Optimization and Scale-Up of Enzymatic Hydrolysis of Wood Pulp for Cellulosic Sugar Production

Binbin Hu, Suiyi Zhu, Shuai Fang, Mingxin Huo, Yunhan Li, Yang Yu, and Mingjun Zhu

With the decreased demand for pulp and paper worldwide, the reorganization of pulp and paper mills for cellulosic sugar production is possible. To maximize cellulosic sugar production from the wood pulp with minimum resources, the effects of pH, buffer system, temperature, enzyme loadings, pulp concentrations, and mixing modes on enzymatic hydrolysis were investigated, one factor at a time. Temperature played an important role in enzymatic hydrolysis. When the temperature was lower than 45 °C, the sugar production declined dramatically to almost half of the maximum value. Increasing enzyme dosage, increasing pulp concentration, and adding xylanase increased sugar production. The intermittent manual mixing mode generated higher concentrations of sugars and could be used for large-scale production. At pilot-scale, the diverted pulp for the pulping process was directly hydrolyzed without any treatment, and the residue after hydrolysis was hydrolyzed by adding fresh enzymes. This study provides insight on economically feasible enzymatic hydrolysis of wood pulp at large-scale cellulosic sugar production.

Keywords: Wood pulp; Enzymatic hydrolysis; Cellulosic sugar production; Condition optimization; Scale-up

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INTRODUCTION

Increasing concerns about greenhouse gases and the depletion of fossil energy have attracted attention for the substitution of fuels and chemicals generated from renewable resources. It was mandated that 36 billion gallons of biofuels would be blended in gasoline in the US by 2022, with 58% of this amount obtained from non-grain products (Sissine 2007). Annually, 56.4 billion tons of lignocellulose are produced, and it is one of the most abundant renewable resources on the planet (Field et al. 1998). Lignocellulose must be broken down physically and/or chemically to allow good accessibility for enzymes (Martin et al. 2014), followed by enzymatic hydrolysis and fermentation to release sugars and generate bioproducts (Hasunuma and Kondo 2012). To make biofuels economically competitive, biorefineries are needed in which lignocellulosic material is fractionated and each component is fully utilized, reducing the overall cost of bioproducts (Bozell 2010).

The enzymatic hydrolysis of lignocellulosic biomass is affected by many substrate-related physical and chemical parameters, such as native (untreated) wood lignin content, the extent of lignin and xylan removed by pretreatment, lignin structure, substrate size,
reaction conditions, and substrate pore surface area or substrate accessibility to cellulose (Lan et al. 2013). It is also affected by the degraded products of hemicellulose and lignin (Mussatto et al. 2008; Kim et al. 2011). Irreversible loss of cellulases is observed with the non-specific binding of lignin, which subsequently reduces the efficiency of hydrolysis (Gao et al. 2014). Lignin-derived products, such as phenolic compounds, have inhibitory effects on cellulases and other enzymes used in cellulose hydrolysis (Ximenes et al. 2010, 2011). Both xylose and xylo-oligomers directly inhibit cellulases, leading to lower yields of glucose (Kim et al. 2011). After inhibitors are removed, there can be significant improvement in enzyme activity; for example it has been found that the sugar conversion of lignin and hemicellulose-removed cellulose pulp was 91.8%, four times that of untreated pulp (Mussatto et al. 2008). Well-fractionated biomass components not only have higher values, but they also favor detoxification of lignocellulosic feedstock, subsequently reducing the enzyme loadings of cellulosic hydrolysis.

The usage of enzymes for cellulose hydrolysis incurs one of the highest costs in the production of cellulosic sugars (Jørgensen et al. 2007; Klein et al. 2012). The enzyme cost is $0.34 for producing a gallon of cellulosic ethanol, which is about 16% of the total cost, and it proportionally increases with enzyme loadings (Humbird et al. 2011). Optimizing the conditions for enzymatic hydrolysis can effectively lower the enzyme loading in the production of cellulosic sugars (Modenbach and Nokes 2013).

