# FRACTIONATION AND CHARACTERIZATION OF EXTRACTED LIGNIN FROM OIL PALM EMPTY FRUIT BUNCH USING SYNTHESIZED DEEP EUTECTIC SOLVENTS

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**FACULTY OF ENGINEERING**
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**2020**

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**THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR IN PHILOSOPHY**

**FACULTY OF ENGINEERING**
**UNIVERSITY OF MALAYA**
**KUALA LUMPUR**

**2020**

## UNIVERSITY OF MALAYA
## ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: Tan Yee Tong

Matric No: 17006136/1

Name of Degree: Doctor of Philosophy

Title of Thesis ("this Work"): Fractionation and Characterization of Lignin from Oil Palm Empty Fruit Bunch Using Synthesized Deep Eutectic Solvents

Field of Study: Bioprocess Engineering

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# FRACTIONATION AND CHARACTERIZATION OF EXTRACTED LIGNIN FROM OIL PALM EMPTY FRUIT BUNCH USING SYNTHESIZED DEEP EUTECTIC SOLVENTS

## ABSTRACT

Deep eutectic solvent (DES), a new generation green solvent has been introduced as an alternative pretreatment solvent due to its ability for lignocellulosic biomass fractionation and lignin extraction. Numerous types of compounds can be adopted as DES constituents, which has resulted in abundant variation of DES with varying properties. Selection of the suitable chemical compound category is therefore essential for optimum DES performance. However, investigation on the performance comparison between the different DES categories is lacking. In addition, despite its widely acknowledged effectiveness in biomass fractionation, the properties of DES-fractionated biopolymers, especially lignin are not well understood. Hence, this project focused on identifying the best performing DES constituents for lignocellulosic biopolymer fractionation and lignin extraction from oil palm empty fruit bunch (EFB). Subsequently, the properties of DES-pretreated EFB solid and DES-extracted lignin (DEEL) were elucidated to assess their potential for downstream conversion. EFB was selected as the source of biomass in this study due to its presence as one of the most abundant agricultural wastes in Malaysia and its rich lignocellulosic biopolymer content. The first stage of DES selection started with identifying the suitable pH range of the solvent. Acidic DES demonstrated great fractionation ability to achieve 100% of glucan retention in DES-pretreated biomass solid, 100% of hemicellulose hydrolysis, and 50.6% of lignin extraction. The fractionation performance of alkaline and neutral DESs were comparatively inefficient. To optimize the DES performance, types of acid constituent hydrogen bond donor (HBD) in DES that promoted EFB fractionation and lignin extraction were next investigated based on the acid's functional group and molar ratio. The results showed that the presence of hydroxyl group (-OH) and short alkyl chain (-CnH2n+1) in the acid HBD could enhance lignin extraction and biomass fractionation performance. As a result, choline chloride:lactic acid (CC-LA) 1:15 and choline chloride:formic acid (CC-FA) 1:2 achieved the highest lignin extraction yield at >60 wt% when compared with the other acidic DESs. The EFB solid fractionated by both acidic DES were enriched with >80 wt% of glucan content. The type of DES solvent applied in lignin extraction process significantly affect the isolated lignin's composition, molecular structure, thermal properties and their compatible downstream conversion technologies. CC-LA DEEL would be more suitable for functional modification as shown through the higher amount of labile β-O-4 linkage (2.52 unit/Ar) and volatile product emission. CC-FA DEEL encompassed a highly condensed structure due to the abundant C-C linkages formed via the extensive hydrolysis of ether bond. A circular biorefinery process was then proposed, in which the process was initiated with DES pretreatment for biopolymer fractionation and followed by the conversion of DES-fractionated biopolymers into bio-based materials or chemicals. This study demonstrates a step-wise DES constituent selection process for lignocellulosic biomass pretreatment. The findings obtained is expected to aid in the selection of DES solvent for efficient biomass fractionation, thus providing an alternative solution to the lagging development of the lignocellulosic biomass conversion, such as oil palm empty fruit bunch into a variety of bio-based products.

Keywords: Deep eutectic solvent, oil palm empty fruit bunch, biomass fractionation, lignin extraction, bio-based products

# PEMECAHAN DAN PENCIRIAN LIGNIN DARI OIL PALM EMPTY FRUIT BUNCH MENGGUNAKAN PELARUT EUTEKTIK

## ABSTRAK

Pelarut eutektik (DES), sejenis pelarut generasi baru yang mesra alam telah diperkenalkan sebagai pelarut alternatif disebabkan kecekapan ia dalam permecahan biojisim lignoselulosa dan permerahan lignin. Perbagai-bagai jenis kompaun boleh digunakan sebagai konstituen DES, justeru DES yang bersifat berbeza-beza boleh dihasilkan. Demikian, pemilihan konstituen yang sesuai adalah amat penting untuk memastikan prestasi DES. Namun begitu, penyiasatan tentang perbandingan antara prestasi kategori DES yang berbeza adalah amat terhad. Lagi, walaupun prestasi DES dalam pemerahan biojisim sudah dibuktikan, sifat biojisim yang diperahkan oleh DES, terutamanya lignin masih belum difahami. Justeru, projek ini menumpu terhadap pengenalpastian konstituen DES yang tersesuai untuk pemecahan tandan buah kelapa sawit (EFB) dan pemerahan lignin. Kemudian, sifat pepejal EFB dan lignin yang diperahkan oleh DES (DEEL) telah didedahkan untuk menyiasat potensi mereka bagi proses penaiktarafan selanjutnya. Biojisim yang terpilih dalam projek ini ialah EFB disebabkan ia adalah salah satu sisa pertanian yang paling banyak di Malaysia dan kandungan biojisim lignoselulose yang tinggi. Taraf pertama pemilihan DES bermula daripada pengenalpastian julat pH yang bersesuaian. DES bersifat asid mempamerkan prestasi yang bagus dalam pemecahan EFB dan mencapai 100% pengekalan selulosa dalam pepejal, 100% hemiselulosa hidrolisis dan 50.6% pemerahan lignin. Sebaliknya, DES yang bersifat neutral and alkali menunjukkan efisiensi pemecahan biojisim yang lebih lemah. Demi mengoptimumkan prestasi DES, jenis konstituen penderma ikatan hidrogen (HBD) asid dalam DES yang dapat menggalakkan permecahan EFB dan pemerahan lignin telah dikajikan berdasarkan kumpulan berfungsi asid dan nisbah molar. Kumpulan hidroksil (-OH) dan rantai alkil (-CnH2n+1) yang pendek pada asid dapat meningkatkan prestasi pemechan biojisim and pemerahan lignin. Justeru, choline klorida:asid laktik (CC-LA) 1:15 dan choline klorida:asid formic (CC-FA) 1:2 telah berjaya mencapai pemerahan lignin sebanyak >60% berbanding dengan DES bersifat asid yang lain-lain. Pepejal EFB yang dihasilkan dengan kedua-dua DES bersifat asid tersebut telah diperkayakan dengan >80% kandungan selulose. Jenis pelarut DES yang digunakan dalam pemerahan lignin menunjukkan pengaruhan ketara terhadap komposisi DEEL, strucktur molekul, sifar terma dan jenis teknologi penaiktarafan yang bersesuaian. CC-LA DEEL akan lebih sesuai untuk pengubahsuaian fungsional disebabkan jumlah talian β-O-4 (2.52 unit/Ar) dan penghasilan produk gas yang lebih banyak. CC-FA DEEL merangkumi struktur yang lebih padat disebabkan pembentukan talian C-C yang banyak melalui hidrolisis talian ether. Selanjutnya, proses kilang pekeliling telah dicadangkan dimanakan proses itu dimulakan dengan pemecahan biojisim dengan menggunakan DES, diikuti oleh penukaran biojisim yang telah dipecahkan kepada bahan-bahan dan kimia-kimia bersifat bio. Projek ini telah menunjukkan proses pemilihan konstituen DES yang melibatkan dua langkah. Hasil kajian ini boleh digunakan sebagai panduan dalam proses pemilihan konstituen DES untuk pemecahan biojisim yang cekap. Justeru, projek ini menawarkan penyelesaian alternatif bagi pembangunan penaiktarafan biojisim seperti EFB yang kini ketinggalan kepada bio-produk yang beraneka ragam.

Kata Kunci: Pelarut eutektik, tandan kelapa sawit, pemecahan biojisim, pemerahan lignin, produk berasaskan bio

# ACKNOWLEDGEMENTS

First of all, I would like to express my immense gratitude to my project supervisors, Assoc. Prof. Ir. Dr. Ngoh Gek Cheng and Assoc. Prof. Ir. Dr. Adeline Chua Seak May who have expertly guided me through the course of my PhD education. I am forever grateful for their guidance and advise, which has fuelled my inspiration throughout the candidacy. I am also highly appreciative towards the given opportunity of conducting my research in Bioprocess Laboratory, University Malaya.

My appreciation extends towards my seniors and colleagues, especially Dr. How Seow Wah who has been offering a great deal of help in making sure smooth daily laboratory operation. Thanks also go to the engineers and laboratory assistants in the Department of Chemical Engineering for their generous assistance, especially in terms of providing infrastructure and equipment support. Without the support from the university staffs, the goal of this study would not have been achieved.

Lastly, I am especially grateful towards my family, partner and friends for their unwavering encouragement and support throughout this journey. Thank you all for being a part of this journey.

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| | | |
|---|---|---|
| AA | : | Acetic acid |
| AAD | : | Absolute average deviation |
| BA | : | Butyric acid |
| CA | : | Citric acid |
| ChCl/CC | : | Choline chloride |
| $C_p$ | : | Heat capacity |
| DEEL | : | DES-extracted lignin |
| DES | : | Deep eutectic solvent |
| EFB | : | Oil palm empty fruit bunch |
| FA | : | Formic acid |
| GLUC | : | Glucose |
| GLY | : | Glycerol |
| HBA | : | Hydrogen bond acceptor |
| HBD | : | Hydrogen bond donor |
| LA | : | Lactic acid |
| LF | : | DES pretreatment liquid fraction |
| MA | : | Malic acid |
| MAE | : | Maleic acid |
| ODW | : | Oven-dried weight of oil palm empty fruit bunch |
| PA | : | Propionic acid |
| PhOH | : | Phenolic hydroxyl content |
| SA | : | Succinic acid |
| SF | : | DES-pretreated solid fraction |

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# CHAPTER 1: INTRODUCTION

## 1.1 Research Background

Lignocellulosic biomass has been widely recognized as an alternative renewable resource to fossil-based feedstock for fuel and chemical production. This type of biomass consists of three main biopolymers namely cellulose, hemicellulose and lignin. Each of these biopolymers has its unique composition and structure, enabling them to be transformed into a wide array of bio-based products. Being the most abundant renewable aromatic resource on Earth, lignin is the most underutilized lignocellulosic biopolymer. The structural complexity and huge variation of extracted lignin existed in the market have increased the difficulty for the downstream conversion of lignin-derived products.

The introduction of deep eutectic solvent (DES) for biopolymer fractionation and lignin extraction process has initiated a new lignocellulosic biomass processing scheme, which emphasizes on lignin isolation prior to carbohydrate upgrading. DES is a new designer solvent constituting of at least one hydrogen bond donor and hydrogen bond acceptor. There is a vast variety of compounds that can be selected to form DES, including certain naturally-existing constituents. The synthesized DES solvents will inherit characteristics of the adopted DES constituents. Thus, their solvents' properties can be tailored to the application need based on the selected constituents.

The use of renewable feedstock and green technology is highly emphasized in the development of new industrial process. Therefore, biomass processing using DES, specifically lignin extraction has been a field of high interest since its pioneering work in year 2012. However, the development of this technology is in its early phase and there are still many research gaps related to DES application that demand answer, which have led to the conception of this study.

## 1.2 Problem Statement

In Malaysia, the oil palm industry generates more than 40 megatonne of lignocellulosic solid wastes annually (Malaysian Palm Oil Board, 2016; Sohni et al., 2018). This demands for a plan to effectively utilize the abundant biomass wastes generated in our country. Among the different solid wastes produced, oil palm empty fruit bunch (EFB) is the most generated solid waste from oil palm mills (Ahmad, Zhang, Doherty, & O'Hara, 2016). Traditionally, EFB was incinerated and then repurposed as fertilizer. However, this practice has been inhibited for its adverse environmental effect (Ibrahim, Zakaria, Sipaut, Sulaiman, & Hashim, 2011). Being one of the most abundant agricultural waste produced in Malaysia, it is of utmost importance to search for alternative EFB application for the production of higher commercial value products.

Due to food-energy conflict, the utilization of lignocellulosic biomass for industrial purpose is preferable over sugar-based crops (Rastogi & Shrivastava, 2017). They are viewed as the potential feedstock replacement for non-renewable fossil fuels to produce bio-based products sustainably (Raman & Gnansounou, 2015). With the ratification of Paris Agreement in year 2016, the implementation of renewable energy and carbon emission reduction have both become more important than ever in curbing global warming issue. Nevertheless, the development of lignocellulosic biomass conversion technology is not as advanced as the sugar-based biomass. The setback in exploiting lignocellulosic biomass mainly lies in their recalcitrant properties, which increases the difficulty for biomass separation (Gillet et al., 2017; U.S.DOE., 2015). Moreover, the solubility of lignocellulosic biopolymers is very limited in many solvents (N. Sun, Rodriguez, Rahman, & Rogers, 2011), imposing additional difficulty in biomass conversion. Thus, these factors necessitate a pretreatment step in the early stage of biorefinery to fractionate the biopolymers for efficient valorisation of these distinctive biopolymers (Davison, Parks, Davis, & Donohoe, 2013). To this day, biomass fractionation remains as one of the major challenges in designing a lignocellulosic biomass refinery process (Gillet et al., 2017).

In conventional pretreatment, carbohydrate portion of the biomass is utilized, often for bioethanol production. The remaining lignin stream is incinerated for power generation (Ragauskas et al., 2014). Lignin is anticipated to be the major aromatic resource in the future bio-economy owing to its natural abundancy, which constitutes 30% of the non-fossil carbon resources on Earth (Laurichesse & Avérous, 2014). Yet, lignin received comparably lesser attention than the carbohydrate in the early development of biorefinery (Zakzeski, Bruijnincx, Jongerius, & Weckhuysen, 2010). It remains as the most underutilized lignocellulosic biopolymer (C. Xu, Arancon, Labidi, & Luque, 2014). This would decrease the economic return of biorefinery as the lignocellulosic biopolymers are not exploited to their full potential (Azadi, Inderwildi, Farnood, & King, 2013).

Valorisation of lignin for high value platform and commodity chemical production has proven to be a very challenging task (Holladay, White, Bozell, & Johnson, 2007). This is due to a number of shortcomings, for instance biomass fractionation process has always been designed to optimize carbohydrate processing at the expense of lignin's quality (Holladay et al., 2007). As a result, the extracted lignin with heavily modified structure is not ideal for valorisation. Furthermore, the high variability of lignin existed in the market also adds challenges to the development of lignin conversion technologies. The composition and properties of the extracted lignin differ substantially from the native lignin, depending on the type of extraction process and biomass source (Constant et al., 2016). Consequently, the highly varied and complex structure of different extracted lignins made it very challenging to devise lignin conversion process that can cater for various lignin types (C. Xu et al., 2014). Though lignin extraction methods aimed at isolation of lignin with structure close to native lignin have been developed, such methods are primarily for analytical purpose due to low lignin yield (Constant et al., 2016). Additionally, most lignin available in the market are sulphur-containing lignin such as Kraft and lignosulfonate due to the remnant of pulping chemicals. However, sulphur compound is a known catalyst poison, which would result in the incompatibility of sulphur-containing lignin for catalytic conversion (Laurichesse & Avérous, 2014; Rinaldi et al., 2016). Thus, to reduce the alteration resulted by carbohydrate processing, the processing scheme of extracting lignin in the early stage of biorefinery operation with conformed method is highly encouraged. The search of an alternative lignin extraction method that is conducive for downstream processing remains a progressive effort.

Deep eutectic solvent (DES) was originally presented by A. P. Abbott, Capper, Davies, Rasheed, and Tambyrajah (2003) as an alternative solvent to ionic liquid (IL). These two types of solvents share some common advantages over conventional molecular solvents in terms of non-flammability, good thermal stability, low vapor pressure and good solubilizing capacity of selected organic compounds (Dai, van Spronsen, Witkamp, Verpoorte, & Choi, 2013a). IL solvents were shown effective in lignocellulosic biomass fractionation and dissolution (Brandt, Gräsvik, Hallett, & Welton, 2013). However, IL synthesis process involves ions with high purity and precise synthesis procedure (Gordon et al., 2008). The synthesis cost of IL is therefore considerably high (Francisco, van den Bruinhorst, & Kroon, 2012). Hence, DES has attracted much attention as a cheaper substitute to IL for biomass processing (Francisco et al., 2012).

Nevertheless, DES pretreatment is a considerably new method introduced in the last decade, thus more studies are required to evaluate the feasibility of this process to be adopted in biorefinery scheme. It is essential to specify the species of DES constituents suitable for lignocellulosic biomass fractionation and lignin extraction since near infinite sorts of DES can be synthesized using different combinations of chemical compounds.

The main DES categories used for biomass processing are amide-based, polyol-based and acid-based DES, with each of the categories displaying varying strength in biomass pretreatment performance. However, the performance comparison between these groups of DES is unclear. Research on the potential of DES-fractionated product for bio-product conversion is also of high importance. Particularly, the properties of DES-extracted lignin and the prospect of how they can fit into current lignin utilization schemes are still not well understood to decide the practicality of DES for lignin extraction. The collective research outcomes would establish the feasibility of DES application and facilitate the integration of DES into biomass conversion process as a fractionation solvent.

## 1.3 Research Objectives and Scope of Works

This study aims to evaluate the applicability of DES pretreatment on EFB biomass for biopolymer fractionation and lignin extraction. This new pretreatment method which can selectively solubilize lignin has the potential to be adopted as a new lignin extraction protocol to facilitate the recovery of lignin for downstream processing. As DES pretreatment is a newly introduced method in recent years, selection of the DES constituent and the understanding of the pretreatment products' properties are imperative to establish the feasibility of this method for biomass conversion process.

The objectives of this project and their respective scopes of works are listed as follows.

i. To synthesize deep eutectic solvents with different pH nature and functional groups.

    - To synthesize different categories of deep eutectic solvents with acidic, neutral and basic pH nature.
    - To synthesize acidic deep eutectic solvent using carboxylic acid with different functional groups as hydrogen bond donors.

ii. To investigate the suitable pH condition of deep eutectic solvent for optimum solvent performance in oil palm empty fruit bunch fractionation and lignin extraction.

    - To elucidate the biopolymer composition of DES-fractionated products, i.e. DES-pretreated liquid fraction, DES-pretreated solid fraction and DES-extracted lignin.
    - To perform mass distribution study on the DES-fractionated products produced by DES with different pH nature.

  - To assess the energy requirement by the DES pretreatment system with varying pH nature.
  - To identify the pH range of DES suitable for oil palm empty fruit bunch pretreatment based on their biopolymer fractionation and lignin extraction efficiency.

iii. To evaluate the effect of functional groups in acidic deep eutectic solvent on the solvent's biopolymer fractionation and lignin extraction efficiency.

  - To evaluate the effect of functional groups in the DES's carboxylic acid hydrogen bond donor on oil palm empty fruit bunch pretreatment performance based on the DES-extracted lignin and DES-pretreated solid fraction properties.
  - To evaluate the effect of DES molar ratio on acidic DES formation and pretreatment performance.

iv. To investigate the properties of DES-pretreated oil palm empty fruit bunch solid and DES-extracted lignin for potential bio-based products conversion.

  - To determine the biopolymer composition, molecular structure and crystal composition of DES-pretreated solid fraction.
  - To evaluate the purity, thermal and structural properties of DES-extracted lignin.
  - To identify the bio-based products that can potentially be produced from DES-pretreated solid fraction and DES-extracted lignin based on their properties.
  - To perform a life-cycle analysis of biomass utilization process involving the application of DES as pretreatment solvent.

## 1.4 Structure of Dissertation

This dissertation is presented in five chapters and the main contents of each chapter are as described below.

**Chapter 1: Introduction** presents the preface to this project, which includes the research background, problem statement, research objectives and scopes of work.

**Chapter 2: Literature Review** provides the detailed background knowledge of lignocellulosic biomass, deep eutectic solvent and lignin biopolymer. The general knowledge, definition and some related research studies are also included in this chapter.

**Chapter 3: Methodology** describes the materials and methods used to achieve the research objectives. The methodology is presented in four subsections: preparation of EFB biomass, synthesis of DES, EFB pretreatment and characterization of DES-fractionated products.

**Chapter 4: Results and Discussion** reports the results obtained via methodology described in Chapter 3. The findings are discussed in four sections, namely effect of pH nature of DES, effect of functional group in hydrogen bond donor of acidic DES, properties elucidation of DES-extracted lignin as well as potential bio-products from DES-fractionated products and their life cycle analysis.

**Chapter 5: Conclusion and Recommendation** concludes the dissertation by the overall conclusion, novelties and the implications of this project. Some recommendations are also proposed for future studies.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Lignocellulosic Biomass and Its Potential

Lignocellulosic biomass is the richest plant material on Earth (Brandt et al., 2013). It can be derived from sources such as agricultural residue, forest species and municipal waste (food or other organic wastes). The main constituents of lignocellulosic biomass are cellulose, hemicellulose and lignin biopolymers, with their relative composition differ depending on the biomass species. Biomass is a valuable renewable feedstock for a vast selection of bio-based products. It has been reported as the only renewable resource of liquid transportation fuel available (U.S.DOE., 2015). In the United States, a national vision which envisions 5% supply of the nation's power demand, 20% of transportation fuel and 25% of commodity chemical being produced from biomass resource by year 2030 was established (BTAC, 2002). This policy has certainly demonstrated the feasibility of biomass in versatile applications.

The exploitation of biomass as energy and chemical feedstock could bring about a lot of positive impacts other than its perquisite of being renewable. Burning it as fuel does not add extra carbon to the atmosphere beyond the amount absorbed during cultivation process (Lucia, 2008). Therefore, the utilization of biofuels can actually reduce greenhouse gas emission while maintaining the regular level of economic activities. Also, many lignocellulosic biomass can be sourced and converted locally in biorefinery. This can reduce the dependence on fossil fuels as it is only available in certain geographical locations in the world. Furthermore, the price fluctuation of biomass commodities will be lessened as compared to petrochemicals since the biomass distribution and accessibility are more evenly dispersed globally (Dapsens, Mondelli, & Pérez-Ramírez, 2012). This could meet the aim of biorefinery development whereby bio-based products are to be equally competitive in the market as oil refinery products and gradually replacing them in the long run (Cherubini, 2010).

One of the early applications of biomass is the production of bioethanol, which had been derived from corn and added to gasoline since the 1980s (Y. Sun & Cheng, 2002). Previously bioethanol was mainly produced from sugar-based biomass, such as sugarcane, corn or wheat. With technological advancement in recent decades, three institutions namely INEOS Bio, POET-DSM and Abengoa Bioenergy have successfully commercialized bioethanol production process from lignocellulosic biomass (U.S.DOE., 2016). Nevertheless, the key to develop a viable biorefinery process offering lucrative economical gain for investors is to utilize the entire biomass by taking advantage of each biopolymers' individual inherent properties. Hence, apart from biofuels production, the development of other bio-products from both sugar and lignin portion in lignocellulosic biomass has also been actively investigated (Holladay et al., 2007; Werpy & Petersen, 2004).

### 2.1.1 Lignocellulosic Biomass Constituents

Lignocellulosic biomass is a complex matrix in which the biopolymers interact strongly with each other through intermolecular linkages (Pérez, Muñoz-Dorado, de la Rubia, & Martínez, 2002). The composition of lignocellulosic biomass varies depending on the type of biomass. Generally, the composition of the major biopolymer constituents are 40-50% cellulose, 20-40% hemicellulose and 18-35% lignin (N. Sun et al., 2011). The minor components include ash, protein, pectin and other extractives (van Osch et al., 2017).

i. Cellulose

Cellulose is the most abundant biopolymers on Earth which presents in plants as the structural component of primary cell wall and also in photosynthetic algae, fungi or bacteria (Agbor, Cicek, Sparling, Berlin, & Levin, 2011). It is a linear homogeneous polymer chain consisting of D-glucose monomer units, linked together by 1-4-β glycosidic bonds (Ioelovich, 2008). The repeating unit of this polymer is cellobiose constituting of two glucose monomers with 180° relative position to each other, as shown in Figure 2.1. The β configuration contributes to the linear and stretched arrangement of this polysaccharide, in contrast to starchy biomass which presents in helical form due to α configuration (Brandt et al., 2013). Thus, cellulose polymer chains are bundled and packed into microfibrils by hydrogen bonds and van der waals intermolecular forces. The microfibrils are then grouped together to form cellulosic fibres (Agbor et al., 2011). These intermolecular interaction present in the cellulose and the microfibrils structure account for the recalcitrant cellulose structure (Siró & Plackett, 2010), resulting in the difficulty to dissolve cellulose in water and common organic solvents.

Cellulose in biomass is presented in two forms: crystalline and amorphous structure (P. Kumar, Barrett, Delwiche, & Stroeve, 2009). The crystalline portion is in organized form while amorphous cellulose has undefined structure. Hence, amorphous cellulose is more prone to degradation or hydrolysis than crystalline fraction when subject to biomass pretreatment (Béguin & Aubert, 1994).

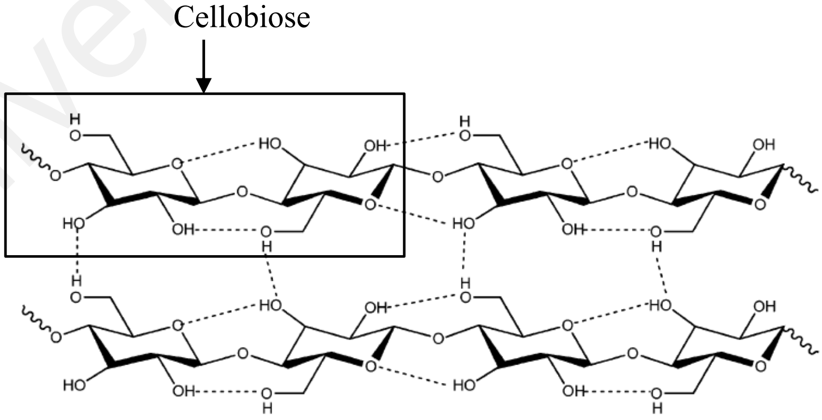


**Figure 2.1: Inter- and intramolecular hydrogen bonds in cellulose biopolymer. Adapted from H. Wang, Gurau, and Rogers (2012).**

### ii. Hemicellulose

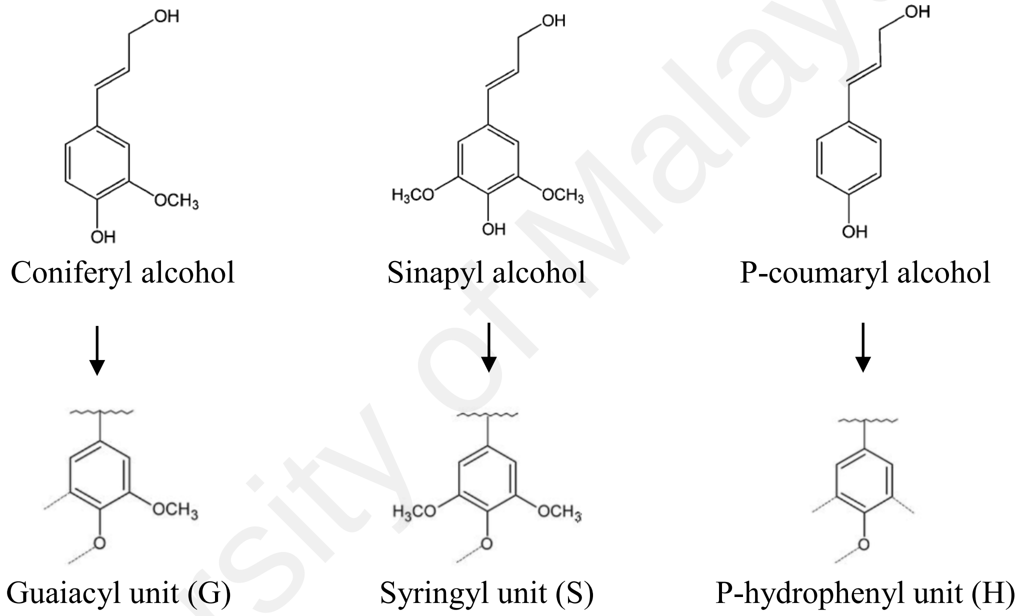
Like cellulose, hemicellulose is another polysaccharide-based biopolymer in lignocellulosic biomass. Hemicellulose is a branched and heterogeneous polysaccharide polymer (Agbor et al., 2011; Pérez et al., 2002). The sugars monomers are linked together by 1-4-β glycosidic bonds and a minor amount of 1-3-β glycosidic bonds (Pérez et al., 2002). Contrast to cellulose which consists only of glucose monomer, hemicellulose composes of different types of sugar monomers including pentoses, hexoses and sugar acids (Hendriks & Zeeman, 2009). The types of hemicellulose present in biomass differ according to the plant species. In grass and hardwood, xylose is more abundant whereas mannose is the dominant type of hemicellulose in softwood (Brandt et al., 2013).

Hemicellulose has a lower degree of polymerization than that of cellulose. Therefore, hemicellulose is comparably more easily to be hydrolysed (van Osch et al., 2017). Among the three major lignocellulosic biopolymers, hemicellulose is most vulnerable to thermal and chemical changes (Hendriks & Zeeman, 2009).

### iii. Lignin

While cellulose exists as the richest biopolymer on Earth, lignin is the most abundant renewable aromatic compound (Alvarez-Vasco et al., 2016). The latter is also the only aromatic biopolymer in lignocellulosic biomass (Berlin & Balakshin, 2014). Lignin's function is to connect the carbohydrate portion (van Osch et al., 2017) and also acts as a resilient protection layer for cellulose and hemicellulose against external impact (Kubo & Kadla, 2005). However, this would unfavorably impede the accessibility of enzymatic action to the carbohydrate fraction of the biomass (Mosier et al., 2005).

Lignin is a complex, heterogeneous polymer comprising of three different monolignol monomers, namely coniferyl, sinapyl and p-coumaryl alcohols (Boerjan, Ralph, & Baucher, 2003; Hatakeyama & Hatakeyama, 2009). When incorporated into lignin polymer, these monomers are respectively identified as guaiacyl (G), syringyl (S) and p-hydroxyphenyl (H) phenylpropanoid units. These monomers are the precursors of lignin polymer (Figure 2.2). The phenylpropanoid units contain a phenyl group with propyl group substitution and their difference are the number of methoxy groups attachment. As a heterogeneous polymer, various functional groups are available in lignin (Laurichesse and Avérous, 2014). In view of its rich functionality and renewability, lignin is regarded as a valuable aromatic resource for versatile applications.



Coniferyl alcohol

Sinapyl alcohol

P-coumaryl alcohol

Guaiacyl unit (G)

Syringyl unit (S)

P-hydrophenyl unit (H)

**Figure 2.2: Lignin monolignol monomers and phenylproponoid units**

The lignin monomers are connected by assorted linkages, with the most common one as β-aryl ether (β-O-4) linkage (Boerjan et al., 2003). Other linkages are such as β-β, β-5, α-O-4, 4-O-5, 5-5, α-O-γ and β-1 (Gillet et al., 2017). The strength of these linkages differ from one another according to the different substituent groups present in the units (J.-b. Huang et al., 2015). Hence, lignin extracted by different extraction methods usually possess different structures and properties owing to the varying amount of aromatic moieties and linkages present.

### 2.1.2 Oil Palm Empty Fruit Bunch (EFB)

Oil palm industry in Malaysia generates more than 40 Mt of solid biomass waste annually, which includes oil palm trunk, frond, empty fruit bunch and palm kernel shell (Malaysian Palm Oil Board, 2016). Among them, oil palm empty fruit bunch (EFB) constitutes to the highest percentage of solid waste produced. Handling this huge amount of bulk biomass is a challenging task for the country in terms of economic and environmental responsibilities. Based on a report by Ministry of Energy, Science, Technology, Environment and Climate Change (MESTECC) Malaysia, EFB is usually utilized for different purposes such as 62% for mulching, 16% being dumped in plantation, 11% being incinerated, 6% in compost and 5% being sold commercially (Ministry of Energy, 2008). As a lignocellulosic biomass, EFB consists mainly of cellulose, hemicellulose and lignin. As such, it can be used as raw materials to produce various bio-products, when subject to suitable technology and process.

As one of the participating countries in Paris Agreement, MESTECC Malaysia has set a target of increasing the nation's electricity supply from renewable sources from the current 2% to 20% by year 2030. Biomass resources, such as oil palm products, would certainly be one of the major sources of renewable energy. In addition, a new policy named "Roadmap Towards No Single-Use Plastics 2018-2030" was launched to address the plastic pollution problem in Malaysia. Being ranked as the eighth worst country in plastic waste mismanagement worldwide (Jambeck et al., 2015), Malaysia government is now actively advocating the development of biodegradable food packaging and bio-plastics from the cellulosic fraction of oil palm industry waste, especially EFB. To convert EFB into a suitable feedstock for energy and bio-plastics production, the development of a more efficient biomass fractionation technology is therefore a prudent action.

## 2.2 Extraction and Application of Lignin Biopolymer

Lignin is the second most abundant biopolymer after cellulose, constituting to 30% of the organic carbon on Earth (Boerjan et al., 2003). Around 50% of native lignin's components are aromatic hydrocarbon, which suggests its potential for fuel and aromatic chemical production (C. Xu et al., 2014). As explained in Section 2.1.1iii, lignin is a heterogeneous biopolymer with various phenylpropanoid structure. Despite having a heterogeneous and complex structure, lignin is viewed as a potential feedstock for industrial application due to the higher carbon and lower oxygen content than the polysaccharides fraction in biomass (Rinaldi et al., 2016).

### 2.2.1 Lignin Extraction Methods

Lignin has been extracted mainly as by-product in pulping process. Lignin that is separated from lignocellulosic material is termed as technical lignin (Shrotri, Kobayashi, & Fukuoka, 2017). The structure of technical lignin is altered from its original form in the biomass matrix, which is the native lignin, based on the extraction process employed. The common pulping methods include kraft pulping, soda pulping and sulphite pulping (Gillet et al., 2017). The fundamental parameters of these lignin extraction methods are tabulated in Table 2.1. The methods can be classified into two groups: sulphur-based process and sulphur free process, depending on the presence of sulphur-containing compound in the pulping solvent used.

Kraft process has been a dominant pulping technology in paper pulping industry (Holladay et al., 2007), contributing to 85% of the world's total lignin production (Gillet et al., 2017). However, this pulping process are operated under the optimized condition for carbohydrate processing. Lignin extracted under such condition is heavily modified and therefore it is very challenging to be utilized. With the growing interest in lignin valorisation in recent decades, processes emphasizing on lignin extraction prior to carbohydrate processing such as organosolv pulping or green solvent extraction have thus been developed (Gillet et al., 2017; Rinaldi et al., 2016).

