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Full Length Research Paper

Saccharification of delignified sawdust from 20 different trees in the Lagos area of Nigeria

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Sawdust produced during the chopping of trees is a major waste product causing pollution of air as well as of the Lagos Lagoon in Nigeria. Sawdust from 20 different trees processed in the wood industry has been delignified successively by the Kraft process and hydrogen peroxide followed by *Trichoderma viride* cellulose catalyzed bioconversion into glucose, a fermentable sugar. Sugars are also released from sawdust during each delignification procedure prior to enzymatic catalyzed bioconversion. A 654% increase in sugar formation was observed from *Entada gigas* and 422% increase from *Nauclea diderrichii* when exposed to both delignification procedures compared to the amount of sugar released during Kraft pretreatment only. Relative high amounts of glucose were released during bioconversion of these waste celluloses when subjected to both delignification procedures compared to the bioconversion of Kraft delignified sawdust. Both delignification procedures resulted in a 175% increase in sugar formation for both *Erythrophleum suaveolens* and *Milicia excels* wood species. Different glucose concentrations were released during biodegradation with the highest at 9.23 mg.ml^{-1} released from *Lophira alata* after Kraft pretreatment and 14.28 mg.ml^{-1} from *E. suaveolens* after both delignification procedures. The concentration of sugar produced during the cellulase catalyzed bioconversion of delignified sawdust was many folds higher than the amount of sugars released during the delignification procedures.

Key words: Bioenergy, sawdust, *Trichoderma viride* cellulase, delignification, saccharification.

INTRODUCTION

Renewable energies are needed mostly because of limited petroleum based fuel supply and the effect of fossil fuel combustion on the environment. Another key concern for many countries is the accumulation of solid waste of which the organic section is a major component. The role of biomass in meeting energy demands will play an important role in the future and such will be the development of organic waste as a resource for bio-product synthesis (Oparaku, 2010).

Lagos the commercial capital of Nigeria is very close to the rain forest of the country which makes it a natural destination of wood felled from this region. Timbers are transported along the creeks and lagoons to Lagos from various sawmills on the shores of the Lagos Lagoon. Large volumes of sawdust, a waste product of the wood processing industry, is produced by more than 2000 sawmills located in Nigeria (FAO, 2002). Due to a lack of waste management procedures, the daily produced volumes of almost 104 000 m^3 sawdust (Aina, 2006) are incinerated, burnt or accumulated on the banks of the Lagos Lagoon where the ocean tide and waves washes these wood residuals into the lagoon causing water pollution (Akparta and Ekundayo, 1983). Sawdust clogs water ways and it enhances the germination of fungal

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spores in the water bodies which results in adverse effects on the aquatic habitat (Akpata, 1986). The high sulphur content of wood results in the formation of sulphur dioxide during incineration and together with uncontrolled burning air pollution is aggravated with ash that is, further degrading air quality. The presence of heavy metals in sawdust could also have a negative effect on the health of the local population (Abulude, 2006).

The molecular structure of wood suggests that cellulose a structural component thereof could be bio-recycled into glucose, a fermentable sugar (Reddy and Yang, 2009). Cellulase, a multi-component enzyme system produced by micro-organism such as *Trichoderma viride* and *Aspergillus niger* exhibits the ability to saccharify cellulose. These enzymes have been proved to be effective in the bioconversion of wood products such as wastepaper into fermentable sugars (van Wyk, 2001).

Sawdust samples 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. Each sawdust sample from the various trees was delignified with the cellulose content and thereof determined (Ndukwe et al., 2009). The various sawdust wood samples were exposed to *T. viride* cellulase action with the delignified cellulose component bioconverted into fermentable sugars such as glucose. The cellulase catalyzed bioconversion of cellulose from wood wastes into fermentable sugars which is well documented, as various pretreatment procedures renders cellulose, more susceptible for bioconversion (Bohdan and Dahman, 2011). The current investigation aimed to determine the amount of sugars released during the pretreatment of waste cellulose from wood mainly used by the wood manufacturing industry along the Lagos Lagoon as well as to quantify the amount of sugars released during the cellulase catalyzed bioconversion of the these different pretreated woods.

