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The Production of Second-generation Bioethanol from Lignocellulosic Biomass using Two Strains of *Sacharomyces cerevisiae*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Concerns about first generation bioethanol's impact on the food chain and biodiversity have shifted research to second generation (2G) bioethanol technologies. The 2G-bioethanol is made from lignocellulosic biomass, which is more sustainable and does not harm food security or the environment. This production process uses non-food crops, food crop residues, wood or food wastes, such as wood chips, skins, or pulp from fruit pressing. The present study examines the bioethanol production potential of three lignocellulosic biomass residues: corn cob, corn husk, and corn stem, as well as their physiochemical and mineral composition before and after fermentation. Before fermentation, the corn waste samples were hydrolyzed into sugar monomer and the hydrolysate was fermented separately to produce bioethanol for five days at 282^{o}C using two Saccharomyces cerevisiae strains: typed yeast ATCC 3585 and Baker's yeast ATCC 204508/S288c. At one-day intervals, the pH, simple sugar and ethanol production were measured. ANOVA was used to find significant differences between the investigated organisms. The results showed that Saccharomyces cerevisiae ATCC 35858 produces more ethanol than the other strain (20.25±0.63). Corn cob also produced more ethanol than stem and husk. During fermentation, the typed yeasts outperformed the Baker's yeast in pH, reducing sugar, and specific gravity. Average dry yeast cell mass (ADM) of Saccharomyces cerevisiae ATCC 35858 and Saccharomyces cerevisiae ATCC 204508/S288c were 1.82±0.07 and 1.98±0.03, respectively. According to

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proximate composition, fermentation lost over 50% of the corn waste's nutrients (ash), while recovering over 50% of the minerals (nitrogen, phosphorus, and potassium). The ability of the two Saccharomyces cerevisiae strains to produce bioethanol was not significantly different at p value ≤ 0.05.

Keywords: Second generation bioethanol; lignocellulosic biomass; fermentation; saccharomyces cerevisiae; corn waste.

1. INTRODUCTION

The production of Bioethanol from second-generation feedstocks, primarily lignocellulosic biomass, offers the possibility of a cleaner, lower-carbon biofuel that can be used as a substitute for fossil fuels [1,2]. Because Fossil fuel is a non-renewable resource that also contributes significantly to greenhouse gas emission, excessive fossil fuel consumption is also the primary cause of today's global energy crisis and climate deterioration [3,4]. However, due to costly pre-treatment technologies, one major constraint is the high cost of production. Bioethanol from first-generation feedstocks, on the other hand, is created from starch- and sugar-based feedstocks such as corn, wheat, and sugarcane, which are commonly used as human and livestock feed [5-7]. However, using food crops to produce bioethanol has resulted in an unbridgeable gap between energy and food security [1]. As a result, the focus of bioethanol research has shifted to the transformation of lignocellulosic biomass, which is a product of plant photosynthesis, such as straw, leaves, and other agricultural or forest wastes, which produces a large amount of bioethanol every year [2,7,8]. Lignocellulosic biomass is one of the potential feedstocks for bioethanol production because it is abundant, renewable, and non-edible [9,10].

Bioethanol can be made from a variety of feedstocks, including sucrose, starch, lignocellulosic and algal biomass, using a microorganism-mediated fermentation process. Yeasts, particularly Saccharomyces cerevisiae, are the most commonly used microbes in ethanol production due to their high ethanol productivity, ethanol tolerance, and ability to ferment a wide range of sugars [6,11].

In a study by G del Río et al. corn waste was used as a substrate to produce bioethanol [12]. Manmai et al. after pretreatment of the biomass using cellulase enzyme, fermented the fermentable sugar for ethanol production in the sorghum stem by Saccharomyces cerevisiae [13]. In another study conducted by Suresh et al. the ethanol production process from potato wastes by pretreated ultrasonication using hydrochloric acid or α-amylase (US enzyme) in the presence of Saccharomyces cerevisiae was investigated [14].

Each year, nearly 1.5 billion tons of biomass feedstock, forestry wastes, and dedicated energy crops are produced around the world, yielding more than 442 billion liters of bioethanol if they are all used. Biomass resources in Nigeria have the potential to generate 2.33 EJ of energy, while agricultural residues have about 1.09 EJ of energy potential, with cassava, maize, oil palm, plantain, rice, and sorghum being the significant contributors [15]. It was also estimated that 20 to 70% of generated waste in Nigeria is collected in different locations, with household waste having great potential for bioconversion, organic waste becomes a viable alternative and promising way for generating renewable energy [16].

Corn is one of the most widely planted crops in the world. During the processing of corn, a large volume of corn-cobs is generated as agricultural waste [17,18].For every grain production of corn, wastes such as corn cobs, corn stalk and corn husk account for 40% of the production [19]. Global production of corns was about 1.13 Gtonnes was the second-highest after sugarcane [20]. Corn wasters cause severe environmental problems as it was the second-highest greenhouse gaseous (GHG) emissions source for landfilling and the highest GHG source among the biomass burning in 2017 [20-22].

In Nigeria, Maize (*Zea mays*) is one of three major grain crops grown all over the states of the country with up to 5.3 million hectares of maize grown annually. Oladeji and Enweremadu [23] reported that according to Federal Office of Statistics (FOS) on Agricultural Survey, Federal Ministry of Agriculture, in 2006, Nigeria was ranked the second largest producer of maize in Africa with about 7.5 million tons after South Africa Republic with 11.04 million tons. The corn cob is one of the major wastes generated from

the corn/maize. After the grain is shelled from the maize and processed for food, the larger portion being the cob forms waste and thrown away thereby causing environmental challenges [23].

Therefore, this work investigated the possibility of transforming corn (*Zea mays)* wastes (stem, husk and cob) to ethanol using two strains of *Saccharomyces cerevisiae*; *S. cerevisiae* from FIIRO and baker's yeast, and also carried out physiochemical analysis of the hydrolysis residue of corn wastes for possible use as biofertilizer and animal feedstock.

