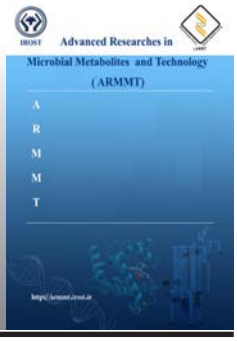
IROST

Advanced Research in
Microbial Metabolites & Technology
(ARMMT)

journal homepage: http://armmt.irost.ir



Efficient xanthan gum production from phosphoric acid–pretreated Cedar wood and Elm wood

Mohammadhadi Jazini [1*], Ali Ameri [1], Dariush Sohrabi [1], Keikhosro Karimi [1,2]

[1] **Department of Chemical Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran**
[2] **Industrial Biotechnology Group, Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan 84156-83111, Iran**

Article Info

Received: 04 December 2018
Received in revised form: 14 January 2019
Accepted: 26 January 2019

Keywords:
Cedar wood
Elm wood
Lignocellulosic biomass
Phosphoric acid pretreatment
Xanthan gum

Abstract

Cedar and Elm woods were investigated for the microbial production of xanthan gum using a commercial strain of Xanthomonas campestris. However, the yields of xanthan gum from untreated woods were inefficient. Thus, a dilute phosphoric acid (1-2% w/v) pretreatment at elevated temperatures (140-180 °C) for 10-20 min and concentrated phosphoric acid (85% w/v) pretreatment at 60 °C for 1-3 h were applied to increase production yields. Concentrated acid pretreatment resulted in the highest yields of 9.9 and 10.4 g xanthan gum per 100 g of raw Cedar and Elm wood, respec-tively, whereas the untreated woods yielded 2.0 and 2.4 g xanthan gum per 100 g of raw woods. The dilute acid pretreatment was not as efficient as the concentrated acid pretreatment, it resulted in 4.2 and 5.2 g xanthan gum per 100 g of Cedar and Elm respectively. Consequently, the woods are suitable substrates for xanthan gum production after pretreatment with concentrated phosphoric acid at 160 °C for 1 h. The quality of produced xanthan gum was also compared with a commercial xanthan gum using Fourier transform infrared spectroscopy, and the results indicated that the produced xanthan gum was similar to the commercial product. Although the pretreat-ments presented in this study increased the xanthan gum yield up to fourfold, it was at the expense of increasing pretreatment costs. This research provides the basis for the economic analysis required for the commercial implementation of these pretreatments.

1. Introduction

Polysaccharides with biological origin, like xanthan gum, have received increasing attention because of their biodegradability and biocompatibility. *Xanthomonas campestris* is a common bacterium used for the production of xanthan gum on an industrial scale (García-Ochoa et al., 2000). Lignocellulosic biomass has been recently introduced as renewable raw material for xanthan gum production (Jazini et al., 2017). The main obstacle in using biomass is its recalcitrant structure, in which the cellulose is surrounded and compacted by lignin and hemicellulose. This three-dimensional structure must be disrupted to make cellulose accessible to hydrolyzing enzymes. Hence, different pretreatment

*Corresponding author. Tel: + 983133915616 E-mail address: m.h.jazini@cc.iut.ac.ir

DOI: 10.22104/ARMMT.2019.3335.1019

methods using dilute and concentrated phosphoric acid in different operational conditions have been proposed. For example, pretreatment of bagasse for production of fermentable sugars was investigated using dilute phosphoric acid (de Vasconcelos et al., 2013). In another work, pretreatment of wheat straw for production of edible fungal biomass at different temperatures (150-210 °C), acid concentrations (0.5-3% w/v), and reaction times (5-20 min) has been reported (Nair et al., 2017). Phosphoric acid pretreatment of olive tree prunings for production of ethanol has been performed. It has been reported that the pretreatment at 170 °C and 0.5% w/v phosphoric acid concentration resulted in maximum sugar recovery (Martínez-Patiño et al., 2015). Dilute sulfuric acid pretreatment of lignocellulosic biomass from animal manure resulted in a 116% increase in methane yield (Nasir & Ghazi, 2015). Phosphoric acid pretreatment of rice straw was studied for production of ethanol. The yield of 0.39 to 0.44 g ethanol/ g rice straw that was reported depended on pretreatment conditions (Beheshti & Karimi, 2016). Interestingly, pretreatment of wheat straw with phosphoric acid plus hydrogen peroxide enhanced hydrolysis efficiency to 96.3% (Yao et al., 2019). Fungal pretreatement followed by dilute phosphoric acid pretreatment of olive tree biomass has been recently reported as an efficent method which could result in the overall total sugar yield of 51% of the theoritical yield (Martínez-Patiño et al., 2018).

Lignocelluloses like Cedar wood and Elm wood are widely available in Iran and the dis-posal of these woods is now a big challenge for municipalities. To our knowledge, they have not been used for microbial production of xanthan gum before, although lignocellulosic material like rice straw has been used as a substrate in microbial production of xanthan gum (Jazini et al., 2017)

The aim of this work was to study the feasibility of using two types of wood, namely, Cedar and Elm woods, as lignocellulosic biomass for the production of xanthan gum. These woods were selected as representatives of soft- and hardwoods, respectively. High and low concentrations of phosphoric acid were implemented to disrupt the structure of the woods. The hydrolysate, obtained after hydrolysis of the pre-treated wood, is a mixture of different sugars, including xylose, mannose, and galactose, in addition to glucose. Additionally, pretreatment of different types of woods results in different extractives. Therefore, the growth of a microor-ganism on the hydrolysate will be different from the growth on glucose alone. This depends on the ability of the microorganism to utilize other sugars. To the authors' knowledge, this work is novel; there is no published work that features both the phosphoric acid pretreatment of Cedar and Elm woods, and the xanthan gum produced from the pretreated woods. Nevertheless, as mentioned before, acid pretreatment of lignocellulosic biomass other than Cedar and Elm woods as well as their application in microbial production of xanthan gum have been reported.

2. Materials and methods

2.1. Wood collection and pretreatment

Cedar and Elm woods were collected from Isfahan University of Technology Forest (32°43'37.1"N 51°31'32.2"E, Isfahan, Iran). They were debarked, cut into small pieces, ball milled, and then sieved to obtain particle sizes of less than 1 mm.

Pretreatment was done using concentrated (85%) and dilute (1 and 2%) phosphoric acid. The experiments with 85% acid were performed at 60 °C at different pretreatment times (1, 2, and 3 h). Pretreatment with dilute acid was conducted at 140, 160, and 180 °C for 10 and 20 min. All pretreatments were done on both Elm and Cedar wood (Table 1).

The pretreatment with concentrated phosphoric acid was performed as follows: An amount of 10 g wood was mixed with 90 g of phosphoric acid solution in a 500 ml blue-

cap bottle (Schott AG, Mainz, Germany). The closed bottle was put into a shaking water bath (Model WNE 14, Memmert GmbH, Schwabach, Germany) at a desired temperature for a certain period of time. The pretreated woods then were filtered and washed with distilled water to remove the remaining acid. Finally, the pH was adjusted to 7 with 1 M NaOH. Afterward, the woods were dried at room temperature for 72 h.

The pretreatments with dilute phosphoric acid were conducted in the same way as the pretreatments with concentrated phosphoric acid. However, a high-pressure stainless steel vessel (500 ml working volume) equipped with a pressure gauge and a temperature sensor was used as a batch reactor to perform the pretreatments. The vessel was submerged in an oil bath whose temperature was controlled by means of a temperature controller and an electrical heater (Amiri et al., 2010).

