Received: 16 August 2024 | Revised: 6 September 2024 | Accepted: 16 October 2024 | Published online: 21 October 2024

RESEARCH ARTICLE

Archives of Advanced Engineering Science
2024, Vol. 00(00) 1–11
DOI: 10.47852/bonviewAAES42024107

Production and Characterization of Bioethanol Using Native Microbial Isolates from Decomposed Maize Cob via Simultaneous Saccharification and Fermentation

Yakubu Atiku[1], Surajudeen Abdulsalam[1], Jibril Mohammed[1], Saeed Ahmed[1] and Ahmed Inuwa[1,*]

[1]Faculty of Engineering and Engineering Technology, Abubakar Tafawa Balewa University, Nigeria

Abstract: Maize cob is a major component of agricultural waste composed mainly of cellulose that can be converted to bioethanol through fermentation as a means of waste management. Achieving enhance bioethanol yield without ascertaining proper conversion pathway may be subjective. Therefore, this research aimed at converting maize cob to bioethanol through fermentation using native microbial isolates from decomposed maize cob via response surface methodology. Microbial strains used were *Pichia kudriavzevii strains* with accession number KP998095.1 and MN861069.1. A pH value of 5.5 corresponded to bioethanol yield of 52.45 g/L, and pH was observed to be sensitive to fermentation, hence influencing the extent of fermentation process. Optical density (O. D_{610nm}) of 2.2 after eight (8) days of fermentation corresponded to bioethanol yield of 52.45 g/L. The growth pattern observed in most of the samples followed a typical microbial growth pattern depicting lag, log, and stationary phases. Reducing soluble sugar of 23.5 mg/L on enzyme hydrolysis corresponded to bioethanol yield of 52.45 g/L, and reducing soluble sugar content of the experimental samples was observed to decrease as the fermentation progresses. The infrared spectra of the optimum sample distillate of bioethanol from the fermentation revealed the presence of OH at 3311.7 cm^{-1} stretching band and C = C at 1636.3 cm^{-1} stretching band. The elemental compositions of the distillate were oxygen (31.03%), hydrogen (9.74%), carbon (56.85%), nitrogen (1.92%), and sulfur (0.46%) which confirmed the presence of bioethanol. The empirical formula of the best distillate using C, H, and O compositions (%) was found to be C_2H_5OH and hence revealed the presence of bioethanol. The result of the gas chromatography mass spectrophotometry analysis showed that mass spectra comparison of the various peaks revealed the presence of bioethanol as the largest peak with 76.38% ethanol by concentration. Maize cob can be said to be a promising feedstock for bioethanol production using native microbial isolates.

Keywords: bioethanol production, characterization, Pichia kudriavzevii, native microbial isolates, maize cob

1. Introduction

In the quest for sustainable and renewable energy sources, there has been a lot of interest in the development of bioethanol as a workable replacement for fossil fuels. Agricultural wastes are one of the many feedstocks that can be exploited to make bioethanol; however, they are a potentially useful but underutilized source [1]. Corn, a widely grown crop worldwide, generates a lot of waste in the form of cobs, which are occasionally carelessly tossed away or left unused. In this paper, the feasibility of using maize cobs more specifically deteriorated ones as a feedstock for bioethanol production is examined [2].

Conversion of lignocellulosic biomass such as maize cobs into bioethanol through fermentation represents combined advantages of waste-to-value conversion as an alternative biofuel generation, production of fermentable sugars from the biomass fractions, namely cellulose, hemicellulose, and lignin, is a challenging process [3]. Bioethanol, identified as a sustainable solution to fossil fuels, has gained significant attention with an annual global production of 29 billion tones [4]. The effect caused by the global warming, acid weather, and urban smog due to carbon dioxide(CO_2) emission from the conventional fossil fuel to the atmosphere has persuaded researchers to look for renewable energy options, such as bioethanol, bioethanol is one of the best natural biofuels, and the need for pre-treatment of biomass is justified to enhance the bioethanol conversion efficiency [1], and its usages in gasoline reduced the CO_2 equivalent greenhouse gas emissions from transportation by 43.5 million metric tonnes in 2016 [5]. Second-generation bioethanol from lignocellulosic biomass such as corn cob has gained significant interest as a potential replacement for fossil fuel-derived source(s) [6].

Second-generation bioethanol production involves a number of consecutive stages such as pre-treatment, hydrolysis, fermentation,

*Corresponding author: A. M. Inuwa, Faculty of Engineering and Engineering Technology, Abubakar Tafawa Balewa University, Nigeria. Email: aminuwa.pg@atbu.edu.ng

© The Author(s) 2024. Published by BON VIEW PUBLISHING PTE. LTD. This is an open access article under the CC BY License (https://creativecommons.org/licenses/by/4.0/).

and distillation and/or separation, and the overall process for any given substrate could potentially have thousands of different permutations [7, 8]. However, further research is needed to take this process beyond fermentable sugar yields, to include the effect of yeast cultures, or indeed other microorganisms, also of particular interest is the potential effect of fermentation inhibitors such as metal ions, H_2S and NH_3 released from the biomass during processing on final alcohol yields, which may be process or substrate-specific [9] Some biomass residues that have been used as precursors of reducing sugars for bioethanol production are rice straw, rice husk, macaranga, bamboo, agave leaves, palm oil, wheat bran, sorghum stalk, sugarcane leaves, and citronella residues, to mention a few [10, 11].

The significance of native microbial isolates becomes apparent in this setting. Native microorganisms may have special enzymatic properties that could enhance the saccharification and fermentation processes since they are innately tuned to the local environment [12].Yeast participation in fermentation is limited by the inhibitory effects of ethanol concentration which increases over the course of fermentation. The adoption of new or emerging fungal isolates towards large-scale ethanol production relies upon its ability to tolerate certain levels of ethanol within the broth, since studies indicated that the generally accepted benchmark for ethanol tolerance amongst yeast isolates is 12% (v/v) [13] Fungal yeast strains are traditionally involved in many food fermentations and manufacturing products such as beers, ciders, wines, sake, baked goods, cheese, sausages, and other fermented foods; fungal strains were reported to have different capacity of stress tolerances [14].

Furthermore, structural alterations that produce false positives when relying solely on biochemical and phenotypic identification approaches make realistic molecular identification of some fungal species still difficult. Nevertheless, the development of DNA-based techniques largely overcomes the drawbacks of traditional techniques, and investigations employing molecular techniques revealed a higher diversity of fungi [15]. It has been reported that based on biochemical and morphological characterization alone, strains of fungi have all been misidentified at one point in research due to a high degree of resemblance (similarity).

A common crop in the world, maize, produces a lot of waste in the shape of cobs, which are sometimes thrown away inefficiently or left unused. The possibility of employing maize cobs more precisely, degraded ones as a feedstock for bioethanol production, is investigated in this work [16]. Nevertheless, the current study focused on the simultaneous saccharification and fermentation (SSF) of native microbial isolates from maize cobs to produce and characterize bioethanol. In a single fermenter, SSF may carry out enzymatic saccharification and fermentation to produce biofuel. As a result, SSF offers a number of benefits, including lowering energy input, working with ease, and streamlining the manufacturing process.

2. Experimental Methods

For the purpose of conducting this research, SSF technique was adopted in conducting the research, established procedures were used in carrying out the experiment, and Box-Behnken statistical tool of response surface methodology was used in designing the experiment for the production of bioethanol as explained in the section below.

