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ORIGINAL RESEARCH ARTICLE

Microbial Bioethanol Production from Locally Sourced Corncobs through Saccharification and Fermentation using *Aspergillus niger* and *Saccharomyces cerevisiae*

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ABSTRACT
The utilisation of agricultural residues, specifically corncobs, as a renewable feedstock for bioethanol production holds promise in sustainable energy generation. This study investigates the feasibility of microbial bioethanol production from locally sourced corncobs through co-digestion involving *Aspergillus niger* and *Saccharomyces cerevisiae*. The process involved washing the corncobs, grinding them, and then hydrolysing the corncob flour with cultured Aspergillus niger. The resulting sugar syrup was then fermented with Saccharomyces cerevisiae to produce ethanol. The process was optimised to obtain the highest ethanol yield. The results indicated that the process developed achieved a maximum ethanol yield of 33.2, 36.7, and 45.5% from the triplicate digesters used with a percentage purity in the range of 62.06% to 87.69% and a mean volume of ethanol recovery of 145 mL per 400 mL of the fermented product. This yield was obtained when the temperature of the hydrolysis using inoculum and saccharification was maintained at 27 °C. Additionally, the optimal substrate concentration for maximum ethanol yield was found to be 50 % (w/v). The study demonstrates the potential of corncobs obtained from local mashing as a source of fermentable sugars for the microbial production of bioethanol. Additionally, the results provide a basis for developing an efficient and economically feasible process for producing bioethanol from corncobs obtained from local mashing.

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Aspergillus niger, Bioethanol, Corncobs, Fermentation, *Saccharomyces cerevisiae*

INTRODUCTION
Bioethanol is ethanol (C_2H_5OH), or ethyl alcohol, produced by biological methods involving fermentation of biological materials such as sugar, starch, or cellulose by microorganisms. It is commonly used as a fuel source in the transportation sector and can be blended with gasoline to create ethanol blends like 10% ethanol or 85% ethanol (Faisal *et al.*, 2020). The escalating demand for renewable energy sources has fueled the interest in utilising agricultural residues as substrates for bioethanol production. Corncobs represent an abundant and underutilised biomass resource with significant potential for bioethanol generation.

Bioethanol has tremendous benefits as it offers a renewable and clean-burning fuel alternative to fossil fuels. Hence, research on bioethanol is significant due to its potential to reduce emissions, enhance energy security, promote rural development, and utilise waste (Oh and Jin, 2018). However, traditional crops like corn and sugarcane can not meet the high demand for ethanol today due to their primary roles as food and animal feed (Zhang, *et al.,* 2021). Consequently, the focus is shifting towards using abundant, renewable, and cost-effective resources like agricultural waste, rich in lignocellulosic materials, for producing bioethanol (Sakar, *et al.,* 2012). However, this approach comes with challenges such as efficient biomass handling, effective pretreatment methods for complete delignification, and the development of fermentation techniques to convert both glucose and xylose into ethanol (Sakar *et al.*, 2012).

Various studies have explored strategies to enhance bioethanol production using various substrates., such as utilising marine microalgae due to its abundance and unique properties, using diverse biomasses like wood and agricultural waste, employing enzyme catalysts and microbial strains for lignocellulosic biomass pretreatment, and utilising genetic engineering to enhance feedstock degradation (Singh *et al.,* 2021). Cai *et al.* (2016) integrate corn cob bagasse for microbial lipid and bioethanol,

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yielding high production and reduced COD. Prasad *et al.* (2019) explore genetic engineering for lignocellulosic feedstock degradation. Mosier *et al.* (2005) optimise cassava peel waste for bioethanol through enzymatic saccharification and integrated processes yielding significant ethanol quantities. Duque *et al.* (2021) propose enzymes and microbes to enhance lignocellulosic biomass pretreatment. Research by Maity and Mallik (2022) highlights marine microalgae's potential as a third-generation biofuel source due to resources, seawater growth, and unique metabolites. Chen *et al.* (2022) note diverse biomasses for bioethanol, including wood and marine algae, with waste biomass recycling.

