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Alkali and glycerol pretreatment of West African biomass for production of sugars and ethanol

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Abstract

KOH (1%) and glycerol (20-40%) pretreatment of bamboo, rubber, elephant grass and Siam weed were investigated with respect to biomass composition, enzymatic hydrolysis and ethanol fermentation. Delignification was higher under alkaline environment and increased considerably with temperature (25-121 °C). Lignin removal had a positive effect on biomass digestibility, sugars production and ethanol yield. The highest sugar and ethanol yields were observed for KOH pretreatment (121 °C, 1 h) on elephant grass which also recorded the highest delignification (67.5%) and consequently the lowest solids recovery (59.5%). The grass gave the highest yield on glucose (19 g/100 g raw material), xylose (8.2 g/100 g raw material) and ethanol (9.8 g/100 g raw material) under KOH pretreatment (121 °C, 1 h). The elephant grass has great potential to be used as a major energy crop in Africa in a biorefinery where production of sugars, ethanol and other valuable biochemicals are optimised.

Key words: KOH pretreatment; glycerol pretreatment; enzymatic hydrolysis; ethanol; elephant grass

1. Introduction

The biofuel industry in Africa is mainly based on ethanol from sugarcane and molasses as well as biodiesel from *Jatropha*. Lignocellulosic feedstocks such as agricultural and forest residues, grasses and energy crops, and municipal solid waste are known to have promising outcomes though they have not been exploited much (Bensah *et al.*, 2015a). Unlike first generation biofuels, second generation biofuels require advanced processing systems, including additional step for biomass pretreatment. The

suitability of a pretreatment method is measured by its ability to breakdown recalcitrant lignocellulosic biomass into simpler and fermentable components at low cost, low energy requirement, low inhibitor concentrations, high sugar yield, and less adverse effects on the environment (Bensah and Mensah, 2013). According to Menon and Rao (2012), only few pretreatment methods – including steam explosion, liquid hot water, ammonium fibre explosion (AFEX), lime, and dilute acid applications – have shown promising results at commercial scale. Further, pretreatment such as extrusion, boiling, alkali-based methods (e.g. potassium hydroxide, lime and soaking in aqueous ammonia) and crude glycerol have been identified to possess favourable characteristics for ethanol production even at small-to-moderate scales in Africa (Bensah *et al.*, 2015a; Thomsen *et al.*, 2015; Bensah and Mensah, 2013).

Alkali pretreatment methods possess unique benefits in their ability to remove lignin, thereby exposing cellulose and hemicellulose to enzymatic attack. They are applied at relatively mild conditions and result in reduced sugar degradation (Kim *et al.*, 2016). Bases such as NaOH, lime and aqueous/anhydrous NH_3 are more frequently used compared to KOH. Recently, KOH has received attention among researchers owing to its benign nature and ability to selectively remove and isolate lignin. The black liquor by-product of KOH pretreatment contains potassium which may find useful applications in soil fertilisation and conditioning, leading to improved economic attractiveness of KOH-based biorefineries (Jaffar *et al.*, 2016; Liu *et al.*, 2015). KOH pretreatment has been applied to biomass such as wheat straw (Tutt *et al.*, 2012a; Liu *et al.*, 2015; Jaffar *et al.*, 2016), rye straw (Tutt *et al.*, 2012b), municipal solid waste (Alqaralleh, 2012), sweet sorghum (Gyalai-Korpos *et al.*, 2008), corn cobs (Wanitwattanarumlug *et al.*, 2012) and switchgrass (Sharma *et al.*, 2013). Concentrations of KOH ranging from 0.5 -

9 % w/w have been used at temperatures of 20 - 130 °C. Generally, moderate temperatures (≥ 60 °C) require shorter retention times (< 1 h) for adequate biomass solubility while lower temperatures (25-60 °C) at longer times (1 h-1 week) favour high sugar and ethanol yields (Gyalai-Korpos *et al.*, 2008).

Like KOH, glycerol is little-known as a solvent for pretreatment of biomass. Glycerol is a by-product of biodiesel production, produced in large quantities and discarded as waste in Africa. However, glycerol can be converted directly to ethanol (Chaudhary *et al.*, 2011) or applied as organic co-solvent in biomass pretreatment (Lynam and Coronella, 2014). Though majority of glycerol pretreatment studies have focused on elevated temperatures (170-400 °C) (Martín *et al.*, 2015; Martín *et al.*, 2011; Romani *et al.*, 2013), their applications at moderate temperatures have not been given much attention.

In this study, KOH and glycerol pretreatments are applied to four West African biomass – elephant grass, Siam weed, rubber wood and bamboo wood – and their effects on biomass conversion to sugars and ethanol are discussed. The results are benchmarked against a previous study based on hydrothermal pretreatment (Bensah *et al.*, 2015b) at 195 °C, 10 min at 6% solids loading using a loop reactor.

2. Materials and methods

2.1. Substrate collection and preparation

Four biomass, namely, rubber wood (*Heavea brasiliensis*), bamboo wood (*Bambusa vulgaris*), Siam weed (*Chromolaena odorata*) and elephant grass (*Pennisetum*

purpureum) were studied. Rubber wood (RW) was obtained from Ghana Rubber Estates Limited (Takoradi, Western Region) and green bamboo wood (BW) was harvested at Kumasi Institute for Tropical Agriculture in Ejisu-Juaben municipality of Ghana in July 2014. Elephant grass (EG) and Siam weed (SW) were both obtained from a farm at Dormeabra in May 2015 in the same municipality. Biomass were washed with tap water, cut into pieces (2-6 cm), sun-dried for a week and stored at room temperature in zip bags until they were studied.

