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OPTIMIZATION OF *SACCHAROMYCES CEREVISIAE* FERMENTATION OF MAIZE BRAN TO ETHANOL

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ABSTRACT

This study explores the optimization of bioethanol production from maize bran through hydrolysis and fermentation processes. Maize bran and sweet potatoes were procured as primary materials, and *Saccharomyces cerevisiae* was employed for fermentation. The maize bran was processed to a powdered form and hydrolyzed using crude β-amylase enzyme extracted from sweet potatoes. The hydrolysis was optimized using the Taguchi method, examining factors such as temperature (30°C, 40°C, 50°C), substrate concentration (2%, 6%, 10%), and duration (1, 3, 6 hours). Glucose content was analyzed using the DNS method. Subsequently, the fermentation process was also optimized with varying yeast loading rates (1%, 10%, 20%), temperatures (30°C, 40°C, 50°C), and durations (1, 3, 6 hours). Ethanol yield was determined using a distillation apparatus and alcohol meter. The results demonstrated that the highest glucose yield (95 mg) was achieved at 6% substrate concentration hydrolyzed at 50°C for 6 hours. Optimal ethanol production (1.60%) occurred at 30°C with a 10% yeast loading rate, fermenting for 6 hours. These findings suggest that precise control of substrate concentration, temperature, and process duration are critical for maximizing biogas yield from maize bran. Further research is recommended to refine these parameters and explore extended hydrolysis and fermentation periods.

Key words: Bioethanol, Optimization, Hydrolysis, Fermentation, Taguchi.

INTRODUCTION

Maize, often known as maize, has a rich and diverse history. Maize was first domesticated in Mesoamerica, now Mexico, almost 9,000 years ago. It was brought to Europe by Spanish conquistadors in the 16th century. It grew rapidly over the world and became a staple crop in many countries, including the United States. Maize is a versatile crop that can be grown for human food, animal feed, and industrial applications. Maize is adaptable to a wide variety of climates and soil types. It has been genetically modified to fight pests and diseases, boost yields, and withstand dry conditions[19]. It is an important source of nourishment, particularly in developing nations where it is frequently a staple food. Because of its high starch content, maize bran has the potential to be used as an ethanol feedstock[7,1]. However, hydrolysis of maize bran into fermentable sugars is difficult due to the raw material's complicated structure and the presence of anti-nutritional components. The complex nature of lignocellulose biomass could lead to low ethanol yields[3,17]. Hence the need for efficient hydrolysis methods. According to [15] converting starch into fermentable sugars continues to be a significant problem for sustainable ethanol production. This fact highlights the need of efficient starch hydrolysis in ethanol generation. To maximize the conversion of maize bran into fermentable sugars, hydrolysis conditions must be optimized [9].Potato-derived amylase enzymes have been

demonstrated to be effective at converting starch-rich materials into fermentable sugars. Sweet potato waste contains a high concentration of amylase enzyme, and using it in ethanol production reduces costs [6]. According [10,21], potato amylases have several advantages over other sources of amylase, including their high enzyme activity , stability and specificity. .The utilization of potato waste for amylase extraction is an effective method to reduce waste and recover valuable products [18]. The use of crude amylase enzymes for hydrolysis is an attractive alternative to pure commercial enzymes, but the optimization of enzyme extraction and purification processes is necessary to maximize their efficacy.[13]. The objective is to determine the optimal conditions for hydrolyzing maize chaff using crude amylase enzymes from potatoes. There will be a conduction of series of experiment s to assess the effects of factors such as enzyme concentration, temperature, pH, and incubation time on the hydrolysis of maize bran. The expectations is that by optimizing the hydrolysis process, there will be a maximum yield of fermentable sugars from maize bran, which will contribute to the development of a more sustainable and cost effective ethanol production process. In addition to assessing the effects of process variables on hydrolysis efficiency, there will be an investigation on the feasibility of immobilizing the crude amylase enzymes on various support materials. Immobilization of enzymes is an effective strategy for improving their stability and reusability, which could lead to significant reduction in the cost and environmental impact of the hydrolysis process. Futhermore, there will be an examination on the potential for scaling up the hydrolysis process using pilot scale reactors. This will enable us to assess the technical and economic feasibility of implementing the optimized hydrolysis process on an industrial scale. Overall, the research aims to provide valuable insights into the optimization of maize bran hydrolysis using potato derived amylase enzymes, with the goal of developing a more efficient and sustainable process for ethanol production. The hydrolysis of maize bran with crude amylase enzymes has various advantages over other methods for turning maize bran to ethanol. For example, compared to typical acid or alkaline pretreatment processes, enzymatic hydrolysis is a more environmentally friendly process that produces fewer byproducts and uses less energy. The utilization of potato waste for amylase extraction is an effective method to reduce waste and recover valuable products. The main objective of this study is to optimize the fermentation of maize bran to ethanol using amylase enzyme hydrolysate and *Saccaromyces cerevisiae* with the following specific objectives;

