

**Toward an Integrated Approach to a
Sustainable Biorefinery using the
IonoSolv Process**

A thesis submitted for the degree of

Doctor of Philosophy

by

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Declaration of Originality

The entirety of the work described in this thesis was carried out at Imperial College London between October 2013 and October 2017. Unless otherwise stated the work is my own and has not been submitted previously for a degree at this or another university. This thesis was revised on 19th February 2021 to remove material that had been included without attribution.

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Abstract

Lignocellulosic biomass and Algae are feedstocks that hold great potential in production of sustainable biofuels, materials and chemicals. This work focusses on the application of ionic liquid based fractionation of lignocellulosic biomass and algae.

Firstly, the pretreatment of *Miscanthus x giganteus* using different ionic liquid with low concentration was performed. The delignification yield increased gradually as concentration of ionic liquids go higher. Dilute triethylammonium hydrogen sulphate with different cation: anion ratio was used in pretreatment of miscanthus, pine and willow. The results showed increased removal of cellulose, hemicellulose and lignin with lower cation: anion ratio. Liquor analysis on liquid phase after pretreatment using triethylammonium hydrogen sulphate on Miscanthus under varies of conditions indicate that hemicellulose removal rate is better under conditions of higher temperatures and longer reaction time.

Secondly, Algae fractionation using IonoSolv process was tested. Among all seaweeds tested, *Posidonia oceanica* showed high saccharification yield after pretreatment under very mild conditions. Finally, 2-stage pretreatment aiming to remove hemicellulose and lignin in two separate steps was carried out. The results showed promising hemicellulose removal and delignification yield with promising saccharification yield. A further study on using recycled ionic liquid in the 2nd stage pretreatment has demonstrated consistent results of effective pretreatment over 5 cycles.

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Glossary

[DMBA]	<i>N,N</i> -dimethylbutylammonium
[HC ₄ im]	<i>N</i> -butylimidazolium
[TEA]	triethylammonium
[HSO ₄]	hydrogen sulphate
AFEX	ammonia fibre expansion
AIL	acid insoluble lignin
ASL	acid soluble lignin
AIR	acid insoluble residue
APIL	aprotic ionic liquid
DA	dilute acid
DMSO	dimethylsulfoxide
CCA	chromated copper arsenate
GHG	greenhouse gases
HMF	5-hydroxymethylfurfural
HPLC	high performance liquid chromatography
IL	ionic liquid
PIL	protic ionic liquid
LA	levulinic acid
LCA	life cycle assessment
LCC	lignin-carbohydrate complex
M	molar
MESP	minimum ethanol selling price
NMR	nuclear magnetic resonance spectroscopy
NREL	National Renewable Energy Laboratory
ppm	parts per million
SE	steam explosion
UV	ultraviolet spectroscopy
wt%	weight percent

Contents

Declaration of Originality	2
Copyright	2
Abstract	3
Acknowledgements	4
Glossary	5
List of Figures.....	9
List of Tables.....	13
Introduction.....	14
Part I. Background	16
1 Ionic liquids	16
1.1 General	16
1.2 Physicochemical Properties.....	17
1.3 Protic Ionic Liquids.....	18
2 Biomass	19
2.1 Lignocellulosic Biomass	19
3 Biorefinery.....	29
3.1 General	29
3.2 Deconstruction of lignocellulose	31
4 Ionic liquid recovery	38
5 Algae.....	39
5.1 General	39
5.2 Algal biorefinery approach	41
5.3 Conversion technologies for algal biofuels	46
5.4 Economic issues and life cycle assessment	50

5.5 Conclusion	51
Research gap	52
Part II. Experimental Methods	54
General Materials and Equipment	54
Ionic Liquids	54
Biomass feedstock	56
Fractionation of Biomass	57
Water content measurement	57
Dilute acid pretreatment	57
IonoSolv Pretreatment	58
Pulp analysis	60
Compositional analysis	60
Saccharification Assay	63
Liquor analysis	64
Part III. Results and discussion	66
Chapter 1: IonoSolv process	66
Lignin extraction at low IL concentration	66
Dilute [TEA][HSO ₄] meets lignocellulosic biomass	68
Liquid phase content	71
Chapter 2: Algae Based Biorefinery	83
Pretreatment of algae from UK	83
Pretreatment of <i>Posidonia oceanica</i> macroalgae	88
Chapter 3: 2-stage Pretreatment	92
Why 2-stage	92
Dilute acid 1 st stage pretreatment	93

Full IL 2-stage pretreatment.....	99
Further improvement of saccharification yield	102
Mini reactor	104
2-stage pretreatment using recycled ionic liquid.....	111
Part IV. Conclusion	118
Future work	120
References.....	123
Appendix.....	140

List of Figures

Figure 1-1. Different cations and anions for ionic liquid combination	17
Figure 1-2. Proton transfer during the synthesis of PILs	19
Figure 1-3. Cellulose, hemicellulose and lignin in lignocellulosic biomass.....	21
Figure 1-4. 1-4- β glycosidic bond found in cellulose (left) and 1-4- α glycosidic bond found in starch (right).....	22
Figure 1-5. Proposed acid catalysed mechanism for the formation of HMF and levulinic acid from hexoses.....	23
Figure 1-6. The hexoses and pentoses typically found in hemicellulose.....	24
Figure 1-7. The main structures of hemicelluloses.....	25
Figure 1-8. The pathways for the conversion of furfural as a platform molecule into chemicals and fuels	26
Figure 1-9. Proposed closed chain mechanism for the dehydration of xylose to furfural via intramolecular rearrangement in acidic solution	26
Figure 1-10. Proposed open chain mechanism for the dehydration of xylose to furfural via 1,2-hydride shift catalyzed by Lewis acid	27
Figure 1-11. The monolignols that made up lignin	28
Figure 1-12. A fragment of Freudenberg's model of the structure of lignin	29
Figure 1-13. First and second generation biorefinery.....	30
Figure 1-14. A conceptual process flow for the large-scale IonoSolv process.....	37
Figure 1-15. Biorefinery approach for algal feedstocks.....	41
Figure 1-16. Potential algal biomass conversion processes	47
Figure 2-1. Schematic representation of IonoSolv biomass deconstruction process.....	60
Figure 3-1. Lignin removal yield of pretreatment with different [TEA][HSO ₄] concentrations.....	66
Figure 3-2. Lignin removal yield of pretreatment with different [N ₄₁₁₀][HSO ₄] concentrations.....	67
Figure 3-3. Lignin removal yield of pretreatment with different [HC _{4im}][HSO ₄] concentrations.....	68

Figure 3-4. Compositional analysis results of pulp after pretreatment of Miscanthus using ILs with different acid: base ratios.....	69
Figure 3-5. Compositional analysis results of pulp after pretreatment of pine using ILs with different acid base ratios.....	70
Figure 3-6. Compositional analysis results of pulp after pretreatment of willow using ILs with different acid base ratio.....	70
Figure 3-7. Pulp yield of pretreatment at 100°C.....	72
Figure 3-8. Pulp yield of pretreatment at 110°C.....	73
Figure 3-9. Pulp yield of pretreatment at 115°C.....	73
Figure 3-10. Compositional analysis of raw Miscanthus and pulp after pretreatment.....	74
Figure 3-11. Percentage of xylose found in liquid phase after pretreatment at 100°C.....	75
Figure 3-12. Percentage of xylose found in liquid phase after pretreatment at 110°C.....	75
Figure 3-13. Percentage of xylose found in liquid phase after pretreatment at 115°C.....	76
Figure 3-14. Furfural content in liquid phase from pretreatment at 100°C.....	78
Figure 3-15. Furfural content in liquid phase from pretreatment at 110°C.....	78
Figure 3-16. Furfural content in liquid phase from pretreatment at 115°C.....	79
Figure 3-17. Percentage of xylan extracted from Miscanthus after pretreatment at 100°C....	81
Figure 3-18. Percentage of xylan extracted from Miscanthus after pretreatment at 110°C....	82
Figure 3-19. Percentage of xylan extracted from Miscanthus after pretreatment at 115°C....	82
Figure 3-20. Photos of different types of algae received.....	83
Figure 3-21. Extractives in algae.....	84
Figure 3-22. Compositional analysis result of algae pretreatment pulps.....	86
Figure 3-23. Saccharification yield of pulps from algae pretreatment.....	87
Figure 3-24. Compositional analysis results of <i>Posidonia oceanica</i> macroalgae pulp after pretreated with 80% IL.....	88
Figure 3-25. Saccharification yields of <i>Posidonia oceanica</i> macroalgae pulp after pretreated with 80% IL.....	89
Figure 3-26. Compositional analysis results of <i>Posidonia oceanica</i> macroalgae pulp after pretreatment with 20% IL.....	90

Figure 3-27. Saccharification yields of <i>Posidonia oceanica</i> macroalgae pulp after pretreated with 20% IL.....	91
Figure 3-28. A very brief flow chat of 2 stage ionoSolv process.....	93
Figure 3-29. Compositional analysis results of pulp after 1 st stage pretreatment at 80°C.....	94
Figure 3-30. Compositional analysis results of pulp after 1 st stage pretreatment at 100°C.....	94
Figure 3-31. Compositional analysis results of pulp from both dry and wet pretreatment, compared with raw Miscanthus and the 1 st stage pulp.....	96
Figure 3-32. Composition of pulp without considering weight lost during pretreatment.....	98
Figure 3-33. Saccharification yield of pulp from both dry and wet pretreatment.....	99
Figure 3-34. Compositional analysis results of pulp from 1 st stage pretreatment using IL, compared with raw Miscanthus.....	101
Figure 3-35. Compositional analysis results of pulp from 2 nd stage pretreatment using IL, compared with raw Miscanthus.....	101
Figure 3-36. Saccharification yield of pulp from both dry and wet pretreatment.....	102
Figure 3-37. Saccharification yield of 2-stage pretreatment on Miscanthus.....	104
Figure 3-38. Mini reactor with adjustable stirring function.....	105
Figure 3-39. Compositional analysis results of 2-stage pretreatment done in pressure tube and mini reactor.....	106
Figure 3-40. Saccharification results of 2-stage pretreatment done in pressure tube and mini reactor.....	107
Figure 3-41. Compositional analysis results of 2-stage pretreatment on wheat straw.....	108
Figure 3-42. Compositional analysis results of 2-stage pretreatment on wheat straw under different conditions.....	110
Figure 3-43. Saccharification results of 2-stage pretreatment on wheat straw under different conditions.....	111
Figure 3-44. Route of hemicellulose degradation.....	112
Figure 3-45. Compositional analysis results of pulp from 1 st stage pretreatment.....	113
Figure 3-46. Mannan mass balance.....	113
Figure 3-47. Compositional analysis results of untreated pine, pulp from 1 st stage	

pretreatment and final pulp of each cycle.....	115
Figure 3-48. Saccharification results of untreated pine, pulp from 1 st stage pretreatment and final pulp of each cycle.....	115
Figure 3-49. HMF content in IL after each cycle.....	116

List of Tables

Table 1-1. Biochemical composition of microalgae expressed on a dry matter basis (%dry weight).....	44
Table 1-2. Biochemical composition of macroalgae expressed on a dry matter basis (%dry weight).....	45
Table 1-3. Potential algal feedstock for bioethanol production.....	49
Table 3-1. Miscanthus composition.....	72
Table 3-2. Water content in wet macro algae.....	84
Table 3-3. Two-stage pre-treatment and saccharification conditions.....	103
Table 3-4. Details of conditions tried on mini reactor.....	108

Introduction

At present, world energy consumption is increasing dramatically, both in developed and developing countries. As the global population keeps growing, it is not likely that energy demand can be controlled in the coming decades. Fossil fuels, including coal, oil and natural gas, have been the major source of energy in recent human history, and are now facing a variety of problems such as resource depletion and contamination of the nearby environment and water (1). Spills could occur during transportation of crude oil, causing long-term damage to the environment especially if it happens on the sea (2). Burning of fossil fuels generates CO₂ which results in an instant increase of the CO₂ concentration in the atmosphere (3). Both the coming shortage of traditional fossil fuels and the serious environmental issues caused by coal and oil has led to increased research into alternative energy sources. One of the most promising new energy sources is biomass.

Biomass is biological material derived from living, or recently living organisms. It most often refers to plants or plant-based materials which are specifically called lignocellulosic biomass (4). As it is a renewable (energy) resource, the consistent supply of biomass supply can be guaranteed. Also, it is a carbon-neutral (5) or even a carbon-negative (6) resource so the concern of CO₂ emissions can be minimised. Another reason for the interest in biomass rather than fossil fuels for combustion is the generation of toxic gases like SO₂ and NO₂ during coal and oil combustion. A disappointing fact is that biomass currently only accounts for about 12% of global energy production (7).

Other than direct combustion, biomass has many more applications and can produce many other products, the most commonly known one is biofuels. Biofuels are fuels derived from biomass, the most widely used biofuels are bioethanol and biodiesel. Biodiesel is especially popular in Europe, it can be produced from oils by transesterification (8). Bioethanol is currently produced from plants that also produce food, which significantly limits the productivity and development of bioethanol due to concerns with competition between fuel

production and food production.

As a result, we must aim toward using some material that is highly abundant or normally considered not very useful. Lignocellulosic biomass (agricultural waste, trees, grass, etc.) therefore becomes our ideal choice. Using lignocellulosic biomass doesn't compete with food, it also has higher yields per land area (9) and the cost of producing them is lower. Less fertilizer will also be consumed than plants that mainly contain sucrose and starch (10). The application of cellulose, hemicellulose and lignin extracted from lignocellulosic biomass is a promising source of future fuels, chemicals and materials for a variety of uses. One drawback of lignocellulosic biomass is its resistance to chemical treatment and it therefore requires a pretreatment step to separate the main components prior to valorisation. This pretreatment could be both physical and chemical; popular methods including using steam explosion (11), dilute acid (12), and other techniques, including the use of ionic liquids, as detailed below.

Algae is another alternative source of biofuels. It has a very short growth cycle, requires no land-use and some algae can be cultivated in contaminated water. Certain algae are edible but the fact that many countries have a problem with cleaning their beaches that are occupied by algae outweighs this shortcoming. For areas without access to a large supply of lignocellulose, algae can be a great option as a bioenergy crop. Good integration of an algae-based biorefinery with algae waste treatment, contamination removal and allocation of by-products could make it a future solution of the renewable energy supply.

This thesis aimed at looking for an improvement of current 2nd generation biorefinery thinking by making better use of current lignocellulose feedstocks using thermally stable, low-cost ionic liquids (ionoSolv) for separations. Also, the possibility of using the ionoSolv process on an algae-based biorefinery is examined. The feasibility of using recycled ionic liquids was also tested.

Part I. Background

1 Ionic liquids

1.1 General

Ionic substances (i.e. salts) normally exist in solid state at room temperature due to the very strong electrostatic bonds between the anion and cation counterparts. Salts like NaCl and KCl have a melting point of 801°C and 770°C. Ionic liquids are salts that are liquid at low temperature, normally under 100°C (13). The first IL, ethylammonium nitrate, was discovered by Gabriel in 1881, which has a melting point of 52-55°C (14). The main reason ILs have low melting points is the bulkiness and low symmetry of their constituent cations and anions. Also, the delocalization of the ionic charge over more than one atom in both the cations and anions (except for halides), helps lower the interactions and hence the melting point. The delocalization of charge causes a reduction in lattice energy, which also contributes to structural flexibility. Cations with more than one alkyl chain also can have a lower symmetry, thus lowering the melting point of the ionic liquid (15)

Figure 1-1 shows common cations and anions used in the synthesis of common ILs. The cation in an IL is usually comprised of a bulky peralkylated organic ion such as (dialkyl)imidazoles, (tetraalkyl)amines or (alkyl)pyridines while the anions are usually polyatomic (except for halides). Anions can be either organic or inorganic such as methyl sulphate, hexafluorophosphate, iodide, trifluoromethanesulfate, dicyanamide, bromide, chloride and acetate (16) (17). Since ILs are made entirely of anions and cations, there are a great number of different combinations to form ILs, and consequently different associated properties. This is the reason why ILs are called “designer solvents” (17) (18). At the moment, only 600 molecular solvents are in use today, in comparison, there are at least a million binary ionic liquids potentially possible to synthesize. In addition, the ILs also have a

hybrid ionic-organic nature which makes them capable of participating in a wide variety of interactions from weak and isotropic forces (e.g., van der Waals, solvophobic) to strong (Coulombic), specific, and anisotropic forces (e.g., hydrogen bonding, electron pair donor/acceptor interactions) (18). The designer characteristics of ILs have made them very popular since they can be tailored and tuned to fit wide a variety of applications. Applications include chemical synthesis (19) (20), electrochemistry (21), and carbon capture (22) as well as lignocellulose pretreatment (23) (24).

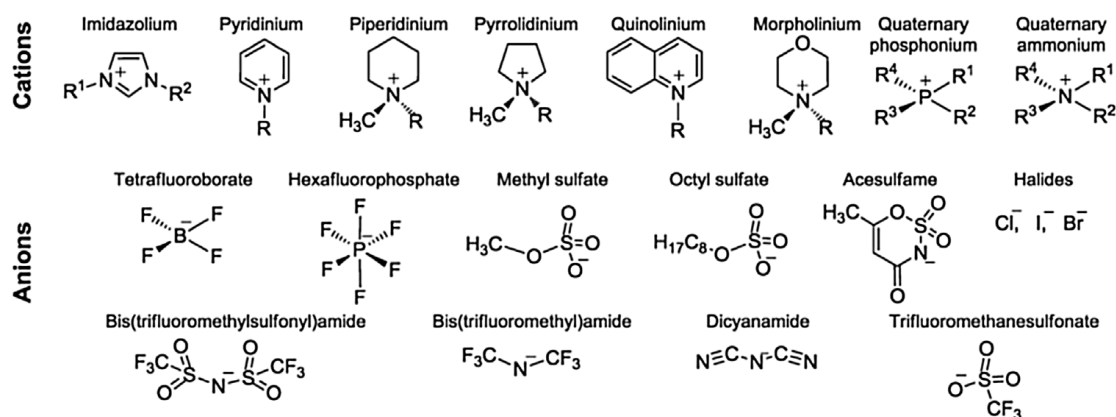


Figure 1-1. Different cations and anions for ionic liquid combination (25)

1.2 Physicochemical Properties

Ionic liquids generally have a melting point lower than 100°C , however certain ILs, including a few 1-alkyl-3-methylimidazolium based ILs (26) and some with formate anions (27), have melting points well below 0°C (28). Thermal stability is important nature of ILs since thermal decomposition temperature of an IL has a significant impact on its application. A good example is ILs used in CO_2 desorption process must have high thermal stability as such process normally happens at high temperatures (29). ILs formed with small or symmetric ions normally shows high melting points (30). Another factor that has an impact on melting points are the alkyl chains interaction. One example is 1-hexyl-3-methylimidazolium $[\text{PF}_6]$ has the melting point of -61°C . which is lower than similar ILs with shorter or longer alkyl chain. The reason is that shorter alkyl chains ($n < 6$) have higher melting points because they form symmetrical ILs with better ion cohesion. On the other hand, Longer alkyl chains ($n > 8$) also

have higher melting points as the cations are more hydrophobic, and form Van der Waals interaction (31).

ILs generally have high viscosities at room temperature which could range from 10 mPa·s to over 1000 mPa·s (13). The high viscosity is normally an issue in IL applications as it can slow down the rate of chemical reactions (32). Therefore the research of IL viscosities is focusing on methods to reduce the viscosity while still maintain the chemical property of the IL. The attempts of using artificial intelligence to estimate viscosities, melting points and other physicochemical properties of ILs is getting more successful in recent years, this approach will provide a new solution for IL testings and modification (33). One easy way to adjust IL's viscosity without having to modify cations and anions are introducing impurities. Researches in this area mainly focus on viscosities of binary and ternary mixtures of ILs with water and ethanol (34) (35).

1.3 Protic Ionic Liquids

Protic Ionic Liquids (PILs) are a class of ILs that are synthesised by a one-step acid-base neutralization reaction. One key characteristic that distinguishes PILs from other ILs is the availability of a proton to form an inter- and an intra- hydrogen bonding network between the anion and the cation as well as dissolved solutes (36). The most important advantages of PILs over other aprotic ILs (AILs) is their low production cost and ease of synthesis (21). For example, [TEA][HSO₄] requires 7 steps to synthesize starting from oil, N₂, H₂, S₈ and O₂, while the synthesis of [C₂C₁im][OAc] needs 29 steps instead (37). Also, PIL synthesis will involve mainly exothermic reactions and therefore minimal energy input will be required and there is the potential to use the excess heat generated during reaction (38). Unlike some APILs, synthesis of PILs will normally require no further purification processes which greatly reduces cost and usage of other solvents (39). Hallett *et al.* estimated the production cost of [TEA][HSO₄] at \$1.24/kg and [HC₁im][HSO₄] is expected to cost \$2.96/kg (37). The cost of producing a PIL is mainly affected by the price of the raw materials (37), typically chemicals that will be used to make the cations (39).

PILs have certainly gained increased attention recently. They have a wide variety of uses in biomass application including pretreatment of lignocellulosic biomass for bioethanol production (40), delignification of corn stover (41), and also pretreatment of macroalgae which will be discussed later in this thesis. The synthesis of PILs is based on the classical acid–base neutralization reaction, which transfers a proton from a Brønsted acid to a Brønsted base as shown in Figure 1-2.

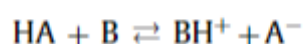


Figure 1-2. Proton transfer during the synthesis of PILs

After synthesis of PILs there will normally be a process to adjust water content in PILs, normally to remove a certain amount of water to achieve the desired water-IL mixture for the application. The ratio of cations to anions can also be adjusted easily to suit certain reactions. The impact is also discussed later in this thesis. PILs are used in most of the experiments which will be talked later as one of the purposes of this PhD is to carry out research on pretreatment with a low cost.

2 Biomass

2.1 Lignocellulosic Biomass

Lignocellulosic biomass consists of 3 main biopolymers. The first one is cellulose which is a highly crystalline form of polymeric glucose. The second major biopolymer is hemicellulose, which is an amorphous polymer consisting of a mixture of pentoses and hexoses, depending on the plant in question. Finally, there is lignin, a heterogeneous polymer containing aromatic subunits (42) (43). These three components account for over 90% of lignocellulosic biomass's dry weight. Figure 1-3 demonstrates the structure of lignocellulose (13). In plant cells wall which accounts for most of the plant's dry weight, we have linear fibres of cellulose presented in yellow. Cellulose fibres are surrounded by hemicellulose (blue). Cellulose and

hemicellulose are further interconnected with lignin which is orange in the figure. Apart from these three major contents, lignocellulose contains smaller amounts of pectins, inorganics, proteins and extractives such as waxes and lipids (5). In terms of carbohydrate content, lignocellulose normally contains up to 70wt%; specific composition depends on which plant species are being investigated (42), different parts of the plant (44), the environment it was grown in (45). There are three types of lignocellulosic biomass: grasses, softwoods and hardwoods. All three types have different content of carbohydrate. Substructures of lignin and hemicellulose are also different in different types (42). Generally, people agree covalent bonds exist between the lignin and hemicellulose fractions, but not between the lignin and cellulose fractions, which is shown in Figure 1-3 (46).

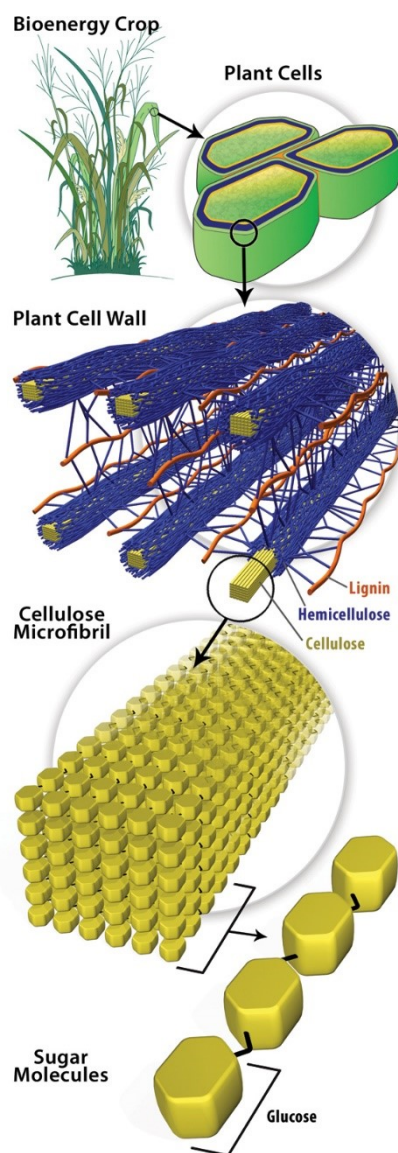


Figure 1-3. Cellulose, hemicellulose and lignin in lignocellulosic biomass (47) Credit: Biological and Environmental Research Information System, Oak Ridge National Laboratory

2.1.1 Cellulose

Cellulose is the largest component of lignocellulosic biomass. It normally accounts for 35-50wt% of the dry weight of biomass. Cellulose is a linear polymer made of glucose units which are linked to each other by 1-4- β glycosidic bonds as shown in Figure 1-4 (43). The degree of polymerization in cellulose can vary from 10,000 to 15,000 (48). As cellulose is made of D-glucose units that undergo condensation reactions to link to each other with a β -configuration such that each successive glucose unit is rotated 180 degrees around the

axis (49). However, glucose units in starch which shares the same chemical formula with cellulose are linked with α -configuration such that each successive unit is oriented in the same direction (50)(Figure 1-4).

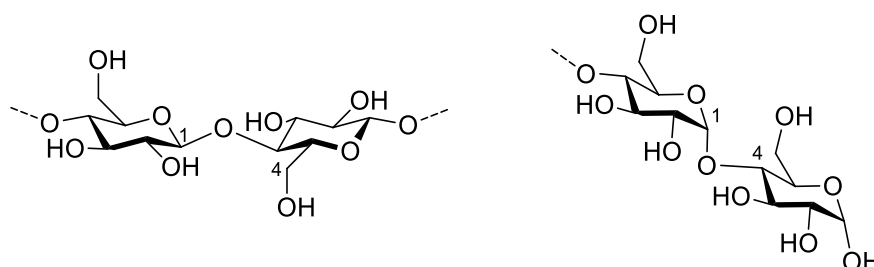


Figure 1-4. 1-4- β glycosidic bond found in cellulose (left) and 1-4- α glycosidic bond found in starch (right)

Chains of linear glucose polymers, which are referred to as glucans, are further connected by two intramolecular hydrogen bonds between neighbouring glucose units and one intermolecular hydrogen bond link the chains into flat sheets (42). Flat sheets mainly interact with each other via van der Waals interactions which lead to a stabilisation of the cellulose fibrils. The microfibrils of cellulose, which are relatively stiff, give the cell wall some rigidity (51). During the synthesis of the microfibrils in the plant cell, some of the hemicelluloses get physically trapped within the microfibril, thereby making this region more amorphous compared to the rest of the crystalline microfibrils (52). There are generally two types of cellulose in lignocellulose. Native cellulose (type 1 cellulose) contains two intramolecular and one intermolecular hydrogen bond. Type 1 cellulose can be converted into type 2 cellulose which is thermodynamically more stable and contains hydrogen bonds between different sheets. Due to its high molecular weight and a high degree of polymerization, cellulose is insoluble in water and most of the inorganic solvents (116). The depolymerisation of cellulose to glucose is investigated in this research. It is a vital process to produce ethanol from biomass. The cellulose depolymerisation can happen at the presence of Brønsted acids (53) (54) (55) and enzymes (56) (57) (58) (59). Enzymes catalysed process is called saccharification and is carried out in this research to evaluate the effectiveness of pretreatment.

Cellulose used in industry today is mainly obtained from the wood pulping process using the Kraft process (51). Paper, paperboard, membranes and textile productions represent the largest end applications of cellulose (60). Cellulose has applications in many fields. In the food industry, cellulose is generally considered a “generally recognized as safe” (GRAS) ingredient (61).

The hydrolysis of cellulose produces glucose which can be converted to a variety of chemicals such as ethanol, levulinic acid (62), lactic acid, 5-hydroxymethyl furfural (5-HMF) (63), sorbitol (64) and gluconic acid (65). Figure 1-5 shows the mechanisms of forming 5-HMF from glucose or mannose (both hexoses). 5-HMF is considered as a versatile platform of chemicals and it is in fact listed as one of the most important renewable chemicals that can be obtained from lignocellulosic biomass (66).

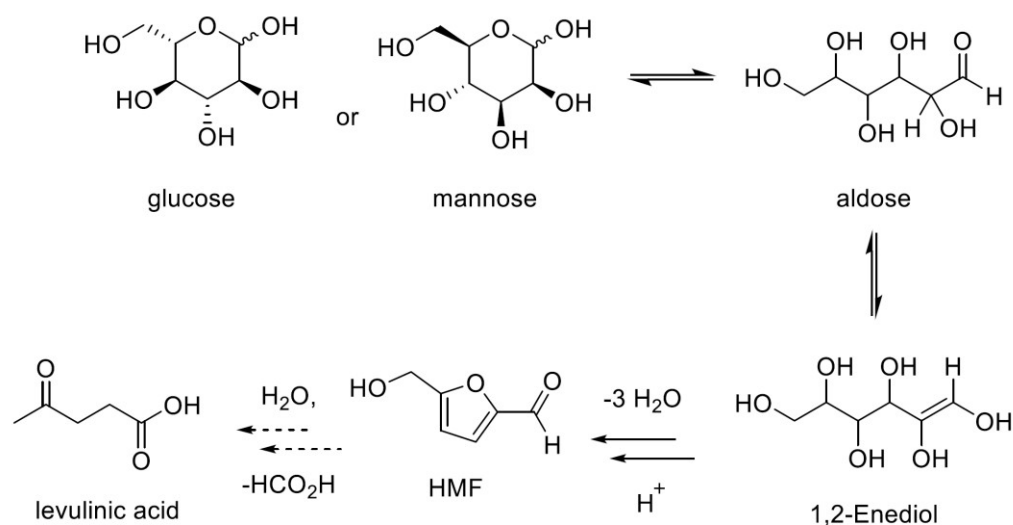


Figure 1-5. Proposed acid catalysed mechanism for the formation of HMF and levulinic acid from hexoses.

Adapted from Reference 66.

2.1.2 Hemicellulose

Hemicellulose is a group of heterogeneous polysaccharides that are made of hexose sugars (C6) (e.g. glucose, mannose, galactose) and pentose sugars (C5)(e.g.xylose, arabinose) as shown in Figure 1-6 (67). Hemicellulose is the second-largest component of lignocellulosic

biomass after cellulose and makes up around 20 to 30% of its dry weight, the exact abundance of which is largely dependent on the plant species (43). The amorphous hemicellulose binds non-covalently the cellulose microfibril surface increasing its overall stiffness (48). Unlike cellulose, hemicellulose is branched and has a much lower degree of polymerization of around 100 to 200 units.

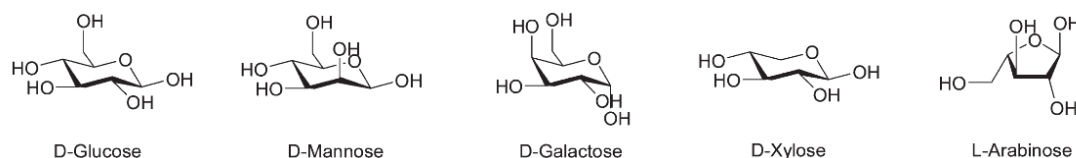
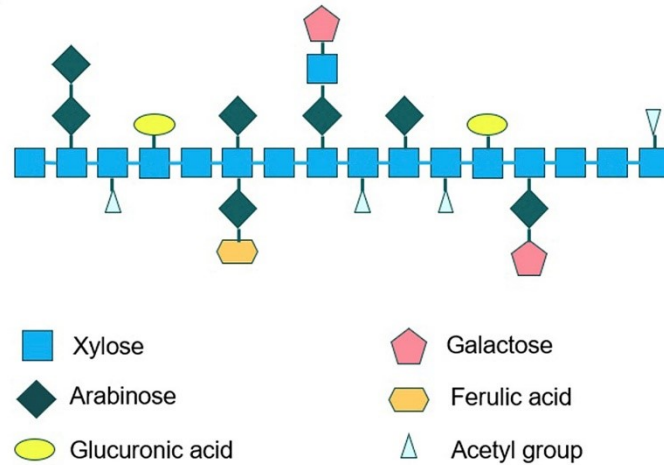


Figure 1-6. The hexoses and pentoses typically found in hemicellulose

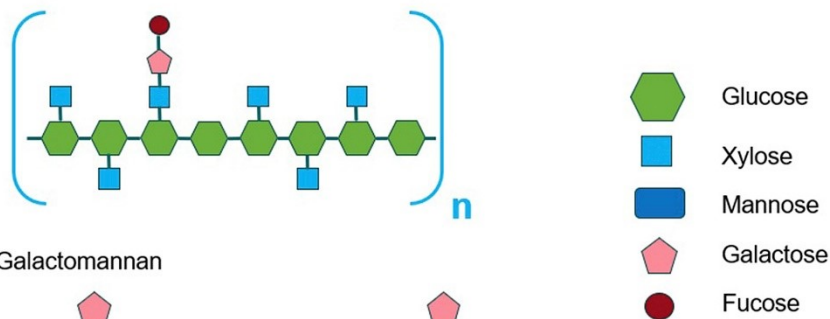
Hemicellulose polysaccharides are much easier hydrolyze under mild conditions compared to cellulose due to the lower degree of polymerization and branching. The characteristics and composition of hemicellulose depend on species. For example, mannan (C6 sugar) is the main component in softwood hemicellulose (e.g. galactoglucomannan polysaccharide) while xylan (C5 sugar) is the dominant component in hardwood and grasses (e.g. glucuronoxylan polysaccharides) (68).

Hemicellulose is the second most abundant polysaccharide of all biomass (69). Among all pentoses and hexoses that made hemicellulose, xylose is the most abundant sugar (70). Therefore, it is important to understand how hemicellulose existed in biomass and the potential applications of it. Figure 1-7 showed some typical hemicellulose structures. Xylan, one of the most common structure, is composed of xyloses units which form a chain with other units like arabinose and galactose (71). Xyloglucan has a main chain of glucose instead, and mostly substituted by xylose units (72). Galactomannan is made of galactose and mannose in the form of a mannan chain. Glucomannan is also a common structure where galactose is replaced with glucose.

- Xylan



- Xyloglucan



- Galactomannan

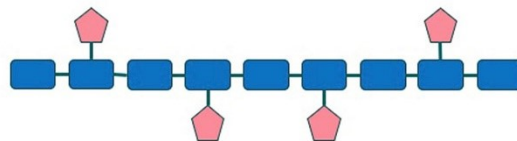


Figure 1-7. The main structures of hemicelluloses: a) xylan, b) xyloglucan and c) galactomannan. Adapted from Reference (73).

Although one of the research goals in this thesis is to avoid hemicellulose sugar degradation to achieve the highest possible sugar yield for further use, ie. Biofuel production, the degradation product of hemicellulose is not necessarily a negative aspect of a pretreatment. Some degradation product can be useful in further production of other chemicals. One good example is furfural (74). Furfural can act as a platform molecule to make many other chemicals as shown in Figure 1-8.

