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Pretreatment of Crop Wastes from Edible Biomass with a Protic Ionic Liquid

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Abstract- The use of protic ionic liquids (PILs) in biomass processing has attracted significant attention in the recent years due to their synthesis in a relatively cheaper and easier way in comparison with their aprotic counterparts. In this study, PIL, triethylammmonium hydrogen sulfate ($TEAHSO_4$) was explored in terms of its effects on the deconstruction of industrial crop wastes; corn cob, corn husk and wheat bran. Characterization of the pretreated biomass samples via scanning electron microscopy (SEM) and compositional analysis demonstrated structural changes and improvements in the cellulose content of the biomass. $TEAHSO_4$ led to favorable modifications in the composition of wheat bran. It enhanced cellulose content of wheat bran from 40% to 60%, through removal of 67% of the lignin present in the biomass. Pretreated biomass samples were evaluated in terms of their conversion to glucose. At the 24th hour of the enzymatic reaction, cellulose in the pretreated corn cob, corn husk and wheat bran samples were converted into glucose with roughly 85%, 71% and 96% yields, respectively. $TEAHSO_4$ pretreatment enhanced the enzymatic accessibility of wheat bran almost 10-fold. Ionic liquid media recovered following the pretreatments were analyzed for their content regarding the platform chemicals; 5-hydroxymethylfurfural (5-HMF) and levulinic acid (LA). The highest yield for 5-HMF was obtained as 47% in the ionic liquid medium obtained upon $TEAHSO_4$ pretreatment of corn husk. On the other hand, corn cob pretreatment resulted in the highest LA yield which was 93%.

Keywords Protic ionic liquid; lignocellulosic biomass; pretreatment; glucose; 5-HMF; levulinic acid.

1. Introduction

Pretreatment technologies have been regarded quite essential among biomass processing strategies for the fractionation of lignocellulosic feedstocks and conversions of the major fractions, cellulose, hemicellulose and lignin to their building blocks [1-4]. These building blocks and their derivatives can readily replace the current petroleum-based products and introduce promising pathways for a wide range of applications [5] such as hydrogen [6] and ethanol [7] production. Accordingly, researchers seek novel technologies that aim to make use of each component of lignocellulose and pursue positive environmental impacts. Among these, ionic liquid (IL) pretreatment offers favorable features including easy handling and recycling due to low vapor pressure of ILs and also, high thermal stability and tunable characteristic of the reagents [8]. Significant progress has been experienced in the use of ILs for biomass processing in the last decade. ILs not only exploited for the deconstruction and fractionation of biomass to enhance enzymatic accessibility of cellulose [9–11], but also served as promising media for the production of platform chemicals such as aromatic chemicals from lignin [12,13], platform chemicals such as 5-HMF [14] and LA [15] from cellulose.

While wheat bran has been principally used as a livestock feed, its nutritive value on human well-being encouraged its use in different food products such as cereals and baby food. Being a significant source of polysaccharides, wheat bran can be also appreciated as a renewable feedstock for the production of cellulose and hemicellulose derived fuels and chemicals. Considering its interaction with ILs, wheat bran has been pretreated with a few ILs and valorized for the extraction of polysaccharides. Wheat bran pretreatment with 1-butyl-3-methylimidazolium acetate (BMIMAc) in combination with the solvent, 1,3-dimethyl-2-imidazolidinone has been quite successful in the separation of polysaccharide from biomass; polysaccharide content of the regenerated material increased to 95% [16]. Another dialkylimidazolium IL, 1,3-dimethylimidazolium methyl methylphosphonate (DMIMMPh) was found to extract polysaccharides from wheat bran with 16% yield [17]. Corn stover is the non-edible residue of corn plant that is left on the field following the harvest; cobs, husks, stalks, leaves, and tassels of the corn plant [18]. Interaction of ILs with wastes derived from corn plant has been studied more extensively compared to wheat bran. Corn cob pretreated with 1-methyl-3-methylimidazolium dimethylphosphite (MmimDMP) was converted into reducing sugars with more than 70% yield [19]. A highly reputable work on the pretreatment of corn stover with ILs showed that 1-ethyl-3-methylimidazolium acetate (EMIMAc) was able to reduce the cellulose crystallinity of corn stover samples at biomass loadings as high as 30-50% and enhance cellulose to glucose conversion roughly with 80% yield [20].