- ✓ To determine the effect of substrate concentration on ethanol yield.
- ✓ To determine the effect of time on ethanol yield.
- ✓ To determine the effect of temperature on ethanol yield.

MATERIALS AND METHOD

MATERIALS

Feedstock: The feedstock used for this work was maize bran. White maize was bought from local vendors in Estako West Local Government Area of Edo State.

Potato: Fresh sweet potato used in this work was bought from Uchi market and transported to the laboratory in clean cellophane bags prior to further treatment. The enzyme; β-amylase was extracted using sodium acetate buffer.

Saccharomyces cerevisiae: Standard grade *S cerevisiae* used for the fermentation process was procured from Mega Corps Nigeria Ltd, Auchi branch.

Blender: A motorized blender (model: CB-8231-J) was used for this work. It was collected from the Department of Basic Sciences Auchi Polytechnic.
Water Bath: The water bath (model: 622OV-60V-40A) used for this work was to maintain a constant temperature for the hydrolysis.
Weighing Balance. The weighing balance (model: KA67/K1918B) used to weigh samples in this work was from the Department of Basic Sciences, Auchi Polytechnic, Auchi.
Heating Mantle: The heating mantle used for the distillation of the fermented broth was from the Department of Basic Sciences, Auchi Polytechnic, Auchi.
Beakers and Test-Tube: The beakers and test-tubes used were collected from the Department of Basic Sciences, Auchi Polytechnic, Auchi.
Spectrophotometer: The Spectrophotometer used was collected from the Department of Basic Sciences, Auchi Polytechnic, Auchi.

METHOD

Processing of Feedstock: The maize was soaked in water for 3 days, it was grinded in a blender (model: CB-8231-J) and the starch sieved using cheese cloth. After de-starching, the bran residue was oven dried at 50^{o}C for 24 hours after which they were blended again to form powdered maize bran (PMB) and stored in plastic containers prior to further use.
Extraction of β-amylase Enzyme from Potato: The β-amylase used in this work was extracted from potato. This was prepared following the procedure described by AAT Bioquest (2022). A 2.93 g portion of sodium acetate was put into a 500 ml beaker into which 400 ml of distilled water was added and the contents stirred and mixed properly. After which 1.62 ml of Acetic acid (glacial) was added to the contents of the beaker and the volume made up to 500 ml by adding distilled water.
The potato was then washed and a 200 g portion grated and blended in the prepared sodium acetate buffer and allowed to stand for 1 hour. The supernatant was syphoned using a 10 ml syringe and centrifuged at 4000 rpm for 1 hour. The supernatant was used as raw β-amylase extract.

Optimization of Crude Amylase Hydrolysis of PMB using Taguchi Method: This was carried out using three factors; temperature, substrate concentration and duration of hydrolysis (Time). The method involves varying these factors in an array of low, medium and high (Orthogonal array). For temperature, the low was 30^{o}C, medium 40^{o}C and high 50^{o}C. For substrate concentration, the low was 2%, medium 6% and the high 10%, while for duration of hydrolysis, the low was 1 hour, medium 3 hours and high 6 hours [18].

Analysis of Hydrolysate for Glucose Content: DNS method was used to estimate the percentage glucose content for all samples hydrolyzed. Standard graph was plotted by using glucose solution (100µg/ml of working standard). Six (6) test tubes were used. 1 ml of the standard glucose solution was added to tube 1. Into tubes 2, 3, 4 5 and 6 were added 0.8 ml, 0.6 ml, 0.4 ml, 0.2 ml and 0.0 ml of the standard glucose solution respectively. The volume of the tubes was then made up to 1.0 ml with distilled water. To each of these tubes was added 2.0 ml of 3,5-Dinitrosalicylic acid (DNS) reagent, shaken properly and placed in a water bath maintained at 90^{o}C for 5 minutes. The tubes were cooled and 7 ml of distilled water added to stabilize the color. The absorbance was measured with a spectrophotometer at 540 nm, with solution in tube 6 taken as blank. The above procedure was repeated for 1.0 ml of extract and 1.0 ml of water for unknown

estimation. The sugar contents of sample extracts were calculated by comparing their absorbance at 540 nm with the standard graph. The individual values were taken in duplicates .