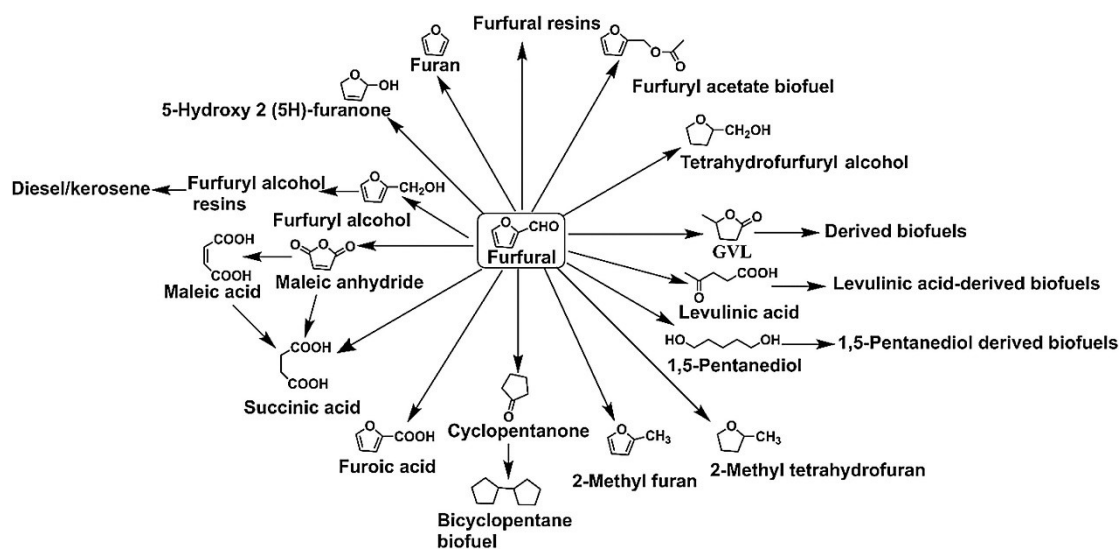


Figure 1-8. The pathways for the conversion of furfural as a platform molecule into chemicals and fuels.

Adapted from Reference (74)

Some novel research is now focusing on ways to enhance the degradation of hemicellulose to furfural by using ILs (75). Other methods to get furfural from hemicellulose rich biomass include using a tin-loaded montmorillonite solid acid catalyst (76), and a two-stage process which is acid hydrolysis followed by dehydration, using response surface methodology (77). The mechanism of xylose degradation to furfural is not entirely clear yet, two most commonly recognised explanations are shown in Figure 1-9 (closed chain mechanism) and Figure 1-10 (open chain mechanism).

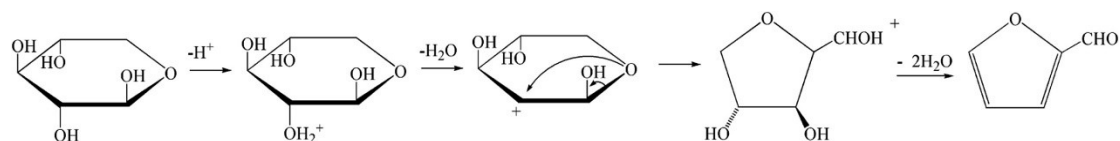


Figure 1-9. Proposed closed chain mechanism for the dehydration of xylose to furfural via intramolecular rearrangement in acidic solution. Adapted from Reference (78)

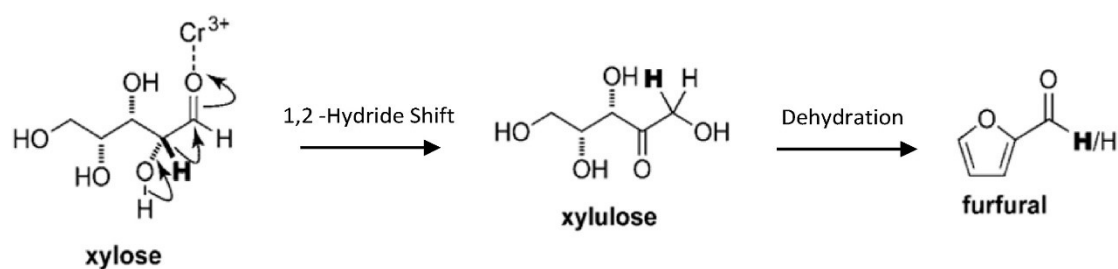


Figure 1-10. Proposed open chain mechanism for the dehydration of xylose to furfural via 1,2- hydride shift catalyzed by Lewis acid. Adapted from Reference (79)

2.1.3 Lignin

Lignin is an aromatic containing polymer that fills the space in the cell wall. Lignin is built up at a mature state of plant growth (50) and plays an important role in providing mechanical reinforcement and structural rigidity to the polysaccharides in biomass (80). Lignin is water insoluble therefore can provide water-proofing for the cell wall. Figure 1-11 showed the monolignols that made up lignin, namely hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units. Each of the unit forms lignin via polymerisation thus lead to the complex structure of lignin. In fact, the properties of lignin from one biomass can be significantly different from another. Lignins derived from different part of the same plant can be different too. This character of lignin introduced obvious difficulties in lignin valorisation (117). The most common way to make use of lignin is burn it directly for energy, which is a huge waste of lignin's potential.

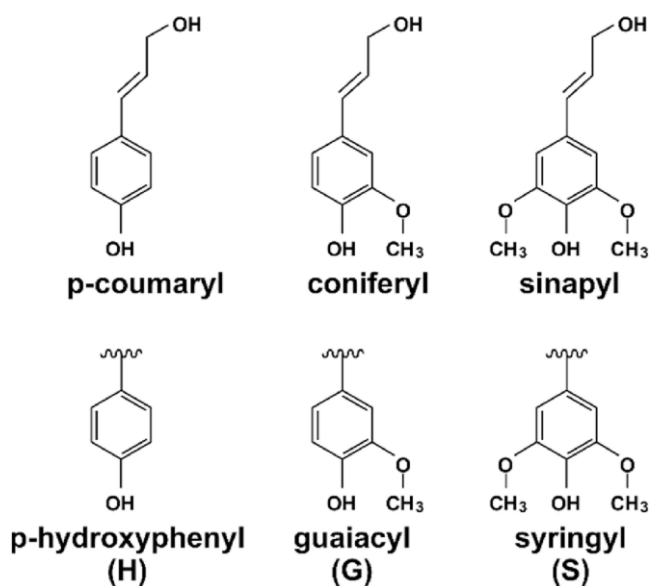


Figure 1-11. The monolignols that made up lignin (81)

Lignin content in lignocellulosic biomass is different from one type to another. Miscanthus has a relatively low lignin content of 9-13 wt% (2) compared to softwood which has 8-30 wt% lignin. While all three subunits are found in grass lignin, softwood lignin is made almost exclusively from G units. Hardwood lignin on the other hand has approximately equal proportions of G and S units (82). In this research, delignification is an important process in the whole experiment as the purpose is to make sure cellulose and hemicellulose are exposed to enzyme after lignin is removed. The percentage of each monomer and the way they are linked to each other plays key roles in delignification. Figure 1-12 showed the most typical linkages between H, G and S units. Delignification of hardwoods is generally easier due to the high abundance of syringyl units in hardwood lignins. As many possible linkages are made impossible due to the OCH_3 group on both C-3 and C-5 position (60).

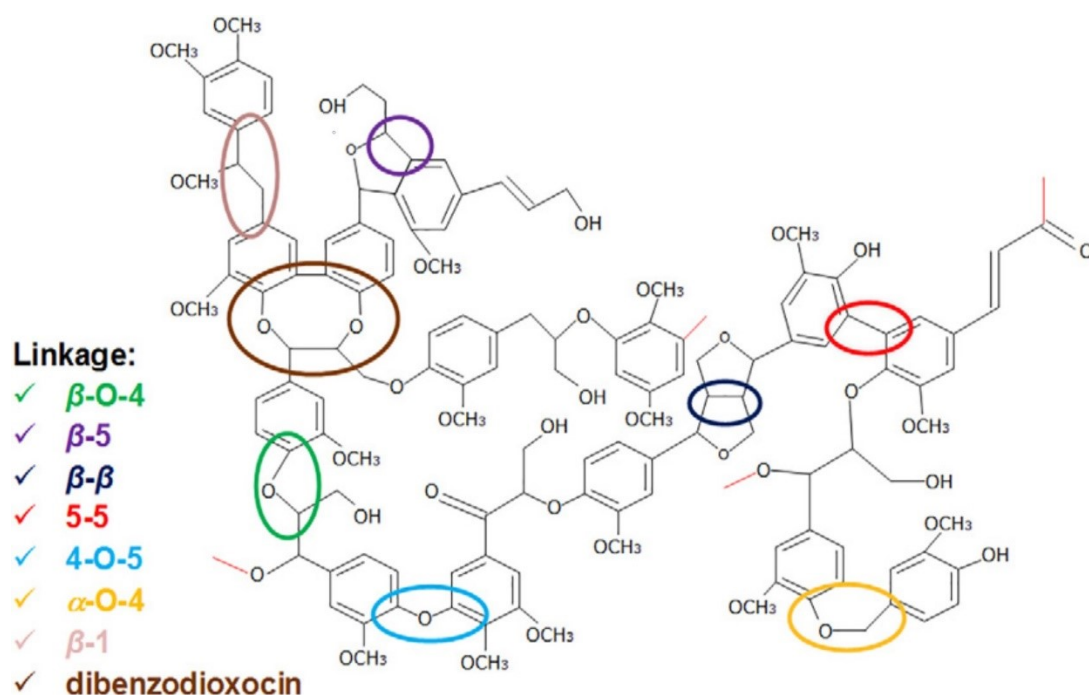


Figure 1-12. A fragment of Freudenberg's model of the structure of lignin (83)

3 Biorefinery

3.1 General

The biorefinery, as defined by NREL, is a “facility that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass” (84). Biorefineries use biomass as raw materials rather than traditional petrochemical methods which use crude oil. The major product of current biorefineries is bioethanol derived from carbohydrates in any plants (85) (86) (87) and biodiesel, obtained from oily plants such as rapeseed and oil palm (88). These carbohydrates, which are composed of complex polymers consisting of different kinds of sugars, will then go through a fermentation process to produce ethanol. This is called the 1st generation biorefinery (50). Bioethanol generated from 1st generation biorefineries is now competitive with petrochemical-derived ethanol. 1st generation biorefineries are a relatively simple set of methods yet require a large amount of biomass which competes with food supplies as many ideal biomass sources are food plants, such as sugarcane (50). Therefore, an alternative source of bioethanol is required.

Lignocellulosic biomasses are therefore envisioned to be the raw material of the 2nd generation of biorefineries (50). Lignocellulosic biomasses are normally non-edible plants and are highly abundant. They normally grow on non-arable land and grow faster than edible plants. Typical lignocellulosic biomass can be grasses like Miscanthus, softwoods like pine and hardwoods like willow. Dry lignocellulosic biomass is made of carbohydrate polymers and aromatic polymers. The product ethanol is called lignocellulosic ethanol, and is believed to hold a promising future. The main barrier of application of 2nd generation biorefinery is it requires a pre-treatment step prior to hydrolysis and fermentation as shown in Figure 1-13 (50).

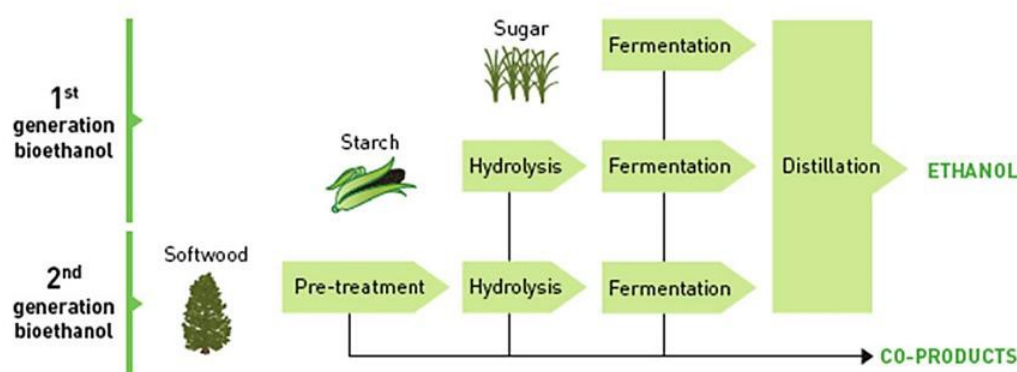


Figure 1-13. First and second generation biorefinery (50).

1st generation bio-ethanol production from sugary plants only requires fermentation and in the case of starchy plants a hydrolysis step while 2nd generation bio-ethanol requires a pretreatment step

One issue with the 1st generation biofuels is high release of nitrous oxide during production. Nitrous oxide generation can be significantly higher compared to traditional fuels due to the use of fertilisers during the growing of raw material biomass (89). The land-use change of cultivation may also promote a decrease in soil carbon when changing (100). On the other hand, raw material biomasses for the production of 2nd generation biofuels, especially grass type biomasses, are much more environmental friendly. One of the biomass used in this research, miscanthus, is a good example. It grows fast, and requires zero care from human and almost zero fertilization. In many areas of the world, the land that miscanthus grows on

has no plan to be used for agricultural purposes, therefore using miscanthus as a raw material for biorefinery does not compete with food production either directly or indirectly.

As mentioned before, the problem of using lignocellulose in a biorefinery is the high recalcitrance of lignocellulosic biomass which necessitates a pretreatment process prior to hydrolysis or fermentation. Pretreatment methods include concentrated acid (90), dilute acid (DA) (91), hot water (92), steam explosion (SE) (93), ammonia fibre expansion (AFEX) (94), organosolv (95) (96) and ionic liquid pretreatments (97) (98) (99). Traditionally, the main purpose of most pretreatment technologies is the isolation of a highly digestible cellulose-rich pulp for better enzymatic hydrolysis, lignin removal and cellulose preservation, which almost completely ignored hemicellulose. In the research, the preserve of hemicellulose during delignification is one of the major research gaps. The saccharification of hemicellulose sugars together with cellulose sugar is also a main task.

3.2 Deconstruction of lignocellulose

The very first deconstruction of lignocellulose was established back in the 19th century for pulping and paper industry (100). The process is called Kraft process, and was the dominating pulping or deconstruction process that has been employed for years in the paper production industry using softwood as a feedstock. During the Kraft process, biomass is heated in an aqueous mixture of sodium hydroxide (NaOH) and sodium hydrogen sulphide (NaHS) or Sodium sulphide (Na_2S) at 130-180°C for several hours, dissolving part of the hemicellulose and most of the lignin by fragmentation and formation of water-soluble lignothiols. The liquor is burnt afterwards for energy generation and regeneration of the sulphide (100). Another deconstruction process is the sulphite pulping which uses sulphurous acid salts to extract lignin. The above process is optimised for high cellulose yield and fibre strength. In the biorefinery process, the requirements of the deconstruction of lignocellulosic biomass are different. In this research particularly, an ideal deconstruction would remove most lignin while still preserves cellulose and hemicellulose.

Traditionally, the priority of the lignocellulose deconstruction process in the biorefinery is providing available glucose for fermentation (50). To achieve this goal, a couple of strategies are applied, disrupting the lignocellulosic structure (e.g. Grinding) (44) (101) (102) (103), decrystallizing the cellulose (90) (104) (105) and selectively removing lignin and/or hemicelluloses (106) (98) (107). After obtaining a cellulose-rich pulp, the next step is saccharification. Glycosidic bonds are hydrolysed during saccharification, usually catalysed by either enzymes (103) (108) or chemicals (90) (109). During the above process, hemicellulose is completely ignored. The result is sugars that account around 20% of the dry weight of the biomass is disposed of. Another issue of ignoring hemicellulose is the condition designed for deconstruction might cause hemicellulose degradation product to accumulate in the cellulose phase, which can lead to a lower saccharification yield. This research therefore focuses on finding a lignocellulose deconstruction process that can remove as much lignin as possible, while still preserves most cellulose and hemicellulose. In this section, some of the most common pretreatment methods will be discussed.

3.2.1 Hot Water based lignocellulose deconstruction

Hot water based processes are also known as hydrothermal processes. Sometimes they are also called chemical-free mechanism. Hydrothermal processes are normally regarded as simple, cost-effective and toxic-free as no harmful chemical is involved (110). The downside of this method is it normally requires the reaction to happen at high temperature and pressure. The specific conditions depend on biomass species but the temperature requirements can be as high as 220 °C and the pressure could go to 5MPa (111). As a result, the capital cost of building the container for a hot water based process can be high due to the conditions. The maintenance cost is also expected to be high due to the corrosion that could happen at high pressures (145). This method generally performs well when the task is delignification, however, such condition is too harsh for hemicellulose to be preserved together with cellulose (165). The formation of furfural and 5-HMF together with

pseudo-lignin will decrease the accessibility of cellulose after pretreatment, thus lead to a higher amount of enzymes required for saccharification and increase the overall cost as enzymes are normally expensive.

3.2.2 Ionic Liquid dissolution

The use of ionic liquid for biomass dissolution has gained great attention in the recent years with the largest data being collected for 1-ethyl-3-methylimidazolium acetate [C₂C₁im][OAc] (112) and 1-butyl-3-methylimidazolium chloride [C₄C₁im][Cl] (113). Both ILs have a sufficiently high hydrogen bond basicity β to decrystallize or dissolve cellulose by disrupting the strong hydrogen bonding network within the cellulose microfibril structure. Cellulose can be dissolved in above ILs without the absence of water, the biomass-IL mixture is heated to between 90 to 160 °C for several hours, depending on the feedstock and IL used. Cellulose is regenerated as an amorphous solid by adding an antisolvent, typically ethanol or water. Hemicellulose and lignin are also partially removed. The advantage of this method is that cellulose can be dissolved directly in ILs, and regenerated cellulose will require much less enzyme to hydrolyse as it has a much lower crystallinity (114). There are several identified disadvantages of this IL dissolution process that needs to be overcome. First is the limited thermal stability of these types of salts (115) (116). Also, the energy input to remove water from IL before cellulose dissolution to ensure full cellulose dissolution cannot be ignored. To summarise, ionic liquid dissolution is a unique method but still has many flaws. As a result, ionosolve process is investigated.

3.2.3 Alkaline pretreatment

Alkaline pretreatment such as using sodium hydroxide, calcium hydroxide or aqueous ammonia can significantly improve lignin removal and enhance enzymatic hydrolysis of the recovered cellulose (117). Under alkaline conditions, the ester and ether linkages between

hemicellulose and lignin can be easily broken which significantly facilitates the solubilization of lignin and hemicellulose into the solvent (118). The alkaline pretreatment can achieve a good lignin removal while providing cellulose that is highly digestible, yet not decrystallized (119). The reaction condition of alkaline pretreatment is mild, normally happens under room temperature and natural atmospheric pressure. Generally, alkaline pretreatment works better on grass type biomasses than softwood and hardwood (120) (121).

3.2.4 Organosolv

Organosolv is short for organsolvation, which is a successful example of processing grasses and hardwoods. In organosolv processing, biomass is pretreated in hot aqueous alcohol with a low concentration of acid catalyst (around 1-2wt%) (96) (122) (123), or concentrated organic acids (124). One of the prime advantages of Organosolv compared to other pretreatment processes is the recovery of high-quality lignin that can be potentially valorized to produce resins. The recovery of organic solvents through distillation is also simple which provides another advantage over acid and alkaline-based pretreatments. The factor that limits a better application of organosolv process is the potential hazards of handling of a large number of organic solvents (125).

There are many other deconstruction methods in the history of exploring the application of biomass rather than burn them for energy directly. The most original way is to simply crush the biomass, this is called mechanical splintered pretreatment. Now, this method is commonly used before any other treatment (126). Other non-chemical methods include microwave treatment pretreatment (127), ultrasonic treatment pretreatment (128) and High-energy electron radiation pretreatment (129). There are also many chemical pretreatment methods to mention. Acid pretreatment (130), oxidation pretreatment (131), CO₂ explosion pretreatment (132), SO₂ explosion pretreatment (133) and biological pretreatment method (134).

3.2.5 IonoSolv Process

Like ionic liquid pretreatment, IonoSolv pretreatment is another IL-based pretreatment for lignocellulose biomass. Certain ionic liquid-water mixtures have been found to be able to effectively remove hemicelluloses and lignin from lignocellulosic biomass (135). The ILs used in the IonoSolv process normally consist of a hydrogensulfate or alkylsulfate anion and an imidazole or amine derived cation. 10-40wt% of water with ionic liquids is also required. Like the organosolv process, the IonoSolv process is also considered as delignification or fractionation processes where the solvent selectively extracts lignin as well as hemicellulose from the biomass, leaving a cellulose-rich pulp as a solid residue (136). The advantage of the IonoSolv process over the organosolv process is that IonoSolv is conducted at atmospheric pressure while organosolv needs operating pressures of 3.4 to 4.1 bar.

Lignin and hemicellulose are partly or fully dissolved during IonoSolv pretreatment. In an ideal pretreatment, lignin will be mostly dissolved in the liquor, while most cellulose remains in the pulp with some hemicellulose. (137). The hypothesis of lignin extraction in ILs is initiated by the hydrolysis of the glycosidic bonds in the lignin-carbohydrate complexes which shorten the lignin polymer length. This is followed by the solubilization of the shorter lignin polymer chains and subsequent fragmentation in the IL solution (138). The IL anion seems to play a vital role in the delignification mechanism, acting as both proton source and nucleophile that breaks β -O-4 bonds, the major ether linkage in lignin (139).

Pretreatment of Miscanthus with [HC₄im][HSO₄] with 20wt% water is a typical example which can achieve 90% of the saccharification yield compared to less than 20% in the ionic liquid process (106). IonoSolv using [C₄C₁im][MeSO₄], [HC₄im][HSO₄] or [C₄C₁im][HSO₄] has been reported to achieve almost full delignification of Miscanthus (50). Saccharification is around 30 times compared to untreated biomass despite the fact that the crystallinity of cellulose in pulp remained unchanged. This is a result of the enlarged surface area of the cellulose following the removal of lignin. The use of cellulose dissolving ILs do suffer from

some problems. They all have low thermal stability (140), low tolerance of water (141), and high production cost (e.g. [C₂C₁im][OAc] production cost range from \$20 to 101 per kg) (142). The ionoSolv ILs do not suffer from these drawbacks.

One issue of the ionoSolv process that cannot be ignored is the potential formation of pseudo-lignin. During the process, solubilized lignin fragments can undergo condensation reactions where the fragments react with each other or with other sugars forming pseudo-lignin. The re-deposition of pseudo-lignin onto the cellulose-rich pulp can affect saccharification yields considerably. Formation of pseudo-lignin can be avoided by carefully choosing conditions of pretreatment according to feedstock type and IL used (136). The extraction of lignin by the IL significantly enhances glucose yield during saccharification due to the greater exposure of cellulose fibrils resulting in 80%, 65% and 80% glucose yield for *Miscanthus* (136), *pinus sylvestris* and *Salix willow* (143), respectively (at 20 wt% loading, 150 °C and 1 hour). Water in the IL-water mixture played the key role for the hydrolysis reactions needed to cleavage several bonds such as ether bonds, ester bonds and the branched glycosidic bonds in hemicellulose. Water also contributes in lowering the viscosity of ILs; an important process advantage since the high ILs viscosity will lead to a higher cost by increasing residence times and therefore process vessel CAPEX.

One big milestone of the ionoSolv process since its discovery by Dr. Agnieszka Brandt is the effective application of low-cost protic ILs in the pretreatment of lignocellulose biomass. The most commonly used ILs normally have a bulk production cost of \$40-80 per kg while the cost of low-cost protic ILs is around only \$1.24 per kg for bulk production (144). Such a reduction in cost proved the potential economic viability and made scale-up possible Figure 1-14 showed a conceptual process flow for the large-scale ionoSolv process.

4 Ionic liquid recovery

As discussed above, ionic liquids have become a promising solution for lignocellulose pretreatment, not only due to their performance in lignin and hemicellulose removal, but also their reusability. Effectiveness of IL recycle, recovery and reuse are crucial to evaluating the overall success of the ionoSolv processes, including requiring the separation of water from the used IL before it can be recycled. This step was considered as an energy-intensive process and probably a cost-limiting step. The current method of water separation is simple evaporation, where in the lab water is evaporated under vacuum at mild temperature (around 40 °C) however thermal-based evaporation needs to be applied in a large scale process. The corrosion issue is also a barrier as many ILs are found to be corrosive especially at high concentrations, as a result, a high maintenance cost can be expected. One possible solution to the highly energy-intensive water separation is to try membrane-based separations.

Pressure-driven membrane technologies such as reverse osmosis (RO) or nanofiltration (NF) cannot be used in the separation of IL-water system due to the high osmotic pressure build up in separation (146). One example is the separation of 1-butyl-3-methylimidazolium bromide and $[C_4C_1im]Br$ and 1-butyl-3-methylimidazolium tetrafluoroborate $[C_4C_1im][BF_4]$ from their aqueous solutions using NF membranes. The maximum rejections obtained were 67% and 60%, respectively (147), which is not perfect.

To overcome such barriers, pervaporation and membrane distillation (MD) are two other membrane-based technologies that are independent of the osmotic pressure. Pervaporation uses a hydrophilic dense or porous membrane and the driving force of transport in pervaporation is a combined effect of concentration and pressure gradient. Membrane distillation uses a hydrophobic porous membrane and the driving force in MD is limited to pressure gradient only (148). Several successful examples of using these methods in IL re-concentration have been reported. In one test using pervaporation technology to re-concentrate $[C_2C_1im][OAc]$ has shown the ability to recover more than 99.9 wt% of IL with

successful 5 times recycle (149). Another study was conducted using membrane distillation to separate and concentrate two imidazolium-based ILs with acetate and formate anions using commercial hydrophobic PTFE and PVDF membranes. A 10-fold concentration was achieved from 5 to 50 wt% IL with PTFE giving better performance (150).

Life cycle and economic considerations of the entire value chain of biorefinery are very crucial. The sustainability of biorefinery process affects the future of not just biorefinery itself, but also the global energy supply. Normally the bioenergy system is considered to be carbon neutral, however, additional energy input is often required during the production/cultivation of feedstock, transportation and conversion (151). Therefore the assessment needs to be carried out carefully and consider as much factor as possible. Life-cycle greenhouse gas (GHG) emissions of biofuels and other bioenergy systems heavily depend on a huge variety of factors therefore made the evaluation of a biorefinery process very difficult (152). i.e. different biomasses require different land-use, fertilizer use, and weather conditions (153). The recycle of ILs will potentially improve the overall life cycle of ILs, however, the energy consumption of IL recovery should not be ignored.

5 Algae

5.1 General

Compared with biomass used in the 1st and 2nd generation biorefinery, algae are photosynthetic aquatic organisms that are responsible for over 50% of primary photosynthetic productivity on earth but rarely used for commercial purposes (154) (155) (156) (157). While lignocellulosic biomass solved the problem of “food versus fuel” competition, they still require relevantly long growth cycle. Land consumption is another factor cannot be ignored (158). To overcome such barriers, algae can provide a very promising route. It does not use any land to grow, its ability of regeneration is also much better. Besides, many countries have the issue of dealing with too much algae in the water

and on shore. The technology of deriving fuel and other by products from algae is referred to as the 3rd generation biorefinery (159).

Algae are simple aquatic organisms that photosynthesize, but there are an estimated approximately 300,000 species, whose diversity is much greater than that of the land plants (160). It represents an environmentally sustainable, renewable source of biomass for the production of biofuels (161). Algae can be cultivated in farms or collected from the sea or lakes. They will absorb CO₂ from the air and contain oils that can be used as raw material for biodiesel production (162). The carbohydrate content in algae can be converted into bioethanol. It is believed that algae have the potential to cover the whole global transportation fuel demand (163).

Apart from the application in biofuel production, algae can also play some other beneficial roles. Removal of CO₂ from industrial flue gases can be achieved by algae bio-fixation which can reduce emissions of greenhouse gas while producing biodiesel. Also this technology does not require CO₂ with high purity therefore no complex CO₂ separation from flue gas will be required (164). Algae can also be fit into wastewater treatment, water contaminants such as NH₄⁺, NO₃⁻, PO₄³⁻ can be effectively used as nutrients for microalgae (165) (166). Algae can normally grow under undefined conditions and require very limited nutrition. Therefore it is possible to carry out algae cultivation in areas which are unsuitable for agricultural purposes, independent of the seasonal weather changes. Saltwater or wastewater can also be used to avoid the need of clean freshwater (165). Due to the huge number of algae species, it also has the potential to produce some fine chemicals. Chemicals with high commercial value include fats, polyunsaturated fatty acids, oil, natural dyes, sugars, pigments, antioxidants and other fine chemicals and biomass (167) (168). Finally, a large number of biotechnology areas including biofuels, nutrition and food additives, cosmetics, aquaculture, pharmaceuticals and prevention of environmental pollution can be revolutionized by algae (168).

5.2 Algal biorefinery approach

As with the 1st and 2nd generation biorefineries, an algae-based biorefinery focuses on the production of biofuels as well as high value co-products from biomasses by the integration of bioprocessing and appropriate low environmental impacting chemical technologies in a cost-effective and environmentally sustainable manner (169). A typical algae-based biorefinery approach is displayed in Figure 1-15 (170).

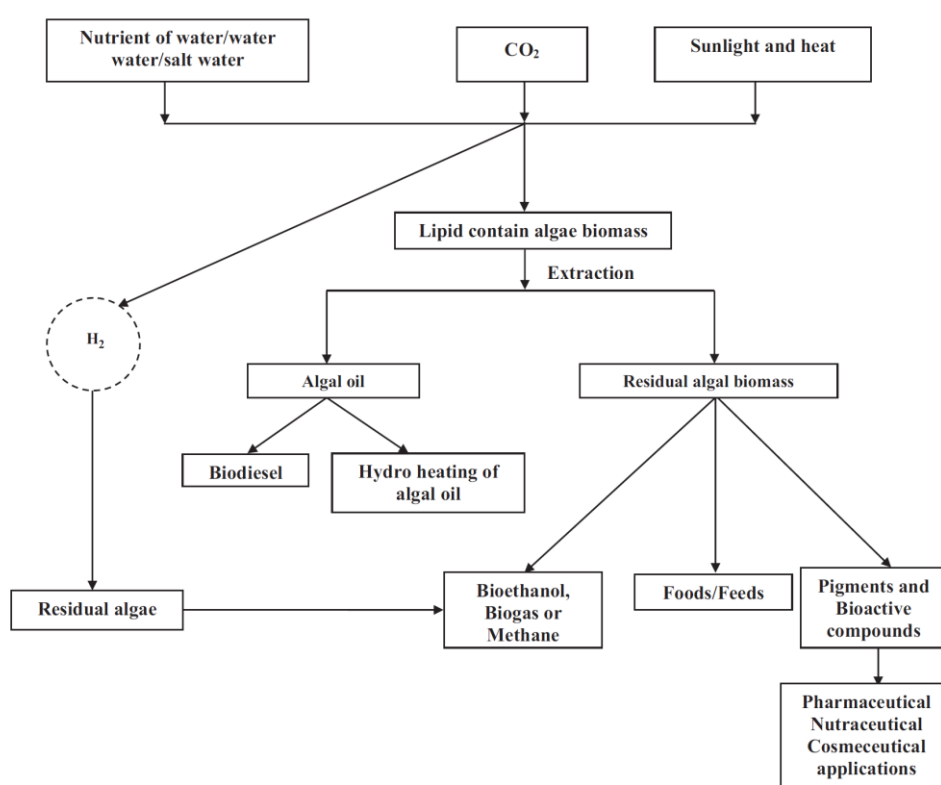


Figure 1-15. Biorefinery approach for algal feedstocks. Adapted from Reference 171

5.2.1 Biology of algae

The term “algae” refers to a polyphyletic, artificial assemblage of organisms (171). Algae can be divided into two major types, unicellular algae are called microalgae while multicellular ones are referred to as macroalgae, which is often call seaweed. Species of algae are very diverse with over 40,000 species already identified and many more yet to be identified.

Algae are classified into multiple major groupings as follows: cyanobacteria (Cyanophyceae), green algae (Chlorophyceae), diatoms (Bacillariophyceae), yellow-green algae (Xanthophyceae), golden algae (Chrysophyceae), red algae (Rhodophyceae), brown algae (Phaeophyceae), dinoflagellates (Dinophyceae) and 'pico-plankton' (Prasinophyceae and Eustigmatophyceae).

5.2.2 Microalgae

Microphytes or microalgae are microscopic algae, typically found in freshwater and marine systems (160). Microalgae are unicellular species, which exist individually, or in chains or groups. Size of microalgae depend on species and can range from a few micrometers to several hundreds of micrometers. One difference between microalgae and plants is microalgae do not have roots, stems and leaves. Microalgae play a crucial role for life on earth as its capability of photosynthesis. Approximately half of the atmospheric oxygen is produced by microalgae while greenhouse gas carbon dioxide is used. The biodiversity of microalgae is enormous. It has been estimated that about 20,000–800,000 species exist of which about 40,000–50,000 species are described (172) (173).

5.2.3 Macroalgae

Macroalgae or seaweed is a macroscopic, multicellular, marine algae that lives near the seabed (benthic) includes some members of the red, brown and green algae (174). A macroalgae, or seaweed, may belong to one of the several groups of multicellular algae: the red algae, green algae and brown algae. Some tuft-forming bluegreen algae (cyanobacteria) are sometimes considered to be seaweeds. A macroalgae normally contains following parts:

Thallus: the algal body

Lamina or Blade: a flattened structure that is somewhat leaf- like

1.Sorus: a spore cluster

2. On *Fucus*, air bladder: a floatation-assisting organ on the blade

3. On kelp, float: a floatation-assisting organ between the lamina and stipe

Stipe: a stem-like structure, may be absent

Holdfast: a specialized basal structure providing attachment to a surface, often a rock or another alga

Haptera: a finger-like extension of the hold fast anchoring to a benthic substrate

The stipe and blade are collectively known as the frond.

5.2.4 Biochemical composition of algae

Algal biomass is made of three main components: proteins, carbohydrates and lipid. The chemical compositions of various microalgae and macroalgae are shown in Table 2-1 and Table 2-2 (175) (176). The percentage of biochemical composition varies with different types of algae. Some microalgae (e.g. *Scenedesmus dimorphus* and *Prymnesium parvum*) have high lipids content can be a good raw material for production of biodiesel. Macroalgae like *Caulerpa cupressoides* and *Caulerpa laetevirens* have over 50wt% of carbohydrates composition and can be a promising bioethanol source.