2.1. Preparation of fermentation medium for the production of bioethanol

The bioethanol production experiment was carried out based on Box-Behnken statistical tool design matrix, the factors considered were concentration of NaOH (g/L), biomass loading (g/L), and pH, whereas the responses were optical density (cell mass), reducing sugar (mg/L), CO_2 (g), and ethanol yield (%); Table 1 shows the experimental factors and the levels of variables.

Further, the fermentation was carried out in liquid state known as liquid state fermentation; twenty-five millimeters (25 mL) of the fungal isolates identified as *Pichia kudrievzevii* strains culture broth medium was aseptically inoculated to the varying experimental samples based on the Box-benhken statistical tool of the design expert version 13 as shown in Table 2. SSF in each of the seventeen (17) single 1000 mL fermenters was adopted. In addition, the fermentation experiment was carried out at room temperature for 8 days, and all the fermenters were shaken on daily basis manually for proper microbial interaction with the substrates. Other fermentation indicators such as cell mass were measured at two (2) days interval; after eight (8) days of fermentation, separation of bioethanol via distillation at 78 °C was carried out. The method used in this work with slight

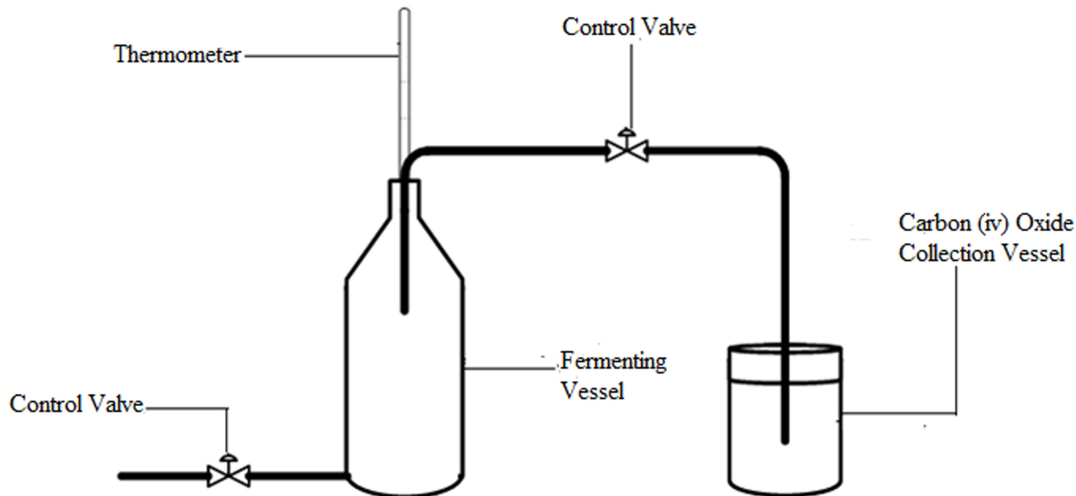
Table 1
Factors and levels of variables for the fermentation experiment

Factor	Level		
	Low (−)	Average (0)	High (+)
Biomass Loading (g/L)	50	55	60
NaOH (g/L)	1	1.5	2
pH	5	5.5	6.0

Table 2
Experimental design matrix for the production of bioethanol

Std	Run	Factor 1 A:A:Biomass loading g/L	Factor 2 B:B:NaOH g/L	Factor 3 C:C:pH –
1	9	50	1	6
2	3	60	1	6
3	6	50	2	6
4	14	60	2	6
5	1	50	1.5	5.5
6	12	60	1.5	5.5
7	13	50	1.5	6.5
8	5	60	1.5	6.5
9	2	55	1	5.5
10	11	55	2	5.5
11	16	55	1	6.5
12	4	55	2	6.5
13	10	55	1.5	6
14	15	55	1.5	6
15	7	55	1.5	6
16	8	55	1.5	6
17	17	55	1.5	6

Figure 1
Schematic process flow diagram for the developed fermenter



modification is in conformity with the literature by Mondal et al. [10] and Germec et al. [17]. Equation (1) was used to calculate the percentage (%) ethanol yields. Figure 1 is the schematic process flow diagram of the developed fermenter used.

$$\text{Ethanol yield}(\%) = \frac{\text{Ethanol produced(mL)}}{\text{Fermentation broth(mL)}} \times 100 \qquad (1)$$

2.2. Adjustment of the pH condition after alkaline hydrolysis

The pH values of all the seventeen (17) experimental samples generated based on the Box-Behnken Statistical tool design matrix after alkaline pre-treatment which had pH values above 10 were adjusted using 10% H_2SO_4 by inserting the electrode of the pH meter into each of the samples after blanking each time before measurement and pH value of the individual samples were observed and recorded. The pH ranged between 5.0 and 6.5 after adjustment.

2.3. Preparation of fungal broth medium

Thirty grams (30 g) of the sabroud destrox broth (SDB) agar was dissolved in 700 mL of distilled water, mixed and made up to 1000 mL with distilled water. The media was autoclaved at the temperature of 121°C for 15 min after which the pure isolates were added and incubated at 37°C for three (3) days. The cultures were kept in the refrigerator at 4°C for two days prior to SSF for the production of bioethanol.

2.4. Preparation of dinitro salicylic acid (DNSA) reagent

An amount of 0.25 g 3, 5-DNSA reagent mixed with 75 g Rochelle salt ((sodium potassium tartrate) Na-K) was added to 50 mL of concentration 2M NaOH followed by addition of 2 mL of liquid phenol red and 2 g sodium sulfate ($NaSO_4$); then, it was finally diluted to 250 mL with distilled water. The prepared reagent was then kept in dark specimen bottle for application. The adopted procedure with slight modification is in agreement with the literature by Megala et al. [7] and Shukla et al. [18].

2.5. Determination of CO_2 by titration

CO_2 produced during the fermentation process was passed through sealed vessel containing 1 M NaOH as depicted in Figure 1 under Section 2.1. Samples were collected from the aqueous solution of NaOH after every two days for titration in order to quantify the amount of CO_2 produced using 1M HCl as titrant. It was a two-step titration: the first was carried out using 3 drops of phenolphthalein as indicator, which resulted in a colorless end point. This was done to neutralize excess NaOH and convert all the sodium carbonate into bicarbonates. The second titration was carried out using 4 drops of methyl orange as indicator and resulted in orange color end point which converted sodium bicarbonates to water and CO_2. The difference in mL between the first and the second end points (volume of the titrant) was used to determine the amount in grams of CO_2 present in the fermentable sugar. The aforementioned procedure is in agreement with the literature by Yang et al. [19]; Equation (2) was used to determine the amount of CO_2 in the fermentation broth samples.

$$\begin{aligned} &\textit{Volume of the Titrant}(mL) \times \text{Molarity of Standard Acid} \\ &\quad \times \text{Molecular Weight of } CO_2 = \text{Mass of } CO_2 \text{ (g)} \end{aligned} \qquad (2)$$

2.6. Determination of growth and residual sugar in the fermentation medium

The growth was determined by measuring the cell density (optical density) at 610 nm using photochem colorimeter manufactured by Aimil LTD India at time intervals. The amount of sugar in the fermentation medium after each period of fermentation was determined following the DNSA method. The DNSA reagent (1 mL) was added to 1 mL of the fermentation broth medium in a test tube and properly mixed; the mixture was boiled for 5 min and cooled under running tap water. Five mL (5 mL) of 40% Rochelle salt solution was added to the mixture

and concentration was read using 752N UV-VIS spectrophotometer at 540 nm as amount of reducing sugar in mg L^{-1}, the method reported with slight modification is in agreement with the literature by Germec et al. [17], Yang et al. [19], de Andrade Silva et al. [20], and Ningthoujam et al. [21].

