Ethanol Production from Hydrothermally-Treated Biomass from West Africa

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Despite the abundance of diverse biomass resources in Africa, they have received little research and development focus. This study presents compositional analysis, sugar, and ethanol yields of hydrothermal pretreated (195 °C, 10 min) biomass from West Africa, including bamboo wood, rubber wood, elephant grass, Siam weed, and coconut husk, benchmarked against those of wheat straw. The elephant grass exhibited the highest glucose and ethanol yields at 57.8% and 65.1% of the theoretical maximum, respectively. The results show that the glucose yield of pretreated elephant grass was 3.5 times that of the untreated material, while the ethanol yield was nearly 2 times higher. Moreover, the sugar released by the elephant grass (30.8 g/100 g TS) was only slightly lower than by the wheat straw (33.1 g/100 g TS), while the ethanol yield (16.1 g/100 g TS) was higher than that of the straw (15.26 g/100 g TS). All other local biomass types studied exhibited sugar and ethanol yields below 33% and 35% of the theoretical maximum, respectively. Thus, elephant grass is a highly promising biomass source for ethanol production in Africa.

Keywords: Ethanol; Hydrothermal pretreatment; Elephant grass; Bamboo; Rubber; Siam weed; Coconut husk

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INTRODUCTION

Globally, several potential biomass-comprising agricultural residues (e.g., wheat straw, maize stover, sugarcane bagasse, and rice straw), grasses (e.g., switchgrass, Miscanthus, and reed), and wood (e.g., pine, spruce, eucalyptus, aspen, poplar, and birch) have received considerable attention as potential substrates for bioethanol production (Limayem and Ricke 2012; Bensah and Mensah 2013; Kang et al. 2014). Most biomass sources widely investigated are those that are native to or grown in the developed and emerging countries where almost all research and development has been undertaken. Comparatively, biomass in Africa has not received significant research attention and thus remains largely unexplored (Bensah et al. 2015). This may be attributed to the general lack of support of research institutions and the absence of partnerships with leading research centres and companies in this emerging area of study. There is, however, a gradual shift occurring in favour of advanced biofuels on the African continent, underlined by recognition of the potential lignocellulosic resources for the sustainable production of biofuels and biochemicals in the near future. Recently, assessment of the ethanol potential from various biomass residues in Ghana and Africa was undertaken by Kemausuor et al.
(2014) and Bensah et al. (2015), respectively. Thomsen et al. (2014) reported on the structural compositional analysis of 14 biomass types from Ghana and estimated their theoretical methane and ethanol potentials. Further experimental studies on ethanol yields have been performed, and materials such as plantain peelings, plantain trunks, maize cobs, and maize stalks have been identified as particularly promising for bioethanol production (Thomsen et al. 2015). Comprehensive reviews on favourable biomass pretreatment methods for ethanol production in Africa have also been undertaken (Bensah and Mensah 2013; Bensah et al 2015).

This work considers other biomass sources that are also abundant in West Africa and are therefore available in large quantities for a future ethanol industry. It investigates the hydrolysis and ethanol yields of elephant grass (*Pennisetum purpureum*), Siam weed (*Chromolaena odorata*), green bamboo (*Bambusa vulgaris*), rubber (*Hevea brasiliensis*) wood, and coconut husk (*Cocos nucifera* L.) and compares them with those of wheat straw, a highly studied material used for ethanol production via hydrothermal pretreatment (HTT). HTT was selected since it has been successfully applied to numerous materials, both agricultural residues and wood waste, and is the basis for many demonstration plants the world over (Tomás-Pejó et al. 2011).

African elephant grass (*Pennisetum purpureum*) is among the potential grasses that can be sustainably cultivated for a future biofuel industry, even though current uses are focused on hay and pasture for livestock (Duku et al. 2011). The grass grows prolifically in both forest and savannah and is usually found in dense bushes together with other, equally promising biomass such as Guinea grass and Siam weed. *Miscanthus*, a related grass, is among the most investigated feedstocks (Chou 2009; Heaton et al. 2010; Han et al. 2011). By contrast, elephant grass has received less attention as a feedstock for bioethanol (Gutiérrez et al. 2012; Cardona et al. 2014).

