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Efficient Pretreatment of Rice Straw by Combination of Screw-Press and Ionic Liquid to Enhance Enzymatic Hydrolysis

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ABSTRACT

The increasing value of lignocellulose-based biorefining has attracted interest from research and development organizations worldwide to find an efficient pretreatment method for dissociating the recalcitrated structure of lignocellulosic biomass. Nowadays, different pretreatment processes have been developed, including chemical, physical or biological processes. In this study, the effects of pretreatment by a combination of screw-press and four different types of ionic liquids (ILs) to enhance the saccharification efficiency were observed to select the best IL. The inhibition effects of ILs were monitored based on the relative cellulase activities, and the results showed that each IL had a different inhibition degree. Based on pretreatment efficiency and inhibition properties, 1-allyl-3-methylimidazolium chloride, [AMIM]Cl, was selected for the optimization experiment using response surface methodology with a three-factor Box-Behnken design (consisting of ionic liquid concentration, reaction temperature and retention time). Under the best conditions (150 °C reaction temperature, 50% concentration), 68.26% of recovered sugar could be achieved from the pretreated rice straw. A mathematical model represented the statistically significant ($P < 0.05$) effects of two treatment parameters (temperature and concentration) on the saccharification efficiency with a coefficient of determination of 0.7460.
Keywords: ionic liquid, screw press, rice straw, pretreatment

INTRODUCTION

Lignocellulosic-based biomass, including agricultural wastes, has gained the interest of researchers and industries worldwide during this decade because it can be utilized as material for biorefining and for biofuel production. The key challenge to utilize the lignocellulosic biomass is the difficulty of efficiently converting lignocellulosic polymers to small fermentable sugars. Pretreatment involves a process to unlock the recalcitrance of lignocellulosic biomass and for enzymatic hydrolysis to release sugars (Agbor *et al.*, 2011). Nowadays, many pretreatment processes, including thermal, mechanical, chemical and biological processes, have been investigated to find

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efficient methods for biorefining. The application of each pretreatment method with different types of lignocellulosic biomass has been analyzed and reviewed in many studies (Kumar *et al.,* 2009; Sousa *et al.,* 2009; Brodeur *et al.,* 2011).

Lignocellulosic biomass is comprised of mainly cellulose—a homologous polymer of glucose molecules connected by β-1,4 linkages—and it also contains hemicellulose—a heterologous polymer of 5- and 6-carbon sugars—and even less lignin—a complex aromatic polymer (Maki *et al.,* 2009). The plant cell wall is composed of lignocellulosic biomass, though with different proportions depending on the plant species. For example, rice straw has 69.2% cellulose, 4.9% lignin and 10.2% hemicellulose (Zhu *et al.,* 2005). The cellulase enzyme plays a key role in the conversion of lignocellulosic biomass into fermentable sugar. The cellulase enzyme complex comprises three classes of soluble extracellular enzymes: 1,4-β-endoglucanase, 1,4-β-exoglucanase and β-glucosidase. The synergistic actions of these three classes of enzymes determine the degree of depolymerization of cellulose, which is reduced by many factors, for example, the presence of inhibitors and the topology of microcellulosic fibrils (Zhang *et al.,* 2006).

After harvesting lignocellulosic biomass, mechanical pretreatment is an important step to reduce the biomass to an appropriate size that can be handled by the downstream processes, for example, pretreatment with other methods or transfer to a reactor. For chemical pretreatment, ionic liquids (ILs) have received growing attention as "ecologically green" solvents for lignocellulosic pretreament as they can dissolve cellulose and remove lignin (Ang *et al.,* 2012; Engel *et al.,* 2012). These chemicals have several advantages over conventional chemicals such as a shorter reaction time, being more environmentally friendly and requiring milder operation conditions; however, the major constraint to using IL is its high cost (Ang *et al.,* 2012; Engel *et al.,* 2012).

This study investigated the application of a sequential pretreatment process using a combination of a screw press and IL pretreatment to enhance the digestibility of rice straw. The efficiency of each pretreatment condition was evaluated by the analysis of the reducing sugars released from the hydrolyzed rice straw. The results of this work will be valuable for further experiments to determine the optimal operating conditions using this sequential pretreatment method to enhance the conversion of lignocellulosic biomass to fermentable sugars.

