World Academy of Science, Engineering and Technology
International Journal of Materials and Metallurgical Engineering
Vol:5, No:4, 2011

Ionic Liquid Pretreatment and Enzymatic Hydrolysis of Wood Biomass

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Abstract**—**Pretreatment of lignocellulosic biomass materials from poplar, acacia, oak, and fir with different ionic liquids (ILs) containing 1-alkyl-3-methyl-imidazolium cations and various anions has been carried out. The dissolved cellulose from biomass was precipitated by adding anti-solvents into the solution and vigorous stirring. Commercial cellulases Celluclast 1.5L and Accelerase 1000 have been used for hydrolysis of untreated and pretreated lignocellulosic biomass. Among the tested ILs, [Emim]$COOCH_3$ showed the best efficiency, resulting in highest amount of liberated reducing sugars. Pretreatment of lignocellulosic biomass using glycerol-ionic liquids combined pretreatment and dilute acid-ionic liquids combined pretreatment were evaluated and compared with glycerol pretreatment, ionic liquids pretreatment and dilute acid pretreatment.

Keywords**—**Cellulase, enzymatic hydrolysis, lignocellulosic biomass, pretreatment.

I. INTRODUCTION

IN a modern bio-refinery, one can expect that lignocellulose would be converted through a number of different processes into mixture of products, including bio-fuels, valuable chemicals, heat and electricity [1]. Ethanol fuel can be produced from biomass through a biochemical platform consisting of sequential or simultaneous steps. These include (i) pretreatment to produce a more easily hydrolyzed substrate; (ii) enzyme hydrolysis to convert cellulose and hemicellulose components to their sugar monomers; and (iii) fermentation of sugars to ethanol [2]. Lignocellulose consists mainly of cellulose, hemicellulose and lignin. The typical percentages of dry weight are 35–50% cellulose, 20–35% hemicellulose, and 5–30% lignin [1]–[3]. As primary component of the lignocellulosic biomass, cellulose is the most abundant organic compound on earth and has the potential to be a renewable source for energy and chemicals [1]. The task of hydrolyzing lignocellulose to fermentable monosaccharides is still technically problematic, since the digestibility of cellulose is hindered by many physico-chemical, structural and compositional factors. Owing to these structural characteristics, pretreatment is an essential step for obtaining potentially fermentable sugars in the hydrolysis step. The aim of the pretreatment is to break down the lignin structure and disrupt the crystalline structure of cellulose for enhancing enzymes accessibility to the cellulose during the hydrolysis step [2], [4].

A large number of pretreatment approaches have been investigated on a wide variety of feedstocks types, and several recent review articles provide a general overview of the field [2], [4], [5]. Many pretreatments, such as dilute acid hydrolysis, pH controlled liquid hot water treatment, and lime result in biomass substrates that lack components (often hemicellulose) and are highly crystalline. Ammonia fiber explosion (AFEX) technique retains hemicellulose, but cellulose remains highly crystalline after pretreatment [2]-[5]. However, the transformation of carbohydrates is still a challenging task because of their low solubilities in almost any solvent. Compared to common organic solvents used in carbohydrate chemistry, such as dimethylacetamide, ILs display interesting properties and potential advantages, as reasonable chemical inertness, good thermal stability, low volatility, or unique salvation abilities [4]. Ionic liquids incorporating anions which are strong, hydrogen-bond acceptors were the most effective, especially when combined with microwave heating [6]. Like concentrated acids, ionic liquids containing chloride, acetate, and other moderately basic anions disrupt the hydrogen bond network of cellulose, and enable its dissolution [2]–[6].

Crude glycerol pretreatment and the dissolution of biomass in ionic liquids are emerging pretreatment technologies. Crude glycerol is a major by-product of biodiesel industry. The rapid growth of global biodiesel production indicates that the crude glycerol from the biodiesel industry will be a costly waste, rather than a valuable by-product in the future. Therefore, the exploitation of crude glycerol for pretreatment of biomass could be an attractive economic route for the utilization of this by-product of biodiesel industry, helping the commercial success of both bioethanol and biodiesel [7].

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II. MATERIALS AND METHODS

Materials

Wood biomass samples were received as shavings and were ground into powder using an electric mill, then dried in an oven (Vaccum Drying Ovens) at 100°C. Celluclast 1.5L from *Trichoderma reesei* CCN 03116 was purchased from Novozyme and Accelerase 1000 was a generous gift from Genencor (U.S.A). Glucose (Merck), 3,5-dinitrosalicilic acid (Merck), phenol (Chimopar Bucureşti), sodium sulphite (Fulka), acetic acid (Chimactiv Bucureşti), sodium hydroxide (Chemapol), were of analytical grade and have been used as purchased.

