Use of Sugarcane Bagasse with Different Particle Sizes to Determine the Relationship between Physical Properties and Enzymatic Hydrolysis

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The supramolecular structures of a substrate, such as crystallinity, specific surface area, average pore size, and cellulase adsorption capacity, *etc.*, affect the enzymatic hydrolysis of a lignocellulosic biomass. It is unclear which of these factors is most important for efficient hydrolysis. To eliminate the influence of the hemicellulose content and the lignin, sugarcane bagasse samples with the same cellulose, hemicellulose, and lignin content but with different particle sizes were used as substrates to investigate the relationship between physical properties and enzymatic conversion efficiency. When the content of hemicellulose and lignin was not significantly different, the decrease in the crystallinity index (CrI) and the increase in the specific surface area (SSA), cellulase adsorption, average pore size, and the cellulase adsorption per SSA could give rise to higher enzymatic convertibility. The effects of the CrI and the average pore size were more pronounced than the effects of the SSA, the cellulase adsorption capacity, and the cellulase adsorption per SSA. According to the developed formula, the CrI was more influential than the average pore size under the specific conditions.

Keywords: Cellulase; Particle size; Crystallinity; Average pore size; Glucose yield; Sugarcane bagasse

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INTRODUCTION

Biofuels from lignocellulosic biomass, such as bioethanol and bio-butanol, are promising alternatives to fossil fuels. Lignocellulose must be hydrolyzed before it can be converted into fuels by fermentation. The efficient hydrolysis of lignocellulosic materials requires an effective pretreatment to change the structure, chemical composition, degree of polymerization, crystallinity, specific surface area, and enzyme adsorption capacity of the selected material (Lee *et al*. 1982; Yeh *et al*. 2010; Del Rio *et al*. 2012). It is difficult to determine which of these factors has the greatest impact on the enzymatic hydrolysis of a lignocellulosic biomass, as almost all of them change during chemical pretreatments (Lee *et al*. 1982; Mansfield *et al*. 1999; Kumar and Wyman 2009; Li *et al*. 2014b).

The cellulose in lignocellulosic biomass is composed of complex crystalline and amorphous regions. The degree of crystallinity affects the efficiency of enzymatic hydrolysis. The amorphous region is hydrolyzed rapidly by cellulases into monosaccharides or oligosaccharides, while the crystalline region is more recalcitrant to cellulolytic attack (Zhang and Lynd 2004). Thus, disorder in the crystalline region is beneficial for efficient saccharification (Ghose and Bisaria 1979; Bertran and Dale 1985).

For example, the high initial rate of hydrolysis of ball milled cotton cellulose is attributed to its reduced particle size and crystallinity index (Yeh *et al*. 2010). The crystallinity index is more influential than specific surface area on the rate of hydrolysis and the degree of polymerization when microcrystalline cellulose is pretreated by combined ball milling and microwave irradiation (Peng *et al*. 2013). However, the increased crystallinity of calcium hydroxide pretreated corn stover did not negatively affect the sugar yield on enzymatic hydrolysis (Kim and Holtzapple 2006). In another instance when different samples were subjected to enzymatic hydrolysis, the sample with the highest initial degree of crystallinity exhibited the highest conversion yield (Peciulyte *et al*. 2015). Hence, the effect of crystallinity on lignocellulose hydrolysis is still controversial.

In addition to crystallinity, specific surface area (SSA) also affects enzymatic hydrolysis (Zhu *et al*. 2009). In pretreated wheat straw, SSA is more influential than CrI and lignin content (Gharpuray *et al*. 1983). However, Peng *et al*. (2013) found that the CrI was more influential than the SSA and the degree of polymerization. Thus, the role of the SSA during enzymatic hydrolysis of biomass is still unclear, possibly because both the chemical composition and the physical structures change at the same time.

Cellulase adsorption on cellulose is important for hydrolysis (Lee *et al*. 1982; Klyosov *et al*. 1986; Piccolo *et al*. 2010; Wiman *et al*. 2012). However, pretreatments alter many parameters, such as the chemical composition and the supramolecular structure, and it is difficult to discern which changes lead to enhanced enzymatic conversion.

In this study, sugarcane bagasse samples with different particle sizes were used in order to eliminate the influence of chemical composition. Two different milling techniques incorporated screen fractionation to obtain sugarcane bagasse (SCB) with different particle sizes. The supramolecular structure parameters such as CrI, SSA, cellulase adsorption, and average pore size were determined. The initial rate of enzymatic hydrolysis and glucose yield was measured, and their dependence on the supramolecular structure parameters were investigated. This strategy helped to differentiate the effects of different physical parameters on enzymatic hydrolysis.