2. MATERIALS AND METHODS

2.1 Collection and Physical Pretreatment of Corn Wastes

Four kilograms (4 kg) of fresh corn wastes (cob, husk, and stem) were collected from Elemere farm, Kwara State University, Malete, Kwara State, in a clean polythene bag. They were identified at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Kwara State Nigeria, the corn wastes were identified as corn cob, corn husk, and corn stem, respectively. All corn wastes required for the study were washed with tap water to remove soil and dirt before being air dried to crispiness on the laboratory bench for one week at room temperature 28 ± 2 ^{0}C. Using a mortar and pestle, the dried corn wastes were crushed into rough particles. Finally, it was milled into powder using an electric blender (Sonik Japan) and stored in labeled substrates bags in the refrigerator at 4 ^{0}C for future use.

2.2 Collection and Characterization of Ethanol Producing Strain of *Saccharomyces cerevisiae*

The ethanol-producing strain of *Saccharomyces cerevisiae* ATCC 36858 was obtained from the Federal Institute of Industrial Research Oshodi (FIIRO) in Lagos, Nigeria and the organism was physically identified on yeast peptone dextrose agar plates. For two days, the organism (typed yeast) was cultured on Yeast Peptone Dextrose agar (YPDA) plate at 30^{0}C.

2.3 Morphological and Microscopic Characteristics

The colonies were observed and described on (YPDA). The morphological and cultural characteristics of the Typed yeasts (ring formation, colony color, colony elevation, colony texture, and colony shape) were observed and recorded. Under the microscope, characteristics such as (shape, ascospore, budding, pseudomycelium or mycelium, etc.) were observed and recorded [24].

2.4 Biochemical Characteristics

The organism was Gram stained following the method of Fawole and Oso (2004). Then, Sugar Fermentation test and Nitrate test were carried out following the method of Tofighi et al. [25].

2.5 Collection and Activation of Baker's Yeast

Dry baker's yeast (*Saccharomyces cerevisiae* ATCC 204508/S288c) was obtained from Dangote flour mill PLC, Ilorin, Kwara State, Nigeria. It was activated on Yeast Peptone Dextrose Agar YPDA containing 2.5 g yeast extract, 5 g peptone water, 5 g dextrose, 3.75 g agar and 250 ml distilled water. The preparation was autoclaved at 121˚C for 15 min and was allowed cool down. 15 ml was dispensed into small plates (60 x15 mm) disposable Petri dishes. 2g of the dry yeast was grown on the agar plate at 30 ^{0}C for two days to activate the yeast. A loopful of the yeast colony was transferred from the agar plate into 100 ml of 5% yeast peptone dextrose broth (which was obtained by dissolving 2 g of dextrose, 2 g of peptone water, 1 g of yeast extract and 95 ml of distilled water) and incubated at room temperature on a shaker (Stuart Orbital Shaker SSL1) at 130 rpm for two days. 7ml of the broth was centrifuged at 4500 rpm for 5 minutes. The supernatant was decanted and the pellet was re-suspended in 10 ml of sterile distilled water twice, centrifuged and the supernatant was decanted. The pellet was re-suspended in 1/10 of 50 ml (5 ml) citrate buffer (1 g of citric acid and 1.47 g of sodium citrate) and was used as inoculum [26].

2.6 Acid Hydrolysis of Corn Wastes

The above-mentioned pretreated corn wastes were degraded using both dilute and concentrated Tetraoxosulphate (vi) acid (H_2SO_4). Each of the milled corn wastes (cobs, husks and stems) was mixed with 25 grams of dilute H_2SO_4 (1.5 percent). The hydrolysis was performed by placing the mixture in an oven at 160 ^{0}C for 30 minutes. The polysaccharide was hydrolyzed into sugar monomers in this process, and the liquid

fraction was recovered by passing it through No 1 Whatman filter paper (90 mm) to separate the filtrate from the residue. The sugar monomers obtained during the hydrolysis process were then subjected to microbial fermentation, while the residues were analyzed proximally [27].

2.7 Ethanol Production (Yeast Fermentation)

Hundred (100) ml of the above hydrolysates (sugar monomers) were transferred into another set of labeled conical flasks, covered, and autoclaved at 121^0C for 15 minutes before cooling. The flasks were inoculated with 2 ml of two days' broth culture containing approximately 1.5 $x10^{12}$ cfu/ml of ethanol producing yeast (EPY) and activated barkers' yeast (BY) and properly covered to ferment for five (5) days at 28 ± 2^0C. At one-day intervals, samples were aseptically taken from the fermenting medium (5 ml) and centrifuged at 4500 rpm for 6 minutes, with the supernatant used for ethanol production, simple sugar, specific gravity, pH, and the residue used for yeast cell mass [28].

2.7.1 Ethanol assay

Using a vinometer, 1 ml of supernatant was poured into the funnel at the top of the vinometer (Vinometer FIW 13 0-25%) until it was about half full. The funnel was used to support the vinometer until 6 drops of sample fell from the tip. The tester was immediately inverted to drain the remaining liquid. The funnel was turned on its side. Until the residual liquid in it no longer drops. The scale's percentage ethanol yield was read and recorded [29].

2.8 pH Determination

During the 5 days of fermentation, the pH of each sample was measured every day with a pH meter (OHAUZ STARTAR 2000). The electrode was immersed in the supernatant and the pH of the solution was read and recorded for each sample after the function selector was switched from standby to pH [29].

2.9 Determination of Reducing Sugar Content (Brix Level) and Specific Gravity

This was accomplished through the use of a refractometer. The refractometer's front end (Refractometer RF 110) was aimed in the direction of a bright light, and the diopter's adjusting ring was adjusted until the reticle was clearly visible. The refractometer was calibrated by opening the cover plate and placing two drops of distilled water on the prism with a dropping pipette. The cover plate was closed, lightly pressed, rotated, and the calibration screw was adjusted to align the light/blue boundary (made up of the brix level and specific gravity scale) with the null line. The cover plate was removed, and the prism's surface was cleaned with cotton wool. Two drops of the sample to be measured were dropped on the prism, the replaced plate was covered and lightly pressed, and the corresponding scale of the light/blue boundary was read and recorded [29].

2.10 Yeast Dry Weight Measurement

The dry weight of yeast cells was determined using the filter paper method. Conical flasks containing fermentation medium were shaken for 1 minute to ensure yeast cell homogenization, 5 ml of the fermenting medium was withdrawn and centrifuged at 4500 rpm for 6 minutes, and the wet weight of the pellets was transferred to pre-weigh filter paper. It was dried in the oven for 10 minutes at 40 ^{0}C . The dried weight of the pellets was determined using an electronic weighing balance (CAS-44). The yeast weight was calculated by subtracting the initial weight of the filter paper from the final weight [30].