MATERIALS AND METHODS

Wood samples and cellulase enzymes

T. viride cellulose (EC. 3.2.1.4) obtained from Duchefa, The Netherlands was dissolved in 0.40 ml Tris (hydroxymethyl) aminomethan (0.005 M) buffer, pH 4.5, at a concentration of 10.0 $mg \cdot ml^{-1}$. The common Nigerian names and botanical names (in brackets) of sawdust bioconverted during this investigation 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*).

Pretreatment of wood sources

Prior to delignification, all wood samples were dehydrated at 105°C (Ndukwe et al., 2009) and these dried samples (2kg, 2.8 to 5.0 mm particle size) were subjected to Kraft-pulping (350 g NaOH and 140 g NaS_2 dissolved in 8 L water) followed by delignification in a rotary steel digester at 170°C at a pressure of 200 kPa for 1.45 h at a cooking liquor to wood ratio of 4:1 (Neto et al., 2002). After pre-treatment, these delignified lignocellulose fibers were washed with deionized water until free of the Kraft reagents. The pulp air dried samples of partially delignified cellulose were then treated with a domestic blender to regain their original sawdust particle size.

To ensure maximum lignin free cellulose fiber, the Kraft pre-treated sawdust materials (10 g) was exposed to hydrogen peroxide (30% : 60 ml) at a temperature of 40°C for 30 min. These pretreated and decolourized cellulose fibers were washed with deionized water until free of hydrogen peroxide and dried at 105°C until a constant mass was recorded which was finally treated with a domestic blender to ensure the original particle size.

Analytical methods

Kraft pulped sawdust samples as well as sawdust samples exposed to both delignification procedures (10 mg) were mixed separately in triplicate with 0.40 ml Tris (hydroxymethyl aminomethan, 0.005 M) buffer solution, at pH 4.5. The enzymatic hydrolysis was carried out with 100 µl of a *T. viride* cellulase solution (10.0 $mg.ml^{-1}$) at an incubation temperature of 40°C for a period of 2 h (Van Wyk, 2001). The amount of total reducing sugars released during cellulase action was determined by the dinitrosalicyclic acid (DNS) method at 546 nm using glucose as a standard (Miller, 1959). The amount of reducing sugars released from all the wood waste materials during the two delignification procedures, prior to cellulase incubation were determined. When determining the amount of sugar released during the delignification procedures, no cellulase enzyme was added to the cellulose samples, instead the volume of enzyme (100 ul) was replaced with the buffer solution.

RESULTS AND DISCUSSION

Pollution and solid waste production are topical issues as many institutions lack procedures to effectively manage these variables ensuring a healthy environment. In Nigeria, sawdust is a major component of solid waste which is currently identified as a resource for potential bioenergy development. The microbial degradation of sawdust cellulose in terms of change in mass has been described whilst the current investigation reflects the relative saccharification of sawdust from 20 different trees. Different pretreatment methods such as Kraft pulping and oxidative delignification with hydrogen peroxide have been described in order to render cellulose more susceptible for cellulase catalyzed bioconversion into fermentable sugars (Kozlowski et al., 2006) as well as to remove carbohydrates from the celluloses (Sixta and Schild, 2009). During hydrolysis, the B-1,4-glucosidic bonds in cellulose are destroyed and reducing sugars such as glucose are released from the complex cellulose

Table 1. Sugars released from lignocellulosic sawdust materials after Kraft and hydrogen peroxide delignification procedures and prior to these waste cellulose materials saccharified with *T. viride* cellulases.

Names of Lignocellulose wood waste (sawdust)	Concentration of sugar (mg.ml^{-1}) released after Kraft pulping	Concentration of glucose (mg.ml^{-1}) released after oxidative delignification cellulose of Kraft pulped celluloses	Increase (%) in sugar concentration after both delignification procedures relative to the Kraft treatment only
E. suaveolens	0.55	3.00	44
S. globulifera	0.85	1.17	37
R. heudelotii	0.69	1.04	51
P. macrocarpa	0.37	1.10	196
M. excelsa	0.36	0.72	98
I. asarifolia	0.79	1.18	50
H. ciliata	0.27	0.58	112
S. gabonensis	0.71	0.83	16
P. angolensis	0.61	0.64	3
T. superba	0.41	1.68	301
A. germinans	0.39	0.61	56
T. scleroxylon	0.67	1.17	73
U. guineensis	0.43	0.48	11
N. diderrichii	0.34	1.77	422
M. altissima	0.28	0.55	94
E. gigas	0.42	3.17	654
C. pentadra	0.54	0.79	47
K. ivorensis	0.35	1.06	196
L. alata	0.61	2.45	297
S. pustulatas	0.29	0.97	234

