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Comparative study on two-step concentrated acid hydrolysis for the extraction of sugars from lignocellulosic biomass

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HIGHLIGHTS

- Two-step concentrated acid hydrolysis has been conducted with oak, pine, and EFB.
- Oak, pine, and EFB have been characterized in the compositional and XRD analysis.
- Crystalline structure change in raw biomass has been clearly shown in XRD analysis.
- Optimum condition was selected on the highest sugar recovery in the shortest time.
- Pine has the highest recalcitrance based on the CrI and sugar recovery correlation.

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ABSTRACT

Among all the feasible thermochemical conversion processes, concentrated acid hydrolysis has been applied to break the crystalline structure of cellulose efficiently and scale up for mass production as lignocellulosic biomass fractionation process. Process conditions are optimized by investigating the effect of decrystallization sulfuric acid concentration (65–80 wt%), hydrolysis temperature (80 °C and 100 °C), hydrolysis reaction time (during two hours), and biomass species (oak wood, pine wood, and empty fruit bunch (EFB) of palm oil) toward sugar recovery. At the optimum process condition, 78–96% sugars out of theoretically extractable sugars have been fractionated by concentrated sulfuric acid hydrolysis of the three different biomass species with 87–90 g/L sugar concentration in the hydrolyzate and highest recalcitrance of pine (softwood) was determined by the correlation of crystallinity index and sugar yield considering reaction severity.

1. Introduction

The world's energy consumption has progressively increased with the rapid population growth and economic development as more countries have intensified their industrial activities over the last century. Problems of fossil fuel sources such as depletion of unevenly distributed resources, environmental damage caused by greenhouse gases emission, and price fluctuation of oil and gas have increased the need for renewable energy sources. Among many options of renewable energy sources, lignocellulosic biomass is still considered as the only large-scale sustainable carbon source currently available for the future energy supply. The great potential of lignocellulosic biomass utilization has still strongly encouraged the researches that can be applied into industrially and economically viable processes for conversion of biomass to energy, fuels, and chemicals (Moe et al., 2012).

Lignocellulosic biomass refers to nonstarch, fibrous part of plant biomass that is composed of three major constituents: cellulose, hemicellulose, and lignin (Basu, 2010). It can be classified into four main sources: (1) agricultural residues (corn stalk, corn stover, sugarcane bagasse), (2) forestry residues (wood waste, sawdust, mill scrap), (3) energy-woody crops (willow, poplar, switch grass), and (4) industrial and municipal solid wastes (paper mill sludges, recycled newspaper, wasted paper) (Sathitsuksanoh et al., 2010). Lignocellulosic biomass can be processed into biofuel production without competing with food production. In addition, biofuel production from lignocellulosic biomass will generate lignin as residue that can be upgraded into valuable fuel additives or used for

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power generation. The process is designed to reuse all aqueous streams and to convert all solids into economically profitable and useful products (Farone and Cuzens, 1996). Among the various lignocellulosic biomass, wood is one of the most potentially used materials in acid hydrolysis since wood and its residues are the dominant form of lignocellulosic biomass resources (Basu, 2010).

The conversion of lignocellulosic biomass includes two processes: hydrolysis of cellulose into fermentable sugars and fermentation of the sugars to ethanol (Sun and Cheng, 2002). The hydrolysis reaction for cellulose conversion into sugar is principally the degradation of chemical bonds in cellulose, involving the hydrolytic cleavage of β-1,4-glycosidic bond which is catalyzed by H^+ ions of an acid or by the action of a cellulolytic enzyme. The homogeneous hydrolysis of a glycoside in an acid aqueous medium is understood as a replacement process of its initial OR group by a hydroxyl group regenerating H^+ ion by the addition and heterolytic cleavage of a water molecule (Klemm et al., 1998). The acid hydrolysis of glycosidic bonds follows a first-order rate law. The reaction rate depends on the H_3O^+ ion concentration, the reaction temperature, and the chemical environment of the glycosidic bond and the rate is increased with the increasing acid ion concentration and temperature (Klemm et al., 1998; Saeman, 1945).

The conventional methods for hydrolysis process currently are based on thermochemical route (acid-catalyzed hydrolysis) and biochemical route (enzyme-catalyzed hydrolysis), both of which require pretreatment step to utilize lignocellulosic biomass. Among various methods of hydrolysis, concentrated acid hydrolysis, one of the promising methods based on thermochemical route, has several advantages with respect to the milder operating condition (the lower operating temperature and pressure), the higher conversion rate, and the higher sugar recovery. Compared to dilute acid hydrolysis, the advantages of this process are the higher efficiency of sugar recovery, which can reach over 90% of theoretical yield for both glucose and xylose (Guha et al., 2010; Shahbazi and Zhang, 2010; Taherzadeh and Karimi, 2007), and the milder operating temperature and pressure (Iranmahboob et al., 2002) thus the decreasing sugar yield due to inhibitors formation can be minimized (Moe et al., 2012). In comparison with enzymatic hydrolysis, concentrated acid hydrolysis has the higher reaction rate, which directly implies the shorter reaction time. The major challenge in employing enzymatic hydrolysis is finding the appropriate pretreatment and less costly enzyme preparation methods to increase the lignocellulosic substrates availability (Moe et al., 2012; Sun et al., 2011). Due to highly complex and strong recalcitrance of lignocellulosic biomass, the thermochemical route has been regarded more preferable in order to overcome slow reaction by multiple cellulase enzymes (Selig et al., 2013). The disadvantages of concentrated acid hydrolysis are the higher toxicity and corrosivity that required corrosive-resistant reactors or specialized non-metallic constructions and the indispensable need for acid recovery process to make the process economically feasible (Moe et al., 2012; Sun and Cheng, 2002).

Despite the disadvantages, concentrated acid hydrolysis is still attractive and relevant today as this process was claimed to have a low overall cost for the ethanol production (Groenestijn et al., 2006). Arkenol, Inc., a technology and project development company in the United States, has reported that the concentrated acid hydrolysis process could be made economically viable and ready for commercial implementation. Arkenol-developed technology has used commercially available ion exchange resins to separate remaining acid-sugar solution into its acid and sugar components without diluting the sugar. This process was claimed to be capable of producing a clean stream of mixed sugar (both hexoses and pentoses) for fermentation, because the separated sulfuric acid was recirculated and reconcentrated to the level required by decrystallization and hydrolysis steps; and the small quantity of acid left in the sugar solution was neutralized with lime to make hydrate gypsum, $CaSO_4{\cdot}2H_2O$, an insoluble precipitate which could be separated from the sugar solution and used as an agricultural soil conditioner. The Masada Resource Group has also developed full-scale cellulosics-to-ethanol projects in North America (Taherzadeh and Karimi, 2007). Although concentrated acid hydrolysis was previously regarded as economically not viable process due to the requirement of the large amounts of acid, the development of effective acid recovery technologies and the high flexibility of this process toward different feedstocks have renewed its interest (Janga et al., 2012; Moe et al., 2012).

