STRATEGIC ORGANOSOLV PRETREATMENT TOWARD ENERGY-EFFICIENT SUGAR AND LIGNIN UTILIZATION IN LIGNOCELLULOSE BIOREFINERY

MD KHAIRUL ISLAM

PhD

The Hong Kong Polytechnic University

2021

The Hong Kong Polytechnic University

Department of Civil and Environmental Engineering

Strategic Organosolv Pretreatment Toward Energy-efficient Sugar and Lignin Utilization in Lignocellulose Biorefinery

Md Khairul Islam

A thesis submitted in partial fulfilment of the requirements for
the degree of Doctor of Philosophy

May 2021

CERTIFICATE OF ORIGINALITY

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

_________________ (Signed)

MD KHAIRUL ISLAM (Name of Student)

Dedicated

To

My parents

ABSTRACT

Biorefinery to convert lignocellulosic biomass into bioenergy and chemicals has become inevitable for the sustainable replacement of the petroleum refinery. However, the progress of the technology development has been limited due to the significant variation of biomass recalcitrance and product values, especially lignin, after pretreatment. Therefore, the modern pretreatment concept has broadened its target from cellulose and hemicellulose utilization to lignin valorization. The main technical gaps are due to the side effects of lignin degradation and condensation during thermochemical processes. In this dissertation, three technical routes using different organosolv were designed and tested to facilitate lignin valorization in biorefinery process. N-Methyl-2-pyrrolidone (NMP) was selected first (Chapter-5) for its highest lignin soluble potential and low-cost. The pretreatment resulted in complete removal of hemicellulose, more than 90% lignin removal, and 98% cellulose digestibility using commercial enzyme. 2D-HSQC NMR analysis showed that the fractionated lignin contained 16.4% of β-o-4 linkage, which was more than double of the conventional ethanol pretreatment. To better separate the lignin and hemicelluloses, staged organosolv (OS)-dilute acid (DA) process (Chapter-6) was developed to successfully facilitate high reactive lignin fractionation and solvent recovery. The staged pretreatment yielded net positive energy (1.5 MJ/kg-wood) in biorefinery conversion of palm tree biomass to bioethanol. Finally, pentanol-water biphasic pretreatment approach (Chapter-7) simplified the fractionation of lignin, hemicelluloses, and cellulose in one-pot, while achieving 70.3% of lignin removal and more than 90% enzyme digestibility of substrates. Biphasic pretreatment process preserved approximately 42.5% of β-o-4 linkages due to the physical separation of lignin from acidic environment, resulting in net positive energy of 1.1 MJ/kg of wood residue.

PUBLICATIONS ARISING FROM THE THESIS

Peer-reviewed articles published

1. **Islam, M.K.**, Thaemngoen, A., Lau, C.Y., Guan, J., Yeung, C.S., Chaiprapat, S., Leu, S.-Y. (2021) Staged Organosolv Pretreatment to Increase Net Energy and Reactive Lignin Yield in Whole Oil Palm Tree Biorefinery. *Bioresour. Technol.*, 2021.
2. **Islam, M.K.**, Wang, H., Rehman S, Dong, C., Hsu, H-Y., Lin, C.S.K., Leu, S-Y. (2020) Sustainability metrics of pretreatment processes in a waste derived lignocellulosic biomass biorefinery. *Bioresour. Technol.*, 298: p. 122558.
3. **Islam, M.K.**, Dong, C., Hsu, H.Y., Lin, C.S.K., Leu, S.-Y. (2020) Valorisation of Woody Biomass. Waste Valorisation: Waste Streams in a Circular Economy. Wiley: p.87-108.
4. **Islam, M.K.**, Rehman, S., Guan, J., TSE, H-Y., Yeung, C.S., Leu, S.-Y. (2021) Biphasic Pretreatment for Energy and Carbon Efficient Conversion of Lignocellulose into Bioenergy and Reactive Lignin. Appl. Energy.
5. **Islam, M.K.**, Lau, C.Y., Guan, J., Yeung, C.S., Leu, S.-Y. (to be submitted) N-Methyl-2-pyrrolidone pre-treatment of lignocellulose for high lignin yield and cellulose digestibility.

Conference proceedings

6. **Islam, M.K.**, Rehman, S., Guan, J., Lau, C.Y., Yeung, C.S., Leu, S.-Y. (2020) Pentanol-water biphasic pretreatment facilitates energy-efficient fractionation of lignocellulose (Conference Paper**)**. 12th International Conference on Applied Energy (ICAE2020), 1 Dec.-10 Dec. 2020.

ACKNOWLEDGMENTS

It is my great pleasure to acknowledge some of the wonderful people in the Hong Kong Polytechnic University for their kind support and guidance that I have received to enhance my theoretical as well as practical knowledge related to my research.

First and foremost, I would like to show my deep gratitude, indebtedness, and profound admiration to my honourable and beloved supervisor **Dr. Shao-Yuan Leu**, Associate Professor for allowing me to work in his group at the Department of Civil and Environmental Engineering in The Hong Kong Polytechnic University, Hong Kong. He has provided expert guidance, constant help and positive motivation throughout my research study and to prepare the final dissertation. His devotion to the research work along with his research integrity has taught me to highly appreciate his depth of knowledge and supportive attitude to all his students. His positive motivation, clear vision, and dynamism have always inspired me for the research. I would also like to appreciate his empathy, friendly attitude, and a great sense of humor. It was a great privilege and honour for me to work with this great personality.

I am also highly grateful to Dr. HSU Shu-Chien and Dr. LENG Zhen, Associate Professors of Civil and Environmental Engineering at The Hong Kong Polytechnic University, Hong Kong, for their kind co-operation and valuable feedback related to research work. I am highly grateful to the "Research Institute for Sustainable Urban Development" of The Hong Kong Polytechnic University for their funding to support my research work.

I would like to extend my warm gratitude to all our group members for their suggestions and friendly co-operation throughout my research work. Particularly, I would like to appreciate Dr. Chi Shun Yeung and Dr. Dong Chengyu for their suggestions and practical lessons in the laboratory at the beginning of my research work. Furthermore, my special appreciation goes to all our lab technicians for their continuous assistance and kind support during the experiment in the laboratory. I want to convey my heartiest facilitation to all my friends that I have made during my postgraduate study at The Hong Kong Polytechnic University. They helped me a lot to overcome bad times and occasional frustration with the strength of friendship and endless love.

Finally, I am highly indebted to my beloved parents for their unbelievable sacrifices to accomplish my postgraduate study in Hong Kong.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Bt	1-Butanol
CED	Cellulose enzymatic digestibility
CHF	Combined hydrolysis factor
CNCs	Cellulose nanocrystals
CSF	Combined severity factor
DMAc	Dimethyl acetamide
DMSO	Dimethyl sulfoxide
DA	Dilute acid
D_p	Degree of polymerization
EHS	Environment and health safety
EFB	Empty fruit branch
ETG	Ethylene glycol
EtOH	Ethanol
FAO	Food and Agriculture Organization
FPU	Filter paper activity
G	Guaiacyl
GHG	Greenhouse gas
GPC	Gel permeation chromatography
GVL	γ-Valoractone
H	p-Hydroxyphenyl
HPLC	High-performance liquid chromatography
KJ	Kilo joule
LCA	Life-cycle assessment
MJ	Mega joule
M_n	Number average molecular weight
M_W	Weight average molecular weight
NREL	National Renewable Energy Laboratory
NFCs	Nano fibrillated celluloses
NMP	N-Methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
OS	Organosolv

Pt	1- Pentanol
R_0	Hansen distance
RED	Relative energy difference
S	Syringyl
SEM	Scanning electron microscopy
SPORL	Sulfite pretreatment to overcome recalcitrance of lignocellulose
THF	Tetrahydrofuran
TKN	Total Kjeldahl nitrogen
δ_D	Dispersion force
δ_P	Dipolar force
δ_H	Hydrogen bonding force
1,4-BDO	1,4- Butanediol
2DHSQC	Two-dimensional heteronuclear single quantum coherence

CHAPTER 1. INTRODUCTION

Fossil fuels are the driven force of modern civilization. In the last one and half-century, petroleum and petrochemical-derived products have led to a sensational advancement in industrial and technological development since industrial revolution. Many researchers in the current centuries have predicted the maximum production and reserve about petroleum, which is still the principal source of energy and chemicals (Aguilera et al., 2009; Bentley, 2002). The increasing demand for petroleum combined with the rapid depletion of the natural reserves is putting more pressure on petroleum products. Moreover, the extreme consumption of fossil fuels has increased greenhouse gas (GHG) emissions which enhance global warming (Rosa et al., 2009). Both the increasing demands and environmental factors have strengthened the desire for sustainable and renewable alternatives for energy and chemical feedstocks.

Lignocellulosic biomass having zero net carbon emission is the most promising feedstocks for a sustainable biorefinery to produce fuels and chemicals (Banerjee et al., 2016; Berlin, 2013). The concept of biorefinery is analogous to a petroleum refinery, capable of producing building-block chemicals from naturally abundant lignocellulosic biomass. The building-block chemicals can be used as the platform to produce biofuel, desired chemicals, and materials through different biochemical process. Global tree has the ability to produce 4.4 billion hectares of canopy indicating its potential to mitigate climate changes. Global forestation can restore more than 200 billion tons of carbon at matured plants, which can reduce 25% of the total atmospheric carbon. It is estimated that approximately 10 metric tons of dry biomass are producing per hectare which can be regarded as the abundant sources of renewable carbon to produce biofuels and biochemicals (Bastin et al., 2019).

In order to utilize biomass in biorefinery, two major conversion processes such as thermo-chemical and bio-chemical processes are conceivable (Sikarwar et al., 2016). The thermochemical route includes pyrolysis, gasification and catalytic conversion to produce syngas, heat, biofuel, fertilizer, and biochar (Din & Zainal, 2016). Syngas is the most common products in thermochemical processes which is used to produce other desirable chemicals using Fischer-Tropsch and other synthesis pathways (Sheldrake & Schleck, 2007). However, the biochemical conversion uses comparatively a mild condition to produce building-block chemicals; is one of the most promising technologies in recent time.

The biochemical conversion of biomass in the biorefinery combines a series of complex operations, starting from the pretreatment of biomass to its conversion into building-block chemicals. Pretreatment is the most energy-intensive process which determines the efficiency of downstream processing. Lignocellulose pretreatment is the key step of biochemical conversion to overcome its inherent recalcitrance which is the main bottleneck of biorefinery process. Biomass pretreatment enhances the reactivity and accessibility of carbohydrates to increase the sugar yields due to structural modifications, and the resulting carbohydrates and lignins fractions are used to convert into the value-added products through different catalytic procedures (Cheng et al., 2015a). The modern pretreatment concept has widened its target from carbohydrate sugar utilization to whole biomass (lignin) valorization, but significant technical gaps still remain mostly due to the unwanted side effects of lignin degradation and condensation during this thermochemical process. Due to the structural complexity of lignin, several condensation products formed during pretreatment depending on cooking conditions and types of lignin/biomasses. Lignin condensation is the long-lasting problem in biorefinery which reduces the potential of fractionated lignin to convert into valuable aromatic chemicals. Lignin sub-units are connected by aryl ether (β-o-4) linkages. During pretreatment, lignin is degraded and dissolved through the cleavage of β-o-4 linkage. However, the cleavage of β-o-4 linkage produced benzylic cation which can undergo condensation reactions in aqueous acid medium to form Hibbert ketone or aldehydes. The benzylic carbocation can also undergo nucleophilic substitution reaction with the electron-rich aromatic units of lignin to form condensed C–C structures (Lancefield et al., 2017). Though conventional organosolv pretreatment provides the option of lignin recyclability, lignin also undergoes non-productive condensation reaction which limits the potential application of fractionated lignin. In addition, the fractionation of dissolved sugars and lignin from pretreatment liquor is highly energy intensive for conventional organosolv process. Therefore, the development of a mild and energy efficient pretreatment strategy to fractionate lignocellulose components with minimal undesirable side reactions is promising to replace the conventional delignification process.

In this dissertation, three alternative routes were proposed and investigated to elucidate the lignin dissociation and condensation chemistry, which further suggest the possible way of process design and operation (**Fig.1.1**). The principles of the pretreatment and key concept of proposed pretreatment processes have been provided as follows, which include the utilization of a new organosolv reagent, a staged pretreatment, and a phase separation pretreatment approach.

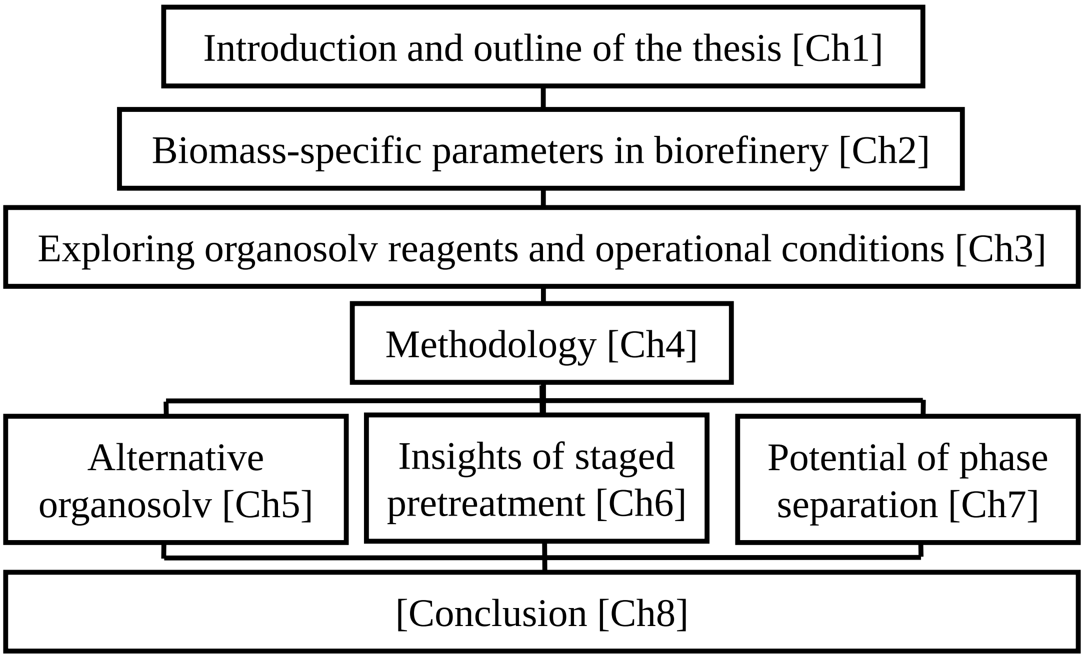


Fig.1.1. Key features and chapter arrangement of the dissertation.

Lignocellulosic biomass is a complex biological product with which its physiochemical structure varied significantly among plant species, organs, parts, and/or even growing environment. Chapter-2 provides a detail on the generation of woody biomass, wood chemistry, and potential values of woody biomass involved in a biorefinery.

Urban biorefinery differs from the widely discussed biorefinery process with the biomass feedstock. The feedstock of urban refinery is mainly contributed by municipal solids wastes, which may include disposed wood furniture, wooden formwork, yard waste, trimmings, or even food processing products. In addition, natural disaster i.e., typhoon produces a huge amount of woody biomass in urban areas. In Hong Kong, approximately 90,000-120,000 tonnes of wood product waste have been disposed of the landfills every year (HKEPD, 2012) which occupies significant space in the landfilling sites. Using urban waste as biorefinery may be more relatively more sustainable than using agriculture or forestry residues as biomass feedstock but is with the challenges of high difference in compositions and species contributed from different waste generation sources.

Some key metrics were established to clarify the biorefinery performance, operational profile, water-and-energy nexus, and health-safety factors for sustainable operation of an urban biorefinery (Chapter-3). The parameters were developed to obtain a high pretreatment yield with minimal inputs of fractionating reagents. The parameters also explained the operational challenges and health-safety factors of the biomass pretreatment especially when practicing the techniques in highly populated cities like Hong Kong. The mechanism of different pretreatment techniques also discussed along with its importance to fractionate and valorize building block chemicals of biomass. The experimental techniques applied throughout this research were detailed in Chapter-4.

A new pretreatment process using N-Methyl-2-Pyrrolidone (NMP) was designed (Chapter-5) based on the developed metrics to treat *Acacia Confusa* wood residues, available biomass with significant bark content, and extractives. The chemical composition was determined before and after pretreatment to determine the efficiency of NMP pretreatment for lignocellulose fractionation. 2D-HSQC and GPC analysis were carried out to determine the integrity of fractionated lignin.

Based on distinguished dissociation mechanisms of lignin and hemicelluloses, an innovatively staged organosolv (OS)-dilute acid (DA) pretreatment process was investigated to achieve a whole oil palm tree residues conversion (Chapter-6). The integrated mass and energy balance were carried out to determine the energy and product benefits of the staged organosolv process. The hydrolysis and fermentation results were used to quantify the energy footprint of the process in the production of a benchmarked biofuel (ethanol) and high-value lignin in one hectare of oil palm farm after one plant life cycle.

A co-solvent pretreatment approach was developed to simplify the fractionation of lignin and hemicelluloses in one pot (Chapter-7). A series of pretreatment and enzymatic saccharification experiments were conducted to determine the pretreatment efficiency of the pentanol-water pretreatment system. The fractionation efficiency and involved mechanisms were investigated using a pentanol-water biphasic system on *Acacia Confusa* wood residues. The effect of physical separation of immiscible aqueous and organic phase was assessed by quantifying energy consumption in fractionation and the amount of β-aryl ether (β-O-4), resinols (β–β), and phenyl-coumaran (β-5) linkages in the fractionated lignin.

CHAPTER 2. GENERATION OF WOODY BIOMASS AND ITS STRUCTURE

2.1. Introduction

Woody biomass derived from municipal wastes, forestry, and agricultural residues is an important source of renewable energy and chemicals. According to Food and Agriculture Organization of the United States (FAO), the global production of wood residues and chips between 1974 and 2018 has increased from 1.85×10^4 to 5.69×10^8 tons (2020). Most of the woody biomass has been generated in Asia (51%), America (20%), and Europe (27%), which is derived from different sources, *e.g.*, woody product wastes (**Fig.2.1(a)**) and yard wastes (**Fig.2.1(b, c)**). It contains different parts of woody plants, such as the trunks, branches, and leaves. Living woody plant cells are generated from mitosis division of the apical and lateral meristems (Evert, 2006). The apical meristems are located in the tips of the stems, roots, and branches and responsible for the primary growth, *i.e.*, lengthening of the plants; and the lateral meristems present under the bark of roots, stem, and branches are responsible for the secondary growth, *i.e.,* thickening of the plant. The stem, or the trunk, is currently the most useful part of the woody plant. The tree trunk consists of four segments, *i.e.,* bark, phloem, vascular cambium, and the secondary xylem (Harada & Côté Jr, 1985). Bark protects the plant from mechanical and biological attacks. The thin layer of cells between the phloem and the xylem is the vascular cambium, which divides the wood trunk into xylem in the inner side and phloem on the outer. Phloem is the living tissue that stores and transports nutrients to different parts of the plants, and the xylem is produced by the multiplication of meristematic cells in the vascular cambium (Lachaud et al., 1999).

Fig.2.1. Examples of woody biomass derived from different sources (a) woody product wastes; (b) yard wastes; and (c) trimmings (Islam et al., 2020a).

2.2. Properties and classification of woods

Lignocellulosic biomass represents the most abundant natural resource originating from woody (Yang et al., 2019) and herbaceous plants (De Bhowmick et al., 2018). It is a complex natural biopolymer and mainly composed of cellulose (35 to 50%), hemicellulose (20 to 35%), and lignin (5 to 30%) with a significant difference in composition as well as properties for different species especially when they are derived from municipal waste (**Table 2.1**) (Chandel et al., 2018). The physiochemical properties and chemical compositions vary significantly with regard to average sizes, plant species, and parts, of which the related impacts are discussed in the following sections.

Table 2.1. Chemical compositions of selected lignocelluloses derived from urban wastes (samples collected from timber wastes recycling facilities in Hong Kong)

Type of wastes	**Form work**	**Furniture**	**Wood pallet**	**Christmas tree (Pine)**	**Eucalyp tus**	***Acacia confusa***	**Grass**
Glucan	41.1 %	32.9 %	40.7 %	41.8 %	40.2 %	39.8 %	28.4 %
Xylan	3.1 %	9.3 %	4.3 %	3.7 %	13.5 %	14.9 %	15.2 %
Galactan	3.4 %	0.0 %	3.5 %	4.6 %	0.5 %	2.8 %	3.0 %
Mannan	13.3 %	3.8 %	12.8 %	12.1 %	0.8 %	3.4 %	2.4 %
Kraft lignin	24.5 %	22.7 %	23.6 %	25.2 %	23.3 %	22.2 %	16.9 %

Woody materials are commonly classified based on its seed structure and reproduction system of the plants **(Fig.2.2)**. Hardwood is known as the angiosperm originates from the deciduous trees, *e.g.*, oak, maple, eucalyptus, and beech. Softwood or gymnosperm originates from coniferous trees, *e.g.*, pine, spruce, Douglas-fir, and redwood. Hardwood and softwood vary in appearance and have different functional plant cells. In softwood, approximately 90% of the wood volume comprises tracheids; which transports liquid and provides mechanical support to the tree. In hardwood, fibres give mechanical support, and vessels transport liquid throughout the plant. The transformations and storage of nutrients are performed by parenchyma cells for both softwood and hardwood (Daniel, 2009).

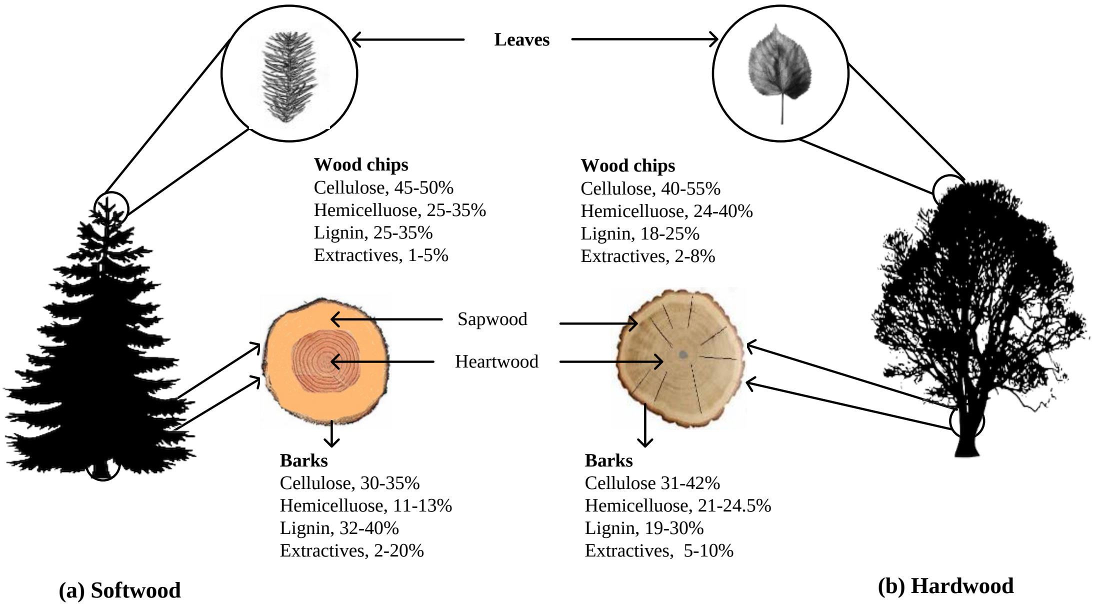


Fig.2.2. Structure and chemical composition of softwood and hardwood (Islam et al., 2020a).

Wood consists of elongated cells (Fujita & Harada, 2000) in the longitudinal direction of the stem and grows radially as concentric rings. The inner part of the wood ring is heartwood, and the outer is sapwood, of which the types and contents of basic chemicals are provided in **Fig.2.2**. Heartwood consists of entirely dead tissues and provides mechanical support to the trees. The dead cells in heartwood contain deposited extractives, which improve biological resistance and darkness of the heartwood. Transportation and storage of water, mineral, and nutrients take place in the sapwood although most of the cells in sapwood are dead. The living cells of sapwood protect against microbial attacks. When the living cells in sapwood die (aging), the sapwood is gradually converted into heartwood.

2.3. Plant cell wall structure and chemistry of lignocellulosic biomass

The plant cell wall is a three-layered structure consisting of primary wall, secondary wall, and middle lamella **(Fig.2.3)**. The primary cell wall entirely consists of cellulose skeleton crosslinked with glycan, and two types of links are found in the primary wall (Li et al., 2016). Type I wall is found in dicotyledonous plants and is composed of equal amount of glucan and xyloglucan located on a pectin matrix. Type II wall is found in grasses and cereals, and is formed by glucuronoarabinoxylan and glucan (Rihouey et al., 2017). The secondary wall is formed with three sub-layers, *i.e.*, outer, middle, and inner lamella of which cellulose and hemicelluloses are embedded and distributed differently to the sublayers (Rihouey et al., 2017). Cellulose and hemicellulose have similar distribution tendency and their concentrations increase from outer to inner lamellae (Höfte & Voxeur, 2017). Lignin is found dominantly in the outer portion of the middle lamellae and the lignin content of the lignocellulose matrix reduces with the increasing distance from the middle lamellae (Welker et al., 2015). The cell wall compositions are the characteristic features of living plants, which are crucial for their survival, metabolism, and degradation (Bacete et al., 2018). In addition to the cell wall composition, structural features of cellulose such as crystallinity index and degree of polymerization are distinctively affecting enzymatic digestibility of biomass (Xu et al., 2019). The crystallinity index is used to demonstrate the proportion of crystalline material in lignocellulose. High crystallinity reduces the accessibility of cellulase to cellulose surfaces and thus reduces substrate enzyme digestibility of lignocellulose (Tu et al., 2017). Degree of polymerization negatively correlated with the hydrolysis of lignocellulose. The reduction in cellulose degree of polymerization remarkably increases biomass digestibility due to the increase of reducing ends in cellulose chain (Cheng et al., 2018b). The harvesting of plants with nitrogen fertilizer supply reduces crystallinity, degree of polymerization, and silicon accumulation of biomass and thus increases the digestibility during hydrolysis (Sun et al., 2017). This provides insight into the sustainable production and utilization of agricultural biomass for the production of energy and chemicals.

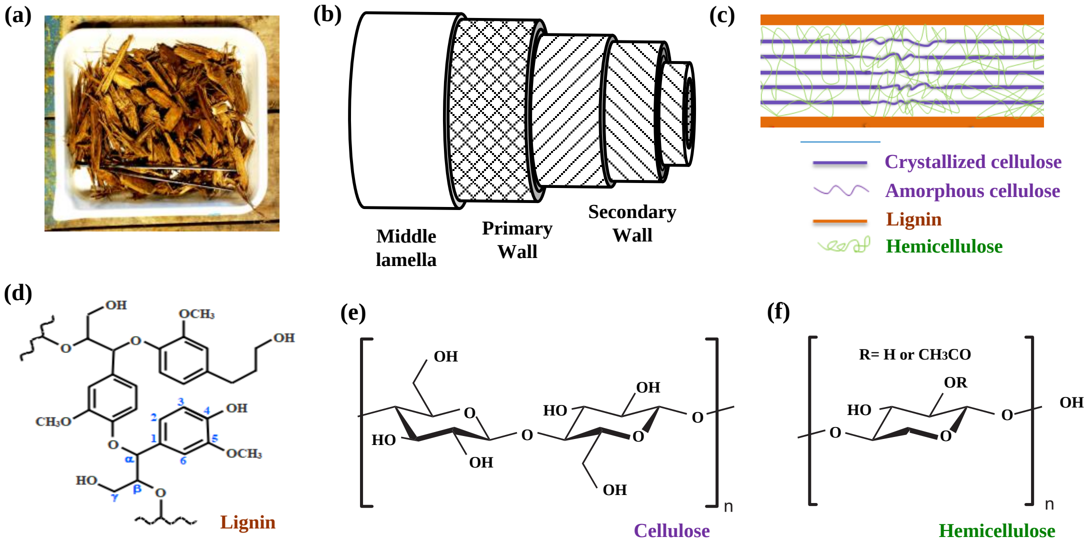


Fig.2.3. Schematic illustration of the physicochemical structure of woody plant cell wall at various scale; (a) wood chips; (b) cell wall structure; (c) chemical composition of the secondary wall; (d) basic structure of lignin; (e) cellulose; and (f) hemicellulose.

Wood is comprised of various heterogeneous chemical constituents, of which cellulose, hemicellulose, and lignin are the main constituents. Those building block chemicals are heterogeneously distributed throughout the wood cell walls **(Fig.2.3)**, and the compositions are varied significantly among different species. Although the cellulose content in hardwood and softwood are almost same; the softwood contains more lignin and less hemicelluloses than that of hardwood (Alén, 2011; Kibblewhite & Brookes, 1976). Low molecular weight compounds such as extractives and some inorganics are nonstructural constituents of wood.

2.3.1. Cellulose

Cellulose is the most abundant and widely applied biopolymer in nature. Glucose monosaccharide is the basic unit of cellulose and is connected with 1-4, β-β-glucosidic linkage to form a large polymer. The degree of polymerization of wood cellulose molecule ranges from 800 to 10,000 (Klemm et al., 2005) which is about 4-5 times higher than the artificial polyester and nylon 66 polymer (Papadopoulou et al., 2016). This high degree of polymerization provides cellulosic fibre superior tensile strength and toughness for advanced application. Cellulose nanocrystals (CNCs) and nano fibrillated celluloses (NFCs) have high strength and stiffness, and thus can be used to enhance the gas barrier properties of film and the mechanical strength in paper-based products (Osong et al., 2016). The linear cellulose molecules have a strong tendency to form intra and intermolecular hydrogen bonds due to the presence of strong electronegative oxygen atoms, making this hydrophilic biopolymer an outstanding candidate for cleaning and packaging. The bundles of cellulose molecules aggregate to form microfibrils, a basic unit of cellulose fibres. Cellulose fibres are formed by crystalline, highly ordered arrangement of microfibrils, or amorphous, less ordered arrangement of microfibrils (Xiang et al., 2016). This characteristic offers cellulose a lightweight fibre with excellent tensile strength (Isogai, 2013), *i.e.,* UV curable acrylic resin, poly-lactide, polyethylene, poly polypropylene, polystyrene, glass fibre, carbon nanotube, phenol-formaldehyde resin, latex, nano clay, and paper. The strong hydrogen bonding also imparts the cellulose molecule's high tensile strength and insolubility in most of the solvents. However, the cellulose is soluble in a strong mineral acid solution in which cellulose molecules are completely hydrolyzed to glucose.

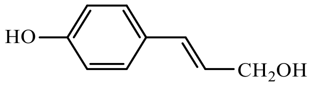
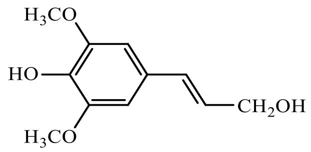
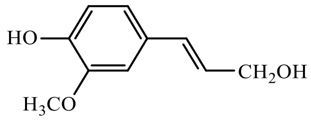
2.3.2. Hemicelluloses

Hemicelluloses are hetero polysaccharides of pentose and hexose sugars. Hemicelluloses are less ordered than cellulose and more easily dissolved in acids. The degree of polymerization of hemicelluloses is usually less than 300. This component easily hydrolyzes to monomer sugars in dilute mineral acids at low temperature (Zhang et al., 2006). The monomers are easily digestible and fermentable to form into desired products by various microorganisms (Kumar et al., 2018). Lower energy consumption in the fractionation of hemicellulose sugars is a key benefit to produce platform chemicals like succinic acid, levulinic acid, xylitol, fumaric acid, malic acid, 2,5-furan dicarboxylic acid, 3-hydroxy propionic acid, aspartic acid, glucaric acid, glutamic acid, and arabinitol (Kumar et al., 2018). There are some basic differences between the hemicellulose structure in the hardwood and softwood. Hardwood hemicellulose is mainly composed of glucuronoxylan and glucomannan, while softwood hemicelluloses are mainly galactoglucomannan and arabinoglucuronoxylan. Combinations of different types and amounts of hexoses and pentoses show different digestibility or fermentability during fermentation in a biorefinery (Wang et al., 2014).

2.3.3. Lignin

Lignin is amorphous, hydrophobic biopolymer constructed by three monolignols (**Table 2.2**), *i.e.,* p-hydroxyphenyl (H-lignin), syringyl (S-lignin) and guaiacyl (G-lignin) which is the main renewable source of aromatic chemicals (Sjostrom, 2013). Lignin has many remarkable properties, like high resistance to chemical, biological, and thermal degradation rather than being only a low-cost fuel. The presence of multiple functional groups in the matrix makes lignin a promising alternative for carbon-based materials (Ho-Yin et al., 2019). Lignin exhibited superior functionality in UV absorbance, amphiphilicity, and anti-oxidation (Tian et al., 2017). The application of lignin and its derivatives in adhesives and green composites has been reported recently (Espinoza-Acosta et al., 2018; Faris et al., 2016). Owing to its low cytotoxicity and pH controllable properties; lignin has also been considered for bio-imaging and drug delivery system (Dai et al., 2017). The exact linkages among those building block components are very complex and have not yet been completely decoded (Özparpucu et al., 2017), but a few major linkages have been confirmed through investigating the dissociated monomers after digestion. β-aryl and α-aryl ether linkages are the representative chemical linkages in the plant cell wall, accounting for at least 43-50% and 6-8% (in number) in softwood, respectively (Li et al., 2015). The potentials to cleave the two linkages in or outside the phenolic structures are process specific.

Table 2.2. Lignin monomers and their composition.

Name	Structure	Softwood	Hardwood
H-lignin		< 5	0-8
S- Lignin		Trace amounts	46– 75
G-Lignin		> 95	25 – 50

Lignins are generally classified as hardwood lignin, softwood lignin, and grass lignin. Hardwood lignins are composed of S and G units which are originated from trans-sinapyl alcohols and trans-coniferyl alcohol respectively. Softwood lignin, on the other hand, are mainly composed of guaicyl unit which comes from the trans-coniferyl alcohol. P-hydroxyphenyl unit of grass lignin is derived from trans-p-coumaryl alcohol and foregoing two precursors. Native lignins are insoluble in water and acids, but isolated lignins show the maximum solubility in different organic solvents such as dimethyl sulfoxide, acetone, dioxane, tetrahydrofuran etcetera. Isolated lignins can be classified as milled wood lignin, kraft lignin, alkali lignin, and enzymatically liberated lignin.

2.3.4. Extractives

Extractives are a wide variety of low molecular weight organic compounds which are present in a small amount but with high functionality, which makes it a high potential for different uses. They have been used in recent years for pharmaceuticals (Constable et al., 2007; Nair et al., 2015), food additives (Moure et al., 2006), and chemical reagents (Eriksson et al., 2018; Mattos et al., 2016; Miranda et al., 2017). Extractives are bio-synthesized as secondary metabolites and protect plants from different insects and microbial attacks (Ayvaz et al., 2010; Gierlinger et al., 2004). Although wood contains less than 10% extractives, they greatly contribute to the physical properties of wood (*e.g.,* colour and odour). Extractives content determines the tensile strength, density, elasticity, permeability, flammability, and wettability of wood (Jankowska et al., 2018; Taylor et al., 2007). The major chemicals of wood extractives can be grouped as terpenoids, phenolics, alkaloids, fats, and waxes. The composition of extractives largely varies in different wood species and in the different parts (i.e. stem, leaves, barks, and roots) of the same wood species. The functions of extractives differ significantly, and different extractives can be discovered in different morphological areas of the plant. For instance, resin acids in the resin canals of plants protect wood from microbial attack; fats and waxes exist in ray parenchyma tissue and act as a power source of woody plants. The majority of the wood-based extractives are low boiling point organic compounds, while a small amount of inorganic extractives has also been reported.

The plant extractives are now being used for the production of flavors, fragrances, oil, and pharmaceutical preparations (Gandini, 2008). Paclitaxel is a plant extractive used for the treatment of different types of cancer (Bernabeu et al., 2016; Foglietta et al., 2018; Tanaka et al., 2017). A significantly anticancer activity of keratin nanoparticles-based paclitaxel in breast cancer models has been reported recently (Bernabeu et al., 2016). Paclitaxel loading on keratin nanoparticles increases the solubility and drug delivery of paclitaxel. The traditional use of turpentines as a solvent is substituted by cheap petrochemicals. Terpene and terpenoids can be used as a feedstock to produce rose alcohols, linalool, and nerol. Linalool is the key intermediate during the production of vitamin E (Langenheim, 2003). Turpentines form low molecular weight polymer having better flexibility and this polymer can be used in papermaking, adhesives, printing inks, and coating (Chen, 1992). Yumrutas reported that turpentines can be used as an additive for gasoline (Yumrutaş et al., 2008). Plant tannins contain molecules of complex phenolic structures obtained mainly in the bark and heartwood of the trees. Tannins have been used in leather industry for vegetable tanning process; but the more convenient synthetic tannin replaced the lengthy use of vegetable tannins (Pizzi, 1983).

CHAPTER 3. SUSTAINABILITY METRICS OF PRETREATMENT PROCESSES IN A WASTE DERIVED BIOMASS BIOREFINERY

3.1. Introduction

The global demand for fuels and chemicals is reaching a critical situation; hence it is with urge to establish new technique in replacing the fossil fuel-based products (Clark, 2019). High energy-dense fossil fuels contribute to 80% of the world's primary energy consumption (Raud et al., 2019), and the relationship between increase of atmospheric carbon dioxide and global warming has been widely recognized (Mercure et al., 2018). Although the current commitments of global leaders and their strategies for energy security to tackle carbon emissions are in progress, our sustainable future has not yet foreseen. The complication between the development of green energy technology and oil price toward circular economy has hindered the process of green energy policy. In addition, the consequence of increasing reliance on fossil fuels, resulting in high energy prices, may also affect the food market and many industries (Taghizadeh-Hesary et al., 2019).

The green biorefinery aims to shift the paradigm of fossil fuels utilization gradually to biofuels and other bio-products. The energy comes from natural and renewable sources i.e., sunlight, wind, biomass, or water that don't have any adverse effect on the environment is regarded as "Green energy". The energy from waste lignocellulosic biomass is green since it uses naturally accumulated atmospheric carbon to produce energy. It is not also associated with atmospheric pollution and deforestation due to the use of agricultural, forest, and other waste residues. The European Union has emphasized the use of biofuels, such as biogas, biodiesel, bioethanol, biomethanol, biohydrogen, and vegetable oil as the alternative fuels to promote green transportation systems (Bórawski et al., 2019). Among many green energy routes, lignocellulosic biomass is the most abundant natural biopolymer and has zero net carbon emission, hence it has become a promising feedstock for sustainable production of fuels and chemicals that are not replaceable through other renewable energy sources (Baral et al., 2019).

Being a new energy and chemical platform that is still under development, many concerns of biorefinery have been raised regarding its full-scale application, such as environmental impacts, stability, and diversification of uses (Chandel et al., 2018). The biofuels dispute was initially based on the advantages and disadvantages of various generations of biofuels. The first-generation biofuels were rapidly developed due to their high sugar and oil contents in the raw materials, but it also created the well-known food versus fuel debates (Callegari et al., 2020). Green biorefinery which utilizes waste product-based feedstock is now the expected technology and the major focus of this study.

Biorefinery is an integrated process of a few unit operations (**Fig.3.1**), such as pretreatment, saccharification, fermentation, and distillation. Based on various design principles, additional processes may be included in the system, such as evaporation, size reduction, and solvent recycling. Green chemistry serves as the fundamental of process design and development to achieve sustainability in the process (Keijer et al., 2019), of which environmentally friendly parameters need to be carefully evaluated.

The quantitative factors of the “greenness” of the biorefinery techniques have not been clearly identified. Many research and review articles have attempted to clarify the concept of lignin chemistry and valorization (Cheng et al., 2018a); the advances, performances, and bottlenecks of different pretreatment techniques (Sankaran et al., 2020); the conceptual principal of green solvents (Soh & Eckelman, 2016); strategies for costs reduction (Ubando et al., 2019); and challenges to produce biofuels and renewable chemicals (Raud et al., 2019).

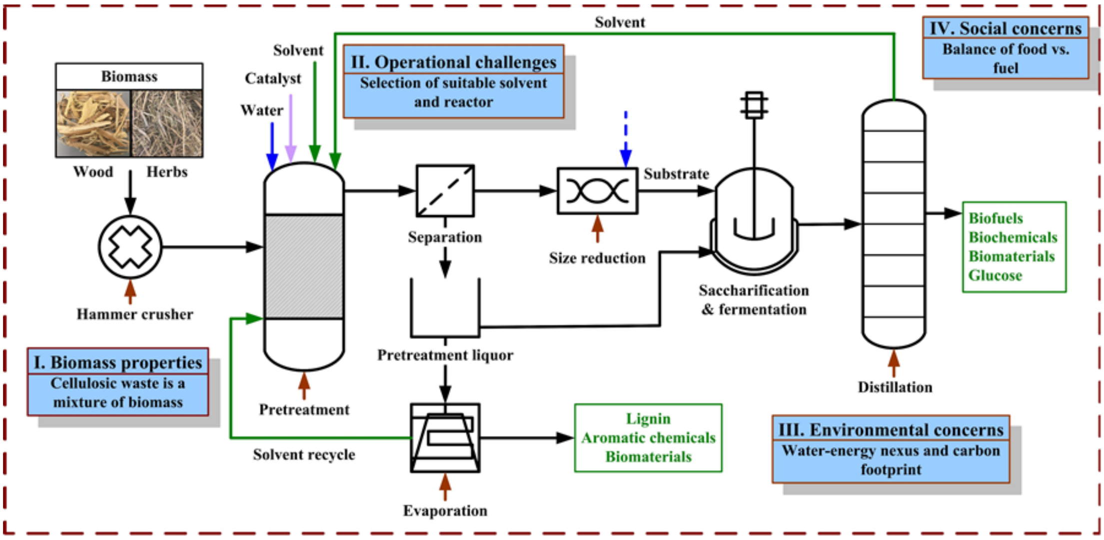


Fig.3.1. Conceptual diagram of an integrated biorefinery and proposed sustainability challenges on (I) biomass properties, (II) operational challenges, (III) environmental challenges, and (IV) social concerns (Islam et al., 2020b).

This chapter provides a comprehensive review about the concept of green production in urban biorefinery with a special focus on the green metrics of pretreatment reagents and processes. The parameters are summarized on a quantitative basis to support future research or process development of feedstock characterization, selection of reagents, requirements of technology background, water-energy nexus, sensitivity to the environment, and social impacts.

3.2. Design concepts of pretreatment techniques

Biorefinery starts with fractionation of biomass to dissolved lignin, hemicelluloses, and solid substrate. Biomass fractionation (known as pretreatment) is carried out to enhance the reactivity and accessibility of carbohydrates with slight modifications in the structure to increase the sugar yields (Leu et al., 2012; Zhao et al., 2017). The resulting sugar and lignin are converted to the desired products through different catalytic procedures. Cleaving of ether and ester linkages are the main reactions in the pretreatment as biomass components are linked and cross-linked with those linkages (Dong et al., 2019). The cost and efficiency of biomass fractionation depend on the reagents or methods, temperature, and the types of biomasses used in the pretreatment.

