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Research Note

Enzymatic Saccharification of Poplar Wood

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In this paper, enzymatic saccharification of poplar wood has been studied. Poplar wood samples were subjected to chemical processing (acidic delignification and swelling by ammonium hydroxide) under selected operational conditions. By removal of lignin along with recovery of cellulose both at the level of 80%, solid residues were obtained that were, then, used as the substrate for enzymatic hydrolysis using a mixture of the following two enzymes, cellulose and cellobiase. Increase in the swelling capacity of the cellulose substrate along with decrease of the polymerization degree of lignocellulosic materials facilitate the action of cellulases enzymes. Considering selected operational conditions in the enzymatic saccharification of the wood residues, glucose was produced at the level of 30 g/l. The experimental results were fitted to the hyperbolic empirical model. There was a close relationship between the experimental and the calculated results in some of the enzymatic treatments.

INTRODUCTION

There have been great interests in recent years for finding efficient ways to utilize lignocellulosic materials (LCM) as the raw material for production of fuels, chemicals and foods [1-3]. Renewable nature, high availability and low price are main reasons for using LCM as the starting material for the bioconversion to sugar [4]. Among the lignocellulosic materials, wood is considered to be the largest resources. On the basis of chemical constituents of the cell wall, the wood system can be classified into soft- and hard- wood [2,3]. The cell wall composition of these woods are significantly different:

- The lignin content of softwoods is generally higher than that of hardwoods,
- The cellulose content of hardwoods is generally higher than that of softwoods,
- The hemicellulose content of hardwoods is rather similar to that of softwoods [2].

Moreover, poplar as the main genus of trees of the willow family has some favorable characters such as rapid growth and ability to grow in semi-dry regions. Poplar is among popular trees in Iran and widely used in the manufacturing of variety items in the related industries [5-7]. Therefore, its production and existence in Iran could be evaluated as following a good trend. However, the present crops and trees in the world have not been optimized for production of digestible cellulose and alternative crops, trees etc. for this purpose may be developed in the future [8]. Meanwhile, there is a great need for provision of data for local lignocellulosic materials to be compared and evaluated as the possible raw material for the enzymatic hydrolysis (action of cellulases) and production of sugars.

Presence of a lignin-hemicellulose shield around the cellulose fraction in plant cell wall has made the LCM highly resistant to the enzymatic depolymerization [1,3]. The complex structure of these plant constituents is thought to confer a wide range of biophysical and biomechanical properties on the plant tissues, as well as on product(s) made from these tissues [1,9]. Performing a proper treatment prior to the enzymatic hydrolysis is, therefore, needed to facilitate the accessibility of cellulose and enhance the action of the hydrolytic enzyme(s) on this substrate [4,10]. Enzymes or biocatalysts are biodegradable compounds and, therefore, harmless to the environment [4]. Variety of physicochemical, mechanical and biological processes have been used as pretreatment step for efficient utilization of LCM [11,12]. In order to have a successful pretreatment, one should consider several factors in detail, such as maximizing the removal of

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lignin, minimizing the carbohydrate loss, etc. [13]. Moreover, an efficient way of performing a pretreatment is reported to be a two-step process:

1. Separation and removal of lignin-hemicellulose layer,
2. Reduction of crystallinity degree of cellulose fraction and extending the amorphous areas [14,15].

The objective of this research is to study and optimize some of the operational variables in the acidic pretreatment of poplar wood chips followed by the swelling treatment using ammonia. The wood samples obtained from the delignification and swelling processes were used as the substrate for the enzymatic hydrolysis (digestible cellulose). Several selected operational variables in the enzymatic saccharification process were, then, evaluated.

MATERIALS AND METHODS

Wood as the Raw Material

Poplar wood, purchased from a local market, was milled and after screening, the fraction of particles with appropriate size were selected and homogenized to make sure that particles have proper size (250-425 μm) and identical composition [5].