This study aimed to investigate the effect of decrystallization acid concentration, hydrolysis temperature, and hydrolysis reaction time toward sugar recovery in various biomass species. Crystallinity degree of the raw biomass has also been analyzed to conduct a preliminary study about the effect of its crystalline structure on the digestibility of substrate in the hydrolysis process. Although two-step concentrated acid hydrolysis has been a commercialized technology, its application on various lignocellulosic biomass is still an interesting subject to study as the resulting data can be contributed to the researches in biomass utilization area as a basis for the selection of potential lignocellulosic feedstocks.

2. Methods

2.1. Materials and apparatus

Raw biomass used in this experiment were obtained from oak wood, pine wood, and empty fruit bunch (EFB) of palm oil which represented hardwood, softwood, and non-woody biomass, respectively. All the raw biomass was first ground and sieved to pass 18 mesh (1 mm) screen before being fed to the reactor. Sulfuric acid (H_2SO_4, 96%) was purchased from Daejung Chemicals and Metals, Co., Ltd., South Korea. The initial concentrations of sulfuric acid used in decrystallization process were 65, 70, 75, and 80 wt%. The solid loading (acid to biomass) ratio was averagely 2.0 in dry weight basis.

The reactor was 250 mL round bottom flask with four necks (DURAN® Schott, Germany), and equipped with an overhead stirrer (Techno Lab-system, Poong Lim, South Korea) and a Teflon-coated stirring rod. A condenser was used to prevent the vapor product escaped from the reactor during the process. An electric heater was connected to a heating mantle and equipped with a digital temperature controller (DX7, Han Young, South Korea) and a thermocouple. Quenching water was provided to suspend the reaction in the samples in order to prevent further degradation of the desired products.

2.2. Lignocellulosic feedstocks characterization

Feedstocks characterization conducted in this experiment includes: (1) ultimate and proximate analysis (to analyze the elemental composition), (2) compositional analysis (to determine the lignocellulosic component), and (3) crystallinity index analysis (to determine the crystallinity degree) of the raw biomass. Alpha–cellulose fiber (approximately 99.5%) and lignin alkali model compounds were purchased from Sigma Chemical Company and Sigma–Aldrich, USA to determine the crystallinity degree of cellulose used as a comparable reference to the crystallinity degree of raw biomass.

2.2.1. Ultimate and proximate analysis

The ultimate (elemental) analysis was conducted by Advanced Analysis Center at KIST, South Korea. It was carried out in Flash 2000 Organic Elemental Analyzer for carbon (C), hydrogen (H),

nitrogen (N), sulfur (S), and in Fisons EA 1108 Elemental Analyzer for oxygen (O).

The proximate analysis was carried out by thermogravimetric analysis (TGA) method in a TA Q600 TGA instrument based on the procedure described elsewhere (Kneller, 1986; Warne, 1996). The system was initially flushed with nitrogen for 10 min at a flow rate of 50 cm^3/min. Sample was placed in a platinum pan and heated up to 110 °C at constant heating rate of 20 °C/min, held isothermally during 15 min to measure the moisture content (*M*) which was the weight loss due to water removal, then continually heated up to 950 °C at 20 °C/min and again held there to constant weight to give the loss due to volatile matters (*VM*). At this point, air was permitted to flow through the system at a flow rate of 50 cm^3/min to oxidize the remaining organic matters yielding the weight loss due to the burning away of the fixed carbon (*FC*). The remaining weight of sample is the amount of residual ash (*ASH*).

2.2.2. Compositional analysis

The lignocellulosic composition of each raw biomass was quantitatively determined based on the laboratory analytical procedure (LAP) developed by National Renewable Energy Laboratory (NREL) with appropriate adjustment (Sluiter et al., 2008, 2010; Templeton et al., 2010). The sample of each biomass (0.3 g, dry basis) was first treated with 72 wt% H_2SO_4 at 30 °C for 1 h in a round bottom flask reactor and stirred every 15 min with a glass stirring rod. Deionized water (84 mL) was added into the decrystallized sample to obtain 4 wt% H_2SO_4 solution. The mixture was then heated to 121 °C in a pressure tube glass (8648-30 ACE Glass) with screw on Teflon caps and o-ring seals (5845-47 plug) and kept static for 1 h in a vacuum oven. After cooling to the room temperature, the liquid products were further purified using a 0.2 μm filter and the dissolved sugars were quantified using high performance liquid chromatography (HPLC). The liquid products were also analyzed in a UV–Vis-NIR Spectrophotometer (Cary 5000, Varian Inc.) to determine the amount of acid-soluble lignin (ASL) by absorbance at 200–205 nm and typical absorptivity of 110 L g^{-1} cm^{-1} at recommended wavelength (Hatfield and Fukushima, 2005). The amount of acid insoluble lignin (AIL) was determined gravimetrically in the mass balance after separating the solid products using vacuum filtration. AIL was calculated as the amount of acid and water-free solid residue after subtraction by the ash amount measured in the proximate analysis.

2.2.3. Crystallinity index (CrI) analysis

XRD analysis was performed on a four circle goniometer (XRD-6000 Lab X Shimatzu) using CuKα radiation generated at 40 kV and 30 mA. Scans were obtained from 5 to 90° 2θ with the scan speed of 4°/min. The samples, consisted of raw biomass (oak wood, pine wood, and EFB) and lignin as the side product from hydrolysis of each raw biomass (oak lignin, pine lignin, and EFB lignin), were crushed and sieved into size of 106–150 μm and they all were mounted onto a quartz holder during the analysis.

2.3. Concentrated acid hydrolysis process stages

In general, the experiment consists of three major stages: decrystallization reaction, hydrolysis reaction, and products separation. The first two reactions can also be considered as two-step concentrated acid hydrolysis in which the combination of both higher and lower concentrated sulfuric acid are used. The overall experimental process stages are represented in the work flow diagram in Fig. 1.

Initially the raw biomass and the concentrated acid solution were gradually fed into the reactor. The stirring was adjusted at 75 rpm to ensure complete biomass wetting (intimate contact between acid and biomass). This feeding process was performed until the wood and acid mixed well, indicated by dark color of the mixture. The decrystallization reaction was carried out at around 30 °C and atmospheric pressure within 30 min. After decrystallization, some amount of deionized water at the room temperature was added to dilute the substrate to ~30 wt% acid concentration. At this point, the sample was taken immediately to investigate the concentration and distribution of sugars at the end of decrystallization stage. Afterwards, the mixture was heated up to the hydrolysis temperature (80 °C and 100 °C) and the hydrolysis reaction continued during two hours. The mixture was sampled every 30 min and the sample was immediately quenched prior to centrifugation to separate the liquid and solid fraction. The reactor was cooled down to the room temperature after the hydrolysis reaction had been completed. The mixture was then separated in a vacuum filtration process. The liquid product (filtrate) contained acid and sugars, while the solid product (acid-insoluble residue) contained some amount of unconverted wood, acid, and water.