MATERIALS AND METHODS

Materials and chemicals

Rice (*Oryza sativa*) straws were obtained from a rice field in the Piedmont region, Italy. Cellulase from *Trichoderma reesei* (Celluclast® 1.5L) and cellobiase from *Aspergillus niger* (Novozyme 188), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals used in this study were purchased from Ajax (Bangkok, Thailand).

Combination pretreatment of screw press and ionic liquids

The screw press used in this study was an oil press (AP08, Reinartz; Neuss, Germany) which consists of a screw, strainer, drive and feed hopper (Sriariyanun *et al.,* 2013). This screw press has a screw outer diameter of 80 mm and a screw length of 450 mm in the compression zone and is driven by a 4 kW electric motor with a fixed speed of 17 revolutions per minute (rpm). The barrel wall temperatures during operation reach 90 °C. Feeding of the straw was done manually with a feed rate of 4 $kg.hr^{-1}$. The specific energy requirement for 1 kg dry rice straw is about 0.5 kWh. All rice straw was sieved through an aluminum mesh to screen for a particle size smaller than 2 mm and then subjected to IL pretreatment. Four types of ILs—1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl), 1-ethyl-3-methylimidazolium acetate ([EMIM]

Ac), 1-allyl-3-methylimidazolium chloride ([AMIM]Cl)—were used in this study. For each reaction, 100 mg of the mechanical pretreated sample was mixed with 2 g IL (5% weight per weight) and heated to 120 °C for 1–6 hr. After heating for the desired time, an equal volume of distilled water was added as an anti-solvent. This solution was left for 30 min to allow precipitation of the dissolved cellulose. The mixture was filtered through a cotton cloth. The residue was dried overnight at 60 °C until constant dry weight and then kept in a sealed bag prior to enzymatic hydrolysis.

Enzymatic hydrolysis

The cellulose contents were determined using quantitative saccharification with acid hydrolysis based on the NREL procedure (Sluiter *et al.*, 2008). Hydrolysis of the pretreated rice straw was performed in a 50 mM sodium citrate buffer (pH 4.8) with a Celluclast 1.5 L loading at 20 filter paper units per gram of substrate and supplemented with 100 cellobiose units per gram substrate of Novozyme 188. Pretreated biomass was mixed with buffer (5% weight per volume, w/v) as a reaction mixture and incubated in a rotary shaker at 50 °C for 48 hr at an agitation rate of 150 rpm. After 48 hr digestion, the reaction mixture was filtered to separate the leftover biomass from the liquid fraction. The liquid fraction remaining was analyzed for reducing sugars using the dinitrosalicylic (DNS) method (Miller, 1959). The yield of fermentable sugar recovery was calculated as the ratio of cellulose content in the rice straw.

Optimization of screw-press and 1-allyl-3-methylimidazolium chloride pretreatment

Optimization of the pretreatment conditions for the efficient conversion of reducing sugars (*Y*) was carried out using response surface methodology. The independent variables were: the [AMIM]Cl concentration (X_1), temperature (X_2) and pretreatment time (X_3). A Box-Behnken design (Bringans *et al.*, 2008) for each variable at three levels (max = +1, mid = 0, min = -1) was selected for the optimization, with a total of 17 runs. Experimental data were analyzed using the statistical software, Design-Expert (version 7.0.0; STAT-EASE Inc.; Minneapolis, MN, USA), to fit the second-order polynomial regression model as shown in Equation 1:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (1)$$

where Y is the response variable (reducing sugar content), β_0 is a constant, β_i is the linear coefficient, β_i is the quadratic coefficient and β_{ij} is the two factor interaction coefficient. The accuracy and general ability of Equation 1 was evaluated by the coefficient of determination (R^2).

RESULTS AND DISCUSSION

Effect of combination of screw press and ionic liquid pretreatment

Rice straw was loaded into the screw press. As the screw turned, the materials were conveyed along the screw from the transport zone to the compression zone. The flight heights decreased, forcing the materials into a smaller volume and causing compression of the biomass. The combination of compression and shearing produced frictional forces between the biomass, the inner surface of the strainer barrel and the screw surface, which generated heat. The dry cake was then discharged via the opening and collected for further analysis. As can be seen from Figure 1, the screw-pressed product displays a changed structure in the rice straw as the pressed straw is more fibrous and fluffy. It is clear that the screw press pretreatment caused shear and frictional force on the mass which helped to disperse the lignocellulose fibrils.

