Surfactant as an Additive for Producing Cellulosic Sugar from Wood Residue

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Due to environmental concerns and the proposed global policies for reducing carbon emissions, lignocellulosic biomass is an attractive renewable feedstock for biofuel and biochemical production. Wood residues from the plywood industry have a lignin content as high as 30.7% ± 0.1%. Diluted acid pretreatment followed by enzymatic hydrolysis is often employed to release sugar from lignocellulosic biomass. A high lignin content limits the accessibility of cellulose. Lignin also binds with enzymes, which reduces the enzymatic hydrolysis efficiency. In this study, different concentrations of polyethylene glycol (PEG) 6000 were used as an additive to inhibit the detrimental effects caused by lignin. The optimal dosage was 1 g/L, which increased the glucose production to 35.8% and 26.6% for solid-to-liquid ratios of 2% and 20%, respectively. The results suggest that PEG 6000 is a suitable potential additive for increasing the bioethanol production from plywood residue in upscale operations.

INTRODUCTION

The worldwide petroleum availability is decreasing, and its price is rising. Moreover, there is increasing awareness regarding the environmental effects of fossil fuels. Many countries have legislative policies or laws to reduce petroleum usage to protect the environment (Gong *et al.* 2018). Unlike central areas of developed countries, developing countries and rural areas of developed countries still use waste wood combustion as a source of energy (Bhutto *et al.* 2011). However, waste wood combustion causes environmental pollution and contributes to climate change (Kjellstrom *et al.* 2006). In addition, the combustion of waste wood is detrimental to human health (Bruce *et al.* 2000; Zhang and Smith 2007; Kim *et al.* 2011a).

Lignocellulose can be used as a biofuel and biochemical source. The three major components of lignocelluloses are cellulose, hemicellulose, and lignin (Zhang *et al.* 2017). Lignocellulosic feedstocks are pretreated by diluted acid to release the lignocellulosic structures prior to enzyme hydrolysis, which results in fermentable cellulosic sugar. The most important part of using lignocellulose to produce biofuel is obtaining enough fermentable monosaccharide from lignocellulose to serve as a carbon source during fermentation (Robak and Balcerek 2018). To obtain monosaccharide from lignocellulose, the raw materials must undergo pretreatment and enzymatic hydrolysis (Capolupo and Faraco 2016). During enzymatic hydrolysis, lignin is an inhibiting factor that reduces the efficiency of the process (Rahikainen *et al.* 2011; Newman *et al.* 2013). In the case of corn stover, the rearrangement of the lignin structure hinders the hydrolysis

effects of cellulose when dilute acid is applied in the pretreatment (Kumar and Wyman 2009). The quantity of lignin varies across various lignocellulosic feedstocks (Buranov and Mazza 2008; Guo *et al.* 2009). Generally, woody plants are composed of 15% to 40% dry weight of lignin. In hardwood (angiosperm) species, the average lignin content is 19% to 28%. In softwood (gymnosperm) species, the average lignin content is 24% to 33% (Fengel and Wegener 1983). Compared with herbaceous plants such as rice straw, which contain a low amount of lignin, the inhibition effects caused by lignin need to be solved in order to facilitate enzymatic hydrolysis when using woody feedstocks.

This study used effective pretreatment conditions to obtain a high hydrolysis efficiency (30 filter paper unit (FPU)/g cellulose for enzyme loading). However, the glucose concentration declined when 15 FPU/g cellulose for enzyme loading was applied. Surfactants can be used to solve the problem of reduced enzymatic hydrolysis efficiency caused by lignin. The use of surfactants during enzymatic hydrolysis represents an economical method to increase the hydrolysis efficiency, as surfactants based on ethylene oxide are commonly used to enhance the enzyme hydrolysis efficiency (Park *et al.* 1992; Kurakake *et al.* 1994). Kristensen *et al.* (2007) and Xi *et al.* (2012) found that the use of polyethylene glycol (PEG) 6000 as an additive during enzymatic hydrolysis can effectively increase the hydrolysis efficiency for wheat straw and cotton stalk. In this study, PEG 6000 was added in different concentrations during the enzymatic hydrolysis process to improve the efficiency and reduce the cost of the process. The change in the enzymatic hydrolysis efficiency with the addition of PEG 6000 was analyzed. Bioethanol was also used to execute the fermentation process to understand the effect of the surfactant.