Despite all the research efforts, several challenges hinder the widespread implementation of microbial bioethanol production. Issues such as low ethanol yield, high production costs, inefficient fermentation processes, substrate limitations, and microbial sensitivity to environmental conditions impede the scalability and cost-effectiveness of bioethanol production. Addressing these challenges is crucial to maximising microbial bioethanol production's efficiency, yield, and economic viability, ultimately advancing its potential as a renewable and eco-friendly alternative to fossil fuels. Although various substrates of sugar origin can be utilised as feedstock, because of the challenges associated with these substrates' availability, the use of cellulosic and other agro waste has become the only option (Bender, *et al.*, 2022). Corncob is a significant feedstock for bioethanol production due to its various benefits. It is considered a valuable agricultural residue with high cellulose content, making it a suitable source of sugars for fermentation into bioethanol. Corncobs are readily available as agricultural waste, reducing the need for dedicated cultivation of energy crops and minimising competition with food production (Zhang *et al.*, 2015).

Based on the foregoing, this study aims to investigate the feasibility and efficacy of harnessing corncobs obtained from local sources for bioethanol production through a co-digestion strategy involving *A. niger* and *S. cerevisiae*. The approach involves enzymatic hydrolysis of corncobs to liberate fermentable sugars, followed by simultaneous saccharification and fermentation facilitated by the co-culture of *A. niger* and *S. cerevisiae*. The outcomes of this research endeavour hold immense significance in sustainable bioethanol production, exploring a novel avenue for valorising corncobs, a locally sourced agricultural residue, while also contributing to renewable energy solutions. The insights gained from this study are poised to advance efficient bioethanol production strategies, aligning with the global pursuit of renewable and environmentally sustainable energy sources.

MATERIALS AND METHODS

Materials used

Ground corncob obtained from local mashing Katsina state metropolis was used as the substrate for bioethanol production, Bama bottle obtained from food vendors with the capacity of 1 litre was used as the digesters, and cultured *Aspergillus niger*, *Saccharomyces cerevisiae* obtained from the stock cultures of microbiology laboratory of Umaru Musa Yar'adua University Katsina were used as the inoculum. Other materials include filtration cloth, plastic bucket, sterile distilled water, syringe, cotton wool, foil paper, measuring cylinder, weighing balance (SF-400 Afrimash, Nigeria) sulfuric acid, calcium hydroxide, pH meter, (Ph100B, Mesulab, China) spectrophotometer (V1200, shanghai yoke instruments, chaina), autoclave (AUT41-18, Labstac, U.S), rotatory evaporator (Sz-96, Wincom, Chaina), viju bottles, plain container.

Sample Collection

The sample was collected in a sterile polythene bag from the farmhouse Kerau quarters in Katsina, katsina state metropolis, and was then transported to the Umaru Musa Yar'adua University microbiology laboratory.

Sample Pretreatment

The sample pre-treatment was carried out following the method of Edeh (2020) with littelw modifications as follows:

Acid treatment

Ground corncob was treated with 0.5% sulfuric acid (50mL H_2S0_4 diluted into 950mL of H_2O) and mixed thoroughly to help breakdown larger components into small particles. It was then taken to autoclave to soften the mixture for the fastest degradation. The mixture of the corncobs with the sulfuric acid was rinsed three times and filtrated using a clean filtration cloth. The softened corncob residue was then spread on a plate container for partial drying to reduce the moisture content.

Neutralisation

Ten gram 10g of calcium hydroxide was diluted into 1000mL of distilled water, and the solution was used to neutralise the acidic content of the corncobs. The corncobs were then rinsed with distilled water three times and filtrated to remove the water. The pH measure of the treated corncob was taken, and a value of 6.5, which is neutral, was obtained.

Bioethanol Production

The bioethanol was produced based on the following steps as described by Amores and Suárez (2019):

Enzymatic hydrolysis

Four sets of bottles (A, B, C, D) were used were used as digesters, and each bottle was treated with 200g of the pre-treated corncobs mixed with 400mL of sterile distilled water. Five millilitres (5 mL) of 10^6 CFU/mL of *Aspergillus niger* was added to each of the first three bottles (A, B, and C). The lids of the bottles were closed and shaken vigorously to ensure a sufficient mixture of the inoculum with the substrate and labelled A–C, respectively. The other bottle (D) is left untreated with the inoculum to

serve as control. All the four digesters were then incubated at room temperature and subjected to periodic agitation for four days.

Fermentation

A 3.5 grams of yeast (*Saccharomyces cerevisiae*) obtained in the UMYUK microbiology laboratory was diluted into 20 mL of water, and 6 mL of the diluent was inoculated into each digester container except for the control. All the containers were incubated at room temperature and subjected to 12 hours of periodic agitation for four days of incubation.

