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Physicochemical and Microbial Characterization of Maize Cob for Bioethanol Production

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ABSTRACT: The overall efficiency of processes designed to convert lignocellulosic biomass to bioethanol depends on determining the compositions of such material. This paper evaluates the physicochemical and microbial characterization of maize cob for bioethanol production. The proximate analysis of the substrate (maize cob) showed a low moisture content of 3.55% which is favourable for biofuel because high moisture content is associated with lower carbon burn rate. In addition, the low crude lipid of 0.96% and low crude protein of 2.63% favours high yield of bioethanol; the remarkable high carbohydrate content of 87.86% also favours high bioethanol yield. The ultimate analysis of the substrate revealed relatively low nitrogen of 0.42% and sulfur content of 1.13% which implies lower amounts of ammonia (NH_3), Hydrogen cyanide (HCN) and Hydrogen Sulphide (H_2S) which are inhibitory to microorganisms. The lower amount of lignin: 10.43% in the dry matter; amount of extractive of 0.15 g was also recorded from the Lignocellulosic composition of the maize cob. Molecular identification showed that microbial isolates were *Pichia kudriavzevii* strains; the identified microbial isolates are capable of producing amylase and cellulase enzymes for the production of bioethanol. From the aforementioned, the analyzed maize cob is a very promising feedstock for converting the fermentable sugar to bioethanol.

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The global conventional energy resources especially fossil fuel is not sustainable (Pothiraj *et al.*, 2015), hence, biofuels such as bioethanol have turned into promising alternatives to the fossil fuels used in today's transportation sector. Alvira *et al*. (2010) reported that ethanol has numerous applications in chemical, pharmaceutical, and food industries in the form of raw material, solvent, and fuel. Due to increasing population and industrialization, global energy demand has increased steadily over the last few decades, and presently, about 80% of this energy is derived from non-renewable fossil fuel supplies (Kumar and Singh, 2016). Elliston *et al*. (2015) reported that second generation bioethanol production involves a number of consecutive stages such as pretreatment, hydrolysis, fermentation and distillation and/or separation; the overall process for any given substrate could potentially have thousands of different permutations. However, further research is needed to take this process beyond fermentable sugar yields, to include the effect of yeast cultures, or indeed other micro-organisms, 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 (Elliston *et al*. 2015). The cost effectiveness of bioethanol production through hydrolysis of starchy substrates by using enzymatic and microbial processes has been proven to be commercially viable (Kim and Dale, 2002).

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However, two broad fermentation techniques have emerged namely submerged fermentation which utilizes free flowing liquid substrates during bioethanol production, such as molasses and broths and solid-state fermentation which is a fermentation method used by several industries like the pharmaceuticals, food, textile etc., to produce metabolites of microorganisms using a solid support in place of liquid medium (Subramaniyan and Vimala. 2012). The biofuels that are generated from various feedstocks are categorized as first, second and third generation biofuels. First generation biofuels are mostly derived from edible food crops such as sugarcane, wheat, barley, corn, potato, soybean, sunflower, and coconut. Second generation biofuels are mostly generated from lignocellulosic materials such as wood, straw and agriculture residues (Naik *et al*., 2010; Katoka *et al*. (2017). Lee *et al*. (2019) reported that another biomass, namely algae used as the feedstock for third generation biofuels can produce large quantity of lipids suited for biodiesel production (Lee *et al*., 2019). The overall efficiency of processes designed to convert lignocellulosic biomass to ethanol depends on determining the compositions of such material. Lignocelluloses mainly consist of cellulose, hemicelluloses, and lignin which are bonded together by covalent bonding, various intermolecular bridges, and van der Waals' forces forming a complex structure, making it resistant to enzymatic hydrolysis and insoluble in water (Ayeni *et al*., 2013; Foyle *et al*., 2007). The use of microorganism in their individual form in the saccharification process always results in partial conversion of the cellulosic material to fermentable sugar which invariably result in low yield of bioethanol. In addition, the application of acid for treatment of biomass prior to bioethanol production may change the composition of the fermentable sugar produced, thereby affecting the yield of bioethanol. In view of the aforementioned, continuous efforts have to be emphasized particularly on bioethanol; therefore, this study evaluates the physicochemical and microbial characterization of maize cob for bioethanol production.