2.4. Product analysis

The liquid product was analyzed in YL9100 HPLC system (Young Lin, Anyang, South Korea) after being cooled at the room temperature and the concentrations of sugars (glucose, xylose, and cellobiose) were determined by an ion exchange chromatography column (Aminex HPX-87 H, Bio-Rad, 300 mm × 7.8 mm) operated at 60 °C. The eluent (mobile phase) used was 5 mM sulfuric acid. The pump was operated at flow rate of 0.06 mL/min in the isocratic mode and the sample injection volume was 40 μL. The filtrate was syringed through a 0.5 μm filter and diluted with distilled water in a volume ratio of 1/30. Sugars (cellobiose, glucose and xylose) were detected by a refractive index detector (YL9170 RID) at 36 °C. Actual concentration of the products can be determined after 20 min analysis time by calculating the concentration of each compound from the peak area and the standard calibration curve of each component. This quantification procedure was performed according to the NREL standardized method (Sluiter et al., 2008) with appropriate adjustment.

Solid product from the filtration process was then neutralized by washing with copious amount of water, dried in the oven at 90 °C until the weight remained constant, and then utilized in the XRD analysis as amorphous lignin sample. The amount of net (acid and moisture-free) solid residue in the final product was determined from the material balance. In the calculation, the filtration process was assumed to be ideal, means the filtrate contained no wood (solid fraction of hydrolyzate) and the solid residue contained no sugars (all sugars were collected in the filtrate), thus there was no material accumulation in the system (zero mass loss).

3. Results and discussion

3.1. Lignocellulosic feedstocks characterization

3.1.1. Ultimate and proximate analysis

The ultimate and proximate analysis data of all the raw biomass are presented in Table 1. The result shows that pine wood has the highest carbon and hydrogen content but the lowest oxygen to carbon (O/C) ratio. EFB contained nitrogen (that contributed to protein content in biomass) and the highest ash amount.

3.1.2. Compositional analysis

The lignocellulosic composition data of all the raw biomass (in dry weight basis) are shown in Table 2. The measurement was performed with duplicate samples and each sample was analyzed three times in HPLC to determine the amount of dissolved sugars

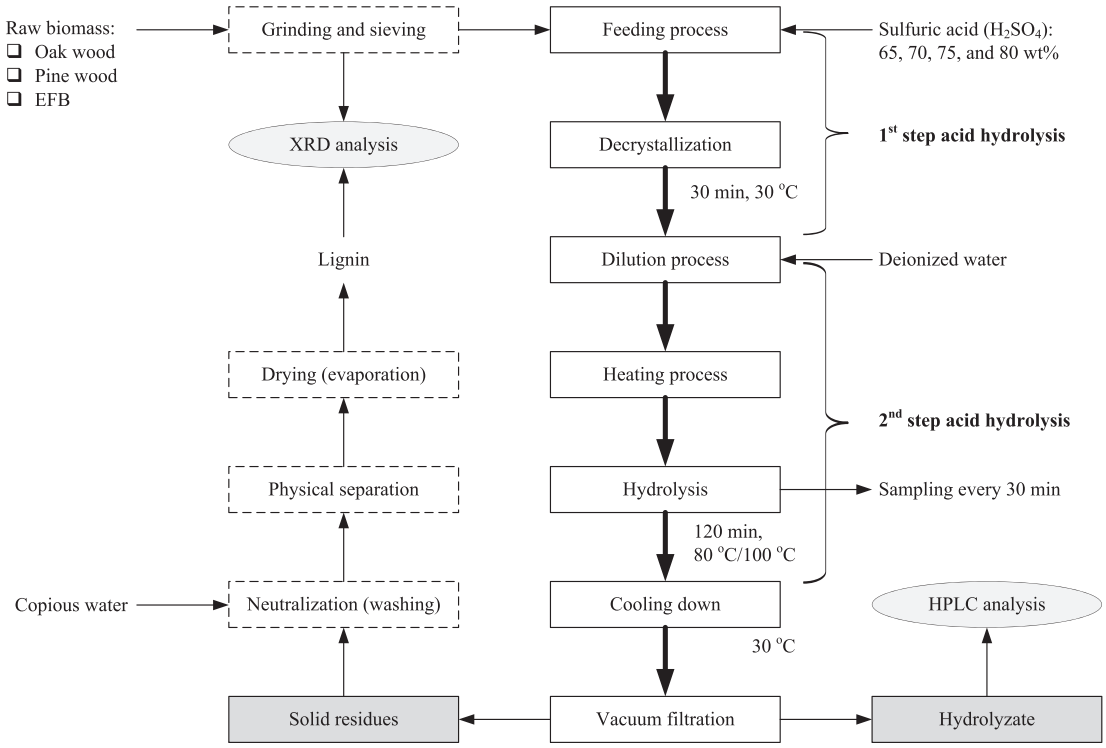

Fig. 1. Overall experimental process stages of the two-step concentrated acid hydrolysis.

Table 1
Ultimate and proximate analysis result for oak wood, pine wood, and EFB (wt%).

Component	Ultimate analysis					Proximate analysis			
	C	H	N	S	O	*FC*	*VM*	*M*	*ASH*
Oak wood	46.5	6.0	0.0	<0.3	45.4	13.4	78.8	5.5	2.3
Pine wood	48.6	6.2	0.0	<0.3	40.6	12.6	81.4	5.3	0.7
EFB	42.6	5.7	1.7	<0.3	39.5	19.1	66.8	6.7	7.4

Table 2
Lignocellulosic composition of oak wood, pine wood, and EFB (wt%).

Component	Cellulose	Hemicellulose	Lignin	
			AIL	ASL
Oak wood	42.5–44.1	15.9–16.7	28.2–33.8	3.31–3.51
Pine wood	41.8–43.6	19.3–19.9	30.5–34.2	0.57–0.91
EFB	39.9–42.8	20.3–22.7	24.0–26.9	2.43–3.20

more precisely. The concentrations of polymeric sugars (cellulose and hemicellulose) were calculated from the concentrations of corresponding monomeric sugars (glucose and xylose, respectively) based on the stoichiometric correlations and formulas described in NREL/TP-510-42618 (Sluiter et al., 2008). Oak wood contained the highest amount of cellulose but the lowest amount of hemicellulose, pine wood contained the highest amount of acid-insoluble lignin, and EFB contained the highest amount of hemicellulose.

3.1.3. Crystallinity index

Since cellulose is the major part of the lignocellulosic biomass, the crystallinity of cellulose in biomass may have important effect to the reactivity of the substrates in hydrolysis process. Crystallinity index (CrI) of cellulose is defined as the percentage of crystalline part presented in the total cellulose (Guha et al., 2010). In this study, the CrI determination was applied to the raw biomass for the comparison of crystallinity degree in each raw biomass, thus the CrI is defined as the percentage of crystalline part presented in the total raw biomass.

X-ray diffraction (XRD) is the most commonly used method to measure the crystallinity of dried cellulose samples, but it has been found that CrI varies significantly depending on the choice of determination method. CrI alone may not adequately explain the cellulose digestibility, because cellulose accessibility is not only affected by crystallinity, but it is also likely to be affected by lignin/hemicellulose content and distribution, particle size, and porosity of the cell wall (Park et al., 2010). Nevertheless, in this study CrI determination was conducted to provide a preliminary insight about the crystallinity degree difference in oak wood, pine wood, and EFB and whether it would give meaningful correlation with their overall conversion. In addition to the CrI determination, the results from XRD analysis were also used to show the change of crystalline structure in each raw biomass before and after hydrolysis.