Table 1. Cellulose, Hemicellulose, and Lignin in Fractionated Samples

Sample	Cellulose (%)	Hemicellulose (%)	Lignin (%)
HMR200	37.74 ± 0.48	25.32 ± 0.17	27.92 ± 5.49
HMR300	39.05 ± 0.38	27.02 ± 0.89	23.95 ± 1.73
HMR400	39.06 ± 0.92	27.40 ± 1.41	21.86 ± 3.79
HMP400	37.72 ± 0.58	26.77 ± 1.22	25.48 ± 1.92
JMP400	36.99 ± 0.32	26.91 ± 0.67	22.85 ± 1.19

Note: Each point in the table is a mean value ± standard deviation from three independent experiments, reprinted with permission from Li *et al*. (2014a)

EXPERIMENTAL

Materials

Sugarcane bagasse (SCB) was acquired from the Qianwu Sugar Refinery, Zhuhai, Guangdong, China. It was air-dried and then stored at room temperature in woven bags. The SCB was ground with a hammer mill (HM) (FY130, Tianjin Taisitie Instrument Co., Ltd, China) or a jet mill (JM) (Jin and Chen 2006) (NADA Superfine Tech. Co., Ltd, Nanjing, China). A very small amount of the samples, which were passed through a 400-mesh sieve (P400), were obtained by the HM. Therefore, a 400-mesh sieve was used in the

jet mill to produce a larger amount of P400. Sodium citrate, citric acid, sulfuric acid, glucose, and xylose were of analytical grade. Table 1 shows the cellulose and hemicellulose contents, which were determined as described by Sluiter *et al*. (2008) and previously published in Li *et al*. (2014a).

The enzyme activities of Celluclast 1.5 L and Novozym 188 (both kindly provided by Novozymes, Beijing, China) were 83.4 FPU/mL and 748 IU/mL, respectively, as measured by the methods of Adney and Baker (1996) and Fujita *et al*. (2002).

Particle Size Fractionation

Particle size fractionation was carried out on a power-driven sieve shaker (8411, Shangyu Screen Factory, Zhejiang, China) equipped with 100-, 200-, 300-, and 400-mesh sieves corresponding to the sieve pore diameters of 150 μm, 75 μm, 48 μm, and 38 μm, respectively. The fractions were named according to their retention on (R) or passage through (P) each specific sieve number. For example, the fraction retained by a 100-mesh sieve was R100, while the fraction that passed through the 400-mesh sieve was P400. Briefly, the stainless steel sieves (20 cm diameter × 5 cm height) were stacked within the grooves on the bottom, with the sieve size increasing from top to bottom (38 μm, 48 μm, 75 μm, and 150 μm). About 150 g of the milled sugarcane bagasse was placed into the top sieve. The sieves were shaken until no more particles passed through. All the prepared samples were stored at room temperature in sealed plastic bags.

Scanning Electron Microscopy (SEM)

Microscopic analysis was carried out as described by Cheng *et al*. (2007). Briefly, the samples were mounted on aluminum stubs and sputtered with a thin gold layer (model SCD005, BAL-TEC, Balzers, Liechtenstein). The prepared samples were imaged using a Philips XL-30ESEM scanning electron microscope (Eindhoven, Netherlands).

Crystallinity Measurement

The crystallinity of the samples was determined using a D8 ADVANCE X-ray diffractometer (Bruker, Karlsruhe, Germany) operated at 40 kV, 40 mA, Cu/Kα radiation (1.54 Å), and a 2θ range of 10° to 40° with a step size of 0.06°. The crystallinity index (*CrI*) of the biomass was calculated with the empirical peak-height method (Segal *et al*. 1959),

$$\boldsymbol{CrI}\ (\%) = \frac{\boldsymbol{I_{crystalline} - I_{amorphous}}}{\boldsymbol{I_{crystalline}}} \times \mathbf{100} \quad (1)$$

where $I_{crystalline}$ is the maximum intensity at 2θ of 22° to 23° and $I_{amorphous}$ is the minimum intensity at 2θ of 18° to 19°.

Measurement of Specific Surface Area

The specific surface area (SSA) was determined by the Brunauer-Emmett-Teller (BET) method (Brunauer *et al*. 1938). The samples were dried at 105 °C for 6 h before analysis. The BET specific surface area was determined using a SSA analyzer (Tristar 3000, Micromeritics, USA), and the SSA was determined by nitrogen gas adsorption-desorption isotherms at the liquid nitrogen temperature of 77 K. The average pore diameters were determined from N_2 desorption at a relative vapor pressure of 0.01 to 0.99 following the Barrett-Joyner-Halenda (BJH) model (Gregg and Sing 1982).