Table 1-1. Biochemical composition of microalgae expressed on a dry matter basis (%dry weight). Data from Reference 176, 177

Microalgae	Protein	Carbohydrates	Lipid
<i>Scenedesmus obliquus</i>	50-56	10-17	12-14
<i>Scenedesmus quadricauda</i>	47	-	1.9
<i>Scenedesmus dimorphus</i>	8-18	21-52	16-40
<i>Chlamydomonas reinhardtii</i>	48	17	21
<i>Chlorella vulgaris</i>	51-58	12-17	14-22
<i>Chlorella pyrenoidosa</i>	57	26	2
<i>Spirogyra</i> sp.	6-20	33-64	11-21
<i>Dunaliella bioculata</i>	49	4	8
<i>Dunaliella salina</i>	57	32	6
<i>Euglena gracilis</i>	39-61	14-18	14-20
<i>Prymnesium parvum</i>	28-45	25-33	22-39
<i>Tetraselmis maculata</i>	52	15	3
<i>Porphyridium cruentum</i>	28-39	40-57	9-14
<i>Spirulina platensis</i>	46-63	8-14	4-9
<i>Spirulina maxima</i>	60-71	13-16	6-7
<i>Synechococcus</i> sp.	63	15	11
<i>Anabaena cylindrica</i>	43-56	25-30	4-7

Table 1-2. Biochemical composition of macroalgae expressed on a dry matter basis (%dry weight) Data from Reference 176, 177

Macroalgae	Protein	Carbohydrates	Lipid
<i>Hypnea valentiae</i>	11.8–12.6	11.8–13.0	9.6–11.6
<i>Acanthophora spicifera</i>	12.0–13.2	11.6–13.2	10.0–12.0
<i>Laurencia papillosa</i>	11.8–12.9	12.0–13.3	8.9–10.8
<i>Ulva lactuca</i>	11.4–12.6	11.6–13.2	9.6–11.4
<i>Caulerpa racemosa</i>	11.8–12.5	16.00	9.0–10.5
<i>Ulva reticulate</i>	12.83	16.88	8.50
<i>Enteromorpha compressa</i>	7.26	24.75	11.45
<i>Chaetomorpha aerea</i>	10.13	31.50	8.50
<i>Chaetomorpha antennina</i>	10.13	27.00	11.45
<i>Chaetomorpha linoides</i>	9.45	27.00	12.00
<i>Cladophora fascicularis</i>	15.53	49.50	15.70
<i>Microdictyon agardhianum</i>	20.93	27.00	9.40
<i>Boergesenia forbesii</i>	7.43	21.38	11.42
<i>Valoniopsis pachynema</i>	8.78	31.50	9.09
<i>Dictyosphaeria cavernosa</i>	6.00	42.75	10.51
<i>Caulerpa cupressoides</i>	7.43	51.75	10.97
<i>Caulerpa peltata</i>	6.41	45.00	11.42
<i>Caulerpa laetevirens</i>	8.78	56.25	8.80
<i>Caulerpa racemosa</i>	8.78	33.73	10.63
<i>Caulerpa fergusonii</i>	7.76	23.63	7.15
<i>Caulerpa sertularioides</i>	9.11	49.50	6.99
<i>Halimeda macroloba</i>	5.40	32.63	9.89
<i>Codium adhaerens</i>	7.26	40.50	7.40
<i>Codium decorticatum</i>	6.08	50.63	9.00
<i>Codium tomentosum</i>	5.06	29.25	7.15

5.3 Conversion technologies for algal biofuels

The conversion of algal biomass-to-energy encompasses the different processes, which depend, to a large extent, on the types and sources of biomass, conservation options and enduses (177). The conversion methods can be divided into two basic types, namely thermochemical and biochemical conversion (178) (179). Type and quantity of algae feedstock, the desired form of the energy; economic consideration, project specific and the desired end form of the product can all be factors that affect the choice of conversion process (180).

5.3.1 Thermochemical conversion

Thermochemical conversion refers to the thermal decomposition of organic components in algal biomass to produce fuel products (178). The thermochemical conversion process includes direct combustion, gasification, liquefaction and pyrolysis. When algae are heated under an oxygen deficient condition, synthesis gas, or syngas, which is mainly made of hydrogen and carbon monoxide will generate. Synthesis gas can be burned as source of energy or used in further processes for production of other products (181).

5.3.2 Biochemical conversion

The biochemical conversion processes of algal biomass into fuels include aerobic digestion, alcoholic fermentation, photobiological hydrogen production, transestrification and in-situ transesterification (182). A detailed route of conversion process of algae and its potential products can be found in Figure 1-16. Among which the alcoholic fermentation process is going to be discussed in this study.

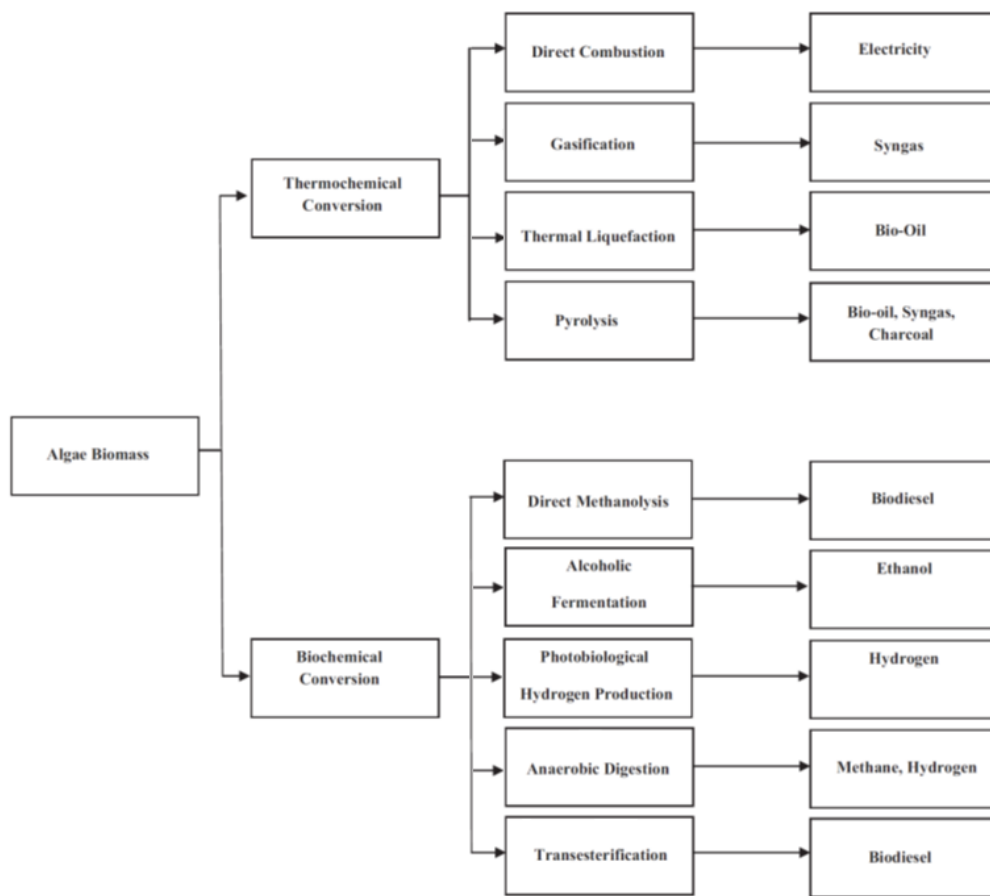


Figure 1-16. Potential algal biomass conversion processes (170)

Alcoholic fermentation is a metabolic process in which an organic substrate undergoes chemical changes due to activities of enzymes, secreted by the micro-organisms. In the case of algae, sugar, starch will be converted into ethanol by yeast (177). An additional pretreatment before fermentation is needed to release more glucose from algae (183). The pretreatment procedure will be discussed in the experiment part of this thesis. In the fermentation process, pulp after pretreatment is mixed with water and *Saccharomyces cerevisiae* yeast are kept warm in large tanks called fermenters (183). The yeast will break down the sugar and convert it into ethanol as shown in Equation 1-1 below.



Equation 1-1

After the fermentation, ethanol will be separated from solid residue. A purification process, usually distillation, is then carried out to remove the water and other impurities in the alcohol product (177). The product can be used in a variety of applications e.g. directly add into petrol for powering cars (184). The solid residue from the process can be used in animal feeding or for gasification process (177) which helps offset feedstock costs which typically make up 55–80% of the alcohol selling price (185).

Current examples of alcoholic fermentation on algae are not difficult to find. Ethanol production by dark fermentation using the marine green alga *Chlorococcum littorale* is investigated, 27% of the cellular starch was consumed within 24h at 25°C, a higher temperature will accelerate the fermentation. The maximum productivity of ethanol of 450 mmol/g of dry weight at 30°C was achieved (186). *C. vulgaris* are another good source of ethanol due to the high starch content (ca. 37% dry wt), ethanol conversion efficiency up to 65% has also been reported (187). Table 2-3 listed some other potential algal feedstock for bioethanol production.

Table 1-3. Potential algal feedstock for bioethanol production

Algae species	Process/Pre-treatment	Bacteria/medium	Yield	Conditions	References
<i>C. vulgaris</i> FSP-E	SHF with dilute acid hydrolysis	<i>Z. mobilis</i> ATCC 29191	0.233 g /g algae	T: 45°C, 200 rpm, 48 h	(188)
<i>Chlamydomonas reinhardtii</i> UTEX 90	SHF	Yeast	0.235 g /g algae	T: 30°C, 40 h, 160 rpm (yeast)	(189)
<i>Gelidium amansii</i>	Continuous dilute-acid hydrolysis	Yeast, soy peptone, bacto peptone, glucose, agar, galactose	0.38 g /g algae	T: 30°C, 16 h, 150 rpm	(190)
<i>Scenedesmus abundans</i> PKUAC	Saccharification & fermentation	Yeast, peptone, glucose	0.103 g /g dry algae	T: 30°C, 48 h, 200 rpm	(191)
<i>Chlorella variabilis</i>	Enzymatic Saccharification & fermentation	<i>E. coli</i> KO11	0.32 g/g carbohydrate	T: 35°C, 150 rpm, 72 h	(192)
<i>Gracilaria verrucosa</i>	Enzymatic hydrolysis	Yeast and (NH ₄) ₂ HPO ₄	0.43 g/g sugars	T: 4°C, 15 min, 10,000 rpm centrifuged after 6 h intervals	(193)
<i>Ulva fasciata</i>	Solid state fermentation	Yeast and peptone	0.47 g/g reducing sugar	T: 28 ± 2°C, 12 h, 120 rpm	(194)
<i>Chlorococcum infusionum</i>	Alkaline pretreatment	Yeast	0.26 g/g algae	T: 30°C, 72 h, 200 rpm	(195)
<i>Laminaria japonica</i>	Acid hydrolysis and simultaneous enzyme treatment	<i>E. coli</i> KO11	0.40 g/g sugars	T: 30°C, 10 min, 1000 rpm	(196)
<i>Saccharina</i>	SSF	Yeast, (NH ₄) ₂ HPO ₄	6.65 g/L	T: 43°C, 48 h, 130 rpm	(197)

<i>japonica</i>		MgSO ₄ · 7H ₂ O		rpm	
Lipid-extracted	Chemo-enzymatic	Yeast, malt, peptone,	0.14 g/g	T: 30°C, 12 h, 200	(198)
<i>Dunaliella</i>	saccharification and	dextrose	biomass	rpm	
<i>tertiolecta</i>	fermentation				
Mixed microalgae	Saccharification using	Clostridium spp.	0.46 g/L	T: 80-90°C, 2 h	(199)
	acid hydrolysis &				
	fermentation				

SHF: Separate hydrolysis and fermentation, SSF: Simultaneous saccharification and fermentation.

5.4 Economic issues and life cycle assessment

The high productivity and valuable composition of algae has showed great potential in making great contribution to fuel production. A rapid growth rate makes algae much faster to produce than lignocellulose. To have better insight into the potential of algae as a large scale feedstock for biofuels, the overall production costs have to be considered. In 2009, Solix was able to demonstrate biofuel production from microalgae, but the cost was about US\$ 33 per gallon. Phase I and phase II of Solix's business plan indicate that algae biofuels will cost about \$3.30 to \$1.57 per gallon by reducing the capital costs for facility construction. An approximate production cost of algal oil ranged from \$10.87 per gallon to \$13.32 per gallon as obtained through a comparative cost analysis (200). Additionally, the production costs of algal biodiesel ranged from \$6.50 to \$8.00 per gallon as suggested by Rosenberg et al. based on economic modelling (201).

Among three major products, biogas production seems to be more economically feasible than biodiesel or bioethanol. The reason is production of biogas uses entire biomass of algae in the process compared with partial use of algae in production of biodiesel and bioethanol. In biodiesel production, the lipids in algae are converted to product while carbohydrates are converted into bioethanol in alcoholic fermentation. The pretreatment process needed prior

to fermentation in many case will also add to the production cost. Biogas production is believed to be closer to commercialization than the other two. There is a study indicating that biogas could be converted from complex carbohydrates (202). Another study showed that biogas production from algae has greater GHG and energy security advantages as compared to liquid fuels production (203).

Algae biofuels as a replacement for traditional fossil fuels is still underdeveloped. Algae-based biofuels production still has to overcome several major barriers, such as reducing the energy input, reducing the carbon footprint and improving the economic feasibility. Cost of cultivation or collection of algae must also be taken into consideration. The process economics can be improved considerably by utilizing the algal residues through biorefinery technologies. With the production of multiple algae based products *via* a biorefinery processes, algae biofuels can be economically competitive to lignocellulosic bioethanol. A combination of algae cultivation together with the ability to reduce the contamination while growing could also cut the initial cost.

5.5 Conclusion

The concurrent production of valuable co-products that have wide applications in medicine, food and cosmetic industries with biofuel production, has significant potential. The production of algae based fuels can be cost competitive with fossil fuels if cultivation is carried out with executing resource efficiency in terms of fertilizer, water and CO₂ source. In conclusion, algae are excellent biomass resource for biofuel production due to its capacity of producing high quality biofuels, great productivity and reduced environmental impact compared to other resources.

Research gap

From the literature review above we can see there is still a long way to go to achieve a commercialised biorefinery using lignocellulose or algae as feedstock. For the production of 2nd generation bioethanol, a pretreatment before hydrolysis to increase accessibility of cellulose in biomass is needed. Several ionic liquids have already shown potential in lignocellulose pretreatment either in the ionic liquid dissolution process or the ionic liquid pretreatment. One major barrier to commercialisation is the high cost to produce ionic liquids. This issue can be directly tackled by reducing the price of ionic liquid used and better allocation of by-products to offset the cost.

As a result, an effective, low-cost alternative ionic liquid for pretreatment of lignocellulose is required. The alternative chosen in this study is protic ionic liquids that can be easily synthesised. Performance of protic ionic liquids also needs to be tested to achieve a good hemicellulose and lignin removal rate and better saccharification yield. Conditions of pretreatment include temperature, time, biomass loading, and ionic liquid concentration need to be confirmed. Further separation to recover lignin and collect cellulose rich pulp is also important. To reach a better allocation of by-products, the possibility to isolate hemicellulose before pretreatment to avoid hemicellulose degradation during delignification need to be discussed. A good hemicellulose recovery is needed while the delignification and fermentation remain not affected by hemicellulose removal. To further reduce the cost of ionic liquid, application of recycled ionic liquid also needs to be examined.

Although lignocellulosics have quite a few advantages over 1st generation biorefinery feedstocks, lignocellulose still has the problems of long life cycle, high recalcitrance towards chemicals, etc. Therefore, it is worthwhile further searching for another feedstock other than lignocellulose. In this study, algae were chosen and the feasibility of an algae based biorefinery towards production of bioethanol needs to be examined.

Key objectives and work to overcome the research gap can be summarised as follows. Liquid

phase analysis needed to be carried out to understand the form of cellulose/hemicellulose existing in the ionic liquid liquor phase. Degradation of hemicellulose under different conditions needed to be investigated to understand the formation of pseudo lignin. A 2-stage pretreatment needed to be developed to separate cellulose, hemicellulose and lignin one by one. Feasibility of using recycled 2nd stage IL in 2-stage pretreatment also needed to be assessed. Algae pretreatment using the ionoSolv process under several conditions was needed to give an overview of how this 3rd generation biorefinery material performs with ionic liquids.

The final goal of this project is to make the ionoSolv process more economically viable and gain deeper understanding in biorefinery to identify the remaining challenges for successful industrial implementation.

Part II. Experimental Methods

Some contents in this section are similar to what is used as standard in the group. i.e. Laboratory Analytical Procedures (LAP) published by the National Renewable Energy Laboratory (NREL).

General Materials and Equipment

Reagents and solvents were purchased from Sigma Aldrich and, unless stated otherwise, used as received. The Karl-Fischer titrator used in this work was a V20 volumetric Titrator (Mettler-Toledo), the vortex shaker a VWR International REAX TOP and the analytical balance a Sartorius CPA 1003 S balance (± 0.001 g).

Ionic Liquids

Starting materials for ionic liquid synthesis were purchased from Sigma Aldrich and, unless stated otherwise, used as received. The minimum purity of the starting materials was as follows: triethylamine $\geq 99\%$, Butyldimethylamine 99%, *N,N*-dimethylbutylamine 99%, *N*-butylimidazole 98%. Sulfuric acid was obtained from Sigma Aldrich as a 5M solution.

Protic ILs were synthesised according to the standard operating procedure of our laboratory (204). In brief, the required amine or imidazole was weighed into a round-bottom flask and cooled with an ice bath. Under stirring, an equimolar amount of the required acid, typically as a 1 to 5M aqueous solution, was added dropwise. Excess water was removed first using a rotary evaporator (Büchi) and the product further dried using a Schlenk line at 40°C overnight. ^1H , ^{13}C and HMQC NMRs were recorded on a Bruker 400 MHz spectrometer and can be found in the Appendix. Chemical shifts (δ) are reported in ppm, referenced to the DMSO signal at 2.500 (^1H dimension) and 39.520 (^{13}C dimension).

Synthesis of triethylammonium hydrogensulfate [TEA][HSO₄]

Triethylamine (75.9 g, 750 mmol) was cooled with an ice bath in a 500 mL round-bottom flask. Under stirring, 150 mL of 5 M H₂SO₄ (750 mmol) were added dropwise. The water was removed using a rotary evaporator and the product dried using a Schlenk line at 40°C overnight. The ionic liquid was recovered as a white, hygroscopic solid.

¹H NMR: δH (400 MHz, DMSO-d₆)/ppm: 3.39 (s (br), HSO₄⁻, N-H⁺), 3.10 (q, J = 7.3 Hz, 6H, N-CH₂), 1.20 (t, J=7.3 Hz, 9H, N-CH₂-CH₃).

Synthesis of 1-butylimidazolium hydrogensulfate [HC₄im][HSO₄]

200 g of freshly distilled *N*-butylimidazole (1.61 mol) was cooled with an ice bath in a 1 L round-bottom flask. Under stirring, 322 mL of 5M H₂SO₄ (1.61 mol) were added dropwise. The water was removed initially using a rotary evaporator and the product further dried using a Schlenk line at 50°C overnight. The ionic liquid was recovered as a slightly pink, viscous liquid which changed colour over time to orange, golden and finally colourless.

¹H NMR: δH (400 MHz, DMSO-d₆)/ppm: 9.14 (s, 1H, N-CH-N), 7.79 (s, 1H, N-CH), 7.68 (s, 1H, N-CH), 4.26 (br, N-H, HSO₄⁻), 4.19 (t, J = 7.2 Hz, 2H, N-CH₂), 1.77 (m, 2H, N-CH₂-CH₂), 1.23 (m, 2H, N-CH₂-CH₂-CH₂), 0.88 (t, J = 7.4 Hz, 3H, N-CH₂-CH₂-CH₂-CH₃).

Synthesis of Butyldimethylammonium hydrogen sulphate [N₄₁₁₀][HSO₄]

5M sulphuric acid (125ml, 0.625mol) was added slowly by a dropping funnel to a 1L round bottom flask which contained 63g (0.625mol) butyldimethylamine. The round bottom flask was cooled by ice bath till sulphuric acid and butyldimethylamine were mixed properly and one liquid phase was formed. The ionic liquid was then dried by rotary evaporator (15bar, 60°C) and further dried by vacuum line with the vacuum of less than 0.1mbar at 40°C. Dried nitrogen was then added into the round bottom flask to avoid contact with moisture.

^1H NMR: δH (400 MHz, DMSO- d_6)/ppm: 0.86 (t, 3H, N-CH₂-CH₂-CH₂-CH₃), 1.25 (2H, N-CH₂), 1.56 (2H, N-CH₂), 2.75 (2H, N-CH₂), 3.03 (s, HSO₄⁻), 9.26 (s, N-H⁺).

Synthesis of *N,N*-dimethylbutylammonium hydrogensulfate [DMBA][HSO₄]

N,N-dimethylbutylamine (75.9 g, 750 mmol) was cooled with an ice bath in a 500 mL round-bottom flask. Under stirring, 150 mL of 5 M H₂SO₄ (750 mmol) were added dropwise. The water was removed initially using a rotary evaporator and the product further dried using a Schlenk line at 70°C overnight. The ionic liquid was recovered as a colourless, viscous liquid.

^1H NMR: δH (400 MHz, DMSO- d_6)/ppm: 9.24 (s, 1H, N-H), 3.02 (dt, J = 12.9, 5.0 Hz, 2H, N-CH₂), 2.76 (d, J = 4.3 Hz, 6H, N-(CH₃)₂), 1.64 – 1.51 (m, 2H, N-CH₂-CH₂), 1.29 (h, J = 7.4 Hz, 2H, N-CH₂-CH₂-CH₂), 0.89 (t, J = 7.4 Hz, 3H, N-CH₂-CH₂-CH₂-CH₃). ^{13}C NMR δC (101 MHz, DMSO- d_6)/ppm: 56.62 (N-CH₂), 42.48 (N-CH₃), 25.82 (N-CH₂-CH₂), 19.40 (N-CH₂-CH₂-CH₂), 13.71 (N-CH₂-CH₂-CH₂-CH₃).

Biomass feedstock

Miscanthus x giganteus was obtained from Silwood Park campus (Imperial College London, UK). *Pinus sylvestris* was obtained from Metla (Finish Forest Research Institute). Willow was obtained from Rothemstead, UK, Wheat straw was obtained from the University of Warwick. Mixed seaweed was collected from Swanage beach, Dorset, UK. Seaweed *Posidonia oceanica* was obtained from beaches of El Campello, province of Alicante, Spain. All lignocellulosic biomass was air-dried, grinded (Retch SM 2000) and sieved (Retsch AS 200) (180-850 μm , 20 + 80 US mesh scale) prior to use and stored in plastic bags at room temperature in the dark.

Fractionation of Biomass

Water content measurement

Pretreatments, determination of oven dried weight and ionic liquid water content measurements were conducted according to the standard operating procedure from our laboratory (205) in triplicates. Pretreatments were conducted with a final water content of 20wt% according to Equation 2-1

$$\%water = \frac{m_{IL} \cdot wc_{IL} + m_{BM} \cdot mc_{BM} + m_{water}}{m_{IL} + m_{water}}$$

Equation 2-1

Where m_{IL} is the mass of the IL solution, w_{IL} is the water content of the IL solution, m_{BM} is the biomass weight, mc_{BM} is the moisture content of the biomass and m_{water} is the weight of the water added in order to reach 20wt%. The moisture content was taken into account in order to make experiments at different loading more comparable since the amount of moisture in the biomass becomes more significant at higher loadings.

Dilute acid pretreatment

All pretreatments were carried out in triplicate. Dilute acid used for pretreatment (1M sulphuric acid), is made from 98vol% sulphuric acid purchased from Sigma-Aldrich. About 1g biomass was weighed on aluminium foil and transferred to a glass tube which can tolerate high temperature and pressure. 10g dilute sulphuric acid was added to each sample in the tube. Such biomass loading was chosen for the purpose of minimising the consumption of acid while biomass can be immersed by liquid phase. The pressure tube was vortexed on vortex shaker a VWR International REAX TOP to reach a perfect mixture of biomass and sulphuric acid.

Pressure tubes with samples were transferred into a preheated convection oven (ThermoScientific HERA THERM OM H60) and heated at the required temperature for a

required time period. After cooking the samples were cooled to room temperature with the pressure tubes unopened. The pressure tubes were then safe to open at room temperature. A filtration was carried out to separate the liquid phase (liquor) from the solid (pulp). The pulp was then washed by Soxhlet extraction using absolute ethanol for 24h. After the Soxhlet extraction the pulp was air dried at room temperature and kept in sealed plastic bags for further analysis.

IonoSolv Pretreatment

1st stage pretreatment (Dilute IL)

All experiments were carried out in triplicate. The ionic liquids were diluted by distilled water if needed. Typically, 1g of biomass was weighed and transferred into a glass pressure tube together with 10g diluted ionic liquid. The pressure tube was sealed and vortexed on vortex shaker a VWR International REAX TOP to ensure all biomass was fully wetted. The tube was then placed in a pre-heated convection oven at the required temperature for a required time period. The pressure tube was then cooled to room temperature before open. Filtration was performed to separate pulp and liquor. The pulp was washed after filtration until no pH changes can be observed from washed water. The liquor was collected and analysed on Aminex HPX-87H column (BioRad, 300 x 7.8 mm) with 0.01 M H₂SO₄ as mobile phase (0.6 mL/min) if needed.

2nd stage (Concentrated IL)

First, an IL-water mix was prepared by adding the required amount of distilled water to dry ionic liquid to obtain an 80 wt% IL solution. Pretreatment experiments were carried out in triplicate at IL solution to biomass ratio of 1:10 g/g in a wide-mouthed 40 mL Ace pressure tube with screw cap and shaken with a vortex shaker to ensure a perfect mixture of biomass and IL. The tubes were placed into a preheated convection oven at chosen temperature for certain time period. After the pretreatment time had elapsed, the tubes were removed from

the oven and allowed to cool. After pretreatment, 40 mL ethanol was added to the pressure tube and the suspension was transferred into a 50 mL Falcon tube, ensuring no pulp was lost. The tube was shaken for 1 min and left to settle at room temperature for at least 1 hour. The tube was shaken again for 1 min and centrifuged at 3,000 rpm for 50 min. The ethanol wash was repeated 3 times in total. The supernatant was carefully decanted into a round-bottom flask with stirrer. The residual pulp was then transferred into a cellulose thimble and Soxhlet extracted with refluxing ethanol (150 mL) for 24 hours. Following extraction, the thimbles containing pulp were air dried and weighed to calculate pulp yield.

The ethanol used for the Soxhlet extraction was combined with the ethanol washings from the previous steps and evaporated under vacuum at 40°C with agitation, leaving a dried ionic liquid/lignin mixture. To this mixture was added 30 mL of distilled water as antisolvent in order to precipitate the lignin. The mixture was transferred into a clean 50 mL Falcon tube, shaken for one minute and left to settle at room temperature for at least 1 hour. The tube was centrifuged as above and the supernatant decanted and collected in a round bottom flask. This washing step was repeated for at least 3 times. The lid of the Falcon tube containing lignin residue was pierced and the tube was placed in a vacuum oven overnight to dry at 40°C under vacuum. The dried lignin was weighed the following day to obtain the lignin yield. Lignin yield can be either expressed relative to the initial lignin content in the biomass (referred to as Klason lignin) as in Equation 2-2 or relative to the untreated biomass as in Equation 2-3. Figure 2-1 made by Aida Rafat from our group shows a schematic representation of the overall biomass deconstruction procedure.

$$\text{Lignin yield (\%)} = \frac{m_{\text{lignin precipitate}}}{m_{\text{Klason lignin}}} \cdot 100\%$$

Equation 2-2

$$\text{Lignin yield (\%)} = \frac{m_{\text{lignin precipitate}}}{m_{\text{oven dried biomass}}} \cdot 100\%$$

Equation 2-3

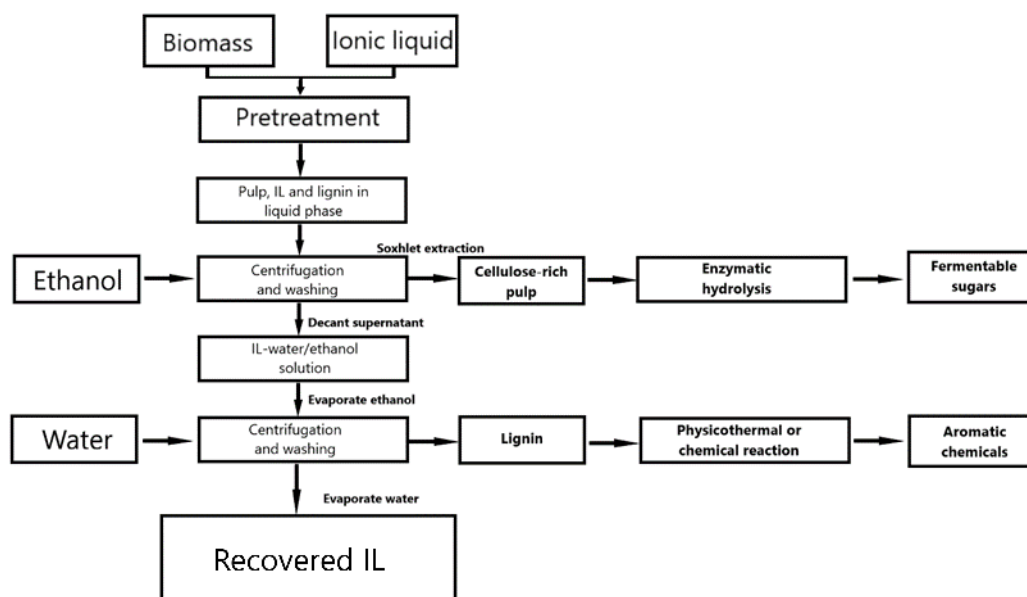


Figure 2-1. Schematic representation of IonoSolv biomass deconstruction process

Pulp analysis

Compositional analysis

The purpose of conducting compositional analysis on pulp samples after pretreatment is to determine the changes that occurred to the biomass before and after pretreatment (i.e. untreated biomass vs. pulp). The pulp and untreated biomass compositions can be compared in terms of the cellulose or glucan content, hemicellulose content (e.g. xylan, manan, galactan) as well as lignin content. The comparison provides a good indication of the pretreatment effectiveness especially in terms of “delignification” which reflects the amount of lignin that was extracted by IL during the pretreatment.

Compositional analysis was carried out according to a published procedure by the National Renewable Energy Laboratory (NREL) (206). 300 mg (calculated on ODW basis) of air-dry biomass or recovered pulp was weighed out into a pressure tube and the weight recorded. 3 mL of 72% sulfuric acid (Fluka) were added, the samples stirred with a Teflon stir rod and the

pressure tubes placed into a preheated water bath at 30°C. The samples were stirred again every 15 min for one hour. They were then diluted with 84 mL distilled water and the lids closed. The samples were autoclaved (Sanyo Labo Autoclave ML5 3020 U) for 1 hour at 121°C and left to cool to close to ambient temperature. The samples were then filtered through filtering ceramic crucibles of a known weight. The filtrate was filled in two Falcon tubes and the remaining black solid washed with distilled water. The crucibles were placed into a convection oven (VWR Venti-Line 115) at 105°C for 24±2 hours. They were then taken out and placed in a desiccator for 15 min before they were weighed and the weight recorded. They were then placed into a muffle oven (Nabertherm + controller P 330) and ashed to constant weight at 575°C. The weight after ashing was recorded. The content of acid insoluble lignin (AIL) was determined according to Equation 2-4. The content of one of the Falcon tubes was used for the determination of acid soluble lignin content (ASL) by UV analysis at 240 nm (Equation 2-5) (Perkin Elmer Lambda 650 UV/Vis spectrometer).

$$\%AIL = \frac{Weight_{crucible\ plus\ AIR} - Weight_{crucible\ plus\ ash}}{ODW_{sample}} \cdot 100$$

Equation 2-4

$$\%ASL = \frac{A}{l \cdot \epsilon \cdot c} \cdot 100 = \frac{A \cdot V_{filtrate}}{l \cdot \epsilon \cdot ODW_{sample}} \cdot 100$$

Equation 2-5

where $Weight_{crucibles\ plus\ AIR}$ is the weight of the oven-dried crucibles plus the acid insoluble residue, $Weight_{crucibles\ plus\ ash}$ is the weight of the crucibles after ashing to constant temperature at 575°C, A is the absorbance at 240 nm, l is the path length of the cuvette in cm (1 cm in this case), ϵ is the extinction coefficient (12 L/g cm), c is the concentration in mg/mL, ODW is the oven-dried weight of the sample in mg and $V_{filtrate}$ is the volume of the filtrate in mL and equal to 86.73 mL.

Delignification can be calculated as:

$$\text{Delignification} = \frac{\text{kalsol lignin (untreated)} - \text{lignin (pulp)}}{\text{kalsol lignin (untreated)}} \times 100$$

Equation 2-6

Where, Klason lignin_(untreated) is the original lignin content in the untreated biomass determined also by compositional analysis as the sum of ASL and AIL lignin. Lignin_(pulp) is the lignin remaining on the pulp calculated as the sum of ASL and AIL as determined by the previous steps.

The other Falcon tube contents were neutralized by careful addition of calcium carbonate until the pH reached 5. After settling, the liquid was filtered through a 0.2 µm PTFE syringe filter and submitted to HPLC analysis for the determination of total sugar content. HPLC analysis of glucose, xylose, mannose, arabinose and galactose was performed on a Shimadzu HPLC with an AMINEX HPX-87P Column (Biorad, 300 x 7.8 mm, prepacked HPLC carbohydrate analysis column) with refractive index detection. The mobile phase was de-ionized water, the column temperature 85°C and the flow rate was 0.6 ml min⁻¹. Calibration standards with concentrations of 0.1, 1, 2 and 4 mg mL⁻¹ of glucose, xylose, mannose, arabinose and galactose and 8 mg mL⁻¹ of glucose were used. The content of carbohydrates, acid-soluble lignin, Klason lignin, ash and extractives (where applicable) was expressed as a fraction of the sum of all components (normalized to 100%). Sugar recovery standards were made as 10 mL aqueous solutions close to the expected sugar concentration of the samples and transferred to pressure tubes. 348 µL 72% sulfuric acid was added, the pressure tube closed and autoclaved and the sugar content determined as described above. The sugar recovery coefficient (SRC) was determined according to Equation 2-7 and the sugar content of the analysed sample using Equation 2-8:

$$SRC = \frac{c_{HPLC} \cdot V}{initial\ weight}$$

Equation 2-7

$$\%Sugar = \frac{c_{HPLC} \cdot V \cdot corr_{anhydro}}{SRC \cdot ODW_{sample}} \cdot 100$$

Equation 2-8

where C_{HPLC} is the sugar concentration detected by HPLC, V is the initial volume of the solution in mL (10.00 mL for the sugar recovery standards and 86.73 mL for the samples), initial weight is the mass of the sugars weighed in, $\text{corr}_{\text{anhydro}}$ is the correction for the mass increase during hydrolysis of polymeric sugars obtained by dividing the molecular weight of one polymeric sugar by its monomeric weight (0.90 for C6 sugars glucose, galactose and mannose and 0.88 for C5 sugars xylose and arabinose) and ODW is the oven-dried weight of the sample in mg. Correlations between delignification and saccharification yields were calculated using least squares linear regression in Excel.

Saccharification Assay

Saccharification assays were carried out according to an adapted procedure by the NREL⁴ in triplicates with blanks (also triplicates). All reagents were purchased from Sigma Aldrich.

For wet samples, moisture contents were determined again directly prior to saccharification. 100±10 mg (on and ODW basis) of air-dried or wet biomass were placed into a Sterilin tube and the weight recorded. Three blanks were run with 100 µL of purified water instead of biomass in order to correct for sugar residues present in the enzyme solutions. The water contained in the biomass sample was (calculated using its moisture content and the total sample mass) subtracted from 1.5 mL. The difference was added as water using a pipette. 8.4 mL solution consisting of 5 mL 1M sodium citrate buffer at pH 4.8, 40 µL tetracycline antibiotic solution (10 mg/mL in 70% ethanol), 30 µL cycloheximide antibiotic solution (10 mg/mL in purified water), 3.38-3.41 mL purified water and 20-50 µL of Novozymes experimental enzyme mixture NS-22201 (kindly provided directly by Novozymes) were added, the tubes closed and placed into a Stuart Orbital Incubator (S1500) for 7 days at 50°C and 250 rpm.