EXPERIMENTAL

Preparation of Hydrolysate from Waste Wood Chips

Dilute acid-catalyzed steam explosion was employed as the pretreatment procedure. Before pretreatment, the waste wood chips were cut to less than 2 cm using a shredder (DHS3-422; Weizhou, Kaohsiung, Taiwan). The shredded chips were used as the raw material and were mixed with 1% sulfuric acid solution to create a solution with 20% solids. The solution was incubated at room temperature overnight. The mixture was then transferred to a steam explosion reactor consisting of a 20-L pressure vessel and a reactor tank with a cooling system to collect the slurry. Saturated steam was flowed into the reactor to heat the impregnated material to the desired temperature, and the material was maintained at a specific residence time before suddenly depressurizing. The pretreatment conditions of the sulfuric acid concentration and the operating temperature were 1% (w/w) and 200 °C, respectively. The residence times were 1 min, 5 min, and 8 min (Table 1). After the pretreatment, the hydrolysates were drained from the reactor and centrifuged to separate the solid and liquid portions. Thus, a cellulose-rich hydrolysate was obtained for the subsequent hydrolysis procedure (Huang *et al.* 2011). The pretreated wood residues were stored at 4 °C.

Enzymatic Hydrolysis in 250-mL Flasks

To decide which pretreated wood hydrolysate was the most appropriate for producing bioethanol, the enzymatic hydrolysis procedure was performed in 250-mL flasks using 50 mM sodium acetate buffer (pH 4.8), PEG 6000 (Sigma-Aldrich, St. Louis,

MO, USA), and 2% dry matter (w/v) with an appropriate quantity of CTec3 cellulase enzyme (Novozymes, Bagsværd, Denmark). The procedure was performed at 50 °C on an orbital shaker at 100 rpm for 72 h. The pretreated wood hydrolysate that was used for enzymatic hydrolysis was not subjected to extra washing or drying processes. For preparing the 2% dry mass slurry, the moisture content of the wood hydrolysate was measured, and the wood hydrolysate was weighed on a wet basis. The wood hydrolysate contained 1 g of dry mass. The weighed pretreated wood hydrolysate was further mixed with sodium acetate buffer, and a final volume of 50 mL was used for the enzymatic hydrolysis. To determine the best pretreatment condition, 15 FPU/g of cellulose of CTec 3 without PEG 6000 was used in this study. To determine the usage amount of surfactant, 15 FPU/g of cellulose of CTec3 was used with 0 g/L, 0.1 g/L, 0.5 g/L, 1 g/L, and 1.5 g/L of PEG 6000. The pH was adjusted to 5.0 by using 10 M NaOH. After the enzymatic hydrolysis test was completed, the sugar concentration in the reaction solution was analyzed through high performance liquid chromatography (HPLC) (Agilent 1200; Agilent, Santa Clara, CA, USA). Triplicate reactions were undertaken for each sample.

Separate Hydrolysis and Ethanol Fermentation in a 5-L Fermenter

Prior to the separate hydrolysis and fermentation process, the enzymatic hydrolysis was performed at 250 rpm in a 5-L fermenter with a working volume of 3 L without pH control. The wood hydrolysates from the pretreated procedure were mixed with deionized water and commercial CTec3. The solid-to-liquid ratio was set at 20% (w/v). The enzyme activity was set at 15 FPU/g for cellulose with or without 1 g/L of PEG 6000 or at 30 FPU/g for cellulose without PEG 6000. The pH of the hydrolysis medium was adjusted to 5.0 by using 10 M NaOH, and the temperature was set at 50 °C. After hydrolysis for 72 h, the glucose-rich liquid fraction was separated from the hydrolysis medium through vacuum filtration. The liquid fraction was then used in the subsequent fermentation process. The fermentation of the glucose-rich liquid was performed in a 5-L fermenter with a working volume of 3 L at 150 rpm. The fermentation temperature was set at 30 °C for *Saccharomyces cerevisiae* YY5A (Ma *et al.* 2012) with an initial dry cell weight of 0.7 g/L. The cultural conditions for the seed cultures were created in accordance with the fermentation method of Ma *et al.* (2012). The pH of the cultural medium was adjusted to 6.0 by using 10 M NaOH. The ethanol concentration in the cultural medium was analyzed by HPLC.

