Journal of Energy Technologies and Policy
ISSN 2224-3232 (Paper) ISSN 2225-0573 (Online)
Vol.10, No.7, 2020

Potential of Brachystegia nigerica Sawdust as Fermentation Medium for Biobutanol Production: A Preliminary Study

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Abstract
The potential of *Brachystegia nigerica* sawdust as both fermentation medium and substrate for biobutanol production was investigated. Simultaneous saccharification and fermentation process using *Clostridium acetobutylicum* and *Aspergillus niger* as fermenting bacterium and hydrolyzing fungus respectively. Standard microbiological and biochemical methods were adopted. Fermentation of the sawdust was carried out for 120 hours. The substrate was prepared for fermentation was divided into three parts each. A part was subjected to acid pretreatment using 5% (v/v) sulphuric acid and the mixture was autoclaved at 121^{o}C for 15 minutes and then filtered using a sterile muslin cloth. The second part was done by alkaline pretreatment by adding 0.1 M of NaOH to the substrates and left to react for 48 hours while the third part was pretreated with 5% (v/v) H_2SO_4 for 48 hours and neutralize with 1M NaOH. The hydrolysates were inoculated with *Aspergillus niger* for saccharification, and left for 48 hours after which it was inoculated with *Clostridium acetobutylicum* for fermentation simultaneously. The hydrolysates were fermented for 120 hours and triplicate readings were taken every 24 hours to quantify the amount of biobutanol produced per day. Temperature and pH of the substrates were monitored daily for 120 hrs. Result showed that total bacterial and fungal count before fermentation was 3.6×10^3 cfu/g and 2.83×10^3 sfu/g respectively. Bacterial isolated were *Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis, Bacillus licheniformis, Proteus vulgaris, Bacillus badius, Bacillus lentus* and *Staphylococcus aureus* and fungal isolated were *Saccharomyces cerevisiae, Rhizopus stolonifer, Aspergills niger, Aspergillus flavus, Penicillium notatum* and *Penicillium digitata.* Biobutanol produced from biomass pretreated with NaOH, H_2SO_4 and Neutralized (H_2SO_4 neutralized with NaOH) as measured by Gas Chromatography Mass Spectrophotometer gives 1.75046 e^{-1}, 1.8367 e^{-1}, 1.31740 e^{-1} respectively, while gravimetric analysis of biobutanol production shows no production at 24 hours, 0.52 ml, 0.75 ml, 0.44 ml at 48 hours, 1.76 ml, 1.86 ml, 0.86 ml at 72 hours, 1.32 ml, 0.62 ml, 0.51 ml at 96 hours and 0.22 ml, 0.61 ml, 0.20 ml at 120 hours respectively. This implies that biobutanol can be produced from the fermentation of *Brachystegia nigerica* sawdust with its highest yield at 72 hrs of fermentation thereby renewing wastes into useful products and reducing environmental pollution.
Keywords: Biobutanol; Fermentation; Sawdust; Production
DOI: 10.7176/JETP/10-7-02
Publication date: November 30th 2020

1. Introduction

Owing to the fast depletion of fossil fuel-reserves and environmental impact caused by the over-consumption of fossil-based energy, there's urgent need to develop a renewable and environmentally friendly substitute for fossil fuels. Production of butanol may be made by fermentation process called ABE (acetone, butanol, ethanol) fermentation, carried out mostly by bacterium *Clostridium acetobutylicum* (Wackett, 2008). The basic problem of wider use of biobutanol hinge on its production with sufficient efficiency and this in turn is limited by separation of butanol from fermentation broth (Fortman *et al.,* 2008).

Brachystegia nigerica Hoyle Jones is of the family Leguminosae. It is endemic to wetter areas of forest in southern Nigeria where trees are abundant in small localized populations. The wood is used for quality furniture and tool handles. It is suitable for light construction, flooring, joinery, interior trim, ship building, vehicle bodies, boxes, crates, food containers, toys, novelties, veneer and plywood. In traditional herbal medicine, leaf and bark extracts are used in the treatment of stomach disorders, fever, urinary infections and gonorrhea (Obeng, 2012).

Sawdust are small particle; timber-industrial waste available in large volume often burnt off resulting in environmental pollution, and often become a valuable commodity which is considered in Manufacturing, Energy and Agricultural utilization (Rominiyi et al., 2017). According to Adegoke and Mohammed (2002), sawdust are common waste in saw milling industries, pulp plants, paper industries and wood processing industries are common in southern part of Nigeria. In relation to environmental concerns, technological advancement has been employed to improve the recycling of sawdust as it is an important desirable and necessary ingredient of economic and social growth

Nevertheless, its consequences of littering the environment can be unpleasant. In recent time, new and more cost-effective processes for breaking down lignocellulose biomass into their constituent sugars have been developed especially in small-scale laboratory production. About 70–90% yields of soluble carbohydrates have been obtained from corn stover, hardwood and softwood following treatment with dilute acid (0.05 % (w/v)

H_2SO_4) (Luterbacher et al., 2014).

Lignocellulose biomass can be converted by saccharification into a mixture of simple sugars (mainly pentose's, hexoses and disaccharides) after pretreatment, which are more suitable for further transformations. The pretreated biomass can be converted into fuels along several different pathways. These include bioethanol production (Dziugan et al., 2013), direct bioconversion into butanol or acetone–butanol–ethanol (ABE) mixtures (Xue et al., 2013).