2.7. Determination of bioethanol quantity

Both yield and concentration can be used interchangeably to quantify bioethanol; in this work, the two approaches were adopted. The distillate collected over a slow heat at 78°C (for a period of 50 min.) which was re-distilled at 78°C (for a period of 40 min.) was measured using a graduated 120 mL capacity white bottles and expressed as the quantity of ethanol produced in g/L by multiplying the volume of distillate collected at 78°C by the density of ethanol (0.93 g/mL). The g/L is equivalent to the yield of 100 g of the dried substrate [22].

2.8. Characterization of bioethanol

In this study, the determination of density of bioethanol was carried out by weighing the empty 120 mL graduated white bottle followed by taking the weight of the white bottle with sample (bioethanol). In addition, the weight of the white bottle with water was also measured separately using weighing balance; Equation (3) was used for the determination of density of bioethanol. The aforementioned procedure with slight modification is in agreement with the related procedure in the literature [23].

$$Density = \frac{Y_2 - Y_1}{Y_3 - Y_1} \tag{3}$$

where $Y1$ = weight (g) of empty density bottle, $Y2$ = weight (g) of empty density bottle + sample, $Y3$ = weight (g) of empty density bottle + water.

However, the Fourier transform infrared (FTIR) characterization was carried out using FTIR machine manufactured by Agilent Technologies, the gas chromatography mass spectrophotometry (GC-MS) characterization was carried out using GC7890B, MSD 5977A, Agilent Technologies, and the sample distillate obtained after distillation was analyzed for elemental compositions using series II CHNS/O analyzer 2, 400 Perkin Elmer.

3. Results and Discussions

3.1. Production of bioethanol

Fermentation is one of the conversion pathways that produces bioethanol due to action of microorganisms. Native microorganisms used in this study for the fermentation of maize cob resulted in the formation of bioethanol after 8 days. Out of the seventeen samples investigated, sample S6 produced the highest bioethanol yield of 6.29% (v/v) which corresponded to 58.5 g/L of bioethanol as shown in Figure 2.

However, it was observed that the quantity of bioethanol of 58.5 g/L obtained in this study was above the value of 20.92 g/L reported by Okuofu et al. [24] and below the value of 104 g/L reported by Decarvalho [25] using corn cob as feedstock; the possible reason for the differences in yields may be attributed to variability in the fermentation condition such as microbial type(s), pH, and temperature. Furthermore, ethanol concentration of 16.8 g/L (1.68%) was obtained after 72 hours fermentation period at 30°C using corn cob as feedstock [2, 26]. The high quantity of bioethanol reported by Tharunkumar et al. (2024) may be due to the fact that there was further addition of substrate and enzyme on the spent fermentation broth after the first stage fermentation that is to say it was a multi-state operation. Other observed variation in the bioethanol quantity in the current study in comparison with the literature may be due to variability in the factors affecting saccharification and fermentation considered [6].

3.2. Effect of pH on the fermentation process

During fermentation, pH is one of the important factors that affect the fermentation reaction; it is generally a known fact that fungi favor strongly to slightly acidic environment, hence the reason for the pH adjustment. The best pH condition of 5.5 by sample S6 gave the highest bioethanol yield of 6.29 % (v/v), the numerical optimization and subsequent validation result revealed a pH of 5.5 and bioethanol yield of 5.64% (v/v) which corresponded to 52.45 g/L of bioethanol as depicted in Figure 3.

Figure 2
Bioethanol yield sample identity for seventeen samples (S1–S17) for the production of bioethanol

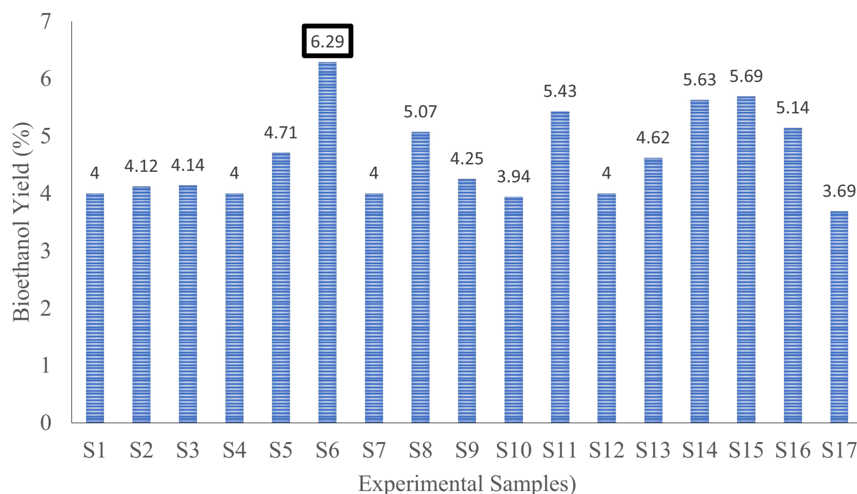
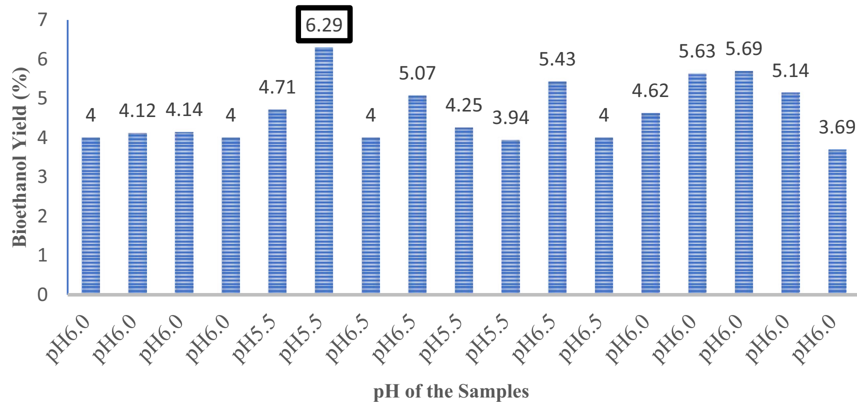


Figure 3
Bioethanol yield (%) against pH of various samples for the experiment



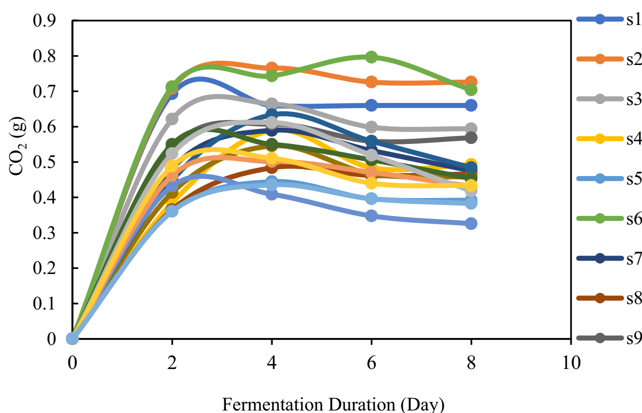
Furthermore, the optimum pH value of 5.5 obtained in this study is in agreement with the literature range of values of 5.5–7.0 [13, 24, 27]. According to Zhu et al. [11], optimal pH for fungal cellulases varies from species to species, though in most cases the optimum pH ranges from 3.0 to 6.0. This is an indication that optimum condition for the activity of cellulases varies among organisms and within different strains of a particular organism [28]. In addition, microorganism has its specific pH that enhances specific enzymes to catalyze certain required reactions [8].

