Handling heterogeneous hybrid poplar particle sizes for sugar production

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Abstract

Five chip sizes of freshly harvested hybrid poplar, ranging from 0.2 x 0.2 cm to 2.0 x 1.5 cm, plus an equal mixture of all the particles, were used to determine the influence of initial particle size on sugar recovery after steam explosion pretreatment and enzymatic hydrolysis. It was found that there is essentially no effect of particle size or particle size heterogeneity on sugar recovery. Enzymatic digestibility of solids from all the particles showed similar yields ranging from 78 to 82% glucan conversion after 72 hours. The overall sugar recovery from all the samples ranged from 87-90% and 61-64% for glucose and xylose respectively and was not influenced by particle size. An unsteady heat transfer model was used to assess the intra-particle temperature profile of the wood during pretreatment. It was found that all the wood chips, regardless of size, are essentially isothermal. This supports the experimental result of no significant difference in sugar yields among different particle sizes. Steam pretreatment appears to be a robust method that can accommodate a wide range of particle sizes.

Keywords: feedstock heterogeneity, particle size, steam pretreatment, hydrolysis, sugar production, hybrid poplar.

1. Introduction

The use of lignocellulosic biomass for the production of fuels and chemicals will be imperative if petroleum is to be replaced by renewable and sustainable alternatives. Production of fuels from lignocellulosic materials has attracted worldwide interest because they do not compete with food, and have significantly lower global warming potentials [1]. The United States has diverse and abundant biomass resources that can potentially be used as fuels and chemicals feedstocks. Biomass in its raw, “as-harvested”, form may not, however, be a good feedstock for fuels and chemicals production [2]. It is low in energy and bulk density, and has variable physical attributes, all of which can reduce the feedstock’s energy value and make supply system logistics complex and expensive [3].

Feedstock cost is a major economic factor in commercial scale biofuels production, contributing 40-50% of the operating costs in a lignocellulosic biomass based biorefinery [4]. The low selling prices of the products generated by a biorefinery do not allow a biomass conversion facility to afford ‘pristine’ feedstock composed of clean and homogeneous high quality raw material [5]. Low cost feedstocks will have heterogeneous chemical compositions [6] and physical characteristics. This variability results from plant physiology, agronomic and environmental conditions, as well as differences in the way biomass is harvested, stored and processed prior to conversion [7]. Heterogeneous biomass, including characteristics such as particle size, moisture content, bark content, leaf/needle content and general chemical attributes of the biomass, will affect the bioconversion process [8]. Nonetheless, the capability for a

biorefinery to handle heterogeneous feedstock – including particle size - is an important criteria for economic viability.

Size reduction has been proposed as a critical operation for preparing physically heterogeneous feedstock for use in a biorefinery [9]. However, size reduction of biomass along with transportation and storage costs can make up between 13 and 28% of total feedstock costs [10]. Thus, to reduce feedstock costs, particle size heterogeneity in the feedstock may need to be tolerated. In order to make the biomass to biofuels and biochemicals process economically feasible and sustainable, a robust bioconversion system should handle different particles sizes without altering the overall sugar yields.

Most bioconversion research has been carried out using high quality, homogeneous biomass. This biomass, such as screened white wood chips or milled agricultural residues, is typically uniformly comminuted. Little attention has been paid to the efficacy of converting variable particle sizes biomass into fermentable sugars and fuels. The influence of particle size on bioconversion had been studied by a few researchers. Brownell et al. [11], analyzed the effect of chip size on bioconversion of aspen wood to better understand the influence of the particle size on lignocellulosic bioconversion. They concluded that smaller chip size (3.2 mm fiber direction) resulted in more rapid chip heating and more extensive decomposition during pretreatment, leading to high enzymatic hydrolysis yields. Brownell et al. [11] also concluded that larger aspen wood chips produced more of the fermentation inhibitors 5-hydroxymethylfurfural (HMF) and furfural, due to slow particle heating. Negro et al. [12] steam pretreated two poplar particle sizes, 2 to 5 mm and 12 to 15 mm, and found no effect on either enzyme digestibility of pretreated solids or on the downstream ethanol fermentation yield. The

effect of using mixed particle sizes on overall sugar production via steam pretreatment and bioconversion has not been investigated.

