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Bio-ethanol production from saccharified sawdust cellulose obtained from twenty different trees along the Lagos Lagoon in Nigeria.

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Lignocellulosic biomass can be adequately utilized in the production of bio-ethanol, a promising alternative energy resource for non-renewable fossil fuels. The development of renewable energy resources involves mainly the hydrolysis of the cellulose component of waste biomass into glucose and the successive fermentation of the resulting sugars into ethanol. An investigation into the use of sawdust waste from twenty different tropical hardwoods for bio-ethanol production has been performed. Kraft-pretreated waste cellulose from the various trees have been saccharified with *T. viride* cellulase as well as sulphuric acid. The resulting sugars were converted into bio-ethanol by means of separate hydrolysis and fermentation (SHF) as well as simultaneous saccharification and fermentation (SSF). The highest alcohol concentration of 3.71 $mg.ml^{-1}$ was obtained during the separate hydrolysis and fermentation process converting *K. ivorensis* wood sawdust whilst the lowest alcohol concentration of 2.54 $mg.ml^{-1}$ was recorded with *Ipomoea asarifolia* cellulose. During SSF the highest alcohol production was calculated at a value of 3.89 $mg.ml^{-1}$, produced from *K. ivorensis* cellulose with the lowest concentration obtained at 2,73 $mg.ml^{-1}$ obtained from cellulose extracted from *Erythropleum suaveolens*. Fermentation of sugars obtained after sulphuric acid catalyzed degradation of these cellulose materials produced the highest ethanol concentration of 3.32 $mg.ml^{-1}$ with the lowest bio-ethanol concentration (1,86 $mg.ml^{-1}$) obtained from *Masonia altissima* cellulose. The highest relative percentage yield of alcohol obtained after acid catalyzed saccharification was 40% calculated with cellulose from *Kyaya ivorensis* whilst a maximum extent of 65% alcohol production was evident during the fermentation of sugars produced from *T. viride* cellulase catalyzed saccharification of *Masonia altissima* cellulose.

Keywords: Bio-ethanol; Lignocellulosic biomass; *Saccharomyces cerevisiae* yeast; *Trichoderma viride* cellulase; Bioconversion; Kraft cellulose.

INTRODUCTION

The bioconversion of abundant lignocellulosic biomass materials into biofuels presents a viable option for improving energy security and reducing greenhouse emissions (Kuhad and Singh, 2007: Wyman, 1999). Various technological developments have improved the bioconversion of biomass into bioethanol (bio-ethanol) (Kapoor,

Chandel, Kuhar, Gupta, and Kuhad, 2007). Enzymatic catalyzed saccharification is one of the promising strategies in the conversion of lignocelluloses into fermentable sugars. This bioconversion process provides several advantages over other chemical and physical conversion techniques as the energy demand is low during the degradation of cellulose, produces minimal inhibitor byproducts and it requires relatively mild operating conditions with less pollution impact on the environment (Van Wyk, 2001; Kadam et al., 2003; Peri et al., 2007). It has been reported that cellulosic ethanol and bio-ethanol produced from other renewable biomass resources have the potential to cut greenhouse gas emissions by 86% (Wang, 2007).

The use of renewable resources, such as lignocellulosic biomasses in bio-ethanol production offers several environmental benefits and averts a grain supply crisis and competition in shortage of food supply (Kumar et al., 2009; Lin and Tanaka, 2006; Lynd et al., 1996). Unlike fossil fuels, which are formed by the decomposition of plant biomass grown millions of years ago, biofuels are produced from plants grown today with a short cycle plant growth and thus no net carbon dioxide is added to the atmosphere. The exhaust emissions from bio-ethanol combustion and its toxicity level are lower than observed during combustion of petroleum based fuels (Wyman et al., 1990; Bull et al., 1992).