As reported by Oktavia et al. [29], cellulases of fungal species such as *Aspergillus niger* isolated produced optimum activity at temperature range of 28 – 40°C and pH range of between 3.0 to 9.0.

3.3. Effect of CO_2 production on the extent of fermentation

The measurement of the amount of CO_2 released due to fermentation through titration enables a real-time determination of the ethanol in the medium. During this study, seventeen (17) experimental samples were used; it was observed from Figure 4 that there was significant increase in the amount of CO_2 produced at day 2 up to day 6 which corresponded to the period of maximum amount of CO_2 generation of 0.7964 g at day 6 by sample S6 which showed that the microorganism in the fermentation medium was able to effectively utilize the growth conditions such as fermentable sugar, pH, and temperature as depicted in Figure 4.

Figure 4
Carbon dioxide produced (g) versus fermentation duration (Day) for the optimization studies



However, there was very slight decrease in the amount of CO_2 produced beyond Day 4 in experimental samples S1–S17 as depicted in Figure 4; the observed changes may be attributed to changes that resulted in growth conditions of the microorganism such as availability of the substrate, microbial load, temperature, and pH [30].

However, the generation of CO_2 due to the fermentation of maize cob as feedstock is in line with the literature by Condor et al. [31]; also, CO_2 has been an inhibitory gas during fermentation process, hence the need to quantify and remove it from the fermentation broth during fermentation process. This explanation is in conformity with the literature by Roozbahani et al. [32].

3.4. Effect of cell mass (optical density) on the extent of fermentation

Continuous measuring of optical density (O.D) is the most basic and powerful tool for providing optimal yields and controlling reproducibility in many fermentation strategies. The higher the light is able to travel through the given medium, the lower the optical density of the medium (sample). Experimental sample S6 revealed optical density $O.D_{610nm}$ of 2.0 after eight (8) days of fermentation corresponding to highest bioethanol yield of 6.29 % (v/v) although the optimization and subsequent validation revealed optical density $O.D_{610nm}$ of 2.2 and bioethanol yield of 5.64% (v/v) which corresponded to 52.45 g/L of bioethanol using maize cob. The growth patterns in samples S6, S15, S13, and S11 followed a typical microbial growth pattern depicting lag, log, and stationary phases which is in agreement with the findings of Mun et al. [33] as depicted in Figure 5. Cellulase and amylase enzymes produced by the isolates using the submerged fermentation method, which is a novel method for the production of bioethanol, were known to yield more bioethanol which also agrees with the literature by Anderson et al. [34] and Tenkolu et al. [35].

However, the fluctuation in growth phases for samples S1–S5, S7–S10, S12, and S16–S17 may be due to non-uniformity in mixing prior to sample collection for routine analysis of O.D. The growth pattern observed in the current study is in compliance with literature by Chongkhong [36].

Figure 5
Optical density (O. D_{610nm}) against fermentation duration (Day) for the optimization studies

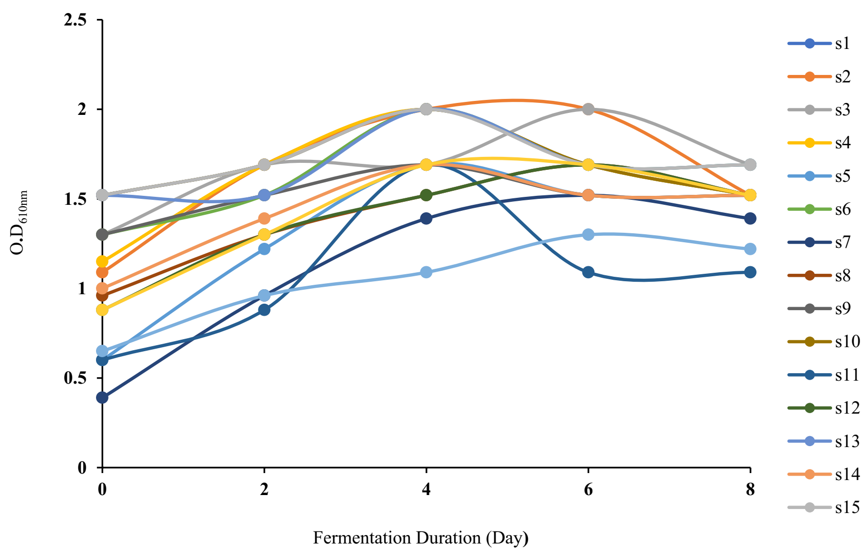
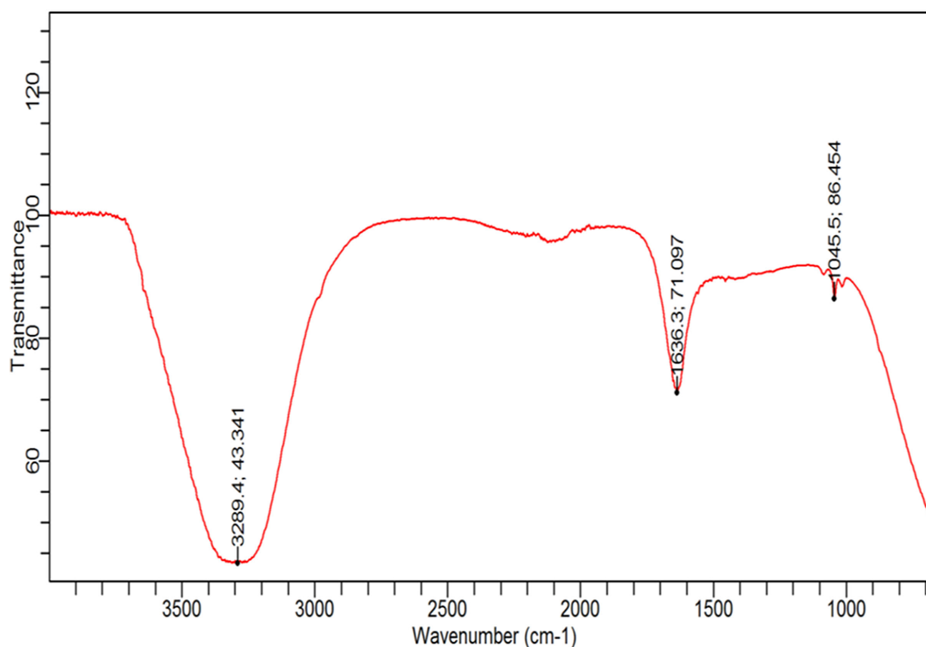


Figure 6
FTIR spectra of the optimum produced bioethanol distillate from fermentation broth



3.5. Characterization of the optimum bioethanol sample distillate

After the optimization experiment, the optimum bioethanol sample distillate was subjected to a number of characterizations so as to ascertain its properties. Results on characterization are presented in Sections 3.5.1, 3.5.2, and 3.5.3.

3.5.1. Characterization via FTIR spectra

The infrared spectra of the optimum distillate end product from the fermentation broth after the optimization experiment were measured between 500 and 4000 cm^{-1} as presented in Figure 6.

The absorption peak for OH stretch was observed in the optimum bioethanol sample distillate in the OH stretching region between 3000 and 3500 cm^{-1} as shown in Figure 6. The peak absorbance of the OH stretching band observed in the wide region of 3311.7 cm^{-1} is identified as OH stretch, while the peak at 1636.3 cm^{-1} is assigned to C = C stretching band; the peak at 1045.5 cm^{-1} is assigned to C-C stretching band, and the result obtained in the present study is in conformity with the literature by Luthra et al. [37] and Nisha & Vidya [38] for lignocellulosic biomass. The confirmation of the presence of OH, C = C, and C-O in the optimum sample distillate is an indication that maize

Table 3
Summary of the FTIR characterization

Stretching region (cm^{-1})	Stretch band (cm^{-1})	Functional group
3000–3500	3311.7	OH
1500–2000	1636.3	C = C
1260–1050	1045.5	C-O

cob used is a good feedstock for bioethanol production. Table 3 is the summary of the FTIR Characterization.

