ETHANOL PRODUCTION FROM MAIZE COBS AND PLANTAIN PEELS BY YEAST SPECIES

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Abstract

Maize cob and plantain peels (agro-waste) were chemically evaluated to explore their potential application in bioethanol production. The proximate composition of maize cob was 7.7% moisture, 4.0% protein, 1.2% lipid, 2.4% ash and 57.5% cellulose. The total dietary fibre content was 26.87%. For the plantain peel there was 11.1% moisture, 7.7% protein, 4.7% lipid, while the fibre and cellulose content were 1.8% and 41.6% respectively. Pretreatment and natural acid saccharification was done using Anacardium occidentale nut shell extract. Result showed maximum reducing sugar yield of 93mg/g and 260mg/g for the maize cob and plantain peel respectively. Bioethanol produced was higher in maize cob (26.35±3.80g/L) at 72 hr of incubation using Pichia caribbica (IMI 398400) and higher in plantain peel (16.27±1.01) with Kluyveromyces marxianus (IMI 398399). The present study revealed that the fermentation of hydrolysates obtained from the pretreatment and natural acid saccharification was found to be best for higher ethanol production at optimized conditions.

Key words: *Agro-waste; bioethanol; natural acid; Pichia caribbica; Kluyveromyces marxianus.*

INTRODUCTION

The most commonly used and widely researched of the biofuel is bioethanol. Bioethanol is a type of alcohol that can be produced primarily through the fermentation of any feedstock containing significant amounts of sugar (Myers and Myers, 2007). Ethanol has widespread use as a solvent of substances intended for human contact or consumption (Chastain, 2006), including scents, flavourings, colourings, and medicines. In Chemistry, it is both an essential solvent and a feedstock for the synthesis of other products such as acetic acid. It has a long history as a fuel for heat and light, and more recently as a fuel for internal combustion engine (Reshamwala et al., 1995). Ethanol can be blended with petrol or burned in nearly pure form in slightly modified spark-ignition engines (Reshamwala et al., 1995). A litre of ethanol contains approximately two thirds of the energy provided by a litre of petrol. Ethanol production processes only use energy from

renewable sources and there is no net CO_2 emission to the atmosphere, thus making ethanol an environmentally beneficial energy source. In addition, ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the green house gas effect. This reduction of green house gas emission is the main advantage of utilizing biomass conversion into ethanol (Anuj et al., 2007).

Maize (*Zea mays* Linn.) is the most important cereal in the world after wheat and rice with regard to cultivation areas and total production (FAO, 2009). It plays an important role in the African diet. In Nigeria, maize is processed into varying products such as pap and 'eko' by using simple traditional methods (Abdulrahaman and Kolawole, 2006). The United States produces 40% of the world's harvest (Purseglove, 1992). Worldwide production was 817 million tonnes in 2009—more than rice (678 million tonnes) or wheat (682 million tonnes) (FAO, 2009). In 2009, over 159 million hectares of maize were planted worldwide, with a yield of 5 million tonnes per hectares. Maize is increasingly used as a feedstock for the production of ethanol fuel (Jacob, 2007). The price of food is affected to a certain degree by the use of maize for biofuel production because farm acreage is diverted from other food crops to maize production. This reduces the supply of the other food crops and increases their prices (Jacob, 2007). However, researches into the possibility of using maize cob in bioethanol production are gaining momentum. Akpan *et al.* (2005) were able to obtain glucose from maize cob hydrolyzed with H_2SO_4 for bioethanol production.

Plantain (*Musa paradisiaca* Linn.) belongs to family Musaceae with about 50 species. The principal centers of cultivation in West Africa are in the humid parts of the Ivory Coast and Ghana (Oliver, 1960). It serves as staple in over 120 countries in the world (Debabandya *et al.*, 2009) and all parts of the plant serve other vital uses (Oliver, 1960; Abiw, 1990; Oke *et al.*, 1998). It is an important component in the general cropping system providing shade and mulch to the ground and a continuity of food (Kobayashi *et al.*, 2001). Scientists are currently looking at the possibility of using plantain peels for the production of varying biofuel on a commercial scale.

The bio-ethanol production in Nigeria targets staple food crops for its derivation, cultivated land to be used are high-value lands and cleared forest that will have a negative impact on the environment (Agba et al., 2009; Agboola *et al.*, 2010). Nigeria as a country is yet to meet the 25% forest cover stipulated by international standards (Agboola *et al.*, 2010). The debate of potential conflict between food and fuel will continue unless the feedstock for the bio-fuel shifts from major crops to production of second generation bio-fuels, which uses waste materials and nonfood crops that can be grown on marginal land as feedstock. Recent developments with cellulosic ethanol production and commercialization may allay some of the concerns on use of large arable farm land for growing energy crops. Rather than allowing agro-wastes such as maize cob and plantain peel to become solid municipal wastes which may habour pathogenic microorganisms (Ledward *et al.*, 2003), it

is necessary to convert them to useful end-products. This study was therefore initiated to explore the possibility of hydrolyzing maize cob and plantain peels with cashew nut shell extract and ethanol fermentation of the obtained hydrolysates using yeast.

