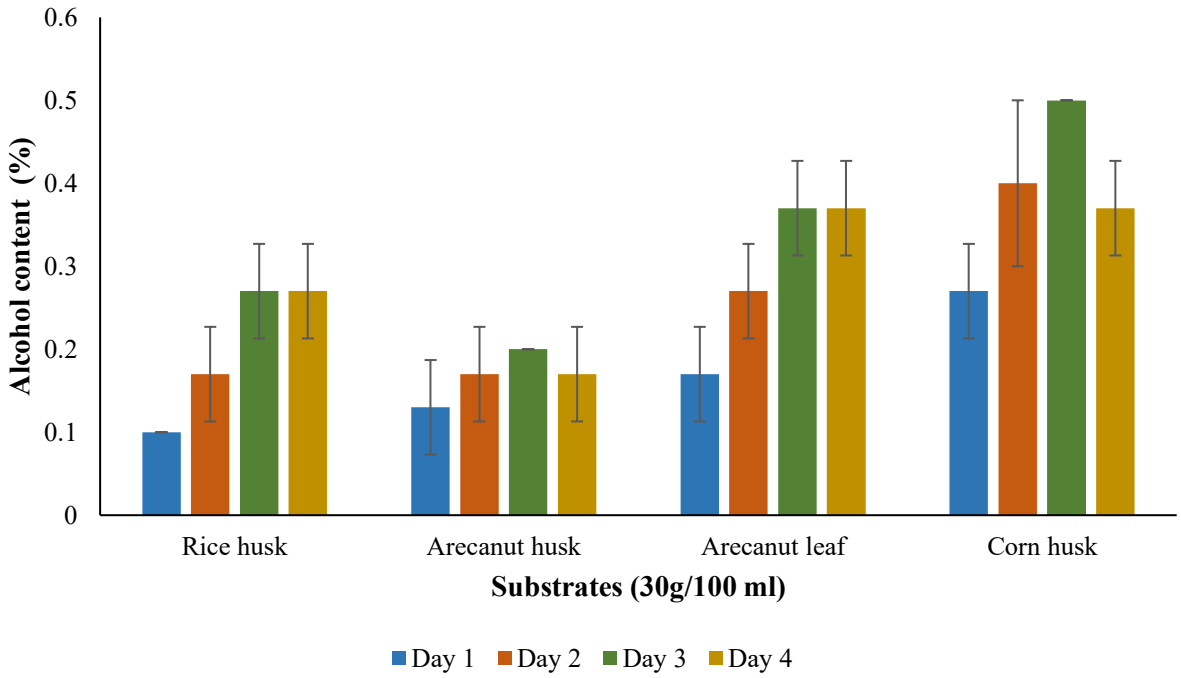

Figure 1: Effect of rice husk, arecanut husk, arecanut leaf, corn husk on bioalcohol production.

The higher ethanol production observed from corn husk compared to the other substrates may be attributed to its relatively high carbohydrate content, which is essential for fermentation by *Saccharomyces cerevisiae* (Simas-Rodrigues et al., 2015). Carbohydrates such as cellulose and hemicellulose in the corn husk likely underwent effective acid hydrolysis and fermentation, leading to the production of alcohol. This finding aligns with previous research that suggests lignocellulosic materials with a higher carbohydrate content are more conducive to bioethanol production (Christy et al., 2023[b]).

However, it is important to note that the ethanol yield from corn husk in this study (0.5%) was lower than that reported for fruit-based substrates, such as grapes (6.08%) and bananas (5.11%), which contain high amounts of fermentable sugars like fructose and sucrose (De Silva et al., 2022). These sugars can be directly fermented by yeast, resulting in higher alcohol yields compared to lignocellulosic materials, which require more complex hydrolysis steps to break down their carbohydrate structures.