2.2. Hydrolysis

A mixture of two commercial enzymes were used as the enzyme solution: Cellic® CTec2 (VCNI0013) (the main source of cellulase and β-glucosidase) and Cellic® HTec2 (VHN00002) (the source of hemicellulase and cellulase). As suggested elsewhere (Hashemi et al., 2016), the enzymes were mixed in the ratio of 9:1 to reach the final activity of 114.8 filter-paper units per milliliter of enzyme solution (PFU/ml). The activity of the cellulase was measured according to the method presented earlier (Adney & Baker, 1996), and was determined to be 125 and 23 filter paper units (FPU/ml), respectively. The release of 2.0 mg of reducing sugar as glucose from 50 mg of filter paper in 60 minutes has been defined as 1 PFU.

An amount of 1 g dried pretreated wood was mixed with 20 ml of sodium citrate buffer solution (0.05 M, 4.8 pH) in 118 ml glass bottles (717561, Pajouhesh Setayesh Sepahan, Isfahan, Iran). Then, the bottles were autoclaved at 121 °C for 20 min. After cooling to room temperature, 176 µL of the enzymatic mixture was poured into each of the glass bottles. Then each was sealed with butyl rubber and an aluminum cap.

The bottles were put in a shaking incubator (JTSL20, Jal Tajhiz, Tehran, Iran) at 45 °C and 120 rpm. The samples were taken from the bottles at 12, 24, and 72 h in order to determine the concentration of released glucose and total sugars.

2.3. Microbial fermentation

X. campestris was purchased from Persian Type Collection Culture (PTCC 1473, Persian Type Collection Culture, Tehran, Iran). The GYC medium (50 g/l glucose, 10 g/l yeast extract, 30 g/l calcium carbonate) was used for the preparation of the inoculum. The medium prepared for cultivation of *X. campestris* on the wood hydrolysate was a mixture containing 45 ml of the enzymatic hydrolysate supplemented with the following components: 2.8 g/l KH_2PO_4, 1.1 g/l $(NH_4)NO_3$, 0.25 g/l $MgSO_4$, 2.1 g/l citric acid, 0.0006 g/l H_3BO_3, 0.0006 g/l $ZnCl_2$, 0.0006 g/l $FeCl_3$, 0.02 g/l $CaCO_3$, 13 mM HCl, and 1M NaOH. The pH of the solution was adjusted to 7.2 before sterilization in an autoclave. The cultivation was performed in a 1000-ml shake flask containing 5 ml of inoculum and 45 ml of the medium (Jazini et al., 2017). The operating conditions were set at 30 °C and 130 rpm in a shaking incubator (JTSL20, Jal Tajhiz, Tehran, Iran).

Xanthan gum was purified according to the method previously developed and reported (Niknezhad et al., 2014). Accordingly, the cells were separated from the fermentation broth by centrifugation (25 min, 15000 rpm). The fermentation broth was then diluted with 0.1% calcium chloride in isopropanol solution at a ratio of 1:2. This caused the precipitation of xanthan gum that was then separated by centrifugation at 15000 rpm for 30 min. The separated xanthan gum was dried in an oven at 50 °C for 48 h. The xanthan gum yield was defined as the amount of xanthan gum obtained per 100 g of raw material.

2.4. Analytics

Solid recovery (SR) was calculated according to the following equation:

$$\text{SR}\ (\%) = \frac{\text{Weight of solids after pretreatment}}{\text{Weight of the dried raw sample}} \times 100 \quad (1)$$

The concentration of glucose in the hydrolysate was measured using a high performance liquid chromatography (HPLC) system. The system was equipped with UV/VIS and RI detectors (Jasco International Co., Japan). An Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at 60 °C with 0.6 ml/min eluent of 5 mM sulfuric acid was implemented. Knowing the glucose concentration, the yield of glucose was calculated (Poornejad et al., 2013):

$$\text{Glucose yield}\ (\%) = \frac{\text{produced glucose}\ \left(\frac{\text{g}}{\text{l}}\right)}{1.111 \times \text{glucan in the sample}\ \left(\frac{\text{g}}{\text{l}}\right)} \times 100 \quad (2)$$

Total reducing sugars was determined according to the method reported earlier (Gusakov et al., 2011).

Lignin and carbohydrate of untreated and pretreated wood were measured according to the standard method given by the National Renewable Energy Laboratory (Beheshti & Karimi, 2016).

In order to compare the quality of the produced xanthan gum with the commercial product, Fourier Transform Infrared Spectroscopy (FTIR, Bruker Tensor 27 FT-IR, Billerica, MA, USA) was implemented. The commercial xanthan gum from *X. campestris* was obtained from Sigma-Aldrich (CAS Number: 11138-66-2, Sigma-Aldrich, St. Louis, USA). FTIR technique was also implemented to compare the crystallinity of the wood samples before and after pretreatment. The crystallinity index was defined as the ratio of absorbance at 1430 cm^{-1} wavenumber to that at 897 cm^{-1}. The ratio for the absorbance at 1375 cm^{-1} and 2900 cm^{-1} wavenumbers was defined as the total crystallinity index (Nieves et al., 2011). These indices were used for quantitative comparison of the impact of pretreatment on the structure of the untreated and pretreated samples. The morphology of the pretreated and untreated woods was compared using Scanning Electron Microscopy (SEM, Zeiss, Jena, Germany).

In order to provide sufficient data for analysis and comparison, all experiments were conducted in triplicate.

3. Results and discussion

3.1. Solid recovery

Table 1 shows solid recoveries obtained from the dilute and concentrated acid pretreatment of Cedar and Elm woods. It can be clearly seen that solid recovery decreased as the temperature increased. For example, solid recovery dropped from 91.0 to 65.7% as the temperature increased from 140 to 160 °C in the pretreatment of ce-dar wood (Table 1). Additionally, this reduction was considerably more when the temperature increased from 140 to 160 °C compared to the increase in temperature from 160 to 180 °C. For instance, a roughly 27% reduction in solid recovery was observed when the temperature increased from 140 to 160 °C, while a 21% re-duction was observed by increasing temperature from 160 to 180 °C in the pretreatment of Cedar wood with 1% phosphoric acid for 10 min (Ta-ble 1). A similar trend was observed in the pre-treatment of Elm wood (Table 1). It was reported that an increase in temperature from 201 to 216 °C can decrease the solid recovery from 93.4 to 83% in the pretreatment of softwoods with 0.4% sulfuric acid (Nguyen et al., 1998). Noori and Karimi (2016) showed that the increase in temperature from 0 to 25 °C in the alkali pretreatment of elm wood decreased the solid recovery from 93.1 to 85.6% (Noori & Karimi, 2016).

Acid concentration showed significant impact on the solid recovery. The greater the acid concentration, the greater the decrease in

solid recovery. The reduction in solid recovery was more effective at higher temperatures. For example, the increase in acid concentration from 1 to 2% at 140, 160, and 180 °C resulted in 6.5, 8.5, and 12.6% decrease in solid recovery in the pretreatment of Cedar wood for 10 min (Table 1). A similar trend was also observed in the case of Elm wood.

Table 1. Solid recoveries obtained after pretreatments of Cedar wood and Elm wood with dilute and concentrated phosphoric acid. Data are mean ± SD of three replicates.