As mentioned previously, the economic success of a potential biorefinery is directly related to the use of low cost biomass. While a more heterogeneous raw material is less expensive, the efficiency of the whole bioconversion process using mixtures of different particles sizes has not yet been investigated. In this research, the effect of feedstock particle size and particle size heterogeneity was evaluated using a single raw material, and one set of pretreatment conditions. Robust statistical analysis with replicates of all performed experiments was applied to quantitatively assess the impact of particle size on sugar recovery. The objectives of this research were twofold. Firstly, to determine the influence of particle size and particle size heterogeneity on sugar production during bioconversion of hybrid poplar via steam explosion and enzymatic hydrolysis. Secondly, to evaluate the robustness of steam explosion as a pretreatment for simultaneously processing different particle sizes ranging from 0.2 x 0.2 cm to 2.0 x 1.5 cm. A heat transfer analysis was performed to provide a theoretical underpinning for the experimental results.

2. Methods

In this study, five particle sizes ranging from 0.2 x 0.2 cm to 2.0 x 1.5 cm, plus an equal weighted mixture of all particles, were used to determine the influence of particle size on sugar production following steam pretreatment and enzymatic hydrolysis. The steam pretreated particles were then chemically characterized and saccharified. A complete mass balance of carbohydrates was determined to assess overall sugar recovery. Figure 1 shows process flow for the bioconversion experiments.

2.1. Material

Freshly harvested 18-year-old hybrid poplar, *Populus deltoides* x *Populus nigra*, from Puyallup, WA was used for this study. The biomass was comminuted using two different processes to obtain five different size fractions: S1 (length: 0.2 x width: 0.2 x thickness: 0.1 cm), S2 (0.4 x 0.4 x 0.4 cm) and S3 (1.2 x 0.4 x 0.4 cm) (referred to as Crumbles™) were made using a patented technology developed by Forest Concepts, LLC and previously described by Dooley et al. [13]. In the Forest Concepts process, Crumbles™ particles are produced by feeding wood veneer in a direction normal to the grain through a counter rotating pair of intermeshing cutting discs aligned axially perpendicular to the direction of veneer travel. S4 (0.7 x 1.0 x 0.2 cm) and S5 (2.0 x 1.5 x 0.4 cm) (chips), were made using a slant disc chipper (Acrowood, Everett, WA). In addition, a mixture was prepared of all the particles in equal proportions. The particles had a moisture content of 60-65% and were stored at -20°C until use.

2.2 Calculation of sugar recovery

Material balances for each particle size were closed for steam pretreatment and enzymatic hydrolysis by measuring the composition and total mass of each liquid and solid stream leaving pretreatment and saccharification and converting this data to amount of sugars recovered. The calculations were based on Wyman et al. [14]. Recoveries were then calculated based on glucose and xylose available in the raw material fed to the systems. Thus, based on hybrid poplar composition and the appropriate increase in mass with hydrolysis, for S1, a maximum of 54 and 15 mass units of glucose and xylose respectively could be produced from 100 mass units of hybrid poplar. After pretreatment and saccharification a monomeric sugar recovery of

87.3 and 62.1% of glucose and xylose were observed for S1. Thus, 47.1 and 9.3 mass units of glucose and xylose were recovered after bioconversion. Identical procedures were completed for the rest of the samples.