3.5.2. Characterization for elemental compositions of the optimum sample distillate

The optimum sample distillate was characterized by its carbon, hydrogen, nitrogen, sulfur, and oxygen content as presented in Table 4.

Table 4
Elemental compositions of the optimum sample distillate

Parameter	Composition
Carbon (%)	56.85
Hydrogen (%)	9.74
Nitrogen (%)	1.92
Sulfur (%)	0.44
Oxygen (%) by difference	31.03

The oxygen content was obtained by difference as presented in Table 4, lower values of nitrogen (1.92%) and sulfur (0.46%) implied lower emission during combustion process. However, the high carbon content of 56.85% relative to percentage oxygen of 31.03% and hydrogen of 9.74% in the optimum sample distillate revealed the potential of maize cob as feedstock for bioethanol production. In addition, lower proportion of the oxygen (31.03%) and hydrogen (9.74%) in comparison with carbon content (56.85%) increases the energy value of a fuel; this explanation is in compliance with the literature by Kaur et al. [15] and Ayala et al. [39]. It was observed based on the results presented in Table 4 for elemental composition that the produced ethanol has an empirical (simplest formula) of C_2H_5OH. Since nitrogen and sulfur contents in the distillate samples were small, it can be considered negligible; therefore, the empirical formula based on that is C_2H_5OH.

3.5.3. Characterization via GC-MS of the optimum sample distillate

GC-MS analyzer with model GC 7890B, MSD 5977A, Agilent Technology was used for the analysis. GC-MS analysis was carried out in the present studies in order to be able to know the possible compounds that might be present in the optimum sample distillate under investigation. Interpretation on mass spectrum of GC-MS was carried out by the database having many patterns; this is available at National Institute of Standard and Technology (NIST) [40]. The mass spectrum of the unknown section was compared with that of the known section stored in the NIST library. The name, molecular weight, and structure of the compounds from the test sample distillate were determined. Figure 7 is the