3.2 Effect of fermentation time

The effect of fermentation time on bioethanol yield ($p < 0.05$) was tested by incubating the hydrolysed corn husk substrate for various durations, with alcohol content measured at 24-hour intervals. The results indicated that the significantly higher ($p < 0.05$) alcohol yield was obtained after 3 days of fermentation, with the ethanol concentration reaching 0.5%. In comparison, alcohol yields after the first, second and fourth days of fermentation were significantly lower ($p < 0.05$) at 0.3%, 0.4%, and 0.4%, respectively (Figure 2). The three-day fermentation period yielded 1.67 times more ethanol than the first day, confirming it as the optimal incubation period for corn husk as a substrate for bioethanol production. This outcome is consistent with previous studies that have reported maximum ethanol production after 72 hours of fermentation with other substrates. For example, Dash et al. (2017) noted that the highest ethanol production was observed from sweet potatoes after 72 hours of fermentation, while Ilangarathna and Kapilan (2022) reported that *Saccharomyces cerevisiae* achieved the highest bioethanol yield from coconut husk fiber after 3 days. These studies further support the notion that fermentation time plays a crucial role in the efficiency of bioethanol production.

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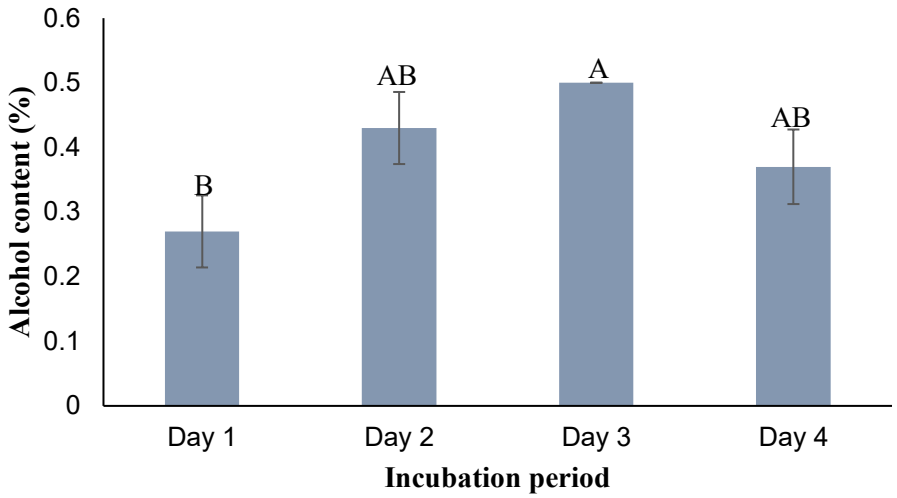

Figure 2: Effect of incubation period on bioalcohol production from corn husk using *Saccharomyces cerevisiae.* Values with different uppercase letters indicate significant differences ($p < 0.05$)

The fermentation process is influenced by the growth and activity of microorganisms, particularly yeast. Yeast cells do not immediately begin fermentation upon inoculation, as they require an adaptation period to acclimatize to the environment (Christy et al., 2023[c]). During the initial stages, the yeast cells begin to grow and activate enzymes necessary for fermentation. A shorter incubation period often results in lower alcohol yields because the yeast cells have insufficient time to multiply and produce alcohol.

In contrast, a longer incubation period, when the environmental conditions become more suitable for yeast growth, leads to a higher ethanol yield. However, beyond a certain point, as the fermentable sugars are depleted, the alcohol yield may plateau or even decrease due to the accumulation of toxic by-products that inhibit yeast activity (Zabed et al., 2014).

3.3 Effect of Hydrolyzing Agent

When corn husk substrate was hydrolyzed with different acids (1 M H_2SO_4, 1 M HNO_3, and 1 M HCl) and alkaline solutions (1 M NaOH and 1 M KOH) separately, a significantly higher ($p < 0.05$) amount of alcohol was obtained in acid hydrolysis (HCl: 0.4%, H_2SO_4: 0.5%, HNO_3: 0.3%) compared to alkaline hydrolysis (NaOH: 0.2%, KOH: 0.3%) (Figure 3). Among the three acids used for acid hydrolysis, the highest alcohol yield was observed with 1M sulfuric acid after the third day of fermentation of the corn husk substrate using *Saccharomyces cerevisiae*. Therefore, 1 M sulfuric acid (H_2SO_4) was selected as the hydrolyzing agent for further optimization studies (Figure 3).