Siam weed is an invasive weed of agriculture and forestry in many countries of Asia, Africa, America, and Australia (McFadyen and Skarratt 1996). It is a perennial shrub that grows in thick bushes, about 1.5 to 2 m in height, with large quantities of wind-dispersed seeds (Ping et al. 2011). This invasive weed has spread to Central and East Africa since its introduction to West Africa in the 1930s (McFadyen and Skarratt 1996). Its introduction in Ghana in 1969 also led to its invasion of new territories and environments, spreading to about two-thirds of the surface area of the country (Uyi et al. 2009). Several biological control mechanisms, such as the use of the leaf-feeding moth, *Pareuchaetes pseudoinsulata*, have resulted in little success in controlling the weed (Timbilla and Braimah 2000), and thus, other sustainable weed management practices are needed. Elsewhere in Nigeria (Ayeni et al. 2014) and China (Zhao et al. 2010), Siam weed has been explored as a sustainable feedstock for bioethanol due to its high cellulose content.

The use of bamboo in Africa is not extensive and only few applications (soil stabilization, pipes for transporting water, construction industry, etc.) are known (Scurlock et al. 2000). In Ghana, bamboo stems are mainly used as props to temporarily support weights in the construction of buildings. Some minor uses are in local industries such as weaving, basketry, furniture, flooring, and ceilings (Obiri and Oteng-Amoako 2007). The use of bamboo as a feedstock for bioethanol has been investigated elsewhere (Leenakul and Tippayawong 2010; Li et al. 2012; Ma et al. 2013), but those growing in West Africa have not received attention, to the knowledge of the authors.

The rubber tree is a major plantation crop cultivated in tropical Africa, Asia, and South America (Nair 2010a). In Africa, plantations are found mainly in the West and Central African regions, with major rubber producing countries including Ghana, Ivory
Coast, Guinea, Nigeria, Liberia, Cameroun, and Gabon. In the Western Region of Ghana where large plantations are located, old rubber trees are processed into chips and pellets for export to Denmark by companies such as the Takoradi Renewable Energy Limited. Research to convert rubber wood to bioethanol has been conducted in Asia (Alhassan and Kuang 2010), but the same cannot be said about rubber wood cultivated in Africa, since no literature has been found.

The coconut palm (Cocos nucifera L.) is a highly resourceful tree to over 80 tropical countries due to the usefulness of its many products: fruits, leaves, husks, shells, trunk/stem, and palm sap (Nair 2010b; Hemstock 2013). The tree is cultivated in lowlands near the sea in many countries in Africa, though it is produced extensively in Mozambique, Tanzania, and Ghana (Agyemang-Yeboah 2011). The meat (white lining inside the shell) and water are mostly taken raw, and the coir (dried meat) is used to produce oil for cooking and medicinal purposes. The husk and shell are usually discarded as waste.

EXPERIMENTAL

Materials

Mature rubber wood (H. brasiliensis) was collected from the Ghana Rubber Estates Limited in Takoradi, Ghana. Green bamboo wood (B. vulgaris) was obtained by cutting fully-grown bamboo culms at the Kumasi Institute for Tropical Agriculture in the Ejisu-Juaben district of Ghana. Mature elephant grass (P. purpureum), Guinea grass (P. maximum), and Siam weed (C. odorata) were harvested from open fields adjacent to the Department of Animal Science of the Kwame Nkrumah University of Science and Technology (KNUST). Coconut husks were obtained from a vendor in Kumasi. All materials were obtained in August 2013. The biomass were washed to remove dirt, chopped into pieces (2 to 6 cm), and air-dried for a minimum of 5 d. Dried biomass was cut-milled (SM 2000, Retsch) to pass through a 2-mm screen. Milled materials were used for further studies as described below.

Biomass Composition

The structural composition of the raw biomass was analysed for cellulose, hemicellulose (xylan and arabinan), lignin, and ash using the laboratory analytical procedures of the National Renewable Energy Laboratory (NREL) with minor modifications (NREL 2011). Lipophilic extractives were removed from the milled biomass (> 9 to 16 g) with ethanol (96% v/v, 300 mL) for 6 h by Soxhlet extraction. The mass of extractives, including volatiles, was expressed as the amount of material lost through extraction. The lipophilic-extracted residues were dried to constant weight at 60 °C and stored in a desiccator. The carbohydrate fraction was determined by first hydrolysing 0.1600 to 0.1699 g samples using H2SO4 (72% w/w, 1.5 mL) at 30 °C for 60 min in a thermostatic water bath with intermittent vortex agitation. The reaction was immediately quenched in an ice bath and diluted to 4% w/w. The samples were autoclaved at 121 °C for 60 min and filtered to separate the solids, which were dried to constant weight at 105 °C and subsequently ashed at 550 °C for 180 min. The Klason lignin fraction was determined as the difference in weight between the dried and ashed solids. The filtrate was collected and quantified for sugars and acetic acid by high-performance liquid chromatography (HPLC) using a BioRad Aminex HPX87H column (USA) at 63 °C, with eluent (4-mM H2SO4) flow of 0.6 mL/min, and
detected by a refractive index detector. The recovery of D-glucose, D-xylose, and L-arabinose was determined through standard addition of sugars to samples before autoclavage.

