

The influence of variety type on dilute acid pretreatment-hydrolysis responses of sugarcane bagasse

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Abstract

This study evaluated the influence of variety type on dilute acid pretreatment and enzymatic hydrolysis responses in terms of xylose, glucose and total combined sugar yields of eight varieties of sugarcane bagasse. The pretreatment was carried out in small tubular reactors. The materials were pretreated under five different conditions and hydrolysed with a standard enzyme dosage (15 FPU/g water insoluble solids, WIS). Pretreatment and enzymatic hydrolysis responses showed xylose, glucose and combined sugar ranged from 7.0 to 19.6; 12.7 to 32.4 and 28.5 to 54.4 g/100g dry raw material, respectively. The variability of sugar yields was attributed to breeding technology and type of varieties. The xylose yield was not always consistent with the variety type or breeding technology. The cellulose digestibility was significantly affected by the variety type for most of classical breeding varieties and was less obvious for precision breeding varieties. It was also established that most of precision breeding varieties were superior in digestibility than many classical breeding varieties. These findings will significant contribution to the sugarcane development with the aim of selecting sugarcane with highly hydrolysable fibres in conjunction with high biomass and sucrose yield per hectare to make lignocellulose to ethanol process affordable.

Key words: sugarcane bagasse, classical breeding, precision breeding, pretreatment, enzymatic hydrolysis.

1. Introduction

The search for alternative fuels such as cellulosic ethanol for transportation sector has been a priority worldwide with the aim of reducing CO₂ emissions (Wyman, 1999; Wheals et al., 1999). Currently, bioethanol production from carbohydrates originated from lignocellulose biomass, in particular, sugarcane bagasse (SCB) has attracted increasing attention due to higher biomass yields (Cardona et al., 2010). In South Africa alone, about 8 million dry tons of SCB is produced every year (Leibbrandt et al., 2011). About 50% of bagasse generated is used for power generation to either power the sugar mills or to run the ethanol distilleries plants (Leibbrandt et al., 2011). The remaining percentage could be potentially available for bioethanol production.

The positive utilisation of SCB as raw material will bring a breakthrough to a complete utilisation of the whole sugarcane (crop) for bioethanol production. This could possibly increase bioethanol production and make it sustainable, in particular, SADC countries looking for a new possible land to expand sugarcane production for bioenergy (Watson, 2011).

Sugarcane bagasse like other lignocellulose biomass is composed of cellulose, hemicellulose and lignin (Peng et al., 2009; Cardona et al., 2010). It also contains minor amounts of ash and extractives (Sanjuan et al., 2001). Cellulose structure is composed of β -D-glucopyranose units linked by (1—4) glucosidic bonds (Klemm et al., 2005). The major part of cellulose is bounded by hydrogen bonds and forms crystalline microfibril structure, with a minor part being amorphous. Its hemicellulose is amorphous mainly composed of polymers of xylose and arabinose with minor amount of galactose and glucose (Lavarack et al., 2002; Sun et al., 2003). Hemicellulose is linked to cellulose and lignin by covalent bonds and fewer hydrogen bonds. Lignin acts like glue and bind cellulose and hemicellulose, which in turn, makes plant lignocellulose structure more moisture resistance and difficulty to break.

Due to this structure matrix, it is difficult for enzymes to access cellulose if the material is in native form (Rivers and Emert, 1988; Palonen, 2004; Chandra et al., 2007; Öhgren et al.,

2007). Consequently, pretreatment step is required to unlock the matrix structure prior to enzymatic hydrolysis. An efficient pretreatment alters the lignocellulosic structure by opening pore size through the removal of either lignin or hemicellulose or both and thereby exposing cellulose for enzyme attack (Sun and Cheng, 2002; Zeng et al., 2007; Hendriks and Zeeman, 2009; Avira et al., 2010). Also pretreatment may reduce cellulose crystallinity thereby enhancing enzymic hydrolysis (Zhu et al., 2008).

Several pretreatment methods have been reported for lignocellulosic pretreatment (Mosier et al., 2005; Wyman, et al., 2005; Lloyd and Wayman, 2005; Alvira et al., 2010). Among these methods, dilute acid and steam explosion have been considered as economically feasible for industrial scale production because they are well established, easily controlled and could handle larger quantities of biomass for shorter time. In general, pretreatment with dilute acid and steam explosion are characterised by hydrolysing hemicellulose and leaving the pretreated solid enrich with cellulose and lignin.