Cellulase Adsorption

The Celluclast 1.5 L and Novozym 188 cellulases were used in adsorption experiments that were performed as previously described (Lee *et al*. 1982; Wiman *et al*. 2012). Briefly, 0.2 g of the dry sample was suspended in 5 mL of 0.1 M sodium citrate buffer (pH 4.8) in 15-mL tubes and pre-incubated at 4 °C for 1 h. The enzyme preparation (0.11 g protein/g dry matter) was added, and the mixture was incubated at 4 °C for 12 h at 100 rpm. Samples of 0.5 mL were taken from the reaction mixture periodically and centrifuged at 12000 rpm for 5 min at room temperature. The supernatant was used for the analysis of the un-adsorbed protein concentration by the modified Lowry method (Dulley and Grieve 1975) using bovine serum albumin (BSA) (Beyotime Institute of Biotechnology, Guangdong, China) as the protein standard. The protein concentrations were determined using a microplate reader (ELx 800, BioTek, Winooski, VT, USA) with 96-well plates at 750 nm. All experiments were performed in triplicate. The maximum absorbed protein was denoted as Am,pro.

Enzymatic Hydrolysis

Enzymatic hydrolysis was conducted at 2% (w/v) of the biomass in 10 mL of 0.1 M sodium citrate buffer (pH 4.8) in 25-mL tubes. Microbial growth was prevented by the addition of 80 μg/mL tetracycline and 60 μg/mL nystatin (dissolved in dimethyl sulfoxide). Dry matter (DM) was supplemented with Celluclast 1.5 L (25 FPU/g DM) and Novozym 188 (45.8 IU/g DM). The reactions were performed at 50 °C at 200 rpm in an orbital incubator (THZ-C, Shenhua Biotechnology Co., Ltd., Guangzhou, China) for 48 h. Samples of 0.5 mL were taken periodically from the reaction mixture and heated in boiling water for 10 min to stop hydrolysis. After cooling, the samples were centrifuged at 12000 rpm for 5 min at room temperature, and the supernatants were filtered through 0.45-μm membrane filters (Sartorius, Gottingen, Germany) prior to HPLC analysis. The sugar yield was calculated by the following equations,

$$\boldsymbol{Glucose}\ (\%) = \frac{\boldsymbol{Glucose\ concentration}\ (\boldsymbol{g/L})}{\boldsymbol{Solid\ loading} \times \boldsymbol{cellulose}\ (\%) \times \mathbf{1.11}} \times \mathbf{100} \quad (2)$$

$$\boldsymbol{Xylose}\ (\%) = \frac{\boldsymbol{Xylose\ concentration}\ (\boldsymbol{g/L})}{\boldsymbol{Solid\ loading} \times \boldsymbol{hemicellulose}\ (\%) \times \mathbf{1.14}} \times \mathbf{100} \quad (3)$$

where the glucose or xylose concentration was the maximum concentration after enzymatic hydrolysis, the percent cellulose or hemicellulose were drawn from Table 1, 1.11 or 1.14 is the coefficient of glucose or xylose that was converted from cellulose or hemicellulose, respectively, and the solid loading was 20 g/L.

High Performance Liquid Chromatography (HPLC)

The concentrations of glucose and xylose were quantified by HPLC on an LC-15 instrument (Shimadzu, Tokyo, Japan) equipped with an Aminex HPX-87H column and a Cation H+ Cartridge Micro-Guard column (Bio-Rad, Hercules, CA, USA) at 55 °C with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min.

Statistical Analysis

All of the experiments were conducted in duplicate, and the data are presented as the mean value ± standard deviation (SD). Statistical analyses were done using the PASW

statistics 18 software using one-way ANOVA and Tukey tests. The results were considered statistically significant at a 95% confidence interval.

RESULTS AND DISCUSSION

Scanning Electron Microscopy

Figure 1 shows the SEM micrographs of SCB with different particle sizes. The particles in the HMR200 fraction were much larger than those of other fractions, and the shape was round. The particles in the HMR300 fraction had a long rod shape. It should be noted that this fractionation method cannot obtain particles with accurate sizes, due to the fact that spheres and rods with the same diameter can both pass thorough the sieve. Compared with those in the HMR300 fraction, the particles in the HMR400 fraction had smaller lengths and widths. A clear reduction of particle size was observed in the HMP400 fraction particles which were rounder and much smaller than particles in the HMR400 fraction. The JMP400 fraction, which was obtained using a jet mill, was more homogeneous than the HMP400 fraction. The jet mill provides more severe breakage than the hammer mill. The powder is fed into a flat circular milling chamber and pressurized with air or nitrogen flowed tangentially through a venturi tube. Also, the milled biomass was separated thorough a specific sieve to obtain product with a desired particle size, which might be the reason that JMP400 was more homogenous. In sum, particle size fractionation was effective in obtaining samples with different particle sizes.

