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Potential of *Milicia excelsa* Sawdust as Fermentation Medium for Bioethanol Production: A Preliminary Study

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Abstract

The potential *Milicia excelsa* sawdust as both fermentation medium and substrate for bioethanol production was investigated. Fermentation of the sawdust was carried out for 120 hours. Three liters of distilled water was added into 500 g of sterilized and unsterilized samples (sawdust) respectively with the addition of 4.5 g of *Saccharomyces cerevisiae* (yeast) and fermentation was terminated every 24 hrs. Microbial population and organisms responsible for the fermentation were determined using standard microbiological technique. Temperature, pH and total titratable acidity of the substrates were monitored daily for 120 hrs. Bacterial population at 0 hr was 7.0×10^3 cfu/ml, it reduced to 0.03×10^3 cfu/ml after 120 hrs of the fermentation while fungal population was 5.0×10^3 sfu/ml at 0 hour and it reduced to 0.01×10^5 sfu/ml at 120 hrs. pH of the sterilized sawdust was between 5.2 and 9.9 while that of the unsterilized was between 5.5 and 9.8. Initial total titratable acidity was 0.001 mol/dm^3, while total titratable acidity during the fermentation of sterilized samples decreased from 0.017 to 0.005 mol/dm^3, that of unsterilized samples from 0.023 to 0.007 mol/dm^3. Bacterial isolated before the fermentation was *Staphylococcus aureus*, Micrococcus spp, Actinomycetes spp., while during fermentation were: *Clostridium cellulovorans*, Bacillus spp, *Lactobacillus plantarum*. The fungi isolated were *Aspergillus niger*, Rhizopus spp, *Mucor mucedo* and *Saccharomyces cerevisiae*. The yield of the bioethanol generated from the fermentation was 105, 205, 295, 239, and 163 ml at 24, 48, 72, 96 and 120 hrs respectively for the sterilized samples and 65, 139, 214, 191, and 168 ml at 24, 48, 72, 96 and 120 hrs respectively for the unsterilized samples. This implies that bioethanol can be produced from the fermentation of *Milicia excelsa* sawdust with its highest yield at 72 hrs of fermentation thereby renewing wastes into useful products and reducing environmental pollution.

Keywords: Bioethanol; Fermentation; Sawdust; Production

Introduction

Lignocelluloses biomass (e.g. sawdust obtained from wood) provides a unique and sustainable resource for environmentally safe organic fuels and chemicals. Furthermore, due to the abundance of lignocelluloses materials, its conversion to ethanol (a biofuel) is considered one of the most important uses of biomass as an energy source in the modern world especially in the United States, Europe and Asia [1]. Ethanol produced from biomass could be of great benefit to the transportation sector where it is assumed that 2/3 of Nigeria's gasoline is consumed. Globally, fossil fuels are being threatened out of dominance over other fuels by: The high international market price of fossil fuels, the negative effects of fossil fuels products on the environment e.g. the release of greenhouse gases like carbon dioxide that contribute to global warming. The pollution of air, water, and soil by fossil fuels products (carbon dioxide from fossil fuel combustion accounted for nearly 80% of global warming in the 1990s [2].

By far, the greatest proportion of the world's energy requirements comes from petroleum exports especially in the Middle East, a region of high political tension. These reasons have necessitated efforts at finding alternatives to fossil fuels. Lignocelluloses wastes (LCW) refer to plant biomass wastes that are composed of cellulose, hemicellulose and lignin as well as other minor components. Both the cellulose and hemicellulose fractions are polymers of sugars and are thereby potential sources of fermentable sugars, which can be converted into other products.

Currently, the second generation bioproducts from lignocelluloses biomass such as bioethanol, biodiesel, biohydrogen and methane are increasingly being produced from wastes (residues) rather than from energy crops (jatropha, switch grass, hybrid poplar and willow) because the latter competes for land and water with food crops that are already in high demand. The use of food crops such as corn and sugarcane to produce biofuels is increasingly being discouraged due to the current worldwide rise in food prices. In order to minimize food-feed-fuel conflicts, it is necessary to integrate all kinds of bio-waste into a biomass economy [3]. Furthermore, the use of lignocelluloses wastes offers a possibility of geographically distributed and greenhouse-gas-favorable sources of products [4].