Pretreatment conditions			Cedar wood	Elm wood
T (°C)	Acid concentration (%)	Time (min)	Solid recovery (%)	Solid recovery (%)
140	1	10	91.0±0.3	89.1±0.2
140	1	20	89.5±0.2	86.5±0.1
140	2	10	85.0±0.4	83.9±0.3
140	2	20	83.2±0.4	78.6±0.4
160	1	10	65.7±0.5	73.4±0.4
160	1	20	64.3±0.3	70.3±0.3
160	2	10	60.1±0.4	68.17±0.5
160	2	20	58.5±0.4	65.55±0.2
180	1	10	51.5±0.2	60.3±0.1
180	1	20	49.7±0.1	57.7±0.2
180	2	10	46.6±0.2	52.4±0.4
180	2	20	45.0±0.3	49.8±0.3
60	85	60	53.4±0.3	58.7±0.2
60	85	120	45.1±0.2	49.6±0.3
60	85	180	33.3±0.4	40.5±0.3

The increase in pretreatment time from 10 to 20 min resulted in reduction of solid recovery (Table 1). However, this effect was considerably more at elevated temperatures. For example, the increase in the pretreatment time from 10 to 20 min resulted in a 2.9% reduction of solid recovery in the pretreatment of Elm wood at 140 °C, while it resulted in a 4.9% reduction in the case of pretreatment at 180 °C. Similar results were observed for Cedar wood (Table 1).

The solid recovery values detected in the pretreatments with 85% phosphoric acid revealed that solid recoveries in these experiments were less than those obtained in the experiments with dilute acid at 140 and 160 °C (Table 1). However, the solid recovery value of 51.5% observed in the dilute acid pretreatment at 180 °C was close to the values obtained in the pretreatment with 85% acid (53.4%). This indicated that the pretreatment at 60 °C could result in a solid recovery value similar to that at 140-160 °C, but with the expense of an increase in the pretreatment time (from 10-20 min to 1 h) and acid concentration (from 1-2 to 85%).

3.2. Chemical composition of lignocellulosic biomass

Table 2 presents the chemical composition of untreated as well as pretreated woods with 85% phosphoric acid. The glucan content of the samples increased as the pretreatment time increased but then decreased. In the case of Cedar wood, this decrease was observed after 2 h, while it was detected after 1 h for Elm wood. The concentra-tion of hemicellulosic sugars, i.e., xylan, galac-tan, and mannan, decreased as the pretreatment time increased. The reduction of hemicellulosic sugars in Elm wood was more pronounced than that in Cedar wood, indicating that the applied pretreatment was more effective to the hardwood than the softwood. Lignin content of the samples increased as pretreatment time increased. The increase in lignin content was higher in the case of Cedar wood than in Elm wood (44.5% com-pared to 36.8% for Cedar wood and Elm wood, respectively). This higher increase in lignin con-tent was probably due to the higher reduction in hemicellulose sugars. The ash content increased by 52% in the pretreatment of Cedar wood and Elm wood (Table 2).

Table 2. Chemical composition of untreated woods as well as woods pretreated with 85% phosphoric acid at 60 °C for 1, 2, and 3 h. Data are mean ± SD of three replicates.

Pretreatment conditions				Chemical composition					
Wood	T (°C)	t (h)	(%) Acid	(%) Glucan	(%) Xylan	(%) Galactan	(%) Mannan	(%) Lignin	(%) Ash
Cedar	Untreated	-	-	47.4±0.2	9.5±0.3	2.1±0.1	11.2±0.5	30.1±0.3	0.41
Cedar	60	1	85	54.7±0.1	4.0±0.2	0.8±0.3	2.4±0.2	37.7±0.2	0.44
Cedar	60	2	85	58.6±0.4	1.4±0.1	ND	ND	40.9±0.4	0.53
Cedar	60	3	85	55.8±0.2	ND	ND	ND	43.5±0.4	0.62
Elm	Untreated	-	-	49.3±0.3	9.4±0.1	1.4±0.2	1.1±0.2	26.6±0.3	0.42
Elm	60	1	85	57.6±0.1	4.8±0.4	ND	ND	27.4±0.2	0.56
Elm	60	2	85	56.2±0.3	1.1±0.2	ND	ND	32.3±0.1	0.60
Elm	60	3	85	51.4±0.2	0.9±0.2	ND	ND	36.4±0.2	0.64

ND= not detected

3.3 Enzymatic hydrolysis

Figure 1 illustrates the total sugar content of the hydrolysate obtained from enzymatic hydrolysis of the pretreated and untreated Cedar and Elm woods using dilute and concentrated phos-phoric acid. It can be clearly seen that the pre-treatments considerably enhanced the carbohy-drate hydrolysis of both woods.

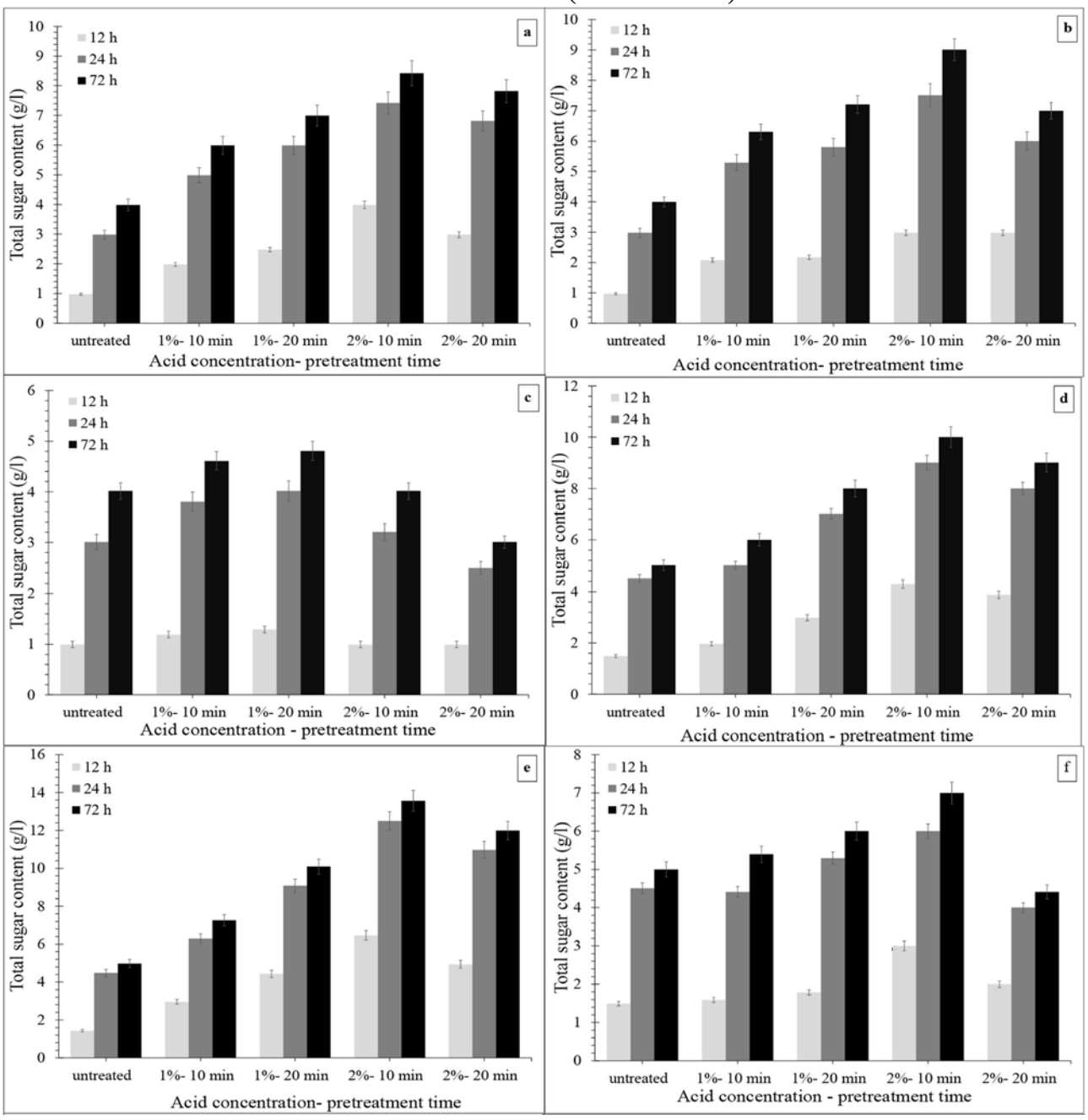
Moreover, Figure 1 shows that the time of hydrolysis had significant effect on the total sugar obtained. In all experiments, higher total sugar was detected at 24 and 72 h compared to that at 12 h. For example, total sugar content was 3.9, 7.4, and 8.4 g/l after 12, 24, and 72 h in the pretreatment of Cedar wood at 140 °C with 2% acid for 10 min. Therefore, it was recommended not to stop the hydrolysis before 24 h.