Lignocellulosic biomass such as agricultural and forestry residues (e.g., wood waste, sawdust, wheat straw, sugarcane bagasse and corn stover), are renewable bio-energy resources that are sufficiently abundant to be utilized as raw materials for bioenergy development. Approximately 90% of the dry weight of most biomass materials is stored in the form of lignocelluloses (Yat et al., 2008). The bioconversion of abundant low-cost lignocellulosic biomass into fermentable sugars has a tremendous potential to be applied as a resource for bioenergy generation. Reports have shown that 1.3×10^{10} metric tons of wood are produced by terrestrial plants globally per year, with an energy equivalent of 7×10^{9} metric tons of coal or about two-thirds of the world's energy requirement. Available lignocellulosic biomass from forestry residues, agriculture wastes and other cellulosic bioresources accounts for about 180 million tons per year (Demain et al., 2005: Perlack et al., 2005).

In Nigeria, a large quantity of wood waste to the amount of $104{,}000m^3$ comprising mainly of sawdust is being generated daily from more than 2000 sawmills located across the country. The forest estate in Nigeria is estimated at about 10 million hectares with over 2 million hectares still in reserve. The unregulated and poor disposable techniques of this wood waste have led to reported incidence of environmental pollution across the country. Effort in the utilization of a vast number of sawdust lignocellulosic wood waste for the development of bioenergy will not only tackle the overwhelming incidence of environmental pollution occasioned by indiscriminate dumping of this biomaterial but will also provide a renewable and sustainable energy for economic and social advancement (Aina, 2006; Akpata, 1986).

During this investigation, sawdust samples from twenty different trees along the Lagos Lagoon were collected, delignified and saccharified with cellulase from *Trichoderma viride.* The cellulose sections of these delignified plant materials were also hydrolyzed into sugars with sulphuric acid. The aim of this investigation was to determine the amount of bio-ethanol produced during fermentations of sugars obtained during saccharification of sawdust cellulose. The information obtained during this investigation would be of value in order to design a waste cellulose to bio-ethanol procedure for each waste cellulose material.

MATERIALS AND METHODS

Cellulose Materials

Commercial cellulase from *Trichoderma viride* obtained from Duchefa, The Nederlands, was dissolved in 50 mM sodium acetate / NaOH buffer, pH 4.5, at a concentration of 10.0 $mg.ml^{-1}$. The common Nigerian names and botanical names (in brackets) of sawdust investigated are as follows (Ndukwe et al., 2009): Erunobo (*Erythropleum suaveolens*), Okilolo (*Symphona globulifera*), Erimado (*Ricindendron heudelotii*), Oporoporo (*Pterygota macrocarpa*), Iroko (*Milicia excels*), Odoko (*Ipomoea asarifolia*), Abura (*Hallea ciliate*), Itara (*Sacoglottis gabonensis*), Akomu (*Pycnanthus angolensis*), Afara (*Terminalia superb*), Ofun (*Avicennia germinans*), Obeche (*Triplochiton scleroxylon*), Akun (*Uapaca guineensis*), Opepe (*Nauclea diderrichii*), Masonia (*Masonia altissima*), Agba (*Entada gigas*), Some (*Ceiba pentadra*), Mahogany (*Khaya Ivorensis*), Eki-Eki (*Lophira alata*) and Itako (*Strombosia pustulata*).

Kraft-Pretreatment Process

To ensure an initial constant and similar cellulose mass for all cellulosic waste during the enzymatic bioconversion all the sawdust samples were dehydrated at 105^0C (Ndukwe et. al, 2009). The dried samples (2kg, 2.8-5.0 mm particle size) were subjected to a kraft-pulping process with an approximately 3:1 ratio of the pulping chemicals (200 g NaOH and 75 g NaS_2 dissolved in 8 L water) and were delignified in a rotary steel digester at 170^0C and a pressure of 200 kPa for 1.45 h at a cooking liquor to wood ratio of 4:1 (Gustafson*, et. al.,* 1983). After the Kraft-pretreatment the resulted cellulose fibers from individual sawdust materials were washed repeatedly with deionized water and dried until a constant weight was obtained at 105^0C. Mechanical pretreatment of the Kraft cellulose was conducted to reduce the fibers to sawdust particle size to increase the substrate surface area for more effective digestibility by cellulase.