Due to the disadvantages of ethanol as a universal fuel and component in fuel blends, biobutanol is getting greater attention (Shi et al., 2012). First of all, butanol has a higher calorific value than ethanol (the energy content of butanol is 29.2 MJ/L, that of ethanol is 19.6 MJ/L). It also has better miscibility with gasoline and diesel, lower miscibility with water, a higher-octane number and is less volatile (Gautam and Martin, 2000). Because of its greater hydrophobicity, butanol can be stored in humid conditions. Moreover, it is noncorrosive and can be used in existing combustion engines, in mixtures with gasoline of up to 30% (v/v) (Mascal, 2012). The properties of butanol closely resemble those of modern gasoline. Butanol also has potential as a starting material for various chemical processes, leading to isoprene, isobutene, butane and others (Kumar et al., 2012).

Butanol production via acetone-butanol-ethanol (ABE) fermentation is a distinct ability of some species of the genus Clostridium; the most popularly known of them are strains of Clostridium acetobutylicum, Clostridium beijerinckii and Clostridium saccharoperbutylacetonicum but there exist others with the same ability. In general, all Clostridium bacteria which are solvent producers share certain common characteristics such as rod-shaped morphology, anaerobic metabolism, formation of heat resistant endospores, incapability of reduction of sulphate as a final electron acceptor and G+ type of bacterial cell wall (Rainey et al., 2009). ABE fermentation consists of two distinct phases, acidogenesis and solventogenesis. While the acidogenesis phase is coupled with growth of cells and production of butyric and acetic acids as main products, the solventogenesis phase started by medium acidification, can be characterized by initiation of sporulation and metabolic switch when usually part of formed acids together with sugar carbon source are metabolized to 1-butanol and acetone.

The present work was carried out principally for the production of biobutanol from sawdust 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 biobutanol through fermentation. Hence, there is need to research on how the yield of the sawdust can be improved upon.

2.0 Materials and Methods

2.1 Source and collection of sample

Sawdust from *Brachystegia nigerica* wood was obtained at the Olaoluwa Sawmill, along Agagu Road, Akure, Nigeria. The sample was collected in a clean plastic bag directly from the milling machine and transferred to the Department of Microbiology laboratory of the Federal University of Technology, Akure, for further analysis.

Isolation and identification of bacterial and fungal isolates

Serial dilution of the (*Brachystegia nigerica*) sawdust was determined before and during fermentation. The pour plate method was used. From the fermented sawdust of (*Brachystegia nigerica*) , 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 hours while plates containing Potato Dextrose Agar were incubated at 24°C for 72 hrs. The analyses were carried out at 0, 24, 48, 72, 92 and 120 hours. All the colonies were counted manually and then multiplied by the corresponding dilution factor. 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, while fungal identification was done based on the cultural, morphological and microscopic examination of the colonies (Fawole and Oso, 2012)

2.2 Proximate composition of samples

The samples were analyzed to determine its fat content, moisture content, ash, crude protein content and carbohydrate content before pretreatment. This helps to know the nutritional composition of the sawdust.

2.3 Determination of minerals content

The mineral content was analyzed from the solution obtained by first dry-ashing the sample ash in 10% (v/v) HCl, filtered and made up to mark in a 100 ml volumetric flask using distilled deionized water. Sodium and potassium were determined by flame photometry while calcium, magnesium and iron were determined by atomic

absorption spectrometer (AAS).

2.4 Preparation of the sample

Fifty grams (50 g) each of the sawdust was weighed into 20 different fermenters. Five set of fermenters each contained acid pretreated samples, alkaline pretreated samples, neutralized pretreated samples and the control respectively. Into each of the fermenters, the biomass was subjected to acid pretreatment for delignification by measuring 50 grams of the substrate each into conical flask containing 5% (v/v) sulphuric acid and the mixture was autoclaved at 121^{o}C for 15 minutes and then filtered using a sterile muslin cloth. The residue was washed with 1% NaOH to neutralize the acid, then with distilled water. The residues were dried in an oven at 70^{o}C for 24 hours. The fermentation was carried out for 120 hours and readings were taken at every 24 hours to quantify the amount of biobutanol produced and to determine the pH and temperature of the fermentation media per day. The second part was determined by alkaline pretreatment by adding 0.1 M of NaOH to the substrates and left to react for 48 hours while the third part was pretreated with 5% (v/v) H_2SO_4 for 48 hours. The hydrolysates in each fermenter were inoculated with *Aspergillus niger* for saccharification, and allowed to stand for 48 hours after which it was inoculated with *Clostridium acetobutylicum* for fermentation simultaneously. The hydrolysates were fermented for 120 hours and triplicate readings were taken every 24 hours to quantify the amount of biobutanol produced per day.

2.5 Microorganism

Clostridium acetobutylicum (GenBank accession No. ATCC824) used in this study was isolated from agriculture soil in Akure, Nigeria. The culture was maintained on 50% glycerol as a spore suspension at 30 °C. The fresh culture was revived by activating the spores in tryptone yeast extract acetate medium (TYA).

2.6 Growth Medium

The TYA medium was used as a fermentation medium to study the effect of culture conditions consisted of the following components: 30 g/L glucose, 6 g/L tryptone, 2 g/L yeast extract, 3 g/L ammonium acetate, 0.5 g/L KH2PO4, 0.3 g/L MgSO4.7H2O, and 0.01 g/L FeSO4.7H2O. The fermentation experiments were conducted with a working volume of 100 mL.