Comparison of Figure 1 a, b, and c pointed out that total sugar content obtained after the pretreatment at 180 °C was less than those at 140 and 160 °C. The highest total sugar content of 4.8 g/l was achieved after the pretreatment of cedar wood at 180 °C with 1% acid for 20 min, while 8.4 and 9.0 g/l total sugar content was observed after the pretreatment with 2% acid for 10 min at 140 and 160 °C, respectively. In other words, the increase in temperature from 140 to 160 °C enhances the accessibility of the enzyme to the cellulose, whereas further increase of temperature to 180 °C had a negative effect on the hydrolysis. This may be because the amount of cellulose retained in the wood material at elevated temperatures is less than that at lower temperatures. This effect has been previously reported (Nguyen et al., 1998; Noparat et al., 2015).

In the pretreatment of Elm wood, the effect of temperature on total sugar content was also similar to the effect observed in the pretreatment of Cedar wood. The highest total sugar content at 140, 160, and 180 °C were 10.0, 13.6, and 7.1 g/l, respectively, indicating that 160 °C was the opti-mum temperature in terms of total sugar content.

It was observed that at 160 °C, total sugar content decreased when the acid concentration and pretreatment time were increased to 2% and 20 min, respectively (Figure 1b). However, in the pretreatment of Cedar wood at 180 °C, this reduc-tion in total sugar content was observed when the pretreatment time was 10 min and 2% acid was used (Figure 1c). This may be due to

the fact that at 180 °C the cellulose has been released from the lignocellulosic biomass during pretreatment since the recalcitrant structure has been removed. After pretreatment at 140 °C, the maximum total sugar content achieved in the pretreatment of Elm wood (10.0 g/l) was higher than that of Cedar wood (8.4 g/l). This may be due to the more recalcitrant structure of softwood Cedar compared with hardwood Elm. The difficulty in the bioconversion of softwoods compared with hardwoods has been previously reported (Palonen et al., 2004). Figure 1g and 1h present the total sugar content obtained after the pre-treatments with 85% phosphoric acid. As shown in these figures, the prolongation of pretreatment to more than 1 h led to a reduction in sugar production. The total sugar content obtained from the pretreated Elm wood (22.1 g/l) was higher than the value obtained in the pretreatment of Cedar wood (20.0 g/l). The maximum total sugar content of 22.1 g/l was observed after the pre-treatment with 85% acid, whereas the maximum was 13.6 g/l after the pretreatment with dilute acid. This pointed out that the pretreatment with concentrated acid (85%) at 60 °C was more effective than diluteacidpretreatmentatelevated temperatures (140 to 180 °C).



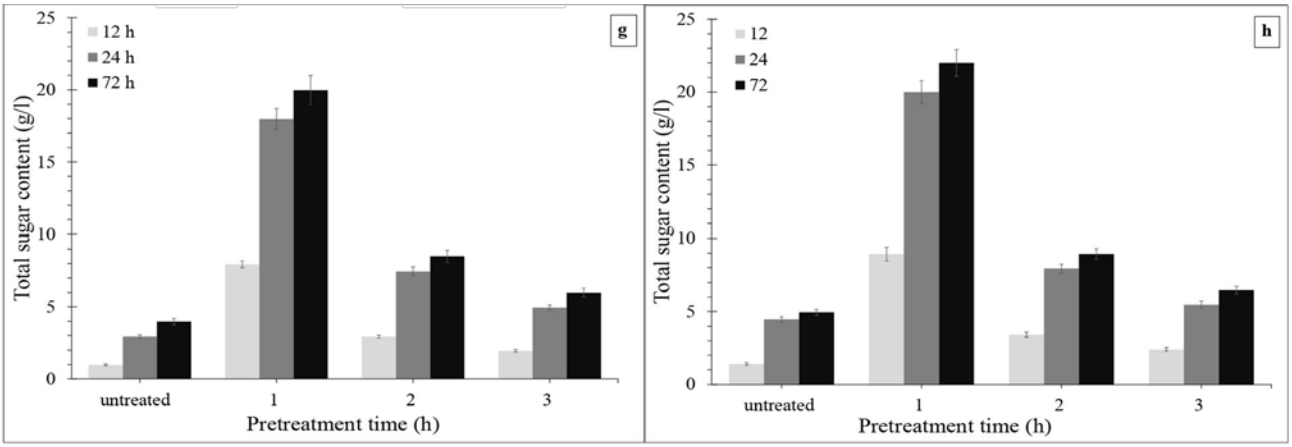


Figure 1. Total sugar content released by hydrolysis of Cedar wood and Elm wood pretreated with diluted and concentrated phosphoric acid. a) pretreatment of Cedar wood with dilute phosphoric acid at 140 °C, b) pretreatment of Cedar wood with dilute phosphoric acid at 160 °C, c) pretreatment of Cedar wood with dilute phosphoric acid at 180 °C, d) pretreatment of Elm wood with dilute phosphoric acid at 140 °C, e) pretreatment of Elm wood with dilute phosphoric acid at 160 °C, f) pretreatment of Elm wood with dilute phosphoric acid at 180 °C, g) pretreatment of Cedar wood with 85% phosphoric acid at 60°C, and h) pretreatment of Elm wood with 85% phosphoric acid at 60°C. Data are mean ± SD of three replicates.

Figure 2a show the glucose yields calculated for all pretreatments with 85% phosphoric acid. Figure 2b presents the yields for the pretreatments resulting in maximum total sugar content at each constant temperature (2%, 10 min at 140 and 160 °C; 1% and 20 min at 180 °C for Cedar wood; 2% and 10 min at 140, 160, and 180 °C for Elm wood). Glucose yields depicted in this figure are in accordance with the total sugar content shown in Figure 1. Similar to the total sugar content, the maximum glucose yield of 89% was obtained after the pretreatment of Elm wood with concentrated phosphoric acid. This yield was higher than the maximum glucose yield observed after the pretreatment of Cedar wood (78%). After the dilute acid pretreatment at 160 °C, the maximum glucose yield was 61 and 39% for Elm wood and Cedar wood, respec-tively. The glucose yields calculated in pretreatments with 85% phosphoric acid for 1 and 2h as well as the glucose yields obtained from the pretreatments with 2% phosphoric acid for 10 min at 140 and 160 °C were higher than the glucose yields obtained from the untreated woods (Figure 2).

