Studies to optimize the process of biofuel production from castor stalk

Abstract: Lignocellulosic biomass is a rich source of cellulose and one of the most promising raw materials for the production of biofuels and other value added chemicals. However, its high lignin content and complex cellular structure represent a significant processing challenge. In this work, the effect of pretreatment using \([\text{EMIM}]\text{[Ac]}\) was studied at various process parameters in order to develop a cost-effective process. In order to minimize the loss of sugars in this process bulk of the solids, comprising both regenerated cellulose and undissolved particles were subjected to the enzymatic hydrolysis. Up to 96\% enzymatic digestibility was achieved, even with relatively coarse particle sizes (0.6–1.0 mm range), at 10\% biomass loading. The enhanced digestibility of CS is attributed to reduction in lignin content, crystallinity of the cellulose coupled with an increase in surface area.

Keywords: biofuel; castor stalk; enzymatic hydrolysis; ethyl methyl imidazolium acetate; ICGC-6; IL recovery.

Introduction

In view of increasing energy demands and rapidly depleting conventional fuel resources as well as their impact on climate change, there is a need to explore alternative renewable energy resources. Lignocellulosic biomass is one of the most promising alternative resources as it is highly abundant in many regions. To obtain value added chemicals and fuels from the lignocellulosic biomass, the components must be depolymerized into monomers. Enzymatic hydrolysis is preferred over other harsh methods of depolymerisation, such as acid treatment, as it is generally more environmentally benign and economical [1]. However it is difficult to hydrolyze the biomass directly, due to the highly complex structure of the lignocellulosic biomass, close association of lignin, carbohydrates and the crystalline nature of the cellulose present [2–4]. Therefore, it is necessary to pretreat the biomass to reduce this recalcitrance for an effective enzymatic hydrolysis [5–8].

Castor (\(\text{Ricinus communis}\) L.) is an important non-edible oil crop of the spurge (\(\text{Euphorbiaceae}\)) family. It is widely distributed throughout the tropics and subtropics [9]. The major castor cultivating countries in
the world are South America, India and Africa. Major castor oil-producing countries include Brazil, China, and India. India is the largest producer of Castor, with annual production fluctuating between 250,000 and 350,000 tons. India is a net exporter of castor oil, accounting for over 90% of castor oil exports, while the United States, European Union, and China are the major importers, accounting for 84% of imported castor oil [9, 10]. Castor oil and its derivatives, besides being employed in medicines, are used in a wide range of sectors such as agriculture and in the production of textiles, paper, high quality lubricants, plastics, rubber and pharmaceuticals. The oil produced from this crop is considered to be of importance to the global specialty chemical industry because it is the only commercial source of a hydroxylated fatty acid [11, 12]. Consequently, there has been a steady increasing demand for castor oil and its products in the market because of their renewable nature, biodegradability and eco-friendliness [13, 14].

Castor is highly drought tolerant and an ideal crop for arid and semi-arid regions which does not compete with other food crops. It imposes only low demand on soil fertility and requires only average rainfall. However, it is not foraged by animals and disposal of the plant wastes after oil extraction is a significant problem in oil producing countries [12, 15, 16]. The studies undertaken by Xiaoping et al. have shown that the physical, mechanical and chemical properties of the castor stalk depends on the position on the stem. Castor stalk has three regions viz, cortex, xylem and marrow (or pith) which are present in the ratio of 10.90%, 83.95% and 5.15% respectively, by weight. The cortex contains long and strong fibers while xylem and pith resembles hardwood and softwood respectively in the structural and mechanical properties [17, 18]. The mechanical properties such as elastic modulus, crystallinity and hardness are higher than those of the lower region due the presence of higher lignin content in the upper region [17]. All regions of CS have significant holocellulose (cellulose and hemicellulose) content, therefore, it has high potential as an alternative for the production of biofuel and other value-added molecules. Therefore, this study focuses on the optimisation of the pretreatment of castor stalk (CS) and its enzymatic hydrolysis, in order to demonstrate the possibilities to scale up for further processing to obtain biofuel or other valuable chemicals.