2.3. Pretreatment and processing conditions

The bioconversion process flow for this research is shown in Fig. 1. Prior to pretreatment, 800 g of dry biomass of each of the six feedstocks was impregnated with gaseous sulfur dioxide (3% w/w) and sealed in airtight plastic bags. Specifically, 24 g of SO₂ was added to the 800 g of dry biomass from a gas cylinder into a plastic bag containing the biomass. After 12 hours, the weight of the biomass was monitored to determine the gas retention for each chip size. Samples were then subdivided into 400 g samples and pretreated using a 2.7 L batch steam gun manufactured by Aurora Technical (Savona, BC, Canada) at 195°C for 5 min. The pretreatment conditions were chosen for previous work done by Ewanick [8]. At the end of the reaction time, a pneumatic valve was opened between the pressurized reaction tank and the collection tank, causing the explosion of the biomass, which was discharged into the collection tank. The resulting slurry was vacuum filtered to separate the liquid fraction from the pretreated solids. Both fractions were analyzed as described below and used to construct a mass balance of carbohydrates and lignin. Solids were washed with deionized water equal to 20 times the mass of solids prior to analysis and hydrolysis. Each steam explosion was performed 3 times for each different size fraction and the mixture, resulting in a total of 18 substrates, all of which were analyzed as described below.

2.4. Instrumental analysis

2.4.1. High Pressure Liquid Chromatography (HPLC)

Carbohydrates were measured on a Dionex (Sunnyvale, CA) HPLC (ICS-3000) system equipped with an AS autosampler, ED electrochemical detector, dual pumps, and anion exchange column (Dionex, CarboPac PA1). Deionized water at 1 mL/min was used as an eluent, and post-column addition of 0.2 M NaOH at a flow rate of 0.5 mL/min ensured optimization of baseline stability and detector sensitivity. After each analysis, the column was reconditioned with 0.25 M NaOH. Twenty microliters of each sample were injected after filtration through a 0.22 µm syringe filter (Restek Corp., Bellefonte, PA). Samples were measured against standards consisting of arabinose, galactose, glucose, xylose, and mannose. In addition, fucose was used as an internal standard.

Acetic acid, furfural and 5-hydromethylfurfural were measured using refractive index detection on a Shimadzu Prominence LC. Separation of these compounds was achieved by an anion exchange column (REZEX RHM-Mono-saccharide H⁺ (8%), Phenomenex, Inc., Torrance, CA) with an isocratic mobile phase that of 5 mM H₂SO₄ at a flow rate of 0.6 mL/min. The column oven temperature was maintained at 63°C. Twenty microliters of each sample were injected after being appropriately diluted in deionized water and filtered through a 0.22 µm syringe filter (Restek Corp., Bellefonte, PA). Standards were prepared and used to quantify the unknown samples.

2.5. Compositional analysis

2.5.1. Insoluble carbohydrates, acetate groups and lignin

Raw hybrid poplar and solids were analyzed gravimetrically for lignin content, photometrically for soluble lignin, and by HPLC for carbohydrate and acetates content using modified TAPPI method T-222 om-98 [15], NREL procedure LAP 004, LAP 002 and LAP 017 [16-18] respectively.

Briefly, 0.2 g of finely ground oven dried sample was treated with 3 mL of 72% H_2SO_4 for 120 minutes at room temperature, then diluted into 120 mL total volume, and then autoclaved at 121°C for 60 minutes. The autoclaved samples were then filtered using a glass crucible to separate the acid insoluble lignin (AIL) from the acid soluble lignin (ASL) and carbohydrates. The AIL was determined by weighting the oven dried crucibles, and the ASL in the filtrate was analyzed by UV at 205 nm and calculated using absorptivity coefficient of 110 L/g-cm [16]. The filtrate was analyzed by HPLC for carbohydrate composition and acetates content.

2.5.2. Soluble carbohydrates

Monomeric and oligomeric soluble carbohydrates were determined using NREL LAP TP-510-42623 [19]. Briefly, 0.7 mL of 70% H_2SO_4 was added to 15 mL of the liquid samples, and the volume made up to 20 mL with water. Samples were autoclaved at 121°C for 60 minutes and analyzed by HPLC as described previously. Monomeric sugars were determined by analyzing the original samples without acid hydrolysis. Oligomeric sugar was calculated by subtracting monomeric sugar content from total sugar content determined after acid hydrolysis.