The CrI of each raw biomass was estimated by means of wide-angle X-ray scattering (WAXS), using the amorphous subtraction method, as also known as the Ruland–Vonk method (Park et al., 2010; Thygesen et al., 2005). In this method, crystallinity was determined by subtracting the amorphous contribution from diffraction spectra using an amorphous standard, i.e. lignin powder obtained from the solid product of hydrolysis after being dried and neutralized. Oak, pine, and EFB lignin were obtained from the hydrolysis of oak wood, pine wood, and EFB, respectively at 70 wt% decrystallization acid concentration, 30 °C decrystallization reaction temperature and 78 °C hydrolysis reaction temperature. CrI was calculated as the ratio between the area of the crystalline contribution and the total (crystalline + amorphous) area (Park et al., 2010; Thygesen et al., 2005; Young and Lovell, 1991). The integration of all peak areas was processed in OriginPro 8.5.1 software after applying Savitzky–Golay method for signal smoothing. The integration limits were selected so only the part of the diffractogram containing visible crystalline intensity was used, from $2\theta = 10°$ to $2\theta = 50°$. From the peak areas calculation, it was found

that pine wood has the highest crystallinity (CrI = 0.66), followed by EFB (CrI = 0.65) and oak wood (CrI = 0.61), respectively. The results are comparable to the alpha–cellulose (CrI = 0.75).

The different states of cellulose crystalline structure in each raw biomass between the pre- and post-hydrolysis are illustrated in Fig. 2. The pre-hydrolysis state is represented by the original raw biomass diffraction spectra while the post-hydrolysis state is represented by the amorphous lignin diffraction spectra. The ratio between amorphous peak area (after hydrolysis) and peak area contained both crystalline and amorphous diffraction (before hydrolysis) has been used to determine CrI. Significant change in the peak intensity can be observed especially around $2\theta = 22.5°$, indicating the cellulose crystallinity was dramatically reduced after hydrolysis. Special notice addressed to EFB diffraction spectra where the abnormal peaks could be seen above $2\theta = 25°$ (specifically at 26.78°, 40°, and 50°) which were likely attributed to the high impurities content in EFB. As also shown in EFB lignin spectra, crystal structure in the impurities was not broken under strong acid condition. The proximate analysis data, clearly showed that EFB contained the highest amount of ash (inorganic solid residues, rock, dirt, and other impurities) among all the raw biomass, can support this XRD result thus the CrI of EFB was determined without calculating these abnormal peak areas.

3.2. Selection of the operating parameters

Several studies have been conducted on acid hydrolysis for biomass conversion to investigate the effect of operating variables on sugar recovery and subsequent sugar degradation (Janga et al., 2012; Lenihan et al., 2010; Moe et al., 2012). Among the possible options of operating variables, including: acid concentration, reaction temperature, and residence time at decrystallization and hydrolysis stage, biomass species, solid loading (acid to biomass) ratio, and solid particle size, there are four input parameters varied in this work: (1) decrystallization acid concentration, (2) hydrolysis temperature, (3) hydrolysis reaction time, and (4) biomass species. In this work, five output parameters were observed to study the effect of decrystallization acid concentration, hydrolysis temperature, hydrolysis reaction time, and biomass species:

(1) Hydrolyzate (sugar) concentration, refers to monomeric sugar concentration, is the sum of glucose and xylose concentration from HPLC analysis.
(2) Glucose/xylose ratio, represents the degree of decrystallization when it is combined with glucose yield as well as the degree of hydrolysis when it is combined with xylose yield. In the fermentation process of sugar streams containing both hexose and pentose sugars, this parameter might be important as the sugars could be fermented simultaneously and thus advantageously obviating the need for the sugars separation (Farone and Cuzens, 1996).
(3) Glucose yield, can be interpreted as the decrystallization degree of cellulose, was calculated on the basis of initial dry raw biomass.
(4) Sugar yield, is the sum of monomeric sugars (glucose and xylose) and oligomers (cellobiose) amount in the hydrolyzate compared to the initial dry raw biomass.
(5) Sugar recovery efficiency, is the ratio between the total amount of sugars in the hydrolyzate and the total amount of sugars in the biomass (when all cellulose and hemicellulose in the biomass could be fully converted into sugars).

3.3. The effect of decrystallization acid concentration

The use of the high sulfuric acid concentration ranged from 65 to 80 wt% was necessary to effectively reduce the cellulose

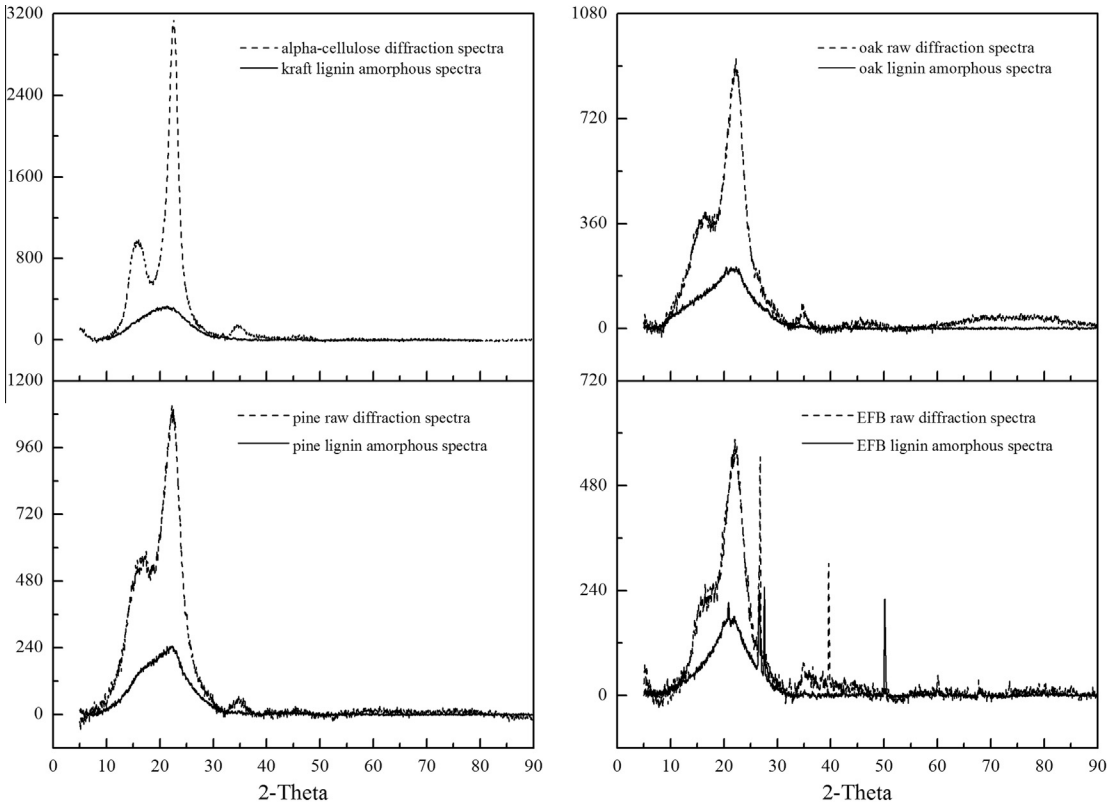

Fig. 2. Comparison of cellulose crystalline structure in alpha–cellulose, oak wood, pine wood, and EFB before and after hydrolysis.

crystallinity, dismantle the lignocellulosic structure, and increase the porosity of lignocellulosic materials (Sun and Cheng, 2002) in the shorter reaction time (which means the faster degradation of cellulose could be achieved), so that the cellulose in biomass would be more easily accessible in the following hydrolysis process.