Hydrothermal Pretreatment (HTT)
Uncatalysed HTT was performed on each biomass type at 195 °C for 10 min at solids loading of 60 g dry matter (DM)/L of water in a 2-L loop reactor, as previously described by Bjerre and Schmidt (1997). The heating time of the reactor was approximately 60 to 90 s, and after pretreatment, the reactor was quickly immersed in a coolant to reduce the temperature to below 70 °C.

Pretreated solids were separated from the liquid in a Buchner funnel with a nylon cloth (1-μm) under vacuum. The solids were washed with water at least three times, dried at 60 °C for a minimum of 48 h, and compositionally analysed as described above. The liquid component was studied as described below.

Weak Acid Hydrolysis of Hydrolysates
The liquid fraction of the pretreated biomass, known to contain high fractions of polysaccharides and oligomers (Thomsen et al. 2006), was hydrolysed by adding 10 mL of 8% w/v H₂SO₄ to 10 mL of filtrate in a 25-mL Pyrex test tube. Four samples were prepared for each biomass (filtrate). 200 μL of ultrapure water was added to two samples, while 200 μL of sugar standards (D-glucose, D-xylose, and L-arabinose) was added to the remaining samples for each biomass (filtrate).

The samples were agitated in a vortex shaker, autoclaved at 121 °C for 10 min, and centrifuged at 4000 rpm for 5 min. H₂SO₄ (8% w/v) was added to the supernatants until the pH fell to 2-3, after which the solution was filtered for HPLC quantification, as described in the biomass compassion analysis.

Enzymatic Hydrolysis
Enzymatic hydrolysis was performed in triplicate on both raw and pretreated biomass at 5% total solids (TS). About 1.25 g DM of biomass was measured into a 50-mL Falcon tube, and the pH was adjusted to 5 using 50-mM sodium acetate buffer. The samples were treated with cellulase (Novozyme Cellic CTec2) at a loading of 11.14 FPU/g DM and supplemented with xylanase (Novozyme Cellic HTec2) at a ratio of 9:1 based on protein loading for all assays. Enzyme blank solutions (control), in triplicate, and substrate-blank (without enzymes) solution for each sample were prepared to enable the accurate determination of sugars produced as a result of enzyme addition. Sodium azide solution (2%, 0.25 mL) was added to each sample to prevent microbial contamination.

The tubes were mounted horizontally in a shaker incubator and hydrolysed at 150 rpm and 50 °C for 72 h. After hydrolysis, the Falcon tubes were centrifuged for 10 min at 4000 rpm, and the supernatants were diluted with 0.08-M H₂SO₄ in Eppendorf tubes until the pH fell to the range 2 to 3. They were centrifuged again for 10 min at 10000 rpm, and the supernatants were finally filtered into vials for HPLC analysis as described above. The glucose and pentose sugar yields are expressed as g/100 g of material used for enzymatic hydrolysis. The percent theoretical yields for glucose (% th₇) and pentose sugars (% th₅) were calculated as (Eq. 1 and 2),
\[
\% \text{th}_G = \frac{Y_G}{C} \times 0.90 \times 100 \tag{1}
\]
\[
\% \text{th}_P = \frac{Y_P}{P} \times 0.88 \times 100 \tag{2}
\]

where \(Y_G\) and \(Y_P\) are glucose and pentose sugar (xylose and arabinose) yields in g/100 g DM, respectively; \(C\) and \(P\) refer to the quantity of glucose and pentose sugar in 100 g DM of biomass, respectively; and the hydration factor is 0.90 for glucose and 0.88 for xylose and arabinose (Kim et al. 2009).

**Inoculum Preparation**

A preculture media containing 50 g/L glucose, 2.5 g/L \((\text{NH}_4)_2\text{SO}_4\), 2 g/L yeast extract, 1 g/L KH\(_2\)PO\(_4\), and 0.3 g/L MgSO\(_4\) was prepared. The sterilised media was inoculated with dry yeast (\textit{Saccharomyces cerevisiae}, “Ethanol Red,” Fermentis) and incubated at 35 °C and 90 rpm for 24 h. The yeast was washed with water and centrifuged at 4000 rpm for 10 min and the supernatant was discarded. This process was repeated two more times, after which the mass and total solids (using Mettler Toledo HR83, USA) were determined.