Figure 7
Chromatogram of the bioethanol sample showing the first eluded peak

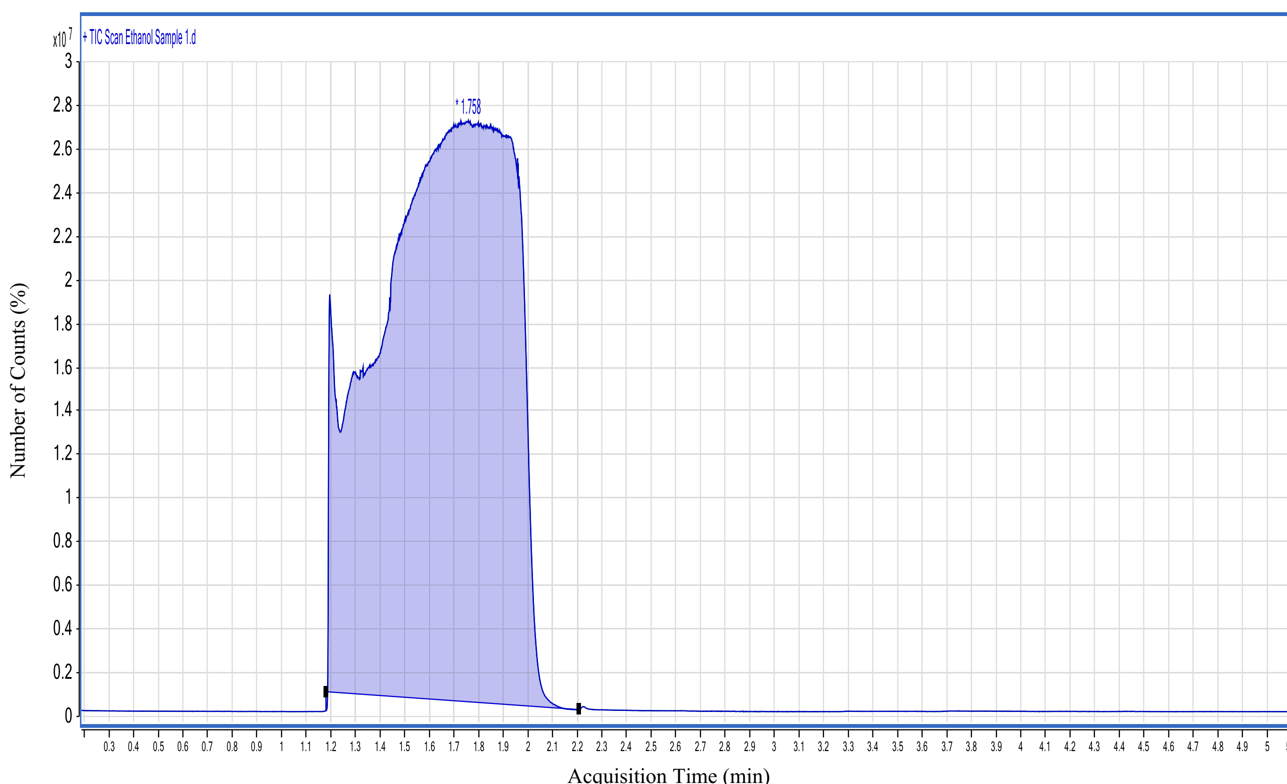


Figure 8
Chromatogram of the bioethanol sample showing the other eluded peaks

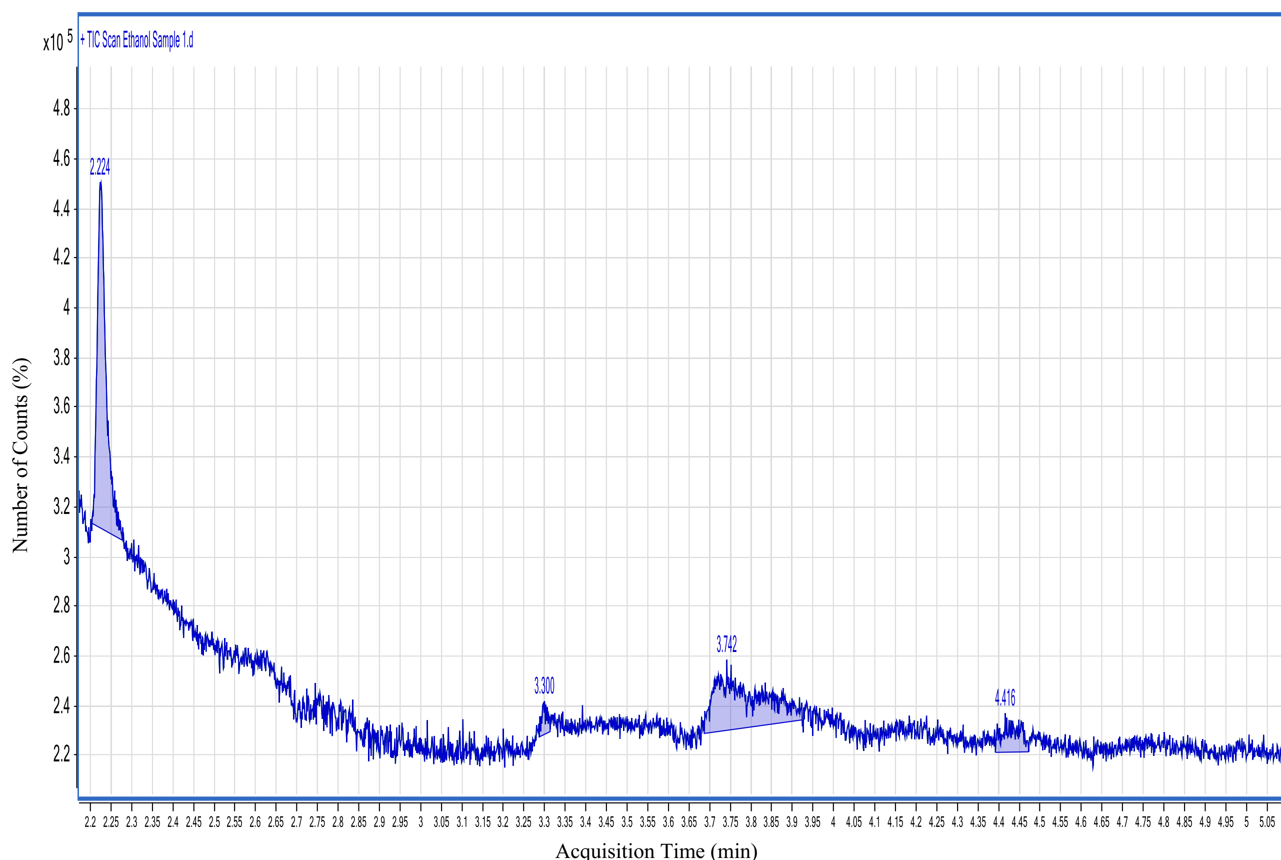


Table 5
Compounds present in the sample distillate

Peak	Rt	Height	Area	Compound	MF	M_W/ EM
1	1.54	22675738.12	972163665.77	Ethanol	C_2H_6O	46/46.042
2	2.22	139115.84	1063156782.96	Hydrazide Acetic Acid	$C_2H_6N_2O$	74/74.048
3	3.3	13843.22	11540.21	1-ethenyl Oxypentane	$C_7H_{14}O$	114/114.1045
4	3.742	28422.22	171211.19	7-methylene Bicyclo [4:1:0] heptane	C_8H_{12}	108/108.094
5	4.416	15682.58	37301.95	2,3-Epoxy butane	C_4H_8O	72/72.058

Rt = Retention time, MF = Molecular formula, M_W = Molecular weight and EM = Exact mass

chromatogram of the distillate sample showing the first eluded peak whereas Figure 8 is the detail chromatogram of the sample distillate showing the other eluded peaks as the sample distillate passes through the stationary phase [41].

The result of the mass spectra comparison of the various peaks showed that the eluded peaks were the compounds present in the sample distillate. In this study, five largest peaks revealed the presence of ethanol, hydrazide acetic acid, 1-ethenyl oxypentane, 7-methylene bicyclo [4:1:0] heptane, and 2, 3-Epoxy butane. Detail on the compounds present in optimum sample distillate is shown in Table 5.

1) Quantification of bioethanol in the optimum sample distillate via GC-MS analyzer

After confirming the identities of the possible compounds present in the optimum sample distillate, the sample was subjected to quantification for ethanol concentration (%). Absolute ethanol was used as standard as a basis for the quantification. The quantification result revealed that the optimum sample distillate gave 76.38 % ethanol concentration, and the presence of other bioactive compounds as indicated in GC-MS result of the optimum sample distillate may be the reason for not getting bioethanol concentration above 76.38% reported in this study.

Figure 9
Chromatogram of the sample used as a standard

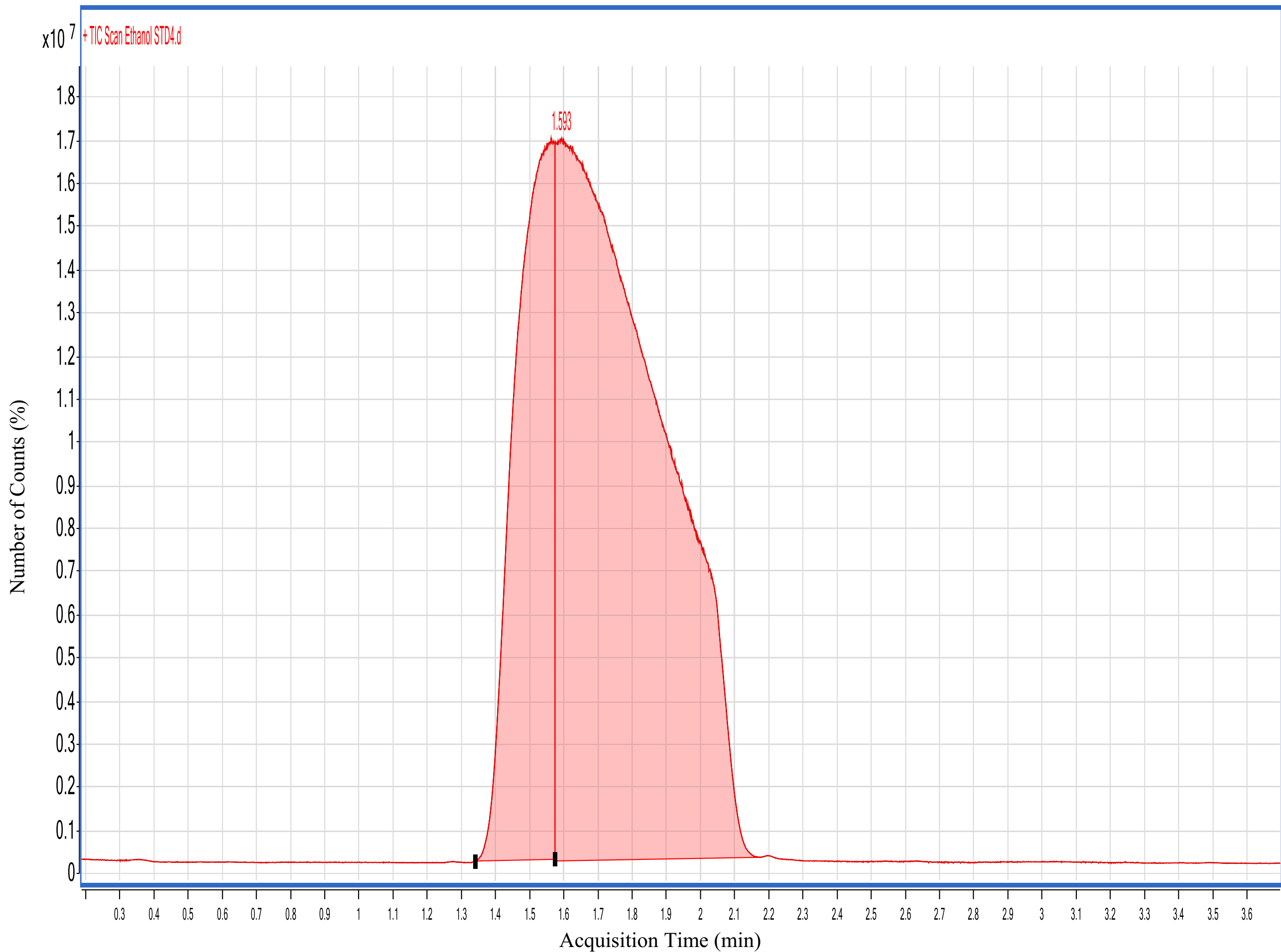


Figure 10
Calibration curve used for the quantification of the bioethanol

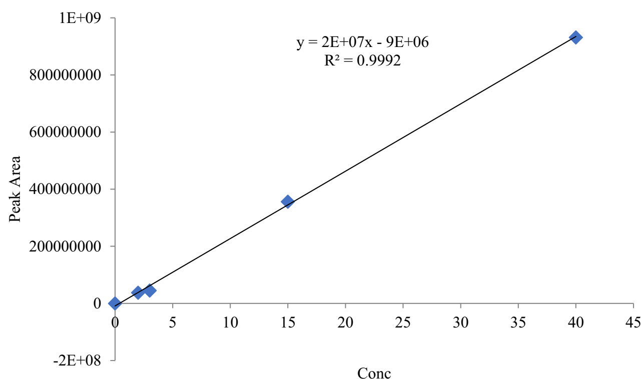


Figure 9 is the chromatogram of the sample used as standard, whereas Figure 10 is the calibration curve used for the quantification of bioethanol; Table 6 shows the detailed information on the bioethanol quantification.