2.6. Bulk density analysis

Bulk density of different particles of hybrid poplar was determined according to the ASAE Standard S269.4 DEC 91 for cubes, pellets, and crumbles [20]. One cylindrical container with a specific inside diameter was used for determination of bulk density of different particle sizes and the particle size mixture. Each measurement was repeated for five times. Biomass was poured into the container from a certain height to facilitate the free flowing of the samples until the container was overflowed. The excess material was removed by striking a straight edge across the top. The loosely filled container was tapped on the laboratory bench five times by dropping the container from a height of 10 cm. Filling and tapping was repeated until the container overflowed. The weight of the material with the container was recorded. The net weight of the sample was obtained by subtracting the weight of the empty container. The filled container was weighed to 0.01g precision. Bulk density was calculated by dividing the mass by the container volume.

2.7. Enzymatic hydrolysis

Enzymatic hydrolysis of washed solids was done at 5% w/v solids in a total volume of 50 mL in 125 mL Erlenmeyer flasks. The solution was buffered at pH 4.8 with 0.05 M citric acid buffer and the hydrolysis was carried out at 50°C and 150 rpm shaking on an orbital shaking incubator (New Brunswick). Cellulase (Celluclast 1.5 L, 26 FPU/mL, Sigma) was added at 5 FPU/g cellulose and supplemental beta-glucosidase (Novozym 188, 492 CBU/mL, Sigma) was added at 10 CBU/g cellulose. 1 mL samples were periodically removed and analyzed for glucose content.

2.8. Statistical analysis

One way ANOVA at 5% alpha was performed for each dependent variable to assess statistical differences as a function of particle size. In the cases where statistically significant differences were found (p value<0.05), a “Tukey test” (multiple comparison of means) was performed to find the specific group or groups of particles that were causing those differences. Data analysis was done using the statistical software R version 2.12.1.

2.9. Heat transfer model

An unsteady state heat conduction in a finite slab with a 4 mm thickness – the thickest chips - was used to investigate the pretreatment reaction uniformity. A heat balance on the chip gives the following differential equation:

$$\frac{\partial T}{\partial t} = \alpha \left(\frac{\partial^2 T}{\partial x^2} \right), \alpha = \frac{k}{\rho C_p} \quad (1)$$

Where T is the temperature, t is the reaction time, x is the position in the slab, α is the thermal diffusivity, k is the thermal conductivity, ρ is the density, and C_p is the heat capacity. With initial and boundary conditions of:

$$t = 0, T = T_i, -b < x < b \quad (2)$$

$$x = -b, \quad T = T_s, \forall t > 0 \quad (3)$$

$$x = b, \quad T = T_s, \forall t > 0 \quad (4)$$

Where, T_i is the particle initial temperature (25° C), b is the particle thickness, x is the position in the slab ($x=0$ chip center) and T_s is the particle surface temperature.

252 The analytical solution to equations (1)-(4) was obtained in the dimensionless form as
 253 follows:

$$\frac{T(x, t) - T_s}{T_i - T_s} = 2 \sum_{n=0}^{\infty} \frac{(-1)^n}{(n + 0.5)\pi} \exp\left[-(n+0.5)^2 \pi^2 \frac{\alpha t}{b^2}\right] \cos\left[(n + 0.5) \pi \frac{x}{b}\right] \quad (5)$$

254 Where, $T(x, t)$ is the temperature in position x at time t .

255 The thermal diffusivity (α) was calculated using the density of hybrid poplar and
 256 the thermo physical properties of wood which were estimated from relations published
 257 by Simpson and TenWolde [21] (Table 1). Equation 5 was solved using the numerical
 258 computing software Matlab version 8.1.0.604 (R2013a).