**Table 2.1: Extraction condition of technical lignin. Adapted from Gillet et al. (2017); Guadix-Montero and Sankar (2018); Laurichesse and Avérous (2014).**

| **Lignin type** | **Sulphur-based process** | | **Sulphur-free process** | |
|---|---|---|---|---|
| | **Kraft** | **Lignosulfonate** | **Soda** | **Organosolv** |
| Pulping/ extraction solvent | Sodium hydroxide (NaOH) and sodium sulphide (NaS) | Sulphite salt $(SO_3^{2-})$ or bisulfite salt $(HSO_3^-)$ | Sodium hydroxide (NaOH) and anthraquinone (AQ) | Organic solvent |
| Pulping pH condition | 13 | 1-2 | 11-13 | Depending on the pulping solvent |
| Pulping temperature | 170°C | 140°C | 150-170°C | 150-200°C |
| Sulphur content in extracted lignin | 1-3% | 3.5-8.0% | - | - |

### 2.2.2 Application of Lignin

The application of lignin can be sorted into three main categories namely fuel, macromolecule and aromatic monomer (Holladay et al., 2007). Despite Kraft lignin constitutes to the largest volume percentage in lignin production, they are not available in the same abundance in the market (Rinaldi et al., 2016). This is because Kraft lignin production is well-integrated into the pulping process as fuel. The black liquor consisting mainly of extracted lignin in Kraft process is used for internal energy supply and the recovery of pulping chemicals for recycling use (Holladay et al., 2007). Other potential higher value fuel applications of lignin include production of alcohol, syngas, pyrolysis oil or biochar (Holladay et al., 2007; Tripathi, Sahu, & Ganesan, 2016).

A small portion of lignin is used commercially in its macromolecular or polymeric form for material applications such as dispersants, emulsifiers, binders, and resins. In these applications, lignin is utilized in its recovered form with little or without modifications (Holladay et al., 2007). Though the amount of possible lignin applications might be limited by its heavily modified properties, the lignin conversion process for these polymeric application is much simpler without the need to break down the polymer into smaller molecules (Rinaldi et al., 2016). The examples of lignin's material applications are phenol-formaldehyde resin, polyurethane foam, epoxy resin and carbon fibre (Laurichesse & Avérous, 2014).

Apart from being applied in fuel or polymeric application, the potential usage of lignin extends into more high value commodity products namely the production of aromatic chemicals and other lignin monomeric compounds (Holladay et al., 2007). The highly functionalized and aromatic nature of lignin makes it a possible feedstock for aromatic chemicals production as a replacement for petrochemicals, such as BTX (benzene, toluene and xylene) and their functionalized aromatic derivatives. Majority of aromatic chemicals are being derived from petrochemicals (Fache, Boutevin, & Caillol, 2016). These aromatic monomers are the essential raw materials in almost all manufacturing industries, for instance pharmaceutical, food, household products (soaps and detergents), synthetic fibres for clothes and construction materials (paints, coatings and insulating materials). To utilize lignin in its monomeric form, it has to go through a depolymerisation process to break down the complex polymer structure into small functional molecules.

Varying types of depolymerisation processes have been developed for example reductive, oxidative, electrochemical, thermal and biological processing (Di Marino, Stöckmann, Kriescher, Stiefel, & Wessling, 2016). The most significant lignin-derived chemical production is vanillin through the oxidation process of lignosulfonates by company Borregaard in Norway (Rodrigues Pinto, Borges da Silva, & Rodrigues, 2012). Despite high interest has been shown towards lignin utilization, limited commodity lignin-derived chemical production processes are established (C. Li, Zhao, Wang, Huber, & Zhang, 2015). There are several setbacks contributing to the difficulties in high-value lignin valorisation, including the heavily modified lignin structure derived from paper pulping industry, high variation of lignin type existed in the market and the incompatibility of sulphur-containing lignin with catalytic conversion technology. Nevertheless, the key to a sustainable and more competitive biorefinery is the complete valorisation of lignocellulosic biomass, especially lignin biopolymer which is often neglected in biorefinery (Schutyser et al., 2018). The search of a biomass fractionation method which focuses on lignin extraction prior to carbohydrate processing is actively investigated.

## 2.3 Pretreatment of Lignocellulosic Biomass for Biopolymer Fractionation

Lignocellulosic biomass is highly recalcitrant to conversion and modification owing to the lignin layer barricading carbohydrate fraction and the presence of highly recalcitrant cellulose (Rastogi & Shrivastava, 2017). Therefore, a pretreatment step is crucial in biorefinery process to increase the efficiency of subsequent conversion process. It is even more desirable when the pretreatment process is capable of fractionating the biopolymers followed by separate processing based on their inherent functionality.

Since the introduction of bioethanol-blended gasoline in 1980s (Y. Sun & Cheng, 2002), the bioenergy production from biomass and municipal waste has been continuously researched (Gossett, Stuckey, Owen, & McCarty, 1982; Saddler, Brownell, Clermont, & Levitin, 1982; Wyman, 1994). The production of bioethanol includes two major processes, namely 1) hydrolysis of polysaccharides to reducing sugar by cellulase enzyme followed by 2) fermentation of the reducing sugars to bioethanol using yeast or bacteria. In the earlier days, biomass pretreatment process was designed to increase the accessibility of enzymatic action to carbohydrates and also to enhance the fermentable sugar yield. The attention has been gradually turning towards biorefinery concept, in which the pretreatment strategy is aimed at biopolymer fractionation for complete biopolymer utilization.

Biomass fractionation technology is considered as the heart of biorefinery, which dictates the possibility of the downstream bio-based product conversion (Schutyser et al., 2018). One of the main challenges in realizing biorefinery is the development of an efficient biomass pretreatment process for biopolymer fractionation (Zakzeski et al., 2010). The deconstruction process of raw biomass represents the biggest cost in lignocellulosic biofuels production (U.S.DOE., 2015). The current paper production is the most optimal commercial fractionation process, yet it only uses up 30% of its feed (van Osch et al., 2017). The remaining feed went into waste or incinerated due to high lignin content with high heating value (Flaxbart, 1999). This statistics shows that the present fractionation technology is inadequate to achieve a competitive and efficient biorefinery scheme.

To break down the recalcitrant biomass structure for the subsequent upgrading process, different pretreatment approaches have been developed such as physical, chemical, thermal, biological pretreatment and etc. (Agbor et al., 2011; Hendriks & Zeeman, 2009). Table 2.2 summarizes the pros and cons of the different pretreatment methods. The selection of pretreatment method depends largely on the type of biomass feedstock and the desired product. In this project, the main targeted biomass pretreatment outcome is to fractionate the lignocellulosic biopolymers for separate upgrading process. Therefore based on this context, the chemical pretreatment method which is known to be efficient for biopolymer fractionation was explored further.

Table 2.2: Pro and con of various biomass pretreatment methods. Adopted from Chaturvedi and Verma (2013) and P. Kumar et al. (2009)

| Pretreatment method | Pros | Cons |
|---|---|---|
| Physical pretreatment | Reduction of cellulose crystallinity | Higher power consumption |
| Thermal pretreatment | Transformation of hemicellulose and lignin | Deconstruction of xylan fraction; incomplete disruption of lignin-carbohydrate complex |
| Chemical pretreatment | Fractionation of biopolymers; enhancement of enzymatic hydrolysis | Formation of enzyme inhibitory organism; need for recycling, high capital cost |
| Biological pretreatment | Eco-friendly | Low rate of hydrolysis; time-consuming process |

### 2.3.1 Conventional Chemical Pretreatment

Many types of solvents have been adopted in chemical pretreatment of lignocellulosic biomass, with acid and alkaline as the most common pretreatment solvents used (P. Kumar et al., 2009).

#### 2.3.1.1 Acid pretreatment

Both concentrated and diluted acid pretreatment have been proven effective in lignocellulosic biomass pretreatment for sugar hydrolysis (P. Kumar et al., 2009). Sulphuric acid is the most studied acid in biomass pretreatment among other types of mineral acids such as nitric acid, hydrochloric acid, phosphoric acid, peracetic acid and oxalic acid (Singh, Suhag, & Dhaka, 2015). Organic acids are not as effective as mineral acids in biomass pretreatment and hence they are not commonly utilized (X. Zhao, Cheng, & Liu, 2009).

During acid pretreatment, the main reaction that occurs is the hydrolysis of hemicellulose (Agbor et al., 2011; Hendriks & Zeeman, 2009). Following acid pretreatment, enzymatic hydrolysis process is usually performed to produce fermentable sugar for biofuel production. During enzymatic hydrolysis, hemicellulose would adsorb to the enzyme which hinders the access of enzyme to cellulose (X. Zhao et al., 2009). Therefore, removing hemicellulose by an efficient pretreatment process such as acid pretreatment could enhance the hydrolysis performance of cellulose.

Even though acid pretreatment can improve the hydrolysis performance of the acid-pretreated biomass, the acidic condition exerted by acid pretreatment brings about a number of drawbacks that prompted researchers to search for other better alternatives. The use of mineral acid poses some concerns in terms of processing costs and downstream conversion efficiency. Highly corrosion-resistant reactor for acid pretreatment is required due to the corrosive nature of acid, which incurs high capital and maintenance costs (P. Kumar et al., 2009). Besides, even though concentrated acid pretreatment is effective in enhancing ethanol yield, the challenge lies in the need to recycle the expensive solvent used. Even though dilute acid is comparably less inexpensive, dilute acid pretreatment requires higher temperature condition than concentrated acid pretreatment. The higher temperature condition promotes the formation of side products such as furfural and HMF which could act as inhibitors to fermentation organisms (Rasmussen, Sorensen, & Meyer, 2014). The inhibitory byproduct removal process is therefore mandatory. Thus, economic trade-off is needed to be considered between the inexpensive material, recycling cost and process efficiency (Wyman, 1994). These factors would give rise to extra cost and complexity on the pretreatment process.

#### 2.3.1.2 Alkaline pretreatment

Alkali pretreatment can remove lignin and hemicellulose from the biopolymer matrix, hence improving the carbohydrate digestibility in the subsequent processing (Mosier et al., 2005). Alkalis such as sodium hydroxide, potassium hydroxide and calcium hydroxide have been adopted in lignocellulosic biomass pretreatment (Singh et al., 2015). The reaction mechanism of alkali pretreatment involves the solvation and saponification of ester bonds that cross-link between the biopolymers. Biomass structure is swollen from the reactions, thus increasing its internal surface area and enhancing the subsequent enzymatic hydrolysis performance (Hendriks & Zeeman, 2009; Y. Sun & Cheng, 2002).

However, alkaline pretreatment method is reported to be ineffective on biomass with high lignin content (Agbor et al., 2011; Y. Sun & Cheng, 2002). Alkali pretreatment also causes peeling effect at the end groups of biomass matrix, resulting in loss of low molecular weight polysaccharides. Moreover, the subsequent downstream process requires large amount of washing water to remove salt formed as by-product from the alkali reaction (Chaturvedi & Verma, 2013; Mosier et al., 2005). Although the process does not produce carbohydrate-based inhibitor species (HMF, furfural) similar to those by acid pretreatment, lignin depolymerization by alkali solvents might also generate certain fermentation inhibitors that are required to be separated before fermentation treatment (Chaturvedi & Verma, 2013).

### 2.3.2 Non-Conventional Chemical Pretreatment

The introduction of 'Green Chemistry' concept by Anastas and Eghbali (2010) instigates the research efforts in material, product and process design in a more environmentally friendly manner. It is unavoidable to employ solvents in industrial processes to dissolve solutes, promote mass and heat transfer as well as facilitate separation and purification processes (Cvjetko Bubalo, Vidović, Radojčić Redovniković, & Jokić, 2015). Solvents are usually consumed in large quantity particularly when different types of solvents are utilized to accommodate diverse processes. For example, approximately 4 x $10^6$ ton of solvents was used in chemical industry in Europe in year 2004 (Capello, Fischer, & Hungerbühler, 2007). The figure represented a single continent is substantial enough to reflect the magnitude of solvent used worldwide annually. Hence, the choice of solvent is critical as it greatly influences the cost, safety and environmental performance of a process.

As the utilization of solvent covers nearly all aspects of human lives, the use of low cost solvents with lowest possible environmental impact is therefore desirable. In replacement of conventional solvents to mitigate the environmental impact, two green solvents namely ionic liquid (IL) and deep eutectic solvent (DES) have been proven to be efficient for lignocellulosic biomass processing (van Osch et al., 2017). Low vapour pressure, thermal stability and non-flammability are among the important characteristics of green solvents in the implementation of sustainable technology (Durand, Lecomte, & Villeneuve, 2016), all of which have been observed in both IL and DES.

#### 2.3.2.1 Ionic liquid (IL) and deep eutectic solvent (DES)

Ionic liquids are organic salts comprising of cation and anion that normally melt below 100°C (Brandt et al., 2013; Ohno & Fukaya, 2009; N. Sun et al., 2011). Differ from conventional solvents which consist of single molecular compound, ILs are formed by combining at least two ionic species. Therefore, the solvent properties of IL can be tailored according to the need of the application based on the solvent constituents selected (van Osch et al., 2017).

ILs were found to be effective at milder pretreatment condition as opposed to the effectiveness of conventional solvents at more extreme condition (N. Sun et al., 2011). The IL solvents were successfully employed for different applications, for example in promoting saccharification of carbohydrate-rich biomass (Lee, Ngoh, & Chua, 2015) and extracting lignin from lignocellulosic biomass (Rashid, Gnanasundaram, Appusamy, Kait, & Thanabalan, 2018). However, there are a number of challenges encountered in commercializing the application of ILs. First and foremost, the expensive cost of this solvent prohibits its large scale industrial application (Ashworth, Matthews, Welton, & Hunt, 2016). Moreover, certain ILs have low tolerance to moisture (Andrew P. Abbott et al., 2001). Presence of water in IL is regarded as impurity which interferes with the thermophysical properties of IL (Florindo, Oliveira, Rebelo, Fernandes, & Marrucho, 2014). This increases the complexity and operating cost of the solvent synthesis process. In addition, toxicity level of a solvent has raised significant concern in solvent industry. Toxicity profiles of some ILs are deemed alarming (H. Wang et al., 2012). The first IL that was found to be able to dissolve cellulose, [$C_4$ mim]Cl, was reported to have toxic effect towards mice in research. Nevertheless, there is a wide variety of anions and cations that can be used to form IL. Thus, one can always choose the combination which will result in benign designer solvent (Fukaya, Iizuka, Sekikawa, & Ohno, 2007).

A new type of synthetic green solvent, deep eutectic solvents (DES) was introduced by A. P. Abbott et al. (2003) as an alternative for ILs. Generally, DES is formed by at least one hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) in simple heating and mixing process (van Osch et al., 2017). DES shares some common green solvent characteristics with IL, for example low vapour pressure, chemical and thermal stability, non-flammability and low melting point (Zdanowicz, Wilpiszewska, & Spychaj, 2018). Similar to IL, DES offers the possibility to tune the solvent's properties as a designer solvent. However, DES has certain advantages over IL such as cheaper price, less sensitivity with water content and simpler synthesis process (van Osch et al., 2017). Therefore, DES is presented as a more attractive alternative green solvent and firstly applied in biomass processing field by Francisco et al. (2012) as an alternative to IL.

## 2.4 Deep Eutectic Solvent (DES)

Deep eutectic solvent (DES) is a mixture of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). The solvent was first synthesized as a mixture of quaternary ammonium salts with amides (choline chloride and urea) (A. P. Abbott et al., 2003). Eutectic mixture is the mixture of two or more compounds displaying minimum melting point at a specific composition ratio (Durand et al., 2016). In the isobaric solid-liquid phase diagram, eutectic point represents equilibrium status between two crystalline phases and a liquid phase (Meng, Ballerat-Busserolles, Husson, & Andanson, 2016) (Figure 2.3a).

Freezing point of this mixture reached as low as 12°C compared with that of choline chloride (302°C) and urea (133°C) (Figure 2.3b). The significant depression in freezing temperature of DES compared to its starting constituents is attributed to the hydrogen bonding formed between halide anion in choline chloride and urea, which also prevents the recrystallization of the DES constituents (A. P. Abbott et al., 2003; Dai, van Spronsen, Witkamp, Verpoorte, & Choi, 2013b).

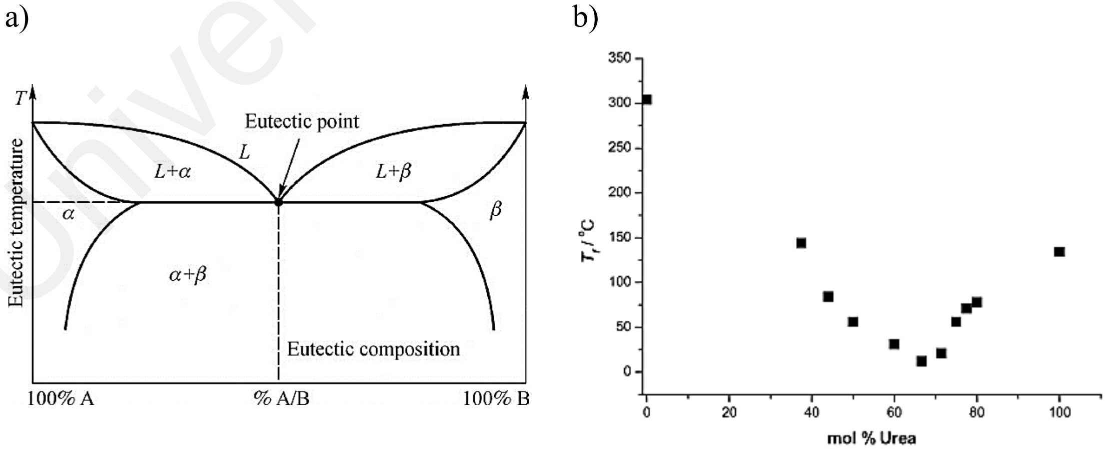


**Figure 2.3: a) Phase diagram of eutectic binary mixture. b) Freezing temperature of choline chloride/urea mixture as a function of composition. Adapted from A. P. Abbott et al. (2003).**

The design of DES is highly flexible as there are a wide array of components to be chosen from such as amides, acids, amines and alcohols as HBD, as long as they are compatible with the selected HBA to form homogeneous liquid. Hydrogen bonds exist in different binding energies and contact distances that depend on more factors other than the nature of HBA and HBD, as stated in Francisco et al. (2012). Therefore, the formation of DES does not solely depend on the nature of the constituents but also their relative composition (Francisco et al., 2012; Lian, Hong, Carranza, Mota-Morales, & Pojman, 2015). This constitutes to extra degree of freedom in the solvent application since the solvent's properties can also be customized by altering the ratio of HBD to HBA. Thus, the applications of DES are very much versatile due to its tunable properties. DES is now a topic of high interest in many research fields, including electrochemistry (Smith, Abbott, & Ryder, 2014), catalysis (Vigier, Chatel, & Jérôme, 2015), or dissolution and extraction process (Francisco et al., 2012).

The first reported HBA, choline chloride (ChCl) ($C_5H_{14}ClNO$), a quaternary ammonium salt with choline cation and chloride anion, is by far the most commonly used HBA. Majority of studies adopted ChCl as HBA in their investigation due to its benign properties as well as affordable pricing. Choline ion is part of B complex vitamins (Yates, Schlicker, & Suitor, 1998). It is synthesized through metabolism process in human body and obtained through diets. ChCl is a nutrient necessary for the formation of various metabolites and thus widely used as animal feed additive for growth promotion, or sometimes as human's vitamin supplement.

Choi et al. (2011) discovered that various combinations of cellular constituents and plant metabolites are capable of forming DES, which they named them as natural deep eutectic solvent (NADES). This finding establishes the theory that DES can be synthesized from naturally existing compounds. It is further evidenced from the successful cases of several groups of researchers synthesizing various DES using natural components like organic acids, amino acids and sugars (Craveiro et al., 2016; Francisco et al., 2012; Yiin et al., 2016). Preparation of DES using naturally occurring components is highly encouraged, as many conventional solvents would pose severe hazard concern towards living organisms. Notably the application of DES is also well received in pharmaceutical and medical fields (Aroso et al., 2015; Mota-Morales et al., 2013; Zainal-Abidin, Hayyan, Ngoh, & Wong, 2019).

### 2.4.1 Synthesis of DES

Unlike ILs, synthesis of DES is comparably easier as it involves simple mixing between the starting constituents (Andrew P. Abbott, Boothby, Capper, Davies, & Rasheed, 2004). Several DES synthesis methods have been developed so far. The most common method used is mixing and heating the DES constituents until they melt and become homogeneous liquid (A. P. Abbott et al., 2003). Alternatively, some researchers prepared the DES solvent by dissolving the starting materials in water prior to mixing then proceeded to vacuum evaporation for excess water removal (Dai et al., 2013b) or freeze-drying the water-containing DES (Yiin et al., 2016). On the other hand, Florindo et al. (2014) developed a grinding method where the selected components were grinded in mortar under room condition until they became homogeneous liquid. Even though various methods have been introduced for DES synthesis, the fundamental principle involves simple mixing without the need for further purification. Preparation of DES is 100% atomic in which all the materials constitute to DES without waste. Moreover, the storage of DES is hassle-free as it is not volatile and flammable. Stable DES will also remain in homogeneous liquid form for an extended period of time without precipitation (AlOmar et al., 2016; Kow & Sirat, 2015).

### 2.4.2 Categorization of DES

There is near infinite variety of possible HBD and HBA combinations that can form DES. With each different pairing, the nature of the synthesized DES varies based on the selected constituents. The usual HBD applied are such as carboxylic acid, amide, polyalcohol, sugar and phenolic compounds while pairing it with choline chloride (ChCl) as HBA (Satlewal, Agrawal, Bhagia, Sangoro, & Ragauskas, 2018; Tang et al., 2017). It is rather subjective on how researchers categorize different types of DES. In this project, the DES will be classified according to their pH nature condition, namely acidic, basic and neutral. According to an early work of DES application in biomass pretreatment, the performance of DES varied with the HBD of different nature applied, which were monocarboxylic acid, dicarboxylic acid and polyalcohol (C. W. Zhang, Xia, & Ma, 2016). The authors attributed the varying performance to the acid strength and nature of the HBD used. Therefore, investigation of DES with different pH category would help significantly to specify the suitable DES constituents for biomass pretreatment purpose. The classification of DES according to their pH nature will also enable the comparison against conventional acid and alkaline solvent in biomass pretreatment performance.

### 2.4.3 Application of DES in Biopolymer Fractionation and Enzymatic Hydrolysis Enhancement

The commonly adopted biomass processing scheme involves acid and alkali pretreatment followed by enzymatic saccharification for the production of fermentable sugar. The introduction of DES has provided another option for biomass pretreatment other than ILs and conventional chemical pretreatment. The possibility of adopting a green solvent in renewable resource utilization is a much attractive prospect. Ability of DES in solubilizing lignocellulosic biomass, particularly lignin was first demonstrated by Francisco et al. (2012). DES was tested to have nearly absolute solubilizing selectivity of lignin with low to negligible solubility of cellulose and hemicellulose (Francisco et al., 2012; Adepu K. Kumar, Parikh, Shah, Liu, & Cotta, 2016; Yiin et al., 2016). The capability of DES on selective biopolymer solubilization has urged researchers to explore more into the application of DES in biopolymer fractionation.

Many types of DESs have been applied to different bulk lignocellulosic biomass pretreatment for biopolymer fractionation or enzymatic hydrolysis enhancement (Loow et al., 2017). Typically, DES pretreatment is efficient in hemicellulose and lignin removal from biomass, which also depends on the types of DES and pretreatment condition applied (Alvarez-Vasco et al., 2016; Satlewal et al., 2018; Zulkefli, Abdulmalek, & Abdul Rahman, 2017). The pretreatment parameters such as temperature, time and solid to liquid ratio could affect the solvent performance, with DES type usually having the most influence over the DES performance. As mentioned previously, there are a wide range of constituents suitable for DES synthesis. The constituents used would determine the nature of the solvent synthesized, and their behaviour in biomass pretreatment. HBD in DES is usually altered in the search of optimum DES for biopolymer fractionation. The HBDs that have been investigated include carbohydrate, acid, polyalcohol, amide and phenolic compound.

To date, most of the DES pretreatment studies focus on the enhancement of fermentable sugar production from DES-pretreated biomass. Many researchers concluded that the improved hydrolysis rate after DES pretreatment was due to extensive hemicellulose or lignin removal or the combination of both (X. D. Hou, Feng, Ye, Huang, & Zhang, 2017; Procentese et al., 2015). Table 2.3 shows glucose and xylose yield of both DES-pretreated and untreated biomass from some reported works. Literature reported that sugar yield resulted from enzymatic hydrolysis was highly dependent on the pretreatment conditions such as biomass type, temperature, duration, as well as solvent type. Most studies demonstrated improved fermentable sugar yield after DES pretreatment. Procentese et al. (2015) compared performance of three types of DES on saccharification of corncob and found that pretreatment using choline chloride:glycerol at 150°C and choline chloride:imadazole at 80°C could recover more than 70% of the sugar content after saccharification. In addition, C. W. Zhang et al. (2016) also obtained similar result where choline chloride:glycerol pretreated corncob achieved higher glucose yield compared to carboxylic-based DES. Using choline chloride:lactic acid DES, 36% sugar yield was achieved on rice straw hydrolysis (Adepu K. Kumar et al., 2016). Rice straw saccharification efficiency in terms of sugar yield was reported to be lower than acid and alkali pretreated biomass. This result is in contrast to that in Gunny, Arbain, Nashef, and Jamal (2015) whereby choline chloride:glycerol and choline chloride:ethylene glycol DES were found to be outperformed dilute NaOH in pretreating rice husk in term of glucose production and energy consumption. This might be due to different biomass source or the loss of cellulase activity in acidic DES. Zulkefli et al. (2017) was also able to obtain 60% glucose yield from highly fibrous oil palm trunk using ethylammonium chloride:ethylene glycol DES. The researchers associated the high sugar yield with ability of DES to remove most hemicellulose in pretreatment step, leaving the pretreated biomass with high relative cellulose content for saccharification.

In general, both acid- and polyol-containing DESs are efficient in enhancing the saccharification performance of DES-pretreated biomass based on the intensive amount of researches conducted. Nevertheless, the efficiency of DES in biomass fractionation and the distribution of each lignocellulosic biopolymer after DES pretreatment are not well investigated.

**Table 2.3: Glucose and xylose yield from enzymatic hydrolysis process of untreated and DES-pretreated biomass**

| Work | DES | Biomass | DES pretreatment condition (S/L ratio, temperature, time) | Glucose yield (%) | | Xylose yield (%) | |
|---|---|---|---|---|---|---|---|
| | | | | Untreated biomass | DES-pretreated biomass | Untreated biomass | DES-pretreated biomass |
| Z. Chen, Reznicek, and Wan (2018) | ChCl:glycerol, 0.9wt% $H_2SO_4$ | Switchgrass | 1:10, 110°C, 1h | 15.13 | 102.02 | 3.15 | 98.78 |
| Zhu Chen, Bai, A, and Wan (2018) | ChCl:ethylene glycol, 1.0wt% $H_2SO_4$ | Switchgrass | 1:10, 130°C, 30mins | 11 | 98 | 2 | 105 |
| Z. Guo, Ling, Wang, Zhang, and Xu (2018) | betaine:lactic acid | Xylose residue | 1:20, 120°C, 2h | 55* | 96.8* | - | - |
| (X.-D. Hou, Lin, Li, Yang, & Fu, 2018) | ChCl:oxalic acid | Rice straw | 1:20, 120°C, 3h | 18.4 | 52.9 | 3.1 | 0.4 |
| Kim, Dutta, Sun, Simmons, and Singh (2018) | ChCl:p-coumaric acid | Switchgrass | 1:20, 160°C, 3h | - | 85.7 | - | 28.8 |
| | ChCl:catechol | | | - | 77 | - | 42.4 |
| Adepu K. Kumar et al. (2016) | ChCl:glycerol | Rice straw | 1:10 | 56.6* | 87.1* | - | - |
| A. L. Li, Hou, Lin, Zhang, and Fu (2018) | ChCl:lactic acid, 10wt% $H_2O$ | Rice straw | 1:20, 120°C, 3h | 24.2 | 66.8 | 6.7 | 21.7 |
| Pan et al. (2017) | ChCl:urea | Rice straw | 1:20, 130°C, 4h | 4.46 | 0.87 | 8.53 | 0.31 |
| Procentese et al. (2015) | ChCl:imidazole | Corncob | 1:16, 80°C, 15h | 32.8 | 85.5 | 15.5 | 63 |
| Procentese et al. (2017) | ChCl:glycerol | Lettuce | 1:16, 150°C, 16h | - | 94.9 | - | 75 |
| Procentese et al. (2018) | ChCl:glycerol | Potato peel | 1:16, 115°C, 3h | 1 | 41 | - | - |
| | | Apple residue | | 2 | 76 | - | - |
| | | Coffee silverskin | | 3 | 29 | - | - |

**Table 2.3 Continued**

| Work | DES | Biomass | DES pretreatment condition (S/L ratio, temperature, time) | Glucose yield (%) | | Xylose yield (%) | |
|---|---|---|---|---|---|---|---|
| | | | | Untreated biomass | DES-pretreated biomass | Untreated biomass | DES-pretreated biomass |
| Tian, Chandra, Lee, Lu, and Saddler (2017) | Betaine:lactic acid | Poplar wood | 1:20, 130°C, 3h | 27.9* | 83* | - | - |
| G. C. Xu, Ding, Han, Dong, and Ni (2016) | ChCl:formic acid | Corn stover | 1:20, 130°C, 2h | | 91.5* | - | - |
| C. W. Zhang et al. (2016) | ChCl:glycerol | Corncob | 1:20, 90°C, 24h | 22.1* | 96.4* | - | - |
| Z. Zhao et al. (2018) | ChCl: monoethanolamine | Wheat straw | 1:20, 70°C, 9h | 20.9 | 84.1 | 8.9 | 35.9 |
| Zulkefli et al. (2017) | Ethylammonium chloride: ethylene glycol | Oil palm trunk | 1:19, 100°C, 48h | 25* | 60* | - | - |

### 2.4.4 Application of DES in Lignin Extraction and Upgrading

The discovery of DES having almost absolute lignin solubility has prompted researchers to explore on lignin extraction using DES. As a new designer solvent, the potential of DESs was first evaluated on the solubilisation or conversion of lignin model compound and existing technical lignin. An extensive solubility study of lignin monomers (syringaldehyde, vanillic acid, syringic acid and ferulic acid) and technical lignin (kraft and organosolv) in different DESs was performed (Soares et al., 2017). Urea:propionic acid DES was found to be able to dissolve high concentration of both lignin monomer and technical lignin. Technical lignin has always known to have limited solubility in many solvents. Using urea:propionic acid DES, solubility of kraft and organosolv lignin achieved 1181 and 228 fold enhancement in comparison to solubility of lignin in water (Soares et al., 2017). This study demonstrates the capability of DES to partake in lignin processing which involves solubilisation process.

Currently, most lignin extraction studies from lignocellulosic biomass involve the use of acidic DES solvent made of organic acid HBD. By using carboxylic acid-based DES, Jablonský, Škulcová, Kamenská, Vrška, and Šima (2015) and A. K. Kumar, Parikh, and Pravakar (2016) managed to decrease the lignin content of wheat and rice straw biomass by 57.9% and 42%, respectively. Alvarez-Vasco et al. (2016) applied acidic DES pretreatment on both hard (poplar) and softwood (Douglas fir). The acidic DESs successfully extracted appreciable amount of lignin while choline chloride:glycerol did not show potential in biomass dissolution nor lignin extractability. The extracted lignin from Douglas fir wood was precipitated with 95% purity. The DES-extracted lignin had narrower and lower molecular weight distribution than that of milled wood lignin, implying that the lignin obtained was rather homogeneous in nature.

Lignin has always known as a very useful macromolecule in material industries (Laurichesse & Avérous, 2014; Northey, 1992). While there are a number of studies on technical lignin modification using DES for material synthesis (Hong et al., 2016; J. Liu, Wang, Fu, & Chang, 2016), application of DES-extracted lignin is scarce. Due to lignin's insolubility in many solvents, the application of lignin nanoparticles for bio-based functional materials has been researched by many (Lievonen et al., 2016; Roopan, 2017). Lyu et al. (2018) produced lignin nanoparticle nanoscale lignin particle with uniform diameter of 200-420 nm and smooth topographic surface by dissolving the DES-extracted lignin in acetone at 0.1 g/L concentration.

To date, the information and studies on the properties of lignin extracted using DES are very limited. Understanding on the DES-extracted lignin structure is essential in order to determine its potential application as an aromatic biopolymer. DES-extracted lignin represents a new class of extracted lignin and its potential is largely untapped. To fully explore the possibility of applying DES-extracted lignin as fuel and aromatic feedstock, it is essential to initiate more comprehensive structural and properties studies of this lignin type. Subsequently, the outlook on the utilization of DES-extracted lignin for current existing fuel, macromolecular and fine chemicals conversion schemes would be highly appreciated.

## 2.5 Research Gaps

Lignocellulosic biomass fractionation and lignin extraction using the new generation green solvent DES is a considerably new research field with a lot of untapped research areas. The studies on value-added processing of oil palm empty fruit bunch is highly encouraged as it is one of the most abundant agricultural solid waste in Malaysia. The potential conversion of EFB using DES will be very rewarding in terms of economical and environmental benefits in the long run if successful. Therefore, this non-conventional pretreatment technology demands for more exploration to explore its possibility to be adopted in the future biorefinery scheme.

DES has shown potential in multiple application fields in replacement of conventional solvents due to the flexibility in designing the solvent properties. There is a wide spectrum of constituents available that can be used to form DES. Hence, one of the important research aspects is to identify the most effective type of DES for efficient lignocellulosic biopolymers fractionation and lignin extraction. Despite that a number of DES types have been used in biomass pretreatment, comparison study on different DES types is insufficient to identify the suitable DES constituents from a wide selection of chemical compounds.

Also, information pertaining to the characterization of DES-fractionated products is inadequate to give a comprehensive understanding on the influence of DES pretreatment on lignocellulosic biopolymers, particularly the DES-extracted lignin. Properties of these products can vary according to the DES used in pretreatment. Hence, it is highly important to evaluate the effect of DES constituents on DES-fractionated products' properties. The potential valorization process of the biopolymers can then be identified based on their distinctive properties.