structure. The degradation of cellulose by means of Kraft pulping (Suckling et al., 2001) and bleaching with hydrogen peroxide (Zeronian and Inglesby, 1995) have been described with the extent of cellulose degradation expressed in terms of change in viscometry or polymerization. The relative sugar concentrations obtained during the two delignification procedures of the different sawdust celluloses are summarizes in Table 1.

After Kraft-pretreatment, the highest amount of glucose (0.86 mg.ml^{-1}) was released from *S. globulifera*. The other wood types rich in glucose was found to be *I. asarifolia* and *S. gabonensis* with 0.79 and 0.71 mg.ml^{-1} released glucose, respectively.The wood species having lowest amount of glucose were found to be *H. ciliate, M. altissima* and *S. pustulata,* with released glucose concentrations of 0.27 mg.ml^{-1}, 0.28 mg.ml^{-1} and 0.29 mg.ml^{-1}, respectively.

Hydrogen peroxide pretreatment performed after Kraft pulping increased the amount of sugars released from wood samples. *E. suaveolens* and *E. giga* species produced the highest concentrations at values of 3.00 and 3.17 mg.ml^{-1}, respectively. Percent increase was calculated to be 441% for *E. suaveolens* and 654% for *E. giga* relative to the amount of sugar released after Kraft pulping. The successive peroxide delignification of the Kraft pretreated L. alatawood biomass materials also resulted in high sugar concentrations of 2.45 mg.ml^{-1} corresponding to 297% increase in sugar release to the Kraft pulping of this material. The lowest concentration of sugars released from wood materials during peroxide treatment and after Kraft pulping was calculated for *U. guineensis* at a concentration of 0.48 mg.ml^{-1} that was 11% higher than the concentration obtained during the Kraft pretreatment of this cellulose.

T. viride cellulase catalysed bioconversion of the Kraft pulped sawdust (Table 2) resulted in hydrolysis of the remained cellulose in the pretreated wood materials into glucose. After enzymatic hydrolysis, highest amount of glucose was obtained from *L. alata* and *C. pentadra* with concentrations of 9.22 and 9.08 mg.ml^{-1}, respectively. These concentrations were 319 and 317% higher than the lowest amount of glucose released by *M. excels* (2.20 mg.ml^{-1}). *P. angolensis, U. guineensis* and *N. diderrichii* wood species were also hydrolyzed efficiently by the cellulase enzyme, producing 7.87, 7.71 and 7.01 mg.ml^{-1}, glucose, respectively.

To increase the susceptibility for cellulase catalysed bioconversion, the Kraft pulped lignocelluloses materials were successively subjected to hydrogen peroxide that resulted in increased lignin removal. After hydrogen peroxide delignification, all cellulose materials were efficiently saccharified by the enzyme action confirming

Table 2. Sugar concentration of delignified waste cellulose after saccharification with *T. viride* cellulase.

Species of Lignocellulose wood waste (sawdust)	Sugar concentration ($mg.ml^{-1}$) after *T.viride* cellulase saccharification of Kraft delignified waste cellulose	Sugar concentration ($mg.ml^{-1}$) after *T. viride* cellulase saccharification of Kraft and peroxide delignified cellulose	Increase (%) in sugar concentration after both delignification procedures relative to saccharification of Kraft pulped materials only
E. suaveolens	5.19	14.28	175
S. globulifera	4.74	7.31	54
R. heudelotii	4.51	9.75	116
P. macrocarpa	5.35	8.23	53
M. excelsa	2.20	6.06	175
I. asarifolia	6.27	9.02	43
H. ciliata	5.76	8.41	45
S. gabonensis	6.84	8.33	21
P. angolensis	7.87	9.85	25
T. superba	6.56	12.83	98
A. germinans	3.95	7.20	82
T. scleroxylon	6.42	9.65	50
U. guineensis	7.72	11.11	43
N. diderrichii	7.01	8.67	23
M. altissima	5.40	11.06	105
E. gigas	5.45	12.36	127
C. pentadra	9.08	10.47	15
K. ivorensis	5.76	9.31	61
L. alata	9.23	13.34	44
S. pustulatas	5.59	9.87	76