ILs 1-ethyl-3-methylimidazolium tetrafluoroborate ([Emim]BF4), 1-butyl-3-methylimidazolium hexafluoro-phosphate ([Bmim]PF6), 1-ethyl-3-methylimidazolium acetate ([Emim]COOCH3) and 1-ethyl-3-methyl-imidazolium trifluoroacetate ([Emim]COOCF3) were obtained from Merck at highest available quality. Glycerol and sulfuric acid 98% were purchased from Chimopar Bucuresti and calcium carbonate from Loba Feinchemie.

Methods

A. Ionic Liquid Pretreatment

A 2% wood biomass solution was prepared in a 50 ml autoclave vial. The glass vials and the contents was heated and sttired in a OmniStation modular reactor at 90^0C for 24 hours. After 24 hours incubation time, 20 ml anti-solvents (deionized water or acetone) were slowly added for regeneration of the dissolved cellulose from biomass. The pretreated biomass and wash solution were separated by filtration. The recovered wood flour was washed with 6 ml ethanol and dried at 80^0C for 1 hours. This method is a modified version of the method used by Lee et al. [8].

B. Glycerol Pretreatment

0.2 grams of wood biomass to be pretreated was mixed with 4 ml glycerol in a bottom flask. Magnetic stirrer was used to stir the mixture. The flask was heated at 130^0C for 24 hours. The mixture was allowed to cool to about 50 ^{0}C. Then, 4 ml water was added in it. The mixture was stirred vigorously for 3 hour and filtered. The pretreated biomass was washed with excess of distilled water and dried overnight at room temperature. This method is modified version of the method used by Guragain et al. [7].

C. Dilute Acid Pretreatment

In this method, 0.3 grams of wood biomass was mixed with 3 ml 0.82% sulfuric acid and autoclave at 150ºC for 30 min. The sample was then filtered and the pretreated biomass was washed with distilled water and dried 600C for 5 hours. The total reducing sugars from filtrate were analyzed by 3,5-DNS spectrofotometric method after netralization with calcium carbonate $CaCO_3$ to a pH between 5 and 6.

D. Glycerol-Ionic Liquids Combined Pretreatment

0.1 grams glycerol-pretreated biomass (see B. Glycerol pretreatment) were mixed with 2 ml ionic liquid $[Emim]COOCH_3$ in a 50 ml autoclave vial. The glass vials and the contents were heated and sttired in a OmniStation modular reactor at 90^0C for 24 hours. After 24 hours incubation time, the pretreatment of lignocellulosic biomass was carried out in the same manner described in section A.

E. Dilute Acid-Ionic Liquids Combined Pretreatment

0.1 grams pretreated biomass with dilute acid (see C. Dilute acid pretreatment) were mixed with 2 ml ionic liquid $[Emim]COOCH_3$ in a 50 ml autoclave vial. The glass vials and the contents were heated and sttired in a OmniStation modular reactor at 90^0C for 24 hours. After 24 hours incubation time, the pretreatment of lignocellulosic biomass was carried out in the same manner described in section A.

F. Enzymatic Hydrolysis of Wood Biomass

Hydrolysis was carried out with 1% pretreated and untreated biomass at 40^0C, in 0.05 M pH 4.8 sodium acetate buffer and 50 µl commercial cellulase enzymes Celluclast 1,5L CCN 03116, Accelerase 1000, in a OmniStation modular reactor at 200 rpm. Samples taken at defined time intervals were analyzed for liberated sugars amount by 3,5-DNS spectrofotometric method [9].

Cellulase activity was determinated according to the method reported by the Commission on Biotechnology of the International Union of Pure and Applied Chemistry (IUPAC) [10].