Biomass samples were sized-reduced using a Retsch knife mill fitted with a 2-mm screen. The total solids (% w/w) of samples, determined in duplicate using convection oven (NREL, 2008), were as follows: EG – 90.6 ± 0 , RW – 90.6 ± 0 , BW – 90.0 ± 0 , SW – 88.4 ± 0 . The composition of bamboo wood and rubber wood was known from previous work (Bensah *et al.*, 2015b) while that of elephant grass and Siam weed was determined using the method described under ‘compositional analysis.’

2.2. Pretreatment

Both KOH and glycerol pretreatment were carried out in 1 L Duran bottles using 45 g dry mass (DM) of biomass (≤ 2 mm) at solids loading of 6% w/w. Pretreatment was performed at room temperature (~ 25 °C) or at 121 °C in an autoclave as shown in Table 1. Pretreated biomass were filtered in vacuum and residual solids were washed with at least 3 litres of water. Solids were dried at 60 °C until the moisture content was less than 8% after which their composition were determined. The solid fractions were further studied under enzymatic hydrolysis and fermentation as described below. The filtrate was also analysed.

Table 1

2.3. Compositional analysis

Organic-soluble extractives in raw and pretreated samples were removed by extracting biomass with 98% v/v ethanol for at least 6 hours in a Soxhlet apparatus. The structural composition of extractive-free biomass was determined using strong acid hydrolysis based on laboratory analytical protocol of National Renewable Energy Laboratory (NREL, 2011), with slight modifications previously described by Bensah *et al.* (2015b). Samples were subjected to sulphuric acid (72 % w/w) treatment at 30 °C for 60 mins in a water bath and subsequently autoclaved in weak sulphuric acid (4 % w/w) at 121 °C for another 60 mins. The samples were vacuum filtered in porous crucibles and the solid residues were dried to constant weight at 105 °C, and afterwards ashed at 550 °C for three hours. The filtrate was collected in 50 ml Falcon tubes and analysed for D-glucose, D-xylose and L-arabinose by High Performance Liquid Chromatography (HPLC). The fraction of Klason lignin in samples was determined based on the dried weight of solid residues and weight of ashed materials.

2.4. Analysis of sugars in pretreatment hydrolysate

10 ml of the liquid fraction, containing glucan and pentosan polymers and oligomers, were hydrolysed using equal volume of 8 % w/v H₂SO₄ at 121 °C for 10 min in an autoclave. Samples were transferred to 50 ml Falcon tubes and centrifuged at 4000 rpm

for 5 minutes. The pH of the supernatants was reduced to 2-3 with H₂SO₄ and 1 ml of the solution was filtered for HPLC analysis as described under analytical methods.

2.5. *Enzymatic hydrolysis of pretreated solid residues*

Raw bamboo and rubber wood were earlier taken through enzymatic hydrolysis and the results are presented by Bensah *et al.* (2015b). In this study, enzymatic hydrolysis of raw EG and SW as well as pretreated biomass was carried out in triplicate at solids loading of 5% w/w in 50 ml Falcon tubes based on laboratory analytical procedures of NREL (NREL, 2011) with minor modifications. The liquor consisted of 24.62 ml of citric acid buffer (50 mM, pH 5), 0.25 ml of sodium azide (to prevent microbial growth), and cellulase (Novozyme Cellic CTec2) loading of 11.14 Filter Paper Units (FPU)/g TS (0.119 ml/1.25 g TS), supplemented with xylanase (Novozyme Cellic HTec2) at a ratio of 9:1 based on protein loading for all assays. A blank solution (without enzymes) was also prepared for each biomass in triplicate in addition to a control (containing only enzyme solution). Enzymes were kindly donated by Novozymes (Denmark) to Danish Technical University.

The tubes were agitated in a shaker incubator at 50 °C and 140 rpm. After 72 h of agitation, samples were centrifuged at 4000 rpm for 10 minutes and the pH of the supernatants was reduced to 2-3, and further centrifuged for 10 min at 8000 rpm. Finally, 1 ml of supernatants was transferred into vials for HPLC analysis as described below.

2.6. *Inoculum preparation and fermentation*

Saccharomyces cerevisiae (Ethanol Red, Fermentis, France) was grown on sterilised medium 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_2PO_4 , and 0.3 g/L MgSO_4 . The yeast was incubated (30 °C, 140 rpm, 18 h) overnight and subsequently used in fermentation at a loading of 2% w/w (20 mg/g DM of sample) or at cell concentration of 2 g/L, without addition of nutrients. Production of ethanol was carried out at 35 °C for 144 hours through simultaneous saccharification and fermentation (SSF), preceded by a six-hour prehydrolysis step (50 °C) using cellulase and xylanase at the same loading as was used in enzymatic hydrolysis.

Samples were fermented in triplicate at 10% total solids and 140 rpm in a shaker incubator using 100 ml Duran bottles. In addition, blank samples containing only fermentation slurry (sodium acetate buffer, 50 mM, pH 5) were included in triplicate. The fermentation bottles (fitted with glycerol-filled yeast locks) were weighed regularly to monitor CO_2 loss. After 144 h, 1 ml samples were diluted with H_2SO_4 to pH of 2-3, centrifuged at 4000 rpm for 5 minutes, and prepared for HPLC analysis for ethanol, sugars, xylitol, and acids.

2.7. Analytical methods

Carbohydrates (glucose, xylose and arabinose) in hydrolysates of pretreated samples and the final liquor in enzymatic hydrolysis and fermentation were quantified by HPLC using appropriate standards. The HPLC, which had an organic acid column (BioRad Aminex HPX-87H) and was equipped with a refractive index detector, was operated at 60°C with a mobile phase (4 mM H_2SO_4) flowrate of 0.6 ml/min. Samples were diluted

using appropriate volumes of deionised water and filtered using microfilters (0.2 μm , 0.4 μm). The same HPLC conditions were also used for detection of acids generated during enzymatic hydrolysis as well as products of fermentation, including ethanol, xylitol and acids.