**Simultaneous Saccharification and Fermentation (SSF)**

Liquefaction (prehydrolysis) of the biomass was carried out prior to SSF. SSF was performed using 10% DM of solid fraction in 50-mM sodium acetate buffer (pH 5) using the same cellulase and xylanase loadings as in the case of enzymatic hydrolysis. A blank sample (without biomass) was also prepared in triplicate.

The samples were prehydrolysed at 50 °C for 6 h in a shaker incubator at 150 rpm. The prepared yeast solution was added to each sample at 20 mg yeast/g DM at temperatures below 32 °C and the flasks (fitted with glycerol-filled yeast locks) were weighed before incubating for 6 d at 35 °C to monitor CO\(_2\) loss. After 144 h, 1 mL of each sample was mixed with 9 mL of 0.01-M H\(_2\)SO\(_4\), centrifuged at 4000 rpm for 5 min, and quantified for ethanol, glucose, xylose, xylitol, arabinose, lactic acid, formic acid, acetic acid, and propionic acid using HPLC (under the same conditions described above, with appropriate standards).

**RESULTS AND DISCUSSION**

**Biomass Characterization**

The compositional analysis of the studied biomass is shown in Table 1. The glucan was highest for bamboo (49.8%) and lowest for coconut husk (21.3%). The glucan content (49.8%) of the bamboo wood was higher than those (41.3 and 40.7%) obtained by Li et al. (2012) and Leenakul and Tippayawong (2010), respectively, while the xylan content was lower, by at least 22.9%. With the exception of coconut husk, which had a hemicellulose (xylose and arabinose) content of 13.3%, all other biomass types had hemicellulose content over 15%. Wheat straw had the highest pentose fraction, 23.9%. Moreover, the coconut husk was observed to have the highest lignin and extractives content, and the cellulose content (21.3%) was comparable to those (18.19 to 21.26%) obtained by Ding et al. (2012) but considerably lower than the value (32%) observed by van Dam et al. (2006). These differences were expected, since the components of biomass vary with species, location,
and time/season of harvest, among others (Bals et al. 2010). The lignin content was very low for elephant grass, at 12.4%, while the ash content was the highest, at 7.9%.

The extent of carbohydrate solubilisation is dependent on the severity factor (Overend and Chornet 1987), which was determined as 3.80 (log $R_0 = 10 \text{ min} \times \exp [(195-100) \, ^\circ\text{C}/14.75]$) for this pretreatment. As shown in Table 1, the glucan fractions in the hydrothermally pretreated biomass were higher than those of the raw biomass due to the partial removal of hemicelluloses and extractives.

Table 1. Chemical Composition of Raw and Pretreated (Solid Fraction) Biomass

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Composition (g/100 g DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucan</td>
</tr>
<tr>
<td>BW Raw</td>
<td>49.8 (0.29)</td>
</tr>
<tr>
<td>Pret</td>
<td>49.4 (0.79)</td>
</tr>
<tr>
<td>RW Raw</td>
<td>43.5 (2.38)</td>
</tr>
<tr>
<td>Pret</td>
<td>56.2 (0.50)</td>
</tr>
<tr>
<td>SW Raw</td>
<td>36.2 (0.53)</td>
</tr>
<tr>
<td>Pret</td>
<td>47.8 (1.03)</td>
</tr>
<tr>
<td>EG Raw</td>
<td>32.0 (0.45)</td>
</tr>
<tr>
<td>Pret</td>
<td>48.4 (0.24)</td>
</tr>
<tr>
<td>CH Raw</td>
<td>21.3 (0.94)</td>
</tr>
<tr>
<td>Pret</td>
<td>38.6 (1.37)</td>
</tr>
<tr>
<td>WS Raw</td>
<td>35.6 (0)</td>
</tr>
<tr>
<td>Pret</td>
<td>57.1 (1.22)</td>
</tr>
</tbody>
</table>

Standard deviations are stated in parenthesis, when applicable. BW: bamboo wood, RW: Rubber wood, SW: Siam weed, EG: elephant grass, CH: coconut husk, WS: wheat straw
Raw: raw biomass, Pret: pretreated biomass. ND: not determined

With the exception of bamboo, the rest exhibited clear increases, and the two biggest margins of increase of 17.3 and 21.5% were observed for coconut husk and wheat straw, respectively. The effect of hydrothermal treatment on biomass composition was minimum for bamboo, suggesting that uncatalysed HTT was ineffective on the woody
grass. All biomass types experienced a decrease in hemicellulose fraction, showing the ease with which pentoses were removed. The percentage removal of pentoses was greatest for coconut husk (51.9%) and least for elephant grass (16.5%).

