Alkaline Hydrogen Peroxide and Deep Eutectic Solvent Sequential Pretreatment of Oil Palm Fronds

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Abstract

Recently, significant focus has been given to the valorization of lignocellulosic biomass, which is usually neglected and not fully utilized despite its abundance and potential use to transform into bioenergy and bio-based products. Cost-effective pretreatments are essential to overcome recalcitrant properties of lignocellulosic biomass in order to reutilize cellulosic components within the biomass for value-added products. Oil palm frond (OPF) is one of the abundantly available lignocellulosic biomass in Malaysia, making it the promising source for biomass valorization. For the past decade, alkaline hydrogen peroxide (AHP) was known to be an effective pretreatment method for lignocellulosic biomass. On the other hand, a new green solvent, namely deep eutectic solvent (DES) received much focus in the field of biomass processing due to its simple synthetization method and promising performance. In this study, AHP at ambient temperature (~25°C) and pressure (1 atm) was found to improve delignification synergically together with subsequent pretreatment using DES. A combination of AHP (0.25 vol%, 90 min) and Type III DES (ChCl:Urea, 120°C, 4 h) at the later stage resulted in a pretreated OPF with a delignification of 18.99%, remarkably improved Type III DES-alone delignification extent by 1.6 folds. By investigating operating conditions in Type III DES (ChCl:Urea) pretreatment, the delignification of OPF was improved further to 35.41%. In order to enhance the performance of sequential pretreatment, novel Type II DES (ChCl:$CuCl_2$•$2H_2O$) was introduced. It has been observed, Type II DES (ChCl:$CuCl_2$•$2H_2O$) has the ability to recover monomeric sugars directly through the DES hydrolysate. Thus, it is of interest to select a recommended condition for delignification while maximizing sugar recovery. The sequential pretreatment of AHP (0.25 vol%, 90 min) at ambient temperature and pressure with Type II ChCl:$CuCl_2$•$2H_2O$ DES (90°C, 3 h) at the later stage resulted in delignification of 55.14% and xylose recovery of 28.96% with extraordinary xylan (80.79%) and arabinan (98.02%) removals. Thus, the application of this novel Type II DES was able to provide effective lignin and hemicellulose removals from the OPF while recovering portion of xylose and arabinose monomeric sugars.

Publications

Journal articles

1. **Ho, M.C.**, Ong, V.Z., Wu, T.Y., 2019. Potential use of alkaline hydrogen peroxide in lignocellulosic biomass pretreatment and valorization–A review. Published in Renewable and Sustainable Energy Reviews (Rank 1 in Green and Sustainable Science and Technology), vol. 112, pp. 75-86.
2. **Ho, M.C.**, Wu, T.Y., Chee, S.W.Q., Ngang, C.Y., Chew, I.M.L., Teoh, W.H., Jahim, J.M., Mohammad, A.W., 2019. An application of low concentration alkaline hydrogen peroxide at non-severe pretreatment conditions together with deep eutectic solvent to improve delignification of oil palm fronds. Published in Cellulose (Rank 1 in Materials Science, Paper, and Wood), vol. 26(16), pp. 8557-8573.
3. **Ho, M.C.**, Wu, T.Y., 2019. Sequential pretreatment of alkaline hydrogen peroxide and choline chloride:copper (II) chloride dihydrate - Synergistic fractionation of oil palm fronds. In press in Bioresource Technology (Rank 1 in Agricultural Engineering), 25 Dec 2019.

Conference proceedings

1. **Ho, M.C.**, Wu, T.Y., 2019. Potential use of alkaline hydrogen peroxide in biomass pretreatment and valorization – a review. In: 6th International Conference on Sustainable Solid Waste Management (NAXOS 2018). Naxos Island, Greece; p. 1-11.
2. **Ho, M.C.**, Wu, T.Y., 2019. Application of novel copper (II) chloride dihydrate deep eutectic solvent with alkaline hydrogen peroxide at non-severe pretreatment conditions in delignification and sugar extraction of oil palm fronds. In: 10th International Conference on Environmental Engineering and Management (ICEEM 2019). Iasi, Romania; p. 1-2.

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 2 original papers published in peer reviewed journals and 1 in press publication. Refer to **Appendix A**, **Appendix B**, and **Appendix C** for the original published papers. The core theme of the thesis is Waste Pretreatment. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Engineering under the supervision of A/P Wu Ta Yeong.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapters 2 and 3 my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status** *(published, in press, accepted or returned for revision, submitted)*	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author(s), Monash student Y/N***
2, 3	*Potential use of alkaline hydrogen peroxide in lignocellulosic biomass pretreatment and valorization–A review*	*Published*	*50%. Concept, collecting data, outline content, structure content, and writing first draft*	*1) Ong Zhenquan, input into manuscript and writing 50%*	*Yes*
3	*An application of low concentration alkaline hydrogen peroxide at non-severe pretreatment conditions together with deep eutectic solvent to improve delignification of oil palm fronds*	*Published*	*80%. Concept, data collecting, data analysis, discussion, and writing*	*1) Samuel Chee Wei Qiang, experimental work and data 10%* *2) Ngang Chia Yee, experimental work and data 10%*	*Yes*
4	*Sequential pretreatment of alkaline hydrogen peroxide and choline chloride:copper (II) chloride dihydrate - Synergistic fractionation of oil palm fronds*	*In press*	*100%. Concept, experimental work, data collecting, data analysis, discussion, and writing*	*N/A*	*N/A*

I have / ~~have not~~ renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student name: Ho Mun Chun

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I hereby certify that the above declaration correctly reflects the nature and extent of the student's and

co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor name: A/P Wu Ta Yeong

Main Supervisor signature: **Date: 1/11/2019**

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List of abbreviations

AHP – Alkaline hydrogen peroxide

ANOVA – Analysis of variance

ATR – Attenuated total reflection

BET – Brunauer-Emmett-Teller

CrI – Crystallinity index

DES – Deep eutectic solvent

DTG – Derivative thermogravimetric

FE-SEM – Field emission scanning electron microscopy

FTIR – Fourier transform infrared spectroscopy

HBA – Hydrogen bond acceptor

HBD – Hydrogen bond donor

HMF – Hydroxymethylfurfural

HPLC – High performance liquid chromatograph

I_{200} – Maximum instensity of 200 peak ($2\theta = 22°$)

I_{am} – Minimum intensity between peaks 200 and 110 ($2\theta = 18°$)

L – Lignin content in pretreated OPF

L_0 – Lignin content in raw OPF

ND – Not detectable

OPF – Oil palm frond

TGA – Thermogravimetric analysis

XRD – X-ray diffraction

Y – Mass yield or mass recovery of pretreatment in fraction

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Chapter 1

Introduction

1.1 Preface

The conversion of biomass into various value-added biochemical, including biofuel, is generally termed as biomass valorization (Loow et al., 2016a). Recent research publications indicate the shift of global research focal point to waste valorization as the alternative solution in chemicals and bioenergy productions. Annually, hundreds billion tonnes of lignocellulosic biomass waste is produced, but they are usually neglected via disposal in a landfill or through burning (Luo et al., 2014). These practices do not contribute to any economic benefits to the industry and at the same time, do not fully utilize the biomass despite its abundance in mass. The availability of lignocellulosic biomass is not limited by geographical distribution, hence making it a promising feedstock for biofuel production and other value-added products (Himmel et al., 2007; Ragauskas et al., 2006). Furthermore, the abundance of agricultural residues, which could be acquired at low cost, making it a better and more promising option as raw feedstock in sugar platform biorefinery (Díaz et al., 2014; Thompson and Meyer, 2013). To date, more and more research on the valorization of agricultural residual into biofuel has attracted significant attention, mainly due to the positive effects from both economic and environmental aspects and long term energy sustainability with greenhouse gas mitigation (da Costa Correia et al., 2013; Li et al., 2013). Therefore, the valorization of lignocellulosic biomass for the value-added products and biofuel productions could potentially relieve the stresses to the natural resources and shift the dependencies of fossil fuel to sustainable energy sources.

Lignocellulosic biomass is a complex structure of carbohydrate polymers which consist of cellulose (38 – 50%), hemicellulose (23 – 32%), and aromatic polymer lignin (15 – 25%) (Mamman et al., 2008). Cellulose is a strong molecular structure in biomass, which is formed by long chains glucose monomeric units connecting via β-glycosidic linkages with reducing and non-reducing ends (Choudhary et al., 2016). These cellulose microfibrils which possess both amorphous and crystalline properties are then stacked in parallel due to the presence of hydrogen bonding and weak van der Waals forces. Thereafter, these microfibrils are interlaced together with hemicellulose and lignin to form macro fibrils (Loow et al., 2017a). As a result, access to cellulose by chemical or enzymatic means is difficult due to the presence of complex linkages network and hydrogen bonding (Balat, 2011). Hemicellulose is considered an amorphous polymer, which is a cluster of heterogeneous polysaccharides with a mix of hexose and pentose monosaccharides (Jia et al., 2014; Li et al., 2014; Si et al., 2015; Wu et al., 2014). When chemicals and/or heat are applied, hemicellulose is relatively easier to dissociate as compared to cellulose (Cherubini, 2010). Typically, softwood based biomass contains mainly glucomannans and mannans, while xylan (β-(1,4)-linked xylose homopolymer) are usually found in hardwood based biomass (Choudhary et al., 2016). Therefore, both cellulose and hemicellulose contribute to most of the sugar present in the biomass.

Lignin is a hydrophobic polymer composed of several cross-linked polymers of phenolic substances (Kumar et al., 2009), bounded to the hemicellulose and cellulose in a complex polymer matrix which acts as a physical barrier against microbial attack as well as provides structural support to the plant cell (Li et al., 2014; Si et al., 2015; Wu et al., 2014; Xu et al., 2012). Lignin contains several functional groups, such as hydroxyl, carbonyl, and methoxyl in the lignin matrix, which contribute to high polarity of lignin macromolecule (Feldman et al., 1991). These recalcitrance properties of plant biomass prevent enzymes from accessing the carbohydrate substrate (Díaz et al., 2014). In general, pretreatments to overcome lignocellulosic recalcitrance are costly and sometimes involve inhibitors to the biofuels conversion (Li et al., 2016b), hence hindering the development of an economically viable process to convert biomass to biofuels or other valuable products (Alvarez-Vasco and Zhang, 2013). Many research has revealed the negative effect of lignin during enzymatic digestion of biomass. Therefore, an effective lignin extraction or disruption could significantly alter the structural matrix and crystallinity of cellulose and ultimately enhance biomass saccharification in fermentation (Li et al., 2014; Si et al., 2015; Wu et al., 2014; Xu et al., 2012).

A typical biorefinery processing of lignocellulosic biomass involves pretreatment step, subsequent enzymatic hydrolysis to disintegrate polysaccharides, and fermentation of released sugar solution to produce biofuels and biochemical (da Costa Correia et al., 2013; Li et al., 2013). In order to facilitate downstream biorefinery of lignocellulosic biomass, a variety of pretreatment stage has been proposed to overcome recalcitrant structure of biomass by disrupting carbohydrate matrix and disintegrate high molecular structures like lignin, hemicellulose, and cellulose into smaller compounds, such as monosaccharides, organic acids, and phenolic compounds (Alvarez-Vasco and Zhang, 2013; Michalska and Ledakowicz, 2014). A wide range of biomass fractionation approaches have been studied, such as chemical (alkali, dilute acid, organic solvent), physical (grinding, milling, irradiation), physiochemical (wet oxidation, auto-hydrolysis), and biochemical methods (Loow et al., 2017a).

To date, the disruption of the complex polymer matrix to release monosaccharides remained as the main challenge for effective utilization of lignocellulosic biomass. Thus, methods that improve the biomass digestibility for the subsequent enzymatic or microbial attack have gain greater attention in recent years (Cabrera et al., 2014). Recent research has revealed the potential of deep eutectic solvent in biomass pretreatment and fractionation (Loow et al., 2017a). On top of that, alkaline hydrogen peroxide was widely used in biomass processing due to its excellent delignification performance and relatively milder operating conditions (Ho et al., 2019a). Thus, it is of interest to study the synergism between alkaline hydrogen peroxide and deep eutectic solvent in sequential pretreatment to improve the overall performance of biomass pretreatment.

1.2 Problem statement

The complex structure of lignocellulosic biomass often make the conversion of carbohydrate into biofuel or chemicals inefficient. As such, pretreatments are required to improve the accessibility of biomass. From the past decades, many chemical pretreatments were introduced for biomass valorization, which include acid and alkali pretreatments. However, extreme temperature and pressure were required in order to alter lignocellulosic structure significantly. Hence, this work explores the incorporation of alkaline hydrogen peroxide (AHP) with deep eutectic solvent (DES) in sequential pretreatment, in order to develop an novel integrated biomass pretreatment process that could achieve comparable delignification under less extreme conditions.

Although AHP has been widely studied for biomass pretreatment, the literature regarding the application of AHP under mild conditions in sequential pretreatment strategies is still very limited. Also, the applications of DESs in biomass processing still require further exploration. To the best of our knowledge, no investigation has been carried out to study the sequential pretreatment of low concentration AHP at mild conditions (ambient temperature and pressure) with DES in pretreating oil palm fronds (OPF). It was hypothesized that the introduction of mild concentration (<1.00 vol%) AHP at ambient conditions could help improve macrostructure alteration and partial lignin solubilization of OPF. Therefore, facilitating DES in accessing the biomass matrix and providing better disruption on the hydrogen-bonding network, which led to a better delignification.

DES has been critically involved in the recent studies of biomass pretreatment. However, the study regarding the application of Type II DES (metal salt hydrate + organic salt) in biomass fractionation is not available. On the other hand, even though inorganic salts such as $FeCl_3$, $AlCl_3$, and $CuCl_2$ have been extensively studied for pretreating biomass and hemicellulose extraction (Loow et al., 2015; Loow et al., 2018), no available literature was found regarding the application of inorganic hydrate salt-based Type II DES. To the extent of our knowledge, no investigation has been carried out to study the potential use of inorganic hydrate salt ($CuCl_2 \cdot 2H_2O$) based Type II DES and its synergistic effects with AHP in the pretreatment of OPF. It was hypothesized that the Type II DES might inherit the ability of inorganic salt ($CuCl_2$) to selectively hydrolyze hemicellulose from the fronds at relatively lower temperature and atmospheric pressure.

1.3 Research objectives

The objectives of this research were:

1) To determine the synergistic effects of low concentration AHP at mild conditions together with Type III DES (choline chloride:urea) in OPF delignification pretreatment (**Chapter 3**).

2) To investigate the potential use of novel Type II DES (choline chloride:copper (II) chloride dihydrate) by comparing to Type III DES (choline chloride:urea) in OPF processing and its synergistic effects with AHP at a non-severe condition in a sequential pretreatment (**Chapter 4**).

1.4 Scopes of study

The experiment study can be divided into following phases:

Phase 1: The proposed study emphasized on the effect of OPF delignification via AHP pretreatment under mild concentration followed by a second stage Type III ChCl:Urea DES to study the synergistic effect of the sequential pretreatment (**Objective 1**). The research scopes are summarized as follows:

a) The AHP was synthesized by mixing hydrogen peroxide with deionized water and adjusting the pH to 11.50 with the use of sodium hydroxide pellets. The AHP was prepared for 5 sets with concentration varies from 0.05 – 1.00 vol% of solution.
b) The efficiency in OPF delignification was evaluated by first undergo AHP pretreatment with various concentration ranges from 0.05 – 1.00 vol%, temperature ranging from room temperature to 90°C and pretreatment duration of 30 to 120 min, followed by a second stage pretreatment of DES with a duration of 4 h at 120°C.

Phase 2: The research scopes focused on the effect of temperature and duration of Type III ChCl:Urea and Type II ChCl:$CuCl_2$•$2H_2O$ DESs in delignification and sugar recoveries of OPF after AHP pretreatment (Objective 2). The following are the scopes of this research study:

a) The ChCl:Urea DES was prepared by mixing ChCl and urea at molar ratio of 1:2, stirred at 200 rpm under 90°C and atmospheric pressure until homogeneous colourless liquid was formed.
b) The ChCl:$CuCl_2$•$2H_2O$ DES was prepared by mixing ChCl and $CuCl_2$•$2H_2O$ at molar ratio of 1:1, stirred at 200 rpm under 45°C and atmospheric pressure until homogeneous clear brown liquid was formed.
c) The efficiency of OPF delignification was evaluated by first undergo AHP pretreatment at recommended conditions obtained from phase 1, followed by DES pretreatment at temperature ranging from 110 to 140°C (Type III); 80 to 100°C (Type II) and duration of 1 to 4 h.

The scope of work involved in this study is summarized and presented in the process flow diagram (**Fig. 1.1**).

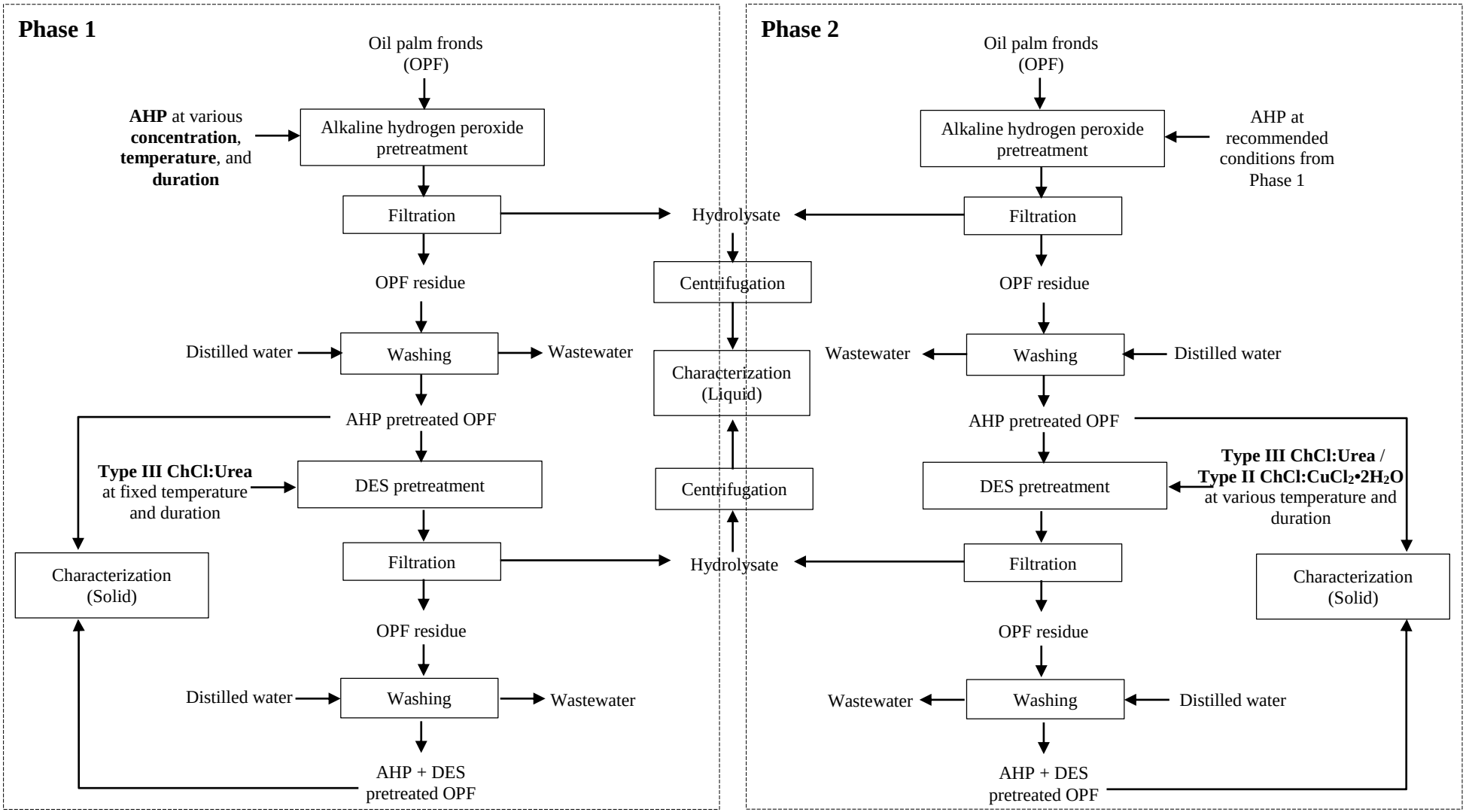

Fig. 1.1 Process flow diagram of the work scope involved in this study.

Chapter 2

Literature review

Publication:

1. **Ho, M.C.**, Ong, V.Z., Wu, T.Y., 2019. Potential use of alkaline hydrogen peroxide in lignocellulosic biomass pretreatment and valorization–A review. Renewable and Sustainable Energy Reviews 112, 75-86. (**Appendix A**)

2.1 The importance of renewable energy and biofuel

Over the past decades, the rapid development of countries and the continuous growth of population over the globe has caused a significant increase in energy consumptions, which has resulted in the grave problem of depleting reserves of non-renewable energy. According to the International Energy Agency, transportation dominates oil consumption over the globe, where it contributes more than 70% of greenhouse gases to the environment. Due to the increasing awareness of sustainable development, it has drawn scientist's attention to find an alternative energy source to replace the uses of non-renewable energy. One of the promising renewable energy sources is biofuel from biomass. Biofuel is a relatively environmental friendly bioenergy as compared to fossil fuel as the former is biodegradable, non-toxic, and low emission profile (Mahapatra and Manian, 2017).

In recent decades, there has been an increasing interest in producing biofuel from palm oil as an alternative sustainable and environmentally-friendly energy source (Kurnia et al., 2016). However, there are several problems associated with such an application, such as the fact that biofuel from palm oil is insufficient to fulfill the global fuel demand, competition between food and energy, and deforestation due to the expansion of oil palm plantations (Mukherjee and Sovacool, 2014). As such, numerous processes and studies have been proposed to produce biofuel from oil palm waste (lignocellulosic biomass).

2.2 Lignocellulosic biomass

There has been growing interest to replace non-renewable fuel to renewable biofuel. Bioethanol derived from cellulosic source attracts much focus from the researcher owing to their high availability. Bioethanol was produced from an energy source named lignocellulosic biomass, where it comprises from three main components which are cellulose (30-50%), hemicellulose (15-35%) and lignin (10-30%) (Krasznai et al., 2018).

Cellulose is a linear homogenous polymer comprising of anhydrous D-glucose which are linked with β-1,4 glycosidic bonds by intramolecular hydrogen bonds. The high degree of hydrogen bonding within cellulose contributes to their structural integrity and formation of highly ordered crystalline network that are not readily accessible by water (Krasznai et al., 2018). It has been found out that the cellulose consists of approximately 50-90% of crystalline cellulose while the remaining are disorganized amorphous cellulose (Foyle et al., 2007). Hemicellulose is a branched heterogeneous polymer of both pentose and hexose. The most common hemicellulose in pentose existing in straws and grasses are xylan, arabino-4-O-methyl glucurono- xylan, arabino-glucurono- xylan while hemicellulose exists in hexose are mainly mannans and β-glucans (Foyle et al., 2007). Hemicellulose interacts with cellulose to form hydrogen bonds with microfibrils, thus enhances the structural

integrity and stability of cellulose-lignin matrix (Krasznai et al., 2018). Lignin functions as a binding and encrusting agent, existing at the external surroundings of lignocellulosic microfibril, providing structural integrity and acts as a protective barrier (Krasznai et al., 2018). This macromolecule consists of three primary monomers, namely Sinapyl alcohol, Coniferyl alcohol, and *p*-Coumaryl alcohol linked by aryl ether or C-C bonds. **Table 2.1** below summarized the overall compositions of each component in various lignocellulosic biomass.

Table 2.1

Compositions of lignocellulosic biomass in various samples.

Biomass Sample	Composition (%)			References
	Lignin	Hemicellulose	Cellulose	
Oil Palm Fronds	14.0	19.2	31.5	(Hong et al., 2012)
Wheat Straw	15.0	50.0	30.0	(Kumar et al., 2009)
Beech Wood	27.3	30.8	48.5	(Kalogiannis et al., 2015)
Corn Cobs	15.0	35.0	45.0	(Kumar et al., 2009)
Oil Palm Trunk	19.1	16.2	56.1	(Zulkefli et al., 2017)

2.3 Oil palm fronds (OPF)

Oil palm frond is one of the lignocellulosic biomass available in Malaysia. Presently, Malaysia has one of the largest oil palm plantations in the world. This industry generates more than 90% of the national total of lignocellulosic biomass (Loow et al., 2017b). Oil palm frond is a by-product produced from the oil palm plantation, which is also the highest solid waste generated from the oil palm industry. Each year, approximately 26.2 million tons of oil palm fronds are produced per million of fresh fruit bunches processed (Yunus et al., 2010). Even though oil palm fronds are readily available sources for a biorefinery, the fronds are typically used as mulches in the plantation (Loow and Wu, 2018). This approach has little economic value to the industry, while environmental problems may arise due to the improper handling of biomass decomposition (Isikgor and Becer, 2015). Thus, the valorization of oil palm fronds may potentially serve as a solution towards sustainable biorefinery.

2.4 Production of biofuel

Biofuel such as bioethanol and biogas can be produced through fermentation and microorganism digestion of the biomass. However, the recalcitrant properties of biomass often hinder the direct fermentation and microorganism attack. Therefore, pretreatments to remove the recalcitrant barrier or fractionate biomass were essential for the productions of biofuel. In recent decades, much attention was given to the innovation of the new pretreatment method to improve the enzymatic sugar

yield and ultimately enhance the conversion to biofuel through fermentation or digestion. Countless research has been attempted to utilize pretreated lignocellulosic biomass to generate the second generation of bioethanol and biogas. It has been found that the sugar yield of the pretreated bamboo chips was 87% higher in comparison to raw bamboo chips, where up to 95% conversion of sugar into bioethanol was achieved (Yuan et al., 2018). From **Table 2.2**, it was observed in many studies, where the incorporation of pretreatment improved the enzymatic hydrolysis and sugar yield of the biomass compared to direct hydrolysis of raw biomass. Thus, pretreatment is crucial in order to realize the valorization of biomass in biorefinery.

2.5 Types of pretreatment process

Lately, thermochemical pretreatment, such as combustion, gasification, pyrolysis, and others, have been introduced in biomass conversion. Due to the recent interest in biofuel production, a wide range of biomass fractionation approaches have been studied, such as chemical (alkali, dilute acid, organic solvent), physical (grinding, milling, irradiation), physiochemical (wet oxidation, auto-hydrolysis), and biochemical methods (Loow et al., 2017a). For the past decade, ionic liquids have been in the spotlight as an alternative solvent for lignocellulosic biomass deconstruction to enhance downstream process-economics (Kumar et al., 2016). However, ionic liquids possess several disadvantages, such as high cost, high toxicity, and long-term recyclability concerns, which only boosted the biorefinery industry to a limited extent (Vigier et al., 2015).

To date, on-going studies have been carried out to discover more advanced and effective methods for biomass pretreatment. However, the applications of technologies in a practical scale are often limited by the cost. Several emerging pretreatment technologies have been proposed, such as microwave irradiation, ultrasound, gamma rays, electron beam irradiation, a pulsed electric field, high hydrostatic pressure, and high pressure homogenization (Hassan et al., 2018). Moreover, promising approaches such as hydrothermal carbonization, supercritical fluid, and ammonia fiber explosion have been critically reviewed in order to create a greener and more sustainable alternatives for industrial lignocellulosic biomass applications (Sankaran et al., 2019). Among the established pretreatment technologies, alkaline pretreatment is one of the most mature pretreatment technologies to delignify biomass without severe polysaccharides degradation (Loow et al., 2016a). Recent applications of alkaline hydrogen peroxide (AHP) have shown promising performance to improve delignification through the radical oxidation route (Ho et al., 2019a). On top of that, a new class of solvents, known as deep eutectic solvents (DESs), has emerged as an alternate promising green solvent in the pretreatment of biomass (Loow et al., 2017a). DESs have been increasingly applied in many studies related to biomass fractionation due to their promising potential to extract bioactive

phenolic compounds (Wei et al., 2015), delignification (Ho et al., 2019b), sugar recovery (Loow et al., 2018), furanic conversion (Lee et al., 2019), and biodiesel processing (Lu et al., 2016).

2.6 Alkaline Hydrogen Peroxide (AHP)

Alkaline hydrogen peroxide has been used extensively in delignification process to the removal of lignin and to alter the biomass structure to allow accessibility of enzymatic hydrolysis to cellulose and hemicellulose (Mittal et al., 2017). Studies have shown that the delignification of corn stover using alkaline hydrogen peroxide resulted in more than 80% of lignin removed while the subsequent process yield more than 90% of carbohydrates. In addition, studies have shown that pretreatment of olive tree biomass using alkaline hydrogen peroxide obtained the highest yield of 79% lignin removal at 80°C and 10% (w/v) solid loading (Martínez-Patiño et al., 2017).

2.6.1 Reaction mechanism

The extent of AHP pretreatments relies on the H_2O_2 decomposition and its intermediate products, which acts as an initiator for various oxidative reactions with biomass components. The decompositions of hydrogen peroxide can be further improved with the absence of stabilizer, such as $MgSO_4$ or sodium silicate and the presence of heavy metal ions (Mittal et al., 2017). Under alkaline conditions specifically close to pH 11.5, hydrogen peroxide will dissociate to produce hydroperoxyl anion (HOO^-), which is responsible for the corresponding carbonyl and ethylene groups' oxidative reactions and initiation of radicals forming. Thus, AHP pretreatment for delignification is strongly pH-dependent with an optimum pH of 11.5-11.6, which is the pKa for the H_2O_2 decomposition reaction (Gould, 1984). These hydroperoxyl anions will react with hydrogen peroxide to produce highly reactive hydroxyl radical (HO•) and superoxide anion radical (O_2^-•) (Lewis et al., 1987). These radicals are known to be strong oxidants in lignin oxidization and depolymerization of biomass into low molecular weight fragments (da Costa Correia et al., 2013; Mittal et al., 2017). According to Cabrera et al. (2014), no significant alteration in biomass structure during lignin oxidation might be observed depending on the pH adopted, as only the aliphatic part of lignin is oxidized in low alkalinity conditions. Therefore, the effectiveness of delignification strongly depends on the pH employed during the reaction in order to promote the H_2O_2 derived radicals.

2.6.2 Solubilization of hemicellulose

Alvarez-Vasco and Zhang (2013) used AHP to pretreat *Douglas fir* at a relatively high temperature of 180°C, which resulted in delignification of 22% and exceptionally high glucomannan removal (hemicellulose) of 78%, while causing little degradation on cellulose. According to Alvarez-

Vasco and Zhang (2013), reducing end groups on glucomannan can occur quickly at a high temperature which can facilitate organic acids formation through endwise depolymerization, accompanied with termination reactions (formation of non-reducing end groups). These series of reactions provide a potential route to convert hemicellulose to valuable organic acids such as lactic acid, glycodic acid, succinic acid, and formic acid while removing the protective barrier of biomass to enhance cellulosic biofuels or biochemical productions.

2.6.3 Delignification of biomass

AHP (4.3 vol% H_2O_2) caused lignin solubilization up to as high as 92.44% when used to pretreat cashew apple bagasse for 6 h at 35°C (da Costa Correia et al., 2013). When da Costa Correia et al. (2013) increased the concentration of H_2O_2 from 0.645 to 4.3 vol% under similar solid loadings (10%) and conditions (35°C, 24 h, pH 11.5), the degree of delignification drastically increased from 16.5 ± 0.9% to 80.2 ± 0.5%. On top of that, da Costa Correia et al. (2013) also revealed that when a lower solid loading was employed (5%), the delignification was substantially increased to a maximum of 96.7 ± 0.7% over a duration of 24 h. Delignification efficiency of AHP in pretreating hardwood based biomass are often lower than in softwood pretreatment. Thus catalyst in AHP seemed to play a vital role in hardwood pretreatment in order to achieve subsequent profitable yield. According to Li et al. (2013), Cu(II)(bpy)-catalysed AHP exhibited an improvement in the lignin solubilisation from 36.6% (uncatalysed) to 50.2%, which resulted in total material loss of 25.9% for *Hybrid poplar* pretreatment under similar conditions (48 h, ambient temperature, pH 11.5, 10 g H_2O_2/L).

2.6.4 Advantages and limitations

Generally, the use of AHP pretreatment does not cause high cellulose degradation and usually operated at ambient conditions. The pretreatment liquids were reported to have an absence of inhibitors (furfural and hydroxymethylfurfural) to fermentation (da Costa Correia et al., 2013). Therefore, hydrolysate detoxification processes can be omitted. Owing to the low operating temperature and atmospheric pressure employed in AHP pretreatment, expensive specialized reactors were not necessary, hence reducing the operating cost (Cabrera et al., 2014). On top of that, the lower operating cost of AHP pretreatment also attributed by the availability of relatively inexpensive industrial chemicals (alkaline peroxides) as compared to ionic liquid and organic solvent. On the other hand, the use of AHP in biomass processing possess several limitations, such as high pH and high peroxide loadings may affect economic viability and relatively long pretreatment time required at ambient conditions (Cabrera et al., 2014; Mittal et al., 2017). Therefore, the realization of implementing AHP pretreatment process in industry would require further exploration of the

opportunities to recycle catalyst and valorize lignin rich hydrolysate (Mittal et al., 2017). **Table 2.2** compiled the key findings from various AHP pretreatment studies. It is interesting to note that the AHP could be customized and adapted to fractionate different biomasses by optimizing the operating conditions to suit different applications. Numerous studies also revealed that, AHP pretreatments are highly compatible with enzymatic hydrolysis to yield a high amount of sugars. These unique features allow AHP to potentially become one of the promising pretreatment methods used in a bio-refinery.

Table 2.2

Summary of various AHP pretreatments in biomass processing.

Biomass	Initial pretreatment	Subsequent pretreatment	Key findings	Ref.
Rice husk	*High pressure alkaline hydrogen peroxide* Solid loading = 3:50 (w/v) T = 90°C Time = 30 min pH = 11.5 P = 30 bar H_2O_2 concentration = 3.0% (w/v)	*Enzymatic hydrolysis* 29.1 mL of citrate buffer (0.05M, pH 5), 0.3 mL of sodium azide (2%), 0.3 mL of NS22086 enzyme mixture, and 0.06 mL of each of the five Novozyme enzyme mixtures (NS22083, NS22118, NS22119, NS22002, NS22035) T = 50°C Time = 72 h Mixing speed = 150 rpm	*With initial pretreatment* Solubilized compounds = 1.55 ± 0.18 g Sugar yield = 98.47 ± 6.35%	(Díaz et al., 2014)
Cashew apple bagasse	*Alkaline hydrogen peroxide* Solid loading = 5% (w/v) T = 35°C Time = 6 h pH = 11.5 H_2O_2 concentration = 4.3% (v/v) Mixing speed = 150 rpm	*Enzymatic hydrolysis* Cellulase complex (Novozymes NS 22074, 108.12 FPU/mL), 0.3 g cellulose equivalent biomass, 15 mL of 0.1M citrate buffer (pH 4.8) and 120 µL of 10 mg/mL tetracycline in 70% v/v ethanol. Final cellulose activity at 60 FPU/g cellulose with volume of 30 mL T = 45°C Time = 72 h Mixing speed = 150 rpm	*With initial pretreatment* Lignin removal = 92.44% Enzymatic disgestibility = 87% Glucose yield = 161 mg/g untreated biomass	(da Costa Correia et al., 2013)
Corn stover	*Alkaline hydrogen peroxide* Solid loading = 5% (w/v) T = 50°C Time = 3.5 h pH = 11.5 H_2O_2 concentration = 2% (v/v)	*Enzymatic hydrolysis* 5% (w/v) solid loading, Cellic CTec2 (64.6 FPU/g) in sodium citrate buffer (50mM, pH 5.4). T = 50°C Time = 72 h	*Without initial pretreatment* Glucan yield = <10% Arabinoxylan yield = < 10% *With initial pretreatment* Lignin removal = 68.7 ± 4.3% Glucan yield = 88.1 ± 7.6% Arabinoxylan yield = 92.5 ± 9.1%	(Mierzejewska et al., 2019)
Banana rachis	*Alkaline hydrogen peroxide* Solid loading = 2% (w/v) T = Room temperature Time = 90 min	*Enzymatic hydrolysis* 1g sample, 50 µL cellulose from *Trichoderma reesei* (Novozyme 2730, ⩾ 700 unit/gram of enzyme), 50 µL of β-glucosidase	*Without initial pretreatment* *Glucose recovery = 2.8 ± 0.2%* Xylose recovery = 9.0 ± 0.7% Arabinose recovery = 1.5 ± 0.0%	(Costa et al., 2018)

	pH = 14 H_2O_2 concentration = 2% (v/v)	from *Aspergillus niger* (Novozyme 2605, ⩾1000 U/g) in citrate buffer (50mM, pH 5.0). T = 50°C Time = 72 h Mixing speed = 150 rpm Supplemented with 0.3g of Tween 80 (1%, v/v) after 2 h.	*With initial pretreatment* Lignin removal ≈ 55% Glucose recovery = 36.5 ± 1.5% Xylose recovery = 5.6 ± 0.3% Arabinose recovery = 1.6 ± 0.0%	
Bamboo chip	*Two-stage Alkaline/Alkaline hydrogen peroxide* Alkaline pre-extraction Solid loading = 10% (w/v) T = 100°C Time = 180 min Alkaline hydrogen peroxide Solid loading = 10% T = 75°C Time = 180 min pH = 11.5 H_2O_2 concentration = 4% (v/w)	*Enzymatic hydrolysis* 20% (w/v) solid loadings, Cellic Ctec2 (113 FPU/mL, 137.6 mg/mL protein content), β-glucosidase (Novozymes 188, 160 CBU/mL, 120 mg/mL protein content), enzyme loading of 30 mg pretein/g glucan in sodium citrate buffer (100mM, pH 5). T = 50°C Time = 120 h Mixing speed = 250 rpm	*Without initial pretreatment* Glucan content = 48.4% Lignin content = 25.1% *With initial pretreatment* Glucan content = 70.1 ± 1.1% Lignin content = 14.5 ± 0.9% Delignification = 65.2% Glucose conversion = 78% Xylose conversion = 77%	(Yuan et al., 2019)

2.7 Deep eutectic solvents

The deep eutectic solvent is a colourless fluid, which is formulated from a combination of two solids comprises of hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA). DES contains large, nonsymmetric ions that have low lattice energy contributes to a lower melting point compared to the individual components that have high melting points (Loow et al., 2018). For example, the melting point of choline chloride and urea are 302°C and 133°C, respectively. When these two components form at a molar ratio of 1:2, the resulting DES has a melting point of 12°C (Loow et al., 2018). The reasons for having low melting points is due to charge delocalization occurring through hydrogen bonding (Smith et al., 2014). The term DES has been used to differentiate it from ionic liquids (ILs) as there are differences in solvent properties while some DESs are not ionic.

2.7.1 Classification of DESs

DESs are categorized into four types according to the nature of the complexing agent used. **Table 2.3** has shown the summarized general formula of each type of DES (Smith et al., 2014).

Table 2.3

Types of DESs and its components and formula.

Type	Components	General Formula
I	Metal Salt + Organic Salt	$Cat^+X^-zMCl_x$; $M = Zn, Sn, Fe, Al, Ga, In$
II	Metal Salt Hydrate + Organic Salt	$Cat^+X^-zMCl_x{\cdot}yH_2O$; $M = Cr, Co, Cu, Ni, Fe$
III	HBD + Organic Salt	Cat^+X^-zRZ; $Z = CONH_2, COOH, OH$
IV	Zinc/Aluminium Chloride + HBD	$MCl_x + RZ = MCl_{x-1}^+{\cdot}RZ + MCl_{x+1}^-$; $M = Al, Zn$ & $Z = CONH_2, OH$

As can be seen from **Table 2.3**, Type I DESs are synthesized using metal halides such as $ZnCl_2$ with an organic salt. For instance, Type I DES include the well-studied ionic liquids produced by imidazolium salts with certain metal halides such as $FeCl_3$, AgCl, CuCl, LiCl, CdCl, $CuCl_2$, $SnCl_2$, $ZnCl_2$, $LaCl_3$, YCl_3, and $SnCl_4$ (Smith et al., 2014). Due to the high melting point of non-hydrated metal chlorides, the applications of Type 1 DES have been limited.

Thus, the scope has been widened by including the usage of hydrated metal halides in combination with choline chlorides, which are known as Type II DESs. The relatively low cost of the hydrated metal salts, as well as their air and moisture insensitivity, allowed them to be applied in large scale industrial manufacturing processes (Smith et al., 2014). Choline chloride is widely used as one of the organic salt in DES synthesization, due to its relatively low material cost, environmentally benign, readily biodegradable, and low toxicity (Zhang et al., 2012).

Type III DESs are synthesized using organic salt and HBD such as carboxylic acids, alcohols, and amides, which are biodegradable and low-cost components. Type III DESs have a huge array of selectable hydrogen bonds in synthesization, which results in high adaptability of such DES as a form of extraction solvent (Smith et al., 2014).

For Type IV DESs, it is synthesized by the combinations of inorganic transition metals and urea. Inorganic transition metals have high charge density and do not form low melting point eutectic solvents under the usual case. However, studies have shown that the combinations of urea and metal halides are able to form eutectic mixtures with a melting point below 150℃, even though those metal salts do not exist in ionic form in non-aqueous media (Smith et al., 2014).

2.7.2 Applications of DESs in biomass processing

The applications of DESs in biomass processing is still in its early development stage as compared to its application in other industry, for example, electroplating processes. Several studies have revealed the excellent results and the potential of DESs in biomass pretreatment conducted in laboratory scale. The applications include extraction of phenolic compounds from various sources of

biomass, delignification and/or sugar recovery of biomass, conversion of furanic derivatives, and biodiesel production.

2.7.2.1 Extraction of phenolic compounds from biomass

The capability of DESs to donate and accept protons and electrons which promote the probability of hydrogen bonds formations and thus increase dissolution capability grant DESs with the ability to extract phenolic compounds from lignocellulosic materials (Bubalo et al., 2014). Therefore, DESs have been widely implemented in the extraction of phenolic compounds such as pigments, carotenoids, and flavonoids, each with distinct polarity from lignocellulosic biomass (Loow et al., 2017a). The extraction efficiency of the various phenolic compound is strongly dependent on the properties of the DESs such as polarity, viscosity, and composition (Duan et al., 2016). Many studies have revealed that the extractability of phenolic compounds (high and low polarity) of DESs have surpassed most of the conventional extraction solvents.

2.7.2.2 Conversion to furanic derivatives

DESs can be used to produce furanic derivatives by favoring dehydration reactions of pentose and hexose sugars to furfural and HMF respectively with high selectivity which can be used as platform chemicals for an extensive range of useful end products (Loow et al., 2017a; Vigier et al., 2015). According to Assanosi et al. (2014), high temperatures used in various acid catalysis system in deriving furanic components was undesirable due to the rehydration of products. Recently, Assanosi et al. (2016) discovered the *N*,*N*-diethylethanolammonium chloride and *p*-TSA mixture (DES) as the reaction medium to achieve HMF yield of 84.8%.

2.7.2.3 Delignification of biomass

In order to enhance the hydrolysis of polysaccharides before subsequently undergoing fermentation into useful end products, a pretreatment stage is usually essential (Loow et al., 2016a). Recent literature has shown the potential of DESs as an effective pretreatment stage in lignocellulosic biomass deconstruction by selectively cleave the ether bonds without affecting the C-C linkages (Alvarez-Vasco et al., 2016). Thus, causing discontinuity of lignin polymer structure, breaching of the protective barrier of biomass and enhance the accessibility to the polysaccharides environments (Loow et al., 2017a). The phenolic nature of lignin allows the use of ChCl-based DESs in separating the lignin from the lignocellulosic matrix. The use of DESs in pretreating biomass caused delignification and reduction in cellulose crystallinity due to its unique ability in donating and accepting protons and electrons, which enabled the disruption of inter and intra hydrogen bonds in

lignocellulosic biomass, leading to better dissolution capability (Paiva et al., 2014; Vigier et al., 2015). Brandt et al. (2013) proposed that the lignin extraction efficiency of DES was governed by the solvent properties based on Kamlet-Taft polarity parameter (hydrogen bond donating ability or acidity α, hydrogen bond accepting ability or basicity β, as well as polarity and polarizability π*) (Jessop et al., 2012). **Table 2.4** summarized the recent applications of DES in delignification of biomass. It is worth noting that, DES could be used to fractionate different biomass by changing its combination of HBA and HBD. Thess traits of DES allowed it to adapt to different applications and potentially become an alternative solvent in the biorefinery.

Table 2.4

Summary of DES pretreatments in delignification of biomass.

Biomass	Pretreatment conditions	Delignification (%)	Reference
Corn stover	(1) ChCl:Formic acid DES Molar ratio: 1:2 Temperature: 130°C Pressure: Atmospheric Time: 2 h Solid loading: 5 wt%	23.8	(Xu et al., 2016)
Oil palm empty fruit bunch	(1) ChCl:Lactic acid DES Molar ratio: 1:5 Temperature: 120°C Pressure: Atmospheric Time: 8 h Solid loading: 10 wt%	88	(Tan et al., 2018)
Oil palm fronds	(1) Ultrasound Amplitude: 70% Time: 30 min (2) ChCl:Urea DES Molar ratio: 1:2 Temperature: 120°C Pressure: Atmospheric Time: 4 h Solid loading: 1:10 (w/v)	36.42	(Ong et al., 2019)
Oil palm fronds	(1) 30 vol% H_2O, 70 vol% ChCl:Urea DES Molar ratio: 1:2 Temperature: 120°C Pressure: Atmospheric Time: 4 h Solid loading: 1:10 (w/v)	16.31	(New et al., 2019)
Switchgrass	(1) Guanidine hydrochloride:ethylene glycol :*p*-toluensulfonic acid Ternary DES Molar ratio: 1:1.94:0.06 Temperature: 120°C Pressure: Atmospheric Time: 6 min Solid loading: 10 wt%	82.07	(Chen et al., 2019)

2.7.2.4 Recovery of lignocellulosic sugar

After undergoing DES pretreatment, biomass surface morphology analysis revealed the formation of porous structure which was mainly due to the destruction of the supramolecular structure

of the biomass by the DES (Zhang et al., 2016). Xu et al. (2016) conducted an experiment to pretreat corn stover using ChCl-formic acid DES. They observed the stiff bundles of fibers initially in the structure of corn stover became looser due to the removal of hemicellulose and lignin which subsequently improved the accessibility of enzymes to the cellulose, and achieved maximum glucose yield of 99%. **Table 2.5** summarized the enzymatic glucose yield of biomass after undergoing the DES pretreatment. It can be observed that, DES is highly compatible with enzymatic hydrolysis to yield a high amount of glucose.

Table 2.5

Summary of enzymatic glucose yield of DES pretreated biomass.

Biomass	Pretreatment conditions	Glucose yield (%)	Reference
Corncob	(1) ChCl:Lactic acid Molar ratio: 5:1 Temperature: 90°C Pressure: Atmospheric Time: 24 h Solid loading: 5 wt%	96.4	(Zhang et al., 2016)
Corncob	(1) ChCl:Malonic acid Molar ratio: 1:1 Temperature: 90°C Pressure: Atmospheric Time: 24 h Solid loading: 5 wt%	61.5	(Zhang et al., 2016)
Corncob	(1) ChCl:Glycerol Molar ratio: 2:1 Temperature: 90°C Pressure: Atmospheric Time: 24 h Solid loading: 5 wt%	83.5	(Zhang et al., 2016)
Corn stover	(1) ChCl:Formic acid DES Molar ratio: 1:2 Temperature: 130°C Pressure: Atmospheric Time: 2 h Solid loading: 5 wt%	99	(Xu et al., 2016)
Switchgrass	(1) Guanidine hydrochloride:ethylene glycol :*p*-toluensulfonic acid Ternary DES Molar ratio: 1:1.94:0.06 Temperature: 120°C Pressure: Atmospheric Time: 6 min Solid loading: 20 wt%	78.4	(Chen et al., 2019)

2.8 Concluding remarks

Although alkaline hydrogen peroxide (AHP) has been widely studied for biomass pretreatment, the literature regarding the application of AHP under mild conditions in sequential pretreatment strategies is still very limited. Also, the applications of DESs in biomass processing still require further exploration. To the best of my knowledge, no investigation has been carried out to

study the sequential pretreatment of low concentration AHP at mild conditions and Type III DES (ChCl:Urea) in pretreating oil palm fronds. In order to investigate the synergistic effect between AHP and Type III DES (ChCl:Urea), a study was carried out to determine the effects of AHP operating conditions (concentration, temperature, and duration) on the delignification of Type III DES (**Chapter 3**).