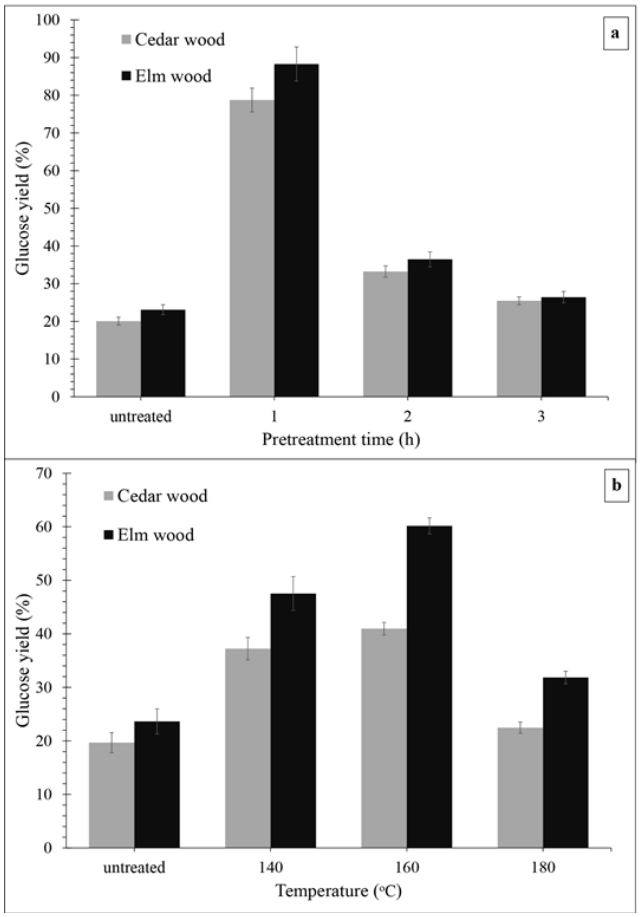


Figure 2. Glucose yield obtained after the pretreatment of Cedar wood and Elm wood using dilute and concentrated phosphoric acid. (a) Pretreatment with 85% phosphoric acid at 60°C and (b) pretreatment with dilute acid (2%, 10 min at 140 and 160 °C; 1% and 20 min at 180 °C for Cedar wood; 2% and 10 min at 140, 160, and 180 °C for Elm wood). Data are mean ± SD of three replicates.

3.4. Scanning electron microscopy

SEM images were captured to investigate the effects of pretreatment on the morphology and surface characteristics of the woods (Figure 3). This analysis was obtained for the untreated Elm wood and Cedar wood as well as the woods pretreated with 85% acid for 1 h at 60° C, from which the highest sugar yields were obtained. Comparison of the pretreated and untreated samples (Figure 3a with 3b; Figure 3c with 3d) showed that the pretreated samples had a more porous structure with comparatively large pores. In other words, the compact and recalcitrant structure of the untreated samples were signifi-cantly opened up and disordered.

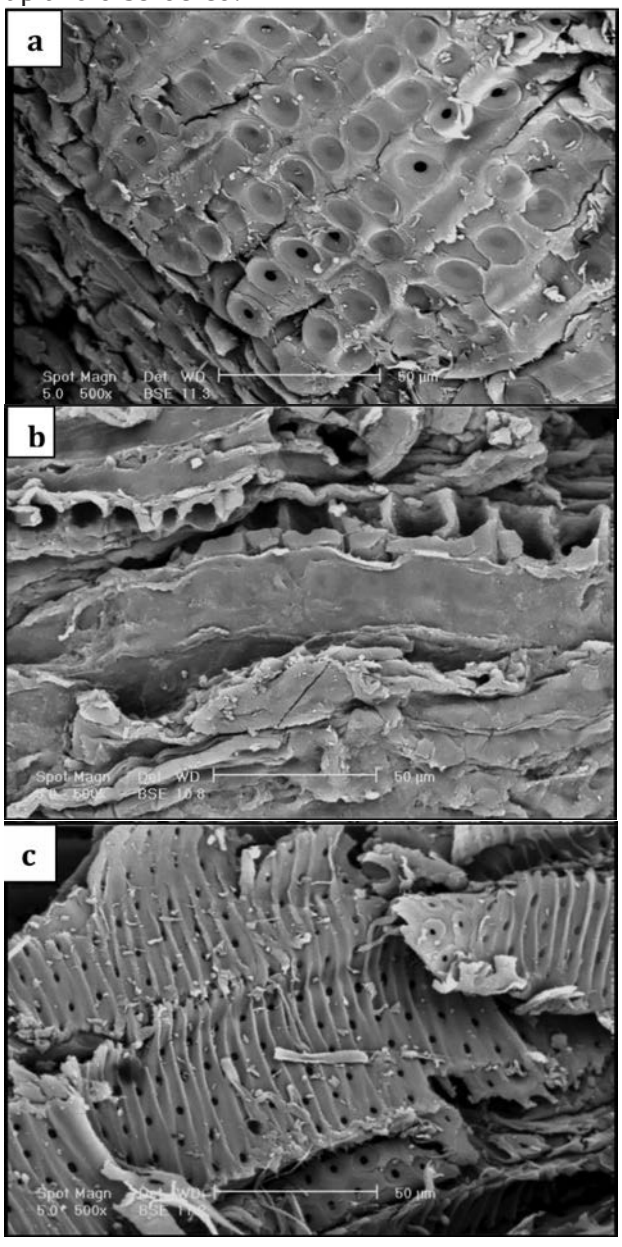


Figure 3. SEM image (1000×magnification) of (a) untreated Cedar wood, (b) pretreated Cedar wood using 85% phosphoric acid at 60 °C for 1 h, (c) untreated Elm wood, and (d) pretreated Elm wood using 85% phosphoric acid at 60 °C for 1 h.

3.5 FTIR analysis

The crystallinity of the pretreated and untreat-ed woods was compared using FTIR analysis (Figure 4 a and b). Figure 4a shows the spectra of the untreated Cedar wood and the pretreated Cedar wood with 85% phosphoric acid at 60°C for 1 h. The crystallinity index was calculated to be 0.68 and 0.56 for untreated and pretreated samples, respectively. Total crystallinity index was determined to be 2.24 and 2.08. The reduc-tion in crystallinity index reflects the reduction in cellulose type I, which is highly resistant to hydrolysis. Hence, the indexes indicated that the pretreatment was able to decrease the crystallinity, and therefore provided more accessible sites to hydrolyzing enzymes. The crystallinity and total crystallinity indexes for Elm wood were 0.70 and 4.75, respectively, which decreased to 0.67 and 2.64 after the pretreatment (Figure 4 b). However, the reduction in total crystallin-ity index in Elm wood (44.4%) was greater than that of Cedar wood (7.1%). This means that the pretreatment created more accessible sites for hydrolytic enzymes in Elm wood compared to Cedar wood. This was also in accordance with the higher total sugar content (Figure 1h) and glucose yield (Figure 2a) as well as lower lig-nin content (Table 2) obtained after the pretreatments of Elm wood.