After the screw press pretreatment, the rice straws were sieved again with an aluminum sieve with a 2 mm opening; then, four types of ILs ([BMIM]Cl, [EMIM]Ac, [EMIM]Cl, [AMIM]Cl) were selected for sequential pretreatment of the rice straw. In this step, the different reaction time of each ionic liquid was varied with the temperature fixed at 120 °C. Apparently, the textures of rice straw pretreated with combination of screw-press and ILs were different from the unpretreated rice straw as there was considerably less evidence of any remaining contact rice straws (Figure 1). After the IL pretreatment, all rice straw residues were hydrolyzed with Celluclast 1.5 L and Novozyme 188. The reducing sugar content released from the lignocellulosic biomass was measured by DNS assay. The highest amount of recovered reducing sugars was 87.21% from the rice straw pretreated with a combination of screw press and [EMIM]Ac (Figure 2), while 8.38% was recovered from unpretreated rice straws. Interestingly, the sequential screw press-IL pretreatment yielded a high sugar recovery with a relatively short reaction time (6 hr), compared to conventional chemicals, such as NaOH (24 hr).

To select the best IL for pretreatment, the amounts of reducing sugars derived from the rice straw pretreated with different ILs were compared. After 6 hr-pretreatment with different ILs, the amounts of reducing sugars were 87.21, 81.55, 75.81 and 71.55% from the rice straw pretreated with [EMIM]Ac, [EMIM]Cl, [AMIM]Cl, and [BMIM]Cl, respectively (Figure 2). These results showed that the selected ILs were efficient in the pretreatment of rice straw biomass resulting in a high percentage of sugar recovery and a short reaction time.

Effect of ionic liquid inhibition on commercial cellulase activities

The cellulase enzyme plays an important role in the recovery of small sugar monomers from lignocellulosic biomass, which requires suitable conditions to achieve the best activity, for example in an optimized citrate buffer (Sluiter

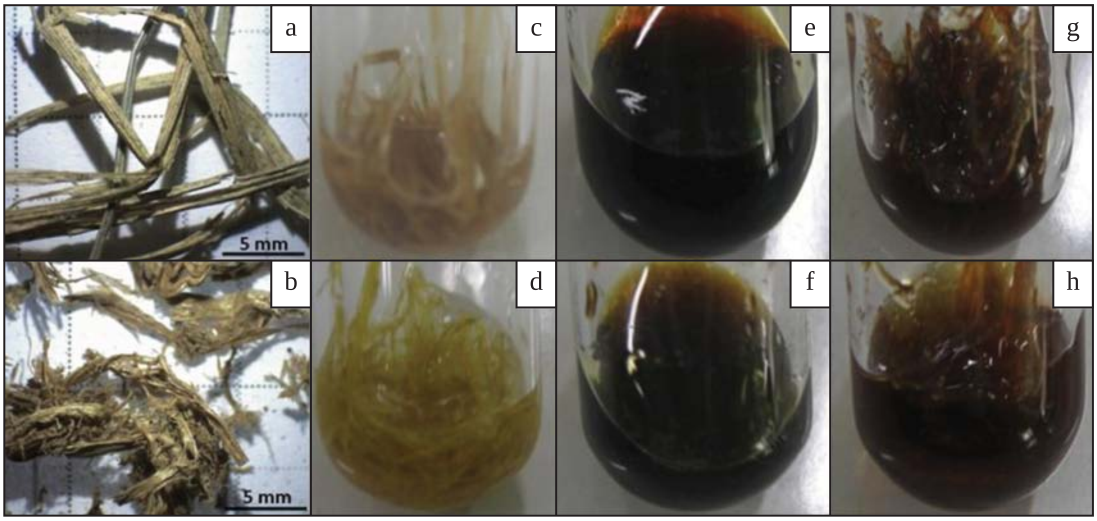

Figure 1 Appearance of rice straw with different pretreatments of ionic liquids—1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl), 1-ethyl-3-methylimidazolium acetate ([EMIM]Ac), 1-allyl-3-methylimidazolium chloride ([AMIM]Cl): (a) Before screw press pretreatment; (b) After screw press pretreatment. Effect of ionic liquid pretreatment after screw press operation and incubation at 120 ^{o}C for 6 hr of: (c) Screw pressed mass; (d) Screw pressed mass pretreated with 24-h-NaOH; (e) Screw pressed mass pretreated with [EMIM]Ac; (f) Screw pressed mass pretreated with [EMIM]Cl; (g) Screw pressed mass pretreated with [BMIM]Cl; (h) Screw pressed mass pretreated with [AMIM]Cl.