DES has been critically involved in the recent studies of biomass pretreatment. However, the study regarding the application of Type II DES (metal salt hydrate + organic salt) in biomass fractionation is not available. To the extent of my knowledge, no investigation has been carried out to study the potential use of inorganic hydrate salt ($CuCl_2•2H_2O$) based Type II DES and its synergistic effects with AHP in the pretreatment of OPF. Thus, it is of interest to study the effect of operating conditions (temperature and duration) of Type II DES (ChCl:$CuCl_2•2H_2O$) and Type III DES (ChCl:Urea) on the delignification and sugar recoveries of AHP-pretreated OPF (**Chapter 4**).

Chapter 3

An application of low concentration alkaline hydrogen peroxide at non-severe pretreatment conditions together with deep eutectic solvent to improve delignification of oil palm fronds

Publication:

1. **Ho, M.C.**, Wu, T.Y., Chee, S.W.Q., Ngang, C.Y., Chew, I.M.L., Teoh, W.H., Jahim, J.M., Mohammad, A.W., 2019. An application of low concentration alkaline hydrogen peroxide at non-severe pretreatment conditions together with deep eutectic solvent to improve delignification of oil palm fronds. Cellulose 26(16), 8557-8573. (**Appendix B**)

3.1 Abstract

Cost-effective pretreatments are essential to overcome the recalcitrant properties of lignocellulosic biomass in order to reutilize cellulosic components within the biomass for value-added products. In this study, a sequential pretreatment of low concentration (<1.00 vol%) alkaline hydrogen peroxide (AHP) at ambient temperature (~25^{o}C) and pressure (1 atm) with Type III deep eutectic solvent (DES) was used to fractionate oil palm fronds (OPF). A combination of AHP (0.25 vol%, 90 min) and DES (120°C, 4 h) at the later stage resulted in a pretreated OPF with a delignification of 18.99%, remarkably improved DES-alone delignification extent by 1.6 folds. The characterizations of pretreated OPF confirmed that mild conditions AHP + DES pretreatment could synergically improve the delignification efficiency. Thus, the use of this sequential pretreatment enabled the lignin extraction and effectively disrupted the recalcitrant structure of OPF, yielding a potential feedstock for a biorefinery process at the later stage.

3.2 Introduction

Over the past decades, the rapid development of countries and continuous growth of population over the globe have caused a significant increase in the energy consumptions, which have resulted in depletion of non-renewable energy. According to the International Energy Agency, transportation dominates oil consumption over the globe, where it contributes more than 70% of greenhouse gases to the environment. Due to the increasing awareness of sustainable development, it has drawn scientist's attention to find an alternative energy source to replace the uses of non-renewable energy. Hence, scientists have found a promising energy source by converting biomass into biofuel. Biofuel is relatively environmental friendly bioenergy as compared to fossil fuel as the former is biodegradable, non-toxic, and low emission profile (Mahapatra and Manian, 2017). Currently, Malaysia is regarded as one of the largest exporters of crude palm oil in the world. This industry yield more than 90% of the country's total lignocellulosic biomass, which is derived from 5.4 million hectares of oil palms in the country (Loow et al., 2017b). Among the oil palm biomass, approximately 26.2 million tonnes per annum of oil palm fronds are produced per million of fresh fruit bunch processed (Yunus et al., 2010), making it the promising source for biofuel productions. Despite the abundance of oil palm fronds, the fronds are commonly used as mulches by applying them to the soil surface around the oil palm trees (Loow and Wu, 2018). However, this typical approach offers limited economical values to the plantation, while the improper handling of this biomass may cause grave environmental problems (Isikgor and Becer, 2015).

Owing to the availability of lignocellulosic biomass, the production of biofuel or chemical building block from this biomass could be a solution towards sustainable development, especially in

the decarbonization of transport industry (Martínez-Patiño et al., 2017). Lignocellulosic biomass consists of three main components, namely cellulose (40 – 50%), hemicellulose (15 – 25%) and lignin (20 – 25%) (Loow et al., 2015). Cellulose and hemicellulose in the biomass make up the polysaccharides available for sugar hydrolysis and fermentation to convert into high-value products. Lignin is an amorphous phenolic polymer with high heterogeneity through the coupling of 4-hydrophenylpropanoids (Loow et al., 2016b). Due to its highly branched and random structure, the recalcitrant lignin wall poses a formidable barrier to digestion via enzymatic routes, which prohibit cellulose and hemicellulose from rapid degradation into fermentable sugars (Foyle et al., 2007). Hence, the fractionation of biomass into its main components (cellulose, hemicellulose, and lignin) could favor the productions of value-added products and biofuel to enhance the biofuel competitiveness (González-García et al., 2016; Kim et al., 2016).

Delignification – a pretreatment strategy aimed to fractionate lignin in the biorefinery process has been attracting attention recently. The effectiveness of delignification is associated with achieving higher cellulose accessibility, which is then impacting on the process yield. By utilizing a suitable pretreatment step, a more energy-efficient and simpler downstream process can be achieved (Loow et al., 2016b). Alkaline pretreatment is one of the most used approaches to recover lignin from the biomass without severe cellulose degradation (Loow et al., 2016a). On the other hand, hydrogen peroxide (H_2O_2) was known to enhance wastewater treatment efficiency (Subramonian and Wu, 2014; Subramonian et al., 2017). Recently, H_2O_2 was used to assist biomass processing in an acidic condition (Loow and Wu, 2018; Loow et al., 2017b). However, the use of H_2O_2 in an alkaline medium has proven to be a more effective approach to improve lignin depolymerization through radical oxidation action (Ho et al., 2019a). For example, the addition of H_2O_2 during alkaline pretreatment of rice husk increased overall sugar recoveries by 75% while no inhibitors such as furfural were found in the hydrolysate (Díaz et al., 2014; Su et al., 2015). Lately, a new green solvent, namely deep eutectic solvent (DES), has received much focus in the biomass valorization. It was first introduced by Abbott et al. (2004), in which a liquid eutectic mixture was formed through combining two solids comprising a quaternary ammonium salt and a hydrogen-bond donor (HBD). DESs possess similar physiochemical properties with conventional ionic liquids. However, the former has better biodegradability and enzyme compatibility while possessing lesser toxicity and lower costs (Loow et al., 2018). To date, DESs have been increasingly involved in studies related to biomass processing due to its ability to extract bioactive phenolic compounds, delignification and/or sugar recovery, furanic conversion, and biodiesel processing (Lee et al., 2019; Loow et al., 2017a). According to Kumar et al. (2016), the use of DESs in biomass processing enabled the depolymerization of lignin

without hydrolyzing the cellulose and hemicellulose, which enhanced the enzymatic hydrolysis with insignificant inhibitor formation.

Although alkaline hydrogen peroxide (AHP) has been widely studied for biomass pretreatment, the literature regarding the application of AHP under mild conditions in sequential pretreatment strategies is still very limited. Also, the applications of DESs in biomass processing still require further exploration. To the best of our knowledge, no investigation has been carried out to study the sequential pretreatment of low concentration AHP at mild conditions with DES in pretreating oil palm fronds. It was hypothesized that the introduction of mild concentration (<1.00 vol%) AHP at ambient conditions could help improve macrostructure alteration and partial lignin solubilization of oil palm fronds. Therefore, DES was facilitated in accessing the biomass matrix and providing better disruption on the hydrogen-bonding network, which led to a better delignification. Thus, the objective of this study was to evaluate the synergistic effects of low concentration AHP at mild conditions together with DES in oil palm fronds delignification pretreatment (**Objectives 1**).

3.3 Materials and methods

3.3.1 Raw material and chemicals

Fresh oil palm fronds were provided by oil palm plantation owned by Universiti Kebangsaan Malaysia. The leaflets were removed while the petiole was pressed in a frond pressing machine to separate the juice from the oil palm fronds. Pressed oil palm fronds were sun-dried for two days before grinding to a smaller size using pulverizer (8000 rpm). After that, grinded oil palm fronds were sieved through a mechanical sieve to recover biomass at a size ≤0.5mm. Sieved oil palm fronds were washed with distilled water and dried in an oven at 60°C for 48 h. The prepared oil palm fronds were termed as OPF throughout this study and stored in a container filled with desiccants at room temperature prior to pretreatments. National Renewable Energy Laboratory (NREL) standard laboratory analytical procedure was adopted and carried out to determine OPF composition in terms of structural carbohydrates and lignin (Sluiter et al., 2008). D(+)glucose, D(-)xylose, and L(+)arabinose were used as standard solutions to calibrate standard curves for composition analysis through high performance liquid chromatography (HPLC). Sodium hydroxide (AR grade, 99%, pellets), hydrogen peroxide (extra pure, 50%), choline chloride (99%), urea (AR grade, 99%) and other chemicals utilized in this study were ensured to be of analytical grades.

3.3.2 Sequential pretreatment

Two-stage sequential pretreatments, namely AHP as the first stage while Type III DES as the second stage, were used in pretreatment of OPF.

3.3.2.1 AHP as a first stage pretreatment

In this first pretreatment, 4.5 g of OPF sample was measured and placed into a 600 mL beaker. The AHP solution was prepared by diluting with deionized water to yield concentration ranging from 0.05 – 1.00 vol%, then adjusted to pH 11.5 using NaOH pellets. The synthesized solution was then charged into the beaker containing OPF sample at a fixed solid to liquid ratio of 1:20 (w/v). The OPF was then soaked in AHP solution at temperature ranging from room temperature to 90°C for different durations from 30 – 120 min. After undergoing the AHP pretreatment, the resulting AHP-pretreated biomass sample was filtered from the liquid fractions. The pretreated sample was washed with distilled water to remove the residual AHP and then dried at 60°C in the oven overnight. Later, the dried sample was collected and stored in a container filled with desiccants prior to subsequent uses.

3.3.2.2 DES pretreatment as a second stage pretreatment

The second stage pretreatment was performed on the pretreated OPF from the first AHP pretreatment stage. 2.5 g of pretreated OPF from AHP was measured and transferred into 50 mL Schott bottle. ChCl:Urea at a molar ratio of 1:2 was used as a Type III DES in this study. DES was prepared (Loow et al., 2018), and added to the Schott bottle at a fixed solid to liquid ratio of 1:10 (w/v). The pretreatment process was performed in an oil bath, maintaining the temperature at 120°C for a duration of 4 h without introducing stirring. The pretreatment conditions (120°C, 4 h) were adapted from Loow et al. (2018) for optimum sugar recovery of OPF through inorganic salt hydrolysis. Upon the completion of the pretreatment, the Schott bottle was removed from the oil bath and rinsed with running tap water to cool down the content and quench the reaction. The resulting AHP + DES-pretreated biomass sample was filtered and washed with distilled water to remove excessive DES. Then, the solid fraction was left to dry in an oven overnight at 60°C before storing in a container filled with desiccants before the subsequent analysis.

3.3.3 Inorganic salt hydrolysis

Inorganic salt hydrolysis was carried out to investigate the hemicellulose recoveries of raw and pretreated biomass. Firstly, 1.0 g of OPF sample was transferred into 50 mL Schott bottle. Inorganic salt solution ($CuCl_2$) was prepared at a concentration of 0.4 mol/L (Loow et al., 2017b), and added to the OPF sample at a solid to liquid ratio of 1:10 (w/v). The Schott bottle containing the reaction mixture was then sent to autoclave (240V, Hirayama HV-85, Japan) at 120°C for a duration of 30 min. Thereafter, the mixture in the Schott bottle was cooled to room temperature to quench reaction before extracting the liquid fraction. The extracted hydrolysate was centrifuged at a speed of

13,500 rpm for 10 min using a Mini 1312M Micro Centrifuge. The centrifuged hydrolysate was filtered using a 0.22 µm syringe filter prior to monomeric sugar analysis.

3.3.4 Analytical methods

3.3.4.1 Lignin quantification

National Renewable Energy Laboratory (NREL) standard laboratory analytical procedure for lignin composition was adopted to quantify lignin in the OPF sample (Sluiter et al., 2008). Acid hydrolysis was done by adding 3 mL of 72% of H_2SO_4 and 0.3 g of dried sample into a 250 mL conical flask. After that, the mixture was incubated at 30°C and 150 rpm for an hour. Then, 84 mL of deionized water was added into the incubated mixture and sent for autoclave at 121°C for an hour. The reacted mixture was transferred for vacuum filtration where the filtrate was sent for acid soluble lignin test by using UV-Vis spectrophotometer at 320 nm in which the deionized water was used as a blank solution. On the other hand, acid insoluble lignin was determined by measuring the difference in dry weight of the filter paper before and after vacuum filtration. Delignification (**Eq. 3.1a**) is defined as the percentage of lignin removed in the samples (New et al., 2019), whereby:

$$Delignification\ (\%) = \frac{(L_0 - LY)}{L_0} \times 100\% \qquad \textbf{(Eq. 3.1a)}$$

Where,

L_0 = *Lignin content in raw OPF,* $\left(\frac{mass\ of\ lignin\ in\ raw\ OPF}{mass\ of\ raw\ OPF}\right)$

L = *Lignin content in pretreated OPF,* $\left(\frac{mass\ of\ lignin\ in\ pretreated\ OPF}{mass\ of\ pretreated\ OPF}\right)$

Y = *Mass yield or mass recovery of pretreatment in fraction,* $\left(\frac{mass\ of\ pretreated\ OPF}{mass\ of\ raw\ OPF}\right)$

DES stage-wise delignification (**Eq 3.1b**) is defined as the percentage increase in DES delignification by comparing the AHP and AHP + DES pretreatment, whereby:

$$DES\ stage\text{-}wise\ delignification\ (\%) = Sequential\ pretreatment\ delignification - AHP\ delignification \qquad \textbf{(Eq 3.1b)}$$

3.3.4.2 Fourier transform infrared spectroscopy (FTIR)

Attenuated total reflection (ATR) tip FTIR (Thermoscientific Nicolet iS10 spectrometer) was used to analyze the chemical structures of the raw and pretreated OPF samples. The sample spectra were obtained via 64 scans over the range of 500 to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1}.

3.3.4.3 Field emission scanning electron microscope (FE-SEM)

The raw and pretreated OPF samples were firstly adhered to a specimen stub using double-coated tape and sputter coated with platinum prior to imaging using Hitachi SU 8010 in order to visualize the microstructural alteration in biomass surface morphology.

3.3.4.4 X-Ray diffraction (XRD)

A Bruker D8 Discover diffractometer was used to determine the crystallinity index (CrI) of raw and pretreated OPF samples with a 2θ ranged from 5 to 51° in steps of 0.041°. The crystallinity index (CrI) in **Eq. 3.2** is defined by Segal et al. (1959) as the relative degree of crystallinity in the specimen, whereby:

$$CrI(\%) = \frac{(I_{200} - I_{am})}{I_{200}} \times 100\% \quad \textbf{(Eq. 3.2)}$$

Where I_{200} is the maximum intensity of the 200 peak (2θ = 22°) and I_{am} is the intensity minimum between peaks at 200 and 110 (2θ = 18°).

3.3.4.5 High performance liquid chromatography (HPLC)

Agilent series 1200 infinity HPLC system equipped with a Refractive Index detector and BioRad Aminex HPX-87H column was used to analyze the concentrations of monomeric sugars in the hydrolysate obtained from inorganic salt sugar recoveries and acid hydrolysis compositional analysis for the dried OPF sample. The mobile phase for the application of HPX-87H column was 0.005 mol/L H_2SO_4 at an operating flow rate of 0.6 mL/min and the column temperature was controlled at 65°C. The concentration of monomeric sugars was presented in g/L, while the sugar recoveries (%) were derived by adapting formula from Loow et al. (2018) as shown in **Eq. 3.3**:

$$\text{Sugar recovery (\%)} = \frac{\text{sugar recovered (g/L)} \times \text{volume of solvent used (L)}}{\text{sample carbohydrate composition} \times \text{mass of sample used (g)}} \times 100\% \quad \textbf{(Eq. 3.3)}$$

3.3.4.6 Brunauer-Emmett-Teller (BET)

BET analysis was carried out with N_2 adsorbate at -195.85°C by using a Micromeritics ASAP 2020 BET model. Degassing was performed prior to analysis with conditions adapted from the literatures (Lim and Wu, 2016; Lim and Wu, 2015), where samples were initially heated to 90°C for 2 h, and subsequently holding at 110°C for 22 h.

3.3.4.7 Thermogravimetric analysis (TGA)

The thermogravimetric analysis was conducted with a Mettler Toledo Thermo Gravimetric Analyzer in a nitrogen environment, adapting the methodology from Marcotullio et al. (2011). The raw and pretreated OPFs were placed in a ceramic sample holder, thereafter the temperature was raised to 50°C at a ramping rate of 10°C/min and kept constant for 45 min. After that, the temperature was risen to 120°C at a ramping rate of 10°C/min and held isothermal for 15 min to dry samples thoroughly before increasing the temperature to 550°C at 10°C/min. The furnace was maintained isothermal for 40 min, in which the residue was regarded as char after the thermogravimetric analysis.

3.3.5 Statistical analysis

One-way analysis of variance (ANOVA) was carried out using IBM SPSS Statistics 24 to statistically process the experimental data for identifying any occurrence of significant variations between pretreatment conditions. This analysis helped in determining the recommended reaction condition of AHP to induce the most synergic effect with DES in delignification of OPF. The homogeneous subsets for experimental data based on a range of parameters were identified using Duncan's test with a significance value of $P < 0.05$.

3.4 Results and discussion

3.4.1 Effect of AHP and DES on delignification

The chemical compositions of OPF by dry weight were comprised of glucan (48.23 ± 0.03), xylan (21.86 ± 0.01), arabinan (1.09 ± 0.06), lignin (18.84 ± 0.09), and the remaining weight of 9.98% were ash as well as water and ethanol extractives. From the previous study by (Loow et al., 2018), the recommended operating conditions for DES (ChCl:Urea) pretreatment of OPF for maximum sugar recoveries were 120°C and 4 h. When the raw OPF was pretreated in a DES pretreatment, the lignin content was reduced to 18.14% from 18.84% (**Table 3.1** and **Table 3.2**). It was hypothesized in this study that the incorporation of AHP pretreatments, though in very mild conditions, with DES pretreatment (ChCl:Urea at 120°C for 4 h) would enhance further the delignification of OPF. In this study, pretreatment of OPF using DES alone was considered as a 'control' in the experiment.

3.4.1.1 Effect of AHP concentration in sequential pretreatment

The results were interpreted from two different perspectives, namely lignin content reduction and overall delignification of OPF. According to one-way ANOVA, the lignin content after the pretreatment of 0.50 vol% AHP was significantly reduced as compared to the raw OPF. Interestingly, it was found that the AHP pretreatment was effective against OPF, despite under a very mild concentration at room temperature. For example, at 1.00 vol% single staged AHP, delignification achieved was 12.75%, which was comparable to the delignification obtained in control (12.16%). The efficiency of lignin removals showed increasing trends with increases in AHP concentrations, which were consistent with the previous study (da Costa Correia et al., 2013). The delignification could be improved from 2.54 to 12.75% when the concentration of AHP increased from 0.05 to 1.00 vol% at room temperature. However, higher AHP concentration could lead to several problems in terms of higher H_2O_2 and NaOH consumptions. Also, safety and environmental issues might arise when the process was scaled up. Therefore, the recommended operating condition was not selected based solely on the delignification performance of AHP but overall delignification efficiency of sequential pretreatment and the synergistic effect between both pretreatments. This could potentially reduce the AHP concentration further.

Table 3.1

Delignification of OPF using AHP + DES sequential pretreatment by varying AHP concentration. Values annotated with different letters represents different significance levels (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Mass yield (%)	Lignin (%)	Delignification (%)
Raw OPF	-	18.84 ± 0.09^{a}	-
AHP duration: 90 minutes			
AHP (0.05 vol%)	99.63	$18.43 \pm 0.05^{b,c}$	2.54 ± 0.35^{A}
AHP (0.10 vol%)	99.77	$18.35 \pm 0.07^{c,d}$	2.83 ± 0.04^{A}
AHP (0.25 vol%)	99.00	18.26 ± 0.04^{d}	4.05 ± 0.05^{B}
AHP (0.50 vol%)	98.97	17.82 ± 0.09^{e}	6.38 ± 0.14^{C}
AHP (1.00 vol%)	97.15	16.92 ± 0.06^{f}	12.75 ± 0.05^{D}
DES (ChCl:Urea; 120°C; 4 h)			
DES	91.22	$18.14 \pm 0.12^{d,g}$	12.16 ± 0.16^{E}
AHP (0.05 vol%) + DES	91.46	17.22 ± 0.05^{g}	14.23 ± 0.13^{F}
AHP (0.10 vol%) + DES	91.46	17.09 ± 0.03^{h}	14.62 ± 0.20^{G}
AHP (0.25 vol%) + DES	93.92	16.25 ± 0.05^{i}	18.99 ± 0.04^{H}
AHP (0.50 vol%) + DES	95.33	$17.15 \pm 0.03^{g,h}$	13.22 ± 0.23^{I}
AHP (1.00 vol%) + DES	93.54	16.63 ± 0.03^{j}	17.44 ± 0.21^{J}

For a fixed DES pretreatment together with AHP pretreatment at various concentrations, the DES stage-wise delignification was improved from 0.05 up to 0.25 vol% AHP. However, a further increase in AHP concentration (> 0.25 vol%) had an adverse effect in the DES stage-wise delignification. The lignin content reduced from 18.26 to 16.25% (2.01% in reduction) when DES pretreatment was carried out using 0.25 vol% AHP-pretreated OPF (**Table 3.1**). On top of that, the combination of 0.25 vol% AHP and DES pretreatment resulted in delignification of 18.99%, which

was approximately 1.6 times higher than the delignification of DES pretreatment alone. The decreasing DES delignification after AHP pretreatment (>0.25 vol%) could be due to the competition in targets components during the delignification of both pretreatment mechanism or excessive alteration of biomass in the AHP pretreatment, which could hinder the delignification performance of DES pretreatment when higher concentration AHP was used (Gould, 1984; Loow et al., 2018). According to Mittal et al. (2017), excessive AHP pretreatment could cause a reduction in the accessibility of biomass due to the collapse of the localized cell wall, which effectively led to poorer sugar conversion. From the experimental results (**Table 3.1**), OPF underwent sequential pretreatment of 0.25 vol% AHP + DES was able to achieve the highest delignification (18.99%), which was higher than the control (12.16%) and the other combination of different AHP concentrations coped with DES, suggesting that the best synergistic effect induced. This combination was able to achieve significant synergistic effect and maximum delignification while reducing the severity of AHP pretreatment concentration from 1.00 to 0.25 vol%. Therefore, the recommended concentration of AHP at 0.25 vol% was used to investigate the subsequent study on the effect of AHP reaction duration on the overall delignification of OPF.

3.4.1.2 Effect of AHP reaction duration in sequential pretreatment

This study was extended to investigate the effect of AHP reaction duration on both lignin content reduction and delignification (**Table 3.2**). In the first stage, the extent of delignification of AHP increased with prolonged duration of pretreatments. One-way ANOVA suggested that the improvement of delignification did not have significant differences when the reaction duration was increased from 90 to 120 min, indicating that the pretreatment had reached its plateau state. The result could imply that the oxidation reaction had reached its completion and any further increase in reaction duration would only lead to a little or negligible effect on the extent of OPF delignification. Under a constant AHP concentration (0.25 vol%), the lignin content could be reduced to 18.17% and achieved delignification of 4.29% for a pretreatment duration of 120 min, despite under significantly less extreme pretreatment conditions (**Table 3.2**).

Table 3.2

Delignification of OPF using AHP + DES sequential pretreatment by varying AHP reaction duration. Values annotated with different letters represents different significance levels (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Mass yield (%)	Lignin (%)	Delignification (%)
Raw OPF	-	18.84 ± 0.09^{a}	-
AHP concentration: 0.25 vol%			
AHP (30 min)	99.01	18.62 ± 0.08^{b}	2.14 ± 0.10^{A}
AHP (45 min)	98.23	18.53 ± 0.03^{b}	3.38 ± 0.23^{B}
AHP (60 min)	99.15	18.34 ± 0.08^{c}	3.48 ± 0.07^{B}
AHP (90 min)	99.00	$18.26 \pm 0.04^{c,d}$	4.05 ± 0.05^{C}
AHP (120 min)	99.23	18.17 ± 0.03^{d}	4.29 ± 0.26^{C}
DES (ChCl:Urea; 120°C; 4 h)			
DES	91.22	18.14 ± 0.12^{d}	12.16 ± 0.16^{D}
AHP (30 min) + DES	93.47	17.54 ± 0.06^{e}	12.98 ± 0.09^{E}
AHP (45 min) + DES	93.13	17.25 ± 0.05^{f}	14.73 ± 0.14^{F}
AHP (60 min) + DES	92.41	16.86 ± 0.06^{g}	17.31 ± 0.07^{G}
AHP (90 min) + DES	93.92	16.25 ± 0.05^{h}	18.99 ± 0.04^{H}
AHP (120 min) + DES	93.80	16.23 ± 0.13^{h}	19.20 ± 0.19^{H}

Delignification of DES pretreatment on each of the AHP-pretreated OPF at different reaction duration ranging from 30 to 120 min showed a similar trend as compared to the AHP pretreatment alone (**Table 2**), whereby the delignification increased as the AHP pretreatment duration increased. For example, the delignification of AHP + DES-pretreated OPF increased from 12.98 to 19.20% as the pretreatment duration for AHP increased from 30 to 120 min. More alterations on OPF biomass were expected when the pretreatment duration increased, which led to the improvement of sequential pretreatment delignification synergically. According to one-way ANOVA, the improvement on the extent of delignification reached its maximum at 90 min duration (18.99%), where further increase in duration had little impact on the pretreatment process. A Plausible explanation of such phenomenon was due to the further increase in AHP pretreatment duration (> 90 min) alone had little effect on the biomass delignification and structural alteration, thus no significant differences in delignification of DES in the second stage as compared to AHP (90 min) + DES pretreatment.

3.4.1.3 Effect of AHP temperature in sequential pretreatment

The study was extended to investigate the effect of AHP temperature on the synergism induced in DES pretreatment. From the results tabulated in **Table 3.3**, delignification of AHP pretreatment increased from 4.05 to 24.18% as temperature increased. However, the increases of AHP temperature have an adverse effect on the subsequent DES pretreatment, where the stage-wise delignification showed decreasing trends as the temperature of AHP increased. This phenomenon could be due to the competition of hydroxyl substrate in the lignin between both pretreatments. As the temperature increased in AHP pretreatment, the rate of formation of radicals increased, thus the delignification was improved. The improvement in delignification would result in better lignin removal, hence the number of hydroxyl groups available in lignin reduced. Thus, lesser substrates

were available for hydrogen bonding in subsequent DES pretreatment. It was observed that the increase in AHP temperature was not compatible with the DES delignification in sequential pretreatment. Therefore, AHP at room temperature was recommended for the best synergism achieved in DES pretreatment. Among all the pretreatment combinations, AHP concentration of 0.25 vol% at room temperature and 90 min was recommended for the best delignification and synergism in DES. **Fig. 3.1**, **3.2**, and **3.3** showed the colour changes after AHP, DES, and AHP + DES pretreatment, respectively at the proposed conditions.

Table 3.3

Delignification of OPF using AHP + DES sequential pretreatment by varying AHP temperature. Values annotated with different letters represents different significance levels (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Mass yield (%)	Lignin (%)	Delignification (%)	DES Stage-wise Delignification (%)
Raw OPF	-	18.84 ± 0.09^{a}	-	-
AHP: 0.25 vol%, 90 minutes				
AHP (Room temperature)	99.00	18.26 ± 0.04^{b}	4.05 ± 0.05^{A}	-
AHP (40°C)	83.36	18.41 ± 0.02^{c}	18.54 ± 0.07^{B}	-
AHP (50°C)	83.36	18.07 ± 0.02^{d}	20.06 ± 0.10^{C}	-
AHP (70°C)	82.00	17.95 ± 0.05^{e}	21.85 ± 0.22^{D}	-
AHP (90°C)	80.36	17.78 ± 0.04^{f}	24.18 ± 0.15^{E}	-
DES (ChCl:Urea; 120°C; 4 h)				
AHP (Room temperature) + DES	93.92	16.25 ± 0.05^{g}	18.99 ± 0.04^{F}	14.94 ± 0.49^{A}
AHP (40°C) + DES	78.67	17.43 ± 0.02^{h}	27.21 ± 0.07^{G}	8.67 ± 0.01^{B}
AHP (50°C) + DES	78.31	17.97 ± 0.04^{e}	25.32 ± 0.15^{H}	5.25 ± 0.06^{C}
AHP (70°C) + DES	77.76	17.68 ± 0.03^{i}	27.02 ± 0.13^{G}	5.17 ± 0.36^{C}
AHP (90°C) + DES	76.11	17.66 ± 0.01^{i}	28.67 ± 0.04^{I}	4.48 ± 0.11^{D}

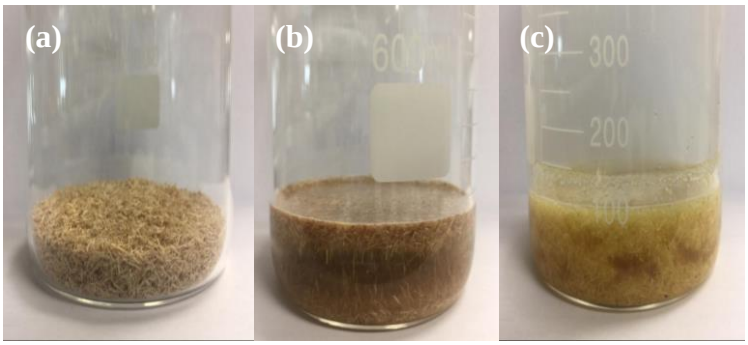

Fig. 3.1 (a) Raw OPF (b) before and (c) after AHP pretreatment.

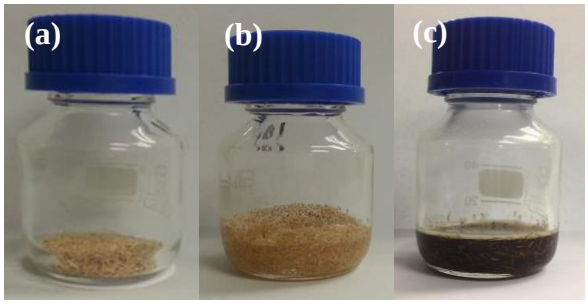

Fig. 3.2 (a) Raw OPF (b) before and (c) after DES pretreatment.

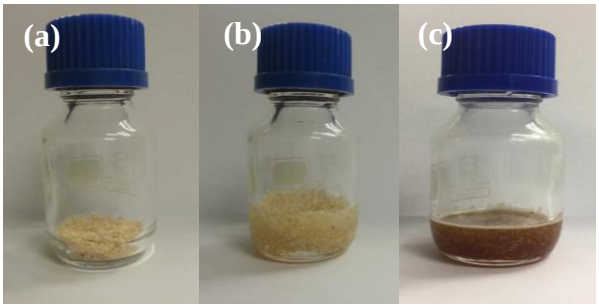

Fig. 3.3 (a) AHP-pretreated OPF (b) before and (c) after DES pretreatment.

3.4.2 Compositional analysis and sugar recoveries from raw and pretreated OPF

Generally, pretreatments specifically target lignin will result in hemicellulose (xylan, arabinan) and cellulose (glucan) enrichment. It was expected that the glucan percentage of pretreated OPF increased as compared to the raw glucan content (**Table 3.4**). For example, OPF underwent AHP + DES pretreatment resulted in an increase in glucan content from 48.23 (raw) to 54.40%, which was higher than the control pretreatment (50.31%) and AHP (48.95%). Furthermore, the glucan enrichment from raw OPF in the sequential pretreatment was greater than the sum of either of the two stages alone, confirming the existence of the synergistic effect. It was interesting to note that, despite under mild AHP pretreatment conditions, glucan content of AHP pretreated OPF showed a slight increase to 48.95%. However, the xylan content decreased (to 21.67%) as compared to raw OPF (21.86%), which could be due to the hemicellulose solubilization capability of AHP pretreatment (Li et al., 2013; Morone et al., 2017; Toquero and Bolado, 2014). From this study, it was revealed that the AHP pretreatment could cause notable hemicellulose removal, despite under mild conditions.

Table 3.4

Compositional analysis of OPF using AHP (0.25 vol%, 90 min) + DES (120°C, 4 h) sequential pretreatment. Values annotated with different letters represents different significance levels (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Compositional analysis (%)			
	Lignin	Xylan	Arabinan	Glucan
Raw	18.84 ± 0.09^{A}	$21.86 \pm 0.01^{A*}$	1.09 ± 0.06^{a}	$48.23 \pm 0.03^{a*}$
DES	$18.14 \pm 0.12^{B,C}$	$22.13 \pm 0.09^{B*}$	0.59 ± 0.02^{b}	$50.31 \pm 0.15^{a*}$
AHP	18.26 ± 0.04^{C}	$21.67 \pm 0.01^{C*}$	$0.53 \pm 0.02^{b,c}$	$48.95 \pm 0.31^{a*}$
AHP + DES	16.25 ± 0.05^{D}	$22.13 \pm 0.07^{B*}$	0.46 ± 0.05^{c}	$54.40 \pm 1.81^{b*}$

An attempt to recover monomeric sugars using the inorganic salt pretreatment on the OPF samples was shown in **Table 3.5.** The monomeric sugar recoveries (glucose and arabinose) were in a relatively smaller quantity because the $CuCl_2$ inorganic salt hydrolysis was specialized in xylan hydrolysis (Loow et al., 2015; Loow et al., 2018). One-way ANOVA also confirmed that the amount of glucose and arabinose produced did not have significant difference across different pretreatment

conditions. As for xylose recoveries, the xylose concentration recovered from raw and DES-pretreated OPF deviated from the values reported by Loow et al. (2018), which could be due to the difference in the maturity of palm trees (Tan et al., 2016). The xylose concentration in the hydrolysate obtained from AHP-pretreated OPF was 7.54 g/L, which was lower than the xylose recovered from the raw OPF (8.90 g/L). This result was expected as the AHP pretreatment of OPF resulted in some xylan removal (**Table 3.5**), thus affecting the xylose recovery. Interestingly, the xylose concentrations of AHP + DES-pretreated OPF differed from the DES-pretreated OPF, which were 12.26 and 11.38 g/L respectively, even though both samples were similar in terms of xylan content (**Table 3.4**). This could be due to the difference in carbohydrate accessibility (Loow et al., 2017b), which implied that the AHP + DES-pretreated OPF was more accessible as compared to the DES-pretreated OPF. Thus, a higher xylose recovery of 55.40% could be achieved for AHP + DES-pretreated OPF as compared to the DES-pretreated OPF (51.42%) and raw OPF (40.71%).

Table 3.5

Sugar recoveries of OPF using AHP (0.25 vol%, 90 min) + DES (120 °C, 4 h) sequential pretreatment. Values annotated with different letters represents different significance levels (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Sugar concentration (g/L)			Sugar Recoveries (%)		
	Xylose	Arabinose	Glucose	Xylose	Arabinose	Glucose
Raw	8.90 ± 0.01^{a}	0.88 ± 0.02^{A}	$0.32 \pm 0.03^{a*}$	40.71	80.73	0.66
DES alone	11.38 ± 0.02^{b}	$0.55 \pm 0.06^{A,B}$	$0.28 \pm 0.01^{a*,b*}$	51.42	93.22	0.56
AHP alone	7.54 ± 0.15^{c}	0.47 ± 0.05^{B}	$0.22 \pm 0.03^{b*}$	34.79	88.68	0.45
AHP + DES	12.26 ± 0.05^{d}	0.40 ± 0.23^{B}	$0.26 \pm 0.07^{a*,b*}$	55.40	86.96	0.48

3.4.3 Characterization studies of raw and pretreated OPF samples

3.4.3.1 FE-SEM

FE-SEM was used to study the morphological changes of OPF after undergoing various pretreatment process (**Fig. 3.4**). The raw OPF exhibited a smooth and well-structured fiber with an enclosed surface (**Fig. 3.4a**). The homogeneous and continuous fibrils structures resulted in a low degree of porosity. Therefore, the penetration of chemicals to internal hemicellulose and cellulose was discouraged, leading to a low hemicellulose sugar recovery. The low degree of porosity of OPF restricted the penetration of DES, which resulted in a rough and disordered surface, but the overall surface of OPF was still considered intact and enclosed, suggesting that the DES pretreatment had minimal effect on cellulose and hemicellulose. After undergoing AHP pretreatment alone at a concentration of 0.25 vol% and duration of 90 min, the overall surface was relatively smoother as compared to the control pretreatment, which concurred with the lignin quantification analysis as slight reduction of lignin content was obtained during the process (**Table 3.3**). On top of that, numerous tiny dots were observed on the outer layer of AHP-pretreated OPF (**Fig. 3.4c**), thus improving the

accessibility and porosity of OPF to the internal layers. A similar observation was reported by Li et al. (2016c), whereby tiny holes were exhibited on Jerusalem artichoke after undergoing alkaline peroxide pretreatment. Hence, even with a majority of lignin components retained in the OPF after the AHP pretreatment (**Table 3.4**), the increased porosity enhanced the subsequent DES penetration to the internal hydrogen bonding network. A drastic rupture of the structure was observed in the OPF after undergoing the synergistic combination of both AHP and DES pretreatments (**Fig. 3.4d**), exposing highly ordered crystalline straps. The effects of AHP in enhancing the porosity, lignin and partial hemicellulose disruption led to better penetration and delignification during the DES pretreatment in a second stage, which eventually destructing the OPF structure more thoroughly.

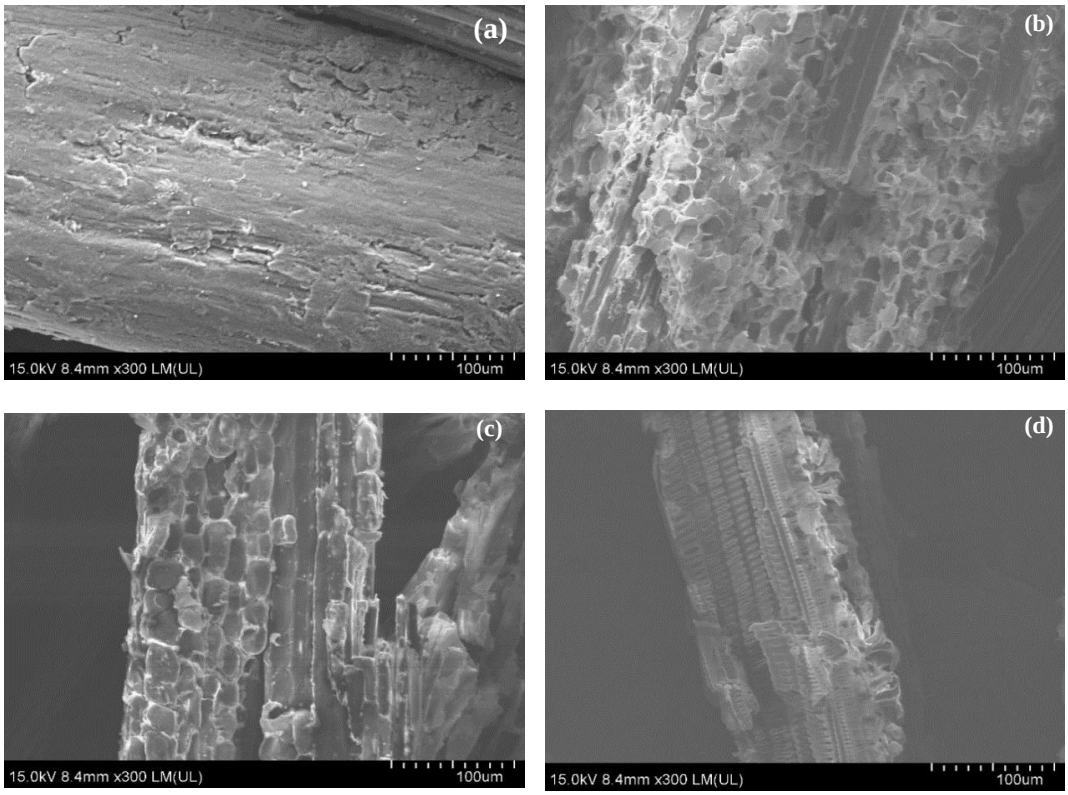

Fig. 3.4 FE-SEM images of (a) raw OPF, (b) DES-pretreated OPF, (c) AHP-pretreated OPF, and (d) AHP + DES-pretreated OPF at x300 magnification.

3.4.3.2 XRD

The intensity of crystalline and amorphous regions was taken at 22 and 18°, respectively, from diffractogram using XRD (Johar et al., 2012). The CrI of raw OPF was obtained to be 37.94% and increased to 43.26% after control pretreatment due to the ability to remove amorphous lignin (Loow et al., 2018). As for AHP pretreatment, the CrI achieved (43.62%) was similar to the control. It was an interesting observation that AHP under room temperature and mild concentration of 0.25 vol% could cause such a significant increase in CrI, as AHP at room temperature had the lower capability to solubilize hemicellulose and expose phenolic ring in lignin (Díaz et al., 2014; Maziero et al., 2012).

Thus, it was deduced that the increase in CrI as a result of AHP pretreatment was mainly attributed by its ability to enhance porosity and accessibility to crystalline region, at the same time partially remove lignin and hemicellulose (Li et al., 2016c). This claim was validated by the increase in crystalline peak (**Fig. 3.5**). After undergoing AHP + DES pretreatment, the CrI increased to 48.94%. It was observed from the XRD spectra of AHP + DES-pretreated OPF, amorphous peak reduced as a result of amorphous component removal, led to a significant increase in CrI. Interestingly, the crystalline peak intensity experienced slight reduction after AHP + DES pretreatment (**Fig. 3.5**). According to Vigier et al. (2015), DES was able to cause a reduction in cellulose crystallinity when it was used to pretreat biomass. Besides, these observations also concurred with the findings from Kumar et al. (2016), where they claimed that the partial disruption of amorphous cellulose caused the CrI of cellulose to decrease. The increase in porosity of OPF after the AHP pretreatment allowed the DES to access the pretreated OPF more effectively, resulting in significant delignification and slight decrystallization of cellulose. As such, the combined sequential pretreatment achieved the best results due to the ability to remove lignin. Thereby, both pretreatments exposed crystalline cellulose, which would potentially enhance the enzymatic accessibility for glycosidic bond cleavage (Li et al., 2013).

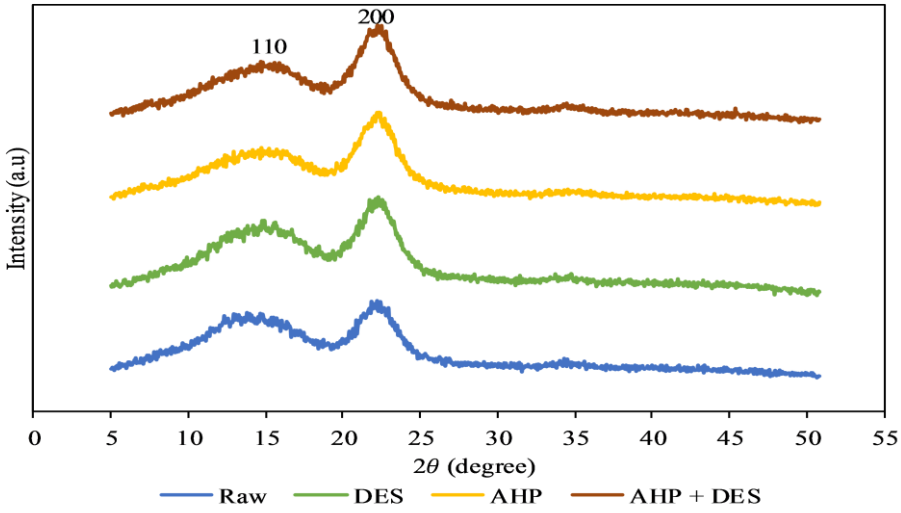

Fig. 3.5 XRD spectrum of raw and pretreated OPFs.

3.4.3.3 FTIR

FTIR was used to determine the changes of chemical functional groups on OPFs after undergoing pretreatment. Consistent peaks at 900 cm^{-1} and 2900 cm^{-1}, representing β-glycosidic linkages and –OH groups in the cellulose, respectively, were observed (**Fig. 3.6**). This observation implied that the cellulose in the OPF was not significantly altered by the pretreatments. Previous

studies reported that ChCl served to stabilize the cellulose system (Loow et al., 2018), while AHP only selectively targeted lignin and hemicellulose (Mittal et al., 2017). Thus, the dissolution of cellulose was unlikely. The most significant differences were observed for the peaks at 1031 cm^{-1} (**Fig. 3.6**), indicating the presence of C–O stretching vibration in cellulose, hemicellulose, and lignin (Ofori-Boateng and Lee, 2014). When comparing with raw OPF, the obvious peak reduction at 1031 cm^{-1} shown in AHP + DES-pretreated OPF implied the significant removal of lignin from the OPF. On top of that, peaks representing aryl-alkyl ether bonds (C–O–C) at 1235 cm^{-1} experienced significant reduction after the pretreatment (**Fig. 3.6**). A more drastic change was observed in OPF after AHP pretreatment as compared to the raw OPF, indicating the capability of AHP and DES in cleaving aryl-alkyl ether bond, also known as β–O–4 linkage. Furthermore, the peaks ranged from 1508 to 1600 cm^{-1}, represented by aromatic skeletal C=C from lignin aromatic ring (Loow et al., 2018), experienced slight reductions especially for OPF after underwent DES pretreatment. This observation was due to the ability of Cl^- ions from the DES to form hydrogen bonding with the aromatic ring in lignin, causing the dissolution of lignin polymer. Besides, the results also suggested that the delignification of AHP pretreatment at mild concentration mainly attributed by the cleavage of β–O–4 linkage and only minimal effect on the phenolic ring. Moreover, a notable decreased in the peak at 1735 cm^{-1} after the AHP pretreatment (**Fig. 3.6**), denoted the ability of AHP to breakdown C=O acetyl group in the hemicellulose.

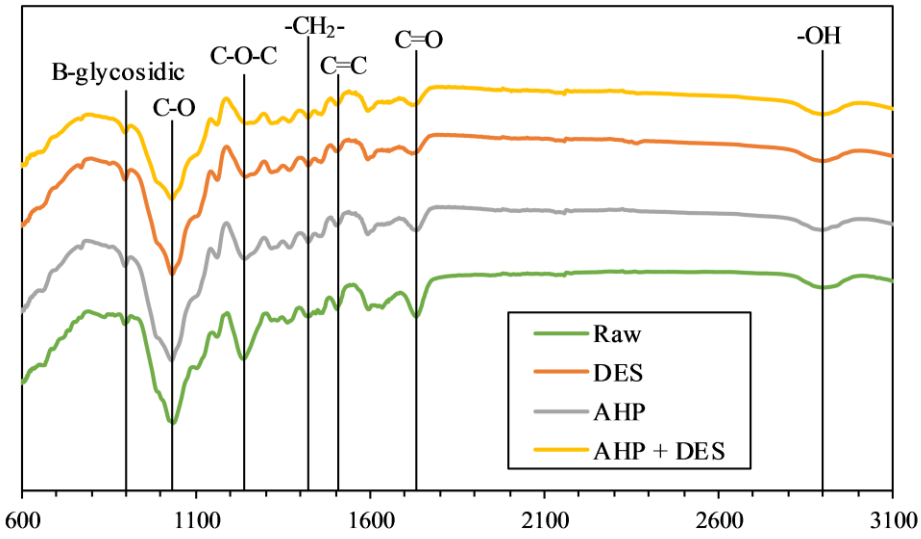

Fig. 3.6 FTIR spectrum of raw and pretreated OPFs.

