

EXECUTIVE SUMMARY

Economic growth, expanding population and accompanying structural changes strongly lead to increase in the world energy demand by 25% from 2014 to 2040. World's liquid fuel demand is expected to grow from 90 million barrels/per day (b/d) in 2012 to 100 million b/d in 2020 and to 121 million b/d in 2040. About 95% of current transportation energy needs are met by petroleum. The burning of these fossil fuels would result in increasing the worldwide energy-related CO₂ emissions from 32.2 billion metric tonnes (BMT) in 2012 to 35.6 BMT in 2020 and 43.2 BMT in 2040 (IEA 2016). Therefore, the transition from a fossil fuel-based economy to renewable fuel based economy will inevitably take place in the foreseeable future.

India is one of the fastest developing countries in the world with an ever-increasing economy and is projected to grow by 7.7% in the fiscal year 2017-18 (World Bank, Global Economic Prospects). The Ministry of Statistics confirms that the country still depends on coal and lignite for its energy requirements followed by petroleum.¹⁻² India imports around 80% of its fuel demand and transportation sector consume around 40% of oil demand. Hence, with the rapid depletion of our available reservoirs, rising environmental hazards and pollution; cheaper and clean energy resources should be considered and exploited to meet the nation's energy requirements and to diminish the greenhouse effect.

Bioenergy is the world's fastest growing energy source over the projection period to meet the increasing energy demand with environmental benefits. Currently, 10% of the world's energy demand is met by bio energy: Austria (7%), Finland (20%), Germany (5%) etc.³ The consumption of renewable will increase by an average of 7.6%/year between 2012 and 2040 (IEA 2017). Biorefinery can play a major role for developing countries, consuming a huge amount of fossil energy to reduce the fuel dependency. In 2014, biofuels provided 4% of world road transport fuel and are expected to rise slowly, reaching almost 4.3% in 2040.

First-generation bioethanol is mainly produced from sugar or starch based material, i.e. corn, wheat, sugarcane juice etc. These first-generation feedstocks supply is insufficient to meet the demand and is in competition with food, posing risks to national food security thus are not ethically acceptable. In this concern, lignocellulosic biomass (LCB) is considered as abundant and potential feedstock for second-generation biofuel production either as gaseous fuel, liquid and/or solid fuel to meet the increasing energy demands. According to a report published by Ministry of New and Renewable Energy (MNRE), India has an enormous biomass potential and produces around 500 Million Metric Tonnes (MMT) of biomass residues per year.⁴ These residues are a potential source of biomass feedstock for energy production.

LCB is biological plant-based materials, i.e. agricultural residues, forestry waste, algae, municipal waste, water bodies and is most abundant, available organic material for the production of liquid fuel, which can also help to mitigate the GHG emission for sustainable development. LCB mainly consists of three biopolymers, i.e. cellulose (30-55%), hemicelluloses (15-35%), lignin (10-30%), besides small amount of ash (1-20 %), extractives (1-15%) and proteins (1-2%).⁵⁻⁶ These biomass components in plant cell wall are organised into highly complex, non-uniform, three-dimensional heterogeneous matrix with different degree of polymerization relative to species, origin, feedstock and climate conditions. These components are architected together via strong inter/intramolecular hydrogen bonding, Van der Waals forces, dipole-dipole interaction, π - π bonding, covalent bonding and via many different cross-links, making a rigid lignin-carbohydrate recalcitrant matrix, which resists any biotic and abiotic degradation.⁵⁻⁶ This recalcitrance nature of LCB hinders the extraction of monomeric sugars for the production of ethanol.

Cellulose in LCB is a linear homopolymer of anhydrous-D-glucose subunits linked together by strong β -(1,4)-glycosidic linkage formed by condensation of glucose units.^{6,7} Cellulose has a high degree of polymerization $[(C_6H_{12}O_5)_n; n=10,000-15,000]$, which aggregate together in 20-300 pairs and forms cellulose microfibrils with a reducing end (hemiacetal hydroxyl group on the pyranose ring) and a non-

reducing end cross-linked with neighbouring chains via inter/intramolecular hydrogen bonding networks, making it highly crystalline in nature.