Protic ionic liquids (PILs) as an important class of ILs, are cheaper and simpler to synthesize when compared to dialkylimidazolium ILs which have long been explored for their impacts on biomass, such as EMIMAc. Unlike their aprotic counterparts, synthesis of PILs is conducted through one step reaction between a BrØnsted acid and a BrØnsted base [21]. Proton exchange mechanism occurring during their synthesis plays an important role in lignin extraction and thus, entails changes in the component's aromatic structure and the production of lignin derived platform chemicals [22–24].Certain PILs were also examined with respect to their impact on the pretreatment of corn stover. The impact of PILs, pyridinium acetate (PyAc), 1-methylimidazolium acetate (MimAc) and pyrrolidinium acetate (PyrrAc) on the lignin extraction of stover was demonstrated [23]. PyrrAc extracted more than 70% of lignin in the biomass. This finding was also evident from the SEM images of the pretreated corn stover in which pore formations were observed due to penetration of PyrrAc in the structure. In another study, cholinium based PILs, which are defined as biocompatible ILs due to being synthesized from renewable resources, were exploited for one-pot corn stover conversion to high-titer cellulosic ethanol [25]. Ethanol production with more than 40 g/L was achieved through the use of cholinium lysinate (ChLys) and corn stover loading at roughly 34%.

Triethylammmonium hydrogen sulfate ($TEAHSO_4$) has shown promise as a low-cost PIL in the conversion of different feedstocks into fermentable sugars. In a previously reported study, Hallett et al. demonstrated Miscanthus pretreatment by aqueous $TEAHSO_4$ solution with IL to H_2O ratio of 80:20 g g^{-1} at 120°C for 8 hours. Biomass, which was delignified with up to 85% yield, was converted into glucose with 80% hydrolysis yield [26]. Similarly, willow was transformed into glucose with 82% yield following $TEAHSO_4$ pretreatment conducted at 150 °C for 1 hour [27]. Recently, the same group reported promising findings for Miscanthus pretreated by $TEAHSO_4$ at elevated temperatures, relatively high biomass loadings and for shorter periods than usual and concluded the possibility of large-scale use of PILs in biomass pretreatment [28].

This study focuses on the effects of PIL, $TEAHSO_4$ with respect to the deconstruction of crop wastes from edible biomass feedstocks; corn cob, corn husk and wheat bran with the aim to appreciate the structural changes in the biomass and improve the enzymatic digestibility of biomass. Besides, the performance of $TEAHSO_4$ regarding the conversion of solubilized cellulose of biomass samples into platform chemicals, 5-hydroxymethylfurfural (5-HMF) and levulinic acid (LA) was demonstrated.

2. Materials and Methods

2.1. Materials

Lignocellulosic feedstocks, corn cob, corn stover and wheat bran, which were ground and sieved to particle size less than 1 cm, were oven-dried prior to pretreatments. Reagents, trisodium citrate dihydrate, citric acid monohydrate, sulfuric acid, calcium carbonate, D-glucose and D-xylose were purchased from Merck (Darmstadt, Germany). Triethylamine, 5-hydroxymethylfurfural and levulinic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). The cellulase enzyme, Cellic Ctec2 was kindly provided by Novozymes (Denmark).

2.2. Synthesis of Protic Ionic Liquid, $TEAHSO_4$

Synthesis of TEAHSO4 (Fig. 1) was conducted through an acid-base reaction using triethylamine and sulfuric acid which were used in equimolar amounts. Triethylamine was put in a round bottom flask and mixed with an equal volume of water. Sulfuric acid solution in combination with three-fold higher volume of water was added dropwise to the base solution that was under constant stirring and cooled with an ice bath. In order to ensure the completion of the reaction, PIL solution was stirred overnight. Following the water removal via rotary evaporator operated at 70°C, the resultant product was obtained as a white hygroscopic solid (Fig. 2). Proton NMR spectrum of $TEAHSO_4$ is also provided (Fig. 3).

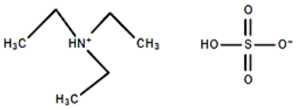


Fig. 1. Chemical structure of the PIL, $TEAHSO_4$.

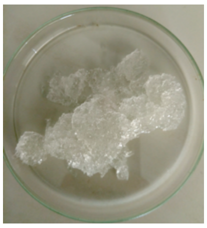


Fig. 2. Appearance of the PIL, $TEAHSO_4$.