The Energy Commission of Nigeria reported that the fuel-wood resource constitutes 2.8% of the total renewable energy resources in Nigeria. Biomass reserve in Nigeria is put at 80 million m^3, which equals to an equivalence of 1.645 billion tons of energy which is predicted to be potentially available for the next 100 years [5]. Lignocellulose is the most abundant renewable biomass with a worldwide annual production of 1×10^{10} MT [6].

A biofuel is a fuel that is produced through contemporary biological processes, such as agriculture and anaerobic digestion, rather than a fuel produced by geological processes such as those involved in the formation of fossil fuels, such as coal and petroleum, from prehistoric biological matter [7]. Biofuels can be derived directly from plants, or indirectly from agricultural, commercial, domestic and/or industrial wastes. Renewable biofuels generally involve contemporary carbon

fixation, such as those that occur in plants or microalgae through the process of photosynthesis [8].

Other renewable biofuels are made through the use or conversion of biomass (referring to recently living organisms, most often referring to plants or plant-derived materials). This biomass can be converted to convenient energy-containing substances in three different ways: thermal conversion, chemical conversion, and biochemical conversion. This biomass conversion can result in fuel in solid, liquid or gas form. This new biomass can also be used directly for biofuels [9].

Bioethanol is an alcohol made by fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn, sugarcane or sweet sorghum waste. Cellulosic biomass, derived from non-food sources, such as trees and grasses, is also being developed as a feedstock for ethanol production. Ethanol can be used as a fuel for vehicles in its pure form, but it is usually used as a gasoline additive to increase octane and improve vehicle emissions. Bioethanol is widely used in the USA and in Brazil. The principle fuel used as a petrol substitute for road transport vehicles is bioethanol [10].

Bioethanol fuel is mainly produced by sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. There is also ongoing research and development into the use of municipal solid wastes to produce ethanol fuel.

Ethanol or ethyl alcohol (C_2H_50H) is a clear colourless liquid; it is biodegradable, low in toxicity and causes little environmental pollution if spilt. Ethanol burns to produce carbon dioxide (CO_2) and water, it is a high octane fuel and has replaced lead as an octane enhancer in petrol. By blending ethanol with gasoline we can also oxygenate the fuel mixture so it burns more completely and reduces polluting emissions [11].

Ethanol fuel is the most common biofuel worldwide, particularly in Brazil. Alcohol fuels are produced by fermentation of sugars derived from wheat, corn, sugar beets, sugar cane, molasses and any sugar or starch from which alcoholic beverages such as whiskey, can be made (such as potato and fruit waste, etc.). The ethanol production methods used are enzyme digestion (to release sugars from stored starches), fermentation of the sugars, distillation and drying [12].

The present work was carried out basically from the production of bioethanol from saw dust as it is important to utilize wastes such as sawdust which are usually burnt and as such polluting our environment. Sawdust can be utilized to produce bioethanol through fermentation. Hence, there is need to research on how the yield of the sawdust can be improved upon.

Materials and Methods

Source and collection of sample

The sawdust from *Milicia excelsa* wood was obtained at the M. O. Ojuola Sawmill opposite Chicken Republic, Road Block, Akure, Nigeria. The sample was collected in a clean plastic bag and transferred to the Department of microbiology laboratory of the Federal University of Technology, Akure, for the experiment.

Preparation of the sample

Five hundred grams (500 g) each of the sawdust was weighed into 10 different fermenters. Five fermenters contained sterilized samples and the remaining five with unsterilized samples. Into each of the fermenters, 3000 ml or 3 L of distilled water was added after which 4.5 grams of *Saccharomyces cerevisiae* was also added. The 2 set of fermenters with the sterilized and unsterilized samples were terminated after 24, 48, 72, 96 and 120 hrs of the fermentation respectively. The period of fermentation of the sawdust lasted for 5 days (120 hrs).

Determination of the viable bacterial count of samples

Serial dilution of the (*Milicia excelsa*) sawdust was done before and during fermentation. The pour plate method was used. From the fermented sawdust of (*Milicia excelsa*), 1 ml of the liquor was drawn aseptically with the aid of a sterile syringe and dispensed into 9 ml of distilled water in the first test tube (10^1). The first test tube was shaken thoroughly and 1 ml taken from it was transferred into the second test tube, which was also shaken thoroughly. The dilutions were repeated up to (10^9). 0.2 ml was then drawn aseptically from this dilution and was transferred into some Petri dishes already labeled.