3. Results and discussion

3.1. Composition of biomass

The structural composition of raw biomass used is shown in Table 2. The glucan fractions in both elephant grass (EG) and Siam weed (SW) are lower than respective values (32.0 and 36.2%) harvested in 2013 (Bensah *et al.*, 2015b). However, the lignin fractions in the current studied biomass were higher, with the fraction in EG being twice as high as the previous one. The significant difference could be attributed to factors such as different times of harvest, different location and soil conditions, and the fact that the earlier harvested-biomass (2013) were at a lower stage of maturity. The glucan fraction in the grass falls on the lower end of the range of values (30.0-41.9%) reported by other researchers while the lignin fraction was closer to the high end of the range (8-20.7%) from literature (Montipo *et al.*, 2018; Santos *et al.*, 2018; Kataria *et al.*, 2017). Regarding bamboo, glucan fraction is higher than those (37.2-48.4%) obtained by other authors while xylan and lignin fractions are slightly lower (Yuan *et al.*, 2018; Jiang *et al.*, 2016; He *et al.*, 2014; Li *et al.*, 2012). For rubber, the glucan (39.0-49.1) and lignin (13.4-28) fractions reported elsewhere are consistent with the value obtained in the present study, but hemicellulose fraction recorded in this study is considerably lower

(Chen *et al.*, 2018); Petchpradab *et al.*, 2009). The glucan and hemicellulose fractions in the SW are lower but lignin is higher, relative to results obtained by Zhao *et al.* (2010).

Table 2

3.2. Effect of pretreatment on biomass composition

The composition of the solid fraction of alkali and glycerol pretreated biomass is shown in Tables 3. Under alkali and glycerol pretreatment, glucan fraction in pretreated substrates was lower than those in the raw material for bamboo and rubber for most reaction conditions. For bamboo, the elevated temperature in alkaline environment led to increased dissolution of lignin, hemicelluloses, extractives and ash, culminating in higher glucan fraction. This is affirmed by other authors who applied various pretreatment on bamboo including microwave-KOH (Li *et al.*, 2012) and NaOH (Yuan *et al.*, 2018; Li *et al.*, 2016).

In contrast, glucan fractions in pretreated EG and SW were largely higher (with respect to the raw material) for all pretreatment conditions. Moreover, the highest glucan fractions (except rubber) were observed for KOH pretreatment at 121 °C /1 h, with EG having the highest glucan-rich substrate (57%).

In the alkali media at constant temperature, increasing reaction time from 24 to 48 h resulted in marginal increase in the glucan component for RW and EG, while an opposite trend was observed for BW and SW. For rubber, the increased reaction time from 24 to 48 h appears to have caused significant reduction (4.8% difference) in the extractives composition, resulting in an increase in the fractional composition of glucan.

For glycerol pretreatment, glucan fraction in the solid residue decreased with glycerol concentration for EG and SW, which recorded 1.3 and 3.7 percent drops, respectively. Glucan composition was not affected for bamboo though it increased marginally for rubber. A general observation shows glucan fractions from glycerol pretreated-biomass were lower compared to KOH pretreatment for biomass studied except SW. Further, glucan fractions were lower relative to respective values obtained from hydrothermal pretreatment of the same biomass (Bensah *et al.*, 2015b) with the exception of bamboo and EG pretreated at 121 °C /1 h under alkaline environment.

Xylan fractions decreased with time under KOH pretreatment at room temperature for BW, EG and SW, but marginally increased for RW (Tables 2 and 3). With the exception of EG, KOH pretreatment at 121 °C yielded the lowest xylan fractions in pretreated solid residues for all other biomass, corroborating the widely observed view that elevated temperatures promote xylose solubilisation. The EG, under KOH treatment at 121 °C /1 h, recorded the highest xylan fraction (relative to other pretreatment on the grass) at 24.1% which may be attributed to the high delignification at that condition (see section on biomass delignification). Moreover, the autocatalytic conditions prevalent at high temperatures (≥ 180 °C) that cause rapid removal of hemicellulose sugars are absent at the conditions used in this study; thus, xylan values recorded are lower than those obtained under hydrothermal pretreatment of the same biomass (Bensah *et al.*, 2015b). With regards to glycerol pretreatment, higher glycerol concentration resulted in lower xylan fractions in the pretreated solid residues for all biomass studied, as shown in Table 3.

Lignin fractions in the solid residues for alkali or glycerol pretreated biomass were lower than respective values from hydrothermal pretreatment on the biomass (Bensah *et*

al., 2015b). Of the alkaline conditions, lignin composition was lowest at 121 °C /1 h for all biomass except rubber where there was a marginal increase. The effect of elevated temperature on lignin composition was mostly visible on the pretreated grass which recorded a value of 10.6% at 121 °C/1 h compared to 19.5% for the raw material. Lignin fractions in pretreated samples were higher than respective values in the raw material for all biomass. Moreover, increasing glycerol concentration from 20 to 40% did not appreciably affect lignin fraction in the pretreated samples.