et al., 2008). Before proceeding to the hydrolysis reaction, the pretreated lignocellulosic biomass requires washing with a large amount of water to remove the remaining chemicals that inhibit the function of the cellulase. The washing is inconvenient and generates wastes. Therefore, the current study observed the effect of IL inhibition on commercial cellulase activity in order to find out if it were possible to reduce the washing step in the biorefining process. For this, filter paper was selected as a substrate for the cellulase, and each IL was added in a mix with sodium citrate buffer to get final concentrations of 10% and 20% w/v. Then enzymatic hydrolysis reactions were performed and the reducing sugar contents were measured. The cellulase activities in the IL-mixed samples were compared with the no-IL sample.

From Figure 3, the cellulase activity in all the IL-mixed buffers was greatly reduced compared to the no-IL buffer. The relative cellulase activities, compared to the no-IL buffer, were reduced to 35.65, 59.68, 49.03 and 61.76% when the buffer was mixed with 10% w/v [EMIM]Ac, [EMIM]Cl, [BMIM]Cl and [AMIM]Cl, respectively. Furthermore the more amounts of ILs in the buffer had a greater negative effect on the cellulase activity, which was reduced to 24.24, 35.53, 28.40 and 33.22% when the buffer was mixed with 20% w/v [EMIM]Ac, [EMIM]Cl, [BMIM]Cl and [AMIM]Cl, respectively. These results suggest that all ILs have a different degree of cellulase inhibition and that these inhibition abilities also depend on the quantities of remaining residues.

Optimization of screw-press and 1-allyl-3-methylimidazolium chloride pretreatment

Based on the results in the previous sections, four types of ILs were tested to pretreat rice straw to find the best IL to convert lignocellulosic biomass to reducing sugars. The order of percentage of recovered reducing sugars derived from hydrolysis reaction from highest to lowest efficiency of the IL was [EMIM]Ac, [EMIM]Cl, [AMIM]Cl and [BMIM]Cl, respectively. On the other hand, the increasing order for the ILs with regard to negative effects on cellulase activity was [AMIM]Cl, [EMIM]Cl, [BMIM]Cl and [EMIM]Ac, respectively. Only one IL—[AMIM]Cl—was selected for the optimization study, as it produced a high efficiency in pretreatment (75.81% recovered sugars) but

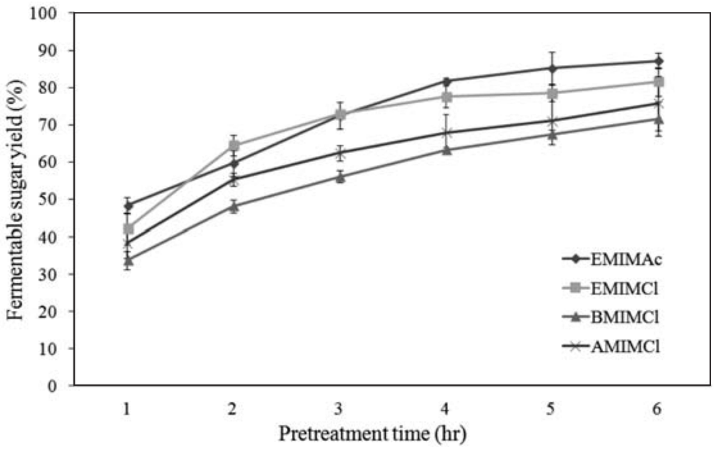

Figure 2 Yield of fermentable sugars after hydrolysis from rice straw pretreated by combination of screw press and four different types of ionic liquids—1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl), 1-ethyl-3-methylimidazolium acetate ([EMIM]Ac), 1-allyl-3-methylimidazolium chloride ([AMIM]Cl). Data represent average of three replicates.

had a low negative effect on the cellulase activity at 10% w/v (61.76% relative cellulase activity compared to the no-IL condition).