However, in term of cost, both pretreatment and enzymatic hydrolysis steps are still bottle necks for industrial scale production process (Zaldivar et al., 2001; Wyman, 2007; Avira et al., 2010). Therefore, these operating costs can be reduced by selection of varieties that are easily hydrolysable and through optimisation of pretreatment conditions for the preferred varieties. Development of such selection methods will enable modification of properties of SCB that will have a positive impact on ethanol yield, such as, higher carbohydrates and lower lignin, through classical and precision breeding.

Moreover, for over 100 years, various sugarcane breeding programs have been focussing on only how to increase the sucrose per unit biomass (Bekker, 2007). With the recent knowledge of producing bioethanol from lignocellulose materials, it is also equally important to increase both fermentable sugar and fibre yields per hectare, to maximise energy production per land used. Following recent development, various research initiatives have been developed to find a way of enhancing bioethanol production from SCB, one of these initiatives is to use classical and precision breeding technologies to produce sugarcane of preferred fibre characteristics, such as, higher biomass yields per hectare and physico-chemical compositions that are easily amenable to hydrolysis, which will significantly reduce the lignocellulosic bioethanol production costs.

In this study, eight varieties of sugarcane bagasse developed by classical and precision breeding technologies were compared, the objective being to study the influence of variety type, or consistency, on dilute acid pretreatment and enzymatic hydrolysis in terms of xylose, glucose and total combined sugar yields.

2. Materials and Methods

2.1. Sugarcane bagasse

The samples of SCB were supplied by South Africa Sugarcane Research Institute (SASRI) breeding program. All varieties were planted in the same area but some were originated from classical breeding labelled 1 to 100, and the rest by precision breeding (genetic engineered) labelled 101 to 115. The sugarcane samples were milled (type of the mill) to extract juice from the SCB, the remained fibres were washed three times with water to remove all residue sucrose and other soluble sugars. Then were pressed to reduce water content and finally were dried at 40°C until dry. The average moisture content of these materials after drying was about 6%. Prior to its use, the milled SCB were sieved to obtain a representative particle size suitable for the raw material composition analysis and for pretreatment studies. The particles retained between 425 and 825 µm were packed in zipped plastic bags and then stored in a temperature and moisture controlled room set at 20°C and relative humidity, 65% until needed. The total storage time of these samples was 12 months.

2.2. Dilute sulphuric acid pretreatment

Dilute sulphuric acid pretreatment was carried out in small tubular reactor (total volume of 14.3 ml) according to Yang and Wyman (2009). 1.5 g dry material (DM) was soaked in 30 ml of dilute sulphuric acid solution or water for 12 hours. Soaked samples were concentrated through filtering to a solid loading of 30% (w/v). The obtained wet biomass was loaded into the reactor and compressed by a metal rod to ensure uniform heat and mass transfer. The reactor was first submerged into a heating-up fluidised sandbath set at 30°C above the target

temperature. The reactor was heated until the target temperature was reached (approximately within 120 seconds), after which it was transferred into the second fluidised sandbath set at the target reaction temperature. After the reaction time completed, the reactor was quenched by submerging into cold water bath. After cooling, the whole slurry was mixed with 100ml of distilled water and vacuum-filtered into a solid and a liquid fraction. One part of filtrate was analysed for monomeric sugars content and the other part was used to determine the total sugars in the pretreated liquor as monomers and oligomers by post-hydrolysis as described elsewhere ([Jacobsen and Wyman, 2002](#)). The pretreated solid was washed (water insoluble solid) to raise the pH up to 5 and then dried at 40 °C for 48 hours. All pretreatments were performed on duplicate and average results are shown.

The bagasse were pretreated at (150°C, 0.96%w/w acid for 15 minutes); (160°C, 0.96 %w/w acid for 15 minutes); (190°C, 0.07%w/w acid for 15 minutes); (200°C, no-acid for 10 minutes and (180°C, 0.5%w/w acid for 15 minutes).

