# Effect of Ionic Liquid Pretreatment on the Structure, Enzymatic Hydrolysis and Fermentation of Energy Cane Bagasse

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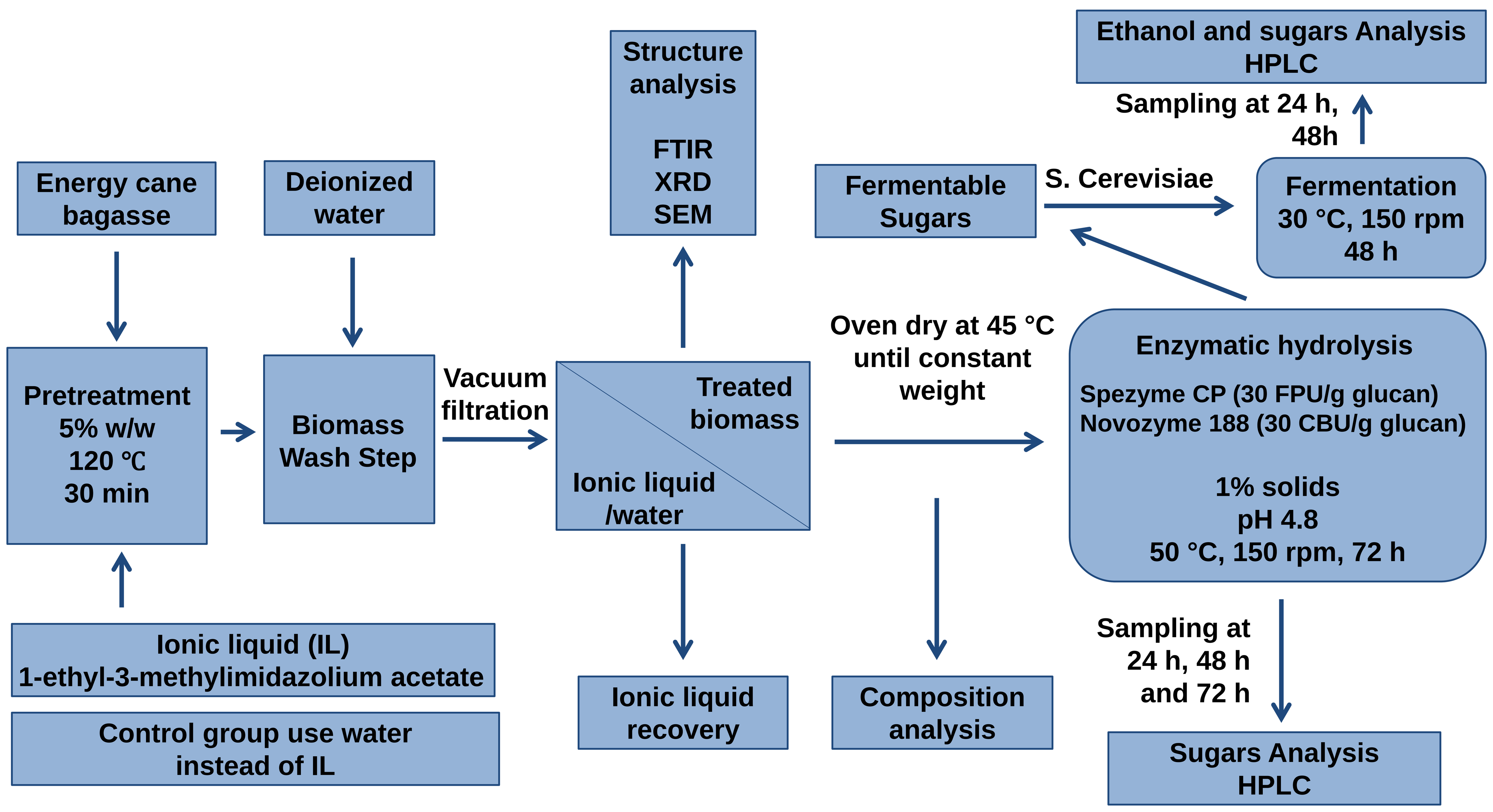
## Abstract

