Bioresource Technology 238 (2017) 254–262

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

A co-production of sugars, lignosulfonates, cellulose, and cellulose nanocrystals from ball-milled woods

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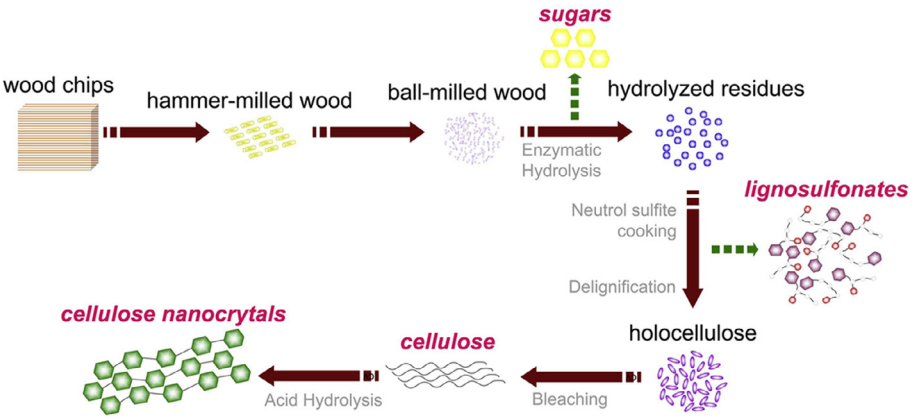
HIGHLIGHTS

- Micronized wood facilitates the co-production procedures.
- The mass balance points out the potential of cellulose and lignin recovery.
- Holistically, the study demonstrated the concept of co-production would be realizable.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 December 2016
Received in revised form 16 March 2017
Accepted 17 March 2017
Available online 22 March 2017

Keywords:
Sugars
Lignosulfonates
Cellulose
Cellulose nanocrystals
Co-production
Particle size
Aspect ratio
Crystallinity
Mass balance

ABSTRACT

This study demonstrated the technical potential for the large-scale co-production of sugars, lignosulfonates, cellulose, and cellulose nanocrystals. Ball-milled woods with two particle sizes were prepared by ball milling for 80 min or 120 min (BMW_{80}, BMW_{120}) and then enzymatically hydrolyzed. 78.3% cellulose conversion of BMW_{120} was achieved, which was three times as high as the conversion of BMW_{80}. The hydrolyzed residues (HRs) were neutrally sulfonated cooking. 57.72 g/L and 88.16 g/L lignosulfonate concentration, respectively, were harvested from HR_{80} and HR_{120}, and 42.6 ± 0.5% lignin were removed. The subsequent solid residuals were purified to produce cellulose and then this material was acid-hydrolyzed to produce cellulose nanocrystals. The BMW_{120} maintained smaller particle size and aspect ratio during each step of during the multiple processes, while the average aspect ratio of its cellulose nanocrystals was larger. The crystallinity of both materials increased with each step of wet processing, reaching to 74% for the cellulose.

1. Introduction

Lignocellulosic biomass is an abundant and sustainable, carbon-neutral resource. It can be converted into biofuels as a promising

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http://dx.doi.org/10.1016/j.biortech.2017.03.097
0960-8524/

renewable energy alternative to fossil fuels (Wyman, 1999). The biochemical conversion of biofuel production from lignocellulosic biomass includes the pretreatment of biomass feedstock, enzymatic hydrolysis of disrupted carbohydrates (saccharification), and the fermentation of sugars by microbes. Since upstream progresses, i.e. pretreatment and saccharification, are the key technological barriers for industrial production of cellulosic ethanol, substantial research efforts have been made in pretreatment of biomass feedstock to improve the cellulose-to-glucose conversion. The versatile pretreatment methods are classified into physical (grinding and milling) (Alvira et al., 2010; Barakat et al., 2014), chemical (acids, alkalis, oxidizing agents, sulfites, sulfates, and organic solvents) (Zhang et al., 2006; Sun and Cheng, 2002; Liu et al., 2016a; Yang et al., 2013), physico-chemical (steam explosion) (Cantarella et al., 2004), biological (bacterial) (Itoh et al., 2003), and a combination of these pretreatment methods (Pan et al., 2004; Zhu et al., 2009). Finally, an over 90% of cellulose-to-glulose conversion was reachable after the above pretreatments. However, these pretreatments to overcome the recalcitrance of biomass require more energy consumption, chemical usage, or damage to residual carbohydrate components and lignin (Agarwal et al., 2013; Miao et al., 2011). For example, physical treatment for the size reduction consumed 0.2–3.1 $\times 10^3$ MJ/ton wood with various milling types, species, and conditions (Zhu et al., 2015). Organic solvent treatment or sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) consumed 1.6–1.8 $\times 10^3$ MJ/ton wood based on sugar yields at 65% carbohydrate equivalent with the optimal conditions, and the consumption in steam explosion with the same sugar yields was as much as 2.0 $\times 10^3$ MJ/ton wood (Zhu and Pan, 2010; Zhu et al., 2015). Therefore, the complicated procedures to produce biofuels as a single-product are currently not cost-competitive for large consumption. To solve this problem, a further biorefinery concept should be designed with processes that produce additional high value-added products from lignocellulosic biomass, generating the maximum value by balancing multi-products distribution.

The traditional biorefinery reported in the literature have focused on only improving the percentage of cellulose-to-glucose conversion. The hydrolyzed residues or unemployed components has been overlooked. Enzymes digest cellulose and hemicellulose into monomeric sugars. Generally, with a physical treatment of feedstock, the integrated lignin and partly recalcitrant cellulose with high crystalline structure are still remained in the hydrolyzed residues. A considerable amount of lignin can be recovered in chemical pretreatment and partly recalcitrant cellulose is remained in hydrolyzed residues. Therefore, a potential to transfer the unemployed components into value-added products is existent. The significant profitability improvement in biorefinery operations is possible.

