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Pretreatment of Wood Biomass with Ionic Liquids: A “Green” Approach to Separate Cellulose for Use in Oilfield Application

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ABSTRACT

Cellulosic polymers have been extensively used as a potential alternative to organic synthetic polymer in oilfields applications due to many unique and attractive properties, including biodegradable, inexpensive, easy to deal with after use and so on. Among many types of celluloses, wood cellulose has gained increased worldwide interest due to growing global environmental awareness and concepts of sustainability and no conflict between food and chemical/materials production. However, extraction of cellulose from wood biomass with “green” methods is important and challenging for the production of cellulose for further chemical processing. This study describe a new approach for the separation of cellulose from wood biomass with a clean efficient method which combined Ionic Liquids (ILs) pretreatment/recovered steps followed by enzymatic delignification. The pretreatment of wood biomass with the hydrophilic IL (emim) (OAc) (1-ethyl-3-methylimidazolium acetate) was conducted at a range of temperatures (50-80°C). The IL was recovered by washing with water-acetone mixture prior to enzymatic delignification in aqueous systems without or with small amount of ILs. Characterizations of untreated and treated wood materials were performed using SEM, XRD, FTIR, TGA and chemical methods. IL pretreatment did alter the structure to render a more accessible surface area for enzyme. Obtained cellulose fibers became more separated into individual microsized fibers, making it suitable for oil field application. We confidently believe that this newly developed process will play a great role in converting lignocellulosic biomass-the most abundant renewable biomaterials in the world-to biomaterials, biopolymers, biofuels, fracture fluids and natural oil sorbents.

Key words: Wood biomass, cellulose, ionic liquids, biofield application

INTRODUCTION

The use of natural cellulosic polymer over petroleum based synthetic polymer in oilfields applications has received increased worldwide interest because of the growing global environmental awareness and concepts of sustainability, green technology and so on. Cellulose-earth’s most abundant biopolymer-and cellulose derivatives (e.g., acetylated cellulose) have been used extensively as natural oil sorbents

(Li *et al.*, 2013). The application of cellulose derivatives in the oil field in various phases of drilling, completion and production of oil is well documented. Generally, native cellulose fibers produced from different sources is modified with organic anhydrides, in particular with acetic anhydride, to produce novel environmentally friendly materials for a wide range of applications including utilization in oilfields. Considering these facts, extraction of cellulose from renewable sources with clean methodology is very important.

Among many types of celluloses, wood cellulose has gained increased attention due to its abundance, inexpensive and no conflict between food and chemical/materials production. Wood-the most abundant lignocellulosic resources on the world-represents an abundant carbon-neutral renewable resource for bioenergy and biomaterials production. Wood consists of up to 50% cellulose that is rigid semi-crystalline embedded in amorphous hemicelluloses and lignin. However, the recalcitrant nature of the wood cell wall represents the biggest challenge in the extraction of cellulose with green technology. In fact, a distinct crystalline structure of cellulose makes it a challenge to find suitable solvents for its dissolution as well as isolation from lignin.

To date, a number of pretreatment approaches including physical (e.g., pyrolysis and mechanical disruption) (Mosier *et al.*, 2005), physico-chemical (e.g., steam explosion and ammonia fiber explosion) (Gabriele *et al.*, 2010), chemical (e.g., acid hydrolysis, alkaline hydrolysis and oxidative delignification) (Zhao *et al.*, 2008) and biological methods (Singh *et al.*, 2009; Lee, 1997) have been investigated for extraction of cellulose. However, except biological pretreatment, all of such processes suffer from severe reaction conditions (e.g., high acid concentrations, high temperatures) which lead to the production of inhibitory byproducts and need of costly neutralization processes (Gable and Zacchi, 2007). In addition, conventional chemicals, such as sulphate and sulfite pulping processes pose serious environmental hazards in air and water. On the other hand, the biological pretreatment for wood delignification is an environmentally safe process. However, this approach in aqueous system has been found to be very slow mainly due to the difficulties in enzyme accessibility to the solid substrate and the poor solubility of lignin (Singh *et al.*, 2009; Sousa *et al.*, 2009). Thus, to make this approach as an efficient method of degrading lignin, limitations associated with aqueous solutions must be overcome. One approach to address these problems is to use ILs as co-solvents during delignification.