Degradation products such as formic, acetic, and propionic acids generated in the hydrolysates of the pretreated materials were quantified, as shown in Table 2. Formic and acetic acids were detected in all samples, while propionic acid was detected only in WS hydrolysate, at a low value of 0.02 g/g of raw material. From Table 2, the percentage of dry biomass that degraded into carboxylic acids ranged from 0.43 to 0.95% for coconut husk and Siam weed, respectively. Degradation of wheat straw into organic acids was also low, but was higher than the total organic acids content (<0.23% w/w) obtained by Ambye-Jensen et al. (2013) for the HTT of wheat straw at severities of 3.06 to 3.65. According to Palmqvist et al. (1999), inhibition of yeast by carboxylic acids is significant if the concentration is above 10 g/L. The total carboxylic acids concentration remained below that critical level in this study. The generation of sugar degradation compounds such as furfural and hydromethylfurfural was not studied. However, the low concentration (≤0.21 g/L) of formic acid could indicate that only negligible degradation of furfural and HMF occurred in all samples (Qi and Xiuyang 2007; Xiao et al. 2013).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total organic acids in (w/w) % of raw material (DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bamboo wood</td>
</tr>
<tr>
<td>Formic acid</td>
<td>0.07 (0.1)</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.48 (0.3)</td>
</tr>
</tbody>
</table>

Standard deviations are stated in parenthesis. Propionic acid was detected only in WS hydrolysate at 0.02 g/g of raw biomass.

**Enzymatic Hydrolysis**

The evaluation of HTT was performed by enzymatic hydrolysis at a solids loading of 5% w/w. In increasing order, the glucose released from the pretreated materials was as follows: bamboo, coconut husk, Siam weed, rubber wood, and elephant grass (Table 3). The glucose yield (30.8 g/g TS) observed for pretreated elephant grass was the highest among the local materials analysed, but lower than that of wheat straw (benchmark) by 2.3 percentage points (Table 3). Nonetheless, the theoretical glucose yield (57.8%) of elephant grass was higher than wheat straw because of its lower glucan fraction in the pretreated biomass (Table 1). In a related work on Napier grass (*P. purpureum* Schumach), uncatalysed HTT (180-250 °C, 8 to 30 min) was ineffective at releasing glucose, but the use of a two-step process involving the application of concentrated H₃PO₄ (85 wt.%, 60 °C, 60 min) and H₃PO₄-catalysed HTT (3 wt.%, 200 °C, 8 min) produced a high glucose yield of 50 g/g glucan in the grass (Takata et al. 2013).

The glucose released from raw rubber was only three-fifth of the level observed from the pretreated rubber, which was second to elephant grass. However, at a yield of 36.0 g glucose/100 g cellulose in pretreated rubber, the glucose released from HTT-pretreated rubber was at least 1.5 times lower than in the results of Alhassan and Kuang (2010), who achieved conversion of 52.5 to 95.1 g/100 g cellulose in pretreated materials after successive mild acid and alkali pretreatment of rubber under temperatures and reaction times not exceeding 100 °C and 60 min, respectively.
The glucose yield of pretreated Siam weed was nearly twice as low as that of pretreated rubber and three times lower than that of pretreated elephant grass, but the convertibility of the pretreated weed was approximately four times that of the native biomass, suggesting the possibility to increase yields further by varying the pretreatment conditions. It was observed that the glucan content of raw Siam weed (36.2%) was higher than that of raw elephant grass (32.0%), but the effect of the pretreatment in terms of sugar yields (Table 3) was less observable on the weed. The high lignin content (34.1%) of the pretreated solids could be responsible for the lower glucose yield as compared to that of wheat straw. This is corroborated by Zhao et al. (2010), who also observed low sugar yields due to low lignin removal by mild acid and alkali applications on the Siam weed stem. They concluded that lignin removal was the main factor important in realizing high sugar yields.