Optimization of Fermentation Process using Orthogonal Array; Taguchi Method: This carried out using three factors; temperature, *Saccharomyces cerevisiae* loading rate and Time of Fermentation. The method involves varying these factors in an array of low, medium and high. For temperature, the low was 30°C, medium 40°C and high 50°C. For yeast loading rate, the low was 1%, medium 10% and the high 20%, while for duration of fermentation, the low was 12 hour, medium 48 hours and high 72 hours [18].

Distillation of Fermented Broth: After fermentation the fermented broth was poured into a distillation flask of a distillation apparatus fitted with a condenser. The flask was then mounted in a heating mantle and the temperature set at 80°C. The percentage ethanol content was determined using an alcohol meter.

RESULT AND DISCUSSION

RESULT

The results of glucose yield after crude amylase enzyme hydrolysis of maize bran (PMB) is shown in Table 4.1.

Table 4.1: Glucose content (mg) of maize bran hydrolysate after 1 hour of hydrolysis at different temperature and substrate concentrations

Concentration (%)	Temperature °C	Glucose content (mg)
2	30	0.0
2	40	5.0
2	50	12.0
6	30	5.0
6	40	10.0
6	50	30.0
10	30	5.0
10	40	15.0
10	50	25.0

The substrate concentration used in the hydrolysis process ranged from 2% to 10%. The temperature at which the hydrolysis was tested were 30°C, 40°C, and 50°C. Glucose yield from the hydrolytic process ranged from 5 mg to 30 mg, with the highest yield recorded at 6% substrate concentration hydrolyzed at 50°C.

In Table 4.2 is shown the result of glucose yield from the crude amylase hydrolysis of powdered maize bran at 50°C for 1 to 6 hours.

Table 4.2: Glucose content of maize bran hydrolyzed with maize bran for 1 to 6 hours at 6% substrate concentration

Time (HR)	Temperature °C	Glu content (mg)

1	50	32
3	50	60
6	50	95

In Table 4.3, the result of the effect of *Saccharomyces cerevisiae* (yeast) loading rate on the ethanol yield from the powdered maize bran hydrolysate is shown. Three loading rates were considered: 1%, 10%, and 20%. Similarly**,** three levels of temperature were used: 30°C, 40°C, and 50°C.

Table 4.3: Percentage ethanol content of fermented broth of maize bran hydrolysate fermented for 1 hour using *Saccharomyces cerevisiae at different loading rate* and fermentation temperature

S cerevisiae loading rate (%)	Temperature °C	Ethanol %
1	30°C	0.25±0.02
1	40°C	0.10±0.01
1	50°C	0.10±0.01
10	30°C	1.60±0.02
10	40°C	0.30±0.02
10	50°C	0.20±0.01
20	30°C	0.20±0.02
20	40°C	0.15±0.01
20	50°C	0.15±0.01

The ethanol yield at 1% loading rate ranged from 0.10% to 0.25%. At 10% and 20% loading rate, the ethanol yield ranged from 0.20% to 1.60% and 0.15% to 0.20% respectively. Highest yield was observed at 10% loading rate fermented at 30°C.

Table 4.4 shows the result of the effect of duration (Time) on the fermentation process using the best outcome of temperature and *S. cerevisiae* loading rate (30°C and 10% respectively). Duration of hydrolysis used ranged from 1 hour to 6 hours.

Table 4.4: Percentage ethanol content of fermented broth of maize bran hydrolysate Fermented, for 1 to 6 hours, using *Saccharomyces cerevisiae at* 10% loading rate.

Time	*S cerevisiae* loading rate (%)	Temperature	Ethanol (%)
1	10	30°C	0.20
3	10	30°C	0.65
6	10	30°C	0.80

The hydrolysates showed an increase in ethanol yield as the duration of fermentation increased from 1 hour to 6 hours.