Therefore, utilisation of lignocellulosic material, which is free from food versus fuel debate is more practicable and sustainable to produce fuels and commodity chemicals. However, these lignocellulosic materials vary from one biomass to another in fibre morphology and chemical composition from one geographical location to another depending upon the climate condition, soil fertility and cultivational practices. Hence, complete physicochemical characterization of each biomass is necessary to determine the overall efficiency of the process prior to any biochemical or thermochemical conversion processes.

We have for the first time completely characterised ten agricultural residues available in North Indian region, i.e. rice straw, rice husk, cotton stalk, wheat straw, sugarcane bagasse, corn stover, sorghum stalk, mustard stalk, corn cob and jatropha pruning. Physical and chemical characterization of biomass samples were carried out by higher heating value, crystallinity index, thermal property, CHNS/O analysis, FT-IR, metal analysis and compositional analysis. We have found that, among all the biomass samples, corn cob has the highest content of cellulose and hemicellulose, i.e. 61.2% (w/w), making it the most potent feedstock for production of biofuels using biochemical process, whereas cotton stalk has relatively higher thermochemical potential due to its higher heating value (19.2 MJ/kg). Rice husk and rice straw have highest ash content, i.e. 17.4 and 13.7% (w/w), respectively, indicating a significant amount of undesirable material. The detailed characteristics of various physicochemical properties of these biomasses facilitate the scientists, researchers and investors to choose the best feedstock for biofuel potential.

LCB can be converted into liquid fuels using biochemical process, which involve mainly three steps; (1) Pretreatment; (2) Saccharification; (3) Fermentation. Pretreatment that costs about 20% of the total biomass to bioethanol conversion process is a pre-requisite and essential step to deconstruct the carbohydrate-lignin complex and make cellulose accessible for enzymatic attack. The associated drawbacks of chemical/ physical pretreatment methods have resulted in a surge to

look for milder and environmental friendly pretreatment agents. While no pretreatment technology can be called a winner due to the different composition and constitution of LCBs. Ionic Liquids (ILs) have been demonstrated as “green solvents” to address the issue of environmental safety and economic viability of pretreatment. These are organic salts, containing organic cation and anion with melting points of $<100\text{ }^{\circ}\text{C}$ and are mainly composed of organic cations (i.e. pyridinium, imidazolium and quaternary ammonium species) and organic/inorganic anion (i.e. halogen, acetate and some other polyatomic inorganic species).⁸⁻¹² These have unique solvation properties and are ‘eco-friendly’ because of negligible vapour pressure, non-flammability, good thermal and chemical stability.¹³⁻¹⁴

In this concern, different imidazolium and choline based ILs were selected by varying cation, anion, alkyl chain, which may be useful for biomass dissolution after a huge literature survey. On the basis of extensive literature survey, we have selected imidazolium and cholinium based IL from our study, i.e. [C₂mim][OAc], [C₄mim][OAc], [C₂mim][Cl], [C₄mim][Cl], [C₄mims][BF₄], [Ch][Lys] and [EOA][OAc] and measured their solvatochromic properties, i.e. hydrogen bond acidity (α), hydrogen bond basicity (β), solvent polarizability (π^*). We have also estimated the kinematic viscosity (η) and surface tension (σ) of these five ILs. A series of experiments with varying ILs, reaction time and temperature have been performed at “DBT IOC Centre for Bioenergy Research” for conversion of lignocellulosic biomass to fermentable sugars. The research was first carried out on crystalline cellulose solubilization, where, we have initially optimised our conditions over Avicel PH 101 (pure cellulose) as a model substrate for biomass. The effect of different cation, anion, IL’s solvatochromic properties was quantified under two conditions, $100\text{ }^{\circ}\text{C}/5\text{h}$ and $130\text{ }^{\circ}\text{C}/2\text{h}$. We have found that ILs treatment of crystalline cellulose (Avicel PH 101) significantly reduced the crystallinity, which resulted in a sharp increase of sugar yields after enzymatic saccharification. The results showed that crystalline cellulose treated at $130\text{ }^{\circ}\text{C}/2\text{ h}$ resulted in better glucose yields as compared to $100\text{ }^{\circ}\text{C}/5\text{ h}$. ILs comprising acetate anion resulted in