Analysis Methods

The waste wood chips were supplied by a plywood factory in Malaysia, the sugarcane bagasse was supplied by the Taiwan Sugar Corporation (Tainan, Taiwan), and the rice straw was collected from a private farm (Taoyuan, Taiwan). The components of these three feedstocks were determined according to the Laboratory Analytical Procedures from the National Renewable Energy Laboratory (Sluiter *et al.* 2008) before and after pretreatment. The carbohydrate contents were determined by measuring the hemicellulose (xylan and arabinan) and cellulose (glucan) of the derived sugars. The lignin content was also determined in the analytical procedure.

Each sample was filtered through a 0.45-μm filter and diluted appropriately with deionized water. The quantitative analysis for xylose, glucose, ethanol, arabinose, acetic acid, and inhibitors such as furfural and hydroxymethylfurfural (HMF) was conducted using the HPLC system equipped with a refractive index detector at 45 °C. The separation was achieved using a Coregel-87H3 column (Transgenomic, San Jose, CA,

USA) which was maintained at 65 °C with 4 mM H_2SO_4 as eluent at a flow rate of 1.0 mL/min (Chen *et al*. 2011). All the data were obtained from the average values of the three independent experiments.

RESULTS AND DISCUSSION

Preparation of Hydrolysate from the Waste Wood Chips

The composition of the waste wood chips was 44.2% ± 0.4% glucan, 15.7% ± 0.1% xylan, and 30.7% ± 0.1% lignin before (Table 2) and after pretreatment (Table 3). The total lignin content of the waste wood chips was considerably higher than that of sugarcane bagasse and rice straw. The pretreatment conditions are presented in Table 1. Different reaction times were applied to pinpoint a pretreatment condition with the maximum glucose production. The results of the enzymatic hydrolysis are illustrated in Fig. 1. The pretreatment with 1% sulfuric acid at 200 °C, 20% solid content, and 15 FPU/g of cellulose of enzyme loading for 5 min exhibited the highest glucose concentration. The concentration of fermentation inhibitors such as furfural and HMF were 2.6 g/L ± 0.01 g/L and 0.9 g/L ± 0.02 g/L with 1 min residence times; 3.1 g/L ± 0.02 g/L and 1.2 g/L ± 0.01 g/L with 5 min residence times; and 3.5 g/L ± 0.01 g/L and 1.6 g/L ± 0.01 g/L with 8 min residence times in the steam explosion reactor during pretreatment, respectively. On a commercial scale, the enzyme loading must be as low as possible to reduce the costs of bioethanol production (Liu *et al.* 2016a). However, a lower enzyme load will decrease the glucose production after the enzymatic hydrolysis process.

Table 1. Steam Explosion Pretreatment Conditions for Wood Residue

Raw Material	Temperature (°C)	Solid Content (%)	Sulfuric Acid Concentration (%)	Reaction Time (min)
Wood Residue	200	20	1	1
Wood Residue	200	20	1	5
Wood Residue	200	20	1	8

Table 2. Major Compositions of Various Feedstocks before pretreatment

Sample	Carbohydrate Contents			Lignin		
	Cellulose	Hemicellulose				
	Glucan (%)	Xylan (%)	Arabinan (%)	ASL (%)	AIL (%)	Total lignin (%)
Rice Straw	33.1 ± 1.6	19.5 ± 1.8	4.0 ± 0.9	8.4 ± 2.9	5.9 ± 2.8	14.3 ± 2.9
Sugarcane Bagasse	38.4 ± 0.6	27.8 ± 1.0	4.0 ± 0.2	7.9 ± 0.5	15.5 ± 1.3	23.4 ± 1.6
Wood Chips	44.2 ± 0.4	15.7 ± 0.1	0.8 ± 0.1	6.5 ± 0.1	24.2 ± 0.1	30.7 ± 0.1