Ionic Liquids (ILs) represent nonvolatile, thermal stable, non-flammable and tunable designer solvents that hopefully can replace highly Volatile Organic Solvents (VOSs) for a wide range of applications (MacFarlane and Seddon, 2007; Moniruzzaman *et al.*, 2010a). Recently, many ILs have been used to dissolve wood and other lignocellulosic biomass at higher temperatures and cellulose rich materials and lignin can readily be separated by the addition of a variety of precipitating solvents (Kilpelainen *et al.*, 2007; Mora-Pale *et al.*, 2011; Sun *et al.*, 2009). However, notable problems of wood biomass dissolution in ILs at higher temperature (over 100°C) are: The significant loss of cellulose and other carbohydrates and only partial delignification (Sun *et al.*, 2009). Singh *et al.* (2009) reported that short time IL pretreatment of switchgrass biomass can easily swollen cell wall to weak the network of biomass components. So, the selective delignification of wood biomass via biological pretreatment of IL-swollen wood materials may be effective for isolating cellulose fibers with minimum modification in structure.

In this study, we have reported a novel pretreatment method of wood biomass for isolating cellulose fibers via enzymatic delignification with prior IL pretreatment/recovery steps. Chemical characterization of untreated and treated wood materials was performed to determine cellulose, hemicellulose and lignin contents. Physical characteristics including cellulose crystallinity, thermal stability and wood cell wall morphology were also investigated using X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmittance Electron Microscopy (TEM) Fourier Transform Infrared Spectroscopy (FTIR) and Thermogravimetric Analysis (TGA). Commercial laccase that are copper-containing oxidase enzymes obtained from white rot fungi was selected as a biocatalyst because it can degrade the lignin of biomass with leaving the other components (e.g., cellulose) virtually untouched (Blanchette, 1991).

MATERIALS AND METHODS

Materials: Wood chips from hinoki cypress (*Chamaecyparis obtusa*) were dried in an oven at 110°C at atmospheric pressure. The IL (emim)(OAc) (1-ethyl-3-methylimidazolium acetate) (>95%) was obtained from Ionic Liquids Technologies GmbH (Heilbronn, Germany) and used as received. Commercial Laccase Y120 (EC.1.10.3.2) (1000 U g⁻¹) from *Trametes* sp., was kindly supplied by Amano Enzyme Inc. (Nagoya, Japan). The 1-hydroxybenzotriazole (HBT) was obtained from Sigma (St. Louis, MO). All other reagents used in the experiments were analytical grade.

Process: A simplified flowchart of the proposed method shown in Fig. 1. In a typical experiment, 0.4 g wood materials

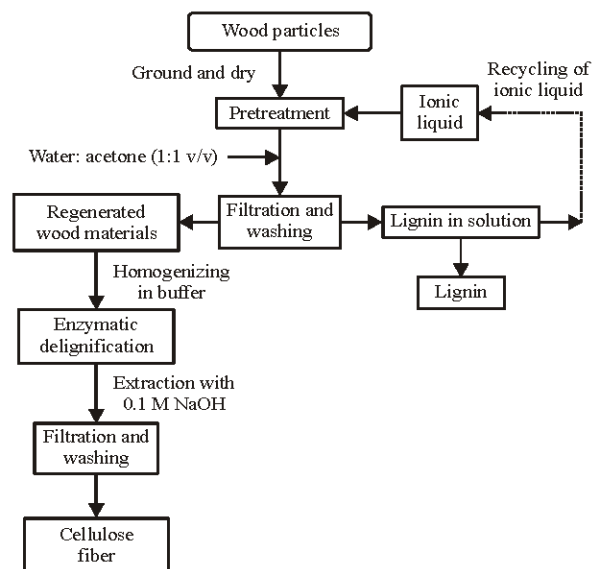


Fig. 1: Simplified schematic diagram for delignifying process of wood biomass. Enzymatic delignification was performed in buffer with or without small amount of IL in O₂ bubbles. The Ionic Liquid (IL) is (emim) (Oac)