Crystallinity Index

Different milling processes affect the crystallinity of lignocellulose (Chang and Holtzapple 2000; Yeh *et al*. 2010; Silva *et al*. 2012). In this study, the crystallinity index decreased with decreased particle size (Table 2), which is in agreement with an earlier report (Yoshida *et al*. 2008). Interestingly, compared with HMP400, the crystallinity index of JMP400 sharply decreased, although both samples passed through the 400-mesh sieve. In a previous report by Yoshida *et al*. (2008), the crystallinity decreased with decreasing particle size smaller than 355 μm for both the untreated and delignified *M. sinensis*. The crystallinity of fractions smaller than 63 μm decreased by 41% compared with the 150-63 μm fraction. However, in the present study, the crystallinity of HMP400 with much smaller particle size (≤ 38 μm) only decreased by 12% compared with HMR200 (150-75 μm). It should be noted that ball milling was used in the study by Yoshida *et al*. (2008), while hammer mill was used to prepared the samples discussed above. Furthermore, the crystallinity index of JMP400 decreased by 30% compared with HMR200.

Taken together, the findings mentioned above indicate that the milling method also shows great influence on the crystallinity of lignocellulose. Yeh *et al*. (2010) investigated the effect of particle size on the crystallinity of microcrystalline cotton cellulose by using a media mill to obtain the substrates. With the increased milling time up to 60 min, milling generated smaller average particles which was accompanied by the decrease in crystallinity. When the milling time was longer than 60 min, the particle size and crystallinity did not decrease. A similar trend of smaller average particle size accompanied by decreased crystallinity was observed in the present study (Table 2). It seems that crystallinity is a dependent factor of particle size when the substrate is pretreated by milling.