2.11 Proximate Analysis Before and After Fermentation of the Corn Wastes

The proximate content of the corn wastes before and after fermentation in terms of total ash, crude fibre, crude fat, crude protein, moisture and crude carbohydrate was carried out according to AOAC [31,32].

2.11.1 Total ash

Total ash content as total inorganic matter was determined before and after fermentation of corn wastes by incineration of a sample at 600^0C [31]. One (1) gram was placed in a pre-weighed crucible and incinerated at 600^0C in a muffle furnace. The crucible was removed from the oven and cooled in a desiccator before being reweighed. The ash content was calculated using the following formula: Percentage ash = $\frac{ashweight}{sampleweight} \times 100$

2.11.2 Crude fat

After extraction with a Soxhlet extraction unit, the crude fat was calculated [31]. Two grams (2 g) of corn waste before and after fermentation were weighed into a labeled thimble in a 250 ml boiling flask. The thimble was plugged with cotton wool, and 300 ml of petroleum ether was added to the boiling flask samples. This was done in a reflux mode with a Soxhlet apparatus for 6 hours at 60 ^{0}C. The petroleum ether containing fat extracts was poured into a container, oven dried for 30 minutes at 102 ^{0}C, and the percentage (percent) fat was calculated using the formula.: Percentage (%) Fat = $\frac{ashweight}{sampleweight} \times 100$

2.11.3 Moisture contents

Moisture content was calculated using [31]. Clean silica Petri dish dried in an oven and cooled in a desiccator. W1 weighed the empty Petri dish, and 5 g of the sample was placed in the Petri dish and recorded (W2). It was placed in a preheated oven at 105 ^{0}C °C for 3 hours. Remove and cool in a desiccator before weighing (W3).

Percentage moisture content = $\frac{W1-W3}{W2-W1} \times 100$

2.11.4 Crude fibre

The AOAC [31] method was used to determine crude fiber. A gram of defatted sample (waste) was placed in a glass crucible and attached to the extraction unit. The sample was digested for 30minutes with 1.25 percent boiling 150 ml sulphuric acid, then drained and washed with boiling distilled water. The sample was digested with 1.25 percent NaOH in 150 ml, then washed with boiling distilled water. The crucible was removed, dried in an oven at 110^{o}C, cooled in a desiccator, and weighed (W1). The sample was ashed for 2 hours in a 550^{o}C muffle furnace, cooled in a desiccator, and reweighed (W2). The formula was used to calculate the percentage of fiber [31].:

Percentage crude fibre = $\frac{Digestedsample(W1)-Ashedsample(W2)}{weightofsample} \times 100 \frac{weightofdigestedsample(W1)-Weightofashsample(W2)}{sampleofweight} \times 100$

2.11.5 Crude protein

Ten (10) grams of waste were placed in 50 ml Kjedhal flasks. The flasks were filled with two milliliters of distilled water and left for 30 minutes. A total of 0.2 g of powdered pumice, 1.33 g of K_2SO_4 catalyst mixture, and 1.5 g of concentrated H_2SO_4 were added. On the digestion rack, this was heated until the frothing stopped. To condense H_2SO_4 to about one-third of the way up the flask's neck, the temperature was raised to gently boil. The isolated particle was washed with a 30% H_2O_2 solution before boiling for 1 hour and cooling. Slowly swirling ten ml of deionized water was added. Total crude protein was determined spectrophotometrically at 550 nm using a two-ml aliquot of each diluted solution [31].

2.11.6 Determination of total carbohydrate

Raimi et al [32] method was used to calculate total carbohydrate. To disperse the sample, two grams (2 g) of the pretreated sample were weighed into a 100 ml measuring cylinder containing 10.0 ml of water and thoroughly stirred with a glass rod. A total of 13ml (13.0 ml) of 52 percent perchloric acid reagent was added. For 25 minutes, it was frequently stirred with a glass rod. The content was prepared to a volume of 100 ml, then transferred to a volumetric flask of 250 ml and prepared to a volume of 250 ml. The flask was shaken, and the contents were filtered into a test tube using filter paper. 1ml of filtrate was pipetted into test tubes, 1.0ml of diluted glucose standard solution was used to make a duplicate standard, and 5.0ml of freshly prepared anthrone reagent was added to each tube. After thoroughly mixing the tubes, they were placed in a boiling water bath for 10 minutes. The tubes were quickly cooled to room temperature by immersing them in a cool water bath. At 620 nm, the optical densities of the sample and the glucose standard were measured against a reagent blank. The absorbance was plotted against a graph of standard.

If the weight of the sample = w, Absorbance of dilute standard = a, Absorbance of dilute sample = b. The percentage total carbohydrate was calculated to be = $\frac{2b \times b}{a \times w}$

2.11.6 Determination of mineral content (NPK) in the waste before and after fermentation

The amount of Nitrogen, Phosphorus and Potassium in the corn waste was determined according to the method described by Onwuka [33].

2.11.7 Phosphorus determination before and after fermentation

Onwuka [33] vanadate colorimetric method was employed. In 20 ml of distilled water, three (3) grams of the ashes obtained before and after hydrolysis were dissolved. 2 ml of the ached mixture were pipetted into a 100 ml volumetric flask, followed by 2.5 milliliters of vanadate molybdate reagent and thoroughly mixed. It was filled to the brim with distilled water and set aside for 10 minutes. In a 100 ml volumetric flask, 2 ml of distilled water and 2.5 ml vanadate reagent were mixed together and made up to the mark with distilled water. At 540 nm, the absorbance of the test and control was measured and compared to a standard curve of potassium dihydrogen phosphate (KH2PO4).

Percentage phosphorus = $A \times \frac{10}{W} \times V$. Where A = concentration of dilute ash, W = weight of original food ashed, V = volume of ashed solution to 100 ml.

2.11.8 Determination of nitrogen in the waste before and after fermentation

The Nitrogen was determined using the Micro Kjeldahl method [33]. Two grams (2 g) of corn wastes labeled before and after fermentation were digested in a tube with 4 ml H2SO4 and 2 Klehdahl tablets for 30 minutes at 420^0C until a clear solution was obtained. It was cooled and watered down. The distillation unit was connected to the tube containing the diluted sample, and the condenser outlet was connected to a conical flask containing 25 ml of boric acid. Distillation was carried out for 4 minutes after 25 milliliters (25 ml) of 40% NaOH was dispensed into the conical flask. The ammonium borate solution was titrated with 0.1M HCl until it reached a purplish – grey end point.