Recently ionic liquids (ILs) have emerged as potential solvents for the dissolution and pretreatment of lignocellulosic biomass. Pretreatments with ILs can be less energy demanding, more easy to handle and more environmental friendly than other pretreatment methods [19]. Among the ILs studied, 1-ethyl-3-methylimidazolium acetate ([EMIM][Ac]) is currently considered as the most effective pretreatment solvent, producing delignification of the biomass, reduction in the crystallinity of the cellulose and disruption of the overall structure of the fiber. In a study [20] on XRD patterns of untreated, pretreated avicel and three other feedstocks, it was shown that [EMIM][Ac] pretreatment resulted in native cellulose I lattice expansion and conversion to cellulose II. They have also showed substantially more rapid hydrolysis along with removal of lignin from the lignin-carbohydrate complex [20]. Rapid enzymatic hydrolysis was observed as a result of [EMIM] [Ac] pretreatment, due to partial removal of lignin and a decrease in the crystallinity of the various lignocellulosic substrates [20–22].

Various practical factors are thought to affect the efficacy of the IL pretreatment in terms of enhanced enzymatic hydrolysis [23–27]. An increase in the biomass loading, impacts the dissolution of the biomass and affects the further enzymatic digestibility of the biomass [28, 29]. However, in an industrial process, the biomass loading and particle size along with recovery of the IL and time of pretreatment are critical factors accounting for the cost of the process, therefore in order to optimize the pretreatment process for a particular IL and biomass combination, these parameters need to be studied in detail [30]. The previous reports on the effects of particle size reduction on the enzymatic digestibility have shown increases in the hydrolysis rate upon size reduction [31, 32].

In order to optimise the process for commercial production of sugars and other value added products including ethanol, there is requirement for a systematic study of different process parameters. A detailed study was carried out on the pretreatment of CS with [EMIM][Ac] at various parameters considering both the practical applicability of the process and the yields of the sugars obtained. The lack of a systematic study, considering all the major parameters at the same time and effective recovery of the IL, has motivated the authors to target optimising a process for ethanol biofuel production from lignocellulosic biomass. In this paper, the impact of biomass loading, biomass particle size and reaction time on the chemical and physical
properties of the biomass was analysed, demonstrating that an effective pretreatment can be achieved at surprisingly coarse particle sizes. Along with the chemical and physical properties, the efficiency of the process was assessed by measuring the enzymatic digestibility of the pretreated biomass and the recovery of the sugars. Methods for the successful recovery of the [EMIM][Ac] have also been investigated and are reported to further improve the commercial applicability of the process.

Materials and methods

Biomass and chemicals

Castor stalk was first washed and dried in a vacuum oven at 85 °C for 24 h before being ground to different mesh size ranges (0.3–0.6 mm, 0.6–1.0 mm, 1.0–2.0 mm). Ground castor stalk samples were stored in a dry cabinet prior to use. The IL, 1-ethyl-3-methylimidazolium acetate ([EMIM][Ac]) was purchased from Sigma-Aldrich. Cellulase enzyme, Ctec2 (Novozyme) was provided by RIL (150 FPU/mL). Citric acid and sodium citrate were purchased from Sisco Research Laboratories, India and sulphuric acid (98%) was purchased from Merck, India.

Pretreatment of CS

CS was incubated with [EMIM][Ac] with biomass loadings of 5%, 10% and 15% at 100 °C at different time intervals (2, 4, 6, 8 and 10 h). In a typical pretreatment experiment, 0.5 g of powdered CS was suspended in 9.5 g of IL in a round bottom flask and the mixture was stirred and heated using a hotplate at 100 °C.

After incubation, the solution was cooled to room temperature. The cellulose was precipitated by adding 10 times the volume of deionized water to the mixture (v/v) with vigorous stirring for 1 h in order to increase the polarity of the medium. The pretreated biomass was collected by vacuum filtration which contained both regenerated biomass as well as undissolved portion of the biomass and thoroughly washed with deionized water and then oven dried at 80 °C for 12 h.