Table 6
The bioethanol quantification

S/N	Sample	Area	X	DF	Conc. (%)	Average Conc. (%)
1		972163665	49.06	1.5	73.59	
2		1063156783	53.61	1.5	80.41	76.38 ± 3.5
3		992839242.9	50.09	1.5	75.14	

X = Exact mass, DF = Dilution factor and Conc. = Concentration.

4. Conclusion

The bioethanol production revealed 6.29% (v/v) bioethanol which corresponded to 58.5 g/L of bioethanol using maize cob. The infrared spectra of the optimum bioethanol distillate revealed the presence of OH, C = C, and C-O at different stretching band which confirmed the presence of bioethanol. The empirical formula determined based on the elemental compositions of the

optimum sample distillate confirms the compound to be C_2H_5OH (ethanol). The GC-MS result revealed the presence of bioethanol as the largest peak; the quantification result revealed 76.38% bioethanol by concentration. Maize cob can be said to be the potential feedstock for the production of bioethanol.

Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

Data Availability Statement

Data available on request from the corresponding author upon reasonable request.

Author Contribution Statement

Yakubu Atiku: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration. **Surajudeen Abdulsalam:** Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing, Visualization Supervision, Project administration. **Jibril Mohammed:** Methodology, Software, Writing – review & editing, Visualization, Supervision, Project administration. **Saeed Ahmed:** Methodology, Writing – review & editing, Visualization, Supervision, Project administration. **Ahmed Inuwa:** Software, Writing – review & editing, Visualization.

References

[1] Liu, Z., Sun, Q., Wang, Y., Liu, J., & Zhu, P. (2024). Preparation and characterization of bacterial cellulose by kombucha using corncob. *Cellulose*, *31*(11), 1–18.

[2] Igwebuike, C. M., Awad, S., & Andrès, Y. (2024). Renewable energy potential: Second-generation biomass as feedstock for bioethanol production. *Molecules*, *29*(7), 1619.

[3] Rajeswari, S., Baskaran, D., Saravanan, P., Rajasimman, M., Rajamohan, N., & Vasseghian, Y. (2022). Production of ethanol from biomass – Recent research, scientometric review and future perspectives. *Fuel*, *317*(2022), 123448.

[4] Sun, C. Z., Shuangfei, G., Binyuan, Y., Tao, W., Yuguang, C., Haina, Z., & Hongbbo, C. Z. (2024). Conversion of lignocellulosic biomass-derived compounds to 2-ethylhexanol: A review. *Environmental Chemistry Letters*, *22*(3), 1093–1113.

[5] Adamu, H., Bello, U., Yuguda, A. U., Tafida, U. I., Jalam, A. M., Sabo, A., & Kamar, M. (2023). Production processes, techno-economic and policy challenges of bioenergy production from fruit and vegetable wastes. *Renewable and Sustainable Energy Reviews*, *186*, 113686.

[6] Bhattacharjee, A., & Nath, R. (2024). Agro-waste valorization and production of bioethanol. In S. P. Saha, D. Mazumdar, S. Roy & P. Mathur (Eds.), *Agro-waste to microbe assisted value added product: Challenges and future prospects* (pp. 211–228). Springer.

[7] Megala, S., Rekha, B., & Saravanathamizhan, R. (2020). Chemical and non-chemical pre-treatment techniques for bio ethanol production from biomass. *International Journal of Energy and Water Resources*, *4*, 199–204.

[8] Nicolaï, T., Arts, W., Calderon-Ardila, S., Smets, R., Vander Borght, M., Thevelein, J. M., & Sels, B. F. (2024). Uncovering challenges for complete carbohydrate to bioethanol utilization in a reductive catalytic fractionation biorefinery. *ACS Sustainable Chemistry & Engineering*, *12*(22), 1–10.

[9] Ramírez-Márquez, C., Segovia-Hernández, J. G., & Mansouri, S. S. (2024). Alcohol production process design and scale-up. In H. Amiri, M. Tabatabaei & A. S. Nizami (Eds.), *Higher alcohols production platforms* (pp. 237–269). Academic Press.

[10] Mondal, S., Neogi, S., & Chakraborty, S. (2024). Optimization of reactor parameters for amplifying synergy in enzymatic co-hydrolysis and microbial co-fermentation of lignocellulosic agro-residues. *Renewable Energy*, *225*(2024), 120281.

[11] Zhu, Q., Dong, H., Yan, D., Gao, D., Xu, K., Cheng, X., . . . , & Lu, X. (2024). High concentration bioethanol production from corn stalk via enhanced pretreatment with ionic liquids. *Chemical Engineering Science*, *283*(2024), 119375.

[12] Raji, R. O., Musa, A. O., Akoh, P. Q., Ibrahim, H. A., Akinsola, R. O., & Oyewole, O. A. (2024). Bioconversion of biomass energy and biological residues: The role of microbes. In N. R. Maddela, S. A. Aransiola, C. I. Ezugwu, L. K. W. Eller, L. Scalvenzi & F. Meng (Eds.), *Microbial biotechnology for bioenergy* (pp. 153–177). Elsevier.

[13] Atiku, Y. M., Abdulsalam, S., Mohammed, J., & Ahmed, S. I. (2023). Physicochemical and microbial characterization of maize cob for bioethanol production. *Journal of Applied Sciences and Environmental Management*, *27*(6), 1193–1201.

[14] Soto, J., Sanhueza, T., Ortiz, J., Luz Mora, M. D. I., Garcia-Romera, I., & Arriagada, C. (2024). Plant growth-promotion and abiotic stress tolerance of dark septate endophyte fungi isolated from roots of native Andean Ericaceae plants colonizing volcanic deposits in Southern Chile. *Journal of Soil Science and Plant Nutrition*, *24*, 1–10.

[15] Kaur, P., Singh, S., Sharma, N., & Agrawal, R. (2024). Filling in the gaps in second-generation biorefineries: Evaluating rice straw and its bioethanol residue for the production of biogenic silica nanoparticles. *Nanotechnology for Environmental Engineering*, *9*(1), 67–76.

[16] Jaiswal, A., Bhattacharya, R., Srivastava, S., Singh, A., & Bose, D. (2024). Bioethanol production and its impact on a future bioeconomy. In S. Mondal, A. Kumar, R. K. Pachauri, A. K. Mondal, V. K. Singh & A. K. Sharma (Eds.), *Clean and renewable energy production* (pp. 375–411). Wiley.

[17] Germec, M., Ozcan, A., & Turhan, I. (2024). Effect of process parameters and media on the production of ethanol by *Scheffersomyces stipitis* in shake flask fermentation. *Biomass Conversion and Biorefinery*, *14*(14), 16415–16424.