(110-550 μM) and 4.0 g IL were placed in a flask and heated at 80°C in an oil bath with magnetic stirring for 1 h. After cooling the wood-IL mixture to Room Temperature (RT), water-acetone (1:1 v/v) was added as an antisolvent and stirred at RT for 20 min to separate solid materials and dissolved lignin and IL. After settling the mixture, Recovered Wood Materials (RWMs), lignin and IL were recovered as reported by Sun *et al.* (2009). Note that RWM was washed several times with distilled water to remove any residual IL. Then, dried RWM and acetate buffer (100 mM, pH 4.5) (ca. 5 wt% biomass) were placed in a three neck flask and homogenized. Laccase (200 U g^{-1} biomass) were added to the flask while 1-hydroxybenzotriazole (HBT) (1.5 wt% of biomass) was added as a mediator. Reaction was carried out with the supply of O_2 bubbles with a small stirrer at 50°C with or without small amount of IL. After 24 h reaction time, 0.1 M NaOH was added and stirred for 1 h to extract lignin from enzymatically delignified RWMs. The mixture was filtered under mild vacuum and finally cellulose rich wood fibers (hereafter referred as Cellulose Rich Fibers (CRFs)) was collected. To remove traces of NaOH, the CRFs were washed with distilled water until pH paper showing the final drops of washing liquid to be pH neutral. The CRFs was oven dried at 70°C, 0.1 MPa for 24 h to attain constant weight.

Chemical compositions of the materials: The α -cellulose and hemicelluloses contents of the untreated wood, RWM and CRFs samples were analyzed according to the procedure that originally pioneered by Wise *et al.* (1946). Briefly, the holocellulose content (α -cellulose+hemicelluloses) of the materials was determined using an acidified sodium chlorite solution at 70°C for an hour and the process was repeated until the product became white. Then, the α -cellulose content of the materials was determined by treatment with 6 wt% sodium hydroxide over night at 80°C in order to leach hemicelluloses. The difference between the values of holocellulose and α -cellulose gives the hemicellulose content of the materials. The lignin content of the materials was determined using TAPPI methods (TAPPI., 1991, 1998) with a scaled down process.

Characterizations of untreated and treated wood materials: The fibers morphology was characterized using a Scanning Electron Microscope (SEM) (S-4700, Hitachi Ltd., Tokyo, Japan). For SEM images, fibers were mounted on metal stubs by double-faced tap and images were taken. Prior to imaging samples were coated with gold-palladium in a sputter coater (E1030 Ion Sputter, Hitachi Ltd.).

The FTIR spectra of the samples were recorded from a KBr disk containing 1% finely ground samples on an IRPrestise-21 FTIR spectrophotometer (Shimadzu, Japan) in the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} . Spectral outputs were recorded in the transmittance mode as a function of wave number.

The crystallinity of the untreated and treated wood materials was investigated by Powder X-Ray Diffractory

(PXRD), using a XRD-6100 diffraction system (Shimadzu, Japan). The diffraction patterns were measured from $2\theta = 8-40^\circ\text{C}$ with scan speed of $0.1^\circ\text{C min}^{-1}$ using Cu K α radiation at 40 kV and 30 mA.

Thermogravimetric analysis was performed to compare the degradation characteristics of untreated and treated wood materials. The thermal stability of each sample was determined using a Pyris 1 Thermogravimetric analyzer by heating 10 mg sample in platinum pan at a rate of 10 min^{-1} in a nitrogen environment.