Wood Compositional Analysis

The extraneous component of LCM refers to all the non-cell wall materials and consists of a wide variety of chemicals [2]. Extractive content of the wood sample in the organic solvent was determined according to the standard procedure (details concerning the analytical techniques are described in [5]). Upon preparing the wood sample, which was free from extractive materials, a solid residue was obtained and lignin and cellulose contents were measured using H_2SO_4 and HNO_3, respectively [5]. The composition of the poplar wood (on a dry basis) used in the experiments was estimated to be about 51.9% cellulose, 25% hemicellulose and 23.1% lignin.

Delignification

Milled, screened and homogenized wood samples were delignified with acetic acid-HCl-water media according to the predetermined conditions [5,10]. Operational variables used in the delignification were as below: temperature of heating, 110° and 130°C; time of heating, $\frac{1}{2}$, 1 and $1\frac{1}{2}$ hour(s); ratio of liquid to solid, 8 and 10; and catalytic level of HCl was 0.2 and 0.45% (Table 1). The reaction was performed in a batch system [5]. The delignification reaction was conducted in an autoclave and at the end of heating time, the solid residue was separated by filteration, washed with water and air-dried. Aliquots from the solid residue were analyzed for cellulose, hemicellulose and lignin using the same procedure described above for untreated wood samples.

Swelling Pretreatments

Delignified samples were treated with NH_4OH solution at 60°C for 3 hours using a liquid/solid ratio of 10 ml/g. At the end of swelling treatments, using appropriate filter units, the solid residues were separated, washed with water and air-dried. Aliquots of the samples obtained in this way were subjected to the chemical analysis outlined above and the composition of the solid residue from the wood samples was determined [5].

Enzymatic Hydrolysis

Saccharification of the pretreated samples was performed using Trichoderma reesi cellulases (CE) (Celluclast, Novo, Denmark) and Aspergillus niger β-glucosidase (cellobiase, CB) (Novozyme, Novo, Denmark). The extracellular cellulose is deficient in β-glucosidase, therefore, a mixture of these two enzymes were used. Activities of these commercial enzyme solutions were determined using usual standard methods [5,16]. The operational conditions used for the enzymatic hydrolysis are listed in Table 2. Sodium azide (NaN_3) was also added to the reaction mixture, as an antimicrobial agent. At appropriate time intervals, the hydrolyzed samples were withdrawn from the reaction mixture and boiled for 15 minutes in caped test tubes to deactivate the enzyme and terminate the reaction. After centrifugation at 3000 rpm for 5 minutes, the supernatant was filtered using 0.45 μm membranes and the resulting samples were analyzed for reducing sugars

Table 1. Operational conditions used for the delignification process of poplar wood.

Operational Condition		
Catalytic level of HCl used (CA, %)		0.2; 0.45
Heating in autoclave	Duration (h),	1
	Temperature (T°C)	110; 130
Liquid:solid (wood) ratio (ml/g)		8; 10

Table 2. Operational conditions used for the enzymatic hydrolysis of the chemically processed poplar wood.

Operational Condition	
pH	4.85
Cellulase level (CE) (FPU/g)	3; 6; 13
Cellobiase level (CB) (IU/g)	80; 169
Temperature (°C)	48.5
Liquid; solid (wood) (L/S) ratio (ml/g)	8:1; 12:1; 20:1
Duration of the enzymatic hydrolysis (h)	56

(glucose) by the glucose oxidase-peroxidase method [5,16].