**Table 3. Sugar Conversion after Enzymatic Hydrolysis of Untreated and Pretreated Biomass**

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Glucose released (g/100 g TS)</th>
<th>Pentose sugars released (g/100 g TS)</th>
<th>% theoretical maximum Glucose</th>
<th>% theoretical maximum Pentose sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Untreated material</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bamboo wood</td>
<td>2.8 (0.7)</td>
<td>0.5 (0.1)</td>
<td>5.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Coconut husk</td>
<td>0 (0.1)</td>
<td>0.0 (0.0)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Siam weed</td>
<td>2.1 (0.4)</td>
<td>0.7 (0.1)</td>
<td>5.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Rubber wood</td>
<td>11.8 (1.2)</td>
<td>0.4 (0.0)</td>
<td>24.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Elephant grass</td>
<td>8.7 (1.2)</td>
<td>2.4 (0.2)</td>
<td>24.8</td>
<td>10.7</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>6.1 (0.1)</td>
<td>2.5 (0.0)</td>
<td>15.7</td>
<td>9.5</td>
</tr>
<tr>
<td><strong>Pretreated material</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bamboo wood</td>
<td>3.5 (0.2)</td>
<td>1.6 (0.0)</td>
<td>6.4</td>
<td>11.7</td>
</tr>
<tr>
<td>Coconut husk</td>
<td>10.1 (3.2)</td>
<td>1.1 (0.1)</td>
<td>23.9</td>
<td>15.8</td>
</tr>
<tr>
<td>Siam weed</td>
<td>11.1 (1.5)</td>
<td>3.0 (0.2)</td>
<td>21.1</td>
<td>32.7</td>
</tr>
<tr>
<td>Rubber wood</td>
<td>20.2 (0.3)</td>
<td>4.7 (0.1)</td>
<td>32.7</td>
<td>44.6</td>
</tr>
<tr>
<td>Elephant grass</td>
<td>30.8 (2.0)</td>
<td>8.4 (0.2)</td>
<td>57.8</td>
<td>44.4</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>33.1 (2.3)</td>
<td>9.8 (0.1)</td>
<td>52.7</td>
<td>55.4</td>
</tr>
</tbody>
</table>

Released glucose or pentose sugars (xylose and arabinose) given as g/100 g of DM in solid fraction after enzymatic hydrolysis. Standard deviations are shown in parentheses.

The enzymatic hydrolysis of pretreated coconut husk gave an average sugar yield of 11.11 g/100 g DM at 5% w/v, which is considerably lower than the maximum value (27.9 g/100 g DM) obtained by Ding et al. (2012) using microwave-assisted alkali pretreatment at 1% (w/v) on husk with similar cellulose fraction. Unlike HTT, the alkali process reduced and degraded the lignin fraction in the solids, exposing the cellulose and hemicellulose to hydrolytic enzymes, which may have caused the higher sugar release during enzymatic hydrolysis (Ding et al. 2012).

On bamboo, both the native and the pretreated biomass were insignificantly hydrolysed. This may be partly attributed to the high density and hardness of bamboo (Li et al. 2012), and to the fact that bamboo is mainly of \( p \)-hydroxyphenyl (H), vanillin (G), and syringaldehyde (S) type lignin (Wen et al. 2010; Bai et al. 2013; Li et al. 2013). Similarly, low yields (≤8.5 g glucose and xylose/100 g of raw material) were observed...
following the dilute acid pretreatment of bamboo (Leenakul and Tippayawong 2010). In contrast, Li et al. (2012) obtained high cellulose-to-glucose conversions of 77.1 g glucose/100g cellulose (~27 g glucose/100 g dry pretreated material) based on acid-catalysed ethanosolv pretreatment due to high lignin removal.

**Ethanol Fermentation**

The effect of HTT (195 °C, 10 min, log $R_0 = 3.80$) on the SSF yields of five West African biomass types, alongside wheat straw, was studied. In terms of the ethanol yields (g eth/100 g DM), the pretreatment was beneficial for elephant grass, Siam weed, and coconut husk but was ineffective on rubber and bamboo (Fig. 1). Moreover, only the ethanol yield obtained from pretreated elephant grass (16.1 g/100 g DM) was higher than that of wheat straw (15.3 g/100 g DM), which was used as a benchmark. It also had the highest theoretical ethanol yield (65.1%) and concentration (15.9 g/L) of all the biomass types studied. Both the ethanol yield and production of the pretreated elephant grass was 1.8 times that of the untreated biomass. However, a recent work by Eliana et al. (2014) on elephant grass, using NaOH pretreatment, achieved 95% of the theoretical ethanol yield and a concentration of 26 g/L.