highest glucose yields and chloride based ILs performed moderately, whereas BF_4^- based IL was ineffective in transforming the cellulose structure. The results showed that, amongst all the properties of ILs, hydrogen bond basicity (β) is relatively more important than kinematic viscosity and surface tension for impacting the structural transformation and subsequent enzymatic hydrolysis. $[\text{C}_2\text{mim}][\text{OAc}]$ with high β value (1.32) and lower viscosity (4.4, cSt/sec) and surface tension (30.3, mN/m) was found to be the most efficient in cellulose transformation (cellulose I to cellulose II) resulting in higher glucose yields (89.8%) upon saccharification.

After physicochemical characterisation, ILs pretreatment optimisation and ILs properties evaluation, the study was further explored to deconstruct the actual biomass, i.e. mustard stalk and wheat straw for the production of fermentable sugars. This study helped to investigate the effect of IL cation and anion on pretreatment of different feed stocks. With that, we have investigated the driving factors responsible for improved enzymatic digestibility of various ILs treated biomass along with postulating a plausible mechanism thereof. Post pretreatment, mainly two factors impacted the enzymatic digestibility (i) structural deformation (cellulose I to II) along with xylan/lignin removal and (ii) properties of ILs; wherein, K-T parameters, viscosity and surface tension had a direct influence on pretreatment.

A systematic investigation of these parameters and their impact on enzymatic digestibility is drawn. $[\text{C}_2\text{mim}][\text{OAc}]$ with 1.32 β -value resulted in 97.7% of glucose yield using 10 FPU/g of biomass. A closer insight into the cellulose structural transformation explained the better digestibility. The impact of these parameters on the digestibility can pave the way to customise the process to make biomass vulnerable to enzymatic attack.

Pretreatment of any biomass helps to open the biomass structure by removing the xylan, lignin and alter the physicochemical behaviour of biomass, hence makes it accessible for enzyme attack to release the fermentable sugars. To prove this methodology, alteration in physicochemical properties of the IL pretreated mustard stalk were studied using Fourier Transform Infrared

Spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC) and SEM to have a mechanistic insight into the pretreated biomass structure. Cellulase enzymes are specific in nature and need to attach to the surface of biomass. This study helped to determine the possible reason to enhance the overall sugar released after ILs pretreatment. We have found that IL pretreatment helps to increase specific surface area, the porosity of biomass, and a reduction in biomass crystallinity. These all properties in together help to enhance the binding capacity of cellulase for improved sugar release. We have found that analysis of composition, cellulose and hemicellulose related properties, viz. total crystallinity index (TCI), lateral order index (LOI), hydrogen bond index (HBI) and an acetyl content of the IL pretreated biomass followed inverse relationship with enzymatic saccharification. The surface area, on the other hand, followed a linear relationship ($R^2=0.97$) with glucose yield (%). DSC helped to quantify the total freezing bound and non-freezing bound water present in the bulk biomass and in the pores. The study was based on the theory that the water present in the pores has a depressed melting point, which helped to find out the pore distribution in pretreated biomass. Interestingly, pore size as calculated by the enthalpy change during the melting of bound water showed all the pretreated biomass to be majorly containing two pore sizes, viz. 19 nm and 198 nm. In conclusion, we reported that among all ILs, a combination of [OAc]⁻ as anion and small alkyl chain [C₂mim]⁺ as cation, i.e. [C₂mim][OAc] was found to be the best for biomass dissolution and hence, gave maximum sugar yield (97.7%). Overall, the above results indicated that the polysaccharide enzymatic digestibility is closely correlated with substrate physicochemical properties.

The insight provided in this thesis could help national/regional governments and academic/international organisations for development and promotion of bioenergy services for the production of bioethanol for sustainable development.