Table 3

3.3. Solids recovery after pretreatment

In general, pretreatment causes biomass components to solubilise in the liquid phase leading to material loss in the solid phase, with the extent of loss dependent on reaction conditions. Solids recovery was determined by dividing the dry mass of pretreated material against that of the raw material. For KOH pretreatment, solids recovery is influenced significantly by temperature as observed in Fig. 1. The influence of reaction time at constant temperature is however mixed; while the solids recovery of the woody biomass was marginally reduced, that of EG and SW increased as the time was varied from 24 - 48 h, with SW recording the highest percent rise of 5. Overall, solids solubilisation follows the following order: EG > SW > BW > RW, which shows that, EG was the least recalcitrant to fractionation under KOH pretreatment. This observation is in agreement to the work of Kim *et al.* (2016) that grasses are more easily fractionated than woody biomass under alkaline pretreatment, since the alkali readily

cleaves *p*-hydroxyphenyl and syringyl lignins which are dominant in herbaceous biomass. The combined effects of high temperature and alkaline condition caused the solids recovery of EG to fall below 60%, which was significantly lower than values recorded for all other pretreatments of the grass. Similarly, recoveries fell below 60% when EG was pretreated with sulphuric acid at 121-190°C/ 5-30 min, and was mainly attributed to solubilization of hemicellulose and non-structural components (Santos *et al.*, 2018; Montipó *et al.*, 2018).

The minimum solids recovery (74.3) of bamboo, obtained under KOH pretreatment (121 °C), is about 6.6% higher than that obtained by Li *et al.* (2016) using NaOH (121°C, 1 h, 1 %) which is similar to conditions used in our study. Expectedly, a minimum recovery of 85.6% was reported by Yuan *et al.* (2018) at a lower temperature (100 °C) using NaOH pretreatment (4-10%, 0.5-3 h) on bamboo.

Unlike the alkali pretreatment, solids recovery exceeded 90% for all biomass under glycerol application, with recovery in BW (under 20% glycerol) and EG reaching over 99% (Fig. 1). It is also observed that solids recovery decreased when glycerol concentration was increased from 20-40% for the woody biomass but marginally increased for EG and SW.

Fig. 1.

3.4. Delignification of pretreated biomass

Alkaline pretreatment effectively disrupts lignin structure resulting in solubilisation, with the degree of dissolution influenced by pH, temperature and reaction time (Kim *et*

al., 2016; Bensah and Mensah, 2013). Lignin removal allows hydrolytic enzymes to have easy access to the carbohydrates of the biomass. In this study, delignification (%) was determined as $L(\%) = 100 \times (L_R - L_P) / L_R$, where L_R and L_P refer to the dry mass of lignin in raw and pretreated samples, respectively.

Delignification was less than 1% under KOH pretreatment at 25 °C (24, 48 h) for bamboo and rubber. However, at 121 °C /1 h, delignification was 39.3% on bamboo though it was still low for rubber (5.9%) and to some extent SW where delignification reached a maximum of 11.6% at 121 °C /1 h. Lignin removal from BW is greatly influenced by temperature as opposed to time or concentration; thus, when Juan *et al.* (2018) applied higher concentrations of NaOH (4-10%) for up to 3 h, the maximum lignin removed was 4.9%. With regards to SW, the percent delignification is close to that (9.1%) reported by Zhao *et al.* (2010) based on NaOH pretreatment (10%, 121 °C, 2 h).

The EG was the most delignified biomass under KOH pretreatment, recording values of 25.7, 33.3 and 67.5% for 25 °C /24 h, 25 °C /48 h and 121 °C /1 h, respectively. The results obtained in this study compare well with those observed by Minmunin *et al.* (2015) where NaOH pretreatment (70 °C /2 h) of the grass produced lignin removal of 8.7, 20.4 and 51.5% for aqueous solutions of 0.5, 3.0 and 5.5 wt.% NaOH, respectively.

Lignin removal was ineffective for all biomass under glycerol pretreatment. Moreover, lignin recovery in pretreated substrates exceeded 100%, with maximum value of 112.8% recorded for rubber in 20% glycerol solution. Higher than normal lignin recoveries may be attributed to formation of pseudo-lignin (Hu *et al.*, 2012).

3.5 Degradation products

Carboxylic acids (lactic, formic, acetic and propionic) formed as a result of degradation of sugars in the hydrolysates of pretreated samples were quantified (Fig. 2). Propionic acid is not shown as it was only detected in a few of the samples at low concentrations, with the highest yield of 0.02 % for bamboo pretreated at 121 °C in KOH solution. Under glycerol pretreatment, only acetic acid was significantly detected in the hydrolysates of RW and EG, at 0.24 ± 0.001 and 0.27 ± 0.002 % respectively (data not shown). The effect of temperature on the production of degradation products was most conspicuous for KOH pretreatment at 121 °C since it gave the highest acid yields on all biomass studied. In the case of bamboo, acetic acid generation was about one-fourth of values obtained by Li *et al.* (2012) who studied microwave-KOH (8-12%) pretreatment of bamboo at 180 °C.

Fig. 2.

3.6. Enzymatic hydrolysis of pretreated biomass

Enzymatic hydrolysis (EH) of untreated materials was assessed in our earlier studies (Bensah *et al.*, 2015b) where it was found that the native materials were generally unsuitable since the maximum glucose yield was below 12 g/100 g of DM in solid fraction. The effect of alkaline and glycerol pretreatment on biomass considered in this work in terms of glucose and xylose yields is shown in Tables 4 and 5, respectively. Alkaline pretreatment removes some amount of lignin and thus loosens the complex biomass structure which results in increased cellulose fractions. However, partial

delignification does not necessarily lead to high sugar yields since factors such as location, surface area and surface properties of the lignin also play significant roles (Kim *et al.*, 2016).

