Evaluation of Corn Stover and Rice Bran as Potential Biomass for Biofuel Production

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Abstract

This research explored the potential of corn stover and rice bran as suitable biomass for biofuel production through microbial investigation, proximate analysis and evaluation of mineral content using conventional techniques. The microbial investigation revealed twenty microorganisms in total. Generally, the most predominant bacteria found in both samples were from the genus *Bacillus*, followed by *Paenibacillus.* The predominant fungi were from the genus *Aspergillus*. The total bacterial and fungal count for corn stover was 4.56 x 10^5 cfu/g and 2.55 x 10^5 sfu/g, while it was 5.97 x 10^5 cfu/g and 1.36 x 10^5 sfu/g for rice bran. The results obtained from the proximate and mineral evaluation showed that both corn stover and rice bran had a low moisture content (6.06%, 5.47%) and ash content (5.23%, 5.18%) which can boost the efficiency of the fuel produced. Corn stover had a crude fibre of 38.09%, crude fat of 1.87%, and crude protein of 3.56% while rice bran had a crude fibre of 29.72%, crude fat of 1.21%, and crude protein of 3.25%. They both had high carbohydrate contents with corn stover having 51.44% and rice bran having 57.89% which can liberate suitable amounts of monomeric sugars for the production of biofuels. Both samples had a moderate mineral content and a comparative study showed that corn stover had a higher potassium (31.51%), phosphorus (23.26%) and magnesium content (14.87%) while rice bran had a higher calcium (32.24%) and iron content (5.79%). Due to their composition and nutritional content, corn stover and rice bran can serve as suitable subtrates and present an economically viable solution for the production of biofuels. Employing them as energy sources will not only eradicate wastes but also create wealth without affecting the food supply.

Keywords: Corn Stover, Rice Bran, Biomass Composition, Biofuel
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1. Introduction

Research is currently inclined towards developing novel processes for the production of biofuels and related products from renewable resources like biomass due to the depletion of fossil fuel reserves and the negative effect of their over-consumption on the environment. Biomass is practically a limitless resource that can be acquired from a wide variety of sources such as wood processing residues, agricultural crop residues, algae, and forestry residues. All over the world, a lot of plant biomass exists as low-value agricultural produce or wastes that needs good disposal. Unfortunately, up to now, most of the biomass residuals are burned directly. This not only has low energy efficiency, but also causes serious environmental pollution such as respiratory aerosols, SO_2 and CO. (Cheng *et al*., 2012). Therefore, the conversion of lignocellulosic biomass to high quality bioenergy by advanced processes rather than combustion is a promising way to substitute fossil-based fuels and decrease greenhouse gas emissions (Kumar and Gayen, 2012). Agricultural byproducts and residues have recently received attention from researchers for biofuel production due to the many benefits associated with their use. They offer an economically viable solution for the production of biofuels because they do not compete with human and animal foods. Rice bran is a residual waste of the rice processing industry that accounts for approximately 10% of rice production. It is available, inexpensive, contains large amounts of carbohydrates and has limited application as an animal feed which makes it a potential substrate for biofuel production (Al-Shorgani *et al*., 2012). Corn stover dominates a lot of agricultural residues in terms of yield per hectare and availability. It is also an abundant and alterantive feedstock for biofuel production because of its ready availability, high residue yield rate and ease of cultivation (DOE, 2011). Generally, agricultural residues are eco-friendly, renewable, and abundant which makes them suitable for the production of a biofuel and highly important in the advent of renewable energy.

2. Materials and Methods

2.1 Study area

The Federal University of Technology, Akure, (FUTA) farm with coordinates 7.3070º N, 5.1398º E is located inside the institution, along Akure-Ilesha Road, Ondo State, South West Nigeria. It is a teaching and research farm located along the Staff road and is operated mainly by agricultural students. The local rice farm utilized is situated along Stateline Road, Off FUTA South Gate, Akure, Ondo State. It is a small scale farm that specializes in the production and processing of local rice.

2.2 Sample Collection from Study Area

Corn stover was collected from FUTA farm while rice bran was collected from a local rice farm at Stateline Road, Akure. The samples were collected, labelled and transported in a polythene bag to the Microbiology Laboratory, FUTA.

2.3 Microbial isolation

Isolation of microorganisms was carried out as described by Fawole and Oso (2012). A gram of each sample was suspended in 9.0 ml of sterile distilled water and 10-fold dilutions were made. One mililitre aliquot of each diluted sample (10^{-3} and 10^{-5}) was plated on freshly prepared Nutrient agar (NA) and Potato Dextrose agar (PDA) using the pour plate technique. The plates were incubated at 35 ± 2°C for 24 hours and 25 ± 2°C for 72 hours respectively. After incubation, the plates were observed for microbial growth. The colonies formed on the NA plates were counted and sub-cultured on a fresh media. The mycelial growth and sporulation of fungi on the PDA plates was also observed and sub-cultured on a fresh media. This was repeated until distinct and pure colonies of each isolate were obtained.