Compositional analysis of castor stalk

The carbohydrate and lignin contents of castor stalk samples were determined according to the standard NREL analytical procedure. Briefly, the samples were treated successively with 72% sulfuric acid at 30 °C for 1 h and 4% sulfuric acid at 121 °C for 1 h. The monosaccharide composition and contents were determined by HPLC equipped with a Bio-Rad Aminex HPX-87H column and utilizing refractive index detector. The mobile phase consisted of 5 mmol/L sulfuric acid aqueous solution and maintained a flow rate of 0.6 mL/min. The column temperature was kept at 60 °C. The cellulose and xylan contents were calculated from glucose and xylose contents multiplied by conversion factors of 0.90 and 0.88, respectively. The content of acid-insoluble lignin was determined gravimetrically by using filtering crucibles. The content of acid-soluble lignin was measured spectrophotometrically at 320 nm using the extinction coefficient of 30 L g⁻¹/cm [33].

Powder X-ray diffraction (PXRD)

The Powder X-ray diffraction (PXRD) patterns were obtained at 22±2° using a powder diffractometer (Philips PW3040/60 X'pert PRO, The Netherlands). For each XRD experiment, approximately 1–2 g of finely ground sample was used. Cu Kα1 radiation (λ = 1.540 Å) was produced at 40 kV and 25 mA. The data were collected
in the Brag–Brentano ($\theta/2\theta$) horizontal geometry using a $2\theta$-range of $3^\circ$ to $50.0^\circ$ with a step size of $0.02^\circ$ and an accompanying scan rate of $0.5^\circ$/min. The fraction of crystalline material in the sample is referred to as the crystallinity index (CrI). The CrI was calculated using the following formula:

$$\text{CrI} (%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (1)$$

where $I_{002}$ is the intensity of the crystalline peak at $2\theta$ in the range of $20–23^\circ$ and $I_{am}$ is the “valley” intensity of amorphous cellulose, hemicellulose and lignin at $2\theta$ in the range of $17–19^\circ$ considering the shift of peaks.

**Scanning electron microscopy (SEM) analysis**

SEM analysis was carried out using a JEOL JSM-840 scanning electron microscope. Prior to examination, samples were platinum sputter-coated to render them electrically conductive. All micrographs were taken at 30 000× magnification.

**Confocal LASER scanning microscopy (CLSM) analysis**

In situ CLSM analysis was carried out using LSM 5 EXCITER laser scanning microscope. For the analysis, samples of biomass treated with IL ([EMIM][Ac]) were taken at different pretreatment times and observed under the microscope.

**Enzymatic hydrolysis**

The cellulase-catalyzed hydrolysis of the different cellulose substrates (untreated or pretreated) was carried out in stirred flasks. In a typical hydrolysis reaction, pretreated biomass was added to 5 mL of citrate buffer (50 mM, pH 4.8) having Cellulase activity at 40 FPU/g loading and incubated for 24 h (50 °C; 200 rpm). Control experiments without enzyme were also carried out. Five hundred microliters of the reaction medium was withdrawn in order to determine the progress of the reaction which was stopped by incubating the withdrawn sample at 90 °C for 10 min to deactivate the enzyme [31]. Then, the sample was diluted in ultrapure water and filtered (0.2 μm) prior to analysis by HPLC.

The enzymatic digestibility/glucan conversion was calculated by using the following formula:

$$\text{Glucan conversion} (%) = \frac{\text{Glucose produced via enzymatic hydrolysis} \times 0.9}{\text{Glucan in pretreated biomass}} \times 100 \quad (2)$$

**Thermogravimetric analysis**

Thermal analysis was performed by using a thermogravimetric analyzer (DTG-60A, SHIMADZU). Samples of 5–10 mg placed in alumina crucibles were heated from 30 to 600 °C at a rate of 10 °C/min in the presence of nitrogen (30 mL/min).

**Fermentation experiments**

IL treated biomass were enzymatically hydrolyzed and the hydrolysate was concentrated to 20 mg/mL using a rotary evaporator. Fermentation was carried out using wild type yeast strain BY4741 at 30 °C in a shaker incubator for 24 h. Fermentation yields were determined using HPLC.
Results and discussion

This study highlighted the importance of the process parameters on the chemical composition, digestibility, crystallinity and surface morphology of the biomass. Furthermore, the thermal stability of CS pre- and post-IL treatment was also investigated to explore deconstruction and other physicochemical changes occurring in the CS. Confocal microscopic imaging was carried out for the first time, to visualize the in situ effect of IL on the biomass fibers during the pretreatment. Additionally, the resulting sugars, after the enzymatic hydrolysis of pretreated CS, were fermented, and the ethanol yield was calculated. After pretreatment the used IL was successfully recovered, giving potential scope for this process to be applicable at an industrial scale.