2.3. Enzymatic hydrolysis

The water insoluble solid (WIS) fraction was subjected to enzymatic hydrolysis to evaluate the effect of pretreatment on the enzyme accessibility for each of SCB varieties. These experiments were conducted in 24 ml glass tubes. The tubes were loaded with 200 mg (dry weight) of WIS and 10 ml of 0.05 M citrate buffer (pH 4.8) with the enzyme solution. Sodium azide was added at a concentration of 0.02% (w/v) to prevent microbial contamination. Two commercial enzymes preparations were used: Spezyme CP (Genencor-Danisco, Denmark) with protein concentration of 140 mg/ml (cellulase activity of 65 FPU/ml) and Novozym 188 (Novozymes A/S, Denmark) with protein concentration of 95 mg/ml (β -glucosidase activity of 700 IU/ml). Protein concentration and activities of undiluted enzymes (Spezyme and Novozym 188) were determined by applying analysis protocol described elsewhere ([Garcia-Aparicio et al., 2011](#)). Cellulase loading of 32.31 mg protein/g WIS (corresponding to 15 FPU/g WIS) of Spezyme CP supplemented with β -glucosidase of 2.02 mg protein/g WIS (equivalent to 15 IU/g WIS) was applied in all the experiments. Tubes loaded with the mixtures were placed in water bath shaker maintained at 50 °C with shaking at 90 revolutions per minute. Samples were withdrawn after 72 hours, prepared as described below and analysed for sugars by High Performance Liquid Chromatography (HPLC) (method described below). All experiments were performed in duplicate.

2.4. Analysis methods

The compositions of all raw SCB were determined by the Standard Methods Analytical Procedure (LAPs) for biomass developed by NREL, USA ([Laboratory Analytical Procedure, 2007](#)). In brief, 5 g of milled and sieved samples was extracted with water for 24 hours in a Soxhlet apparatus. The water extractives free sample was then extracted with 95% ethanol for another 24 hours. The extractives free samples were dried at 40°C for 48 hours minutes. Thereafter, 0.3 g of dried sample was hydrolysed with 3 ml sulphuric acid (72% sulphuric acid) in a heating water bath set at 30°C for 60 minutes. The sample was then diluted with 84 ml of de-ionised water to make the final concentration 4% w/w H₂SO₄ and the mixture was autoclaved at 121°C for 60 minutes. The resulting mixture was filtered in a porous crucible. The filtrate was taken for monomeric sugars analysis by HPLC. The solid fraction was dried at 105°C for 12 hours and then put in the furnace set at 575°C for four hours. The remaining material was cooled in desiccators and weighed to determine the amount of insoluble lignin. Soluble lignin in the liquid fraction was measured by UV-spectrophotometer at a wavelength of 280 nm. The composition of raw material was performed in four replicates.

Liquid fractions resulting from: unpretreated and pretreated materials compositional analysis, pretreated liquor, post-hydrolysis and enzymatic hydrolysis were analysed by HPLC for sugars. The oligomers concentrations were quantified as the difference between monomeric sugars obtained before and after post-hydrolysis. Liquid fractions were analysed with an Aminex HPX-87H Ion Exclusion Column equipped with a Cation-H Cartridge (Bio-Rad, Johannesburg, South Africa). Sugars concentrations were measured with a RI detector at 220 nm and 280 nm (Shodex, RI-101) operated at 65 °C with a mobile phase of 5 mM sulphuric acid and a flow rate of 0.6 ml/min.

2.5. Statistical analysis

One-Way-Analysis of Variance (ANOVA) was determined to evaluate whether there were statistical differences on sugar yield during pretreatment and enzymatic hydrolysis processes between varieties or among pretreatment conditions. The analysis of the variance (ANOVA) was carried out using Design Expert software version 8.0.3. The hypothesis was accepted or rejected at 95% confidence interval. Likewise, the correlation coefficients were calculated using STATISTICA (software, version 10).

3. Results

3.1. Raw material composition

The chemical composition of SCB varieties from eight is summarised in Table 1. A pairwise comparison indicated no significant ($P > 0.05$) differences in the contents of: glucan, xylan, lignin and total carbohydrates between varieties 20 and 34, 55 and 89, 87 and 63, 102 and 114. When compared to varieties from classic al breeding, Table 1 shows that varieties originated from precision breeding (102 and 114) were characterised by higher xylan and higher total carbohydrates. In addition, varieties from precision breeding were also characterised by lower lignin than classical breeding varieties (20, 34, 55, 57, 63 and 89). However, no significant difference was observed on average glucan content between varieties 57, 63, 102 and 114. Because varieties 20 and 34, 55 and 89, 87 and 63, 102 and 114 did not show significant compositional difference were then pretreatment and enzymatic hydrolysis responses to assess the influence of the variety.