2.3. Pretreatment of Biomass with $TEAHSO_4$

Pretreatment of biomass with aqueous solution of $TEAHSO_4$ was conducted in a similar manner reported in the Ionosolv protocol [29]. All pretreatments were performed in Pyrex tubes with Teflon-lined screw caps which were placed in an oven operated at 120°C. 10 g of $TEAHSO_4$ solution with $TEAHSO_4$:H_2O weight ratio of 4:1 was mixed with 1.5 g of biomass and incubated for 2 hours. At the end of the reaction, the samples were allowed to cool to room temperature and ethanol was added to precipitate the pretreated biomass. Pretreated biomass, which was washed with ethanol three times for 15 minutes in a shaker to remove the residual $TEAHSO_4$, was recovered by filtration. Finally, the recovered biomass was dried at 70°C under vacuum overnight before it was subjected to enzymatic reaction and characterization. Weight of the pretreated biomass was recorded to determine the percentage solid recovery (SR), which was obtained according to Eq. (1).

$$SR\ (\%) = (W_{PRT} / W_{UT}) \times 100 \qquad (1)$$

Here, W_{PRT} is the weight of biomass recovered after pretreatment (g) and W_{UT} is the weight of untreated biomass subjected to pretreatment (g). All pretreatments were performed in duplicate [30].

5-HMF yield obtained from the cellulose solubilized in $TEAHSO_4$ during pretreatment was determined according to Eq. (2).

$$5\text{-}HMF\ (\%) = [C_{5\text{-}HMF} / (Cellulose_{sol} \times 1.11 \times 0.7)] \times 100 \qquad (2)$$

Here, $C_{5\text{-}HMF}$ is the concentration of 5-HMF (g/L) in the IL medium, 1.11 is the stoichiometric conversion factor of cellulose to equivalent glucose and 0.7 is the stoichiometric conversion factor of glucose to 5-HMF conversion.

$Cellulose_{sol}$ is the concentration of cellulose solubilized in IL medium (g/L) which is determined according to Eq. (3).

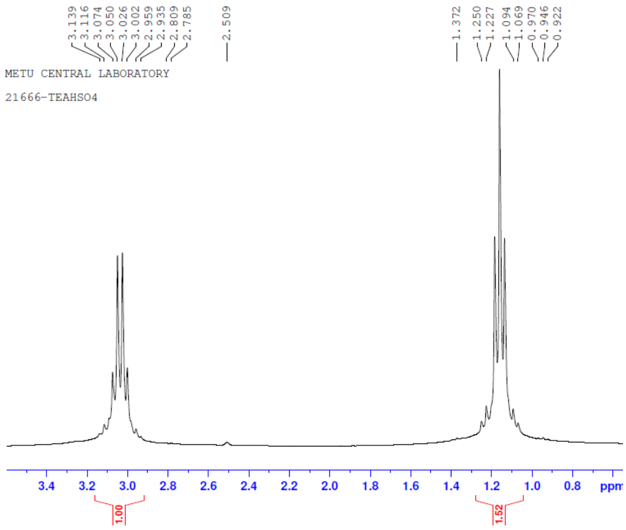


Fig. 3. ^{1}H-NMR spectrum of the PIL, $TEAHSO_4$.

$$Cellulose_{sol}\ (g/L) = [(C_S \times Cellulose_U\ (\%))/100) \times (Cellulose_{sol}(\%)/100)] \qquad (3)$$

Here, C_S is the concentration of biomass subjected to pretreatment, $Cellulose_U$ (%) is the percentage cellulose content of the untreated biomass, $Cellulose_{sol}$ (%) is the percentage of cellulose in the untreated biomass unrecovered after pretreatment.

Levulinic acid (LA) yield obtained from the cellulose solubilized in $TEAHSO_4$ during pretreatment was determined according to Eq. (4).

$$LA\ (\%) = [C_{LA} / (Cellulose_{sol} \times 1.11 \times 0.644)] \times 100 \qquad (4)$$

Here, C_{LA} is the concentration of LA (g/L) in the IL medium, 1.11 is the stoichiometric conversion factor of cellulose to equivalent glucose and 0.644 is the stoichiometric conversion factor of LA to glucose conversion.

5-HMF and LA concentrations (g/L) in IL media were determined with HPLC analysis. The relevant operation conditions for the analysis are given in the following subsection.

2.4. Enzymatic Hydrolysis of Biomass

Enzymatic hydrolysis of untreated and pretreated biomass samples was conducted in 0.05 M sodium citrate buffer at pH 4.8 and 50°C in a shaker incubator. Biomass was used at 3% (w/v) loading. Cellic Ctec2 having 225 FPU/ml of cellulase activity was used at 2 % (v/v) loading. The reaction was monitored for 48 hours. The samples were incubated at 100°C for 5 min in order to stop the enzymatic reaction and finally centrifuged at 6000 rpm for 20 minutes prior to analysis.