2.7 Production of Cellulase

High cellulase enzyme production by a local fungal isolate of *Aspergillus niger* on the medium with following ingredients in g/L: $NaNO_3$—1.0; K_2HPO_4—1.0; $MgSO_4.7H_2O$—0.5; KCl—0.5; $FeSO_4.7H2O$—0.01 (Sridevi *et al*. 2009). Spores of *Aspergillus niger* were produced on potato dextrose agar slants after 72 hours of growth at 28 ^{o}C. Three milliliters of spore suspension of *Aspergillus niger* with density of 2×10^8 spores/ml was prepared from slants with sterile distilled water and was inoculated aseptically into enzyme production medium. The submerged culture was incubated at 30 ^{o}C on a rotary shaker (180 rpm) under axenic conditions. The filtrate, obtained after removal of mycelial mat by filtration through Whatman filter paper No.1 and followed by centrifugation at 10,000 rpm for 10 min, was used as a crude enzyme source.

Analysis Methods

Fermentation samples were terminated after 120 hours for analysis and centrifuged at 7000 rpm for 5 min. Biobutanol were analyzed by gas chromatography mass spectrophotometer (GCMS) equipped with a flame ionization detector (FID) and 30 m capillary column (Equity 1TM; 30 m × 0.32 mm × 1.0 µm).

2.8 Determination of Reducing Sugar Produced

The reducing sugar was determined by titrimetric methods. 30ml of fermentation broth was weighed into the burette. 10 ml of mixed Fehling's solution was pipetted into a conical flask and 4 drops of 1% methylene blue indicator was added. The solution was heated and the broth in the burette was titrated against the solution on the conical flask until the colour disappears. The reducing sugar was calculated as follows: Percentage Reduced Sugar = Titre value × Weight of the sample

2.9 Physiochemical parameters and gravitational determination of biobutanol content using separating funnel

The physiochemical parameters measures during fermentation were temperature, pH and total reducing sugar produced. The upper layer of the immiscible layer of biobutanol with water due to its low density compare to water, fermentation broths were separated with separating funnel and measured using micropipette.

Data analysis

The experimental data were analysed statistically using one-way analysis of variance (ANOVA) in Statistical Package for Social Sciences (SPSS) analytical tool. The statistical analysis was conducted in order to determine whether there are any significant differences between the means of the obtained data.

3.0 Result

Table 1: Total Bacterial and Fungal Count of *Brachystegia nigerica* sawdust before fermentation

Samples	Brachystegia nigerica *sawdust*
Bacteria (cfu/g)	3.60×10^3
Fungi (sfu/g)	2.83×10^3

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

Table 2: Biochemical characterization profile of the bacterial isolates from *Brachystegia nigerica* sawdust

Cell shape	Oxidase	Catalase	MR	VP	Citrate	Starch hydrolysis	Glucose	Mannitol	Sucrose	Lactose	Indole	Motility	H_2S	Probable bacteria
Rod	+	+	-	-	+	ND	-	+	-	-	-	+	-	*Pseudomonas aeruginosa*
Rod	-	+	-	+	+	+	+	+	+	-	-	+	-	*Bacillus cereus*
Rod	-	+	-	+	+	+	+	+	+	-	-	-	-	*Bacillus subtilis*
Rod	-	+	-	+	+	ND	+	+	+	+	-	+	+	*Bacillus licheniformis*
Rod	-	+	-	-	-	+	+	+	+	-	+	+	+	*Proteus vulgaris*
Rod	-	+	-	-	+	+	-	-	-	-	-	+	-	*Bacillus badius*
Rod	-	+	-	-	+	+	+	+	+	+	-	+	-	*Bacillus lentus*
cocci	+	+	+	+	+	ND	+	+	+	+	-	-	-	*Staphylococcus aureus*

Keys: - = negative to the test, + = positive to the test, ND = not determined, MR = methyl red, VP= voges prosker, H_2S = Hydrogen Sulphide gas production.

Table 3: Morphological and Microscopy Characterization of Fungal Isolated from *Brachystegia nigerica* sawdust

Morphological and microscopy characterization	**Fungal isolates**
Mycelium, sporangiophore and spores grow outside the Petri dish, Whitish growth and later become greyish-brown. Sporangiophores are tall, irregular, ovoid, solitary, smooth walled stolons opposite the branched rhizoids	*Rhizopus stolonifer*
Mycelium growing fast, with black conidia, the reverse of the plate is creamy. Conidiophores is smooth, black, long and coarse	*Aspergillus niger*
Dark colony colour, rough surface, no sclerotia, brown-green conidia with velvety surface. Conidiophores irregularly branched, consisting of short stipes. Conidia are cylindrical and smoth-walled	*Penicillum digitatum*
Yellowish green colony, present of metular, biseration, sclerotia, smooth surface	*Aspergillus flavus*
Colonies are white to cream, smooth, glabrous, pseudo-hyphae are formed with large globose to ellipsoidal cells with multilateral budding.	*Saccharomyces cerevisiae*

Table 4: Proximate composition of *Brachystegia nigerica* sawdust before pretreatment

Parameters	***Brachystegia nigerica* sawdust %**
Protein content	7.25±0.01
Moisture content	19.55±0.05
Ash content	1.72±0.02
Crude fat content	0.46±0.01
Fibre content	4.69±0.01
Carbohydrate content	72.10±0.10

Table 5: Mineral composition of the *Brachystegia nigerica* sawdust before fermentation.

Biomass	*Brachystegia nigerica* sawdust g/l
H_2SO_4	0.957±0.01
NaOH	0.800±0.01
Neutral	0.750±0.02
Control	ND

Table 6: Reducing Sugar Produced by the Hydrolysates (g/l)

Parameters	*Brachystegia nigerica* sawdust %
Potassium (K)	96.30±0.10
Iron (Fe)	6.44±0.01
Cadium (Cd)	18.73±0.02
Calcium (Ca)	44.63±0.04
Magnesium (Mg)	81.42±0.01

ND = Not Detected; probably because the reducing sugar in the sample is below the limit of detection.