Percentage Nitrogen = $\frac{0.14 \times A}{weight of the corn wastes \in gram.}$A = volume (ml) of 0.1M HCl used in the titration.

2.11.8 Determination of potassium in the waste before and after fermentation

Flame photometry was used to determine potassium [33]. Five (5) grams of waste were ashed and digested with 10 mL of HCl. The ash was transferred to a 100 ml volumetric flask and filled to the line with deionized water. Absolute KCl standards of varying concentrations were prepared. The absorbance of both the sample and the standard was measured using a flame photometer set to potassium wavelength. The KCl concentration was plotted against the sample absorbance, and the percentage potassium was calculated.

2.12 Data Analysis

The mean and standard error of mean replicates are presented as the results. To establish significant differences, a one-way analysis of variance (ANOVA) and Duncan's multiple range test (DRMT) were used with the statistical R- app and Graph pad prism version 6. P 0.05 was used to determine significance.

3. RESULTS AND DISCUSSION

3.1 Morphological, Microscopic and Biochemical Characteristics of *Saccharomyces cerevisiae* ATCC 36858

The characteristics of the ethanol producing strain of *Saccharomyces cerevisiae* obtained from the Federal Institute of Industrial Research Oshodi (FIIRO) are shown in Tables 1-3. The FIIRO yeast were cream-white in color, flat, with a smooth surface, small oval shape, fission cell types, and no filament was observed. The sugar utilization test showed that the isolates were capable of utilizing a wide range of sugars as carbon and energy sources but could not utilize nitrate.

Table 1. Colonial morphology of the yeasts

Characteristics	Typed Yeast
Color	cream white
Elevation	Flat
Texture	Moist smooth
Margin	Entire

Table 2. Microscopic characteristic of the yeasts

Characteristics	Typed Yeast
Cell – Shape	Small pointed oval
Gram stain	Ascospores G –ve, Vegetative cell G +ve
Ascospore	Present
Pseudohyphae	Absent
True mycillium	Absent
Cell types	Fission
Organism	*Saccharomyces cerevisiae* ATCC 36858

Table 3. Biochemical characteristics of yeast

Characteristics	Typed Yeast
Ferment glucose	Yes
Ferment Sucrose	Yes
Ferment Galactose	Yes
Ferment Maltose	Yes
Ferment fructose	Yes
Nitrate ability	NO
Organism	*Saccharomyces cerevisiae* ATCC 36858

3.2 Ethanol Yield

The percentage (%) yield of bioethanol after five days of fermentation of *Zea mays* wastes with *Saccharomyces cerevisiae* ATCC 36858 and *Saccharomyces cerevisiae* ATCC 204508/S288c is presented in Figs. 1 and 2 respectively: The highest ethanol yield of 12.53% (123.3g/L), 18.5% (185g/L) and 20.13% (201.3g/L) was recorded after 96 hours of fermentation for Corn stem, husk and cob respectively using *Saccharomyces cerevisiae* ATCC 36858. While, 6.5% (65g/L), 8.23% (82.3g/L) and 8.53% (85.3g/L) was also recorded after 96 hours of fermentation for Corn stem, husk and cob respectively using *Saccharomyces cerevisiae* ATCC 204508/S288c. *Saccharomyces cerevisiae* ATCC 36858 was observed to have the higher ethanol yield compared to the baker's yeast. Also, corn cobs have the highest ethanol yield among the corn wastes. This could be due to the ease with which Saccharomyces can convert its carbohydrate content to sugar monomers due to its lower density, making it easier for Saccharomyces to begin ethanol production, or it could be due to the hydrolysis process used for waste pretreatment.; this is in line with the findings of Boonchuay et al. [34]; Katsimpouras et al. [35]. This work contradicts Tanbuwal et al. 2018's findings, which found a low concentration of ethanol (0.331 mg/l) in corn cob wastes, which could be due to differences in the hydrolysis process used in the work. The volume of ethanol yielded in this study is higher than that reported by other researchers using corn waste such as corn stover [36], corn stalk juice [37], and waste corn [37,38].

The ability of these yeasts to produce ethanol can be attributed to an enzyme found in Saccharomyces cerevisiae that can break down sugar monomers into ethanol [39]. At a p value of 0.05, statistical analysis revealed no significant difference in ethanol yield. On the basis of each organism's yield on the three substrates, the means were compared.

As previously stated, the ability of Saccharomyces cerevisiae ATCC 35858) to produce more ethanol than Saccharomyces cerevisiae ATCC 204508/S288c (Figs. 1 & 2) was not unusual. Saccharomyces cerevisiae ATCC 35858 is a distinct yeast strain that has been identified and classified as an ethanol producing yeast that contains the enzyme alcohol dehydrogenase. Alcohol dehydrogenases are important enzymes in the ethanol production process; they also aid in the continuation of fermentation at high ethanol concentrations; and they improve alcohol tolerance. This study supports the findings of Oyeleke et al. [40] and Raj et al. [39]. Corn wastes can be considered a convenient substrates for ethanol production, as shown in Figs. (1 & 2), with corn cob having the highest yield among the wastes (Figs. 1 & 2).

3.3 Simple Sugar Content During Fermentation

Reducing sugar of the corn cob which produces the highest volume of ethanol decreased

generally throughout the fermentation period, with the least sugar level observed at day 5. The result is presented in Fig. 3. At p value ≤ 0.05 there were no significant differences between the reducing sugar contents during fermentation.

