Structural changes of PEG assisted 1-butyl 3-methylimidazolium chloride pretreated sugar cane bagasse for enhanced enzymatic hydrolysis

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Abstract
In this study, sugar cane bagasse was pretreated by [BMIM]Cl and polyethyleneglycol (PEG) to enhance the enzymatic conversion and consequently the fermentation yield of the lignocelluloses. The structural changes of the raw and PEG assisted ionic liquid pretreated lignocellulosic sample was investigated to understand that how this combination is efficient for lignnocelluloses pretreatment. The process was optimized and the structural analysis was conducted on samples pretreated at optimum condition. Fourier transform- infrared spectroscopy was mainly used to evaluate the various structural changes in the sugar cane bagasse.

Keywords: Ionic liquid, Polyethylene glycol, pretreatment, lignocelluloses, Ft-IR

Introduction

Pretreatment of lignocelluloses is an essential step in the production of bioethanol from the lignocellulosic biomass which is the most abundant carbon resource on the Earth [1]. Different pretreatment methods have been proposed for the lignocelluloses pretreatment catagorized as physical, chemical, or physico-chemical methods. Among the chemical methods, ionic liquids have been suggested as efficient agents for the pretreatment of lignocelluloses [2]. The capability of various ionic liquids for pretreatment of lignocellulosic biomass has been evaluated in many studies [1, 3, 4] and the resulted structural changes of the pretreated lignocelluloses were evaluated through different methods. Ft-IR, raman spectroscopy, XRD, SEM, NMR are the most commonly used analysis for tracking the structural changes. In this study we applied 1-butyl-3-methyl imidazolium chlordie and PEG for the pretreatment of sugarcane bagasse (SCB) as the model lignocelluloses and Ft-IR for assessing the variation in chemical bonds and cellulose type of the pretreated SCB.

There are four different cellulose crystalline allomorphs identified by their characteristic X-ray diffraction (XRD) patterns and solid-state 13C nuclear magnetic resonance (NMR) spectra: celluloses I, II, III and IV. Cellulose I is the most abundant form found in nature. Cellulose II can be prepared by two distinct routes: alkali treatment and regeneration of solublized cellulose. Celluloses IIII and IIIII can be formed from celluloses I and II
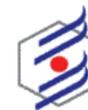
reversibily, respectively, by treatment with liquid ammonia. By heating celluloses IIII and IIIII, celluloses IVI and IVII are produced, respectively [5].

The optimization of the process has been reported in our previously published paper [6], here the main structural changes in Ft-IR spectrum due to pretreatment is studied.

Experimental
Materials
The Iranian Research Organization for Science and Technology (IROST) provided sugarcane bagasse (SCB). A cutter mill (Moulinex, AR1044) was used for SCB grinding. A cutter mill (Moulinex, AR1044) was used for SCB grinding. Different mesh number sieves were used to size SCB in three particle sizes named as fine, medium and coarse. Fine particles which were smaller than 0.21 mm were obtained by sieve of mesh number 70. Medium particles which were in size range between 0.21 and 0.595 mm were separated by sieves of mesh numbers 30 and 70 and coarse particles that were in size between 0.595 and 1.41 mm were provided by using sieves of mesh numbers 14 and 30. These different particle sizes of SCB were kept at room temperature in capped containers. Glucose, xylose, phloroglucinol, sulfuric acid (98%) and glacial acetic acid were purchased from Merck. Ionic liquid of [BMIM] Cl, Celluclast 1.5 L and Novozyme 188 enzymes were purchased from Sigma-Aldrich.

Ionic liquid pretreatment
0.5 g pre-milled SCB based on dry weight was pretreated with IL. The samples were soaked in PEG solution over night. The IL was added to the sample tubes until the ratio of IL to solid content was 10:1. The mixture was heated in an oil bath according to the predicted optimum pretreatment conditions reported in Table 1[6].

Following pretreatment, 5 ml deionized water was added to the reaction mixture. The solution was mixed and centrifuged (Vifion, VF550) at 12000 rpm for 10 min. To remove the residual IL from the regenerated SCB, the biomass was washed 3 times with 10 ml deionized water. The wet regenerated SCB was then freeze-dried for 24 h before enzymatic hydrolysis and other analysis.

Table 1 characteristic of different samples evaluated for structural changes

samples	PEG per. (%)	Temperature (°C)	Time(min)	Particle size	Color of the line in spectrum
Raw SCB	-	-	-	-	Black
1	4.85	153	120	Fine	Blue
2	1.97	141	119	Medium	Red
3	3.08	148	99	Coarse	Green

Fourier transform infrared spectroscopy
Fourier transform infrared spectroscopy (FT-IR) was carried out using a Perkin Elmer system (Massachusetts, USA) to determine the chemical structure of the untreated and pretreated SCB. The samples were mixed with potassium bromide (KBr) and then pressed uniformly into a disc. The samples then were scanned at a range of 4000-450 cm^{-1} with a spectral resolution of 2 cm-1. The rubber band correction method was used for base line correction following the spectrum minima.
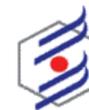
Results and discussion