Table 7: Fibre contents of the *Brachystegia nigerica* sawdust before pretreatment

	Fibre Content		
Brachystegia nigerica	**Lignin %**	**Hemicellulose %**	**Cellulose %**
Before pretreatment	15.624±0.01	38.925±0.01	49.911±0.01
NaOH pretreated	12.231±0.03	29.514±0.01	39.414±0.01
H_2SO_4 pretreated	10.424±0.01	25.474±0.01	35.413±0.01

Each value is a mean ±standard deviation of three replicates

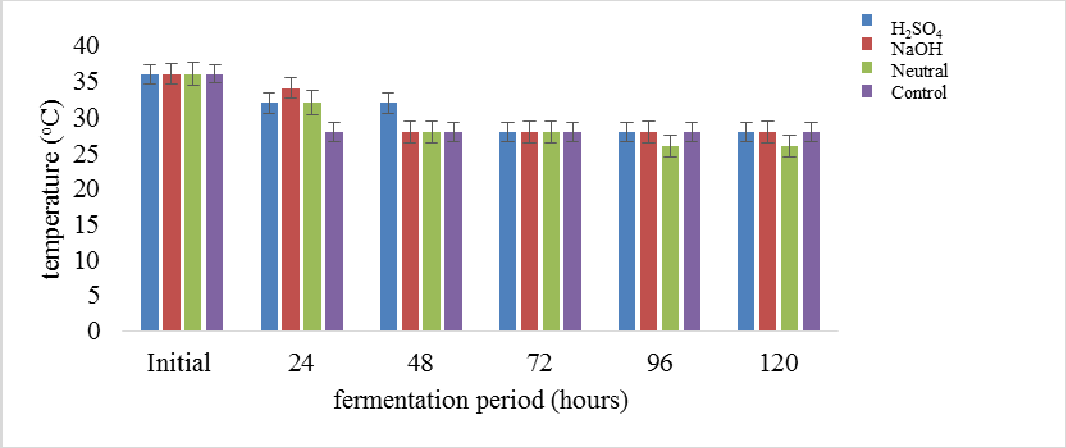

Figure 1: Temperature during fermentation of *Brachystegia nigerica* sawdust (°C)

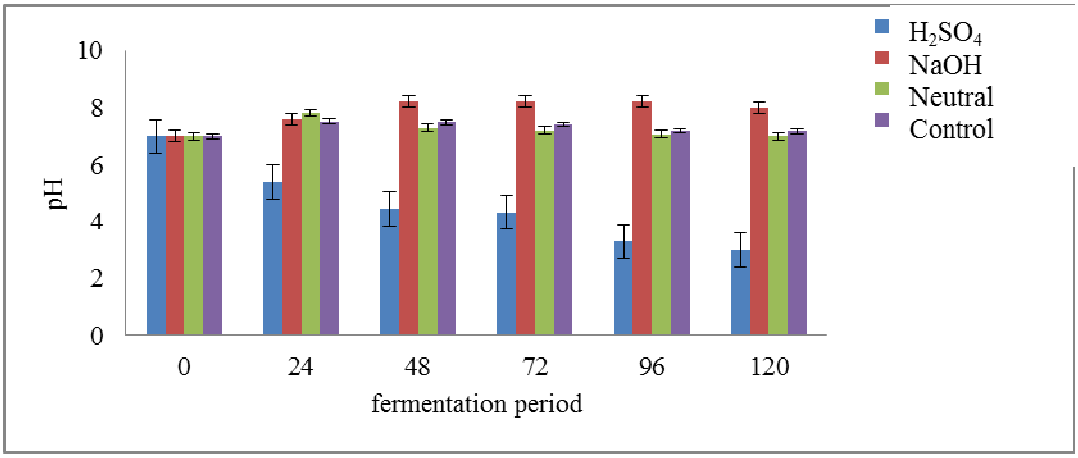

Figure 2: pH medium of *Brachystegia nigerica* sawdust during fermentation

Table 8: Quantity of biobutanol produced by *Brachystegia nigerica* sawdust using GCMS

Brachystegia nigerica **Sawdust Pretreatments**	**Biobutanol produced (g/100ml)**
H_2SO_4	1.75046 e^{-1}
NaOH	1.8367 e^{-1}
Neutral	1.31740 e^{-1}
Control	ND

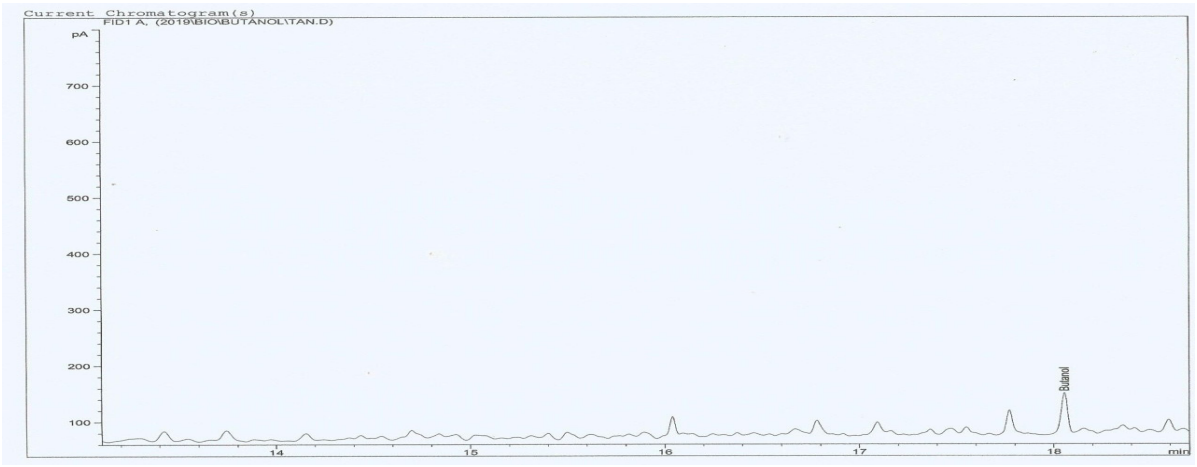

Figure 3: Biobutanol produced from *Brachystegia nigerica* sawdust pretreated with H_2SO_4