Prominence LC-20A Modular HPLC System with RI detector was used to determine the concentration of glucose in the hydrolysates. Biorad Aminex HPX-87H column was employed at 55°C with 5 mM H2SO4 as the mobile phase at a flow rate of 0.5 ml/min. Hydrolyzates were filtered through 0.20 m membrane PTFE filter before their examination in HPLC. Identical operation conditions were used to determine 5-HMF and LA concentrations in IL media and also, for the compositional analysis of biomass samples.

Percentage glucose yield based on the cellulose content of the pretreated cotton stalks were calculated according to Eq. (5).

$$\text{Glucose Yield (\%)} = C_G / (C_B \text{ x Cellulose}_P \text{ (\%) x} 1.11) \text{ x } 100 \qquad (5)$$

Here, C_G is the glucose concentration in the hydrolysates (g/L), C_B is the initial concentration of the biomass in the hydrolysis buffer that is subjected to enzymatic hydrolysis (g/L) and Cellulose_P (%) is the cellulose content of the pretreated biomass. 1.11 is the stochiometric conversion factor of cellulose to equivalent glucose.

2.5. Biomass Characterization

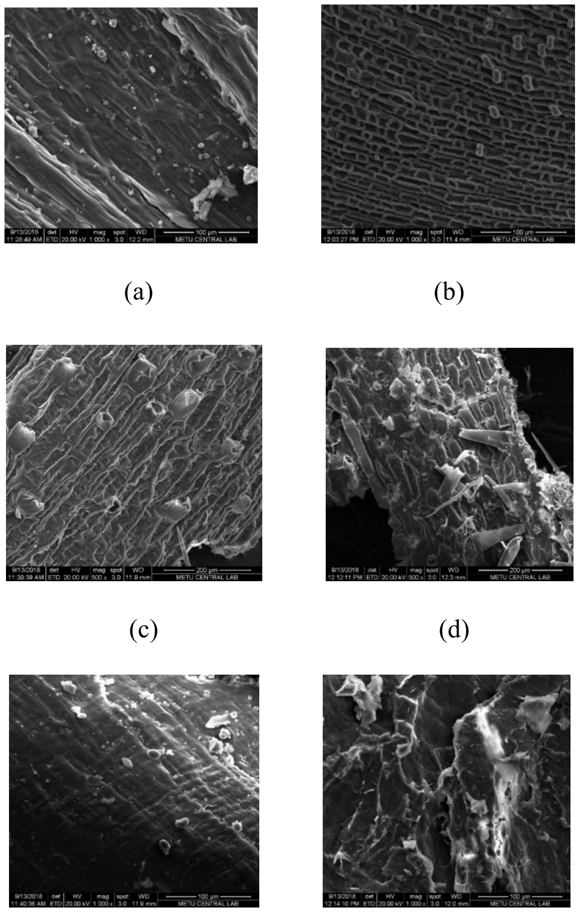
Scanning electron microscopy (SEM) images were obtained using a QUANTA 400F Field Emission SEM operated at 10 kV. All samples were sputter coated with gold/palladium (Au/Pd) prior to analysis. SEM images of the samples were taken at magnifications; 500X and 1000X. Compositions of the untreated and pretreated samples were determined through two step acid hydrolysis reported by NREL [31]. Percentage lignin extracted was determined according to the previously reported study [32].

3. Results and Discussion

3.1 Compositional and Morphological Changes in the Biomass

In this part, biomass samples were investigated regarding the compositional and morphological changes obtained after pretreatment with $TEAHSO_4$. According to Table 1, corn cob and corn husk were obtained with roughly 64% and 68% solid recoveries, respectively. On the other hand, pretreatment of wheat bran with $TEAHSO_4$ resulted in a much lower solid recovery, 45%, indicating that wheat bran was more susceptible to pretreatment. Cellulose content of all samples increased with $TEAHSO_4$ pretreatment which was more profound for wheat straw. Its cellulose content increased almost from 40% to 60%. This finding was related to the lignin extraction capacity of $TEAHSO_4$ from wheat bran. Almost 67% of the lignin present in the wheat bran was extracted upon pretreatment. Cellulose contents of corn cob and corn husk were increased from 32% to 39% and from 32% to 42%, respectively. These results were also correlated with the lignin extraction from the corresponding feedstocks.