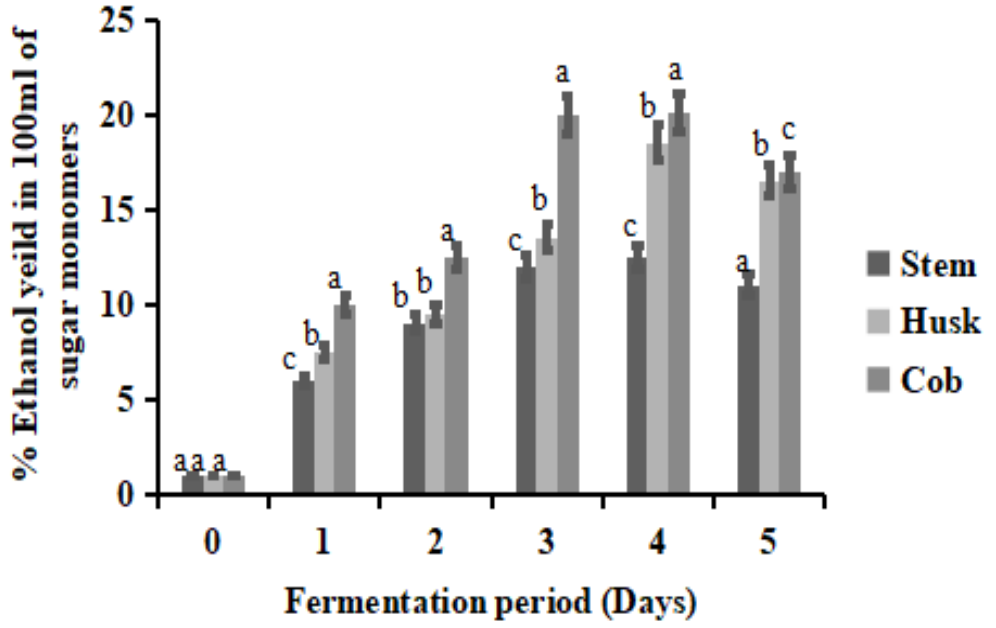


Fig. 1. Ethanol Yield During the Fermentation of Corn Waste Using *Saccharomyces cerevisiae* ATCC 36858

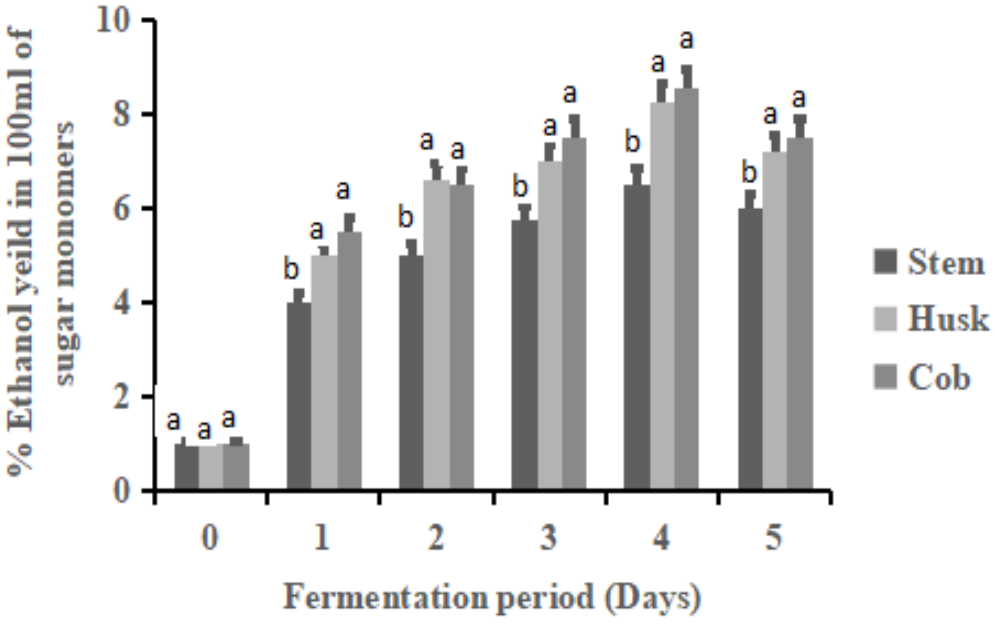


Fig. 2. Ethanol yield during the fermentation of corn waste using *Saccharomyces cerevisiae* ATCC 36858

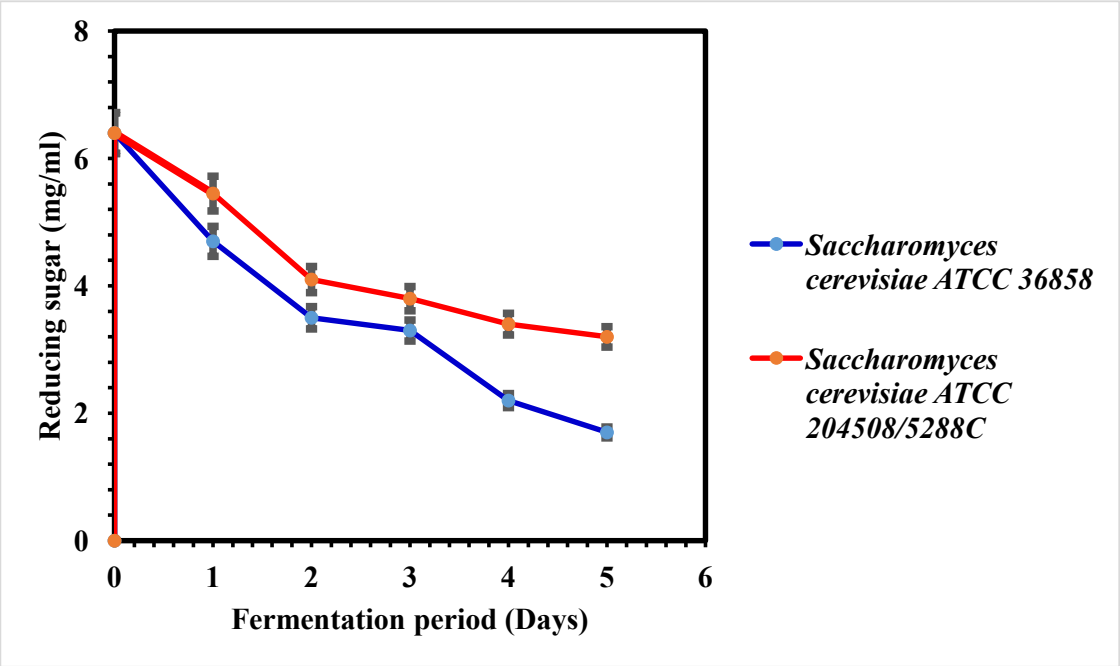


Fig. 3. Simple Sugar Content During the Fermentation of Corn Waste by the Three Strains of *Saccharomyces cerevisiae*

Values are means of replicates with standard error of mean (SEM)

3.4 Specific Gravity During Fermentation

Specific gravity of the fermenting substrate gradually decreases as the fermentation days increased; the least specific gravity was with *Saccharomyces cerevisiae* ATCC 36858. Detailed of this result is presented in Fig. 4. Statistical analysis reveals that the average specific gravity of the yeasts during fermentation was insignificant at P value ≤ 0.05.