# CHAPTER 3: METHODOLOGY

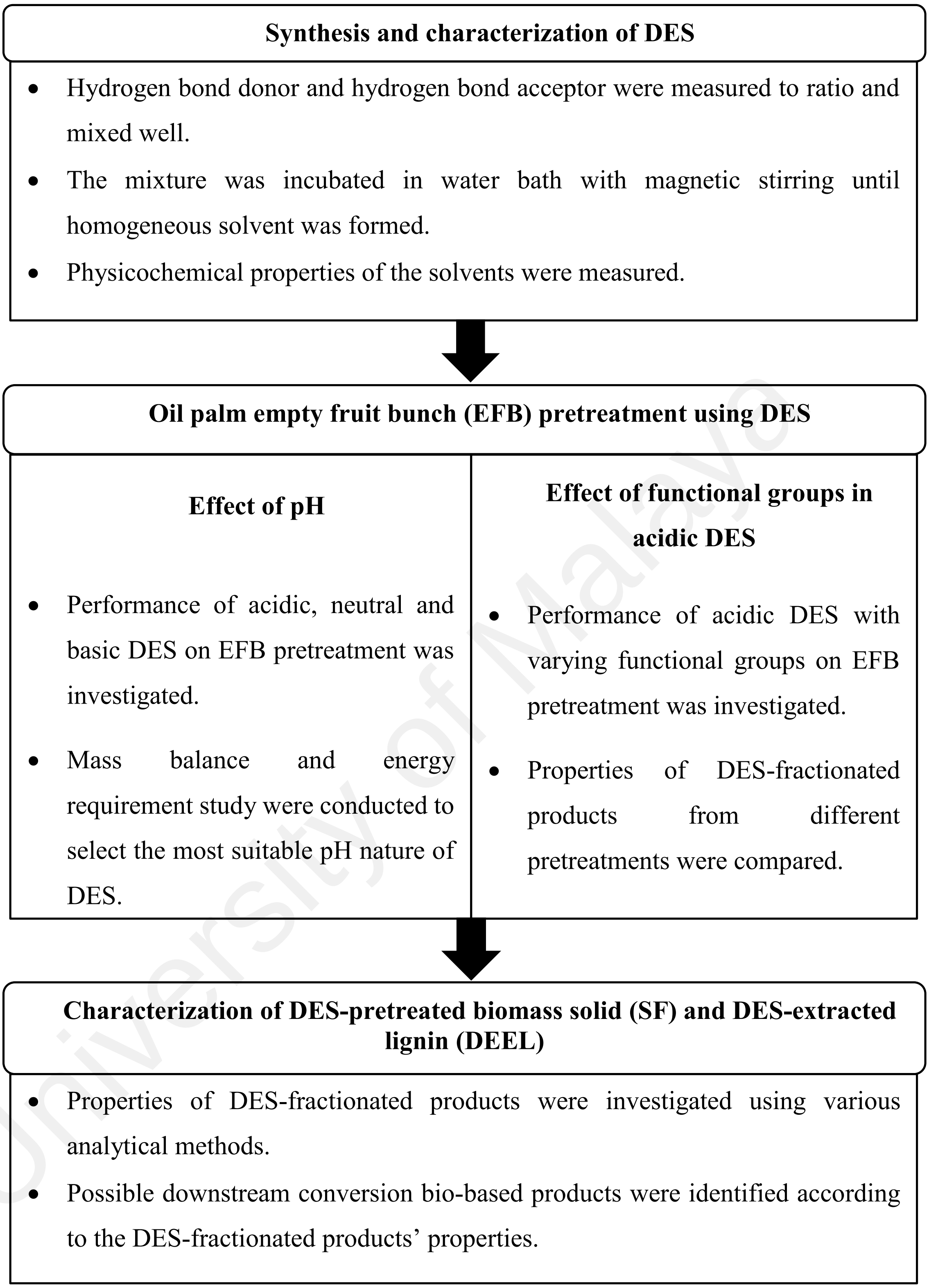
Research activities of this project are categorized into three main sections: synthesis of green solvents and their characterization, oil palm empty fruit bunch (EFB) pretreatment using DES and characterization of DES-fractionated products. Based on these research activities, this project was conducted in two main phases namely investigation on the effect of pH nature of DES and the effect of functional groups in acidic DES on EFB pretreatment performance. Figure 3.1 summarizes the activities of this work.

## 3.1 Preparation of EFB Biomass Material and Its Characterization

Oil palm empty fruit bunch (EFB) was collected from a local plantation in Pahang, Malaysia. The biomass was first rinsed with water and dried in oven at 40°C. Next, the EFB stalks were disintegrated into individual fibres using granulator (Rapid, Sweden) and sieved to mesh size between 0.250 mm and 0.707 mm.

### 3.1.1 Determination of Oven-Dried Weight (ODW)

To determine the solid content of EFB biomass, a representative amount (1 g) of biomass was dried in an oven at 40°C for 24 hours. The sample was then removed from the oven and placed into a desiccator for 15 minutes to cool down to room temperature. The weight of the sample was then recorded. The sample was returned to the drying oven for another 1 hour before transferred to a desiccator and weighed after 15 minutes. The same procedure was repeated until the change in EFB weight was less than 1% in 1 hour.



**Synthesis and characterization of DES**

- Hydrogen bond donor and hydrogen bond acceptor were measured to ratio and mixed well.
- The mixture was incubated in water bath with magnetic stirring until homogeneous solvent was formed.
- Physicochemical properties of the solvents were measured.

**Oil palm empty fruit bunch (EFB) pretreatment using DES**

**Effect of pH**

- Performance of acidic, neutral and basic DES on EFB pretreatment was investigated.
- Mass balance and energy requirement study were conducted to select the most suitable pH nature of DES.

**Effect of functional groups in acidic DES**

- Performance of acidic DES with varying functional groups on EFB pretreatment was investigated.
- Properties of DES-fractionated products from different pretreatments were compared.

**Characterization of DES-pretreated biomass solid (SF) and DES-extracted lignin (DEEL)**

- Properties of DES-fractionated products were investigated using various analytical methods.
- Possible downstream conversion bio-based products were identified according to the DES-fractionated products' properties.

**Figure 3.1: Activities flow chart**

The total solid content in EFB and oven dried weight (ODW) of EFB were computed using Equation 3.1 and Equation 3.2, respectively. The calculation of all biomass components was based on ODW.

$$\text{Total solid (\%)} = 100\% - \left(\frac{\text{EFB weight}_{\text{before drying}} - \text{EFB weight}_{\text{after drying}}}{\text{EFB weight}_{\text{before drying}}}\right)\% \tag{3.1}$$

$$\text{Oven dry weight, ODW (g)} = \text{weight}_{\text{EFB extractive free}}\ (\text{g}) \times \frac{\text{total solids (\%)}}{100} \tag{3.2}$$

### 3.1.2 Removal of Extractives

The EFB fibre was first processed using a two-step extraction process to remove the non-structural materials that might interfere with the subsequent pretreatment and analytical procedure. The extraction protocol used was according to US National Renewable Energy Laboratory (NREL) protocol for extractives removal (Sluiter, Ruiz, Scarlata, Sluiter, & Templeton, 2005). The extraction process was conducted using Soxhlet extraction method using water followed by ethanol as extraction solvents.

Ten gram (10 g) of EFB fibre was first placed into a thimble, which was then inserted into a Soxhlet tube. A 250 mL round bottom flask was filled with 200 mL of extraction solvent i.e. water, and placed on a heating mantle. The reflux duration of each extraction was set at 24 hours to ensure complete extractives removal. The collected extraction solvent which contained removed extractives was recovered using rotary evaporator. The flask containing dried extractives was weighed and recorded. The extractive removal process was then repeated on the same batch of EFB fibre using ethanol as extraction solvent.

The percentage of water-soluble and ethanol-soluble extractives were calculated using Equation 3.3.

$$\text{Extractives (wt\%)} = \frac{\text{Weight}_{\text{flask+extractives}} - \text{Weight}_{\text{flask}}}{\text{ODW}_{\text{EFB}}} \times 100\% \qquad (3.3)$$

### 3.1.3 Biopolymer Compositional Analysis

Biopolymer compositional analysis of raw EFB biomass was performed using standard NREL protocol (Sluiter et al., 2008). After the extractives removal process as described in Section 3.1.2 was performed, the extractive-free EFB was then subjected to two-stage acid hydrolysis process. 0.3 g of EFB fibre was first added to 3 mL of 72% $H_2SO_4$ and mixed well in a conical flask. Next, the mixture was incubated in a 30°C water bath for 1 hour. The mixture was stirred every 7-8 minutes. After 1 hour of incubation, 84 mL of ultrapure water was added to the flask to dilute the acid-hydrolysed mixture to a 4% $H_2SO_4$ solution. For the second stage of hydrolysis, the diluted EFB and acid mixture was autoclaved for 1 hour at 121°C under liquid condition setting.

Upon completion of the two-stage hydrolysis process, the acid-hydrolysed mixture was separated into acid-hydrolysed filtrate and solid residue using vacuum filtration. The filtrate was then used for carbohydrate content and acid soluble lignin (ASL) analysis while acid insoluble lignin (AIL) and ash content were determined from the solid residue.

#### 3.1.3.1 Measurement of glucan and xylan content

Twenty (20) mL of the filtrate was transferred to a conical flask. Calcium carbonate was gradually added to the filtrate to neutralize the solution to pH 5-6. The pH value was monitored constantly using a pH meter (Metrohm, Switzerland). The flask was swirled constantly throughout the process. Once the predetermined pH value was achieved, the precipitate in the flask was allowed to settle for around approximately 5 minutes. The mixture was filtered using a 3 mL syringe through 0.2 µm syringe filter (Agilent, Captiva Econofilter nylon membrane, United States) into HPLC autosampler vial.

Glucose and xylose standards with concentration of 0-2.5 mg/mL were also prepared to construct a calibration curve in HPLC. The carbohydrate content of the filtrate and sugar standards was measured using high performance liquid chromatography instrument (HPLC Waters, USA) equipped with refractive index detectors and a Hi-Plex H column (Agilent, USA). Ultrapure water with 18.2 MΩ conductivity was used as mobile phase at the flow rate of 0.6 mL/min. The runtime of each analysis was set at 35 minutes.

To correctly calculate the polymeric sugar concentration in the biomass based on the concentration of monomeric sugar as measured by HPLC, an anhydro correction of 0.88 for xylose and 0.90 for glucose was applied to the calculation of glucan and xylan content as expressed in Equation 3.4 and 3.5.

$$\text{Conc}_{\text{glucan}} = \text{Conc}_{\text{glucose}} \times 0.9 \tag{3.4}$$

$$\text{Conc}_{\text{xylan}} = \text{Conc}_{\text{xylose}} \times 0.88 \tag{3.5}$$

Where

$\text{Conc}_{\text{glucose}}$ = Glucose concentration measured in HPLC based on the calibration curve, mg/mL

$\text{Conc}_{\text{xylose}}$ = Xylose concentration measured in HPLC based on the calibration curve, mg/mL

The percentage of glucan and xylan content in EFB biomass were calculated using Equation 3.6 and Equation 3.7, respectively.

$$\text{Glucan content (\%)} = \frac{\text{Conc}_{\text{glucan}}(\text{mg/mL}) \times V_{\text{filtrate}}(\text{mL})}{\text{ODW (mg)}} \times 100\% \tag{3.6}$$

$$\text{Xylan content (\%)} = \frac{\text{Conc}_{\text{xylan}}(\text{mg/mL}) \times V_{\text{filtrate}}(\text{mL})}{\text{ODW (mg)}} \times 100\% \tag{3.7}$$

Where

$V_{\text{filtrate}}$ = Volume of acid-hydrolysed filtrate from two-stage acid hydrolysis process, mL

#### 3.1.3.2 Acid soluble lignin (ASL), acid insoluble lignin (AIL) and ash content

Acid soluble lignin (ASL) in the acid-hydrolysed filtrate was measured using UV-spectrophotometer at the wavelength of 320 nm with absorptivity, ε of 30 L/g.cm (Raman & Gnansounou, 2015). The percentage of ASL in EFB biomass was calculated using Equation 3.8.

$$\text{Acid soluble lignin, ASL (\%)} = \frac{\text{UV}_{\text{abs}} \times V_{\text{filtrate}}(\text{mL})}{\varepsilon\ (\text{L/g} \cdot \text{cm}) \times \text{ODW (g)} \times \text{pathlength (cm)}} \times 100\% \tag{3.8}$$

Where

$\text{UV}_{\text{abs}}$ = Absorbance of the sample detected in UV-Vis spectrophotometer at 320 nm

ε = Absorptivity of EFB biomass at 320 nm, L/g.cm

Pathlength = pathlength of cuvette, 1 cm

Meanwhile, the solid residue collected was dried in an oven at 103°C. The solid residue was left in the oven for 1 hour and cooled down in a desiccator for 15 minutes before weighing. The heating and cooling process was repeated until a constant weight was obtained. The final weight was recorded as acid insoluble residue.

Subsequently, the dried solid residue was ashed in a furnace at 550°C. The solid residue was incinerated in the furnace for 1 hour. The residue was then placed into the oven at 103°C for 30 minutes and cooled down in the desiccator for 15 minutes before weighing. The heating and cooling process was also repeated until a constant weight was obtained. The remaining substance was recorded as acid insoluble ash. Acid insoluble lignin (AIL) was calculated as the difference between acid insoluble residue and ash as shown in Equation 3.9. The percentage of AIL in EFB biomass was calculated using Equation 3.10. The total lignin content in EFB biomass is the sum of ASL and AIL as expressed in Equation 3.11.

$$\text{Acid insoluble lignin, AIL (mg)} = \text{acid insoluble residue} - \text{acid insoluble ash} \tag{3.9}$$

$$\text{Acid insoluble lignin, AIL (\%)} = \frac{\text{weight}_{\text{AIL}}}{\text{ODW}} \times 100\% \tag{3.10}$$

$$\text{Total lignin content (\%)} = \text{ASL (\%)} + \text{AIL (\%)} \tag{3.11}$$

## 3.2 Synthesis of Deep Eutectic Solvents

DES was prepared by mixing the hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) in a 80°C water bath on hot plate with magnetic stirrer until a homogeneous liquid was formed, as illustrated in Figure 3.2. All DESs were kept overnight under ambient room condition before use. Only DESs that remained as homogeneous liquid were used for EFB pretreatment and the solvent that crystallized or precipitated was considered as a non-homogeneous mixture.

**Figure 3.2: Illustration for DES synthesis setup**

Table 3.1 tabulates a list of chemicals used as DES constituent for DES synthesis. Fourteen different DESs were synthesized for the two phases of study in this project. More information regarding the selected constituents in the respective studies are elaborated in Section 3.3.1 and 3.3.2.

**Table 3.1: Purity and supply of chemicals used in DES synthesis**

| No | Chemicals | Purity (%) | Company |
|---|---|---|---|
| 1 | Choline chloride | ≥98% | Sigma Aldrich |
| 2 | Urea | ≥98% | Sigma Aldrich |
| 3 | D(+)-glucose | - | Merck |
| 4 | Lactic acid | ≥85% | R&M Malaysia |
| 5 | Glycerol | ≥99.8% | R&M Malaysia |
| 6 | Potassium carbonate | - | R&M Malaysia |
| 7 | Formic acid | 98-100% | Merck |
| 8 | Acetic acid | 99-100% | Merck |
| 9 | Propionic acid | ≥99% | Merck |
| 10 | Butyric acid | ≥99% | Sigma Aldrich |
| 11 | Succinic acid | Bioreagent grade | Sigma Aldrich |
| 12 | Maleic acid | ≥99% | Merck |
| 13 | Malic acid | ≥99% | Sigma Aldrich |
| 14 | Citric acid | ≥99% | R&M Malaysia |

## 3.3 Oil Palm Empty Fruit Bunch Pretreatment Using DES

The EFB pretreatment procedure using DES is presented in Figure 3.3. Biopolymer fractionation and lignin extraction from EFB were carried out in a single step pretreatment using DES. Every pretreatment was performed in three replicas and the reported results are the average value from the three replicas. Extractive-free EFB (0.45 g) was mixed thoroughly with DES (4.5 g) at 10 wt% solid loading and then incubated in an oil bath at 120°C for 8 hours. The pretreated biomass solid fraction (SF) and the liquid fraction (LF) were separated through filter paper (Advantec 4A, Japan) using vacuum filtration.

The SF was rinsed with ethanol until the washing liquid was no longer turbid. The ethanol washing liquid was mixed with LF to form the mixture LF1. Ultrapure water was added to the LF1 at a volume ratio of ethanol:water 1:2 for lignin precipitation. The supernatant fraction of the mixture (LF2) was separated from precipitated lignin using centrifugation at 3500 rpm for 10 minutes (Figure 3.3).

The precipitated lignin obtained was labelled as DES-extracted lignin (DEEL). The lignin was washed three times with ultrapure water then centrifuged for further purification. The DEEL was dried overnight into powder in oven at 40ºC and weighed.

Using the procedures as described, EFB pretreatment study was conducted in two phases: EFB pretreatment using DESs with different pH nature and EFB pretreatment using acidic DESs with different HBDs.

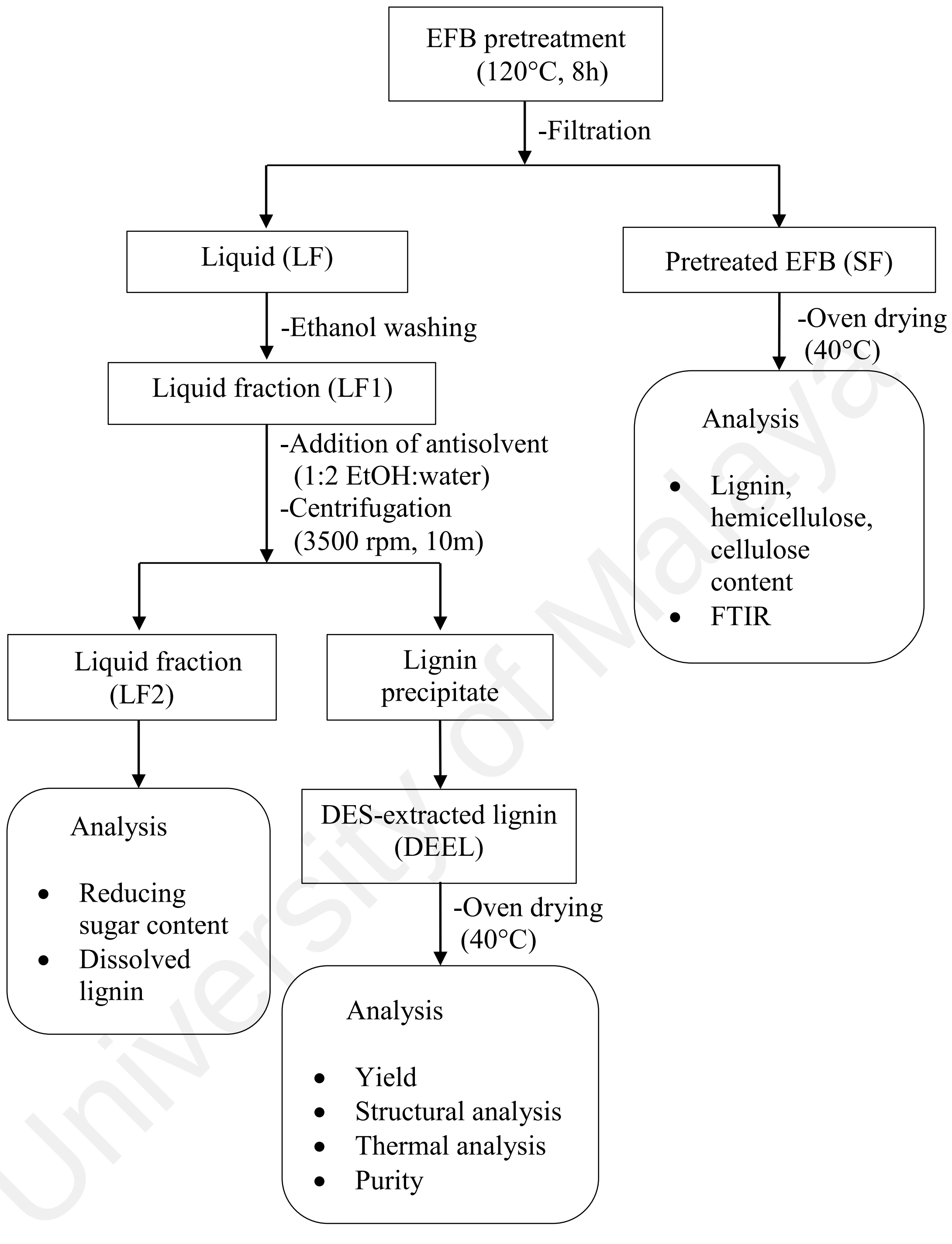


**Figure 3.3: Oil palm empty fruit bunch (EFB) pretreatment flow chart**

### 3.3.1 Effect of pH Nature of DES on Biomass Fractionation and Lignin Extraction Efficiency

The EFB pretreatment performances of DESs with different pH nature were evaluated and compared. The DESs used were of acidic, neutral and basic pH nature.

#### 3.3.1.1 Synthesis and characterization of DES with different pH nature

The synthesized DESs with acidic, near-neutral and basic pH and their categories are tabulated in Table 3.2. Their pH value, viscosity and heat capacity were also measured.

**Table 3.2: DESs with different pH nature for EFB pretreatment**

| pH nature | HBA | HBD | DES Combinations | Abbrevia-tion | Molar ratio |
|---|---|---|---|---|---|
| **Acidic** | Ammonium salt | Carboxylic acid | Choline chloride : lactic acid | CC-LA | 1 : 5 |
| | Sugar | Carboxylic acid | D(+)-glucose: lactic acid | GLUC-LA | 1 : 5 |
| **Neutral** | Ammonium salt | Sugar | Choline chloride : D(+)-glucose | CC-GLUC | 1 : 1 |
| | Ammonium salt | Polyol | Choline chloride : glycerol | CC-GLY | 1 : 2 |
| **Basic** | Ammonium salt | Amide | Choline chloride : urea | CC-UREA | 1 : 2 |
| | Inorganic carbonate salt | Polyol | Potasssium carbonate : glycerol | [$K_2CO_3$]-GLY | 1 : 6 |

##### (a) ***Viscosity and pH***

Viscosity of different DES combination i.e. CC-LA, GLUC-LA, CC-GLUC, CC-GLY and CC-UREA were measured using LV viscometer (Brookfield, USA) and [$K_2CO_3$]-GLY using HAAKE VT550 (Thermo Scientific, USA) at ambient temperature in the laboratory (27°C). The pH value of the solvents was determined using pH meter (Metrohm, Switzerland) and pH indicator strips (Merck, USA).

### (b) ***Heat capacity***

Heat capacity ($C_p$) of DES was measured using differential scanning calorimeter (Mettler Toledo, DSC1, USA) based on the standard sapphire method. Around 10 mg of DES was crimped in a hermetic aluminium sample pan (ME-27331) and analysed under nitrogen atmosphere. The measurement began with a 5-minute isothermal period at 20°C, followed by a dynamic ramp to 90°C at 5°C/min heating rate, and ended with a 5-minute isothermal period at 90°C. The heat flow curves of both sample and sapphire standard were blank-corrected and the sample curve was compared against the standard sapphire curve to determine the sample's $C_p$ value.

Relationship between the measured heat capacity, $C_p$ value of DES and temperature was correlated using linear and higher-order polynomial equations. Least squares method was adopted to determine the regression coefficients and data fitting into the equation model was evaluated using the absolute average deviation (AAD). Equation model with the lowest AAD value was selected to represent the $C_p$ model for energy requirement study. Based on the model developed in Section 4.1.4, second-order polynomial equation was found to be best-represented the $C_p$ correlation for all DES. Therefore, $C_p$ value of the DES was calculated using Equation 3.12 for energy requirement study.

$$\text{Heat capacity}, \mathrm{C_P}\ (\mathrm{J \cdot mol^{-1} {}^{\circ}C^{-1}}) = \mathrm{a_0 + a_1 T + a_2 T^2} \tag{3.12}$$

Where

$\mathrm{a_0, a_0, a_0}$ = Coefficients determined by regression of experimental data

$\mathrm{T}$ = DES pretreatment temperature, K

#### 3.3.1.2 Evaluation of DES performance – mass balance and energy requirement study

The mass balance of the DES pretreatment system was developed using the DES-fractionated product characterization procedures and calculation methods described in detailed in Section 3.4. The weight distribution of glucan, xylan and lignin in DES-fractionated streams namely SF, LF1, LF2 and DEEL were tabulated to assess the DES fractionation efficiency.

In addition, energy requirement by the different DES pretreatment system of varying pH nature was also evaluated to ensure the process feasibility in terms of energy consumption. Energy required, Q for DES pretreatment per unit biomass was calculated based on the Equation 3.13 and Equation 3.14.

$$\text{Q per unit solvent } (\text{J}/\text{g}_{\text{solvent}}) = \int_{\text{T}_1}^{\text{T}_2} \text{C}_\text{P}\, \text{dT} \tag{3.13}$$

$$\text{Q per unit biomass } (\text{J}/\text{g}_{\text{biomass}}) = \text{Q per unit solvent} \times \frac{\text{g}_{\text{solvent}}}{\text{g}_{\text{biomass}}} \tag{3.14}$$

Where

$g_{solvent}/g_{biomass}$ = ratio of solvent to biomass in DES pretreatment

$T_1$ = initial temperature = 28°C

$T_2$ = pretreatment temperature = 120°C

$C_p$ = specific heat capacity of DES at $T_1$ and $T_2$ (J/$g_{solvent}$ °C) (Section 3.3.1.1(b))

### 3.3.2 Effect of Functional Groups and Molar Ratio in Acid HBD on Biomass Fractionation and Lignin Extraction Efficiency

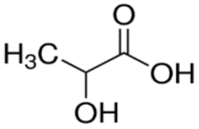
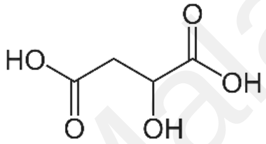
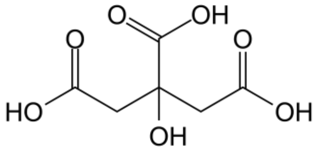
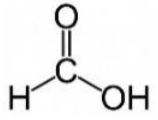
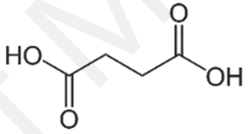
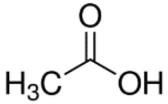
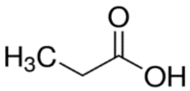
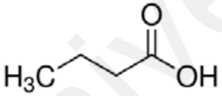
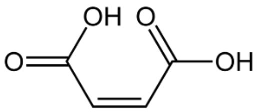
Results from the pH screening study showed that acidic DES are more efficient for EFB fractionation and lignin extraction (Section 4.1 ). Hence, further investigation on the effect of functional groups in carboxylic acid HBD was carried out using nine different types of acidic DESs. The acidic DES pretreatment performance was evaluated based on their biopolymer fractionation efficiency and lignin extraction yield, as well as the properties of pretreated EFB SF and DEEL.

As DES formation occurred at different molar ratio (Section 4.2.1), effect of acidic DES molar ratio on pretreatment performance was also investigated using the selected best performing acidic DESs.

#### 3.3.2.1 Synthesis of acidic DES with different acid HBD and molar ratio

Nine carboxylic acids selected as the HBD in acidic DES synthesis and their respective acid categories are tabulated in Table 3.3. Choline chloride was used as HBA for all the acidic DESs. The acids used are categorized into three major groups, which are alpha-hydroxy (lactic, malic and citric acid), linear saturated (formic, acetic, propionic, butyric and succinic acid) and unsaturated carboxylic acids (maleic acid). The acids are further categorized into mono-, di- and tricarboxylic acids. The DESs were synthesized at various molar ratio combinations including 2:1, 1:1, 1:2, 1:5, 1:10 and 1:15 (HBA:HBD).

**Table 3.3: Categories of carboxylic acid HBD (Number of carbons of each structure is stated in bracket)**

| | **Monocarboxylic acid** | | **Dicarboxylic acid** | | **Tricarboxylic acid** | |
|---|---|---|---|---|---|---|
| **Alpha-hydroxy** | Lactic acid (C3) |  | Malic acid (C4) |  | Citric acid (C6) |  |
| **Linear Saturated** | Formic acid (C1) |  | Succinic acid (C4) |  | | |
| | Acetic acid (C2) |  | | | | |
| | Propionic acid (C3) |  | | | | |
| | Butyric acid (C4) |  | | | | |
| **Unsaturated** | | | Maleic acid (C4) |  | | |

#### 3.3.2.2 Evaluation of DES performance – lignin extraction yield and properties of DES-fractionated products

To select the most suitable acidic DES for biopolymer fractionation, the performance of the DES pretreatment was evaluated based on DES-extracted lignin (DEEL) yield and the properties of DES-fractionated products i.e. SF and DEEL. The measurement of lignin extraction yield is detailed in Section 3.4.3.1. The functional group distribution and phenolic hydroxyl content in DEEL as well as the solid recovery percentage and functional group distribution in DES-pretreated EFB SF were also evaluated to assess their potential for downstream bio-products conversion. The detailed methodology of DES-fractionated product characterization is included in Section 3.4.

## 3.4 Characterization of DES-Fractionated Products

Following DES pretreatment, the EFB was fractionated into three fractions, namely solid fraction (SF), liquid fraction (LF) and DES-extracted lignin (DEEL) as presented previously in Figure 3.3. These DES-fractionated products were characterized separately using different analytical procedures and tools to determine the EFB pretreatment performance.

### 3.4.1 Liquid Fraction (LF)

#### 3.4.1.1 Dissolved sugar

The dissolved sugar in LF2 was determined as reducing sugar using 3,5-Dinitrosalicylic acid (DNS) assay (Miller, 1959). The glucose and xylose contents were analysed as total reducing sugar content in LF2. The DNS reagent was prepared based on the following composition (w/v): 1% 3,5-dinitrosalicylic acid, 0.2% phenol, 1% sodium hydroxide and 0.05% sodium sulphite. The reagent was first prepared without sodium sulphite and stored in dark bottle at 4°C. Sodium sulphite was added into the reagent prior to the assay.

The assay was conducted by adding 0.5 mL of the LF2 sample into 3 mL DNS reagent in a capped test tube. The mixture was incubated in a 95°C water bath for 15 minutes. Next, the tubes were cooled to room temperature in tap water and 1 mL of 40% (w/v) Rochelle salt solution was simultaneously added to each test tube for colour stabilisation. 0.5 mL of the reaction mixture was diluted with 0.75 mL of ultrapure water in cuvette (2.5 times dilution factor). Absorbance of this mixture was measured at 540 nm in UV-vis spectrophotometer.

The same procedure was conducted using DES solvent as sample for blank measurement and glucose standard (0.25-1.25 mg/mL) for calibration curve preparation. The dissolved sugar content in LF2 was corrected to actual sugar content in LF using a 2.5 times dilution factor for the dilution in cuvette. The percentage of sugar content in LF was calculated using Equation 3.15.

$$\text{Reducing sugar in LF}(\%) = \frac{\text{Conc}_{\text{sugar}} \times \text{V}_{\text{LF2}} \times \text{dilution factor}}{\text{ODW}} \qquad (3.15)$$

Where

$\text{Conc}_{\text{sugar}}$ = Concentration of reducing sugar based on glucose calibration curve, mg/mL

$\text{V}_{\text{LF2}}$ = volume of LF2, mL

### 3.4.1.2 Dissolved lignin

The dissolved lignin in LF2 was measured using UV-vis spectrophotometer at 290 nm. The calibration curve of dissolved lignin was prepared using alkali lignin (Low sulfonate content, Sigma Aldrich). The percentage of dissolved lignin in LF was calculated using Equation 3.16.

$$\text{Lignin in LF(\%)} = \frac{\text{Conc}_{\text{lignin}} \times \text{V}_{\text{LF2}} \times \text{dilution factor}}{\text{ODW}} \tag{3.16}$$

Where

$\text{Conc}_{\text{lignin}}$ = Concentration of lignin based on lignin calibration curve, mg/mL

## 3.4.2 DES-Pretreated EFB Solid Fraction (SF)

### 3.4.2.1 Solid recovery and biomass dissolution

The percentage of solid recovery in SF indicated the extent of biopolymer dissolution efficiency of DES. The lower the SF solid recovery percentage, the higher the dissolution efficiency. The recovery percentage of SF after DES pretreatment was determined using Equation 3.17 whereas the biomass dissolution percentage was calculated using Equation 3.18.

$$\text{SF recovery (\%)} = \frac{\text{weight}_{\text{SF}}}{\text{ODW}_{\text{raw EFB}}} \times 100\% \tag{3.17}$$

$$\text{Biomass dissolution (\%)} = 100\% - \text{SF solid recovery (\%)} \tag{3.18}$$

#### 3.4.2.2 Biopolymer composition

Lignin, cellulose, hemicellulose and ash content in SF were characterized using the same NREL protocol used for raw EFB compositional analysis as described in Section 3.1.3. The percentage of biopolymer in SF was calculated using Equation 3.19.

$$\text{Biopolymer in SF (\%)} = \frac{\text{cellulose}_{\text{SF}} \;/\; \text{hemicellulose}_{\text{SF}} \;/\; \text{lignin}_{\text{SF}}\ (\text{g})}{\text{ODW}_{\text{SF}}\ (\text{g})} \times 100\% \tag{3.19}$$

#### 3.4.2.3 Fourier-transform infrared spectroscopy (FTIR)

Investigation on the functional groups of the SF provides the understanding on the varying effect brought upon the biomass by different DESs pretreatment. The functional groups in the SFs were determined using FTIR analysis. SF was mixed with potassium bromide and pressed into pellets. The infrared spectra were recorded from 4000 $\text{cm}^{-1}$ to 450 $\text{cm}^{-1}$ with the spectral resolution of 4 $\text{cm}^{-1}$ on FTIR spectrometer (Perkin Elmer, Spectrum 400, USA). Two infrared ratios were calculated from the FTIR spectra to measure the crystallinity of SF, namely crystallinity ratio (CR) (Nelson & O'Connor, 1964) and lateral order index (LOI) (Hurtubise & Krassig, 1960). The ratios were calculated using Equation 3.20 and Equation 3.21.

$$\text{CR} = \text{a}_{1372\text{cm}^{-1}} / \text{a}_{2900\text{cm}^{-1}} \tag{3.20}$$

$$\text{LOI} = \text{a}_{1430\text{cm}^{-1}} / \text{a}_{900\text{cm}^{-1}} \tag{3.21}$$

### 3.4.3 DES-Extracted Lignin (DEEL)

#### 3.4.3.1 DEEL yield

The DES-extracted lignin (DEEL) yield was calculated using Equation 3.22.

$$\text{DEEL yield }(\%) = \frac{\text{weight}_{\text{DEEL}}}{\text{weight}_{\text{lignin in raw EFB}}} \times 100\% \tag{3.22}$$

#### 3.4.3.2 Phenolic hydroxyl content (PhOH)

Phenolic hydroxyl group (PhOH) is the most reactive functional groups in lignin polymer (Lai, 1992; Zakis, 1994). It is an important indicator of lignin's reactivity in the subsequent processing. The PhOH content was measured using Δε method based on two level ionization of lignin under alkaline condition (Goldmann et al., 2016; Zakis, 1994).

One (1) mg of lignin was dissolved in a mixture of 5 mL dioxane and 5 mL 0.2 M NaOH. The solution was filtered through filter paper (Whatman, No. 1, United Kingdom) to remove possible undissolved solids. Two portions of 2 mL filtered solution was diluted to 25 mL (final concentration= 0.08 g/L) using pH 6 buffer solution and 0.2 M NaOH, respectively. Using pH 6 lignin solution as the reference, UV-vis spectrum of 0.2 M NaOH lignin solutions was recorded with UV-vis spectrophotometer in the wavelength range of 250-400 nm. PhOH content of the different acidic DES-extracted DEELs was calculated using the absorbance value of 300 nm and 360 nm peaks in UV-vis spectrum. The formula is listed as Equation 3.23.

$$\text{Total PhOH (mmol/g)} = 0.250 \times \Delta a_{300}(\text{NaOH}) + 0.107 \Delta a_{360}(\text{NaOH}) \tag{3.23}$$

Where Δa = absorbance / (lignin solution concentration, g/L* cuvette pathlength, cm)

### 3.4.3.3 Fourier-transform infrared spectroscopy (FTIR)

Similar to the FTIR testing on SF (Section 3.4.2.3), functional group study on DEEL can identify the effect of varying DES pretreatment on DEEL. Functional groups distribution in DEEL was also observed using FTIR spectroscopy. The DEEL was mixed with potassium bromide and then pressed into pellets. Infrared spectra were recorded from 4000 $cm^{-1}$ to 450 $cm^{-1}$ with the spectral resolution of 4 $cm^{-1}$ on FTIR spectrometer (Perkin Elmer, Spectrum 400, USA).