DISCUSSION

Optimization of hydrolysis and fermentation of maize bran using crude amylase enzyme and *Saccharomyces cerevisiae* was carried out. The substrate concentration used in the hydrolysis process ranged from 2% to 10%. The temperature at which the hydrolysis was tested were 30°C, 40°C, and 50°C. Glucose yield from the hydrolytic process ranged from 5 mg to 30 mg, with the highest yield recorded at 6% substrate concentration hydrolyzed at 50°C. In Table 4.3, the result of the effect of *Saccharomyces cerevisiae* loading rate on the ethanol yield from the powdered maize bran hydrolysate is shown. Three loading rates were considered: 1%, 10%, and 20%. Similarly, three levels of temperature were used: 30°C, 40°C, and 50°C. The ethanol yield at 1% loading rate ranged from 0.10% to 0.25%. At 10% and 20% loading rate, the ethanol yield ranged from 0.20% to 1.60% and 0.15% to 0.20% respectively. Highest yield was observed at 10% loading rate fermented at 30°C. Table 4.4 shows the result of the effect of duration (Time) on the fermentation process using the best outcome of temperature and *S. cerevisiae* loading rate (30°C and 10% respectively). Duration of hydrolysis used ranged from 1 hour to 6 hours. The hydrolysates showed an increase in ethanol yield as the duration of fermentation increased from 1 hour to 6 hours. As the concentration of maize bran increases from 2% to 10%, the glucose yield generally increases. This is evident from the rise in glucose content at each temperature level as the concentration moves from 2% to 6% to 10%. For instance, at 50°C, the glucose yield increases from 12 mg at 2% concentration to 30 mg at 6% concentration, then slightly decreases to 25 mg at 10% concentration.

Higher temperatures favor glucose yield up to a point. At 2% concentration, glucose yield rises from 0 mg at 30°C to 12 mg at 50°C. Similarly, at 6% and 10% concentrations, the yields at 50°C are the highest compared to 30°C and 40°C.

Extending the hydrolysis duration significantly boosts glucose yield. At a constant 50°C and 6% substrate concentration, glucose yield increases from 32 mg after 1

hour to 95 mg after 6 hours. This indicates that longer hydrolysis allows for more extensive breakdown of maize bran into glucose.

The ethanol yield is highest at a 10% loading rate, with the peak yield being 1.60% at 30°C. Increasing the yeast concentration to 20% does not lead to higher ethanol yields; instead, it slightly decreases. This suggests that an optimal yeast concentration exists for maximum ethanol production. Ethanol yields are highest at the lowest temperature (30°C) across all yeast loading rates. Higher temperatures (40°C and 50°C) result in significantly lower ethanol yields. This implies that 30°C is the optimal fermentation temperature for *Saccharomyces cerevisiae* in this process.

Prolonging fermentation increases ethanol yield. At the optimal conditions (10% yeast loading rate and 30°C), ethanol yield rises from 0.20% after 1 hour to 0.80% after 6 hours. This trend suggests that extending fermentation time allows more conversion of glucose to ethanol. Both glucose and ethanol yields are influenced by temperature, with higher glucose yields at 50°C for hydrolysis and optimal ethanol yields at 30°C for fermentation. This indicates distinct optimal temperatures for hydrolysis and fermentation processes, necessitating temperature adjustments between the stages. For hydrolysis, a moderate substrate concentration (6%) at high temperature (50°C) maximizes glucose yield. In fermentation, an intermediate yeast concentration (10%) at low temperature (30°C) yields the highest ethanol. Balancing substrate and yeast concentrations is crucial for maximizing overall process efficiency. Both processes benefit from longer durations, with glucose yield increasing over 6 hours of hydrolysis and ethanol yield rising over 6 hours of fermentation. However, practical limits on time must be balanced with the rate of return on yield. Specifically, the hydrolysis stage requires high temperatures and moderate substrate concentrations, while the fermentation stage benefits from lower temperatures, optimal yeast loading, and extended time. Further research is required to explore longer hydrolysis and fermentation durations, intermediate temperatures, and fine-tuning substrate and yeast concentrations to further enhance yields.

