CHAPTER 1

INTRODUCTION

In this chapter, an overview of primary energy demand and significance of transportation fuels in India is described along with the ethanol production technologies. With that, a brief overview of lignocellulosic biomass, their type, availability, chemical constituents, recalcitrance nature, with their biofuel potential and routes for conversion into biofuels are introduced. The concept of an ionic liquid for the valorization of lignocellulosic biomass as a green solvent along with the motivation, research objectives with proposed chapter is also described. In the end, the outlines of the thesis are provided.

1.1 PRIMARY ENERGY DEMAND AND TRANSPORTATION FUELS

1.1.1 GLOBAL PERSPECTIVE

The increase in industrialization has led to an increase in worldwide energy demand in the past three decades. The population is growing rapidly in many parts of the world and it is expected to reach approximately 8.5 billion by the year 2030 and 9.7 billion in 2050 (UNDP, 2013). Figure 1.1 shows that the total world energy consumption in 2012 was 1.384×10^{19} million tonnes of oil equivalent (Mtoe), and is estimated to reach 1.586×10^{19} Mtoe, and 2.055×10^{19} Mtoe in 2020 and 2040 respectively, almost a 48% increase from 2012 to 2040.[3]

Out of total global primary energy demand, the fuel exclusively accounts in terms of crude oil (31.3%), followed by coal (28.6%), natural gas (21.2%), biomass (10.3%), nuclear (4.8%), hydro (2.4%) other renewable (1.4%) (IEA 2016). Current world demand for crude petroleum is approximately 97 million barrels/day, which is estimated to increase to 116 million barrels/day by 2030, out of which transportation sector accounts for 60% (IEA 2016).

Burning of fossils fuels, i.e. coal, petroleum results in the emission of nitrogen oxides (NO_X), Sulfur dioxide (SO_2) and carbon dioxide (CO_2) etc., which are primarily responsible for GHG emissions and resulting to the climate change (IPCC, 2014). U.S. Energy Information Administration in 2015 reported that U.S. motor gasoline and diesel (distillate) fuel consumption released about 1,105 and 440 million metric tons of CO_2 respectively, for a total of 1,545 million metric tons of CO_2, which was equivalent to 83% of total U.S. transportation sector CO_2 emissions and equivalent to 29% of total U.S. energy-related CO_2 (U.S. Energy Information Administration).[15]

Hence, the concerns regarding the rising world energy demand, continuous depletion of fossil fuel reserves, negative impact on the environment due to GHG emissions and energy security due to international affairs, urge many countries to implement new alternative renewable energy policies. In this regard, many countries such as the US, Europe, Brazil, India etc. have mandated new biofuel policy as summarised in Table 1.1.
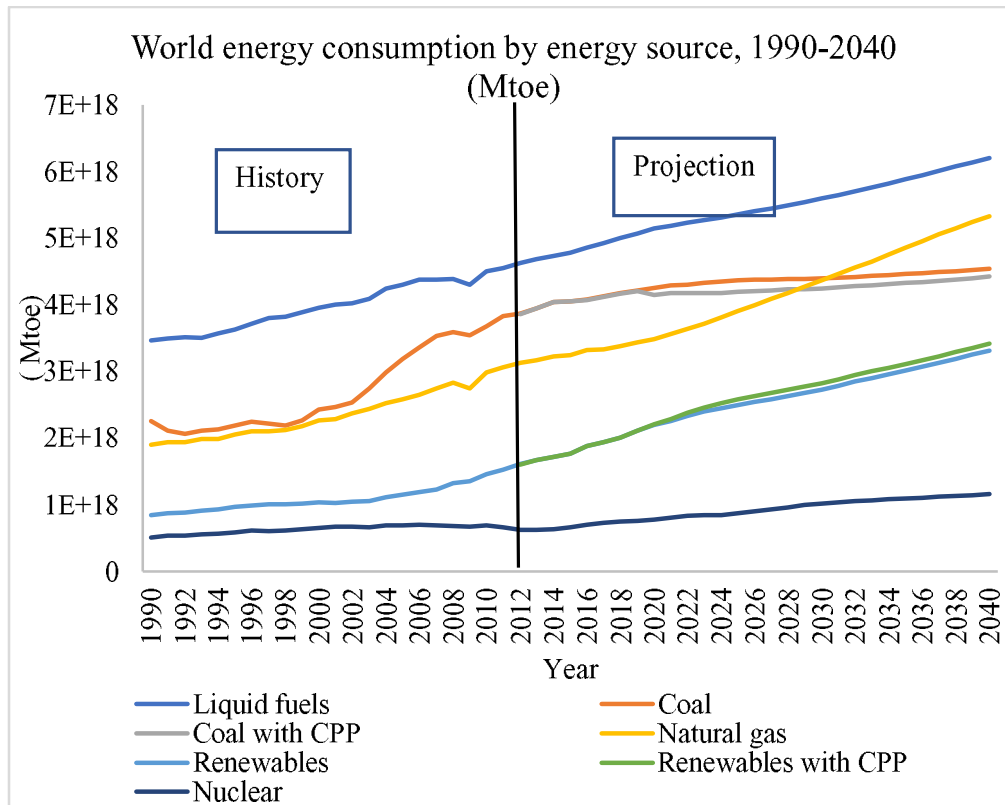
Figure 1.1 World energy consumption by energy source, 1990-2040 (Mtoe)

Biorefineries in this concern can provide a significant contribution to sustainable development by utilising the bio-based energy resources, i.e. forestry waste, energy crops, algae, municipal solid waste, agricultural residues etc. as an alternative to replace the fossil fuels for the production of liquid fuels, materials, power, and/or heat etc. It can also help to supplement the fossil fuel and can eventually result in independent future with bio-economy.

According to a report published by Sandia Laboratory, United States can produce 90 billion gallons of biofuels from plant-based feedstock using biorefinery to replace crude oil and currently using about 7.1% of biofuels in all of its transportation fleets. Similarly, European Commission in 2007 decided that out of total energy use, 20% to be achieved from renewable sources and with a target of 10% renewable energy use in transportation by 2020 (Renewable Energy Road Map, 2007). Similarly, the Indian government has also drawn an ambitious plan to utilised biofuels.

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Table 1.1 World fuel mandate

	Early policies	**Current policies**	**Planned changes**
EU	Biofuels Directive (2003/30/EC) required that 5.75% of all transport fuel by volume is biofuel by 2010. There were no sustainability considerations in the directive.	The Biofuels Directive was superseded by the Renewable Energy Directive (EU RED, 2009/28/EC), which suggested that ~20% of all energy delivered to EU consumers by 2020 comes from renewable sources. The EU RED does not specify the proportion that has to come from individual countries, the transport sector, or indeed from biofuel. Mandatory environmental sustainability criteria to reduce the use of land having high biodiversity value (e.g., rainforests) with an aim to reduce the transport fuels GHG emissions by 6% by 2020.	Incorporation of iLUC factor penalty for biofuels. Double or triple credits for second-generation biofuels, including those made from a lignocellulosic material, and algae.
USA	The US Renewable Fuel Standard (RFS1), effective from 2005, required 7.5 billion gallons (34 billion litres) of renewable fuel to be blended into gasoline (petrol) by 2012. There were no sustainability standards described for the biofuels.	Under the Energy Independence and Security Act (2007), the programme was revised (RFS2) and expanded to require 36 billion gallons of biofuels on the market by 2022. RSF2 includes the use of new energy feedstock to limit GHG thresholds for renewable fuels.	Several states (e.g., California) are adopting Low Carbon Fuel Standards, which have more stringent requirements than those of RFS2.
Brazil	Brazil National Alcohol Program (Pro´ -A´ cool) decreed by the President (Decreto No. 76.593) in 1975 set a goal of 3.5 billion litres of ethanol to be produced by		Brazil's 25% blend the mandate was increased to E27 in early 2015.