When acid hydrolysis was performed using sulfuric acid, *Chara globularis* substrate produced a significantly higher amount of bioethanol after the second day of fermentation with *S. cerevisiae* (Christy et al., 2023[a]). Additionally, maximum alcohol production was observed when rice straw was hydrolyzed with sulfuric acid at 121 °C for one hour (Ren et al., 2010).

The selection of the best hydrolyzing agent for pretreatment plays a vital role in alcohol production from cellulose/starch substrates, as it facilitates the conversion of polysaccharides into monomers. Alkaline hydrolysis resulted in comparatively lower ethanol yields than acid hydrolysis because mono- and dimeric carbohydrates, such as glucose, fructose, and cellobiose, are severely degraded by alkalis at temperatures below 100°C (Rabelo et al., 2011). Furthermore, alkaline hydrolysis is a slower process and requires neutralization.

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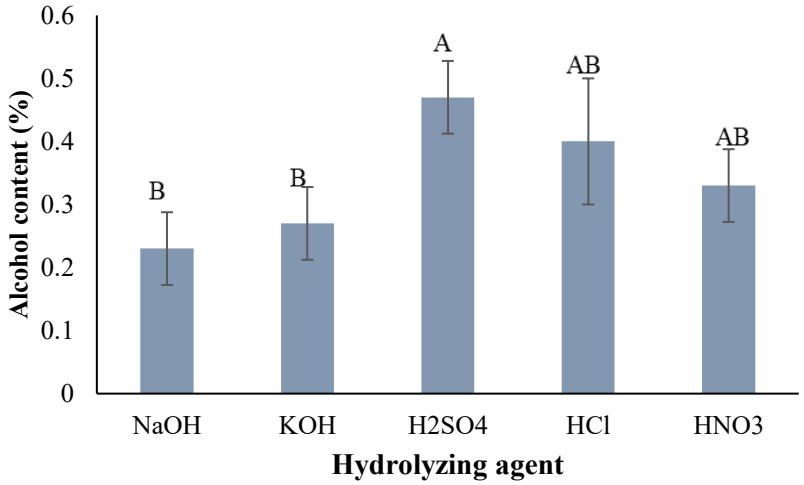

Figure 3: Effect of different Hydrolyzing agents on bioalcohol production from corn husk using *Saccharomyces cerevisiae* in 3-day fermentation. Values with different uppercase letters indicate significant differences ($p < 0.05$)

In acid hydrolysis, significantly higher alcohol yields were obtained with sulfuric acid compared to nitric acid. This could be attributed to the formation of toxic substances or inhibitors in samples hydrolyzed with nitric acid (Christy et al., 2023[a]).

3.4 Effect of pH

An initial pH of 6.0 resulted in a significantly higher bioalcohol yield from the corn husk substrate, with pH optimization increasing the yield from 0.5% to 0.7% (Figure 4). Thus, a pH of 6.0 was selected as the optimum value for fermenting corn husk substrate using *Saccharomyces cerevisiae* and was used in further studies. When sour banana fruit juice was fermented at pH 7.0 with *S. cerevisiae*, a significantly higher bioethanol yield was observed (Vivekanandaraja and Kapilan, 2021). Similarly, maintaining a pH of 6.0 during fermentation resulted in significantly higher bioethanol yields with the Dahanala red Nadu rice substrate (Christy et al., 2024).

The pH is a key factor in fermentation because it directly impacts the microorganisms and their cellular biochemical activities. Furthermore, the level of hydrogen ions (H^+) in the fermentation medium can affect how easily vital nutrients pass into the cells (Zabed et al., 2014).