A pulp/paper mill could be converted to an integrated forest biorefinery (IFBR) (Machani et al. 2014). Typically it is located close to forest resources and has ready-to-use facilities and personnel. With the current significant reduction of paper demand, many pulp/paper mills in North America are struggling, and they are willing to diversify their products and generate value-added biofuels and biochemicals (Machani et al. 2014). The kraft pulping process removes most lignin and hemicelluloses, leaving cellulose as the dominant constituent in the pulp. Cellulose accounts for over 80% of dry mass in most pulps (Puls et al. 2006; Lahtinen et al. 2014). The reduction of hemicelluloses and lignin in wood pulp minimizes the lignin matrix and non-specific binding of cellulolytic enzymes and increases the mean pore size of the substrate, leading to good accessibility, digestibility, and efficiency in enzymatic hydrolysis (Alvira et al. 2010).

**Fig. 1.** Proposed flowchart to incorporate a process for cellulosic sugar production into a pulp mill.
Reorganization of a kraft mill to produce cellulosic sugars and further biofuels and biochemicals is feasible because of the compatible supply chains, facilities, and workforce (Jin et al. 2010); enzymatic hydrolysis is the only technology that would need to be added to the pulping process (Fig. 1). In this study, the enzymatic hydrolysis of well-fractionated wood pulp for cellulosic sugar production was investigated. The conditions of the hydrolysis were optimized to minimize the cost for large-scale production. This study provided insight for the diversion of pulp from the pulping process for large-scale cellulosic sugar production.

**EXPERIMENTAL**

**Raw Materials**

The wood pulp was produced from chips of mixed woods of maple, birch, aspen, and beech from a kraft process to remove hemicellulose and lignin. The typical constituents of the mixed hard woods are listed in Table 1. The pulp for the experiments was taken after the washing step of the process at 10% concentration, 60 °C, and a pH of 8.5 to 9. After the pulp was mixed with the enzymes for hydrolysis, the pH dropped to about 7.5.

| Table 1. Major Constituents of the Mixed Hard Woods for Pulping |
|-----------------|-----------------|-----------------|
| Sugar Components | Percentage (%)  | Non-Sugar Components | Percentage (%)  |
| Glucose         | 35.87 ± 1.73    | Acetic Acid      | 9.59 ± 0.67    |
| Xylose          | 17.24 ± 0.20    | 4-O-Methylgallic Acid | 6.23 ± 0.4 |
| Arabinose       | 0.49 ± 0.01     | Klason Lignin    | 20.84 ± 0.05   |
| Mannose         | 1.72 ± 0.05     | Acid-Dissolved Lignin | 3.66 ± 0.33 |
| Galactose       | 1.06 ± 0.02     | Ash              | 0.22 ± 0.01    |

**Enzymes**

AlternaFuel®CMax (15,000 CMCase/g, Dyadic International Inc., Jupiter, USA), a liquid cellulase prepared from the submerged fermentation of *Myceliophthora thermophile*, was used for hydrolysis. β-glucosidases and other hemicellulases were added to the enzyme cocktails. The xylanase AlternaFuel®100L (30,000 Xylanase Units /g) was supplemented to facilitate the release of glucose and xylose in the trials unless otherwise stated. AlternaFuel®CMax and AlternaFuel®100L were abbreviated as CMax and AF100L in the text.

**Enzymatic Hydrolysis**

For lab-scale trials, 50 g of the desired concentrations of pulp were added to a 125-mL Erlenmeyer flask containing 20 to 30 glass beads (4 mm in diameter) to facilitate mixing. The flask was covered with aluminum foil to prevent evaporation and pre-heated to 55 °C in a temperature-adjustable shaking incubator (Biomega Incu-Shaker, Spectra Service Inc.). Hydrolysis was initiated by adding the enzymes, and 1.5-mL samples were taken from the flask for the desired period of time for sugar analysis. The sample was analyzed directly or stored at -20 °C to stop hydrolysis. Each sample was analyzed in duplicate. For the effect of pH on hydrolysis, 0.5 M phosphate buffer was used to provide strong buffering capacity. Basically the pulp for this experiment was allocated to buffering and non-buffering fractions. The non-buffering fraction was added to enzymes directly for...
hydrolysis. For the buffering fraction, the liquid in the pulp was squeezed out and replaced with the buffer. The pH of the slurry was measured using a bench-top pH meter (Fisher Scientific Accumet pH meter 915, Pittsburgh, USA) during sampling. The pulp consistency was 10%, and the enzymatic hydrolysis was carried out at 55 °C unless otherwise stated. The initial mixing was performed at 250 rpm and reduced to 150 rpm after liquefaction.