The effect of various pretreatment conditions on the chemical composition, enzymatic digestibility and physical structure of the biomass

The resulting biomass posttreatment was rich in the cellulose content compared to untreated biomass (as shown in the Tables S1–S3 in Supplementary Information), with the partial removal of lignin from the biomass. Lignin and xylan remained in the solution after treatment with anti-solvent. The precipitated biomass and undissolved portions after treating with anti-solvent, contained a higher proportion of glucans. Firstly, the impact of each parameter on chemical composition and enzymatic digestibility of CS (also referred to as cellulose conversion in this report) has been described.

Further these changes were explained by investigating the changes occurring in the crystallinity and surface morphology of the biomass.

Impact of different biomass loadings

Impact on chemical composition and enzymatic digestibility

It is important, to the economics of an industrially viable process, to utilize the combination of a high-solids pretreatment at large particle size to improve the process economics by increasing sugar and ethanol yields while decreasing capital costs along with the recovery of ionic liquid [34–36].

However, utilizing high-solid loadings in this conversion process is still relatively new, and more detailed investigation is required to overcome certain challenges, such as high concentrations of inhibitors and equipment to handle mass transfer limitations that are not as apparent at the low- and moderate solids loadings [37]. Different biomass loadings were utilized to investigate the efficacy of the IL pretreatment. The temperature of the reaction mixture was kept relatively low at 100 °C, taking into account the stability of the IL, and the pretreatment time was varied from 2 h to 10 h at the interval of 2 h. The biomass loading was increased from 5% to 15% (w/w) with the interval of 5.

A techno-economic analysis, done by Klein et al., has shown that increasing the biomass loading is more important than increasing the rate of IL recycling, as it brings several simultaneous advantages including lower capital cost, lower electricity use, and lower working capital required [38–41]. Increasing the biomass loading to 40–50 wt% during IL pretreatment was shown to have a prominent effect on the overall economics of bio-refinery operation. However, Cruz et al. reported significant drop in the glucan digestibility of glucan present in the biomass at these biomass loadings [28, 42]. The aim of the current study was to assess the maximum biomass loading that can be achieved while retaining high pretreatment efficiency for a given amount of IL.

The increase in the glucan digestibility is an important marker of lignocellulose pretreatment quality. Glucose is regarded as a major product of bio-refineries for its use in fermentation process to produce biofuels and other chemicals [43, 44]. The pretreated CS was subjected to hydrolysis by commercial CTec2 enzyme at 40 FPU/g loading for 24 h at 50 °C and it was observed that the pretreated CS was significantly more digestible than the untreated CS in all sets of experiments.
As expected with the increase in the biomass loading, the biomass digestibility was decreased as a general pattern (Fig. 1), similar to the relative glucan content present in the pretreated biomass (Table S1). Interestingly, the glucan recovery was more at 10 % biomass loadings as compared with 5 % biomass loadings in the pretreated biomass for the lesser pretreatment periods (up to 6 h). Lignin removal was most efficient in the lower biomass loadings while for high biomass loading of 15 %, the lignin removal was significantly lower. In the pretreated biomass, the lignin removal was 18.4 % and 14.5 % respectively for 5 % and 10 % biomass loading after the pretreatment time of 10 h with a particle size of 0.3–0.6 mm. It is interesting to note that the cellulose conversion after 24 h of enzymatic hydrolysis for 10 % biomass loading was similar to that of the 5 % biomass loading with values of 96 % and 97 % respectively (Fig. 1). This aspect can possibly reduce the

![Graphs showing enzymatic digestibility of biomass](image)

**Fig. 1:** Enzymatic digestibility of the biomass of particle size 0.3–0.6 mm (a), 0.6–1.0 mm (b) and 1.0–2.0 mm (c), after pretreatment with [EMIM][Ac] for various time intervals with 5 %, 10 % and 15 % biomass loadings and 100 °C temperature.
cost of the process by employing higher biomass loadings. It was observed that there was a significant drop in the biomass digestibility at 15% biomass loading, which could be explained by the less efficient removal of the lignin, as a result of the pretreatment along with the other physical parameters. This suggested that the optimum biomass loading was 10% where there was no significant loss of sugars observed along with the high yields of sugars.