There is a general trade-off between the fractionation efficiency of biomass and the amounts of reagents/solvents in various pretreatment processes is shown in **Fig.3.2**. Dilute acid pretreatment dissolves all hemicelluloses by chemical hydrolysis with a solid-liquid (S/L) ratio of 1:3 to make cellulose more accessible to enzyme (Liu et al., 2018a), and sulfuric acid has been most commonly applied in the process (Loow et al., 2016; Wyman, 2018). Whereas, alkali pretreatment aims lignin dissolution than hemicellulose removal and has been widely used in treating agricultural residues (wheat straw, switchgrass), but maybe less effective on treating woody biomass for saccharification (Kim et al., 2016; Kumari & Singh, 2018). Alkali reacts with acetyl and uronic acid groups resulting in the structural alteration of lignin, hemicellulose, and cellulose. Further to low lignin-containing biomass (herbs), ammonia fibre explosion (AFEX) pretreatment is a method of choice with low solid-liquid ratio (1:2) at high pressure. It alters the structure of lignocellulose other than dissolving any component and increases digestibility due to recrystallization of cellulose, hydrolysis of hemicellulose, and depolymerization of lignin (da Costa Sousa et al., 2016; Maurya et al., 2015). Concerning softwood, SPORL pretreatment is considered as an effective pretreatment method for removing the recalcitrance of softwood biomass at low solid-liquid ratio (1:3-4) (Zhang et al., 2016a; Zhu et al., 2009). SPORL dissolves almost all hemicelluloses and partial lignin through lignin sulfonation (Zhang et al., 2016a). Another effective method is the organosolv pretreatment, which involves the digestion of the lignocellulose in a mixture of organic solvents and water in the presence of acid, alkali or salt catalyst which can effectively separate lignocellulosic components by removing hemicellulose and lignin barriers (Bajpai, 2016a; Nitsos et al., 2017). The organosolv pretreatment is very effective for woody biomass with a high solid-liquid ratio (1:7 or more) (Dong et al., 2019). Recently, urea pretreatment of corn stover with high solid loading has been reported (Wang et al., 2018a). Urea is converted into gaseous ammonia during the pretreatment process which can alter the chemical linkages between hemicellulose and lignin. The porosity increases the enzyme accessibility to the internal surfaces of the substrate to improve digestibility (Yoo et al., 2016). The highest glucose and xylose recovery along with the maximum lignin removal were obtained at 50% solid loading (Wang et al., 2018a).

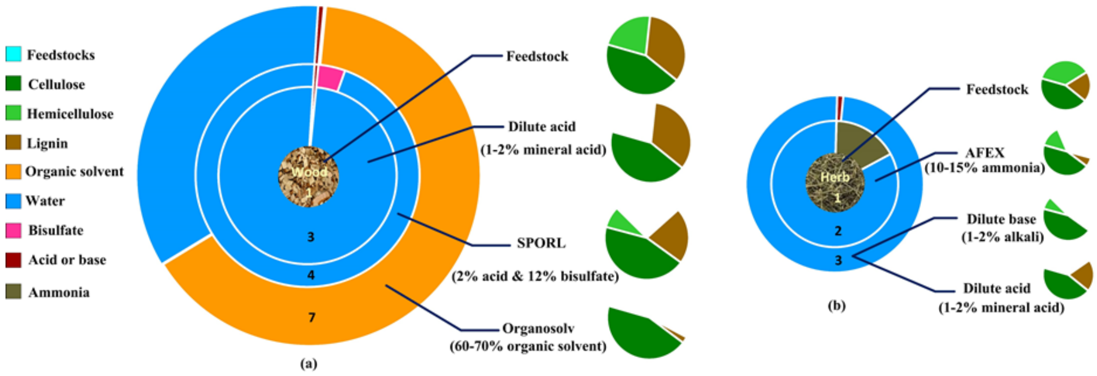


Fig. 3.2. Effectivity of different pretreatment methods along with their solid-liquid ratios for (a) wood residue and (b) herbs. The number on the circle represents the pretreatment liquor ratios per unit of biomass weight (Islam et al., 2020b).

3.2.1. Major pretreatment technologies

Conversion of woody biomass into useful products relies on a serious of complex processes, *i.e.*, pretreatment, hydrolysis, fermentation, and separation. Pretreatment aims to alter and modify the basic structure of lignocellulosic biomass for functional application. The main purpose of pretreatment is to increase the biomass conversion yield through increasing the enzyme accessibility to the pretreated substrates.

Lignocellulosic biomass is a complex matrix of the introduced building block chemicals, which provides strong recalcitrance to cellulose degradation (Himmel et al., 2007). Woody biomass contains more lignin and possesses greater recalcitrance than herbaceous biomass. To overcome the biomass recalcitrance, it is necessary to fractionate all the components at the very beginning of the bioconversion process. Different pretreatment techniques have been designed to remove the recalcitrance of lignocellulosic biomass (Baker et al., 2017; Brosse et al., 2009; Park et al., 2017; Zhao et al., 2017). Pretreatment removes lignin and hemicellulose and increases the surface area and digestibility of cellulose. It can also prevent the degradation of pentose sugar and minimize the formation of inhibitory components for fermentation. Physical, biological, and chemical methods have been reported for the pretreatment of plant biomass, while biological pretreatment is less preferred industrially due to its low efficiency and processability (Abd-Aziz et al., 2018; Mussatto, 2016). Physical pretreatment like steam explosion is usually used for woody biomass, but the process alone has not been extensively used commercially in comparison with the chemical pretreatment approach (Zhu & Pan, 2010).

Chemical pretreatments are most extensively studied which utilize a wide range of chemicals at elevated temperatures (Terán Hilares et al., 2017; Wang et al., 2018b). Acid, alkali, organosolv, peroxide, sodium hypochlorite, sodium sulfide, ozone, and ammonia have all been used in chemical pretreatments (Leu & Zhu, 2013). The major chemical pretreatment concepts are classified into three categories (*i.e.,* organosolv, acid, and alkaline pretreatment), of which the key mechanisms have been presented in **Fig.3.3**.

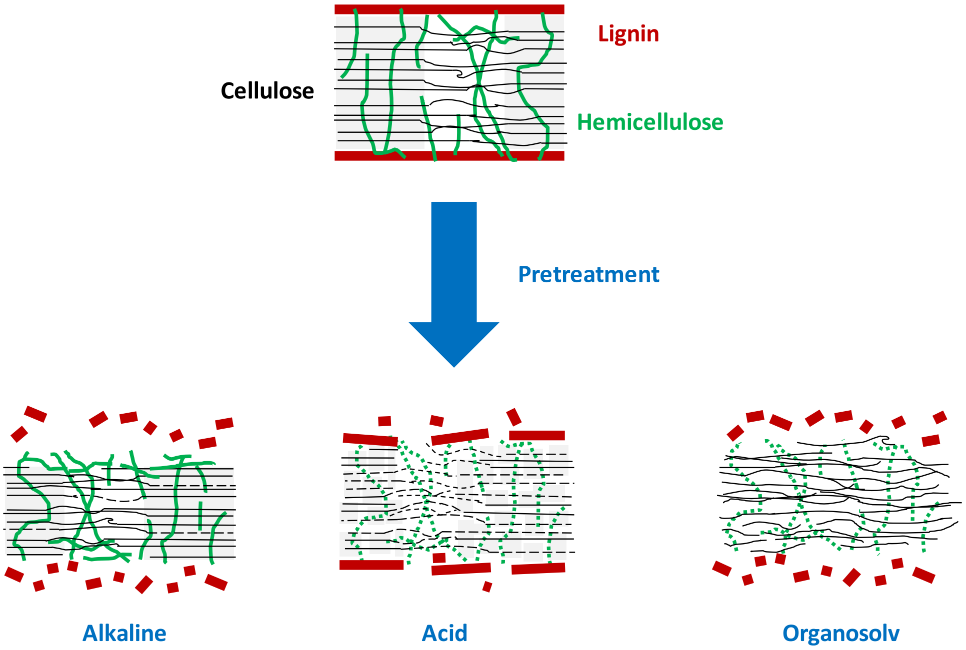


Fig.3.3. Mechanism of different chemical pretreatments (Islam et al., 2020b).

Acid pretreatments have been successfully applied to solubilize hemicellulose and efficiently used to remove the recalcitrance of biomass. Both dilute and concentrated H_2SO_4 acids are most commonly used for pretreatment of lignocellulosic biomass (Mosier et al., 2005). Dilute acid pretreatment processes are more preferable process than concentrated acid process because concentrated acids mediate the generation of fermentation inhibitors (Taherzadeh & Karimi, 2008). Acid pretreatment can be done at low temperature (<120 °C) for a longer retention time or at high temperature (>180 °C) for shorter retention time. Other acids such as nitric acid, hydrochloric acid, phosphoric acid, acetic acid, and formic acid are also successfully studied in biomass pretreatment (Gámez et al., 2006; Rodrıguez-Chong et al., 2004).

Alkali preferentially removes lignin over hemicellulose from biomass (Leu & Zhu, 2013). Alkali reacts with acetyl and uronic acid group in the hemicellulose and thus increases the accessibility of enzyme (Chang & Holtzapple, 2000). The hydrolysis of ester linkage more effectively removes lignin with minimum cellulose and hemicellulose solubility (Sun & Cheng, 2002). However, NaOH is more effective than other alkalis in reducing cellulose crystallinity and degree of polymerization and altering the structure of lignin (Kumar & Wyman, 2009).

Organosolv techniques are mainly used for the extraction of high-quality lignin. Ethanol, methanol, propanol, butanol, butanediol, ethylene glycol, glycerol, acetic acid, acetone, and tetrahydrofuran are most commonly used in combination with inorganic acids, salts, or alkali catalysts (Bajpai, 2016b; Zhao et al., 2009a). Organic solvents are most effective in lignin dissolution than other chemical pretreatments. Lignin removal increases the accessible surface of the substrate for enzyme saccharification (Agbor et al., 2011). Temperature, reaction time, solvent concentration, and catalyst concentration greatly determine the crystallinity, fibre length, and surface area of pretreated biomass. The effect of the different catalysts such as H_2SO_4, NaOH, and $MgSO_4$ has been studied on ethanol organosolv of pine wood. The results showed that H_2SO_4 is the most effective catalyst for fractionation of pine (Park et al., 2010a; Park et al., 2010b). The combination of ethanol organosolv to ball milling increased the lignin separation and glucose yield while decreasing the pretreatment severity of organosolv pretreatment (Hideno et al., 2013). Ionic liquid and deep eutectic solvents are recyclable organic compounds, have been widely exploited as solvent for pretreatment of lignocellulose for further processing to energy and chemicals (Kanbayashi & Miyafuji, 2016; Moniruzzaman & Goto, 2018). The presence of solvent in the substrate reduces the enzyme activity of cellulose hydrolysis (Mosier et al., 2005).

3.2.2. Lignin dissociation and distribution

Lignin imparts recalcitrance to biomass structure and inhibits the enzyme activity during saccharification and fermentation process of carbohydrates. It acts as a barrier between cellulose and enzyme; thus promoting non-specific binding of enzyme on lignin (Jung et al., 2018). The hydrophobic interaction, electrostatic interaction, and hydrogen bonding are accountable for the non-productive interaction of lignin and cellulase (Cheng et al., 2018a). Since lignin valorization is related to the ether linkages, mild delignification techniques are used to preserve the ether linkages in lignin and to limit lignin condensation (Dong et al., 2019). To overcome the inhibitory effect of lignin, diverse pretreatment techniques have been applied to reduce lignin content or modify lignin structure with minimal loss of sugars (Kumar et al., 2016). In the pretreatment process, lignin undergoes depolymerization to form monomers with small molecular weight and redistributes on the surface of plant cell wall (Jung et al., 2018). Delignification reaction is mainly attributed to the cleavage of both α and β ether linkages. The chemical reactions in cleaving the two representative ether linkages under different types of biorefinery processes are illustrated in **Fig.3.4(a)** and **3.4(b)**. The presented model lignin structures include two G-lignins linked at the α-o-4 position. The monolignol at the bottom right contains a -OH group linking to its phenolic structure at the 4th position while its α- and β- positions both linked with other lignins. The monolignol at the upper left is linked with other lignins at the 4th and β-positions, but its α- position is linked only with a -OH group. The concept diagram demonstrates the major functions and limitations of applied reagents and/or catalysts to cell wall decomposition during thermochemical processes. The cleavage of the two linkages under alkaline pretreatment process is shown in **Fig.3.4(a)**. At elevated pH, the cleavage reactions can initiate most rapidly at the -OH group at the 4th position of the phenolic structure, while can be carried out only very slowly when the 4th position is linking to another lignin. During the pretreatment process more -OH structures can be created to continue the cleavage of the linkages. Alkali can also cleave some hydrolysable linkages in lignin and polysaccharides, which result in a reduction in the degree of polymerization and crystallinity of the substrate (Kim et al., 2016). For acid pretreatment processes, bond cleavage can start randomly at either the α- or β-positions disregarding whether there is a hydroxyl group bonding with the aromatic ring at the 4th carbon. With different reagents the α- and β- linkages can be cleaved and the original functional groups are replaced by hydrogen, ethyl, or sulfonate group. In addition, acid-based biorefinery processes can effectively cleave glucosidic linkages in hemicelluloses and amorphous cellulose, which further improve the accessibility of enzymes to cellulose to promote enzymatic hydrolysis (Leu & Zhu, 2013).

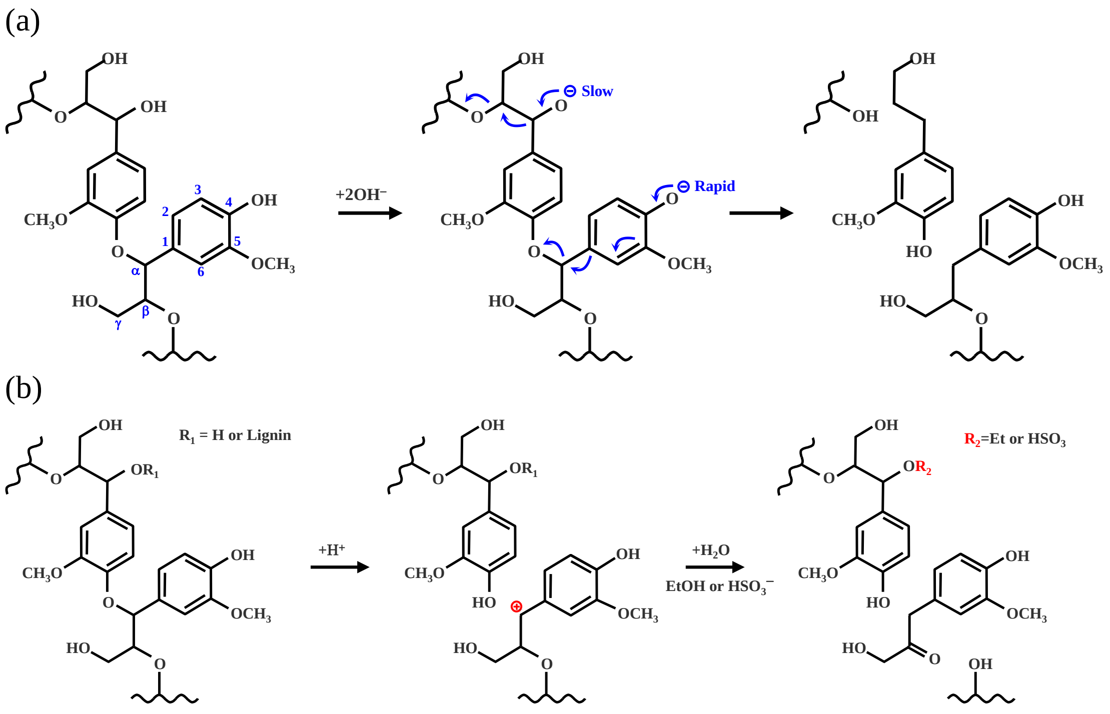


Fig.3.4. (a) Cleavage of α-o-4 and β-o-4 bonds in alkaline pretreatment process; (b) cleavage of α-o-4 and β-o-4 bonds in dilute acid, organosolv, and sulfite pretreatment processes (Islam et al., 2020a).

Distribution of native and pretreated lignin is as crucial as removal to reduce the biomass recalcitrance since the enzyme activity is surface phenomena. During thermochemical pretreatments, lignin extrudes form the cell wall corners and middle lamella to outside surface at the glass transition temperature of lignin. The molten lignin coalesces into larger droplets that migrate within and outside surface of the cell wall (Donohoe et al., 2008). Trajano *et al.* revealed, depolymerization and condensation as the primary mechanisms of lignin removal and re-deposition (Trajano et al., 2013).

3.3. Saccharification and fermentation

Saccharification is the conversion process to break down polysaccharides into monomeric sugars (Zhu et al., 2017). Acid hydrolysis and enzymatic hydrolysis are commonly applied procedures for saccharification of lignocellulosic biomass. Acid hydrolysis can directly depolymerize the polysaccharide chain in the lignocellulosic biomass without any pretreatment, which can effectively release more than 90% of monomer sugars from the feedstock (Frederick Jr et al., 2008; Lavarack et al., 2002). However, concentrated acid hydrolysis can further react with the dissolved substrates (*i.e*., carbohydrates), resulting in a high concentration of by-products, such as organic acids, furfural, and hydroxymethylfurfural (HMF) (Badger, 2002).

Enzymatic hydrolysis is currently the most widely applied/discussed approach for biomass conversion. This process accounts for approximately 27% of the total operation cost of the overall bioconversion processes and therefore needs to be optimized with the pretreatment processes for cost reduction (Bbosa et al., 2018). Saccharification efficiency can be affected by the enzyme-related (Gupta et al., 2016) and substrate-related factors (Leu & Zhu, 2013). The mechanisms of the interactions between dissociated and residual components to enzyme activities are the functions of biomass and pretreatment process. **Fig.3.5(a)** to **Fig.3.5(d)** show some images of the pretreated substrate before and after enzymatic hydrolysis. The surface of woody biomass before pretreatment (**Fig.3.5(a)**) is smooth with the inner surface well protected from hydrolysis. The pretreatment by ethanol, alkaline, and SPORL opened the plant cell wall (red arrow) and created different levels of dissociation (**Fig.3.5(b)** to **Fig.3.5(d)**, respectively). With increased severity of pretreatment condition and degree of lignin removal, the damage in cell wall becomes observable, together with lignin precipitation on the substrate surface (**Fig.3.5(d)**). The cell wall structure is further decomposed after hydrolysis, while enzymes may be captured under a scanning electron microscope (SEM) within 24 hours of hydrolysis (arrow mark in **Fig.3.5(e)**). The cell wall can be completely decomposed after enzymatic hydrolysis (**Fig.3.5(f)**).

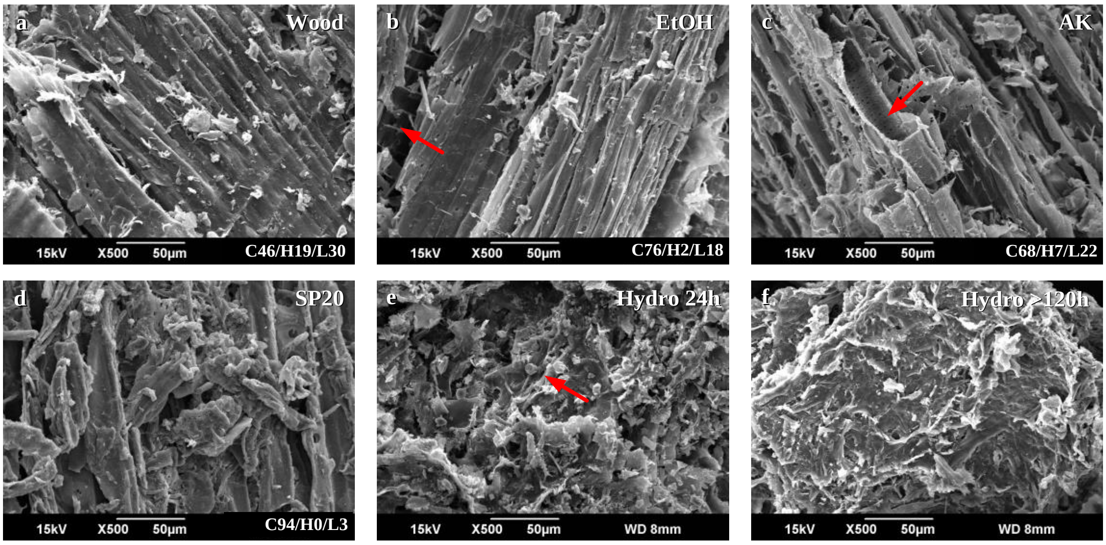


Fig.3.5. SEM images of substrate at different stages of pretreatment or hydrolysis.

Enzyme hydrolysis of biomass suffers from a low reaction rate due to the high crystallinity of cellulose, lower accessible surface area for enzyme, and non-productive binding to cellulase enzyme (Dadi et al., 2006). Enzymatic hydrolysis requires a pretreatment to break the regular structure of cellulose and to increase surface area for enzyme access (Badger, 2002). The yield of enzymatic hydrolysis increased from 20% to 90% after proper pretreatment of biomass **(Table 3.1)**. The efficiency of both acid and enzyme hydrolysis depends on the crystallinity, molecular structure, degree of polymerization, surface area, swelling, lignin content, and hemicellulose content (Dadi et al., 2006). The extent of hydrolysis to be done depends on the targeted chemicals. Complete hydrolysis is necessary to produce ethanol. On the other hand, complete hydrolysis reduces efficiency to produce butanol and lactic acid from the biomass.

Fermentation is a metabolic process that consumes sugars and produces alcohols or other chemicals depending upon the strains used in the fermentation. Fermentable sugars from the biomass can be converted to different chemicals by different enzymes. In the United States (US), less than 5% of the total chemical consumptions is obtained from the fermentation of lignocellulosic biomass. Common fermentation products in the recent literatures include ethanol, butanol, and lactic acid (Macedo & Brigham, 2014; Wang et al., 2015; Zheng et al., 2015).

Table 3.1. Pretreatment condition and saccharification yield for different biomass feedstocks.

Feedstocks	(%) Solvent	(%) Catalyst	Solid/l iquid	Composition of raw substrate			Temp. (°C)	Time (min)	Composition of pretreated substrate			Enzyme load (FPU)	CED (%)	Ref.
				Cell.	**Hemi.**	**Lignin**			**Cell.**	**Hemi.**	**Lignin**			
Lodgepole pine	65 EtOH	1.1 H_2SO_4	0.14	49.0	21.7	26.4	170	60	74.8	3.58	17.59	20	100	(Del Rio et al., 2010)
Lodgepole pine	65 BuOH	1.1 H_2SO_4	0.14	49.0	21.7	26.4	170	60	74.64	2.35	18.25	20	90	
Pitch pine	50 EtOH	1 $MgCl_2$	0.1	44	30	26	210	10	46	0	25		61.1	(Park et al., 2010b)
Pitch pine	50 EtOH	2 NaOH	0.1	44	30	26	210	20	34	0	12		85.4	
Hemp hurd	45 MeOH	3 H_2SO_4	0.04	43	21	23	165	20	48	18	22		67.9	(Gandolfi et al., 2014)
Eucalyptus	25w EtOH	1 CH_3COOH	0.2	42.2	34.1	28.1	200	60	83	3.5	13.5	9.5	~100	(Teramoto et al., 2009)
Japanese cypress	50 EtOH	0.4 HCl	0.18	41.5	25	32	170	45	75	0	25	22.8	~70	(Hideno et al., 2013)
Liriodendron tulipifera	50w EtOH	1 NaOH	-	41.1	21.2	21.4	140	50	60	22.3	20		67	(Koo et al., 2012)
Miscanthus	80 EtOH	1 H_2SO_4		49.09	24.18	25.11	170	60	48.09	8.5	22.01		45	(Obama et al., 2012)
Norway spruce	65 EtOH	-	0.07	38.8	22.2	26.4	210	15	35.7	2.9	3.9	15	87	(Agnihotri et al., 2015)
Palm EFB	78 FA	-	0.1	37.01	15	17.4	107	90	82.44	5.6	4.91	15	99	(Cui et al., 2014)
Wiilow wood	55w EtOH	0.1 H_2SO_4	0.10	32.9	14.2	28.5	187	180	46.6	12.2	26.6	33	87	(Huijgen et al., 2011)
Bamboo	75 EtOH	2 H_2SO_4	0.2	41.3	24	22.8	180	60	89.7	6.4	3.3	15	83.4	(Li et al., 2012)
Alfaalfa stem	9w PAA	-	0.16	30.2	18	15.2	100	120	30.1	16.3	14.9	60	47	(Xu & Tschirner, 2011)
Crofton wood	40wPAA	-	0.25	37.6	22.4	16.4	90	90	73	33.27	1	20	38	(Zhao et al., 2008)
Siam wood	50wPAA	-	0.25	41	22.3	20.7	90	90	64.6	26.7	6.1	10	70	(Zhao et al., 2010)
Oil palm trunk	7 H_2SO_4	4 Na_2SO_3	0.2	38.1	24.4	21.4	170	30	68.8	4.9	30.1	15	66	(Noparat et al., 2017)

where, BuOH- Butanol, EtOH- Ethanol, FA- Formaldehyde, MeOH- Methanol, PAA- Peracetic acid, SED- Substrate enzyme digestibility.

3.4. Green parameters of pretreatment solvents

The conversion of biomass into chemicals and materials involves separation, extraction, and chemical conversion reactions (Cheng, 2017). The chemical conversions require some solvents *i.e.*, ethanol, methanol, butanol, hexane, tetrahydrofuran (THF), and dimethyl sulfoxide (DMSO); which have hazards related to toxicity and flammability. Biomass must be depolymerized or partially deoxygenated to convert to fuels and chemicals through pretreatment and conversion steps (Khoo et al., 2016). Many pretreatment solvents have been reported such as alcohols, dilute mineral acid, acetone, acetic acid, alkali, ionic liquid, deep eutectic solvents and water (Hassan et al., 2018; Kumari & Singh, 2018). The application of these solvents depends on the intended solute *e.g.*, cellulose dissolution needs the breaking of the cellulose chain whereas sugar production needs active species (Esposito & Antonietti, 2015; Liebert, 2010). Selection of appropriate solvents requires consideration of green metrics of solvent with respect to (i) solubility or functionality (ii) environmental and health safety (EHS), (iii) recyclability, and (iv) vapour pressure (Soh & Eckelman, 2016).

3.4.1. Solubility

Solubility parameters are very effective practical tools for determining the functionality of a solvent for a particular purpose. Selectivity and reaction compatibility are also important criteria for solvent selection which include physicochemical properties and solubility parameters (Byrne et al., 2016; Tobiszewski et al., 2015). Hildebrand solubility parameter (δ_T) is a metric based on the energy of vaporization of the liquid solvents and is defined as the square root of cohesive energy density (Barton, 2018). The energy of vaporization of a solvent was a function of hydrogen bonding forces (δ_H), dispersion forces (δ_D), and intermolecular forces (δ_P) to determine the solubility of solvent on the solute (Barton, 2017). Hansen solubility parameters are treated as coordinates in a three-dimensional system called Hansen space and the distance (R_a) between the Hansen parameters are calculated by the following formula:

$$R_a^2 = 4(\delta_{D2} - \delta_{D1})^2 + (\delta_{P2} - \delta_{P1})^2 + (\delta_{H2} - \delta_{H1})^2 \quad (3.1)$$

The Hansen distance (R_a) value is compared with the experimentally determined solubility radius (R_0) and the ratio of R_a/R_0 is called the relative energy differences (RED) (Zhang et al., 2019). The smaller the value of RED, the better the solubility of a solute in the solvent. The Hansen solubility parameters of common biorefinery solvents to lignin are represented in **Table 3.2**. Among all of these solvents, NMP has the least relative energy difference with respect to lignin and has the highest solubility of lignin which indicates the potential of NMP for lignin dissolution. Water, on the other hand, has the lowest solubility of lignin due to the highest relative energy difference. However, the small relative energy gaps between lignin and different alcohols indicate its applicability to fractionate lignin from biomass.

Table 3.2. Solubility parameters of some common biorefinery solvents

Solvents	**Hansen Solubility Parameters (Parameters, 2007)**				
	δ_D	δ_P	δ_H	R_0	**RED**
1,4- Dioxane	19	1.8	7.4	16.58	1.210
Acetic acid	14.5	8	13.5	16.36	1.19
Acetone	15.5	10.4	7	16.59	1.21
Butanol	16	5.7	15.8	14.52	1.06
Choloform	17.8	3.1	5.7	17.71	1.29
Diethyl ether	15.49	2.9	4.6	21.0	1.53
DMAC	16.8	11.5	9.4	12.92	0.94
Ethanol	15.8	8.8	19.4	13.53	0.98
Ethyl acetate	15.8	5.3	7.2	17.89	1.30
Ethylene glycol	17	11	26	13.72	1.00
Formic acid	14.3	11.9	16.6	15.36	1.12
Glycerol	17.4	12.1	29.3	15.45	1.12
GVL	16.9	11.5	6.3	14.80	1.08
Methanol	15.1	12.3	22.3	14.74	1.07
Methyl acetate	15.5	7.2	7.6	17.26	1.25
NMP	18	12.3	7.2	11.82	0.91
Pentanol	15.9	5.9	13.8	14.86	1.08
Pyridine	19	8.8	5.9	13.51	0.98
THF	16.8	5.7	8	15.93	1.16
Water	15.1	16	42.3	28.87	2.10

The values of R_0 and RED were calculated with respect to lignin.

3.4.2. Environmental and health safety

The use of a solvent in the biorefinery determines the efficiency, economic viability, and environmental sustainability of the process. In addition to solvent efficiency, selection of the solvents must be balanced with the cost, toxicity, environmental, and health safety (**Fig.3.6**). The concept of a green solvent/green process demonstrates the desire to minimize the adverse health and environmental implications during production (Byrne et al., 2016). Therefore, the most crucial question is how to measure the greenness of a solvent. The most widely accepted procedures are (i) EHS assessment method and (ii) the life-cycle assessment (LCA) method (Sheldon, 2017).

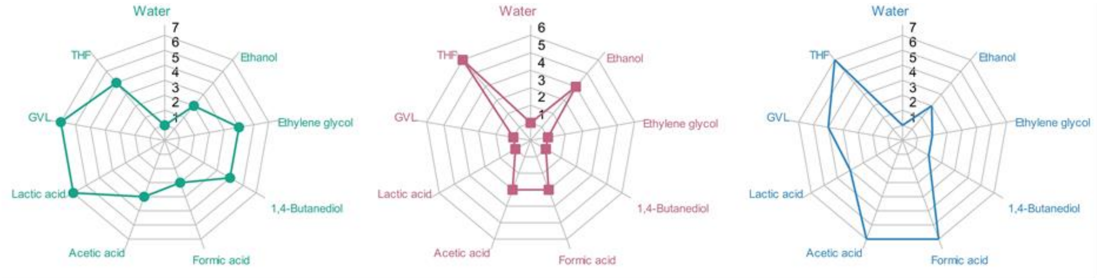


Fig. 3.6. Environmental (a), health (b), and safety (c) scores of common biorefinery solvents.

The EHS assessment is a screening method to early phase identification of possible hazards of solvents in a chemical process. The utilization of organic solvents can create a number of potential hazards *i.e.*, flammability, explosiveness, toxicity, and non-degradability (Sheldon, 2017). Process chemists and engineers are curious to check flammability and explosion potential as the key metrics. Flammability and fire hazards mostly depend on the flashpoint with the combination of the boiling point when flashpoint is less than 24°C (Sheldon, 2017).

However, human health metrics are based on the occupational hazards and depend on both the hazards and exposure of the chemicals. Referring to the solvent selection guides of leading pharmaceuticals, acute and chronic toxicity along with the carcinogenicity and mutagenicity are the prior concerns for solvent selection mainly in the Pfizer (Alfonsi et al., 2008), Sanofi (Prat et al., 2015), and GlaxoSmithKline (GSK) (Alder et al., 2016) solvent selection guides. In addition, the boiling point is another essential health metrics since the primary exposure route for a solvent is inhalation.

Furthermore, the environmental assessment includes ecotoxicity (*i.e.,* toxicity to aquatic life, bioaccumulation, volatile organic carbon emissions etc) and ozone layer depletion potential of the chemicals. Data needed for the environmental scoring system is still unavailable. Sanofi assessed environmental safety by the boiling point where high boiling solvents are penalized since they consume high energy during recycling (Prat et al., 2015). Low boiling solvents require less energy in distillation but have a high risk of exposure and volatile organic carbon (VOC) emissions. Life cycle assessment was introduced by considering various types of effects which are sometimes synergistic (Amelio et al., 2014). Organic alcohols are the least toxic among common biorefinery solvents which are using in the fractional for lignin first concept.

3.4.3. Recyclability

Recycle and reuse of the solvents is one of the crucial green criteria of biorefinery. Recycling not only reduces the cost of the process but also significantly reduces eco-footprint or life cycle impacts to make the process sustainable. Life cycle impacts largely depend on the decision whether waste solvents from biorefinery may be discarded or recycled (Byrne et al., 2016; Kralisch et al., 2015). The separation methods *i.e.,* liquid-liquid extraction, distillation, incineration, recrystallization, evaporation, scrubbing, etc are generally applied for recycling of chemicals.

A study reported the 84% recovery of NaOH was achieved by concentrating alkaline pretreatment liquor to 60% solid content (García et al., 2011). However, in an ammonia pretreatment process, NH_3 was evaporated directly from the pretreated slurry and obtained 99.6% recovery of NH_3 used in the biorefinery process (da Costa Sousa et al., 2016). Ethanol was used as a favourable solvent in the biorefinery process by Araceli and co-workers due to its high recoverability (García et al., 2011). The distillation process of ethylene glycol-water mixture was simulated and optimized with 70 and 80% recovery of ethylene glycol and water respectively (Alriols et al., 2009). A modified liquid-CO_2 extraction process recovered about 99.5% of gamma-valerolactone (GVL) from pretreatment slurries (Shuai et al., 2016b). Liquid CO_2 continuously flowed through the slurry at high pressure to recover the low-boiling point solvent into a low-pressure vessel. This approach can separate the solvent from dissolved sugars, lignin, and solid materials and can be applied to other solvents with similar characteristics. Bian et al. (2017) recycled p-toluene sulfonic acid (p-TsOH) using a commercially proven crystallization technology by repeated cooling of concentrated liquor to ambient temperature. Since p-TsOH has relatively low water solubility than sugars; the process can easily be conducted in a whole slurry process. Furthermore, direct contact membrane distillation is a low temperature and ambient pressure process which can be applied to separate water from ionic liquids (ILS) (Lynam et al., 2016). In some pretreatment processes, solvents or reagents are recovered as by-products to reduce the process cost and to make the process sustainable (Dong et al., 2018; Xie et al., 2018). Alkaline pretreatment reagents such as KOH and urea were recovered as fertilizer for agricultural plants (Dong et al., 2018; Wang et al., 2018a). A study (Liu et al., 2017) investigated the recyclability of the DBN-based ionic-liquid ([Dbne][Dep]) from wool keratin liquor. The condensation of ionic liquid (IL) rich liquor at 120°C increased the recyclability of IL by five times. Another study (Liu et al., 2018b) developed a conjugative freeze crystallization and evaporation process for recycling of ILs from a cellulose spinning process. Compared to evaporation only process, the conjugative process consumed 25% less energy for recycling of [Emim][Ac] and [Emim][Dep]. There is a trade-off between recyclability of solvents and different LCA metrics. For instance, the production of dichloromethane has a low carbon footprint but releases ozone layer depleting compounds (Soh & Eckelman, 2016). The efficient use of solvent and energy has advantages through all the ways of product life cycle *i.e.,* GHG emissions and upstream processing. Hence, recyclability of solvent is a key parameter for the sustainability of a process and in measuring life cycle impacts of the solvent (Wilson et al., 2019).

3.4.4. Vapour pressure

Vapour pressure of a solvent is a vital parameter for determining the process safety of the biorefinery operation. The vapour pressure of a liquid is the equilibrium pressure exerted by its vapour at a particular temperature in equilibrium with the condensed phase (Speight, 2016). It is the indication of the volatility of the substance *i.e.,* the substance with high vapour pressure at the room temperature is regarded as the volatile substance, which increases with increasing temperature. Hence, the vapour pressure determines the selection of process equipment and safety measures have to be taken. Even though the proper reactor is used to withstand the vapour pressure of solvent, still there is a risk of expansion due to the increase of solvent volume at the high temperature. To avoid this risk, the reactor should not be filled up completely, allowing some space for the volume expansion of the solvent at high temperature.

3.5. Processability of lignocellulose in urban biorefinery

The rapid increase of the generation of urban lignocellulosic biomass has become a new-century-challenge as it is expected that by 2050 approximately 70 % of the global population will be moving to the cities (Hassan et al., 2019; Satchatippavarn et al., 2016). Urbanization increases the consumption of resources and generation of solid wastes, but it also creates new opportunity of new technologies. Development of urban biorefinery creates outstanding benefits in reducing transportation costs for both the feedstock and the products, which is the major barrier to the conventional secondary biorefinery using forestry and agricultural residues as feedstock. Energy, water, and chemicals can be more effectively utilized and recycled in various facilities in either a centralized or decentralized fundamental.

The technological feasibility of urban biorefinery, however, relies heavily on the process safety, health, and environmental controls than the conventional approaches applied in rural areas. The waste stream and the potential emission of volatile organic compounds require sophisticated downstream treatment before releasing to the receiving bodies. Pretreatment of biomass has been carried out at high temperature (165-220°C), which often time resulted in elevated pressure at a critical liquid-vapour environment. Operation and maintenance of the high-pressure vessels and its risk control are site-specific, of which many techniques are not available in certain areas. The operational pressures of the recently introduced pretreatment solvents are demonstrated over reactor temperature in **Fig.3.7(a)**. The operational pressure of different fractionation processes ranged from 10 kPa (GVL) to nearly 10,000 kPa (ammonia) at 120°C. Typical autoclaves can withstand the pressure of 100-110 kPa which is approximately equal to the vapour pressure of water at 100-120°C. The use of low boiling point solvents like ammonia, THF, and ethanol requires a special reactor to withstand at least 1-1.2 MPa pressure at a temperature between 150-210 °C. The safety requirement limits the processability of certain pretreatment processes in cities.

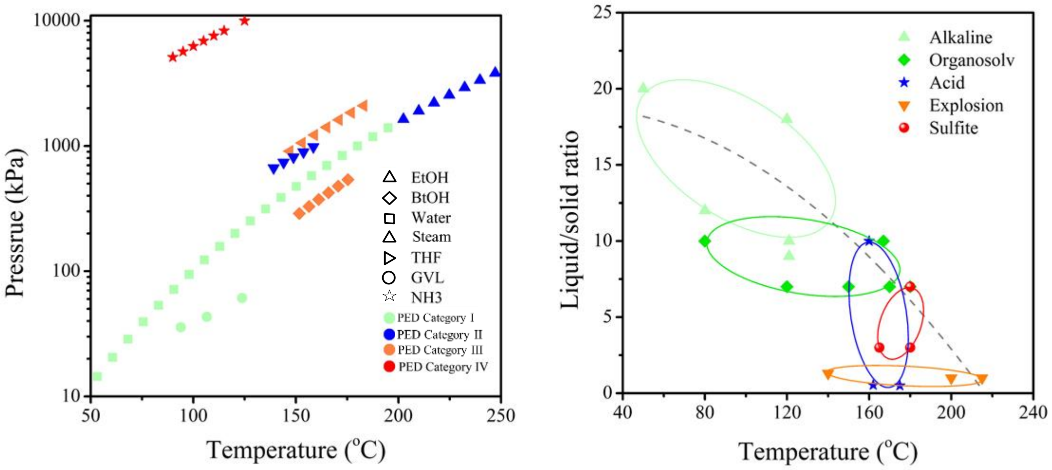


Fig. 3.7. Relationship between (a) temperature and pressure exerted by different solvents in the reactor (shapes represent solvent types and colours represent PED category), and (b) temperature and solid-liquid ratio for different pretreatments.

The applicability and hazard of pressure equipment (pretreatment reactor) can be determined by using the well-known pressure equipment directive (PED) calculator (2019). The PED calculator classifieds the hazard Category I, II, III, and IV of a reactor or process using pressure and volume of the reactor **Fig.3.7(a)**. The higher the category, and therefore the greater the hazards, the more demanding the conformity assessment modules are. Hence, the equipment or reactor in category I, is subjected to go through only the manufacturers own quality control but categories II, III & IV require the direct inspection (license) of the notified body for the approval and monitoring of the manufacturer. PED calculator considers the type of reagents along with the volume and pressure of the reactor. For ethanol (volatile liquid) and ammonia (gas) pretreatment of biomass, reactors fall in the category of II and IV respectively (highly hazardous). On the other hand, the reactors which are using water as a solvent are much safer than volatile liquids.

3.6. Water-energy nexus

In a biorefinery, pretreatment and conditioning of substrates are the most energy and water-intensive process (Pan et al., 2015). The direct energy footprint of pretreatment and the downstream process is related to the amount of water used in the production method. Water is the greenest solvent for biorefinery, and it has a strong influence on the efficiency of thermochemical conversion of biomass during pretreatment. It has been used with most of the other solvents in the pretreatment processes. The excessive use of water, however, increases the energy consumption during heating, evaporation, and distillation (Dong et al., 2018). Water consumption in the pretreatment process is a function of solvent solubility, delignification mechanism, and impacts of pretreatment solvents to the enzyme (Chen et al., 2015; Liu et al., 2016). The general pattern of the published solid-liquid ratios for effective fractionation over temperature is shown in **Fig.3.7(b)**. In general, less amount of water was needed in a high temperature process such as steam and ammonia explosion, and more water is needed at relatively lower temperature (Minnick et al., 2016). The energy consumptions of different process units using different pretreatment techniques are calculated in **Fig.3.8(a).** Diluted acid and alkali pretreatment consumed higher amount of energy for cooking, washing, and fermentation than all other processes. SPORL and steam explosion consumed relatively less water and energy (**Fig.3.8(a)** and **Fig.3.8(b)**) due to the suitability of whole slurry simultaneous saccharification and fermentation (SSF). Recently, a low-temperature method of pretreatment using sodium-hydroxide has been reported for ethanol production from sugarcane bagasse without washing process (Wang et al., 2019b). This process significantly reduces the water and energy consumption but suffers from comparatively lower hydrolysis efficiency than organosolv pretreatments (Dong et al., 2018).

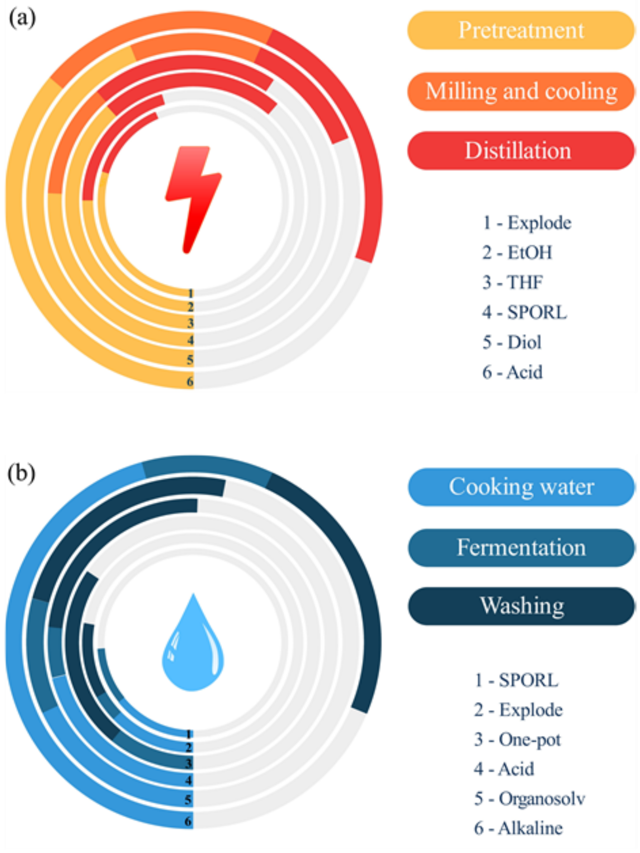


Fig. 3.8. (a) Energy and (b) water consumption for different pretreatment techniques.