RESULTS AND DISCUSSION

Chemical composition of untreated and IL treated wood materials: Pretreatment of wood materials with IL was carried out at moderate conditions (80°C for 1 h) to swollen the wood cell by partial dissolution and the yield of solids was recovered through the addition of an anti-solvent as stated in the experimental section. We have selected moderate conditions during IL pretreatment so that the major components of wood particularly, cellulose loss were minimized. Another aim was to obtain Recovered Wood Material (RWM) containing cellulose with minimum altered crystallinity. It has been reported that the crystallinity of RWMs highly depends on the time and temperature during IL pretreatment. Generally, elevated temperatures (100°C or higher) and longer pretreated times lead to complete dissolution of wood biomass in which the crystallinity of regenerated cellulose rich materials decreased significantly (Lucas *et al.*, 2011). The chemical composition of Untreated Wood Materials (UWMs) is cellulose = 41.2%, hemicellulose = 25.2% and lignin = 29.3%. After IL treatment, the change of chemical compositions was very insignificant. This implies that IL pretreatment did not significantly change the chemical composition of wood materials but did alter the structure to render a more accessible surface area for enzyme. At the end of the enzymatic delignification of RWMs the α -cellulose content of cellulose rich materials was increased from 41.2-73.1% while hemicellulose and lignin content were significantly decreased to 8.5 and 9.8%, respectively. The yield of RWMs after IL treatment was about 87% of the weight of the untreated wood biomass. During the IL treatment, the swelling of wood cell wall was occurred due to partially break the bonds between major biopolymers in the wood matrix (Lee *et al.*, 2009). Consequently, a small portion of the hemicellulose and lignin was solubilized, resulting the decrease of their content of the IL treated wood materials. Compared to the untreated sample, RWMs after IL treated had somewhat higher cellulose content due to the removal of hemicellulose, lignin and water/IL/acetone soluble extractives during the pretreatment/recovering processes.

Enzymatic delignification of RWMs after IL treatment: After IL treatment, RWM was subjected to biological pretreatment (at 50°C for 24 h) using laccase as a biocatalyst. The reaction was carried out using acetate buffer containing