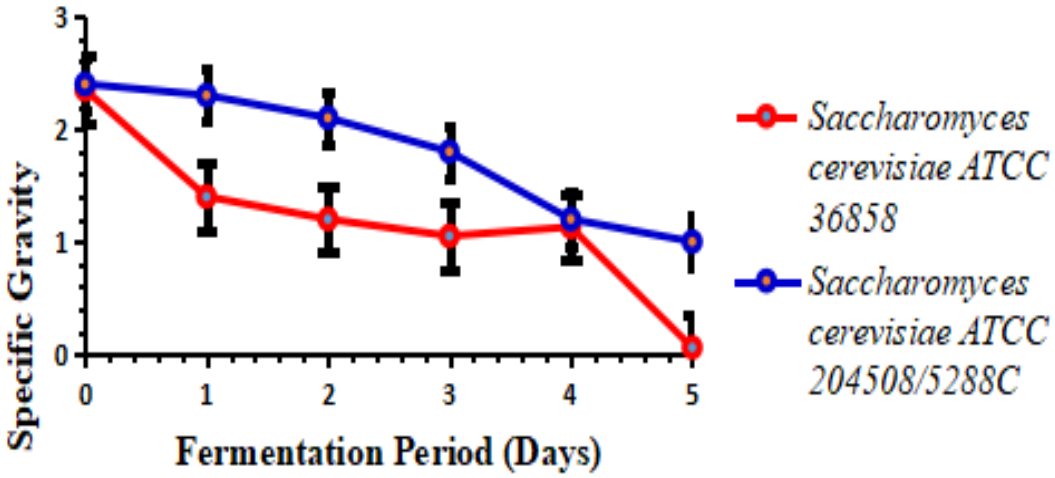


Fig. 4. Specific gravity during the fermentation of corn waste using the two strains of *Saccharomyces cerevisiae*

Values are means of replicates with standard error of mean (SEM)

Sugar and specific gravity were reduced throughout the fermentation period (Figs. 3 and 4). The total soluble solids usually decreased as the sugar in the medium fermented to ethanol [41-43]. On day four (4) of fermentation, the sugar depletion was very rapid, and the ethanol yield was highest; this period corresponds to the exponential phase of yeast growth, and product formation is usually at its peak during late exponential growth [7].

3.5 pH of Sample During Fermentation

During fermentation, the pH value fluctuated, This is the same with the two yeast strains. The two *Saccharomyces* strains were found to produce ethanol most at pH levels between 4.6 and 5.6. (Fig. 5). At p value ≤ 0.05, there was no statistically significant difference between the pH values of the yeasts during fermentation. The fluctuation in the pH do not affect yeast growth, but instead promotes yeast growth and ethanol formation while also acting as a deterrent to bacterial contaminants and favoring more catabolic reactions [44,45].

3.6 Dry Weight of Yeasts Cell During Fermentation

Fig. 6 shows how the dry weight of yeast cells increases during ethanol production. The cell mass of *Saccharomyces cerevisiae* ATCC 36858 decreased on the last day of fermentation, whereas the cell mass of *Saccharomyces cerevisiae* ATCC 204508/S288c (Baker's yeast) increased throughout the fermentation period. Statistical analysis revealed that the difference in yeast cell average dry weight was insignificant. The increase in dry yeast cell mass indicated rapid cell growth (Fig. 6). The increase in cell mass was proportional to the increase in ethanol yield. The ability of an organism to produce metabolic products determines its growth [46].

3.7 Proximate Composition Before and After Fermentation of the Corn Wastes

Figures 7 and 8 show the approximate composition of dried milled corn (Zea mays) wastes before and after fermentation. Before and after fermentation, the cob had the highest carbohydrate content, the stem had the highest crude fiber content, and the leaf had the highest protein content. Before and after fermentation, the average ash contents, moisture contents, and total fat in the samples were all within a narrow range of values. The differences in average ash, moisture content, total fat, protein, and crude fibre before and after fermentation were insignificant at p value ≤ 0.05, whereas carbohydrate values were significantly different (p value ≤ 0.05).

The mineral composition of Zea mays wastes before and after fermentation was measured to determine the presence of important minerals. Figs. 9 and 10 depict nitrogen, phosphorous, and potassium (NPK). Nitrogen was the most abundant mineral in all of the corn wastes. Corn husk contained high levels of nitrogen, potassium, and phosphorous. It was also discovered that cob had the lowest mineral composition value in corn wastes, and this difference was insignificant at p value ≤ 0.05.

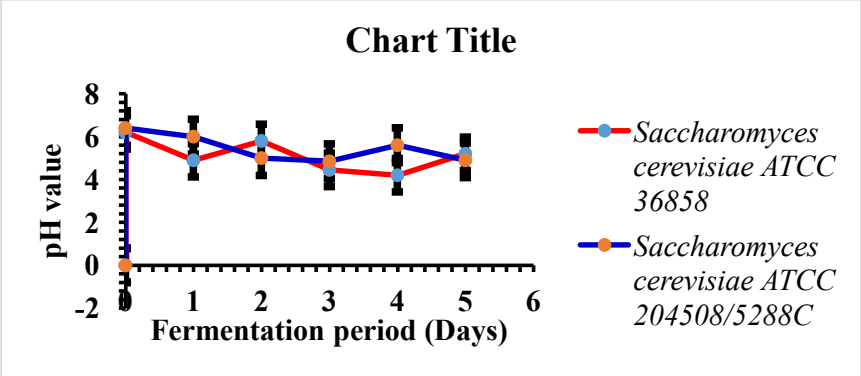


Fig. 5. Change in the pH value during the fermentation of corn waste using the two strains of *Saccharomyces cerevisiae*

Values are means of replicates with standard error of mean (SEM)