**Correlation with the changes in crystallinity and surface morphology of CS**

In order to investigate the mechanism of IL action on the digestibility of the cellulose at various pretreatment parameters, it is important to study the effect of IL treatment on the crystallinity and surface morphology of CS, because these properties are directly related to the accessibility of the enzymes into the highly organized structure of the biomass.

In particular, there remains a critical need to efficiently and economically break down the complex and highly recalcitrant structure consisting of tightly held cellulose, hemicellulose and lignin components in order to enhance enzyme accessibility. IL treatment causes the swelling in the biomass and disrupts the overall structure.

The lower crystallinity index indicates a higher amount of amorphous cellulose present in the biomass [45]. As expected the crystallinity index was found to decrease after IL treatment compared to untreated CS. However, at the 15% biomass loading the reduction in CrI was not significant which explains the low digestibility of the pretreated biomass and a reason of decreased enzymatic digestibility of the pretreated biomass (Fig. 2).

In scanning electron microscopy, the pretreated biomass showed a highly disrupted structure along with presence of non-uniform deconstructed form. At 15% biomass loading less deconstructed surfaces were observed, indicating less penetration of IL and presence of native structures (Fig. S1).

**Impact of different biomass particle size**

**Impact on chemical composition and enzyme digestibility**

Particle size is one of the crucial factors which directly impacts the contact and diffusion of ionic liquid into the lignocellulosic material and in turn, the solubilisation of lignocellulosic biomass. The effect of particle size on the pretreatment process was examined for three particle size ranges of castor stalk residue (0.3–0.6 mm, 0.6–1.0 mm and 1.0–2.0 mm) treated with ionic liquid at 100 °C for 24 h. The three particle sizes show certain influence on sugar conversion and yield of regenerated biomass (Fig. 2a and b). The relative glucan content was more in the pretreated biomass as compared to the untreated biomass due to partial removal of lignin. It was observed that the sugar conversion decreases with increasing of biomass particle size as a general trend. CS of larger particle size was not effectively solubilized in the ionic liquid. As expected at the largest particle size, 1.0–2.0 mm, there was a significant drop in the digestibility as well as in the sugar recovery. However, at relatively smaller particle sizes of 0.3–0.6 mm and 0.6–1.0 mm the ionic liquid pretreatment efficiently resulted in the high yields of sugars. As expected the highest amount of glucan was observed in 0.3–0.6 mm particle size CS at the lowest of biomass loading of 5% (after 10 h of IL pretreatment). However, at larger particle size (1.0–2.0 mm) the reduction in the lignin content was significantly low, 1.4% at 10% biomass loading. Lignin removal was significantly affected by the particle size of the biomass. This may be caused by the fact that the pretreatment of larger size particles of biomass could have the limitation of heat and mass transfer due to higher temperature gradient and increase in the diffusion path of ionic liquid penetrating inside the particle. While for the CS of the smallest particle size, the lignin removal was maximum 18.4% at 5% biomass loading at 10 h of pretreatment time. At the particle size of 0.6–1.0 mm the lignin removal was not as significant as that at smallest particle size, however the enzymatic digestibility was similar for both particle sizes, suggesting possible impact of reduction in CrI and disruption of biomass in enhancing the accessibility of the enzyme.
Previous studies showed that the larger size particle possesses lower bulk density therefore it requires more amount of ionic liquid contacting larger volume of biomass to achieve the same effectiveness with that of smaller size particle [32]. Moreover, the higher crystallinity index of untreated biomasses with larger size particles (Fig. 1) could directly decrease the sugar conversion.