Morphological changes in the biomass samples before and after $TEAHSO_4$ pretreatment were visualized by SEM analysis. SEM images of the biomass samples were taken at magnifications; 500X and 1000X. As shown in Fig. 4, the structural changes in each biomass were revealed with a disruption of the surface morphology. Pore formations along with an increase in the surface area were observed for corn cob pretreated by $TEAHSO_4$ (Fig. 4a) which indicated the penetration of PIL through the structure. In addition to pore formations, fibril breakages were obtained for corn husk after pretreatment (Fig. 4d). Wheat bran possessing an intact surface before pretreatment (Fig. 4e) was destroyed and an irregular surface structure was attained (Fig. 4f). Besides, larger pores were observed for the wheat bran pretreated with $TEAHSO_4$. Although the precise extent of pretreatment could not be appreciated from SEM images of different feedstocks, we can conclude that the most distinctive change was observed for wheat bran. This finding was in accordance with the compositional changes in the biomass; 67% of lignin was removed from the structure.



(a) (b)

(c) (d)

(e) (f)

Fig. 4. SEM images of untreated corn cob (a), $TEAHSO_4$ pretreated corn cob (b), untreated corn husk (c), $TEAHSO_4$ pretreated corn husk (d), untreated wheat bran (e), $TEAHSO_4$ pretreated wheat bran (f).

3.2 Effect of PIL pretreatment on enzymatic hydrolysis of biomass

Enzymatic hydrolysis of untreated and pretreated biomass samples was examined for 48 hours. Percentage glucose yields for untreated and pretreated biomass were determined on the basis of the glucose content of their corresponding cellulose content. As seen in Fig. 5, enzymatic accessibility of all samples was enhanced due to the pretreatment. The most significant change was observed for wheat bran in which roughly 96% glucose yield was achieved at the 24^{th} hour of hydrolysis; being 10-fold higher than the yield obtained for the untreated biomass. Considering the hydrolysis of other biomass samples at the 24^{th} hour of the enzymatic reaction, 9- and 4-fold enhancement were obtained for corn cob and corn husk, respectively. Besides, the complete hydrolysis of pretreated corn cob and wheat bran within 48 hours of the reaction implied the efficiency of lignin removal from biomass samples being 40% and 67%, respectively. Similar results obtained upon PILs pretreatment were also reported in the literature. Bagasse pretreatment with PIL, 1-hexylpyridinium chloride HpyCl for 10-60 minutes at 80-100°C resulted in roughly 3-fold increase in the hydrolysis of the biomass [33]. Besides, enzymatic hydrolysis of bagasse increased 5-fold when bagasse was subjected to 2-hydroxy ethylammonium acetate (2-HEAA) pretreatment at 150°C for 3.5 h [34]. This study also represented a linear relationship between cellulose hydrolysis and lignin removal from the biomass.

3.3 Conversion of solubilized cellulose in PIL into platform chemicals

Studies concerning ILs use for levulinic acid (LA) production from biomass is scarce in the literature and the impacts of PILs have not yet been explored, as well. Dialkylimidazolium ILs in the presence or absence of heterogeneous catalysts have been preferred for this purpose. IL, 1-butyl-3-methylimidazolium bromide (BMIMBr) employed in the presence of Fe/HY zeolite catalyst converted oil palm fronds into LA at 120°C for 3 hours with 68.2% yield [35]. 1-ethyl-3-methylimidazolium methylphosphonate (EMIMP), which decreased crystallinity of pulverized cedar, yielded a better LA production (65%) compared to acid pretreatment [36]. Besides, rice straw subjected to the pretreatment with IL, 1-methyl-3-(3-sulfopropyl)imidazolium hydrogen sulfate ($C_3SO_3HmimHSO_4$) resulted in roughly 22 % LA yield based on the amount of C6 sugars in the biomass [37].

In this study, we have shown that PIL, $TEAHSO_4$ was capable of converting the solubilized cellulose into 5-HMF and LA during the pretreatment. While PIL removed a fraction of lignin from the biomass, it also catalyzed the reactions for cellulose to glucose, glucose to 5-HMF and 5-HMF to LA conversion which are hydrolysis, dehydration and hydration, respectively. Based on the amount of cellulose solubilized in $TEAHSO_4$, corn cob pretreatment resulted in roughly 40% and 93% of 5-HMF and LA yields, respectively (Table 2).

Table 1. Solid recovery and chemical composition of corn cob, corn husk and wheat bran.