Ionic liquids (ILs) are promising solvents for the pretreatment of lignocellulose as they are thermally stable, environmentally friendly, recyclable, and have low volatility. This study evaluated the effect of 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) for the pretreatment of energy cane bagasse in terms of biomass composition, structural changes, enzymatic digestibility, and ethanol yield. IL-treated energy cane bagasse resulted in significant lignin removal with slight glucan and xylan losses, and exhibited a much higher enzymatic digestibility and ethanol yield than untreated or water-treated energy cane bagasse. The enhanced digestibilities of IL-treated biomass can be attributed to delignification and reduction of cellulose crystallinity as confirmed by FTIR and XRD analysis.

## Introduction

Ionic liquids pretreatment can reduce the crystallinity of cellulose and partially remove hemicellulose and lignin while not generating degradation products which are inhibitory to enzymes or fermenting microorganisms[1]. Pretreatment with ILs are less energy demanding, easier to handle and more environmentally friendly than other pretreatment methods such as mechanical milling, steam explosion, acid, base, or organic solvent processes[2]. Energy cane is a cold tolerant hybrid of commercial and wild sugarcane varieties bred for high fiber content and low sucrose[3]. This study aims to assess the effect of an acetate-based ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) during the pretreatment of energy cane bagasse by monitoring changes in biomass chemical composition (cellulose, hemicellulose and lignin), lignocellulose structure, enzymatic digestibility and ethanol yield.