Abbreviations: ASL, acid-soluble lignin; AIL, acid-insoluble lignin

Table 3. Major Compositions of Various Feedstocks after pretreatment

Sample	Carbohydrate Contents			Lignin
	Cellulose	Hemicellulose		
	Glucan (%)	Xylan (%)	Arabinan (%)	Total lignin (%)
Rice Straw	51.0 ± 1.8	1.5 ± 0.2	0.7 ± 0.1	31.6 ± 1.0
Sugarcane Bagasse	43.1 ± 0.5	2.3 ± 0.2	0.4 ± 0.1	35.2 ± 0.8
Wood Chips	46.2 ± 0.2	0.5 ± 0.1	0.3 ± 0.1	47.1 ± 0.3
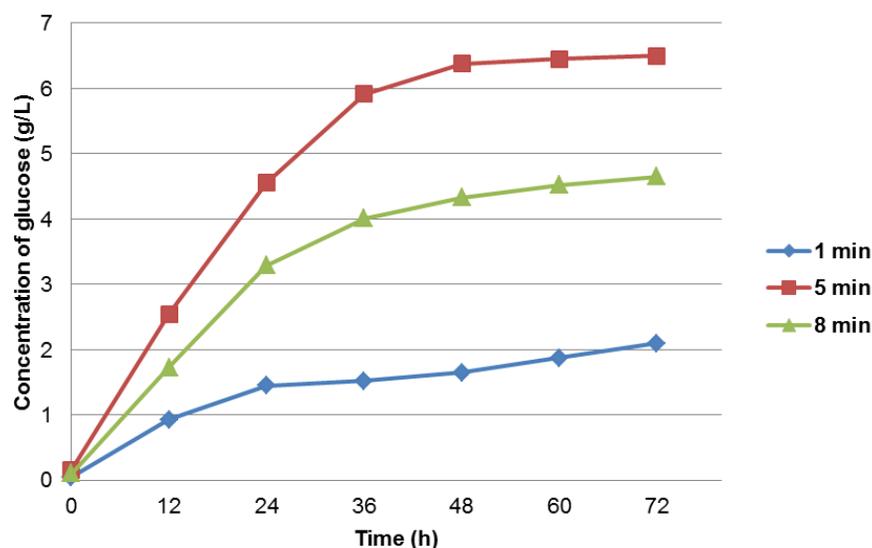
Fig. 1. The increment of glucose concentration with different pretreatment conditions. The residence times of waste wood chips at 200 °C during pretreatment were 1 min, 5 min, and 8 min.

In a previous study, the enzymatic hydrolysis efficiency for sugarcane bagasse and rice straw hydrolysates was lower than that for wood chip hydrolysates (Yuan *et al.* 2017). This phenomenon may result from the fact that wood chips have a higher lignin content than sugarcane bagasse and rice straw. During the enzymatic hydrolysis process, the lignin content is a critical inhibiting factor due to the irreversible adsorption of lignin onto the cellulase. This phenomenon reduces the enzymatic hydrolysis efficiency and reduces the release of fermentable monosaccharide (Zhu *et al.* 2008).

Effect of Surfactant Addition during Enzymatic Hydrolysis

Some mechanisms dictate how lignin becomes an inhibitor during the enzymatic hydrolysis process. To investigate the interaction between lignin and cellulase, researchers have proposed that lignin may strongly bind to the cellulase by hydrophobic interactions and electrostatic repulsions. This phenomenon reduces the enzymatic hydrolysis efficiency and the concentration of the released monosaccharide (Zhu *et al.* 2008; Rahikainen *et al.* 2011). After the pretreatment procedure, phenolic compounds, such as syringaldehyde, vanillin, and aromatic aldehydes derived from lignin, always are present in lignocellulosic hydrolysates. These derived compounds can reduce the enzymatic hydrolysis efficiency and increase the cost of the cellulosic sugar production (Kim *et al.* 2011b; Qin *et al.* 2016).