	1980. In 1979, the Brazilian car manufacturers signed an agreement with the federal government to produce vehicles that ran on ethanol only (rather than a fossil fuel blend). By 1984, the sale of ethanol-powered cars had reached 84% of total vehicle sales in Brazil. Bioethanol produced from sugar cane in Brazil has been reported to have a global warming potential (GWP) 70% lower than gasoline [14].		
India	GOI in 2009 mandated blending of 5% ethanol in gasoline and has an ambitious plan to blend 20% by 2017.		

1.1.2 INDIAN PERSPECTIVE

India's energy demand has been increased from 441 Mtoe in 2009 to 775 Mtoe in 2013 by 10% and is projected to increase to 1469 Mtoe by 2030 (IEA, 2014). Oil consumption surged to 3.8 million barrels/day (mb/d) by 2014, out of which 40% was used in transportation sector.[16] Figure 1.2 shows that out of total 775 Mtoe use of energy in 2013, coal and petroleum accounted for 67% followed by biomass (24%), natural gas (6%), nuclear (1%), hydroelectric (2%) and other renewable (1%). At the end of 2013, India consumed about 141 MMT of crude oil, out of which 77% was imported, indicating the crude oil dependency of India for energy demand and supply.[17] The transport sector's share of the country's total primary energy consumption will increase from 8.1% in 2010 to 11.3% in 2030 (IEA 2014).[18]

Total transportation energy consumption has been increased from 2012 (83 Mtoe) and is projected to 274 Mtoe in 2040, with per-capita energy emissions ranging from 1.6-1.7 tCO_2.[19] It is estimated in Intended Nationally Determined Contributions (INDC) Scenario, that India's energy related CO_2 emission would be 30% higher in 2022 as compared to 2013, reaching 2.4 GT, and, it will exceed 3.0 GT by 2030 if stronger action will not be forthcoming after 2030.[15]
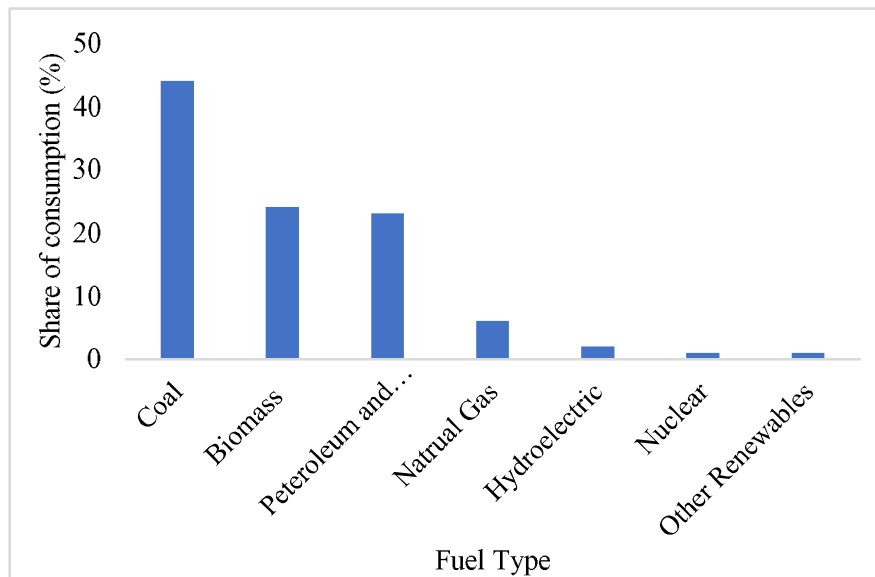

Figure 1.2 Type of energy resource and the share of energy consumption in India by 2013 (Source: IEA)[16]

1.1.2.1 NATIONAL BIOFUEL POLICY

Considering the oil dependency and environmental issues, Ministry of Petroleum & Natural Gas (MoPNG), Government of India (GOI) in 2003 initiated the Ethanol Blended Petrol Programme (EBPP) to promote 5% ethanol blending in gasoline in nine States and four Union territories, which was further expanded up to 20 states in 2006-2007. The Government of India formally announced a national policy on biofuel in 2009 with a mandate of E10 (10% blending) with gasoline in an effort to boost Indian economy and to reduce to fossils fuels dependency, national security and GHG emission reductions. Furthermore, the policy

encourages the use of renewable fuels and proposes a 20% biofuel (ethanol and biodiesel) mandate by the end of 2017.[4]

However, only above 3% blends could be achieved due to non-availability of ethanol. Due to vis-a-vis food, debate government does not allow to use food-based material like sugarcane juice and food grains for the production of ethanol. The main reason to encourage cellulosic ethanol was the abundant availability of biomass across the country. Ethanol in India is predominantly produced from sugarcane molasses, a by-product of sugar production, which could not meet even 5% ethanol blending demand. Moreover, in June 2015, Government of India decided not to use the sugar cane juice for the production of ethanol. However, to strengthen the India energy security, it was decided to utilise renewable energy resources, i.e. lignocellulosic biomass for production of ethanol.

1.1.2.2 BIOMASS AVAILABILITY

India being a 7th largest country in the world with 328 million hectare's land produces about 500 MMT of biomass/year as per Ministry of New and Renewable energy. Out of which, 120-150 MMT is available as agricultural crops per annum. It is estimated that 164.5 MMT of surplus crop residue was available in 2007-2008. Total surplus agricultural biomass available in 2013 was estimated to be 382.7 MMT annually, which could transform into 129.6 billion litres of ethanol.

1.2 FUEL ETHANOL TECHNOLOGY

Ethanol is generally classified into two groups, based on the feedstock used, i.e. First generation fuels and second-generation fuels (Figure 1.3). Ethanol produced from sugar/starch, grains such as wheat, barley, rice, potato etc. is known as "First generation ethanol". In such feedstocks, starch is simply hydrolyzed using certain enzymes, i.e. amylase, which convert polymeric starch to monomeric glucose units. These monomeric sugars upon fermentation by yeast can produce ethanol. Molasses is the liquid residue left after sugar production from sugarcane juice contains approximately 50% of sugar, which is used for the production of ethanol using fermenting yeast.