that the number of cellulose fibers exposed to enzyme attack increased after the peroxide treatment. The successive hydrogen peroxide delignification process resulted in *E. suaveolens* and *L. alata* again releasing the highest sugar concentration (14.28 and 13.34 $mg.ml^{-1}$, respectively) at 135 and 120% more than the lowest amount released by *Milicia excelsa* at a concentration of 6.06 $mg.ml^{-1}$. The third highest sugar producing celluloses after hydrogen peroxide pre-treatment was calculated for *T. superba* and *E. gigas* woods with glucose concentrations of 12.83 and12.36 $mg.ml^{-1}$, respectively. Similar sugar concentrations were calculated for *S. globulifera* and *A. germinans* at values of 7.31 and 7.20 $mg.ml^{-1}$, respectively, indicating a resemblance in their susceptibility for cellulase catalyzed degradation. A large majority of the sawdust materials exposed to both delignification procedures showed a resemblance in their sugar releasing profile during biodegradation with *T. viride* cellulase as an average glucose concentration of 9.04 $mg.ml^{-1}$ was obtained. The successive pre-treatment of Kraft pulped sawdust with hydrogen peroxide resulted in cellulose from all the trees to be increasingly biodegraded. The highest increase in saccharification after both delignification procedures compared to the biodegradation of the Kraft pulped materials only was observed with *E. suaveolens* and *M. excelsa* (174 and 175%, respectively) followed by *R. heudelotii* (115%) and *E. gigas* (127%). The lowest increase in saccharification was observed with *C. pentadra* (15%) followed by *S. gabonensis* (21%) and *N. diderrichii* (23%).

The average concentration of sugars released from the various sawdust samples during Kraft pulping was equal to 0.48 $mg.ml^{-1}$ with concentrations varying between values of 0.27 and 0.79 $mg.ml^{-1}$. The average sugar concentration increased to 1.25 $mg.ml^{-1}$ after hydrogen peroxide delignification of Kraft pulped lignocellulosic materials. As expected, enzymatic hydrolysis increased the amount of released sugar. The cellulase catalyzed bioconversion of Kraft-pulped materials produced, resulted in an average sugar concentration of 5.90 $mg.ml^{-1}$ which was 12 times higher than the average sugar released after the Kraft delignification procedure. After exposure to both Kraft pulping and hydrogen peroxide, *T. viride* catalyzed bioconversion of these waste lignocelluloses released sugars at an average concentration of 9.70 $mg.ml^{-1}$ that was 20 times higher than the average concentration of sugar released during the Kraft delignification; eight times higher than the amount of sugars released during peroxide delignification of Kraft pulped materials and 1.6 times more than sugars released when the Kraft delignified celluloses were saccharified with the cellulases.

This investigation on the saccharification of sawdust is

topical as sawdust is described as a suitable bio-source for the production of glucose (Chin et al., 2011) and xylitol (Rafiqui and Sakinah, 2102) as well as a lignocellulosic biomass with a potential for bioethanol production (Limayem and Ricke, 2012).

Conclusion

The cellulase catalyzed bioconversion of cellulose into fermentable sugars would become the focus in the future as the demand for bioenergy and strategies to limit environmental pollution increases. Sawdust is a major concern at the Lagos Lagoon and this investigation concluded that the bioenergy potential of waste cellulose is currently a major pollutant. The complexity of lignocellulose structure is illustrated by the different amount of sugars released after different delignification processes for the same material. Information obtained from this study would be useful for future research on bioenergy production from waste lignocellulose accumulated in the Lagos area of Nigeria.

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