The concentration and distribution of sugars resulted from the end of decrystallization reaction are shown in Fig. 3. The results are quite different among the raw biomass, but still comparable tendencies can be observed. At the lower acid concentration (65–70 wt%), the production of glucose was less significant than of xylose as glucose was still existed in its oligomers form (cellobiose), indicating that xylose, the derivative of hemicellulose, was liberated at the higher production rate. However, the production of xylose was decreased with the increasing acid concentration, while the production of glucose started to increase when the acid concentration was increased, from 70 to 75 wt%.

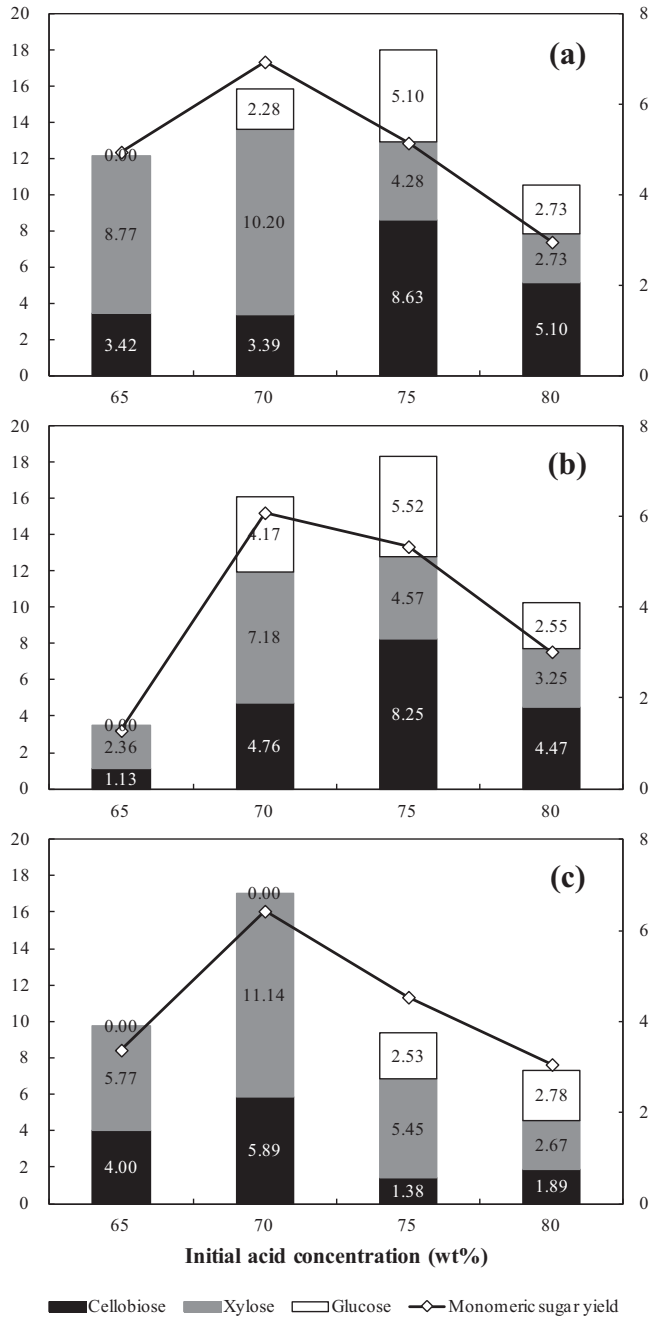

Fig. 3. Product distribution at the end of decrystallization reaction from: (a) oak wood, (b) pine wood, (c) EFB, at various initial acid concentration. The primary and secondary vertical axis represents sugar concentration (g/L) and monomeric sugar yield (wt%), respectively.

The effect of decrystallization acid concentration toward sugar concentration, glucose to xylose ratio, glucose and sugar yield are presented in Fig. 4. All the output parameters were calculated at the end of hydrolysis process after the sugars were collected in the filtrate.

In oak wood hydrolysis, the increasing acid concentration from 65 to 75 wt% resulted in the increasing sugar concentration and sugar yield (significantly at 80 °C and slightly at 100 °C), as shown in Fig. 4(a) and (b), but when the acid concentration was increased further to 80 wt%, the sugar concentration was decreased, mainly related to the decreasing xylose concentration at the more acidic environment due to the formation of smaller chemicals. Glucose/xylose ratio tended to increase with the increasing acid concentration. The highest value of glucose/xylose ratio was 7.92 that achieved at 80 wt% and 100 °C, which was the most severe condition in this experiment. The highest sugar concentration was 80.02 g/L with glucose/xylose ratio of 2.27, glucose yield of 31.50 wt%, and sugar yield of 52.39 wt%, achieved at 75 wt% and 80 °C.

In pine wood hydrolysis, comparable with those happened in oak wood hydrolysis as shown in Fig. 4(c) and (d), the amount of sugar yield also tended to increase with the increasing acid concentration but slightly decrease at the more acidic environment. The increasing acid concentration also contributed to the increasing glucose/xylose ratio, but the values were not as high as those obtained in oak wood or EFB hydrolysis, indicating the more stable hemicellulose structure of softwood which contains mostly glucomannans (Saha, 2003). Some portions of glucose could also be derived from the glucomannans thus in the decomposition of hemicellulose some of the glucoses were also reduced together with the xyloses. The highest value of glucose/xylose ratio was only 2.77 that was also achieved at 80 wt% and 100 °C. The highest sugar concentration was 84.62 g/L with glucose/xylose ratio of 2.04, glucose yield of 29.83 wt%, and sugar yield of 50.47 wt%, achieved at 80 wt% and 80 °C.

In EFB hydrolysis, the results were quite different as shown in Fig. 4(e) and (f) where the increasing acid concentration from 75 to 80 wt% still significantly increased the sugar yield. It was related to the highest moisture content in EFB which contributed to the lowering acid concentration used compared to those used in oak or pine wood hydrolysis. Therefore, the less reaction severity at 80 °C resulted in the zero inhibitors formation, as confirmed in the HPLC analysis results. Similar with those happened in oak and pine wood hydrolysis, glucose/xylose ratio in EFB hydrolysis also increased with the acid concentration. The highest value of glucose/xylose ratio was 7.53 that was also achieved at 80 wt% and 100 °C. The highest sugar concentration was 81.63 g/L with glucose/xylose ratio of 1.95, glucose yield of 32.23 wt%, and sugar yield of 57.03 wt%, achieved at 80 wt% and 80 °C.

In summary, among all biomass species, there has been a comparable evidence with some results from dilute acid hydrolysis process (Hong et al., 2013; Lenihan et al., 2010) that increasing the acid concentration is generally more effective way to maximize overall sugar yield than increasing the operating temperature. In the use of 65–75 wt% sulfuric acid and especially at 80 °C, no sugar degradation products were identified. Acid concentration is the most important parameter for release of sugar (Rahman et al., 2006; Roberto et al., 2003). However, it is always necessary to consider the upper limit of acid concentration where the sugar can still remain formed effectively, because beyond that limit the reaction environment will be more severe and the sugar decomposition into smaller species will inevitably happen.