![Fig. 1. Ethanol yield (bar chart) and final ethanol concentration from raw (straight line) and pretreated biomass (broken line). Error bars show standard deviations from mean values.](image)

Except for elephant grass and pretreated wheat straw, the theoretical yields were low (<35%). For bamboo, the lower yield for pretreated relative to raw biomass suggests the inability of hydrothermal treatment at 195 °C and 10 min to open the structure for the release of sugars for fermentation. Apart from ethanol, byproducts such as xylitol, lactic acid, and acetic acid were also produced in low concentrations. Propionic acid was detected in very low levels, at a maximum of 0.067 g/100 g DM in raw elephant grass, while formic
acid was detected only in raw wheat straw at 0.2 g/100 g DM. Lactic acid and acetic acid concentrations reached maximum values of 0.29 and 0.09% w/v for raw elephant grass and pretreated rubber, respectively; the levels were generally too low to inhibit yeast at the reaction conditions (Narendranath et al. 2001; Graves et al. 2006).

**Further Studies on Elephant Grass**

Due to the relatively high sugar and ethanol potential shown by the elephant grass, further hydrothermal investigations at lower severities were performed. The grass was pretreated at the same solids loading of 6 wt.% in 1-L Duran glass bottles by autoclaving at 121 and 145 °C for 15, 30, and 60 min. The pretreated solids and filtrate were compositionally analysed, after which the solids were submitted to enzymatic hydrolysis and SSF as described in previous sections. The composition of the solid fraction of the pretreated elephant grass is given in Table 4, and the results of enzymatic hydrolysis and fermentation are shown in Table 5.

In general, the autoclave pretreatment increased the hemicellulose content, in contrast to a decrease for the HTT (Table 4), ostensibly as a result of the absence of autohydrolysis of hemicellulose into oligomers, monomers, and other products at low temperatures (<150 °C). The glucan and lignin fractions of both autoclaved and HTT-treated material were higher than those of the native material; however, the values recorded by HTT (glucan, 48.4%; lignin, 24.3%) were higher than the maximum (glucan, 42.1%; lignin, 23.2%) obtained from the autoclave pretreatment (Table 4).

**Table 4. Chemical Composition of Pretreated Elephant Grass (Solid Fraction) Based on Hydrothermal (Autoclave) Pretreatment**

<table>
<thead>
<tr>
<th>Pretreatment conditions</th>
<th>Composition (g/100 g DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucan</td>
</tr>
<tr>
<td>121 °C, 15 min</td>
<td>38.0 (2.39)</td>
</tr>
<tr>
<td>121 °C, 30 min</td>
<td>38.2 (1.77)</td>
</tr>
<tr>
<td>121 °C, 60 min</td>
<td>39.8 (0.08)</td>
</tr>
<tr>
<td>145 °C, 15 min</td>
<td>40.6 (0.47)</td>
</tr>
<tr>
<td>145 °C, 30 min</td>
<td>42.1 (0.10)</td>
</tr>
<tr>
<td>145 °C, 60 min</td>
<td>39.4 (0.41)</td>
</tr>
<tr>
<td>HTT, 195 °C, 10 min</td>
<td>48.4 (0.24)</td>
</tr>
<tr>
<td>Untreated material</td>
<td>32.0 (0.45)</td>
</tr>
</tbody>
</table>

Standard deviations are stated in parenthesis when applicable. Analogous results from the hydrothermal pretreatment (HTT) and the native material are shown in the last two rows.

The maximum glucan and pentosan conversion to monomers after enzymatic hydrolysis, 38.98 and 21.41%, respectively (Table 5), were significantly less than respective values obtained from the HTT (57.8% for glucan and 44.4% for pentosan). This indicates that the uncatalysed autoclave pretreatment was unable to adequately expose the carbohydrates in the grass to the action of the hydrolytic enzymes. Moreover, the
autohydrolytic effect associated with the high temperatures of the HTT was absent, resulting in low sugar yields, similar to findings with other West African biomass by Thomsen et al. (2015), who employed autoclave pretreatment at 100 °C for 10 min. The highest ethanol concentration of 5.62 g/L occurred at a severity of 2.8 (145 °C, 30 min), well below the value recorded for hydrothermally-pretreated grass (15.58 g/L). Moreover, the theoretical ethanol conversion of the autoclave pretreatment, at a maximum of 27.81%, was also far lower than that of HTT-pretreated grass (65.09%). It can be concluded that simple, uncatalysed hydrothermal methods such as boiling and steaming at moderate temperatures are unsuitable for the use of elephant grass. Thus, further studies on elephant grass should focus on two scenarios: 1) varying the treatment conditions under HTT, and 2) incorporating chemicals at low concentrations and moderate temperatures.