It is well known that lignin derivatives are popular used in the practical application, since natural lignin is difficult to be isolated from lignocellulosic biomass. Sulfonated lignin (lignosulfonate) is a commercial value-added product, which is widely used in construction, animal feed, drilling, packaging, leather, and agriculture (Cheng et al., 2015; Aitcin, 2000; Suparno et al., 2005; Lora et al., 2008). The sulfonation is performed under alkaline or acid conditions, and a over 50% conversion of lignin was obtained (Xu et al., 2015; Liu et al., 2016a). Nevertheless, the usage of alkaline or acid is inconsistent with the minimizing environmental risk. An alternative milder method was developed to use hydrolysis and neutral sulfite processes, reported by Liu et al. (2016a) Liu et al. (2016b). Unlike the traditional approach of alkaline and acid sulfite cooking by the extraction of lignin at the cost of the degradation of a considerable amount of another components, this neutral sulfite process may maintain cellulose and hemicellulose and produce non-toxic byproducts, like furfural, acetic acid (Prusas, 1984; Zhu et al., 2009; Jin et al., 2013; Gu et al., 2012). This treatment can be acted as an optimization to recover lignosulfonates in biorefinary.

The other potential recalcitrant cellulose in hydrolyzed residuals can be also theoretically and practically co-produced. Enzymes hydrolyze the amorphous cellulose and then crystalline cellulose, finally remaining crystalline cellulose in hydrolyzed residuals. Their properties are similar to the cellulose in pulping (Frederick et al., 2008; Filson et al., 2009). For economic assessment, the cellulose nanofibers/nanocrystals are highly value-added and achievable in a biorefinery. They can be survived in diluted acid with mechanical force or concentrated acid. In fact, Oksman et al. (2011) isolated cellulose nanocrystals and nanofibers from the industrial biofuel residuals, which exhibited a more thermal stability with a lower surface charge than commercial cellulose nanocrystal isolated from cotton (Herrera et al., 2012). A handful researches have paid attention on co-producting biofuels and biomaterials. The approaches recover only lignin or cellulose combined with the biofuels, and do not recover other components. Therefore, at present, no research attempted to recover sugars, lignin and recalcitrant cellulose in a biorefinary progress.

The aim of this study was to transfer the traditional biorefinery for a single-product to a new biorefinery for producing multi-products. We demonstrate the feasibility of maximizing total value by co-producing multiple products through utilizing all components of a lignocellulosic material. This new biorefinery is designed by using ball milling pretreatment, neutral sulfite delignification, and cellulose upgradation, generating four target products (sugars, lignosulfonates, cellulose, and cellulose nanocrystals). The product yields are balanced to provide an intuitive view of the integrated profitability of multi-products. In order to co-produce more products and maximize their values of these products, recent advances in bioprocessing through medium ball milling pretreatment allows the improved co-production and less energy consumption. Because 92% of sugar conversion spent over 50 days for ball milling, which was a low-efficiency and high energy-consuming process (Agarwal et al., 2013). A moderate/mild mechanical energy may release most accessible cellulose in a low-cost pathway, and balance the remaining intact lignin and less accessible cellulose, which are further chemically processed into value-added products (Zhu et al., 2011; Jiang et al., 2016). Furthermore, we evaluated the effects of micronized wood (ball-milled wood) on the facilitation of co-production and the balance of each product distribution in this study. Neutral sulfite treatment are introduced to recover lignosulfonates in an environmental way. The loosened lignin generated from the medium ball milling pretreatment is also prone to extraction during the neutral sulfite treatment (Lawther et al., 1996; Lu and Ralph, 2003). Cellulose and cellulose nanocrystals are obtained by common bleaching method to accurately learn the product yield. Douglas-fir wood chips were used as a model biomass feedstock to demonstrate the utility of the micronized wood for co-production of multiple products in a biorefinery. The wood chips were first micronized by a pilot planetary ball mill and were further subjected to a sequence of fractionation procedures that were designed to maximize product yield with minimal energy consumption. The mass balance of products or residuals was monitored in each step. This biorefinery can be improved or optimized on an industrial scale based on the best current technologies. Different methods may generate a slightly different balance of materials and products, but the general trend should be the same.

2. Materials and methods

2.1. Ball-milled wood preparation

The preparation of milled wood was subjected to a two-stage milling. Fresh Douglas-Fir wood chips were hammer-milled to

wood flours with particle size of 235 μm (D(50)) using Bliss Eliminator Fine Grind Hammer mill (No. EMF-24115-TFA). The hammer-milled wood was designated as HMW. A gear drive planetary ball mill (Across International Co., USA) was used with a 5-liter capacity steel container and steel balls of ø5 mm, ø10 mm, and ø20 mm for 80 min and 120 min milling time, respectively. One jar was loaded with a ball-to-material ratio (BMR) of 29.6:1. The rotation speed of the disc was maintained at 270 rpm with one rotation direction.

2.2. Enzymatic hydrolysis

Enzymatic hydrolysis of the ball-milled wood (120 g with moisture content of 5.6%) was performed in a 1000 mL flask. 4.74 mL CTec2 enzyme and 0.475 mL HTec2 enzyme (Novozymes Co., USA) were mixed with 60 mL sterile water and added into the milled slurry with a liquor-to-solid ratio of 3.6–1. This gave a milled-wood titer of 19% with an enzyme does of 5.5% on wood, allowing good condition for both hydrolysis yield and high sugar titer achievement. The samples were hydrolyzed at 50 °C with 150–200 rpm shaking speed on an orbital shaker, keeping the pH being around 4.8–5.3 by addition of 20% KOH solution. The hydrolysis was completed after 72–96 h with sugar titer leveling off. The solid/liquid sample was then separated by filtration. The solid sample (hydrolysis residues) produced from the hydrolysis was then stored in a refrigerator at −20 °C for subsequent neutral sulfite cooking.