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While, 1st generation fuels show a net benefit in terms of GHG reduction and energy balance, potentially they have a negative impact on biodiversity and are in competition with food and fuel industries. Hence, these feedstocks are facing ethical, political and environmental concerns for biofuel production. Hence, this part has not been considered in the current study. On the other hand, 2nd generation ethanol is produced from non-edible feedstocks derived from lignocellulosic biomass, i.e. I.) energy crops, *Switchgrass*, *Miscanthus*, II.) agricultural residues, i.e. rice straw, wheat straw, cotton stalk, residues from forestry etc., which are greener, replenishable and are free from competition between food and feed unlike 1st generation ethanol. In 2nd generation, the lignocellulosic biomass can be converted into ethanol using three step processes, i.e. pretreatment, saccharification and fermentation as shown in Figure 1.3. However, in the case of 2nd generation ethanol production, there are certain obstacles in each step such as cost reduction, technology advancement, enzyme cost that need to be overcome over a period of time.
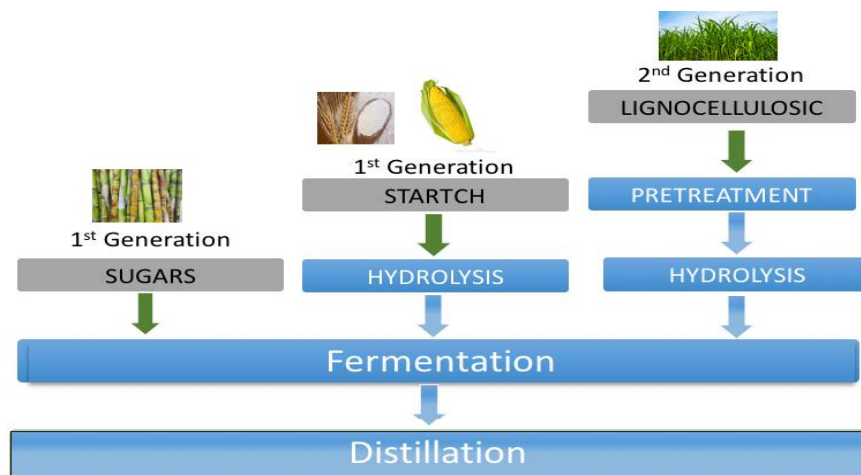

Figure 1.3 Steps involve in 1st and 2nd generation fuel conversion processes

1.3 BIOMASS TO ETHANOL CONVERSION PROCESS

Ethanol can be produced from lignocellulosic biomass using biochemical conversion processes typically involving three steps: (1) **Pretreatment-**helps to deconstruct the intrinsic recalcitrant architect of cell wall matrix in lignocellulose; (2**) Enzymatic hydrolysis-**release of monomeric sugars from pretreated biomass

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by certain enzymes; (3) **Fermentation**-metabolizing the glucose to ethanol by microorganisms, which is followed by distillation to separate the ethanol from fermented broth (Figure 1.4).
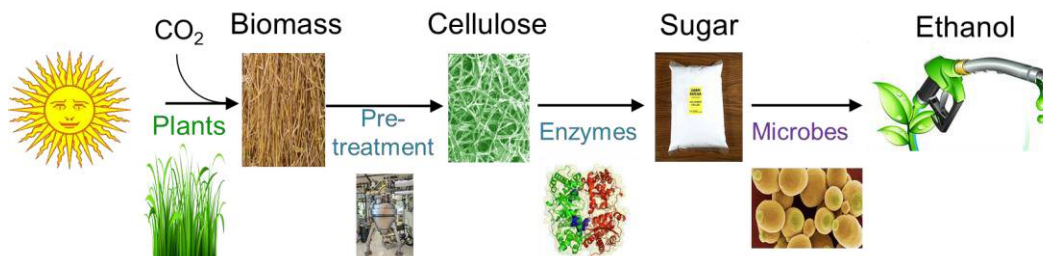

Figure 1.4 Steps involved in biochemical conversion of biomass to ethanol

Lignocellulosic plant cell wall mainly consists three biopolymers, i.e., cellulose (30-40%), hemicelluloses (15-30%), lignin (10-25%), besides small amount of ash (1-20%), extractives (1-15%) and proteins (1-2%) are also present.[5-6] These components together are organised into complex non-uniform three-dimensional heterogeneous cell wall matrix with different degree of polymerization relative to species, origin, feedstock and climate conditions. They 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]

Cellulose is most abundant, renewable organic biopolymer on the planet by making 25-55% of dry weight of biomass followed by hemicellulose and lignin in plant cell wall, which provide structural support to plant.[6] Cellulose is a linear homopolymer of anhydrous-D-glucose units linked together by strong β-1,4-glycosidic linkage formed by condensation of glucose units (Figure 1.5).[7] These anhydroglucose units are distorted 180° with each other making a strong inter/intramolecular hydrogen bonding between two cellulose chains in cellulose fibrils, which makes it highly ordered crystalline regions that are not accessible to water or any hydrolytic enzymes. Cellulose has a high degree of polymerization

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having "n" numbers of anhydroglucose units, i.e. $(C_6H_{10}O_5)n$; n = 10,000 to 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. The glucose in cellulose chain possesses three free OH groups at C2, C3, and C6 positions, which are high hydrogen donors, capable of undergoing series of chemical reactions, making it highly recalcitrant.

There are two types of intra-molecular hydrogen bonds between O3-H---O5, O2-H---O6 and one inter-molecular hydrogen bonds: O6-H-H---O3. The hydrophobic interactions and C-H---O bonds hold the hydrogen-bonded sheet together in cellulose crystals[20-21] and have a limited accessibility towards water
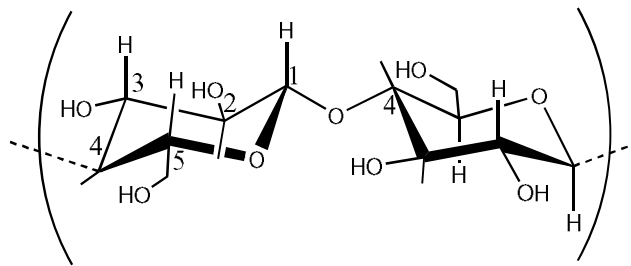

Figure 1.5 Cellobiose unit in cellulose chains (β-(1-4)-glycosidic bond)

Hemicellulose is 2nd most abundant, complex carbohydrate hetero biopolymer after cellulose in nature by making 25-30% of total wood dry weight. It mainly consists of D-glucose, D-xylose, D-galactose, 4-O-methyl-D-glucuronic acid, L-arabinose D-mannose D-galacturonic and D-glucuronic acids. These monomeric sugar units are polymerised by β-(1-4)-glycosidic linkage and have low degree of polymerization (only 50-300). Hardwoods mainly consist of arabinoglucuronoxylans and galactoglucomannans (O-acetyl-O-4-methylglucuronoxylan) (Figure 1.6), while hardwoods predominant in glucuronoxylan. The structure consists of a linear backbone of β-(1-4)-D-xylopyranose with some of the xyloses acetylated and about one-tenth of them carrying a uronic acid (4-O-methylglucuronic acid) with α-1,2 linkages (Pereira et al., 2003). Galactoglucomannans (O-acetyl-galactoglucomannans) are another predominant hemicelluloses found in softwoods, consisting of β-D-glucopyranosyl

and β-D-mannopyranosyl units, linked by β-(1-4)-glycosidic bonds, partially acetylated at C2 or C3 and substituted by α-D-galactopyranosyl units. Xyloglucans consist of a β-(1-4)-D-glucose chain with 75% of the residues carrying an O-6 D-xylose branch.
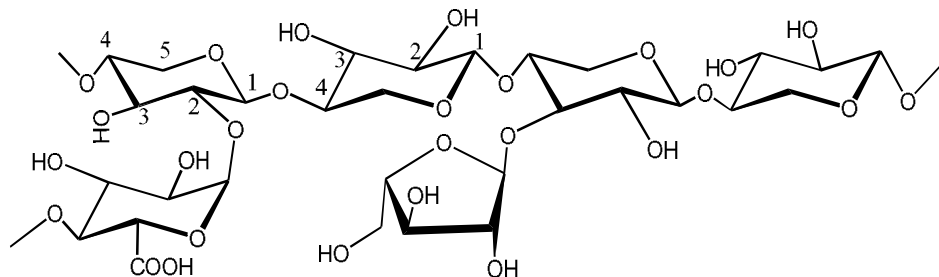