The trials scaling up about 140 times for selecting mixing mode were carried out in 20-L buckets covered with lids. The pulp with 15% concentration was completely mixed with the enzymes and separated into two equal fractions of 7 kg. In one trial, the pulp was continuously mixed with a custom-made propeller, which was fixed in the center of the lid (propelling continuous mixing). In the other trial, the pulp was mixed with the same type of propeller that was manually operated for 10 min every 3 to 4 h (manual intermittent mixing) (Fig. 2).

![Fig. 2. The trials for mixing mode selection included (A) continuous mixing or (B) manually intermittent mixing every 3 to 4 h.](image)

The pilot-scale trial, scaling up about 20,000 times, was carried out in a custom-made reactor similar to the intermittent mixing mode in Fig. 2(B), but with a top-removed 1400-L intermediate bulk container (IBC tote), to which 1000 kg of 10% concentration pulp was added. After the enzymes were added, they were completely mixed with the pulp using a custom-made propeller to continuously mix every 3 to 4 h until liquefaction. After that, the propeller was located in the center of the reactor, constantly mixing the pulp at 150 rpm. The temperature was controlled during the working hours, with a heating blanket, especially for the first 6 to 7 h at the beginning of hydrolysis in the pilot-scale trial, but it was not controlled after working hours. Instead, the tote was covered with polystyrene foam to maintain the heat.

**Enzymatic Conversion Rate**

The pulp consistency before hydrolysis was determined with dry weight analysis. About 5 g of pulp was placed in a pre-weighed aluminum tray. The wet weight of the pulp was read to 0.1 mg on an analytical balance. After drying at 105 °C overnight, the dry weight of the pulp was determined with the same balance. The content of the biomass in the pulp was determined by Eq. 1, and the dry mass \( m \) before hydrolysis was determined by Eq. 2.

\[
\text{Mass content} \% = \frac{\text{Dry weight}}{\text{Wet weight}}
\]

\[
m = \text{Initial wet weight} \times \text{Mass content} \%
\]
After hydrolysis, the solid fraction (residue) was separated from the liquid with a pre-weighed Whatman No. 1 filter paper in a Buchner funnel mounted on a Buchner flask, which was connected to a vacuum pump. The solid was washed twice with 50 mL of deionized water and dried at 105 °C overnight with the filter paper, and the dry mass after hydrolysis was read at the analytical balance. The enzymatic conversion rate was calculated with Eq. 3.

\[
\text{Enzymatic conversion rate (\%) = } \left(1 - \frac{\text{Dry mass after hydrolysis}}{\text{Dry mass before hydrolysis}}\right) \times 100
\]

For sludge residue recycling, the residue from the previous hydrolysis was filtered and separated into two equal parts. One part was washed 3 times with deionized water, and the other part was not washed. The two equal parts were then adjusted to about 10% concentration, and the pH was adjusted to about 8 with 1 M NaOH to simulate the original pH of the pulp. The enzymes used for hydrolysis were 5 mg/g dry mass.

**Analytical Procedures**

Glucose and xylose concentrations were determined by using a high-performance liquid chromatography device (HPLC; 1260 Infinity, Agilent Technologies, Santa Clara, USA) equipped with an Aminex HPX-87H column (Bio-Rad, Berkeley, USA) and a refractive index detector (RID). The mobile phase was 5 mM H₂SO₄, and the flow rate was 0.5 mL/min. The temperature of the column and the detector were kept at 60 °C and 35 °C, respectively. The sample was centrifuged at 15,000 \(\times\) g for 1 min, and the supernatant was passed through a 0.2-μm syringe filter before HPLC.

The constituents of wood were analyzed following the National Renewable Energy Laboratory (NREL) protocol (Sluiter et al. 2008). Wood chips were air-dried overnight at room temperature and broken down to 1-mm wood dust using a Wiley mill (Thomas Scientific, Swedesboro, USA); 0.30 g of the wood dust was added to a pressure tube, followed by 3.00 mL of 72% H₂SO₄. The wood dust was fully mixed with the H₂SO₄, incubated at 30 °C for 60 min, and stirred every 5 to 10 min. After incubation, the acid was diluted to a 4% concentration and autoclaved at 121 °C for 1 h. The solid fraction was filtrated for Klason lignin analysis, and the liquid fraction was used for acid soluble lignin (ASL) and structural carbohydrate analysis. ANOVA was adopted for statistics analysis and the P-value represented the significance of difference.