Figure 4: Biobutanol produced from *Brachystegia nigerica* sawdust pretreated with NaOH

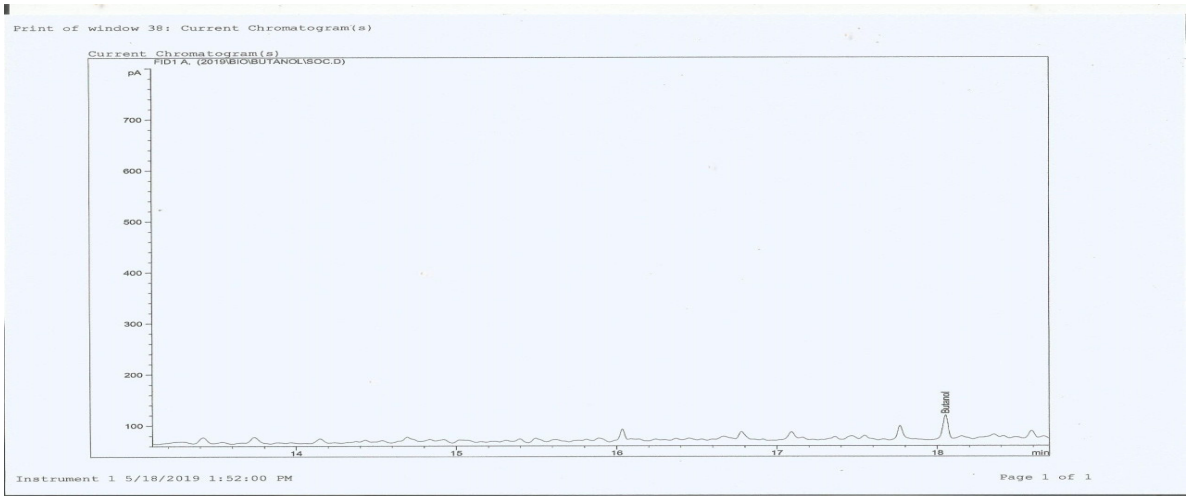

Figure 5: Biobutanol produced from *Brachystegia nigerica* sawdust with neutral pretreatment

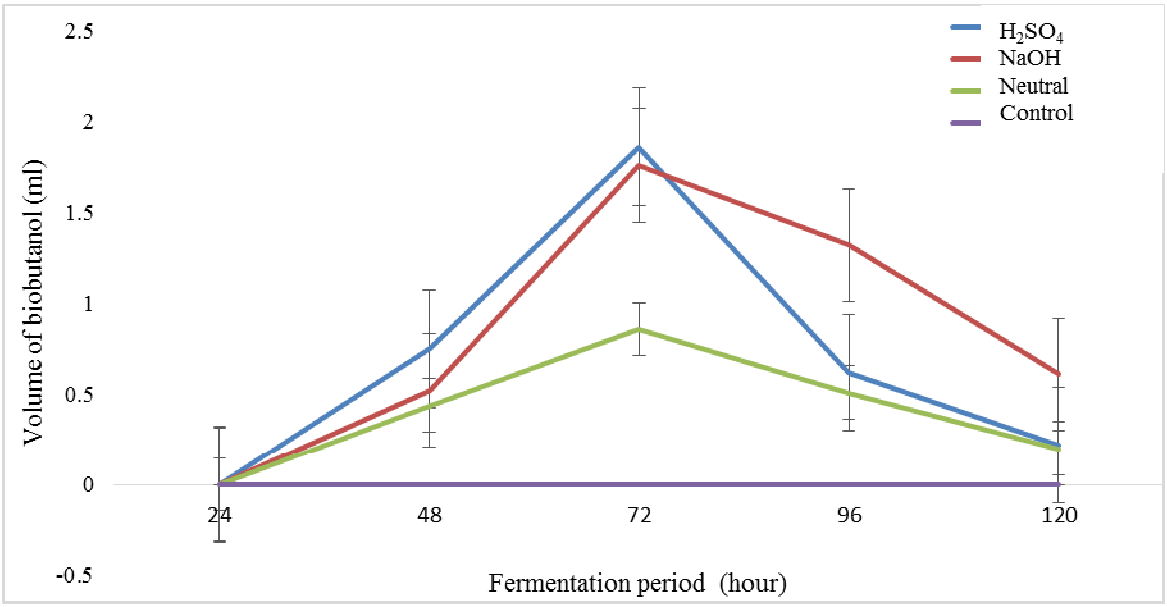

Figure 6: Volume of biobutanol produced from Brachystegia nigerica sawdust using gravimetric method

Table 9: Bacterial load of *Brachystegia nigerica* sawdust during fermentation (cfu/ml x10^3)