3.4.3.4 BET

From the BET analysis, the specific surface area of raw and pretreated OPFs could be obtained. According to Loow et al. (2018), the delignification of OPF could increase the surface area

of OPF, leading to an increase in accessibility. The initial specific surface area of raw OPF was 0.4096 m^2/g. After control pretreatment, the area increased slightly to 0.4310 m^2/g. Furthermore, when the raw OPF was pretreated using 0.25 vol% AHP under ambient conditions for 90 min, the BET surface area increased to 0.4249 m^2/g. Interestingly, the surface area of AHP-pretreated OPF was only slightly lower in comparison with control pretreatment. This result was unexpected as the degree of delignification (**Table 3.4**) was anticipated to increase the surface area significantly. Based on the FE-SEM image of AHP-only pretreatment, although the surface was relatively smoother as compared to the control pretreatment, there was a notable amount of tiny dots. This observation indicated that the internal structure (cellulose) was readily accessible. Thus, the ability of AHP in creating small holes during biomass structure alteration was likely to cause the increase in the surface area similar to the DES-pretreated OPF, despite lower delignification (**Table 3.4**). The OPF pretreated with both AHP and DES achieved the highest surface area of 0.5117 m^2/g, which was 18.72% increase in surface area in comparison with raw OPF. The increase in surface area of AHP + DES-pretreated OPF was significantly higher than the DES-pretreated OPF and AHP-pretreated OPF. Thus, the BET analysis validated the presence of synergistic effects when AHP was integrated with DES in OPF pretreatment.

3.4.3.5 TGA

TGA and derivative thermogravimetric (DTG) spectra were obtained for raw and pretreated OPFs. The TGA spectrum showed a slight decrease in weight at the temperature range of 50 – 125°C (**Fig. 3.7a**), representing the evaporation of volatile components and physically adsorbed water (Darji et al., 2015). Furthermore, consistent with the previous study done by Subhedar and Gogate (2014), a significant decomposition was observed at 200 – 375°C, indicating the liberation of volatile hydrocarbons owing to the decays of hemicellulose, cellulose, and some portions of lignin. Lignin decomposed slowly over the temperature ranging from 200 – 900°C (Phitsuwan et al., 2016). Thus, the weight remaining after the decomposition of hemicellulose and cellulose (> 375°C) was mainly lignin and ash. Raw OPF has the highest residue, followed by AHP-only, control, and lastly AHP + DES-pretreated OPF in descending order. (Jablonský et al., 2015) reported that a small amount of ash was able to solubilized in DES during the delignification process. Therefore, this result was consistent with the chemical composition of pretreated OPFs, in terms of containing higher cellulose and hemicellulose but lower lignin and ash content as compared to the raw OPF. From the DTG analysis of raw OPF (**Fig. 3.7b**), two distinct peaks were observed at 280 and 330°C, representing hemicellulose and cellulose, respectively. This observation was consistent with the previous study (Loow et al., 2017b). All pretreated OPFs showed a slight shift to the right for both maximum

degradation temperatures of cellulose and hemicellulose, similar to the findings obtained by Phitsuwan et al. (2016) after pretreatment of Napier grass. The temperature shift of DTG decomposition peaks could be possibly explained by the large proportion of crystalline cellulose, as a result of removing lignin and a fraction of hemicellulose. According to Braga et al. (2014), the high degree of polymerization, rich in hydrogen bonds, and strong interactions between cellulose attributed to the thermally stable properties of cellulose. Moreover, the AHP + DES-pretreated OPF obtained the highest derivative weight at 360 °C, indicating the greater removal of lignin and with an increase of cellulose proportion. The claim was backed by the compositional analysis carried out in this study (**Table 3.4**).

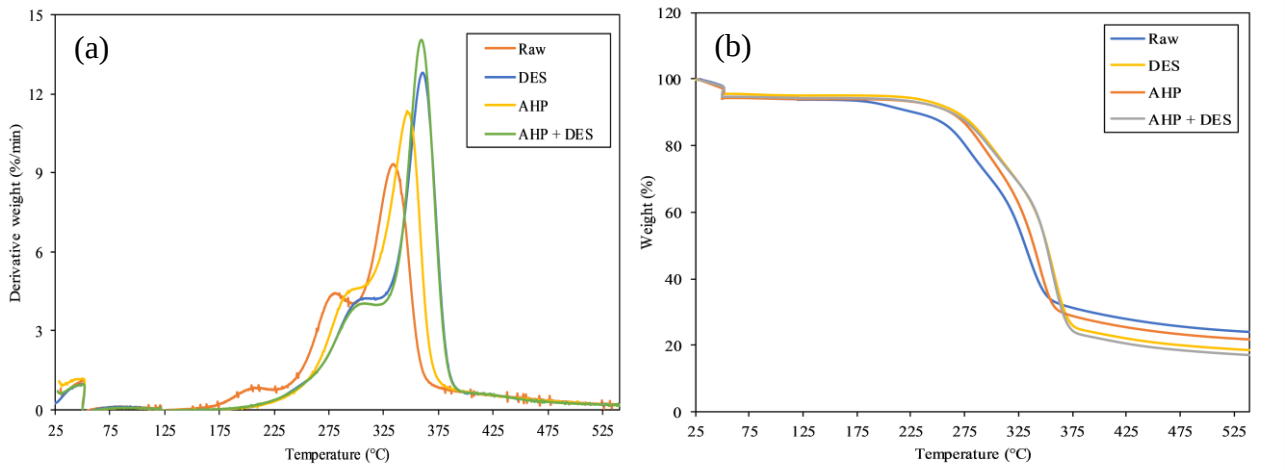

Fig. 3.7 Spectrum for (a) TGA and (b) DTG of raw and pretreated OPFs.

3.4.4 Proposed mechanism for pretreatments of OPF

The mechanism of AHP in the pretreatment relied on decomposition products. At pH closer to the pKa of H_2O_2 (pH 11.5), hydrogen peroxide decomposes into hydroperoxy anion (HOO^-) in primary decomposition, hydroxyl radical (•OH), and superoxide anion ($•O_2^-$) in secondary decomposition as shown in **Eq. 3.4** and **Eq. 3.5** (Gould, 1984). These lignin acting species are responsible for the delignification in AHP pretreatment. According to (Mittal et al., 2017), aromatic rings exposure and cleavage were not seen in mild peroxide concentrations (90mg H_2O_2/g dry corn stover). As the aromatic rings alteration were mainly attributed by the •OH, thus it was presumed that the HOO^- anion was the dominant reactive oxidant species in mild concentration AHP. This claim was backed by the FTIR analysis, where the peaks representing aromatic rings remained relatively unchanged. HOO^- enabled the cleavage of internal alkyl aryl ether bonds in the lignin and destruction of C-C bonds in the aliphatic region, which contained an adjacent phenolic hydroxyl moiety (Li et al., 2012). As stated by Halma et al. (2015b), the scission of β-O-4 linkages by HOO- anion occurred through an internal nucleophilic reaction in the absence of free-phenolic hydroxyl groups (hardwood).

Therefore, a hydroxyl group was generated on the side chain of the aromatic ring. The increase in free hydroxyl groups would plausibly improve the solubilization of lignin and lead to more site-specific delignification.

$$H_2O_2 + HO^- \rightarrow HOO^- + H_2O \quad \textbf{(Eq. 3.4)}$$

$$H_2O_2 + HOO^- \rightarrow HO^{\bullet} + O_2^{-\bullet} + H_2O \quad \textbf{(Eq. 3.5)}$$

On the other hand, ChCl:Urea delignification properties were mainly due to the ability of strong electronegativity halogen anion (Cl^-) to form hydrogen bonds with hydroxyl groups in the lignin (Loow et al., 2018). This capability of Cl^- enabled the disruption of the hydrogen bonding network in the OPF, thus extracting the lignin. It was proposed that the generation of hydroxyl groups as a result of β-O-4 scission in AHP, encouraging the hydrogen bonds forming in DES pretreatment of OPF. Thus, the hydroxyl groups enhanced the lignin extraction ability of Cl^- anion and better disruption of hydrogen bonds network as illustrated in **Fig. 3.8**. This proposed mechanism could explain the synergistic improvement of DES pretreatment on AHP-pretreated OPF. However, it was observed that a further increase in AHP concentration (>0.25%) affected DES delignification negatively. As AHP concentration increased, more lignin fragmentation and aromatic rings alterations were expected. Consequently, stronger hydrogen bond interactions were imposed on the fragmented lignin and newly formed hydroxyl groups by the OPF. Besides, the aromatic rings alterations by the AHP could lead to the removal of hydroxyl groups, which reduced the site of attacks for DES delignification. The collective effect of both proposed reasons could contribute to the reduction in DES delignification performance by hindering the hydrogen bonds forming with lignin.

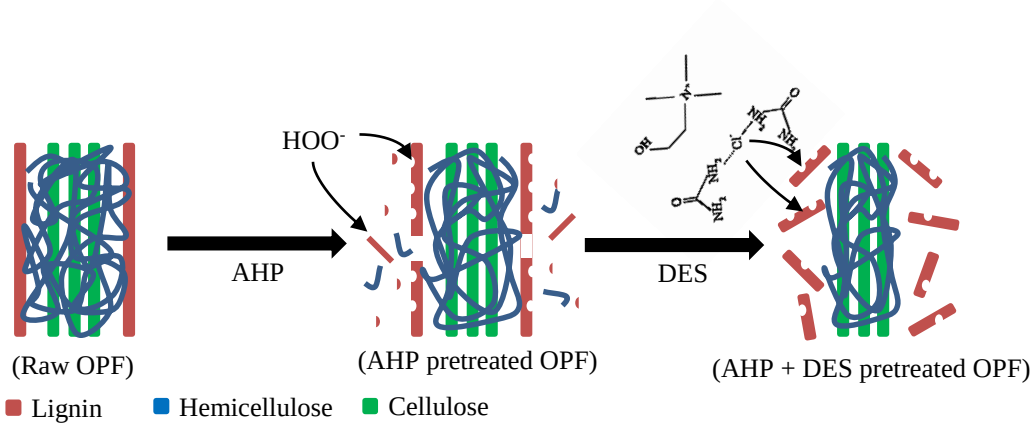

Fig. 3.8 Simple illustration of pretreatment mechanism.

3.4.5 Comparison with past studies using AHP in pretreatment of biomass

The findings from this study were compared with various pretreatment integrated with AHP (**Table 3.6**). The lignin content reduction obtained in this study was relatively lower than all of the previous studies as hardwood (OPF) is typically more difficult to pretreat. It is worth noting that the AHP used in this study was utilized at a relatively mild concentration, shorter duration, and less energy intensive. The use of novel DES also claimed to be less hazardous, and stable at elevated temperature as compared to the sulphuric acid, and organosolv. Interestingly, the present study was able to achieve better delignification as compared to the findings obtained by Ayeni et al. (2013) in shea tree sawdust biomass pretreatment, although the conditions employed in this study were significantly less severe by incorporating sequential pretreatment approach. However, it was found that the delignification efficiency of AHP + DES pretreatment was inferior as compared to the established methods. Thus, further works are needed to improve the performance of DES pretreatment. It was deduced that the use of a more effective DES would potentially improve delignification and synergistic effect more significantly. The application of DES in biomass processing is still in its infancy, more improvement is expected as the DESs develop further in the future.

3.5 Conclusion

The synergistic effects of AHP and DES pretreatments were investigated for OPF delignification. The combination of AHP (0.25 vol%, 90 min) at room temperature and DES (ChCl:Urea) at fixed operating conditions (120°C, 4 h) achieved highest synergistic effect in DES delignification (18.99%) as compared to DES-alone pretreatment (12.16%). The concentration of AHP greatly influenced the subsequent delignification of DES pretreatment. The improvement of AHP + DES sequential pretreatment in delignification was attributed by the ability of AHP to enhance biomass accessibility and susceptibility to hydrogen bonds forming, which were verified via various characterization studies. Hence, the use of mild concentration AHP at ambient conditions with DES pretreatment could improve lignin extraction and yield glucan-rich OPF. Thus, facilitating the downstream conversion of OPF to biofuel and chemical building block. The results obtained indicate AHP (0.25 vol%, 90 min) at ambient conditions was able to synergistically improve the delignification of DES from 12.16 to 18.99% in OPF sequential pretreatment (**Objective 1**). The recommended condition of AHP for the best synergistic effect will be used in phase 2, which will be discussed in **Chapter 4**.

Table 3.6

Performance of various pretreatments integrated with AHP in lignocellulosic biomass delignification.

Biomass	Pretreatment conditions	Lignin content (%)		Delignification (%)	Reference
		Before pretreatment	After pretreatment		
Jerusalem artichoke stem	(1) 5.0% (w/v) of H_2O_2 with 2.0% (w/v) of NaOH, ultrasonic at 50°C for 2 h.	26.00	21.60	40.3	(Li et al., 2016c)
Jerusalem artichoke stalk	(1) 2.0% (v/v) of H_2O_2 with 2.0% (w/w) of NaOH, heated to 121℃ for 90 min.	17.26	11.78	53.4	(Li et al., 2016a)
Olive tree biomass	(1) 10.0% S/L; H_2O at 120°C for 60 min. (2) 2.4% (w/v) of H_2SO_4, autoclave at 130°C for 84 min. (3) 1.0% (w/v) of H_2O_2, adjusted to pH 11.5 using NaOH, heated to 80°C for 90 min.	18.50	36.30	19.7	(Martínez-Patiño et al., 2017)
Olive tree biomass	(1) 10.0% S/L; H_2O at 120°C for 60 min. (2) 2.4% (w/v) of H_2SO_4, autoclave at 130°C for 84 min. (3) 7.0% (w/v) of H_2O_2, adjusted to pH 11.5 using NaOH, heated to 80°C for 90 min.	18.50	15.02	77.8	(Martínez-Patiño et al., 2017)
Shea tree sawdust	(1) 1.0% (v/v) of H_2O_2, adjusted to pH 11.5 using $Ca(OH)_2$, stirring at 21 rad/s, at 150°C for 45 min.	29.90	30.25	9.64	(Ayeni et al., 2013)
Tea oil fruit hull	(1) Acetic acid organosolv (53% acetic acid, 0.6% H_2SO_4) at 125°C for 1.7 h. (2) 1.0% of H_2O_2, adjusted to pH 11.5 using NaOH at ambient condition for 12 h.	24.60	21.00	79.0	(Tang et al., 2017)
Oil palm fronds (OPF)	(1) 0.25% (v/v) of H_2O_2, adjusted to pH 11.5 using NaOH, at ambient condition for 90 min. (2) DES (ChCl:Urea) at 120°C for 4 h under atmospheric pressure.	18.84	16.25	19.0	This study

Chapter 4

Comparison of sequential pretreatment between alkaline hydrogen peroxide/Type III DES choline chloride:urea and alkaline hydrogen peroxide/Type II DES choline chloride:copper (II) chloride dihydrate

Publication:

1. **Ho, M.C.**, Wu, T.Y., 2019. Sequential pretreatment of alkaline hydrogen peroxide and choline chloride:copper (II) chloride dihydrate - Synergistic fractionation of oil palm fronds. Bioresource Technology, In press. (**Appendix C**)

4.1 Abstract

Deep eutectic solvents (DESs) have rapidly emerged in the field of biomass processing as promising solvents in biomass valorization and processing. In this study, Type III DES, Choline chloride:Urea and novel Type II DES, namely Choline chloride:Copper(II) chloride dihydrate (ChCl:$CuCl_2$•$2H_2O$) were used to pretreat oil palm fronds (OPFs). The sequential pretreatment of alkaline hydrogen peroxide (0.25 vol%, 90 min) at ambient temperature and pressure with Type II DES (90°C, 3 h) at the later stage resulted in a delignification of 55.14% with an extraordinary xylan (80.79%) and arabinan (98.02%) removals. The characterizations of pretreated OPF confirmed the excellent performance of DES in OPF fractionation. Thus, the application of Type II DES at ambient pressure and relatively lower temperature was able to improve the lignin and hemicellulose removals from OPF.

4.2 Introduction

Agricultural lignocellulosic residues are biomass resources abundantly available in many countries (Spyridon et al., 2016). Presently, Malaysia has one of the largest oil palm plantations in the world. This industry generates more than 90% of the national total of lignocellulosic biomass (Loow et al., 2017b). Oil palm frond is a by-product produced from the oil palm plantation, which is also the highest solid waste generated from the oil palm industry. Each year, approximately 26.2 million tons of oil palm fronds are produced per million of fresh fruit bunches processed (Yunus et al., 2010). Even though oil palm fronds are readily available sources for a biorefinery, the fronds are typically used as mulches in the plantation (Loow and Wu, 2018). This approach has little economic value to the industry, while environmental problems may arise due to the improper handling of biomass decomposition (Isikgor and Becer, 2015).

Recently, lignocellulosic biomass has been attracting focus from various research groups in its valorization potential to form value-added products such as biofuel. Lignocellulosic biomass is a complex structure of polymers, which mainly comprised of cellulose, hemicellulose, and lignin. Due to the recalcitrant nature of lignocellulosic biomass, a pretreatment stage is often required to effectively fractionate biomass and improve enzymatic digestion of cellulose and hemicellulose. Along the years, numerous pretreatment methods have been introduced, which aimed to improve process yield and efficiency of the downstream process (Loow et al., 2016b). Alkaline pretreatment is one of the most established pretreatment technologies to delignify biomass without severe polysaccharides degradation (Loow et al., 2016a). Recent applications of alkaline hydrogen peroxide (AHP) have shown promising performance to improve lignin depolymerization through radical oxidation route (Ho et al., 2019a).

To date, on-going studies were carried out to innovate more advanced and effective methods in biomass pretreatment (Loow et al., 2017a). However, the applications of technologies in a practical scale are often limited by the cost. Recently, a new class of solvent, known as deep eutectic solvent (DES), has emerged as an alternative solvent in biomass pretreatment. DES was first introduced by Abbott et al. (2004) by combining a quaternary ammonium salt (HBA) and a hydrogen-bond donor (HBD) to form a liquid eutectic mixture. To date, DESs have been increasingly applied in many studies related to biomass fractionation due to their promising potential to extract bioactive phenolic compounds (Wei et al., 2015), delignification (Ho et al., 2019b), sugar recovery (Loow et al., 2018), furanic conversion (Lee et al., 2019), and biodiesel processing (Lu et al., 2016).

Although DES has been critically involved in the recent studies of biomass pretreatment, the study regarding the application of Type II DES (metal salt hydrate + organic salt) in biomass fractionation is not available. On the other hand, even though inorganic salts such as $FeCl_3$, $AlCl_3$, and $CuCl_2$ have been extensively studied for pretreating biomass and hemicellulose extraction (Loow et al., 2015; Loow et al., 2018), no available literature was found regarding the application of inorganic hydrate salt-based Type II DES. To the best of our knowledge, no investigation has been carried out to study the potential use of inorganic hydrate salt ($CuCl_2 \bullet 2H_2O$) based Type II DES and its synergistic effects with AHP in the pretreatment of oil palm fronds. It was hypothesized that the Type II DES might inherit the ability of inorganic salt ($CuCl_2$) to selectively hydrolyze hemicellulose from the fronds at relatively lower temperature and atmospheric pressure. On top of that, the implementation of 0.25 vol% AHP at room temperature for 90 min (**Chapter 3**) could help to alter macrostructure and partially solubilize lignin in the oil palm fronds (Ho et al., 2019b). Thus, facilitating the DES in accessing the biomass matrix, which led to better delignification and hemicellulose extraction. Therefore, the objectives of this study were to evaluate the potential use of novel Type II DES (choline chloride:copper (II) chloride dihydrate) by comparing to Type III DES (choline chloride:urea) in oil palm fronds processing and its synergistic effects with AHP at a non-severe condition in a sequential pretreatment.

4.3 Materials and methods

4.3.1 Raw material and chemicals

Fresh oil palm fronds used in this study were provided by Universiti Kebangsaan Malaysia oil palm plantation. After the leaflets were separated, the petioles were sent to fronds pressing machine to remove juices from the oil palm fronds. The pressed petioles were left drying under the sun for two days before grinding to a smaller size using pulverizer (8000 rpm). After that, the mechanical sieve was used to isolate ground petioles at a size of $\leq$0.5mm. These $\leq$0.5mm sieved and

ground petioles were washed with distilled water and dried in an oven at 60°C for 48 h. The prepared oil palm fronds were herein referred to as OPF throughout this study and stored in a tight-fitting lid container filled with desiccants at room temperature prior to experiments. Compositions of OPF in terms of structural carbohydrates and lignin were determined by adopting standard laboratory analytical procedure from National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008). D(+)glucose (AR grade, 99%), D(-)xylose (AR grade, 99%), L(+)arabinose (AR grade, 99%), acetic acid (HPLC grade, ≥ 99.8%), and formic acid (AR grade, ≥ 98%) were used for high performance liquid chromatography (HPLC) standard curves calibration. Sodium hydroxide (AR grade, 99%, pellets), hydrogen peroxide (extra pure, 50%), choline chloride (99%), urea (AR grade, 99%), copper (II) chloride dihydrate (AR grade, 99%), and other chemicals were ensured to be of analytical grades.

4.3.2 Sequential pretreatment

Two-stage sequential pretreatments, namely AHP as the first stage while Type II or Type III DES as the second stage, were used in the pretreatment of OPF.

4.3.2.1 AHP pretreatment

In AHP pretreatment, 4.5 g of OPF sample was weighed and placed into a 600 mL beaker. The AHP solution was prepared by diluting with deionized water to yield 0.25 vol% of hydrogen peroxide solution and adjusted to a pH 11.5 using NaOH pellets based on the past study (Ho et al., 2019b). The AHP solution was then added to the beaker containing OPF at a solid to liquid ratio of 1:20 (w/v). This non-severe pretreatment was carried out at room temperature and ambient pressure for 90 min (Ho et al., 2019b). The AHP-pretreated OPF sample was then filtered and washed with distilled water to remove any residual AHP. Then, the AHP-pretreated sample was dried at 60°C in the oven overnight before storing the sample in a container with desiccants prior to further uses.

4.3.2.2 DES pretreatment

Type III and Type II DES was used to pretreat OPF samples obtained from the AHP pretreatment. Type III DES, namely choline chloride:urea (ChCl:Urea) and Type II DES, namely choline chloride: copper (II) chloride dihydrate (ChCl:$CuCl_2$•$2H_2O$) was synthesized at a molar ratio of 1:2 and 1:1 respectively. The Type III and Type II DES were synthesized at 90°C and 45°C respectively. The mixture were stirred at 200 rpm until a homogeneous clear liquid was formed. Fresh DES was synthesized each time the pretreatment was conducted.

Firstly, 1.0 g of AHP-pretreated OPF was weighed and transferred into a 50 mL Schott bottle. DES was added to the Schott bottle at a fixed solid to liquid ratio of 1:10 (w/v). Prior to the

pretreatment process, oil baths were heated to the stable temperature of either 80, 90, or 100°C for Type II DES and 110, 120, 130, or 140°C for Type III. When the desired oil bath temperature was achieved and stable, the Schott bottle containing both OPF and DES was placed in the oil bath. Effects of different operating temperatures and reaction durations (1, 2, 3, and 4 h) were investigated in this study. For Type II DES, further increase in temperature (> 100°C) was not attempted as pretreatment process was not feasible due to the occurrence of precipitation. Upon the completion of pretreatment, the Schott bottle was removed from the oil bath and quenched using running tap water. The resulting liquid fraction (DES hydrolysate) was collected, centrifuged, and diluted prior to HPLC analysis. After that, the solid fraction was filtered and washed with distilled water to remove any excessive DES. The AHP + DES-pretreated OPF sample was dried at 60°C overnight inside an oven and stored in a transparent seal bag before undergoing subsequent analysis.

4.3.3 Analytical methods for liquid hydrolysate

4.3.3.1 High performance liquid chromatography (HPLC)

The concentrations of monomeric sugars, structural carbohydrates, and inhibitors in the centrifuged hydrolysate obtained from DES pretreatment and acid hydrolysis of OPF samples were analyzed using an Agilent series 1200 infinity HPLC system equipped with a Refractive Index (RI) detector and BioRad Aminex HPX-87H column. 0.005 mol/L H_2SO_4 was used as mobile phase for the application of HPX-87H column at an operating flow rate of 0.6 mL/min, while the column temperature was set at 65°C. The sugar recoveries (%) from DES hydrolysate were evaluated using formula adapted from Loow et al. (2018) as shown in **Eq. 4.1**.

$$\textit{Sugar recovery (\%)} = \frac{\textit{sugar recovered in hydrolysate (g/L)} \times \textit{volume of solvent used (L)}}{\textit{sample carbohydrate fraction} \times \textit{mass of sample used (g)}} \times 100\% \quad \textbf{(Eq. 4.1)}$$

4.3.4 Analysis of solid fractions

4.3.4.1 Structural carbohydrates (glucan, xylan, and arabinan) and lignin quantification

Standard laboratory analytical procedure for the lignin composition was adopted from the National Renewable Energy Laboratory (NREL) to quantify lignin in the OPF sample (Sluiter et al., 2008). Acid hydrolysis was done by adding 3 mL of 72% H_2SO_4 to 0.3 g of dried sample in a 250 mL conical flask. Next, the conical flask was incubated at 30°C and 150 rpm for an hour. After that, 84 mL of deionized water was added into the incubated mixture and before sending to autoclave at 121°C for 1 h. The autoclaved mixture was transferred to vacuum filtration where the filtrate was collected and analyzed for structural carbohydrates concentration in HPLC and acid soluble lignin in UV-Vis

spectrophotometer at 320 nm. Lastly, acid insoluble lignin was determined by evaluating the difference in dry weight of the filter paper before and after vacuum filtration. Lignin (**Eq. 4.2**) and structural carbohydrates (**Eq. 4.3**) removals were evaluated as follows (New et al., 2019):

$$\textit{Lignin removal (\%)} = \left(1 - \frac{\textit{Lignin content in pretreated OPF}}{\textit{Lignin content in raw OPF}} \times \textit{yield}\right) \times 100\% \quad \textbf{(Eq. 4.2)}$$

$$\textit{Structural carbohydrate removal (\%)} = \left(1 - \frac{\textit{Structural carbohydrate content in pretreated OPF}}{\textit{Structural carbohydrate content in raw OPF}} \times \textit{yield}\right) \times 100\% \quad \textbf{(Eq. 4.3)}$$

Where,

Structural carbohydrates = Glucan, xylan, and arabinan

Yield = Mass recovery of pretreatment in fraction, $\left(\frac{\textit{mass of pretreated OPF}}{\textit{mass of raw OPF}}\right)$

4.3.4.2 Fourier transform infrared spectroscopy (FTIR)

Chemical structures of the raw and pretreated OPF were analyzed using attenuated total reflection (ATR) tip FTIR (Thermoscientific Nicolet iS10 spectrometer). The spectra were obtained via 64 scans over the range of 500 to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1}.

4.3.4.3 Field emission scanning electron microscope (FE-SEM)

OPF samples were adhered to a specimen stub using double-coated tape and sputter-coated with platinum prior to imaging using Hitachi SU 8010 in order to visualize the microstructural alteration in biomass surface morphology.

4.3.4.4 X-Ray diffraction (XRD)

The OPF samples were scanned with a 2θ ranged from 5 to 51° in steps of 0.041° using a Bruker D8 discover diffractometer to determine the crystallinity index (CrI). The crystallinity index (CrI) as shown in **Eq. 4.4** is defined by Segal et al. (1959) as the relative degree of crystallinity in the specimen, where:

$$\textit{Crystallinity index (\%)} = \frac{I_{200} - I_{am}}{I_{200}} \times 100\% \quad \textbf{(Eq. 4.4)}$$

Where I_{200} is the maximum intensity of the 200 peak (2θ = 22°) and I_{am} is the intensity minimum between peaks at 200 and 110 (2θ = 18°) (French, 2014).

4.3.4.5 Brunauer-Emmett-Teller (BET)

Micromeritics ASAP 2020 BET model with N_2 as the adsorbate at -195.85°C was used to carry out BET analysis. Degas conditions were adapted from Lim and Wu (2016); Lim and Wu (2015), where samples were initially heated to 90°C for 2 h and subsequently holding at 110°C for 22 h.

4.3.4.6 Thermogravimetric analysis (TGA)

Mettler Toledo Thermo Gravimetric Analyzer in nitrogen environment was used to conduct thermogravimetric analysis of samples. The methodology was adapted from Marcotullio et al. (2011). The OPF samples were placed in a ceramic pan, thereafter the temperature was increased to 50°C at ramping rate of 10°C/min and kept isothermal for 45 min. Then, the temperature was raised to 120°C at ramping rate of 10°C/min and held constant for 15 min to remove moisture content before increasing the temperature to 550°C at same ramping rate. Once the temperature has reached 550°C, the furnace was maintained isothermal for 40 min.

4.3.5 Statistical analysis

The experimental data were statistically analyzed using IBM SPSS statistics 24. One-way analysis of variance (ANOVA) was carried out to identify any significant variation between pretreatments. The homogeneous subset for a range of parameters was verified via Duncan's test with a significance level of $P < 0.05$.

4.4 Results and discussion

4.4.1 Compositional analysis of raw OPF

The composition of raw OPF by dry weight was comprised of glucan (44.46 ± 0.49%), xylan (21.49 ± 0.06%), arabinan (2.04 ± 0.02%), lignin (17.20 ± 0.10%), and the remaining weight of 14.81% of ash, water, and ethanol extractives. The compositions were found to have slightly deviated from other literature findings (Loow et al., 2018; New et al., 2019; Tan et al., 2016). These variations were expected due to the different maturity and geographical source of the palm tree in which the OPF was harvested (Loow and Wu, 2018).

4.4.2 Effect of AHP and type III DES on solid fractions

The recommended concentration for AHP pretreatment of OPF for best synergism was determined to be 0.25 vol% (Ho et al., 2019b). When OPF was pretreated in AHP at room temperature and 90 min, the delignification of Type III DES could be improved by 1.6 times. Thus, this study was proposed to determine the recommended operating conditions for ChCl:Urea pretreatment when integrated with AHP pretreatment (0.25 vol% of H_2O_2 at room temperature, pH 11.5 and 90 min), in order to achieve best delignification while revealing the sugar recoveries potential of DES.

The composition of pretreated OPFSs (solid fractions) was tabulated in **Table 4.1**. The results were interpreted from three main perspectives, namely, solid recovery, delignification, and sugar content. According to one-way ANOVA, the solid yield of Type III DES pretreatment was found to reduce as the temperature and duration increased. The solid yield of OPF was reduced to as low as 84.81% after Type III DES pretreatment at 140°C and 4 h. The reduction in solid yield was not severe, although a relatively high temperature was used to pretreat the OPF. This observation implied that the Type III DES (ChCl:Urea) pretreatment was not able to extract a significant amount of OPF components to the hydrolysate. However, it is worth noting that, pretreatment with high solid yield and high selectivity in delignification may serve as an effective fractionation of biomass in the biorefinery. Thus, the composition of pretreated OPFs was extensively studied at various temperature and duration.

4.4.2.1 Lignin

It was found that the lignin removal of AHP + Type III DES (ChCl:Urea) pretreated OPF showed an increasing trend as temperature and duration increased. According to one-way ANOVA analysis, the lignin removal of OPF reached a plateau state as the duration of pretreatment increased. This observation can be seen from **Table 4.1**, where the lignin removal of AHP + Type III DES (ChCl:Urea) pretreatment of 130 and 140°C, at the duration of 3 – 4 h do not show significant differences. Besides, the lignin content of pretreated OPFs was found to decrease as the duration increased at temperature above 120°C. The lignin content was found not to exhibit obvious trend below 120°C, which may be due to the poorer delignification and higher viscosity of DES at low temperature (Loow et al., 2017a). This observation suggested that the rate of delignification was more dominant at temperature above 120°C, which the rate increased as temperature increased.

Table 4.1

Composition of OPF after undergoing AHP + Type III DES (ChCl:Urea) sequential pretreatment at different temperature and duration. Values annotated with different letters represents the difference in significance levels for each category (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Yield (%)	Lignin		Glucan		Xylan		Arabinan	
		Content (%)	Removal (%)	Content (%)	Removal (%)	Content (%)	Removal (%)	Content (%)	Removal (%)
Raw	-	17.20 ± 0.10^{a}	-	44.46 ± 0.49^{a}	-	21.49 ± 0.06^{a}	-	2.04 ± 0.02^{a}	-
AHP alone	95.90 ± 0.18^{a}	16.68 ± 0.06^{b}	6.98 ± 0.35^{a}	45.48 ± 0.06^{b}	$1.88 \pm 0.14^{a,b}$	21.45 ± 0.13^{a}	4.25 ± 0.57^{a}	$1.94 \pm 0.03^{a,b}$	8.63 ± 1.30^{a}
DES temperature: 110°C									
AHP + DES (1 h)	92.92 ± 0.45^{b}	$15.57 \pm 0.15^{c,d,e}$	15.89 ± 0.79^{b}	$46.24 \pm 1.07^{b,c,d}$	$3.34 \pm 2.24^{b,c,d}$	21.52 ± 0.31^{a}	$6.92 \pm 1.33^{b,c}$	$1.94 \pm 0.05^{a,b}$	$11.34 \pm 2.24^{a,b}$
AHP + DES (2 h)	90.10 ± 1.02^{c}	15.79 ± 0.06^{c}	$17.26 \pm 0.31^{b,c}$	$46.80 \pm 0.36^{c,d,e}$	$5.16 \pm 0.74^{d,e,f}$	21.61 ± 0.06^{a}	$9.37 \pm 0.26^{c,d,e,f}$	$1.91 \pm 0.01^{a,b,c}$	$15.52 \pm 0.15^{b,c,d}$
AHP + DES (3 h)	89.49 ± 1.34^{c}	$15.18 \pm 0.10^{d,e,f}$	$21.00 \pm 0.54^{d,e,f}$	$47.49 \pm 0.73^{e,f}$	$4.40 \pm 1.48^{c,d,e}$	$21.78 \pm 0.25^{a,b}$	$9.29 \pm 1.05^{c,d,e,f}$	$1.89 \pm 0.05^{b,c}$	$17.07 \pm 2.13^{c,d}$
AHP + DES (4 h)	89.36 ± 0.92^{c}	$15.23 \pm 0.04^{c,d,e,f}$	$20.88 \pm 0.20^{d,e}$	49.25 ± 0.02^{g}	1.00 ± 0.05^{a}	$22.41 \pm 0.17^{c,d}$	$6.79 \pm 0.72^{b,c}$	1.80 ± 0.11^{c}	21.07 ± 4.79^{d}
DES temperature: 120°C									
AHP + DES (1 h)	90.29 ± 0.83^{c}	$15.44 \pm 0.20^{c,d,e,f}$	$18.94 \pm 1.04^{c,d}$	$46.03 \pm 0.11^{b,c}$	6.52 ± 0.23^{f}	21.58 ± 0.03^{a}	$9.32 \pm 0.13^{c,d,e,f}$	$1.93 \pm 0.01^{a,b}$	$14.56 \pm 0.23^{b,c}$
AHP + DES (2 h)	89.35 ± 0.63^{c}	$15.45 \pm 0.19^{c,d,e,f}$	$19.73 \pm 1.00^{c,d}$	$47.01 \pm 0.05^{d,e,f}$	$5.52 \pm 0.11^{e,f}$	21.61 ± 0.41^{a}	$10.16 \pm 1.71^{d,e,f}$	$1.82 \pm 0.11^{b,c}$	$20.19 \pm 4.75^{c,d}$
AHP + DES (3 h)	87.16 ± 0.29^{d}	$15.24 \pm 0.28^{c,d,e,f}$	$22.75 \pm 1.42^{e,f}$	48.70 ± 0.10^{g}	$4.53 \pm 0.20^{c,d,e}$	$22.09 \pm 0.39^{a,b,c}$	$10.41 \pm 1.58^{d,e,f}$	1.34 ± 0.03^{d}	42.66 ± 1.35^{e}
AHP + DES (4 h)	86.70 ± 1.01^{d}	$15.13 \pm 0.05^{d,e,f,g}$	$23.73 \pm 0.25^{f,g}$	50.79 ± 0.19^{h}	0.96 ± 0.38^{a}	$22.36 \pm 0.36^{b,c,d}$	$9.78 \pm 1.47^{d,e,f}$	1.18 ± 0.02^{e}	49.80 ± 0.75^{f}
DES temperature: 130°C									
AHP + DES (1 h)	87.24 ± 1.32^{d}	$15.61 \pm 0.47^{c,d}$	$20.80 \pm 2.39^{d,e}$	47.75 ± 0.06^{f}	$6.30 \pm 0.12^{e,f}$	$22.69 \pm 0.22^{c,d,e}$	$7.90 \pm 0.91^{b,c,d,e}$	1.41 ± 0.12^{d}	39.63 ± 5.10^{e}
AHP + DES (2 h)	$85.36 \pm 1.03^{d,e}$	$14.96 \pm 0.54^{f,g}$	$25.76 \pm 2.67^{g,h}$	49.58 ± 0.32^{g}	$4.81 \pm 0.62^{c,d,e,f}$	$22.62 \pm 0.15^{c,d}$	$10.14 \pm 0.59^{d,e,f}$	1.34 ± 0.01^{d}	43.89 ± 0.52^{e}
AHP + DES (3 h)	$84.03 \pm 1.10^{e,f}$	$13.63 \pm 0.25^{i,j}$	$33.42 \pm 1.22^{i,j}$	$51.22 \pm 0.11^{h,i}$	$3.19 \pm 0.20^{b,c}$	$23.31 \pm 0.26^{f,g}$	$8.84 \pm 1.02^{c,d,e,f}$	0.84 ± 0.10^{g}	65.15 ± 4.27^{h}
AHP + DES (4 h)	81.37 ± 0.27^{g}	$13.65 \pm 0.34^{i,j}$	35.41 ± 1.62^{j}	54.38 ± 0.18^{k}	0.47 ± 0.34^{a}	$23.52 \pm 0.44^{f,g}$	10.94 ± 1.66^{f}	0.72 ± 0.07^{h}	71.26 ± 2.69^{i}
DES temperature: 140°C									
AHP + DES (1 h)	$85.30 \pm 1.14^{d,e}$	$14.61 \pm 0.12^{g,h}$	27.56 ± 0.60^{h}	50.92 ± 0.90^{h}	$2.31 \pm 1.73^{a,b}$	$23.25 \pm 0.47^{e,f,g}$	$7.69 \pm 1.87^{b,c,d}$	1.00 ± 0.04^{f}	58.15 ± 1.72^{g}
AHP + DES (2 h)	$83.42 \pm 1.21^{e,f}$	$14.14 \pm 0.20^{h,i}$	31.41 ± 0.97^{i}	52.08 ± 0.08^{i}	$2.27 \pm 0.16^{a,b}$	23.62 ± 0.17^{g}	$8.29 \pm 0.66^{b,c,d,e,f}$	0.84 ± 0.04^{g}	65.69 ± 1.82^{h}
AHP + DES (3 h)	$82.24 \pm 1.42^{f,g}$	13.52 ± 0.37^{j}	35.37 ± 1.78^{j}	53.08 ± 0.14^{j}	$1.81 \pm 0.26^{a,b}$	24.35 ± 0.16^{h}	$6.82 \pm 0.59^{b,c}$	$0.61 \pm 0.02^{h,i}$	$75.19 \pm 0.75^{i,j}$
AHP + DES (4 h)	81.34 ± 2.05^{g}	13.60 ± 0.18^{j}	35.68 ± 0.84^{j}	54.12 ± 0.56^{k}	0.99 ± 1.03^{a}	24.92 ± 0.26^{h}	$5.67 \pm 0.97^{a,b}$	0.54 ± 0.01^{i}	78.38 ± 0.50^{j}

4.4.2.2 Structural carbohydrates

It was observed from **Table 4.1**, Type III DES (ChCl:Urea) was unable to remove glucan and xylan effectively. The unusual trends obtained for glucan and xylan removal may be due to the release of free-bound sugars (Loow et al., 2018), while the effect on structural carbohydrates was not obvious. Interestingly, Type III DES (ChCl:Urea) was able to remove arabinan with removal up to 78.38% at a temperature of 140°C and duration of 4 h. In general, Type III DES (ChCl:Urea) was able to selectively target lignin, while the majority of the structural carbohydrates were not extracted. Therefore, when lignin removal increased as pretreatment temperature and duration increased, enrichment on glucan and xylan content were observed. For example, the glucan and xylan content were able to increase to 54.12 and 24.92% respectively at pretreatment condition of 140°C and 4 h.

4.4.3 Effect of type III DES on liquid fractions

Type III DES (ChCl:Urea) hydrolysate after pretreatment was collected and analyzed to determine sugars and inhibitors content in the liquid fractions. It was observed in solid fractions compositional analysis, whereby Type III DES (ChCl:Urea) mainly target lignin and arabinan. Thus, it is expected that the Type III DES (ChCl:Urea) hydrolysate was able to recover some of the arabinose. **Table 4.2** summarized the sugars and inhibitors content found in hydrolysate after pretreatment at various operating conditions.

4.4.3.1 Sugar recovery

It was observed in **Table 4.2**, the glucose and xylose recoveries from OPFs were not detected, even though a small amount of glucan and xylan removal were found in solid fraction compositional analysis. According to Loow et al. (2018), Type III DES (ChCl:Urea) mainly target lignin, whereas the degradation of cellulose and hemicellulose were limited. Thus, it was proposed that the released glucan and xylan were mainly attributed by the free-bound sugars in the OPFs. These released glucan and xylan could plausibly retain in the form of an oligomer or further degraded into other products due to the heating environment at high temperature. On the other hand, the recovery of arabinose was strongly dependent on pretreatment duration. For example, the arabinose recovery was increased from 7.62 (1 h) to 8.71% (2 h) then decreased to 6.99% (4 h) at pretreatment temperature of 110°C. This result was indicating that the sugar degradations might have occurred under prolonged pretreatment. However, at temperature above 120°C, the recovery of arabinose exhibited an unusual trend, which could be due to the thermal degradation of arabinose.

4.4.3.2 Inhibitors

Acetic acids was formed by degradation of acetyl groups in hemicellulose (xylose and arabinose). From **Table 4.2**, acetic acid concentrations increased as the temperature and duration of pretreatment increased for temperature below 140°C. Thus, it was proposed that the decrease in arabinose recovery was owing to the degradation of sugars due to the high temperature heating environment. However, a decreasing trend was observed for acetic acid concentration at a pretreatment temperature of 140°C. This phenomenon could be due to the evaporation of acetic acid (boiling point = 118°C), whereby the rate of evaporation surpassed the rate of acetic acid formations at a pretreatment temperature of 140°C. Thus, acetic acid concentration decreased.

4.4.4 Recommended conditions for Type III DES

The recommended conditions for Type III DES (ChCl:Urea) pretreatment was 130°C and 4 h. This condition was able to achieve lignin content of 13.65% with delignification of 35.41% while yielding glucan-rich OPF (54.38%). Further increase in temperature to 140°C does not have significant effect on the delignification and glucan enrichment of OPFs, thus lower temperature (130°C) pretreatment was selected. Pretreatment at a lower temperature could potentially reduce the risk associated with processes, while savings on the operating cost. **Fig. 4.1**, **4.2**, and **4.3** showed the colour changes after AHP, Type III DES, and AHP + Type III DES pretreatment, respectively at the recommended conditions.

From the previous study (Ho et al., 2019b), it was found that AHP pretreatment could help to promote the accessibility of OPF in the later pretreatment stage. Comparison of pretreated OPF after sequential pretreatment and Type III DES (ChCl:Urea) only pretreatment at recommended conditions were tabulated in **Table 4.3a**. It was found that the lignin, xylan and arabinan removals after sequential pretreatment with AHP were generally higher as compared to single-stage pretreatment. It is worth highlighting that, the lignin removal obtained in Type III DES (ChCl:Urea) pretreatment was increased from 26.89 to 35.41% after incorporating AHP in sequential pretreatment. This improvement suggested the occurrence of synergism between AHP and Type III DES (ChCl:Urea) in delignification. From the hydrolysate perspective (**Table 4.3b**), arabinose content was found to increase slightly in AHP + Type III DES (ChCl:Urea) pretreatment. Besides, acetic acid was found lesser in sequential pretreatment. From Ho et al. (2019a) review, AHP pretreatment was claimed to exhibit excellent acetyl group removal, thus lesser substrate (acetyl group) was available for degradation in Type III DES (ChCl:Urea) pretreatment.

Table 4.2

Type III DES (ChCl:Urea) hydrolysate after pretreating AHP-pretreated OPF at different temperature and duration. Values annotated with different letters represents the difference in significance levels for each category (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Sugar recovery						Formic acid		Acetic acid	
	Glucose (mg)[ζ]	Recovery (%)	Xylose (mg)[ζ]	Recovery (%)	Arabinose (mg)[ζ]	Recovery (%)	(g/L)	(mmol/L)	(g/L)	(mmol/L)
110°C										
DES (1 h)	ND[Ψ]	-	ND	-	$1.48 \pm 0.20^{a,b,c}$	$7.62 \pm 1.01^{a,b,c}$	ND	-	ND	-
DES (2 h)	ND	-	ND	-	$1.69 \pm 0.11^{b,c,d,e}$	$8.71 \pm 0.58^{b,c,d,e}$	ND	-	0.36 ± 0.01^{a}	5.99 ± 0.13^{a}
DES (3 h)	ND	-	ND	-	$1.62 \pm 0.03^{b,c}$	$8.34 \pm 0.17^{b,c}$	ND	-	$0.57 \pm 0.02^{a,b}$	$9.27 \pm 0.36^{a,b}$
DES (4 h)	ND	-	ND	-	$1.36 \pm 0.02^{a,b,c}$	$6.99 \pm 0.09^{a,b,c}$	ND	-	$0.86 \pm 0.06^{c,d,e}$	$14.34 \pm 1.07^{c,d,e}$
120°C										
DES (1 h)	ND	-	ND	-	$1.76 \pm 0.36^{b,c,d,e}$	$9.09 \pm 1.85^{b,c,d,e}$	ND	-	0.40 ± 0.03^{a}	6.71 ± 0.56^{a}
DES (2 h)	ND	-	ND	-	$1.83 \pm 0.14^{c,d,e}$	$9.44 \pm 0.70^{c,d,e}$	ND	-	$0.86 \pm 0.07^{c,d,e}$	$14.28 \pm 1.09^{c,d,e}$
DES (3 h)	ND	-	ND	-	$1.37 \pm 0.11^{a,b,c}$	$7.09 \pm 0.57^{a,b,c}$	ND	-	$1.07 \pm 0.02^{e,f,g}$	$17.74 \pm 0.32^{e,f,g}$
DES (4 h)	ND	-	ND	-	1.02 ± 0.13^{a}	5.26 ± 0.67^{a}	ND	-	1.20 ± 0.10^{g}	19.92 ± 1.75^{g}
130°C										
DES (1 h)	ND	-	ND	-	$1.42 \pm 0.25^{a,b,c}$	$7.31 \pm 1.29^{a,b,c}$	ND	-	$0.65 \pm 0.10^{b,c}$	$10.81 \pm 1.63^{b,c}$
DES (2 h)	ND	-	ND	-	$1.36 \pm 0.13^{a,b,c}$	$7.01 \pm 0.66^{a,b,c}$	ND	-	$0.88 \pm 0.05^{d,e}$	$14.67 \pm 0.88^{d,e}$
DES (3 h)	ND	-	ND	-	$1.64 \pm 0.01^{b,c,d}$	$8.48 \pm 0.02^{b,c,d}$	ND	-	$0.92 \pm 0.11^{d,e,f}$	$15.25 \pm 1.84^{d,e,f}$
DES (4 h)	ND	-	ND	-	$1.45 \pm 0.17^{a,b,c}$	$7.47 \pm 0.85^{a,b,c}$	ND	-	1.12 ± 0.20^{g}	18.66 ± 3.32^{g}
140°C										
DES (1 h)	ND	-	ND	-	$1.40 \pm 0.17^{a,b,c}$	$7.21 \pm 0.87^{a,b,c}$	ND	-	1.14 ± 0.01^{g}	18.99 ± 0.19^{g}
DES (2 h)	ND	-	ND	-	$2.14 \pm 0.38^{d,e}$	$11.03 \pm 1.98^{d,e}$	ND	-	$1.12 \pm 0.02^{f,g}$	$18.66 \pm 0.33^{f,g}$
DES (3 h)	ND	-	ND	-	$1.45 \pm 0.12^{a,b,c}$	$7.62 \pm 0.64^{a,b,c}$	ND	-	$0.89 \pm 0.14^{d,e}$	$14.79 \pm 2.37^{d,e}$
DES (4 h)	ND	-	ND	-	2.16 ± 0.58^{e}	11.15 ± 2.99^{e}	ND	-	$0.80 \pm 0.04^{c,d}$	$13.26 \pm 0.64^{c,d}$

[Ψ] ND = Not detectable

[ζ] Sugar mass in milligram per gram of OPF in dry basis

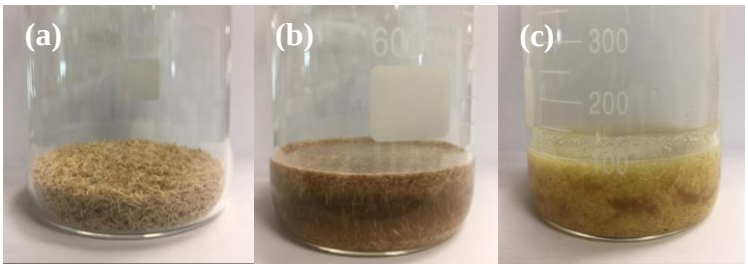

Fig. 4.1 (a) Raw OPF (b) before and (c) after AHP pretreatment.