Table 4

This study shows that increased delignification favours high glucose yields in enzymatic hydrolysis. Of the pretreatment methods and conditions considered for each sample, KOH pretreatment gave the highest glucose yield at 121 °C /1 h. Further, pretreatment was most effective on the grass which recorded the highest glucose yield of 20.4 g/100 g of untreated material, representing 47.3% of the theoretical maximum. This was lower than the yield (22.2 g/100 g of untreated DM) obtained under hydrothermal pretreatment (195 °C, 10 min) in an earlier study under the same hydrolysis conditions (Bensah *et al.*, 2015b), but higher than that (24.8 g/100 g DM of pretreated material or 19.3 g/100 g DM of raw material) obtained by Scholl *et al.* (2015) based on steam explosion (190 °C /10 min) of EG under 48 h of enzymatic hydrolysis using enzyme loading of 10 FPU/g DM and solids loading of 4% w/v. In another study, Li *et al.* (2015) obtained cellulose-to-glucose conversion of at least 55% from dilute acid pretreated EG cultivars, using a low enzyme loading of 7 FPU/g DM. Kataria and coworkers (2017) also obtained 55% conversion on steam explosion (161 °C, 11.5 min) of acid-soaked EG using 20 FPU/g in 96 h of saccharification while a higher yield of 76% (glucose + cellobiose) was reported based on sulphuric acid pretreatment (121 °C, 30 min), with 20 FPU/g and 15 U/g β -glucosidase (Santos *et al.*, 2018).

In general, longer pretreatment and/or higher temperatures correlated positively with glucose yields. The effect of temperature was however the deciding factor as observed in glucose yield increase of about three times for bamboo, one and half times for RW and SW, and one and quarter times for EG, compared with pretreatment condition producing the second highest sugar yield for each biomass. The elevated temperature also led to higher delignification of all samples under alkaline conditions which was favourable to enzymatic saccharification. Between the two woody samples, RW gave higher sugar yields (at least three-quarters higher) under alkaline conditions at 25 °C. However, at 121 °C, BW recorded sugar yield of 16.8 g/100 g (36.9% conversion) which was marginally higher than that for RW. The glucose yield of bamboo was found superior to result obtained by Li *et al.* (2012) who measured 21% conversion using microwave-KOH pretreatment of bamboo, but inferior by nearly two-thirds of glucose yield of NaOH pretreated bamboo (Jiang *et al.*, 2016). Bamboo is a woody grass that contains p-hydroxyl, guaiacyl, and syringyl that is typical of hardwood lignin found in materials like RW, nonetheless it was less prone to releasing sugars at mild conditions. Therefore, when Jiang *et al.* (2016) applied ultra-high pressure in addition to NaOH, as high as 87.3% conversion was attained. Another two-stage pretreatment involving NaOH (8%, 100 °C, 3 h) and H₂O₂ (6%) gave about 95% cellulose conversion (as opposed to about 38% using only NaOH) at 18 FPU/g cellulose in 72 h (Yuan *et al.*, 2018).

Only few studies on conversion of rubber wood to sugars have been cited in literature. A study by Petchpradab *et al.* (2009) using autoclave pretreatment (130-240 °C) gave maximum glucan conversion of 8% using enzyme loading of 0.3 units/mg solid, lower than values obtained under KOH pretreatment in this study. A lower yield

than this study was also reported by Nazarpour *et al.* (2013) based on fungi pretreatment of rubber.

Contrary to KOH pretreatment, glycerol application was ineffective in releasing glucose from biomass studied at the conditions considered in this work. An objective of this study was to ascertain whether glycerol pretreatment at moderate temperatures and times could produce sugars at rates that make it economically possible to implement low-tech plants in African countries using glycerol from biodiesel plants. However, glycerol pretreatment at mild conditions is unfavourable perhaps due to the high boiling point and the low basicity of glycerol, and as such higher temperatures ($\geq 180^\circ\text{C}$) are thus required. This observation confirms the work of Lynam and Coronella (2014) where glycerol-pretreated of rice hulls at 110°C failed to release glucose in enzymatic hydrolysis. In contrast, high cellulose to glucose conversion of 98% was realised by Romaní *et al.* (2013) on *Eucalyptus* wood pretreated at 200°C /69 min in glycerol solution (56%) using enzyme load of 20 FPU/g solid.

In terms of increasing glucose yields under alkaline and glycerol pretreatment, the following order can be gleaned from Table 4: SW < RW < RW < EG, meaning SW is the most recalcitrant while EG is the least resistant to fractionation and enzymatic action. As corroborated by other authors (e.g. Zhao *et al.*, 2010), SW is more difficult to fractionate than other herbaceous plants; nonetheless, total reducing sugar conversion (28.8%) obtained under this study (KOH, 121°C) was over 5% point greater than values reported by the authors under NaOH and mild acid pretreatments.

Among pretreatment options used, xylose yields were highest for samples pretreated with KOH at 121°C /1 h as shown in Table 5. Though xylan to xylose conversion was higher for BW (58.6%) compared to EG (57.2%), the grass gave a higher yield of

xylose (10.8 g/100 g of untreated material) because it had a bigger xylan fraction in the native material. The yield from EG was more than three times higher than that obtained by Scholl *et al.* (2015) at 3 g/100 g under steam pretreatment (190 °C /10 min) but lower than the result from Bensah *et al.* (2015b) by a third under hydrothermal pretreatment. As shown in Table 5, glycerol pretreated samples under the conditions used were generally inferior to those from KOH pretreatment in releasing xylose under enzymatic hydrolysis.

Table 5

3.7. Fermentation

SSF of the solid fraction of pretreated substrates was performed at 32 °C at solids loading of 6 % w/w and yeast concentration of 2 g/L (or 2 g/100 g DM of sample). No nutrients were added. Fig. 3 gives the mass percentage of products detected in the fermentation liquor. Glucose was fully consumed in the liquor (data not shown), signifying efficient conversion to ethanol. On the other hand, xylose conversion was poor and could be attributed to inability of yeast to convert C5 sugars to ethanol. Xylose concentrations were relatively high in all KOH-pretreated samples (121 °C/1 h), with elephant grass recording the highest at 14.94%. Xylose and other hemicelluloses in waste fermentation broth may be converted into useful biochemicals such as lactic acid (in addition to lactic acid from the liquor) to improve overall economics (Zhang *et al.*, 2014).