Many mechanisms have been proposed to result from the addition of surfactants, including the stabilization of the enzyme, changes to the ultrastructure of the substrate, and effects on the enzyme-substrate interactions. However, the most common mechanism involves the combination of the free chemical groups released from lignin to impede the adsorption of cellulase onto lignin and facilitate the access of cellulase to cellulose during the enzymatic hydrolysis process (Zheng *et al.* 2008; Wang *et al.* 2018). Different concentrations of PEG 6000 were used during the enzymatic hydrolysis process, as shown in Fig. 2. The final concentrations of glucose were 6.50 g/L, 8.83 g/L, and 8.96 g/L in the hydrolysates when using 0 g/L, 1 g/L, and 1.5 g/L of PEG 6000, respectively.

When 1 g/L of PEG 6000 was used, the glucose production increased by 35.8% compared to the case where PEG 6000 was not used. Because there was no significant difference in the glucose production when 1 g/L and 1.5 g/L of PEG 6000 were used, 1 g/L was the optimal PEG 6000 concentration for wood residue in this study. When the concentration of PEG 6000 exceeds 1 g/L, it's enough to reduce the detrimental effects caused by lignin then elevate the enzymatic hydrolysis efficiency.
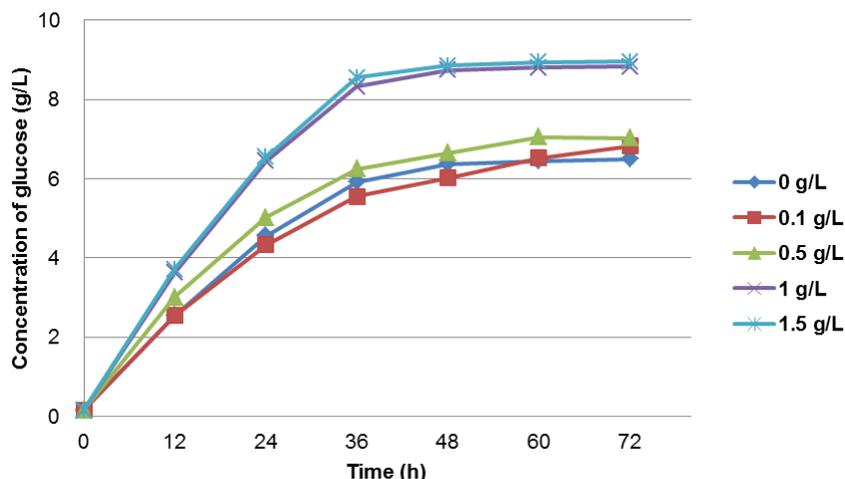
Fig. 2. Enzyme hydrolysis with different pretreatment conditions. In order to determine the optimal amount of surfactant, 15 FPU/g of cellulose of CTec3 was used with 0 g/L, 0.1 g/L, 0.5 g/L, 1 g/L, and 1.5 g/L of PEG 6000