**RESULTS AND DISCUSSION**

Optimizing conditions for enzymatic hydrolysis of wood pulp can effectively lower enzyme loadings in the production of cellulosic sugars (Yang et al. 2011; Modenbach and Nokes 2013). Thus, the hydrolytic conditions, such as buffer system, pH, temperature, agitation, substrate concentration, enzyme loadings, and hydrolytic time were optimized.

**Effects of pH, Buffer System, and Temperature on the Enzymatic Hydrolysis**

*Effect of pH on the enzymatic hydrolysis*

The effect of pH on hydrolysis was initially investigated with the bleached pulp cardboard, which had a pH of 4.8 after soaking in deionized water overnight. The pulp was adjusted to 10% concentration, and the pH dropped further to 4.1 after it was mixed with the enzymes. The mixture of the pulp and the enzymes was separated into two parts. One
part of the mixture was pH-adjusted, in which the pH was initially adjusted to 5.6 and was kept between 4.2 and 5.6 during hydrolysis. The pH of the other part of the mixture was not controlled, and it dropped to 3.6 at the end of hydrolysis. The sugar production for the non-pH-adjusted trial was 71.0 g/L at the end of hydrolysis, compared with 74.4 g/L for the pH-adjusted trial (Fig. 3). This result indicated that the hydrolysis of the pulp by CMax and AF100L was not noticeably affected by pH ($P=0.03>0.01$).

The pulp taken from the process had a moderately high pH of 8.5 to 9.0. After the pulp was mixed with the enzymes, the pH was neutralized to about 7.5. Because CMax had a broad pH range for its activities from 3.5 to 8.0, the hydrolysis was not noticeably affected by the initial pH, which continued to decrease due to the release of acetic acid and other organic acids during hydrolysis. The chemical residues in the pulp from the kraft process, such as HCO$_3^-$/CO$_3^{2-}$, may have had a buffer function to keep the pH stable.

In addition, the surface charges caused by the higher pH of the pulp may have altered the surface hydrophobicity and electrostatic interactions of cellulose, making it favorable to binding cellulases (Leu and Zhu 2013). Therefore, pH-induced surface properties, such as hydrophobicity, show greater differences between cellulosic and lignocellulosic substrates than among different lignocelluloses. Lou et al. (2014) found that the effects of lignosulfonate with different molecular weights (MW) on the enzymatic hydrolysis of pure cellulose were influenced by pH and that the optimal pH was different from that of fractions of commercial sodium lignosulfonate. In the present study, the higher pH of the wood pulp may have altered the surface charges of the pulp, improving enzymatic activity. Thus, the pH-adjusted hydrolysis was actually facilitated by charges from the pH.

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**Fig. 3.** The effect of pH on enzymatic hydrolysis. pH changes in trials without pH adjustment (A) and with pH adjustment (B). Sugar productions without enzymes (C), with enzymes and pH adjustment (D), and with enzymes but without pH adjustment (E). The enzyme dosage was 15 mg/g dry mass of each one of CMax and AF100L.

**Effect of buffer system on the enzymatic hydrolysis**

The effect of pH on hydrolysis was further tested with a 0.5 M phosphate buffer system, pH 5.8. The unbleached pulp was separated into two fractions, one for the buffered trial, and the other for the non-buffered control. The liquid in the pulp of the buffered trial was squeezed out and replaced by the same amount of 0.5 M buffer. After adding CMax and AF100L, the pH of the buffered trial and the non-buffered control were 5.8 and 7.7, respectively. At the end of the hydrolysis, the pH was 4.8 for the buffered trial and 4.3 for
the non-buffered control. The total sugar production was 91.5 g/L for the buffered trial and 87.2 g/L for the non-buffered control. The enzymatic conversion rate of the buffered trial was 64.0%, compared to 61.5% for the non-buffered control (Fig. 4). The result indicated that the hydrolysis of the pulp by CMax and AF100L was not significantly affected by the buffer system (P=0.02>0.01). Because pH adjustment and buffering did not have a significant impact on sugar production and yield, and also for economic reasons in large-scale application, pH adjustment was not considered in the following experiments.

Fig. 4. Effect of buffer on enzymatic hydrolysis. (a) pH changes in the non-buffered (A) and buffered (B) trials; sugar production in the non-buffered (C) and buffered (D) trials. (b) enzymatic conversion rates at 48 h. A 0.5 M phosphate buffer, pH 5.8, was used for buffering. The enzyme dosage was 15 mg/g dry mass of each one of CMax and AF100L.