The sugar concentration-time series of the data obtained in the saccharification reactions were determined and the results were fitted to the hyperbolic (empirical) model of cellulose hydrolysis:

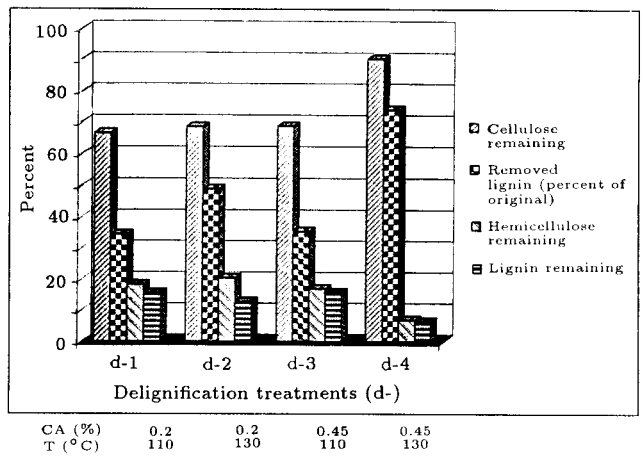
$$SC = y_1 \frac{t}{t + t_{1/2}}, \quad (1)$$

where SC is the sugar concentration obtained at time t, y_1 is the sugar concentration that should be reached at infinite reaction time and $t_{1/2}$ is the time to reach 50% of y_1 [10,12,17]. A commercial software (Excel 97) was used for the evaluation of the data.

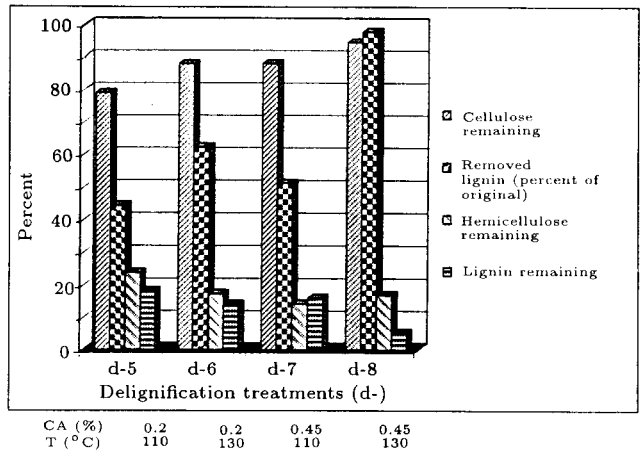
RESULTS AND DISCUSSION

Chemical Treatment of the Wood Samples

The chemical processing of several wood samples for improving their potential as substrate for the enzymatic hydrolysis has been studied rather extensively. One of the popular treatments is conducting the processes in two steps: delignification in an acidic medium followed by swelling by alkali [2,3]. On the basis of the results of several studies reported in the literature, the operational conditions, listed in Table 1, are selected for the delignification of the poplar wood [5]. Effects of heating temperature (110° and 130°C for one hour) and the catalytic level of HCl (0.2 and 0.45%) in the delignification medium (acetic acid-HCl-H_2O) were studied at two levels of liquid/solid (wood) ratios (8:1 and 10:1). Although raising the temperature of heating process from 110° to 130°C at the catalytic level (HCl) of 0.2% had little effect on recovery of cellulose, the level of lignin removal increased from 34 to 48% (Figure 1a, operational conditions used to obtain data for this figure were the same as those described in Table 1, from which also the definition of symbol used can be found). Increasing the level of HCl catalyst from 0.2 to 0.45% while keeping the heating temperature at 110°C had no considerable effect on cellulose recovery as well as on the level of lignin removal (Figure 1a). However, increasing the