MATERIALS AND METHODS

Proximate and Biochemical Properties of the Maize Cob: The biomass characterization was carried out in order to determine the composition of the maize cob (feedstock). Moisture content (MC) was determined according to the method reported by Al-mamun and Torii (2015), crude fibre, lipid content, carbohydrate and energy content were determined following the method reported by Khan *et al*. (2013). The pH of the substrates was measured in accordance with similar works reported by Jyothilakshmi and Prakash, (2016) and Saitawee *et al.* (2014). The total solid content was measured in accordance to the work reported by (Muzenda, 2014). The volatile solids and fixed solids were determined based on the procedure well detailed in Ngumah *et al.* (2013), bulk density of maize cob was determined based on the method reported by Bhagwanrao and Singaravelu *et al*. (2014).

Ultimate Analysis of Maize Cob: The ultimate analysis or organic elemental composition of the substrate (maize cob) was carried out to determine carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) analytically (Verma, 2014). The oxygen (O) content was obtain by subtracting the percentage of carbon, hydrogen, nitrogen and sulphur from 100 (Adekunle *et al.*, 2015).

Lignocellulosic Composition of Maize Cob: The cellulosic composition of the maize cob such as amount of extractives, hemicellulose, cellulose and lignin were determined as shown in the sections below.

Determination of the amount of extractives in maize cob solvent extraction: The amount of extractives was determined by adding sixty millimeters (60 mL) of acetone to 1 g of the ground maize cob (A) in a container, the content was heated at 90°C using a hot plate for 2 h. After 2 h, the sample was dried in an oven at 110°C until constant weight was obtained (B). By using Equation (1), the amount of extractives was then determined (Mansor *et al*., 2019).

$$\text{Amount of Extractive (g)} = (\text{A} - \text{B}) \qquad (1)$$

Where; A = amount of biomass in gram; B = weight of the sample after oven drying

Determination of the amount of hemicellulose in maize cob: One hundred and fifty (150) mL of 0.5 mol/L sodium hydroxide (NaOH) solution was added to 1 g of maize cob with extractives free (B) in a container. The content was heated at 80°C using a hot plate for 3.5 h. After that, the sample was then washed with deionised water until it was free from Na^+. The Na^+ was detected by using pH paper and the reading was 6.9. The sample was then dried in an oven at 105°C until constant weight was obtained (C). The amount of hemicellulose was then determine using Equation (2) (Mansor *et al*., 2019).

$$\text{Amount of Hemicellulose (g)} = (\text{B} - \text{C}) \qquad (2)$$

Where; B = amount of extractive in gram; C = weight of the sample washed with deionized water after oven drying

Determination of the amount of lignin in maize cob: The amount of lignin was determined where thirty (30) mL of 98 % sulphuric acid was added to 1 g of maize cob with extractives free (B). The sample was left at 27°C for 24 h before it was boiled at controlled temperature of 100°C using a hot plate for 1 h. The mixture was filtered and the solid residue washed using deionised water until sulfate ion undetectable. Detection of sulfate ion was done via titration process with 10% of barium chloride solution. The sample was dried in an oven at 105°C until constant weight obtained (D). The final weight of residue was then recorded as lignin content (Mansor *et al*., 2019). The acid insoluble lignin was determined by drying the residues at 105°C and accounting for ash by incinerating the hydrolyzed samples at 575°C in a muffle furnace (Ayeni *et al*., 2015).
(D) = Amount of Lignin (g)

Determination of the amount of cellulose in maize cob: Equation three (3) is the total lignocellulosic component inside the biomass. One gram (1 g) was the total amount of biomass sample used in the experiment. The difference between the initial weights (amount of extractive) of the sample with the three other components weight calculated from the experimental process, the content of cellulose (E) was calculated as shown in equation (3) (Mansor *et al*., 2019).

$$(A– B) – (B –C) + D + E = 1 g \qquad (3)$$

Microbial Analysis: The microbiological analysis was centered on getting the fungal isolates from the decomposed maize cob and their types for application in the bioethanol production.