2.3. Neutral sulfite cooking

The samples of the hydrolysis residual (HR) solids were mixed with a cooking liquor consisting of 16% Na_2SO_3 and 6% Na_2CO_3 (-w/w feed) with a liquid-to-solid ratio of 6–1. The mixture was heated to 160 °C with a temperature increase of 1.3 °C/min. The cooking temperature was maintained at 160 °C for 180 min. The sodium carbonate was used as the buffer to control the pH close to neutral. After this treatment, filtering was performed to collect the neutral sulfite cooking liquor and the lignosulfonate concentration was calculated. The neutral sulfite cooking residues were designated as NSCR.

2.4. Preparation of cellulose and cellulose nanocrystal

The delignification procedure was conducted at 70 °C for 7 h using 6 g of sodium chlorite and 5 mL of acetic acid with addition every hour. The resulting holocellulose was separated by filtration through a filter paper (Whatman, USA), freeze dried, weighted into a flask at ambient temperature for 30 min. Next, 200 mL of 2% sodium hydroxide was added and allowed to react for 2 h at 90 °C. This delignification and bleaching process was repeated for improved purity of the cellulose. The purified cellulose samples were filtered by a sintered glass filter and washed with deionized water. This prepared cellulose was then freeze dried and was designated as C in this study.

Freeze-dried cellulose (1 g per 9.8 mL of acid) was mixed with 64% H_2SO_4 with strong mechanical stirring at 44 °C for 30 min. The suspension was diluted 10 times with deionized water and stored in a refrigerator to prevent further reaction. The supernatant was removed by repeated centrifuge (Sorvall, 5000 rpm for 5-min) until it was turbid. Then supernatant was then collected and sonicated for 1 h, dialyzed against deionized water for a week. The cellulose nanocrystals was designated as CNCs.

2.5. Characterization

2.5.1. Particle size measurement

Particle size distributions of the ball-milled wood were characterized by using Malvern Mastersizer 3000 based on the light scattering pattern of wood particles in water flowing through a lighted cell. Quantitative oven/frozen dried samples were suspended in deionized water by sonication (Branson, USA), keeping the volume density in the range of 16–24% after pouring this suspension into the testing tank. Deionized water was used as dispersion medium and sonicated for 1 min with samples in tank before characterization.

2.5.2. Carbohydrates analysis

A 300.0 ± 10.0 mg portion of the sample was weighed into a tared pressure tube with 3.00 ± 0.01 mL of 72% sulfuric acid. The mixture was placed in a water bath set at 30 ± 3 °C and incubated for 60 ± 5 min with stirring every 5–10 min. This acid was diluted to a 4% concentration by addition of 84.00 ± 0.04 mL deionized water before sealed. A set of sugar recovery standards (SRS), including glucose, xylose, galactose, arabinose, and mannose, was prepared to correct for losses due to the degradation of sugars during dilute acid hydrolysis. The selection of sugars should be chosen to most closely resemble the concentrations of sugars in the test samples. The mixture of sealed sample and SRS were then autoclaved the sealed sample and SRS for one hour at 121 °C. After completion of the autoclave cycle, they were allowed to hydrolyze to slowly cool to near room temperature. The acidic solution was then filtered and diluted to 100 times to analyze carbohydrate composition by ion exchange chromatography (Dionex ICS-3000, Dionex Corp., Sunnyvale, CA, USA). The sugars were calculated by combination of Eqs. (1) and (2):

$$\%Sugar = \frac{C_{anhydro} \times V_{filtrate} \times \frac{1g}{1000mg}}{ODW_{sample}} \times 100 \quad (1)$$

$$C_{anhydro} = \frac{C_{HPLC} \times dilutionfactor}{\%R_{ave.sugar}/100} \quad (2)$$

where ODW_{sample} was the weight of sample in milligrams, $C_{anhydro}$ referred to the concentration in mg/mL of a sugar in the hydrolyzed sample after the correction of loss on 4% hydrolysis, $V_{filtrate}$ was the volume of the filtrate, 86.73 mL, C_{HPLC} was the concentration of a sugar as determined by HPLC, mg/mL, and $\%R_{ave.sugar}$ was the average recovery of a specific SRS component.

2.5.3. Lignin and lignosulfonate measurement

The lignin isolated in the above acid hydrolysis includes acid soluble lignin (ASL) and acid insoluble residue (AIR). A portion of the filtered acidic solution was diluted to 2.5 times by deionized water using a pipette. The absorbance of this diluted solution at 240 nm (wavelength) was measured by UV–Visible spectrophotometer (PerkinElmer Lambda 35, PerkinElmer, Inc., Waltham, MA, USA). ASL determination must be done within 6 h of hydrolysis, otherwise it should be stored in a refrigerator for a maximum of two weeks. The equation of the amount of ASL was calculated according to following Eq. (3):

$$\%ASL = \frac{UVabs \times Volume_{filtrate} \times Dilution}{\varepsilon \times ODW_{sample} \times Pathlength} \times 100 \quad (3)$$

$$LSg/L = \frac{UVabs \times Dilution}{\varepsilon \times Pathlength} \quad (4)$$

where UVabs was the average UV–Vis absorbance for the sample at appropriate wavelength, $Volume_{filtrate}$ was the volume of the acid

hydrolysis filtrate, 86.73 mL, ε was the absorptivity of biomass at specific wavelength, ε_{lignin} was 24, L/g cm, $\varepsilon_{lignosulfonate}$ was 12, L/g cm, ODW_{sample} was the weight of sample in milligrams, and pathlength was the pathlength of UV–Vis cell in cm.