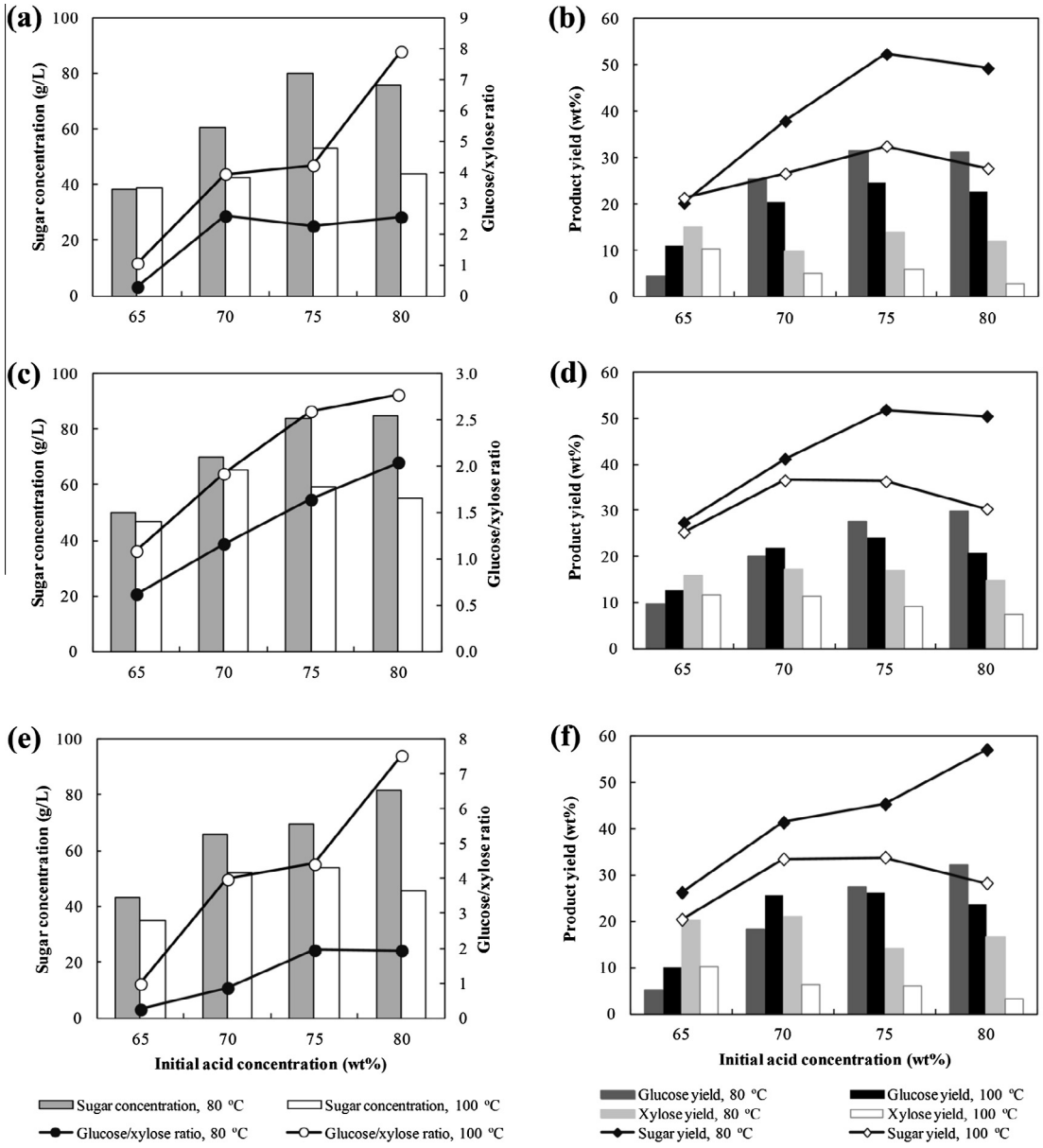

Fig. 4. The effect of decrystallization acid concentration on sugar concentration, glucose/xylose ratio (left), and sugar yield (right) in the hydrolysis of: (a and b) oak wood, (c and d) pine wood, (e and f) EFB, at various hydrolysis temperature.

3.4. The effect of hydrolysis temperature

The hydrolysis reaction was preceded by the dilution of the mixture aimed to hydrolyze the formed oligosaccharides to a mixture of hexoses and pentoses. In this process, water cleaved the glycosidic bonds in polysaccharides so that the cellulose and hemicellulose would be broken into simple sugars (monosaccharides). Fig. 4 also represents the effect of hydrolysis temperature toward sugar concentration, glucose/xylose ratio, glucose yield, and sugar yield.

In all biomass species, especially at the acid concentration range from 70 to 80 wt%, the increasing temperature generally decreased sugar concentration, glucose yield, and sugar yield, as well as increased glucose/xylose ratio. However, the increasing temperature at 65 and 70 wt% acid concentration slightly increased the glucose yield. This might indicate that the use of below 70 wt% acid concentration was not strong enough to completely decrystallize the cellulose thus it is necessary to add the severity of the reaction environment by increasing the temperature in order to obtain more glucoses.

Glucose/xylose ratio tended to increase with the temperature. At all acid concentrations, the values of glucose/xylose ratio at 100 °C are always higher than at 80 °C because of the sugar degradation at the higher temperature. Even though at both hydrolysis temperatures the increasing acid concentration always contributed to the increasing glucose/xylose ratio, but the reason behind this evidence was not exactly the same. At 80 °C the increasing glucose/xylose ratio was mainly attributed to the increasing of glucose amount. However, at 100 °C the increasing glucose/xylose ratio was attributed to the decreasing of xylose amount that was greater than the decreasing of glucose amount.

The glucose yield at 100 °C was always lower than at 80 °C, especially at the higher acid concentration (75–80 wt%). It was an evidence that at the higher acid concentration, the higher temperature promoted the decreasing of glucose yield while at the lower acid concentration (65–70 wt%) some data still showed the

higher glucose yield at 100 °C. There are some possible reasons behind this evidence. After decrystallization reaction, cellulose (which is the major source of glucose) existed in the substrate as high molecular cellulose (partially broken cellulose), oligomers (more broken high molecular cellulose), monomers, and hydrolyzed monomers to smaller chemicals. At the lower acid concentration, high molecular cellulose existed more than oligomers while at the higher acid concentration, oligomers existed more than high molecular cellulose. During hydrolysis, after the lower decrystallization acid concentration treatment, substrates were hydrolyzed to have more oligomers and also monomers at both 80 °C and 100 °C. After the higher decrystallization acid concentration treatment, substrates were hydrolyzed to have more monomers at 80 °C while to have monomers as well as over-hydrolyzed chemicals from monomers at 100 °C, as confirmed in the HPLC chromatogram after 20 min analysis time. Some studies have reported that the higher temperature leads to the decomposition of glucoses into hydroxymethylfurfural (HMF) which, on continued heating, will yield levulinic acid and formic acid (Hong et al., 2013; Lenihan et al., 2010; Saeman, 1945; Taherzadeh and Karimi, 2007).