III. RESULTS AND DISCUSSION

A. Effect of Ionic Liquid Pretreatment

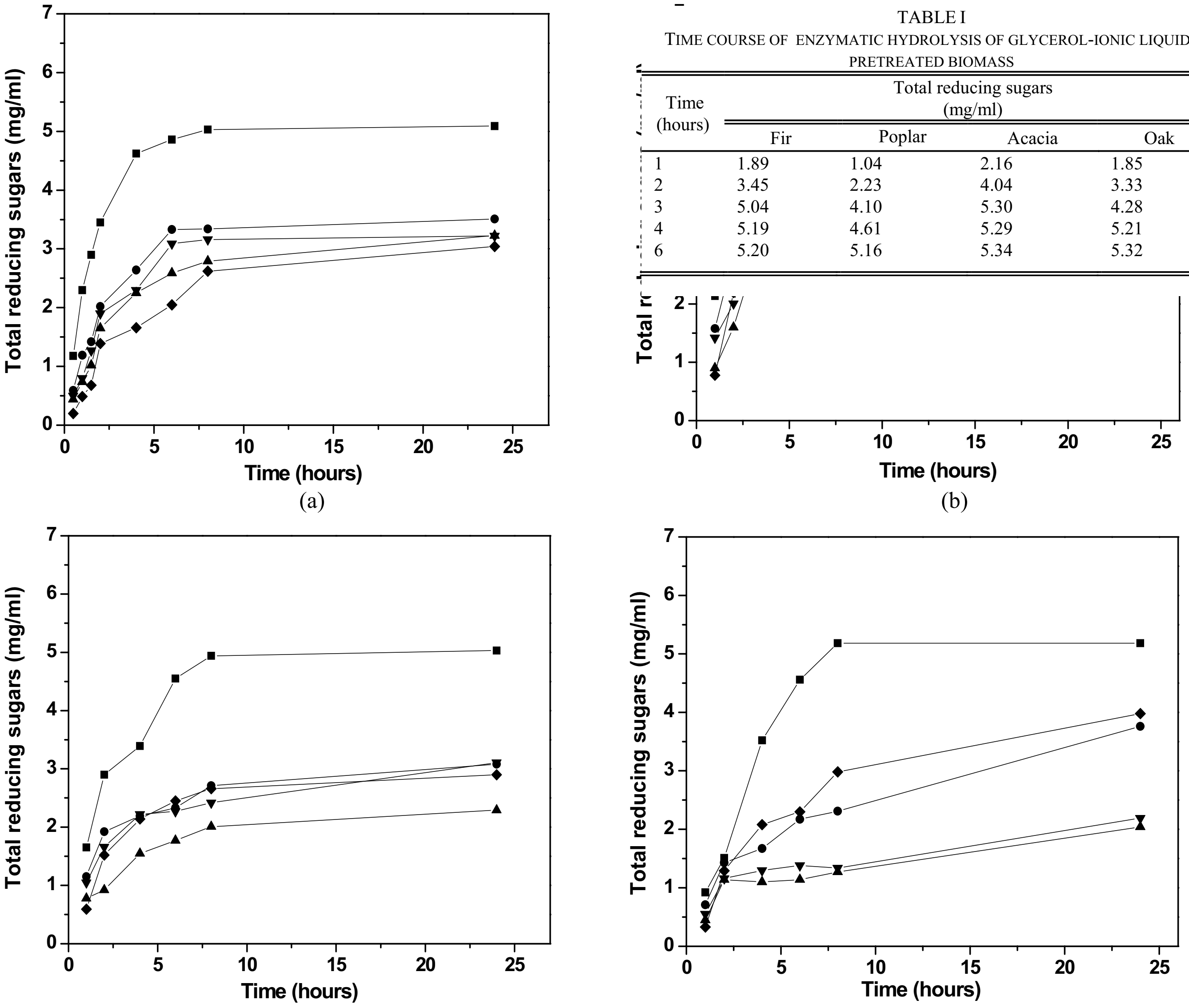
Lignocellulosic biomass (poplar, acacia, oak, and fir) was pretreated with four ionic liquids $[Emim]BF_4$, $[Bmim]PF_6$, $[Emim][COOCF_3$ and $[Emim][COOCH_3$. As the pretreatment process is for accelerating enzymatic hydrolysis, we further evaluated the solvents based on the yield of total reducing sugars from pretreated biomass. ILs display interesting properties: thermal stability, low volatility, unique salvation abilities, disrupting and removing the cross-linked matrix of lignin and disrupting hydrogen bonds in crystalline cellulose [11]-[13].

The reaction was monitored by sampling 0.2 mL supernatant at specific time intervals, followed by centrifugation at 5000g for 10 min, and measuring the released reducing sugars by the DNS method.