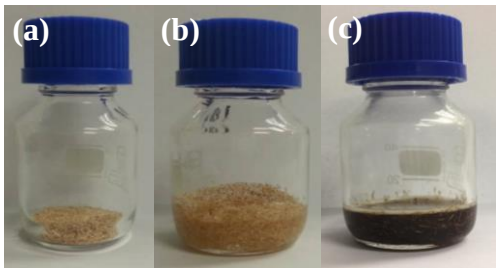

Fig. 4.2 (a) Raw OPF (b) before and (c) after Type III DES (ChCl:Urea) pretreatment.

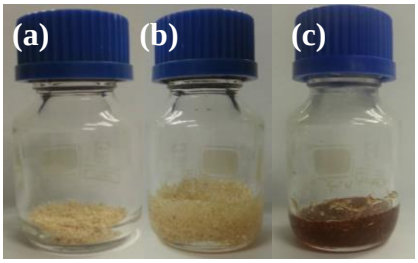

Fig. 4.3 (a) AHP-pretreated OPF (b) before and (c) after Type III DES (ChCl:Urea) pretreatment.

Table 4.3a

Compositional analysis of OPF after undergoing AHP (0.25 vol%, 90 min) and Type III ChCl:Urea DES (130°C, 4 h) pretreatments. Values annotated with different letters represents the difference in significance levels for each category (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Yield (%)	Lignin		Glucan		Xylan		Arabinan	
		Content (%)	Removal (%)	Content (%)	Removal (%)	Content (%)	Removal (%)	Content (%)	Removal (%)
Raw	-	17.20 ± 0.10^{a}	-	44.46 ± 0.49^{a}	-	21.49 ± 0.06^{a}	-	2.04 ± 0.02^{a}	-
AHP	95.90 ± 0.18^{a}	16.68 ± 0.06^{b}	6.98 ± 0.35^{a}	45.48 ± 0.06^{b}	1.88 ± 0.14^{a}	21.45 ± 0.13^{a}	4.25 ± 0.57^{a}	1.94 ± 0.03^{a}	8.63 ± 1.30^{a}
DES	87.35 ± 0.33^{b}	15.01 ± 0.08^{c}	26.89 ± 0.41^{b}	52.50 ± 0.16^{c}	1.07 ± 0.30^{a}	$22.98 \pm 0.13^{b,c}$	$10.44 \pm 0.51^{b,c}$	0.88 ± 0.05^{b}	63.92 ± 2.01^{b}
AHP + DES	81.37 ± 0.27^{c}	13.65 ± 0.34^{d}	35.41 ± 1.62^{c}	54.38 ± 0.18^{d}	0.47 ± 0.34^{a}	23.52 ± 0.44^{c}	10.94 ± 1.66^{c}	0.72 ± 0.07^{c}	71.26 ± 2.69^{c}

Table 4.3b

AHP and Type III ChCl:Urea DES (130°C, 4 h) hydrolysates after pretreatment. Values annotated with different letters represents the difference in significance levels for each category (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Sugar recovery						Formic acid		Acetic acid	
	Glucose (mg)$^{\zeta}$	Recovery (%)	Xylose (mg)$^{\zeta}$	Recovery (%)	Arabinose (mg)$^{\zeta}$	Recovery (%)	(g/L)	(mmol/L)	(g/L)	(mmol/L)
AHP	0.07 ± 0.01	0.02 ± 0.01	0.14 ± 0.01	0.07 ± 0.01	0.09 ± 0.01^{a}	0.42 ± 0.05^{a}	ND$^{\Psi}$	ND	0.01 ± 0.01^{a}	0.15 ± 0.01^{a}
DES	ND	-	ND	-	1.27 ± 0.02^{b}	6.52 ± 0.13^{b}	ND	-	1.41 ± 0.07^{b}	23.40 ± 1.14^{b}
AHP + DES	ND	-	ND	-	$1.45 \pm 0.17^{b,c}$	$7.47 \pm 0.85^{b,c}$	ND	-	1.12 ± 0.20^{c}	18.66 ± 3.32^{c}

$^{\Psi}$ ND = Not detectable

$^{\zeta}$ Sugar mass in milligram per gram of OPF in dry basis

4.4.5 Effect of AHP and type II DES on solid fractions

From past study (Ho et al., 2019b), the recommended concentration for AHP pretreatment of OPF for the best synergism was determined to be 0.25 vol% (one-way ANOVA). When OPF was pretreated in AHP at room temperature and 90 min, the delignification of DES could be improved by 1.6 times. Thus, this study was emphasized to investigate the recommended operating conditions for a novel Type II DES ChCl:$CuCl_2$•$2H_2O$ pretreatment after undergoing AHP pretreatment (0.25 vol% of H_2O_2 at room temperature, pH 11.5 and 90 min), in order to achieve the best delignification and/or sugar recoveries from OPF.

The composition of pretreated OPFs was summarized in **Table 4.4**, and interpreted from various perspectives, such as solid recovery, delignification, cellulose enrichment in the biomass, and sugar removal from the biomass. According to one-way ANOVA, the solid yield of Type II DES pretreatment was significantly reduced as the temperature and duration increased. For example, the solid yield of OPF was reduced from 84.39 to 70.77% as the Type II DES pretreatment duration increased from 1 to 4 h at a constant temperature of 80°C. This result implied a significant amount of OPF components being released to the hydrolysate as the temperature and duration of Type II DES pretreatment increased. However, it is worth noting that, low solid yield as a result of extreme operating conditions may not be a favorable solution towards effective fractionation of biomass in the biorefinery. Therefore, the composition of pretreated OPFs was carefully investigated at various temperature and duration during Type II DES sequential pretreatment.

4.4.5.1 Lignin

It was found that the lignin removal of AHP + Type II DES pretreated OPF exhibited an increasing trend, where lignin removal increased as temperature and duration increased. According to one-way ANOVA analysis, the lignin removal of OPF reached a plateau as the duration of pretreatment increased. This trend could be seen from **Table 4.4**, where the lignin removal of AHP + DES pretreatment at 100°C and duration of 3 – 4 h did not show any significant differences. This trend also suggested that the delignification of Type II DES was highly temperature driven as compared to the duration. Generally, the lignin content of the pretreated OPFs was reduced as the temperature of pretreatment increased at constant duration (New et al., 2019), which indicated an increase in the rate of delignification. Nonetheless, the present study showed that the lignin content increased when the temperature of Type II DES pretreatment increased from 90 to 100°C at a fixed duration of 4 h. Similar result was observed by Huerta and Saldaña (2019), where the lignin content increased from 21.4 to 27.5% when the temperature of the pressurized hot water pretreatment increased from 140 to 180°C at fixed duration of 40 min. The increase in lignin content indicated that

the increase in the rate of sugar removal was more dominant as the temperature increased. In general, the lignin content of pretreated OPFs was observed to exhibit increasing trends as the duration of pretreatment increased. However, a different trend was obtained at pretreatment temperature of 100°C, whereby the lignin content was first decreased from 1 (15.87%) to 2 (11.34%) h, then increased to 22.34% at 4 h. At 80 and 90°C, the sugar removal was more dominant as compared to the delignification. When the temperature was raised to 100°C, the rate of delignification was improved, thus reducing the lignin content. However, as the pretreatment prolong, lignin removal reached the plateau, while accessibility to the sugar improved. Thus, the rate of sugar removal was more dominant as compared to the delignification after 2 h, hence the lignin content increased.

4.4.5.2 Structural carbohydrates

Remarkably, Type II DES (ChCl:$CuCl_2$•$2H_2O$) was able to exhibit extraordinary xylan and arabinan removal at operating condition of 100°C and 3 h (**Table 4.4**). It is worth noting that the operating conditions employed in these studies were relatively less severe as compared to the conventional inorganic salt hydrolysis studied by Loow et al. (2018). However, the inorganic salt in the form of Type II DES in this study was able to achieve remarkable xylan and arabinan removals efficiencies. It was observed that the sugar removal was strongly dependent on the pretreatment temperature and duration. While the removal of hemicellulose (xylan and arabinan) may be favorable, the degradation and extraction of cellulose were not favorable in most biorefinery process. Generally, as the pretreatment temperature and duration increased, the hemicellulose removal increased. This observation concurred with the recent findings reported by Manzanares et al. (2020), where an increase in pretreatment temperature caused an increase in xylan removal. Glucan enrichment was observed as temperature and duration of pretreatment increased, as most of the glucan was not degraded during the process. It can be shown by a previous study, where cellulose enrichment was observed after undergoing sequential pretreatment of elephant grass (Toscan et al., 2019). However, as the pretreatment conditions were increased to 100°C and 4 h, the glucan removal showed a drastic increase and resulted in the reduction of glucan content. This phenomenon was due to the exposure of cellulose when the recalcitrant barrier (lignin and hemicellulose) were disrupted. Thus, prolong pretreatment duration might cause the degradation of cellulose due to the increase in accessibility to the cellulose.

4.4.6 Effect of type II DES on liquid fractions

Type II DES hydrolysate after the pretreatment was collected and analyzed to obtain sugars and inhibitors content in the liquid fractions. It was observed in the solid fractions compositional

analysis (**Table 4.4**) that a high amount of structural carbohydrates were released to the liquid fractions during the pretreatment process. Thus, it is expected that the Type II DES hydrolysate was able to retain a certain amount of reducing sugars. **Table 4.5** summarized the sugars and inhibitors content found in the DES hydrolysate after the pretreatments at various operating conditions.

4.4.6.1 Sugar recovery

It was observed in **Table 4.5**, the glucose recoveries from the OPFs were found to be relatively low as compared to the other sugars. Although a significant amount of glucan was released from the biomass during the pretreatment process (**Table 4.4**), the glucose could potentially be converted to other form of degradation products due to prolonged exposure in the heating environment of Type II DES. On the other hand, xylose and arabinose recoveries were found to be strongly dependent on pretreatment duration. While the increase in pretreatment temperature could potentially improve sugar recoveries, the unfavorable sugar degradation rate was also improved (Loow et al., 2015). It is worth highlighting that maximum xylose recoveries were obtained in each pretreatment temperature at different duration (**Table 4.5**). This results indicated the occurrence of xylose degradations surpassing the xylose extractions. For example, maximum xylose recoveries of 16.56, 28.96, and 27.51% were obtained in 80°C (4 h), 90°C (3 h), and 100°C (1 h) respectively.

4.4.6.2 Inhibitors

Indeed, the recovery and utilization of the liquid fractions to form value-added products could potentially improve overall biorefinery process-economic. However, the inhibitors content in the liquid fractions may be hindering effective fermentation or bioconversion in the later stage. Thus, it is crucial to examine the inhibitors formed in the hydrolysate after the pretreatment. The inhibitors focused in this study were weak acids such as formic and acetic acids, while the furfural and 5-hydroxymethylfurfural (HMF) were not within the scope of study. According to Ho et al. (2019a), furfural and HMF would cause the formation of undesired alcohols, thus creating a lag phase in the bioethanol formation. However, the yield of bioethanol formation was not affected after prolong fermentation. On the other hand, weak acids were claimed to enhance the overall bioethanol formation due to the ability to reduce intracellular pH. Nevertheless, high concentrations of these acids (exceeding 100 mmol/L) would result in acidification of cytoplasm and cell death during the fermentation process (Larsson et al., 1999).

Table 4.4

Composition of OPF after undergoing AHP + Type II DES (ChCl:$CuCl_2$•$2H_2O$) sequential pretreatment at different temperature and duration. Values annotated with different letters represents the difference in significance levels for each category (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Yield (%)	Lignin		Glucan		Xylan		Arabinan	
		Content (%)	Removal (%)	Content (%)	Removal (%)	Content (%)	Removal (%)	Content (%)	Removal (%)
Raw	-	17.20 ± 0.10^{a}	-	44.46 ± 0.49^{a}	-	21.49 ± 0.06^{a}	-	2.04 ± 0.02^{a}	-
AHP alone	95.90 ± 0.18^{a}	$16.68 \pm 0.06^{b,c}$	6.98 ± 0.35^{a}	45.48 ± 0.06^{a}	1.88 ± 0.14^{a}	21.45 ± 0.13^{a}	4.25 ± 0.57^{a}	1.94 ± 0.03^{b}	8.63 ± 1.30^{a}
DES temperature: 80°C									
AHP + DES (1 h)	84.39 ± 0.81^{b}	$16.88 \pm 0.07^{a,b,c}$	17.17 ± 0.35^{b}	51.70 ± 0.15^{b}	1.87 ± 0.29^{a}	21.50 ± 0.28^{a}	15.57 ± 1.08^{b}	0.16 ± 0.01^{c}	93.32 ± 0.57^{b}
AHP + DES (2 h)	79.11 ± 1.37^{c}	$17.07 \pm 0.14^{a,b}$	21.47 ± 0.63^{c}	55.74 ± 0.62^{c}	0.81 ± 1.11^{a}	19.12 ± 0.53^{b}	29.59 ± 1.96^{c}	0.12 ± 0.02^{d}	95.22 ± 0.68^{c}
AHP + DES (3 h)	74.42 ± 1.22^{d}	$17.05 \pm 0.15^{a,b}$	26.21 ± 0.66^{d}	59.53 ± 0.57^{d}	0.35 ± 0.96^{a}	18.07 ± 0.10^{c}	37.42 ± 0.34^{d}	$0.10 \pm 0.01^{d,e}$	$96.39 \pm 0.24^{c,d}$
AHP + DES (4 h)	70.77 ± 1.90^{e}	$17.09 \pm 0.27^{a,b}$	29.65 ± 1.11^{e}	62.53 ± 0.41^{e}	0.44 ± 0.65^{a}	16.15 ± 0.04^{d}	46.79 ± 0.20^{e}	$0.07 \pm 0.02^{e,f}$	$97.52 \pm 0.65^{d,e}$
DES temperature: 90°C									
AHP + DES (1 h)	$72.90 \pm 1.11^{d,e}$	15.82 ± 0.14^{d}	32.95 ± 0.59^{f}	57.75 ± 1.35^{d}	5.30 ± 2.22^{b}	14.21 ± 0.43^{e}	51.80 ± 1.45^{f}	$0.11 \pm 0.01^{d,e}$	$96.19 \pm 0.19^{c,d}$
AHP + DES (2 h)	$57.64 \pm 0.81^{f,g}$	16.02 ± 0.20^{d}	46.32 ± 0.68^{g}	65.52 ± 1.91^{f}	15.05 ± 2.47^{c}	9.98 ± 0.78^{f}	73.24 ± 2.10^{g}	$0.09 \pm 0.01^{d,e}$	$97.50 \pm 0.07^{d,e}$
AHP + DES (3 h)	48.09 ± 0.75^{h}	16.04 ± 0.08^{d}	55.14 ± 0.23^{h}	69.27 ± 1.09^{g}	25.06 ± 1.18^{d}	8.58 ± 0.41^{g}	80.79 ± 0.92^{h}	$0.08 \pm 0.01^{e,f}$	98.02 ± 0.30^{e}
AHP + DES (3.5 h)	$45.36 \pm 0.41^{h,i}$	16.54 ± 0.11^{c}	56.37 ± 0.28^{i}	$69.96 \pm 0.17^{g,h}$	28.62 ± 0.17^{e}	6.97 ± 0.49^{h}	85.29 ± 1.03^{i}	$0.08 \pm 0.01^{e,f}$	98.23 ± 0.22^{e}
AHP + DES (4 h)	43.83 ± 2.85^{i}	$16.69 \pm 0.18^{b,c}$	57.47 ± 0.45^{i}	71.75 ± 0.32^{h}	29.27 ± 0.32^{e}	3.57 ± 0.03^{i}	92.72 ± 0.07^{j}	$0.08 \pm 0.02^{e,f}$	98.34 ± 0.47^{e}
DES temperature: 100°C									
AHP + DES (1 h)	59.67 ± 2.74^{f}	15.87 ± 0.02^{d}	44.95 ± 0.08^{j}	69.51 ± 1.20^{g}	6.71 ± 1.62^{b}	11.01 ± 0.54^{j}	69.44 ± 1.49^{k}	ND[Ψ]	-
AHP + DES (2 h)	$45.64 \pm 2.30^{h,i}$	11.34 ± 0.43^{e}	69.91 ± 1.14^{k}	81.54 ± 0.63^{i}	16.28 ± 0.65^{c}	2.11 ± 0.10^{k}	95.52 ± 0.21^{l}	ND	-
AHP + DES (3 h)	34.64 ± 0.43^{j}	13.22 ± 0.01^{f}	73.36 ± 0.02^{l}	93.45 ± 0.27^{j}	$27.19 \pm 0.21^{d,e}$	0.32 ± 0.03^{l}	99.48 ± 0.04^{m}	ND	-
AHP + DES (4 h)	20.86 ± 0.61^{k}	22.34 ± 0.40^{g}	72.91 ± 0.48^{l}	75.25 ± 1.72^{k}	64.69 ± 0.81^{f}	ND	-	ND	-

[Ψ] ND = Not detectable

Formic acid was one of the degradation products of sugars, while the acetic acid was formed by the degradation of acetyl groups in hemicellulose (xylose and arabinose) (Graça et al., 2018). **Table 4.5** shows that, formic acid concentrations were increased along with the increases of both temperature and duration of pretreatment. These results were in agreement with the glucan removal in **Table 4.4**. Glucose recovery in the hydrolysate was relatively less as compared to the glucan removal, suggesting that most of the glucose released was degraded into various degradation products, such as formic acid. As a case in point, at pretreatment conditions of 100°C and 4 h, high glucan was removed due to the over exposure of cellulose during pretreatment. Thus, more formic acid (119.79 mmol/L) was formed in the hydrolysate, which could be due to the prolonged heating in the Type II DES (ChCl:$CuCl_2$•$2H_2O$) system.

Furthermore, acetic acid showed a similar trend, whereby increases of temperature and duration encouraged the formation of acetic acid. Thus, it was proposed that the decrease in xylose and arabinose recovery were owing to the degradation of sugars and reduced rate of xylan and arabinan removals. This study indicated that the increase in pretreatment conditions of Type II DES might not be favorable for the sugar recoveries in the hydrolysate (**Table 4.5**). For example, at 100°C and 4 h, the recoveries of glucose, xylose, and arabinose were relatively small (< 5%) in comparison with 90°C and 3 h. Loow et al. (2015) also reported that, sugar degradation drastically increased as temperature increased. The present study showed that at high temperature of 100°C and 4 h, inhibitors of exceeding 100 mmol/L were formed during the pretreatment process (**Table 4.5**).

4.4.7 Recommended conditions for type II DES

The range of temperature studied in this paper were 80, 90, and 100°C. Although an increase in delignification was observed in higher temperature and shorter duration (**Table 4.4**), several disadvantages along with difficulties were shown when the temperature was increased further. It was observed that the glucan removal from OPF and inhibitors formation in the hydrolysate were increased together with an increase of pretreatment temperature. On top of that, when the temperature was raised to 100°C, the ability of Type II DES to recover xylose and arabinose (**Table 4.5**) deteriorated. This phenomenon could be due to the thermal degradation of sugars induced during the heating of pretreatment.

Table 4.5

Type II DES (ChCl:$CuCl_2$•$2H_2O$) hydrolysate after pretreating AHP-pretreated OPF at different temperature and duration. Values annotated with different letters represents the difference in significance levels for each category (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Sugar recovery						Formic acid		Acetic acid	
	Glucose (mg)[ζ]	Recovery (%)	Xylose (mg)[ζ]	Recovery (%)	Arabinose (mg)[ζ]	Recovery (%)	(g/L)	(mmol/L)	(g/L)	(mmol/L)
80°C										
DES (1 h)	ND[Ψ]	-	12.26 ± 2.33[a]	5.72 ± 1.08[a]	5.50 ± 0.09[a,b]	28.32 ± 0.47[a,b]	ND	ND	0.19 ± 0.08[a]	3.13 ± 1.42[a]
DES (2 h)	ND	-	19.38 ± 3.27[b]	9.03 ± 1.53[b]	4.37 ± 0.12[c]	22.49 ± 0.63[c]	ND	ND	0.59 ± 0.18[a,b]	9.83 ± 2.95[a,b]
DES (3 h)	ND	-	26.91 ± 3.46[c]	12.54 ± 1.61[c]	4.58 ± 0.17[c,d]	23.63 ± 0.86[c,d]	ND	ND	1.00 ± 0.19[b,c]	16.58 ± 3.16[b,c]
DES (4 h)	ND	-	35.53 ± 2.17[d]	16.56 ± 1.01[d]	4.32 ± 0.59[c]	22.23 ± 3.03[c]	ND	ND	1.31 ± 0.10[c]	21.80 ± 1.73[c]
90°C										
DES (1 h)	1.49 ± 0.09[a]	0.33 ± 0.02[a]	33.51 ± 0.64[d]	15.62 ± 0.30[d]	7.39 ± 0.23[e]	38.10 ± 1.19[e]	ND	ND	0.89 ± 0.07[b,c]	14.75 ± 1.16[b,c]
DES (2 h)	3.07 ± 0.12[b]	0.68 ± 0.02[b]	52.17 ± 0.43[e]	24.32 ± 0.21[e]	6.87 ± 0.16[e]	35.42 ± 0.78[e]	0.23 ± 0.01[a]	4.83 ± 0.16[a]	2.04 ± 0.03[d]	33.97 ± 0.55[d]
DES (3 h)	5.40 ± 0.19[c,d]	1.19 ± 0.04[c,d]	62.13 ± 1.67[f]	28.96 ± 0.78[f]	6.08 ± 0.49[b]	31.34 ± 2.55[b]	0.36 ± 0.01[a]	7.77 ± 0.18[a]	2.56 ± 0.03[d,e]	42.67 ± 0.54[d,e]
DES (3.5 h)	6.20 ± 0.47[d]	1.37 ± 0.11[d,h]	57.31 ± 1.62[f]	26.72 ± 0.76[f]	5.26 ± 0.13[a]	27.10 ± 0.67[a]	0.43 ± 0.03[a]	9.27 ± 0.61[a]	2.72 ± 0.01[e]	45.33 ± 0.16[e]
DES (4 h)	9.25 ± 0.01[e]	2.03 ± 0.01[e]	50.21 ± 0.42[e]	23.41 ± 0.20[e]	5.49 ± 0.11[a,b]	28.29 ± 0.59[a,b]	1.15 ± 0.07[b]	24.93 ± 1.47[b]	3.21 ± 0.02[e]	53.42 ± 0.39[e]
100°C										
DES (1 h)	4.58 ± 0.48[c]	1.01 ± 0.11[c]	59.01 ± 1.87[f]	27.51 ± 0.87[f]	4.89 ± 0.30[a,c,d]	25.17 ± 1.56[a,c,d]	0.53 ± 0.01[a]	11.48 ± 0.08[a]	2.02 ± 0.08[d]	33.53 ± 1.24[d]
DES (2 h)	8.26 ± 0.68[f]	1.82 ± 0.15[f]	21.59 ± 1.61[b]	10.07 ± 0.76[b]	2.31 ± 0.40[f]	11.91 ± 2.06[f]	1.86 ± 0.34[c]	40.43 ± 7.35[c]	2.85 ± 0.54[e]	47.34 ± 9.09[e]
DES (3 h)	14.78 ± 0.84[g]	3.25 ± 0.18[g]	18.61 ± 4.98[b]	8.68 ± 2.32[b]	ND	-	4.25 ± 0.58[d]	92.30 ± 12.54[d]	5.16 ± 0.88[f]	85.84 ± 14.81[f]
DES (4 h)	14.27 ± 0.13[g]	3.14 ± 0.03[g]	9.23 ± 3.24[a]	4.30 ± 1.51[a]	ND	-	5.52 ± 0.04[e]	119.79 ± 0.86[e]	5.99 ± 0.35[g]	99.79 ± 5.87[g]

[Ψ] ND = Not detectable

[ζ] Sugar mass in milligram per gram of OPF in dry basis

Besides, several difficulties in handling the Type II DES occurred when the temperature was increased further (> 100°C). At higher temperature, precipitation was observed in the Type II DES hydrolysate upon contact with water. According to Loow et al. (2017a), DES ability in delignification was mainly governed by the hydrogen bonds formations between chloride anion and biomass components. This proposed mechanism suggested that there would be a saturation of hydrogen bonds formed in the Type II DES. As the pretreatment temperature increased, a drastic decrease of solid yield was also observed. This observation indicated a high amount of biomass components were released during the pretreatment, thus hydrogen bonding in Type II DES hydrolysate approached its saturation. It was proposed that the hydrogen bonds between chloride anions and biomass components were reversible, whereby the addition of water would disrupt the hydrogen bonds network (Ma et al., 2018), thus forming the precipitation. On top of that, the addition of water could have acidified the solvent through the Brønsted acids pathway (Loow et al., 2015), which may also favor the precipitation. Similar findings were observed by García et al. (2012), where the addition of acidified water enabled the precipitation of lignin.

The recommended conditions for Type II DES may be varied to adapt to different needs of pretreatment. In the case of liquid fractions not recovered, the recommended condition for Type II DES pretreatment was 100°C and 2 h. This condition was able to achieve the lowest lignin content with 69.91% of delignification, while causing relatively less removal of glucan to yield glucan-rich OPF. However, it is important to note that this condition resulted in lower sugar recoveries and higher inhibitors content in the liquid fractions. Thus, the valorization of liquid fractions may not be feasible. In the present study, the aim was to identify the recommended conditions to achieve both reasonable sugar recoveries and delignification in liquid and solid fractions, respectively. Therefore, pretreatment condition of 90°C and 3 h was selected, as it was able to achieve 28.96 and 31.34% of xylose and arabinose recoveries respectively while generating lesser formic acids (0.36 g/L) in the liquid fraction. Furthermore, this condition was able to achieve 55.14% of delignification with glucan enrichment up to 69.27%, while removing the majority of xylan and arabinan from the OPF. An additional study was carried out at pretreatment condition of 90°C and 3.5 h to confirm the best duration for the recommended condition (**Table 4.4** and **Table 4.5**). Although a slight increase in delignification (~1%) can be achieved with 3.5 h, sugar recoveries were found to slightly decrease. Thus, a further increase in duration may not be economically viable. **Fig. 4.4**, **4.5**, and **4.6** showed the colour changes after AHP, Type II DES, and AHP + Type II DES pretreatment, respectively at the recommended conditions.

From a previous study (Ho et al., 2019b), it was found that AHP pretreatment could help promote the accessibility of OPF in the later pretreatment stage. Comparisons of compositional

analysis of pretreated OPF using a single-stage pretreatment (AHP alone or Type II DES alone) and a sequential pretreatment (AHP + Type II DES) were tabulated in **Table 4.6a**. It was found that the lignin and structural carbohydrates removals after undergoing sequential pretreatment coupled with AHP were generally higher in comparison with the single-stage pretreatment. However, high glucan removal was not desired in the downstream processing of biorefinery. On the other hand, a significant improvement on the lignin removal was obtained by integrating AHP into the sequential pretreatment. For example, the lignin removal of Type II DES (37.56%) was increased to 55.14% by incorporating an AHP pretreatment as an initial pretreatment process. This phenomenon indicated the synergism effect of AHP with Type II DES (ChCl:$CuCl_2$•$2H_2O$) in delignification of biomass.

From the hydrolysate perspective (**Table 4.6b**), xylose and arabinose contents were increased in sequential pretreatment. Nonetheless, glucose content was decreased in this sequential pretreatment, which could be owing to the multiple pretreatment stages that caused the glucose to be removed in the earlier pretreatment. On top of that, free bound sugar in the raw OPF was directly released to the Type II DES hydrolysate in a single-stage pretreatment, hence more glucose was observed (Loow et al., 2018). Beside sugar recoveries, acetic acid was found lesser in the sequential pretreatment. This observation was expected because AHP pretreatment was claimed to exhibit excellent acetyl group cleavage, in which less substrate (acetyl group) was available for degradation in the later DES pretreatment (Ho et al., 2019a).

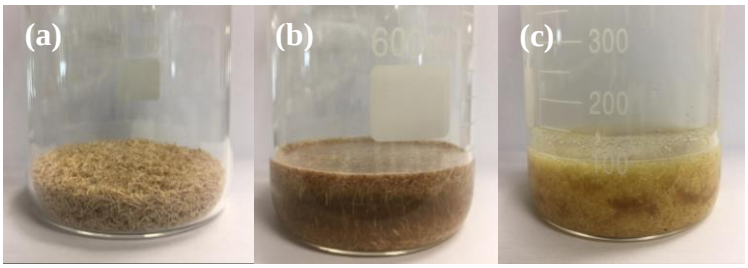

Fig. 4.4 (a) Raw OPF (b) before and (c) after AHP pretreatment.

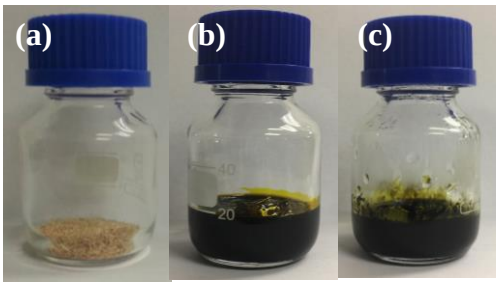

Fig. 4.5 (a) Raw OPF (b) before and (c) after DES pretreatment.

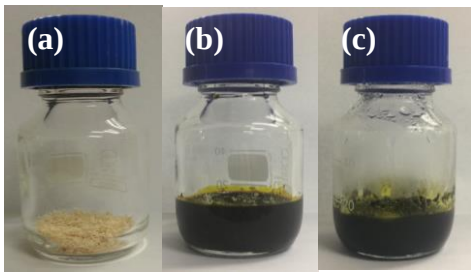

Fig. 4.6 (a) AHP-pretreated OPF (b) before and (c) after DES pretreatment.

4.4.8 Comparison between Type II and Type III DES

Sequential pretreatment using Type III DES (ChCl:Urea) was found to be highly selective to lignin. AHP + Type III DES (ChCl:Urea) was able to achieve delignification of 35.41% while retaining the majority of glucan and xylan (**Table 4.3a**). However, the glucan and xylan content were not significantly enriched, which may affect the accessibility and efficiency of downstream bioconversion. Additional pretreatment to fractionate xylan and glucan may be required to yield glucan-rich and xylan-rich fractions. The sugar recovery obtained suggested that the Type III DES (ChCl:Urea) was unable to significantly extract and retain sugars in the form of reducing sugars in the hydrolysate (**Table 4.3b**), thus its application in sugar recovery was not feasible. Overall, more improvements on the delignification efficiency were required to release its potential as an effective and selective delignification pretreatment.

On the other hand, sequential pretreatment using Type II DES (ChCl:$CuCl_2$•$2H_2O$) was able to exhibit remarkable delignification and hemicellulose removal performance (**Table 4.6a**). AHP + Type II DES (ChCl:$CuCl_2$•$2H_2O$) was able to achieve delignification up to 55.14%, while removing majority of xylan (80.79%) and arabinan (98.02%). However, undesired glucan removal of 25.06% has occurred during pretreatment. Although glucan was partially extracted during pretreatment, approximately 75% of glucan was retained. On top of that, Type II DES (ChCl:$CuCl_2$•$2H_2O$) was able to yield glucan-rich OPFs with glucan content up to 69.27%, which may favor the downstream bioconversion. Besides, Type II DES (ChCl:$CuCl_2$•$2H_2O$) hydrolysate was able to recover 28.96 and 31.34% of xylose and arabinose, respectively (**Table 4.6b**). The inhibitors content in the hydrolysate were well below the inhibitory concentration of typical fermentation process (Ho et al., 2019a), thus the hydrolysate may be utilized to produce value-added products to improve overall process economics. It is worth noting that, the sugar recoveries of Type II DES (ChCl:$CuCl_2$•$2H_2O$) were not optimized, thus more works were needed to enhance the sugar recoveries further. By comparing Type II (ChCl:$CuCl_2$•$2H_2O$) to Type III (ChCl:Urea) DES, ChCl:$CuCl_2$•$2H_2O$ DES was selected due to its remarkable delignification and ability to fractionate hemicellulose effectively, while able to recover a significant amount of sugars in the hydrolysate for further utilization.

Table 4.6a

Compositional analysis of OPF after undergoing AHP (0.25 vol%, 90 min) and Type II ChCl:$CuCl_2$•$2H_2O$ DES (90°C, 3 h) pretreatments. Values annotated with different letters represents the difference in significance levels for each category (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Yield (%)	Lignin		Glucan		Xylan		Arabinan	
		Content (%)	Removal (%)	Content (%)	Removal (%)	Content (%)	Removal (%)	Content (%)	Removal (%)
Raw	-	17.20 ± 0.10^{a}	-	44.46 ± 0.49^{a}	-	21.49 ± 0.06^{a}	-	2.04 ± 0.02^{a}	-
AHP	95.90 ± 0.18^{a}	16.68 ± 0.06^{b}	6.98 ± 0.35^{a}	45.48 ± 0.06^{a}	1.88 ± 0.14^{a}	21.45 ± 0.13^{a}	4.25 ± 0.57^{a}	1.94 ± 0.03^{b}	8.63 ± 1.30^{a}
DES	56.90 ± 0.01^{b}	19.01 ± 0.06^{c}	37.56 ± 0.19^{b}	66.98 ± 0.28^{b}	14.87 ± 0.35^{b}	8.39 ± 0.18^{b}	77.95 ± 0.47^{b}	0.05 ± 0.01^{c}	98.68 ± 0.10^{b}
AHP + DES	48.09 ± 0.75^{c}	16.04 ± 0.08^{d}	55.14 ± 0.23^{c}	69.27 ± 1.09^{c}	25.06 ± 1.18^{c}	8.58 ± 0.41^{b}	80.79 ± 0.92^{c}	$0.08 \pm 0.01^{c,d}$	98.02 ± 0.30^{b}

Table 4.6b

AHP and Type II ChCl:$CuCl_2$•$2H_2O$ DES (90°C, 3 h) hydrolysates after pretreatment. Values annotated with different letters represents the difference in significance levels for each category (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Sugar recovery						Formic acid		Acetic acid	
	Glucose (mg)$^{\zeta}$	Recovery (%)	Xylose (mg)$^{\zeta}$	Recovery (%)	Arabinose (mg)$^{\zeta}$	Recovery (%)	(g/L)	(mmol/L)	(g/L)	(mmol/L)
AHP	0.07 ± 0.01^{a}	0.02 ± 0.01^{a}	0.14 ± 0.01^{a}	0.07 ± 0.01^{a}	0.09 ± 0.01^{a}	0.42 ± 0.05^{a}	ND$^{\Psi}$	ND	0.01 ± 0.01^{a}	0.15 ± 0.01^{a}
DES	6.95 ± 0.58^{b}	1.56 ± 0.13^{b}	57.61 ± 0.80^{b}	26.81 ± 0.37^{b}	$5.44 \pm 0.15^{b,c}$	$26.70 \pm 0.74^{b,c}$	0.52 ± 0.01^{a}	11.19 ± 0.24^{a}	3.90 ± 0.19^{b}	64.93 ± 3.14^{b}
AHP + DES	5.40 ± 0.19^{c}	1.19 ± 0.04^{c}	62.13 ± 1.67^{b}	28.96 ± 0.78^{b}	6.08 ± 0.49^{c}	31.34 ± 2.55^{c}	0.36 ± 0.01^{a}	7.77 ± 0.18^{a}	2.56 ± 0.03^{c}	42.67 ± 0.54^{c}

$^{\Psi}$ ND = Not detectable

$^{\zeta}$ Sugar mass in milligram per gram of OPF in dry basis

4.4.9 Proposed mechanism for type II DES pretreatment

The combination of ChCl and $CuCl_2 \cdot 2H_2O$ at the specific molar ratio of 1:1 formed a Type II DES, which was used in this study. The mechanism of Type II DES pretreatment in a delignification of OPF mainly relied on the formation of hydrogen bonds between lignin and halogen anion in the DES. According to Dai et al. (2014), halogen anions in the DES were able to form hydrogen bonding with hydroxyl groups in the lignin after the donation of protons. On top of that, hydrogen bonds formation between Cl^- anions and lignin aromatic ring could result in extraction of aromatic rings and lignin dissolution (Loow et al., 2018). It was proposed that, the delignification efficiency mainly driven by the ability of strong electronegativity halogen anion (Cl^-) to establish hydrogen bonding with the lignin. Multiple sources of Cl^- anions from ChCl and $CuCl_2 \cdot 2H_2O$ DES system could be the reason of the enhanced delignification observed in this study as compared to the single source Type III DES system (New et al., 2019). Moreover, the AHP pretreatment could generate free hydroxyl groups in the aromatic side chain (Halma et al., 2015a), plausibly improving site-specific delignification. Thus, this effect induced the synergistic effect in the delignification of OPF by combining AHP and DES pretreatments.

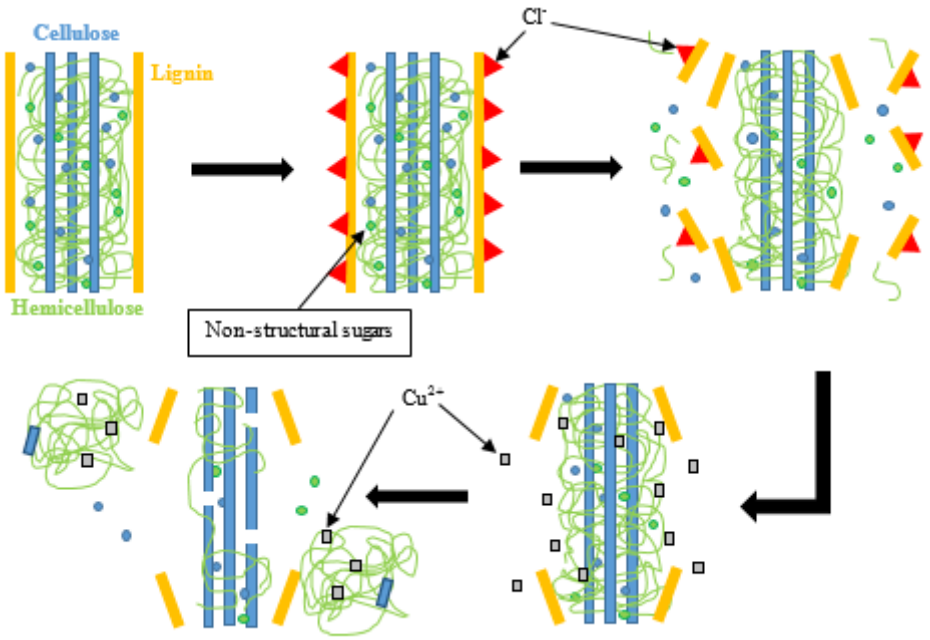

Fig. 4.7 Simple illustration of the proposed mechanism for Type II DES ($ChCl:CuCl_2 \cdot 2H_2O$) pretreatment.

The mechanism of inorganic hydrate salt-based Type II DES pretreatment in a sugar recovery can be categorized into Lewis and Brønsted acids pathway (Loow et al., 2015). However, in the current Type II DES system, water molecules from ligands were limited. Thus, the Lewis acid pathway was proposed to be more dominant as compared to the Brønsted acid pathway, in which

excess water was needed to produce H_3O^+ ions in the latter (Loow et al., 2015). According to Román-Leshkov and Davis (2011), the metal cations Cu^{2+} would coordinate with water ligands to form metal ion ligand complexes $[M(H_2O)_n]^{z+}$ (where M is the metal ion, z is the cation oxidation state, and n is the solvation number). The metal ions complexes acted as Lewis acids would then aid in the cleavage of glycosidic linkages, while the coordinated water molecules were used to depolymerize structural carbohydrates (cellulose and hemicellulose) into monosaccharides by participating as nucleophiles (Amarasekara and Ebede, 2009; Cotton et al., 1988; Peng et al., 2010). The simplified proposed mechanism was illustrated in **Fig. 4.7**. Thus, it was also possible for Type II DES to depolymerize cellulose into glucose with prolong exposure to Type II DES and removals of protective barriers. As the temperature and duration increased for Type II DES pretreatment, it was observed that Type II DES was able to degrade glucan from the OPF significantly (**Table 4.4**). This phenomenon could be due to the significant removal of the protective barrier (lignin and hemicellulose), hence Type II DES was allowed to depolymerize cellulose through the proposed mechanism pathway.

4.4.10 Characterization studies of raw and pretreated OPF samples

4.4.10.1 FE-SEM

FE-SEM was used to observe the morphological alterations on OPF through different pretreatments (**Fig. 4.8**). The untreated OPF (**Fig. 4.8a**) exhibited a smooth and enclosed surface, which resulted in a lower degree of porosity (Loow et al., 2018). Thus, the penetration of chemicals to the internal structure was not favorable, leading to a lower delignification and sugar recoveries (**Table 4.4**). After AHP pretreatment, the outer layer of OPF was slightly stripped, leaving a relatively rough surface (**Fig. 4.8b**). However, the overall structure of OPF was noticeably enclosed and intact, indicating that the mild AHP pretreatment had little effect on the cellulose and hemicellulose (Ho et al., 2019b). Moreover, tiny holes were found on the surface of AHP-pretreated OPF, thus potentially enhancing the accessibility and porosity to the internal structure. This finding concurred with Li et al. (2016c) and Ho et al. (2019b), whereby small holes were found on the biomass after undergoing AHP pretreatment. While the majority of lignin was not removed from the OPF, the increase in porosity could potentially enhance subsequent DES penetration. Due to a low degree of porosity in the raw OPF, the cellulose was not effectively exposed after undergoing Type II DES (ChCl:$CuCl_2$•$2H_2O$) pretreatment at 90°C and 3 h (**Fig. 4.8c**). However, the major disruption of the structure was observed, owing to the extraordinary hemicellulose removal by Type II DES (**Table 4.6a**). The synergistic combination of AHP and Type II DES pretreatments resulted in the most drastic rupture of OPF structure, exposing ordered crystalline structures (**Fig. 4.8d**). The effects of AHP in OPF

modifications coupled with subsequent delignification and hemicellulose removal during Type II DES pretreatment led to the destruction of the OPF structure.

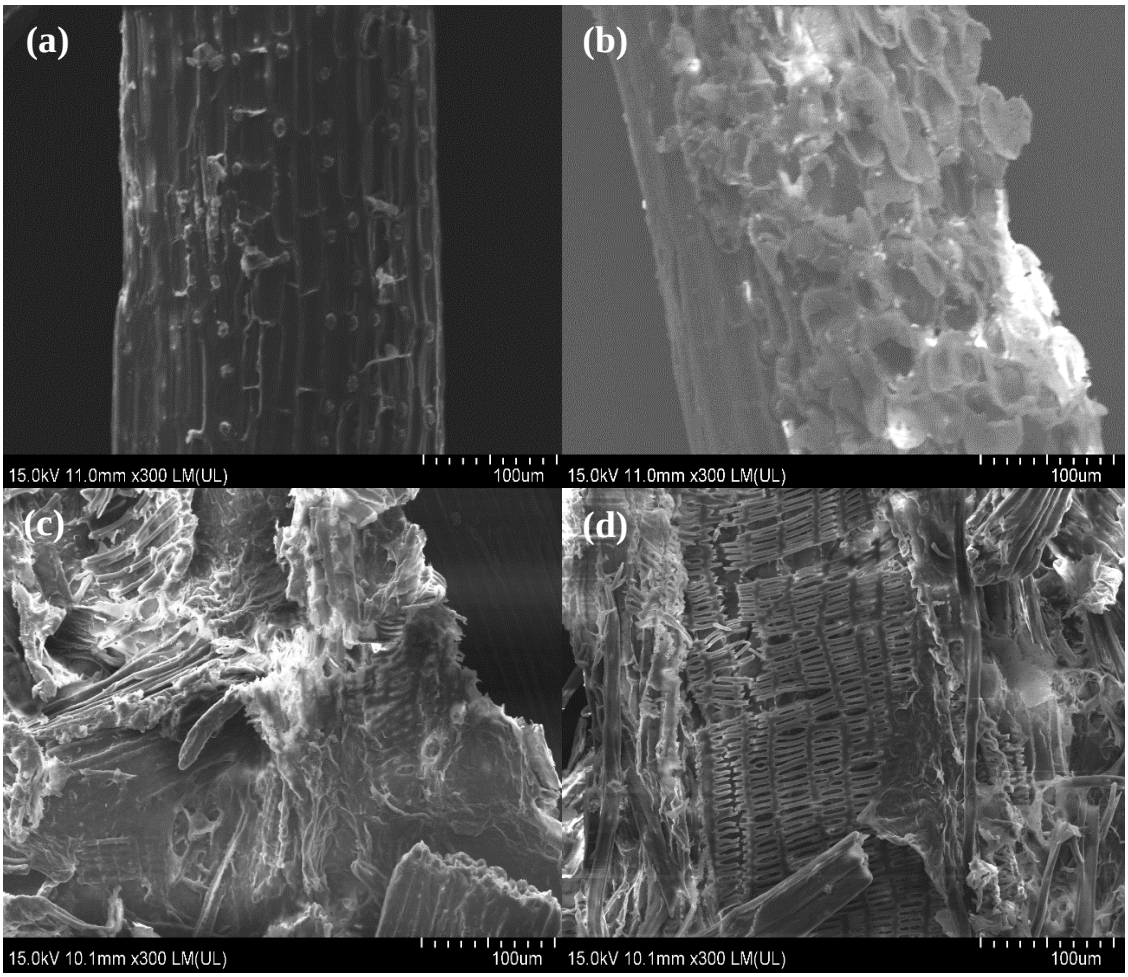

Fig. 4.8 FE-SEM images of (a) raw OPF, (b) AHP-pretreated OPF, (c) Type II DES-pretreated OPF, and (d) AHP + Type II DES-pretreated OPF at x300 magnification.