	Bacterial load cfu/ml by pretreatment			
Period of fermentation	**H_2SO_4**	**NaOH**	**Neutral**	**Control**
24	$1.37^{b}\pm0.06$	$2.03^{b}\pm0.15$	$1.53^{a}\pm0.06$	$3.83^{c}\pm0.29$
48	$1.33^{ab}\pm0.15$	$1.83^{b}\pm0.06$	$1.53^{a}\pm0.12$	$3.37^{bc}\pm0.55$
72	$1.87^{c}\pm0.12$	$1.27^{a}\pm0.06$	$1.43^{a}\pm0.06$	$3.07^{b}\pm0.12$
96	$1.90^{c}\pm0.00$	$1.17^{a}\pm0.21$	$3.17^{b}\pm0.21$	$3.10^{b}\pm0.17$
120	$1.13^{a}\pm0.15$	$1.36^{a}\pm0.06$	$5.33^{c}\pm0.35$	$1.33^{a}\pm0.15$

Values are represented as mean ± standard deviation. Mean values with same superscript along the same column are not significantly different at 95% confidence interval.
Key: Control is the sample without pretreatment
Neutral is the sample acidified with H_2SO_4 and neutralized with NaOH

4.0 DISCUSSION

The results of the total bacterial and fungal counts of Brachystegia nigerica sawdust are presented in Table 1. The results revealed that total bacterial and fungal count are 3.6 x 10^3 cfu/g and 2.83 x 10^3 sfu/g respectively. The morphological and biochemical characterization of the bacterial isolates associated with Brachystegia nigerica sawdust are shown in Tables 2. The eight isolates were identified as; Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis, Bacillus licheniformis, Proteus vulgaris, Bacillus badius, Bacillus lentus and Staphylococcus

aureus. Proximate composition of the biomasses prior to pretreatment is shown in Tables 4 with percentage of protein, moisture, ash, crude fat, fibre, and carbohydrate contents for Brachystegia nigerica sawdust are 7.25±0.01, 19.55±0.05, 1.72±0.02, 0.46±0.01, 4.69±0.01 and 72.10±0.10

Table 5 showed the result of the mineral composition of the biomasses and it was observed that Brachystegia nigerica sawdust possesses higher minerals percentage of Potassium, Iron, Cadium and Magnesium. Results showed that acid hydrolysis is more effective for the breaking down of the lignocellulose bond of Brachystegia nigerica sawdust, cellulose content reduces to 35.413±0.01% as compared to the results before pretreatment which is 49.911±0.01% (table 7). These results is similar with the report of Nanda et al., 2014 in terms of the high cellulose content been reported from pinewood, timothy grass and wheat straw. Also, the effectiveness of acid hydrolysis on lignocellulose biomass was due to the fact that hemicelluloses (primarily xylose) are easily hydrolysable in dilute acid than cellulose (glucose) because of their amorphous nature and lower degree of polymerization (Hu and Ragauskas, 2012). Results of the this study on composition of cellulose, hemicellulose and lignin were comparable to the results of other studies on composition of sawdust as reported earlier (Song et al., 2012; Amiri et al., 2014). However, cellulose content of Brachystegia nigerica in this study was on the higher side in comparison to other studies (Wang et al., 2014; Hui et al., 2014). Variation in composition of lignocellulosic masses in different studies may as well be attributed to different agronomic and cultural practices adopted for growth of the plants and different methods employed for analysis of composition. The results thus obtained in the present study are in agreement with Thakur et al., (2013) who also reported dilute NaOH pretreatment as an effective method. Similarly, pretreatment of substrates with alkali may result in the swelling of the particle causing easy lignin removal and cellulose depolymerization (Damisa et al., 2008)

Therefore, this observation of both pretreatment methods showed that pretreatment had effect on the cellulose and hemicellulose contents of the biomasses, the reduction in the cellulose and hemicellulose can be traced to the ability of the pretreatment in breaking them down into simple sugars. The hemicellulosic and cellulosic contents of the biomass before pretreatment fall within the range of expected values for hardwood meanwhile lignin values were a little lower than expected (Cao *et al*., 2016). The bacterial load of the *Brachystegia nigerica* sawdust during fermentation as shown in table 9 reveals the change in the microbial loads observed in the various pretreatments during the fermentation. The bacterial load ranges from 1.37±0.06 x10^3 cfu/ml at 24 hours, and reaches its peak at 96 hours with result ranging from 3.17±0.10 x10^3 cfu/ml and 5.33±0.35 x10^3 cfu/ml at 120 hours. The quantity of reducing sugar in g/l detected in the hydrolysates produced from the different pretreatments reveals that hydrolysates gotten from H_2SO_4 pretreated *Brachystegia nigerica* sawdust has the highest value with 0.957±0.01 for the reducing sugar produced. The pH decreases for fermenting *Brachystegia nigerica* hydrolysates pretreated with H_2SO_4, Neutral and control from 5.40 to 3.00, 7.83 to 7.01 and 7.53 to 7.18 respectively while for the pretreatment with NaOH, the pH increases from 7.58 to 8.00 as shown in Figure 2. The reduction in pH may have been as a result of release metabolic activity of microorganisms (*Clostridium acetobutylicum* and *Aspergillus niger*) as well as subsequent release of CO_2 and ABE production during fermentation period, this study on reduction in pH during fermentation process has also been reported by Mbajiuka *et al*., 2015. The analysis of the data confirmed the typical two-phase behaviour of the fermentation (Jones and Woods 1986): acidogenic phase and solventogenic phase. The solventogenic phase was characterized by: the gradual reduction of cell concentration as a result of cell lysis, steady increase in solvent concentration up to a constant value and the gradual decrease in acid concentration as a result of their reassimilation (Raganatia *et al*.,2013). Similar to Areesirisuk *et al*., (2006) report, acidogenesis phase of *Clostridium beijerinckii* JCM 1390 occurred since the beginning of the fermentation and solventogenesis phase was observed after approximately 20 hours of the fermentation (Areesirisuk *et al*., 2006).