Saccharification yields were obtained by filtering 1 mL of the saccharification mixture through a PTFE syringe filter. Samples were run on Shimadzu HPLC with an AMINEX HPX-97P column (Bio rad, 300 x 7.8 mm) with purified water as mobile phase (0.6 mL/min). The column temperature was 85°C and acquisition was run for 20 min. A representative chromatogram can be found in the Appendix. Calibration standards with concentrations of 0.1, 1, 2 and 4 mg/mL of glucose, xylose, mannose, arabinose and galactose and 8 mg/mL of glucose were used.

Liquor analysis

Ionic liquid solutions were analysed directly after pretreatment by collecting a certain amount of the solution with a pipette into an Eppendorf micro centrifuge tube. The tube was shaken and centrifuged with a VWR MICRO STAR 17R centrifuge at 4°C and 13.3 G for 10 min to remove any water-insoluble material. The supernatant was pipetted off into a HPLC vial and submitted for analysis on a Shimadzu HPLC system with RI and UV/Vis detector and an Aminex HPX-87H column (BioRad, 300 x 7.8 mm) with 0.01 M H₂SO₄ as mobile phase (0.6 mL/min). The column temperature was 55°C and acquisition was run for 60 min. A representative chromatogram can be found in the Appendix. Calibration was carried out using standards with concentrations of 0.1, 1, 2 and 4 mg/mL of glucose, xylose, arabinose, furfural (99%), 5-HMF (99%), levulinic acid (≥98%), glacial acetic acid and formic acid (≥95%). Analyte concentrations in the HPLC sample were calculated using the resulting calibration curves. The mass fraction w/w of analytes detected in the ionic liquid solution (in mg/g of dried biomass) was determined using the following equation:

$$w/w = \frac{C_{HPLC} \cdot (m_{sample} + m_{water}) \cdot m_{IL}}{\rho_{HPLC} \cdot m_{sample} \cdot (1 - w_{C_{sample}}) \cdot ODW_{BM}}$$

Equation 2-9

C_{HPLC} : analyte concentration as determined by HPLC analysis in mg/mL; m_{sample} : mass of ionic liquid solution sampled in mg; m_{water} : mass of water added in order to dilute IL sample in mg;

m_{IL} : dry mass of ionic liquid used for pretreatment in g; ρ_{HPLC} : density of HPLC sample in g/mL (value used 1.045 g/mL); $w_{C_{sample}}$: water content of the sample; ODW_{BM} : oven dried weight of the biomass used for pretreatment in g.

Part III. Results and discussion

Chapter 1: IonoSolv process

Lignin extraction at low IL concentration

In the normal IL lignocellulosic biomass pretreatment carried out in our group, a high concentration IL, normally 80wt%, is used to remove lignin from the biomass. In the hemicellulose pre-extraction it is preferred to have the hemicellulose removed and keep lignin in the pulp for further pretreatment, thereby achieving further fractionation. Therefore, it is important to examine if there is a break point of IL concentration where most of lignin can still be held in the pulp while hemicellulose gets dissolved in the liquid phase.

This experiment was performed by using $[\text{HC}_4\text{im}][\text{HSO}_4]$ from low concentration of 10wt% to a moderate concentration of 50wt%. 10g ILs was cooked with 1g pre-weighed Miscanthus in pressure tubes at 120°C for 3h. Pulp was washed by DI water until pH neutral then airdried overnight for compositional analysis.

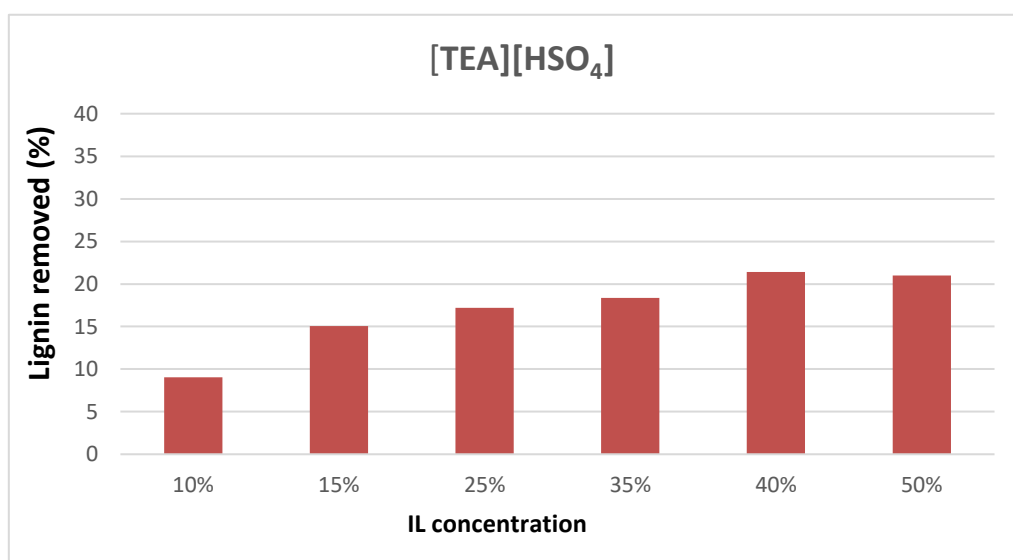


Figure 3-1. Lignin removal yield of pretreatment with different $[\text{TEA}][\text{HSO}_4]$ concentrations

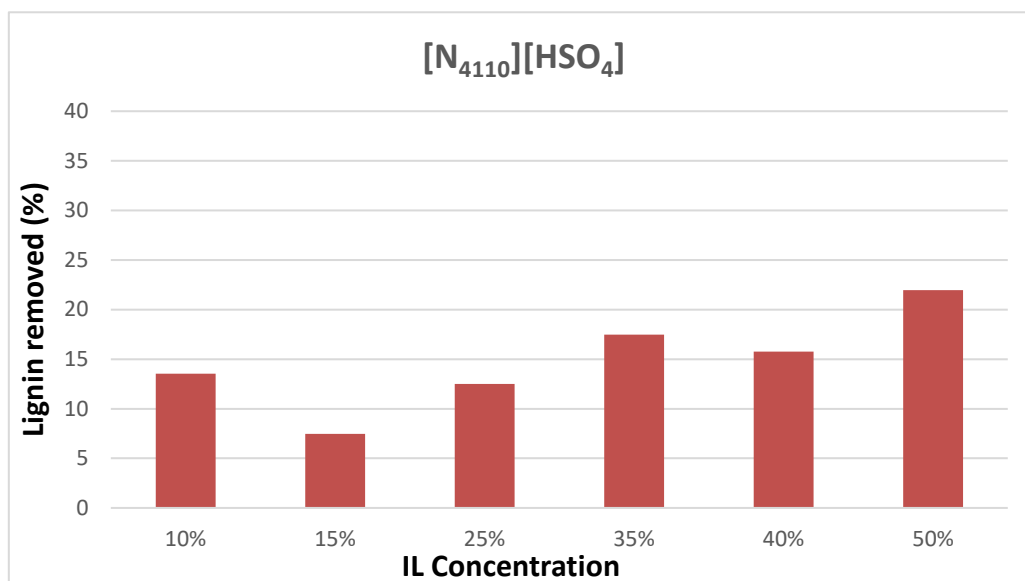


Figure 3-2. Lignin removal yield of pretreatment with different $[N_{4110}][HSO_4]$ concentrations

As can be seen from Figure 3-1, the lignin removal rate increased slightly with $[TEA][HSO_4]$ concentration used. We already know a very high lignin removal rate can be achieved using 80wt% $[TEA][HSO_4]$ (145). Therefore, it is expectable to observe a significant increase in lignin removal occurs between 50wt% to 80wt% IL concentration. Also, if we add lignin removal data acquired from 2-stage pretreatment experiments reported in chapter 3 in this thesis, there is a huge increase of lignin removal from 3.5% using 1wt% $[TEA][HSO_4]$ to 15% using 15wt% $[TEA][HSO_4]$. This may not be a huge issue in terms of lignin recovery after high concentration IL pretreatment, but may become a problem when IL is recycled and used again in pretreatment or pre-extraction. Similar lignin removal changes were also observed in Figure 3-2 when $[N_{4110}][HSO_4]$ was used. The unstable increase of lignin removal in Figure 3-2 could be a result of poor performance of Karl-Fischer titrator at high water content. The Karl-Fischer titrator used works more accurately when sample tested has a water content of less than 20% and could give unstable results when high water content sample was measured.

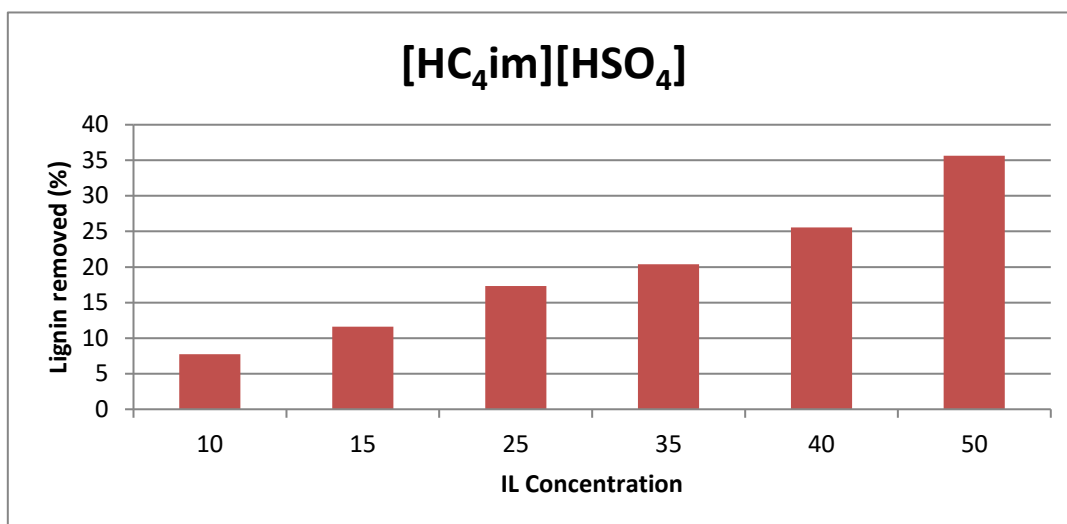


Figure 3-3. Lignin removal yield of pretreatment with different $[\text{HC}_4\text{im}][\text{HSO}_4]$ concentrations

$[\text{HC}_4\text{im}][\text{HSO}_4]$ experiments showed a slightly different result. While the lignin removal rate kept the same trend and increased with higher IL concentration, the climbing rate was more linear. Also lignin removal increased from 7.5% to 36%, which is a much bigger increase compared to results from the other two ILs. This suggests $[\text{HC}_4\text{im}][\text{HSO}_4]$ has a better lignin solubility at low concentration, and may not be a good choice for 2-stage pretreatment (sequential hemicellulose and lignin extraction).

Lignin removal from the pulp increased with increasing IL concentration in water. This is due to the low solubility of lignin in water, and good solubility in IL. There is no obvious break point of lignin solubility in IL. Therefore, it is best to use IL at a low concentration in order to prevent having too much lignin dissolved in IL after pre-extraction which will cause a contamination of IL and inhibit recycling.

Dilute $[\text{TEA}][\text{HSO}_4]$ meets lignocellulosic biomass

As previously mentioned, ILs are tunable solvents. Therefore, an IL's acidity/basicity ratio can be adjusted to receive different performance in reaction (by acid: base ratio in the case of PILs). To test IL pre-extraction on different types of lignocellulosic biomass, Miscanthus, pine

and willow were chosen as typical grass, softwood and hardwood types of biomass. [TEA][HSO₄] was used in this experiment at 3 different acid/base ratio of 1:2, 1:1 and 2:1. 1:2 and 2:1 acid base ratio [TEA][HSO₄] was made by adding calculated amount of triethylamine or sulphuric acid to create [TEA][HSO₄]. Concentrations of all ILs were adjusted to 10wt% (90wt% water). The pre-extraction was carried out at 120°C for 3h at a loading of 1g biomass with 10g IL. The pulp was washed with DI water till pH neutral after pre-extraction and cooling, then air dried for compositional analysis to determine the lignin content.

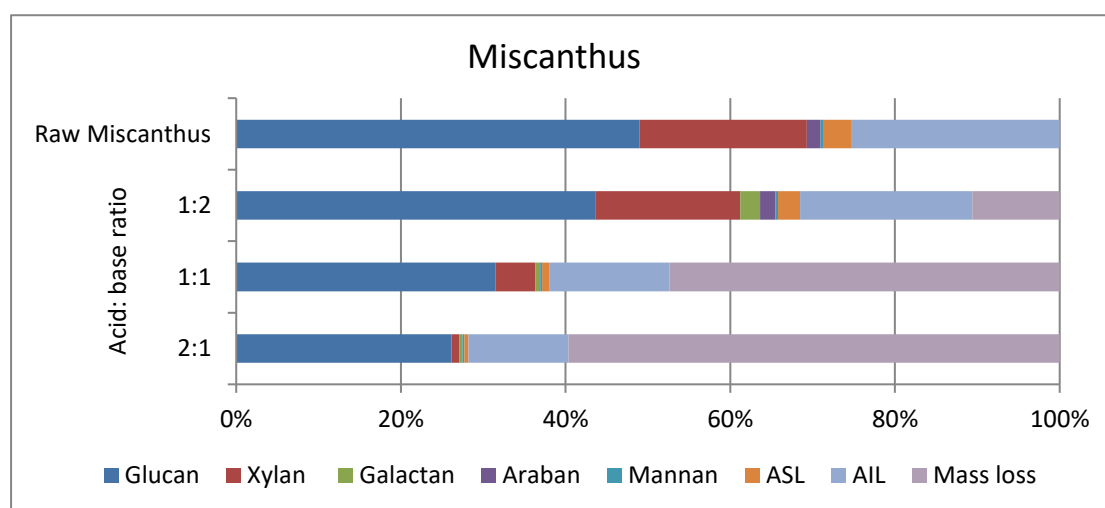


Figure 3-4. Compositional analysis results of pulp after pretreatment of Miscanthus using ILs with different acid :
base ratios

Figure 3-4 showed percentage of lignin removed from Miscanthus after experiment. At 1:2 acid: base ratio, a pulp yield of 89% was achieved, which suggests little occurred during the pretreatment. Hemicellulose remained untouched, while 5% of cellulose and lignin were removed. The results indicate a low acid: base ratio is not suitable for hemicellulose removal at all. Much better hemicellulose removal was achieved at 1:1 and 2:1 acid: base ratios. At a 1:1 ratio, the pulp yield reached 53% with 80% hemicellulose dissolved in IL. Almost 1/3 of lignin was also dissolved in IL while 1/3 of cellulose was also lost. At 2:1 acid base ratio, the lowest pulp yield was achieved. The hemicellulose removal was slightly better compare to

1:1 ratio. However, we lost nearly half of cellulose in pulp, which will cause a significantly lower final saccharification yield in the end. Therefore the 1:1 acid base ratio reached a balance point of removing hemicellulose and keeping as much cellulose in pulp as possible.

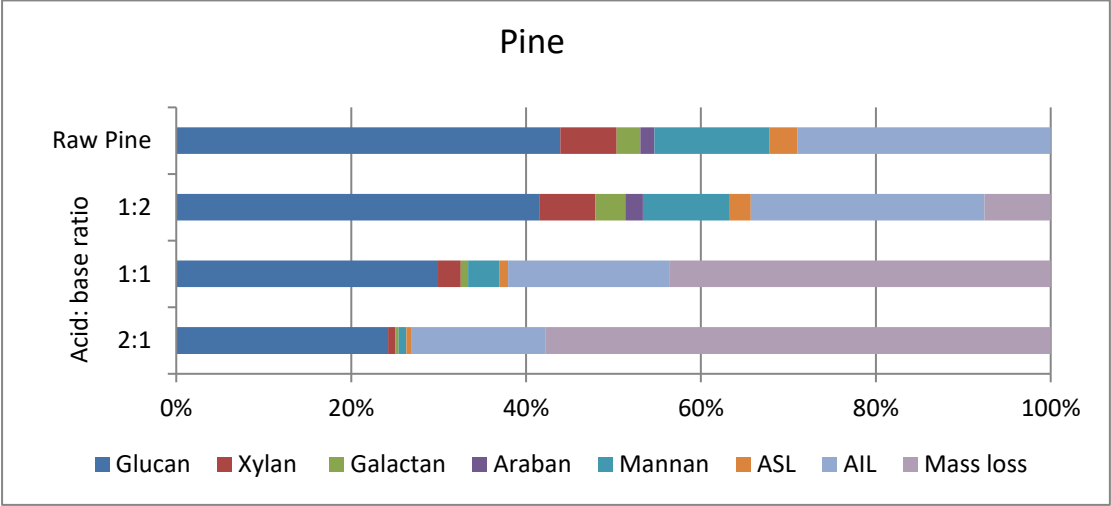


Figure 3-5. Compositional analysis results of pulp after pretreatment of pine using ILs with different acid base ratios

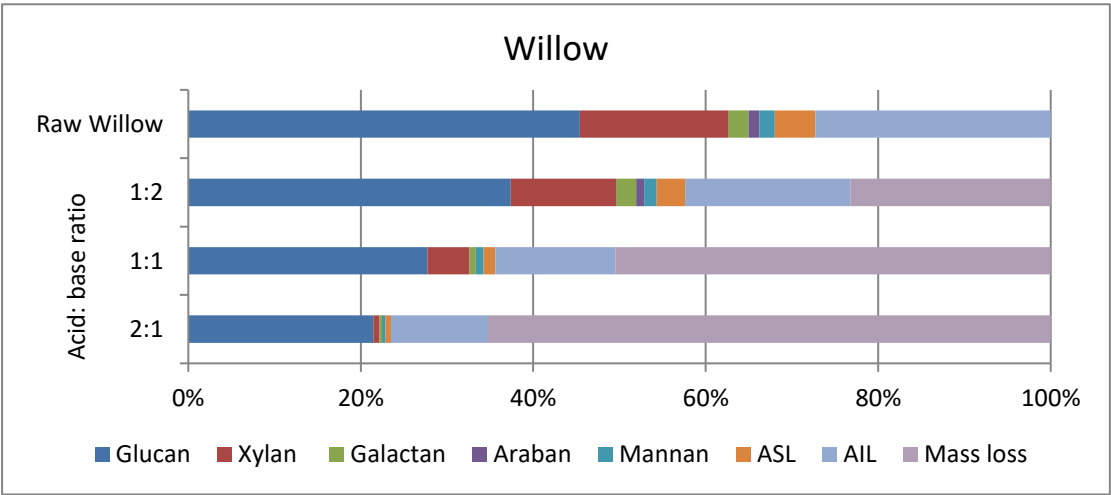


Figure 3-6. Compositional analysis results of pulp after pretreatment of willow using ILs with different acid base ratio

Figure 3-5 and Figure 3-6 show similar results for hemicellulose pre-extraction of pine and willow. The hemicellulose removal was best with a 2:1 acid base ratio, but it was

overshadowed by the fact that the hemicellulose removal at 1:1 acid base ratio was already very good while cellulose left in the pulp was much better at a 1:1 ratio compared with a 2:1 ratio. To conclude, the best acid base ratio for hemicellulose pre-extraction is 1:1. The lignin dissolved in IL from all three biomasses at 1:1 acid base ratio is about 10-20%, which is not a negative sign in terms of further application of lignin after lignin removal and recovery. However, it is not clear the effect of lignin accumulation in IL if we try to recycle IL used in here after several cycles.

Liquid phase content

As the main product of pretreatment, pulp was carefully isolated, collected and analysed. Compositional analysis tells us the changes of sugar content, lignin content and ash content in pulp, while saccharification shows the potential glucan available for fermentation in pulp. The other pretreatment product, the liquid phase, also requires analysis. The liquid phase after pretreatment will contain water, IL, hemicellulose sugar (as monomers/oligomers) and lignin depending on the conditions. Sugar dissolved in liquid phase will also have degradation issues. Degradation products could be useful chemicals, however they will also cause problems such as lower sugar recovery and reduced purity of the recycled IL.

Therefore, it is necessary to analyse the liquid phase after pretreatment. In this experiment, the effect of pretreatment temperature and IL concentration of hemicellulose removal and sugar degradation products is inspected. [TEA][HSO₄] was chosen to extract hemicellulose from Miscanthus. The concentrations tested were 5wt%, 10wt% 15wt% and 20wt%. Temperatures of 100°C, 110°C and 115°C were chosen. Reaction times of 1-12h at 100°C and 110°C was tested while 1-8h at 115°C were performed. Due to the huge amount of data, compositional analysis was not performed on all samples. The liquid phase after pretreatment was collected and analysed through the HPLC using the Aminex H column. All experiments were performed in triplicate.

The compositional analysis was conducted to measure the sugar and lignin content of raw Miscanthus. The results of analysing raw Miscanthus are presented in Table1, where the main hemicellulose in the Miscanthus is xylan. The raw Miscanthus xylan content is used to normalise the xylose and furfural concentration. And in this case xylan is chosen to represent hemicellulose, all hemicellulose data in this section will be based on xylan content.

Table 3-1. Miscanthus composition

	Glucan	Xylan	Galactan	Arabinan	Mannan	Sugar	Hemi	ASL	AIL	Ash
	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	
Miscanthus	44.4	19.6	0.0	2.8	0.0	66.8	22.4	4.6	25.2	0.7

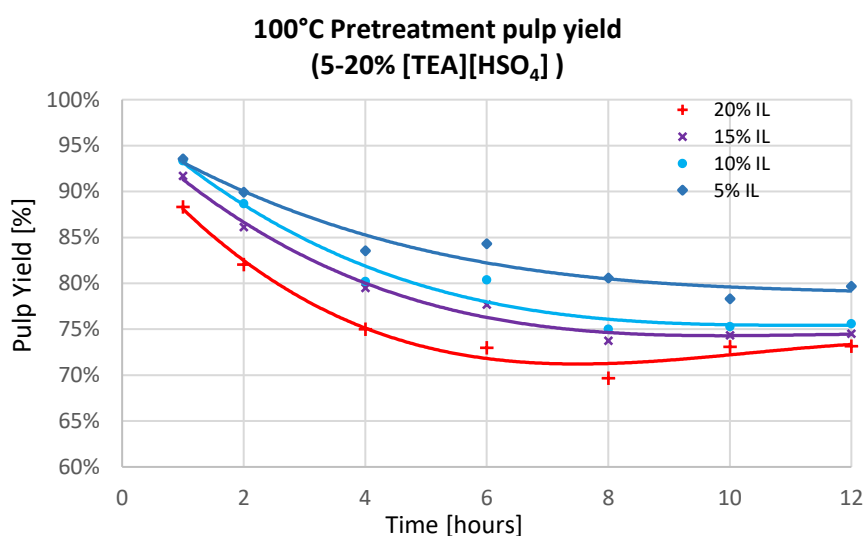


Figure 3-7. Pulp yield of pretreatment at 100°C

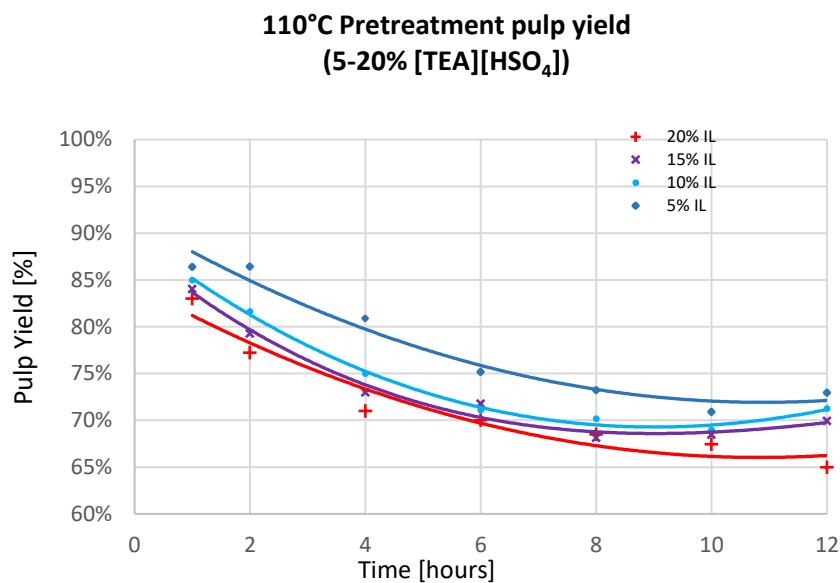


Figure 3-8. Pulp yield of pretreatment at 110°C

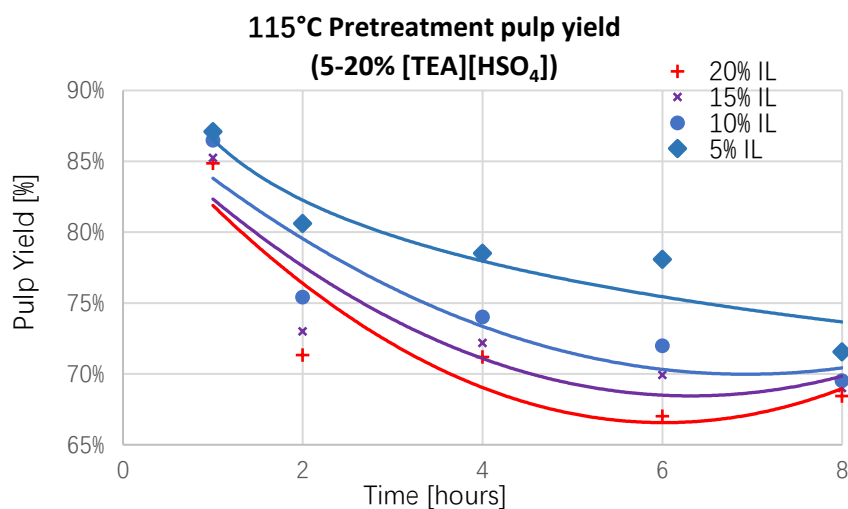


Figure 3-9. Pulp yield of pretreatment at 115°C

The pulp yield for the 100°C, 110°C and 115°C pretreatment are shown in Figures 3-7, 3-8 and 3-9. As can be seen from all 3 figures, a higher IL concentration results in a lower pulp yield and the pulp yield decreases with time until it reaches a constant (72% pulp yield using 20wt% IL) value at eight hours. The decreased pulp yield for the first eight hours is due to the removal of xylan and ASL. As the reaction goes on, most of the xylan and ASL are

hydrolysed, leaving cellulose and AIL in the pulp. As cellulose and ASL hydrolysis is slow at low IL concentrations, the pulp yield remains unchanged after most hemicellulose got removed. The hydrolysis of xylan and ASL can be confirmed by compositional analysis of pulp shown in Figure 3-10, where significant amounts of xylan (in grey) and ASL (in yellow) were removed in the first-stage pre-treatment compared to the raw Miscanthus.

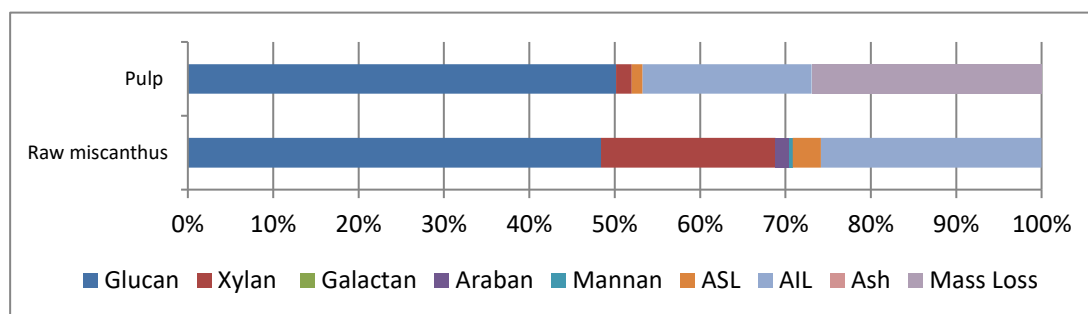


Figure 3-10. Compositional analysis of raw Miscanthus and pulp after pretreatment using 20wt% [TEA][HSO₄] at 100°C for 12h

Xylose in liquid phase

During the pre-treatment, the xylan was hydrolysed to form xylose. In this study, the liquid phase after the pretreatment was collected and analysed together with pulp washing liquid. The mixture of liquid phase which contains IL and xylose was analysed on HPLC-H column. Xylose concentration in the liquid phase was obtained with the total volume of liquid phase. The xylose content dissolved in the liquid phase was then calculated and normalised using the xylan content in the raw Miscanthus. In the liquid phase, xylose content and xylose concentration in the liquid phase were interchangeable, as the same amount of IL was used for all pre-treatment.

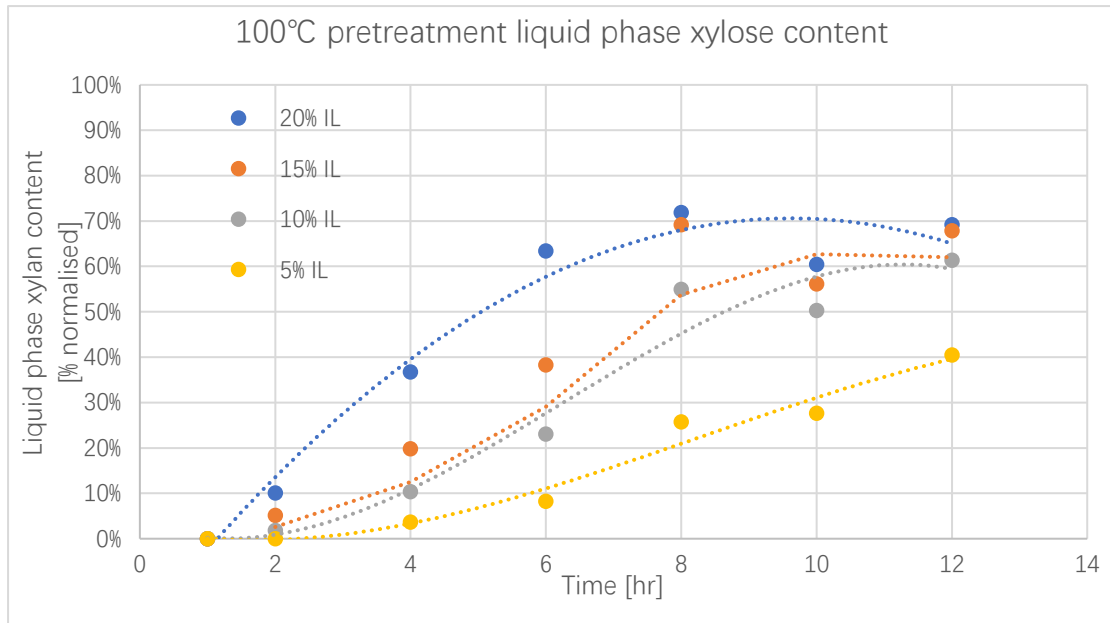


Figure 3-11. Percentage of xylose found in liquid phase after pretreatment at 100°C

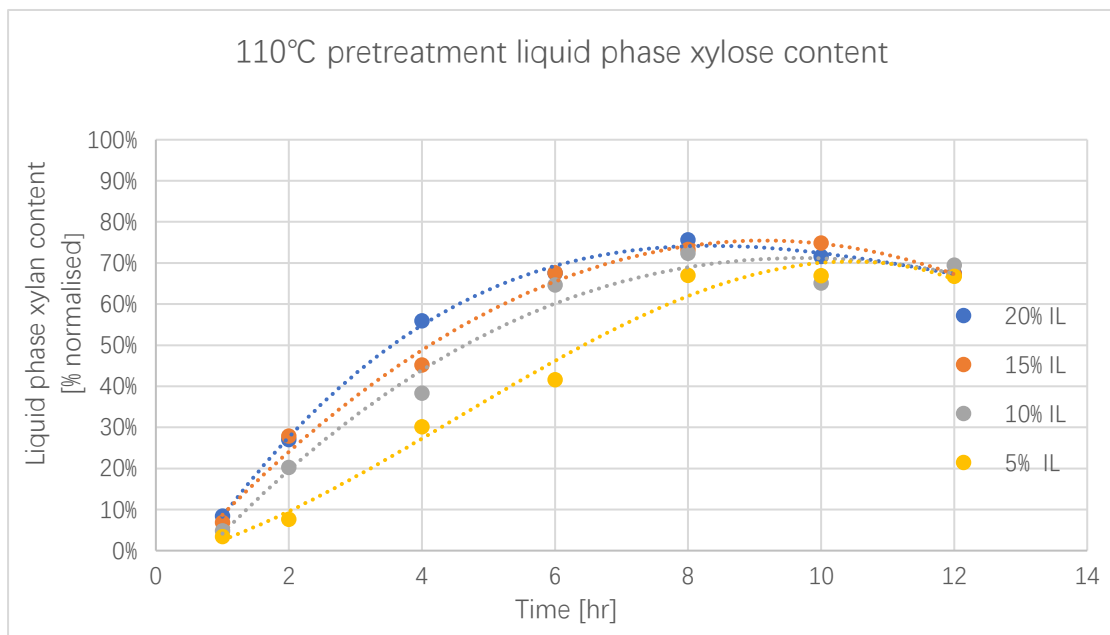


Figure 3-12. Percentage of xylose found in liquid phase after pretreatment at 110°C

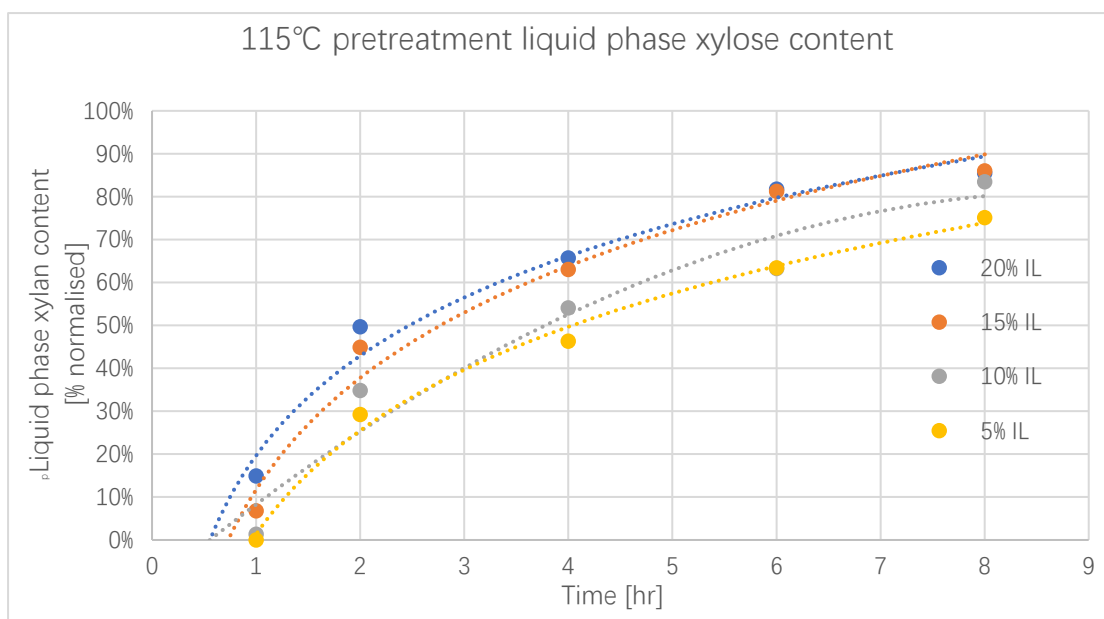


Figure 3-13. Percentage of xylose found in liquid phase after pretreatment at 115°C

The xylose content in liquid phase for 100°C, 110°C, and 115°C were shown in Figure 3-11, Figure 3-12, and Figure 3-13. Polynomial trend lines were plotted, to make it easier to compare temperature and IL concentration trends. As can be seen in Figure 3-11, the xylose content in the liquid phase reached a constant value of 68% at eight hours with IL concentration of 20wt%. For 15wt% and 10wt% IL concentration, a similar xylose concentration was achieved for longer reaction time. A similar trend can also be observed in 110°C pretreatment in Figure 3-12, where slightly higher xylose content of 73% was achieved for 20wt% and 15wt% IL concentration. This suggests some of the xylan cannot be hydrolysed at 100°C and 110°C with 20wt% [TEA][HSO₄]. Also can be seen from data of 110°C pretreatment is a slight decrease of xylose content in liquid phase after 8h, which could be a sign of overcook when xylose started to degrade and form furfural.