3.7. Current challenges and opportunities

In recent years, prodigious developments have been achieved in biorefinery technology to produce biofuels and chemicals. Many of the biorefineries are now in operation in America, Europe, and Asia (Valdivia et al., 2016). Despite these successes, some biorefinery projects have been postponed and many plants have been closed (Chandel et al., 2018) (Dale, 2018). Up to now, biorefinery technologies are still in developing (Raud et al., 2019); and one main barrier is the development of highly efficient and cost-effective pretreatment technology to fractionate lignocellulosic biomass (Xu et al., 2013).

Among all the pretreatment methods, organosolv pretreatment is an essential technique to produce high-value lignin, but the concerns are processibility, toxicity, and recyclability. The green solvents have higher potential due to its reduced health and environmental impacts. Hence, the introduction of non-toxic and effective solvents along with the recovery of solvents favours reaching green chemistry principle. Despite some reports of almost complete lignin removal (Chen et al., 2017; Dong et al., 2019); the development of low temperature and cost-effective delignification technology is still a big challenge to the researcher at the current stage.

Urban biorefinery receives biomass with great diversity, which creates another challenge to pretreatment. Mild pretreatments with suitable mechanisms are in study to overcome the lignin condensation and degradation issues, which reduce the yields of lignin monomers. Inconsistent sizes of the waste-derived biomass also resulted in mass transfer issues which affect the designed conditions of the pretreatment. New monitoring techniques and dynamic simulation tools are in needed to overcome those issues.

3.8. Summary

The high technological barriers and reliance on non-renewable resources for the conversion of lignocellulosic biomass indicates that biorefinery has not yet an economic practice in industrial scale. Applying municipal waste-derived biomass as the feedstock, biorefinery is environmentally and economically attractive in supporting waste management, but its sustainability relies on careful identification of the reagents and overall processes. With the recent advances after significant research efforts, green biorefinery may be soon realized in the very near future after the successful developments of mild pretreatment, low toxicity/high recyclability reagents, low-profile reactors, and low water/energy facilities.

CHAPTER 4. METHODS

This chapter summarizes all the analytical techniques and experiment /calculation procedures applied to evaluate the pretreatment processes and lignin quality over this study (Chapter 5-7).

4.1. Chemical composition analysis

The chemical composition of biomass and substrate samples was analyzed before and after pretreatment. The analytical procedure of structural carbohydrates and lignin has been well-documented by National Renewable Energy Laboratory (NREL) of the United States (Sluiter et al., 2008). Before analysis, wood samples were air-dried and ground into a fine powder to ensure maximum reagent penetration. A part of the sample was dried in an oven at 105 °C for at least 12 hours to a constant weight. The moisture content was determined by calculating the loss in weight of the wood sample. The sample was mixed with 72% H_2SO_4 and hydrolyzed at 30°C temperature in a water bath for 60 minutes. The solution was diluted to 3.97 % H_2SO_4 (w/w) and autoclaved at 121°C for another 60 minutes for complete hydrolysis. During primary hydrolysis, polysaccharides are completely separated from the lignin whereas secondary hydrolysis depolymerizes oligomers to monomer sugars. The autoclaved masses were filtered to separate lignin residue and filtrate containing cellulose and hemicellulose sugars. An aliquot was used to determine the structural sugars while lignin was gravimetrically determined from the dried mass of the residue. The analytical procedure for structural and non-structural components of wood is demonstrated in **Fig.4.1**.

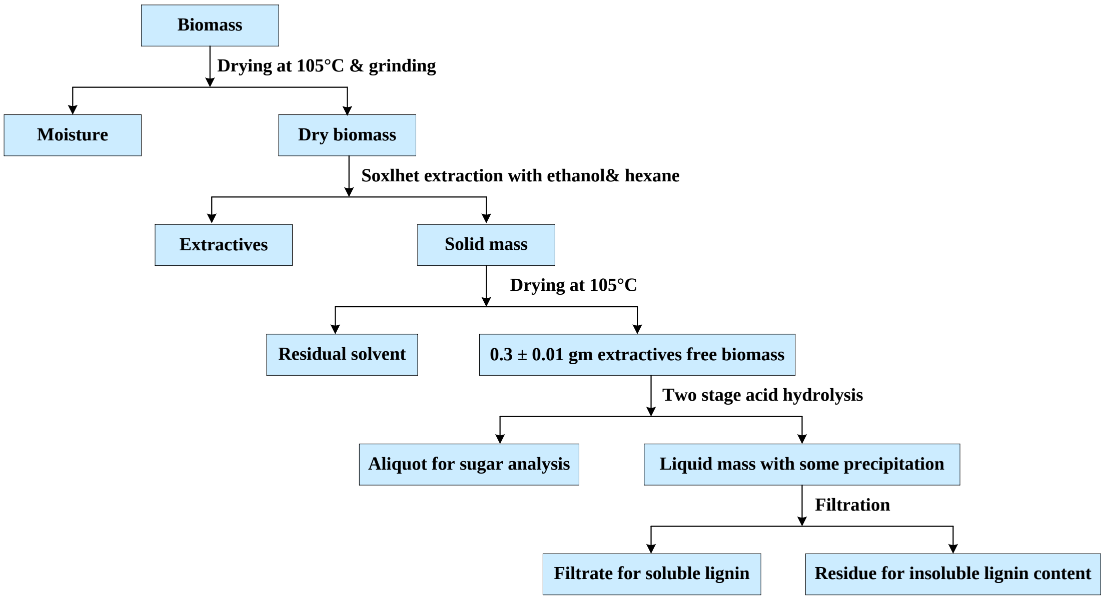


Fig.4.1. Chemical composition analysis scheme of lignocellulosic biomass.

Extractives can be analyzed by soaking the biomass samples in polar and nonpolar organic solvents, such as methanol, ethanol, acetone, hexane, benzene, and diethyl ether at the desired temperature with continuous solvent refluxing. Soxhlet extraction is the most effective method of extractive separation from plants and reported comprehensively (Ramluckan et al., 2014; Schwanninger & Hinterstoisser, 2002). The crude protein contents of the biomass sample were analyzed by the Total Kjeldahl Nitrogen (TKN) approach.

4.2. Organosolv hydrolysis factor (OHF)

The efficiency of biomass fractionation depends on the reagents or methods, temperature, and the types of biomass used in the pretreatment (Jung et al., 2018). The OHF as a pretreatment severity was used to predict hemicellulose removal from pretreated substrates (Ji et al., 2017) which is expressed as-

$$OHF = e^{(\alpha - E/RT\ +\ \beta C + \gamma C)}\, Ct \quad (4.1)$$

where C is the molar concentration of chemicals used in reaction (mole/L), t is the reaction time in minute, E (J/mole) is activation energy, T is temperature in degree Kelvin, R = 8.314 (J/mole/K) is the universal gas constant, α and β are adjustable parameters. The contents of residual hemicellulose (X_R) were fitted as a function of OHF using following equation-

$$X_R = (1-\theta)e^{OHF}\ + \theta e^{-fOHF} \qquad (4.2)$$

Where, θ represents the fraction of slow degrading hemicelluloses and f represents the proportion of the rate constant between slow and fast rate hydrolysis of hemicellulose.

4.3. Enzymatic hydrolysis of pretreated substrate

The saccharification experiments were conducted to determine the digestibility of celluloses in pretreated substrates to monomer sugars. The saccharification experiments were carried out at 3% solid content with 15 filter paper unit (FPU)/g-cellulose enzyme loads, using 150 mL Erlenmeyer flasks incubated in an orbital shaker at 50°C with a speed of 150 rpm. The substrate was mixed with 1M sodium acetate buffer at pH 4.80, and 1 mL of solutions were taken at 3, 6, 9, 12, 24, and 72 hours of saccharification. The cellulose enzyme digestibility (CED) of pretreated substrates were determined by taking the ratios of measured glucose concentrations over the maximum glucose concentrations to be produced from fully digested substrates.

4.4. Simultaneous saccharification and fermentation (SSF)

SSF of pretreated substrates was carried out in a 250 mL Erlenmeyer flask with a total working volume of 100 mL at 5% (w/v) substrate loading. The pH of the mixture was adjusted at 5.5-6.2 by auto addition of KOH using BLBIO-2020 model pH controller. The enzyme Cellic-Ctec2 at 15 FPU/g-cellulose and precultured yeast at 5 g/L were added to the mixture for hydrolysis and fermentation. The yeasts were precultured in yeast extract peptone dextrose (YPD) medium (2% (w/v) glucose, 1% (w/v) yeast extract, and 2% (w/v) peptone extract) at 37℃ for 9 hrs. SSF experiments were conducted for 108 hours and aliquots were collected periodically to estimate ethanol concentration in fermentation medium to determine the efficiency of the process.

4.5. Isolation of ball milled lignin

Biomass powder of 20 mesh treated overnight with ethanol and toluene (2:1) to remove wood extractives. Extractive free wood samples were milled for 6 hours using vertical lab planetary ball mill rotating at 600 rpm in a 500 ml zirconium dioxide (ZrO_2) bowl containing ZrO_2 balls (10 mm × 10 mm). The milling time was adjusted for 10 minutes per cycles after with a 5-minute interval. Lignin was extracted from milled wood powder with 80% p-dioxane for 48 hours in an orbital shaker. The extracted liquid was separated and concentrated to one third of its volume by rotary evaporation. Lignin was precipitated by dropping the concentrated solution into water. Lignin residue was centrifuged and dried in the freeze dryer to get dried lignin fraction. Lignin was also regenerated after dissolving in acetic acid and washed with petroleum ether to purify it.

4.6. Lignin characterization

4.6.1. NMR analyses

Lignin structure and its transformation during pretreatment were determined by 2D-HSQC NMR. It also provided quantitative information of different linkages in lignin samples. For the test, around 50 milligrams of lignin sample were dissolved in deuterated DMSO. 2D-HSQC spectra were recorded using a JEOL ECZR 500 MHz spectrometer equipped with a 5 mm ROYAL probe. For the 1H dimension, the number of complex points was 1024 with a spectral width of 11 ppm and recycle delay of 1.5 seconds. In the 13C dimension, the number of transients was 64, and time increments 256 with 1.5 s interscan delay. The peaks from S and G aromatic units of lignin were used as the internal reference. The quantitative values were determined by comparing the integral volume of HSQC correlations (Wang et al., 2017a). JEOL's Delta 5.2.1 software was used to determine the volume integrals of HSQC correlations.

The amount of hydroxyl groups in fractionated lignin samples were quantified by 31P NMR using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) as the phosphorylating agent (Wang et al., 2017a). Approximately, 20 mg of lignin sample was dissolved in 500 µL of anhydrous pyridine and deuterated chloroform mixture (1.6:1.0, v/v). Then, 100 µL of chromium-III acetylacetonate solution (5 mg/mL in anhydrous pyridine and deuterated chloroform 1.6:1, v/v) was added to the dissolved lignin as the relaxation reagent; followed by the addition of 100 µL of cyclohexanol (10.85 mg/mL in anhydrous pyridine and deuterated chloroform 1.6:1, v/v) as an internal standard. The phosphorylation reaction was carried out for 15 minutes after the addition of 100 µL of TMDP and transferred to the NMR tube for analysis. The 31P NMR spectra were recorded on the JEOL ECZR 500 MHz spectrometer at 90° pulse, 25 s pulse delay with 16 scans.

4.6.2. Infrared spectroscopy

Infrared (IR) spectra of lignin samples were recorded using a Perkin Elmer Fourier-transform infrared (FTIR) spectrometer with a diamond crystal attenuated total reflectance (ATR) accessory. Powdered lignin samples were placed on top of a diamond crystal and exposed to an infrared beam in the region between 4000 and 400 cm^{-1} with 12 scans and 4 cm^{-1} resolution. The infrared beam penetrated and reflected off the surface of the sample through the diamond crystal in the form of an evanescent wave. The room temperature and relative humidity were around 26°C and 35% respectively.

4.6.3. Gel permeation chromatography (GPC)

The number-average (Mn) molecular weights of all lignin samples were determined by GPC (Agilent 1200, USA)(Wang et al., 2017a). Approximately 10 milligrams of lignin samples were dissolved in 2 mL 0.11 M LiCl solution in N, N-dimethylacetamide and filtered mixture through 0.45 mm filter. An aliquot of 10μL for each sample was injected for GPC analysis. The column temperature was maintained at 80°C and eluted with the mobile phase N, N-dimethylacetamide at the rate of 1 ml/min. The analysis was carried out by the HPLC equipped with Agilent PLgel MIXED-B column and refractive index detector.

4.6.4. Scanning electron microscopy (SEM)

SEM images of freeze-dried lignin samples were captured by an ultra-high resolution Tescan MAIA3. The samples were mounted on aluminum stubs with the support of conductive carbon tape to reduce sample charging. The mounted samples were then gold-coated using high-resolution sputter-coater. Images were recorded at a beam voltage of 25 kilovolts (kV).

The crystallinity or carbon structure of substrates was determined by X-ray diffraction (XRD) using powdered X-ray (Bruker D8-Advance). The data were recorded at a scanning speed of 3° per minute from 2θ = 10 to 50° with a step size of 0.020. The diffractometer was operated at 30 mA and 40 KV with Cu/Kα radiation. The peak intensity was used to determine the crystallinity index (CrI) of the samples-

$$CrI(\%) = \frac{I_c - I_{am}}{I_c} \times 100 \qquad (4.3)$$

Where, I_{am} = amorphous peak intensity at 2θ = 18.5 and I_c = the maximum peak intensity at around 2θ = 22.5.

4.7. Energy balance

4.7.1. Energy consumption

The total energy consumption was calculated for the biorefinery conversions including digester heating, hydrolysis, fermentation, and distillation to separate final products. The energy consumptions were calculated based on enthalpy change of related system (Cambero & Sowlati, 2016; Huang & Zhang, 2011) using the following equations:

$$E_H = \frac{1}{\eta}\left[\int_{T_0}^{T_t}\left(\sum_{i=1}^{n} m_i^{T_j} \cdot Cp_i^{T_j}\right)dT + \sum_{i=1}^{n} m_i^{T_k} \cdot L_i^{T_k}\right] \qquad (4.4)$$

where E_H is the total energy consumption during conversion (kJ); η is the efficiency of the heat transfer; T_0 is the initial temperature of heating or cooling system (°C); T_t is the desired temperature (°C); m is the total mass of heating or cooling substance (kg); C_t is the heat capacity of the substance (kJ/kg/°C); L is the latent heat of the substance (kJ/kg); T_j is the specific temperature of selected substance (°C); T_k is the boiling points of the selected substance (°C); subscripted '*i*' indicates the type of substance in the mixture; and n is the total number of the compounds. The components interaction was neglected to heat capacity and latent heat since the components mass ratios are very small compared to the water content of the system. The energy consumption in the pretreatment was calculated using liquid to solid ratio, pretreatment temperature, and pretreatment time (Zhu & Pan, 2010).

Distillation energy (E_{Dstl}) was calculated based on the equilibrium curve of organosolv-water system with necessary liquid/volume (Madson, 2003) and can be expressed as:

$$E_{Dstl} = L \cdot e_{steam} = R_c \cdot V \cdot e_{steam} \quad (4.5)$$

where V and L are the vapour and liquid flow rates, respectively (kg-mole/h); e_{steam} is the energy content of the stream (kJ/kg); and R_c is the specific constant for L/V ratios (Madson, 2003).

4.7.2. Bioenergy and net energy calculation

Heating value is the key parameter to determine the potential of bioenergy production from lignocellulosic biomass. Heating value is practically determined by bomb calorimeter using ASTM-D2015 as standard method. However, it can be determined using mathematical equation based on chemical compositions and ultimate analysis of lignocellulose (Cordero et al., 2001; Sheng & Azevedo, 2005). The mathematical evaluation of heating value is based on the energy release or requirement to recombine all chemical bonds between elements. Dulong equation is the most popular method and commonly used to determine the heating value of coal (Lowry, 1963). However, Dulong equation resulted significant deviation in heating value when compared to value obtained from calorimeter. It was due to the higher oxygen content of the substrate which reduced the heating value. Therefore, Dulong equations was modified to accurately determine the heating value of solid fuel as follows-

$$\text{Energy value }\left(\text{kJ/kg}\right) = 337\cdot \text{C} + 1418\cdot(\text{H} - \frac{\text{O}}{8}) + 93\cdot\text{S} + 23\cdot\text{N} \qquad (4.6)$$

where C, H, O, S, and N are the weight percentage of carbon, hydrogen, oxygen, sulfur, and nitrogen in the biomass, respectively.

The net energy production was calculated by the following equation:

$$E_H = \sum_{i=1}^{4} M_i \cdot Y_i \cdot E_{EtOH}^{i} - E_{pre} - E_{mill} - E_{dis} \quad (4.7)$$

where M_i and Y_i are the mass fraction and ethanol yields of the specific biomass type from the oil palm tree (tons/ha), respectively; E_{EtOH}^{i} is the energy production from bioethanol (GJ/ton); E_{pre} , E_{mill} , and E_{dis} are energies used in pretreatment, hammer milling, and distillation, respectively.

4.7.3. Carbon flow analysis

The carbon efficiency of wood biorefinery determines the potential of mitigating GHG emissions of the process. The carbon flow was evaluated based on mass flow of the biorefinery process and molecular formula of the components. The amount of carbon in each processing step was calculated based on the amount of carbon recovery in the products using the following equation-

$$Mc = \sum_{i=i} M_i \cdot C_i \qquad (4.8)$$

Where, M is the mass obtained at a particular step and C is the carbon content of the respective mass.

CHAPTER 5. PRETREATMENT OF LIGNOCELLULOSE FOR HIGH LIGNIN YIELD AND CELLULOSE DIGESTIBILITY

5.1. Introduction

Lignocellulosic biomass is an abundant and low-cost natural biopolymer which demonstrates great potential for sustainable production of biofuels and chemicals . However, lignocellulosic biomass is a complex polymer in which cellulose, hemicellulose, and lignin formed the biomass recalcitrance against its chemical and biological degradation (Leu & Zhu, 2013; Yoo et al., 2017). The biomass recalcitrance varies greatly among plant species, origins, ages, harvesting and storing methods (Zhao et al., 2012a), hence the complexity of bioconversion increases when mixed wastes are applied as the feedstock of the biorefinery. Pretreatment is a critical procedure to reduce the biomass recalcitrance, which modifies the physiochemical properties of the biomass, and enhances the accessibility of biomass components to enzymes and chemicals (Leu & Zhu, 2013). Pretreatment also helps to separate the biomass components into different fractions, which facilitate complete valorization of whole biomass (Galbe & Wallberg, 2019; Ilanidis et al., 2020).

A diverse range of physical and chemical pretreatment approaches have been practiced overcoming biomass recalcitrance for the effective utilization of biomass components. Organosolv pretreatment has recently received increased attention due to its high lignin recyclability for valorization (Meng et al., 2020a). Acid-catalyzed organosolv is the most widely studied pretreatment approach which targets to the cleavage of the ether linkages for solubilizing both hemicellulose and lignin, leaving cellulose as an insoluble substrate (Pan et al., 2005). However, the use of mineral acid can lead to the formation of benzylic carbocation intermediate which then undergoes hydrolysis to form condensed lignin structures (Hibbert ketones & homo-benzaldehyde)(Lee & Jeffries, 2011; Wyman, 2013). Increasing the severity of pretreatment for high recalcitrant biomass could also increase the possibility of lignin condensation hence demand the value of lignin in downstream process (Dong et al., 2019). Searching new organosolv/catalyst systems have attracted significant attentions in the community in order to achieve complete delignification, hemicellulose dissociation, while keeping the structural integrity of lignin, which has been known as the lignin-first pretreatment principle (Renders et al., 2017).

Lignin is composed of phenolic and alcoholic hydroxyl monomers with some carboxyl and carbonyl functional groups connected through intermolecular hydrogen bondings (Islam et al., 2020a). The solubility of lignin is a function of solvent parameters which can be divided into major three components *i.e.,* dispersion forces, solvent polarity, and hydrogen bond ability (known as Hansen solubility parameters, R_a Equation 3.1) (Islam et al., 2020b). The combined effects of these solvent parameters, or smaller R_a, helps to overcome the intermolecular interaction of lignin to dissolve in the solvent. As introduced in Section 3.4.1, NMP has the lowest R_a with respect to lignin, implying its high solubility and potential to be applied in the fractionation of lignocellulosic biomass (Ma et al., 2018). NMP is a widely used industrial solvent in paint, pharmaceuticals, plastics, petrochemical processing, and cleaning for its strong dissolution power and high thermal stability (Roche-Molina et al., 2020). It is currently producing from petrochemicals, but could also be sustainably produced from γ-aminobutyric acid which is a plant-based protein (Lammens et al., 2010). The biological production of NMP opens up the possibility of its use in the fractionation of lignocellulosic biomass. During pretreatment, NMP can serve as an aprotic and neutral organic solvent that can be acidified with a small amount of

Bronsted acid in the solution which is crucial to break lignin carbohydrate linkages. As NMP does not form azeotrope with water and is with significant different boiling temperature versus water, it may be easily separated and recovered from the pretreatment spent liquor in the distillation process. The parameters implies that NMP may possess high potential for lignocellulose fractionation, when applied in an acid-catalyzed organosolv-water system.

In biorefinery application, NMP has been applied in ionic liquid (IL) for lignin solubilization and biomass fractionation. Xie et al. (2012) reported a [NMP][EmimAc] (1-ethyl-3-methyl imidazolium acetate) IL that dissolved approximately 10 % corn stover at 140 °C in 60 min. The pretreated substrate showed reasonable digestibility at approximately 83% of total reducible sugars. An exceptional solubility of fractionated lignin was reported in [NMP][C_2 carboxylic acid] solvent system (Mu et al., 2015). The solubility of lignin was enhanced by the hydrogen bonding and π-π interaction between solvent and lignin molecules. Asakawa et al. (2016) reported that NMP can be applied as a co-solvent and work with choline acetate [Cho][OAc] IL for similar digestibility at using IL alone. Interestingly, no study has been conducted to investigate the potential of using NMP directly as a pretreatment reagent for biomass pretreatment. As the basic principle of organosolv pretreatment relies on lignin solubilisation with the solvent associated with acid catalysts to hydrolyse the hemicelluloses, NMP alone may offer significant benefit to lignin removal which simplify the whole biorefinery process, without the need of IL. Besides, none of the aforementioned studies have evaluated the structural integrities of fractionated lignin.

In this chapter, we investigated the fractionation efficiency of *Acacia Confusa* wood using acid-catalyzed NMP, with attention to high lignin removal, reduced lignin condensation, and saccharification yield of the substrate. The hydrolyzing effect of acid with the combination of high lignin solubility of NMP facilitated the dissolution of hemicellulose and lignin. The experimental results were also applied to construct the modified severity factor OHF (see Chapter 4) which is similar to the combined hydrolysis factor (CHF) reported by Leu et al. (2013). The OHF has been introduced to quantify the pretreatment efficiency of hemicellulose removal over different pretreatment conditions (*i.e.*, chemical/acid doses, treatment time, and temperature), and may offer important insights of lignin removal. Due to complex reaction pathways and kinetics, severity factors have never been successfully applied in lignin simulation previously. With the attempt to clarify the factors in organosolv pretreatment process, a numerical strategy was developed upon properly controlled parameters to quantify and predict the impacts of lignin dissociation and lignin condensation to pretreatment efficiency. 2D-HSQC NMR and GPC analysis were also performed to determine the changes in the structural integrity of fractionated lignin during pretreatment.

5.2. Experimental Design

5.2.1. Biomass samples

Acacia Confusa wood residue samples were provided by a local supplier (The RecycleGreen Project Limited Company, Hong Kong) after urban forestry management (for removal of intruding species). The samples were stored in a cool room after drying and size reduction to 1-2 cm using a disc mill as shown in **Fig.5.1.**

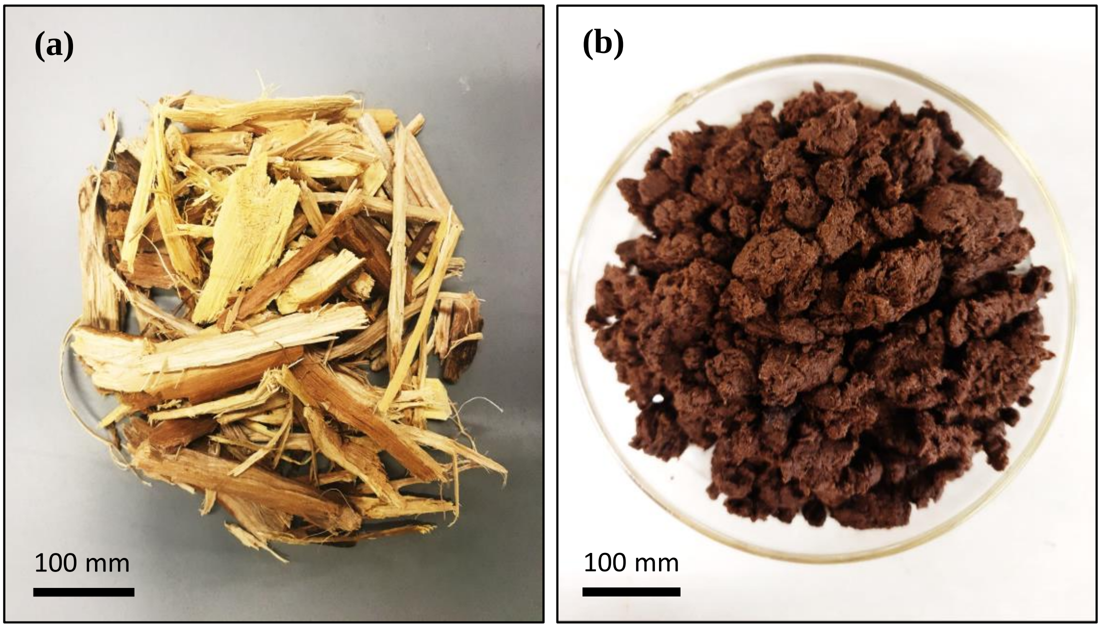


Fig.5.1. The appearances of *Acacia Confusa* (a) raw wood residue and (b) pretreated solid substrate after size reduction.

5.2.2. Experimental facilities

Pretreatment of wood samples was carried out in a rotating stainless-steel reactor which can withstand high temperature (250-300℃) and pressure (50 MPa). The sugar content of raw and pretreated substrates was determined by using an aminex HPX-87P column (Bio-Rad, Hercules, CA, USA) in high-performance liquid chromatography coupled with a refractive index detector (Shimadzu, Japan). Lignin samples were characterized by GPC and 2D-HSQC NMR spectroscopy. The 2D-HSQC NMR spectra were recorded on a JOEL 400 MHz conducted spectrometer.

5.2.3. Pretreatment

The pretreatment solvent was prepared by mixing the desired amount of NMP, catalyst (H_2SO_4), and deionized water. The prepared solvent and biomass were mixed according to a fixed solid-liquid ratio of 1:7 and transferred to 400 mL stainless steel reactor. The reactors were sealed and mounted on the 15 L rotating cylinder for pretreatment. The reactors were heated to 170℃ and continued for 60 minutes to accomplish the pretreatment. The reactors were allowed to cool to room temperature, and pretreated pulps were separated from the liquor using a vacuum filter. The pulp was washed with small amount of NMP to dissolve the residual lignin from the surface of pulp. The pulp was then further washed with deionized water thoroughly and stored in a cool room for further evaluation to determine pretreatment, saccharification and fermentation yields. Lignin was precipitated and harvested from the pretreatment liquor by adding excess water as an anti-solvent. Parallel ethanosolv pretreatments were conducted to compare the feasibility of NMP pretreatment over conventional ethanol process.

5.3. Results and discussion

5.3.1. Chemical composition and pretreatment efficiency

The results of chemical composition analysis before and after pretreatment are shown in **Fig.5.2**. The untreated *Acacia Confusa* woody biomass contained 37.6% cellulose, 19.0% hemicellulose, 30.7% lignin, and some other minor ingredients. With the increases of acid doses, the pretreatment resulted in higher degrees of lignin and hemicellulose dissociation (except for 65% EtOH, 75-100 mM H_2SO_4), resulting in higher cellulose content in the substrates.

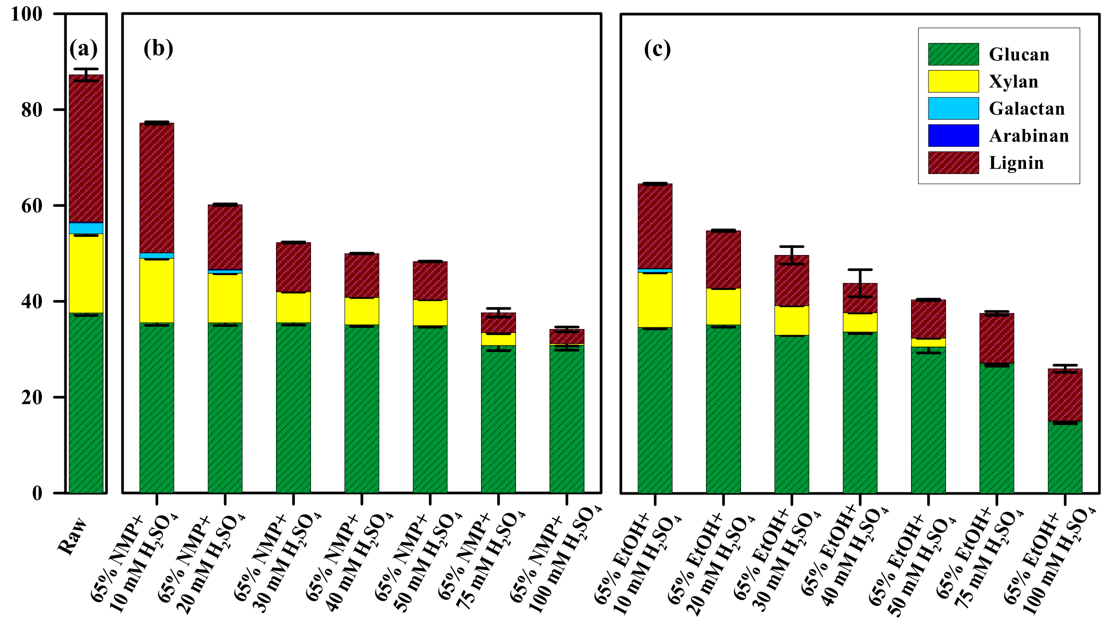


Fig.5.2. Chemical compositions of (a) untreated (b) NMP and (c) EtOH pretreated *Acacia Confusa* wood chips at 170 °C for 60 minutes.

Both NMP and H_2SO_4 played important roles in the pretreatment process. Sulfuric acid catalyzed the cleavage of the lignin-carbohydrate linkages of lignocellulose components, which were subsequently dissolved in NMP. The residual cellulose content in the pretreated substrate increased with the increasing acid concentrations, as expected. Kalogiannis et al. (2018) reported that the increase of H_3O^+ in organosolv can subsequently increase the fractionation efficiency regardless the type of organosolv used in the processes. The hydrolyzing effects of the acid catalyst facilitates effective hydrolysis of hemicellulose, which in turn, makes the lignin removal easier due to the framework structure of hemicellulose and lignin (Yoo et al., 2017). In absence of a catalyst, pretreatment efficiency is very low as the biomass fractionation can be only initiated by the autoionization of the solvent or acid produced from the dissociation of biomass. The pretreated substrate contained up to

81.4% cellulose with 89.2% cellulose recovery at 100 mM acid for 65% NMP solution. These values are similar to previous publications (Chen et al., 2017; Dong et al., 2019).

The performances of hemicellulose and lignin removal increased with the acid concentration in the liquor, as shown in **Fig.5.3**. For ethanosolv pretreatment, lignin removal started decreasing after 40 mM of acid concentration because of lignin condensation at high acid severity. The maximum lignin removal (80.3%) in EtOH pretreatment was obtained at 40 mM acid concentration, which was significantly lower than what reported in the literatures. At 65% EtOH and 20mM H_2SO_4 Dong et al. (2019) achieved more than 90% lignin removal for *Eucalyptus* wood. The lignin in *Acacia Confusa* seems to be easily condensed due to the influence of extractives in the biomass (Yeh et al., 2014).

For NMP pretreatment, unlike the EtOH process, approximately 90% lignin removal was achieved with 100 mM of H_2SO_4. The increase in lignin removal may be due to the formation of 4-methylamino butyric acid from NMP which increased polarity of the solvent system (Lennon et al., 2020). The increased polarity synergistically increased lignin removal and reduced the amount of lignin condensation.

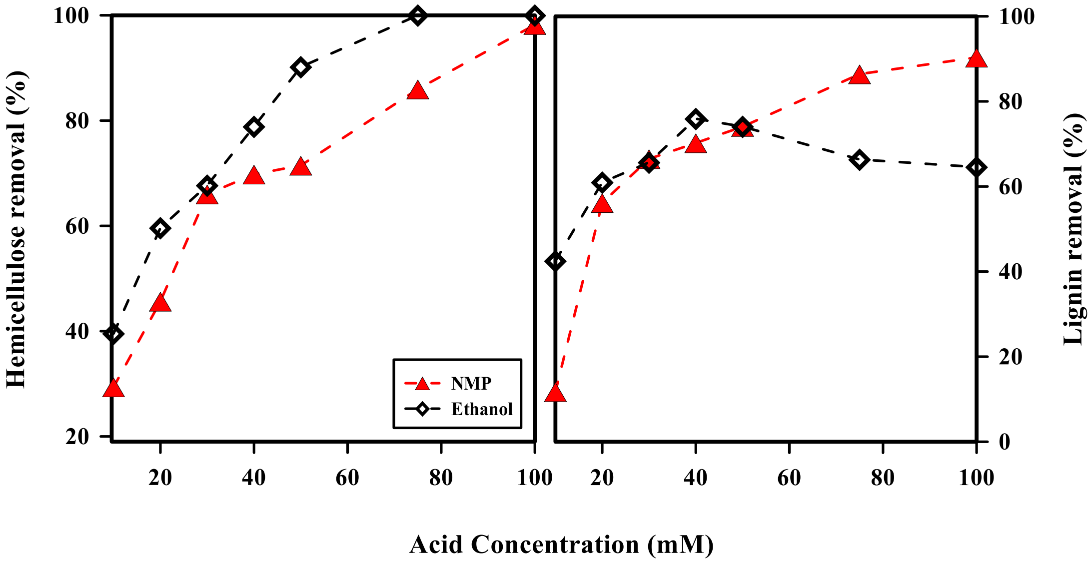


Fig.5.3. The effects of pretreatment on (a) hemicellulose removal and (b) lignin removal from substrates for ethanol and NMP with different acid concentrations.

5.3.2. Prediction of hemicellulose removal by OHF

The OHF versus X_R was constructed to predict the hemicellulose removal in the pretreatment, which are shown in **Fig.5.4**. The adjustable parameters *e.g.*, α, β, γ, θ, and f were obtained by fitting the value of residual hemicellulose content in pretreated substrates using equations (4.1) and (4.2). The adjustable parameters are listed in the **Table 5.1**. The graph shows two different trends for residual hemicellulose contents in the pretreated substrates.

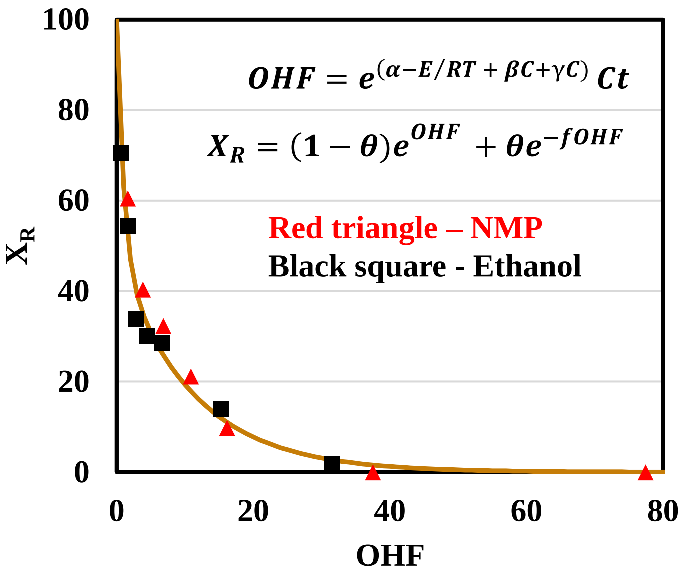


Fig.5.4. Measured residual hemicellulose X_R as a function of pretreatment severity evaluated as the OHF with model predictions.

The initial hemicellulose degradation was very fast as an exponential function of the OHF, followed by a slow rate of hemicellulose degradation. Excellent fitting was obtained, and the results showed that hemicellulose degradation was very slow at hemicellulose content ≤10% in the pretreated substrates. The value of activation energy and other adjustable parameters was approximately similar to the previous reports (Leu et al., 2013). The OHF successfully predicted the pretreatment severity and hemicellulose degradation in this study.

Table 5.1: Fitting parameters for hemicellulose dissolution to Equation (5.2).

	α	**β**	**γ**	**θ**	**f**
NMP	28.5	17.5	-0.2130	0.48	0.091
EtOH	28.5	17.5	-0.0486	0.48	0.091

5.3.3. Enzymatic hydrolysis of solid cellulosic residues

The pretreated substrates were hydrolyzed to determine cellulose digestibility at 15 FPU of enzyme load per gram cellulose. The CED's were compared for NMP and ethanol pretreated substrates as demonstrated in **Fig.5.5.**

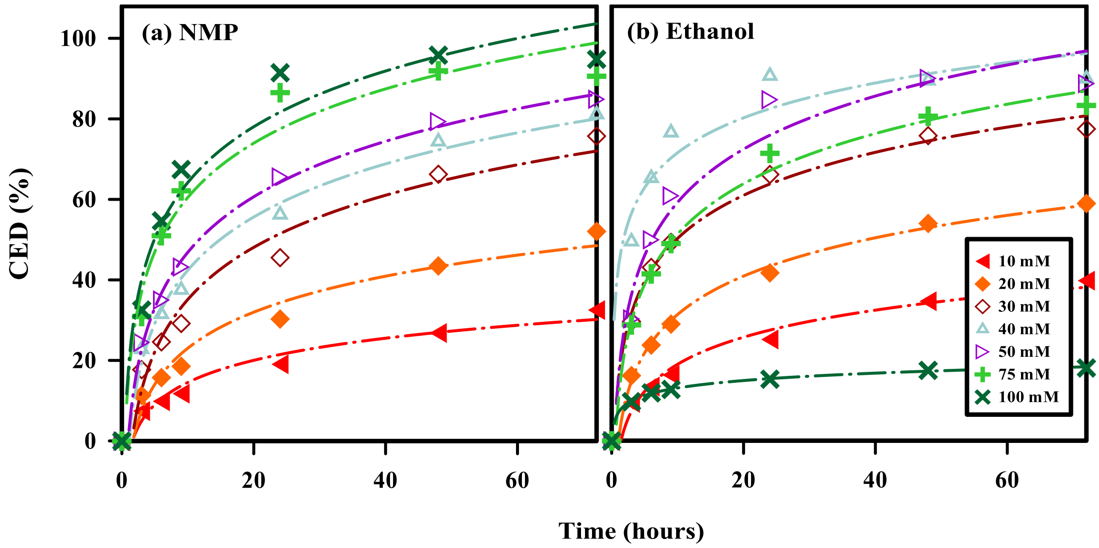


Fig.5.5. Glucose yields of (a) NMP and (b) ethanol pretreated *Acacia Confusa* wood substrates during enzymatic hydrolysis by Cellic Ctec2.

The untreated wood cellulose is inaccessible to cellulolytic enzyme due to the high content of lignin and its complex network with cellulose and hemicellulose (Kim et al., 2019). The digestibility of cellulose was significantly increased regardless of the solvent and pretreatment conditions due to the high disintegration of the substrates (*i.e.*, hemicellulose and lignin) to increase enzyme accessible area of substrates. For NMP pretreated substrate, the digestibility was increased from 32.6% to 94.9% of cellulose as acid concentration increased from 10 mM to 100 mM in NMP solution (**Fig.5.5**), which was mostly due to high lignin (90.4%) and hemicellulose (98.2%) dissolution. The hydrolysis yield was directly related to lignin removal and structure due to the non-productive binding between lignin and hydrolyzing enzyme.

Almost complete digestibility (94.9%) of NMP pretreated substrates indicated the lignin stabilization during pretreatment which reduced formation of condensed lignin. For ethanol pretreated substrate, cellulose digestibility increased from 38.1% to 89.0% when acid dose was increased from 10 mM to 40 mM (**Fig.5.6**). However, the CED of ethanol pretreated substrate significantly decreased at high acid severity (>40 mM) although the complete removal of hemicellulose was achieved. The digestibility of ethanol pretreated substrate decreased to 18.12% at high pretreatment severity (100 mM). This supported the phenomena of lignin condensation at high pretreatment severity for ethanol pretreatment which increased the hydrophobic interaction between lignin and enzyme during hydrolysis (Shen et al., 2019). Ethanol pretreatment resulted in severe lignin condensation when acid concentrations were higher than the optimal acid dose, thus reducing lignin removal from pretreated substrates. The condensed lignin contains a high amount of C-C and C=C linkages which increases the hydrophobic interaction between lignin and hydrolyzing enzyme (Wang et al., 2019c). In addition, lignin condensation reduces the lignin solubility which in turn reduces the enzyme-accessible area of the substrates.

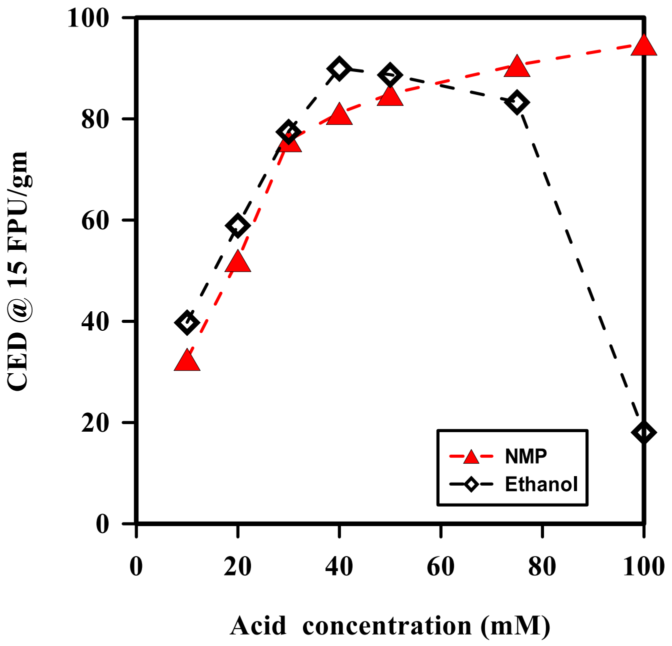


Fig.5.6. CED_{72} of pretreated substrates in comparison with the increased pretreatment acid concentrations.