	Untreated biomass			**Pretreated biomass**				
	Cellulose (%)	**Hemicellulose (%)**	**Lignin (%)**	**Solid recovery (%)**	**Cellulose (%)**	**Hemicellulose (%)**	**Lignin (%)**	**Lignin extracted (%)**
Corn cob	31.6±0.7	33.3±0.3	17.6±0.0	64.2±1.6	38.8±0.4	19.3±0.0	16.7±0.5	39.1±0.3
Corn husk	31.8±0.1	19.7±0.1	12.2±0.1	67.5±2.1	42.1±1.0	20.3±0.1	12.4±0.2	31.2±0.2
Wheat bran	40.2±0.9	20.5±0.2	16.5±0.2	45.0±2.2	59.7±0.0	31.2±0.4	12.0±0.0	67.4±0.1

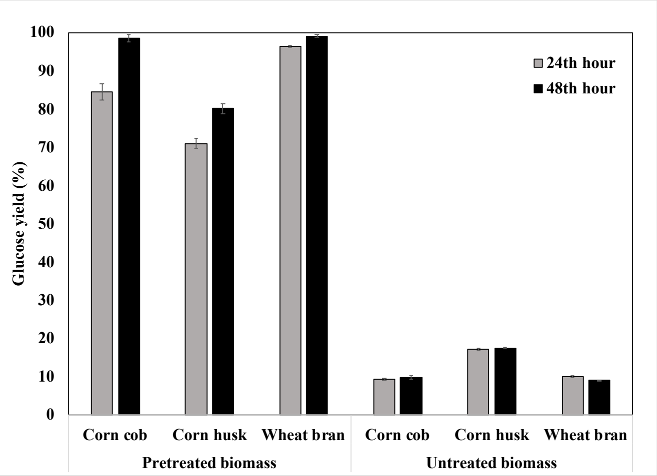


Fig. 5. Effect of $TEAHSO_4$ pretreatment on the glucose yields (%).

Table 2. 5-HMF and LA percentage yields obtained upon $TEAHSO_4$ pretreatment of corn cob, corn husk and wheat bran.

	5-HMF (%)	**LA (%)**
Corn cob	38.9±4.5	93.1±0.8
Corn husk	47.2±2.5	81.1±1.5
Wheat bran	7.2±0.3	45.0±0.6

4. Conclusion

This work demonstrated the use of low-cost PIL, $TEAHSO_4$ with respect to its potential in both the deconstruction of biomass and the production of glucose and platform chemicals. Taking PIL's efficiency in lignin removal and valorization of cellulose into 5-HMF and LA into consideration, $TEAHSO_4$ could be a promising pretreatment agent for the transformation of different lignocellulosic feedstocks into fuels and chemicals through a multi-product approach.

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References

[1] N. Mosier, C. Wyman, B. Dale, Y. Lee, M. Holtzapple, M. Ladisch, "Features of promising technologies for pretreatment of lignocellulosic biomass", Bioresource Technology, DOI: 10.1016/j.biortech.2004.06.025, Vol. 96, pp. 673–86.

[2] B. Yang, C.E. Wyman, "Pretreatment: The key to unlocking low-cost cellulosic ethanol", Biofuels, Bioproducts and Biorefining, DOI: 10.1002/bbb.49, Vol. 2, No.1, pp. 26-40.

[3] M.D. Moniruzzaman, M.D. Zahangir Alam, S.M.A. Sujan, M. Hossain, M.S. Jamal, "Enzymatic Saccharification of Bagasse: Effects of Different Pre-treatment Methods", International Journal of Renewable Energy Research, Vol.3, No.2, 2013.

[4] M.P. Gundupalli, D. Bhattacharyya, N. Senthilkumar, "RSM Based Modelling for Mineral and Organic Acid Pretreatment of Coconut Pith using High Pressure Batch Reactor", 7th International Conference on Renewable Energy Research and Applications, 2018, DOI: 10.1109/ICRERA.2018.8567025.

[5] F. Cherubini, A.H. Strømman, "Chemicals from lignocellulosic biomass: opportunities, perspectives, and potential of biorefinery systems", Biofuels, Bioproducts and Biorefining, DOI: 10.1002/bbb.297, Vol. 5, No. 5, pp. 548-561.

[6] O. Nakagoe, Y. Furukawa, S. Tanabe, Y. Sugai, R. Narikiyo, "Hydrogen production from steam reforming of woody biomass with cobalt catalyst" 1st International Conference on Renewable Energy Research and Applications, 2012, DOI: 10.1109/ICRERA.2012.6477356.

[7] P.R. Castelló, J.M.P.León, M.A.S. Bobi "Bioethanol industrial production optimization", 2nd International Conference on Renewable Energy Research and Applications, 2013, DOI: 10.1109/ICRERA.2013.6749885.

[8] H. Tadesse, R. Luque, "Advances on biomass pretreatment using ionic liquids: An overview", Energy&Environmental Science, DOI: 10.1039/C0EE00667J, Vol. 4, pp. 3913-3929.