Of the carboxylic acids, formic acid was most noticeable especially on glycerol pretreated samples where values ranged from 2.89 to 6.63%. The grass gave the highest yield of xylitol at 3.64% for KOH-pretreated samples (121 °C /1 h) while those pretreated at room temperature generated considerable quantities of lactic acid at an average of 4.33%. Acetic acid and propanoic acid were generally low (< 1%) in the final fermented liquor (data not shown).

Fig. 3.

The final ethanol concentration, conversion efficiency and yield are shown in Table 6. The final ethanol yield is based on the dry mass of the original untreated biomass. The results show that ethanol concentration, conversion efficiency and yield from the alkali pretreatment were considerably higher than those from glycerol pretreated substrates, likely as a result of the failure of glycerol solution to cause biomass fractionation under the moderate conditions used in this study. Under alkaline conditions, the greatest effect on ethanol production for each biomass occurred under pretreatment at 121 °C. The grass recorded the highest ethanol yield of 9.8 g/100 g of dry untreated material in alkaline pretreatment at 121 °C which is comparable to the value (10.1 g/100 g DM) realised under hydrothermal pretreatment in a different study (Bensah *et al.*, 2015b). Further, ethanol yield (16.4 g/100 g pretreated mass) from EG is found higher than the maximum yield (107.7 µL/g of pretreated EG) obtained by Scholl *et al.* (2015) which by our calculation is equivalent to 7.1 g ethanol/100 g raw EG. Further, the effect of increasing pretreatment time from 24 to 48 h at room temperature was not felt as far as ethanol yields are concerned. In another study, the ethanol yield of

elephant grass ranged from 0.11-0.29 g/100g at solids loading of 4-20 %, with separate fermentation performed for 48 h on samples that had been concomitantly ball-milled and hydrolysed at 10 FPU/g (Menegol *et al.*, 2016). A high yield, representing 74.6% of the theoretical maximum, was obtained by Montipo *et al.* (2018) based on fermentation of steam-exploded EG at 20% solids loading and enzyme dosage of 20 FPU/g sample.

KOH pretreatment at ambient conditions as well as glycerol pretreatment at moderate temperatures produced lower ethanol concentrations (< 5 g/L) on BW, RW and SW. But, ethanol concentration exceeding 5 g/L was realised for the grass at room temperature under KOH application, suggesting the need to do further investigating at longer pretreatment times. Overall, ethanol yields and conversion in decreasing order for the samples follows the order EG > RW > BW > SW. The results indicate Siam weed was the least amenable to fractionation under the pretreatment conditions studied, suggesting harsher conditions such as longer times, higher temperatures and higher KOH or glycerol concentrations may be more suitable. Two-stage pretreatments under mild conditions appear to give high ethanol yields for recalcitrant biomass such as bamboo (Yuan *et al.*, 2018).

Clearly, the EG possesses the greatest potential among the materials studied and at the conditions considered as it gave the highest yield of ethanol, 9.8 g /100 g of untreated EG, at a final concentration of 16.4 g/L. This was achieved at solids and glucan loading of 10.0 and 6.1%, respectively.

Table 6

4. Conclusions

KOH-pretreated bamboo wood, rubber wood, elephant grass and Siam weed gave higher delignification compared to glycerol-pretreated samples, with delignification increasing significantly with pretreatment temperature (25, 121 °C). KOH pretreatment at 121 °C/1 h on elephant grass gave the lowest solids recovery (59.5%) and highest delignification (67.5%), and consequently produced the highest sugar (27.2 g glucose and xylose /100 g of raw material) and ethanol yields (9.8 g/100 g raw material). Higher glucose ($\geq 35\%$ theoretical maximum) and xylose ($\geq 52\%$ theoretical maximum) yields were also observed for bamboo and rubber compared to Siam weed for KOH pretreatment at 121 °C/1 h.

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Fig. 1. Solids recovery after pretreatment of biomass samples

Recovery is obtained as grams of solids in hydrolysate after pretreatment per gram of dry biomass before pretreatment. A – KOH/25 °C/24 h, B – KOH/25 °C /48 h, C – KOH/121 °C /1 h, D – glycerol (20%)/121 °C, E – glycerol (40%)/121 °C /1 h.

Fig. 2. Carboxylic acids in hydrolysates of KOH pretreated samples

Conditions: A (25 °C, 24 h), B (25 °C, 48 h) and C (121 °C, 1 h).

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A – KOH/25 °C /24 h, B – KOH/25 °C /48 h, C – KOH/121 °C /1 h, D – glycerol (20%)/121 °C, E – glycerol (40%)/121 °C /1 h.

Fig. 3. Weight percentage (g/100 g DM) of hemicellulose and fermentation products after 144 h of SSF.

Hemicellulose comprises xylose and arabinose. Error bars show standard deviations from mean values.