Separate Hydrolysis and Fermentation
The results of the separate hydrolysis processes are displayed in Figs. 3 and 4.
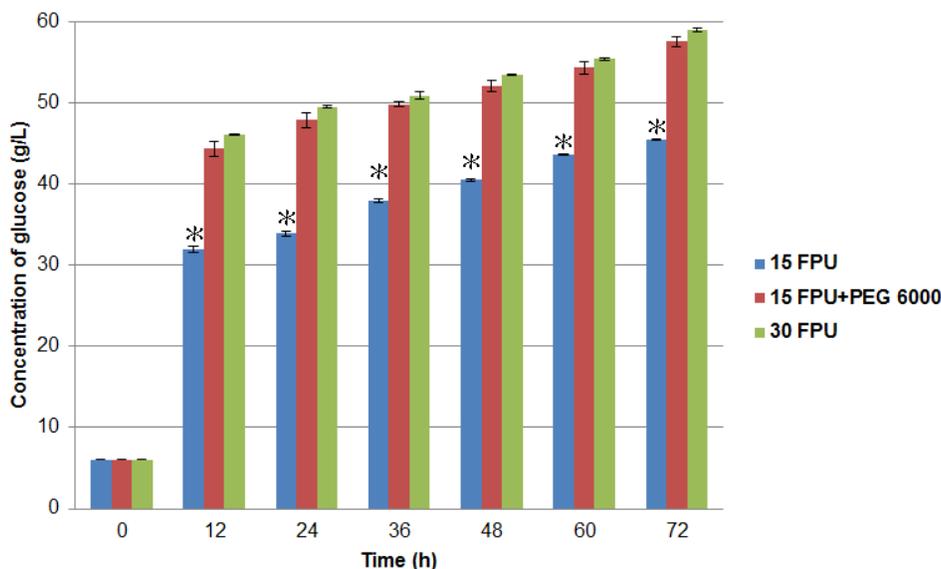
Fig. 3. Results of separate hydrolysis for glucose. The enzyme activity was set at 15 FPU/g for cellulose with or without 1 g/L of PEG 6000 or at 30 FPU/g for cellulose without PEG 6000. Data were expressed as the mean ± the standard error (n = 3); *P < 0.01 compared with the other groups
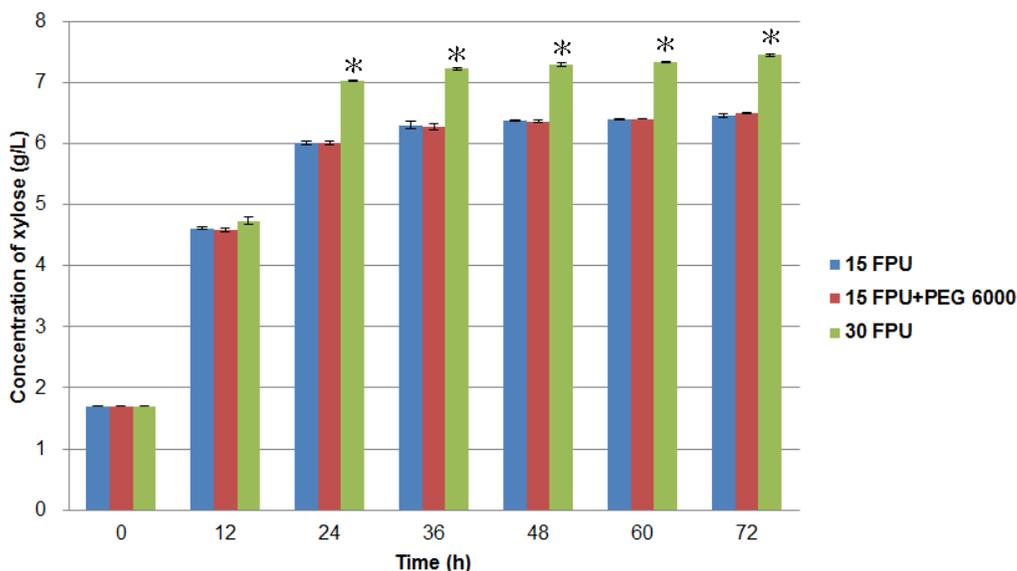
Fig. 4. Results of separate hydrolysis of xylose. The enzyme activity was set at 15 FPU/g for cellulose with or without 1 g/L of PEG 6000 or at 30 FPU/g for cellulose without PEG 6000. Data were expressed as the mean ± the standard error (n = 3); *$P < 0.01$ compared with the other groups.