4.4.10.2 BET

From the BET analysis, the specific surface areas of raw and pretreated OPFs were determined. The initial specific surface area of raw OPF was 0.4071 m^2/g. After undergoing AHP pretreatment alone, the area increased slightly to 0.4220 m^2/g. This increase in surface area was mainly due to the increase in OPF porosity as a result of biomass structure alteration observed in the FE-SEM (Ho et al., 2019b). Furthermore, when the raw OPF was pretreated using Type II DES at 90°C and 3 h, the specific surface area increased to 0.5801 m^2/g, which was increased by 42.50% as compared to raw OPF. This result was expected, as the majority of the OPF structure was disrupted. Remarkably, the combination of AHP and Type II DES was able to achieve the best surface area of 0.7577 m^2/g, resulting in 86.12% increase in the surface area from the initial area of raw OPF. The AHP was able to enhance the accessibility of OPF. Thus, Type II DES was able to disrupt the structure better, which likely to cause a significant increase in surface area (Loow et al., 2018). The specific

surface area obtained through BET was able to highlight the synergistic effects of AHP + Type II DES pretreatment when comparing with single-stage Type II DES pretreatment.

4.4.10.3 XRD

XRD (**Fig. 4.9**) results indicated that the CrI of raw OPF was 37.30%. After undergoing AHP pretreatment, the CrI was increased to 43.85% due to the ability to enhance porosity and accessibility to the crystalline region while partially removing amorphous components (Ho et al., 2019b). In the case of Type II DES pretreatment alone, the CrI achieved as high as 59.91%. The substantial increase in CrI was mainly attributed by its ability to remove amorphous hemicellulose effectively. On top of that, this increase in CrI could be caused by DES ability to partially disrupt and decrease the amorphous regions of cellulose (Loow et al., 2018). After undergoing AHP + Type II DES pretreatment, the CrI was remarkably increased to 66.32%. It was observed from the diffractogram of AHP + Type II DES-pretreated OPF, the intensity of the amorphous region reduced owing to the higher delignification and hemicellulose removal (**Table 4.6a**), thus increasing CrI. Although it was observed that the AHP + Type II DES-pretreated OPF had higher glucan (cellulose) content as compared to Type II DES-pretreated OPF (**Table 4.6a**), the crystalline peak intensity for the former OPF showed a slight reduction. This finding concurred with Vigier et al. (2015), where DES was claimed to cause a reduction in cellulose crystallinity when the cellulose was readily exposed to the DES. As such, the combined sequential pretreatment achieved best results due to the better accessibility to the OPF and effective removal of lignin and hemicellulose. This claim was validated by the noticeable increase in CrI after the combined pretreatment, implying higher exposure of cellulose, which would potentially enhancing enzymatic hydrolysis (Li et al., 2013).

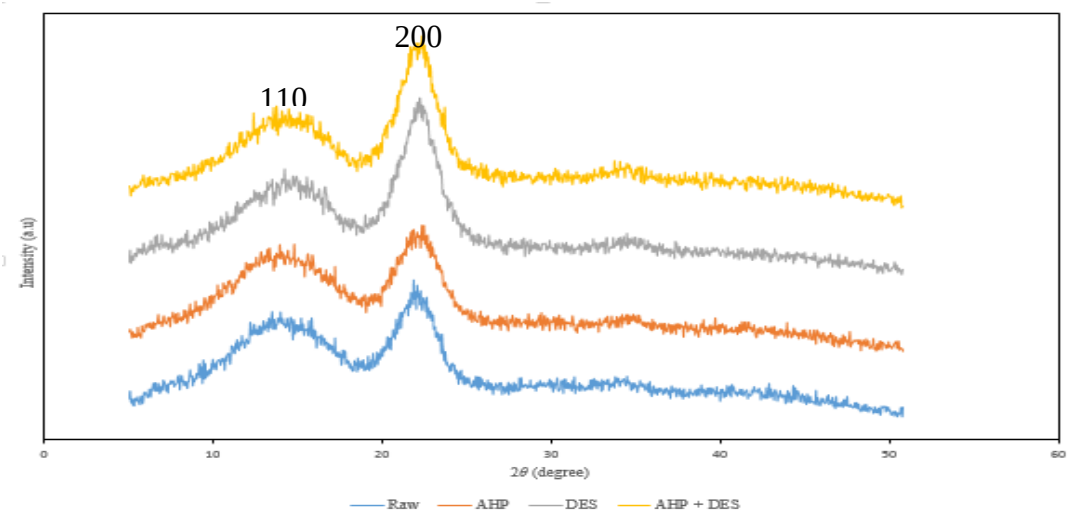

Fig. 4.9 XRD diffractograms of raw and pretreated OPFs.

4.4.10.4 FTIR

FTIR was used to identify the chemical functional groups on OPFs after the pretreatments (**Fig. 4.10**). Peaks at 900 cm^{-1} and 2900 cm^{-1} representing β-glycosidic linkages and –OH groups in the cellulose respectively, were increased after DES and AHP + Type II DES pretreatments. This observation implied that more cellulose in OPF was exposed as a result of Type II DES pretreatments. Under the recommended conditions (**Table 4.4**), the dissolution of cellulose was unlikely, while only lignin and hemicellulose were selectively targeted in both AHP and Type II DES pretreatments (Loow et al., 2018; Mittal et al., 2017). Moreover, the changes were observed for peaks at 1031 cm^{-1}, indicating the presence of C-O stretching vibration in cellulose, hemicellulose, and lignin (Ofori-Boateng and Lee, 2014). When comparing with raw OPF, the peak at 1031 cm^{-1} showed a significant increase in both Type II DES and AHP + Type II DES-pretreated OPFs, which implied the significant enrichment of cellulose. Nonetheless, the most drastic changes peaks representing hemicellulose and lignin. The presence of aryl-alkyl ether bonds (C–O–C) was shown by peaks 1235 cm^{-1}, experienced significant reduction especially after Type II DES pretreatments, highlighting the capability of Type II DES ($ChCl:CuCl_2•2H_2O$) in cleavage of aryl-alkyl ether bond, also known as β–O–4 linkages. Moreover, peaks ranged from 1508 to 1600 cm^{-1}, represented by aromatic skeletal C=C from lignin aromatic ring (Loow et al., 2018), were found to flatten for OPF underwent Type II DES pretreatments. This observation was due to the ability of DES to form hydrogen bonding with lignin and other phenolic compounds, leading to lignin dissolution. On top of that, results also confirm that mild conditions AHP employed in this study had minimal effect on the phenolic ring, while delignification was mainly attributed by β–O–4 linkage (Ho et al., 2019b). As a result of effective hemicellulose dissolution, almost complete disappearance of peaks at 1735 cm^{-1} after Type II DES and AHP + Type II DES pretreatments were observed, denoted the breakdown of C=O acetyl group in hemicellulose (Loow et al., 2018).

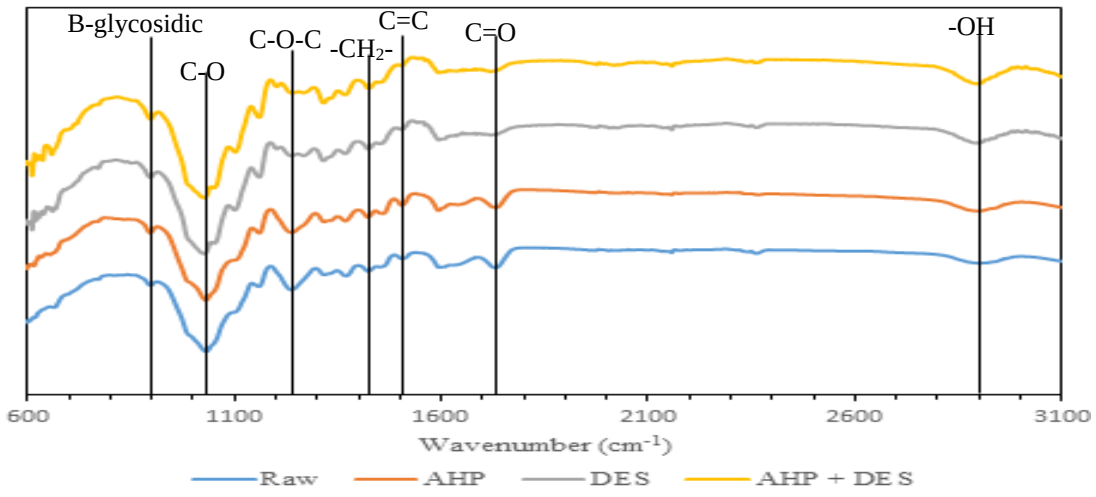

Fig. 4.10 FTIR spectrum of raw and pretreated OPFs.

4.4.10.5 TGA

TGA and derivative thermogravimetric (DTG) were obtained for raw and pretreated OPFs. A slight decrease in weight at the temperature of 50 to 125°C indicated the evaporation of volatile components and water (**Fig. 4.11a**). Significant decomposition of OPF was observed at 200 to 375°C due to the decays of hemicellulose, cellulose, and some portions of lignin (Subhedar and Gogate, 2014). Raw OPF has the highest residue, followed by AHP, Type II DES, and lastly AHP + Type II DES- pretreated OPF in descending order, which implied the reduction of lignin and ash after pretreatment (Jablonský et al., 2015). From DTG analysis (**Fig. 4.11b**), two distinct peaks were observed at 280 and 330°C in Raw and AHP-pretreated OPF, representing hemicellulose and cellulose respectively. Both Type II DES and AHP + Type II DES pretreatments resulted in the disappearance of hemicellulose peak, due to high hemicellulose removal capabilities as observed in the compositional analysis (**Table 4.4**). Moreover, the AHP + Type II DES-pretreated OPF obtained the highest derivative weight at 320°C, which implied significant removal of lignin and hemicellulose, at the same time higher degree of cellulose enrichment. This observation was consistent with the compositional analysis in **Table 4.4**.

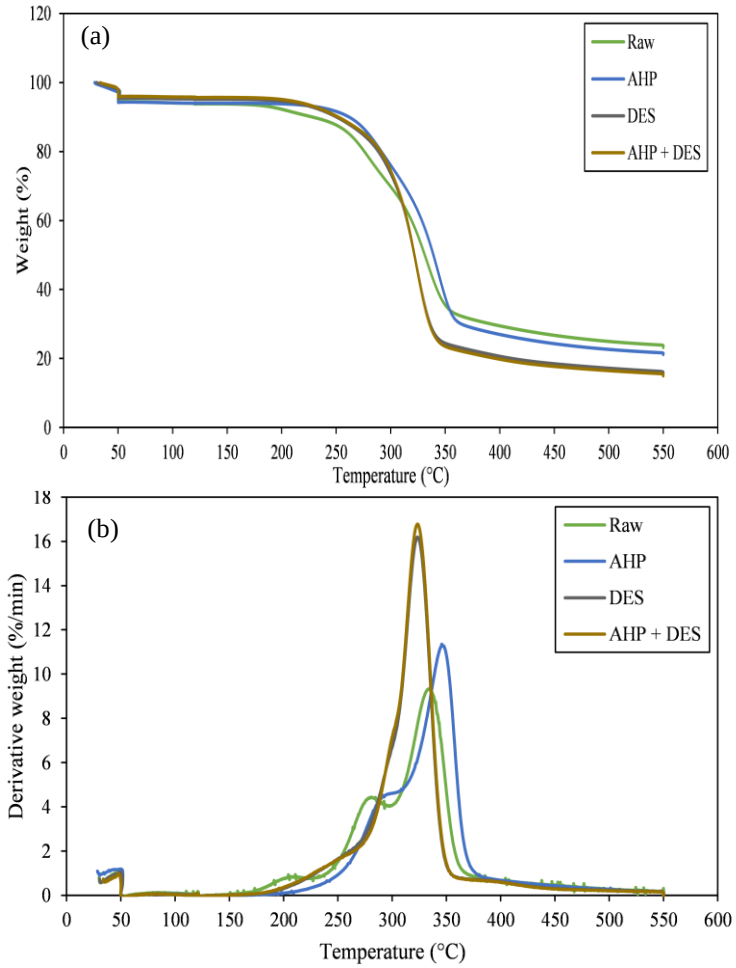

Fig. 4.11 TGA (a) and DTG (b) spectra of raw and pretreated OPFs.

4.5 Comparison with various DES pretreatment of biomass

The result obtained from this study were compared with various types of DES pretreatments (**Table 4.7**). It is worth noting that the pretreatment conditions used in this study were relatively less severe as compared to the other applications. The present study was able to achieve better delignification and xylan extraction as compared to the previous study (Ho et al., 2019b), despite lower temperature and shorter duration. However, the delignification achieved in this study was inferior as compared to the findings obtained by Tan et al. (2018) (ChCl:Lactic acid) and Chen et al. (2019) (Ternary DES). It is important to note that, Type II ChCl:$CuCl_2$•$2H_2O$ DES has the potential of achieving higher delignification but with the expense of sugar recoveries. Thus, further works are needed to develop Type II ChCl:$CuCl_2$•$2H_2O$ DES in order to achieve high delignification without severe sugar degradation.

The result was then compared with the pretreatments investigated in this thesis (**Chapter 3** and **Chapter 4**). **Fig. 4.12** summarized the performance of different pretreatments carried out in this thesis. These studies have revealed the potential of sequential pretreatment and Type II DES (ChCl:$CuCl_2$•$2H_2O$) to adapt to various applications, such as delignification, biomass fractionation, sugar recoveries, and furfural productions. The synergism between AHP and DES could potentially provide an opportunity to further enhance the downstream conversion of biomass into value-added products. Thus, contributing towards a new chapter in lignocellulosic biomass biorefinery.

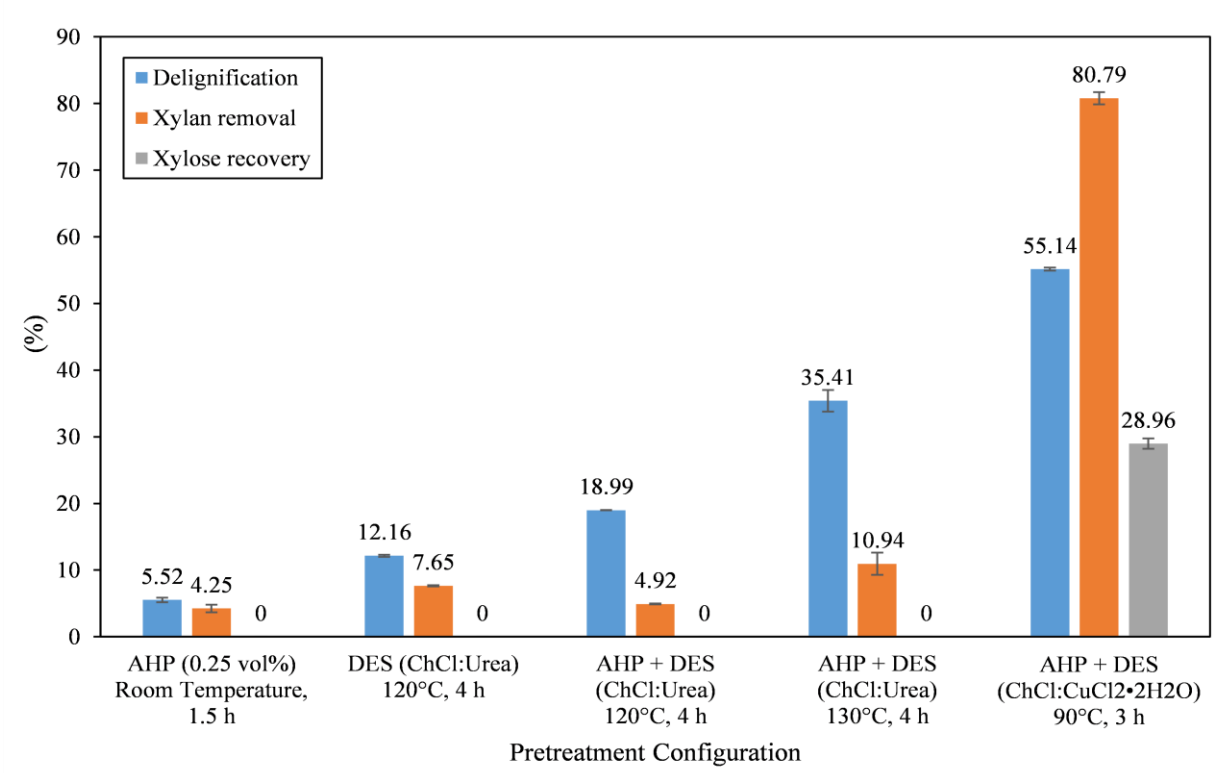

Fig. 4.12 Pretreatment performance of various configuration studied in the thesis.

Table 4.7

Performance of various DES in lignocellulosic biomass pretreatments.

Biomass	Pretreatment conditions	Delignification (%)	Xylan Removal (%)	Reference
Oil palm empty fruit bunch	(1) ChCl:Lactic acid DES Molar ratio: 1:5 Temperature: 120°C Pressure: Atmospheric Time: 8 h Solid loading: 10 wt%	88	100	(Tan et al., 2018)
Oil palm fronds	(1) 0.25% (v/v) AHP Temperature: Room Temperature Pressure: Atmospheric Time: 90 min (2) ChCl:Urea DES Molar ratio: 1:2 Temperature: 120°C Pressure: Atmospheric Time: 4 h Solid loading: 1:10 (w/v)	19.00	4.92	(Ho et al., 2019b)
Oil palm fronds	(1) Ultrasound Amplitude: 70% Time: 30 min (2) ChCl:Urea DES Molar ratio: 1:2 Temperature: 120°C Pressure: Atmospheric Time: 4 h Solid loading: 1:10 (w/v)	36.42	-	(Ong et al., 2019)
Oil palm fronds	(1) 30 vol% H_2O, 70 vol% ChCl:Urea DES Molar ratio: 1:2 Temperature: 120°C Pressure: Atmospheric Time: 4 h Solid loading: 1:10 (w/v)	16.31	-	(New et al., 2019)
Switchgrass	(1) Guanidine hydrochloride:ethylene glycol :*p*-toluensulfonic acid Ternary DES Molar ratio: 1:1.94:0.06 Temperature: 120°C Pressure: Atmospheric Time: 6 min Solid loading: 10 wt%	82.07	79.36	(Chen et al., 2019)
Oil palm fronds	(1) 0.25% (v/v) AHP Temperature: Room Temperature Pressure: Atmospheric Time: 90 min (2) ChCl:$CuCl_2$•$2H_2O$) DES Molar ratio: 1:1 Temperature: 90°C Pressure: Atmospheric Time: 3 h Solid loading: 1:10 (w/v)	55.14	80.79	This study

4.6 Conclusion

The potential of novel Type II DES (ChCl:$CuCl_2$•$2H_2O$) in delignification and sugar recoveries of OPF was investigated. AHP pretreatment was found to improve delignification of DES pretreatment in sequential pretreatment synergically, but little effect was observed in sugar recoveries. The combination of AHP (0.25 vol%, 90 min, room temperature) and Type II DES (ChCl:$CuCl_2$•$2H_2O$) at recommended conditions (90°C, 3 h) achieved the delignification of 55.14% and maximum xylose recoveries of 28.96% with xylan and arabinan removals up to 80.79 and 98.02% respectively. During Type II DES pretreatment, the temperature and duration play pivotal roles on the OPF delignification, hemicellulose removal, and subsequently sugar recoveries from the hydrolysate. The effectiveness of ChCl:$CuCl_2$•$2H_2O$ in delignification and hemicellulose removal was attributed by its abilities to generate more chloride anions to form hydrogen bonds with lignin, at the same time forming metal hydrate cation complexes to breakdown hemicellulose. Therefore, ChCl:$CuCl_2$•$2H_2O$ pretreatment could improve biomass fractionation and yield glucan-rich solid fraction, while recovering xylose from the liquid fraction.

Chapter 5

Conclusion

5.1 Overall conclusion

In conclusion, mild concentration AHP (0.25 vol%) at ambient condition for 90 min was able to improve DES delignification synergistically in subsequent pretreatment (**Chapter 3**). A combination of AHP (0.25 vol%, 90 min) and Type III DES (ChCl:Urea, 120°C, 4 h) at the later stage resulted in a pretreated OPF with a delignification of 18.99%, approximately 1.6 folds compared to Type III DES alone (**Objective 1**). AHP was found able to enhance OPF porosity and accessibility, thus making it more susceptible to DES delignification and sugar recoveries. By integrating mild AHP at ambient conditions in sequential pretreatment with Type III DES (ChCl:Urea) at 130°C and 4 h, delignification was increased to 35.41% from 26.89% (single-stage Type III DES at 130°C and 4 h). Similar synergism was observed in sequential pretreatment of AHP and Type II DES (ChCl:$CuCl_2$•$2H_2O$) at recommended conditions (**Objective 2**). This configuration was able to achieve delignification of 55.14% with remarkable xylan (80.79%) and arabinan (98.02%) removal efficiencies while a maximum xylose recovery of 28.96% was obtained in the hydrolysate.

5.2 Recommendations for future work

Preliminary study on the DES hydrolysate obtained from the recommended conditions in this study revealed the ability of Type II DES (ChCl:$CuCl_2$•$2H_2O$) in producing furfural (0.05 ± 0.01 g/L). It is important to note that the recommended conditions employed in this study were not optimized for furfural production. Furfural generally formed at an elevated temperature (> 100°C) (Lee et al., 2019). Thus it was suggested to examine the potential of DES in furfural formation at a higher temperature. Furthermore, aqueous DES may potentially improve the overall delignification further in the OPF pretreatment (New et al., 2019). In order to maximize the potential uses of DES to adapt to different applications, it was also worth studying the effect of DES at a higher temperature (> 100°C) but shorter duration. This novel Type II inorganic hydrate salt-based DES may be a promising green solvent in the biorefinery. However, more in-depth researches on the fundamental mechanism, applications, recyclability, process scale-up, and cost-benefits feasibility were needed to release its potential to the fullest as a sustainable and effective pretreatment solvent in biomass fractionation.

5.3 Contributions of research findings

The pretreatment processes are required to disrupt lignocellulosic biomass in order to facilitate the hydrolysis of cellulose and hemicellulose into fermentable sugars. However, the pretreatment process of lignocellulosic biomass is often costly. Thus, hindering the valorization of lignocellulosic biomass to produce valued chemicals (Isikgor and Becer, 2015). AHP at high concentration, temperature, and pressure has been implemented in an existing industry as a pretreatment process. However, the corrosive

properties of AHP at harsh conditions imposed additional capital cost for the corrosion resistant reactor (Michalska and Ledakowicz, 2016). Recently, a simpler and safer pretreatment process by using DES has been introduced. The simple synthesization and promising performances in biomass pretreatment of DESs have been attracting much attention. The incorporation of mild conditions AHP pretreament into the integrated sequential pretreatment was found to induce synergism in the delignification of the subsequent DES pretreatment. The mild conditions AHP pretreatment was implemented as a biomass conditioning stage, which could facilitate the subsequent DES pretreatment in terms of biomass accessibility. The uses of AHP and DES sequential pretreatment were able to reduce the overall process hazard and energy consumption, while maintaining comparable delignification efficiency. In this work, Type II ChCl:$CuCl_2$•$2H_2O$ DES was found to exhibit delignification, hemicellulose removal, and xylose recovery properties. It is possible for Type II ChCl:$CuCl_2$•$2H_2O$ DES to adapt to different applications by adjusting the pretreatment conditions. From the present study, the Type II ChCl:$CuCl_2$•$2H_2O$ DES was able to show promising biomass fractionation efficiency, hence could potentially plays a vital role in the biorefinery processes.

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Appendix A

Potential use of alkaline hydrogen peroxide in lignocellulosic biomass pretreatment and valorization–A review (Original Published Paper)

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Potential use of alkaline hydrogen peroxide in lignocellulosic biomass pretreatment and valorization – A review

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ABSTRACT

Alkaline hydrogen peroxide (AHP) was first introduced in 1984. Its ability to selectively attack carbonyl and ethylene groups is proven to enhance the delignification process of lignocellulosic biomass. The capabilities of AHP in dissociating the recalcitrant matrix of biomass indicate its potential in improving the accessibility of biomass to achieve valorization. In this paper, the background information of AHP and its reaction pathways are provided. Moreover, the recent applications of AHP in biomass processing and biofuel production are discussed by highlighting the effects of AHP in process selectivity and biomass dissolution. This paper also aims to provide some insights on the advantages and disadvantages of utilizing AHP in a pretreatment process. Lastly, a technoeconomic and cost benefit analysis of two scenarios of AHP production are critically reviewed to highlight the feasibility of implementing AHP in large scale production. It was reported that, AHP pretreatments were compatible with wide range of biomass and subsequent enzymatic hydrolysis. Besides, it was found that the application of AHP sequential pretreatment of biomass could increase the production yield of various bio-products such as silica and lignin. On top of that, AHP pretreatment was found to be a mild pretreatment method that promoted inherent safer and greener process. However, the mild operation condition would require a prolonged period to achieve the necessary delignification. Through technoeconomic analysis, the high chemical cost has rendered the installation of recycle stream compulsory to achieve economic feasibility.

1. Introduction

The conversion of biomass into various value-added biochemical, including biofuel, is generally termed as biomass valorization [1]. This phrase has been widely used in recent research publications, indicating the shift of global research focal point to the alternative sources for producing chemicals and bioenergy, which could be derived from the waste. Annually, hundreds billion tonnes of lignocellulosic biomass waste are produced but they are usually neglected via disposal on landfill or through burning [2]. These practices do not contribute to any economic benefits to the industry and at the same time do not fully utilize the biomass despite its abundance in mass. The availability of lignocellulosic biomass is not limited by geographical distribution, hence making it a promising feedstock for biofuel production and other value-added products [3,4]. Furthermore, the abundance of agricultural residues, which could be acquired at low cost, making it a better and more promising option as a raw feedstock in sugar platform biorefinery [5,6]. To date, more and more research on the valorization of agricultural residual into biofuel has attracted great attention, mainly due to the positive effects from both economic and environmental aspects and long- term energy sustainability with greenhouse gas mitigation [7,8]. Therefore, the valorization of lignocellulosic biomass for the value-added products and biofuel productions could potentially relieve the stresses to the natural resources and shift the dependencies of fossil fuel to sustainable energy sources.

Lignocellulosic biomass is a complex structure of carbohydrate polymers which consist of cellulose (38–50%), hemicellulose (23–32%), and aromatic polymer lignin (15–25%) [9]. Cellulose is a strong molecular structure in biomass, which is formed by long chains glucose monomeric units connecting via β-glycosidic linkages with reducing and non-reducing ends [10]. These cellulose microfibrils which possess both amorphous and crystalline properties are then stacked in parallel

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due to the presence of hydrogen bonding and weak van der Waals forces. Thereafter, these microfibrils are interlaced together with hemicellulose and lignin to form macrofibrils [11]. As a result, access to cellulose by chemical or enzymatic means is difficult due to the presence of complex linkages network and hydrogen bonding [12]. Hemicellulose is considered an amorphous polymer, which is a cluster of heterogeneous polysaccharides with a mix of hexose and pentose monosaccharides [13–16]. When chemicals and/or heat are applied, hemicellulose is relatively easier to dissociate as compared to cellulose [17]. Typically, softwood based biomass contains mainly glucomannans and mannans, while xylan (β-(1,4)-linked xylose homopolymer) are usually found in hardwood based biomass [10]. Therefore, both cellulose and hemicellulose contribute to most of the sugar present in the biomass.

Lignin is a hydrophobic polymer composed of several cross-linked polymer of phenolic substances [18], bounded to the hemicellulose and cellulose in a complex polymer matrix which acts as a physical barrier against microbial attack as well as provides structural support to the plant cell [14–16,19]. Lignin contains a number of functional groups, such as hydroxyl, carbonyl, and methoxyl in the lignin matrix, which contribute to high polarity of lignin macromolecule [20]. These recalcitrance properties of plant biomass prevent enzymes from accessing the carbohydrate substrate [5]. In general, pretreatments to overcome lignocellulosic recalcitrance are costly and sometimes involve inhibitors to the biofuels conversion [21], hence hindering the development of an economically viable process to convert biomass to biofuels or other valuable products [22]. A lot of research has revealed the negative effect of lignin during enzymatic digestion of biomass. Therefore, an effective lignin extraction or disruption could significantly alter the structural matrix and crystallinity of cellulose and ultimately enhance biomass saccharification in fermentation [14–16,19].

A typical biorefinery processing of lignocellulosic biomass involves pretreatment step, subsequent enzymatic hydrolysis to disintegrate polysaccharides, and fermentation of released sugar solution to produce biofuels and biochemical products [7,8]. In order to facilitate downstream biorefinery of lignocellulosic biomass, a variety of pretreatment stage has been proposed to overcome the recalcitrant structure of biomass by disrupting carbohydrate matrix and disintegrate high molecular structures like lignin, hemicellulose, and cellulose into smaller compounds, such as monosaccharides, organic acids, and phenolic compounds [22,23]. A wide range of biomass fractionation approaches have been studied, such as chemical (alkali, dilute acid, organic solvent), physical (grinding, milling, irradiation), physiochemical (wet oxidation, auto-hydrolysis), and biochemical methods [11].

To date, the disruption of the complex polymer matrix to release monosaccharides remained as the main issue for effective utilization of lignocellulosic biomass. Thus, methods that improve the biomass digestibility for subsequent enzymatic or microbial attack have gain greater attention in the recent years [24]. Recent research discovers that alkaline peroxide is widely used in biomass saccharification process due to the effective generation of radicals and its extraordinary performance in delignification in most crop residue with little sugar degradation and furan derivatives production as compared to the other pretreatment approaches [13,15,16,25–28]. Hydrogen peroxide (H_2O_2) is commonly used in pulping and bleaching industries. By tuning the peroxide solution to alkaline using hydroxide ions (OH^-), radical species (e.g., HO· and HOO·) and molecular oxygen are formed as a result of H_2O_2 decomposition, which in turn selectively react with lignin in several reaction pathways, resulting in complicated total oxidation reaction mechanism and leading to high delignification [5,29]. A lot of research has confirmed the rate and extent of enzymatic hydrolysis or microbial digestion are inversely proportional to lignin content in the biomass [5,26]. The excellent delignification using alkaline peroxide is not the only reason why researchers are paying more attention in biomass valorization for the past decades. Unlike acid or alkaline pretreatment, alkaline peroxide pretreatment can be performed at relatively milder conditions (concentration, temperature) and at atmospheric pressure, while effectively removing lignin from various agricultural residues [24]. Furthermore, alkaline peroxide is a relatively "green" reagent with low environmental impact as it can be easily decomposed to yield water and oxygen as end products.

To the best of our knowledge, although alkaline hydrogen peroxide has received significant attention and been applied in various research field, in-depth literature reviews specifically target on the recent applications of alkaline hydrogen peroxide in biomass valorization are still limited [30,31]. Thus, this review paper serves to compile, highlight, and discuss the vast potential, technoeconomic feasibility and role of alkaline hydrogen peroxide as one of the pretreatment technologies in biomass transformation and processing.

2. Alkaline hydrogen peroxide (AHP) in biomass processing

2.1. Background information of AHP

There is often solutions available in the nature to tackle most of the research problem, which offer the potential approaches that mimic the natural processes in the biosphere as effective strategies. According to Li et al. [8], oxidative deconstruction of plant cell wall by aerobic saprophytes is commonly seen in the nature, which is potentially important to offer a direct effective pretreatment method. However, the understanding to the carbon and energy propagation pathways is still incomplete. Particularly, brown rot fungi was proposed to use a non-enzymatic mechanism to selectively alter lignin and cellulose by using hydroxyl radicals generated from hydrogen peroxide through Fenton chemistry [32] as a result of plant cell wall polymer oxidation [8].

Oxidative pretreatment approaches that implement effective trait of biological deconstruction routes of biomass are more likely to induce synergism with biological conversion [8]. Over the past decades, various oxidizing agents used in the pretreatments are developed, such as peracids [33], organic peroxides [34], oxygen [35], ozone [36], and hydrogen peroxide [37] (Table 1). Recent research has shown inclination towards the uses of AHP in biomass pretreatments due to its excellent delignification effect in lignocellulosic biomass while retaining carbohydrates that are highly susceptible to enzymatic hydrolysis and fermentation [38]. Under normal circumstances, hydrogen peroxide can only react with aliphatic part of lignin, while no changes and degradation of phenolic compounds are observed [39]. However, hydrogen peroxide is able to attack the phenolic compounds when it is used under alkaline conditions and heated to a relatively high temperature, which will expose the phenolic ring and cause carboxylic

Table 1
Type of oxidizing agents and their applications.

Oxidizing agent	Conditions	Applications	Ref.
Peracids	15% peracetic acid Liquid to solid: 6 to 1 Time: 7 d	Hybrid poplar enzymatic digestibility improvement	[33]
Organic peroxide	Refluxed boiling using 2 ml of 70% *Tert*-butyl hydroperoxide Liquid to solid: 500 to 1 Time: 24 h	Delignification of wood chips and pulps	[34]
Oxygen	Wet oxidation pretreatment Temperature: 200 °C Time: 10 min	Softwood (*Picea abies*) enzymatic hydrolysis improvement	[35]
Ozone	Ozone Temperature: 4 °C	Delignification of pine and eucalyptus kraft pulps	[36]
Hydrogen peroxide	1% (w/v) H_2O_2 Liquid to solid: 50 to 1 pH: 11.5 Time: 18–24 h Temperature: 25 °C	Delignification of various agricultural residue and enzymatic saccharification improvement	[37]

groups to be added to the macromolecular structure [5,40]. Such alteration of biomass structure by AHP pretreatment has led to effective lignin and xylan removals, while decreasing cellulose crystallinity as well as swelling of biomass due to the insertion of polar groups into the molecule [41].

2.2. Reaction mechanism

The extent of AHP pretreatments relies on the H_2O_2 decomposition and its intermediate products which act as an initiator for various oxidative reactions with biomass components. The decompositions of hydrogen peroxide can be further improved with the absence of stabilizer, such as $MgSO_4$ or sodium silicate and the presence of heavy-metal ions [42]. Under alkaline conditions specifically close to pH 11.5, hydrogen peroxide will dissociate to produce hydroperoxyl anion (HOO^-), which is responsible for the corresponding carbonyl and ethylene groups' oxidative reactions and initiator for radicals forming. Thus, AHP pretreatment for delignification is strongly pH-dependent with optimum pH of 11.5–11.6 which is the pKa for the H_2O_2 decomposition reaction [37]. These HOO^- will react with hydrogen peroxide to produce highly reactive hydroxyl radical ($HO\cdot$) and superoxide anion radical ($O_2^{-}\cdot$) [43], which are known to be strong oxidants for promoting lignin oxidization and depolymerizing biomass into low molecular weight fragments [7,42]. According to Cabrera et al. [24], no significant lignin alteration in biomass structure under low alkalinity condition was reported. It was claimed that only aliphatic part of lignin was oxidized in low alkalinity conditions. Therefore, the effectiveness of delignification strongly depends on the pH employed during the reaction in order to promote the H_2O_2 derived radicals. Fig. 1 shows the reactions illustration of hydrogen peroxide decomposition in alkaline conditions and the generation of highly reactive radicals as described above.

These radicals generated from the H_2O_2 dissociation in alkaline conditions are responsible for depolymerizing lignin through cleaving and fragmenting lignin macrostructure into smaller compounds [7,24,42]. As mentioned by Li et al. [8], such pretreatment was effective in enhancing the dissolution of grass lignins due to the destruction of ester, ether cross-links as well as cleavage of β-O-4 bonds within the lignin. On top of that, AHP pretreatment can also cause a significant amount of hemicellulose solubilization [22]. However, undesired cellulose depolymerization is also present in delignification, depending on the operational variables used. Thus, optimizations of peroxide loading, reaction duration, and temperature are to be carried out for different biomass to achieve efficient biomass fractionation [42].

2.3. Recent applications of AHP in biomass processing

Generally, one or more pretreatment stages are essential for effective hydrolysis of biomass to overcome the recalcitrance nature of biomass before introducing subsequent fermentation. A past study showed that biomass without undergoing a pretreatment stage would result in a sugar yield of 21% [8]. However, the sugar yield could be increased up to 98% by implementing a pretreatment step prior to hydrolysis [5]. Recent literature has shown an inclined trend of AHP in biomass dissolution application and revealed the potential of AHP as one of the economically viable pretreatment process. An effective pretreatment stage is achieved through the disintegration of lignin in the biomass structure, leading to the increase in accessibility of polysaccharides during hydrolysis. AHP is particularly effective in enhancing the glucose yield in the subsequent hydrolysis due to its ability to solubilize lignin and hemicellulose, which are the two protective barrier to cellulose [8,22]. Table 2 summarizes several available past studies regarding the application of AHP in biomass delignification and sugar recovery.

AHP is highly dependent on the operational variables whereby undesired cellulose depolymerization can occur. Therefore, optimizations are carried out to specifically target different biomass for efficient fractionation [42]. Cabrera et al. [24] reported that AHP showed no significant effect in biomass structure alteration under low alkalinity conditions, as only aliphatic part of lignin was oxidized. The efficiency of AHP in delignification was highly dependent on the promotions of H_2O_2 derived radicals, which could be affected by the reaction conditions. Moreover, Li et al. [21] revealed that AHP caused delignification and increase in cellulose crystallinity in pretreated biomass because AHP was able to selectively target lignin via radical formation under alkaline conditions. These intermediate products formed as a result of decomposition of H_2O_2 under alkaline condition (pH 11.5), enabling the disruption of complex matrix in lignocellulosic biomass, thereby exposed cellulose and enhanced its dissolution capability.

According to da Costa Correia et al. [7], AHP (4.3 vol% H_2O_2) caused lignin solubilization up to 92.44% when it was used to pretreat cashew apple bagasse for 6 h at 35 °C. When the concentration of H_2O_2 was increased from 0.645 to 4.3 vol% under similar solid loadings (10%) and conditions (35 °C, 24 h, pH 11.5), the degree of delignification drastically increased from 16.5 to 80.2% [7]. On top of that, da Costa Correia et al. [7] also revealed that when a lower solid loading was employed (5%), the maximum delignification was substantially increased to 96.7% over a duration of 24 h. The delignification efficiency of AHP in the pretreatment of hardwood based biomass is often lower than in softwood pretreatment. Catalyst, which was employed in AHP seemed to play a vital role in hardwood pretreatment in order to achieve subsequent profitable yield. For example, Li et al. [8] reported that Cu(II)(bpy)-catalyzed AHP exhibited an improvement in lignin solubilization from 36.6 (uncatalyzed) to 50.2%, leading to a total material loss of 25.9% for *Hybrid poplar* pretreatment under similar conditions (48 h, ambient temperature, pH 11.5, 10 g H_2O_2/L). The highly ordered structure of the cell wall matrix could be limiting oxidative radicals and caused disproportionation reactions. However, the employment of small and diffusible homogenous catalyst such as Cu(II)(bpy) provided an alternative route to improve site-specific reactions with cell wall polymer, thus improving the lignin and xylan solubilization [8]. On top of that, Li et al. [21] explored the efficiency of ultrasonic (40 kHz, 500 W) assisted AHP in delignification of Jerusalem artichoke and was able to improve lignin removal from 37.5 (AHP only) to 40.3%. Despite a minor improvement of delignification via ultrasonication, degree of polymerization by amorphous substances

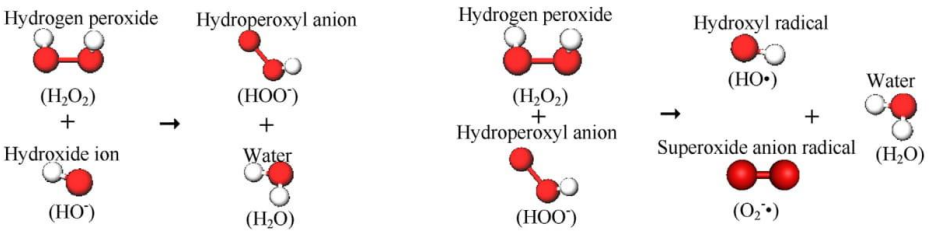

Fig. 1. Illustration of hydrogen peroxide derived radicals' generation reactions.

Table 2
Applications and performance of AHP in delignification and/or sugar recovery from lignocellulosic biomass.

Biomass	Initial pretreatment	Subsequent pretreatment	Key findings	Ref.
Rice husk	*Alkaline hydrogen peroxide* Solid loading = 3:50 (w/v) T = 90 °C Time = 1 h pH = 11.5 H_2O_2 concentration = 7.5% (w/v)	*Enzymatic hydrolysis* 29.1 mL of citrate buffer (0.05 M, pH 5), 0.3 mL of sodium azide (2%), 0.3 mL of NS22086 enzyme mixture, and 0.06 mL of each of the five Novozyme enzyme mixtures (NS22083, NS22118, NS22119, NS22002, NS22035) T = 50 °C Time = 72 h Mixing speed = 150 rpm	*With initial pretreatment* Solubilized compounds = 1.58 ± 0.07 g Sugar yield = 81.60 ± 2.63%	[5]
Rice husk	*High pressure alkaline hydrogen peroxide* Solid loading = 3:50 (w/v) T = 90 °C Time = 30 min pH = 11.5 P = 30 bar H_2O_2 concentration = 3.0% (w/v)	*Enzymatic hydrolysis* 29.1 mL of citrate buffer (0.05 M, pH 5), 0.3 mL of sodium azide (2%), 0.3 mL of NS22086 enzyme mixture, and 0.06 mL of each of the five Novozyme enzyme mixtures (NS22083, NS22118, NS22119, NS22002, NS22035) T = 50 °C Time = 72 h Mixing speed = 150 rpm	*With initial pretreatment* Solubilized compounds = 1.55 ± 0.18 g Sugar yield = 98.47 ± 6.35%	[5]
Cashew apple bagasse	*Alkaline hydrogen peroxide* Solid loading = 5% (w/v) T = 35 °C Time = 6 h pH = 11.5 H_2O_2 concentration = 4.3% (v/v) Mixing speed = 150 rpm	*Enzymatic hydrolysis* Cellulase complex (Novozymes NS 22074, 108.12 FPU/mL), 0.3 g cellulose equivalent biomass, 15 mL of 0.1 M citrate buffer (pH 4.8) and 120 μL of 10 mg/mL tetracycline in 70% v/v ethanol. Final cellulose activity at 60 FPU/g cellulose with volume of 30 mL T = 45 °C Time = 72 h Mixing speed = 150 rpm	*With initial pretreatment* Lignin removal = 92.44% Enzymatic digestibility = 87% Glucose yield = 161 mg/g untreated biomass	[7]
Hybrid poplar	*Cu^{II}(bpy)-catalyzed alkaline hydrogen peroxide* Solid loading = 1:10 (w/v) T = Ambient temperature Time = 48 h pH = 11.5 H_2O_2 concentration = 10 g/L	*Enzymatic hydrolysis* 1 M (pH 5.0) Na-citrate buffer (500 μL), 10 mM tetracycline (40 μL), mixed enzyme of Novozyme Cellic C-Tec (227 FPU/mL) and H-Tec (1090 FXU/mL) T = 50 °C Time = 72 h	*Without initial pretreatment* Lignin solubilization = None Hemicellulose solubilization = None Glucose yield = 21% *Uncatalysed pretreatment* Lignin solubilization = 36.6% Hemicellulose solubilization = 14.9% Glucose yield = 27% Material loss = 14.1% *Catalyzed pretreatment* Lignin solubilization = 50.2% Hemicellulose solubilization = 32.7% Glucose yield = 50.0% Material loss = 25.9%	[8]
Jerusalem artichoke	*Alkaline hydrogen peroxide* Solid loading = 1:20 (w/v) T = 50 °C Time = 120 min NaOH concentration = 2% (w/v) H_2O_2 concentration = 5% (w/v)	*Enzymatic hydrolysis* Pretreated residue (1 g), mixed-cellulase reaction buffer (0.2 M acetic acid – sodium acetate, pH 4.8) supplemented with mixed cellulose (β-glucanase ≥ 3.6×10^4 U, cellulose ≥ 360 U, and xylanase ≥ 6×10^4 U) to 40 mL T = 50 °C Time = 48 h Mixing speed = 150 rpm	*Without initial pretreatment* Cellulose content = 37.7 ± 0.6% Lignin Removal = None Degree of polymerization = 2199.2 Cellulose crystallinity ≈ 45% Total sugar yield = 4.1 g/L *With initial pretreatment* Cellulose content = 43.8 ± 0.8% Lignin Removal = 37.5% Degree of polymerization = 1292.5 Cellulose crystallinity ≈ 58.8% Total sugar yield = 8.4 g/L	[21]
Jerusalem artichoke	*Ultrasonic assisted alkaline hydrogen peroxide* Ultrasonic frequency = 40 kHz Ultrasonic power = 500 W Solid loading = 1:20 (w/v) T = 50 °C Time = 120 min NaOH concentration = 2% (w/v) H_2O_2 concentration = 5% (w/v)	*Enzymatic hydrolysis* Pretreated residue (1 g), mixed-cellulase reaction buffer (0.2 M acetic acid – sodium acetate, pH 4.8) supplemented with mixed cellulose (β-glucanase ≥ 3.6×10^4 U, cellulose ≥ 360 U, and xylanase ≥ 6×10^4 U) to 40 mL T = 50 °C Time = 48 h Mixing speed = 150 rpm	*Without initial pretreatment* Cellulose content = 37.7 ± 0.6% Lignin Removal = None Degree of polymerization = 2199.2 Cellulose crystallinity ≈ 45% Total sugar yield = 4.1 g/L *With initial pretreatment* Cellulose content = 44.3 ± 0.5% Lignin Removal = 40.3% Degree of polymerization = 785.3 Crystallinity = 62.5% Total sugar yield = 10.4 g/L	[21]
Douglas fir	*Alkaline hydrogen peroxide* Solid loading = 1:10 (w/v) T = 180 °C Time = 60 min pH = 11.6 H_2O_2 concentration = 4% (w/w)	None	*With initial pretreatment* Delignification = 22% Glucomannan removal = 78%	[22]
Corn stover	*Alkaline hydrogen peroxide* Solid loading = 1:10 (w/v) T = 50 °C Time = 3 h pH = 11.5	*Enzymatic hydrolysis* 1% solid loading, CTec3 enzyme (16 mg protein per gram of glucan) with 50 mM citrate buffer (pH 4.8), supplemented with HTec3 (4 mg protein per gram of glucan) T = 50 °C	*Without initial pretreatment* Lignin removal = None Glucose yield = 23% Xylose yield = 17% *With initial pretreatment*	[42]

(*continued on next page*)

Table 2 (*continued*)

Biomass	Initial pretreatment	Subsequent pretreatment	Key findings	Ref.
	H_2O_2 concentration = 250 mg H_2O_2/g dry biomass	Time = 120 h Mixing speed = 130 rpm	Lignin removal = 80% Glucose yield = 90% Xylose yield = 80%	
Rice straw	*Alkaline hydrogen peroxide-assisted wet air oxidation* Soaking in alkaline hydrogen peroxide Time = 14 h pH = 11.9 H_2O_2 concentration = 0.5% (w/v) Pressurized with 6 bar air at 190 °C for 20 min with mixing at 200 rpm	*Enzymatic hydrolysis* Cellulase from *Trichoderma reesei* (60 FPU/mL) and β-glucosidase from almonds (10CBU/mg), solid loading at 60 g/L in sodium citrate buffer (0.1 M, pH 4.8) with 2% sodium azide. T = 50 °C Time = 72 h	*Without initial pretreatment* Lignin removal = None Glucose yield = 83.25 ± 7.34 g/kg untreated rice sraw *With initial pretreatment* Lignin removal = 77.29% Cellulose recovery = 83.01% Glucose yield = 200.34 g/kg untreated rice straw	[44]
Agave bagasse	*Alkaline hydrogen peroxide* Solid loading = 1:10 (w/v) T = 23 °C Time = 48 h pH = 11.5 H_2O_2 concentration = 500 g/kg of biomass Mixing speed = 9.43 rad s^{-1}	*Enzymatic hydrolysis* Celluclast 1.5 L (30 FPU g^{-1} of glucan) and Novozyme 188 (60 CBU g^{-1} of glucan), enzyme loading normalized to 5 g glucan dm^{-3} in citrate buffer (0.05 M, pH 4.8). T = 55 °C Time = 72 h Mixing speed = 15.71 rad s^{-1}	*Without initial pretreatment* Lignin content = 19.6% Glucan content = 40.5% Reducing sugar = 1.6 g dm^{-3} *With initial pretreatment* Lignin content = 17.6% Glucan content = 44.5% Reducing sugar = 6.9 g dm^{-3}	[45]
Coconut husk	*Alkaline hydrogen peroxide* Solid loading = 4% (w/v) T = Room temperature Time = 1 h pH = 11.5 H_2O_2 concentration = 7.35% (v/v) Mixing speed = 100 rpm	*Enzymatic hydrolysis* 5% (w/v) solid loading, Celluclast 1.5 L (20 FPU/g biomass) in citrate buffer (50 mM, pH 4.8). T = 50 °C Time = 72 h Mixing speed = 150 rpm	*Without initial pretreatment* Lignin content = 39.9% Glucose yield = 3.0 g/L *With initial pretreatment* Lignin content = 33.78% Glucose yield = 6.0 g/L	[46]
Corn stover	*Alkaline hydrogen peroxide* Solid loading = 5% (w/v) T = 50 °C Time = 3.5 h pH = 11.5 H_2O_2 concentration = 2% (v/v)	*Enzymatic hydrolysis* 5% (w/v) solid loading, Cellic CTec2 (64.6 FPU/g) in sodium citrate buffer (50 mM, pH 5.4). T = 50 °C Time = 72 h	*Without initial pretreatment* Glucan yield = < 10% Arabinoxylan yield = < 10% *With initial pretreatment* Lignin removal = 68.7 ± 4.3% Glucan yield = 88.1 ± 7.6% Arabinoxylan yield = 92.5 ± 9.1%	[47]
Banana rachis	*Alkaline hydrogen peroxide* Solid loading = 2% (w/v) T = Room temperature Time = 90 min pH = 14 H_2O_2 concentration = 2% (v/v)	*Enzymatic hydrolysis* 1 g sample, 50 μL cellulose from *Trichoderma reesei* (Novozyme 2730, ≥700 unit/gram of enzyme), 50 μL of β-glucosidase from *Aspergillus niger* (Novozyme 2605, ≥1000 U/g) in citrate buffer (50 mM, pH 5.0). T = 50 °C Time = 72 h Mixing speed = 150 rpm Supplemented with 0.3 g of Tween 80 (1%, v/v) after 2 h.	*Without initial pretreatment* *Glucose recovery = 2.8 ± 0.2%* Xylose recovery = 9.0 ± 0.7% Arabinose recovery = 1.5 ± 0.0% *With initial pretreatment* Lignin removal ≈55% Glucose recovery = 36.5 ± 1.5% Xylose recovery = 5.6 ± 0.3% Arabinose recovery = 1.6 ± 0.0%	[48]
Bamboo chip	*Two-stage Alkaline/Alkaline hydrogen peroxide* Alkaline pre-extraction Solid loading = 10% (w/v) T = 100 °C Time = 180 min Alkaline hydrogen peroxide Solid loading = 10% T = 75 °C Time = 180 min pH = 11.5 H_2O_2 concentration = 4% (v/w)	*Enzymatic hydrolysis* 20% (w/v) solid loadings, Cellic Ctec2 (113 FPU/mL, 137.6 mg/mL protein content), β-glucosidase (Novozymes 188, 160 CBU/mL, 120 mg/mL protein content), enzyme loading of 30 mg protein/g glucan in sodium citrate buffer (100 mM, pH 5). T = 50 °C Time = 120 h Mixing speed = 250 rpm	*Without initial pretreatment* Glucan content = 48.4% Lignin content = 25.1% *With initial pretreatment* Glucan content = 70.1 ± 1.1% Lignin content = 14.5 ± 0.9% Delignification = 65.2% Glucose conversion = 78% Xylose conversion = 77%	[49]

(hemicellulose, lignin, and pectin) removal was significantly reduced, leading to crystallinity index (CrI) of Jerusalem artichoke increased from 45% to 62.5%. Thus, the biomass porosity and accessibility were greatly improved.