Table 1 Average compositional analysis of eight varieties of sugarcane bagasse. (A) Chemical composition % dry weight. (B) The ANOVA analysis to test the statistical difference on bagasse composition between varieties at a significance level of 0.05

Variety ID	20	34	55	89	57	63	102	114
A: Chemical composition								
Glucan (%)	39.2	38.8	37	36.6	42.3	42.9	42.5	42.6
Xylan (%)	28.0	28.3	27.9	27.6	26.7	26.4	32	30.9
Total carbohydrates (%)	69.4	69.6	67.8	66.6	70.8	71.7	77.0	76.7
Lignin (%)	22.8	22.6	20.4	21.6	17.4	17.7	16.2	16.1
B: ANOVA t-test								
	20 vs 34		57 vs 63		55 vs 89		102 vs 114	
Glucan	0.6098		0.3326		0.8162		0.8387	
Xylan	0.6135		0.685		0.8873		0.1316	
Total carbohydrates	0.4641		0.052		0.1966		0.0711	
Lignin	0.7344		0.3428		0.0554		0.8712	

The components with p-value less than 0.05 are considered to be significantly difference between the varieties at 95% confidence interval

3.2. Effect of pretreatment on sugarcane bagasse varieties

Hemicellulose solubilisation is a prime indicator of the dilute acid pretreatment efficiency. In this study, xylose yield was used to describe hemicellulose solubilisation because xylan was the major component in the SCB hemicellulose. The average xylose yield is depicted in Table 2, with the values ranging from 19.6 g/100g DRM at (160°C, 0.96%, for 15 min) to 7.3g/100g RM at (200°C, no acid, for 15 min). At (200°C, no-acid for 10 min) pretreatment condition, xylose yield was relatively low, in particular for precision breeding varieties compared to classical breeding varieties. At this condition (200°C, no-acid for 10

min), the low xylose yield observed is due to the fact that hemicellulose hydrolysis depends on organic acid (acetic acid or formic acid) liberated from the biomass during pretreatment to increase the hydrogen ion that drives the hydrolysis (Jacobsen and Wyman, 2002). Moreover, xylose yield among varieties was substantially enhanced when the temperature was changed from 150 to 160°C. No significant improvement was observed when the temperature was increased from 160°C to 180°C and the acid loading lowered to 0.5%. Temperatures higher than 190°C implied the reduction of xylose yield even at low acid loading (0.07%).

An interesting observation here is how varieties with similar chemical composition responded differently in respect to pretreatment conditions. For example, in more than two pretreatment conditions, variety 34 released significantly higher xylose compared to xylose release by variety 20. At 180°C, 0.5% for 15 min these varieties (20 and 34) showed similar xylose yield. Similar results were observed when varieties 55 and 89, 57 and 63, 102 and 114 were compared. In general, the variety type did not seem to consistently impact xylose yield. However, the xylose yield variability between varieties was significantly influenced by temperature and acid loading.

Table 2 Total xylose yields of eight varieties of sugarcane bagasse at different pretreatment conditions. (A) Xylose yields (g/100 g dry raw materials). (B) The ANOVA analysis to test the statistical difference on xylose yield between varieties with similar chemical composition at a significance level of 0.05

Variety ID	20	34	55	57	63	89	102	114
A	Xylose yield (g/100g dry raw materials)							
150°C, 0.96%, 15 min	12.7	15.4	13.5	13.3	7.5	8.4	8.0	16.0
160°C, 0.96%, 15 min	14.8	17.4	17.0	17.1	14.8	17.0	16.1	19.6
180°C, 0.5%, 15 min	14.5	13.9	15.2	15.6	14.3	16.3	16.9	15.1
190°C, 0.07%, 15 min	7.0	10.8	8.3	9.8	11.0	14.3	15.7	15.7
200°C, no-acid, 10 min	12.3	12.8	11.1	12.0	9.7	9.9	7.4	7.3
B	ANOVA t-test, p-values							
	20 vs 34	57 vs 63	55 vs 89	102 vs 114				
150°C, 0.96%, 15 min	0.0089	0.0001	0.0002	0.0001				
160°C, 0.96%, 15 min	0.0059	0.0127	0.9614	0.001				
180°C, 0.5%, 15 min	0.6243	0.0232	0.3278	0.047				
190°C, 0.07%, 15 min	0.0004	0.1108	0.0001	0.9999				
200°C, no-acid, 10 min	0.4558	0.0041	0.0738	0.9046				

The bold values are p-values less than 0.05. The p-value less than 0.05 are considered to be significantly difference between the varieties at 95% confidence interval.