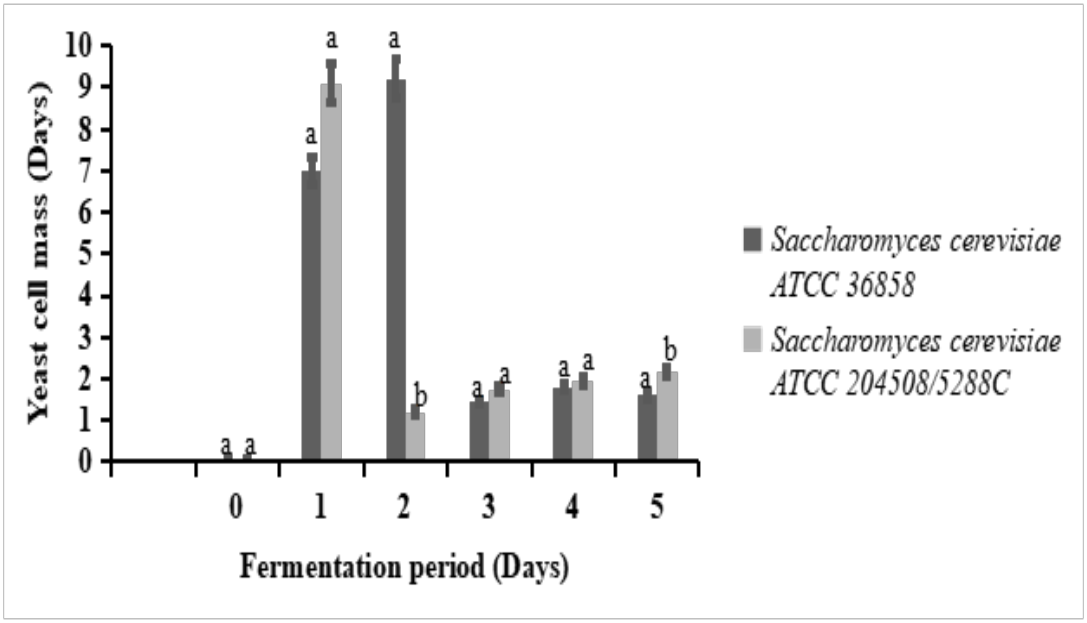


Fig. 6. Change in dry cell #weight of the two strains of *S. cerevisiae* during the fermentation

Values are means of replicates with standard error of mean (SEM)

Means with the same letter are not significantly different at 5% level of probability using Duncans Multiple Range Test (DMRT)

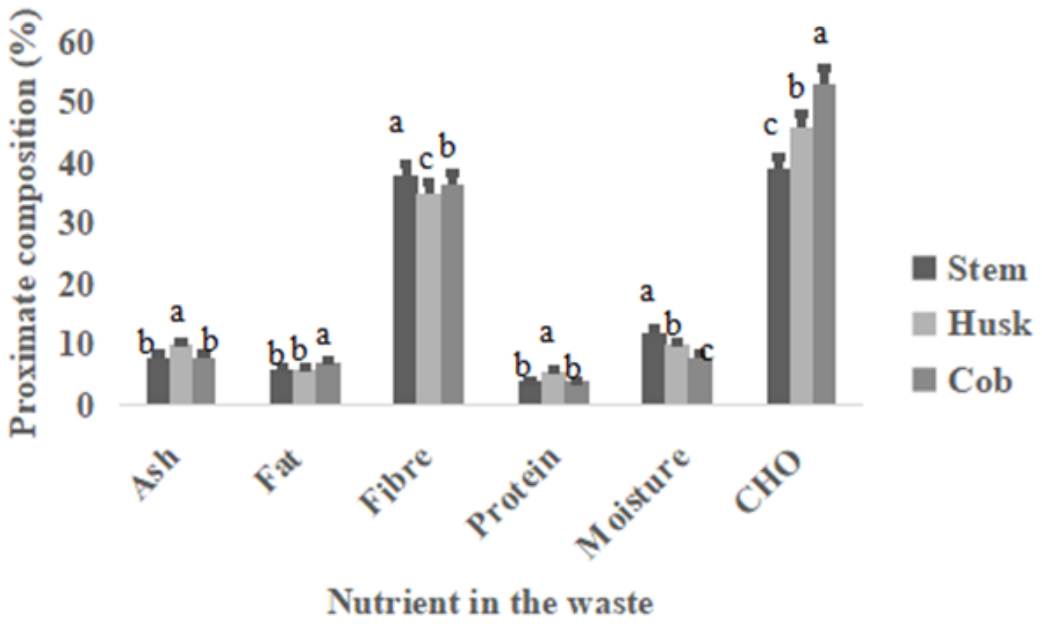


Fig. 7. Proximate composition of corn wastes before fermentation

Values are means of replicates with standard error of mean (SEM)

Means with the same letter are not significantly different at 5% level of probability using Duncans Multiple Range Test (DMRT)

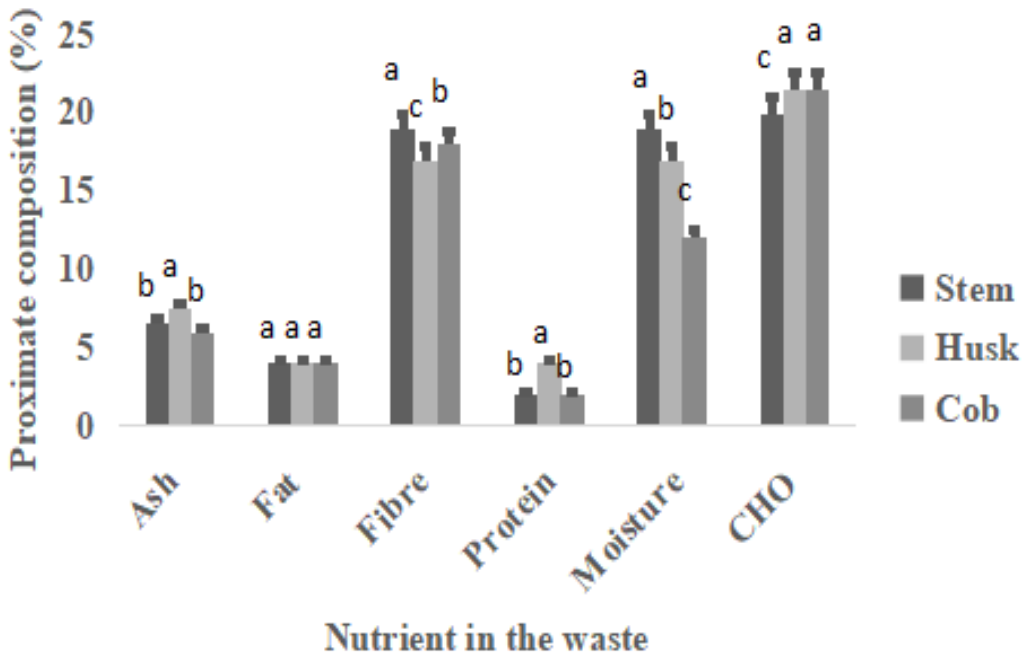


Fig. 8. Proximate composition of corn wastes after fermentation

Values are means of replicates with standard error of mean (SEM)