CONCLUSION AND RECOMMENDATIONS

SUMMARY

Hydrolysis Process:

- **Substrate Concentration and Temperature**: Glucose yields from maize bran hydrolysate were measured at various substrate concentrations (2%, 6%, and 10%) and temperatures (30°C, 40°C, and 50°C). Results indicated that both higher substrate concentrations and higher temperatures generally led to increased glucose yields, with the highest glucose yield (30 mg) observed at 6% substrate concentration and 50°C after 1 hour of hydrolysis.
- **Hydrolysis Duration**: At 6% substrate concentration and 50°C, extending the hydrolysis time from 1 to 6 hours significantly increased glucose yield, reaching 95 mg after 6 hours.

Fermentation Process:

- **Yeast Loading Rate and Temperature**: The ethanol yield from fermented maize bran hydrolysate was analyzed at different yeast loading rates (1%, 10%,

and 20%) and temperatures (30°C, 40°C, and 50°C). The highest ethanol yield (1.60%) was achieved with a 10% yeast loading rate at 30°C. Increasing the yeast loading rate to 20% did not improve ethanol yield, suggesting an optimal yeast concentration for fermentation.

- **Fermentation Duration**: Using the optimal conditions of 10% yeast loading rate and 30°C, extending the fermentation time from 1 to 6 hours increased ethanol yield from 0.20% to 0.80%.

CONCLUSION

This study investigates the hydrolysis of maize bran using crude amylase enzyme and the subsequent fermentation of the hydrolysate to produce ethanol. The key variables analyzed include substrate concentration, temperature, hydrolysis duration, yeast (Saccharomyces cerevisiae) loading rate, and fermentation duration. The optimal conditions for hydrolysis were identified as 6% substrate concentration and 50°C for up to 6 hours, while for fermentation, the optimal conditions were 10% yeast loading rate and 30°C for up to 6 hours. The study highlights the need to optimize both stages (hydrolysis and fermentation) for efficient bioethanol production from maize bran. These findings provide valuable insights into the bioconversion of maize bran, showcasing the importance of parameter optimization in achieving high yields of glucose and ethanol.

RECOMMENDATIONS

Based on the results of this study on the hydrolysis of maize bran using crude amylase enzyme and the subsequent fermentation of the hydrolysate to produce ethanol, the following recommendations are proposed:

1. **Optimize Substrate Concentration and Temperature for Hydrolysis**:
    - Use a substrate concentration of 6% for hydrolysis as it provided the highest glucose yield at 50°C.
    - Maintain the hydrolysis temperature at 50°C to maximize glucose production.
2. **Extend Hydrolysis Duration**:
    - Implement a hydrolysis time of up to 6 hours to achieve higher glucose yields. While 1 hour provides some yield, longer durations significantly increase the glucose concentration.
3. **Optimize Yeast Loading Rate and Fermentation Temperature**:
    - Use a yeast loading rate of 10% for fermentation as it resulted in the highest ethanol yield.
    - Maintain fermentation at a temperature of 30°C for optimal ethanol production.
4. **Extend Fermentation Duration**:
    - Extend fermentation duration to at least 6 hours to maximize ethanol yield. Initial yields at 1 hour are significantly lower than those achieved at 6 hours.
5. **Monitor and Adjust Process Parameters**:
    - Continuously monitor glucose and ethanol concentrations throughout the hydrolysis and fermentation processes to ensure optimal conditions are maintained.
    - Adjust substrate concentration, temperature, yeast loading rate, and processing time as needed based on real-time data.
6. **Consider Scale-Up Implications**:

- When scaling up the process, ensure that the reactor design and process control systems can maintain the recommended optimal conditions for substrate concentration, temperature, and time.
- Pilot-scale studies should be conducted to confirm that lab-scale results are replicable at larger scales.

7. **Explore Further Enhancements**:
   - Investigate the use of additional enzymes or enzyme combinations to potentially increase hydrolysis efficiency and glucose yield.
   - Explore genetic modification or selective breeding of Saccharomyces cerevisiae strains to enhance ethanol production.
8. **Economic and Environmental Assessment**:
   - Conduct a cost-benefit analysis to determine the economic viability of the process at commercial scale.
   - Assess the environmental impact of the process, including the use of resources and waste management, to ensure sustainability.
9. **Integration with Existing Biorefineries**:
   - Evaluate the possibility of integrating this process with existing biorefinery operations to utilize maize bran more efficiently and improve overall biofuel production.

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