The initial concentrations of glucose in the three different hydrolysis conditions were all 6.0 g/L. After 72 h of enzymatic hydrolysis for 15 FPU/g of cellulose with 1 g/L of PEG 6000, 15 FPU/g of cellulose without 1 g/L of PEG 6000, and 30 FPU/g of cellulose without using PEG 6000, the final concentrations of glucose reached 45.4 g/L, 57.5 g/L, and 58.9 g/L, respectively. The initial concentrations of xylose in the three hydrolysis conditions were all 1.70 g/L after 72 h of enzymatic hydrolysis. However, the final concentrations of xylose reached 6.45 g/L, 6.50 g/L, and 7.45 g/L for the three different hydrolysis conditions, respectively. Because we used dilute acid-catalyzed steam explosion as the pretreatment procedure, the hemicellulose part was easily dissolved in the hydrolysate liquid. Thus, the addition of PEG 6000 primarily affected the hydrolysis of cellulose in this study.

Because the solid-to-liquid ratio was set at 20% (w/v), the theoretical yield of glucose was approximately 211 g. The highest concentration of glucose was obtained when 30 FPU/g of cellulose was used. Compared with the use of 15 FPU/g of cellulose of CTec3 without PEG 6000, the use of 15 FPU/g cellulose of CTec3 with 1 g/L of PEG 6000 effectively increased the enzymatic hydrolysis efficiency and increased the concentration of the fermentable glucose. Furthermore, PEG 6000 is cheaper than commercial cellulase (CTec3). Thus, the use of small quantities of PEG 6000 as an additive during the enzymatic hydrolysis process can increase the enzymatic hydrolysis efficiency without using double the quantity of cellulase, which reduces the production cost of bioethanol.

Fermentation Performance after the Addition of Surfactant

The theoretical yield of ethanol from glucose fermentation is 0.51 g/L of glucose

(Krishnan *et al.* 1999). In this study, the initial concentration of glucose in the separated hydrolysate medium with PEG 6000 was 55.9 g/L, and the final concentration of ethanol in the separated hydrolysate medium was 29.8 g/L (Fig. 5); the initial concentration of glucose in the separated hydrolysate medium with PEG 6000 was 45.5 g/L, and the final concentration of ethanol in the separated hydrolysate medium without PEG 6000 was 24.2 g/L (Fig. 6). However, the final concentration of ethanol (29.8 g/L and 24.2 g/L) exceeded the theoretical yield from glucose fermentation. Adding PEG 6000 during enzymatic hydrolysis didn't affect the yield ratio from glucose to ethanol. In another study, it was observed that *S. cerevisiae* YY5A could also use xylose alone to produce ethanol. This may explain why the production of ethanol exceeded the theoretical value. When standard xylose and glucose were used as a carbon source, the ethanol yield of *S. cerevisiae* YY5A was 0.36 g/L ± 0.01 g/L (Ma *et al.* 2012).
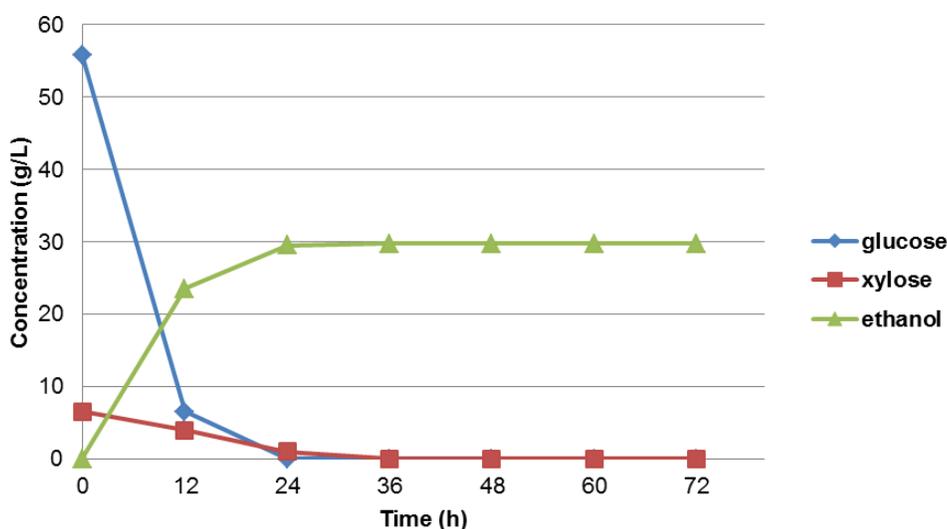
Fig. 5. The concentration variation of glucose, xylose, and ethanol from the acid-pretreated wood chips by using the separated hydrolysates with PEG 6000
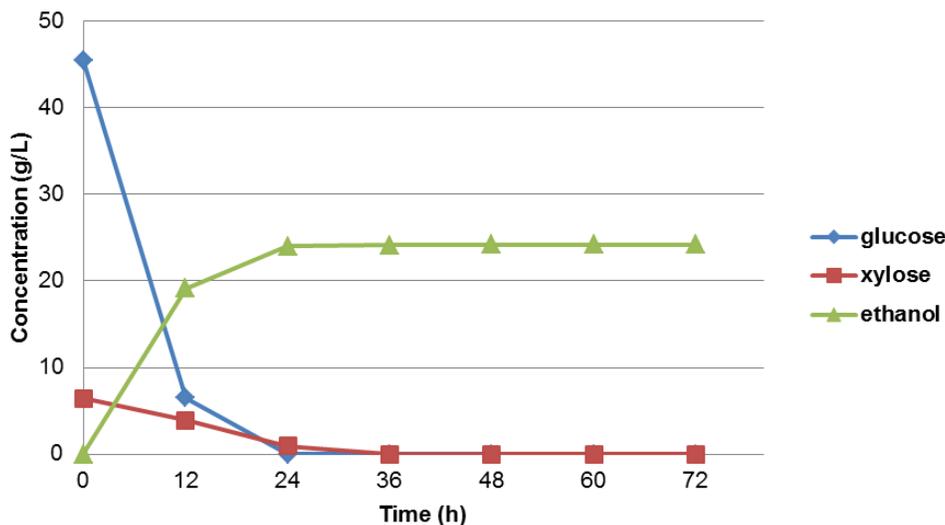
Fig. 6. The concentration variation of glucose, xylose, and ethanol from the acid-pretreated wood chips by using the separated hydrolysates without PEG 6000