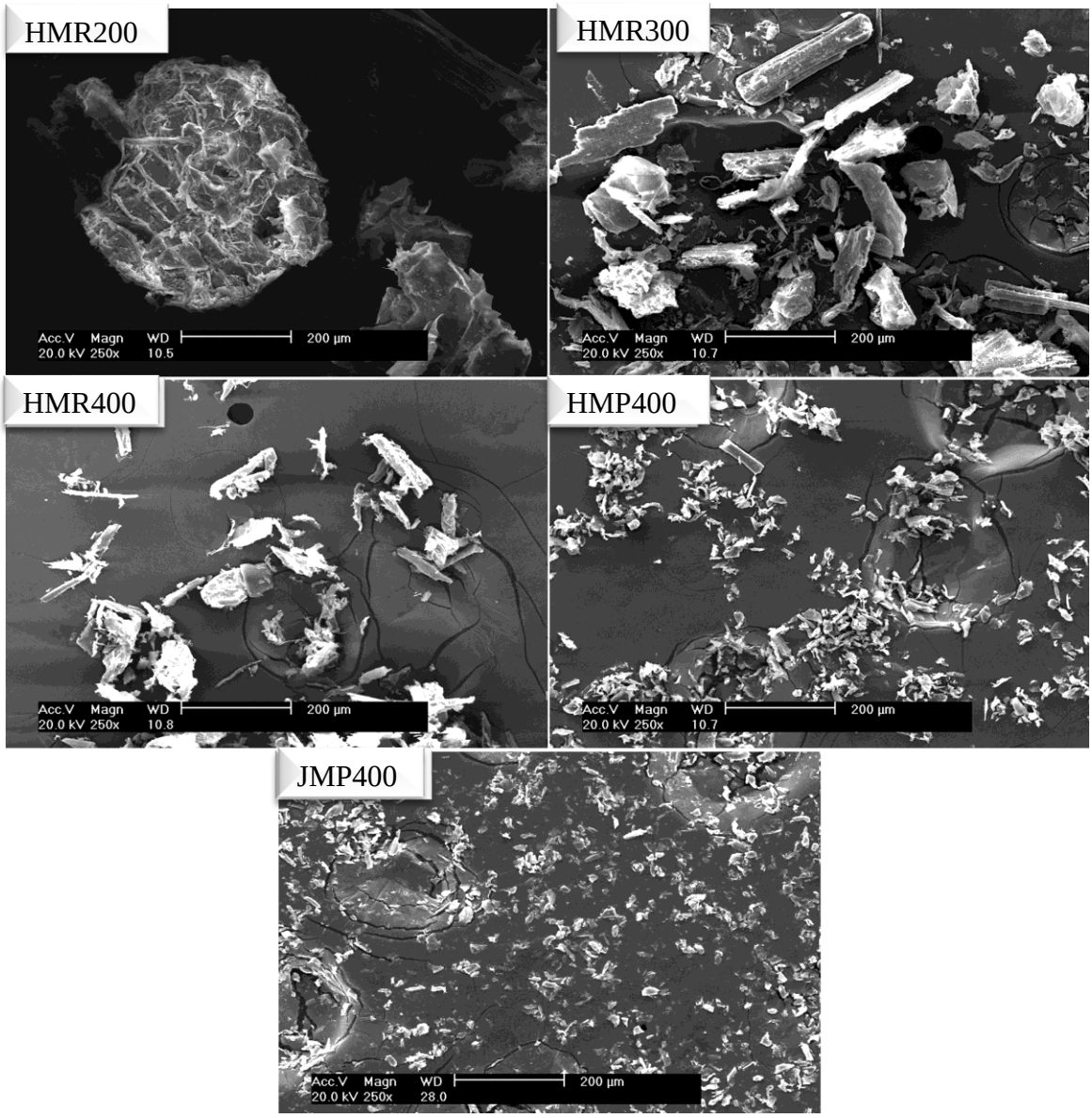

Fig. 1. Scanning electronic microscope images of sample fractions HMR200, HMR300, HMR400, HMP400, and JMP400. HM, prepared by a hammer mill; JM, prepared by a jet mill

Table 2. Characteristics of Sugarcane Bagasse Powder and Monosaccharide Hydrolysis

Sample	Particle size (µm)	CrI (%)	Glucose release rate (g/L/h)*	Glucose yield (%)	Xylose release rate (g/L/h)*	Xylose yield (%)
HMR200	75 to 150	48.95	0.11±0.00a	23.57±0.46a	0.03±0.00a	9.21±0.23a
HMR300	48 to 75	47.86	0.13±0.00a	28.21±0.04b	0.04±0.00b	12.23±0.01b
HMR400	38 to 48	43.86	0.17±0.01b	35.99±0.94c	0.06±0.00c	16.24±0.71c
HMP400	≤38	43.08	0.18±0.01b	35.59±1.35c	0.06±0.00c	15.91±0.51c
JMP400	≤38	34.11	0.23±0.01c	40.01±0.96d	0.06±0.00c	16.22±0.23c

* Monosaccharide hydrolysis rates were calculated from the sugar concentration at 12 h. Different letters within the same column indicate significant difference ($P < 0.05$).

Specific Surface Area

The accessible surface area (ASA) for cellulase also affects enzyme efficiency. The ASA is related to several factors that are altered by pretreatments, including porosity structure factors (the pore size, volume, and SSA), chemical composition, and cellulose structure factor (Liu *et al*. 2015). Thus, SSA was investigated in the milled and fractionated SCB. Samples with a smaller particle size showed increased SSA. The increase in SSA of HMR300, HMR400, and HMP400 was 1.48-, 1.49-, and 1.60-fold, respectively, that of HMR200 (Table 3). Similar to the pattern of CrI, the SSA of JMP400 was 2.17- and 1.35-fold higher than that of HMR200 and HMP400, respectively. The BJH adsorption average pore diameter is also presented in Table 3. The average pore diameter increased with decreased particle size. Compared with HMP400 and HMR200, the average pore diameter of JMP400 was increased by 1.87- to 3.27-fold. A higher SSA often accompanies a higher average pore diameter, which was also observed by other researchers (Liu *et al*. 2015).

Table 3. Enzyme Adsorption Capacities of Sugarcane Bagasse Powder

Sample	SSA (m^2/g)	Am,pro (mg/g SCB)	Am,pro/SSA (mg/m^2)	BJH Adsorption average pore diameter (nm)
HMR200	0.99 ± 0.00	7.51 ± 3.97	7.55	14.74
HMR300	1.47 ± 0.01	22.90 ± 13.53	15.59	17.08
HMR400	1.48 ± 0.00	28.85 ± 9.28	19.50	22.60
HMP400	1.59 ± 0.00	37.67 ± 12.12	23.69	25.85
JMP400	2.15 ± 0.02	75.80 ± 15.07	35.22	48.24

Am,pro: maximum total protein adsorption, SSA: specific surface area
Each point in the table is a mean value ± standard deviation from two independent experiments

Cellulase Adsorption

Cellulase adsorption on a solid cellulose substrate is an important parameter governing the enzymatic hydrolysis rate (Lee *et al*. 1982). Cellulases initially adsorb quickly onto the substrate (Ryu *et al*. 1984). The source of cellulose, pretreatment method, crystallinity, and SSA affect cellulase and xylanase adsorption (Lee *et al*. 1982), which results in different enzymatic conversion. The enzyme adsorption on SCB particles was analyzed (Table 3). As expected, the samples with smaller particles or higher SSA adsorbed more cellulase. JMP400 showed the strongest cellulase adsorption ability. Compared with HMR200, the adsorbed protein of HMR300, HMRR400, HMP400, and JMP400 was increased by 3.05-, 3.84-, 5.02-, and 10.09-fold, respectively. The increased protein adsorption was attributed partly to the increased specific surface area, as SSA and protein absorption displayed a linear relationship. The amount of protein adsorbed per area was also increased. Compared with HMP400, JMP400 showed higher protein adsorption and higher adsorbed enzymes per m^2.