[9] N.I. Haykir, E. Bahcegul, N. Bicak, U. Bakir, "Pretreatment of cotton stalk with ionic liquids including 2-hydroxy ethyl ammonium formate to enhance biomass digestibility", Industrial Crops and Products, DOI: 10.1016/j.indcrop.2012.04.04, Vol. 41, 430-436.

[10] C. Li, G. Cheng, V. Balan, M.S. Kent, M. Ong, S.P.S. Chundawat, et al., "Influence of physico-chemical changes on enzymatic digestibility of ionic liquid and AFEX pretreated corn stover", Bioresource Technology, DOI: 10.1016/j.biortech.2011.04.005, Vol. 102, No. 13, pp. 6928-6936.

[11] C. Li, B. Knierim, C. Manisseri, R. Arora, H. V. Scheller, M. Auer, et al., "Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification", Bioresource Technology, DOI: 10.1016/j.biortech.2009.10.066, Vol. 101, No. 13, pp. 900-4906.

[12] J.B. Binder, M.J. Gray, J.F. White, Z.C. Zhang, J.E. Holladay, "Reactions of lignin model compounds in ionic liquids", Biomass and Bioenergy, DOI: 10.1016/j.biombioe.2009.03.006, Vol. 33, No. 9, pp. 1122-1130.

[13] G. Chatel, R.D. Rogers, "Review: Oxidation of lignin using ionic liquids-an innovative strategy to produce renewable chemicals", ACS Sustainable Chemistry&Engineering, DOI: 10.1021/sc4004086, Vol. 2, No. 3, pp. 322–339.

[14] M.E. Zakrzewska, E. Bogel-Łukasik, R. Bogel-Łukasik, "Ionic liquid-mediated formation of 5-hydroxymethylfurfural-A promising biomass-derived building block", Chemical Reviews, DOI: 10.1021/cr100171a, Vol. 111, No. 2, pp. 397-417.

[15] H. Ren, Y. Zhou, L. Liu, "Selective conversion of cellulose to levulinic acid via microwave-assisted synthesis in ionic liquids", Bioresource Technology, DOI: 10.1016/j.biortech.2012.12.132, Vol. 129, pp. 616-619.

[16] Q. Hou, W. Li, M. Ju, L. Liu, Y. Chen, Q. Yang, et al., "Separation of polysaccharides from rice husk and wheat bran using solvent system consisting of BMIMOAc and DMI", Carbohydrate Polymers, DOI: 10.1016/j.carbpol.2015.07.059, Vol. 133, 517-523.

[17] K. Kuroda, H. Kunimura, Y. Fukaya, N. Nakamura, H. Ohno, "^{1}H NMR evaluation of polar and nondeuterated ionic liquids for selective extraction of cellulose and xylan from wheat bran", ACS Sustainable Chemistry&Engineering, DOI: 10.1021/sc500407a, Vol. 2, No. 9, pp. 2204–2210.

[18] J.L. Thompson, W.E. Tyner, "Corn stover for bioenergy production: Cost estimates and farmer supply response", Biomass and Bioenergy, DOI: 10.1016/j.biombioe.2013.12.020, Vol. 62, pp. 166-173.

[19] Q. Li, X. Jiang, Y. He, L. Li, M. Xian, J. Yang, "Evaluation of the biocompatible ionic liquid 1-methyl-3-methylimidazolium dimethylphosphite pretreatment of corn cob for improved saccharification", Applied Microbiology and Biotechnology, DOI:10.1007/s00253-010-2484-8, Vol. 87, No. 1, pp. 117-126.

[20] H. Wu, M. Mora-Pale, J. Miao, T.V. Doherty, R.J. Linhardt, J.S. Dordick, “Facile pretreatment of lignocellulosic biomass at high loadings in room temperature ionic liquids”, Biotechnology and Bioengineering, DOI:10.1002/bit.23266, Vol. 108, No. 12, pp. 2865-2875.

[21] T.L. Greaves, C.J. Drummond, “Protic ionic liquids: Properties and applications”, Chemical Reviews, DOI: 10.1021/cr068040u, Vol. 108, No. 1, pp. 206–237.

[22] E.C. Achinivu, “Protic ionic liquids for lignin extraction—A lignin characterization study”, International Journal of Molecular Sciences, DOI: 10.3390/ijms19020428, Vol. 19, No. 2, pp. 428-442.

[23] E.C. Achinivu, R.M. Howard, G. Li, H. Gracz, W.A. Henderson, “Lignin extraction from biomass with protic ionic liquids”, Green Chemistry, DOI: 10.1039/C3GC42306A, Vol. 16, No. 3, pp. 1114-1119.