a) Liquid:solid ratio: 8:1



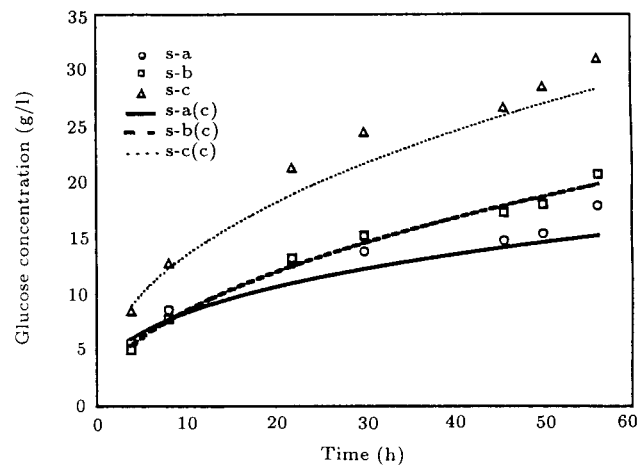
b) Liquid:solid ratio: 10:1

Figure 1. Chemical composition of solid (wood) residues obtained from the delignification treatments on poplar wood samples along with lignin removal- the values are calculated as the percent of original amount of each component in the sample before treatment.

catalytic level of HCl (from 0.2 to 0.45%) along with raising the heating temperature from 110° to 130°C improved both levels of cellulose recovery and lignin removal significantly (see Figure 1a). Therefore, one may conclude that when the poplar wood samples at

the liquid to solid ratio of 8:1 were subjected to this delignification process, HCl catalyst at the higher level (0.45%) and heating temperature at 130°C both act together and show mutual effect(s) on the recovery of cellulose and removal of lignin (delignification treatment (d-): d-4; Figure 1a). On both cellulose recovery and lignin removal, more improvements were observed when the liquid/solid ratio was set at the level of 10:1 (see Figure 1b). The data presented in Figure 1 (a and b) illustrate that performing the delignification process at 130°C for 1 hour at the liquid/solid ratio of 10 with 0.45% HCl catalytic level results in more than 80% recovery of cellulose and removal of lignin.

Delignified poplar wood sample was, then, subjected to the treatment with ammonium hydroxide under conditions specified previously. Table 3 presents the chemical composition of the solid (wood) residue obtained. It can be seen that little changes in the chemical composition were caused by the swelling treatment. Upon the chemical pretreatment of the poplar wood samples, hemicellulose content, for example, has changed from 25% to 14.21% for the delignified wood and to 12.97% for the delignified and swollen sample (Table 3). These data, which are considered as the average values, show that considerable decrease in the amount of hemicellulose along with significant reduction of lignin, both were accompanied with the recovery of cellulose fraction at the level of 80% or more (see Table 3). In the literature, discussions over the role(s) of hemicellulose on the enzymatic susceptibility of LCM are rather controversial [1,9]. For example, approximately 60% of xylose component (mainly pentose in hemicellulose fraction) in typical hardwood species have O-acetyl groups [1,3]. During acidic hydrolysis, the cleavage of the acetyl side chains occurs and the resulting acetic acid appears to promote the observed hydrolytic action [3]. On the other hand, results presented in some reports indicate that the presence of hemicellulose does not play an important role in susceptibility of cellulose toward the enzymatic attack [3]. Considering these facts, there will be many researches into finding an effective way for LCM conversion to useful chemicals, and hemicellulose has been said to have a key role in solving the problem [1]. Approaches taken toward the importance of relationship between lignin removal and the enzymatic hydrolysis of the cellulosic fraction of LCM is rather straight-forward [2,11,18]. In the present study, hemicellulose content of the poplar wood samples obtained from different delignification treatments did not show to change significantly (Figure 1). Although, increasing the level of lignin removal is accompanied with higher levels of cellulose recovery (Figure 1; d-4 and d-8). In the study conducted on Eucalyptus wood, when 90% of lignin along with 87% of hemicellulose were removed, great improvement on cellulose recovery was achieved (87 to 92% of the cellulosic fraction was recovered) [17]. The amount of total reducing sugars which were obtained upon the saccharification of the treated Eucalyptus wood was about 45 g/1 [17]. It appears that removal of hemicellulose along with lignin separation each at the level of 80% or higher could contribute to a noticeable change in the amount of reducing sugars obtained upon the enzymatic hydrolysis of the cellulose recovered (e.g., the level of glucose obtained in the present work (Figure 2) can be compared with the total reducing sugars produced from the Eucalyptus wood [17]). In



* the levels of the enzymes in the reaction mixtures were:
s-a; CE 13 FPU/g and CB 80 IU/g, and liquid:solid ratio, 8:1
s-b; CE 13 FPU/g and CB 169 IU/g, and liquid:solid ratio, 20:1
s-c; CE 13 FPU/g and CB 169 IU/g, and liquid:solid ratio, 12:1

Figure 2. Dependence of the concentration of glucose formed on the reaction time during the enzymatic saccharification-selected treatments (for definition of the symbols used in this figure see Table 2).