Effect of temperature on enzymatic hydrolysis

The optimal activity of cellulases is between 50 and 60 °C according to the manufacturer’s application sheet (Product #346, Dyadic International). But lowering temperature was desirable for reduction of energy consumption in hydrolysis for industrial application of sugar production. In this research three different temperatures, room temperature (about 25 °C), 35 °C, and 55 °C, were tested to determine the effect of temperature on hydrolysis (Fig. 5).

Fig. 5. Effect of temperature on enzymatic hydrolysis at (A) room temperature, (B) 35 °C, and (C) 55 °C. The enzyme dosage was 15 mg/g dry mass of each one of CMax and AF100L.
The sugar production at room temperature was 33.1 g/L after hydrolysis for 48 h, compared with 53.4 g/L and 86.8 g/L at 35 °C and 55 °C, respectively. The sugar production at room temperature was only 38.1%, and at 35 °C was only 61.5% of that at 55 °C. The result indicated that the enzymatic hydrolysis of the pulp was significant affected by temperature \((P=3.51\times10^{-6}<0.01)\). Lower temperature reduced energy consumption, but sugar production was obviously decreased. This result indicated that temperature played an important role in hydrolysis and a temperature near the optimum was necessary for hydrolysis.

### Effects of Enzyme Dosage on the Enzymatic Hydrolysis

To evaluate the effect of enzyme dosage on hydrolysis and the possibility to reduce the amount of enzymes used, trials with 5, 7.5, 10, and 15 mg/g dry mass of each one of CMax and AF100L were tested. As shown in Fig. 6, sugar production and yield were directly correlated with enzyme doses, and they were improved by increased loading. At the highest enzyme dose (15 mg/g dry mass), the sugar production and yield were 79.9 g/L and 62.0%, respectively, after 48 h of hydrolysis. The result indicated that the enzyme dosage played a significant role on enzymatic hydrolysis of the pulp \((P=2.47\times10^{-6}<0.01)\). For the larger-scale application, the enzyme dose of 15 mg/g dry mass was used.

![Fig. 6. Effect of enzyme dosage on enzymatic hydrolysis. (a) Sugar production with (A) 5 mg/g, (B) 7.5 mg/g, (C) 10 mg/g, or (D) 15 mg/g dry mass of each one of CMax and AF100L. (b) Total enzymatic conversion rate at 48 h with different enzyme doses](image)

Enzymatic hydrolysis of wood pulp by CMax was dramatically improved by supplementing with AF100L. Without AF100L, sugar production was 60.0 g/L after 48 h of hydrolysis, a 47.7% reduction compared with the 88.6 g/L sugar production of the trial supplemented with AF100L (Fig. 7). This result indicated that the enzymatic hydrolysis of the pulp was significant affected by supplementing with AF100L \((P=0.001<0.01)\). Thus, xylanases were needed for effective hydrolysis. By adding xylanases, the hemicellulose barrier around cellulose was removed, which increased the accessibility of cellulose to cellulases (Hu et al. 2011; Qing and Wyman 2011).
To investigate whether the enzyme dosage ratio of CMax and AF100L had an effect on the hydrolysis of wood pulp, sugar production from the reduced 10 mg/g dry mass of CMax was then investigated with different amounts of AF100L. As shown in Fig. 8, sugar production increased with increased AF100L loading. The result indicated that the enzymatic hydrolysis of the pulp was significantly affected by enzyme dosage ratio of CMax and AF100L (P=5.72×10^{-7}<0.01). However, even with the highest AF100L loading of 15 mg/g dry mass, the sugar production was only 65.8 g/L after 48 h, which was far lower than the sugar production using 15 mg/g dry mass of each one of CMax and AF100L, indicating that 15 mg/g dry mass of both enzymes was the limit for hydrolysis. The synergy between xylanase and cellulases may have enhanced the proximity and access of the substrate to the enzymes (Moraïs et al. 2010).