Table 1

Pretreatment conditions

Pretreatment	Concentration of KOH or glycerol in pretreatment liquor (% w/w)	Temperature, (°C)	Reaction time (h)
KOH			
A	1	~25	24
B	1	~25	48
C	1	121	1
Glycerol			
D	20	121	1
E	40	121	1

Table 2

Composition of raw bamboo and rubber wood (% dry weight)

Biomass	Glucan	Xylan	Arabinan	Klason lignin	Extractives	Ash	Sum
EG	29.8 ± 0.3	14.0 ± 0.6	1.9 ± 0.6	19.5 ± 0.7	18.1	4.3 ± 0.1	87.6
SW	28.5 ± 0.5	13.7 ± 0.1	1.3 ± 0	24.7 ± 0.6	14.5	1.6 ± 0.1	84.3
BW ^a	49.8 ± 0.3	17.9 ± 0.3	0.8 ± 0	23.5 ± 0.5	7.1	1.5 ± 0	100.6
RW ^a	43.5 ± 2.4	15.0 ± 0.3	0.3 ± 0	20.2 ± 0.4	6.5	0.9 ± 0	86.4

EG - elephant grass, SW - Siam weed, BW – bamboo wood, and RW – rubber wood;

^a Source: Bensah *et al.* (2015b).

Table 3

Composition of solid fraction of samples after pretreatment

Biomass	Pretreatment	Conditions, °C/h	Glucan	Xylan	Arabinan	Klason lignin	Extractives	Ash	Sum
Bamboo	KOH	25/24	44.1 ± 0.4	19.2 ± 0.5	1.8 ± 0.2	26.8 ± 0.6	3.7	2.8 ± 0.1	98.3
	KOH	25/48	43.2 ± 0.5	18.2 ± 0.1	1.8 ± 0.1	25.7 ± 0.3	2.6	2.5 ± 0	94.0
	KOH	121/1	53.1 ± 0.2	17.8 ± 0.1	1.2 ± 0	19.2 ± 1.5	1.1	1.6 ± 0	94.0
	G20	121/1	40.7 ± 1.5	18.4 ± 0.1	1.5 ± 0.1	26.1 ± 0.8	4.8	0.7 ± 0	92.2
	G40	121/1	40.9 ± 2.2	18.2 ± 1.1	1.5 ± 0.2	26.6 ± 1.1	3.6	0.6 ± 0.1	91.4
	Raw bamboo ^a		49.8 ± 0.3	17.9 ± 0.3	0.8 ± 0	23.5 ± 0.5	7.1	1.5 ± 0	100.6
Rubber	KOH	25/24	41.7 ± 0.6	16.1 ± 0.2	1.0 ± 0.2	23.0 ± 0.6	5.8	2.9 ± 0.2	90.6
	KOH	25/48	43.9 ± 0.4	16.4 ± 0.5	0.8 ± 0.1	25.7 ± 0.4	1.0	2.6 ± 0.2	90.4
	KOH	121/1	43.4 ± 1.2	13.1 ± 0.4	0.4 ± 0	24.1 ± 0.6	3.0	3.1 ± 0.2	87.2
	G20	121/1	30.0 ± 0	14.3 ± 2.1	0.9 ± 0.2	23.4 ± 1.3	6.9	0.6 ± 0.1	76.1
	G40	121/1	31.0 ± 2.1	12.7 ± 0	0.8 ± 0.2	18.4 ± 0.7	13.3	0.2 ± 0.1	76.3
	Raw rubber ^a		43.5 ± 2.4	15.0 ± 0.3	0.3 ± 0	20.2 ± 0.4	6.5	0.9 ± 0	86.4
EG	KOH	25/24	42.9 ± 0.2	20.2 ± 0.1	3.0 ± 0.2	20.6 ± 0.9	3.2	3.1 ± 0.1	93.1
	KOH	25/48	43.3 ± 0	19.5 ± 0	3.5 ± 0	17.9 ± 1.2	5.2	5.4 ± 0.3	94.8
	KOH	121/1	60.6 ± 0.6	24.1 ± 0	2.5 ± 0.1	10.6 ± 0	0	2.5 ± 0.2	100.3
	G20	121/1	37.7 ± 1.4	19.3 ± 0.7	2.4 ± 0.1	22.0 ± 0.7	8.3	2.1 ± 0.1	91.9
	G40	121/1	36.4 ± 1.4	17.8 ± 0.7	2.5 ± 0.1	21.6 ± 1.0	8.3	1.9 ± 0.1	88.4
	Raw EG		29.8 ± 0.3	14.0 ± 0.6	1.9 ± 0.6	19.5 ± 0.7	18.1	4.3 ± 0.1	87.7
Siam weed	KOH	25/24	36.1 ± 0	16.4 ± 0.1	1.1 ± 0.1	29.7 ± 1.5	1.7	3.0 ± 0	87.9
	KOH	25/48	35.0 ± 1.1	15.5 ± 0.4	1.1 ± 0.2	29.6 ± 0.4	4.4	3.3 ± 0.3	88.8
	KOH	121/1	39.5 ± 1.7	15.5 ± 0.1	0.7 ± 0.1	28.6 ± 0.8	5.3	4.5 ± 0.1	94.1

G20	121/1	36.3 ± 2.4	17.4 ± 1.1	1.4 ± 0.1	27.6 ± 0.8	7.6	1.0 ± 0.3	91.3
G40	121/1	32.6 ± 0.6	15.6 ± 0.1	1.3 ± 0.1	27.9 ± 1.1	6.1	0.9 ± 0.1	84.5
Raw SW		28.5 ± 0.5	13.7 ± 0.1	1.3 ± 0	24.7 ± 0.6	14.5	1.6 ± 0.1	84.3

G20 - glycerol (20 % w/w), G40 – glycerol (40 % w/w), ^a Source: Bensah *et al.* (2015b).