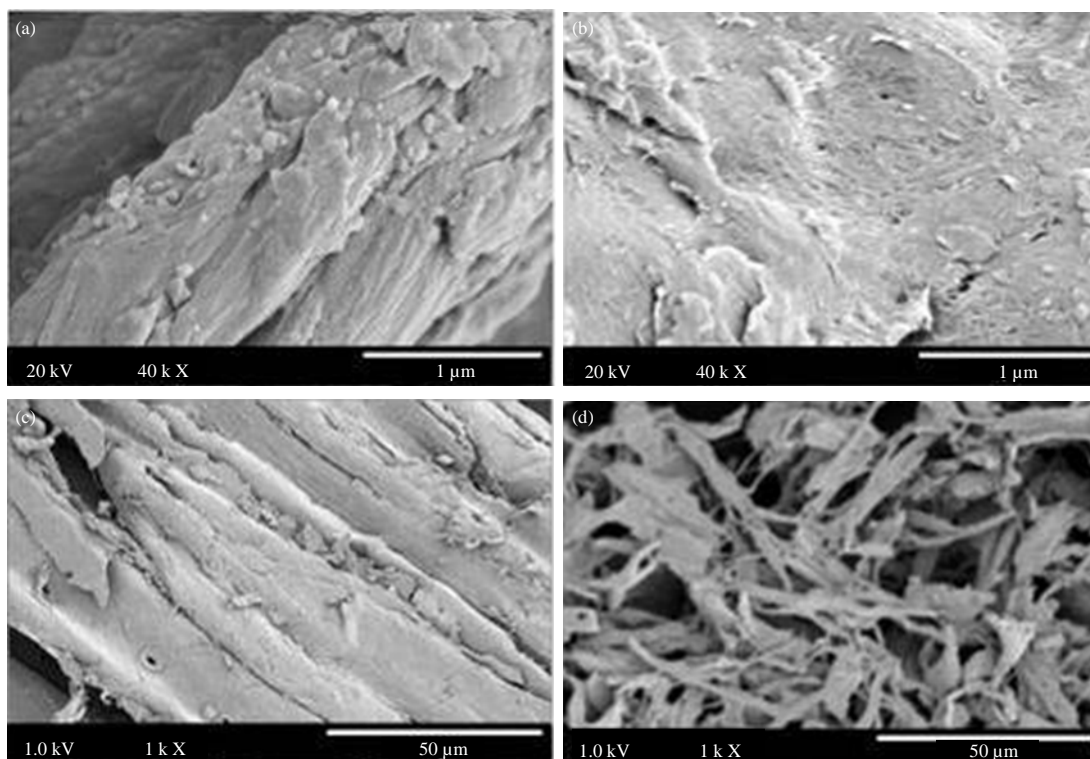


Fig. 2(a-d): (a-c) SEM images of untreated wood materials and the corresponding RWMs after IL pretreatment, respectively and (d) CRFs obtained by combination of IL and enzymatic treatments

2.5 wt% IL (emim) (OAc). Here, we have used small amount of IL during delignification because it was reported that the presence of such IL at low concentration enhances the stability of many enzymes in aqueous media (Moniruzzaman *et al.*, 2010a, b). Another possible advantage of using IL is to favor the dissolution of substrates and products, resulting better process efficiency. To check these phenomena, we investigated the delignification efficiency of RWMs in the aqueous media without IL. The results indicated that produced cellulose fibers obtained with 16.5 wt% lignin whereas, 9.8 wt% lignin was remained in the fibers obtained from aqueous medium containing 2.5% IL. This is as expected because IL pretreatment did the swollen wood structure to incorporate enzyme and catalysis deep into the wood biomass for improved delignification. Furthermore, the presence of IL may accelerate the delignification efficiency by favoring the dissolution of substrate and products (Pu *et al.*, 2007). As shown in the process flow chart, after finishing the enzymatic delignification, the sample was diluted with 0.1 M NaOH to extract the lignin. We expected that a portion of the hemicelluloses was also leached in this step which is reflected in the finally produced CRFs. This chemical compositional change in the wood fiber may result in a better crystalline degree of cellulose and hence improved thermal properties and higher strength of the fibers. To confirm these, our next aim

was to characterize wood fibers and cellulose rich fibers using different techniques in order to better understand compositional and structural impacts.

Characterization of treated and untreated wood fibers:

SEM images of the morphology of untreated wood, RWM and CRF samples were taken to investigate the structure of these fibers and are shown in Fig. 2. It was seen that the surface of the untreated wood fibers is very irregular due to the coating of cellulose fibers by lignin and other non-cellulosic substances (Fig. 2a). However, RWMs (Fig. 2b) after IL treatment have a relatively homogeneous macrostructure with more porosity possibly due to the fusing the wood fibers during IL pretreatment (Sun *et al.*, 2011). This implies that pretreatment with IL may reconstitute wood structure to render a more accessible surface area, leading to the enhancement of enzymatic delignification. Interestingly, CRFs obtained from enzymatic delignification have smooth and clean surfaces (data not shown) because most of the non-cellulosic materials (e.g., lignin) were removed during IL and biological treatments. It was also found that wood cell networks composed of cellulose, hemicellulose and lignin were broken down and cellulose fibers were partially separated into individual micro-sized fibers (Fig. 2d) like as cellulose fibers obtained from wood by chemical pretreatment (Chen *et al.*,