The Petri dishes were agitated gently in circular motion to ensure even distribution and uniform growth of the organisms. The media were allowed to solidify. Plates containing Nutrient agar were incubated in an inverted position at 37°C for 24 hrs while plates containing Potato Dextrose Agar were incubated at 24°C for 72 hrs. MRS plates were incubated anaerobically. The analyses were carried out at 0, 24, 48, 72, 92 and 120 hrs. All the colonies were counted manually and then multiplied by the corresponding dilution factor. The experiment was replicated.

Isolation and identification of bacteria and lactobacillus isolates

Pure cultures were obtained from the various bacteria colonies that grew on each plate. The cultures were transferred to double strength Nutrient agar slant for identification and stored at refrigerated temperature. The identification of bacteria was based on morphological characteristic and biochemical tests. Morphological characteristics were observed for each bacteria colony after 24 hours of growth. Biochemical characterization was performed accordingly [13].

Morphological and physiological characterization

The appearance of the colony of each isolate on the agar media was studied and characteristics such as shape, colour and size were observed. The physiological characteristics were done by Gram staining, motility test [13] and spore staining [14].

Biochemical characteristics

Biochemical Tests such as Catalase, coagulase, oxidase, urease and indole, were carried out according to Chessbrough M [15]. Methyl red test was carried out according to Olutiola PO et al. [16] while sugar fermentation and starch hydrolysis test were carried out according to Fawole MO and Oso BA.

Identification of fungi

This was done based on the cultural, morphological and microscopic examination of the colonies [17].

Enumeration of fungi

Spore counting was carried out by counting the number of visible spores that appears on the plates. Calculation of spore forming unit (sfu) per ml for fungi was based on the volume of the sample used.

Physiochemical parameters

The physiochemical parameters measures were temperature, pH and total titratable acidity.

Proximate composition of samples

The unfermented and fermented samples were analysed to determine its fat content, moisture content, ash, crude protein content and carbohydrate content. This helps to know the nutritional composition of the sawdust [18].

Determination of minerals content

The mineral content was analysed from the solution obtained by first dry-ashing the sample ash in 10% (Vol/Vol) HCl, filtered and made up to mark in a 100 ml volumetric flask using distilled de-ionised water.

Sodium and potassium were determined by flame photometry while calcium, magnesium and iron were determined by atomic absorption spectrometer (AAS) [18].

Results

The microbial populations of *Milicia excelsa* saw dust before and during fermentation were observed in Table 1. pH before and during the fermentation of the sample is shown in Table 2.

Tables 3 and 4 shows the temperature and titratable acidity before and during fermentation of the wood saw dust *Milicia excelsa* while Table 5 shows the microorganisms that are responsible for the fermentation of *Milicia excelsa* saw dust. The percentage proximate composition and mineral of the unfermented *Milicia excelsa* wood sawdust (*Milicia excelsa*) is shown in Figures 1 and 2.

Fermentation time (hours)	Bacteria Count (cfu/ml)	Fungi (sfu/ml)
0	7.0×10^3	5.0×10^3
24	5.0×10^5	4.0×10^3
48	3.2×10^5	2.2×10^5
72	8.2×10^4	5.6×10^4
96	3.5×10^4	2.5×10^4
120	3.0×10^3	1.0×10^3

Table 1: Microbial population of *Milicia excelsa* wood saw dust before and during fermentation.

The occurrence of microorganisms before and during the fermentation was observed in Table 5. Table 6 reported bioethanol generated from 500 g of *Milicia excelsa* wood sawdust (*Milicia excelsa*)

Table 7 reported the comparison of the bioethanol produced from the fermented *Milicia excelsa* saw dust in relations with conventional ethanol.

Period of Fermentation (hours)	pH of sterilized sawdust	pH of unsterilized sawdust
0	ND	5.5
24	6.3	7.5
48	6.8	7.3
72	9.9	9.8
96	8	7.7
120	6	6.8
ND-Not determined		

Table 2: pH before and during the fermentation of *Milicia excelsa* wood sawdust.