Figure 1. Arabinoglucuronoxylan in softwood hemicellulose

Lignin is a highly-branched, aromatic, amorphous, water-insoluble heteropolymer, synthesized by three monomers, i.e., coumaryl alcohol (p-hydroxyphenyl propanol), sinaply alcohol (syringyl propanol) and coniferyl alcohol (guaiacyl propanol)[22], by free radical mechanism which gives rise to a random sequence of p-hydroxyphenyl (H-lignin), guaicyl (G-lignin), and syringyl (S-lignin) subunits as shown in Figure 1.7. Lignin forms a 3D network via coupling of these subunits at monolignols β positions resulting in arylglycerol-β-aryl ether (β-O-4), pinoresinol (β-β'), phenyl coumarin (β-5'), spirodienone (SD) and diphenylethane (β-1') dimer formation (Figure 1.7). Lignin is always present in association with cellulose and hemicellulose, which provides waterproofing, structural reinforcement and resistance towards any biological and physical attack and the composition differ between softwood, hardwood and grasses, softwood mainly consists of guaiacyl units while hardwood consists of syringyl units in the majority.

Perineal grasses and agricultural residues contain minor amounts of p-hydroxyphenyl groups attached with guaiacyl units. Guaiacyl units in lignin are more likely to form C-C cross-link at the C-5 position of the ring, during delignification.

Thus, efficient deconstruction/pretreatment of lignocellulose is essentially required to disrupt the complex architect and is an essential step involve in the

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biological conversion of lignocellulosic biomass to biofuels (Figure 1.8). Pretreatment increases the enzyme accessibility towards cellulose for better enzymatic saccharification.[23]
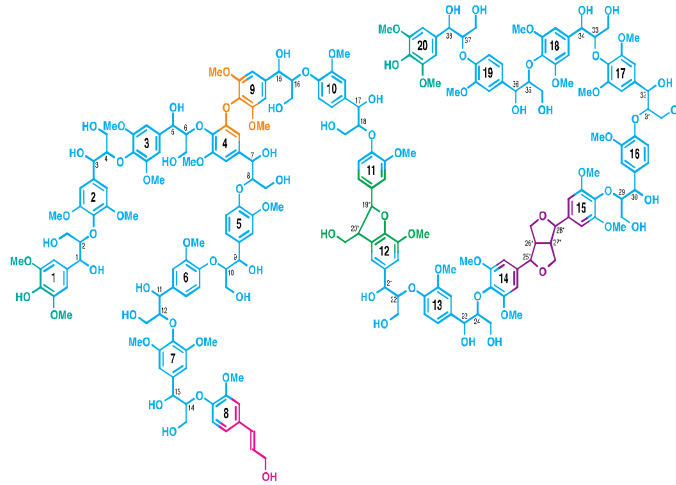
Figure 1.7 Lignin dimeric linkage and 3D structure
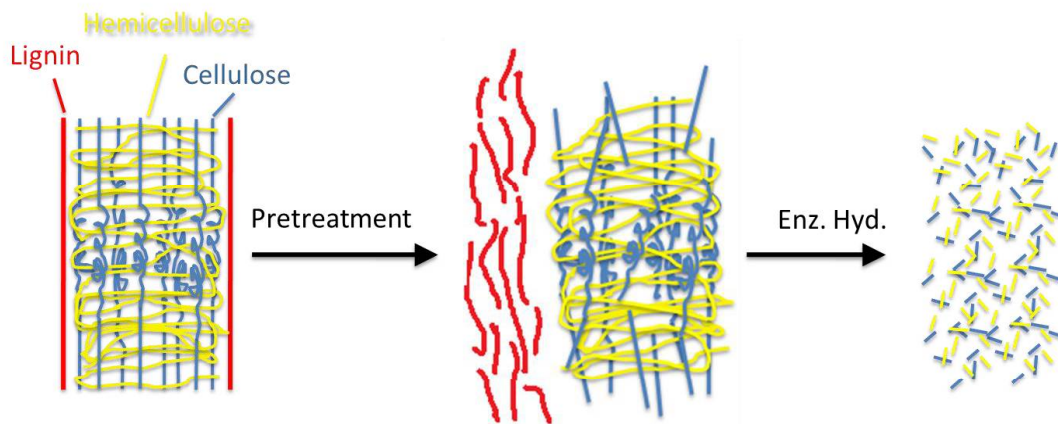
Figure 1.8 Biomass pretreatment and saccharification

Till date, different pretreatment technologies have been developed over the years including physical (e.g. milling, grinding, irritation), chemical (e.g. dilute acid hydrolysis, alkali treatment and ionic liquid), physicochemical (e.g. steam explosion and ammonia fiber explosion, pH controlled water pretreatment, ammonia fiber expansion, ammonia recycle percolation (ARP, steam explosion (CO_2 explosion) and biological pretreatment to fractionate, solubilize, hydrolyze

cellulose, hemicellulose & lignin components. Chemical pretreatments are typically conducted under pressure at temperatures significantly above the boiling point of the solvent (e.g., water or organic liquids), with or without the addition of catalysts. For example, dilute acid[24-25], sulphur dioxide (SO_2), hydrogen peroxide (H_2O_2), steam explosion (autohydrolysis), ammonia fibre expansion (AFEX), wet oxidation, lime[26], liquid hot water, carbon dioxide (CO_2) explosion[27] and organic solvent treatments.[28-29] However, all these methods have several drawbacks, like high energy demands, extreme process parameters.

A pretreatment method can be characterised as good with following benefits:

- Non-corrosive and low cost of the chemicals
- Increasing the surface area, porosity, and accessibility of enzymes
- With minimum formation of inhibitors products for downstream processes
- Remove maximum amount of lignin and reduce total crystallinity
- Promote high sugar yields after enzyme hydrolysis
- To be economical and cost effective and scalable
- Capable of treating multiple biomass

Recalcitrant lignocellulosic biomass can be comminuted by size reduction, cutting, chipping, to reduce open up the cell wall matrix, which is helpful to increase the accessible surface area and pore volume, decreasing the degree of polymerization of cellulose and crystallinity of lignocellulosic materials and its crystallinity, hydrolysis of hemicelluloses, and partial depolymerization of lignin. The size of the materials is usually 10–30 mm after chipping and 0.2–2 mm after milling or grinding. Vibratory ball milling has been found to be more effective in breaking down the cellulose crystallinity of spruce and aspen chips and improving the digestibility of the biomass than ordinary ball milling. Different milling processes (two-roll milling, hammer milling, colloid milling and vibratory milling) can be used to improve the digestibility of the lignocellulosic materials compared to ordinary ball milling (Taherzadeh and Karimi 2008). This process is generally

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not economically feasible because of high-energy consumption for obtaining desired particle size. The energy requirements for physical pretreatments depend on the final particle size and reduction in crystallinity of lignocellulosic biomasses. In most cases when physical pretreatment is the only option, the required energy is higher than the theoretical energy content available in the biomass. Physical pretreatment methods are expensive and usually cannot be used in a large-scale process. Biological pretreatment employs microorganism to perform the pretreatment task. The employed microorganisms specifically possess the capability to degrade the lignocellulosic components of the feedstock to amorphous form.