[18] Shukla, A., Kumar, D., Girdhar, M., Sharma, A., & Mohan, A. (2023). Steam explosion pretreatment with different concentrations of hydrogen peroxide along with citric acid: A former step towards bioethanol production. *International Journal of Energy Research*, *2023*(1), 2492528.

[19] Yang, R., Su, Y., Ren, T., Li, Y., Deng, Y., & Zheng, C. (2023). Automatic and matrix interference-free acid–base titration by coupling CO_2 vapor generation with microplasma carbon optical emission spectrometry. *Analytical Chemistry*, *95*(47), 17238–17245.

[20] de Andrade Silva, C. A., Oka, M. L., da Silva, P. G. P., Honma, J. M., Leite, R. S. R., & Fonseca, G. G. (2024). Physiological evaluation of yeast strains under anaerobic conditions using glucose, fructose, or sucrose as the carbon source. *Journal of Bioscience and Bioengineering*, *137*(6), 420–428.

[21] Ningthoujam, R. M., Pankaj, Y., Virendra, K. A., Daoud, A., Saud, P., Ashish, D., & Harish, K. (2024). Fermentation of rice straw hydrolyzates for bioethanol production and increasing its yield by applying random physical and chemical mutagenesis. *Waste and Biomass Valorization*, *15*, 1–19.

[22] González-Guerra, G. M., Guillermo, M. G. R., Jose, R. S. T., Kazuhiro, R., Maria-del Pilar, G., Fernando, I. H., . . . , & Juan, C. G. Z. (2024). Synthesis of bioethanol from mixed vegetable wastes: Experimental methodology and characterization. *Engineering Reports*, *6*(7), e12784.

[23] De Almeida, M. A., & Colombo, R. (2023). Production chain of first-generation sugarcane bioethanol: Characterization and value-added application of wastes. *BioEnergy Research*, *16*(2), 924–939.

[24] Okuofu, S. I., Bhagwat, P., Gerrano, A. S., Singh, S., & Pillai, S. (2023). Simultaneous saccharification and bioethanol production from underutilized biomass, cowpea haulm using co-cultures of *Saccharomyces cerevisiae* (BY4743) and *Scheffersomyces stipitis* (PsY633). *Biomass Conversion and Biorefinery*, *13*(9), 7505–7519.

[25] Vinotha, T., Umamaheswari, N., Pandiyan, J., Al-Ghanim, K. A., Nicoletti, M., & Govindarajan, M. (2023). Biofuel production from mango and orange peel and tapioca shells by fermentation using consortium of bacteria: Agricultural and food waste valorization. *Fermentation*, *9*(7), 678.

[26] Tharunkumar, J., Arosha, V., Bajhaiya, A. K., & Rakesh, S. (2024). Optimizing alkaline pretreatment for delignification of paddy straw and sugarcane bagasse to enhance bioethanol production. *Biomass Conversion and Biorefinery*, 1–11.

[27] Zhang, M. L., Yunyun, L., Wentao, W., Wen, L., Cuiyi, Z., Yu, J., & Bao, Q. W. (2024). Bioethanol production from alkali-treated corn stover via acidic adjustment by furfural residue. *BioEnergy Research*, *17*, 1–8.

[28] Piergiovanni, M., Gosetti, F., Rocío-Bautista, P., & Termopoli, V. (2024). Aroma determination in alcoholic beverages: Green MS-based sample preparation approaches. *Mass Spectrometry Reviews*, *43*(4), 660–682.

[29] Oktavia, F. D., Steven, S., Putri, A. H. I., Hernowo, P., Restiawaty, E., & Bindar, Y. (2024). Vacuum distillation of raw bio-crude oil (RBCO) from empty fruit bunches (EFB) pyrolysis to produce light distillate containing acetic acid and phenol for sustainable chemical purposes. *Brazilian Journal of Chemical Engineering*, 1–18.

[30] Sadalage, P. S., Dar, M. A., Paiva-Santos, A. C., & Pawar, K. D. (2024). Enhanced bioconversion of grass straw into bioethanol by a novel consortium of lignocellulolytic bacteria aided by combined alkaline-acid pretreatment. *Biomass Conversion and Biorefinery*, 1–17.

[31] Condor, B. E. D., Mark, D. G. L., Carl, F. Z. A., Paula, I. G. A., Ralf, R. M. N., Dillirani, L., & Duu-Jong, C. J. (2024). Effects of carbon dioxide concentration and swine wastewater on the cultivation of *Chlorella vulgaris* FSP-E and bioethanol production from microalgae biomass. *Applied Energy*, *369*, 123617.

[32] Roozbahani, P. A., Behnejad, H., & Hamzehloo, M. (2024). Synthesis, analysis, and application of zinc oxide and bismuth-based nanocomposites ($ZnO/BaBi_2O_6$) as a photocatalyst for organic dyes degradation. *Inorganic Chemistry Communications*, *168*, 112821.

[33] Mun, W., Choi, S., Jang, H., & Mitchell, R. J. (2024). One-Pot fermenter processing for the mass cultivation of predatory bacteria. *Chemical Engineering Journal*, *480*, 148054.

[34] Anderson, A., Van der Mijnsbrugge, A., Cameleyre, X., & Gorret, N. (2024). From yeast screening for suitability as single cell protein to fed-batch cultures. *Biotechnology Letters*, *46*, 1–16.

[35] Tenkolu, G. A., Kuffi, K. D., & Gindaba, G. T. (2024). Optimization of fermentation condition in bioethanol production from waste potato and product characterization. *Biomass Conversion and Biorefinery*, *14*(4), 5205–5223.

[36] Chongkhong, S. (2024). Integration of ensiled corncob to diluted molasses as carbon and microbial sources for ethanol production. *Trends in Sciences*, *21*(1), 7015–7015.

[37] Luthra, V., Bhushan, K., Kocher, G. S., & Singh, A. (2024). Quality evaluation and fourier transform infrared (FTIR) spectroscopy characterization of Vodka produced from malted and un-malted potatoes using *Saccharomyces cerevisiae* (MK680910). *Potato Research*, *67*, 1–15.

[38] Nisha, J., & Vidya, P. (2024). Immobilized *Saccharomyces pastorianus* cells in electro-fermentation: A novel approach to bioethanol production from mustard straw. *Uttar Pradesh Journal of Zoology*, *45*(17), 87–97.

[39] Ayala, J. R. M., Gesela, C., Marcos, A. G., Conrado, C., Mario, A. L., Jose, A. S., . . . , & Daniela, G. (2021). Characterization of orange peel waste and valorization to obtain reducing sugars. *Molecules*, *26*(5), 1348.

[40] Kennepohl, D., Farmer, S., & Reusch, W. (2020). 12:9 infrared spectra of some common functional groups. *LibreTexts: Chemistry*, *14*(2021).

[41] Ran, Y., Elsayed, M., Eraky, M., Dianlong, W., & Ai, P. (2024). Sequential production of biomethane and bioethanol through the whole biorefining of rice straw: Analysis of structural properties and mass balance. *Biomass Conversion and Biorefinery*, *14*(2), 2693–2705.

How to Cite: Atiku, Y., Abdulsalam, S., Mohammed, J., Ahmed, S., & Inuwa, A. (2024). Production and Characterization of Bioethanol Using Native Microbial Isolates from Decomposed Maize Cob via Simultaneous Saccharification and Fermentation. *Archives of Advanced Engineering Science*. https://doi.org/10.47852/bonviewAAES42024107