Decomposition of maize cob: One hundred grams (100 g) of the macerated maize cob was soaked in 100 mL of distilled water and left for 24 h to aid decomposition of maize cob. It was observed that there was 30% reduction in the water content after 24 h. The maize cob sample was then incubated at 39°C for 72 h after which it was removed and placed inside an aluminium foil paper where it was further incubated at 39°C under anaerobic condition for six (6) days.

Isolation and characterization of fungi: The decomposed maize cob sample prior to isolation of the native fungi was placed in hot air oven at 45°C to remove excess moisture for 48 h, it was removed and macerated in a ceramic pestle and mortar. The fine macerated samples were taken for serial dilution. One gram (1 g) of the decomposed maize cob was added to 9 mL of sterile distilled water in a beaker and mixed thoroughly. This served as the stock solution for the isolation of the fungi, serial dilution of the sample was carried out by pipetting 1 mL of the stock solution into another 9 mL of distilled water. The sample suspension was further diluted to 10^{-6}, from 10^{-6} serially diluted (fungal sources) stock solution, 0.1 mL inoculum was pipetted into five different petri dishes containing freshly prepared potato dextrose agar with inclusion of streptomycin/chloramphenol (antibiotics) at 50°C on alcohol sterilized bench. Spreading of inoculum was done by the pour plate method followed by gentle agitation to enable uniform spread. This was carried out using standard sterilization techniques in the presence of gentle Bunsen flame. However, after inoculation the plates were incubated in a laboratory incubator at temperature of 38°C after 5 days. Growth was monitored daily and identification of the various fungal colonies were carried out. Subcultures (3 times for each identified colony) from the various plates were carried out by aseptically transferring each independently identified colony isolate into other potato dextrose agar slants (containing antibiotics) until pure fungal strains was obtained (for any batch, the incubation was done at 38°C and then recorded for 5 days). Pure fungal isolates were stored in culture tubes plugged with cotton wool in a refrigerator at 4°C for further use. Subsequent culturing of the identified pure fungal strains were carried out using potato dextrose agar (PDA) in Petri dishes with the inclusion of streptomycin/chloramphenol (mixture of 6 g in 10 mL) after autoclaving which was then followed by incubation at room temperature for 6 days before use. Morphological and microscopic characteristics were used in the identification of the fungal isolates, the method used was a modified method of Ezeonu *et al*. (2016), and this method is in compliance with the work reported by Pitt and Hocking (2002) and Zafar *et al*. (2017)

Morphological Characterization: For the purpose of purifying the fungal isolates, the cultures were carefully and aseptically sub cultured on Potato Dextrose Agar (PDA) and stored on PDA slants for further analysis at - 4°C. Characterization of the fungal isolates were done on the basis of cultural characteristics and morphological characteristics including spore type, mycelia and other fruiting bodies in a lactophenol cotton blue wet mount by compound microscope at magnification of ×1000. Observed characteristics were recorded and comparison made with the established identification key as reported by Barnett and Hunter (1972).

Molecular Characterization: Isolates obtained were genetically identified via amplification and sequence analysis of the ribosomal ribonucleic acid (RNA) gene internal transcribed spacer region (ITS) (Sugita *et al*.,

2004). Daan Gene deoxyribonucleic acid (DNA) extraction protocol for the DNA extraction and Kapa PCR (polymerase chain reaction) mix protocol were adopted, total reaction volume of PCR used was 15 micro litre. Products in between first well 100 bp ladder and second well 100 bp ladder in size were successfully amplified by PCR. Sequences results of 18srRNa1_18srRNa_F_G10_3730XLab1 and 18srRNa2_18srRNa_F_H10_3730XLab1 respectively of the PCR product were compared with known ITS region sequences deposited in the GenBank (http://www.ncbi.nlm.nih.gov) and the percentage of resemblance (similarity), query coverage and Expect (E) value noted using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). Plate I is the gel electrophoresis micrograph of amplified PCR products.