TABLE I

TIME COURSE OF ENZYMATIC HYDROLYSIS OF GLYCEROL-IONIC LIQUIDS PRETREATED BIOMASS

Time (hours)	Total reducing sugars (mg/ml)			
	Fir	Poplar	Acacia	Oak
1	1.89	1.04	2.16	1.85
2	3.45	2.23	4.04	3.33
3	5.04	4.10	5.30	4.28
4	5.19	4.61	5.29	5.21
6	5.20	5.16	5.34	5.32



(a)

(b)

(c)

(d)

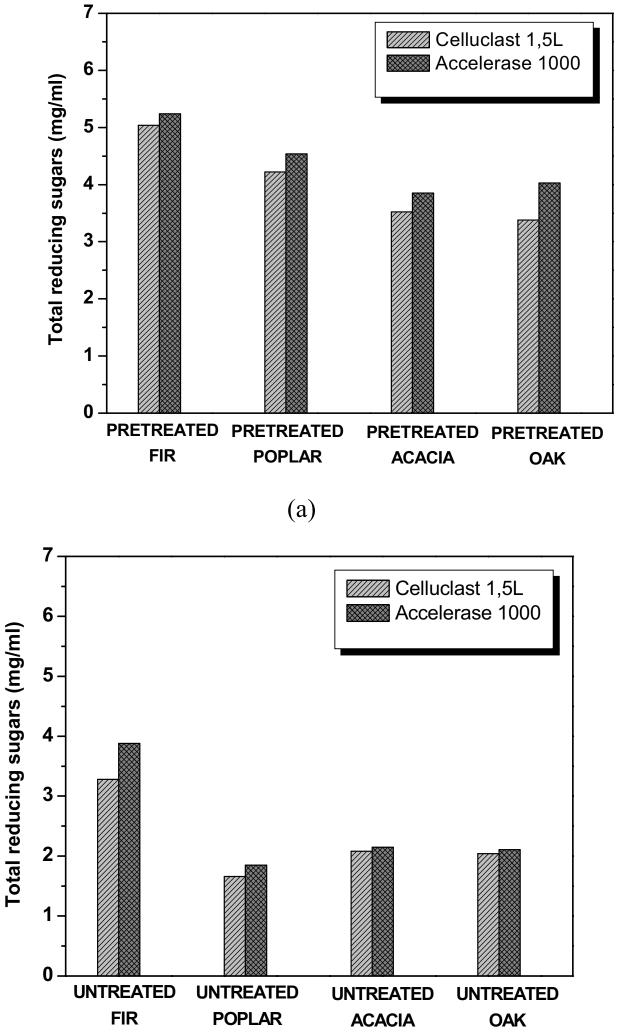
Fig. 1 Effect of ionic liquids pretreatment on enzymatic hydrolysis.
(a) poplar, (b) fir, (c) oak, (d) acacia
-■- [Emim] COOCH$_3$, -●-[Emim]COOCF$_3$, -▲- [Emim]BF$_4$, -▼- [Bmim]PF$_6$, -◆- untreated biomass
Hydrolysis conditions: 10 mg/ml pretreated and untreated biomass, pH 4,8 sodium citrate buffer, cellulase Celluclast 1,5L, 40^{0}C.

Figure 1 summarizes the various ILs pretreatment results and the amounts of sugars released by enzymatic hydrolysis with and without pretreatment.

Compared with [Emim]BF$_4$, [Bmim]PF$_6$ and untreated biomass, [Emim]COOCH$_3$ pretreatment showed the best efficiency, reduced the crystallinity of cellulose, partialy removed lignin and produced higher sugars content. The fir biomass pretreated with [Emim]COOCH$_3$ and [Emim]COOCF$_3$ was rapidly hydrolyzed by Celluclast 1,5L, this hydrolysis being completed after ca. 4 hours. In case of poplar, oak and acacia pretreated with [Emim]COOCH$_3$, the hydrolysis was completed after ca. 6 hours. Pretreated poplar with all ILs were hydrolyzed much faster than untreated poplar. As concerns the influence of different anions to the pretreatment, ILs with acetate anion produced higher sugars content.

B. Comparision of Enzymatic Hydrolysis of Pretreated and Untreated Biomass by Two Different Cellulases

Biomass samples, obtained before and after pretreatment with [Emim]COOCH3, were hydrolyzed at 0.5 ml/g enzyme/substrate ratio, at 40^{0}C. In this work, commercial cellulases such as Celluclast 1.5L (Novo) and Accelerase 1000 (Genencor) have been used for hydrolysis. The resulting hydrolysis for pretreated and untreated biomass as measured by total soluble reducing sugars (DNS) are shown in Figure 2. As already observed, hydrolysis yields for biomass samples were higher when the Accelerase 1000 enzymatic complex was used.