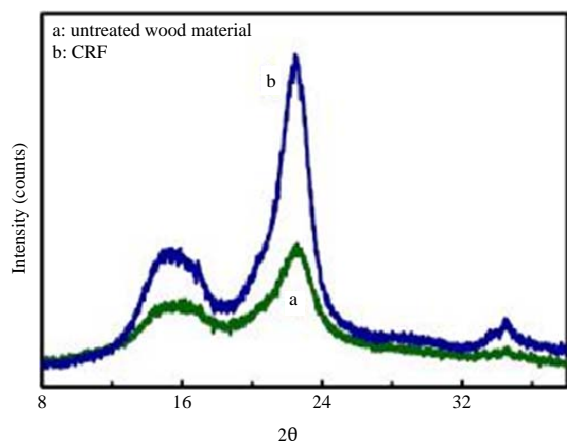


Fig. 3: Powder X-ray diffraction patterns for untreated wood materials and CRFs after enzymatically delignification of RWMs

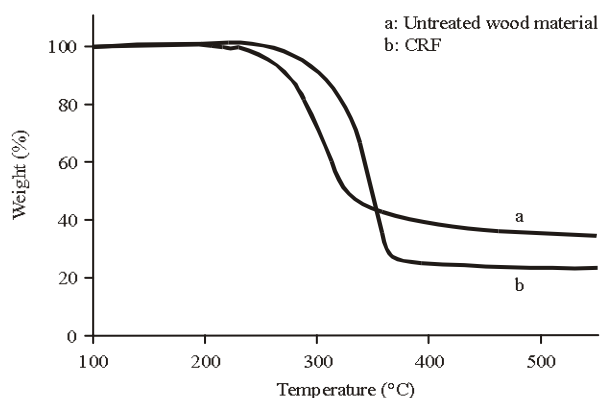


Fig. 4: Thermogravimetric analysis for untreated wood materials and CRFs after enzymatically delignification of RWMs

2011). Since the produced cellulose fibers became more separated into individual microsized fibers, they will provide more hydroxyl groups to attach functional groups during modification of cellulose form (e.g., acetylation). Note that modification of cellulose fibers to form their derivatives (carboxymethyl cellulose, hydroxyethyl cellulose, cellulose acetate) is required to use them in oil files.

The Powder X-Ray Diffraction (PXRD) patterns of the untreated wood materials and CRFs were conducted to investigate the crystalline behavior of the fibers and are shown in Fig. 3. Both samples showed major intensity peaks at 2θ values around 15.5 and 22.2° related to their crystalline structure. It has been shown that IL pretreatment did not change the crystallinity significantly of the wood fibers (data not shown). The intensity of IL treated sample was slightly increased compared to the untreated sample. This is in contrast with other published literature where it is reported that the degree of crystallinity of regenerated wood materials after

dissolution in ILs at higher temperatures reduced significantly (Lee *et al.*, 2009; Lucas *et al.*, 2011). Regarding the increased intensity in our study, one possible explanation is that the crystalline regions of cellulose were unaffected but amorphous parts are partially destroyed during IL pretreatment due to the mild pretreatment conditions (80 for 1 h). As shown in Fig. 3, the peaks around $2\theta = 15.5$ and 22.2°C are sharper for enzymatically treated wood fibers which are expected to represent typical cellulose I form (Alemdar and Sain, 2008). This result indicates higher crystallinity degree in the structure of the treated fibers which is undoubtedly due to the removal of most of hemicellulose and lignin through the IL pretreatment followed by enzymatic delignification (Alemdar and Sain, 2008; Chen *et al.*, 2011). The increase in the number of crystallinity regions enhances the rigidity of cellulose. Higher crystallinity in the isolated cellulose fibers is associated with higher tensile strength of the fibers which would be beneficial for producing high strength composite materials.