259 **3. Results and discussion**

260 *3.1. Bulk density and raw material composition*

261 Bulk density and chemical composition of the original six different groups of
 262 particles are presented in Table 2. The bulk density of all of the different particle sizes
 263 was low and not substantially different among the different particles sizes, ranging from
 264 0.15 to 0.19 g/cm³. Glucan and xylan made up the majority of the carbohydrates in the
 265 raw material, ranging from 47 to 52% and from 12 to 15% respectively. Acetate content
 266 varied from 3.5 to 3.8%. The presence of both acid soluble lignin (ASL) and acid
 267 insoluble lignin (AIL) were similar among the different samples, ranging from 25 to 26%.
 268 No statistical differences were found among the groups in terms of glucan, xylan,

acetates and lignin content. The chemical composition of raw poplar was found to be similar to results obtained by Negro et al. [12]; and Bura et al. [22].

3.2. Solids composition after pretreatment

The influence of particle size on the chemical composition of solids after steam pretreatment was measured. The chemical composition of solids after steam pretreatment, expressed as % dry matter, for different particle sizes and the mixture are shown in Fig. 2. The glucan and lignin content ranged from 61 to 65% and from 32 to 36%, respectively. No statistically significant differences were found for either glucan or lignin content for all the particles tested, including the mixture of particles. As expected, only glucan and lignin were found in solids, since all the hemicellulose was solubilized into the liquid fraction. Similar values were obtained by Bura et al. [22], who pretreated poplar using steam pretreatment under similar conditions, reporting values for glucan and lignin of 68 and 31% respectively. Negro et al. [12] performed uncatalyzed steam pretreatment using poplar feedstock with particle sizes of 2 to 5 mm and 12 to 15 mm. They found the cellulose, hemicellulose and lignin content of the washed solid fraction ranged from 56-59%, 4-9% and 30-38% respectively. The high levels of hemicellulose observed in the solid fractions were likely due to the lack of acid catalyst during pretreatment. Negro et al. [12] also reported no significant influence of chip size on the chemical composition of solids after pretreatment.

The chemical composition of the mixed particle size after steam explosion was determined to test whether steam pretreatment was a suitable fractionation method for processing a heterogeneous mixture of different particle sizes. It was found that the glucan and lignin content of the pretreated mixed sample was identical to those

produced with the uniform particle size samples. No statistical differences were found between the mixed sample and other particles sizes (S1-S5). Steam pretreatment was able to produce a consistent slurry regardless of particle size and particle size heterogeneity, demonstrating the robustness of this method for biomass fractionation.

3.3. Liquid stream composition after pretreatment

Concentration of sugar, acetic acid, furfural and 5-hydroxymethylfurfural (HMF) in the liquid fractions after pretreatment were measured and the results are shown in Table 3. Table 3 shows that the concentration of glucose and xylose, expressed as % of untreated biomass weight, ranged from 6 to 9% and from 8 to 10%, respectively. The majority of the sugars were in monomeric form, ranging from 84 to 88% and 90 to 94% for glucose and xylose respectively, showing the efficiency of SO₂ in hydrolyzing carbohydrates to monomeric sugars [23]. A statistically significant difference was found in the glucose content in the liquid streams, where the smallest size (S1) showed the lowest glucose recovery (6.4%). Similar trends were found by Ballesteros et al. [24], who steam pretreated herbaceous agricultural waste (*Brassica carinata*) using three particle sizes (2-5, 5-8 and 8-12 mm), where the smallest size showed the lowest cellulose recovery. In the present study, no statistically significant differences were observed in xylose content of the liquid stream among the different particle sizes, including the mixture of particles. Furthermore, particle size did not influence the presence of sugars in monomeric or oligomer form in the liquid stream. As expected, more xylose than glucose was found in the liquid streams, because hemicellulose is more labile than cellulose and becomes solubilized during pretreatment. Similar results were found by Negro et al. [12], but they found that particle size did affect the liquid

stream xylose content, with a higher xylose concentration resulting from the smaller particles.