In terms of xylose removal (xylose removal calculated as: xylose content in the raw SCB minus xylose in the pretreated solid and the results obtained is divided to the xylose content in the raw material of that particular variety), the highest removal was 93% at (180°C, 0.5%, 15 min) for variety 57 and the lowest was 74.1% at 150°C, 0.96%, 15 min for variety 102. Xylose removal was enhanced by the increase of pretreatment severity until certain extent where there is degradation of pentose into by-products and other low molecular weight products. The highest xylose recovery by achieved here was 64% for variety 57 at (160°C, 0.96%, 15 min) and a lowest was 23% for variety 102 at (200°C, no-acid, 15 min).

3.2.1. Enzymatic hydrolysis of pretreated sugarcane bagasse varieties

The effect of different pretreatment conditions was assessed in terms of glucose yield from enzymatic hydrolysis of the obtained pretreated solids. The glucose yield after enzymatic hydrolysis of the pretreated solids is depicted in Table 3. The untreated raw bagasse glucose yield ranged from 6.6—14.6 g/100g DRM with the highest being on variety 63 and variety 20 gave the lowest value. The highest glucose yield of pretreated solid was 32.6 g/100g DRM for variety 102 at (160°C, 0.96%, for 15 min) and lowest was 12.5 g/100g DRM for variety 57 at (150°C, 0.96%, for 15 min). Glucose yield was substantially enhanced when temperature was raised from 150°C to 160°C. For example at 150°C, glucose yield for variety 57 was 12.5 g/100g RM and was improved to 18.8 and 21.2 g/100g DRM when the temperature was increased to 160 and 180°C, respectively. In addition, glucose yield among varieties was also enhanced at (200°C, no-acid, for 10 min) pretreatment condition compared to the results obtained at (160°C, 0.96%, for 15 min). It was interesting also to see high glucose yield when no acid was used (200°C, for 10 min) compared to xylose yield (Table 2).

Table 3 Average glucose yields at 15 FPU/g WIS of eight varieties of sugarcane bagasse at different pretreatment conditions. (A) Glucose yields (g/100 g dry raw materials). (B) The ANOVA analysis to test the statistical difference on glucose yield between varieties with similar chemical composition at a significance level of 0.05

Variety ID	20	34	55	57	63	89	102	114
A								
	Glucose yield at 15 FPU/g WIS (g/100g dry raw materials)							
Untreated	6.6	11.9	6.7	8.3	14.6	8.7	12.4	7.8
150°C, 0.96%, 15 min	15.3	18.3	15.0	12.7	25.6	16.6	25.2	27.8
160°C, 0.96%, 15 min	19.8	21.7	18.4	18.8	26.0	18.2	29.5	27.6
180°C, 0.5%, 15 min	22.9	31.5	27.6	21.2	29.8	27.0	32.6	25.4
190°C, 0.07%, 15 min	20.4	25.0	27.1	22.4	22.6	20.6	23.6	24.5
200°C, no-acid, 10 min	19.5	29.1	28.7	18.8	30.4	25.0	27.2	32.3
B								
	ANOVA t-test, p-values							
	20 vs 34		57 vs 63		55 vs 89		102 vs 114	
Untreated	0.0001		0.0001		0.0063		0.0002	
150°C, 0.96%, 15 min	0.0498		0.0001		0.2536		0.0486	
160°C, 0.96%, 15 min	0.0496		0.0001		0.757		0.054	
180°C, 0.5%, 15 min	0.0001		0.0001		0.5587		0.0001	
190°C, 0.07%, 15 min	0.0034		0.889		0.0004		0.4969	
200°C, no-acid, 10 min	0.0001		0.0001		0.0012		0.0002	

The bold values are p-values less than 0.05. The p-value less than 0.05 are considered to be significantly difference between the varieties at 95% confidence interval.