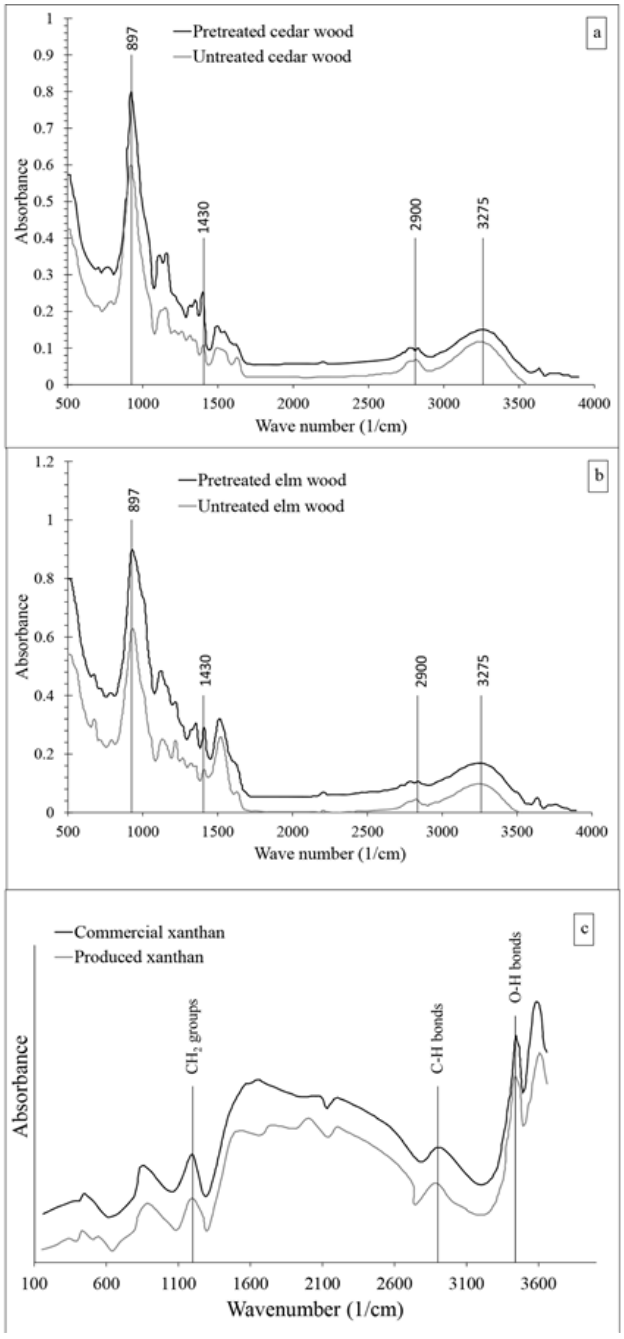


Figure 4. The FTIR spectrum of (a) untreated Cedar wood as well as pretreated Cedar wood with 85% phosphoric acid at 60 °C for 1 h, (b) untreated and pretreated Elm wood using 85% phosphoric acid at 60 °C for 1 h, and (c) the produced xanthan gum from Elm wood pretreated with 85% phosphoric acid at 60 °C for 1 h as well as the commercial xanthan gum.

3.6. Xanthan gum production and qualification

Figure 5 shows the xanthan gum yield obtained from untreated and pretreated Cedar wood and Elm wood. This yield was calculated for all samples pretreated with 85% phosphoric acid and the samples pretreated with dilute acid which resulted in maximum total sugar content (2%, 10 min at 140 and 160 °C; 1% and 20 min at 180 °C for Cedar wood; 2% and 10 min at 140, 160, and 180 °C for Elm wood). It was observed that the xanthan gum yield was high for pretreated samples with the high glucose yield. This is due to the availability of more substrate. The maximum xanthan gum yield of 5.2 g xanthan gum per 100 g of raw Elm wood was attained after dilute acid pretreatment at 160 °C. In pretreatments with 85% phosphoric acid, the maximum xanthan gum yield of 10.1 g xanthan gum per 100 g of Elm wood was observed after 1h pretreatment. Similar to the results observed in total sugar content and glucose yield, the maximum xanthan gum yield obtained from Cedar wood after the pretreatment with 85% phosphoric acid (9.9 g xanthan gum per 100 g of raw Cedar wood). This yield was higher than the maximum yield obtained after the pretreatment with dilute acid (4.3 g xanthan gum per 100 g). The maximum xanthan gum yields attained by the researchers from molasses, cheese whey, palm date, and waste dates were 30, 15.8, 51.1, and 13.4 g xanthan gum per 100 g of raw material, respectively (Kalogiannis et al., 2003; Moshaf et al., 2011; Salah et al., 2010; Silva et al., 2009). The maximum xanthan gum yield from rice straw, a lignocellulosic biomass, after pre-treatment with 2 M NaOH was reported as 10.1 g xanthan gum per 100 g of raw material (Jazini et al., 2017). Thus, the maximum yield obtained in this study was lower than the yields obtained from non-lignocellulosic sources, while it was close to the value obtained from rice straw (Jazini et al., 2017). The highest yield of xanthan gum obtained from Cedar wood in the pretreatment with 85% phosphoric acid was very close to that of Elm wood (9.9±0.2 g compared to 10.1±0.2 g xanthan gum per 100 g for Cedar wood and Elm wood, respectively). The maximum xanthan gum yield obtained in concentrated phosphoric acid pretreatments was two times

higher than the maximum yield achieved in dilute acid pre-treatment (10.4 compared to 5.2 g xanthan gum per 100 g of raw Elm wood).

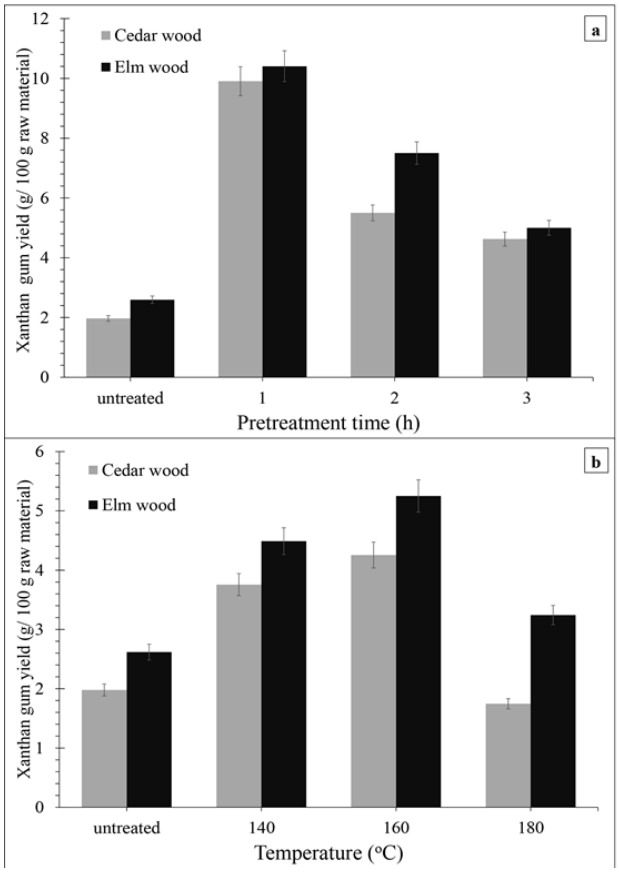
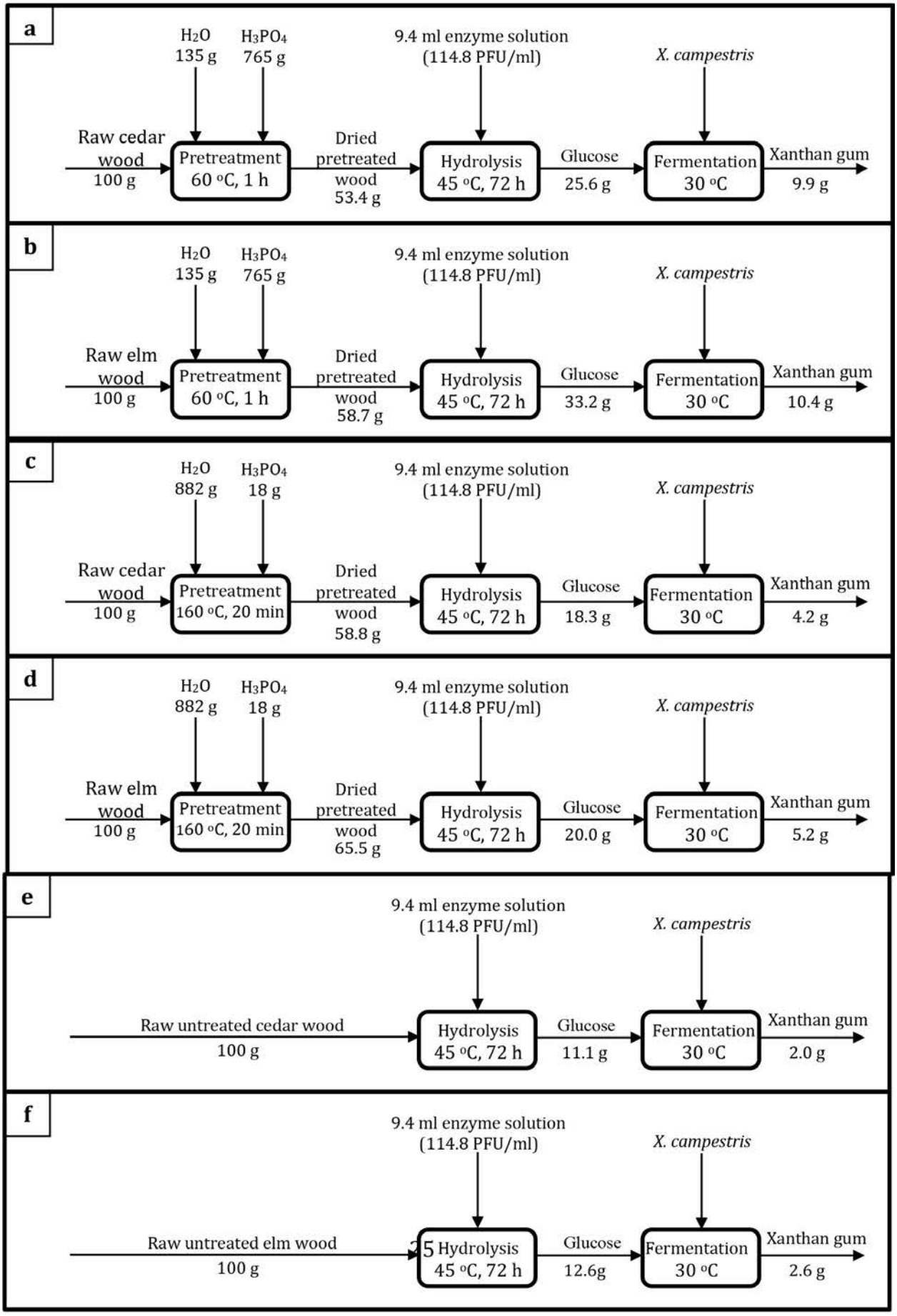