5.3.4. Lignin characterization

In pulp and paper industry, lignin has been treated as a waste product or low-value fuel for power generation. Here, lignocellulose is subjected to alkaline pretreatment to remove lignin and to produce strong carbohydrate pulp. During pulping, lignin structure is altered and chemically degraded by hydroxyl (OH) and hydrosulfide (SH) ions (Giummarella et al., 2019). Lignin also experiences severe condensation reactions which make lignin difficult depolymerization and valorization. The effective valorization of lignin is the key parameter for the economic viability and sustainability of lignocellulose biorefinery. The effective utilization of lignin requires a deep understanding of lignin structure and beneficiary reactive functional groups. The content of original ether linkages in the fractionated lignin is a crucial factor to produce aromatic monomers and other value-added products (Bhagia & Ragauskas, 2019). The lignin quality and integrity analyzed by semi-quantitative 2D-HSQC NMR

($_1$H-$_{13}$C) spectra were illustrated in **Fig.5.7**, which demonstrated the major substructures (S and G units) and lignin inter-unit linkages such as β-O-4, β-β, and β-5. The NMR peaks were assigned by comparing with benchmarked ball mill lignin and reported literatures (Amiri et al., 2019; Dutta et al., 2018; Schneider et al., 2020). The position and colour of correlations represented corresponding sub-structures which are demonstrated in legends, *i.e.,* β-o-4 (green) , β-5 (brown), and β-β (pink), S unit (grass green), G unit (light orange), and H unit (purple). The semi-quantitative values of major lignin were determined using the aromatic units as the internal standard.

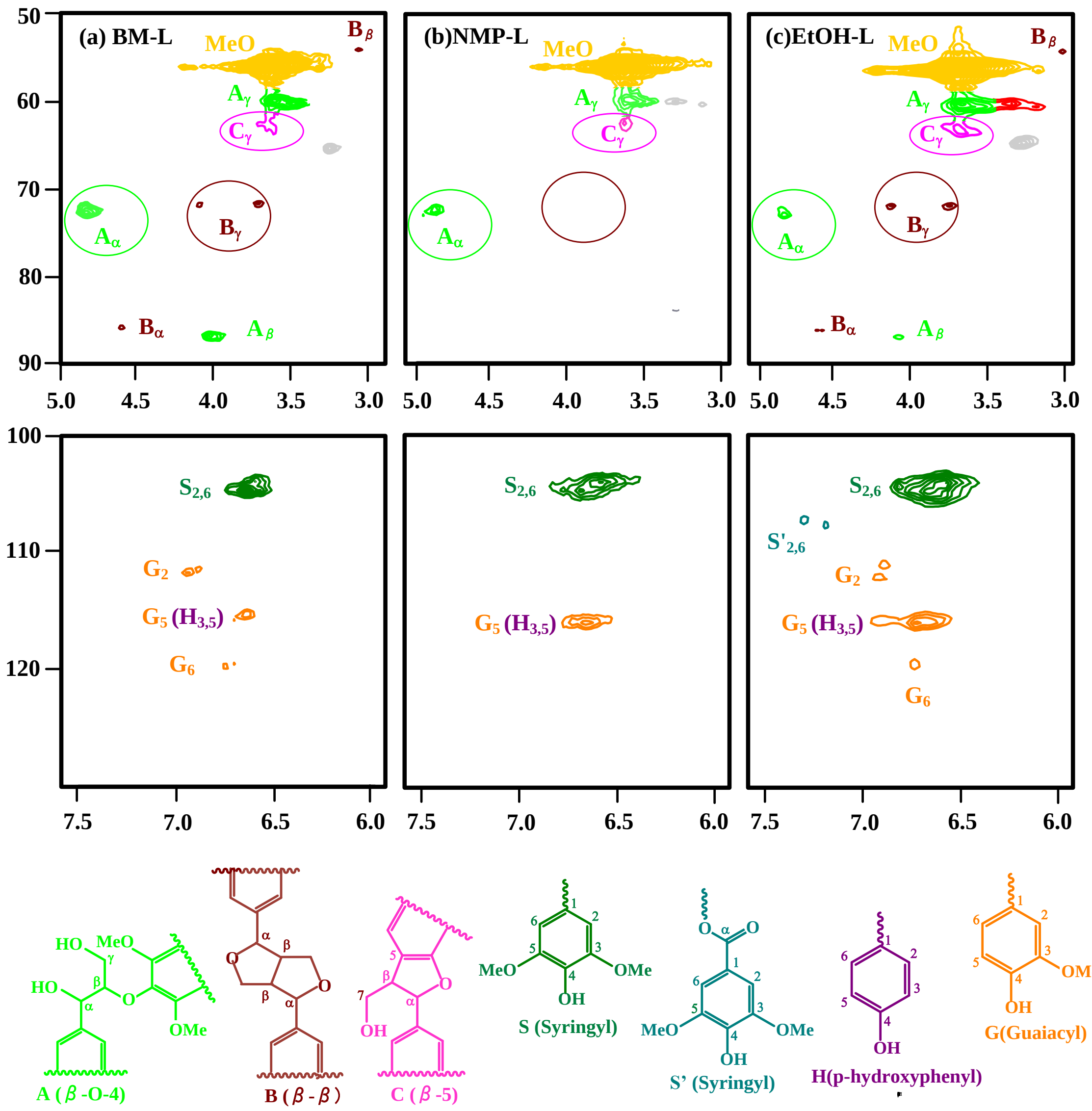


Fig.5.7. 2D-HSQC NMR spectra of sidechain and aromatic regions in the (a) ball mill lignin, (b) NMP lignin, and (c) ethanol lignin from *Acacia Confusa* wood.

For ball mill lignin, α-position of β-O-4, β–β, and β-5 linkages appeared at δC/δH 72.56/4.82 ppm, δC/δH 85.66/4.60 ppm, and δC/δH 86.1/4.1 ppm respectively. The corresponding peaks were observed for ethanol and NMP pretreatment at the same position with different intensities. The β-o-4 is the predominant linkage of lignin, which determines the integrity and potential of fractionated lignin for effective valorization to produce aromatic chemicals (Zijlstra et al., 2020). The most available craft lignin contains high proportion of condensed C-C linkages and requires harsh condition to depolymerize into aromatic chemicals. The depolymerization products of kraft lignin suffer from lack of selectivity and contain of mixture complex structures (Raikwar et al., 2019). The preservation of maximum β-o-4 linkages in the fractionated lignins is desirable C-O bonded linkage of native lignin for mild and selective depolymerization to aromatic phenols. The higher proportion of β-o-4 linkages facilitates reductive depolymerization of lignin into specific phenolics in high selectivity and yield. The acid concentration and the type of organosolv played a significant role to preserve β-o-4 linkages in the fractionated lignins. The delignification reaction starts with the cleavage of β-o-4 linkage, which is also the main pathway for lignin degradation during pretreatment. For ball mill lignin, α-position of β-o-4 appeared at δC/δH 72.56/4.82 ppm. The corresponding peaks were appeared in NMP and ethanol pretreated lignin spectra at reduced intensities. The semi-quantitative analysis showed approximately 16.36% of original β-o-4 linkages for NMP pretreated lignin, which was much higher than the conventional ethanol pretreatment (11.4%). This may be due to high acid severity in ethanol-water solvent system. The presence of H_3O^+ is very crucial for hydrolysis of both hemicellulose and lignin linkages. However, acid induced formation carbocation which led the formation of condensed structure in lignin to decrease solubility in the solvent system (Lancefield et al., 2017). The stabilization of carbocation by physical factor or epoxy formation can reduce the formation of condensed structure. Although certain amount of ethoxylation was observed at δC/δH 60.44/3.28 ppm for ethanol pretreatment, it also induced severe condensation reactions due to high acid activity in ethanol medium.

The number average (Mn) and weight average (Mw) molecular weights of ball mill, NMP, and ethanol fractionated lignins of *Acacia Confusa* wood were compared in **Fig.5.8**. The highest Mn (12,796) and Mw (16,761) were obtained for ball lignin which was used as the reference of original lignin. During pretreatment, lignin was fractionated due to the cleavage of inter-unit aryl ether linkages and thus reduced in its average molecular weight. The molecular weight of fractionated lignins decreased rapidly with increasing pretreatment severity (acid) due to the strong hydrolyzing capability of acid. Both the Mn and Mw of NMP fractionated lignins were much higher than that of ethanol fractionated lignins regardless of the conditions of lignin fractionation. This supported the positive effects of NMP and/or its degradation product to stabilize lignin during fractionation and to maintain the high lignin integrity.

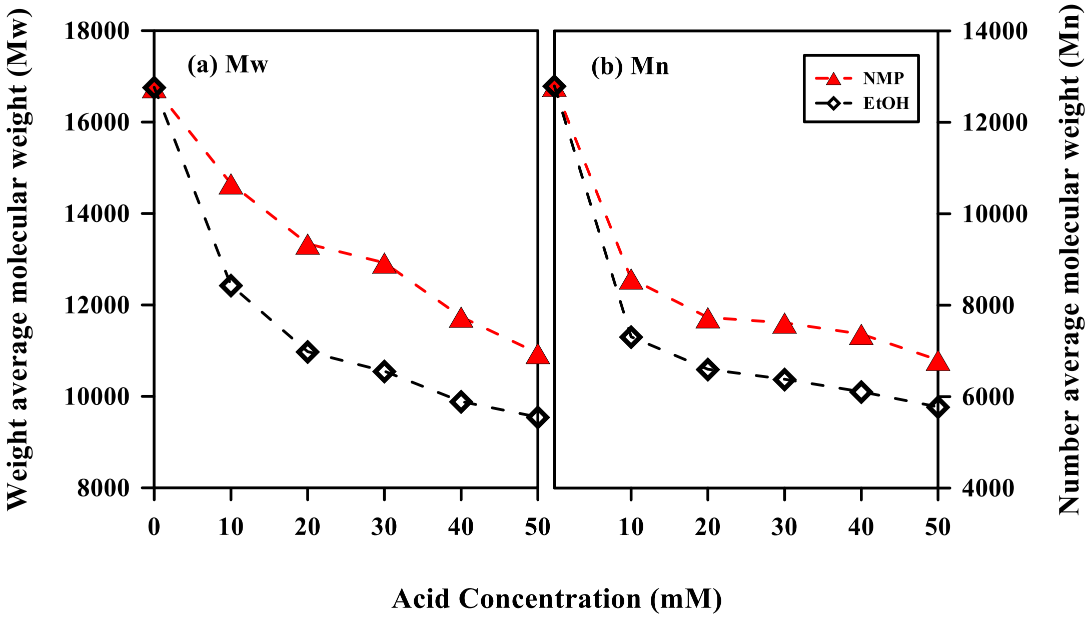


Fig.5.8. The changes in (a) weight-average and (b) number average molecular weight with respect to acid concentration during pretreatment.

The comparison of functional groups in the fractionated lignins was carried out by quantitative ^{31}P NMR analysis. The values were quantified using peak integration of cyclohexanol which is used as the internal standard. The ^{31}P NMR spectra are demonstrated in **Fig.5.9**, and the quantitative values of corresponding peaks are listed in **Table 5.2**.

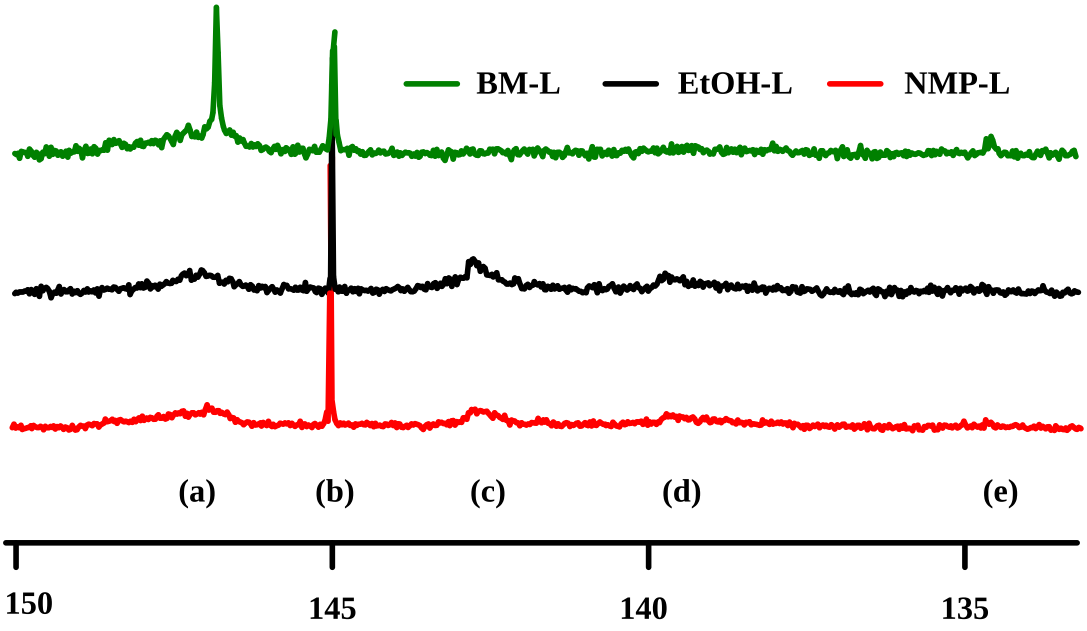


Fig.5.9. The quantitative ^{31}P NMR spectra of the BML (green), EtOH (black), and NMP (red) fractionated lignins. The ^{31}P NMR peaks represent (a) aliphatic OH (b), cyclohexanol -internal standard, (c) syringyl OH, (d) guaiacyl OH, and (e) carboxyl OH, respectively.

Notably, it was found that BML contained more aliphatic OH groups (2.26 mmol/g-lignin) and very few phenolic groups (0.11 mmol/g-lignin) than the ethanol and NMP fractionated lignins (Wang et al., 2017a). The fewer phenolic OH contents of BML indicated the lower cleavage of β-O-4 linkages during lignin fractionation. Therefore, BML is rich in β-O-4 linkages which supports the values obtained from

2D-HSQC NMR analysis. The cleavage of β-O-4 linkages during pretreatment resulted in the higher contents of phenolic-OH (syringyl OH and guaiacyl OH) for ethanol (2.17 mmol/g) and NMP (1.68 mmol/g) fractionated lignins. The higher amount of phenolic OH for ethanol fractionated lignin indicated the higher cleavage of β-O-4 linkages during pretreatment. The aliphatic OH content of ethanol fractionated lignin (1.25 mmol/g) was lower than the NMP fractionated lignin (1.70 mmol/g) due to severe lignin condensation during ethanol pretreatment.

Table 5.2. The quantitative values of different lignin fractions from ^{31}P-NMR analysis (mmol/g)

Fractions	**(a) Aliphatic OH**	**(c) Syringyl OH**	**(d) Guaiacyl OH**	**(e) Carboxyl OH**
BML	2.26	0.0102	0.1	0.039
EtOH	1.25	1.33	0.84	0.0014
NMP	1.7	0.91	0.77	0.039

NMP is highly soluble in water due to the strong hydrogen bond between NMP and water molecule, which is beneficial to break the lignin-lignin and lignin-carbohydrate linkages (Meng et al., 2020b). It also has a very high hydrogen bond acceptability (0.77) which is one of the key parameters for effective dissolution of lignin (Sherwood et al., 2014). NMP is a complex molecule (having both hydrophilic and hydrophobic part) and its mechanism of high dissolution ability is still unknown (Basma et al., 2018). The strong hydrophobic interaction between NMP and lignin helped to solubilize fractionated lignin, and the hydrophilic part of NMP were supposed to be formed a protective layer surrounding lignin aggregate to prevent lignin condensation as shown in **Fig.5.10**.

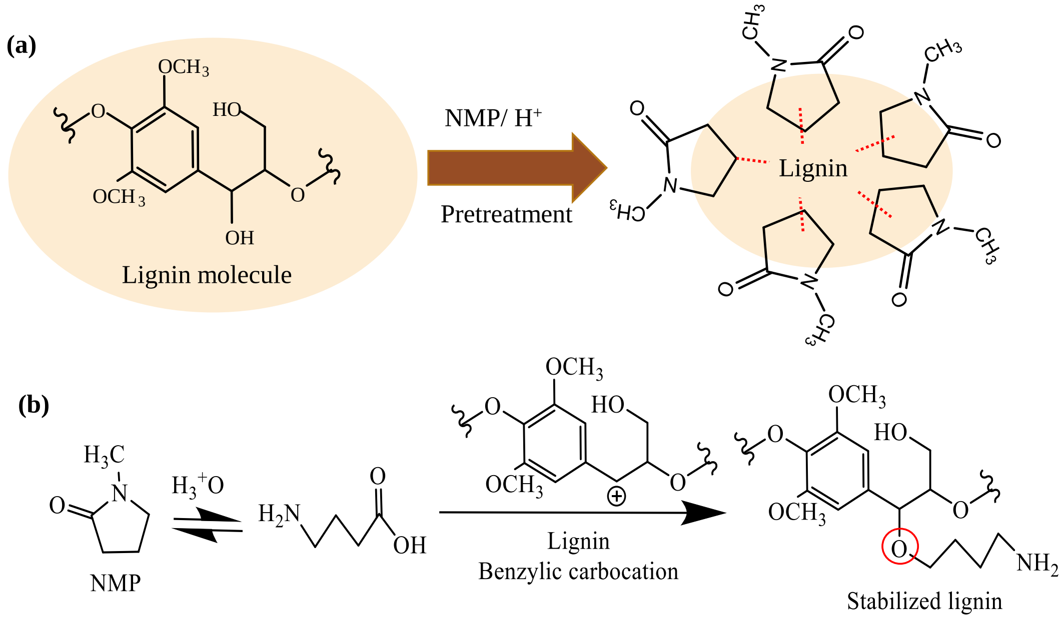


Fig.5.10. Proposed mechanism of lignin dissolution by (a) hydrophobic interaction and (b) stabilization pathway for NMP pretreatment of lignocellulosic biomass.

A small portion of NMP can undergo ring opening reaction to form 4-methylamino butyric acid in aqueous acidic medium, which increases the polarity of the solvent system to synergistically increase the lignin solubility (Lennon et al., 2020). The amount of β-o-4 linkages for NMP pretreatment supported the phenomena of lignin stabilization during pretreatment by NMP or its degradation products. The less intensive correlations of β-β and β-5 linkages in the NMP fractionated lignin also supported the stabilization of lignin structure to prevent its condensation. The possible formation of 4-methylamino butyric acid in water-NMP solution and a stabilization reaction of reactive benzyl carbocation with solvent could happen, reducing the probability of further reactions to form more condensation products (Hibbert ketones, aldehydes, or aromatic polymers) (Meng et al., 2020b).

5.4. Summary

NMP were reported to be a promising candidate for pretreatment in the biorefinery. It demonstrated more than 90% of lignin solubility and 98.2% of hemicellulose removal from the woody biomass. The increased lignin removal was reflected in the almost complete (95%) enzyme digestibility of the pretreated substrates, even at high acid concentrations. The high lignin solubility in NMP and its degradation products stabilized lignin during fractionation to prevent condensation reactions. The quantitative HQSC results showed that the NMP pretreatment preserved 16.4% of original β-o-4 lignin linkages which was much higher than the conventional ethanol pretreatment (11.4%), which suffered from severe lignin condensation in the processes. In comparing to EtOH, NMP seems to be an alternative solvent to fractionate lignocellulosic biomass with less severe degradation and condensation, even when the process was applied to treat the "tougher" *Acacia Confusa* samples with high concentrations of extractives. The methodology and analytical platform established in this chapter shall serve as a benchmark which were further applied to compare with other newly developed pretreatment process in the next chapters.

CHAPTER 6. STAGED ORGANOSOLV PRETREATMENT TO INCREASE NET ENERGY AND REACTIVE LIGNIN YIELD IN WHOLE OIL PALM TREE BIOREFINERY

6.1. Introduction

Palm oil industry is a growing business for cooking oil and biodiesel. According to FAO of the United Nations, the global production of palm oil has doubled in the last decade from 42.4 million tons in 2008 to 71.5 million tons in 2018 (2020). The increased production of palm oil results in a huge amount of palm tree biomass, which possesses economic and environmental concerns for its disposal and management. Empty fruit branch (EFB), trunk, frond, and leaves are the major waste biomass produced from oil palm processing mill, and the accumulated amount of biomass from different parts of oil palm crops per hectare are all significant. The production of EFB generally starts after three years of planting palm tree and seven years to reach the stable production, resulting in approximately 18 to 35 tons per hectare per year for the planting density of 144 trees per hectare (Garcia-Nunez et al., 2016). After a 25-30 years' life cycle, the palm trees need to be eradicated due to the difficulty in harvesting oil fruit from a tall tree. The production of trunks and leaves during the eradication of palm garden is approximately 86.6 and 16.5 tons per hectare (oven-dried basis), respectively for a planting density of 143 palms per hectare at the age of 29 years (Garcia-Nunez et al., 2016). Therefore, the total biomass produced from a palm garden after a cycle is approximately 646 tons per hectare, of which approximately 543, 87, and 17 tons are harvested from EFBs, trunks, and leaves, respectively. In the current practices, oil palm biomass is either slash-and-burnt on-site, or used only for family cooking, which poses serious concerns of greenhouse gas emissions and air pollution

(Dhandapani & Evers, 2020). In 2013, approximately 1.96 million tons of oil palm tree residues, or equivalent 14,757 tera joule (TJ) of heat energy, were burnt in Thailand without sophisticated monitor or control (Prokurat, 2013).

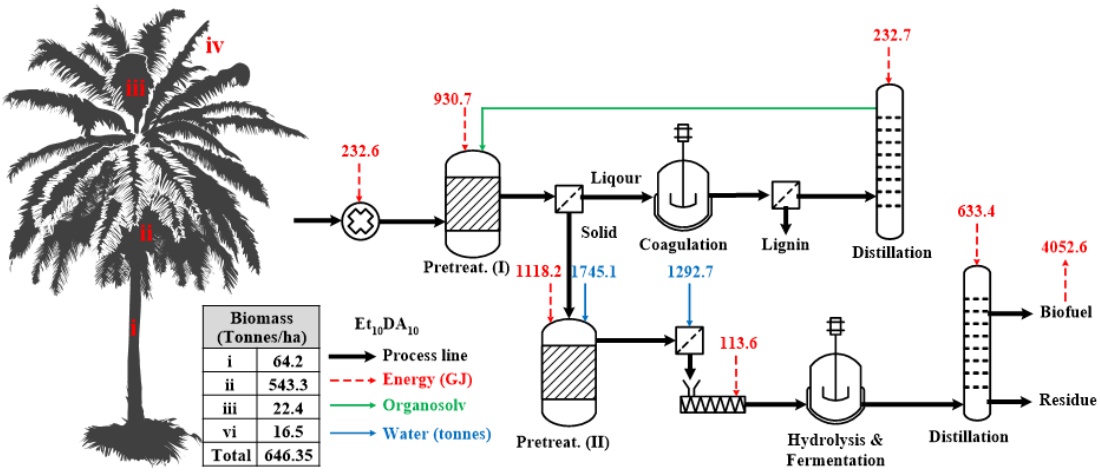
Developing biorefinery technique to fractionate oil palm biomass and convert them into valuable products is an environmentally attractive approach to support the sustainable development of oil palm industry. Palm tree residues is a ready-to-apply feedstock that can be converted into bioethanol or other fermentation products, such as citric acid, lactic acid, and butanol. Bioconversion of palm residues starts with biomass pretreatment, hydrolysis, fermentation, and separation, among which pretreatment is the most crucial and energy-intensive step as it determines the efficiency of conversion process and the type of formed products. The modern pretreatment strategies have paid significant attention on two different scopes, *i.e.*, the whole slurry application which applied pretreatment spent liquors with hydrolysate for co-fermentation (Dong et al., 2018), and fractionation of reactive lignin for further application (Islam et al., 2020b). Based upon the design principles a wide variety of pretreatment technologies, *i.e.*, physical, chemical, biological, and physicochemical pretreatment of lignocellulosic biomass have been extensively studied (Dong et al., 2019; Galbe & Wallberg, 2019). However, the feasibility of the ideal process to treat the oil palm residues collected from different parts of the palm tree has not been clarified. The potential values of lignin, possibility of solvent loss during distillation, and overall energy balance to fit into the oil palm factory is still unclear.

Pretreatments is a biomass-specific process of which the performances vary significantly when different types and forms of biomass apply (Meng et al., 2020a; Xiao et al., 2017; Xu et al., 2017). Thermochemical reaction at 80-220°C is the most popular approach for plant cell wall fractionation; and the common reagents include dilute acid, alkali, ionic liquors, and organosolv (Barakat et al., 2014; Wang et al., 2019a). Among all the pretreatment processes, dilute acid pretreatment is a widely tested approaches for biomass conversion due to its outstanding performance in hemicellulose removal at low cost, however this process also leads into the well-known lignin condensation problems which seriously limits its potential of lignin valorization (Islam et al., 2020b). Acid-catalyzed organosolv pretreatment was therefore developed for its high lignin dissolution (Zhang et al., 2016b; Zhao et al., 2017) and preservation of β-o-4 linkages for downstream utilization (Dong et al., 2019).

The feasibility of organosolv pretreatment to some part of oil palm residues (*i.e.*, EFB) has been demonstrated in a few studies. A study investigated an excellent glucose and lignin recovery using ethanol organosolv at the optimum condition of 160°C, 2% (w/w) sulfuric acid, and 78 minutes cooking time, respectively (Goh et al., 2011). Another study reported an alkaline-based ethylene glycol pretreatment process to fractionate the EFB and achieved a nearly 90% sugar yield in two-staged acid hydrolysis (Chin et al., 2019). The application of alcohol in pretreatment showed outstanding lignin dissociation while providing easily hydrolysable substrates. However, organosolv pretreatments possess critical economic challenges in the costs of the chemicals, although many reports announced that the solvents are theoretically recyclable (Zhao et al., 2009b). In a “lignin-first” biorefinery (Cao et al., 2018), the fractionated lignin dissolved in the organosolv has been harvested through the application of anti-solvent (*e.g.*, large amount of water) followed by solid-liquid separation. The precipitated lignin and dissolved hemicelluloses mixed in the diluted organosolv can be both applicable in chemical processing (but not for fermentation).

As distillation energy is directly proportional to the quantity of the treated liquids, high energy cost is expected in the biorefinery with single-stage pretreatment.

Staged pretreatment is an innovative approach to conditioning the fractionation of the building block chemicals from biomass (Cheng et al., 2015b). As organosolv and acid possess different mechanisms on lignin and hemicelluloses, the two reagents may be applied in separated order to react with the targeted linkages as demonstrated in **Fig.6.1.** In the process, biomass is first pretreated by organosolv and sent to solid/liquid separation. The organosolv with higher purity and dissolved compounds may be more easily processed and recycled through distillation. The pretreated biomass can then be treated in a second pretreatment process to improve the digestibility of the substrate, which can be further hydrolyzed and fermented to form into biofuel and separated by the second distillation tower.



Biomass (Tonnes/ha)	
i	64.2
ii	543.3
iii	22.4
vi	16.5
Total	646.35

Fig. 6.1. Whole oil palm tree and the production rates of different biomass (left) and the potential process units studied in the biorefinery process (a) chipping; (b) pretreatment digester; (c) solid/liquid separation; (d) anti-solvent reactor; (e) filtration and size reduction; (f) saccharification and fermentation; and (g) distillation.

In this chapter, EFB, trunk, frond, and palm leaves were investigated individually in both the one-step and staged pretreatment processes to quantify the values of the whole palm biomass in biorefinery. The changes of chemical compositions, substrate digestibility, and valuable lignin for each oil palm compartments were studied before and after the pretreatment. The results of the study were used to quantify the energy footprint of the process in the production of a benchmarked biofuel (ethanol) and high-value lignin in one hectare of oil palm farm after one plant life cycle (from seeding to clear-cut). The outcomes of the study provide insights to oil palm chemistry, enzymatic hydrolysis, lignin valorization, and whole tree biorefinery.

6.2. Biomass feedstock

The oil palm tree (*Elaeis guineensis*) residues were collected from Satun province of southern Thailand. The EFB, trunk, frond, and leaves were collected after separated harvesting activities and the appearances of the biomass samples after basic size reduction are shown in **Fig.6.2**. The separated samples were oven-dried, milled to 1-2 cm in size using disc mill, and stored in an air-tight plastic container before further experiments.

Fig.6.2. The appearances of oil palm tree biomass after size reduction of (i) trunk; (ii) EFB; (iii) frond; and (iv) leaves.

6.3. Pretreatment design

The biomass samples were pretreated in both single-step and two-stage processes with changing combinations of solvents, reagents, and pretreatment sequence presented in **Table 6.1**. The comparison was also made for ethanol and butanol pretreatment with four major fractions of palm tree residues. The abbreviations of the single-step pretreatment; organosolv followed by dilute acid; and dilute acid followed by organosolv were presented as the type of solvents (*i.e.*, DA for dilute acid, Et for ethanol, and Bt for butanol, respectively) followed by cooking time in subscription. The reactor was loaded with 25 g palm residues mixing with various combinations of solvent/reagents. The loaded reaction temperature was 160ºC and the targeted ratio of solid to total liquid was 1:7. For the single-step pretreatment, organosolv was mixed with dilute sulfuric acid and treated for 20 minutes. For the staged pretreatment, the biomass was first treated with one solvent (dilute H_2SO_4 acid or organosolv) for 10 minutes; then the pretreatment spent liquors were separated from the pretreated biomass by Buchner filter; and remaining solid were introduced to another solvent for pretreatment for 10 additional minutes. For each pretreatment condition, 5g solid sample were collected from the single-step process and two samples were tested for the staged pretreatment. The samples were washed thoroughly with distilled water and stored at 4°C before chemical composition analysis and saccharification experiments.

Table 6.1. Pretreatment conditions for the fractionation of palm residues

Sample	First stage Pretreatment				Second stage Pretreatment			
	Temp. (°C)	Time (min)	Solvent	L/S Ratio	Temp. (°C)	Time (min)	Solvent	L/S Ratio
DA_{10}	160	10	Dilute acid	3	-	-	-	-
Et_{10}	160	10	Ethanol	4	-	-	-	-
Bt_{10}	160	10	Butanol	4	-	-	-	-
$DA_{10}Et_{10}$	160	10	Dilute acid	3	160	10	Ethanol	4
$DA_{10}Bt_{10}$	160	10	Dilute acid	3	160	10	Butanol	4
$Et_{10}DA_{10}$	160	10	Ethanol	4	160	10	Dilute acid	3
$Bt_{10}DA_{10}$	160	10	Butanol	4	160	10	Dilute acid	3
$EtDA_{20}$	160	20	Ethanol + Dilute acid	7	-	-	-	-
$BtDA_{20}$	160	20	Butanol + Dilute acid	7	-	-	-	-

DA_{10}: Dilute acid pretreatment; Et_{10}: Ethanol pretreatment; Bt_{10}: Butanol pretreatment; $DA_{10}Et_{10}$: Dilute acid followed by ethanol pretreatment; $DA_{10}Bt_{10}$: Dilute acid followed by butanol pretreatment; $Et_{10}DA_{10}$: Ethanol followed by dilute acid pretreatment; $Bt_{10}DA_{10}$: Butanol followed by dilute acid pretreatment; $EtDA_{20}$: Acid catalyzed ethanol pretreatment; $BtDA_{20}$: Acid catalyzed ethanol pretreatment. Subscripts represents the pretreatment time.

6.4. Results and discussion

6.4.1. Chemical composition of Oil palm tree residues

The chemical compositions of different parts of the raw and pretreated palm biomass, *i.e.*, (a) EFB, (b) trunk, (c) frond, and (d) leaves are demonstrated in **Fig.6.3(a)** to **Fig.6.3(d)**. All the experiments were conducted twice, and the results were expressed as an average. EFB contained the highest amounts of cellulose (36.1%), hemicellulose (25.8%), and lignin (23.39%) while the leaves contained the lowest amount of cellulose (25.14%), hemicellulose (18.80%), and lignin (15.53%). The minor ingredients composition (extractives & ash) of trunk and frond were almost similar, while leaves contained significantly higher amounts of extractives (14.2%, mostly water extractable), crude protein (8.0%), and ashes (5.1%). The chemical compositions of the palm biomass confirmed with the previous reported data (Ahmad et al., 2019). It should be notified that some publications reported very high lignin contents in similar samples which may be resulted from uncalculated extractives and crude protein during the two-stage acid hydrolysis. The presence of small amount of crude protein does not significantly affect the performance in the organosolv pretreatment since the acidic aqueous medium enhances the extraction of nitrogen from biomass (Jazrawi et al., 2015). Polar and non-polar extractives can easily be withdrawn from the substrates by the water and organosolv during pretreatment (Sluiter et al., 2005). However, the high content of crude protein and extractives may reduce the pretreatment efficiency due to competitive dissolution with the targeted components towards the saturation point of solvent.

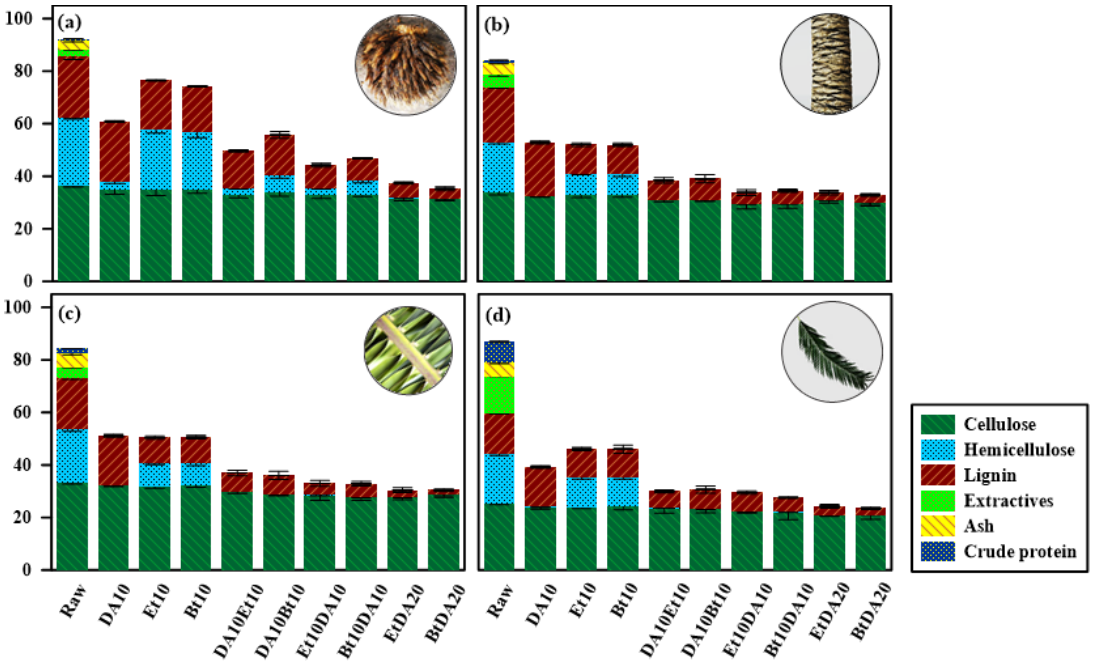


Fig.6.3. Chemical composition of the raw and pretreated palm tree residues for (a) EFB; (b) trunk; (c) frond; and (d) leaves.

In addition, different biomass with varied chemical compositions showed its potential in producing different products. For example, leaves contain low lignin so it can be more suitable to produce cellulose fibre or bioethanol production (Fareez et al., 2018). High-lignin containing empty fruit branch may be more suitable to produce high-quality lignin (Shuai et al., 2016a) and the trunk may be used for construction purpose. This study evaluated only the feasibility of the various biomass in a biorefinery, using the proposed stage pretreatment concept.

Among the three groups of primary pretreatments of the two-step processes (*i.e.*, DA_{10}, Et_{10}, Bt_{10}), DA_{10} showed complete hemicellulose removal for the trunk, frond, and leave biomass samples, while approximately 90% hemicellulose removal was obtained for EFB. DA pretreatment is a well-recognized technology for effectively solubilizing hemicelluloses in the biomass, leaving cellulose and lignin as water-insoluble residues (Mesa et al., 2017). However, EFB showed the lowest hemicellulose removal at same pretreatment conditions, which may be due to the highest amount of cellulose and recalcitrant lignin in the biomass. Highly ordered structure of cellulose as well as the highest amount of lignin limited the excess of reagents to the targeted linkages (Xu et al., 2019). The OS (Et_{10} & Bt_{10}) pretreatment cannot completely remove the hemicelluloses but can dissociate various and considerable amounts of lignin from 20.8-24.1% (EFB) to 46.2-49.1% (frond). This lignin may offer great values to the downstream applications as detailed in the next section.

Acid-catalyzed single step organosolv pretreatment showed the highest performance in lignin and hemicellulose removal, as expected. The lignin removal of DA-first process was the lowest (2-3%), which may be mostly due to condensation (Dong et al., 2019), which is initiated by acidolysis of lignin to the formation of condensed structure. Furthermore, it should be emphasized that the condensation effects were not observed in the first stage of OS-DA process which may be due to the lower tendency of lignin acidolysis and stabilization of lignin carbocation by organosolv. Using trunk as an example, the lignin removal in the OS-DA process may be classified into two stages. In the OS process, approximately 50% of lignin has been removed with the cleavage of the ether linkages by the ions created from the solvolytic cleavage of organosolv. The second stage of the lignin removal occurred in the DA process, which may be mostly originated from the removal of lignin-hemicellulose structure. Lignin condensation may occur in this process, resulting in reduced quality of the fractionated lignin. The single-stage process created approximately 40% additional lignin removal than the staged process ($Et_{10}DA_{10}$ and $Bt_{10}DA_{10}$), which was mostly due to the synergetic effects of simultaneous cleavages of hemicellulose-lignin and lignin-lignin linkages (Leu & Zhu, 2013). The strong hydrolysing effect of the acid catalyst resulted in effective hydrolysis and effective removal of both hemicellulose and lignin. However, the presence of acid catalyst also led to the formation of condensed structure of lignin which reduced the digestibility of substrate and valorization potential of lignin (Dong et al., 2019). Therefore, the lignin produced in single-stage process contained lignin with significant amount of condensed structure which resulted the lower valued lignin than the staged process. Although the hydrolysis performance of the two-stage process approached to the single-step process but three types of lignins with various reactivity can be recovered. This facilitated the overall fractionation efficiency of staged pretreatment process.

6.4.2. Enzymatic hydrolysis

Saccharification is the process of hydrolyzing cellulose polymer to building block monosaccharides. The saccharification efficiency or digestibility of pretreated cellulose is influenced by the pretreatment efficiency and structural features of substrate. Enzymatic hydrolysis was carried out at 3% solid loading and at 15 FPU enzyme loading per gram of cellulose in the substrate. The CEDs of all four parts of palm residues showed a similar trend of first-order kinetics which is illustrated in **Fig.6.4(a)** to **Fig.6.4(d)**. The cellulose digestibility of pretreated substrate increased sharply at the beginning and almost plateaued after 24 hours of hydrolysis. For DA pretreated EFB, the CED was 49% at 72 hours of hydrolysis which was higher than the OS pretreated EFB (20%). DA pretreatment can effectively reduce the biomass recalcitrance through the removal of hemicellulose, cleavage of β-o-4 linkage, structural modification of biomass, and enhance the surface area accessibility of cellulose to enzyme due to the complete hemicellulose removal (Leu & Zhu, 2013). Since almost complete hemicellulose removal was achieved in DA pretreatment, the hydrolysis yield of the substrate was primarily affected by the lignin content and its structure. With only partial removal of hemicellulose and lignin, the OS substrates resulted in the lowest hydrolysis efficiencies.

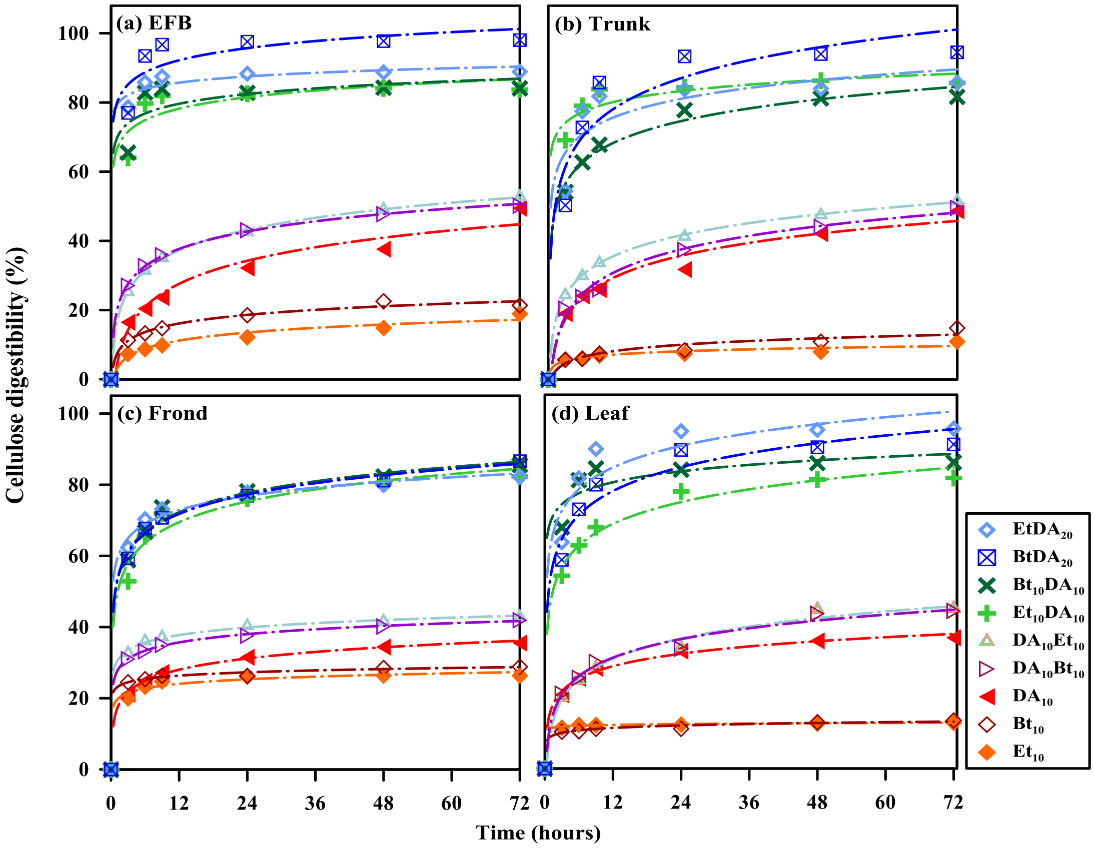


Fig.6.4. Cellulose digestibility over time for pretreated oil palm tree residues of (a) EFB; (b) trunk; (c) frond; and (d) leaves.

The CEDs of different biomass samples at 72 hours of enzymatic hydrolysis are demonstrated in **Fig.6.5**. The hydrolysis efficiencies of different samples collected from different parts of the oil palm varied significantly among the pretreatment processes even though the chemical compositions of some samples were quite close. The CED_{72} of DA-OS pretreated EFB was only 50.2% but the OS-DA one was as high as 84.1%. DA-OS pretreated substrate experienced lignin condensation in the first stage that significantly reduced the CED of the substrate. Condensed lignin is more hydrophobic due to the removal of aliphatic hydroxyl groups and the formation of resinous coproducts (Dong et al., 2019), hence interfere the productive interactions between the substrate and enzyme (Wang et al., 2019c). Although the OS-DA pretreatment resulted in comparatively the lower lignin removal but produced a similar hydrolysis yield as the single-stage pretreatment, indicating that the lignin structure is more influential than lignin content for enzymatic hydrolysis.

