CHAPTER 7

CONCLUSIONS AND FUTURE DIRECTIONS

7.1. CONCLUSIONS

This thesis gives emphasis on the production of bioethanol from lignocellulosic biomass using a "green" ionic liquid pretreatment process. Lignocellulosic biomass is renewable, cheap, worldwide available resource of biopolymers, which can be converted into liquid fuel either by thermochemical or biochemical pathways; alternate to those produced from non-renewable fossil resources. In this context, second-generation biorefineries that utilise lignocellulosic biomass as feedstock for the production of bioethanol are considered to be of primary importance.

Lignocellulosic biomass can be converted into liquid fuels using biochemical process, which involve mainly three steps; (1) Pretreatment; (2) Saccharification; (3) Fermentation. In lignocellulosic biomass, the cell wall components, i.e. cellulose, hemicellulose, and lignin are attached together in an intricate inter/intra molecular hydrogen bond network. This carbohydrate-lignin complex makes cellulose highly crystalline in nature, and have a high degree of polymerization. These all together makes lignocellulosic biomass recalcitrant in nature and inaccessible to cellulases to interact with cellulose and hemicellulose. Thus, pretreatment is a prerequisite step prior to enzymatic saccharification specific to overcome these restrictions, which improves biomass bio-digestibility, increase overall surface area and make it more accessible for cellulases for biomass conversion to biofuels.

The insight provided in this thesis could help industry and academic institutions to promote the concept of bioenergy services for the development of sustainable biofuels from lignocellulosic biomass and GHG reductions.

Following conclusions are drawn from the research work of the present thesis.

1. The detailed characteristics of various physicochemical properties of 10 agricultural biomass will facilitate the scientists, researchers and investors to choose the best feedstock and assist their biofuel potential. We found that holo-cellulose present in biomass plays an important role in deciding the bioethanol conversion. Physicochemical characterization

of all these biomass shows that cotton stalk, rice straw, and mustard stalk are the potential candidates for biofuel production due to their higher heating value, devolatilization, cellulose and hemicellulose content and are thus good for both biochemical and thermochemical conversion platforms. Although many of this biomass is found with a higher amount of inorganic salt yet, all of the biomass shows good potential for biofuels production. The highest content of polysaccharide present in corn cob and cotton stalk makes them a material of choice for bioethanol production. The higher heating value (calorific value) of the cotton stalk and jatropha pruning make them suitable for thermochemical conversion to biofuels, which may fulfil the biofuels demand.

2. Ionic liquids are molten salts below 100 °C comprising organic cations and organic or inorganic anions and have excellent solvation properties such as hydrogen bond basicity, hydrogen bond acidity, and polarizability and are liquid at room temperature. Certain imidazolium-based ILs, i.e. [C_2mim][OAc], [C_4mim][OAc], [C_2mim][Cl], [C_4mim][Cl] and [C_4mim][BF_4] were demonstrated to have a promising ability for efficient dissolution of lignocellulosic biomass and cellulose precipitation and regeneration upon the addition of an anti-solvent such as water. Initially, the study was optimised over Avicel, a pure cellulose, which was further conducted using real biomass samples. The study showed the cellulose transformation efficiencies in terms of improving the enzyme hydrolysis by five ILs and the effect of Kamlet-Taft parameters including hydrogen bond acidity, hydrogen bond basicity and polarity, viscosity and surface tension and their glucose yields had good correlation for better pretreatment efficiency. Among the Kamlet-Taft parameters, β-value (hydrogen bond basicity) stands as the best predictor and the first parameter to be considered while checking ILs pretreatment efficiency. We have found that $[OAc]^-$ anion based ILs such as [C_2mim][OAc] with $\beta > 0.72$ causes in the maximum reduction of CrI and gave 89.7% glucose yield after 72 h of cellulase hydrolysis and ILs with $\beta \leq 0.72$ like [C_4mim][BF_4] neither induced any structural transformation in cellulose nor improve glucose yields. With that we have also found that viscosity and surface tension have a negative correlation with glucose yields, lower the viscosity/surface tension higher the sugar yields in saccharification. This correlation is applicable only if IL has $\beta > 0.72$, hence, could also be an important parameter to predict the treatment efficiency. ILs with smaller anion and alkyl chain on cation is efficient for the treatment and enzymatic hydrolysis. If the variation is induced either in anion or cation, keeping the other constant, the surface tension and viscosity could be an indicator of treatment efficiency.

$[C_2mim][OAc]$ having higher β-value, lower viscosity, lower surface tension and smaller size of anion and shorter alkyl chain gave higher glucose yields. This study will help to design a novel IL with above properties to enhance cellulose in ionic liquid for efficient lignocellulosic biomass treatment and better saccharification.