### 3.4.3.4 Biopolymer and elemental composition

Biopolymer compositional analysis i.e. cellulose, hemicellulose and lignin content of lignin was performed using standard NREL protocol used in raw EFB analysis (Section 3.1.3). The carbon, hydrogen and nitrogen content was analysed using a CHN elemental analyser (Perkin Elmer, CHNS/O 2400 Series II, USA). The percentage of oxygen was calculated using Equation 3.24.

$$\text{Oxygen content in DEEL, O (\%)} = 100\% - (\text{C} + \text{H} + \text{N} + \text{S})\% \qquad (3.24)$$

Where

C, H, N and S = Carbon, hydrogen, nitrogen and sulphur content in DEEL, respectively

### 3.4.3.5 2D Heteronuclear single quantum coherence spectroscopy nuclear magnetic spectroscopy (2D HSQC NMR)

Lignin samples were first acetylated by mixing 100 mg of lignin in a mixture of 1 mL of pyridine and 1 mL of acetic anhydride (Hu, Xiao, Shen, & Zhang, 2013). The mixture was left under room condition for 24 hours followed by the addition of 10 mL of diethyl ether solvent to the mixture to precipitate the dissolved lignin. The precipitated lignin was further washed three times with 5 mL of diethyl ether to remove the remaining solvent. The lignin was then dried in an oven at 40°C for further analysis.

The acetylated EFB lignin was dissolved in deuterated DMSO-d6 solvent for structural characterization on NMR spectrometer (Bruker, Advance 500 mHz, United States). The two dimensional heteronuclear single quantum coherence spectroscopy nuclear magnetic spectroscopy (2D HSQC NMR) spectra of the DEEL were obtained at 25°C and a total of 40 scans were recorded for each lignin spectrum.

### 3.4.3.6 Thermogravimetric analysis (TGA) and Thermogravimetric-Fourier transform infrared spectroscopy analysis (TG-FTIR)

The thermal behaviour and volatiles evolution trend of DEEL are essential for the development of thermochemical application of lignin. The absorbance value of the volatiles is linearly dependent on the evolving gas concentration as according to Beer-Lambert law (Gao, Li, Quan, Du, & Duan, 2013). The thermal behavior of DEEL was investigated using thermal analyser (TA Instrument, TGA Q500, USA) coupled with FTIR (Thermo Fisher Scientific, Nicolet iS10, USA). The thermal analysis was conducted from 30°C to 900°C with heating rate of 10°C/min. Nitrogen gas was used as the carrier gas with gas flow rate at 20 mL/min. FTIR scans of the volatiles were recorded simultaneously from 300-4000 cm$^{-1}$.

### 3.4.3.7 Pyrolyzer-gas chromatography mass spectrometry (PY-GCMS)

PY-GCMS analysis was conducted to identify the volatiles products of lignin. The pyrolysis process was conducted on pyrolyzer at 650°C (Shimadzu, Frontier Lab PY-2020 iD, Japan). The chromatographic separation of volatile products was performed on GCMS (Agilent, GC 7890A/MS 5975C, United States) under helium condition at 1 mL/min flowrate. The GC column used was DB-5ms (30 m x 250 µm x 0.25 µm). The oven programme was set at 50°C for 5 minutes then ramped up to 300°C at 10°C/min for 10 minutes.

# CHAPTER 4: RESULTS AND DISCUSSION

The application of deep eutectic solvent (DES) as pretreatment solvent for biopolymer fractionation and lignin extraction from oil palm empty fruit bunch (EFB) was investigated. The performance of varying types of DES were assessed in two phases, namely screening of suitable pH nature of DES and selection of carboxylic acid hydrogen bond donor (HBD) of DES. In the first phase of study, DES with acidic, near-neutral and basic pH nature were synthesized and applied on EFB pretreatment. The suitable pH range was selected based on the biopolymer fractionation and delignification efficiency of DES, which was measured by recovery of DES-pretreated EFB solid fraction (SF), mass distribution of DES-fractionated biopolymers, as well as energy requirement level by each DES pretreatment.

The result from the pH nature screening showed that acidic DES had higher efficiency in EFB fractionation and lignin extraction. Therefore, the assessment of suitable carboxylic acid as HBD in acidic DES was subsequently performed in the second phase of study. Acidic DESs were synthesized using different carboxylic acids with varying functional groups as HBD. The EFB fractionation and lignin extraction efficiency of acidic DES was evaluated based on the yield recovery and functional group distribution of both SF and DEEL. Following that, the selection of optimum molar ratio of the best performing acidic DESs to achieve high lignin extraction yield was conducted.

To further assess the potential of DEEL for lignin-derived products’ conversion, the properties of DEEL produced with the highest yield from acidic DES pretreatment were examined. The investigated lignin properties included lignin composition, structural and thermal properties. Based on the properties study of DEEL and SF, the bio-products that can potentially be produced was suggested and their life cycle analysis which involves DES pretreatment as the first step in biorefinery process was also discussed.

## 4.1 Screening and Selection of Suitable pH Nature of DES for EFB Fractionation and Lignin Extraction

pH nature is a fundamental characteristics of solvent which has great impact over the solvent's chemical reaction (Skulcova, Russ, Jablonsky, & Sima, 2018). In conventional biomass pretreatment, one of the most commonly applied process is chemical pretreatment using acid or alkaline solvent. In this study, the EFB pretreatment performance of DESs with acidic, neutral or basic pH nature were evaluated based on their biopolymer fractionation and lignin extraction efficiency. With the obtained results on the recovery percentage and biopolymer composition of DES-pretreated EFB solid fraction (SF), the mass balance of glucan, xylan and lignin in DES-fractionated product streams, namely solid fraction (SF), liquid fraction (LF) and DES-extracted lignin (DEEL) were determined to elucidate the DES fractionation efficiency. The energy requirement of each DES pretreatment system was also assessed as an additional selection criterion for the suitable type of DES.

### 4.1.1 Synthesis and Characterization of DES

Diverse combinations of DES constituent in solvent synthesis result in DES with varying properties. The dissolution performance of a solute depends largely on the properties of solvent used. It is therefore crucial to evaluate the effect of pH of DES due to the dependence of solvent performance on its ionic strength. In addition, viscosity is also an important property of solvent to be taken into account when developing a solvent-solute extraction process, specifically in the case of DES pretreatment process. Considering that pretreatment process involves mass transfer operation of biopolymer in solvent, it is imperative to understand how the viscosity of the different DES solvent influences their performance in biomass fractionation.

pH value of the synthesized DESs varied according to the solvent's constituents as presented in Table 4.1. The DESs that were synthesized to represent each pH nature category are choline chloride:lactic acid (CC-LA) and glucose:lactic acid (GLUC-LA) for acidic DES category, choline chloride:glucose (CC-GLUC) and choline chloride:glycerol (CC-GLY) for near-neutral DES category, as well as choline chloride:urea (CC-UREA) and potassium carbonate:glycerol ([$K_2CO_3$]-GLY) for basic DES category. Expectedly, the two lactic acid-based DESs (CC-LA and GLUC-LA) are acidic in nature with pH value ranged between 0-2. Whereas CC-GLY and CC-GLUC have pH value of 6.69 and pH 5.37, respectively, attributing to their HBD i.e. glycerol and glucose having neutral pH. In contrast, urea and potassium carbonate salt act dominantly over choline chloride and glycerol, contributing to the basic nature of CC-UREA and [$K_2CO_3$]-GLY with pH respectively registered at 9.45 and 12.83.

The viscosity of DES was determined from linear regression analysis between shear stress and shear rate, which ranged from tenth mPa.s to thousandth of mPa.s as listed in Table 4.1. The viscosity of [$K_2CO_3$]-GLY at $0.709\times10^4$ mPa.s is 8000 folds higher than that of water (0.852 mPa.s) while the least viscous investigated DES, CC-LA is only 90 times more viscous than water ($0.818\times10^2$ mPa.s). The highly viscous DES might impede the mass transport phenomena during biomass pretreatment and eventually affecting its fractionation performance. The viscosity analysis also showed that all six DESs are Newtonian fluids. Newtonian fluid exhibit constant viscosity at different shear rates and constant temperature. Therefore, when applying to industrial application which usually involves the use of stirring reactor to facilitate the material transfer for more efficient reaction, DES as fractionation solvent will display consistent viscosity regardless of the temperature and stirring speed. As a result, the reaction will be more controllable. The effect of pH nature and viscosity of DES on biomass pretreatment performance will be explained later in Section 4.1.2.

**Table 4.1: Physical properties of synthesized DES in comparison with water under ambient condition**

| Solvents | Molecular formula/Acronym | Viscosity at 27°C (mPa.s) | pH value | pH condition |
|---|---|---|---|---|
| Water | $H_2O$ | 0.852 | 7.00 | Neutral |
| Choline chloride:lactic acid | CC-LA | $0.818\times10^{2}$ | 0 – 1 | Acidic |
| Glucose:lactic acid | GLUC-LA | $0.359\times10^{3}$ | 1 – 2 | |
| Choline chloride:glycerol | CC-GLY | $0.258\times10^{3}$ | 6.69 | Near neutral |
| Choline chloride:glucose | CC-GLUC | $0.290\times10^{4}$ | 5.37 | |
| Choline chloride:urea | CC-UREA | $0.540\times10^{3}$ | 9.45 | Basic |
| Potassium carbonate:glycerol | $[K_2CO_3]$-GLY | $0.709\times10^{4}$ | 12.83 | |

**Table 4.2: Solid recovery percentage and compositional analysis of raw EFB and DES-pretreated EFB SF**

| pH of DES | EFB | SF recovery (%) | SF weight (mg)[a] | Biopolymer compositions in SF (wt %) | | | Delignification (%) |
|---|---|---|---|---|---|---|---|
| | | | | Lignin | Glucan | Xylan | |
| | Raw EFB | - | - | 17.9 ± 0.3 | 32.1 ± 0.4 | 14.4 ± 0.2 | - |
| **Acidic** | CC-LA SF | 53.1 ± 0.5 | 222 | 4.7 ± 0.2 | 71.4 ± 1.3 | n.d.[b] | 88 |
| | GLUC-LA SF | 69.6 ± 0.7 | 291 | 13.6 ± 0.1 | 46.3 ± 1.0 | 5.1 ± 0.3 | 55 |
| **Near neutral** | CC-GLY SF | 96.2 ± 0.2 | 403 | 16.9 ± 0.2 | 30.9 ± 1.0 | 14.7 ± 1.0 | 22 |
| | CC-GLUC SF | 96.5 ± 0.8 | 404 | 18.0 ± 0.1 | 29.7 ± 0.9 | 14.1 ± 0.8 | 17 |
| **Basic** | CC-UREA SF | 93.5 ± 0.2 | 392 | 14.7 ± 0.1 | 30.7 ± 0.1 | 14.4 ± 0.9 | 34 |
| | $[K_2CO_3]$-GLY SF | 74.8 ± 3.3 | 313 | 13.7 ± 0.4 | 37.6 ± 0.6 | 14.4 ± 1.2 | 51 |

[a] 450 mg EFB was used in pretreatment (ODW=419 mg).

[b] n.d. = not detectable

### 4.1.2 Fractionation and Delignification Efficiency of DES Pretreatment

The biopolymer fractionation and delignification efficiency of EFB pretreatment using DES with varying pH nature are presented in this section. A pretreatment process with high biopolymer fractionation efficiency helps to achieve complete utilization of biomass as the fractionated biopolymers could be processed individually in downstream process. The performance of DES will be discussed from three perspectives: a) recovery of DES-pretreated EFB solid fraction (SF), b) biopolymer composition analysis of SF, and c) biopolymer mass distribution of each DES pretreatment system.

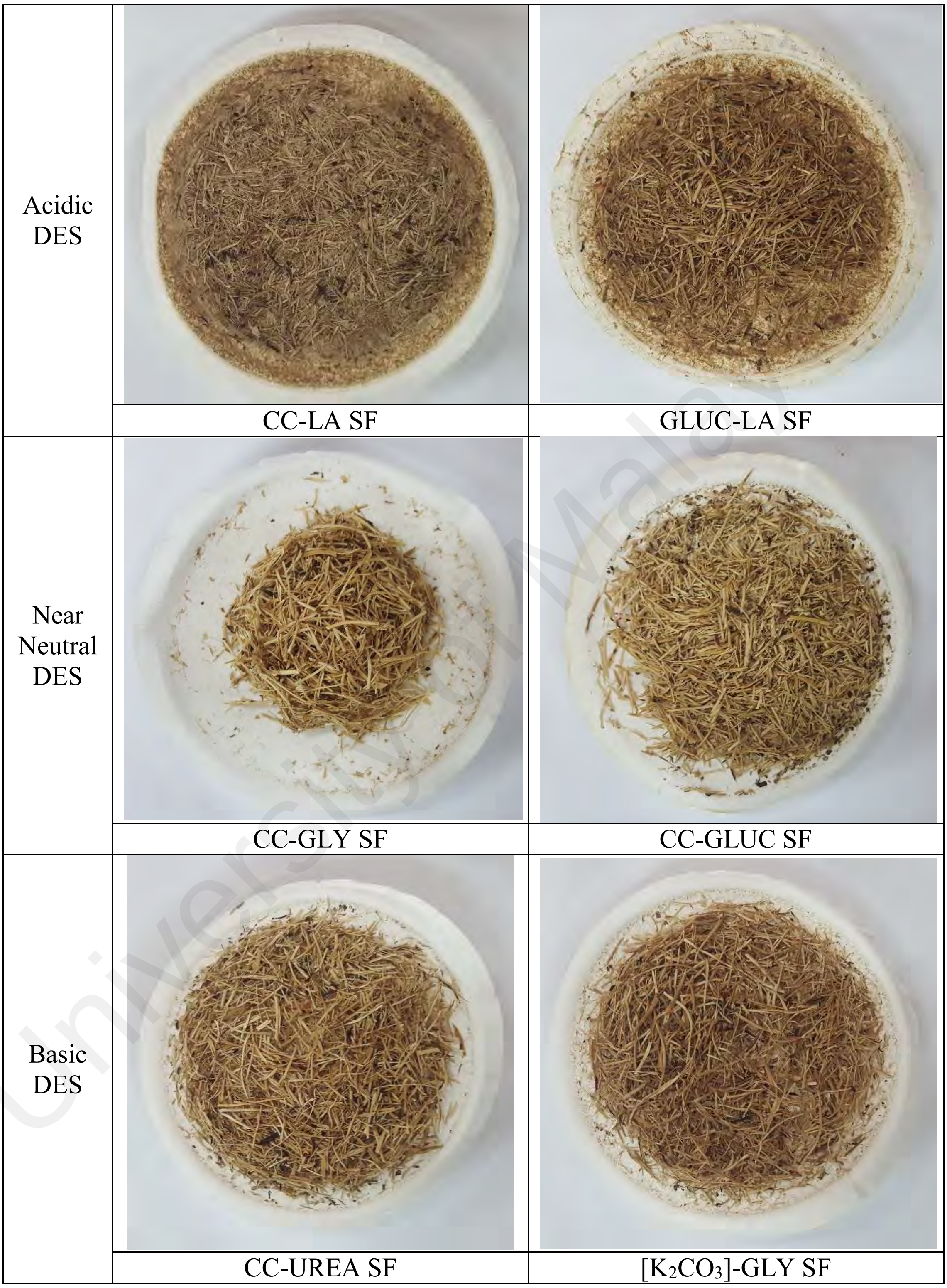
The solid recovery of SF and the biopolymer mass distribution in the SF, pretreatment liquid fraction (LF) and DES-extracted lignin fraction (DEEL) revealed the biopolymer fractionation efficiency of DES. Whereas the delignification efficiency of DES was evaluated through the biopolymer composition of SF to indicate the percentage of lignin being removed from the SF. The evaluation of fractionation and delignification efficiency of DES pretreatment are helpful in selecting the suitable pH range of DES for EFB processing.

#### 4.1.2.1 Solid recovery of DES-pretreated EFB solid fraction

The solid recovery percentage of DES-pretreated EFB solid fraction (SF) can reflect the fractionation efficiency of DES. Low solid recovery percentage of SF usually suggests that the DES is effective in separating the biomass into solid and liquid fraction. The recovery percentage of SF produced by DES with varying pH nature and their biopolymer compositions are presented in Table 4.2. Among the acidic, near-neutral and basic DES categories, acidic DESs managed to dissolve the highest portion of the biomass components, thereby achieving the lowest SF recovery percentage. The solid recovery of SF by CC-LA and GLUC-LA pretreatments were 53.1% and 69.6%, respectively. Comparatively, the SF recovered from [$K_2CO_3$]-GLY basic DES pretreatment was higher at 74.8% while another basic DES, CC-UREA exhibited limited biopolymer dissolving capacity like in the near-neutral DESs i.e. CC-GLY, CC-GLUC, showing more than 93% of SF recovery percentage (Table 4.2).

The solid recovery percentage of SF from each DES pretreatment correlates well to the DES's pH value. The more extreme the pH condition, the lower the SF recovery. DES with stronger ionic strength can dissolve the recalcitrant biomass matrix more efficiently, which explains for the low SF recovery in CC-LA, [$K_2CO_3$]-GLY and GLUC-LA pretreatments, as well as the near complete solid recovery in pretreatments using near-neutral DES. This signifies that DES with more extreme pH condition is better than DES with mild pH condition in fractionating the biopolymer into solid and liquid fraction in the pretreatment mixture. Similar findings were also observed in other studies adopting different types of biomass whereby the solid recovery of DES-pretreated wood and corncob using carboxylic acid-based DES, especially lactic acid-based DES, were lower than that of CC-GLY pretreatment (Alvarez-Vasco et al., 2016; C. W. Zhang et al., 2016).

The solid recovery trend also matches the physical observation of the recovered DES-pretreated SFs. Figure 4.1 shows the images of SF produced from DES pretreatment. The EFB fibres pretreated by CC-LA are visibly thinner, which correlated well with its lowest solid recovery percentage at 53.1% (Table 4.2). GLUC-LA and [$K_2CO_3$]-GLY, which had the second and third lowest solid recovery at 69.6% and 74.8%, respectively, produced a mixture of thin and coarse fibres after the DES pretreatments. Meanwhile, the SFs recovered from CC-UREA, CC-GLY and CC-GLUC pretreatments remained as coarse fibres, signifying that the fibres were largely intact after the pretreatment process, as portrayed through their high solid recovery at > 90 wt%.



CC-LA SF
GLUC-LA SF
CC-GLY SF
CC-GLUC SF
CC-UREA SF
$[K_2CO_3]$-GLY SF

**Figure 4.1: Pretreated EFB solid fraction after DES pretreatment**

#### 4.1.2.2 Biopolymer composition of DES-pretreated EFB solid fraction

Apart from the solid recovery percentage, the biopolymer composition of DES-pretreated SF also reveals the fractionation efficiency of DES and their delignification performance. The biopolymer composition of SF produced by the DES with varying pH are tabulated in Table 4.2. The biopolymer compositional modification by each DES are discussed according to their respective pH category.

(a) ***Acidic DES pretreatment***

CC-LA and GLUC-LA pretreated SFs displayed the greatest compositional modification when compared with that of untreated EFB (Table 4.2). The most significant compositional change lies in the hemicellulose content. Hemicellulose is usually more susceptible to modification under chemical and thermal treatment owing to their lower degree of polymerization as compared to cellulose (van Osch et al., 2017). After the pretreatment, CC-LA DES was able to remove all the hemicellulose in the EFB as indicated by the absence of hemicellulose detected in CC-LA SF. Also, 79% of the original hemicellulose content was removed from the EFB by GLUC-LA, leaving 21% of the hemicellulose remained in the GLUC-SF pretreated SF. The high hemicellulose removal rate of the acidic DES pretreatment displayed similar tendency as the conventional acid pretreatment in which the main reaction occured was hemicellulose hydrolysis, particularly xylan (Hendriks & Zeeman, 2009).

Other than depolymerization of hemicellulose, the two acidic DESs, CC-LA and GLUC-LA were also capable of performing substantial delignification on EFB. About 88% and 55% of lignin were removed from CC-LA and GLUC-LA SF, respectively. The high delignification efficiency of acidic DES could be attributed to its ability in cleaving the β-O-4 ether bond, the most abundant inter-unit linkage in lignin (Alvarez-Vasco et al., 2016). This is further verified through DES-extracted lignin (DEEL) characterization analysis using nuclear magnetic resonance (NMR) spectroscopy, which will be elaborated later in Section 4.3.3. The delignification performance difference between CC-LA and GLUC-LA could be due to the different hydrogen bond acceptor (HBA) in the DES. Hydrogen bonds are formed between chloride ion in DES and hydroxyl group in biomass, which could cleave the lignin-carbohydrate complex in biomass and result in the separation of lignin and hemicellulose (Y. Liu et al., 2017). Therefore, CC-LA which contained choline chloride (ChCl) managed to achieve higher lignin removal performance than GLUC-LA with glucose as HBA.

Cellulose i.e. glucan was largely remained in the SFs after DES pretreatment. As shown in Table 4.2, the acidic DES-pretreated SFs were enriched with cellulose content due to the dissolution of hemicellulose and lignin. From 32.1% of glucan in the raw EFB, the relative glucan content in CC-LA and GLUC-LA SFs increased to 71.4% and 46.3%, respectively. In this context, acidic DES is an efficient fractionation solvent to isolate cellulose for potential carbohydrate biopolymer conversion, particularly CC-LA SF which consisted predominantly of cellulose (71.4%), a minimal amount of lignin (4.7%) and total absence of hemicellulose.

### (b) ***Near-neutral DES pretreatment***

There was only a minor difference between the biopolymer composition of SF produced by CC-GLY and CC-GLUC in the near-neutral pH DES preteatments and that of raw EFB (Table 4.2). In addition to the high SF recovery at 96%, the relatively unchanged biopolymer composition of near-neutral DES pretreated SF indicated that their fractionation and delignification efficiency were not as prominent as in the case of acidic DES. The delignification percentage of CC-GLY and CC-GLUC were respectively at 22% and 17%. Other than EFB, CC-GLY at pH 6.69 was also not effective in dissolving other types of lignocellulosic biomass as evidenced in other studies using wood biomass

(Alvarez-Vasco et al., 2016; Y. Liu et al., 2017). However, the minor change of DES-pretreated SF composition does not necessarily imply that the DES is inefficient in altering the biomass structure. Studies have shown that CC-GLY was able to enhance enzymatic hydrolysis performance of biomass in producing high glucose yield (Procentese et al., 2017; C. W. Zhang et al., 2016). The structure of biomass matrix in the above study must have been altered by the DES pretreatment that enhances the hydrolysis performance. Therefore, it is essential to recognize the pretreatment purpose as to whether for biomass dissolution or hydrolysis enhancement in order to employ the suitable solvent.

### (c) ***Basic DES***

There was a notable discrepancy in the biopolymer fractionation efficiency of the two basic DESs CC-UREA[$K_2CO_3$]-GLY applied in EFB pretreatment, owing to their difference in ionic strength at pH 9.45 and at pH 12.83, respectively. Referring to Table 4.2, the compositional analysis correlates well with the solid recovery finding in which basic DES with more extreme pH condition i.e. [$K_2CO_3$]-GLY was more efficient in fractionating biopolymer than the milder DES CC-UREA. The delignification efficiency of [$K_2CO_3$]-GLY was 51%, which was higher than that of CC-UREA at 34%. With regards to hemicellulose depolymerisation, [$K_2CO_3$]-GLY also achieved 36% of removal rate, which was more significant that the 20% achieved by CC-UREA. Nevertheless, both basic DESs showed low cellulose removal rate at around 23% and 25% due to the recalcitrant structure of cellulose biopolymer. Comparing the compositional changes of glucan, lignin and xylan biopolymers, lignin removal effect was the most noticeable in basic DESs, which is similar to the trend of conventional alkaline pretreatment.

The relative xylan content in basic DES pretreated SFs remained the same as raw EFB at 14.4% (Table 4.2). The glucan content in CC-UREA pretreated SFs was also maintained at around 30% as that in raw EFB while in the case of [$K_2CO_3$]-GLY pretreatment, the glucan content increased slightly to 37.6%. Evidently the degree of fractionation efficency of basic DES was comparatively lesser than that of acidic DES pretreatment.

DES pretreatment performance trend in the present work on oil palm empty fruit bunch also coincides with that of other types of biomass reported in the literatures. Acidic DES was found to be able to extract substantial amount of hemicellulose and lignin from wood biomass while preserving the cellulose in DES-pretreated solid fraction (Alvarez-Vasco et al., 2016). Neutral pH DESs with different HBDs (ethylene glycol and xylitol) also displayed limited fractionation efficiency on rice straw biomass (X. D. Hou et al., 2018). In the same work, basic choline chloride:formamide DES facilitated higher lignin removal compared to hemicellulose and cellulose (X. D. Hou et al., 2018). These literature reported findings further ascertain that DES pretreatment outcome differs according to the nature of the solvent used.

From the analysis of SF recovery and biopolymer composition, it can be inferred that the effect of individual HBD or HBA in DES was not the sole determination factor of their dissolution capacity. The CC-LA, CC-GLY, CC-GLUC and CC-UREA DESs sharing the same HBA (choline chloride) exhibited significant difference in solid recovery percentage. The phenomenon has also been witnessed in the pretreatments of CC-GLY and [$K_2CO_3$]-GLY with the same HBD (glycerol). This suggests that both HBD and HBA contribute synergistically to biomass dissolution. Further elaboration on the solubilisation of each biopolymer in DES is presented in the subsequent mass distribution study (Section 4.1.2.3).

### 4.1.2.3 Mass distribution of glucan, xylan and lignin biopolymers

The investigation of mass distribution of biopolymers i.e. lignin, glucan and xylan in the DES-fractionated solid and liquid fractions after DES pretreatment will lead to in-depth understanding of the biopolymer fractionation performance of the DES with varying pH nature. The biopolymers mass distribution in solid fraction (SF), liquid fraction (LF) and DES-extracted lignin (DEEL) of the varying pH DES pretreatment are tabulated in Table 4.3. The biopolymers' mass recovery varied greatly between the different DES pretreatment systems. DES in ascending order of their total biopolymer mass recovery after pretreatment are CC-LA (75%), [$K_2CO_3$]-GLY (79%), GLUC-LA (82%), CC-UREA (98%), CC-GLY (99%) and CC-GLUC (101%). Three DES with more extreme pH condition i.e. acidic CC-LA (pH 0-1), GLUC-LA (pH 1-2) and basic [$K_2CO_3$]-GLY (pH 12.83), experienced greater mass loss (or lower mass recovery) compared to the other investigated DES with moderate pH value. The total carbohydrate recovery was more than 70% in all six DES pretreatments. However, mass recovery of lignin content had registered rather big discrepancy, with the lowest recovery at 59% in [$K_2CO_3$]-GLY pretreatment to the highest at 91% in CC-GLUC pretreatment.

### Table 4.3: Mass balance and recovery percentage of biopolymer after DES pretreatment

| | Mass (mg) | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | Carbohydrate[d] | | | | |
| | Ash | Lignin[b] | Glucan | Xylan | Reducing sugar by DNS | Other | Total mass recovery |
| **CC-LA** | | | | | | | |
| **Raw** | 28 | 87 | 157 | 70 | | 78 | 419 |
| **LF** | | 4 | | | 44 | | 48 |
| **SF** | 20 | 10 | 159 | 0 | | 33 | 222 |
| **DEEL[a]** | | 44 | | | | | 44 |
| **Recovery[c](%)** | | **67** | | **89** | | | **75** |
| **GLUC-LA** | | | | | | | |
| **Raw** | 28 | 87 | 157 | 70 | | 78 | 419 |
| **LF** | | 5 | | | 14 | | 19 |
| **SF** | 20 | 40 | 135 | 15 | | 83 | 291 |
| **DEEL[a]** | | 35 | | | | | 35 |
| **Recovery[c](%)** | | **90** | | **72** | | | **82** |
| **CC-GLY** | | | | | | | |
| **Raw** | 28 | 87 | 157 | 70 | | 78 | 419 |
| **LF** | | 2 | | | 11 | | 13 |
| **SF** | 20 | 68 | 124 | 59 | | 132 | 403 |
| **DEEL[a]** | | 0 | | | | | 0 |
| **Recovery[c](%)** | | **80** | | **86** | | | **99** |
| **CC-GLUC** | | | | | | | |
| **Raw** | 28 | 87 | 157 | 70 | | 78 | 419 |
| **LF** | | 7 | | | 13 | | 20 |
| **SF** | 21 | 73 | 120 | 57 | | 134 | 404 |
| **DEEL[a]** | | 0 | | | | | 0 |
| **Recovery[c](%)** | | **91** | | **84** | | | **101** |
| **CC-UREA** | | | | | | | |
| **Raw** | 28 | 87 | 157 | 70 | | 78 | 419 |
| **LF** | | 7 | | | 11 | | 18 |
| **SF** | 22 | 58 | 120 | 56 | | 136 | 392 |
| **DEEL[a]** | | 0 | | | | | 0 |
| **Recovery[c](%)** | | **74** | | **83** | | | **98** |
| **$[K_2CO_3]$-GLY** | | | | | | | |
| **Raw** | 28 | 87 | 157 | 70 | | 78 | 419 |
| **LF** | | 8 | | | 11 | | 20 |
| **SF** | 20 | 43 | 118 | 45 | | 87 | 313 |
| **DEEL[a]** | | 0 | | | | | 0 |
| **Recovery[c](%)** | | **59** | | **77** | | | **79** |

[a] DEEL is assumed to have no impurities

[b] Lignin is calculated as sum of acid insoluble lignin (AIL) and acid soluble lignin (ASL).

[c] Recovery (%) = (biopolymer $_{\text{LF}}$ + biopolymer $_{\text{SF}}$ + biopolymer $_{\text{DEEL}}$) / biopolymer $_{\text{Raw}}$ *100%. The standard deviation is ±1%.

[d] Percent recovery of carbohydrate (%) = [glucan (mg) + xylan (mg) + reducing sugar detected by DNS (mg)] / carbohydrate $_{\text{Raw}}$ (mg) *100%

### (a) ***Acidic DES pretreatment***

In CC-LA pretreatment, lignin content accounted for a major portion of the total mass loss. From 87 mg of total lignin in raw EFB, 67% (58 mg) total recovery was achieved, which includes 10 mg recovery in SF (11%), 44 mg in DEEL (51%) and 4 mg in LF (5%) (Table 4.3). Under acidic condition, lignin will undergo fragmentation reaction and cleavage of β-O-4 linkage (L. Zhang et al., 2015). The lignin biopolymer might have depolymerized into smaller monomeric compounds and was not detectable as dissolved lignin in the liquid fraction, resulting in high lignin mass loss in the CC-LA pretreatment system. For carbohydrate recovery, the recovered carbohydrate in CC-LA LF might consist entirely of hemicellulose, which amounted to 63% hemicellulose recovery as total recovery of cellulose fraction in SF was achieved. From 157±1 mg of glucan in raw EFB, around 159±1 mg of glucan was recovered in CC-LA SF after subjected to DES pretreatment.

In GLUC-LA pretreatment, a significantly higher lignin recovery percentage was obtained at 90%, as compared to CC-LA pretreatment with 67% lignin recovery. This could be attributed to the less acidic condition of GLUC-LA, exerting a lower degree of lignin depolymerisation and therefore giving a less efficient delignification performance (Table 4.2). Hence, 40 mg out of the 87 mg of lignin in raw EFB was remained in the GLUC-LA pretreated EFB (Table 4.3). Nevertheless, the carbohydrate recovery in GLUC-LA pretreatment was lower compared to CC-LA pretreatment. Unlike CC-LA pretreatment which obtained almost 100% of glucan recovery in SF, a small portion of glucan was removed from GLUC-LA SF. Therefore, from the mass distribution study, it is evident that CC-LA can selectively dissolve the different biopolymers, thereby constituting to high biomass fractionation efficiency.

### (b) ***Near-neutral DES pretreatment***

Comparing between the CC-GLY and CC-GLUC pretreatments, their biopolymer components distribution trend are highly similar (Table 4.3). There is only ±5 mg difference in the lignin, glucan and xylan biopolymer recovery in both SF and LF between the two pretreatment systems. The biopolymers were largely remained in the SF, signifying that DES pretreatment had minimal impact on the fractionation of biopolymer. Hence, the total mass recovery of both pretreatments was close to 100% as a result of poor DES dissolution performance. The biopolymer solubilisation capability of DES is dependent on the formation of hydrogen bonding network between DES and solutes (Dai et al., 2013b). The large number of hydroxyl groups on HBD (polyol and sugar) of near-neutral pH DES could form an extensive hydrogen bonding network within the solvent, which resulted in minimal interaction and dissolution of the solutes (Y. Wang et al., 2016). Therefore, the mass distribution study has further verified that near-neutral DESs are unsuitable for biomass fractionation and delignification application.

### (c) ***Basic DES pretreatment***

In respect with basic DES, the mass distribution of CC-UREA pretreatment appeared to be more similar to near-neutral pH DES pretreatments than the high alkalinity [$K_2CO_3$]-GLY pretreatment (Table 4.3). This is probably due to CC-UREA is less basic than [$K_2CO_3$]-GLY. In both the basic DES pretreatments, the major mass loss was resided in lignin biopolymer. The unrecoverable lignin accounted for 36 mg (41%) of the total lignin content in [$K_2CO_3$]-GLY pretreatment and 21 mg (26%) in CC-UREA pretreatment. The phenolic groups in lignin are solubilized under alkaline pretreatment condition (Zhu & Theliander, 2015), which might result in the higher loss of lignin in the more basic [$K_2CO_3$]-GLY pretreatment system.

The result indicates that DES at harsher pH were able to dissolve biopolymer better than DES with near-neutral pH (Table 4.3). Cellulose is most resistant during DES pretreatment process and the dissolution of lignin and hemicellulose is dependent on the types of DES. Thus, high biopolymer fractionation efficiency is achievable through DES pretreatment with suitable type of DES. The study also shows that acidic DES pretreatment can dissolve hemicellulose effectively as in the conventional acid pretreatment. Likewise; basic DES pretreatment resembles the conventional alkaline pretreatment with the major biopolymer dissolution occurs in lignin fraction (Singh et al., 2015). In view of the similarities, DES has the potential of replacing conventional solvents in biomass processing.

In the mass distribution study, one interesting observation was that despite [$K_2CO_3$]-GLY having very high viscosity, lignin dissolution by the solvent was rather high, which can be attributed to the strong ionic strength of [$K_2CO_3$]-GLY. Conventionally, low solvent's viscosity promotes mass transfer operation of solute into the solvent. However, the mass distribution study shows that low solvent's viscosity might not necessarily be proportional to high biomass dissolution during DES pretreatment. Despite of its low viscosity at $0.258\times10^3$ mPa.s, CC-GLY did not show significant dissolution capacity whereas the most viscous DES [$K_2CO_3$]-GLY at $0.709\times10^4$ mPa.s displayed appreciable extent of dissolution. This suggests that the type of DES adopted in the biomass pretreatment exerts higher influence over viscosity in contributing to the dissolution performance.

Out of the six DES pretreatments investigated in the study, the mass distribution effect of CC-LA was especially noticeable. In the CC-LA pretreatment, a hemicellulose-rich liquid fraction, relatively pure cellulosic solid fraction and DES-extracted solid lignin were produced. This implies that DES with naturally-existing components is also capable fractionating the biomass. These DES-fractionated products can subsequently be processed into fuels or chemicals. The value of each biopolymer can thus be fully valorised by customizing the conversion process condition according to the nature of the biopolymers.

### 4.1.3 Recovery of DES-Extracted Lignin (DEEL)

DES was introduced into the biomass processing field as a green solvent with selective lignin solubilisation ability (Francisco et al., 2012). The solvent can potentially be adopted in biomass pretreatment process that focuses on lignin isolation prior to carbohydrate processing. Through this pretreatment scheme, lignin can be produced as a targeted product instead of by-product like in the pulping process. This could improve the extracted lignin's quality to enhance its potential as an aromatic feedstock for higher-value product conversion.