**Effects of Pulp Concentration on Enzymatic Hydrolysis**

A high concentration of wood pulp for hydrolysis increases sugar and ethanol production, which subsequently reduces the cost for ethanol distilling. To make cellulosic ethanol feasible in the market, ethanol production must be at least 76.5 g/L, which requires an initial sugar concentration higher than 150 g/L (Gurram et al. 2015). In this experiment, pulp concentrations of 10%, 15%, and 20% were compared; sugar production increased...
with higher concentrations (Fig. 9-(a)). The sugar production of the 10% concentration pulp was 87 g/L at 48 h. When the concentration increased to 15% and 20%, the production increased to 115 g/L and 149 g/L, respectively, which were 32% and 71% increases. Although the sugar production was significant affected by pulp concentration (P=6.84×10^{-5}<0.01), the enzymatic conversion rate of 10% pulp was 61.5% at 48 h, and it decreased to 58.0% and 54.1% respectively when the pulp concentration increased to 15% and 20% (Fig. 9-(b)). The results were consistent with the observation by Kristensen et al. (2009) and may be caused by the inhibition of enzyme absorption to cellulose by the hydrolytic products, such as glucose or celllobiose. Although fermentation processes benefit from higher sugar production, lower conversion rate may become significant when the pulp concentration increased. In addition, mixing became a problem when the pulp concentration was increased to 15%. Considering that the raw pulp concentration from the pulping process was also about 10%, and could be directly used for hydrolysis without further adjustment, 10% pulp was chosen for the pilot-scale trial.

![Graph](image)

**Fig. 9.** Effect of pulp concentration on enzymatic hydrolysis. (a) Sugar production with (A) 10%, (B) 15%, and (C) 20% pulp concentration. (b) Total enzymatic conversion rate at 48 h with different pulp concentration.

**Different Mixing Modes**

Because pulp is water-insoluble, effectively increasing the contact of the enzymes with the pulp is critical for enzymatic hydrolysis and sugar production. To select a suitable mixing mode, two different trials were carried out for hydrolysis with 7 kg of pulp. The rates of sugar production were the same at the beginning of hydrolysis, probably due to the complete mixing before hydrolysis for both trials (Fig. 10). After 6 h, sugar production in the two trials was different. The sugar production was 18% more for the manual intermittent mixing at 15 h, indicating that it had an advantage in sugar production over the propelling continuous mixing. At 48 h, the sugar production reached 102.0 g/L for the intermittent mixing, but only reached 95.6 g/L for the continuous mixing. The production gap between the two modes was reduced to 6.7%, indicating that sugar production wasn’t significant affected by mixing mode (P=0.06>0.01). Thus, intermittent mixing blended the pulp and enzymes more completely. This result was confirmed by pH, another indicator for the degree of hydrolysis. The initial pH for both trials was 6.4 after adding the enzymes. At 12 h, the pH for the intermittent mixing dropped to 4.0, and further down to 3.8 at 48 h. However, the pH was 4.6 for the continuous mixing at 12 h and 4.2 at 48 h. With more complete hydrolysis, more acids are produced. Therefore intermittent mixing was more complete, it was chosen for further pilot-scale trials.
Fig. 10. Effect of mixing mode on enzymatic hydrolysis and sugar production. (A and B) pH of manual intermittent mixing and propeller continuous mixing, respectively; (C and D) sugar production of manual intermittent mixing and propeller continuous mixing. The pulp concentration was 15%, and the enzyme dosage was 15 mg/g dry mass of each one of CMax and AF100L. pH was not controlled during hydrolysis.

**Pilot-scale Trial**

Given that the conditions of the pilot-scale trial may be different from a lab-scale trial (Roche et al. 2009; Jones et al. 2014), the combination of the optimized parameters was validated by a one-ton pilot-scale trial (Fig. 11). In the pilot trial, the temperature was controlled at about 55 °C at the beginning of the hydrolysis, and it dropped to about 35 °C overnight. The mixing was intermittent, with a high intensity of mixing at the beginning, but reduced intensity when the pulp was liquefied. The sugar production reached 66.4 g/L and 70.3 g/L, respectively, at 48 h and 72 h, and the enzymatic conversion rate increased to 59.0% and 65.0%, respectively in the same period. Hydrolysis at the pilot-scale was scaled up from the lab-scale, but it showed 19.2% and 12.3% less in sugar production and enzymatic conversion rate respectively, probably caused by poor mixing and temperature control. The initial mixing of the pulp with the enzymes was important but laborious before the pulp was liquefied, and a twin-shaft mixer may be considered for the large-scale production.