Table 5. Results on Enzymatic Hydrolysis and Fermentation of Autoclave Pretreatment of Elephant Grass

<table>
<thead>
<tr>
<th>Pretreatment conditions</th>
<th>Glucose released in EH, g/100 g DM</th>
<th>Pentose sugars released in EH, g/100 g DM</th>
<th>% theoretical maximum</th>
<th>Eth. yield, g/100 g DM</th>
<th>Eth. conc. after SSF, g/L</th>
<th>% theoretical eth. conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>121 °C, 15 min</td>
<td>12.21 (0.69)</td>
<td>3.07 (0.13)</td>
<td>29.21</td>
<td>11.62</td>
<td>4.93 (1.00)</td>
<td>25.43</td>
</tr>
<tr>
<td>121 °C, 30 min</td>
<td>12.56 (0.52)</td>
<td>3.42 (0.08)</td>
<td>29.86</td>
<td>13.64</td>
<td>3.90 (0.37)</td>
<td>20.01</td>
</tr>
<tr>
<td>121 °C, 60 min</td>
<td>12.98 (2.20)</td>
<td>3.85 (0.21)</td>
<td>29.67</td>
<td>13.75</td>
<td>6.58 (0.61)</td>
<td>32.44</td>
</tr>
<tr>
<td>145 °C, 15 min</td>
<td>14.91 (0.55)</td>
<td>4.66 (0.09)</td>
<td>33.39</td>
<td>15.15</td>
<td>4.52 (1.59)</td>
<td>21.84</td>
</tr>
<tr>
<td>145 °C, 30 min</td>
<td>15.29 (0.26)</td>
<td>4.95 (0.06)</td>
<td>33.00</td>
<td>16.10</td>
<td>5.75 (0.05)</td>
<td>26.74</td>
</tr>
<tr>
<td>145 °C, 60 min</td>
<td>16.91 (0.34)</td>
<td>5.79 (0.06)</td>
<td>38.98</td>
<td>21.41</td>
<td>5.59 (0.58)</td>
<td>27.81</td>
</tr>
<tr>
<td>HTT, 195 °C, 10 min</td>
<td>30.8 (2.0)</td>
<td>8.41 (0.40)</td>
<td>57.8</td>
<td>44.4</td>
<td>16.07 (1.43)</td>
<td>65.09</td>
</tr>
</tbody>
</table>

Standard deviations are stated in parenthesis. EH – enzymatic hydrolysis; * [g ethanol/ (0.51 × g glucan)] × 100; Analogous results from the hydrothermal pretreatment (HTT) are shown in the last column. All yields are based on dry mass of pretreated material.

CONCLUSIONS

1. Hydrothermal pretreatment was most effective on elephant grass: the pretreated material exhibited the highest cellulose digestibility of 30.7 g glucose/100 g DM at 57.8% percent of the theoretical yield of glucose. It also gave the highest ethanol yield of 16.1 g/100 g dry pretreated biomass, representing about 65% of the theoretical yield. Both the glucan conversion to glucose and ethanol yields for the grass were higher than wheat straw.

2. Apart from elephant grass, the sugar and ethanol yields of all other biomass types were low, with both theoretical glucose and ethanol yields falling below 35%.
3. Additional autoclave pretreatments of the elephant grass were performed at moderate temperatures (121, 145 °C) and times (15, 30, 60 min), but the sugar and ethanol yields were significantly lower than those obtained from hydrothermal pretreatment.

4. Due to their high cellulose content (>43%), both bamboo and rubber could play key roles as feedstocks considered for ethanol production, as they thrive in warm, moist tropical weather and are found extensively in West Africa.

5. Coconut husk, given its relatively high lignin (25.3%) and extractive (24.2%) contents, could be of interest as a substrate for producing other biochemicals. Further, its lignin content makes it potentially useful for the production of compressed solid fuels.

6. The fractions of cellulose in Siam weed and elephant grass are comparable to that of wheat straw, suggesting the high potential of extracting sugars from both crops using other pretreatment methods. In Ghana and other countries, both biomass types are found growing together and as such could be exploited together, in addition to other weeds and grasses such as Guinea grass (*Panicum maximum*), for ethanol production.

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