MATERIALS AND METHODS

Collection and Processing of Substrates Used

Maize cob and plantain peel were collected from dumping sites (Mushin Local Government Area) in Lagos metropolis. The samples were transported to the laboratory, washed, dried and ground to powder form using a blender (Binatone). The particles were then sieved to obtain average particle sizes of 300 im in diameter.

Chemical Analysis

The moisture content of the agro-waste was determined by drying the sample to a constant weight at 100°C for 17 h and total ash content was estimated according to the methods described by FAO (1986) by heating the residue of moisture determination at 560°C for 7 h. The crude protein content was determined by the standard AOAC (1990) method. The total lipid content was determined by solvent extraction method. The cellulose was isolated by known procedure (Updegraff 1969).

Isolation and Characterization of Microorganism

Kluyveromyces marxianus and *Pichia caribbica* were isolated from cassava tuber steep and maize steep respectively. They were identified as yeast by a fungi expert in the Botany laboratory of the University of Lagos, Akoka, Lagos. The isolates were characterized based on standard procedures of colonial morphology, cultural characteristics and biochemical tests as described (Seeliger 1956; El-Zaatari *et al.* 1990; Olutiola *et al.*. 2000).. The biochemical tests carried out include sugar fermentation test, germ-tube test, resistance to chloramphenicol and urea hydrolysis test. The identities were further confirmed by comparing the characteristics with those of known taxa. (Rhodes and Hartman 1980, Ellis *et al.* 2007). The pure isolates were also sent on malt extract agar (MEA) slants in 5.0 mL McCartney bottle to Centre for Agriculture and Bioscience International (CABI) Kew Garden, England for molecular identification, where the accession numbers were respectively given as IMI 398399 and IMI 398400.

Pretreatment Methods

The modified method (Ocloo and Ayernor 2010) was used. The ground cob and peels were slurried with distilled water using a solid to liquid ratio of 10% (w/v). The mixture was allowed to boil until gelatinized at 70°C and allowed to cool.

Natural Acid Saccharification (NAS)

The natural acid saccharification (NAS) was done by soaking mashed cashew nut shell in ethanol at the ratio of 5:6 (w/v) for 72 h (Sofowora, 1993). The filtrate was concentrated using a rotary evaporator under reduced pressure and temperature (50 <"C), and the pH was obtained as 3.2 using a pH meter. About 20 mL of the cashew extract was added to the gelatinized mash, stirred and the mixture allowed to cool

gradually to 50°C for the conversion of the gelatinized mash to sugars. The mixture was autoclaved after an hour at 121°C for 15 min to arrest enzyme action and immediately filtered using muslin cloth. The reducing sugar content in the hydrolysates was determined by Dinitro salicyclic acid (DNS) method of Miller (1959).

Identification of Specific Simple Sugars in the Hydrolysate

The hydrolysates were analyzed on an HP 6890 Series GC powered with an HP ChemStation Rev. A 09.01 (1206) and a flame ionization detector (FID). Sample (2-3 μL) was injected from slit injector. The carrier gas was hydrogen at the flow rate of 1.0 mL/min. the fractionation was carried out in an isothermal temperature of 210°C. The injector and detector temperatures were 250°C and 325°C respectively. Typical coefficient of correlation for standard curve was 0.95-0.99. Peaks were identified by comparison of retention times with those of standard glucose, xylose, arabinose, maltose, rhamnose, lactose, sucrose, ribose and fructose.

Fermentation of Hydrolysate

The fermentation studies were carried out using *K. marxianus* (IMI 398399) and *P. carribica* (IMI 398400) in the hydrolysate obtained from pretreated, enzymatic hydrolyzed agro-waste. The yeast were separately added at a rate of 1mL yeast broth/50ml of mixture. In order to study the effect of enzymatic saccharification on ethanol yield in the agro-waste, a separate set of fermentation experiment was carried out in a similar manner using the hydrolysates obtained from the pretreatment without natural acid saccharification. Fermentation was allowed for 72 h at 28-30°C.