All remaining solids after acid hydrolysis were transferred into the filtering crucible and rinsed with at least 50 mL of deionized water. This residue was dried at 105 ± 3 °C until a constant weight was achieved. The dried weight was as the amount of AIR.

The neutral sulfite cooking liquor was filtered and diluted 2000 times. The lignosulfonate concentration (LS, g/L) was calculated according to Eq. (4). UVabs was average UV–Vis absorbance for the sample at appropriate wavelength. The absorbance of this diluted liquor at the wavelength of 232.5 nm with the extinction coefficient of 24.5 was measured using UV–Visible spectrophotometer (Liu et al., 2016a).

2.5.4. Imaging

The morphologies of BMWs, HRs, cellulose, and cellulose nanocrystals were imaged by scanning electronic microscope (SEM), and transmission electron microscopy (TEM). SEM (FEI SEM Quanta 200F, FEI Company, Hillsboro, OR, USA) was used in high vacuum mode with an accelerating voltage of 5 and 10 kV. Before the test, oven-dried BMWs, frozen-dried HRs and cellulose were fixed on aluminium stubs with carbon adhesive disks. In order to determine the particle sizes of BMWs, HRs, and cellulose, SEM micrographs at low magnification were analyzed using the public domain software ImageJ. The TEM (JEOL 1200 EX, Tokyo, Japan) was operated at 100 kV. A drop of sample suspensions was introduced on a formvar- and carbon-coated copper grid. Samples were stained with 2 wt% uranyl acetate in deionized water for 30 min. The water was completely evaporated under ambient condition before TEM testing. The size and aspect ratio of CNCs were calculated by the combination of TEM and ImageJ. POM (Olympus BX-51, Tokyo, Japan) was used to observe and calculate the aspect ratio of oven dried HMW, BMWs, freezing dried HRs, and cellulose.

2.5.5. Crystallinity by XRD

X-ray powder Diffraction (XRD) patterns of HMW, BMWs, HRs, and cellulose were recorded using a Rigaku Miniflex 600 X-ray Diffractometer (Rigaku Co., Tokyo, Japan) with Ni-filtered Cu Kα radiation (λ = 0.15418 Å) at 45 kV and 40 mA. X-ray diffraction data were collected from 2θ = 10° to 40° at a scanning rate of $0.02°s^{-1}$ at room temperature.

The crystallinity index (CrI) for CNCs was determined by the following Eq. (5):

$$Cr(\%) = (I_{Max} - I_{Am})/I_{Max} \times 100 \quad (5)$$

where I_{Max} was the maximum intensity of the diffraction peak, and I_{Am} was the intensity of diffraction attributed to amorphous cellulose.

3. Results and discussion

3.1. Products and mass balance

The flowing chart in Fig. 1 shows detailed mass balances of the processes of enzymatic hydrolysis, neutral sulfite cooking, and bleaching for BMW_{80} and BMW_{120}, respectively. The degradation products were not measured in this work. The sugar yields of enzymatic hydrolysis, the lignin recovery in neutral sulfite cooking based on the ball-milled woods, and cellulose recovery based on the ball-milled woods were calculated by following equations:

$$\text{monomeric sugar yield } (\%) = \frac{\text{monomeric sugar(g) released in enzymatic hydrolysis}}{\text{total monomeric sugar(g) in ball – milled wood}} \times 100 \quad (6)$$

$$\text{monomeric sugar recovery } (\%) = \frac{\text{monomeric sugar(g) in liquor} + \text{sugar(g)in residues}}{\text{total monomeric sugar(g) in ball – milled wood}} \times 100\% \quad (7)$$

$$\text{lignin recovery } (\%) = 100 - \frac{\text{lignin in neutral sulfite cooking residues(g)}}{\text{total lignin(g) in ball – milled wood}} \times 100 \quad (8)$$

$$\text{cellulose recovery } (\%) = \frac{\text{glucose(g)after delignification and bleaching}}{\text{total glucose(g) in ball – milled wood}} \times 100 \quad (9)$$

where monomeric sugar refers to glulose, xylose/mannose, arabinose, or galactose. Cellulose consists of glucose, and hemicellulose included xylose/mannose, arabinose, and galactose. The original BMW_{80} contained 459 g glucan, 168 g xylan/mannan, 15 g arabinan, 36 g galactan, and 322 g lignin per 1000 g of sample. The smaller particle size of the BMW_{120} led to a similar component distribution but lower values of 446 g glucan, 167 g xylan/mannan, 14 g arabinan, 35 g galactan, and 320 g lignin, resulting from the degradation of cellulose into acetylpropionic acid and formic acid during sulfuric acid hydrolysis in chemical analysis. However, there was a considerable difference in monomeric sugar yields between those two BMWs. As can be seen in Fig 1a, 23.7% glucan, 21.0% xylan/mannan, 29.3% arabinan, and 7.5% galactan in BMW_{80} were digested during enzymatic hydrolysis to monomeric sugars with the sugar recovery of 96.9%, 87%, 89.3%, and 68.6%, respectively (Table 2). BMW_{120} showed a high level of saccharification. 78.3% glucose, 57.4% xylose/mannose, 69.1% arabinose, and 12.9% galactose with the sugar recovery of 102.5%, 87.3%, 104.9%, and 61.4%, respectively, were obtained, nearly three times as high as monomeric sugar yields of BMW_{80} (Table 2). This indicated that sufficient ball milling reduced the particle size and damaged the ultrastructure of cellulose, and crystallinity, obviously enhancing the production of monomeric sugars under the same enzymatic hydrolysis conditions, especially the cellulose to glucose conversion. This result confirmed the findings of Millett et al. (1979) obtained from vibratory ball milled wood (Douglas Fir). Ball milling combined with chemical pretreatment increased the total sugar yield from 65% to 92% (Liu et al., 2016b). The cellulose to glucose conversion of 78.3% (BMW_{120}) was equivalent or slightly higher than the conversion obtained by stream explosion under a good performed condition (Biswas, 2015). Both methods used direct enzyme hydrolysis. The limitation of hemicellulose as a physical barrier around the cellulose made their sugar yields be lower than the yields (over 90%) obtained by enzymatic hydrolysis after removal of hemicellulose (Penttilä et al., 2013; Zhu et al., 2015). The sugar recovery over 100% might be due to the mass loss in the chemical test of BMW_{120} and the decimal conservation in calculation.