## Materials and Methods



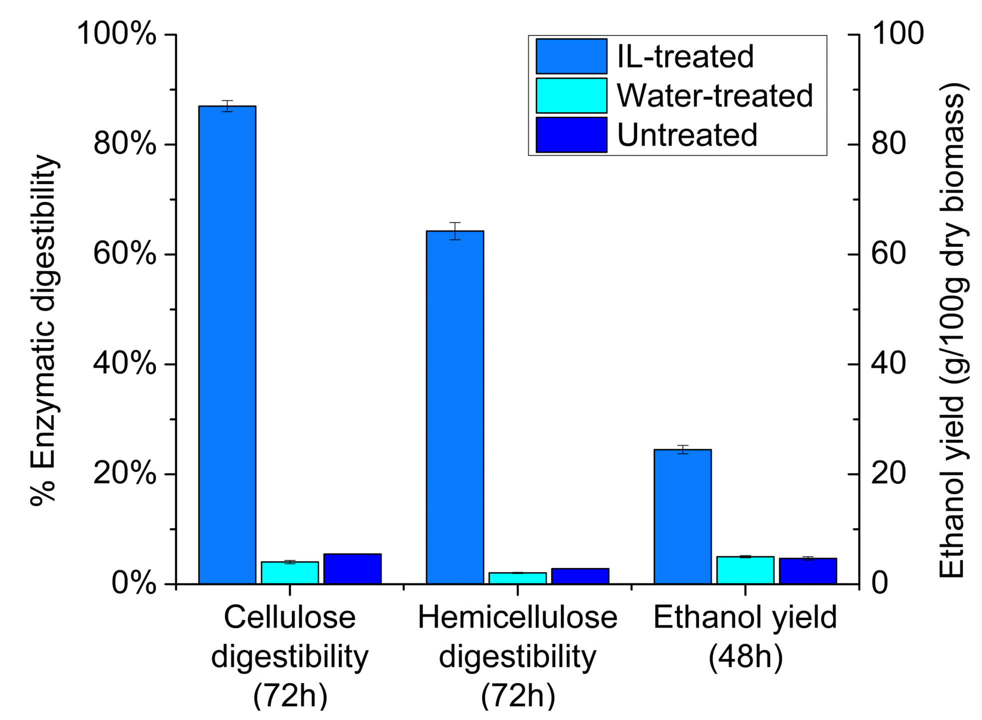

## Results and Discussion

- Composition analysis (Table 1) revealed that 32.0% of the initial lignin was removed in IL-treated energy cane bagasse, whereas only 2.3% of the initial lignin was removed in water-treated bagasse.
- IL treated samples retained 91.2% of the initial glucan and 86.0% of the initial xylan.
- Compared to a previous study on dilute ammonia-treated energy cane bagasse[4], IL pretreatment exhibited better xylan retention (86.0% versus 69.9%) and less lignin removal (32.0% versus 54.8%).

**Table 1. Chemical Composition Analysis**

| Biomass component (%, dry basis) | Untreated | Water-treated | IL-treated |
|---|---|---|---|
| Acid soluble lignin | 3.98 ± 0.08 | 4.15 ± 0.14 | 5.54 ± 0.36 |
| Acid insoluble lignin | 20.83 ± 0.22 | 21.10 ± 0.14 | 14.31 ± 1.06 |
| Total lignin | 24.81 ± 0.14 | 25.25 ± 0.01 | 19.85 ± 1.45 |
| Glucan | 40.87 ± 0.22 | 43.41 ± 0.27 | 43.89 ± 0.21 |
| Xylan | 20.82 ± 0.10 | 21.85 ± 0.18 | 21.10 ± 0.33 |
| Arabinan | 1.53 ± 0.05 | 1.59 ± 0.06 | 2.05 ± 0.27 |
| Recovered solids | 100 | 95.99 ± 1.67 | 84.89 ± 2.32 |

**Fig. 1 Enzymatic Digestibility and Ethanol Yield**

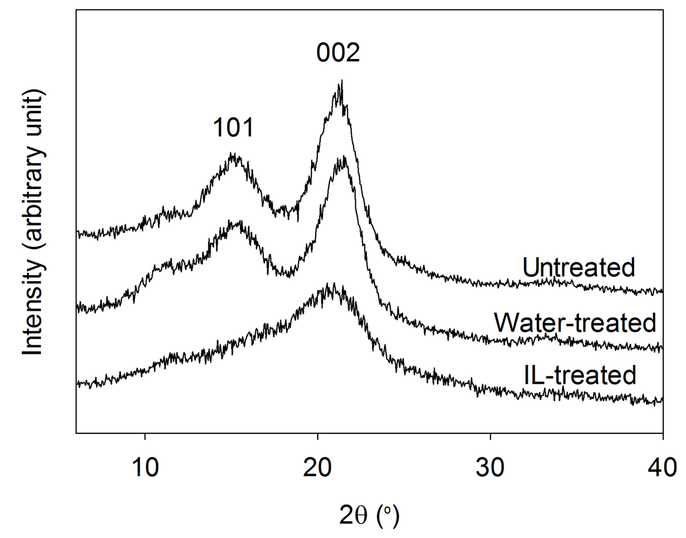



- IL-treated biomass exhibited significantly higher cellulose and hemicellulose digestibility (87.0% and 64.3%, respectively) than untreated (5.5% and 2.8%, respectively) and water-treated (4.0%, and 2.1%, respectively) biomass (Fig. 1).
- Ethanol yield of IL-treated biomass (24.5 g/100 g dry biomass) was significantly higher than water-treated (5.0g/100g dry biomass) and untreated (4.7g/100g dry biomass) biomass. Data comparable to the ethanol yield (23 g/100 g dry biomass) observed for dilute ammonia-treated energy cane bagasse[4].