Means with the same letter are not significantly different at 5% level of probability using Duncans Multiple Range Test (DMRT)

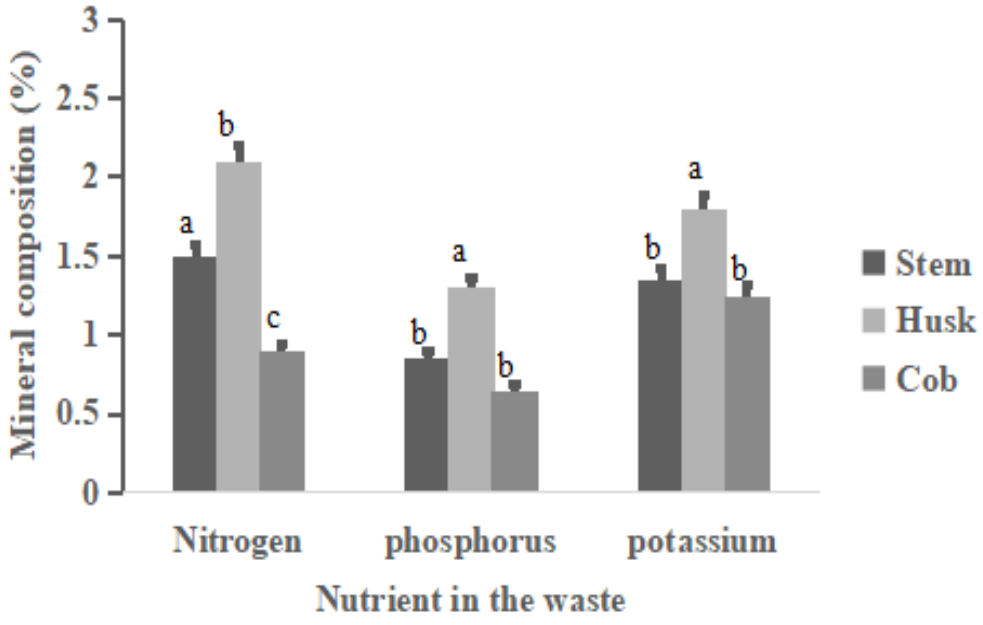


Fig. 9. Nitrogen, Potassium and phosphorus in the waste before fermentation

Key: Values are means of replicates with standard error of mean (SEM)

Means with the same letter are not significantly different at 5% level of probability using Duncans Multiple Range Test (DMRT)

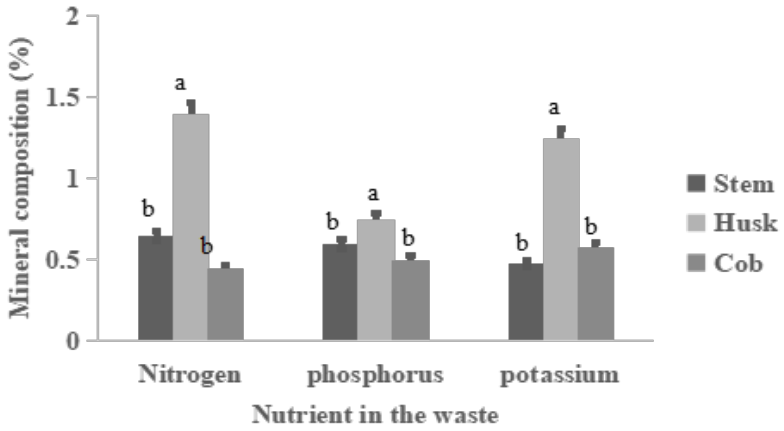


Fig. 10. Nitrogen, Potassium and phosphorus in the waste before and after fermentation
Key: Values are means of replicates with standard error of mean (SEM)
Means with the same letter are not significantly different at 5% level of probability using Duncans Multiple Range Test (DMRT)

After fermentation, the residue can still be used in livestock feed as a source of total CHO. This is because the available total CHO (Figs. 7 & 8) still meets livestock's daily nutritional needs, as recommended by Akinfemi et al. [47], Salah et al. [48], Abubakar et al. [49], and Mekuanint & Girma, [50]. There was a loss of about 50% of the nutrients in the corn wastes (Figs. 7 & 8). The nutrient uptake by fermenting yeast for growth and metabolism, as well as bioconversion into ethanol, could account for the lost [51]. Though, the crude fiber and net CHO levels were still relatively high. This could be due to residual or unhydrolyzed cellulose and hemicellulose in the corn wastes, which became trapped in the fiber and carbohydrate matrix [52]. If used as supplements, the nutrients available after fermentation of the corn wastes could be advantageous to livestock and fish farming [28,49].

The observed reduction in minerals (NPK) was due to the yeast cell metabolism (Figs. 9 & 10). The observation is similar to Bušić et al., (2018). Similar findings have been reported earlier [53-55]. However, there was noticeable mineral conservation after fermentation of corn wastes, with about half of the mineral composition recovered, thus, fermentation residues could be used to supplement livestock feed or as part of biofertilizer components [56].

4. CONCLUSION

The findings in this work supported the use of lignocellulosic biomass such as corn waste (corn stem, husk and cob wastes) to produce bioethanol using the two strains of *Saccharomyces cerevisiae* studied. The residual wastes after fermentation were found to still meet the total CHO requirements of livestock feed and could be used to augment feed for fish and livestock and as well as production of biofertilizer. Therefore, corn wastes could be potential wastes to wealth that are key to creating marketable value-added products. The prospective knowledge of waste management and wastes to wealth may be extended to farmer as these can gear them toward increasing agricultural produce, and proper handling of wastes rather than leaving the wastes unexploited or allowing them to constitute havoc to the environment. Furthermore, the nutritional and mineral value of the corn wastes after fermentation can be improved by supplementing with other cheap sources of high nutritional compounds from leguminous and other agricultural waste to formulate livestock feed and biofertilizer [57-62].

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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