Distillation and Determination of Quantity of Ethanol

The fermented broth was dispensed into a round-bottomed flask fixed to a distillation column that was cooled by running tap water. A conical flask was connected to the other end of the distillation column to collect the distillate. A heating mantle with the temperature adjusted to 78°C was used to heat the round-bottomed flask containing the fermented broth. The distillate collected was measured using a measuring cylinder, 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 (0.8033 g/mL). g/L is equivalent to the yield of 100 g of dried substrate (Oyeleke and Jibrin, 2009).

RESULTS

Proximate composition

The data representing the proximate composition of the agro-waste are shown in Table 1. These agro-wastes were selected based on the abundance and availability as their produce are major common staple tropical food crops in Nigeria. The plantain peel was observed to have higher moisture, ash, protein and lipid contents than the maize cob while the cob showed higher fibre and cellulose content than the plantain peels.

Table 1: Proximate composition (%) of agro-waste.

Sample	Moisture	Ash	Protein	Lipid	Fibre	Cellulose
Maize cob	7.71	2.35	3.99	1.20	26.87	57.50
Plantain peel	11.09	4.57	7.70	4.66	1.81	41.60

Cultural, morphological and biochemical characteristics of isolates

The organisms used for fermentation were *K. marxianus* and *P. caribbica*. *P. caribbica* showed flat colonial growth with cloudy white, entire, smooth surface. Cells are ellipsoidal in cluster, measuring 1.0–3.5 μm in diameter. *K. marxianus* grew moderately slow in culture, without covering a 9 cm Petri dish after 72 h of growth at 28-30°C. The colonies matured to form creamy-white clusters. The cells are short ovoid to elongate with some at the point of constriction measuring 2.0 - 5.0 μm in diameter. The cells were produced singly. Table 2 shows the phenotypic characteristics.

Table 2: Phenotypic characterization of organisms

Biochemical test	Organism	
	K. marxianus	*P. caribbica*
Cell morphology	Elongated	Ellipsoid
Gram reaction	+	+
Sucrose	+	+
Glucose	+	+
Fructose	+	+
Dextrose	+	-
Maltose	-	+
Xylose	-	+
Galactose	+	-
Lactose	V	+
Urea	-	+
Resistance to chloramphenicol	+	+

+ = PRESENT
- = ABSENT
V = Variable

Reducing Sugar Content

The reducing sugar content obtained from enzymatic hydrolysis of the pretreated agro-waste samples are shown in the Figure 1. The results on sugar recovery after natural acid hydrolysis showed a maximum sugar release of 93mg/g in maize cob and 260mg/g in plantain peel. It was observed from the present study that valuable amounts of reducing sugars are liberated only after the enzymatic hydrolysis.

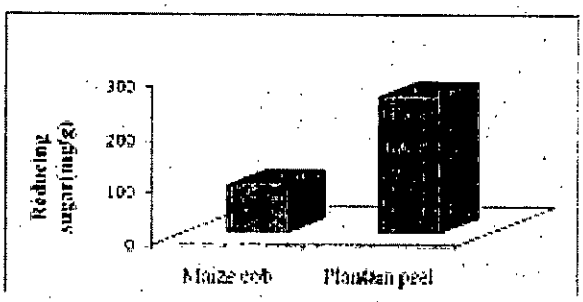

Figure1: Reducing sugar yield in hydrolysates

Type and Concentration of Specific Simple Sugars in the Hydrolysates

The hydrolysates were subjected to Gas Chromatographic analysis in order to obtain the type and amount of sugars present. The standards used include glucose, fructose, sucrose, arabinose, xylose among others.

The correlation co-efficient of each selected standard is 0.99 g/L. The results showed the presence of the test sugars in varying concentrations. Maize cob had the highest concentration of glucose, fructose and xylose as shown in Table 3.

Table 3: Concentration of different sugar components

Sugar component	Sample (mg/g)	
	Maize cob	Plantain peel
Ribose	2.06×10^{-4}	2.06×10^{-4}
Xylose	3.20	1.28×10^{-4}
Arabinose	3.80	2.06×10^{-4}
Rhamnose	3.55×10^{-5}	3.55×10^{-5}
Fructose	11.42	7.09
Glucose	7.27	5.44
Maltose	8.05	2.07×10^{-5}
Lactose	9.96	1.67×10^{-4}
Sucrose	4.25×10^{-5}	14.02

Determination of quantity of ethanol produced

The fermentation efficiencies and volume of ethanol production by the isolates in the hydrolysates of the agro-waste are presented in Table 4. Ethanol yield varied significantly between the microorganisms and the highest yield was 26.35±3.80 g/L with *Pichia caribbica*, followed by 16.27±1.01 g/L while the lowest yield was 02.62±2.61 by *K. marxianus* in the plantain peel. It was observed that maize cob produced higher amount of bioethanol despite the higher amount of reducing sugar in the plantain peel.