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Chandel *et al.* (2013) proposed that for the dilute acid pretreatment procedure, the concentration of fermentation inhibitors such as furfural, HMF, and phenolic compounds in the hydrolysates was approximately 1 g/L to 3 g/L. In this study, furfural and HMF were detected in the separated hydrolysate medium. The concentrations of furfural and HMF were 3.1 g/L ± 0.02 g/L and 1.2 g/L ± 0.01 g/L with 5 min residence times in the steam explosion reactor during pretreatment, respectively. This was not the lowest inhibitor concentration within three pretreatment conditions. The reduced efficiency of enzymatic hydrolysis might refer to the existence of lignin after pretreatment. Adding PEG 6000 did not affect the concentrations of furfural or HMF in the hydrolysate. In these conditions, *S. cerevisiae* YY5A can continue to use glucose as a carbon source for producing ethanol. Thus, *S. cerevisiae* YY5A can effectively use lignocelluloses from waste wood residues to produce bioethanol.

Using different pretreatment procedures can increase the production of xylose after enzymatic hydrolysis (Kim *et al.* 2016; Liu *et al.* 2016b). The increase in the enzymatic hydrolysis efficiency caused by PEG 6000 may increase the concentration of the fermentable sugars and the efficiency of the fermentation process.

CONCLUSIONS

1. Wood residue is waste generated by the plywood industry. During a 5 min reaction at 200 °C, 1% sulfuric acid easily destroyed the structure of wood residue.

2. At an enzyme loading of 15 FPU/g cellulose, the addition of 1 g/L PEG 6000 increased the amount of fermentable glucose released from cellulose by 35.8%.

3. The improvement caused by adding PEG 6000 mainly affected the hydrolysis of cellulose rather than xylose. The addition of PEG 6000 increased the production of glucose by up to 26.6% when the solid-to-liquid ratios were set at 20% (w/v).

4. Using different pretreatment conditions and adding PEG 6000 as an additive may increase the concentration of fermentable sugars, such as glucose and xylose, from wood hydrolysates and increase the productivity of the fermentation process.

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