The typical microorganisms used as the liberator of the lignocellulosic structure are brown rot, soft rot and white rot fungi. However, pretreating the lignocellulosic feedstock by this method results in a slower production rate and high retention time. Hence, this approach is unfavourable for the large-scale production.

Chemical pretreatments include primarily alkalis and acids that work on the biomass through delignification, decreasing the crystallinity of cellulose and reducing the degree of polymerization, i.e., dilute H_2SO_4, HCl, H_3PO_4 and HNO_3 are used for pretreatment of lignocellulosic biomass. Among these chemicals, H_2SO_4 is the most commonly used acid, while NaOH is the commonly used alkali.[30-33]

In alkaline pretreatment, various bases, i.e. sodium, potassium, calcium, and ammonium hydroxide are used to dissolve the hemicellulose and lignin present in lignocellulosic biomass. Alkaline pretreatment attributes the degradation of ester and glycosidic linkage in lignocellulosic cell wall matric, lignin solubilization and chemical modification with cellulose decrystallization and reduction in crystallinity index. Sodium hydroxide and lime have been extensively studied for many years, and it has been shown to disrupt the lignin structure of the biomass, increasing the accessibility of enzymes to cellulose and hemicellulose. Compared with acid processes, alkaline processes cause less sugar degradation, and many of the caustic salts can be recovered and/or regenerated.[5, 34-38]

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Acid pretreatment is conducted using phosphoric acid (H_3PO_4), nitric acid (HNO_3), sulfuric acid (H_2SO_4) and hydrochloric acid (HCl) in low concentration (0.1-2.5% w/w) to disrupt the crystalline structure of the lignocellulosic material by solubilization of hemicellulose component into xylose. With dilute H_2SO_4, pretreatment has been commercially used to treat all type of biomass, i.e., switchgrass, corn stover, spruce (softwood), and poplar over a temperature range of 120-210 °C, with 0.2- 4.0 wt % acid loading for a time interval (1-4 h). Dilute acid pretreatment swells the biomass and helps in removal of amorphous hemicellulose and rupture the cell wall matrix and hence, increases the surface area of pretreated biomass.[5, 39-44]

The major demerits of dilute acid pretreatment are a generation of sugar degraded products, i.e. acetic acid, furfural, HMF, humins, which cause inhibition of cellulase enzymes, hence, washing step is essential. Besides being toxic and corrosive nature of acid, huge metallurgy is required and difficult to recover after pre-treatment that may affect process economics.

Steam explosion is one of the most widely used methods for pretreating LCB for ethanol production. In this method, the biomass is subjected to pressurised steam (1 to 3.5 Mpa) for a short period of time (5 to 10 min) at high temperature (180-240 °C) followed by explosive decompression. The treatment combines mechanical forces with autohydrolysis caused by the formation of acetic acid from the acetyl groups on the hemicelluloses. There are several positive sides to the steam explosion technology such as low environmental impact and lower capital cost as well as higher energy efficiency and the possibility of using a non-hazardous reaction medium. The pretreatment is suitable both for agricultural residue and for hardwoods and has high efficiency, but for softwood raw materials addition of an acid catalyst is needed. The main drawbacks of the process are hemicellulose degradation causing the formation of compounds that work as inhibitors for the following enzymatic hydrolysis and fermentation steps. Major structural changes include hydrolysis of hemicellulose by organic acids produced during the process of autohydrolysis as well as relocation and degradation of lignin and

hemicellulose.[45-49]

Xylo-oligomer formation is another disadvantage that steam explosion offers. These are known to be inhibitory to cellulase activity. However, use of acid catalyse may prevent their formation. Therefore, exogenous acid catalysts such as H_2SO_4, oxalic, maleic and fumaric acids, and another acid generating compounds such as SO_2 and CO_2 need to be combined with steam pretreatment for woody biomass sample. The addition of acids decreases time and temperature of reaction, leading to a complete removal of hemicellulose.[50-52]. Steam explosion results in the generation of toxic components, i.e. levulinic acid, hydroxymethylfurfural, furfural etc. from degradation of carbohydrates and re-condensation of lignin that could affect the enzymatic hydrolysis and fermentation process. Hence, detoxification step is essential before enzymatic hydrolysis.

Liquid hot water pretreatment is a hydrothermal pretreatment, in which the biomass is treated with water at elevated temperatures similar to a steam explosion, but uses a lower temperature and lower dry matter content. Liquid hot water process solubilizes most of the hemicelluloses while leaving the cellulose and lignin mostly unaffected. Some of the advantages of this pretreatment are that no catalyst is needed and the production of inhibitors are low.[53-56]

The AFEX pretreatment involves treating lignocellulosic biomass with liquid anhydrous ammonia at temperatures between 60 and 100 °C at high pressures. The pressure is then released causing the ammonia to vaporise. The effect of the pretreatment is physical disruption and swelling of the fibres, partial decrystallisation of cellulose, and breakdown of the lignin-carbohydrate linkage and reduces cellulose crystallinity and increases the digestibility of biomass by removing acetyl linkage by deacetylation method. The pretreatment is more efficient on agricultural residues than on wood and other high-lignin feedstocks.[57-59]

Another new method of pretreatment is microwave irradiation, which produces mechanical waves. High heating, have higher efficiency and lower residence time (5 to 20 min) are the advantages of this technique. This method is

helpful to transform ultra-structure of cellulose by degrading lignin and hemicelluloses and hence increases the enzymatic susceptibility of lignocellulosic biomass.[60]

CO$_2$ as a supercritical fluid is also employed to remove lignin and open the biomass structure. This is done by the sudden release of CO$_2$ pressure, which disrupts the cellulose and hemicellulose structures, giving an increase in the accessible surface area. The addition of co-solvents like ethanol can improve dissolution. Low reaction temperatures reduce the sugar degradation, but the sugar yield is low compared to steam and ammonia explosion.[61]

Organosolv pulping includes the extraction of lignin from lignocellulosic biomass with organic or aqueous organic solvents mixtures (e.g. methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfurylalcohol, oxalic, acetylsalicylic and salicylic acid alcohol with inorganic acid (e.g. hydrochloric acid, sulfuric acid, and phosphoric acid) in temperature range of 100-250 °C. Organosolvent used in pretreatment is too expensive and need high pressure vessels for pretreatment and the solvent after process need to recovered for cost reduction and economic viability.[62] Moreover, all the merits and demerits are summarised in Table 1.2. Hence, exploring other methods like use of ionic liquids as a reaction media could be useful.