Enzymatic Hydrolysis

Enzymatic hydrolysis by Celluclast 1.5 L and Novozym 188 was investigated (Fig. 2). The glucose yield from the hydrolysis of HMR300 was significantly higher than that from HMR200. Additionally, the glucose yield from HMR400 was significantly higher than that from both HMR200 and HMR300. However, glucose released from HMR400 and HMP400 was not significantly different although the particle size of the two samples was different. The JMP400 showed the highest cellulose digestion among all samples. The pattern for xylose formation was not the same as that for glucose. The xylose from JMP400,

HMR400, and HMP400 was not significantly different while was significantly higher than xylose from HMR200 and HMR300. The size reduction from 200-mesh to 400-mesh enhanced xylose formation greatly.

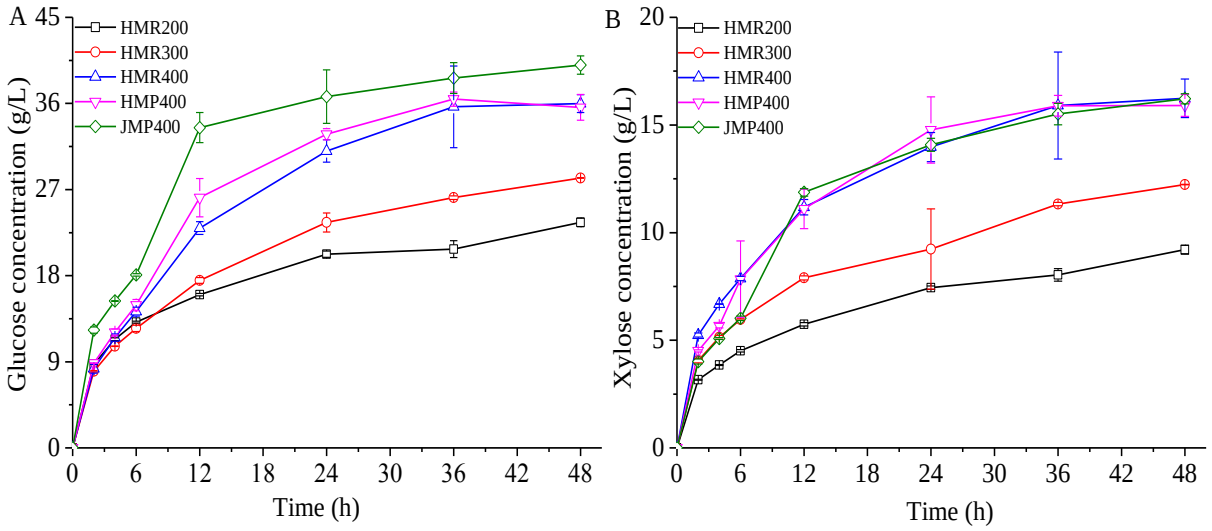

Fig. 2. Sugar release during enzymatic hydrolysis. Hydrolysis conditions: substrate, 20 g/L; Celluclast 1.5 L, 25 FPU/g; Novozym 188, 45.8 IU/g substrate; 50 °C; 48 h; and 200 rpm

The glucose and xylose release rates during the first 12 h are shown in Table 2. The glucose release rate during the first 12 h of HMR200 and HMR300 was the same. For the other samples, the glucose release rate during the first 12 h increased with the reduced particle size. The glucose yield at 48 h was also higher with smaller particles. Furthermore, JMP400 obtained a higher glucose release rate and glucose yield compared with HMP400. Under the given conditions, the glucose yield of JMP400 reached 40.01%. The xylose release rate in the first 12 h increased with decreased particle size until 48 μm. The xylose yield also showed the same pattern. The xylose yield for all the samples was lower than 20%.

Glucose yield increased with decreased crystallinity (Fig. 3). Compared with HMR200, the CrI of HMR300 was reduced by 2.23%, correspondingly the increase in glucose yield was 19.67%. The CrI of HMR400 deceased by 8.3% compared with HMR300, with the glucose yield increased by 27.57%. Meanwhile, a CrI decrease of 20.82% resulted in a 12.43% increase of glucose yield if HMP400 and JM400 were compared. The CrI between HMR400 and HMP400 was almost the same, which led to no significant enhancement of glucose yield. Although no proportional linear relation was found between CrI and cellulose convertibility, decrease of CrI gave increased glucose yield. The CrI became less and less influential to the glucose yield when it was further decreased to lower than 34.11 (Fig. 3).