To optimize the conditions of the [AMIM]Cl pretreatment, as shown in Table 1, three independent variables ([AMIM]Cl concentration, pretreatment temperature and pretreatment time) were tested at three different levels (high, medium and low) based on a Box-Behnken design. The percentage of sugar recovery was set as the response factor for each of the pretreatment conditions (Table 1). Then, all the experimental data were analyzed using the Design-Expert software to generate the model that represented the relationship between the independent and dependent variables. The range and levels of independent variables and code values are shown in Table 1.

The results of the analysis showed that all independent and dependent variables were fitted to different mathematic models with different *P*–values and each model was validated by lack-of-fit tests. In this step, only the two-factor interaction model was statistically significant ($P = 0.0762$). This model was validated by lack-of-fit tests that yielded a *P*-value of 0.1247 (not significant) indicating the good fit of this model to the experimental data. The models were then processed using analysis of variance (ANOVA) to eliminate all terms that were not statistically significant ($P > 0.05$) for the dependent variables by backward regression (alpha = 0.100). The generated model was significant ($P = 0.0030$) and had a high R^2 value (0.7460). The mathematical model representing the relationship between the independent and dependent variables is presented as Equation 2:

Percentage of recovered sugar = 46.03916 – 1.91397× Concentration – 0.081854 × Temperature + 0.015886 × Concentration × Temperature (2)

Next, the relationship between the percentage of recovered sugar with two independent variables (concentration and temperature) was represented as a contour plot as shown in Figure 4. Based on this model, the maximum percentage of recovered sugar was 57.74% with a 50% concentration of [AMIM]Cl at 150 °C. Interestingly, within the range of design parameters in this study, the pretreatment time had no significant effect on the percentage of recovered sugar or the pretreatment efficiency.

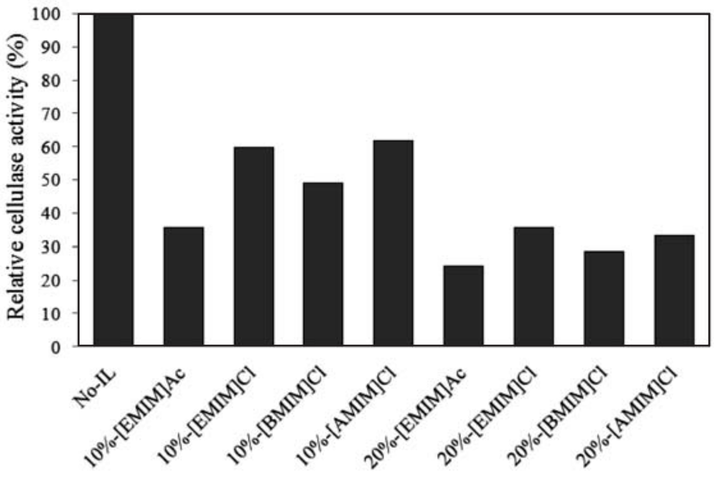

Figure 3 Relative cellulase activity of commercial cellulase enzyme in buffer containing different types of ionic liquid (IL)—1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl), 1-ethyl-3-methylimidazolium acetate ([EMIM]Ac), 1-allyl-3-methylimidazolium chloride ([AMIM]Cl)—at 10% and 20% weight per volume concentration.

Table 1 Experimental design for 1-allyl-3-methylimidazolium chloride ([AMIM]Cl) pretreatment.

Run	Factor 1: X_1 [AMIM]Cl concentration (% weight per volume)	Factor 2: X_2 Pretreatment temperature (°C)	Factor 3: X_3 Pretreatment time (hr)	Response (Y) % sugar recovery
1	100 (+1[A])	125 (0[A])	6 (-1[A])	31.18
2	50 (+1)	125 (+1)	3.5 (0)	38.75
3	0 (0)	125 (-1)	1 (+1)	38.45
4	50 (0)	125 (-1)	3.5 (-1)	32.29
5	50 (0)	100 (+1)	6 (+1)	27.34
6	100 (0)	100 (0)	3.5 (0)	31.88
7	50 (0)	150 (0)	1 (0)	37.74
8	50 (-1)	125 (0)	3.5 (-1)	30.97
9	0 (+1)	125 (-1)	6 (0)	36.63
10	0 (0)	100 (0)	3.5 (0)	39.46
11	50 (-1)	125 (0)	3.5 (+1)	38.75
12	50 (0)	100 (+1)	1 (-1)	28.75
13	0 (0)	150 (0)	3.5 (0)	36.13
14	100 (+1)	150 (0)	3.5 (+1)	68.26
15	50 (-1)	125 (+1)	3.5 (0)	40.57
16	100 (0)	125 (0)	1 (0)	33.70
17	50 (-1)	150 (-1)	6 (0)	48.35

A = Numbers in parentheses represent the level of tested factor; +1 = high, 0 = medium, and -1 = low.