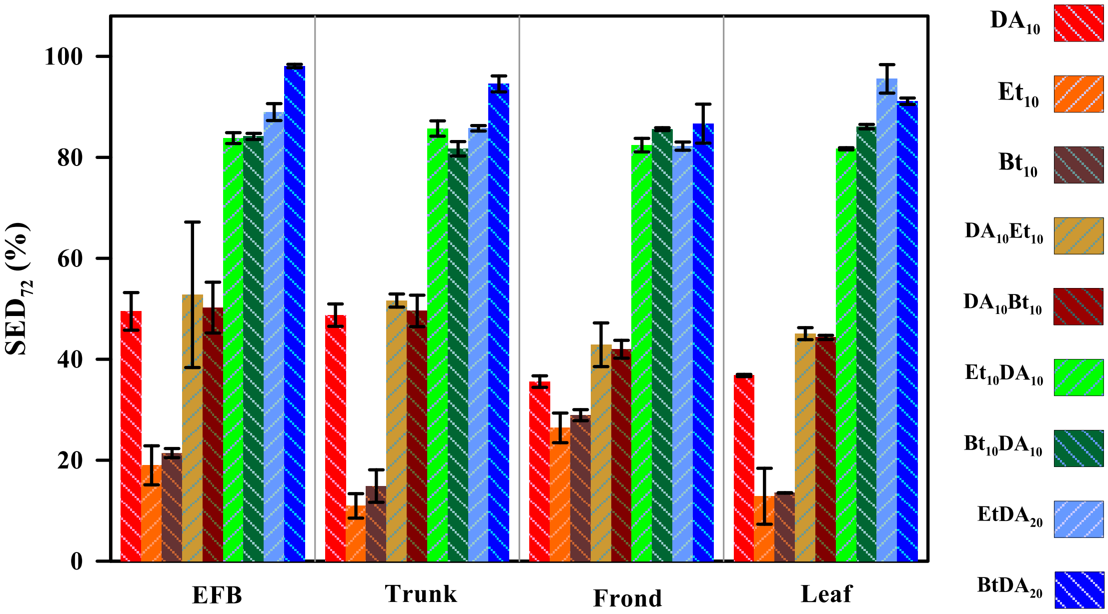


Fig.6.5. The CED at 72 hours for pretreated oil palm tree residues such as (a) EFB; (b) trunk; (c) frond; and (d) leaves.

6.4.3. Characterization of residual lignin

The aliphatic sidechain (δC/δH 50−75/3.0−5.0) and aromatic (δC/δH 100-135/5.0-8.0) regions of 2D-HSQC NMR spectra ($_1H_{13}C$) of the residual lignin harvested from the $DA_{10}Bt_{10}$ and $Bt_{10}DA_{10}$ processes are shown in **Fig.6.6(a)** and **Fig.6.6(b)**, respectively. The integration of the contours volume of peaks was used to determine the relative abundance of corresponding peaks. The aromatic units of lignin were used as an internal standard to determine the relative abundance of all other linkages.

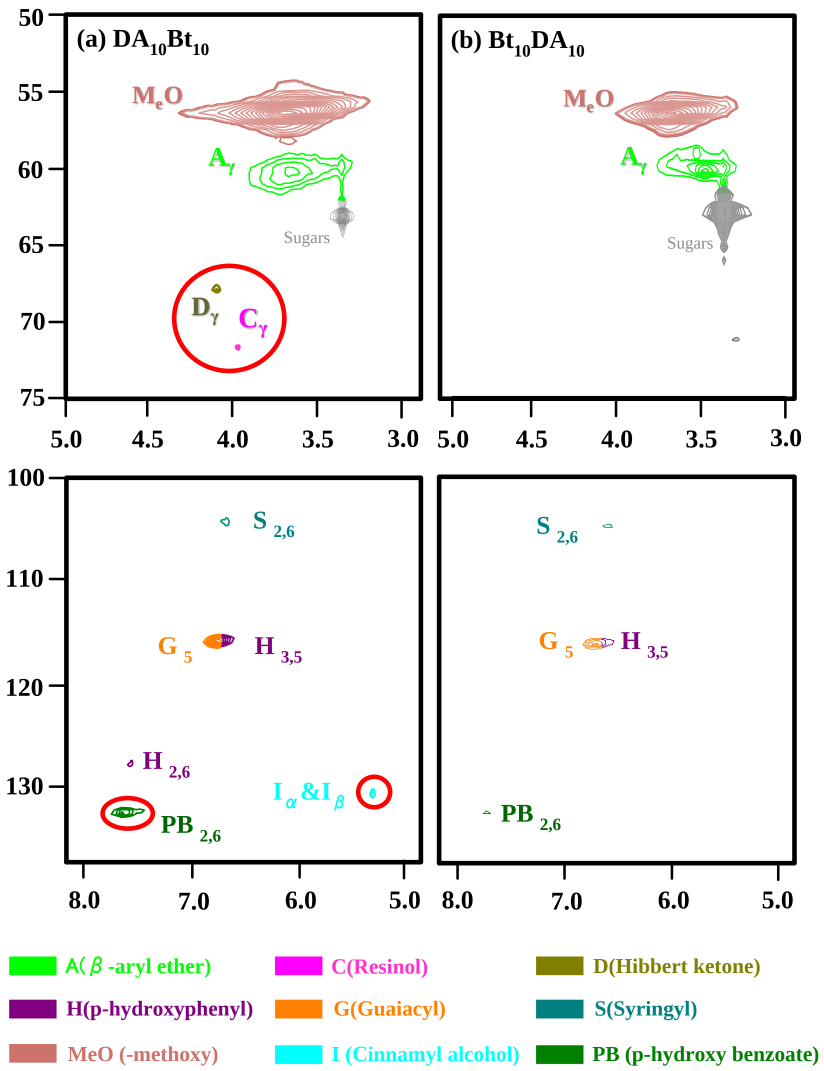


Fig.6.6. 2D-HSQC NMR spectrum of residual lignin isolated after hydrolysis of (a) $DA_{10}Bt_{10}$ (DA-OS) and (b) $Bt_{10}DA_{10}$ (OS-DA) pretreatments.

The structural difference of DA-OS and OS-DA pretreated lignin of palm biomass was obvious due to lignin condensation through different pretreatment approaches. In the NMR spectra, the α position of β-o-4, β-5, and β−β interunit linkages was not detected in the aliphatic region of the residual lignin due to the presence of these groups under the detection limit. During pretreatment, lignin experienced subsequent cleavage of these linkages producing highly depolymerized lignin with quinoid end-group in spirodienone which prevents cross-linking of lignin fractions and thus facilitates dissolution of fractionated lignin in the organosolv liquor (Lancefield et al., 2018; Zhao et al., 2020). This result revealed that both pretreatment sequences resulted in nearly complete removal of β-o-4 linkages of the residual lignins with varying degrees of acidolysis in lignin. The structure of residual lignin was also altered by the cleavage of linkages and cross-linkages of lignin polymer. The alteration of structure enhanced the fractionation of biomass components and hydrolysis efficiency of the substrate, while the condensed structure had a strong negative effect on both parameters. The distinctive signals in the side chain region appeared at δC/δH 67.88/4.10 and 71.60/3.96 ppm may be due to the presence of Hibbert ketone (D) and resinol (C) structure in the pretreatment (Wang et al., 2017a). The Hibbert ketone was formed by acidolysis of lignin started with the formation of carbocation by protonation of the benzylic hydroxyl groups and ended up with the subsequent loss of proton and hydrolysis of oxonium intermediates (Zhao et al., 2020). Hibbert ketone and resinol are condensed form of lignin and are responsible for the lower digestibility of the substrates. The condensed structures contributed to approximately 21.7% of the total DA-OS pretreated lignin which decreased its potential to produce aromatic chemicals. On the other hand, organosolv pretreatment prevents lignin condensation by stabilizing lignin carbocation formed during fractionation (Zhao et al., 2017). In aromatic region, the syringyl, guaiacyl, p-hydroxyphenyl, and para-hydroxybenzoate correlations appeared at δC/δH 104.28/6.65, 116.28/6.82, 129.80/7.68, and 132.13/7.63 ppm respectively from DA-OS pretreated lignin. The corresponding signals of building block lignin units (S, G, and H) were also found in the aromatic region of OS-DA lignin at the lower intensity. However, the cinnamyl alcohol (δC/δH 130.76/5.27) peak did not show up in OS-DA lignin which indicated the insignificant amount of derivatives formation and the higher integrity of reactive lignin for OS-DA pretreatment. High integrity of lignin increases the solubility of lignin fractions under the reaction conditions as well as facilitated the production of valuable aromatic monomers (Zhang et al., 2017).

The morphological changes of lignocellulosic structure for palm trunk before pretreatment, lignin after OS-DA pretreatment, and DA-OS pretreatment under SEM are demonstrated in **Fig.6.7(a)** through **Fig.6.7(c)**, respectively. The pretreated lignin harvested from substrate residues, after repeated enzymatic hydrolysis, showed significant differences in particle size and shapes. The biomass before pretreatment demonstrated the complex cell wall structure of which lignin was embedded in the building-block matrix (**Fig.6.7(a)**). In OS-DA pretreatment, lignin extruded out of the plant cell wall and dissolved in the pretreatment liquor (Donohoe et al., 2008), and the residual lignin formed into a homogenously distributed small particles (approximately 50-100 µm in diameter) after enzymatic hydrolysis. With the removal of soluble lignin and carbohydrates, the lignin residue showed a rough surface in comparison with the cell wall. The pretreated lignin in DA-OS process (**Fig.6.7(c)**) demonstrated smoother surface and larger particles than ones harvested in OS-DA process. The condensed lignin settled as droplets during isolation and purification process (Donohoe et al., 2008).

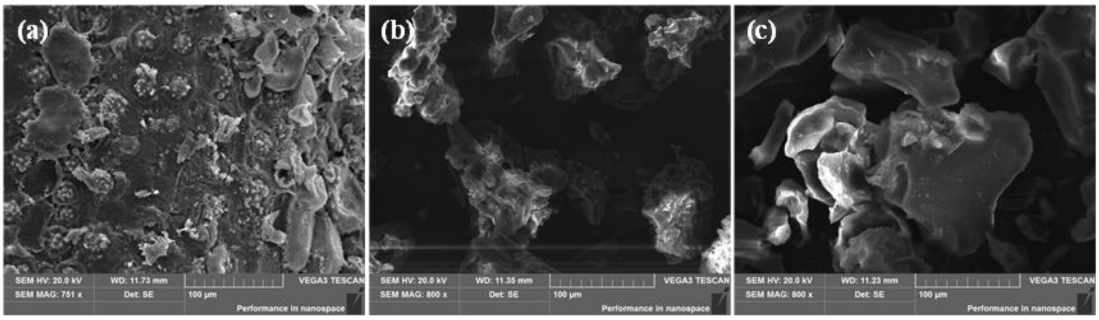


Fig.6.7. Scanning Electron Microscopic images of (a) palm trunk before pretreatment (b) residual lignin after OS-DA pretreatment, and (c) residual lignin (condensed

lignin) after DA-OS pretreatment. Magnification is provided in the left bottom and scale bar in the right.

6.4.4. Integrated biorefinery process for bioethanol and lignin production

The mass balance of the integrated whole palm tree biorefinery was conducted to determine the overall production of ethanol and lignin products (**Fig.6.8)**. The calculation was based on the total biomass produced per hectare of palm oil farm after one harvesting cycle (*i.e.*, seedling, oil/biomass harvesting, and then clear-cut). The OS-DA pretreatment facilitated the fractionation of biomass by removing lignin and hemicellulose while keeping cellulosic substrate in the water-insoluble fraction. The lignin and hemicelluloses were reproduced from the pretreatment liquor, respectively.

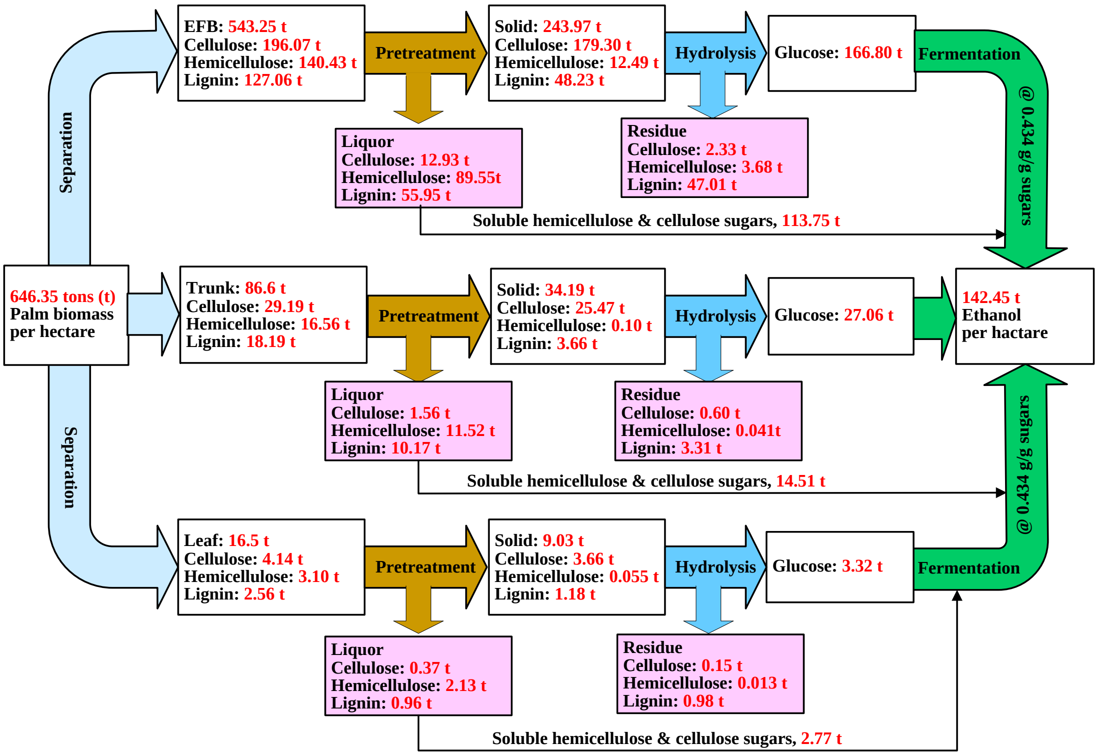
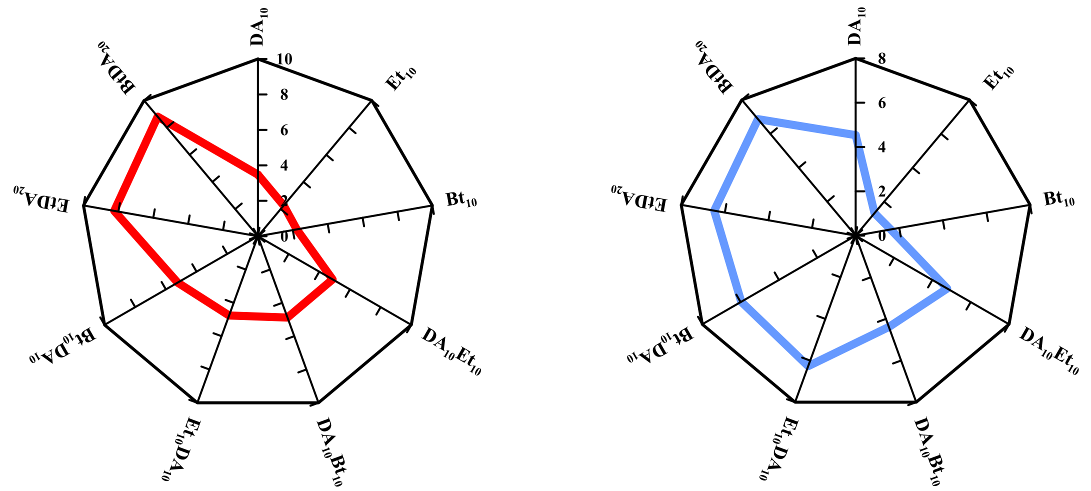


Fig.6.8. Mass flow of biomass in the integrated process for bioethanol, lignin, and other products from the whole palm residues per hectare per oil harvesting cycle. The mass in each step were quantified using conversion yields and the percentage of desired ingredients. All numbers are expressed in tons.

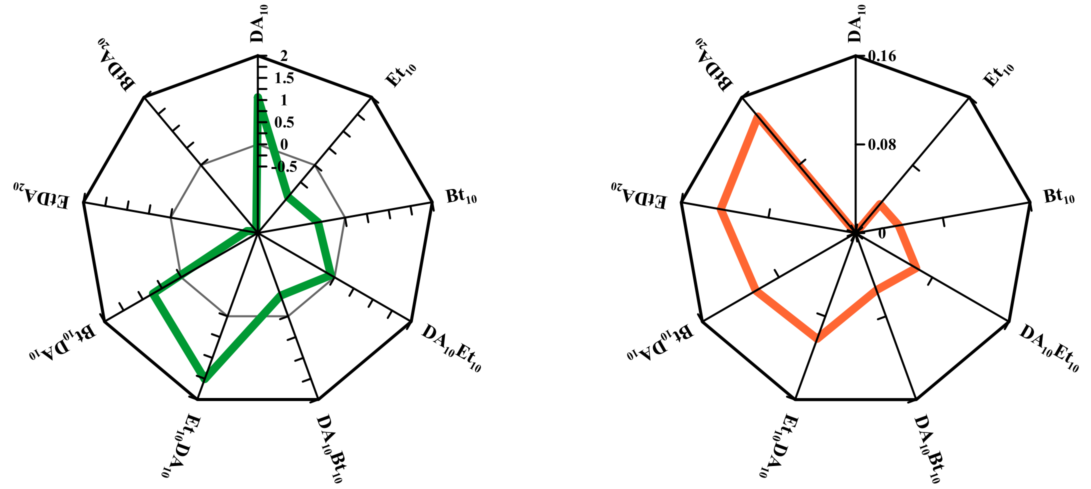
A total dry biomass of 645.4 tons can be produced in the farm, of which include 543.3 tons, 86.6 tons, and 16.5 tons of EFB, trunk, and leaf, respectively (Garcia-Nunez et al., 2016). Process integration of pretreatment and saccharification resulted 197.2 tons of glucose from cellulose-rich substrate while 131.03 tons of sugars (hemicellulose and cellulose) recovered from the pretreatment liquor. Co-fermentation produced 4.34 g/L of ethanol from 10 g/L of total sugars (cellulose and hemicellulose) in the medium with yield of 0.434 g/g of total sugars. At this rate, about 142.45 tons of ethanol can be produced from the overall process. Approximately 67.1 tons of OS lignin can be harvested from the pretreatment liquor which can be used to produce other value-added chemicals. Lignin quality is a determining factor for selection of proper pretreatment approaches. The OS-DA pretreatment produced the highest amount of reactive and non-condensed lignin that indicated the benefit of two-stage pretreatment.

6.4.5. Energy balance of overall process

Total energy consumptions, net energy, and products output of palm tree biomass conversion are demonstrated in **Fig.6.9(a)** to **Fig.6.9(d)**, and the values were compared with the different pretreatment approaches. The energy was calculated for the conversion of 1-ton dry biomass and expressed in giga-joule (GJ). The energy consumption in the pretreatment stage is a function of the desired temperature and the amount of solvent used in the fractionation biomass components.


(a) Energy Consumption (GJ)

(b) Biofuel Energy (GJ)


(c) Net Energy Output (GJ)

(d) High Quality Lignin (ton)

Fig.6.9. Energy and product yields per ton of whole palm tree biomass for different bioconversion scenarios.

Pretreatment was the most energy-intensive process of bioconversion of palm residues, which was calculated for 47.4% to 69.6% of the total energy consumption for different pretreatment approaches. The substrate was cooled and washed with water to remove residual solvent and inhibitory components of enzyme hydrolysis. The energy consumption in size reduction for hydrolysis and fermentation processes was the least, and it was accounted for 5.75% to 24.25% of the total energy consumption, which is a function of biomass components during pretreatment process.

The net energy consumption of distillation was affected by the types and compositions of the solvents (water-organosolv mixture) used in the pretreatment processes, which varied upon the heat capacities and latent heats of the individual solvent. A wide range of 0.28-2.92 GJ energy was consumed per ton of dry biomass in the distillation processes depending on the pretreatment processes, accounting for 12.47% to 46.80% of the overall energy consumption. The energy consumption of different biomass fractions showed similar trends, and the maximum energy was obtained from EFB conversion. Although the total bioethanol production of staged OS-DA process was similar to that of single-step pretreatment, much higher net energy production was found for the staged OS-DA due to the less energy consumption in the distillation and solvent recovering process **Fig.6.9(b,c)**. Staged pretreatment facilitated separation of fractionated components with minimum amount of solvents and thus reduced the energy consumption in the recovery process. The pretreatment also produced very high-quality lignin compared to single-step process and thus increased the feasibility of staged OS-DA pretreatment in the bioconversion process **Fig.6.9(d)**. The use of ethanol as organosolv was more energy efficient in compared to butanol due to the lower energy consumption in the distillation and product separations. The net energy production in staged process was higher in comparison with the reported literature for DA (Mesa et al., 2017), dilute alkali (Park & Kim, 2012), deep eutectic solvent (Ai et al., 2020)(Ai et al., 2020), organosolv (Pan et al., 2007), and ionic liquid (An et al., 2015) pretreatment using the same assumptions.

It should be noted that the mass balance presented in **Fig.6.8** and energy balance in **Fig.6.9** both applied the results of lab-scale experiments to support the prediction of full-scale operation which can produce at least 200 tons ethanol per day (**Fig.6.1**). Certain simulation errors may be expected with varied heat and mass transfer between different reactor systems at increased scales. As similar performances for different reactor scales have long been studied in pulping industry (Sewsynker-Sukai et al., 2020), however, it was assumed that the presented results among different pretreatment designs shall still project into reasonable comparison in full-scale. In addition, it should also note that the inclusion of additional processes (*i.e.*, coagulation tank, solid/liquid separation unit, and distillation tower) will unavoidably increase the capital, operational and maintenance costs of the overall system, but this investment would hopefully be compensated by long term environmental benefits, *i.e*., increased energy production, lignin yield, and solvent recycle potential. The high-cost pretreatment unit may be carried out in one digestion vessel under a sequestration operational mode to reduce the cost.

6.5. Summary

The goal of this chapter was to develop a staged pretreatment process to increase the yield of reactive lignin, recycle solvent, and save energy in oil palm biomass biorefinery. OS-DA pretreatment resulted in approximately 969.5 GJ of net energy per hectare of oil palm farm and also offered opportunity to harvest high-quality lignin as confirmed by 2D-HSQC NMR. With the same chemical and energy inputs, OS-DA pretreatment offered 30-40% higher hydrolysis yield than that of DA-OS process due to significant different lignin structures.

CHAPTER 7. BIPHASIC PRETREATMENT FOR ENERGY AND CARBON EFFICIENT CONVERSION OF LIGNOCELLULOSE INTO BIOENERGY AND REACTIVE LIGNIN.

7.1. Introduction

Lignocellulose biorefinery is expected to be an alternative to petroleum refinery for sustainable production of biofuel and chemicals (Banerjee et al., 2016). Cellulose, hemicellulose, and lignin are the major components of lignocellulosic biomass which can be used as the building-block chemicals for many other chemicals (Islam et al., 2020a; Wang et al., 2019a). For example, cellulose and hemicellulose sugars can be used to produce bioethanol, biobutanol, furfural, lactic acid, GVL etc. Lignin can be utilized to produce polyphenol, polyurethanes, phenol resin, carbon fibres, and other valuable aromatic chemicals (Berlin, 2013). However, lignocellulosic biomass possesses strong recalcitrance to biodegradation due to the complex structure of cell wall components *i.e.* cellulose, hemicellulose, and lignin (McCann & Carpita, 2015). The network structures and strong interaction between hemicellulose and lignin limit the cellulose accessibility due to the strong interaction between hemicellulose and lignin. Therefore, pretreatment is the most critical step in the biorefinery which can fractionate lignocellulose components by altering the structure of cell wall components to enhance the accessibility of cellulose to different chemicals and enzymes for efficient conversion (Zhao et al., 2012b). In addition, pretreatment is the most energy-intensive process in biorefinery which largely determines the efficiency of lignocellulose conversion (Kumar et al., 2009).

Over the decades, numerous studies have been devoted to effective fractionation to maximize the efficiency of the biomass valorization process (Rezania et al., 2020;

Yoo et al., 2020). Liquid hot water, dilute acid, dilute alkali, organosolv, ionic liquid, steam explosion, ammonia fibre expansion pretreatment are the most common pretreatment methods for lignocellulose (Chiang et al., 2012; Kumari & Singh, 2018). Among these methods, dilute acid pretreatment is a cost-effective method to remove biomass recalcitrance to produce energy and chemicals (Kapoor et al., 2017). However, DA pretreatment encounters low digestibility of substrate and low-value residual lignin due to severe condensation during pretreatment (Islam et al., 2021). With the benefit of lignin recyclability, organosolv among other chemical methods is getting more attention in lignocellulosic biorefinery (Pan et al., 2007). In organosolv pretreatment, lignocellulose is fractionated by dissolving hemicellulose and lignin in the water-organosolv mixture leaving cellulose as an insoluble pulp which has high digestibility (Zhao et al., 2017). To implement a sustainable lignocellulosic biorefinery, not only fractionation of major components is important, the energy and carbon efficiency of the fractionation process must be considered. After organosolv pretreatment, the separation of lignin from the spent liquor and the recovery of organic solvent usually complicates the overall process. Lignin and hemicellulose are commonly fractionated through the use of large amount of water as an antisolvent (Pan et al., 2007). The addition of water significantly increases the energy consumption in distillation to concentrate dissolved sugars in the liquor and organic solvent recovery (Dong et al., 2018; Islam et al., 2021).

Lignin-first biorefinery has been receiving increasing interest recently as this holistic biorefinery concept focuses on reducing later lignin depolymerization/modification steps during the fractionation process which broadens the application of lignin (Calvaruso et al., 2017; Renders et al., 2017; Van den Bosch et al., 2017). Organosolv pretreatment with water and immiscible organic solvent facilitates the physical separation of biomass components in three different phases. Hydrolyzed hemicellulose and lignin are dissolved in the aqueous and organic phases respectively while cellulose fraction remained as a solid residue (Zimmermann et al., 2018). A previous study using one-pot catalytic n-butanol/water biphasic system was investigated to convert eucalyptus sawdust into a cellulose pulp, hemicellulose-derived polyols, and lignin-derived phenolics (Renders et al., 2018). The valorization of hemicellulose and lignin was achieved at 200℃ temperature in presence of Ru/C catalyst with hydrogen pressure. The phase separation at lower temperature offers a facile strategy to separate lignin-derived chemicals in butanol and polyols in water. Another example is an acid-catalyzed water/n-butanol biphasic system which promoted the separation of biomass components in three different phases of which lignin can be recovered and cellulose residues have 96% enzyme digestibility (Schmetz et al., 2019). Wang et al., reported a one-pot mild fractionation technique for poplar wood using a phenol/water system to increase the processibility of wood components (Wang et al., 2020). The study reported about 90% lignin removal with 73% cellulose retained in the solid substrate under the optimized condition at 120℃.

Although biphasic organosolv pretreatment can ease the fractionation of dissolved sugars and lignin, the challenges still remains due to the poor mass transfer between solvents (Zimmermann et al., 2018). The partial solubility of butanol and phenol (8-10%) in water (Liu et al., 2005) at room temperature showed that a significant portion of solvent will be wasted during separation. Also, the aqueous phase may contain inhibitors such as phenol which may need additional extraction before biorefinery application. Moreover, the lignin integrity and modification after pretreatment would highly affect its further application. To address these issues, an integration of lignin-first fractionation and biphasic organosolv pretreatment was proposed which can realize the effective fractionation of lignin with high energy and carbon efficiencies to produce bioethanol and reactive lignin from the whole biomass.

Herein, an integrated biorefinery was designed and investigated using pentanol-water biphasic pretreatment of *Acacia Confusa* wood to simultaneously produce reactive lignin as well as a carbohydrate pulp which was amenable to bioethanol production. Pentanol is almost immiscible (1.7-2%) with water which can facilitate more efficient solvent recovery to physically separate hemicellulose and lignin fractions (as shown in **Fig.7.1**). The major process units of biorefinery *i.e.*, pretreatment, fractionation/distillation, saccharification, and fermentation were fully studied to calculate energy footprints for bioethanol production and reactive lignin recovery. The energy benefit was determined for the proposed fractionation process by comparing homogenous organosolv pretreatment (ethanol pretreatment) and dilute acid pretreatment. The advantageous effect of pentanol-water pretreatment on lignin quality/reactivity was also assessed by quantifying the amount of different linkages and molecular weight of the fractionated lignin. Finally, carbon efficiency was quantified to determine the potential of mitigating GHG emissions from biorefinery conversion.

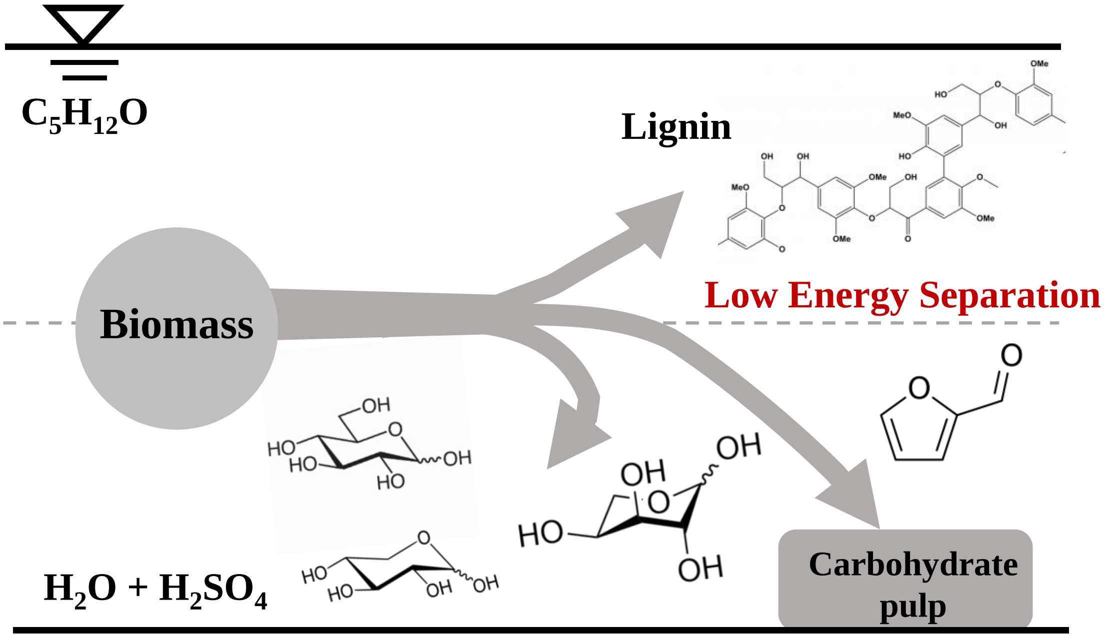


Fig.7.1. Conceptual diagram of pentanol-water biphasic pretreatment for integrated biorefinery process to produce fuels and chemicals.

7.2. Pretreatment design

About 50 g of *Acacia Confusa* wood residues were cooked in each digester with various combinations of temperature, solid to liquid ratio, solvent, and catalyst compositions to optimize pentanol-water biphasic solvent (**Table 7.1)**. For comparison and optimization, one parameter was varied while keeping all other parameters constant.

Table 7.1. Pretreatment conditions for optimization of pentanol-water biphasic solvent system for *Acacia Confusa* wood residues.

Sample	**Pretreatment conditions**			
	(%) Pentanol	**(mM) H_2SO_4**	**°C Temp.**	**Liquid/ solid**
Untreated	-	-	-	-
$P_{00}C_{45}T_{170}S_7$	0	45	170	7
$P_{10}C_{45}T_{170}S_7$	10	45	170	7
$P_{20}C_{45}T_{170}S_7$	20	45	170	7
$P_{35}C_{45}T_{170}S_7$	35	45	170	7
$P_{50}C_{45}T_{170}S_7$	50	45	170	7
$P_{65}C_{45}T_{170}S_7$	65	45	170	7
$P_{80}C_{45}T_{170}S_7$	80	45	170	7
$P_{95}C_{45}T_{170}S_7$	95	45	170	7
$P_{65}C_{00}T_{170}S_7$	65	0	170	7
$P_{65}C_{15}T_{170}S_7$	65	15	170	7
$P_{65}C_{30}T_{170}S_7$	65	30	170	7
$P_{65}C_{45}T_{170}S_7$	65	45	170	7
$P_{65}C_{60}T_{170}S_7$	65	60	170	7
$P_{65}C_{85}T_{170}S_7$	65	85	170	7
$P_{65}C_{100}T_{170}S_7$	65	100	170	7
$P_{65}C_{120}T_{170}S_7$	65	120	170	7
$P_{65}C_{45}T_{170}S_5$	65	45	170	5
$P_{65}C_{45}T_{170}S_9$	65	45	170	9
$P_{65}C_{45}T_{140}S_7$	65	45	140	7
$P_{65}C_{45}T_{200}S_7$	65	45	200	7

The pretreated samples were abbreviated as $P_iC_jT_kS_l$; where P_i was the pentanol composition; C_j was the catalyst concentration; T_k was the temperature, and S_l was the liquid to solid ratio. The values of the respective parameters were shown in the subscription.

To compare pentanol-water with conventional solvents, three different biorefinery scenarios were investigated to produce building-block sugars/basic fuels and reactive lignin for value-added chemicals from *Acacia Confusa* wood residues.

The major process units and products obtained from all three approaches are demonstrated in **Fig.7.2**.

Scenario-I (S-I): Dilute acid was used to fractionate lignocellulose sugars due to the strong hydrolyzing effect of acid in the aqueous medium. Wood chips were mixed and heated at 170°C for 30 minutes in a stainless-steel reactor with a solid-liquid ratio of 1:3. Carbohydrate sugars were hydrolyzed and fermented to produce bioethanol while lignin was obtained as a solid residue after SSF of pretreated substrates.

Scenario-II (S-II): Ethanosolv pretreatment fractionates lignocellulose components and provides an option to recycle fractionated lignin. The solvent was prepared by mixing 65% ethanol and 45 mM H_2SO_4 as catalyst. Pretreatment was carried out at 170°C for 60 minutes with a solid-liquid ratio of 1:7. Lignin was precipitated from the pretreatment liquor using water as anti-solvent. Liquor was distilled to recover solvent (ethanol) and dissolved sugars for further use. Carbohydrate pulp and liquor sugars were subjected to saccharification and fermentation to produce bioethanol.

Scenario-III (S-III): Pentanol-water biphasic pretreatment was carried out under optimized conditions (65% pentanol, 170°C, 45mM H_2SO_4, and 1:7 solid-liquid ratio), aiming for energy-efficient fractionation of lignocellulose. The pretreatment liquor was physically separated into aqueous (dissolved sugars) and organic fractions (containing lignin) by a separatory funnel while leaving cellulose as a solid residue. The aqueous phase was used for fermentation after neutralization by KOH using the BLBIO-2020 model pH controller. Pentanol was recycled by rotary vacuum evaporation from organic fractions to for reuse in the pretreatment process. The organic phase containing lignin can be directly utilized to produce lignin foam or lignin monomer for high-value applications (Zimmermann et al., 2018).

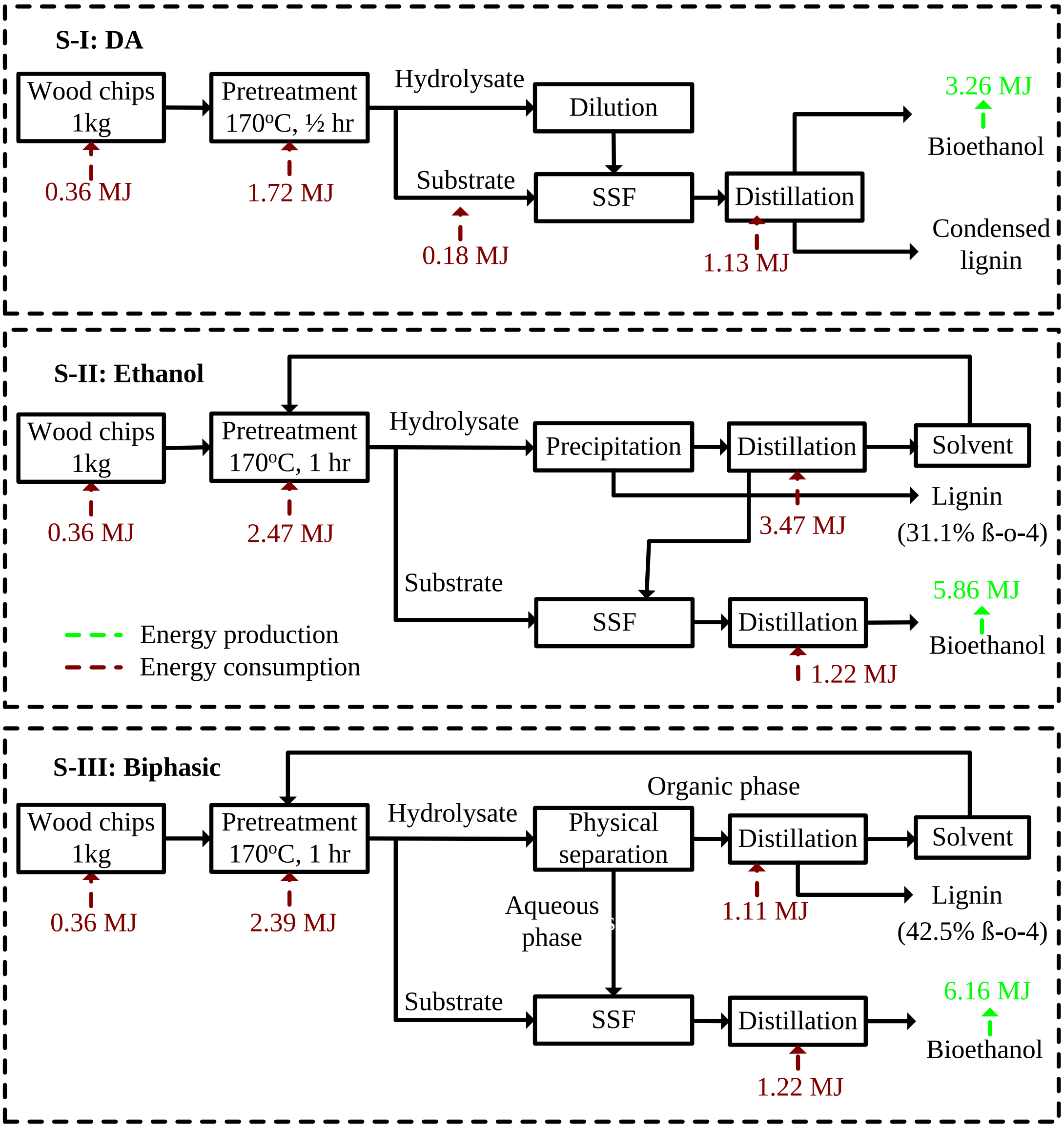


Fig.7.2. Demonstration of major unit operations and unit process for (S-I) dilute acid, (S-II) ethanol pretreatment, and (S-II) pentanol-water biphasic pretreatment system.

7.3. Results and discussion

The pretreatment of biomass reduces inherent lignocellulose recalcitrance which ultimately increases the enzymatic digestibility of cellulose residue (Leu & Zhu, 2013). The ultimate objective of this pretreatment design was to remove lignocellulose recalcitrance by dissolving hemicellulose and lignin in the pretreatment liquor while leaving the maximum amount of cellulose as a solid residue. Three scenarios, namely DA (S-I), ethanol (S-II), and pentanol-water (S-III) pretreatments were performed to compare and demonstrate effective fractionation of lignocellulose.

7.3.1. Optimization of the pentanol-water solvent system

The efficiency of the proposed process was determined in terms of hemicellulose removal, lignin removal, cellulose recovery, and cellulose digestibility of the pretreated substrates. The results are shown in **Fig.7.3(a)** to **Fig.7.3(d)**. Hemicellulose removal increased with the pretreatment severity which included acid concentration, cooking temperature, and solid-liquid ratios used in the pretreatment. All these parameters increased the hydrolyzability of hemicellulose linkages during pretreatment. Besides, the higher acid concentration reduced the pretreatment time and temperature for effective/ complete removal of hemicellulose. The high acid concentration increased the availability of protons (H_3O^+) in the system which was mainly responsible for the cleavage of all lignocellulose linkages (Islam et al., 2021). Complete hemicellulose removal was obtained at 60 mM acid concentration in the pretreatment liquor after cooking for 60 minutes at 170°C. There was a slight increase (92.63% to 92.98%) of hemicellulose removal when the temperature was increased from 170 °C to 200 °C at 45 mM acid concentration. On the other hand, the increase of pentanol composition reduced the hemicellulose removal due to the reduced mobility of H_3O^+ in concentrated pentanol solution. The hemicellulose removal reduced from 93.92% to 59.15% as the pentanol composition increased from 10% to 95% in the liquor.

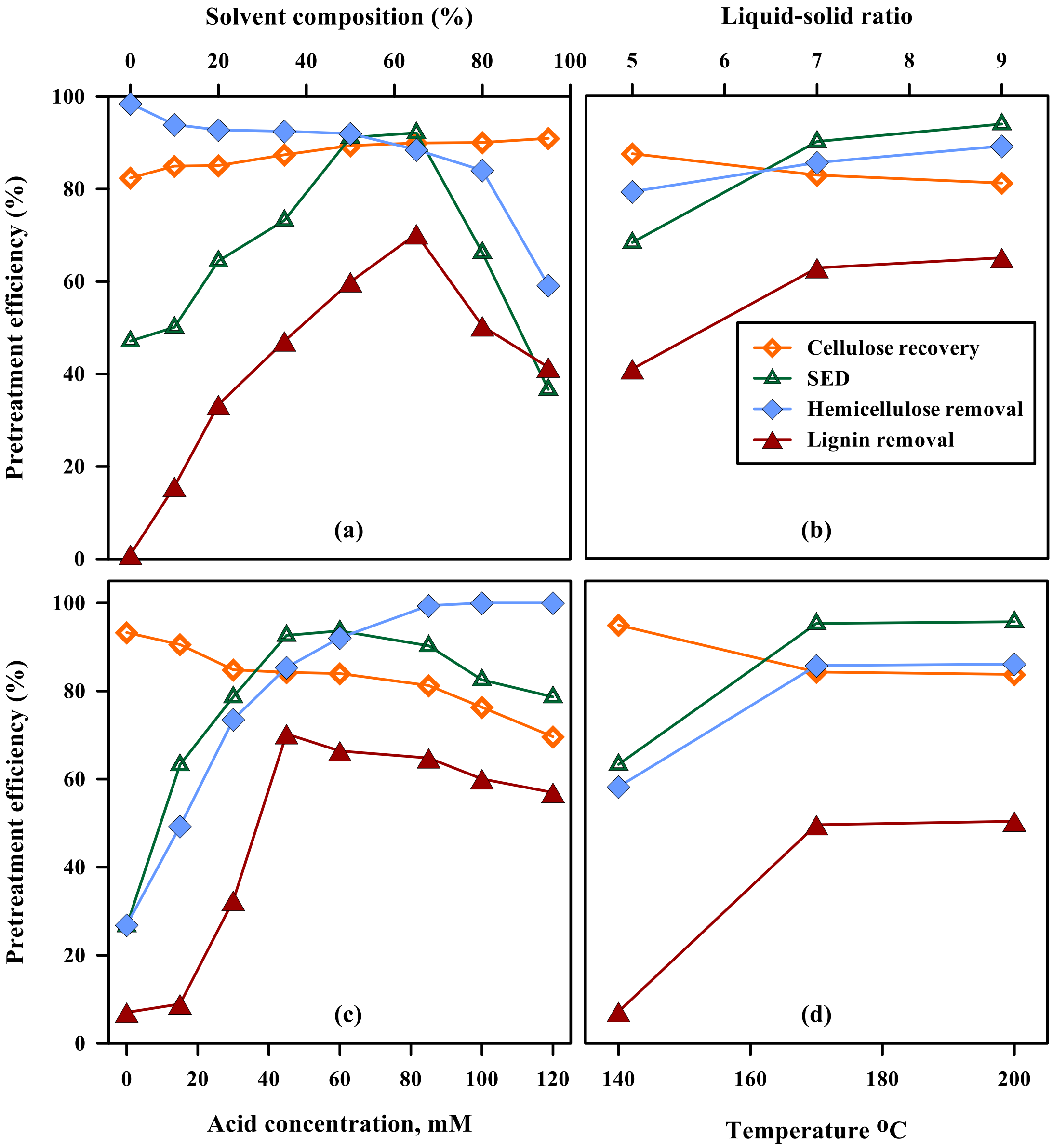


Fig.7.3. Process optimization for pentanol-water biphasic solvent using pretreatment efficiency for varying (a) solvent composition, (b) liquid to solid ratio, (c) catalyst concentration, and (d) pretreatment temperature.