Table 1.2 Advantages and disadvantages of different pretreatment methods of lignocellulosic biomass

Pretreatment method	Advantages	Disadvantages	Ref.
Milling	-Decrease of cellulose crystallinity and degree of polymerization -Reduction of particle size to increase specific surface area and pore size	-High power and energy consumption	63-66
Steam explosion	-Causes lignin transformation and hemicellulose solubilization -Lower cost -Higher yield of glucose and hemicellulose in the two-step method	-Generation of toxic compounds -Partial hemicellulose degradation	5, 45, 47, 52, 67-69
Liquid hot water	-Size reduction of the biomass is not needed -No chemicals are generally required -No requirement of corrosion-resistant materials	-High energy and high-water requirement -Formation of toxic compounds	53-54, 56, 70-72
Ammonia fibre expansion (AFEX)	-Increases accessible surface area -Fewer inhibitors formation -Does not require small particle size of biomass	-Not very effective for the biomass with high lignin content -High cost of large amount of ammonia	55, 73-76
CO_2 explosion	-Increase accessible surface area -Availability at relatively low cost -Do not form inhibitory compounds -Non-flammability	-Very high-pressure requirements	61
Wet oxidation	-Easy recovery after extraction and environmental acceptability -High degree of solubilization of hemicellulose and lignin -Avoid formation of degradation compounds	-High cost of oxygen and alkaline catalyst	77-78
Concentrated acid	-High glucose yield -Ambient temperatures	-High cost of acid and need to be recovered -Corrosion-resistant equipment is required -Concentrated acids are toxic and hazardous	40, 79-82
Diluted acid	-High recovery of sugars at the end of the process -Low formation of toxic products	-Concentration of reducing sugars is relatively low -Generation of degradation products	5, 42, 49, 82-99
Alkali	-Decrease in the degree of polymerization and crystallinity of cellulose -Disruption of lignin structure	-High cost -Not used for large-scale plant	35, 38, 79, 100-102
Ozonolysis	-Effectively removes lignin content -Does not produce toxic residues -Reaction is carried out at room temperature and pressure	-High cost of large amount of ozone	
Organosolv	-Causes lignin and hemicellulose hydrolysis	-Solvents need to be drained and recycled -High cost	62, 103-104
Biological	- Mild environmental conditions and low-energy requirements -Delignification, Partial hydrolysis of hemicelluloses -Reduction in degree of polymerization of cellulose	-Slow process rate -Very low treatment rate -Not very effective for commercial application	105-107

1.4 IONIC LIQUID AS A MEDIA TO EXTRACT SUGARS

Ionic liquids (ILs), are organic salts with melting points of <100 °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) (Figure 1.9) [8-12]. Ionic liquids have emerged as an alternative "green solvent" in chemicals synthesis, catalysis[108], biocatalysis[109], in electrochemical devices, or as engineering fluids, and now have been used as a solvent for lignocellulosic biomass deconstruction due to unique solvation properties.[13-14]

Cations:

Imidazolium   Pyridinium   Pyrrolidinium   Ammonium   Phosphonium
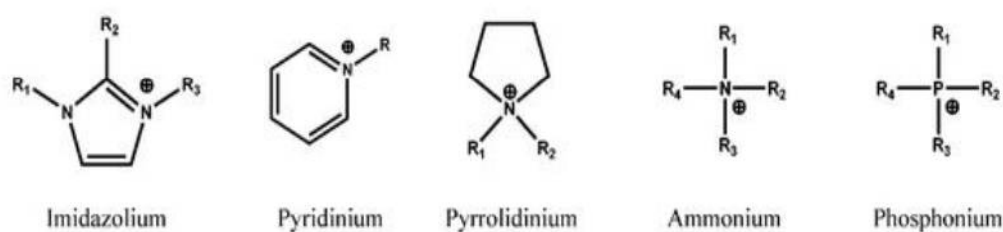
Anions:

hydrophilic   hydrophobic

Chloride   Trifluoromethane-sulfonate   Tetrafluoro-borate   Bis(trifluoromethane-sulfonyl)amide   Hexafluoro-phospate
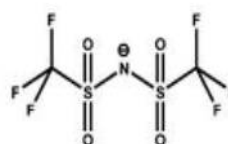
Figure 1.9 Cation and anions of ionic liquids

Figure 1.10 shows ILs can vary by different combinations i.e., negligible vapour pressure, tunable solubility, designability, thermal stability, biocompatibility.[11] ILs are highly polar in nature with varying degree of hydrogen bonding ability, and in general, anion poses high hydrogen bonding basicity in order to solubilize crystalline cellulose.[110-112] Dissolution of cellulose is desired in biomass-to-fuels.[113]

IL pretreatment, when compared with other pre-treatment techniques (ammonia fibre expansion or diluted acid), has shown to decrease biomass crystallinity by breaking inter/intra-chain-H bonds linkage in cellulose fibrils

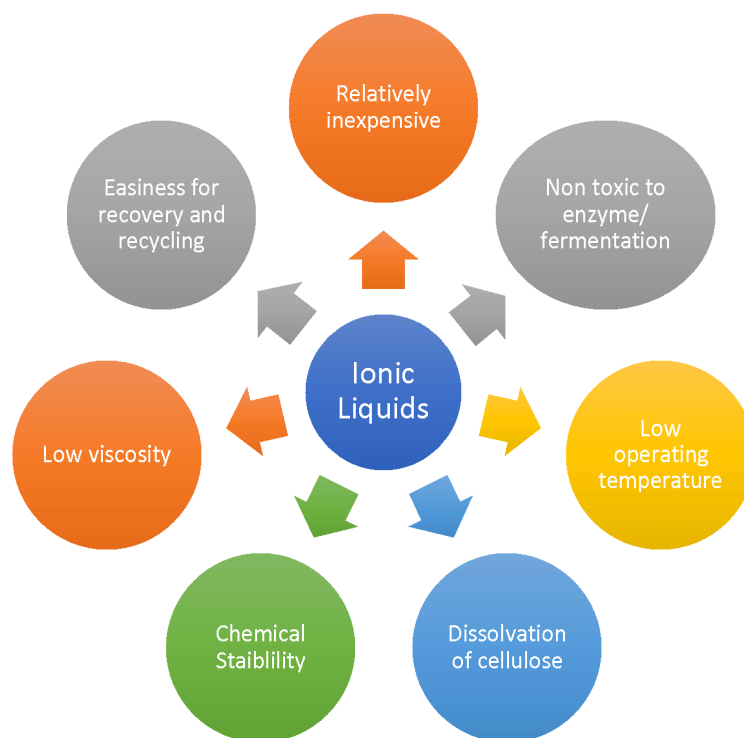more than other pre-treatment techniques.[114]

Figure 1.10 Properties of liquid for biomass processing

Anion of ionic liquid, e.g. trialkylphosphonates chloride, dialkyl phosphates, carboxylates, propionate, lactate, acetate, dialkyl, amino acid and formate imparts unique property to ILs to interact with -OH groups present in cellulose chains and helps to disintegrate the inter/intra molecular hydrogen bonding in cellulose chains.[115-117] Ionic liquid with low hydrogen bond capability is unable to dissolve cellulose. The dissolving power of these relevant ILs can be tuned by varying cation alkyl chain, anion in addition to viscosity and working temperature is attributed to strong hydrogen-bonding interactions between the anions and equatorial -OH groups on the cellulose fibrils.[118]

1.5 RESEARCH OBJECTIVES AND QUESTIONS

The main objective of the current research is the physicochemical characterisation of different biomass residues available in North India to assess their biofuel potential either by thermochemical or biochemical pretreatment pathways, across different agricultural residues. The second objective of the research is to use "ionic liquid" for lignocellulosic biomass pretreatment

production of fermentable sugars for ethanol production. The thesis is subdivided into four research projects with a particular aim and attempts to answer the following research questions.

(i) Physicochemical characterisation of different agricultural residues available in Northern India to assess their biofuel potentials.