2.4 Identification of isolates

The cultural and morphological identifications of the bacteria isolates were carried out in accordance with Bergey's Manual of Determinative Bacteriology. Further identification was done using standard biochemical techniques described by Hemraj *et al*. (2013). Fungi isolates were identified morphologically and microscopically. The nature of conidia, sporangiospore, arthrospore, spore head, rhizoid and hyphae were observed as described by Khalid *et al*. (2009).

2.5 Proximate analysis

Proximate composition of the moisture content, ash content, crude fat, crude protein, crude fibre and carbohydrate content of the samples was determined using the AOAC (2012) method. Moisture and ash content was determined through a thermogravimetric approach. Crude fibre was determined gravimetrically after chemical digestion and solubilization of other materials present in the samples and removal of ash. Crude fat was determined using the Soxhlet method while the crude protein was determined using the Micro-Kjeldahl method in which the organic matter of the samples were digested with sulfuric acid in the presence of a catalyst. The carbohydrate content of each sample was determined by calculating the percent remaining after all other components have been measured.

2.6 Determination of Mineral Content

This was determined by wet-ashing method, followed by the reading of the level of mineral as described by AOAC (2012).

3. Results

3.1 Microbial population of corn stover and rice bran

The total bacterial and fungal counts of the samples were recorded and presented in Table 1. The results shows that the total bacterial and fungal count for corn was 4.56 x 10^5 cfu/g and 2.55 x 10^5 sfu/g, while it was 5.97 x 10^5 cfu/g and 1.36 x 10^5 sfu/g for rice bran. Generally, corn stover had a higher fungal load while rice bran had a higher bacterial load

Table 1. Total Bacterial and Fungal Count of Substrates before Fermentation

Samples	Bacteria (cfu/g)	Fungi (sfu/g)
Corn stover	4.56 x 10^5	2.55 x 10^5
Rice bran	5.97 x 10^5	1.36 x 10^5

Key: cfu/g= colony forming unit per gram, sfu/g= spore forming unit per gram

3.2 Morphological and Biochemical Characterization of Bacterial Isolates

The morphological and biochemical characterization of the bacterial isolates obtained from the samples are shown in Table 2 and Table 3. The isolates from corn stover were identified as *Paenibacillus mobilis, Bacillus cereus, Enterobacter cloacae, Streptococcus lactis, Bacillus anthracis, Staphylococcus piscifermentans, Pediococcus acidilactici, Pseudomonas andropogonis* and *Bacillus subtilis.* The isolates from rice bran were identified as *Streptococcus pyogenes, Bacillus cereus, Bacillus simplex, Paenibacillus thiaminolyticus, Staphylococcus aureus, Bacillus subtilis, Staphylococcus hominis, Paenibacillus mobilis,* and *Bacillus anthracis*.

Table 2. Biochemical Characterization of Bacterial Isolates from Corn Stover

MR	VP	H_2S	Indole	Motility	Catalase	Coagulase	Citrate	Urease	Oxidase	Lactose	Sucrose	Glucose	Arabinose	Mannitol	Galactose	Xylose	Starch Hydrolysis	Gran reaction	Probable organism
+	-	-	+	+	+	-	-	+	-	+	+	+	-	-	-	-	+	-ve rods	*Paenibacillus mobilis*
-	+	-	-	+	+	-	+	+	-	-	+	+	-	-	-	-	+	+ve rods	*Bacillus cereus*
-	+	-	-	-	+	-	+	-	-	-	+	+	+	+	-	+	+	-ve rods	*Enterobacter cloacae*
+	-	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+ve cocci	*Streptococcus lactis*
-	+	-	+	+	+	-	-	-	-	-	+	+	-	-	-	-	+	+ve rods	*Bacillus anthracis*
-	-	-	+	+	+	-	-	+	-	+	+	+	-	-	-	-	+	+ve cocci	*Staphylococcus piscifermentans*
+	-	-	+	+	-	-	-	+	-	+	+	+	-	-	+	+	+	+ve cocci	*Pediococcus acidilactici*
-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-ve rods	*Pseudomonas andropogonis*
-	+	-	-	+	+	-	+	-	-	+	+	+	+	+	-	+	+	+ve rods	*Bacillus subtilis*

Keys: - = negative to the test, + = positive to the test, MR = Methyl Red, VP = Voges Proskauer, H_2S = Hydrogen sulphide gas production.