Fig. 11. The pilot-scale trial with the optimized conditions. The pulp concentration was 10%, and the enzyme dosage was 15 mg/g dry mass of each one of CMax and AF100L. The pH was not controlled during hydrolysis. The temperature was intermittently controlled to 55 °C during working hours. In (a), (A) temperature; (B) sugar production. (b) enzymatic conversion rates at 48 and 72 h.
The sugar production and enzymatic conversion rates of the lab-scale, scaling-up and pilot-scale trials are compared in Table 2. With increased pulp concentration from 10% to 15% in the scaling-up trial, sugar production increased from 87.0 g/L to 102.0 g/L, but the enzymatic conversion rate decreased from 77.3% to 60.4%, which was consistent with our previous results (Fig. 9) even though more vigorous mixing was applied in the scaling-up trial. In the pilot trial, the sugar production reduced from 87 g/L to 70.3 g/L, and the conversion rate reduced from 77.3% to 65%, which was probably caused by the poor mixing and temperature control and could be overcome by proper engineering design in application.

**Table 2.** Sugar Production and Enzymatic Conversion Rates in Lab-scale, Scaling-up and Pilot-scale Trials

<table>
<thead>
<tr>
<th>Type of Trial</th>
<th>Pulp Concentration</th>
<th>Sugar Production (g/L)</th>
<th>Enzymatic Conversion Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab-scale</td>
<td>10%</td>
<td>87.0</td>
<td>77.3</td>
</tr>
<tr>
<td>Scaling-up</td>
<td>15%</td>
<td>102.0</td>
<td>60.4</td>
</tr>
<tr>
<td>Pilot-scale</td>
<td>10%</td>
<td>70.3</td>
<td>65.0</td>
</tr>
</tbody>
</table>

**Sludge Residue Recycling**

After each round of hydrolysis, about 65% of the pulp was converted to sugars, and the remaining was filtered out as residue. To fully utilize the pulp, the residue was recycled for further sugar production. The hydrolysis of residues was carried out with and without washing. As shown in Fig. 12, sugars were released from both the non-washed and washed residues, but the sugar production from the washed residues was noticeably higher than that from the non-washed residues. After 72 h, the sugar production was 43.9 g/L for the non-washed residues and 66.8 g/L for the washed residues, increasing by 52.2%, indicating that washing remarkably improved sugar production from the residues. This result indicated that washing might remove most of the end-products of hydrolysis (glucose, cellobiose, or other oligosaccharides), and relieve the cellulosic fibers from end-product inhibition (Tengborg *et al.* 2001; Gan *et al.* 2003; Himmel *et al.* 2007).

**Fig. 12.** Sugar production from the residues of enzymatic hydrolysis. (A) Washed residues and (B) unwashed residues. The enzyme dosage was 5 mg/g dry mass of each one of CMax and AF100L.
CONCLUSIONS

1. In this study, the hydrolytic conditions of wood pulp were optimized. The optimum conditions for hydrolysis were 55 °C, 15 mg/g dry mass of each one of CMax and AF100L, and 10% concentration.

2. The hydrolysis was not greatly affected by pH. This result was significant because pH adjustment and buffering were costly and less environmentally friendly for large-scale production. Keeping the temperature at 55 °C and utilizing good mixing were important, especially at the beginning of the hydrolysis. The addition of xylanase improved enzymatic conversion rate.

3. The optimized parameters were applied to a pilot-scale operation, and the results were consistent with the lab-scale trials with lower sugar production and enzymatic conversion rates, indicating that the hydrolysis was scalable after proper designing for mixing and temperature control. After washing, the hydrolytic residues were able to be recycled for further sugar production.

4. This study provides insight for economical, large-scale cellulosic sugar production from wood pulp. Maple, birch, aspen, and beech wood pulps can be hydrolyzed under the optimized low-cost conditions and are good candidates for renewable energy production. The present results provide reliable data for industrial application.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support from the National Natural Science Foundation of China [grant nos. 51278200 & 51478190], Guangdong Provincial Natural Science Foundation Key Project [grant no. 2014A030311014], Guangdong Provincial Science and Technology Program [grant no. 2012B091100163], and Guangzhou Science and Technology Program [grant no. 201510010288].

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Article submitted: April 13, 2016; Peer review completed: May 22, 2016; Revised version received: June 28, 2016; Accepted: June 29, 2016; Published: July 13, 2016. DOI: 10.15376/biores.11.3.7242-7257