Period of fermentation (hours)	Temperature of sterilized sawdust (°C)	Temperature of unsterilized sawdust (°C)
0	ND	27
24	31	32
48	30	28
72	31	30
96	31	29
120	31	30
ND-Not Determined		

Table 3: Temperature before and during the fermentation of *Milicia excelsa* wood Sawdust.

Time in hours	Total titratable acid of sterilized (mol/dm3)	Total titratable acid of unsterilized (mol/dm3)
0	ND	0.001
24	0.017	0.023
48	0.011	0.014
72	0.009	0.014
96	0.007	0.009
120	0.005	0.007
ND-Not Determined		

Table 4: Total titratable acidity before and during the fermentation of *Milicia excelsa* wood sawdust.

Fermentation Time (hours)	Microorganisms
0	*Aspergillus niger*, *Staphylococcus aureus*, Micrococcus spp
24	*Lactobacillus plantarum*, *Saccharomyces cerevisiae*, *Aspergillus niger*
48	*Lactobacillus plantarum*, *Saccharomyces cerevisiae*, *Mucor mucedo*
72	*Lactobacillus plantarum*, *Saccharomyces cerevisiae*, *Mucor mucedo*, *Clostridium cellulovorans*
96	*Lactobacillus plantarum*, *Saccharomyces cerevisiae*, *Aspergillus niger*
120	*Lactobacillus plantarum*, *Saccharomyces cerevisiae*, *Mucor mucedo*, Rhizopus spp

Table 5: Occurrence of microorganisms before and during the fermentation of *Milicia excelsa* wood sawdust.

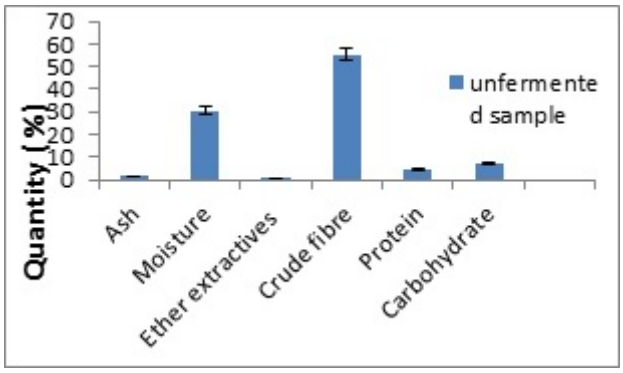

Figure 1: Percentage proximate composition of the unfermented *Milicia excelsa* wood sawdust.

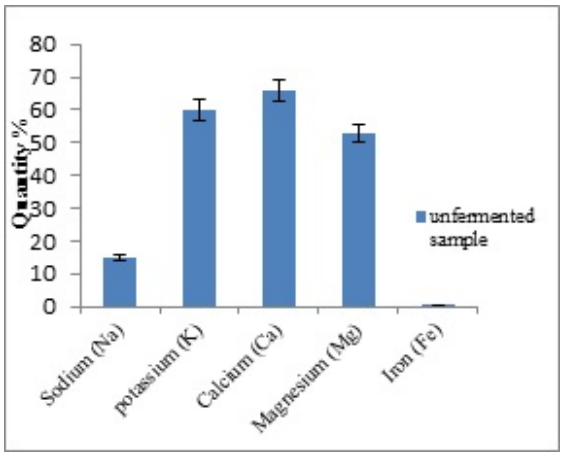

Figure 2: Mineral composition of unfermented *Milicia excelsa* wood sawdust.

Figures 3 and 4 explain percentage proximate composition and mineral of fermented *Milicia excelsa* wood sawdust (*Milicia excelsa*) at 24 to 120 hours and mineral composition of fermented *Milicia excelsa* wood sawdust (*Milicia excelsa*) at 24 to 120 hours respectively.

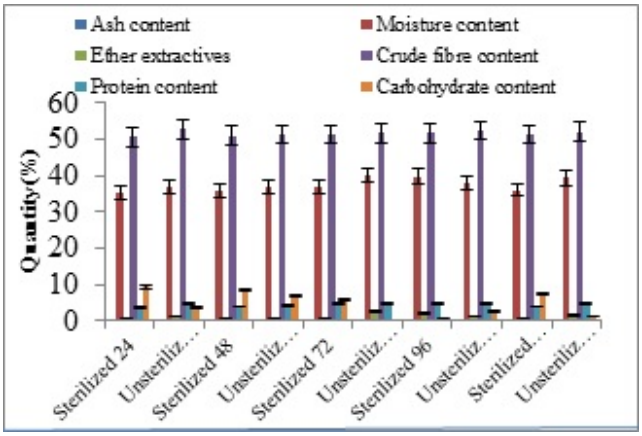

Figure 3: Percentage proximate composition of fermented Milicia excelsa wood sawdust at 24 to 120 hours.