From the results, it is apparent that there is a minimum particle size up to which the effect of particle size acts as limiting factor. In castor stalk it was 0.6–1.0 mm particle size above which the cellulose digestibility is greatly affected. This is an important result in terms of the economics of the process of biofuel production, as it can save the energy requirement for grinding. Biomass of particle size 0.6–1.0 mm was as efficiently digestible as biomass of particle size 0.3–0.6 mm up to a biomass loading of 10% (w/w). The chemical composition

![Crystallinity index (CrI) of the biomass of particle size 0.3–0.6 mm (a), 0.6–1.0 mm (b) and 1.0–2.0 mm (c), after pretreatment with [EMIM][Ac] for 2, 4, 6, 8 and 10 h at 5%, 10% and 15% biomass loadings.](image-url)
also influences the physical and mechanical properties, as the lignin content increases the hardness of the cell wall increases [46]. The cellulose content of the cortex region is highest 35.76\% and hemicellulose and lignin of the cortex are lowest, 7.23\% and 6.09\%, respectively. While lignin and hemicellulose of xylem are highest (13.43\% and 7.23\%) and the cellulose content is in between cortex and pith, 33.76\%. The chemical composition of the pith is; cellulose (32.9\%), hemicellulose (9.61\%) and lignin (8.77\%). The composition not only varies at different positions on the stem, such as lower stem region, upper stem region or branch region but also with harvest seasons [18, 47]. The composition reported by Li et al. [18] was cellulose (33.19\%), hemicellulose (11.12\%) and lignin (15.48\%).

**Correlation with the changes in crystallinity and surface morphology of CS**

Our studies indicate that smaller particle size and lower biomass loading result in lowering the crystallinity of the biomass, coupled with higher sugar conversion. This shows that the decrease of crystallinity disrupted the crystalline structure of cellulose leading to more accessibility of enzymes into the biomass leading to a higher content of sugar product. As shown in Fig. 2, at 5\% biomass loading the particle size of 0.3–0.6 mm and 0.6–1.0 mm showed CrI = 0.26 and 0.29, respectively while the particle size range of 0.1–0.2 mm showed CrI = 0.38 after 10 h of pretreatment time, suggesting ineffective penetration of IL into the crystalline form.

The pretreated biomass with particle size 0.6–1.0 mm showed a similar reduction in the CrI value to biomass of smaller particle size of 0.3–0.6 mm. This is consistent with the higher yields of sugars obtained for the particle size range of 0.6–1.0 mm with 10\% loadings. The pretreated biomass of 1.0–2.0 mm particle size range showed quite high values of crystallinity indicating poor accessibility of enzymes into the highly organized complex of lignocellulose resulting in lower yields of sugars.

Surface morphological studies with particle size of 0.3–0.6 mm for all biomass loadings and for 0.6–1.0 mm up to 10\% biomass loadings indicate mostly disrupted structures, thus facilitating the higher digestibility of the biomass, whereas for 1.0–2.0 mm particle size displayed a less disrupted surface morphology (Fig. S1).

**Impact of reaction time**

**Impact on chemical composition and enzyme digestibility**

Previous studies showed that prolonged pretreatment at high temperatures possesses the risk of degrading the dissolved cellulose into undesirable by-products [21], therefore it is necessary to optimize the duration of IL pretreatment. To investigate the effect of reaction time on CS pretreatment, castor stalk was stirred in IL at 100 °C for five different residence times viz. 2, 4, 6, 8 and 10 h. As expected, an increase in the digestibility was observed with increasing the pretreatment time (Fig. 1).

At particle size of 0.3–0.6 mm, the lignin removal was significantly higher, even at lesser duration of pretreatment at 5\% and 10\% biomass loadings, while at 15\% biomass loading the lignin removal was more at longer pretreatment duration (Table S1–S3). Long pretreatment time intervals were reported to favor lignin extraction for CS of particle size 0.6–1.0 mm, while for largest particle size the lignin removal was very less. As the pretreatment time was increased the diffusion of ionic liquid into the biomass was improved and in turn increased the dissolution and extraction of lignin from the biomass. However, the increase in the cellulose conversion was not significant in the biomass of larger particle size (1.0–2.0 mm). At smaller particle size there was a successive increase in the digestibility. The biomass of particle size of 0.6–1.0 mm showed similar cellulose conversion yields as in the biomass of particle size 0.3–0.6 mm at the pretreatment time periods of 4 h and above. After 6 h of pretreatment time and 10\% of biomass loading the biomass with particle sizes of 0.3–0.6 mm and 0.6–1.0 mm respectively showed 95\% and 94\% of cellulose conversion along with the glucan loss of 8.2\% and 8.4\%. This resulted the possibility of scaling up of the IL based pretreatment method due to high yields of sugars at 10\% biomass loading and lesser glucan loss (comparable to 5\% biomass loadings) at the end of 6 h heating time.
Correlation with the changes in crystallinity and surface morphology of CS