Nevertheless, compared to glucose, xylose is more susceptible to the harsh condition, due to its amorphous structure (Rahman et al., 2007) and reactivity. Therefore, xylose is easier and faster to degrade than glucose, which is more resistant to harsh condition (Taherzadeh and Karimi, 2007). Xylose is liberated during the decomposition of hemicellulose. At the higher temperature, xylose degrades into furaldehydes and the acetylxylan in hemicellulose was hydrolyzed into acetic acid (Shahbazi and Zhang, 2010). It was clearly observed that in all biomass species and at all acid concentrations, the increasing hydrolysis temperature contributed to the decreasing xylose yield.

At above the 65 wt% acid concentration, the sugar yield resulted from hydrolysis at 100 °C was always lower than at 80 °C. This evidence has confirmed that hydrolysis temperature is the main

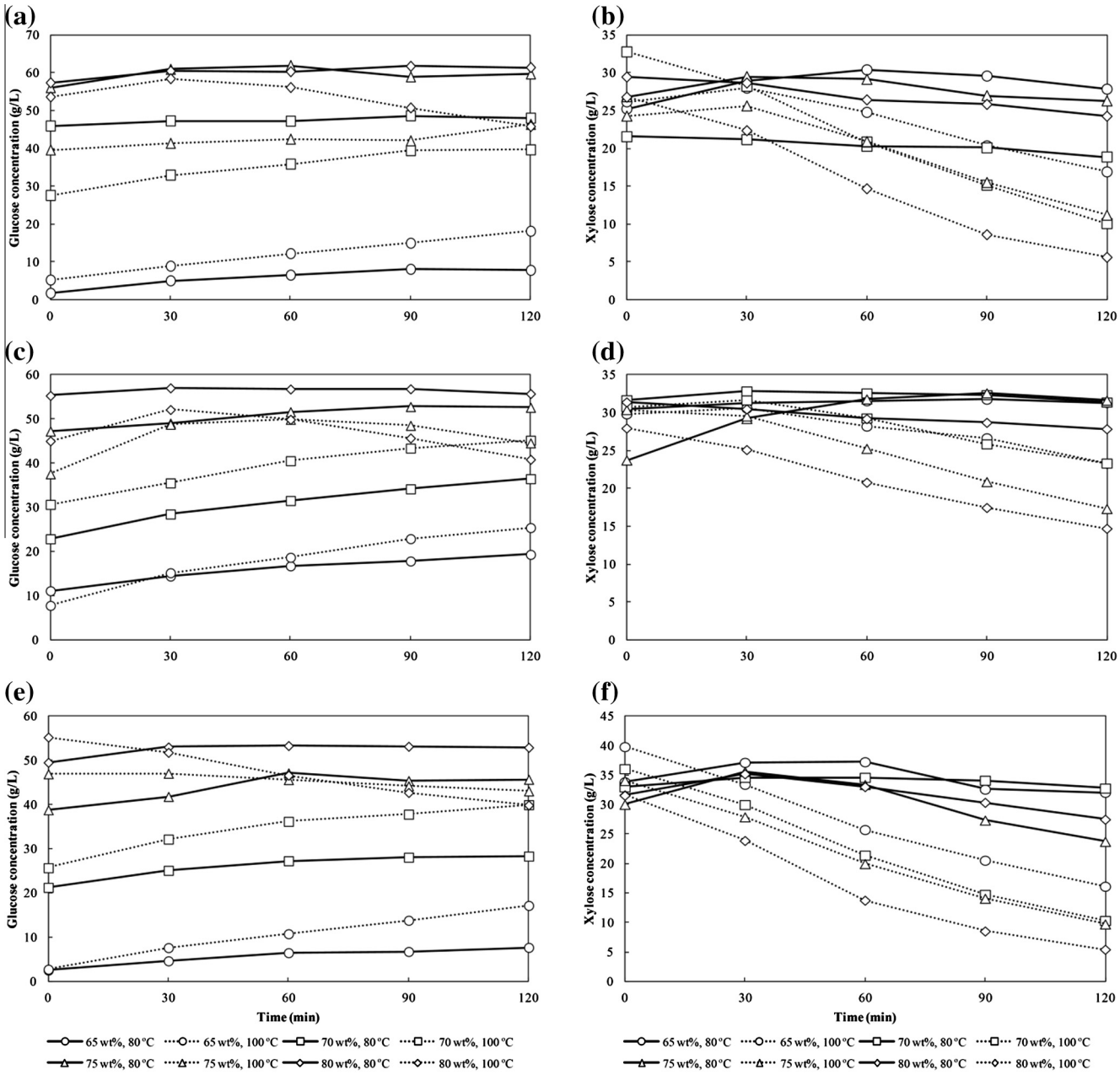

Fig. 5. Comparison of glucose and xylose concentration profile from the hydrolysis of: (a and b) oak wood, (c and d) pine wood, (e and f) EFB, at all process conditions.

parameter responsible for the sugar degradation into various byproducts. It has more significant detrimental effect than acid concentration in the decrystallization stage.

3.5. The effect of hydrolysis reaction time

In Fig. 5, it is clearly seen that sugar concentration tended to decrease over time after two hours reaction, especially at the more severe reaction condition, i.e. 80 wt% acid concentration and 100 °C hydrolysis temperature. The decreasing sugar concentration was mainly associated with the degradation of xylose, which was faster than glucose, as indicated by the sharper curve slope of xylose concentration profile.

The starting point (t = 0 min) was actually counted as the time when the hydrolysis reaction had just started at the desired temperature. It was the time when the temperature had reached 80 °C (or 100 °C). When monomeric sugar yields are compared between Figs. 3 and 4, the sugar concentrations at the end of decrystallization stage were still far below the sugar concentrations at the beginning of hydrolysis stage, thus it implies that the sugars production mainly occurred at the heating stage with the highest hydrolysis reaction rate of cellobiose into glucose.

At the lower acid concentration (65–70 wt%) and 100 °C, glucose concentration tended to increase over time while at the higher acid concentration (~80 wt%) and 100 °C, glucose concentration significantly decreased over time. This happened because of the different reaction rate between those two conditions. The higher acid concentration could significantly convert cellulose into glucose in the shorter time while the lower acid concentration needed the longer time to hydrolyze as much as possible cellulose into glucose, before it was finally decomposed afterwards. That is why at the lower acid concentration, glucose concentration seemed to increase over time. Hydrolysis reaction time affected sugar (especially xylose) concentration more significantly at the higher temperature (~100 °C). It was also observed that at above 70 wt% acid concentration, the maximum sugar concentration generally could have been achieved after 30 min reaction. Extending the hydrolysis reaction time had just decreased the overall sugar yield. Compared to decrystallization acid concentration and hydrolysis temperature, hydrolysis reaction time also played a role as severity factor but with the less impact.