Saccharomyces cerevisiae generally prefers slightly acidic conditions, thriving best within a pH range of 4.0 to 6.0, though this can vary depending on temperature, oxygen levels, and the specific yeast strain. Enzymes involved in glucose fermentation perform optimally in mildly acidic environments (Christy et al., 2024). If the pH falls below 4.0, the fermentation process takes significantly longer. On the other hand, when the pH rises above 5.0, ethanol production decreases considerably (Staniszewski et al., 2007).

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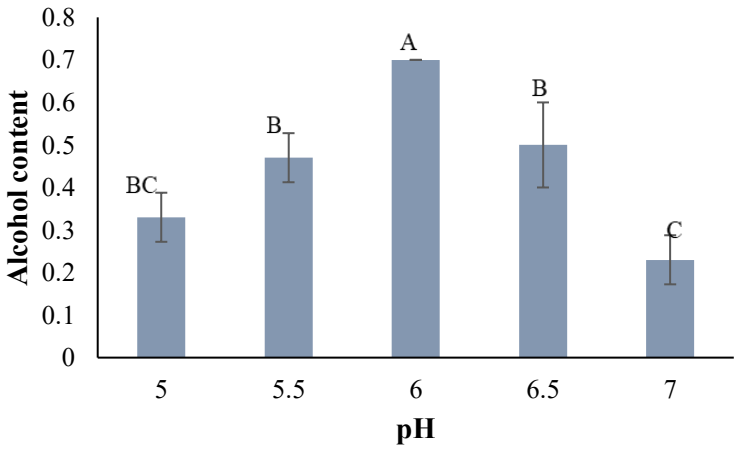

Figure 4: Effect of pH of the fermentation medium on bioalcohol production from corn husk using *Saccharomyces cerevisiae* in 3day fermentation. Values with different uppercase letters indicate significant differences ($p < 0.05$)

3.5 Effect of Substrate amount

A substrate concentration of 12.5 g per 100 ml of *Parthenium hysterophorus* plant resulted in a significantly higher ethanol yield on the fourth day of fermentation (Gnanasegaram and Kapilan, 2024). Similarly, ethanol production increased when 90% concentration of Dahanala red naadu rice substrate was obtained (Christy et al., 2024). In this study, bioalcohol yield reached 1.1% at a substrate concentration of 40 g per 100 ml (Figure 5).

The quantity of substrate available during fermentation has a direct effect on microbial growth, cell multiplication, and fermentation rate. Increasing the initial sugar content up to a certain level enhances the fermentation process (Zabed et al., 2014). However, when sugar concentration becomes excessively high, fermentation tends to stabilize as microbial cells can no longer absorb the excess sugar, leading to a steady fermentation rate (Laopaiboon et al., 2007).

A decline in bioethanol yield beyond an optimal substrate concentration may be caused by decreased cell viability and metabolic inactivity. Rapid ethanol production early in fermentation can result in leakage of intracellular metabolites into the medium (Tse et al., 2021).

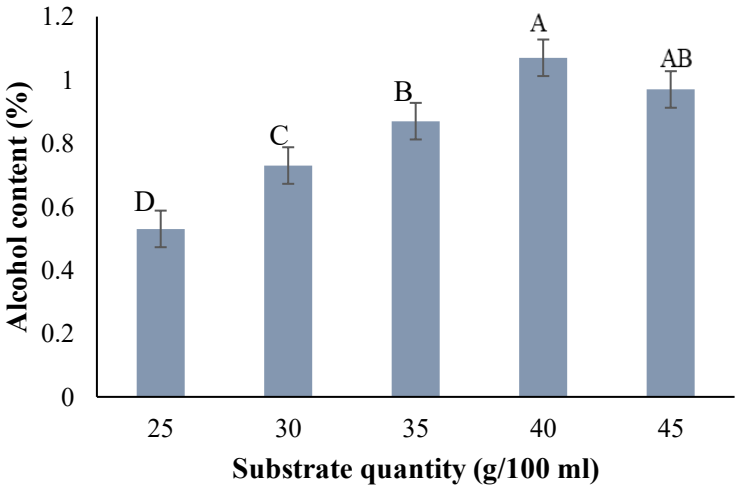

Figure 5: Effect of the amount of corn husk substrate on bioalcohol production using *Saccharomyces cerevisiae* after 3 days of fermentation. Values with different uppercase letters indicate significant differences ($p < 0.05$)