Nonetheless, it is important to take note that the reaction conditions are dependent on the type of biomass. For example, the optimum conditions for corn stover in delignification were 3 h, 50 °C, and 250 mg H_2O_2/g dry biomass [42], whereas for Jerusalem artichoke were 2 h, 50 °C, and 5% H_2O_2 (w/v) [21]. On the contrary, Alvarez-Vasco and Zhang [22] used AHP to pretreat *Douglas fir* at relatively high temperature of 180 °C, which resulted in delignification of 22% and exceptionally high glucomannan removal (hemicellulose) of 78%, while causing little degradation on cellulose. According to Alvarez-Vasco and Zhang [22], reducing end groups on glucomannan could occur quickly at high temperature, which facilitated organic acids formation through endwise depolymerization, together with termination reactions (formation of non-reducing end groups). These series of reactions provided a potential route to convert hemicellulose to valuable organic acids such as lactic acid, glycodic acid, succinic acid and formic acid, while removing protective barrier from the biomass to enhance cellulosic biofuels or biochemical productions. On the other hand, Morone et al. [44] demonstrated the use of pressurized air (6 bar, 190 °C) oxidation, which was assisted by AHP for reducing the H_2O_2 dosage in the pre-treatment. By employing wet air oxidation on AHP pre-soaked rice straw, the H_2O_2 concentration could be reduced until 0.5% (w/v) while achieved maximum lignin removal and cellulose recovery of 77.29% and 83.01%, respectively [44]. High temperature treatment could lead to higher cellulose enrichment in the biomass and better solubilization of hemicellulose and lignin. However, high temperature environment also promoted sugar degradation and formation of carboxylic acids such as acetic acid as a result of acetyl groups removal from the hemicellulose. The formation of such acids remarkably change the pH of the reaction content from pH 11.9 to pH 5.63 [44]. Mittal et al. [42] further revealed that the lignin extraction efficiency depended on the peroxide concentration, whereby under mild peroxide concentrations, only side chain structures of lignin were oxidized, while no significant

changes on aromatic rings. At 250 mg H_2O_2/g dry biomass, both side chain structures and aryl-ether bonds were dissociated, suggesting the disruptions of lignin aromatic rings and β-O-4 units.

Recent applications of AHP in biomass processing reported excellent delignification and high affinity with enzymatic hydrolysis. It was found that, the AHP pretreated corn stover was able to enhance saccharification to a maximum of 92.5% [47]. On top of that, the application of AHP under mild temperature (< 100 °C) was able to achieve significant delignification and high glucan enrichment [48,49]. It is interesting to note that the AHP could be customized and adapted to fractionate different biomass by optimizing the operating conditions to suit different applications. Numerous studies also revealed that, AHP pretreatments were highly compatible with enzymatic hydrolysis to yield high amount of sugars. These unique features allow AHP to potentially become one of the promising pretreatment methods used in a biorefinery.

2.3.1. Morphological alteration

High sugar recovery from the biomass was predominately affected by effective removals of lignin and hemicellulose, which ultimately altered the cellulose features. According to Li et al. [21], biomass after undergoing AHP pretreatment showed transparent in colour with a reduction in particle size. Surface morphology analysis displayed disordered fibrils and formation of tiny holes on the pretreated biomass, which was caused by the removal of non-cellulosic polymers. AHP process disrupted polymers matrix and resulted in a serious collapse of supramolecular structure, enabling easier accessibility of enzymes towards the biomass. Ordered and intact structures were observed in an untreated rice straw (Fig. 2a). However, after undergoing both pressurized air and AHP pretreatments, the pretreated biomass became loosened with a formation of cracks and cavities (Fig. 2b arrows), along with some debris which could be originated from the fragmentation of hemicellulose and lignin [44]. A similar observations (Fig. 3) was obtained from the surface morphology analysis done by Mittal et al. [42], whereby the corn stover after undergoing AHP pretreatment resulted in finer particles (Fig. 3d arrow). Transmission electron micrographs of the pretreated corn stover revealed less densely stained (lignin loss) and multiple delaminations (Fig. 3f arrows), plus cell disjoining at the middle lamella with dimmer cell wall (Fig. 3e arrows). The collective structure alteration which was caused by the removals of hemicellulose and lignin subsequently increased the exposing area and accessibility of cellulose. The AHP displayed excellent performance in a pretreatment of corn stover, in which more than 80% delignification could be achieved after 3 h at 50 °C, while retaining more than 90% of carbohydrates initially available in the corn stover [42]. A maximum yield of glucose and xylose at 90% and 80%, respectively were attained when AHP (250 mg H_2O_2/g dry corn stover) and enzymatic hydrolysis were applied, in which the hydrolysate could be utilized for biofuel production at the later stage in a biorefinery [42]. Analogous observations were reported by Díaz et al. [5], whereby a maximum sugar yields of 98.5% and 81.6% were achieved after AHP-pretreated biomass underwent saccharification at 30 bar and atmospheric pressure, respectively.

2.3.2. Inhibitors

AHP pretreatment of lignocellulosic biomass has been known to generate small amount of acetic acid as a result of hemicellulose acetyl group removal. Most of the lignocellulosic pretreatment methods tend to generate 5-hydroxymethylfurfural (5-HMF) and furfural as a result of sugar degradation to different extent. Interestingly, Li et al. [21] revealed that no formation of 5-HMF and furfural could be detected using any of the four alkali-based pretreatments, namely dilute sodium hydroxide, alkaline peroxide, ultrasonic assisted dilute sodium hydroxide, and ultrasonic assisted alkaline peroxide. Li et al. [21] found that the concentration of acetic acids was significantly reduced by 38.5% to 1.15 g/L when H_2O_2 was used in the pretreatment, contrary to the negative results from ultrasonic assisted pretreatment. This result suggested that H_2O_2 possessed strong hydrolysate detoxification effect. Also, Morone et al. [44] reported a maximum acetic acid concentration of 1.2 g/L with the absence of 5-HMF and furfural productions using pretreated rice straw via AHP. The absence of these inhibitors suggested that no (or very minimum) sugar degradation happened at an elevated temperature because the sugars were mainly preserved in an oligosaccharides form [44]. It is worth noting that, weak acids could reduce the intracellular pH which requires ATP to release protons out of the cell. This phenomena would dissipate the proton-motive force across membrane and enhance ethanol formation. However, high concentrations of acetic, formic, and levulinic acid exceeding 100 mmoL/L would result in depletion of cell energy reserves, acidification of cytoplasm and cell death during fermentation [50]. Furthermore, the total phenolic content produced from the pretreated biomass (using AHP and pressurized air pretreatments) was found to be in the range from 0.29 g/L to 0.36 g/L, which was lower than the inhibitory concentration of most microbes [44]. However, contradicting results were obtained by Banerjee et al. [51], whereby most studies on alkaline peroxide assisted wet air oxidation (APWAO) of different lignocellulosic biomass reported the presence of furfural and HMF, which are the most potent inhibitors for subsequent bioconversion process. According to Larsson et al. [50], furfural and HMF would be consumed by yeast cells to form undesired alcohols, creating a lag phase in ethanol formation. However, the yield of ethanol was not reduced in the presence these inhibitors. Therefore, additional hydrolysate detoxification may be omitted for AHP pretreatment technologies.

3. AHP pretreatment for subsequent biofuel production

Countless research has been attempted to utilize pretreated lignocellulosic biomass to generate second generation of bioethanol and biogas. Among those proposed methods, AHP-pretreated biomass has

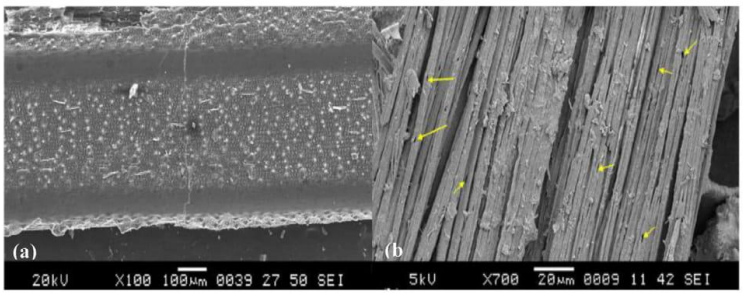

Fig. 2. Scanning electron micrograph for (a) untreated rice straw and (b) APWAO pretreated rice straw.
Reprinted with permission from Morone et al. [44]. Copyright 2017 Springer Nature.

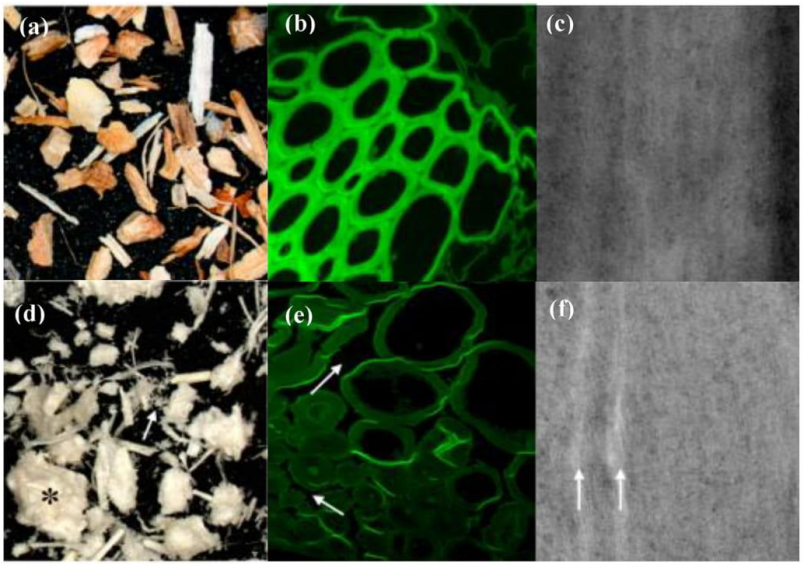

Fig. 3. (a) Stereoscope, (b) confocal scanning laser, and (c) transmission electron micrographs of untreated corn stover; (d) stereoscope, (e) confocal scanning laser, and (f) transmission electron micrographs of pretreated corn stover.
Reprinted with permission from Mittal et al. [42]. Copyright 2017 American Chemical Society.

gained traction within the scientific community in the production of biofuels (Table 3). The effectiveness of AHP process was its ability to remove substantial amount of lignin on the biomass, while retaining most of the polysaccharide in the solid biomass. This is because the presence of lignin in the biomass would prevent effective hydrolysis of hemicellulose and cellulose due to the nonproductive binding of enzymes [52]. In a study conducted by Yuan et al. [60], it was found that the sugar yield from NaOH–AHP pretreated bamboo chips was 87% higher in comparison to raw bamboo chips, this factor enables AHP pretreated bamboo chip to be more readily converted to bioethanol. The use of AHP pretreatment lead to the removal of lignin and silica from the biomass, thus, increasing the accessibility of enzymes to substrates for the hydrolysis of carbohydrates. Furthermore, the higher ethanol yield could also be explained by the low production of inhibitory products such as furfural and 5-HMF after the AHP pretreatment. In a study conducted by de Fátima Rodrigues de Souza et al. [53], AHP-pretreated sugarcane bagasse was found to have mass loss of 20% and 44% for hemicellulose and lignin, respectively. This high selectivity for lignin increased cellulose content within the biomass by 43%, benefitting a high solid-liquid ratio loading for enzymatic hydrolysis and fermentation to biofuels at the later stage [54]. In a separate study, AHP-pretreated corn stalk was able to produce 22% more biogas, while reducing biogas production by a third of the time required in comparison to untreated corn stalk [55]. However, it was worth noticing that both lignin solubilization and polysaccharide retention were not the only parameters affecting enzymatic hydrolysis and subsequent fermentation efficiency. de Souza Filho et al. [56] noticed that AHP-pretreated cactus pear had greater water retention ability during enzymatic hydrolysis, which was the main reason of low cellulose conversion into glucose. The same result was also observed by Vu et al. [57], where AHP and alkaline only pretreatment on gooseweed did not show any significant sugar recovery. Therefore, AHP might not be a suitable pretreatment method for certain biomass such as cactus pear and gooseweed. Besides, more attention should be placed on ensuring that every parameter affecting nominal sugar hydrolysis and fermentation was investigated during the application of AHP pretreatment.

In addition to the use of AHP in biofuel production, sequential AHP pretreatment was widely applied to various biomass as a novel approach to further improve fermentation and enzymatic hydrolysis [58]. The use of sequential AHP pretreatment in combination with other pretreatment is shown in Table 3. Sequential pretreatments were employed to generate less inhibitory byproducts such as acetic acid, furfural and silica, to improve the digestibility of enzymes [59]. For example, alkaline pre-extraction followed by sequential AHP pretreatment showed the benefit of removing silica from the biomass because the residual silica could hinder hydrolysis of substrate as well as prevent an efficient lignin recovery [58]. A mild sodium hydroxide pre-extraction was found to be effective in converting insoluble silica particles into a soluble form, namely sodium silicates. This was demonstrated by Yuan et al. [60], where the complete conversion of glucose and about 95% conversion of xylose into ethanol was achieved in 96 h. Another example was a sequential acid pretreatment followed by AHP pretreatment to reduce the production of acetic acid, which was produced by deacetylation of hemicelluloses under harsh conditions [61]. This sequential method was crucial as AHP pretreatment was highly selective towards lignin, while retaining considerable concentration of hemicellulose within the biomass. Additionally, it was found that lignin could be effectively removed from the olive tree pruning biomass after undergoing both formic acid and AHP pretreatments. With enzymatic hydrolysis and fermentation, the delignified olive tree pruning biomass could be reused as a feedstock for the production of ethanol up to 46 g/L [62]. Sequential AHP pretreatment was also widely investigated due to the synergetic effects as compared to an individual pretreatment. For example, in a study done by Phan and Tan [63], super critical carbon dioxide pretreatment followed by AHP pretreatment resulted in an excellent glucose recovery up to 97.8%, in comparison to a single stage AHP pretreatment which resulted in recovery of 22.9%. The high fermentable sugar concentration is crucial for later stage ethanol production in a biorefinery [64].

The utilization of AHP for the production of second generation biofuel showed a promising route towards a larger scale commercial production. However, biofuel productions are often not economically

Table 3
Compilation of AHP pretreated biomass in production of biofuel.

Lignocellulosic biomass	Pretreatment	Enzyme		Fermentation			Biofuel		Ref.
		Type	Loading	Time (h)	Strain	Time (h)	Type	Concentration (g/L)	
Corn Stover	AHP pretreatment only	Accellerase 1000 Multifect-Xylanase Multifect-Pectinase Novozyme 188	15.0 mg enzyme protein/g glucan	48	*S. cerevisiae* GLBRC Y35	120	Ethanol	13.70	[38]
Sweet sorghum bagasse	AHP pretreatment only	Celluclast β-glucosidase	60.0 FPU/g dry biomass 80.0 IU/g dry biomass	96	*S. cerevisiae*	96	Ethanol	19.33	[52]
Sugarcane bagasse	AHP pretreatment only	FPase Xylanase β-glucosidase Avicelase CMCase	10.0 FPU/g of substrate	72	*S. passalidarum* UFMG-CM-Y473	72	Ethanol	24.00	[53]
Jerusalem artichoke stalk	AHP pretreatment only	Cellic CTec2	20.0 FPU/g substrate	12	*S. cerevisiae* SPSC01	84	Ethanol	55.60	[54]
Corn stalk	AHP pretreatment only	N/R	N/R	N/R	Mesophilic anaerobic microorganisms	264	Biogas	622.40 $L_{biogas}.kgVS_{sd}^{-1}.d^{-1}$	[55]
Maize cob	AHP pretreatment only	N/R	N/R	N/R	Anaerobic microorganisms	N/R	Methane	3941.00 mL/d	[65]
Sugarcane Bagasse	AHP pretreatment only	Cellulase β-glucosidase	3.5 FPU/g WIS 25.0 IU/g WIS	N/R	Anaerobic microorganisms	960	Methane	152.00 L/g COD	[66]
Wheat straw	Alkaline pre – extraction and AHP pretreatment	Cellic CTec2 β-glucosidase	10.0 mg protein/g glucan	72	*S. cerevisiae* SR8u	96	Ethanol	31.10	[58]
Bamboo chips	Alkaline pre – extraction and AHP pretreatment	Cellic CTec2 β-glucosidase	5.5–18.0 FPU/g cellulose	96	*S. cerevisiae* SR8u	96	Ethanol	46.00	[60]
Rape straw	Sulphuric acid and AHP pretreatment	Cellic CTec3 β-glucosidase	15.0 FPU of Cellic CTec3 and 15 IU of β-glucosidase/g substrate	72	*S. cerevisiae*	72	Ethanol	53.00	[61]
Olive tree pruning	Sulphuric acid and AHP pretreatment	Cellic CTec3 β-glucosidase	15.0 FPU of Cellic CTec3 and 15.0 IU of β-glucosidase/g solid	72	*S. cerevisiae*	72	Ethanol	46.00	[62]
Matured coconut fiber	AHP pretreatment and alkaline pretreatment	Cellic CTec2 HTec2	30.0 FPU of Cellic CTec2 and 130.0 IU of HTec2/g solid	48	*P. stipitis* *S. cerevisiae* *Z. mobilis*	48	Ethanol	89.15	[67]
Rapeseed straw	AHP pretreatment and steam treatment	Celluclast β-glucosidase	1.5 L of Celluclast/g of cellulose 0.04 g β-glycosidase/g cellulose	48	Yeast	48	Ethanol Hydrogen Methane	0.42 g ethanol/g glucose 45.00 mLH_2/gVS 347.00 $mLCH_4/gVS$	[68]

[a]N/R Not reported.

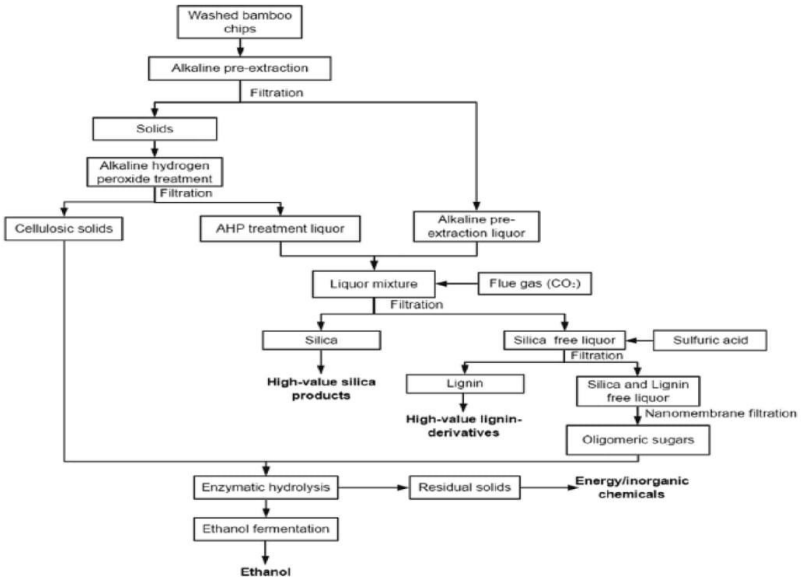

Fig. 4. Block flow diagram of an integrated bamboo chip biorefinery process. Reprinted with permission from Yuan et al. [60]. Copyright 2018 Elsevier.

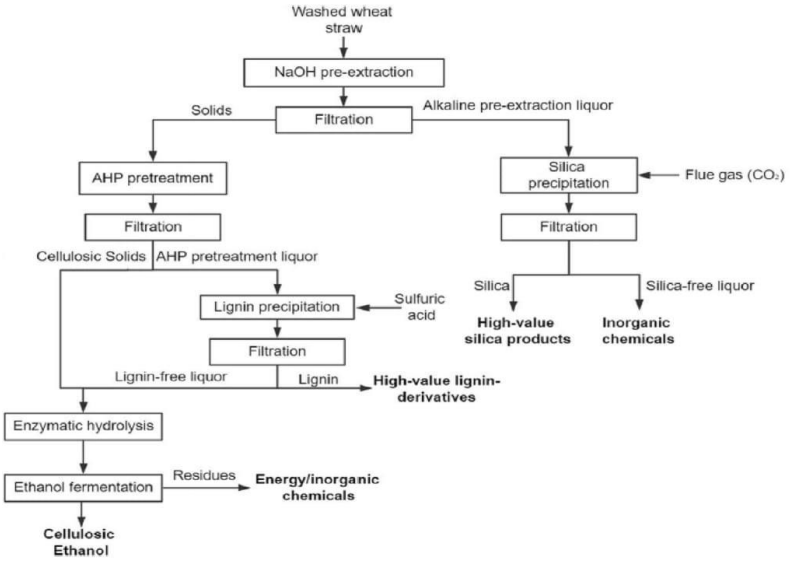

Fig. 5. Block flow diagram of an integrated wheat straw biorefinery process. Reprinted with permission from Yuan et al. [58]. Copyright 2018 Elsevier.

feasible without government support in the form of subsidies [69]. Therefore, there is a need to fully exploit the usefulness of biomass to provide a more competitive market for spurring biorefinery sector. In a study done by Yuan et al. [60], the processed liquor obtained from the AHP-pretreated bamboo chips was further processed to recover high-valued silica products and lignin derivatives. The detailed experimental flow is shown in Fig. 4. The dissolved silica and lignin were precipitated by carbon dioxide treatment and 72% H_2SO_4 acidification, respectively.

In a separate study, wheat straw was pretreated with AHP prior to the production of bioethanol. An integrated biorefinery process to fractionate and fully utilize the biomass was demonstrated through the recovery of silica and lignin (Fig. 5). In this study, 10 g of wheat straw underwent alkaline pre-extraction (0.25 moL/L NaOH, 30 °C for 6 h), followed by sequential AHP pretreatment (40 mg H_2O_2/g biomass, 50 °C for 15 h), achieving a total ethanol production up to 31.1 g/L. Additionally, 0.47 g of silica and 1.04 g of lignin were successfully

recovered [58]. This study demonstrated AHP ability in bioethanol production, in parallel to silica and lignin recovery. AHP was found to be suitable for the production of second generation biofuel due to its ability to selectively remove lignin, while enriching cellulose content. Furthermore, the utilization of sequential AHP pretreatment is a novel approach to compensate for an individual pretreatment limitation. This combination of pretreatment methods was also found to promote the extraction of various valuable byproducts, which was expected to improve the economic and cost competitiveness in the biorefinery using AHP as a pretreatment step.

4. Advantages and limitations of AHP

The use of AHP for biomass pretreatment has its own set of advantages and limitations in comparison to other type of pretreatment methods. Some of the advantages of utilizing AHP pretreatment method include the ability to reduce and even prevent (in some cases) the formation of 5-HMF and furfural throughout the pretreatment process. Both inhibitors are known to be detrimental to the fermentation process in later stages of bioprocessing to produce biofuels. Furthermore, contrary to ultrasound pretreatment, AHP was able to reduce the generation of acetic acid, which showed a positive effect on hydrolysate detoxification [21]. Besides, it was reported that AHP pretreatment showed a low polysaccharide degradation in comparison to other pretreatment method. In two separate studies done by Michalsk and Ledakowicz [23] and da Costa Correia et al. [7], it was found that the cellulose and hemicellulose degradations of biomass were largely unaffected by AHP pretreatment for a range of AHP concentrations. This characteristic was useful in the later stages of bioprocessing where high polysaccharide concentration was crucial in effective fermentation. Utilizing AHP also displayed numerous advantages which would increase the feasibility of large scale production. Firstly, AHP was found to be effective in pretreating biomass even at milder operating condition. For example, Douglas-fir wood was successfully delignified with AHP at temperature of 20 °C [70]. Michalsk and Ledakowicz [23] further supported the finding when it was reported that the biogas production for *Sida hermaphrodita* was optimized at 25 °C. Besides a lower operating temperature, AHP pretreatment was successfully conducted at atmospheric pressure in an open vessel for the pretreatment of oil palm empty fruit bunch [71]. The mild pretreatment condition provided an economical advantage as less energy would be utilized during the pretreatment. The mild operating conditions would further reduce the capital cost, as the need of constructing vessels out of expensive and sturdy material was not compulsory [38]. Most conventional pretreatment method would require particle sizes to be screened prior to pretreatment because smaller size particles have larger surface area for efficient pretreatment. However, Rabelo et al. [72] reported that bagasse screening was not necessary for AHP pretreatment because no significant pretreatment advantages were found for both screened and un-screened bagasse. This finding was crucial in reducing operation cost considerably. On an industrial scale, higher solid loadings are desirable since they lead to lower costs with reactors and larger product recovery [73]. Some previous studies showed that the use of AHP led to satisfactory results in improving enzymatic digestibility at high solid loadings [73] but higher solid loadings could also present challenges during AHP pretreatment for effective mixing of H_2O_2 and base and for pH adjustment in a stirred tank reactor [38].

Although AHP allowed the pretreatment process to be operated at a milder temperature and pressure conditions, a high pH condition (11.5) was required to initiate an effective pretreatment. The high pH condition was needed to deprotonate the H_2O_2 effectively [74]. This will pose a safety risk to operator and potential environmental hazard in the event of improper handling. Besides, the corrosive nature of AHP pretreatment will impose the need to construct reaction vessel out of exotic material, thus, increasing capital cost. Neutralization should be carried out as a post pretreatment to ensure the biomass was suitable to be used for fermentation process. Consequently, the need for acid in neutralization will increase the chemical costs and safety risk for biorefineries. Moreover, the process of neutralization will generate high amount of salt between acid and AHP. It was found that salt such as NaCl could act as an inhibitor in the fermentation process by disrupting the osmotic pressure of cells and preventing a desired growth condition during the fermentation process. For example, the productions of acetone-butanol-ethanol from the alkaline peroxide wheat straw hydrolysate with and without the removal of salt were 22.17 g/L and less than 2.59 g/L, respectively [75]. In addition to the cost of chemical for neutralization and salt removal, the relatively higher cost of H_2O_2 posed an economic challenge for the industry. The chemical cost estimation for both alkaline peroxide and NaOH to process one metric ton of biomass was in the range of US$ 138 to 141 at a H_2O_2 loading of 0.125 g/g biomass [76]. Therefore, the utilization of AHP in an industrial scale would require an effective recycling process and significant yield of value-added products for achieving a feasible process [42]. Also, one drawback from utilizing an ambient operating condition for AHP could be the long residence time required for a successful pretreatment [24]. This could be resolved by implementing various strategies such as sequential or catalyzed pretreatment.

AHP presents a unique pretreatment method which promotes polysaccharide yield and lower production of inhibitors. Furthermore, the mild conditions required promote an inherently safer and greener process design through implementation of low operating temperature and ambient pressure. However, the drawback from operating at such mild conditions is the long residence time required for an effective pretreatment. Besides, the high cost of chemical acts as a major obstacle in the economic competitiveness of AHP. Therefore, a comprehensive technoeconomic and cost benefit analysis will need to be conducted to ensure the feasibility of the process.

5. Techno-economic analysis of AHP

A comprehensive techno–economic analysis (TEA) of AHP pretreatment is needed to provide a bigger picture on the feasibility of employing such technologies in a large scale production of biofuel. In the case study conducted by Stoklosa et al. [77], lignocellulosic biomass was first subjected to alkaline pre-extraction followed by AHP pretreatment. At the end of the pretreatment the biomass was filtered for further hydrolysis into bioethanol, while the liquid stream containing water, alkali and solubilized organic was separated for disposal. Through TEA, it was shown that the utilization of AHP pretreatment would require a high minimum ethanol selling price (MESP) of 2.73 US $/gal. The high MESP was mainly contributed by the large quantity of chemicals used during the pretreatment stages. Hence, justifying the need for an alkali recovery stream to reduce the operational cost for improving the economic viability of AHP pretreatment.

In the same TEA, the recovery stream scenario was scrutinized as well. This was achieved through the installation of recovery boiler, re–causticization plant, and multi–effect evaporator. Through the recovery and re–causticizing of Na_2CO_3 to regenerate NaOH, the MESP was successfully lowered to 2.27 US$/gal. While the MESP obtained after implementing recovery stream to reduce chemical cost was still found to be higher than ammonia fiber explosion, $2.09/gal (Table 4). The obtained MESP was found to be comparable to the MESP targeted by National Renewable Energy Laboratory (NREL), which was found to be at 2.15 US$/gal [78]. Furthermore, AHP pretreatment MESP and capital was among the lowest in comparison to most conventional pretreatment methods (Table 4).

While the utilization of recovery stream was able to reduce MESP, this process had its drawbacks. During the causticization process, solubilized sugars would be sent to the boiler instead of being fermented. This would greatly impact bioethanol yield, as removal of the liquid stream resulted in a loss of 26.5% of xylan and 9% of glucan. Furthermore, this setup had a much higher capital cost, at 17.21 million

Table 4
TEA summary of various pretreatment.

Type of pretreatment	CAPEX ($ × 10^6)	OPEX ($ × 10^6/yr)	MESP ($/gal)	Ref.
AHP	9.76	N/R	2.73	[77]
AHP with recycle stream	17.21	N/R	2.27	[77]
Organosolv	164.10	210.35	3.07	[79]
Ionic liquid	96.00	90.00	5.00	[80]
Ammonia fiber explosion	25.60	N/R	2.09	[81]
Dilute acid	114.63	50.06	3.18	[82]
Dilute alkali	102.77	52.70	3.35	[82]
Liquid hot water	101.89	48.10	3.07	[82]
Steam explosion	91.36	45.83	3.24	[82]

*N/R Not reported.

US$, and higher thermal requirement to fully operate the entire system [77]. Therefore, AHP pretreatment has been shown to be both a technologically and economically feasible process. In the case study provided above, the high cost of chemicals could be overcome through implementation of recycle stream. However, the recycle process could be further improved through separation of sugar to increase bio–ethanol yield.

6. Practical implication of this study

From various literature reviews, it is worth noting that, the application of AHP may not be suitable for all type of biomass. For instance, the effectiveness of AHP in enhancing enzymatic hydrolysis was stifled when cactus pear and gooseweed were pretreated with AHP pretreatment. A lot of past studies indicated that sequential AHP pretreatment had the capability to synergistically enhance both enzymatic hydrolysis as well as fermentation yield. The synergism between pretreatment strategies could potentially provide an opportunity to enhance further the production of biofuels at the later stage and effectively improve process-economic feasibility. In the technoeconomic analysis, it was found that the AHP pretreatment biorefineries would require a recycle stream to be economically sustainable. It is also highly recommended to invest and optimize technology that could separate sugar from the recycle stream for AHP pretreatment biorefineries to increase sugar yield. Therefore, future research on the applications of sequential pretreatment strategies, recyclability and process scale up using AHP are required to release its potential to the fullest as a cost effective pretreatment.

7. Conclusion

This review highlights the various applications of AHP, particularly for the purpose of lignocellulosic biomass processing to produce biofuels. The advancement of biomass processing technologies is often limited by cost, environmental impact, and toxicity. AHP pretreatment has shown promising potentials in the biomass valorization due to its compatibility with subsequent bioconversion, relatively safe decomposition products, and flexibility in adjusting process conditions to suit different scope of biomass fractionation. Sequential AHP pretreatment is found to be capable of extracting various bio–products such as silica and lignin. The benefit of utilizing AHP pretreatment under a mild operating condition is an inherently safer process, but the drawback is that a longer residence time is required to achieve satisfactory delignification of biomass. Lastly, the installation of recycle stream to recover spent chemical is necessary to reduce the cost of production of bio-ethanol. Therefore, more in-depth studies on the feasibility of sequential pretreatment strategies, recyclability study and process scale up using AHP are required to contribute towards a new chapter in biorefinery using lignocellulosic biomass as a sustainable feedstock.

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Appendix

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Appendix B

An application of low concentration alkaline hydrogen peroxide at non-severe pretreatment conditions together with deep eutectic solvent to improve delignification of oil palm fronds (Original Published Paper)

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ORIGINAL RESEARCH

An application of low concentration alkaline hydrogen peroxide at non-severe pretreatment conditions together with deep eutectic solvent to improve delignification of oil palm fronds

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Abstract Cost-effective pretreatments are essential to overcome the recalcitrant properties of lignocellulosic biomass in order to reutilize cellulosic components within the biomass for value-added products. In this study, a sequential pretreatment of low concentration (< 1.00 vol%) alkaline hydrogen peroxide (AHP) at ambient temperature (~ 25 °C) and pressure (1 atm.) with Type III deep eutectic solvent (DES) was used to fractionate oil palm fronds (OPF). A combination of AHP (0.25 vol%, 90 min) and DES (120 °C, 4 h) at the later stage resulted in a pretreated OPF with a delignification of 18.99%, remarkably improved DES-alone delignification extent by 1.6 folds. The characterizations of pretreated OPF confirmed that mild conditions AHP + DES pretreatment could synergically improve the delignification efficiency. Thus, the use of this sequential pretreatment enabled the lignin extraction and effectively disrupted the recalcitrant structure of OPF, yielding a potential feedstock for a biorefinery process at the later stage.

Keywords Biomass valorization · Biorefinery · Green solvent · Lignocellulosic biomass · Waste management

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Introduction

Over the past decades, rapid development of countries and the continuous growth of population over the globe has caused a significant increase in the energy consumptions, which have resulted in grave problem of depleting reserves of non-renewable energy. According to International Energy Agency, transportation dominates oil consumption over the globe, where it contributes more than 70% of greenhouse gases to the environment. Due to the increasing awareness of sustainable development, it has drawn scientists' attention to find an alternative energy source to replace the use of non-renewable energy.

One of the promising energy sources is by converting biomass into biofuel. Biofuel is relatively environmental friendly bioenergy as compared to fossil fuel as the former is biodegradable, non-toxic, and low emission profile (Mahapatra and Manian 2017). Currently, Malaysia is regarded as one of the largest exporters of crude palm oil in the world. This industry yield more than 90% of the country's total lignocellulosic biomass, which is derived from 5.4 million hectares of oil palms in the country (Loow et al. 2017b). Among the oil palm biomass, approximately 26.2 million tonnes per annum of oil palm fronds are produced per million of fresh fruit bunch processed (Yunus et al. 2010), making it the promising source for biofuel productions. Despite the abundance of oil palm fronds, the fronds are commonly used as mulches by applying them to the soil surface around the oil palm trees (Loow and Wu 2018). However, this typical approach offers limited economical values to the plantation, while the improper handling of this biomass may cause grave environmental problems (Isikgor and Becer 2015).

Owing to the availability of lignocellulosic biomass, the production of biofuel or chemical building block from this biomass could be a solution towards sustainable development, especially in decarbonization of transport industry (Martínez-Patiño et al. 2017). Lignocellulosic biomass consists of three main components, namely cellulose (40–50%), hemicellulose (15–25%) and lignin (20–25%) (Loow et al. 2015). Cellulose and hemicellulose in the biomass make up the polysaccharides available for sugar hydrolysis and fermentation to convert into high-valued products. Lignin is an amorphous phenolic polymer with high heterogeneity through coupling of 4-hydrophenylpropanoids (Loow et al. 2016a). Due to its highly branched and random structure, the recalcitrant lignin wall poses a formidable barrier to digestion via enzymatic routes to prohibit cellulose and hemicellulose from rapid degradation into fermentable sugars (Foyle et al. 2007). Hence, the fractionation of biomass into its main components (cellulose, hemicellulose, and lignin) could favor the productions of value-added products on top of carbon–neutral biofuel productions to enhance the biofuel competitiveness (González-García et al. 2016; Kim et al. 2016).

Delignification—a pretreatment strategy aimed to fractionate lignin in the biorefinery process has been attracting attention recently. The effectiveness of delignification is associated to achieve higher cellulose accessibility, which is then impacting on the process yield. By utilizing a suitable pretreatment step, a more energy-efficient and simpler downstream process can be achieved (Loow et al. 2016b). Alkaline pretreatment is one of the most used approaches to recover lignin from the biomass without severe cellulose degradation (Loow et al. 2016a). On the other hand, hydrogen peroxide (H_2O_2) was known to enhance wastewater treatment efficiency (Subramonian and Wu 2014; Subramonian et al. 2017). Recently, H_2O_2 was used to assist biomass processing in an acidic condition (Loow and Wu 2018; Loow et al. 2017b). However, the use of H_2O_2 in an alkaline medium has proven to be a more effective approach to improve lignin depolymerization through radical oxidation action (Ho et al. 2019). For example, the addition of H_2O_2 during alkaline pretreatment of rice husk increased overall sugar recoveries by 75% while no inhibitors such as furfural were found in the hydrolysate (Díaz et al. 2014; Su et al. 2015). Lately, a new green solvent, namely deep eutectic solvent (DES), has received much focus in the biomass valorization. It was first introduced by Abbott et al. (2004), in which a liquid eutectic mixture was formed through combining two solids comprising a quaternary ammonium salt and a hydrogen-bond donor (HBD). DESs possess similar physiochemical properties with conventional ionic liquids. However, the former has better biodegradability and enzyme compatibility, while possessing lesser toxicity and lower costs (Loow et al. 2018). To date, DESs have been increasingly involved in studies related to biomass processing due to its ability to extract bioactive phenolic compounds, delignification and/or sugar recovery, furanic conversion, and biodiesel processing (Lee et al. 2019; Loow et al. 2017a). According to Kumar et al. (2016), the use of DESs in biomass processing enabled the depolymerization of lignin without hydrolyzing the cellulose and hemicellulose, which enhanced the enzymatic hydrolysis with insignificant inhibitor formation.

Although alkaline hydrogen peroxide (AHP) has been widely studied for biomass pretreatment, the literature regarding the application of AHP under mild conditions in sequential pretreatment strategies is still very limited. Also, the applications of DESs in biomass processing still require further exploration.

To the best of our knowledge, no investigation has been carried out to study the sequential pretreatment of low concentration AHP at mild conditions (ambient temperature and pressure) and DES in pretreating oil palm fronds. It was hypothesized that the introduction of mild concentration (< 1.00 vol%) in AHP at ambient conditions could help improve macrostructure alteration and partial lignin solubilization of oil palm fronds. Therefore, DES was facilitated in accessing the biomass matrix and providing better disruption on the hydrogen-bonding network, which led to a better delignification. Thus, the objective of this study was to evaluate the synergistic effects of low concentration AHP at mild conditions together with DES in oil palm fronds delignification pretreatment.

Materials and methods

Raw material and chemicals

Fresh oil palm fronds were provided by oil palm plantation owned by Universiti Kebangsaan Malaysia. The leaflets were removed and the petiole was pressed in a fronds pressing machine to separate juice from the oil palm fronds. Pressed oil palm fronds were sun-dried for two days before grinding to a smaller size using pulverizer (8000 rpm). After that, grinded oil palm fronds were sieved through a mechanical sieve to recover biomass at a size $\leq$ 0.5 mm. Sieved oil palm fronds were washed with distilled water and dried in an oven at 60 °C for 48 h. The prepared oil palm fronds were termed as OPF throughout this study and stored in a container filled with desiccants at room temperature prior to pretreatments. National Renewable Energy Laboratory (NREL) standard laboratory analytical procedure was adopted and carried out to determine OPF composition in terms of structural carbohydrates and lignin (Sluiter et al. 2008). D(+)glucose, D(−)xylose, and L(+)arabinose were used as standard solutions to calibrate standard curves for composition analysis through high performance liquid chromatography (HPLC). Sodium hydroxide (AR grade, 99%, pellets), hydrogen peroxide (extra pure, 50%), choline chloride (99%), urea (AR grade, 99%) and other chemicals utilized in this study were ensured to be of analytical grades.

Sequential pretreatment

Two-stage sequential pretreatments, namely AHP as the first stage while Type III DES as the second stage, were used in a pretreatment of OPF.

AHP as a first stage pretreatment

In this first pretreatment, 4.5 g of OPF sample was measured and placed into a 600 mL beaker. The AHP solution was prepared at a range of 0.05–1.00 vol% adjusted to pH 11.5 using NaOH pellets. The synthesized solution was then charged into the beaker containing OPF sample at a fixed solid to liquid ratio of 1:20 (w/v). The OPF was then soaked in AHP solution at room temperature for different durations from 30 to 120 min. After undergoing the AHP pretreatment, the resulting AHP-pretreated biomass sample was filtered from the liquid fractions. The pretreated sample was washed with distilled water to remove the residual AHP and then dried at 60 °C in the oven overnight. Later, the dried sample was collected and stored in a container filled with desiccants prior to subsequent uses.

DES pretreatment as a second stage pretreatment

The second stage pretreatment was performed on the pretreated OPF from the first AHP pretreatment stage. 2.5 g of pretreated OPF from AHP was measured and transferred into 50 mL Schott bottle. ChCl:Urea at a molar ratio of 1:2 was used as a Type III DES in this study. DES was prepared (Loow et al. 2018), and added to the Schott bottle at a fixed solid to liquid ratio of 1:10 (w/v). The pretreatment process was performed in an oil bath, maintaining temperature at 120 °C for a duration of 4 h without introducing stirring. The pretreatment conditions (120 °C, 4 h) were adapted from Loow et al. (2018) for optimum sugar recovery of OPF. Upon the completion of the pretreatment, the Schott bottle was removed from the oil bath and rinsed with running tap water to cool down the content and quench reaction. The resulting AHP + DES-pretreated biomass sample was filtered and washed with distilled water to remove excessive DES. Then, the solid fraction was left to dry in an oven overnight at 60 °C. Finally, the dried AHP + DES-pretreated biomass sample was stored in a

container filled with desiccants before the subsequent analysis.

Inorganic salt hydrolysis

Inorganic salt hydrolysis was carried out to investigate the hemicellulose recoveries of raw and pretreated biomass. Firstly, 1.0 g of OPF sample was transferred into 50 mL Schott bottle. Inorganic salt solution ($CuCl_2$) was prepared at a concentration of 0.4 mol/L (Loow et al. 2017b), and added to the OPF sample at a solid to liquid ratio of 1:10 (w/v). The Schott bottle containing the reaction mixture was then sent to autoclave (240 V, Hirayama HV-85, Japan) at 120 °C for a duration of 30 min. Thereafter, the mixture in the Schott bottle was cooled to a room temperature to quench reaction before extracting the liquid fraction. The extracted hydrolysate was centrifuged at a speed of 13,500 rpm for 10 min using a Mini 1312M Micro Centrifuge. The centrifuged hydrolysate was filtered using a 0.22 µm syringe filter prior to monomeric sugar analysis.

Analytical methods

Lignin quantification

National Renewable Energy Laboratory (NREL) standard laboratory analytical procedure for lignin composition was adopted to quantify lignin in the OPF sample (Sluiter et al. 2008). Acid hydrolysis was done by adding 3 mL of 72% of H_2SO_4 and 0.3 g of dried sample into a 250 mL conical flask. After that, the mixture was incubated at 30 °C and 150 rpm for an hour. Then, 84 mL of deionized water was added into the incubated mixture and sent for autoclave at 121 °C for an hour. The reacted mixture was transferred for vacuum filtration where the filtrate was sent for acid soluble lignin test by using UV–Vis spectrophotometer at 320 nm in which the deionized water was used as a blank solution. On the other hand, acid insoluble lignin was determined by measuring the difference in dry weight of the filter paper before and after vacuum filtration. Delignification is defined as the percentage of lignin removed in the samples (New et al. 2019), whereby:

$$Delignification(\%) = \frac{L_0 - LY}{L_0} \times 100\%$$

where L_0 = Lignin content in raw OPF, $\left(\frac{\text{mass of lignin in raw OPF}}{\text{mass of raw OPF}}\right)$, L = Lignin content in pretreated OPF, $\left(\frac{\text{mass of lignin in pretreated OPF}}{\text{mass of pretreated OPF}}\right)$, Y = Mass yield or mass recovery of pretreatment in fraction, $\left(\frac{\text{mass of pretreated OPF}}{\text{mass of raw OPF}}\right)$

Fourier transform infrared spectroscopy (FTIR)

Attenuated total reflection (ATR) tip FTIR (Thermoscientific Nicolet iS10 spectrometer) was used to analyze the chemical structures of the raw and pretreated OPF samples. The sample spectra were obtained via 64 scans over the range of 500 to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1}.

Field emission scanning electron microscope (FE-SEM)

The raw and pretreated OPF samples were firstly adhered to a specimen stub using double-coated tape and sputter coated with platinum prior to imaging using Hitachi SU 8010 in order to visualize the microstructural alteration in biomass surface morphology.