The crystallinity index decreases with the increase in pretreatment time. In the smallest particle size of biomass 0.3–0.6 mm, the effect of pretreatment time was the most significant, while for the biomass of larger particle size, it was least effective. In the biomass of particle size 0.6–1.0 mm, the CrI decreased significantly up to 10% of biomass loading supporting the high yields of glucan conversion (Fig. 1). In the surface morphology of the pretreated biomass, the more disrupted structures were observed for biomass of particle size 0.3–0.6 mm and 0.6–1.0 mm with increase in the pretreatment time.

Ionic liquid pretreatment effect on the thermal stability of the biomass

In order investigate the impact of the IL pretreatment on the thermal stability of the biomass, differential thermogravimetric analysis was carried out for the smallest particle size and lowest biomass loading as the representative set of parameters. Decreased biomass thermal stability was reported due to changes in cellulose crystal structure and degradation of biomass components after IL pretreatment [48]. Thermal properties vary among different cellulose sources as shown in the studies by Zhang et al. [48], where avicel samples exhibited higher decomposition temperatures due to the transformation from cellulose I into cellulose II, while samples of switchgrass and corn stover showed an improved thermal stability as a result of removal of minerals by [C4mim][OAc]. As a result of pretreatment the castor stalk samples showed two distinct thermal decomposition peaks (shown as $T_{\text{max},L}$ and $T_{\text{max},H}$ in Fig. 3). The presence of two distinct peaks could be explained by the previous studies carried out by Zhang et al. [48], where the peaks corresponding to the degradation of hemicellulose and the amorphous form of cellulose and crystalline cellulose, respectively, which further confirmed the significant impact of the IL on the structure of cellulose. Longer pretreatment decreased the thermal resistance due to the degradation of biomass components and decrystallization of cellulose [48].

In this experiment biomass of particle size 0.3–0.6 mm at 5% loading was taken as a representative sample for thermal analysis. Thermal decomposition of lignocellulosic biomass samples often presents two distinct peaks in the DTG due to the degradation of hemicelluloses, followed by cellulose [48]. Decomposition of lignin occurs in a wide range that overlaps partially with that of hemicelluloses and cellulose [49, 50]. In Fig. 3, the thermal decomposition peaks became clearly distinct due to partial removal of bound lignin from

<table>
<thead>
<tr>
<th>Biomass</th>
<th>$T_{\text{max},L}$ ($^\circ$C)</th>
<th>$T_{\text{max},H}$ ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>327</td>
<td>351</td>
</tr>
<tr>
<td>Pretreated for 2 h</td>
<td>300</td>
<td>351</td>
</tr>
<tr>
<td>Pretreated for 4 h</td>
<td>300</td>
<td>349</td>
</tr>
<tr>
<td>Pretreated for 6 h</td>
<td>298</td>
<td>347</td>
</tr>
<tr>
<td>Pretreated for 8 h</td>
<td>297</td>
<td>345</td>
</tr>
<tr>
<td>Pretreated for 10 h</td>
<td>296</td>
<td>339</td>
</tr>
</tbody>
</table>

Fig. 3: Differential thermogravimetric curves of untreated and IL pretreated CS of particle size 0.3–0.6 mm at 5% biomass loading.
the fibres in IL treated CS. The peaks shift to lower temperatures for the IL pre-treated samples, indicating lower thermal stability of the IL treated biomass due to disruption of crystal structure, disruption of biomass and partial delignification (Fig. 3).

** Ionic liquid separates lignocellulose fibres upon pretreatment **

Figure 4b shows a confocal image of the material during IL pretreatment of CS of 0.3–0.6 mm particle size at 5% biomass loading for 10 h while Fig. 4a shows untreated CS. It can be clearly observed that the IL treatment disrupts the structure in such a way that the fibres become separated and the particle size is also reduced. Thus it appears that the ions interact with the fibres, disrupting the hydrogen bonds and van der Waals forces present between the lignocellulosic fibres.