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3.6 Effect of Inoculum size

Increasing *Saccharomyces cerevisiae* inoculum concentration from 25 g/L to 125 g/L significantly enhanced bioalcohol yield, peaking at 75 g/L (Figure 6), which was selected as the optimized concentration for further studies. Similarly, Christy et al. (2023[c]) reported higher bioethanol yields at 100 g/L inoculum concentration with *Chara globularis*. Gnanasegaram and Kapilan (2024) achieved maximum bioethanol yield with 5 g/100 mL yeast inoculum concentration for 4 days using *Parthenium hysterophorus*. Ojewumi et al. (2018) found that 6% (v/v) yeast inoculum concentration yielded the highest ethanol from sweet potato peel.

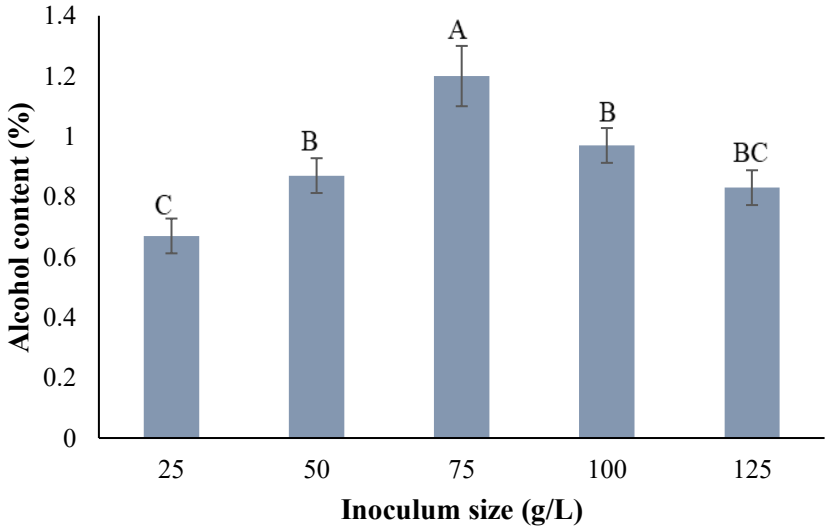

Figure 6: Effect of inoculum size on the production of bioalcohol from corn husk substrate after 3 days of fermentation using *Saccharomyces cerevisiae*. Values with different uppercase letters indicate significant differences ($p < 0.05$)

While inoculum concentration does not affect overall ethanol production, it impacts sugar consumption rates and ethanol productivity (Laopaiboon et al., 2007). Higher cell concentrations reduce fermentation time by accelerating sugar utilization (Zabed et al., 2014). However, excessive inoculum concentrations decrease ethanol yield due to nutrient competition, limited cell growth, and system saturation with biocatalysts (Ojewumi et al., 2018; Laopaiboon et al., 2007).

4. Conclusions

Corn husk was identified as the most effective substrate for bioalcohol production among the agro-wastes evaluated in this study. The optimization of key factors such as fermentation time, hydrolyzing agent, pH, substrate concentration, and inoculum size significantly enhanced bioalcohol production by *Saccharomyces cerevisiae*. Specifically, the yield increased fourfold, from 0.3% under non-optimized conditions to 1.2% following optimization. These results highlight the significant potential of corn husk as a sustainable and efficient feedstock for bioalcohol production under optimized fermentation conditions.

5. Acknowledgment

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6. Declaration of conflict of interest

All authors of this study declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the current work.

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