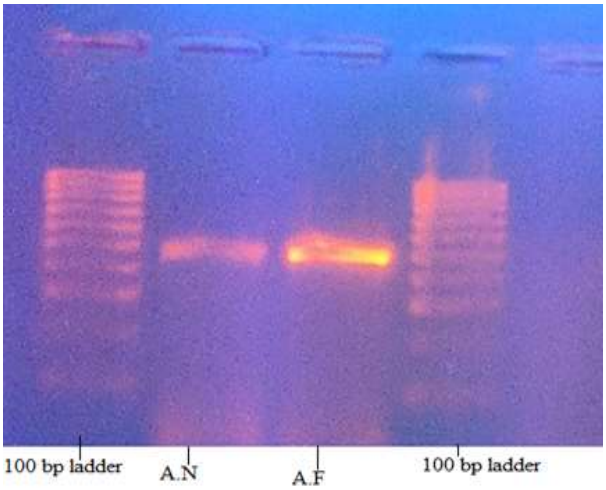

Plate I: Gel Electrophoresis Micrograph of Amplified PCR Products

Screening Isolates for Enzymatic Activity: Screening isolates for enzymes activity test is an important aspect of biological processes; the isolates were screened for enzymatic activity for cellulase and amylase respectively. Detail on the experimental procedure for amylolytic and cellulolytic activity is presented below.

Determination of amylolytic activity: Starch hydrolysis for amylolytic activity was carried out using the method of Castro (1993) as reported in Kiama (2015) as follows: Starch agar was used to determine the hydrolytic activities of the exo-enzyme by introducing the grown colony of the pure isolates to the petri dishes containing the prepared media from starch agar. The detection of the hydrolytic following the growth period was made possible by performing the starch test to determine the presence or absence of starch in the medium. The starch in the presence of iodine showed a blue black colour to the medium, indicating the absence of enzyme. When starch has been hydrolyzed, a clear zone of hydrolysis surrounding the colonies was observed hence, indicating a positive test.

Determination of cellulolytic activity: Cellulase production for cellulolytic activity was carried out using the modified method of Cappuccino & Sherman (2002) as reported in Kiama (2015). Cellulase production was determined by using the media containing 0.7 g KH_2PO_4, 0.2 g K_2HPO_4, 0.01 g $MgSO_4.7H_2$ 0, 0.1 g kNO_3, 0.06g yeast extract, 1 g carboxymethyl cellulose (CMC) and 1.5 g agar per liter. The two (2) plates were inoculated using the grown colony of the pure isolates and incubated at 29°C for 5 days. For best viewing of the clear halo, the plates were stored at 50°C for 12 h after 5 days of incubation. The use of CMC in place of microcrystalline cellulose is in agreement with the literature (Mrudula *et al*., 2012).

RESULTS AND DISCUSSIONS

Proximate Analysis and Biochemical Properties of maize cob: In order to ascertain the suitability of the maize cob (feedstock) for the production of bioethanol proximate analysis and biochemical properties of the maize cob were determined as presented in Table 1.

Table 1: Proximate Analysis and Biochemical Properties of Maize Cob (Dry weight (g/100))

Parameter	Present studies Mean value	Previous studies (Abubakar *et al*., 2016),	Previous studies (Sulaiman *et al*., 2019)
Moisture Content (%)	3.55±0.22	6.00	3.05
Crude Fibre (%)	61.92 ±0.12	33.33	-
Crude Protein (%)	2.63 ±0.11	4.19	-
Crude Lipid (%)	0.96±0.09	4.72	-
Ash Content (%)	5. 01±0.13	2.49	2.7
Total Solid (%)	96.45±0.22	-	-
Volatile Solid (%)	87.86±0.09	-	75.00
Fixed Solid (%)	7.23±0.13	-	-
Total Carbohydrate (%)	87.86±0.01	48.56	-
Energy (kCal/100 g)	370.56±0.00	-	-
Bulk Density (g/cm^3)	0.72±0.00	-	-