Extraction of the ethanol

After the fermentation period, the fermented product's liquid portion was separated from the undigested solid by filtration method through a clean cloth. Next, the liquid mixture was distilled at 78°C to separate the ethanol from the water mixture. The distilled ethanol was then collected, and the volume was measured and recorded before storing the ethanol. The percentage yield of each digester was calculated using the formula below:

$$Ethanol\ yield = \frac{Volume\ of\ Recovered\ ethanol}{Total\ volume\ of\ the\ Mixture} \times 100\% \quad (1)$$

Qualitative confirmation of the ethanol

Confirmatory tests for bioethanol typically involve using chemical reagents to verify the presence of ethanol in a sample using potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4) as reagents. A small amount of the sample was collected in the test tube. A few drops of potassium dichromate solution and a few drops of concentrated sulfuric acid were added to the sample using a Pasteur pipette. The mixture was heated in a water bath and colour change was observed.

If ethanol is present, it will react with the reagents, causing a colour change from orange to green as the ethanol is oxidised to acetic acid; the intensity of the green colour in the mixture increases with the increase in the concentration of the ethanol in the sample (Faisal *et al.,* 2020).

Percentage purity of the ethanol

A standard solution was created with a known ethanol concentration to establish a calibration curve. The initial absorbance of the blank was measured at approximately 340nm before the addition of the bioethanol sample. Final absorbance was measured, calculated and recorded (Kumar and Sharma, 2017). The percentage purity was then calculated by substituting the absorbance of the extracted ethanol in the calibration curve equation obtained by plotting the graph of the absorbance against the various concentrations (percentages of the standard ethanol).

RESULTS

Ethanol Recovery

The total volume of the ethanol recovered from each of the three digesters is depicted in Figure 1. Approximately 133 mL, 147 mL and 182 mL of ethanol were recovered from digesters 1, 2 and 3, respectively. However, no ethanol was recovered from the negative control. Overall, the mean volume of ethanol recovery per 400 mL of the fermented product in this study was 145 mL. On the other hand, the percentage yield of the ethanol from the digesters ranges from 33.20% to 45.50% (Table 1).

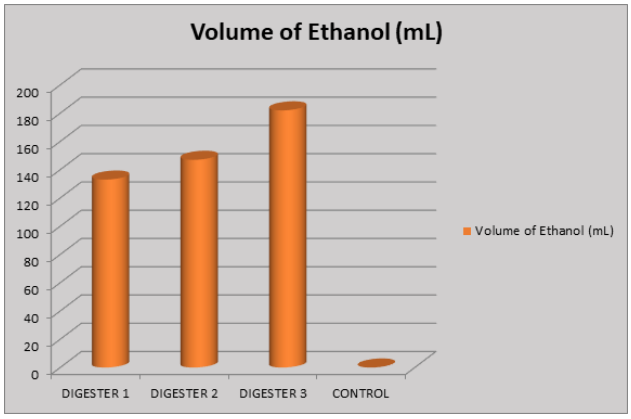

Figure 1: Volume of bioethanol recovered from the digesters

Table 1: Percentage yield of ethanol from the four digesters

Digester	Volume of Fermented Product (mL)	Volume of Ethanol Recovered (mL)	Percentage Yield of the Ethanol
Digester 1	400	133	33.2%
Digester 2	400	147	36.7%
Digester 3	400	182	45.5%
Digester 4 (Control)	400	0	0%

Percentage Purity of the Synthesised Bioethanol

The UV-spectroscopic calibration curve of the absorbance units at 214nm of standard solutions of ethanol is shown in Figure 2. the high R-square value obtained indicates good accuracy and precision of the measurements. Using the calibration curve of the standard, the percentage purity of the synthesised ethanol from the four digesters was determined (Table 2). The result showed that the percentage purity of the obtained ethanol ranges from 62.06% to 87.69%. Although digester three has the highest percentage yield of ethanol, the purest ethanol was obtained from digester two.