[24] T. Rashid, C.F. Kait, I. Regupathi, T. Murugesan, “Dissolution of kraft lignin using Protic Ionic Liquids and characterization”, Industrial Crops Products, DOI: 10.1016/j.indcrop.2016.02.017, Vol. 84, pp. 284-293.

[25] F. Xu, J. Sun, N.V.S.N.M. Konda, J. Shi, T. Dutta, C.D. Scown, et al., “Transforming biomass conversion with ionic liquids: Process intensification and the development of a high-gravity, one-pot process for the production of cellulosic ethanol”, Energy Environmental Science, DOI: 10.1039/C5EE02940F, Vol. 9, No.3, pp. 1042-1049.

[26] A. Brandt-Talbot, F.J.V. Gschwend, P.S. Fennell, T.M. Lammens, B. Tan, J. Weale, et al. “An economically viable ionic liquid for the fractionation of lignocellulosic biomass”, Green Chemistry, DOI: 10.1039/C7GC00705A, Vol. 19, No. 13, pp. 3078-3102.

[27] L. Weigand, S. Mostame, A. Brandt-Talbot, T. Welton, J.P. Hallett, “Effect of pretreatment severity on the cellulose and lignin isolated from: Salix using IonoSolv pretreatment”, Faraday Discussions, DOI: 10.1039/c7fd00059f, Vol. 202, pp. 331-349.

[28] F.J.V. Gschwend, F. Malaret, S. Shinde, A. Brandt-Talbot, J.P. Hallett, “Rapid pretreatment of: Miscanthus using the low-cost ionic liquid triethylammonium hydrogen sulfate at elevated temperatures”, Green Chemistry, DOI: 10.1039/C8GC00837J, Vol. 20, pp. 3486-3498.

[29] F.J.V. Gschwend, A. Brandt, C.L. Chambon, W.C. Tu, L. Weigand, J.P. Hallett, “Pretreatment of Lignocellulosic Biomass with Low-cost Ionic Liquids”, Journal of Visualized Experiments, DOI: 10.3791/54246, Vol. 114.

[30] N.I. Haykir, E. Bahcegul, N. Bicak, U. Bakir, “Pretreatment of cotton stalk with ionic liquids including 2-hydroxy ethyl ammonium formate to enhance biomass digestibility”, Industrial Crops Products, DOI: 10.1016/j.indcrop.2012.04.041, Vol. 41, pp. 430-436.

[31] A. Sluiter, D. Hyman, C. Payne, J. Wolfe, B. Hames, D. Hyman, et al. “Determination of Structural Carbohydrates and Lignin in Biomass Determination of Structural Carbohydrates and Lignin in Biomass”, National Renewable Energy Laboratory, 2008.

[32] N.I. Haykir, U. Bakir, “Ionic liquid pretreatment allows utilization of high substrate loadings in enzymatic hydrolysis of biomass to produce ethanol from cotton stalks”, Industrial Crops Products, DOI: 10.1016/j.indcrop.2013.10.017, Vol. 51, pp. 408-414.

[33] Uju, A. Nakamoto, Y. Shoda, M. Goto, W. Tokuhara, Y. Noritake, et al. “Low melting point pyridinium ionic liquid pretreatment for enhancing enzymatic saccharification of cellulosic biomass” Bioresource Technology, DOI: 10.1016/j.biortech.2012.06.096, Vol. 135, pp. 103-108.

[34] E.G.A. Rocha, T.C. Pin, S.C. Rabelo, A.C. Costa, “Evaluation of the use of protic ionic liquids on biomass fractionation”, Fuel, DOI: 10.1016/j.fuel.2017.06.014, Vol. 206, pp. 145-154.

[35] N.A.S. Ramli, N.A.S Amin, “Catalytic hydrolysis of cellulose and oil palm biomass in ionic liquid to reducing sugar for levulinic acid production”, Fuel Processing Technology, DOI: 10.1016/j.fuproc.2014.08.011, Vol. 128, pp. 490-498.

[36] Y. Muranaka, T. Suzuki, H. Sawanishi, I. Hasegawa, K. Mae, “Effective production of levulinic acid from biomass through pretreatment using phosphoric acid, hydrochloric acid, or ionic liquid”, Industrial Engineering Chemistry Research, DOI: 10.1021/ie501811x, Vol. 53, No. 29, pp. 11611-11621.

[37] L. Liu, Z. Li, W. Hou, H. Shen, “Direct conversion of lignocellulose to levulinic acid catalyzed by ionic liquid”, Carbohydrate Polymers, DOI: 10.1016/j.carbpol.2017.11.078, Vol. 181, pp. 778-784.