Table 4: Amount of bioethanol from agro-waste

	Amount of bioethanol (g/L)		
Microorganism	Maize cob	Plantain peel	Control
Pichia caribbica	26.35±3.80	10.85±1.21	0.00±0.00
K. marxianus	02.62±2.61	16.27±1.01	0.00±0.00

Values are expressed in mean ±SEM

DISCUSSION

In the proximate composition of maize cob and plantain peel, it was observed that plantain peel had higher moisture, ash, crude lipid and crude protein content than maize cob. This is in consonant with the literature findings of (Hammond *et al.* 1996; Emaga *et al.* 2007; Arumugam and Manikandan 2011) who had reported high content of these components in the peels of fruits such as banana, plantain, tomato and mango. However, the fibre and cellulose content was observed to be higher in the maize cob which could be attributed to the fibrous nature of the cob. Generally, lignocellulosic biomass should undergo numerous processes consisting of pre-treatment, hydrolysis, enzymatic saccharification and fermentation of the sugar to produce bioethanol (Sun and Cheng, 2002). It is shown from the results that a combination of pretreatment and enzymatic saccharification could be a promising approach for extracting fermentable sugars from agro-waste as feedstock for ethanol production. As in most ethanol production using various lignocellulosic biomasses, the main process of pre-treatment involves alkaline or acid hydrolysis and enzyme saccharification to produce glucose and xylose followed by fermentation with yeast (Patle and Lal, 2007).

The reducing sugar yields from the maize cob and plantain peels was significantly affected by the enzymatic saccharification where the yield of fermentable sugars was high. Acid and alkaline pretreatment of biomass have been extensively studied. In previous experiment using agricultural wastes, (Patle and Lal 2007) reported the liberation of reducing sugar with a yield of 36-123 g/L and ethanol production of 11-54 g/L. The concentrations of the hemicellulosic (xylose, arabinose) and cellulosic (glucose, fructose) monomers were observed to be higher in maize cob than plantain peels which could be due to the enzymatic activities of the extract. The fermentation of enzymatic hydrolysates showed better fermentation efficiencies in comparison to acid hydrolysates of agricultural residues. (Singh *et al.* 1984) It was also reported that an initial pretreatment of fibrous peel residues breakdown the structure to make them more susceptible to enzymatic reactions. (Arumugam and Manikandan 2011).

The cashew nut shell extract used in the present study exhibited a high efficiency in the conversion of cellulose and hemicellulose from the agro-waste, which was comparable with the results earlier reported by (Oyeleke and Jibrin, 2009; Arumugam and Manikandan 2011; Mohd *et al.*, 2011). The saccharification of different agro-wastes had been reported by other workers employing enzymes from different microorganisms. Adesanya et al. (2008) achieved starch conversion in cassava peel by using amylase and glucoamylase, simultaneously. Previous authors were also able to achieve similar results. Abouzied and Reddy (1986); Narasimha et al. (2006) Using rice malt, Ocloo and Ayernor (2010) produced 8.30 ± 0.76 %v/v alcohol. Hammond et al. (1996) reported an increased sugar recovery and ethanol production from bananas and banana wastes using commercial amylase and glucoamylase.

Maize cob and plantain peel were used to produce bioethanol through pretreatment, enzymatic saccharification and fermenting with *P. caribbica* and *K. marxiamus*. In the fermentation process the organisms behaved differently according to the nutritional requirement. *P. caribbica* was capable of producing higher amount of ethanol from maize cob hydrolysate while *K. marxiamus* was able to produce higher amount of ethanol from plantain peel hydrolysate. *P. caribbica* was able to utilize the monomeric sugars of maize cob because species of the genus have been reported to be better fermenters of pentose sugars (Toivola et al., 1984; Prior et al., 1989). The maximum amount of bioethanol (26.35±3.80 g/L) produced from maize cob showed that the organism effectively utilize the substrate. Previously, it has been reported that 26.31± 1.41 g/L of bioethanol from guinea corn husk and 9.55-10.32 g/L of bioethanol from empty bunch of fruit. (Oyeleke and Jibrin 2009 and Mohd *et al.* 2011)

CONCLUSION

In this present study, the potential of producing bioethanol from agro-wastes such as maize cob and plantain peel hydrolyzed with plant extract was evaluated. Substantial amount of fermentable sugars were obtained and corresponding high amount of

bioethanol. This shows that agro-waste which are diverse and commonly pose significant disposal problems can be used for the production of bioethanol when properly hydrolyzed. This will be of great help in reducing the colossal effects of environmental pollution by fossil fuel as well as reduce the pressure on crop plants as sources of materials for biofuel production.

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