Acetic acid is formed by hydrolysis of acetyl groups in the hemicellulose when using hydrothermal pretreatments to fractionate hardwood and agricultural residues. Table 3 shows that the acetic acid content in the water soluble fraction ranged from 3.8 to 4.6% w/w of initial raw material (15-18 g/L) and did not show any significant statistical difference among the different particle size, including the mixture of particles. Similar results were found by Vajzovic [25], who steam pretreated hybrid poplar with 3% SO₂ at 195° C for 5 minutes, and reported an acetic acid content of 3.7%. Schutt et al. [26], steam pretreated poplar using more severe pretreatment conditions, and reported lower values of acetic acid ranging from 2.8-3 %. The reduced acetic acid content can be explained by the use of dry biomass, which can result in ineffective SO₂ catalysis and incomplete hemicellulose dissolution [27].

Furans such as furfural from pentoses and 5-hydroxymethylfurfural (HMF) from hexoses, which are the products of dehydration reactions during pretreatment under severe acidic conditions [23], were also found in the liquid fractions (Table 3). The furfural content was lower than 1.6% (6.5 g/L) and HMF was less than 0.3% (1.2 g/L) for all the six groups of particles. Similar results were obtained by Vajzovic [25], who pretreated hybrid poplar using steam pretreatment under the same conditions used in this present study, and reported values for of 1.3% for furfural and 0.3% for HMF.

As shown in Table 3, the chemical composition of the liquid stream coming from the mixture of particles was similar to that coming from the different particle sizes. No

statistical differences were found for acetic acid and xylose contents of the liquid streams from the mixed particle size and the other particle sizes.

3.4 Enzymatic hydrolysis

The influence of raw material particle size on enzymatic digestibility of the washed pretreated solids was evaluated. Figure 3 shows the cellulose conversion of the six particle sizes investigated. It can be seen that the enzymatic hydrolysis of the all steam pretreated particles showed similar glucan to glucose conversion. A conversion range from 78 to 82% was observed during 72 hours of enzymatic hydrolysis for all the particle sizes, and no statistically significant differences were found among the different pretreated substrates, including the mixture of particles. This trend has been previously observed by Negro et al. [12], who enzymatically hydrolyzed uncatalyzed steam pretreated poplar coming from two particle size fractions, 2 to 5 mm and 12 to 15 mm. Negro found no significant effect of particle size on enzymatic digestibility. Similarly, Monavari et al. [28], steam pretreated 3 different spruce chip sizes (1-2, 3-4 and 5-6 mm in thickness with an average length of 30-45 mm) and concluded that particle size did not significantly influence the enzymatic hydrolysis of the substrates, suggesting that feedstock initial particle size is not an important factor for enzymatic saccharification of biomass.

Chandra et al. [29] have examined the properties of various lignocellulosic substrates following pretreatment and hydrolysis. They found that hemicellulose and lignin content, crystallinity, degree of polymerization, and available surface area, are important characteristics for subsequent enzyme hydrolysis. In the current study, the similar chemical composition of all solids after pretreatment, in which no xylan and

acetic acid were present, might have contributed to the similar saccharification for all sizes. When xylan is present on the surface of cellulose the action of cellulases can be hindered [30]. According to Chang and Holtzapple [31], lignin and acetyl groups function as parallel barriers for enzymatic hydrolysis. The absence of acetyl groups as well the similar lignin content in solids for all the particle sizes resulted in similar saccharification curves for all the sizes, including the mixture of particles. It appears that a wide range of particle sizes can be effectively processed during steam pretreatment, resulting in pretreated substrates with similar chemical properties. Therefore the initial particle size heterogeneity of hybrid poplar is not an important factor to consider if the fractionation process is sufficiently robust to complete hemicellulose and acetyl groups hydrolysis.