The result of the proximate analysis carried out on the substrate (maize cob) showed that moisture content was 3.55%, which is below the values of 6.00% reported by Abubakar *et al*. (2016), 11.74% reported by Danish *et al*. (2015), and 4.6% reported by Danje (2011). In a similar work carried out on corn cob by Anukan *et al*. (2017), reported a moisture content of 5.1%, variation in these values of moistures may be due to the source of the substrate (maize cob) used. However, the 3.55% moisture content obtained in this work as shown in Table1 is within the values of 3.05% and 6.00% obtained by Abubakar *et al*. (2016) and Sulaiman *et al*. (2019) and in close agreement with the work of the later which is desirable for proper combustion of maize cob because high moisture content has a significantly lower carbon burn rate. The maize cob used in this work was characterized by ash content of 5.01% which is above the value of 2.49% reported by Abubakar *et al*. (2016) and 2.70% reported by Sulaiman *et al*. (2019). In addition, a related literature by Anukan *et al*. (2017) on corn cob reported a value of 8.5% ash content. The variation in the ash content of the substrate used in comparison with those of other researchers may be due to the source of the feedstock, high ash content reduces the energy value of the fuel. Furthermore, the high crude fibre of 61.92% and high total carbohydrate of 87.86% obtained in this work, is above the value of 33.33% crude fibre and 48.56% total carbohydrate reported by Abubakar *et al*. (2016) which showed the potential of the maize cob for bioethanol production. The low crude lipid of 0.96% recorded in this work, is below the value of 4.72% reported by Abubakar *et al*. (2016). This also revealed the possibility of using the maize cob (substrate) for bioethanol production, the aforementioned finding is in compliance with a similar work reported by Inobeme *et al*. (2014). This is due to the fact that high lipid content makes biomass unsuitable for bioethanol production. The low crude protein content of 2.63% obtained in this study is below the value of 4.19% reported by Abubakar *et al*. (2016) also showed the potential of the proposed substrate for bioethanol production. High crude protein content makes biomass unsuitable for bioethanol production.

Ultimate Analysis of the Maize Cob: The ultimate characterization of the feedstock (maize cob) was carried out based on its elemental composition and lignocellulosic composition, detail information is presented below.

Elemental composition of the maize cob: Products formed during fermentation of biomass are usually influenced not only by its physical characteristics but also by the chemical composition of the biomass fuel and the operating conditions of the fermenter. The chemical properties of maize cob were studied in order to obtain information regarding the relative proportions of the major elemental compositions of the material and to predict the impact of these compositions on the bio-ethanol quality and yield.

Table2: Ultimate Analysis for the Maize Cob (Dry weight basis (%))

	Composition		
Parameter	This Study	*Previous Studies*	
		(Sulaiman *et al*., 2019)	Anukam *et al*., 2017)
Sulphur (%)	1.13	0.21	1.3
Nitrogen (%)	0.42	0.22	0.43
Hydrogen (%)	3.80	6.02	5.60
Carbon (%)	49.83	44.78	44.40
Oxygen (by difference)	39.82	48.77	48.27

The results in Table 2 show that maize cob is composed of three major elements with a higher proportion of carbon than oxygen. Increased biomass oxygen content is an indication of decreased biochemical conversion processes and increased thermal reactivity of biomass during thermochemical conversion processes, the finding is in agreement with the literature as reported by Anukan *et al*. (2017). Furthermore, the value of 39.82% oxygen content obtained in this study is low compared with values of 48.77% and 48.27% reported by Sulaiman *et al*. (2019) and Anukam *et al*. (2017) respectively. This can be attributed to the low moisture content value of the maize cob used. In addition, higher carbon content of 49.83% obtained which is above value of 44.78% and 44.40% reported by Sulaiman *et al*. (2019) and Anukam *et al*. (2017) respectively showing biomass had higher carbon content, hence it is a good feedstock for bioethanol production. The low hydrogen (3.80%) and oxygen (39.82%) contents obtained were below values of 6.02% hydrogen and 48.77% oxygen; 5.60% hydrogen and 48.27% oxygen reported by Sulaiman *et al*. (2019) and Anukam *et al*. (2017) respectively. Higher proportion of the oxygen and hydrogen in comparison with carbon content, reduces the energy value of a fuel, due to lower energy contained in carbon-oxygen and carbon-hydrogen bonds than in carbon-carbon bonds. The relatively low nitrogen content of 0.42% and sulfur content of 1.13% obtained in this study was lower than 0.43% nitrogen and 1.30% sulphur reported by Anukam *et al*. (2017), the results of the present study may imply lower

amounts of NH_3, HCN, and H_2S (which are environmentally harmful compounds) presence during bioethanol production. In summary, the ultimate analysis of the maize cob in the present work is in close agreement with those reported by previous researchers (Sulaiman *et al*., 2019; Anukan *et al*., 2017) except for the slight variations in some elements such as hydrogen, nitrogen and sulphur.