The rate of xylose formation (rate of reaction) can be derived from the gradient of the xylose content trend line. Comparing Figure 3-11 and Figure 3-12, a higher xylose formation rate can be observed at higher IL concentrations and higher temperatures. Due to the fact that the xylan to xylose reaction is an acid catalytic hydrolysis, high IL concentration will give a more acidic therefore the rate of reaction increases.

The gap of reaction rates between different IL concentrations is smaller at higher temperatures, suggesting the IL concentration contribution in the rate equation was reduced at higher temperatures. The 70% xylose content barrier at 100°C was surpassed at a higher reaction temperature, as shown in Figure 3-12 and Figure 3-13 of 110°C and 115°C. The xylose concentration reached 87% in pretreatment at 115°C using 20wt% IL, suggesting more xylan was extracted from the hemicellulose in the Miscanthus. There are two possible explanations for this 70% xylose content barrier.

This phenomenon can be explained by the xylose formation and degradation rate. At 100°C and 110°C, the xylose formation is equal to the degradation; thus, the xylose concentration was constant after eight hours. As the temperature increased to 115°C (Figure 3-13), the xylose formed at a faster rate than its degradation rate, causing the xylose concentration to increase and reach a higher mark.

Furfural formation experimental results and discussion

During the pre-treatment, the main xylose degradation product furfural was formed. The content of furfural in the liquid phase was also obtained using an HPLC-H column. The furfural content is normalised using the furfural content when all xylan in the raw Miscanthus is converted into furfural. Similar to the xylose, the furfural content and the furfural concentration in the liquid phase are interchangeable because the liquid phase volume is constant throughout the experiment.

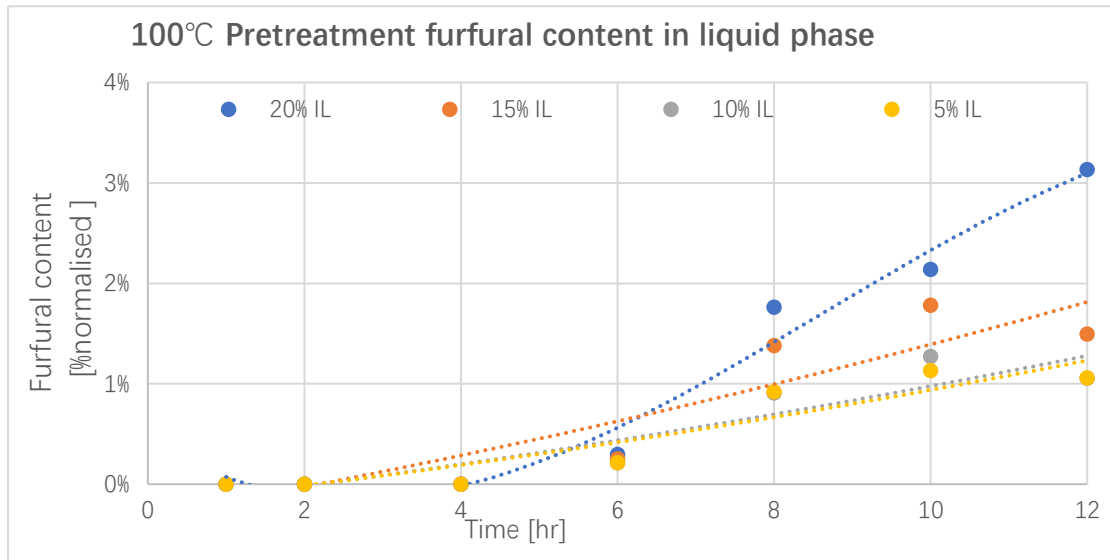


Figure 3-14. Furfural content in liquid phase from pretreatment at 100°C

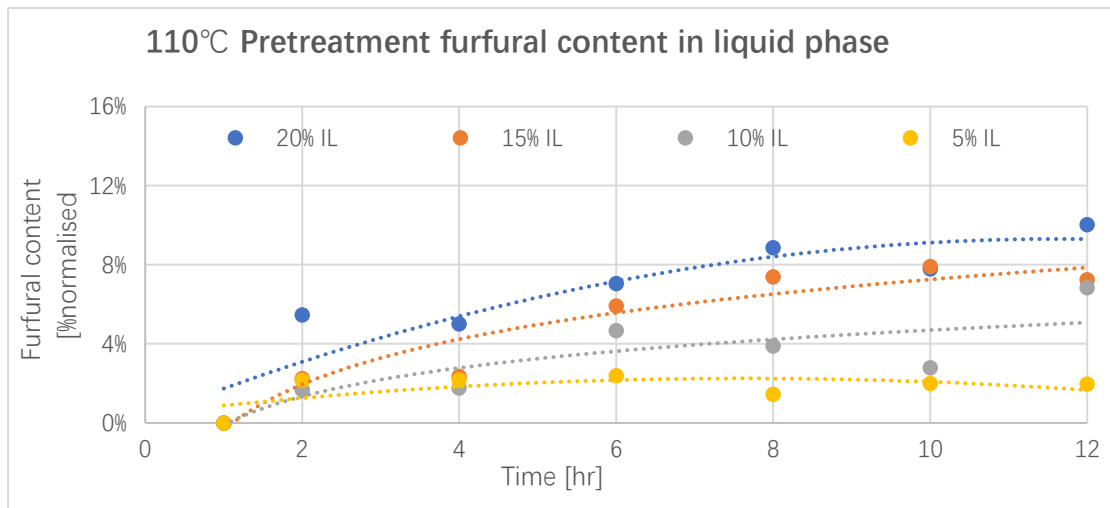


Figure 3-15. Furfural content in liquid phase from pretreatment at 110°C

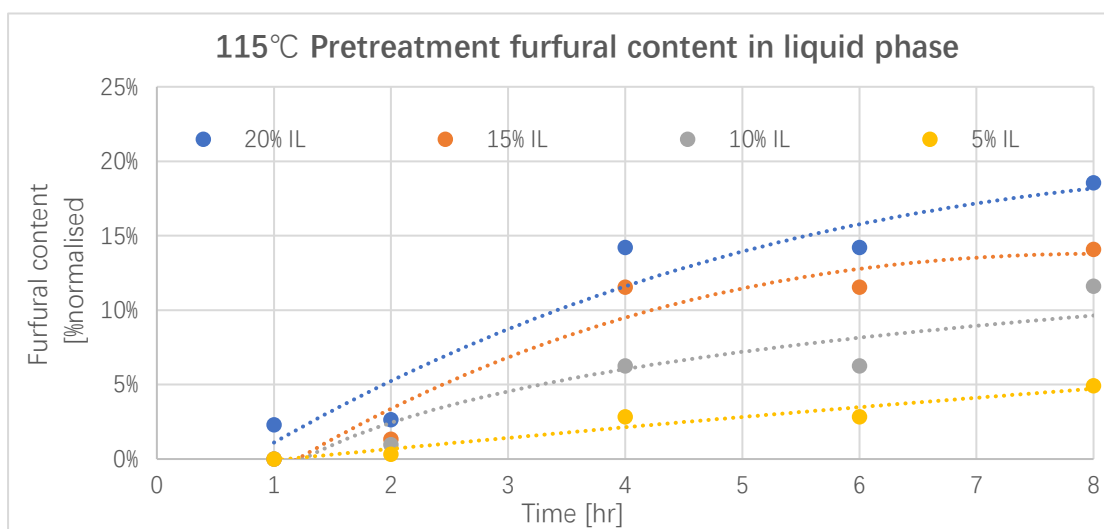


Figure 3-16. Furfural content in liquid phase from pretreatment at 115°C

The furfural concentration profiles for 100°C, 110°C, and 115°C pretreatment were plotted in Figure 3-14, Figure 3-15, and Figure 3-16. Polynomial trend lines were added, so the trend for different temperatures and IL concentrations could be observed. In Figure 3-15 and Figure 3-16, the furfural concentration in the liquid phase increased with the reaction time. This is because the xylose concentration increases over time. The trend line suggested that more furfural could form if the reaction time increased. A rough estimate of the reaction rate can be derived from the gradient of the concentration-time profile, where the reaction rate is greater for higher IL concentrations. This is because of the increasing IL acidity alongside the increasing IL concentration result in dehydration of xylose (207). The data in Figure 3-14 doesn't look as stable as data in Figures 3-15 and 3-16. The trend lines are also less clear. This is due to the resolution of HPLC at low furfural concentration. Data smoothness is better at higher temperatures as more furfural will form at harsher conditions.

Comparing data from all figures, the furfural yield increased significantly when the reaction temperature increases and the maximum furfural concentration (18%) was obtained at 115°C and 20wt% IL concentration. The rate of formation of furfural also increases dramatically,

this indicates that furfural production is very sensitive to temperature and might have a high activation energy.

The high furfural conversion rate will affect hemicellulose sugar recovery if hemicellulose sugar, in this case, xylose is going to be isolated for further use. However, it may not be a negative impact on overall process as furfural is also a valuable product of biorefinery. A deeper economical evaluation will be needed to balance the cost of applying higher temperature, losing hemicellulose recovery product and getting more furfural as a by-product.

Xylan extraction results and discussion

The xylan extraction results can be obtained by combining data from previous sections. As each experimental data point is normalised based on xylan content in raw *Miscanthus*, the xylose content and furfural content in these sections can be added directly to give the percentage xylan extracted. This is the best alternative way to measure the overall hemicellulose removal rate other than compositional analysis. The results are shown in Figures 3-17, 3-18 and 3-19. 100% xylan extraction was achieved at 115°C and 20wt% IL concentration. Although a very high extraction rate was achieved, the percentage of xylose converted to furfural is also high.

One fact which must be mentioned is xylose is not the only possible source of furfural. The other C5 hemicellulose sugar arabinose can also degrade to furfural. As shown in Table 3-1, there is 2.8% of arabinose in untreated *Miscanthus*. Figure 3-10 demonstrated no arabinose left in pulp after pretreatment. Also, no arabinose was found in liquid phase analysis, which suggests arabinose could have converted into furfural. It is hard to calculate how much arabinose degraded to form furfural due to the low resolution of HPLC H column which may not be able to detect the existence of arabinose in the liquid phase. The only arabinose data we have is provided by compositional analysis which is analysed by HPLC P column. As a

result, the percentage of xylan converted to furfural explained here should always be slightly higher than the actual number.

A slight decrease of traceable xylan can be observed in Figure 3-17 and 3-18 after 8h. This could be the result of further degradation of furfural to formic acid when cooked longer than 8h. The xylan removal rate reached 100% at 115°C when cooked for 8h. The 100% traceable xylan under this condition means no furfural degradation was happening. Therefore pretreatment time should be controlled within 8h.

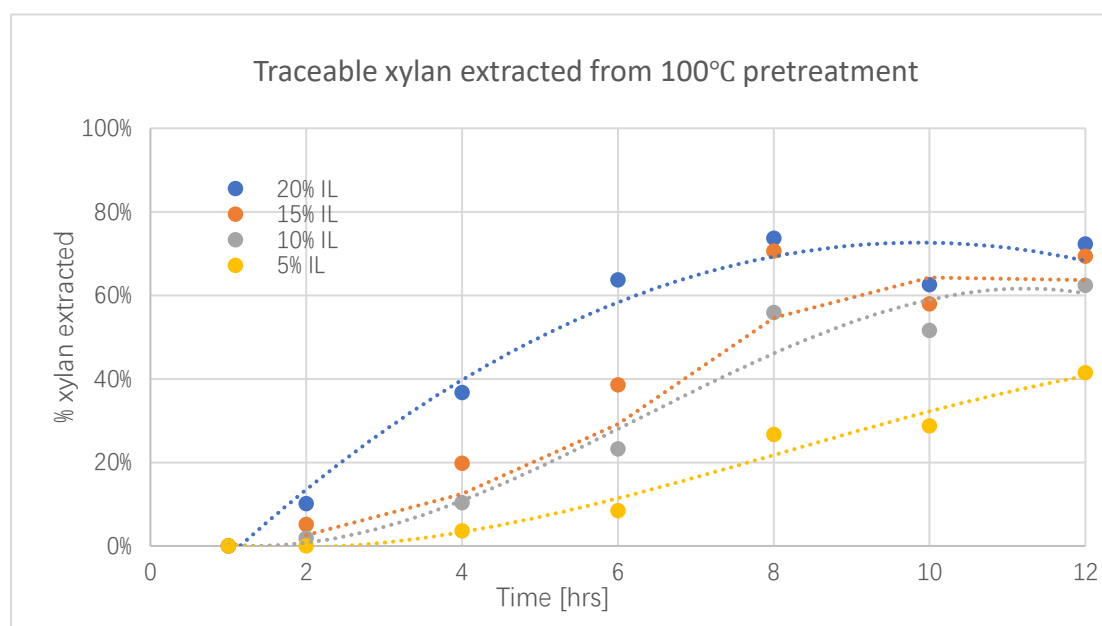


Figure 3-17. Percentage of xylan extracted from Miscanthus after pretreatment at 100°C

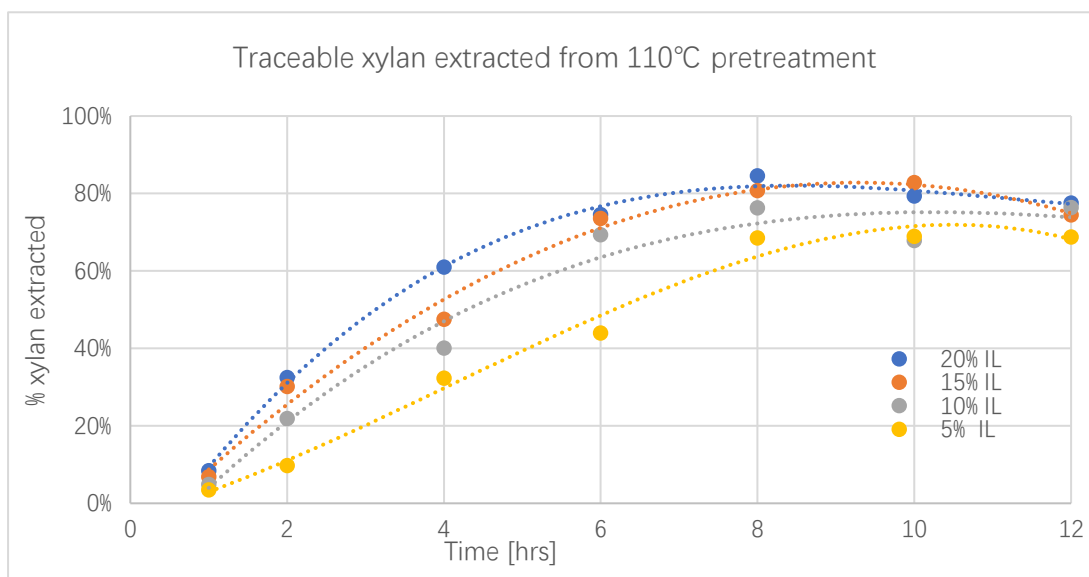


Figure 3-18. Percentage of xylan extracted from Miscanthus after pretreatment at 110°C

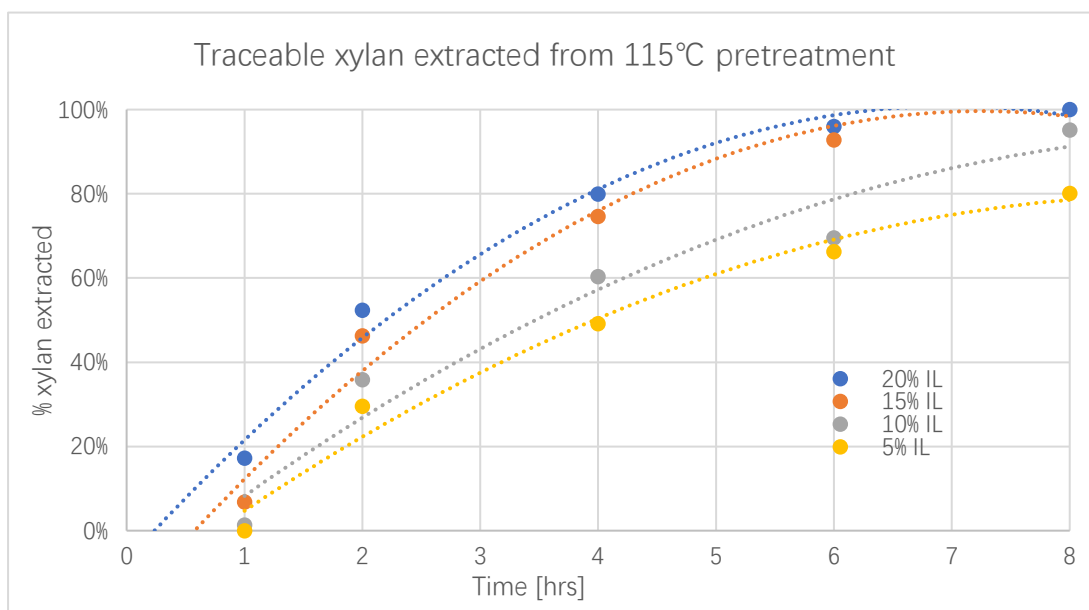


Figure 3-19. Percentage of xylan extracted from Miscanthus after pretreatment at 115°C

Chapter 2: Algae Based Biorefinery

Pretreatment of algae from UK



Type 1



Type 2



Type 3



Type 4

Figure 3-20. Photos of different types of algae received

A mixture of algae collected from the seashore of the UK was received. All algae were wet when they were collected and delivered to the lab. A wash using tap water to remove mud and sand was done after delivery, and the samples were carefully sorted into 4 types of algae. Species of each type are listed below.

Type 1: *Fucus spiralis*

Type 2: *Fucus serratus*

Type 3: *Saccorhiza polyschides*

Type 4: *Laminaria digitata*

First, the water content of all types of algae was measured by drying them at 105°C overnight. Oven-dried samples were transferred immediately into a desiccator to cool down to room temperature and weighed on a balance. Water content of algae can be found in Table 3-2. All types of algae had a very high water content of around 80%.

Table 3-2. Water content in wet macro algae

	Type 1	Type 2	Type 3	Type 4
Water content %	76.57	76.50	83.33	75.78

Extractive levels in algae was measured followed the LAP “Determination of extractives” (NREL-TP-510-42619) (208). Samples were washed in a soxhlet extractor by water for 24h and followed by ethanol for 24h. As can be seen from Figure 3-21, algae normally have a much higher extractive content than lignocellulosic biomass. Type 1 and 2 algae consist an extractive content of over 25%, while Type 4 algae have an extractives content of 45%.

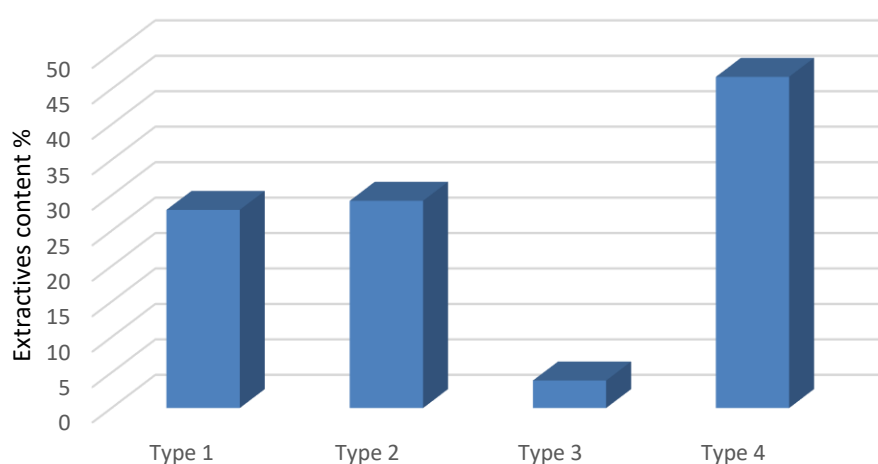


Figure 3-21. Extractives in algae

As all algae was received wet, a wet pretreatment was conducted alongside normal (dry) pretreatment. For the normal pretreatment, all 4 types of macro algae were dried in oven at 105°C overnight to remove all water content. The reason the algae was not air dried is due to the condition of the algae when received, as all algae had been kept wet in packaging for several days and needed to be oven dried as soon as possible to avoid decomposition. All algae used in dry pretreatments was transferred immediately into a desiccator when taken out of the oven and cooled for 30min to reach room temperature. 1g of oven dried algae was then weighed and transferred into a pressure tube. 20g of 80wt% [TEA][HSO₄] was then added in to the pressure tube. Such algae loading was chosen for the purpose of minimising the consumption of IL while algae can be immersed by liquid phase. The tube was then sealed and vortexed till an even mixture was achieved.

For wet pretreatment, the idea is to reach a same biomass dry matter loading as the dry pretreatment. Due to the high water content of wet algae, IL used in this experiment must be as dry as possible to reach a dry algae to IL to water ratio of 1g:16g:4g. [TEA][HSO₄] was dried first under vacuum on rotary evaporator to remove as much water as possible, then dried on Schlenk line under vacuum. The final water content of [TEA][HSO₄] used in this experiment was 4.49%. Type 1 and Type 4 was chosen to do wet pretreatment as there was not enough Type 2 and 3 algae left. The weight of wet algae needed to match 1g ODW was calculated and wet algae was weighed and transferred into a pressure tube. The pressure tube was sealed immediately to avoid change of water content of wet algae. [TEA][HSO₄] with 4.49% water content was solid instead of liquid and will absorb moisture in air very fast once exposed in air, therefore it is not easy to weigh and add IL into pressure tube without change water content of IL. A glovebox was used in this case. All sealed tubes with wet algae in were transferred into the glovebox together with [TEA][HSO₄]. Weighing and adding IL was performed in the glovebox to make sure dry algae to IL to water ratio was controllable. IL absorbs water in wet algae very quick and become liquid again once the pressure tube was vortexed.

One factor that couldn't be accurately controlled here is the particle size of algae. Wet algae can be ground but cannot be sieved. To make sure the particle size of algae used in both dry and wet pretreatment are as close as possible, algae was ground in the same grinder for the same amount of time. Estimated particle size of wet algae are below 1mm which is similar to the particle size of dry algae used (180µm-850µm).

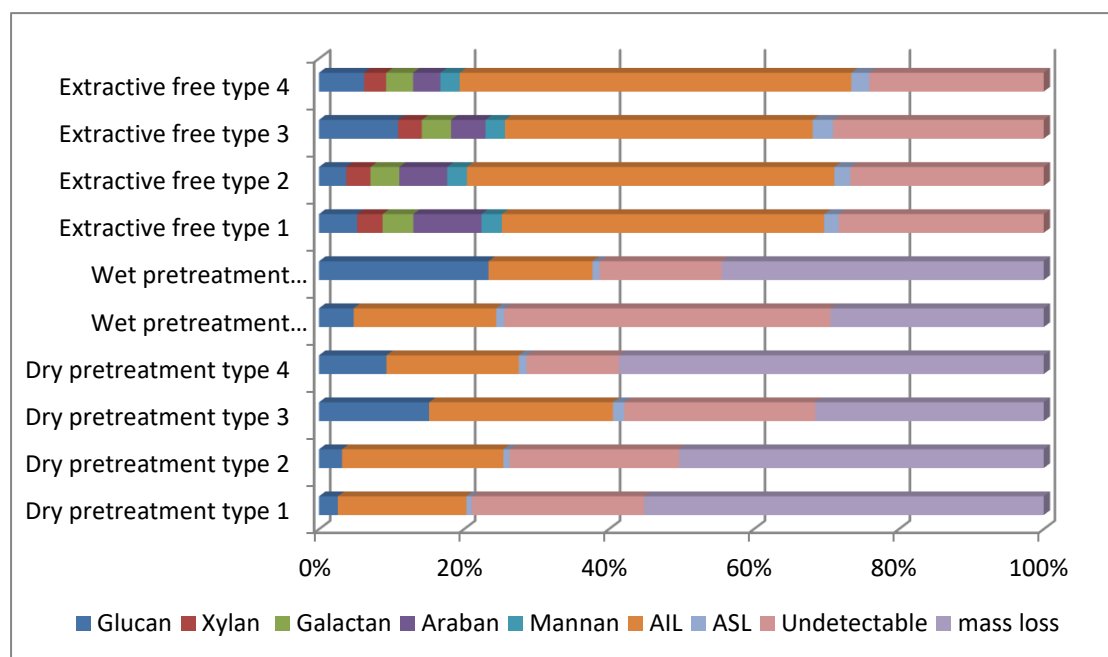


Figure 3-22. Compositional analysis result of algae pretreatment pulps.

The pretreatment was done at 120°C for 6h. Pressure tubes were cooled down to room temperature before pulp washing. Ethanol was used to wash IL off the pulp. Pulp was then dried for compositional analysis and enzymatic saccharification. As can be seen from Figure 3-22, around 30% weight of extractive free algae was accounted for as undetectable, which is likely glyceride ester content that cannot be measured by HPLC. Around 50% of lignin was also detected as acid insoluble residue. Considering macro algae do not have lignin content, the composition of AIL left needs to be addressed in the future for more comprehensive analysis. Glucan content in all 4 types of algae was very low, the highest glucan content was only 10% in type 3. Therefore, the ethanol production potential will not be high. Hemicellulose contents were between 10-20% for all 4 types and was removed after

pretreatment. One issue of the compositional analysis data is the inconsistency of HPLC results here due to low resolution of the machine and lack of proper analysis method for algae. Glucan content of pulp from wet pretreatment of type 4 was even higher than it was in extractive free untreated algae.

Hemicellulose was completely removed during the pretreatment while AIL removal rate was not ideal. Removal of around 50% indicates the condition of pretreatment might need to be harsher, and glucan left in pulp will not be easily accessible by enzymes. Low saccharification yield was achieved after 7 days of incubating. Type 3 algae reached 30% saccharification yield, while type 1 and 2 algae get only 13% and 15% respectively. The saccharification result also suggests it makes no obvious difference to do the wet pretreatment comparing with dry one. As a result, dry pretreatment is preferred here. It gives the same saccharification yield as wet pretreatment while the execution of dry pretreatment is much easier and should cost less. First, the cost of drying ILs to a very low water content can be avoided; also weighing and mixture of algae and IL can be much easier as a glove box will not be needed. The other advantage of dry pretreatment over wet pretreatment is controllable particle size for more consistent results as particle size is one factor influences pretreatment.

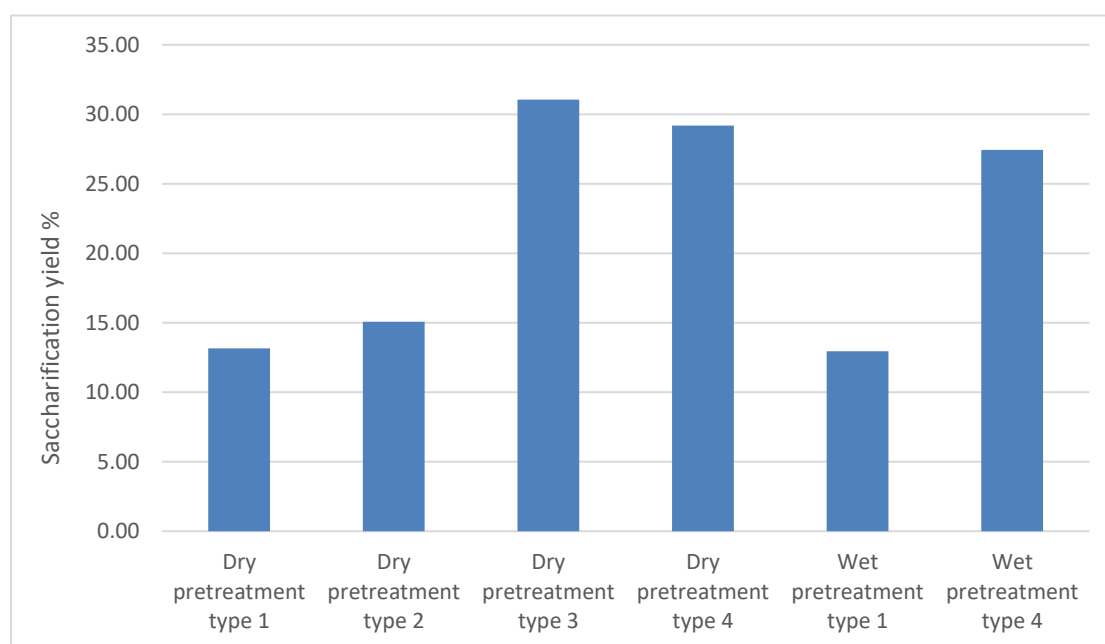


Figure 3-23. Saccharification yield of pulps from algae pretreatment

Further experiments were not performed due to lack of algae left available. According to data obtained, it is possible low concentration IL will also do a similar job and achieve similar saccharification yield. To gain further understand of macroalgae pretreatment in ionoSolv process, another more commonly researched seaweed, *Posidonia oceanica* was chosen.

Pretreatment of *Posidonia oceanica* macroalgae

Posidonia oceanica was obtained from Spain as dried leaf. PO algae was grinded using and sieved on sieving machine (Retsch AS 200). Particle size of *Posidonia oceanica* used in pretreatment was controlled in between 180µm to 850µm. The first batch of experiments was done by using high concentration IL like the usual pretreatment on lignocellulosic biomass. In this case, 80wt% [TEA][HSO₄] was used. A time course of 1-5h at 150°C was conducted. 1g of air dried *Posidonia oceanica* was weighed and transferred into a pressure tube. 10g of 80wt% [TEA][HSO₄] was added to the tube. The tube was sealed and vortexed to make a good mixture of algae and IL. All samples were then cooked in a preheated oven at 150°C for 1-5h. All samples were cooled to room temperature after pretreatment and washed by ethanol to remove IL from pulp. Washed pulp was air dried and analysed by compositional analysis and saccharification. Compositional analysis here was only done to analyse sugar content in pulp. Fatty acid ester content was not measured.

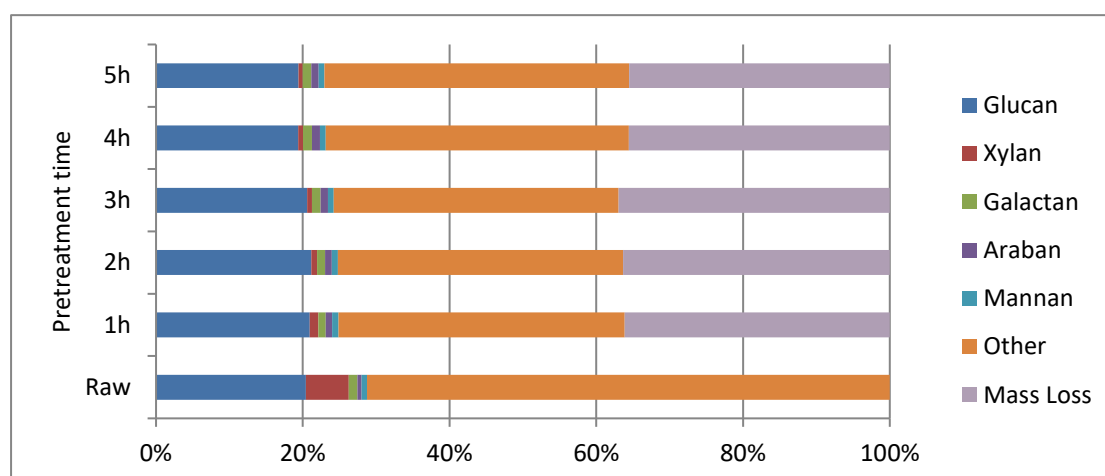


Figure 3-24. Compositional analysis results of *Posidonia oceanica* macroalgae pulp after pretreated with 80wt%

Compositional analysis result of untreated *Posidonia oceanica* indicates the algae has just over 20% glucan content which is not as good as lignocellulosic biomass used in previous experiments, but a lot higher than algae obtained from UK beach, which means *Posidonia oceanica* have a good potential to become a source of bioethanol production. Fig 3-24 showed the compositional analysis result of pulp from pretreatment using 80wt% IL. Results from 1h to 5h is very similar, which indicates all reactions happened in the first hour, and pretreatment time of *Posidonia oceanica* using 80wt% [TEA][HSO₄] does not need to be longer than 1h. A pulp yield of 65% was achieved and all glucan stayed in the pulp while half of undetectable content (fatty acid esters, etc) dissolved in the IL. The saccharification results showed in Fig 3-25 also suggests the pretreatment should not be longer than 1 hour. The saccharification yield of pulp reached nearly 100% at 1h pretreatment time. The saccharification yield dropped to 82% as pretreatment time went longer and reached 5h. This suggests the pulp got overcooked. Although the compositional analysis results of glucan content did not change from 1-5h, the saccharification yield dropped by 18%.

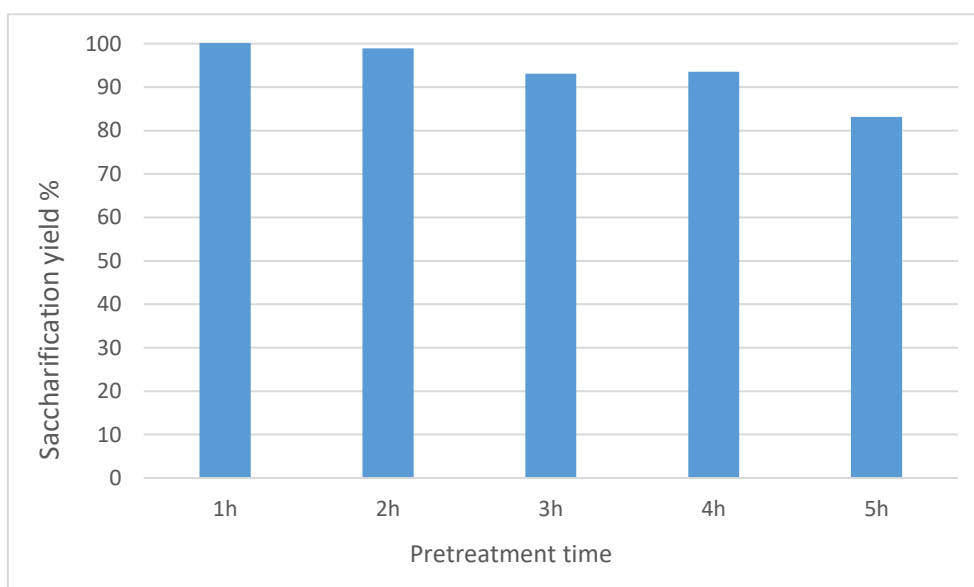


Figure 3-25. Saccharification yields of *Posidonia oceanica* macroalgae pulp after pretreated with 80wt% IL.