The amount of DES-extracted lignin (DEEL) from each DES pretreatment is as shown in Table 4.3. Upon addition of water anti-solvent, lignin could only be precipitated and recovered in acidic DES pretreatment. No lignin precipitate was recovered in near-neutral and basic DES pretreatments. It has been observed that 50.6 wt% and 40.2 wt% of the total lignin content in EFB were recovered as DEEL after subjected to CC-LA and GLUC-LA pretreatments, respectively. Despite that the basic DESs CC-UREA and [$K_2CO_3$]-GLY were able to remove significant amount of lignin from SF at 34% and 51%, respectively (Table 4.2), there was no lignin precipitate upon the addition of anti-solvent as opposed to what was observed in the acidic DES pretreatment.

In the case of acid pretreatment, the addition of anti-solvent to the pretreatment liquid fraction enabled the condensation and precipitation of lignin that was solubilized during the pretreatment (Hendriks & Zeeman, 2009). On the other hand, under alkaline pretreatment condition, the phenolic groups in lignin were ionized which has resulted in lignin remained solubilized in the solution (Zhu & Theliander, 2015). This has prevented water from precipitating the dissolved lignin from the basic DES pretreatment mixture.

From this comparison study between DES with varying pH nature, acidic DES has been identified as the suitable DES type for lignin extraction. After acidic DES pretreatment, lignin could be extracted and recovered through a simple water precipitation method. Nevertheless, the DEEL yield obtained by using CC-LA and GLUC-LA differs from one another at 50.6 wt% and 40.2 wt%, respectively. It is possible that different hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) combination in DES has constituted to varying strength in lignin extraction. Therefore, the search of the suitable acid HBD was extended to the next phase of study as elaborated in Section 4.2.

### 4.1.4 Energy Requirement Study

DES pretreatment is a relatively new biomass processing method and hence investigation on its energy requirement level is important to assess the solvent's viability for biomass pretreatment. To determine the energy consumption of DES pretreatment on EFB, the heat capacity value, $C_p$ of each DES adopted was measured and tabulated in Table 4.4. Among the DES studied, CC-GLUC which has the highest molar mass (159.89 g/mol), has the highest $C_p$ value between 251.17 – 282.70 J/mol.°C at temperature between 30 to 80°C. On the contrary, CC-UREA has the lowest molar mass (86.58 g/mol) and $C_p$ value at 196.54 – 212.99 J/mol.°C within the same temperature range. This shows that $C_p$ value of DES is proportionally related to their molar mass value, which was also observed in the other study (Leron & Li, 2012).

**Table 4.4: Molar mass and molar heat capacities, $C_p$ of the investigated DES**

| | CC-LA | GLUC-LA | CC-GLY | CC-GLUC | CC-UREA | [$K_2CO_3$]-GLY |
|---|---|---|---|---|---|---|
| **Molar mass*** (g/mol) | 98.34 | 105.09 | 107.94 | 159.89 | 86.58 | 98.68 |
| **T (°C)** | **Molar heat capacity (J/mol. °C)** | | | | | |
| 30 | 234.04 | 251.17 | 225.59 | 346.96 | 196.54 | 223.02 |
| 40 | 239.94 | 256.43 | 236.38 | 354.95 | 199.14 | 226.97 |
| 50 | 245.84 | 262.73 | 239.62 | 362.95 | 202.60 | 231.90 |
| 60 | 251.74 | 269.04 | 239.62 | 374.14 | 206.06 | 238.81 |
| 70 | 257.64 | 276.39 | 245.02 | 385.33 | 209.53 | 244.73 |
| 80 | 261.58 | 282.70 | 250.41 | 393.33 | 212.99 | 248.68 |

*molar mass = $x_{HBD}$ $M_{HBD}$ + $x_{HBA}$ $M_{HBA}$, where x = mole ratio; M = molecular weight

However, this inferred observation is not entirely applicable to all DESs in this study. The $C_p$ value of CC-LA, GLUC-LA, CC-GLY and [$K_2CO_3$]-GLY do not correlate to their molar masses. Nevertheless, the molar masses of these four DESs are highly similar, ranging from 98.34 – 107.94 g/mol. Therefore, the influence of DES constituents might have overwhelmed the effect of molar mass in the heat storage capacity of the solvents.

Table 4.4 also shows that the $C_p$ of these six DESs tabulated increases with elevating temperature. To develop a heat capacity model as a function of temperature, the experimental measured $C_p$ data was fitted into polynomial equations of different orders. Table 4.5 tabulates the absolute average deviation (AAD) of three $C_p$ models i.e. linear, second-order and third-order polynomial model which indicates the deviation of experimental heat capacity data with the developed model. All three models provide good correlation to the experimental $C_p$ data with low AAD at 0.07 – 1.73%. Among the three models, second-order polynomial equation is best representing the $C_p$ correlation for all DESs based on the lowest AAD value, as shown in Table 4.5. Therefore, the $C_p$ values at initial and operating temperature of the pretreatment were determined using Equation 4.1 based on second-order polynomial equation for energy requirement study.

$$C_P\ (\mathrm{J mol^{-1} {}^\circ C^{-1}}) = a_0 + a_1T + a_2T^2 \tag{4.1}$$

**Table 4.5: Absolute average deviation (AAD) of $C_p$ models, regression coefficient of selected 2nd order $C_p$ model and the calculated energy consumed, Q**

| **DES** | **AAD (%)** | | | **Regression Coefficient of 2nd order polynomial equation** | | | **Q ($J/g_{biomass}$)** |
|---|---|---|---|---|---|---|---|
| | 1st order | 2nd order | 3rd order | $a_o$ | $a_1 \times 10^2$ | $a_2 \times 10^2$ | |
| CC-LA | 0.19 | 0.11 | 0.14 | 212.76 | 75.51 | -0.18 | 2409 |
| GLUC-LA | 0.18 | 0.09 | 0.18 | 235.85 | 45.38 | 0.17 | 2451 |
| CC-GLY | 0.84 | 0.84 | 0.87 | 206.91 | 78.91 | -0.33 | 2087 |
| CC-GLUC | 0.24 | 0.21 | 1.62 | 323.34 | 70.35 | 0.23 | 2242 |
| CC-UREA | 0.10 | 0.07 | 0.04 | 188.22 | 24.89 | 0.08 | 2248 |
| [$K_2CO_3$]-GLY | 0.31 | 0.31 | 1.73 | 207.99 | 46.10 | 0.07 | 2297 |

By using second-order polynomial model, energy consumption of each DES pretreatment was evaluated and presented in Table 4.5. The highest amount of energy consumption per unit biomass was by GLUC-LA at 2451 $J/g_{biomass}$ and the lowest was by CC-GLY at 2087 $J/g_{biomass}$. The energy required by acidic and basic DES are generally higher than DES with near-neutral pH. DES CC-LA that yielded the highest fractionation and delignification efficiency consumed 2409 J per unit biomass, the second highest among the six DES pretreatments. Nonetheless, the six investigated DESs have similar energy requirement between 2.1 to 2.5 kJ/g in EFB pretreatments. Conventional alkaline pretreatment using 2M and 0.5M of NaOH solution were reported to have energy consumption level at 7 and 3 kJ/g, respectively (Procentese et al., 2017). A dilute acid pretreatment using sulphuric acid developed by US National Renewable Energy Laboratory required 1.692 kJ/g energy input for the process (Mafe, Davies, Hancock, & Du, 2015). Based on the findings, the energy consumption level of DES pretreatment is fallen in the middle range as compared with the conventional acid and alkaline pretreatments. In view of that, the energy consumption of DES pretreatment was not considered as a crucial factor in the DES selection process.

### 4.1.5 Summary

The collective results obtained from solid recovery, biopolymer composition and mass distribution studies confirm that pH of DES plays an important role in determining the biopolymer fractionation trend. The trend demonstrates some resemblance to that of acid and alkaline conventional solvent pretreatment. Among the acidic, near-neutral and basic DES, acidic DES displayed the most promising performance in both biomass fractionation and delignification. CC-LA DES achieved 88% of delignification efficiency and 100% of hemicellulose removal, while retaining nearly 100% of the cellulose content in the pretreated EFB solid.

On the other hand, high viscosity of DES does not necessarily lead to low fractionation and delignification performance and vice versa. For instance, [$K_2CO_3$]-GLY DES with the highest viscosity was able to achieve 51% of delignification while CC-GLY with the second lowest viscosity only managed to reach 22% of delignification from EFB. Selection of less viscous DES is indeed important for easy handling and energy saving in terms of stirring operation in large scale reactor. Yet, based on this study, the nature of DES prevails over its viscosity in determining the solvent's performance in biomass pretreatment. Other than viscosity, energy consumption was also not considered as a major factor in DES selection as the DES pretreatments in this study display minimal difference in their energy requirement level.

Organic acids are not usually adopted in biomass pretreatment due to their ineffectiveness in swelling the polysaccharides (X. Zhao et al., 2009). Nevertheless, DES incorporating organic acid i.e. lactic acid in the current study is proven to have great potential in biomass processing, with its ability to extract lignin in pellet form, to produce hemicellulose-rich liquid and relatively pure cellulosic fraction through a simple single stage process.

## 4.2 Selection of Carboxylic Acid Hydrogen Bond Donor with Varying Functional Groups in Acidic DES for EFB Fractionation and Lignin Extraction

The effect of pH nature of DES on EFB pretreatment presented in Section 4.1 demonstrates that acidic DES has greater biopolymer fractionation and delignification capability than the near-neutral and basic DESs. In addition, the lignin dissolved during acidic DES pretreatment could be recovered by using simple water precipitation method. The two acidic DESs investigated exhibited varying strength in lignin extraction efficiency. Hence, in the subsequent phase of study, the performance of acidic DESs synthesized using varying type of carboxylic acids as hydrogen bond donor (HBD) were compared to determine the most suitable acid HBD for lignin extraction and EFB fractionation. In this study, choline chloride (ChCl) was used as the common hydrogen bond acceptor (HBA) for the synthesis of all acidic DESs.

The evaluation of acidic DES performance on EFB fractionation and lignin extraction were mainly based on the yield and properties analysis of the DES-fractionated products, such as acidic DES-pretreated solid fraction (SF) and DES-extracted lignin (DEEL). The biopolymer fractionation performance of the acidic DES was evaluated through the solid recovery percentage, biopolymer composition and functional group distribution analysis of SF. The production yield of DEEL and their functional group distribution analysis were performed to assess the DES's lignin extraction performance. The effect of HBA:HBD ratio of DES as well as functional groups in the carboxylic acid HBD of DES was also revealed through the properties analysis of these DES-fractionated products.

### 4.2.1 Synthesis of DES with Various Carboxylic Acid Hydrogen Bond Donor

Choline chloride:lactic acid (CC-LA) yielded the best biomass fractionation and delignification efficiency, as demonstrated previously in Section 4.1. Pairing with the similar hydrogen bond acceptor (HBA) i.e. choline chloride (ChCl), acidic DESs were synthesized using carboxylic acids with different functional groups as hydrogen bond donors (HBD). The types of carboxylic acid used include alpha-hydroxy, linear saturated and unsaturated carboxylic acids with monocarboxylic, dicarboxylic and tricarboxylic (COOH) groups structure (Table 3.3). It was found that the formation of DES occurred at different HBA to HBD ratio, depending on the types of DES constituents used. Thus, the synthesis of acidic DESs were performed at varying HBA to HBD ratio (HBA:HBD), such as 2:1, 1:1, 1:2 and 1:5, and the findings are listed in Table 4.6.

Different DESs were present as homogeneous solvent under various HBA to HBD molar ratio due to the influence of varying functional groups in the acid. Generally, DESs with alpha-hydroxy acids (AHA) as HBDs which include lactic acid, malic acid and citric acid were formed at ratio 1:1 while the linear saturated acids namely formic acid, acetic acid, propionic acid and butyric acid formed DESs at ratio 1:2. The presence of hydroxyl group (-OH) in AHA might be responsible for its ability to form DES with ChCl at ratio 1:1 as opposed to linear saturated acids. OH group in AHA could construct extra hydrogen bond with HBA to stabilize the solvent, which would lead to the formation of DES even when the acid HBD content is in proportional to ChCl at ratio 1:1. Unsaturated maleic acid which contains double bond could also form DES at ratio 1:1. The presence of double bond enabled the acid HBD to undergo substitution reaction and formed extra bond with the HBA. Contrarily, owing to the saturated structure, linear saturated acid-based DES required higher relative amount of HBD to form a stable DES network with the HBA. Thus, the linear saturated-based DES can only be formed at the HBA:HBD ratio of 1:2.

**Table 4.6: Synthesis of acidic DES with varying types of carboxylic acid as hydrogen bond donor and different molar ratios**

| **HBD** | | | **DES** | **DES Acronym** | **Molar ratio (HBA:HBD)*** | | | |
|---|---|---|---|---|---|---|---|---|
| Category | Name | Molecular Formula | | | 2:1 | 1:1 | 1:2 | 1:5 |
| Alpha-hydroxy (AHA) | Lactic acid | $C_3H_6O_3$ | Choline chloride:lactic acid | CC-LA | x | ✓ | ✓ | ✓ |
| | Malic acid | $C_4H_6O_5$ | Choline chloride:malic acid | CC-MA | ✓ | ✓ | ✓ | x |
| | Citric acid | $C_6H_8O_7$ | Choline chloride:citric acid | CC-CA | ✓ | ✓ | x | - |
| Linear Saturated | Formic acid | $CH_2O_2$ | Choline chloride:formic acid | CC-FA | x | x | ✓ | ✓ |
| | Acetic acid | $C_2H_4O_2$ | Choline chloride:acetic acid | CC-AA | x | x | ✓ | ✓ |
| | Propionic acid | $C_3H_6O_2$ | Choline chloride:propionic acid | CC-PA | x | x | ✓ | - |
| | Butyric acid | $C_4H_8O_2$ | Choline chloride:butyric acid | CC-BA | x | x | ✓ | - |
| | Succinic acid | $C_4H_6O_4$ | Choline chloride:succinic acid | CC-SA | ✓ | x | x | - |
| Unsaturated | Maleic acid | $C_4H_4O_4$ | Choline chloride:maleic acid | CC-MAE | - | ✓ | - | - |

* ✓ – homogeneous DES at room temperature; x – non-homogeneous mixture.

On the other hand, only multiple carboxyl group (COOH) containing acids can form DES when the relatively higher HBA composition was utilized in the DES synthesis. Monocarboxylic acid namely lactic, formic, acetic, propionic and butyric acid were all failed to form homogeneous DES with ChCl on 2:1 ratio. On the contrary, dicarboxylic acids (i.e. malic acid and succinic acid) and tricarboxylic acid (i.e. citric acid) managed to form DES when HBA to HBD ratio of 2:1 was used. This is probably due to the extra COOH groups in dicarboxylic and tricarboxylic acids that are available for hydrogen bond formation with the excess HBA at higher HBA constituent ratio.

When comparing the DES with similar carbon chain length, despite that malic, butyric, succinic and maleic acids have the similar $C_4$ structure, their respective DESs were formed at different molar ratios (Table 4.6). The same observation is also applied to lactic and propionic acids with $C_3$ structure. This further confirms that the functional groups of HBD in DES play a major role in DES formation.

To ensure consistent comparison basis, DESs with the same acid HBD category as stated in Table 4.6 were utilized in similar molar ratio for EFB pretreatment. The molar ratio of 1:1 was adopted for AHA-based DESs namely choline chloride:lactic acid (CC-LA), choline chloride:malic acid (CC-MA) and choline chloride:citric acid (CC-CA). Whereas the linear saturated monocarboxylic acid-based DESs such as choline chloride:formic acid (CC-FA), choline chloride:acetic acid (CC-AA), choline chloride:propionic acid (CC-PA) and choline chloride:butyric acid (CC-BA) were utilized at the ratio of 1:2 and dicarboxylic acid-based DES choline chloride:succinic acid (CC-SA) at ratio 2:1. The unsaturated choline chloride:maleic acid (CC-MAE) was utilized at 1:1 ratio.

### 4.2.2 Analysis of Acidic DES-Pretreated EFB Solid Fraction (SF)

The varying types of acidic DES synthesized as described in Section 4.2.1 were applied to EFB pretreatment for biopolymer fractionation and lignin extraction. Similar to the pH nature screening study in Section 4.1, the properties of DES-fractionated EFB solid fraction (SF) produced from acidic DES pretreatment were elucidated to examine the fractionation efficiency of the different types of acidic DESs. The evaluation aspects were SF recovery percentage, biopolymer composition and functional group distribution. Through these properties analysis of SF, the effect of the carboxylic acid HBD's different functional groups on the DES fractionation performance trend was also discussed. The functionalities that could enhance DES performance can therefore be determined. Subsequently, the functional group distribution analysis using FTIR which revealed the crystal structure of SF has provided a preliminary understanding on the suitability of SF as a carbohydrate feedstock.

#### 4.2.2.1 Solid recovery and biopolymer composition of acidic DES-pretreated SF

The solid recovery percentage of SF and the DES-extracted lignin (DEEL) yield obtained from the acidic DES pretreatments are as shown in Figure 4.2. There is an evident trend in the solid recovery of each acidic DES category. In the alpha hydroxy (AHA)-based DES group, the SF recovery increased with the increase in COOH group number in the acid HBD. The solid recovery of CC-LA, CC-MA and CC-CA with one, two and three COOH groups were respectively at 60.2%, 62.5% and 72.6%. Meanwhile, the linear saturated acid group investigated comprised of monocarboxylic acids with different aliphatic chain length i.e. CC-FA, CC-AA, CC-PA and CC-BA and dicarboxylic acid CC-SA (Table 3.3). Figure 4.2 showed an increased trend in solid recovery (SF) from 50.8% to 75.9% when going across the linear-saturated acid-based DES category from CC-FA, CC-AA, CC-PA, CC-BA to CC-SA. This shows that with the increase in number of COOH group and aliphatic carbon chain length, the fractionation efficiency of acidic DES was weakened.

The solid recovery trend corresponds well to the yield of DEEL obtained. Generally, the higher the lignin yield, the lower the SF recovery (Figure 4.2). As explained previously in Section 4.1.2.1, low solid recovery percentage of SF usually indicates that the DES is efficient in biomass fractionation, which would lead to high solid lignin yield. Hence, CC-SA which achieved the highest solid recovery at 75.9% had extracted the lowest lignin yield at a mere 10.7%. Contrarily, CC-FA with the second lowest solid recovery at 50.8% managed to obtain the highest lignin yield at 61.9%. Nevertheless, CC-MAE pretreatment that achieved a low DEEL yield at 20.7% recovered the least SF, which might be due to its lowest $pKa_1$ among all the acid HBDs. Therefore, it might have the ability to break down the higher percentage of carbohydrate biopolymer than the other DESs.

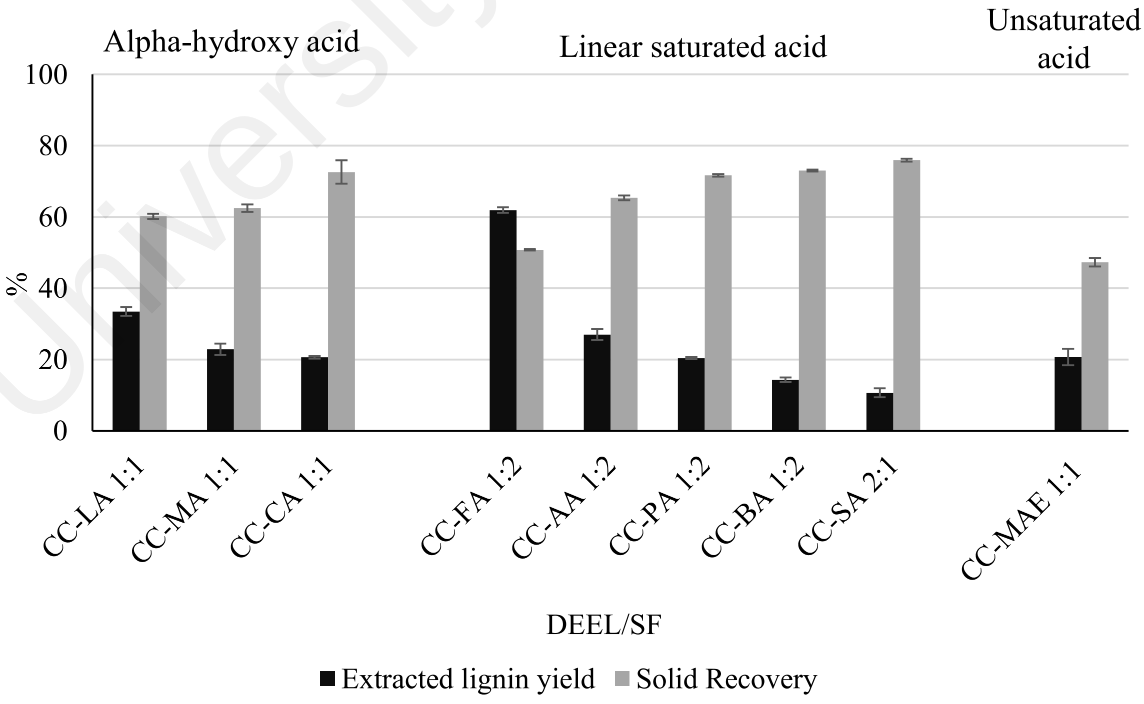


**Figure 4.2: Solid recovery percentage of SF and DEEL yield from acidic DES pretreatment**

Subsequently, biopolymer composition of the acidic DES-pretreated SFs were analysed to further examine the fractionation efficiency of DES and the results are presented in Figure 4.3. Expectedly, extensive xylan removal was observed in all acidic DES pretreatments. Under acidic environment, hemicellulose is most susceptible to hydrolysis among all three lignocellulosic biopolymers (Chaturvedi & Verma, 2013; Singh et al., 2015).

In the AHA-based DES pretreatments, complete xylan removal from the SF was achieved. However, some difference was noticed in the DES delignification performance within the AHA-based DES category. Decrease in COOH groups from CC-CA to CC-LA enhanced the DES's delignification efficiency. Whereas in linear saturated acid-based DESs, lignin and xylan removal efficiency decreased with increasing aliphatic chain length from CC-FA to CC-BA. As a result of extensive lignin and xylan removal, SF which produced from DES containing less COOH group, shorter aliphatic chain or unsaturated structure, such as CC-LA, CC-FA and CC-MAE were enriched with glucan content as shown in Figure 4.3. Among the nine acidic DES pretreatments, CC-SA which contained dicarboxylic and linear saturated $C_4$ structure, displayed the weakest fractionation performance, with its SF having the closest composition to the raw EFB. Further interpretation to the effect of acid functional group on DES performance is elaborated in the Section 4.2.3.1.

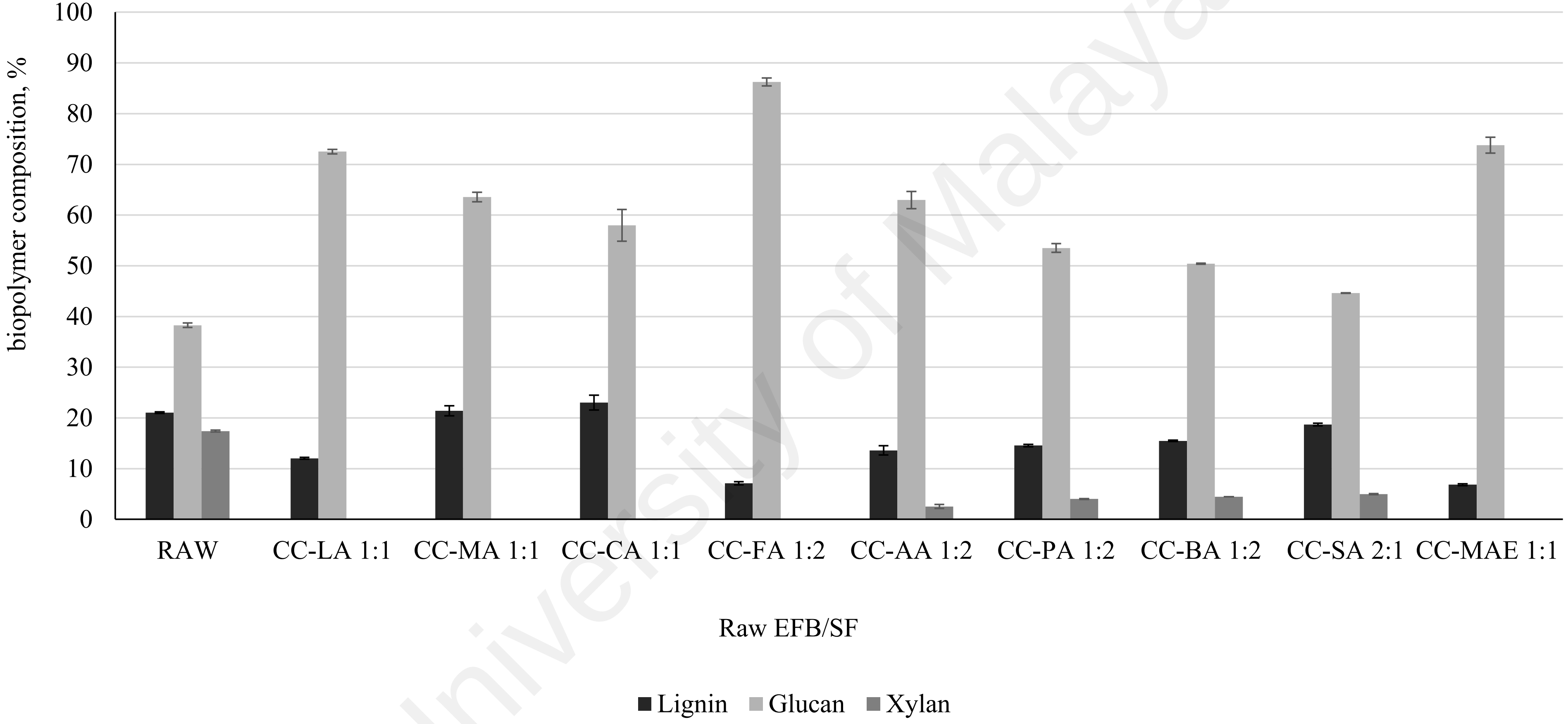


**Figure 4.3: Biopolymer composition in acidic DES-pretreated SF**

### 4.2.2.2 Functional groups distribution in SF

The functional groups in SF was investigated to reveal the effect of varying types of acidic DES on the pretreated EFB structure in this study. Through FTIR analysis, the peaks that appear at a particular frequency in the spectrum can pinpoint the presence of specific functional groups in the SF. The data was then used to calculate the crystallinity index of the different SFs to determine their potential for carbohydrate processing.

The FTIR spectra of the raw EFB and the acidic DES-pretreated SF are illustrated in Figure 4.4 and the peaks assignment are tabulated in Table 4.7. The peaks are assigned based on Lynam, Kumar, and Wong (2017), Hurtubise and Krassig (1960) and Ditzel, Prestes, Carvalho, Demiate, and Pinheiro (2017). The FTIR spectra of selective SFs including CC-LA, CC-MA, CC-CA, CC-FA, CC-AA, CC-SA, and CC-MAE were compared with that of raw EFB.

**Table 4.7: FTIR peaks determination of SFs**

| Absorption frequency/peaks ($cm^{-1}$) | Functional groups | Assignment |
|---|---|---|
| 3330-3310 | O-H | Intramolecular hydrogen bonding in cellulose 1 |
| 2900 | C-H | Cellulose |
| 1640 | O-H | Adsorbed water by cellulose |
| 1430 | C-H | $CH_2$ symmetric bending in cellulose |
| 1372 | C-H | C-H deformation |
| 1320 | C-H | C-H deformation or $CH_2$ wagging |
| 1161 | C-C | Amorphous stretching of cellulose type 1 and 2 |
| 1109 | C-O-C | Glycosidic ether bond |
| 1030 | C-O-C | Pyranose ring |
| 896 | C-C | Cellulosic B-glycosidic linkage |

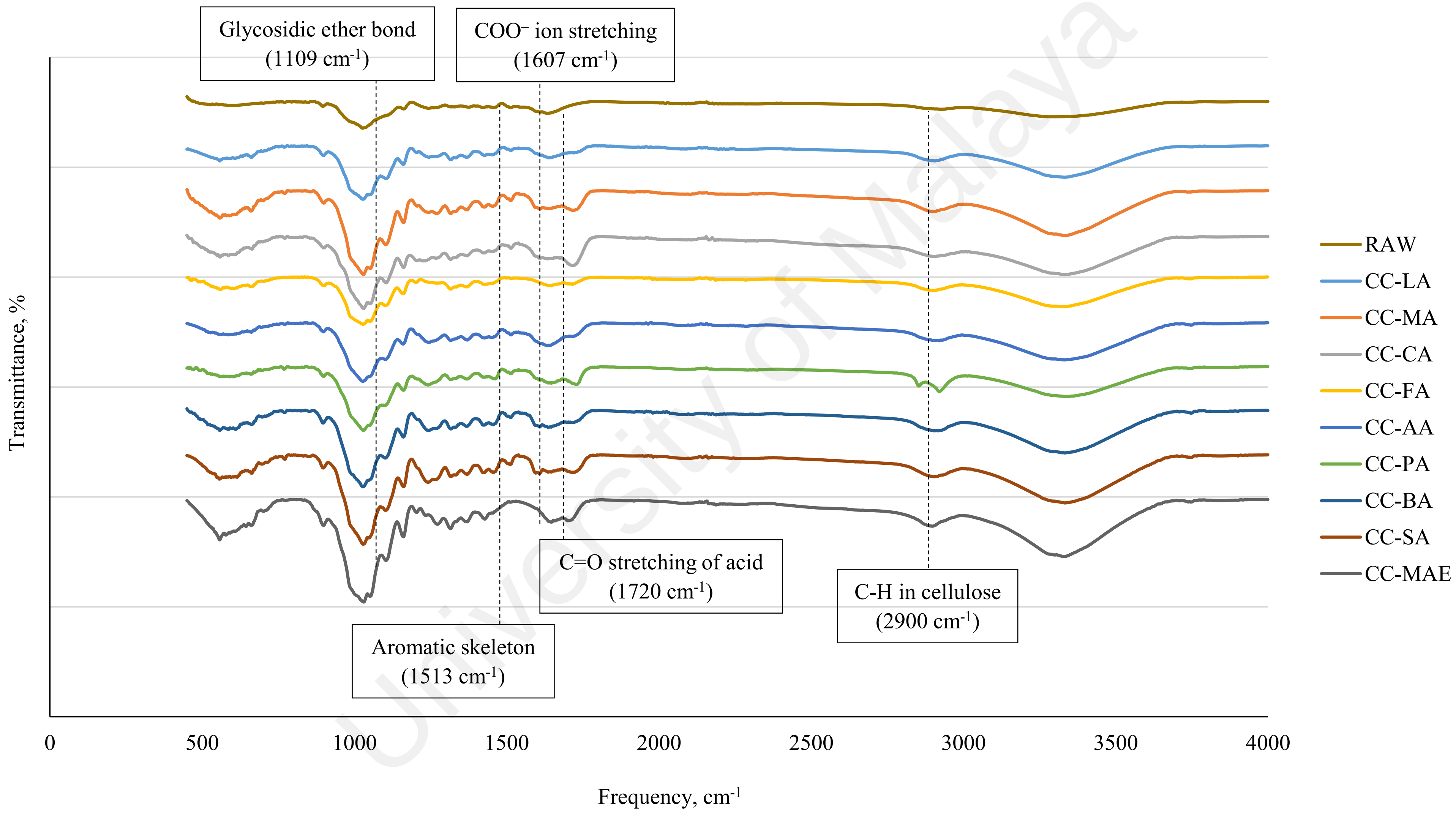


**Figure 4.4: FTIR spectra of SF from acidic DES pretreatment**

Generally, signals near the frequency band of 1320 cm$^{-1}$ and 1372 cm$^{-1}$ that accounts for C-H deformation were enhanced in all SF spectra when compared with that of raw EFB spectra. These band regions are the manifestation of crystallinity degree of cellulose (Nelson & O'Connor, 1964). Moreover, a signal related to the glycosidic ether bond appeared in all SF spectra at 1109 cm$^{-1}$. The signal occurrence might be due to the removal of amorphous hemicellulose and lignin biopolymer after DES pretreatment. Considering that these peaks are more distinct in the acidic DES-pretreated SF, it can be deduced that the cellulose content are richer in the SFs than in the raw EFB.

Owing to the extensive delignification occurred during the DES pretreatment, the peaks assigned to lignin's aromatic skeleton at 1513 cm$^{-1}$ and C-O linkage between lignin and hemicellulose at 1250 cm$^{-1}$ were diminished or disappeared in the SFs of CC-LA, CC-FA, CC-AA and CC-MAE pretreatments. However, the lignin peaks remained in the SF spectra of CC-MA, CC-CA and CC-SA pretreatments, which are corresponded well to the higher lignin content in these SFs as a result of weak delignification performance as shown in Figure 4.3.

All SF spectra, except for CC-LA and CC-AA, displayed a new peak around 1720 cm$^{-1}$ which is attributed to the C=O stretching of acid. In addition, the appearance of another peak at 1607 cm$^{-1}$ in CC-MA, CC-CA, CC-BA and CC-SA spectra manifested the presence of $COO^-$ ion stretching. The affinity of these acidic DES solvents to the SFs which resulted in the binding of solvents to the pretreated biomass solid even after washing procedure might be responsible for the emerged peaks. Remnants of pretreatment solvent in the extracted lignin are undesirable as they could possibly hinder the efficiency of downstream processing.

Figure 4.3 exhibits that the biopolymer composition of the glucan-rich SF was modified significantly after DES pretreatment. Thus, further assessment on the crystallinity alteration of the SF was performed using two infrared ratio indexes calculated from the absorbance of peaks attributed to glucan in FTIR spectrum, namely crystallinity ratio (CR) and lateral order index (LOI). Both CR and LOI of the investigated SFs are depicted in Figure 4.5.