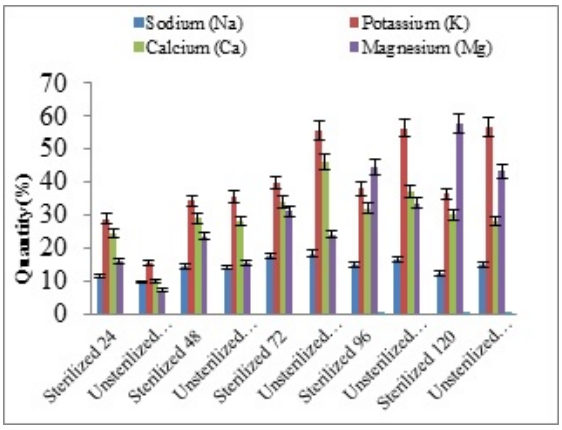

Figure 4: Mineral composition of fermented *Milicia excelsa* wood sawdust at 24 to 120 hours.

Fermentation time (hours)	Weight of sawdust (grams)	volume of Ethanol from sterilized sawdust (ml)	volume of Ethanol from unsterilized sawdust (ml)
24	500	105	65
48	500	205	139
72	500	295	214
96	500	239	191
120	500	163	168

Table 6: Bioethanol generated after the fermentation of *Milicia excelsa* wood sawdust.

S. No	Parameter	Ethanol	24 HOFMS	US	48 HOFMS	US	72 HOFMS	US	96 HOFMS	US	120 HOFMS	US
1	Specific gravity (g/cm^3)	0.787	0.999	0.996	0.999	0.996	0.999	0.995	0.999	0.995	0.999	0.995
2	Refractive index (@30°C)	1.362	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A
3	Viscosity (Absolute, Pas/sec)	1.2	1.026	0.926	0.82	0.58	0.614	0.466	0.613	0.32	0.613	0.598
4	Flash point (°C)	13-14	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
5	Boiling point (°C)	78.4	99.5	98.4	99.4	98.2	99.3	98	99.2	97.7	98.9	97.4
6	Alcohol content (By vol. %)	95.6	0.26	0.15	0.33	0.26	0.4	0.32	0.47	0.399	0.53	0.49
7	Appearance	CLS	CLS	CLS	CLS	CLS	CLS	CLS	CLS	CLS	CLS	CLS
NA- Not available; HOFM - Hours of Fermentation; CL- coloured; CLS- colourless; S- Sterile; US- Unsterile												

Table 7: Comparison of bioethanol produced from fermented *Milicia excelsa* wood sawdust with conventional ethanol.

Discussion

The potential of *Milicia excelsa* tree saw dust as both fermentation medium and substrate for bioethanol production was investigated. The bacterial population was noticed to be lowest on the third day of the fermentation (at 72 hours) (Table 1). This may be as a result of the dominance of the lactic acid producing bacteria inhibiting the growth of other non-desirable organisms. The fungi were observed to be few colonies but with yeast being present throughout the fermentation which may be as a result of the presence of *Saccharomyces cerevisiae* added into the substrate for the fermentation.

The temperature was increasing and decreasing throughout the period of fermentation. This may be as a result of the environmental impact (weather condition) on the substrate being fermented. Because the temperature was within the mesophilic range, the growth of mesophilic bacteria was favoured. The temperature range is as shown in Table 3 with sterilized sample in the range of 27°C -31°C and unsterilized in the range of 28°C - 30°C.

Some of the organisms isolated before and during the fermentation process belong to the lactic acid producing bacteria i.e., *Lactobacillus plantarum*. Others include *Staphylococcus aureus*, *Clostridium cellulovorans*, Micrococcus spp, Bacillus spp, *Saccharomyces cerevisiae* (yeast), *Aspergillus niger*, Rhizopus spp, and *Mucor mucedo* as represented in Table 5.