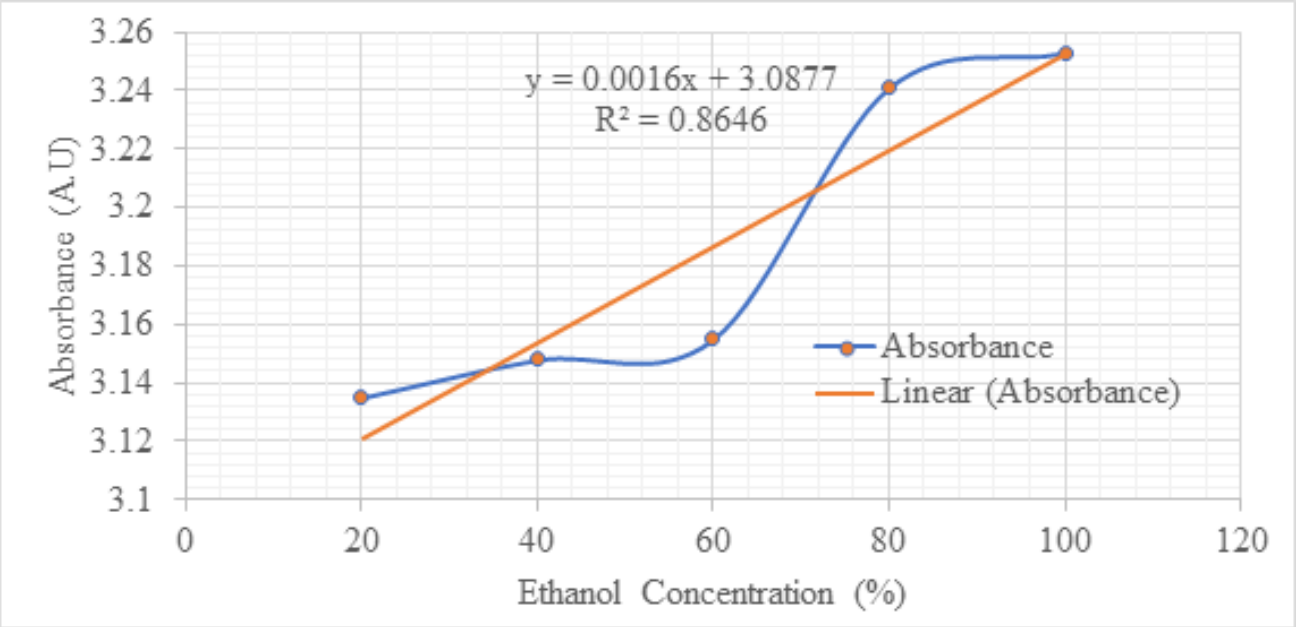

Figure 2: Calibration of the absorbance units obtained by UV- visible spectrophotometer at 214nm against the standard ethanol concentrations.

Table 2: Percentage purity of ethanol from the four digesters

Digester	Volume of Fermented Product (mL)	Absorbance at 214 nm	Percentage Purity of the Ethanol
Digester 1	400	3.198	68.94%
Digester 2	400	3.228	87.69%
Digester 3	400	3.187	62.06%
Digester 4 (Control)	400	3.085	-1.69%

DISCUSSION

Based on the knowledge that bioethanol can be produced from complex and simple sugars by microbial fermentation, this study evaluated the potential of using cellulosic materials as corncob, in particular, for bioethaol production. The study demonstrates a successful outcome. Both chemical and physical pretreatment options hastened the degradation of the complex organic matter into smaller or simpler molecules for easy utilisation of these molecules by fermenting organisms, as opined by Edeh, (2020). The acid pre-treatment made the corncob softer and any unwanted contamination by biological properties were killed. The physical pretreatment using steam explosion incorporates moisture into the hardened corncob so that it will become softer and easily fermented (Sigh *et al.,* 2022)

The enzymatic hydrolysis of the cellulose was successfully achieved using *Aspergillus niger*. This is due to the ability of *A. niger* to rapidly grow and secrete extracellular cellulase and amylase enzymes that catalyse complex carbohydrates into simpler sugars. Similarly, cultured *Saccharomyces cerevisiae* has been the inoculum of choice for many experiments to produce bioethanol (Ede *et al.* 2020). This is due to its renowned ability to breakdown glucose into alcohol and H_2O, as described in the findings of Oh and Jin (2020). The co-digestive effects of *A. niger* and *S. cerevisiae* resulted in maximum fermentation leading to the maximum ethanol recovery in this study. This inference corroborated the finding of Edeh (2020), who also reported high ethanol yield using the same organisms.

CONCLUSION

This study investigates the feasibility of microbial bioethanol production from locally sourced corncobs through co-digestion involving *Aspergillus niger* and *Saccharomyces cerevisiae*. A very high yield of ethanol was obtained (mean = 145 mL per 400 mL of the fermented product) with a percentage purity above 80%. The findings demonstrate promising potential for sustainable ethanol production using corncobs. Future studies may investigate further process optimisation to enhance ethanol yield and efficiency, potentially exploring variations in mashing techniques or fermentation conditions.

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