Table 3. Chemical composition of the solid (wood) residues obtained from the chemical processing.

Wood Sample	Content (Weight Percent, on a Dry Basis)		
	Lignin	Hemicellulose	Cellulose
Untreated wood	23.1	25	51.9
Delignified wood	4.16	14.21	81.2
Delignified + swollen wood*	4.05	12.97	82.97

* Operational conditions in the delignification process: temperature of heating 130°C; time of heating 1 hour; liquid/solid (wood) ratio 10:1; level of HCl catalyst 0.45%.

continuation process for use of LCM for fermentation reactions (for example ethanol production), it would be better to separate pentose sugars from the cellulosic fraction; possible conversion of hemicellulose fraction to pentose sugars during acidic-alkali pretreatments of LCM has been addressed in [1,9,15]. Most of the yeast strains are unable to use pentose sugars [9]; therefore, it is suggested that appropriate technology should be developed to separate hemicellulose sugars [1]. In the present study, no attampts have been made to separate hemicellulose from the delignified residues.

Enzymatic Hydrolysis of Chemically Treated Wood

The solid residues obtained from the chemical processing on the wood samples were used as the substrate for the hydrolytic reaction which was conducted in the media containing cellulose and cellobiase mixtures. The results from the enzymatic saccharification for liquid:solid (wood) ratio of 12:1 are presented in Figure 3. At the constant level of CE enzyme, increasing the level of CB enzyme from 80 to 169 IU/g had rather little improving effect on the amount of glucose produced while at the constant level of CB enzyme, increasing the level of CE enzyme from 3 to 13 FPU/g resulted in a significant increase in the glucose concentration (saccharification treatments (s-1, s-5; Figure 3). Similar trends for glucose production was observed when liquid:solid (wood) ratio was set at two different levels of 8:1 and 20:1; details and related figures could be found in [5]. Delignification and swelling of Eucalyptus wood were performed under similar operational conditions and at the end of the processing time, removal of lignin and recovery of the cellulosic fraction were at a 90% level [17]. The enzymatic susceptibility of the solid residues obtained

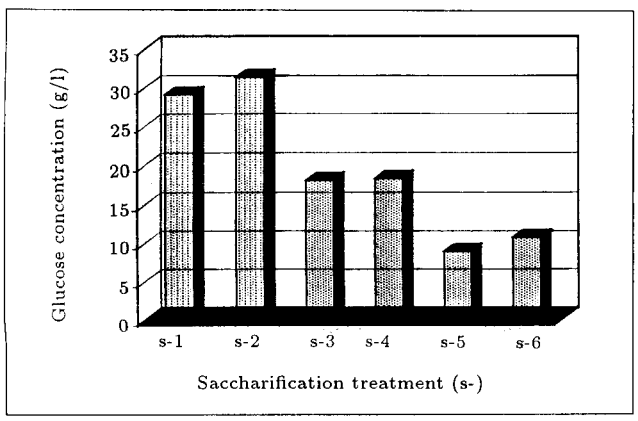


Figure 3. Enzymatic saccharification of the chemically delignified-swelled poplar wood samples. Liquid:solid (wood) ratio was set at 12:1, other operational conditions were same as those given in Table 1 (for definition of symbols used in this figure see Table 2.)

was high and using the mixtures of the enzymes (CE and CB) resulted in the production of total reducing sugars at the concentration of about 45 g/l [10,17].