Saccharification and Fermentation of Kraft-pretreated Sawdust (300 mg)

During the separate hydrolysis and fermentation process, each of the Kraft cellulose materials (300 mg) were prepared in triplicate and hydrolyzed with 3,0 ml of the *T. viride* cellulase enzyme (10.0 $mg.ml^{-1}$) in 17.0 ml Tris-buffer (0.05 M, pH 4.0). This procedure was followed by vortex agitation of the cellulose-cellulase mixture with the incubation carried out at 40^0C for a period of 6 h. After the saccharification process, the fermentation was performed at 37^0C, pH of 5.5 ± 0.2 while the fermentation medium was inoculated with 10% (w/v) solution of *S. cerevisiae* (baker's yeast, 15.0 ml) and 5.0 ml each of YMP (0.3% yeast extract, 0.3 % malt extract, and 0.5% peptone) concentrations respectively, to provide a 50.0 ml fermentation broth which results in a 6.0 $mg.ml^{-1}$ concentration with respect to the 300 mg Kraft cellulose material. During the simultaneous saccharification and fermentation process, the cellulase catalyzed biodegradation of cellulose and fermentation of the resulting sugars was carried out concurrently in a shaking water bath at 180 rev/min for a period of 72h in anaerobic conditions.

Acid Hydrolysis of Kraft Pretreated Cellulose and Fermentation of Acid Hydrolysate

The various Kraft-celluloses (0,3 g) were mixed separately in triplicate with 3.0 ml H_2SO_4 (72%) in glass test tubes and the samples were hydrolyzed at different incubation periods of 1,2 h and 3,0 h in a water bath at 210^0C. The acid hydrolysates were transferred into a glass bottle and diluted to 4.0 % by adding 84.0 ml of distilled water (Emmel *et. al*., 2003). The hydrolysates were autoclaved at 12°C for 1.0 h and neutralized with $CaCO_3$ to a pH-value between 5-6 which resulted in a 6.0 ml biomass-sugar solution. The fermentation of acid hydrolysates was performed with 6.0 ml of the acid catalyzed sugars from each of the twenty Kraft-treated cellulose (0.3 g) inoculated with 15.0 ml *S. cerevisiae* and 15.0 ml of YMP resulting in 36,0 ml total fermentation medium with a final concentration of 8.3 $mg.ml^{-1}$ (with respect to the 300 mg Kraft pretreated cellulose). The fermentation procedures were also performed in a shaker water bath at 180 rev/min for 72 h under anaerobic conditions.

Distillation of the Fermentation Syrup

After the fermentation period, the fermented broth from each of the sawdust biomaterials was subjected to a distillation process using a reflux condenser producing a colorless and clear filtrate of bio-ethanol produced from the fermented syrup. Boiling chips (glass chips) were added to the distillation flasks in order to reduce side swerving of the filtrate during boiling. The bio-ethanol was collected from the distillation flasks at temperatures between 80^oC and 85^oC.

Determination of Bio-Ethanol Concentration

A Cobas Integra Ethanol Quantitative Determination Method (Cobas Integra 400/700/800; Ethanol Gen.2) measured the amount of bio-ethanol produced from delignified sawdust cellulose. This analytical method is based on an enzymatic catalyzed procedure using alcohol dehydrogenase (ADH) which oxidizes ethanol to acetaldehyde using the coenzyme nicotinamide adenine dinucleotide (NAD), which is concurrently reduced to form NADH according to the following reaction.

$$\text{Ethanol} + NAD^+ \xrightarrow{\text{ADH}} \text{acetaldehyde} + NADH + H^+$$

Unlike gas chromatographic, HPLC and osmometric methods, the Cobas Integra method is specific, fast and simple to perform and the technique does not need any reagent preparation or sample volatilization as a reagent cassette specific for ethanol identification and quantification is available. The NADH formed during the reaction was measured by a spectrophotometric method at 340 nm as a rate of change in absorbance and is directly proportional to fermented ethanol concentration. The Cobas

software is Windows-based and result could be calculated on a desktop computer.