Lactic acid producing bacteria are a group of Gram positive bacteria, non-respiring, non-spore forming, cocci or rods that produce lactic acid as the major end product of the fermentation of carbohydrates. Lactic acid bacteria carry out this reaction by the conversion of carbohydrate to lactic acid plus carbon dioxide and other organic acids without the need for oxygen. They are described as microaerophiles as they do not utilize oxygen. *Lactobacillus plantarum* a homofermenter produces high acidity in all vegetable or plant fermentation and this plays the major role. The homofermenter convert sugars primarily to lactic acid [19]. Yeast however is a unicellular fungus which reproduces asexually by budding or division especially the genus Saccharomyces. Most yeast require an abundance of oxygen for growth, therefore by controlling the supply of oxygen, their growth can be checked. In addition to oxygen, they require a basic substrate such as sugar. Some yeast can ferment sugars to alcohol and carbon dioxide in the absence of air but require oxygen for growth. They produce ethyl alcohol and carbon dioxide from simple sugars such as glucose and fructose. Yeasts are fairly tolerant of high concentration of sugar and grow well in solution containing 40% sugar [20].

The presence of lactic acid during the fermentation helps to produce an acidic medium in the substrate and the presence of yeast helps to ferment the sugar in the sawdust for the effective production of bioethanol. The sugars in wood are polysaccharides (cellulose, hemicellulose and lignin) and cannot be broken down easily by the yeast. For the yeast to be able to act on them effectively, the sugar has to be broken down to simpler sugars (monosaccharides) such as glucose which the yeast can ferment to produce high yield of bioethanol [21]. And to do this, the sawdust must be hydrolyzed in chemicals such as Sulfuric acid to convert the cellulose to glucose (pre-treatment). Hydrolysis is the process of breaking the glucosidic bonds that hold the glucose basic unit together to form a large cellulose molecule [22]. The pre-treatment is carried out to increase the surface area and accessibility of the plant fiber to enzymes and thus achieve sugar yield for ethanol fermentation [23].

From the result presented on Table 6, the bioethanol yield produced from the fermentation of sterilized samples was found to be 105, 205, 295, 239, and 163 ml at 24, 48, 72, 96 and 120 hours respectively while the bioethanol yield from the fermentation of the unsterilized samples was found to be 65, 139, 214, 191 and 168 ml at 24, 48, 72, 96 and 120 hours respectively. The bioethanol yield from the fermentation of sterilized samples was majorly higher than those of unsterilized samples. This may majorly higher than those of unsterilized samples. This may be as a result of dominance of undesirable organisms during

the fermentation of the unsterilized samples. The bioethanol produced was not as potent as the conventional one and needs to go through several purification processes to make it potent and effective for commercial purposes or use. Without pretreatment of the sawdust, this research showed that production of bioethanol is still possible. The yield obtained is as a result of the lignocellulose and cellulose present in the sawdust which made access to the glucose in the sawdust limited. The percentage proximate composition of the sample showed that there was increase in the moisture, crude, protein and carbohydrate content of the sample after fermentation as compared to before the fermentation of the substrate. It was equally observed that the fermentation of the substrate decreased the ash content of the substrate. In the mineral analysis, it was observed that the fermentation of the substrate drastically reduced its mineral content. The sample was initially observed to be rich in calcium, magnesium and potassium with little quantity of sodium and very low iron. All of these minerals content in the sample reduced drastically after the fermentation of the sample.

Conclusion

Organisms such as the Lactic acid producing bacteria and yeast have brought about the fermentation of *Milicia excelsa* sawdust. Saw dusts are often regarded as waste and are mostly discarded or burnt causing environmental pollution. But based on this experiment, this research showed that *Milicia excelsa* wood sawdust has the potential for Bioethanol production. Therefore, can be concluded that sawdust is not waste that should be discarded but can be renewed into useful products such as bioethanol through the process of fermentation. The use of lignocellulose (sawdust) for bioethanol production reduces greenhouse gas and soot (black carbon) emission and this means a greatly reduced global warming impact. Further research is ongoing as to increasing the yield of the bioethanol produced through utilization of the total sugar in sawdust which is largely composed of cellulose lignocellulosic.

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