Table 3. Biochemical Characterization of Bacterial Isolates from Rice bran

MR	VP	H_2S	Indole	Motility	Catalase	Coagulase	Citrate	Urease	Oxidase	Lactose	Sucrose	Glucose	Arabinose	Mannitol	Galactose	Xylose	Starch Hydrolysis	Gran reaction	Probable organism
+	-	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+ve cocci	*Streptococcus pyogenes*
-	+	-	-	+	+	-	+	+	-	-	+	+	-	-	-	-	+	+ve rods	*Bacillus cereus*
-	+	-	-	+	+	-	+	-	-	+	+	+	+	+	-	+	+	+ve rods	*Bacillus simplex*
+	-	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+ve rods	*Paenibacillus thiaminolyticus*
+	+	-	-	+	+	+	-	+	-	+	+	+	-	+	+	-	+	+ve cocci	*Staphylococcus aureus*
-	+	-	-	+	+	-	+	-	-	+	+	+	+	+	-	+	+	-ve rods	*Bacillus subtilis*
+	-	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	+ve cocci	*Staphylococcus hominis*
+	-	-	+	+	+	-	-	+	-	+	+	+	-	-	-	-	+	-ve rods	*Paenibacillus mobilis*
-	+	-	+	+	+	-	-	-	-	-	+	+	-	-	-	-	+	+ve rods	*Bacillus anthracis*

Keys: - = negative to the test, + = positive to the test, MR = Methyl Red, VP = Voges Proskauer, H_2S = Hydrogen sulphide gas

3.3 Morphological and Micro-morphological Characterization of Fungal Isolates

The morphological and micro-morphological characterizations of the fungal isolates obtained from the samples are shown in Table 4 and Table 5. The isolates from corn stover were identified as *Aspergillus flavus, Aspergillus niger, Penicillum notatum, Rhizomucor pusillus* while the isolates from rice bran were identified as *Rhizopus microsporus, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum*..

Table 4. Morphological and Micro-morphological Characterization of Fungal Isolates from Corn Stover

Morphological and microscopy characterization	Fungal isolates
Colonies appeared as powdery mass of bright lime-green spores. Hyphae occured as thread-like septate branches that form mycelium. Conidiophores were rough-textured, colorless, thick-walled and bearing vesicles while the conidia were very finely roughened.	*Aspergillus flavus*
Colonies were initially white but rapidly became black with conidial production. Reverse was pale yellow with radial fissures. Hyphae were septate and hyaline. Conidiophores were long, smooth and hyaline, becoming darker at the apex and terminating in a globose vesicle. Conidia were black and very rough.	*Aspergillus niger*
Colonies were whitish, flat, rapid growing and cottony in texture. Reverse was pale to yellowish. Hyphae were septate and hyaline. Conidiophores were brached and the conidia were round, unicellular and visualized as unbranching chains at the tips of the phialides.	*Penicillum notatum*
Colonies were white initially and turned yellowish brown later. Texture appeared cottony and the reverse was pale. Hyphae was broad and aseptate. Sporangiophores were irregularly branched while the sporangiospores were small, unicellular, and ellipsoidal in shape. Sporangia were brown in color and round in shape.	*Rhizomucor pusillus*

Table 5. Morphological and Micro-morphological Characterization of Fungal Isolates from Rice Bran

Morphological and microscopy characterization	Fungal isolates
Colonies were initially white and rapidly became grayish-brown. Hyphae were broad, irregularly branched, hyaline and sparsely septate. Sporangiophores were unbranched, brown, and bear globose sporangia. Sporangiospores are subglobose, uniform in size and slightly striated. Rhizoids are hyaline, dark brown and occur at the junctiions of the stolons and nodal sporangiophores.	*Rhizopus microspores*
Colonies appeared as powdery mass of bright lime-green spores. Hyphae occured as thread-like septate branches that form mycelium. Conidiophores were rough-textured, colorless, thick-walled and bearing vesicles while the conidia were very finely roughened.	*Aspergillus flavus*
Colonies were initially white but rapidly became black with conidial production. Reverse was pale yellow with radial fissures. Hyphae were septate and hyaline. Conidiophores were long, smooth and hyaline, becoming darker at the apex and terminating in a globose vesicle. Conidia were black and very rough.	*Aspergillus niger*
Colonies were initially white but became pigmented with a pinkish coloration. Hyphae were septate and hyaline. Conidiophores are simple, short and unbranched. Microconidia was abundant, aseptate and ellipsoidal in shape.	*Fusarium oxysporum*

3.4 Proximate composition of corn stover and rice bran

The proximate composition of the both samples is shown in Table 6. Corn stover had a moisture content of 6.06%, ash content of 5.23%, crude fibre of 38.09%, crude fat of 1.87% crude protein of 3.56% and carbohydrate content of 51.44% while rice bran had a moisture content of 5.47%, ash content of 5.18%, crude fibre of 29.72%, crude fat of 1.21%, crude protein of 3.25% and carbohydrate content of 57.89%.