In the present study, data from three selected enzyme treatments, corresponding to one of the three different ratios of liquid:solid in the reaction mixture of the saccharification process, were used to prepare Figure 2 as the representative plot. The representative sugar concentration-time plot also corresponds to the highest level of glucose formed at each of three ratios of L/S used to set the saccharification treatments (Figure 2). The related plots from 18 different saccharification treatments could be found in [5]. Raising the liquid:solid (wood) ratio from 8:1 to 12:1 caused the concentration of glucose to increase from 16 to 31.5 g/l; however, the glucose concentration decreased to 21 g/l as the level of the liquid to solid ratio increased to 20:1 (see Figure 2). At lower levels of liquid:solid ratio, the enzyme(s) might not have enough access to the wood as the substrate of the reaction; however, at higher levels of the L/S ratio, there might not be enough substrate (cellulose surfaces) available for the enzymatic reaction to proceed. The kinetics of the enzymatic hydrolysis of cellulose is a complex process and among various approaches regarding this matter, more emphasize has been placed on the adsorption of cellulase on the cellulose surface, a prerequisite for the hydrolysis [19-22]. After passing some initial stages and supersaturation of cellulose with cellulase molecules, little uptake of the enzymes occurs and the concentration of cellulase in the reaction mixture first remains constant and enventually increases, indicating that digestion of cellulose results in release of the adsorbed cellulase to the bulk solution (desorption of the enzyme) [19]. The released enzymes are, then, ready to approach the surface of the cellulose particles and start the catalytic action [19]. With this approach, one may interpret the results which are shown in Figure 2 (i.e., increase in the concentration of glucose in the reaction mixture after 40-45 hours could be due to the action of the released cellulase molecules from the cellulose surface to the bulk solution). In continuation of the matter, it would be better to also consider the model developed on the basis of shrinking particle theory and Langmuir isotherm concept, in which the activity of the cellulases adsorbed at the cellulose surface decreases with time and this is mainly due to the formation of complexes formed between substrate and inactive enzymes [23]. The shrinking particle theory has not been used in the present study. The hyperbolic empirical model used in this study (Equation 1) has been developed during the course of the organosolvent (alcohol/water/catalyst) pretreatment of poplar wood followed by the enzymatic hydrolysis [12]. In that study, the concentration of cellulose used was the same for all experiments and

the researchers were able to describe the dependence of the maximum amount of sugar obtained on the type of pretreatment experiment conducted [12]. The model was also used in the experiment with Eucalyptus wood [10,17]. Different chemical pretreatments and different enzyme concentrations were used, although the researchers described a statistical design to find a proper predictor (independent variable) for the variable to be modeled (amount of the sugar produced in each experiment) [10,17]. The hyperbolic model described in those experiments was used in the present study and it seems to provide relatively good predictions for the amount of sugar (glucose) produced during the saccharification process. It can be seen from Figure 2 that there is a close relationship between the experimental and calculated results. However, there is a definite need to optimize the operational variables all together, using an appropriate statistical model.

CONCLUSION

The chemical processing of the poplar wood samples in acetic acid-HCl media resulted in the separation of the main chemical fractions of the wood. Removal of lignin along with the recovery of cellulose, at levels above 80% were achieved at the optimal conditions; heating at 130°C for 1 hour at liquid/solid ratio of 10:1 and HCl catalytic level of 0.45%. As suggested in many studies, swelling treatment was also performed to overcome some unfavorable structural properties of the delignified materials (woods) such as high degree of crystallinity. Ammonium hydroxide was used as a swelling agent with the temperature and time of treatment being 60°C and 3 hours, respectively. The enzymatic saccharification was performed using the mixture of the two enzymes, cellulase and cellobiase, at three different liquid:solid (wood) ratios. The highest concentration of glucose obtained in this study was about 30 g/l. This value is comparable with the concentration of glucose obtained from other wood samples such as Eucalyputs reported in [10,17].

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