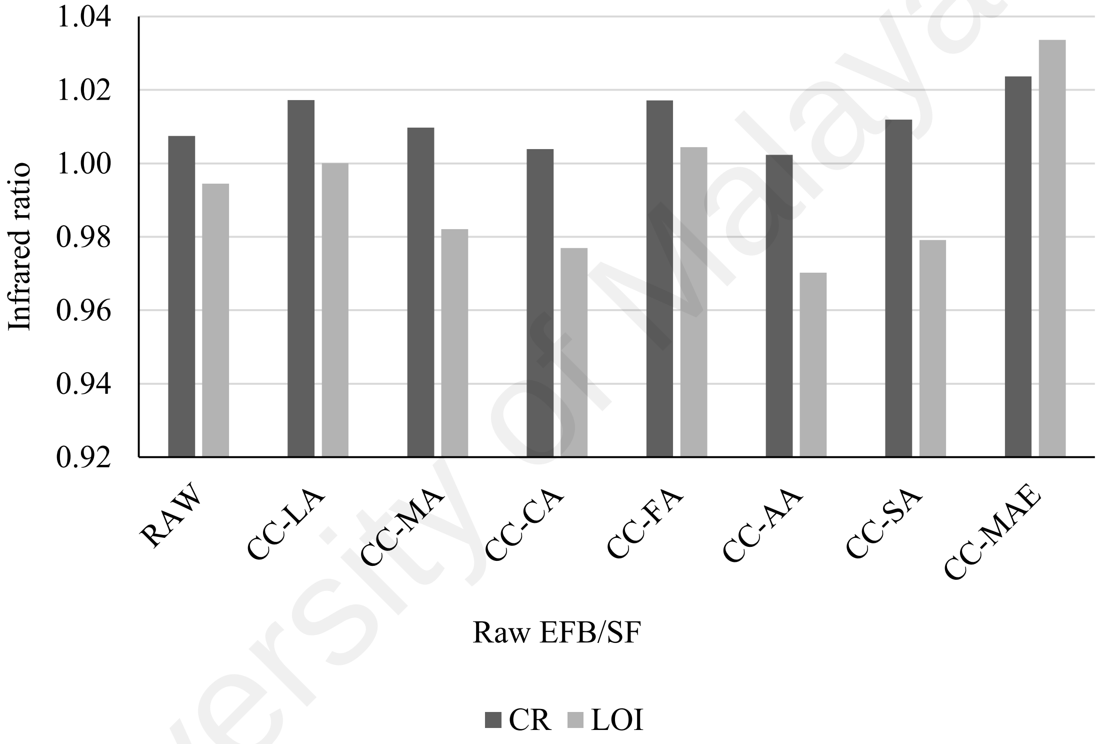


**Figure 4.5: Infrared ratio of raw and pretreated EFB SF**

High infrared ratio implies the presence of high quantity of crystalline structure in the biomass solid (Hurtubise & Krassig, 1960). Figure 4.5 shows that the increment of the both CR and LOI indexes in CC-LA, CC-FA and CC-MAE SFs are most noticeable and higher than that of raw EFB, which signifies enhanced relative glucan content in the SFs after DES pretreatment. This finding corresponds well to the biopolymer composition result given in Figure 4.3 in which the glucan content of the three mentioned SFs was highest among all the SFs. Therefore, these indexes can reflect the relative glucan content in the pretreated biomass.

It was portrayed through Figure 4.3 that lignin removal and hemicellulose hydrolysis performance of the acidic DES pretreatments were highly extensive. Removal of these amorphous biopolymers has contributed to the enrichment of the highly crystalline cellulose content (Pan et al., 2017), which is reflected through the increased infrared ratio. This further portrays the ability of DES pretreatment to retain glucose in the solid biomass and separate it from lignin and hemicellulose. Hence, the DES-pretreated SF is potentially a good candidate for carbohydrate processing, for example as a substrate for bioethanol production.

### 4.2.3 Analysis of DES-extracted lignin (DEEL)

From the screening study on pH nature of DES presented in Section 4.1, acidic DES was found to have excellent efficiency in delignification. The dissolved lignin during acidic DES pretreatment was easily recovered through water precipitation process. Hence, the potential of acidic DES for lignin extraction application was further assessed using various types of acidic DES. To identify the suitable acid HBD for optimum lignin extraction, the analyses performed were DES-extracted lignin (DEEL) yield and the functional group distribution in DEEL as observed in FTIR. The effect of assorted functional groups in the HBD on the DES performance trend was scrutinized. Through the selection process, acidic DES that has the highest lignin extraction yield performance was determined. The basic lignin's structure as an aromatic biopolymer was also examined and compared between the different DEELs through the functional group analysis.

#### 4.2.3.1 DEEL yield

DES-extracted lignin (DEEL) yield obtained from the assorted acidic DES pretreatments are depicted in Figure 4.2. Similar to solid recovery of SF, the result portrays an evident pretreatment performance trend for the same category of DESs. In the AHA-based DES group, monocarboxylic acid-based DES achieved higher lignin extraction yield than dicarboxylic and tricarboxylic acid-based DESs. The DEELs yield produced from choline chloride:lactic acid (CC-LA), choline chloride:malic acid CC-MA and choline chloride:citric acid (CC-CA) pretreatments were 33.5%, 22.9% and 20.6%, respectively. The similar performance trend was also observed in other study in which the delignification efficiency of dicarboxylic acid-based DES was lower than the monocarboxylic acid-based DES in rice straw pretreatment (X. D. Hou et al., 2018).

Despite having the similar type of functional groups i.e. hydroxyl (OH) and carboxyl (COOH) groups, the extra COOH groups in malic acid and citric acid HBD promote the formation of hydrogen bonds with the choline chloride HBA. Moreover, apart from the interaction with HBA, the two COOH groups in dicarboxylic acid-based DES can also form extensive chains of dimer which restricts the mobility of solvent molecules, thereby hindering the solvent-solute interaction with lignin. The above theories were also agreed by Dai et al. (2013b) and D'Agostino, Harris, Abbott, Gladden, and Mantle (2011). Similar to AHA-based DES, the lignin extraction efficiency of linear saturated choline chloride:succinic acid (CC-SA) with dicarboxylic structure was much lower than all the monocarboxylic acid-based DESs used in this study and also the lowest among all nine DES pretreatments at 10.7%. This further confirms that monocarboxylic acid is more suitable as DES constituents to promote high lignin extraction performance.

Next, the effect of aliphatic chain length in acid HBD on lignin extraction was investigated using linear saturated acids with $C_1$ to $C_4$ carbon chain as shown in Figure 4.2, namely choline chloride:formic acid (CC-FA), choline chloride:acetic acid (CC-AA), choline chloride:propionic acid (CC-PA) and choline chloride:butyric acid (CC-BA). When the chain length increased from $C_1$ to $C_4$, the lignin extractability of DES decreased. CC-FA with one-carbon alkyl group achieved 61.9% of DEEL yield while CC-BA with four-carbon achieved significantly lower yield at 14.3%. Similar findings was also observed in another study in which CC-FA exhibited higher lignin solubility than CC-AA (Lynam et al., 2017). As an electron donating group, alkyl group increases the electron density around the oxygen in OH group of acid. This intensifies the hydrogen bond strength between hydrogen and oxygen. As a result, a weaker acid is formed due to the reduced acid ionization strength. Thus, decrease in aliphatic chain length of acid HBD enhances the ability of DES to donate protons in solvent-solute interaction (Teles et al., 2017). The enhanced ability of DES to donate protons is ideal for breaking lignin-carbohydrate complex in lignocellulosic biomass and leading to significant lignin extraction, as observed in this study.

To elucidate the effect of functional groups in HBD, the performance of DESs with similar aliphatic chain length were compared. For monocarboxylic acids with $C_3$ carbon structure, CC-LA achieved higher DEEL yield at 33.5% than CC-PA at 20.4%. The same goes to $C_4$ dicarboxylic acids, in which CC-MA with 22.9% lignin yield outperformed CC-SA with 10.7% yield. The presence of OH group in AHA HBD could have enhanced the lignin extraction performance of DES. When comparing all the $C_4$ dicarboxylic acids, DEEL yield decreased in the order of alpha-hydroxy acid CC-MA (22.9%) > unsaturated acid CC-MAE (20.7%) > linear saturated acid CC-SA (10.7%). This sequence corresponds to the decreasing polarity of HBD. The OH group in malic acid and double bond in maleic acid contributed to their higher polarity nature as compared to malic acid, the linear saturated acid. This might facilitate the solvent interaction with lignin solute through hydrogen bonding and promote the lignin extraction efficiency.

Thus far, the varying DEEL yield across the different acid HBD categories suggests that the functional groups in acid HBD have significant impact on the lignin extraction ability of DES. Short alkyl chain, OH group and double bond in carboxylic acid HBD could improve the acidic DES performance in lignin extraction. However, the presence of more than one COOH groups weakens the DES performance. This exhibits the importance of selecting the suitable DES constituent for DES synthesis to achieve enhanced lignin production efficiency.

#### 4.2.3.2 Functional groups distribution in DEEL

Apart from the quantitative aspect in terms of lignin yield, investigation on the DEEL properties is also crucial to determine the viability of DES for lignin extraction. The functional groups in DEEL were analyzed using FTIR and the spectra are illustrated in Figure 4.6. The FTIR peak assignments are tabulated in Table 4.8. The main peaks obtained from FTIR spectra of DEELs are assigned based on Constant et al. (2016), Lynam et al. (2017) and Tejado, Pena, Labidi, Echeverria, and Mondragon (2007). The FTIR spectra of all DEELs were rather similar, except that obtained using CC-MAE.

All DEEL spectra displayed the presence of both guaiacyl, G (1269, 850 cm$^{-1}$) and syringyl, S (1328, 1118 cm$^{-1}$) lignin units. This observation is as expected since the source of lignin and extraction method are identical. Hence, the similar basic aromatic lignin structure can be observed in the EFB-derived lignin. In spite of that, intensity of the peaks varied from one another, possibly due to the different acid constituent HBD used in the lignin extraction process.

**Table 4.8: FTIR peaks determination of DEELs**

| Absorption frequency/peaks (cm$^{-1}$) | Functional groups | Assignment |
|---|---|---|
| 3371 | O-H st | Phenolic and aliphatic hydroxyl |
| 2939 | C-H st | Methyl $CH_3$ and methylene $CH_2$ |
| 1700 | C=O st | Unconjugated carbonyl |
| 1593 | C-C st | Aromatic ring skeleton |
| 1508 | C-C st | Aromatic ring skeleton |
| 1457 | C-H asymmetric | Methyl $CH_3$ and methylene $CH_2$ |
| 1421 | C-C st | Aromatic ring skeleton |
| 1365 | O-H ip | Phenolic hydroxyl |
| 1328 | C-O st | Syringyl unit |
| 1269 | C-O st | Guaiacyl unit |
| 1221, 1164 | C-O st | Acyl and phenyl (sp$^2$) |
| 1118 | C-H (Ar) ip | Syringyl unit |
| 1032 | C-O st | Alkoxy (sp$^3$) |
| 850 | C-H (Ar) op | Guaiacyl unit |

*st: stretching vibration, ip: in-plane deformation vibration, op: out-of-plane deformation vibration, Ar: aromatic.

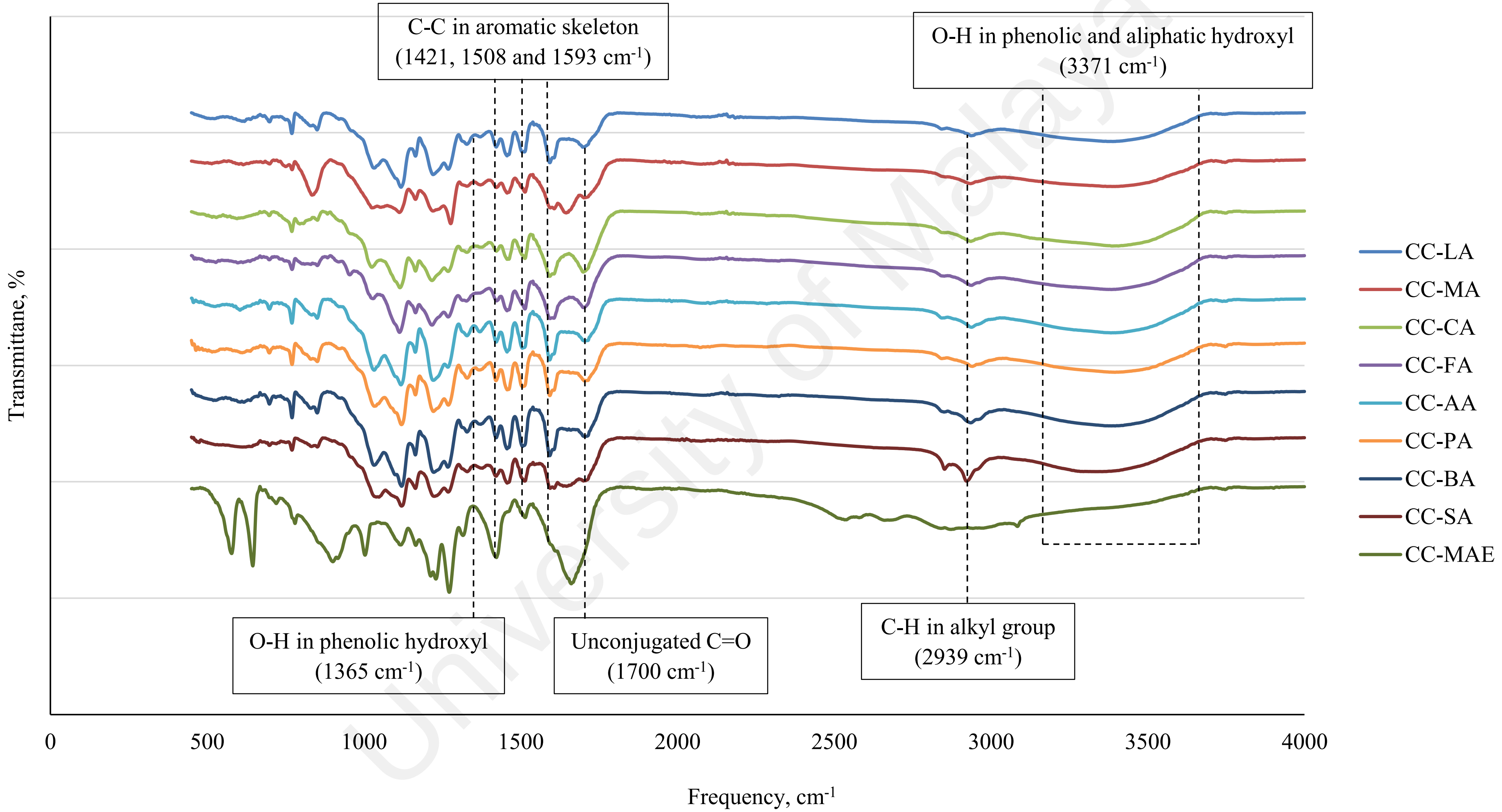


**Figure 4.6 FTIR spectra of DES-extracted lignin (DEEL)**

CC-LA DEEL spectrum depicted the most intense aromatic skeleton vibration (C-C) signals at 1421, 1508 and 1593 cm$^{-1}$, indicating that the DEEL consists of rich aromatic structure. Aromatic C-H bending was also shown in the range of 850-650 cm$^{-1}$ in the CC-LA DEEL spectrum. The wide absorption band focused at 3371 cm$^{-1}$ was attributed to phenolic and aliphatic OH in the DEEL, along with the presence of phenolic OH at 1365 cm$^{-1}$. A strong signal at 1700 cm$^{-1}$ due to unconjugated C=O was observed in the CC-LA DEEL spectrum. It was reported that that hydroxyl and carbonyl functional groups are the important characteristics of a reactive lignin for efficient modification processing (S. Wang, Luo, & Media, 2017). In view of CC-LA DEEL spectrum encompassed the peaks of important functional group for potential downstream conversion, the spectrum was used to compare against the FTIR spectrum of other DEELs.

The aromatic skeleton vibration and the C-H bending signals of both CC-MA and CC-CA DEELs spectra were weaker, which might indicate the existence of less aromatic moieties in the lignin structure than that of CC-LA. Also, the PhOH peak at 1365 cm$^{-1}$ was diminished in the CC-CA DEEL spectrum. This corresponds to the result in Section 4.3.1 where the PhOH content of CC-CA DEEL was the lowest among all the DEELs investigated in this study. Similarly diminished peak was also observed in the CC-FA DEEL spectrum which has second lowest PhOH content as measured in Section 4.3.1. Also, the peaks for guaiacyl and syringyl lignin units in CC-FA, CC-MA and CC-CA were comparatively less intense than that of CC-LA. On the other hand, the FTIR spectra of CC-AA, CC-PA and CC-BA DEELs were generally similar to the spectrum of CC-LA DEEL.

For CC-SA DEEL spectrum, the peak intensity in the aromatic region band were much reduced, as well as the C=O group peak. Yet, the signal attributed to C-H stretching vibration of alkyl group at 2939 cm$^{-1}$ was the strongest among all DEELs. These results might imply that CC-SA DEEL contains more of aliphatic structure than aromatic structure. Other than CC-SA, CC-MAE DEEL also showed a distinct FTIR bands than the other DEELs. The difference was probably due to its high affinity for carbohydrate or DES solvent. Thus, both the G and S lignin structure were less distinct compared to that in other DEELs.

Based on the collective findings, the abundance of functional groups present in DEEL is considerably influenced by the type of DES acid constituent, as indicated by the difference in FTIR peak intensity. The presence of aromatic structure, hydroxyl and carbonyl groups are associated with the potential of lignin as an aromatic feedstock for aromatic product conversion. Hence, DEELs that contain all the essential functional structures with high intensities might be fitting for aromatic chemical production. To ascertain that, more extensive assessment on the DEEL properties performed was discussed in Section 4.3.

### 4.2.4 Selection of Optimum Molar Ratio of Acidic DES for Lignin Extraction

It was discussed previously in Section 4.2.1 that the relative composition ratio of HBD and HBA could influence DES formulation (Table 4.6). Hence, the effect of DES molar ratio on DES pretreatment for biopolymer fractionation and lignin extraction was closely investigated using the five DESs with the highest DEEL yield, namely CC-LA, CC-MA, CC-CA, CC-FA and CC-AA. Figure 4.7 depicts the solid recovery percentage of SF and DEEL yield from EFB pretreatment using DES with varying HBA:HBD ratio. The biopolymer composition of SF produced is presented in Figure 4.8. The findings presented in both figures suggest that the molar ratio of HBA to HBD in DES has a significant impact on SF recovery and lignin extractability.

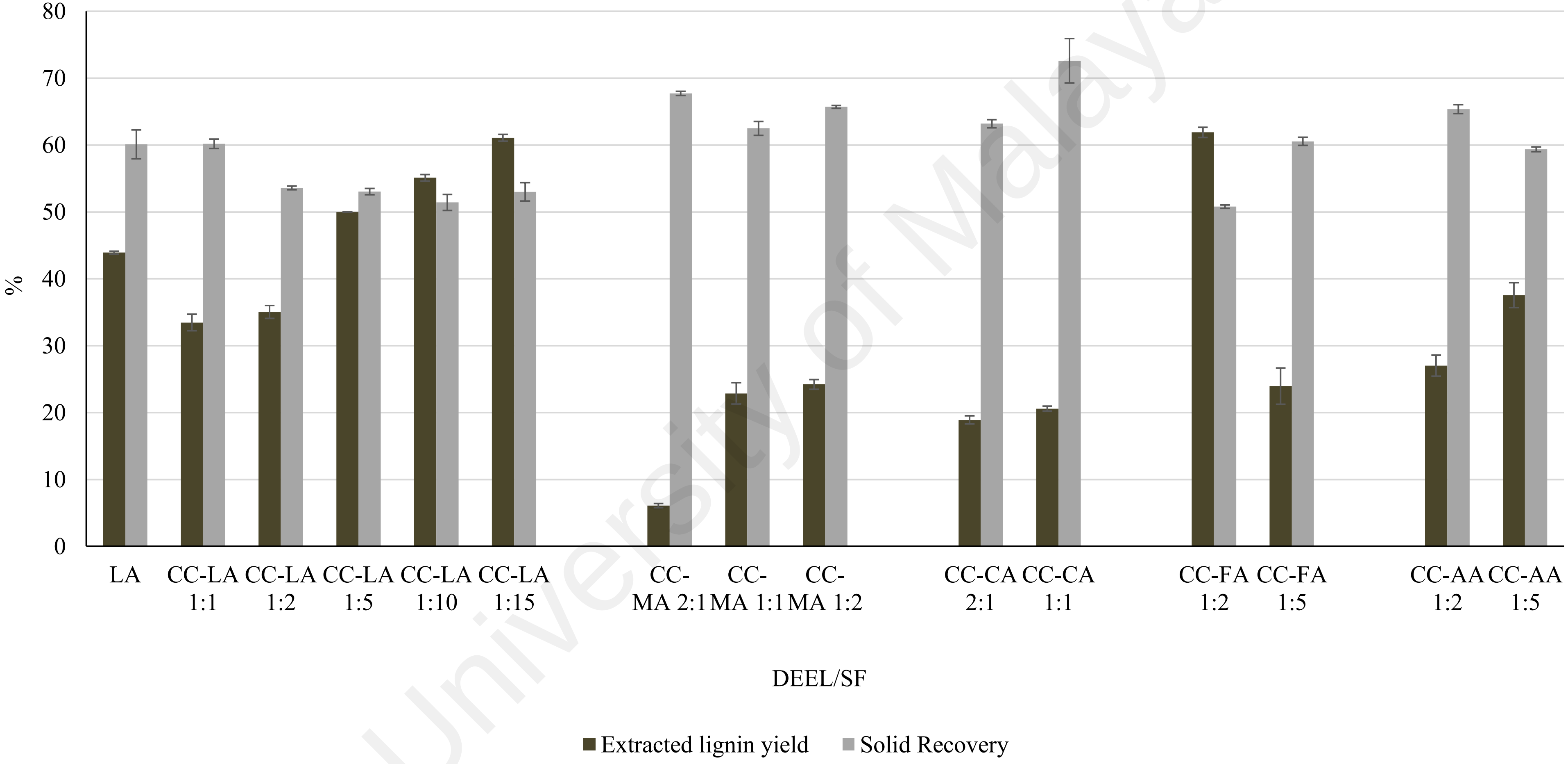


Figure 4.7: Extracted DEEL yield and solid recovery of SF produced by DES with varying molar ratios

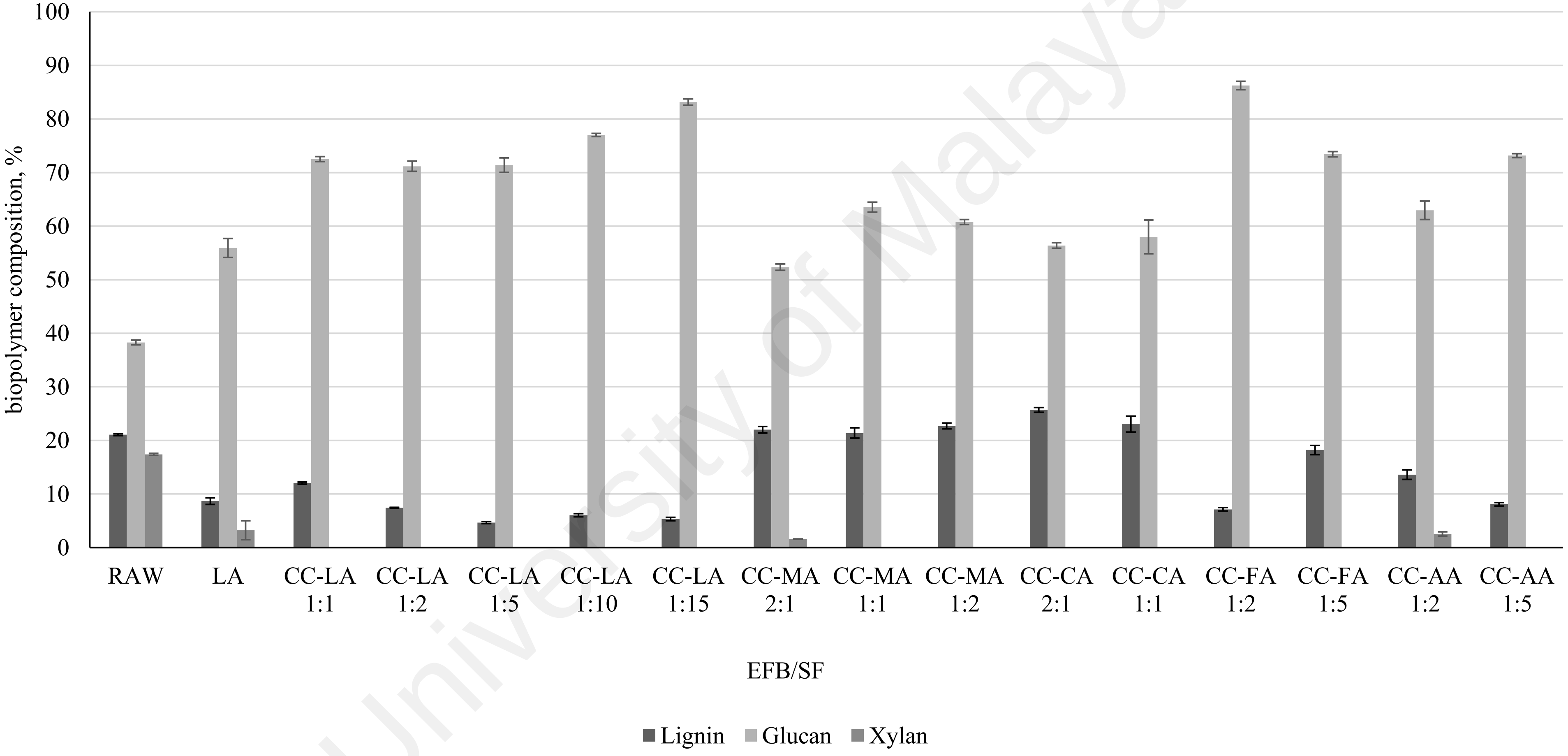


**Figure 4.8: Biopolymer composition of SF produced by DES with varying molar ratio**

Weakened delignification performance and thus decrement in DEEL yield was observed when the relative content of HBA in the DES was increased from the HBA:HBD ratio of 1:1 to 2:1 ratio in CC-MA and CC-CA pretreatments. Due to the poorer fractionation performance of DES with 2:1 ratio, small amount of xylan remained in the SF from CC-MA 2:1 pretreatment, as opposed to complete xylan removal in CC-MA 1:2 pretreatment (Figure 4.8).

In contrast, the DEEL yield increased as the relative HBD ratio was increased in CC-LA, CC-MA and CC-AA pretreatment. Steep increment in lignin yield from 33.6% to 61.1% was observed in CC-LA pretreatment when the ratio was increased from 1:1 to 1:15. Other than CC-LA, the lignin extraction performance of CC-AA pretreatment was also improved significantly by 10.6% from 27.0% to 37.6% whereas there was only a minor improvement of 1.3% in the CC-MA pretreatment when the HBA:HBD ratio was altered from 1:1 to 1:2 (Figure 4.7). A control experiment using lactic acid (LA) as pretreatment solvent was performed to compare the extraction efficiency of LA-based DES and LA. LA pretreatment managed to yield 43.9% lignin, which was lower than that of CC-LA 1:5 to 1:15 (Figure 4.7). Despite more than 90 wt% of CC-LA 1:15 solvent comprised of LA, its extraction efficiency is 17.2% higher than pure LA solvent. This suggests that the synergistic effect of HBA and HBD in DES could enhance the lignin extraction efficiency.

On the contrary, lignin yield from CC-FA pretreatment showed a drastic decline from 61.9% to 24.0% when the DES molar ratio was increased from 1:2 to 1:5 (Figure 4.7). This might be due to 1:5 ratio is not the suitable eutectic composition for CC-FA DES formation. As shown in the FTIR spectrum of CC-FA in Figure 4.9, 3313 cm$^{-1}$ peak that indicated the presence of OH stretch in carboxylic acid was not found in CC-FA 1:5. Furthermore, the peaks at 1082 and 1053 cm$^{-1}$ were diminished in the spectrum, indicating that C-O bond of alcohol group in CC-FA with 1:5 ratio was less intense than that in CC-FA with 1:2 ratio due to weaker hydrogen bond formation in the former solvent. When acid content was increased from ratio 1:2 to 1:5, insufficient HBA was available to interact with the acid present in excess amount to form DES. Hence the solvent might behave more like an acid than a DES. Also, since the boiling point of formic acid is around 101°C, the synthesized DES CC-FA at ratio of 1:5 might have vaporized at the higher pretreatment temperature of 120°C and impeded the DES's efficiency in lignin extraction.

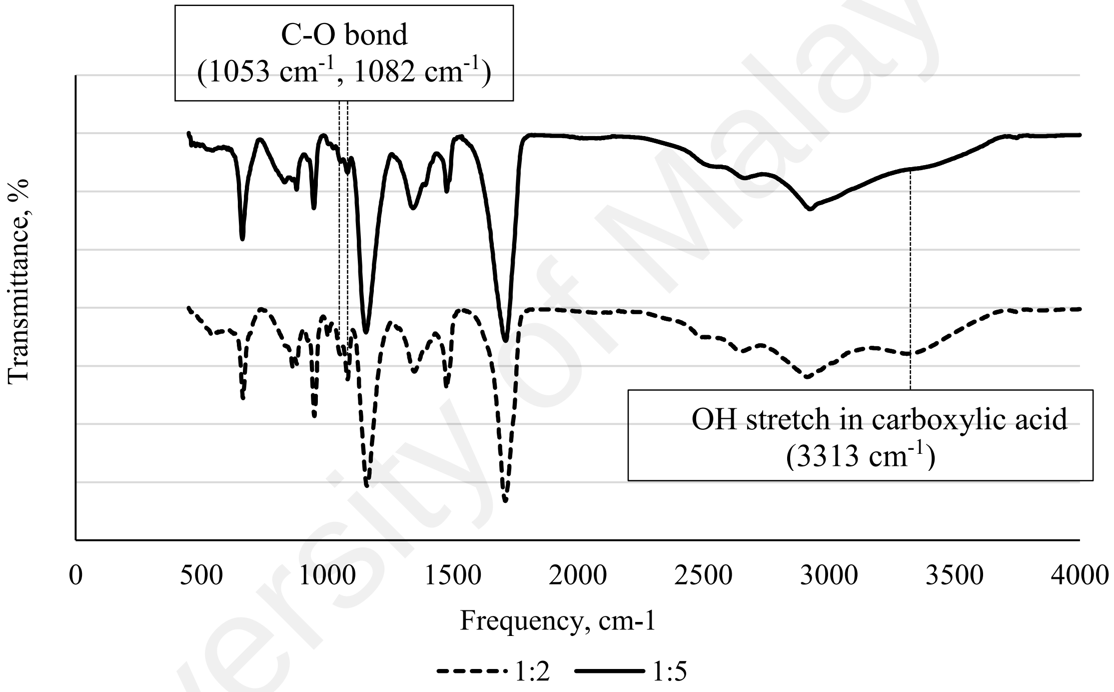


**Figure 4.9: FTIR spectrum of CC-FA**

The alteration in molar ratio of HBA to HBD has demonstrated great influence in lignin extraction performance of DES. Hence, with suitable HBA to HBD ratio employed, high lignin production through DES pretreatment could be achieved. Pretreatments using CC-LA with 1:15 ratio and CC-FA with 1:2 ratio managed to achieve similarly high lignin yield at 61.1% and 61.9%, respectively. Thus, for DES solvent to perform at its best in lignin extraction and biopolymer fractionation, an optimized HBA to HBD molar ratio is needed to be identified for specific type of DES.

### 4.2.5 Summary

This work demonstrates the pronounced impact of functional groups in carboxylic acid HBD constituent and the molar ratios of HBA to HBD on DES formation and their pretreatment performance. The acidic DESs with varying type of carboxylic acid as HBD constituent were formed under different HBA to HBD ratio. The properties analyses on the DES-fractionated SF and DEEL revealed that hydroxyl group, double bond, short alkyl chain and monocarboxylic structure on acid HBD could enhance the DES performance for biopolymer fractionation and lignin extraction. This shows the significance of the selection of suitable acid constituent with compatible HBA counterpart for producing DES with excellent lignin production efficiency.

Among the acidic DESs investigated, CC-FA and CC-LA at 1:2 and 1:15 molar ratio, respectively, displayed the highest biopolymer fractionation and lignin extraction efficiency. The DESs showed promising result in glucan isolation where the DES-fractionated SFs were comprised of up to 86% of glucan content and minimal amount of lignin. Based on the functional group analysis, the SFs obtained were enriched with highly crystalline cellulose structure due to the removal of amorphous biopolymers. The acidic DES-pretreated EFB SF has the potential to be used as a substrate for sugar-based products transformation.

Other than the glucan-rich SF fraction, CC-FA and CC-LA pretreatments also achieved >60 wt% of lignin yield. The FTIR spectrum of DEELs produced exhibited the presence of important functional groups for lignin downstream conversion. Therefore, based on the collective analysis of SF and DEEL, CC-FA and CC-LA DESs have shown promising performance in EFB pretreatment for biomass fractionation and lignin extraction.

## 4.3 Assessment of DES-Extracted Lignin Properties and Their Potential Bio-Products Conversion

As described in the previous Section 4.2, DES-extracted lignin (DEEL) with varying yield and properties could be obtained using acidic DES with different acid HBD constituents. Other than the quantitative aspect i.e. yield percentage, it is of utmost importance to determine the composition and properties of DEELs. It has been reported that the characteristics of lignin differ according to the source of lignin and extraction approaches (Tejado et al., 2007). Thus, properties analyses on DEEL can present an insight into the influence of different DES on the quality of extracted lignin, as well as to determine the potential of DEELs for downstream conversion.

In this section, DEELs which were produced with high extraction yield have been selected for extended characterization analyses. The analysis performed on DEEL can be categorized into structural and thermal analyses. The structural analyses include measurement of phenolic hydroxyl group content, biopolymer and elemental composition and also nuclear magnetic resonance spectroscopy (NMR). Thermogravimetric analysis (TGA), thermogravimetry-Fourier transform infrared spectroscopy (TG-FTIR) and pyrolyzer-GCMS (PY-GCMS) were also performed to assess the thermal properties of DEEL. The DEEL properties were compared with lactic acid (LA)-extracted lignin to evaluate the influence of DES and acid solvent over extracted lignin. Based on the distinctive properties of DEEL, the potential lignin-derived bioproducts conversion are proposed.

### 4.3.1 Phenolic Hydroxyl Content Analysis (PhOH)

The properties of lignin can be altered depending on the lignin extraction method adopted. During the extraction process, lignin biopolymer can be depolymerized into subunits with different structures, resulting in the extracted lignin with varying level of reactivity for its subsequent processing. The extent of lignin's reactivity is determined by the abundance of phenolic hydroxyl group (PhOH), the most reactive functional group in lignin biopolymer (Lai, 1992; Zakis, 1994). The phenolic phenylpropane unit can form quinonmethide intermediates that are prone to nucleophilic reactions (Mansouri & Salvadó, 2006). As a result, they are more vulnerable to modification during a chemical reaction (Hong et al., 2016). Thus, the quantification of PhOH is essential to assess the lignin's potential for modification. The PhOH content of DEEL obtained from DES pretreatments with high lignin yield (Section 4.2.3.1) are shown in Figure 4.10.

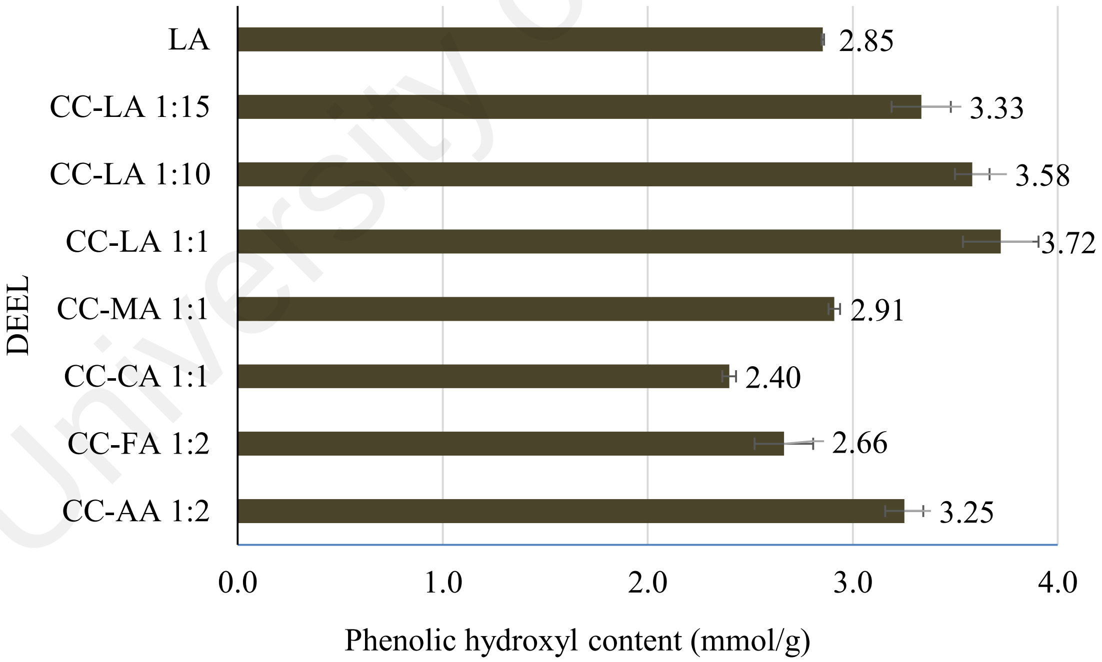


**Figure 4.10: Phenolic hydroxyl content of different DEELs**

CC-LA DEELs had the highest PhOH content among the selected DEELs at 3.33-3.72 mmol/g while CC-CA 1:2 DEEL the lowest at 2.40 mmol/g. There was no correlation observed between the types of HBD in DES and PhOH content. As the molar ratio of CC-LA increased from 1:1 to 1:15, the PhOH content of DEEL decreased gradually. The decrement might be due to lignin condensation as a result of increased acidity in the solvent system (McDonough, 1993). This is further verified by the lower PhOH content of lignin extracted by pure lactic acid (LA) solvent at 2.85 mmol/g when compared with that of CC-LA DEELs (Figure 4.10).