Figure 5. Xanthan gum yield obtained from (a) Cedar and Elm woods pretreated with 85% phosphoric acid at 60 °C for 1-3 h and (b) Cedar wood and Elm wood pretreated with dilute phos-phoric acid (2%, 10 min at 140 and 160 °C; 1% and 20 min at 180 °C for Cedar wood; 2% and 10 min at 140, 160, and 180 °C for Elm wood). Data are mean ± SD of three replicates.

Figure 6 shows the overall input-output diagram for all the pretreatment methods based on 100 g raw wood. In this figure, the amounts of pretreated wood that were obtained from the pretreatment, the amounts of glucose that were released from the pretreated wood, and the amount of xanthan gum that was produced via fermentation are presented. For example, 53.4 g dried pretreated wood was obtained from 100 g raw Cedar wood (Table 1). The pretreated wood contained 54.7% glucan (Table 2) that was con-verted to 25.6 g glucose (78.7% glucose yield, Figure 2). An amount of 9.9 g xanthan gum was resulted by the fermentation of 25.6 g glucose (Figure 5). It can be seen that the xanthan gum yields obtained from the pretreatments with concentrated acid (Figure 6, a and b) were about twice those of dilute acid pretreatment (Figure 6, c and d). Xanthan gum yields in the pretreatments of woods with dilute acid (Figure 6, c and d) were about double those for untreated woods (Figure 6, e and f). This revealed that it was possible to reach about a fourfold increase in xanthan gum yield compared to untreated woods while eliminating the severe pretreatment temperatures (140 to 180 °C).

FTIR technique was used to detect the differences and similarities in the chemical structure between produced xanthan gum and the commercial xanthan gum. Figure 4c depicts the spectra of xanthan gum produced from the sample pretreated with 85% acid at 60 °C for 1 h and commercial xanthan gum. The absorbance at wave numbers around 3400 cm^{-1}, 2939 cm^{-1}, and 1200 cm^{-1} represent O–H bonds, C–H bonds of CH_2 groups, and saccha-rides, respectively (Faria et al., 2011). The observed absorbance at 3400 cm^{-1} for produced and commercial xanthan gum were 0.1948 and 0.1795, respectively. The detected values at 2939 cm^{-1} were 0.3087 and 0.3416, and those at 1200 cm^{-1} were 0.1401 and 0.0941 for produced and commercial xanthan gum, respectively. The close proximity of the absorbance observed at characteristic wave numbers indicated that the produced xanthan gum had very similar characteristics to those of the commercial product.

Figure 6. Overall input-output diagram for different pretreatment of Cedar wood, Elm wood, and untreated wood for pro-duction of xanthan gum. (a) Cedar wood pretreated with 85% phosphoric acid at 60 °C for 1h, (b) Elm wood pretreated with 85% phosphoric acid at 60 °C for 1h, (c) Cedar wood pretreated with 2% phosphoric acid at 160 °C for 20 min, (d) Elm wood pretreated with 2% phosphoric acid at 160 °C for 20 min, (e) untreated Cedar wood, and (f) untreated Elm wood. (see next page)


a
H2O 135 g
H3PO4 765 g
9.4 ml enzyme solution (114.8 PFU/ml)
X. campestris
Raw cedar wood 100 g
Pretreatment 60 °C, 1 h
Dried pretreated wood 53.4 g
Hydrolysis 45 °C, 72 h
Glucose 25.6 g
Fermentation 30 °C
Xanthan gum 9.9 g
b
H2O 135 g
H3PO4 765 g
9.4 ml enzyme solution (114.8 PFU/ml)
X. campestris
Raw elm wood 100 g
Pretreatment 60 °C, 1 h
Dried pretreated wood 58.7 g
Hydrolysis 45 °C, 72 h
Glucose 33.2 g
Fermentation 30 °C
Xanthan gum 10.4 g
c
H2O 882 g
H3PO4 18 g
9.4 ml enzyme solution (114.8 PFU/ml)
X. campestris
Raw cedar wood 100 g
Pretreatment 160 °C, 20 min
Dried pretreated wood 58.8 g
Hydrolysis 45 °C, 72 h
Glucose 18.3 g
Fermentation 30 °C
Xanthan gum 4.2 g
d
H2O 882 g
H3PO4 18 g
9.4 ml enzyme solution (114.8 PFU/ml)
X. campestris
Raw elm wood 100 g
Pretreatment 160 °C, 20 min
Dried pretreated wood 65.5 g
Hydrolysis 45 °C, 72 h
Glucose 20.0 g
Fermentation 30 °C
Xanthan gum 5.2 g
e
9.4 ml enzyme solution (114.8 PFU/ml)
X. campestris
Raw untreated cedar wood 100 g
Hydrolysis 45 °C, 72 h
Glucose 11.1 g
Fermentation 30 °C
Xanthan gum 2.0 g
f
9.4 ml enzyme solution (114.8 PFU/ml)
X. campestris
Raw untreated elm wood 100 g
Hydrolysis 45 °C, 72 h
Glucose 12.6g
Fermentation 30 °C
Xanthan gum 2.6 g