X-ray diffraction (XRD)

A Bruker D8 Discover diffractometer was used to determine the crystallinity index (CrI) of raw and pretreated OPF samples with a 2θ ranged from 5° to 51° in steps of 0.041°. The crystallinity index (CrI) is defined by Segal et al. (1959) as the relative degree of crystallinity in the specimen, whereby:

$$CrI(\%) = \frac{(I_{200} - I_{am})}{I_{200}} \times 100\%$$

where I_{200} is the maximum intensity of the 200 peak (2θ = 22°) and I_{am} is the intensity minimum between peaks at 200 and 110 (2θ = 18°).

High performance liquid chromatography (HPLC)

Agilent series 1200 infinity HPLC system equipped with a Refractive Index detector and BioRad Aminex HPX-87H column was used to analyze the concentrations of monomeric sugars in the hydrolysate obtained

from inorganic salt sugar recoveries and acid hydrolysis compositional analysis for the dried OPF sample. The mobile phase for the application of HPX-87H column was 0.005 mol/L H_2SO_4 at an operating flow rate of 0.6 mL/min and the column temperature was controlled at 65 °C. The concentration of monomeric sugars was presented in g/L, while the sugar recoveries (%) were derived by adapting formula from Loow et al. (2018):

$$Sugar\ recovery(\%) = \frac{sugar\ recovered(g/L) \times volume\ of\ solvent\ used\ (L)}{Sample\ carbohydrate\ composition \times mass\ of\ sample\ used\ (g)} \times 100\%$$

Brunauer–Emmett–Teller (BET)

BET analysis was carried out with N_2 adsorbate at − 195.85 °C by using a Micromeritics ASAP 2020 BET model. Degasing was performed prior to analysis with conditions adapted from the literatures (Lim and Wu 2015, 2016), where samples were initially heated to 90 °C for 2 h, and subsequently holding at 110 °C for 22 h.

Thermogravimetric analysis (TGA)

The thermogravimetric analysis was conducted with a Mettler Toledo Thermo Gravimetric Analyzer in nitrogen environment, adapting the methodology from Marcotullio et al. (2011). The raw and pretreated OPFs were placed in a ceramic sample holder, thereafter the temperature was raised to 50 °C at a ramping rate of 10 °C/min and kept constant for 45 min. After that, the temperature was risen to 120 °C at ramping rate of 10 °C/min and held isothermal for 15 min to dry samples thoroughly before increasing the temperature to 550 °C at 10 °C/min. The furnace was maintained isothermal for 40 min, in which the residue was regarded as char after the thermogravimetric analysis.

Statistical analysis

One-way analysis of variance (ANOVA) was carried out using IBM SPSS Statistics 24 to statistically process the experimental data for identifying any occurrence of significant variations between pretreatment conditions. This analysis helped in determining the optimum reaction condition of AHP to induce the most synergic effect with DES in delignification of OPF. The homogeneous subset for experimental data based on a range of parameters were identified using Duncan's test with a significance value of $P < 0.05$.

Results and discussion

Effect of AHP and DES on delignification

The chemical compositions of OPF by dry weight were comprised of glucan (48.23% ± 0.03%), xylan (21.86% ± 0.01%), arabinan (1.09% ± 0.06%), lignin (18.84% ± 0.09%), and the remaining weight of 9.98% were ash as well as water and ethanol extractives. From the previous study by Loow et al. (2018), the recommended operating conditions for DES (ChCl:Urea) pretreatment of OPF for maximum sugar recoveries were 120 °C and 4 h. When the raw OPF was pretreated in a DES pretreatment, the lignin content was reduced to 18.14% from 18.84% (Tables 1 and 2). It was hypothesized in this study that the incorporation of AHP pretreatments, though in very mild conditions, with DES pretreatment (ChCl:Urea at 120 °C for 4 h) would enhance further the delignification of OPF. In this study, pretreatment of OPF using DES alone was considered as a 'control' in the experiment.

Effect of AHP concentration in sequential pretreatment

The results were interpreted from two different perspectives, namely lignin content reduction and overall delignification of OPF. According to one-way ANOVA, the lignin content after the pretreatment of 0.50 vol% AHP was significantly reduced as compared to the raw OPF. Interestingly, it was found that

Table 1 Delignification of OPF using AHP + DES sequential pretreatment by varying AHP concentration

Pretreatment conditions	Mass yield (%)	Lignin (%)	Delignification (%)
Raw OPF	–	18.84 ± 0.09^{a}	–
AHP duration: 90 min			
AHP (0.05 vol%)	99.63	$18.43 \pm 0.05^{b,c}$	2.54 ± 0.35^{A}
AHP (0.10 vol%)	99.77	$18.35 \pm 0.07^{c,d}$	2.83 ± 0.04^{A}
AHP (0.25 vol%)	99.00	18.26 ± 0.04^{d}	4.05 ± 0.05^{B}
AHP (0.50 vol%)	98.97	17.82 ± 0.09^{e}	6.38 ± 0.14^{C}
AHP (1.00 vol%)	97.15	16.92 ± 0.06^{f}	12.75 ± 0.05^{D}
DES (ChCl:Urea; 120 °C; 4 h)			
DES	91.22	$18.14 \pm 0.12^{d,g}$	12.16 ± 0.16^{E}
AHP (0.05 vol%) + DES	91.46	17.22 ± 0.05^{g}	14.23 ± 0.13^{F}
AHP (0.10 vol%) + DES	91.46	17.09 ± 0.03^{h}	14.62 ± 0.20^{G}
AHP (0.25 vol%) + DES	93.92	16.25 ± 0.05^{i}	18.99 ± 0.04^{H}
AHP (0.50 vol%) + DES	95.33	$17.15 \pm 0.03^{g,h}$	13.22 ± 0.23^{I}
AHP (1.00 vol%) + DES	93.54	16.63 ± 0.03^{j}	17.44 ± 0.21^{J}

Values annotated with different letters represents different significance levels (one-way ANOVA, Duncan's test; $P < 0.05$)

Table 2 Delignification of OPF using AHP + DES sequential pretreatment by varying AHP reaction duration

Pretreatment conditions	Mass yield (%)	Lignin (%)	Delignification (%)
Raw OPF	–	18.84 ± 0.09^{a}	–
AHP concentration: 0.25 vol%			
AHP (30 min)	99.01	18.62 ± 0.08^{b}	2.14 ± 0.10^{A}
AHP (45 min)	98.23	18.53 ± 0.03^{b}	3.38 ± 0.23^{B}
AHP (60 min)	99.15	18.34 ± 0.08^{c}	3.48 ± 0.07^{B}
AHP (90 min)	99.00	$18.26 \pm 0.04^{c,d}$	4.05 ± 0.05^{C}
AHP (120 min)	99.23	18.17 ± 0.03^{d}	4.29 ± 0.26^{C}
DES (ChCl:Urea; 120 °C; 4 h)			
DES	91.22	18.14 ± 0.12^{d}	12.16 ± 0.16^{D}
AHP (30 min) + DES	93.47	17.54 ± 0.06^{e}	12.98 ± 0.09^{E}
AHP (45 min) + DES	93.13	17.25 ± 0.05^{f}	14.73 ± 0.14^{F}
AHP (60 min) + DES	92.41	16.86 ± 0.06^{g}	17.31 ± 0.07^{G}
AHP (90 min) + DES	93.92	16.25 ± 0.05^{h}	18.99 ± 0.04^{H}
AHP (120 min) + DES	93.80	16.23 ± 0.13^{h}	19.20 ± 0.19^{H}

Values annotated with different letters represents different significance levels (one-way ANOVA, Duncan's test; $P < 0.05$)

the AHP pretreatment alone was effective against OPF, despite under a very mild concentration at room temperature. For example, at 1.00 vol% single staged AHP, delignification achieved was 12.75%, which was comparable to the delignification obtained in the control (12.16%). The efficiency of lignin removals showed increasing trends with increases in AHP concentrations, which were consistent with the previous study (da Costa Correia et al. 2013). The delignification could be improved from 2.54 to 12.75%, when the concentration of AHP increased from 0.05 to 1.00 vol% at room temperature. However, higher AHP concentration could lead to several problems in terms of higher H_2O_2 and NaOH consumptions. Also, safety and environmental issues might arise when the process was scaled up. Therefore, the recommended operating condition was not selected based solely on the delignification performance of AHP but overall delignification efficiency of sequential pretreatment and the synergistic effect between both pretreatments. This could potentially reduce the AHP concentration further.

For a fixed DES pretreatment together with AHP pretreatment at various concentrations, the DES stage-wise delignification were improved from 0.05 up to 0.25 vol% AHP. However, further increase in AHP

concentration (> 0.25 vol%) had adverse effect in the DES stage-wise delignification. The lignin content reduced from 18.26 to 16.25% (2.01% in reduction) when DES pretreatment was carried out using 0.25 vol% AHP-pretreated OPF (Table 1). On top of that, the combination of 0.25 vol % AHP and DES pretreatment resulted in delignification of 18.99%, which was approximately 1.56 times higher than the delignification of DES pretreatment alone. The decreasing DES delignification after AHP pretreatment (> 0.25 vol%) could be due to the competition in targets components during the delignification of both pretreatment mechanism or excessive alteration of biomass in the AHP pretreatment, which could hinder the delignification performance of DES pretreatment when higher concentration AHP was used (Gould 1984; Loow et al. 2018). According to Mittal et al. (2017), excessive AHP pretreatment could cause reduction in accessibility of biomass due to the collapse of localized cell wall, which effectively led to a poorer sugar conversion. From the experimental results (Table 1), OPF underwent sequential pretreatment of 0.25 vol% AHP + DES was able to achieve the highest delignification (18.99%), which was higher than the control (12.16%) and the other combination of different AHP concentrations coped with DES, suggesting that the best synergistic effect induced. This combination was able to achieve significant synergistic effect and maximum delignification while reducing the severity of AHP pretreatment concentration from 1.00 to 0.25 vol%. Therefore, the optimum concentration of AHP at 0.25 vol% was used to investigate the subsequent study on the effect of AHP reaction duration on the overall delignification of OPF.

Effect of AHP reaction duration in sequential pretreatment

This study was extended to investigate the effect of AHP reaction duration on both lignin content reduction and delignification (Table 2). In the first stage, the extent of delignification of AHP increased with prolonged duration of pretreatments. One-way ANOVA suggested that the improvement of delignification did not have significant differences when the reaction duration was increased from 90 to 120 min, indicating that the pretreatment had reached its plateau state. The result could imply that the oxidation reaction had reached its completion and any further increase in reaction duration would only led to a little or negligible effect on the extent of OPF delignification. Under a constant AHP concentration (0.25 vol%), the lignin content could be reduced to 18.17% and achieved delignification of 4.29% for a pretreatment duration of 120 min, despite under a significantly less extreme pretreatment conditions (Table 2).

Delignification of DES pretreatment on each of the AHP-pretreated OPF at different reaction duration ranging from 30 to 120 min showed a similar trend as compared to the AHP pretreatment alone (Table 2), whereby the delignification increased as the AHP pretreatment duration increased. For example, the delignification of AHP + DES-pretreated OPF increased from 12.98 to 19.20% as the pretreatment duration for AHP increased from 30 to 120 min. More alterations on OPF biomass were expected when the pretreatment duration increased, which led to the improvement of sequential pretreatment delignification synergically. According to one-way ANOVA, the improvement on the extent of delignification reached its maximum at 90 min duration (18.99%), where further increase in duration had little impact on the pretreatment process. Plausible explanation of such phenomenon was due to the further increase in AHP pretreatment duration (> 90 min) alone had little effect on the biomass delignification and structural alteration, thus no significant differences in delignification of DES in the second stage as compared to AHP (90 min) + DES pretreatment. Among all the pretreatment combinations, AHP concentration of 0.25 vol% at 90 min was found to be the most recommended conditions for the best delignification of OPF.

Compositional analysis and sugar recoveries from raw and pretreated OPF

Generally, pretreatments specifically target lignin will result in hemicellulose (xylan, arabinan) and cellulose (glucan) enrichment. It was expected that the glucan percentage of pretreated OPF increased as compared to the raw glucan content (Table 3). For example, OPF underwent AHP + DES pretreatment resulted in an increase in glucan content from 48.23 (raw) to 54.40%, which was higher than the control pretreatment (50.31%) and AHP (48.95%). Furthermore, the glucan enrichment from raw OPF in the sequential pretreatment was greater than the sum of either of the two stages alone, confirming the existence of

Table 3 Compositional analysis of OPF using AHP (0.25 vol %, 90 min) + DES (120 °C, 4 h) sequential pretreatment

Pretreatment conditions	Compositional analysis (%)			
	Lignin	Xylan	Arabinan	Glucan
Raw	18.84 ± 0.09^{A}	21.86 ± 0.01^{A}	1.09 ± 0.06^{a}	48.23 ± 0.03^{a}
DES	$18.14 \pm 0.12^{B,C}$	22.13 ± 0.09^{B}	0.59 ± 0.02^{b}	50.31 ± 0.15^{a}
AHP	18.26 ± 0.04^{C}	21.67 ± 0.01^{C}	$0.53 \pm 0.02^{b,c}$	48.95 ± 0.31^{a}
AHP + DES	16.25 ± 0.05^{D}	22.13 ± 0.07^{B}	0.46 ± 0.05^{c}	54.40 ± 1.81^{b}

Values annotated with different letters represents different significance levels (one-way ANOVA, Duncan's test; $P < 0.05$)

synergistic effect. It was interesting to note that, despite under mild AHP pretreatment conditions, glucan content of AHP pretreated OPF showed a slight increase to 48.95%. However, the xylan content decreased (to 21.67%) as compared to raw OPF (21.86%), which could be due to the hemicellulose solubilization capability of AHP pretreatment (Li et al. 2013; Morone et al. 2017; Toquero and Bolado 2014). From this study, it was revealed that the AHP pretreatment could cause some hemicellulose removals, despite under mild conditions.

An attempt to recover monomeric sugars using the inorganic salt pretreatment on the OPF samples was shown in Table 4. The monomeric sugar recoveries (glucose and arabinose) were in a relatively smaller quantity because the $CuCl_2$ inorganic salt hydrolysis was specialized in xylan hydrolysis (Loow et al. 2015, 2018). One-way ANOVA also confirmed that the amount of glucose and arabinose produced did not have significance difference across different pretreatment conditions. As for xylose recoveries, the xylose concentration recovered from raw and DES-pretreated OPF was deviated from the values reported by Loow et al. (2018), which could be due to the difference in the maturity of palm trees (Tan et al. 2016). The xylose concentration in the hydrolysate obtained from AHP-pretreated OPF was 7.54 g/L, which was lower than the xylose recovered from the raw OPF (8.90 g/L). This result was expected as the AHP pretreatment of OPF resulted in some xylan removal (Table 3), thus affecting the xylose recovery (Table 4). Interestingly, the xylose concentrations of AHP + DES-pretreated OPF differed from the DES-pretreated OPF, which were 12.26 and 11.38 g/L respectively, even though both samples were similar in terms of xylan content (Table 3). This could be due to the difference in carbohydrate accessibility (Loow et al. 2017b), which implied that the AHP + DES-pretreated OPF was more accessible as compared to the DES-pretreated OPF duirng the inorganic salt hydrolysis. Thus, a higher xylose recoveries of 55.40% could be achieved for AHP + DES-pretreated OPF as compared to the DES-pretreated OPF (51.42%) and raw OPF (40.71%).

Characterization studies

FE-SEM

FE-SEM was used to study the morphological changes of OPF after undergoing various pretreatment process (Fig. 1). The raw OPF exhibited a smooth and well-

Table 4 Sugar recoveries of OPF using AHP (0.25 vol%, 90 min) + DES (120 °C, 4 h) sequential pretreatment

Pretreatment conditions	Sugar concentration (g/L)			Sugar recoveries (%)		
	Xylose	Arabinose	Glucose	Xylose	Arabinose	Glucose
Raw	8.90 ± 0.01^{a}	0.88 ± 0.02^{A}	0.32 ± 0.03^{a}	40.71	80.73	0.66
DES	11.38 ± 0.02^{b}	$0.55 \pm 0.06^{A,B}$	$0.28 \pm 0.01^{a,b}$	51.42	93.22	0.56
AHP	7.54 ± 0.15^{c}	0.47 ± 0.05^{B}	0.22 ± 0.03^{b}	34.79	88.68	0.45
AHP + DES	12.26 ± 0.05^{d}	0.40 ± 0.23^{B}	$0.26 \pm 0.07^{a,b}$	55.40	86.96	0.48

Values annotated with different letters represents different significance levels (one-way ANOVA, Duncan's test; $P < 0.05$)

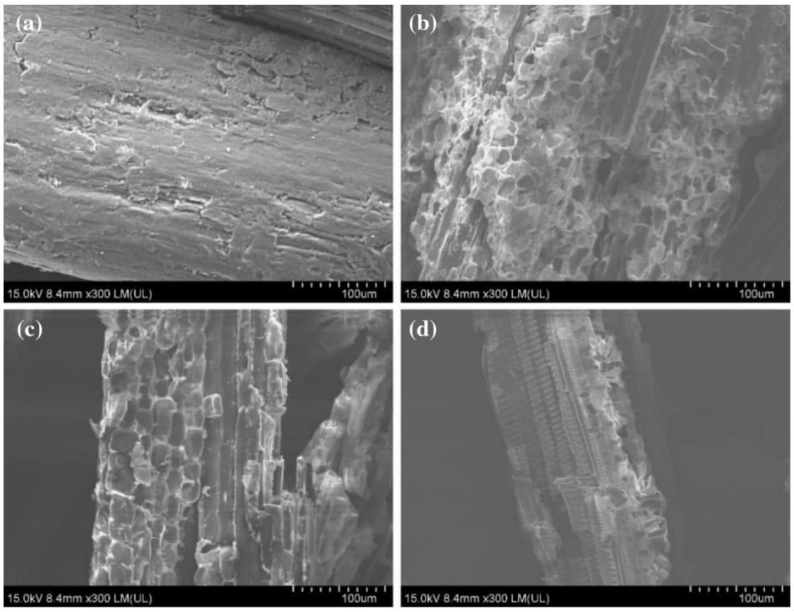

Fig. 1 FE-SEM images of **a** raw OPF, **b** DES-pretreated OPF, **c** AHP-pretreated OPF, and **d** AHP + DES-pretreated OPF at × 300 magnification

structured fiber with enclosed surface (Fig. 1a). The homogeneous and continuous fibrils structures resulted in a low degree of porosity. Therefore, the penetration of chemicals to internal hemicellulose and cellulose was discouraged, leading to a low sugar recovery. The low degree of porosity of OPF restricted the penetration of DES, which resulted in rough and disordered surface, but the overall surface of OPF was still considered intact and enclosed, suggesting that the DES pretreatment had minimal effect on cellulose and hemicellulose. After undergoing AHP pretreatment alone at a concentration of 0.25 vol% and duration of 90 min, the overall surface was relatively smoother as compared to the control pretreatment, which concurred with the lignin quantification analysis as slight reduction of lignin content was obtained during the process (Table 3). On top of that, numerous of tiny dots were observed on the outer layer of AHP-pretreated OPF (Fig. 1c), thus improving the accessibility and porosity of OPF to the internal layers. A similar observation was reported by Li et al. (2016b), whereby tiny holes were exhibited on Jerusalem artichoke after undergoing alkaline peroxide pretreatment. Hence, even with a majority of lignin components retained in the OPF after the AHP pretreatment (Table 3), the increased porosity enhanced the subsequent DES penetration to the internal hydrogen bonding network. A drastic rupture of structure was observed in the OPF after undergoing the synergistic combination of both AHP and DES pretreatments (Fig. 1d), exposing highly ordered crystalline straps. The effects of AHP in enhancing the porosity, lignin and partial hemicellulose disruption led to a better penetration and delignification during the DES pretreatment in a second stage, which eventually destructing the OPF structure more thoroughly.

XRD

Intensity of crystalline and amorphous regions were taken at 22° and 18°, respectively from diffractogram using XRD (Ong et al. 2019). The CrI of raw OPF was obtained to be 37.94% and increased to 43.26% after control pretreatment due to the ability to remove amorphous lignin (Loow et al. 2018). As for AHP pretreatment, the CrI achieved (43.62%) was similar to the control. It was an interesting observation that

AHP under room temperature and mild concentration of 0.25 vol% could cause such a significant increase in CrI, as AHP at room temperature had lower capability to solubilize hemicellulose and expose phenolic ring in lignin (Díaz et al. 2014; Maziero et al. 2012). Thus, it was deduced that the increase in CrI as a result of AHP pretreatment was mainly attributed by its ability to enhance porosity and accessibility to crystalline region at the same time partially remove lignin and hemicellulose (Li et al. 2016b). This claim was validated by the increase in crystalline peak (Fig. 2). After undergoing AHP + DES pretreatment, the CrI increased to 48.94%. It was observed from the XRD spectra of AHP + DES-pretreated OPF, the amorphous peak reduced owing to the significant amount of amorphous component removed, leading to significant increase in CrI. Interestingly, the crystalline peak intensity experienced slight reduction after AHP + DES pretreatment (Fig. 2). According to Vigier et al. (2015), DES was able to cause reduction in cellulose crystallinity when it was used to pretreat biomass. Besides, this observations also concurred with the findings from Kumar et al. (2016), where they claimed that the partial disruption of amorphous cellulose caused the CrI of cellulose to decrease. The increase in porosity of OPF after the AHP pretreatment allowed the DES to access the pretreated OPF more effectively, resulting in significant delignification and slight decrystallization of cellulose. As such, the combined sequential pretreatment achieved the best results due to the ability to removal of lignin. Thereby, both pretreatments exposed crystalline cellulose, which would potentially enhance the enzymatic accessibility for glycosidic bond cleavage (Li et al. 2013).

FTIR

FTIR was used to determine the changes of chemical functional groups on OPFs after undergoing pretreatment. Consistent peaks at 900 cm^{-1} and 2900 cm^{-1}, representing β-glycosidic linkages and –OH groups in the cellulose respectively, were observed (Fig. 3). This observation implied that the cellulose in the OPF was not significantly altered by the pretreatments. Previous studies reported that ChCl served to stabilize cellulose system (Loow et al. 2018), while AHP only selectively targeted lignin and hemicellulose (Mittal et al. 2017). Thus, the dissolution of cellulose was unlikely. The most significant differences were observed for the peaks at 1031 cm^{-1} (Fig. 3), indicating the presence of C–O stretching vibration in cellulose, hemicellulose, and lignin (Ofori-Boateng and Lee 2014). When comparing with raw OPF, the obvious peak reduction at 1031 cm^{-1} shown in AHP + DES-pretreated OPF implied the significant removal of lignin from the OPF. On top of that, peaks representing aryl–alkyl ether bonds (C–O–C) at

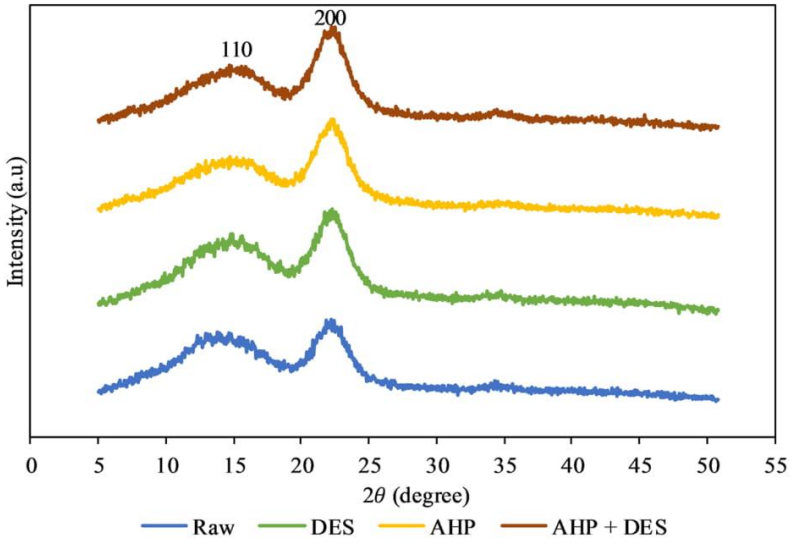

Fig. 2 XRD spectrum of raw and pretreated OPFs

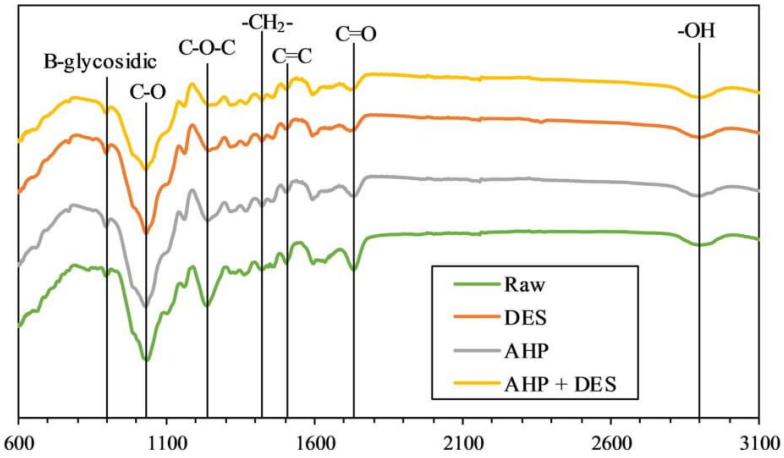

Fig. 3 FTIR spectrum of raw and pretreated OPFs

1235 cm^{-1} experienced significant reduction after the pretreatment (Fig. 3). A more drastic change was observed in OPF after AHP pretreatment as compared to the raw OPF, indicating the capability of AHP and DES in cleaving aryl–alkyl ether bond, also known as β–O–4 linkage. Furthermore, the peaks ranged from 1508 to 1600 cm^{-1}, represented by aromatic skeletal C=C from lignin aromatic ring (Loow et al. 2018), experienced slight reductions especially for the OPF which was undergone DES pretreatment. This observation was due to the ability of Cl^- ions from the DES to form hydrogen bonding with aromatic ring in lignin, causing dissolution of lignin polymer. Besides, the results also suggested that the delignification of AHP pretreatment at mild concentration mainly attributed by the cleavage of β–O–4 linkage and only minimal effect on the phenolic ring. Moreover, a notable decrease in peak at 1735 cm^{-1} after undergoing AHP pretreatment (Fig. 3), denoted the ability of AHP to breakdown C=O acetyl group in the hemicellulose.

BET

From the BET analysis, the specific surface area of raw and pretreated OPFs could be obtained. According to Loow et al. (2018), the delignification of OPF could increase the surface area of OPF, leading to an increase in accessibility. The initial specific surface area of raw OPF was 0.4096 m^2/g. After the control pretreatment using DES alone, the area increased slightly to 0.4310 m^2/g. Furthermore, when the raw OPF was pretreated using 0.25 vol% AHP under ambient conditions for 90 min, the BET surface area increased to 0.4249 m^2/g. Interestingly, the surface area of AHP-pretreated OPF was only slightly lower in comparison with control pretreatment. This result was unexpected as the degree of delignification (Table 3) was anticipated to increase the surface area significantly. Based on the FE-SEM image of AHP-only pretreatment, although the surface was relatively smoother as compared to the control pretreatment, there was notable amount of tiny dots. This observation indicated that the internal structure (cellulose) was readily accessible. Thus, the ability of AHP in creating tiny holes during biomass structure alteration, was likely to cause the increase in surface area, despite low delignification achieved (Table 3). The OPF pretreated with both AHP and DES achieved the highest surface area of 0.5117 m^2/g, which was 18.72% increase in the surface area in comparison with raw OPF. The increase in surface area of AHP + DES-pretreated OPF was significantly higher than the DES-pretreated OPF and AHP-pretreated OPF. Thus, the BET analysis validated the presence of synergistic effects when AHP was integrated with DES in OPF pretreatment.

TGA

TGA and derivative thermogravimetric (DTG) spectra were obtained for raw and pretreated OPFs. The TGA spectrum showed a slight decrease in weight at the

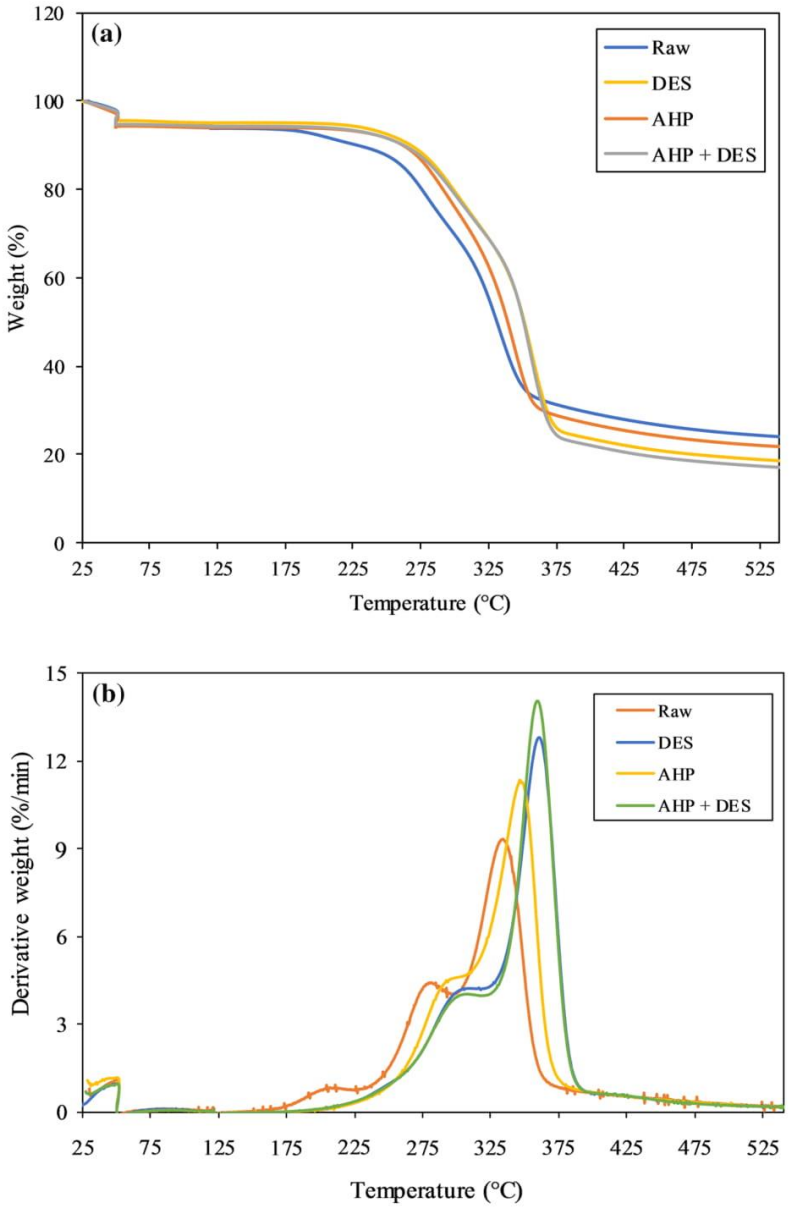

Fig. 4 Spectrum for **a** TGA and **b** DTG of raw and pretreated OPFs

temperature range of 50–125 °C (Fig. 4a), representing the evaporation of volatile components and physically adsorbed water (Darji et al. 2015). Furthermore, consistent with the previous study done by Subhedar and Gogate (2014), a significant decomposition was observed at 200–375 °C, indicating the liberation of volatile hydrocarbons owing to the decays of hemicellulose, cellulose, and some portions of lignin. Lignin decomposed slowly over the temperature ranging from 200 to 900 °C (Phitsuwan et al. 2016). Thus, the weight remaining after the decomposition of hemicellulose and cellulose (> 375 °C)

was mainly lignin and ash. Raw OPF had the highest residue, followed by AHP-only, control, and lastly AHP + DES-pretreated OPF in descending order. Jablonský et al. (2015) reported that small amount of ash was able to solubilized in DES during the delignification process. Therefore, this result was consistent with the chemical composition of pretreated OPFs, in terms of containing higher cellulose and hemicellulose but lower lignin and ash content as compared to the raw OPF. From the DTG analysis of raw OPF (Fig. 4b), two distinct peaks were observed at 280 and 330 °C, representing hemicellulose and cellulose, respectively. This observation was consistent with the previous study (Loow et al. 2017b). All pretreated OPFs showed a slight shift to the right for both maximum degradation temperatures of cellulose and hemicellulose, similar to the findings obtained by Phitsuwan et al. (2016) after a pretreatment of Napier grass. The temperature shift of DTG decomposition peaks could be possibly explained by the large proportion of crystalline cellulose, as a result of removing lignin and a fraction of hemicellulose. According to Braga et al. (2014), high degree of polymerization, rich in hydrogen bonds, and strong interactions between cellulose, attributed to the thermally stable properties of cellulose. Moreover, the AHP + DES-pretreated OPF obtained the highest derivative weight at 360 °C, indicating the greater removal of lignin and with an increase of cellulose proportion. The claim was backed by the compositional analysis carried out in this study (Table 3).

Proposed mechanism for pretreatments of OPF

The mechanism of AHP in the pretreatment relied on the decomposition products. At pH closer to the pKa of H_2O_2 (pH 11.5), hydrogen peroxide decomposes into hydroperoxy anion (HOO^-) in primary decomposition, hydroxyl radical (·OH), and superoxide anion ($\cdot O_2^-$) in secondary decomposition as shown in Eqs. 1 and 2 (Gould 1984). These species were responsible for the delignification in AHP pretreatment. According to Mittal et al. (2017), aromatic rings exposure and cleavage were not seen in mild peroxide concentrations (90 mg H_2O_2/g dry corn stover). As the aromatic rings alteration were mainly attributed by the ·OH, thus it was presumed that the HOO^- anion was the dominant reactive oxidant species in mild concentration AHP. This claim was backed by the FTIR analysis, where the peaks representing aromatic rings remained relatively unchanged. HOO^- enabled the cleavage of internal alkyl aryl ether bonds in the lignin and destruction of C–C bonds in aliphatic region, which contained an adjacent phenolic hydroxyl moiety (Li et al. 2012). As stated by Halma et al. (2015), the scission of β–O–4 linkages by HOO– anion occurred through internal nucleophilic reaction in the absence of free-phenolic hydroxyl groups (hardwood). Therefore, a hydroxyl group was generated on the side chain of the aromatic ring. The increase in free hydroxyl groups would plausibly improve the solubilization of lignin and lead to more site-specific delignification.

$$H_2O_2 + HO^- \rightarrow HOO^- + H_2O \quad (1)$$

$$H_2O_2 + HOO^- \rightarrow HO^{\cdot} + O_2^{-\cdot} + H_2O \quad (2)$$

On the other hand, ChCl:Urea delignification properties were mainly due to the ability of strong electronegativity halogen anion (Cl^-) to form hydrogen bonds with hydroxyl groups in the lignin (Loow et al. 2018). This capability of Cl^- enabled the disruption of hydrogen bonding network in the OPF, thus extracting the lignin. It was proposed that the generation of hydroxyl groups as a result of β–O–4 scission in AHP, encouraging the hydrogen bonds forming in DES pretreatment of OPF. Thus, the hydroxyl groups enhanced the lignin extraction ability of Cl^- anion and better disruption of hydrogen bonds network as illustrated in Fig. 5. This proposed mechanism could explain the synergistic improvement of DES pretreatment on AHP-pretreated OPF. However, it was observed that further increase in AHP concentration (> 0.25 vol%) affected DES delignification negatively. As AHP concentration increased, more lignin fragmentation and aromatic rings alterations were expected. Consequently, stronger hydrogen bond interactions were imposed on the fragmented lignin and newly formed hydroxyl groups by the OPF. Besides, the aromatic rings alterations by the AHP could lead to the removal of hydroxyl groups, which reduced the site of attacks for DES delignification. The collective effect of both proposed reasons could contribute to the reduction in DES delignification performance by hindering the hydrogen bonds forming with lignin.

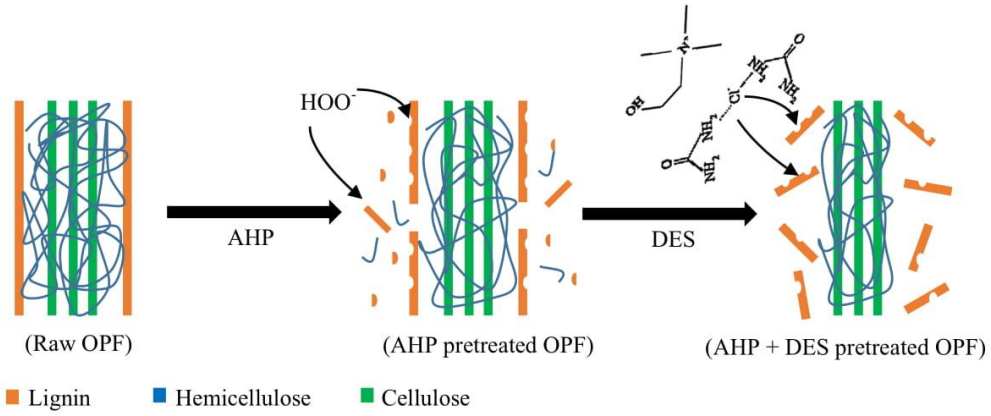

Fig. 5 Simple illustration of pretreatment mechanism

Table 5 Performance of various pretreatments integrated with AHP in lignocellulosic biomass delignification

Biomass	Pretreatment conditions	Lignin content (%)		Delignification (%)	References
		Before pretreatment	After pretreatment		
Jerusalem artichoke stem	(1) 5.0% (w/v) of H_2O_2 with 2.0% (w/v) of NaOH, ultrasonic at 50 °C for 2 h	26.0	21.6	40.3	Li et al. (2016b)
Jerusalem artichoke stalk	(1) 2.0% (v/v) of H_2O_2 with 2.0% (w/w) of NaOH, heated to 121 °C for 90 min	17.26	11.78	53.42	Li et al. (2016a)
Olive tree biomass	(1) 10.0% S/L; H_2O at 120 °C for 60 min (2) 2.4% (w/v) of H_2SO_4, autoclave at 130 °C for 84 min (3) 1.0% (w/v) of H_2O_2, adjusted to pH 11.5 using NaOH, heated to 80 °C for 90 min	18.5	36.3	19.7	Martínez-Patiño et al. (2017)
Olive tree biomass	(1) 10.0% S/L; H_2O at 120 °C for 60 min (2) 2.4% (w/v) of H_2SO_4, autoclave at 130 °C for 84 min (3) 7.0% (w/v) of H_2O_2, adjusted to pH 11.5 using NaOH, heated to 80 °C for 90 min	18.5	15.0	77.8	Martínez-Patiño et al. (2017)
Shea tree sawdust	(1) 1.0% (v/v) of H_2O_2, adjusted to pH 11.5 using $Ca(OH)_2$, stirring at 21 rad/s, at 150 °C for 45 min	29.90	30.25	9.64	Ayeni et al. (2013)
Tea oil fruit hull	(1) Acetic acid organosolv (53% acetic acid, 0.6% H_2SO_4) at 125 °C for 1.7 h (2) 3.0% of H_2O_2, adjusted to pH 11.5 using NaOH at ambient condition for 12 h	24.6	10.0	91.9	Tang et al. (2017)
Oil palm fronds (OPF)	(1) 0.25% (v/v) of H_2O_2, adjusted to pH 11.5 using NaOH, at ambient condition for 90 min (2) DES (ChCl:Urea) at 120 °C for 4 h under atmospheric pressure	18.84	16.25	18.99	This study

Comparison with past studies using AHP in pretreatment of biomass

The findings from this study were compared with various pretreatment integrated with AHP (Table 5). The lignin content reduction obtained in this study was relatively lower than all of the previous studies as hardwood (OPF) is typically more difficult to pretreat. It is worth noting that the AHP used in this study was utilized at a relatively mild concentration, shorter duration, and less energy intensive. The use of novel DES also claimed to be less hazardous, and stable at elevated temperature as compared to the sulphuric acid, and organosolv. Interestingly, the present study was able to achieve better delignification as compared to the findings obtained by Ayeni et al. (2013) in shea tree sawdust biomass pretreatment, although the conditions employed in this study were significantly less severe by incorporating sequential pretreatment approach. However, it was found that the delignification efficiency of AHP + DES pretreatment was inferior as compared to the other established methods. Thus, further work are needed to improve the performance of DES pretreatment. It was deduced that the use of a more effective DES would potentially improve delignification and synergistic effect more significantly.

Conclusion

The synergistic effects of AHP and DES pretreatments were investigated for OPF delignification. The combination of AHP (0.25 vol%, 90 min) at room temperature and DES (ChCl:Urea) at fixed operating conditions (120 °C, 4 h) achieved highest delignification (18.99%) as compared to DES-alone pretreatment (12.16%). The concentration of AHP greatly influenced on the subsequent delignification of DES pretreatment. The improvement of AHP + DES sequential pretreatment in delignification was attributed by the ability of AHP to enhance biomass accessibility and susceptibility to hydrogen bonds forming, which were verified via various characterization studies. Hence, the use of mild concentration AHP at ambient conditions with DES pretreatment could improve lignin extraction and yield glucan-rich OPF. Thus, facilitating downstream conversion of OPF to biofuel and chemical building block.

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Appendix C

Sequential pretreatment of alkaline hydrogen peroxide and choline chloride:copper (II) chloride dihydrate - Synergistic fractionation of oil palm fronds (Original Published Paper)

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Sequential pretreatment with alkaline hydrogen peroxide and choline chloride:copper (II) chloride dihydrate– Synergistic fractionation of oil palm fronds

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ABSTRACT

In this study, a novel Type II deep eutectic solvent (DES) namely, choline chloride:copper(II) chloride dihydrate ($ChCl:CuCl_2{\cdot}2H_2O$) was used to pretreat oil palm fronds (OPFs). The sequential pretreatment with alkaline hydrogen peroxide (0.25 vol%, 90 min) at ambient conditions and a Type II DES (90 °C, 3 h) at a later stage resulted in a delignification of 55.14% with high xylan (80.79%) and arabinan (98.02%) removals. The characterizations of pretreated OPFs confirmed the excellent performance of DES in OPF fractionation. Thus, the application of a Type II DES at ambient pressure and relatively lower temperature was able to improve the lignin and hemicellulose removals from OPFs.

1. Introduction

In recent decades, there has been an increasing interest in producing biofuel from palm oil as an alternative sustainable and environmentally-friendly energy source (Kurnia et al., 2016). However, there are several problems associated with such an application, such as the fact that biofuel from palm oil is insufficient to fulfill the global fuel demand, competition between food and energy, and deforestation due to expansion of oil palm plantations (Mukherjee and Sovacool, 2014). As such, numerous processes and studies have been proposed to produce biofuel from oil palm waste. Presently, Malaysia has one of the largest oil palm plantations in the world. The oil palm industry produces up to 90% of the national total of lignocellulosic residues (Loow et al., 2017b). Oil palm frond is a byproduct produced from the oil palm plantation, which is also the highest solid waste generated from the oil palm industry. Each year, with every millions of fresh fruit bunches processed, approximately 26.2 million tons of oil palm fronds are produced (Yunus et al., 2010). Even though oil palm fronds are readily available sources for a biorefinery, the fronds are typically used as mulches in the plantation (Loow and Wu, 2018). This approach has little economic value to the industry, while environmental problems may arise due to the improper handling of biomass decomposition (Isikgor and Becer, 2015).

Recently, lignocellulosic biomass has been attracting focus from various research groups due to its potential to form value-added products such as biofuel. Lignocellulosic biomass is a complex structure of polymers, which is mainly comprised of lignin, hemicellulose, and cellulose. Due to the recalcitrant nature of lignocellulosic biomass, a pretreatment stage is often required to effectively fractionate biomass and improve the enzymatic digestion of cellulose and hemicellulose. Throughout the years, numerous pretreatment methods have been introduced, which aimed to improve the process yield and efficiency of the downstream process (Loow et al., 2016b). On top of that, several emerging pretreatment technologies have been proposed, such as microwave irradiation, ultrasound, gamma rays, electron beam irradiation, a pulsed electric field, high hydrostatic pressure, and high pressure homogenization (Hassan et al., 2018). Moreover, promising approaches such as hydrothermal carbonization, supercritical fluid, and ammonia fiber explosion have been critically reviewed in order to create greener and more sustainable alternatives for industrial lignocellulosic biomass applications (Sankaran et al., 2019). Among the established pretreatment technologies, alkaline pretreatment is one of the most mature pretreatment technologies to delignify biomass without severe polysaccharides degradation (Loow et al., 2016a). Recent applications of alkaline hydrogen peroxide (AHP) have shown promising performance to improve delignification through the radical oxidation route (Ho et al., 2019a).

To date, on-going studies have been carried out to discover more advanced and effective methods for biomass pretreatment. However, the applications of technologies in a practical scale are often limited by the cost. Recently, a new class of solvents, known as deep eutectic solvents (DESs), has emerged as a promising type of solvent in the pre-

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treatment of biomass (Loow et al., 2017a). The first DES was found by Abbott et al. (2004) by combining a quaternary ammonium salt (HBA) and a hydrogen-bond donor (HBD) to form a liquid eutectic mixture. Recently, DESs have been increasingly applied in many research works associated with biomass fractionation due to their promising potential for removing bioactive phenolic compounds (Wei et al., 2015), delignification (Ho et al., 2019b), sugar recovery (Loow et al., 2018), furanic conversion (Lee et al., 2019), and biodiesel processing (Lu et al., 2016).

Although DESs have been critically involved in the recent studies of biomass pretreatment, a study regarding the application of a Type II DES (metal salt hydrate + organic salt) in biomass fractionation is not available. Conversely, even though inorganic salts such as $FeCl_3$, $AlCl_3$, and $CuCl_2$ have been widely applied in biomass pretreatment and hemicellulose recovery (Loow et al., 2015; Loow et al., 2018), no available literature was found regarding the application of an inorganic hydrate salt-based Type II DES. To the extent of our knowledge, limited studies have been carried out to investigate the potential use of an inorganic hydrate salt ($CuCl_2{\cdot}2H_2O$) based Type II DES and its synergistic effects with AHP in the pretreatment of oil palm fronds. It was postulated that the Type II DES might inherit the ability of the inorganic salt ($CuCl_2$) to selectively hydrolyze hemicellulose from the fronds at relatively lower temperatures and atmospheric pressures. On top of that, the implementation of a mild concentration of AHP may help to alter the macrostructure and partially solubilize lignin in the oil palm fronds (Ho et al., 2019b), thus facilitating the ability of the DES to access the biomass matrix, leading to better delignification and hemicellulose removal. Therefore, the objectives of this study were to investigate the potential use of a novel choline chloride:copper(II) chloride dihydrate (Type II DES) in oil palm fronds processing and its synergistic effects with AHP under non-severe conditions in a sequential pretreatment method.

2. Materials and methods

2.1. Raw material and chemicals

Fresh oil palm fronds used in this study were provided by the oil palm plantation owned by Universiti Kebangsaan Malaysia . After the leaflets were separated, a frond pressing machine was used to remove juices from the petioles of oil palm fronds. A pulverizer (8000 rpm) was used to grind the pressed petiole to a smaller size after it has been left drying under the sun for 2 d. Thereafter, a mechanical sieve was used to isolate ground petioles at a size of ≤0.5 mm. Distilled water was used to wash these ≤0.5 mm sieved and ground petioles before drying in an oven for 2 d at 60 °C. The processed oil palm fronds are herein referred to as OPFs throughout this study and stored using a container with a tight-fitting lid with desiccants at ambient conditions prior to the experiments. The compositions of OPFs in terms of the structural carbohydrates and lignin was determined by adopting the standard laboratory analytical procedure from the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008). D(+)glucose, D(−)xylose, L(+)arabinose, acetic acid, and formic acid were used for standard curve calibration by high-performance liquid chromatography. Choline chloride, copper(II) chloride dihydrate, hydrogen peroxide, sodium hydroxide pellets, and other chemicals used were ensured to be of analytical grade.

2.2. Sequential pretreatment

Two-stage pretreatment with AHP followed by a Type II DES was used to pretreat the OPFs.