Lignocellulosic Composition of the Maize Cob: The estimation of composition of lignocellulosic biomass as feedstock for bioethanol production is a crucial step in order to determine the efficiency of overall biochemical conversion to bioethanol, lignocellulosic residues such as maize cob can be used as substrates for the production of bioethanol.

Table 3: Lignocellulosic Composition of the Maize Cob (Dry weight (%))

Parameter	**Present studies**	**Previous studies**	
		(Pointner *et al*., 2019)	(Wanitwattanarumlug *et al*., 2012)
Cellulose (%)	34.75	38.80	41.27
Hemicellulose (%)	39.31	44.40	46.00
Lignin (%)	10.43	11.90	7.40
Extractive (g)	0.15	-	-

The fibre composition of the maize cob in this study was determined with respect to the proportions of cellulose, hemicellulose and lignin. The cellulose content of 34.75% obtained is in close agreement with the value of 38.80% reported by Pointner *et al.* (2019). Also, the hemicellulose content of 39.31% obtained is close to the value of 44.40% reported by Pointner *et al*. (2019). The lignin content of 10.43% obtained fell within the values of 7.40% and 11.90% as reported by Wanitwattanarumlug *et al*. (2012) and Pointner *et al*. (2019) respectively. The low lignin content and extractives in this study (impurities or other chemical compounds) is advantageous in ease of microbial degradation of the biomass for the fermentation process. High content of lignin represents an obstacle for saccharification process for bioethanol production. Therefore, the lower the lignin content of biomass, the easier the biochemical reaction. In addition, low amount of extractive of 0.15 g reflect low amount of impurities that may be inhibitory to microorganisms. In addition, in a related research carried out a value of 17.51% lignin was reported by cavalaglio *et al*. (2020) for cardoon biomass. Furthermore, lignocelluloses mainly consist of cellulose, hemicelluloses, and lignin which are bonded together by covalent bonding, various intermolecular bridges, and van der Waals' forces forming a complex structure, making it resistant to enzymatic hydrolysis and insoluble in water, this explanation is in agreement with the work of Ayeni *et al*. (2013); Singh *et al*. (2017).

Interpretation of Molecular Identification of the Microbial Isolates: Conventional identification of fungal organism mostly relies on the identification by means of morphological characters specific to the genus and species; this is sometimes unsuccessful, due to the typical features of some isolates. Summary of the nearest phylogenetic strain from the blast nucleotide sequences 18srRNa1_18srRNa_F_G10_3730XLab1 and 18srRNa2_18srRNa_F_H10_3730XLab1 for the identified microbial strains is presented in Table 4.

Table 4: Identities of Fungal Isolates Based on Sequence Alignment (BLAST)

Culture code	Nearest phylogenetic strain	Accession number	Sequence similarity (%)
A.N	*Pichia kudriavzevii*	KP998095.1	99.34
A.F	*Pichia kudriavzevii*	MN861069.1	99.32

However, molecular biological identification systems for microorganisms have been suggested as a solution to this problem. These polymerase chain reaction (PCR) systems are useful only in identifying the genus of the isolates used for the molecular identification as a whole or the single specie (strain). The colour change observed from red to yellow was indicative of the presence of an array of organic acids which may include phytic acid, a compound recently reported to be produced by *Pichia* species. This observation suggests that the cultured organisms possess good fermentative capabilities. This result is similar to the finding of Walker *et al.* (2006).Furthermore, the nucleotide BLAST query revealed that all isolates share extremely high/complete identity with corresponding sequences that are deposited in GenBank data base library; Query samples A.N and A.F initially suspected to be *Aspergillus Niger* (A.N) and *Aspergillus Flavus* (A.F) showed different strains on molecular identification. Microbial strains identified were *Pichia kudriavzevii* with accession number KP998095.1 and MN861069.1. Percentage resemblance of the two identified *Pichia kudriavzevii* KP998095.1 and MN861069.1 were 99.34 and 99.32% respectively as presented in Table4. The identification of the aforementioned isolate was made

possible through the use of internal transcribed spacer (ITS) region which contains variable elements that allow for sequence-based identification of fungi therefore, the region offers a possible template for design of species-specific primers for identification of the major fungal species (Maxwell *et al*., 2016).