The effect of temperature on the fermenting Brachystegia nigerica hydrolysates were determined during the fermentation periods for each pretreatment method employed shown in Figure 1, during the fermentation, the temperature of Brachystegia nigerica biomass pretreated with H_2SO_4, NaOH and control decreased daily from 36^{o}C to 28^{o}C at 72 hours which remain static at 28^{o}C till 120 hour, while neutralized hydrolysates further decreased to 26^{o}C at 96 and 120 hours respectively. The quantity of biobutanol produced from Brachystegia nigerica sawdust pretreated with NaOH, H_2SO_4 and Neutralized (H_2SO_4 neutralized with NaOH) produced 1.451 g/ml, 1.561 g/ml and 1.317 g/ml of biobutanol respectively as quantified by Gas Chromatography Mass Spectrophotometer (figure 3,4 and 5). Figures 7 show the quantity of biobutanol measured gravimetrically using separating funnel. Pretreated biomass with NaOH, H_2SO_4 and Neutralized has no production at 24 hours, 0.52 ml, 0.75 ml and 0.44 ml at 48 hours respectively. At 72 hours, the highest production volume extracted from Brachystegia nigerica biomass pretreated with NaOH, H_2SO_4 and Neutralized with 1.76 ml, 1.86 ml and 0.86 ml respectively. At 96 and 120 hours, 1.32 ml, 0.62 ml, 0.51 ml and 0.22 ml, 0.61 ml, 0.20 ml was extracted respectively. Highest biobutanol productivity was obtained at 72 hours, denoting that at this period, productivity is more accurate indicator of the efficiency of the process. This result does not agree with the findings of Lépiz-Aguilar et al., (2013). They also reported that poor solvent production was obtained without pretreatment of the biomass which is in agreement with the findings of this study. In comparison to ethanol production, there is a

maximum ethanol yield at 72 hours fermentation period in each of the raw materials at all concentration of the substrates which increased steadily reaching the peak at 72 hours of fermentation and then declined (Wang et al., 2011). The reason for this could be that the yeast was progressing to the stationary phase and could no longer utilize the limited sugar present in the sample (Foyle et al., 2007). When the compositions of the hydrolysates are limited, an energy deprivation would occur and the fermentative capacity will drastically reduce, this co-relates with the work of Martin et al. (2002). However, it was observed that it is sufficient to terminate the fermentation process at 72 hours for Brachystegia nigerica biomass as at this period, biobutanol production from the hydrolysates was at its peak and production started diminishing.

5.0 Conclusion

Biobutanol was successfully produced from *Brachystegia nigerica* sawdust through simultaneous saccharification and fermentation of their hydrolysates with *Aspergillus niger* and *Clostridium acetobutylicum* in 120 hours fermentation period. However, the highest biobutanol yield was recorded at 72 hours, this indicates the sufficient times to terminate the fermentation process. The presence of *Pseudomonas aeruginosa* and *Bacillus cereus* in the substrate is of public health concerns as they can pose great health risk to man. However, it is acknowledged that one of the biggest challenges for industrialization of biobutanol fermentation is low biobutanol yield due to the formation of byproducts acetone and ethanol and low biobutanol tolerance capability of the microorganism. Based on this research, it is discovered that to achieve cost effectiveness and increase volume in the production of biobutanol, the use of alkaline and acidic pretreatment could be used since they produced highest volume of biobutanol.

References

Adegoke, C.O. and Mohammed, T.I. (2002) Investigation of Sawdust Briquettes as High Grade Fuels. *The West Indian Journal of Engineering*, Faculty of Engineering, University of West Indies.

Amiri, H., Karimi, K. and Zilouei, H. (2014) Organo-solvent pretreatment of rice straw for efficient acetone, butanol, and ethanol production. *Bioresource Technology*, **152**:450–456

Areesirisuk, A., Laopaiboon, L., Leelavacharamas, V. and Laopaiboon, P. (2006). Improvement of gas chromatographic analysis for organic acids and solvents in acetone-butanol ethanol fermentation from sweet sorghum juice. *African Journal of Biotechnology* **9**(38): 6422-6429

Cao, G., Sheng, Y., Zhang, L., Song, J. and Cong, H. (2016). Biobutanol Production from Lignocellulosic Biomass: Prospective and Challenges. *Journal of Bioremediation Biodegradation* **7**: 363:1-6.

Damisa, D. I., Ameh, J. B. and Umoh, V. J. (2008). Effect of chemical pretreatment of some lignocellulosic wastes on the recovery of cellulase from Aspergillus nigerAH3 mutant. *African Journal of Biotechnology*, **7**:2444–2450

Dziugun, P., Balcerek, M., Pielech-Przybylska, K. and Patelski, P. (2013) Evaluation of the Fermentation of High Gravity Thick Sugar Beet Juice Worts for Efficient Bioethanol Production. *Biotechnology Biofuels*, **6**: 158–168.

Fawole, M. O. and Oso, B. A. (2012). Laboratory Manual of Microbiology, Spectrum Books Limited. **2**: 112-121.

Fortman, J. L., Chhabra, S., Mukhopadhyay, A., Chou, H., Lee, T. S., Steen, E. and Keasling, J.D. (2008). Biofuel alternatives to ethanol: pumping the microbial well. *Trends in Biotechnology*, **26**(7), 375-381.