Despite CC-LA 1:15 DEEL having the lowest PhOH content among the other CC-LA-extracted lignin, its hydroxyl content was still higher than the lignin extracted by other DESs. Considering its high lignin yield and high PhOH content, CC-LA DEEL extracted at 1:15 ratio was used as the basis of comparison with other literature reported works (Table 4.9).

The characteristics of lignin are influenced by not only the extraction method, but also the botanical source of lignin (Tejado et al., 2007). As shown in Table 4.9, PhOH content of DEEL obtained in this study is comparable to lignin extracted using different methods from selected published works that range from 0.17 to 4.50 mmol/g, as well as the commercialized technical lignin. Although the PhOH content of DEEL obtained in this study was not significantly high compared to the lignin reported in the literatures, this analysis validates the suitability of using DES as an alternative lignin extraction solvent to produce comparably reactive lignin.

**Table 4.9: Comparison table of PhOH content of DEEL with common technical lignin obtained from literatures**

|  | Lignin source[a] | Phenolic hydroxyl content of various lignin (mmol/g) |  |  |  |
|---|---|---|---|---|---|
|  |  | Kraft | Soda | Organosolv | DEEL |
| Mansouri and Salvadó (2006) | Softwood; fiber plant; Miscanthus Sinensis | 4.50 | 4.40 | 2.66 | - |
| Serrano et al. (2018) | Pine; wheat straw; wheat straw | 2.80<br>3.40 (Indulin[b]) | 2.60 (Protobind[b]) | 1.70 | - |
| Constant et al. (2016) | Softwood; mixture of wheat straw and Sarkanda grass; spruce; mixture of hardwoods | 2.77 (Indulin[b]) | 2.86 | 2.73<br>3.30 (Alcell[b]) | - |
| Nitsos et al. (2016) | Spruce and birch wood | - | 0.17-2.0 | 1.68-3.99 | - |
| Ibrahim et al. (2011) | EFB | 4.11 | 2.58 | - | - |
| This work | EFB | - | - | - | 3.33 |

[a] Multiple lignin sources is listed in the order of lignin types from left to right (Kraft, Soda and Organosolv).
[b] Indulin, Protobind and Alcell lignin are commercialized technical lignin using the stated extraction processes.

The measurement of PhOH content identifies CC-LA 1:1 DEEL as the most reactive lignin whereas CC-FA 1:2 DEEL as lignin with the least amount of reactive PhOH group. The PhOH content of CC-LA 1:15 DEEL at 3.33 mmol/g is slightly lower than that of CC-LA 1:1 DEEL at 3.72 mmol/g, confirming that HBA to HBD ratio of DES could influence the DEEL properties. Therefore, the structural and thermal properties of CC-LA 1:1 DEEL with the most reactive structure, as well as CC-LA 1:15 and CC-FA 1:2 DEELs with the highest extraction yield, were further investigated in the subsequent sections to assess their potential for downstream conversion. The direct comparison of most reactive CC-LA DEEL and least reactive CC-FA DEEL can then be performed. To compare the performance of DES and its respective acid solvent in lignin extraction, the properties of lactic acid-extracted lignin was also examined.

### 4.3.2 Biopolymer and Elemental Composition Analysis

As lignin is extracted from a complex biomass matrix, analysis on the lignin's composition is important to assess its purity and possible impurities in the lignin structure. This can also identify the solvent used to extract lignin biopolymer with the highest purity and quality for downstream processing. The biopolymer and elemental composition of EFB-derived lignin extracted using lactic acid solvent (LA), choline chloride:lactic acid with the ratio of 1:1 (CC-LA 1:1) as well ratio 1:15 (CC-LA 1:15) and choline chloride:formic acid with the ratio of 1:2 (CC-FA 1:2) are tabulated in Table 4.10.

**Table 4.10: Biopolymer and elemental composition of LA-extracted lignin and DEELs**

| **Extracted lignin** | **Biopolymer composition (wt%)** | | | | **Elemental composition (%)** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Lignin | Glucan | Xylan | Other | C | H | N | O | S |
| LA | 75.15 ± 0.99 | 1.42 ± 0.26 | 5.94 ± 1.62 | 17.49 | 52.87 | 5.78 | 0.00 | 41.98 | 0.49 |
| CC-LA 1:1 | 78.89 ± 0.22 | 1.94 ± 0.15 | 0.62 ± 0.07 | 18.55 | 52.50 | 5.39 | 1.36 | 40.00 | 0.75 |
| CC-LA 1:15 | 80.64 ± 1.00 | 0.00 ± 0.00 | 1.02 ± 1.25 | 18.34 | 53.89 | 5.17 | 0.77 | 38.80 | 1.37 |
| CC-FA 1:2 | 85.84 ± 1.07 | 1.39 ± 0.02 | 0.00 ± 0.00 | 12.77 | 47.86 | 5.44 | 2.48 | 42.83 | 1.40 |

The finding clearly showed that the lignin composition varied with the choice of extraction solvent. The lignin composition, which also represents lignin's purity, ranged from 75.15% to 85.84%. LA-extracted lignin contained more residual carbohydrates than the other three DEELs at a total carbohydrate content of 7.36%, which is the sum of 1.42% glucan and 5.94% xylan. However, the amount of carbohydrate residue varied from 1.02% to 2.56% in the DEELs. In a reported literature, lactic-acid based DES was also able to obtain DEEL with the similar carbohydrate residue percentage at 2.46-2.62% from corncob biomass under the same pretreatment condition of 120°C and 8 h (Zongwei Guo et al., 2019). This shows that lactic acid-based DESs are capable of extracting lignin with low carbohydrate impurities from diverse types of biomass, which would be useful in developing a biomass fractionation scheme suitable for assorted biomass.

Carbohydrate content in extracted lignin is considered as an impurity as it has been reported to impact on the lignin downstream processing negatively (H. Wang, Duan, Zhang, & Yang, 2018). In biomass matrix, carbohydrate links with lignin in the form of lignin-carbohydrate complex (LCC) (Y. Liu et al., 2017). In the current study, lactic acid solvent might have a lower efficiency in breaking the LCC linkages than its corresponding DESs (CC-LA 1:1 and CC-LA 1:15), as evidenced by the higher carbohydrate residue in LA-extracted lignin. Applying DES in lignin extraction has increased the lignin product's purity and lowered the carbohydrate impurities than applying the acid solvent. The synergistic effect between HBA and HBD in DES could lead to stronger hydrogen bonding network with the biopolymers which enhances the extraction efficiency.

Due to their similar botanical origin i.e. oil palm empty fruit bunch (EFB), the elemental composition which refers to the carbon, hydrogen, nitrogen, oxygen and sulphur content of all the extracted lignins are rather similar (Table 4.10). Kraft and soda lignin extracted from EFB also had the similar composition in other reported work with 57% carbon, 6% hydrogen and 35% oxygen (Ibrahim et al., 2011). The comparatively lower carbon and higher oxygen content in CC-FA DEEL than the other lignins might be due to the active production of new hydroxyl groups through hydrolysis of ether bond and methoxyl groups (H. Guo et al., 2017). The occurrence of extensive hydrolysis of ether bond was further evidenced by the absence of β-O-4′ ether bond in the CC-FA DEEL as shown in Table 4.11, which will be discussed in Section 4.3.3.

As presented in Table 4.10, sulphur-containing compound was still retained in the EFB biomass matrix in small amount after DES pretreatment. Even though sulphur has been reported to be an inhibitory species for lignin modification (Laurichesse & Avérous, 2014), it is an essential nutrient in plant for protein synthesis and other metabolic activities (Maathuis, 2009). Therefore, this compound is still present in DEEL in small percentage after extraction process.

### 4.3.3 2-Dimensional Heteronuclear Single Quantum Coherence Nuclear Magnetic Resonance Spectroscopy (2D HSQC NMR) Analysis

Lignin is comprised of various monolignol structures that cross link at different positions in the aromatic ring and aliphatic chain, forming assorted linkages and subunits in the biopolymer. These linkages and subunits have different properties, and therefore the relative abundance of these structures can indicate the lignin-derived products that can potentially be produced in the downstream conversion process. These monolignol units and linkages can be identified through signal in response to the correlation between $^{13}C$ and $^{1}H$ using 2-Dimensional heteronuclear single quantum coherence nuclear magnetic resonance spectroscopy (2D HSQC NMR). The NMR spectra were evaluated according to two regions of interest, namely the aliphatic side chain region ($\delta C/\delta H$ 50-90/2.5-5.8) and aromatic region ($\delta C/\delta H$ 90-150/5-8.5). The NMR spectra of the four investigated lignins are as shown in Figure 4.11. The signals' assignment and quantification are tabulated in Table 4.11.

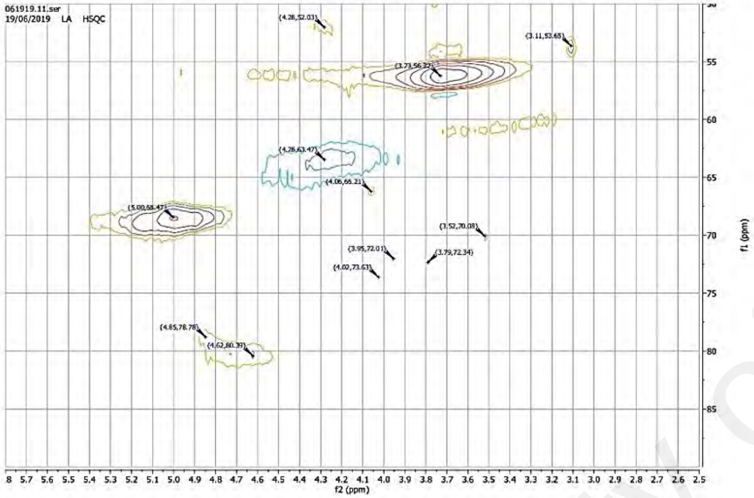
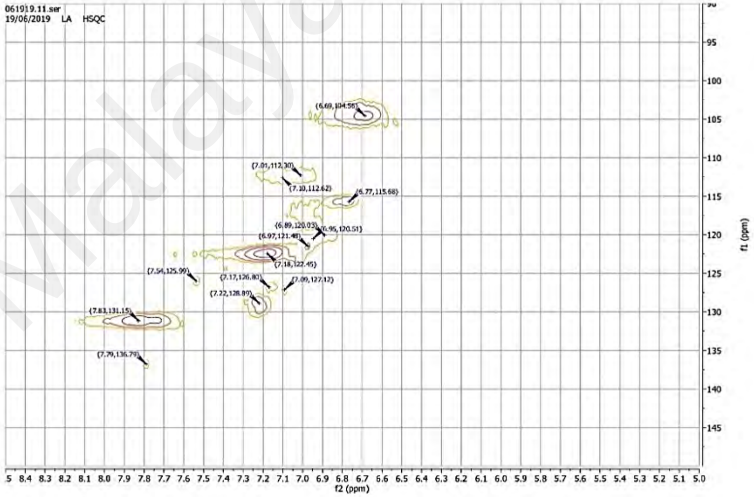
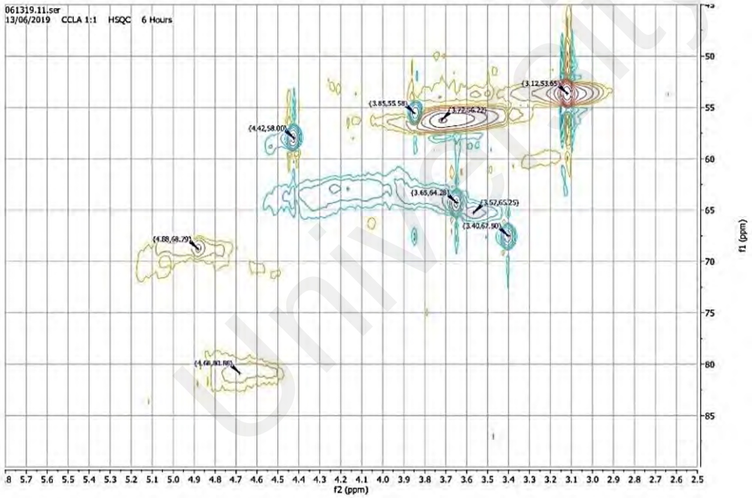
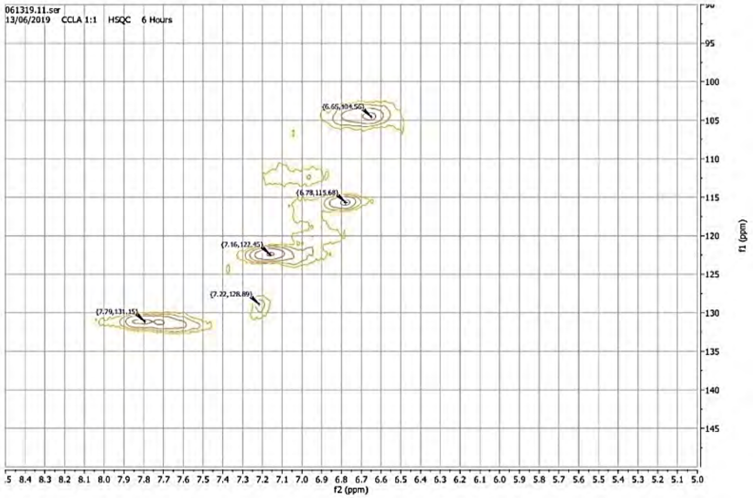
<table>
<tr><th></th><th>Aliphatic side chain region</th><th>Aromatic region</th></tr>
<tr><td>LA-extracted lignin</td><td>
</td><td>
</td></tr>
<tr><td>CC-LA 1:1 DEEL</td><td>
</td><td>
</td></tr>
</table>

**Figure 4.11: 2D HSQC-NMR spectra of the EFB-derived lignins**

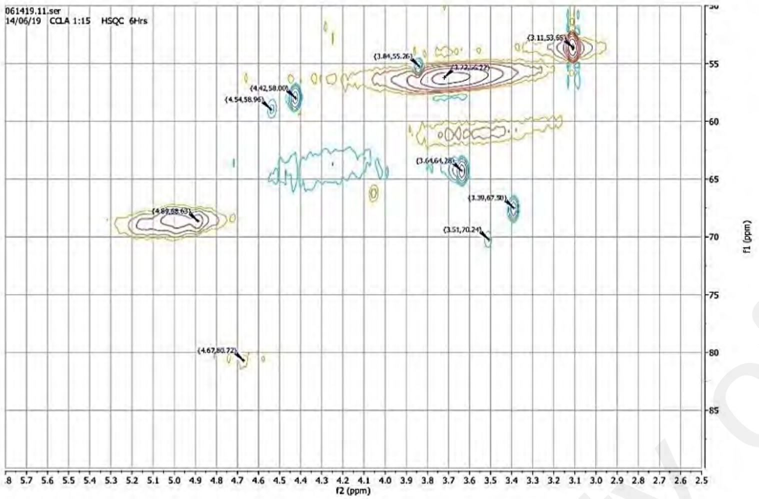
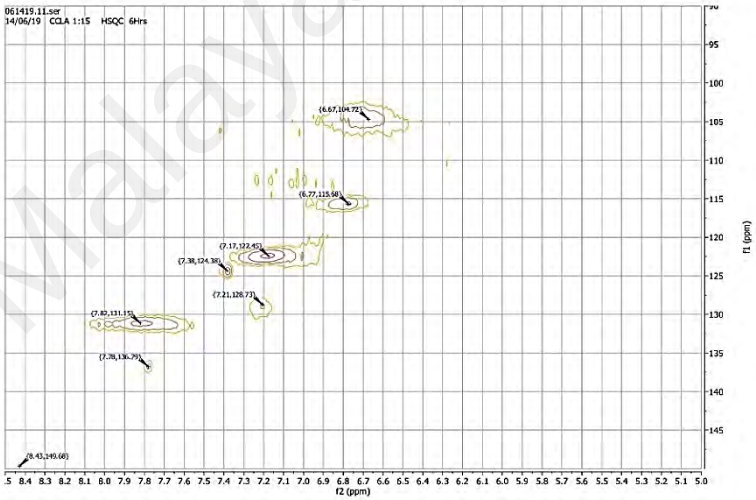
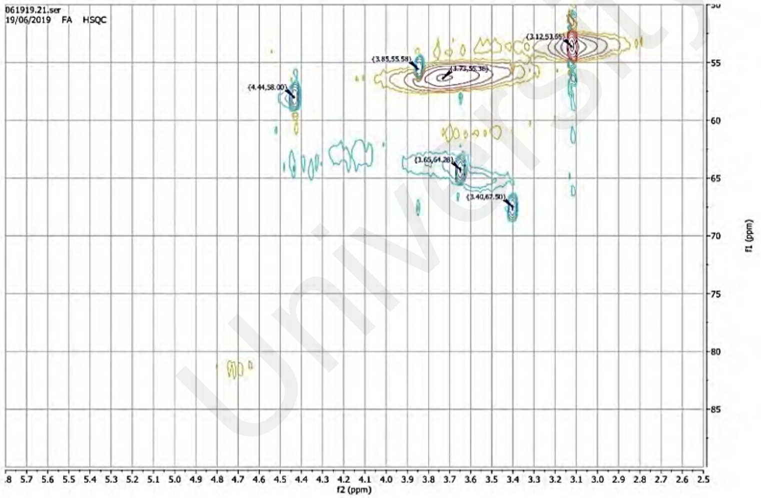
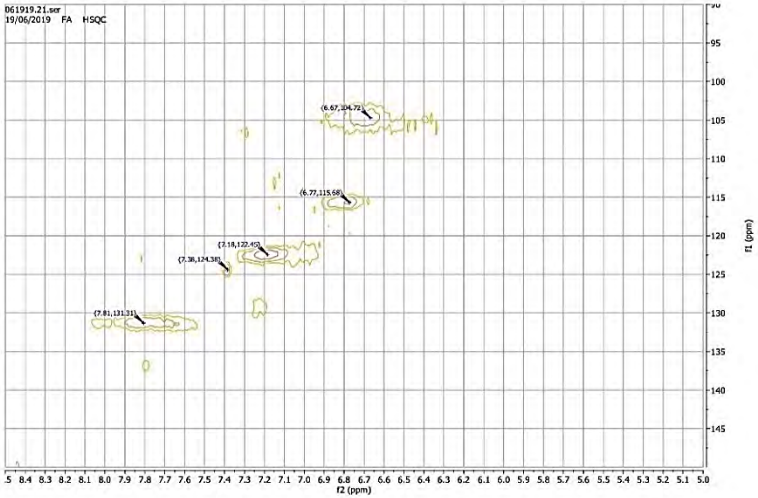
| | Aliphatic side chain region | Aromatic region |
|---|---|---|
| CC-LA 1:15 DEEL |  |  |
| CC-FA DEEL |  |  |

**Figure 4.11: Continued**

### Table 4.11: Quantification and assignment of aromatic unit and aliphatic side chains in the extracted lignins

| | δC/δH | LA | | CC-LA 1:1 | | CC-LA 1:15 | | CCFA | |
|---|---|---|---|---|---|---|---|---|---|
| | | Unit/Ar [a] | % | Unit/Ar | % | Unit/Ar | % | Unit/Ar | % |
| Aromatic units | | | | | | | | | |
| Syringyl, S (%) | 104.5/6.7 | 1.65 | 83.85 | 1.83 | 64.78 | 1.04 | 50.09 | 1.69 | 62.01 |
| Guaiacyl, G (%) | 115.7/6.8 | 0.27 | 13.97 | 0.84 | 29.79 | 1.04 | 49.91 | 0.97 | 35.65 |
| P-hydroxyphenyl, H (%) | 117.3/7.0 | 0.04 | 2.18 | 0.15 | 5.43 | ND.[b] | - | 0.06 | 2.34 |
| S+G+H | - | 1.96 | - | 2.82 | - | 2.08 | - | 2.73 | - |
| $C_6$-$H_6$ in ferulate units | 122.4/7.2 | 1.95 | - | 1.89 | - | 2.94 | - | 2.50 | - |
| $C_{2,6}$-$H_{2,6}$ in p-hydroxybenzoate substructures | 131.2/7.7 | 0.59 | - | 1.05 | - | 0.54 | - | ND. | - |
| | | | | | | | | | |
| Aliphatic linkages | | | | | | | | | |
| $C_\alpha-H_\alpha$ in β-O-4′ substructures, $A_\alpha$ | 68.7/5.0 | 1.74 | 89.92 | 0.45 | 2.35 | 2.52 | 12.71 | ND. | - |
| $C_\beta-H_\beta$ in β-O-4’ substructures, $A_\beta$ | 80.2/4.7 | 0.10 | 5.11 | 0.13 | 0.69 | ND. | - | ND. | - |
| $C_\gamma-H_\gamma$ in γ−hydroxylated β-O-4′ substructures, $A_\gamma$ | 59.9/3.2 | ND. | - | 0.04 | 0.22 | ND. | - | ND. | - |
| $C_\beta-H_\beta$ in β−5′ phenylcoumaran substructures, $B_\beta$ | 55.6/3.9 | ND. | - | 1.17 | 6.04 | 0.36 | 1.81 | 1.70 | 3.17 |
| $C_\gamma-H_\gamma$ in β−5′ phenylcoumaran substructures, $B_\gamma$ | 64.3/3.6 | ND. | - | 0.57 | 2.95 | 2.29 | 11.55 | 6.17 | 11.54 |
| $C_\beta-H_\beta$ in β−β′ resinol substructures, $C_\beta$ | 53.7/3.1 | 0.10 | 4.96 | 16.89 | 87.53 | 12.82 | 64.58 | 40.45 | 75.60 |
| $C_\gamma-H_\gamma$ in p-hydroxycinnamyl alcohol end group | 58.0/4.4 | ND. | - | 0.04 | 0.22 | 1.86 | 9.35 | 5.18 | 9.69 |
| | | | | | | | | | |
| Functional groups | | | | | | | | | |
| Methoxyl group | | 14.16 | | 10.32 | | 29.04 | | 25.95 | |

[a] The aromatic units and linkages are expressed as unit per aromatic ring (Unit/Ar)

[b] ND. = not detected

In the NMR spectra, the C-H cross peak signal of syringyl (S) unit at position $C_2$ and $C_6$ in the aromatic ring was obtained at δC/δH 104.5/6.7. The signal of guaiacyl (G) at position $C_2$, $C_5$ and $C_6$ was registered at δC/δH 112.6/7.0, 115.7/6.8 and 117.3/7.0, respectively. $C_5$–$H_5$ in G unit was selected for quantification of G unit as this signal was observed in all lignins. Weak signal of p-hydroxyphenyl (H) was also detected at δC/δH 126.8/7.2. From the NMR spectra, the oil palm empty fruit bunch (EFB)-derived lignin was identified to be a SGH type of lignin due to the presence of syringyl, guaiacyl and p-hydroxyphenyl units. LA-extracted lignin and the three DEELs were composed mainly of S and G units, along with a small amount of H as shown in Table 4.11.

(a) ***Aromatic functional group region***

Lignin is the only aromatic biopolymer in lignocellulosic biomass, hence the assessment of its aromatic subunits is important to identify its potential use. All the extracted lignins appeared to have a much higher ratio of syringyl (S) unit in relative to guaiacyl (G), except CC-LA 1:15 DEEL with almost equal percentage in both units (Table 4.11). The H unit was absent from CC-LA 1:15 DEEL and accounted for a low portion in the other three extracted lignins at 2.18% – 5.44%. The relative percentage of these aromatic units in the three DEELs was largely similar to each other than that of LA-extracted lignin. S and G units fell within the range of 50.09% – 64.78% and 29.79% – 35.65% in the DEELs, and 83.85% and 13.97% in the LA-extracted lignin, respectively.

Owing to the availability of $C_5$ position in G unit for coupling with other monolignols, lignin comprising of higher percentage of G units has more resistant linkages such as β-5’ than lignin with more S units (Boerjan et al., 2003). Having a higher percentage of S unit, lignin derived from EFB might be more susceptible to modification when subjected to valorisation process. The dominance of S unit in EFB lignin is likely to be due to the plant species as lignins extracted using different processes namely alkaline or organosolv reported in other studies also had the similar S and G unit distribution (Hussin, Rahim, Mohamad Ibrahim, & Brosse, 2013; Sun, Fang, Tomkinson, & Bolton, 1999).

A strong signal at around δC/δH 122.4/7.2 was assigned to ferulate, an aromatic acid or hydroxycinnamate that generates nucleation site (from where lignin polymer can grow) for lignin-polysaccharide cross linking (Boerjan et al., 2003; Zeng, Helms, Gao, & Chen, 2013). Hydroxyl groups in polysaccharide are activated by ferulic acid to form ester bonds with lignin polymer, resulting in the formation of lignin-carbohydrate complex (LCC) between the polysaccharide and lignin (Ralph & L Landucci, 2010). Hence ferulate was reported to be representing the abundance of LCC linkages remained in the lignins (Zeng et al., 2013). In this study, ferulate signal was present in all lignins in elevated amount (1.95 to 2.94 unit per Ar) despite minimal amount of carbohydrate was detected (Table 4.10). The intense signal might be caused by the overlapping signal between ferulate and the oxidized S and G units (Constant et al., 2016). Therefore, the value registered cannot represent the abundance of LCC linkages adequately.

Another hydroxycinnamate group, p-hydroxybenzoate structure was also manifested in the NMR spectra of LA-extracted lignin, CC-LA 1:1 and CC-LA 1:15 DEELs (δC/δH 131.2/7.7). It was reported that p-hydroxybenzoate typically exists when the native lignin is comprised of both G and S units (Constant et al., 2016). This accords with the findings in this study in which the EFB-derived lignin is of GSH type (Table 4.11). This aromatic structure was also found in significant quantity in alkaline soluble EFB (Sun et al., 1999). On the other hand, the absence of p-hydroxybenzoate in CC-FA DEEL in the current study signifies that CC-FA might be more destructive towards the native lignin structure than the other DESs.

### (b) ***Aliphatic linkage region***

Type of linkages in lignin and their respective abundance can affect lignin's reactivity as the strength of these linkages differs from one another (C. Li et al., 2015). Table 4.11 shows the main aliphatic linkages present in the lignins, namely β-aryl ether (β-O-4′, label A), phenylcoumaran (β-5′, label B) and resinol (β-β′, label C). The relative amount of these linkages varied with the type of solvent used in the lignin extraction process.

In native lignin, β-O-4′ usually accounts for the highest percentage of the total inter-unit linkages (Boerjan et al., 2003). However, as shown in Table 4.11, β-O-4′ linkage was mostly cleaved, which is as expected since it is the most labile linkage in lignin as reported in Boerjan et al. (2003). This labile bond in the DEEL obtained from other biomass species such as pinewood sawdust, peach pit and walnut shell, was also reported to be cleaved significantly (W. Li et al., 2018; Muley et al., 2019). This shows that DES exerts the similar influence to the extracted lignin from other biomass sources in this aspect. Comparing the four lignins obtained in the current study, LA-extracted lignin contained the highest relative percentage of β-O-4′ at 89.92%. Nevertheless, CC-LA 1:15 DEEL preserved the most β-O-4′ per aromatic unit at 2.52 unit/Ar. It was also reported by other researcher that β-O-4 bonds in lignin were cleaved faster by the lactic acid-based DES than by pure lactic acid when similar pretreatment conditions were applied (120°C, 8 h) (Smink, Juan, Schuur, & Kersten, 2019). Findings in Table 4.11 also showed that the linkage signal disappeared completely in CC-FA DEEL. In addition to the absence of p-hydroxybenzoate, it is justifiable to conclude that CC-FA had altered the most of EFB lignin's native structure as compared with the other solvents.

β-5' was the second most abundant linkage in the DEELs and it was not detected in the LA-extracted lignin. The precursor of this structure is G unit. The linkage is formed through the coupling of G unit's vacant $C_5$ position in the aromatic ring with other monolignols’ β position (Zeng et al., 2013). β-5’ usually presents in low to negligible abundance in S-dominant lignin (Ralph & L Landucci, 2010), which explains for its absence in the LA-extracted lignin with 83.85% S unit of the total aromatic units.

β-β’ was the predominant linkage in all three DEELs, which accounted for 64.58%-87.53% of their total linkages. Whereas in LA-extracted lignin, it accounted for a mere 4.96% of the total linkages. W. Li et al. (2018) observed similar composition in the lignin extracted from endocarp biomass whereby β-β’ percentage increased significantly after DES extraction as opposed to β-O-4′ and β-5.

C-C bond i.e. β-5’ and β-β’ is harder to break based on the higher bond dissociation energy than that of C-O ether bond (β-O-4’) (J.-b. Huang et al., 2015). Hence, the cleavage of C-C bond is still very challenging to this day. However, the cleavage of C-C bonds is vital for lignin utilization schemes as most technical lignins contain elevated amount of C-C bonds after the extraction process. Selective modification of β-β’ linkage in Kraft lignin was achieved using oxidant 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Tran, Lancefield, Kamer, Lebl, & Westwood, 2015). Also in a catalytic hydrogenolytic process, softwood lignin with more β-5’ was less suitable to be converted as they contributed to lower bio-oil yield (H. Guo et al., 2017). Even though β-O-4′ linkages are present in minimal amount in DEELs, both the literature findings above pointed that lignin with elevated amount of β-β’ and lower amount of β-5′ might also be conducive for depolymerisation process.

The p-hydroxycinnamyl alcohol end group detected at δC/δH 58.0/4.4 is a product from monolignol dimerization reactions (coupling of two monolignols) that normally present in small amount in lignin. It was found disappeared from LA-extracted lignin spectra and remained in minor amount in the DEELs.

Apart from the linkages native to lignin, some other signals attributed to the carbohydrate contaminants, LCC linkages and degradation products were also identified. The high xylan content in LA-extracted lignin and CC-LA 1:15 DEEL as presented in Table 4.10 was further substantiated by the presence of xylan signals at around δC/δH 70-73/3.5-4.0. The same region in the other two DEELs (CC-LA 1:1 and CC-FA) was found clear of any xylan signal. There are three main types of LCC linkages usually found in lignocellulosic biomass, namely benzyl ether, benzyl ester and phenyl glycoside (Yue et al., 2017). Signal at δC/δH 80.3/4.6 attributed to benzyl ether linkage was manifested in the LA-extracted lignin spectrum. The observation is expected due to the large amount of xylan residue remained in the LA-extracted lignin (Table 4.10). Also, phenyl glycoside (δC/δH 100.1/4.7) was present in CC-LA 1:1 DEEL, possibly from the cross linking of lignin with glucan (Yue et al., 2017). Furthermore, furanic derivatives was detected at δC/δH 124/7.4 in all lignins, which could be developed from the sugar dehydration reaction during the extraction process (Constant et al., 2016). Hibbert’s ketones (δC/δH 66.2/4.0), a known degradation product from acid-catalyzed process, was observed in all lignins in this study. The occurrence of the same degradation product was also reported in another CC-LA DEEL structural study (Alvarez-Vasco et al., 2016).

Based on the lignin structure information obtained from NMR spectra (Table 4.10), CC-FA is a less suitable lignin extraction solvent as it produces highly altered lignin product. The lack of β-O-4′ linkage and high β-5′ percentage in the CC-FA DEEL indicates that the DEEL might be highly recalcitrant in depolymerisation processes. More elaboration on the suitable application of CC-FA DEEL is provided in Section 4.3.4. On the contrary, CC-LA 1:15 retained higher amount of β-O-4′ linkage at 2.52 Unit/Ar than the other DEELs and LA-extracted lignin with only 0 – 1.74 Unit/Ar β-O-4′ linkage content. In this regard, CC-LA 1:15 DEEL can be considered as a candidate for possible lignin depolymerisation conversion due to the more abundant modification sites.

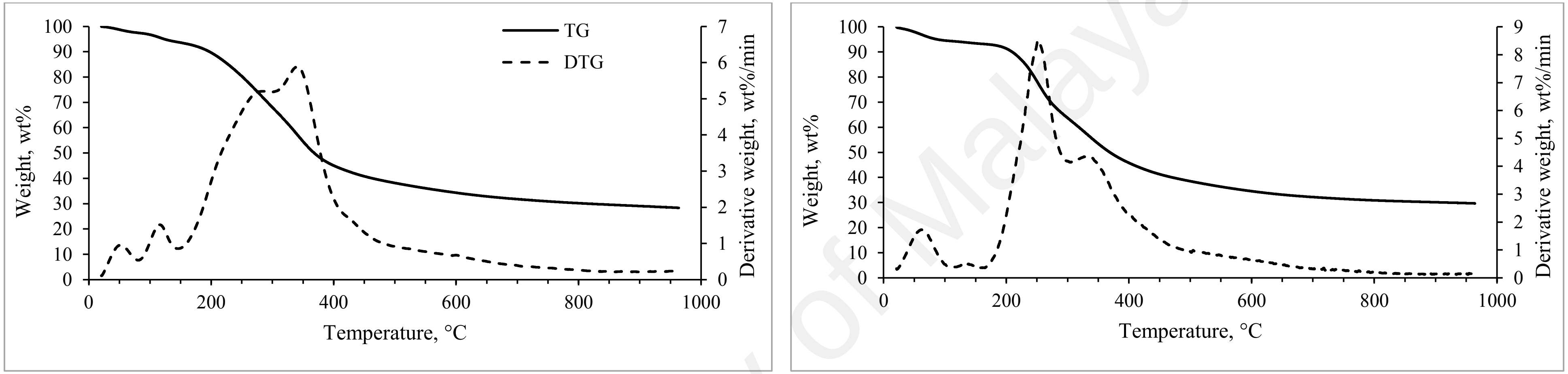
### 4.3.4 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) provides information on the thermal properties of lignin, which will be useful to predict the behaviour of lignin under thermochemical treatment. As a heterogeneous polymer, lignin decomposes over a wide range of temperature since the different functional groups in lignin have varying degree of thermal stability (Y. Huang, Wei, Qiu, Yin, & Wu, 2012; Laurichesse & Avérous, 2014). Figure 4.12 displays the thermogravimetric (TG) and derivative thermogravimetric (DTG) curves of the four investigated lignins in the present study. Despite extracted using different solvents, the lignins exhibited similar degradation trend in general. Three stages of thermal degradation observed were: <200°C, 200-350°C and >350°C. The maximum rate of weight loss occurred at 340°C in LA-extracted lignin, 254°C in CC-LA 1:1, 342°C in CC-LA 1:15 and 274°C in CC-FA DEELs, indicating that most of the lignin components turned to volatiles during the second stage of degradation.