The result of pretreatment using 80wt% IL showed the *Posidonia oceanica* can be treated under a mild condition. Therefore, another set of experiments was carried out using lower concentration IL, in this case, 20wt% [TEA][HSO₄] was used. The first batch of time course experiments was done at 150°C from 1h to 3h. The compositional analysis results showed in Fig 3-26 demonstrate that the reaction also happened in the first hour. To further investigate, another batch of pretreatments was carried out and time points of 15min, 30min and 45min was chosen. As can be seen from Fig 3-26, glucan remained very stable and stayed in the pulp. Hemicellulose removal using 20wt% IL is better compare with using 80wt% IL, and almost completely dissolved in the IL after 1h. Lower IL concentration also give out a higher removal of undetectable content, dissolving 80% at 1h, 30% more than result get using 80wt% IL. This indicates 30% of undetectable content can only be dissolved in low concentration of IL.

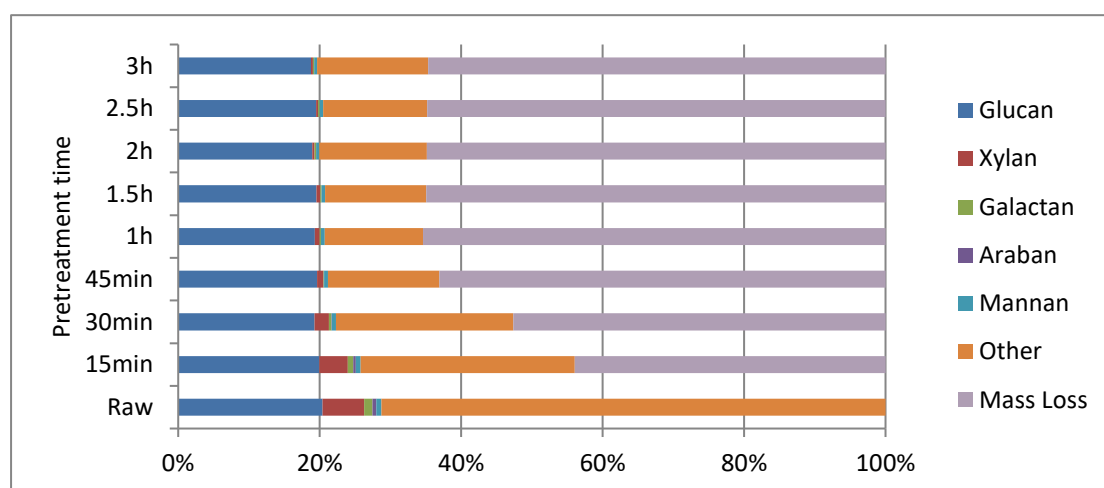


Figure 3-26. Compositional analysis results of *Posidonia oceanica* macroalgae pulp after pretreatment with 20wt% IL.

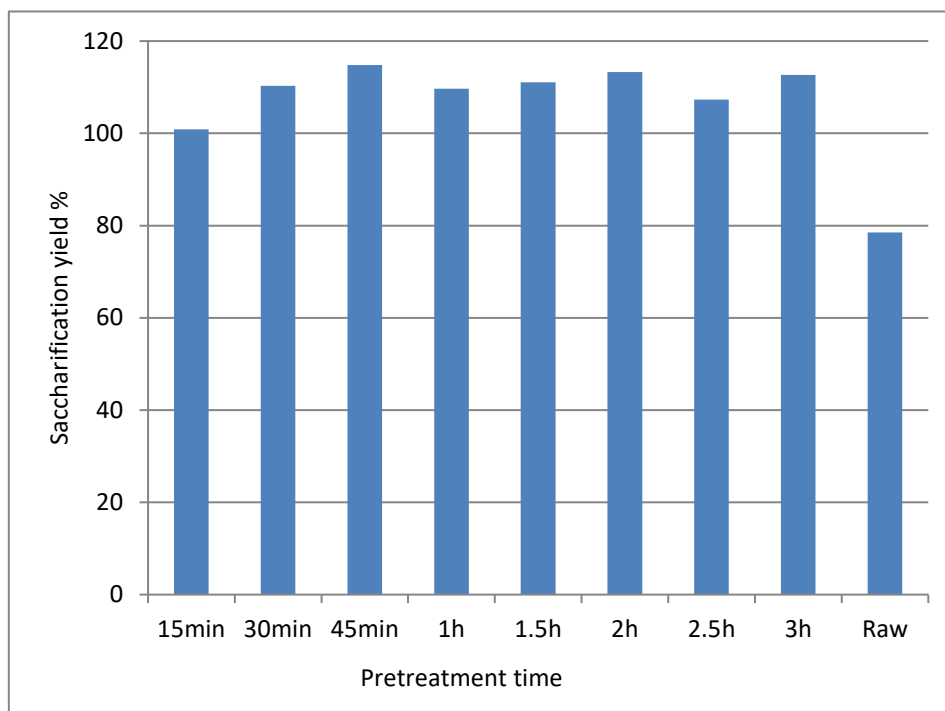


Figure 3-27. Saccharification yields of *Posidonia oceanica* macroalgae pulp after pretreated with 20wt% IL.

The saccharification results in Figure 3-27 showed a generally very high saccharification yield. The untreated *Posidonia oceanica* is already giving 78% saccharification yield which is already even better than some pretreated lignocellulosic biomasses (50). In Figure 3-27, treated pulps achieved saccharification yields over 100%; this is due to the inconsistent performance of the HPLC. But clearly 1h of pretreatment using 20wt% [TEA][HSO₄] can give a very high saccharification yield.

In conclusion, the *Posidonia oceanica* is a promising macroalgae to be used in ethanol production. Quite a few further studies need to be followed. The undetectable part of compositional analysis data need to be determined. A technoeconomic evaluation need to be done to assess whether it is worth it to actually do a pretreatment before fermentation as untreated *Posidonia oceanica* is already giving 78% saccharification yield. It is also possible a better condition can be achieved by using lower IL concentration and cook the seaweed at a lower temperature to reduce cost and avoid overcooking of the pulp.

Chapter 3: 2-stage Pretreatment

Why 2-stage

The ionoSolv pretreatment of lignocellulosic biomass to remove lignin and improve saccharification yield of biomass has been successful. However, there are possible improvements of the process to be made. Using concentrated IL to remove lignin at relevantly high temperature (150°C or 170°C) will also completely or partially dissolve hemicellulose in the IL. The result is that the hemicellulose in biomass, which typically accounts for around 25% of its dry weight, will be wasted. Hemicellulose in IL can also form degradation products and further produce pseudo lignin which will be isolated together with lignin once antisolvent is added. It is fine if the lignin will only be burned to produce energy, but further application of lignin to produce much more valuable chemical products will get more difficult as pseudo-lignin is added.

In lab scale experiments, reuse of IL is not yet discussed, however IL recycling is needed in an actual biorefinery processes to minimise production cost. Figure 3-28 contains a brief introduction of an industrial 2G biorefinery process with 2-stage pretreatment. The IL will be recycled after lignin is recovered for the next cycle of pretreatment. Obviously minimum impurities in recycled ILs is preferred. In a normal pretreatment, there will be hemicellulose and hemicellulose degradation products in the IL together with lignin after pretreatment. After lignin was precipitated together with pseudo lignin, hemicellulose and its degradation products will be left in the recycled IL. Some of them can be removed by evaporation, which will require heat and increase cost, some of them cannot be isolated easily. Impurities left in the recycled IL could potentially cause a lower performance of pretreatment in the next cycle.

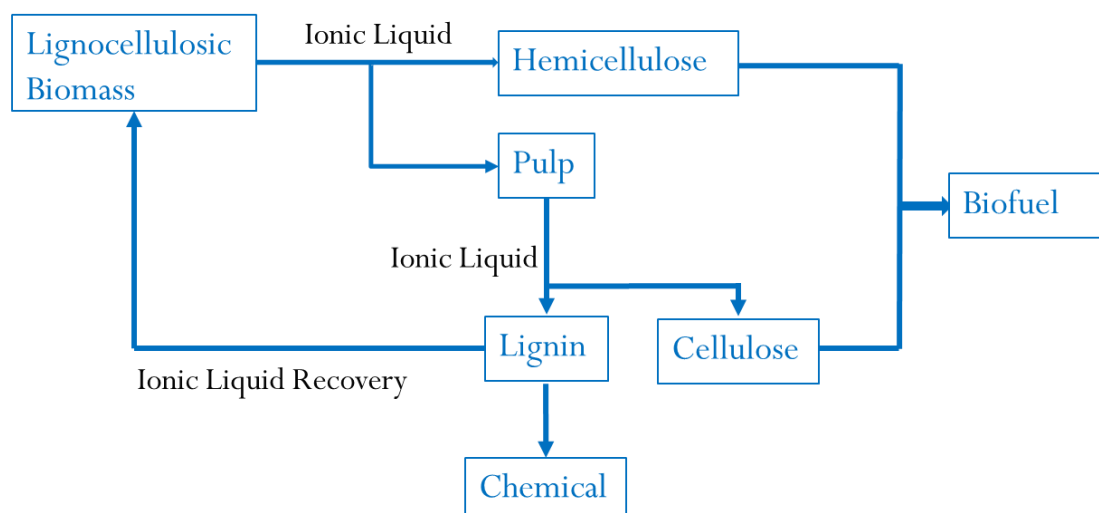


Figure 3-28. A very brief flow chat of 2 stage ionicSolv process

Therefore, it would be advantageous to be able to remove hemicellulose and lignin separately. Also, due to the fact that hemicellulose removal requires a less harsh condition than lignin removal, a 2-stage pretreatment would be a good solution here. In a 2-stage pretreatment, a hemicellulose pre-extraction was performed under mild conditions which will be able to remove as much hemicellulose as possible from pulp, and also produce minimum hemicellulose degradation products. The 1st stage pulp which is hemicellulose free but still rich in cellulose and lignin will then be pretreated like in a normal pretreatment to remove lignin and leave a cellulose rich pulp for fermentation.

Dilute acid 1st stage pretreatment

In this experiment, dilute sulphuric acid was used in a 1st stage pretreatment. Sulphuric acid is generally cheaper than most ILs. To try to determine a condition as mild as possible, a time course experiment was carried out at 80°C from 1h to 8h. 1g of Miscanthus was added into a pressure tube, 10g of 1% H₂SO₄ was then added into the pressure tube. The tube was sealed and vortexed to ensure a good mixture of sulphuric acid and biomass. All tubes were transferred to a preheated oven and cooked at 80°C. After the pretreatment, tubes were

cooled to room temperature and pulp was filtered and then washed by DI water. Washed pulp was then air dried overnight until no further weight change and analysed by compositional analysis. All experiments were done in triplicates.

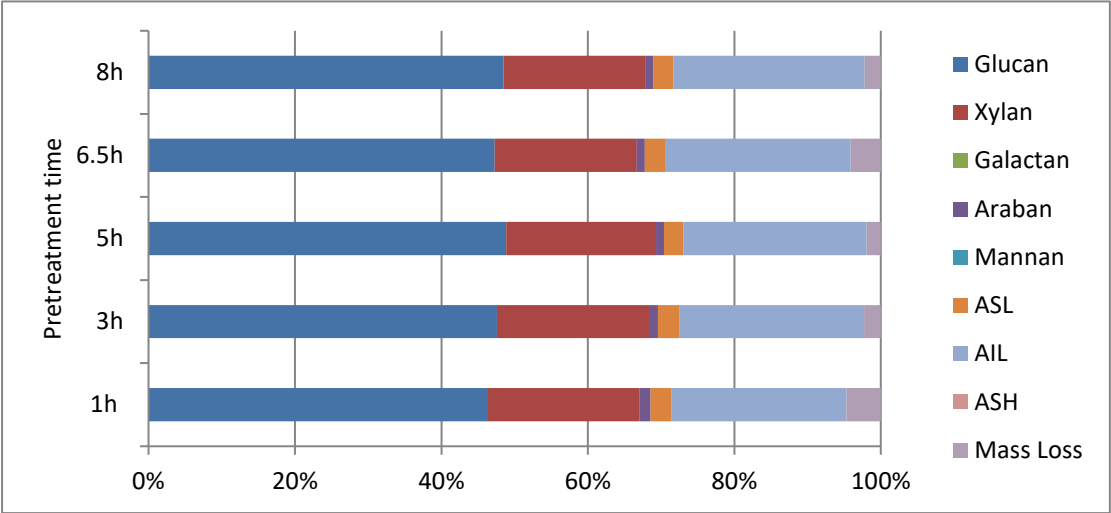


Figure 3-29. Compositional analysis results of pulp after 1st stage pretreatment at 80°C

Figure 3-29 shows compositional analysis result of pulp. A pulp yield of over 90% was achieved and the condition was clearly too mild. Nearly all hemicellulose stayed in the pulp, indicating a harsher condition is needed.

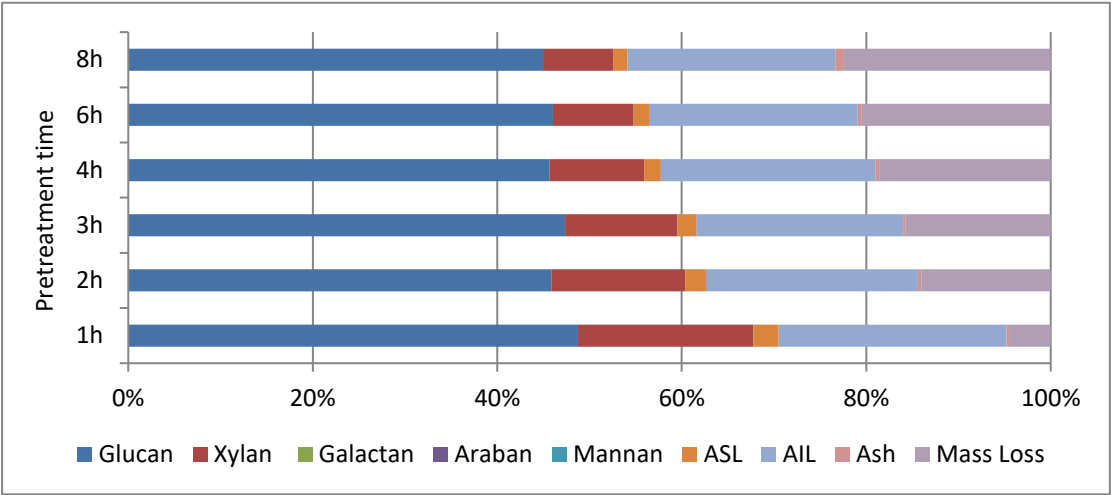


Figure 3-30. Compositional analysis results of pulp after 1st stage pretreatment at 100°C

The second time course experiment of 1st stage pretreatment was carried out at 100°C; all other conditions are same as previous experiments. Figure 3-30 indicates the pretreatment is working at 100°C. Hemicellulose removal increased gradually as pretreatment time gets longer. A good hemicellulose removal was achieved at 8h. Cellulose and lignin stayed in the pulp throughout the pretreatment. Therefore a 1st stage pretreatment using 1% sulphuric acid at 100°C for 8h was chosen for full 2-stage pretreatments.

A full 2-stage pretreatment was performed to test its feasibility. The first stage was done by cooking biomass with 1% H₂SO₄ at 1:10 weight ratio at 100°C for 8h. After pretreatment, a mixture of pulp and H₂SO₄ was filtered and pulp was further washed by DI water to remove H₂SO₄. Pulp was then transferred in aluminum foil and air dried for further analysis and 2nd stage pretreatment. The second stage was done by using 80wt% [TEA][HSO₄] at 150°C and cooked for 30,60 and 90min at the same biomass loading. The final pulp was washed with ethanol to remove IL and lignin before air dried for compositional analysis and saccharification.

Hornification is a phenomenon happens when adhesion of fiber surfaces to each other occur as a result of drying, leading to lower porosity and solvent accessibility. Hornification has been a problem in the biorefinery and could potentially cause decline of saccharification. By adding a hemicellulose pre-extraction before normal pretreatment also means adding one more drying process which could potentially have negative effect on accessibility of cellulose in final pulp. Therefore, it is important to investigate if drying 1st stage pulp before 2nd stage pretreatment will reduce saccharification yield.

A wet 2-stage pretreatment was designed. The wet 2-stage pretreatment avoided drying 1st stage pulp so the pulp was kept in a wet condition throughout the whole 2-stage pretreatment. At the end of the 1st stage pretreatment, instead of drying the pulp after wash, wet pulp was quickly weighed and transferred into a pressure tube and sealed to prevent further change of water content. To determine the actual dry weight of pulp in

pressure tube, a series of tests of water content of wet pulp was carried out. Samples of wet pulp were weighed immediately after filtration and wrapped in aluminium foil to dry in oven at 105°C overnight. Dried pulp was then transferred into a desiccator and cooled to room temperature to measure oven dried weight. A wet 1st stage pulp right after filtration and wash normally have a water content of 67%.

In the dry 2-stage pretreatment, the 2nd stage tube contains 1g pulp with 2g of water and 8g of IL. To achieve the same pulp:water:IL ratio, a very dry IL was used in wet 2nd stage pretreatment as the wet pulp already has a very high water content. The IL used in wet 2nd stage pretreatment was dried on a Schlenk line and a water content of 4.5% was achieved. In the end, 2.79g wet 1st stage pulp which contains same weight of 1g air dried pulp was weighed, and mixed with 8.38g IL which contains 8g dry IL. The final pulp: water: IL ratio was 0.93:2.24:8 which is very close to the ratio in dry pretreatment. All samples were then mixed on vortex and cooked in a preheated oven at 150°C for 30, 60 and 90min and the final pulp was washed by ethanol to remove IL and lignin and then dried for compositional analysis and saccharification.

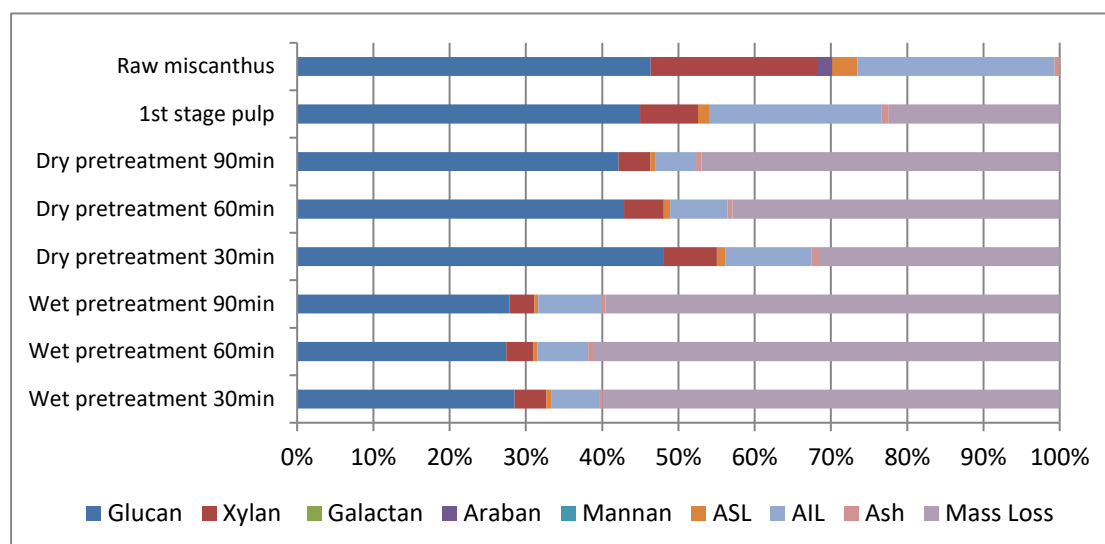


Figure 3-31. Compositional analysis results of pulp from both dry and wet pretreatment, compared with raw Miscanthus and the 1st stage pulp.

Figure 3-31 showed the results of compositional analysis on 1st stage pulp and final pulps. The first stage pretreatment removed 70% hemicellulose as expected and kept lignin in the pulp. Cellulose also remained in pulp so no glucan loss happened at this stage. The 2nd stage pretreatment achieved high lignin removal. Approximately half of the lignin dissolved into the IL at 30min; as the pretreatment time reached 90min, approximately ¾ of the lignin was dissolved. The 2nd stage pretreatment also further dissolved hemicellulose left in pulp, made cellulose more accessible. Some cellulose loss in the 2nd stage pretreatment was observed, but losing 10% cellulose might be acceptable as the pretreatment gives us a much better saccharification yield which will be demonstrated later.

The final pulps of wet 2nd stage pretreatment were also analysed, However, it is not very straightforward to compare the compositional analysis results of both methods. Due to the nature of wet pretreatment, wet 1st stage pulp need to be mixed with IL as soon as possible to avoid further change of water content. Therefore, water content of wet 1st stage pulp can only be estimated so calculation of pulp yield is very difficult. As a result, a figure like Figure 3-31 can't compare results from wet 2-stage pretreatment and dry pretreatment. An alternative way is to normalise compositional analysis results from both pulps directly to 100% without considering pulp yield. Figure 3-32 showed the composition of pulp without considering weight lost during pretreatment. Both methods showed similar performance, with good hemicellulose and lignin removal rate. Cellulose content in the pulp from dry pretreatment method is even slightly higher than in pulp from wet method. The compositional analysis results suggest drying of 1st stage pulp will not introduce hornification effects that can have negative impact on lignin removal.

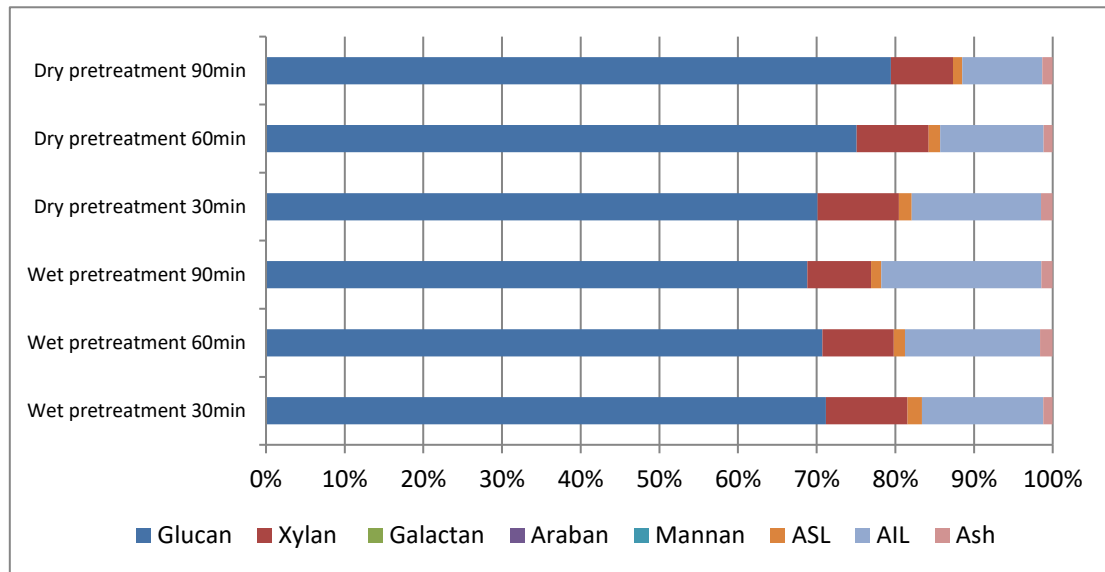


Figure 3-32. Composition of pulp without considering weight lost during pretreatment

Figure 3-33 gives the saccharification results of both wet and dry pretreatment. Although glucan in the pulp from all conditions of both methods was similar, huge gaps of saccharification yield was obtained. The saccharification yield becomes higher when pretreatment time went longer, which also means better lignin removal. In the case of wet 2-stage pretreatment, saccharification yield increased from 25% at 30min pretreatment time to 65% at 90min. An unexpected result is the dry pretreatment actually gives a better saccharification yield, which reached 79% when pretreated for 90min. The saccharification results suggest a dry 2-stage pretreatment is better. It gives better saccharification yield, the whole procedure is simpler therefore less error will be introduced, and it is more controllable.

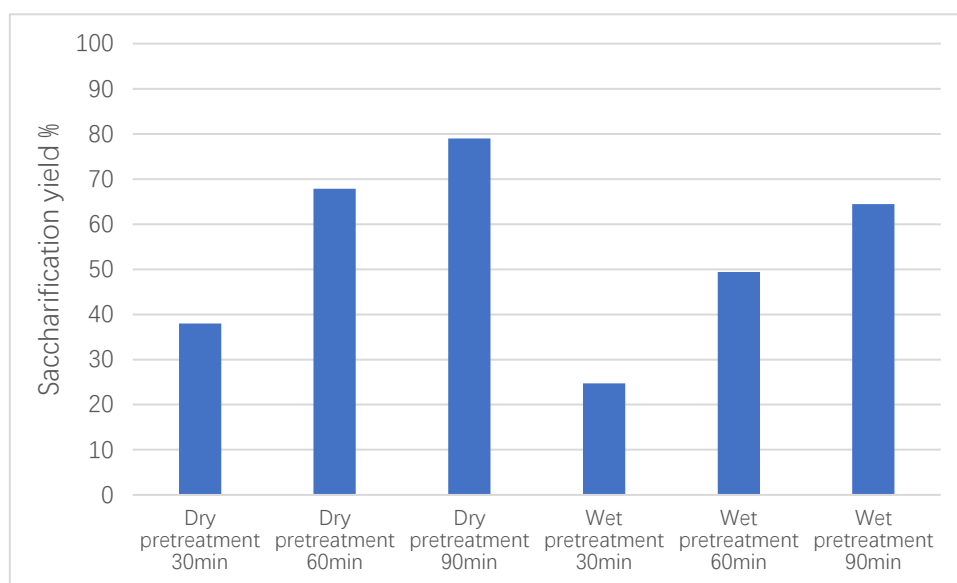


Figure 3-33. Saccharification yield of pulp from both dry and wet pretreatment

Full IL 2-stage pretreatment

As shown previously, ILs can be recycled in a biorefinery process. After adding water as antisolvent into a lignin rich IL to precipitate lignin, a very low concentration IL solution will be collected after lignin recovery. To use IL in the next cycle of pretreatment, water needs to be evaporated to obtain a high concentration IL. This will require extra energy input and increase the cost. Therefore, if a low concentration IL can be directly used in the 1st stage pretreatment to remove hemicellulose, energy cost can be offset by the higher sugar yield. A full IL 2-stage pretreatment was carried out to test if dilute IL can be used in the 1st stage pretreatment.

The question here is what concentration should be chosen. In this case, 4 concentrations were applied from 1wt% to 20wt%. The IL used in this experiment was [TEA][HSO₄]. The 1st stage pretreatment was done by cooking 1g of Miscanthus with 10g of dilute IL at 100°C for 12h. After pretreatment, the whole system was cooled to room temperature and filtered to separate pulp and liquid phase. Pulp was then further washed by DI water to wash IL off. After drying, 1st stage pulp was collected for 2nd stage. The second stage was done by using

80wt% IL at the same biomass loading of 1g Miscanthus to 10g 80wt% IL. After cooking in a preheated oven at 150°C for 90min, the mixture of final pulp and IL was cooled to room temperature before washed by ethanol to remove IL and lignin from pulp. Pulp was then dried for compositional analysis and saccharification.

Figure 3-34 and Figure 3-35 showed compositional analysis results of pulps from both stages. In the 1st stage, glucan and lignin remained intact in the pulp, while hemicellulose removal rate increased with higher IL concentration. Pretreatment using 1wt% IL achieved 95% pulp yield and only a little hemicellulose dissolved. Using 20wt% IL can remove over 60% of hemicellulose. The compositional analysis of final pulp showed nearly identical data. Lignin had 90% dissolved in IL. Hemicellulose in final pulp reached a very low level using all 4 concentrations. Although same final composition was obtained, the purity of lignin recovered will be better and hemicellulose dissolved in low concentration IL at lower temperature will be less likely to degrade. The data from saccharification is provided in Figure 3-36. Despite the fact that all 4 final pulps have similar glucan content and identical lignin/hemicellulose removal, the saccharification yield of the final pulps are quite different. Pretreatment using 1wt% IL gave a saccharification result of over 83% while 20wt% IL 1st stage achieved 78%. The gap between all saccharification yields was not large, but there is a clear trend which indicates lower IL concentration in the 1st stage pretreatment can result in higher saccharification yields.

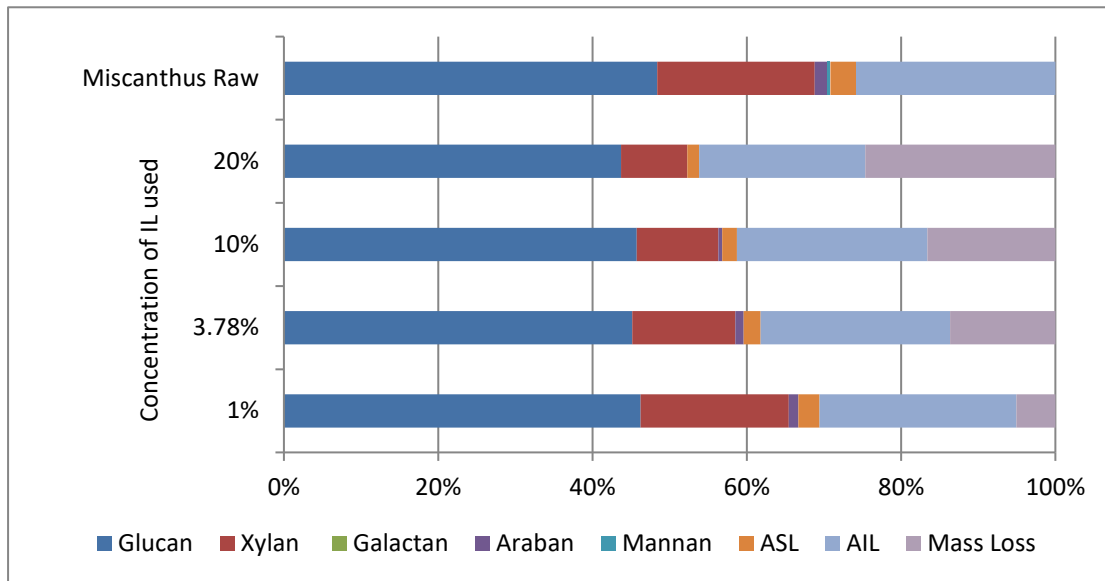


Figure 3-34. Compositional analysis results of pulp from 1st stage pretreatment using IL, compared with raw Miscanthus.

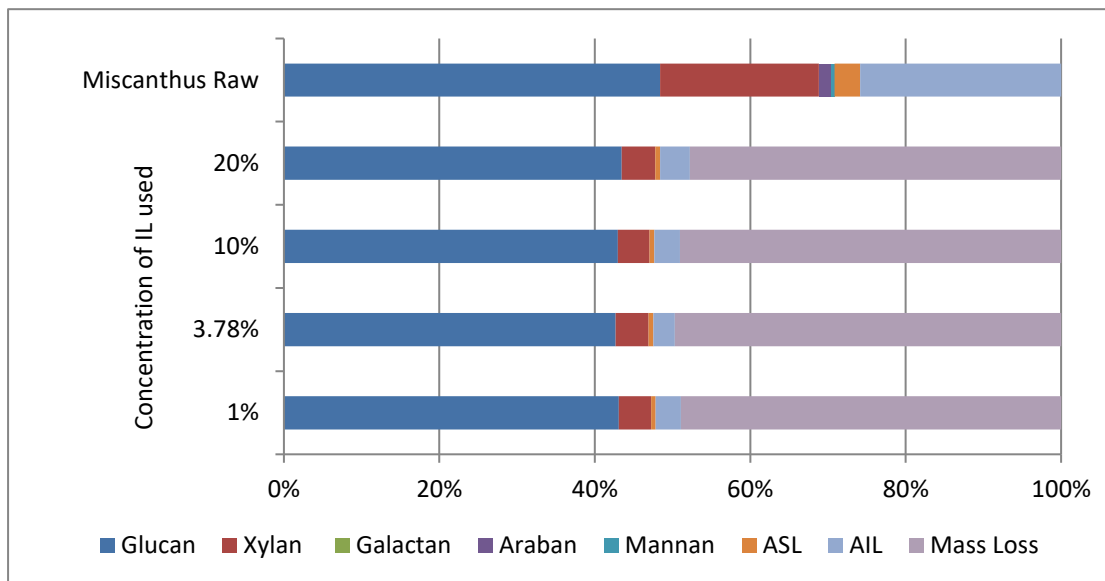


Figure 3-35. Compositional analysis results of pulp from 2nd stage pretreatment using IL, compared with raw Miscanthus.

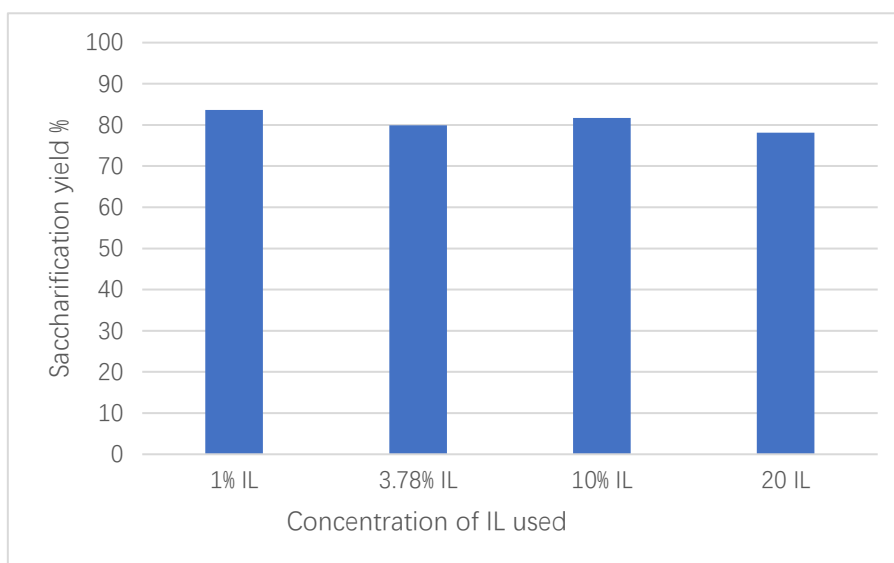


Figure 3-36. Saccharification yield of pulp from 2-stage pretreatment

Further improvement of saccharification yield

The saccharification yields achieved in full IL 2-stage pretreatment were around 80%, however higher saccharification yield in normal pretreatment has been reported (145). Therefore, it is worth trying some modification of the pretreatment process. In this case, pulp from experiments in Chapter 1 was collected and wet saccharification was conducted. Although previous data from wet 2-stage pretreatment does not show any advantage of saccharification yield over dry 2-stage pretreatment, it is still possible that drying of the final pulp will have a greater hornification effect than drying of 1st stage pulp. As lignin provides support of lignocellulose biomass structure, removal of hemicellulose in the 1st stage may not have a strong effect on the rigidity of biomass structure therefore drying of 1st stage pulp will have little hornification impact. After lignin was dissolved during the 2nd stage pretreatment, drying of the final pulp will have a much stronger impact on pulp structure and cause a decrease in accessibility of glucan. The hypothesis is that it is fine to dry the pulp before delignification, but best to avoid drying of pulp after lignin removal.

For the 1st stage pre-treatments, conditions were selected based on the optimum xylan extraction achieved at each temperature. The second stage pre-treatment condition is the

optimum condition for Miscanthus lignin extraction, provided by Dr Florence Gschwend (Hallett Group Imperial College London). The pretreatment time for the second stage was reduced from 1.5 hours to 1 hour to prevent overcooking the pulp.

Saccharification was carried out on the raw Miscanthus and pulps after first and second-stage pre-treatment. Dry pulp saccharification was used for the raw Miscanthus and 1st stage pulp. For the pulp from the 2nd stage pretreatment, wet saccharification was performed to prevent hornification. Three sets of 2-stage pretreatments was carried out and conditions used were summarised in Table 3-3.