3.5 Total sugar recovery

The overall sugar recovery after pretreatment and enzymatic hydrolysis was calculated and is shown in Table 4. Table 4 shows the combined overall recovery of glucose and xylose from solid and liquid fractions, expressed as % of the theoretical amount available in the raw material. It can be seen that 87 to 90% of glucose was recovered in all samples. 61 to 64% of the xylose was recovered, all of it after steam pretreatment. No statistical differences were found in the total sugar recovery among the particle sizes, including the mixture of particles. These results parallel the equivalent chemical composition of solid and liquid streams among the different pretreated substrates after steam explosion, as well as the similar enzymatic hydrolysis conversion for all the particles tested including the mixture of particles. The initial size or size heterogeneity of the different particles did not significantly affect the overall sugar yields.

Previous research has shown mixed effects of particle size on sugar recovery. Negro et al. [12], observed higher cellulose recovery for larger sizes and higher xylose recovery for smaller particles. Monavari et al. [28], established that the glucose recovery decreased with increasing chip size after steam explosion and enzymatic hydrolysis. Conversely, Cullis et al. [32], reported an improvement in glucose and hemicellulose derived sugar recovery by increasing chip size and moisture content. Identical effects were found by Ballesteros et al. [33], where higher cellulose and hemicellulose recovery were obtained for larger particles (8-12 mm). Similar results were found by Liu et al. [34], who pretreated five different particles sizes of corn stover ranging from 0.5 to 2.5 cm. They established that high pressure steam penetrated the interior of the bed of corn stover easier with larger particle size due to the high porosity or low bulk density. The improved steam penetration with larger particles resulted in higher conversion yields compared to those obtained with smaller particles and higher bulk density. In the current study, the bulk density was low for all the particle sizes and not substantially different. High pressure steam should equally penetrate the interior of the different bed of particles, resulting in the efficient biomass steam explosion for all the particles tested. While previous studies reported an effect of chip size on sugar recovery, the statistical significance on the sugar recovery and the effect of simultaneously processing different particle sizes were never established.

The sugar recovery results show that the efficacy of pretreatment and saccharification was independent of particle size. An unsteady state heat transfer model was developed to better understand the insensitivity of steam explosion pretreatment to particle size. Figure 4 shows the calculated temperature trajectory at the center of the

thickest chip (4 mm) when the temperature in the reactor is held at 195°C. It can be seen that the center of the thickest chips reaches the target temperature in approximately 27 seconds. Thinner chips, of course, will reach the target temperature quicker. The total reaction time in the steam gun was 5 minutes so it can be safely assumed that all the particles are isothermal during pretreatment. The absence of significant temperature gradients, regardless of particle size, is consistent with the uniformity of sugar recovery for all the particles investigated.

4. Conclusions

The influence of initial particle size and particle size heterogeneity of hybrid poplar on sugar recovery and enzymatic hydrolysis after steam explosion pretreatment were investigated. The solid fractions after pretreatment for all the particle sizes had equivalent chemical compositions, including an absence of acetyl groups and xylans. Saccharification of the solids resulted in similar cellulose to glucose yields ranging from 78% to 82%. All the particle sizes investigated also had similar sugar recoveries in the liquid stream after pretreatment. The overall sugar recovery after pretreatment and saccharification ranged from 87-90% and 61-64% for glucose and xylose, respectively, and there were no significant differences among the different particle size samples. An unsteady heat transfer analysis shows that all the particle sizes investigated are essentially isothermal during pretreatment, providing theoretical support of the experimental results. Consequently, the initial chip size ranging from 0.2 x 0.2 cm to 2.0 x 1.5 cm does not significantly affect the bioconversion process, from steam explosion to overall sugar recovery after saccharification. Steam pretreatment is therefore a robust

method that can accommodate different particle dimensions in the same reactor, without significantly altering downstream conversion processes.

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Table 1

Hybrid poplar density and thermophysical properties.

Property	Value	Unit
Thermal conductivity (k)	0.23	W/(m K)
Hybrid poplar density (ρ)	355	Kg/m ³
Heat capacity (C_p)	1255.47	J/(kg K)

Table 2

Bulk density and compositional analysis of untreated biomass for the different particle sizes. Error intervals are standard deviations from triplicate measurements.