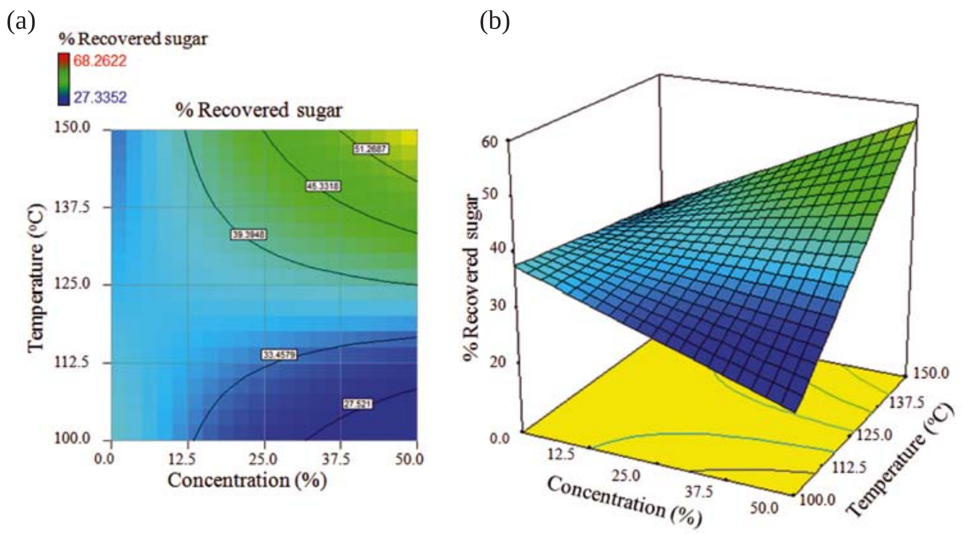

Figure 4 Percentage of recovered sugar derived from hydrolyzed rice straw. Effects of two independent variables (temperature and concentration) on the dependent variable (% Recovered sugar) in: (a) Contour plot; (b) Three-dimensional-view plot.

CONCLUSION

The effect was investigated of a combination pretreatment of screw press and ionic liquid to enhance saccharification of rice straw. Compared to the conventional chemical pretreatment (diluted alkaline or acid), the combination of screw press and ionic liquid pretreatment had a much higher efficiency and a shorter reaction time (Brodeur *et al.*, 2011; Guragain *et al.*, 2011). Of the four types of IL tested, the best IL yielding the highest percentage of recovered sugar from the hydrolyzed rice straw was [EMIM]Ac. However it had the largest negative effect on cellulase activities. Based on the goal to apply the use of IL pretreatment to the biorefining process, state of the art procedures are required to find the point that both maximizes the hydrolysis efficiency and minimizes the inhibition of cellulase activity.

With regard to both preferred characters of the IL, [AMIM]Cl was selected for the optimization experiment because it had moderate efficiency in the pretreatment but also had a minimal negative effect on the cellulase activity. The parameters and conditions chosen in the testing were motivated by the industrial-level economic concerns of temperature and the time of operation. Moreover, the most important parameter was the IL concentration because the cost of IL is still relatively high compared to other chemicals. The optimization experiment was a suitable method to evaluate the viability of IL application in the biorefining pretreatment process.

The results of the optimization experiment showed that a lower IL concentration (up to 50% w/v) resulted in a good pretreatment efficiency (68.26%), which was not much different from that achieved using the 100% IL concentration (75.81%). Moreover, the reduced use of IL in the pretreatment reduced the amount of water required in the washing step (before hydrolysis). The concept of using aqueous-IL for pretreatment is generating greater interest although there is a trade-off with reduced efficiency (Fu and Mazza G, 2011; Hou *et al.*, 2013). Additionally, it should lead to the adaptation of process development to consolidate processing so that the pretreatment and hydrolysis process can be performed simultaneously in one reactor and contribute positively to the technology becoming economically viable.

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