Table 3-3. Two-stage pre-treatment and saccharification conditions

	First stage conditions			Saccharification		Second-stage conditions			Saccharification	
	IL %	T	Time	Type	Time	IL %	T	Time	Type	Time
	[wt%]	[°C]	[hrs]		[days]	[wt%]	[°C]	[hrs]		[days]
Set 1	20%	100	8	dry	7	80%	150	1	wet	7
Set 2	20%	110	8	dry	7	80%	150	1	wet	7
Set 3	20%	115	8	dry	7	80%	150	1	wet	7

The saccharification yields for the three sets of two-stage pre-treatments are shown in Figure 3-37. The saccharification yields for first stage pre-treated pulps (orange column) were slightly improved compared to the raw Miscanthus (blue column). This increase in saccharification yield is because of the removal of hemicellulose. However, the yields were low because the lignin in the pulps remained intact. After the second-stage pre-treatment, in which most of the lignin is removed, the saccharification yield improves significantly. The highest yield (93.3 %) was obtained from condition SET 2 second-stage pulp.

The saccharification yield of final pulp from 1st stage pretreatment at 100°C using 20wt% IL showed in Figure 3-37 was 85%, which is 7% higher than results showed in Figure 3-36. The wet saccharification improved the yield by 7%. The result suggests it is fine to dry pulp in between the 1st stage and 2nd stage, but drying of final pulp needs to be avoided in order to reach better saccharification yields.

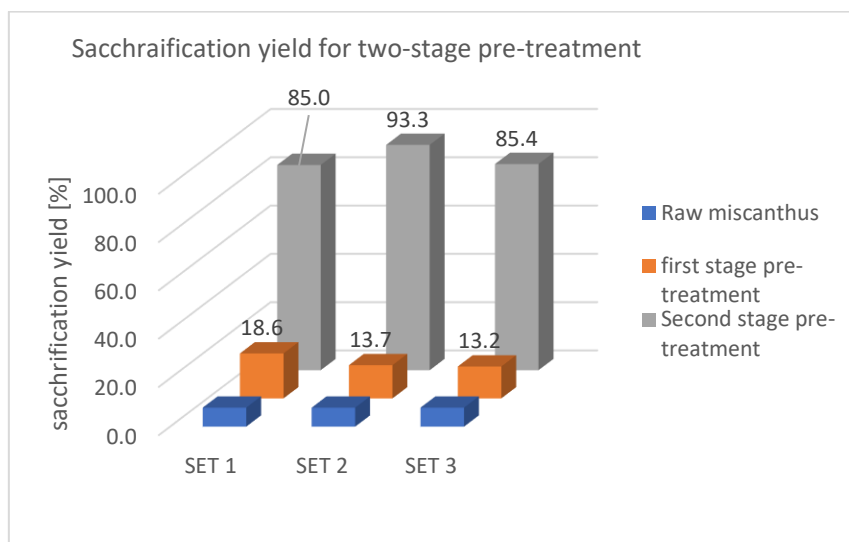


Figure 3-37. Saccharification yield of 2-stage pretreatment on Miscanthus

Mini reactor

All previously described experiments were done in pressure tubes without stirring during pretreatment. Although the mixture of biomass and IL/acid was mixed properly on a vortex, bubbles will still form during pretreatment and the even mixture will be disturbed. Another concern with the 2-stage pretreatment is the 1st stage didn't quite remove all hemicellulose from biomass, some hemicellulose dissolved during the 2nd stage which could still form sugar degradation products in the IL after pretreatment. Therefore, it is good to know if adding stirring during the 1st stage pretreatment can help improve hemicellulose removal. In a larger scale experiment which is closer to actual scale biorefinery process, stir during pretreatment will be required to make sure an even mixture throughout the whole pretreatment. It is better to understand how stirring would affect the performance of ionoSolv process starting from a slightly larger scale in the mini reactor.

One issue with the reactor used is the size and design of stir bar. With a biomass loading of 1g biomass to 10g IL, a minimum of 3g biomass is needed to ensure enough surface between the liquid phase and the stir bar. Another issue is the reactor can be sealed but cannot hold pressure. Therefore, it is not possible to run the 2nd stage pretreatment on the reactor as it requires 150°C. The 2nd stage pretreatment will be carried out in pressure tubes. A picture of mini reactor used in this experiment can be found below (Figure 3-38).

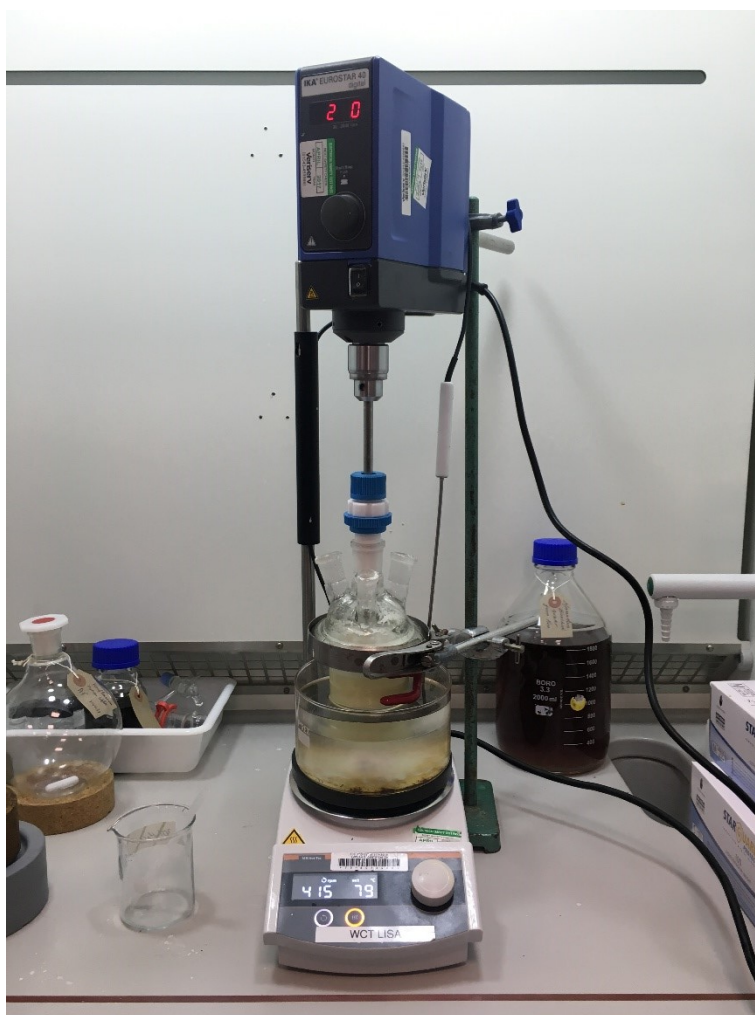


Figure 3-38. Mini reactor with adjustable stirring function

Miscanthus in mini reactor

6g of Miscanthus was weighed and transferred into the reactor, 60g of 20wt% [TEA][HSO₄] was then added. Stir was set at 66rpm and reactor was heated in an oil bath at 100°C for

12h. Reactor was cooled to room temperature after 12h. The pulp was filtered and washed by DI water then dried for compositional analysis and 2nd stage pretreatment. The 2nd stage pretreatment was done by mixing 1g of 1st stage pulp with 10g of 80wt% [TEA][HSO₄] in a pressure tube. The tube was cooked at 150°C for 90min, pulps were washed by ethanol to remove lignin and IL then air dried for compositional analysis and saccharification.

Figure 3-39 and Figure 3-40 compare the results of 2-stage pretreatment done in pressure tube like previously described and result obtained using reactor with stir. The results are quite similar, as the reactor with stirring function did not show a better hemicellulose removal. Saccharification results from both methods are also very close. This indicates at the chosen scale for reactor pretreatment (6g biomass) and with the particle size of 180-850µm, stirring is not necessary. Accessibility of hemicellulose and lignin are already good enough for reaction in tubes without stirring.

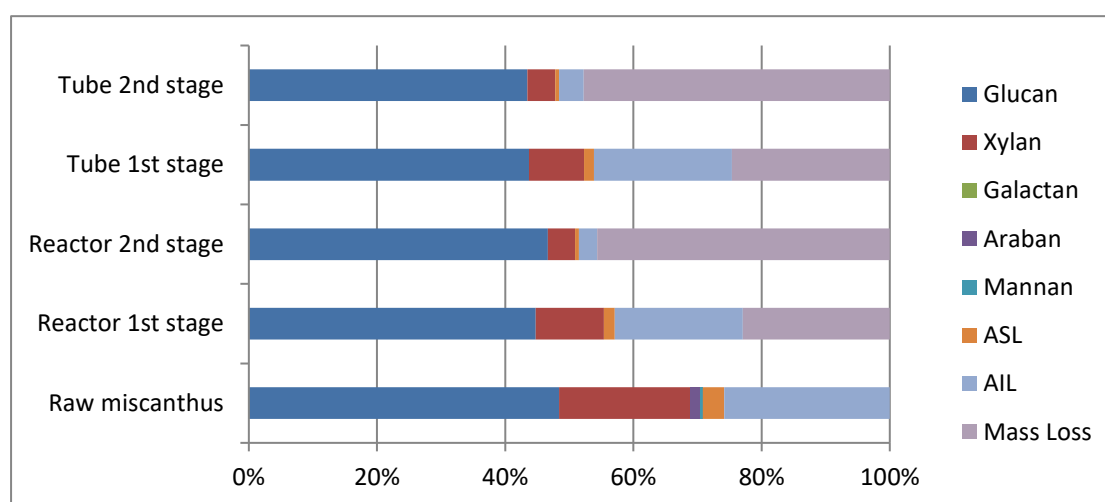


Figure 3-39. Compositional analysis results of 2-stage pretreatment done in pressure tube and mini reactor

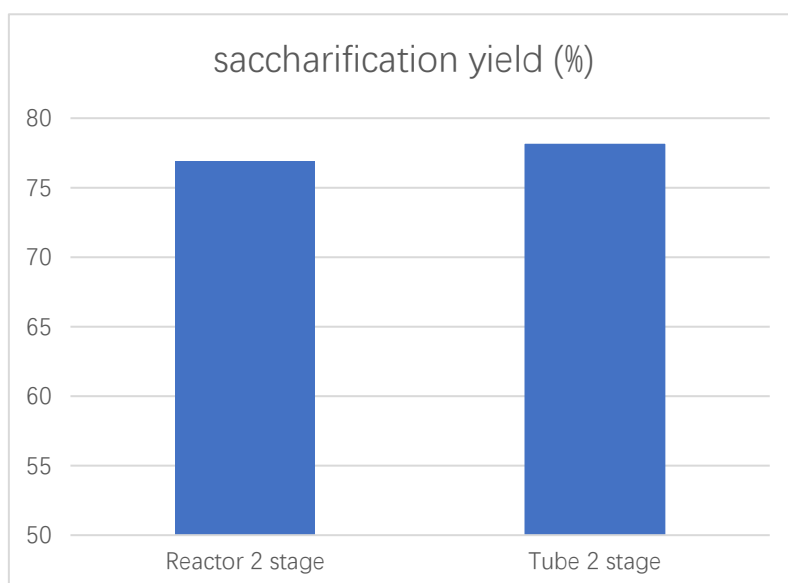


Figure 3-40. Saccharification results of 2-stage pretreatment done in pressure tube and mini reactor

Wheat straw in reactor

Wheat straw is another popular source of carbohydrates. It has over 40% of cellulose and could be another promising biorefinery raw material. In this case, a 1st stage pretreatment of wheat straw in a reactor with stirring was tested. The concentration of sulphuric acid used for wheat straw pretreatment was 2% as suggested by Jeong et al (209). A different biomass loading was used here due to the low weight to volume ratio of wheat straw used, with a particle size of 180-850µm, the wheat straw is very fluffy and a 1:20 biomass loading must be used. As a result, 3g of wheat straw was mixed with 60g 2% H₂SO₄. The reactor was heated to 100°C for 12h. Miscanthus was also tested under this condition with a 1: 10 biomass loading. The 2nd stage pretreatment also happened in tube using 80wt% [TEA][HSO₄] at 150°C for 90min.

The compositional analysis of pulps can be found in Figure 3-41. As can be seen from the results, untreated Miscanthus and wheat straw have very similar composition. Hemicellulose removal from the 1st stage pretreatment are also quite similar. A difference here is wheat straw will lose 20% glucan during the 1st stage pretreatment, which implies there are more

glucose-hemicellulose linkages in wheat straw. Lignin removal is better in Miscanthus pretreatment. Saccharification yield will be discussed later in this chapter.

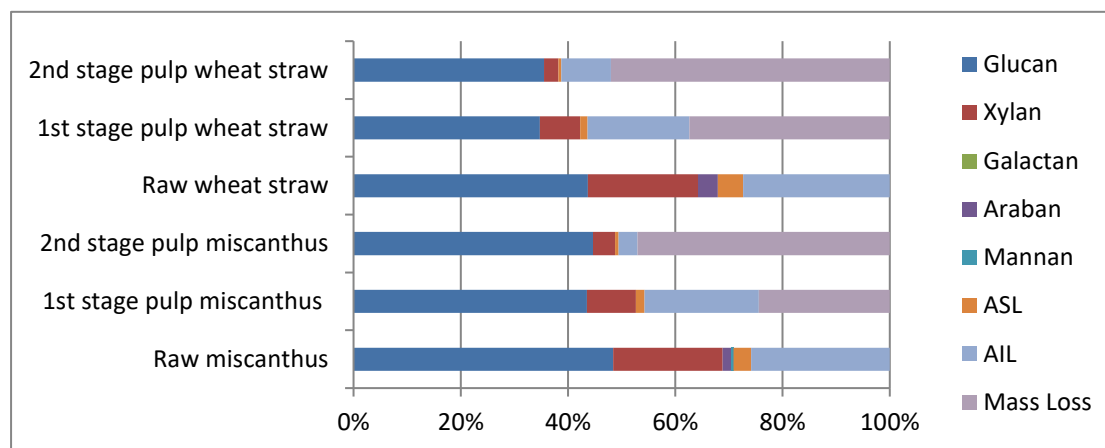


Figure 3-41. Compositional analysis results of 2-stage pretreatment on wheat straw

Systematic variations of procedure

Four conditions were tried in this experiment to observe effect of temperature and speed of stirring. Details of conditions can be found in Table 3-4. All conditions applied a 1:20 biomass loading in which 3g of wheat straw and 60g 2% sulphuric acid was mixed. 2nd stage pretreatments were happened in pressure tubes using 1g of 1st stage pulp and 10g 80wt% [TEA][HSO₄] at 150°C for 90min.

Table 3-4. Details of conditions tried on mini reactor

	Temperture (°C)	Time (h)	rpm
Condition 1	100	12	66
Condition 2	120	12	66
Condition 3	100	12	150
Condition 4	90	12	150

Figure 3-42 shows the compositional results of all pulps. Comparing condition 1 with condition 2, we can see condition 2 achieved a much better hemicellulose removal during the 1st stage, but also lost slightly more glucan at the same time. The hemicellulose removals in the final pulp were about the same, but lignin removal of condition 1 was slightly better. 10% more glucan content was lost in condition 2. The saccharification yield showed in Figure 3-43 gave slightly higher yields from condition 2 which means pulps from condition 2 will offer slightly better cellulose accessibility to enzymes. In conclusion, condition 1 will leave about 10% more glucan in pulp with only 1.5% less saccharification yield, the result is a better glucose potential using condition 1. Condition 2 at 120°C will provide purer recycled IL after pretreatment with less sugar degradation product in, but will cost more energy to maintain higher reaction temperature and produce less glucose.

By comparing condition 1 and condition 3, we can observe very similar 1st stage pulp composition. But the 1st stage pulp got shredded by high stirring speed, visually the pulp have much smaller particle size than untreated wheat straw will condition 1 with 66rpm does not seem to have an effect on particle size of pulp. The fact that pulps created from different particle sizes have the same compositional analysis result suggests the particle size of 180-850µm already offered full potential accessibility to hemicellulose for sulphuric acid. A smaller particle size will not provide better hemicellulose removal. The final pulp result showed similar lignin removal, but different glucan content. There were 20% more glucan left in pulp from condition 1. Saccharification result of both conditions showed a slightly higher yield obtained from condition 3 pulp, however due to the large amount of glucan lost in condition 3, the condition 1 pulp will provide much more glucose per untreated unit of wheat straw. Overall, condition 1 is favoured over condition 3.

Condition 4 used a lower reaction temperature compared to condition 3. The result is a similar 1st stage pulp composition, but the lignin removal in the final pulp from condition 3 is slightly higher than from condition 4. However, condition 4 does have more glucan left in the final pulp. This could be the result of the less harsh condition of condition 4 resulting in a

slightly higher particle size of the 1st stage pulp. Saccharification yield of the pulp from condition 4 is the lowest of all 4 conditions while condition 3 has the highest. But the small gap between saccharification yields of condition 3 and 4 means overall both conditions will provide similar glucose per unit of untreated wheat straw. As a result, condition 4 is slightly better than condition 3 due to its lower reaction temperature and therefore lower cost of energy input. In conclusion, condition 1 is the best among all conditions tested. It requires less energy input at mild temperature and lower stirring speed, gives good hemicellulose and lignin removal and offers the best glucose produced per unit of untreated wheat straw.

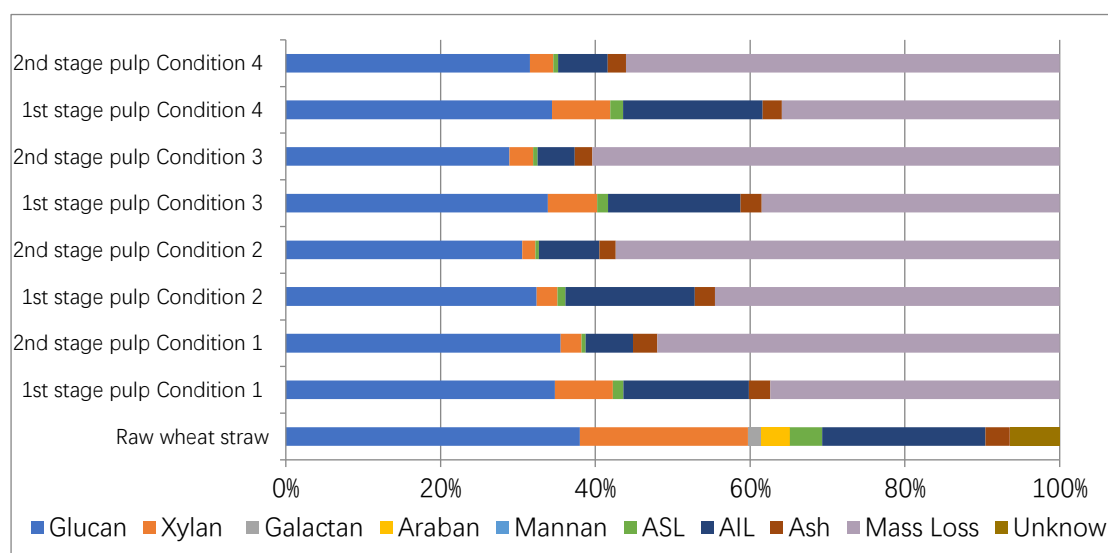


Figure 3-42. Compositional analysis results of 2-stage pretreatment on wheat straw under different conditions

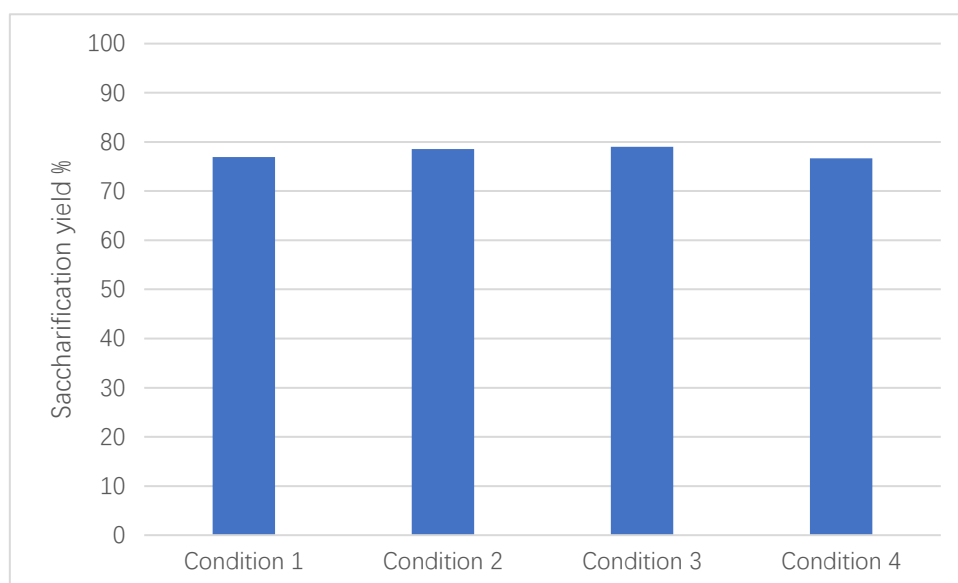


Figure 3-43. Saccharification results of 2-stage pretreatment on wheat straw under different conditions

2-stage pretreatment using recycled ionic liquid

One of the advantages of ILs is it can be recycled. It will greatly reduce the cost of biorefinery using IL if IL can be recycled and reused in pretreatment. 1-step pretreatment of *Miscanthus* using recycled [TEA][HSO₄] has been proved to be feasible previously in our group (145). Delignification yield of 85% was achieved from using fresh IL to cycle 4. Saccharification yield also remained around 70% through all the cycles. IL recovery yield of each cycle was above 98% with low hemicellulose degradation product accumulation. Reuse of IL from pretreatment of softwood can be a different story. Unlike hardwoods and grasses, softwoods have different hemicellulose composition which typically contains more C6 sugars as glucomannan is one of the major hemicellulose components. C5 degradation products, which includes furfural, will be easily evaporated during re-concentration of IL. HMF as C6 degradation product cannot be removed in the same way. Therefore, it is suspected that HMF will be accumulated over each cycle and will have some impact on the performance of pretreatment.

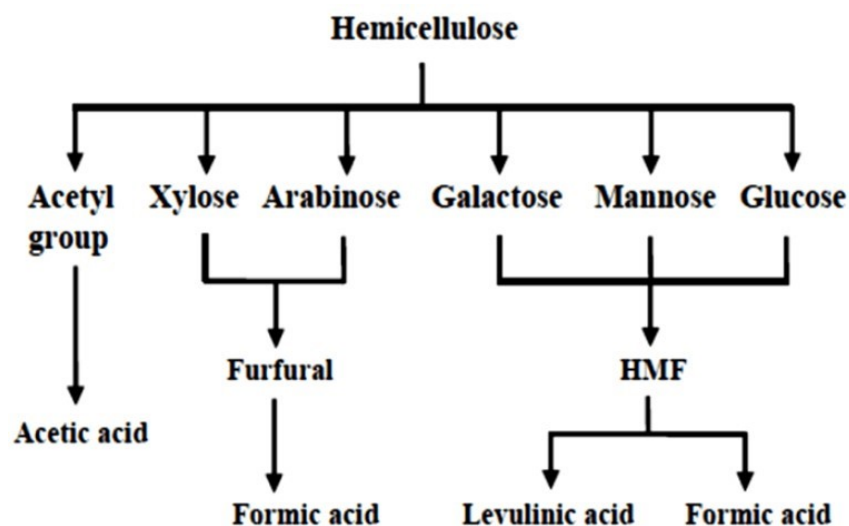


Figure 3-44. Route of hemicellulose degradation

One way to prevent accumulation of HMF is to remove C6 as much as possible before pretreatment. Therefore, it is worth trying if 2-stage pretreatment could result in a good delignification and saccharification yield. First of all, the condition of the 1st stage pretreatment needs to be determined. In this experiment, pine was used as a typical softwood. Pine was ground and sieved; the particle size of grinded pine used was between 180 μ m and 850 μ m. [DMBA][HSO₄] was chosen here as [TEA][HSO₄] does not work well on softwoods (210). 1g of pine was mixed with 10g 20wt% DMBA HSO₄ and cooked in a preheated oven at 150°C. Pretreatment time was varied from 45min to 3h. The pulp was cooled to room temperature before being filtered and washed. Compositional analysis results of the pulps are shown in Figure 3-45. All experiments were done in triplicate.

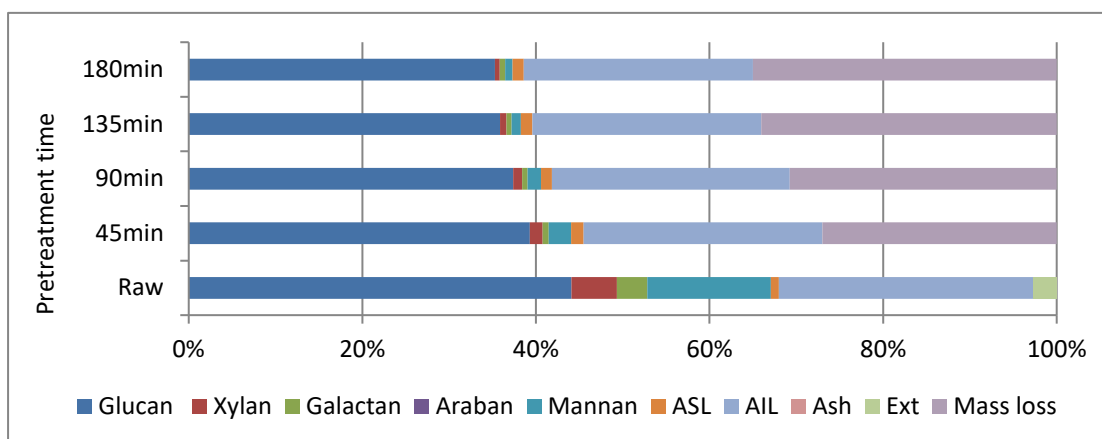


Figure 3-45. Compositional analysis results of pulp from 1st stage pretreatment

As can be seen from Figure 3-45 lignin remained in the pulp. A good hemicellulose removal was achieved at 45min of pretreatment and get slightly better with longer pretreatment time. Glucan left in pulp also declined as pretreatment time gets longer. Therefore 90min is a balance point where hemicellulose especially mannan was dissolved and most glucan still remained in the pulp. A closer look of mannan mass balance can be found in Figure 3-46. Mannan left in the pulp was only a little more at 90min than longer pretreatment time while degraded mannan increased much more rapidly after 90min. Since prevention of forming of C6 degradation products is crucial in the experiment. 90min is chosen to be the condition of the 1st stage pretreatment.

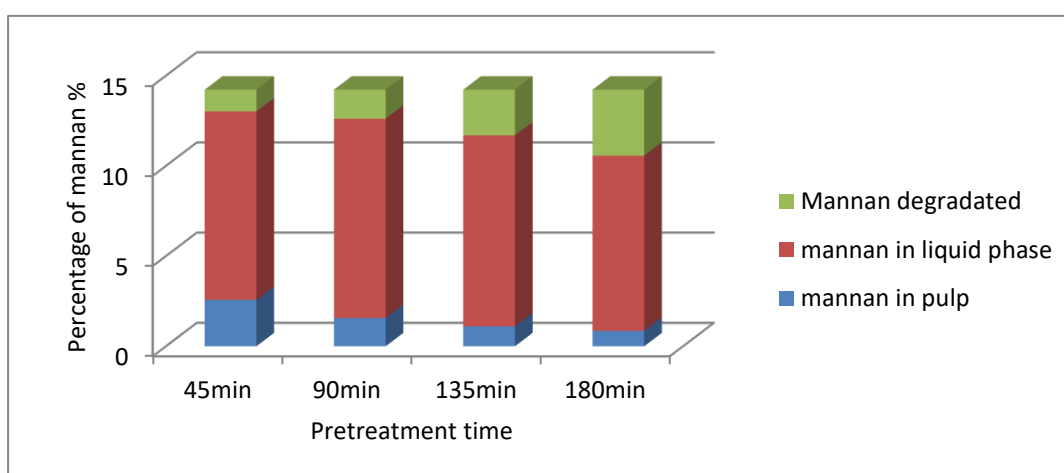


Figure 3-46. Mannan mass balance

In this experiment, the 1st stage pretreatment was not involved in using of recycled IL. The hemicellulose free 1st stage pulp was prepared under conditions described previously. In the first cycle, 1g of air dried 1st stage pulp was mixed with 10g 80wt% fresh [DMBA][HSO₄] and cooked at 150°C for 90min. After pretreatment, the mixture of glucan rich pulp and IL was cooled to room temperature. The pulp was then washed properly by ethanol and dried for compositional analysis and saccharification. IL together with ethanol used in pulp wash and lignin was collected and ethanol was evaporated, leaving IL with lignin and little water content. DI water was then added to precipitate lignin. After lignin was precipitated and separated from IL, it was re-concentrated by evaporation to 20wt% water content and ready for the next cycle. Recycled IL was also tested on HPLC for its HMF content. 5 cycles were done in total. All experiments were done in triplicate.

Figure 3-47 showed compositional analysis results of raw pine, 1st stage pulp and final pulps from each cycle. The first information noticed is loss of glucan. 20% of glucan was lost in the first 3 cycles, which will affect glucose availability. The glucan left in the pulp started to increase from cycle 4 and in cycle 5 90% of glucan stayed in pulp. This could be the result of the change of cation and anion ratio after each cycle which lead to loss of acidity in recycled IL. Although IL used in this experiment was designed to have a 1:1 cation to anion ratio, later tests have suggested a slight excess of acid in this batch of IL, which may account for some glucan hydrolysis during the first 3 cycles. Elemental analysis from previous work of our group suggests there will be more sulphate (assuming the sulphur did not change its oxidation state) deposited than dimethylbutylammonium cations, through biomass neutralisation (i.e. acetate hydrolysis). It is possible at cycle 4 the acid base ratio of recycled IL reached a point where less glucan will be dissolved. This hypothesis suggests if IL is reused for more cycles, eventually the cation to anion ratio will be too high and acidity of IL will be too low. The result will be less delignification yield and lower saccharification yield. A small amount of sulphuric acid can be added after each cycle to maintain the cation to anion ratio, and this will not be costly as the acid is much cheaper than the amine used here.

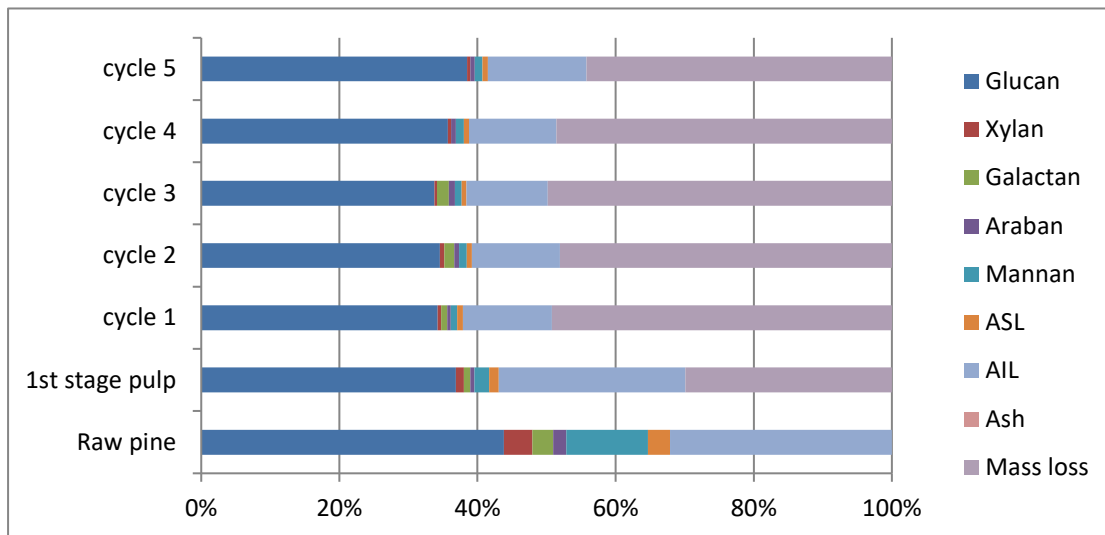


Figure 3-47. Compositional analysis results of untreated pine, pulp from 1st stage pretreatment and final pulp of each cycle

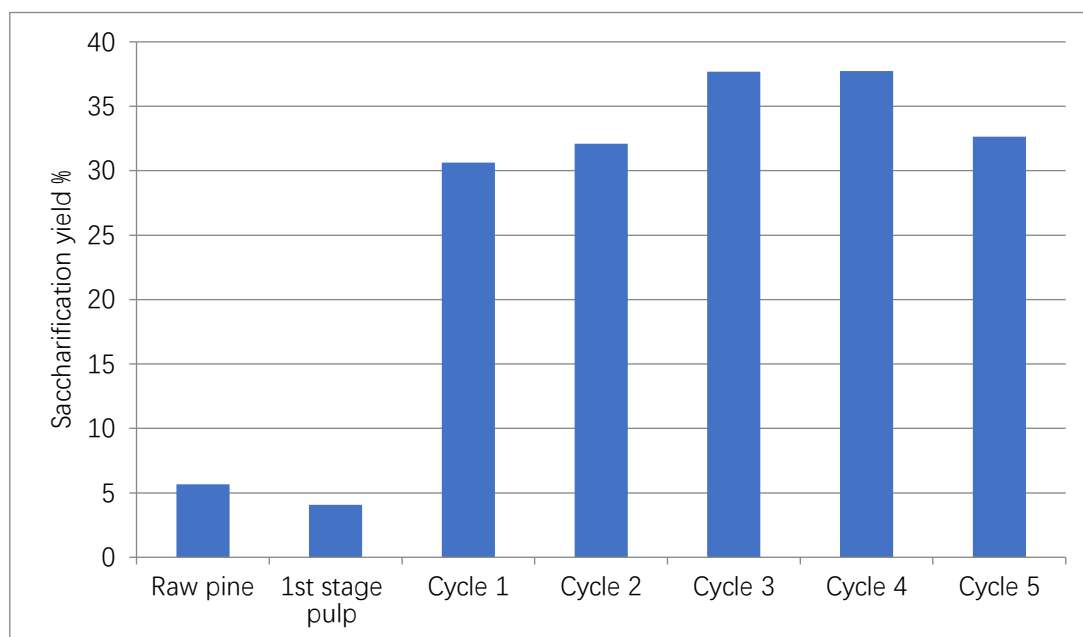


Figure 3-48. Saccharification results of untreated pine, pulp from 1st stage pretreatment and final pulp of each cycle

Delignification yield was not as high as expected. 50% of the lignin dissolved into the IL and delignification was very stable throughout all cycles. The result of insufficient delignification

was low saccharification yield. As can be seen from Figure 3-48, the saccharification yield was generally not very high. The best saccharification yield of 37% was achieved at cycle 3 and cycle 4. The low delignification could be the result of the 1st stage pretreatment. A normal pretreatment under the same condition on untreated pine will have better lignin removal. It is possible although the 1st stage pretreatment did not remove lignin, a small part of lignin dissolved in IL and redeposited on surface of 1st stage pulp, which have made lignin less accessible and more difficult to dissolve.

Figure 3-49 indicated HMF accumulation in IL. At cycle 4 there is only 1.23mg HMF in 1ml of recycled IL which have 20wt% water. The HMF as expected does accumulate but at a very slow rate.