2.2.1. AHP pretreatment

In AHP pretreatment, 4.5 g of OPFs was weighed and transferred into a beaker with a size of 600 mL. A 0.25 vol% hydrogen peroxide solution was prepared and adjusted to a pH of 11.5 using sodium hydroxide based on the past study (Ho et al., 2019b). Then, the AHP solution was added to the beaker containing the OPFs at a solid to liquid ratio of 1:20 (w/v). This non-severe pretreatment was carried out at room temperature and ambient pressure for 90 min (Ho et al., 2019b). The AHP-pretreated OPF sample was then separated and washed with distilled water to remove any residual AHP. After that, an oven was used to dry the AHP-pretreated sample at 60 °C overnight before storing the sample in a container with desiccants prior to further use.

2.2.2. DES pretreatment

A type II DES was used to pretreat the OPF samples obtained from the AHP pretreatment. The Type II DES namely, choline chloride:copper(II) chloride dihydrate (ChCl:$CuCl_2{\cdot}2H_2O$), was synthesized at a molar ratio of 1:1. The mixture was synthesized at 45 °C and stirred at 200 rpm until a homogeneous clear brown liquid was formed. Fresh ChCl:$CuCl_2{\cdot}2H_2O$ was prepared whenever the pretreatment was carried out.

First, AHP-pretreated OPFs were weighed (1.0 g) and placed into a 50 mL Schott bottle. DES (ChCl:$CuCl_2{\cdot}2H_2O$) was charged into the Schott bottle at a fixed solid to liquid ratio of 1:10 (w/v). A targeted temperature of either 80, 90, or 100 °C in the oil bath was controlled and stabilized prior to the pretreatment process. The Schott bottle containing both OPFs and ChCl:$CuCl_2{\cdot}2H_2O$ was placed in the oil bath after the temperature reached its target and stabilized. Effects of various operating temperatures (80, 90, and 100 °C) and reaction durations (1, 2, 3, and 4 h) were investigated in this study. Further increasing the temperature above 100 °C was not attempted because precipitation was observed at high pretreatment temperatures (>100 °C), making the pretreatment process not feasible. The Schott bottle was removed from the oil bath and quenched using running tap water upon the completion of the pretreatment. The resulting liquid fraction (DES hydrolysate) was collected, centrifuged, and diluted prior to HPLC analysis. After that, the solid fraction was filtered and washed with distilled water to remove any residual ChCl:$CuCl_2{\cdot}2H_2O$. The AHP + ChCl:$CuCl_2{\cdot}2H_2O$-pretreated OPF sample was dried at 60 °C overnight inside an oven and stored in a sample bag before sending it for subsequent analysis.

2.3. Analysis of the liquid hydrolysate using high-performance liquid chromatography

The concentrations of inhibitors, monomeric sugars, and structural carbohydrates in the centrifuged hydrolysate obtained from the DES pretreatment and acid hydrolysis of OPF samples were analyzed using an Agilent HPLC system (series 1200 infinity) equipped with a BioRad Aminex column (HPX-87H) and a refractive index (RI) detector. The mobile phase for the HPX-87H column was 0.005 mol/L H_2SO_4 at an operating flow rate of 0.6 mL/min, while the column temperature was controlled at 65 °C. The sugar recoveries (%) were evaluated using formula (Eq. (1)) adopted from Loow et al. (2018).

$$\text{Sugar recovery (\%)} = \frac{\text{sugar recovered in hydrolysate (g/L)} \times \text{volume of solvent used (L)}}{\text{sample carbohydrate fraction} \times \text{mass of sample used(g)}} \times 100\% \quad (1)$$

2.4. Analysis of solid fractions

2.4.1. Structural carbohydrates (glucan, xylan, and arabinan) and lignin quantification

The standard laboratory analytical methodology from NREL was used to determine the lignin contents in the OPF samples (Sluiter et al., 2008). 72% H_2SO_4 at solid to liquid ratio of 1:10 (w/v) was added to 0.3 g of sample in a conical flask. Next, the conical flask was incubated at 30 °C and 150 rpm for 1 h. The incubated mixture was then combined with 84 mL of deionized water before autoclaving at 121 °C for 1 h. The autoclaved mixture underwent vacuum filtration, where the filtrate was collected and analyzed for the structural carbohydrate concentration by HPLC and acid soluble lignin by a UV–Vis spectrophotometer at 320 nm. Last, the difference in dry weight of the filter paper before and after vacuum filtration was evaluated to quantify the acid insoluble lignin. The lignin (Eq. (2)) and structural carbohydrates (Eq. (3)) removals were evaluated as follows (New et al., 2019):

$$\text{Lignin removal}\,(\%) = \left(1 - \frac{\text{Lignin content in pretreated OPF}}{\text{Lignin content in raw OPF}} \times \text{yield}\right) \times 100\% \quad (2)$$

$$\text{Structural carbohydrate removal}\,(\%) = \left(1 - \frac{\text{Structural carbohydrate content in pretreated OPF}}{\text{Structural carbohydrate content in raw OPF}} \times \text{yield}\right) \times 100\% \quad (3)$$

where

Structural carbohydrates = glucan, xylan, and arabinan
Yield = mass recovery of pretreatment in fraction, $\left(\frac{\text{mass of pretreated OPF}}{\text{mass of raw OPF}}\right)$

2.4.2. Fourier transform infrared spectroscopy (FTIR)

Attenuated total reflection (ATR) tip FTIR (Thermoscientific Nicolet iS10 spectrometer) was used to analyze the chemical structures of the raw and pretreated OPF samples. A spectral resolution of 4 cm^{-1} with 64 scans over the range of 500–4000 cm^{-1} was applied for each spectrum collected.

2.4.3. Field emission scanning electron microscopy (FE-SEM)

Double-coated tape was used to adhere the OPF sample on a specimen stub, and it was sputter-coated with platinum prior to imaging using a Hitachi SU 8010 instrument in order to capture the OPF surface morphology and microstructural changes.

2.4.4. X-ray diffraction (XRD)

A Bruker D8 discover diffractometer was used to determine the crystallinity index (CrI) of the OPF samples with a 2θ ranging from 5 to 51° in steps of 0.041°. The CrI was defined as the relative degree of crystallinity in the sample by Segal et al. (1959), as shown in Equation (4).

$$\text{Crystallinity index}\,(\%) = \frac{I_{200} - I_{am}}{I_{200}} \times 100\% \quad (4)$$

where

I_{200} = Maximum intensity of the 200 peak (2θ = 22°)
I_{am} = Intensity minimum between peaks at 200 and 110 (2θ = 18°) (French, 2014).

2.4.5. Brunauer-Emmett-Teller (BET) analysis

The Micromeritics ASAP 2020 BET model with N_2 as the adsorbate at −195.85 °C was used to carry out BET analysis. The degassing conditions were adapted from Lim and Wu (2016), Lim and Wu (2015), where samples were initially heated to 90 °C for 2 h and subsequently held at 110 °C for 22 h.

2.5. Statistical analysis

The experimental data were statistically analyzed using IBM SPSS statistics 24. One-way analysis of variance (ANOVA) was carried out to identify any significant variation between pretreatments. The homogeneous subset for a range of parameters was verified via Duncan's test with a significance level of $P < 0.05$.

3. Results and discussion

3.1. Compositional analysis of raw OPFs

Raw OPFs on a dry basis were comprised of lignin (17.20 ± 0.10%), arabinan (2.04 ± 0.02%), xylan (21.49 ± 0.06%), and glucan (44.46 ± 0.49%) (Table 1), with the remaining weight of 14.81% composed of ash, water, and ethanol extractives. The compositions were found to slightly deviate from other literature findings (Loow et al., 2018; New et al., 2019; Tan et al., 2016). These variations were expected due to the different maturities and geographical sources of the palm trees from which the OPFs were harvested (Loow and Wu, 2018).

3.2. Effect of AHP and the type II DES on the solid fractions

From a past study (Ho et al., 2019b), the recommended concentration for AHP pretreatment of OPFs for the best synergism was determined to be 0.25 vol% (one-way ANOVA). When OPFs were pretreated with AHP at room temperature for 90 min, the delignification of DES could be improved by 1.6 times. Thus, this study emphasized the determination of the recommended conditions for a novel ChCl:$CuCl_2{\cdot}2H_2O$ pretreatment after undergoing AHP pretreatment (0.25 vol% of H_2O_2 at room temperature, pH 11.5 and 90 min) in order to achieve the best delignification and/or sugar recoveries from OPFs.

The composition of pretreated OPFs is summarized in Table 1 and interpreted from various perspectives, such as the solid recovery, delignification, cellulose enrichment in the biomass, and carbohydrate removal from the biomass. According to one-way ANOVA, the solid yield of DES pretreatment was significantly reduced as the temperature and duration increased. For example, the solid yield of OPF was reduced from 84.39 to 70.77% as the DES pretreatment duration increased from 1 to 4 h at a constant temperature of 80 °C. This result implied that a significant amount of OPF components were released into the hydrolysate as the temperature and duration of DES pretreatment increased. However, it is worth noting that a low solid yield as a result of extreme operating conditions may not be a favorable solution towards the effective fractionation of biomass in the biorefinery. Therefore, the composition of pretreated OPFs was carefully investigated at various temperatures and durations during DES sequential pretreatment.

3.2.1. Lignin

It was found that the lignin removal of AHP + DES pretreated OPFs exhibited an increasing trend, where lignin removal increased as the temperature and duration increased. According to one-way ANOVA, the lignin removal of OPFs reached a plateau as the duration of pretreatment increased. This trend could be seen from Table 1, where the lignin removal of AHP + DES pretreatment at 100 °C for a duration of 3–4 h did not show any significant differences. This trend also sug-

Table 1
Compositional analysis of the raw OPF and pretreated OPFs after undergoing various pretreatments at different temperatures and durations. Values annotated with different letters represent the difference in significance levels for each category (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Yield (%)	Lignin		Glucan		Xylan		Arabinan	
		Content (%)	Removal (%)	Content (%)	Removal (%)	Content (%)	Removal (%)	Content (%)	Removal (%)
Raw	–	17.20 ± 0.10 ^{a}	–	44.46 ± 0.49 ^{a}	–	21.49 ± 0.06 ^{a}	–	2.04 ± 0.02 ^{a}	–
AHP alone	95.90 ± 0.18 ^{a}	16.68 ± 0.06 ^{b,c}	6.98 ± 0.35 ^{a}	45.48 ± 0.06 ^{a}	1.88 ± 0.14 ^{a}	21.45 ± 0.13 ^{a}	4.25 ± 0.57 ^{a}	1.94 ± 0.03 ^{b}	8.63 ± 1.30 ^{a}
DES temperature: 80 °C									
AHP + DES (1 h)	84.39 ± 0.81 ^{b}	16.88 ± 0.07 ^{a,b,c}	17.17 ± 0.35 ^{b}	51.70 ± 0.15 ^{b}	1.87 ± 0.29 ^{a}	21.50 ± 0.28 ^{a}	15.57 ± 1.08 ^{b}	0.16 ± 0.01 ^{c}	93.32 ± 0.57 ^{b}
AHP + DES (2 h)	79.11 ± 1.37 ^{c}	17.07 ± 0.14 ^{a,b}	21.47 ± 0.63 ^{c}	55.74 ± 0.62 ^{c}	0.81 ± 1.11 ^{a}	19.12 ± 0.53 ^{b}	29.59 ± 1.96 ^{c}	0.12 ± 0.02 ^{d}	95.22 ± 0.68 ^{c}
AHP + DES (3 h)	74.42 ± 1.22 ^{d}	17.05 ± 0.15 ^{a,b}	26.21 ± 0.66 ^{d}	59.53 ± 0.57 ^{d}	0.35 ± 0.96 ^{a}	18.07 ± 0.10 ^{c}	37.42 ± 0.34 ^{d}	0.10 ± 0.01 ^{d,e}	96.39 ± 0.24 ^{c,d}
AHP + DES (4 h)	70.77 ± 1.90 ^{e}	17.09 ± 0.27 ^{a,b}	29.65 ± 1.11 ^{e}	62.53 ± 0.41 ^{e}	0.44 ± 0.65 ^{a}	16.15 ± 0.04 ^{d}	46.79 ± 0.20 ^{e}	0.07 ± 0.02 ^{e,f}	97.52 ± 0.65 ^{d,e}
DES temperature: 90 °C									
AHP + DES (1 h)	72.90 ± 1.11 ^{d,e}	15.82 ± 0.14 ^{d}	32.95 ± 0.59 ^{f}	57.75 ± 1.35 ^{d}	5.30 ± 2.22 ^{b}	14.21 ± 0.43 ^{e}	51.80 ± 1.45 ^{f}	0.11 ± 0.01 ^{d,e}	96.19 ± 0.19 ^{c,d}
AHP + DES (2 h)	57.64 ± 0.81 ^{f,g}	16.02 ± 0.20 ^{d}	46.32 ± 0.68 ^{g}	65.52 ± 1.91 ^{f}	15.05 ± 2.47 ^{c}	9.98 ± 0.78 ^{f}	73.24 ± 2.10 ^{g}	0.09 ± 0.01 ^{d,e}	97.50 ± 0.07 ^{d,e}
AHP + DES (3 h)	48.09 ± 0.75 ^{h}	16.04 ± 0.08 ^{d}	55.14 ± 0.23 ^{h}	69.27 ± 1.09 ^{g}	25.06 ± 1.18 ^{d}	8.58 ± 0.41 ^{g}	80.79 ± 0.92 ^{h}	0.08 ± 0.01 ^{e,f}	98.02 ± 0.30 ^{e}
AHP + DES (3.5 h)	45.36 ± 0.41 ^{h,i}	16.54 ± 0.11 ^{c}	56.37 ± 0.28 ^{i}	69.96 ± 0.17 ^{g,h}	28.62 ± 0.17 ^{e}	6.97 ± 0.49 ^{h}	85.29 ± 1.03 ^{i}	0.08 ± 0.01 ^{e,f}	98.23 ± 0.22 ^{e}
AHP + DES (4 h)	43.83 ± 2.85 ^{i}	16.69 ± 0.18 ^{b,c}	57.47 ± 0.45 ^{i}	71.75 ± 0.32 ^{h}	29.27 ± 0.32 ^{e}	3.57 ± 0.03 ^{i}	92.72 ± 0.07 ^{j}	0.08 ± 0.02 ^{e,f}	98.34 ± 0.47 ^{e}
DES temperature: 100 °C									
AHP + DES (1 h)	59.67 ± 2.74 ^{f}	15.87 ± 0.02 ^{d}	44.95 ± 0.08 ^{j}	69.51 ± 1.20 ^{g}	6.71 ± 1.62 ^{b}	11.01 ± 0.54 ^{j}	69.44 ± 1.49 ^{k}	ND $^{\Psi}$	–
AHP + DES (2 h)	45.64 ± 2.30 ^{h,i}	11.34 ± 0.43 ^{e}	69.91 ± 1.14 ^{k}	81.54 ± 0.63 ^{i}	16.28 ± 0.65 ^{c}	2.11 ± 0.10 ^{k}	95.52 ± 0.21 ^{l}	ND	–
AHP + DES (3 h)	34.64 ± 0.43 ^{j}	13.22 ± 0.01 ^{f}	73.36 ± 0.02 ^{l}	93.45 ± 0.27 ^{j}	27.19 ± 0.21 ^{d,e}	0.32 ± 0.03 ^{l}	99.48 ± 0.04 ^{m}	ND	–
AHP + DES (4 h)	20.86 ± 0.61 ^{k}	22.34 ± 0.40 ^{g}	72.91 ± 0.48 ^{l}	75.25 ± 1.72 ^{k}	64.69 ± 0.81 ^{f}	ND	–	ND	–

$^{\Psi}$ ND = Not detectable.

gested that the delignification of DES was highly temperature driven compared to the duration. Generally, the lignin content of the pretreated OPFs was reduced as the temperature of pretreatment increased for a constant duration (Liu et al., 2018), which indicated an increase in the rate of delignification. Nonetheless, the present study showed that the lignin content increased when the temperature of DES pretreatment increased from 90 to 100 °C at a fixed duration of 4 h. A similar result was observed by Huerta and Saldaña (2019), where the lignin content increased from 21.4 to 27.5% when the temperature of the pressurized hot water pretreatment increased from 140 to 180 °C at a fixed duration of 40 min. The increase in lignin content indicated that the increase in the rate of carbohydrate removal was more dominant as the temperature increased. In general, the lignin content of pretreated OPFs was observed to exhibit an increasing trend as the duration of pretreatment increased. However, a different trend was obtained at a pretreatment temperature of 100 °C, whereby the lignin content first decreased from 1 (15.87%) to 2 (11.34%) h and then increased to 22.34% at 4 h. At 80 and 90 °C, the carbohydrate removal was more dominant compared to the delignification. When the temperature was raised to 100 °C, the rate of delignification was improved, thus reducing the lignin content. However, as the pretreatment was prolonged, lignin removal reached a plateau, while accessibility to the carbohydrate improved. Thus, the rate of carbohydrate removal was more dominant compared to the delignification after 2 h, and hence the lignin content increased.

3.2.2. Structural carbohydrates

Remarkably, the DES (ChCl:$CuCl_2{\cdot}2H_2O$) was able to exhibit extraordinary xylan and arabinan removals at operating conditions of 100 °C and 3 h (Table 1). It is worth noting that the operating conditions employed in these studies were relatively less severe compared to the conventional inorganic salt hydrolysis studied by Loow et al. (2018). However, the inorganic salt in the form of DES in this study was able to achieve remarkable xylan and arabinan removal efficiencies. It was observed that the carbohydrate removal was strongly dependent on the pretreatment temperature and duration. While the removal of hemicellulose (xylan and arabinan) may be favorable, the degradation and extraction of cellulose were not favorable in most biorefinery processes. Generally, as the pretreatment temperature and duration increased, the hemicellulose removal increased. This observation concurred with the findings reported by Zhang et al. (2017), where an increase in the pretreatment temperature and duration caused an increase in xylan removal. Glucan enrichment was observed as the temperature and duration of pretreatment increased, as most of the glucan was not degraded during the process. This can be shown by a previous study, in which cellulose enrichment was observed after undergoing the sequential pretreatment of elephant grass (Toscan et al., 2019). However, as the pretreatment conditions were increased to 100 °C and 4 h, the glucan removal showed a drastic increase and resulted in the reduction of the glucan content. Similar results were also obtained by Zhang et al. (2017), where increases in the temperature and duration caused a decrease in the glucan content. This phenomenon was due to the exposure of cellulose when the recalcitrant barrier (lignin and hemicellulose) was disrupted. Thus, prolonging the pretreatment duration might cause the degradation of cellulose due to the increase in accessibility to the cellulose.

3.3. Effect of AHP and the Type II DES on the liquid fractions (hydrolysates)

The DES hydrolysate after the pretreatment was collected and analyzed to obtain the sugar and inhibitor contents in the liquid fractions. It was observed in the compositional analysis of the solid fractions (Table 1) that a high amount of structural carbohydrates was released into the liquid fractions during the pretreatment process. Thus, it is expected that the DES hydrolysate was able to retain a certain amount of reducing sugars. Table 2 summarizes the sugar and inhibitor contents found in the DES hydrolysate after the pretreatments at various operating conditions.

3.3.1. Sugar recovery

It was observed in Table 2 that the glucose recoveries from the OPFs were found to be relatively low compared to the other sugars. Although a significant amount of glucan was released from the biomass during the pretreatment process (Table 1), the glucose could potentially be converted to other forms of degradation products due to prolonged exposure to the heating environment of DES. On the other hand, xylose and arabinose recoveries were found to be strongly dependent on the pretreatment duration. While an increase in the pretreatment temperature could potentially improve sugar recoveries, the unfavorable sugar degradation rate was also improved (Loow et al., 2015). It is worth highlighting that maximum xylose recoveries were obtained for each pretreatment temperature at a different duration (Table 2). These results indicated the occurrence of xylose degradation surpassing the xylose extraction. For example, the maximum xylose recoveries of 16.56, 28.96, and 27.51% were obtained in 80 °C (4 h), 90 °C (3 h), and 100 °C (1 h) conditions, respectively.

3.3.2. Inhibitors

Indeed, the recovery and utilization of the liquid fractions to form value-added products could potentially improve the economics of the overall biorefinery process. However, the inhibitors content in the liquid fractions may be hindering effective fermentation or bioconversion in the later stage. Thus, it is crucial to examine the inhibitors formed in the hydrolysate after pretreatment. The inhibitors focused in this study were weak acids such as formic and acetic acids, while furfural and 5-hydroxymethylfurfural (5-HMF) were not within the scope of this study. According to Ho et al. (2019a), the formation of undesired alcohols was caused by the furfural and 5-HMF, thus creating a lag phase in the bioethanol formation. Nevertheless, the yield of bioethanol formation was not affected after prolonged fermentation. On the other hand, weak acids were claimed to enhance the overall bioethanol formation due to their ability to reduce the intracellular pH. Nevertheless, high concentrations of these acids (exceeding 100 mmol/L) would result in the acidification of cytoplasm and cell death during the fermentation process (Larsson et al., 1999).

Formic acid was one of the degradation products of sugars, while the acetic acid was formed by the degradation of acetyl groups in hemicellulose (xylose and arabinose) (Graça et al., 2018). Table 2 shows that formic acid concentrations were increased along with increases of both the temperature and duration of pretreatment. These results were in agreement with the glucan removal in Table 1. Glucose recovery in the hydrolysate was relatively less compared to the glucan removal, suggesting that most of the glucose released was degraded into various degradation products, such as formic acid. As a case in point, at pretreatment conditions of 100 °C and 4 h, a large amount of glucan was removed due to the over exposure of cellulose during pretreatment. Thus, more formic acid (119.79 mmol/L) was formed in the hydrolysate, which could be due to the prolonged heating in the DES (ChCl:$CuCl_2{\cdot}2H_2O$) system.

Furthermore, acetic acid showed a similar trend, whereby increases in the temperature and duration encouraged the formation of acetic acid. Thus, it was proposed that the decreases in xylose and arabinose recoveries were due to the degradation of sugars and reduced rate of xylan and arabinan removal. This study indicated that the increase in pretreatment conditions of DES might not be favorable for the sugar recoveries in the hydrolysate (Table 2). For example, at 100 °C and 4 h, the recoveries of glucose, xylose, and arabinose were relatively small

Table 2
Analysis of the DES hydrolysates after undergoing various pretreatments at different temperatures and durations. Values annotated with different letters represents the difference in significance levels for each category (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Sugar recovery						Formic acid		Acetic acid	
	Glucose (mg) ζ	Recovery (%)	Xylose (mg) ζ	Recovery (%)	Arabinose (mg) ζ	Recovery (%)	(g/L)	(mmol/L)	(g/L)	(mmol/L)
DES temperature: 80 °C										
AHP + DES (1 h)	ND ᵠ	–	12.26 ± 2.33 ^{a}	5.72 ± 1.08 ^{a}	5.50 ± 0.09 ^{a,b}	28.32 ± 0.47 ^{a,b}	ND	ND	0.19 ± 0.08 ^{a}	3.13 ± 1.42 ^{a}
AHP + DES (2 h)	ND	–	19.38 ± 3.27 ^{b}	9.03 ± 1.53 ^{b}	4.37 ± 0.12 ^{c}	22.49 ± 0.63 ^{c}	ND	ND	0.59 ± 0.18 ^{a,b}	9.83 ± 2.95 ^{a,b}
AHP + DES (3 h)	ND	–	26.91 ± 3.46 ^{c}	12.54 ± 1.61 ^{c}	4.58 ± 0.17 ^{c,d}	23.63 ± 0.86 ^{c,d}	ND	ND	1.00 ± 0.19 ^{b,c}	16.58 ± 3.16 ^{b,c}
AHP + DES (4 h)	ND	–	35.53 ± 2.17 ^{d}	16.56 ± 1.01 ^{d}	4.32 ± 0.59 ^{c}	22.23 ± 3.03 ^{c}	ND	ND	1.31 ± 0.10 ^{c}	21.80 ± 1.73 ^{c}
DES temperature: 90 °C										
AHP + DES (1 h)	1.49 ± 0.09 ^{a}	0.33 ± 0.02 ^{a}	33.51 ± 0.64 ^{d}	15.62 ± 0.30 ^{d}	7.39 ± 0.23 ^{e}	38.10 ± 1.19 ^{e}	ND	ND	0.89 ± 0.07 ^{b,c}	14.75 ± 1.16 ^{b,c}
AHP + DES (2 h)	3.07 ± 0.12 ^{b}	0.68 ± 0.02 ^{b}	52.17 ± 0.43 ^{e}	24.32 ± 0.21 ^{e}	6.87 ± 0.16 ^{e}	35.42 ± 0.78 ^{e}	0.23 ± 0.01 ^{a}	4.83 ± 0.16 ^{a}	2.04 ± 0.03 ^{d}	33.97 ± 0.55 ^{d}
AHP + DES (3 h)	5.40 ± 0.19 ^{c,d}	1.19 ± 0.04 ^{c,d}	62.13 ± 1.67 ^{f}	28.96 ± 0.78 ^{f}	6.08 ± 0.49 ^{b}	31.34 ± 2.55 ^{b}	0.36 ± 0.01 ^{a}	7.77 ± 0.18 ^{a}	2.56 ± 0.03 ^{d,e}	42.67 ± 0.54 ^{d,e}
AHP + DES (3.5 h)	6.20 ± 0.47 ^{d,h}	1.37 ± 0.11 ^{d,h}	57.31 ± 1.62 ^{f}	26.72 ± 0.76 ^{f}	5.26 ± 0.13 ^{a}	27.10 ± 0.67 ^{a}	0.43 ± 0.03 ^{a}	9.27 ± 0.61 ^{a}	2.72 ± 0.01 ^{e}	45.33 ± 0.16 ^{e}
AHP + DES (4 h)	9.25 ± 0.01 ^{e}	2.03 ± 0.01 ^{e}	50.21 ± 0.42 ^{e}	23.41 ± 0.20 ^{e}	5.49 ± 0.11 ^{a,b}	28.29 ± 0.59 ^{a,b}	1.15 ± 0.07 ^{b}	24.93 ± 1.47 ^{b}	3.21 ± 0.02 ^{e}	53.42 ± 0.39 ^{e}
DES temperature: 100 °C										
AHP + DES (1 h)	4.58 ± 0.48 ^{c}	1.01 ± 0.11 ^{c}	59.01 ± 1.87 ^{f}	27.51 ± 0.87 ^{f}	4.89 ± 0.30 ^{a,c,d}	25.17 ± 1.56 ^{a,c,d}	0.53 ± 0.01 ^{a}	11.48 ± 0.08 ^{a}	2.02 ± 0.08 ^{d}	33.53 ± 1.24 ^{d}
AHP + DES (2 h)	8.26 ± 0.68 ^{f}	1.82 ± 0.15 ^{f}	21.59 ± 1.61 ^{b}	10.07 ± 0.76 ^{b}	2.31 ± 0.40 ^{f}	11.91 ± 2.06 ^{f}	1.86 ± 0.34 ^{c}	40.43 ± 7.35 ^{c}	2.85 ± 0.54 ^{e}	47.34 ± 9.09 ^{e}
AHP + DES (3 h)	14.78 ± 0.84 ^{g}	3.25 ± 0.18 ^{g}	18.61 ± 4.98 ^{b}	8.68 ± 2.32 ^{b}	ND	–	4.25 ± 0.58 ^{d}	92.30 ± 12.54 ^{d}	5.16 ± 0.88 ^{f}	85.84 ± 14.81 ^{f}
AHP + DES (4 h)	14.27 ± 0.13 ^{g}	3.14 ± 0.03 ^{g}	9.23 ± 3.24 ^{a}	4.30 ± 1.51 ^{a}	ND	–	5.52 ± 0.04 ^{e}	119.79 ± 0.86 ^{e}	5.99 ± 0.35 ^{g}	99.79 ± 5.87 ^{g}

ᵠ ND = Not detectable.
ζ Sugar mass in milligram per gram of OPF in dry basis.

(<5%) in comparison with those at 90 °C and 3 h. Loow et al. (2015) also reported that sugar degradation drastically increased as the temperature increased. The present study showed that at a high temperature of 100 °C and long duration of 4 h, inhibitors exceeding 100 mmol/L were formed during the pretreatment process (Table 2).

3.4. Recommended conditions for the type II DES

The temperatures studied in this paper were 80, 90, and 100 °C. Although an increase in delignification was observed at higher temperature and a shorter duration (Table 1), several disadvantages along with difficulties were found when the temperature was increased further. It was observed that the glucan removal from OPFs and inhibitor formation in the hydrolysate were increased together with an increase of the pretreatment temperature. On top of that, when the temperature was raised to 100 °C, the ability of the DES to recover xylose and arabinose (Table 2) deteriorated. This phenomenon could be due to the thermal degradation of sugars induced during the heating of pretreatment.

In addition, several difficulties in handling the DES occurred when the temperature was increased further (>100 °C). At higher temperature, precipitation was observed in the DES hydrolysate upon contact with water. According to Loow et al. (2017a), the ability of the DES in delignification was mainly governed by the hydrogen bonds formed between chloride anions and biomass components. This proposed mechanism suggested that there would be a saturation of hydrogen bonds formed in the DES. As the pretreatment temperature increased, a drastic decrease of the solid yield was also observed. This observation indicated that a high amount of biomass components were released during the pretreatment, and thus hydrogen bonding in the DES hydrolysate approached its saturation. It was proposed that the hydrogen bonds between chloride anions and biomass components were reversible, whereby the addition of water would disrupt the hydrogen bonds network (Ma et al., 2018), thus causing the precipitation. On top of that, the addition of water could have acidified the solvent through the Brønsted acid pathway (Loow et al., 2015), which may also favor the precipitation. Similar findings were observed by García et al. (2012), where the addition of acidified water enabled the precipitation of lignin.

The recommended conditions for DES may be varied to adapt to different needs of pretreatment. In the case of liquid fractions not recovered, the recommended condition for DES pretreatment were 100 °C and 2 h. This condition was able to achieve the lowest lignin content with 69.91% delignification, while causing relatively less removal of glucan to yield glucan-rich OPF. However, it is important to note that this condition resulted in lower sugar recoveries and higher inhibitors content in the liquid fractions. Thus, the valorization of liquid fractions may not be feasible. In the present study, the aim was to identify the recommended conditions to achieve both reasonable sugar recoveries and delignification in the liquid and solid fractions, respectively. Therefore, pretreatment conditions of 90 °C and 3 h were selected, as they were able to achieve xylose and arabinose recoveries of 28.96 and 31.34%, respectively, while generating less formic acid (0.36 g/L) in the liquid fraction. Furthermore, this condition was able to achieve 55.14% of delignification with glucan enrichment up to 69.27% while removing the majority of xylan and arabinan from the OPFs. An additional study was carried out at pretreatment condition of 90 °C and 3.5 h to confirm the best duration for the recommended condition (Tables 1 and 2). Although a slight increase in delignification (~1%) can be achieved with 3.5 h, the sugar recoveries were found to slightly decrease. Thus, a further increase in duration may not be economically viable. It is worth highlighting that the pretreatment conditions proposed in this study were relatively less severe compared to the conventional pretreatments. Although, the delignification achieved in this study was inferior compared to the results reported by Tan et al. (2018) (ChCl:Lactic acid, 88%) and Chen et al. (2019) (Ternary DES, 82.07%), it is possible to recover sugar directly from the ChCl:$CuCl_2{\cdot}2H_2O$ DES hydrolysate. It is important to note that ChCl:$CuCl_2{\cdot}2H_2O$ DES has the potential of achieving higher delignification but at the expense of sugar recoveries. Thus, further optimization is possible to adapt to different applications.

From a previous study (Ho et al., 2019b), it was found that AHP pretreatment could help promote the accessibility of OPF in the later pretreatment stage. Comparisons of the compositional analysis of pretreated OPFs using a single-stage pretreatment (AHP alone or DES alone) and a sequential pretreatment (AHP + DES) were tabulated in Table 3a. It was found that the lignin and structural carbohydrates removals after undergoing sequential pretreatment coupled with AHP were generally higher in comparison with the single-stage pretreatment. However, high glucan removal was not desired in the downstream processing of the biorefinery. On the other hand, a significant improvement in the lignin removal was obtained by integrating AHP into the sequential pretreatment. For example, the lignin removal of DES (37.56%) was increased to 55.14% by incorporating AHP pretreatment as an initial pretreatment process. This phenomenon indicated the synergistic effect of AHP with DES (ChCl:$CuCl_2{\cdot}2H_2O$) in the delignification of biomass.

From the hydrolysate perspective (Table 3b), the xylose and arabinose contents were significantly increased in sequential pretreatment compared to the AHP pretreatment alone. Nonetheless, the glucose content was decreased in this sequential pretreatment, which could be due to the multiple pretreatment stages that caused the glucose to be removed in the earlier pretreatment. On top of that, free bound sugar in the raw OPFs was directly released into the DES hydrolysate in a single-stage pretreatment, and hence, more glucose was observed (Loow et al., 2018). In addition to sugar recoveries, acetic acid was found to be less in the sequential pretreatment. This observation was expected because AHP pretreatment was claimed to exhibit excellent acetyl group cleavage, in which less substrate (acetyl group) was available for degradation in the later DES pretreatment (Ho et al., 2019a). The formic acid concentration was found to be slightly higher in DES pretreatment compared to sequential pretreatment, which was due to the presence of more free bound sugars associated with the OPFs. According to the results in Table 3b, it was found that the sugar recoveries were not improved significantly by incorporating AHP in sequential pretreatment. However, a substantial improvement in delignification was observed after sequential pretreatment with AHP and DES (Table 3a) compared to the DES pretreatment alone. This observation suggested that the use of AHP in sequential pretreatment was able to improve delignification synergistically but not the sugar recoveries.

3.5. Proposed mechanism for DES pretreatment

The combination of ChCl and $CuCl_2{\cdot}2H_2O$ at the specific molar ratio of 1:1 formed a Type II DES, which was used in this study. The mechanism of DES pretreatment in the delignification of OPFs mainly relied on the formation of hydrogen bonds between lignin and halogen anions in the DES. According to Loow et al. (2018), halogen anions in the DES were able to establish hydrogen bond with hydroxyl groups in the lignin after the donation of protons. On top of that, hydrogen bonds formation between Cl^- anions and the lignin aromatic ring could result in the extraction of aromatic rings and lignin dissolution (Loow et al., 2017a). It was proposed that the delignification efficiency was mainly driven by the ability of a strongly electronegative halogen anion (Cl^-) to establish hydrogen bonding with the lignin. Multiple sources of Cl^- anions from the ChCl and $CuCl_2{\cdot}2H_2O$ DES system could be the reason for the enhanced delignification observed in this study compared to the single source Type III DES system (New et al., 2019). Moreover, the

Table 3a
Compositional analysis of the raw OPF and pretreated OPFs after undergoing pretreatments via AHP alone (0.25 vol%, 90 min), DES alone (90 °C and 3 h) and AHP (0.25 vol%, 90 min) plus DES (90 °C, 3 h). Values annotated with different letters represents the difference in significance levels for each category (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Yield (%)	Lignin		Glucan		Xylan		Arabinan	
		Content (%)	Removal (%)	Content (%)	Removal (%)	Content (%)	Removal (%)	Content (%)	Removal (%)
Raw	–	17.20 ± 0.10 ^{a}	–	44.46 ± 0.49 ^{a}	–	21.49 ± 0.06 ^{a}	–	2.04 ± 0.02 ^{a}	–
AHP alone	95.90 ± 0.18 ^{a}	16.68 ± 0.06 ^{b}	6.98 ± 0.35 ^{a}	45.48 ± 0.06 ^{a}	1.88 ± 0.14 ^{a}	21.45 ± 0.13 ^{a}	4.25 ± 0.57 ^{a}	1.94 ± 0.03 ^{b}	8.63 ± 1.30 ^{a}
DES alone	56.90 ± 0.01 ^{b}	19.01 ± 0.06 ^{c}	37.56 ± 0.19 ^{b}	66.98 ± 0.28 ^{b}	14.87 ± 0.35 ^{b}	8.39 ± 0.18 ^{b}	77.95 ± 0.47 ^{b}	0.05 ± 0.01 ^{c}	98.68 ± 0.10 ^{b}
AHP + DES	48.09 ± 0.75 ^{c}	16.04 ± 0.08 ^{d}	55.14 ± 0.23 ^{c}	69.27 ± 1.09 ^{c}	25.06 ± 1.18 ^{c}	8.58 ± 0.41 ^{b}	80.79 ± 0.92 ^{c}	0.08 ± 0.01 ^{c,d}	98.02 ± 0.30 ^{b}

Table 3b
Analysis of the hydrolysates after undergoing pretreatments via AHP alone (0.25 vol%, 90 min), DES alone (90 °C, 3 h) and AHP (0.25 vol%, 90 min) plus DES (90 °C, 3 h) . Values annotated with different letters represents the difference in significance levels (one-way ANOVA, Duncan's test; $P < 0.05$).

Pretreatment conditions	Sugar recovery						Formic acid	Acetic acid
	Glucose (mg) [ζ]	Recovery (%)	Xylose (mg) [ζ]	Recovery (%)	Arabinose (mg) [ζ]	Recovery (%)	(mmol/L)	(mmol/L)
AHP alone	0.07 ± 0.01 [a]	0.02 ± 0.01 [a]	0.14 ± 0.01 [a]	0.07 ± 0.01 [a]	0.09 ± 0.01 [a]	0.42 ± 0.05 [a]	ND	0.15 ± 0.01 [a]
DES alone	6.95 ± 0.58 [b]	1.56 ± 0.13 [b]	57.61 ± 0.80 [b]	26.81 ± 0.37 [b]	5.44 ± 0.15 [b,c]	26.70 ± 0.74 [b,c]	11.19 ± 0.24 [a]	64.93 ± 3.14 [b]
AHP + DES	5.40 ± 0.19 [c]	1.19 ± 0.04 [c]	62.13 ± 1.67 [b]	28.96 ± 0.78 [b]	6.08 ± 0.49 [c]	31.34 ± 2.55 [c]	7.77 ± 0.18 [a]	42.67 ± 0.54 [c]

[Ψ]ND = Not detectable.
[ζ] Sugar mass in milligram per gram of OPF in dry basis.

AHP pretreatment could generate free hydroxyl groups in the aromatic side chain (Ho et al., 2019b), plausibly improving site-specific delignification. Thus, this effect induced the synergistic effect in the delignification of OPFs by combining AHP and DES pretreatments.

The mechanism of inorganic hydrate salt-based DES pretreatment on the sugar recovery can be categorized into Lewis and Brønsted acid pathways (Loow et al., 2015). However, in the current DES system, water molecules from ligands were limited. Thus, the Lewis acid pathway was proposed to be more dominant compared to the Brønsted acid pathway, as excess water was needed to produce H_3O^+ ions in the latter (Loow et al., 2015). According to Román-Leshkov and Davis (2011), the metal cation Cu^{2+} would coordinate with water ligands to form metal ion ligand complexes $[M(H_2O)_n]^{z+}$ (where n is the solvation number, z is the cation oxidation state, and M is the metal ion). The metal ion complexes acted as Lewis acids and would then assist in the cleavage of glycosidic linkages, while the coordinated water molecules were used to depolymerize structural carbohydrates (cellulose and hemicellulose) into monosaccharides by participating as nucleophiles (Peng et al., 2010). The simplified proposed mechanism is illustrated in Fig. 1. Thus, it was also possible for DES to depolymerize cellulose into glucose with prolonged exposure to DES and the removal of protective barriers. As the temperature and duration increased for DES pretreatment, it was observed that DES was able to significantly degrade glucan from the OPFs (Table 1). This phenomenon could be due to the significant removal of the protective barrier (lignin and hemicellulose), hence, DES was allowed to depolymerize cellulose through the proposed mechanistic pathway.

3.6. Characterization studies

FE-SEM was used to observe the alterations of the OPF morphology after different pretreatments. The untreated OPFs exhibited a smooth and enclosed surface, which resulted in a lower degree of porosity (Loow et al., 2018). Thus, the penetration to the internal structure by chemical means was not favorable, resulting in lower delignification and sugar recoveries (Tables 3a and 3b). After AHP pretreatment, the outer layer of OPFs was slightly stripped, leaving a relatively rough surface. However, the overall structure of OPFs was noticeably enclosed and intact, indicating that the mild AHP pretreatment had a limited effect on the carbohydrates (Ho et al., 2019b). Moreover, tiny holes were found on the surface of the AHP-pretreated OPFs, thus potentially enhancing the accessibility and porosity to the internal structure. This finding concurred with those of Li et al. (2016) and Ho et al. (2019b), whereby small holes were found in the biomass after undergoing AHP pretreatment. While the majority of lignin was not removed from the OPFs, the increase in porosity could potentially enhance subsequent DES penetration. Due to a lower degree of porosity in the raw OPFs, the cellulose was not effectively exposed after undergoing DES ($ChCl:CuCl_2{\cdot}2H_2O$) pretreatment at 90 °C for 3 h. However, the major disruption of the structure was observed due to the extraordinary hemicellulose removal by DES (Table 3a). The synergistic combination of

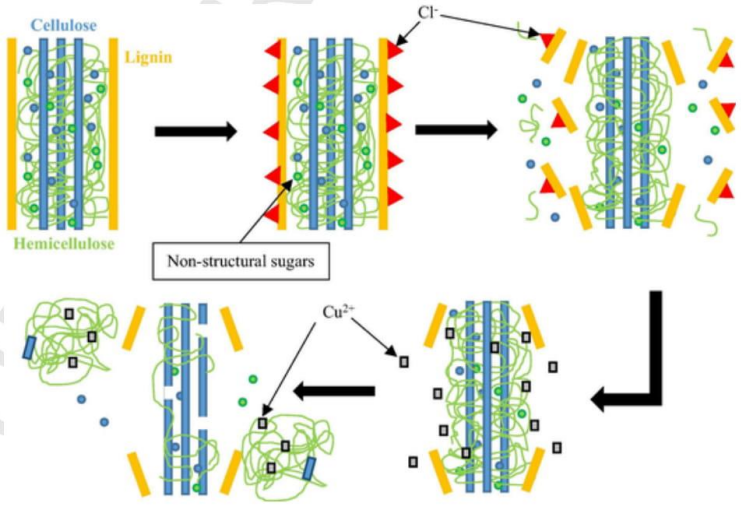

Fig. 1. Simple illustration of the proposed mechanism for Type II DES ($ChCl:CuCl_2{\cdot}2H_2O$) pretreatment.

AHP and DES pretreatments resulted in the most drastic rupture of the OPF structure, exposing ordered crystalline structures as suggested in the proposed mechanism for this sequential pretreatment (Fig. 1). The effect of AHP in OPF modifications coupled with subsequent delignification and hemicellulose removal during DES pretreatment led to the destruction of the OPF structure.

From the BET analysis, the specific surface areas of raw and pretreated OPFs were determined. The initial specific surface area of raw OPFs was 0.4071 m^2/g. After undergoing AHP pretreatment alone, the area increased slightly to 0.4220 m^2/g. This increase in surface area was mainly due to the increase in the OPF porosity as a result of the biomass structural alteration observed in the FE-SEM (Ho et al., 2019b). Furthermore, the specific surface area increased to 0.5801 m^2/g when the raw OPFs were pretreated using DES alone at 90 °C and 3 h, which was increased by 42.50% compared to raw OPFs. This result was anticipated, as the majority of the OPF structure was disrupted. Remarkably, the combination of AHP and DES was able to attain the best surface area of 0.7577 m^2/g, resulting in an 86.12% increase in the surface area from the initial area of raw OPFs. The AHP was able to enhance the accessibility of OPFs. Thus, DES was able to disrupt the structure better, which is likely to cause a significant increase in surface area (Loow et al., 2018). The specific surface area obtained through BET was able to highlight the synergistic effects of AHP + DES pretreatment when compared with single-stage DES pretreatment.

The XRD results indicated that the CrI of raw OPFs was 37.30%. The CrI was increased to 43.85% after going through AHP pretreatment due to the capability of AHP to improve the porosity and accessibility to the crystalline structure while partially removing amorphous components (Li et al., 2016). In the case of DES pretreatment alone, the CrI reached up to 59.91%. This substantial increase in the CrI was mainly due to its ability to effectively remove amorphous hemicellulose. On top of that, this increase in CrI could be caused by the capability of DES to partially disrupt and remove the amorphous regions of cellulose (Loow et al., 2018). After undergoing the pretreatment with AHP and DES, the CrI was remarkably increased to 66.32%. It can be seen from the diffractogram of AHP + DES-pretreated OPFs that the intensity of the amorphous region decreased due to the higher delignification and hemicellulose removal (Table 3a), thus increasing CrI. Although it was observed that the AHP + DES-pretreated OPFs had a higher glucan (cellulose) content compared to DES-pretreated OPFs (Table 3a), the crystalline peak intensity for the former OPFs showed a slight reduction. This finding concurred with that of Vigier et al. (2015), where DES was claimed to reduce the crystallinity of cellulose when the cellulose was readily exposed to the DES. As such, the AHP + DES pretreatment attained the best results due to the better accessibility to the OPFs and effective removal of lignin and hemicellulose. This claim was validated by the noticeable increase in CrI after the sequential pretreatment, indicating the higher exposure of cellulose, which would potentially enhance enzymatic hydrolysis (Li et al., 2013).

The chemical functional groups in OPFs after the pretreatments were identified using FTIR. Hydroxyl groups and β-glycosidic linkages in the cellulose represented by peaks at 2900 cm^{-1} and 900 cm^{-1} respectively, were increased after DES and AHP + DES pretreatments. This result suggested that more cellulose in OPF was exposed as a result of DES pretreatments. Under the recommended conditions (Table 1), the dissolution of cellulose was unlikely, while only lignin and hemicellulose were selectively targeted in both AHP and DES pretreatments (Loow et al., 2018; Mittal et al., 2017). Moreover, the changes were detected for peaks at 1031 cm^{-1}, indicating the existence of the CO stretching vibration in the OPFs (Ofori-Boateng and Lee, 2014). When comparing with raw OPFs, significant increases in the peaks at 1031 cm^{-1} for both DES and AHP + DES-pretreated OPFs were showed, which implied the significant enrichment of cellulose. Nonetheless, the most drastic changes were peaks representing hemicellulose and lignin. The presence of aryl–alkyl ether bonds (C—O—C) was shown by the peak at 1235 cm^{-1}, and it showed noticeable reduction especially after DES pretreatments, highlighting the capability of DES (ChCl:$CuCl_2{\cdot}2H_2O$) in the cleavage of aryl–alkyl ether bonds. Moreover, wavenumbers from 1508 to 1600 cm^{-1} representing aromatic skeletal C═C in the lignin aromatic ring (Loow et al., 2018), were found to flatten for OPFs that underwent the DES pretreatment. This observation was due to the ability of DES to form hydrogen bond with the lignin and other phenolic compounds, leading to lignin dissolution. On top of that, the results also confirmed that the mild conditions AHP employed in this study had a minimal effect on the phenolic ring, while the delignification was mainly attributed to the β-O-4 cleavage (Ho et al., 2019b). As a result of effective hemicellulose dissolution (Table 3a), almost complete disappearance of the peaks at 1735 cm^{-1} were observed after DES and AHP + DES pretreatments, denoting the breakdown of the C═O acetyl group in hemicellulose (Loow et al., 2018).

4. Conclusion and future prospects

AHP was found to improve the delignification of DES in sequential pretreatment, but little effect was observed on the sugar recoveries. The combination of AHP and DES at the recommended conditions achieved a delignification of 55.14% as well as xylan and arabinan removals up to 80.79 and 98.02%, respectively. Even though DES may seem to be a promising solvent, it is also worth studying the sustainability aspect of the process using life cycle analysis, exergoeconomics or their combinations. More in-depth research on DES optimization, recyclability, and cost-benefit feasibility is also needed to realize its application on an industrial scale.

CRediT authorship contribution statement

Mun Chun Ho: Investigation, Validation, Writing - original draft. **Ta Yeong Wu:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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