**Table 2. Crystallinity Index**

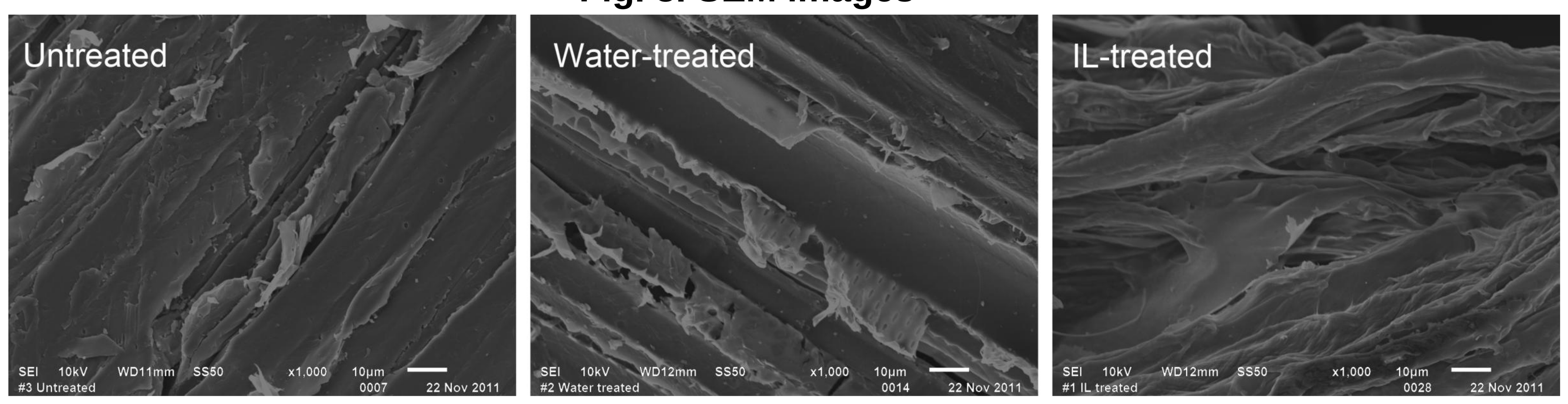
| Sample | Lateral order index (LOI) | Total crystallinity index (TCI) | Crystallinity index (CrI) |
|---|---|---|---|
| | FTIR (1426/896 cm$^{-1}$) | FTIR (1373/2917 cm$^{-1}$) | XRD |
| Untreated | 0.9593 | 0.4057 | 0.5628 |
| Water-treated | 0.8174 | 0.3747 | 0.5338 |
| IL-treated | 0.3718 | 0.1937 | 0.2452 |

**Fig. 2 XRD patterns**




- As shown in Fig. 2, two typical diffraction peaks were observed at 2θ=15° and 21°, which correspond to (101) and (002) lattice planes of crystalline cellulose type I. After IL pretreatment, the peak (101) disappeared and the peak (002) became broader and weaker.
- Compared to water-treated and untreated biomass, IL-treated biomass exhibited significantly lower LOI, TCI (based on FTIR data) and CrI (based on XRD data), which indicated a reduction of cellulose crystallinity (Table 2).
- In terms of surface morphology, IL-treated biomass exhibited a loose, disordered and curly structure; whereas, untreated and water-treated biomass both showed compact, ordered and rigid fibril structures (Fig. 3).

**Fig. 3. SEM images**




## Conclusions

IL-treated energy cane bagasse resulted in significant lignin removal (32.0%) with slight glucan and xylan losses (8.8% and 14.0%, respectively), and exhibited significantly higher cellulose and hemicellulose digestibility and ethanol yield (87.0%, 64.3% and 24.5 g/100 g dry biomass, respectively) than untreated (5.5%, 2.8% and 4.7g/100g dry biomass, respectively) and water-treated (4.0%, 2.1% and 5.0g/100g dry biomass, respectively) biomass. SEM images revealed a loose and disordered structure of biomass post pretreatment. FTIR and XRD analysis indicated that IL-treated biomass exhibited a significant loss of native cellulose crystalline structure.

## Acknowledgements

The authors would like to thank Dr. Lee Madsen, Ms. Chardcie Verret, Dr. Amitava Roy, Dr. Eizi Morikawa and Ms. Ying Xiao for their help with the analytical work.

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