Comparing all four investigated lignins, CC-FA DEEL remained with the most char residue at 42 wt% of the initial lignin weight at 960°C, followed by CC-LA 1:15 with 37% of char. NMR analysis in Section 4.3.3 revealed that CC-FA DEEL had the highest amount of C-C inter-unit linkage per aromatic unit namely $\beta-\beta'$ and $\beta-5'$, the highly resistant linkages in lignin (Table 4.11). This could lead to the high percentage of non-volatile residue that remained stable under high temperature pyrolysis treatment (Ibrahim et al., 2011). This finding suggests that CC-FA would be a suitable solvent for lignin extraction if biochar is one of the targeted pyrolysis products in thermochemical application. Biochar, which can be produced from whole biomass or lignin, has many applications such as fuel, soil quality improvement by decreasing nutrient and water run-off or adsorption surface in water treatment processes (Tripathi et al., 2016).

(a) LA

(b) CC-LA 1:1



(c) CC-LA 1:15

(d) CC-FA

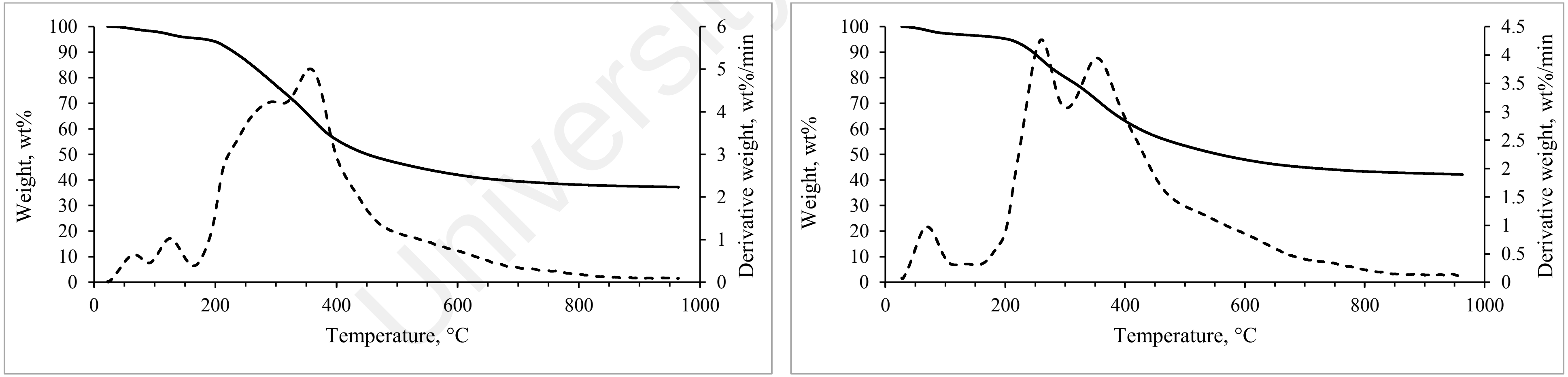
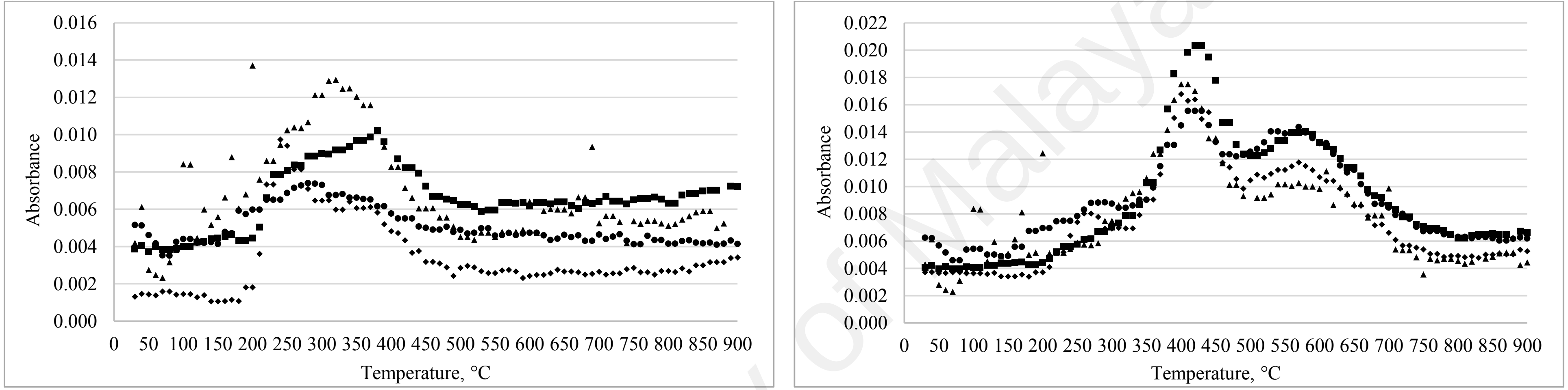


**Figure 4.12: TG and DTG of the lignins extracted using (a) LA (b) CC-LA 1:1 (c) CC-LA 1:15 (d) CC-FA.**

### 4.3.5 Thermogravimetric-Fourier Transform Infrared Spectroscopy (TG-FTIR) Analysis

Thermogravimetric-Fourier transform infrared spectroscopy (TG-FTIR) is a dynamic analysis tool used to monitor the time-dependent volatile organic compounds evolution. This analysis can help to forecast the pyrolysis product yield in the thermochemical valorisation process. The main functional groups of the evolved volatiles identified in the FTIR spectrum were OH from $H_2O$ at 3400-4000 $cm^{-1}$, C-H from $CH_4$ at 2800-3100 $cm^{-1}$, C=O from $CO_2$, CO at 2250-2400 $cm^{-1}$ and 2000-2250 $cm^{-1}$, respectively, C=O from aldehydes, ketones and acids at 1650-1900 $cm^{-1}$, C-O-C from aromatics compounds at 1238 $cm^{-1}$ as well as O-H from alcohol at 1112 $cm^{-1}$. All of which varied in amount when pyrolysis temperature increased from 30°C to 900°C. The absorbance of these products with respect to increasing temperature are depicted in Figure 4.13.

At low temperature below 200°C, the emission of all volatile products were very low in concentration with absorbance at around 0.008. Most of the products reached the highest absorbance i.e. highest emission concentration at around 200°C to 400°C. Above temperature of 500°C, all the volatiles evolution became minimal with absorbance dropped to around 0.008, except for $CH_4$. This result trend implies that the thermochemical process aimed for producing volatile products is best to be conducted under moderate temperature condition between 200°C to 400°C. Information in Figure 4.13 is in accordance with the TGA analysis as shown in Figure 4.12 in which the solid mass loss (which turned into volatiles) was minimal in the low (<200°C) and high (>500°C) temperature range.


(a) $H_2O$ (b) $CH_4$

(c) $CO_2$ (d) CO

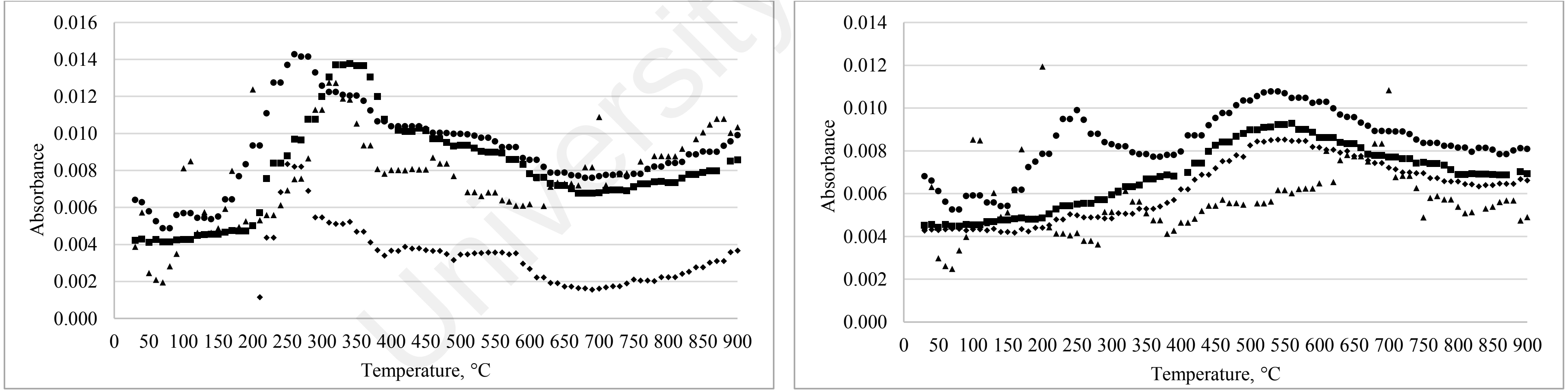
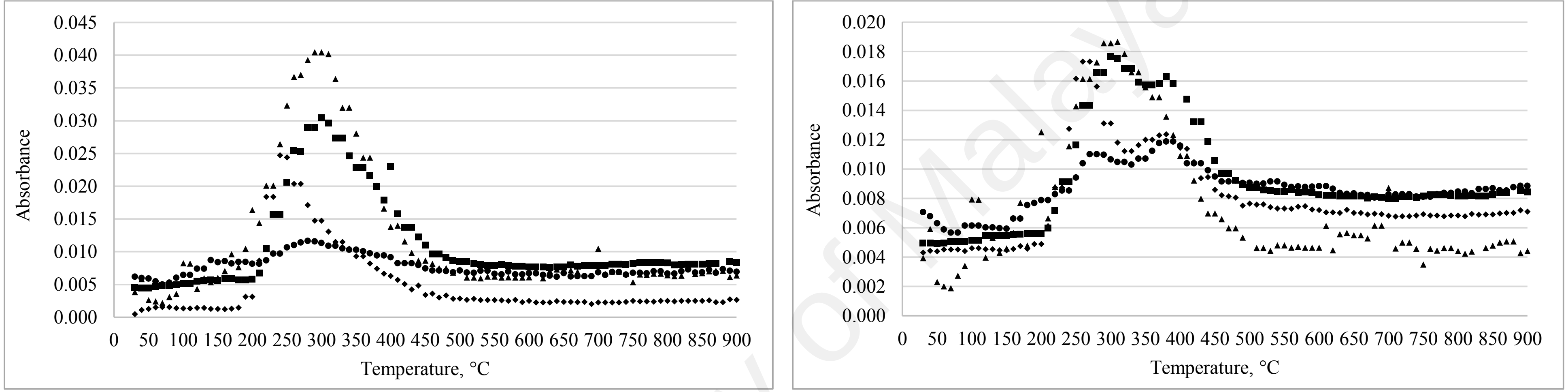
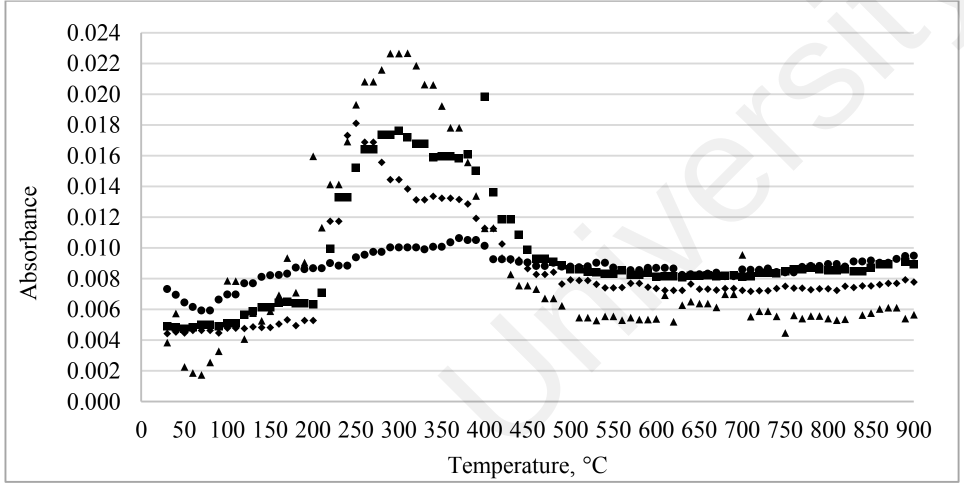


**Figure 4.13: Evolution of volatile products from lignin pyrolysis with increasing temperature. ▲: LA-extracted lignin, ♦: CC-LA 1:1 DEEL, ■: CC-LA 1:15 DEEL, ●: CC-FA DEEL**



(e) C=O aldehydes, ketones and acids

(f) C-O-C aromatic



(g) alcohol

**Figure 4.13 Continued**

As shown in Figure 4.13, evolution trend of the different gas species from all four lignins are rather similar. However, some lignins emitted certain volatiles species at higher amount than the others. Generally, LA-extracted lignin and CC-LA 1:15 DEEL released higher concentration of gaseous products. This is possibly to be due to the higher percentage of the β-O-4 linkages present in the two extracted lignins (Table 4.11). Therefore, the lignins are more prone to depolymerisation into smaller compounds when subjected to thermal treatment.

Small amount of water was still bounded to the lignins even after drying, which has led to $H_2O$ emission with absorbance lower than 0.008 (Figure 4.13a). Evolution of $CH_4$ was found to have two absorption maxima of C-H stretching in Figure 4.13b. The first peak at around 420°C could be from the pyrolysis products of the more weakly-bonded methoxyl or methylene groups, whereas the breakage of aromatic rings at higher temperature (570°C) in lignin structure could lead to the appearance of second C-H peak (Y. Huang et al., 2012). The CC-LA 1:15 lignin which contained the highest amount of methoxyl group (Table 4.11), had a much higher first maxima in $CH_4$ emission than the rest of the lignins. Also, CC-LA 1:15 and CC-FA with the highest amount of total aromatic moieties per aromatic ring (2.82 and 2.73 unit/Ar) had the highest peak for the second maxima.

Furthermore, $CO_2$ and CO were emitted during the pyrolysis process due to the degradation of carboxyl and ether functional groups located at the aliphatic side chains of lignin structure (Y. Huang et al., 2012). Their released amount were comparatively lower than the other volatile species as shown in Figure 4.13c and d. Also, the carbonyl groups containing compounds, such as aldehyde, acid and ester represented by the C=O stretching absorbance peaks were released as the most concentrated volatiles among the other gas species, possibly due to the acid solvent contaminants remained in the extracted lignins (Figure 4.13e). In addition, the extracted lignins which are rich in phenolic compounds also contributed to the appearance of C-O-C bending arising from aromatics and O-H functionalities in the volatiles FTIR spectra, as shown in Figure 4.13f and g, respectively.

TG-FTIR exhibits the potential of DEEL to be applied as fuel in other forms apart from internal energy generation in paper mill based on the emission of $CO_2$, CO and $CH_4$. Lignin can be used as feedstock in gasification or pyrolysis to produce syngas, which is currently mainly derived from coal and natural gas (Holladay et al., 2007). As an intermediate material for the production of other fuels and commodity chemicals such as hydrogen, methanol or ammonia, syngas derived from biomass is envisioned as an important feedstock in the future industry development (Centi & Perathoner, 2019).

### 4.3.6 Pyrolyzer-GCMS (PY-GCMS) Analysis

While TG-FTIR reveals the functional groups of the volatile products of lignin, pyrolyzer-GCMS (PY-GCMS) serves to identify the volatile compounds being released during pyrolysis process. This analytical approach was used in the current study to establish the understanding on lignin's structure and verify the possible products from lignin through thermochemical route.

The categories of pyrolysis products and their respective percentages are tabulated in Table 4.12. The selectivity of pyrolysis products differs from each lignins. The products present in the lignins were mainly aromatic-containing compounds, contributing to between 74.08% to 77.20% of the total pyrolysis products. Majority of the aromatic compounds discovered were phenolic compounds with the weightage around 53.69% to 61.71%. Other products were carbonyls (aldehyde, ketone, acid, ester), hydrocarbons, furanic derivatives and etc.

### Table 4.12: Relative area of different pyrolysis product categories and the distribution of GSH type phenolic compounds

| | Relative area (%) | | | |
|---|---|---|---|---|
| | **LA** | **CC-LA 1:1** | **CC-LA 1:15** | **CC-FA** |
| Phenolic compounds | 61.71 | 57.80 | 56.50 | 53.69 |
| Carbonyl | 11.81 | 17.19 | 16.75 | 5.45 |
| Hydrocarbon | 1.64 | 1.24 | 0.63 | 1.29 |
| Alcohols, ethers | 1.44 | 2.37 | 0.45 | 0.57 |
| Aromatic carbonyl | 8.68 | 8.43 | 7.06 | 9.16 |
| Aromatic hydrocarbon | 3.12 | 2.95 | 1.67 | 1.72 |
| Furanic derivatives | 2.84 | 3.72 | 4.01 | 5.40 |
| Aromatic nitrogen-containing | 0.00 | 2.46 | 4.07 | 1.30 |
| Aromatic alcohol and ether | 0.71 | 1.84 | 0.77 | 3.93 |
| Total aromatic compound | 77.06 | 77.20 | 74.08 | 75.20 |
| **Phenolic compound distribution** | | | | |
| G | 20.40 | 11.39 | 18.11 | 20.45 |
| S | 8.30 | 7.46 | 5.56 | 7.15 |
| H | 71.30 | 81.14 | 76.34 | 72.40 |

Other than lignin-derived compounds, some detected pyrolysis products were originated from carbohydrate residue in the lignins, for example acetic acid and furanic derivatives. Small amount of acetic acid and its derivatives (2.0%-5.5%) were resulted from the elimination of acetyl group from xylose (Gao et al., 2013). Furfural, which is a derivative from glucose degradation, was also present. CC-LA 1:1 with highest glucose content was presented with the highest furfural yield at 0.65% compared with the other lignins. Also, the presence of fatty acids originated from the plants signifies that the natural acids are resistant to DES extraction condition (Constant et al., 2016).

The H unit in general accounted for most of the phenolic compounds (71-81%), followed by G (11-20%) then S (5-8%), as presented in Table 4.12. The remarkably high percentage of H detected suggests that demethoxylation reaction might have occurred during the pyrolysis process, which explains for the high $CH_4$ emission resulting from methoxyl group (Figure 4.12). The GSH distribution information obtained from PY-GCMS analysis highly contrasts with the NMR result suggesting S unit was the most abundant aromatic unit present in lignin (Section 4.3.3). The discrepancy could be due to demethoxylation reaction that occurred during pyrolysis process, resulting in the conversion of S unit to G unit, as well as S and G units to H unit. The degree of demethoxylation determines the GHS unit selectivity in the product distribution. When comparing between the different lignins, LA-extracted lignin was found to contain the highest percentage of S unit and the lowest H unit content than the three DEELs. This indicates that DES can promote demethoxylation reaction better than lactic acid. It also suggests that CC-LA is a better demethoxylation promoting reagent than CC-FA, which would be helpful in deriving p-hydroxyphenyl- and guaiacyl-based aromatic compounds.

Phenol was found to be the richest compound in all four lignins investigated (9.3-15.4%). Other abundant phenolic compounds were such as catechol (2.8-4.2%), 1,2-Benzenediol, 3-methyl- (3.5-4.5%), 1,2-Benzenediol, 4-methyl- (3.7-3.8%). This exhibits the potential of lignin for phenolic compound production, particularly phenol which is an important platform chemical for many petroleum-based chemicals. Also, the hydrocarbon produced from the lignins could be refined as the secondary products in the future lignin product refinery.

### 4.3.7 Summary

The properties analysis of various EFB-derived lignin demonstrated the degree of influence of pretreatment solvent on the extracted lignin. Comparing to lactic acid solvent, DES has better performance in terms of extracting lignin with higher purity and phenolic hydroxyl content. Nevertheless, the basic structure of lignin extracted using lactic acid and DES are rather similar, as shown in the molecular structure detected in NMR and the thermal degradation trend in TGA analysis due to the same biomass species used.

When comparing the different DEELs, CC-LA 1:15 DES has a more labile structure suitable for downstream functional modification to produce smaller aromatic chemicals. This is as evidenced in its more abundant β-O-4’ linkages at 2.52 unit/Ar, higher phenolic hydroxyl content at 3.33 mmol/g, and higher concentration of volatile products released. Contrarily, CC-FA DEEL contained more condensed structure and high percentage of pyrolysis char residue. Thus, due to the varying amount of aromatic structures and linkages, the DEELs obtained using different DESs have the potential for diverse applications. The GSH aromatic unit distribution exhibited in PY-GCMS analysis also demonstrated the possibility of monitoring the selectivity of different aromatic compounds produced from thermochemical process by altering the DES solvent adopted for lignin extraction process.

## 4.4 Potential Bio-products from DES-Pretreated SF and DES-Extracted Lignin – Life Cycle Perspective

In this study, the efficiency of fourteen different DES pretreatments on oil palm empty fruit bunch (EFB) fractionation and lignin extraction have been investigated. CC-FA and CC-LA DESs emerged as the solvents with the highest biopolymer fractionation efficiency and lignin extraction yield. Lignin yield of >60 wt% were achieved and their pretreated SFs were enriched with glucan content to >80 wt%.

Figure 4.14 illustrates the flowchart of a proposed circular biorefinery process centered around the use of lactic acid-based DES as biopolymer fractionation solvent. A biorefinery process can be initiated with DES pretreatment for biomass fractionation, then proceed to the production of a vast variety of bio-products from the DES-fractionated carbohydrate and lignin biopolymers according to their distinctive properties. The highlight of this integrated process is that the bio-products produced not only can be marketed for commodity purpose, but also utilized as part of the production process.

Lactic acid has been identified as one of the top 30 potential candidates of sugar-derived building block chemicals for biorefinery (Holladay et al., 2007). To date, 90% of the lactic acid is produced worldwide via bacterial fermentation from glucose (Bozell & Petersen, 2010; Hofvendahl & Hahn–Hägerdal, 2000). As lactic acid is resulted from glucose fermentation, its production can potentially be incorporated into lignocellulosic biomass biorefinery scheme. For example, an integrated process of ethanol and lactic acid productions from sugarcane bagasse was recommended (Mandegari, Farzad, van Rensburg, & Görgens, 2017). Other than being marketed as commercial product, lactic acid generated in the process can be used for DES synthesis. The possibility of integrating the application of lactic acid in biomass processing with its production in a single process scheme is very attractive. This will ensure a sustainable provision of the hydrogen bond donor material for DES synthesis within the facility instead of outsourcing it.

As mentioned in Section 2.2.2, lignin can be utilized in material, fuel and chemical application. Similar to carbohydrate-based products, the produced lignin-derived products can also potentially be utilized in the production line itself. Other than lactic acid, some lignin monolignols were found suitable to be the hydrogen bond donor to form DES for biomass pretreatment purpose, such as 4-hydroxybenzyl alcohol, catechol, vanillin and p-coumaric acid (Kim et al., 2018). This provides more opportunities to synthesize chemicals from lignocellulosic biomass that are compatible as raw material in DES synthesis. In addition, TGA analysis revealed that certain DES like CC-FA can produce a high percentage of biochar following pyrolysis thermochemical treatment process. Other than using it as fuel, the biochar can also be employed to improve the soil quality in oil palm plantation.

In short, Figure 4.14 presents a preliminary life cycle perspective manifesting the possibility to design a circular biomass utilization process through the application of DES. Nevertheless, biomass upgrading process using DES still requires more extensive researches to reach the stage of commercialization. More recommendations to move forward on this research field are discussed in the next chapter.

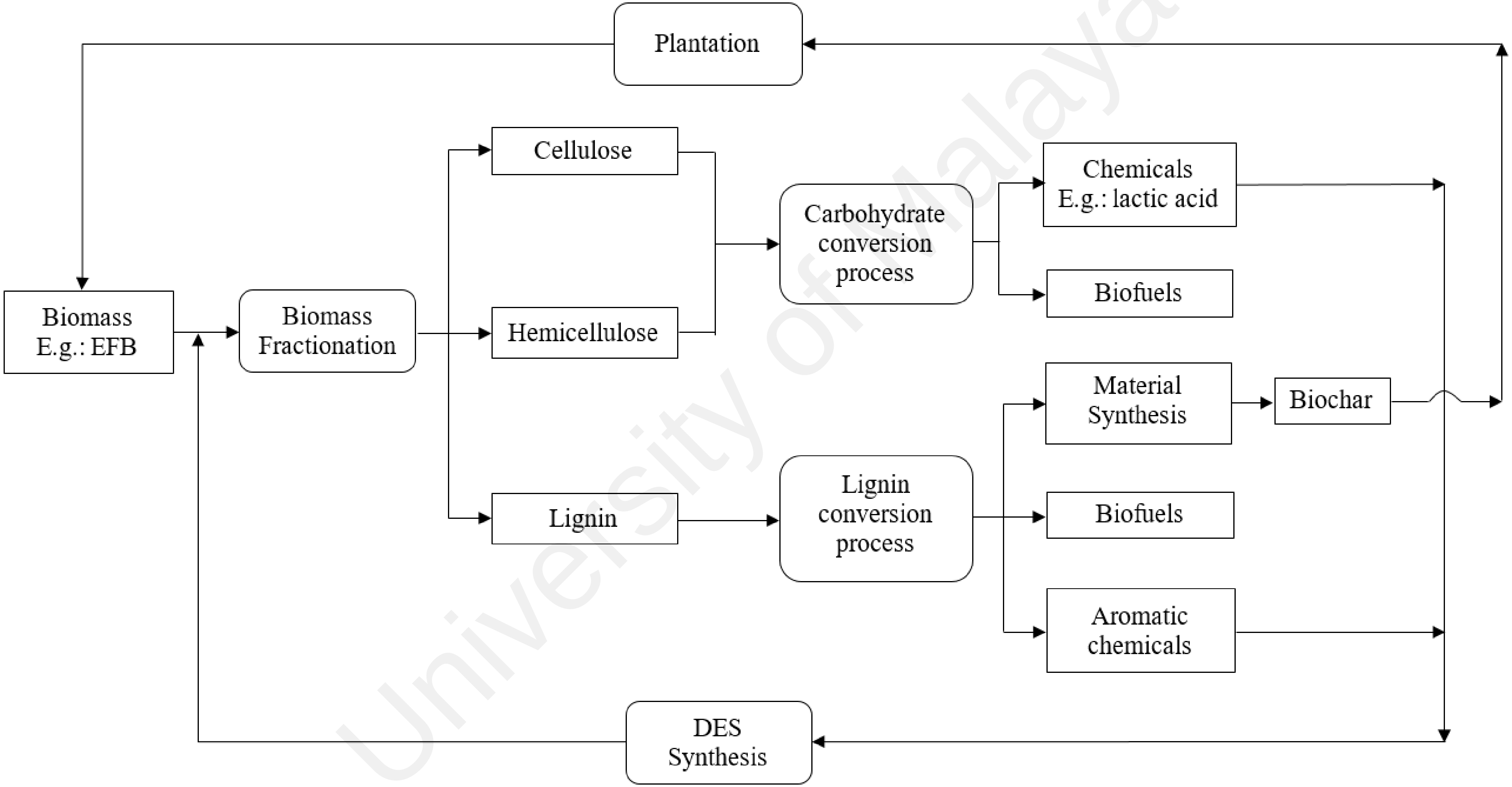


Figure 4.14: Proposed circular system of biomass fractionation and upgrading process using DES

# CHAPTER 5: CONCLUSION AND RECOMMENDATION

## 5.1 Conclusions

This project investigated the performance of varying type of deep eutectic solvents (DES), an alternative green designer solvent in replacement of conventional solvent, on oil palm empty fruit bunch (EFB) fractionation and lignin extraction. The research findings are concluded as follows.

i. Fourteen different DESs with varying pH nature and acid functional groups stable under ambient room condition were successfully synthesized in-house. The different combinations of chemical constituents were found to form DES under varying hydrogen bond acceptor to hydrogen bond donor molar ratio condition. When subjected to EFB pretreatment, the synthesized DESs demonstrated different level of efficiency in biopolymer fractionation and lignin extraction based on the selected constituent.

ii. In the screening study of suitable DES type for EFB pretreatment, the influence of solvent viscosity on the performance of DES, as well as the energy consumption level of DES pretreatment were determined to be less prominent than the effect of pH nature. The investigated DESs which were constituted of different raw constituents consumed similar range of energy at 2.1 to 2.5 kJ/g in EFB pretreatment. Acidic DES exhibited significantly enhanced biopolymer fractionation and lignin extraction efficiency than the near-neutral and basic DESs, owing to its ability in hemicellulose hydrolysis and extraction of lignin that could be precipitated into solid form. Therefore, acidic DES was determined as the suitable DES type for biomass fractionation and lignin extraction.

iii. In the selection of carboxylic acid hydrogen bond donor, the presence of hydroxyl group, double bond, short alkyl chain and monocarboxylic functional group structure on carboxylic acid demonstrated positive influence on the acidic DES's biomass fractionation and lignin extraction performance. With hydrogen bond donor encompassing the stated functional groups, choline chloride:formic acid (CC-FA) with 1:2 molar ratio and choline chloride:lactic acid (CC-LA) with 1:15 molar ratio achieved the highest lignin extraction yield from EFB at 62 wt% and 61 wt%, respectively. Additionally, the DES-pretreated EFB biomass from both DES pretreatments were respectively enriched with 86 wt% and 83 wt% of glucan content, which signifies the efficiency of the DESs in separating lignin and carbohydrate fraction from the biomass matrix. The findings confirm that the selection of suitable acid constituent and DES molar ratio are important to ensure optimum biopolymer fractionation and lignin production efficiency.

iv. The DES-pretreated EFB solid can be employed as carbohydrate feedstock for polysaccharide-based product conversion due to its enriched cellulose content (>80 wt%) and minimal lignin remnants. Diverse type of lignin-derived products can also be potentially produced by engineering the type of DES for lignin extraction. For instance, CC-FA DEEL which has more condensed structure as indicated by higher percentage of C-C linkages is suitable to be applied as carbon source. CC-LA 1:15 DEEL is recommended for smaller aromatic chemical production through functional modification process as it contains the higher amount of β-O-4′ linkage (the usual site for modification) and volatile products. Based on the life cycle analysis conducted, the application of DES in biomass pretreatment enables the possibility of a circular biomass utilization process by producing the raw material for DES synthesis within the facility.

In conclusion, this project introduces an alternative value-added application for EFB which capitalizes on its rich lignocellulosic biopolymer content. This pretreatment scheme using DES has proved to be highly effective to overcome the biomass recalcitrance for biopolymer fractionation and lignin extraction. The lignin extracted with varying properties using different types of DES will be useful for a wide variety of applications, therefore increasing the economic attractiveness of the biorefinery. This project can be used as a guideline in the selection of suitable DES constituents for lignocellulosic biomass fractionation and lignin extraction. With the use of DES as biomass fractionation solvent, the design of a circular system in biorefinery, which is becoming a prerequisite of the industry for sustainable development, is achievable in the future.

## 5.2 Novelties and Implications of This Project

Some novelty and implication aspects resulted from this study are listed as below.

i. This study has proposed an alternative application of oil palm empty fruit bunch (EFB), one of the most abundant agricultural wastes in Malaysia, as feedstock for fuel and chemical production. EFB is a recalcitrant biomass source encompassing of rich lignocellulosic biopolymers. In recent years, the production of EFB pellet to be used as solid fuel is getting more common. While it is an efficient way to utilize this abundant agricultural waste, finding more value-added applications to increase the revenue to oil palm industry would be very beneficial to our country. The application of DES in fractionating EFB will provide the opportunity to maximize each biopolymer's potential for product conversion according to their inherent properties.

ii. This study has performed a comprehensive evaluation on the performance of 14 types of DES with varying pH nature and functional groups. The pH nature and functional group of DES were revealed to impact on their fractionation and lignin extraction efficiency. The outcome of this project would be helpful in aiding the selection of suitable DES solvent for efficient lignocellulosic biomass fractionation and lignin extraction.

iii. This study focused on evaluating the lignin extraction performance of varying type of DES. Currently, most of the reported works on DES application targeted to enhance the enzymatic hydrolysis performance of DES-pretreated biomass, and often oversighted the valuable aromatic lignin biopolymer. The structural and thermal properties of DEEL elucidated in this project with discussion on their potential for downstream conversion can promote the understanding on DEEL's structure and also motivate the valorization of DEEL in downstream processing.

iv. This study addressed the possibility of designing a circular biorefinery system through a preliminary life cycle analysis. A biorefinery process can start with DES pretreatment for biopolymer fractionation and lignin extraction. The fractionated biopolymers can then be converted into commodity chemical, DES constituent material or fuel for internal heating. A circular biorefinery system incorporated with the application of green solvent in biomass processing can certainly play an important role in the future sustainable development.

## 5.3 Recommendations

Following the completion of this project, some research areas associated with the application of DES in biomass processing are recommended for future studies.

i. To develop a complete and precise DES pretreatment protocol, including pretreatment scheme, washing and recycling procedure. Standardizing these protocols is a crucial step to ensure the DES-fractionated products have consistent quality and properties. Large volume of solvents is usually required in washing procedure. Research on minimizing the amount of solvents would reduce the operating cost and environmental impact.
ii. To perform optimization study on the operating parameters based on the desired end-products. The adopted pretreatment operating parameters such as temperature, duration, solid to liquid ratio or pressure would affect the properties of DES-fractionated products. Researches with well-defined pretreatment goal, for instance enhancement of enzymatic hydrolysis performance or preparation of lignin for depolymerisation conversion are advisable. The response to the pretreatment optimization should be aimed to optimize the efficiency of commodity product conversion.
iii. To perform comprehensive techno-economic assessment of the DES pretreatment process. Extended techno-economic assessment is also a pressing research topic to further establish the feasibility of this alternative technology. To ensure the accuracy of the assessment, the use of mathematical model or simulation software such as MATLAB, ASPEN HYSYS or HINT is highly encouraged. The outcome of these assessment would aid in establishing large scale setup to move towards commercialization.

iv. To apply DEEL as feedstock for lignin product conversion. The lignin properties study suggested that DEEL extracted using different DES solvents are suitable for different applications. Therefore, the applicability of DEEL in varying types of conversion schemes such as fuel, polymeric application or chemical production should be explored further. Since lignin is the most abundant renewable aromatic resource on Earth, researching on the possible DEEL applications, which are yet to be accomplished by the existing type of lignin, will be a very valuable research direction.

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# LIST OF PUBLICATIONS AND PAPERS PRESENTED

## Published Journal Articles

1. **Tan, Y. T.**, Ngoh, G. C., & Chua, A. S. M. (2018). Evaluation of fractionation and delignification efficiencies of deep eutectic solvents on oil palm empty fruit bunch. *Industrial Crops and Products, 123*, 271-277. doi:10.1016/j.indcrop.2018.06.091
2. **Tan, Y. T.**, Ngoh, G. C., & Chua, A. S. M. (2019). Effect of functional groups in acid constituent of deep eutectic solvent for extraction of reactive lignin. *Bioresource Technology, 281*, 359-366. doi:10.1016/j.biortech.2019.02.010
3. **Tan, Y. T.**, Chua, A. S. M., & Ngoh, G. C. (2020). Deep eutectic solvent for lignocellulosic biomass fractionation and the subsequent conversion to bio-based products - A review. *Bioresource Technology, 297*. doi:10.1016/j.biortech.2019.122522
4. **Tan, Y. T.**, Chua, A. S. M., & Ngoh, G. C. (2020). Evaluation on the properties of deep eutectic solvent-extracted lignin for potential aromatic bio-products conversion. *Industrial Crops and Products*, 154. doi:10.1016/j.indcrop.2020.112729