Neutral sulfite cooking controls the pH of liquor in a range of 5–9 by using sodium carbonate and sodium sulfite. The pH value was much milder than the conventional alkaline cooking ($pH \geq 11.5$) (Gould, 1984; Yang et al., 2002). This approach not only effectively controlled the alkaline degradation of cellulose and avoided corrosion of the reactor, but also efficiently sulfonated the lignin aliphatic chain to lignosulfonate with a high yield (Sjöström, 1993; Zhu et al., 2009; Liu et al., 2016b). However, there was still a degradation of 20.5% glucan, 45.9% xylan/mannan, 44.4% arabinan, 63.6% galactan in neutral sulfite cooking for HR_{80}. Additionally, 30.2%, 48%, 60%, 52.9% of glucan, xylan/mannan, arabinan, and galactan, respectively, were removed in HR_{120}. The absence of sugars in neutral sulfite cooking might be contributed to the loosen structure and the removal of lignin, resulting in a low protection around cellulose and hemicellulose. They were degraded under the high treatment temperature (160 °C) and long duration time

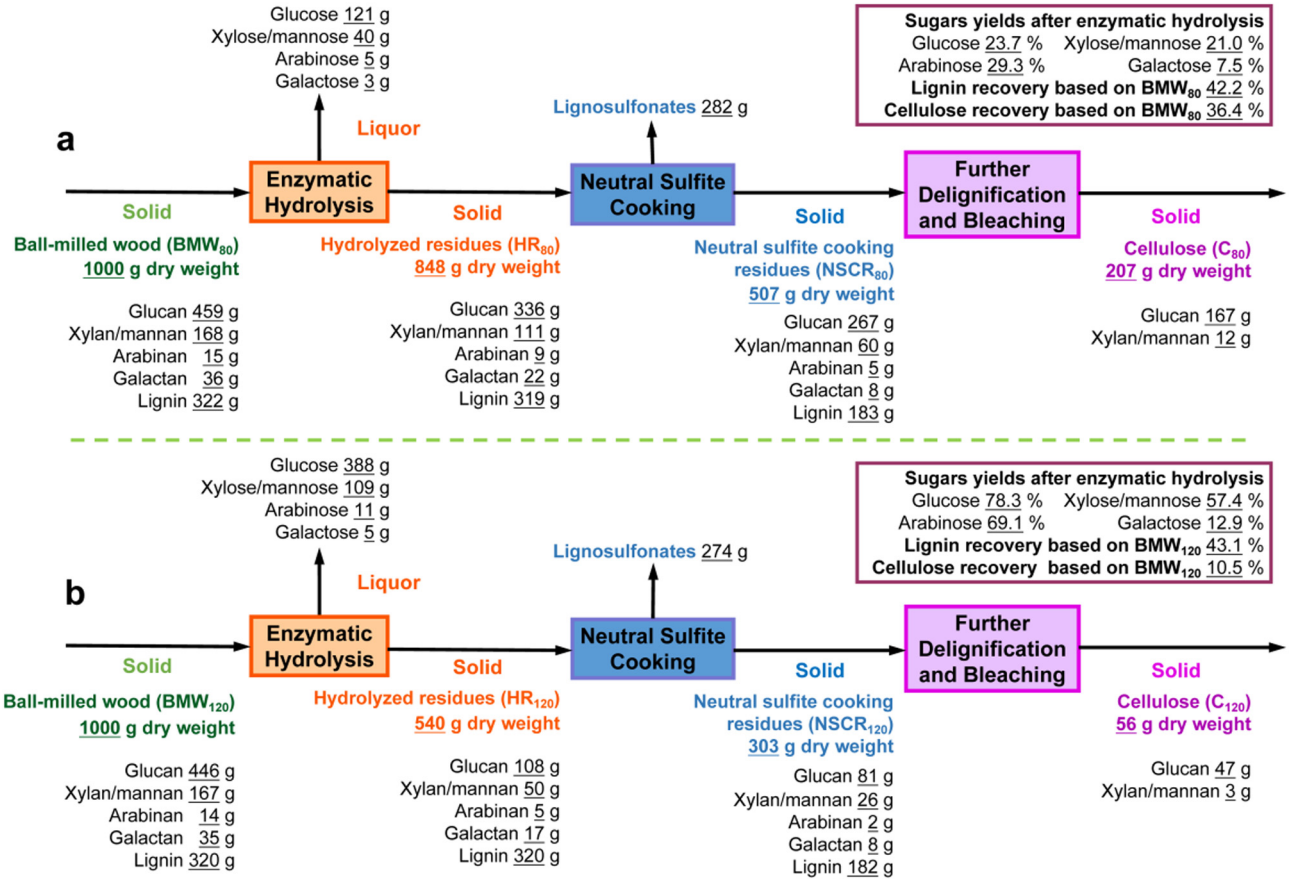

Fig. 1. The mass balance of the processes with enzymatic hydrolysis, neutral sulfite cooking, further delignification, and bleaching on ball-milled woods to co-produce sugars, cellulose and lignin.