RESULTS AND DISCUSSION

The search for alternative and renewable energy resources are topical issues researched by many scientific institutions. Also of major importance is the conservation of the environment and development of procedures that would sustain the environment. Pollution of water ways by organic discharges in Nigeria is a serious threat posed to the Nigerian inland waters and most pollution sources arise from the dumping of untreated or partially treated sewage into the river (Adokole and Anunne, 2003) brewery effluents into the river and discharge of bio-degradable wood wastes from sawmill located along the lagoon (Nwankwo *et.al*., 1994). Manufacturing operations that produces raw wood, such as sawmill, paper mills and furniture manufacturers contributes largely to the pollution in the Nigeria water ways. Others include agricultural and domestic wastes which find their ways into the river body (Vega, et.al., 1996). The wood residue decomposition is a slow process that can result in decades of leachate production and during these periods substances found naturally in wood, such as resin acids, lignin, terpenes, fatty acids and tannins, dissolve from the wood substance into the water. Wood wastes deposited into or near water can alter, disrupt or destroy fish habitat which could result in decreasing fish variety and abundance (FAO, 1991). The city of Lagos generates 294,979.9 tons/year of wood waste, an estimated 2,288 m^3 is generated in Abeokuta, Ogun state and 104,000 m^3 is generated daily in Nigeria. In Nigeria and most developing countries in Africa, this residue is generally regarded as waste and this has led to open burning practices, dumping in water bodies or dumping in an open area which constitutes environmental pollution (Aina, 2006; Aiyeloja et.al. 2013).

Delignified sawdust samples (Ndukwe et al., 2009) from 20 different tree species which are currently used in the wood industry in Nigeria were collected from Okobaba a major sawmill on the bank of the Lagos Lagoon, for biodegradation of their cellulose content into fermentable sugars by *Trichoderma viride* cellulase. (Ndukwe et al., 2012). Sugars obtained after acid catalyzed degradation of the various sawdust materials were also fermented into bio-ethanol. The current investigation aimed to determine the relative amount of bio-ethanol, a resource of energy, produced during fermentation of sugars released during different saccharification procedures of cellulose from sawdust obtained from the twenty different trees.

Table 1 reflects the concentration of bio-ethanol produced during SHF of the various sawdust materials. The concentration of the alcohol solutions varies between a maximum value of 3,71 $mg.ml^{-1}$ obtained from *K. ivorensis* and the lowest concentration of 2,54 $mg.ml^{-1}$ calculated for ethanol produced from *I. asarifolia*.

Table 1: Bio-ethanol production during Separate Hydrolysis and Fermentation (SHF) of Kraft pretreated cellulose obtained from different types of wood sawdust.

Name of Tree (cellulose source)	Bio-ethanol Concentration (mg/mL)
Erythropleum suaveolens	2.69 ± 0.04
Symphona globulifera	2.66 ± 0.05
Ricindendron heudelotii	2.77 ± 0.01
Pterygota macrocarpa	2.74 ± 0.03
Milicia excels	2.67 ± 0.02
Ipomoea asarifolia	2.54 ± 0.01
Hallea ciliate	2.92 ± 0.02
Sacoglottis gabonensis	2.83 ± 0.04
Pycnanthus angolensis	2.86 ± 0.01
Terminalia superb	3.07 ± 0.03
Avicennia germinans	2.61 ± 0.01
Triplochiton scleroxylon	2.81 ± 0.02
Uapaca guineensis	2.93 ± 0.03
Nauclea diderrichii	2.85 ± 0.05
Masonia altissima	2.66 ± 0.03
Entada gigas	2.73 ± 0.02
Ceiba pentadra	2.80 ± 0.01
Khaya ivorensis	3.71 ± 0.04
Lophira alata	2.75 ± 0.03
Strombosia pustulata	2.61 ± 0.01

The highest amount of alcohol produced was 46 % higher than the lowest value with the average concentration at a value of 2,81 $mg.ml^{-1}$. Bio-ethanol produced from the waste cellulosic materials during SHF is indicated in table 2 showing the highest concentration of 3,90 $mg.ml^{-1}$ obtained from *M. altissima*. The lowest ethanol concentration was obtained from *L. alata* cellulose at a value of 2,88 $mg.ml^{-1}$ that was 35 % less than the maximum amount of ethanol produced. The average concentration of alcohol produced was 3,3 $mg.ml^{-1}$ that was 18 % higher than the

average alcohol produced during the SHF of these cellulosic materials. The highest alcohol concentration produced during the SSF process was 3 % higher than the maximum alcohol produced during SHF. The lowest amount of alcohol produced during SSF was also higher than the lowest concentration of alcohol produced during SHF.