Table 4

Glucose yield and conversion after pretreatment and enzymatic hydrolysis

Pretreatment	Glucose released, g/100 g DM of pretreated solids	Glucose yield, g/100 g DM of untreated material	Glucose conversion, % theoretical maximum
Bamboo			
A	6.2 ± 0.2	5.6	12.6
B	6.4 ± 0.3	5.8	13.4
C	21.8 ± 1.6	16.2	36.9
D	0.8 ± 0.1	0.8	1.7
E	0.8 ± 0	0.8	1.8
Rubber			
A	11.5 ± 1.9	10.2	24.7
B	11.0 ± 1.2	9.7	22.5
C	17.2 ± 1.6	13.6	35.7
D	7.3 ± 2.2	7.2	22.1
E	7.8 ± 0.2	7.3	22.7
Elephant grass			
A	17.3 ± 0.3	12.2	36.3
B	18.8 ± 1.0	13.6	39.0
C	31.9 ± 1.4	19.0	47.3
D	6.0 ± 0.1	6.0	14.4
E	5.6 ± 0.7	5.5	13.8
Siam weed			
A	5.4 ± 0.3	4.5	13.6
B	5.8 ± 0.5	5.0	14.9
C	10.5 ± 0.5	8.0	24.0
D	1.1 ± 0.1	1.0	2.6
E	0.9 ± 0.1	0.8	2.5

A – KOH/25 °C /24 h, B – KOH/25 °C /48 h, C – KOH/121 °C /1 h, D – glycerol (20%)/121 °C, E – glycerol (40%)/121 °C /1 h. Glucose released is expressed as g glucose per 100 g DM of the solid fraction of pretreated samples after enzymatic hydrolysis. Glucose yield is given as g glucose per 100 g DM of untreated samples after enzymatic hydrolysis. Cellulose conversion to glucose is expressed as g glucose released in enzymatic hydrolysis per 100 g cellulose in pretreated solids, multiplied by the hydration factor of glucose (0.9).

Table 5

Xylose yield and conversion after pretreatment and enzymatic hydrolysis

Pretreatment	Xylose released, g/100 g DM in pretreated solids	Xylose yield, g/100 g DM of untreated material	Xylose conversion, % theoretical maximum
Bamboo			
A	4.3 ± 0	3.9	22.5
B	4.5 ± 0	4.1	24.9
C	10.5 ± 0.4	7.8	58.6
D	0.2 ± 0	0.2	1.3
E	0.3 ± 0	0.3	1.4
Rubber			
A	4.1 ± 0.2	3.7	25.6
B	4.1 ± 0.1	3.7	25.3
C	7.0 ± 0.3	5.5	52.9
D	0.4 ± 0.1	0.4	3.1
E	0.4 ± 0	0.4	2.8
Elephant grass			
A	7.1 ± 0.1	5.0	35.3
B	8.0 ± 0.2	5.8	41.1
C	13.8 ± 0.4	8.2	57.2
D	1.2 ± 0	1.2	6.2
E	1.0 ± 0.1	1.0	5.6
Siam weed			
A	4.8 ± 0.1	3.9	29.0
B	5.0 ± 0.2	4.3	32.0
C	7.2 ± 0.2	5.5	46.7
D	0.2 ± 0	0.1	0.8
E	0.1 ± 0	0.1	0.7

A – KOH/25 °C /24 h, B – KOH/25 °C /48 h, C – KOH/121 °C /1 h, D – glycerol (20%)/121 °C, E – glycerol (40%)/121 °C /1 h. Xylan conversion to xylose in pretreated biomass at 5% DM using cellulase (Cellic Ctec2) and xylanase (Cellic Htec2) loadings of 11.14 FPU and 0.44 FPU/g TS, respectively. Xylan conversion to xylose is expressed as g xylose released in enzymatic hydrolysis per 100 g xylan in pretreated solids, multiplied by the hydration factor of xylose (0.88).

Table 6

Ethanol concentration, conversion efficiency and yield

Pretreatment condition	Final ethanol concentration, g/L in fermentation	Conversion efficiency (% theoretical ethanol conversion), g/100 g of potential ethanol from pretreated biomass	Ethanol yield, g/100 g DM of untreated material
Bamboo			
A	2.4 ± 0.1	10.5	2.1 ± 0.1
B	2.3 ± 0.4	10.5	2.1 ± 0.4
C	7.8 ± 2.4	28.9	5.8 ± 2.4
D	0.3 ± 0.2	1.3	0.3 ± 0.2
E	0.3 ± 0.1	1.5	0.3 ± 0.1
Rubber			
A	4.4 ± 1.4	20.9	3.9 ± 1.4
B	4.2 ± 0.2	18.6	3.7 ± 0.2
C	7.4 ± 0.2	33.4	5.8 ± 0.2
D	2.2 ± 1.1	14.5	2.2 ± 1.1
E	1.5 ± 0.3	9.6	1.4 ± 0.3
Elephant grass			
A	5.9 ± 0.9	26.8	4.1 ± 0.9
B	5.4 ± 1.8	24.8	4.0 ± 1.8
C	16.4 ± 1.1	53.2	9.8 ± 1.2
D	2.0 ± 0.3	10.2	2.0 ± 0.3
E	1.9 ± 0.5	10.2	1.9 ± 0.5
Siam weed			
A	1.3 ± 0.5	6.9	1.0 ± 0.5
B	1.5 ± 0.2	8.7	1.3 ± 0.2
C	2.2 ± 0.6	11.1	1.7 ± 0.6
D	0.4 ± 0.1	2.3	0.4 ± 0.1
E	0.3 ± 0.1	2.1	0.3 ± 0.1

Graphical abstract

Highlights

- Mild KOH and glycerol pretreatment (25-121 °C) of four biomass were studied.
- Highest delignification (67.5%) was observed for elephant grass.
- Glucan conversion (47.3%) was highest for the grass under alkaline conditions.
- Xylan conversion was 46.7-58.6% for KOH pretreatment at 121 °C.
- KOH pretreatment gave the highest ethanol conversion on the grass at 53.2%.

KOH pretreated samples

Glycerol pretreated samples

Figure 1

Figure 2

Figure 3