The increase in SSA gave rise to an increased glucose yield (Fig. 3). A 1.48-fold increase in the SSA of HMR300 compared with HMR200 only gave an increase of 19.67% in glucose yield. However, the same SSA resulted in a great increase (27.57%) in glucose yield when HMR400 and HMR300 were compared. Another sharp increase in SSA (1.35-fold) in the JMP400 sample compared with HMP400 contributed to an increase in glucose yield of 12.43%. This result suggests that the increase in SSA could generally enhance the cellulose digestibility. However, the correlation between the two was not profound when the SSA was in the range from 1.48 to 1.59.

In another report (Peciulyte *et al*. 2015) no correlation was found between the conversion yield and SSA in substrates resulting from different types of chemical pretreatments, which may be because the SSA (> 90 m^2/g) was beyond its critical value. Furthermore, different pretreatment technologies lead to products with different chemical compositions and altered physical properties, which influence enzymatic hydrolysis concurrently. Gharpuray *et al.* (1983) developed a formula to describe the relationship between the extent of hydrolysis at 8 h and SSA, CrI, and lignin content for pretreated wheat straw with peracetic acid, caustic soda, ethylene glycol, or milling. According to the formula, SSA is the most influential structural feature. The SSAs of the samples in that study are almost in the same range as those reported here. However, no correlation was found with the SSA from 1.48 to 1.59 in the present work. It is possibly because different pretreatments were used in that study (Gharpuray *et al.* 1983) and the present study.

The relationship between glucose yield and cellulase adsorption was also plotted (Fig 3). Compared with HMR200, the increase of Am,pro and Am,pro/SSA of HMR300 by 3.05- and 2.06-fold give 1.20-fold glucose yield. However, a slight increase of Am,pro of HMR400 compared with HMR300 (1.26-fold) resulted in an 1.28-fold increase in glucose yield. Further increase in Am,pro, in relation to HMR400 and HMP400, gave rise to the same enzymatic digestibility. The Am,pro/SSA showed a similar pattern with that of the Am,pro.

The trend that increased Am,pro results in enhanced glucose yield but not in a proportional manner could be concluded. Additionally, it should be noted that further increase of Am,pro may become less important when JMP400 and HMP400 were compared. The 24 h glucan hydrolysis rate data had a strong relationship to cellulase adsorption capacities, but 72 h yields did not relate well to the adsorption capacities (Kumar and Wyman 2009). They concluded that enzyme effectiveness dropped over time because of changes of substrates, enzyme features, and possible inhibitions.

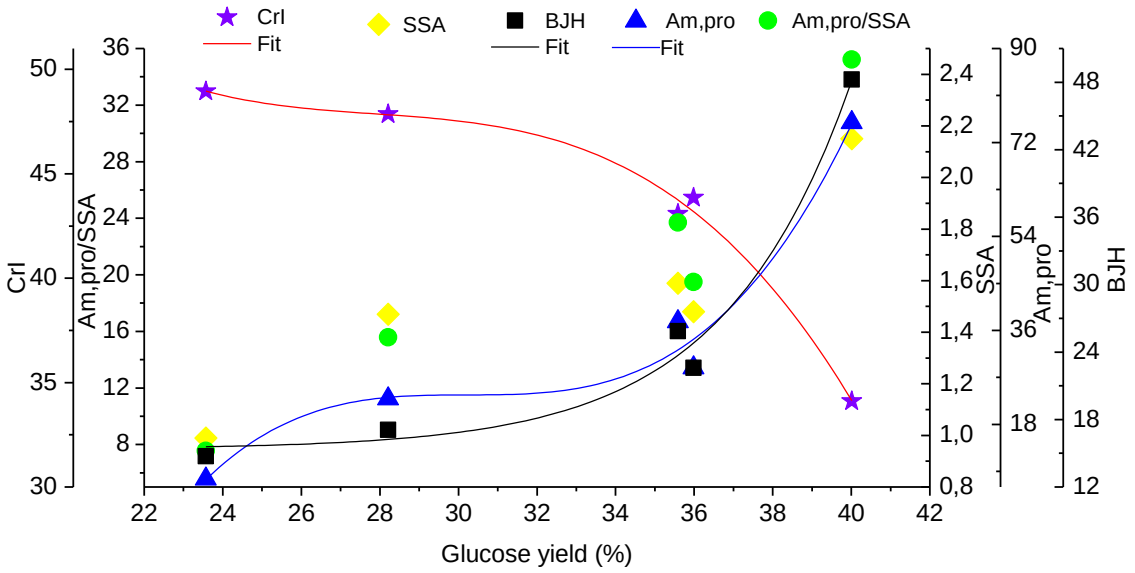

Fig. 3. Relationships between glucose yield and other parameters. Am,pro, maximum adsorbed cellulase protein on substrate; SSA, specific surface area; BJH, adsorption average pore diameter (nm); CrI, crystallinity index; Am,pro/SSA, maximum adsorbed cellulase protein per m^2 of substrate