Figure 1 shows the Ft-IR spectrum of different raw and pretreated samples, the characteristics of which are reported in Table 1. In general, aaccording to this figure, a great change has occurred in the pretreated samples compared with untreated SCB. The first obvious change is the change in O-H stretching band in 3300 cm^{-1} which have moved to higher frequencies in all pretreated samples. This can be interpreted as an increase in the number of free hydroxyl groups and thus a decrease in intermolecular hydrogen bonding. The peaks appeared in 2870 cm^{-1} have changed to multiple peaks in pretreated samples which is the indicator of free cellulose fibrils in the structure. Some peaks apperaed between 2800 and 3300 cm^{-1} are not related to lignocelluloses structure, their apperance in the spectrum can be due to the remaining of IL in lignocelluloses structure which is in agreement with the FT-IR spectrum of pure 1-butyl-3-methyl imidazolium chloride. The peak at 1734 cm^{-1} related to ester bonds of acetyl, feryolul and p-comaryl between hemicellulose and lignin. However, this peak has enhanced in fine particles. Peak at 1512 cm^{-1} related to aromatic structure (C=C stretching in aromatic ring) has weakened in medium and coarse samples spectrum, however, for fine particles there is no obvious change. Peak at 1603 cm^{-1} related to the aromatic oscillation has been completely removed in the spectrum of sample 2, while it has enhanced for other samples. The shift in peak 1428 to 1420 cm^{-1} which means change in cellulose allomorph type from I to II, has not happened in none of samples. The peak at 1246 cm^{-1} has removed in medium and coarse particles. However, peak at 897 cm^{-1} has weakened in all samples without showing any shift.
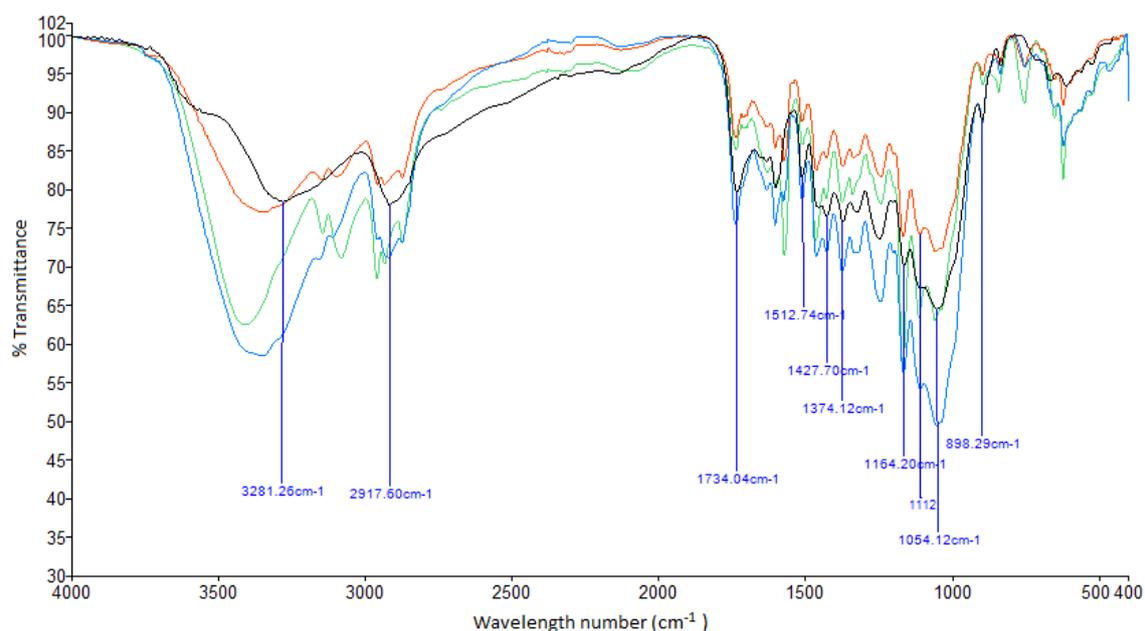

Figure 1 Ft-IR spectrum of raw and various pretreated sugar cane bagasse; sample 1 (blue), sample 2 (red), sample 3 (green)

Table 1 shows the values of lateral order index (LOI) and total crystallinity index (TCI) calculated from Ft-IR data as the method developed by Nelson and O'Conner [7]. The values of LOI and TCI are considerably decreased for pretreated samples.
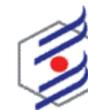
Table 1 the values of lateral order index (LOI) and total crystallinity index (TCI)

Sample	LOI	TCI
Raw SCB	6.32	0.88
1 (fine)	1.74	0.29
2 (medium)	3.35	0.42
3 (coarse)	3.89	0.49

Conclusions

This study shows that the structural changes of lignocelluloses due to pretreatment, in addition to the applied method is highly dependent on operational conditions of the process and the particle size. The Ft-IR spectrum of medium and coarse particles have shown more similarity in comparison to fine particles, despite the intense decrease in LOI and TCI of the fine particles.

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