Lignin removal showed a similar response as hemicellulose removal and the lignin solubility increased with the pentanol composition up to 65% which enhanced the hydrophobic interaction between lignin and solvent. However, lignin removal started decreasing after 65% since lignin dissolution is highly dependent on hemicellulose dissolution. At the very high acid concentration (≥ 85 mM acid) or pretreatment severity, lignin removal from the solid pulp was reduced due to lignin condensation reaction. The formation of condensed C=C bond in the lignin reduced its dissolution from the solid pulp. The maximum lignin removal was obtained at 170℃ and 45 mM of acid concentration which was about 70.30% of the original lignin which was comparatively lower than some of the reported studies (Pan et al., 2007; Wang et al., 2020). Maybe, it was due to the limited mass transfer between biomass lignin and pentanol phase. However, pentanol-water system facilitated a much easier separation of lignin with the higher amount of original β-o-4 linkages which was discussed later in the lignin characterization section.

The solid recovery reduced due to the removal of hemicellulose and lignin in the pretreatment process as shown in **Fig.7.3(a)** to **Fig.7.3(d)**. The solid residues obtained after pretreatment, and fractionation mainly contained cellulose which was about 72.10% of the total solid. Biorefinery efficiency largely depends on the cellulose recovery as a readily usable solid residue which can be used to produce building-block sugars or bioethanol. The cellulose recovery decreased with increasing acid doses and cooking temperature in the pretreatment. The higher cooking temperature and acid doses increased the severity of pretreatment that was responsible for higher cellulose degradation. For example, the cellulose recovery reduced from 90.56% to 69.62% as the acid doses increased from 15 mM to 120 mM at the same cooking temperature (170℃). At same acid dose and solid-liquid ratio, the cellulose recovery also reduced from 92.36% to 83.83 % when the cooking temperature was increased from 140 ℃ to 200℃. However, the effects of solvent composition and solid-liquid ratios were minimal on the cellulose recovery.

Therefore, the optimum conditions were found to be 65% pentanol, 45 mM acid concentration, and 170 ℃ temperature with a 1:7 solid-liquid ratio. The optimized pretreatment conditions resulted in 92% hemicellulose and 70.27% lignin solubilization in the solvent system while resulting in an insoluble fraction with 88.49% cellulose recovery.

7.3.2. Pretreatment efficiency of different solvents

The compositions of pretreated wood residues and hydrolysates are illustrated in **Fig.7.4,** where the composition of pretreated substrates is expressed in terms of raw substrate and solid yield. S-I pretreatment removed 98.47% of hemicellulose from substrate while leaving all the lignin in the solid cellulosic residues. For organosolv pretreatment, S-II and S-III, the solid yield was significantly reduced (53.74% and 52.08% of its initial weight respectively) due to the removal of easily hydrolysable hemicellulose and lignin. S-II pretreatment removed 90.19% of hemicellulose and 74.01% of lignin from *Acacia Confusa* wood residue. S-III approach resulted in a similar performance for hemicellulose (89.49%) and lignin (70.27%) removal from raw wood. In addition, approximately 90% of the original cellulose was recovered from solid residue which suggested that S-III pretreatment was not too harsh to hydrolyze cellulose.

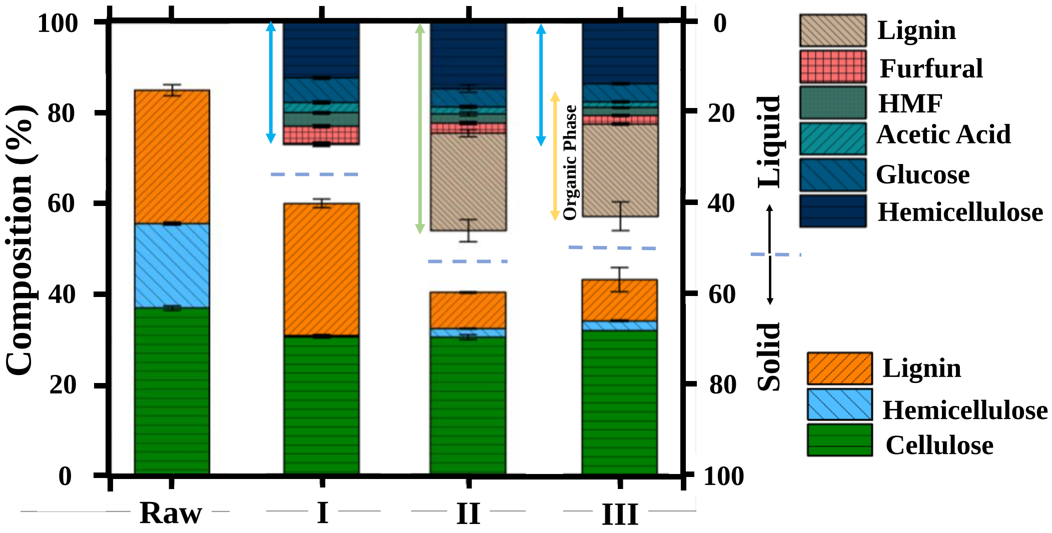


Fig.7.4. Chemical compositions of pretreated *Acacia Confusa* wood residues and pretreatment liquor. The data are represented as the percentage per 100 g of raw biomass used for pretreatment.

There was a trade-off between substrate-related factors such as hemicellulose and lignin removal from pretreated substrates in pretreatment (Leu & Zhu, 2013). Pentanol served as a solvent for hydrophobic lignin in the fractionation process while dilute acid cleaved inter-unit ester and ether linkages of hemicellulose and lignin. The use of mineral acid significantly increases H_3O^+ ion in pentanosolv liquor and subsequently increases the fractionation efficiency of the solvent system. The hydrolyzing effect of the acid catalyst accelerates effective hydrolysis of hemicellulose to its sugars and dissolves in the aqueous phase. The effective hydrolysis of hemicellulose, in turn, makes lignin removal easier due to the framework structure of hemicellulose and lignin.

Dissolved hemicellulose and cellulose were recovered in liquor as building block sugars and the sugar yields in the liquor were determined. S-III pretreatment recovered 4.02% and 13.81% of raw biomass as cellulose and hemicellulose, respectively. Approximately, 1.70%, 1.93%, and 1.28% of raw biomass were degraded to HMF, furfural, and acetic acid, respectively in the pretreatment liquor. S-II pretreated liquor yielded similar amounts of carbohydrate sugars and by-products in the liquor.

S-I pretreatment produced a relatively higher amount of HMF (2.95%), furfural (3.80%), and acetic acid (2.28%) while reducing hemicellulose sugar recovery (12.65%) in the pretreatment liquor. The degradation of sugar into fermentation inhibitors also increased as the acid concentration increased. This was due to the strong hydrolyzing effect of acid during pretreatment which led to degradations of hemicellulose sugars to fermentation inhibitory products (Delbecq et al., 2018).

The sugar content of fractionated lignin was determined by the NREL procedure (Sluiter et al., 2008). No lignin was fractionated for the S-I process and thus all lignin appeared as the residual content of pretreated substrates. For S-II, lignin was precipitated by adding access water while leaving all the dissolved sugars in aqueous solution. Therefore, S-II lignin contained only 1.1% sugars after a few fractionation steps (washing, centrifugation, and drying in a freeze dryer). On the other hand, S-III lignin was directly obtained from the pentanol phase by evaporation of solvent which contained 4.7% total sugars. Although this value is slightly higher than S-II fractionated lignin, the sugar content can be further reduced by water washing if additional purity is required. This indicated a high and automatic partition of sugars and lignin between aqueous and pentanol phases through biphasic pretreatment.

7.3.3. Prediction of hemicellulose removal by OHF

The residual hemicellulose was plotted against the OHF to find the relationship between hemicellulose removal and pretreatment severity as shown in **Fig.7.5**. By fitting the OHF versus X_R curve, the adjustable parameters α, β, θ, and f were determined using equations (4.1) and (4.2). Hemicellulose dissolution was very fast in the beginning and followed an exponential function of OHF. However, hemicellulose dissolution rate decreased when residual hemicellulose content reached ≤20% in the pretreated substrates indicating two kinds of hemicellulose degradation in pretreatment process. The excellent fitting of equation 4.1 and 4.2 showed that the OHF can predict hemicellulose removal and pretreatment severity for *Acacia Confusa* wood.

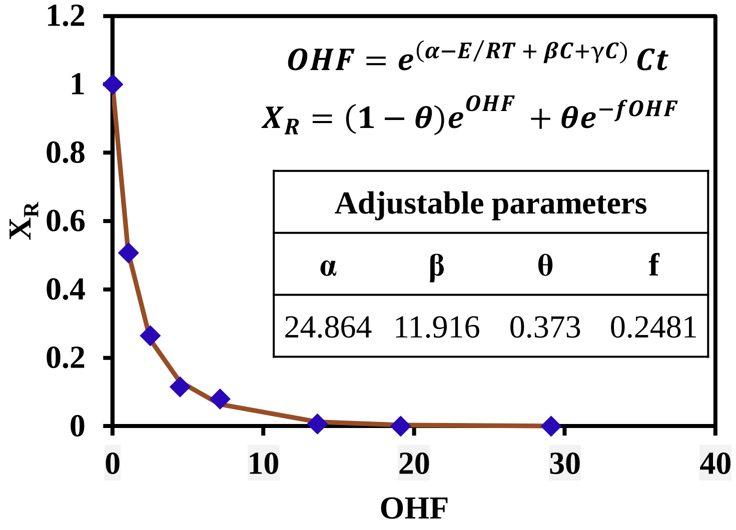


Fig.7.5. Experimentally determined hemicellulose residue in the solid substrate as a function of reaction severity evaluated as the OHF with model prediction.

The initial hemicellulose degradation was very fast as an exponential function of the OHF and followed by a slow rate of hemicellulose degradation. Excellent fitting was obtained, and the results showed that hemicellulose degradation was very slow at hemicellulose content ≤10% in the pretreated substrates. The value of activation energy and other adjustable parameters was approximately similar to the previous reports. Therefore, the OHF can predict the pretreatment severity and hemicellulose degradation in this study.

7.3.4. Simultaneous saccharification and Fermentation

Effective pretreatment facilitates the separation of cellulosic fractions that are readily digestible during enzymatic hydrolysis. High cellulose digestibility is a prerequisite for high sugar/ethanol yield in biorefinery conversion. SSF is the most promising and time-saving process for saccharification and fermentation in one reactor. To investigate the effect of three different pretreatment scenarios on the digestibility and fermentation, SSF was carried out in fermentation broth with yeast at 5% solid loading and 15 FPU/gm of enzyme loading. **Fig.7.6** shows the digestibility, glucose and ethanol concentration from solid residues using S-I, S-II, and S-III pretreatment method.

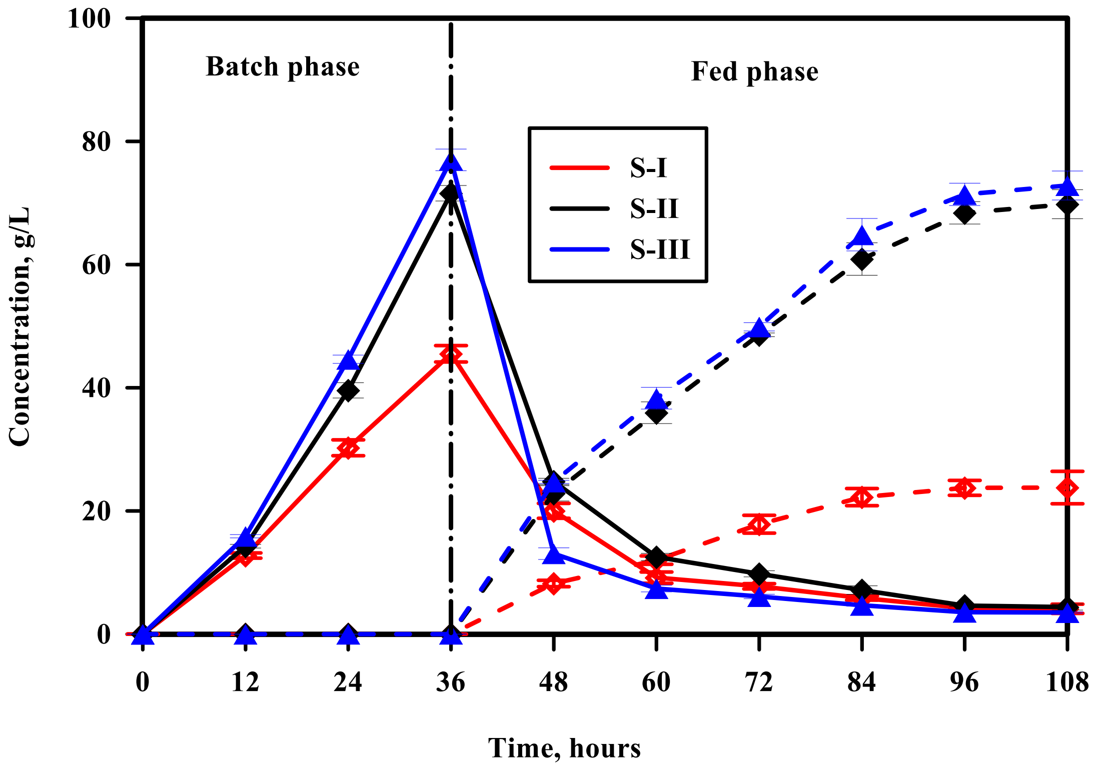


Fig.7.6. SSF yield of S-I, S-II, and S-III pretreated substrates using Saccharomyces Cerevisiae yeast to produce ethanol. Solid lines represent sugar concentrations and dashed lines represent ethanol concentrations.

Both the S-II and S-III pretreated substrates exhibited increased digestibility and glucose yields compared to the S-I pretreated sample. The related calculations from leftover sugars and ethanol showed 47.12%, 88.74%, and 92.14% cellulose digestibility of S-I, S-II, and S-III pretreated substrates, respectively. It is well-known that a higher removal of lignin usually leads to higher enzymatic hydrolysis rates and higher glucose yields for both S-II and S-III. Hydrophobic interaction between enzyme and condensed residual lignin significantly reduced the digestibility (sugar release) of S-I pretreated substrate (Chen et al., 2015). The released sugars resulted in approximately 76.0, 82.4, and 82.0% fermentation yields for S-I, S-II, and S-III, respectively. The related calculation showed that the ethanol yield was approximately

0.20, 0.39 and 0.41 g/g-glucan for S-I, S-II, and S-III, respectively. The hydrolysis and fermentation efficiency of this study (S-III) is highly competitive with the latest reports (**Table 7.2)** for different lignocellulosic feedstocks and pretreatment techniques.

Table 7.2. Saccharification and fermentation yields of various lignocelluloses for different pretreatment methods.

Feedstock	Pretreat. Process	Time, min	Temp. ℃	L/S ratio	CED (%)	F. yield, g/g-glucan	Ref.
Sugarcane straw	Dilute acid	30	155	10	66	0.24	(Mesa et al., 2017)
Softwood pine	Dilute alkali	300	180	10	83.5	0.33	(Safari et al., 2017)
Mixed sawdust	Organosolv	60	175	10	80	0.33	(Alio et al., 2020)
Rice straw	Acetic acid	50	100	10	74.09	0.28	(Tsegaye et al., 2020)
Hardwood	Hydrothermal	10	200	10	77.93	0.36	(Lee & Yu, 2021)
Sugarcane bagasse	DES	240	140	15	88.23	0.37	(Liu et al., 2021)
Walnut	Ionic Liquid	180	160	6.66	82	0.38	(Das et al., 2021)
Acacia wood	**S-III**	**60**	**170**	**7**	**92.14**	**0.41**	**This study**

7.3.5. Characterization of solid cellulosic fraction

The morphological changes of raw and pretreated *Acacia Confusa* wood residues are demonstrated using SEM micrographs in **Fig.7.7(a) to Fig.7.7(d)**. The longitudinal view of raw wood chips showed a densely packed and smooth surface layer of wood fibre which limits enzyme accessibility (**Fig.7.7(a)**). The morphology of biomass microfibrils significantly changed (became distorted and rough) during all the three pretreatment approaches due to the removal of hemicellulose and lignin. However, S-I pretreated substrates resulted in minimum changes of surface morphology due to hemicellulose removal only (**Fig.7.7(b)**). On the other hand, the surface of S-II and S-II pretreated substrates demonstrated drastic rapture and channelling showing the effective removal of both hemicellulose and lignin from substrates (**Fig.7.7(c-d)**). The effective removal of hemicellulose and lignin exposed the surface to enzyme which significantly increased the digestibility of cellulose in the substrates.

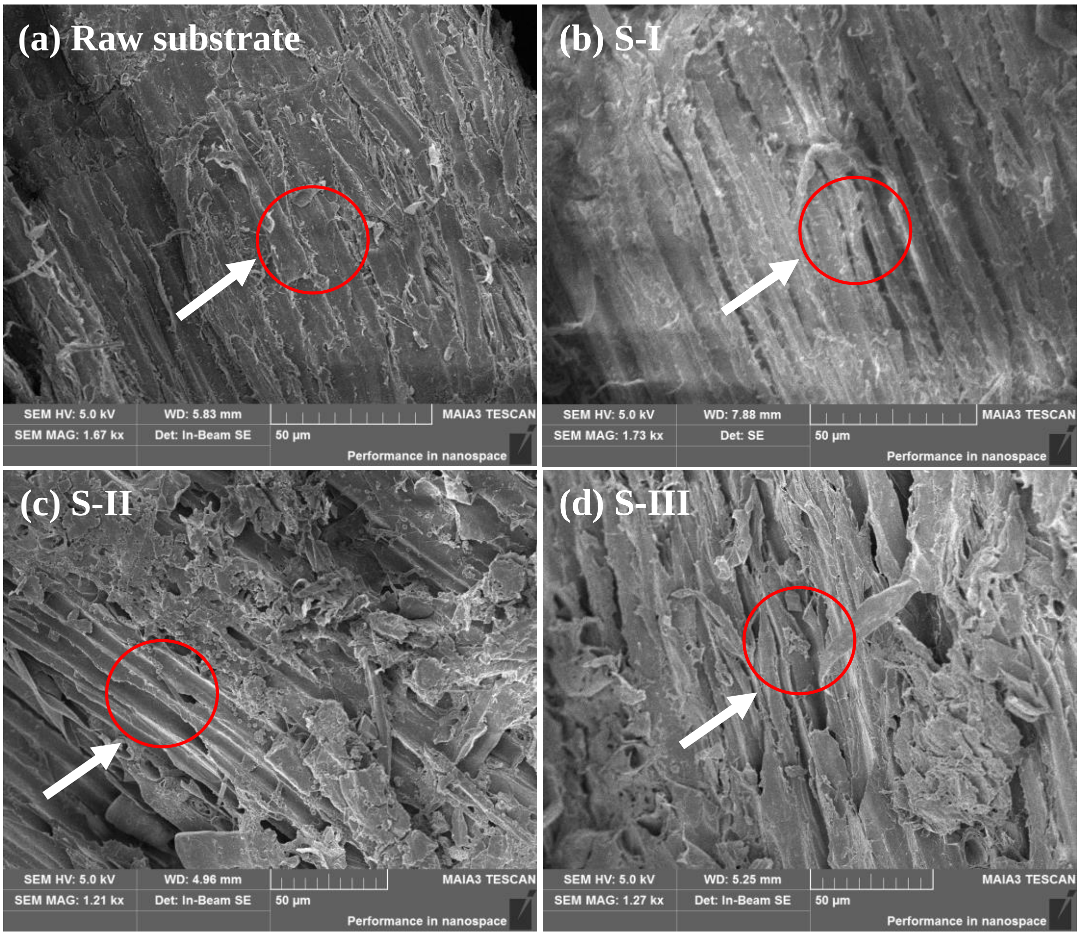


Fig.7.7: Scanning Electron Microscopic images of (a) raw biomass, (b) S-I, (c) S-II, and (d) S-III pretreated *Acacia Confusa* wood chips.

Crystallinity indicates the amount of cellulose in the substrates. The higher the crystallinity of the substrate indicates the higher removal of amorphous hemicellulose and lignin. Therefore, cellulose crystallinity is one of the indicators of an effective pretreatment process (Wang et al., 2017b). The crystallinity of raw wood substrate increased from 45.83% to 47.62% after S-I pretreatment due to removal of amorphous hemicellulose (**Fig.7.8 (a,b)**). However, S-II and S-III pretreatment resulted in 54.17% and 63.16% crystallinity in pretreated substrates, respectively (**Fig.7.8 (c,d)**). This was due to the effective removal of both hemicellulose and lignin during S-II and S-III pretreatment. Results indicated the precedence of S-III pretreatment over S-I and S-II approaches.

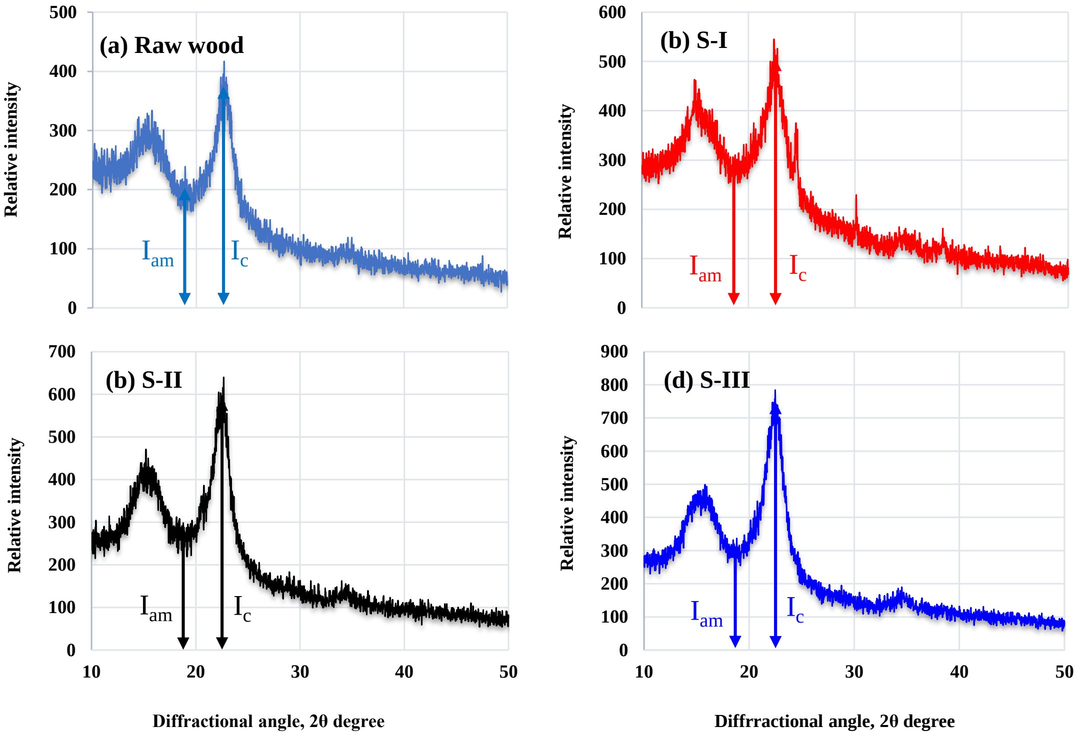


Fig.7.8: X-ray diffractogram illustrating the calculation of crystallinity index for (a) raw biomass, (b) S-I, (c) S-II, and (d) S-III pretreated *Acacia Confusa* wood chips.

7.3.6. Fractionation and solvent recovery

Fractionation and solvent recovery are key metrics for biorefinery performance as described in our previous publication (Islam et al., 2020b). The major drawback of organosolv pretreatment is the cost of reagents which emphasizes effective fractionation and solvent recovery. However, organic solvent can be recovered by distillation which can make up the high cost of solvent. The acid-catalyzed monophasic organosolv pretreatment, *i.e.,* S-II overcomes biomass recalcitrance by dissolving lignin, hemicellulose, and a part of cellulose sugars in the pretreatment liquor as shown in the liquor composition. Lignin precipitation facilitates harvesting dissolved sugars (hemicellulose and cellulose) and helps to preserve high lignin integrity which are prerequisites of integrated biorefinery. Lignin is precipitated from the pretreatment liquor using water as anti-solvent while keeping all the sugars in the liquid phase. Another approach is the direct distillation of pretreatment liquor, but this approach could limit the recovery of dissolved sugars from lignin residue obtained after distillation. The lignin and dissolved sugars appeared as a semi-solid residue after ethanol distillation. When water was added to harvest dissolved sugars, 43% of dissolved sugars got trapped in the clusters of hydrophobic lignin which cannot be leached out with water. The liquor analysis showed that approximately 57% of dissolved sugars were recovered when compared with the precipitation-first method. The significant loss of sugars limits the ultimate purpose of integrated biorefinery as well as the lignin purity.

In such circumstances, ethanol in S-II was recovered by distillation after precipitating lignin from the pretreatment liquor. For S-III, the lignin-containing pentanol phase was easily separated by traditional phase separation equipment while leaving cellulosic solid residues and an aqueous phase containing dissolved sugars.

Both solid residues and aqueous part can be used for saccharification and fermentation to produce building-block chemicals while the lignin part (pentanol phase) can be directly used to produce lignin foam (Li & Ragauskas, 2012). Pentanol can also be recovered from the pretreatment liquor by distillation. To determine the amount of solvent recovered in the organic phase, lignin-containing liquor was evaporated at a reduced pressure to collect pentanol. The result showed that approximately 89% of pentanol was recovered after pentanol-water biphasic pretreatment which allowed physical separation. The solvent loss (~11%) was due to the pentanol's solubility in water (2-3%), residual solvent in substrate and system loss during the fractionation and recovery process.

7.3.7. Characterization of Lignin

Fractionated lignins were analyzed by 2D-HSQC NMR after harvesting at optimized pretreatment conditions and compared with the ball-milled lignin and ethanol pretreated lignin. The NMR spectrum was analyzed in terms of major lignin sub-units (S and G units), inter-unit linkages (β-o-4, β-5, and β-β), and lignin-carbohydrate complex (ester and benzyl ether linkages). The major peak correlations were assigned by comparing with the published literature (Amiri et al., 2019; Dutta et al., 2018; Schneider et al., 2020). In addition, the semi-quantitative analysis was carried out using the integral value of α-position of the linkages at the same contour level. The aromatic units (S and G units) were used as the internal standards for quantitative determination. The aliphatic sidechain (δC/δH 50-90/3.0-5.0) and aromatic (δC/δH 100-130/6.0-7.5) regions of 2D-HSQC NMR spectra are shown in **Fig.7.7(a)** to **Fig.7.7(c)**.

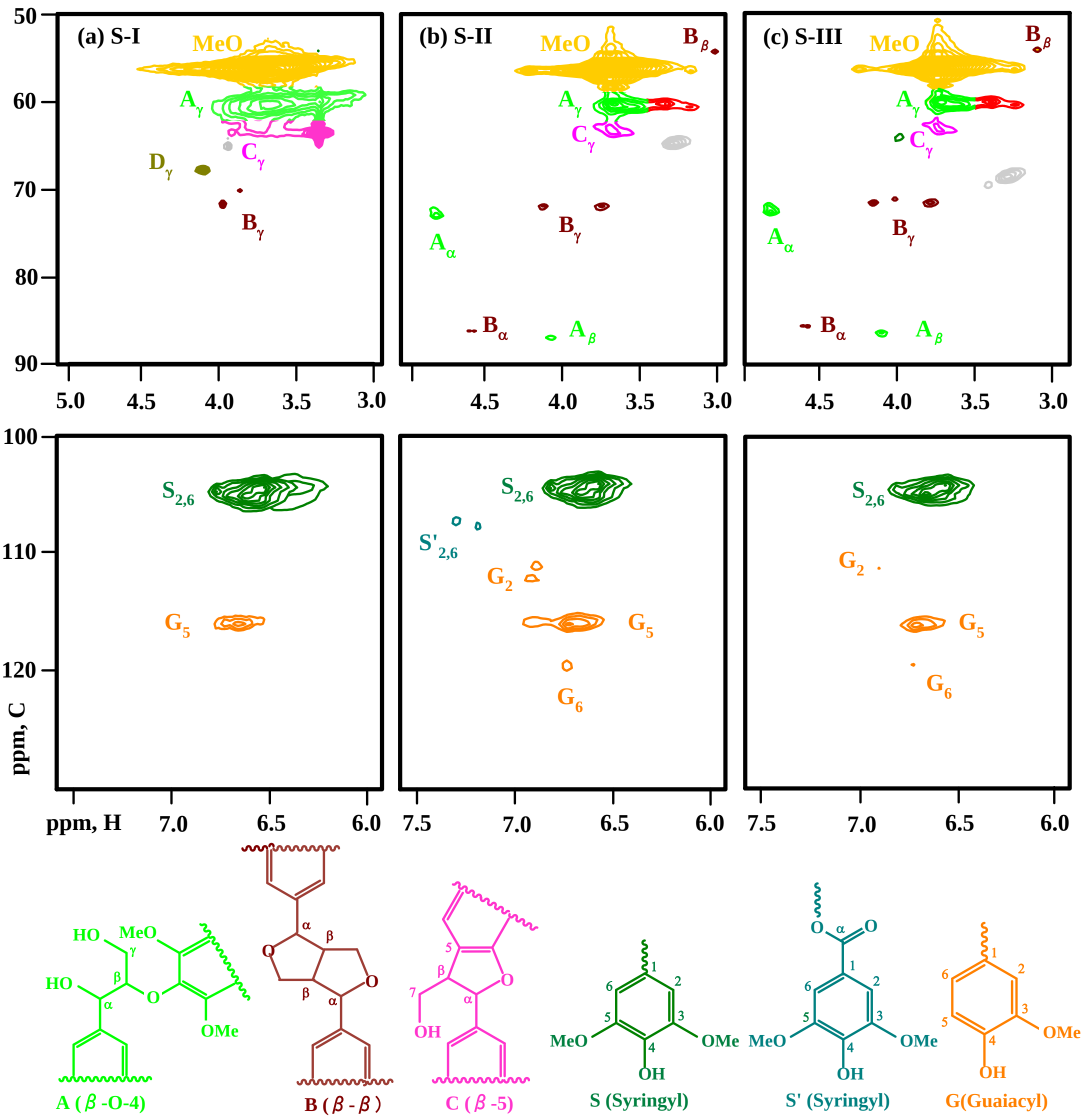


Fig.7.7. Structural elucidation of (a) S-I, (b) S-II, and (c) S-III fractionated lignins by 2D-HSQC NMR analysis.

For S-III lignin, α-position of β-o-4, β–β, and β-5 linkages appeared at δC/δH 72.56/4.82 ppm, δC/δH 85.66/4.60 ppm, and δC/δH 86.1/4.1 ppm respectively. The corresponding signals were determined for S-II and S-I pretreatment at the same position with reduced peak intensities. In the aromatic region, the syringyl, and guaiacyl units appeared at δC/δH 104.24/6.62, and 111.61-119.64/6.64-6.92 ppm respectively in S-I lignin. Similar peaks were found in the S-II and S-III lignin with higher intensity of syringyl units.

The potential of the fractionated lignins to produce value-added chemicals depends on the structural properties which largely depends on the pretreatment conditions of lignocellulose. The harsh (highly acidic) pretreatment induces the formation of detrimental -C=C- linkages (condensed lignin) which ultimately reduces the amount of beneficiary β-o-4 linkages in the fractionated lignin as shown in **Fig.7.7.** Therefore, in the lignin-first biorefinery, the prime concern is the fractionation of lignocellulose components while keeping the maximum amount of beneficiary β-o-4 linkages of lignin.

Lignin experienced structural modification through the cleavage of β-o-4 linkage which is regarded as the main pathways for lignin degradation during fractionation. The cleavage of β-o-4 linkage produced benzylic cation (**Fig.7.8(a)** and **Fig.7.8(b))** which undergoes condensation reaction aqueous acid medium to form Hibbert ketone or aldehydes (C–C bond) in the presence of acid as shown in **Fig.7.8(d)** and **Fig.7.8(f)**. The benzylic cation can also react with the electron-rich aromatic units of lignin to repolymerize into condensed C–C structures. Pentanol reduced the formation of C-C linkages and further lignin degradation reaction by trapping the reactive benzylic cations and preserved the higher amount of beneficiary β-o-4 linkages in the fractionated lignin **Fig.7.8(c)**. Besides, the auto separation of lignin in pentanol phase blocked/inhibited the reaction pathway of **Fig.7.8(b) to Fig.7.8(d-f)** for aqueous medium. The amount of S and G units determine the extent of α-OH substitution by an alkyl group of solvent since the G-lignin unit is more reactive in the substitution than the S-lignin. Pentoxylation at α-hydroxyl group of lignin was confirmed at δC/δH 60.44/3.18 ppm which reduced the condensation of fractionation lignin. Thus, pentanol pretreatment preserved 42.47% of original β-o-4 linkages in the fractionated lignin which was much higher than the conventional ethanol pretreatment

(11.35%) as shown in **Fig.7.9(a)**. Besides, the reduced acid severity on lignin due to the physical separation of acid and lignin was responsible to preserve the higher proportion of the most important β-o-4 linkages. In addition, the lowest amount of condensed β-5, and β-β linkages also supported the phenomena of preservation of a high proportion of reactive lignin S-III fractionation.

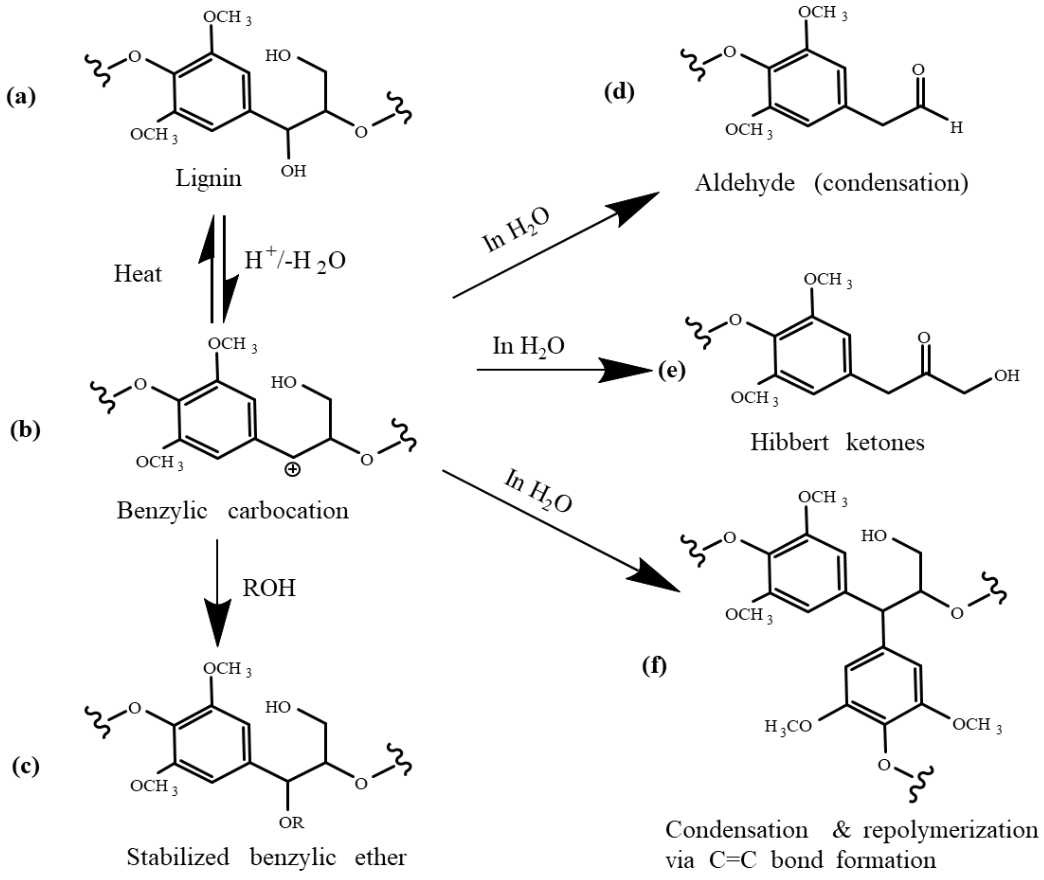


Fig.7.8. Lignin degradation pathway for acidic organosolv medium. Aqueous acid hydrolysis leads the reaction pathway (b) to form aldehyde (d), Hibbert ketones (e), and (f) aromatic C-C bond. Pentanol trapped the benzylic carbocation and dominated the reaction path towards the formation of benzylic ether (c).

Hardwood naturally contains about 46–75% S-lignin and 25–50% G-lignin (Islam et al., 2020a). G-lignin contains more reactive sites and can be easily reacted with some other groups to form condensed structure. This conversion depends on the pretreatment conditions and solvent used for fractionation. S-I fractionated lignin contained the lowest amount of G-lignin and the highest amount of S-lignin indicating severe condensation during pretreatment (Yoo et al., 2018). On the other hand, the highest amount of G-lignin in S-III fractionated lignin supported lignin stabilization and protection during biphasic pretreatment **Fig.7.9(a)**.

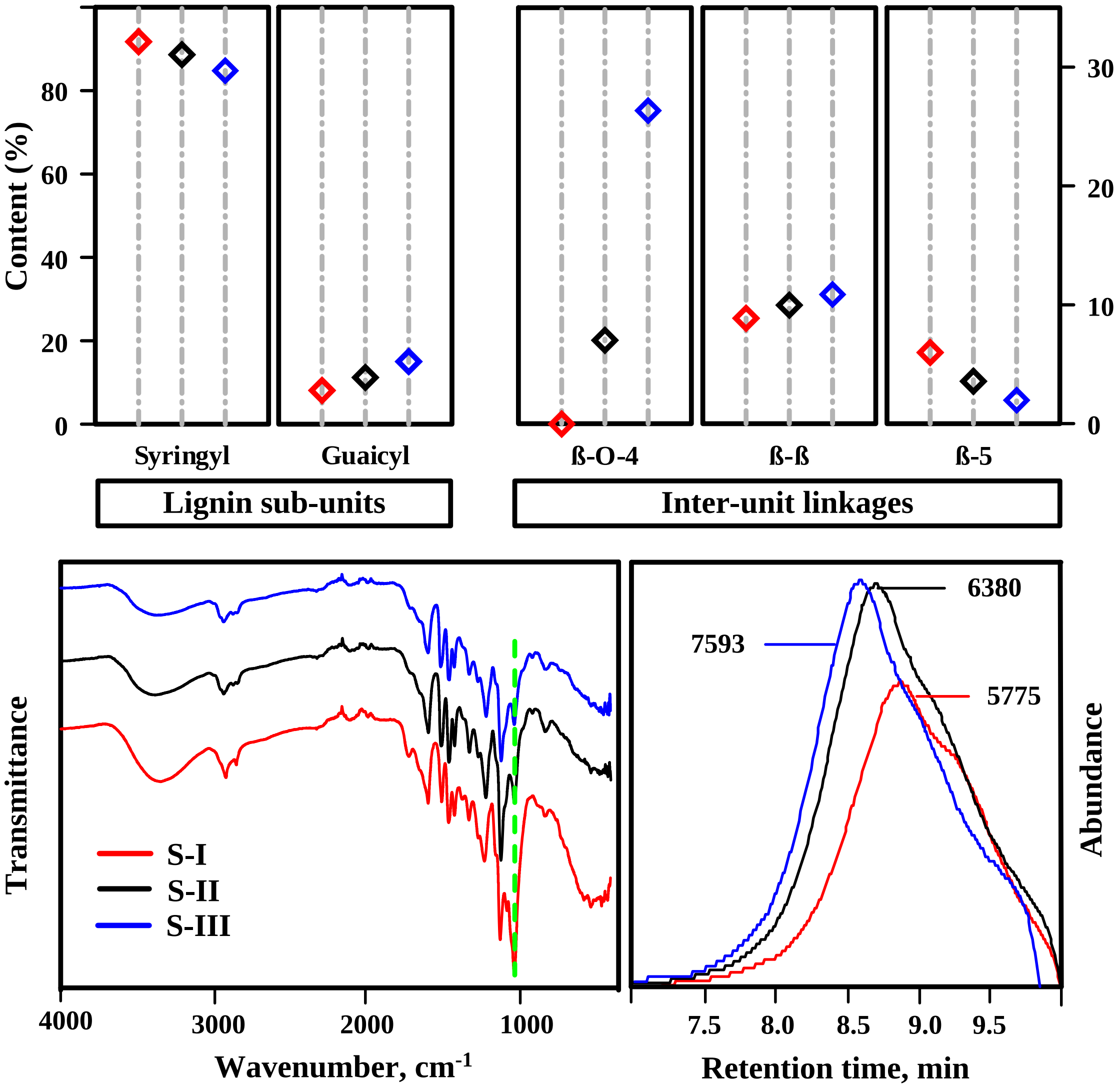


Fig.7.9. Characterization of fractionated lignin in terms of (a) semi-quantitative values of major sub-units and linkages 2D-HSQC NMR analysis (b) infrared responses and (a) molecular weight distribution.