Variation in the chemical constituents, and properties of biomass due to agro-climate conditions, species and from one geographical location to other dictates that a full physicochemical characterization needs to be done for any process development.

Hence, the overarching objective of this research is to investigate the physicochemical properties of lignocellulosic biomass and effect of different parameters of lignocellulosic biomass. Literature pertaining to the major focus of each chapter is outlined in the introduction section and conclusions for the work are provided at the end of each chapter.

Q1: Understanding the structural and compositional variability of biomass.

Q2: What are the factors responsible for biomass recalcitrance?

Q3: What are the analytical techniques used for characterisation of lignocellulosic biomass in terms of proximate, ultimate analysis, crystallinity Index, surface morphology etc?

(ii) Ionic liquid treatment of Avicel as a cellulose substrate for better enzymatic saccharification and correlation of ionic liquid properties with sugars released.

The cellulose in biomass cell wall are connected with intra/intermolecular hydrogen bonding and can be converted into biofuel. Hence, the aim of the current study is to use an imidazolium-based ionic liquid for cellulose solubilization and regeneration using water as antisolvent. The idea of conduction experiments on avicel was so as we can replicate the process parameters on real biomass as cellulose is the main component of biomass.

Q4: What are the properties of ionic liquids, which are helpful for cellulose solubilization?

Q5: What is the mechanism for cellulose solubilization in of ionic liquids?

Q6: How cellulose solubilization are affected by physical and chemical properties of ILs, cation, anion combinations and alkyl chain length?

Q7. What is the impact if ILs properties on sugar recovery?

(iii) Ionic liquid pretreatment of biomass for sugars production: Driving factors with a plausible mechanism for higher enzymatic digestibility

After optimisation and understanding the effect of ionic liquid properties over Avicel as a model substrate, the study was further extended to deconstruct biomass samples at optimised pretreatment conditions and investigating of the driving factors and a plausible mechanism for improved enzymatic digestibility.

Q7: How these ionic liquids behave with real biomass samples for pretreatment and effects on xylan and lignin removals?

Q8: How ILs properties affect the overall sugar yields and mechanism of alteration of cellulose structure?

1.6 GENERAL RESEARCH METHODOLOGY

General research methodology for whole research work is presented in figure 1.11. Research is initiated with literature survey followed by complete physicochemical characterization of lignocellulosic biomass for sugar component, energy content, thermal stability etc. to determine the biofuel potential of biomass.

After complete characterization, the further study was focused on the application of ionic liquids for lignocellulosic biomass pretreatment and saccharification of cellulose-rich material for sugars production. The last step involves the ionic liquid recycling using pervaporation method for economic sustainability.

1.7 ORGANIZATION OF THESIS

The whole work conducted within the thesis is subdivided into three main parts; (1) Introduction, (2) Literature review and (3) Experimental. The thesis layout is shown in Figure 1.12.

Chapter 1 introduces the present research topic and the motivation towards the

research work behind complete physicochemical characterization, and the concept of ionic liquid as a "green solvent" for lignocellulosic biomass pretreatment. With that, it also mentioned the aim of current research work, major objectives/outlines and proposed chapter of the thesis in upcoming context.
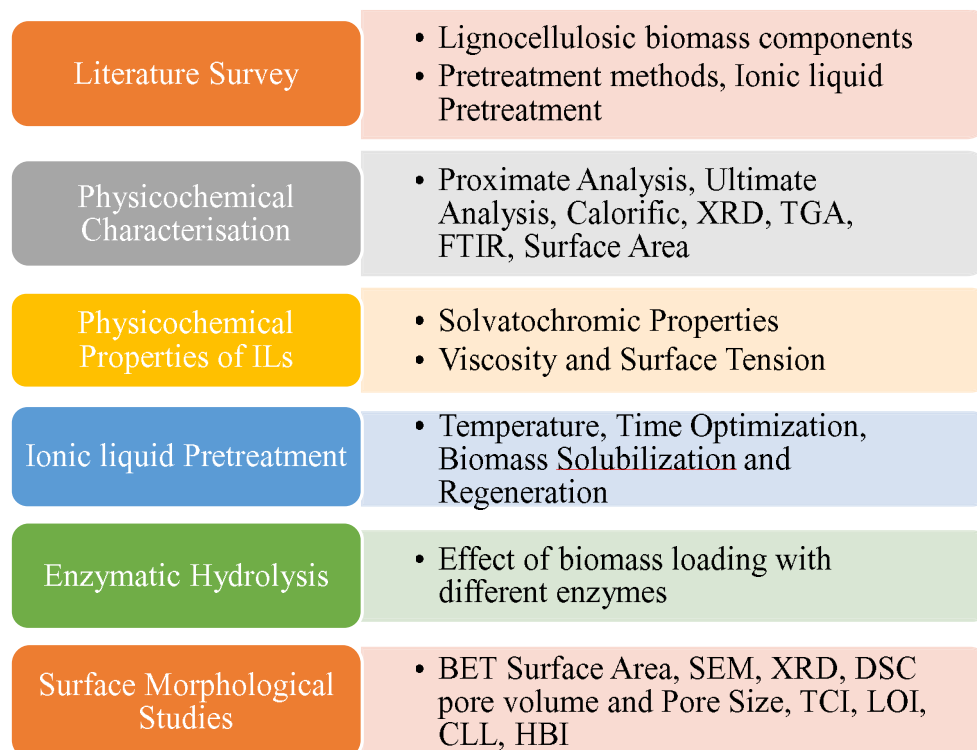

Literature Survey	• Lignocellulosic biomass components • Pretreatment methods, Ionic liquid Pretreatment
Physicochemical Characterisation	• Proximate Analysis, Ultimate Analysis, Calorific, XRD, TGA, FTIR, Surface Area
Physicochemical Properties of ILs	• Solvatochromic Properties • Viscosity and Surface Tension
Ionic liquid Pretreatment	• Temperature, Time Optimization, Biomass Solubilization and Regeneration
Enzymatic Hydrolysis	• Effect of biomass loading with different enzymes
Surface Morphological Studies	• BET Surface Area, SEM, XRD, DSC pore volume and Pore Size, TCI, LOI, CLL, HBI

Figure 1.11 General research methodology

Chapter 2 this chapter reviews the literature of last ten years (1997-2017) that motivated and explains the need for using lignocellulosic biomass for the production of biofuels. It provides a detailed literature of biomass feedstock availability, biomass chemical composition, and different techniques for biomass characterisation and research conducted on ionic liquids (ILs) as a "green solvent" for biomass dissolution. The major inferences and gaps found from the literature review are also covered at last.

Chapter 3 this chapter objective addresses the physicochemical characterization of common lignocellulosic agricultural residues available in

India, i.e., rice straw, rice husk, cotton stalk, wheat straw, bagasse, corn stover, sorghum stalk, mustard stalk, corn cob, and jatropha pruning. Physical and chemical characterization of lignocellulosic samples is carried out by higher heating value, crystallinity index, thermal properties, CHNS/O analysis, FTIR, metal analysis, and compositional analysis. Understanding of the biomass characteristics is very important for the overall efficiency of processes designed to convert the lignocellulosic materials into the desired biofuel. For example, high sugar content of any biomass represents the biochemical potential for ethanol production, similarly, high energy content helps are helpful from thermochemical perspective.