(3 h). HR_{80} contained 37.6% lignin and 56.4% sugars and HR_{120} contained 59.2% lignin and 33.3% sugars. The different chemical component distribution in HRs had barely effects on removal of lignin. The neutral sulfite cooking treatment achieved 42.6% and 43.1% lignin removal based on HR_{80} and HR_{120}, respectively, 42.2% and 43.1% overall lignin recovery based on BMW_{80} and BMW_{120}, respectively (Table 2). This was attributed to the retention of integrated lignin in HRs and action of equivalent sulfite (SO_3^{2-}) to break the lignin unit.

The lignosulfonate contents of HMWs, BMWs, and HRs under the same cooking conditions are shown in Fig. 2. The concentration of lignosulfonate increased slightly with an increased of ball milling time. The reason was that intensive force broke the cellulose located at the interface of lignin, cellulose and hemicellulose, exposing lignin to carbonate (CO_3^{2-}) and sulfite (SO_3^{2-}). Neutral sulfite cooking resulted in a higher lignosulfonate concentration from HRs than from BMWs. Because a portion of cellulose was hydrolyzed by cellulase, and HRs contained a higher percentage of lignin, facilitating sulfonation (Yang et al., 2013). This explanation was proved by the mass balance shown in Fig. 1. 57.72 g/L and 88.16 g/L lignosulfonate concentration were collected from HR_{80} and HR_{120}, respectively. However, the practical mass of lignosulfonate from those two HRs was almost the same based on the equal mass of lignin in HRs.

The aim of delignification/bleaching is to collect cellulose from the neutral sulfite cooking residues. The intensive purification removed the majority of hemicellulose and lignin, resulting in 62.5% and 58.0% glucan yield from $NSCR_{80}$ and $NSCR_{120}$, respectively. The cellulose recovery based on BMWs reached to 36.4% (BMW_{80}) and 10.5% (BMW_{120}).

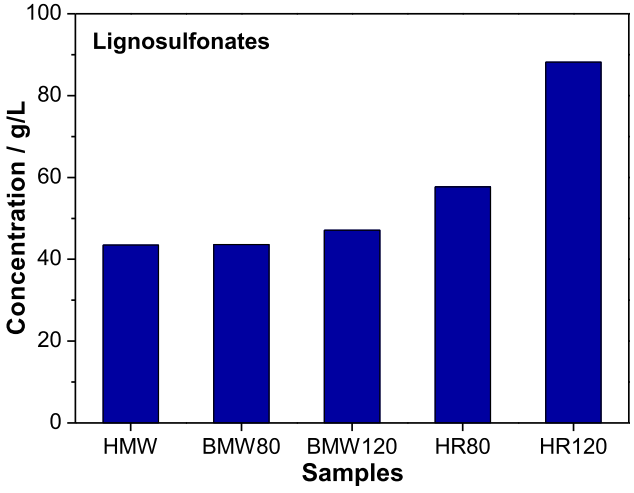

Fig. 2. Effect of ball milling treatment and enzymatic hydrolysis on the lignosulfonate concentrations of the materials.

Holistically, medium ball milling pretreatment makes hydrolysis residual solids contain nearly intact uncontaminated lignin and recalcitrant glucose that are amenable to further processing. This moderate pretreatment controls the whole product yield balance except lignosulfonates. The low cellulose-to-glucose conversion of 23.7% lead to a high cellulose yield of 35.4%. On the contrary, 78.3% cellulose-to-glucose conversion results in a 10.5% cellulose yield. The lignin removal and the obtained lignosulfonates have the same conversion and yield. Therefore, the medium ball milling

Table 1
Particle size of BMW, HR, Cellulose, and CNC.

Samples	Length	Width	Samples	Length	Width
BMW_{80}	74 ± 68 μm	19 ± 9 μm	BMW_{120}	23 ± 20 μm	14 ± 13 μm
HR_{80}	67 ± 46 μm	15 ± 12 μm	HR_{120}	12 ± 7 μm	6 ± 5 μm
C_{80}	25 ± 12 μm	6 ± 4 μm	C_{120}	13 ± 4 μm	4 ± 2 μm
CNC_{80}	188 ± 88 nm	12 ± 6 nm	CNC_{120}	135 ± 59 nm	6 ± 4 nm

pretreatment allowed balance of the different product yields, which could be changed to meet design and market needs. To summarize the total overall glucose recovery, 60.1% (BMW_{80}) and 88.8% (BMW_{120}) glucose were utilized. 42.2% and 43.1% lignin recovery were obtained in the whole procedures.

3.2. Particle size

The original hammer-milled wood had the D(50) particle size of 234 μm, ranging from 45.3 μm (D(10)) to 709 μm (D(90)). In this stage, the tight fiber bundles and the well-organized structure were almost integrated. As the impact force and shear force continuously acted on wood fibers during the ball milling treatment, the resulted BMWs showed an intuitive view of particle size reduction with increasing milling time, breaking the surface of fiber layers along the direction perpendicular to the fiber axis (Avolio et al., 2012). The dimension of 74 μm length and 19 μm width was the particle size of BMW_{80}. A treatment time of 120 min disintegrated the fiber cell wall into micronized fragments and was pulverized into a quasi-circular shape with a length size of 23 μm and a width size of 14 μm, which was much smaller than 80 min ball milling treatment. The results also indicated that size reduction in length was more significant than in width. The fragments were identified as amorphous microparticles. These amorphous microparticles facilitated significant sugars conversion during the enzymatic hydrolysis (Zhang et al., 2007; Zhu et al., 2011). HR_{120} had a smaller length size of 12 μm and a width size of 6 μm (Table 1), the cellulose in HRs were almost the recalcitrant cellulose. With the deficient milling, well-organized structure was still remained in HR_{80}, resulting in a slight size reduction. This structure limited the access of the enzymes into the internal fibrous structure. As a result, only a portion of the surface layers were exfoliated from fibers, resulting in low digestion. C_{80} had the particle size with a length size of 25 μm and a width size of 6 μm (Table 1), which was attributed to the removal of lignin and hemicellulose, and the exposure of oriented cellulose in NSCR. C_{120} showed a length size of 13 μm and a width size of 4 μm, and the individual cellulose were twisted and stick together made the size nearly unchanged (Table 1).