Glucose yield was also closely correlated with the average pore diameter. When HMR300 was compared with HMR200, the increased average pore diameter gave rise to higher glucose yield (Fig. 3). The glucose yield was increased by 27.57% corresponding to an increase of average pore diameter by 32.32% (HMR400 and HMR300). For efficient hydrolysis, the enzyme accessibility to the surface of cellulosic substrates required that the diameters of the pores in the fiber walls need to be larger than the typical diameters of enzyme molecules, which are around 10 nm (Liu *et al*. 2011). Regardless of the substrate, the initial rate of hydrolysis using cellulase from *Trichoderma reesei* is linearly correlated with the pore volume of the substrate accessible to a nominal diameter of 51 Å (Grethlein 1985). Peciulyte *et al*. (2015) investigated the impact of supramolecular structure of cellulose on the efficiency of enzymatic hydrolysis, but no significant correlations were observed between the digestibility and supramolecular characteristics, such as SSA and lateral fibril dimensions. However, a strong correlation was found between the average pore size of the substrate and the enzymatic convertibility, which agrees with the results obtained in the present work. Taken together, a pore size larger than the cellulase molecules accelerates their accessibility and thus enhances enzymatic digestion.

The CrI and average pore size showed higher correlation than the others as discussed above. A formula (Equation 4) was therefore regressed to express the relationship between glucose yield, CrI, and average pore size. The square of correlation coefficient was 0.9084, indicating the model could express 90.84% variability of the response variable (Fig. 4). The formula indicated that glucose yield could be enhanced with the decrease of CrI and the increase of BJH. Additionally, the influence of CrI on the glucose yield is more obvious than that of BJH.

$$Glucose\ yield\ (\%) = 0.574(1 - \mathrm{CrI})^{0.69}(\mathrm{BJH})^{0.18} + 9.3358 \qquad (4)$$

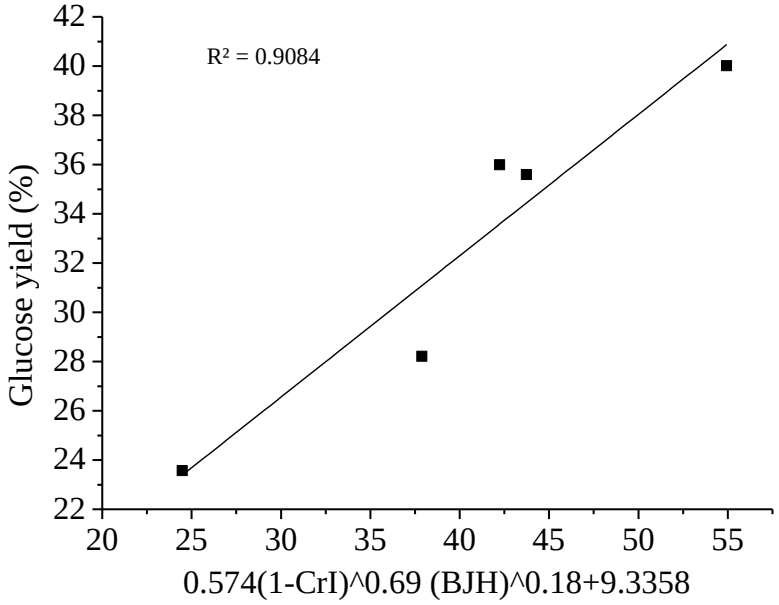

Fig. 4. Correlation of glucose yield of the theoretical after 48 h with CrI and BJH

CONCLUSIONS

1. Smaller particle size resulted in lower crystallinity index (CrI), higher specific surface area (SSA), cellulase adsorption, cellulase adsorption per SSA, and larger average pore size of sugarcane bagasse.
2. When chemical composition was not a factor, higher enzymatic convertibility was achieved by lower CrI and higher SSA, cellulase adsorption, average pore size, and cellulase adsorption per SSA of lignocellulosic biomass.
3. CrI and average pore size under specific conditions displayed strong correlation with the glucose yield.
4. The importance of all the investigated parameters for the cellulose digestibility became less according to the non-linear correlation.
5. The correlation of glucose yield, CrI, and average pore size was expressed as the following formula. $Glucose\ yield\ (\%) = 0.574(1 - \mathrm{CrI})^{0.69}(\mathrm{BJH})^{0.18} + 9.3358$

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