Fig 1: Chromatograms of the Blast Nucleotide Sequence (18srRNa1_18srRNa_F_G10_3730XLab1)

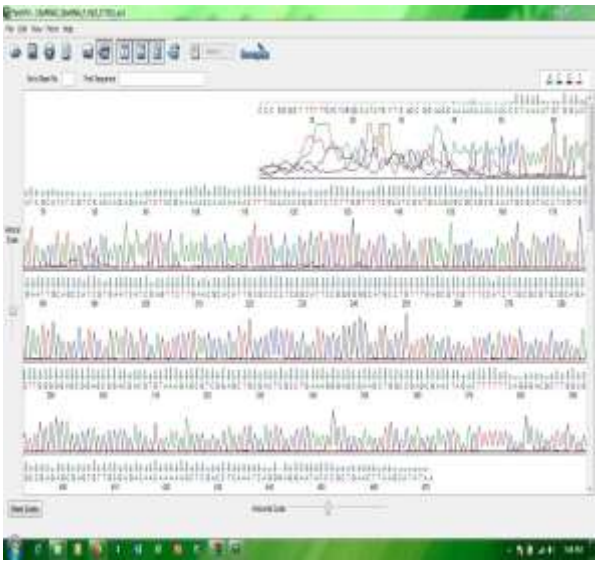

Fig 2: Chromatograms of the Blast Nucleotide Sequence (18srRNa1_18srRNa_F_G10_3730XLab1)

The data obtained from this study supports the use of DNA-based identification to avoid misidentification. Chromatograms on the blast nucleotide sequences are presented in Figures 1 and 2. The results of the blast nucleotide sequences alignments of the identified microbial strains revealed lower expect (E) value of 0.0. It was observed that there was a significant alignment in the blast nucleotide sequence in comparison with the queried samples (*Aspergillus Niger* and *Aspergillus Flavus*).

Enzymatic Activity of Fungal Isolates: Information regarding the secretion of enzymes by the microorganism for the fermentation of substrates is an important aspect for the bioconversion process. Detail on the enzymatic activity test on the fungal isolates is presented in Table 5.

Table 5: Enzymatic Activity Test on the Fungal Isolates

Isolate Identity	Amylase	Cellulase
Aspergillus niger	+	+
Aspergillus flavus	+	+

Key: '(+)' Denotes hydrolysis and '(-)' Denotes no hydrolysis.

All isolates were positive for amylase as shown in Table 5 due to the appearance of halo transparent zone of hydrolysis surrounding the pure isolate of the grown colony of *Aspergillus flavus and Aspergillus niger* respectively, hence starch was hydrolyzed. In addition, all isolates tested were also positive for cellulase due to the appearance of halo transparent zone of hydrolysis surrounding the pure isolate of the grown colony of *Aspergillus flavus and Aspergillus niger* respectively and so cellulose was well hydrolyzed. A similar finding as reported by Bulai *et al*. (2021) also revealed that *Aspergillus* species can degrade substrates that are carbohydrate based. Cellulases secreted by the fungal isolates that is *Aspergillus flavus* and *Aspergillus niger* have many potential in industrial application, in the present study fermentable sugar produced from the maize cob (cellulosic substrate) could be further used as substrate for subsequent fermentation to produce bioethanol, this explanation provided is in line with the related literature as reported by Sadaf *et al*. (2005).

Conclusion: The potential of maize cob as feedstock for bioethanol production was assessed through characterization via proximate, ultimate and microbial characteristics. The finding revealed total carbohydrate content of 87.86% indicating the potential of maize cob for bioethanol production; the feedstock revealed low nitrogen and sulphur contents of 0.42% and 1.13% respectively and the lignocellulosic composition showed low amount of extractive of 0.15 g. Based on the enzymatic activity test carried out, the identified isolates can be used for the production of bioethanol.

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