The glucose yields of varieties with similar chemical composition showed interesting results. Variety 34 was more digestible than variety 20 at all pretreatment conditions investigated. Similar observation was obtained when 63 and 57 also were compared. Nevertheless, there was glucose yields did not show consistence when varieties 55 and 89, 102 and 114 were compared, indicating the glucose yield to these varieties was not influenced by variety type. The results also indicate that, precision breeding varieties released relatively higher sugar compared to classical breeding varieties.

3.2.2. The combined sugar yield of sugarcane bagasse varieties

The combined sugar yield of eight varieties of sugarcane bagasse pretreated at five different conditions and pretreated solid hydrolysed by enzyme at a dosage at 15 FPU/g WIS is depicted in Table 4. The highest combined sugar was 54.4 g/100g RM for variety 102 at (180°C, 0.5%, 15 min) and the lowest was 28.5 g/100 g RM for variety 89 at (150°C, 0.96%, 15 min). The sugars yield was improved significantly when the temperature was changed from 150 to 160°C (same heating time and acid loading) for instance variety 20, combined sugars yield was increased by 22.6%. In general, based on the overall average the yield at each pretreatment condition, no significant improvement was observed when pretreatment condition was changed from (160°C, 0.96%, 15 min) to (180°C, 0.5%, 15 min). However, for some varieties for instance 34, 55 and 89, the combined sugar yield was substantially enhanced compared to variety 114, where the yield was significantly reduced. The yield was also substantially lowered at (190°C, 0.07%, for 15 min) compared to the yield obtained at (160°C, 0.96%-acid, for 15 min). Similar trend was observed when the yield obtained at (200°C, no-acid for 10 min) and (160°C, 0.96%-acid, for 15 min) were compared. It was also interesting to see varieties 34, 63, 102 and 114, consistently, released higher combined sugar yield than varieties 20, 55, 57, and 89.

Table 4 Average combined sugar yields of eight varieties of sugarcane bagasse at different pretreatment conditions. (A) Combined sugar yield (g/100g DRM). (B) The ANOVA analysis to test the statistical difference on combined sugar yield between varieties with similar chemical composition at a significance level of 0.05

Variety ID	20	34	55	57	63	89	102	114
A	Combined sugar yield (g/100g dry raw materials)							
Unpretreated	10.5	16.7	10.5	12.6	20.2	12.2	17.6	11.6
150°C, 0.96%, 15 min	32.3	39.1	33.4	30.8	37.7	28.5	38.2	51.6
160°C, 0.96%, 15 min	39.6	46.4	43.1	44.1	49.1	39.8	54.1	53.7
180°C, 0.5%, 15 min	41.6	51.0	49.0	43.1	51.7	48.4	54.4	48.1
190°C, 0.07%, 15 min	34.2	42.5	40.3	38.0	39.5	41.5	44.9	49.5
200°C, no-acid, 10 min	36.7	47.7	44.2	39.8	46.0	39.3	47.2	45.2
B	ANOVA t-test, p-values							
	20 vs 34	57 vs 63	55 vs 89	102 vs 114				
Unpretreated	0.0001	0.0001	0.0352	0.0002				
150°C, 0.96%, 15 min	0.0001	0.0001	0.0001	0.0001				
160°C, 0.96%, 15 min	0.0001	0.0001	0.0007	0.5334				
180°C, 0.5%, 15 min	0.0001	0.0001	0.3222	0.0001				
190°C, 0.07%, 15 min	0.0001	0.1285	0.2065	0.0007				
200°C, no-acid, 10 min	0.0001	0.0001	0.0001	0.0234				

The bold values are p-values less than 0.05. The p-value less than 0.05 are considered to be significantly difference between the varieties at 95% confidence interval.

4. Discussions

4.1. SCB chemical composition

Characterisation of lignocellulose to identify its components is a fundamental criterion to assess the suitability of the feedstock for bioethanol production. Varieties 57, 63, 102 and

114 had highest glucan content than other varieties. Higher glucan content is more beneficial to ethanol production because (currently) glucose can be converted to ethanol at a high yields than other types of sugars for instance xylose. The varieties from precision breeding technology showed higher xylan content, with lower lignin contents compared to those from classical breeding technology (Table 1). This finding indicates that the use of precision breeding technology probably could reduce lignin contents, leading to lower pretreatment requirements. Improving total carbohydrates could also increase bioethanol production per unit biomass. Huang et al., (2009) reported an increase in ethanol production (linearly) with increase in total polymeric sugars components of the fibre.