Ball milling reduced the cellulose size, which shortened the necessary hydrolysis time for harvesting the CNCs. Compared with previous studies that produced CNCs by sulfuric acid hydrolysis of the microcrystalline cellulose for 2 h (Du et al., 2017), the cellulose hydrolyzed for 30 min in this study produced the maximum yield of CNCs. CNC_{120} displayed shorter and narrower crystals with a length size of 135 nm and a width size of 6 nm (Table 1). The resulting particle sizes were smaller than the CNCs from microcrystalline cellulose (270 nm length × 17 nm width) (Du et al., 2017). In conclusion, the substrate subjected to 120 min ball milling had smaller particle sizes throughout the process.

3.3. Aspect ratio

The aspect ratio (length to diameter, L/d) was calculated based on more than 10 thousand individual particles of BMW, HR, and C,

Table 2
Comparison of different ball milling treatment time on product yields.

Component	Ball milling pretreatment (80 min)			Ball milling pretreatment (120 min)		
	Enzymatic hydrolysis			Enzymatic hydrolysis		
	Hydrolyzed liquor (g)	Monomeric sugar yield (%)	Monomeric sugar recovery (%)	Hydrolyzed liquor (g)	Monomeric sugar yield (%)	Monomeric sugar recovery (%)
Glucose	121	23.7	96.9	388	78.3	102.5
Xylose/mannose	40	21.0	87.0	109	57.4	87.3
Arabinose	5	29.3	89.3	11	69.1	104.9
Galactose	3	7.5	68.6	5	12.9	61.4
	Neutral Sulfite Cooking Treatment			Neutral Sulfite Cooking Treatment		
	Cooking residual (g)	Lignin recovery (based on BMW_{80}) (%)		Cooking residual (g)	Lignin recovery (based on BMW_{120}) (%)	
Lignin	136	42.2		138	43.1	
Lignosulfonates	282			274		
	Delignification and Bleaching			Delignification and Bleaching		
	Purified solid (g)	Cellulose recovery (based on BMW_{80}) (%)		Purified solid (g)	Cellulose recovery (based on BMW_{120}) (%)	
Glucan	167	36.4		47	10.5	

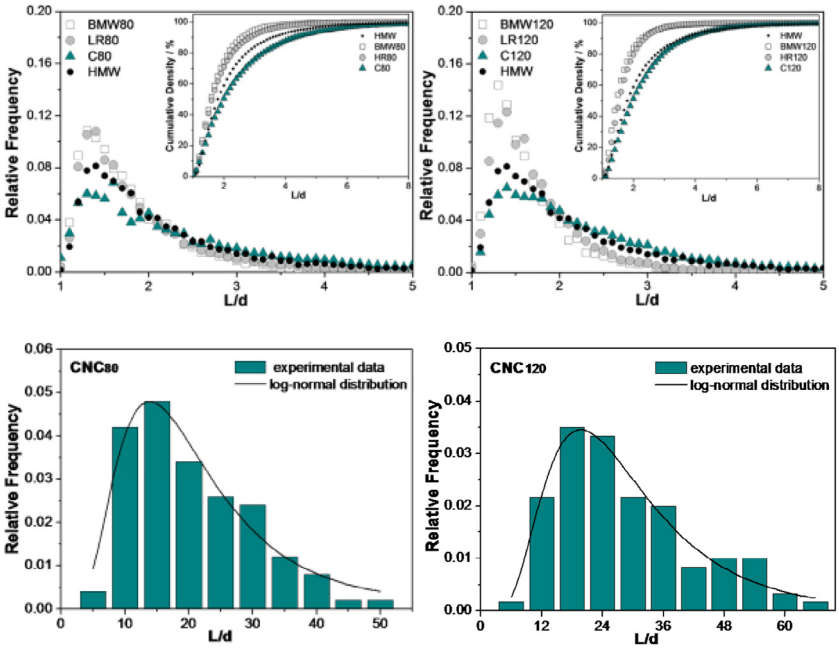

Fig. 3. The aspect ratio distribution of BMW, HR, Cellulose, and CNC, and fitted log-normal distribution of CNC.

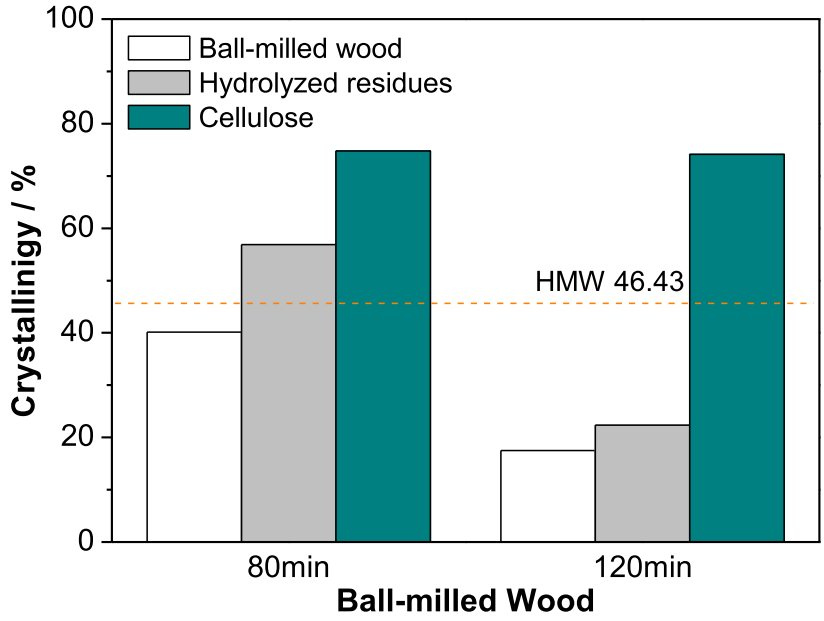

Fig. 4. Crystallinity index based on the Segal method for the ball-milled wood, lignin residues, and cellulose.