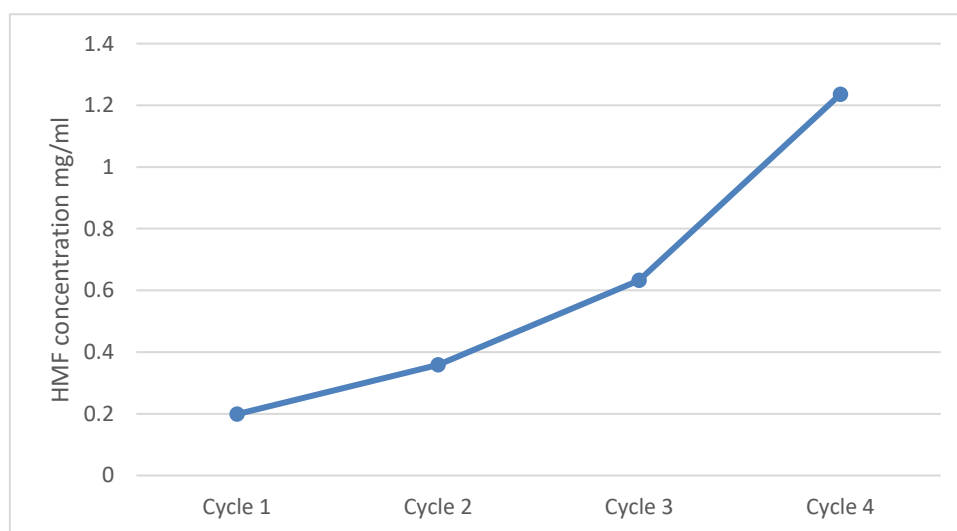


Figure 3-49. HMF content in IL after each cycle

In conclusion, the 2-stage pretreatment using recycled IL on the 2nd stage does work but requires improvements. The slightly increased glucan percentage in pulp starting from cycle 4 indicates the possibility of IL losing acidity after each cycle. The saccharification yield in cycle 5 decreased while the glucan in pulp increased indicates a drop of enzyme accessibility of glucan. This could be the result of

pseudo-lignin accumulation in IL over cycles followed by redeposition on pulp. The future research of using recycled IL lies in finding a better condition for both stages and also using recycled IL in the 1st stage pretreatment.

Part IV. Conclusion

The initial purpose of this project when it started 4 years ago was to selectively extract hemicellulose from lignocellulose and make use of it since the separation and application of hemicellulose was ignored in ionic liquid pretreatments back. The direction changed as the research proceeded. Other than dissolving lignin together with hemicellulose in the ionic liquid process using high concentrations of ionic liquids, the possibility of dissolving only hemicellulose using dilute ionic liquids without delignification has been established. A 2-stage pretreatment was conducted and partially achieved the desired goal. Hemicellulose dissolved in dilute ionic liquid in the 1st stage pretreatment without too much degradation. Lignin was removed and recovered in the 2nd stage pretreatment with a decent delignification yield. Cellulose stayed in the pulp during both stages and showed better accessibility by achieving good saccharification yield. The feasibility to use recycled ionic liquid in the 2nd stage of a 2-stage pretreatment has been established. Seaweed as a next generation feedstock has been tested. Pretreatment on a variety of seaweed was done and saccharification was carried out. *Posidonia oceanica* showed good potential as a source of sugar production. Despite the relevantly low cellulose content in raw *Posidonia oceanica*, cellulose in *Posidonia oceanica* has great accessibility and requires easy pretreatment to achieve a very high saccharification yield.

Apart from all of the achievements above, questions are also emerging along with findings. Hemicellulose is isolated from biomass without a further indication of the final product. To choose between production of furfural/HMF or bioethanol depends on the hemicellulose sugars in certain biomass types. Conversion of C5 sugar to ethanol is more complicated and a more comprehensive evaluation of production cost and product value will be necessary. The saccharification yield of softwood pulps is still far lower than the yield of Miscanthus pulp pretreated under the same conditions. A further research on the reason PILs with certain cations have better delignification on softwoods needs to be done for better choice of ILs. Recycled IL experiment could be performed for several further cycles to further observe composition changes of pulps and their saccharification yields. The hypothesis of

pseudo lignin formation and redeposition affecting saccharification yield might be further proved with more exploration. Despite the fact *Posidonia oceanica* requires only a mild pretreatment and can achieve a high saccharification yield, the fact that certain algae could be more profitable in biodiesel production or cosmetic industry cannot be ignored.

In conclusion, the work presented in this thesis demonstrates the potential of the ionoSolv process. The cost of ionic liquids can be reduced. A huge variety of biomass feedstocks including lignocellulose and seaweed will work with the ionoSolv process. The process itself is robust with the ability to maintain its performance over at least 5 cycles without any additional purification on recycled ionic liquid. A lot of work still needs to be done if a commercialized industrial scale ionoSolv biorefinery plant is to be built.

Future work

As stated in the conclusion, the project did provide some encouraging result. However, there is still a long way to go to build the first ionoSolv plant. Oil refineries are still far away from being replaced by biorefineries. The pretreatment of lignocellulose is now better understood, yet improvements can still be made in the process. Hemicellulose mainly exist in the form of sugar monomers in the liquor of the 1st stage pretreatment and are unused at the moment. The isolation and application of removed hemicellulose can be the next step of improving cost efficiency of the ionoSolv process. While the C6 sugars can undergo the transformation to HMF, C5 sugars can be dehydrated to produce furfural. Both can be valuable by-products. The HMF and furfural in 1st stage pretreatment liquor together with hemicellulose sugars, although not in a large amount, can also be isolated in the future. The 1st stage pretreatment is not yet perfected. The long reaction time will significantly increase the cost and affect the feasibility of the 2-stage pretreatment. Therefore, a new balance point could be found where lignocellulose can be cooked at a harsher condition to reduce reaction time. The new condition will almost certainly achieve a better hemicellulose removal, with a larger percentage of hemicellulose sugars transformed into HMF and furfural. With a proper isolation method of both hemicellulose sugar and sugar degradation products, the preservation of hemicellulose sugar in the form of sugar monomers will not be necessary.

The 2nd stage pretreatment also has the possibility to be better. As the only method to be able to pretreat softwood, ionic liquid based pretreatment (ionic liquid dissolution process and ionoSolv process) have certainly got a huge potential particularly in a softwood biorefinery. Currently, the saccharification yield of pulp after ionoSolv pretreatment of softwood feedstock is noticeably lower than the yield of grass pulp. Therefore, the future work of ionoSolv can definitely focus on deconstruction of softwood. Right now in the lab, the process uses ethanol to wash pulp after pretreatment. Such huge amount of ethanol usage is not likely to be repeated in an actual ionoSolv plant due to the cost of purchasing ethanol, evaporation of ethanol to recover lignin and ionic liquid. As a result, the wash step needs to be updated. One possible solution is to separate the pulp and lignin rich liquor

before pulp wash to reduce the amount of ethanol needed. Also there has been suggestions in our group to use the final liquor after lignin precipitation (mainly consisting of water and ionic liquid) to wash the pulp before using ethanol or simply to perform a water wash after washing with the final liquor. This will affect lignin recovery yield but wash water can then be used in lignin precipitation instead of using DI water to further cut the cost. But again the increased lignin fragments in the wash water will be taken together with recycled ionic liquid into the next cycle of pretreatment and might affect the performance of recycled ionic liquids.

At the moment, the re-concentration of recycled ionic liquid is done by evaporating water under vacuum. In an IonoSolv plant it is more likely to be carried out under atmospheric pressure which will require a lot of heat input. Membrane-based separation can be the solution. Also, 80wt% ionic liquid is now used in our pretreatment, however it is possible to reduce the concentration slightly to sacrifice a little delignification and saccharification yield to achieve a slightly lower ionic liquid usage together with an easier processing and IL-water separation due to the decreased viscosity of ionic liquid with lower concentration and less water needed to be removed.

The use of recycled ionic liquid in 2-stage pretreatments has not been fully tested. The results from this project only examined the recycle of ionic liquid in the 2nd stage while the 1st stage uses fresh ionic liquid. One future work that needs to be done immediately is to use recycled ionic liquid in the 1st stage. Since very low concentration ionic liquid is used in the 1st stage pretreatment, the energy required to re-concentrate the ionic liquid could be a lot lower. In fact, the final liquor might be able to be used directly in the 1st stage. If in some case the concentration of ionic liquid needed for 1st stage pretreatment is lower than the final liquor from the previous cycle, the liquor after hemicellulose isolation can be mixed with final liquor resulting in an easy reuse of the 1st stage liquor.

There are several other issues that need to be addressed. The high viscosity of ionic liquids is always a barrier in industrial processes which will require more energy input or higher temperature to overcome. The current experiments performed at lab scale are all in glass container. In the case of commercialisation, corrosion is an unavoidable problem. At the moment, the study on corrosion of ionic liquid on metal is not as popular and common as the lab scale ionoSolv process, which leaves ionic liquid corrosion research with a long way to go.

The ionoSolv process has an amazing potential to overcome many barriers not only in biorefinery, but also in areas not biomass or biofuel related. There are alternatives to fossil fuels. Many forms of renewable energy like wind, solar and tidal power generation are great options to supply energy. But oil derived chemicals cannot be produced by these technologies. The ionoSolv process holds a great advantage here as the potential to produce biomass derived chemicals. Actually, renewable power generation mentioned above might be a more feasible way towards an environmental friendly sustainable energy supply. IonoSolv might find more value in producing chemicals in the future. Other possible applications include removing metal in crude oil, regeneration of engine oil and extracting metal from electronic waste. All are potential uses of ionic liquid can lead us to a greener and more sustainable future.

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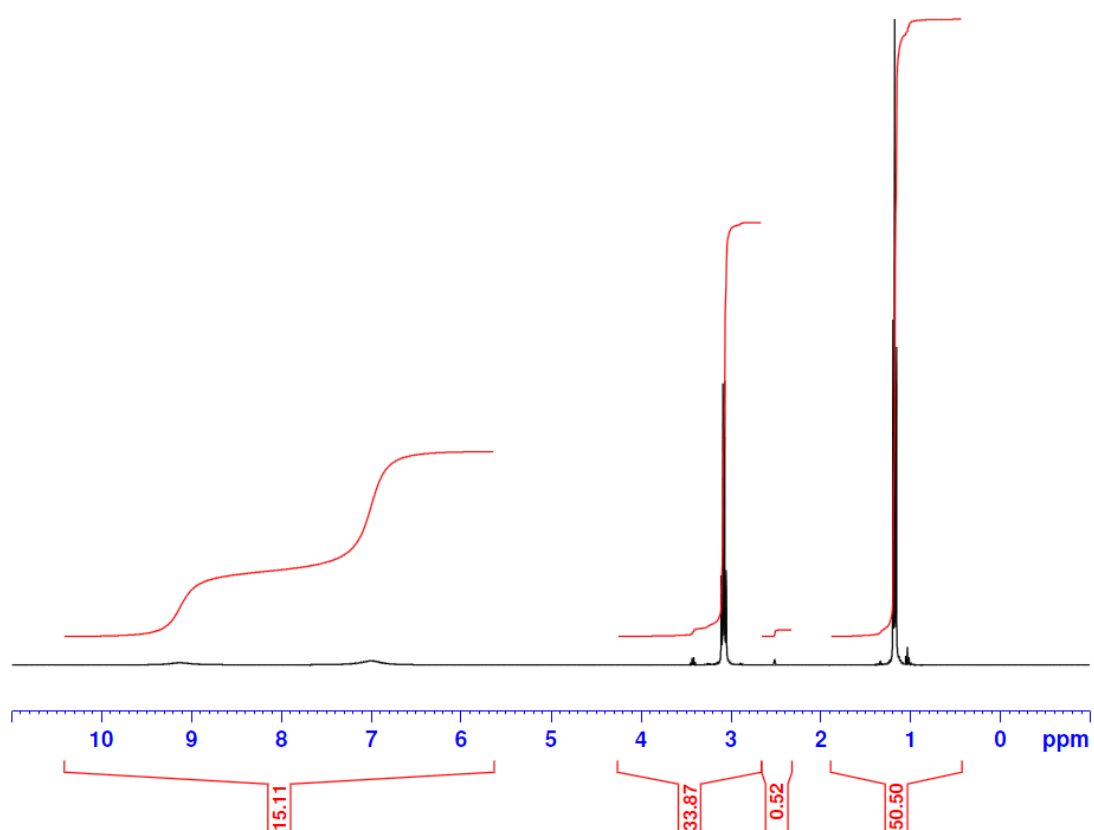
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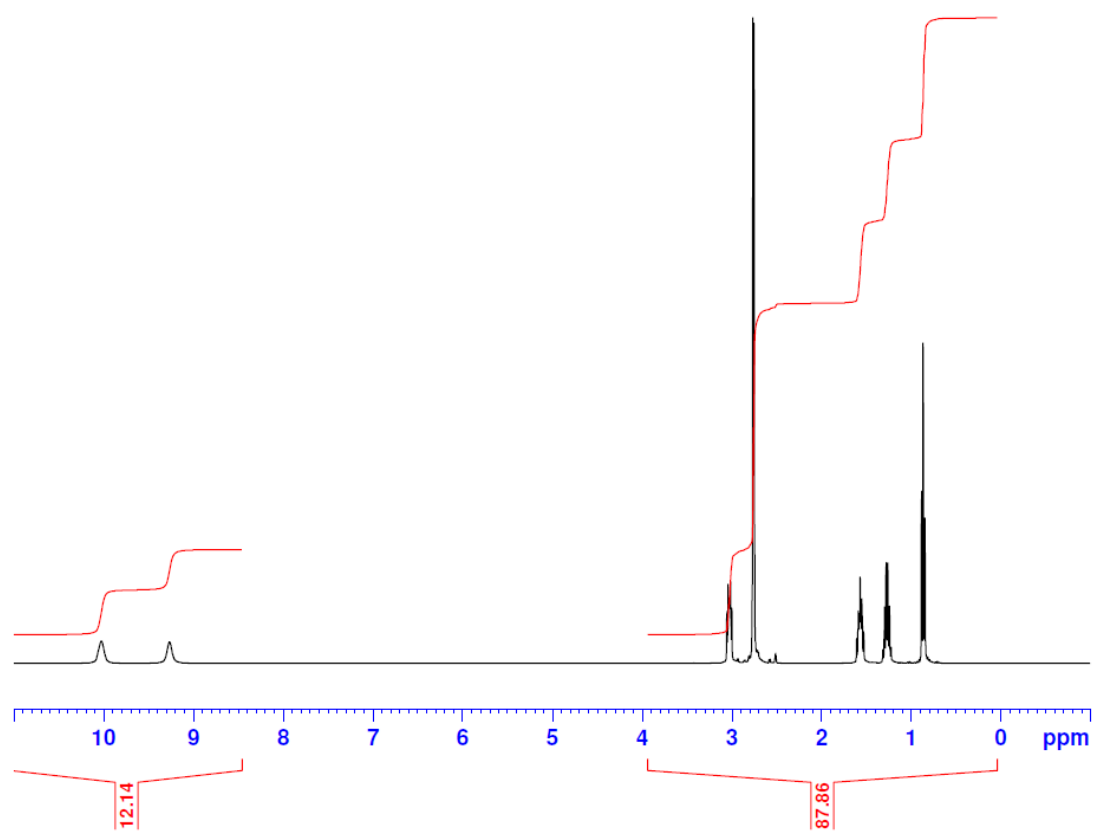
Appendix

Ionic Liquid Synthesis NMRs

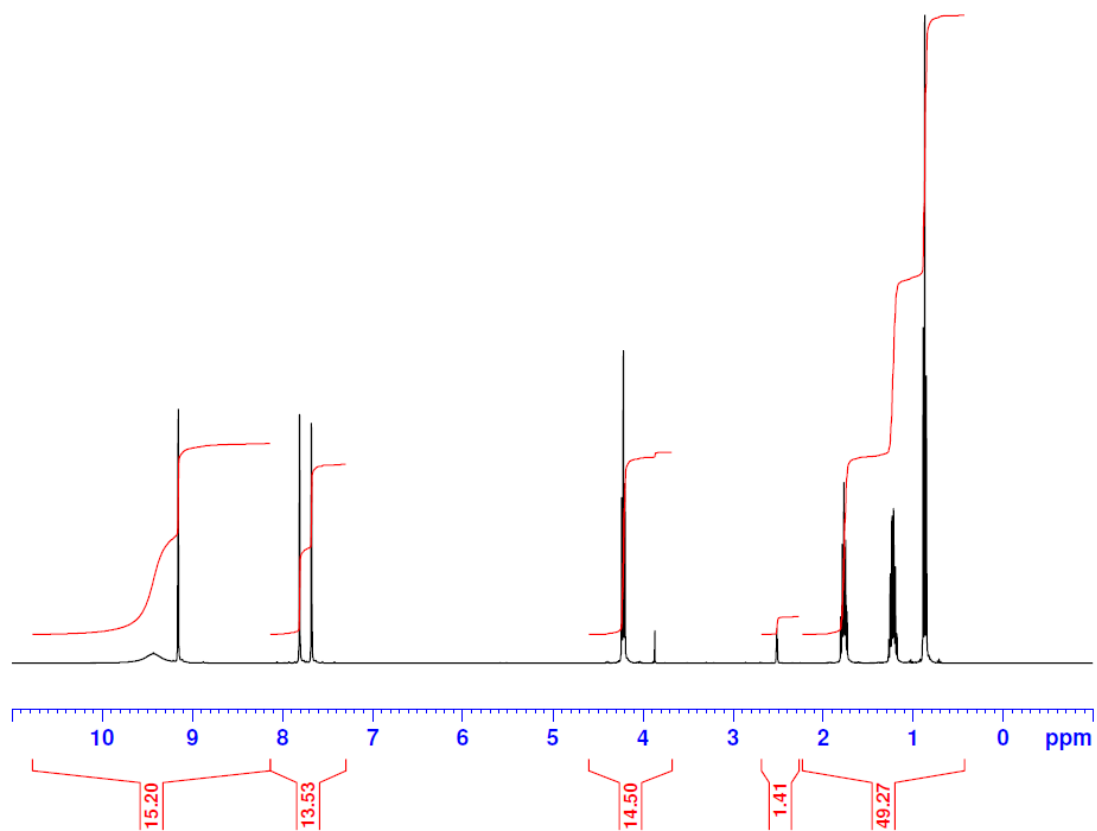
^1H NMR spectrum of $[\text{TEA}][\text{HSO}_4]$



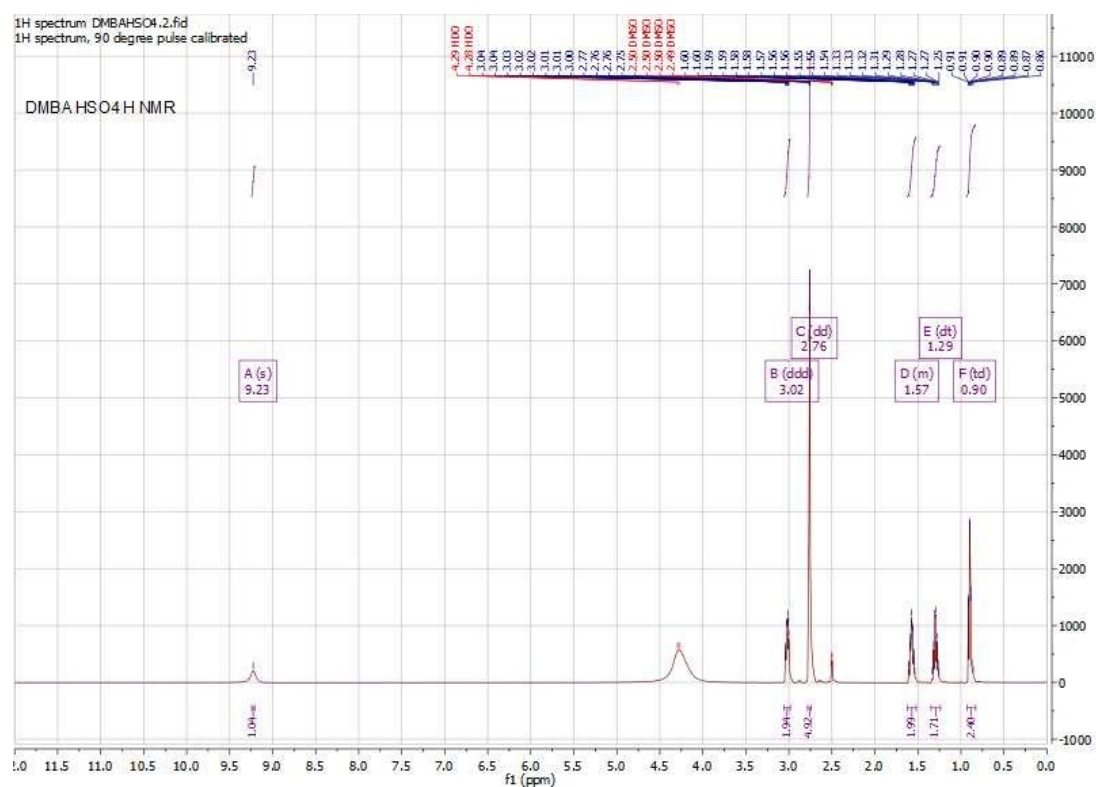
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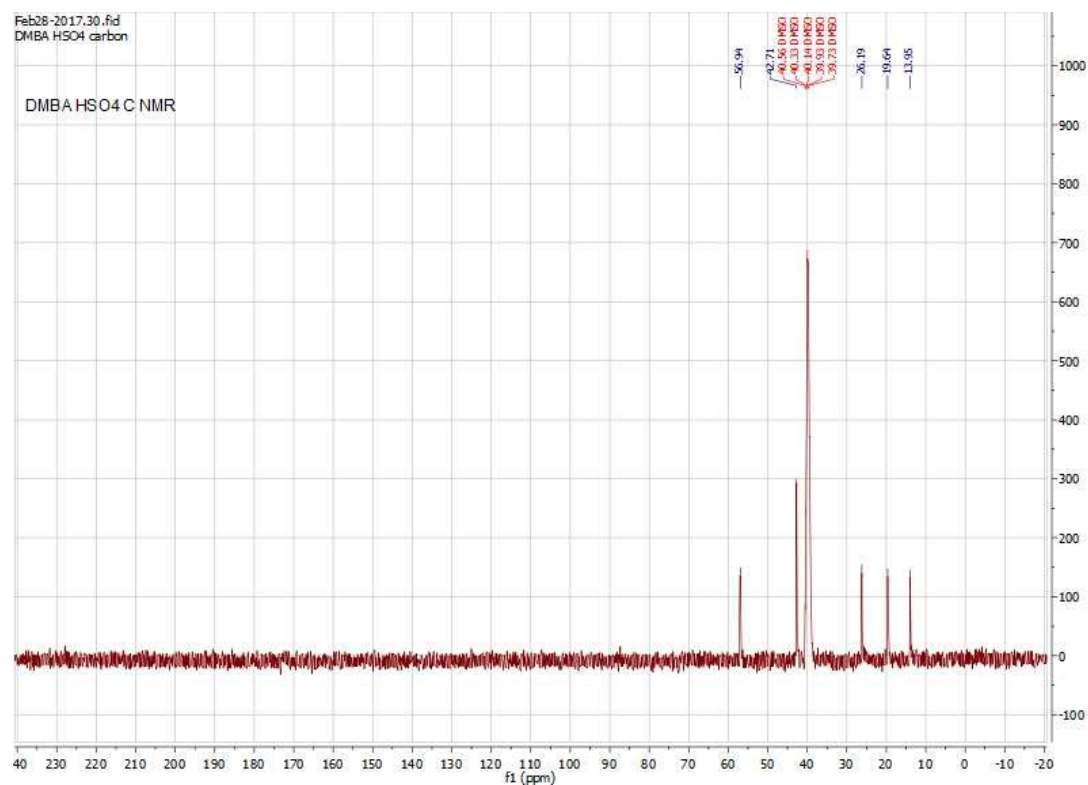
^1H NMR spectrum of $[\text{HC}_4\text{im}][\text{HSO}_4]$



¹H NMR spectrum of [DMBA][HSO₄]

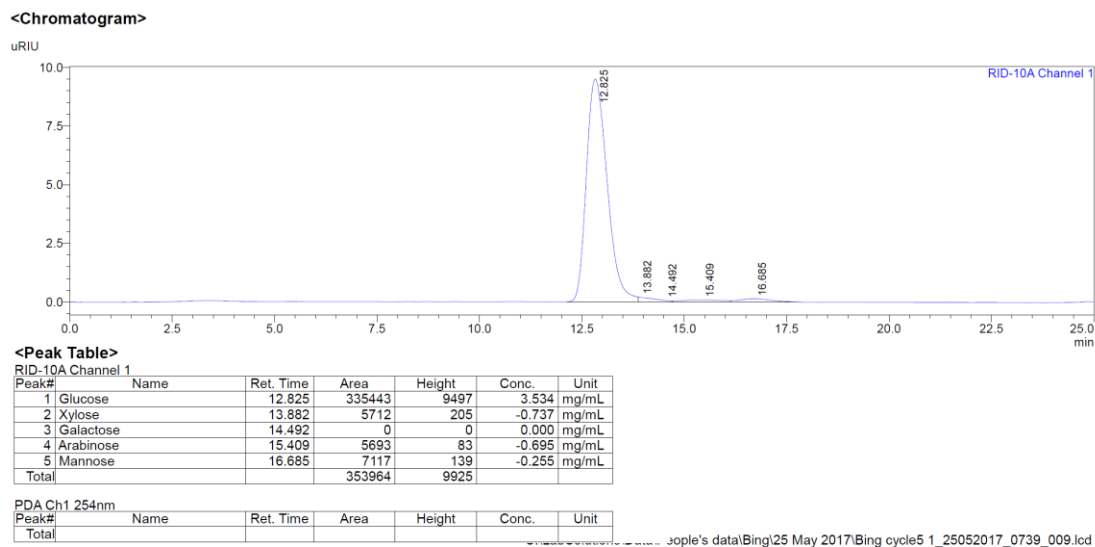
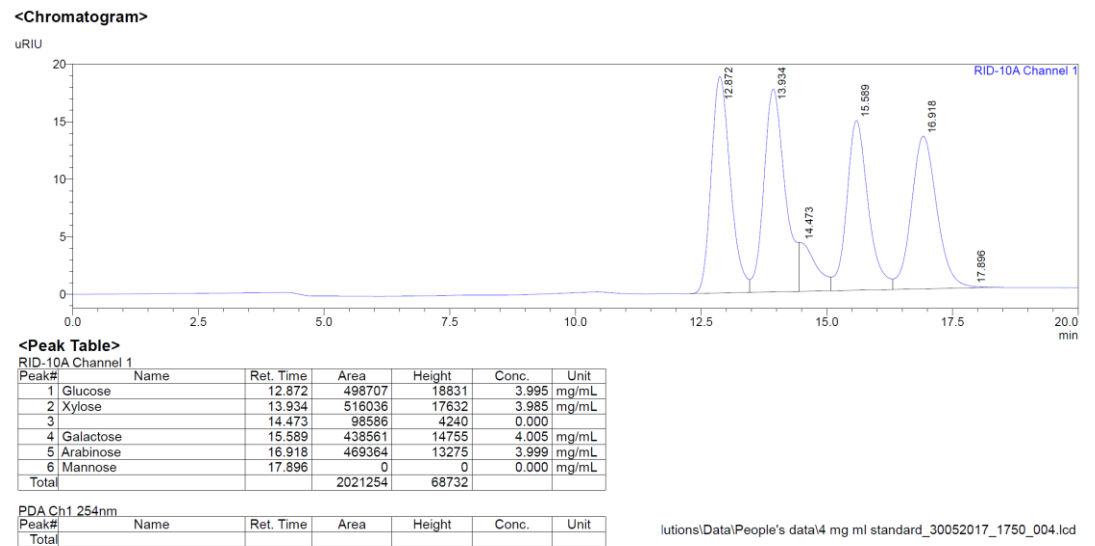


¹³C NMR spectrum of [DMBA][HSO₄]

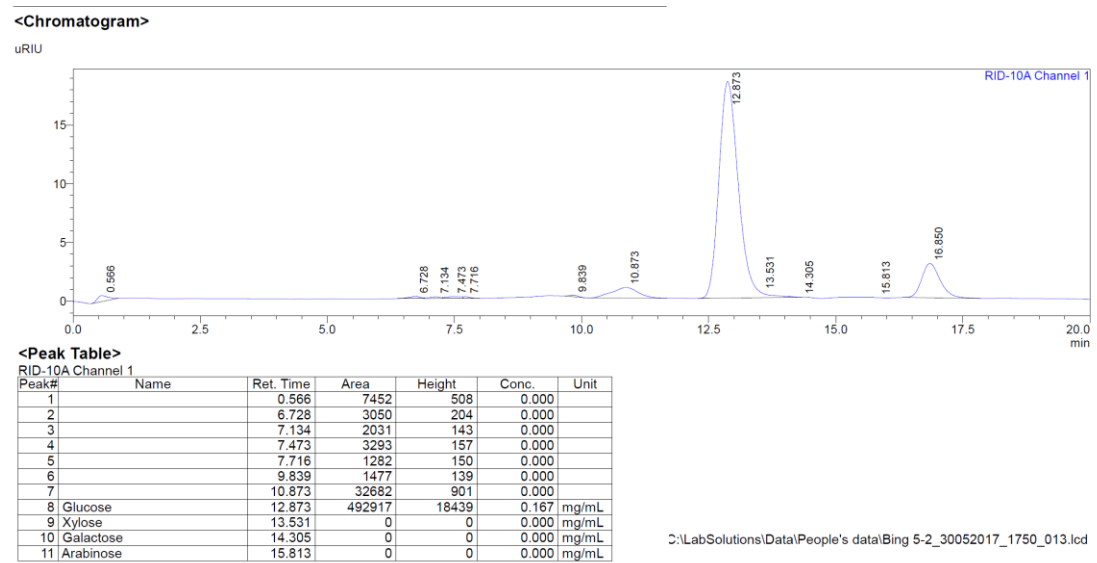


Representative chromatograms

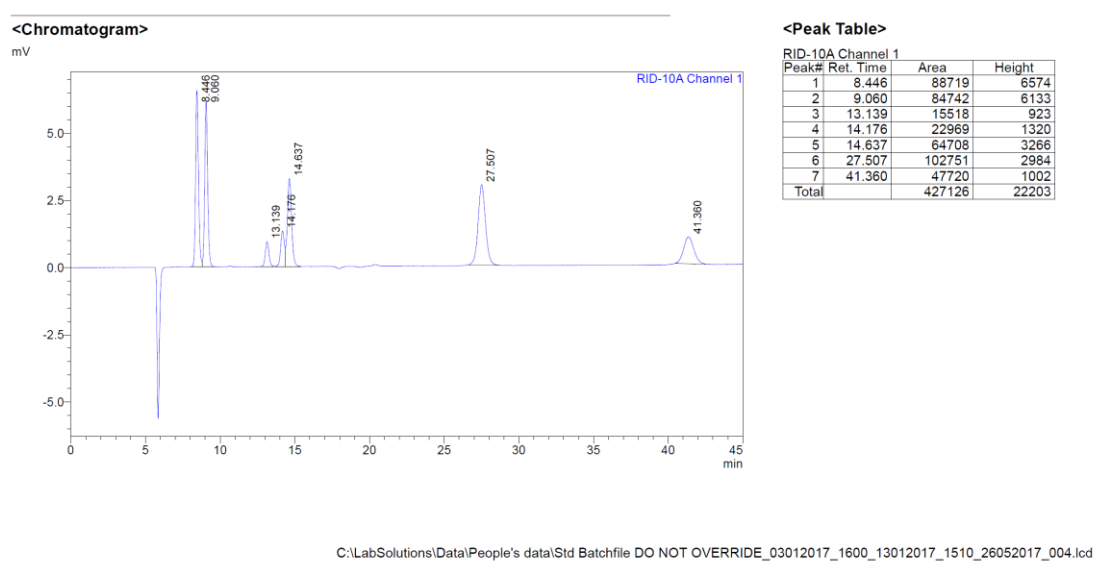
Compositional analysis



Saccharification



Liquor analysis



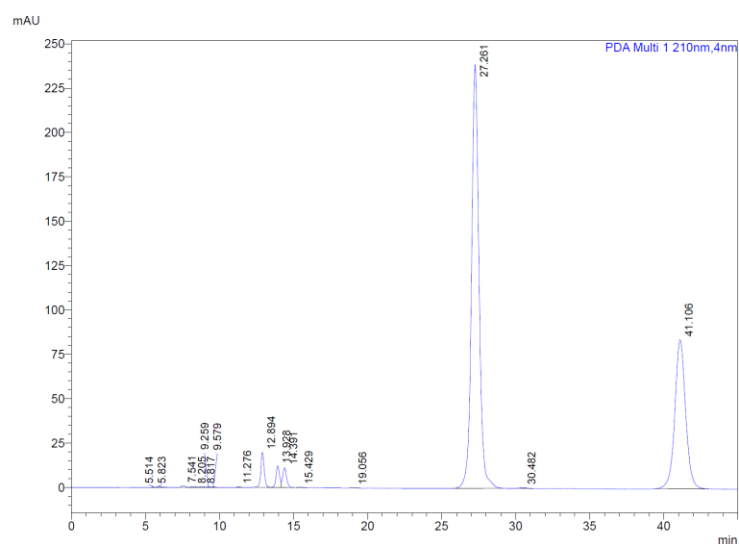
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<Peak Table>

PDA Ch1 210nm			
Peak#	Ret. Time	Area	Height
1	5.514	2333	340
2	5.823	11488	466
3	7.541	14430	902
4	8.205	9019	414
5	8.817	7871	398
6	9.259	3710	295
7	9.579	4143	282
8	11.276	5192	389
9	12.894	314241	19756
10	13.928	200784	12087
11	14.391	203279	11142
12	15.429	5390	320
13	19.056	3259	149
14	27.261	8352577	238844
15	30.482	11972	380
16	41.106	4248326	84049
Total		13398014	370212

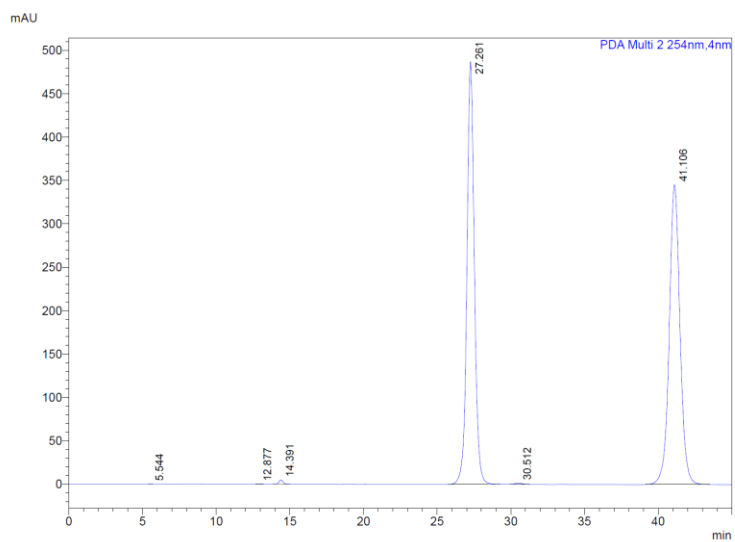
PDA Ch2 254nm			
Peak#	Ret. Time	Area	Height
1	5.544	1324	169
2	12.877	2508	160
3	14.391	91439	4904
4	27.261	17036438	486971
5	30.512	42008	1242
6	41.106	17514676	345421
Total		34688393	838867

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26/05/2017 19:29:07 Page 3 / 4

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26/05/2017 19:29:07 Page 4 / 4

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