4. Conclusion

A huge amount of waste Cedar wood and Elm wood are produced in Iran. There should be a method for bioconversion of these wastes to value-added products. Cedar wood and Elm wood, as lignocellulosic biomass, cannot be used directly for efficient microbial xanthan gum production because of their recalcitrant structure. Therefore, they should be pretreated to make cellulose accessible to hydrolyzing enzymes. Phosphoric acid pretreatment of Cedar wood and Elm wood was able to disrupt the structure of such lignocellulosic biomass. Application of high concentration of acid (85%) at 60 °C resulted in at least an approximate twofold increase in xanthan gum yield compared to the application of dilute acid (1-2%) at elevated tem-peratures (140-180 °C). In the pretreatment with 85% acid, Elm wood, a hardwood, yielded about 10% more xanthan gum compared to Cedar wood, a softwood. The maximum xanthan gum yield in pretreatments with 85% acid was 10.4 g xanthan gum per 100 g of raw Elm wood, while the maximum yield obtained in dilute acid pretreatments was 5.2 g xanthan gum per 100 g of raw Elm wood. From all pretreated woods, the xanthan gum yield obtained from Elm wood was higher than the yield from Cedar wood. The quality of the produced gum was similar to the commercial product. Although the pretreatments presented in this study increased the xanthan gum yield up to fourfold, it was at the expense of increasing pretreatment costs. This research provides the basis for the economic analysis required for the commercial implementation of these pretreatments.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

The financial support provided for this project by Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, is gratefully acknowledged. The authors also appreciate Novozymes, Denmark, for providing Cellic® HTec2 and Cellic® CTec2 enzymes for this study.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Open access

References

[1] Adney, B., & Baker, J. (1996). Measurement of cellulase activities. Laboratory Analytical Procedure (LAP), 6, 19-27.

[2] Amiri, H., Karimi, K., & Roodpeyma, S. (2010). Production of furans from rice straw by single-phase and biphasic systems. Carbohydrate Research, 345(15), 2133-2138.

[3] Beheshti, H., & Karimi, K. (2016). Ethanol and value-added byproducts from rice straw by dimorphic fungus *mucor hiemalis*. Engineering in Life Sciences, 16(8), 750-761. doi:doi:10.1002/elsc.201600023

[4] de Vasconcelos, S. M., Santos, A. M. P., Rocha, G. J. M., & Souto-Maior, A. M. (2013). Diluted phosphoric acid pretreatment for production of fermentable sugars in a sugarcane-based biorefinery. Bioresource technology, 135, 46-52.

[5] Faria, S., de Oliveira Petkowicz, C. L., de Morais, S. A. L., Terrones, M. G. H., de Resende, M. M., de França, F. P., & Cardoso, V. L. (2011). Characterization of xanthan gum produced from sugar cane broth. Carbohydrate polymers, 86(2), 469-476.

[6] García-Ochoa, F., Santos, V. E., Casas, J. A., & Gómez, E. (2000). Xanthan gum: Production, recovery, and properties. Biotechnology advances, 18(7), 549-579.

[7] Gusakov, A. V., Kondratyeva, E. G., & Sinitsyn, A. P. (2011). Comparison of two methods for assaying reduc-

ing sugars in the determination of carbohydrase activities. International Journal of Environmental Analytical Chemistry, 2011, 4-9.

[8] Hashemi, S. S., Karimi, K., Nosratpour, M. J., & Sárvári Horváth, I. (2016). Efficient biogas and ethanol production from safflower straw using sodium carbonate pretreatment. Energy Fuels, 30(12), 10592-10601.

[9] Jazini, M., Fereydouni, E., & Karimi, K. (2017). Microbial xanthan gum production from alkali-pretreated rice straw. RSC Advances, 7(6), 3507-3514.

[10] Kalogiannis, S., Iakovidou, G., Liakopoulou-Kyriakides, M., Kyriakidis, D. A., & Skaracis, G. N. (2003). Optimization of xanthan gum production by *xanthomonas campestris* grown in molasses. Process Biochemistry, 39(2), 249-256.

[11] Martínez-Patiño, J. C., Lu-Chau, T. A., Gullón, B., Ruiz, E., Romero, I., Castro, E., & Lema, J. M. (2018). Application of a combined fungal and diluted acid pretreatment on olive tree biomass. Industrial Crops and Products, 121, 10-17.

[12] Martínez-Patiño, J. C., Romero-García, J. M., Ruiz, E., Oliva, J. M., Álvarez, C., Romero, I., ... Castro, E. (2015). High solids loading pretreatment of olive tree pruning with dilute phosphoric acid for bioethanol production by *escherichia coli*. Energy Fuels, 29(3), 1735-1742.

[13] Moshaf, S., Hamidi-Esfahani, Z., & Azizi, M. (2011). Optimization of conditions for xanthan gum production from waste date in submerged fermantation. World Academy of Science, Engineering and Technology, 57, 521-524.

[14] Nair, R., Lundin, M., Lennartsson, P. R., & Taherzadeh, M. J. (2017). Optimizing dilute phosphoric acid pretreatment of wheat straw in the laboratory and in a demonstration plant for ethanol and edible fungal biomass production using *neurospora intermedia*. Journal of Chemical Technology & Biotechnology, 92(6), 1256-1265. doi:doi:10.1002/jctb.5119

[15] Nasir, I. M., & Ghazi, T. I. M. (2015). Pretreatment of lignocellulosic biomass from animal manure as a means of enhancing biogas production. Engineering in Life Sciences, 15(7), 733-742.

[16] Nguyen, Q., Tucker, M., Boynton, B., Keller, F., & Schell, D. (1998). Dilute acid pretreatment of softwoods In M. Finkelstein & H. Davison (Eds.), Biotechnology for fuels and chemicals. Springer, pp. 77-87.

[17] Nieves, D. C., Karimi, K., & Horváth, I. S. (2011). Improvement of biogas production from oil palm empty fruit bunches (opefb). Industrial Crops and Products, 34(1), 1097-1101.

[18] Niknezhad, S., Asadollahi, M., Zamani, A., & Biria, D. (2014). Production of xanthan gum by *xanthomonas* species using starch as carbon source. Minerva Biotecnologica, 26(3), 191-197.

[19] Noori, M. S., & Karimi, K. (2016). Chemical and structural analysis of alkali pretreated pinewood for efficient ethanol production. RSC Advances, 6(70), 65683-65690.

[20] Noparat, P., Prasertsan, P., O-Thong, S., & Pan, X. (2015). Dilute acid pretreatment of oil palm trunk biomass at high temperature for enzymatic hydrolysis. Energy Procedia, 79, 924-929.

[21] Palonen, H., Thomsen, A. B., Tenkanen, M., Schmidt, A. S., & Viikari, L. (2004). Evaluation of wet oxidation pretreatment for enzymatic hydrolysis of softwood. Applied biochemistry and biotechnology, 117(1), 1-17.

[22] Poornejad, N., Karimi, K., & Behzad, T. (2013). Improvement of saccharification and ethanol production from rice straw by nmmo and [bmim][oac] pretreatments. Industrial Crops and Products, 41, 408-413.

[23] Salah, R. B., Chaari, K., Besbes, S., Ktari, N., Blecker, C., Deroanne, C., & Attia, H. (2010). Optimisation of xanthan gum production by palm date (*phoenix dactylifera l.*) juice by-products using response surface methodology. Food Chemistry, 121(2), 627-633.

[24] Silva, M. F., Fornari, R. C., Mazutti, M. A., de Oliveira, D., Padilha, F. F., Cichoski, A. J., ... Treichel, H. (2009). Production and characterization of xantham gum by *xanthomonas campestris* using cheese whey as sole carbon source. Journal of Food Engineering, 90(1), 119-123.

[25] Yao, F., Tian, D., Shen, F., Hu, J., Zeng, Y., Yang, G.,... Zhang, J. (2019). Recycling solvent system in phosphoric acid plus hydrogen peroxide pretreatment towards a more sustainable lignocellulose biorefinery for bioethanol. Bioresource technology, 275, 19-26.