**Fermentation of sugars obtained from the enzymatic hydrolysis of pretreated CS**

In order to develop a commercial process, it is important to know the fermentability of the sugars obtained upon enzymatic hydrolysis of the IL pretreated biomass.

The enzymatic hydrolysate of pretreated biomass was subjected to *S. cerevisiae* fermentation and the yield of ethanol was measured using HPLC. It was observed that glucose obtained from biomass after IL pretreatment was equally fermentable as commercial glucose.

It has been reported that [EMIM][Ac] is toxic to *Clostridium* sp. fermentation at >2.5 g/L concentration, which is expected to be present as a residue after IL pretreatment [51]. Therefore, in this study, 2 g/L of [EMIM][Ac]

---

**Table 1:** Fermentation yields of various substrates.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Glucose (g/L)</th>
<th>Ethanol (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial glucose</td>
<td>20</td>
<td>7.1 ± 0.7</td>
</tr>
<tr>
<td>Commercial glucose + 2 g/L [EMIM][Ac]</td>
<td>20</td>
<td>7.0 ± 0.8</td>
</tr>
<tr>
<td>Enzymatic hydrolysate of IL pretreated CS</td>
<td>20</td>
<td>6.8 ± 0.6</td>
</tr>
</tbody>
</table>
was added to the fermentation liquor, in order to investigate any inhibitory effect on fermentation. It was observed that [EMIM][Ac] was not toxic to yeast fermentation and the same yield of ethanol was obtained (Table 1).

Ionic liquid recovery

In addition to biomass loading and particle size, the recovery of IL is an important measure for the development of a sustainable process for the biofuel production. In previous reports up to a maximum of 89% of IL was recovered with the impurity of water [52]. In our study 95% of IL was recovered by a method in which the filtrate was thoroughly mixed with silica gel (in acetonitrile) and then passed through a sintered crucible (see Suppl. Information for details). In addition, the reusability of ILs is also critical for practical applications, and thus was also investigated in this work. The remarkable increase in biomass loading coupled with IL reuse improves the outlook of using ILs in cost-effective pretreatment of lignocellulosic biomass.

Conclusions

We have shown that an IL pretreatment enriched the biomass in glucan content by partially removing the lignin. At 0.6–1.0 mm particle size, the pretreated CS was up to 96% digestible at 10% biomass loading, which was comparable to 5% biomass loading (97% digestible). This showed that IL pretreatment was effective for up to 10% biomass loading in this particle size range, by efficiently disrupting the complex structure, thereby producing more glucose. Relatively lower yields of glucose for the particle size 1.0–2.0 mm at higher biomass loadings could be explained by mass transfer limitation and low penetration of IL into the biomass. Upon treatment of CS in the IL, penetration of the ions into the cellular structure causes separation the fibrils of the cellulose, causing disruption of the structure, as shown by confocal images. Conversion of the crystalline form into an amorphous form was indicated by a reduction in the CrI. This resulted in increased enzymatic

Scheme 1: A sustainable process for biofuel production utilizing biomass waste.
digestedibility of the pre-treated biomass. Disruption of the structure was further confirmed by DTG curves. Of particular significance is the high digestibility at larger particle sizes and high biomass loadings. In this parameter dependant study we have demonstrated the basis of optimisation of an industrially viable process for utilisation of castor biomass, a globally important biomass source as shown in the Scheme 1.

**Associated content**

This material is available free of charge via the Internet at. Tables S1–S3, chemical composition of untreated and pretreated CS; Fig. S1, scanning electron micrograph images of untreated and pretreated CS; Fig. S2 and S3 proton NMR spectra of pure and recovered [EMIM][Ac], respectively.

**Acknowledgments:** The authors gratefully acknowledge Reliance Industry Limited for financial support and VK gratefully acknowledges the IITB-Monash Academy for the award of a postgraduate PhD scholarship. The authors acknowledge the central facility and SAIF, IIT Bombay for characterization facilities. DRM is grateful to the Australian Research Council for funding under the Australian Laureate Fellowship program.

**Author contributions:** The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

**References**

V. Kotia et al.: Studies to optimize the process of biofuel production from castor stalk


Supplemental Material: The online version of this article offers supplementary material (https://doi.org/10.1515/pac-2017-0406).