Table 6. Proximate Composition of Corn Stover and Rice Bran

Parameters	Corn Stover (%)	Rice Bran (%)
Moisture	6.06	5.47
Ash	5.23	5.18
Crude fibre	38.09	29.72
Crude fat	1.87	1.21
Crude protein	3.56	3.25
Carbohydrate	51.44	57.89

3.5 Mineral content of corn stover and rice bran

Table 7 shows the result for the mineral composition of each sample. Corn stover had 31.51% of potassium, 23.26% of phosphorus, 9.11% of calcium, 14.87% of magnesium, 3.83% of iron and 3.68% of zinc while rice bran had 14.51% of potassium, 13.16% of phosphorus, 32.24% of calcium, 8.67% of magnesium, 5.79% of iron and 3.70% of zinc.

Table 7. Mineral Content of Corn Stover and Rice Bran

Parameters	Corn Stover (%)	Rice Bran (%)
Potassium	31.51	14.51
Phosphorus	23.26	13.16
Calcium	9.11	32.24
Magnesium	14.87	8.67
Iron	3.83	5.79
Zinc	3.68	3.70

5. Discussion

The results from the microbial load investigation showed that corn stover had a higher fungal load while rice bran had a higher bacterial load. The reason for this may be due to the horizontal and vertical transmission of fungi in maize plants while the higher bacterial load in rice bran could be as a result of cross-contamination from the local rice farmers during the course of processing. The results of the biochemical characterization of the bacterial isolates showed that *Paenibacillus mobilis, Bacillus cereus, Enterobacter cloacae, Streptococcus lactis, Bacillus anthracis, Staphylococcus piscifermentans, Pediococcus acidilactici, Pseudomonas andropogonis* and *Bacillus subtilis* can be isolated from corn stover while, *Streptococcus pyogenes, Bacillus cereus, Bacillus simplex, Paenibacillus thiaminolyticus, Staphylococcus aureus, Bacillus subtilis, Staphylococcus hominis, Paenibacillus mobilis,* and *Bacillus anthracis* can be isolated from rice bran. Generally, the most predominant bacteria found in both substrates are from the genus *Bacillus*, followed by *Paenibacillus*. This discovery is in agreement to that of Degani *et al*. (2021) where the genus *Bacillus*, *Burkholderia, Enterobacteria* and *Paenibacillus* was stated as most common maize-associated bacteria. These findings are also similar to the work of Yu *et al*. (2021) where five different species of *Bacillus* were isolated from rice bran. Different species of fungi were also isolated from corn stover and identified as *Aspergillus flavus, Aspergillus niger, Penicillum notatum, Rhizomucor pusillus* while those isolated from rice bran were identified as *Rhizopus microsporus, Aspergillus flavus, Aspergillus niger,* and *Fusarium oxysporum.* This result agrees with the findings of Abdulaziz *et al*. (2021) where he also isolated species of *Aspergillus* and *Penicillium* from corn stover. Similarly, Vadamalaikrishnan and Jayaraman (2021) isolated species of *Rhizopus, Aspergillus* and some other non-sporulating fungi from rice bran, paddy and other products.

A comparative study of the results from the proximate analysis of corn stover and rice bran showed that the percentage moisture, ash, crude fat, fibre, and protein of corn stover was higher than that of rice bran. The moisture content (6.06%) and ash content (5.23%) obtained from corn stover was in line with the findings of Mensah *et al* (2021) who reported an average moisture content of 6.69% and ash content of 5.03% in corn stover. However, these findings disagree with that of Sujarwanta *et al*. (2021) who reported higher values of moisture, ash, crude fat, fibre, and protein in rice bran. In this study, it was observed that rice bran had a higher carbohydrate content of 57.89% as compared to 51.44% gotten from corn stover. The results of the mineral composition of both samples showed that corn stover was generally higher in potassium, phosphorus and magnesium while rice bran was higher in calcium and iron. It was however noted that both samples had an almost equivalent amount of zinc in them. In summary, the overall results obtained from the evaluation showed that each sample has relatively low moisture and ash content which is preferable because high values have an effect on bioconversion and combustion efficiency of the fuel produced. Both samples have a moderate mineral content which will prevent fouling and clinkering in combustion systems. These findings are in agreement with the work of Pradhan (2015). Lastly, both corn stover and rice bran had a high carbohydrate content which can liberate suitable amounts of monomeric sugars for the production of biofuels.

6. Conclusion

The findings of this research shows that corn stover and rice bran can serve as suitable substrates for biofuel production due to their adequate proximate and mineral composition which can ensure the production of a quality biofuel. They are relatively cheap substrates available in abundance and their use in renewable energy will avoid direct fuel-versus-food competition and prevent environmental pollution.

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