Sample	BD ^a (g/cm ³)	Glucan (%)	Xylan (%)	Acetate (%)	Lignin (%)
S1	0.16 ± 0.01	48.6 ± 0.8	13.5 ± 1.6	3.7 ± 0.1	25.6 ± 0.3
S2	0.15 ± 0.01	49.0 ± 1.0	13.0 ± 1.0	3.8 ± 0.1	26.1 ± 0.4
S3	0.17 ± 0.01	51.9 ± 1.5	12.6 ± 1.5	3.5 ± 0.1	24.7 ± 0.3
S4	0.17 ± 0.01	49.1 ± 0.6	14.6 ± 1.6	3.8 ± 0.4	26.4 ± 0.3
S5	0.19 ± 0.01	46.9 ± 0.8	12.3 ± 0.8	3.5 ± 0.3	25.1 ± 0.3
Mix	0.18 ± 0.01	49.1 ± 0.6	13.2 ± 1.0	3.6 ± 0.1	25.6 ± 0.8

^a Bulk density.

Table 3

Concentration of glucose, xylose, acetic acid and furans in the liquid fraction after pretreatment of biomass for the different particle sizes (expressed as % of untreated biomass weight). Error intervals are standard deviations from triplicate measurements.

	Glucose (%)		Xylose (%)		Acetic acid (%)	Furfural (%)	HMF ^b (%)
	Total	Mon ^c	Total	Mon ^c			
S1	6.4 ± 0.2	87	8.9 ± 0.8	93	3.80 ± 0.23	1.08 ± 0.05	0.21 ± 0.01
S2	7.3 ± 0.7	87	8.2 ± 1.3	94	3.87 ± 0.56	1.20 ± 0.06	0.26 ± 0.03
S3	8.5 ± 0.4	88	8.2 ± 0.7	95	4.24 ± 0.39	1.34 ± 0.17	0.29 ± 0.02
S4	7.5 ± 0.6	88	10.1 ± 0.3	93	4.61 ± 0.32	1.54 ± 0.06	0.28 ± 0.03
S5	8.0 ± 0.1	84	9.3 ± 0.6	90	4.30 ± 0.31	1.59 ± 0.12	0.29 ± 0.01
Mix	8.1 ± 0.4	84	8.9 ± 0.1	93	4.12 ± 0.12	1.44 ± 0.15	0.25 ± 0.01

^b 5-hydroxymethylfurfural, ^c Monomeric sugar.

Table 4

Combined overall recovery of monomeric glucose and xylose of solid and liquid fractions after pretreatment and enzymatic hydrolysis, expressed as % of the theoretical available in the raw material. Error intervals are standard deviations from triplicate measurements.

Sample	Glucose (%)	Xylose (%)
S1	87.3 ± 2.0	62.1 ± 1.1
S2	87.7 ± 1.5	62.0 ± 1.8
S3	90.2 ± 1.3	61.6 ± 1.4
S4	89.8 ± 2.4	63.7 ± 1.8
S5	88.6 ± 2.7	64.4 ± 1.7
Mix	88.5 ± 1.6	61.1 ± 0.9

Figure

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Figure caption

Fig. 1. Process flow diagram for bioconversion of hybrid poplar into carbohydrates.

Fig. 2. Composition of solid fractions of pretreated hybrid poplar, expressed as % dry matter, for different particle sizes. Error bars indicate standard deviations from triplicate measurements.

Fig. 3. Cellulose to glucose conversion for steam pretreated hybrid poplar during enzymatic hydrolysis at 5% (w/v) solids consistency and 5 FPU/g cellulose and 10 CBU/g cellulose enzymes loading. Error intervals indicate standard deviations from triplicate measurements.

Fig. 4. Calculated temperature profile at the center of a 4 mm thick poplar particle.