All fractionated lignins showed similar IR spectrum with a distinctive signal at 1029 cm^{-1} as shown in **Fig.7.9(b)**. It was attributed to the complex deformation of C–C, C=C bending, and C–O–H groups. For S-I, the intense peak at 1029 cm−1 may be due to excessive formation of condensed structure (C–C and C=C) in fractionated lignin. In the ATR-FTIR spectra of all lignin samples, absorption bands were found at 1546, 1505, and 1459 cm^{-1} corresponding to the vibrations of the phenyl alkane aromatic ring. The wide absorption band at 3382 cm^{-1} is attributed to both the aromatic and aliphatic OH groups, while the absorption bands for C–H vibration of the $-CH_2$ and $-CH_3$ groups appeared at 2933, 2885, and 1459 cm^{-1} respectively (Lawther et al., 1996). The band at 1694 cm^{-1} may be due to non-conjugated carbonyl linkages while the conjugated carbonyl groups with aromatic rings appeared at two different positions at 1630 and 1694 cm^{-1}, which were probably due to p-coumaric acid and ferulic acid group in the lignin macro-molecules. The characteristic band of the syringyl ring appeared at 1392 cm^{-1}, while the stretching vibrations of C−O and C−C in the syringyl ring were assigned at 1122 cm^{-1} (Boeriu et al., 2004). The presence of p-hydroxyphenyl groups appeared as an absorption band at 815 cm^{-1}. The vibration bands of C–C, C–O, and C=O stretching could be merged at 1225 cm^{-1}. A complex vibration band relating to the C–H, C–O, and C–C deformation was assigned at 1029 cm^{-1} (Lawther et al., 1996; Sun et al., 2002).

The molecular weight distribution of fractionated lignins was demonstrated with respect to retention time of the GPC column (**Fig.7.9(c))**. The number average molecular weight of S-III lignin (7593 Da) was slightly higher than the S-II fractionated lignins (6380 Da). Lignin molecular weight is directly proportional to the amount of β-o-4 linkages in the lignin (Wang et al., 2017a). This supported the positive effect of S-III pretreatment to stabilize lignin during fractionation to obtain high lignin integrity. Although dilute acid (S-I) cleaved almost all β-o-4 linkages of fractionated, condensation and repolymerization resulted high molecular weight (5775 Da). The properties of lignin fractions and downstream processibility for proposed S-III pretreatment are illustrated in **Fig.7.10.**

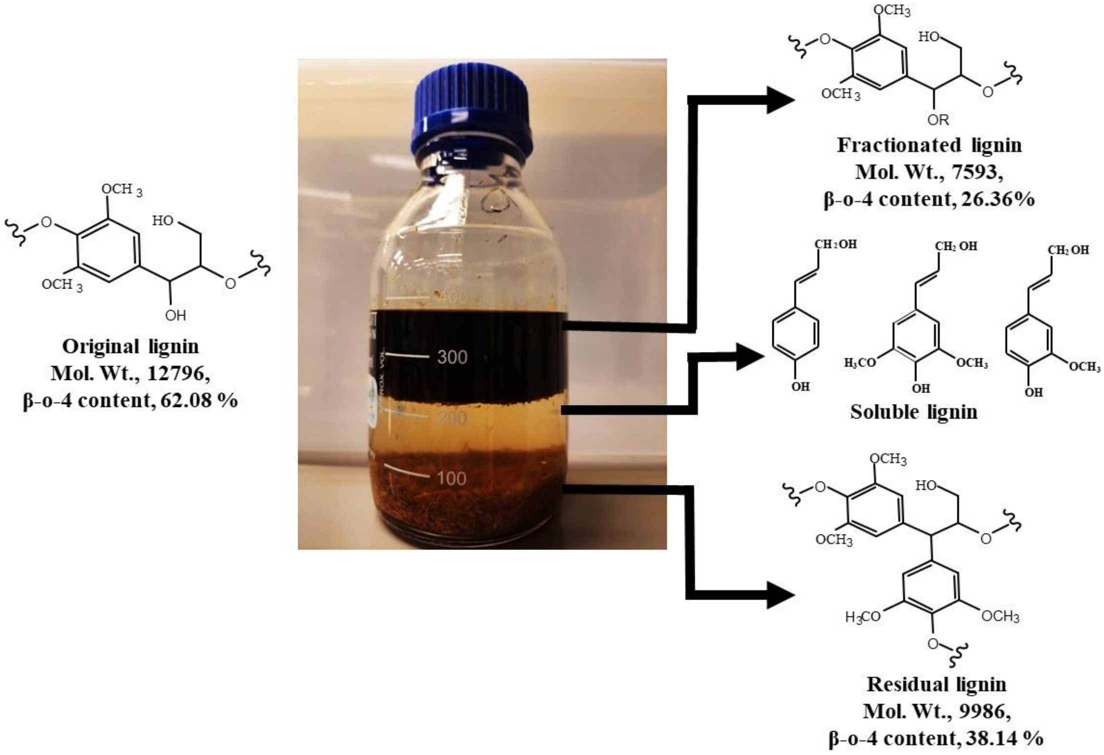


Fig.7.10. Lignin structure and its characteristics for different fractions of pentanol-water pretreatment.

7.3.8. Carbon efficiency of the process.

The flow of carbon is demonstrated in **Fig.7.11** for each pretreatment approach for *Acacia Confusa* wood biorefinery. The desired carbon output streams were ethanol and fractionated lignin with reactive functional groups/linkages. Sugar degradation products such as HMF, furfural, and acetic acid appeared as byproduct streams.

In S-I pretreatment, only 17.10% of wood carbon was converted to bioethanol while recovering 40.96% carbon as condensed residual lignin. Carbon remains in residual lignin can only be used for low-value applications due to severe condensation during pretreatment. In addition, only 24.15% of carbon can be obtained in the form of reducible sugars due to lower digestibility (47.12%) of dilute acid pretreated substrate. Approximately 8.7% of wood carbon was converted to CO_2 which is a metabolic product during fermentation. The amount of CO_2 formation was directly proportional to ethanol production from reducible sugars. A small proportion of wood carbon converted to fermentation inhibitors such as HMF (3.85%), furfural (5.42%), and acetic acid (2.08%).

Carbon efficiency was significantly increased due to higher amount of bioethanol (27.84%) and reactive lignin (21.75%) in product stream of S-II. As compared to S-I, an additional 10.74% of carbon was obtained in the product stream as bioethanol and 21.75% of carbon as reactive lignin which can be converted to value-added aromatic chemicals. Besides, the higher digestibility (21.75%) of the pretreated pulp produced a higher carbon conversion rate (49.60%) in *Acacia Confusa* wood biorefinery. A similar carbon efficiency was obtained in S-III pretreatment, the proposed S-III pretreatment preserved 49.29% of carbohydrate carbon to ethanol. However, fractionated lignin in S-III showed higher structural integrity and functionality due to the higher proportion of reactive functional groups/linkages compared with the lignin recovered from S-II. In addition, S-III pretreatment was advantageous in fractionation and energy consumption which are discussed in the next sections. Considering pretreated pulp and fractionated lignin as the products, approximately 70% carbon efficiency was achieved for *Acacia Confusa* wood biorefinery by the S-III process which is comparable to the recent report (77.6%) in Science. (Liao et al., 2020).

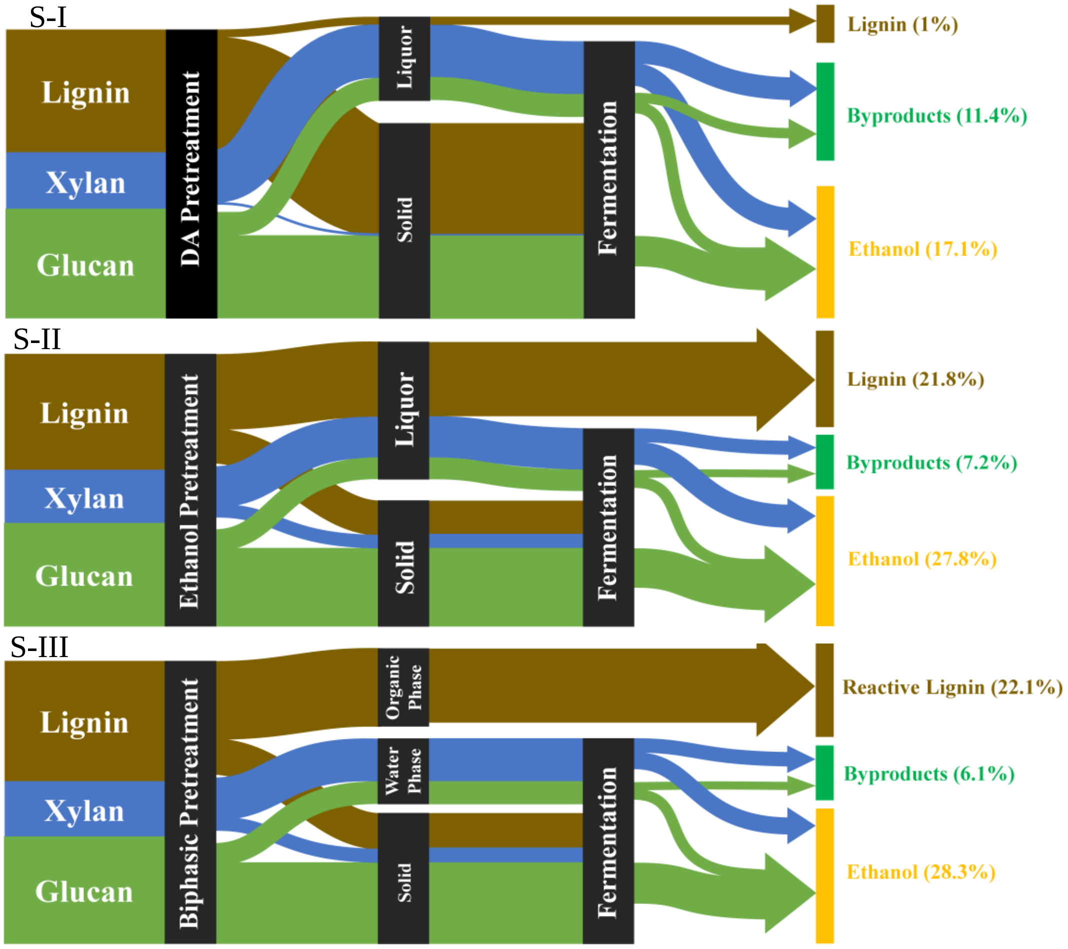


Fig.7.11. Carbon flow diagram of S-I, S-II, and S-III for biorefinery conversion of *Acacia Confusa* wood residues. The values were expressed as the percentage of carbon based on the carbon content of the entire wood.

7.3.9. Energy balance

The overall energy footprint was calculated for different process units to convert 1 kg of dry wood to bioethanol as shown in **Table 7.3.** The total energy consumption for size reduction (chipping & milling) was similar for S-II, and S-III (0.36 MJ), while S-I consumed higher energy (0.54 MJ) due to intensive milling after pretreatment.

Pretreatment is the most energy-intensive process which consumed 32.90% to 60.14% of the total energy required for the conversion process. The energy required in pretreatment is directly proportional to solvent volume and pretreatment temperature. Again, solvent volume depends on the mechanism of pretreatment and solubility of targeted lignocellulose components/solutes (Chen et al., 2015). Dilute acid (S-I) pretreatment only dissolves readily hydrolysable hemicellulose and therefore, requires less amount (1:3) of solvent/water (Castro et al., 2014). On the other hand, organosolv requires a relatively higher solvent volume (1:7); since it aims to dissolve both lignin and hemicellulose in the liquor while leaving cellulose (Islam et al., 2020b; Zhu & Pan, 2010). Therefore, the energy consumption of S-I (1.72 MJ) was much lower than S-II (2.47 MJ) and S-III (2.39 MJ) pretreatments. The energy consumption difference between S-II and S-III was due to the difference in specific heats of ethanol and pentanol. The higher energy consumption of S-II and S-III pretreatment was compensated by the high energy yield in products. The product energies of S-I, S-II, and S-III pretreatment approaches were 3.261 MJ, 5.59 MJ, and 5.74 MJ, respectively.

For lignin first integrated biorefinery (S-II and S-III), fractionation of lignocellulose components, products, and solvent recovery are major challenges which consumes the major portion of total energy required (Islam et al., 2021). For S-II (conventional organosolv) pretreatment, dissolved sugars and lignin fractionated the addition of water (three volume) as anti-solvents to the pretreatment liquor. The addition of anti-solvent increases the total volume of pretreatment liquor which in turn consumes a large extent of energy for solvent recovery and enrichment of liquors. Thus, S-II process consumed 4.69 MJ of energy for fractionation and solvent recovery.

Table 7.3. Energy footprints for bioethanol production from 1 kg of *Acacia Confusa* wood chips for S-I, S-II, and S-III processes.

Process	Energy footprints, MJ/kg of wood					
	Pretreatment	**Milling**	**Distillation**	**Total consumed**	**Product (ethanol)**	**Net Energy**
S-I	1.747	0.540	1.134	3.421	3.261	-0.160
S-II	2.479	0.360	4.696	7.535	5.865	-1.670
S-III	2.391	0.360	2.340	5.091	6.166	1.075

S-III pretreatment fractionated lignin and hemicellulose sugars with a high distribution coefficient between pentanol and water phase, respectively. The physical separation of aqueous and organic phases provided energy-saving fractionation while maintaining the almost complete fractionation of hemicellulose sugars and lignin in the respective phases. The S-III process reduced about 32.43% of the total energy consumed than S-II, due less energy requirement in the liquor enrichment and solvent recovery. The organic phase containing lignin can be directly used to produce lignin foam and high-value lignin aromatic chemicals to strengthen the concept of integrated biorefinery. Thus, the S-III process yielded 1.1 MJ of net positive energy from 1 kg of *Acacia Confusa* wood. It should be noted that the calculation was based on the results of laboratory experiments to evaluate the potential of full-scale biorefinery. Certain errors may be expected at large scale due to different rates of mass and heat transfer in different reactors.

7.4. Summary

The proposed pentanol-water biphasic (S-III) pretreatment can be a budding candidate for pretreatment of lignocellulose in biorefinery, which was found to save

32.43% of the total energy consumption compared to the conventional S-II process by reducing hemicellulose and lignin fractionation. The S-III process yielded net positive energy 1.1 MJ/kg of wood with 50.3% carbon recovery in the product stream as ethanol and reactive lignin. It resulted in approximately 70.3% lignin removal and 92.1% enzyme digestibility of substrates. S-III pretreatment preserved up to 42.5% of the original β-o-4 linkages due to the physical separation of lignin from a vulnerable acidic environment. Thus, the use of pentanol as a pretreatment solvent is possible, and thereby can achieve the objectives of high energy output and saccharification yield in biorefinery. The productivities of both lignin and carbohydrates are both close to all state-of-the-art techniques and may be further increased. However, the successfulness of the biphasic (S-III) process will rely on effective solvent recovery, lignin removal, and recovery on a large scale, which need to be further optimized.

CHAPTER 8. CONCLUSION

The main objective of developing new pretreatment process is to reduce the inherent recalcitrance of lignocellulosic biomass and to fractionate it for effective valorization of the whole biomass. This study proposed three different pretreatment approaches (*i.e.*, NMP, staged-organosolv, and biphasic pretreatment) for lignin-first biorefinery to effectively fractionate three major lignocellulose components (i.e., lignin, cellulose, and hemicelluloses) while keeping the high integrity of fractionated lignin (**Fig 8.1**).

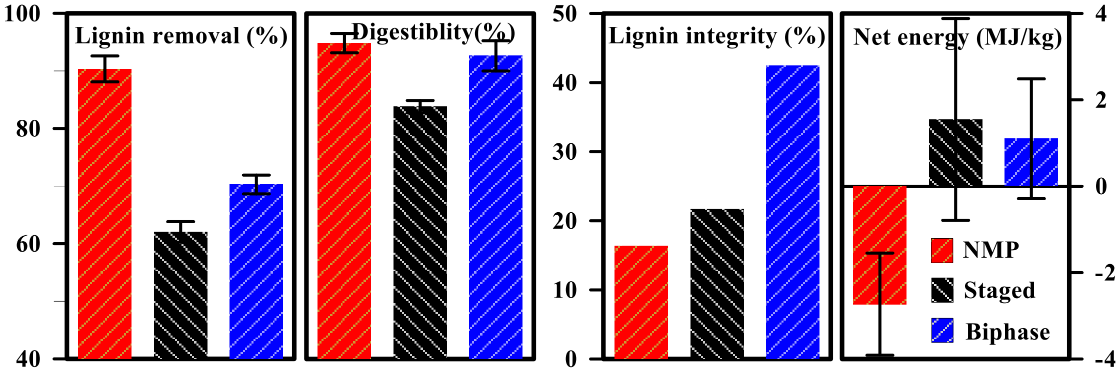


Fig.8.1. The comparative efficiency of NMP, staged, and biphasic pretreatment.

The efficiency of pretreatments was evaluated by lignin removal, cellulose digestibility of pretreated substrates, lignin integrity, and net energy yield in biorefinery. NMP pretreatment resulted in almost complete removal of hemicellulose and more than 90% lignin removal with approximately 95% digestibility of cellulose using commercial enzyme at a moderate load (15 FPU/gm-glucan). The NMP pretreatment process yielded net negative energy (-2.72 MJ/kg) due to the high energy requirement in the solvent recovery. The excellent fitting of residual hemicellulose versus OHF showed the potential of the OHF to predict hemicellulose removal. The quantitative 2D-HQSC NMR analysis showed that the NMP pretreatment preserved 16.4% of original β-o-4 lignin linkages, which was much higher than the conventional ethanol pretreatment (11.4%) possibly due to the strong hydrophobic interaction of NMP helped to dissolve fractionated lignin. The hydrophilic part of NMP was supposed to be formed a protective layer surrounding lignin aggregate to prevent lignin condensation. Further, OS-DA pretreatment process can simplify the separation of different building-block chemicals in the processes without sacrificing the pretreatment performance. The staged pretreatment produced three different types of lignin with reactive functional groups. The fractionated lignin in the first stage (OS) reduced 21.7% condensed structure due to the separate application of organosolv and acid for pretreatment. The net positive energy (1.5 MJ/kg-wood) was obtained due to potential energy saving on solvent recovery and less water consumption for fractionation.

Finally, biphasic pretreatment further simplified the whole pretreatment /separation processes in one-pot. It preserved up to 42.5% of β-o-4 linkages due to the auto separation of lignin from a vulnerable acidic environment. The use of the pentanol-water biphasic solvent system for the pretreatment of lignocellulose saved 32.43% of fractionation energy compared to the conventional organosolv process, due to direct and simple separation of water and organosolv containing lignin fractions. The energy calculation showed that the staged organosolv process yielded net positive energy of 1.5 MJ/kg-wood, which was higher than NMP (-2.72 MJ/kg) and biphasic process (1.1 MJ/kg) pretreatment approaches. The energy yield of the staged process was much higher when comparing to conventional ethanol pretreatment process (-1.94 MJ/kg). Balancing among the fractionation efficiencies, easiness of downstream operation, and energy consumption, three organosolv pretreatment processes offered different options for sustainable bioconversion of the biomass produced from different industries and/or regions of the world.

REFERENCES

Abd-Aziz, S., Ibrahim, M.F., Jenol, M.A. 2018. Biological Pretreatment of Lignocellulosic Biomass for Volatile Fatty Acid Production. *Emerging Areas in Bioengineering*, **1**, 191-201.

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A., Levin, D.B. 2011. Biomass pretreatment: fundamentals toward application. *Biotechnology advances*, **29**(6), 675-685.

Agnihotri, S., Johnsen, I.A., Bøe, M.S., Øyaas, K., Moe, S. 2015. Ethanol organosolv pretreatment of softwood (Picea abies) and sugarcane bagasse for biofuel and biorefinery applications. *Wood science and technology*, **49**(5), 881-896.

Aguilera, R.F., Eggert, R.G., Lagos CC, G., Tilton, J.E. 2009. Depletion and the future availability of petroleum resources. *The Energy Journal*, 141-174.

Ahmad, F.B., Zhang, Z., Doherty, W.O., O'Hara, I.M. 2019. The outlook of the production of advanced fuels and chemicals from integrated oil palm biomass biorefinery. *Renewable and Sustainable Energy Reviews*, **109**, 386-411.

Ai, B., Li, W., Woomer, J., Li, M., Pu, Y., Sheng, Z., Zheng, L., Adedeji, A., Ragauskas, A.J., Shi, J. 2020. Natural deep eutectic solvent mediated extrusion for continuous high-solid pretreatment of lignocellulosic biomass. *Green Chemistry*, **22**(19), 6372-6383.

Alder, C.M., Hayler, J.D., Henderson, R.K., Redman, A.M., Shukla, L., Shuster, L.E., Sneddon, H.F. 2016. Updating and further expanding GSK's solvent sustainability guide. *Green Chemistry*, **18**(13), 3879-3890.

Alén, R. 2011. *Biorefining of forest resources*. Paperi ja Puu Oy.

Alfonsi, K., Colberg, J., Dunn, P.J., Fevig, T., Jennings, S., Johnson, T.A., Kleine, H.P., Knight, C., Nagy, M.A., Perry, D.A. 2008. Green chemistry tools to influence a medicinal chemistry and research chemistry based organisation. *Green Chemistry*, **10**(1), 31-36.

Alio, M.A., Tugui, O.-C., Rusu, L., Pons, A., Vial, C. 2020. Hydrolysis and fermentation steps of a pretreated sawmill mixed feedstock for bioethanol production in a wood biorefinery. *Bioresource technology*, **310**, 123412.

Alriols, M.G., Tejado, A., Blanco, M.a., Mondragon, I., Labidi, J. 2009. Agricultural palm oil tree residues as raw material for cellulose, lignin and hemicelluloses production by ethylene glycol pulping process. *Chemical Engineering Journal*, **148**(1), 106-114.

Amelio, A., Genduso, G., Vreysen, S., Luis, P., Van der Bruggen, B. 2014. Guidelines based on life cycle assessment for solvent selection during the process design and evaluation of treatment alternatives. *Green Chemistry*, **16**(6), 3045-3063.

Amiri, M.T., Bertella, S., Questell-Santiago, Y.M., Luterbacher, J.S. 2019. Establishing lignin structure-upgradeability relationships using quantitative 1 H–13 C heteronuclear single quantum coherence nuclear magnetic resonance (HSQC-NMR) spectroscopy. *Chemical science*, **10**(35), 8135-8142.

An, Y.-X., Zong, M.-H., Wu, H., Li, N. 2015. Pretreatment of lignocellulosic biomass with renewable cholinium ionic liquids: Biomass fractionation, enzymatic digestion and ionic liquid reuse. *Bioresource technology*, **192**, 165-171.

Asakawa, A., Oka, T., Sasaki, C., Asada, C., Nakamura, Y. 2016. Cholinium ionic liquid/cosolvent pretreatment for enhancing enzymatic saccharification of sugarcane bagasse. *Industrial Crops and Products*, **86**, 113-119.

Ayvaz, A., Sagdic, O., Karaborklu, S., Ozturk, I. 2010. Insecticidal activity of the essential oils from different plants against three stored-product insects. *Journal of Insect Science*, **10**(1), 21.

Bacete, L., Mélida, H., Miedes, E., Molina, A. 2018. Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. *The Plant Journal*, **93**(4), 614-636.

Badger, P. 2002. Ethanol from cellulose: a general review. *Trends in new crops and new uses*, **1**, 17-21.

Bajpai, P. 2016a. Pretreatment of Lignocellulosic Biomass. in: *Pretreatment of Lignocellulosic Biomass for Biofuel Production*, Springer, pp. 17-70.

Bajpai, P. 2016b. *Pretreatment of lignocellulosic biomass for biofuel production*. Springer.

Baker, P., Charlton, A., Hale, M. 2017. Fungal pre-treatment of forestry biomass with a focus on biorefining: A comparison of biomass degradation and enzyme activities by wood rot fungi across three tree species. *Biomass and Bioenergy*, **107**, 20-28.

Banerjee, A., Dick, G.R., Yoshino, T., Kanan, M.W. 2016. Carbon dioxide utilization via carbonate-promoted C–H carboxylation. *Nature*, **531**(7593), 215.

Barakat, A., Chuetor, S., Monlau, F., Solhy, A., Rouau, X. 2014. Eco-friendly dry chemo-mechanical pretreatments of lignocellulosic biomass: Impact on energy and yield of the enzymatic hydrolysis. *Applied Energy*, **113**, 97-105.

Baral, N.R., Sundstrom, E.R., Das, L., Gladden, J., Eudes, A., Mortimer, J.C., Singer, S.W., Mukhopadhyay, A., Scown, C.D. 2019. Approaches for More Efficient Biological Conversion of Lignocellulosic Feedstocks to Biofuels and Bioproducts. *ACS Sustainable Chemistry & Engineering*, **7**(10), 9062-9079.

Barton, A.F. 2017. *CRC handbook of solubility parameters and other cohesion parameters*. Routledge.

Barton, A.M. 2018. *Handbook of Poylmer-Liquid Interaction Parameters and Solubility Parameters*. Routledge.

Basma, N.S., Headen, T.F., Shaffer, M.S., Skipper, N.T., Howard, C.A. 2018. Local structure and polar order in liquid N-methyl-2-pyrrolidone (NMP). *The Journal of Physical Chemistry B*, **122**(38), 8963-8971.

Bastin, J.-F., Finegold, Y., Garcia, C., Mollicone, D., Rezende, M., Routh, D., Zohner, C.M., Crowther, T.W. 2019. The global tree restoration potential. *Science*, **365**(6448), 76-79.

Bbosa, D., Mba-Wright, M., Brown, R.C. 2018. More than ethanol: a techno-economic analysis of a corn stover-ethanol biorefinery integrated with a hydrothermal liquefaction process to convert lignin into biochemicals. *Biofuels, Bioproducts and Biorefining*.

Bentley, R.W. 2002. Global oil & gas depletion: an overview. *Energy policy*, **30**(3), 189-205.

Berlin, A. 2013. No barriers to cellulose breakdown. *Science*, **342**(6165), 1454-1456.

Bernabeu, E., Gonzalez, L., Legaspi, M.J., Moretton, M.A., Chiappetta, D.A. 2016. Paclitaxel-loaded TPGS-b-PCL nanoparticles: in vitro cytotoxicity and cellular uptake in MCF-7 and MDA-MB-231 cells versus mPEG-b-PCL nanoparticles and Abraxane®. *Journal of nanoscience and nanotechnology*, **16**(1), 160-170.

Bhagia, S., Ragauskas, A.J. 2019. Preserving Aryl Ether Linkages and Higher Yields of Isolated Lignin through Biomass Fibrillation. *ACS Sustainable Chemistry & Engineering*, **8**(1), 34-37.

Bian, H., Chen, L., Gleisner, R., Dai, H., Zhu, J. 2017. Producing wood-based nanomaterials by rapid fractionation of wood at 80 C using a recyclable acid hydrotrope. *Green Chemistry*, **19**(14), 3370-3379.

Boeriu, C.G., Bravo, D., Gosselink, R.J., van Dam, J.E. 2004. Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy. *Industrial crops and products*, **20**(2), 205-218.

Bórawski, P., Bełdycka-Bórawska, A., Szymańska, E.J., Jankowski, K.J., Dubis, B., Dunn, J.W. 2019. Development of renewable energy sources market and biofuels in The European Union. *Journal of cleaner production*, **228**, 467-484.

Brosse, N., Sannigrahi, P., Ragauskas, A. 2009. Pretreatment of Miscanthus x giganteus using the ethanol organosolv process for ethanol production. *Industrial & Engineering Chemistry Research*, **48**(18), 8328-8334.

Byrne, F.P., Jin, S., Paggiola, G., Petchey, T.H., Clark, J.H., Farmer, T.J., Hunt, A.J., McElroy, C.R., Sherwood, J. 2016. Tools and techniques for solvent selection: green solvent selection guides. *Sustainable Chemical Processes*, **4**(1), 7.

Callegari, A., Bolognesi, S., Cecconet, D., Capodaglio, A.G. 2020. Production technologies, current role, and future prospects of biofuels feedstocks: A state-of-the-art review. *Critical Reviews in Environmental Science and Technology*, **50**(4), 384-436.

Calvaruso, G., Clough, M.T., Rinaldi, R. 2017. Biphasic extraction of mechanocatalytically-depolymerized lignin from water-soluble wood and its catalytic downstream processing. *Green Chemistry*, **19**(12), 2803-2811.

Cambero, C., Sowlati, T. 2016. Incorporating social benefits in multi-objective optimization of forest-based bioenergy and biofuel supply chains. *Applied Energy*, **178**, 721-735.

Cao, Z., Dierks, M., Clough, M.T., de Castro, I.B.D., Rinaldi, R. 2018. A convergent approach for a deep converting lignin-first biorefinery rendering high-energy-density drop-in fuels. *Joule*, **2**(6), 1118-1133.

Castro, E., Nieves, I.U., Mullinnix, M.T., Sagues, W.J., Hoffman, R.W., Fernández-Sandoval, M.T., Tian, Z., Rockwood, D.L., Tamang, B., Ingram, L.O. 2014. Optimization of dilute-phosphoric-acid steam pretreatment of Eucalyptus benthamii for biofuel production. *Applied Energy*, **125**, 76-83.

Chandel, A.K., Garlapati, V.K., Singh, A.K., Antunes, F.A.F., da Silva, S.S. 2018. The path forward for lignocellulose biorefineries: bottlenecks, solutions, and perspective on commercialization. *Bioresource technology*, **264**, 370-381.

Chang, V.S., Holtzapple, M.T. 2000. Fundamental factors affecting biomass enzymatic reactivity. *Applied biochemistry and biotechnology*, **84**(1), 5-37.

Chen, G.-F. 1992. Developments in the field of rosin chemistry and its implications in coatings. *Progress in organic coatings*, **20**(2), 139-167.

Chen, H., Zhao, J., Hu, T., Zhao, X., Liu, D. 2015. A comparison of several organosolv pretreatments for improving the enzymatic hydrolysis of wheat straw: substrate digestibility, fermentability and structural features. *Applied Energy*, **150**, 224-232.

Chen, L., Dou, J., Ma, Q., Li, N., Wu, R., Bian, H., Yelle, D.J., Vuorinen, T., Fu, S., Pan, X. 2017. Rapid and near-complete dissolution of wood lignin at≤ 80° C by a recyclable acid hydrotrope. *Science Advances*, **3**(9), e1701735.

Cheng, F., Zhao, X., Hu, Y. 2018a. Lignocellulosic biomass delignification using aqueous alcohol solutions with the catalysis of acidic ionic liquids: a comparison study of solvents. *Bioresource technology*, **249**, 969-975.

Cheng, G., Zhang, X., Simmons, B., Singh, S. 2015a. Theory, practice and prospects of X-ray and neutron scattering for lignocellulosic biomass characterization: towards understanding biomass pretreatment. *Energy & Environmental Science*, **8**(2), 436-455.

Cheng, J. 2017. *Biomass to renewable energy processes*. CRC press.

Cheng, J., Leu, S.-Y., Zhu, J., Gleisner, R. 2015b. High titer and yield ethanol production from undetoxified whole slurry of Douglas-fir forest residue using pH profiling in SPORL. *Biotechnology for biofuels*, **8**(1), 22.

Cheng, S., Yu, H., Hu, M., Wu, Y., Cheng, L., Cai, Q., Tu, Y., Xia, T., Peng, L. 2018b. Miscanthus accessions distinctively accumulate cadmium for largely enhanced biomass enzymatic saccharification by increasing hemicellulose and pectin and reducing cellulose CrI and DP. *Bioresource technology*, **263**, 67-74.

Chiang, K.-Y., Chien, K.-L., Lu, C.-H. 2012. Characterization and comparison of biomass produced from various sources: suggestions for selection of pretreatment technologies in biomass-to-energy. *Applied energy*, **100**, 164-171.

Chin, D.W.K., Lim, S., Pang, Y.L., Lim, C.H., Lee, K.M. 2019. Two-staged acid hydrolysis on ethylene glycol pretreated degraded oil palm empty fruit bunch for sugar based substrate recovery. *Bioresource technology*, **292**, 121967.

Clark, J.H. 2019. Green biorefinery technologies based on waste biomass. *Green Chemistry*, **21**(6), 1168-1170.

Constable, D.J., Jimenez-Gonzalez, C., Henderson, R.K. 2007. Perspective on solvent use in the pharmaceutical industry. *Organic process research & development*, **11**(1), 133-137.

Cordero, T., Marquez, F., Rodriguez-Mirasol, J., Rodriguez, J. 2001. Predicting heating values of lignocellulosics and carbonaceous materials from proximate analysis. *Fuel*, **80**(11), 1567-1571.

Cui, X., Zhao, X., Zeng, J., Loh, S.K., Choo, Y.M., Liu, D. 2014. Robust enzymatic hydrolysis of formiline-pretreated oil palm empty fruit bunches (EFB) for efficient conversion of polysaccharide to sugars and ethanol. *Bioresource technology*, **166**, 584-591.

da Costa Sousa, L., Jin, M., Chundawat, S.P., Bokade, V., Tang, X., Azarpira, A., Lu, F., Avci, U., Humpula, J., Uppugundla, N. 2016. Next-generation ammonia pretreatment enhances cellulosic biofuel production. *Energy & Environmental Science*, **9**(4), 1215-1223.

Dadi, A.P., Varanasi, S., Schall, C.A. 2006. Enhancement of cellulose saccharification kinetics using an ionic liquid pretreatment step. *Biotechnology and bioengineering*, **95**(5), 904-910.

Dai, L., Liu, R., Hu, L.-Q., Zou, Z.-F., Si, C.-L. 2017. Lignin nanoparticle as a novel green carrier for the efficient delivery of resveratrol. *ACS Sustainable Chemistry & Engineering*, **5**(9), 8241-8249.

Dale, B. 2018. Time to rethink cellulosic biofuels? *Biofuels, Bioproducts and Biorefining*, **12**(1), 5-7.

Daniel, G. 2009. Wood and fibre morphology. *Pulp and Paper Chemistry and Technology*, **1**, 49-71.

Das, L., Achinivu, E.C., Barcelos, C.A., Sundstrom, E., Amer, B., Baidoo, E.E., Simmons, B.A., Sun, N., Gladden, J.M. 2021. Deconstruction of Woody Biomass via Protic and Aprotic Ionic Liquid Pretreatment for Ethanol Production. *ACS Sustainable Chemistry & Engineering*, **9**(12), 4422-4432.

De Bhowmick, G., Sarmah, A.K., Sen, R. 2018. Lignocellulosic biorefinery as a model for sustainable development of biofuels and value added products. *Bioresource technology*, **247**, 1144-1154.

Del Rio, L.F., Chandra, R.P., Saddler, J.N. 2010. The effect of varying organosolv pretreatment chemicals on the physicochemical properties and cellulolytic hydrolysis of mountain pine beetle-killed lodgepole pine. *Applied biochemistry and biotechnology*, **161**(1-8), 1-21.

Dhandapani, S., Evers, S. 2020. Oil palm 'slash-and-burn'practice increases post-fire greenhouse gas emissions and nutrient concentrations in burnt regions of an agricultural tropical peatland. *Science of The Total Environment*, 140648.

Din, Z.U., Zainal, Z. 2016. Biomass integrated gasification–SOFC systems: Technology overview. *Renewable and Sustainable Energy Reviews*, **53**, 1356-1376.

Dong, C., Meng, X., Yeung, C.S., Ho-Yin, T., Ragauskas, A.J., Leu, S.-Y. 2019. Diol pretreatment to fractionate a reactive lignin in lignocellulosic biomass biorefineries. *Green Chemistry*, **21**(10), 2788-2800.

Dong, C., Wang, Y., Chan, K.-L., Bhatia, A., Leu, S.-Y. 2018. Temperature profiling to maximize energy yield with reduced water input in a lignocellulosic ethanol biorefinery. *Applied Energy*, **214**, 63-72.

Donohoe, B.S., Decker, S.R., Tucker, M.P., Himmel, M.E., Vinzant, T.B. 2008. Visualizing lignin coalescence and migration through maize cell walls following thermochemical pretreatment. *Biotechnology and bioengineering*, **101**(5), 913-925.

Dutta, T., Papa, G., Wang, E., Sun, J., Isern, N.G., Cort, J.R., Simmons, B.A., Singh, S. 2018. Characterization of lignin streams during bionic liquid-based pretreatment from grass, hardwood, and softwood. *ACS sustainable chemistry & engineering*, **6**(3), 3079-3090.

Eriksson, D., Arshadi, M., Kataria, R., Bergsten, U. 2018. Lipophilic extractives in different tree fractions and forestry assortments of Pinus sylvestris due for thinning or final cutting. *Scandinavian Journal of Forest Research*, 1-9.

Espinoza-Acosta, J.L., Torres-Chávez, P.I., Olmedo-Martínez, J.L., Vega-Rios, A., Flores-Gallardo, S., Zaragoza-Contreras, E.A. 2018. Lignin in storage and renewable energy applications: A review. *Journal of energy chemistry*, **27**(5), 1422-1438.

Esposito, D., Antonietti, M. 2015. Redefining biorefinery: the search for unconventional building blocks for materials. *Chemical Society Reviews*, **44**(16), 5821-5835.

Evert, R.F. 2006. *Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development*. John Wiley & Sons.

FAO. 2020. Food and Agriculture Organization of the United Nations. 2020 ed.

Fareez, I.M., Ibrahim, N.A., Yaacob, W.M.H.W., Razali, N.A.M., Jasni, A.H., Aziz, F.A. 2018. Characteristics of cellulose extracted from Josapine pineapple leaf fibre after alkali treatment followed by extensive bleaching. *Cellulose*, **25**(8), 4407-4421.

Faris, A.H., Rahim, A.A., Ibrahim, M.N.M., Alkurdi, A.M., Shah, I. 2016. Combination of lignin polyol–tannin adhesives and polyethylenimine for the preparation of green water-resistant adhesives. *Journal of Applied Polymer Science*, **133**(20).

Foglietta, F., Spagnoli, G.C., Muraro, M.G., Ballestri, M., Guerrini, A., Ferroni, C., Aluigi, A., Sotgiu, G., Varchi, G. 2018. anticancer activity of paclitaxel-loaded keratin nanoparticles in two-dimensional and perfused three-dimensional breast cancer models. *International journal of nanomedicine*, **13**, 4847.

Frederick Jr, W., Lien, S., Courchene, C., DeMartini, N., Ragauskas, A., Iisa, K. 2008. Production of ethanol from carbohydrates from loblolly pine: A technical and economic assessment. *Bioresource technology*, **99**(11), 5051-5057.

Fujita, M., Harada, H. 2000. Ultrastructure and formation of wood cell wall. *Wood and cellulosic chemistry*, 1-49.

Galbe, M., Wallberg, O. 2019. Pretreatment for biorefineries: a review of common methods for efficient utilisation of lignocellulosic materials. *Biotechnology for biofuels*, **12**(1), 1-26.

Gámez, S., González-Cabriales, J.J., Ramírez, J.A., Garrote, G., Vázquez, M. 2006. Study of the hydrolysis of sugar cane bagasse using phosphoric acid. *Journal of food engineering*, **74**(1), 78-88.

Gandini, A. 2008. Polymers from renewable resources: a challenge for the future of macromolecular materials. *Macromolecules*, **41**(24), 9491-9504.

Gandolfi, S., Ottolina, G., Consonni, R., Riva, S., Patel, I. 2014. Fractionation of hemp hurds by organosolv pretreatment and its effect on production of lignin and sugars. *ChemSusChem*, **7**(7), 1991-1999.

Garcia-Nunez, J.A., Ramirez-Contreras, N.E., Rodriguez, D.T., Silva-Lora, E., Frear, C.S., Stockle, C., Garcia-Perez, M. 2016. Evolution of palm oil mills into bio-refineries: literature review on current and potential uses of residual biomass and effluents. *Resources, Conservation and Recycling*, **110**, 99-114.

García, A., Alriols, M.G., Llano-Ponte, R., Labidi, J. 2011. Energy and economic assessment of soda and organosolv biorefinery processes. *Biomass and bioenergy*, **35**(1), 516-525.

Gierlinger, N., Jacques, D., Schwanninger, M., Wimmer, R., Pâques, L.E. 2004. Heartwood extractives and lignin content of different larch species (Larix sp.) and relationships to brown-rot decay-resistance. *Trees*, **18**(2), 230-236.

Giummarella, N., Lindén, P.r.A., Areskogh, D., Lawoko, M. 2019. Fractional profiling of kraft lignin structure: Unravelling insights on lignin reaction mechanisms. *ACS Sustainable Chemistry & Engineering*, **8**(2), 1112-1120.

Goh, C.S., Tan, H.T., Lee, K.T., Brosse, N. 2011. Evaluation and optimization of organosolv pretreatment using combined severity factors and response surface methodology. *Biomass and bioenergy*, **35**(9), 4025-4033.

Gupta, V.K., Kubicek, C.P., Berrin, J.-G., Wilson, D.W., Couturier, M., Berlin, A., Filho, E.X.F., Ezeji, T. 2016. Fungal Enzymes for Bio-Products from Sustainable and Waste Biomass. *Trends in Biochemical Sciences*, **41**(7), 633-645.

Harada, H., Côté Jr, W. 1985. Structure of wood. *Biosynthesis and biodegradation of wood components*, 1-42.

Hassan, S.S., Williams, G.A., Jaiswal, A.K. 2018. Emerging technologies for the pretreatment of lignocellulosic biomass. *Bioresource technology*, **262**, 310-318.

Hassan, S.S., Williams, G.A., Jaiswal, A.K. 2019. Moving towards the second generation of lignocellulosic biorefineries in the EU: Drivers, challenges, and opportunities. *Renewable and Sustainable Energy Reviews*, **101**, 590-599.

Hideno, A., Kawashima, A., Endo, T., Honda, K., Morita, M. 2013. Ethanol-based organosolv treatment with trace hydrochloric acid improves the enzymatic digestibility of Japanese cypress (Chamaecyparis obtusa) by exposing nanofibers on the surface. *Bioresource technology*, **132**, 64-70.

Himmel, M.E., Ding, S.-Y., Johnson, D.K., Adney, W.S., Nimlos, M.R., Brady, J.W., Foust, T.D. 2007. Biomass recalcitrance: engineering plants and enzymes for biofuels production. *science*, **315**(5813), 804-807.

HKEPD. 2012. Monitoring of Solid Waste in Hong Kong - Waste Statistics for 2011, Hong Kong Environmental Protection Department.

Ho-Yin, T., Cheng, S.-C., Yeung, C.S., Lau, C.-Y., Wong, W.-H., Dong, C., Leu, S.-Y. 2019. Development of a waste-derived lignin-porphyrin bio-polymer with enhanced photoluminescence at high water fraction with wide pH range and heavy metal sensitivity investigations. *Green Chemistry*, **21**(6), 1319-1329.

Höfte, H., Voxeur, A. 2017. Plant cell walls. *Current Biology*, **27**(17), R865-R870.

Huang, W.-D., Zhang, Y.-H.P. 2011. Analysis of biofuels production from sugar based on three criteria: thermodynamics, bioenergetics, and product separation. *Energy & Environmental Science*, **4**(3), 784-792.

Huijgen, W.J., Smit, A.T., Reith, J.H., Uil, H.d. 2011. Catalytic organosolv fractionation of willow wood and wheat straw as pretreatment for enzymatic cellulose hydrolysis. *Journal of Chemical Technology and Biotechnology*, **86**(11), 1428-1438.

Ilanidis, D., Wu, G., Stagge, S., Martín, C., Jönsson, L.J. 2020. Effects of redox environment on hydrothermal pretreatment of lignocellulosic biomass under acidic conditions. *Bioresource Technology*, **319**, 124211.

Islam, M.K., Dong, C., Hsu, H.Y., Lin, C.S.K., Leu, S.Y. 2020a. Valorisation of Woody Biomass. *Waste Valorisation: Waste Streams in a Circular Economy*, 87-108.