Chapter 4 this chapter focused the study over ionic liquid treatment of Avicel PH 101 on determining cellulose transformation efficiencies for five different combinations of ionic liquids varying cation, anion with different alkyl chain length, i.e. [C_2mim][OAc], [C_4mim][OAc], [C_2mim][Cl], [C_4mim][Cl] and [C_4mim][BF$_4$] with different correlation between Kamlet-Taft parameters, i.e., hydrogen bond acidity, hydrogen bond basicity and polarity, viscosity and surface tension with glucose yields after enzymatic hydrolysis. It also describes the changes in the structure of avicel pure cellulose with ionic liquid pretreatment. These changes were tracked with X-ray powder diffraction, FT-IR etc. A comparative enzymatic hydrolysis was performed using different commercial cellulases through saccharification of various IL pretreated biomass at low biomass loadings and specific enzyme to link the changes in the enzymatic hydrolysis with respect to the cellulose structure.

Chapter 5 describes the ionic liquid assisted deconstruction and regeneration of mustard stalk and wheat straw at optimised pretreatment conditions and investigating of the driving factors and a plausible mechanism for improved enzymatic digestibility. Effect of severity of pretreatment conditions has been studied at 100 °C for 5 h and 130 °C for 2 h. The efficiency of pretreatment was determined and correlated with sugar released by enzymatic hydrolysis of ILs pretreated lignocellulosic biomass using commercial cellulases. Cellulose structural deformation was analysed and quantified by different analytical

techniques, i.e., X-Ray diffractometry (XRD) and FT-IR spectroscopy, whereas, the lignin and xylan content in pretreated and native biomass were quantified by the chemical compositional analysis. With that, the effect of the different physicochemical properties of ionic liquids, i.e. Kamlet-Taft Parameters, Viscosity and surface tension were correlated in terms sugar release after enzymatic hydrolysis. The results are further validated using different biomass such as wheat straw with a plausible mechanism of hydrolysis of pretreated biomass.

Chapter 6 describes the impact on ILs pretreatment on physicochemical properties of the mustard stalk, where, we have characterised the lignocellulosic biomass for porosity, surface area, crystallinity and their impact on enzymatic saccharification. In this chapter, we used differential scanning calorimetry (DSC) to quantified the pore size distribution in pretreated biomass, for which we have measured the depression in the melting point of water present in bulk and pores. Freezing bound water to represent the pore size and pore volume, specific surface area, and reduced crystallinity was further correlated with sugar recovery, which in term represents the enhanced accessibility of enzymes towards polysaccharides surface. With that, we have also an insight of cellulose crystal transformation by FT-IR related properties by total crystallinity index (TCI), lateral order index (LOI) and hydrogen bond index (HBI) values and correlated with sugar released.

Chapter 7 Briefly outlines conclusions and recommendation with future challenges of the investigation.
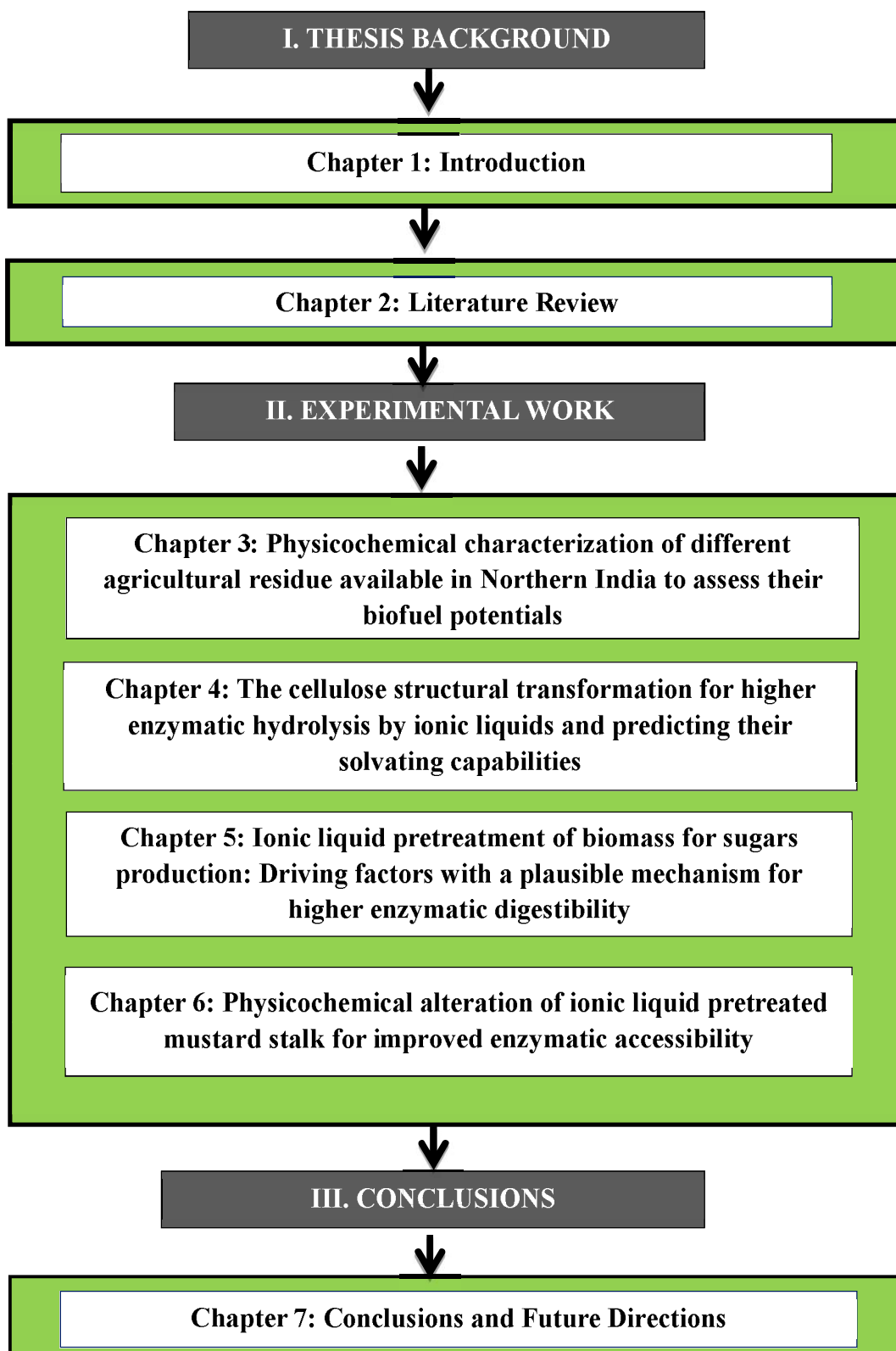
I. THESIS BACKGROUND

Chapter 1: Introduction

Chapter 2: Literature Review

II. EXPERIMENTAL WORK

Chapter 3: Physicochemical characterization of different agricultural residue available in Northern India to assess their biofuel potentials

Chapter 4: The cellulose structural transformation for higher enzymatic hydrolysis by ionic liquids and predicting their solvating capabilities

Chapter 5: Ionic liquid pretreatment of biomass for sugars production: Driving factors with a plausible mechanism for higher enzymatic digestibility

Chapter 6: Physicochemical alteration of ionic liquid pretreated mustard stalk for improved enzymatic accessibility

III. CONCLUSIONS

Chapter 7: Conclusions and Future Directions

Figure 1.12 Thesis layout