Table 2: Bio-ethanol production during the Simultaneous Hydrolysis and Fermentation of 0.3g of Kraft pretreated cellulose obtained from different types of wood sawdust.

Name of Tree (cellulose source)	Bio-ethanol Concentration (mg/mL)
Erythropleum suaveolens	2.73 ± 0.01
Symphona globulifera	3.26 ± 0.03
Ricindendron heudelotii	2.90 ± 0.05
Pterygota macrocarpa	3.26 ± 0.01
Milicia excels	3.04 ± 0.04
Ipomoea asarifolia	3.57 ± 0.05
Hallea ciliate	3.12 ± 0.02
Sacoglottis gabonensis	3.52 ± 0.01
Pycnanthus angolensis	3.06 ± 0.04
Terminalia superb	3.29 ± 0.05
Avicennia germinans	3.42 ± 0.06
Triplochiton scleroxylon	3.35 ± 0.01
Uapaca guineensis	3.25 ± 0.03
Nauclea diderrichii	3.88 ± 0.01
Masonia altissima	3.90 ± 0.02
Entada gigas	3.25 ± 0.01
Ceiba pentadra	3.06 ± 0.03
Khaya ivorensis	3.89 ± 0.01
Lophira alata	2.88 ± 0.04
Strombosia pustulata	3.65 ± 0.02

The relative change in ethanol production between the SSF process and the separate hydrolysis and fermentation is indicated in table 3. The SSF-procedure produced the highest concentration of alcohol from all twenty cellulosic materials. The highest increase in alcohol production was obtained from *M. altissima* cellulose which produced 46 % more ethanol during SSF than SHF. The lowest extent of increase in ethanol production was obtained during the fermentation of sugars obtained from *E. suaveolens* cellulose at a percentage of 1,49 %. The average percentage increase was calculated at a value of 18,44 %. Nine cellulosic materials resulted in values higher than the average value of increase in alcohol production.

Bio-ethanol produced during fermentation of sugars released during sulphuric acid catalyzed saccharification of the cellulose materials is indicated in table 4. The highest alcohol concentration (3,32 $mg.ml^{-1}$) was obtained during the fermentation of *K. ivorensis* cellulose followed by an alcohol concentration of 3,28 $mg.ml^{-1}$ obtained from *S. pustulata* cellulose. The lowest bio-ethanol concentration (1,86 $mg.ml^{-1}$) was obtained during fermentation of sugars released from *M. altissima*. The highest alcohol concentration was 1,8 times higher than the lowest sugar concentration obtained during this fermentation procedure. The average concentration of alcohol production was calculated at a value of 2,83 $mg.ml^{-1}$ with seven of the wood materials producing bio-ethanol at a concentration lower than the average bio-ethanol concentration.

CONCLUSION

Increased in the development of lignocellulose for bioconversion into biofuels would contribute to sustainable development by reducing greenhouse-gas emissions and the use of grain and competitive feedstock for bioethanol production. Lignocel-lulosic biomass, including agricultural and forestry residues, sawdust, instead of traditional feedstock (starch crops, wheat, grain etc.) have proven to be an ideally inexpensive, renewable and abundantly available source of fermentable sugars for bioenergy development. The development of sawdust as a renewable energy resource would not only address the issue of environmental pollution but will also limit the utilization of fossil fuels as an energy resource.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

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AUTHOR CONTRIBUTIONS

Sample preparations were done by NAD, WOO, BIO, AND CCI while NAD and TMN

performed the incubations, analyses and calculations were performed by NAD. JPHvW wrote the manuscript with all authors read and approved the submission of the document.

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