3.6. The effect of biomass species

Among all the raw biomass, the lowest sugar yield was found in pine wood hydrolysis as it was the most difficult to convert. As observed at the decrystallization stage, the pine wood was relatively hard to be uniformly wetted and penetrated by acid solution. Crystallinity has been reported to affect cellulose accessibility by acids (Zhao et al., 2006), thus the high recalcitrance of pine wood (softwood) in hydrolysis may also be attributed to the higher cellulose crystallinity of pine wood as compared to oak wood (hardwood) (Janga et al., 2012; Newman, 1994). The results from XRD analysis have confirmed this report. Moreover, it has also been reported that softwoods are less easily treated than hardwoods and usually need a combined chemical, such as SO_2 or H_2SO_4 and steam-aqueous treatment for fractionation (Janga et al., 2012; Overend et al., 1987). Hemicellulose solubilization was also affected by the dominance of a thermally stable glucomannan backbone in softwood hemicellulose, compared to simply xylan backbone in hardwood hemicellulose (Janga et al., 2012; Saha, 2003). During hydrolysis, the presence of higher amount of condensed lignin in softwoods may also hinder the swelling of the cell wall (Janga et al., 2012; Phaiboonsilpa et al., 2010).

3.7. Determination of optimum process condition for each biomass species

Summarizing all the experimental results, the effect of decrystallization acid concentration, hydrolysis reaction temperature, and hydrolysis reaction time toward sugar yield among the different raw biomass have been recognized well. These operating

Table 3
Summary of the optimum results for the two-step concentrated acid hydrolysis of oak wood, pine wood, and EFB.

Input parameter				Output parameter				
Raw material	$C_{H_2SO_4}$ (wt%)	$T_{hydrolysis}$ (°C)	$t_{hydrolysis}$ (min)	G/X ratio	C_{sugar} (g/L)	$Y_{glucose}$ (wt%)	Y_{sugar} (wt%)	Sugar recovery (%)
Oak wood	65	80	90	0.27	37.66	4.12	20.67	33.15
		100	60	0.49	37.01	6.75	20.40	32.72
	70	80	30	2.23	68.45	27.41	43.60	69.94
		100	30	1.17	61.18	19.72	39.54	63.43
	75	80	30	2.06	90.39	34.53	59.98	96.23
		100	30	1.61	66.97	23.69	41.56	66.68
	80	80	30	2.11	89.07	34.46	59.51	95.48
		100	30	2.61	80.76	34.18	52.67	84.50
Pine wood	65	80	120	0.62	50.63	9.84	27.37	41.10
		100	90	0.86	49.53	11.77	26.75	40.18
	70	80	120	1.16	67.82	19.54	39.64	59.54
		100	60	1.38	69.79	20.78	38.72	58.16
	75	80	90	1.62	85.36	27.99	51.91	77.97
		100	30	1.65	78.29	27.31	48.45	72.78
	80	80	30	1.87	87.48	29.94	52.28	78.53
		100	30	2.08	77.20	26.53	43.58	65.45
EFB	65	80	60	0.17	43.72	3.78	26.87	39.77
		100	30	0.23	41.04	4.46	24.77	36.67
	70	80	60	0.79	61.86	16.27	39.15	57.96
		100	30	1.07	62.14	19.67	39.21	58.04
	75	80	60	1.41	80.52	28.11	50.56	74.85
		100	30	1.69	74.95	27.87	48.21	71.37
	80	80	30	1.51	88.20	31.68	61.36	90.84
		100	30	2.17	75.67	30.50	48.10	71.20

Notation: $C_{H_2SO_4}$ = initial sulfuric acid concentration, $T_{hydrolysis}$ = hydrolysis temperature, $t_{hydrolysis}$ = hydrolysis reaction time, G/X ratio = glucose to xylose ratio, C_{sugar} = sugar (glucose and xylose) concentration, $Y_{glucose}$ = glucose yield, Y_{sugar} = sugar (glucose, xylose, and cellobiose) yield.

Table 4
Summary of the optimum process condition for the two-step concentrated acid hydrolysis of oak wood, pine wood, and EFB.

	Oak wood	Pine wood	EFB
Initial acid concentration (wt%)	75	80	80
Hydrolysis temperature (°C)	80	80	80
Hydrolysis reaction time (min)	30	30	30
Sugar concentration (g/L)	90.39	87.48	88.20
Glucose/xylose ratio	2.06	1.87	1.51
Glucose yield (wt%)	34.53	29.94	31.68
Sugar yield (wt%)	59.98	52.28	61.36
Sugar recovery efficiency (%)	96.23	78.53	90.84

parameters can actually affect the sugar recovery, either individually or synergistically. In order to enhance the sugar yield, one parameter should be kept in a low value if the value of other parameters was desired to rise, unless the sugar decomposition would be unavoidable.

The optimum process condition for each raw biomass was determined based on the highest sugar recovery in the shortest hydrolysis reaction time after comparing the values of sugar yield in all process conditions. Based on the calculation corresponding to the carbohydrate composition data in Table 2, the average amount of holocellulose (cellulose and hemicellulose) in oak wood, pine wood, and EFB was 60.68, 61.86, and 65.35 wt%, respectively. The average amount of sugars theoretically obtained from the carbohydrate conversion of these three consecutive biomass was 62.33, 66.58, and 67.56 wt%. Table 3 summarizes the optimum results of the two-step concentrated acid hydrolysis overall experimental results.

The different results could be recognized, especially in terms of glucose and sugar yield, and were associated with the different reactivity among the different biomass species. Reactivity is the combined effect of crystallinity (in all biomass species), hornification (in EFB), and lignin composition (in pine wood and EFB), which is related to the cellulose accessibility by sulfuric acid. In other words, crystallinity, hornification (Diniz et al., 2004), and lignin composition interfere the solvent diffusivity into the cellulose structure. At the lower sulfuric acid concentration (~65 wt%), crystallinity is the dominant factor for the reducing solvent diffusivity, as shown by the low glucose production in all the raw biomass. As the sulfuric acid concentration increased, lignin composition and hornification might affect the degree of cellulose conversion where cellulose crystallinity had been reduced by the higher sulfuric acid concentration (>75 wt%). As previously mentioned, the lowest sugar recovery efficiency in pine wood hydrolysis has been attributed to the highest crystallinity as one of the possible reactivity factors while oak wood which showed the lowest crystallinity gained the highest sugar recovery efficiency. Finally, the optimum process conditions are summarized in Table 4.

Even though the results have indicated that there could be an important correlation between CrI and sugar yield to explain the cellulose digestibility in lignocellulosic biomass, it is still necessary to conduct further studies about the effect of other factors affecting cellulose accessibility such as lignin/hemicellulose content and distribution, hornification, particle size, and porosity of cell wall in order to gain more comprehensive understanding on the cellulose digestibility in various lignocellulosic biomass.

4. Conclusions

Concentrated acid hydrolysis has been successfully applied to break cellulose crystal structure and extract sugars without further hemicellulose degradation from oak wood, pine wood, and EFB. Reaction severity, which is mainly the combination between decrystallization sulfuric acid concentration and hydrolysis temperature, affected the sugar yield showing the compromise between glucose and xylose yield. At the optimum process condition, 78–96% sugars out of theoretically extractable sugars have been fractionated with 87–90 g/L sugar concentration in the hydrolyzate. Pine showed the highest recalcitrance followed by EFB and oak based on the correlation between CrI and sugar recovery efficiency.

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