4.2. Pretreatment and enzymatic hydrolysis responses

This study has demonstrated the use of dilute acid as effective way pretreatment responses of different varieties of SCB. Xylose yield signified hemicellulose hydrolysis. The varieties with similar chemical composition indicated significant changes in xylose yields at the same pretreatment condition (Table 2). However, there was no clear consistence variety type factor or composition factors contributed these variations. It was also interesting to note that higher xylan content in the raw SCB did not enhance xylose yield (Tables 1, 2). This finding infers within the range of conditions tested, xylose yield did not depend on one variety type or higher xylan content in the biomass alone rather the combination of components factor contributed to the observed differences. It was also not possible to differentiate the two breeding technology just based on pretreatment results.

The enzymatic hydrolysis response of both untreated and pretreated SCB varieties showed significant variation on glucose yield (Table 3). Without pretreatment, the glucose conversion of different SCB varieties was between 14–31%. The glucose yield was substantially improved after pretreatment, representing a conversion of between 28 and 78% of the possible glucose in the respective WIS. However, varieties 20, and 57 consistently gave lower glucose yield before and after pretreatment compared to other varieties like 34, 63, 102, and 114. The recalcitrant of some of the varieties like 20 and 57 to enzymatic hydrolysis indicates that the pretreatment conditions applied were inadequate to unlock their respective structures for higher enzymatic hydrolysis response. Their respective glucose yields were improved by increasing the pretreatment severity. Evidently, more severe pretreatment conditions were required to enhance their subsequent enzymatic hydrolysis response.

The effect of variety type could also contribute to the observed glucose yield variability (Table 3). For instance glucose yield of variety 34 was substantially higher when compared to the yield of variety 20 (both these varieties were classical breeding with similar fibre composition). Similarly, variety 63 was significantly more digestible than variety 57. However, no clear consistence on glucose yields when varieties of precision breeding (102 and 114) were compared. In essence this finding show that glucose yield was not only affected by fibre composition variability and by breeding technology applied but also depended on variety type factor and hence brought obvious glucose yield variations.

The total sugars yield of untreated SCB varieties after enzymatic hydrolysis showed significant variation, 10.5–20.2 g/100 g DRM, corresponding to 10–28.2% of the total carbohydrates presents in the variety 20 and 63, respectively. The combined sugar yield was significantly improved after pretreatment. The highest yield obtained being 54.4 g/100 g RM, corresponding 70% of total carbohydrates in variety 102. Glucose yield after enzymatic hydrolysis was the major constituent in the combined sugar yield followed by xylose. Thus, the low digestibility of some of varieties like 20 and 57 substantially lowered their combined sugar yields. The pretreatment at low severity condition (150°C, 0.96%, for 15 minutes) enhanced xylose yield but failed to liberate high glucose upon enzymatic hydrolysis, thus, lowered the combined sugar yield (Tables 2, 3, 4). The combined sugar yield was significantly improved at higher pretreatment severity (180°C, 0.5%, for 15 minutes). Therefore, mutual balance of pretreatment and enzymatic hydrolysis steps is very essential to maximise both xylose and glucose and minimise by-product formation (HMF and furfural), hence, maximising the total sugar yield. The use of dilute sulphuric acid is necessary to boost pentose sugars recovery, consequently, increasing ethanol production provided that the yeast capable of fermenting pentoses is well developed.

5. Conclusions

Eight varieties of sugarcane bagasse of similar chemical composition from classical and precision breeding technologies were evaluated for their response to dilute acid pretreatment and enzymatic hydrolysis. This work has clearly established how variety type factor could substantially impact sugar yields during pretreatment and enzymatic hydrolysis. However, the variation of xylose yields was not always consistent with the variety type factor and fibre composition, breeding technology. The contribution of variety type to digestibility variations was statistically significant for most of classical breeding varieties assessed and was less consistent to precision breeding varieties. The findings obtained from this study have significant contribution to the sugarcane development with the aim of selecting sugarcane with highly hydrolysable fibres in conjunction with high biomass and sucrose yield per hectare to make lignocellulose to ethanol process affordable.

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