Foyle, T., Jennings, L. and Mulcahy, P. (2007) Compositional analysis of lignocellulose materials: evaluation of methods used for sugar analysis of waste paper and straw, *Bioresource Technology.,* **98** (16), 3026–3036.

Gautam, M. and Martin, D. W. (2000). Combustion Characteristics of Higher-Alcohol/gasoline Blends. Proc. Inst. Mech. Eng. *A Journal of Power Energy*, **214**: 497–511.

Hu, F., and Ragauskas, A. (2012). Pretreatment and lignocellulosic chemistry. *Bioenergy Resources.* **5**:1043–1066.

Hui, L., Huangwei, X. and Kunlun, D. (2014). Optimum bioconversion of glucose: ethanol pretreatment. *Bioresources*, **9**(2):3163–3173

Izabela A. W. and Dorota K., (2019). Butanol Synthesis Routes for Biofuel Production: Trends and Perspectives, *Materials*. **12**: 350- 372

Jones, D. T. and Woods, D. R. (1986). Acetone-butanol fermentation revisited. *Microbiology Reviews* **50**:484–524.

Kumar, M., Goyal, Y., Sarkar A. and Gayen K. (2012) Comparative Economic Assessment of ABE Fermentation Based on Cellulosic and Non-Cellulosic Feedstocks. *Applied Energy*, **93**: 193–204.

Lépiz-Aguilar, L., Rodríguez-Rodríguez, C. E., Arias, M. L. and Lutz, G. (2013). Acetone-Butanol-Ethanol (ABE) Production in Fermentation of Enzymatically Hydrolyzed Cassava Flour by Clostridium beijerinckii BA101 and Solvent Separation. *Journal of Microbiology and Biotechnology,* **23**(8): 1092–1098.

Martín, C., Galbe, M., Wahlbom, C. F., Hahn-Hägerdal, B. and Jönsson, L. J. (2002) Ethanol production from

enzymatic hydrolysates of sugarcane bagasse using recombinant xylose-utilizing *Saccharomyces cerevisiae*. *Enzyme and Microbial Technology*, **31**: 274–282.

Mascal, M. (2012). Chemicals from Biobutanol: Technologies and Markets. *Biofuel Bioproduction Biorefined*, **6**:483–493.

Mbajiuka, C. S., Onwuakor, C. E., Ifediora, A. C. and Nwokoji L. I. (2015). Fermentation of Pods of kolanut Using Palm Wine Yeasts for the Production of Alcohol and Biomass. *American Journal of Microbiological Research.* **3**(2); 80-84.

Nanda, S., Ajay, K. D. and Janusz, A. K. (2014). Butanol and ethanol production from lignocellulose feedstock: biomass pretreatment and bioconversion. *Energy Science and Engineering*, 2(**3**): 138–148

Obeng, E.A., (2012). *Brachystegia nigerica* Hoyle & A.P.D.Jones. [Internet] Record from PROTA4U. Lemmens, R.H.M.J., Louppe, D. & Oteng-Amoako, A.A. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. <http://www.prota4u.org/search.asp>.

Raganatia, F., Olivieri, G., Procentese, A., Russo, M.E., Marzocchella, A. and Salatino, P. (2013). Butanol production by bioconversion of cheese whey in a continuous packed bed reactor, Bioresource Technology, **138**:259-265.

Rainey, F. A., Hollen, B. J. and Small A. (2009). Genus I. Clostridium *Prazmowski 1880, 23AL. In* Bergey`s manual of Systematic Bacteriology*,* The Firmicutes*, Second Edition, Volume Three, yield of glucose and xylose.* Biotechnology Biofuels *4:16.*

Rominiyi, O.L., Adaramola, B.A., Ikumapayi, O.M., Oginni, O.T. and Akinola, S.A. (2017) Potential Utilization of Sawdust in Energy, Manufacturing and Agricultural Industry; Waste to Wealth. World Journal of Engineering and Technology, **5**: 526-539.

Shi, S., Yue, C., Wang, L., Sun, X. and Wang, Q. (2012) A Bibliometric Analysis of Anaerobic Digestion for Butanol Production Research Trends. *Procedia Environmental Science*, **16**; 152–158.

Song, Z., Yang, G., Guo, Y. and Zhang, T. (2012) Comparision of two chemical pretreatments of rice straw for biogas production by anaerobic digestion. *Bioresources* **7**(3):3223–3236

Thakur, S., Bhuvnesh, S., Snehal, I., Kuhad, R. C. and Akshaya, G. (2013) Degradation and selective ligninolysis of wheat straw and banana stem for an efficient bioethanol production using fungal and chemical pretreatment. *Biotechnology* **3**:365–372

Wackett, L. P. (2008). Biomass to fuels via microbial transformations. *Current Opinion in Chemical Biology,* **12**:187-193.

Wang, G. S., Lee, J.W., Zhu, J. Y. and Jeffries, T. W. (2011) Dilute acid pretreatment of corncob for efficient sugar production. *Applied Biochemistry and Biotechnology*, **163**: 658–668.

Wang, W., Yuan, T. and Cui, B. (2014) Biological pretreatment with White rot fungi and their Co-Culture to overcome lignocellulose recalcitrance for improved enzymatic digestion. *BioResources* **9**(3):3968–3976

Xue, C., Zhao, X. Q., Liu, C. G., Chen, L. J. and Bai F. W. (2013). Prospective and Development of Butanol as an Advanced Biofuel. *Biotechnology Adv*ancement, **31**: 1575–1584.