3. After optimisation over avicel as cellulose substrate, we further conducted our study using real lignocellulosic biomass in which cellulose is embedded in the complex recalcitrance lignin-carbohydrate heterogeneous matrix which is highly recalcitrant in nature. For that, we have focused our study on the driving factors for effective ILs mediated pretreatment of two lignocellulosic biomasses such as wheat straw and mustard stalk. We have found a clear contribution of the ILs properties and optimum conditions for efficient hydrolysis of pretreated biomass and acceleration in enzymatic hydrolysis of pretreated biomass with higher hydrogen bond basicity of ILs; which facilitated the delignification, hemicellulose removal and significantly reduced the cellulose crystallinity with the transition from cellulose I to cellulose II resulting in more amorphous nature. A combination of temperature and the specific ILs was the factor responsible for allomorph transformation. Therefore, higher enzymatic hydrolysis was the consequence of primarily xylan removal supported by the lignin removal with cellulose structural transformation to cellulose II allomorph. Therefore, efforts should be made to customise ILs having more hydrogen bond basicity (β) either by external induction or by structural manipulation. Hence, IL pre-treatment could be customised to target the removal of hemicellulose and lignin both, as most of the methods developed, either target lignin or xylan preferentially. Removal of both of these recalcitrant could help to reduce the processing load and to improve glucose yields during commercialization.
4. IL pretreatment resulted in an alteration in structure and properties of regenerated biomass. We have found that IL pretreatment helps to reduce the cellulose crystallinity measured by LOI, TCI and HBI. LOI and TCI and a negative correlation of these parameters with glucose yield (R^2>0.80) were observed across five ILs at both the reaction temperatures 100 and 130 °C. Residual acetyl content also had a negative correlation with glucose yield (R^2=0.89). IL pretreatment helps to remove xylan and lignin and hence created pores and increases surface area, which is assumed to increase the accessibility of enzymes towards cellulose fibrils. The highest surface area was also achieved (7.05 m^2/g) after $[C_2mim][OAc]$ pretreatment resulting in highest glucose yield (97.7%) among the five ILs employed. The pore size distribution versus pore volume profile determined by DSC

showed all the pretreated biomasses to be predominately containing two pore sizes viz. 19 nm and 198 nm.

5. Imidazolium-based ILs are toxic for cellulases and yeast in downstream processing of biomass for bioethanol production. So, for economic and sustainable development the use of cheaper and biocompatible ILs are necessary. Hence, in the next study, we conducted ionic liquid pretreatment using biocompatible ILs, i.e. cholinium lysinate and ethanol amine acetate for biomass pretreatment. We have found that cholinium lysinate being highly basic in nature have the unique capability to dissolve lignocellulosic biomass and have higher biocompatibility as compared to imidazolium-based ionic liquids and are less toxic to cellulase and yeast. Hence, to better exploit, we have used three combinations of IL mixture [EOA][OAc]:[Ch][Lys]; i.e. 50:50, 80:20, 20:80 using cholinium lysinate and ethanol amine acetate to reduce the concentration of costly [Ch][Lys] for pretreatment for sustainable production of biofuel. We have found that cholinium lysinate being costly can be replaced by ethanolamine acetate by 50%, which is as effective as 100% pure cholinium lysinate. We have found that this [EOA][OAc]:[Ch][Lys] (50:50) mixture is much more efficient in removing a high amount of lignin and can be tolerated by the yeast upto 10% concentration.
6. ILs recycling study was conducted using compact membrane system using pervaporation methods. We have successfully recycled 95-95% of IL using 20% ionic liquid-water system using pervaporation method. The purity of recycled ionic liquid was monitored from conductivity measurement.

The overall study is helpful for understanding the properties of LCB and IL pretreatment followed by enzymatic hydrolysis of regenerated biomass for production of fermentation sugars. This study will be helpful for the understanding of the properties of lignocellulosic biomass for any process design and helpful to design biocompatible ionic liquids for biomass pretreatment for bioethanol production.

7.2 FUTURE DIRECTIONS

Several challenges remain to be overcome for ILs production processes with greater industrial value.

1. IL pretreatment technology is relatively recent and has demonstrated several advantages relative to the conventional pre-treatments methods, i.e. acid and alkali-based. At the current cost of the ILs, large-scale biomass processing seems unlikely to be economical in

the near future. Thus, a vast research is still strongly required for. Hence, a complete evaluation of IL pretreatment will further enhance our understanding of efficient conversion of lignocellulose materials.

2. Imidazolium-based ILs are toxic for enzymes and fermenting yeast and hence, further research will be focused on the use of protic and cholinium based IL utilisation in biomass processing in order to make IL-biomass processing completely ‘green’, low-cost ILs manufactured from renewable sources of carbon are needed.
3. Recovery and reusability of IL are still needed to be addressed after IL pretreatment. ILs are not yet ready for large-scale industrial application when it comes to cellulose processing. However, in order for the biorefinery concept to be successful, it needs to meet two criteria; firstly, the biomass processing needs to be efficient and cost-effective enough to compete with current energy alternatives.
4. Techno-economic analysis and modelling for IL deconstruction will be investigated to understand the overall benefits, cost analysis and environmental effects.

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