Islam, M.K., Thaemngoen, A., Lau, C.Y., Guan, J., Yeung, C.S., Chaiprapat, S., Leu, S.-Y. 2021. Staged organosolv pretreatment to increase net energy and reactive lignin yield in whole oil palm tree biorefinery. *Bioresource Technology*, **326**, 124766.

Islam, M.K., Wang, H., Rehman, S., Dong, C., Hsu, H.-Y., Lin, C.S.K., Leu, S.-Y. 2020b. Sustainability metrics of pretreatment processes in a waste derived lignocellulosic biomass biorefinery. *Bioresource Technology*, **298**, 122558.

Isogai, A. 2013. Wood nanocelluloses: fundamentals and applications as new bio-based nanomaterials. *Journal of wood science*, **59**(6), 449-459.

Jankowska, A., Boruszewski, P., Drożdżek, M., Rębkowski, B., Kaczmarczyk, A., Skowrońska, A. 2018. The Role of Extractives and Wood Anatomy in the Wettability and Free Surface Energy of Hardwoods. *BioResources*, **13**(2), 3082-3097.

Jazrawi, C., Biller, P., He, Y., Montoya, A., Ross, A.B., Maschmeyer, T., Haynes, B.S. 2015. Two-stage hydrothermal liquefaction of a high-protein microalga. *Algal research*, **8**, 15-22.

Ji, H., Song, Y., Zhang, X., Tan, T. 2017. Using a combined hydrolysis factor to balance enzymatic saccharification and the structural characteristics of lignin during pretreatment of Hybrid poplar with a fully recyclable solid acid. *Bioresource technology*, **238**, 575-581.

Jung, S., Trajano, H.L., Yoo, C.G., Foston, M.B., Hu, F., Tolbert, A.K., Wyman, C.E., Ragauskas, A.J. 2018. Topochemical understanding of lignin distribution during hydrothermal flowthrough pretreatment. *ChemistrySelect*, **3**(32), 9348-9352.

Kalogiannis, K., Matsakas, L., Aspden, J., Lappas, A., Rova, U., Christakopoulos, P. 2018. Acid assisted organosolv delignification of beechwood and pulp conversion towards high concentrated cellulosic ethanol via high gravity enzymatic hydrolysis and fermentation. *Molecules*, **23**(7), 1647.

Kanbayashi, T., Miyafuji, H. 2016. Effect of ionic liquid treatment on the ultrastructural and topochemical features of compression wood in Japanese cedar (Cryptomeria japonica). *Scientific reports*, **6**, 30147.

Kapoor, M., Soam, S., Agrawal, R., Gupta, R.P., Tuli, D.K., Kumar, R. 2017. Pilot scale dilute acid pretreatment of rice straw and fermentable sugar recovery at high solid loadings. *Bioresource technology*, **224**, 688-693.

Keijer, T., Bakker, V., Slootweg, J.C. 2019. Circular chemistry to enable a circular economy. *Nature chemistry*, **11**(3), 190-195.

Khoo, H., Ee, W., Isoni, V. 2016. Bio-chemicals from lignocellulose feedstock: sustainability, LCA and the green conundrum. *Green Chemistry*, **18**(7), 1912-1922.

Kibblewhite, R.P., Brookes, D. 1976. Distribution of chemical components in the walls of kraft and bisulphite pulp fibres. *Wood Science and Technology*, **10**(1), 39-46.

Kim, D.H., Park, H.M., Jung, Y.H., Sukyai, P., Kim, K.H. 2019. Pretreatment and enzymatic saccharification of oak at high solids loadings to obtain high titers and high yields of sugars. *Bioresource technology*, **284**, 391-397.

Kim, J.S., Lee, Y., Kim, T.H. 2016. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresource technology*, **199**, 42-48.

Klemm, D., Heublein, B., Fink, H.P., Bohn, A. 2005. Cellulose: fascinating biopolymer and sustainable raw material. *Angewandte Chemie International Edition*, **44**(22), 3358-3393.

Koo, B.-W., Min, B.-C., Gwak, K.-S., Lee, S.-M., Choi, J.-W., Yeo, H., Choi, I.-G. 2012. Structural changes in lignin during organosolv pretreatment of Liriodendron tulipifera and the effect on enzymatic hydrolysis. *Biomass and bioenergy*, **42**, 24-32.

Kralisch, D., Ott, D., Gericke, D. 2015. Rules and benefits of life cycle assessment in green chemical process and synthesis design: a tutorial review. *Green Chemistry*, **17**(1), 123-145.

Kumar, P., Barrett, D.M., Delwiche, M.J., Stroeve, P. 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial & engineering chemistry research*, **48**(8), 3713-3729.

Kumar, R., Tabatabaei, M., Karimi, K., Sárvári Horváth, I. 2016. Recent updates on lignocellulosic biomass derived ethanol-A review. *Biofuel Research Journal*, **3**(1), 347-356.

Kumar, R., Wyman, C.E. 2009. Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. *Biotechnology progress*, **25**(2), 302-314.

Kumar, V., Binod, P., Sindhu, R., Gnansounou, E., Ahluwalia, V. 2018. Bioconversion of pentose sugars to value added chemicals and fuels: recent trends, challenges and possibilities. *Bioresource technology*, **269**, 443-451.

Kumari, D., Singh, R. 2018. Pretreatment of lignocellulosic wastes for biofuel production: a critical review. *Renewable and Sustainable Energy Reviews*, **90**, 877-891.

Lachaud, S., Catesson, A.-M., Bonnemain, J.-L. 1999. Structure and functions of the vascular cambium. *Comptes Rendus de l'Académie des Sciences-Series III-Sciences de la Vie*, **322**(8), 633-650.

Lammens, T.M., Franssen, M.C., Scott, E.L., Sanders, J.P. 2010. Synthesis of biobased N-methylpyrrolidone by one-pot cyclization and methylation of γ-aminobutyric acid. *Green chemistry*, **12**(8), 1430-1436.

Lancefield, C.S., Panovic, I., Deuss, P.J., Barta, K., Westwood, N.J. 2017. Pre-treatment of lignocellulosic feedstocks using biorenewable alcohols: towards complete biomass valorisation. *Green Chemistry*, **19**(1), 202-214.

Lancefield, C.S., Wienk, H.L., Boelens, R., Weckhuysen, B.M., Bruijnincx, P.C. 2018. Identification of a diagnostic structural motif reveals a new reaction intermediate and condensation pathway in kraft lignin formation. *Chemical science*, **9**(30), 6348-6360.

Langenheim, J.H. 2003. *Plant resins*. Oregon, etc.: Timber Press.

Lavarack, B., Griffin, G., Rodman, D. 2002. The acid hydrolysis of sugarcane bagasse hemicellulose to produce xylose, arabinose, glucose and other products. *Biomass and bioenergy*, **23**(5), 367-380.

Lawther, J.M., Sun, R.-C., Banks, W. 1996. Rapid isolation and structural characterization of alkali-soluble lignins during alkaline treatment and atmospheric refining of wheat straw. *Industrial Crops and products*, **5**(2), 97-105.

Lee, I., Yu, J.-H. 2021. Design of hydrothermal and subsequent lime pretreatment for fermentable sugar and bioethanol production from acacia wood. *Renewable Energy*, **174**, 170-177.

Lee, J.-W., Jeffries, T.W. 2011. Efficiencies of acid catalysts in the hydrolysis of lignocellulosic biomass over a range of combined severity factors. *Bioresource technology*, **102**(10), 5884-5890.

Lennon, G., Willox, S., Ramdas, R., Funston, S.J., Klun, M., Pieh, R., Fairlie, S., Dobbin, S., Cobice, D.F. 2020. Assessing the oxidative degradation of N-methylpyrrolidone (NMP) in microelectronic fabrication processes by using a multiplatform analytical approach. *Journal of analytical methods in chemistry*, **2020**.

Leu, S.-Y., Yang, T.-H., Lo, S.-F., Yang, T.-H. 2012. Optimized material composition to improve the physical and mechanical properties of extruded wood–plastic composites (WPCs). *Construction and Building Materials*, **29**, 120-127.

Leu, S.-Y., Zhu, J. 2013. Substrate-related factors affecting enzymatic saccharification of lignocelluloses: our recent understanding. *Bioenergy Research*, **6**(2), 405-415.

Leu, S.-Y., Zhu, J., Gleisner, R., Sessions, J., Marrs, G. 2013. Robust enzymatic saccharification of a Douglas-fir forest harvest residue by SPORL. *biomass and bioenergy*, **59**, 393-401.

Li, C., Zhao, X., Wang, A., Huber, G.W., Zhang, T. 2015. Catalytic transformation of lignin for the production of chemicals and fuels. *Chem. Rev*, **115**(21), 11559-11624.

Li, S., Bashline, L., Zheng, Y., Xin, X., Huang, S., Kong, Z., Kim, S.H., Cosgrove, D.J., Gu, Y. 2016. Cellulose synthase complexes act in a concerted fashion to synthesize highly aggregated cellulose in secondary cell walls of plants. *Proceedings of the National Academy of Sciences*, **113**(40), 11348-11353.

Li, Y., Ragauskas, A.J. 2012. Ethanol organosolv lignin-based rigid polyurethane foam reinforced with cellulose nanowhiskers. *RSC advances*, **2**(8), 3347-3351.

Li, Z., Jiang, Z., Fei, B., Pan, X., Cai, Z., Yu, Y. 2012. Ethanol organosolv pretreatment of bamboo for efficient enzymatic saccharification. *BioResources*, **7**(3), 3452-3462.

Liao, Y., Koelewijn, S.-F., Van den Bossche, G., Van Aelst, J., Van den Bosch, S., Renders, T., Navare, K., Nicolaï, T., Van Aelst, K., Maesen, M. 2020. A sustainable wood biorefinery for low–carbon footprint chemicals production. *Science*, **367**(6484), 1385-1390.

Liebert, T. 2010. Cellulose solvents–remarkable history, bright future. in: *Cellulose solvents: for analysis, shaping and chemical modification*, ACS Publications, pp. 3-54.

Liu, F., Liu, L., Feng, X. 2005. Separation of acetone–butanol–ethanol (ABE) from dilute aqueous solutions by pervaporation. *Separation and Purification Technology*, **42**(3), 273-282.

Liu, H., Sun, J., Leu, S.Y., Chen, S. 2016. Toward a fundamental understanding of cellulase-lignin interactions in the whole slurry enzymatic saccharification process. *Biofuels, Bioproducts and Biorefining*, **10**(5), 648-663.

Liu, W., Chen, W., Hou, Q., Wang, S., Liu, F. 2018a. Effects of combined pretreatment of dilute acid pre-extraction and chemical-assisted mechanical refining on enzymatic hydrolysis of lignocellulosic biomass. *RSC Advances*, **8**(19), 10207-10214.

Liu, Y., Meyer, A.S., Nie, Y., Zhang, S., Thomsen, K. 2018b. Low energy recycling of ionic liquids via freeze crystallization during cellulose spinning. *Green chemistry*, **20**(2), 493-501.

Liu, Y., Zheng, X., Tao, S., Hu, L., Zhang, X., Lin, X. 2021. Process optimization for deep eutectic solvent pretreatment and enzymatic hydrolysis of sugar cane bagasse for cellulosic ethanol fermentation. *Renewable Energy*.

Loow, Y.-L., Wu, T.Y., Jahim, J.M., Mohammad, A.W., Teoh, W.H. 2016. Typical conversion of lignocellulosic biomass into reducing sugars using dilute acid hydrolysis and alkaline pretreatment. *Cellulose*, **23**(3), 1491-1520.

Lowry, H.H. 1963. Chemistry of coal utilization: supplementary volume.

Lynam, J.G., Chow, G.I., Coronella, C.J., Hiibel, S.R. 2016. Ionic liquid and water separation by membrane distillation. *Chemical Engineering Journal*, **288**, 557-561.

Ma, J., Nan, X., Liu, J., Zhu, W., Qin, W. 2018. Dispersion of pristine and polyaniline functionalized carbon nanotubes in designed solvent mixtures by Hansen solubility parameters. *Materials Today Communications*, **14**, 99-105.

Macedo, N., Brigham, C.J. 2014. From Beverages to Biofuels: The Journeys of Ethanol-Producing Microorganisms. *International Journal of Biotechnology for Wellness Industries*, **3**(3), 79-87.

Madson, P. 2003. Ethanol distillation: the fundamentals. *The Alcohol Textbook*, **4**.

Mattos, B.D., Lourençon, T.V., Gatto, D.A., Serrano, L., Labidi, J. 2016. Chemical characterization of wood and extractives of fast-growing Schizolobium parahyba and Pinus taeda. *Wood Material Science & Engineering*, **11**(4), 209-216.

Maurya, D.P., Singla, A., Negi, S. 2015. An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. *3 Biotech*, **5**(5), 597-609.

McCann, M.C., Carpita, N.C. 2015. Biomass recalcitrance: a multi-scale, multi-factor, and conversion-specific property. *Journal of experimental botany*, **66**(14), 4109-4118.

Meng, X., Bhagia, S., Wang, Y., Zhou, Y., Pu, Y., Dunlap, J.R., Shuai, L., Ragauskas, A.J., Yoo, C.G. 2020a. Effects of the advanced organosolv pretreatment strategies on structural properties of woody biomass. *Industrial Crops and Products*, **146**, 112144.

Meng, X., Pu, Y., Li, M., Ragauskas, A.J. 2020b. A biomass pretreatment using cellulose-derived solvent Cyrene. *Green Chemistry*, **22**(9), 2862-2872.

Mercure, J.-F., Pollitt, H., Viñuales, J.E., Edwards, N.R., Holden, P.B., Chewpreecha, U., Salas, P., Sognnaes, I., Lam, A., Knobloch, F. 2018. Macroeconomic impact of stranded fossil fuel assets. *Nature Climate Change*, **8**(7), 588-593.

Mesa, L., Martínez, Y., Barrio, E., González, E. 2017. Desirability function for optimization of Dilute Acid pretreatment of sugarcane straw for ethanol production and preliminary economic analysis based in three fermentation configurations. *Applied Energy*, **198**, 299-311.

Minnick, D.L., Flores, R.A., DeStefano, M.R., Scurto, A.M. 2016. Cellulose solubility in ionic liquid mixtures: temperature, cosolvent, and antisolvent effects. *The Journal of Physical Chemistry B*, **120**(32), 7906-7919.

Miranda, I., Sousa, V., Ferreira, J., Pereira, H. 2017. Chemical characterization and extractives composition of heartwood and sapwood from Quercus faginea. *PloS one*, **12**(6), e0179268.

Moniruzzaman, M., Goto, M. 2018. Ionic Liquid Pretreatment of Lignocellulosic Biomass for Enhanced Enzymatic Delignification.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y., Holtzapple, M., Ladisch, M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource technology*, **96**(6), 673-686.

Moure, A., Gullón, P., Domínguez, H., Parajó, J.C. 2006. Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals. *Process Biochemistry*, **41**(9), 1913-1923.

Mu, L., Shi, Y., Chen, L., Ji, T., Yuan, R., Wang, H., Zhu, J. 2015. [N-Methyl-2-pyrrolidone][C1–C4 carboxylic acid]: a novel solvent system with exceptional lignin solubility. *Chemical Communications*, **51**(70), 13554-13557.

Mussatto, S.I. 2016. *Biomass fractionation technologies for a lignocellulosic feedstock based biorefinery*. Elsevier.

Nair, S.G., Shah, J.V., Shah, P.A., Sanyal, M., Shrivastav, P.S. 2015. Extractive spectrophotometric determination of five selected drugs by ion-pair complex formation with bromothymol blue in pure form and pharmaceutical preparations. *Cogent Chemistry*, **1**(1), 1075852.

Nitsos, C., Rova, U., Christakopoulos, P. 2017. Organosolv fractionation of softwood biomass for biofuel and biorefinery applications. *Energies*, **11**(1), 50.

Noparat, P., Prasertsan, P., Sompong, O., Pan, X. 2017. Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose for Enzymatic Hydrolysis of Oil Palm trunk. *Energy Procedia*, **138**, 1122-1127.

Obama, P., Ricochon, G., Muniglia, L., Brosse, N. 2012. Combination of enzymatic hydrolysis and ethanol organosolv pretreatments: Effect on lignin structures, delignification yields and cellulose-to-glucose conversion. *Bioresource technology*, **112**, 156-163.

Osong, S.H., Norgren, S., Engstrand, P. 2016. Processing of wood-based microfibrillated cellulose and nanofibrillated cellulose, and applications relating to papermaking: a review. *Cellulose*, **23**(1), 93-123.

Özparpucu, M., Rüggeberg, M., Gierlinger, N., Cesarino, I., Vanholme, R., Boerjan, W., Burgert, I. 2017. Unravelling the impact of lignin on cell wall mechanics–a comprehensive study on young poplar trees downregulated for CINNAMYL ALCOHOL DEHYROGENASE (CAD). *The Plant Journal*.

Pan, S.-Y., Lin, Y.J., Snyder, S.W., Ma, H.-W., Chiang, P.-C. 2015. Development of low-carbon-driven bio-product technology using lignocellulosic substrates from agriculture: Challenges and perspectives. *Current Sustainable/Renewable Energy Reports*, **2**(4), 145-154.

Pan, X., Arato, C., Gilkes, N., Gregg, D., Mabee, W., Pye, K., Xiao, Z., Zhang, X., Saddler, J. 2005. Biorefining of softwoods using ethanol organosolv pulping: Preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products. *Biotechnology and bioengineering*, **90**(4), 473-481.

Pan, X., Xie, D., Yu, R.W., Lam, D., Saddler, J.N. 2007. Pretreatment of lodgepole pine killed by mountain pine beetle using the ethanol organosolv process: fractionation and process optimization. *Industrial & engineering chemistry research*, **46**(8), 2609-2617.

Papadopoulou, E.L., Pignatelli, F., Marras, S., Marini, L., Davis, A., Athanassiou, A., Bayer, I.S. 2016. Nylon 6, 6/graphene nanoplatelet composite films obtained from a new solvent. *RSC Advances*, **6**(8), 6823-6831.

Parameters, H.C.H.S. 2007. A User’s Handbook. *4–5](CRC Press, 2007)*.

Park, J.-y., Shiroma, R., Al-Haq, M.I., Zhang, Y., Ike, M., Arai-Sanoh, Y., Ida, A., Kondo, M., Tokuyasu, K. 2010a. A novel lime pretreatment for subsequent bioethanol production from rice straw–calcium capturing by carbonation (CaCCO) process. *Bioresource technology*, **101**(17), 6805-6811.

Park, N., Kim, H.-Y., Koo, B.-W., Yeo, H., Choi, I.-G. 2010b. Organosolv pretreatment with various catalysts for enhancing enzymatic hydrolysis of pitch pine (Pinus rigida). *Bioresource technology*, **101**(18), 7046-7053.

Park, Y.C., Kim, J.S. 2012. Comparison of various alkaline pretreatment methods of lignocellulosic biomass. *Energy*, **47**(1), 31-35.

Park, Y.C., Kim, T.H., Kim, J.S. 2017. Effect of organosolv pretreatment on mechanically pretreated biomass by use of concentrated ethanol as the solvent. *Biotechnology and Bioprocess Engineering*, **22**(4), 431-439.

PED. 2019. Pressure equipment directive category calculator.

Pizzi, A. 1983. Tannin-based wood adhesives, Chapter 4 in Wood Adhesives Chemistry and Technology (A. Pizzi, Ed.), Dekker, New York Google Scholar.

Prat, D., Wells, A., Hayler, J., Sneddon, H., McElroy, C.R., Abou-Shehada, S., Dunn, P.J. 2015. CHEM21 selection guide of classical-and less classical-solvents. *Green Chemistry*, **18**(1), 288-296.

Prokurat, S. 2013. Palm oil–strategic source of renewable energy in Indonesia and Malaysia. *Journal of Modern Science*(3), 425-443.

Raikwar, D., Majumdar, S., Shee, D. 2019. Thermocatalytic depolymerization of kraft lignin to guaiacols using HZSM-5 in alkaline water–THF co-solvent: a realistic approach. *Green Chemistry*, **21**(14), 3864-3881.

Ramluckan, K., Moodley, K.G., Bux, F. 2014. An evaluation of the efficacy of using selected solvents for the extraction of lipids from algal biomass by the soxhlet extraction method. *Fuel*, **116**, 103-108.

Raud, M., Kikas, T., Sippula, O., Shurpali, N. 2019. Potentials and challenges in lignocellulosic biofuel production technology. *Renewable and Sustainable Energy Reviews*, **111**, 44-56.

Renders, T., Cooreman, E., Van den Bosch, S., Schutyser, W., Koelewijn, S.-F., Vangeel, T., Deneyer, A., Van den Bossche, G., Courtin, C., Sels, B. 2018. Catalytic lignocellulose biorefining in n-butanol/water: a one-pot approach toward phenolics, polyols, and cellulose. *Green Chemistry*, **20**(20), 4607-4619.

Renders, T., Van den Bosch, S., Koelewijn, S.-F., Schutyser, W., Sels, B. 2017. Lignin-first biomass fractionation: the advent of active stabilisation strategies. *Energy & environmental science*, **10**(7), 1551-1557.

Rezania, S., Oryani, B., Cho, J., Talaiekhozani, A., Sabbagh, F., Hashemi, B., Rupani, P.F., Mohammadi, A.A. 2020. Different pretreatment technologies of lignocellulosic biomass for bioethanol production: An overview. *Energy*, 117457.

Rihouey, C., Paynel, F., Gorshkova, T., Morvan, C. 2017. Flax fibers: assessing the non-cellulosic polysaccharides and an approach to supramolecular design of the cell wall. *Cellulose*, **24**(5), 1985-2001.

Roche-Molina, M., Hardwick, B., Sanchez-Ramos, C., Sanz-Rosa, D., Gewert, D., Cruz, F.M., Gonzalez-Guerra, A., Andres, V., Palma, J.A., Ibanez, B. 2020. The pharmaceutical solvent N-methyl-2-pyrollidone (NMP) attenuates inflammation through Krüppel-like factor 2 activation to reduce atherogenesis. *Scientific reports*, **10**(1), 1-16.

Rodrıguez-Chong, A., Ramírez, J.A., Garrote, G., Vázquez, M. 2004. Hydrolysis of sugar cane bagasse using nitric acid: a kinetic assessment. *Journal of Food Engineering*, **61**(2), 143-152.

Rosa, L.P., Pires de Campos, C., Muylaert de Araujo, M.S. 2009. Biofuel contribution to mitigate fossil fuel CO 2 emissions: Comparing sugar cane ethanol in Brazil with corn ethanol and discussing land use for food production and deforestation. *Journal of Renewable and Sustainable Energy*, **1**(3), 033111.

Safari, A., Karimi, K., Shafiei, M. 2017. Dilute alkali pretreatment of softwood pine: A biorefinery approach. *Bioresource technology*, **234**, 67-76.

Sankaran, R., Cruz, R.A.P., Pakalapati, H., Show, P.L., Ling, T.C., Chen, W.-H., Tao, Y. 2020. Recent advances in the pretreatment of microalgal and lignocellulosic biomass: A comprehensive review. *Bioresource technology*, **298**, 122476.

Satchatippavarn, S., Martinez-Hernandez, E., Hang, M.Y.L.P., Leach, M., Yang, A. 2016. Urban biorefinery for waste processing. *Chemical Engineering Research and Design*, **107**, 81-90.

Schmetz, Q., Teramura, H., Morita, K., Oshima, T., Richel, A., Ogino, C., Kondo, A. 2019. Versatility of a dilute acid/butanol pretreatment investigated on various lignocellulosic biomasses to produce lignin, monosaccharides and cellulose in distinct phases. *ACS Sustainable Chemistry & Engineering*, **7**(13), 11069-11079.

Schneider, W.D.H., Fontana, R.C., Baudel, H.M., de Siqueira, F.G., Rencoret, J., Gutiérrez, A., de Eugenio, L.I., Prieto, A., Martínez, M.J., Martínez, Á.T. 2020. Lignin degradation and detoxification of eucalyptus wastes by on-site manufacturing fungal enzymes to enhance second-generation ethanol yield. *Applied Energy*, **262**, 114493.

Schwanninger, M., Hinterstoisser, B. 2002. Comparison of the classical wood extraction method using a Soxhlet apparatus with an advanced extraction method. *European Journal of Wood and Wood Products*, **60**(5), 343-346.

Sewsynker-Sukai, Y., David, A.N., Kana, E.G. 2020. Recent developments in the application of kraft pulping alkaline chemicals for lignocellulosic pretreatment: Potential beneficiation of green liquor dregs waste. *Bioresource Technology*, 123225.

Sheldon, R.A. 2017. Metrics of green chemistry and sustainability: past, present, and future. *ACS Sustainable Chemistry & Engineering*, **6**(1), 32-48.

Sheldrake, G.N., Schleck, D. 2007. Dicationic molten salts (ionic liquids) as re-usable media for the controlled pyrolysis of cellulose to anhydrosugars. *Green Chemistry*, **9**(10), 1044-1046.

Shen, X.-J., Wen, J.-L., Mei, Q.-Q., Chen, X., Sun, D., Yuan, T.-Q., Sun, R.-C. 2019. Facile fractionation of lignocelluloses by biomass-derived deep eutectic solvent (DES) pretreatment for cellulose enzymatic hydrolysis and lignin valorization. *Green chemistry*, **21**(2), 275-283.

Sheng, C., Azevedo, J. 2005. Estimating the higher heating value of biomass fuels from basic analysis data. *Biomass and bioenergy*, **28**(5), 499-507.

Sherwood, J., Constantinou, A., Moity, L., McElroy, C.R., Farmer, T.J., Duncan, T., Raverty, W., Hunt, A.J., Clark, J.H. 2014. Dihydrolevoglucosenone (Cyrene) as a bio-based alternative for dipolar aprotic solvents. *Chemical Communications*, **50**(68), 9650-9652.

Shuai, L., Amiri, M.T., Questell-Santiago, Y.M., Héroguel, F., Li, Y., Kim, H., Meilan, R., Chapple, C., Ralph, J., Luterbacher, J.S. 2016a. Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization. *Science*, **354**(6310), 329-333.

Shuai, L., Questell-Santiago, Y.M., Luterbacher, J.S. 2016b. A mild biomass pretreatment using γ-valerolactone for concentrated sugar production. *Green Chemistry*, **18**(4), 937-943.

Sikarwar, V.S., Zhao, M., Clough, P., Yao, J., Zhong, X., Memon, M.Z., Shah, N., Anthony, E.J., Fennell, P.S. 2016. An overview of advances in biomass gasification. *Energy & Environmental Science*, **9**(10), 2939-2977.

Sjostrom, E. 2013. *Wood chemistry: fundamentals and applications*. Elsevier.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. 2008. Determination of structural carbohydrates and lignin in biomass. *Laboratory analytical procedure*, **1617**, 1-16.

Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. 2005. Determination of extractives in biomass. *Laboratory analytical procedure (LAP)*, **1617**.

Soh, L., Eckelman, M.J. 2016. Green solvents in biomass processing. *ACS Sustainable Chemistry & Engineering*, **4**(11), 5821-5837.

Speight, J.G. 2016. *Environmental organic chemistry for engineers*. Butterworth-Heinemann.

Sun, D., Li, Y., Wang, J., Tu, Y., Wang, Y., Hu, Z., Zhou, S., Wang, L., Xie, G., Huang, J. 2017. Biomass saccharification is largely enhanced by altering wall polymer features and reducing silicon accumulation in rice cultivars harvested from nitrogen fertilizer supply. *Bioresource technology*, **243**, 957-965.

Sun, R., Sun, X., Fowler, P., Tomkinson, J. 2002. Structural and physico-chemical characterization of lignins solubilized during alkaline peroxide treatment of barley straw. *European Polymer Journal*, **38**(7), 1399-1407.

Sun, Y., Cheng, J. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource technology*, **83**(1), 1-11.

Taghizadeh-Hesary, F., Rasoulinezhad, E., Yoshino, N. 2019. Energy and food security: Linkages through price volatility. *Energy policy*, **128**, 796-806.

Taherzadeh, M.J., Karimi, K. 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *International journal of molecular sciences*, **9**(9), 1621-1651.

Tanaka, S., Miyazaki, H., Shiozaki, A., Ichikawa, D., Otsuji, E., Marunaka, Y. 2017. Cytosolic Cl-Affects the Anticancer Activity of Paclitaxel in the Gastric Cancer Cell Line, MKN28 Cell. *Cellular Physiology and Biochemistry*, **42**(1), 68-80.

Taylor, A.M., Gartner, B.L., Morrell, J.J. 2007. Heartwood formation and natural durability—a review. *Wood and fiber science*, **34**(4), 587-611.

Teramoto, Y., Lee, S.-H., Endo, T. 2009. Cost reduction and feedstock diversity for sulfuric acid-free ethanol cooking of lignocellulosic biomass as a pretreatment to enzymatic saccharification. *Bioresource technology*, **100**(20), 4783-4789.

Terán Hilares, R., Swerts, M.P., Ahmed, M.A., Ramos, L., da Silva, S.S.r., Santos, J.l.C.s. 2017. Organosolv Pretreatment of Sugar Cane Bagasse for Bioethanol Production. *Industrial & Engineering Chemistry Research*, **56**(14), 3833-3838.

Tian, D., Hu, J., Bao, J., Chandra, R.P., Saddler, J.N., Lu, C. 2017. Lignin valorization: Lignin nanoparticles as high-value bio-additive for multifunctional nanocomposites. *Biotechnology for biofuels*, **10**(1), 192.

Tobiszewski, M., Tsakovski, S., Simeonov, V., Namieśnik, J., Pena-Pereira, F. 2015. A solvent selection guide based on chemometrics and multicriteria decision analysis. *Green Chemistry*, **17**(10), 4773-4785.

Trajano, H.L., Engle, N.L., Foston, M., Ragauskas, A.J., Tschaplinski, T.J., Wyman, C.E. 2013. The fate of lignin during hydrothermal pretreatment. *Biotechnology for biofuels*, **6**(1), 110.

Tsegaye, B., Balomajumder, C., Roy, P. 2020. Organosolv pretreatments of rice straw followed by microbial hydrolysis for efficient biofuel production. *Renewable Energy*, **148**, 923-934.

Tu, Y., Wang, L., Xia, T., Sun, D., Zhou, S., Wang, Y., Li, Y., Zhang, H., Zhang, T., Madadi, M. 2017. Mild chemical pretreatments are sufficient for complete saccharification of steam-exploded residues and high ethanol production in desirable wheat accessions. *Bioresource technology*, **243**, 319-326.

Ubando, A.T., Rivera, D.R.T., Chen, W.-H., Culaba, A.B. 2019. A comprehensive review of life cycle assessment (LCA) of microalgal and lignocellulosic bioenergy products from thermochemical processes. *Bioresource technology*, **291**, 121837.

Valdivia, M., Galan, J.L., Laffarga, J., Ramos, J.L. 2016. Biofuels 2020: Biorefineries based on lignocellulosic materials. *Microbial biotechnology*, **9**(5), 585-594.

Van den Bosch, S., Renders, T., Kennis, S., Koelewijn, S.-F., Van den Bossche, G., Vangeel, T., Deneyer, A., Depuydt, D., Courtin, C., Thevelein, J. 2017. Integrating lignin valorization and bio-ethanol production: on the role of Ni-Al 2 O 3 catalyst pellets during lignin-first fractionation. *Green Chemistry*, **19**(14), 3313-3326.

Wang, G., Qi, S., Xia, Y., Parvez, A.M., Si, C., Ni, Y. 2020. Mild One-Pot Lignocellulose Fractionation Based on Acid-Catalyzed Biphasic Water/Phenol System to Enhance Components' Processability. *ACS Sustainable Chemistry & Engineering*, **8**(7), 2772-2782.

Wang, H.-M., Wang, B., Wen, J.-L., Yuan, T.-Q., Sun, R.-C. 2017a. Structural characteristics of lignin macromolecules from different Eucalyptus species. *ACS Sustainable Chemistry & Engineering*, **5**(12), 11618-11627.

Wang, L., Zhang, K., Xu, Y., Zhang, M., Wang, D. 2018a. High-solid pretreatment of corn stover using urea for enzymatic saccharification. *Bioresource technology*, **259**, 83-90.

Wang, P., Chen, Y.M., Wang, Y., Lee, Y.Y., Zong, W., Taylor, S., McDonald, T., Wang, Y. 2019a. Towards comprehensive lignocellulosic biomass utilization for bioenergy production: Efficient biobutanol production from acetic acid pretreated switchgrass with Clostridium saccharoperbutylacetonicum N1-4. *Applied Energy*, **236**, 551-559.

Wang, Q., Wang, W., Tan, X., Chen, X., Guo, Y., Yu, Q., Yuan, Z., Zhuang, X. 2019b. Low-temperature sodium hydroxide pretreatment for ethanol production from sugarcane bagasse without washing process. *Bioresource technology*, **291**, 121844.

Wang, S., Li, F., Zhang, P., Jin, S., Tao, X., Tang, X., Ye, J., Nabi, M., Wang, H. 2017b. Ultrasound assisted alkaline pretreatment to enhance enzymatic saccharification of grass clipping. *Energy Conversion and Management*, **149**, 409-415.

Wang, S., Zhao, W., Lee, T.S., Singer, S.W., Simmons, B.A., Singh, S., Yuan, Q., Cheng, G. 2018b. Dimethyl Sulfoxide Assisted Ionic Liquid Pretreatment of Switchgrass for Isoprenol Production. *ACS Sustainable Chemistry & Engineering*, **6**(3), 4354-4361.

Wang, Y., Abdel-Rahman, M.A., Tashiro, Y., Xiao, Y., Zendo, T., Sakai, K., Sonomoto, K. 2014. l-(+)-lactic acid production by co-fermentation of cellobiose and xylose without carbon catabolite repression using Enterococcus mundtii QU 25. *RSC Advances*, **4**(42), 22013-22021.

Wang, Y., Leng, L., Islam, M.K., Liu, F., Lin, C.S.K., Leu, S.-Y. 2019c. Substrate-related factors affecting cellulosome-induced hydrolysis for lignocellulose valorization. *International journal of molecular sciences*, **20**(13), 3354.

Wang, Y., Tashiro, Y., Sonomoto, K. 2015. Fermentative production of lactic acid from renewable materials: Recent achievements, prospects, and limits. *Journal of bioscience and bioengineering*, **119**(1), 10-18.

Welker, C., Balasubramanian, V., Petti, C., Rai, K., DeBolt, S., Mendu, V. 2015. Engineering plant biomass lignin content and composition for biofuels and bioproducts. *Energies*, **8**(8), 7654-7676.

Wilson, A.N., Dutta, A., Black, B.A., Mukarakate, C., Magrini, K., Schaidle, J.A., Michener, W.E., Beckham, G.T., Nimlos, M.R. 2019. Valorization of aqueous waste streams from thermochemical biorefineries. *Green Chemistry*.

Wyman, C.E. 2013. *Aqueous pretreatment of plant biomass for biological and chemical conversion to fuels and chemicals*. John Wiley & Sons.

Wyman, C.E. 2018. Ethanol production from lignocellulosic biomass: overview. in: *Handbook on Bioethanol*, Routledge, pp. 1-18.

Xiang, Z., Gao, W., Chen, L., Lan, W., Zhu, J., Runge, T. 2016. A comparison of cellulose nanofibrils produced from Cladophora glomerata algae and bleached eucalyptus pulp. *Cellulose*, **23**(1), 493-503.

Xiao, K., Yuan, Y., Xiao, N., Zhou, W., Yang, Q., Feng, W., Zhu, D. 2017. Enzymatic saccharification responses of Eichhornia crassipes, sugarcane bagasse and Metasequoia glyptostroboides to two oxidation pretreatments for biofuel production. *Industrial Crops and Products*, **107**, 22-29.

Xie, H., Shen, H., Gong, Z., Wang, Q., Zhao, Z.K., Bai, F. 2012. Enzymatic hydrolysates of corn stover pretreated by a N-methylpyrrolidone–ionic liquid solution for microbial lipid production. *Green chemistry*, **14**(4), 1202-1210.

Xie, X., Feng, X., Chi, S., Zhang, Y., Yu, G., Liu, C., Li, Z., Li, B., Peng, H. 2018. A sustainable and effective potassium hydroxide pretreatment of wheat straw for the production of fermentable sugars. *Bioresource Technology Reports*, **3**, 169-176.

Xu, F., Shi, Y.-C., Wang, D. 2013. X-ray scattering studies of lignocellulosic biomass: a review. *Carbohydrate polymers*, **94**(2), 904-917.

Xu, H., Che, X., Ding, Y., Kong, Y., Li, B., Tian, W. 2019. Effect of crystallinity on pretreatment and enzymatic hydrolysis of lignocellulosic biomass based on multivariate analysis. *Bioresource technology*, **279**, 271-280.

Xu, L., Tschirner, U.W. 2011. Peracetic acid pretreatment of alfalfa stem and aspen biomass. *BioResources*, **7**(1), 0203-0216.

Xu, Q.-Q., Zhao, M.-J., Yu, Z.-Z., Yin, J.-Z., Li, G.-M., Zhen, M.-Y., Zhang, Q.-Z. 2017. Enhancing enzymatic hydrolysis of corn cob, corn stover and sorghum stalk by dilute aqueous ammonia combined with ultrasonic pretreatment. *Industrial Crops and Products*, **109**, 220-226.

Yang, H., Zhang, X., Luo, H., Liu, B., Shiga, T.M., Li, X., Im Kim, J., Rubinelli, P., Overton, J.C., Subramanyam, V. 2019. Overcoming cellulose recalcitrance in woody biomass for the lignin-first biorefinery. *Biotechnology for biofuels*, **12**(1), 1-18.

Yeh, T.-F., Chang, M.-J., Chang, W.-J. 2014. Comparison of dilute acid and sulfite pretreatments on Acacia confusa for biofuel application and the influence of its extractives. *Journal of agricultural and food chemistry*, **62**(44), 10768-10775.

Yoo, C.G., Dumitrache, A., Muchero, W., Natzke, J., Akinosho, H., Li, M., Sykes, R.W., Brown, S.D., Davison, B., Tuskan, G.A. 2018. Significance of lignin S/G ratio in biomass recalcitrance of Populus trichocarpa variants for bioethanol production. *ACS Sustainable Chemistry & Engineering*, **6**(2), 2162-2168.

Yoo, C.G., Kim, H., Lu, F., Azarpira, A., Pan, X., Oh, K.K., Kim, J.S., Ralph, J., Kim, T.H. 2016. Understanding the physicochemical characteristics and the improved enzymatic saccharification of corn stover pretreated with aqueous and gaseous ammonia. *BioEnergy Research*, **9**(1), 67-76.

Yoo, C.G., Meng, X., Pu, Y., Ragauskas, A.J. 2020. The critical role of lignin in lignocellulosic biomass conversion and recent pretreatment strategies: A comprehensive review. *Bioresource Technology*, **301**, 122784.

Yoo, C.G., Yang, Y., Pu, Y., Meng, X., Muchero, W., Yee, K.L., Thompson, O.A., Rodriguez, M., Bali, G., Engle, N.L. 2017. Insights of biomass recalcitrance in natural Populus trichocarpa variants for biomass conversion. *Green Chemistry*, **19**(22), 5467-5478.

Yumrutaş, R., Alma, M.H., Özcan, H., Kaşka, Ö. 2008. Investigation of purified sulfate turpentine on engine performance and exhaust emission. *Fuel*, **87**(2), 252-259.

Zhang, C., Gleisner, R., Houtman, C., Pan, X., Zhu, J. 2016a. Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses for Bioconversion of Woody Biomass. in: *Biomass Fractionation Technologies for a Lignocellulosic Feedstock Based Biorefinery*, Elsevier, pp. 499-541.

Zhang, J.-w., Lu, G.-p., Cai, C. 2017. Self-hydrogen transfer hydrogenolysis of β-O-4 linkages in lignin catalyzed by MIL-100 (Fe) supported Pd–Ni BMNPs. *Green Chemistry*, **19**(19), 4538-4543.

Zhang, K., Pei, Z., Wang, D. 2016b. Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: a review. *Bioresource technology*, **199**, 21-33.

Zhang, P., Hu, H., Shi, S. 2006. Application of hemicellulose. *Tianjin Pap Mak*, **2**, 16-8.

Zhang, Q., Tan, X., Wang, W., Yu, Q., Wang, Q., Miao, C., Guo, Y., Zhuang, X., Yuan, Z. 2019. Screening solvents based on Hansen solubility parameter theory to depolymerize lignocellulosic biomass efficiently under low temperature. *ACS Sustainable Chemistry & Engineering*, **7**(9), 8678-8686.

Zhao, C., Hu, Z., Shi, L., Wang, C., Yue, F., Li, S., Zhang, H., Lu, F. 2020. Profiling of the formation of lignin-derived monomers and dimers from Eucalyptus alkali lignin. *Green Chemistry*.

Zhao, H., Jones, C.L., Baker, G.A., Xia, S., Olubajo, O., Person, V.N. 2009a. Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis. *Journal of Biotechnology*, **139**(1), 47-54.

Zhao, X., Cheng, K., Liu, D. 2009b. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Applied microbiology and biotechnology*, **82**(5), 815.

Zhao, X., Li, S., Wu, R., Liu, D. 2017. Organosolv fractionating pre-treatment of lignocellulosic biomass for efficient enzymatic saccharification: chemistry, kinetics, and substrate structures. *Biofuels, Bioproducts and Biorefining*.

Zhao, X., Zhang, L., Liu, D. 2012a. Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. *Biofuels, Bioproducts and Biorefining*, **6**(4), 465-482.

Zhao, X., Zhang, L., Liu, D. 2012b. Biomass recalcitrance. Part II: Fundamentals of different pre-treatments to increase the enzymatic digestibility of lignocellulose. *Biofuels, Bioproducts and Biorefining*, **6**(5), 561-579.

Zhao, X., Zhang, L., Liu, D. 2010. Pretreatment of Siam weed stem by several chemical methods for increasing the enzymatic digestibility. *Biotechnology journal*, **5**(5), 493-504.

Zhao, X.B., Wang, L., Liu, D.H. 2008. Peracetic acid pretreatment of sugarcane bagasse for enzymatic hydrolysis: a continued work. *Journal of chemical technology and biotechnology*, **83**(6), 950-956.

Zheng, J., Tashiro, Y., Wang, Q., Sonomoto, K. 2015. Recent advances to improve fermentative butanol production: genetic engineering and fermentation technology. *Journal of bioscience and bioengineering*, **119**(1), 1-9.

Zhu, J., Pan, X. 2010. Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation. *Bioresource technology*, **101**(13), 4992-5002.

Zhu, J., Pan, X., Wang, G., Gleisner, R. 2009. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresource technology*, **100**(8), 2411-2418.

Zhu, X., Tong, S., Li, X., Gao, Y., Xu, Y., Dacres, O.D., Ashida, R., Miura, K., Liu, W., Yao, H. 2017. Conversion of Biomass into High-Quality Bio-oils by Degradative Solvent Extraction Combined with Subsequent Pyrolysis. *Energy & Fuels*, **31**(4), 3987-3994.

Zijlstra, D.S., Lahive, C.W., Analbers, C.A., Figueirêdo, M.B., Wang, Z., Lancefield, C.S., Deuss, P.J. 2020. Mild organosolv lignin extraction with alcohols: the importance of benzylic alkoxylation. *ACS Sustainable Chemistry & Engineering*, **8**(13), 5119-5131.

Zimmermann, C.J., Bollar, N.V., Wettstein, S.G. 2018. Liquid phase conversion of lignocellulosic biomass using biphasic systems. *Biomass and Bioenergy*, **118**, 163-171.