(a)

(b)

Fig. 2 Total reducing sugars released by enzymatic hydrolysis of pretreated (a) and untreated (b) biomass, at 4h reaction time. Hydrolysis conditions: 10 mg/ml biomass, pH 4,8 (sodium citrate buffer), cellulases: Celluclast 1,5L, Accelerase 1000, 40^0C.

C. The Effect of Different Pretreatments of Enzymatic Hydrolysis

Three different pretreatment processes, including ionic liquids, glycerol and dilute acid, were used for enzymatic hydrolysis of lignocellulosic biomass materials from poplar, acacia, oak and fir. Pretreatment of lignocellulosic biomass using glycerol-ionic liquids combined pretreatment and dilute acid-ionic liquids combined pretreatment were evaluated and compared with glycerol pretreatment, ionic liquids pretreatment and dilute acid pretreatment. Commercial cellulase Celluclast 1.5L has been used for hydrolysis.

Liberated sugars amount at defined time intervals after enzymatic hydrolysis of pretreated biomass with glycerol-ionic liquids combined method are shown in Table I. At 3 h, the concentrations of total reducing sugars released were 5.04 mg/ml for fir biomass and 5.30 mg/ml in the case of acacia biomass, that means a practically complete enzymatic hydrolysis of cellulose from the biomass.

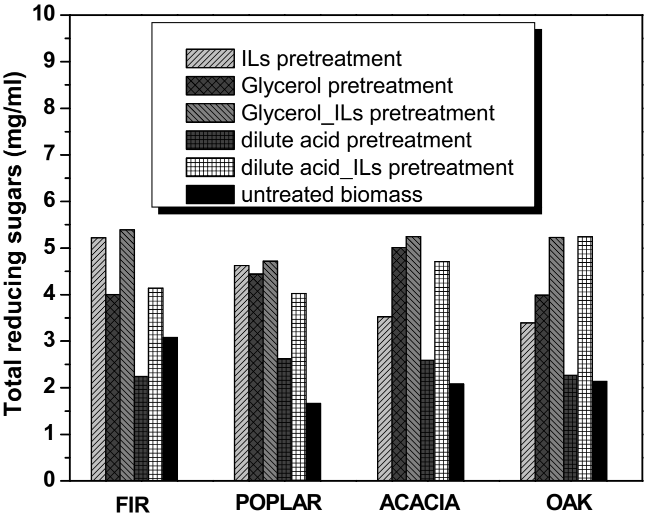


Fig. 3 Effect of different pretreatments on hydrolysis of cellulose.

The results of enzymatic hydrolysis of untreated and pretreated biomass at 4 hours reaction time are depicted in Fig. 3. The enzymatic hydrolysis biomass pretreated by ILs and glycerol showed higher cellulose conversion rates compared to the dilute acid pretreated biomass. The main role of dilute acid pretreatment is to solubilize hemicellulose from the biomass and to make cellulose more accessible for cellulases [14]-[16]. The pretreatment of biomass using glycerol effectively removed lignin with significantly higher cellulose and lower lignin content [7].

In the combined method, ILs steeping was applied as an auxiliary step to enhance the efficiency of glycerol and dilute acid treatments. In ILs pretreatment a more elevated temperature (90^0C) was used, to obtain the effective dissolution of cellulose and partial delignification without sugar degradation. For all type of substrates the combined glycerol-ionic liquids pretreatment and dilute acid-ionic liquids pretreatment were more effective as pretreatments by only ILs, glycerol or dilute acid.

Compared with the dilute acid pretreatment, the combined method with ILs produced higher sugars content, ranging from 2.27 mg/ml to 5.10 mg/ml for oak biomass, at 4 hours hydrolysis time.

IV. CONCLUSION

The potential of several lignocellulosic materials for bioethanol production was investigated. Different pretreatment of biomass was used to facilitate the conversion of cellulose into fermentable sugar (glucose). Accelerase 1000 was more efficient that Celluclast 1,5 L in the same hydrolysis conditions. In the study of ILs pretreatment, hydrolysis of cellulose was facilitated by the pretreatment with [Emim]$COOCH_3$ and [Emim]$COOCF_3$.

The present study demonstrated that enzymatic hydrolysis using combined pretreatment teachniques could result in significant improvements of enzymatic hydrolysis efficiency. Further studies from our group will focus on recovery and reuse the expensive ionic liquids used in pretreatments.

ACKNOWLEDGMENT

This work was partially supported by the strategic grants POSDRU/88/1.5/S/50783, project ID 50783, and POSDRU 107/1.5/S/77265, co-financed by the European Social Fund – Investing in People, within the Sectorial Operational Programme Human Resources Development 2007 – 2013.

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