respectively, and 100 individual particles of CNC (Fig. 3). These particles were measured by POM and analyzed using ImageJ software (National Institute of Health). The L/d distributions of BMW_{80}, HR_{80}, and C_{80} were boarder than that for BMW_{120}, HR_{120}, and C_{120}. These results are consistent with the effect of the ball milling treatment to cut the wood fibers in length and initially break down the amorphous region. Increasing milling time led to sufficient mechanical destruction and decreased the aspect ratio significantly. HR_{80} and HR_{120} showed a similar L/d distribution and peak L/d values of nearly 1.4, and enzymatic hydrolysis varied practical dimensions of lengths and widths, as shown in Table 1. Pure cellulose weakened their relative frequency at peak value for a boarder L/d distribution, due to the separation of fiber bundles by removal of the remaining lignin and hemicellulose.

The histograms of CNC aspect ratio are shown in Fig. 3. They are well fitted to a classical model of log-normal distribution (Elazzouzi-Hafraoui et al., 2008). The equation and average aspect ratio ($\bar{L}/d$) are described as follows:

$$f(x) = \frac{1}{x\sigma\sqrt{2\pi}} e^{-\frac{(\ln x-\mu)^2}{2\sigma^2}} \tag{10}$$

$$\bar{L}/d = e^{\mu+\sigma^2/2} \tag{11}$$

where μ and σ correspond to the location parameter and the scale parameter, respectively. The average aspect ratio of CNC_{80} and CNC_{120} based on 100 individual fibers was 18.00 ± 9.23 and 25.61 ± 13.16, respectively, close to the model values of 18.60 and 25.93, respectively. This close fit proved that the log-normal distribution can accurately describe the aspect ratio distribution of CNC samples. The aspect ratio distribution of CNC_{120} presented a boarder aspect distribution and average value. This indicated that the intensity of the acids that acted on C_{120} led to a stronger hydrolysis effect, peeling the cellulose nanocrystal from microfiber cellulose.

3.4. Crystallinity

Fig. 4 shows the X-ray crystallinity index (Cr) of HMW, BMWs, HRs, and cellulose. Ball milling is an intensive mechanical action, this treatment damaged the crystalline structure, decreased the crystalline phase and their crystalline size, while it did not alter the cellulose crystalline structure (Zhang et al., 2007; Liao et al., 2011). BMW_{80} had the crystallinity of 40.15%, a little lower than HMW's, 46.43%. With increasingly continuous 40 min milling time, the BMW_{120} reduced to only 16.52%. The significantly reduced Cr in further milling indicated that the sufficient ball milling treatment time or enough small particle size resulted in the effective destruction of the crystalline structure in cellulose (Zhang et al., 2015; Jiang et al., 2016). The Cr_{HR80} was higher than the Cr_{HMW}, and Cr_{HR120} have an improvement as well. Because the ends of the crystalline cellulose chains were connected with amorphous cellulose, the ending cellulose were easily attacked by cellulase in early hydrolysis stage, removing cellobiase units from free chain ends, and finally resulting in the hydrolysis of cellobiose units to glucose (Sarkar et al., 2012; Banerjee et al., 2010). As the limited hydrolysis time and some integrated cell wall in BMW_{80}, the high crystalline cellulose was remained in HRs, and most amorphous cellulose was hydrolyzed. However, the high sugars conversion of BMW_{120} decreased the cellulose percentage in HR_{120}. Although there was more crystalline cellulose as a percentage of the total cellulose of HR_{120}, the Cr showed a slight increase. The purified cellulose exhibited a high crystalline characters after delignification and bleaching, due to recrystallization after lignin and hemicellulose removal (Kargarzadeh et al., 2012). Cr_{C80} was 74.8%, and Cr_{C120} was 74.2%. This phenomenon was consist with the results reported by Oksman et al. (2011). Alkali treatment with a low concentration of NaOH solution at high temperature removed the hemicellulose, keeping its main structure and causing a formation of recrystallizing cellulose. However, the alkaline treatment might cause the occurrence of transformation from cellulose I to cellulose II, in which a parallel natural cellulose was converted into an antiparallel one by unhinging some cellulose (Okano and Sarko, 1985).

4. Conclusion

In this work, the concept of co-production of sugars, lignosulfonates, cellulose, and cellulose nanocrystals was realizable, which was performed by employing the mechanical pretreatment, neutral sulfite delignification, and cellulose upgradation. The multi-products distribution would be balanced through controlling processing conditions. It depends on the market needs. Ball milling with a reasonable energy consumption improved the enzymatic digestibility of Douglas-fir, while reduced the cellulose yields. The neutral sulfite process converted 42.6 ± 0.5% lignin to lignosulfonate. Each individual process might not be the best methods. However, it can be optimized on an industrial scale based on the best current technologies.

Acknowledgements

The enzymatic hydrolysis was performed by Gevo Co. The authors appreciate the help of Yalan Liu and Jinxue Jiang. The authors gratefully acknowledge the Northwest Advanced Renewables Alliance (NARA), supported by the Agriculture and Food Research Initiative Competitive Grant no. 2011-68005-30416 from the USDA National Institute of Food and Agriculture, United States, and the China Scholarship Council (CSC) fundings, China, for Lanxing Du's living expenses.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.03.097.

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