To determine any chemical changes of wood materials that occurred during IL pretreatment as well as enzymatic delignification, FTIR spectra of the untreated, RWMs and CRMs samples were measured and are compared (data not shown). The dominant peaks at ca. 3346 cm^{-1} (O-H stretch) and ca. 2892 cm^{-1} (C-H stretch) represent the aliphatic moieties in major biopolymers of wood materials. The prominent peak at 1731 cm^{-1} in the untreated wood materials is attributed to a C = O stretching vibration in acetyl groups of the hemicelluloses (Labbe *et al.*, 2005). After IL pretreatment, this peak disappeared, indicating that some of hemicellulose was removed in the recovered step. The lignin characteristic peaks at $1592/1503$ (C = C stretching vibration) and 1256 cm^{-1} (asymmetric bending in CH_3) and 1251 cm^{-1} (C-O vibration in the syringyl ring) (Labbe *et al.*, 2005) were remained with lower intense (compare to untreated sample) in the RWM sample, indicating that lignin in wood biomass was not removed significantly rather than swollen the biomass during IL pretreatment. However, these peaks disappeared after enzymatic delignification due to the removal most of lignin. The absorbance bands at 1150 , 1052 and 896 cm^{-1} , corresponding to C-O-C asymmetric bridge stretching vibration in cellulose/hemicellulose, C-O stretching vibration in cellulose/hemicellulose and C-H deformation vibration in cellulose, were more resolved in CRM sample, indicating that produced cellulose rich wood fibers are richer in carbohydrates what is consistent with our chemical composition study.

Investigation of the thermal properties of the natural fibers is important in order to gauge their applicability for biocomposite processing, in which processing temperature for thermoplastic polymers rises above 200°C . The TGA results obtained for untreated and CRF wood chips are shown in Fig. 4. These results clearly illustrate that the thermal stability of the wood fibers increases after IL treatment followed enzymatic delignification. After heating to 550°C , the fibers

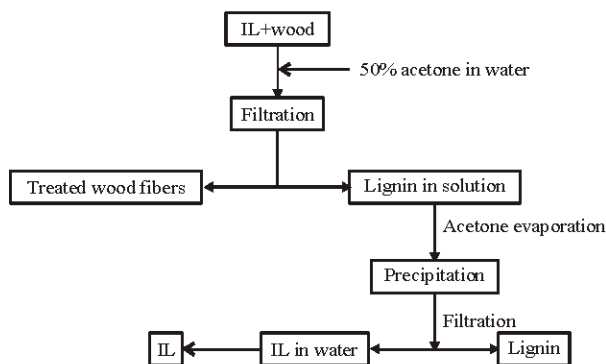


Fig. 5: Simplified schematic diagram for recovery of IL (emim) (OAc)

residue of all three samples remained, indicating the presence of the carbonaceous materials in the wood biomass in the nitrogen atmosphere. However, there is a distinction between the amounts of the residues of the fibers remaining after 550°C heating for untreated and treated wood fibers. It can be concluded from these results that the higher temperature of thermal decomposition and lesser residual mass of the fibers obtained after IL-enzymatic treatment has been related to partial removal of hemicelluloses and lignin from the fibers and higher crystallinity of the cellulose rich materials. These results are very consistent with results obtained from the XRD studies.

Recovery and reuse of IL: IL (emim) (OAc) was recycled and reused (Fig. 5). To this approach, after evaporation of the acetone from wood and acetone-water mixture and filtration of the lignin precipitate, the aqueous solutions were dried using a rotary evaporator and then a vacuum oven (12 h at 80°C). Over 90% of IL was recovered and reused for a second pretreatment and separation process in the same manner as in the first cycle.

CONCLUSION

This study described a highly effective and clean pretreatment method for isolation of cellulose fibers from wood biomass with minimum structural alteration. The delignification efficiency of IL treated wood was improved notably in presence of 2.5 wt% IL in aqueous system. Characterization studies clearly indicated that although IL pretreatment did not change the cellulose chemical composition, crystal structure and thermostability significantly, it did change the structure to render a more accessible surface area, leading to enhanced enzymatic delignification. Obtained cellulose fibers had a higher degree of porosity and thermal stability than that of the native wood fibers. We confidently believe that produced natural cellulose fibers from wood-the most abundant lignocellulosic resources on the world-have useful applications in oilfields.

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